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UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

BEFORE THE HONORABLE MARILYN HALL PATEL, JUDGE

E.I. DU PONT DE NEMOURS & CO.,

PLAINTIFF,

VS.

NO. C 89-2860 MHP

CETUS CORPORATION, A DELAWARE CORPORATION,

DEFENDANT.

SAN FRANCISCO, CALIFORNIA
WEDNESDAY, FEBRUARY 6, 1991

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E.I. DU PONT DE NEMOURS & CO., INC.
LEGAL DEPARTMENT
WILMINGTON, DELAWARE 19898

BY: GEORGE A. FRANK, ESQ
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(APPEARANCES CONTINUED ON NEXT PAGE)

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WEDNESDAY, FEBRUARY 6TH, 1991 8:00 A.M.

(PROCEEDINGS HELD IN OPEN COURT, JURY PRESENT:)

THE COURT: GOOD MORNING, LADIES AND GENTLEMEN.

GOOD MORNING. AND YOU'RE GOING TO KEEP DOWN TO THE SPEED OF LIGHT AT LEAST?

THE WITNESS: I'VE HAD DISCUSSIONS WITH THE REPORTER.

THE COURT: OKAY.

GOOD MORNING, COUNSEL.

MR. FIGG: GOOD MORNING.

MR. PASAHOW: GOOD MORNING.

KARY BANKS MULLIS, DEFENDANT'S WITNESS, PREVIOUSLY SWORN

RESUMED

DIRECT EXAMINATION RESUMED

BY MR. PASAHOW:

Q. GOOD MORNING, DR. MULLIS.

A. GOOD MORNING, MR. PASAHOW.

Q. AT THE END OF YESTERDAY WE WERE TALKING ABOUT THE PERIOD AFTER YOU HAD RECEIVED YOUR AUTOMATED EQUIPMENT FOR MAKING THESE OLIGONUCLEOTIDES PIECES OF DNA, AND YOU HAD DONE YOUR COMPUTERIZATON AND YOU FOUND YOURSELF WITH SOME EXTRA TIME IN THE LABORATORY.

YOU WERE EXPLAINING WHAT YOU WERE DOING TO OCCUPY THAT TIME, AND WE CONCLUDED WITH MY ASKING YOU TO DRAW SOMETHING THAT YOU WERE DOING WITH YOUR FINGER AND OVERNIGHT YOU'VE DONE THAT

FOR US.

AND COULD I ASK YOU TO COME DOWN AND EXPLAIN WHAT YOU'VE DRAWN HERE.

A. YES. THIS WAS A -- THIS DIAGRAM REPRESENTS SORT OF A GENERAL PICTURE OF REPRESENTING A LOT OF LITTLE EXPERIMENTS THAT I DID.

I HAD -- SEVERAL OLIGONUCLEOTIDES SYNTHESIZED THAT WOULD BE COMPLIMENTARY TO EACH OTHER, SO THAT I WANTED TO STUDY IN A VERY SIMPLE WAY THE -- THE PROCESS BY WHICH YOU -- THEY ARE DOUBLE-STRANDED WITH EACH OTHER AT LOW TEMPERATURE THEN THEY BECOME SINGLE-STRANDED AS YOU RAISE THE TEMPERATURE.

I WOULD TAKE THE OLIGONUCLEOTIDES AT CERTAIN CONCENTRATION THAT WOULD MAKE THEM EASY TO SEE ON THE SPECTROMETER AND I HAD A LITTLE -- THE SPECTROMETER THAT I HAD HAD A DEVICE TO RAISE OR LOWER THE TEMPERATURE IN THE VAT AUTOMATICALLY.

IT WOULD PLOT THE CHANGE IN THE OPTICAL DENSITY WITH WHICH WE TALKED ABOUT YESTERDAY, SORT OF LIKE BEING THE SHADOW, THE THINGS MAKE THE LIGHT THEY ABSORB.

I COULD START THE EXPERIMENT DOWN AT 30 DEGREES SAY AND I COULD RAISE THE TEMPERATURE UP TO 80 AND WATCH IT. SO THERE WOULD BE A CERTAIN POSITION WHERE THE OPTICAL DENSITY WOULD CHANGE RATHER RAPIDLY. THAT POSITION IS WHAT I WAS MOST INTERESTED IN.

IT REPRESENTS THE PLACE WHERE HALF THE OLIGONUCLEOTIDE

ARE SINGLE-STRANDED AND HALF OF THEM ARE BOUND TO EACH OTHER AND THEY'RE DOUBLE-STRANDED. THAT'S THE THING I WANTED TO BE ABLE TO TELL PEOPLE.

I WANT TO BE ABLE TO PREDICT THAT THERE IS SEQUENCE OLIGONUCLEOTIDE, SO WHEN I MADE A OLIGONUCLEOTIDE AND DELIVERED TO IT SOME SCIENTISTS AT CETUS TO USE I COULD TELL HIM WHAT THAT TEMPRETURE WAS. I WAS DOING THESE EXPERIMENT HOPING IF I DID ENOUGH OF THEM, THAT I COULD FIGURE OUT A SORT OF GENERAL RULE WHERE I COULD GO FROM THE SEQUENCE TO THE TM WHICH IS WHAT THEY CALL THAT, THE MELTING TEMPERATURE.

I DIDN'T NEVER FIND THAT PARTICULAR RULE, BUT I NOTICE IN DOING THOSE EXPERIMENTS THAT I COULD RUN THE TEMPRETURE BACK AND FORTH I COULD DO THE EXPERIMENT, FREQUENT THE SINGLE STRANDS AT A HIGH TEMPERATURE DOWN, I COULD LOWER THE TEMPERATURE OR I COULD START FROM A DOUBLE-STRAND AND GO UP AND THIS CURVE WOULD APPEAR JUST ABOUT THE SAME (INDICATING).

IN FACT -- AND I REASONED FROM THAT I COULD DO THIS QUITE RAPIDLY, IN A MINUTE OR TWO. I COULD RAISE THE TEMPERATURE OR LOWER IT. AND I REASONED THAT IF, IN FACT, I GOT THE SAME SHAPE OF CURVE COMING DOWN MOVING RAPIDLY AS I GOT GOING UP, THAT THE PROCESS OF THEM COMING BACK TOGETHER WITH A RAPID PROCESS -- IT WAS NOT SOMETHING THAT SHOULD TAKE 15 OR 20 MINUTES BECAUSE IF IT TOOK 15 MINUTES AND I DROPPED THE TEMPERATURE AND JUST SAY THREE MINUTES ACROSS HERE (INDICATING) AND FOLLOWED THIS CURVE, IF IT HAD TAKEN 15 MINUTES THE CURVE

WOULD COME OUT HERE (INDICATING) AND THEN SLOWLY DROPPED DOWN.

THAT'S WHAT I REASONED. SO THAT WAS ONE OF THE LITTLE EXPERIMENTS THAT I DID AND I DIDN'T MAKE A BIG DEAL OUT OF IT AT THE TIME, BUT I CAME AWAY WITH THE IDEA THAT OLIGONUCLEOTIDES HYBRIDIZE FAIRLY RAPIDLY.

Q. WHEN YOU MADE THIS OBSERVATION THAT THE OLIGONUCLEOTIDES WOULD COME TOGETHER RATHER RAPIDLY, WAS THAT THE CONVENTIONAL WISDOM ON THE SUBJECT?

A. WELL, THE REASON THAT I SORT OF NOTICED IT IN A SENSE WAS THAT IT WAS NOT THE CONVENTIONAL WISDOM. AND, IN FACT, MOST PROTOCOLS FOR MY HYBRIDIZING OLIGONUCLEOTIDES AT THE CONCENTRATION SIMILAR TO WHAT I WAS USING LIKE, TEN MICROMOLES THE PROTOCOLS THAT PEOPLE FOLLOWED AT THE TIME TREATED THE PROCESS AS THOUGH IT TOOK A LONG TIME.

AND THEY -- PEOPLE WOULD HYBRIDIZE -- A TYPICAL PROTOCOL PEOPLE WOULD USE TO BUY NUCLEIC ACID TO MAKE IT SINGLE-STRANDED AND HAVING THE PROBE, IF THERE, OR A PRIMER, THE PROBES AND PRIMERS ARE THE SAME THING IN A WAY, THEY'RE OLIGONUCLEOTIDES.

HAVE -- THIS IS A GOOD WAY OF DESCRIBING IT: TAKE A BIG BEAKER OF WATER THEY WOULD HAVE A LITTLE TUBE FLOATING WITH A THING, LITTLE STYROFOAM COLLAR THAT KEPT IT FLOATING IN THE WATER. THEY HAD THAT BOILING TO MELT IT, THEN THEY JUST TAKE THE -- TURN OFF THE HOT PLATE AND LET THE TUBES SLOWLY COME DOWN TO ROOM TEMPERATURE.

AS THE LARGE BEAKERS THEY'RE FULL OF WATER SLOWLY COOL, RATHER THAN PULLING THE TUBE OUT AND LETTING IT DROP RAPIDLY OR PULLING IT OUT AND STICKING IT IN COLD WATER, THEY LET THE WHOLE THING SLOWLY COOL DOWN.

THOSE ARE THE KIND OF PROCEDURES THAT ARE IN THESE ARTICLES FROM BACK IN THE EARLY 70'S AND LATE 60'S. THAT'S THE WAY PEOPLE WERE DOING IT.

THEY HAD THE FEELING OLIGONEUCLEOTIDES ARE NEEDED TO INCUBATE, IS THE WORD THEY USED TO DESCRIBE THAT KIND OF THING, AND IT WAS -- IT SORT OF PORTRAYED THEIR FEELING ABOUT OLIGONUCLEOTIDES. THEY WERE VERY COMPLICATED LITTLE CREATURES AND THEY NEEDED TIME TO FIND THE RIGHT SPOT.

THEY DIDN'T THINK OF THEM AS BEING MOLECULES THAT EXIST IN A TIME FRAME THAT'S QUITE SHORT COMPARED TO WHAT THEY THINK OF, WERE HUGE AND SLOW COMPARED TO MOLECULES. AND SO MOST OF THE PROCEDURES THAT WERE AROUND AT THAT TIME, IN FACT, INVOLVED SLOW CAREFUL HYBRIDIZATION OF LONG TIMES.

WHAT WAS HAPPENING THE OLIGONUCLEOTIDES WERE REALLY BINDING QUICKLY AND PEOPLE WERE JUST OUT FOR LUNCH WHILE THAT WAS HAPPENING AND IT DIDN'T MATTER TO MOST PROTOCOLS, THEY GAVE MORE TIME FOR THAT PROCESS THAN WAS REALLY REQUIRED FOR MOST PURPOSES. PEOPLE NEEDED TO HYBRIDIZING OLIGONUCLEOTIDES IT DIDN'T MATTER THAT THEY WAITED AN EXTRA HOUR.

Q. DURING THE TIME YOU WERE DOING EXPERIMENTS IN YOUR LABORATORY, IN THIS PERIOD AFTER YOU HAD YOUR AUTOMATIC

EQUIPMENT, DOCTOR, DID YOU INVESTIGATE SEQUENCING METHODS THAT MIGHT BE USED IN YOUR LABORATORY AT CETUS?

A. WE DID. LARGELY AS -- IN RESPONSE TO THE SCIENTISTS WHO WE WERE MAKING THE OLIGONUCLEOTIDES FOR. THEY WEREN'T AS EASY TO CONVINCE AS WE WERE OURSELVES. THAT WE WERE QUITE CONFIDENT IN MAKING THE OLIGONUCLEOTIDES PRECISELY THE WAY WE WANTED THEM TO BE AND WE WEREN'T FOR A LONG TIME CONVINCED. IT TOOK US A WHILE TO BELIEVE IN OUR ABILITIES TO DO THAT.

BUT IN THE PERIOD OF WHERE THIS PROCESS WAS STILL FAIRLY NEW TO US WE GENERALLY HAD TO PROVIDE A SEQUENCE FOR EACH OF THE PRODUCTS THAT WE MADE. PARTICULARLY FOR VERY -- FOR ONES -- AND SO WE -- WE INVESTIGATED THE USE OF MAXIM GILBERT SEQUENCING AND ALSO SANGER SEQUENCING, BUT NEITHER OF THOSE METHODS REALLY WORKED VERY WELL WELL WITH OLIGONUCLEOTIDES WE FINALLY SETTLED WITH MAXIM GILBERT.

Q. WHY DIDN'T THE SANGER METHOD WORK WELL WITH THE OLIGONUCLEOTIDES?

A. WELL, THE SANGER METHOD REQUIRED A PRIMING SITE THAT YOU COULD USE WHERE YOU COULD PUT DOWN A PRIMER AND IF YOU'RE MAKING PRIMERS THEN YOU HAVE TO MAKE A PRIMER SITE ON THEM. WE WOULD HAVE HAD TO -- IT WAS NOT AN INSOLVABLE PROBLEM.

IT WAS LITTLE CLUMSY TO TRY TO USE THE SANGER METHOD. YOU HAD TO BUILD A -- HAVE TO MAKE A DONOR SORT OF TO FIT ONTO EVERY OLIGO THAT WE HAD MADE. IT WAS GOING TO TAKE A LOT OF WORK TO USE THE SANGER METHOD.

Q. THAT'S BECAUSE THEY WERE SO SHORT?

A. WELL, BECAUSE THEY WERE SHORT AND WHEN YOU'RE DOING SANGER SEQUENCING YOU WANT -- YOU NEED THIS PART THAT YOU WANT TO HAVE THE SEQUENCE FROM, NEEDS TO BE ADJACENT TO A SITE THAT YOU CAN PRIME. YOU WANT TO SAY START HERE AND SEQUENCE ACROSS THE UNKNOWN SEQUENCE.

WHEREAS WHEN YOU'RE MAKING AN OLIGONUCLEOTIDE IT'S ONLY GOING TO BE 20 BASE LONG IN THE FIRST PLACE. WE COULD HAVE PRIMED WITH PROBABLY A PENTAMER WE HAD MADE, BUT WE WOULD HAVE TO MAKE THAT SEPARATELY. IT WAS GOING TO BE A LOT OF SORT OF JUST LITTLE BIT MORE WORK THAN WE WANTED TO DO.

AFTER AWHILE WE WEREN'T REALLY WILLING TO INVEST A LOT OF WORK IN ANALYZING THE PRODUCTS WE WERE MAKING IN THAT MANNER BECAUSE WE WERE CERTAIN WE WERE MAKING THEM CORRECTLY AND WE HAD MORE ABREVIATED METHODS FOR FIGURING OUT FOR SURE WE HAD MADE THEM RIGHT. THAT BASE COMPOSITIONS AND SIZE AND STUFF LIKE THAT.

Q. COULD YOU EXPLAIN WHAT METHOD YOU DID USE TO FIGURE OUT THAT YOUE WERE MAKING THE RIGHT SHORT PIECES OF DNA FOR THE OTHER SCIENTISTS IS AT CETUS?

A. OKAY. WHAT WE FINALLY SETTLED ON WAS JUST -- WE DECIDED THAT WE -- WE COULD -- IF WE WOULD JUST PUT A RADIOACTIVE PHOSPHATE ON THE FIVE-PRIME END OF THESE THINGS, LIKE YOU HEARD ABOUT PUTTING IN PHOSPHATE LABELS, IF WE -- WE USED AN ENZYME CALLED OLIGONUCLEOTIDE KINASE, GET SOME RADIOACTIVE -- WHAT

WAS -- RADIOACTIVE ATP IS WHAT WE USED SORT OF WHAT THEY'VE BEEN TALKING ABOUT ONE OF THE A'S, THAT ENZYME WILL ALLOW US TO STICK A RADIOACTIVE PHOSPHORUS, ADD THEM ON THE FIVE-PRIME END OF THE OLIGONUCLEOTIDE.

HAVING DONE THAT WE KNEW THAT IT HAD FIVE-PRIME END, BUT WE COULD NOT DO THAT TO IT, SO THAT WAS PART OF THE CHARACTERIZATION WE COULD DO THAT TO IT.

THEN WE WOULD TAKE THE OLIGONUCLEOTIDE, WE TAKE A MIXTURE THAT CONTAINED THE RADIOACTIVE OLIGONUCLEOTIDE, PUT IT ON A GEL, ELECTRPHORETIC GEL, WHICH IS A METHOD FOR SEPARATING THINGS ACCORDING TO SIZE.

IF WE PUT IT ON THERE WITH A COUPLE OF PREVIOUSLY MADE OLIGOS THAT WE KNEW THE SIZE OF, THEN WE RAN IT BESIDE THAT, SAID THIS IS SUPPOSED TO BE 20 LONG; THAT ONE THAT WE MADE LAST WEEK WAS 15; THE ONE WE MADE A MONTH AGO WAS 25, IT'S RUNNING RIGHT NEAR THOSE TWO, RIGHT BETWEEN THEM. THAT WOULD BE GOOD EVIDENCE IT WAS THE RIGHT SIZE.

THEN IF WE DIGEST THE OLIGONUCLEOTIDE WITH AN ENZYME THAT BROKE IT DOWN TO THE BASIC NEUCLEOTIDE BUILDING BLOCK AND ANALYZE THAT PICTURE WE WOULD GET -- WE COULD BREAK IT DOWN INTO THE FOUR BASIC BLOCKS AND WE COULD SAY WHAT RATIOS SHOULD THOSE NOW BE.

IN OTHER WORDS, IF WE SUPPOSE TO HAVE FOUR A'S AND THREE T'S, TWO C'S AND G IN THERE ONCE WE LOOKED AT THOSE BLOCKS AFTER BREAKING DOWN THE OLIGO DOWN WITH AN ENZYME THEY SHOULD

APPEAR IN THOSE RATIOS. AND WE HAD A LITTLE DEVICE THERE IN THE LAB THE CALLED AN HPLC. WE HAD SOME FANCY EQUIPMENT. THAT WAS ONE OF THE THINGS THAT WAS REALLY PLEASANT ABOUT WORKING AT CETUS.

WE COULD EASILY DETERMINE THE COMPOSITION, SAY, IN BASES OF NEUCLEOTIDES, OF A MIX OF NEUCLEOTIDES THEY'RE TEN P'S FOUR G'S, THREE C'S AND AN A, AND THAT INFORMATION WOULD SAY THAT -- PLUS THE SIZE THAT WE ALREADY DETERMINED PRETTY WELL CHARACTERIZE THE SEQUENCING.

WE STILL WOULDN'T KNOW FOR SURE IF THEY WERE ARRANGED IN THE RIGHT ORDER THAT WE HAD TRIED TO ARRANGE THEM IN, BUT THE PROBLEMS THAT WE WOULD HAVE IN OLIGONUCLEOTIDE SYNTHESIS AT THAT POINT WERE NOT PROBLEMS OF ARRANGING THEM IN ORDER.

IF WE'D HAD A PROBLEM WITH AN OLIGO THE PROBLEM WOULD USUALLY BE WE LEAVE OUT ONE, SO THAT WOULD AFFECT THE RATIO, WE'D SEE THAT. WE WOULDN'T KNOW WHICH ONE WE LEFT OUT, WE WOULD KNOW WE LEFT ONE OUT.

THE PROBLEMS WERE PRETTY FAR, WE REALLY DIDN'T HAVE ANY PROBLEMS AFTER AWHILE SO THAT BECAME A VERY STANDARD THING. IT WAS -- WE WERE VERY SURPRISED IF WE EVER CAME UP WITH ONE THAT WAS WRONG.

Q. WE'RE GOING TO BE TALKING ABOUT GELS LATER, SO COULD I ASK YOU TO COME DOWN HERE AND SHOW US HOW A GEL TOLD YOU THE DIFFERENT SIZE OF THESE OLIGONUCLEOTIDES?

A. OKAY. PEOPLE HAVE PROBABLY ALREADY HEARD THIS THREE OR FOUR

TIMES IN DIFFERENT VERSIONS.

THEY SORT OF -- I'LL DRAW YOU JUST LIKE -- THESE THINGS ARE MADE BETWEEN TWO GLASS PLATES. JUST REAL THIN. THEY CALL IT GELS FOR THE SAME REASON YOU CALL IT JELLO. IT'S -- GEL IS SOMETHING THAT'S NOT QUITE A SOLID AND IT'S NOT QUITE A LIQUID EITHER. SO IT'S STIFF ENOUGH TO STAY BETWEEN THESE TWO GLASS PLATES. IF YOU WERE TO PULL THE GLASS PLATES AWAY FROM IT IT WOULDN'T STAND UP BY ITSELF.

YOU MAKE IT SO IT HAS THESE LITTLE WELLS AT THE TOP OF IT AND I'M NOT REALLY MUCH OF AN ARTIST. THESE ARE -- THEY ARE LITTLE SPACES BETWEEN THE GLASS PLATE. GET THE IDEA? IT'S LIKE THERE'S A PLATE HERE (INDICATING) ON THE FRONT AND ONE IN THE BACK, THEN THESE LITTLE WELLS HERE (INDICATING).

SO YOU CAN TAKE A SYRINGE AND AND FILL THIS WITH -- PUT A LITTLE LIQUID DOWN INTO THIS SPOT RIGHT THERE (INDICATING), SO YOU MAKE THE GEL LIKE THAT. YOU MAKE IT BY LETTING IT SOLIDIFY WITH A LITTLE THICK LIKE A CONE IT'S LEFT WITH THESE LITTLE WELLS SO YOU CAN NOW ADD THE SAMPLE TO.

LET'S SAY WE ADDED A SAMPLE TO THAT ONE SAMPLE WE DON'T KNOW WHAT IS IS. YOU PUT ONE IN HERE CALLED OUR MARKERS OR OUR GEL MARKERS. THE MARKERS WOULD BE SOME PREVIOUSLY MADE OLIGONUCLEOTIDES THE SIZES OF WHICH WE ALREADY ARE FAMILIAR WITH.

SO WE PUT A COUPLE OF NEUCLEOTIDES HERE (INDICATING) THAT HAVE BEEN FIVE-PRIME LABELS AND ARE RADIOACTIVE. THE

REASON WE LABEL THEM IS BECAUSE IT'S HARD TO SEE THEM OTHERWISE, BUT IF YOU LABEL THEM WITH RADIOACTIVE, WITH P32, THEN HELD A PIECE OF, LIKE, X-RAY FILM UP TO THIS THING, WHEREVER THE P32 WAS IT LEAVES SPOTS ON THE FILM. SO WE CALL THAT AN AUTORADIOGRAM. SOUNDS LIKE SOMETHING YOU GET IN THE MAIL.

ANYHOW, ONCE YOU PUT THESE SAMPLES IN HERE YOU WOULD STICK THIS ASSEMBLY INTO A THING CALLED A GEL BOX, AND THAT WOULD ALLOW YOU TO HAVE -- TO HAVE AN ELECTRIC FIELD SETTLE ACROSS THIS, SO THIS WOULD BE A VERY NEGATIVE -- SAY, THIS WOULD BE 1,000 VOLTS NEGATIVE COMPARED TO THIS DOWN HERE (INDICATING) WHICH WOULD BE -- THIS IS THE POSITIVE SIDE.

THE OLIGONUCLEOTIDES ALL HAVE A NEGATIVE CHARGE ON THEM, SO THEY'RE GOING TO BE ATTRACTED TO THE BOTTOM OF THE GEL OR TO WHEREVER THIS PART IS. IT CAN BE UP, IT'S NOT GRAVITY THAT'S PULLING THEM DOWN, IT'S THE FIELDS, SO EVERYTHING NEGATIVE IS GOING TO MOVE IN THAT DIRECTION (INDICATING) WHEN YOU TURN ON THE POWER. YOU GET YOUR FINGERS OUT OF THE BOX.

SO AFTER ABOUT AN HOUR THE OLIGONUCLEOTIDES IN THE MARKER LANE WOULD, SAY, DISTRIBUTE THEMSELVES DOWN HERE ACCORDING TO HOW BIG THEY WERE. THIS WOULD BE A 32 NEUCLEOTIDE ONE; THIS WOULD BE A 25 ONE; THIS WOULD BE 25 AND IF YOU HAD A 15 ONE IT WOULD RUN RIGHT OFF INTO THE WATER. OR IF YOU STAYED TO LONG IN THE LUNCH ROOM WHILE THESE WERE RUNNING ALL OF THEM WOULD RUN OFF IN THE WATER.

THERE WAS LIKE WATER DOWN IN THE BOTTOM THAT'S HOW YOU

PUT THE FIELD ON IT. IF YOU CATCH THEM JUST THE RIGHT TIME THEY'RE RUNNING DOWN THE PLATE, THEN YOU CAN PULL THIS WHOLE THING OFF OF THE GEL BOX, TURNING OFF THE POWER, TAKE ONE OF THE PLATES CAREFULLY OFF.

YOU HAVE TO PULL THE PLATES APART NOW BECAUSE THE RADIOACTIVE PARTICLES COMING OFF OF THESE THINGS WON'T GO THROUGH THE GLASS, YOU SO YOU PULL THE PLATES APART, TAKE A PIECE OF SARAN WRAP, LAY IT OVER THE SORT OF MOIST JELLY KIND OF THING, THAT'S THE GEL MATERIAL, SO THAT YOU DON'T WET THE X-RAY FILM.

YOU NOW TAKE IT IN THE DARKROOM, LAY A PIECE OF X-RAY FILM ON TOP OF IT AND JUST LET IT SIT THERE QUIETLY FOR MAYBE AN HOUR OR TWO, DEPENDING HOW RADIOACTIVE THINGS ARE.

WHEN YOU COME BACK YOU DEVELOP THAT X-RAY FILM, IF YOU'RE LUCKY YOUR TWO OLIGONUCLEOTIDES WILL BE SOMEWHERE IN THE RANGE THAT YOU HAVE STANDARDS FOR.

IF ONE OF THEM IS WAY UP HERE (INDICATING) IT WAS THE FIRST 40 MER I HAD MADE YOU SAY, AH-HAH, THE BIGGER THEY ARE THE SLOWER THEY RUN ON THE THING. THEY'RE TRYING TO FIGHT THEIR WAY THROUGH THIS GEL MATERIAL WHICH IS KIND OF GIVING THEM SOME RESISTANCE AND THE LITTLE ONES GO THROUGH IT EASIER THAN THE BIGGER ONES.

SO I THINK THAT'S A LESSEN IN GELELECTROPHORESIS.

Q. ONE MORE THING, SOMETIMES WE'VE HEARD YOU LOOK AT THEM UNDER ULTRAVIOLET LIGHT, HOW DOES THAT WORK?

A. FEW HAVE A LARGE ENOUGH AMOUNT -- REMEMBER WE WERE TALKING ABOUT PUTTING THEM IN A SPECTROMETER, AND THEY DO ABSORB ULTRAVIOLET LIGHT -- FEW HAVE ENOUGH OF THE MATERIAL ON THERE THAT IT WOULD ABSORB AN APPRECIABLE AMOUNT OF, SAY, ULTRAVIOLET LIGHT, THAT WOULD COME OUT OF A HAND-HELD -- ONE OF THESE LITTLE -- YOU HAVE BLACK LIGHT HAND-HELD LAMP THAT WILL MAKE MINERALS GLOW, THAT SORT OF THING.

IF YOU HAVE ENOUGH MATERIAL THERE YOU CAN DETERMINE THAT THEY'RE IN AND THEY'RE ABSORBING THE ULTRAVIOLET LIGHT BY THE FACT THAT THEY -- THEY SORT OF WILL THROW A SHADOW ON SOMETHING BEHIND IT.

YOU CAN'T SEE ULTRAVIOLET LIGHT, YOU WOULDN'T SEE THE SHADOW, BUT IF YOU HAVE SOMETHING BEHIND THE -- THE GEL THAT WOULD ABSORB ULTRAVIOLET LIGHT WOULD FLUORESCE IF YOU TAKE THEM IN A DARKROOM. YOU HAVE A WHITE T-SHIRT ON YOU GO UNDER A BLACK LIGHT IT BRIGHTLY FLUORESCES, IT LOOKS A LITTLE ELECTRIC.

THERE'S A LOT OF MATERIAL THAT YOU CAN PUT BEHIND A GEL AND THEN SHINE A LIGHT ON TOP OF, IF THERE WAS SOMETHING ABSORBING THE -- YOU'D HAVE LIGHT YOU SEE DARK SPOT ON WHAT'S OTHERWISE A BRIGHT WHITE BACKGROUND YOU HAVE -- GETS ABSORBED BASICALLY THROWING A SHADOW ON THE BACKGROUND.

MR. PASAHOW: BEFORE WE GO ON, MS. MORIYAMA HAS POINTED OUT I OMITTED TO MARK THE LAST DRAWING OF THE OLIGONUCLEOTIDES ANNEALING AND COMING APART, SO I SHOULD DO THAT FIRST.

THE CLERK: DEFENDANTS B-252 MARKED FOR IDENTIFICATION

ONLY.

(DEFENDANT'S EXHIBIT B-252
MARKED FOR IDENTIFICATION)

MR. PASAHOW: THEN THE DRAWING DR. MULLIS JUST DID OF THE GEL SHOULD ALSO BE MARKED.

THE CLERK: DEFENDANTS B-253 MARKED FOR IDENTIFICATION ONLY.

(DEFENDANT'S EXHIBIT B-253
MARKED FOR IDENTIFICATION)

Q. (BY MR. PASAHOW) NOW, DURING THIS PERIOD, DR. MULLIS, WERE YOU AWARE OF WORK THAT WAS GOING ON IN THE OTHER LABORATORIES AT CETUS?

A. I WAS VERY MUCH AWARE OF IT. PARTICULARLY THE LABORATORIES THAT WERE CLOSE TO MINE, BUT SINCE OUR GROUP WAS MAKING OLIGONUCLEOTIDES FOR ANY OF THE LABORATORY AT CETUS THAT NEEDED THEM, I WAS -- I MEAN, I WOULD GO AND HAVE CONFERENCES WITH OTHER SCIENTISTS TO TALK TO THEM ABOUT HOW MANY OLIGONUCLEOTIDES THEY WERE GOING TO WANT US TO MAKE AND TRY TO TALK THEM INTO HAVING LESS AND THEY TRY TO TALK ME INTO MORE.

SO I USUALLY, IN THE PROCESS OF THAT KIND OF NEGOTIATION, I WOULD FIND OUT EXACTLY WHAT IT WAS THEY WERE GOING TO USE THE OLIGONUCLEOTIDES FOR.

I REALLY WAS MUCH MORE AWARE OF THE WORK AROUND THAT HAD TO DO WITH OLIGONUCLEOTIDES THEN IN GENERAL, BUT I KNEW EVERYTHING THAT WAS GOING ON DUE TO THE FACT THAT -- I MEAN, IN

SOME WAY BECAUSE WE HAD -- THE SCIENTISTS AT CETUS WERE CALLED ON EVERY THREE MONTHS OR EVERY FOUR MONTHS, I CAN'T REMEMBER EXACTLY HOW OFTEN, BUT EVERY FEW MONTHS YOU WOULD HAVE TO GIVE A SORT OF AN ACCOUNTING OF WHAT YOUR LAB HAD BEEN DOING FOR THE LAST THREE OR FOUR MONTHS.

SO WE HAD TO HAVE A SEMINAR IN FRONT OF THE REST OF THE COMPANY AND TELL THEM WHAT YOU BEEN DOING, SO I WENT TO ALL OF THOSE. THAT WAS ANOTHER WAY THAT I WAS INFORMED.

Q. WERE YOU AWARE OF THE WORK THAT WAS GOING ON IN DOCTOR ~~ERLICH~~'S LABORATORY?

A. HIS LABORATORY WAS RIGHT NEXT TO MINE AND THERE WAS -- WE HAD A PARTICULARLY ACTIVE INTER-COMMUNICATION BECAUSE WE HAD A DOOR BETWEEN THE TWO LABS. AND ALSO HIS -- ONE OF HIS TECHNICIANS OR ONE OF THE PEOPLE WORKING IN THAT LAB, RANDY ~~SAIKI~~, WAS SORT OF AN NIGHT OWL AND I WAS, TOO, WE END UP BEING IN THE BUILDING 10:00 OR 11:00 O'CLOCK AT NIGHT. SO WE TALKED TO EACH OTHER A LOT ABOUT WHAT WAS GOING ON IN BOTH LABS.

Q. WHAT WAS GOING ON IN DOCTOR ERLICH'S LABORATORY AT THIS TIME?

A. THEY WERE INTERESTED IN -- THERE WERE SEVERAL PROJECTS, BUT THE ONE MOST INTERESTING TO ME WAS A PROJECT THAT WAS -- HAD AS ITS ULTIMATE AIM TO DEVELOP METHODS WHEREBY OLIGONUCLEOTIDES COULD BE USED TO DETERMINE THE -- LIKE A SINGLE BASE PAIR IN A PARTICULAR GENE THAT MIGHT BE ONE THAT WAS KNOWN AT THE TIME TO SOMETIMES HAVE MUTATION THAT WOULD CAUSE A GENTIC DISEASE,

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THAT'S SORT OF A ROUND ABOUT WAY.

LET ME GIVE A SPECIFIC EXAMPLE. THE THING THEY WERE USING AS A MODEL SYSTEM, WHEN WE TALK ABOUT MODEL SYSTEMS I'M NOT SURE IF THAT'S CLEAR, IT'S SORT OF HERE'S A NICE TEST SYSTEM THAT CAN YOU MAKE, DEVELOP SOMETHING IN. FIRST -- A FIRST TRY AT IT KIND OF THEN YOU CAN, HAVING DEVELOPED IT IN ONE SYSTEM, YOU CAN NOW USE THAT PROCESS IN ANOTHER.

THE MODEL SYSTEM WHICH WAS A REAL CLINICAL PROBLEM WASN'T JUST A MODEL SYSTEM THEY HAD CHOSEN, WAS A VERY WELL STUDIED GENETIC DISORDER CALLED SICKLE CELL ANEMIA. THAT WAS ONE OF THE VERY FIRST DISEASE THAT -- FIRST GENTIC DISEASE, I THINK, TO BE CHARACTERIZED AT THE MOLECULAR LEVEL.

PEOPLE BY THEN HAD KNOWN FOR SOMETIME THAT PRECISELY WHICH NUCLEOTIDE WOULD BE CHANGED IN THAT TO CAUSE THAT DISEASE. ONE PARTICULAR BASE PAIR THAT WERE A T WOULD GO TO AN A AND THAT WOULD MAKE -- THAT WAS THE MUTATION THAT CAUSED THAT DISEASE.

SO WHAT THEY WERE TRYING TO DO WAS TO DEVELOP A METHOD THEY COULD FIND OUT QUICKLY FROM A CLINICAL SAMPLE THAT CONTAINED DNA WHETHER OR NOT THE -- THAT -- IF GENES IN THAT PARTICULAR SAMPLE CONTAINED THAT MUTATION, WOULD BE A WAY TO SCREEN PEOPLE FOR -- WHETHER -- FIND OUT WHETHER OR NOT THEY WERE A CARRIER OF THAT.

MOST OF THE PEOPLE THAT HAVE THAT MUTATION HAVE IT IN A FORM THAT'S COMPLETELY -- IT DOESN'T CAUSE THEM TO HAVE THE DISEASE BECAUSE THEY ONLY CARRY ONE GENE LIKE THAT AND THERE'S

ANOTHER GENE WAS CALLED THE WILD TYPE, BUT IT'S IMPORTANT TO KNOW WHO THOSE CARRIERS ARE BECAUSE IF THEY MATE WITH EACH OTHER THERE'S SOME CHANCE THEIR OFFSPRING WILL HAVE BOTH COPIES OF THE MUTANT GENE.

WE WANTED TO DEVELOP A REALLY RAPID AND RELIABLE METHOD THAT COULD BE USED TO SCREEN FOR THAT GENE.

Q. WHAT'S CALLED THE WILD TYPE IS THE NORMAL GENE FOR HEMOGLOBIN?

A. RIGHT. THEY CALL IT WILD TYPE FOR HISTORICAL REASONS, I GUESS. THE PEOPLE THAT HAVE IT IN THE WILD.

Q. BEFORE WE TALK ABOUT YOUR INVENTION OF PCR, COULD I ASK YOU TO SUMMARIZE FOR US WHAT YOU BELIEVE TO BE THE ABILITIES THAT YOU BROUGHT TO THAT WHICH MADE IT POSSIBLE FOR YOU TO MAKE THE INVENTION?

A. WELL, YOU WANT ME TO REALLY START AND WAX PHILOSOPHIC HERE?

I HAD A LOT OF NICE EQUIPMENT AVAILABLE, BUT I CAME -- WHEN I CAME TO CETUS I CAME WITH A LONG -- I MEAN, MY BACKGROUND WAS VARIED IN LOTS OF FIELDS, BUT WHEREVER I HAD BEEN I HAD ALWAYS BEEN INVENTIVE. SINCE -- THAT I THINK WAS THE KEY TO THE WHOLE THING IS THAT I ALWAYS SINCE I WAS A KID LIKED TO MAKE THINGS.

I ENJOYED, YOU KNOW, PICKING APART AN OLD WASHING MACHINE AND FINDING SOMETHING TO DO WITH THE SOLENOIDS IN THERE BESIDES PUTTING IN A WASHING MACHINE. OR TAKING SUGAR AND TESTING NITRIDE AND MELTING IT IN MY BACKYARD AND MAKING ROCKET

FUEL. I HAD A LONG TRADITION IN MY LIFE IN THAT AND I WAS NEVER VERY GOOD IN FOLLOWING RECIPES. WHEN I COOK I DON'T HAVE A COOKBOOK, I DON'T LIKE TO DO THAT.

WHEN I DO A PROCEDURE IN A LABORATORY I WOULD RESIST GOING TO SOMEBODY SAYING, HOW EXACTLY DO YOU DO THIS? GIVE ME YOUR EXACT WAY OF DOING IT. I JUST LIKE TO SIT DOWN AND THINK HOW COULD THIS BE DONE IN A LOT EASIER WAY USUALLY.

I WAS NEVER GOOD. YOU KNOW, I PLAY THE PIANO BUT I CAN'T READ MUSIC. I'M NOT VERY -- THAT'S A ORNERY TRAIT ALMOST IN A WAY.

YOU WANT ME TO GO ON ABOUT THAT?

Q. NO. I ACTUALLY HAD IN MIND --

THE COURT: I THINK WE GOT THE PICTURE.

Q. (BY MR. PASAHOW) -- WHAT SCIENTIFIC BACKGROUND THAT YOU BROUGHT?

A. I HAD ORGANIC CHEMISTRY, OBVIOUSLY, AND I HAD SORT OF A -- SINCE I HAD BEEN INTERESTED IN PHYSICS, I'VE ALWAYS BEEN INTERESTED IN MATHEMATICS.

SO MY ORGANIC CHEMISTRY WAS SUPPLEMENTED BY MY KNOWLEDGE OF THE PHYSICAL SIDE -- WHAT THEY CALL PHYSICAL ORGANIC CHEMISTRY.

I WAS INTERESTED IN THOSE KIND OF EXPERIMENTS, LIKE I WAS DOING THOSE MELTINGS. I WAS REALLY INTERESTED IN THE MATHEMATICS OF THAT PROCESS, HOW COULD YOU CALCULATE WHAT THAT CURVE WAS GOING TO LOOK LIKE, JUST KNOWING THE SEQUENCE OF THE

OLIGONUCLEOTIDE. SO I TAKEN A LOT OF MATH COURSES AND PHYSICS COURSES AND STUFF, SO I HAD A GOOD FEELING FOR THAT.

I ALSO HAD -- WITH REGARD TO PCR, I HAD BEEN DOING SOMETHING AT THE TIME I WAS -- OBVIOUSLY, I WASN'T THINKING I'M PREPARING MYSELF TO INVENT PCR, BUT -- I DIDN'T REALLY KNOW I WAS GOING TO, BUT I WAS WRITING A LOT OF COMPUTER PROGRAMS.

AND FROM WRITING THE PROGRAMS, I HAD DEVELOPED A REAL APPRECIATION FOR WHAT HAPPENS WHEN YOU MAKE A LOOP IN A COMPUTER PROGRAM. IN FACT, THE BEAUTY OF COMPUTER PROGRAMS IN A LOT OF WAYS IS THAT YOU CAN BUILT UP LOOPS IN THEIR REITERATIVE PROCESS.

YOU ASK THE COMPUTER -- YOU SAY, "DO THIS," AND YOU WRITE DOWN THE CODE; SAY, "DO THIS AND DO THAT," AND YOU CAN DO THAT; AND THEN CHANGE THIS COUNTER FROM A-1 TO 2; THEN GO BACK AND DO THIS AGAIN.

THE WHOLE THING, YOU HAVE TO WRITE IT ONCE AND THEN THE COMPUTER DOES IT OVER AND OVER AND OVER AGAIN. THAT'S ONE OF THE BEAUTIES OF THE COMPUTER, IS THAT THEY DON'T MIND DOING THINGS OVER AND OVER AGAIN.

BIOCHEMISTS IN 1984, AND FOREVER BEFORE THAT, IF SOMETHING CHANGED IN '84, BUT BEFORE '84, BIOCHEMISTS WOULD NEVER THINK ABOUT DESIGNING AN EXPERIMENT THAT REQUIRED THEM TO DO SOMETHING OVER AND OVER AND OVER AGAIN. THEY JUST DIDN'T THINK OF THEMSELVES AS AUTOMATONS, TO SAY, "OKAY. I CAN DO SOMETHING 20 TIMES."

YEAH, SURE. I WOULD NEVER DO ANYTHING 20 TIMES.

I WOULD HAVE NEVER HAVE THOUGHT ABOUT THAT EXCEPT FOR THE FACT THAT I HAD SEEN HOW EFFECTIVE THAT CAN BE IN A COMPUTER PROGRAM, AND HOW -- AND I UNDERSTOOD FROM THE SORT OF MATHEMATICAL STANDPOINT REALLY HOW RAPIDLY AN EXPONENTIAL PROCESS, WHEN, WHERE YOU TAKE A THING AND YOU DO SOMETHING TO IT, AND THEN YOU CAN -- THAT BECOMES THE THING THAT YOU DID SOMETHING TO, AND YOU DO THAT TO IT AGAIN AND AGAIN, LIKE THAT REITERATIVE PROCESS.

I UNDERSTOOD THE MATHEMATICS. IT WAS NOT LIKE ANY DEEP PROFOUND WISDOM. IT'S JUST THAT I HAD PLAYED WITH ENOUGH SYSTEMS WHERE THAT HAPPENED TO SEE THAT YOU CAN REALLY GET TO -- QUICKLY GET TO BIG NUMBERS.

IF YOU SET UP THE LITTLE PROCESS THAT IS REITERATIVE, THEY CAN GENERATE VERY LARGE NUMBERS VERY QUICKLY. AND THAT WAS -- THAT WAS SOMETHING IN MY HEAD THAT WAS HANGING AROUND.

AND WHEN I HAD MY MAJOR SORT OF INSIGHT INTO HOW THE FACT THAT PCR COULD HAPPEN, THAT WAS SOMETHING THAT IMMEDIATELY CAME TO BEAR, AND I SAID, "HOLY" WHATEVER. AND I UNDERSTOOD SUDDENLY -- I WAS QUICKLY ABLE TO REALIZE THAT TEN CYCLES OF A PROCESS THAT DOUBLED SOMETHING WOULD GIVE YOU A THOUSAND COPIES OF IT.

THAT'S SOMETHING THAT WAS ALREADY IN MY -- I DIDN'T HAVE TO REALLY GO THROUGH IT VERY SLOWLY.

I THINK THOSE --

Q. NOW, YOU DESCRIBED THE INVENTION OF PCR IN AN AR
WROTE FOR SCIENTIFIC AMERICAN?
A. YES.
Q. LET ME SHOW YOU WHAT'S IN EVIDENCE AS EXHIBIT A-52.
IS THAT A COPY OF THE ARTICLE?
A. YES, THIS IS A RATHER POOR COPY OF IT. I'M GETTING USED TO LOOKING AT POOR COPIES OF THINGS IN THIS TRIAL.
THE COPIES OVER AND OVER AGAIN, YOU FINALLY -- YES, IT LOOKS LIKE TO ME, BUT THEY ALL LOOK PRETTY MUCH THE SAME.
Q. AND THAT WAS PUBLISHED IN THE APRIL 1990 ISSUE OF SCIENTIFIC AMERICAN?
A. YES.
Q. ~~NOW, WHERE WERE YOU WHEN YOU CONCEIVED THE PCR INVENTION?~~
A. I WAS DRIVING ONE NIGHT IN MY CAR, AND I WAS ON HIGHWAY 128 HEADED FROM CLOVERDALE TO A LITTLE CABIN THAT I HAVE IN MENDOCINO COUNTY.
Q. WHAT WERE YOU THINKING ABOUT THAT NIGHT?
A. I WAS THINKING ABOUT WHAT I WAS WORKING ON IN THE LAB AND WHAT I WAS GOING TO BE WORKING ON THE NEXT WEEK.
THAT PARTICULAR WEEK, I HAD BEEN THINKING ABOUT TRYING TO FIND A BETTER WAY TO DO THE PROBLEM THAT HENRY ERLICH'S LAB WAS WORKING ON.
THE WAY THEY WERE GOING ABOUT THE PROBLEM WAS A REASONABLE WAY -- SORT OF A REASONABLE WAY, BUT I DIDN'T PARTICULARLY LIKE IT BECAUSE IT REQUIRED -- IT WOULD WORK FOR

SICKLE CELL BECAUSE THERE WAS A -- FORTUNATELY THERE WAS A RESTRICTION SITE RIGHT WHERE THE CHANGE IN THE BASE PAIRS IS.

BUT THE RESTRICTION SITES -- THERE'S AN ENZYME THAT WILL CUT IN THAT SITE WHEN ITS PARTICULAR -- WHEN IT HAS A PARTICULAR SEQUENCE. WHEN IT DOESN'T HAVE THAT PARTICULAR SEQUENCE, THAT ENZYME WON'T CUT.

THEIR METHOD WAS DEPENDING UPON THE FACT THERE WAS A RESTRICTION SITE RIGHT WHERE THE MUTATION LAY, SO YOU CAN USE THAT RESTRICTION ENZYME AS A METHOD FOR DETERMINING WHETHER IT WAS THERE OR NOT.

THAT WAS A COMPLICATED SITUATION THEY WERE SETTING UP. I WAS TRYING TO THINK OF WHAT I WOULD THINK WAS A BETTER APPROACH, BECAUSE IT WOULD WORK ANYWHERE. IT WOULDN'T REQUIRE THE RESTRICTION. SO YOU COULD USE IT FOR ANY PARTICULAR DISEASE.

AND I WAS -- DO YOU WANT ME TO DESCRIBE THAT PROCESS?

Q. PLEASE.

A. THAT'S GOING TO REQUIRE ME TO COME DOWN THERE. EITHER THAT OR USING THE DIAGRAMS.

Q. YOU HAVE A DIAGRAM IN THE SCIENTIFIC AMERICAN ARTICLE OF YOUR IDEA?

A. IF YOU HAVE A CLEAN VERSION IN THERE, WE CAN PROBABLY USE THAT.

Q. I'LL SEE IF I CAN PUT THIS ON THE TELEVISION AND WE CAN ALL SEE IT.

(PAUSE IN THE PROCEEDINGS)

THE WITNESS: IF I CAN GET TO -- CAN I GET IN THE JURY BOX, YOUR HONOR?

THE COURT: NO, NO.

THE WITNESS: WELL, LET'S TURN THIS AROUND A LITTLE SO I CAN SEE IT.

THIS IS SORT OF SYMBOLIC OF THE -- THIS IS NOT THE BETA-GLOBIN GENE I WAS ACTUALLY WORKING WITH, SO THE SEQUENCE THERE IS JUST AN ARBITRARY SEQUENCE, BUT IT REPRESENTS THE NOTION THAT WE KNEW THESE SEQUENCES. IF YOU KNOW THIS SEQUENCE, YOU KNOW THIS ONE. I GUESS YOU GATHERED THAT SO FAR.

SO YOU ONLY HAVE TO KNOW ONE OF THEM. YOU CAN -- THAT IMPLIES THE OTHER ONE.

BUT WE -- IN THE CASE OF THE SICKLE CELL GENE, OR IN THE BETA-GLOBIN GENE, WHICH HAS A SICKLE CELL MUTANT IN IT, WHICH ALSO HAS A WILD TYPE, THE -- THERE'S ONE PARTICULAR BASE PAIR THAT YOU DON'T KNOW, THAT'S WHAT YOU'RE TRYING TO FIND OUT.

IF YOU WANT TO KNOW -- IN THIS PARTICULAR CASE, I THINK THAT'S A G UP THERE AN A C THERE, BUT IN THE CASE OF THE B BETA-GLOBINS, IT'S AN A-T -- IT'S EITHER AN A-T OR THE A, WE DON'T KNOW WHICH. WE'RE TRYING TO FIND OUT.

THE PROCEDURE I WAS PONDERING, WELL, YOU KNOW, IF WE CAN MAKE AN OLIGONUCLEOTIDE THAT WILL BIND SPECIFICALLY TO THIS STRAND AND, FOR THE TIME BEING, JUST THINK ABOUT THIS SETUP RIGHT HERE, DON'T WORRY ABOUT THIS, IT'S THE MIRROR IMAGE OF IT,

KIND OF.

IF I CAN MELT THE STRANDS APART IN THE DNA SAMPLE AND THEN HYBRIDIZE CAREFULLY AN OLIGONUCLEOTIDE RIGHT ADJACENT TO THE BASE PAIR THAT I'M INTERESTED IN, OR TO THE BASE, AND SO THIS WOULD BE THE THREE-PRIMED END OF THE OLIGONUCLEOTIDE, THE ONE THAT IT CAN ADD NUCLEOTIDE UNITS TO, AND PUT POLYMERASE IN THERE, IF I CAN DO THAT, AND THEN I COULD -- IF I COULD DEVISE A METHOD WHEREBY I COULD EXTEND IT BY JUST ONE BASE AND THEN RE-ISOLATE THE EXTENDED OLIGONUCLEOTIDE AND FIGURE OUT WHICH BASED HAD BEEN ADDED TO THE END OF IT, THEN I SHOULD BE ABLE TO KNOW WHAT THAT BASE WAS, BECAUSE IF IT ADDED A C, THEN I WOULD KNOW THAT WAS A G, OKAY? IF IT ADDED A T, I WOULD KNOW THAT WAS AN A.

I THOUGHT THAT PROCESS -- IT SEEMED TO ME LIKE -- REASONABLE THAT THAT MIGHT WORK, BUT LOTS OF THINGS ON PAPER LOOKED LIKE THEY MIGHT WORK. AND I THOUGHT IT MIGHT BE KIND OF MESSY BECAUSE THERE'S SO MUCH -- THERE'S SO MANY GENES AND HUMAN DNA THAT MY OLIGONUCLEOTIDE, ALTHOUGH IT MATCHES THE SEQUENCE PERFECTLY HERE MIGHT ALSO FALL IN OTHER PLACES AND PICK UP THE WRONG BASE.

SO I THOUGHT THERE MIGHT BE A LOT -- IT MIGHT BE ALL KIND OF THINGS ACTUALLY.

SO WHAT I DECIDED TO DO AS A WAY OF ASSURING MYSELF THAT WHAT I THOUGHT WAS GOING TO HAPPEN WOULD HAPPEN AND AS A WAY OF -- I WAS THINKING OF PUTTING THIS IN. EVERY TIME I DO

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THIS, I'LL USE A CONTROL OLIGONUCLEOTIDE WHICH WILL --

THIS THING IS MAKING A TERRIBLE NOISE HERE.

THE COURT: HOLD IT JUST A LITTLE WAYS AWAY FROM YOUR MOUTH.

THE WITNESS: OKAY. I'VE THOUGHT IF, IN DOING THIS PROCEDURE, IF I WOULD EMPLOY ALWAYS A CONTROL OLIGONUCLEOTIDE, THEN -- ON THE OTHER STRAND, THEN IF THE ONE OLIGONUCLEOTIDE WERE TO PICK UP A G, IF I PUT ANOTHER OLIGONUCLEOTIDE ON THE OTHER STRAND POINTED TOWARDS THE SAME BASE PAIR, BUT THE OTHER SIDE OF IT, THEN IF THIS ONE PICKS UP A C, THEN THIS ONE OUGHT TO PICK UP A G; IF IF THIS ONE PICKS UP AN A, THIS ONE OUGHT TO PICK UP A T, BECAUSE THESE TWO BASES WERE ORIGINALLY COMPLEMENTS OF EACH OTHER.

SO THE TWO THAT WOULD GO IN THERE WOULD BE COMPLEMENTARY TO THEM, WOULD THEN BE COMPLEMENTARY TO EACH OTHER ALSO.

SO I SAID I'LL SET UP AN EXPERIMENT WHERE I PUT TWO OLIGONUCLEOTIDES IN, BOTH POINTED TOWARDS THE SAME BASE PAIR, BUT ON DIFFERENT STRANDS AND I'LL ADD -- INSTEAD OF ADDING THE NORMAL NUCLEOTIDES WHICH, CAN YOU REMEMBER, THEY HAVE TWO ENDS. THEY HAVE A FIVE-PRIME AN THREE-PRIME END, SO THEY CANNOT ONLY BE STUCK ON THERE BUT ALSO HAVE SOMETHING STUCK ON THEM.

THERE ARE THESE CHEMICALLY AVAILABLE THINGS CALLED DIDEOXYNUCLEOTIDES. THAT'S WHAT THESE LITTLE RED THINGS ARE THAT SAY DDC. YOU'VE CAN BUY THE DIDEOXYNUCLEOTIDES.

IF YOU ADD ONE OF THOSE, IT STOPS THE CHAIN; IT WON'T GROW ANY MORE. THAT'S A MECHANISM YOU CAN USE IF YOU JUST WANT TO GET ONE THING STUCK ON THERE.

THESE WILL GO IN JUST LIKE THE NORMAL NUCLEOTIDES. THEY'LL GO IN C'S ACROSS FROM G'S AND A'S ACROSS FROM T'S.

I WAS THINKING, IF YOU MADE THESE RADIOACTIVE SO THAT ONCE THEY WENT IN, THEY MAKE THE OLIGONUCLEOTIDE RADIOACTIVE, AND BY USING THE GEL TO SEPARATE THE OLIGONUCLEOTIDES IN AN EXPERIMENT, I COULD FIGURE OUT WHICH RADIOACTIVE TRIPHOSPHATE THEY HAD TAKEN UP.

AND I COULD GO INTO THAT, BUT I DON'T THINK IT'S NECESSARY FOR THIS EXPLANATION.

THERE WAS A SIMPLE WAY I WOULD BE ABLE TO TELL WHICH ONE HAD GONE IN.

SO BASICALLY I HAD TO DECIDE WHETHER OR NOT THAT SORT OF THING WOULD WORK. AND I WAS THINKING ABOUT THAT THAT NIGHT WHEN I WAS DRIVING IN THE CAR, AND I REMEMBERED FROM, LIKE, WHEN I WAS AT WORKING AT UC, IS IF -- THERE WAS A GUY THERE IN THE LAB WHO WAS -- HE WAS DOING A PROJECT TRYING TO MEASURE PRESENCE OF THE NUCLEOTIDE DERIVATIVES IN HUMAN BLOOD.

I HAD THIS FEELING, I SAID, WELL, I MIGHT GET A CLINICAL SAMPLE HERE, LIKE A BLOOD SAMPLE USUALLY IS THE SORT OF THING THEY WILL USE. I'LL PURIFY THE DNA FROM THAT TO DO THIS AS A CLINICAL PROCEDURE, AND IT MIGHT HAVE ALREADY IN IT AS AN IMPURITY SOME OF THE NORMAL NUCLEOTIDES.

SO THAT WOULD COMPLICATE MY EXPERIMENT. THE WAY I HAD SET THIS UP IT DEPENDED COMPLETELY ON THE FACT THAT ONLY THE DIDEOXY OR THE TERMINATING NUCLEOTIDE DERIVATIVES WOULD BE IN THE SOLUTION. IF I PUT SOME -- IF I PUT SOME NORMAL NUCLEOTIDES IN THERE, THREE OR FOUR OF THOSE MIGHT ADD BEFORE THE RADIOACTIVE TERMINATING NUCLEOTIDE ADDED, SO IT MIGHT BE IN FRONT OF THE WRONG BASE WHEN IT DID THAT.

IF IT MOVED, IF THE CHAIN GREW DOWN HERE, THEN WHEN THE THING WENT IN, IT MIGHT NOT BE -- REFLECT THE BASE -- IT WOULDN'T REFLECT THE BASE PAIR AT THIS LOCATION.

SO I HAD TO BE CONCERNED. I SAID, WELL, HOW CAN I GET RID OF THEM IN AN EASY WAY?

IN A CLINICAL PROCEDURE THAT PEOPLE ARE GOING TO DO IN A HOSPITAL, YOU DON'T WANT IT TO BE REALLY DIFFICULT.

I WAS THINKING, WHAT WOULD BE A SIMPLE WAY TO SET THIS THING UP SO THAT IF THERE WAS A NEUCLEOTIDE IN THERE THAT CAME IN WITH THE SAMPLE, THAT THEY COULD BE DESTROYED WITHOUT THE DIDEOXYNUCLEOTIDES THAT I WAS GOING TO PUT IN BEING DESTROYED?

SINCE THEY'RE VERY SIMILAR CHEMICALLY, I THOUGHT, WELL, LET'S SEE, THERE'S A LOT OF ENZYMES THAT WILL DESTROY NUCLEOTIDE DERIVATIVES. THEY'RE -- LIKE THE POLYMERASE HAPPENS TO BE ONE OF THOSE; IN FACT, IT DOESN'T DESTROY THEM BUT CHANGES THEM FROM BEING A FLOATING-AROUND FREE BASE TO ONE THAT'S STUCK INTO A GROWING CHAIN.

THAT DIDN'T OCCUR TO ME IMMEDIATELY. FIRST, I WENT

THROUGH A BUNCH OF OTHER ALTERNATIVES. I'VE THOUGHT OF OTHER ENZYMES I DO ADD TO THE CLINICAL SAMPLE THAT WOULD GET RID OF ANY NUCLEOTIDES THAT WERE IN THERE.

THE ONLY REALLY CHEAP AND RELIABLE ENZYME THAT I KNEW ABOUT WAS ONE CALLED BACTERIAL ALKALINE PHOSPHATATES, BAP. THAT'S THE ONLY ONE THAT I KNEW ABOUT. AND THAT HAD A REALLY NASTY LITTLE PROPERTY, WHICH WAS THAT YOU COULD DESTROY IT BY HEATING IT.

BUT IT WAS KNOWN IN THE LITERATURE AT THE TIME THAT IF YOU COOLED THE SOLUTION OFF, ONCE YOU DESTROYED IT, IT WOULD LOSE ITS STRUCTURE WHEN YOU HEATED IT. BUT WHEN YOU COOL THE SOLUTION OFF IN SIMILAR FASHION TO HOW SOME OF THESE OLIGONUCLEOTIDES ROLL UP, AGAIN, WHEN YOU COOL, THEY'LL HAVE OR FORM STRUCTURES. THIS PROTEIN WOULD REFORM ITSELF INTO A FORM THAT WAS STILL ACTIVE.

SO IF I HAD PUT THE BACTERIAL PHOSPHATATES INTO A T CLINICAL SAMPLE TO GET RID OF THE NUCLEOTIDES, THEN I WOULD -- AND I COULD HAVE THEN HEATED THE SAMPLE TO DESTROY THE PHOSPHATATES.

BUT WHEN I COOLED IT OFF AND TRIED TO ADD MY DIDEOXY THE PHOSPHATATES WOULD REFORM AND IT WOULD DESTROY MY DIDEOXY, WHICH WERE FAIRLY EXPENSIVE, AND I DIDN'T WANT ANY OF THEM TO GET DESTROYED.

SO I THOUGHT THAT THAT'S NOT A GOOD WAY TO DO IT. I'VE THOUGHT OF A LOT OF OTHER WAYS.

I REMEMBER I SAID, WAIT, THE POLYMERASE ENZYME I'M GOING TO END UP USING HERE DOES JUST THAT: IT TAKES NUCLEOTIDES OUT OF SOLUTION AND STICKS THEM ONTO OLIGONUCLEOTIDES.

AND SO I THOUGHT, WHY DON'T I TAKE THE CLINICAL SAMPLE, PUT THE OLIGONUCLEOTIDES IN IT, DON'T PUT THE RADIOACTIVE TERMINATING NUCLEOTIDES IN, AND JUST PUT THE POLYMERASE IN AND JUST LET IT SIT FOR AWHILE, SORT OF DO A MOCK REACTION.

SO I LET IT SIT FOR A WHILE.

IF THERE ARE ENOUGH NUCLEOTIDES IN THERE, THAT WOULD GET IN THE WAY. IT WOULD ADD THEM TO THE ENDS OF THE PRIMER.

BUT NOW IF I BOIL THE SOLUTION AGAIN AFTER IT HAD TIME FOR THAT TO HAPPEN, I CAN -- I WILL MELT WHATEVER -- WHATEVER NUCLEOTIDES HAD BOUND TO WHATEVER OLIGONUCLEOTIDES HAD BOUND AND BE EXTENDED ON THE DNA, THEY WOULD MELT OFF.

NOW, IF I HAD A VAST EXCESS LIKE ABOUT A HUNDRED THOUSAND- OR MILLION-FOLD, WHAT I WAS THINKING OF, THE OLIGONUCLEOTIDES, WHEN I COOLED THE SOLUTION, THE NEW OLIGONUCLEOTIDES WOULD COME BACK TO THE SITES THAT I WAS INTERESTED IN EXPLORING, RIGHT?

THE BETA-GLOBIN SITE, BECAUSE THE OLD ONES WOULD MELT AWAY AND WOULD BE LOST IN THE SOLUTION, AND THE NEW ONES THAT HAD NOT BEEN EXTENDED BY ANY BASES WOULD COME IN, AND SO THEY WOULD NOW HAVE THE THREE-PRIME END POSITION EXACTLY OVER THE BASE PAIR THAT I WAS INTERESTED IN DISCOVERING.

THE OTHER ONES WOULD JUST BE OVER IN THE SOLUTION

SOMEWHERE.

THEN I COULD ADD MY DEOXY. I COULD DO MY SINGLE BASE POSITION, AND I COULD ILLUSTRATE THESE TWO AND TELL ME WHAT WAS THE BASE PAIR AT THAT POSITION. NEAT. BECAUSE NOW I ONLY NEED ONE ENZYME. I DON'T HAVE TO HAVE TWO ENZYMES IN THIS PROCEDURE. I WOULD HAVE TO HAVE ONE ENZYME AND USE IT TWICE.

WHEN I CAN SEE -- SINCE YOU HAVE ALL BEEN STEEPED NOW IN THE POLYMERASE CHAIN REACTION, THIS LITTLE DIAGRAM UP HERE LOOKS A LOT LIKE A POLYMERASE CHAIN REACTION.

OKAY. I'VE NOW HAD THAT IN MY HEAD, BECAUSE I HAD DESIGNED IT FOR ANOTHER PURPOSE, BUT IT WAS STILL FLOATING THERE.

AND I SAID, WOULD THERE BE ANY PROBLEMS? I WAS KIND OF PLEASED WITH MYSELF, HAVING SOLVED THAT PROBLEM SO NEATLY.

WELL, I'LL USE THE SAME ENZYME TWICE. ANY PROBLEMS THAT ARE GOING TO BE CAUSED BY THE FACT THAT THERE MIGHT BE A LOT OF NUCLEOTIDES IN THERE AT FIRST, AND THESE OLIGONUCLEOTIDES THAT BIND IN THERE MIGHT EXTEND QUITE A WAYS ON DOWN, MAYBE THEY'LL PICK UP 40 OR 50 BASES BEFORE THE SUPPLY OF NUCLEOTIDES THAT CAME IN WITH THE SAMPLE RUNS OUT.

SO IF THEY PICK UP 40 OR 50 BASES AND THEY'RE STILL IN MY SOLUTION, ALTHOUGH THEY'RE MELTED OFF THE SITE, THEY'LL STILL BE FLOATING AROUND. WILL THEY CAUSE ANY TROUBLE? ARE THEY LIKELY TO INTERFERE WITH THE PROCEDURE?

IT OCCURRED TO ME THIS WAS VERY MUCH OF A FLASH OF

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INSIGHT. IT SEEMS OBVIOUS BECAUSE WE'VE SEEN THIS HERE.

I WAS DRIVING AT NIGHT. I HADN'T SEEN THIS BEFORE IT OCCURRED TO ME THAT IF IN FACT THERE WERE ENOUGH NUCLEOTIDES IN THERE TO EXTEND THE OLIGONUCLEOTIDE LONG ENOUGH THAT THE EXTENSION PRODUCT OF THIS OLIGONUCLEOTIDE WOULD FORM -- HAVE ON IT THIS SEQUENCE BECAUSE IT WOULD BE COMPLEMENTARY TO THAT AND THEREFORE IT WOULD OFFER ANOTHER SITE FOR THIS OLIGONUCLEOTIDE TO BIND.

SO I WOULD HAVE GENERATED BASICALLY A COPY OF THIS WHOLE SEQUENCE BY TAKING AN OLIGONUCLEOTIDE AND EXTENDING ON IT. AND THAT COPY OF THIS SEQUENCE WOULD LOOK LIKE THAT SEQUENCE, AND THAT SEQUENCE WOULD BIND THIS OLIGONUCLEOTIDE.

AND I SAID, I WILL HAVE MY -- I SAID SOMETHING REALLY LOUD, THAT THAT WILL DOUBLE THE SEQUENCE. IT WON'T INTERFERE. IT WILL RAISE THE LEVEL OF RADIOACTIVE NUCLEOTIDES THAT I'LL BE ABLE TO INCORPORATE.

AND THE NASTIEST PROBLEM WITH ANY KIND OF DNA DIAGNOSTICS AT THAT POINT WAS THE FACT THAT THE SIGNAL THAT YOU COULD EXPECT TO GET THE AMOUNT OF RADIOACTIVITY THAT YOU COULD ACTUALLY EXPECT TO HAVE INCORPORATED INTO AN OLIGONUCLEOTIDE BY A PROCEDURE LIKE THIS WAS EXTREMELY LOW BECAUSE, ALTHOUGH THERE WAS A LOT OF -- A DNA SAMPLE HAD A LOT OF NUCLEOTIDES IN IT BUT NOT HAVE MANY REPRESENTING ANY ONE SITE.

SO YOU COULD EXPECT TO GET, LIKE, ONE OR TEN MICROGRAMS, AT THE MOST, OF DNA FROM A CLINICAL SAMPLE AND THERE

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WOULD BE IN THERE A CERTAIN NUMBER OF COPIES OF ANY PARTICULAR WHAT'S CALLED A SINGLE-COPIED GENE. YOU CAN GET ONE PER CELL, SO THERE WOULDN'T BE MANY COPIES OF IT.

SO ANY DNA PROCEDURE YOU WERE PLANNING LIKE THIS HAD TO DEAL WITH THE FACT YOU NEEDED AN EXTREMELY SENSITIVE SYSTEM TO BE ABLE TO SEE IT AT ALL.

AND I HAD JUST REALIZED THAT I COULD MAKE -- I COULD DOUBLE THE SIGNAL. IT WAS REALLY A NEAT LITTLE THING THAT THIS DOES.

AND THEN IT OCCURRED TO ME, OF COURSE, THAT NUCLEOTIDES WERE FAIRLY CHEAP, AND THEY WERE SOLUBLE IN WATER, AND THEY WERE LEGAL IN CALIFORNIA, AND THAT I COULD PUT THEM IN THERE MYSELF -- I COULD PUT IN ALL THE NUCLEOTIDES I WANTED TO -- AND THAT I COULD REPEAT THIS PROCESS OVER AND OVER AGAIN MYSELF.

AND I HAD -- I WOULD HAVE A LOOP REITERATIVE PROCESS THAT WOULD GO AROUND AND AROUND, AND EVERY TIME IT DID, IT WOULD DOUBLE THE AMOUNT OF THE SITE THAT WAS AVAILABLE IN THE SOLUTION. IT WOULD JUST GO UP, JUST LIKE TEN TIMES WOULD GIVE ME 1,000; 20 TIMES -- I HAD TO SIT DOWN AND MAKE SURE 20 TIMES WOULD GIVE ME A MILLION. I KNEW TEN TO 1,000, IT WOULD. IT WOULD GIVE ME A MILLION COPIES. I JUST HAD TO DO IT 20 TIMES AND I HAVE A MILLION COPIES OF A PARTICULAR SITE I WAS INTERESTED IN.

I HAD PULLED THE CAR OFF THE ROAD, AND I WAS JUST DRAWING LITTLE THINGS, LIKE THESE LITTLE DIAGRAMS, THINGS SORT

OF LIKE THIS WITH JUST LINES AND STUFF. I DIDN'T HAVE A LOT OF PAPER IN THE CAR, EITHER. I WAS WRITING ON ENVELOPES.

AND IT JUST OCCURRED TO ME PRETTY QUICKLY THAT WHAT WOULD ACCUMULATE WOULD NOT BE JUST SOME NUMBER OF NUCLEOTIDES LONG, LIKE THE VERY FIRST TIME I DID THIS, THAT THERE WOULD BE A PRODUCT FORMED WHICH WOULD HAVE ANY NUMBER OF -- THERE WOULD BE NO WAY OF STOPPING IT.

Q. (BY MR. PASAHOW) WE HAVE A BETTER DIAGRAM FOR THAT.

A. THANK YOU, MR. PASAHOW.

THIS IS MORE LIKE THE KIND OF THINGS I WAS DRAWING. THIS IS ALSO A LITTLE MORE ELABORATE. THIS ILLUSTRATES THE IDEA.

NOW, THIS IS WHERE WE WERE BACK THERE, EXCEPT IT WAS IN YELLOW.

THESE TWO OLIGONUCLEOTIDES, THE FIRST TIME THAT THEY GOT EXTENDED WOULD BE -- THEY WOULD BE ON A TEMPLATE THAT HAD AN INDEFINITE LENGTH DNA THAT YOU ISOLATE NATURALLY ON REALLY, REALLY LONG MOLECULES. WE HAVE HAVE NOT PICTURED HERE VERY ADEQUATELY, BUT WE HAD -- THEY WERE REALLY LONG, AND THE ENDS ARE NOT WELL DEFINED. THEY DON'T HAVE LITTLE DOTS ON THE END, SO THEY GO OFF IN BOTH DIRECTIONS FOR THOUSANDS OF BASES.

AND WHEN YOU START -- I EXPECTED EXTENDING AN OLIGONUCLEOTIDE ON IT WOULD PRODUCE SOMETHING THAT WENT OFF SOME NUMBER OF BASES UNTIL THE ENZYME FELL OFF OR UNTIL SOMETHING HAPPENS.

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SO I'D BE GETTING A LOT. FROM ONE EXTENSION ON THE DNA, YOU GET THE THING OF INDEFINITE LENGTH. IT'S SOMETHING I LATER ENDED UP CALLING THE LONG PRODUCT, BUT AT THE TIME, I JUST HAD A PICTURE OF THIS LONG THING BEING CALLED -- BUT I REALIZED THAT WHEN, IN THE SECOND ROUND OF THIS PROCESS, A -- THE OTHER PRIMER MADE -- NOT THE ONE THAT MADE THE FIRST STRAND.

THIS STRAND RIGHT HERE IN THIS DIAGRAM WAS MADE FROM THIS PRIMER BEING SENT TO THERE; THIS STRAND RIGHT HERE WOULD HAVE ONE END DEFINITE, BECAUSE THAT WOULD BE THE END THAT CAME FROM THE FIVE-PRIME END OF THE PRIMER IT MADE.

SO THAT ONE I KNOW, BECAUSE WE SYNTHESIZED IT IN THE LAB TO STOP RIGHT THERE.

WHEN THE OTHER OLIGONUCLEOTIDE PRIMED THAT AND EXTENDED DOWN, IT -- WHEN IT CAME TO THAT END THAT WAS DEFINITE, IT WOULD MAKE SOMETHING THAT HAD BOTH ENDS FIXED, SO IT HAD A DEFINITE LENGTH, AND THE LENGTH WOULD BE THE DISTANCE BETWEEN THE FIVE-PRIME END OF ONE OF THE OLIGONUCLEOTIDES AND THE FIVE-PRIME END OF THE OTHER ONE.

WHILE I WAS -- WHEN I STARTED DRAWING THESE LITTLE THINGS, THESE DIAGRAMS, ON A PIECE OF PAPER, I REALIZED, I DON'T HAVE TO HAVE THESE OLIGONUCLEOTIDES SITUATED DIRECTLY BESIDE THE BASE PAIR. ~~BY THEN, I'D FORGOTTEN ALL ABOUT THE PROBLEM OF DIAGNOSIS~~.

I REALIZED THIS IS A PROCESS THAT I CAN USE ANYWHERE I WANT TO AMPLIFY A LITTLE SECTION OF DNA AND I CAN TAKE THE

OLIGOS AND I CAN MOVE THEM JUST BY CHANGING THEIR SEQUENCE. I CAN HAVE THEM BE AS FAR APART AS I WANT TO. I COULD MAKE THEM 400 BASE PAIRS APART.

EVEN THEN, IF I DO THIS PROCESS OVER AND OVER, I'LL GENERATE A PIECE OF DOUBLE-STRANDED DNA THAT WILL HAVE A 400 BASE PAIR SIZE. IT WILL REPRESENT THAT SEQUENCE THAT IS BETWEEN THE SPOT THAT THOSE TWO OLIGONUCLEOTIDES SAT DOWN.

LIKE THESE THINGS START POPPING UP, THESE LITTLE PIECES HERE THAT HAVE EXACTLY A DEFINE SITE, IT TOOK A LOT OF SORT OF THINKING ABOUT THAT BEFORE IT REALLY SANK INTO ME WHAT I HAD. BUT IT -- BY THE WEEKEND -- BY THE END OF THE WEEKEND, I HAD DRAWN ALL OVER EVERYTHING IN MY CABIN. I GOT IN THE CABIN FINALLY AND I STAYED UP ALL NIGHT DRAWING LINES ALL OVER EVERYTHING AND TRYING TO FIGURE OUT WHY THIS WOULDN'T WORK.

BECAUSE I REALLY COULDN'T BELIEVE THAT SOMEBODY HAD NOT DONE THIS ALREADY. I SAID, WHY, THERE'S SOMETHING I'M NOT SEEING HERE. MAYBE TOMORROW MORNING, I'LL FIGURE THIS OUT.

BUT THE NEXT DAY, IT WAS STILL THERE, AND IT WILL WORK. I KNOW IT WILL WORK.

AND I THINK THAT'S PRETTY MUCH HOW IT HAPPENED. IT WAS FUN.

Q. I TAKE IT YOU WERE EXCITED BY YOUR INVENTION?

A. I FELT I WAS REAL WIZARD, ALL OF A SUDDEN.

Q. NOW, WHEN YOU WENT BACK TO THE CETUS LABORATORY OVER IN EMERYVILLE ON MONDAY MORNING AFTER THIS WEEKEND, DID YOU

CONTINUE TO WORK ON YOUR INVENTION?

A. WELL, BY WORKING ON IT AT THAT POINT, I WAS BASICALLY THINKING ABOUT IT, AND THE FIRST THING I DID IN TERMS OF WORKING WAS TO GO UP TO THE LIBRARY AND HAVE A LITERATURE SEARCH DONE TO SEE IF I COULD FIGURE OUT WHY IT HADN'T BEEN DONE, FOR ONE THING, AND IF IT HAD BEEN DONE.

I KNEW IF IT HAD BEEN DONE SUCCESSFULLY, I WOULD HAVE HEARD ABOUT IT. IT WAS TOO PHENOMENAL. IF THAT HAD ACTUALLY EVER BEEN DONE, NOBODY WOULD HAVE FORGOTTEN ABOUT IT.

THAT WOULD HAVE BEEN IN THE WORLD, AND PEOPLE WOULD BE USING IT. I KNEW THAT.

I THOUGHT ABOUT IT AND REALIZED I WAS GOING TO BE A FAMOUS PERSON. AND YOU DON'T TAKE AN INVENTION THAT WOULD MAKE YOU FAMOUS AND JUST THROW IT AWAY.

I SAID, SOMETHING MUST BE WRONG. THERE MUST BE SOMETHING THAT KEEPS IT FROM WORKING, SO LET'S LOOK THROUGH THE LITERATURE AND FIND SOMEBODY THAT'S DONE THIS AND FAILED, AND FAILED IN A BIG ENOUGH WAY THEY WOULD HAVE SAID, HERE'S WHY IT DIDN'T WORK. IT WOULD BE NEAT IF IT WOULD WORK, BUT HERE'S WHY.

SO I LOOKED THROUGH THE LITERATURE, THE TITLES OF ALL THE PAPERS I COULD GET IN THE LITERATURE SEARCH, AND LOOKING FOR SOMETHING LIKE, HERE'S HOW WE COULDN'T DO THIS.

YOU DON'T USUALLY FIND THAT. I THOUGHT, IF SOMEBODY HAD PUBLISHED ON IT AND REALLY REALIZED WHY IT WOULDN'T WORK, THEY WOULD HAVE WRITTEN SOME KIND OF PUBLICATION. AND I LOOKED

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FOR IT AND I COULDN'T FIND ANYTHING.

IT'S DIFFERENT FROM WHAT YOU NORMALLY DO. IN A SITUATION LIKE THAT, YOU GO TALK TO PEOPLE. I AM FAIRLY TALKATIVE ANYHOW, AND A LOT OF PEOPLE COMING THROUGH MY LAB LOOKING TO SEE IF WE FINISHED THEIR OLIGONUCLEOTIDES. IF WE HADN'T, WHAT WAS I DOING WRITING ALL THIS WEIRD STUFF ON MY DESK?

I TOLD EVERYONE I COULD FIND AT CETUS ABOUT THE IDEA AND ASKED THEM IF THEY EVER HEARD OF ANYBODY DOING IT AND TRIED TO EXPLAIN TO THEM IN FACT, WHEN THEY SAID NO, WE HADN'T, I SAID --

MR. FIGG: YOUR HONOR, WE'RE GETTING INTO HEARSAY HERE.

THE COURT: WELL, IN A MANNER OF SPEAKING.

THE WITNESS: I COULDN'T FIND --

THE COURT: WHY DON'T WE HAVE ANOTHER QUESTION?

MR. PASAHOW: WELL, YOUR HONOR, AS TO THE RESPONSE OF HIS COLLEAGUES, THAT IS ONE OF THE THINGS WE INTEND TO BRING OUT TO SHOW THAT IN FACT THE CONVENTIONAL WISDOM WAS THAT THIS NOTION WOULDN'T WORK.

THE COURT: I UNDERSTAND. WE'RE REALLY GETTING INTO LONG NARRATIVES. MAYBE SHORT PUNCHY QUESTIONS AND ANSWERS.

Q. (BY MR. PASAHOW) DID YOU DISCUSS THE INVENTION WITH YOUR COLLEAGUES IN THOSE FIRST FEW DAYS AFTER YOU GOT BACK?

A. WITH MANY OF THEM, WITH EVERYTHING, WITH EVERYBODY THAT I COULD GET TO LISTEN.

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Q. WERE ANY OF YOUR COLLEAGUES AS EXCITED AS YOU WERE ABOUT THIS NEW IDEA?

A. NO. THEY WERE MORE INTERESTED IN THEIR OLIGONUCLEOTIDES THAT WERE PERHAPS LATE, OR WHATEVER, BUT THEY WEREN'T INTERESTED. THEY WERE AMAZINGLY RESILIENT TO THE IDEA, I THOUGHT. I JUST COULDN'T BELIEVE NO ONE ELSE GOT EXCITED ABOUT IT.

Q. DID ANY OF THEM SUGGEST TO YOU THAT IT WOULDN'T WORK?

A. MANY OF THEM SUGGESTED THAT IT WOULDN'T WORK. AND THEY WOULD SORT OF -- THEY WOULD FIRST, I THINK, JUST THINK THAT IT WOULDN'T WORK; THEN THEY TRIED TO THINK OF A REASON WHY IT WOULDN'T WORK. BUT SOMETIMES THEY OFFERED REASONS WHY IT WOULDN'T WORK. BUT EVEN MY GIRLFRIEND DIDN'T THINK IT WOULD WORK.

Q. WAS YOUR GIRLFRIEND A SCIENTIST?

A. SHE WAS AN OLIGONUCLEOTIDE CHEMIST.

Q. WHEN DID YOU FIRST FORMALLY PRESENT THIS IDEA TO YOUR COLLEAGUES AT CETUS?

A. WELL, IT WOULD HAVE BEEN IN A TALK THAT I GAVE IN ~~AUGUST~~ OF ~~1983~~, WHICH WAS ONE OF THE ONES THAT I HAD TO GIVE. EVERY THREE OR FOUR MONTHS, I HAD TO GO DOWN AND TELL THEM WHAT I HAD BEEN DOING, AND I -- IN THE COURSE OF THAT, I MENTIONED THIS. I DESCRIBED THIS IDEA.

I PUT SOME DIAGRAMS UP ON THE SCREEN AND EXPLAINED WHAT I THOUGHT WAS A MAGNIFICENT CREATION AND I -- IT WAS THE FIRST

TIME I HAD TALKED ABOUT IT IN A MEETING WERE THERE WERE A LOT OF PEOPLE, AND I EXPECTED THERE WOULD BE THEN -- BY THEN, I THOUGHT A LOT OF PEOPLE WOULD SEE IT AND I'D DESCRIBE IT IN A WAY THEY WOULD GET EXCITED ABOUT IT.

Q. WHAT WAS THE RESPONSE?

A. A COUPLE OF TECHNICIANS ACTUALLY DID COME DOWN AFTER THE TALK AND THEY WERE EXCITED ENOUGH TO SAY THEY'D BE INTERESTED IN -- YOU KNOW, THAT THEY DIDN'T -- NEITHER OF THEM WORKED FOR ME, BUT THEY WERE INTERESTED IN HEARING MORE ABOUT IT.

MOST OF THE PEOPLE EITHER LEFT THE ROOM BEFORE I WAS DONE OR IN A -- SORT OF STAYED AROUND TO HECKLE. YOU KNOW, THEY WERE FRIENDS OF MINE. THEY WERE USE TO ME HAVING REALLY BIZARRE IDEAS, AND THEY JUST THOUGHT THIS WAS ONE MORE OF THOSE.

Q. DID YOU START DOING SOME WORK TOWARDS ACTUALLY FINDING OUT WHETHER THE IDEA WOULD WORK IN THE LAB?

A. WELL, I HAD SOME OLIGONUCLEOTIDES SYNTHESIZED THAT -- THE FIRST -- I ~~DID THE FIRST EXPERIMENT, I THINK, IN SEPTEMBER 1983.~~

Q. WHEN WAS THE DRIVE THAT YOU TOLD US ABOUT?

A. I THINK IT WAS APRIL, MAY, JUNE. THE BUCKEYES WERE BLOSSOMING, I KNOW THAT. AND THAT HAPPENS AROUND -- I DIDN'T -- YOU KNOW, I LOT OF THE -- MOST OF MY RECORDS ON THIS THING I WAS WRITING ON MY COMPUTER. I WAS IN LOVE WITH MY COMPUTER BY THEN AND I AM NOT SURE EXACTLY WHEN, BUT I THINK IT'S APRIL, MAY 1983.

Q. NOW, BETWEEN THAT TIME AND WHEN YOU DID YOUR FIRST

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LABORATORY EXPERIMENT, DID YOU DO ANY KIND OF LITERATURE SEARCH OR RESEARCH TO TRY AND FIND OUT HOW YOU MIGHT GO ABOUT DOING THIS?

A. I DID A LOT OF JUST, YOU KNOW, QUESTIONING MY FRIENDS WHO KNEW THINGS, YOU KNOW, TO FIND OUT HOW THEY WOULD GO ABOUT IT. I WENT TO THE LITERATURE AND I LOOKED UP POLYMERASE AND, YOU KNOW, TRIED TO FIGURE OUT WHAT ITS PROPERTIES WERE, AND VARIOUS SOURCES AND BOOKS AND PAPERS.

I DID A LOT OF THINKING ABOUT WHAT SORT OF RATES OF REACTION I WOULD EXPECT, AND MOST OF THAT WAS JUST EDUCATED GUESSING. I DON'T THINK THERE WAS AVAILABLE -- OR AT LEAST I COULDN'T FIND IT. THERE WERE NO ANSWERS AVAILABLE TO QUESTIONS THAT -- A LOT OF THE QUESTIONS THAT I HAD TO SORT OF GUESS AT.

AND THEY WERE THINGS TO DO WITH: HOW FAST DOES THIS ENZYME WORK? HOW LONG SHOULD YOU HAVE TO WAIT FOR THIS TO HAPPEN? WHAT -- HOW MUCH OF THE ENZYME SHOULD YOU ACTUALLY TRY TO USE?

PEOPLE HAD RECIPES FOR VARIOUS PROCEDURES THAT THEY DID, BUT THOSE RECIPES WERE NOT RELEVANT, REALLY, TO WHAT I WAS INTERESTED IN.

Q. DID YOU COME UP WITH A SET OF CONDITIONS THAT YOU HOPED WOULD WORK?

A. WELL, I CAME UP WITH LOTS OF DIFFERENT POSSIBILITIES, AND ~~THE FIRST EXPERIMENT THAT I TRIED DEFINITELY DID NOT WORK~~. AND IT WAS -- IT WAS -- MY STANDARD PRACTICE WHEN I WAS TRYING TO DO

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SOMETHING NEW WOULD BE TO TRY THE EASIEST POSSIBLE THING, THE THING THAT I THOUGHT GIVEN, YOU KNOW, THE BLESSINGS FROM ALL THE CHEMICALS AND EVERYTHING, IF EVERYTHING WORKS PERFECTLY, WAS THE EASIEST THING TO DO BECAUSE, IF THAT WORKS, YOU DON'T HAVE TO DO ANY HARDER ONES.

SO I TOOK A COUPLE OF OLIGONUCLEOTIDES THAT I HAD SYNTHESIZED THAT WOULD BIND -- THAT SHOULD BIND TO -- WHAT HAD JUST BEEN PUBLISHED BY GENENTECH, A SEQUENCE FOR A THING CALLED HUMAN NERVE GROWTH FACTOR.

I PICKED A FOUR HUNDRED BASE REGION OF THAT; I MAKE TWO OLIGONUCLEOTIDES THAT WOULD BIND TO IT; I PUT THEM TOGETHER WITH SOME HUMAN DNA, SOME TRIPHOSPHATES, SOME DNA POLYMERASE, AND I WENT HOME.

I SAID LET IT SIT OVERNIGHT AND MAYBE THE WHOLE PROCESS WILL HAPPEN BY ITSELF. MAYBE I WON'T REALLY HAVE TO HEAT AND COOL AND HEAT AND COOL, BECAUSE MAYBE THERE WILL BE -- SOME OF THE STRANDS AT ANY PARTICULAR TIME WILL BE SINGLE-STRANDED. IF YOU LEAVE THINGS AT 37 DEGREES FOR LONG TIMES, DNA WILL BE STABLE AT 37. IT'S DOUBLE-STRANDED BUT SOMETIMES, ACCIDENTALLY, ONE OF THEM WILL SHAKE ITSELF APART.

I'VE NOT -- IF IT DOES, MY OLIGONUCLEOTIDES MAY BE PRIMED AND GET EXTENDED. MAYBE THAT WILL JUST SHAKE ITSELF APART; THEN THE PRIMING PROCESS WILL GO OVER AND OVER BY ITSELF.

IT WAS THE -- IT WAS ASKING A LOT, BUT I TRIED THE EXPERIMENT, AND I COULD EXAMINE IT REAL EASILY BY JUST PUTTING

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IT ON A GEL AND SEEING IF I HAD A FOUR HUNDRED BASE PAIR BAND.

SO I CAME BACK THE NEXT DAY. I STARTED THAT THING AT MIDNIGHT, I THINK, AND CAME BACK THE NEXT DAY. AND AROUND NOON, I PUT IT ON THE GEL, AND I DIDN'T SEE ANYTHING. SO I SAID, WELL, THAT DIDN'T WORK. IT WASN'T THAT DISCOURAGING. I WAS ASKING A LOT.

Q. WHEN DID YOU DO THAT EXPERIMENT?

A. THAT WOULD HAVE BEEN SEPTEMBER -- AROUND SEPTEMBER, A COUPLE OF WEEKS PROBABLY AFTER MY TALK.

Q. SO SEPTEMBER 1983?

A. YES.

Q. YOU REFERRED TO 37 DEGREES. IS THAT A FEW DEGREES ABOVE FREEZING YOU WERE TALKING ABOUT?

A. NOT IN A BIOLOGICAL SENSE. IT'S A LONG WAY ABOVE FREEZING. IT'S THE TEMPERATURE OF YOUR BODY.

Q. THAT'S 37 DEGREES CENTIGRADE?

A. 37 DEGREES CENTIGRADE.

Q. CELSIUS.

A. OKAY.

Q. AFTER THIS SEPTEMBER EXPERIMENT DIDN'T WORK, WHAT DID YOU DO NEXT?

A. I THINK I WENT TO A MEETING, AND THEN -- A MEETING IN BOSTON.

THE NEXT TIME I GOT AROUND TO DOING AN EXPERIMENT, IT WAS IN OCTOBER. THIS TIME, I TOOK THE SAME SYSTEM, BASICALLY,

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THE SAME TWO OLIGONUCLEOTIDES AND HUMAN DNA, AND I -- THIS TIME, I DID WHAT I WOULD CALL ~~FIVE PCR CYCLES~~.

NOW, I HEATED THE DNA TO DENATURE; I ALLOWED THE PRIMERS TO ANNEAL OR SOME TIME FOR THEM TO ANNEAL; AND I PUT IN SOME THE POLYMERASE THAT I WAS USING CALLED THE KLENOW FRAGMENT OF THE POLYMERASE IN THE ENZYME. THAT'S WHAT WE HAD AVAILABLE IN THE LAB. THAT WAS THE ENZYME I WAS USING. SO I PUT THAT IN, AND I HAD THE TRIPHOSPHATES.

I DID THAT FIVE TIMES, SO -- FIVE TIMES IS ABOUT AS MANY AS I WANTED TO COMPLETE IT. MAYBE I DID IT TEN TIMES, SOMETHING LIKE THAT. PROBABLY DID IT FIVE TIMES AND MAYBE FIVE MORE TIMES.

IT SEEMED LIKE KIND OF A LOT OF EFFORT TO GO TO, BUT I THOUGHT IT MIGHT WORK.

SO I LOOKED AT THAT ON A GEL, AND I STILL DIDN'T HAVE ANY 400 BASE PAIR PIECE.

SO I THOUGHT, WELL, SOMETHING ELSE IS GOING ON THERE BESIDES WHAT I INTENDED.

Q. SO THIS WAS IN OCTOBER 1983?

A. YEAH, THAT WOULD HAVE BEEN OCTOBER.

Q. THIS TIME, YOU WERE DOING A CYCLING REACTION USING --

A. RIGHT.

Q. -- PRIMERS?

A. RIGHT.

Q. YOU KEEP TALKING ABOUT TRIPHOSPHATES. MAYBE YOU'D BETTER

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TELL US WHAT ~~TRIPHOSPHATES~~ ARE.

A. THAT'S THE NUCLEOTIDE -- THE SORT OF SINGLE MOLECULE FORM OF A NUCLEOTIDE THAT IS -- YOU ACTUALLY USE IN A POLYMERASE REACTION CALLED A NUCLEOTIDE TRIPHOSPHATE. IT'S LIKE A SPRING-LOADED LITTLE DERIVATIVE OF THE NUCLEOTIDE THE ENZYME CAN GRAB AND STICK IN GROWING CHAIN AND, BOING, IT POPS IN, TAKING A LITTLE BIT OF ENERGY TO GET THE BOND FORMED, ALMOST LIKE A LITTLE SPRING IN THERE.

SO WHEN IT STICKS IT IN THE RIGHT PLACE, THE MOLECULE FLYS APART AND CAPTURES INTO THE GROWING CHAIN. THAT'S JUST OUR JARGON FOR THEM TO CALL THEM TRIPHOSPHATES INSTEAD OF CALLING THEM NUCLEOTIDE TRIPHOSPHATES. I GUESS, FOR PURPOSES HERE, WE SHOULD CALL THEM NUCLEOTIDES, FOR THE TIME BEING.

Q. ARE THESE THE THINGS WE'VE BEEN CALLING A'S AND G'S AND C'S AND T'S?

A. YES.

Q. WHAT DID YOU DO AFTER BOTH THE SEPTEMBER EXPERIMENT AND THEN THE OCTOBER EXPERIMENT DIDN'T WORK?

A. I DECIDED -- I THOUGHT ABOUT IT, WHEN I WAS DOING IT. I SAID, YOU KNOW, MULLIS, YOU'RE ASKING A LOT HERE TO BE STARTING RIGHT ONTO HUMAN DNA AND MAKING A FOUR HUNDRED BASE PAIR PIECE. WHY DON'T YOU TRY A DNA THAT'S MUCH LESS COMPLICATED THAN HUMAN DNA AND TRY TO MAKE A SMALLER PIECE AND SEE IF YOU CAN DO THAT.

IF YOU CAN DO THAT, YOU CAN WORK BACK UP TO THE 400 BASE PAIR PIECE. THE 400 BASE PAIR PIECE WOULD BE VERY, VERY

IMPRESSIVE TO MY SKEPTICAL COLLEAGUES. THAT'S ONE OF THE REASONS I WANTED TO DO THAT.

I REALIZED THAT WAS REALLY PUSHING MY LUCK. I COULD GET THIS LITTLE ROUND CYCLE OF DNA CALLED PLASMID, AND THIS ONE HAD A PBR322, AND THAT YOU COULD -- EVERYBODY HAD A LITTLE BIT OF IT. I HAD SOME IN THE REFRIGERATOR.

I HAD A COUPLE OF OLIGONUCLEOTIDES SYNTHESIZED BY COREY LEVINSON, WHO WAS MY ASSOCIATE IN THE LAB, THAT WOULD BIND TO THAT PBR322 AND THEY WOULD BIND ABOUT 25 BASE PAIRS. THEIR FIVE-PRIME ENDS WOULD BE ABOUT 25 BASE PAIRS APART.

AND SO THEN I HAD A MUCH SIMPLER KIND OF SYSTEM. I TOOK -- THIS IS IN DECEMBER OF 1983. I TOOK THOSE TWO AND THE PLASMID AND THE NUCLEOTIDES, AND I DID ABOUT TEN CYCLES JUST ABOUT THE SAME AS I HAD DONE FOR THE HUMAN DNA THE LAST TIME, TEN CYCLES, AND THEN I ADDED -- WELL, I TOOK -- I LOOKED AT THAT STUFF ON A GEL AFTER ABOUT TEN CYCLES AND NOW I WAS LOOKING FOR A 25 BASE PAIR PIECE, AND I THOUGHT I COULD SEE A BAND.

IT WAS ON ONE OF THESE GELS, LIKE AN ETHIDIUM STAIN GEL. I DON'T KNOW IF WE'VE TALKED ABOUT ETHIDIUM. IT'S NOT NEARLY AS SENSITIVE AS RADIOACTIVE TRACER-TYPE GELS WHERE YOU'VE PUT THEM ON AN AUTORADIOGRAM AND MAKE A BLACK SPOT WHEREVER THE P32 IS.

FOR AN ETHIDIUM STAIN GEL, YOU CAN GET THE DNA TO HAVE A COLOR YOU'VE ELECTED TO GLOW, RED. I PUT IT ON THAT KIND OF GEL. IT TAKES A LOT MORE DNA TO SEE AN ETHIDIUM BAND THAN IT

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DOES TO SEE A RADIOACTIVE ONE.

YOU'RE SITTING THERE IN THE DARKROOM. YOU SHINE THE LIGHT AT VARIOUS ANGLES AND YOU SAY, I THINK I SEE A BAND DOWN THERE, AND YOU TRY TO TAKE A PICTURE OF IT.

I TOOK A PICTURE. IT DIDN'T SHOW UP VERY MUCH ON THE PICTURE. I DECIDED, WELL, I'LL DO TWO MORE CYCLES, AND THIS TIME I'LL PUT IN SOME RADIOACTIVE C. I'LL PUT IT ON A NEW GEL AND LOOK AT IT ON MY RADIOGRAM. IF THERE'S ANY OF IT ON THERE AT ALL, IT WILL SHOW UP.

I DID THAT. I WAS WORKING LATE IN THE AFTERNOON WHEN I DID THAT, AND I PUT IT ON A GEL THAT WAS -- ACTUALLY LOADED THE REACTION ON A GEL THAT WAS GRINDING ALREADY IN THE LABORATORY, AND I -- FOR ANOTHER REASON.

AND I TOOK THAT OFF AFTER ABOUT AN HOUR AND PUT IT UP AGAINST A PIECE OF X-RAY PAPER, WAITED FOR, I THINK, 30 MINUTES, AND THEN I DEVELOPED IT. AND, LO AND BEHOLD, RIGHT WHERE I WANTED IT TO BE WAS THIS BEAUTIFUL BAND.

I WAS THRILLED, BUT THERE'S NOBODY IN THE LAB TO TELL. THE LAB WAS EMPTY BY THEN.

AND HAL HALLUIN, WHO WAS PATENT ATTORNEY AT CETUS, HAPPENED TO STILL BE IN HIS OFFICE. IT WAS ABOUT 7:00 O'CLOCK. HIS OFFICE WAS RIGHT DOWN THE HALL FROM MINE. I THOUGHT, WELL, I'LL TELL HAL. BEST THING THAT WAS AVAILABLE AT THE TIME.

AND A PATENT ATTORNEY IS ALWAYS WILLING TO BELIEVE IN A NEW INVENTION, WHICH IS A REALLY -- I TOLD A LOT OF PEOPLE ABOUT

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THE IDEA AND THEY HAD JUST SAID, "FORGET IT. IT WON'T WORK."

BUT WHEN I WENT DOWN TO HAL, I SAID, "I JUST INVENTED SOMETHING I THINK IS PHENOMENAL."

HE SAID, "OH, YEAH? GREAT. THAT'S WHAT MY BUSINESS IS." AND HE WANTED TO SEE THE DATA.

I SAID, "I JUST GOT ONE BAND ON AN AUTORADIOGRAM. I THINK IT REALLY WORKED, HAL."

AND I EXPLAINED TO HIM THE INVENTION. HE REALIZED -- HE SAID, "IF THIS WORKS, IT WILL BE PHENOMENAL. THIS WILL BE THE MOST FUN THING WE PATENTED HERE."

I TOOK HIM IN THE DARKROOM. THE THING WAS STILL WET. AFTER YOU DEVELOP THEM, YOU HANG THEM UP. THEY WERE DRYING.

HE LOOKED AT IT AND IT WAS JUST -- THERE WERE NO CONTROLS; VERY SLOPPY EXPERIMENT. BUT IT WAS THERE.

AND HE CONGRATULATED ME, WHICH I WAS IMPRESSED, BECAUSE NO ONE HAD CONGRATULATED ME ON HAVING THE IDEA.

HAL FINALLY BELIEVED NOT ONLY DO YOU HAVE IT, BUT YOU GOT IT TO WORK. AND I FELT REALLY GOOD.

AND I TOOK THE GEL TO FRED FALOONA'S HOUSE, WHO WAS A TECHNICIAN THAT WORKED FOR ME AT THE TIME AND HELPED ME SET THE THING UP AND REALLY DIDN'T KNOW MUCH ABOUT BIOCHEMISTRY AND DIDN'T KNOW WHETHER TO TRUST ME WHEN I TOLD HIM THIS REACTION WOULD CHANGE THE COURSE OF MOLECULAR BIOLOGY.

WE DRANK A FEW BEERS. BY THE TIME WE WERE THROUGH, I THINK HE BELIEVED IT WOULD HAVE AN EFFECT.

Q. DID YOU SAVE THE FIRST PICTURE YOU DID OF THAT ETHIDIUM STAIN GEL IN YOUR NOTEBOOK?

A. I -- THERE'S COPIES OF IT AROUND.

Q. LET ME SHOW YOU WHAT IS A PORTION AT LEAST OF PAGE 174 OF EXHIBIT A-53.

A. YOU CAN'T SEE IT.

Q. I'M SORRY. YOU NEED THE MICROPHONE.

A. THESE THINGS DO AGE IN TIME, BUT ACTUALLY SOMEWHERE RIGHT ABOUT THERE (INDICATING)IS WHERE I THOUGHT THERE OUGHT TO BE A BAND.

AND WHEN I THOUGHT I COULD SEE IT, I DID MY NOTATION. THERE WAS -- THE ARROW MARKS THE SPOT WHERE TRUE BELIEVERS SEE A BAND. YOU CAN'T SEE THAT AT THE BOTTOM THERE. THERE WEREN'T TOO MANY TRUE BELIEVERS AT THAT POINT.

THIS IS THE ETHIDIUM STAIN GEL. YOU SEE A BAND ABOUT 24 BASE PAIRS, AND I COULD TELL IT WAS ABOUT 24 BASE PAIRS BY ALL THESE OTHERS. THESE ARE THE KIND OF THINGS I WAS LOOKING FOR.

THIS IS THE STANDARD SORT OF THINGS I PUT ON THERE. THIS IS ACTUALLY A DIGEST OF THE PLASMID I HAD USED.

AND I KNEW SOMEWHERE AROUND HERE, THERE OUGHT TO BE A NEW BAND. IT REALLY WAS HARD TO TELL.

I'VE GOT A BETTER RADIOGRAM -- CAN YOU SEE THAT?

Q. THIS IS THE RADIOGRAM YOU DID A FEW DAYS LATER?

A. YEAH. THIS IS -- THIS IS THE -- THAT IS WHERE -- I DIDN'T

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HAVE STANDARDS ON THIS ONE.

THESE ARE THE STANDARDS. THEY RAN OUT AT ABOUT 45 BASE PAIRS, SO I DIDN'T HAPPEN TO HAVE A STANDARD AVAILABLE THAT WAS A 25 BASE PAIR DOUBLE-STRANDED PIECE.

I WOULD HAVE PUT THAT IN THIS LANE AND SAID THAT IS 25, BUT THE FACT THERE WAS SOMETHING THERE IN THE GENERAL NEIGHBORHOOD OF 25, THERE WERE A COUPLE OF OTHER LITTLE BANDS. I COULD ALWAYS EXPLAIN BY REFERRING BACK TO MY -- THERE WAS A WAY THAT A 36 COULD HAPPEN IN A 41 IF THE PROCESS WAS REALLY WORKING.

SO I SAID, WELL, THAT COULD BE IT, AND THAT COULD BE THE 41. I DIDN'T NEED THOSE, BUT THE FACT THAT WAS THERE, IT WAS VERY BRIGHT AND I DIDN'T HAVE TO FIND A TRUE BELIEVER TO SAY, IS THERE SOMETHING THERE?

WITH THE MAGIC OF RADIOACTIVITY, YOU CAN SEE MINISCULE LITTLE THINGS. THIS WAS PROBABLY A VERY INEFFICIENT REACTION THE WAY I SET IT UP. IT DID MAKE A PRODUCT THAT WAS NOT THERE BEFORE. I COULD DETECT A SMALL AMOUNT OF PCR BY THE FACT THAT THERE WAS NO 25 BASE PAIR PIECE IN THERE BEFORE. THERE WAS ONE.

Q. WHY DO YOU HAVE A QUESTION MARK THERE BY THOSE NUMBERS?

A. JENNIFER PROBABLY PUT THOSE IN. I DIDN'T KNOW FOR SURE THAT WAS 41, AND THAT WAS 36, AND THAT WAS 25. THOSE WERE MY GUESSES.

IF I JUST LEFT THE NUMBERS THERE, I KNEW FOR SURE THAT COULD HAVE BEEN MISLEADING. I WAS STILL IN A STAGE WHERE THIS

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WAS AN INDICATION IT WAS WORKING BUT CERTAINLY DIDN'T PROVE IT. I MEAN, THAT WAS EXCITING TO ME, BUT IT WAS A LONG WAY FROM THAT. IN FACT, HAL HAD MENTIONED -- HE SAID, YOU'RE GOING TO HAVE TO GET TO WORK NOW DOING SOME EXPERIMENTS THAT REALLY PROVES THAT THIS WORKS AND WE'LL WRITE A PATENT.

Q. HOW COULD YOU TELL THE 25 WAS SOMETHING THAT HAD BEEN MADE BY THE REACTION AND THAT IT WASN'T SOMETHING THAT HAD ALREADY BEEN THERE?

A. WELL, BY -- THE ONE CONTROL -- SET OF CONTROLS I HAD ON THERE WAS THE LABEL, LIKE I WAS DOING THAT REACTION OUT OF WHAT WAS A RESTRICTION DIGEST OF A PLASMID, AND THAT WAS THE LANE THAT WAS RIGHT BESIDE THAT -- WHAT WAS THE LABELED RESTRICTION FRAGMENTS THAT WOULD HAVE BEEN IN THERE ALREADY.

AND I CLEARLY HAD SOMETHING NEW, SEE, IN THAT LANE ON THE LEFT THERE. THERE IS NO BIG BLACK SPOT ON THE BOTTOM.

SO I GOT A NEW SPECIES PRODUCED, AND I COULDN'T TELL HOW MUCH I HAVE OF IT. I HAD -- I COULD TELL THAT IT WAS THERE AND HADN'T BEEN IN THERE BEFORE.

THE PCR PROCESS WILL GENERATE A FRAGMENT WITHOUT HAVING THE FRAGMENT BE DEFINED ALREADY. YOU DON'T USUALLY START -- IN FACT, YOU ALMOST NEVER START WITH A FRAGMENT THAT YOU'VE ALREADY MADE TO BE A CERTAIN SIZE. USUALLY, THIS PROCESS GOES IN AND SNATCHES IT OUT, BASICALLY, THEN AMPLIFIES IT UP.

SO THE 25 BASE PAIRS HAD NOT EXISTED BEFORE SO NOW IT EXISTED, AND I DIDN'T HAVE TO KNOW HOW MUCH EXISTED TO KNOW THE

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PROCESSED WORK. I'VE SAID IT; MADE IT. NOW I CAN FIGURE OUT HOW TO MAKE MORE OF IT.

Q. WHAT IS THIS STREAK OF DARK COLOR?

A. THAT, MR. PASAHOW, IS THE STREAK OF DARK COLOR, AND YOU ALWAYS GET STREAKS OF DARK COLOR, JUST ABOUT, WHEN YOU USE RADIOACTIVE THINGS. I DON'T KNOW WHAT ALL THAT IS. WHAT IS -- THAT WHOLE SIDE?

Q. YES.

A. THAT IS MY STANDARDS, AND THEY'RE KIND OF BLOBS. IF I HAD NOT -- IF I HAD NOT DEVELOPED -- I OVEREXPOSED IT, SO THERE WOULD BE DISCREET LITTLE BANDS THERE, BUT THEY'RE SHOWING ME WHAT WAS IN THE REACTION TO START WITH.

YOU CAN SEE SOME OF THOSE AFTER THE REACTION IS OVER, BUT THAT'S SHOWING ME THE FRAGMENTS THAT I BEGAN WITH. THE ONE ON THE RIGHT IS SHOWING ME THE FRAGMENTS THAT I ENDED UP WITH AND SHOWING ME THE NEW ONES THAT I HAD SYNTHESIZED USING THE PCR.

Q. SO THIS WAS THE SOLUTION BEFORE YOU DID THE PCR ON IT?

A. THAT WAS AS CLOSE AS I COULD GET TO WHAT WOULD HAVE BEEN THERE BEFORE IT. IT WOULDN'T -- THAT WAS NOT A PERFECT EXPERIMENT, EITHER. THE PROCESS OF HEATING AND COOLING AND HEATING AND COOLING BY ITSELF WOULD HAVE DONE SOMETHING TO THOSE BANDS, TOO. THAT WAS PUT ON THERE SO I KNEW WHERE 44 WAS. WHAT I WAS LOOKING AT WAS SOMETHING TOTALLY NEW.

Q. DID YOU THINK THESE RESULTS WERE GOING TO CONVINCE YOUR

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COLLEAGUES AT CETUS YOU HAD MANAGED TO MAKE THIS IDEA WORK?

A. I THINK IT CONVINCED HAL. HAL WAS THE PATENT ATTORNEY. HE HAD A VESTED INTEREST IN THAT, WANTING TO SEE A NICE INVENTION.

MOST PEOPLE, NO, THEY WERE NOT. THIS WAS IN DECEMBER OF '83, AND IT WAS STILL A LONG, UPHILL BATTLE TO GET ANYBODY AT CETUS EXCEPT -- I THINK FRED FALOONA HAD ALSO STARTED BELIEVING BY THEN, WHO WAS MY TECHNICIAN AND MY FRIEND, BUT IT WAS NOT CONVINCING. I WOULDN'T EXPECT IT TO BE.

Q. DO YOU RECALL THE NEXT EXPERIMENT YOU DID INVOLVING PCR?

A. I THINK I WENT BACK TO EITHER TRY HUMAN EXPERIMENT AGAIN -- NO. I TRIED AN EXPERIMENT -- YES. THIS WAS EVEN MORE AMBITIOUS.

I HAD A DNA -- AN ORGANISM CALLED LAMBDAPHAGE, L-A-M-B-D-A, P-H-A-G-E. IT HAS -- WHAT I HAD STARTED WITH WITH THIS EXPERIMENT WAS FIVE NOW BASE PAIR CYCLE.

I'VE SAID, OKAY. I'LL GO UP BY AN ORDER OF MAGNITUDE TO LAMBDA, WHICH HAS 50,000 BASE PAIRS, A LITTLE MORE COMPLICATED THAN PLASMID AND WAY LESS COMPLICATED THAN THE HUMAN GENE WHICH I STARTED WITH.

I'LL TRY THAT. I SHOULD HAVE GONE TO A 25 BASE PAIR PRODUCT THERE, TOO.

I SAID, WELL, THERE'S THIS CONVENIENT 2,000 BASE PAIR PIECE I COULD PRODUCE BY ANOTHER METHOD USING A RESTRICTION ENZYME. I COULD USE THAT AS A STANDARD ON A GEL.

I WANTED TO MAKE A PIECE THAT I SORT OF ALREADY HAD SO

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I COULD PUT IT SIDE BY SIDE AND SAY, HEY, THIS IS A 2,000 BASE PAIR PIECE. I'VE SHOULD HAVE FOUND A LITTLER PIECE. I JUST HAPPENED TO HAVE A DIGESTED LAMBDA. I DESIGNED A COUPLE OF PRIMERS TO MAKE A TWO THOUSAND BASE BEAR PIECE LAMBDA. I TRIED THAT EXPERIMENT AND IT DID NOT WORK.

Q. WHAT DO YOU MEAN IT DIDN'T WORK?

A. I DIDN'T GET THE TWO THOUSAND BASE PAIR PIECE. IN FACT, I -- IT'S HARD TO GET A TWO THOUSAND BASE PAIR PIECE WITH THE ENZYME I WAS USING THEN. I DECIDED, WELL, THAT'S TOO AMBITIOUS.

Q. GOING BACK TO THIS EXPERIMENT THAT WORKED IN DECEMBER:

WHEN YOU DID THIS PCR ON THAT, YOU SAID THE DNA WAS A CYCLE OF DNA. DID YOU DO THE PCR ON THE CYCLE?

A. NO. IT ACTUALLY CAME FROM A CYCLE OF 5,000 BASE PAIR CYCLE.

WHAT I DID IT ON WAS A RESTRICTION ENZYME DIGEST OF THAT CYCLE. SO YOU TAKE ONE OF THESE ENZYMES, LIKE DR. SMITH WAS TALKING ABOUT, AND YOU CAN CUT THEM. YOU TAKE A PIECE THAT HAS 5,000 BASE PAIRS IN A CYCLE AND CUT IT INTO MAYBE TEN FRAGMENTS.

EVERYWHERE THE SEQUENCE OCCURS THAT THAT ENZYME RECOGNIZES, IT WILL MAKE A CUT IN THE CYCLE. SO IT WOULD SNIP IT UP INTO SOME MANAGEABLE NUMBER OF PIECES. THAT'S WHAT I ACTUALLY HAD USED AS A BEGINNING DNA.

Q. DO YOU RECALL THE SIZE OF THE PIECE OUT OF WHICH YOU AMPLIFIED THE 25 BASE PAIR PIECE?

A. YES. I THINK IT WAS 44 BASE PAIRS. I HAD A PICTURE BECAUSE

WE HAD THE SEQUENCE OF PBR, SO I COULD HAVE A PICTURE OF THE WHOLE SEQUENCE.

AND WHERE THE CUTS WOULD BE FROM THE ENZYME, I KNEW THIS PARTICULAR ENZYME CALLED POK -- P-O-K -- 1, THAT ONE WOULD CUT AT A SET CERTAIN NUMBER OF PLACES.

ONE OF THE FRAGMENTS THAT CAME OUT OF THAT WOULD BE 4 BASE PAIRS LONG. SO I HAD SAID, I'M GOING TO GO INTO THAT. I'M GOING TO AMPLIFY THAT BECAUSE THAT'S HOW I DESIGNED THE EXPERIMENT. I THOUGHT THAT WOULD BE THE EASIEST.

BREAKING THE WHOLE PLASMID APART AND HYBRIDIZING, TO ME, SEEMED A LITTLE MORE COMPLICATED THEN BREAKING A LITTLE PIECE APART.

Q. YOU DESCRIBED THIS EXPERIMENT YOU DID AFTER THE 25 BASE PAIR PIECE WITH THE TWO THOUSAND BASE PAIR PIECE, AND THAT DIDN'T WORK.

DO YOU RECALL THE NEXT PCR EXPERIMENT THAT YOU DID?

A. I WENT BACK AND REPEATED THIS ONE AGAIN. I JUST WANTED -- I SAID, WHOOPS, GO BACK AND START WITH SOMETHING YOU KNOW.

I REPEATED THIS EXPERIMENT AGAIN, JUST THE 25 BASE PAIR PIECE AND THE PBR. IF I HAD ANOTHER SYSTEM QUITE THAT SIMPLE, I WOULD HAVE TRIED IT AGAIN. I WANTED TO TRY TO REPEAT THOSE.

Q. DO YOU RECALL HOW THAT EXPERIMENT CAME OUT?

A. WELL, IT DID MAKE THE PIECE -- IT WASN'T QUITE AS NICE AS THIS RESULT, BUT I THINK, YOU KNOW, ~~SOME DANG YOUR CELS JUST DON'T COME OUT NICE~~. AND WHEN I -- I WAS A LITTLE -- I DECIDED

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TO MAKE THE PIECE AND ALSO I DECIDED TO CUT THAT LITTLE 25 BASE PAIR PIECE WITH SEVERAL ENZYMES WHO -- I MEAN, I TREATED WITH ENZYMES THAT WOULD CUT WITHIN IT IF IT WERE THE RIGHT 25 BASE PAIRS.

IN OTHER WORDS, IF I KNEW WHAT I WAS DOING, I KNEW WHAT THE SEQUENCE OF THAT 25 BASE PAIRS WAS AND IF, THEREFORE, AND IF I CUT IT WITH AN ENZYME THAT WOULD CUT A SEQUENCE WITHIN THAT LIKE A FIVE BASE LONG SEQUENCE, THAT THAT ENZYME RECOGNIZED IF I TREAT MY REACTION WITH THAT IT SHOULD BREAK THE THING UP AND IT SHOULDN'T SHOW UP ANYMORE ON THE GEL AND THAT'S WAS SORT OF BEGINNING TO CHARACTERIZE THE FRAGMENTS.

THAT'S REALLY WHAT I THINK IT IS AND HAS IT GOT THE RIGHT SEQUENCE. THAT EXPERIMENT WAS KIND OF INCONCLUSIVE BECAUSE THE GEL WAS SO MESSY. I COULD BELIEVE THAT IT WORKED BUT I REALLY COULDN'T PROVE TO MUCH FROM IT.

Q. NOW, IN ~~JUNE 1984~~ THERE WAS A SCIENTIFIC MEETING OF THE CETUS SCIENTISTS; IS THAT RIGHT?

A. YES, THAT WAS OUR ANNUAL SCIENTIFIC MEETING. COULD I HAVE SOME WATER HERE?

(PAUSE IN THE PROCEEDINGS)

THE WITNESS: WE HAD A MEETING JUST EVERY YEAR BACK THEN WHERE ALL OUR ADVISERS WHICH WOULD -- WHERE WE HAD SOME REALLY NICE ADVISORS LIKE HAMMILTON SMITH WHO YOU TALKED TO YESTERDAY WAS ONE OF THEM. WE HAD -- OUT OF 20 OR 30 REALLY PROMINENT SCIENTISTS THAT WOULD COME TO CETUS ONCE A YEAR AND WE

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WOULD USUALLY GO OFF ON A LITTLE RETREAT WITH THEM SOMEWHERE AND --

Q. AT THIS MEETING IN JUNE 1984 DID YOU PRESENT THE RESULTS OF YOUR PCR EXPERIMENTS UP TO THAT TIME?

A. YES, I DID.

Q. DO YOU RECALL WHAT KIND OF RESULTS YOU WERE SHOWING AT THAT MEETING?

A. I THINK THAT I -- ONE OF THE RESULTS I SHOWED WAS THIS GEL AND I HAD ANOTHER ONE THAT WAS AN EXPERIMENT WE HAD DONE IN THE THE TIME BETWEEN JANUARY AND JUNE, WE FINALLY DONE AN EXPERIMENT WITH HUMAN DNA LOOKING FOR A 58 BASE PAIR FRAGMENT PRODUCED FROM THE -- I WAS BACK TO THE BETA-GLOBIN THE SAME SITE I HAD BEEN PLAYING WITH ORIGINALLY WITH THE SICKLE CELL THING.

I WANT TO AMPLIFY THAT REGION THAT CONTAINS THAT MUTATION. SO I HAD A GEL WHERE WE HAD SOME POLYMERASE, BUT I THOUGHT ENCOURAGING RESULTS FROM IT. WE AMPLIFIED A LITTLE FRAGMENT OUT OF HUMAN DNA, SO I HAD THOSE TWO THINGS ON A POSTER, KIND OF LIKE A LITTLE BIGGER THAN THAT KIND OF POSTER THERE.

ALSO HAD A WRITTEN DESCRIPTION OF THE EXPERIMENTS, IT'S A TYPICAL KIND OF MECHANISM THAT SCIENTISTS USE FOR COMMUNICATING LIKE YOU HAVE A MEETING AND EVERYBODY BRINGS THEIR RESULT, YOU PUTS THEM ON A POSTER AND YOU STANDBY YOUR POSTER. PEOPLE COME IN AND LOOK AT YOUR STUFF AND THEY ASK YOU QUESTIONS AND WHATEVER AND MAKE SUGGESTIONS. SO IT WAS A POSTER SESSION

WHERE I PRESENTED IT.

Q. AND OTHER SCIENTISTS FROM CETUS HAD THEIR POSTERS THERE AS WELL?

A. YES.

Q. NOW, THE PIECE OF HUMAN DNA THAT YOU AMPLIFIED THAT WAS THE PIECE THAT'S INVOLVED IN CAUSING SICKLE CELL?

A. IT IS. IT'S THE PIECE -- I AMPLIFIED THE WHOLE 58 BASE PAIRS -- IT WAS LIKE 25 OR 30 ON BOTH SIDES, 30 ON BOTH SIDES OF IT, WAS ONLY ONE BASE PAIR THAT'S INVOLVED, DIRECTLY INVOLVED IN THAT SINGLE MUTATION.

Q. WHAT WAS THE RESPONSE OF YOUR PEERS TO THE POSTER SHOWING THESE RESULTS?

A. THEY IGNORED THEM. THEY BASICALLY IGNORED THEM. I WAS THINKING THEY WERE NOW -- SEE IT ALL THESE FAMOUS MEN COMING HERE, IF MY OWN FELLOW SCIENTISTS DON'T APPRECIATE THE MAGNITUDE OF WHAT THIS DISCOVERY IS, AT LEAST PEOPLE FROM -- REAL SCIENTISTS FROM OUTSIDE WILL CERTAINLY UNDERSTAND.

AND NOBODY REALLY PAID -- IT IS REALLY A SORT OF A -- IT'S SOMETHING I NOW KIND OF COME TO ACCEPT THAT SCIENTISTS LIKE OTHER PEOPLE ARE NOT ALWAYS INTERESTED IN SOMETHING THAT IS TOTALLY OUT OF THEIR EXPERIENCE AND NEW.

THEY REALLY -- AND THOSE POSTER SESSIONS THEY TEND TO GRAVITATE TOWARD THINGS THEY ALREADY KNOW ABOUT. THEN THEY JUST FIND OUT NEW DETAILS ABOUT THAT, BUT YOU KNOW THAT'S WHERE THEIR FRIENDS WILL BE STANDING AROUND WHEN SEEING SOMETHING TOTALLY

BRAND NEW THEY'RE NOT ATTRACTED TO THAT LIKE SOME WEIRDOS ARE LIKE ME. I LIKE POSTER SESSIONS, I LOOK AT THE NEW STUFF, WHATEVER I DON'T KNOW.

AT ANY RATE THERE WAS THIS GUY JOSH LETTERBURG ONE OF OUR SUPERVISORS, I GRABBED HIM I WANTED TO HEAR WHAT JOSH HAD TO SAY ABOUT THIS. HE WAS A FAMOUS MAN WHO HAD BEEN A MOLECULAR BILOLGIST FOR A LONG, LONG TIME HE WAS A NOBEL LAUREAT.

MR. FIGG: YOUR HONOR, EXCUSE ME, WE'RE REALLY GETTING INTO HEARSAY TESTIMONY NOW.

THE COURT: LET'S JUST STICK AND TRY TO PROVIDE CONCISE ANSWERS TO THE QUESTIONS.

THE WITNESS: OKAY.

THE COURT: AND WE'RE BETTER OFF.

Q. (BY MR. PASAHOW) TO SHORTEN THIS, DID DR. LETTERBURG LOOK AT YOUR POSTER AND DISCUSS IT WITH YOU?

A. YES, HE DID.

Q. WERE THERE ANY OTHER SCIENTIST WHO SHOWED ANY SUBSTANTIAL INTEREST IN THE POSTER?

A. NO, I SORT OF HAD TO FORCE JOSH TO LOOK AT IT, BUT WHEN SAW IT HE DEFINITELY AGREED WITH ME IT WAS PRETTY EXCITING.

Q. NOW, AFTER THIS MEETING IN JUNE 1984 DID SOME CHANGE HAPPEN IN THE GROUP THAT WAS WORKING ON PCR OVER THE COURSE OF THE NEXT FEW MONTHS?

A. WELL, YES . . . WE START -- FRED FALOONA, WHO WAS THE TECHNICIAN WHO WAS SORT OF MY RIGHT-HAND MAN AT THAT TIME, HE

AND I STARTED GOING TO MEETINGS THAT WERE HELD ABOUT EVERY WEEK BY HENRY ERLICH'S LABORATORY IN AN ATTEMPT IT WAS -- WE DECIDED THAT THE PCR PROCEDURE WOULD BE A REAL KNOWLEDGABLE WAY TO SOLVE THE PROBLEM THEY WERE WORKING ON.

WE WOULD TRY TO AMPLIFY THE DNA SAMPLES THAT THEY WERE USING AND THEN THEY WOULD APPLY A PROCEDURE THAT THEY WERE WORKING ON AT THE TIME CALLED OLIGER (PHONETIC) RESTRICTION, CALLED OR, THAT PROCEDURE WOULD WORK ALL RIGHT IF THERE WAS A LOT OF DNA.

IT WAS NOT WORKING IN THEIR HANDS ON HUMAN DNA THERE WAS AN AMOUNT OF THE SEQUENCE THAT WAS THERE WAS NOT SUFFICIENT. SO WE DECIDED WE TRY TO MAKE THE TWO PROCEDURES WE WOULD USE THE PCR, AMPLIFY THE DNA SIGNAL AND THEN THEY WOULD PERFORM THE OR ON THE RESULTS OF THAT.

Q. AND AT SOME POINT WAS AN EXPERIMENT ARRANGED WHERE YOU DID WHAT YOU DESCRIBED THAT IS YOUR GROUP AMPLIFIED SOME HUMAN DNA AND THE OR PROCEDURE WAS TRIED TO THE RESULT?

A. YES. FRED FALOONA FINALLY -- I THINK IT WAS PROBABLY IN OCTOBER OR SOMEWHERE IN THERE, FRED AMPLIFIED SOME DNA. WE DID 25 CYCLES OF AMPLIFICATION AND GAVE IT TO STEVE OR RANDY, ONE OF THE PEOPLE IN HENRY'S LAB, THEY DID THE OR PROCEDURE ON IT.

Q. WHAT WAS THE RESULTS?

A. IT WAS ASTONISHING TO THEM. I REMEMBER SAYING DON'T USE ALL OF THIS ON THAT -- ON THE PROCEDURE JUST TAKE A SMALL AMOUNT BECAUSE THE GENE IS GOING TO HAVE BEEN AMPLIFIED ABOUT A HUNDRED

THOUSAND TIMES. SO IF YOU USE THE WHOLE SAMPLE YOU'RE GOING TO, YOU KNOW, VERY TOO MUCH.

THEY WERE LOOKING AT LITTLE SPOTS THEY WERE TRYING TO SEE TINY LITTLE SPOTS, THIS IS GOING TO BLACKEN THE WHOLE THING. SO THE RESULT WAS THEY DID IT. THEY'VE USED THE WHOLE THING, OF COURSE, BECAUSE THEY DIDN'T BELIEVE IT REALLY WAS GOING TO AMPLIFY IT.

THE FIRST RESULT WAS REALLY A HUGE LIKE AN OVEREXPOSED AUTORADIOGRAM.

Q. DID SOMEONE TRY IT AGAIN?

A. YEAH, THEY WENT BACK AND SAID, OKAY, LET'S TRY WHAT HE SAYS, USE A SMALL AMOUNT SO THEY USED A SMALL AMOUNT, IT AMPLIFIED IT SUCCESSFULLY AND THEN WE WERE ALL REALLY EXCITED ABOUT IT AND WE STARTED DOING THOSE KIND OF EXPERIMENTS A LOT.

Q. DID SCIENTISTS IN DOCTOR ERLICH'S LAB THEN START TRYING TO DO PCR REACTIONS?

A. YES. THEY DEVELOPED A SUDDEN INTEREST IN PCR AND I THINK FRED WENT DOWN THERE WITH THEM AND SHOWED THEM OR THEY CAME UP TO OUR LAB, I CAN'T REMEMBER HOW IT WAS DONE, THEY STARTED DOING THEM ALSO.

Q. WHAT THEN DID THE SCIENTISTS IN DOCTOR ERLICH'S LAB FOCUS ON IN CONNECTION WITH THE PCR REACTION?

A. WELL, THE IMMEDIATE GOAL WAS TO GET THE PCR REACTION AND THE OR REACTION TO WORK TOGETHER AND TO JUST BASICALLY TRY TO DEVELOP METHODS THAT WOULD INCREASE THE EFFICIENCY OF THE

REACTION JUST, YOU KNOW, MAKE IT WORK BETTER AND BETTER.

THEY WERE INTERESTED IN SOLVING THE PROBLEM OF THE SICKLE CELL MUTATION BECAUSE THAT'S WHAT THEY WORKED ON, PUTTING THOSE TWO PROCEDURES TOGETHER.

Q. OVER THE COURSE OF TIME DID THEY COME UP WITH A PROCEDURE THEY WERE SATISFIED WITH?

A. YEAH. WE WERE ALL WORKING ON IT TOGETHER AND WE CAME -- FINALLY GOT TO THE POINT WHERE WE COULD BE CONFIDENT THAT A METHODOLOGY THAT WE WORKED OUT WOULD PRODUCE RESULTS EVERY TIME. THAT WAS -- LET'S SEE, IT TOOK QUITE AWHILE. IT WAS A LOT OF MESSING AROUND THAT TOOK PLACE TO TRY TO GET THOSE SYSTEMS TO WORK. BOTH THOSE SYSTEMS WERE REALLY COMPLICATED AND WE WERE TRYING TO FIT THE TWO OF THEM TOGETHER.

Q. DO YOU RECALL ABOUT WHEN YOU STARTED HAVING RELIABLE REPRODUCIBLE RESULTS?

A. I THINK IT WOULD HAVE BEEN PROBABLY -- IT WAS REALLY PROBABLY BEEN -- WORK ON IT NINE MONTHS OR MORE, SOMEWHERE AROUND IN THE SUMMER OF 19 -- THAT WOULD HAVE BEEN . . . 1985, SOMEWHERE AROUND THERE.

I THINK WE WERE STARTING TO GET CONFIDENT WE COULD WRITE DOWN A PROCEDURE AND FOLLOW IT AND IT WOULD PRODUCE THE SAME RESULTS EVERY TIME. WE HAD HAD A LOT OF IRREPRODUCIBLE THINGS. WE FINALLY GOT IT TO WHERE IT WAS RELIABLE.

IT WAS VERY CLUMSY, BUT IT WAS RELIABLE.

THE COURT: MR. PASAHOW, I THINK WHENEVER YOU CAN FIND

A TIME TO BREAK, I'M SURE THIS WOULD BE APPROPRIATE TO DO THAT.

MR. PASAHOW: THIS IS FINE.

THE COURT: CORRECT, MR. YEOMANS?

FINE, WE'LL TAKE ABOUT TEN, 15 MINUTES. PLEASE FOLLOW THE INSTRUCTIONS YOU'VE BEEN GIVEN PREVIOUSLY.

YOU MAY STEP DOWN.

(RECESS TAKEN)

(CONTINUED ON THE NEXT PAGE, NOTHING OMITTED)

(OPEN COURT, JURY PRESENT, AT 10:16 A.M.:)

MR. PASAHOW: SHALL I PROCEED?

THE COURT: YOU CAN CONTINUE, YES.

Q. (BY MR. PASAHOW) DR. MULLIS, BEFORE THE BREAK, WE WERE TALKING ABOUT THE WORK THAT WAS GOING ON ON THE COMBINED PCR/OR METHOD, AND I HAD JUST SHOWN YOU A COPY OF WHAT WE'VE MARKED AS EXHIBIT B-38, WHICH IS AN ARTICLE THAT APPEARED IN THE DECEMBER 1985 -- OR ONE OF THE DECEMBER 1985 ISSUES OF SCIENCE; IS THAT CORRECT?

A. YES.

Q. WHAT IS THAT ARTICLE ABOUT?

A. THAT'S AN ARTICLE REPORTING THE WORK THAT WE HAD BEEN DOING, THE -- WE HAD BEEN DOING ON THE SICKLE CELL DIAGNOSTIC PROBLEM.

IT DESCRIBES THE MATING OF THE -- THE USE OF PCR TO AMPLI -- PREAMPLIFY THE DNA BEFORE THE OLIGOMER RESTRICTION, THE OR RESTRICTION, WAS PERFORMED.

AND IT SHOWS THAT THOSE TWO THINGS COMBINED CAN NOW SOLVE THE PROBLEM OF LOOKING AT A VERY SMALL AMOUNT OF DNA AND BEING ABLE TO READ A SEQUENCE OR BASICALLY ONE SINGLE BASE PAIR IS ALL WE WERE LOOKING AT.

Q. AND THE -- THE FIRST NAMED PERSON ON THAT ARTICLE WAS RANDY SAIKI; IS THAT CORRECT?

A. YES.

Q. AND WHY WAS THAT?

A. WELL, HE WAS ONE OF THE PEOPLE THAT WAS DOING A GOOD BIT OF

THE WORK THAT THE GROUP WAS INVOLVED WITH. HE WORKED IN HENRY ERLICH'S LAB, AND -- AND THERE WERE A WHOLE BUNCH OF US THAT WERE ACTUALLY DOING THE WORK INVOLVED.

THIS PAPER WAS NOT DESIGNED TO -- TO DESCRIBE THE ENTIRE PCR PROCESS -- THE WHOLE PROCESS. IT WAS BASICALLY TO DEMONSTRATE THAT IT HAD AN APPLICATION HERE IN DOING FETAL -- IN LIKE DIAGNOSTIC -- DIAGNOSIS OF SICKLE CELL ANEMIA.

Q. AND AS THE LIST OF AUTHORS GOES ON, YOU, OF COURSE, ARE ONE OF THEM AS WELL.

A. YES.

Q. NOW, WHILE THE -- DR. ERLICH'S LABORATORY, AND WITH YOUR CONSULTING AND MR. FALOONA'S CONSULTING AND HELP, WAS WORKING ON COMBINING PCR WITH OR, WHAT WERE YOU FOCUSING YOUR TIME ON?

A. MOSTLY I WAS EXPLORING THE POSSIBILITIES FOR PCR. I WAS STARTING TO AMPLIFY DIFFERENT SIZED FRAGMENTS TO TRY TO UNDERSTAND -- JUST PLAYING WITH IT AGAIN, SAYING, WELL, WHAT IF I DO THIS, WHAT IF I DO THAT, MOSTLY WORKING IN VERY SIMPLE MODEL SYSTEMS WHERE IT WAS NOT REQUIRED THAT I DO 20 OR 30 CYCLES AT A TIME, PLACES WHERE I COULD GET AWAY WITH FIVE OR 10 AND STILL GET A RESULT.

AND JUST EXPLORING THE -- WHAT THE PROCEDURE -- WHAT ARE SOME OF THE THINGS IT COULD DO, TRY TO SEE HOW BIG A PIECE I COULD MAKE. AND ALSO JUST DETERMINING -- GETTING DATA TOGETHER FOR -- I WAS GOING TO SUBMIT -- THERE WAS A PATENT INVOLVED AND I NEEDED GOOD CLEAN DATA THAT SHOWS THAT IT COULD DO WHAT I SAY

IT CAN DO.

Q. NOW, WE'VE HEARD SOME TALK ABOUT A PROCESS CALLED NESTED PRIMERS.

ARE YOU FAMILIAR WITH THAT?

A. YES.

Q. WHEN DID YOU FIRST START WORKING WITH NESTED PRIMERS?

A. OKAY. THAT -- THE NESTED PRIMER TECHNIQUE WAS SOMETHING THAT I . . . I'M NOT SURE WHEN I VERY FIRST STARTED WORKING ON THAT, BUT IT WAS IN . . . IT WAS AS A -- AS A WAY -- SEE, I COULD AMPLIFY -- WHEN I FIRST WENT -- WHEN I FIRST DID THE POSTER SESSION ON THIS, I'D TRIED TO AMPLIFY HUMAN DNA TO GET A SINGLE BAND OUT OF IT, AND I -- I HAD GOTTEN A SORT OF FUZZY LITTLE BAND THAT LOOKED LIKE IT WAS FAIRLY -- FAIRLY -- IT WAS REPRESENTATIVE OF THE BETA-GLOBIN LOCUS.

BUT IT WAS -- THERE WAS A -- SOMETIMES IT WOULDN'T BEHAVE JUST EXACTLY LIKE I WANTED TO, AND IT WAS BECAUSE I -- I THINK THAT IT WAS BECAUSE OF THE NON -- SOME OTHER THINGS BESIDES THE BETA-GLOBIN LOCUS RESEMBLED THE BETA-GLOBIN LOCUS.

AND WHEN I SAY "LOCUS," THAT REGION OF THE BETA-GLOBIN GENE THAT I WAS TRYING TO AMPLIFY.

AND THERE WERE -- THERE WERE OTHER SEQUENCES IN HUMAN DNA THAT WERE SIMILAR TO THAT, AND SO SOME OF THEM WOULD ALSO GET AMPLIFIED, SO THAT EVERYTHING THAT WAS THERE IN THAT BAND WAS NOT NECESSARILY FROM THE BETA-GLOBIN GENE.

AND I -- I -- WHERE THE NESTED PRIMER IDEA CAME UP,

I -- I THINK IT WAS PROBABLY IN THE SPRING SOMETIME OF '85, OR SOMEWHERE IN THERE, I STARTED REALIZING THAT I COULD . . . I GET THE YEARS CONFUSED SOMETIMES.

PROBABLY IN THE SPRING OF '85, SOMEWHERE AROUND THERE, I REALIZED -- IT OCCURRED TO US THAT IF WE DID TWO SEQUENTIAL AMPLIFICATIONS, LIKE DO ONE AMPLIFICATION -- NOW, I PROBABLY SHOULD DRAW THIS, YOU KNOW.

Q. LET ME GET YOU A MICROPHONE FIRST.

A. OKAY. I -- I DON'T KNOW. DO YOU THINK I NEED ONE?

Q. FOR THE REPORTER'S BENEFIT.

A. OKAY. I THINK SOMETIMES IT DISTORTS WHAT I'M SAYING.

THIS REALLY -- THE CONCEPT IS REALLY EASY. IT HAS TO DO WITH THE FACT THAT THE PROCEDURE OF AMPLIFICATION REALLY SELECTS A CERTAIN REGION AND TRIES TO AMPLIFY IT, BUT IT CAN'T DO THAT ABSOLUTELY PERFECTLY IF WHAT IT'S STARTING WITH IS A VERY COMPLEX BUNCH OF DNA.

THAT'S WHY I WAS DOING MOST OF MY EXPLORING WITH PCR ON VERY SIMPLE SYSTEMS THAT ONLY HAD, SAY, 5,000 BASE PAIRS. IF YOU GO FROM 5,000 UP TO THREE TIMES TEN TO THE NINTH, WHICH IS LIKE THREE BILLION BASE PAIRS, IT'S A BIG JUMP. AND SO A LOT OF LITTLE THINGS MIGHT HAPPEN THAT LOOKED -- HAVE IT LOOK SORT OF LIKE THE BETA-GLOBIN AREA, AND SO THOSE THINGS GET AMPLIFIED, TOO.

BUT IN THE COURSE OF A SINGLE AMPLIFICATION, YOU DO RELATIVELY PURIFY THE REGION YOU'RE LOOKING AT; RIGHT? IT'S --

IN OTHER WORDS, IT WAS THERE AT ONE IN A HUNDRED THOUSAND OR ONE IN A MILLION TO START WITH, AND IF I AMPLIFIED A HUNDRED THOUSAND-FOLD, IT'S NOT -- IT COMES UP TO BE LIKE ONE IN A THOUSAND, SO IT'S -- IT HAS BEEN PURIFIED BY A SINGLE AMPLIFICATION.

HERE'S WHAT -- THIS, I'M GOING TO USE TO SORT OF SYMBOLIZE A DOUBLE-STRANDED DNA THING HERE.

WE'RE GOING -- IF I MAKE TWO OLIGONUCLEOTIDES THAT WILL BIND, POINTED WHERE THE THREE-PRIME END IS TOWARDS EACH OTHER, TO THE STRANDS, THAT -- AND DO PCR, I CAN GET A BAND. OKAY. I CAN GET A PIECE OF DNA.

OKAY. IT'S IN A MIXTURE NOW THAT HAS THE ORIGINAL DNA AND A LOT OF OTHER THINGS IN THERE, TOO, AND A LOT OF THINGS IN THERE ON THE GEL LOOK JUST LIKE THIS BAND.

OKAY. NOW, IF I TAKE -- SO IF I PUT THAT ON A GEL AND I TRY TO ANALYZE IT AND SAY, "THIS IS THE BETA-GLOBIN LOCUS," I CAN GET CONFUSED BECAUSE THERE'S SO MANY OTHER THINGS THERE.

BUT IF I JUST TAKE THE TUBE THAT THIS IS IN AND SUBJECT IT TO A FURTHER AMPLIFICATION WITH TWO MORE PRIMERS, THAT NOW INSTEAD OF BEING THESE TWO -- THESE TWO PRIMERS ARE NOW INCORPORATED IN THE SEQUENCE OF THIS THING, THEY GET -- IN THE PROCESS OF AMPLIFICATION, THE PRIMERS GET INCORPORATED INTO THE FRAGMENT THAT'S AMPLIFIED. I DON'T KNOW IF -- I GUESS YOU'VE NOTICED THAT.

SO IF I TAKE TWO MORE THAT ARE LOCATED INSIDE -- AND

THAT'S WHY THIS IS CALLED NESTED . . . PRIMER. OKAY. CALL IT NESTED PRIMER TECHNIQUE -- I CAN MAKE -- BECAUSE THESE TWO -- I'LL MAKE TWO INSIDE HERE. THESE ARE KIND OF NESTED INSIDE OF THOSE; OKAY?

AND WHAT THIS HAS -- THIS HAS THE EFFECT OF SAYING, GIVEN THAT THESE PIECES OF DNA THAT YOU'VE MADE SO FAR DO NOT ALL HAVE THE SAME INTERNAL SEQUENCE, THEY'RE THE SAME SIZE PRETTY MUCH, AND THEY'VE BEEN MADE BY THESE TWO, THEY HAD SEQUENCES THAT THOSE TWO COULD USE -- COULD FIND, BUT THEY MAY NOT HAVE SEQUENCES THAT THESE TWO CAN FIND UNLESS, IN FACT, THEY REALLY ARE BETA-GLOBIN.

IF THEY'RE REALLY WHAT I -- SO THIS THING CAN -- THESE TWO WILL NOW DISTINGUISH BETWEEN THE MEMBERS OF THIS SET THAT ARE BETA-GLOBIN AND ONES THAT ARE NOT. SO IT GIVES ME A -- IT MAKES ME A SMALLER FRAGMENT.

IN THE CASE OF THE EXPERIMENT THAT I -- THE FIRST TIME I DID IT, IT MADE A 58-BASE PAIR FRAGMENT, SEE, OUT OF THIS 110-BASE PAIR FRAGMENT THAT I HAD ORIGINALLY MADE, WHICH ITSELF CAME OUT OF AN AMORPHOUS COLLECTION OF DNA THAT WAS A WHOLE HUMAN DNA. SO IT HAD THE EFFECT OF ALLOWING ME TO INTRODUCE MORE SPECIFICITY INTO THE PROCESS.

EACH STEP INTRODUCED A CERTAIN AMOUNT, AND THE TWO STEPS TAKEN TOGETHER MADE THE PROCESS VERY, VERY SPECIFIC.

BUT I COULD NOW -- WITH THE NESTED PRIMER THING, I COULD TAKE -- START WITH HUMAN DNA AND I COULD GET A VERY CLEAN

BAND ON THE GEL THAT WAS REPRESENTATIVE OF -- JUST OF ONE LOCATION, AND IT -- ALL IT MEANT WAS, I JUST STOPPED HALFWAY THROUGH, TOOK A SMALL AMOUNT OF THIS REACTION, PUT IT IN A NEW TUBE WITH NEW PRIMERS, AND JUST CARRIED THE REACTION ON, SAY, TEN MORE CYCLES OR SOMETHING AND THEN I WOULD -- I WOULD GET A NICE CLEAN RESULT.

(WITNESS RESUMES WITNESS STAND.)

(PAUSE IN PROCEEDINGS)

Q. (BY MR. PASAHOW) WAS THE -- WAS THE USE OF NESTED PRIMERS USEFUL IN MAKING PCR INTO A TECHNIQUE THAT COULD BE USED IN ~~CLINICAL~~ LABORATORIES?

A. YES, IT WAS.

Q. WHY?

A. WELL, IT MEANT THAT YOU COULD AMPLIFY A SINGLE -- A SINGLE GENETIC LOCUS OUT OF WHOLE HUMAN DNA AND YOU COULD GET -- YOU COULD ARRIVE AT A -- AT A CLEAN -- A PRODUCT THAT WAS CLEAN ENOUGH AND THAT YOU HAD ENOUGH OF, AND YOU COULD SEE IT ON THE GEL WITHOUT RADIOACTIVITY.

I DIDN'T MENTION THAT WHEN I WAS DOWN THERE, BUT I HAD DONE -- MOST OF THE WORK I WAS DOING IN 1984, AFTER I GOT PCR TO REALLY WORK RELIABLY, WAS, I WOULD WORK WITH -- I DIDN'T HAVE TO USE P32 ANYMORE.

RADIOACTIVITY IS A REAL MESS IN A LAB. IT'S . . . THERE'S ALL KIND OF REGULATIONS ABOUT HOW YOU CAN -- HOW YOU HAVE TO DISPOSE OF IT, HOW YOU HAVE TO TREAT IT. YOU HAVE TO

WEAR GLOVES; YOU HAVE TO -- IT'S NOT -- IT'S SORT OF A POISONOUS THING.

AND SO IF YOU CAN DEVELOP A TECHNIQUE THAT REPLACES A RADIOACTIVE TECHNIQUE WITH ONE THAT'S NOT RADIOACTIVE, THEN YOU'VE MADE A BIG ADVANCE.

AND THAT'S WHAT I WAS TRYING -- THIS PROCESS HAS GOT THE POTENTIAL TO BE ABLE TO DO THE GENETIC DIAGNOSTIC STUFF THAT WE PUBLISHED IN THIS PAPER WITHOUT THE RADIOACTIVITY. AND THAT'S WHAT I WAS WORKING ON BASICALLY WAS TO TRY TO DEVELOP THAT.

Q. JUST TO CLARIFY THAT: THE DECEMBER 1985 PAPER, DID THAT USE RADIOACTIVITY?

A. YES. YEAH, WE WERE STILL USING P32 PROBES. EVEN THOUGH WE WERE AMPLIFYING, WE WERE STILL USING A PROCESS THAT INVOLVED THE RADIOACTIVE PROBES.

Q. AND WHEN YOU HAD YOUR BANDS ON THE GELS WITH THE NESTED PRIMERS, DID THAT USE RADIOACTIVITY?

A. NO, THERE WAS NONE -- NONE USED THERE.

Q. NOW, YOU ATTENDED A MEETING AT THE COLD SPRING HARBOR LABORATORY IN ~~JUNE 1986~~; IS THAT CORRECT?

A. YES.

Q. IN FACT, YOU SPOKE AT THAT MEETING.

A. YES, I DID.

Q. HOW DID YOU COME TO BE SPEAKING AT THAT MEETING?

A. JIM WATSON, WHO'S THE DIRECTOR OF THE COLD SPRING HARBOR,

INVITED ME TO COME AND SPEAK ABOUT PCR.

Q. WHO'S DR. WATSON?

A. WATSON AND -- CRICK-WATSON. THE PEOPLE THAT, IN 19 -- THE MIDDLE OF THE '50'S, FIGURED OUT WHAT THE STRUCTURE OF DNA WAS. THEY'RE -- LIKE, DR. WATSON WENT ON TO BECOME A VERY . . . A VERY WISE ADMINISTRATOR OF THIS LABORATORY CALLED COLD SPRING HARBOR, WHICH IS SORT OF A -- IT'S A LABORATORY, I THINK, THAT DR. SMITH TALKED ABOUT. IT'S A PLACE WHERE A LOT OF SCIENTISTS COME FROM ALL OVER THE WORLD AND TAKE COURSES AND STUFF.

AND HE HAD DEVELOPED -- HE IS ALSO A REAL GOOD FUND RAISER, DR. WATSON IS. HE KNEW HOW TO HIT UP THE LONG ISLAND MEDICAL SOCIETY FOR LOTS OF MONEY, SO . . .

Q. WAS THE SPEECH YOU GAVE AT COLD SPRING HARBOR IN JUNE 1986 YOUR FIRST PUBLIC PRESENTATION OF THE PCR RESULTS?

A. IT WAS MY FIRST PUBLIC PRESENTATION TO A REALLY -- A BIG GROUP OUTSIDE OF JUST A SMALL KIND OF THING AT CETUS.

Q. HAD YOU PRESENTED IT OUTSIDE OF THE CETUS GROUP BEFORE THAT TIME?

A. ONE -- I GAVE ONE SEMINAR AT MY OWN -- AT THE BIOCHEMISTRY DEPARTMENT AT THE UNIVERSITY OF CALIFORNIA AT BERKELEY. I WENT OVER THERE AND GAVE IT TO MY DEPARTMENT. I THOUGHT -- THEY THOUGHT THAT WOULD BE NICE, BEFORE I WENT TO COLD SPRING HARBOR, TO TELL THEM SORT OF AHEAD OF TIME.

Q. WHAT KIND OF INFORMATION DID YOU PROVIDE AT THE UNIVERSITY OF CALIFORNIA SEMINAR?

A. WELL, I GAVE -- I TOLD THEM HOW PCR WORKED. I GAVE THEM ALL KINDS OF INFORMATION.

Q. WHAT WAS THE REACTION OF THAT AUDIENCE?

A. THEY WERE -- THERE WERE -- THERE WAS A COUPLE OF PEOPLE THERE THAT WERE REALLY STUNNED AND EXCITED BY THE TECHNIQUE, AND THEY WANTED TO KNOW HOW TO DO IT THEMSELVES. BUT IT WAS -- IT WAS -- I MEAN, MOST OF THOSE PEOPLE WERE -- THEY WERE IMPRESSED ONCE THEY -- YOU KNOW, THEY DIDN'T CARRY ME AWAY OR ANYTHING.

Q. IN JUNE 1986, THEN, YOU PRESENTED THESE RESULTS AT THE GATHERING AT COLD SPRING HARBOR?

A. YES. THAT . . . AT THE --

Q. AND WHAT TYPE OF INFORMATION DID YOU PROVIDE AT THAT GATHERING?

A. WELL, THERE, I -- I SHOWED THEM ALL THE EXPERIMENTS THAT I HAD BEEN WORKING ON, INCLUDING THE STUFF THAT I HAD DONE IN CONJUNCTION WITH HENRY'S GROUP, AND I DEMONSTRATED THERE THAT YOU COULD SEE A SINGLE-COPIED HUMAN GENE WITHOUT RESORTING TO P32.

AND THAT WAS A PRETTY PHENOMENAL THING. I MEAN, THE WHOLE THING ACTUALLY WAS MAYBE. ALL OF THE THINGS IT COULD DO WERE.

BUT THAT WAS A VERY SPECIFIC POINT THAT MADE A BIG IMPACT, I THINK. IT HAD NOT BEEN -- NO ONE HAD EVER SEEN THE DIRECT -- DIRECTLY A BAND THAT CAME FROM A SINGLE-COPIED HUMAN GENE WITHOUT USING P32.

Q. WHAT WAS THE RESPONSE OF THE COLD SPRING HARBOR AUDIENCE TO YOUR PRESENTATION?

A. IT WAS VERY GOOD. IN FACT, I GOT A STANDING OVATION.

Q. WAS THAT UNUSUAL?

A. IT WAS.

Q. NOW, WHO ATTENDS THESE COLD SPRING HARBOR MEETINGS?

A. THE -- THE SORT OF -- THEY'RE -- THE COLD SPRING HARBOR IS SORT OF THE INTELLECTUAL CENTER FOR MOLECULAR BIOLOGY, AND JIM WATSON IS KIND OF A SPIRITUAL LEADER, IN A SENSE, IF YOU THINK OF IT AS AN INTERNATIONAL CHURCH OF MOLECULAR BIOLOGY.

AND THE PEOPLE -- IT'S A -- IT'S A VERY IMPORTANT GATHERING ACADEMICALLY, ALSO SOCIALLY. IT'S LIKE ALL THE REAL HOTSHOTS ARE GOING TO BE THERE.

IF YOU WANT TO FIND OUT WHAT'S GOING ON IN MOLECULAR BIOLOGY, YOU GO TO THE COLD SPRING HARBOR, THE ANNUAL SYMPOSIUM. THEY PUBLISH A BIG RED VOLUME EVERY YEAR, AND IT'S NICE TO HAVE A PAPER IN THAT.

Q. AND IS WHAT WE'VE MARKED EARLIER AS EXHIBIT B-192 A COPY OF THE VOLUME FROM THE JUNE 1984 COLD SPRING HARBOR MEETING?

A. YES, IT IS.

Q. AND DO YOU HAVE A PAPER IN THERE?

A. I DO. IT'S CALLED, "THE MOLECULAR BIOLOGY OF HOMO SAPIENS." THAT WAS THE NAME OF THAT MEETING, WHICH WAS A LITTLE BIT PRESUMPTUOUS, I THINK.

BUT, YES, I DO HAVE A PAPER IN THERE.

Q. NOW, WAS -- WAS DR. BRUCE WALLACE ALSO A SPEAKER ON THE PANEL WHERE YOU SPOKE?

A. YES, HE WAS. THE -- IT WAS THE PANEL ON -- THE PANEL THAT THEY PUT ME ON HAD TO DO WITH THE DIAGNOSTIC KIND OF ROUTINES, LOOKING AT SINGLE-COPIED GENES, AND BRUCE HAD BEEN DOING WORK IN THAT SAME AREA, SO HE WAS -- HE SPOKE EITHER RIGHT BEFORE ME OR RIGHT AFTER ME.

Q. DO YOU RECALL IF HE MENTIONED ANYTHING ABOUT PCR IN HIS SPEECH?

A. NO, HE DIDN'T MENTION ANYTHING ABOUT PCR. HE DIDN'T KNOW ANYTHING. HE KNEW A LITTLE BIT ABOUT IT BY THEN, BUT HE HADN'T DONE ANYTHING. HIS PAPER HAD COME OUT EARLIER.

Q. AFTER THE GROUP OF SPEAKERS THAT WERE ON YOUR PANEL FINISHED, DID ANYONE DISCUSS PCR WITH YOU?

A. AFTER THE MEETING? AFTER THE SESSION WAS OVER, THERE WAS A LOT OF REAL EXCITED PEOPLE CAME DOWN FRONT WHERE I WAS WALKING OUT THE DOOR AND WE -- A SORT OF LITTLE KNOT OF PEOPLE MOVED OUTSIDE.

A LOT OF PEOPLE THERE REALLY WANTED TO KNOW HOW TO DO IT. THEY WANTED MORE DETAILS. AND THEY WANTED TO CONGRATULATE ME, TOO. THERE WAS A LOT OF JUST LIKE, YOU KNOW, BACKSLAPPING AND, "HEY, THAT WAS REALLY COOL. WOW," YOU KNOW. THAT WAS -- THERE WAS A LOT OF WOWS.

Q. NOW, IN DECEMBER 1988, YOU WERE BACK AT COLD SPRING HARBOR SPEAKING AGAIN; IS THAT RIGHT?

A. YES, AT THE BANBURY (PHONETIC) CENTER AT COLD SPRING HARBOR.
Q. AND THAT'S A SMALLER CONFERENCE CENTER THERE?
A. YES.
Q. THE DECEMBER 1988 CONFERENCE WAS SOLELY ON THE SUBJECT OF PCR?
A. YES, IT WAS.
Q. AND SO THAT WAS WHAT ALL OF THE SPEAKERS AT THAT SESSION SPOKE ABOUT.
A. YES.
Q. HAVE YOU SPOKEN AT ANY OTHER CONFERENCES ON THE SUBJECT OF PCR?
A. I'VE SPOKEN ALL OVER THE WORLD NOW, IN LIKE PROBABLY 40 OR 50 DIFFERENT MEETINGS WHERE PCR WAS EITHER ONE OF THE THINGS THAT WAS DISCUSSED THERE OR A COUPLE OTHERS WHERE THERE WAS NOTHING ELSE BUT PCR DISCUSSED.
Q. HAVE YOU RECEIVED ANY AWARDS FOR YOUR WORK ON PCR?
A. YES. THE FIRST ONE I GOT, I SHOULD HAVE SAID, AT COLD SPRING HARBOR: I GOT FREE BEER ALL WEEK.

(LAUGHTER)

THE WITNESS: AND THE NEXT ONE WAS THE BERRINGER MANHEIM (PHONETIC) AWARD THAT HENRY ERLICH AND I SHARED. THAT WAS GIVEN BY A BIG GERMAN COMPANY CALLED BERNER LEINLAM (PHONETIC). EVERY OTHER YEAR, THEY GIVE A BIOCHEMICAL ANALYSIS AWARD. IT'S A VERY SIGNIFICANT AMOUNT OF MONEY. IT'S A COVETED AWARD. IT'S A NICE AWARD TO GET.

THE NICEST THING -- THE NICEST AWARD I'VE GOTTEN SO FAR IS THE ALLEN AWARD OF THE AMERICAN SOCIETY FOR HUMAN GENETICS, WHICH IS A FAIRLY LARGE GROUP OF PEOPLE THAT DO -- THEY ARE REALLY IMPRESSED BY PCR BECAUSE IT'S TOTALLY CHANGED WHAT YOU CAN DO WITH HUMAN GENETICS, AND THEY GAVE ME THAT IN OCTOBER OF THIS YEAR AT THEIR ANNUAL MEETING.

Q. (BY MR. PASAHOW) WERE YOU A MEMBER OF THAT SOCIETY?

A. NO. I -- THEY MADE ME AN HONORARY MEMBER, ACTUALLY. THEY USUALLY GIVE THAT AWARD TO SOMEBODY IN THEIR SOCIETY, BUT . . . AND THEY POINTED THAT OUT WHILE THEY WERE GIVING IT TO ME, THAT THEY'D MAKE ME AN HONORARY MEMBER OF THE SOCIETY.

Q. DID YOU ALSO SPEAK BEFORE THE AMERICAN THORACIC SOCIETY AND THE AMERICAN LUNG ASSOCIATION?

A. I -- YES. I GAVE THE -- WHAT WAS CALLED THE PRESIDENTIAL LECTURE. IT WAS THE PRESIDENT OF THE -- THE OUTGOING PRESIDENT OF THE SOCIETY MADE -- SORT OF HAD A GALA KIND OF A RECEPTION AND A -- A LECTURE THAT WAS THE SORT OF -- THE KEYNOTE SORT OF LECTURE FOR THE MEETING. I GAVE THAT. THAT WAS REALLY FUN.

Q. YOU DESCRIBED YESTERDAY THAT YOU NOW HAVE A PRIVATE CONSULTING PRACTICE. DOES ANY OF THAT WORK INVOLVE PCR?

A. OH, MOST OF MY -- MY CLIENTS -- I GO TO LOTS OF COMPANIES AND TO VARIOUS GROUPS THAT ARE DOING RESEARCH MOSTLY IN PCR. A COUPLE OF PEOPLE THAT I'M WORKING WITH IN OTHER DNA AREAS, BUT . . . ALMOST ALL DNA RESEARCH TODAY HAS TO DO WITH PCR, ANYHOW. IT'S AMAZING.

Q. CAN YOU GIVE US SOME IDEA OF THE COMPANIES YOU ARE DOING OR HAVE DONE PCR-RELATED RESEARCH OR CONSULTING?

A. I CAN ALMOST DO IT IN ALPHABETICAL ORDER. AMGEN (PHONETIC) IS A BIG ONE YOU MIGHT HAVE HEARD OF; EASTMAN KODAK. I'VE -- THE NATIONAL INSTITUTES OF HEALTH; THE CENTER FOR -- A THING IN ATLANTA CALLED THE CENTER FOR DISEASE CONTROL, WHICH IS A PART OF THE NATIONAL INSTITUTES OF HEALTH.

IN CALIFORNIA, THERE'S A COMPANY KIRON (PHONETIC). I'VE CONSULTED FOR BIOSEARCH, MILLAGEN (PHONETIC). THERE'S A WHOLE LOT OF DIFFERENT -- I COULD LOOK AT MY CV AND LOOK AT IT AND FIND THEM IF YOU WANTED MORE.

Q. I DON'T THINK WE NEED THAT.

HAVE YOU CONSULTED FOR ANY OF THE HEALTH AGENCIES OF THE GOVERNMENT?

A. WELL, I -- LIKE I SAY, THE NATIONAL CANCER INSTITUTE AT -- WHICH IS PART OF THE NATIONAL INSTITUTES OF HEALTH, I'VE CONSULTED FOR THEM, AND THEN THE CENTER FOR DISEASE CONTROL IN ATLANTA. THAT WAS ANOTHER.

Q. NOW, YOU'VE BEEN HERE SINCE THE BEGINNING OF THE TRIAL AND, OF COURSE, YOU'VE HEARD THE TESTIMONY ABOUT CERTAIN ARTICLES FROM THE KHORANA LABORATORY.

LET ME SHOW THEM TO YOU.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. PASAHOW) STARTING WITH THE PANET ARTICLE WHICH WE'VE MARKED AS EXHIBIT A-19 --

A. YES.
Q. -- ARE YOU FAMILIAR WITH IT?
A. I AM NOW, YEAH. I'M QUITE FAMILIAR WITH IT.
Q. WHEN DID YOU FIRST BECOME AWARE OF THAT ARTICLE?
A. IN THE SUMMER -- EARLY SUMMER, I THINK, OF 1989, I WAS BACK AT CETUS TO SEE KEVIN KASTER, AND I CAN'T REMEMBER WHAT EXACTLY THE NATURE OF THE VISIT WAS, BUT HE MET ME OUT IN THE HALL IN FRONT OF HIS OFFICE AND HE HAD THIS PAPER IN HIS HAND.
Q. WHO IS MR. KASTER?
A. HE'S THE PATENT ATTORNEY AT CETUS WHO HAS HANDLED MOST OF THE PCR PATENTS.
Q. BEFORE BEING SHOWN THAT ARTICLE BY THE CETUS ATTORNEY, DID YOU HAVE ANY KNOWLEDGE OF PRIMER EXTENSION WORK THAT HAD BEEN DONE IN THE KHORANA LABORATORY DURING THE LATE 1960'S OR EARLY 1970'S?
A. I HAD HEARD OF THE KHORANA LABORATORY, AND I KNEW THAT KHORANA HAD GOTTEN THE NOBEL PRIZE FOR BREAKING THE GENETIC CODE, WHICH WAS -- THAT'S WHAT I WAS THINKING OF AS BEING -- AND I KNEW THAT -- I MEAN, THAT WAS HIS MAIN SORT OF ACCOMPLISHMENT.

I KNEW THAT HE HAD ALSO USED A VERY -- ONE OF THE ORIGINAL OLIGONUCLEOTIDE SYNTHESIS METHODS TO SYNTHESIZE A TRNA GENE. THAT'S -- THAT'S ABOUT ALL I KNEW ABOUT THE LAB.

I -- I DIDN'T . . . I DIDN'T READ THAT LITERATURE, BECAUSE IT WAS AN OLD -- I MEAN, THOSE WERE OLD PAPERS. IT WAS A VERY ANTIQUATED METHOD OF SYNTHESIS THAT I WAS NOT INTERESTED

IN LEARNING THE DETAILS OF IT.

Q. NOW, WE HEARD SOME TESTIMONY YESTERDAY ABOUT THE PANET METHOD, AND MR. FIGG SHOWED US THIS CHART, WHICH HAS BEEN MARKED AS EXHIBIT A-182.

DOES THIS CHART REPRESENT THE METHOD THAT'S DESCRIBED IN THE PRINCIPAL PART OF THE PANET PAPER?

A. IT . . . IT RESEMBLES IT. THERE'S -- THERE IS ONE FAIRLY MAJOR DIFFERENCE IN THE WAY THAT PANET DESCRIBED WHAT HE DID AND WHAT THAT CHART DESCRIBES. I DON'T KNOW HOW IMPORTANT THAT IS.

Q. CAN YOU SHOW US THAT DIFFERENCE?

A. IT'S . . . I THINK I CAN TALK WITHOUT A MICROPHONE BECAUSE I'M NOT GOING TO BE DOWN HERE FOR LONG.

RIGHT HERE IN THE PANET ARTICLE, AND IN THE WORK THAT HE DID, THERE WAS -- THIS IS NOT A VERY SHORT STRETCH OF T'S. THERE'S A PARENTHESES AND AN END, AND THERE'S PROBABLY A HUNDRED OR MORE T'S BETWEEN THE -- THIS END AND THIS SEQUENCE OUT HERE. IT'S SLIGHTLY -- I DON'T THINK THAT'S ALL THAT CRITICAL.

Q. BUT THE POINT IS, THIS IS A VERY LONG, LONG STRING OF T'S?

A. YES. YES, IT IS.

Q. NOW, THERE WAS SOME TALK YESTERDAY, WHEN DR. SMITH WAS TESTIFYING, ABOUT THE RATIO OF PRIMERS TO TEMPLATES.

DO YOU RECALL THAT?

A. OH, YEAH. THAT WAS -- THAT WAS A -- I THINK A LITTLE CONFUSION ON THE PART OF THE OPPOSING COUNSEL.

Q. WELL, LET'S NOT GET INTO THAT.

THE QUESTION IS: DID YOU HAVE AN OPPORTUNITY TO CALCULATE WHAT THAT RATIO IS?

A. I THINK IT WAS CALCULATED IN -- IN THE COURTROOM, AND IT WAS CALCULATED WRONG.

I MEAN, IF YOU LOOK IN THE ARTICLE, WHEN THEY WERE DESCRIBING THE -- THE LEVELS OF THE PRIMER THEY PUT IN, THEY WERE -- THE PRIMER THAT MR. FIGG WAS REFERRING TO WAS A POLYMER OF DEOXYADENOSINE, AND IT'S A POLYMER OF DEOXYADENOSINE THAT THE CONCENTRATION OF IT WAS -- WAS.

SINCE THEY DIDN'T KNOW EXACTLY WHAT THE COMPOSITION OF THE POLYMER WAS, THEY HAD TO GIVE THE CONCENTRATION IN TERMS OF THE INDIVIDUAL NUCLEOTIDES, WHICH IS A COMMON NOT -- IT'S A PRACTICE THAT'S NOT UNCOMMON IN TALKING ABOUT DNA IF YOU DON'T REALLY KNOW HOW MANY INDIVIDUAL MOLECULES YOU HAVE BUT -- BECAUSE OF THE FACT THAT YOU'RE DEALING WITH A COLLECTION OF MANY DIFFERENT SIZES, WHICH IS WHAT THEY WERE DOING THERE.

AND A LOT OF TIMES WHEN YOU HAVE POLYMERS OF SOMETHING, IF YOU HAVE A HUNDRED OR 200 BASES IN A ROW, THE PROCESS YOU MAKE THAT POLYMER FROM GIVES YOU A WHOLE BUNCH OF DIFFERENT SIZES. SO YOU -- BY KNOWING HOW MUCH MATERIAL YOU HAVE, YOU CAN'T REALLY KNOW HOW MANY INDIVIDUAL MOLECULES THERE ARE, BUT YOU CAN MEASURE HOW MANY INDIVIDUAL NUCLEOTIDES THERE ARE, AND THAT'S THE NUMBER.

Q. SO THAT WOULD BE HOW MANY A'S OR G'S OR T'S OR C'S?

A. RIGHT, HOW MANY TOTAL THERE ARE.

AND THAT WAS THE NUMBER THAT THEY'D HAD IN THIS PAPER, AND THAT'S WHERE THE ONE NANOMOLE CAME FROM, SO . . . THE ACTUAL NUMBER OF NANOMOLES OF THE PRIMER THERE WAS PROBABLY A HUNDRED OR 200 TIMES LESS THAN THAT.

Q. SO THE NUMBER OF NANOMOLES THAT'S GIVEN IN THE ARTICLE, THAT'S THE NUMBER OF NANOMOLES OF ONE T; IS THAT RIGHT?

A. IT'S ACTUALLY -- IN THIS CASE, IT WAS A -- OF A'S.

Q. OKAY.

A. BUT THEY WERE BINDING TO THOSE T'S. AND THEY GAVE IT IN -- IN TERMS OF NUCLEOTIDES, AND THEY SAID THERE IN THE LEGEND THAT IT WAS IN TERMS OF NUCLEOTIDES.

THERE . . . THAT WAS -- IT WAS A LITTLE MISLEADING TO SAY THAT THERE WAS A NANOMOLE OF PRIMER THERE. THERE WAS ONLY A NANOMOLE OF A AND THE PRIMERS WERE EACH MADE OUT OF PROBABLY A HUNDRED OR 200 A'S YOU COULD TELL.

Q. SO THE PRIMERS THEY WERE USING HERE WERE A HUNDRED OR 200 OF THESE A'S LONG?

A. YES, THEY WERE. THEY DESCRIBED HOW THEY MADE THOSE, BUT THEY DIDN'T TELL YOU EXACTLY HOW LONG THEY WERE, BUT IT'S . . . THEY -- THERE'S -- THEY MAKE SOME REFERENCES IN THE PAPER TO THE FACT THAT THEY WERE LONG. THEY WERE A HUNDRED OR 200.

Q. AND TO GET THE NUMBER -- THE NUMBER OF MOLECULES, THEN, OF A PRIMER, YOU HAVE TO DIVIDE THE NUMBER OF A'S BY THE NUMBER OF A'S ON AVERAGE IN EACH PRIMER?

A. YEAH. IT'S A COMPLICATED -- TO GET THE ACTUAL NUMBER OF

PRIMERS IS VERY DIFFICULT, BUT THAT'S THE BASIC IDEA.

YOU'D SAY WHAT'S THE AVERAGE LENGTH OF THEM, AND THEN WHAT'S . . . WHAT'S -- THAT GIVES YOU A NUMBER. YOU SAY DIVIDE THAT INTO THE NUMBER OF NUCLEOTIDES; THAT'LL TELL YOU THE NUMBER OF PRIMERS.

YEAH. IT TURNS OUT WE SHOULD HAVE DIVIDED THAT NUMBER BY A HUNDRED OR SO.

Q. NOW, DOES THE -- DOES THE PANET ARTICLE, INCLUDING THE SECTION ABOUT THE UNPUBLISHED WORK FROM DR. MOLINEUX, DOES THAT DESCRIBE OPERABLE CONDITIONS THAT COULD BE USED FOR THE PCR PROCESS?

A. NO.

Q. WHY NOT?

MR. FIGG: EXCUSE ME, YOUR HONOR, WE'RE GETTING INTO EXPERT TESTIMONY NOW BY DR. MULLIS.

THIS IS SOMETHING SPECIFICALLY DISCUSSED AT THE PRETRIAL, AND I THINK WE DECIDED THAT HE WOULD NOT BE TESTIFYING AS AN EXPERT WITNESS.

MR. PASAHOW: I THINK THAT'S WRONG, YOUR HONOR. I THINK WE DISCUSSED WITNESSES AT THE PRETRIAL. I REPEATEDLY SAID DR. MULLIS WOULD TESTIFY.

THIS SUBJECT MATTER IS EXPLICITLY SET OUT IN THE DESCRIPTION OF WHAT HE'D TESTIFY TO, AND YOUR HONOR'S ORDER WAS THAT YOU WERE NOT GOING TO LIMIT THE TESTIMONY FROM WITNESSES WHO WOULD BE TESTIFYING.

MR. FIGG: WELL, I DON'T BELIEVE THAT WAS THE WAY IT CAME OUT.

THE COURT: WELL, THE LATTER IS NOT QUITE -- THE LATTER IS NOT QUITE TRUE.

THE OBJECTION IS OVERRULED.

YOU MAY PROCEED.

Q. (BY MR. PASAHOW) DO YOU RECALL THE QUESTION, DR. MULLIS?

A. WERE THERE CONDITIONS IN THIS ARTICLE THAT COULD HAVE BEEN USED FOR PCR? IS THAT WHAT --

Q. THE QUESTION WAS -- THE QUESTION WAS THAT, AND COULD YOU EXPLAIN YOUR ANSWER.

A. OH. WELL, THE -- THERE ARE NO CONDITIONS IN HERE THAT WOULD BE -- THAT YOU COULD USE FOR PCR.

AND I -- I MEAN, THERE -- THERE AREN'T ANY CONDITIONS AT ALL OFFERED, IN FACT, IN REGARD TO THEIR DISCUSSION OF THE WORK THAT HAD SUPPOSEDLY BEEN DONE PREVIOUSLY BY MOLINEUX.

THAT -- THIS PAPER'S NOT ABOUT THAT WORK. I MEAN, IT JUST MENTIONS IT AS A WAY -- IT'S A SPRINGBOARD IN THIS PAPER TO WHAT THEY ARE PRESENTING IN THIS PAPER.

THEY SAID, WE TRIED THIS METHOD AND THIS GUY MOLINEUX IN THE LAB DID IT. IT'S SOMETIME AGO, AND WE DISCOVERED THAT IT WOULDN'T WORK. AND AS WE HAD DESCRIBED EARLIER IN THIS KLEPPE PAPER, THAT WE -- WE THOUGHT IT MIGHT NOT WORK, AND WHEN IT DIDN'T WORK, WE DID THIS WORK WHICH WE'RE REPORTING IN THIS PAPER.

AND THAT'S -- THAT'S WHAT -- I MEAN, THAT'S WHAT -- THAT'S HOW THAT PARAGRAPH COMES TO BE IN THIS PAPER. THIS PAPER'S NOT ABOUT THAT METHOD. IT'S JUST SAYING, WE TRIED IT AND IT DIDN'T WORK.

Q. NOW, YOU ALSO HAVE THE KLEPPE PAPER UP THERE, EXHIBIT A-18?

A. YES.

Q. WHEN DID YOU FIRST SEE THAT PAPER?

A. THAT WAS PROBABLY A LITTLE LATER IN TIME, MAYBE . . . AFTER AUGUST MAYBE.

Q. AFTER THIS LAWSUIT WAS --

A. AUGUST '89 SOMEWHERE.

Q. AFTER THIS LAWSUIT WAS FILED?

A. AFTER THE LAWSUIT WAS FILED.

Q. NOW, DOES THE KLEPPE PAPER HAVE WITHIN IT OPERABLE CONDITIONS THAT COULD BE USED FOR A PCR PROCESS?

A. NO. NO, IT DOES NOT.

Q. WHY NOT?

A. WELL, IT -- WELL, BECAUSE THEY COULDN'T FIND THEM. I MEAN, THEY DIDN'T HAVE CONDITIONS FOR PCR PROCESS WHEN THEY WROTE THAT PAPER.

THEY -- THEY -- AT THE END OF THAT PAPER, THEY -- THEY HAVE A PASSAGE IN THERE THAT SAYS, WE HOPE WE CAN FIND SOMETHING THAT WILL ALLOW US TO REPLICATE THIS GENE THAT WE THINK WE'RE GOING TO HAVE MADE PRETTY SOON. AND WE THINK IT MIGHT BE SOMETHING LIKE THIS, AND IF IT ISN'T, IT'LL BE SOMETHING LIKE

THIS.

THAT'S KIND OF WHAT THAT PAPER SAYS. IT EITHER IS GOING TO INVOLVE . . . EXTENDING OLIGONUCLEOTIDES ON A . . . DNA DUPLEX, OR IT'S GOING TO INVOLVE ATTACHING THE STRANDS, OR -- IT DOESN'T SAY "ATTACHING" THERE; IT SAYS SEPARATING THEM, PHYSICALLY PUTTING THEM IN TWO PLACES AND DOING THEM IN SOME OTHER WAY. AND I THINK THAT OTHER WAY IS WHAT WAS DESCRIBED IN THE PANET ARTICLE.

AND THE FIRST ALTERNATIVE WHICH THEY SUGGESTED, RATHER VAGUELY BUT SUGGESTED, THAT THEY MIGHT TRY TO DO THEM TOGETHER -- AT THE SAME TIME, THEY DECIDED THAT WOULDN'T WORK BY THE TIME THEY WROTE THE PANET ARTICLE.

BUT THERE'S NOTHING IN THE KLEPPE PAPER, THERE'S NO EXPERIMENTAL DETAIL IN THE KLEPPE PAPER, THAT REALLY RELATES AT ALL TO EITHER OF THOSE METHODS. AND IN THE PANET PAPER, THERE'S ONLY EXPERIMENTAL DETAIL RELATING TO THE LATTER, THE ONE THAT DID NOT INVOLVE A PCR EVEN LIKE PROCESS.

Q. NOW, IN THE KLEPPE PAPER, THERE ARE EXPERIMENTAL DETAILS ABOUT SINGLE STRANDS OF DNA AND PRIMERS BEING EXTENDED ONTO THOSE SINGLE STRANDS; IS THAT RIGHT?

A. YES, THERE IS SOME WORK IN THERE ON -- THAT'S WHAT THE PAPER IS ABOUT.

Q. AND AMONG THE DESCRIPTIONS OF THOSE EXPERIMENTS, THEY EXPLAIN THE CONDITIONS THEY USED FOR THOSE PRIMER EXTENSION REACTIONS.

A. YES. THEY HAVE -- THEY HAVE GOOD DETAIL DESCRIBING THE EXPERIMENTS THAT THEY HAD ACTUALLY DONE, AND THEY PUT THAT IN THAT PAPER.

Q. DO YOU RECALL WHAT THE CONCENTRATION WAS OF THE PRIMERS -- I'M SORRY.

DO YOU RECALL WHAT THE CONCENTRATION WAS OF THE TEMPLATE THAT WAS BEING USED IN THOSE EXPERIMENTS?

A. I BELIEVE IT'S SOMETHING LIKE -- IT'S FROM TENTH MICROMOLAR UPWARDS, SOMETIMES -- MAYBE A LITTLE LOWER, BUT AROUND THE RANGE OF MICROMOLAR.

Q. AND DO YOU RECALL THE -- THAT THERE'S AN EXAMPLE IN YOUR PATENT THAT WAS DISCUSSED DURING MR. -- DR. SMITH'S TESTIMONY YESTERDAY?

A. YEAH. WELL, I REMEMBER THE ONE THAT WAS DISCUSSED, YES.

Q. AND THAT WAS THE EXAMPLE 1, I BELIEVE IT WAS?

A. IT WAS THIS SAME EXPERIMENT THAT'S STILL ON THE MONITORS.

Q. THAT WAS --

A. EXAMPLE 1, YES.

Q. EXAMPLE 1 WAS THE VERY FIRST PCR EXPERIMENT YOU EVER DID?

A. IT WAS -- IT WAS BASICALLY THAT. I THOUGHT IT WAS APPROPRIATE TO PUT IT IN THERE.

Q. NOW, DR. SMITH TESTIFIED YESTERDAY, YOU'LL RECALL, THAT THE CONCENTRATION OF THE TEMPLATE IN THOSE TWO EXPERIMENTS WAS SIMILAR, AT LEAST, IF NOT EXACTLY THE SAME.

A. WELL, THE CONCENTRATION OF THE TEMPLATE WASN'T THAT

DIFFERENT IN THE TWO -- IN THE -- FROM THE -- THE ONES THAT WERE IN THE KLEPPE PAPER, YOU MEAN?

Q. UH-HUH.

A. IT WAS AROUND -- IT WAS IN THE MICROMOLAR RANGE, NANOMOLAR -- LOW -- HIGH NANOMOLAR RANGE.

I'D HAVE TO LOOK AT THE ACTUAL . . .

Q. COULD --

A. I THINK THEY DECIDED YESTERDAY -- THEY CALCULATED IT OUT TO BE 700 NANOMOLARS, OR SOMETHING LIKE THAT.

Q. IF IT'S TRUE THAT THE CONCENTRATION OF THE TEMPLATE AND THE TWO WERE THE SAME, WHY COULDN'T SOMEONE HAVE USED THE CONDITIONS IN THE KLEPPE PAPER AS A GUIDELINE FOR CONDITIONS THAT COULD BE USED IN A PCR REACTION?

A. WELL, YOU HAVE TO HAVE MORE THAN JUST THE CONCENTRATION. THE PCR REACTION IS A FAIRLY INTERESTING -- IT'S AN -- THERE'S A LOT OF DIFFERENT THINGS. I MEAN, CONCENTRATION OF TEMPLATE IS ONE.

THE TIME -- THE MOST CRITICAL THING THAT -- THE BIG -- REAL BIG DIFFERENCE, THE THING THAT KLEPPE DIDN'T QUITE CATCH ON, WAS THAT THE TIME THAT IT TAKES BEFORE -- THAT IT TAKES FOR HYBRIDIZING -- THE TIME YOU ALLOW FOR HYBRIDIZING THE OLIGONUCLEOTIDES, THE TIME YOU ALLOW A PASS BEFORE YOU PUT IN THE ENZYME THAT EXTENDS THE OLIGONUCLEOTIDES, THAT HAS TO BE SHORT ENOUGH SO THAT THE TEMPLATE STRANDS DON'T GET TO REHYBRIDIZE WITH EACH OTHER.

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NOW, IF YOUR TEMPLATE STRANDS ARE AT A CONCENTRATION LIKE IN KLEPPE OR LIKE IN EXAMPLE 1 OF THE PATENT, YOU HAVE TO BE FAIRLY QUICK. YOU CAN'T WAIT 15 OR 20 MINUTES. IF YOU WAIT 15 OR 20 MINUTES, THE TEMPLATE AT THOSE CONCENTRATIONS HAS HAD TIME TO FIND ITSELF AND RE-ESTABLISH A DUPLEX TEMPLATE. IT WON'T ALLOW THE PRIMERS IN THERE AT THAT POINT.

Q. I THINK YOU SUGGESTED AN ANALOGY IN THE PAST THAT MIGHT HELP EXPLAIN THIS INVOLVING MAGNETS?

A. OH, YEAH. THAT -- THERE'S A -- THIS IS A -- YOU SORT OF IMAGINE -- IF YOU IMAGINE A -- JUST IMAGINE A SWIMMING POOL THAT'S GOT KIND OF CHOPPY WATER IN IT. AND THIS IS TO SIMULATE THE FACT THAT THE TEMPERATURE IS AT A CERTAIN -- THERE'S A CERTAIN TEMPERATURE INSIDE OF A TUBE. I THINK THE CHOPPINESS OF THE WATER MOVES THINGS AROUND.

AND JUST THINK -- YOU CAN THINK OF A BUNCH OF BAR MAGNETS. THINK ABOUT SOMETHING ABOUT THIS LONG (INDICATING) THAT WERE PLASTIC AND THEY WOULD FLOAT ON THE SURFACE OF THE WATER.

OKAY. NOW, THE BAR -- A BAR MAGNET HAS THE PROPERTY, IF YOU STICK IT UP ASIDE ANOTHER BAR MAGNET, IT WILL CLUMP TOGETHER AND HOLD FAIRLY TIGHTLY.

IF YOU TAKE A WHOLE HANDFUL OF BAR MAGNETS AND THROW THEM ON THE SURFACE OF THE SWIMMING POOL AND ALLOW THE CHOPPY WATER TO SLOWLY MOVE THEM AROUND RANDOMLY, AFTER A CERTAIN AMOUNT OF TIME HAS ELAPSED, THE BAR MAGNETS WILL CLUMP TOGETHER.