UNITED STATES DISTRICT COURT
FOR THE SOUTHERN DISTRICT OF NEW YORK

ASSOCIATION FOR MOLECULAR PATHOLOGY; AMERICAN COLLEGE OF MEDICAL GENETICS; AMERICAN SOCIETY FOR CLINICAL PATHOLOGY; COLLEGE OF AMERICAN PATHOLOGISTS; HAIG KAZAZIAN, MD; ARUPA GANGULY, PhD; WENDY CHUNG, MD, PhD; HARRY OSTRER, MD; DAVID LEDBETTER, PhD; STEPHEN WARREN, PhD; ELLEN MATLOFF, M.S.; ELSA REICH, M.S.; BREAST CANCER ACTION; BOSTON WOMEN'S HEALTH BOOK COLLECTIVE; LISBETH CERIANI; RUNI LIMARY; GENAE GIRARD; PATRICE FORTUNE; VICKY THOMASON; KATHLEEN RAKER, Plaintiffs, v. UNITED STATES PATENT AND TRADEMARK OFFICE; MYRIAD GENETICS; LORRIS BETZ, ROGER BOYER, JACK BRITTAIN, ARNOLD B. COMBE, RAYMOND GESTELAND, JAMES U. JENSEN, JOHN KENDALL MORRIS, THOMAS PARKS, DAVID W. PERSHING, and MICHAEL K. YOUNG, in their official capacity as Directors of the University of Utah Research Foundation, Defendants	Civil Action No. 09-4515 (RWS) ECF Case **DECLARATION OF ROBERT L. NUSSBAUM, M.D.**

I, Robert L. Nussbaum, declare under penalty of perjury that the following is true and correct:

1. I offer my opinion herein on the science of genetics and the biology of DNA, including methods for its extraction and isolation. My opinion is based on the facts or

data cited in this declaration, and on my own original research. A true and correct copy of my Curriculum Vitae is attached hereto as an Exhibit.

2. My qualifications as an expert are as follows. I received my A.B. *cum laude* in Applied Mathematics from Harvard College in 1971. I received my M.D. Medicine from Harvard Medical School in 1975.

3. I currently serve at the University of California, San Francisco (UCSF) as a tenured Professor of Medicine In Residence, as a Professor of Neurology (Joint Appointment), and as the Holly Smith Distinguished Professor of Science and Medicine. I am Chief of the Division of Medical Genetics in the UCSF Department of Medicine, and Executive Committee Member of the UCSF Institute for Human Genetics. Since 2006, I have served as Consultant in Genetics at UCSF Medical Center as well as Faculty in the Biomedical Sciences Graduate Group at UCSF. Since 2008 I have been the Director of the Cancer Risk Program of the Diller Family Comprehensive Cancer Center at UCSF, which provides clinical care and genetic counseling for families with hereditary cancer syndromes and the Director of the Program in Cardiovascular Genetics of the Heart and Vascular Center of the UCSF Medical Center.

4. Prior to my current appointments at UCSF, I was Assistant Professor of Medicine at Baylor College of Medicine from 1981 to 1984. Later, at the University of Pennsylvania School of Medicine (Primary), I was Assistant Professor of Human Genetics from 1984 to 1989, Associate Professor of Human Genetics from 1989 to 1993, and Professor of Genetics from 1993-1994, as well as an Associate Investigator of the Howard Hughes Medical Institute. From 1994 to 2006 I was a Principal Investigator and Chief of the Genetic Disease Research Branch in the Intramural Research Program of the National Human Genome Research Institute.

5. I have been invited to give lectures and present papers at various genetics conferences, including "Genomics in Social and Behavioral Research" in September 2008 at the NHGRI, Bethesda, MD; "Molecular Genetic Approach to Parkinson Disease" in April 2008 at the Gallo Institute, Emeryville, CA; "What Human Genetics has taught us about Alzheimer Disease" in June 2007 at the Alzheimer's Association Medical Scientific Advisory Council Research Symposium, Stanford University; "Genetic Testing" in November 2006 at the President's Council on Bioethics; and "Direct to Consumer Genetic testing" in September 2006 at the National Genetic Policy Summit, Washington, D.C. In 2004, I was elected to the Institute of Medicine of the National Academy of Sciences.

6. My recent publications include "Genomics and the Human Genome Project" in The Molecular and Genetic Basis of Neurological and Psychiatric Disease, 4th edition (eds R. Rosenberg, S. Prusiner, S. DiMauro, R. Barchi, E. Nestler), Butterworth Heinemann, Woburn, MA, 2007; "Genetics of disease: Recent advances in the genetics of human disease offer something new for every scientific interest", Curr. Opin. Genet. Develop. 17:1-3, 2007; and "2004 Presidential Address: What is so Special about the 'Human' in 'Human Genetics', Amer. J. Hum. Genet. 76(2):198-202, 2005. I am also the lead author on the previous (6^{th}) and most recent (7^{th}) edition of a widely used medical genetics textbook entitled "Genetics in Medicine", published in 2007.

7. In addition to my academic practice, I also have a clinical practice and see children and adults for the diagnosis, management and counseling of hereditary diseases, including Hereditary Breast and Ovarian cancers caused by mutations in *BRCA1* and *BRCA2*. I am currently certified by three Specialty Boards: the American Board of Internal Medicine, the American Board of Medical Genetics in Clinical Genetics, and the American Board of Medical

Genetics in Clinical Molecular Genetics. From 1984 to 1993, I was Physician in the Division of Human Genetics at Children's Hospital of Philadelphia. From 1994 to 2006, I was Clinical Staff at the National Institute of Health (NIH) Clinical Center. At UCSF, I currently spend approximately 80% of my time on academic work and 20% of my time on clinical work.

8. I have also engaged in government service with respect to the field of human genetics. For example, I have served the National Center for Human Genome Research Institute (NHGRI) in various roles since 1989 to the present. In 2001, I chaired the Subgroup on Genotyping Methods for the SNP Mapping Advisory Committee at NHGRI. From 1995 to 2006, I was Acting Chief of the Inherited Disease Research Branch at NHGRI. From 1996 to 2006, I also was the lead Project Officer for the Center for Inherited Disease Research, an inter-Institute genotyping facility that provides up-to-date genetic analysis to NIH-funded researchers around the United States.

9. At NIH, I was a regular member of three Initial Review Groups: the Genetic Basis of Disease, NIGMS (1987-1991), the Human Genome Research Study Section (DRG) (1992-1996), and Mammalian Genetics (CSR) (1998-2000), as well as serving on numerous ad hoc Review Panels.

10. I taught human genetics for Medical Students at the University of Pennsylvania from 1986 until 1996 and was Course Director from 1997-2003. I also taught human genetics at National Human Genome Research Institute from 1999 to 2006, including the courses "Introduction to Human Genetics for Genetic Counseling Students" and "Introduction to Clinical Molecular Genetics." I currently teach genetics to medical students and graduate students at UCSF and to genetic counseling students at the California State University, Stanislaus.

11. I have reviewed the Declaration of Dr. Mark A. Kay and the Defendants' Memorandum of Law (1) in support of their motion for summary judgment and (2) in opposition to plaintiffs' motion for summary judgment. In this declaration, I explain how the definition of "isolated DNA" in Defendants' patents covers both naturally-occurring DNA and synthetic DNA, and how the DNA that has been patented is not structurally and functionally different from genes in nature.

DEFINITION OF "ISOLATED" DNA AND THE PROCESS OF ISOLATION

12. When the Defendants argue that an isolated segment of genomic DNA is a man-made substance that is substantially different from the DNA that constitutes a native gene inside a cell, they blur the distinction between three entities: "DNA", "chromatin" and "gene". Their patent defines "isolated DNA" as follows:

> An "isolated" or "substantially pure" nucleic acid (*e.g.*, an RNA, DNA or a mixed polymer) is one which is substantially separated from other cellular components which naturally accompany a native human sequence or protein, *e.g.*, ribosomes, polymerases, many other human genome sequences and proteins. The term embraces a nucleic acid sequence or protein which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems. '473 Patent, col. 19:6-15; '282 Patent, col. 19:8-18; and '492 Patent, col. 17:62 – col. 18:5.

(I must point out that such a broad claim that the term "'isolated' or 'substantially pure'" nucleic acid "embraces…. a protein" is an impossibility since protein is not a nucleic acid.)

13. Although this definition of "isolated" equates the terms "isolated" and "substantially pure" with reference to nucleic acids, "isolated" nucleic acid and "substantially pure" nucleic acid do not mean the same thing. In addition, this definition blurs the distinction between DNA isolated from the cell and synthetic DNA, such as primers made using chemical

reactions or biochemical procedures.

14. I would like to clarify that some of the "isolated DNA" covered by the patent claim -- a DNA segment of interest from a natural source (as opposed to synthetic DNA) – results from a process that occurs in two steps: (1) extracting the DNA away from other non-DNA substances including the proteins that make up the "chromatin" in which DNA is packaged, and (2) separating the segment of human DNA with the sequence of interest away from the rest of the genomic DNA. These two steps are functionally distinct.

15. When DNA is obtained from a natural source, extraction of cellular DNA is a routine biochemical procedure in which all the DNA in a sample of cells or tissue is separated away from other non-DNA substances including the proteins that make up the "chromatin" in which DNA is packaged. Extraction is a chemical process that takes advantage of the chemical properties of DNA (such as its solubility in various liquids) and does not discriminate between one segment of DNA containing a sequence of interest from other segments containing other sequences. With extraction, there is no separation of a particular DNA segment of interest away from the rest of cellular DNA.

16. Separating a DNA segment containing a sequence of interest away from the rest of the DNA relies on the sequence. Although separation may be accomplished by biochemical methods, such as excising that segment or amplifying it by PCR, it is also possible to use biological methods to separate the DNA containing a gene away from other genes without extracting it. Random pieces of DNA can be transferred into non-human cells or microorganisms and the subset of the cells or microorganisms that have acquired a novel property as a result of having taken up the segment of interest containing specific information encoded in the DNA sequence within that segment can be identified and isolated as pure colonies. In this way, the

DNA segment containing a sequence of interest of human DNA in an individual colony of bacteria or cells is in fact separated away from all the other human DNA of the original cell from which the DNA had been obtained, even though it has not been extracted away from bacterial proteins or separated from bacterial DNA.

"ISOLATED" DNA IS NOT MAN-MADE OR STRUCTURALLY DISTINCT FROM DNA IN ITS NATURAL STATE IN THE CELL

17. Isolating a DNA segment of interest therefore depends on extraction of cellular DNA and separation of the DNA segment of interest away from other genomic DNA. This distinction is important when assessing the validity of the claim Dr. Kay makes in paragraph 137 of his declaration:

> "The isolated DNAs are excised, extracted or synthetic chemical compounds made by the hands of molecular biologist, not by nature. They are structurally distinct from any substance found in the human body—indeed, in all of nature."

18. To the contrary, I would argue that isolated DNA is not made by the hands of a molecular biologist, but is instead simply extracted from the chromatin. Moreover, when isolating a DNA segment of interest, neither extracting total cellular DNA nor separating a particular DNA segment away from the rest of DNA produces a substance that is "structurally distinct from any substance found in the human body". Instead, an isolated DNA segment, as DNA, is not significantly different from the same segment of DNA in the cellular DNA from which it was derived.

19. With regards to the extraction step, Dr. Kay has confused chromatin and DNA. The structural difference he points to between DNA in a cell and DNA isolated from a cell are actually differences between chromatin and DNA, not between the DNA in a cell and the

DNA isolated from a cell.

20. Any differences between isolated DNA and DNA in chromatin are actually "epigenetic" changes (note, they are not "genetic"), which means they are superimposed upon genes and not part of a gene itself. The one epigenetic modification of DNA that is not part of chromatin, the modification of DNA by the covalent attachment of methyl groups to the cytosine ring, is preserved in genomic DNA after it is extracted and so does not distinguish the DNA in a cell from the DNA extracted from a cell.

21. DNA swathed in proteins as a component of chromatin inside a cell is not structurally different from DNA after it has been extracted away from other cellular substances, including chromatin. By analogy, the element gold is still gold whether it is a streak of metal embedded in low grade ore or is a nugget that has been extracted away from the silicates in which it is embedded. Thus, with regards to the extraction step required to do what the Defendants claim is to "isolate" a segment of DNA from natural sources, I reject the argument that this "isolated" DNA is "structurally different" from natural DNA.

22. With regards to the separation step, once total DNA has been extracted from a cell, the isolation of any particular segment of interest occurs on the basis of the particular sequence of DNA bases it contains. It is the DNA sequence that distinguishes the segment being isolated from all the other segments of genomic DNA from which it is being separated. Fundamentally, then, a segment of extracted DNA can be separated from the rest of the genomic DNA because it contains genetic information of interest, e.g. it contains the information that comprises a gene. The very same sequence of bases in the DNA that allows scientists to distinguish one segment of extracted DNA and makes that segment of interest is also contained in the DNA when it is in its natural state in the cell.

23. Therefore, with regards to the isolation of a DNA segment of interest from natural sources, such as a cell, the claim that "isolated DNAs …. are structurally distinct from any substance found in the human body—indeed, in all of nature" is incorrect on two grounds. First, in extracting cellular DNA, the structural distinction claimed by Dr. Kay is between chromatin and DNA, not between extracted DNA and cellular DNA. Second, isolating a segment of DNA of interest by separating it from the rest of the extracted genomic DNA depends on the sequence of DNA bases that is present in both the isolated segment and the natural segment in the cell. Thus, "isolated DNA", meaning DNA that has been extracted away from other cellular components and then separated from the rest of the DNA in the cell, is not made by the hand of the scientist; it is extracted away from chromatin and then separated from the rest of the genomic DNA based on its sequence of bases and/or the information the bases contain. A segment of isolated DNA is no more a product of the hand of man than is the gold that has been extracted from other minerals based on its natural, and not man-made, physical and chemical characteristics.

AN "ISOLATED" GENE IS NOT STRUCTURALLY AND FUNCTIONALLY DISTINCT FROM GENES IN NATURE

24. "Gene" is a term that came into use long before it was known what genes were made of. A gene was defined as a unit of heredity that carries the information necessary to pass whatever trait or function the gene is responsible for from one generation to the next. Thus, I agree with Dr. Kay's heredity-based definition of a gene in paragraph 142 of his declaration, where he states:

> "Historically, the term "gene" has been used to describe the unit that is responsible for the inheritance of a discrete trait, such as the color of peas in a peapod."

25. Although Dr. Kay uses the term "historically", this definition is still as valid as any other up to the present day. With progress in molecular genetics, however, genes can now also be defined in molecular terms. Dr. Kay writes in paragraph 143 of his Declaration:

> "In molecular terms, a gene is an aggregate of *several segments of a chromosome* (emphasis added). Some segments regulate the activity of the gene. From other segments, various types of RNA are produced".

This molecular definition of a gene is ambiguous. When speaking of "several segments of a chromosome", does Dr. Kay mean that a gene is an aggregate of several segments of the DNA in a chromosome, some of which regulate the activity of the gene, such as promoter or enhancer elements within the DNA, and others which contain the triplet code? Or, is he saying a gene is an aggregate of several segments of the chromatin that make up a chromosome and that a gene also includes the epigenetic modifications, such as methylation or proteins that are involved in regulating the gene? Furthermore, when a gene is regulated by a protein such as a transcription factor or a regulatory non-coding RNA encoded by a separate, distinct gene on that chromosome, Dr. Kay's definition would then include the DNA sequence of that second gene as part of the first gene, thereby converting two distinct genes into one. Dr. Kay goes on in paragraph 173 to draw a major distinction between isolated DNAs and the naturally occurring genes in the cell.

> "Isolated DNAs are structurally and functionally distinct from any DNA found in nature. The isolated *BRCA1* and *BRCA2* DNA molecules claimed in the *BRCA1* and *BRCA2* patents are likewise extracted, purified, or synthetic, and are structurally distinct from any substance found in the human body, or elsewhere in nature. For example, native DNAs are physically connected to DNA regulatory sequences and proteins that determine which DNA sequences are expressed, how and where they expressed, and their level of expression. In contrast, the claimed isolated *BRCA1* and *BRCA2* DNAs are not associated with these regulators and do not contain this information."

26. Taking the definition of a gene as a unit of heredity that carries the information necessary to pass a trait or function from one generation to the next, the correct view is that a gene such as *BRCA1* or *BRCA2* is composed of a segment of DNA in a chromosome and

not the chromatin or other regulatory proteins.

27. In claiming a patent on isolated DNA sequences that contain the information that comprises the *BRCA1* or *BRCA2* gene, the Defendants have attempted to patent a naturally occurring entity, a gene, basing their claim on alleged and, at best, highly irrelevant differences between the naturally occurring gene and the isolated DNA sequences. That this is the correct interpretation of the coverage of their patent claim is supported by a number of important biological facts, explained as follows.

28. During reproduction, DNA is unpackaged, stripped of chromatin proteins and other epigenetic modification such as some or all of the cytosine methylation, partially or substantially remethylated and repackaged into chromatin as it is passed through the germ line from one generation to the next. Although these modifications are more striking in the production of sperm compared to ova, they occur in both parents. Most importantly, they make clear that it is indeed the DNA that constitutes a gene because the proteins and other epigenetic modifications that surround DNA in chromatin are substantially changed every generation while it is the DNA that remains constant (save for rare spontaneous mutation) and is responsible for passing along the genetic information.

29. With the one notable exception of genomic imprinting, it is DNA -- and *only* DNA, and the information in it -- that is responsible for the transmission of traits from one generation to the next and, therefore, constitutes the genes. Since neither *BRCA1* nor *BRCA2* has been shown to undergo imprinting, the *BRCA1* and *BRCA2* genes are also wholly contained in the DNA, whether in chromatin or in an isolated state without epigenetic modification. The DNA containing the *BRCA1* or *BRCA2* genes are responsible for how a trait determined by either the *BRCA1* and *BRCA2* genes is passed on from one generation to the next.

30. Second, there are cell culture experiments that show that genomic DNA can transmit a trait, regardless of whether it is isolated or present inside the cell in chromatin. There are two classic experiments, one published 65 years ago, the other 27 years ago, that prove that DNA is the essence of genes.

31. In the first experiment, isolated DNA was shown to contain all the genetic information necessary to change a non-pathogenic living bacterial organism into a pathogenic bacterium. Certain strains of the bacteria *Streptococcus pneumoniae* are distinguished by whether the surface of their cell wall, the capsule, is "smooth" or "rough" when observed under the microscope. These two different cell walls correlate with how disease-producing the bacteria are: the smooth bacteria cause rapid death when injected into mice, bacteria with the rough capsule do not. In 1944, Avery, MacLeod and McCarty reported that DNA extracted from a sample of a killed pathogenic smooth strain of the bacteria *S. pneumoniae* was able to change a rough non-pathogenic strain into a smooth pathogenic strain by the process known as transformation, the introduction of DNA or RNA into a cell (Avery OT, MacLoed CM and McCarty M Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types, J. Exp. Med. 79:137, 1944). DNA from a sample of killed pathogenic smooth *S. pneumoniae* was extracted away from the non-DNA substances of the bacteria to a level of purity of 99.98%. The extracted DNA was dissolved in growth medium into which a non-pathogenic strain of *S. pneumoniae* were inoculated and grown. In these cultures, live smooth, pathogenic bacteria appeared because they were "transformed" by the extracted DNA. These originally rough bacteria were permanently changed to smooth and passed on their smooth character to all subsequent offspring. Thus, all the genetic information necessary to change the genes of a non-pathogenic living bacterial organism into those of a pathogenic bacterium was

present in the extracted DNA.

32. In the second set of experiments, extracted whole genomic DNA was shown to contain all the genetic information necessary to change a non-cancerous cell into a cancerous cell. One characteristic of cancer cells in culture is that they lose "contact inhibition". Contact inhibition is a phenomenon in which non-cancer cells growing in a petri dish will continue to divide and the new daughter cells will form attachments to the surface of the dish and spread out on the surface, until they touch other cells in the culture. Upon touching other cells, non-cancerous cells stop dividing and become quiescent. Contact inhibition is an important characteristic of non-cancerous cells. Many cancer cells in culture lose their contact inhibition and continue to divide even after touching other cells, lose their firm attachment to the petri dish surface, and pile up one on top of the other, thereby creating mounds of poorly attached cells.

33. In 1982, three laboratories independently reported that isolated genomic DNA from a cancer cell could convert cultured mouse fibroblast cells that were non-cancerous and showed contact inhibition, into cancerous cells that had lost contact inhibition. (Shih, C. & Weinberg, R. A. Isolation of a transforming sequence from a human bladder sarcoma cell line. Cell 29, 161–169, 1982; Goldfarb, M., Shimizu, K., Perucho, M. & Wigler, M. Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells. Nature 296, 404–409, 1982); Pulciani, S. et al. Oncogenes in human tumor cell lines: molecular cloning of a transforming gene from human bladder carcinoma cells. Proc. Natl Acad. Sci. USA 79, 2845–2849, 1982). In these three experiments, genomic DNA was extracted away from the rest of the cellular constituents of cells that had been derived from human cancers. The extracted DNA was applied to cultures of non-cancerous mouse fibroblast cells under conditions that encouraged the cells to take up the naked DNA. A small amount of the human DNA made its

way into the nucleus of these non-cancerous cells and became incorporated into their chromosomes. The cell cultures were then watched over time. Small areas of heaped-up transformed cells developed on a lawn of otherwise non-transformed contact-inhibited mouse cells. These areas of heaped up cells were isolated, grown up, and DNA was again extracted from these cells. Most of this DNA in fact was made up of mouse cell DNA with a small proportion that was human in origin. The extracted DNA was reapplied to another lawn of non-transformed mouse cells, which again took up this genomic DNA and once again developed small foci of heaped-up cancerous cells on a lawn of otherwise non-cancerous contact-inhibited mouse cells. These foci could again be isolated, grown, and the DNA extracted and re-applied to a new lawn of untransformed cells. This approach of repeated serial transformation of the mouse cells by total genomic DNA extracted from transformed foci led to the discovery that a particular segment of human DNA was responsible for causing the non-cancerous mouse cells to become cancerous and lose contact inhibition. The serial transformation with extracted total cellular DNA in effect separated the human gene responsible for transformation away from the rest of the human DNA. This DNA was found to contain the human ras (H-Ras) oncogene.

34. These experiments with bacteria and cultured mouse cells clearly demonstrate that the same information in the DNA responsible for the smooth capsule or the cancerous behavior of cultured human bladder cancer cells when inside the organism is present in DNA extracted from that organism.

35. The physical embodiment of a gene is DNA and the information contained within that gene is comprised of the arrangement of the bases in the DNA. That is the same whether the DNA is inside the cell or isolated in a test-tube. To claim otherwise is to confuse a gene with the machinery that regulates how that gene is expressed. Thus, I reject the claim that

the genes represented by isolated DNAs that are the focus of this patent are "structurally and functionally distinct" in any significant way from the genes in DNA found in nature.

THE PRIMARY PURPOSE OF ISOLATING DNA IS TO MAKE AN INFERENCE ABOUT THE DNA IN A PERSON'S BODY

36. In paragraphs 138 and 139 of his Declaration, Dr. Kay argues that isolated DNA:

> "…acquires new properties not shared by its native counterpart…. Native DNA does not have the chemical, structural, functional properties that make isolated DNA so useful to the molecular biologist. Native DNA cannot be used as molecular tools, such as probes and primers, and cannot be used to detect mutations. Nor can it be used in sequencing reactions to determine the structure of a DNA molecule."

37. The argument that the isolated DNA can be put to uses that the native DNA cannot forms important underpinning to the arguments the Defendants make in their "Memorandum of law (1) in support of their motion for summary judgment and (2) in opposition to plaintiffs' motion for summary judgment" in which they refer to the case of *Parke-Davis & Co. v. H.K. Mulford Co.* concerning the patentability of a purified natural product, in this case, adrenaline, and quote Judge Hand who stated:

> "even if [the adrenaline] were merely an extracted product without change, the*re is no rule that such products are not patentable.* [The Patentee] was the first to make [adrenaline] available for any use by removing it from the other gland-tissue in which it was found, and, *while it is of course possible logically to call this a purification of the principle, it became for every practical purpose a new thing commercially and therapeutically.* That was a good ground for a patent."

Once again referring to the analogy of gold mining, trace amounts of gold embedded in gangue rock cannot be used for making jewelry or for applications in electronics, the way purified gold can be used. Nonetheless, gold embedded in gangue rock is still gold and is not transmuted into some other novel substance by virtue of its being extracted and used in ways the gold in ore

cannot be used.

39. There is also an important distinction between the case of the purification of adrenaline for commercial or therapeutic use and the isolation of DNA containing the *BRCA1/2* genes for sequencing. The isolation of adrenaline provided a substance that was itself useful for injection into any patient. In contrast, being able to sequence isolated DNA in a test tube in order to find mutations in that isolated *BRCA1/2* gene is by itself of no particular value. The true value only comes from inferring that any mutation found in the isolated material is also present in the DNA of the person from whom the DNA was isolated. This observation underscores that the clinical value of obtaining the sequence and finding a mutation in an isolated segment of a *BRCA1* or *BRCA2* gene relies on the fact that the physical embodiment of a gene is DNA and the information contained within that gene is comprised of the arrangement of the bases in the DNA, whether the DNA is inside the cell of a particular individual or isolated from that individual and placed in a test-tube.

40. Dr. Kay's argument that "isolated" genomic DNA segments can be used as probes and primers is also mistaken. An isolated genomic DNA segment is too large and cannot be used as a "primer" in any practical applications. Primers are short chains of nucleotides, often approximately 20 basepairs in length but may range up to 100 base pairs in length, that are complementary to a region in the DNA. They are ordinarily used as an anchor point on which DNA synthesis can be initiated by extending the primer, such as in PCR reactions to replicate a segment of DNA. Primers are manufactured; they are not extracted from native DNA. Probes are pieces of DNA or RNA corresponding to a gene or sequence of interest. Probes are labeled either radioactively or with some other detectable molecule, are made single-stranded if they were originally double stranded, and then allowed to form stable base pairing (A

with T or U, G with C) with a "target", which is commonly a fragment of DNA or RNA that is complementary to the probe and has been fixed to a solid substrate (such as a membrane or plastic). While it is possible that a segment of genomic DNA the size of a gene such as *BRCA1* or *BRCA2* could be used as a probe, it is never used in this way for any clinical applications.

THE EXONIC SEQUENCES IN cDNA ARE THE SAME AS THOSE OF THE EXONS OF NATIVE DNA AND cDNAS OCCURRING NATURALLY IN THE HUMAN BODY

41. With regards to cDNA, Dr. Kay states in paragraph 154 of his Declaration

> "Thus, contrary to Dr. Leonard's statement that "the coding effect of a cDNA is the same as that of the original DNA from which it was originally derived despite having a shorter sequence," alternative splicing can give rise to many different mRNAs from the same native DNA molecule (*see* Leonard, ¶75). cDNAs can be prepared from mRNAs as discussed below. However, the cDNA captures that one mRNA from which it was synthesized and not all other splice variants that result from that one gene."

Dr. Kay is correct that more than one type of mRNA, and therefore more than one cDNA, can be made from a single genomic copy of a gene due to alternative splicing. However, it remains true that the exonic sequences present in a cDNA, which are a reflection of which exons were retained in the mRNA after splicing of the primary transcript, are present in the same order as are the exons in the genomic copy of the gene. There may be differences between cDNAs due to gaps or insertions representing exons that were spliced out or retained respectively in some mRNAs and not in others, but there are no inversions or swapping of positions of exon sequence between different cDNAs.

42. In paragraph 164 of his Declaration, Dr. Kay states that

> "an isolated cDNA molecule is an artificial construct that does not exist in the body and hence is structurally and functionally different from both native DNA and RNA."

On the contrary, it has been known for decades that cDNAs in the form of "processed pseudogenes" are natural products and exist in the cells of the body. Processed pseudogenes are

double-stranded DNA sequences in the human genome that resulted from the same biochemical process, RNA-templated DNA synthesis (reverse transcription) presumably carried out in our evolutionary past by viruses containing a reverse transcriptase enzyme. These naturally occurring cDNAs were inserted randomly into the human genome and passed down through the generations to the present day. Processed pseudogenes contain largely the same bases, in the same order, as the cDNA made from the mRNA derived from transcription of a gene. Alignments of a cDNA made from the mRNA of a gene and a processed pseudogene of that gene can be identical over 80-90% of the length of the molecule. Like cDNAs made artificially in a test tube, processed pseudogenes can include segments of the polyA tail, but not the 5' cap, that are typically present in mRNA. For example, my coworkers and I reported in 1984 that 14 cDNA copies of the argininosuccinate synthetase gene exist in the human genome, distributed on 12 different human chromosomes (Su TS, Nussbaum RL, Airhart S, Ledbetter DH, Mohandas T, O'Brien WE, Beaudet AL Human chromosomal assignments for 14 argininosuccinate synthetase pseudogenes: cloned DNAs as reagents for cytogenetic analysis. Am J Hum Genet. 1984 Sep;36(5):954-64). Thirteen of these pseudogenes contain >80% of the sequence present in the complete mRNA and, over this shared region, more than 85% of the bases are identical. Attached as an Exhibit is a diagram showing just one of the argininosuccinate synthetase pseudogenes that aligns with 80% of the cDNA of argininosuccinate synthetase mRNA (NM_000050.4 in Genbank at NCBI) with over 90% identity of the nucleotides between the cDNA and the pseudogene.

I declare, pursuant to 28 U.S.C. § 1746, under penalty of perjury under the laws of the United States, that the foregoing is true and correct to the best of my knowledge and belief.

Robert L. Nussbaum, MD

Executed on January 18, 2010