

COPY

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

BEFORE THE HONORABLE MARILYN HALL PATEL, JUDGE

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

PLAINTIFF,)

VS.)

NO. C 89-2860 MHP

CETUS CORPORATION, A DELAWARE)
CORPORATION,)

DEFENDANT.)

SAN FRANCISCO, CALIFORNIA
MONDAY, FEBRUARY 4, 1991

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FOR PLAINTIFF:

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LEGAL DEPARTMENT
WILMINGTON, DELAWARE 19898

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I N D E X

	<u>PAGE</u>	<u>VOL.</u>	
<u>DEFENDANT'S WITNESSES</u>			
<u>FALKINHAM, II, JOSEPH OLIVER</u>			
DIRECT EXAMINATION BY MR. LEWIS	1506	11	
CROSS-EXAMINATION BY MR. FIGG	1573	11	
REDIRECT EXAMINATION BY MR. LEWIS	1610	11	
RE-CROSS-EXAMINATION BY MR. FIGG	1620	11	
<u>SMITH, HAMILTON OTHANEL</u>			
DIRECT EXAMINATION BY MR. PASAHOW	1625	11	
<u>PLAINTIFF'S EXHIBITS</u>	<u>IDEN</u>	<u>EVID</u>	<u>VOL.</u>
A-180A	1625		11
A-180B	1625		11
<u>DEFENDANT'S EXHIBITS</u>	<u>IDEN</u>	<u>EVID</u>	<u>VOL.</u>
B-46		1629	11
B-158		1572	11
B-168		1572	11
B-169		1572	11
B-184		1572	11
B-242		1573	11
B-243		1573	11

1 MR. LEWIS: THANK YOU, YOUR HONOR.

2 THE COURT: OKAY. AND WE'LL CLEAN UP THE EXHIBITS AT
3 SOME POINT DURING ONE OF THE RECESSES.

4 MR. FIGG: YES. THANK YOU, YOUR HONOR.

5 MR. LEWIS: YOUR HONOR, OUR FIRST WITNESS THIS MORNING
6 IS DR. JOSEPH FALKINHAM.

7 THE COURT: OKAY. YOU MAY PROCEED.

8 THE CLERK: PLEASE RAISE YOUR RIGHT HAND.

9 JOSEPH OLIVER FALKINHAM, III, DEFENDANT'S WITNESS, SWORN

10 THE CLERK: PLEASE TAKE THE STAND.

11 PLEASE STATE YOUR FULL NAME AND SPELL YOUR LAST NAME
12 FOR THE COURT.

13 THE WITNESS: JOSEPH OLIVER FALKINHAM, THE THIRD. LAST
14 NAME IS SPELLED F-A-L-K-I-N-H-A-M.

15 THE CLERK: WOULD YOU PLEASE SPELL YOUR MIDDLE NAME?

16 THE WITNESS: OLIVER, O-L-I-V-E-R.

17 THE CLERK: OKAY.

18 THE COURT: YOU MAY PROCEED.

19 DIRECT EXAMINATION

20 BY MR. LEWIS:

21 Q. DR. FALKINHAM, WHAT DO YOU DO FOR A LIVING?

22 A. I'M AN ASSOCIATE PROFESSOR OF MICROBIOLOGY AT VIRGINIA
23 POLYTECHNIC INSTITUTE AND STATE UNIVERSITY.

24 Q. LET ME HAND YOU A DOCUMENT THAT'S BEEN MARKED AS EXHIBIT
25 B-157. CAN YOU IDENTIFY THAT, PLEASE.

1 A. YES. THIS IS A COPY OF MY CURRICULUM VITA.

2 Q. WOULD YOU TELL THE JURY BRIEFLY ABOUT YOUR BACKGROUND.

3 A. I WAS BORN AND RAISED IN OAKLAND, CALIFORNIA. I WENT TO THE
4 UNIVERSITY OF CALIFORNIA, STARTED IN 1959 AS A FRESHMAN AND THEN
5 GRADUATED IN 1969 WITH A PH.D. IN MICROBIOLOGY.

6 AFTER BEING AT THE UNIVERSITY OF CALIFORNIA, 1969, I
7 JOINED THE UNITED STATES AIR FORCE, WAS IN THE UNITED STATES AIR
8 FORCE BIOMEDICAL SCIENCES CORPS FOR ALMOST FOUR YEARS AT TRAVIS
9 AIR FORCE BASE, JUST UP THE ROAD IN FAIRFIELD, WHERE I RAN
10 HOSPITAL LABS FOR THE AIR FORCE AT THAT MEDICAL CENTER.

11 THE LAST YEAR THAT I WAS IN THE SERVICE, I WAS DOWN IN
12 MERCED AT CASTLE AIR FORCE BASE DIRECTING A HOSPITAL LABORATORY
13 THERE.

14 Q. WHAT DID YOU DO THEN?

15 A. AFTER BEING IN THE SERVICE, I WANTED TO GO BACK AND TEACH AT
16 A UNIVERSITY, AND FIRST WENT BACK AND DID A POST-DOCTORAL
17 FELLOWSHIP WITH ROY CURTIS, WHO'S A MICROBIOLOGIST AND
18 GENETICIST. HE WAS THEN AT THE UNIVERSITY OF ALABAMA IN
19 BIRMINGHAM, AND I JOINED HIS LABORATORY WHERE I GOT BACK INTO
20 RESEARCH.

21 Q. AND AFTER THAT?

22 A. I WAS WITH DR. CURTIS FROM 1972, LATE IN 1972, TO 1974.

23 1974, IN AUGUST, I JOINED THE FACULTY AT THE
24 UNIVERSITY, VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY,
25 WHERE I'VE BEEN EVER SINCE.

1 Q. YOU SAID YOUR PH.D. WAS IN MICROBIOLOGY. WHAT IS
2 MICROBIOLOGY?

3 A. MICROBIOLOGY IS THE STUDY OF ORGANISMS WHICH WE NORMALLY
4 LOOK AT THROUGH A MICROSCOPE. IT INCLUDES BACTERIA, THE
5 ORGANISMS WHICH CAUSE DISEASE, SUCH AS THE ORGANISM WHICH CAUSES
6 TUBERCULOSIS, MYCOBACTERIUM TUBERCULOSIS. IT INCLUDES OTHER
7 ORGANISMS -- FOR EXAMPLE, YEAST -- WHICH IS A DIFFERENT CLASS OF
8 ORGANISMS, ALTHOUGH THEY ARE ALSO MICROSCOPIC.

9 Q. IS THERE ANY RELATIONSHIP BETWEEN MICROBIOLOGY AND MOLECULAR
10 BIOLOGY?

11 A. MICROBIOLOGY IS MORE A DISCIPLINE IN WHICH WE LOOK AT A
12 NUMBER OF DIFFERENT ORGANISMS. MOLECULAR BIOLOGY IS ONE OF THE
13 TOOLS THAT WE USE IN THE LABORATORY TO INVESTIGATE THE
14 CHARACTERISTICS AND BEHAVIOR. AND THE TOOLS THAT HAVE BEEN
15 DEVELOPED IN MOLECULAR BIOLOGY HAVE HELPED US UNDERSTAND HOW
16 MICROORGANISMS WORK, HOW THEY CAUSE DISEASE, HOW WE CAN USE
17 THEM, IN CLEANING UP OIL SPILLS, FOR EXAMPLE.

18 Q. IS THERE SOME PARTICULAR AREA IN MICROBIOLOGY IN WHICH YOU
19 HAVE CONCENTRATED?

20 A. SINCE 1975, MY LABORATORY HAS FOCUSED ON A GROUP OF
21 ORGANISMS WHICH CAUSE A TUBERCULOSIS-LIKE DISEASE. THEY ARE
22 RELATED TO THE ORGANISM WHICH CAUSES TUBERCULOSIS, MYCOBACTERIUM
23 TUBERCULOSIS. BUT UNLIKE MYCOBACTERIUM TUBERCULOSIS,
24 MYCOBACTERIA WE STUDY -- MYCOBACTERIUM AVIUM, AS IT'S
25 PARTICULARLY NAMED -- IS NOT TRANSMITTED FROM PERSON-TO-PERSON,

1 AND WE HAVE BEEN ATTEMPTING TO DISCOVER HOW PEOPLE ARE INFECTED
2 WITH IT.

3 THE IMPORTANCE FOR OUR STUDY NOW CAME AFTER WE HAD
4 SHOWN THAT THE ORGANISMS ARE IN THE ENVIRONMENT AND IN NATURAL
5 WATERS, NOT ONLY WATERS THAT YOU FIND DOWN IN THE ENVIRONMENT
6 BUT ALSO PIPED-IN WATER AS WELL.

7 THE IMPORTANCE NOWADAYS IS THAT APPROXIMATELY 50
8 PERCENT OF PATIENTS WITH ACQUIRED IMMUNODEFICIENCY SYNDROME,
9 AIDS, HAVE MYCOBACTERIUM AVIUM INFECTIONS, AND IT DOES SHORTEN
10 THEIR LIFE-SPAN; IT DOES CAUSE THEM GREAT DISCOMFORT.

11 THE UNFORTUNATE SITUATION THAT WE FACE OURSELVES IN,
12 AND A CHALLENGE FOR THOSE OF US IN THE LABORATORY, IS THAT THE
13 ORGANISMS ARE RESISTANT TO VERY MANY MICRO -- EXCUSE ME, I
14 MISSPOKE. NOT THE MICROORGANISMS.

15 MYCOBACTERIA ARE RESISTANT TO ANTIBIOTICS AND SO THERE
16 ISN'T MUCH TREATMENT OR THERAPY FOR THESE, AND SO A NUMBER OF US
17 IN THE AREA LOOKING AT THESE ORGANISMS HAVE UNDERTAKEN STUDIES
18 OF THE MECHANISM OF THE RESISTANCE SO WE CAN USE ANTIBIOTICS FOR
19 THEM.

20 Q. HAVE YOU PUBLISHED IN YOUR FIELD?

21 A. YES, I HAVE. THOSE PUBLICATIONS ARE INCLUDED IN MY
22 CURRICULUM VITA.

23 Q. NOW, YOU'RE NOT GOING TO BE OFFERING OPINIONS TODAY ABOUT
24 THE VALIDITY OF THE PATENTS AS SOME OF THE OTHER SCIENTISTS
25 HAVE; IS THAT RIGHT?

2

1 A. THAT'S CORRECT.

2 Q. WHAT ARE YOU GOING TO TESTIFY ABOUT?

3 A. I'M A TEACHER AT A UNIVERSITY. I TEACH UPWARDS OF, IN SOME
4 YEARS, 300 STUDENTS. I TEACH AN INTRODUCTORY GENETICS CLASS; I
5 TEACH MICROBIAL GENETICS; I TEACH MICROBIOLOGY.

6 IN THOSE COURSES, PARTICULARLY MICROBIAL GENETICS AND
7 MOLECULAR BIOLOGY, THE MATERIAL INCLUDES THAT ON POLYMERASE
8 CHAIN REACTION. AND SO I HAVE BEEN TEACHING THE PRINCIPLES OF
9 POLYMERASE CHAIN REACTION TECHNOLOGY, AND THAT IS WHAT I
10 PERCEIVE MY ROLE HERE TODAY.

11 Q. HAVE YOU TAUGHT IN THE BIOTECHNOLOGY FIELD OUTSIDE OF THE
12 UNIVERSITY?

13 A. YES, I HAVE. I'VE BEEN GIVEN THE OPPORTUNITY TO LECTURE TO
14 THE UNITED STATES CONGRESS OFFICE OF TECHNOLOGY ASSESSMENT, THE
15 UNITED STATES -- THE UNITED STATES COMMERCE DEPARTMENT PATENT &
16 TRADEMARK OFFICE ON GENETIC ENGINEERING AND BIOTECHNOLOGY.

17 IN ADDITION, I HAVE LECTURED WITH A CONTINUING
18 EDUCATION GROUP FOR PATENT ATTORNEYS, ATTORNEYS AND BUSINESS
19 PEOPLE. THE ORGANIZATION IS OUT OF WASHINGTON, D.C. IT IS
20 PATENT RESOURCES GROUP, INCORPORATED.

21 AND PATENT RESOURCES GROUP, INCORPORATED, HAS TWO
22 LECTURES, ONE IN THE FALL AND ONE IN THE SPRING, THAT I GIVE A
23 LECTURE ON GENETIC ENGINEERING, BIOTECHNOLOGY, AND IN THE LAST
24 SEVERAL YEARS, WE HAVE INCLUDED THE POLYMERASE CHAIN REACTION
25 TECHNOLOGY AS PART OF THOSE LECTURES.

2 1 THOSE LECTURES STARTED IN, GOSH, 1981 I FIRST GAVE
2 THOSE LECTURES, AND WE'VE BEEN GIVING THEM TWICE A YEAR SINCE
3 THEN.

4 Q. OTHER THAN YOUR TEACHING, DO YOU HAVE ANY CONTACT WITH PCR?

5 A. I HAVE A NUMBER OF OTHER INSTANCES -- I'VE HAD A NUMBER OF
6 OTHER INSTANCES WHERE I HAVE BEEN CALLED UPON TO SERVE AS A
7 REVIEWER OF GRANT PROPOSALS TO THE NATIONAL INSTITUTES OF HEALTH
8 AND TO THE UNITED STATES AGENCY FOR INTERNATIONAL DEVELOPMENT.

9 IN BOTH THOSE INSTANCES, THE POLYMERASE CHAIN REACTION
10 HAS BEEN PROPOSED AS A METHOD OF USE TO SOLVE CERTAIN PROBLEMS.
11 OF PARTICULAR INSTANCE IS THAT THE THIRD -- DEVELOPING WORLD HAS
12 A MUCH HIGHER INCIDENCE OF TUBERCULOSIS THAN THE UNITED STATES,
13 AND LEPROSY. THE ORGANISMS THAT WE WORK WITH ARE VERY SLOWLY
14 GROWING, AND SO IT TAKES A LONG TIME BETWEEN DIAGNOSIS OF THE
15 DISEASE BEFORE WE CAN INSTITUTE TREATMENT.

16 AS A RESULT, THERE'S A GREAT NEED OF US TO DEVELOP
17 RAPID METHODS FOR THE DETECTION OF INFECTION EITHER OF -- BY
18 MYCOBACTERIUM TUBERCULOSIS OR MYCOBACTERIUM LEPRAE. LEPRAE IS
19 THE CAUSATIVE AGENT OF LEPROSY. THERE'S SOME 10 MILLION CASES
20 OF LEPROSY IN THE WORLD.

21 WE HAVE BEEN -- I HAVE HAD THE OPPORTUNITY TO REVIEW
22 PROPOSALS IN WHICH THE POLYMERASE CHAIN REACTION TECHNOLOGY WILL
23 BE TESTED AND EMPLOYED FOR DIAGNOSIS IN THE DEVELOPING WORLD.

24 Q. ARE YOU AN EXPERT IN PCR?

25 A. I HAVE FOLLOWED THE POLYMERASE CHAIN REACTION LITERATURE

2

1 SINCE, I THINK, DECEMBER 20TH OF 1985 WHEN AN ARTICLE APPEARED
2 IN THE SCIENTIFIC JOURNAL SCIENCE. MY OBJECTIVE WAS THAT, AT
3 THE TIME -- AND IT'S BEEN REINFORCED -- THAT THIS IS ONE OF THE
4 TECHNIQUES AND TECHNOLOGIES THAT WE NEED TO MAKE OUR STUDENTS
5 AWARE OF AS THEY GO OUT INTO THE WORLD TO TAKE JOBS, DO RESEARCH
6 FOR -- BOTH IN THE ACADEMIC SETTING, THE MEDICAL SETTING AND
7 INDUSTRY, AND SO I HAVE KEPT ABREAST OF THE TECHNOLOGY AND THE
8 PRINCIPLES OF ITS OPERATION.

9 I AM NOT A PERSON WHO, BECAUSE OF CONTINUOUS USE,
10 CONSIDERS MYSELF AS GOOD AS THE OTHER WITNESSES WHO ARE GOING TO
11 BE TESTIFYING LATER TODAY AND IN THE ENSUING WEEKS. SO YOU'LL
12 HEAR FROM OTHER PERSONS WHO I FEEL ARE FRANKLY MORE SKILLED AND
13 MORE KNOWLEDGEABLE ABOUT THE OPERATION THAN AM I. BUT I'M QUITE
14 FAMILIAR WITH THE PRINCIPLES AND PRACTICE OF THIS TECHNOLOGY.

15 Q. ARE YOU BEING COMPENSATED FOR YOUR WORK TODAY?

16 A. YES, I AM. I'M BEING PAID MY STANDARD CONSULTING FEE THAT I
17 CHARGE TO CLIENTS.

18 Q. WE HAVE A VIDEODISK, A SERIES OF VIDEO IMAGES ON A
19 VIDEOTAPE, THAT'S BEEN MARKED AS EXHIBIT B-158. ARE YOU
20 FAMILIAR WITH IT?

21 A. YES, I AM.

22 Q. WHAT IS IT?

23 A. IT IS A VIDEODISK PRESENTATION, VERY MUCH LIKE A VIDEOTAPE,
24 BUT THE VIDEODISK GIVES US A LITTLE OPPORTUNITY TO -- TO USE IT
25 IN DIFFERENT WAYS THAT I HAVE, IN WORKING WITH THE ATTORNEYS,

3

1 MR. LEWIS AND OTHERS, DEVELOPED TO ILLUSTRATE THE PRINCIPLES AND
2 PRACTICE OF POLYMERASE CHAIN REACTION TECHNOLOGY.

3 Q. WERE YOU INVOLVED IN ITS PREPARATION?

4 A. YES, I WAS. WHEN I WAS FIRST CONTACTED BY MR. LEWIS WITH
5 REGARD TO THIS CASE, I MADE THE SUGGESTION THAT IT WOULD BE
6 ADVANTAGEOUS FOR US BECAUSE OF THE NATURE OF THE TECHNOLOGY TO
7 PREPARE A VIDEO PRESENTATION SO THAT WE COULD ILLUSTRATE THE
8 PRINCIPLES OF THIS.

9 AND, AT THE TIME, I VOLUNTEERED TO PROVIDE AN OUTLINE,
10 WHICH CONSISTED OF A SERIES OF STATEMENTS, AS WELL AS MY
11 POORLY-DRAWN, HAND DRAWN, FIGURES WHICH I THEN PRESENTED TO MR.
12 LEWIS, AND THE VIDEODISK THAT WE WILL BE REVIEWING TODAY IS A
13 RESULT OF THAT.

14 Q. IS THE MATERIAL ON THE DISK A SUFFICIENTLY ACCURATE
15 DEPICTION OF THE THINGS IT ATTEMPTS TO PORTRAY TO THE JURY?

16 A. YES, IT IS.

17 MR. LEWIS: YOUR HONOR, WITH YOUR PERMISSION, WE'D LIKE
18 TO START THE TAPE.

19 THE COURT: YES, YOU MAY.

20 IS THIS A LASER DISK?

21 MR. LEWIS: YES, IT IS, YOUR HONOR. WE'VE PUT IT ON A
22 LASER DISK BECAUSE IT MAKES IT EASIER TO PAUSE AND MOVE BACK AND
23 FORTH BETWEEN THE SEGMENTS.

24 THE COURT: WITH A LITTLE BAR GRAPH AND ALL THAT?

25 MR. LEWIS: PARDON?

3
1 THE COURT: WITH A BAR, BAR CODE, AND ALL OF THAT?

2 MR. LEWIS: YES, WE HAVE A BAR CODE READER. MISS
3 SREJOVIC WILL BE HELPING US WITH THAT.

4 (PAUSE IN PROCEEDINGS)

5 THE WITNESS: YOUR HONOR?

6 THE COURT: YES.

7 THE WITNESS: COULD I HAVE PERMISSION TO COME DOWN FROM
8 HERE, GET A MICROPHONE, AND GO TO THAT?

9 THE COURT: SURELY. SURELY.

10 THE WITNESS: THANK YOU VERY MUCH.

11 THE COURT: I THINK THAT MIKE OVER THERE WILL EXTEND
12 FAR ENOUGH FOR YOU.

13 MR. LEWIS, DO YOU WANT THE LIGHTS DIMMED AT ALL?

14 MR. LEWIS: I THINK IT'S GOING TO BE VISIBLE WITHOUT
15 THAT, YOUR HONOR.

16 THE COURT: OKAY.

17 MR. LEWIS: IF I MAY:

18 Q. DR. FALKINHAM, THE SUBJECT OF YOUR TESTIMONY IS POLYMERASE
19 CHAIN REACTION.

20 WHAT IS POLYMERASE CHAIN REACTION DONE ON?

21 A. THE POLYMERASE CHAIN REACTION IS DONE ON A MOLECULE CALLED
22 DEOXYRIBONUCLEIC ACID OR, AS WE'LL ABBREVIATE IT, DNA. AND THAT
23 WILL APPEAR ON THE SCREEN HERE.

24 Q. WHERE IS DNA FOUND?

25 A. DNA IS FOUND IN CELLS. EVERY INDIVIDUAL, WHETHER A

3

1 MICROORGANISM OR MAMMAL, MAN, CONSISTS OF A LARGE NUMBER OF
2 CELLS -- BACTERIA MAY BE ONLY ONE CELL -- IN WHICH THERE IS A
3 NUCLEUS, AND THE DNA IS LOCATED INSIDE THE NUCLEUS.

4 AND WE SEE -- IF WE GET A CLOSEUP OF THE NUCLEUS, WE
5 SEE THAT THE NUCLEUS CONSISTS OF THIS SORT OF AMORPHOUS GROUP OF
6 STRANDS.

7 IF WE COULD PAUSE AT THIS POINT.

8 WE CALL THESE INDIVIDUAL STRANDS CHROMOSOMES. HUMAN
9 BEINGS HAVE 22 PAIRS OF THESE CHROMOSOMES, ONE FROM MOTHER, ONE
10 FROM FATHER. IN ADDITION, WE INHERIT ONE SEX-DETERMINING
11 CHROMOSOME FROM ONE PARENT, ONE FROM ANOTHER. THE FEMALES HAVE
12 TWO X CHROMOSOMES; MALES HAVE AN X AND A Y CHROMOSOME.

13 CHROMOSOMES ARE REALLY VERY LONG INFORMATIONAL
14 MOLECULES. I THINK OF THEM AS -- AS LONG BOOKS, IN A WAY, IN
15 WHICH THEY'RE INDIVIDUAL DISCRETE UNITS.

16 AND THOSE INDIVIDUAL DISCRETE UNITS WE WOULD THINK OF
17 AS SENTENCES OR, IN GENETICS, WE CALL THEM GENES, AND THESE
18 GENES THEN ENCODE. THEY HAVE A LITTLE CODE THAT IS READ, BUT
19 THEY REALLY CONTAIN INFORMATION, AND THAT INFORMATION IS WHAT IS
20 TURNED INTO THE ACTIVITIES THAT WE SEE IN CELLS.

21 FOR EXAMPLE, ONE GENE IS THE GENE FOR HUMAN INSULIN, A
22 PROTEIN HORMONE, A SMALL MOLECULE, WHICH CIRCULATES THROUGH OUR
23 BODY, WHICH HAS TO DO WITH THE REGULATION OF SUGAR IN OUR
24 BLOODSTREAM.

25 Q. HOW MANY GENES ARE THERE IN A CELL FROM A HUMAN BEING?

3
1 A. IN A HUMAN BEING, THE ESTIMATES -- AND THESE ARE ONLY
2 ESTIMATES AT THE PRESENT TIME -- THE ESTIMATES HAVE BEEN AS HIGH
3 AS 100,000 INDIVIDUAL HUMAN GENES. SOME ESTIMATES HAVE GONE
4 DOWN TO SOMETHING ON THE ORDER OF 10,000 GENES.

5 SO THAT WITH RESPECT TO THE INSULIN GENE, THERE'S ONLY
6 ONE INSULIN GENE INHERITED FROM EACH PARENT, SO YOU HAVE ONE
7 FROM MOTHER AND ONE FROM FATHER, OUT OF PERHAPS A HUNDRED
8 THOUSAND, OR YOU GET A HUNDRED THOUSAND FROM ONE PARENT, YOU GET
9 A HUNDRED THOUSAND FROM ANOTHER PARENT.

10 SO THE FREQUENCY OF AN INDIVIDUAL GENE MAY BE AS LOW AS
11 ONE IN A HUNDRED THOUSAND.

12 Q. IS THERE A LOT OF THIS MATERIAL IN A CELL?

13 A. WELL, ALTHOUGH THERE ARE LOTS OF GENES, THERE STILL IS A
14 VERY SMALL AMOUNT OF DNA IN A CELL. THERE'S NOT A GREAT AMOUNT
15 OF DNA IN TERMS OF -- WE THINK OF THE AMOUNTS THAT WE NEED IN,
16 SAY, TEST TUBES IN A LABORATORY TO DIAGNOSE A PARTICULAR HUMAN
17 DISEASE OR SOMETHING LIKE THAT. WE DON'T HAVE VERY MUCH.

18 MR. LEWIS: EXCUSE ME. CAN YOU HEAR ME ALL RIGHT?

19 THE COURT: OH, YES. YOU WERE TALKING TO THE COURT
20 REPORTER AS WELL --

21 MR. LEWIS: YES, YOUR HONOR.

22 THE COURT: -- OR ME?

23 ARE YOU ABLE TO HEAR?

24 THE REPORTER: (NODDING HEAD.)

25 Q. (BY MR. LEWIS) WE'VE HEARD -- THE JURY HAS ALREADY HEARD

4
1 SOMETHING ABOUT THE STRUCTURE OF DNA, BUT I'D LIKE YOU TO REVIEW
2 THAT, IF YOU WOULD. DO YOU HAVE A DIAGRAM OF THAT?

3 A. YES. IN THE FOLLOWING DIAGRAM, WE GO AND MOVE FROM THE
4 INDIVIDUAL -- ALL THE CHROMOSOMES TO, SAY, AN INDIVIDUAL
5 CHROMOSOME.

6 STARTING UP HERE (INDICATING) WE HAVE THE SORT OF LONG
7 SPAGHETTI-LIKE THING WHICH YOU SAW. AND NOW YOU SEE IT HAS MORE
8 AND MORE STRUCTURE UNTIL FINALLY WE'VE STRETCHED IT OUT HERE.

9 DNA IN CELLS EXISTS AS A DOUBLE-STRANDED MOLECULE. YOU
10 WOULD INHERIT ONE DOUBLE-STRANDED MOLECULE FROM ONE PARENT, ONE
11 DOUBLE-STRANDED MOLECULE FROM ANOTHER PARENT.

12 THESE TWO STRANDS ARE WRAPPED AROUND ONE ANOTHER AS ONE
13 WOULD WRAP TWO BEADS -- STRINGS OF BEADS OR STRINGS OF PEARLS
14 AROUND ONE ANOTHER. SO AS THEY'RE WRAPPED AROUND ONE ANOTHER IN
15 THIS STRUCTURE, WE TALK ABOUT THE HELICAL DNA, AND SINCE IT'S
16 MADE UP OF TWO STRANDS, WE TALK ABOUT IT AS THE DOUBLE HELIX.

17 Q. NOW, IS THERE MORE DETAILS IN THESE?

18 A. YES. WE CAN LOOK AT THE INDIVIDUAL STRANDS TO FIND OUT WHAT
19 THIS MOLECULE IS MADE OF. AND IN THE FOLLOWING DIAGRAM, WE HAVE
20 A REPRESENTATION OF THAT, WHICH WE'VE NOW BLOWN UP.

21 WE'VE TAKEN THE HELICAL TURNS, THE COIL-LIKE TURNS, OUT
22 OF THE MOLECULE, BUT AS YOU CAN SEE, IT IS STILL A
23 DOUBLE-STRANDED MOLECULE. HERE ARE THE UPPER STRAND AND THE
24 LOWER STRAND.

25 Q. EXCUSE ME, DR. FALKINHAM. THAT MAY BE A LITTLE HARD TO SEE.

4 1 WE HAVE A POSTER OF THOSE IMAGES THAT MAY BE HELPFUL.

2 THIS IS EXHIBIT B-242.

3 A. HERE IS A REPRESENTATION OF WHAT WE HAVE DONE IN THESE
4 FIGURES. IN THE FIRST PANEL, YOU SAW THE CELL AND THE NUCLEUS;
5 THE CLOSE-UP VIEW OF THE NUCLEUS WITH THE FIBERS OF THE
6 CHROMOSOMES.

7 WE'RE LOOKING AT AN INDIVIDUAL CHROMOSOME, AND WE FIND
8 THAT IT'S ACTUALLY A DOUBLE-WOUND FIBER, AND HERE ARE THE
9 INDIVIDUAL COMPONENTS.

10 NOW, DNA IS A POLYMER, "POLY" MEANING MANY, "MER"
11 MEANING UNITS.

12 THE INDIVIDUAL UNITS IN DNA ARE FOUR MOLECULES. THEY
13 CARRY A NUMBER OF NAMES. SOME OF THEM ARE NAMES THAT WE USE IN
14 THE LABORATORY AS SHORTHAND NAMES. SOME OF THEM ARE THE
15 CHEMICAL NAMES. WE'LL USE THE SHORTHAND NAMES. THE CHEMICAL
16 NAMES DON'T NECESSARILY HELP US UNDERSTAND WHAT'S HAPPENING IN
17 THIS PARTICULAR INSTANCE. BUT WE HAVE G, A, C AND T. WE TALK
18 ABOUT THOSE AS THE BASES IN DNA.

19 AND DNA, SURPRISINGLY, FOR ALL OF THE THINGS THAT IT'S
20 CAPABLE OF DOING, CONSISTS OF A DOUBLE-STRANDED MOLECULE AND THE
21 INDIVIDUAL COMPONENTS IN EACH ONE OF THESE STRANDS ARE G, A, C
22 AND T. SO THERE'S NOT A GREAT DEAL OF VARIETY IN TERMS OF WHAT
23 THE MOLECULE IS MADE OF.

24 ACTUALLY, THE INFORMATION THAT IS -- THAT'S IN THIS,
25 REALLY LIKE A CODE, LIKE A -- SOME KIND OF SECRET CODE THAT ONE

4 1 USES, BUT THE -- THE INFORMATION IS LITERALLY IN THE SEQUENCE OF
2 THESE BASES IN THE MOLECULE.

3 Q. YOU CALL THEM BASES. DO THEY HAVE OTHER NAMES THAT WOULD --

4 A. THE OTHER NAMES THAT YOU MAY HAVE HEARD ALREADY IN THIS
5 TRIAL OR WILL HERE: NUCLEIC ACIDS, BECAUSE THESE MOLECULES ARE
6 IN THE NUCLEUS. THEY'RE CALLED NUCLEIC ACIDS. THEY'RE ALSO
7 ACIDIC MOLECULES.

8 ALSO, WHEN THEY ARE READY TO BE INCORPORATED INTO A DNA
9 MOLECULE, WE REFER TO THEM ALSO -- THEY HAVE A DIFFERENT --
10 SLIGHTLY DIFFERENT FORM. THEY'RE CALLED NUCLEOTIDES.

11 SO YOU'LL HEAR THOSE WORDS INTERCHANGEABLY. SOMETIMES
12 I'LL LAPSE INTO SOME OF THOSE WORDS.

13 Q. NOW, HOW DO THESE BASES FIT TOGETHER? THE JURY'S HEARD SOME
14 OF THIS, BUT IF YOU COULD REVIEW IT, I'D APPRECIATE IT.

15 A. WELL, ONE OF THE IMPORTANT THINGS THAT WAS DISCOVERED EARLY
16 ON ABOUT THE STRUCTURE OF DNA WAS THAT THERE SEEMED TO BE IN
17 LIVING CELLS A VERY INTERESTING AGREEMENT BETWEEN THE AMOUNT OF
18 A BASE AND T. THEY WERE ALWAYS EQUAL. THE AMOUNT OF G BASE AND
19 THE AMOUNT OF C BASE, THEY WERE ALWAYS EQUAL.

20 IT'S NOW BEEN SHOWN THAT THESE BASES G AND C ARE ALWAYS
21 PAIRED OPPOSITE ONE ANOTHER IN THE STRANDS, NOT NEXT TO ONE
22 ANOTHER BUT, RATHER, OPPOSITE ONE TO ANOTHER, AND OPPOSITE AN A
23 IS ALSO -- ALWAYS A T; OPPOSITE A T IS ALWAYS AN A.

24 AS A CONSEQUENCE OF THAT, IF I TELL YOU THE SEQUENCE OF
25 ONE OF THE STRANDS OF DNA AND YOU KNOW THAT WHEREVER THERE'S A

5

1 C, THERE'S A G IN THE OPPOSITE STRAND, WHERE THERE'S A T,
2 THERE'S AN A AND SO ON, YOU CAN TELL ME WHAT THE SEQUENCE IS IN
3 THE OPPOSITE STRAND.

4 Q. CAN YOU GO OVER THAT AGAIN? I THINK YOU HAVE A PICTURE.

5 A. YES. WE HAVE A CONTINUATION HERE FROM THIS PANEL THAT WE'VE
6 JUST SHOWN YOU.

7 AND HERE YOU SEE THE BASES A, T, G AND C. A BINDS WITH
8 T; G BINDS WITH C.

9 IN ORDER TO SHOW THIS A LITTLE BETTER, YOU SEE THAT
10 IT'S ONLY THE A FITS IN TO THE T, SORT OF SLOT HERE, AND THE G
11 ONLY FITS IN WITH THE C.

12 Q. IS THAT WHAT THE BASES REALLY LOOK LIKE?

13 A. NO. THAT'S -- THIS IS JUST A SCHEMATIC DIAGRAM OF WHAT THE
14 BASES LOOK LIKE. MY STUDENTS GET VERY DISTRESSED WHEN WE MOVE
15 FROM THIS SCHEMATIC TO THE REAL STRUCTURE OF THE BASES. THIS IS
16 WHEN THEIR EYES SORT OF ROLL BACK IN THEIR HEADS AND THEY GET A
17 LITTLE UPSET. WE DO HAVE SOME CHEMISTRY IN GENETICS.

18 Q. OKAY. YOU WERE TALKING ABOUT THE STRANDS THAT THESE BASES
19 FORM. THEY CAN COME APART?

20 A. YES, THEY CAN.

21 IN THE FOLLOWING DIAGRAMS HERE, I'LL SHOW YOU THAT THE
22 DOUBLE-STRANDED DNA MOLECULE CAN BE SEPARATED INTO TWO SINGLE
23 STRANDS.

24 NOW, THIS IS AN IMPORTANCE FOR CELLS BECAUSE, AS I
25 SAID, THE SEQUENCE IS WHAT'S IMPORTANT.

5 1 THIS PROCESS OF SEPARATING THE DOUBLE-STRANDED DNA
2 MOLECULE INTO SINGLE STRANDS IS CALLED DENATURATION. A FAIRLY
3 BIG WORD, BUT IT'S A SIMPLE ONE, BECAUSE THE NATURAL STRUCTURE
4 OF DNA IS THE DOUBLE-STRANDED, AND SO WHEN WE SEPARATE IT INTO
5 SINGLE-STRANDED PIECES, WE TALK ABOUT THAT AS DENATURATION OR
6 REVERSING THE NATURAL CONDITION.

7 Q. HOW IS DENATURATION ACCOMPLISHED?

8 A. WE CAN SEPARATE -- AFTER WE ISOLATE DNA, WE CAN SEPARATE THE
9 DOUBLE-STRANDED MOLECULE INTO A SINGLE -- INTO ITS SINGLE
10 STRANDS BY A VARIETY OF TECHNIQUES. THE ONE MOST COMMONLY USED
11 IS -- IS HEAT. WE CAN SEPARATE THE STRANDS BY RAISING THE
12 TEMPERATURE, SO THAT THEY NOW CAN NO LONGER INTERACT.

13 THESE -- WHAT HOLDS THESE TWO STRANDS TOGETHER IS NOT A
14 VERY TIGHT, TIGHT BOND. WE USE THE WORD "BOND." IT'S NOT AS
15 TIGHT AS THE BOND HOLDING THE BASES IN THE INDIVIDUAL STRANDS.

16 IN ADDITION TO HEAT, WE CAN PUT THE DNA IN AN ACID
17 SOLUTION WHICH ALSO SEPARATES THE TWO STRANDS, OR WE CAN PUT IT
18 IN AN ALKALINE SOLUTION AND SEPARATE THE TWO STRANDS.

19 Q. IS THERE ANOTHER TERM THAT'S SOMETIMES USED FOR THIS
20 PROCESS?

21 A. BECAUSE WE CAN USE TEMPERATURE TO SEPARATE THESE STRANDS,
22 YOU MAY HEAR PEOPLE TALKING ABOUT MELTING DNA. AND THAT
23 REFERS -- THAT'S A JARGON WORD, SORT OF A MUMBO JUMBO SHORTENED
24 WORD, THAT WE USE IN THE LABORATORY TO INDICATE THAT THE TWO
25 STRANDS HAVE BEEN SEPARATED FROM ONE ANOTHER.

5

1 Q. AND THOSE STRANDS CAN COME BACK TOGETHER?

2 A. THE STRANDS CAN COME BACK TOGETHER AGAIN. ONE OF THE NICE
3 THINGS ABOUT DNA IS, WE CAN MANIPULATE IT IN THE LABORATORY.

4 HERE IS THE PROCESS OF BRINGING THE TWO STRANDS BACK
5 TOGETHER AGAIN. IT'S CALLED RENATURATION, OR ANNEALING,
6 A-N-N-E-A-L-I-N-G. "ANNEALING," OR BRINGING THE TWO STRANDS
7 TOGETHER, SORT OF A REVERSAL OF THE USE OF THE WORD "MELTING."

8 THIS RENATURATION PROCESS, HOWEVER, IS RATHER SPECIFIC,
9 BECAUSE, AS YOU SEE IN THE DIAGRAM HERE, THE SEPARATED TOP
10 STRAND IS NOW ASSOCIATED WITH THE ORIGINALLY-SEPARATED BOTTOM
11 STRAND, AND WHEN THEY CAME BACK TOGETHER AGAIN, THEY FORMED THE
12 ORIGINAL DOUBLE-HELICAL MOLECULE, THE DOUBLE-STRANDED MOLECULE,
13 AGAIN FOLLOWING THE T BINDS WITH A, C BINDS WITH G RULES
14 THROUGHOUT.

15 SO IF ONE SINGLE-STRANDED DNA IS COMPLEMENTARY BY THESE
16 A-T, G-C SEQUENCES, THEN THE TWO MOLECULES CAN COME BACK
17 TOGETHER AGAIN.

18 Q. CAN STRANDS OF DNA FROM TWO DIFFERENT SOURCES COME TOGETHER
19 LIKE THAT?

20 A. YES, IT'S POSSIBLE TO DO THAT AS WELL.

21 ONE CAN ISOLATE DNA FROM ONE INDIVIDUAL, MAKE IT
22 SINGLE-STRANDED, AND NOW BRING THAT TOGETHER WITH
23 SINGLE-STRANDED DNA FROM ANOTHER SOURCE, AND SEE IF YOU CAN GET
24 THE FORMATION OF THE SINGLE-STRAND BINDING WITH ANOTHER
25 SINGLE-STRAND TO MAKE A DOUBLE-STRANDED DNA, AND THE DNA'S CAN

6

1 BE FROM THE TWO DIFFERENT SOURCES.

2 WE'VE BORROWED A WORD FROM THE BREEDERS, ANIMAL AND
3 PLANT BREEDERS, WHO TALK ABOUT HYBRIDS. THESE ARE ANIMALS OR
4 PLANTS THAT ARE THE RESULT OF MATING BETWEEN TWO UNRELATED,
5 PERHAPS, ORGANISMS, OR DISTANT RELATIVES OF ONE ANOTHER.

6 AND SO WE'VE MADE A NEW INDIVIDUAL COMPOSED OF DNA OF
7 ONE PARENT AND DNA OF ANOTHER PARENT. AND SO WE REFER IN THIS
8 CASE -- ALTHOUGH WE'RE NOT DEALING WITH A WHOLE ORGANISM BUT
9 JUST DNA, WE REFER TO THIS AS HYBRIDIZATION. AND WE WOULD TALK
10 ABOUT A MOLECULE IN WHICH THE DNA STRAND IS FROM ONE SOURCE AND
11 THE OPPOSITE STRAND IS FROM ANOTHER SOURCE. THE OPPOSITE STRAND
12 I REFERRED TO AS THE COMPLEMENT. IF THEY ARE FROM TWO DIFFERENT
13 SOURCES, WE WOULD REFER TO THAT DNA AS A HYBRID.

14 Q. WHAT'S NECESSARY FOR STRANDS LIKE THAT FROM DIFFERENT
15 SOURCES TO BIND TOGETHER?

16 A. THE MOST IMPORTANT THING, AGAIN, IS THAT THE SEQUENCE IN ONE
17 OF THE STRANDS IS COMPLEMENTARY TO THE SEQUENCE IN THE OPPOSITE
18 STRAND.

19 AND HERE, WE HAVE A FIGURE SHOWING A SMALLER PIECE OF
20 DNA, HERE FOR ILLUSTRATIVE PURPOSES, FINDING ITS COMPLEMENT.

21 NOW, THIS LARGER MOLECULE HAS OTHER REGIONS WHICH ARE
22 NOT COMPLEMENTARY, AND THAT WAS SHOWN IN THE BEGINNING OF THE
23 DIAGRAM BY THIS SMALLER PIECE HERE ON THE BOTTOM ATTEMPTING TO
24 PAIR AT ONE REGION, AND IT COULDN'T FIND THE RIGHT A-T, G-C
25 SEQUENCE PAIRING, BUT IT WAS ABLE TO IN ANOTHER REGION.

6

1 Q. HOW LONG DOES IT TAKE A SHORT SEGMENT LIKE THIS TO -- TO
2 FIND A MATCHING PLACE, IF THERE IS ONE, IN, SAY, A HUMAN -- A
3 HUMAN'S GENES?

4 A. WELL, IT WOULD DEPEND VERY MUCH ON HOW FREQUENT THESE TWO
5 PIECES OF DNA WERE. IF THIS SMALLER PIECE HERE ON THE BOTTOM
6 HAD TO LOOK THROUGH A VERY LARGE MOLECULE OF DNA, SAY, IT HAD TO
7 FIND ITS COMPLEMENT AND THERE WAS ONLY ONE OF THOSE COMPLEMENTS
8 OUT OF A HUNDRED THOUSAND SEQUENCES, YOU COULD IMAGINE THAT THAT
9 WOULD OCCUR SLOWLY.

10 ON THE OTHER HAND, IF THERE WAS LOTS OF THESE
11 TWO-COMPLEMENTARY SEQUENCES, THAT REACTION WOULD OCCUR VERY
12 QUICKLY. AND, IN SOME CASES IN THE LABORATORY, WE CAN HAVE
13 THESE REACTIONS OCCUR VERY QUICKLY, ON THE -- ON THE TIME ORDER
14 OF SECONDS, BECAUSE THE CONCENTRATION OF THE COMPLEMENTARY
15 SEQUENCE IS SUCH THAT IT'S VERY LIKELY THAT THEY'LL ENCOUNTER
16 SOME -- SOME -- THE COMPLEMENT.

17 ON THE OTHER HAND, YOU MIGHT HAVE TO USE LONGER PERIODS
18 OF TIME IF THESE SEQUENCES WERE VERY RARE AND YOU HAD TO FIND A
19 COMPLEMENT FROM LOTS OF OTHER SEQUENCES, NONE OF WHICH WERE
20 COMPLEMENTARY.

21 Q. ARE THERE OTHER CONDITIONS THAT AFFECT THE SPEED?

22 A. YES. THERE ARE A VARIETY OF CONDITIONS THAT AFFECT THE
23 SPEED OF THIS REACTION. IT WOULD HAVE TO DO WITH THE
24 TEMPERATURE. WHAT WAS THE TEMPERATURE THIS -- WHEN THIS
25 REACTION WAS CARRIED OUT?

6 1 IT'S BEEN DISCOVERED THAT THE CONCENTRATION OF THINGS
2 LIKE SALTS IN THE SOLUTION -- AND WHEN I SAY "SALT," I MEAN
3 SODIUM CHLORIDE -- OTHER KINDS OF SALTS INFLUENCE THIS PROCESS
4 AS WELL.

5 SO WE CAN CHANGE THE CONDITIONS TO EITHER ACCELERATE OR
6 RETARD THIS REACTION.

7 Q. WHAT HAPPENS IF THERE'S NOT A COMPLEMENTARY SEQUENCE IN, FOR
8 INSTANCE, THE TOP STRAND THAT YOU HAVE THERE?

9 A. IN THE FOLLOWING PORTION OF THE PRESENTATION, WE SHOW WHAT
10 HAPPENS WHEN WE DON'T HAVE COMPLEMENTARY SEQUENCES. THEY SIMPLY
11 DO NOT BIND.

12 HERE, ON THE TOP, WE HAVE A PARTICULAR DNA SEQUENCE,
13 AND HERE WE HAVE A SMALLER PIECE, AGAIN FOR ILLUSTRATIVE
14 PURPOSES, AND YOU SEE THAT IT'S TRYING HERE ON THE LEFT TO PAIR
15 UP. IT DOESN'T FIND ITS COMPLEMENT.

16 IT NOW WILL MOVE TO ANOTHER AREA AND FIND THAT -- AND
17 YOU CAN LOOK AT THIS FOR A LONG PERIOD OF TIME, AND YOU'LL SEE
18 THAT THERE ARE NO COMPLEMENTARY REGIONS BETWEEN THIS SMALLER OR
19 LOWER BAND HERE AND THE UPPER BAND. SO WE WOULD NOT GET THE
20 FORMATION OF A DOUBLE-STRANDED MOLECULE IN THIS PARTICULAR
21 INSTANCE.

22 Q. IN THIS DIAGRAM -- ACTUALLY, WE'VE GOT ANOTHER POSTER, I
23 BELIEVE, THAT SUMMARIZES THIS PART.

24 (PAUSE IN PROCEEDINGS)

25 MR. LEWIS: IS THAT EXHIBIT B-243?

6

1 MS. SREJOVIC: YES.

2 MR. LEWIS: YES.

3 Q. YOU CAN SEE IT MORE CLEARLY HERE, BUT WE'VE PUT LITTLE
4 POINTS ON THESE BASES.

5 WHAT DOES THAT SIGNIFY?

6 A. THOSE AGAIN ARE SCHEMATIC, BUT THEY DO REPRESENT SOMETHING
7 IMPORTANT ABOUT THE DNA STRUCTURE THAT YOU WILL HEAR AS WE MOVE
8 THROUGH NOT ONLY THE PRESENTATION BUT THE PRESENTATION OF THE
9 OTHERS FOLLOWING.

10 EACH ONE OF THE BASES, AS YOU SEE, HAS A POINTED END
11 HERE ON THE RIGHT OF THE G AND HERE SORT OF INDENTED PORTION.
12 ALL OF THESE MOLECULES HAVE THAT PARTICULAR STRUCTURE.

13 WE CAN SEE IT AS WELL ON THE VIDEO HERE. ON THE
14 LEFT-HAND END OF THE LOWER SINGLE-STRANDED PIECE OF DNA, YOU SEE
15 THAT THERE'S A SMALL ARROWHEAD HERE AT THIS C ON THE LEFT-HAND
16 END, AND HERE ON THE RIGHT-HAND END, THE T HAS AN INDENTATION IN
17 IT.

7

18 IN THE UPPER STRAND, YOU SEE THAT THE ARROWHEAD HERE IS
19 ON THE OPPOSITE END OF THE MOLECULE, WITH RESPECT TO THE WAY
20 WE'VE DRAWN THE LOWER, AND THE ARROWHEAD NOW IS AT THE G END.

21 Q. DR. FALKINHAM, DO THE BASES ACTUALLY LOOK LIKE THIS?

22 A. NO, THE BASES DO NOT LOOK LIKE THIS. THEY HAVE A VERY
23 COMPLICATED STRUCTURE. WE'VE ATTEMPTED, IN A SCHEMATIC WAY, TO
24 SHOW SOME UNIQUE CHARACTERISTIC ABOUT THE MOLECULES, AND I'VE
25 SHOWN THAT IN THE FOLLOWING PRESENTATION IN THE VIDEO HERE, THE

7

1 NATURE OF THESE ENDS.

2 WE TALK ABOUT DNA AS HAVING ENDS. WE TALK ABOUT IT AS
3 HAVING A THREE-PRIMED END -- P-R-I-M-E -- AND A FIVE-PRIMED END.

4 AND HERE WE HAVE A DOUBLE-STRANDED DNA MOLECULE, AND WE
5 WILL SEE THE LABELING ON THIS MOLECULE NOW.

6 AT ONE END OF ONE OF THE STRANDS -- HERE I'M
7 ILLUSTRATING THE UPPER STRAND -- WE'VE INDICATED THAT ONE END OF
8 THE MOLECULE IS THE FIVE-PRIMED END; THE OTHER END OF THIS LONG
9 STRAND IS THE THREE-PRIMED END.

10 IN THE OPPOSITE STRAND -- AND THIS IS A RULE ABOUT
11 DNA -- THE OPPOSITE STRAND WHERE, INSTEAD OF GOING IN THE
12 FIVE-PRIME ON THE LEFT TO THE THREE-PRIME ON THE RIGHT, AS IT IS
13 IN THE UPPER STRAND IN THIS VIDEO, THE LOWER STRAND GOES FROM
14 THE THREE-PRIMED END TO THE FIVE-PRIMED END IN MOVING FROM LEFT
15 TO RIGHT.

16 WE TALK ABOUT THAT AS DNA HAVING ANTI-PARALLEL STRANDS.
17 I DON'T KNOW IF THAT WORD WILL COME UP, BUT IT'S ONE OF THE
18 WORDS THAT COMES UP ABOUT DNA.

19 THIS FIVE-PRIME AND THREE-PRIME HAVE TO DO WITH THE
20 REAL CHEMICAL STRUCTURE OF THE NUCLEIC ACIDS, OR THE BASE, AND
21 IT HAS TO DO WITH THE POSITION OF THE -- THE LINKAGE OR THE BOND
22 BETWEEN THE INDIVIDUAL BASES IN ONE OF THE STRANDS.

23 SO THE LINKAGE GOES FROM THE FIVE-PRIME POSITION OF ONE
24 TO THE THREE-PRIME POSITION OF THE OTHER. AND DEPENDING UPON
25 WHICH STRAND IT IS, ONE GOES IN A LEFT-TO-RIGHT DIRECTION; THE

7

1 OTHER GOES IN THE RIGHT-TO-LEFT DIRECTION.

2 Q. THE JURY'S HEARD ABOUT SOMETHING CALLED A GROWING END. DOES
3 THAT CORRESPOND TO ONE OF THESE?

4 A. YES. DNA IS MADE BY THE ADDITION OF INDIVIDUAL BASES, AND
5 THE INDIVIDUAL BASES THAT IT ADDS TO HAVE TO HAVE A THREE-PRIMED
6 END FREE.

7 SO IN A MOLECULE, IT WILL START BUILDING AT A FREE
8 THREE-PRIMED END; IT WILL NOT START BUILDING AT A FREE
9 FIVE-PRIME END.

10 NOW, THAT'S ONLY PART OF THE STORY. YOU'LL SEE LATER
11 THAT NOT ONLY DO WE HAVE TO HAVE THAT FREE END, BUT WE ALSO HAVE
12 TO HAVE SOMETHING TO GUIDE OR SERVE AS A PATTERN FOR THE
13 SYNTHESIS.

14 Q. ALL RIGHT. WITH THIS BACKGROUND, LET'S -- LET'S CHANGE THE
15 SUBJECT A LITTLE BIT:

16 I'D LIKE YOU TO EXPLAIN WHAT IT IS THAT PCR DOES. WHAT
17 DOES IT ACCOMPLISH?

18 A. ALL RIGHT. IN THE FIRST PORTION NOW OF THE VIDEO, WE MOVED
19 FROM THE SORT OF BASIC BACKGROUND INFORMATION TO PCR.

20 IF WE PAUSE RIGHT HERE AT THE BEGINNING OF THE PROCESS.

21 (PAUSE IN PROCEEDINGS)

22 THE WITNESS: POLYMERASE CHAIN REACTION STARTS OFF
23 WITH, IN THIS PARTICULAR EXAMPLE, WHICH IS A TYPICAL EXAMPLE, WE
24 SORT OF HAVE A MICROSCOPIC VIEW OF WHAT WE WOULD HAVE IN A TEST
25 TUBE, SO TO SPEAK.

7
1 WE HAVE DNA STRANDS, DOUBLE-STRANDED DNA, AND IN THIS
2 PARTICULAR CASE, WE HAVE A LARGE NUMBER OF STRANDS HERE SHOWN IN
3 THIS LITTLE CIRCLE. THOSE ARE ALL COLORED IN PINK. AND IN ONE
4 OF THE STRANDS, WE SEE A BLUE SEQUENCE, WHICH IS A TARGET
5 SEQUENCE.

6 THIS WOULD BE A SEQUENCE THAT WE ARE PARTICULARLY
7 INTERESTED IN. FOR EXAMPLE, IT COULD BE A PARTICULAR HUMAN GENE
8 THAT WE WISH TO STUDY -- WE MIGHT WISH TO STUDY, IN ORDER TO
9 DIAGNOSE OR TO DETERMINE WHETHER AN INDIVIDUAL HAS SOME
10 INHERITED DISEASE.

11 FOR EXAMPLE, WE NOW KNOW THAT CYSTIC FIBROSIS, A
12 DISEASE WHICH OCCURS IN HUMANS -- IT'S INHERITED -- IS DUE TO A
13 CHANGE IN A PARTICULAR DNA SEQUENCE, A GENE. THAT GENE WAS JUST
14 ISOLATED AND CHARACTERIZED ABOUT -- WITHIN THE LAST YEAR BY A
15 GROUP OF INVESTIGATORS FROM DETROIT AND TORONTO.

16 AND WE WOULD LIKE TO KNOW WHETHER OR NOT AN INDIVIDUAL
17 HAS INHERITED THE NORMAL GENE OR AN ABNORMAL GENE. IF THEY'VE
18 INHERITED THE ABNORMAL GENE, WE WOULD LIKE TO START SOME KIND OF
19 THERAPY RIGHT OFF THE BAT TO TRY TO HELP THAT INDIVIDUAL.

20 AT THE END -- IF WE WOULD NOW CONTINUE THE VIDEO -- YOU
21 WILL SEE THAT AT THE END OF THE PCR PROCESS, WE GO FROM A
22 SITUATION IN WHICH THERE'S, IN THIS LEFT-HAND CIRCLE, ONE TARGET
23 DNA SEQUENCE WITH LOTS OF OTHER SEQUENCES.

24 NOW, ON THE RIGHT-HAND SIDE, YOU SEE THE FIELD IS
25 FILLED WITH LITTLE DNA MOLECULES, SCHEMATICALLY DRAWN. THEY'RE

1 MOSTLY ALL BLUE, AND THERE ARE VERY FEW OTHER SEQUENCES COMPARED
2 TO THE TARGET SEQUENCE.

3 WHAT THIS IS, IS THE -- AND WHAT PCR DOES -- IS
4 SPECIFICALLY AMPLIFY THIS TARGET SEQUENCE, MAKING MANY COPIES OF
5 THIS TARGET SEQUENCE, SO THAT ALTHOUGH, REMEMBER, WE START OFF
6 IN CELLS WITH PERHAPS A GENE THAT WE MIGHT BE INTERESTED IN,
7 THERE MIGHT BE ONLY ONE COPY OF THAT WITH RESPECT TO A HUNDRED
8 THOUSAND OTHER COPIES.

9 AT THE END OF PCR, WE CAN MAKE COPIES OF THAT
10 INDIVIDUAL GENE, SO MANY, IN FACT, THAT WE WILL BE ABLE TO STUDY
11 THIS PARTICULAR GENE IN THIS MIXTURE SO THAT WE CAN THEN USE A
12 SMALL SAMPLE, AMPLIFY THAT PARTICULAR TARGET, AND THEN STUDY THE
13 AMPLIFIED PRODUCT FOR DIAGNOSIS.

14 Q. (BY MR. LEWIS) LET'S TURN TO HOW YOU DO PCR:

15 WHAT DOES IT TAKE TO DO IT?

16 A. ALL RIGHT. THE FOLLOWING PORTION OF THE VIDEO WILL BE A
17 REVIEW OF HOW POLYMERASE CHAIN REACTION IS CARRIED OUT.

18 AND, FIRST, WE LIST THE KEY INGREDIENTS: THERE'S THE
19 DNA TEMPLATE, THE TARGET DNA OR ANY OTHER DNA THAT ONE STARTS
20 WITH; THE DNA BASES; THE DNA PRIMERS; AND FINALLY THE DNA
21 POLYMERASE, AN ENZYME WHICH MAKES DNA.

22 WE'LL TURN TO EACH ONE OF THOSE NOW. FIRST OF ALL, THE
23 DNA TEMPLATE.

24 Q. WHY IS IT CALLED A TEMPLATE?

25 A. IT'S CALLED A TEMPLATE BECAUSE IT IS USED TO GUIDE OR . . .

8

1 USED AS A PATTERN TO MAKE COMPLEMENTARY STRANDS, SO THAT WE
2 DUPLICATE THE STRANDS OF THAT MOLECULE.

3 AND SO SINCE IT'S USED AS THE GUIDE, THEN WE START WITH
4 THAT DNA MOLECULE, AND WE CALL IT THE TEMPLATE FOR THAT REASON.

5 Q. AND THIS CONTAINS THE TARGET THAT WE WERE TALKING ABOUT
6 EARLIER?

7 A. THIS DNA TEMPLATE WOULD CONTAIN THE PARTICULAR TARGET.

8 TYPICALLY, THAT -- THAT IS MAYBE THE DNA OF AN ENTIRE
9 CELL, AND THERE'S ONLY ONE TARGET SEQUENCE IN IT. THERE ARE
10 OTHER APPLICATIONS IN WHICH YOU MIGHT START WITH SOME DIFFERENT
11 KIND OF PRODUCT, BUT FOR ILLUSTRATION HERE TODAY, WE'LL START
12 WITH A VERY LARGE MOLECULE AND THE TARGET SEQUENCE IS JUST A
13 SMALL SEQUENCE WITHIN THAT.

14 Q. YOUR NEXT INGREDIENT WAS DNA BASES?

15 A. YES, THE NEXT INGREDIENT ARE THE DNA BASES. YOU'VE SEEN
16 THOSE NOT ONLY IN THE VIDEO BUT IN THE PANELS. THEY CONSIST OF
17 THE T, G, A AND C BASES.

18 AN IMPORTANT COMPONENT, THE THIRD OF THE POLYMERASE
19 CHAIN REACTION, ARE PRIMERS. THESE ARE SMALL PIECES OF
20 SINGLE-STRANDED DNA. THEY'RE ALSO CALLED OLIGONUCLEOTIDES,
21 "OLIGO" MEANING A FEW, "NUCLEOTIDES," A WORD THAT WE USE
22 SYNONYMOUSLY WITH BASES. AND SO THE PRIMERS ARE A SEQUENCE OF A
23 FEW BASES.

24 THESE ARE USED TO START THE DNA SYNTHESIS OR THE MAKING
25 OF DNA. AND EACH ONE OF THESE, AS IS TYPICAL OF A DNA MOLECULE,

8
1 HAS ONE END OF -- THE FIVE-PRIMED END AND THE THREE-PRIMED END.

2 Q. I'M SORRY. WHICH END WAS WHICH?

3 A. IN THIS PARTICULAR EXAMPLE, I'D HAVE TO -- I THINK WE HAD
4 HERE THAT THE ARROWHEAD WAS THE . . . I'D HAVE TO GO BACK AND
5 LOOK AT MY -- LOOK AT MY EARLIER FIGURE HERE TO MAKE SURE I KNOW
6 WHAT --

7 Q. THAT'S THE DIRECTION OF GROWTH?

8 A. THAT'S THE DIRECTION OF -- OF -- THE FIVE-PRIMED END IS NOT
9 THE DIRECTION OF GROWTH. IT'S THE THREE-PRIMED END THAT'S THE
10 DIRECTION OF GROWTH.

11 I'M SORRY. I HAVE TO REMEMBER WHAT THE LITTLE
12 ARROWHEADS MEAN AT EACH END. WE DON'T HAVE THEM LABELED. LATER
13 ON, YOU'LL SEE THAT WE HAVE THEM LABELED AND WE'LL POINT OUT.

14 BUT EACH ONE HAS A FIVE-PRIMED END AND A THREE-PRIMED
15 END. IT'S IMPORTANT TO REMEMBER THAT THE THREE-PRIMED END IS
16 WHERE DNA BASES CAN BE ADDED, NOT THE FIVE-PRIMED END.

17 Q. WILL ANY RANDOM STRAND OF BASES DO AS A PRIMER?

18 A. NO. THE PRIMERS ARE CHOSEN BECAUSE THEY SPECIFICALLY ARE
19 COMPLEMENTARY TO A REGION OF THE TARGET SEQUENCE, SO THEY'RE NOT
20 JUST ANY SEQUENCE OF DNA BUT, RATHER, THEY ARE COMPLEMENTARY TO
21 A VERY SPECIFIC PORTION OF THAT TARGET.

22 Q. WHAT WAS THE LAST INGREDIENT?

23 A. THE LAST INGREDIENT IS DNA POLYMERASE.

24 Q. NOW, THE JURY'S HEARD QUITE A BIT ABOUT POLYMERASE, BUT
25 COULD YOU REVIEW BRIEFLY WHAT IT IS.

8
1 A. HERE WE JUST SHOWED DNA POLYMERASE AS KIND OF A LITTLE
2 BULLET-SHAPED MOLECULE.

3 DNA POLYMERASES ARE FOUND IN CELLS. SOME CELLS EVEN AS
4 SIMPLE AS A LITTLE BACTERIUM E. COLI, WHICH IS A NORMAL
5 INHABITANT OF HUMANS. WE USE IT IN MOLECULAR BIOLOGY RESEARCH.

6 E. COLI HAS THREE DNA POLYMERASES, ROMAN NUMERAL I,
7 ROMAN NUMERAL II, ROMAN NUMERAL III.

8 SOME VIRUSES HAVE BEEN SHOWN TO HAVE THEIR OWN DNA
9 POLYMERASE AS WELL. THE VIRUS T4, FOR EXAMPLE, WHICH ACTUALLY
10 INFECTS A LITTLE BACTERIAL CELL, HAS ITS OWN DNA POLYMERASE.

11 Q. DO THESE DIFFERENT KIND OF POLYMERASES HAVE DIFFERENT
12 CHARACTERISTICS?

13 A. YES, THEY DO. THE -- FOR EXAMPLE, THE ENZYME DNA
14 POLYMERASE -- OR ONE OF THE DNA POLYMERASES FROM E. COLI WORKS
15 BEST, FOR EXAMPLE, AT 37 DEGREES CENTIGRADE, OR OUR BODY
16 TEMPERATURE OF 98.6. IT'S AN INHABITANT OF US, AND SO IT'S
17 EVOLVED TO THE POINT WHERE IT GROWS BEST AT OUR BODY
18 TEMPERATURE.

19 ON THE OTHER HAND, IF YOU GO TO YOSEMITE AND YOU GO AND
20 FIND THE SULPHUR HOT SPRINGS, YOU FIND OTHER ORGANISMS THAT ARE
21 CAPABLE OF GROWING AT THOSE ELEVATED TEMPERATURES.

22 FROM ONE OF THOSE -- THE ORGANISM'S NAME IS THERMUS
23 AQUATICUS -- THEY ISOLATED A DNA POLYMERASE. THAT DNA
24 POLYMERASE OPERATES AT ELEVATED TEMPERATURES, TEMPERATURES
25 ALMOST AS HIGH AS WATER THAT YOU AND I COULDN'T PUT OUR FINGERS

9

1 IN. SO THAT ORGANISM, WHICH HAS ADAPTED TO GROWING AT ELEVATED
2 TEMPERATURE HAS A DNA POLYMERASE WHICH IS ACTIVE AT ELEVATED
3 TEMPERATURE.

4 Q. DOES POLYMERASE DO ANYTHING BUT MAKE DNA?

5 A. DNA POLYMERASES ARE FASCINATING ENZYMES, NOT ONLY FOR PEOPLE
6 WHO STUDY ENZYMES BUT ALSO FOR PEOPLE WHO STUDY ORGANISMS AND
7 GENETICS.

8 DNA POLYMERASES MAKE DNA BY ADDING SINGLE BASES AT A
9 TIME TO A THREE-PRIMED END OF A PRIMER. IN ADDITION, THEY CAN
10 UNMAKE DNA. THEY CAN BACK UP ON THE STRAND THAT THEY'VE MADE
11 AND UNMAKE IT.

12 THAT'S NOT TOO HARD TO IMAGINE, BECAUSE IF SOMETHING IS
13 CAPABLE OF MAKING SOMETHING, IT'S CAPABLE OF UNMAKING IT AS
14 WELL.

15 THAT ACTIVITY IS CALLED AN EXONUCLEASE, "NUCLEASE"
16 BECAUSE IT'S BREAKING DOWN NUCLEIC ACIDS, AND WE USE AS A SUFFIX
17 OR ENDING TERM FOR ENZYMES "ASE".

18 IN ADDITION, DNA POLYMERASES, IF THEY ENCOUNTER, AS
19 THEY'RE MAKING A STRAND A STRAND IN FRONT OF THEM, NOT THE
20 TEMPLATE STRAND BUT A STRAND THAT THEY WOULD -- THAT'S ON --
21 THAT'S COMPLEMENTARY TO THE TEMPLATE, IN OTHER WORDS, WOULD BE
22 JUST IN FRONT OF THEM, THEY DEGRADE OR BREAK DOWN THAT BY AN
23 EXONUCLEASE ACTIVITY AS WELL.

24 SO DNA POLYMERASES, SOME BUT NOT ALL NECESSARILY, HAVE
25 NOT ONLY THE POLYMERIZING ACTIVITY BUT THE EXONUCLEASE ACTIVITY

9
1 THAT ALLOWS THEM TO UNMAKE DNA, EITHER DNA THAT'S IN FRONT OF
2 THEM OR UNMAKE DNA THAT THEY'VE ALREADY SYNTHESIZED AND THEN
3 RESYNTHESIZE IT.

4 THE CONSEQUENCE OF THAT IS VERY IMPORTANT TO -- TO YOU
5 AND I, ALTHOUGH I DON'T THINK WE THINK OF THAT ALL THE TIME. BY
6 GOING BACK OVER THE DNA THAT THEY'VE MADE, UNMAKING IT, AND THEN
7 REMAKING IT AGAIN, THEY DO SOMETHING THAT ALL OF US WHO TYPE
8 HAVE TO DO, AND THAT IS PROOFREAD.

9 THEY GO BACK OVER, BREAK DOWN WHAT THEY'VE MADE, AND
10 REMAKE IT, SO THAT THEY LOWER THE CHANCE OF MAKING AN ERROR.
11 AND WE CALL THAT PROOFREADING OR AN EDITING FUNCTION OF THE DNA
12 POLYMERASE.

13 SO IT'S A VERY FASCINATING ENZYME, AND IT MAKES SURE
14 THAT WHEN THE MOLECULE MAKES A COMPLEMENTARY STRAND, THE
15 COMPLEMENTARY STRAND IS MORE CLOSELY ACCURATE OF WHAT EXACTLY
16 SHOULD HAVE HAPPENED.

17 Q. WHEN POLYMERASE RUNS INTO A STRAND AHEAD OF IT AND DEGRADES
18 IT AND REPLACES IT, IS THERE A TERM FOR THAT?

19 A. THAT -- WE FIND THAT THERE ARE A NUMBER OF TERMS IN THE
20 LITERATURE. WE TALK ABOUT THAT AS NICK TRANSLATION, BECAUSE WE
21 SORT OF MOVE A LITTLE SPACE ALONG FORWARD IN THE DNA AS IT'S
22 BEING BROKEN DOWN.

23 Q. NOW THAT WE'VE TALKED ABOUT THE INGREDIENTS, LET'S START
24 TALKING ABOUT THE PROCESS.

25 WHERE DO WE BEGIN?

9
1 A. ALL RIGHT. THE FIRST STEP OF THE PROCESS HERE -- WE NOW
2 MOVE TO THE FIRST CYCLE OF THE POLYMERASE CHAIN REACTION --
3 CONSISTS OF, HERE, A STRAND OF DNA WITH THE TARGET SEQUENCE.
4 HERE, THE OTHER DNA, IN KEEPING WITH WHAT WE SHOWED EARLIER,
5 WAS -- THE OTHER DNA IS PINK AND OUR TARGET SEQUENCE HERE IS IN
6 KIND OF A BLUE COLOR. THIS IS THE TARGET SEQUENCE WHICH IS PART
7 OF THE DNA MOLECULE.

8 AND IF WE CONTINUE TO FOLLOW THAT NOW, WE WILL SEE THAT
9 WE FOCUS PRINCIPALLY ON THE TARGET SEQUENCE HERE. IT'S A
10 DOUBLE-STRANDED DNA MOLECULE. AND IN THE EXAMPLE THAT WE'LL BE
11 USING HERE, IT'S EMBEDDED IN PART OF A LARGER MOLECULE.

10
12 IN THE FIRST CYCLE, THE FIRST STEP OF THE FIRST CYCLE
13 IS TO SEPARATE THE DOUBLE-STRANDED DNA MOLECULE INTO SINGLE
14 STRANDS, AND THAT'S SHOWN IN THE FOLLOWING.

15 HERE WE HAVE THE TWO STRANDS SEPARATED FROM ONE
16 ANOTHER.

17 NOW, REMEMBER, THOSE ARE COMPLEMENTARY STRANDS; IN
18 OTHER WORDS, THEY -- THEY CAN REFORM A PAIR. BUT IN THIS CASE,
19 WE'VE TAKEN THE CONDITIONS OF THIS DNA SAMPLE AND WE'VE ADJUSTED
20 IT SUCH THAT IT'S NOW DNA -- DNA SINGLE-STRAND.

21 THE NEXT STEP OF THIS PROCESS INVOLVES THE ADDITION OF
22 THE PRIMERS, AND THAT'S SHOWN IN THE FOLLOWING:

23 HERE ARE THE TWO PRIMERS. ON THE LOWER ORIGINAL
24 STRAND, YOU'LL HEAR ME TALK ABOUT THE PARENTAL STRANDS. THOSE
25 ARE THE ONES THAT WE STARTED WITH.

10 1 HERE, THE PARENTAL STRANDS ARE HERE ON THE BOTTOM WITH
2 THE TARGET SECTION AND THE OTHER DNA, HERE ON THE TOP THE TARGET
3 SECTION OF THE DNA.

4 THE PRIMERS, AS WE SAID BEFORE, ARE VERY SPECIFICALLY
5 CHOSEN. THEY ARE CHOSEN SUCH THAT ONE OF THEM IS COMPLEMENTARY.
6 HERE, I'M ILLUSTRATING THE BOTTOM STRAND. THIS PRIMER IS
7 COMPLEMENTARY TO ONE END OF ONE OF THE SINGLE-STRANDED TARGET
8 SEQUENCES; THE OTHER PRIMER IS COMPLEMENTARY TO THE OPPOSITE END
9 HERE, THE RIGHT-HAND END OF THE -- OF THE UPPER STRAND. IT'S
10 COMPLEMENTARY TO THE UPPER -- TO THE RIGHT-HAND END OF THE UPPER
11 TEMPLATE STRAND.

12 SO THESE PRIMERS ARE CHOSEN SO THAT THEY ONLY BIND TO
13 THESE PARTICULAR ENDS.

14 Q. WHAT HAPPENS NEXT?

15 A. NEXT WE'LL SHOW -- CONTINUE TO RUN THE VIDEO HERE.

16 TWO THINGS THAT I WANT TO ILLUSTRATE HERE: AGAIN, THE
17 THREE-PRIMED AND FIVE-PRIMED ENDS. THE PRIMER HAS A FIVE-PRIMED
18 END AND A THREE-PRIMED END.

19 HERE, I'M ILLUSTRATING THE UPPER PRIMER. THE LOWER
20 PRIMER AGAIN HAS A FIVE-PRIMED AND A THREE-PRIMED END.

21 WHAT IS IMPORTANT HERE IS THAT A SMALL LITTLE BULLET
22 HERE ON THE LOWER PRIMER AND ON THE UPPER PRIMER WHICH
23 REPRESENTS DNA POLYMERASE, DNA POLYMERASE BINDS TO THE
24 THREE-PRIMED END.

25 SO TWO THINGS: FIRST, THE POLYMERASE BINDS TO THE

10 1 THREE-PRIMED END; AND THESE PRIMERS ARE CHOSEN SO THAT THE
2 THREE-PRIMED END ALLOWS THE POLYMERASE TO SYNTHESIZE A
3 COMPLEMENTARY STRAND WHICH CONSISTS OF THE TARGET SEQUENCE AND
4 DNA CONTINUING ON.

5 THE PRIMER FIVE-PRIMED END HERE ON THE BOTTOM STRAND ON
6 THE LEFT DOES NOT SERVE AS A SOURCE FOR ADDING NEW BASES OR
7 SOMETHING TO ADD NEW BASES TO BY THE POLYMERASE. THE POLYMERASE
8 JUST DOES NOT DO THAT.

9 Q. I THINK YOU HAVE SOME PICTURES, AND I'D LIKE YOU TO EXPLAIN
10 IN MORE DETAIL HOW THE PRIMER BINDS WHEN THE POLYMERASE ADDS THE
11 BASES.

12 A. AT THIS POINT, WE'RE GOING TO MOVE TO A SMALLER PORTION AND
13 FOCUS JUST ON ONE OF THE ORIGINAL TARGET SEQUENCES AND THE BOUND
14 PRIMER.

15 YOU SEE HERE THAT THIS PRIMER HAS BOUND TO A SPECIFIC
16 REGION, AND WE WOULD SAY THAT FROM THIS . . . THIS SECOND BASE
17 ON THE RIGHT-HAND END, THE C, MOVING NOW TOWARDS THE LEFT, THAT
18 WOULD CONSIST OF THE TARGET REGION THAT WE WOULD LIKE TO HAVE
19 AMPLIFIED.

20 REMEMBER THAT THIS IS ALSO GOING ON WITH THE BOTTOM
21 STRAND. WE'RE JUST FOCUSING ON THE UPPER STRAND.

22 THIS IS THE FIVE-PRIMED END OF THE PRIMER HERE ON THE
23 LEFT, THIS G. THE T HERE ON THE LEFT-HAND END OF THE PRIMER IS
24 THE THREE-PRIMED END.

25 AFTER WE GET THE PRIMERS, THEY THEN -- THEN DNA

10

1 POLYMERASE CAN BIND AND THAT'S SHOWN NEXT.

2 AND HERE IS THE DNA POLYMERASE.

3 READY NOW, IF WE GIVE IT THE DNA BASES, TO ADD THE
4 BASES ONE AT A TIME TO THE THREE-PRIMED END, AND THAT'S SHOWN IN
5 THE NEXT PANEL.

6 I'VE SORT OF CHANGED WHERE WE WERE A LITTLE BIT TO ADD
7 A LITTLE MORE ROOM FOR THE INDIVIDUAL BASES.

8 AND THAT'S ANIMATED. AND YOU CAN SEE NOW THAT WHERE
9 THERE'S A T, THE DNA POLYMERASE INCORPORATES AN A; THREE G'S IN
10 A ROW, SO WE HAVE THE INCORPORATION OF THREE C'S; A T, AND NOW
11 AN A IS BEING BROUGHT IN; AND A T IS BEING BROUGHT IN.

12 NOW, THIS MOVED OFF THE SCREEN. THE POLYMERASE WILL
13 CONTINUE ADDING BASES TO A THREE-PRIMED END.

14 SOMETIMES THE POLYMERASE CAN SEPARATE, JUST
15 SPONTANEOUSLY, EVEN THOUGH IT STILL HAS SOME TEMPLATE TO USE AS
16 A GUIDE OR A PATTERN. THERE'S A THREE-PRIMED END WHICH IS
17 AVAILABLE FOR IT; THE BASES ARE AVAILABLE. SOMETIMES THE
18 POLYMERASE CAN SEPARATE.

19 ALTERNATIVELY, WE CAN CHANGE THE CONDITIONS SO THAT THE
20 POLYMERASE IS NO LONGER ACTIVE. WE COULD RAISE THE TEMPERATURE
21 FOR, SAY, THE E. COLI POLYMERASE ABOVE -- SIGNIFICANTLY ABOVE 37
22 DEGREES CENTIGRADE, OR 98.6, SO THAT IT NO LONGER IS ACTIVE.

11

23 ALTERNATIVELY, IF THE POLYMERASE RUNS OFF SO THAT
24 THERE'S NO MORE TEMPLATE STRAND, IT WILL STOP MAKING THE
25 COMPLEMENTARY STRAND.

11

1 Q. HOW -- HOW FAST DOES THIS ADDITION OF BASES GO?

2 A. THE ADDITION OF BASES DEPENDS AGAIN ON THE CONDITIONS.

3 Q. PERHAPS I SHOULD ASK: HOW FAST CAN IT GO?

4 A. IT CAN GO ENORMOUSLY FAST, SO FAST THAT I CAN'T IMAGINE HOW
5 IT'S DONE AS RAPIDLY AS IT'S DONE.

6 SOME OF THE NUMBERS THAT I'VE SEEN IN THE LITERATURE
7 HAVE STARTED AT 100 BASES ADDED PER SECOND. ON THE OTHER HAND,
8 IF THE CONDITIONS ARE ADJUSTED, THE RATE OF INCORPORATION OF
9 BASES CAN BE RATHER SLOW.

10 DEPENDING UPON THAT RATE OF ADDITION OF NEW BASES, YOU
11 WILL NEED TO JUST EITHER HAVE A SHORT OR RAPID INCUBATION TIME
12 IN ORDER TO MAKE THE ENTIRE PRODUCT THAT YOU'D LIKE TO MAKE.

13 Q. SOME OF THE TESTIMONY HERE HAS CONCERNED USING RADIOACTIVE
14 BASES IN EXPERIMENTS. CAN YOU EXPLAIN HOW THAT WORKS.

15 A. YES. IN THE NEXT PANEL, WE DIAGRAM IN THIS PARTICULAR
16 EXAMPLE WITH THE CLOSE-UP OF ONE OF THE TEMPLATE STRANDS AND THE
17 PRIMER AND POLYMERASE. HERE WE HAVE TWO G'S. THESE TWO G'S IN
18 DARK BLUE ARE RADIOACTIVE.

19 Q. I'M SORRY. ARE THOSE C'S OR G'S?

20 A. C'S. I'M SORRY. I MISSPOKE. THANK YOU.

21 THESE TWO C'S ARE RADIOACTIVE. IN OTHER WORDS, PART OF
22 THE MOLECULE CONTAINS AN ATOM WHICH IS ACTUALLY RADIOACTIVE.

23 WE USE THAT SO WE CAN FOLLOW WHERE THE MOLECULE IS.
24 THE REASON IS, IS THAT WE DON'T WANT TO ISOLATE LARGE, LARGE
25 AMOUNTS OF DNA, BUT IF WE HAVE THIS RADIOACTIVE ELEMENT SENDS

11 1 OUT A VERY STRONG SIGNAL FOR A VERY SMALL AMOUNT OF DNA.

2 SO IT'S SORT OF LIKE A LITTLE FLAG IN A CROWD OF A TOUR
3 GUIDE, OR A -- OR A CHILD OR A CAR IN A PARKING LOT. IT'S A
4 SMALL -- IT'S A SIGNAL THAT ALLOWS YOU TO FIND A PARTICULAR
5 MOLECULE THAT YOU'RE INTERESTED IN.

6 NOW, WE'VE ANIMATED THAT NOW.

7 THE POLYMERASE WILL AGAIN START MAKING THE
8 COMPLEMENTARY STRAND, USING THE TEMPLATE AS A GUIDE, AND IN THIS
9 CASE THE POLYMERASE JUST RANDOMLY PICKS FROM THE C'S THAT ARE
10 AVAILABLE TO IT. FIRST IT PICKED A RADIOACTIVE C, PUT IT
11 OPPOSITE A G. THE SECOND TIME, IT PICKED UP A NON-RADIOACTIVE
12 C. THE THIRD TIME, IT'S PICKED UP A RADIOACTIVE C.

13 DEPENDING UPON HOW FREQUENT THE RADIOACTIVE BASE IS IN
14 AMONGST THE TOTAL AMOUNT OF BASES OF THAT TYPE, YOU'LL GET MORE
15 OR LESS OF THE BASES INCORPORATED; IN THIS PARTICULAR CASE, THE
16 C'S WILL BE RADIOACTIVE. SO THE ENZYME SIMPLY PICKS FROM SORT
17 OF A RANDOM POOL MADE UP OF RADIOACTIVE AND NON-RADIOACTIVE C'S.

18 IF ALL THE MOLECULES WERE RADIOACTIVE C'S, THEN
19 WHEREVER THERE WAS A C, IT WOULD HAVE TO BE A RADIOACTIVE C.
20 IF, ON THE OTHER HAND, THERE WERE VERY LOW NUMBER OF RADIOACTIVE
21 C'S RELATIVE TO THE NORMAL -- OR THE NON-RADIOACTIVE C'S, THEN
22 THE MOLECULE WOULD HAVE RADIOACTIVE C'S IN IT BUT THEY WOULD BE
23 VERY INFREQUENT.

24 Q. I'M SORRY. I'VE SIDETRACKED YOU WITH THIS QUESTION ABOUT
25 RADIOACTIVITY.

11

1 WE WERE BACK IN CYCLE ONE OF A PCR REACTION.

2 A. YES.

3 NOW, MY POINT IN FOCUSING NARROWLY HERE IS TO SHOW YOU
4 WHAT IS HAPPENING IN THIS PARTICULAR CASE -- IF WE WOULD PAUSE
5 RIGHT HERE -- WHAT'S HAPPENING IN THIS UPPER RIGHT-HAND TEMPLATE
6 PRIMER WHERE DNA POLYMERASE HAS BEEN.

7 SO WE'VE BEEN SORT OF FOCUSING ON THAT. WE KIND OF
8 USED THE MICROSCOPE AND LOOKED UP CLOSE AT THAT.

9 NOW, THAT IS AS WELL HAPPENING SIMULTANEOUSLY ON THIS
10 LOWER STRAND AS WELL.

11 BUT WHAT WE WANT TO DO NOW IS TO MOVE TO A LARGER
12 SCALE. AND NOW IF WE'LL CONTINUE, YOU WILL SEE THE EXTENSION AT
13 THE THREE-PRIMED END HERE ON THE UPPER AND LOWER PRIMERS BY DNA
14 POLYMERASE.

15 NOW, THIS IS THE -- EXCUSE ME.

16 Q. THIS IS THE -- AT THE END OF THE FIRST CYCLE NOW?

17 A. YES, THIS IS THE END OF THE FIRST CYCLE.

18 NOW, I'D LIKE TO POINT OUT WHAT WE HAVE.

19 WE HAVE THE ORIGINAL TWO STRANDS. HERE, THE LOWER
20 STRAND WITH THE TARGET SEQUENCE; THE UPPER STRAND WITH TARGET
21 SEQUENCE.

22 AND NOW THE PRODUCTS OF THAT DNA REPLICATION, WHICH
23 WERE STARTED WITH THE PRIMERS, EXTENDED BY DNA POLYMERASE IN THE
24 PRESENCE OF THE BASES. THEY -- BECAUSE OF THE NATURE OF THE
25 PRIMERS, THEY WERE STARTED AT A UNIQUE SPOT AND CONTINUED SO

11 1 THAT THEY -- WE HAVE NOW A COMPLEMENT OF A TARGET AND A
2 COMPLEMENT OF THE DNA ON ONE SIDE OF THE TARGET SEQUENCE.

3 WE WOULD CALL THIS THE LONG PRODUCT AS DISTINGUISHED
4 FROM A PRODUCT WHICH IS JUST THE TEMPLATE HERE.

12 5 Q. ALL RIGHT. WE'VE GOTTEN TO THE END OF CYCLE ONE. WE MOVE
6 INTO A NEW CYCLE?

7 A. YES. THE SECOND CYCLE IS INITIATED WITH THE SEPARATION OF
8 THE DOUBLE-STRANDED MOLECULES INTO SINGLE STRANDS.

9 I'D POINT OUT RIGHT HERE THAT JUST BECAUSE OF THE
10 MECHANICS OF DOING THIS, WE ENDED UP WITH THESE TWO STRANDS IN
11 THE MIDDLE AS BEING RATHER CLOSE TOGETHER, CLOSE TO ONE ANOTHER.
12 THEY ARE ISOLATED SEPARATE STRANDS. I WOULD LIKE THEM TO BE A
13 LITTLE FURTHER APART, BUT WE NEED MORE ROOM IN HERE BETWEEN THE
14 STRANDS TO SHOW ALL THE DETAIL IN TERMS OF THE PRIMERS AND THE
15 POLYMERASE.

16 SO THE FIRST STEP OF THIS PROCESS IS TO SEPARATE THESE
17 TO -- DOUBLE-STRANDED MOLECULES NOW INTO FOUR SINGLE STRANDS.

18 AND THE NEXT STAGE OF THIS IS ILLUSTRATED, IS THE
19 BINDING OF THE PRIMERS.

20 NOW, HERE, AGAIN, HERE IS THE ORIGINAL UPPER. I'M
21 ILLUSTRATING THE UPPER STRAND WITH ITS BOUND PRIMER NOW.

22 HERE WE HAVE THE LONG PRODUCT, SLIGHTLY DIFFERENT
23 MOLECULE IN TERMS OF THE FACT THAT IT DOESN'T CONTINUE HERE ON
24 THE RIGHT. WE'RE LOOKING AT THE UPPER OF THE TWO MIDDLE LONG
25 PRODUCTS.

12

1 THE PRIMER HAS BOUND TO THE OPPOSITE END OF THIS LONG
2 PRODUCT, WHICH, IN FACT, IS THE COMPLEMENT OF THIS UPPER
3 PARENTAL STRAND.

4 AND THE SAME THING IS TRUE HERE AT THE BOTTOM.

5 AGAIN, THE PRIMERS BIND TO SPECIFIC REGIONS WHICH
6 YOU'VE CHOSEN AND ARE HERE IN THIS PARTICULAR EXAMPLE WITHIN
7 THAT TARGET SEQUENCE. AND THEY BIND WITH THE RIGHT THREE-PRIMED
8 END, SO WHEN POLYMERASE BINDS, AS WE SEE IN THE FOLLOWING,
9 POLYMERASE BINDS TO THE THREE-PRIMED END, AND THAT'S SHOWN HERE.

10 Q. OKAY. BEFORE YOU GO ON, DR. FALKINHAM, LET ME ASK A
11 QUESTION:

12 IS THE PRIMER THAT BINDS TO THE LONG PRODUCT, THE
13 THINGS IN THE MIDDLE, THE SAME PRIMER THAT BINDS TO THE ORIGINAL
14 STRANDS?

15 A. YES, IT IS. NOW -- BUT I HAVE TO MAKE IT CLEAR THAT THESE
16 TWO PRIMERS ARE DIFFERENT FROM ONE ANOTHER.

17 ONE PRIMER HERE BONDS TO THE RIGHT-HAND END OF ONE OF
18 THE COMPLEMENTS, AND HERE THE PRIMER BINDS TO THE LEFT-HAND END
19 OF THE UP -- OF THE LOWER ORIGINAL TARGET SEQUENCE.

20 THIS PRIMER, AS A MATTER OF FACT, IS THE SAME AS THE
21 PRIMER BINDING HERE TO THIS LONG PRODUCT.

22 THIS LONG PRODUCT WAS THE COMPLEMENT OF THIS PARENTAL
23 STRAND. THIS PARENTAL STRAND IS THE COMPLEMENT -- ON THE BOTTOM
24 IS THE COMPLEMENT OF THE ONE ON THE TOP. THEREFORE, THIS --
25 THESE TWO PRIMERS ARE BINDING TO THE SAME REGION OF DNA.

12
1 Q. WE'VE HAD THE BINDING AND WE WERE SHOWING THE POLYMERASE
2 COMING ON BEFORE I INTERRUPTED YOU.

3 WHAT GOES ON NEXT?

4 A. AFTER POLYMERASE HAS BOUND AGAIN TO THE THREE-PRIMED END IN
5 THE PRESENCE OF THE BASES, AND IF THE CONDITIONS ARE
6 APPROPRIATE, WE HAVE THE EXTENSION OF THAT AND THE VIDEO WILL
7 SHOW NOW THE EXTENSION OF THE MOLECULES.

8 AND THIS IS AT THE END OF THE SECOND CYCLE. BUT I NEED
9 TO POINT A FEW THINGS OUT THAT TYPIFY AND MAKE THIS SECOND CYCLE
10 UNIQUE:

11 HERE, JUST BEFORE WE LEAVE, WE HAVE THE BRIGHTER
12 STRANDS.

13 THE THIRD STEP OF THE CYCLE WOULD BE AGAIN THE
14 SEPARATION OF THE STRANDS. AND HERE WE HAVE THE SEPARATED
15 STRANDS, SO WE HAVE EIGHT SEPARATED STRANDS.

16 THE SECOND STEP OF THAT WOULD BE THE BINDING OF THE
17 PRIMERS, AND THAT'S SHOWN HERE.

18 IF WE WOULD HOLD THAT FOR A SECOND, I WOULD POINT OUT
19 THAT THE PRIMERS ARE BINDING TO THE ORIGINAL TEMPLATE STRANDS.
20 WE'VE ALREADY SEEN THAT. THEY'RE BINDING TO THE LONG PRODUCTS.
21 WE'VE SEEN THAT.

22 HERE, THE PRIMER IS BINDING TO THIS PRODUCT OF THE
23 SECOND CYCLE, WHICH IS JUST THE TARGET SEQUENCE, THE TARGET
24 SEQUENCE LITERALLY BEING DEFINED BY THOSE TWO PRIMERS FROM THE
25 RIGHT-HAND AND THE LEFT-HANDED ENDS.

1 NOW, IF WE CONTINUE.

2 AGAIN, THE DNA POLYMERASE BINDS TO THE THREE-PRIMED END
3 AND, WITH ANIMATION, YOU'LL SEE THAT THEY'RE EXTENDED.

4 AND AT THIS POINT, I WANT TO ILLUSTRATE SOMETHING, AND
5 I THINK IT'S WELL ILLUSTRATED HERE AND WILL BE THE NEXT CYCLE:

6 ONE OF THE CHARACTERISTICS OF THE PCR REACTION IS THAT
7 YOU START SEEING NOW JUST GRAPHICALLY MORE OF THE TARGET REGION
8 DEFINED HERE BY THE ENDS OF THE PRIMER THAN YOU DO OF THE OTHER
9 DNA HERE SHOWN IN PINK.

10 AT THE VERY BEGINNING OF PCR, I SAID: WHAT DOES PCR
11 DO? ONE OF THE THINGS THAT PCR DOES IS MAKE MORE COPY OF A
12 PARTICULAR TARGET SEQUENCE, HERE SHOWN IN THE MIDDLE HERE, AND
13 NOT AS MANY COPIES OF THE OTHER DNA.

14 NOW, THIS IS AT THE END OF THE THIRD CYCLE.

15 Q. YOU HAVE ONE MORE CYCLE TO SHOW US, DR. FALKINHAM.

16 A. YES.

17 Q. WOULD YOU EXPLAIN WHAT THAT ONE DOES.

18 A. YES. LET'S LOOK AT NOW THE FOURTH CYCLE. OUR SCREEN WILL
19 BE PRETTY WELL FILLED UP AS WE'RE GOING THROUGH HERE.

20 HERE, AT THE BEGINNING OF THE FOURTH CYCLE, WE HAVE THE
21 SEPARATION OF THE STRANDS, THE DENATURATION EVENT.

22 HERE THEY ARE ALL SEPARATED. EVEN THOUGH SOME OF THEM
23 ARE TOGETHER, THEY ARE STILL SEPARATED STRANDS. THE PRIMERS
24 BIND.

25 WE KEEP CHANGING SCALES HERE BECAUSE THE FIGURE GETS

1 BUSIER AND BUSIER.

2 HERE ARE THE PRIMERS. NOW DNA POLYMERASE BINDS. UNDER
3 THE RIGHT CONDITIONS, WE GET EXTENSION, THE INCORPORATION OF THE
4 BASES AT THE THREE-PRIMED END.

5 AND AGAIN YOU CAN SEE THAT WHAT WE'VE DONE IS, WE'VE
6 MADE MORE OF THE TARGET SEQUENCES. THE NUMBER OF LONG PRODUCTS
7 ARE NOT KEEPING UP NOW WITH THE NUMBER OF AMPLIFICATIONS OF THE
8 TARGET SEQUENCE, HERE SHOWN IN WHITE IN THE CENTER HERE.

9 AND AT THE END OF THIS FOURTH CYCLE, YOU SEE WE HAVE
10 SUBSTANTIAL INCREASE IN THE TARGET SEQUENCES AND NOT AS GREAT AN
11 INCREASE IN THE OTHER DNA OR THE DNA NEIGHBOR.

12 MR. LEWIS: YOUR HONOR, WE HAVE ONE MINOR TECHNICAL
13 DIFFICULTY.

14 THE COURT: YES.

15 MR. LEWIS: COULD WE HAVE A MOMENT WHERE WE COULD TURN
16 OFF THESE MONITORS? IT WILL TAKE NO MORE THAN A MINUTE TO DO.

17 THE COURT: SURELY.

18 (PAUSE IN PROCEEDINGS)

19 Q. (BY MR. LEWIS) NOW, DR. FALKINHAM, YOU WERE REMARKING ON
20 THE WAYS IN WHICH THE LONG --

21 THE REPORTER: I'M SORRY, MR. LEWIS, I CAN'T HEAR YOU.
22 COULD YOU SPEAK UP, PLEASE?

23 Q. (BY MR. LEWIS) BEFORE OUR LITTLE DIFFICULTY, YOU WERE
24 REMARKING ON THE WAY THAT THE LONG PRODUCTS WERE BEING COPIED AS
25 COMPARED TO THE TARGET SEQUENCE.

1 A. YES.

2 Q. ARE THERE TERMS THAT DESCRIBE THOSE RATES OF INCREASE?

3 A. YES. THE TARGET SEQUENCE, AS YOU SAW GRAPHICALLY, APPEARED
4 TO BE MULTIPLYING FASTER THAN THE OTHER SEQUENCES. AS A MATTER
5 OF FACT, IT'S INCREASING -- THE TERM THAT WE USE TO DESCRIBE ITS
6 INCREASE IS EXPONENTIAL. IT'S INCREASING BY LITERALLY POWERS OF
7 TWO.

8 THE FIRST CYCLE, WE HAD TWO STRANDS; THEN WE HAD FOUR
9 STRANDS; THEN WE HAD EIGHT STRANDS; THEN WE HAD 16 STRANDS, IN
10 ENSUING CYCLES, 32, 64, 128. THAT'S AN EXPONENTIAL INCREASE AND
11 IS CONTRASTED TO THE SLOWER OR ARITHMETIC OR LINEAR INCREASE OF
12 THE OTHER DNA STRANDS IN THAT MIXTURE IN THE TWO.

13 AND I'M ILLUSTRATING THIS IN THE FOLLOWING
14 REPRESENTATION. FOR SIMPLICITY, RATHER THAN STARTING WITH TWO
15 ORIGINAL STRANDS, I'M ONLY STARTING WITH SIMPLY ONE STRAND, ONE
16 PARENTAL STRAND.

17 THE EXPONENTIAL HERE ON THE RIGHT-HAND SIDE WOULD BE
18 REPRESENTATIVE OF THE INCREASE IN THE TARGET SEQUENCES; ON THE
19 LEFT-HAND SIDE, THE -- WITH THE HEADING "LINEAR," THIS IS
20 REPRESENTATIVE OF THE OTHER DNA AND ITS RATE OF INCREASE.

21 AT THE BEGINNING OF THE CYCLE, WE'LL START OFF WITH THE
22 SAME TWO STRANDS AND WE HAVE ONE EACH.

23 SO LET'S LOOK AT WHAT HAPPENS AT THE END OF THE FIRST
24 CYCLE. WE'LL HAVE THAT SHOWN IN A MOMENT HERE.

25 (PAUSE IN PROCEEDINGS)

1 THE WITNESS: I THINK WE HAVE A 10-SECOND DELAY THAT
2 WE'VE BUILT INTO THIS. HERE WE ARE.

3 THE END OF THE FIRST CYCLE -- IF WE COULD PAUSE HERE --
4 WE HAVE THE TWO PRODUCTS MADE FROM THE LINEAR AND TWO PRODUCTS
5 MADE FROM THE EXPONENTIAL.

6 Q. (BY MR. LEWIS) NOW, ONE OF THOSE IS THE ORIGINAL STRAND
7 THAT YOU HAD?

8 A. YES. HERE AT THE BOTTOM IS THE ORIGINAL STRAND, BOTH LINEAR
9 AND EXPONENTIAL.

10 HERE IS THE COMPLEMENT OF THAT STRAND, BOTH UNDER THE
11 LINEAR OR EXPONENTIAL MODEL, ITS COMPLEMENT, AND THAT'S THE ONE
12 ABOVE IT.

13 AT THE --

14 Q. SO --

15 A. EXCUSE ME.

16 BUT AT THE END OF THE FIRST CYCLE, WE CAN'T REALLY TELL
17 AT ALL WHETHER OR NOT THE MULTIPLICATION IS A LINEAR OR
18 EXPONENTIAL, BECAUSE THE NUMBER OF PRODUCTS IS THE SAME.

19 Q. OKAY. GO AHEAD -- GO ON. I'M SORRY. I INTERRUPTED YOU.

20 A. LET'S LOOK AT THE SECOND CYCLE.

21 HERE AT THE SECOND CYCLE, WE HAVE THE FOLLOWING: AT
22 THE BOTTOM ON THE LINEAR SIDE, WE HAVE THE PARENTAL STRAND. WE
23 HAVE THE FIRST PRODUCT MADE FROM THAT, USING IT AS A COMPLEMENT.
24 THEN WE ALSO HAVE A PRODUCT NOW WHICH WAS THE COMPLEMENT OF THIS
25 ORIGINAL PARENTAL STRAND AT THE BOTTOM, GIVING US THREE STRANDS.

2

1 OVER HERE ON THE RIGHT-HAND SIDE, WE HAD THE ORIGINAL
2 PARENTAL STRAND AND THE ONE THAT WAS SYNTHESIZED. BUT NOW WE
3 HAVE TWO OTHER STRANDS. THIS IS BECAUSE, UNDER THE EXPONENTIAL
4 MODEL IN WHICH WE SAW THE TARGET DNA AMPLIFYING, IT'S NOT JUST
5 THE ORIGINAL TEMPLATE STRAND THAT'S BEING USED TO GUIDE THE
6 SYNTHESIS OF ITS COMPLEMENT BUT ALSO WE HAVE THE SYNTHESIZED
7 STRAND FROM THE FIRST CYCLE BEING USED TO MAKE A COMPLEMENT,
8 USED AS A TEMPLATE TO MAKE ANOTHER STRAND.

9 SO IN THE EXPONENTIAL ONE, ONE OF THE KEYS IS TO
10 UNDERSTAND THAT IT'S NOT JUST THE ORIGINAL STRAND BUT THE
11 ORIGINAL STRAND AND ITS PRODUCT WHICH CAN BE USED.

12 SO WE GO FROM TWO STRANDS AT THE END OF THE FIRST CYCLE
13 TO FOUR STRANDS HERE, AND WE WOULD START SEEING THE DIFFERENCE
14 BETWEEN LINEAR AND EXPONENTIAL DUPLICATION.

15 AT THE END OF THE THIRD CYCLE, YOU'LL SEE THAT NOW WE
16 START SEEING A GREATER DIFFERENCE, FOUR UNDER LINEAR MODEL;
17 EIGHT UNDER AN EXPONENTIAL MODEL.

18 AT THE END OF THE FOURTH CYCLE -- WE'LL START GOING
19 THROUGH CYCLES -- YOU'LL SEE THAT WE HAVE FIVE NUMBER OF STRANDS
20 HERE UNDER THE LINEAR MOLECULE; 16 UNDER THE EXPONENTIAL MODEL.

21 WE MOVE TO THE FIFTH CYCLE. START MOVING THROUGH THESE
22 A LITTLE QUICKER. YOU CAN SEE SIX HERE; 32 STRANDS ON THE OTHER
23 SIDE.

24 WE FRANKLY ENDED UP WITH A LOT OF PROBLEMS BECAUSE WE
25 COULDN'T PUT THE STRANDS HERE, AND SO YOU'LL SEE A SORT OF BAR

2
1 GRAPH THAT GETS LARGER AND LARGER.

2 HERE, SIX CYCLES; WE HAVE 64 UNDER EXPONENTIAL
3 REPLICATION.

4 AND CONTINUING. THE SEVENTH CYCLE, WE HAVE EIGHT UNDER
5 THE LINEAR MODEL.

6 EIGHT CYCLES, NINE; AND 250 UNDER EXPONENTIAL.

7 THE NINTH CYCLE -- WE PAUSE RIGHT THERE.

8 AT THE NINTH CYCLE, WE SEE 10 COPIES UNDER LINEAR; 500
9 COPIES UNDER EXPONENTIAL. 50 MORE COPIES UNDER THE EXPONENTIAL
10 MODEL.

11 OKAY. CONTINUING ON NOW IN THE 10TH AND ENSUING
12 CYCLES, YOU'LL SEE THAT NOW WE GO -- THERE ARE A HUNDRED MORE
13 COPIES EXPONENTIALLY. THESE ONE-THOUSANDS ARE ACTUALLY
14 APPROXIMATIONS. THE NUMBER IS ACTUALLY, I THINK, 1,056, BUT WE
15 WERE JUST IMPRESSED. I WANTED TO GIVE YOU THE IDEA OF THE
16 MAGNITUDE OF THIS INCREASE.

17 LET'S CONTINUE WITH A FEW MORE CYCLES.

18 AT THE END OF THE 20TH CYCLE, WE HAVE ALMOST SOME
19 NUMBER CLOSE TO A MILLION; UNDER THE LINEAR MODEL ONLY 21.

20 AND FINALLY, I THINK WE END WITH 30 CYCLES IN WHICH WE
21 HAVE A BILLION STRANDS OF THE TARGET SEQUENCE AND ONLY, IN OUR
22 EXAMPLE, IT WOULD BE 31 STRANDS OF THE OTHER DNA.

23 SO WE HAVE ACHIEVED WITH THE POLYMERASE CHAIN REACTION
24 THIS ENORMOUS INCREASE OR AMPLIFICATION OF THE TARGET SEQUENCE,
25 AND THOUGH WE HAVE SOME INCREASE IN THE OTHER SEQUENCES, IT

2 1 ISN'T NEAR AS MUCH.

2 Q. I THINK WE HAVE A COUPLE OF POSTERS TO SUMMARIZE THIS
3 PROGRESSION, AND I HAVE A COUPLE MORE QUESTIONS.

4 (PAUSE IN PROCEEDINGS)

5 THE WITNESS: THE POSTERS SEEM TO BE GETTING BIGGER AND
6 BIGGER HERE.

7 Q. (BY MR. LEWIS) THEY'RE EXPONENTIAL.

8 A. THEY'RE EXPONENTIALLY INCREASING. I SEE.

9 (LAUGHTER)

10 Q. (BY MR. LEWIS) THESE HAVE BEEN MARKED AS EXHIBITS B-168 AND
11 B-169.

12 AND THESE ARE ESSENTIALLY THE SAME THINGS THAT WERE
13 SHOWN IN THE VIDEO?

14 A. YES. WHAT I'VE ATTEMPTED TO DO HERE IS, RATHER THAN SHOW
15 THEM TO YOU SORT OF TIME LAPSE WITH THE VIDEO, WE HAVE ON YOUR
16 LEFT-HAND SIDE HERE THE BEGINNING WITH THE SINGLE-STRAND AND YOU
17 CAN SEE THAT THAT SINGLE-STRAND IS HIGHLIGHTED IN RED ALL THE
18 WAY ACROSS, AND THE NUMBER OF CYCLES.

19 WE'VE SHOWN IN DETAIL THE NUMBER OF STRANDS UP TO SIX
20 CYCLES, AND THEN HERE WE GO FROM A THOUSAND TO A MILLION TO A
21 BILLION STRANDS AT THE 10TH, 20TH, AND 30TH CYCLE.

22 AND THAT'S CONTRASTED HERE ON THE JURY'S RIGHT. AGAIN,
23 THE PARENTAL STRAND IS SHOWN HERE IN RED ACROSS THE BOTTOM,
24 FOLLOWING THE LEGEND, AND THEN THE COPIES HERE ARE MADE.

25 AND AT THE END OF 10, 20 AND 30 CYCLES, WE HAVE 11, 21

3
1 AND 31 STRANDS COMPARED TO 1,000, ONE MILLION, AND ONE BILLION
2 STRANDS BY AMPLIFICATION THROUGH AN EXPONENTIAL INCREASE.

3 I WOULD POINT OUT THAT PROBABLY THESE HEIGHTS OF THESE,
4 1,000, ONE MILLION AND A BILLION, ARE PROBABLY NOT TO SCALE.
5 THEY WOULD JUST KIND OF ZIP OUT OF THE CEILING HERE.

6 Q. THIS IS FAIRLY ABSTRACT, BUT IN PRACTICAL TERMS, HOW DOES
7 THE DIFFERENCE BETWEEN A LINEAR AND EXPONENTIAL INCREASE AFFECT
8 THE SCIENTIST'S WORK?

9 A. WELL, THE -- ONE OF THE PROBLEMS THAT WE HAVE, AS I SAID
10 BEFORE, HAS TO DO WITH DIAGNOSIS AND THINGS LIKE THAT.

11 LET ME ILLUSTRATE: PARTICULARLY WITH A -- WITH A
12 PATIENT WITH -- WITH AIDS WHO IS SUSPECTED OF HAVING A
13 MYCOBACTERIUM AVIUM INFECTION, OR A PATIENT IN THE DEVELOPING
14 WORLD WHO IS SUSPECTED OF HAVING TUBERCULOSIS OR OF LEPROSY, THE
15 ORGANISM MYCOBACTERIUM LEPRAE CAN'T BE GROWN, SO THE DIAGNOSIS
16 IS BASED ON MANIFESTATION OF SYMPTOMS, WHICH IS QUITE FAR BEYOND
17 THE POINT WHERE YOU WANT TO INSTITUTE ANTIBIOTIC THERAPY.

18 TUBERCULOSIS AND MYCOBACTERIUM CAN BE CULTURED IN THE
19 LABORATORY. BUT FROM THE POINT OF VIEW OF A PATIENT IN THE
20 PHYSICIAN'S OFFICE COMPLAINING OF WEIGHT LOSS, NIGHT SWEATS AND
21 FEVERS, AND PERSISTENT COUGH FOR A LONG PERIOD OF TIME, IT MAY
22 BE UPWARDS OF SIX TO EIGHT WEEKS BEFORE WHEN THAT CULTURE IS
23 TAKEN.

24 IT'S NOT LIKE A THROAT SWAB FOR STREP THROAT, WHERE YOU
25 CAN GET IT WITHIN 24 HOURS OR LESS, BUT, RATHER, IT CAN BE AS

3
1 LONG AS SIX TO EIGHT WEEKS.

2 NOW, IF THE PERSON HAS TUBERCULOSIS, YOU WOULD LIKE TO
3 INSTITUTE THERAPY RIGHT THEN AND THERE. IN AN AIDS PATIENT, THE
4 SAME KIND OF DELAY OCCURS BECAUSE MYCOBACTERIUM AVIUM, THAT WE
5 FIND IN PERHAPS 50 PERCENT OF AIDS PATIENTS, IS A SLOW-GROWING
6 ORGANISM AS WELL.

7 ONE OF THE ADVANTAGES WOULD BE TO TAKE A SPECIFIC
8 TARGET SEQUENCE OF, SAY, THOSE THREE ORGANISMS -- AND I'M
9 SPEAKING OF THOSE THREE ORGANISMS BECAUSE I'M VERY FAMILIAR WITH
10 THE RESEARCH AND THE CLINICAL PRACTICE IN MICROBACTERIOLOGY IN
11 TUBERCULOSIS.

12 WE TAKE AND IDENTIFY A TARGET SEQUENCE, HAVE PRIMERS
13 WHICH AMPLIFY THAT TARGET SEQUENCE, AND HOPEFULLY WE MIGHT BE
14 ABLE TO FIND A PATIENT IN WHICH THEY MAY HAVE ONE -- HERE WE'RE
15 ILLUSTRATING HERE WITH THESE ORIGINAL STRANDS -- ONE PIECE OF
16 DNA; IN OTHER WORDS, ONE ORGANISM IN A PATIENT. WE MIGHT BE
17 ABLE TO AMPLIFY THAT THROUGH ENOUGH CYCLES SO THAT WE'D BE ABLE
18 TO DETECT WHETHER OR NOT THAT PIECE OF DNA WAS THERE.

19 WE CAN'T DO IT NORMALLY BECAUSE WE CAN'T FIND THAT ONE
20 LITTLE PIECE OF DNA IN AMONGST ALL THE CELLS OF THE BODY.

21 SO IF WE CAN AMPLIFY WITH PCR, WE'D BE ABLE TO PROVIDE
22 A VERY EARLY IMPOSITION OF ANTIBIOTIC THERAPY OR SOME KIND OF
23 SUPPORTIVE THERAPY TO TREAT THIS PATIENT. AND, AS I THINK ALL
24 OF US KNOW, THE EARLIER YOU INSTITUTE TREATMENT, THE BETTER THE
25 PROGNOSIS FOR THE PATIENT, THE BETTER THE PATIENT -- THE SOONER

3
1 THE PATIENT GETS WELL.

2 SO THIS IS SOMETHING THAT'S VERY IMPORTANT TO US
3 WORKING NOT ONLY WITH MYCOBACTERIA BUT MANY, MANY DISEASES
4 THROUGHOUT THE WORLD AND IN AMERICA AS WELL.

5 Q. DO YOU HAVE A VIDEO SEGMENT THAT ILLUSTRATES WHAT YOU WERE
6 TALKING ABOUT?

7 A. YES. LET'S MOVE ON IN THE VIDEO.

8 AND IF WE FREEZE RIGHT HERE, LET ME DESCRIBE WHAT I'M
9 ATTEMPTING TO SHOW.

10 WHAT I WANT TO SHOW HERE IS THAT IT ISN'T STRANDS THAT
11 SORT OF GET STACKED UP SOMEWHERE, BUT WE ACTUALLY WORK WITH
12 TUBES.

13 YOU'VE SEEN PRESENTATIONS WHERE TUBES ARE INVOLVED, I'M
14 SURE.

15 BUT HERE I HAVE TWO BEAKERS, ONE ON THE LEFT SHOWING
16 THE LINEAR AMPLIFICATION. HERE WE'VE GOT THAT -- IN THIS
17 RIGHT-HAND PANEL THAT YOU'RE LOOKING AT ON THE VIDEO, THE
18 RIGHT-HAND BEAKER SHOWS THE EXPONENTIAL INCREASE.

19 AND HERE, WHAT I WANTED TO SHOW YOU AND ILLUSTRATE IS
20 THAT THE TARGET SEQUENCE WILL BE -- WILL END UP FILLING THIS
21 BEAKER WITH MANY, MANY COPIES WHERE THE LINEAR DOES NOT, AND
22 THAT LITERALLY IS WHAT'S HAPPENING IN THE TUBE.

23 IF WE COULD CONTINUE.

24 HERE WE'RE AT THE FIFTH CYCLE WITH SIX STRANDS MADE BY
25 THE LINEAR REPLICATION MODEL; 32 BY THE EXPONENTIAL.

3
1 AND CONTINUING ON, WE'LL JUST LET IT RUN THROUGH THE
2 CYCLES.

3 CYCLE 10, YOU SEE MORE AND MORE STRANDS THERE ON THE
4 RIGHT-HAND SIDE.

5 THESE ARE ATTEMPTS BY ME JUST TO ILLUSTRATE THIS
6 MAGNIFICATION, AND AMPLIFICATION IS THE WORD THAT IS COMMONLY
7 ASSOCIATED WITH THE POLYMERASE CHAIN REACTION.

8 Q. ALL RIGHT. I'M GOING TO CHANGE THE SUBJECT A LITTLE BIT.
9 LET ME GET THE POSTERS OUT OF THE WAY.

10 (PAUSE IN PROCEEDINGS)

11 Q. (BY MR. LEWIS) DR. FALKINHAM, YOU DESCRIBED HOW THE PCR
12 PROCESS WORKS.

13 ARE YOU FAMILIAR WITH DIFFICULTIES, COMPLICATIONS THAT
14 ONE CAN RUN INTO IN TRYING TO DO A PCR REACTION?

15 A. ONE OF THE THINGS THAT I DID IN NOT ONLY PREPARING FOR CLASS
16 AND TALKING ABOUT THE POLYMERASE CHAIN REACTION BUT ALSO IN
17 PREPARING FOR THIS PRESENTATION WAS NOT ONLY TO ILLUSTRATE THE
18 PRINCIPLES BUT ALSO TO ILLUSTRATE SOME PROBLEMS THAT CAN OCCUR.

19 THERE IS ALREADY PUBLISHED, AS YOU CAN IMAGINE, SINCE
20 1985 PAPERS DESCRIBING THE POLYMERASE CHAIN REACTION, ITS USE,
21 AND ALSO WHAT I WOULD CONSIDER TO BE REVIEW TYPE ARTICLES IN
22 WHICH THE PROCESS AND THE TECHNOLOGY WAS REVIEWED AND PEOPLE
23 IDENTIFIED PROBLEMS THAT CAN GO WRONG IN THE POLYMERASE CHAIN
24 REACTION.

25 AND SO WHAT I'VE DONE IS PUT THREE OF THESE TOGETHER

4
1 WHICH ATTEMPT TO ILLUSTRATE PROBLEMS WITH REGARD TO EITHER THE
2 TEMPLATES OR THE PRIMERS THAT SORT OF FALL IN A GENERAL CATEGORY
3 TO GIVE YOU A LITTLE MORE UNDERSTANDING OF THIS REACTION.

4 Q. OKAY. IS THERE A PROBLEM -- YOU DESCRIBED THE PRIMERS
5 BINDING TO THE TEMPLATES AS PART OF REACTION. IS THERE A
6 PROBLEM IN OBTAINING THAT BINDING?

7 A. YES, THERE CAN BE A PROBLEM. AND THAT'S ILLUSTRATED IN THIS
8 FIRST INSTANCE. THESE ARE ALL HEADED AS: "WHAT CAN GO WRONG?"

9 HERE WE HAVE A VERY SCHEMATIC PRESENTATION OF THE TWO
10 ISOLATED TEMPLATE STRANDS ABOVE AND BELOW IN BLUE AND TWO
11 PRIMERS WHICH WOULD BE COMPLEMENTARY TO EITHER -- TO OPPOSITE
12 ENDS OF THE COMPLEMENTARY STRANDS.

13 AND IT WOULD BE OUR OBJECTIVE AFTER THE DENATURATION
14 STEP TO ADD THE PRIMERS AND HAVE THEM ANNEAL OR RENATURE AT THE
15 SPECIFIC SITES. AND THAT'S SHOWN HERE.

16 DON'T WORRY THAT THIS DNA APPEARS TO BE BENT. THAT'S
17 ALL RIGHT. DNA IS NOT REALLY A LONG, STRAIGHT, RIGID THING LIKE
18 MY POINTER.

19 THIS IS WHAT ONE WANTS AND THIS IS WHAT I'VE
20 ILLUSTRATED, BUT LET ME SHOW YOU HERE AS THIS VIDEO MOVES WHAT
21 CAN GO WRONG.

22 HERE, THE PRIMERS HAVE NOT BOUND TO THE TEMPLATE BUT,
23 RATHER, THE TWO TEMPLATE STRANDS HAVE BOUND.

24 NOW, THE QUESTION IS, WHAT -- UNDER WHAT CONDITIONS?
25 WELL, IF THE TEMPLATE STRANDS ARE IN EXCESS, THERE ARE LOTS OF

4
1 THOSE TEMPLATE STRANDS, WE WOULD EXPECT THAT TO HAPPEN, BECAUSE
2 THEY COULD FIND ONE ANOTHER MORE EASILY.

3 IF THE PRIMERS WERE VERY INFREQUENT, THEIR
4 CONCENTRATION WAS VERY LOW, THERE WERE VERY FEW OF THEM IN THAT
5 MIXTURE, THEY, TOO, WOULD HAVE A DIFFICULT TIME FINDING THESE
6 TEMPLATES, AND PERHAPS WE WOULD FAVOR TEMPLATE-TEMPLATE
7 REASSOCIATION OR RE-ANNEALING OR ANNEALING OR RENATURATION OVER
8 PRIMER BINDING TO THE TEMPLATE.

9 WE CAN CURE THAT BY KEEPING THE CONCENTRATION OF
10 PRIMERS LOW RELATIVE TO --

11 Q. I'M SORRY. YOU SAID PRIMERS.

12 A. I'M SORRY. I DID MISSPEAK.

13 -- BY KEEPING THE CONCENTRATION OF THE TEMPLATE STRANDS
14 LOW AND, COUPLED WITH THAT, KEEPING THE CONCENTRATION OF THE
15 PRIMERS HIGH SO THAT WE HAVE RATHER HIGH RATIOS OF THE NUMBER OF
16 PRIMERS TO THE NUMBER OF TEMPLATES.

17 Q. ARE THERE PROBLEMS THAT CAN OCCUR WITH THE PRIMERS
18 THEMSELVES?

19 A. YES, AND THAT'S ILLUSTRATED IN THE FOLLOWING FIGURE HERE.

20 AGAIN I ENTITLED IT: "WHAT CAN GO WRONG?"

21 AND HERE WE HAVE TWO PRIMERS. THREE-PRIMED,
22 FIVE-PRIMED, FIVE-PRIMED, THREE-PRIMED. HERE'S THE THREE-PRIMED
23 END OF ONE PRIMER AND FIVE OF THE OTHER; HERE'S THE FIVE WITH
24 ONE, THE THREE-PRIME OF THE OTHER ON THE LOWER.

25 WE LOOK AT THOSE VERY QUICKLY. WE CAN SAY, WELL, THOSE

4
1 WOULD BE PRIMERS. THEY ARE A FEW NUCLEOTIDES LONG SO THEY --
2 LIKE THESE OLIGONUCLEOTIDES.

3 BUT AS ILLUSTRATED IN THE FOLLOWING HERE, YOU SEE THAT
4 THERE'S A PROBLEM, AND THAT IS THAT IF YOU LOOK CLOSELY ENOUGH
5 AT THESE, AND WE FIND THAT ONE END OF THE UPPER PRIMER, THE
6 THREE BASES, THE G, C, A AT THE LEFT-HAND END OF THE UPPER
7 PRIMER CAN PAIR WITH THE RIGHT-HAND END OF THE OTHER PRIMER.

8 NOW, I HAVE FULFILLED THE REQUIREMENTS OF MAKING THE
9 DOUBLE-STRANDED DNA MOLECULE, BECAUSE ONE OF THE STRANDS, THE
10 UPPER HERE, GOES -- IN GOING FROM LEFT-TO-RIGHT, FIVE -- EXCUSE
11 ME, I MISSPOKE -- THREE TO FIVE, THE LOWER STRAND GOES FROM FIVE
12 TO THREE. SO THE STRANDS ARE -- HAVE THIS OPPOSITE POLARITY, A
13 WORD THAT WE'VE USED.

14 Q. AND WHAT HAPPENS?

15 A. WELL, IN THE PRESENCE OF DNA POLYMERASE, AND THE BASES,
16 THESE TWO PRIMERS CAN BE EXTENDED AT THE THREE-PRIMED END, AND
17 WE WOULD HAVE THIS KIND OF INTERESTING MOLECULE. IT'S NOT AN
18 AMPLIFIED TARGET SECTION PARTICULARLY. IT'S NOT JUST A
19 SINGLE-PRIMER.

20 SINCE IT'S COMPOSED OF TWO PRIMERS, WE'VE COME UP WITH
21 A SORT OF WHIMSICAL MNEMONIC AND CALL IT A PRIMER DIMER --
22 P-R-I-M-E-R, PRIMER, DIMER D-I-M-E-R, "DI" MEANING TWO, "MER"
23 MEANING UNITS -- SO IT'S MADE UP OF TWO PRIMER UNITS.

24 Q. YOU POINT OUT SOME PROBLEMS WITH TEMPLATES AND PRIMERS. ARE
25 THERE PROBLEMS THAT CAN OCCUR WITH THE TEMPLATES ALONE?

5 1 A. YES, THERE ARE. I WOULD POINT OUT, JUST BEFORE WE LEAVE
2 THIS, THAT -- THAT THIS REALLY ISN'T -- ISN'T PCR, BECAUSE WE
3 HAD NO INTERACTION OF THESE PRIMER MOLECULES WITH THE TEMPLATE.

4 LET'S GO ON NOW AND TAKE A LOOK AT THIS THIRD PROBLEM
5 WHERE I HAVE TWO PRIME -- TWO TEMPLATES. I HAVE TO BE CAREFUL.
6 I'M GETTING TOO MANY WORDS HERE.

7 WE HAVE THE TWO TEMPLATE STRANDS NOW HAVE BEEN
8 SEPARATED, WHICH IS THE FIRST STEP OF ANY PCR REACTION.

9 AND AGAIN LOOKING AT THESE, WE WOULD SAY, WELL, THOSE
10 ARE JUST TEMPLATE SEQUENCES. BUT IN LOOKING AT THOSE FURTHER,
11 AND HERE, TO ILLUSTRATE SOME PROBLEMS, THIS UPPER TEMPLATE
12 STRAND HAS A REGION -- HAS TWO REGIONS WHICH ARE COMPLEMENTARY
13 TO ONE ANOTHER. ACTUALLY, IT CAN FORM THIS HAIRPIN-LIKE
14 STRUCTURE.

15 WE TALK ABOUT THESE STRUCTURES IN DNA AS HAVING A STEM,
16 HERE WITH THE WHITE BONDS HOLDING THESE TOGETHER, WITH A LOOP ON
17 THE LEFT-HAND END. THIS STEM-LOOP STRUCTURE THEN COULD FORM
18 WITH THESE ISOLATED TEMPLATES.

19 NOW, HERE IS THE THREE-PRIMED END OF THE TEMPLATE AND,
20 AS YOU CAN SEE, THE FIVE-PRIMED END HERE ON THE RIGHT-HAND SIDE
21 OF THIS UPPER TEMPLATE CAN SERVE AS A TEMPLATE, AND WE WOULD
22 ACTUALLY EXTEND FROM THIS THREE-PRIMED END TO THE RIGHT-HAND
23 SIDE AS SHOWN IN THE VIDEO.

24 Q. IS THAT PCR?

25 A. THIS ISN'T PCR, EITHER, BECAUSE YOU HAVEN'T SEEN THE PRIMERS

5

1 INTERACTING WITH THIS MOLECULE, EITHER.

2 Q. WHAT HAPPENS TO THE BOTTOM STRAND?

3 A. THE BOTTOM STRAND, SINCE IT'S THE COMPLEMENT OF THE UPPER
4 STRAND, THE SAME THING COULD HAPPEN. THE SAME THING IN -- BY
5 THAT I MEAN, WE COULD FORM A HAIRPIN, AND THAT'S SHOWN HERE.

6 BUT WHAT I WANT TO POINT OUT WITH THIS HAIRPIN MOLECULE
7 IS THAT, ALTHOUGH THERE IS A REGION DOWN HERE AT THE VERY BOTTOM
8 OF THE THREE-PRIMED END OF THE TEMPLATE THAT COULD BE USED AS A
9 TEMPLATE, THIS IS A FIVE-PRIMED END OF THE MOLECULE, ON THE
10 UPPER PART, THAT COULD NOT BE EXTENDED BY DNA POLYMERASE, SO WE
11 WON'T GET AN INCORPORATION OF BASES INTO THAT MOLECULE.

12 IF WE WERE, SAY, MEASURING, FOR EXAMPLE, THE
13 INCORPORATION OF A RADIOACTIVE ELEMENT, HERE WE HAVE A G, WE'D
14 LOOK FOR THE RADIOACTIVE INCORPORATION OF A C HERE. WELL, IT
15 WOULDN'T HAPPEN BECAUSE DNA POLYMERASE WILL NOT ADD AT A
16 FIVE-PRIMED END.

17 Q. IF YOU HAD RADIOACTIVE BASES WHEN YOU WERE WORKING WITH THIS
18 TOP STRAND, WOULD THEY GET ADDED?

19 A. YES. THE RADIOACTIVE BASES WE EXTENDED DOWN HERE. HERE
20 THERE ARE TWO C'S ON THE LOWER STRAND OF THE THREE-PRIMED END OF
21 THE UPPER TEMPLATE MOLECULE. THEY WOULD, DEPENDING UPON THE
22 AMOUNT OF RADIOISOTOPE -- TOTAL RADIOISOTOPE, WE WOULD PROBABLY
23 GET ONE OF THOSE MOLECULES RADIOACTIVE.

24 Q. HOW COULD YOU TELL, THEN, WHETHER YOU'RE GETTING SOMETHING
25 LIKE THIS OR PCR?