Q. YOU DIDN'T READ A SINGLE PAPER OUT OF THAT ISSUE?

A. NOT AT THAT TIME. I CAN'T. YOU KNOW, I HAVE TO LOOK AT THE JOURNAL TO SEE WHAT'S IN THERE.

Q. WHEN DO YOU RECALL THAT YOU FIRST HEARD OF THE KLEPPE PAPER?

A. I HEARD OF IT IN RELATION TO THIS CASE.

Q. WHEN DID YOU FIRST HEAR OF THE PANET PAPER?

A. I THINK . . . I WOULD SAY IT WAS AROUND 1989, MAYBE LATE '89 AND THE SAME FOR THE PANET PAPER.

Q. WHEN DID YOU FIRST HEAR OF DR. KHORANA'S NATIONAL SCIENCE FOUNDATION GRANT?

A. SOMEWHAT FURTHER ALONG.

Q. LATER?

A. LATER, YES.

Q. SO YOU DIDN'T KNOW ABOUT THESE PAPERS IN 1984?

A. NO, I DID NOT.

Q. DO YOU KNOW OF ANYONE -- HAVE YOU EVER SPOKEN WITH ANYONE WHO DID KNOW ABOUT THEM IN 1984?

A. NO, I HAVE NOT.

Q. WOULD IT BE FAIR TO SAY, DOCTOR, THAT BY 1984 THE LITERATURE THAT WAS PUBLISHED IN THE AREA OF PRIMER EXTENSION REACTIONS IN 1972 WOULD HAVE BEEN SOMEWHAT DIFFICULT TO FIND?

A. PRIMER EXTENSION IN 1982?

Q. YES.

A. I COULDN'T SAY DEFINITELY, BUT I WOULD . . . I THINK IT WOULD BE SPARSE COMPARED TO THE LAST TEN YEARS OR SO.

Q. IF 1984 SCIENTISTS WERE LOOKING FOR ART RELATING TO PRIMER EXTENSION IT MIGHT VERY WELL -- HE MIGHT VERY WELL -- EXCUSE ME -- HE MIGHT VERY WELL MISS LITERATURE THAT WAS PUBLISHED IN THE EARLY 1970'S?

A. I THINK SO. MOST OF IT WOULD BE SUPERSEDED BY MORE RECENT WORK.

Q. YOU INDICATED, I BELIEVE, THAT YOU TEACH PCR I THINK TO SOME OF YOUR STUDENTS?

A. YES.

Q. AND YOU ALSO TEACH OTHER TECHNIQUES THAT INVOLVE THE PRIMER EXTENSION REACTION SUCH AS SEQUENCING?

A. YES. I TEACH THE SANGER SEQUENCING METHOD AND OTHER TECHNIQUES.

Q. AM I CORRECT, THAT ONE OF THE THINGS YOU TEACH YOUR STUDENTS IS THAT -- THAT A T PRIMER EXTENSION REACTION IN DNA POLYMERASE IS PRETTY FAITHFUL IN REPLICATING OR COPYING A STRAND?

A. I HAVE GIVEN LECTURES OF THE FIDELITY OF REPLICATION MECHANISMS. I DON'T -- I DON'T THINK I'VE TALKED -- WHEN I TALK ABOUT SANGER SEQUENCING, I DON'T USUALLY COMMENT ON THE ACCURACY OF THE PROCESS IN TERMS OF --

Q. ISN'T IT TRUE THAT IT'S THE AVERAGE SIZE OF PIECE OF DNA THAT'S BEING SEQUENCED BY SANGER SEQUENCING IS 300 TO 400 BASES?

A. IN MY LAB HUNDREDS, ABOUT 300.

Q. SO 300 OF THESE BUILDING BLOCKS?

A. UH-HUH.

Q. ISN'T IT TRUE THAT IT'S VERY RARE TO OBSERVE A MISINCORPORATION?

A. YOU CAN'T TELL.

Q. BUT IT'S A RARE EVENT, RIGHT?

A. WELL, BASED ON THE TEACHINGS OF DR. KORNBERG'S BOOK I WOULD GUESS IT WOULD BE ONE IN 10,000, SOMETHING LIKE THAT.

Q. THE WHOLE POINT OF SEQUENCING IS THAT THIS IS A VERY FAITHFUL REACTION AND YOU CAN RELY ON ITS FAITHFULNESS AND DEDUCE FROM THAT WHAT THE SEQUENCE OF A PIECE OF DNA 300 BASES LONG IS?

A. YES, BUT THAT DOESN'T REALLY RELY ON THE ACCURACY OF THE ENZYME. I MEAN, IF YOU'RE DOING 300 AND YOU -- EVEN IF YOU'VE GOT ONE MISINCORPORATION OUT OF 300, IT WOULD NOT AFFECT THE SEQUENCE WHATSOEVER THAT YOU READ.

Q. BUT THE ENZYME HAS TO BE QUITE FAITHFUL FOR YOU TO RELY ON IT IN GENERAL FOR DETERMINING THE SEQUENCE OF A PIECE OF DNA 300 BASES LONG. WOULDN'T YOU AGREE WITH THAT?

A. IT'S ADEQUATELY FAITHFUL, YES.

Q. NOW, WE'VE HEARD OTHER TESTIMONY THAT SEQUENCING REALLY BECAME AVAILABLE -- YOU USED THE TERM THE SANGER SEQUENCING METHOD -- THAT BECAME AVAILABLE IN THE LATE 70'S, RIGHT?

A. THAT'S RIGHT. I WENT TO THE -- I WENT TO THE JORDAN CONFERENCE IN 1975 WHERE BOTH THE MAXIM GILBERT AND THE SANGER SEQUENCING METHODS WERE PRESENTED AT THAT MEETING.

THE ACTUAL SANGER SEQUENCING METHOD WAS AN EARLIER

VERSION OF THE ONE THAT WE USE TODAY. I THINK IT WAS DEVELOPED LATER ON, MAYBE '77, '78.

Q. THAT SANGER SEQUENCING METHOD REQUIRES THE USE OF A PRIMER?

A. THAT'S CORRECT.

Q. AND VARIOUS PRIMERS WERE USED FOR SANGER SEQUENCING IN THE EARLY 1980'S, RIGHT?

A. YES.

Q. HOW LONG WERE THOSE PRIMERS?

A. WELL, YOU COULD -- IN THE EARLY 1980'S YOU WOULD PURCHASE PRIMERS IN GENERALLY RANGE FROM 15 TO 20 UP TO 25.

Q. SO PEOPLE KNEW THAT FOR USING PRIMER EXTENSION REACTION IN SEQUENCING THAT PRIMERS IN THE ORDER OF 15 TO 25 WERE APPROPRIATE?

A. THAT'S CORRECT.

Q. YOU SAID IN THE EARLY '80S, YOU COULD PURCHASE PRIMERS. HOW DID YOU GET THEM BEFORE THEN?

A. WELL, PRIMERS BEFORE THAT USUALLY WERE OBTAINED BY SIMPLY CUTTING OUT A PIECE -- YOU COULD OBTAIN A PRIMER BY CUTTING OUT A PIECE OF DNA AND PURIFYING IT. OR YOU COULD CALL SOMEONE WHO, FOR EXAMPLE, HAD BEEN TRAINED BY KHORANA OR EVEN KHORANA HIMSELF AND CONVINCE HIM THAT HE SHOULD SYNTHESIZE THAT FOR YOU.

Q. DID YOU EVER DO THAT?

A. I'M SORRY.

Q. DID YOU EVER DO THAT?

A. YES. I DID, YES.

Q. SO YOU EITHER HAD TO CLIP IT OUT OF SOMETHING ELSE OR YOU HAD TO TALK SOMEBODY OUT OF IT WHO KNEW HOW TO SYNTHESIZE IT?
A. YES. I'D SAY BEFORE 1980, YEAH.
Q. AND THE MOST COMMON PROCEDURE WAS YOU WOULD CLIP IT OUT OF THIS SOMETHING ELSE, RIGHT?
A. THAT'S RIGHT.
Q. AND I WANT TO MAKE SURE I UNDERSTAND WHAT YOU'RE TALKING ABOUT THERE. YOU WOULD HAVE BACTERIA GROWING THAT HAD IN THEM A CIRCULAR PIECE OF DNA CALLED A PLASMID?
A. THAT'S RIGHT.
Q. IN THAT PLASMID IS A LITTLE PIECE OF DNA THAT YOU COULD SNIP OUT WITH YOUR RESTRICTION ENZYMES?
A. THAT'S RIGHT, YES.
Q. SO YOU HAVE TO GROW UP THAT BACTERIA AND YOU HAVE TO OPEN UP THE CELLS AND LET THAT DNA OUT. IS THAT RIGHT SO FAR?
A. UH-HUH.
Q. THEN YOU HAVE TO DIGEST THAT CIRCULAR PIECE OF DNA WITH SOME ENZYMES TO SNIP OUT THE PIECE THAT YOU WANT. RIGHT SO FAR?
A. THAT'S CORRECT.
Q. THEN YOU HAVE TO SEPARATE THAT OUT ON SOMETHING CALLED A GEL; IS THAT RIGHT?
A. THAT'S CORRECT.
Q. AND YOU HAVE TO THEN EXTRACT THAT PRIMER FROM THAT MATERIAL IN THE GEL?
A. THAT'S CORRECT.

Q. NOW, YOU FINALLY GOT PRIMER YOU CAN USE FOR SEQUENCING?
A. YES. ACTUALLY THAT WAS CONSIDERABLY EASIER THAN SYNTHESIS, IF YOU HAD SUCH A PROCEDURE, IF YOU HAD THE APPROPRIATE PRIMER IN A PLASMID.
Q. NOW, IN 1984 WERE PEOPLE STILL RELYING ON THAT AS THEIR PRINCIPAL WAY OF GETTING PRIMERS FOR SEQUENCING?
A. NO, BY 1984 IT WAS ROUTINE TO MAKE THEM BY AUTOMATED SOLID STATE SYNTHESIS.
Q. BY 1984 THESE MACHINES HAD BECOME AVAILABLE AND YOU COULD PUSH A BUTTON AND GET THE PRIMER YOU WANTED?
A. YES.
Q. YOU AND MR. PASAHOW TALKED ABOUT, I THINK, A POST-DOC OF ORDINARY SKILL?
A. UH-HUH.
Q. NOW, I'M CORRECT, AREN'T I, THAT A POST-DOC IS SOMEONE WHO IS STILL WORKING IN THE LABORATORY OF A MORE SENIOR SCIENTIST AND BASICALLY WORK UNDER THE DIRECTION OF THAT SCIENTIST?
A. THAT'S CORRECT.
Q. WOULD YOU AGREE WITH ME THAT THE PERSON OF ORDINARY SKILL IN THE ART IN 1984 IS SOMEONE WHO'S ALREADY DONE HIS POST-DOCTORAL WORK AND IS OUT NOW WORKING MORE OR LESS INDEPENDENTLY?
A. I WAS -- I WAS GROUPING ORDINARY SKILL AS BEING SOMEONE WHO IS EITHER -- WHO HAS A PH.D. DEGREE AND IS NOW IN POST-DOCTORAL TRAINING OR I WOULD EXTEND IT UP TO A VERY YOUNG ASSISTANT PROFESSOR.

THERE'S A BROAD RANGE. ACTUALLY SOME POST-DOCS TRAIN FOR FIVE YEARS, OTHERS TRAIN FOR TWO, BECOME ASSISTANT PROFESSORS. SO I'D SAY REALLY IN THAT FIVE YEARS THEY'RE STILL ACCUMULATING A LOT OF EXPERIENCE. THEY WOULD BE PEOPLE OF ORDINARY SKILL.

Q. SO AT SOME POINT AFTER THEY HAD WORKED AS A POST-DOC FOR A FEW YEARS; IS THAT RIGHT?

A. THAT'S RIGHT.

Q. NOW, DR. SMITH, I WANTED TO GO THROUGH SOME OF THE COMMENTS MADE IN KHORANA PRIOR ART WITH YOU AS MR. PASAHOW DID AND SEE IF I CAN CLARIFY A FEW QUESTIONS THAT ARE STILL IN MY MIND ON THAT.

A. ALL RIGHT.

(PAUSE IN THE PROCEEDINGS)

Q. (BY MR. FIGG) I'D LIKE TO START WITH THIS NATIONAL SCIENCE FOUNDATION GRANT THAT YOU'VE TALKED ABOUT WITH MR. PASAHOW.

YOU HAVE YOUR COPY THERE?

A. I HAVE MY COPY. IT'S A LITTLE TOO FAR TO SEE WELL WITHOUT MY GLASSES.

Q. I HAVE THE SAME PROBLEM.

I'VE PLACED ON THE EASEL A COPY OF EXHIBIT A-170, WHICH IS A BLOW UP OF PAGE 18 OF THAT GRANT APPLICATION. YOU HAVE THAT THERE?

A. I HAVE IT RIGHT IN FRONT OF ME HERE.

Q. I BELIEVE YOU SAID TO MR. PASAHOW THAT THIS DOES NOT COVER WHAT YOU CONSIDER TO BE PCR?

A. THAT'S CORRECT.

Q. I'D LIKE TO GO THROUGH IT STEP BY STEP AND SEE WHAT IS AMBIGUOUS ABOUT IT.

DO YOU AGREE, DOCTOR, THAT THIS LITTLE DIAGRAM WE HAVE HERE ON THIS EASEL, WHICH IS EXHIBIT A-156, IS A FAIR REPRESENTATION OF A DNA DUPLEX?

A. A SHORT ONE, YES.

Q. IN OTHER WORDS, DOUBLE-STRANDED DNA?

A. DOUBLE-STRANDED DNA.

Q. SO THIS WOULD BE A FAIR REPRESENTATION OF WHAT IS SAID UP HERE ON THE TOP OF THIS GRANT, PAGE 18, REPLICATION OF A DOUBLE-STRANDED DNA?

A. YEAH. HE'S PROBABLY TALKING ABOUT SOMETHING SLIGHTLY LONGER THAN THAT, BUT REASONABLY FAIR.

Q. WE'VE SELECTED ONE OF THIS LENGTH BECAUSE IT'S EASY TO ILLUSTRATE, BUT WE UNDERSTAND IT MIGHT BE SOMEWHAT LONGER.

A. PROBABLY BE TWO OR THREE TIMES LONGER THAN THAT.

Q. ONE OF THIS LENGTH WOULD ALSO BE A DNA DUPLEX?

A. IT COULD BE, YES.

Q. NOW, HE SAYS: "DENATURE THE BIHELICAL STRUCTURE."

ISN'T THAT JUST ANOTHER WAY OF SAYING DENATURE OF DOUBLE-STRANDED DNA?

A. THAT IS CORRECT.

Q. AND A POST-DOC OR PERSON OF -- A SCIENTIST OF ORDINARY SKILL IN 1984 WOULD HAVE UNDERSTOOD THAT TO MEAN THE SAME THING?

A. YES, HE WOULD KNOW VERY WELL.
Q. HOW HE WOULD ACCOMPLISH THAT?
A. WELL, EITHER BY RAISING THE TEMPERATURE OF THE SOLUTION OF THE DOUBLE-STRANDED DNA TO THE DENATURE, THE POINT OF THE DNA AND WELL ABOVE THAT OR BY USING ALKALINE TREATMENTS.
Q. DO YOU AGREE A COMMON TECHNIQUE THAT AN ORDINARY SCIENTIST IN 1984 WOULD HAVE USED WOULD HAVE BEEN SIMPLY TO PLACE THE LITTLE TUBE CONTAINING THIS DNA IN A BOWLING WATER VAT?
A. THAT'S CORRECT.
Q. WATER BOILS AT A HUNDRED DEGREES CENTIGRADE MOST PLACES?
A. YEAH.
Q. NOW, I'VE SEPARATED THESE TWO STRANDS, IS THAT WHAT ORDINARY SCIENTIST OF ORDINARY SKILL IN 1984 WOULD UNDERSTAND WOULD HAPPEN IF YOU DENATURED BIHELICAL STRUCTURE?
A. THAT'S CORRECT.
Q. NOW, IT ALSO SAYS TO DO THAT IN THE PRESENCE OF AN EXCESS OF THE TWO APPROPRIATE PRIMERS. AND I BELIEVE YOU INDICATED TO MR. PASAHOW THAT THE SCIENTIST OF ORDINARY SKILL IN 1984 WOULD UNDERSTAND THOSE WOULD BE PRIMERS THAT WOULD GO ON OPPOSITE ENDS OF THE SEQUENCE?
A. I THINK SOME ONE LOOKING AT THIS ALONE WOULD NOT KNOW THAT. THERE'S NO WAY THEY WOULD KNOW THAT JUST FROM LOOKING AT THIS PAGE THAT I'M LOOKING AT HERE.
Q. WELL, DO YOU UNDERSTAND THAT THE GOAL OF THIS PROCEDURE IS TO, AFTER ONE CYCLE, TO COME UP WITH TWO COPIES OF THIS PIECE OF

DNA?

A. ALL RIGHT. OKAY. WITH THAT PROVISO, THEN WE COULD ASSUME IF YOU'RE SAYING TWO COMPLETE COPIES --

Q. RIGHT.

A. -- THEN YOU WOULD HAVE TO BE AT ONE END FOR THE PRIMER TO THEN EXTEND ALL THE WAY TO THE OTHER END. I DON'T THINK THERE'S ANY OTHER WAY YOU COULD DO IT.

Q. POST-DOCTORAL SCIENTIST OF ORDINARY SKILL IN 1984 WOULD HAVE KNOWN IF YOU WERE GOING TO GET TWO COPIES YOU HAD TO PUT PRIMER AT EACH END?

A. TWO COMPLETE COPIES, YES.

Q. WE HAVE SOME LITTLE ILLUSTRATIONS HERE OF PRIMERS. SO ONE OF THEM WOULD GO ON THAT END (INDICATING) AND THE OTHER ONE WOULD GO ON THIS END (INDICATING). IS THAT A FAIR REPRESENTATION OF WHAT THIS REPRESENTS?

A. I DON'T THINK SO BECAUSE I DON'T THINK WE -- YOU'RE SAYING THAT THIS WOULD HAPPEN IF THE TEMPLATE IS IN A HIGH CONCENTRATION IN THE SOLUTION AND YOU ADDED THE PRIMER. DEPENDS ON THE EXCESS OF THE PRIMER. DEPENDS ON THE ABSOLUTE CONCENTRATION OF THE TEMPLATE AND SOLUTION.

IT MIGHT WELL BE THAT YOU WOULD END UP WITH TWO, THREE PRIMERS AND THE TWO STRANDS BACK TOGETHER AGAIN.

Q. BUT THIS IS WHAT DR. KHORANA IS EXPLAINING AS THE PROCESS, RIGHT?

A. YES, BUT HE'S NOT TELLING YOU HOW TO DO THAT PROCESS. SO,

IN FACT, HE CAN SAY IT IN WORDS, BUT I DON'T KNOW IT'S GOING TO OCCUR.

Q. YOU SAID THAT YOU DIDN'T THINK THIS DESCRIPTION DESCRIBED PCR AND I'M TRYING TO FOCUS ON THAT FOR THE TIME BEING.

A. OKAY.

Q. SO DOES THE DESCRIPTION THAT IS CONTAINED HERE DESCRIBE PRODUCING THESE TWO STRUCTURES?

A. OKAY. WHERE ARE WE AT, ON NUMBER 1 HERE?

WE HAVE JUST FINISHED NUMBER 1; IS THAT CORRECT?

Q. RIGHT.

A. OKAY. NUMBER 1 ITSELF DOESN'T TELL YOU ANYTHING. BUT IF YOU LOOK AT THE FIGURE ITSELF DOWN BELOW WHICH IS ILLUSTRATING THE FIRST STEP, THEN INDEED HE'S DRAWN EXACTLY WHAT YOU HAVE ON THE BOARD THERE, SO I'D HAVE TO AGREE.

Q. WELL, THE NEXT STEP SAYS: "REPAIR IN THE PRESENCE OF THE ENZYME."

YOU UNDERSTAND THAT TO MEAN THAT DR. KHORANA IS TELLING THE READER TO CONDUCT A PRIMER EXTENSION REACTION --

A. YES.

Q. -- ONCE THE PRIMERS HAVE BEEN ANNEALED?

A. YEAH.

Q. SO YOU WOULD AGREE WITH ME, WOULDN'T YOU, THAT IT'S IMPLICIT IN PARAGRAPH TWO AT THE TOP OF THIS PAGE 17 THAT THE PRIMERS WOULD BE ANNEALED TO THE ENDS OF THE TEMPLATES?

A. I ALREADY STATED THAT, YES. THE PRIMERS ARE AT THE END.

Q. IT'S IMPLICIT HERE AND IT'S EXPLICIT IN HIS DIAGRAM?

A. YES.

Q. NOW, THIS STEP 2 SAYS: "REPAIR SO AS TO COMPLETE THE DOUBLING OF THE ORIGINAL DUPLEX."

A. OKAY. HE'S SAYING -- HE'S ASSUMING HE CAN DO THAT. NOW, AGAIN WE DON'T HAVE THE -- ANY OF THE CONDITIONS. IT WOULD BE VERY LIKELY HE WOULD PERFORM THIS UNDER CONDITIONS WHERE THAT WOULDN'T NECESSARILY GO.

Q. BUT WHAT I'M TRYING TO FOCUS ON IS, WHAT WOULD THIS TELL THE ORDINARY SKILLED SCIENTISTS IN 1984?

A. WOULD TELL THEM -- IT WOULD TELL THEM TO CARRY THIS OUT, BUT WOULDN'T TELL THEM ANYTHING ABOUT HOW TO ACCOMPLISH IT.

Q. IF THEY DID CARRY IT OUT THIS IS THE RESULT THEY WOULD GET, ISN'T THAT CORRECT?

THAT THEY WOULD FILL OUT OR COMPLETE THE DOUBLING. THEY WOULD FILL THIS OUT TO THE ENDS LIKE THIS (INDICATING)?

A. THAT'S CORRECT, YES. WE WOULD ASSUME -- WE CAN ASSUME THAT PROVIDING THEY'RE DOING THIS UNDER APPROPRIATE CONDITIONS.

Q. RIGHT. AND THE DOUBLING OF THE ORIGINAL DUPLEX YOU UNDERSTAND TO MEAN THAT AFTER HE COMPLETED THIS PROCEDURE, THIS ORDINARY SKILLED SCIENTISTS WOULD NOW HAVE TWO COPIES OF THAT ONE IDENTICAL TO THE DUPLEX THAT HE BEGAN WITH?

A. YES.

Q. AND THE LAST STEP IS REPAIR WITH THE POLYMERASE -- EXCUSE ME. "REPEAT DENATURATION AND THE WHOLE CYCLE."

AND DO YOU AGREE THAT WHAT THAT REFERS TO IS THAT EACH OF THESE DUPLEXES WOULD NOW BE DENATURED IN THE PRESENCE OF AN EXCESSIVE PRIMERS, THEY WOULD BE REPAIRED AND EACH OF THESE WOULD NOW BE DOUBLED?

IS THAT THE WAY YOU UNDERSTAND IT?

A. YOU HAVE TO MAKE THE ASSUMPTION HE'S NOW NOT JUST DOING THIS IN ANOTHER TUBE FOR ONE CYCLE. YOU HAVE TO MAKE THE ASSUMPTION THAT HE'S NOW CONTINUING WITH THAT PARTICULAR TUBE IN A SECOND CYCLE. I GUESS THAT'S A REASONABLE ASSUMPTION.

Q. THAT'S THE ASSUMPTION YOU WOULD MAKE?

A. THAT'S -- IT'S HARD TO PUSH YOURSELF IN THE FRAME OF THIS IF YOU DIDN'T KNOW ABOUT PCR, BUT I THINK THAT'S ONE OF THE ALTERNATIVE WAYS YOU WOULD LOOK AT THAT.

Q. THAT'S A REASONABLE INTERPRETATION?

A. I'M LOOKING AT THIS FROM THE STANDPOINT OF SOMEONE IN 1981, NOT IN 1972 HERE. IT MAY NOT HAVE BEEN OBVIOUS TO SUCH PERSON EXACTLY -- BECAUSE THESE ARE NOT AS FAMILIAR REACTIONS TO THOSE PEOPLE.

Q. BUT EVEN TO A SCIENTIST OF ORDINARY SKILL IN 1984 --

A. 1984.

Q. -- IT WOULD BE A REASONABLE ASSUMPTION THAT'S WHAT HE'S TALKING ABOUT?

A. RIGHT. I THINK THAT'S PROBABLY CORRECT, YES.

Q. AND -- NOW, AM I CORRECT, DOCTOR, THAT . . . LET'S FOCUS ON THIS PRIMER HERE (INDICATING).

A. UH-HUH.
Q. THIS IS WHAT IS REFERRED TO IN CLAIM 1 AS THE EXTENSION PRODUCT, RIGHT?
A. RIGHT. LET ME GO BACK JUST TO A POINT FOR A MINUTE HERE. YOU SAID REPEAT THE WHOLE CYCLE, WE'RE TALKING ABOUT WHAT YOU WOULD GET IN THE NEXT CYCLE?
Q. I THOUGHT I DID. I THOUGHT I ASKED YOU TO CONFIRM HE WOULD THEN BE DOUBLING AGAIN.
A. I THINK AGAIN HERE IT'S GOING TO BE AN ASSUMPTION BASED ON THE CONDITIONS. BECAUSE BY THE TIME YOU REACH THE SECOND CYCLE YOU'RE NOW MUCH MORE CONCENTRATED IN TERMS OF THE -- YOU'VE DOUBLED THE AMOUNT OF TEMPLATE AND YOU HAVE ALSO USED UP SOME OF YOUR PRIMER, SO YOU'VE CHANGED THE CONDITIONS OF THE REACTION.

IT'S NO LONGER THE SAME AS THE FIRST CYCLE, SO I DON'T THINK I COULD DEFINITELY PREDICT THAT YOU WOULD HAVE THE SAME PROCESS IN THE SECOND CYCLE.

Q. BUT HE IS TELLING YOU IN STEP ONE TO DO THIS DENATURING IN THE PRESENCE OF AN EXCESS, AND HE EMPHASIZES EXCESS?
A. SURE. HE'S TELLING YOU TO DO IT, BUT YOU HAVE TO ASSUME HE ACTUALLY ACCOMPLISHED IT.
Q. WHAT I'M TRYING TO DO IS FOCUS ON WHAT THIS DESCRIBES AND WE'LL GET TO THE OTHER POINT.
A. IF YOU MAKE ALL THOSE ASSUMPTIONS, YES. IF YOU SAY THIS IS WHAT YOU GET, I GUESS I'D HAVE TO TAKE IT.
Q. BUT I'M NOT SUGGESTING TO YOU THAT I SAY IT'S WHAT YOU GET.

THAT'S WHAT DR. KHORANA HAS SAID IN HERE, RIGHT?

A. YES. BUT AGAIN, YOU KNOW, HE DOESN'T SAY HOW HE'S GOING TO ACCOMPLISH THIS. VERY EASY TO SAY WE'RE GOING TO DO THE FOLLOWING THINGS, BUT IT'S ENTIRELY DIFFERENT.

HE'S ACTUALLY ASKING FOR MONEY FROM THE NATIONAL SCIENCE FOUNDATION TO TRY TO DO THIS. IT'S NOT ALL APPARENT HE CAN ACCOMPLISH THIS.

Q. THAT MAY OR MAY NOT BE, DOCTOR, BUT WHAT I'M FOCUSING ON --

MR. PASAHOW: I THINK MR. FIGG CUT OFF THE WITNESS.

THE COURT: HAVE YOU COMPLETED YOUR ANSWER?

THE WITNESS: YES, I THINK SO.

MR. FIGG: I'M SORRY. I DIDN'T MEAN TO INTERRUPT YOU.

THE COURT: BE CAREFUL ABOUT TALKING OVER EACH OTHER.

MR. FIGG: AND ALSO YOUR HONOR'S COMMENT.

Q. DR. SMITH, WHAT I WANT TO FOCUS ON NOW IS YOUR COMMENT TO MR. PASAHOW THAT THIS DOES NOT DESCRIBE A PCR REACTION.

I UNDERSTAND THAT YOU HAVE SOME QUESTIONS ABOUT HOW IT WOULD WORK -- HOW IT WOULD WORK AND THE CONDITIONS. I'M GOING TO GET TO THAT LATER.

BUT FOR THE TIME BEING, WHAT HE HAS DESCRIBED HERE IS GOING THROUGH ONE CYCLE AND DOUBLING, RIGHT?

A. YEAH.

Q. AND IF THE STEP WERE REPEATED USING THE REASONABLE INTERPRETATION THAT YOU REFERRED TO EARLIER, EACH OF THESE WOULD THEN BECOME THE TARGET IN THE NEXT CYCLE AND EACH OF THEM WOULD

AGAIN BE DOUBLED; IS THAT CORRECT?

A. IF -- IF WE MAKE ALL OF THOSE ASSUMPTIONS, THEN I THINK YOU WOULD HAVE TO SAY THAT -- I MEAN, YOU'RE ESSENTIALLY SAYING THIS IS WHAT'S HAPPENING AND DESPITE THE FACT THAT THERE'S NO DESCRIPTION OF HOW IT CAN BE DONE OR WHETHER IT CAN BE DONE. LET'S ASSUME IT IS BEING DONE.

Q. RIGHT.

A. OKAY. I CAN'T OBJECT TO THAT, I GUESS.

Q. SO YOU AGREE WITH THAT THEN, AFTER THE FIRST CYCLE HE WOULD HAVE TWO COMPLETE COPIES AND AFTER THE SECOND HE WOULD HAVE FOUR COMPLETE COPIES IF IT WORKED AS SET FORTH HERE?

A. YES.

Q. NOW, WHEN YOU RESPONDED TO ONE OF MR. PASAHOW'S QUESTIONS YOU INDICATED THAT THE KEY ELEMENT OF CLAIM 1 WAS THAT THE EXTENSION PRODUCT OF ONE PRIMER BE CAPABLE OF SERVING AS THE TEMPLATE FOR THE OTHER PRIMER IN THE NEXT CYCLE. DO YOU RECALL THAT?

A. THAT'S RIGHT.

Q. I HAVE CLAIM 1 HERE IF YOU WOULD LIKE TO SEE IT, BUT YOU REMEMBER THAT, RIGHT?

A. UH-HUH.

Q. AND JUST FOCUSING ON THIS PRIMER HERE (INDICATING) --

A. UH-HUH.

Q. -- THIS IS WHAT IS REFERRED TO AS THE EXTENSION PRODUCT IN CLAIM 1, RIGHT? THIS BOTTOM STRAND HERE THAT'S IN BLUE

(INDICATING).

A. THAT'S THE EXTENSION PRODUCT, YES.

Q. NOW, WHEN IT SAYS THE EXTENSION PRODUCT OF ONE CYCLE CAN SERVE AS THE TEMPLATE FOR THE PRIMER IN THE NEXT CYCLE . . . THIS IS THE OTHER -- I'LL JUST LEAVE THAT OFF -- THIS IS THE OTHER PRIMER, RIGHT?

A. UH-HUH.

Q. AND, ISN'T IT TRUE, THAT IF YOU DOUBLE THE ORIGINAL DUPLEX THAT IT IS -- IT'S AXIOMATIC?

IT'S UNAVOIDABLE THAT THAT EXTENSION PRODUCT WILL SERVE AS THE TEMPLATE FOR THE OTHER PRIMER?

A. WELL, I THINK AGAIN YOU MAY -- YOU MIGHT GET NO REACTION, BUT IF YOU'RE ASSUMING THAT EVERYTHING IS GOING TO GO --

Q. RIGHT.

A. -- THEN I DON'T THINK THE MOLECULES ARE GOING TO KNOW WHICH MOLECULE IS WHICH, THEY'RE JUST GOING TO REACT.

BUT MY POINT IN MY PREVIOUS TESTIMONY IS THAT THERE'S NO EXPLICIT STATEMENT HERE THAT THE PRODUCT STRAND WE'RE USING IS SECOND CYCLE. NOR IS THERE ANY USE OF THE WORD EXPONENTIAL OR ANY OTHER PROPERTIES SUGGESTING THAT HE'S LOOKING AT IT IN TERMS OF THIS KIND OF REACTION AND HE DOESN'T GO INTO THE DETAILS OF THE SECOND STEP.

FURTHERMORE, WHEN I'M READING THIS, WHEN I'M MAKING THOSE STATEMENTS, I'M NOT MAKING ALL THOSE ASSUMPTIONS THAT YOU'RE MAKING.

Q. THE ASSUMPTIONS THAT WE'RE MAKING IS JUST THAT THE REACTION WILL WORK AS IT'S SET FORTH HERE FOR THE TIME BEING.

A. WE KNOW THAT PCR WORKS, SO IF YOU DESIGN CONDITIONS SO THAT THESE STEPS GO, THEN OBVIOUSLY IT'S GOING TO WORK.

Q. I'M SORRY.

A. I MEAN, IF YOU'RE CHOOSING -- WE KNOW THAT YOU CAN CARRY OUT AMPLIFICATION OF DNA IF YOU USE APPROPRIATE CONDITIONS. AND THE -- SO ESSENTIALLY IF YOU ASSUME THAT ALL OF THESE CONDITIONS ARE KNOWN AHEAD OF TIME AND ALL THESE REACTIONS ARE GOING TO TAKE PLACE, THEN I THINK YOU HAVE SOMETHING THAT IS VERY SIMILAR TO CLAIM 1.

Q. WELL, SO WHAT YOU'RE SAYING IS IF WE ASSUME THAT WHAT DR. KHORANA HAS DESCRIBED HERE IS DONE IT IS PCR; IS THAT CORRECT?

A. I THINK IF YOU -- IF YOU DO ASSUME THAT IT'S CARRIED OUT UNDER THE CONDITIONS THAT MAKE THAT REACTION POSSIBLE; NAMELY, PCR CONDITIONS, THOSE THAT WERE WORKED OUT IN THE INVENTION OF PCR, IF YOU IN EFFECT HAVE KNOWLEDGE OF WHAT THOSE CONDITIONS ARE SO THAT CAN YOU GET THIS THING TO GO AND GET THOSE PRODUCTS, THEN I WOULD HAVE TO AGREE THAT IT'S FORMALLY A -- IT AGREES WITH THE CLAIM 1.

BUT IT LEAVES -- IT'S VERY, VERY VAGUE. IT HAS NO EXPLICIT STATEMENTS. IT'S POSSIBLE HE COULD HAVE WRITTEN THIS IN MUCH MORE DETAIL AND FASHION WHERE IT WOULD BE CLEAR TO THE READER EXACTLY WHAT'S GOING ON, BUT IT'S NOT THERE.

Q. DR. SMITH --

A. WE'RE FILLING IN A LOT OF THIS.

Q. WE'RE FOCUSING NOW ON THE REACTIONS STEPS THAT ARE DESCRIBED HERE.

A. BUT WE'RE FILLING IN A LOT OF THINGS THAT ARE REALLY EXPLICITLY --

Q. ARE WE FILLING IN ANY REACTION STEPS?

MR. PASAHOW: EXCUSE ME, YOUR HONOR. I THINK DR. SMITH WAS TALKING WHEN MR. FIGG STARTED TALKING AGAIN. DR. SMITH DOES --

THE COURT: I ASSUME YOU FINISHED YOUR ANSWER?

THE WITNESS: YES.

THE COURT: I'M NOT SURE IF THE JURY HEARD IT.

THE WITNESS: WE'RE FILLING IN ESSENTIALLY ALL OF THE STEPS THAT ARE NOT GIVEN IN DETAIL HERE. THIS IS A VERY BARE BONES DESCRIPTION AND I'M NOT SURE THAT SOMEONE IN 1984 WHO DOESN'T KNOW WHAT PCR IS WOULD BE ABLE TO UNDERSTAND THIS AT ALL.

Q. (BY MR. FIGG) BUT THE STEPS THAT ARE SET FORTH HERE ARE THE SAME STEPS SET FORTH IN CLAIM 1?

A. YES. I MEAN, IF YOU -- IF YOU ESSENTIALLY SAY, WELL, WE'RE GOING TO DO IT UNDER SUCH A WAY THAT IT WILL HAPPEN.

Q. RIGHT.

A. THEN YOU'VE DONE PCR.

Q. OKAY. THIS KEY ELEMENT THAT YOU REFERRED TO WHEN YOU RESPONDED TO MR. PASAHOW'S QUESTION IS A NECESSARY CONSEQUENCE

OF MAKING TWO COMPLETE COPIES OF THE ORIGINAL DNA DUPLEX?

A. YOU'RE ASSUMING THAT'S GOING TO HAPPEN. I MADE THE POINT THAT IN THE ACTUAL REAL WORLD REACTIONS THAT SECOND CYCLE IS DIFFERENT FROM THE FIRST CYCLE.

THERE'S NO WAY OF KNOWING IT'S GOING TO HAPPEN THE SAME AS IT WAS IN THE FIRST CIRCLE. SO THAT'S AN IMPORTANT PART OF THE INVENTION OF PCR, TO DETERMINE WHAT THOSE CONDITIONS ARE.

Q. RIGHT. BUT IF IT'S DONE THE WAY IT'S SET FORTH, THEN THE EXTENSION PRODUCT OF THIS PRIMER ABSOLUTELY WILL SERVE AS THE TEMPLATE FOR THE OTHER PRIMER?

A. I THINK -- IF YOU SAY THAT THAT'S WHAT'S GOING TO HAPPEN, THEN IF WE MAKE THAT ASSUMPTION, THEN I AGREE.

Q. THAT'S WHAT THIS SAYS WILL HAPPEN WHETHER I UNDERSTAND --

A. IT DOESN'T SAY --

THE COURT: OKAY. WHAT IS HAPPENING IS THAT -- LET HIM FINISH HIS QUESTION, BUT THEN LET HIM FINISH HIS ANSWER. OKAY. I THINK SOMETIMES YOU ADD TO YOUR ANSWER AFTER YOU STARTED TO ASK THE NEXT QUESTION.

THE REPORTER IS NOT GOING TO BE ABLE TO GET BOTH OF YOU DOWN AT THE SAME TIME.

MR. FIGG: WE'LL BOTH TRY TO CLEAN UP OUR ACT.

THE WITNESS: I'LL SLOW DOWN.

(PAUSE IN THE PROCEEDINGS)

Q. (BY MR. FIGG) NOW, DR. SMITH, WHEN YOU WERE RESPONDING TO MR. PASAHOW'S QUESTION YOU INDICATED THAT YOU THOUGHT THERE

WOULD BE PROBLEMS WITH THE REACTION DESCRIBED HERE AND THE REACTION I DESCRIBED IN THE KLEPPE PAPER BECAUSE IT IS A TRNA GENE?

A. THAT'S CORRECT.

Q. AND TRNA AS YOU DREW OUR LITTLE DIAGRAM . . . THE JURY CAN'T SEE IT, BUT I'M SURE THEY REMEMBER THE CLOVER LEAF STRUCTURE. IT'S BECAUSE OF THAT CLOVER LEAF STRUCTURE THAT YOU FELT THAT THERE MIGHT BE SOME PROBLEMS IN GETTING IT TO WORK?

A. YES. ACTUALLY KLEPPE, ET AL. THOUGHT THERE WOULD BE PROBLEMS.

Q. KLEPPE ACTUALLY DID A LOT OF WORK TO IDENTIFY PRIMERS THAT WOULD COMPLETELY OVERWHELM THAT HAIRPIN STRUCTURE, DIDN'T THEY?

A. OVERWHELM ONE HAIRPIN, BUT IF YOU HAVE SEVERAL YOUR PRIMER IS EXTENDED TO ELIMINATED ONE HAIRPIN, YOU STILL HAVE THE OTHERS TO GO THROUGH.

Q. BUT WHAT DR. KLEPPE WAS REPORTING IN THIS PAPER WAS THE LENGTH OF A PRIMER TO COMPLETELY OVERWHELM ONE OF THE HAIRPINS?

A. I THINK SO. ONE SPECIFIC ONE?

Q. RIGHT.

A. I MEAN, IT ISN'T NECESSARILY THE CASE FOR ALL THE OTHERS.

Q. RIGHT. I BELIEVE WHEN YOU RESPONDED TO MR. PASAHOW'S QUESTION YOU INDICATED THAT YOU THOUGHT THAT THIS MIGHT NOT WORK, BUT YOU MIGHT GET A LITTLE BIT OF IT. DO YOU RECALL THAT?

A. OKAY. NO, I DON'T RECALL EXACTLY WHAT I SAID.

Q. WELL, YOU'RE NOT SAYING THAT WOULD BE ABSOLUTELY IMPOSSIBLE

TO PREPARE A COMPLEMENT OF THIS STRUCTURE USING THE PRIMER EXTENSION REACTION IN 1984, ARE YOU?

A. IN 1984?

Q. RIGHT.

A. I THINK EVEN IN 1984 IT MIGHT BE DIFFICULT FOR THIS PARTICULAR MOLECULE.

Q. HE WOULD GET SOME -- THE SCIENTIST OF ORDINARY SKILL WOULD GET SOME PRODUCT?

A. IT'S HARD TO PREDICT. I HAVE THE FEELING HE WOULDN'T BE ANYMORE SUCCESSFUL THAN KHORANA WAS ESSENTIALLY USING THE SAME ENZYMES. IT WOULDN'T GO 37 DEGREES, I DON'T THINK.

Q. DOCTOR, YOU INDICATED THAT YOU LEARNED OF PCR THROUGH THE SAIKI PAPER; IS THAT RIGHT?

A. THAT WAS THE TIME WHEN I REALLY HAD A CHANCE TO SIT DOWN AND THINK ABOUT THE PROCESS AND FULLY UNDERSTAND IT, YES.

Q. AND THE SAIKI PAPER, AS I RECALL, DESCRIBED A METHOD FOR AMPLIFYING DNA FROM A GENE CALLED THE BETAGLOBIN GENE?

A. THAT'S RIGHT.

Q. NOW, WERE YOU INTERESTED IN AMPLIFYING THE BETAGLOBIN GENE?

A. NO.

Q. YOU HAD NO INTEREST IN THE BETAGLOBIN GENE AT ALL, DID YOU?

A. NO.

Q. DID YOU READ THE SAIKI PAPER AS BEING LIMITED TO AMPLIFYING THE BETAGLOBIN GENE?

A. NO, IT WOULD NOT BE A VERY IMPORTANT CONTRIBUTION IF IT WAS

LIMITED.

Q. YOU READ IT AS A GENERAL PROCEDURE THAT MIGHT BE APPLIED TO ALL SORTS OF DNA?

A. THAT'S RIGHT.

Q. AND, AM I CORRECT THAT IN 1984 A SCIENTIST OF ORDINARY SKILL READING DR. KHORANA'S PUBLICATIONS PROBABLY WOULDN'T -- MOST OF THEM PROBABLY WOULDN'T HAVE ANY INTEREST IN AMPLIFYING THE YEAST ALANINE TRNA GENE, WOULD THEY?

A. I WOULD AGREE.

Q. BUT THEY MIGHT VERY WELL HAVE AN INTEREST IN AMPLIFYING SOME OTHER KIND OF GENE?

A. I THINK WITH THE KIND OF MIND SET THAT WAS IN THE KHORANA PAPER; NAMELY, TO MANUFACTURER MORE -- SOME PIECE OF DNA OR SOME GENE, THEY WOULDN'T HAVE HAD MUCH INTEREST IN IT. EVEN SOMATICALLY THE CLONING WOULD BE NORMALLY AT THAT POINT.

Q. YES. WHAT I'M SAYING IS, THE SCIENTIST OF ORDINARY SKILL IN 1984 WOULD HAVE, HAD THAT SCIENTIST BEEN AWARE OF DR. KHORANA'S PUBLICATIONS, WOULD HAVE REALIZED THAT THIS TECHNIQUE WAS APPLICABLE TO OTHER GENES IN ADDITION TO THE TRNA GENE?

A. YES. IF IT WORKED IT MIGHT BE APPLICABLE TO OTHER PIECES OF DNA, YES.

Q. NOW, THESE HAIRPINS AND CLOVER LEAVES THAT YOU'VE DRAWN OUT . . . I'LL PUT YOUR DRAWINGS BACK OVER HERE.

THESE ARE CHARACTERISTIC OF THAT GENE THAT WE'RE CALLING THE TRNA GENE?

A. THAT'S RIGHT.

Q. AND TRNA IS CHARACTERIZED BY THIS VERY COMPLEX SECONDARY STRUCTURE?

A. THAT'S CORRECT.

Q. THERE ARE A LOT OF OTHER GENES THAT DON'T HAVE THESE KIND OF STRUCTURES?

A. THAT'S CORRECT.

Q. AND IF YOU -- DO YOU KNOW WHETHER THE BETA GLOBIN GENE HAS THESE KIND OF STRUCTURES?

A. I DON'T KNOW FOR CERTAIN.

Q. BUT IF SOMEONE WAS FOLLOWING, A SCIENTIST OF ORDINARY SKILL IN 1984 WERE FOLLOWING DR. KHORANA'S PROCEDURE WITH A GENE OTHER THAN THE TRNA GENE, ONE THAT DID NOT HAVE THE SECONDARY STRUCTURES, THEN THE PROBLEM THAT YOU WERE EXPLAINING IN THIS POSTER WOULD NOT BE A PROBLEM AT ALL, RIGHT?

A. I THINK THAT'S CORRECT. I MEAN, DEPENDS ENTIRELY ON THE PIECE OF DNA, BUT IT WOULD BE VERY UNLIKELY THE SAME PROBLEMS AS WITH THE TRNA GENE.

BUT YOU SAID FOLLOWING THE PROCEDURE OF KHORANA, THERE'S NO PROCEDURE GIVEN HERE. THERE'S A BARE BONES OUTLINE OF SOMETHING, SOME KIND OF PROCESS, BUT THERE'S NO PROCEDURAL INFORMATION IN THE SENSE OF THE KIND OF THING THAT'S IN HERE THAT AN ORDINARY PERSON COULD FOLLOW.

Q. BUT IF A SCIENTIST OF ORDINARY SKILL IN THE ART ATTEMPTED TO FOLLOW THE PROCEDURE USING HIS OWN KNOWLEDGE OR HER OWN

KNOWLEDGE WITH THE PRIMER EXTENSION REACTION, THESE KINDS OF PROBLEMS AT LEAST WOULD NOT BE PROBLEMS THAT THAT SCIENTIST WOULD ENCOUNTER?

A. THERE WOULD BE LESS CHANCE THERE WOULD BE THAT KIND OF PROBLEM, THAT'S RIGHT.

Q. NOW, IT'S ALSO TRUE THAT IN 1984 SCIENTISTS OF ORDINARY SKILL IN THE ART KNEW ABOUT THESE KIND OF STRUCTURES?

A. THAT'S ABSOLUTELY CORRECT.

Q. THEY KNEW THEY EXISTED IN TRNA?

A. THAT'S CORRECT. AND THERE WERE COMPUTER PROGRAMS IF YOU KNEW THE SEQUENCE OF THE GENES YOU WERE INTERESTED IN YOU COULD, OF COURSE, GET SOME NOTION WHAT KIND OF STRUCTURES MIGHT BE PRESENT.

Q. SO IF THE SCIENTIST OF ORDINARY SKILL WERE LOOKING FOR SEQUENCES OF A GENE TO AMPLIFY, THAT SCIENTIST WOULD KNOW HOW TO AVOID THESE KIND OF STRUCTURES?

A. YES. PRESUMABLY.

Q. IF ONE WERE SELECTING DNA TO USE AS A MODEL FOR TESTING WHETHER PCR WOULD WORK OR NOT, THIS TRNA GENE WOULD HAVE BEEN ABOUT THE WORST THING ONE COULD HAVE SELECTED, ISN'T IT?

A. I WOULD HAVE TO AGREE.

Q. AND UNFORTUNATELY THIS WAS THE GENE THAT DR. KHORANA HAD AVAILABLE WHEN STARTING HIS SYNTHESIS WORK IN THE MIDDLE 1960'S?

A. THAT'S HOW HE MISSED THE INVENTION.

(LAUGHTER)

Q. (BY MR. FIGG) DOCTOR, YOU'VE TOLD ME THAT, AT LEAST IN THE NSF GRANT APPLICATION DR. KHORANA DESCRIBED WAS IN CLAIM 1, WHETHER IT WOULD WORK OR NOT IS SOMETHING WE'RE GOING TO COME TO?

A. I THINK I QUALIFIED IT BY SAYING THERE WERE MANY, MANY ASSUMPTIONS THERE.

Q. BUT THE ASSUMPTIONS ARE ON WHETHER OR NOT THE SKILLED SCIENTIST COULD MAKE EACH OF THESE STEPS THAT HE SET OUT WORK?

A. THAT'S CORRECT. AND I MIGHT ADD, THAT THE PERSON WOULD HAVE TO READ IT WITH SOME NOTION IN MIND -- HE WOULD HAVE TO HAVE SOME MOTIVATION HIMSELF AS TO WHAT HE WAS GOING TO DO WITH THIS.

YOU WOULDN'T JUST READ THAT AND SAY, WELL, I'M GOING TO DO THIS. YOU HAVE TO HAVE SOME NOTION OF WHAT HE WAS GOING TO DO, SOME MOTIVATING REASON.

Q. THE PERSON WOULD HAVE TO WANT TO DO WHAT IS SET FORTH IN THE PUBLICATIONS, RIGHT?

A. IN THE GRANT, YES.

Q. I'D LIKE TO TURN NOW TO THE KLEPPE PAPER.

(PAUSE IN THE PROCEEDINGS)

Q. (BY MR. FIGG) I'D LIKE TO FOCUS ON ONE ASPECT OF THIS FIRST. DO YOU HAVE THE PAPER THERE BEFORE YOU?

A. YES, I DO.

Q. I'M LOOKING AT THE PARAGRAPH AT THE BOTTOM OF PAGE 360. DO YOU HAVE THAT?

A. THAT'S CORRECT, YES.

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Q. I'VE PLACED ON THE EASEL EXHIBIT A-158. YOU INDICATED WHEN YOU WERE TALKING ABOUT THE GRANT APPLICATION SOME QUESTION ABOUT WHETHER TO REPEAT THE ENTIRE CYCLE WOULD MEAN TO USE THE PRODUCT FROM THE PRECEDING CYCLE AS THE STARTING MATERIAL IN THE NEXT CYCLE. DO YOU RECALL OUR DISCUSSION OF THAT?

A. YES. I MEAN, THERE'S NO EXPLICIT EXPLANATION THERE, YOU HAVE TO FILL IT IN.

Q. RIGHT. YOU INDICATED YOU THOUGHT THAT WAS A REASONABLE INTERPRETATION OF THAT DOCUMENT?

A. WELL, MAKING A LOT OF ASSUMPTIONS.

Q. IN THE KLEPPE PAPER THERE'S A SENTENCE THAT SAYS:

"THE WHOLE CYCLE COULD BE REPEATED, THERE BEING ADDED EVERY TIME A FRESH DOSE OF THE ENZYME."

A. YEAH.

Q. DO YOU AGREE WITH ME THAT THAT INDICATES MUCH MORE CLEARLY THAT YOU'RE TAKING THE PRODUCT OF THIS FIRST CYCLE, ADDING MORE ENZYME TO IT AND REPEATING THE STEPS?

A. THAT'S CORRECT.

Q. AND SIMILARLY IN THE PANET PAPER. I'VE PLACED ON THE EASEL EXHIBIT A-159, WHICH IS THE BLOW UP OF PAGE 5220.

DO YOU HAVE THAT?

A. YES, I HAVE IT HERE.

Q. NOW, THIS PAPER ACTUALLY REFERS TO THE PERFORMANCE OF MULTIPLE CYCLES, RIGHT?

A. YES. OKAY. IN PART C IN ORDER TO PERFORM MULTIPLE CYCLES.

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Q. AND IT SAYS:

"AFTER EACH CYCLE, FRESH AMOUNTS OF PRIMER ARE ADDED TO MAINTAIN THE APPROPRIATE PRIMER TEMPLATE RATIOS."

AM I CORRECT, THAT THAT WOULD INDICATE TO A PERSON OF ORDINARY SKILL IN 1984 THAT THE PRODUCT OF THE FIRST CYCLE -- OR LET ME START OVER.

THAT THIS WOULD INDICATE TO A PERSON OF ORDINARY STILL IN 1984 THAT THE PRODUCT OF A CYCLE WOULD THEN SERVE AS THE STARTING MATERIAL IN THE NEXT CYCLE?

A. YOU KNOW, I DON'T THINK YOU COULD NECESSARILY INFER THAT. BECAUSE YOU'RE USING UP PRIMER, EVEN IF YOU WERE LOOKING AT IT AS STILL USING THE ORIGINAL STRANDS, YOU WOULD HAVE TO SUPPLY ADDITIONAL PRIMERS JUST TO KEEP THE CONDITIONS WHICH HAD -- SO I -- AGAIN, I MEAN, I THINK ONE WOULD HAVE TO FIRST, OF ALL, HAVE TO ASSUME THE THINGS WORK IN THE FIRST PLACE.

YOU'RE ESSENTIALLY -- I THINK WHAT HE'S SAYING, HE'S REPLENISHING THE AMOUNT OF PRIMER THAT WAS USED IN PREVIOUS CYCLES.

Q. THAT WAS -- I THINK YOU WERE ACTUALLY ADDRESSING A QUESTION I DIDN'T MEAN TO ASK.

MY ONLY POINT HERE IS THAT HE'S INDICATING THAT HE'S ADDING PRIMERS AFTER EACH CYCLE. WHAT'S HE ADDING THEM TO?

A. WELL, I WOULD HAVE TO ASSUME THAT HE'S ADDING THEM TO THE REACTION MIXTURE.

Q. RIGHT. THEN THAT REACTION MIXTURE THAT'S NOW BEEN REPLENISHED WITH PRIMERS IS GOING TO BE TAKEN THROUGH THE CYCLE AGAIN?

A. THAT'S RIGHT.

Q. DOCTOR, YOU INDICATED THAT YOU DIDN'T SEE ANY -- I GUESS YOU QUESTIONED WHETHER THIS REACTION WORKED.

A. YES.

Q. BUT AM I NOT CORRECT THAT THIS PARAGRAPH OF THE PANET PAPER DOES INDICATE THAT REPLICATION OCCURRED ONLY BY PRIMER ELONGATION? THAT'S NOT PAST TENSE, RIGHT?

A. YES, THE REPLICATION OCCURRED ONLY BY PRIMER ELONGATION.

Q. YOU DON'T INFER FROM THAT HE WAS HOPING TO HAVE REPLICATION OCCUR BY PRIMER ELONGATION, BUT THAT THE AUTHOR IS REPORTING HERE THAT THAT'S WHAT HE ACHEIVED?

A. THAT'S WHAT HE'S SAYING, YES, I DON'T SEE THE DATA THERE.

Q. THAT'S WHAT HE'S SAYING?

A. YEAH.

Q. THAT'S HOW A SCIENTIST OF ORDINARY SKILL IN THE ART WOULD HAVE UNDERSTOOD THIS IN 1984?

A. CERTAINLY IN 1984 I WOULD HAVE KNOWN THAT WITHOUT READING --

Q. RIGHT. BECAUSE OF HIS EXPERIENCE WITH PRIMER EXTENSION?

A. THAT'S CORRECT.

Q. AND IT INDICATES THAT TO FORM THESE PRIMER TEMPLATE COMPLEXES -- IN OTHER WORDS, THESE STRUCTURES THAT WE HAD ILLUSTRATED ON THE VELCRO CHART HERE -- THAT IT WAS NECESSARY TO

HEAT AND COOL IN THE PRESENCE OF AN EXCESS TEN TIMES OR MORE OF THE APPROPRIATE PRIMERS.

SO DOESN'T THAT CONVEY TO YOU THAT THE AUTHOR HERE WAS SAYING THAT THIS WAS DONE, THAT IT WAS HEATED AND COOLED IN THE PRESENCE OF AN EXCESS OF PRIMERS?

A. WAIT A MINUTE. YEAH, I WOULD ASSUME HE'S SAYING IT WAS NECESSARY TO HEAT AND COOL. HE'S SAYING IT WAS NECESSARY TO HEAT AND COOL IN THE PRESENCE OF AN EXCESS OF TEN TIMES OR MORE OF THE APPROPRIATE PRIMERS.

BUT THE PROBLEM HERE, HE'S SAYING HE'S GOT -- LET'S SAY WHAT HE'S SAYING HERE, REPLICATION OCCURRED BY PRIMER ELONGATION. SO WE COULD ASSUME THAT HE'S SAYING THAT SOME -- HE'S SAYING ESSENTIALLY THAT THAT OCCURRED.

THAT COULD BE ONE OR TWO NUCLEOTIDES AND HE'S ESSENTIALLY SAYING, IN FACT, I THINK THAT'S THE CASE BECAUSE WHEN WE COME DOWN FURTHER DOWN HE SAYS HE GOT VERY LITTLE SYNTHESIS.

SO I THINK WE COULD MAKE THE ASSUMPTION FROM THIS THAT HE'S GOING THROUGH THESE CYCLES AND ADDING ONE OR TWO NUCLEOTIDES ON THE END OF EACH PRIMER AND THERE'S NO WAY ON EARTH THAT THAT PRODUCT IS GOING TO SERVE IN THE SUBSEQUENT REACTION AS A TEMPLATE.

Q. BUT HE DID INDICATE IN THE NEXT SENTENCE THAT MULTIPLE CYCLES WERE PERFORMED?

A. SO WHAT.

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Q. HE SAID THAT?

A. SO WHAT, THOUGH. YOU'RE GOING THROUGH THESE CYCLES AND GETTING LITTLE BIT OF EXTENSION ONTO EACH PRIMER IN EVERY CYCLE, BUT NONE OF THAT, NONE OF THOSE PRODUCTS ARE CAPABLE OF BEING USED AS TEMPLATE IN SUBSEQUENT REACTIONS. HE'S SEEING A LITTLE BIT OF INCORPORATION AND THAT'S WHAT HE SAYS HERE.

Q. YOU'RE SAYING THAT WHEN HE SAYS REPLICATION OCCURRED, THAT HE REALLY DIDN'T MEAN REPLICATION, HE MEANT JUST A COUPLE OF NUCLEOTIDES?

A. IT DOESN'T MEAN REPLICATION OF THE WHOLE STRAND HERE AND THAT'S BACKED UP BY KHORANA'S STATEMENT IN THE 1985 REPORT.

Q. DOCTOR, HAVE YOU HAD A CHANCE TO SEE ANY OF THE DOCUMENTS THAT WERE FILED IN THE PATENT AND TRADEMARK OFFICE IN CONNECTION WITH THE RE-EXAMINATION OF THE CETUS PATENTS?

A. DIDN'T SEE -- WELL, WHAT KIND OF DOCUMENTS?

Q. YOU SAW SOME OF DU PONT'S SUBMISSION TO THE PATENT AND TRADEMARK OFFICE, DIDN'T YOU?

A. OH, YES, I HAVE A COPY OF IT.

Q. DO YOU RECALL THAT?

AND I'LL HAND YOU A COPY OF EXHIBIT A-84, WHICH HAS BEEN ADMITTED INTO EVIDENCE.

DID YOU NOTICE THAT THERE WAS ALSO A NIH GRANT DOCUMENT THAT WAS SUBMITTED TO THE PATENT AND TRADEMARK OFFICE BY DU PONT?

A. I DIDN'T NOTICE THAT, NO.

Q. DO YOU SEE THAT THERE NOW?

A. WAIT A MINUTE. WHAT ARE YOU REFERRING TO HERE?

OKAY. THERE IS THE -- I'M NOT FAMILIAR WITH THIS DOCUMENT VERY WELL, BUT THIS IS THEN A PROGRESS SUMMARY REPORT -- THIS IS THE NIH -- THE NCI PROGRESS REPORT, YES, I THINK.

Q. I BELIEVE THIS ONE IS A DIFFERENT ONE THAN THE ONE WE'VE BEEN TALKING ABOUT, THOUGH.

A. I SEE.

Q. WOULD YOU TURN TO THE PAGE THAT'S LABELED PAGE TWO AT THE TOP UNDER PROGRESS REPORT. DO YOU SEE THAT?

A. WHAT YEAR IS THIS ONE?

Q. THIS ONE IS, I BELIEVE, 1972.

A. WELL, IT'S FROM '71, '72, SO THIS MUST HAVE BEEN SUBMITTED IN 1971 PERHAPS.

Q. DO YOU SEE THE SECTION IN THIS PROGRESS REPORT ENTITLED "REPLICATION OF THE GENE FOR YEAST ALANINE TRNA?"

A. UH-HUH, YES.

Q. AM I CORRECT THAT THIS STATEMENT IN THE SECOND PARAGRAPH OF THAT SECTION THAT I JUST ASKED YOU TO LOOK AT INDICATES THAT DR. KHORANA WAS SUCCESSFUL IN ACHIEVING SEVERAL-FOLD NET SYNTHESIS OF THE ORIGINAL DNA? AND LET ME READ IT. IT SAYS:

"USING SHORT SYNTHETIC DNA DUPLEXES, PARTS OF THE STRUCTURAL GENE FOR THE ALA TRNA, AND PROVIDING APPROPRIATE PRIMERS CONTAINING THE THREE-PRIME

HYDROXYL END GROUPS, SEVERAL FOLD NET SYNTHESIS OF THE ORIGINAL DNA HAS BEEN ACCOMPLISHED. A RATHER LARGE EXCESS OF THE PRIMERS IS MAINTAINED THROUGHOUT THE REPLICATION CYCLES."

DO YOU SEE THAT?

A. YES, I SEE THAT.

Q. DOESN'T THAT INDICATE TO YOU THAT DR. KHORANA WAS SUCCESSFUL IN ACHIEVING SEVERAL FOLD NET SYNTHESIS USING THE PROCEDURE THAT WE'VE BEEN TALKING ABOUT?

A. NO, IT DOESN'T TELL ME WHAT THE PROCEDURE IS. I DON'T THINK -- THIS IS 1972, BUT PRIOR TO THESE PUBLICATIONS, AND WHERE IS THE DATA?

WHY DIDN'T HE PUBLISH THIS? I DON'T KNOW THIS IS ANYWAY RELATED TO THAT OTHER WORK.

THE COURT: CAN YOU KEEP YOUR VOICE UP. YOU DROPPED OFF AT THAT LAST SENTENCE.

THE WITNESS: WHAT I'M SAYING IS, THAT I DON'T SEE -- I CAN'T DEFINITELY STATE THIS IS IN ANY WAY RELATED TO THE KLEPPE AND PANET WORK. THERE'S NO DESCRIPTION OF HOW THIS WAS DONE.

(PAUSE IN THE PROCEEDINGS)

Q. (BY MR. FIGG) LET'S LOOK AT WHAT HE'S SAYING IN THERE, DR. SMITH. HE SAYS: "USING SHORT DNA DUPLEXES, PARTS OF THE STRUCTURAL GENE OF ALA TRNA."

NOW, SHORT DNA DUPLEX IS REPRESENTED BY THIS ILLUSTRATION THAT WE'VE BEEN REFERRING TO, RIGHT?

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A. THAT IS A SHORT DUPLEX. I DON'T KNOW WHETHER HE'S USING A SHORTER ONE. IT COULD BE FIVE OR SIX LENGTHS, COULD BE LONGER THAN THAT.

Q. RIGHT.

A. WE DON'T KNOW.

Q. AND PRIMERS WITH THE . . . PRIMERS CONTAINING THE THREE-PRIME OH IN GROUPS ARE THE PRIMERS WE'VE BEEN TALKING ABOUT, RIGHT?

THEY'RE PRIMERS THAT HAVE A THREE-PRIME END THAT IS CAPABLE OF BEING EXTENDED?

A. THAT'S CORRECT.

Q. SO YOU WOULD AGREE WITH ME, AT LEAST, WHAT HE SAID HERE IS NOT INCONSISTENT WITH THE INTERPRETATION THAT HE'S TALKING ABOUT, THE SAME REACTION THAT HE DESCRIBED IN THE KLEPPE PAPER, THE NSF GRANT APPLICATION AND THE PANET PAPER?

IT'S NOT INCONSISTENT WITH IT, IS IT?

A. IT'S NOT INCONSISTENT. IT COULD BE THAT HE CARRIES OUT A CYCLE, ISOLATED THINGS, CARRIES OUT ANOTHER CYCLE, SEPARATE STRANDS. IT COULD BE THAT THE PANET WORK --

Q. BUT, IN FACT, PANET ONLY USED -- OR PANET ONLY USED ONE PRIMER IN HIS WORK, DIDN'T HE?

A. YEAH. BUT HE'S NOT SAYING HE'S USING THE DUPLEXES IN THE REACTION, AS FAR AS I CAN SEE. I MEAN, I COULD HAVE SEPARATED THE STRANDS WITHOUT DUPLEX AND ATTACHED TO EACH STRAND TO A CELLULOSE PARTICLE THEN CARRIED OUT THE PANET REACTION.

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Q. LET'S TALK ABOUT THAT.

A. BUT I DON'T THINK WE HAVE A REAL INDICATION AS TO WHAT'S GOING ON HERE. HE COULD -- I'M SORRY, I'M INTERRUPTING YOU. HE COULD IN FACT -- THAT'S OKAY.

Q. WHEN YOU WERE TALKING ABOUT PANET AND WHEN YOU RESPONDED TO SOME OF MR. PASAHOW'S QUESTIONS CONCERNING THE LAST PART OF THE LAST PARAGRAPH OF PAGE 360, I BELIEVE YOU INDICATED WHAT THAT DESCRIBED WAS SEPARATING THE STRANDS INTO -- PUTTING THEM INTO TWO DIFFERENT TUBES; IS THAT RIGHT?

A. YES.

Q. THEN YOU WOULD SYNTHESIZE DNA ON EACH OF THOSE TUBES AND MAKE COPIES IN EACH OF THE TUBES?

A. YOU'RE MAKING -- ALL RIGHT, YOU HAVE THE STRAND SEPARATED IN TWO SEPARATE TUBES. LET'S TAKE ONE TUBE. YOU WOULD THEN -- SO NOW YOU HAVE A SINGLE STRAND, YOU WOULD DO ONE CYCLE BY PRIMER EXTENSION TO COMPLETE THE SYNTHESIS OF THE COMPLEMENTARY COPY TO THAT TEMPLATE.

THEN WHAT HE'S SAYING IS THAT YOU WOULD SEPARATE THOSE TWO INTO SEPARATE TUBES AND NOW TAKE THE TEMPLATE AGAIN AND REPEAT THE PROCESS.

Q. THEN YOU SEPARATE THEM INTO SEPARATE TUBES AGAIN?

A. ALWAYS A PHYSICAL SEPARATION, YES.

Q. IF YOU DID THAT YOU GET TWO COPIES OF THE DUPLEX YOU STARTED WITH AFTER THE FIRST CYCLE, YOU HAVE TO HAVE TWO TUBES?

A. OKAY. YOU'RE DOING BOTH TUBES TOGETHER?

Q. RIGHT.

A. RIGHT. AFTER THE FIRST CYCLE YOU HAVE TWO COPIES, THEY'RE SEPARATE. SO YOU'RE GOING TO HAVE TO AMPLIFY TUBES HERE.

Q. SO THE NEXT ROUND YOU'RE GOING TO HAVE FOUR TUBES?

A. THAT'S CORRECT.

Q. DO YOU SEE ANYTHING IN CLAIM 1 THAT SAYS YOU CAN'T CARRY OUT THIS PROCESS BY THAT PROCEDURE?

A. I NEVER READ IT IN THAT -- FROM THAT ASPECT.

Q. THE CLAIM IS ACTUALLY BROAD ENOUGH TO COVER THAT PROCESS, ISN'T IT?

A. LET ME REFRESH MYSELF HERE. I CAN'T READ IT REALLY FROM THAT DISTANCE.

Q. SURE. LOOK AT THE ORIGINAL.

A. I'M GETTING QUITE A PILE HERE.

(PAUSE IN THE PROCEEDINGS)

THE WITNESS: WELL, THE LANGUAGE HERE IS NOT THAT OF A USUAL SCIENTIFIC PAPER. IT'S A LITTLE HARD FOR ME TO APPRECIATE ALL THE IMPLICATIONS OF THIS.

I DON'T THINK I COULD MAKE A STATEMENT ON THERE AT THIS POINT.

Q. (BY MR. FIGG) WELL, STEP B SAYS, SEPARATING THE PRIMER EXTENSION PRODUCTS ON THE TEMPLATES WHICH THEY WERE SYNTHESIZED, AND IT DOESN'T SAY SEPARATE THEM AND PUT THEM BACK INTO THE SAME TUBE, DOES IT?

A. WELL, WAIT A MINUTE. LET'S SEE, IF YOU PUT THEM INTO FOUR

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TUBES . . . ALL RIGHT. YOU DO THE EXTENSION -- YOU HAVE THE SEPARATED STRANDS, YOU DO THE EXTENSION, NOW YOU GO INTO TWO TUBES AND . . . OKAY. WELL, BUT PANET DOES NOT SAY THAT.

Q. WELL, I'M NOT ASKING YOU ABOUT PANET.

A. WHAT?

Q. I WASN'T ASKING YOU ABOUT PANET. I WAS ASKING YOU IF THAT PROCESS WOULD BE COVERED BY THE PROCESS THAT YOU DESCRIBED AS BEING WHAT IS EXPLAINED IN THE LAST PARAGRAPH OF THE KLEPPE PAPER?

A. THE PROCESS I DESCRIBED IMPLIES THAT YOU'RE ALWAYS USING THE SAME TWO TUBES FOR YOUR REPLICATION. THESE OTHER TUBES YOU JUST ACCUMULATE.

Q. YOU'VE CHANGED YOUR EXPLANATION NOW, BEFORE WE WERE GOING FROM TWO TUBES TO FOUR TUBES AND SEPARATING THE PRODUCTS EACH TIME.

A. I'M SORRY, I MADE A MISTAKE. IT WOULD BE TWO TUBES, FOUR TUBES, SIX TUBES. IT WOULD BE A LINEAR, I BELIEVE.

Q. THE PROCESS AS YOU ORIGINALLY DESCRIBED IT WOULD NOT BE EXCLUDED BY CLAIM 1, WOULD IT?

A. IN WHICH YOU WERE JUST ON THE RECONSTRUCTION, POSSIBLY NOT. BUT I MISSPOKE. I DIDN'T MEAN THAT THAT'S NOT WHAT PANET WAS SAYING.

Q. WHEN YOU DESCRIBED THAT REACTION, SIR, YOU WEREN'T REFERRING TO PANET; YOU WERE REFERRING TO THE LAST SENTENCE IN THE KLEPPE PAPER?

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A. YES.

Q. NOW, ONE OF THE COMMENTS YOU MADE ABOUT DR. KHORANA'S WORK, I BELIEVE, OR THE KLEPPE PAPER, WAS THE CONCENTRATIONS OF THE TEMPLATE WERE -- WOULD BE TO HIGH; IS THAT CORRECT?

A. YES.

Q. IN OTHER WORDS, YOU'RE READING THE EXPERIMENTS THAT ARE DESCRIBED IN THE KLEPPE PAPER WHERE KLEPPE WAS ANNEALING SINGLE-STRANDED TEMPLATES AND SINGLE-STRANDED PRIMERS AND YOU'RE ASSUMING THAT A PERSON READING THE PAPER WOULD USE THOSE SAME CONCENTRATIONS IF THEY WERE CARRYING OUT THE REACTION DESCRIBED IN THE LAST PARAGRAPH?

A. WOULD BE A NATURAL STARTING POINT.

Q. AS I RECALL THOSE CONCENTRATIONS ARE IN THE RANGE OF 100 TO 500 NANOMOLARS?

A. I THINK THAT'S THE CASE, YES.

Q. THERE IS A REFERENCE TO THAT IN THE KLEPPE PAPER. IF YOU'RE UNSURE I CAN REFER YOU TO IT.

A. I DON'T THINK THERE WERE LESS THAN A HUNDRED.

Q. YOU REFER TO THE TOP OF PAGE 246, I BELIEVE, DR. KLEPPE POINTS OUT THE CONCENTRATIONS HE WAS USING WERE IN THAT RANGE. I'M SORRY, 346, MY COPY IS BAD.

A. OKAY. YEAH. USUALLY WAS IN THE RANGE OF A HUNDRED TO 500, YES.

Q. DOCTOR, YOU'RE FAMILIAR WITH THE '202 PATENT, I BELIEVE, RIGHT?

A. YES.

Q. AND YOU'RE FAMILIAR WITH EXAMPLE 1 OF THAT PATENT. I'VE JUST PLACED AN OVERHEAD OF EXAMPLE 1 SO THAT THE JURY CAN SEE IT, BUT I THINK YOU HAVE THE PATENT THERE BEFORE YOU.

A. OKAY.

Q. I JUST WANT TO --

A. I WAS TRYING TO TRANSLATE THIS INTO -- OKAY.

Q. I KNOW WHAT YOU WERE DOING. WE'RE LOOKING AT THE PATENT NOW.

A. OKAY.

Q. YOU WERE DOING A LITTLE QUICK ARITHMETIC IN YOUR HEAD?

A. YES, I DIDN'T COMPLETE THE ARITHMETIC, BUT . . .

Q. NOW, DOCTOR, EXAMPLE 1 IS AN EXAMPLE THAT PATENTEES PROVIDED TO EXEMPLIFY THE INVENTION THEY WERE CLAIMING, IS THAT THE WAY YOU UNDERSTAND IT?

A. YES.

Q. NOW, EXAMPLE 1 USED A CONCENTRATION OF TEMPLATE OF 72 NANAMOLAR, DO YOU AGREE WITH THAT?

A. YES, I THINK THAT'S THE CALCULATION.

Q. IN THE SCIENCE OF MOLECULAR BIOLOGY SCIENTISTS REALLY WOULDN'T CONSIDER 72 TO BE SIGNIFICANTLY DIFFERENT FROM 100, WOULD THEY?

A. PROBABLY NOT.

Q. SCIENTISTS USUALLY TALK IN TERMS OF ORDERS OF MAGNITUDE, RIGHT?

A. AT LEAST IN TERMS OF FACTORS OF TWO, OR FOUR, OR SIX.
Q. WHAT'S IN THE ORDER OF MAGNITUDE?
A. FACTOR OF TEN.
Q. SO IF SCIENTISTS WERE CHANGING THE CONCENTRATION OF A PARAMETER TO SEE WHAT AFFECT THAT HAS, IT WOULDN'T BE UNUSUAL TO SPAN A FEW ORDERS OF MAGNITUDE, WOULD IT?
A. I THINK SO. I'M SORRY.
Q. THAT WOULD BE FAIRLY COMMON, RIGHT?
A. TO DO WHAT AGAIN?
Q. TO SELECT CONCENTRATIONS SPANNING A FEW ORDERS OF MAGNITUDE.
A. DEPENDS VERY MUCH ON WHAT PARAMETER YOU'RE DEALING WITH. IF IT WERE MAGNESIUM CONCENTRATION NORMALLY YOU WOULD CONFINE IT TO PROBABLY ONE ORDER OF MAGNITUDE.
Q. WHICH IS A FACTOR OF TEN?
A. OR IF YOU'RE DEALING WITH LENGTH OF TIME FOR ANNEALING REACTIONS PROBABLY ONE AND A HALF, SOMETHING LIKE THAT.
Q. JUST SO WE UNDERSTAND, AN ORDER OF MAGNITUDE MEANS THAT ONE CONCENTRATION OR ONE NUMBER IS TEN TIMES MORE THAN THE OTHER ONE YOU'RE USING?
A. YES. FOR EXAMPLE, YOU MIGHT DO IT IN THE RANGE FROM ONE TO TEN OR TWO TO 20, SOMETHING LIKE THAT.
Q. OR ONE TO TEN TO 100 TO 1,000?
A. IF YOU WERE DOING THREE ORDERS OF MAGNITUDE.
Q. DR. SMITH THE PATENT EXAMPLE 1 IS USING 72 NANOMOLARS, WHEREAS DR. KLEPPE USED 100 TO 500 NANOMOLARS. IF WE ASSUME

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THAT EXAMPLE 1 WAS WORKING AS DESCRIBED, AM I CORRECT THAT THE CONCENTRATION OF TEMPLATE IN THE NEXT CYCLE WOULD BE A HUNDRED AND 40 NANOMOLARS, ROUGHLY?

A. IN THIS EXAMPLE HERE (INDICATING)?

Q. YES.

A. THIS PARTICULAR EXAMPLE IS PROBABLY THE WORST CASE EXAMPLE IN THE WHOLE -- WHAT THEY'RE ATTEMPTING TO DO HERE IS GIVE THE COMPLETE RANGE OF CONDITIONS UNDER WHICH THE THING MIGHT WORK, EVEN THOUGH IT MIGHT WORK VERY MARGINALLY.

AND THIS IS THE HIGHEST LEVEL. MOST OF THE LEVELS ARE 1,000 TIMES OR EVEN A MILLION TIMES LESS THAN THIS AMOUNT.

Q. THIS IS AN EXAMPLE --

A. THIS IS AN EXAMPLE, THAT'S CORRECT.

Q. AN EXAMPLE OF WHAT IS CLAIMED IN THE PATENT?

A. THAT IS CORRECT.

Q. GOING BACK TO MY EARLIER QUESTION, AFTER THE FIRST CYCLE YOU RAN THIS REACTION YOU'D HAVE A HUNDRED AND 40 NANOMOLARS?

A. NO, I DON'T THINK YOU WOULD.

Q. IF IT WORKED AS IS BEING EXPLAINED IN THE PATENT THAT'S WHAT YOU WOULD HAVE, ISN'T IT?

A. NO, I THINK THE PATENT ALLOWS FOR LESS THAN MAXIMUM EFFICIENCY.

Q. WHAT EFFICIENCY DOES THE PATENT ALLOW FOR?

A. I THINK ANY EFFICIENCY -- THE PATENT ALLOWS IT TO BE A PCR REACTION IF THE PRODUCT STRANDS, EVEN IF THEY'RE NOT PRODUCED AT

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THE FULLEST POSSIBLE NUMBER, AS LONG AS THOSE PRODUCT STRANDS CAN BE DEMONSTRATED TO BE UTILIZED IN SUBSEQUENT CYCLES THAT'S ALL THAT'S REQUIRED.

SO THAT'S COMPATIBLE WITH RATHER LOW EFFICIENCY REACTIONS. ALTHOUGH, IT WOULD BE HARD IF THEY'RE VERY LOW EFFICIENCY, IT WOULD BE HARD TO TELL IT'S ACTUALLY A PCR REACTION RATHER THAN SOMETHING ELSE.

Q. WOULD IT COVER A REACTION THAT'S 10 PERCENT EFFICIENT?

A. YES, IT WOULD. BUT YOU'D HAVE TO DEMONSTRATE, YOU MIGHT HAVE TO CARRY OUT A HUNDRED CYCLES TO DEMONSTRATE THAT YOU ACTUALLY WERE EXCEEDING WHAT YOU WOULD GET BY THE DELAYING LINEAR REACTION. UNLESS YOU ASSUME THE LINEAR REACTION WAS EQUALLY NON-EFFICIENT THEN IT WOULD BE AN EASIER DEMONSTRATION.

Q. WELL, THE EXAMPLE HERE STARTS OUT WITH 72 NANOMOLARS. YOU'LL AGREE WITH ME, AFTER THE FIRST CYCLE, THE CONCENTRATION SHOULD BE HIGHER THAN 72?

A. SHOULD BE HIGHER, YES.

Q. IF IT WAS 10 PERCENT EFFICIENT IT WOULD BE ROUGHLY 80?

A. SOMETHING LIKE THAT.

Q. IF IT WAS 50 PERCENT EFFICIENT IT WOULD BE A HUNDRED AND TENOR SO, A HUNDRED AND FIVE?

A. YES, UH-HUH.

Q. SO YOU'LL AGREE WITH ME, WON'T YOU, THAT THE CONCENTRATION OF TEMPLATE USED IN EXAMPLE 1 IS NOT SIGNIFICANTLY DIFFERENT FROM THE CONCENTRATIONS THAT DR. KLEPPE USED?

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A. IT'S SLIGHTLY LOWER THAN THE LOWEST CONCENTRATION USED IN THAT PAPER.

Q. SLIGHTLY LOWER?

A. SLIGHTLY LOWER, YES.

Q. DOCTOR, WHEN YOU WERE ANSWERING SOME OF MR. PASAHOW'S QUESTIONS YOU INDICATED THAT YOU THOUGHT ANOTHER PROBLEM WAS THE LONG ANNEALING TIME THAT DR. KLEPPE DESCRIBES IN HIS EXPERIMENTS. DO YOU RECALL THAT?

A. I DO.

Q. WHAT YOU MEAN BY THAT IS THE LENGTH OF TIME THAT ONE . . . THAT ONE ALLOWS FOR THESE PRIMERS TO COME BACK IN AND SEAT ON THE TEMPLATE AND FIND A TEMPLATE? THAT'S WHAT YOU MEAN BY ANNEALING TIME?

A. NO. ANNEALING TIME IS THE ACTUAL AMOUNT OF TIME YOU CHOOSE FOR THE REACTION. IT MAY BE SO SHORT THAT YOU DON'T GET ANNEALING, OR IT COULD BE A HUNDRED TIMES LONGER THAN IS ACTUALLY NEEDED.

TO DETERMINE THE ACTUAL ANNEALING TIME WOULD REQUIRE EXPERIMENT.

Q. I UNDERSTAND YOUR POINT, SOMETIMES THIS HAPPENS VERY FAST. WHEN YOU REFER TO ANNEALING TIME YOU'RE REFERRING TO THE AMOUNT OF TIME THE SCIENTIST GIVES THAT REACTION TO OCCUR?

A. THAT IS CORRECT.

Q. NOW, WHEN DR. KLEPPE WAS USING ONE HOUR ANNEALING TIME, AM I CORRECT, THAT IN EVERY INSTANCE WHERE THAT FIGURE WAS USED DR.

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KLEPPE WAS ANNEALING A SINGLE-STRANDED TEMPLATE AND A SINGLE-STRANDED PRIMER?

A. THAT'S CORRECT.

Q. DR. KLEPPE WAS NEVER -- IN ANY OF THOSE EXPERIMENTS WHERE DR. KLEPPE WAS USING ONE HOUR ANNEALING TIMES, HE WAS NEVER ANNEALING A PRIMER TO A TEMPLATE THAT HAD BEEN CREATED BY DENATURING A DOUBLE-STRANDED MOLECULE?

A. NOT IN THAT PAPER, NO.

Q. NOW, DOCTOR, DO YOU THINK A SCIENTIST OF ORDINARY SKILL IN 1984 WOULD HAVE KNOWN THAT IF YOU'RE ANNEALING A PRIMER TO A TEMPLATE THAT'S BEEN CREATED BY DENATURING A DOUBLE-STRANDED TEMPLATE, THAT ONE SHOULD USE MUCH SHORTER ANNEALING TIME THAN ONE HOUR?

A. YOU'RE GETTING COMPLICATED HERE NOW. I DON'T THINK THAT -- YOU KNOW, IN MY EXPERIENCE THE SCIENTIST OF ORDINARY SKILL DON'T HAVE TO GET APPRECIATION OF THE KINETICS OF THESE REACTIONS. YOU FIND HIGHLY SKILLED SCIENTISTS USING ANNEALING TIMES THAT ARE 50 TIMES LONGER THAN YOU REQUIRE.

THE TENDENCY IS ALWAYS TO USE MORE THAN ENOUGH; IN OTHER WORDS, OVERKILL. AND I THINK ALSO PARTICULARLY IN THE EXAMPLE YOU'RE GIVING NOW THE KINETICS OF THE PRIMER ANNEALING WOULD BE VERY SIMILAR TO THE KINETICS OF THE TEMPLATE.

IT'S A VERY DELICATE QUESTION AS TO WHAT WOULD HAPPEN THERE.

Q. WELL, ALL I'M GETTING AT IS WOULD A SCIENTIST IN 1984 HAVE

HAD GUIDANCE TO FOCUS ON ANNEALING TIME AS A PARAMETER TO INVESTIGATE?

A. I THINK THAT WOULD BE ONE OF THE PARAMETERS INVESTIGATED, YES.

Q. A SCIENTIST WOULD HAVE HAD SOME CONSIDERABLE LITERATURE FROM WHICH TO DRAW FROM TO DETERMINE THE ANNEALING TIME?

A. RIGHT, BUT NONE OF THAT LITERATURE WOULD REALLY . . . POINT HIM IN THE DIRECTION OF WHAT'S NEEDED FOR THE PCR REACTION, I DON'T BELIEVE.

I DON'T RECALL, IN THE PCR EVERY ANNEALING TIME IN HERE IS OF THE ORDER OF TWO MINUTES OR LESS. YOU COULD FIND VERY LITTLE IN THE LITERATURE IN TERMS OF THE ANNEALING TIMES OF THAT LENGTH.

Q. THE ANNEALING TIME ON EXAMPLE 1 DOESN'T SAY IT'S TWO MINUTES OR LESS, DOES IT?

A. I THINK THAT'S THE ONLY ONE WHERE IT'S NOT SPELLED OUT. IT'S ALLOWED TO COOL DOWN THE TEMPERATURE IN THAT SMALL TUBE, THAT PROBABLY OCCURRED IN THE ORDER OF ABOUT A MINUTE.

Q. THERE'S NOTHING IN THE --

A. BUT THERE'S NOTHING THERE TO PROVE THAT.

Q. THERE'S NOTHING IN THE BODY OF THE PATENT THAT TELLS YOU THAT ANNEALING TIMES ARE IMPORTANT AT ALL, IS THERE, IF YOU EXCLUDE THE EXAMPLES?

A. YES, SIR. I THINK THE FACT THAT THE PROTOCOL ITSELF SPECIFIES A SHORT ANNEALING TIME IS SUFFICIENT.

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Q. SOME OF THE PROTOCOLS SPECIFY A SHORT ANNEALING TIME AND SOME DON'T?

A. THIS IS BACK -- I THINK A POST-DOCTORAL FELLOW OF ORDINARY SKILL WOULD REALIZE THAT THAT IS A VERY RAPID COOLING.

Q. HOW WOULD THE POST-DOC OF ORDINARY SKILL RECOGNIZE THAT?

A. ONE LITTLE SIMPLE EXPERIMENT WOULD DETERMINE THAT.

Q. AND, DOCTOR, LET ME SHOW YOU AN EXHIBIT WE'VE MARKED AS A-47. I DON'T KNOW IF YOU'RE FAMILIAR WITH THAT. IT'S AN ARTICLE THAT WAS PUBLISHED BY DOCTOR BRUCE WALLACE.

HAVE YOU SEEN THAT BEFORE?

A. I HAVEN'T READ THIS PAPER, NO.

Q. I JUST WANT TO FOCUS ON A PART OF IT.

MR. PASAHOW: YOUR HONOR, IF DR. SMITH IS GOING TO BE CALLED UPON TO DISCUSS THIS PAPER, I WOULD ASK HE BE GIVEN A CHANCE TO READ IT.

THE COURT: ARE YOU GOING TO FOCUS ON A VERY DISCREET PORTION OF IT?

MR. FIGG: THAT'S RIGHT.

THE COURT: LET'S SEE WHAT THE QUESTION IS. IF HE NEEDS TIME WE'LL TAKE OUR SECOND MORNING RECESS, SO HE HAS TIME TO REVIEW IT.

(PAUSE IN THE PROCEEDINGS)

Q. (BY MR. FIGG) DR. SMITH, WHAT I'D LIKE TO FOCUS YOUR ATTENTION ON IS PAGE 22 OF THIS ARTICLE. THERE'S A SECTION THERE CALLED DNA SEQUENCING PROCEDURE.

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DO YOU SEE THAT ON PAGE 22?

A. YES.

Q. AND DR. WALLACE IS DESCRIBING THE PROCEDURE HERE WHERE HE'S STARTING WITH PLASMID DNA TO BE SEQUENCED. DO YOU UNDERSTAND THE PLASMID DNA IS DOUBLE-STRANDED DNA?

A. THAT'S CORRECT.

Q. DR. WALLACE IS USING ONE OF YOUR RESTRICTION ENZYMES HERE TO CLIP OUT A PORTION OF THAT DOUBLE-STRANDED DNA; IS THAT RIGHT?

A. THAT'S CORRECT. WELL, TO CUT THE MOLECULE, YES. HE'S NOT CUTTING OUT ANYTHING.

Q. HE'S BREAKING OPEN THE CYCLE SO HE NOW HAS A LINEAR PIECE OF DOUBLE-STRANDED DNA?

A. THAT'S RIGHT.

Q. AND DO YOU SEE THAT DR. WALLACE IS TAKING THAT LINEAR STRAND OF DNA AND PUTTING IT IN A LITTLE TUBE CALLED A CAPILARY ALONG WITH SOME OTHER THINGS AND OLIGONUCLEOTIDE PRIMER. DO YOU SEE THAT?

A. YES.

Q. AND THEN HE'S HEATING THIS TO A HUNDRED DEGREES FOR THREE MINUTES, WHAT WAS THE PURPOSE OF THAT?

A. TO SEPARATE THE STRANDS OF THE PLASMID.

Q. SO SEPARATE THE STRANDS?

A. UH-HUH.

Q. THEN HE SAYS HE IMMEDIATELY COOLED IT IN ICE WATER. WHAT WAS THE PURPOSE OF THAT?

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A. THAT'S TO PREVENT THE TWO STRANDS FROM READILY COMING BACK TOGETHER AGAIN.

Q. THAT'S TO PREFERENTIALLY ANNEAL THE PRIMER IN PLACE OF THE TWO TEMPLATE STRANDS COMING BACK TOGETHER, RIGHT?

A. OKAY. HAS THE FRAMEWORK IN THERE.

Q. THAT'S HIS POINT OF QUICK COOLING ON ICE, RIGHT?

A. I DON'T KNOW THE PRIMER IS ANNEALING AT THAT POINT, NO.

Q. BUT THAT'S THE REASON FOR DOING IT THIS WAY, IS THAT YOUR UNDERSTANDING?

A. I THINK HE -- THAT'S NOT THE ANNEALING STEP FOR THE PRIMER THERE, I DON'T THINK.

HE'S SIMPLY IMMEDIATELY CHILLING IT SO YOU'RE NOT -- SEE IF YOU WERE TO COME DOWN FROM A HUNDRED DEGREES EVEN REASONABLY RAPIDLY, BUT NOT INSTANTANEOUSLY THOSE PLASMID STRANDS, SOME OF THE THEM WOULD COME BACK TOGETHER AGAIN.

SO BUT ALMOST INSTANTANEOUSLY COOLING IT WAY DOWN SO THAT IT'S VERY UNFAVORABLE FOR -- REALLY VERY UNFAVORABLE FOR THE STRANDS TO COME BACK TOGETHER AGAIN, BUT I DON'T -- AS FAR AS I KNOW, I DON'T THINK HE'S GETTING THE PRIMER ANNEALING IN THAT VERY SHORT TIME.

Q. HE IS USING THAT QUICK COOLING TECHNIQUE TO AVOID THE TEMPLATE STRANDS FROM COMING BACK TOGETHER?

A. THAT'S THE WAY I WOULD INTERPRET IT.

Q. AND HIS REASON FOR DOING THAT IS BECAUSE HE WANTS ULTIMATELY FOR THE PRIMER TO ANNEAL TO THE TEMPLATE STRAND?

A. THAT'S RIGHT. I GUESS THAT IS WHAT HE'S DOING HERE.

Q. THEN HE DOES WHAT'S CALLED A SEQUENCING REACTION, RIGHT?

A. YES.

Q. HE'S USING THE METHOD DESCRIBED BY SANGER IN 1987 --

A. THAT'S RIGHT.

Q. -- WAS THE ONE YOU REFERRED TO EARLIER THAT YOU SOMETIMES HAVE USED?

A. YEAH.

Q. THAT'S USING DNA POLYMERASE AND THE FOUR NUCLEOTIDES?

A. UH-HUH.

Q. AND CARRIES OUT THE PRIMER EXTENSION REACTION?

A. YEAH. I WOULD ASSUME THE PRIMER IS ANNEALING SOMETIME DURING THAT EXTENSION REACTION OR I DON'T KNOW WHETHER HE KEEPS IT AT ZERO DEGREES UNTIL HE BRINGS IT UP TO THE TEMPERATURE FOR THE EXTENSION REACTION OR WHAT.

BUT I THINK THE PRIMER PROBABLY MOST OF IT WOULD ANNEAL AFTER IT'S OUT OF THE ICE WATER. THAT WOULD BE A CONSIDERABLE TIME, I PRESUME.

(PAUSE IN THE PROCEEDINGS)

THE WITNESS: IT'S A TOTAL OF 30 MINUTES ACTUALLY IN WHICH ANNEALING COULD TAKE PLACE.

(PAUSE IN THE PROCEEDINGS)

Q. (BY MR. FIGG) DOCTOR, LET ME SHOW YOU ANOTHER EXHIBIT THAT WE'VE MARKED AS A-109. THIS IS A PAPER BY HONG.

I BELIEVE YOU'VE SEEN THIS ONE BEFORE, HAVEN'T YOU?

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A. YES, I HAVE SEEN THIS PAPER.

Q. AND, AM I CORRECT, THAT DR. HONG WAS DESCRIBING A METHOD HERE FOR SEQUENCING DNA USING THE PRIMER EXTENSION REACTION?

A. THAT'S CORRECT.

Q. AND THIS PAPER WAS PUBLISHED BACK IN THE EARLY 1980'S?

A. '82, I THINK. YES, 1982.

Q. DOCTOR HONG, IS IT YOUR UNDERSTANDING, HE WORKED AT M.R.C. IN DR. SANGER'S LABORATORY?

A. I DON'T KNOW HIM, BUT . . . HE'S IN THE RIGHT SPOT, YES.

Q. NOW, CAN YOU LOOK AT PAGES 245 AND 246 OF THIS ARTICLE.

A. MY PAGES ARE 900 SOMETHING HERE.

Q. MAYBE I'VE GIVEN YOU THE WRONG HONG ARTICLE.

LET ME GIVE YOU A-110.

A. I'M CONFUSED NOW. ARE THESE TWO SEPARATE --

Q. YES, THEY'RE TWO SEPARATE PAPERS.

A. I HAVE TO SEE WHICH ONE I READ.

Q. OKAY. WHY DON'T YOU LOOK AT THEM BRIEFLY.

A. OKAY. I READ THE 1982 PAPER. I HAVEN'T READ THIS PAPER HERE (INDICATING).

Q. THE ONE THAT YOU HAVE NOT READ IS A-110?

A. THAT'S CORRECT.

Q. WELL, LET ME FOCUS YOU ON A COUPLE OF PARAGRAPHS, AND IF YOU NEED TO READ MORE OF IT WE'LL DO THAT. BUT . . . ON PAGE 246 THERE'S A SECTION ENTITLED DENATURING DOUBLE STRAND AND ANNEALING SECOND PRIMER TO THE TEMPLATE. DO YOU SEE THAT?

18

A. YES.

Q. AND DO YOU SEE THAT THE WAY DR. HONG DID THAT WAS HE PUT THE TUBE IN A BOILING WATER VAT FOR THREE MINUTES AND THEN IMMEDIATELY TRANSFERRED TO A DRY ICE ETHANOL MIXTURE FOR ABOUT ONE MINUTE?

A. OKAY. LET ME JUST READ THIS FOR A SECOND.

Q. SURELY.

A. PART OF MY COPY IS CUTOFF HERE, TOO. THE DNA WAS SUSPENDED --

(PAUSE IN THE PROCEEDINGS)

MR. FIGG: EXCUSE ME, DOCTOR.

MR. PASAHOW: THE PROBLEM SEEMS TO BE THE EXHIBIT COPY HAS PART OF THE TEXT CUT OFF DUE TO THE COPYING.

THE COURT: HOW ABOUT YOUR COPY?

MR. PASAHOW: HAS THE SAME PROBLEM. THAT'S HOW I BECAME AWARE IT.

MR. FIGG: UNFORTUNATELY THE POSTER IS MORE LEGIBLE, DOCTOR, IF YOU FEEL MORE COMFORTABLE READING THE POSTER YOU MAY DO THAT.

THE WITNESS: IT'S NOT EASY TO SEE THAT EITHER.

THE COURT: DO YOU HAVE A CLEANER COPY OF THE EXHIBIT?

MR. FIGG: I'M SORRY, YOUR HONOR. APPARENTLY THIS IS A A PHOTOCOPYING MISTAKE THAT PERMEATED ALL OF OUR COPIES IN THE COURTROOM.

THE COURT: WOULD IT BE OF ANY HELP TO TAKE THE RECESS

NOW?

MR. FIGG: WE CAN DO THAT.

THE COURT: WOULD YOU INDICATE TO DR. SMITH WHICH PORTIONS YOU WANT HIM TO REVIEW.

THE WITNESS: IS IT JUST THAT ONE PARAGRAPH?

MR. FIGG: YES, I THINK THAT ONE PARAGRAPH WOULD BE SUFFICIENT.

THE WITNESS: ALL RIGHT. LET ME SEE IF I CAN READ THIS AND SEE IF IT'S ALL HERE. DNA WAS SUSPENDED --

MR. FIGG: THIS DOESN'T REALLY HAVE TO BE ON THE RECORD, I DON'T BELIEVE.

THE COURT: WOULD IT HELP IF WE TOOK A RECESS NOW AND YOU HAD A CHANCE TO READ IT OVER?

THE WITNESS: YES, IT WOULD.

THE COURT: EVEN THOUGH IT'S ONLY A PARAGRAPH.

WE'LL TAKE ABOUT TEN, 15 MINUTES RECESS. PLEASE FOLLOW THE INSTRUCTIONS AND WE'LL SEE YOU AT THE CLOSE OF THE RECESS.

(RECESS TAKEN)

(CONTINUED ON THE NEXT PAGE, NOTHING OMITTED)

(OPEN COURT, JURY PRESENT, AT 11:26 A.M.:)

MR. FIGG: THANK YOU.

THE COURT: HOW ARE WE DOING ON OUR TIME?

MR. FIGG: I'M TRYING TO EXPEDITE HERE. I'M SORRY. I'M GOING A LITTLE OVER MY ESTIMATE.

Q. DR. SMITH, HAVE YOU HAD AN OPPORTUNITY TO LOOK OVER EXHIBIT A-110?

A. YES.

Q. AND AM I CORRECT THAT, ON PAGE 246, THE SECTION ENTITLED "DENATURING DOUBLE STRANDS AND ANNEALING THE SECOND PRIMER TO THE TEMPLATE" BASICALLY DESCRIBES THE PROCEDURE THAT WE WERE TALKING ABOUT IN CONNECTION WITH DR. WALLACE'S PAPER, THAT WHAT -- WHAT IS BEING ACCOMPLISHED THERE IS DENATURING THE STRANDS AND, AS THEY'RE DENATURED, OR WHILE -- WHEN THEY COME BACK TOGETHER, ANNEALING PRIMER TO ONE OF THE STRANDS.

IS THAT WHAT IS BEING DESCRIBED IN THAT SECTION?

A. YES. WELL, YOU'RE . . . IF I UNDERSTAND THE PAPER CORRECTLY HERE, YOU HAVE A CIRCULAR SINGLE-STRANDED PLASMID AND YOU HAVE SYNTHESIZED A DOUBLE-STRANDED REGION, SO YOU HAVE A LINEAR MOLECULE AND A CIRCULAR MOLECULE AND THEY'RE JOINED BY DUPLEX REACTION.

AND YOU'RE THEN DENATURING A LINEAR STRAND FROM A CIRCULAR STRAND. THEY DO THAT BY HEATING IN A BOILING WATER BATH FOR THREE MINUTES AND THEN IMMEDIATELY TRANSFERRING TO ICE-ETHANOL AGAIN FOR THE SAME REASON, I PRESUME, TO PREVENT

THOSE STRANDS FROM COMING BACK TOGETHER AGAIN.

Q. TO -- TO PREVENT THE TWO TEMPLATE STRANDS FROM COMING BACK TOGETHER AGAIN.

A. THAT'S RIGHT. AND THEN ALLOWING 20 MINUTES AT ROOM TEMPERATURE FOR THE PRIMER TO BIND.

Q. UH-HUH. NOW, YOU INDICATED THAT THERE WERE ACTUALLY TWO PORTIONS OF THIS REACTION, I GUESS. THE FIRST ONE WAS, HE SYNTHESIZED A -- HE DID A PRIMER EXTENSION ON A SINGLE-STRANDED MOLECULE.

A. THAT'S RIGHT.

Q. AND THAT'S -- THAT'S DESCRIBED IN THIS PARAGRAPH AT THE BOTTOM OF PAGE 245; RIGHT?

A. YES, IN ONE OF THE DIAGRAMS.

Q. WHEN HE WAS ANNEALING A PRIMER TO A SINGLE-STRANDED TEMPLATE, DR. HONG DIDN'T USE THIS TECHNIQUE OF QUICKLY CHILLING IT IN AN ICE BATH; DID HE? HE SAYS HE LEFT IT AT ROOM TEMPERATURE FOR 10 MINUTES.

A. YES.

Q. SO DR. HONG RECOGNIZED THE DIFFERENCE BETWEEN ANNEALING A PRIMER TO A SINGLE-STRANDED TEMPLATE AND ANNEALING A PRIMER TO A TEMPLATE THAT WAS THE RESULT OF DENATURING A DOUBLE STRAND.

A. WELL, HE'S USING DIFFERENT CONDITIONS, THAT'S TRUE.

Q. RIGHT.

A. YES.

Q. AND HE USED A QUICK-CHILLING METHOD.

(PAUSE IN PROCEEDINGS)

THE WITNESS: BUT THE -- NO. THE LENGTH OF TIME HERE IS 20 MINUTES AT ROOM TEMPERATURE FOR THE ANNEALING REACTION IN -- IN THIS DOUBLE-STRANDED . . . METHOD HERE.

Q. (BY MR. FIGG) RIGHT. BUT HE QUICKLY CHILLED IT TO PREVENT THE TWO TEMPLATE STRANDS FROM FINDING ONE ANOTHER.

A. AT LEAST DURING THAT MINUTE THAT HE --

Q. YEAH.

A. -- THAT TIME SUBSEQUENTLY, IMPOSSIBLE TO COME BACK TOGETHER.

Q. DOCTOR --

MR. FIGG: LET ME ASK THE CLERK TO MARK THIS AS OUR NEXT EXHIBIT. I THINK IT'S 181.

THE CLERK: PLAINTIFF'S A-181 MARKED FOR IDENTIFICATION ONLY.

(PLAINTIFF'S EXHIBIT A-181
MARKED FOR IDENTIFICATION)

MR. PASAHOW: YOUR HONOR, BY STATUTE, PRIOR -- 30 DAYS PRIOR TO THE START OF THIS TRIAL, WE WERE REQUIRED TO BE ADVISED BY DU PONT WHAT ART IT INTENDED TO RELY UPON, AND I'M ADVISED BY MR. NEWLAND -- AND I BELIEVE IT TO BE THE CASE -- THAT WHAT I'VE JUST BEEN HANDED WAS NOT ON THAT STATUTORILY-REQUIRED LIST. AND IN LIGHT OF THAT, I DON'T THINK IT HAS ANY ROLE IN THIS TRIAL.

MR. FIGG: WELL, YOUR HONOR, SECTION --

THE COURT: WHAT PURPOSE IS THIS BEING USED FOR?

MR. FIGG: IT'S STRICTLY REBUTTAL. THIS IS ANOTHER

ARTICLE SHOWING THAT SCIENTISTS IN 1984 RECOGNIZED THE NEED FOR RAPID -- RAPIDLY ANNEALING -- OR RAPID COOLING, SO IT'S DIRECTLY REBUTTAL TO WHAT DR. SMITH TESTIFIED TO ON HIS DIRECT EXAMINATION.

MR. PASAHOW: YOUR HONOR, THE STATUTE --

THE COURT: WELL, IT'S NOT THEN -- YES?

MR. PASAHOW: THE STATUTE MAKES NO EXCEPTION FOR REBUTTAL MATERIAL. IT SAYS THAT THE COURT MAY, IN ITS DISCRETION, ALLOW ADDITIONAL MATERIAL, PROVIDED WE'RE ALLOWED AN OPPORTUNITY TO REVIEW AND PREPARE ON THE ADDITIONAL ART.

AND THE WHOLE POINT OF THIS IS TO STOP JUST WHAT'S HAPPENING HERE, WHICH IS SCIENTIFIC ARTICLES BEING SHOVED IN FRONT OF AN EXPERT AND HIM BEING ASKED TO OPINE ABOUT IT.

THE COURT: WELL, IS THIS BEING PROFFERED AS FINAL -- AS PRIOR ART?

MR. FIGG: IT'S NOT, YOUR HONOR. IT'S BEING -- I MEAN, IT IS PRIOR ART, OF COURSE. IT'S AN ARTICLE THAT WAS PUBLISHED MORE THAN ONE YEAR BEFORE THE FILING DATE OF THE CETUS PATENTS.

BUT IT IS BEING OFFERED TO REBUT DR. SMITH'S TESTIMONY ON DIRECT EXAMINATION.

THE COURT: WELL, I KNOW IT'S A FINE LINE. I WILL ALLOW IT FOR REBUTTAL, BUT THE JURY IS INSTRUCTED THAT IT'S NOT TO BE CONSIDERED FOR PRIOR ART PURPOSES IN ANY RESPECT, AND ONLY FOR REBUTTAL OR IMPEACHMENT PURPOSES OF THIS WITNESS AND WHAT HE MAY OR MAY NOT HAVE BEEN AWARE OF.

MR. FIGG: OKAY. THANK YOU, YOUR HONOR.

Q. DR. SMITH, WE'VE MARKED AS EXHIBIT A-181 A COPY OF AN ARTICLE BY -- ONE OF THE AUTHORS IS MESSING.

DO YOU KNOW WHO DR. MESSING IS?

A. YES.

Q. WHO IS HE?

A. WELL, HE'S A SCIENTIST WHO DEVELOPED SOME VERY USEFUL M13 CLONING VECTORS THAT ARE WIDELY USED IN SEQUENCING AND . . . CLONING.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. FIGG) HIS WORK IN DEVELOPING SYSTEMS FOR SEQUENCING IS . . . IS WIDELY KNOWN AMONG SCIENTISTS IN THIS FIELD OF MOLECULAR BIOLOGY?

A. THAT'S CORRECT.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. FIGG) DOCTOR, WOULD YOU REFER TO PAGE 261 OF THE MESSING ARTICLE.

DO YOU SEE THE SECTION THERE ENTITLED "PLASMID SEQUENCING WITH CHAIN TERMINATORS"?

(PAUSE IN PROCEEDINGS)

THE WITNESS: OKAY. THAT'S PAGE 262 IN MY COPY.

Q. (BY MR. FIGG) I'M SORRY. YOU'RE RIGHT. 262.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. FIGG) AGAIN, MY -- MAYBE I CAN GIVE YOU MY QUESTION AND THEN YOU CAN REVIEW THAT PARAGRAPH --

A. UH-HUH.

Q. -- WITH MY QUESTION IN MIND:

AM I CORRECT THAT THAT PARAGRAPH, LIKE THE WALLACE PAPER AND LIKE THE HONG PAPER THAT WE DISCUSSED BEFORE, DESCRIBES A METHOD OF SEQUENCING USING A PRIMER EXTENSION REACTION ON DOUBLE-STRANDED DNA?

A. THAT APPEARS TO BE THE CASE.

Q. AND AM I CORRECT THAT THE WAY DR. MESSING DESCRIBED THIS IS THAT THE DOUBLE-STRANDED DNA WAS MIXED WITH A PRIMER AND THEN BOILED FOR THREE MINUTES AND THEN QUICKLY CHILLED IN ICE WATER?

A. YES. THE PLAS -- IT'S IMPORTANT HERE THAT THE PLASMID WAS CLEAVED WITH THE RESTRICTION ENZYME AROUND IT.

Q. RIGHT.

A. IN FACT, THAT'S CRUCIAL.

Q. YES.

A. OTHERWISE, IT'S ANOTHER METHOD.

OKAY. MIXED IT WITH AN EXCESS -- 10 MOLAR EXCESS OF THE PRIMER, AND THEN HEATED . . . BOILED FOR THREE MINUTES AND THEN CHILLED IN ICE WATER. THAT MEANS THREE MINUTES AND THEN DUMP IT IN ICE.

Q. RIGHT.

A. THE PRIMER EXTENSION WAS CARRIED OUT IMMEDIATELY, AS PREVIOUSLY DESCRIBED. I ASSUME THAT WAS 15 TO 30 MINUTES, EXTENSION REACTION. SO THE PRIMER IS ANNEALING DURING THAT 15- TO 30-MINUTE PERIOD, I WOULD ASSUME.

Q. YEAH. AND THE REASON, AGAIN, FOR THE QUICK CHILLING WAS TO PREVENT THE TWO TEMPLATE STRANDS FROM COMING BACK TOGETHER --

A. YES.

Q. -- SO THE --

A. IF YOU SLOW COOL, YOU GET QUITE A BIT OF -- IT DOESN'T -- I SHOULD EMPHASIZE, IT DOES NOT PREVENT THEM . . . THEY WOULD BE LARGELY PREVENTED IN THE IMMEDIATE CHILLING STEP, BUT IN THE SUBSEQUENT REACTION, WHICH TAKES PLACE AT ANYWHERE FROM USUALLY 20 -- ROOM TEMPERATURE TO 37 DEGREES, ONE COULD HAVE EXTENSIVE REASSOCIATION OF TEMPLATE STRANDS.

Q. OKAY.

A. I DON'T THINK MANY OF THESE METHODS ARE CURRENTLY BEING USED.

Q. WELL, I UNDERSTAND THAT -- LET'S JUST MAKE SURE I DO UNDERSTAND THIS CORRECTLY:

THAT THE POINT OF THIS QUICK-CHILLING PROCEDURE THAT HAS BEEN DESCRIBED IN THESE PAPERS ULTIMATELY IS TO FAVOR THE FORMATION OF THE PRIMER-TEMPLATE COMPLEX OVER THE FORMATION OF THE TEMPLATE-TEMPLATE COMPLEX.

A. YES. IT SIMPLY MAKES SURE THAT YOU DON'T GO THROUGH A TEMPERATURE -- SEE, ANNEALING OF THE TEMPLATE STRANDS TOGETHER AGAIN OCCURS VERY RAPIDLY, PERHAPS A HUNDRED TO A THOUSAND TIMES MORE RAPIDLY, AT . . . FOR EXAMPLE, 65 DEGREES THAN IT DOES IN ICE.

SO THE IDEA IS TO BRING IT THROUGH THAT 65-DEGREE

TEMPERATURE RANGE IN A MATTER OF SECONDS SO THAT THERE'S VERY LITTLE CHANCE TO RE-ANNEAL IN THAT VERY HIGH KINETIC RATE AREA OF TEMPERATURE. SO THAT'S THE REASON FOR THE RAPID COOLING.

IN ONE CASE THAT WE JUST DEALT WITH, THEY USED, IN FACT, ETHANOL, DRY ICE, WHICH IS EXTREMELY COLD, SO IT WOULD BE ALMOST INSTANTANEOUS, AND YOU'D GET -- YOU'D ALLOW VERY LITTLE OPPORTUNITY FOR THE TEMPLATE TO ANNEAL DURING THAT -- THAT COOLING PERIOD WHERE YOU'RE GOING THROUGH THAT -- THAT CRITICAL 65-DEGREE RANGE.

BUT ANNEALING CAN OCCUR SUBSEQUENTLY DURING THE SEQUENCING REACTION. AND I THINK GENERALLY IN THESE METHODS THAT YOU'RE SHOWING ME, THAT HAS CONTINUED TO BE A VARIABLE PROBLEM AND MOST PEOPLE HAVE -- DO NOT ANY LONGER USE THESE METHODS BUT USE THE CHEN AND SEEBURG.

Q. BUT YOU'RE NOT SUGGESTING THAT WHEN THESE AUTHORS WROTE THAT THEY SUCCESSFULLY ACCOMPLISHED THESE PROCEDURES, THAT THEY WERE SOMEHOW MISREPRESENTING WHAT THEY DID; ARE YOU?

A. THE EXPERIMENTS THEY DID CLEARLY WORKED. BUT WHAT I'M SAYING IS THAT OTHER PEOPLE USING THESE METHODS -- I CAN'T NAME ANY PARTICULAR INDIVIDUALS, BUT I KNOW THAT THESE METHODS ARE NOT IN GENERAL USE CURRENTLY IN MOST -- CERTAINLY NOT IN MY DEPARTMENT. AND THAT'S BECAUSE THERE ARE BETTER METHODS WHICH PREVENT THE STRANDS FROM -- THE TEMPLATE STRANDS FROM HAVING ANY CHANCE OF COMING BACK TOGETHER, EVEN THOUGH THEY'RE BOTH PRESENT.

HERE, I THINK IF YOU WERE TO DO 10 SEQUENCING REACTIONS ON DIFFERENT STRETCHES OF DNA, DIFFERENT LENGTHS AND SO ON, DIFFERENT SEQUENCES, G-C COMPOSITIONS AND SO ON, THAT YOU MIGHT WELL HAVE A GOOD FRACTION OF THOSE THAT WOULD NOT WORK WELL. THAT'S ALL I'M SAYING.

Q. UH-HUH. AND A FRACTION THAT WOULD WORK.

A. BUT CLEARLY IT DID WORK WELL IN THESE PARTICULAR EXAMPLES.

Q. UH-HUH.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. FIGG) DOCTOR, I'D LIKE TO TURN TO A DIFFERENT SUBJECT:

YOU . . . YOU RESPONDED TO SOME OF MR. PASAHOW'S QUESTIONS CONCERNING THE COLD SPRING HARBOR CLONING MANUAL. DO YOU RECALL THAT?

A. YES.

Q. AND YOU REFERRED TO A STATEMENT BY THE AUTHORS, DR. SAMBROOK AND MANIATIS AND FRITSCH, IN THE PREFACE OF THAT BOOK. DO YOU RECALL THAT?

A. YES.

Q. YOU INDICATED THAT YOU KNOW DR. SAMBROOK.

A. I'VE MET HIM ON OCCASION. I DON'T KNOW HIM VERY WELL. HE'S IN A DIFFERENT AREA OF RESEARCH.

Q. WHAT KIND OF REGARD DO YOU HAVE FOR HIS SCIENTIFIC ABILITIES?

A. VERY HIGH.

Q. DO YOU KNOW WHETHER DR. SAMBROOK WAS AWARE OF THE KHORANA PRIOR ART THAT WE'VE BEEN TALKING ABOUT WHEN HE WROTE THAT PREFACE TO THE CLONING MANUAL?

A. I DON'T KNOW, TO BE HONEST.

Q. HAVE YOU SEEN ANYTHING FROM DR. SAMBROOK THAT ATTRIBUTES PCR TO DR. KHORANA AND THE KLEPPE PAPER?

A. I SAW A DECLARATION THAT -- IN WHICH HE REFERRED TO THE KLEPPE PAPER AND THE KHORANA WORK.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. FIGG) YOU'RE REFERRING TO THE DECLARATION THAT DR. SAMBROOK SIGNED FOR DU PONT'S SUBMISSION TO THE PATENT & TRADEMARK OFFICE.

A. I BELIEVE THAT'S -- WAS THE PURPOSE OF IT, YES.

Q. YES.

AND YOU'VE READ THAT DOCUMENT.

A. I READ IT A NUMBER OF MONTHS AGO.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. FIGG) DO YOU RECALL READING THE PARAGRAPH 10 THAT I'VE PUT ON THE OVERHEAD HERE THAT SAID:

"I HAVE READ AND UNDERSTAND THE KLEPPE, ET AL., AND PANET, ET AL., ARTICLES CITED IN DU PONT'S REQUEST FOR RE-EXAMINATION. IN MY OPINION, EACH OF THESE PUBLICATIONS FULLY DISCLOSE THE PROCESS FOR NUCLEIC ACID AMPLIFICATION DESCRIBED IN CLAIM 1 OF THE '195 PATENT"?

A. IF IT'S THE SAME . . . I READ THROUGH THE ENTIRE THING. I DON'T RECALL READING THAT SPECIFIC SENTENCE, BUT --

Q. BUT DO YOU RECALL THAT THAT WAS THE --

A. BUT I DID READ THE DECLARATION AND . . . OKAY. PROBABLY. I MUST HAVE READ THAT.

Q. THAT WAS THE GENERAL TENOR OF WHAT YOU RECALL READING IN THAT DECLARATION.

A. THAT'S RIGHT, YES.

Q. YES.

AND DO YOU AGREE THAT THE METHOD OF AMPLIFICATION DESCRIBED IN THE '195 PATENT IS THE SAME METHOD OF AMPLIFICATION THAT'S DESCRIBED IN THE '202 PATENT?

A. THAT'S RIGHT.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. FIGG) DOCTOR, YOU ALSO TESTIFIED ON DIRECT EXAMINATION ABOUT A 1975 NIH DOCUMENT THAT DR. KHORANA HAD SUBMITTED.

A. YES.

Q. DO YOU RECALL THAT?

A. THE -- YES. THE NCI 1985 GRANT APPLICATION.

Q. YES.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. FIGG) DOCTOR, HAVE YOU -- ARE YOU AWARE OF THE WORK THAT DR. MOLINEUX ACTUALLY DID IN DR. KHORANA'S LAB? HAVE YOU REVIEWED THAT WORK?

A. NO, I DIDN'T.
Q. SO YOU'RE NOT AWARE THAT --
A. IT'S NOT PUBLISHED, AS FAR AS I KNOW.
Q. AND YOU HAVEN'T SEEN THE WORK THAT IS REPORTED IN --
A. NO.
Q. -- DR. MOLINEUX'S SUMMARY, FOR EXAMPLE.
A. NO. YOU MEAN LABORATORY NOTES OR WHATEVER.
Q. RIGHT.
A. NO.
Q. YOU HAVEN'T SEEN THAT.
A. NO.
Q. SO YOU'RE NOT AWARE THAT DR. MOLINEUX ACHIEVED REPLICATION THROUGH THREE CYCLES OF AMPLIFICATION.
A. NO.
MR. PASAHOW: EXCUSE ME, YOUR HONOR.
THE COURT: YES.
MR. PASAHOW: THE QUESTION IS WITHOUT FOUNDATION AND ARGUMENTATIVE AND I OBJECT TO IT.
MR. FIGG: IT DOES HAVE FOUNDATION, YOUR HONOR. DR. MOLINEUX SO TESTIFIED.
THE COURT: THE OBJECTION IS OVERRULED.
YOU MAY ANSWER THE QUESTION.
THE WITNESS: OKAY. I'M SORRY. RESTATE THE --
Q. (BY MR. FIGG) YOU'RE NOT AWARE, THEN, THAT DR. MOLINEUX DID ACHIEVE COMPLETE REPLICATION OF A DNA THAT HE CALLED DNA-I

THROUGH THREE COMPLETE CYCLES OF HEATING, COOLING, PRIMER EXTENSION.

A. I'M NOT AWARE OF THAT, NO.

Q. NO. YOU WEREN'T TOLD ABOUT THAT WHEN YOU WERE ASKED TO OPINE ABOUT THE STATEMENT IN THE NIH DOCUMENT.

A. NO. I -- I'M TOTALLY UNAWARE OF THAT --

Q. OKAY.

A. -- TESTIMONY PRIOR.

Q. AND WERE YOU AWARE THAT DR. MOLINEUX DID ATTEMPT TO WORK WITH MATERIALS CALLED DNA UNWINDING PROTEINS THAT HAD BEEN PROVIDED TO HIM BY DR. BRUCE ALBERTS?

A. YES. WELL, I -- I DIDN'T KNOW THAT, BUT I WOULD INFER IT FROM THE . . . MENTION OF THE HELICASE IN THE 1985 GRANT.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. FIGG) SO YOU'RE NOT AWARE OF WHETHER DR. MOLINEUX WAS ABLE TO SUCCEED OR NOT SUCCEED WITH THE DNA UNWINDING PROTEIN, I TAKE IT.

A. THAT'S CORRECT.

Q. DO YOU AGREE WITH ME, DOCTOR, THAT CLAIM 1 -- THE PROCESS DESCRIBED IN CLAIM 1 OF THE '202 PATENT ONLY REQUIRES TWO CYCLES OF AMPLIFICATION?

A. CLAIM 1 ITSELF IS TWO CYCLES, THAT'S CORRECT.

Q. RIGHT. IT MIGHT BE MORE BUT IT ONLY REQUIRES TWO.

A. FOR CLAIM 1, YES.

Q. AND CLAIM 2 REQUIRES THREE CYCLES OF AMPLIFICATION.

A. OR MORE, YES.

Q. YES.

ARE YOU AWARE OF ANY CLAIM THAT REQUIRES MORE THAN THREE CYCLES OF AMPLIFICATION?

(PAUSE IN PROCEEDINGS)

THE WITNESS: NO.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. FIGG) DOCTOR, YOU MADE SOME REFERENCE TO DR. PANET'S WORK -- OR, EXCUSE ME, THE PANET PAPER.

AND, AGAIN, I'D LIKE TO REFER YOU TO PAGE 5220 OF THE PANET PAPER.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. FIGG) I BELIEVE YOU TESTIFIED THAT THE PROCESS DESCRIBED IN THE PARAGRAPH AT THE TOP OF THE RIGHT-HAND COLUMN OF PAGE 5220 DESCRIBES A DIFFERENT TYPE OF AMPLIFICATION THAN THE TYPE OF AMPLIFICATION DESCRIBED IN THE REST OF THE PAPER; IS THAT RIGHT?

A. THAT'S CORRECT.

Q. THEY'RE TWO DIFFERENT PROCESSES.

A. TWO DIFFERENT PROCESSES, YES.

Q. AND SCIENTISTS OF ORDINARY SKILL IN 1984 WOULD HAVE RECOGNIZED THAT TWO DIFFERENT PROCESSES WERE BEING DESCRIBED HERE.

A. THAT'S CORRECT.

Q. OKAY. AND THE OTHER PROCESS THAT DR. PANET AND DR. KHORANA

DESCRIBED IN THIS PAPER IS ONE THAT INVOLVES BINDING A . . . DNA TO CELLULOSE PARTICLES; RIGHT?

A. THAT'S RIGHT.

Q. I'VE PLACED ON THE EASEL A POSTER THAT I'M GOING TO ASK YOU SOME QUESTIONS ABOUT.

MR. FIGG: I GUESS WHEN MS. MORIYAMA GETS BACK, YOUR HONOR, WE'LL MARK THIS POSTER FOR IDENTIFICATION AS A-182.

THE COURT: A-182? IS THAT YOUR --

MR. FIGG: THAT'S THE NEXT.

THE COURT: -- NEXT IN ORDER?

OKAY. FINE. THANK YOU.

(PLAINTIFF'S EXHIBIT A-182 MARKED FOR IDENTIFICATION)

Q. (BY MR. FIGG) DOCTOR, I UNDERSTAND -- I REALIZE THAT WE'VE SOMEWHAT SIMPLIFIED THINGS HERE, BUT DO YOU AGREE THAT THIS GENERALLY ILLUSTRATES THE PROCESS THAT DR. PANET DESCRIBES IN THE OTHER PARTS OF THE PAPER?

A. YES, IT'S . . . IT'S CLOSE.

Q. THIS (INDICATING) WOULD BE THE TEMPLATE; RIGHT?

A. RIGHT. WELL, THE WHOLE THING IS TEMPLATE, BUT THAT WAS THE SECTION HE WAS INTERESTED IN.

Q. THIS SECTION HERE THAT BEGINS WITH A, C, G IS THE SECTION HE WAS INTERESTED IN --

A. THAT'S RIGHT.

Q. -- REPLICATING.

A. THAT'S RIGHT.

Q. AND THE WAY HE REPLICATED IT WAS, FIRST, HE LINKED IT TO THIS LONG SERIES OF T'S; RIGHT?

A. THAT'S RIGHT.

Q. AND THEN HE HOOKED THOSE T'S ONTO A CELLULOSE PARTICLE.

A. RIGHT. IT WASN'T QUITE IN THAT ORDER, BUT THAT WAS THE RESULT.

Q. I MAY HAVE IT A LITTLE MIXED UP ON THE SEQUENCE IN WHICH HE DID THINGS --

A. YEAH.

Q. -- BUT THIS IS HOW HE ENDED UP.

A. RIGHT.

Q. OKAY. AND THEN TO REPLICATE THIS SEQUENCE HE WAS INTERESTED IN, HE USED THIS PRIMER THAT'S ALL A'S; RIGHT?

A. THAT'S RIGHT.

Q. AND THAT PRIMER THAT'S ALL A'S WOULD STICK TO THIS SECTION THAT'S ALL T'S, AND THEN WHEN HE ADDS POLYMERASE, IT WOULD EXTEND OUT AND COPY THE PART HE WAS INTERESTED IN; RIGHT?

A. THAT'S CORRECT, YES.

Q. AND THEN HE WOULD HEAT THIS, AND THAT PART THAT HE HAD JUST SYNTHESIZED WOULD SEPARATE, AND THIS HE COULD -- HE COULD THEN RECYCLE AND DO MULTIPLE CYCLES OF THAT.

A. THAT COULD BE REUSED, YES.

Q. RIGHT.

NOW, DO YOU HAVE AN UNDERSTANDING AS TO THE RELATIVE

EASE OR DIFFICULTY IN 1972, '74, OF PREPARING THESE KINDS OF PRIMERS AS COMPARED TO PREPARING A PRIMER OF 10 OR 15 NUCLEOTIDES THAT HAD A DEFINED SEQUENCE?

A. MUCH EASIER.

Q. MUCH EASIER.

A. (NODDING HEAD.)

Q. SO IN TERMS OF ECONOMIZING, LET'S SAY, ON THE EFFORTS OF THE ORGANIC ECONOMISTS IN DR. KHORANA'S LABORATORY, THIS PROCEDURE WOULD BE MUCH MORE ECONOMICAL WITH RESPECT TO THE PRIMER THAN A PROCEDURE WHICH REQUIRES DEFINED PRIMERS.

A. YES, IT WOULD.

Q. OKAY. NOW, REFERRING BACK TO PAGE 5220, AT THE TOP OF THE LEFT-HAND COLUMN, DR. PANET AND DR. KHORANA ACTUALLY DESCRIBE THE REACTION MIX THAT THEY USED FOR PUTTING THAT PRIMER ON THAT TEMPLATE.

DO YOU SEE THAT?

A. YES, I DO.

Q. DO YOU SEE THAT THEY USED . . . THEY USED THAT POLY(DA) PRIMER THAT WE'RE TALKING ABOUT HERE.

A. UH-HUH. YES.

Q. AND DO YOU SEE THAT THEY USED .1 PMOLES -- THAT'S PICOMOLES; RIGHT -- OF THE TEMPLATE?

A. LET'S SEE. TEMPLATE IS . . .

(PAUSE IN PROCEEDINGS)

THE WITNESS: YES. POINT -- I'M SORRY. YEAH. .1

PICOMOLE OF THE CELLULOSE-ATTACHED TEMPLATE.

Q. (BY MR. FIGG) RIGHT.

NOW, PICOMOLE IS . . . IS A VERY SMALL AMOUNT OF MATERIAL.

A. (NODDING HEAD.)

Q. LET'S GO ON AND MAYBE I CAN RELATE THIS TO THE AMOUNT OF PRIMER:

DO YOU SEE THEY USED ONE NMOLE -- WHICH I THINK IS NANOMOLE -- OF THE PRIMER?

A. THAT'S RIGHT.

Q. NOW, AM I CORRECT THAT THERE ARE A THOUSAND PICOMOLES IN A NANOMOLE?

A. THAT'S RIGHT.

Q. SO AM I CORRECT THAT THE RATIO OF PRIMER TO TEMPLATE THAT DR. PANET USED WITH THESE POLY(A) PRIMERS, WHICH WERE MUCH EASIER FOR THEM TO MAKE, WAS 10,000 TO ONE?

A. THAT APPEARS TO BE THE CASE HERE. OF COURSE, THIS IS NOT -- NOT A PCR-TYPE REACTION.

Q. NO. IT'S A DIFFERENT REACTION.

A. DIFFERENT REACTION, YES.

Q. AND IT USES PRIMERS THAT WERE MUCH EASIER TO COME BY.

A. UH-HUH.

(PAUSE IN PROCEEDINGS)

MR. FIGG: THANK YOU, DR. SMITH. THAT'S ALL.

THE COURT: THANK YOU.

MR. PASAHOW: I HAVE JUST A FEW THINGS, YOUR HONOR.

THE COURT: YES.

I HAVE YET TO BE ABLE TO IDENTIFY WHAT THE TERM "FEW" MEANS, SO . . .

MR. FIGG: I WAS JUST THINKING, THAT'S PROBABLY A PHRASE YOU'VE HEARD BEFORE.

THE COURT: WELL, AT LEAST WHEN SOMEBODY SAYS, "I HAVE ONLY ONE QUESTION," I KNOW WHEN TO CUT THEM OFF, BUT A FEW IS A LITTLE HARDER.

REDIRECT EXAMINATION

BY MR. PASAHOW:

Q. DR. SMITH, DO YOU STILL HAVE UP THERE THE LARGE EXHIBIT MR. FIGG GAVE YOU WITH THE 1980 -- 1971 GRANT PROPOSAL IN IT?

(PAUSE IN PROCEEDINGS)

THE WITNESS: LET'S SEE, THIS IS . . .

Q. (BY MR. PASAHOW) EXHIBIT A-84.

A. HUH. MUST BE IN . . .

(PAUSE IN PROCEEDINGS)

THE WITNESS: A-84, YES.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. PASAHOW) AND I'LL ALSO SHOW YOU THE LATER GRANT PROPOSAL FROM 1975, WHICH WE'VE MARKED AS EXHIBIT B-159.

NOW, THESE TWO ARE BOTH SUBMISSIONS CONCERNING THE SAME GRANT FROM THE NATIONAL CANCER INSTITUTE; IS THAT CORRECT?

A. THEY'RE BOTH TO THE NATIONAL CANCER INSTITUTE, BUT ONE . . .

ONE IS SEVERAL YEARS LATER THAN THE OTHER ONE.

Q. BUT THEY WERE BOTH FOR THE SAME GRANT NUMBER; IS THAT CORRECT?

A. YES. YES, IT'S A -- RIGHT. IT'S A LATER CONTINUATION OF THE . . . THE SAME GRANT, YES.

Q. SO WHAT MR. FIGG SHOWED YOU WAS A 1971 REPORT THAT DR. KHORANA MADE CONCERNING THAT GRANT?

A. THAT'S CORRECT.

Q. AND WHAT WE LOOKED AT EARLIER, WHERE DR. KHORANA INDICATED HE WAS UNABLE TO GET EXTENSIVE OR COMPLETE REPLICATION OF THE STARTING DNA, THAT WAS THE REPORT HE MADE IN 1975 ON THE SAME GRANT.

A. THAT'S RIGHT.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. PASAHOW) NOW, MR. FIGG AND YOU DISCUSSED A YOUNG PROFESSOR WHO YOU THOUGHT MIGHT, ALONG WITH THE POST-DOC, BE IN THE CATEGORY OF THE ORDINARILY-SKILLED SCIENTIST IN 1974?

A. THAT'S RIGHT.

Q. I'M SORRY. 1984.

A. 1984.

Q. GET OUR TIME PERIODS RIGHT HERE.

WOULD THE -- THE YOUNG PROFESSOR HAVE A DIFFERENT VIEW THAN WE DISCUSSED OF WHAT THE KHORANA PAPERS THAT WE WENT THROUGH MEAN? THAT IS, WOULD THE YOUNG PROFESSOR INTERPRET THE KLEPPE ARTICLE DIFFERENTLY FROM THE -- THE POST-DOC WE

DISCUSSED?
A. I DON'T THINK SO, NO.
Q. WOULD THE YOUNG PROFESSOR INTERPRET THE PANET ARTICLE DIFFERENTLY FROM THE POST-DOC?
A. I DON'T BELIEVE SO.
MR. FIGG: YOUR HONOR, MR. PASAHOW'S LEADING THE WITNESS.
THE COURT: OBJECTION IS OVERRULED.
YOU MAY ANSWER. AND YOUR ANSWER WAS?
THE WITNESS: I DON'T BELIEVE THERE WOULD BE ANY DIFFERENCE IN THEIR INTERPRETATIONS.
Q. (BY MR. PASAHOW) AND WOULD THE YOUNG POST-DOC HAVE A DIFFERENT INTERPRETATION OF THE NATIONAL -- I'M SORRY.
WOULD THE YOUNG PROFESSOR HAVE A DIFFERENT INTERPRETATION FROM THE YOUNG POST-DOC OF THE NATIONAL SCIENCE FOUNDATION PAPER?
A. NO. I THINK IT WOULD BE VERY SIMILAR, YES.
(PAUSE IN PROCEEDINGS)
Q. (BY MR. PASAHOW) NOW, GOING BACK TO THE PARAGRAPH IN KLEPPE THAT WE'VE BEEN DISCUSSING . . .
A. UH-HUH.
Q. DOES THAT PARAGRAPH ANYWHERE EXPLICITLY PROVIDE THAT THE PRODUCT OF ONE CYCLE IS TO BE USED AS TEMPLATE IN THE NEXT CYCLE?
A. NO. IT -- IT NEVER MAKES THE EXPLICIT STATEMENT THAT

THE . . . THAT ANY OF THE PRODUCTS WOULD BE USED IN SUBSEQUENT CYCLES.

Q. WOULD THE ASSISTANT PROFESSOR THAT YOU DISCUSSED WITH MR. FIGG FIND IT ANY EASIER THAN THE POST-DOC WE DISCUSSED TO TRY TO DO PCR BASED UPON WHATEVER GUIDANCE COULD BE FOUND IN THIS PARAGRAPH?

A. I THINK THEY'D HAVE THE SAME PROBLEMS. I DON'T THINK THERE'D BE ANY DIFFERENCE.

Q. NOW, LET ME SHOW YOU THE NATIONAL SCIENCE FOUNDATION GRANT SECTION WE DISCUSSED.

DOES THAT ANYWHERE EXPLICITLY PROVIDE THAT THE PRODUCT OF ONE CYCLE IS TO BE USED AS TEMPLATE IN THE NEXT CYCLE?

A. NO.

Q. WOULD THE --

A. YEAH.

Q. GO AHEAD.

A. IT NEVER MENTIONS WHAT HAPPENS TO THE PRODUCT STRANDS.

Q. WOULD THE ASSISTANT PROFESSOR FIND IT ANY EASIER THAN THE POST-DOC WE DISCUSSED TO DO PCR BASED UPON WHATEVER INFORMATION COULD BE GLEANED FROM THE NATIONAL SCIENCE FOUNDATION SECTION?

A. I DON'T THINK THEY -- THERE WOULD BE ANY DIFFERENCE IN HOW THEY WOULD VIEW THAT.

Q. NOW, WHEN MR. FIGG WENT THROUGH THAT WITH YOU, HE HAD YOU ASSUME CERTAIN THINGS AND HAD THE MOLECULES MOVING A CERTAIN WAY, AND I'M TRYING TO FIND THE BASE TO THESE MOLECULES HERE.

HERE IT IS.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. PASAHOW) NOW, IF YOU WENT THROUGH THE PROCESS THAT YOU UNDERSTAND TO BE DESCRIBED IN THAT NATIONAL SCIENCE FOUNDATION GRANT, WHAT DO YOU ASSUME WOULD HAPPEN TO THE TWO TEMPLATE STRANDS AND THE PRIMER?

(PAUSE IN PROCEEDINGS)

Q. (BY MR. PASAHOW) I GUESS WE'D START OUT BY -- WHAT WOULD HAPPEN?

A. WELL, AS HE DESCRIBES IT IN THE GRANT APPLICATION THERE, THE FIRST STEP IS TO SEPARATE THE STRANDS.

(PAUSE IN PROCEEDINGS)

THE WITNESS: I NEED THE POSTER IN FRONT OF ME HERE, I GUESS.

Q. (BY MR. PASAHOW) LET ME SEE IF I CAN ARRANGE THAT.

CAN YOU SEE IT IF I SET IT HERE?

A. YES. THAT'S GOOD.

ALL RIGHT. THE FIRST STEP WAS SIMPLY TO DENATURE THE . . . THE DOUBLE-STRANDED TEMPLATE IN THE PRESENCE OF AN EXCESS OF PRIMERS.

Q. SO THERE'D BE PRIMERS IN HERE AS WELL.

A. THAT'S CORRECT.

Q. OKAY.

A. NOW, I DON'T THINK WE CAN TELL ANYTHING ABOUT WHAT WOULD ACTUALLY BE THE FINAL RESULT OF THAT WITHOUT KNOWING ABOUT

CONCENTRATIONS OF THE PRIMERS, ABSOLUTE CONCENTRATIONS OF THE TEMPLATE, THE RATES OF -- HOW MUCH TIME YOU WOULD ALLOW FOR THESE THINGS, THE RATIO OF THE TWO. I THINK ALL OF THOSE DIFFERENT FACTORS ARE TOTALLY UNSPECIFIED HERE, SO . . . I DON'T THINK YOU CAN READ THAT AND KNOW EXACTLY WHAT HAPPENED.

Q. NOW, YOU --

A. BUT -- BUT I MADE THE ASSUMPTIONS THAT MR. FIGG WISHED ME TO MAKE.

Q. NOW, YOU DISCUSSED SOMETHING CALLED TEMPLATE RE-ANNEALING.

WOULD DOES THAT MEAN?

A. TEMPLATE RE-ANNEALING WOULD BE SIMPLY THE COMING BACK TOGETHER OF THE TWO SEPARATED TEMPLATE STRANDS.

Q. SO THESE TWO LONG PIECES COME BACK TOGETHER.

AND WHAT HAPPENS TO THE PRIMER?

A. THE PRIMER WOULD -- IF IT WERE ATTACHED TO ONE OF THOSE STRANDS, IT WOULD VERY LIKELY BE DISPLACED BECAUSE THE STABILITY OF THE LONGER STRUCTURE IS MUCH GREATER.

IF THE PRIMER WERE NOT ATTACHED IN THE FIRST PLACE, THEN THE TWO STRANDS WOULD JUST COME BACK TOGETHER.

Q. SO CAN -- CAN ONE OF THESE BUMP OFF ONE OF THE LONGER ONES?

A. NO.

Q. WILL YOU GET ANY EXTENSION IF THESE TWO TEMPLATES REATTACH TO EACH OTHER?

A. I DON'T THINK YOU'D GET A MEASURABLE REACTION.

Q. NOW, LOOKING AT THE -- THE THIRD OF THESE ITEMS WE'VE BEEN

LOOKING AT IN THE PANET ARTICLE, DOES THE PARAGRAPH THAT WE'VE BEEN REFERRING TO OR, I SHOULD SAY, THE PORTION OF THE PARAGRAPH WE'VE BEEN REFERRING TO IN THE PANET ARTICLE ANYWHERE EXPLICITLY INDICATE THAT THE PRODUCT OF ONE CYCLE SHOULD BE USED AS TEMPLATE IN LATER CYCLES?

A. IT SAYS NOTHING ABOUT IT.

Q. AND DO YOU THINK THE ASSISTANT PROFESSOR -- THE HYPOTHETICAL ASSISTANT PROFESSOR IN 1984 WOULD BE ANY BETTER ABLE THAN THE HYPOTHETICAL POST-DOCTORAL FELLOW TO USE THE INFORMATION IN THE PANET ARTICLE AS A GUIDE FOR DOING PCR IN 1984?

A. I DON'T THINK HE'D BE ANY MORE SUCCESSFUL. IN FACT, I THINK EITHER ONE OF THEM, READING THIS PARAGRAPH HERE, WOULD BE DISCOURAGED FROM EVEN TRYING IT.

Q. NOW, YOU INDICATED, WHEN YOU WERE DISCUSSING THE KLEPPE ARTICLE WITH MR. FIGG, THAT KLEPPE MADE IT CLEARER THAN DID THE NATIONAL SCIENCE FOUNDATION PAPER THAT, "AFTER THE FIRST CYCLE . . . THE WHOLE CYCLE WOULD BE REPEATED, THERE BEING ADDED EVERY TIME FRESH DOSE OF ENZYME."

A. YES. AND I INTERPRET THAT TO MEAN THAT THEY'RE ADDING JUST FRESH DOSE OF ENZYME, NOT -- NOT ADDITIONAL PRIMER, BECAUSE, IN THE FIRST CYCLE, THEY MENTION THE PRESENCE OF A SUFFICIENTLY LARGE EXCESS OF APPROPRIATE PRIMER, AND THEN THE ADDITION OF THE ENZYME.

IN THE . . . WHEN THEY TALK ABOUT REPEATING THE WHOLE CYCLE, THEY'RE SPECIFICALLY SAYING ONLY ADD FRESH ENZYME. SO

THAT IT'S VERY CLEAR HERE THAT YOU WOULD BE USING UP THE PRIMER, AND THAT WOULD DISFAVOR SUBSEQUENT SUCCESSFUL REACTIONS.

Q. APART FROM TELLING US THAT WE ADD ENZYME FOR THE NEXT CYCLE, DOES THE KLEPPE PAPER PROVIDE ANY MORE INFORMATION ABOUT THE SECOND CYCLE THAN DOES THE NATIONAL SCIENCE FOUNDATION GRANT PROPOSAL?

A. NO.

Q. THERE WAS SOME DISCUSSION OF THE MEDICAL RESEARCH CENTER LABORATORY IN CAMBRIDGE, THE MRC LABORATORY.

A. YES.

Q. AND THAT'S THE MRC LABORATORY OF MOLECULAR BIOLOGY?

A. I THINK IT WAS, YES.

(WITNESS EXAMINES DOCUMENT.) YES.

Q. THAT'S WHERE MR. HONG WAS, THE AUTHOR OF TWO OF THOSE PAPERS THAT MR. FIGG REVIEWED WITH YOU?

A. THAT'S RIGHT.

Q. WHAT IS THE REPUTATION OF THAT LABORATORY?

A. IT'S -- IT HAS A . . . OUTSTANDING INTERNATIONAL REPUTATION, ONE OF THE VERY BEST PLACES IN THE WORLD.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. PASAHOW) NOW, YOU INDICATED TO MR. FIGG THAT THERE IS A DOUBLE-STRANDED SEQUENCING METHOD THAT IS NOT THE ONE DISCUSSED IN THE WALLACE ARTICLE OR IN THE HONG ARTICLE THAT HAS COME TO BE USED. WHAT IS THAT DOUBLE-STRANDED SEQUENCING METHOD?

A. OKAY. LET ME TRY AND EXPLAIN IT HERE:

IT'S -- THIS IS THE CHEN AND SEEBURG, WHICH EMPLOYS CIRCULAR DNA MOLECULES RATHER THAN ONES THAT YOU CAUGHT AND OPENED UP INTO LINEAR MOLECULES.

NOW, IF YOU HAVE CIRCULAR MOLECULES, THE TWO DNA STRANDS ARE INTERTWINED SUCH THAT EVEN WHEN YOU HEAT AND SEPARATE THE STRANDS, THEY DON'T REALLY COME APART IN THE SOLUTION. THEY CAN'T COME APART LIKE THAT (INDICATING).

THEY -- IF THEY'RE DOING LIKE THAT (INDICATING), THEY JUST COME APART ABOUT THAT MUCH (INDICATING) AND THEN THEY JUMBLE AROUND. THE TWO CIRCLES SLIP OUT OF REGISTER, SO THAT THE BASES ARE NO LONGER MATCHED IN A COMPLEMENTARY FASHION.

AND THEN WHEN YOU LOWER THE TEMPERATURE, OR -- GENERALLY, IT'S DONE WITH ALKALI -- WHEN YOU BRING IT BACK TO NORMAL NON-DENATURED CONDITIONS, THE TWO CIRCLES ARE NOW OUT OF REGISTER AND THEY'RE LOCKED OR COLLAPSED INTO THAT DENATURED STATE SO THAT YOU DON'T HAVE TO WORRY ANY MORE IN THE COURSE OF A NORMAL REACTION, EVEN AN HOUR'S REACTION AT ROOM TEMPERATURE OR 20 -- 37 DEGREES, WHERE ONE NORMALLY RUNS THESE . . . SEQUENCING REACTIONS.

SO YOU ESSENTIALLY REMOVE THE POSSIBILITY OF THE TEMPLATE STRANDS RE-ANNEALING.

AND, TO MY KNOWLEDGE, ALMOST EVERYONE IS USING THIS PROCEDURE. I USE IT IN MY OWN LABORATORY EXTENSIVELY. IT'S ONE OF OUR MAJOR SEQUENCING METHODS NOW. AND AT LEAST TWO OTHERS IN

OUR DEPARTMENT USE IT ALL THE TIME.

Q. IS THERE A NAME THAT THAT METHOD GOES UNDER?

A. WELL, YOU -- NOW YOU CAN BUY KITS, YOU KNOW, THAT HAVE ALL THE INGREDIENTS. IT'S -- I REFER TO IT AS THE SLIP CIRCLE METHOD, BUT OTHER PEOPLE USE OTHER -- OR THE CHEN-SEEBURG.

Q. THE CHEN-SEEBURG?

A. YEAH.

Q. WHY IS IT CALLED THE CHEN-SEEBURG?

A. WELL, THOSE WERE THE FIRST PEOPLE TO DESCRIBE IT, AS FAR AS I KNOW.

MR. PASAHOW: I HAVE NOTHING FURTHER, YOUR HONOR.

THE COURT: MR. . . .

(PAUSE IN PROCEEDINGS)

MR. PASAHOW: OH, EXCUSE ME. SORRY.

(PAUSE IN PROCEEDINGS)

MR. PASAHOW: COULD I HAVE A MOMENT?

(PAUSE IN PROCEEDINGS)

MR. PASAHOW: IF I MAY, YOUR HONOR.

THE COURT: YES. JUST A FEW MORE?

(LAUGHTER)

Q. (BY MR. PASAHOW) DO YOU STILL HAVE THE PAPER INVOLVING THE PANET METHOD THERE?

A. YES.

THE COURT: I SUSPECT SO. HE'S GOT EVERYTHING ELSE UP THERE, IT LOOKS LIKE.

(LAUGHTER)

Q. (BY MR. PASAHOW) CAN YOU DIG IT OUT FOR ME, PLEASE?

A. YES, I HAVE IT HERE.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. PASAHOW) HAVE YOU GOT THE SECTION ON PAGE 5,220 THAT MR. FIGG REFERRED YOU TO?

A. THE . . . AT THE TOP OF THE LEFT COLUMN THERE?

Q. YES.

A. YES.

Q. NOW, IN THE CALCULATION OF THE MOLES OF PRIMER, MR. FIGG REFERRED TO THE FACT THAT IT IS GIVEN . . .

A. YES, I SEE. IT'S IN NUCLEOTIDES. I DIDN'T REALIZE THAT. SO THAT WOULD TEND TO GIVE A MUCH -- EXPRESSED THAT WAY, ONE NANOMOLE IS ACTUALLY QUITE A BIT LESS IN TERMS OF FULL-LENGTH MOLECULES.

Q. WHAT DO YOU MEAN IT'S GIVEN IN NUCLEOTIDES?

A. SO IF . . . IF YOU TOOK A QUANTITY OF THIS PRIMER AND THEN SIMPLY, INSTEAD OF MEASURING THE NUMBER OF WHOLE MOLECULES, YOU BREAK IT DOWN TO ITS SEPARATE BASES, AND THEN TELL HOW MANY OF THE TOTAL BASES YOU HAVE -- IN OTHER WORDS, IF IT WERE 100 IN LENGTH, THEN . . . INSTEAD OF ONE NANOMOLE OF NUCLEOTIDES, IF YOU WANTED TO EXPRESS IT IN TERMS OF THE INTACT MOLECULE, IT WOULD BE 100TH THE AMOUNT, WHICH WOULD BE THEN 100TH OF A NANOMOLE OR 10 PICOMOLES, WHICH, FOR EXAMPLE, WOULD BE A HUNDRED-FOLD EXCESS.

NOW, WE DON'T . . . IT DOESN'T SAY THERE WHAT THE LENGTH IS. IT COULD BE UP TO 180 IN LENGTH, OR A HUNDRED AND -- WELL, IT COULD BE VERY LARGE. IT COULD BE VERY LARGE.

Q. THE POINT IS, YOU'D HAVE TO FIGURE OUT WHAT THE LENGTH WAS AND --

A. I'D HAVE TO KNOW WHAT THE LENGTH WAS, YES.

Q. AND THEN THERE'S A CALCULATION YOU DO TO FIND THE RATIO OF THE PRIMERS TO TEMPLATE?

A. THAT'S RIGHT, YES. YES.

SO, FOR EXAMPLE, IF IT -- IF I KNEW IT WAS 1,000 IN LENGTH, THEN IT WOULD BE A 10-FOLD EXCESS OVER THE TEMPLATE.

Q. MAYBE WE CAN DO THAT CALCULATION SOMETIME WITH ANOTHER WITNESS WHEN WE HAVE MORE TIME.

A. YES.

MR. PASAHOW: THANK YOU.

THE WITNESS: ALL RIGHT.

THE COURT: MR. FIGG.

RECROSS-EXAMINATION

BY MR. FIGG:

Q. DR. SMITH, I THINK WE AGREED THAT THE SEQUENCING OF DOUBLE-STRANDED DNA, AS DESCRIBED IN THE HONG PAPERS AND THE WALLACE PAPER AND THE MESSING PAPER, DID WORK.

A. THEY DID WORK, YES, ON THOSE SPECIFIC EXAMPLES THAT THEY GAVE.

Q. YEAH.

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AND THEY -- THEY WEREN'T ALL WORKING ON THE SAME MOLECULE. THEY WERE WORKING ON DIFFERENT --

A. THAT'S RIGHT.

Q. -- DIFFERENT THINGS.

A. YES.

Q. AND EACH CASE DESCRIBED, THEY DID GET POSITIVE RESULTS.

A. THAT'S CORRECT.

Q. AND IF THE TEMPLATE STRANDS HAD COME BACK TOGETHER IN THOSE REACTIONS AND PREVENTED THE PRIMER FROM ANNEALING, THEY WOULD NOT HAVE GOTTEN POSITIVE RESULTS; WOULD THEY?

A. YES. I DIDN'T LOOK EXACTLY HOW FAR THE EXTENSION WAS. I MEAN, IF IT WENT A HUNDRED AND THEY GOT GOOD SEQUENCE FOR A HUNDRED, MOST PEOPLE WOULDN'T FIND IT VERY USEFUL.

Q. WELL, NOT -- PERHAPS NOT FOR SEQUENCING. BUT A HUNDRED WOULD CERTAINLY FALL WITHIN CLAIM 1 OF THE '202 PATENT; WOULDN'T IT?

A. YEAH, BUT -- YOU'RE CORRECT THAT, IN THOSE SPECIFIC EXAMPLES, CLEARLY THE EXTENSION DID OCCUR.

Q. AND YOU WOULDN'T DOUBT THAT THEY GOT EXTENSION OF AT LEAST A HUNDRED.

A. WELL, I'D HAVE TO LOOK AT THE --

Q. OKAY.

A. -- ACTUAL GELS.

I DON'T THINK THEY PUBLISHED WITH ANYTHING TOO MUCH LESS THAN THAT, THOUGH.

Q. YEAH.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. FIGG) DOCTOR, YOU REFERRED TO THE CHEN AND SEEBURG PROCEDURE.

A. UH-HUH.

MR. FIGG: AND . . . IF IT'S OKAY WITH COUNSEL, I'LL SHOW DR. SMITH A COPY OF THE CHEN AND SEEBURG PAPER. I THINK IT'S BEEN MARKED AS EXHIBIT B-152 AND HAS BEEN ADMITTED INTO EVIDENCE ALREADY. JUST TO EXPEDITE THINGS.

THE COURT: YES.

MR. PASAHOW: I'M PREPARED TO RELY ON THAT REPRESENTATION.

MR. FIGG: OKAY.

Q. THAT IS THE CHEN AND SEEBURG PAPER THAT YOU WERE REFERRING TO?

A. YES, IT IS.

Q. COULD YOU TURN TO PAGE 166 OF THAT PAPER.

(PAUSE IN PROCEEDINGS)

Q. (BY MR. FIGG) DO YOU AGREE THAT BOTH THE . . . COLLAPSED DNA METHOD THAT YOU WERE REFERRING TO AND HEAT DENATURING ARE DESCRIBED IN THE PARAGRAPH ON THE LEFT-HAND COLUMN ABOUT HALFWAY DOWN?

A. OKAY. THEY'RE USING TWO SEPARATE MEANS FOR DENATURATION, ALKALI AND HEAT. THESE ARE THE TWO MOST --

Q. RIGHT.

A. -- COMMONLY USED METHODS, THAT'S RIGHT.
Q. AND IN THAT SECOND METHOD, THEY TOOK A PIECE OF CIRCULAR DOUBLE-STRANDED DNA; RIGHT?
A. THAT'S RIGHT.
Q. AND THEY COOKED IT WITH AN ENZYME AND MADE IT A LINEAR PIECE OF DOUBLE-STRANDED DNA.
A. WELL, WAIT A MINUTE NOW.
(PAUSE IN PROCEEDINGS)
THE WITNESS: OH, YES. LINEARIZED . . . OKAY. THEY USED LINEARIZED OR CO-VALENTLY CLOSED CIRCULAR DNA IN THAT SECOND PART.
Q. (BY MR. FIGG) UH-HUH. AND WHEN THEY USED LINEARIZED, THEY . . . THEY ADDED PRIMER TO THAT.
A. UH-HUH.
Q. THEY HEATED IT TO A HUNDRED DEGREES CENTIGRADE FOR THREE MINUTES; RIGHT?
(PAUSE IN PROCEEDINGS)
THE WITNESS: THREE MINUTES, YES.
Q. (BY MR. FIGG) AND THEN THEY QUICK COOLED IT ON ICE.
A. THAT'S RIGHT.
Q. AND THEN THEY USED THAT PRODUCT IN THE SEQUENCING REACTION THAT USED PRIMER EXTENSION.
A. THAT'S RIGHT.
Q. AND THEY GOT POSITIVE RESULTS WITH THAT PROCEDURE; DIDN'T THEY?