SEMINAL GENOMIC TECHNOLOGIES:

ILLUMINA, INC. & HIGH-THROUGHPUT SNP GENOTYPING BEADARRAY TECHNOLOGY

A CASE STUDY

by

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Date: 19 November 2007

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Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Molecular Genetics & Microbiology in the Graduate School of Duke University

2007
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List of Abbreviations

Allele Specific Oligonucleotide .......................................................... ASO
Allele Specific Primer Extension ......................................................... ASPE
Applied Biosystems ........................................................................... ABI
Association of University Technology Managers ............................... AUTM
Charged-Coupled Device ................................................................. CCD
Cystic Fibrosis Transmembrane Conductance Regulator ................. CFTR
Department of Defense ...................................................................... DOD
Dexoyribonucleic Acid ...................................................................... DNA
Dideoxynucleotide Triphosphate ....................................................... ddNTP
Food and Drug Administration ......................................................... FDA
General Accounting Office ............................................................... GAO
Genome Wide Association ............................................................... GWA
Human Genome Project .................................................................... HGP
Human Leukocyte Antigen ............................................................... HLA
Health, Education, and Welfare ......................................................... HEW
Information Technology ................................................................... IT
Initial Public Offering ........................................................................ IPO
Institutional Patent Agreement ........................................................ IPA
International Haplotype Mapping Project ........................................ HapMap
Introduction

Innovation drives scientific research. In the field of genomics, technological developments have enabled biomedical discoveries to pave the way towards diagnostic testing and personalized medicine. Since the invention of the microarray in the 1990s, genomic technologies such as high-throughput DNA sequencing, genotyping, gene expression profiling, proteomics, and metabolomics have taken the world by storm.

I came to Duke in fall 2006 to pursue a graduate career in pharmacogenomic research. I wanted to understand how the discovery of genetic variants could predict an individual’s likelihood of developing a disease or metabolizing a drug. Shortly into my research, I found that I could no longer perform laboratory research. This was due to a respiratory allergy that I had developed to a common laboratory chemical. It was an ironic twist of fate that my interest in personalized medicine caused me to end my laboratory career because of a personal predisposition to chemical sensitivity.

I began to consider other options for my graduate career that would enable me to use my interests in personalized medicine in a manner outside of laboratory research. I became interested in the process by which genomic-based medicine was becoming a realistic part of the healthcare system. Specifically, I wanted to know how the technological advances
were being used to create personalized profiles of individuals for medical treatment. This curiosity led me to create a graduate project to investigate one particular technology, high-throughput single nucleotide polymorphism (SNP) genotyping, which has made possible genome-wide scanning for individual genetic variation. The information obtained from an individual’s genome-wide SNP scan can be pooled with the information of thousands of others to generate hypotheses of individual sites of genetic variation correlating to disease predisposition.

High-throughput SNP genotyping is one of many key genomic technologies that have become a focal point for research projects (and investor dollars) in recent years, largely due to the completion of the Human Genome Project and the subsequent excitement for a potential wave of personalized medicine. The technology encompasses scanning of an individual’s sample of genetic material, such as blood, followed by an assessment of the identity of several thousands of nucleotide bases within the individual’s genome with a high degree of variability. The identity of the specific genetic variant at each position within the genome can provide information regarding an individual’s disease predisposition and ancestry. Many companies have sprung up to develop platforms that can accurately detect and analyze genetic material from individuals to uncover such exciting information. However, the challenge to accurately detect variation at the resolution of a single nucleotide base in the context of the whole human genome has prevented many companies and individual researchers from succeeding.
This case history began as an inquiry into the development of Illumina’s BeadArray technology, which is regarded as state-of-the-art for its high-throughput SNP genotyping capabilities. Further investigation into this invention uncovered something as distinctive as the technology itself: the series of propitious events that led to the creation and disclosure of this technology by a researcher at an academic institution. The story of the development of the core Illumina technology is one that can be used to illustrate “best practices” of academic invention disclosure, technology transfer to a startup, and marketing due diligence.

Successful development of a core genomic technology began with the insights of an academic scientist with an entrepreneurial spirit who took all the necessary steps to see that his invention was appropriately commercialized. David Walt’s scientific innovation of a bead-based fiber optic array that can accurately detect small molecules was the core technology on which Illumina was built. The uniqueness of the invention enabled Illumina’s initial competitive advantage. Walt’s invention and disclosure of the core technology were critical contributions, and his role as consultant for the overall business plan of Illumina also ensured his continued engagement in the commercialization of his invention. The decisions he made to protect his inventions without hindering ongoing research projects idealize how successful technology transfer on the part of the academic scientist should be executed.
David Walt can be classified as a “star scientist”, based on the criteria originally designed by Zucker & Darby [1]: he had a prestigious faculty position, was author or co-author on several academic articles, and named inventor or co-inventor on many patents prior to his seminal invention. After the invention of his bead-based fiber optic technology, Walt played an integral role in overseeing its development into a commercial product. This role came in the form of both direct consultation to the startup, Illumina, and by the transfer of his laboratory members, such as postdocs and former graduate students, to work as full-time employees for Illumina.

Fiber optic cables have been used as sensors in a variety of applications\(^1\), although the coupling of beads with etched fibers to generate a diverse array for chemical detection was a wholly unique idea. Moreover, this invention had important applications to the scientific community.

The application of the technology was a critical factor to its initial success. David Walt and John Stuelpnagel, a venture capitalist from Channing Weinberg Group, carefully considered the competitive landscape before entering the genomics market in the late

\(^{1}\) Applications of fiber optic sensors include uses as hydrophones for seismic or SONAR capabilities, monitors of temperature and pressure, and uses as optical gyroscopes.
1990s. The characteristics of Walt’s invention provided a level of flexibility, scalability and throughput for genotyping studies that was previously unknown.

The exclusive license that Tufts University, the academic institution from which the invention hailed, attracted sufficient venture capital funds to successfully start a company based on the core technology. Venture capital funding, in turn, leveraged a public stock offering, which provided sufficient funds to purchase other technologies. Since the company’s inception, it has consistently updated its platforms to advance the technological state-of-the-art for conducting genetic studies. The following chapters will cover the history of the inventor, the technology and the company to investigate how these three components have delivered a powerful method for performing high-throughput SNP genotyping studies.

Chapter 1 will discuss the research methodology used to perform this case study. Chapter 2 will discuss the history of conducting studies to understand genetic variation. It will conclude with a discussion of Illumina’s state-of-the-art of genotyping technology. Chapter 3 will discuss the process by which universities have learned to patent and license their technologies. The chapter will include an overview of the trends in university patenting and licensing before and after the passage of the Bayh-Dole Act of 1980. The chapter will conclude with a history of tech licensing at Tufts University. Chapter 4 will discuss the events that led up to David Walt’s disclosure of the bead-based
fiber optic sensor array. It will discuss his motivations for patenting the invention. The chapter will also include an overview of the fiber optic technology. Chapter 5 will begin with the historical account of the founding of Illumina. It will proceed to highlight the events in Illumina’s ten-year history. Chapter 6 will seek to describe how each part of the story contains lessons for successful technology transfer. The chapter will begin with the proposal that David Walt exemplifies the ideals of a “star scientist” in his commitments to both academic and entrepreneurial activities. The chapter will highlight some key issues, such as due diligence, knowledge of intellectual property, venture capital financing, government funding and timing that enabled Illumina’s success. It will also discuss the publications and patents that Illumina has produced. Importantly, Chapter 6 will discuss the impact that the Illumina platforms have had on academic research.
1. Research Methodology

The nature of the research topic lent itself to an initial qualitative research approach. Interpretation of the impact of the technology required additional investigation through quantitative methods. In order to get an initial scope of the project, I first began by searching the Illumina website to learn about the technology and seek an insight about its origins. I then set up telephone interviews with the appropriate personnel. I consulted primary research articles describing the core technology from David Walt’s laboratory, the various Illumina assays, and the publications that have used Illumina products. I then used various databases to construct a framework of the impact of the technology transfer.

1.1 Website Searches

The Illumina website (http://www.illumina.com) provided the initial information used to begin my project. On the history page of the website, there was a mention of the core technology deriving from David Walt’s Tufts University laboratory. From there, the Securities and Exchange Commission (SEC) files (also available on the Illumina website) described an exclusive license on the core technology from Tufts.

The Illumina website also provided useful information on the Illumina products and services, as well as events that have occurred since the Company was incorporated. The publications section of the website listed the publications describing Illumina’s assays, as
well as the publications that have been produced from other institutions as a result of using Illumina products.

Additional websites accessed included:

- David Walt’s website at Tufts University: http://ase.tufts.edu/chemistry/walt/
- The International HapMap Project website: http://www.hapmap.org

1.2 Personnel Interviews

The following telephone interviews were conducted in order to understand (1) the motivations of various parties and sequence of events that led to the development of the bead-based array technology, (2) the decision to exclusively license the technology, and (3) the creation of Illumina, Inc. All of these interviews were approved by an Institutional Review Board. The interviewees were read a letter of informed consent at the beginning of each recorded interview. The recorded files and written transcripts of these interviews can be obtained by request through the Center for Genome, Ethics, Law & Policy at Duke University, Durham, North Carolina.

- On 27 July 2007, Bob Cook-Deegan and I spoke with Nina Green and Martin Son of the Tufts University OTLIC. Our conversation focused on the nature of the exclusive license granted from Tufts to Illumina.
• On 31 July 2007, Bob Cook-Deegan and I spoke with Marc Goldberg of BioVentures Investors. He was the founder, President and CEO of the MBRI. He recounted the initial events that led to the creation of Illumina through the exclusive license of David Walt’s inventions at Tufts University.

• On 7 August 2007, Shubhashini Chaskehandaran and I spoke with Tufts University Vice Provost Peggy Newell. Newell was charged with developing an independent technology transfer office for Tufts. She gave us her thoughts on how successful technology transfer should occur, from a university perspective.

• On 16 August 2007, Bob Cook-Deegan and I spoke with Illumina co-founded and COO John Stuelpnagel and Senior Vice President of Marketing Todd Dickinson. Stuelpnagel gave us a historical perspective on the development of Illumina since before the Company’s inception. Stuelpnagel also provided us with the motivations behind the various strategic moves that the Company has made in order to grow and diversify its capabilities.

• On 31 August 2007, Bob Cook-Deegan and I spoke with David Walt, inventor of the core Illumina technology. Walt gave us an in-depth insight into the development of the bead-based sensor technology and his motivations for sequence of events leading to the disclosure and licensing of the technology.
1.3 Database Searches

In order to track the number of grants, publications and patents that David Walt and Illumina have received, the following databases were accessed through the Duke University Library server:

- **CRISP**: The CRISP (Computer Retrieval of Information on Scientific Projects) database – a database of biomedical research federally funded by the National Institutes of Health – was used to obtain grant information for David Walt and various principal investigators at Illumina. The names of the principal investigators at Illumina were selected based on Illumina publications and news releases. The CRISP database gave a brief description of the project funded and the duration of the funding period, but did not provide information on the amount of money granted.

- **RaDiUS**: The RAND Database of Research & Development in the U.S. (RaDiUS) was accessed to gain information on federal funding granted to Tufts University and Illumina, Inc. from other funding agencies, but also including the NIH. The RaDiUS database contained information on the funding amounts provided per year and a brief description of the project funded.

- **DELPHION**: The Delphion Research intellectual property database was used to obtain patent information on both international and US patents held by David Walt, Illumina, Solexa, CyVera, and Affymetrix. The database provided complete records of patents, including assignee information, grants used to fund the intellectual property, patent claims, and forward and backward references for the patents.
- **SCOPUS**: The SCOPUS abstract and citation database (available through subscription by Duke University Library) was used to access both journal articles pertaining to the Illumina technology, as well as citation information on the publications produced by David Walt and Illumina researchers. This database was also used to track the human intellectual capital transfer of scientists from David Walt’s lab to Illumina (Chapter 6).

- **ISI Web of Science**: The Thomson Corporation’s ISI Web of Science database was accessed through subscription by the Duke University Library to track citation information on author and technology publications. Citations per publication per year were tracked using this website as displayed in Chapter 6. Additionally, impact factors for different scientific journals were obtained through the database’s Journal Citation Report feature. The database was also used to access articles describing key findings in the history of the development of technology to detect genetic variation, as described in Chapter 2.

- **ABI/INFORM**: The ABI/INFORM database was also accessed through subscription by the Duke University Library in order to obtain business news articles on Illumina.

- **Lexis/Nexis**: The Lexis/Nexis database was accessed (also through Duke University Library) in order to obtain articles pertaining to Illumina, including information regarding legal cases and business cases, as well as articles pertaining to university technology transfer and the Bayh-Dole Act.
2. A history of identifying genetic variants

2.1 Technological advances enable scientific discoveries

Genetic variation is the fundamental reason for differences among groups as large as species, populations and individuals, and as small as minute functions of enzymes. Detection of variation at the level of the genome has been approached from many angles over the past 150 years. In the last decade alone, the rapid expansion of technology has exponentially increased the amount of information that can be obtained about genetic variation in less time and at less cost than ever imagined. This chapter aims to discuss the development of the field of high-throughput genotyping, from its origins in genetic trait analysis with pea plants to the development of the Illumina 1M BeadChip and Infinium™ whole genome SNP genotyping assay – industry’s current state-of-the-art.

One theme that this case study seeks to emphasize is that technology breeds scientific breakthroughs. As a technology becomes more sophisticated, discoveries relying on that technology increase in rate, quality and quantity. This chapter will cover the technological progressions in history that have changed the way biomedical research is conducted. High-throughput whole genome SNP genotyping traces its roots to Gregor Mendel, the famous Moravian monk who used his garden pea plants to understand inheritance differences.
This massive improvement has progressed in a manner not unlike punctuated equilibrium; periods of relative calm are shaken up by a technological improvement. Statistics-based studies dominated the field of inheritance until Thomas Hunt Morgan observed the physical crossing over of chromosomes during recombination through his microscope [2]. Genetics research took a new turn in the 1950’s when Oliver Smithies developed a gel-based mechanism to observe molecular differences. It was not until the advent of polymerase chain reaction (PCR) technology in 1987 that automated technologies began to rise in popularity over “gel-based” analysis. With the study of inheritance hinging upon a convergence of science and statistics, the Information Technology (IT) revolution of the ‘70s and ‘80s led to the genetics/genomics revolution of the ‘90s and ‘00s. As a result, the majority of SNP discoveries have come out within the past fifteen years. Today, researchers around the globe use fully-automated machines developed at Illumina to run, decode and analyze over one million SNP genotypes in parallel.

It is important to understand how technological improvements dramatically enhanced the scientific community’s knowledge of genetics and genomics in order to appreciate the type of research that the Illumina platforms have enabled researchers to perform today. Thus, we turn to a historical analysis of the origins of genetic variation inquiry.
2.2 The origins of genetic variation inquiry

The first recorded Western inquiry into the causes of differences in physical characteristics among individuals was carried out in the late nineteenth century. The results of the experiments were wholly ignored until several decades later when statisticians began their own investigations into the matter. As the field of medicine emerged in the early part of the twentieth century, physicians and biochemists began to formulate their own hypotheses of disease origin, basing them on inheritance patterns. Pioneers in genetic research used model organisms to uncover the physical basis of inheritance. Through rigorous analysis, they discovered a correlation between chromosomal recombination and resultant physical characteristics. This correlation established the fundamental basis for linkage.

2.2.1 Mendel – Morgan (1865 – 1911)

Moravian monk Gregor Mendel published a largely unappreciated paper on the genetic variation among pea plants in 1865 [3]. Mendel systematically crossed varieties of pea plants – seven pairs of different varieties – and recorded the observed results. What Mendel uncovered about inheritance from one generation of plants to the next was that the characteristics within each variety do not blend; in essence, the ‘dominant’ trait of one variety would appear three times more often in the following generation than the
‘recessive’ trait. As Matt Ridley explained, Mendel had “proved the atomic theory of biology” [4].

The discovery of chromosomes by Walter Flemming in 1879, and the investigation, by Hugo DeVries, Karl Correns and Erich Tschermak, of the “nuclear component[s]” integral to inheritance paved the way for further examination of Mendel’s postulations [2]. At the turn of the twentieth century, cytologists Walter Sutton and Theodor Boveri independently stated that chromosomal segregation during meiosis could serve as guiding force behind inheritance patterns [2]. Thus, it was thought that the chromosomes themselves, not the genes encoded with the chromosomes, were the “unit factors” that Mendel claimed to control genetic inheritance [2].

It was not until thirty-five years after Mendel’s publication when Archibald Garrod, who was studying the inheritance patterns of alkaptonuria in families in London, revisited Mendel’s theories of inheritance in the context of human disease. Garrod observed that alkaptonuria afflicted individuals within families, but not necessarily individuals in every generation. Using his knowledge of chemistry, Garrod surmised that the disease was caused by the inheritance of a defective catalyst, or enzyme, that was unable to break down a natural byproduct of metabolism. Individuals who had this disease inherited the defective protein from both of their parents – a classic Mendelian ‘recessive’ trait [4]. Thus, Garrod proposed that ‘inborn errors in metabolism’ observed in humans obeyed the
same laws that Mendel observed with his pea plants [5-8]. The “unit factors” that were transmitted from parent to offspring contained information controlling chemical reactions within the body; individuals who inherited defective unit factors would develop a disease [2]. Garrod’s work coincided with William Bateson’s 1909 publication on *Mendel’s Principles of Heredity*, in which Bateson coined the “unit factor” as a “gene”, and variations of the gene – what Mendel referred to as the ‘dominant’ or ‘recessive’ forms – as “alleles.” Bateson proposed that disease inheritance was caused by a lack of a “critical substance” [2].

Around that same time, German physician Wilhelm Weinberg and British mathematician Godfrey Harold Hardy concluded independently that the genetic makeup of populations could be predicted based on the statistical distribution of frequencies of the ‘dominant’ and ‘recessive’ variations of different traits [9, 10]. The variations of the traits, Bateson’s “alleles”, were observed to be inherited in a predictable fashion from generation to generation, barring external forces, such as selection, mutation or migration [11]. Statisticians Francis Galton, a cousin of Charles Darwin, and his student Karl Pearson further denoted that some traits were not inherited in a Mendelian fashion, but rather, the traits appeared in the offspring as a ‘blend’ of those from both parents [6]. (Galton was also known for his support of eugenics, a movement which he named, that attempted to control human matings by encouraging individuals with positive traits to create large families, while simultaneously preventing individuals with “negative traits” from
reproducing [2]). These contributions, as well as those by G. Undy Yule and William Castle, formed the basis for the study of the frequencies of alleles: modern-day population genetics [2].

The statistical evaluation of patterns of genetic inheritance along with the concurrent investigation of the chromosomal theory of heredity drove early geneticist Thomas Hunt Morgan and his undergraduate student, Alfred Sturtevant, to uncover the sources and frequencies of gene separation during meiosis [2]. Morgan’s and Sturtevant’s experiments with *Drosophila melanogaster* (fruit fly) X chromosomes revealed that genes existed in a linear order along a chromosome and that exchange of genetic information can occur between the pairs of homologous (identical) chromosomes during meiosis. Morgan suggested that genes in close proximity were more likely to segregate in concert than genes separated by a greater distance [2, 12, 13]. These experiments made way for the beginnings of linkage studies and map-based analysis of genetic inheritance.

### 2.2.2 Linking genes (1918 – 1954)

As the study of genetic transmission and inheritance progressed, it became clear that the results of Mendel’s experiments were so compelling because of his fortuitous selection of the traits themselves: Mendel unknowingly chose to study traits that were associated with only one gene. The presence of the ‘dominant’ allele in one or both copies of the gene
within a plant generated the dominant trait, otherwise known as the ‘wild-type’ phenotype; the presence of two copies of the ‘recessive’ trait resulted in the ‘recessive’, or ‘mutant’ phenotype. While the phenotype referred to the observed variation of a trait, the genotype referred to the underlying genetic composition of the alleles that caused the physical variation. Mendelian traits could easily correlate a genotype to a given phenotype. But genes were soon identified that had more than two variations of an allele (such as the ABO blood groups by Nobel Laureate for Medicine Karl Landsteiner in 1900 [14]) and traits were found to be controlled by more than one gene (or locus). In 1918, Ronald Fisher resolved the previous debates on inheritance in humans between Garrod and Galton and Pearson when he published his findings on ‘polygenic’ inheritance patterns. Such patterns, he determined, were controlled by the underlying genetic basis of the trait [6].

Further investigation into ‘polygenic’ traits led Fisher and his colleague, Robert Race, to uncover the genes that controlled the production of the Rhesus antigen in 1947 [2]. Through the first demonstration of linkage disequilibrium mapping, Fisher uncovered the order of loci associated with Rh antigen production based on the allele frequencies. He concluded that the types of alleles that got inherited (based on the outcomes of meiotic recombination) determined the presence or absence of the Rh antigen [15].
Linkage disequilibrium (LD) mapping techniques were expanded to understanding the variability of human leukocyte antigen (HLA) proteins produced from the major histocompatibility complex region on chromosome 6. Add-on experiments from the original investigation of the HLA region led to the association of the region with immunological-related diseases [16]. In 1954, the first study linking a marker to a human disease, myotonic dystrophy, was performed [17]. The demonstration that LD could be used to associate a disease with a polymorphic locus gave rise to many association studies that are still performed today [16].

2.2.3 Satellites, SNPs and Southerns (1955 – 1987)

Molecular polymorphisms were originally limited to those that could be assayed with immunogenetic techniques until 1955, when Nobel Laureate Oliver Smithies developed starch gel electrophoresis to investigate polymorphisms within the alpha-globulin locus [18]. By the 1950s, it was known that protein polymorphisms existed, and it was assumed that these variations derived from the underlying nucleic acid sequence [16]. Clinical studies documenting correlations between adverse reactions to a variety of drugs and metabolic deficiencies during that same time also suggested that the cause was due to genetic variation [19-21].
The development of the Southern blot in 1975 finally provided researchers with the technology to visualize polymorphisms at the DNA level and thus confirm that variation was not limited to the coding region of a gene [14, 22]. Detection of DNA variants was achieved by David Botstein and colleagues through the discovery of restriction fragment lengths polymorphisms (RFLPs) on Southern blots of genomic DNA [14, 23]. The technique exploited the ability of restriction enzymes to cleave DNA at very specific sequences (generally 4-6 bases in length). The presence or absence of a cleavage site would change the size of the band that was visualized on the gel and indicate a polymorphic site at that specific sequence. In 1978, Yuet Wai Kan and A.M. Dozy applied RFLP analysis to patient blood samples to uncover a ‘marker variant’ that linked it to the mutant sickle cell gene [24, 25]. The researchers concluded that RFLP analysis could be a tool to track the indirect inheritance patterns of the genes in families. That same year, Alec Jeffreys at the University of Leicester reported that the new technique could be used to study genetic variation among human populations [23].

By 1980, Botstein and colleagues had generated a map of the human genome that used RFLP analysis to set the basis for establishing linkage of DNA variants to genes [26]. The RFLP analysis technique, however, only captured a small percentage of genetic variation [23, 27-29]. One gene locus of particular interest was the β-globin locus, due to its role in sickle cell anemia. The conserved yet variable short sequence motifs were identified around the β-globin locus in 1981, although the significance of the findings
was not fully understood at that time [30-32]. By 1982, Hiroshi Hamada and Takeo Kakunaga at the National Cancer Institute found highly-repetitive alternating pyrimidine-purine bases within the human genome that had the potential to form Z-DNA [33].

In 1985, Jeffreys identified a second ‘common’ class of restriction fragment length polymorphic markers defined by a “variable number of tandem repeats” (VNTRs) [34]. These markers were similar to the larger repetitive regions of DNA – albeit much smaller in overall length – and contained a greater degree of variability at each locus, with up to one hundred alleles per site [14]. This ‘minisatellite’ discovery predated the discovery of the ‘microsatellite’, or dinucleotide repeats, by Weber and May in 1989 [35]. Dinucleotide repeats have since been located randomly throughout the genome in up to thousands of copies and demonstrate a high level of variability at each locus, allowing variability to be scored by polymerase chain reaction (PCR) [14, 36].

The use of microsatellite markers quickly became a popular tool for genetic analysis. Each individual was assumed to have a unique pattern of restriction sites at a particular genomic region. This pattern, known as a haplotype and used a proxy for a smaller genotype, was originally used to define the major histocompatibility locus. Its utility has since been extended to describing the patterns of alleles found within an individual genome [37].
2.2.4 Genome Revolution

The Human Genome Project (HGP) officially launched in October 1990 [25]. Techniques for sequencing large units of human DNA had been successfully demonstrated through a number of methods. HGP researchers used a combination of approaches to work on their assigned sections of the Project.

However, the title of the HGP implied that only a single version of the human genome existed [38]. Scientists realized that results obtained from the initial sample sequences would have to account for individual variation, as there was no one unique sequence for the entire human species. This fact motivated researchers to develop novel techniques to detect genetic variation with a high degree of throughput.

Between 1990 and 2002, scientists were beginning to carry out important studies to understand genetic variation, but the methods use to screen for variation were not capable of handling the scale or accuracy required of a genome-wide, international effort. In order to meet the demands to perform quality studies on genetic variation, it was necessary to determine what characteristics the state-of-the-art technologies would encompass. In a review of genotyping techniques, Pui-Yan Kwok succinctly delineated such criteria for a successful high-throughput whole genome SNP genotyping assay to become marketable: a) the assay must be easily and quickly developed from sequence information; b) the cost of development must be kept low in terms of both ‘marker-specific’ reagents and
manpower; c) the reaction must be robust, successful for even suboptimal samples; d) the assay must be automated with little human intervention; e) the data analysis must be simple, with automated, accurate genotype calling; f) the reactions format must be flexible and scalable, capable of performing a few hundred to a million assays per day; and, g) once optimized, the total assay cost per genotype (including equipment, reagents, and personnel) must be low [39]. Techniques that met these requirements were developed over time through a series of studies.

### 2.2.5 Enzymes in action

This section reviews the history of the technological approaches developed to detect variation and the systems built based on those detection methods. The methods for delivering high-throughput genotyping results have been based largely on the incorporation of microarray technology, which came out of the invention to detect nucleic acid sequences through exploitation of nucleotide base pairing.

In 1979, Wallace et al. demonstrated a technique to hybridize allele-specific oligonucleotides (ASOs) to DNA to detect single-base mismatches. The medical application of this technique was revealed four years later when a polymorphic site was uncovered in the beta-globin gene that predisposed carriers of the minor allele to sickle-cell disease [40]. Although the Southern blot could be considered the first official array
of any kind, oligonucleotide microarrays enable an unparalleled level of high throughput [41].

The invention of polymerase chain reaction (PCR) by Kary Mullis and colleagues in 1987 profoundly impacted studies of genetic variation [42, 43]. The technology enabled the detection of SNPs within the context of whole genomes for the first time [44]. The following year, Leroy Hood and colleagues at Caltech developed a solid-phase assay for DNA ligase-assisted genotyping [45]. The new technology was the first to demonstrate that an enzyme could be used as a tool to detect SNPs and paved the way for the modern-day oligonucleotide ligation assay [44].

In 1989, Cetus Corporation (Sunnyvale, CA) researchers exploited the capabilities of the new in vitro DNA amplification technique to generate one of the first immobilized sequence-specific oligonucleotide probe arrays to detect genetic variation [46, 47]. The format, known as ‘DNA reverse dot blot’, allowed oligonucleotides spotted onto nylon membranes to hybridize to DNA amplified with biotinylated primers. Sequences that hybridized to the oligos were detected nonradioactively through the binding of streptavidin-horseradish peroxidase followed by a colorimetric reaction. Alleles of the β-globin gene and the HLA locus were detected through this format to demonstrate proof-of-principle [46]. Also that year, add-on improvements to PCR technology enabled allele-specific amplification and genotyping of SNPs [48].
As the Human Genome Project began its official launch, data from linkage studies identifying genes responsible for monogenic disorders such as cystic fibrosis (1989), fragile-X syndrome (1991) and Huntington’s chorea (1993) emerged, further strengthening the support for larger throughput studies [49-52]. In 1990, an assay to detect SNPs through single nucleotide primer extension using DNA polymerase on a solid-phase platform was introduced. The technology incorporated a “double-labeling strategy”, whereby colorimetric detection on microtiter plate wells and duplex detection of two SNPs significantly increased the accuracy of detection [53]. The enzyme-assisted techniques using DNA ligase and DNA polymerase proved to be more robust with a greater sensitivity to single nucleotide variation than ASO hybridization [54]. That same year, another group of researchers proposed an ELISA-based oligonucleotide ligation genotyping assay [55]. Much of the technological bases for the high-throughput SNP genotyping platforms today employ similar enzymatic-linked steps.

### 2.2.6 Advances in PCR technology

PCR technology enabled rapid, low-cost detection of microsatellite polymorphisms [35]. By 1992, microsatellites markers successfully derived the first map of the human genome [56], and by 1994, microsatellite markers led researchers to develop a more comprehensive linkage map such that the resolution was at the centiMorgan-level [57-
The linkage maps enabled the mapping of simple traits, but gel-based technologies were neither easily automated nor capable of handling the throughput required to investigate complex diseases that required a large number of individual samples [60].

PCR technology also drove the invention process of other genotyping assays. In 1991, Holland and colleagues exploited the nick-translation 5’ nuclease function of Taq polymerase to generate assays that incorporated fluorogenic probes containing both a reporter dye and a quencher dye into the PCR amplification step; when the probe hybridizes to its target complementary sequence, Taq polymerase will cleave the probe, releasing a fluorescent signal [61]. The assay was further refined by Lee and colleagues at Applied Biosystems who used probes containing two different reporter dyes to distinguish between the mutant and wild type alleles of the human cystic fibrosis gene [62].

2.2.7 SNPs as markers

As researchers sought alternative higher throughput methods to assess variation, SNPs as genetic markers became their target. SNPs have low polymorphism rates on par with RFLPs, but their sheer abundance and ability to be assayed more easily than microsatellites propelled SNP genotyping platform innovations. The biallelic nature of SNP markers – having only two variations per SNP – enabled the development of
‘plus/minus’ assays that broke away from the earlier length measurement methods [60]. Kenneth Livak and colleagues at Applied Biosystems modified the probe design protocols by Lee et al. described above such that the two alleles of the human insulin gene (INS) – designated as type 1 diabetes-associated, or ‘+’, allele, and the non-associated ‘−’ allele – could be detected by a single A-T base substitution [63]. Furthermore, their novel method incorporated multicomponent analysis – a technique that sorted fluorescent data from samples into three clusters based on the intensities of the different dyes. The clusters served as proxies for the different genotypes – homozygous for either allele (AA or BB) or heterozygous (AB) [63]. This new method for detecting genetic variation enabled the development of the lower cost, higher throughput techniques that Kwok stated would be necessary for large-scale genotyping studies.

2.3 Microarrays in the mainstream

Although non-gel-based assays emerged in the late 1980s/early 1990s [53, 55, 63-65], those assays that held the most potential for large-scale SNP genotyping were developed in the mid-1990s: the high-density parallel oligonucleotide arrays [66, 67]. The concept of the microarray was introduced by Edwin Southern of the University of Oxford as a way to run parallel genomic analyses [68]. Microarray technology was developed as a method to perform intrinsically-parallel assays (known also as gene-expression profiling assays) to detect levels of mRNA [68-73]. These intrinsically-parallel platforms for
whole-genome genotyping, epigenetic profiling and sequencing are still being used today [69, 73, 74].

The earlier ‘high-throughput’ assays (i.e., immunoassays, enzymes assays, DNA reverse dot blots) required manual preparation and suffered from limited miniaturization potential. In 1991, Affymax researchers developed a technique to synthesize chemicals onto a solid support, enabling the creation of fluorescently-labeled oligonucleotide probe arrays [75]. The power of this novel format was enhanced two years later when Stephen Fodor and colleagues coupled the arrays to a laser confocal fluorescent scanning detector. The knowledge of the location of specific oligo probes combined with the ability to accurately measure the fluorescence intensity generated when the probes bound to the target DNA sequences created a potent multiplex assay for a variety of biological applications [70, 76].

Variations of the microarray fabrication included the immobilization of pre-synthesized oligonucleotides onto a solid surface [77] and into a three-dimensional gel microchip [ref. 78]. Other popular techniques included the immobilization of the DNA itself to a solid surface followed by exposure to a set of labeled probes either individually [79] or within a mixture [71]. These techniques and others were particularly successful in assaying gene expression variation, the first type of genomic assay to become ‘parallelized’ [69, 80].
2.3.1 Creating microarrays for genotyping

Gene expression profiling was the earliest of the genomic-based assays to achieve parallel design [81-83]. The challenges of running genotyping assays in parallel rested on the fact that resolution had to be achieved at the single nucleotide level [69]. This required the detection of analytes at low concentrations in the context of the whole genome [69].

Geneticists, known for rallying around one organism or system to understand complex processes, chose the cystic fibrosis gene (CFTR) as one of the model genes on which to develop SNP genotyping techniques. This was primarily because cystic fibrosis is a monogenic recessive disease, with over 500 various mutation types [84]. Up until that point, genotyping experiments were conducted via a two-step process: the regions around the mutation of interest were initially amplified by PCR, and then the mutation itself was characterized by some mechanism, including restriction fragment sizing [85], ASO hybridization [86], denaturing gradient gel electrophoresis [87], and single-stranded conformation polymorphism [84, 88]. Other techniques incorporated allele specific primers directly into the PCR step [89, 90].
2.3.2 Affymetrix advances

In 1996, several innovations and discoveries at Affymetrix advanced the field of SNP genotyping. The light-generated DNA probe arrays made by Affymetrix researchers were modified to detect CFTR gene mutations through two separate approaches. The tiling array was comprised of 428 overlapping sets of four oligonucleotide probes designed to scan one exon of the gene, such that each set of four was identical except at a single base position. The probe that emitted the strong hybridization signal would identify the target DNA base at that variable position. The second approach was a mutation array of over 1,480 probes run in parallel pairs, one probe matching the complementary wild-type gene sequence, the other, the mutant gene sequence. The experiment demonstrated the analytical power that probe arrays gave researchers [84].

That same year, Affymetrix researchers successfully analyzed the entire human mitochondrial genome for sequence polymorphisms. They achieved this unprecedented accomplishment through exploiting DNA hybridization as an event that could occur in parallel over a large genomic sequence. While recognizing that either the target DNA sequence itself or the oligo probes could be immobilized, the researchers opted to generate an ‘ordered,’ tiled array. The highly scalable, automated technique abolished the need for gel analysis, through a two-color labeling scheme that quickly analyzed the sequences. The only limiting factor of the array was the physical distance that the photolithographic process needed to lay down the probes [91]. 1996 was also the year
that Affymetrix produced its compact, high-density array of 65,000 20-mer oligonucleotides for gene expression – its first version of the company’s GeneChip[81]. Researchers had also begun to experiment with electromagnetic properties to increase the hybridization rate 10-fold [92].

2.3.3 Choosing from the state-of-the-art

By the end of the year, there were three competing methods to perform high-throughput genotyping analysis [93]: the gain-of-hybridization signal approach, the loss-of-signal approach, and the enzyme-dependent minisequencing approach. The gain-of-signal, as described above [84], compared hybridization signals relative to reference samples, such that a ‘gain’ indicated a sequence change. The loss-of-signal approach, also described above [91], quantified the relative loss of hybridization signal to perfectly match oligonucleotide probes in query samples relative to wild-type reference targets. This approach was used by Hacia et al. to genotype BRCA1 mutations within the gene’s 3.45 kb exon 11 with over 96,600 oligonucelotide probes [94].

The enzyme-dependent minisequencing approach represented an alternative, powerful approach to assay genetic variation through the exploitation of probe hybridization followed by enzymatic primer extension [54, 95-97]. Labeled dideoxyribonuceloside triphosphates (ddNTPs) – each of the four bases corresponded to a unique dye – were
added to the arrays once the target DNA hybridized to the 5’-tethered oligonucleotide probes. The ddNTPs incorporated themselves to the final 3’OH position of the oligo probes and emitted a fluorescent signal that was detected and analyzed [54].

Each of the three methods had its drawbacks. Overall, the approaches required complex screening processes and suffered from inaccurate calls, with ‘modest’ false negative error rates. These complications posed the greatest challenges [93]. Furthermore, the techniques were not suited for detecting mutations in highly repetitive regions or regions with significant secondary structure [98]. Hacia outlined several proposals to improve array hybridization, specificity, sensitivity and accuracy [93].

By 1997, over 1,000 ‘PCR-amplifiable SNP markers’ had been uncovered and mapped [60]. Researchers at the University of Minnesota generated mathematical models for linkage-disequilibrium mapping that took into account the discoveries of ‘multilocus and multiallelic markers and mutational processes at the marker and disease loci’ [99]. Their models further enabled researchers to predict locations of disease genes, as demonstrated by data for cystic fibrosis, Huntington’s disease, Friedreich ataxia and progressive myoclonus epilepsy.

Positional cloning – the process of identifying genes responsible for a phenotype through the physical cloning of a linkage region – was a common – albeit, laborious – technique
to identify DNA polymorphisms in family-based genetic studies [100]. Linkage mapping provided insight into the chromosomal region responsible for a particular disease, although the regions had the potential to be megabases in size. As such, researchers understood the limitations that positional cloning posed in mapping ‘multifactorial’ diseases. The success of understanding such complex traits and diseases lay in the identification of more DNA markers and the development of technology to screen large populations [14].

2.3.4 Reemergence of association-based research

Interest in genome-wide association studies (GWA) increased in 1996 when Neil Risch and Kathleen Merikangas praised the approach for its superior power over linkage analysis in examining genetic variants with modest phenotypic effects [101]. Genome-wide association studies could be performed by direct or indirect approaches [102], although the direct strategy required the identification of all human genes and common variants. Furthermore, the direct method was limited by the lack of sequence knowledge of non-exonic (i.e., non-coding and regulatory) regions of genes, where, the presence of SNPs could influence a phenotype [103].

As a consequence, indirect LD-driven population studies emerged as an alternative form of genetic analysis [104]. LD studies mapped risk loci and neutral polymorphic markers
in proximity to the functional (disease-contributing) variants, in contrast to linkage studies, which identified a variant and a disease inherited in family studies. The physical proximity of the functional and neutral markers necessitated that many generations of recombination would have to occur before the markers were separated. Thus, the markers could occur within the same haplotype blocks within populations [14].

Association studies within populations held the potential to screen for ‘functionally significant’ variations and to test candidate genes for phenotype contribution [102]. Although around since the early 1970s with the investigations of the HLA loci [105, 106], association studies gained popularity in the mid-1990s when researchers used the technique to link the apolipoprotein-E ɛ4 allele with Alzheimer’s disease [107, 108]. As more association studies were performed, experimental design and results became increasingly more reliable due to the emergence of population databases from which ‘control’ populations could be established [109, 110]. Alan Schafer and J. Ross Hawkins suggested that using populations to carry out association studies could be best achieved by understanding haplotype structure within the queried population, and from there, by using a subset of polymorphisms to distinguish among haplotypes [14].
2.3.5 Technological hurdles

By the late 1990s, throughput of genetic screens was significantly augmented by the use of fluorescence technology. Single nucleotide primer extension and DNA microchip methods promised even better results [14, 54, 111-113]. Limitations to the population-based studies rested on PCR-based SNP detection and scoring methods; PCR amplification required additional steps and depended on the use of primers, which often formed primer dimers, both of which introduced room for error. The tiling arrays, although powerful, still rendered ‘imperfect’ results: homozygous variants were accurately detected, while heterozygotes were sometimes missed [41].

The first SNP map was generated in 1998 through gel-based DNA sequencing and high-density-variation DNA chips of 2.3 megabases of human genomic DNA. The sequences came from sequence-tagged sites (STSs) across the human genome. Of 3241 candidate SNPs identified, 2227 were used to construct a SNP map. The results confirmed the ability to perform large-scale SNP mapping experiments [95].

Two multi-institutional, private-public studies released in 1999 demonstrated the power of population-based studies and confirmed the need for add-on research and technological developments. Michele Cargill, David Altshuler and colleagues from MIT and Harvard Massachusetts General Hospital performed a ‘systematic survey’ of SNPs in the coding regions of 106 genes related to cardiovascular disease, endocrinology and
neuropsychiatry via two independent screening methods. The research identified 560 SNPs with varying polymorphic rates, with non-synonymous coding SNPs occurring at a lower rate with lower allele frequencies than ‘silent’ substitutions. Their data confirmed purifying selection during human evolution – fewer non-synonymous coding SNPs than predicted – and demonstrated that some SNPs occurred more frequently in certain populations. The latter results suggest that SNPs can be a marker of identification for population substructure or an indication of a predisposition to a disease. The authors concluded that more signatures of selection were necessary to ‘define the neutral rate of human DNA diversity’ and that a more comprehensive SNP database from varying populations would ‘shed light’ on human population structure [114].

Marc Halushka and colleagues at Case Western Reserve and Affymetrix, Inc. investigated the patterns, frequencies and characteristics of SNPs in 75 ‘candidate’ human genes related to blood-pressure homeostasis and hypertension using the high-density variant detection arrays (VDAs), described earlier [80, 91]. Out of 874 identified SNPs, 387 were located in coding sequences (cSNPs), of which, 54% result in changes in the amino acid sequence. Interestingly, the researchers noted that the overall number of non-synonymous cSNPs identified was 38% of the total number of cSNPs predicted based on statistical metrics; this fact implied that such SNPs undergo selection and were more likely to be specific to certain populations [115]. Gene-based SNPs scans confirmed that SNPs are the ‘etiologic’ cause of disease-susceptibility differences. The study
reinforced the need for more accurate and cost-effective assays for a great collection of SNP surveys in varying populations [115, 116].

2.3.6 Alternatives to GeneChips

Although the Affymetrix GeneChip appeared to be the most successful commercial chip-based array to perform SNP genotyping assessments during the 1990s, it was not the only approach being used. Pharmaceutical companies such as Glaxo Wellcome developed their own SNP genotyping methods [117]. Their development of a multiplex SNP genotyping system used an oligonucleotide ligation assay (OLA) followed by flow cytometry. While previous OLAs used two adjacent probes that became linked together via DNA ligase when the probes perfectly matched the target DNA sequence [ref. 117], the novel approach attached a fluorescent oligo reporter sequence to “capture” probes that hybridized both to the genomic targets and to complementary reporter DNA sequences linked to microspheres. The reporter sequences, designated as “ZipCodes” [118], corresponded to the complementary sequences on the microspheres, such that, because each microsphere contained a different ratio of dyes, the dye ratio observed inferred the SNP genotype. Ye and colleagues at Glaxo Wellcome’s R&D division further expanded this technique to employ single-base extension as a means to streamline the analysis protocol and conduct multiplex reactions [119].
In 2000, researchers at Affymetrix, Inc. and Case Western Reserve introduced a novel method for performing high-density SNP genotyping [120]. The TAG-SBE method operated on a standardized, generic probe array of pre-selected 20-mer oligonucleotide tags [80, 91, 95, 118] independent of the markers to be genotyped. Addition of marker-specific primers added to genomic DNA first amplified the region of interest; subsequent addition of single-base extension (SBE) chimeric primers with 3’ sequences complementary to the selected SNP loci and 5’ sequences complementary to the synthetic oligo probes then annealed to the DNA. Dideoxyribonucleic triphosphate nucleotides, labeled with dyes unique to each SNP allele, extended the SBE primers by one base pair; and then the amplified regions were pooled and hybridized to the probe array, where the hybridization patterns were detected. The new method not only demonstrated that allele frequencies could be quantitatively measured in a highly parallel format, but that flexible multiplex arrays could be designed through a tag-based approach [120].

Other technological developments emerged that sought a method to deliver highly multiplexed high-throughput SNP genotyping capabilities. Han and colleagues at the University of Indiana cited David Walt’s key invention when they proposed a multicolor optical coding assay of quantum dots embedded in microspheres [121]. Researchers at the University of Texas Health Science Center at Houston used matrix-assister laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry to perform production-scale SNP genotyping of PCR-amplified samples [122]. Affymax, Inc.
researchers developed a method to combine PCR amplification with four-color minisequencing on microspheres, followed by fragment detection and analysis using an automated sequencer. The technique exploited the sensitivity of PCR with the multiplex capabilities of a universal fluorescent read-out step [123].

2.3.7 Limitations to the state-of-the-art

Although the development of the microarray technology enabled massive parallel assays of whole genomes, with oligonucleotide probes surpassing the antiquated spotted arrays [ref. 41], changes were necessary to progress the knowledge of genetic variation. Eric Lander of the Whitehead Institute suggested that these changes included a reduction in array size, a circumvention of PCR amplification, and an increase of labeling sensitivity and detection. Furthermore, he suggested that molecular bar codes could serve to generate generic arrays of ‘tag sequences’ – a technique demonstrated in yeast – which would enable large sets of DNA variants to be genotyped [118]. Aravinda Chakravarti commented that the ‘ideal’ technology to detect genomic variation among populations is that which can assay all types of sequence change – SNPs, insertions, deletions, and copy number variations – with a small amount of biological material such that it can resolve variation to the nucleotide level ‘with very high accuracy’ [124].
As SNP genotyping detection methods advanced, it became apparent that the technological process itself could be broken down into four basic functions: reaction principle, assay format/separation step, detection method, platform/assay [44]. By 2001, at least eleven biotechnology companies had developed unique platforms for assaying SNP genotyping [ref. 44].

The advent of SNP discovery led to a variety of methods to collect, run and analyze samples. The complexity of the research techniques for SNP detection grew as platforms became more sophisticated. SNP detection methods such as restriction fragment length polymorphism (RLFP) analysis [26], single-strand conformation polymorphism (SSCP) analysis [125], allele-specific oligonucleotide hybridization (ASO) [64], oligonucleotide ligation assay (OLA) [45], primer extension assay [54, 111], Taqman [126], molecular beacons [127] and structure-specific FLP nuclease technology [128] were developed out of a growing understanding of the field of genetics and previous scientific techniques. SNP reaction platforms began as gel-based products, but have since exploited the scientific achievements of fluorescence polarization [129], semiconductor chips [130], mass spectrometry [131, 132] and high-density oligonucleotide arrays [120, 133].

As the need for larger, closely-matched populations grew, the research designs to account for variables and select appropriate markers became increasingly intricate. Linkage studies were successful for tracking monogenic traits, but were not the appropriate
medium for studying complex traits due to multiple confounding variables. Traits formed by multiple genes required a more “biometrical” approach to their analysis [6].

By the end of the twentieth century, questions regarding the nature of genetic variation of complex diseases remained to be answered: how many genes were involved in these diseases? What was the frequency of mutation? Were the alleles rare or common? How old were the mutations themselves? How does natural selection contribute to their inheritance patterns? Do individuals develop complex diseases due to the lack of protective alleles or due to susceptibility alleles? [124]. The prospect of uncovering answers to these questions laid in the growing potential of large-scale genotyping projects.

2.3.8 SNP maps

While scientists in the mid ‘90s focused on finding SNPs within the gene coding and promoter regions that held functional significance [101], researchers saw the future as a time to investigate non-coding or “evenly spaced” SNPs that could track disease loci through linkage disequilibrium [ref. 6, 102]. The actual number of SNPs needed to conduct such studies was widely debated. This predicament was based on the discovery that SNPs in close proximity to each other could be tightly linked, such that genotyping the SNPs across samples would give redundant results [134]. Researchers saw this
phenomenon as a means to reduce the actual number of SNPs to be genotyped, if the pattern of the SNPs (or, haplotype blocks) could be resolved.

Since the reemergence of SNPs as the preferred marker for genetic studies [103], scientists diligently pursued multiple methods to discover the frequency and characteristics of the SNPs. One particular goal of the SNP map construction was to describe the specific combinations of alleles at closely linked sites (haplotypes). In 2001, two highly publicized articles in *Nature* reported the first attempts to construct genome-wide SNP maps [135, 136]. At that time, it was estimated that over 5.3 million common SNPs existed within the human genome and accounted for much of the sequence variation between individuals [135].

Both public efforts, like the Human Genome Project, and private efforts, like the SNP Consortium, contributed to the first generation of a high-density, evenly-spaced SNP map [136]. The team reported the discovery of 1.4 million unique SNP sites. Data was pooled from multiple databases and also uncovered through the analysis of overlapping clones from bacterial artificial chromosomes (BACs). A small portion of SNPs was discovered through gene-based studies and resequencing projects. The authors concluded that the majority of identified SNPs lay in gene-centric regions (i.e. exons or within 5kb of exons) with varying levels of nucleotide diversity [136]. The surge in identification of genetic
variants provided ample SNPs from which to test for disease susceptibility, quantifying trait markers, and environmental stimuli responses [ref. 16].

Researchers at Perlegen Sciences, Inc. and Affymetrix, Inc., led by David Cox and Stephen Fodor, respectively, uncovered over 36,000 SNPs within 21.7 Mb of unique sequence on human chromosome 21[135]. With a greater number of sample chromosomes surveyed than the International SNP Map Working Group and the SNP Consortium, the team synthesized $3.4 \times 10^9$ oligonucleotides to scan the samples, and performed ‘brute force’ long-range PCR (LR-PCR) amplification of the unique sites identified along the chromosome[73]. Altered hybridization patterns indicated the presence of nucleotide variation. The authors reported a 97% accuracy rate, due to stringent threshold requirements. Construction of haplotype structure was generated from rodent-human somatic cell hybrids. The initial results revealed common haplotype patterns across multiple chromosomes – over 80% of the haplotype structure could be defined by “less than 10% of the total SNPs in the block”[135]. The results held potential for whole-genome association studies; such studies could identify specific haplotype blocks that account for disease susceptibility. The limitation to haplotype analysis was that the haplotype structure was largely ‘unpredictable’ across the genome and that a high density SNP map would be required to improve the resolution of each haplotype block[135].
Once researchers uncovered how to scan an individual genome for genetic variation, a shift towards understanding how individuals within and among populations emerged. Research into the underlying genetic structures of human populations took a dramatic leap forward in 2002 with the initiation of the International Haplotype Mapping Project. This project was made possible due in large part to Illumina’s unique technology. For more information regarding Illumina’s contribution to the HapMap Project, see chapter 5.

\subsection*{2.3.9 Before Illumina technology}

In 2000, the contemporary methods for detecting variations of DNA by using high throughput technology were described in a paper published by Walt in Science [137]. A method of particular promise was the Affymetrix GeneChip. The Affymetrix GeneChip was a probe array, using patterned, light-directed combinatorial chemical synthesis on a glass surface. The technique required painstaking build up of oligos on quartz wafers, one unit at a time through a photolithographic process, based on methods employed in the computer chip making industry [138]. These spotted arrays with a 100-\(\mu\)m center-to-center spacing had \(~400\)-fold lower packing density than that of the early Illumina BeadArray (see next section and Chapter 4) [139].

The Molecular Probe Inversion assay (MIP), developed by ParAllele Biosciences, was another successful PCR-based high-throughput SNP genotyping assay to be highlighted
as a promising technique by Walt in his 2000 *Science* article [137, 140]. Although the system initially matched the GoldenGate assay in its ability to demonstrate multiplexed genotyping for up to 1,500 SNPs [140, 141], the MIP assay still required the generation of immobilized tag arrays on glass, which limited its multiplex levels to 10,000 SNPs [142].

### 2.4 BeadArray breakthrough

Illumina’s first SNP genotyping platform was officially released in 2002 after a soured deal with Appelera Corporation’s Applied Biosystems. The deal rupture turned in Illumina’s favor, as the event forced the young company to rework its strategy and deliver a product to market within months. Chapter 4 will thoroughly explore the history of the company from its founding to the current events of 2007. What is important to note here is the impact that the initial and subsequent BeadArray platforms have had on high-throughput SNP genotyping studies.

When Illumina’s BeadArray technology platform entered the market, no company or research institution had yet devised a technique to overcome the need for PCR amplification of samples. PCR amplification required extensive optimization and presented challenges for multiplexing, given the prevalence of primer-dimer formation [ref. 73]. By the mid-2000s, it was determined that linkage disequilibrium-based
association studies were the best approach for locating susceptibility alleles for disease [143]. Such studies have been significantly enhanced by Illumina’s recent launch of the Infinium™ assay for whole genome genotyping (see Chapter 5).

The following sections will briefly outline Illumina’s platform and assays designed for SNP genotyping. The development of commercial products using these platforms will be discussed in Chapter 4. The impact that the Illumina technology has had on high-throughput SNP genotyping will be discussed in Chapter 4 and analyzed in Chapter 5.

### 2.4.1 Illumina’s Array of Arrays™ platform design

The original Illumina platform combined four basic technologies: 1) an assay to bind and fluorescently label the samples, 2) the oligonucleotide-encoded bead sensors that bound the labeled amplified targets within an array, 3) a scanning mechanism to detect the fluorescent signals from each position within the array, and 4) a decoding mechanism to determine the position of each bead within the array and the fluorescent signal that it was emitting. While the BeadArray technology was the core technology on which the company was built, the other technologies were acquired or developed to generate a powerful platform that efficiently and effectively measures genetic variation. Chapter 4 will discuss the history of how each technology became part of Illumina’s platform. The following paragraphs will explore the advantages of the original GoldenGate assay
design (and the recently developed Infinium whole-genome assay) that have overcome the challenges that previous high-throughput SNP genotyping assays struggled with.

### 2.4.2 Platform basics and background

The initial platform was similar to that which is used today. A standard genotyping assay is performed in the following manner: DNA from the patient is purified and introduced to the array in a manner corresponding to the medium on which the sample is being tested. An Array Matrix needs to be dipped into a sample, whereas the BeadChip is loaded with the samples on a microscope slide-sized glass plate. Sample DNA molecules that are complementary to the oligonucleotide probes on the beads will bind to the beads. Molecules in either the sample or on the bead are labeled with a fluorescent dye either before or after binding. The BeadArray Reader then detects fluorescent dye by shining a laser on fiber optic bundle or on the BeadChip. The detection of the molecules results in a quantitative analysis.

The initial assembly of the BeadArray was as described by the original papers from Walt’s lab [144]. The Array of Arrays™ platforms were designed in two different formats, the Array Matrix and the BeadChip — both marketed under the Sentrix™ trademark. The Sentrix™ array platforms were designed to analyze multiple facets of genomic and genetic analysis, including SNP genotyping, gene expression and,
ultimately, protein expression, through highly multiplexed, scalable assays. Such applications require platforms capable of quantitatively detecting fluorescent signals over a large, linear and dynamic range [145].

The design of the Sentrix formats (BeadArry and Array Matrix) provides the highest density microarray in commercial use. The formats are based on fiber optic technology. Each sample location on the Sentrix™ format rests on top of one fiber optic cable. This cable is composed of a bundled cable, containing thousands of individual fibers fused together. The proximal end of each fiber is etched by techniques invented at Tufts University and exclusively licensed to Illumina. The etching process creates wells at the end of each fiber. Oligonucleotide-coated polymeric beads three-microns in diameter fit snugly in each well, with one bead to a well [Figure 2.1]. The oligos are designed to bind to target sequences of DNA. The fiber bundles, containing 49,777 fibers each, with up to 1520 different bead types for each sample in a 96-bundle array was capable of generating 145,920 assays simultaneously [145]. The Array Matrix incorporated these fiber optic bundles that were cut into lengths of less than one inch, which were then affixed onto an aluminum plate. The BeadChips were fabricated in slides resembling the size used for conventional light microscopy, with varying numbers of sample sites per slide.
2.4.2.1 Sensor design

Sensors were designed through affixing a specific type of oligo to each of the microscopic beads. Different batches of beads were prepared, with beads in a given batch coated with one particular type of oligonucleotide [Figure 2.2]. The oligo sequence on a bead defined the bead’s function as a sensor. The pooled batches of coated beads were brought into proximity with array surface where they were randomly drawn into wells, one bead per well.
2.4.2.2 Detection

In order to measure the fluorescence intensities emanating from each fiber optic cable, Illumina developed the Sherlock™ scanner system. The laser-scanning confocal imaging system was capable of scanning all 96 bundles of an Array Matrix down to 0.8 micron resolution [145]. Lasers at 532 and 635 nm scan the plates simultaneously in order to collect two fluorescence images. The scanning system employs patented macro scan lens technology that is licensed to Illumina by Molecular Dynamics, the company founded by current Illumina CEO Jay Flatley [146].

2.4.2.3 Decoding

In the earliest model of the bead-based DNA sensor developed by David Walt at Tufts University, the different bead types were impregnated with different ratios of fluorescent
dyes. The ratio of dye colors could be detected and the corresponding bead type could be
determined. When Illumina gained exclusive rights to the technology, the company’s
researchers found that this method was riddled with problems when applied to large-scale
experiments. The accuracy of the bead location and identity were compromised due to the
variability in quantitation, instability and the reliance on dye impregnation [144].
Consequently, a technique was devised based on the decoding of fluorescent signals
[139].

The decoding mechanism exploited the specificity and reversibility of the nucleic acid
hybridization to the complementary nucleotides. Upon self-assembly of the pooled bead
libraries on the microwell substrates, serial hybridization steps with fluorescently-labeled
complementary oligonucleotides map the physical location of each bead. A by-product of
this system is the ability to validate each bead in the array by identifying and eliminating
the use of any empty wells. Furthermore, multiple bead copies of up to 30X redundancy
improve reliability and accuracy of resulting data to improve statistical processing [139].
The overall benefit of the novel proprietary decoding scheme was evidenced in the lack
of physical addressing of each bead. The technology helped the company develop
miniaturized, high packing density arrays of greater throughput capacity than other
commercial alternatives [75].
2.4.3 GoldenGate assay development

Although GoldenGate, the initial SNP genotyping assay developed by Illumina, did
require a PCR amplification step, the protocol was designed in a way such that only
universal primers would amplify target regions [ref. 69]. The assay, still in use today, is
the first step to detecting SNPs in queried samples. A combination of oligonucleotide
probes and primers convey genetic variation information from samples to
oligonucleotide-encoded beads through hybridization and fluorescent signal detection.

The GoldenGate assay works as follows [Figure 2.3]: Genomic DNA (gDNA) is
immobilized to a solid support and exposed to oligonucleotides, carefully designed by
Illumina’s proprietary Oligator technology to contain both genomic complementarity and
universal PCR primer sites. Two of the oligos are allele-specific (ASOs) to the SNP sites;
the third is specific to the SNP locus (LSO), which also contains a unique address
sequence complementary to a particular bead type. The LSO hybridizes to the gDNA
between 1-20 base pairs downstream of the annealing ASOs – this requirement enables
flexible oligo selection [147]. Hybridization of the LSO and ASO to the immobilized
gDNA is followed by several washing steps. A polymerase with high specificity for
matching at the 3’ end of the ASO will only extend the ASO primer with perfect
complementation. The polymerase fills the gap between the ASO and LSO and drops off
upon completion. A DNA ligase joins the polymerase-extended sequence with the 5’ end
of the ASO, forming complementary PCR templates that can be amplified with universal
primers [147].

The templates are denatured so that two of three universal PCR primers can amplify the
region for detection. Universal primers P1 and P2 are fluorescently labeled with Cy3 and
Cy5 dyes, respectively. Introduction of the primers to the template will result in the
hybridization of only one of the primers. That primer, along with the universal primer P3
that hybridizes to the LSO address sequence, amplifies the SNP region. The dye-labeled
products are then hybridized to the corresponding bead type that is located on one of the
Sentrix Arrays. The fluorescent dye can be detected through either a fiber optic cable or a
scanner – both mechanisms employ a sophisticated proprietary decoding mechanism to
resolve the identity of the bead type and associated SNP [69].
Figure 2.3: **GoldenGate assay overview.** Image source: [http://www.illumina.com/images/aboutTechAssayGGWorkflowSm.gif](http://www.illumina.com/images/aboutTechAssayGGWorkflowSm.gif)
2.4.3.1 Advantages of the GoldenGate technology

The GoldenGate assay succeeded on its ability to overcome amplification bias by introducing the hybridization step prior to PCR thermal cycling [147]. Furthermore, the assay used Illumina’s core proprietary BeadArray technology (described in Chapter 3) to generate random arrays that were no longer confined to specific locations on an array panel. The advantages of the bead system range from the scale of preparation to the internal quality control mechanisms: Using a bead-based array, one-milliliter of a 3-μm bead solution contained up to $10^{10}$ beads. With a guaranteed redundancy of each bead type of at least 15-fold, based on Poisson statistics, false negatives and false positives could be easily eliminated. The redundancy also enhanced the sensitivity through an increase in signal-to-noise ratios [145].

The small scale of the fiber optic arrays brings the detector directly in contact with the DNA sample. The small size of each bead also increases the number of probes that can fit on each array. The high, local concentration of fluorescent probes enables detection limits of femtomolar concentrations and absolute detection limits of zeptomoles of DNA. Degradation of DNA is reduced over several hybridization-rehybridization cycles with fiber-optic arrays. Stringency can be adjusted using temperature or solvent changes [145].

Flexibility of the assay is demonstrated in that new DNA probe sequences can be easily added to the probe library, providing custom designs for the individual researcher. Zip-
codes for universal fabrication and molecular beacons for label-less detection can also be used [145].

2.4.4 Infinium Whole-Genome Genotyping assay development

In 2005, Illumina released its array-based Infinium™ whole-genome genotyping (WGG) assay – an industry first [148]. Modifying the underlying principles of the GoldenGate assay, Illumina researchers developed a unique assay to perform direct genome-wide SNP genotyping from a single-tube sample preparation without the need for PCR amplification.

Human genomic DNA had been successfully genotyped directly by sequence-specific oligonucleotide probes [149], and the feasibility of genotyping a single human locus directly from gDNA had also been demonstrated [150]. Demonstrations of whole-genome SNP genotyping of human gDNA without PCR amplification occurred in 2003 and 2004 by separate research groups [151, 152].

The Infinium™ assay employs whole-genome amplification, hybridization capture of the amplified genomic loci to an oligo probe array, an array-based enzymatic SNP scoring assay, and a sensitive signal amplification step [Figure 2.4] [148]. Whole-genome amplification is followed by sample hybridization to an array of capture probes. Each
probe is 75 bases long, of which 50 bases function as the capture probe and 25 are used for decoding purposes. Captured SNPs are then scored directly on the array surface by either an allele-specific primer extension (ASPE) or a single base extension (SBE) approach [148, 153].
Figure 2.4: Infinium whole genome genotyping assay schematic. (Above) Infinium I assay uses an ASPE method; (Below) Infinium II assay uses a SBE method.

The ASPE approach is advantageous because of the ease of SNP scoring it provides: only two bead types (A and B) are required per assay. The bead types have oligo probe sequences that only differ at the 3’ terminal base – the base that complements the SNP. When the genomic DNA is hybridized to the array, only DNA sequences that perfectly hybridize to their complementary probes will be extended and labeled. This is due to the polymerase enzyme with high 3’ specificity. The genotypes of the various SNP loci are then scored by analysis of the intensity ratio between the two bead types [148].

The SBE approach requires only one probe per SNP, rather than two. This facet increases the amount of information that can fit on an array. According to a later paper published by Gunderson and colleagues [148], the SBE approach may be preferred over the ASPE approach because of its “inherently robust biochemistry”. SBE is an end-point assay, where dideoxynucleotides compete for incorporation. The disadvantage of this approach, however, is that it requires all four labeled ddNTPs types. This means that a sophisticated signal amplification design is a necessity.

The value of the Infinium™ assay model lies in its unlimited scalability and its “unconstrained” ability to choose highly informative SNPs [148]. Because the technology can be scaled to any size, studies requiring a high density of markers, such as association studies, are feasible. These highly flexible characteristics of Illumina’s platforms combined with its high accuracy rates are some features that have enabled the company
to dramatically grow over the past ten years. Chapter 6 will analyze the scientific impact of these technological innovations.

2.5 Conclusion

The quest to explore the impact of genetic variation on disease susceptibility and drug metabolism has encouraged technological innovation. Likewise, technological innovation has enhanced the quality of and rate at which genetic information can be obtained. The development of the BeadArray technology propelled the state-of-the-art of high-throughput SNP genotyping forward, as its unique design bypassed the previous challenges facing other genomic instrument manufacturers.

The next chapter will explore the development of the institution that enabled the transfer of the invention that became Illumina’s core BeadArray technology from an academic laboratory to a biotechnology startup.
3 University Inventions: the Bayh-Dole Act of 1980 and its impact (or lack thereof) on faculty inventions.

3.1 Overview

Illumina’s core BeadArray technology exemplifies a case of successful technology transfer. While industrial developments have been made to the original technology (see Chapter 4 for more details), the core technology was developed at an academic research lab at Tufts University. In 1998, the university, seeking to enable the many applications of the technology, exclusively licensed the patent portfolio for the invention to Illumina, a startup company.

This chapter begins by discussing the policies that promoted universities to patent and license inventions deriving from federally-funded research. The discussion on the development of formal university technology transfer offices leads to an overview of the Massachusetts Biomedical Research Initiatives (MBRI), a regional technology transfer office developed for Central Massachusetts institutions in the 1990s. The MBRI worked closely with Tufts University to set up the exclusive license to Illumina, the startup company co-founded by chemistry professor David Walt and venture capitalist John Stuelpnagel in 1998. The chapter concludes with a discussion of the subsequent development of the Tufts technology licensing office.
3.2 The rise of university inventions vs. open science policy

The education system in the United States established itself in the late nineteenth century as a bastion for applied scientific inquiry. This contrasted starkly with its European counterparts, which focused on the “theoretical and abstract potion of human knowledge” [154]. European institutions were centralized and regarded as training programs to prepare graduates for governmental service [154]. American universities, specifically those established in light of the Morrill Act of 1862, focused on training students for farming and agricultural duties, with an emphasis on generating research to develop regional economies [154]. The practical nature of American curricula encouraged a higher percentage of individuals ages eighteen to twenty-two to enroll in universities compared with the enrollment rates for the same age group at European institutions [154].

American universities depended largely on local support for sustained growth. Thus, early training focused on providing courses to enhance the local economy [154]. Universities around the country emerged as institutions with specialized expertise in particular areas of research. As a result, faculty were not regarded by their contributions to the overall university, but by their research contributions to their department [154, 155]. This trend encouraged a culture of high mobility among the faculty who sought positions at institutions that were well-regarded in particular areas of research. This
mobility, in turn, also gave rise to a free flow of new ideas and research approaches, as faculty moved among the institutions [154].

Faculty, especially those employed at land grant institutions where engineering disciplines were rapidly expanding, began to seek ways to apply their scientific theories in practice. The liberal exchange of ideas promoted academic relationships with industries that could develop the ideas generated within the universities into products that could support the local economy. This dissemination of knowledge from academia to industry became more fluid over the twentieth century as university graduates sought employment within industry [154].

### 3.2.1 Early academic patenting trends

As academic-industrial collaborations rose in the early twentieth century, specifically within the fields of engineering, so did the number of patent applications by U.S. universities. The rise in university patent applications post-World War I sparked a debate among academic administrators that led to publication of the 1933 American Association for the Advancement of Science (AAAS) Committee of Patents, Copyrights, and Trademarks [156]. The report addressed issues that would be discussed over forty years later during the creation of the Bayh-Dole Act. Namely, the report discussed whether or
not patents were required for technology to be transferred from academic research to an industrial product [156].

The arguments in support of university patenting still dominate debates on the subject today. One argument ran,

“ordinarily no manufacturer or capitalist would be willing to-day to risk his money, and expend time and energy in developing on a commercial scale a new product or process without being assured that his investment in developing the new invention would be protected in some measure” [156].

Other proponents of patenting argued that allowing universities to patent inventions prevented other individuals from “patent pirating” ideas and charging monopoly prices on inventions that they themselves did not invent. Furthermore, it was argued that patents could be viewed as “quality control” devices to prevent “unsuccessful or harmful exploitation” of university inventions that could damage the institution’s credibility [154].

Several of the AAAS Committee report’s concerns about university patenting are still at issue today. It was assumed that research advances hinged on cumulative discoveries. If over-used, patents could potentially block other subsequent downstream research “by men who subsequently do fundamentally important work in the same field” [156]. The report suggested that this problem could be avoided through judicious use of patents. Perhaps one of the most controversial issues addressed by the committee was the idea of
patents disturbing the culture of “open science” norms in academic institutions [154]. Many universities were reluctant to permit patents to be filed by their faculty primarily because of the fears that patenting would “bias academic inquiry away from basic research” [157].

The early instances of patenting by academic institutions were a means to protect public interest and preserve university reputations [154]. Land-grant institutions were among the first universities to submit patents on inventions that could be applied in industry and agriculture. In 1924, the Wisconsin Alumni Research Foundation (WARF) became the first established foundation to manage the patents of its university faculty [154]. The WARF is a separate entity from the University of Wisconsin. This apparent legal distancing of the University that filed faculty patents and the Foundation that received the royalties from patent licenses was in part an attempt to assuage those who felt that universities should not benefit monetarily from faculty inventions [154]. Because of the stigma that patenting inventions represented in academic circles, few institutions actively sought patents.

The dearth of patent activity prior to the 1940s meant that universities handled patents on a case-by-case basis. Few institutions had documented patent policies before World War II. Interestingly, among those that did have patent policies, some barred patent submissions on inventions within the field of medicine. The ban was a reflection of
academia’s concern that patenting of small molecules or other biologics would be a disservice to public health, as patent holders could charge monopoly prices on potentially life-saving drugs [154]. Still, those institutions that permitted patenting viewed the process as a new means to recoup revenue lost during the Great Depression, and an effective method to clarify ownership of faculty inventions [154].

The surge in federal funding for university research after World War II encouraged many other universities to adopt official patenting policies. The increase in funding implied that more research would generate more ‘patentable’ inventions. Federal funding agencies also began to require formal patent policies [154]. Of all of the federal funding agencies, the National Institutes of Health (NIH) within the Department of Health, Education, and Welfare (HEW)² championed the greatest increase of funding to academic institutions, primarily funding biomedical research projects [154]. At this point, however, filing patents on medically-relevant inventions was still prohibited. It would not be until the 1970s when a confluence of events would alter the patenting trends in academia, particularly in fields pertaining to biomedical research.

² The HEW was the forerunner to the Department of Health and Human Services.
3.2.2 The decade that changed patenting trends: the 1970s

During the 1970s, four events shifted the attitudes and trends in university patenting from ambivalence to active engagement.

3.2.2.1 The Research Corporation

The Research Corporation was an independent organization founded in 1912 by Frederick Gardner Cottrell of the University of California at Berkeley for the purposes of, among other goals, managing patents. University patents were donated to the organization by faculty who wanted to commercialize their inventions through channels independent of their institutions’ administration [154].

By 1970, the Research Corporation had agreements with over 200 institutions. But the Corporation was experiencing problems that persist in many technology transfer offices to this day. These problems include diseconomies of scale, unpredictable “arrival rates” of “home run” patents, lack of close faculty ties, and challenges in balancing licensing revenues with other objectives. However, the scope of the Research Corporation’s problems was grander, given the number of patents it was managing [154].

In 1970, Research Corporation began to divest some of its responsibilities to the universities themselves, offering assistance in setting up the early stages of the
technology transfer process, such as screening and evaluation of patent applications [154]. In fact, the efforts that the Research Corporation made to encourage more universities to patent their inventions might have sabotaged the Corporation’s own future activity, as universities began to set up their own independent technology licensing offices [154].

3.2.2.2 The birth of molecular biology

The emergence of the field of molecular biology in the ‘70s also hinted at a goldmine of potential discoveries that could be developed into commercial applications. Many universities adopted patenting policies in order to cash in on research that could bring in revenues during a time of slowed federal funding and rising research costs [154, 158].

3.2.2.3 Institutional Patent Agreements

A third motivating factor was the emergence of Institutional Patent Agreements (IPAs) between federal funding agencies and universities. IPAs generally gave rights to seek patents to grantees and contractors. The IPAs made a transition to the university patenting process on a case-by-case basis to a more uniform process to assign patents as the default [154].
3.2.2.4 Stanford’s OTL

Finally, the conspicuous success of Stanford University’s Office of Technology Licensing (OTL), founded by Niels Reimers, gave other universities incentives to learn to manage their own patents. The technology licensing program was designed by Reimers to concentrate on the marketing rather than administrative and legal aspects of patent management. During its first year of operation, the OTL raised revenues twelve times from what Stanford had received through Research Corporation [154].

The decade prior to the passage of the Bayh-Dole Act was marked by a steady increase in the ratio of total U.S. patents filed by universities to total prior-year academic R&D expenditures [154]. Among the universities involved in patenting, the ‘70s saw a dramatic increase in the percentage of patents in the biomedical arena [154]. These factors all suggest that universities were patenting and managing their patents well before the passage of the Bayh-Dole Act of 1980.

3.3 The Bayh-Dole Act of 1980 and its ramifications

In a broad sense, the Bayh-Dole Act of 1980 streamlined the patenting and licensing process across the federal funding agencies [159]. The Act has often been mistakenly labeled as the Act that enabled universities to patent and license faculty inventions. Before the Act’s passage, each agency had its own rules for granting patents on
inventions derived from its funding. Mowery and colleagues have argued that Act is primarily responsible for initiating the latest phase in university patenting and licensing, which is characterized by direct involvement in patent management [154].

### 3.3.1 Policy debates

Conflicts over the ownership of patents deriving from federal funding emerged during the postwar 1940s. In one Senate debate, many different viewpoints were presented. Democratic West Virginia Senator Harley Kilgore vehemently argued that assigning patents to private corporations represented a “giveaway” of taxpayer dollars [160]. Vannevar Bush, Director of the Office of Scientific Research and Development, countered the senator’s claims, arguing that allowing private contractors to retain patent rights would incentivize participation in federal R&D projects and spur development of commercial products [154]. While the debate focused on whether permitting corporations to retain intellectual property rights would raise prices and sacrifice small businesses, another key issue was disparate practices among the various federal funding agencies. Presidents Kennedy and Nixon both decided to maintain agency-specific patent assignment decisions, allowing the debate to go on into the 1970’s [154].

The debate surrounding patent assignments of federally funded research to universities came to the forefront in 1968 after reports on the National Institutes of Health’s (NIH)
Medicinal Chemistry program by the U.S. General Accounting Office (GAO) and by Harbridge House were released [161, 162]. The reports examined the relationships of pharmaceutical firms and universities as they pertained to research collaborations. The reports noted that while pharmaceutical firms initially screened compounds developed at academic institutions without charge during the 1940s and 1950s, trends towards firms adopting exclusive rights to develop and market the compounds emerged in the 1960s [154, 162]. In an effort to counteract this trend, the Department of Health, Education, and Welfare (HEW) issued a declaration in 1962 ordering universities that collaborated with pharmaceutical firms to sign patent agreements prohibiting corporations from patenting NIH-funded research [161]. The 1968 reports argued that this measure deterred research, as pharmaceutical firms halted screening of compounds generated with federal funds [161, 162]. That same year, HEW established the IPAs that permitted universities to transfer their technology while retaining the rights to federally-funded inventions. The National Science Foundation (NSF) and Department of Defense (DOD) followed suit, permitting universities to retain patent rights [154].

The 1968 revision by HEW sparked an internal debate, led by the Office of the General Counsel, over the concern that the university patents and licenses would contribute to higher health care costs [163]. In response to the concerns, HEW conducted a twelve-month internal review on whether to permit exclusive licenses by universities. During that time, thirty petitions for patent rights were put on hold – a move that greatly upset
the affected universities [154]. Senators Birch Bayh (D-Ind.) and Robert Dole (R-Kans.) were approached by a patent attorney from Purdue University and a congressional staffer affiliated with the University of Arizona. They were asked to introduce a bill creating “a uniform patent policy that gave universities and small businesses rights to any patents resulting from government-funded research” [154]. The Bayh-Dole Bill did not require the approved technology transfer capabilities or restrictions on exclusive licensing agreements, as dictated by previous IPAs [154].

3.3.2 Introduction of the Bayh-Dole Act

The University and Small Business Patent Act (S. 414) was introduced to Congress on 13 September 1978 without much opposition [154]. University representatives lobbied for the bill’s passage and aided in crafting the bill’s language for both the House and Senate versions [164]. Senators Bayh and Dole focused on the lack of uniformity among funding agencies of patent policies and the difficulty of patent title retention by contractors as their primary issues of concern. Other supporters of the bill noted that the “characteristics” of university inventions strongly favored the need for patents and exclusive licenses for product development and commercialization [154]. Specifically, university disclosures primarily described “embryonic” inventions, requiring significant additional investment to become commercially viable. Supporters claimed that companies would not invest in the development of such inventions without clear intellectual property
rights, such as exclusive licenses [154]. These arguments are still made today, to justify the exclusive licensing of university patents to companies.

What was largely missing from the debates of the Bayh-Dole Act was a discussion of the potential risks involved with the Act’s passage. The risks included the potential threat to open science norms and the political ramifications of direct university management of patents [154]. However, the argument that patents on university research would reap “taxpayer benefit[s]” as inventions were commercialized instead prevailed [154].

### 3.3.3 Passage of the Bayh-Dole Act

The Bayh-Dole Act was passed by Congress in the winter of 1980 and signed into law by President Jimmy Carter in December 1980. Effective 1 July 1981, the Act afforded universities and small businesses a uniform patent policy, providing them the rights to patent any invention deriving from federal grants or contracts, subject to certain provisions. The government is allowed a nonexclusive royalty-free license to any patent and retained “march-in” rights in the event that commercialization of a licensed patent failed or that public health or safety was at stake. Furthermore, universities are required to share royalties with inventors and grant preference of licenses to small businesses [154].
Since the passage of the Act, there has been little empirical evidence one way or the other that the Act itself was pivotal. Trends of increased academic patenting were already emerging. There remains mixed support for whether patenting and licensing are required for the technology transfer process to occur [154]. Some scholars contend that university patenting and licensing have harmed disclosure norms of academic research, leading to secrecy within the academic community [165]. Others, such as the Association of University Technology Managers (AUTM), argue for the value of the Bayh-Dole Act in fostering academic-industrial collaborations. According to Mowery and colleagues, the Bayh-Dole Act was “an effect as well as a cause” of the trend of university patenting and licensing [154].

3.3.4 Impact of the Act

Universities around the country differed in their patenting activities and policies on the eve of the Bayh-Dole Act. WARF and Stanford, for example, had well-established technology transfer offices and were patenting inventions well before the 1970s. Some, such as the University of California system, developed offices for technology licensing in the 1970s. Other universities of equivalent scientific prowess, such as Columbia University, adopted patenting policies only after the Act went into effect [154]. Mowery and colleagues explored the inventive activity at Columbia University prior to its adoption of a patenting and licensing policy. They concluded that the potential for
revenue generation by its inventions was so great that Columbia would have developed “some administrative machinery for patenting and licensing in the absence of the Bayh-Dole Act” [154]. In fact, licensing revenue from Columbia’s patents grew sixty-fold between 1985 – 1995 [154].

Success of university patents, measured in licensing revenues, can be attributed to the ability of universities to learn to patent. Those universities that adopted patenting and licensing policies after the passage of the Bayh-Dole Act tended to learn from interinstitutional knowledge via personnel transfer [154]. In addition, AUTM has shared best practice information with its members through publications and conferences.

But a declining trend of the yield that patents from the University of California and Stanford were bringing in after the passage of the Act was also observed. Despite their intensified marketing efforts, one reason suggested for the decline of the incumbent universities was that their earlier patents were the most successful, based on the types of inventions that the patents covered. The Cohen-Boyer patents for recombinant DNA technology, for example, were filed prior to 1980. These patents accounted for a large share of licensing revenue to Stanford and the University of California until the patents expired in 1997 [166].

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3 For more information on AUTM, see the website: [http://www.autm.net](http://www.autm.net)
Technology transfer from academia to industry has indeed increased since 1980. Mowery and colleagues concluded that the overall effects of the Bayh-Dole Act have been exaggerated, given that the “patent propensity” grew steadily since 1945 with no sharp spike post-1980 [154]. The overall economic growth from university inventions over the past three decades are no more pronounced than those innovations of the 1930s or 1950s [154]. This means that the Act probably did not dramatically change the rate of university patenting. This makes sense because transfer of innovation can occur through channels other than patents and licenses. Through personal communications and academic publications, ideas can flow from academia to industry without patents ever being involved [167]. Thus, the patenting and licensing activities within academic institutions does not imply that the passage of the Bayh-Dole was essential for technology transfer to occur. Furthermore, the fact that university patenting and licensing were on the rise and the lack of evidence for low commercialization rates before the passage of the Bayh-Dole Act weaken claims that the Bayh-Dole Act had a dramatic effect on university technology transfer [154]. The passage of the Act cemented processes that were already occurring within the United States and formally endorsed patenting and licensing by universities and public laboratories [154].

Biomedical science was the research area that saw the most significant increases in patenting activity after 1980. This was due to a combination of many factors, including advances in molecular biology techniques. Increases in patenting also resulted from the
decision in *Diamond v. Chakrabarty*, which permitted the patenting of genetically modified organisms[168]. This case profoundly changed the landscape of intellectual property rights in biotechnology and may have been at least as important as the Bayh-Dole Act in increasing academic patenting and licensing activities.

### 3.3.5 Unresolved issues

What remain unresolved are the issues surrounding the effects of patents on scientific research. University research that both contributes to the common scientific knowledge and has practical applications lies at the crux of what should/should not be ‘patentable.’ Ownership of “knowledge inputs” may hinder scientific progress. The potential for significant revenue from patenting and licensing of university inventions may also encourage universities to withhold information. This may take the form of delaying faculty publications until a patent application has been filed, restricting sharing of materials (a common theme of “open science” culture), or limiting sharing of results at conferences and presentations [154, 157, 169]. As a consequence, more restrictions on access to scientific inputs may occur.

The rise of material transfer agreements (MTAs) and formal contracts among academic institutions and between industrial partners is one indication that the cost of performing research is growing [170]. As a resolution to this problem, Mowery and colleagues
propose the following amendments to the U.S. patent system: increased skepticism on discoveries describing natural phenomena, stricter interpretation of the “utility” criteria, and limitations to the scope of claims [154].

Not all universities established technology transfer offices immediately in the wake of the Bayh-Dole Act. Some universities were unable to justify a full-scale internal office, given the lack of patenting activity, interest or funds. For example, the development of the Tufts University Office of Technology Licensing and Industrial Collaboration (OTLIC) emerged from a collaborative multi-institution tech transfer office, which served ten other universities in the Central Massachusetts region.

3.4 The MBRI & Tufts OTLIC

Massachusetts Biomedical Research Initiatives (MBRI) was established after the Bayh-Dole Act passed in 1980. The regional tech transfer office promoted the essential goal of the Act: to bring together emerging technologies and products for the creation of new companies. As a mid-state and Western Massachusetts counterpart to the technology haven in the Cambridge-Boston area, the MBRI was created with the hopes of generating an “industry cluster” (in the words of Harvard Business School Professor Michael Porter) for Central Massachusetts. Marc Goldberg, one of the founders of the MBRI, described the development of the organization’s creation as an “enlightened thought process”. The
MBRI, he explained, “was designed with the intent that emerging companies would become successful and stay in the region to further promote innovation” [171].

The MBRI’s initial board members contained college presidents and administrators from eleven institutions within the region. The board members unanimously agreed that each of their respective institutions was capable of generating great technologies. The challenge, however, lay in understanding how best to commercialize the inventions [171]. This insight could only come from individuals with industrial know-how and a keen eye towards commercial applications. The smaller institutions in Central Massachusetts lacked the day-to-day contact with the licensing officers. What the board hoped to gain from the MBRI was a simulation of what MIT had across its entire campus: a “very powerful” technology licensing and development office [171]. MIT’s success in identifying of the best applications for the university’s technology can be largely attributed to the business insights of Lita Nelsen, Director of the MIT Technology Licensing Office. As such, Goldberg recruited colleagues who, along with his own expertise, brought with them industrial and business acumen that enabled the consortium of institutions to “cherry pick” inventions that had the potential to become core technologies of new start-ups [171].

The MBRI became the sponsor of the Unified Office of Technology Transfer – a project that Goldberg named “the Great Experiment” – in 1992. The patenting and licensing
activities of the eleven institutions were handled directly through the MBRI, as if it were an internal office within each university. The MBRI also played the role of a “closed-end venture capital operation,” providing all the necessary operations to generate new companies; this included regulatory affairs, human resources, and financial backing. In return, MBRI took stock in each of its newly backed companies. The advantage of such an operation was that the scope of the office was broad enough to attract attention of industry and of potential employees [171].

3.4.1 Technology transfer at Tufts with the MBRI

Of the new hires, Goldberg recruited Skip Irving, now a senior partner at Health Advances, a health care consulting firm, to run the office. Goldberg cites the creation of ViaCell\(^4\) and Illumina as by far the most successful of Irving’s many accomplishments [171]. The creation of Illumina, championed by Irving and others who strongly lobbied for the exclusive license from Tufts to the startup, came at a time of change for both MBRI and Tufts. While the exclusive license became effective in early May 1998 and the Tufts-MBRI relationship did not formally end until that July, the events surrounding the licensing deal highlighted the issues that the Tufts administration were already beginning to consider as they rethought their licensing strategy.

\(^4\) As of 11 November 2007, ViaCell was purchased by PerkinElmer. For more information on ViaCell, see the company website at: http://www.viacell.com.
In 1997-1998, several events resulted in Tufts University formally ending its relationship with the MBRI. The federal subsidies that the MBRI received kept the costs for patent filing and management low, but by 1998, MBRI had lost its subsidy so it would have been forced to charge to its clients four times its previous price [172]. In addition, the movement towards creating technology licensing offices within universities was a national trend, as discussed by Mowery et al. [154]. While the relationship between Tufts and MBRI had been an amicable and profitable one, Tufts had been responsible for a substantial amount of the MBRI revenue; and, from the University’s perspective, sharing the resources with the other institutions no longer was the best model [172]. On 1 July 1998, Tufts ended its contract with MBRI [173].

Today, the MBRI has been renamed the Massachusetts Biomedical Initiatives and operates as an independent, tax-exempt corporation to support the development of medical device companies in Central Massachusetts. Tufts University continues to run a successful in-house technology transfer office, starting up five new companies in 2006 alone [174].

### 3.5 Technology licensing at Tufts University

Tufts University had begun its technology transfer activities through licensing patents in the mid-1980s. The Chemistry Department within the School of Arts and Science and the
College of Engineering had the most patents. These patents were managed internally by the respective School or College, a pattern that continued through the early 90s. When Tufts began collaborations with MBRI in 1992 to promote and exploit its technology transfer, the University did not yet have the capacity to manage its intellectual property\textsuperscript{5}. The relationship was mutually beneficial: the MBRI received an annual fee and ten percent of net income derived from licenses negotiated by MBRI, and Tufts saw an increase in faculty invention disclosure and commercialization [173].

In the late 1990s, it had become clear to the Tufts administration that the University needed to redesign its licensing strategy. After the MBRI informed Tufts of the potential price hike, the University opted to build its own tech licensing office on campus with its own internal staff. The University initiated the development of a fully staffed five-member technology transfer office in the summer of 1998. The project was spearheaded by Peggy Newell, then the Associate Provost for Research at Tufts. Newell’s initial actions included pulling names of people from her Roladex whom she knew had chosen alternative career paths and could guide her to people experienced with technology transfer [172]. In addition to contacting universities around the country for advice, Newell met up with technology transfer expert Lita Nelsen at MIT, who gave her guidance [172].

\textsuperscript{5} For more information on the Massachusetts Biomedical Initiative, see: http://www.massbiomed.org/index.htm.
3.5.1 The opening of the OTLIC

The Office’s first director, Frances Toneguzzo, former Associate Director of Technology Transfer at Harvard University’s Office of Technology and Trademark Licensing, arrived in the spring of 1999, bringing with her experience in both industry and academia. Thomas McVarish, Assistant Director of Operations, was hired to oversee the legal expense budget, the collection and distribution of income generated from licensing agreements, as well as the government compliance and the OTLIC database management. His background in research and intellectual property management through positions at Bristol-Myers Squibb and Harvard University were key reasons for his hire. Ojas Mehta, who came with industrial experience in biotechnology and academic experience with technology transfer at the University of Rochester, was appointed Senior Licensing Manager in charge of the School of Medicine and the School of Dental Medicine in the summer of 1999. In the fall of 1999, the Office welcomed Justyna Lipinska who became the Licensing Associate for the School of Veterinary Medicine, Clinical Trial and Material Transfer Agreements. The Office staff was completed in early 2000 when Martin Son arrived to manage the licensing agreements for the College of Engineering [173].
3.5.2 Tufts OTLIC post-Illumina

From the Tufts University side, it appears that Illumina, arguably the University’s most successful startup, was actually created when no one from the University was directly involved. According to current Vice Provost, Peggy Newell, the exclusive license of David Walt’s patent portfolio was signed over to the venture-backed startup during the interim between administrators; Newell did not take her position until 1 July 1998 and the spot had been vacant for the prior six months [172]. Marc Goldberg, President and CEO of the MBRI between 1991 and 1997, was also unable to provide details of the exclusive license, but suggested that Skip Irving’s role in the deal was critical in convincing the Tufts administration to license the portfolio [171]. The portfolio was not the first from Tufts University to be licensed, but the royalties gained from it were the first to be liquidated. After the 180-day lock-up post Illumina’s wildly successful initial public offering in 2000, Tufts University liquidated its shares of Illumina common stock, for a value of approximately $7.2 million[175]. The continued success of the Tufts University OTLIC will be discussed further in Chapter 5.

3.6 Conclusion

In conclusion, university inventions have spurred economic and industrial growth, although the specific role of patenting and licensing remains a subject of active debate. The passage of the Bayh-Dole Act of 1980 created uniform patenting policies of federally
funded university inventions and endorsed the trend of university patenting and licensing. Prior to the passage of the Act, most universities had no policies, inconsistent policies, or delegated those activities to patent management corporations, such as the Research Corporation.

While Tufts University and the MBRI made joint decisions based on which faculty inventions to patent and license, faculty members were simultaneously making their own decisions about their research. In the following chapter, I will discuss the patenting decisions that an individual faculty member at Tufts University made, in order to maximize the utility of his inventions and protect Tufts’ intellectual property. The actions that David Walt, Ph.D., took to balance his academic duties with his entrepreneurial interests sheds light on how best practices of patenting and licensing can be learned from the inventors themselves. Walt’s past experiences, combined with keen insight, taught him valuable lessons that he took into account when disclosing his unique bead-encoded fiber optic technology. His story serves as an example for entrepreneurial scientists who aim to commercialize their inventions without compromising their academic values.
4 David Walt & fiber optic sensors.

David Walt is well known in two very different circles: he is the prestigious Robinson Professor of Chemistry at Tufts University and he is the co-founder, a Director and Chairman of the Scientific Advisory Board of Illumina, Inc. The following chapter will discuss Walt’s background in the context of events that shaped his professional career and made him decide to pursue commercial applications of his technologies. The development of his fiber optic sensor with encoded microspheres technology, the core technology of the Illumina platforms, will also be discussed. The chapter will conclude with a technical discussion on the state of the technology at the time that the exclusive license of Walt’s patents to Illumina was being established.

4.1 Background

David Walt graduated from the University of Michigan, Ann Arbor, with a B.S. in Chemistry in 1974. He pursued a Ph.D. in Chemical Biology – a joint degree in Chemistry and Pharmacology – at SUNY Stony Brook in New York under the guidance of Professor Francis Johnson. His primary research investigated anthracyclines. Upon receipt of his doctoral degree in 1979, Walt traveled up to Cambridge, MA to study as a
postdoctoral research associate for Professor George M. Whitesides at the Massachusetts Institute of Technology.

Walt’s research under Professor Whitesides examined the synthesis of nicotinamide adenine dinucleotide and its cofactors. More specifically, the fellowship gave Walt the training to become an expert in immobilizing enzymes – a relatively new field of inquiry back in the late 70s, early 80s[176]. This revolutionary ability to attach enzymes to a solid support not only saved valuable research money due to the fact that the enzymes could now be washed and reused, but also, on a broader scale, paved the way for other research arenas to develop, one of which being sensor-based research.

Walt’s training at MIT had given him enough expertise in the field of immobilization chemistry that he was highly recruited by Tufts University to take on a role as an Assistant Professor in the Department of Chemistry. Upon completion of his postdoctoral fellowship in 1981, Walt moved due west of Boston to Medford, MA, which is where he remains as an active faculty member and a supportive advisor to undergraduate, graduate, and post-doctoral students to this day.
4.2 Research interests

When Walt initially began to set up shop at Tufts, the field of sensor research was at a primitive stage. Walt described initial attempts to measure pH changes due to enzymatic activity as an illustration of the state-of-the-art in the early 1980s:

“so what people would do was to take a little plastic bag and stick it over the end of a pH electrode with an enzyme inside the bag and then molecules would go through the membrane to the inside of the bag to where this enzyme was and it would – the enzyme would catalyze the reaction, change the pH of the solution and that would be kind of an indirect way to measure the presence of different substances that would occur in a biological context.” [176]

Although, the primary research focus in the early Walt lab at Tufts concentrated on the synthesis of various organic molecules, Walt opted to form a group within his lab that would begin to investigate better ways to develop sensors. Citing his experience with immobilization chemistry, he decided that a more suitable approach to detect molecules of interest rather than using Saran warp and other makeshift instruments would be to directly attach molecules that are known to bind to the target molecules to a particular surface [176].

The group initially experimented by attaching dye indicators to the surfaces of fiber optics. Enzyme immobilization to an insoluble polymeric matrix was demonstrated by entrapping an enzyme in an acrylamide polymer. The immobilized enzyme assayed could still catalyze the reaction, which was measured through changes in the dye indicator color. Such measurements were taken by sending light down the optical fibers [176].
Walt’s research on sensors based on immobilizing enzymes began to get published and agencies began to fund projects for Walt and his laboratory to develop sensors for particular projects. Walt cited his work with the Environmental Protection Agency (EPA) as a project that involved developing a fiber optic sensor-based assay for pollutants in ground water. His group measured various contaminants in ground water through the use of optical fibers, which provided the ease of bringing the instrument directly to the sample at its source. Such a system enabled the accurate measurement of a sample without the need for sampling [176].

Walt’s laboratory also collaborated with medical researchers to develop microsensors. The relatively new medical diagnostic technique was thought to improve critical care treatment of patients by delivering miniature sensors to the bloodstream to measure various blood constituents. The primary focus was to assay the blood of heart transplant and bypass surgery patients for oxygen and carbon dioxide levels – the delicate levels of both molecules must be rigorously monitored given the already weakened state of the patient. At that time, monitoring was done by deep artery blood sampling, a painful process. The microsensors would have used a less painful approach. Walt recounted later that the research eventually developed into a product, but for various reasons, it did not become a commercial successs [176].
The sensor-based research projects were met with reasonable success [Appendix 1]; however Walt felt limited by the number of measurements that could be taken at a time. For each data point, one optical fiber sensor was required. The time to fabricate each sensor was significant. The resolution to such an experimental roadblock would come a few years later after some experimentation and collaboration [176].

4.3 A turning point in sensor research

While working in his laboratory with a graduate student one day in 1989, Walt began to openly ponder the applications for the new diode array technology that had recently emerged in the sensor-based research market. The small photo detectors were capable of measuring light in a straight line. The array represented hundreds of little detectors on a microchip, where each signal represented one spot. The graduate student, Steven Barnard, was working on a project at the time that employed the new detectors and asked a sales representative to come out to the lab to demonstrate the technology.

As Walt observed the demonstration, he asked whether it would be possible to attach different “spots” on the end of an optical fiber. Walt excused himself for his technical ignorance on the operating principle of the fiber [176]. The sales representative replied that, “[if] you put a bunch of different spots on the end of an optical fiber and it would turn out that all the signals would scramble and you wouldn’t figure anything [out].”
Then he added, “[but] you wouldn’t want to use any of these diode arrays anyway. You would want to use something called a CCD camera.” [176]

Walt did not end up purchasing the diode array. Instead, he called a colleague whom he had met in various contexts over his academic career. J. Wilbur (Will) Hicks is most well known for his pioneer research on fiber optic cables. He had worked for a number of years on the development of the original Corning Glass technology and had become a serial entrepreneur with fiber optic-based technology companies in his later years. At that moment, Hicks was working at a company in western Massachusetts. Walt asked him about what he knew of maintaining an image through a fiber [176].

As Walt recounts, Hicks’ immediate reaction was, “How did you find out about this?!?” Walt replied, “I didn’t find out about anything; I’m just asking a question.” Hicks immediately invited Walt and his graduate student, Barnard, out to his company, Optilec, in Southbridge, MA. The following week, the trio convened and Walt explained the type of application that he wanted to configure in order to improve his research. “I’m just interested in putting a few spots on the end of a fiber because that way I could measure a few things simultaneously if I could figure out a way to couple these fibers to this new fangled thing called a CCD detector.” [176]
Hicks replied, “Whoa, well now that’s a really good idea.” He then offered some support. “As a matter of fact, we just developed this new kind of fiber that maintains images – it’s called one of these imaging bundles. What we’re trying to use it for, we’re trying to image things in the body, because we’d like to use this as an endoscope, because it’s very flexible and we can just send it down to a patient and you know, put it up against a tissue and image that tissue. And I like your idea of being able to do this with multiple sensors.”

Hicks handed Walt a piece of fiber with a gentle warning, “I want this back. This piece costs $10,000 to fabricate.” Nervously, Walt accepted the fiber and took it back to the laboratory where he and Barnard immediately set to work [176].

### 4.4 Bundled fiber research

The price of a CCD camera at that time was approximately $60,000, a price that Walt could not afford as an assistant professor with a limited budget. In desperate need of the costly equipment, Walt asked a company to come to the lab to demo the Quantex QX7-QFM Quantitative Fluorescence Microscopy System. The System, composed of an image-processing computer, monitor, RGB video monitor, intensified charge-coupled device (CCD) camera, microscope and video printer was left under Walt’s careful watch for three days. With no intention to purchase the System, let alone the CCD camera, Walt
and Barnard worked twenty-four hours a day during that short time span to collect the as proof of the new applications for the optical fiber [176].

Their work paid off. The fiber was capable of simultaneously measuring multiple molecules. The operating principle was that distinct detectors placed at precise locations on the distal face of an optical fiber could be distinguished based on the unique optical pathways of the imaging fiber and the spatial discrimination of the CCD video camera. Thus, a spot registered by the camera would infer which analyte has been detected by the distal sensors based on the position of the spot relative to that of the detector.

The fiber itself was composed of 1,500 10-μm individual imaging fibers, melted and spun together, creating a 400-μm diameter fiber capable of thousands of discrete light-transmitting units [177]. Cone-shaped polymers were fabricated with fluorescein, a pH-sensitive indicator (pKₐ = 6.7), and attached to the distal ends of the fibers. The sensors were exposed to phosphate-buffered solutions of varying pH and the resulting fluorescence was captured by the CCD camera. The amount of fluorescence produced increased as the solutions became more basic, due to the increased release of dianions from the fluorescein molecules [177]. The discrete sensing regions tested on the fiber demonstrated the precise control that the optical fibers had in maintaining individual images. Walt and Barnard then demonstrated proof-of-principle by swapping one of the pH-sensitive cones for one coated with photopolymerized silicone layer, capable of
detecting carbon dioxide concentrations, $p\text{CO}_2$. The results were published that fall in *Nature* [177].

Work on optical sensors proceeded on a smooth course over the following years. Walt received full tenure in 1992 and took active roles on various academic and governmental panels committees. He received two extensive research grants, providing funding for seven years each. With the infusion of research money from the various funding agencies (Chapter 6 will analyze the actual grants Walt has received since his tenure at Tufts), Walt and his team expanded their research efforts, developing sensors for various applications. He eventually published those results and patented a few of the innovations (see Appendices 1 and 2 for a list of Walt’s patents and publications).

4.5 Expanding possibilities

By 1996, Walt’s sensor-based research had progressed to the point where his researchers could accurately measure up to ten different target analytes in a sample. However, the process to fabricate the fibers was tedious – there were three distinct chemical dipping and etching procedures that each fiber had to undergo to attach one sensor for every target analyte. This meant that for ten different target molecules to be sensed by one fiber, the fiber had to go through thirty rounds of etching and dipping in order to be fully functional. Despite the long fabrication process, the interest in applying sensors to
different areas of research continued and Walt continued to expand his base of collaborators in both academic and industrial laboratories [176].

One of Walt’s industrial collaborators was Robert (Bob) Matson, a scientist who worked at Beckman Instruments in Orange County, California. Matson was working on arrays for DNA molecules, a field that was rapidly expanding in the late 1990s, largely due to the excitement of the Human Genome Project. Matson contacted Walt in 1996 to suggest a research project for his sensors: Matson wanted to see if Walt could attach strings of DNA sequences to his optical fibers. Walt had used short sequences of DNA molecules, known as oligonucleotides (or oligos, for short), to detect the complementary sequences of DNA molecules before; he had designed a biosensor array to measure increasing levels of fluorescence as a proxy to quantify the number of copies of a particular gene were present in various solutions [178]. Walt agreed to the idea, and he and Matson collaborated on the project. Eventually, they published an article demonstrating the capability of the sensors to detect genetic variation at the single nucleotide-base level [179].

At the same time that Walt was collaborating with Matson on the DNA-sensing project, he returned to thinking about the optical fibers given to him by Will Hicks. These fibers, capable of maintaining images through the length of the fiber, were beginning to make headway in the field of optical memories – an alternative way to store information. Walt
wanted to build upon what was known about generating optical memories with these fibers. He reasoned that if the individual fibers in bundles could be etched to fine point, resembling thousands of little pencil points, then each point could be individually addressed and used as a point of memory for data storage [176].

Walt assigned this project to one of his postdocs, Paul Pantano, to see if the idea could be further explored. Pantano diligently worked in the laboratory, trying to etch the optical fibers to Walt’s ideals. But efforts to fabricate the fibers continually backfired. Instead of generating thousands of pencil points, Pantano generated fibers with thousands of indentations, resembling tiny wells. His results were the opposite of what he and his boss had intended. Not wanting to concede defeat to a fiber, Pantano and Walt set aside the failed attempts and secured different kinds of optical fibers from other manufacturers. One of the newly acquired fibers eventually was able to be etched into the desired points [180].

Meanwhile, Karri Michael, a PhD candidate in the lab, was looking for a new project to take on. One day, while informally discussing of her project on lithi-optical fibers, she and Walt decided to return to Pantano’s indented fibers, relics of the postdoc’s puzzling experiment, to see if there was any value in saving the material. Walt suggested that Michael find something to put into the well-like indentations [176].
Michael located some beads that fit snugly into the tiny wells. She used a scanning-force microscope to image beads resting consecutively in the wells. To Walt, the image of three beads in the wells resembled that of the two ears and head of Mickey Mouse [Figure 4.1 - courtesy of D. Walt]. The picture was called the “Mickey Mouse image.” Unfortunately, the usefulness of the experiment was not yet apparent. The fact that there were beads that could fit into the wells of the imaging fibers was interesting, but there was no direct research application at that time. So the project was again put on the sideline, without much regard for whether or not its results would ever bear fruit [176].

![Figure 4.1: Microspheres in etched wells of bundled fiber optic cables.](image)

But only a few months after Michael’s brief experiment was set aside, its research application became stunningly clear. During a brainstorming session in Walt’s office one
day in 1996, Bob Matson from Beckman remarked, “You know, David, it looks like people are going to be more interested in measuring more than just a few things. They might be interested in measuring a hundred things at a time instead of ten.” He went on, “And it’s even possible that there might be, on the outside chance, that they might even be interested in measuring a thousand things at a time.” Walt looked at Matson in disbelief, thinking, “Well, we’re out of luck here” [176]. Despite the newly developed pointed fibers, the etching and protecting processes to fabricate the fibers was still labor-intensive. To generate fibers with an increased number of sensors attached would be a grueling task were the fabrication process to remain as it was.

As Walt sat in the meeting with Matson thinking about the laborious challenge that Matson was proposing, a solution to the problem came rushing to Walt’s mind: “So, all of a sudden I could feel my face getting hot because I realized exactly how to solve the problem. Which, I did not say anything because Bob was from Beckman and it wasn’t his idea; he just asked the right question.” [176]

Immediately after Matson left, Walt “did something that [he] never did before and [he] never did after.” He pointed to four students working in his lab and said, “Come over here.” He motioned for them to come into his office. He shut the door and said to them, “I just had an idea and I’d like you all to drop your research projects right now and focus on this project.” [176]
While Walt conceded that the request might have caused some students to balk at the idea of dropping their current project without warning, he proudly reported that three out of the four students happily agreed to the task. The fourth student, who Walt described as an independent person, was not interested in participating. The other three students were eager to work as a team to accomplish the goal that Walt had proposed to them [176].

The basic idea that Walt had in mind was as follows: if different dyes could be attached to different bead types and different DNA sequences could be attached to each of the different bead, then:

“… you could do exactly what Karri had demonstrated: You could put the beads in the fiber and figure out exactly which bead carried which DNA sequence because every bead carried a different dye on it. And so the dye – so what it did was kind of turned the idea of making arrays on its head. You didn’t have to make them with a particular probe in an exact location. You could make everything ahead of time then spread them out and every array would be different – we called them random arrays but you could figure out what you made after you made it because we had a way of decoding what you made after you made the array.” [176]

4.6 Preparing for disclosure

As the team of students set to work on the project – including the fourth student whose interests in nucleic acids research was reason for her to help out – Walt began to realize the significance of this new innovation. He had been scheduled to give a talk at the upcoming Spring 1997 Pittsburgh Conference – a bi-annual conference held to highlight
new research and trends in the field of analytical chemistry. The conference was the most
highly regarded one of its nature for scientists interested in making measurements. All of
the instrument manufactures would be present to introduce their new product line and
world-renowned chemists would give talks on their research.

Walt was slated for a talk during one of the high profile plenary sessions at the
conference. He was eager to share his new discoveries at the talk, despite his previous
commitment to speak on a different topic. His interest to share his exciting results with
the scientific community, however, won, but with significant conditions attached: in
order to present his findings in a public forum, he would have to file a patent application
on his invention before the presentation which, by that point, was only days away.

The night before the conference, Walt and Michael worked on the patent application for
the fiber optic sensor with the bead-encoding capabilities; the publication describing the
work had been recently submitted but was not yet in press. The next day Walt’s speech
on the random arrays generated a lot of excitement. Walt commented that that conference
was the lead up to where the science ended and the business began [176].

When probed for his knowledge to submit the patent application in advance of any public
disclosure of his invention, Walt conceded that it came from previous business dealings
that had not gone as planned. Walt referred back to his research on microsensors to
measure blood samples of patients with cardiac or respiratory concerns. The technology that he developed for the project was licensed through Tufts University and initially used for some of the monitoring tasks. For several reasons – including the lack of health care reimbursement and the objection by the laboratory running the analyses of having the patients handle the data – the product was unsuccessful [176].

He went on to cite other inventions that he licensed and issues that had cropped up throughout each process. “…[O]ne of the licenses for example, I learned that we had forfeited our – well when we had published the paper and we were approached by the company, they say, ‘Well, it’s too bad you forfeited your foreign rights on this, because that’s about fifty percent of the market here.’ So that was an easy lesson to remember because we had screwed it up once before.” [176]

Walt took from the experiences the understanding of how to protect intellectual property. “…and so I at least understood enough to know that if you talk about anything publicly, you forfeit foreign rights; if you publish something, you forfeit foreign rights. If you publish something, you have a year to file in the U.S.” [176]

The Pittsburgh Conference generated much scientific buzz about Walt’s new invention. Publications describing the technology were issued shortly afterwards, although the focus was not primarily on DNA-based applications [144]. Walt and his researchers continued
to pursue the technology aggressively over the spring and summer of 1997. Overall, the summer was remarkably quiet, which provided ample time to account for the parameters and the capabilities of the technology at that time. The calm summer months rolled over to an active series of events that fall.

4.7 Faculty patenting at Tufts

Tufts University was one of the last university’s of its size in the New England region to establish its own technology licensing office. The University had outsourced much of its technology transfer efforts since the 1980s, when, under the auspices of the Bayh-Dole Act of 1980, federally funded research assigned intellectual property rights to the universities that got federal grants and contracts. Universities and the faculty inventors received royalties for licensed inventions. The Bayh-Dole Act encouraged universities to open their own technology licensing offices, to ensure that their inventions were properly protected and commercialized. Other smaller universities took advantage of localized tech licensing consortia. MBRI was one such company; it received a federal subsidy to inexpensively disclose university inventions and find means to fund startups deriving from the university-based technology [172].

In 1992, Tufts University opened up an agreement with the MBRI, to establish them as its sole provider of technology transfer [173]. At the time, one MBRI employee was
responsible for carrying out the due diligence of all faculty disclosures coming out of Tufts. One MBRI employee assigned to Tufts in 1997. He made a point of regularly speaking with faculty to better understand the type of inventions that they were disclosing [176].

That fall, the MBRI licensing officer approached Walt one day to discuss the bead-based fiber optic sensor invention that Walt had disclosed earlier that spring. “You know,” he began, “I think that I have somebody that might be interested in this. Would you might if I wrote this up as kind of a one-page thing and sent it off?” [176]

Walt replied, “No, by all means, feel free.” [176] His schedule had picked up since the summer and he had resumed teaching classes and training undergraduate chemistry students. The MBRI officer’s offer to write up the idea and present it to potential licensees and funders spared Walt the effort, and provided a crucial link that might otherwise never have been made.

Aside from running his independent research, Walt committed to a position as the principal investigator on a Multi-University Research Initiative (MURI) grant, sponsored by the Department of Defense. The grant funded a group of investigators from multiple universities to collaborate on a project to develop sensor arrays. (While the original
sensor array construction did not employ microwells, as the project evolved over the years, the technology was slowly adopted.)

One of the collaborators on the project was a well-known Columbia University Professor of Chemistry, Clark Still. Still was the founder of Pharmacopeia, a biotechnology company based on his academic inventions. He approached Walt during the fall and asked his permission to pass on information about his bead-based sensor technology to the same venture capital firm that had helped him start up Pharmacoepia. Again, Walt nonchalantly agreed to the request [176].

At that point, the technology was – at its core – capable of etching wells into optical fiber bundles that could hold 3-5-μm-sized beads, such that only one bead would fit in one well, creating an array of encoded beads. The Walt Lab had demonstrated what Illumina’s Chief Operations Officer John Stuelpnagel called ‘technology feasibility’ – the idea that the technology could provide a particular function without being targeted for any specific application [181].

Below is a brief discussion on the development of David Walt’s fiber optic sensor research up to the point where the technology described became incorporated into what would become the exclusively licensed patent portfolio from Tufts University to Illumina (see Chapter 5 for more details on the licensing agreement).
4.8 Fiber optic technology basics

The fibers used for fiber optic technology are composed of two types of glass or plastic, one that makes up the inner ring, known as the core, and the other that makes up the outer ring, known as the cladding. The design is based on the law of refraction: if the core has a high refractive index than that of the cladding, then light emitted in the core will be totally internally reflected, and no light will enter the cladding. This principle allows the light to be transmitted through the core over long distances. When a fiber optic cable is used as a sensor, a sensor molecule placed at the distal end of a fiber will emit light that will travel down the cable [Figure 4.2]. The light will then be detected by a camera at the other end of the cable. This was the design that Walt used to build his initial sensors.

![Figure 4.2: Total internal reflection using fiber optic technology. A) Standard optical fiber construction. B) A sensor attached to the distal end of an optical fiber can emit light, which will be reflected through the core fiber to the proximal end and be detected by a camera.](image-url)
4.8.1 Different optical fiber arrangements

Optical fibers could be used individually to sense targets. The fiber with an attached sensor probe could be introduced directly into a sample containing fluorescently-labeled molecules of interest. Upon binding of the probe to its target molecule, hybridization could be detected. This happened when light emitted at an appropriate excitation wavelength from the proximal end of the fiber would excite the fluorescent label from the target molecule at the distal end of the core. Isotropic light emitted from the fluorophore was then captured by a detection system back at the proximal end of the fiber, indicating that the probe had bound the target [Figure 4.3].

Figure 4.3: Fiber-optic-based sensor design. Walt’s original inventions attached sensors to the end of optical fibers. Binding of the target to the sensor would induce a change in the sensor which could be detected as light passed down the fiber.
The process to attach sensor molecules to the distal ends of fibers entailed several complex steps. In early attempts to measure multiple targets within a sample simultaneously, one fiber cable probe for each target of interested had to be individually manufactured.

Walt’s contact with Will Hicks introduced him to the bundled fiber optic cable. These bundles of fibers can be densely packed together by the thousands to form arrays [182]. The fiber bundles can be easily synthesized by bundling larger fibers into a “perform” that is melted and pulled into a resulting fiber “thread”. The thread has a reduced diameter but identical structure and aspect ratio as the initial perform [Figure 4.4].

According to Walt’s initial experiments, 5000 to 50,000 individual fibers, each 3 – 7 μm in diameter, created a fiber diameter of 300 to 1000 μm. Like an insect’s compound eye, each fiber is linked to its own light signal so each array can build a pixel-by-pixel image reconstruction. Through a series of experiments, Walt generated more complex sensors using these bundled optical fibers [183].

### 4.8.2 Etching fibers

In one of his landmark papers, Walt and his postdoc, Paul Pantano, explain that the distal ends of the fibers can be selectively etched relative to the cladding to create wells of
different depths [183]. Each fiber within the bundled cable was etched to contain micrometer- or nanometer-sized diameter wells.

The engineering process behind the etching was straightforward – soaking the fibers in hydrofluoric acid for increasing amounts of time would increase the amount of the core that was dissolved, thereby creating deeper wells. A standard 1000-μm diameter imaging fiber could be polished with various lapping films and subjected to hydrofluoric acid and then tapered to the desired final diameter through simultaneous heating and pulling with a standard glass capillary tube pipet puller. Distal imaging-fiber faces could then be mounted on aluminum stubs and sputter coated with gold to minimize charging. Resulting bundled fiber comprised approximately 70,000 individually cladded, coherently fused optical fibers, each with a ~2.6 μm diameter for a packing density of ~1 x 10^7 cores/cm^2. Smaller diameter arrays could be engineered through tapering of the imaging fiber’s distal tip – the packing architecture of the individual cladding was not altered.

Using one particular type of fiber bundle (not the one that Hicks had provided), ordered arrays were fabricated through a wet, chemical etching process. Given that each fiber corresponded to one individual well, each well could be individually tested. Pantano and Walt concluded that the ordered nanowell arrays could be used as polymeric pattern-transfer templates. Furthermore, given that each well was connected to its own optical
channel, the wells could be dipped in various polymers to create an array of probes [183]. This idea led Walt to develop more complex probe arrays based on the etched fiber design.

### 4.8.3 Optical fiber array fabrication

The bundled optical fiber that Will Hicks gave Walt was capable of having each fiber individually addressed, such that multiple analytes could be detected simultaneously. However, in order to achieve detection of multiple analytes two other challenges had to be overcome. First, as explained earlier, the bundled optical fibers required a CCD camera to detect the varying levels of signals coming from each individual fiber. Second, the way in which sensors were initially attached would not be appropriate for the bundled fiber cable. Each sensor required three unique steps for attachment. Thus, to add ten unique sensors onto the bundled fiber, thirty steps had to be carried out.

Walt’s initial approach to solving the problem of addressing the fibers was to etch each fiber to a point [Figure 4.4]. As described earlier, filing each fiber to a point was an impossible task using the bundled optical fiber. As a result, Walt’s lab members generated the optical fibers with etched wells. It was from that design that the original experiments to generate sensor-encoded microspheres that could be inserted into individual wells to create an array of probes derived.
4.8.4 Using DNA as sensor probes

The use of DNA as a molecular sensor occurred a few years before the conception of the sensor-encoded microsphere array. The first molecular sensor experiments using fiber optics began as a by-product of Walt’s expertise in immobilization chemistry. Walt and colleagues exploited the basic principles of fiber optic technology to construct sensors for various chemical analytes. Early project included sensor for carbon dioxide, glucose, penicillin and pH [180, 184, 185].
Walt’s lab published its first paper describing the application of its fiber-optic sensor research for DNA-based purposes in December 1996 in *Nature Biotechnology* [186]. Using DNA as probes, the lab exploited the hybridization properties of DNA – that nucleic acids base pair together based on covalent bonding. (Adenine binds to thymine; guanine binds to cytosine). The article proposed that DNA probes can be attached to the distal end of the fiber optic cable, through removal of the cladding and attachment of the probe to the outside of the core.

The article suggested that hybridization of target DNA to the oligonucleotide probe could occur in one of two ways: either the target DNA or the probe could be fluorescently labeled. The article demonstrated the former approach. Using a single core fiber attached to an oligonucleotide probe for interleukin-4 (IL-4), a common molecule found within the human immune system, the manufactured sensor was introduced to a solution containing the fluorescently-labeled IL-4 oligos.

Briefly, the methods involved treatment of the distal ends with saline, followed by a glutaraldehyde solution in phosphate buffer, then a polyethyleneimine treatment. Oligonucleotides (primers to amplify the human IL-4 cDNA) were dissolved in buffer and activated with cyanuric chloride in acetonitrile. The modified fibers’ distal ends were then washed and immersed in 10-μl solutions of cyanuric chloride-activated oligos. The activated oligonucleotides covalently bound to the fiber ends and served as sensors, with
the activated oligos serving as the probes. The individual sensors were grouped together and collectively placed in solutions containing either complementary or non-complementary labeled target sequences. When the complementary labeled target bound to the probe, fluorescence was released. This was because the target solutions contained amplified regions of human IL-4 cDNA plasmid, where the double stranded primary amplification products were internally labeled during an asymmetric PCR using fluorescein-labeled dGTP. Therefore, as the cDNA bound to the probes, fluorescein was released. The more the target molecules bound to the probes, the greater the amount of fluorescence detected. The initial array was designed such that fluorescence was measured with a modified Olympus epifluorescence microscope/charged coupled device camera. The results of the initial experiment revealed that the detection limit of the system was 10nM and required 10 minutes to generate a detectable signal. Longer incubation times resulted in photobleaching.

The study demonstrated the proof-of-principle that the fiber optic cable could act as a DNA biosensor array by probing for specific human cytokine mRNA sequences that were immobilized on the tips of single core fibers. Walt stated that the assay could be expanded by functionalizing the distal ends of different fibers with different cytokine oligo probes. The primary drawback of the initial design was that labeled sample targets are required. This problem could be overcome if competitive hybridization was
employed. Alternatively, Walt suggested that labeled targets could be avoided if self-reporting hybridization probes were used [187].

4.8.5 Making microsphere-based sensor arrays

The development of the bundled fiber enabled the individual monitoring of multiple sensors simultaneously with only one optical fiber. This bundled fiber could form an array of sensors. The array had the advantage that its small size and flexibility permitted the sensors to be directly immersed in the solutions containing target molecules. Initially, sensors were covalently attached to the fiber optic cable via its core through a tedious wet etching process. The development of the microsphere-based arrays dramatically altered the way in which the sensor arrays were generated [Figure 4.5] [144].
Walt employed microspheres to generate increased flexibility and higher throughput of his sensors. Microspheres were encoded and addressed using fluorescent reporter dyes capable of being spectrally resolved due to a particular dye signature. A two-dye system enabled the encoding microspheres with different concentration ratios of the two dyes. This method has been used before in flow cytometry [188].

A one-step procedure was developed to disperse tens of thousands of chemically modified microspheres in an etched fiber to create a sensor array. Optical encoding
schemes using fluorescent dyes permitted the identification of each sensor in the array. The encoding scheme initially permits the preparation of five distinct ratio pairs. Greater diversity can be attained with larger combinations of dyes and other discriminants.

4.8.5.1 Methods

Fiber: A 1000-μm-diameter imaging fiber containing ~20,600 individually cladded optical fibers was polished with lapping films and the distal end was submerged in buffered HF solution and then rinsed. The wet etching procedure was described in Pantano & Walt (1996) was carried out to produce wells ~3μm deep with a volume of ~36 fL (3.6 x 10^{-14} L).

Beads: The microspheres were generated by the following steps: Three 200-μl aliquots of stock 3.1-μm-diameter poly(methylstyrene)-divinylbenzene microspheres (5.8 x 10^9 microspheres/mL) were prerinsed. Three different concentrations of labeling dyes (DiTC/TRC) were added to the microspheres. The solvent swells the polymeric microspheres and allows the dyes to penetrate into the microspheres’ cores.

DiTC and TRC were chosen because the dyes are excited by the same wavelength (577 nm) and give rise to two separate emission wavelengths (670 and 610 nm, respectively).
Also, the dyes have distinct wavelengths from that of fluorescein (ex 490 nm/ em 530 nm) which is used as a target reporter dye for initial proof of concept.

The dye/microsphere suspensions were agitated and excess dyes were removed. Dyed microspheres were immobilized with various sensing chemistries on the outer surfaces:

- Microspheres (from group “A”) were activated with buffered glutaraldehyde and then coupled to alkaline phosphatase.
- The dye-labeled microspheres (from group “B”) were treated with NHS-biotin solution
- Microspheres from group “C” were biotinylated (as described for group B beads) and then treated with Avidin.

**Oligo probes:** The oligonucleotide probes can be either built up in a base-by-base method by using standard phosphoramidite chemistry or *preformed* oligos can be added directly to surface-activated microspheres.

### 4.8.5.2 Generation of random arrays

Individual populations of microspheres were pooled together to form stock solutions containing many copies of the probe-bound beads to create a dilute suspension (≤ 3.9 x 10^6 microspheres/μL). The etched microwell array fiber was held vertically and a 1-μL
drop of the suspension was pipetted onto the fiber’s distal surface such that individual microspheres randomly settle into each well. Microspheres attached to the well due to either a) electrostatic attractions between the untreated glass surface of the wells and the amino-functionalized microspheres; or, b) via addition of a thin polymer film.

4.8.5.3 Sensing analytes

Measurements were taken through an epifluorescence microscope coupled to a CCD detector, such that each individual optical fiber can be analyzed without scanning. The array was exposed to FDP, fluorescein-labeled avidin, and fluorescein-labeled biotin sequentially.

The reactions were monitored at 530-nm using 490-nm excitation light, followed by a buffer. Binding or enzyme activity giving rise to localized fluorescence signals were then decoded by switching to a second excitation wavelength (577 nm) to excite the encoding dyes. The individual encoded ratios were distinguished by taking the mean fluorescence intensity minus background intensity at each emission wavelength and then dividing the two values (670 nm/610 nm) to get a signature of each particular ratio.

In the presence of substrate Fluorescein diphosphate (FDP), ratio A microspheres produced a localized, enzyme-generated fluorescence signal. Ratio B microspheres
produced a signal when fluorescein-labeled avidin reacts with NHS-biotin. Ratio C microspheres also produced a signal when fluorescein-labeled avidin reacted with fluorescein-labeled biotin. Despite the exposure of the array to six different solutions (three target molecules and three buffer washes) 92% of the microspheres were accurately decoded and neither loss nor rearrangement of the microspheres was observed with the application of multiple liquid samples [144].

4.8.5.4 Decoding of the array

A key feature of the study was that the identity of each microsphere was not determined by its position in the array but by its encoded signature (so, any unforeseen microsphere relocation would be immaterial). Thus, only those microspheres giving rise to an analytical signal need to be decoded [144].

The randomly ordered array design dictated the necessity for a decoding system, as opposed to the standard coding system used in other ink-based or chip-based array platforms. Two methods were considered. The first was an optical bar code method. Each bead type could be labeled with a unique combination of two (or more) fluorescent dyes, with different excitation and emission wavelengths and intensities, either before or after probe attachment. The discrepancies between the emissions would allow each bead to be individually identified. This method resembled the Luminex method used for
multiplexed flow cytometry assays, but increased flexibility existed with fiber optic
cables, given the broad range of excitation and emission wavelengths that existed for the
various fluorophores. The optical bar coding technique would use conventional image
processing software.

The other technique involved hybridizing labeled DNA molecules to the array. The
process required sequential hybridization-dehybridization cycles with different decoding
solutions. Image processing techniques could evaluate and register the positions of each
bead type.

Beaded optical arrays are *unique* in that the position of each probe in the array is not
registered by deliberate positioning during array fabrication. Thus, the positions are
spectrally registered after random distribution. This permits each array to be unique. The
drawback to this process is that every array has the sensing probes and must be decoded.
This also requires that each array must be synthesized individually, as opposed to the
combinatorial synthesis approaches as w/ light-directed or ink-jet.

### 4.9 Conclusion

The bead-based sensor described above was the state of the technology when the first
steps towards technology transfer were being taken. The next chapter will explore the
history of Illumina, starting with the exclusive license of the patents in David Walt’s patent portfolio.


5 The Illumina Story.

This chapter will detail the events of Illumina’s history since its inception in 1998 to present day. Key events in the Company’s history, the development of Illumina products, and industrial and academic collaborations are listed separately at the end of the chapter.

5.1 Decision to launch

In the fall of 1997, John Stuelpnagel was working as an associate at New York-based venture capital firm Channing Weinberg (CW) Group. A letter arrived for him one day from a colleague he knew at the Harvard Technology Licensing Group, who was currently working for a venture-backed technology licensing office, MBRI [181]. The MBRI began as an answer to help universities outside of the Boston/Cambridge area enhance their intellectual property management. Tufts University, based in Medford, was one of the largest customers of MBRI’s services, which included performing the due diligence and filing patent applications as well as serving as an incubator for start up biotechnology companies. Stuelpnagel’s contact was representing Tufts at that time and wanted the CW Group to come up to Medford to look at a new technology.

While Stuelpnagel was contemplating the journey northward, he and his colleagues received another message, this time coming from Columbia University Professor Clark
Still, with whom CW Group had helped launch the biotechnology firm, Pharmacopeia. Still, coincidently, was alerting the CW Group to the same technology at Tufts University.

Larry Bock, Stuelpnagel’s boss at CW Group, put in a phone call to Walt to set up a meeting. As it turned out, Walt was scheduled to give a talk at the Scripps Institute in San Diego at the same time that Bock was in town to meet up with his firm’s San Diego office. The two men agreed to meet up for breakfast the morning of the talk.

Over breakfast, Walt learned that, unbeknownst to him, both his MBRI contact and his collaborator, Clark Still, had simultaneously sent the information about Walt’s invention to Bock. Bock was interested to hear about the technology directly from the inventor himself. Walt discussed his work with Bock and the two arranged for a tour of the laboratory to see the research firsthand in the coming weeks. Walt then left to give his talk at Scripps and shortly afterwards returned to the East Coast.

When Walt arrived home from his trip to California, he learned that he had received a voice message from another venture capitalist who happened to be in the audience during the Scripps talk and was interested in Walt’s technology. Even more coincidently, he was also Larry Bock’s former colleague from their time at another venture capital firm.
The following week, John Stuelpnagel and Larry Bock traveled up from New York to Medford to meet with David Walt to see the laboratory in action. As Walt recounted, “John came out and sort of put on the hard sell, such as ‘…this technology really has potential’ and ‘here’s what we’d like to do…’ And so it kind of spiraled at that point. Larry and John were pretty much single-minded in getting me to agree to start a company based on the technology” [176]. Stuelpnagel and Bock spent time meeting the laboratory members (including several current employees of Illumina, Inc., such as the current Director of Product Development, Todd Dickinson), looking at the experimental data, and seeing the actual imaging instruments and arrays that the lab had developed [181].

After Stuelpnagel and Bock left, Stuelpnagel was given the charge of performing due diligence on the markets, on potential competitors, and on the opportunities and the potential competitive advantages for the array technology. The due diligence process, which included an extensive amount of intellectual property due diligence that required the hiring of a third party attorney, continued until approximately January of 1998 [181].

5.2 Timing of events

In the meantime, from fall 1997 until about 15 June 1998, CW Group funded the initial startup costs, providing enough capital to fund the traveling expenses, due diligence costs to assess whether the development and commercialization of the technology would be a
worthwhile investment, and the overall costs of putting together what would be the exclusive license on the technology [181].

In January 1998, the CW Group, Walt, the MBRI and Tufts University opened up the licensing discussion [181]. The parties agreed to a worldwide exclusive license for the technology to the new startup company, named Illumina, which would be effective as of May 6, 1998. Illumina was incorporated in April of that year [189].

The due diligence process continued between January and May 1998 as the market investigation process took hold. The applications for the technology included five potential areas, most of which involved the life sciences: genotyping, gene expression, proteomics, drug and pharmaceutical screening, and chemical/odor sensing. Stuelpnagel applauded the technology for its capability to adapt to sensing any chemical of interest by attaching a detector chemical to the surface of the beads [181].

5.2.1 The exclusive license

Effective 6 May 1998, Illumina entered into a worldwide exclusive licensing agreement with Tufts University [Appendix 3 for a complete copy of the exclusive license]. Under the agreement, Illumina was granted the right to sublicense, to develop, market and sell products deriving from the licensed patents and “Know How” [190]. Citing the Bayh-
Dole Act of 1980 [159], the exclusive license gave Illumina and its affiliates permission “to make, have made, import, use, lease, sell and offer for sale, and otherwise commercialize and exploit Licensed Products, and to practice any method, process or procedure within the Exclusive Technology” [190].

In return, Illumina, Inc. granted Tufts University the right to practice the “Exclusive Technology” for the University’s own research or in collaboration with other third party academic or non-profit research institutions, “solely for non-commercial purposes […] not for sale, license, or other distribution” [190]. The “Exclusive Technology” explicitly referred to a patent portfolio containing thirteen patents developed from research performed by David Walt and colleagues, where Walt is named as one of the inventors [Table 5.1].
Table 5.1: Patents by David Walt and exclusively licensed to Illumina. This table lists the patents included in the patent portfolio at the time of the exclusive license (6 May 1998). Illumina holds a worldwide exclusive license on the use of these patents. Information found in Exhibit 10.8 of Illumina’s S-1 Filing in 2000. Disclosure of the exact patents was made in later SEC filings. All filings were obtained through the SEC EDGAR database.

<table>
<thead>
<tr>
<th>Inventors</th>
<th>Title</th>
<th>Patent No.</th>
<th>Date Issued</th>
</tr>
</thead>
<tbody>
<tr>
<td>WALT, David; BARNARD, Steve</td>
<td>Imaging Fiber Optic Array Sensors, Apparatus and Methods for Concurrently Detecting Multiple Analytes of Interest in a Fluid Sample</td>
<td>US Patent: 5,244,636</td>
<td>9/14/93</td>
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<td>WALT, David; BARNARD, Steve</td>
<td>Fiber Optic Sensor, Apparatus and Methods for Detecting an Organic Analyte in a Fluid or a Vapor Sample (OSCI)</td>
<td>US Patent: 5,244,813; CA Patent: 2,128,413</td>
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<td>WALT, David; HEALEY, Brian</td>
<td>Photodeposition Methods for Fabricating a Three-Dimensional, Patterned Polymer Microstructure</td>
<td>USSN: 08/519,062 (now US Patent: 6,200,737)</td>
<td>Filed: 8/24/1995</td>
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<td>WALT, David</td>
<td>Far-Field Viewing Optical Apparatus for Making Optical Determinations and Analytical Measurements</td>
<td>USSN: 08/572,005 (now US Patent: 5,814,524)</td>
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<td>WALT, David; MICHAEL, Keri</td>
<td>Fiber Optic Sensor with Encoded Microspheres (Analyte Detection System)</td>
<td>USSN: 08/818,199 (now US Patent: 6,023,540)</td>
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<td>WALT, David; DICKINSON, Todd</td>
<td>Self- Encoding Microspheres</td>
<td>USSN: 08/944,850 (now US Patent: 7,115,884)</td>
<td>Filed: 10/6/97</td>
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<td>WALT, David; HEALEY, Brian; FERGUSON, Jane</td>
<td>Fiber Optic Biosensor for Selectively Detecting Oligonucleotide Species in a Mixed Fluid Sample</td>
<td>Application Being Prepared (now US Patent: 6,482,593)</td>
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<td>WALT, David; TAYLOR, Laura</td>
<td>Fiber Optic Biosensor Array Comprising of Cell Populations Confined to Microcavities</td>
<td>Application Being Prepared (now US Patent: 6,377,721)</td>
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</table>
5.2.2 Tech Transfer Financing

The licensing agreement between Tufts and Illumina contained detailed goals and milestones that were required for Illumina to achieve in order to maintain its exclusive license [Appendix 3]. For example, Illumina agreed to raise $500,000 in equity financing from third parties between May 1998 and May 1999. Overall, the company agreed to raise $2 million in total financing, referred to as “First Financing”, by May 2000. Financing sources, as detailed in the agreement, included but were not limited to equity or debt financing, government grant funding, and sponsored research and development funding [190].

5.2.3 Licensing terms

The terms of the licensing agreement were “standard” among exclusive licenses deriving from University inventions, according to Tufts University Vice Provost Peggy Newell, who spoke to us about the licensing negotiations from Tufts’ perspective [172]. Tufts University agreed to the license on the grounds that it would encourage Illumina to use its best efforts to bring “one or more Licensed Products to market” [190]. As part of the plan

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1 The details of the Exclusive License agreement between Tufts University and Illumina were made largely confidential until recently. The 10-Q for the first quarter of 2007 is the first time that Illumina disclosed the exact patents and R&D spending specifications. This information can be located at http://www.sec.gov/Archives/edgar/data/1110803/000093639207000368/a29658e10vq.htm#tocpage.
to ensure the startup was on-track with its goals, Illumina was required to provide Tufts with an operating plan, detailing the “amount of money, number and kind of personnel, and time budgeted and planned for each phase of development of the Licensed Products” within one year of signing the agreement [190].

In addition, Illumina and its affiliates or sublicensees were required to spend above threshold amounts of money on a calendar-year basis to develop and commercialize the Licensed Products in order to maintain the exclusive license [Table 5.2]. The agreement also stipulated that of the minimum expenditure required to develop and commercialize the Licensed Products”, 25% of the total annual expenditure must be specifically devoted towards development of the technology from US Patent No. 5, 512, 490 (hereafter, the ‘490 patent). Failure to meet any of the milestones within a 90-day grace period would permit Tufts the right to change the license to a non-exclusive one [190].

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Expenditure</th>
<th>US 5,512,490 Expenditure</th>
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</thead>
<tbody>
<tr>
<td>1999</td>
<td>$1,000,000</td>
<td>$250,000</td>
</tr>
<tr>
<td>2000</td>
<td>$1,500,000</td>
<td>$375,000</td>
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<tr>
<td>2001</td>
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</tr>
<tr>
<td>2002</td>
<td>$2,500,000</td>
<td>$625,000</td>
</tr>
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</table>

Table 5.2: Breakdown of R&D funds Tufts required Illumina to devote to patents licensed from the Walt portfolio.
5.2.4 Payments and Royalties

Illumina agreed to pay Tufts royalties equal to three percent (3.0%) of the net sales (detailed as gross revenue received from sales of the Licensed Products minus all taxes, duties, insurance and transportation costs, normal and customary rebates, and any credits given). In terms of foreign sales, Illumina agreed to pay royalties to Tufts based on foreign currency conversion rates according to the rates listed at the Bank of America, San Francisco. If Illumina opted to sublicense the Licensed Products, the company would be required to pay Tufts a sublicensing fee equal to twenty-five percent (25%) of the net revenue received from the sublicenses [190].

In return for Tufts’ assistance with the foundation of Illumina, Illumina granted Tufts the right to purchase 500,000 shares of Illumina common stock, totaling 10.0% of the company’s founding capitalization [Table 5.3]. At the time of the signing of the license agreement, the fair market value of Illumina common stock was listed at $0.01 per share. A separate stock purchase agreement date was determined. Illumina also agreed to allow an independent Certified Public Accountant appointed by Tufts to audit Illumina’s records to verify that the company was practicing due diligence [190].
### Table 5.3: Founding Capitalization for Illumina, Inc. [190].

<table>
<thead>
<tr>
<th>Organization</th>
<th>Shares</th>
<th>% Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBRI/Tufts University</td>
<td>500,000</td>
<td>10.0%</td>
</tr>
<tr>
<td>Founders</td>
<td>1,275,000</td>
<td>25.5%</td>
</tr>
<tr>
<td>CW Group</td>
<td>375,000</td>
<td>7.5%</td>
</tr>
<tr>
<td>Reserve</td>
<td>2,825,000</td>
<td>57.0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>5,000,000</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

#### 5.2.5 Warranties and Indemnity

Tufts assumed no responsibilities or warranties in the signing of the licensing agreement. The University stipulated that it could not be held liable to Illumina or Illumina’s affiliates or sublicensees for “any matter regarding this Agreement, Licensed Patents, the Know How, the Licensed Products or any product or services furnished by Tufts” [190]. To Illumina, Tufts only warranted an exclusive license to the Licensed Patents. In return, Illumina vowed to indemnify Tufts, its trustees, officers and employees of any action that might result from the licensing agreement. Illumina also agreed to maintain liability insurance, including product liability, protecting Tufts against all claims [190].

#### 5.2.6 Patent Prosecution and Infringement

Tufts maintained the primary responsibility for prosecution of the Licensed Patents, including enforcement against infringement. Illumina assumed the ability to advise and
consult Tufts in the event that Illumina believed it necessary to enforce and protect of the Licensed Patents. Should Tufts elect to abandon patents or patent applications in any country, Tufts granted Illumina the opportunity to continue prosecution or maintenance at Illumina’s own expense. Upon validation of the licensing agreement, Illumina became responsible for “payment [of] all reasonable fees and costs relating to the filing, prosecution and maintenance of Licensed Patents which are incurred by Tufts … (including interference and/or opposition, nullity and revocation proceedings)” [190].

In the event that either Tufts or Illumina suspected infringement of the Licensed Patents by a third party, Tufts agreed to take primary responsibility to prosecute the offender at its own expense. Illumina was granted the right to proceed with prosecution at the company’s own expense, in the event that Tufts chose not to prosecute the infringing party. In the event that an action alleging invalidity or non-infringement of any of the Licensed Patents was to be brought against Illumina, Tufts claimed the right to intervene and take over sole defense of the action at its expense. Any legal action against either Tufts or Illumina would result in the other party cooperating fully and acting in defense of the other party [190].

Should Illumina be forced to pay fees or expenses to cover the costs to maintain, prosecute or bring or defend any proceeding relating to any infringement by a third party of any of the Licensed Patents, Tufts agreed that 50% of the amount of such costs would
be credited to Illumina in terms of royalties due to Tufts. This aspect of the licensing agreement was called into practice in 2006 when Affymetrix, Inc. sued Illumina, Inc., for patent infringement. The Tufts University Office of Technology Licensing & Industry Collaboration representatives stated that the company had indemnified Tufts during the proceedings and Tufts cooperated with Illumina in sharing information pertaining to its exclusively licensed patent portfolio [191].

The exclusive license to Illumina by Tufts was granted until the expiration of the last to expire of all Valid Claims included in the Licensed Patents. “Following such an expiration, Licensee shall have a non-exclusive, royalty-free, irrevocable license in such country to the Know-How.” The patents in the Exclusive License are set to expire between 2010 and 2017 [190].

5.3 Illumina, the startup

On 15 June 1998, CW Group sponsored a seed round of investment for Illumina. The Series A financing, which was co-sponsored by ARCH Venture partners, raised three quarters of a million dollars, which was used to support the development of the business model and to cover the incubation stage of the company [192, 193].
In the summer of 1998, serious efforts to organize the company began. Walt spent that summer making several trips out to the San Diego office of CW Group, which was where Illumina was housed in its infancy before any real estate decisions were made. His trips involved an extensive amount of consulting and brainstorming, and as the summer rolled into the fall, his priorities shifted away from helping the startup efforts to preparing for classes. Walt chose to maintain his faculty position at Tufts for a number of reasons, which included his enjoyment of performing independent research and his overall lack of interest in leaving for California [176].

Walt’s decision meant that other top scientific talent would have to be selected to run the research and product development activities on-site. One of the first recruits was Dr. Mark S. Chee, who was working as Director of Genetics at Affymetrix at the time and considered one of the top experts in the field of microarray technology. He signed on as Vice President of Genomics and his addition was a boon to the recruitment of other top talent [194].

As recruitment to the startup began, with Illumina’s first employee officially signing on in June, Stuelpnagel continued to critically evaluate the feasibility of the applications for the technology. The business model was written to account for the five applications previously mentioned, but as time passed, the founders began to see several advantages in focusing on genotyping as the company’s initial application:
“One is we felt that we had more competitive advantage in that space because as we continued to develop our own technology at Illumina, it became clear that we had ideas for assay development that would allow very robust genotyping to occur. The other was a competitor avoidance strategy, where, at the time, no one was really using arrays very successfully for genotyping.” [181]

The paucity of direct competition in the genotyping market, compared to the plethora of players entrenched in gene expression market, such as Applied Biosystems, Affymetrix, Clontech Laboratories, PE Biosystems, etc., was reason enough for Illumina to stake its ground in uncharted territory.

5.3.1 The money

From June until November 1998, while the founders worked on the business plan, several presentations were made to potential investors. The presentations highlighted the novelty and feasibility of the technology, as well as the management and scientific talent that the company had already successfully recruited [181].

The chosen title for Walt’s fiber optic sensor array platform was the Array of Arrays™ platform, with the ‘BeadArray’ technology, referring to the fiber optic sensor with encoded bead technology. The goal of the platform was to produce millions of
genotyping experiments on a single microtiter plate – an experiment that could be extended to array-based gene and protein expressions upon product development [193].

By November 1998, the first major round of financing for Illumina closed, raising $8.5 million. CW Group and ARCH Venture Partners recruited venture capital firms Venrock Associates and Tredegar Investments to contribute to the fund [193]. The funding provided extensive expansion into actual laboratory work and experimental proof-of-principle [181]. More research scientists, including graduate students and postdoctoral fellows from David Walt’s lab, began to filter into the newly acquired laboratory spaces to work on the initial phases of product development.

One of the first strategic moves was to acquire a small company called nGenetics in the fall of 1998 [189]. The acquisition brought in a key component of intellectual property that maintains Illumina’s competitive technological edge to this day. The decoding technology allowed the company to expand the complexity of the arrays – from attaching hundreds to millions of objects on a single substrate – that the original Tufts model could not provide [181].

The funding also enabled recruitment of the core management team, as well as the Scientific Advisory Board (SAB) [195]. In addition to Stuelpargel – who would serve as Illumina’s acting President and CEO until October 1999, when he transitioned to CFO –
and Chee, other key members of the core management team included: Anthony W. Czarnik, who was brought in as Chief Scientific Officer, after leaving his post as Vice President of Chemistry at IRORI Quantum Microchemistry in San Diego, CA, and Richard J. Pytelewski, who was brought in as Vice President of Product Development. ARCH Venture Partners venture capitalist Robert Nelsen described the core scientific management team at the newly-formed company: “…[it brought] immediate strength … in the critical areas of array technologies, high throughput assay development, analytical and combinatorial chemistry, systems integration, and product development” [193].

David Walt, being the inventor of the key technology, was the obvious choice for the position as Chairman of the SAB, a position that he still holds today, in addition to his contribution to Illumina as a member of the Board of Directors. Other members of the Scientific Advisory Board were: Professors Christopher C. Goodnow of the Australian National University, Leroy Hood of the University of Washington, John S. Kauer of Tufts University, Paul S. Schimmel of the Scripps Institute, Terrence J. Sejnowski of the Salk Institute, and W. Clark Still of Columbia University [193]. Of the Scientific Advisory Board, Bryan Roberts of Venrock Associates remarked, “As internationally recognized experts in genomics, immunology, molecular biology, analytical and combinatorial chemistry, information processing artificial olfaction and fiber optic sensors, these individuals bring a vast amount of knowledge to the company. This
knowledge will be important as Illumina sets its scientific focus and begins to capitalize on the many potential applications of its technology” [193].

5.3.2 The first collaborations

Corporate partnerships have been touted in the biotech industry as a means for startups to attract more investors – through increased investor confidence – on the way towards an initial public offering [196]. In June 1999, Illumina entered into a research collaboration with Dow Chemical to develop a BeadArray for chemical solvent identification. Dow Chemical hoped that the array technology would serve as a quality control mechanism to assess raw materials entering its plants. Illumina was to retain all rights to commercialize any resulting products from the collaboration [194]. This collaboration was the first of several that soon followed.

In November 1999, Jay Flately came on board, after being appointed that September, and has since served Illumina as its President, Chief Executive Officer and Director [194]. At the time that Stuelpnagel recruited him to Illumina, Flatley was serving as CEO and President of Molecular Dynamics, a company that he had founded. Flatley told Fortune magazine that the “phenomenal technology” was enough to bring him on-board almost at once. “I fell in love” [138]. He conceded that he did consider the following factors before joining Illumina:
“First, I wanted to make sure that BeadArray technology, its benefits, and cost advantages were both powerful and sustainable. Second, the derivative commercial products (arrays, and reagents) have to be readily manufacturable from the standpoints of cost, quality, and scalability. And third, the amenable markets have to be large and growing, with additional growth available from new markets and applications. Finally, on a personal level, I had tremendous respect for both John [Stuelpnagel] and Mark [Chee].” [192].

Addressing Flatley’s concerns, by November 1999, Illumina had established a connection with biotechnology giant, Applera Corporation. A non-exclusive license emerged with Applera’s Applied Biosystems (ABI) division to commercialize genotyping. According to the licensing terms, ABI was charged with developing the assay and the instruments, while Illumina agreed to develop the array. In addition, ABI was responsible for marketing the system worldwide. The agreement was designed to allow the implementation of ABI’s proprietary oligonucleotide ligation assay (OLA) ZipCode assay format for Illumina’s proprietary technology platform that became marketed as “Array of Arrays” for SNP genotyping[194]. The products were to be co-branded, while distribution was to be handled by ABI’s well-oiled sales pipelines. Illumina was to gain the rights to use any instrument developed out of the agreement to use for other applications. Applera Corporation paid $5 million up front in the form of 1.25 million
shares of Series C convertible preferred stock at a price of $4.00 a share [197], with an
agreement to provide Illumina with non-refundable research and development funds of
$10 million over the following two years. Illumina and ABI agreed to split the profits
from the collaborative products, including “instruments, array cassettes and reagent kits”
[194]. This agreement would result in dueling lawsuits two years later, a conflict over
fulfilling terms of this agreement.

Also that month, Illumina joined forces with PyroSequencing in a research collaboration
effort to determine whether the proprietary PyroSequencing assay could be formatted to
the BeadArray platform. PyroSequencing made instrumentation and the necessary
chemicals to perform DNA sequencing and SNP genotyping. Should the collaboration to
be successful, Illumina and PyroSequencing agreed to discussions on generating a
commercial product [194].

A similar deal emerged in December when Illumina agreed to a research collaboration
with ThirdWave Technologies, Inc., whereby Illumina would use the proprietary assay
format, known as Invader, to be formatted to the Illumina BeadArray platform. The
collaboration, if favorable, was to determine whether a product or service of some sort
could be developed [194]. Illumina received an SBIR grant from the National Institute for
General Medical Sciences to use the Invader™ assay in combination with the BeadArray
platform to develop a high-throughput, highly-specific enzymatic assay for analysis of genomic DNA without prior PCR amplification.

5.3.3 Preparing to go public

During that time, the capital provided by the ABI deal was enough to set up the next round of financing, which occurred in November 1999. Illumina raised a total of $28 million, $5 million of which came from ABI while $23 million came from venture capital funds. As Stuelpnagel remarked, the funding was “an important milestone… partly stimulated by the fact that the genomics field was heating up in the 1999-2000 time period” [181]. He added he and Flately immediately saw the potential for the technology in the genomics market. It was at that point that they decided to take the company public, if given the opportunity [181].

Work moved rapidly after that decision to build the infrastructure capable of handling a public offering and of supporting a public company [181]. In December of 1999, Illumina recruited Robert Kain as Vice President of Engineering from his post at Molecular Devices, where he served as Senior Director of Engineering. In March of 2000, the Company appointed David L. Barker to the charge of Vice President and Chief Scientific Officer. Dr. Barker hailed from the position of Vice President and Chief Science Advisor.
at Amersham Pharmacia Biotech. Two months later, Illumina brought Noemi C. Espinosa on board as the Company’s new Vice President of Intellectual Property [194].

In the spring of 2000, Illumina acquired Spyder Instruments. The acquisition of the privately held company provided Illumina the intellectual property surrounding high-throughput peptide synthesis to be converted to high-throughput oligo synthesis [181]. Through this acquisition, the company re-branded the technology to be called the Oligator technology.

5.4 Initial Public Offering

On 3 July 2000, with Goldman Sachs being the company’s lead banker, Illumina announced its initial public offering (IPO). John Stuelpnagel, who was Illumina’s Chief Financial Officer at the time, reported, “the company was in the fortunate position to be selective when choosing underwriters. It chose Goldman Sachs to lead the deal because of its reputation for managing complex stories… Their ability to put us in front of the top investors in the world was just terrific” [198]. Goldman Sachs analysts Steven McGarry and Maykan Ho were particularly instrumental in executing the IPO. According to Stuelpnagel, the analysts were highly regarded in the industry. Illumina chose Chase H&Q and SG Cowen to co-manage the firm based on similar reasoning [198].
On 27 July 2000, Illumina commenced its initial public offering pursuant to a filing Form S-1 Registration Statement (File No 333-33922) [189]. The company increased the size of the IPO from 5 million to 6 million shares. CFO John Stuelpnagel cited an increased interest in genomic research as the reason for the increase [198]. Likewise, the share price that was initially set at $9 - $11 per share was raised to $13 - $15 the week before the IPO, which was eventually priced at $16. The net offering proceeds were $101.3 million [199]. The IPO also contained a “15% greenshoe [option] which is expected to be exercised” [198]. The greenshoe option, also known as an over-allotment provision, would grant the company’s underwriter’s permission to buy up to an additional 15% of the Illumina shares at the offering price, given the fact that public demand for the stock exceeded expectations, with the stock trading above its offering price. Stuelpnagel noted that Illumina, Inc. was “the last successful biotech [IPO] of that market period” [181].

In October of 2000, Illumina succeeded in recruiting Arnold Oliphant, Ph.D. as Vice President of Scientific Operations. Prior to Illumina, he was Vice President of Functional Genomics at Myriad Genetics. David C. Douglas arrived at Illumina in January 2001 to take the position of Vice President of Manufacturing [194].

The success of the IPO can be largely attributed to the investor excitement in the overall market at that time. The IPO marked an important turning point in the company.
Stuelpnagel admitted that Illumina was “very fortunate” to have had the tremendous monetary capital influx when it did, “…because had we not been that successful in raising money, Illumina wouldn’t exist today – at least not in the form, and not with the success that we’ve experienced” [181]. As of 31 December 2006, Illumina has used $46.0 million of the initial funds to purchase property, plant and equipment, approximately $2.4 million for the CyVera acquisition and approximately $52.9 million for general operating expenses [200].

By the end of 2000, the company maintained exclusive ownership to the 13 licensed U.S. patents from the Tufts patent portfolio, and boasted 55 pending U.S. patent applications, including “two allowed applications, some of which derive from a common parent applications” [194]. The issued patents cover the fiber optic arrays, bead array technology and chemical detection. These patents are set to expire between 2010 and 2017 [194].

5.5 Product Development to Product Delivery

Illumina’s first commercial application of its proprietary BeadArray technology was introduced to the market in 2001 with as a SNP genotyping service. The ‘BeadArray’ technology, which referred specifically to the fiber optic sensor with encoded microspheres that derived from David Walt’s chemistry lab, was originally developed into two different Array of Arrays™ formats – the Array Matrix and the BeadChip. The
Array Matrix, the first “consumable” BeadArray product used for SNP genotyping, provided a universal format, capable of analyzing any given set of SNPs. Reagents based on Illumina’s GoldenGate assay protocols and the BeadArray Reader, a laser scanner, were also introduced as part of the initial platform [194].

In January 2001, Illumina entered into a deal with ChevronTexaco Energy Research & Technology Company to build a BeadArray application based on the detection of leaks and gasoline grade for the petroleum energy [201]. During the first fiscal year of public operation, Illumina also struck research collaborations – in addition to its ongoing agreement with Tufts University – with the Australian National University, Stanford University, and the University of California, San Diego to develop novel applications for its BeadArray technology [194].

In June of 2001, Illumina initiated its Fast-Track SNP genotyping service through its first service agreement with pharmaceutical giant, GlaxoSmithKline. Illumina would provide SNP genotyping services on samples collected from GSK. Illumina CEO Jay Flatley endorsed the commercialization of the genetic analysis business as a means to improve the genotyping assay development and to refine the core BeadArray technologies [192].
5.6 Early troubles

Following the success of its IPO, Illumina hit a few low points during its first year as a publicly traded company. The project to develop a genotyping platform with Applera Corp.’s ABI was originally scheduled to begin in 2001, but ABI requested to delay the product launch until summer 2002. In July 2002, ABI announced that the company wanted to delay the release again, citing the company’s inability to optimize and multiplex the SNP assay reagents for commercial release [197]. Illumina, not wanting to delay product launch any longer, forced the company to resort to its Plan B – developing a genotyping platform entirely of its own design. Fortunately, the company had already been preparing contingency plans and had developed a working assay and a working instrument, in addition to a working array. The non-exclusive nature of the collaboration with ABI allowed Illumina to go forward with launching its independent system [181].

The news of the foiled collaboration sent Illumina’s stock price down to the $2 per share range. The cash stockpile from the IPO helped the company stay afloat and continue with its own product development despite the low stock price [181]. By mid-summer 2002, Illumina released its own array-based genotyping system using the Sentrix BeadArray technology with the Sherlock scanner [197]. The launch was a major boon to the company.
5.7 The BeadLab system design

Illumina’s first commercial product, launched in 2002, was the BeadLab system. The BeadLab system contained all the necessary components to isolate, assay, detect and analyze samples for genotyping. The platform was built such that the Illumina’s GoldenGate assay for SNP genotyping could be performed on 96 samples, which were formatted in micotitre plates, known as the Sentrix Array Matrix. Each sample was an individual bundled optical fiber containing over 1500 fused fibers. Each of individual fiber within the bundle was etched according to Walt’s original design, such that one bead encoded with oligonucleotide probes could fit snugly in a well [197].

The BeadLab also was formatted with an automated, laboratory information management system (LIMS). This technology enabled sample tracking and workflow management\(^2\). Fluorescence was monitored by the Sherlock™ confocal laser scanner. The scanner was incorporated into the BeadLab as a technology exclusively licensed from Molecular Dynamics\(^3\) [197].

\(^2\) For more information on any of the Illumina products, see the company website at: http://www.illumina.com.

\(^3\) Molecular Dynamics was the company from which Illumina CEO Jay Flatley hailed. It has since been acquired by Amersham, PLC, which has been incorporated into the GE Healthcare system.
Illumina also offered a custom oligonucleotide manufacturing service as part of the newly established business. Oligonucleotide synthesis would evolve in later years to become a collaboration with Invitrogen [197].

5.8 HapMap Collaboration

In 2002, Illumina had entered into 18 other service contracts [197]. Of significance, Illumina also found a market for itself with the advent of the International HapMap Project [202]. Launched in October 2002, the Project began as an approximately $100 million public-private effort to develop a next-generation map of the human genome [203]. The aim for the Project was to uncover the variations in genetic sequences that predispose individuals to common illnesses such as asthma, cancer, diabetes and heart disease.

Illumina wanted to lend its support in the Project. However, the Project was setting up as Illumina’s collaboration with ABI to generate systems capable of the high throughput analysis was deteriorating. ABI claimed that its assays were not at the level of multiplex that Illumina believed its new platform could handle [192].

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4 For more information on the HapMap Project, see the website at: [http://www.hapmap.org](http://www.hapmap.org).
By the time that the HapMap Project began officially, Illumina had developed its commercial BeadLab system with GoldenGate assay to support the Sentrix Array Matrix platforms. The new technology had the multiplex capabilities to handle the level of throughput demanded of the HapMap Project [138].

Illumina decided to submit its BeadLab protocols as part of the HapMap grant application. In October 2002, the Company was selected to play a significant role in the Project. The International HapMap Project granted Illumina $9 million, through funds from the NIH, to assist the Company in the project [192].

Overall, Illumina remained only one of five funded U.S. HapMap Project participants. The BeadArray technology was used to genotype approximately 15% of the total human HapMap. Illumina’s direct effort counted for one half of the U.S. effort towards the Project. Since major research institutions abroad were also contributing to the HapMap Project through the use of the Illumina BeadLab system, upwards of 60% of the genotyping performed for the entire project might have been done with the Illumina technology [192]. (See Chapter 6 for a more detailed analysis of Illumina’s impact on the HapMap Project).

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Information regarding all federal funding to Illumina is listed in Table 6.6 of Chapter 6.
As a result of Illumina’s contribution to the International HapMap Project, the Genome Quebéc Innovation Centre and the Wellcome Trust Sanger Institute were the first few research institutes to purchase the production-scale SNP genotyping system [204, 205]. The purchases reflected the decision of the Canadian and British funding agencies overseeing the International HapMap Project to use the Illumina system to generate genotyping data. The Sanger Institute was involved in a study to map haplotype blocks in defined chromosomal regions, which largely employed Illumina’s genotyping services. Additional studies in collaboration with Illumina run by the Sanger Institute and the Wellcome Trust’s Centre for Human Genetics, Oxford University, included the design, SNP selection and data analysis for Illumina’s contribution to HapMap [205]. As a result, the Sanger Institute ended up producing data for 25% of the entire HapMap Project [192].

5.9 Applied Biosystems Litigation

In December 2002, Illumina notified ABI that it was in breach of contract. ABI immediately filed a patent infringement suit in the U.S. District Court in San Francisco and a demand for arbitration for breach of contract in the San Diego Superior Court. ABI demanded $30 million in damages and an order to bar Illumina from future sales on genotyping systems or services, insisting that Illumina’s new system infringed on patents owned by ABI. Illumina retaliated with a preliminary injunction on the demand for
arbitration and a counter-suit for the “breach of contract, breach of good faith, fraudulent inducement and unfair competition” in the San Diego Superior Court [206].

By January 2003, both firms had launched their own genotyping systems. ABI launched a chemistry-based genotyping system, the SNPlex. In February 2003, the Judge granted a preliminary injunction, dismissing the arbitration demand. Three months later, ABI responded to Illumina’s suit and filed a cross-complaint for breach of contact and unfair competition [206].

Analysts following the case worried that a loss for Illumina would result in a loss of the company’s core technology, in addition to $30 million in damages. Illumina alleged that ABI had “no intention of fulfilling the joint agreement” and the lawsuit was done in order to “squash” the perceived competition [206]. Larger companies have been charged in the past with stalling projects in a strategic effort to assess the competition – a move that has been observed in the information technology and medical diagnostics industry, according to some leading analysts [206].

In August 2004, Illumina and ABI had reached a settlement, whereby Illumina agreed to return $8.5 million of the $10 million it had received up-front [207]. Illumina was permitted to keep the $1.5 million for its contribution to the collaboration. Under the settlement, the two companies agreed to cross-license each other on the disputed
intellectual property without the exchange of any fees [207]. The ABI SNPllex system is in direct competition with Illumina’s Bead Station.

5.10 BeadChip Platform Launch

By June 2003, Illumina released its Sentrix™ BeadChip Platform [208]. The BeadChips, although processed in the same methods as the Array Matrix, were manufactured with highly flexible MEMS (microelectronic mechanical systems) technology. The advantage of the BeadChip was the creation of a highly flexible chip, capable of handling any number of bead-containing wells clustered into discrete regions. The regions could be used to assay individual samples and can be configured in different sizes and densities [208]. The microscope slide-sized array introduced an increasing amount of power to the high throughput Array of Arrays® format [208]. While the BeadChip still used the same sets of beads and immobilized probe sequences found in the Array Matrix to also assay SNP genotyping and RNA profiling, the new configuration enhanced the system’s flexibility, through its smaller scale and increased range of samples and probes [208].

Later that month, Illumina announced that the National Center for Biochip Technology (NCBT) in Shanghai, China purchased the production-scale SNP Genotyping BeadLab system [209]. The NCBT stated that the BeadLab would enable large-scale genotyping
projects to better understand patient population stratification as well as to support China’s participation in the International HapMap Project.

By the end of summer 2003, Illumina had launched a third-generation, genome-wide SNP-based linkage panel for linkage mapping. The new Linkage III Panel displayed increased robustness and sample throughput than previous panels, as well as a “more powerful and cost-effective approach for discovery than alternate methods using short-tandem repeat (STR) marker maps” [210].

In September 2003, Illumina launched a gene expression product line for both the Sentrix™ Array Matrix and BeadChip platforms. In November 2003, the company launched the BeadStation 500G, a medium-sized benchtop SNP genotyping system. Ideal for individual research laboratories, the BeadStation could process 16 samples per BeadChip assayed using the Linkage III Mapping Panel, interrogating up to 1536 SNP loci per sample. The system could perform up to eight runs per day, equal to 200,000 genotypes [211].

5.11 Volume-based Pricing

In December 2003, Illumina announced that its Oligator® oligonucleotide synthesis technology had made significant strides in reducing the costs of per-base synthesis. The
Company attributed the drop in costs to “improved operational efficiency and capacity”. The volume-based pricing program decreased the price per base 31%, or to $0.11 down from $0.16 [212].

In early 2004, Illumina introduced products to measure whole genome gene expression and began shipments of the BeadStation systems. By mid-April, the Company announced its progress in developing one of many “fixed-content” genotyping products, a revolutionary way to interrogate “high-value” genetic regions of the human genome [213]. The new assay would enable the analysis of a robust sample of haplotype tag SNPs, based on those identified by the International HapMap Project. The assay would also provide unlimited multiplexing, a unique advantage over other PCR amplification-dependent techniques [213].

In May 2004, the BeadArray technology was extended to address a more moderate level of SNP genotyping projects. The Sentrix arrays were reconfigured into two formats to assay either 384 or 768 SNP loci per sample. The products, which still operated on the Illumina BeadLab and BeadStation systems, were specifically designed for researchers whose projects did not require the used of the standard 1536-plex array products [214].

That same month, Illumina announced that a select group of institutional investors agreed to purchase approximately $30.7 million of Illumina common stock through a registered
The Company decided to sell approximately 4.5 million shares of common stock priced at $6.75 per share on May 14, 2004 [215].

In July 2004, Illumina branched out to form a strategic alliance with Genomas, Inc., a healthcare company dedicated to developing personalized medicines to treat obesity and metabolic disorders. The alliance would focus on the design and development of a gene marker panels, to be called PhysioTypes, as haplotype-based predictors of an individual’s response to metabolic syndrome treatments [216].

The end of July was met with troubling news for the Company. Affymax spin-off, Affymetrix, Inc., issued Illumina with a notification of patent infringement on six Affymetrix patents that were issued between 1996 and 2003. The patents-in-suit related to various aspects of the array, software and hardware designs of Illumina products. According to an Illumina press release, CEO Jay Flatley responded to the patent infringement notification by stating, “It is disappointing that Affymetrix has chosen to attempt to compete in the courtroom rather than in the marketplace” [217]. (See section “Affymetrix Litigation” for more information)
5.12 More collaborations

While the Illumina’s legal team set to work to respond to the Affymetrix allegations, the Company found new means to grow and expand its influence. In mid-August, Illumina received a $1.2 million Phase 2 SBIR grant from the National Institutes of Health (NIH) National Institute of Allergy and Infectious Diseases for the continuation of research to develop bead-based proteomic arrays. The grant provided the company funds to drive the development of the third application of the BeadArray technology that had been set out in Illumina’s original business plan [218].

Illumina also announced a collaboration with the Wellcome Trust Centre whereby Illumina would produce over 25 million mouse genotypes for the Centre, which would use the SNPs to investigate potential quantitative trait loci (QTL) for a wide range of characteristics and disease predispositions. The Wellcome Trust would send Illumina SNP loci and mouse samples that Illumina would use to generate high-multiplex genotyping assays based on Illumina’s GoldenGate protocol [219]. Illumina announced that it would use the results of the study to develop a standard mouse panel capable of generating genotypes for various disorders [219].

Later that month, Illumina struck a deal exceeding $1.5 million with Galileo Genomics Inc. (now Genzion BioSciences), a Montreal-based gene and drug target discovery company, which agreed to purchase the bench top BeadStation 500GX for the
development of therapeutics, diagnostics and pharmacogenomics services. Galileo aimed to collect DNA samples from the Quebec Founder Population Databank. The databank could supply Galileo with samples from nearly 6 million people with high levels of genetic homogeneity and extended linkage disequilibrium [220]. The project aimed to investigate five diseases in depth, one of which was osteoarthritis. Galileo agreed to license the resulting diagnostic rights to Illumina for the development of diagnostic products using key SNP markers discovered through the study [220].

That fall, Illumina also partnered with the North American Rheumatoid Arthritis Consortium (NARAC), led by Peter Gergersen, M.D. from the Robert S. Boas Center for Genomics & Human Genetics at the Institute for Medical Research, part of the North Shore-Long Island Jewish Health System, to conduct a large-scale two-phase genotyping study on rheumatoid arthritis. Phase One of the project incorporated Illumina’s Linkage IV Mapping Panel for mapping 3125 samples. From the candidate gene regions identified from Phase One, Illumina will develop a custom panel of SNP loci for a dense mapping of specific gene regions. This project is still in progress [221].

The collaborations with Galileo Genomics and NARAC were early examples of many projects that Illumina sought to capitalize on in order to expand the Company’s circle of influence into untapped markets. The molecular diagnostics model, explained COO John Stuelpnagel, is strikingly similar to the business model that Illumina currently executes in
partnering with academic research studies: after performing whole-genome genotyping on millions of SNPs, researchers inevitably focus on only a “handful” of markers that they test over millions of people to determine whether the markers represent causative SNPs [181]. Diagnosing a particular susceptibility to disease also boils down to genotyping patient samples in the context of a limited number of markers that represent genes that influence a particular disease or disorder.

By the end of 2004, Illumina’s core technology had significantly impacted the way high-throughput genotyping studies were performed and were financed. Chapter 6 will analyze the grants received for and the results reported from studies done with Illumina technology.

### 5.13 Invitrogen collaboration

In 2005, Illumina ceased direct sales of oligos into the market as part of a collaboration with Invitrogen [200]. The collaboration became official in December 2004, when the two companies agreed to combine Illumina’s expertise in oligo manufacturing with Invitrogen’s success in sales, marketing and distribution in order to develop a next generation Oligator DNA synthesis technology [222]. Under the agreement, Invitrogen agreed to pay Illumina up to $3.4 million, of which $2.3 million was paid as an upfront non-refundable collaboration payment in the first quarter of 2005 [199]. The payment...
went towards the implementation of fourth-generation Oligator technology as well as the transfer of the technology into two Invitrogen facilities outside of North America. Collaboration profits are divided equally between the two companies [223].

5.14 CyVera Acquisition

Illumina’s product and services line further expanded into previously untapped markets in April of 2005 when Illumina fully acquired privately-held Connecticut-based CyVera Corporation (CyVera, hereafter) [199]. The technology company offered Illumina the chance to develop assays with mid- to low-multiplex capabilities, based on the acquisition of its intellectual property.

CyVera was spun-off from the privately-held CiDRA Corporation [224] in November 2003 as a new venture-backed company to commercialize life sciences applications of its digitally encoded bead technology [225]. The CyVera platforms provided an unlimited degree of multiplexing, high sample throughput, increased flexibility/customizability compared to contemporary genomic technologies and low measurement costs [225].

CyVera’s bead-based technology employed a holographic imprinting approach to digitally address the rod-shaped beads. When excited with a laser, each bead emitted a unique code image, which was detected by a CCD camera and analyzed [Figure 5.1]. The
digital bar coding technique served the same function as the encoded microspheres of the Illumina BeadArray technology. The CyVera technology was also capable of supporting both nucleic acid and protein probes [226].

![Figure 5.1: CyVera VeraCode technology.](http://www.illumina.com/pages.ilmn?id=6)

Of particular interest to Illumina was CyVera’s VeraCode technology. The BeadArray technology is most effective in applications with mid- to high levels of multiplexing from low to high levels of throughput. VeraCode technology, in contrast, provides low-cost high-performance multiplexing from 1 to 384-plex in a single well [225].

Illumina announced that the acquisition would give Illumina entrance into the molecular diagnostics market by providing a platform capable of high throughput and cost effective in the mid- to low-multiplex range. The CyVera acquisition also brought with it a
proprietary scanning system, known as the Virtual Cytometer, that provided the readout of randomly ordered arrays [200].

Illumina paid $17.5 million for the acquisition, including 1.6 million shares of Illumina common stock and $2.3 million of CyVera’s liabilities at closing [199]. The Company reported that it would incorporate the CyVera technology into the BeadXpress system, although the technology would not be ready for launch until 2006.

5.15 Product development through collaborations

The year 2005 was marked with exponential success for Illumina. The Company advanced the throughput, scalability and reliability of its assays, reduced its operating costs, and saw spectacular increases in the price of its common stock.

Illumina announced in March its plans to release a one-million-SNP genotyping chip by the following year. The new BeadChip would use the Company’s proprietary Infinium™ whole-genome assay, a “PCR-less, fully automatable, single-tube genotyping assay” capable of unlimited scalability [227]. The development of the BeadChip would also take advantage of information obtained from the near-completed HapMap Project, as well as the cSNP and ncSNP content from published research. Additionally, Illumina would employ data uncovered from its research and development of its to-be-released exon-
centric genotyping BeadChip, the Sentrix Human-1 BeadChip, which covers 100,000 SNPs, of which 30,000 are within genes, and 40,000 are within 10KB of genes [227]. Illumina acknowledged its collaborations with customers provided key information to ensure the highest caliber of content to assay for disease association, linkage disequilibrium and related studies [227].

In June, Illumina launched the industry’s first SNP assay panel targeted for fine mapping of the Major Histocompatability Complex (MHC) regions of the human genome – genes tightly linked with the human body’s ability to combat infection and control autoimmunity and inflammation [228]. The creation of the MHC Exon-Centric Panel of 1228 SNPs represented the Company’s move to provide geneticists with tools to interrogate some of the most complex, genetically diverse regions of the human genome [223].

By the end of the month, Illumina had successfully begun shipment of both its exon-centric Sentrix® Human-1 Genotyping BeadChip and its Infinium™ Whole-Genome Genotyping Assay. The Infinium assay provided researchers with multiplexing capabilities and unconstrained locus selection. The new assay protocol also eliminated PCR and ligation steps – steps that often introduced more time and potential for sample-
handling errors. The Sentrix Human-1 BeadChip reported call rates of 99.40%, as well as high reproducibility (99.99%) and accuracy (99.99%). The BeadChip and assay were evaluated by multiple research groups or beta sites prior to official launch [228].

That fall, the National Cancer Institute and Max-Planck Institute of Psychiatry both made significant investments in the Illumina platforms in order to conduct large-scale genotyping studies [229, 230]. Illumina also entered into a blanket deal with GlaxoSmithKline (GSK), one of its earliest industrial collaborators, for a commercial, multi-year, genotyping services agreement whereby Illumina would conduct genetic studies for samples provided by GSK [231]. Illumina also reaffirmed its relationship with the Wellcome Trust Sanger Institute (WTSI) in September when Illumina agreed to develop custom Sentrix® BeadChips for the WTSI and the Wellcome Trust Case-Control Consortium (WT-CCC) as part of a study on SNPs that lead to changes in amino acid sequences, known as non-synonymous SNPs. The study aimed to examine four common disease phenotypes with over 5000 samples. The study would also be the first of its kind to leverage the functionality of the Illumina Infinium™ assay design [232].

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6 Call rates are defined as the percentage of SNPs that can be accurately assigned to a genotype. SNPs that do not give clear signals are designated as “no calls.”
5.16 Emerging business – Diagnostic testing

In October 2005, Illumina announced a collaboration with PharmacoDesign, the largest genetic services company in Korea [233]. PharmacoDesign purchased a BeadStation system and the genotyping arrays and reagents in order to genotype various Korean populations for pharmacogenomic-based studies. Illumina obtained access to the biomarkers discovered by PD and announced that it will incorporate the newly discovered markers into proprietary SNP panels under a worldwide royalty-bearing exclusive license [233].

By end of 2005, Illumina had enhanced its software performance to use the Infinium™ assays to measure DNA copy number variation and loss of heterozygosity, important phenomena that frequently occur in cancer [234]. The completion of the International HapMap Project in October meant that the data collected from the various research centers around the world would be analyzed and soon be publicly available. The fact that Illumina’s BeadArray technology had played a crucial role in the Project further meant that the data obtained would be one of the first major indications of the technology’s impact [138].
5.17 2006 – A year of exponential growth

The year 2006 was marked by an exponential increase in the number of collaborations between Illumina and academic/non-profit organizations, a major expansion in the product line for genotyping capabilities and the returns of its technological prowess through published results in high-impact peer-reviewed journals.

In early January, Illumina commenced shipment of its Setrix® HumanHap300 BeadChip with the Infinium™ whole genome assay. The BeadChip and assay supported the analysis of over 317,000 SNP loci per sample. Each SNP was selected as a “tagSNP” from the International HapMap Project database, making the selection of the SNPs highly informative. Beta testers from the Genotyping Laboratory at the Center for Inherited Disease Research (CIDR) and the Max Planck Institute for Psychiatry praised the HumanHap300 BeadChip for its overall efficient, high-quality results. The new BeadChip complemented the exon-centric Human-1 BeadChip that was released the prior spring [235].

By March of 2006, the Company had expanded the GoldenGate genotyping portfolio to include three panels to aid scientists in understanding how genetic variation affects disease. One Cancer SNP Panel, developed in collaboration with the National Cancer Institute, was capable of genotyping 1421 loci on over 400 genes related to cancer
progression. Illumina also release two GoldenGate SNP genotyping panels specific to the mouse genome, a key model organism in studying human disease [236]. By the end of that same month, Illumina had also expanded the coverage of its standard whole genome genotyping panels through the introduction of the HumanHap550 Genotyping BeadChip, containing over 550,000 SNPs on a single array. A second BeadChip, the HumanHap240S, was launched simultaneously with the HumanHap550. The HumanHap240S complemented the newest additions to the HumanHap550 that the HumanHap300 lacked [237].

In April, Illumina introduced a product that would improve scanning throughput by 400% and would minimize labor costs. The Illumina AutoLoader could load up to 40 BeadChips for scanning on a BeadStation, allowing the machine to run for up to 15 hours unattended. The upgraded software also provided customers with increased scan speed [238]. The additions complemented the growing trend of customers who were performing high-end association studies that required thousands of samples to be read with a high degree of accuracy.

Illumina proceeded to advance its collaborations with companies willing to develop diagnostic tests when it announced its strategic alliance with Iceland-based deCODE genetics in May. The companies planned to co-developed and commercialize DNA-based
diagnostics for risk of heart attack, type-2 diabetes, and breast cancer. Under the alliance, Illumina would use its platform to develop high-multiplex SNP genotyping panels containing common gene variants that deCODE previously demonstrated to correlate with risk of developing one of the diseases. Specifically, the partnership will develop tests for gene variants in well-defined disease-related pathways: leukotriene A4 hydrolase, linked to heart attack; transcription factor 7-like 2 (TCF7L2), linked to type-2 diabetes; and, BARD1, linked to breast cancer [239].

By the end of June, a new addition to the whole-genome genotyping BeadChip was introduced. Illumina unveiled the Sentrix® HumanHap650Y Genotyping BeadChip as the “most comprehensive genomic coverage and highest data quality of any whole-genome genotyping product” on the market [240]. The new product contained genomic coverage of all four populations studied by the International HapMap Project, including the Yoruba in Ibadan, Nigeria, a population which has demonstrably greater genetic diversity than European and Asian populations [240]. The diversity of the Yoruba dictated that a higher number of SNP markers be added to the BeadChips for an increasingly accurate genetic readout. One particularly immediate consequence of the increasingly diverse panels was that researchers began to design studies based on uncovering disease-related genetic variations among different populations. Duke University’s Institute for Genome Sciences & Policy and Center for HIV/AIDS Vaccine Immunology (CHAVI), for example, has designed a study based on the HumanHap650Y to analyze a 2000-sample cohort of HIV-
exposed African individuals to determine whether there exists genetic variation between those who have and those who have not been infected with the virus [240].

One month later, Illumina introduced the HumanHap550+ BeadChip and iSelect Infinium™ genotyping assay. The new BeadChip enabled customers to add up to 120,000 custom SNP markers onto the standard HumanHap550 BeadChip, for a total of 670,000 markers. The iSelect assay permitted the ease of design of “focused-content” arrays whereby up to 60,000 SNPs per sample could be created. The Company predicted that the new products would be used by researchers who have already used whole-genome approaches to select SNPs of interest to further investigate the relevance of those SNPs to disease susceptibility [241].

In July 2006, Illumina announced the formation of diagnostic collaboration with ReaMetrix, Inc. in which the two companies would co-develop molecular diagnostic panels for various diseases. ReaMetrix will gain nonexclusive rights to market the product to the country of India, where it will drive development from Bangalore, India. Illumina will retain the rights to market the finished product to the rest of the world [242]. This collaboration marks an important step for India’s growing healthcare industry. The project will allow the country to “leapfrog current diagnostics and chart a new path with innovative molecular and pharmacogenomic testing,” according to
ReaMetrix’s CEO, Bala Manian, Ph.D [242]. The CEO also commented that the collaboration would target a wide open market of personalized medicine in India [242].

That August, the Company set another industry first by initiating the Illumina CSPro™ Program. Otherwise known as the Certified Service Provider Program, the Illumina CSPro Program proposed a collaborative relationship with organizations that were willing to provide genetic analysis services to the research community with the Illumina technologies [243]. This Program had significant academic input; McGill University’s Genome Quebec Innovation Center validated the Illumina CSPro Program process [243].

That same month, as news from the Markman Ruling in the patent litigation battle with Affymetrix was met with conflicting reviews [see section “Affymetrix Litigation”], Illumina found itself in a new collaboration that opened up door into a previously untapped market. Researchers at the United States Department of Agriculture (USDA) Agricultural Research Service (ARS), the University of Missouri-Columbia (MU) and the University of Alberta (UA) enlisted the Company to develop a multi-sample Bovine BeadChip in order to genotype over 10,000 cattle. The results of the genotyping would be employed for quantitative trait loci (QTL) mapping and breeding selection. Researchers stated that the Bovine BeadChip would “expand gene discovery for better meat and milk production and quality… [and] shed light on the relationships between different populations of animals” [244].
An important report issued in early September from the FDA-led MicroArray Quality Control (MAQC) project demonstrated that the microarray gene expression data generated from the various commercial platforms tested returned high degrees of correlation. The study examined the leading platforms in the industry, concluding that researchers could choose their platform of choice based on “cost, ease of use and quality of content, with little to no legacy data concerns” [245].

That same month Illumina introduced two new additions to its product collection. One was the RatRef-12 Whole-Genome Expression BeadChip, capable of analyzing up to twelve individual rat samples in parallel of over 22,000 rat transcripts derived from the NCBI RefSeq database (Release 16) [246]. The other breakthrough was a multi-sample whole-genome genotyping BeadChip – an industry first. The Infinium™ Whole-Genome Genotyping HumanHap300-Duo and the Human Hap300-Duo+ BeadChips introduced the capability of researchers to analyze two individual samples in parallel, covering over 634,000 total tag SNPs on a single BeadChip. The HumanHap300-Duo+ gave researchers an additional 60,000 SNP loci to custom select within a given genetic region [247]. A HumanHap550-Duo BeadChip would be released a year later [248].
5.18 Solexa Merger

On November 13, 2006, Illumina announced that the company would enter into a merger agreement with Solexa whereby Illumina would acquire Solexa in a stock-for-stock merger. The merger, unanimously approved by the Boards of Directors of both companies, would grant Solexa stockholders shares of Illumina stock valued at $14.00 per share, equating to approximately $600 million, subject to certain provisions. Illumina also entered a securities purchase agreement with Solexa, signifying that Illumina will invest an additional $50 million in Solexa in exchange for newly issued Solexa shares. Solexa, headquartered in Hayward, CA, with operations in Cambridge, England, developed a proprietary next generation genetic analysis system, capable of whole genome sequencing, targeted resequencing, digital gene expression and microRNA analysis. According to an Illumina press release, the market for sequencing is estimated at $1 billion. The acquisition of Solexa by Illumina would create a combined company with a $2.25 billion market opportunity. The merger would create the only company with genome-scale technology for genotyping, gene expression and sequencing. Both companies hope that the merger accomplishes the following goals [200]:

- Expand Illumina’s genetic analysis product offering to include Solexa’s next generation sequencing platform, the 1G Genome Analyzer
- Create the only company to offer both analog and digital gene expression, enhancing Illumina’s rapidly emerging gene expression franchise
• Add to Illumina’s emerging opportunity in molecular diagnostics and content discovery
• Dramatically increase Illumina’s addressable markets
• Drive Solexa’s manufacturing and commercialization
• Leverage Illumina’s global sales and support infrastructure; and
• Accelerate development of future products, leveraging the combination of core technologies

The combined company was expected to give Illumina as much success into expansion into the complementary market of sequencing as Illumina’s acquisition of CyVera Corporation successfully expanded Illumina’s penetration into the lower multiplex, clinical diagnostics market. In return, Solexa would benefit from Illumina’s proven business model, worldwide direct sales network and support infrastructure [190].

According to the press release, Illumina would still maintain Solexa’s operations in California and Cambridge. In addition, two members from the Solexa Board of Directors would join the Illumina Board of Director, increasing the size of Illumina’s Board to ten [249]. The merger agreement maintained that Solexa stockholders, options holders and warrant holders would receive newly issued shares of Illumina common stock, options and warrants, respectively, based on an exchange ratio at the time of the closing of the deal [249].
The official acquisition would not go through until January 26, 2007. According to the original terms of the deal, if the Illumina Average Price ($14.00 divided by the volume weighted average trading price of the Illumina common stock as reported by NASDAQ during 10 randomly selected days during the 20-day trading period ending five days prior to the closing of the merger) was equal to or less than $40.70, then the exchange ratio would be fixed at 0.344 [249]. At the time of the merger, Solexa shareholders received 0.344 shares of Illumina common stock for each share of Solexa common stock [250]. The appointed members from Solexa’s Board of Directors to take seats on Illumina’s Board were Blaine Bowman and Roy A. Whitfield [250].

5.18.1 Sequencing Technology

DNA sequencing technology was acquired as part of the Solexa merger is based on use of sequencing-by-synthesis (SBS) biochemistry [Figure 5.2]. The method exploits single-stranded DNA extension from a priming site one base at a time, with reversible terminator nucleotides. The advantage of these DNA nucleotides is that they can be added to a growing second strand, but initially cannot be further extended. At the end of each cycle, a nucleotide is added, incorporating with it a fluorescent label unique to that particular base, which is imaged in order to infer the base identity. The fluorescent label is then removed and the cycle can continue, adding a new nucleotide base. This process
can continue for up to 50 cycles. The reversible terminator technology is developed using novel synthetic molecules, requiring the use of a proprietary synthetically-derived enzyme. The molecules and enzyme contributed to a significant portion of the IP that Solexa brought with it to its merger with Illumina [190].
In the Solexa platforms, SBS biochemistry finds its application in the presence of macroscopic islands of DNA, called DNA clusters. The clusters form from single DNA molecules attached to the inside surface of a flow cell. Proprietary amplification
techniques generate copies of the starting DNA molecules that become covalently linked to the cell surface. These DNA molecules release fluorescence upon each round of synthesis, thus generating a greater fluorescent signal after each cycle. Tens of millions of such DNA clusters can be independently formed from a single flow cell. These larger clusters can then be sequenced simultaneously, by alternate cycles of SBS biochemistry and electronic imaging [223].

By the end of 2006, Illumina had reported a 151 percent growth in revenue to $184.6 million, marking 22 consecutive quarters of revenue growth [223]. This was fueled largely by the exponential influx of industrial and academic collaborations, studies and strategic alliances that signed on to take advantage of Illumina’s proprietary and highly-innovative products and services. Illumina had introduced eight new products to the Infinium whole-genome assay family alone [251]. The benefits of the technology emerged as peer-reviewed articles in high-impact science journals – academia’s equivalent of soaring stock prices for research groups – began to publish highly-cited, highly-significant results from studies using the Illumina products and services [252]. Chapter 6 will further discuss the impact of Illumina’s technologies on the Science Commons.
5.19 2007 – A year of mixed reviews, thus far

The beginning of 2007 was met with mixed news for the Company. It officially acquired Solexa in late January. It also introduced two new revolutionary products – a high-throughput DNA methylation profiling panel and a BeadChip capable of analyzing one million SNPs in a single run [251, 253]. The GoldenGate Methylation Cancer Panel I introduced an industry standard panel by which researchers could analyze up to 1,536 methylation sites across 96 DNA samples simultaneously [253]. DNA methylation markers are analyzed based on their correlation to gene activity levels and cancer states. A Customized version of the Methylation Cancer Panel would be released later that spring [254]. The Human 1M BeadChip delivered an unprecedented amount of genetic content on a single microarray chip, providing higher-density coverage through the incorporation of additional tag SNPs identified by the International HapMap Project [251].

In March, Illumina launched the HumanCNV370-Duo DNA Analysis BeadChip. A product of a collaboration with deCODE Genetics, the HumanCNV370-Duo became the world’s first microarray chip designed to analyze genomic regions that display copy number variation – a phenomena linked to cancers, congenital disorders, and other diseases [255]. Two weeks following the launch of the HumanCNV370-Duo, the Company announced its release of the BeadXpress System. The platform complemented the larger, high-multiplex BeadLab platforms with its low- to mid-multiplex capabilities.
based on the proprietary VeraCode digital microbead technology acquired from the CyVera acquisition in 2005 [256]. The technology, through its two-color laser detection system and digital holographic codes, enabled high accuracy, precision and speed, paving the way for Illumina’s further expansion into the molecular diagnostics market [256].

5.20 Affymetrix Litigation

With great success has come great struggles. The biotechnology industry is at the center of the struggle between two industries that view intellectual property rights in diametric ways. The pharmaceutical industry uses the patent system as its lifeblood to keep a competitive edge by excluding other companies from manufacturing its blockbuster drugs. The computer science/information technology industry takes a more lax approach to defending intellectual property (IP), as technological inventions require a compilation of multiple technologies to progress. Navigating how biotechnology should treat IP imports significant consideration by industry experts and policy makers, as effects could impact public health measures [257].

5.20.1 Litigation overview

As a holder of valuable proprietary technology, Illumina has sought to defend its patents and protect its scientific integrity. In 2004, Affymetrix filed a lawsuit alleging infringement on a string of six patents by Illumina. The 26 July complaint filed at the
U.S. District Court for the District of Delaware alleged that the use, manufacture and sale of the Illumina Array Matrix and BeadChip counted as patent infringement. Affymetrix sought an injunction against the sale of the products, as well as unspecified monetary damages, interest and attorneys’ fees [223].

Illumina responded on 15 September claiming that the Company was not practicing infringement, and that such patents were invalid. The Company further responded with counterclaims that Affymetrix was practicing “unfair competition and interference with actual and prospective economic advantage.” [223]

### 5.20.2 Illumina’s retaliation

While the documents were being prepared by the court, Illumina retaliated further against the Affymetrix claims of 2005. Affymetrix claimed that Illumina challenged ownership rights for a patent covering the ParAllele Technology acquired by Affymetrix [258]. Filing an Interference request with the United States Patent and Trademark Office (USPTO), Illumina claimed that the U.S. Patent Number 6,858,412 (the ‘412 Patent, hereafter), which relates to the molecular inversion probe genotyping technology, was invented by Illumina scientists during the early development of the company.
Illumina sought interference based on a patent application that described the same invention and that was filed prior to the ‘412 Patent. Affymetrix claimed that it picked up the patent through acquisition of ParAllele Bioscience, Inc. The patent application was filed in 2001 and assigned by the USPTO to Stanford University in February 2005. The University then exclusively licensed the patent to ParAllele [259]. Illumina CEO Jay Flatley cited that a patent filed by Illumina in 2002, entitled “Detection of nucleic acid reactions on bead arrays” details the same invention predating the ParAllele patent [260].

5.20.3 Illumina’s counterclaims

By February 2006, Illumina issued answers and counterclaims to the allegations originally cited by Affymetrix. The Company cited “inequitable conduct with respect to all six asserted Affymetrix patents, violation of Section 2 of the Sherman Act, and unclean hands.” In March 2006, Affymetrix notified Illumina of the decision to drop one of the six patents from the lawsuit, while adding additional claims of infringement of the remaining five patents. U.S. Patent No. 6,607,887 (the ‘887 patent) was dropped from the suit. On 30 June 2006 the court dismissed the patent Affymetrix had sought to withdraw from the suit [261, 262]. At that point, the patents-in-suit were U.S. Patent Nos. 6,355,432 (the ‘432 patent), 6, 646,243 (the ‘243 patent), 5,545,531 (the ‘531 patent), 6,399,365 (the ‘365 patent) and 5,795,716 (the ‘716 patent) [261, 262].
5.20.4 Markman hearing

Affymetrix and Illumina filed summary judgment motions by 14 July 2006, the deadline decided by the court, and all “such motions have been stayed or denied” [190]. On 16 August 2006 District Court Judge Joseph J. Farnan of the United States District Court for the District of Delaware issued a ruling on the Markman hearing [263]. Out of 15 total claim construction disputes, the Judge ruled in favor of Affymetrix 12 times, while siding with Illumina twice, and neither party once [263].

Dueling press releases issued on 17 August 2006 by Illumina and Affymetrix claimed that each respective party predicted a favorable outcome for the overall case to their own advantage, based on the interpretations that resulted from the Markman Order. The 16 August Order addressed 15 disputed claim terms in the five patents-in-suit. Affymetrix asserted that the Court sided with the Affymetrix proposed constructions for “most terms” and adopted only two of Illumina’s proposed constructions [262]. Specifically, Affymetrix reported that the Judge rejected Illumina’s contentions and instead found that [264]:

- The patents-in-suit are not limited to in situ synthesis
- The patents-in-suit are not limited to probes chemically linked to a single surface

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7 To see the specifics of each claim and how each claim was interpreted, read: Affymetrix v. Illumina, J. Farnan.
• The patents-in-suit are not limited placement of probes at predetermined locations (and therefore cover random assembly of arrays)

Affymetrix claimed that the Court’s decision affirms the breadth of the Affymetrix patent portfolio in covering DNA microarray field and related technology. Furthermore, the Court rejected Illumina’s motion to dismiss the ‘716 patent for lack of standing, affirming Affymetrix’s ownership of and right to sue on the ‘716 patent [262]. Illumina released a statement indicating that the Court’s decision to adopt the Company’s position as to the “proper interpretation of certain key terms in the dispute” might have a “favorable” outcome towards Illumina [261].

Affymetrix was represented by Jack B. Blumenfeld and Maryelle Noreika, both attorneys from Morric, Nichols, Arsht & Tunnell, LLP. Illumina was represented by Robert G. Krupka, Esquire; Mark A. Pals, Esquire, and Marcus E. Sernel, Esquire; Terry L. Tang, Esquire from Kirkland & Ellis, LLP, as well as Richard K. Herrmann, Esquire of Morris, James, Hitchens & Williams LLP [264].

5.20.5 Pretrial hearing

A pretrial hearing on 8 February 2007 left industry analysts reporting that Affymetrix would triumph on at least one patent, and most likely on other patents-in-suit. Analysts also predicted that the interpretation decided on by the Judge would define Illumina’s
products to be out of scope, and thus, Affymetrix was predicted to lose on that particular patent. The report issued predicted that Affymetrix would be the overall winner in this case, given the tiered nature of the trial, and the rumors that Judge Joseph Farnan has traditionally ruled in favor of the patentee [264].

The pretrial hearing addressed the specifics of some of the patents-in-suit. In particular, it was revealed that one particular patent, the ‘716 patent, was one in which Mark Chee, was named inventor while he was an employee at Affymetrix, before he left to be a founder of Illumina [264].

The plaintiff (Affymetrix) argued that if Chee was owner of the patent and assigned the patent to Affymetrix, then, citing the doctrine of assignor estoppel, Chee nor any of his colleagues could challenge the validity or enforceability of the patent. Illumina filed a motion to dismiss the claim of infringement of the ‘716 patent on the grounds that Affymetrix did not have the “proper legal title” to it [264]. The defendant (Illumina) claimed that the original invention belonged to Dr. Robert Lipshutz. Dr. Lipshutz worked at Daniel H. Wagner Associates, Inc., and consulted for Affymetrix based on 1991 work agreement whereby all productive work was assigned to Affymax (predecessor to Affymetrix). Illumina argued that Dr. Lipshutz’s invention did not qualify as work produced under the agreement [264].
Analysts theorized that the argument would not stand up in court, given the fact that Dr. Chee is still named as an inventor on the patent [264]. The Markman Order of 16 August proved in favor of Affymetrix. Judge Farnan stated that the 1991 agreement led to a present assignment of the patent to Affymetrix. Thus, the doctrine of assignor estoppel would apply for this particular patent, and Illumina will be unable to challenge the ‘716 patent’s enforceability or validity [264].

The ‘716 patent itself is “indispensable” for a company that manufactures gene chips. The invention describes a decoding process by a computer program and a computer system to infer nucleotide sequences based on fluorescent intensities of nucleic acid probes. Analysts predict that the only way to avoid infringement of this patent would be to track fluorescent intensities manually, which would be an unrealistic approach, given the extremely high throughput nature of the gene chips [264].

5.20.6 Other patents-in-suit

Analysts privy to the pretrial hearing further calculated that Illumina would prevail on demonstrating noninfringement or invalidity on the other patents-in-suit, despite the Judge’s denial of Illumina’s prior interpretation of claims [264].
On the ‘432 patent – a patent that describes the use of beads coupled to “coding system” that is linked to “binding polymers” having different “target specific” DNA sequences – analysts believe that the Markman ruling would put Illumina’s products out of scope [263].

The ‘531 patent detailing the use of “chip plates with probe arrays and wells” describes a similar invention that is described in an Illumina European patent application, filed a year and a half prior to the ‘531 priority date. The PCT Patent Application WO93/17126 provides Illumina evidence that the ‘531 patent infringement claim is invalid [263].

Similar to the ‘531 patent, Illumina believes that the ‘365 patent infringement allegation should be deemed invalid. This argument rests on the fact that there exists a prior British patent application published in 1984, a year prior to the ‘365 patent’s earliest filing date. The patent covers the use of a bar code technique to determine the identity of each “housing” [263].

The final patent-in-suit is the ‘243 patent, describing an invention to assess nucleic acid binding to a substrate with “at least 1000 different spheres, beads, or particles…” Attorneys for Illumina at the Markman hearing declared that synthesizing of the polymers had to actually occur on such substrate. The judge, instead, felt that Illumina’s construction was too limited. Analysts predicted that Illumina might be found guilty of
infringement. Based on the pretrial rulings and the Markman hearing, analysts assumed that Illumina would be found guilty of infringement on all of the patents, save the ‘432 patent [264].

5.20.7 Part one of the trial

The first part of the trial was rescheduled from 16 October 2006 to 5 March 2007 at the request of both parties. The court scheduled a multi-phase trial structure in order to address Illumina’s defenses of invalidity and enforceability of the patents-in-suit, as well as the claims for unfair competition and antitrust violations, in subsequent trials [190].

The first phase of the trial promptly commenced on 5 March 2007 to address the issues of infringement and damages. On 13 March, the jury “returned a verdict finding infringement of the five patents asserted by Affymetrix” [190]. Illumina asserts that the verdict was made without regard to the validity and enforceability of the five Affymetrix patents.

The jury awarded damages retroactive for the sale of certain products to the end of 2005 at a royalty rate of 15%. This amount equaled $16.7 million. Illumina wrote that the first-phase verdict remains subject to post-trial motions and appeals. Illumina believes that a judgment on the verdict has been entered into the case and that such judgment, along with
any final damages aware, will be entered until after the subsequent phases of the trial are completed [190].

5.20.8 Settlement talks

Initial settlement talks fell through in January 2007. Affymetrix has tentatively agreed to forego damages for “price erosion” [190]. Affymetrix will most likely seek damages based on the amount of the royalty it would have received had it licensed the use of its patents to Illumina. While Affymetrix most likely hopes to receive a large damage award and an injunction against Illumina to essentially eliminate the company from the marketplace, Illumina’s recent exponential growth and diversity in its products and services have ensured the company’s success in the coming years [265]. The second phase of the trial was scheduled to occur in the summer of 2007. As of this writing, no news has been released regarding the status of the trial.

5.21 Moving forward

Since the summer, Illumina has released a new software program to streamline data integration and analysis [266] and launched iControlDB, the first industry-hosted controls database for genetic research [267]. The Company has also released its products its has designed out of the intellectual property obtained from the Solexa merger; in July Illumina unveiled Digital Gene Expression (DGE) applications Tag Profiling and Small
RNA Analysis that employ Solexa Sequencing technology to discover novel RNAs, to quality messenger RNA and to measure gene expression without sequence information [268]. By September, the fifth multi-sample Infinium BeadChip product, the Infinium HumanLinkage-12, was introduced at the industry’s lowest cost [269].

5.22 Illumina’s future plans

In our conversation with COO John Stuelpnagel and Vice President of Marketing Todd Dickinson, we asked them what the Company’s five-year plan entailed. With the recent collaborations between Illumina and international companies, Illumina aims to go into the molecular diagnostics market. Stuelpnagel commented that the panels required to perform diagnostic testing match the multiplex levels provided by the Bead Xpress platforms [181]. Illumina also aims to develop its sequencing unit based on its recent merger with Solexa.

It seems that Illumina’s technological advances has already propelled some of its researchers to spin-off their own companies. Mark Chee, VP of Genomics, launched Prognosys BioSciences, Inc. in 2005 and has received federal grants from the National Human Genome Research Institute for the company’s development

8 For more information on Prognosys Biosciences, see the company’s website at: http://www.prognosysbio.com .
The following chapter will investigate the events and accounts reported over the past four chapters to synthesize the recipe for successful technology transfer. The chapter will also analyze the impact of Illumina’s technologies on the advances in high-throughput SNP genotyping research.
**Figure 5.3: Timeline of events in Illumina’s business history.** Data collected from personal communications and the Illumina website news releases ([http://www.illumina.com](http://www.illumina.com)).

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar-97</td>
<td>Fiber optic sensor with encoded microspheres invention is disclosed</td>
</tr>
<tr>
<td>Apr-97</td>
<td>PITCON</td>
</tr>
<tr>
<td>Fall 1997</td>
<td>Meeting with David Walt, Larry Bock &amp; John Stuelpnagel</td>
</tr>
<tr>
<td>Jan-98</td>
<td>Licensing discussions begin with Tufts University</td>
</tr>
<tr>
<td>5/6/1998</td>
<td>Illumina founded by John Stuelpnagel and David Walt</td>
</tr>
<tr>
<td>6/15/1998</td>
<td>Seed round of investment, co-sponsored by ARCH partners</td>
</tr>
<tr>
<td>Jun-98</td>
<td>First employee joins Illumina</td>
</tr>
<tr>
<td>Nov-98</td>
<td>First round of financing raises $8.5 Million</td>
</tr>
<tr>
<td>Fall 1998</td>
<td>Illumina acquires nGenetics</td>
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<tr>
<td>Jun-99</td>
<td>Illumina enters research collaboration with Dow Chemical</td>
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<tr>
<td>Nov-99</td>
<td>Jay Flatley joins Illumina as CEO</td>
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<tr>
<td>Nov-99</td>
<td>Illumina establishes collaboration with Applied Biosystems</td>
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<tr>
<td>Nov-99</td>
<td>Illumina raises $28 Million in second round of venture financing</td>
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<tr>
<td>Spring 2000</td>
<td>Illumina acquires Spyder Instruments</td>
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<tr>
<td>7/3/2000</td>
<td>Illumina IPO raises $103 Million</td>
</tr>
<tr>
<td>Jan-01</td>
<td>Illumina enters deal with ChevronTexaco Energy for BeadArray technology</td>
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<tr>
<td>Jun-01</td>
<td>Illumina initiates FastTrack SNP genotyping service with GlaxoSmithKline</td>
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<tr>
<td>Jul-02</td>
<td>Applied Biosystems announces its decision to forego collaboration</td>
</tr>
<tr>
<td>Jul-02</td>
<td>Illumina releases first product - the BeadLab system with Sentrix Array Matrix</td>
</tr>
<tr>
<td>Oct-02</td>
<td>Illumina wins grant to participate in HapMap Project</td>
</tr>
<tr>
<td>Dec-02</td>
<td>Illumina files suit against Applied Biosystems for breach of contract</td>
</tr>
<tr>
<td>Jun-03</td>
<td>Illumina releases Sentrix BeadChip</td>
</tr>
<tr>
<td>Jul-04</td>
<td>Affymetrix alerts Illumina to patent infringement suit on six patents</td>
</tr>
<tr>
<td>Aug-04</td>
<td>Illumina and Applied Biosystems reach settlement</td>
</tr>
<tr>
<td>Dec-04</td>
<td>Illumina enters deal with Invitrogen for oligo synthesis</td>
</tr>
<tr>
<td>Apr-05</td>
<td>Illumina acquires CyVera Corporation</td>
</tr>
<tr>
<td>Jun-05</td>
<td>Illumina begins shipment of Infinium whole-genome genotyping assay - industry's first</td>
</tr>
<tr>
<td>Oct-05</td>
<td>Completion of HapMap Project</td>
</tr>
<tr>
<td>Aug-06</td>
<td>Markman ruling for Illumina v. Affymetrix trial</td>
</tr>
<tr>
<td>11/13/2006</td>
<td>Illumina announces Solexa merger for early 2007</td>
</tr>
<tr>
<td>1/26/2007</td>
<td>Illumina merges with Solexa</td>
</tr>
<tr>
<td>3/5/2007</td>
<td>Part one of Illumina-Affymetrix trial finds Illumina guilty of infringement</td>
</tr>
</tbody>
</table>
Figure 5.4: **Illumina product development timeline.** Information obtained from press releases publicly available on the Illumina website. Source: [http://www.illumina.com](http://www.illumina.com).

<table>
<thead>
<tr>
<th>Date</th>
<th>Product Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>FastTrack Genotyping Services launched w/ Golden Gate</td>
</tr>
<tr>
<td>2002</td>
<td>BeadLab launched</td>
</tr>
<tr>
<td>6/12/03</td>
<td>Sentrix BeadChip Platform launched</td>
</tr>
<tr>
<td>8/19/03</td>
<td>Linkage III Panel for linkage-mapping</td>
</tr>
<tr>
<td>11/5/03</td>
<td>BeadStation announced</td>
</tr>
<tr>
<td>1/13/04</td>
<td>Whole Genome Gene Expression BeadChips</td>
</tr>
<tr>
<td>4/21/04</td>
<td>100,000 SNPs on BeadChips</td>
</tr>
<tr>
<td>5/4/04</td>
<td>Extends SNP genotyping to 384 and 768 SNPs</td>
</tr>
<tr>
<td>6/28/05</td>
<td>Launches Infinium WGG assay w/ exon-centric Sentrix Human-1</td>
</tr>
<tr>
<td>12/15/05</td>
<td>Launches new software (BeadStudio 2.0) to measure copy number variation and chromosomal changes</td>
</tr>
<tr>
<td>1/10/06</td>
<td>Ships Sentrix HumanHap300</td>
</tr>
<tr>
<td>3/28/06</td>
<td>Launches HumanHap550 and HumanHap240S</td>
</tr>
<tr>
<td>4/17/06</td>
<td>Launches AutoLoader; updated software</td>
</tr>
<tr>
<td>6/29/06</td>
<td>Introduces HumanHap650Y</td>
</tr>
<tr>
<td>7/15/06</td>
<td>Introduces HumanHap550+ and iSelect Infinium assay</td>
</tr>
<tr>
<td>9/21/06</td>
<td>RatRef-12 Whole Genome Expression BeadChip</td>
</tr>
<tr>
<td>9/25/06</td>
<td>Introduces world's first multi-sample WGG BeadChips</td>
</tr>
<tr>
<td>12/1/06</td>
<td>Science &amp; The Lancet publish results using Illumina's HumanHap300 BeadChip</td>
</tr>
<tr>
<td>1/10/07</td>
<td>Introduces High-throughput DNA Methylation panel</td>
</tr>
<tr>
<td>1/10/07</td>
<td>Introduces Human 1M BeadChip</td>
</tr>
<tr>
<td>3/12/07</td>
<td>Introduces HumanCNV370-Duo</td>
</tr>
<tr>
<td>3/21/2007</td>
<td>Launches BeadXpress platform</td>
</tr>
<tr>
<td>4/16/2007</td>
<td>Launches custom methylation application</td>
</tr>
<tr>
<td>5/1/2007</td>
<td>Launches Illumina Connect</td>
</tr>
</tbody>
</table>

**Continued on next page**
<table>
<thead>
<tr>
<th>Date</th>
<th>Product Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/11/2007</td>
<td>Launches iControlDB</td>
</tr>
<tr>
<td>7/24/2007</td>
<td>Launches Digital Gene Expression applications: Tag Profiling; Small RNA Analysis with Genome Analyzer</td>
</tr>
<tr>
<td>8/2/2007</td>
<td>Introduces HumanHap500-Duo</td>
</tr>
<tr>
<td>9/6/2007</td>
<td>Introduces HumanLinkage-12 Genotyping BeadChip</td>
</tr>
</tbody>
</table>
**Figure 5.5: Timeline of collaborations with academic and industrial organizations.** Information collected from news releases made publicly available on the Illumina website (http://www.illumina.com).

<table>
<thead>
<tr>
<th>Date</th>
<th>Name</th>
<th>Date</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>May-01</td>
<td>GSK, PLC</td>
<td>Feb-06</td>
<td>Center for Inherited Disease Research (CIDR)</td>
</tr>
<tr>
<td>May-01</td>
<td>ChevronTexaco Energy/Research</td>
<td>Apr-06</td>
<td>Cancer Research UK</td>
</tr>
<tr>
<td>Jan-03</td>
<td>Genome Quebec Innovation Centre</td>
<td>May-06</td>
<td>Shafallah Center</td>
</tr>
<tr>
<td>Feb-03</td>
<td>The Wellcome Trust Sanger Institute</td>
<td>May-06</td>
<td>deCODE</td>
</tr>
<tr>
<td>Jun-03</td>
<td>National Center Biochip Technology</td>
<td>Jun-06</td>
<td>The Children's Hospital of Philadelphia</td>
</tr>
<tr>
<td>Jul-03</td>
<td>Johns Hopkins University</td>
<td>Jul-06</td>
<td>Johnson &amp; Johnson Pharmaceutical R&amp;D, LLC</td>
</tr>
<tr>
<td>Sep-03</td>
<td>Whitehead Institute</td>
<td>Jul-06</td>
<td>ReaMetrix</td>
</tr>
<tr>
<td>Jan-04</td>
<td>University of Tokyo</td>
<td>Aug-06</td>
<td>Massachusetts General Hospital; Harvard Medical School</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>The United States Department of Agriculture (USDA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Agricultural Research Service (ARS), the University of</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Missouri-Columbia (MU) and the University of Alberta (UA)</td>
</tr>
<tr>
<td>Feb-04</td>
<td>France's Centre National de Genotypage</td>
<td>Aug-06</td>
<td>MicroArray Quality Control (MAQC) project:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FDA, NIH, EPA and USDA</td>
</tr>
<tr>
<td>Jun-04</td>
<td>University of Southern California</td>
<td>Sep-06</td>
<td>MicroArray Quality Control (MAQC) project:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FDA, NIH, EPA and USDA</td>
</tr>
<tr>
<td>Jul-04</td>
<td>University of California, Los Angeles</td>
<td>Oct-06</td>
<td>Duke University</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Top 5 Pharmaceutical companies</td>
</tr>
<tr>
<td>Aug-04</td>
<td>The Wellcome Trust Sanger Institute</td>
<td>Oct-06</td>
<td>Amgen / Brigham &amp; Women's Hospital</td>
</tr>
<tr>
<td>Aug-04</td>
<td>Galileo Genomics, Inc. / Genzion Biosciences, Inc.</td>
<td>Nov-06</td>
<td>Erasmus MC</td>
</tr>
<tr>
<td>Sep-04</td>
<td>North American Rheumatoid Arthritis Consortium</td>
<td>Nov-06</td>
<td>Johns Hopkins</td>
</tr>
<tr>
<td>Dec-04</td>
<td>Invitrogen, Inc.</td>
<td>Nov-06</td>
<td>The Genome Institute of Singapore</td>
</tr>
<tr>
<td>Sep-05</td>
<td>National Cancer Institute</td>
<td>Dec-06</td>
<td>GSK</td>
</tr>
<tr>
<td>Sep-05</td>
<td>Max-Planck Institute of Psychiatry</td>
<td>Jan-07</td>
<td>Children's Hospital of Eastern Ontario</td>
</tr>
<tr>
<td>Sep-05</td>
<td>GSK</td>
<td>Jan-07</td>
<td>Mayo Clinic</td>
</tr>
<tr>
<td>Sep-05</td>
<td>The Wellcome Trust Sanger Institute</td>
<td>Feb-07</td>
<td>Asuragen</td>
</tr>
<tr>
<td>Oct-05</td>
<td>PARC Team</td>
<td>Feb-07</td>
<td>deCODE</td>
</tr>
<tr>
<td>Oct-05</td>
<td>PharmacogenoDesign</td>
<td>Apr-07</td>
<td>National Genome Research Network</td>
</tr>
<tr>
<td>Nov-05</td>
<td>Cancer Research UK; Edinburgh University Colon Cancer Genetics Group;</td>
<td>May-07</td>
<td>Golden Helix</td>
</tr>
<tr>
<td>Nov-05</td>
<td>Institute of Cancer Research; Cancer Research UK; London Research</td>
<td></td>
<td>IntegraGen</td>
</tr>
<tr>
<td>Nov-05</td>
<td>Institute</td>
<td></td>
<td>National Institute of Genomic Medicine (INMEGEN)</td>
</tr>
</tbody>
</table>

*Continued on next page*
<table>
<thead>
<tr>
<th>Date</th>
<th>Institution/Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan-06</td>
<td>North Shore-Long Island Jewish Health System</td>
</tr>
<tr>
<td>Jan-06</td>
<td>Genzion BioScience</td>
</tr>
<tr>
<td>Feb-06</td>
<td>SAIC-Frederick, Inc.</td>
</tr>
<tr>
<td>Jul-07</td>
<td>University of Pennsylvania, the Broad Institute, the CARe Consortium</td>
</tr>
<tr>
<td>Sep-07</td>
<td>SNP Genetics</td>
</tr>
<tr>
<td>Sep-07</td>
<td>Cancer Research UK</td>
</tr>
</tbody>
</table>
6 Analysis.

6.1 Overview

This case study of Illumina’s high-throughput SNP genotyping technology cannot be evaluated without acknowledgment of the people and factors that contributed to it. Specifically, I find that five factors were significant influences: (1) David Walt and his entrepreneurial insights; (2) the Tufts University/MBRI tech transfer office and the exclusive license; (3) the fiber optic sensor with encoded microsphere technology itself; (4) Illumina, Inc. and its overall business model; and, (5) the timing of market entry and the initial public offering.

This chapter will synthesize the effects of such contributions and highlight the ways in which each contribution teaches a lesson about successful genomic technology development and commercialization. The impact of the core Illumina BeadArray technology on high-throughput SNP genotyping studies can be evaluated by many metrics, and score well on all of them, as related later in each section [see ref. 270].
6.2 David Walt

David Walt initiated the process to commercialize his inventions. His ability to attract venture capital funding for his inventions was a result of a couple of factors. First, his expertise in the field of analytical chemistry prior to the invention of his bead-based fiber optic array gave him academic credibility. This credibility enabled him to present the information about his invention to the scientific community, to publish an article disclosing his invention in a peer-reviewed journal [144], to have the licensing office invest in filing a patent application for the invention, and to obtain venture capital interest in the invention. Second, Walt’s seminal invention and other supporting inventions were properly disclosed to ensure intellectual property rights. This understanding of the steps required to secure ownership rights for his inventions came as a result of trial and error from previous commercial endeavors. Clear property rights encouraged those individuals interested to invest in the technology to seek worldwide exclusive licensing.

This section will analyze the academic and industrial impacts of David Walt’s inventions. Specifically, this section will discuss how expertise in a technological field combined with insights about technology transfer classifies Walt as a “star scientist”. Furthermore, this section will suggest that by enabling industry experts to identify the most commercially viable markets for his technology, Walt was able to balance academic duties and industrial work. Another important component to the commercial success of the bead-based array technology was Walt’s decision to remain in academia whereas
industry experts identified the most proving markets and developed the business plans.
This section will conclude by transitioning to a brief analysis the institution that enabled
the transfer of technology and then to a detailed analysis of the successful startup
compány, Illumina.

6.2.1 The role of a “Star Scientist” in the development of commercial
technology

David Walt represents a class of high-achieving highly entrepreneurial scientists that
Zucker and Darby designate “stars” [1]. Although the “stars” initially were defined as
bio-scientists who had reported “more than 40 genetic-sequence discoveries on or more
than 20 articles reporting any genetic-sequence discoveries by 1990” [1], the meaning has
since been used to characterize scientists who have excelled in both academic and
industrial arenas. The extent to which these “stars” have impacted scientific research and
private ventures has been evaluated by measuring the number of patents and publications
that the “star” has produced, and the extent to which each has been cited by other
academic and/or industrial researchers [1, 271, 272].

Stars can positively affect the quality of both academic and industrial outputs. Zucker and
Darby conclude that the influence of these scientists in the transfer of research from the
bench to commercial ventures has a “significant positive effect” on many factors in the
biotechnology industry, such as the number and quality of patents [271]. Firms with ties to “stars” also boast a shorter time to IPO and an increase in the amount of IPO proceeds [271]. Likewise, as stars seek collaborations with industrial partners, the nature of the academic research tends to become more commercially relevant.

Zucker, Darby and Armstrong demonstrated that the “Ten Most Highly Valued Biotechnology Firms In 1994” named leading academic scientists on their IPO prospectuses and as joint authors on publications. Star scientists can strongly influence a company’s “knowledge capture” through the licensing of patents. Patents signal a degree of success within the biotechnology industry and indicate a company’s “knowledge production function” [273]. Patents licensed from star scientists can also impact the amount of venture capital funding a new company can raise, based on the financial and non-financial implications that patent acquisition signifies [271].

More importantly, however, star scientists have direct effects on the transfer of intellectual human capital [271]. Initial surveys of star scientists revealed that the pace of scientific discovery process and diffusion of ideas via technology transfer is directly linked to the amount of input from the stars themselves [1]. This is largely because new techniques/inventions require “tacit knowledge” that is hard to transfer in its initial stages. The extent to which other scientists can be trained to carry out tasks makes some aspects of technology transfer naturally “appropriable” [271]. Thus, technology transfer
can be made easier by the physical transfer of scientists who developed a technology in the academic setting into the commercial organization.

6.2.2 Academic and industrial outputs

David Walt would certainly fall into this classification as a “star scientist.” The following sections will review the grants, publications and patents that Walt has received. The decisions that Walt made regarding a balance between academic and entrepreneurial endeavors are then addressed. These insights have been pivotal to his success in both arenas.

6.2.2.1 Governmental grants

Although not a metric used to classify a “star”, the quality and quantity of government funding that an academic researcher receives is a valuable indicator of a researcher’s production function. David Walt has been a recipient of many federal grants since his initial years on the Tufts University faculty. He continues to be rewarded with multi-year contracts, in addition to his research funding agreement with Illumina. It was through his work on a multi-institution grant that he met Columbia University professor Clark Still, who introduced him to the venture capitalists at Channing Weinberg Group, who co-founded Illumina.
Table 6.1 tracks Walt’s federal funding since his initial years as a member of the Tufts faculty. He has successfully obtained funding from a variety of federal funding agencies. It is interesting to note that subjects of the grants for which Walt has obtained funding (based on the grant titles) have shifted from general chemical sensing to biomedical- and diagnostics-based sensing.
Table 6.1: Federal funding awarded to David Walt, Ph.D. Awards were found using the NIH’s CRISP database and the RaDiUS Database for federal funding by searching for “David R. Walt” or “fiber optic sensor technology”.

<table>
<thead>
<tr>
<th>Funder</th>
<th>Start Date</th>
<th>End Date</th>
<th>Avg FY Fund (K)</th>
<th>Award/Task Number</th>
<th>Title</th>
<th>Brief Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>National Institute of General Medical Sciences</td>
<td>8/1/1993</td>
<td>7/31/2001</td>
<td>170.05</td>
<td>R01GM048142</td>
<td>Image-Ready Fiber-Optic Sensors for Biomedical Analysis</td>
<td>Develop techniques to use fiber-optic sensors to monitor chemicals for clinical and basis research</td>
</tr>
<tr>
<td>Department of the Navy - 0601153N - Defense Research Sciences</td>
<td>1/1/1994</td>
<td>1/12/1996</td>
<td>Restricted</td>
<td>DN054391</td>
<td>Use of Monoclonal and Engineered Cross-Reactive Antibodies to Detect Polynuclear Aromatic Hydrocarbons</td>
<td>Devise bundled fiber optic biosensors, based on cross-reacting antibody arrays, to monitor environmental contaminants.</td>
</tr>
<tr>
<td>Department of Energy - Basic energy science</td>
<td>2/1/2000</td>
<td>2/1/2001</td>
<td>125</td>
<td>FG0200ER62923</td>
<td>Time Resolved Sequence Analysis on High Density Fiber Optic DNA Probe Arrays</td>
<td>Study the preparation of high-density fiber-optic arrays as sensors for rapid analysis of DNA sequences</td>
</tr>
<tr>
<td>National Institute of Dental &amp; Craniofacial Research</td>
<td>9/30/2002</td>
<td>6/30/2006</td>
<td>756.56</td>
<td>U01DE014950</td>
<td>Microsensor Arrays for Saliva Diagnostics</td>
<td>Create platforms to analyze saliva content using fiber optic imaging sensors</td>
</tr>
<tr>
<td>National Science Foundation - MPS - CHE</td>
<td>8/1/2005</td>
<td>7/1/2007</td>
<td>46.67</td>
<td>518293</td>
<td>Fabrication of High Aspect Ratio Optical/Electrochemical Hybrid Sensor Arrays</td>
<td>Demonstrate the feasibility of performing multiplexed analysis using electrochemiluminescence (ECL)</td>
</tr>
</tbody>
</table>

Continued on next page
<table>
<thead>
<tr>
<th>Funder</th>
<th>Start Date</th>
<th>End Date</th>
<th>Avg FY Fund (K)</th>
<th>Award/Task Number</th>
<th>Title</th>
<th>Brief Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>National Institute of Biomedical Imaging and Bioengineering</td>
<td>9/1/2005</td>
<td>8/31/2008</td>
<td>73.31</td>
<td>R01EB003985</td>
<td>Optical Tweezer Arrays Based on Optical Imaging Fibers</td>
<td>Develop a platform for simultaneous and parallel micromanipulation of thousands of particles</td>
</tr>
<tr>
<td>National Institute of Dental &amp; Craniofacial Research</td>
<td>9/1/2006</td>
<td>6/30/2011</td>
<td>Unavailable</td>
<td>U01DE017788</td>
<td>Microsensor Arrays for Saliva Diagnostics</td>
<td>Develop a portable, diagnostic system for pulmonary diseases using saliva as specimen sample</td>
</tr>
</tbody>
</table>
6.2.2.2 Publications

Academia awards merit based on publications in peer-reviewed journals. Before David Walt was a successful entrepreneur, he was a successful analytical chemist, who had publications in several journals. By the year his seminal paper describing a fiber optic sensor with encoded microspheres (Michael et al., 1998) was published, he had 59 publications in 24 unique journals and already was an inventor or co-inventor on 16 combined patents and patent applications [Appendix 1]. Figure 6.1 tracks his peer-reviewed publications by year. Figure 6.2 tracks the years that independent peer-reviewed publications cited any of Walt’s publications. It is evident that the citations of his publications have increased over the past twenty years. However, there is a marked up-tick in citations between the years 2000-2002. This trend is further examined in Figure 6.3.

---

1 Information regarding Walt's publication trends was taken from SCOPUS and ISI Web of Science databases.
Figure 6.1: Journal article publications by David Walt by year. Publication information obtained from ISI Web of Science database.
Figure 6.2: Citations of Walt’s publications by year. Number of citations of Walt’s 121 articles were tracked over a 22-year period, adjusted for self-citation. Data obtained from ISI Web of Science.

In order to determine whether the increase in his publication citations was a result of his bead-based fiber optic sensor array publications, citations of the top 5 publications pertaining to the technology (by citation count) were selected and tracked. Figure 5.3 shows that the combined citations from the top 5 publications accounted for 57%, 58%, and 44% of the total number of citations of Walt’s publications for the years 2000, 2001
and 2002, respectively. These are the years in which Walt’s publications become more highly cited.

![Citation trends by publication](image)

**Figure 6.3: Independent citations of journal articles by Walt by year.** This tracks the number of independent journal articles citing Walt’s publications by the year the citing article was published. Data obtained from ISI Web of Science and SCOPUS databases.
6.2.2.3 Citation analysis

In analyzing publication citations, it is important to evaluate the types of publications, the types of fields citing the publications, and the journals in which those citing papers are published, we can look at how publications of the technology has had an impact.

To date, Walt has published 121 journal articles. Of the 2,198 non-self citations of those 121 articles, the majority of publications are published within the field of analytical chemistry (771 out of 2,198) while only 23 citing publications are within the field of genetics & heredity\(^2\). This trend is in stark contrast to the characteristics of publications citing articles from Illumina. More information regarding the institutions citing the BeadArray technology for SNP genotyping will be covered in Section 6.4.

Walt’s top 15 publications sorted by forward patent citations are listed in Table 6.2. His publications on average have received more forward publication citations, although in a few instances, a particular publication has been cited more by patents than by publications.

\(^2\) This information is publicly available from the ISI Web of Science database.

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Table 6.2: David Walt’s top 15 publications. Information obtained from SCOPUS and ISI Web of Science databases.

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Title</th>
<th>Source title</th>
<th>Forward Publication Citations</th>
<th>Forward Patent Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>Steemers, F.J., Ferguson, J.A., Walt, D.R.</td>
<td>Screening unlabeled DNA targets with randomly ordered fiber-optic gene arrays</td>
<td>Nature Biotechnology</td>
<td>120</td>
<td>60</td>
</tr>
<tr>
<td>2000</td>
<td>Mandal, T.K., Fleming, M.S., Walt, D.R.</td>
<td>Production of hollow polymeric microspheres by surface-confined living radical polymerization on silica templates</td>
<td>Chemistry of Materials</td>
<td>90</td>
<td>7</td>
</tr>
<tr>
<td>1998</td>
<td>Walt, D.R.</td>
<td>Fiber Optic Imaging Sensors</td>
<td>Accounts of Chemical Research</td>
<td>89</td>
<td>37</td>
</tr>
<tr>
<td>2000</td>
<td>Lee, M., Walt, D.R.</td>
<td>A fiber-optic microarray biosensor using aptamers as receptors</td>
<td>Analytical Biochemistry</td>
<td>82</td>
<td>26</td>
</tr>
</tbody>
</table>

*Continued on next page*
<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Title</th>
<th>Source title</th>
<th>Forward Publication Citations</th>
<th>Forward Patent Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>Walt, D.R.</td>
<td>Convergent, self-encoded bead sensor arrays in the design of an artificial nose</td>
<td>Analytical Chemistry</td>
<td>78</td>
<td>7</td>
</tr>
</tbody>
</table>
The publication of David Walt’s microsphere-encoded fiber optic sensor has received 140 total publication citations and 134 patent citations (including 100 USTPO patents; 30 WIPO patents; 4 EPO patents). The fact that the number of patents citing this particular publication is roughly equivalent to the number of publication citations indicates that the invention described held direct, technical applications.

6.2.2.4 Patents

While publications define a researcher’s contribution to the Science Commons, patents are the currency of industry. The fiber optic sensor with encoded microspheres technology has been one of Walt’s most highly cited patents [274]. It, however, was not the only patent included in the exclusive license to Illumina. Table 6.3 details the patents that were included within the exclusive license and the forward patent citations, adjusted to eliminate self-citation by either Illumina or David Walt. Forward patent citations by other institutions suggest that the inventions have been incorporated into other technological applications. Appendix 2 shows the trends of forward patent citations for the 78 patents on which Walt is listed as inventor or co-inventor.
Table 6.3: Forward patent citation trends of patents in the Illumina Exclusive License Patent Portfolio. Data were obtained from Illumina, Inc. SEC filings and the Delphion Intellectual Property database.

<table>
<thead>
<tr>
<th>Inventors</th>
<th>Title</th>
<th>Patent No.</th>
<th>Date Issued</th>
<th>Forward Patent Citations</th>
<th>Adjusted Forward Patent Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>WALT, David; BARNARD, Steve</td>
<td>Imaging Fiber Optic Array Sensors, Apparatus and Methods for Concurrently Detecting Multiple Analytes of Interest in a Fluid Sample</td>
<td>US Patent: 5,244,636</td>
<td>9/14/93</td>
<td>94</td>
<td>62</td>
</tr>
<tr>
<td>WALT, David; BARNARD, Steve</td>
<td>Fiber Optic Sensor, Apparatus and Methods for Detecting an Organic Analyte in a Fluid or a Vapor Sample (OSCI)</td>
<td>US Patent: 5,244,813; CA Patent: 2,128,413</td>
<td>9/14/1993</td>
<td>87</td>
<td>59</td>
</tr>
<tr>
<td>WALT, David; HEALEY, Brian</td>
<td>Photodeposition Methods for Fabricating a Three-Dimensional, Patterned Polymer Microstructure</td>
<td>USSN: 08/519,062 (now US Patent: 6,200,737)</td>
<td>Filed: 8/24/1995</td>
<td>49</td>
<td>45</td>
</tr>
</tbody>
</table>

Continued on next page
<table>
<thead>
<tr>
<th>Inventors</th>
<th>Title</th>
<th>Patent No.</th>
<th>Date Issued</th>
<th>Forward Patent Citations</th>
<th>Adjusted Forward Patent Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>WALT, David</td>
<td>Far-Field Viewing Optical Apparatus for Making Optical Determinations and Analytical Measurements</td>
<td>USSN: 08/572,005 (now US Patent: 5,814,524)</td>
<td>Filed: 12/14/95</td>
<td>85</td>
<td>62</td>
</tr>
<tr>
<td>WALT, David; MICHAEL, Keri</td>
<td>Fiber Optic Sensor with Encoded Microspheres (Analyte Detection System)</td>
<td>USSN: 08/818,199 (now US Patent: 6,023,540)</td>
<td>Filed: 3/14/97</td>
<td>100</td>
<td>77</td>
</tr>
<tr>
<td>WALT, David; DICKINSON, Todd</td>
<td>Self-Encoding Microspheres</td>
<td>USSN: 08/944,850 (now US Patent: 7,115,884)</td>
<td>Filed: 10/6/97</td>
<td>49</td>
<td>31</td>
</tr>
<tr>
<td>WALT, David; HEALEY, Brian; FERGUSON, Jane</td>
<td>Fiber Optic Biosensor for Selectively Detecting Oligonucleotide Species in a Mixed Fluid Sample</td>
<td>Application Being Prepared (now US Patent: 6,482,593)</td>
<td>N/A</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>WALT, David; TAYLOR, Laura</td>
<td>Fiber Optic Biosensor Array Comprising of Cell Populations Confined to Microcavities</td>
<td>Application Being Prepared (now US Patent: 6,377,721)</td>
<td>N/A</td>
<td>20</td>
<td>18</td>
</tr>
</tbody>
</table>
6.2.2.5 Transfer of Human Intellectual Capital

Application of a scientific invention can sometimes be “excludable” based on tacit knowledge that must be acquired through first-hand training [271, 272]. The knowledge boundary between academia and biotechnology firms can be crossed by people acting as “information carriers” [272]. Zucker, Darby and Brewer note that “at least for the first 10 to 15 years, the innovations which underlie biotechnology are properly analyzed in terms of naturally excludable knowledge held by a small initial group of discoverers, their co-workers, and others who learned the knowledge from working at the bench-science level with those possessing the requisite know-how” [272]. Collaborations between academia and industry are one “information highway;” transfer of researchers from academic to industrial posts provides another [272].

Since Illumina’s inception in 1998, David Walt has served as the Chairman of Illumina’s Scientific Advisory Board and has lent his scientific expertise to the Company. The ongoing research agreement between his laboratory at Tufts and Illumina has also served to create a two-way information flow. But perhaps the most salient example of human intellectual capital transfer is illustrated by the scientists who have worked in Walt’s laboratory at Tufts and have since become (and remain as of this publication) employees at Illumina.
Todd Dickinson, Frank Steemers, and Steven Barnard are some of the researchers who have moved from Walt’s laboratory to Illumina. The fact that each of these individuals has produced both patents and publications from Tufts and Illumina exemplifies this intellectual transfer “in vivo”. Table 6.4 shows the number of publications that each researcher has been co-authored while affiliated with Tufts or Illumina.

<table>
<thead>
<tr>
<th></th>
<th>Tufts University</th>
<th>Illumina, Inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Publications</td>
<td>Patents</td>
</tr>
<tr>
<td>Todd Dickinson</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Frank Steemers</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Steven Barnard</td>
<td>8</td>
<td>11</td>
</tr>
</tbody>
</table>

The impact that this personnel transfer has is significant in other ways, as well. Researchers who have used the technology since its incubation phases – and, therefore, are most familiar with experimental details – may also be the people most capable of developing complementary technologies. Todd Dickinson, for example, who had been one of the first researchers to transfer from Walt’s laboratory to Illumina, was influential in helping the young company identify a key technological acquisition that dramatically impacted Illumina’s growth. John Stuelpnagel cites Dickinson as the person who suggested acquiring CyVera Corporation for its VeraCode technology [181]. The VeraCode technology has the low- to mid-multiplex capabilities that the BeadArray
technology cannot provide. Dickinson currently holds the office of Director of Product Marketing at Illumina.

6.2.3 David Walt as an entrepreneurial scientist

David Walt’s status as a “star scientist” is one of the key components to this success story. As an extension of the definition of a “star”, Zucker and Darby noted that the “very best scientists were centrally important in affecting both the pace of diffusion of the science and the timing, location, and success of its commercial applications” [1]. Knowledge of intellectual property, proper disclosure, appropriate due diligence and timing were all essential ingredients in this particular case study. David Walt used past experiences with intellectual property disclosure to understand the know-how of successful technology transfer. The creation of the core technology and the steps taken to properly disclose the invention were decisions executed by Walt. Had he neither the scientific expertise nor the business foresight to carefully consider how and when to share his fiber optic sensor with encoded microsphere technology, this story would likely have turned out less successfully.

6.2.4 Lessons learned by a scientist

Most importantly, we find that David Walt serves as a role model for academic scientists wishing to see their inventions commercialized without having to give up their faculty
posts. Walt currently holds positions as Robertson Professor of Chemistry at Tufts University and as Chairman of the Scientific Advisory Board at Illumina, Inc.

6.2.5 Lessons learned: impact of IP knowledge

David Walt had experienced patenting other inventions well before he developed his fiber optic sensor with encoded microspheres that formed the core Illumina technology. Although the first patents he received were from his work at MIT, he commented that he learned how to patent while at Tufts [176]. Furthermore, he remarked that the process of filing a patent application was largely an experimental one. For example, during his initial research with fiber optic sensors, Walt collaborated with individuals interested in developing sensors for medical diagnostic purposes. One of the collaborations he was involved in resulted in a loss of worldwide rights because of public disclosure before patent application [176].

We find it important that he did make these mistakes with his earlier inventions, because, as he noted “[It was] an easy lesson to remember because we had screwed it up once before” [176]. We believe that had he made similar mistakes with the patents in the Illumina portfolio, certainly the ‘540 patent, Illumina may have been unable to attract the venture capital investment that it did.
6.2.6 Market identification

When speaking with Vice Provost Peggy Newell about the Illumina licensing deal, I asked her for insights on the recipe for success of technology transfer. She replied:

“I can tell you that there is oftentimes an inverse relationship between the sophistication of the investigator and the likelihood of their succeeding and the amount of time it takes for technology transfer. I think that part of the reason that the Illumina deal was as easy and as successful as it was is because David Walt and the people he was working with had realistic expectations for what a deal needs to look like” [172].

Walt’s understanding of his ultimate goals were certainly helpful in enabling John Stuelpnagel to perform appropriate market due diligence when writing up the business proposal. In Newell’s experience, when faculty members are “uninformed” as to the realities of the technology transfer process, the ability to create the best business strategy can be hampered [172]. According to the Tufts Provost, educating faculty on these technology transfer realities is something best taught by those successful academic entrepreneurs themselves [172]. The next section will cover a brief analysis of the Tufts OTLIC since its inception in 2000.

6.3 OTLIC – Technology transfer at Tufts University

The Tufts University OTLIC was not its own independent office during the 1990s. Faculty disclosures and licensing agreements were handled through a regional management office, known as the Massachusetts Biotechnology Research Initiatives...
(MBRI). Of the eleven universities that used the MBRI, Tufts provided the most business, based on the number of disclosures and licenses. The increase in faculty disclosures within Tufts, combined with the extraordinary success of Illumina as a faculty startup, encouraged Tufts University to establish its own technology licensing office [172].

The following section will discuss the activities of the OTLIC in educating faculty about intellectual property. The section will also analyze some trends within Tufts University of technology transfer.

### 6.3.1 Faculty knowledge of IP disclosure

The lessons that Walt learned as an academic scientist about intellectual property disclosure practices exemplify the need for universities to educate their faculty. Vice Provost Newell commented that the technology transfer office at Tufts, the OTLIC, has used David Walt as a ‘role model’ for budding entrepreneurial scientists. While the OTLIC offers seminars for faculty, Newell says that the process is still an ‘informal’ one, since many faculty members are wholly unaware of what to expect from the process [172].
6.3.2 An ‘in-house’ technology transfer office

Between 1995 and 2000, seven new companies based on Tufts faculty inventions were formed, giving Tufts and the MBRI equity positions in six of seven [Table 6.5; Figure 6.4] [173]. Illumina was by far Tufts’ most lucrative start-up. The University’s stock in the company was the first that Tufts was able to liquidate, reaping $7,682,762 from Illumina’s IPO in 2000 [175].

Tufts OTLIC administrators commented that the Illumina liquidation did not fund the formation of the independent licensing office, as the preparation for the office was already in the works well before Illumina’s IPO. Furthermore, they commented that the decision to fund the independent office and the establishment of Illumina occurred almost simultaneously, in part because the University began to recognize a ‘pent-up demand’ for an ‘in-house’ licensing office [191].
Figure 6.4: Companies started from Tufts University by year. Startup companies are listed by the year that the licenses were signed. Data obtained from the Tufts OTILC website (http://techtransfer.tufts.edu).

Since the inception of Tufts University’s independent licensing office, revenues on patent royalties have become a welcome, albeit somewhat unpredictable, source of income to the University. Opening in 2000, the OTILC of Tufts University brought together the necessary factors required to integrate “better service” both to the University’s faculty and to external collaborators\(^1\) [173].

\(^1\) For more information on the Tufts University Office of Technology Licensing & Industry Collaboration, see: http://techtransfer.tufts.edu/index.shtml
The OTLIC has served two distinct roles for Tufts. First, it has kept IP management costs down. Tufts cites that its accurate records of patents filings, applications and industry licensing agreements have moderated costs, as Tufts pays “out-of-pocket costs associated with the protection of [its] intellectual property” [173]. Second, the presence of an accessible technology transfer office has encouraged more Tufts faculty members to participate in technology transfer process, given the potential for royalties gained from the sale of products or the use of products derived from faculty inventions provides financial incentive for Tufts personnel to participate in the technology transfer process.

Some trends have emerged over the past seven years since the inception of the OTLIC that are similar to other university technology licensing ventures: 1) income generation from equity sales of startups and patent royalties are often unpredictable; 2) academic inventions are often licensed early-stage with little or no established proof-of-principle; and 3) exclusive licenses can hinder ultimate success due to high failure rates of product development².

Tufts OTLIC has taken a multi-lateral approach to these issues. It has focused on marketing the technologies, with an emphasis on securing non-exclusive licenses to many

industrial partners. The OTLIC has also prepared seminars to educate faculty on proper
IP disclosure. Companies started up through Tufts are listed in Table 5.5 \(^3\) [174].

Table 6.5: Startup companies from technology licensed by Tufts University.
This is a representative list of the companies that have been started as a result of
technology licensed from Tufts. Companies were started with either exclusive or non-
exclusive licenses (exact details not given). Information obtained from Tufts OTLIC
website Annual Reports.

<table>
<thead>
<tr>
<th>Date</th>
<th>Company</th>
<th>Founder</th>
<th>School/College</th>
<th>Market Focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr-96</td>
<td>Ion Signature Technology</td>
<td>A. Robbat</td>
<td>A&amp;S</td>
<td>Software for signal analysis</td>
</tr>
<tr>
<td>Feb-97</td>
<td>Paratek Pharmaceuticals</td>
<td>S. Levy</td>
<td>Medical</td>
<td>Antibiotic therapy Blood cell treatment Devices for bone fixation</td>
</tr>
<tr>
<td>May-97</td>
<td>Point Therapeutics</td>
<td>W. Bachovchin</td>
<td>Medical</td>
<td></td>
</tr>
<tr>
<td>Jul-97</td>
<td>Securos</td>
<td>K. Kraus</td>
<td>Veterinary</td>
<td></td>
</tr>
<tr>
<td>May-98</td>
<td>Illumina</td>
<td>D. Walt</td>
<td>A&amp;S</td>
<td>Therapeutic compound identification Fiber optic sensors</td>
</tr>
<tr>
<td>Sep-99</td>
<td>Arisaph (Triad)</td>
<td>W. Bachovchin</td>
<td>Medical</td>
<td></td>
</tr>
<tr>
<td>Jun-00</td>
<td>Serica</td>
<td>Kaplan</td>
<td>Engineering</td>
<td>Tissue generation Detection of airborne compounds Chiral separations science Music synchronization</td>
</tr>
<tr>
<td>Jun-02</td>
<td>CogniScent</td>
<td>J. Kauer, J. White</td>
<td>Engineering</td>
<td></td>
</tr>
<tr>
<td>Dec-04</td>
<td>Evolved Nanomaterial</td>
<td>N/A</td>
<td>Engineering</td>
<td>Chiral separations science</td>
</tr>
<tr>
<td>Mar-07</td>
<td>Tempo Control</td>
<td>J. Cerra, M. Visconti</td>
<td>Engineering</td>
<td>Music synchronization Hemolytic-uremic syndrome therapy Novel antimicrobial compounds</td>
</tr>
<tr>
<td>Mar-07</td>
<td>Technologies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar-07</td>
<td>Lakewood Biomedical</td>
<td>S. Tzipori</td>
<td>Veterinary</td>
<td>Hemolytic-uremic syndrome therapy Enhanced data processing</td>
</tr>
<tr>
<td>Jun-07</td>
<td>Centegen</td>
<td>N. Balaban, K. Panetta, S. Agalan</td>
<td>Veterinary</td>
<td></td>
</tr>
<tr>
<td>Jun-07</td>
<td>BA Logix</td>
<td>D. Walt</td>
<td>A&amp;S</td>
<td>Single molecule detection</td>
</tr>
</tbody>
</table>

\(^3\) As of the revisions on this document, Tufts has released information that David Walt has started a new company. Future research should investigate the funding and IP status of this new company.
In fiscal year 2007, OTLIC generated over $3.44 million for Tufts. The office reported 56 new invention disclosures, 22 US patent applications, 11 new patent approvals, and 5 new startup companies. Figure 6.5 shows the licensing trends of the Tufts University OTLIC (and MBRI) through 2006.

Figure 6.5: Patenting and licensing trends at Tufts University. Tufts University’s patents and licenses were managed through the MBRI until 1998.
The data from Figure 6.5 reveal an up-tick in foreign patent application filings. This trend could be an indication of better invention disclosure practices by Tufts faculty. David Walt explained that he forfeited foreign rights on one of his earlier inventions due to mistakes in the sequence of disclosure steps. He said that by learning the lesson first-hand, it was “easy” to remember the proper order of events the next time [176]. Thus, as faculty become savvier at disclosing inventions, they are more likely to become aware of situations in which patent protection would be useful, and how to secure it without hindering ongoing research or foregoing worldwide rights.

Figure 6.6 shows the royalties that Tufts has received per year. The windfall payment of over $7 million in 2001 came from the liquidation of the Illumina stock. According to Peggy Newell, Tufts holds equity in a majority of its startups. Illumina has been the first startup company whose stock Tufts has been able to liquidate [172]. Tufts has not disclosed the amount of stock it holds in its other companies.
Figure 6.6: Royalty income for the Tufts University Office of Technology Licensing and Industry Collaboration. Liquidation of the Illumina stock came in 2001. It made of $7,682,762 of the royalty income from that year.

6.3.3 Technology transfer by other means

The commercialization and development of university inventions is commonly thought to derive from the licensing of patents to industrial partners. This is not, however, the only means by which scientific knowledge is transferred. Industry draws on academic peer-reviewed publications more often than licensing agreements [154]. Furthermore,
conference proceedings and informal social networks also provide a transfer of intellectual capital independent of patents.

In return for technological transfer into industry from academia, industry contributes to its scholarly partners through publications and data in the “Science Commons.” See below for Illumina’s collaborations with academic and non-profit institutions.

6.3.4 The success of technology transfer: The Exclusive License

The transfer of the fiber optic sensor technology to Illumina was pivotal in the company’s overall success. Exclusive licenses serve a multitude of purposes and in the case of Illumina, the exclusive license was “essential” to secure venture capital funding. John Stuelpnagel, co-founder of Illumina, stated, “…if we were not able to get an exclusive license, then we would not have started Illumina” [181]. David Walt expressed similar opinions on the decision to pursue an exclusive license [176].

As Stuelpnagel went onto explain, the decision of universities to dole out patent licenses exclusively presents a “trade-off”; exclusive licenses are only as valuable as the commercial product that the licensing company develops [181]. Non-exclusive licenses, on the other hand, can bring in a steady stream of royalty payments. Exclusive licenses are commonly issued when a particular technology has the potential to become the core
technology upon which a company can develop many products [181]. Stuepnagel and Bock used this reasoning to convince Tufts University to exclusively license David Walt’s patent portfolio.

Technology transfer, if properly handled by the university, presents a powerful tool for academic inventions to be developed into commercial products. However, the quality of the patent applications filed, awarded and issued are equally important factors. The fact that the patents within Walt’s portfolio were downstream technologies that had limited impact on the development of other fiber optic-based sensor inventions suggests that appropriate due diligence was performed by MBRI/Tufts in filing the initial patents. This means that those in charge of filing the patents probably ensured that the claims were broad enough to protect the inventions created by David Walt, but not too broad to preclude others from using fiber optic cables as sensors. This can be supported by the fact that since the “Fiber Optic Sensor with Encoded Microspheres” patent was granted [274], over 554 other US patents on fiber optic sensor technology have been granted\(^4\). Therefore, execution of the technology transfer by MBRI/Tufts enabled Walt to protect his specific inventions without blocking others from creating other, similar inventions.

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\(^4\) To determine how many US patents pertaining to fiber optic sensor technology since David Walt’s ’520 patent, a search was performed in the Delphion database for: (((fiber optic sensor)) AND PD>1997/10/29).
6.4 Illumina, Inc. & BeadArray technology

This case study initially began because of the understanding that Illumina’s BeadArray technology was state-of-the-art for performing high-throughput SNP genotyping analysis. This next section will begin by discussing important business and financial aspects of Illumina that have enabled its success as a market leader in manufacturing analytical tools for genomics research. These aspects include performing market due diligence, venture capital funding, obtaining federal grants, acquiring complementary technologies and timing the IPO for maximum cash inflow. The latter half of this section will investigate the “outputs” of a successful business model. First, we will look at the company’s financial status since its inception. We will then examine “output” through a two pronged-approach: first, by analyzing the patents and publications that Illumina has produced as a result of its technology; and, second, by analyzing the publications that Illumina customers have produced as a result of using Illumina platforms. Specifically, we will try to analyze the impact that the BeadArray technology has had on performing high-throughput SNP genotyping studies. For this reason, we will pay particular attention to Illumina’s contribution to the HapMap Project – a private-public project to understand genetic variability. This section will conclude with a suggestion for future research endeavors regarding the process of university technology transfer.
6.4.1 Lessons for university startups – a business model for success

The exclusive license on the technology may have been critical for venture capital investment, but it alone did not guarantee commercial success. Several other factors enabled the development of the BeadArray technology.

6.4.1.1 Quality people

When asked what one factor or event cemented Illumina’s success, John Stuelpnagel replied that it was not one event in particular, but the people involved in the events that formed the intellectual capital to develop a winning company [181]. This thought was echoed in an earlier speech by David Walt to a group of collegiate entrepreneurs when he said that venture capital funding afforded the startup the financial capability to recruit the top scientific and managerial talent from other companies [275]. The influx of scientists from academic and industrial posts and the managerial staff collaborated to identify applications for the licensed technology. Thus, although the exclusive license on the intellectual property was essential, it was the application of the technology, which could only be identified by a team effort of experts in business and science, that laid out the company’s appropriate business plan.


6.4.1.2 Venture capital funding

The ability to raise venture capital funding was a direct result of the creation of an innovative technology that could become the cornerstone of a company. Equally important in getting venture capital funding was patent protection, at least in the early phases of Illumina’s development. The exclusive licensing of David Walt’s patent portfolio to Illumina was a signal to investors that the technology would have worldwide protection. Amassing venture capital funds was critical to Illumina’s pre-IPO stages, given that the manufacturing process of analytical tools was capital-intensive with fast cash-burn.

Venture capital funding raised pre-IPO was sufficient to purchase of add-on technologies to the core inventions licensed from Tufts. These purchases came through the outright acquisition of two other technology companies, nGenetics and Spyder Instruments. Illumina recognized the importance of the synthesis technology by Spyder Instruments, and the decoding technology by nGenetics in enhancing its core technologies’ capabilities. The acquisition is part of a trend in the pharmaceutical and biotechnology industries; complications in licensing deals have often given way to outright acquisitions of smaller companies rather than licensing deals [276].
6.4.1.3 Due diligence and timing

The execution of due diligence and the providence of good timing were also important elements in Illumina’s development. Walt had demonstrated that his technology could be applied to several areas of research, including environmental, industrial, chemical, and medical arenas [144, 180, 183-185, 187, 277], so Stuelpnagel and Bock had several options from which to choose the initial direction of the company. Stuelpnagel flagged two of the primary goals for the startup: (1) to find the best business model and (2) to minimize cash burn [181]. In that decision process, partnerships between agricultural and pharmaceutical companies were considered as potential options. However, Stuelpnagel stated that the ultimate business model implemented – the creation of “integrated systems for a large number of customers that span from academic institutions to pharmaceutical companies to biotech companies” – was the best choice [181].

The decision was a smart move given the state of the biotechnology market at that time. As Big Pharma underwent a restructuring process in the late 1990s, public interest turned towards the field of biotechnology as a potential source of therapeutic discoveries [278]. The Human Genome Project had generated much public excitement and Wall Street investment for genomics, which was evidenced by the income generated from initial public offerings of many biotechnology firms [196]. Some companies, such as Illumina, spotted not only the enormous potential for revenue by entering the market, but also the value in capitalizing on the manufacture of genomic tools for science that did not entail
costly clinical trials and FDA regulations – a risk reduction approach, as companies marketing drugs and devices were subject to a long FDA approval process and a high rate of failure [196].

The strategy of entering the genomics market through the sale of genotyping platforms was based on competitor avoidance; Affymetrix, Inc. had been the lead supplier of gene expression platforms and second behind Millennium Pharmaceuticals in market capitalization ($4.2 billion in 1999) for the genomics market [196]. The genotyping market, in contrast to gene expression analysis, was fragmented with no clear winning technology to identify SNP markers in an efficient, accurate or cost-effective manner.

Despite immediate interest in the novel technology from other industrial partners, Illumina, like other biotech companies, struggled with cash flow during its early years [181]. Taking the company public was a joint decision by Stuelpnagel and Flatley, who both understood the risks involved and the potential gains. The timing of Illumina’s IPO was auspicious; its $103.3 million IPO in July 2000 was one of the last successful offerings before the stock bubble burst at the end of the year [278]. The success of Illumina’s IPO also helped the company stay afloat during its first two years of public trading, as news of a soured deal with Applied Biosystems and negative investor returns dropped the stock price to ordinarily troublesome levels[181].
6.4.1.4 Government funding

To obtain additional sources of revenue, Illumina capitalized on its scientific expertise to apply for government grants to fund some of its initial projects. Table 6.6 lists the grants that have been earned by principal investigators while employed at Illumina. Stuelpnagel commented that the government funding, including the SBIR grants, was one of the “best investments [that] the government has ever made” [181].

Investment in development of specific products, such as a high-throughput oligo synthesis machine – enabled Illumina to create a technology that lowered overall production costs. Since academic and non-profit institutions, who rely on federal funding to carry out research projects, make up a significant portion of Illumina’s customer base, the ability to keep prices down because of federal funds may, in turn, have saved the government money.
Table 6.6: Federal funding to Illumina Principal Investigators (PIs). This tracks the federal grants and contracts that scientists at Illumina received. Information obtained from CRISP and RaDiUS databases by searching directly by PI name.

<table>
<thead>
<tr>
<th>PI</th>
<th>Funder</th>
<th>Start Date</th>
<th>End Date</th>
<th>Avg FY Fund ($)</th>
<th>Award/Task Number</th>
<th>SBIR (Y/N)</th>
<th>Title</th>
<th>Brief Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chee, Mark S.</td>
<td>NHGRI</td>
<td>2/1/1999</td>
<td>1/31/2000</td>
<td>117.71</td>
<td>R21HG001911</td>
<td>N</td>
<td>Randomly Ordered DNA Arrays for SNP Discovery and Typing</td>
<td>Develop randomly-ordered array-based SNP analysis technology; develop highly parallel, low-cost SNP genotyping assay; develop probe array for SNP discovery</td>
</tr>
<tr>
<td>Chee, Mark S.</td>
<td>NCI</td>
<td>3/10/1999</td>
<td>3/9/2000</td>
<td>99.6</td>
<td>R43CA081952</td>
<td>Y</td>
<td>Gene Expression Analysis on Randomly Ordered DNA Arrays</td>
<td>Develop bead-based mRNA profiling technology</td>
</tr>
<tr>
<td>Chee, Mark S.</td>
<td>NCI</td>
<td>7/1/2001</td>
<td>6/30/2003</td>
<td>325.64</td>
<td>R44CA081952</td>
<td>Y</td>
<td>Random Arrays for Gene Expression Profiling</td>
<td>Further develop mRNA profiling arrays using BeadArray technology by increasing number of unique probes per array, reducing costs, developing panels for mouse gene expression analysis</td>
</tr>
<tr>
<td>Chee, Mark S.</td>
<td>NCI</td>
<td>7/23/1999</td>
<td>9/1/1999</td>
<td>162.11</td>
<td>R43CA083398</td>
<td>Y</td>
<td>Parallel Array Processor</td>
<td>Develop and test parallel processing of sensor arrays for analysis of biological samples</td>
</tr>
<tr>
<td>Chee, Mark S.</td>
<td>NCI</td>
<td>4/1/2002</td>
<td>3/31/2004</td>
<td>342.35</td>
<td>R44CA083398</td>
<td>Y</td>
<td>Parallel Array Processor</td>
<td>Further develop a technology for parallel processing of arrays based on a matrix of arrays in a microtiter format Test feasibility of combining pyrosequencing with bead-based arrays for SNP typing, EST analysis, molecular diagnostics and re-sequencing projects</td>
</tr>
<tr>
<td>Chee, Mark S.</td>
<td>NHGRI</td>
<td>3/1/2000</td>
<td>8/31/2000</td>
<td>101.56</td>
<td>R43HG002119</td>
<td>Y</td>
<td>Pyrosequencing Arrays</td>
<td>Develop high-throughput highly-sensitive assay for DNA analysis without prior PCR amplification</td>
</tr>
<tr>
<td>Chee, Mark S.</td>
<td>NIGMS</td>
<td>7/1/2000</td>
<td>5/31/2001</td>
<td>49.72</td>
<td>R43GM062094</td>
<td>Y</td>
<td>Invader TM Arrays for Nucleic Acid Analysis</td>
<td>Develop bead-based array technology for measuring proteins and post-translational modifications</td>
</tr>
<tr>
<td>PI</td>
<td>Funder</td>
<td>Start Date</td>
<td>End Date</td>
<td>Avg FY Fund ($K)</td>
<td>Award/Task Number</td>
<td>SBIR (Y/N)</td>
<td>Title Brief Description</td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------</td>
<td>------------</td>
<td>-----------</td>
<td>------------------</td>
<td>-------------------</td>
<td>------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Chee, Mark S.</td>
<td>NCI</td>
<td>4/1/2002</td>
<td>2/1/2005</td>
<td>290.07</td>
<td>R33CA088197</td>
<td>N</td>
<td>Protein Profiling Arrays, Develop bead-based array technology for measuring proteins and post-translational modifications.</td>
<td></td>
</tr>
<tr>
<td>Chee, Mark S.; Barker, David; Oliphant, Arnold</td>
<td>NHGRI</td>
<td>9/30/2002</td>
<td>8/1/2005</td>
<td>2,308</td>
<td>U54HG002753</td>
<td></td>
<td>Cooperative Agreements, Highly Parallel SNP Genotyping for a Haplotype Map, Create a haplotype made of the genome by genotyping SNPs on a novel, large-scale genotyping system.</td>
<td></td>
</tr>
<tr>
<td>Gunderson, Kevin</td>
<td>NCI</td>
<td>9/30/2003</td>
<td>3/1/2007</td>
<td>116.8</td>
<td>R43CA103406</td>
<td>Y</td>
<td>Representational analysis of DNA copy number/methylation Allelic expression monitoring by array-based genotyping, Develop a high-throughput array-based commercial technology to scan the entire genome for DNA copy number and methylation states.</td>
<td></td>
</tr>
<tr>
<td>Gunderson, Kevin</td>
<td>NCI</td>
<td>5/1/2004</td>
<td>4/30/2005</td>
<td>97.48</td>
<td>R43CA108391</td>
<td>Y</td>
<td>High-Throughput Methylation Profiling System, Develop a robust and ultra high-throughput technology for simultaneously assaying methylation at many specific sites in a genome.</td>
<td></td>
</tr>
<tr>
<td>Fan, Jian-Bing</td>
<td>NCI</td>
<td>7/1/2002</td>
<td>12/31/2002</td>
<td>49.79</td>
<td>R43CA097851</td>
<td>Y</td>
<td>High-Throughput Methylation Profiling System, Develop a quantiative DNA genotyping platform for measuring allelic gene expression.</td>
<td></td>
</tr>
<tr>
<td>Fan, Jian-Bing</td>
<td>NCI</td>
<td>7/1/2002</td>
<td>10/31/2006</td>
<td>248.56</td>
<td>R44CA097851</td>
<td>Y</td>
<td>High-Throughput Methylation Profiling System, Develop a robust and ultra high-throughput technology for simultaneously assaying methylation at many specific sites in a genome.</td>
<td></td>
</tr>
<tr>
<td>Dickinson, Todd</td>
<td>NIEHS</td>
<td>9/27/1999</td>
<td>9/30/2000</td>
<td>98.4</td>
<td>R43ES010080</td>
<td>Y</td>
<td>Compact Device for Solvent Indentification, Develop a miniaturized, hand-held vapor-sensing device that is robust and reproducible, has commercial appeal by any personnel that routinely encounter solvent-saturated environments.</td>
<td></td>
</tr>
<tr>
<td>Barnard, Steven M.</td>
<td>NHGRI</td>
<td>6/7/2000</td>
<td>5/31/2001</td>
<td>79.72</td>
<td>R41HG002182</td>
<td>N</td>
<td>Optical Binary Encoding of Assembled Arrays, Develop technology platform based on optical binary encoding and randomly assembled arrays for application in SNP genotyping.</td>
<td></td>
</tr>
</tbody>
</table>

Continued on next page
<table>
<thead>
<tr>
<th>PI</th>
<th>Funder</th>
<th>Start Date</th>
<th>End Date</th>
<th>Avg FY Fund ($K)</th>
<th>Award/Task Number</th>
<th>SBIR (Y/N)</th>
<th>Title</th>
<th>Brief Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kruglyak, Semyon</td>
<td>NHGRI</td>
<td>9/30/2003</td>
<td>1/31/2007</td>
<td>96.23</td>
<td>R44HG003096</td>
<td>Y</td>
<td>Development of a Multi-State Decoding Framework</td>
<td>Increase the capability and decrease the cost of decoding the Illumina bead array platform by adding decode states for multiple states.</td>
</tr>
<tr>
<td>Zhao, Chanfeng</td>
<td>NIGMS</td>
<td>3/1/2005</td>
<td>2/28/2006</td>
<td>50.78</td>
<td>R43GM071272</td>
<td>Y</td>
<td>Kinase Substrate Array</td>
<td>Develop a novel, highly miniaturized peptide microarray for the simultaneous profiling of multiple protein kinases in a high throughput, multiplexed format.</td>
</tr>
<tr>
<td>Lebl, Michal</td>
<td>NIAID</td>
<td>4/1/2003</td>
<td>7/1/2006</td>
<td>345.38</td>
<td>R44AI056869</td>
<td>Y</td>
<td>Protease Substrate Array</td>
<td>Design, construct, and evaluate a prototype for a new instrument for the semi- and fully automated, parallel solid-phase synthesis of proteases in small volumes of cells or biological fluids.</td>
</tr>
</tbody>
</table>
6.4.2 Growing a company – Mergers & Acquisitions

COO John Stuelpnagel commented on Illumina’s intent to constantly develop its products in terms of price points and quality [181]. Illumina continues to improve its products and services through the acquisition of technologies. In some instances, acquisitions of technologies have come through outright acquisition of companies. In its ten year history, Illumina has acquired three companies (Spyder Instruments, nGenetics, and CyVera) and merged with one (Solexa).

The decision to acquire certain companies in lieu of creating licensing deals for the use of their technologies holds potential significance. Illumina probably viewed the technologies as highly important to the improvement of its own platforms, if not critical. Some technologies, on the other hand, may have been valuable to Illumina for the Company to expand its product and service lines.

It is reasonable to assume that the values of the core technologies of nGenetics (the decoding technology), Spyder Instruments (the high-throughput peptide synthesis technology) and CyVera (the multiplex genotyping technology) were deemed significant enough to warrant full acquisition by Illumina. Solexa and its unique sequencing platforms was a different story. Solexa was already a highly successful company when it merged with Illumina. The decision to merge Solexa and Illumina was viewed as one that
would create a technology company capable of taking on larger competitors, such as Affymetrix and Applied Biosystems, according to Solexa chief executive John West [279]. So far, the merger has been successful, based on Illumina’s consistently escalating earnings over the past three financial quarters of 2007 [Figure 6.7].

![Illumina 2007 Quarterly Earnings](image)

**Figure 6.7: Earnings over first three quarters of 2007.**

Details on the intellectual property acquired from these transactions is only partially public. Information regarding the intellectual property from Spyder Instruments and nGenetics was made confidential on Illumina’s S-1 Filing with the SEC. Patent information from CyVera Corporation and Solexa Inc., however, was obtained from the Delphion Intellectual Property Database. Illumina obtained 41 patents from its CyVera Corporation acquisition. Of those, 29 are world patents; 10 are granted European patents;
and two are granted US patents. From the Solexa merger, two US and three European patents were assigned to Illumina [Appendix 3].

6.4.3 Financial analysis of Illumina

6.4.3.1 Revenues and Profits.

Illumina was one of half-dozen companies to arise from the completion of the Human Genome Project. Such companies, including Affymetrix, which was founded in 1992 as an Affymax spin off- Agilent Technologies, Nanogen, NimbleGen Systems, ParAllele BioScience, and Perlegen Sciences were developed in an effort to glean information out of the genomic data being published in the National Center for Biotechnological Information database. The industry leader, Affymetrix, only became profitable in 2003, while Illumina proudly assumed that it would take less than half the time that Affymetrix took from its inception to reap a profit [138].

Fortune magazine reported that one reason for the stiff competition lies in the fact that there is no one “correct” way to make a gene chip. While Affymetrix invented the gene chip that was used in the late 1990’s, the platform that emerged from Illumina was a newly ‘evolved’ design that lent itself to greater flexibility, accuracy, throughput and cost efficiency – qualities that the report suggested would determine the ultimate leader [138].
Over the course of Illumina’s first decade, the company has become one of the leaders in the SNP genotyping market, due to the proprietary BeadArray platform. The technological success has brought in business. Illumina reported $97.5 million in the third quarter of 2007, an 82% increase from the third quarter of 2006 and a 15% increase from the second quarter of 2007. The company has reported 25 consecutive quarters of revenue growth [280]. Financial trends are shown in Figure 6.8.

Figure 6.8: Profits and revenues of Illumina (1999 – 2007). All financial data obtained from the Securities and Exchange Commission’s (SEC) EDGAR on-line database (http://www.sec.gov/edgar.shtml).

6.4.4 Illumina spawning – Prognosys Biosciences

Another output of a company that can indicate a firm’s success is the presence of a “spin-off” or a “spawn”. While “spinning off” is an event defined by divestiture by a
corporation of a division in the form of issuing new shares to create a new, independent company [281], “entrepreneurial spawning” is defined by Gompers, Lerner, and Scharfstein as a process whereby “young firms prepare employees to be entrepreneurs by educating them about the entrepreneurial process and by exposing them to a network of entrepreneurs and venture capitalists” [282]. This might be the case for Prognosys Biosciences, Inc. – a startup company founded by Mark Chee, former VP of Genomics of Illumina. The company aims to commercialize haploinsufficiency profiling¹ and has received four SBIR grants since the company’s inception in 2005².

6.4.5 Patents

The ability for companies to obtain rights to intellectual property dramatically increases the likelihood and the amount of venture capital funding during the initial phases of development. University technology transfer is one way in which companies can obtain intellectual property. Companies can also file patent applications for their own in-house inventions. Illumina currently lists 207 US patents, patent applications, European patents and world patents³. The list is available in Appendix 3.

¹ Haploinsufficiency refers to the phenomenon when a diploid genome only has one functional copy of a gene.

² Data obtained by searching for "Chee, Mark S." on the NIH CRISP Database.

³ All patent information was obtained from the Delphion intellectual property network database by searching “Illumina” as “inventor/assignee”.

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6.4.6 Publications

Publications enhance the two-way knowledge transfer. Industrial publications indicate private research returns to the Science Commons. Illumina has made public a complete bibliography of publications that have been written by Illumina scientists and those researchers around the world who have used Illumina technology to perform genetic analysis. The complete list is available in Appendix 4.

6.4.7 Collaborations – Networks

Illumina’s core operations have been significantly enhanced by the company’s collaborations with academic and industrial institutions. The company has taken an active role in working with top research institutions to contribute to large-scale scientific research projects. Some of these collaborations have resulted in technologies licensed exclusively to Illumina. For example, as Illumina moves into the molecular diagnostics market, it has partnered with international companies, such as ReaMatrix, PharmacoDesign and deCODE, to develop profiling panels based on their specific populations [see Chapter 5 for more information]. ReaMatrix and PharmacoDesign, in particular, represent two companies seeking to develop diagnostic products suited for the Indian and Korean populations, respectively. Illumina has been granted worldwide rights to develop diagnostic products based on the results from the two studies to be sold, with
the exception that the tests cannot be sold within the countries in which the companies are based.

Illumina has expanded its network of influence. Figure 5.6 lists the academic and industrial relationships that Illumina has created. Since Illumina’s inception, the company has developed strives to maintain relationships with institutions and organizations around the globe. One particular project that exemplifies this was the HapMap Project, for which Illumina provided a significant share of the data. See Section 6.5.3. for more information.

6.5 High-throughput SNP genotyping technology

The following section will analyze the impact that Illumina has made on high-throughput SNP genotyping research. It will analyze the publication citations from the core papers describing the BeadArray technology in its context of the GoldenGate and Infinium genotyping assays. It will also review the characteristics of the publications citing the core paper. This section will also examine how use Illumina technology is being funded by the US government. Finally, this section will examine Illumina’s impact on the HapMap Project, as the Project’s central tenet was to understand genetic variation among populations.
6.5.1 Publications describing high-throughput SNP genotyping technology by Illumina

To investigate the impact that the Illumina publications have had on academic research, the seminal papers from Illumina scientists describing the BeadArray technology for GoldenGate and Infinium high-throughput SNP genotyping analysis have been further analyzed below.

Illumina cites Steemers et al. (2006) as its core publication for its Infinium whole-genome genotyping assay using single-base extension [283]; Fan et al. (2006) as its core publication describing the GoldenGate technology [69]; and, Gunderson et al. (2004) as its core publication describing its proprietary decoding technology [139]. In addition to these papers, we will also examine Gunderson et al. (2005) and Steemers et al. (2000) for their citation trends [148, 277]. Article citation trends listed in Table 6.7.
Table 6.7: Five articles describing core Illumina technology for high-throughput SNP genotyping. Information regarding patent and publication citations obtained from SCOPUS database.

<table>
<thead>
<tr>
<th>Publication title</th>
<th>Journal</th>
<th># publication citations</th>
<th># patent citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steemers <em>et al.</em> (2000) &quot;Screening unlabeled DNA targets with randomly ordered fiber-optic gene arrays&quot;</td>
<td>Nat Biotech</td>
<td>120</td>
<td>60</td>
</tr>
<tr>
<td>Gunderson <em>et al.</em> (2004) &quot;Decoding Randomly Ordered DNA Arrays&quot;</td>
<td>Genome Research</td>
<td>65</td>
<td>16</td>
</tr>
<tr>
<td>Gunderson <em>et al.</em> (2005) &quot;A genome-wide scalable SNP genotyping assay using microarray technology&quot;</td>
<td>Nat Genetics</td>
<td>76</td>
<td>7</td>
</tr>
<tr>
<td>Fan <em>et al.</em> (2006) &quot;Highly parallel genomic assays&quot;</td>
<td>Nat Rev Genetics</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>Steemers <em>et al.</em> (2006) &quot;Whole-genome genotyping with the single-base extension assay&quot;</td>
<td>Nat Methods</td>
<td>30</td>
<td>2</td>
</tr>
</tbody>
</table>

6.5.1.1 Shift from Analytical Chemistry to Genetics & Heredity

While Illumina is listed as one of the top institutions to cite the key Illumina papers, the characteristics of citing institutions vary widely by location and between academic and industrial settings (data not shown). Additionally, the classification of the citing publications varies; “Genetics & Heredity” and “Biochemical Research Methods” compete for the top source type but many other fields of research and development cite the key papers. This trend towards biomedical uses contrasts with the classification types of the papers that cited Walt’s seminal paper. Walt’s work was highly cited by publications within the analytical chemistry field more often than biomedical-based articles. This shift in the types of articles citing the bead-based sensor technology is
exemplified by in the comparison between one of Walt’s highly cited publications (Steemers et al. (2000)) and the publication describing the Infinium assay (Gunderson et al. (2005)) [Figures 6.9 and 6.10]⁴.

This trend suggests that by David Walt enabling John Stuelpnagel to identify a market for his technology, Stuelpnagel essentially created a demand for the bead-based sensors within the field of genetics and genomics. Furthermore, the fields that are citing the bead-based sensor technology have moved from analytical chemistry to a diverse array of categories, especially in medicine and genomics.

⁴ All information regarding citation trends have been taken from the ISI Web of Science database. Cited references can be analyzed based on citing institution, source title, subject category, etc.
Figure 6.9: Subject categories of articles citing Steemers et al. (2000). This article represents the earliest design of the BeadArray technology, while it was still being developed by Walt. The article serves as a “landmark” article to understand how research based on the BeadArray technology has shifted from analytical chemistry-based studies to those related to genetics and heredity.
Figure 6.10: Subject categories for articles citing Gunderson et al. (2005). This particular article is the first to describe whole-genome genotyping (WGG) using the Infinium assay developed by Illumina. The citations represent the variety of fields using WGG to conduct research.

6.5.1.2 Institutions citing core Illumina publications

The five cited publications have been further analyzed to reveal the characteristics of the citing institutions and the results are displayed in Table 6.8.
Table 6.8: Institutions citing “core” Illumina publications. Institutions having two or more citations for the five selected articles citing core Illumina technology are listed below. The list of citing institutions was obtained from the ISI Web of Science database.

<table>
<thead>
<tr>
<th>Institution</th>
<th>Total Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ILLUMINA INC</td>
<td>30</td>
</tr>
<tr>
<td>TUFTS UNIV</td>
<td>15</td>
</tr>
<tr>
<td>HARVARD UNIV</td>
<td>11</td>
</tr>
<tr>
<td>UNIV OXFORD</td>
<td>10</td>
</tr>
<tr>
<td>UNIV PENN</td>
<td>9</td>
</tr>
<tr>
<td>JOHNS HOPKINS UNIV</td>
<td>8</td>
</tr>
<tr>
<td>STANFORD UNIV</td>
<td>8</td>
</tr>
<tr>
<td>CHILDRENS HOSP</td>
<td></td>
</tr>
<tr>
<td>PHILADELPHIA</td>
<td>7</td>
</tr>
<tr>
<td>UNIV FLORIDA</td>
<td>7</td>
</tr>
<tr>
<td>UNIV MICHIGAN</td>
<td>7</td>
</tr>
<tr>
<td>UNIV TEXAS</td>
<td>7</td>
</tr>
<tr>
<td>ALBANOVA UNIV CTR</td>
<td>6</td>
</tr>
<tr>
<td>MASSACHUSETTS GEN HOSP</td>
<td>6</td>
</tr>
<tr>
<td>MIT</td>
<td>5</td>
</tr>
<tr>
<td>NATL UNIV SINGAPORE</td>
<td>5</td>
</tr>
<tr>
<td>PENN STATE UNIV</td>
<td>5</td>
</tr>
<tr>
<td>UNIV HEIDELBERG</td>
<td>5</td>
</tr>
<tr>
<td>UNIV TORONTO</td>
<td>5</td>
</tr>
<tr>
<td>BRIGHAM &amp; WOMENS HOSP</td>
<td>4</td>
</tr>
<tr>
<td>UNIV CALIF LOS ANGELES</td>
<td>4</td>
</tr>
<tr>
<td>UNIV LONDON</td>
<td>4</td>
</tr>
<tr>
<td>UNIV N CAROLINA</td>
<td>4</td>
</tr>
<tr>
<td>AFFYMETRIX INC</td>
<td>3</td>
</tr>
<tr>
<td>CTR NATL GENOTYPAGE</td>
<td>3</td>
</tr>
<tr>
<td>DEUTSCH</td>
<td></td>
</tr>
<tr>
<td>KREBSFORSCHUNGSZENTRUM</td>
<td>3</td>
</tr>
<tr>
<td>HUNAN UNIV</td>
<td>3</td>
</tr>
<tr>
<td>INDIANA UNIV</td>
<td>3</td>
</tr>
<tr>
<td>NHGRI</td>
<td>3</td>
</tr>
<tr>
<td>UNIV ALBERTA</td>
<td>3</td>
</tr>
<tr>
<td>UNIV BIELEFELD</td>
<td>3</td>
</tr>
<tr>
<td>BOSTON UNIV</td>
<td>2</td>
</tr>
<tr>
<td>BOYCE THOMPSON INST PLANT RES</td>
<td>2</td>
</tr>
<tr>
<td>CHINESE UNIV HONG KONG</td>
<td>2</td>
</tr>
<tr>
<td>CNRS</td>
<td>2</td>
</tr>
<tr>
<td>COLUMBIA UNIV</td>
<td>2</td>
</tr>
<tr>
<td>CORNELL UNIV</td>
<td>2</td>
</tr>
<tr>
<td>CTR STAT GENET</td>
<td>2</td>
</tr>
<tr>
<td>ECOLE NORMALE SUPER</td>
<td>2</td>
</tr>
<tr>
<td>EMORY UNIV</td>
<td>2</td>
</tr>
<tr>
<td>GEORGIA INST TECHNOL</td>
<td>2</td>
</tr>
<tr>
<td>JAPAN SCI &amp; TECHNOL AGCY</td>
<td>2</td>
</tr>
<tr>
<td>KAROLINSKA INST</td>
<td>2</td>
</tr>
<tr>
<td>MCGILL UNIV</td>
<td>2</td>
</tr>
<tr>
<td>NATL PUBL HLTH INST</td>
<td>2</td>
</tr>
<tr>
<td>NORTHWESTERN UNIV</td>
<td>2</td>
</tr>
<tr>
<td>POHANG UNIV SCI &amp; TECHNOL</td>
<td>2</td>
</tr>
<tr>
<td>PROGNOSYS BIOSCI INC</td>
<td>2</td>
</tr>
<tr>
<td>RIKEN</td>
<td>2</td>
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<tr>
<td>S OSTRO bothnia CENT HOSP</td>
<td>2</td>
</tr>
<tr>
<td>SE UNIV</td>
<td>2</td>
</tr>
<tr>
<td>SOUTHEAST UNIV</td>
<td>2</td>
</tr>
<tr>
<td>UNIV CALIF DAVIS</td>
<td>2</td>
</tr>
<tr>
<td>UNIV CALIF IRVINE</td>
<td>2</td>
</tr>
<tr>
<td>UNIV CALIF SAN DIEGO</td>
<td>2</td>
</tr>
<tr>
<td>UNIV CALIF SAN FRANCISCO</td>
<td>2</td>
</tr>
<tr>
<td>UNIV HELSINKI</td>
<td>2</td>
</tr>
<tr>
<td>UNIV LONDON IMPERIAL COLL SCI TECHNOL &amp; MED</td>
<td>2</td>
</tr>
<tr>
<td>UNIV OTAGO</td>
<td>2</td>
</tr>
<tr>
<td>UNIV QUEENSLAND</td>
<td>2</td>
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<tr>
<td>UNIV ROCHESTER</td>
<td>2</td>
</tr>
<tr>
<td>UNIV SO CALIF</td>
<td>2</td>
</tr>
<tr>
<td>UNIV TWENTE</td>
<td>2</td>
</tr>
<tr>
<td>UNIV WASHINGTON</td>
<td>2</td>
</tr>
<tr>
<td>USN</td>
<td>2</td>
</tr>
<tr>
<td>VERIDEX LLC</td>
<td>2</td>
</tr>
</tbody>
</table>

252
Table 6.9 demonstrated that Illumina technology has been cited by a diverse group of national and international, public and private institutions.

Another way to evaluate the core Illumina publications (as a proxy for the core Illumina technology) is to look at the characteristics of the citing publications. This may be an indirect approach since the journals in which articles are published are dependent on many factors. However, it is expected that because publications undergo a rigorous peer-review process, only the best articles with sound research methods and results are accepted into the top academic journals. These academic journals are rated by impact factors. Journals bearing higher impact factors are those in which articles are highly cited by other publications, and, thus, are considered “of higher impact”.

Using the five articles describing core Illumina technology above, I summed citations of by journal source. Impact factors were obtained from the ISI Web of Science database. The top journals in which articles citing core Illumina technology for high-throughput genotyping are published are listed in Table 6.9. Generally, articles citing Illumina technology (and, presumably using Illumina technology) are cited in high-impact journals.
Table 6.9: Journals with articles citing core Illumina high-throughput genotyping articles. All data obtained from ISI Web of Science database.

<table>
<thead>
<tr>
<th>Journal</th>
<th>Number of articles</th>
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6.5.2 Output evaluation: Grants based on Illumina technology for genotyping

The products that Illumina sells are tools for genomic analysis. The company’s primary consumers are industrial, academic and nonprofit institutions, which use these products to perform scientific experiments. The results of these experiments are then reported in publications and used as primary data to obtain sources of funding. Although the abilities to publish a scientific article or to obtain a grant are based on numerous factors, the experimental data are dependent on the caliber of the research instruments used. Another way to evaluate the impact of the overall Illumina product line (of which the core technology is the bead-based array developed by David Walt) is to examine the grants that have been awarded to these institutions for genotyping studies based on Illumina technology.

Federal grants are awarded for many purposes. The National Institutes of Health, for instance, is responsible for funding much of the biomedical research that occurs in for-profit and non-profit institutions across the country. Grants are funded after passing through a stringent review process, where committees carefully evaluate the aims, preliminary data, and research design and methods of each proposal.

Research designs are primarily structured around association- or genome-wide-based studies. Candidate genes for a trait are tested within the subject population for association
between a genetic variant and a phenotype. Genome-wide association studies are constructed to scan the genomes of case and control populations for sequence differences that may account for a trait. Case-control studies have emerged as a means to take population substratification into account. The high-throughput scanning technologies have enabled the rapid analysis of the large populations needed to run these studies. As evidenced by the institutions being funded for Illumina-based genotyping studies, Illumina platforms have been particularly effective in delivering high-quality SNP genotyping results. Tables 6.10 and 6.11 present the grants funded by the NIH for purchases of or studies using Illumina high-throughput genotyping products. The lists presented may not accurately represent all studies conducted using Illumina platforms, as it is only based on NIH grants and those grants that mention “Illumina” by name in the abstract. However, the tables demonstrate that top universities around the nation (and the globe) are being funded for conducting genotyping studies with Illumina products. This is an indication of the value of the Illumina products and services.
Table 6.10: Federal grants to institutions for using Illumina technology – based on information obtained from the RaDiUS database. Information regarding grants obtained by searching “Illumina” as keyword on RaDiUS database.

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<th>End Date</th>
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Table 6.11: Grants funded by the NIH for studies conducting genotyping studies using Illumina platforms. Data collected from the CRISP database by searching for “Illumina” in keywords. Data filtered for grants for Illumina researchers and grants not specifically using Illumina panels/assays.

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<td>GULCHER, JEFFREY</td>
<td>DECODE GENETICS, INC.</td>
<td>Genome-wide association study of MI in Caucasians</td>
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<td>HAIMAN, CHRISTOPHER</td>
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<td>A Comprehensive Analysis of Genetic Variation in DNA Repair Genes in Relation to</td>
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<td>UNIVERSITY OF IOWA</td>
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<td>Fine mapping 8q24 in Familial Bipolar Disorder</td>
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6.5.3 HapMap Project analysis

One important collaboration of which Illumina, Inc. was a part was the International Haplotype Mapping (HapMap) Project. Table 6.12 lists all of the participants from the United States. Table 6.13 lists the participant by country. Importantly, Illumina’s overall contribution to the Project was significant, based on the global academic institutions that employed the Illumina platforms to perform the majority of their research.

Table 6.12: United States HapMap participants. Data collected from the HapMap website (http://www.hapmap.org).

<table>
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<tr>
<th>Principal Investigator</th>
<th>Affiliation</th>
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<td>Arnold Oliphant</td>
<td>Illumina</td>
<td>Genotyping - Illumina BeadArray</td>
<td>NHGRI</td>
<td>U54HG002753</td>
<td>Highly Parallel SNP Genotyping for a Haplotype Map</td>
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<td>David Atshuler</td>
<td>Broad Institute of Harvard and MIT</td>
<td>Genotyping - Sequenom MassExtend, Illumina BeadArray</td>
<td>NHGRI</td>
<td>U54HG002750</td>
<td>Design/Production of Haplotype Map of the Human Genome</td>
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<td>Richard Gibbs</td>
<td>Baylor College of Medicine with ParAllele Bioscience</td>
<td>Genotyping - ParAllele</td>
<td>NHGRI</td>
<td>U54HG002755</td>
<td>A Human Haplotype Map by a Highly Multiplexed Method</td>
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<td>Pui-Yan Kwok</td>
<td>University of California, San Francisco, Washington University in St. Louis</td>
<td>Genotyping - PerkinElmer AcycloPrime-FP</td>
<td>NHGRI</td>
<td>R01HG001720</td>
<td>Molecular Haplotyping By Single Molecule Detection</td>
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<tr>
<td>Kelly Frazer</td>
<td>Perlegen Sciences</td>
<td>Genotyping- High Density Oligonucleotide Array</td>
<td>NHGRI</td>
<td>U54HG003642</td>
<td>Large-Scale Low-Cost Genotyping for the Haplotype Map</td>
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Table 6.13: Total HapMap participants by country. Data collected from HapMap website.

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<tr>
<th>Country</th>
<th>PI Name</th>
<th>Institution</th>
<th>Role</th>
<th>Percent Genome</th>
<th>Chromosomes</th>
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<tr>
<td>Japan</td>
<td>Yusuke Nakamura</td>
<td>RIKEN and University of Tokyo</td>
<td>Genotyping -Third Wave</td>
<td>24.30%</td>
<td>5, 11, 14, 15, 16, 17, 19</td>
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<td>United Kingdom</td>
<td>David Bentley</td>
<td>Wellcome Trust Sanger Institute</td>
<td>Genotyping -Illumina BeadArray</td>
<td>23.70%</td>
<td>1, 6, 10, 13, 20</td>
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<tr>
<td>Canada</td>
<td>Thomas Hudson</td>
<td>McGill University and Genome Québec Innovation Centre</td>
<td>Genotyping -Illumina BeadArray</td>
<td>10.10%</td>
<td>2, 4p</td>
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<tr>
<td>China</td>
<td>Huanming Yang; Yan Shen</td>
<td>Beijing Genomics Institute (HY); Chinese National Human Genome Center at Beijing (YS)</td>
<td>Genotyping - Sequenom MassExtend, Illumina BeadArray</td>
<td>5.90%</td>
<td>3q, 8p, 20 Mb of 3p</td>
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<tr>
<td>China</td>
<td>Lap-Chee Tsui; Paul Kwong-Hang Tam &amp; William Wai-Nam Mak; Jeffrey Tze-Fei Wong; Mary Miu Yee Waye</td>
<td>Hong Kong HapMap Group (LCT); The University of Hong Kong (PKHT &amp; WWNM); Hong Kong University of Science and Technology (JTFW); The Chinese University of Hong Kong (MMYW)</td>
<td>Genotyping -Sequenom MassExtend</td>
<td>2.50%</td>
<td>70 Mb of 3p</td>
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<tr>
<td>China</td>
<td>Wei Huang</td>
<td>Chinese National Human Genome Center at Shanghai</td>
<td>Genotyping -Illumina BeadArray</td>
<td>1.10%</td>
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<td>United States</td>
<td>Arnold Oliphant</td>
<td>Illumina</td>
<td>Genotyping -Illumina BeadArray</td>
<td>16.10%</td>
<td>8q, 9, 18q, 22, X</td>
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<tr>
<td>United States</td>
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<td>Broad Institute of Harvard and MIT</td>
<td>Genotyping -Sequenom MassExtend, Illumina BeadArray</td>
<td>9.70%</td>
<td>4q, 7q, 18p, Y</td>
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<tr>
<td>United States</td>
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<td>Genotyping -ParAllele</td>
<td>4.60%</td>
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<td>United States</td>
<td>Pui-Yan Kwok</td>
<td>University of California, San Francisco with Washington University in St. Louis</td>
<td>Genotyping - PerkinElmer AcycloPrime-FP</td>
<td>2.00%</td>
<td>7p</td>
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<tr>
<td>United States</td>
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<td>Perlegen Sciences</td>
<td>Genotyping- High Density Oligonucleotide Array</td>
<td>Genome-Wide, 2.25 M SNPs</td>
<td>All Chromosomes</td>
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6.6. Future Research

The business model chosen by Stuelpnagel presents an interesting situation that goes beyond the scope of this paper. University inventions are often funded by government grants and licensed to industrial partners to be developed into commercial products to be marketed to private or public customers. What I find here in the case of Illumina, is a company that develops tools to be used for genomic research. The products are both the inputs for future research and outputs of post academic innovation. The core Illumina technology was developed solely from government funds of academic research. It was exclusively licensed to a startup company. This startup then used government funds to develop and purchase add-on technologies. Its products were then marketed to academic and non-profit institutions. These institutions have used the platforms to generate data that could spur more academic innovation. The core technologies are expected to have broad industrial and other commercial uses.

Illumina was born of academic analytical chemistry and engineering, produced products widely used in academic research and expected to be introduced in industry R&D. In their need, academic research is the “opening market” for what is expected to become a much larger industrial sector. A study investigating the overall benefit that this cyclical industrial-academic relationship has had on the field of health care would certainly provide an added dimension of impact evaluation to this case history.
7 Conclusion

The development of the BeadArray technology that lies at the core of Illumia’s high-throughput SNP genotyping platforms provides an ideal case study for technology transfer. We hope to find similar histories that demonstrate the best practices for technology transfer.

The success of this story is marked by several key events/issues:

The unique qualities of the bead-based fiber optic sensor array. The invention was particularly unique in its design. Although it was not initially designed to detect genetic variation, the flexibility and the sensitivity of the sensor overcame the major issues that other SNP genotyping techniques contained.

David Walt’s understanding of intellectual property disclosure. Walt’s prior experiences with IP – and lessons learned for those that did not turn out as expected – were critical factors in how and when he chose to disclose his bead-based sensor invention. Had he not filed a patent prior to his talk at the Pittsburgh Convention, it is certain that the story would have turned out differently. Furthermore, Walt’s prestigious faculty position and distinguished career, which he held prior to his invention, exposed him to networks that he might not have had access to otherwise, such as, had he been at another institution or had he been a less successful academic researcher.
The appropriate due diligence on the part of MBRI and Tufts University. The partnership between MBRI and Tufts University was successful in identifying a faculty invention that held tremendous potential to become a core technology for a company. Rather than licensing the technology to an already existing company, MBRI/Tufts opted to grant a startup exclusive rights to the patents on the technology.

The financing by venture capital firms. The exclusive license was a boon for the startup in securing venture capital funding. Had Stuelpnagel not been able to convince MBRI/Tufts to grant to exclusive license on the patent portfolio, it is almost certain that the venture capital funding raised would have paled in comparison to what was raised with the exclusive license.

The proper market identification by John Stuelpnagel. Determining that the genotyping market would be the best place to introduce the technology was a key factor to Illumina’s initial success. Although the technology had many applications, the genotyping market needed a technology to overcome previous problems of accuracy and high-througput.

Recruitment of a top scientific advisory board. Attracting qualified individuals to serve on the scientific advisory board was one way that Illumina gained confidence as a
company in the eyes of scientists who would be using the technology and potential investors who would infuse money into the growing business.

**The awarding of federal grants for small businesses.** Illumina COO Stuelpnagel said that the federal grants for projects were valuable sources of money for the startup to develop its platforms. Additionally, the influx of federal funds to Illumina legitimized the company’s technology for potential customers and investors.

**The acquisition of other technologies.** Although Illumina’s core technology came from David Walt’s laboratory at Tufts University, additional technologies were acquired to improve the quality and throughput of the platforms. The ability to build upon the success of the initial technology through acquisitions of key technologies was important to secure significant venture capital funding.

**The transfer of human intellectual capital from Tufts to Illumina.** The technology transfer process of the bead-based fiber optic array technology from Tufts to Illumina was aided by the parallel movement of researchers from working as postdocs in Walt’s laboratory to working as staff scientists at Illumina. These individuals were familiar with the technology and could offer suggestions for potential acquisitions of complementary technologies.
The appropriate timing for the initial public offering. The decision to perform an initial public offering at the height of the biotechnology ‘bubble’ in the summer of 2000 helped Illumina earn a tremendous amount of money. Had the IPO been offered later than that time period, Illumina might not have profited as much.

Collaboration with academic and industrial institutions. To ensure that the company constantly improves its products and services to best meet the needs of its customers, specifically, the scientific community, Illumina has used academic researchers as beta-testers and collaborators on most of its projects. These industry-academic collaborations are mutually beneficial and have gained financial success for Illumina and scientific success for the impact of the results of the various projects.

The impact of the actions taken early in the disclosure and licensing of the core technology is significant: the BeadArray technology on which Illumina was built has revolutionized high-throughput SNP genotyping. Results from studies using the technology are promising a bright future for the field of personalized medicine.
Appendix 1: David Walt publications (1981 – 2007) by year in reverse chronological order. Publication (and citations) information obtained from SCOPUS and ISI Web of Science databases.

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<th>Title</th>
<th>Source title</th>
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<tr>
<td>2007</td>
<td>Kuang, Y., Walt, D.R.</td>
<td>Detecting oxygen consumption in the proximity of Saccharomyces cerevisiae cells using self-assembled fluorescent nanosensors</td>
<td>Biotechnology and Bioengineering</td>
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<td>2007</td>
<td>DragÅ,y Whitaker, R., Walt, D.R.</td>
<td>Fiber-based single cell analysis of reporter gene expression in yeast two-hybrid systems</td>
<td>Analytical Biochemistry</td>
<td></td>
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<td>2006</td>
<td>Rissin, D.M., Walt, D.R.</td>
<td>Duplexed sandwich immunoassays on a fiber-optic microarray</td>
<td>Analytica Chimica Acta</td>
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<tr>
<td>2006</td>
<td>Song, L., Ahn, S., Walt, D.R.</td>
<td>Fiber-optic microsphere-based arrays for multiplexed biological warfare agent detection</td>
<td>Analytical Chemistry</td>
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<td>2005</td>
<td>Monk, D.J., Ueberfeld, J., Walt, D.R.</td>
<td>Progress toward the determination of Sr2+ in highly basic solutions using imaging optical fiber sensor arrays</td>
<td>Journal of Materials Chemistry</td>
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<td>2005</td>
<td>Kuang, Y., Walt, D.R.</td>
<td>Monitoring &quot;promiscuous&quot; drug effects on single cells of multiple cell types</td>
<td>Analytical Biochemistry</td>
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<td>2005</td>
<td>Bencic-Nagale, S., Walt, D.R.</td>
<td>Extending the longevity of fluorescence-based sensor arrays using adaptive exposure</td>
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<td>7</td>
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<td>2005</td>
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<td>Detecting biological warfare agents</td>
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<td>2005</td>
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<td>Fabrication and optical characterization of imaging fiber-based nanoarrays</td>
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<td>2005</td>
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<td>Development of a microfluidic platform with an optical imaging microarray capable of attomolar target DNA detection</td>
<td>Analytical Chemistry</td>
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<td>2005</td>
<td>Dicesare, C., Biran, I., Walt, D.R.</td>
<td>Individual cell migration analysis using fiber-optic bundles</td>
<td>Analytical and Bioanalytical Chemistry</td>
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<td>2005</td>
<td>Shepard, J.R.E., Danin-Poleg, Y., Kashi, Y., Walt, D.R.</td>
<td>Array-based binary analysis for bacterial typing</td>
<td>Analytical Chemistry</td>
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<td>2004</td>
<td>Kuang, Y., Biran, I., Walt, D.R.</td>
<td>Simultaneously monitoring gene expression kinetics and genetic noise in single cells by optical well arrays</td>
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<td>2004</td>
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<td>Fabrication of gold microtubes and microwires in high aspect ratio capillary arrays</td>
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<td>2004</td>
<td>Bencic, S., Walt, D.R.</td>
<td>Increasing lifetimes of fiber-optic sensor arrays for chemical warfare detection</td>
<td>Proceedings of SPIE - The International Society for Optical Engineering</td>
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<td>2003</td>
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<td>Information coding in artificial olfaction multisensor arrays</td>
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<td>2003</td>
<td>Stitzel, S.E., Stein, D.R., Walt, D.R.</td>
<td>Enhancing vapor sensor discrimination by mimicking a canine nasal cavity flow environment</td>
<td>Journal of the American Chemical Society</td>
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<td>2002</td>
<td>Schauer, C.L., Stitzel, S.E., Walt, D.R.</td>
<td>Cross-reactive optical sensing arrays</td>
<td>ACS Symposium Series</td>
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<td>2002</td>
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### Appendix 2: David Walt patent portfolio.

Patents where David Walt is listed as inventor or co-inventor. Forward patent citations are listed and adjusted for forward references by David Walt (Tufts University) or Illumina, Inc. All data obtained from the Delphion Intellectual Property database.

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Appendix 3: The Exclusive License from Tufts University to Illumina. Source is the Illumina 10-Q from 2007 (3 May 2007).

EX-10.5 2 a29658exv10w5.htm EXHIBIT 10.5
Exhibit 10.5

LICENSE AGREEMENT

Effective as of May 6, 1998 (the “Effective Date”), TUFTS UNIVERSITY, a body having corporate powers under the laws of the State of Massachusetts (“TUFTS”), and ILLUMINA, Inc., a California corporation having a principal place of business at 2187 Newcastle Avenue, Suite 101, Cardiff, California 92007, (“LICENSEE”) enter into this license agreement (“Agreement”) and thereby agree as follows:

1 BACKGROUND

1.1 TUFTS is the owner of the patents and patent applications listed in Exhibit 1 and any Licensed Patents, as hereinafter defined, which may issue therefrom.
1.2 TUFTS desires to have its technology developed and marketed in order that products resulting therefrom may be available for public use and benefit. 
1.3 LICENSEE desires a worldwide, exclusive license, including the right to sublicense, to develop, market and sell products under the Licensed Patents and Know How (collectively, “Exclusive Technology”) in all fields.

2 DEFINITIONS

2.1 “Affiliate” means any corporation or other entity that is directly or indirectly controlling, controlled by or under common control with LICENSEE. For the purpose of this definition, “control” shall mean the direct or indirect beneficial ownership of at least fifty percent (50%) in the income or stock of such corporation or business.
2.2 “Exclusive” means that, subject to the provision in Section 3.3, TUFTS shall not grant further licenses to the Licensed Patents.
2.3 “Know-How” means trade secrets, know-how, data and other information (whether or not patentable or qualifying as a trade secret) relating to the field of use relating to Licensed Patents discovered or developed at Tufts, or revealed to LICENSEE pursuant to the research agreement referred to in Section 3 of the Master Agreement of even date herewith (“Research Agreement”) between Tufts and LICENSEE. Know-How shall not include Licensed Patents.
2.4 “Licensed Product” means any product, the manufacture or sale of which is within a Valid Claim within the Licensed Patents in the country of manufacture or sale.

2.5 “Licensed Patents” means (i) the U.S. and foreign patents and patent applications listed on Exhibit 1 hereto, (ii) all U.S. or foreign patent applications filed after the Effective Date owned by TUFTS or which TUFTS has the right to license which claim one or more inventions which would be dominated by any patent issuing on a patent application within the Licensed Patents pending as of the Effective Date (or a division, or continuation in whole or part of such a pending application), (iii) all divisions, and continuations in whole or part of any of the preceding, (iv) all foreign patent applications corresponding to or claiming priority from any of the preceding, and (v) all U.S. and foreign patents issuing on any of the preceding, including patents of addition, reexaminations, and reissues.
2.6 “Licensed Territory” means worldwide.
2.7 “LICENSEE” shall mean Illumina, Inc. and its Affiliates.
2.8 “Net Sales” means the gross revenue actually received by LICENSEE from sales of Licensed Products, less the following items, but only insofar as they are included in such gross revenue and are separately stated on the invoice:
   (a) Import, export, value-added, excise and sales taxes, and custom duties, all to the extent separately identified on the invoice;
   (b) Cost of insurance, packing, and transportation from the place of manufacture to the customer’s premises;
   (c) normal and customary rebates, and cash and trade discounts, actually taken; and
   (d) Credit for returns, allowances, or trades actually allowed.
2.9 “Valid Claim” means a claim of (i) an issued, unexpired patent which has not been held unenforceable or invalid by a court or other governmental entity of competent jurisdiction, and which has not been disclaimed, or withdrawn or found invalid or unenforceable in a reissue application or re-examination proceeding; or (ii) a patent application, provided that not more than five (5) years have elapsed from the date the claim takes priority for filing purposes.
3 GRANT
3.1 Subject to Public Law 96-517 and Public Law 98-620, TUFTS hereby grants, to the extent that it lawfully may, to LICENSEE and LICENSEE hereby accepts an exclusive license under the Exclusive Technology to make, have made, import, have imported, use, lease, sell and offer for sale, have sold and otherwise commercialize and exploit Licensed Products, and to practice any method, process, or procedure within the Exclusive Technology, in the Licensed Territory.
3.2 Said license is Exclusive, including the right to sublicense pursuant to Section 12, in the Licensed Territory for a term commencing as of the Effective Date, and ending upon expiration of the last to expire of Licensed Patents.
3.3 LICENSEE agrees that TUFTS shall have the right to practice the Exclusive Technology both on its own and/or in collaboration with third party academic or not-for-profit research institutions, solely for non-commercial purposes, and not for sale, license, or other distribution.
4 DILIGENCE
4.1 LICENSEE will use reasonable best efforts to diligently and continuously commercialize the Exclusive Technology. To support the commercialization of the Exclusive Technology, LICENSEE will raise $500,000 in equity financing from third parties during the first year after the Effective Date and use its best efforts to raise $2,000,000 in total financing (including but not limited to equity or debt financing, government grant funding, sponsored research and development funding, etc.) (“First Financing”) during the second year after the Effective Date. If LICENSEE fails to meet any one of the foregoing milestones within the time specified, TUFTS shall have the right to terminate the license granted hereunder, provided that such action by TUFTS is consistent with a determination of the arbitrators pursuant to Section 15 hereof that LICENSEE has failed to exercise due diligence in the commercialization of the Exclusive Technology pursuant to its obligations under this Section 4.1.
4.2 LICENSEE shall further use its best efforts to bring one or more Licensed Products to market through a thorough, vigorous and diligent exploitation of Licensed Patents and to continue thereafter active, diligent marketing of more Licensed Products throughout the life of this Agreement.
4.3 In addition LICENSEE shall adhere to the following milestones:
   (a) LICENSEE shall deliver to TUFTS on or before the first anniversary of this Agreement an operating plan showing the amount of money, number and kind
of personnel, and time budgeted and planned for each phase of development of the Licensed Products and shall provide similar reports to TUFTS on or before each subsequent anniversary. TUFTS agrees to keep this operating plan confidential.

(b) The following expenditures shall be made by the LICENSEE, its Affiliates or its sublicensees on a calendar-year basis in order to develop and commercialize Licensed Products:

1999 – an expenditure of $1,000,000
2000 – an expenditure of $1,500,000
2001 – an expenditure of $2,000,000
2002 – an expenditure of $2,500,000

(c) Of the expenditures listed in Section 4.3(b), the following minimum expenditures shall be made by LICENSEE, its Affiliates or its sublicensees on a calendar-year basis in order to develop and commercialize a product dominated by US Patent Number 5,512,490:

1999 – an expenditure of $250,000
2000 – an expenditure of $375,000
2001 – an expenditure of $500,000
2002 – an expenditure of $625,000

(d) LICENSEE shall permit an in-plant inspection by TUFTS on or before July 1, 1999 and thereafter permit in-plant inspections by TUFTS at regular intervals with at least six (6) months between inspections.

(e) LICENSEE shall provide TUFTS with an annual report of research and development expenditures required under this Section 4.3.

4.4 If LICENSEE fails to meet any of the milestones in this Section 4, and the default has not been remedied within ninety (90) days after the date of notice in writing of such default by TUFTS, TUFTS shall have the right to change the license granted hereunder to a non-exclusive license.

5 PAYMENTS

5.1 LICENSEE shall pay to TUFTS royalties equal to three percent (3.0%) of the Net Sales received by LICENSEE from the sale of Licensed Products. In the event that a Licensed Product under this Agreement is sold in a combination product containing one or more other active ingredients or components which are or could be separately available on a commercial basis, then Net Sales on the combination product shall be calculated as follows: By multiplying the net selling price of the combination product by the fraction A/A+B, where A is the gross selling price, during the royalty-paying period being considered, of the Licensed Product sold separately, and B is the gross selling price, during the royalty period in question, of the other active ingredients or components sold separately.

5.2 In the event that LICENSEE is required to take a license from any third party in order to commercialize any Licensed Product, and LICENSEE must make royalty payments to such third party (“Third Party Royalty Payment”), the royalties payable to TUFTS pursuant to Section 5.1 above shall be reduced by an amount equal to fifty percent (50%) of the Third Party Royalty Payment, provided, however, that such reduction shall not reduce the royalty payment owed to Tufts.
in any single year to an amount which is less than fifty per cent (50%) of that which would have been due to TUFTS in the absence of Third Party Royalty Payments.

5.3 LICENSEE shall pay TUFTS a sublicensing fee (the “Sublicensing Fee”) equal to twenty five percent (25%) of the net revenue received from sublicensing of Licensed Patents and Licensed Products covered by one or more valid claims of the Licensed Patents in the country in which such Product is sold. The Sublicensing Fee shall be based upon the amount actually paid to LICENSEE by a sublicensee, including fees, royalties and milestone payments, provided that the Sublicensing Fee shall not include research and development support payments, payments in compensation for the grant of rights to any other intellectual property of LICENSEE, or equity or debt financing received by LICENSEE from such sublicensee.

5.4 LICENSEE hereby grants to TUFTS the right to purchase 500,000 shares of LICENSEE’S common stock which represents 10.0% of the founding capitalization (see Exhibit 2), at fair market value as determined by LICENSEE’s Board of Directors as of the date of purchase (such fair market value is currently $0.01 per share) pursuant to a separate stock purchase agreement (“Stock Agreement”).

5.5 The royalty on Net Sales made in currencies other than U.S. Dollars shall be calculated using the appropriate foreign exchange rate for such currency quoted by the Bank of America (San Francisco) foreign exchange desk, on the close of business on the last banking day of each calendar quarter. Royalties and payments to TUFTS shall be made in U.S. Dollars.

5.6 Within thirty (30) days after receipt of a statement from TUFTS, LICENSEE shall reimburse TUFTS for all costs incurred by TUFTS after the Effective Date in connection with the preparation, filing and prosecution of all patent applications and maintenance of Licensed Patents.

5.7 In the event that in any country all of the valid claims within the Licensed Patents which cover a particular Licensed Product are held invalid or unenforceable, then LICENSEE’s obligation to pay royalties on Net Sales with respect to such Licensed Product shall terminate in such country. LICENSEE’s obligation to pay royalties on Net Sales shall terminate on a country-by-country basis upon the expiration of the last to expire of any issued Licensed Patent in each country.

6 ROYALTY REPORTS, PAYMENTS AND ACCOUNTING

6.1 Beginning with the first sale of a Licensed Product, LICENSEE shall make written reports (even if there are no further sales) of royalty payments due, if any, to TUFTS within thirty (30) days after the end of each calendar quarter. This report shall state the number, description, and aggregate Net Sales of Licensed Products during such completed calendar quarter by LICENSEE, its Affiliates and Sublicensees, and resulting calculations of earned royalty payments due TUFTS pursuant to Section 5 for such completed calendar quarter. Each such statement shall be certified by an officer of the LICENSEE as being true, correct and complete. Concurrent with the submission of each such report, LICENSEE shall pay TUFTS any royalties due for the calendar quarter covered by such report.

6.2 LICENSEE agrees to keep and maintain records for a period of three (3) years showing the manufacture, sale, use and other disposition of products sold or otherwise disposed of under the license herein granted. Such records will include sufficient detail to enable the royalties payable hereunder by LICENSEE to be determined. LICENSEE further agrees to permit its books and records to be examined by an independent certified public accountant selected by TUFTS and acceptable to LICENSEE once per calendar year during the term of this Agreement, for the sole purpose of verifying the reports and royalty payments made by LICENSEE. Such examination shall be made at LICENSEE’S place of business during ordinary business hours with at least thirty (30) days prior written notice. The accountant shall
report to TUFTS only whether there has been a royalty underpayment and, if so, the amount thereof. Such examination is to be at the expense of TUFTS except in the event that the results of the audit reveal an underreporting of royalties due TUFTS of five percent (5%) or more, then the audit costs shall be paid by LICENSEE within thirty (30) days of notice by TUFTS to LICENSEE.

7 REPRESENTATIONS AND WARRANTIES

7.1 TUFTS Disclaimer. TUFTS MAKES NO REPRESENTATIONS, EXTENDS NO WARRANTIES OF ANY KIND, EITHER EXPRESS OR IMPLIED (INCLUDING, WITHOUT LIMITATION, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR PURPOSE), AND ASSUMES NO RESPONSIBILITIES WHATSOEVER, WITH RESPECT TO THE LICENSED PATENTS OR KNOW-HOW OR THE USE THEREOF, OR THE MANUFACTURE, POSSESSION, USE, MARKETING, SALE, OR OTHER DISPOSITION BY TUFTS, LICENSEE, OR ANYONE ELSE, OF LICENSED PRODUCT(S) OR ANY OTHER PRODUCTS OF SERVICES (INCLUDING, WITHOUT LIMITATION, PRODUCTS MADE BY TUFTS, AND TUFTS SERVICES, THAT ARE OR WERE FURNISHED TO LICENSEE AT ANY TIME BEFORE, ON, OR AFTER THE DATE HEREOF), EXCEPT ONLY AS EXPRESSLY STATED HEREIN. Without limitation of the foregoing generality, nothing contained herein or in any disclosure of the Licensed Patents or Know-How made by or on behalf of TUFTS shall be construed as extending any representation or warranty with respect to the Licensed Patents or Know-How or Licensed Products or the results to be obtained by the use of the Licensed Patents or Know-How or any Licensed Products, or that anything made, used, or sold by use of the Licensed Patents or Know-How or any part thereof, alone or in combination, will be free from infringement of patents of third parties. TUFTS SHALL NOT BE LIABLE TO LICENSEE, ITS AFFILIATES, ITS SUBLICENSEES, OR ANY OTHER PARTY, REGARDLESS OF THE FORM OR THEORY OF ACTION (WHETHER CONTRACT, TORT, INCLUDING NEGLIGENCE, STRICT LIABILITY, OR OTHERWISE), FOR ANY SPECIAL, INCIDENTAL, CONSEQUENTIAL, PUNITIVE, OR OTHER EXTRAORDINARY DAMAGES ARISING OUT OF OR RELATED TO THIS AGREEMENT, LICENSED PATENTS, THE KNOW-HOW, THE LICENSED PRODUCTS, OR ANY PRODUCTS OR SERVICES FURNISHED OR NOT FURNISHED BY TUFTS, EVEN IF TUFTS HAS BEEN ADVISED OF THE POSSIBILITY THEREOF.

LICENSEE agrees that all warranties, if any, in connection with the sale or other disposition of any Licensed Products (or any products made by TUFTS and furnished at any time to LICENSEE) by LICENSEE, its Affiliates, or its sublicensees will be made by them and will not directly or impliedly obligate TUFTS.

7.2 TUFTS Representations. Notwithstanding the first sentence of Section 7.1, TUFTS:

(a) Warrants to LICENSEE that TUFTS has good title to the Exclusive Technology and any tangible personal property furnished hereunder by TUFTS to LICENSEE, including any quantities of materials similar to the products to be made by LICENSEE as Licensed Products (but TUFTS makes no infringement or other representations or warranties with respect thereto).

(b) Represents that TUFTS is a corporation organized and existing under the laws of the Commonwealth of Massachusetts and has the power and authority to enter into this Agreement and the right to grant all the rights described in this Agreement, including the rights to the Licensed Patents and Know-How described herein.

(c) Represents that TUFTS has taken all necessary action to authorize its execution and delivery of this Agreement by the representatives of TUFTS who carried out such execution and delivery, and to authorize the performance by TUFTS of its obligations hereunder.

(d) Represents that execution and delivery of this Agreement and its performance by TUFTS will not result in any breach or violation of, or constitute a default under, any agreement, instrument, judgment, or order to which TUFTS is a party or by which it is bound.

(e) Represents that TUFTS is not aware of any other intellectual property right owned by TUFTS for which a license is necessary to practice the rights to the Licensed Patents as set forth herein.
7.3 LICENSEE Representations. LICENSEE represents and warrants to TUFTS that:
   (a) LICENSEE is a corporation organized and existing under the laws of California and has the power and authority to enter into this Agreement.
   (b) LICENSEE has taken all necessary action to authorize its execution and delivery of this Agreement by the representatives of LICENSEE who carried out such execution and delivery, and to authorize the performance by LICENSEE of its obligations hereunder.
   (c) Execution and delivery of this Agreement and its Agreement and its performance by LICENSEE will not result in any breach or violation of, or constitute a default.

8 INDEMNITY

8.1 Indemnity. LICENSEE agrees to indemnify and hold harmless TUFTS, its trustees, officers and employees, from all costs, expenses (including reasonable attorneys’ fees), interest, losses, obligations, liabilities, and damages paid or liability for which is incurred by any of said parties (“Losses”), and which arise out of or are in connection with or are for the purpose of avoiding any and all claims, demands, actions, causes of action, suits, appeals, and proceedings (“Claims”), all whether groundless or not, or the settlement thereof, based on any actual or alleged injuries, damages, or liability of any kind whatsoever (including, without limitation, personal injury, death, property damage, breach of warranty, or breach of contract) arising, directly or indirectly, out of any one or more of: any breach of LICENSEE of its representations, warranties, or agreements hereunder; or out of any manufacture, marketing, possession, use, sale or other disposition of Licensed Products or products furnished by TUFTS to LICENSEE in connection herewith or in connection with the Research Agreement (whether same occurs during or after the License or during or after the License Period) by LICENSEE, its Affiliates, its sublicensees, or anyone claiming by, through, or under any of them; or any acquisition, possession, disclosure, or use of the Exclusive Technology or any thereof, by LICENSEE, its Affiliates, its sublicensees, or anyone claiming by, through, or under any of them; or the presence of LICENSEE’s or its Affiliates’ or its sublicensees’ officers, agents, employees, invitees, or property or any thereof on TUFTS’ premises, provided that the obligations of LICENSEE under this Section 8.1 shall not apply if the Claims and any Losses resulted in whole or in part from the intentional misconduct or gross negligence of TUFTS or any other party indemnified under this Section.

8.2 Defense; Settlement. LICENSEE shall defend and control negotiation of settlement of any Claim, as defined in Section 8.1. TUFTS agrees to cooperate fully in the defense of any Claim and may participate in the defense with counsel of TUFTS’s choosing, such separate counsel to be at TUFTS’s expense unless a conflict of interest exists between LICENSEE and TUFTS with respect to the defense in which case LICENSEE shall pay the reasonable fees and expenses of TUFTS’s separate counsel. Any settlement by which TUFTS would incur any obligation or liability, whether for the payment of money, the taking of any action, the refraining from any action, or otherwise, shall require the advance written consent of TUFTS, which may be withheld in the sole discretion of TUFTS without relieving LICENSEE of any of its indemnification or other obligations hereunder.

8.3 Insurance. Not later than thirty (30) days before the time when LICENSEE, any subsidiary, or any sublicensee of LICENSEE shall use in humans or sell any Licensed Products or any products furnished to LICENSEE by TUFTS at any time (before, on or after the date hereof) in connection herewith or in connection with the Research Agreement, and at all times thereafter until the expiration of all applicable statutes of limitation pertaining to any such use, sale or other disposition of any Licensed Products or the aforesaid products furnished by TUFTS (whether same
occurs or exists before or after the Effective Date), LICENSEE will at LICENSEE’s expense, obtain and maintain in full force and effect, comprehensive general liability insurance, including product liability insurance, protecting TUFTS against all claims, suits, obligations, liabilities and damages, based upon or arising out of actual or alleged bodily injury, personal injury, death, or any other damage to or loss of persons or property, caused by any such use, sale, or other disposition. Such insurance policy or policies shall be issued by companies rated by A. M. Best as A VIII or better (or other companies acceptable to TUFTS), shall name TUFTS as an additional named insured, shall have limits of at least one million dollars ($1,000,000) per occurrence with an aggregate of three million dollars ($3,000,000), shall be non-cancelable except upon thirty (30) days prior written notice to TUFTS, and shall provide that as to any loss covered thereby and also by any policies obtained by TUFTS itself, LICENSEE’s policies shall provide primary coverage for TUFTS and TUFTS’ policies shall be considered excess coverage for TUFTS.

8.4 Certificates; Policies. LICENSEE will forthwith after the obtaining of such insurance required by Section 8.3, obtain and deliver to TUFTS certificates of and copies of, and at all times thereafter deliver without further demand replacement certificates and copies of, all such insurance policies that are in force and effect, as reasonably requested by TUFTS.

9 MARKING
Prior to the issuance of patents under Licensed Patents, LICENSEE agrees to mark Licensed Product(s) (or their containers or labels) made, sold, or otherwise disposed of by it under the license granted in this Agreement with the words “Patent Pending,” and following the issuance of one or more patents, with the numbers of any applicable Licensed Patents.

10 USE OF NAMES
10.1 Use of Names. LICENSEE, its Affiliates and sublicensees agrees not to use the name of TUFTS or any TUFTS participant in the Research, as defined in the Research Agreement, in any form of publicity or disclosure without TUFTS’ prior written consent, which may be withheld or withdrawn in TUFTS’ discretion at any time, provided however, that no such consent will be required with regard to: (i) any proper reference by LICENSEE to published technical publications by such participants; (ii) disclosures to potential investors and corporate collaborators; and (iii) TUFTS will make no objection to LICENSEE’s such other disclosures as are required as a matter of law (including disclosures made under applicable securities regulation) and such general disclosures of this Agreement as may be desired by LICENSEE for purposes of grant solicitations from governmental authorities.

11 PATENT PROSECUTION AND INFRINGEMENT
11.1 TUFTS shall have the primary responsibility for the prosecution, filing and maintenance of all Licensed Patents, including the conduct of all interference, opposition, nullity and revocation proceedings, using counsel of its choice; provided, however, that LICENSEE shall have reasonable opportunity to advise and consult with TUFTS on such matters and may recommend TUFTS to take such action as LICENSEE reasonably believes necessary to protect the Licensed Patents. Counsel shall concurrently provide TUFTS and LICENSEE with copies of all material correspondence related to the prosecution of the patent applications within the Licensed Patents. Should TUFTS elect to abandon any patent or patent application in any country, it shall give timely notice to LICENSEE, who may continue prosecution or maintenance, at its sole expense and TUFTS shall have no further rights with respect to such patent application or patent in such country. In the event that a conflict arises with respect to patent counsel selected by TUFTS, LICENSEE may, with just cause and after consulting with TUFTS, select new patent counsel reasonably acceptable to TUFTS.
11.2 Payment of all reasonable fees and costs relating to the filing, prosecution and maintenance of Licensed Patents which are incurred by TUFTS after the Effective Date (including interference and/or opposition, nullity and revocation proceedings) shall be the responsibility of LICENSEE. TUFTS shall periodically send LICENSEE invoices for any such patent expenses incurred by TUFTS and LICENSEE shall pay such invoices within thirty (30) days of receipt thereof.

11.3 Each party shall inform the other promptly in writing of any alleged infringement of the Licensed Patents by a third party, including all detail then available. TUFTS shall have the right, but shall not be obligated, to prosecute at its own expense any such infringements, and LICENSEE agrees that TUFTS may join LICENSEE as a plaintiff at the expense of TUFTS. In any infringement action commenced or defended solely by TUFTS, all expenses and all recovery for infringement shall be those of TUFTS.

11.4 If TUFTS has not taken legal action or been successful in obtaining cessation of the infringement, within one hundred eighty (180) days of written notification from LICENSEE of such infringement, or if TUFTS elects not to continue prosecuting any legal action against an infringer, LICENSEE shall have the right (while the LICENSEE is the exclusive licensee), but shall not be obligated, to prosecute at its own expense any such infringement. LICENSEE may join TUFTS as a plaintiff in any such infringement suit at LICENSEE’s expense. No settlement, consent judgment or other voluntary final disposition of the suit may be entered into without TUFTS’ consent, which shall not be unreasonably withheld or delayed. In any such action by LICENSEE, after LICENSEE is first reimbursed for LICENSEE’s costs and expenses (including attorney’s and expert fees) and then TUFTS is reimbursed for any credited royalties pursuant to Section 11.8, TUFTS shall be entitled to receive an amount equal to the applicable royalties on any recovery of profits and damages that is in excess of LICENSEE’s costs and expenses and TUFTS’ royalty reimbursement. LICENSEE shall indemnify TUFTS against any order for costs or other payments that may be made against TUFTS in such proceedings.

11.5 If any declaratory judgment action alleging invalidity or non-infringement of any of the Licensed Patents shall be brought against LICENSEE, TUFTS shall have the right at its election made within sixty (60) days after commencement of that action, to intervene and take over the sole defense of the action at its expense.

11.6 In any infringement suit that either party brings to enforce the Licensed Patents, the other party shall at the request and expense of the party bringing the suit, cooperate in all reasonable respects, including, to the extent possible, obtaining the testimony of its employees and making available physical evidence in the possession of that party.

11.7 LICENSEE, during the exclusive period of this Agreement, shall have the exclusive right in accordance with the provisions of Section 12, to sublicense any alleged infringer in the Licensed Territory for future use of the Licensed Patents.

11.8 If LICENSEE pay any amounts in fees, expenses or costs to maintain, prosecute, bring or defend any proceeding relating to any infringement by a third party of any Licensed Patents, any declaratory action alleging invalidity or non-infringement of any Licensed Patents, or any interference, opposition, nullity or revocation proceeding relating to any Licensed Patents pursuant to this Section 11 (the “Section 11 Costs”), TUFTS agrees that 50% of the amount of such Section 11 Costs may be credited as they are incurred by LICENSEE against royalties due to TUFTS under Section 5 of this Agreement.

12 SUBLICENSES

12.1 LICENSEE may grant sublicenses under the Exclusive Technology to make, have made, import, have imported, use, lease, sell and offer for sale, have sold and otherwise commercialize and exploit Licensed Products, and to practice any method, process or procedure within the Exclusive Technology in the Licensed Territory. The terms and conditions of each sublicense shall be consistent with the terms and conditions of this Agreement and shall contain, among other things (by way of
example but not limitation), provisions substantially similar to and consistent with: the “Net Sales” definition; Section 6; Section 7.1 (so that no representations or warranties inconsistent with that Section shall be extended to or by any sublicensee); Section 10; and Section 18.

12.2 Any sublicense granted by LICENSEE under this Agreement shall remain in effect in the event of any termination of this Agreement and shall provide for the assignment of such sublicense to TUFTS or its designee in the event that this Agreement is terminated; provided, that the financial obligations of each sublicensee to TUFTS shall be limited to the amounts such sublicensee would be obligated to pay to LICENSEE had this Agreement not been terminated.

12.3 Each sublicense shall provide that the obligations to TUFTS of Sections 6, 7.1, 8.1, 10.1, 11.3, 11.4, 13.4, and 18 shall be binding on the sublicensee and enforceable by both TUFTS and LICENSEE.

12.4 LICENSEE shall furnish to TUFTS a true and complete copy of each sublicense agreement and each amendment thereto, promptly after the sublicense or amendment has been agreed upon. TUFTS agrees that it will keep each agreement confidential.

12.5 No sublicense shall relieve LICENSEE of any of its obligations hereunder, and LICENSEE shall be responsible for the acts or omissions of its Affiliates and sublicensees and for compliance by them with their obligations, and LICENSEE shall take all steps necessary to enforce that compliance to the extent required to allow LICENSEE to fully comply with all of its obligations under this Agreement.

13 TERM AND TERMINATION

13.1 Unless sooner terminated in a manner provided herein, this Agreement shall continue in force on a country-by-country and Licensed Product-by-Licensed Product basis until the expiration of the last to expire of all Valid Claims in such country included in the Licensed Patents. Following such an expiration, LICENSEE shall have a non-exclusive, royalty-free, irrevocable license in such country, to the Know-How.

13.2 LICENSEE shall have the right to terminate the Agreement at any time following ninety (90) days written notice to TUFTS. LICENSEE may terminate this Agreement with respect to any country or any Licensed Patent by giving TUFTS notice in writing at least sixty (60) days in advance of the effective date of termination selected by LICENSEE.

13.3 TUFTS may terminate this Agreement if LICENSEE:

(a) Is in default in payment of royalty;

(b) Is in material breach of any provision hereof;

and LICENSEE fails to remedy any such default, or breach, or fails to act reasonably to remedy any default, or breach, within thirty (30) days after receipt of written notice thereof by TUFTS.

13.4 TUFTS may terminate this Agreement if LICENSEE fails to cure any default on the diligence milestones in Section 4 after a twelve month period commencing upon LICENSEE’s receipt of written notification from TUFTS of LICENSEE’s default of such milestones.

13.5 Surviving any termination are:

(a) LICENSEE’s obligation to pay royalties accrued or accruable;

(b) Any cause of action or claim of LICENSEE or TUFTS, accrued or to accrue, because of any breach or default by the other party; and
14 ASSIGNMENT
Neither party may assign this Agreement or any part hereof without the express written consent of the other, which consent shall not be unreasonably withheld; provided, however, LICENSEE may assign this Agreement or any portion hereof to an Affiliate or to a successor of all or substantially all its business relating to the Licensed Patents or Know-How without the written consent of TUFTS and shall provide TUFTS notice of any such assignment. However, no assignment or other transfer by LICENSEE shall relieve LICENSEE of any obligations hereunder and LICENSEE shall continue to be primarily and jointly and severally liable (along with such assignee or other transferee) for the performance of all obligations of LICENSEE and such assignee or other transferee hereunder.

15 ARBITRATION
15.1 Any controversy arising under or related to this Agreement, and any disputed claim by either party against the other under this Agreement excluding any dispute relating to patent validity or infringement arising under this Agreement, shall be settled by arbitration in accordance with the Rules of Commercial Arbitration of the American Arbitration Association.

15.2 Upon request by either party, arbitration will be initiated by a third party arbitrator mutually agreed upon in writing by LICENSEE and TUFTS within thirty (30) days of such arbitration request. Judgment upon the award rendered by the arbitrator shall be final and nonappealable and may be entered in a court having jurisdiction thereof. The parties agree that any provision of applicable law notwithstanding, they will not request and the arbitrators shall have no authority to award punitive or exemplary damages against any party. The costs of the arbitration, including administrative fees and fees of the arbitrators shall be shared equally by the parties. Each party shall bear the cost of its own attorneys’ fees and expert fees.

15.3 The parties shall be entitled to discovery in like manner as if the arbitration were a civil suit in a Superior Court of the Commonwealth of Massachusetts; provided, however, the arbitrator may limit the scope, time and/or issues involved in discovery.

15.4 Any arbitration shall be held at a location mutually agreed upon in writing by LICENSEE and TUFTS.

16 NOTICES
All notices under this Agreement shall be deemed to have been fully given when done in writing and deposited in the United States mail, registered or certified, or overnight deliver service (e.g., DHL, Federal Express) and addressed as follows:

To TUFTS:
Tufts University
136 Harrison Avenue (75K-1520)
Boston, Massachusetts 02111
Attention: Associate Provost for Research

with a copy to: Massachusetts Biomedical Initiatives
20 Hampden Street
Roxbury, Massachusetts 02119

Attention: Director, Unified Office for Technology Transfer

To LICENSEE: Illumina, Inc.
2187 Newcastle Ave
Suite 101
Cardiff, California 92007

Attention: John R. Stuelpnelagel

Either party may change its address upon written notice to the other party.

17 CONFIDENTIALITY

TUFTS shall maintain this Agreement and the reports and any information provided by LICENSEE to TUFTS in confidence and not disclose such information or reports to any third party, except as required by law and disclosed after notice to LICENSEE and after requesting confidential treatment and a protective order, if available. TUFTS may, however, disclose to third parties total annual royalty payments and general statistical information regarding payments made hereunder in the context of disclosing statistical information pertaining to the performance of the TUFTS Office of Technology Licensing.

18 COMPLIANCE WITH LAWS

18.1 Export Controls. The Export Control Regulations of the U.S. Department of Commerce prohibit, except under special validated license, the exportation from the United States of technical data relating to certain commodities (listed in the Regulations), unless the exporter has received certain written assurance from the foreign importer. In order to facilitate the exchange of technical information under this Agreement, LICENSEE therefore hereby agrees and gives its assurance to TUFTS that LICENSEE will not, unless any required prior authorization is obtained from the U.S. Office of Export Control, re-export directly or indirectly any technical data received from TUFTS under this Agreement and will not export directly the Licensed Products or such technical data to any country listed on either the Commodity Control List or Militarily-Critical Technologies List. TUFTS makes no representation as to whether any such license is required or, if one is required, as to whether it will be issued by the U.S. Department of Commerce.

18.2 Other Laws. In addition to the foregoing export control requirements, LICENSEE agrees that it, its Affiliates, and its sublicensees will comply with all applicable mandatory or permissive patent marking laws, rules, and regulations and comply with all other laws, rules, and regulations of all governmental authorities applicable to any of their activities contemplated by this Agreement, and will comply with all necessary and desirable practices in connection and compliance with safety recommendations of trade associations or governmental authorities.

19 MISCELLANEOUS

19.1 Governing Law. This Agreement shall be governed by the laws of in the Commonwealth of Massachusetts, without reference to principles of conflicts of laws.
19.2 **Waiver.** None of the terms of this Agreement can be waived except by the written consent of the party waiving compliance.

19.3 **Entire Agreement.** This Agreement and any Exhibits attached hereto (each of which is hereby made part hereof by this reference), and the Master Agreement entered into by the parties on even date herewith constitute the entire agreement between the parties concerning the subject matter hereof, and all prior negotiations, representations, warranties, agreements, and understandings related thereto superseded hereby.

19.4 **Force Majeure.** Neither party shall not be considered in breach of this Agreement to the extent any failure to perform any term or provision is caused by any reason beyond such party’s reasonable control, or by reason of any of the following circumstances: labor or employee disturbances or disputes of any kind; accidents; laws, rules or regulations of any government (including, without limitation, export and import regulations); failure of any government approval required; disease; failure of utilities, mechanical breakdowns, material shortages or other similar occurrences; civil disorders or commotions, acts of aggression, vandalism or other similar occurrences; or fire, floods, earthquakes, or acts of God.

19.5 **Independent Contractors.** The parties hereto shall be independent contractors with respect to each other, and neither shall be deemed to be the agent, principal, employee, servant, joint venturer, or partner of the other for any purpose.

19.6 **Severability.** If any provision of this Agreement shall to any extent be found to be invalid or unenforceable, the remainder of this Agreement shall not be affected thereby, and any such invalid or unenforceable provision shall be reformed so as to be valid and enforceable to the fullest extent permitted by law.

19.7 **Headings.** Headings of Articles, Sections, and subsections included herein for convenience for reference only and shall not be used to construe this Agreement.

19.8 **Counterparts.** This Agreement may be executed in two counterparts, each of which shall be deemed an original and which together shall constitute one instrument.

IN WITNESS WHEREOF, the parties have executed this Agreement effective as of the Effective Date set forth above.
Exhibit 1

**Patents and Patent Applications**

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<tr>
<td>WALT, David</td>
<td>Imaging Fiber Optic Array Sensors, Apparatus and Methods for</td>
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<td>Method of Making Imaging Fiber Optic Sensors to Concurrently</td>
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<td>Superresolution Imaging Fiber for Subwavelength Light Energy</td>
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WALT, David  
BARNARD, Steve  
Fiber Optic Sensor, Apparatus and Methods for Detecting an Organic Analyte in a Fluid or Vapor Sample (OSCI)  
Pat #5,244,813  
Issued: 9/14/93  
Canadian Serial No. #2,128,413

Pending U.S. Patent Applications

WALT, David  
HEALEY, Brian  
Photodeposition Methods for Fabricating a Three-Dimensional, Patterned Polymer Microstructure  
USSN #08/519,062  
Filed: 8/24/95

WALT, David  
FARFIELD VIEWING OPTICAL APPARATUS FOR MAKING OPTICAL DETERMINATIONS AND ANALYTICAL MEASUREMENTS  
USSN #08/572,005  
Filed: 12/14/95

WALT, David  
MICHAEL, Keri  
Fiber Optic Sensor with Encoded Microspheres (Analyte Detection System)  
USSN #08/818,199  
Filed: 3/14/97

WALT, David  
DICKINSON, Todd  
Self-Encoding Microspheres  
USSN #08/944,850  
Filed: 10/6/97

WALT, David  
HEALEY, Brian  
FERGUSON, Jane  
Fiber Optic Biosensor for Selectively Detecting Oligonucleotide Species in a Mixed Fluid Sample  
Application Being Prepared

U.S. Patent Application to be Filed

WALT, David  
TAYLOR, Laura  
Fiber Optic Biosensor Array Comprising of Cell Populations Confined to Microcavities  
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Exhibit 2
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METHOD AND APPARATUS FOR ALIGNING ELONGATED MICROBEADS IN ORDER TO INTERROGATE THE SAME

METHOD OF MANUFACTURING OF A DIFFRACTION GRATING-BASED OPTICAL IDENTIFICATION ELEMENT

DIFFRACTION GRATING-BASED OPTICAL IDENTIFICATION ELEMENT

ASSAY STICK COMPRISING CODED MICROBEADS

CHEMICAL SYNTHESIS USING DIFFRACTION GRATING-BASED ENCODED OPTICAL ELEMENTS

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DIFFRACTION GRATING-BASED OPTICAL IDENTIFICATION ELEMENT

DIFFRACTION GRATING-BASED ENCODED MICRO-PARTICLES FOR MULTIPLEXED EXPERIMENTS

COMPOSITIONS AND METHODS FOR DETECTING PROTEASE ACTIVITY

Method of manufacturing of a diffraction grating-based optical identification element

MULTIPLEX DECODING OF ARRAY SENSORS WITH MICROSPHERES
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WO06069346A3  METHYLATION-SENSITIVE RESTRICTION ENZYMEN ENDONUCLEASE METHOD OF WHOLE GENOME METHYLATION ANALYSIS  ILLUMINA INC.  2006-06-29

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US20060134324A1  Filament with easily removed protective coating and methods for stripping the same  ILLUMINA INC.  2006-06-22

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US20060118630A1  Holographically encoded elements for microarray and other tagging labeling applications, and method and apparatus for making and reading the same  ILLUMINA INC.  2006-06-08

US20060119913A1  Fourier scattering methods for encoding microbeads and methods and apparatus for reading the same  ILLUMINA INC.  2006-06-08

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US7040959  Variable rate dispensing system for abrasive material and method thereof  ILLUMINA INC.  2006-05-09

US7033754  Decoding of array sensors with microspheres  ILLUMINA INC.  2006-04-25
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