PCR: The Accidental Discovery of Something Obvious

Clever new techniques for working with DNA have made advances in genetic research possible. One of these techniques, called PCR, takes advantage of the way DNA is copied.

Kary Mullis was driving to his cabin in northern California one weekend in 1983, but he kept thinking about his work as a molecular biologist making DNA probes. Having a large supply of probes is essential to genetic research. Researchers often have only a tiny DNA fragment to work with, and yet they need to perform many tests on that fragment. Kary knew the pace of research was slowed by the tedious process of making copies of DNA fragments for study. He wanted to find a faster, easier way.

He was thinking about how the DNA polymerase enzyme might help solve this problem. In the cell, this enzyme plays an important role in duplicating DNA. In his laboratory, Kary used it to make DNA copies for probes, one at a time. Kary suddenly realized he could take advantage of the enzyme's natural ability and make many copies of DNA at one time. He was so excited that he almost drove off the road!

How is PCR like a chain letter?

How PCR Multiplies DNA

Each cycle of PCR doubles the number of copies. If a cycle takes four minutes, here is how the number of copies grows over time.

Cycle	Doubles the Number		Number of Copies	Minutes
start	2 (initial double helix)	=	2	0
1	2x2	=	4	4
2	2x2x2	=	8	8
3	2x2x2x2	=	16	12
4	2x2x2x2x2	=	32	16
5	2x2x2x2x2x2	=	64	20
6	2x2x2x2x2x2x2	=	128	24
7	2x2x2x2x2x2x2x2	=	256	28
8	2x2x2x2x2x2x2x2x2	=	512	32
9	2x2x2x2x2x2x2x2x2x2	=	1,024	36
10	2x2x2x2x2x2x2x2x2x2x2	=	2,048	40
11	2x2x2x2x2x2x2x2x2x2x2x2	=	4,096	44
12	2x2x2x2x2x2x2x2x2x2x2x2x2	=	8,192	48
13	2x2x2x2x2x2x2x2x2x2x2x2x2x2	=	16,384	52
14	2x2x2x2x2x2x2x2x2x2x2x2x2x2x2	=	32,768	56
15	2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2	=	65,536	60
16	2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2	=	131,072	64
17	2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2	=	262,144	68
18	2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2	=	524,288	72
19	2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2x2	=	1,048,576	76

What is the next cycle? How many copies would there be after one hour and forty minutes?

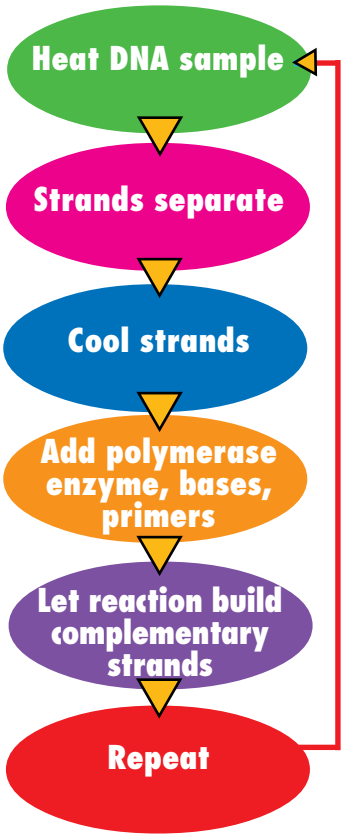
Note: The growth in the number of copies is "geometric" while the growth in time is "arithmetic."

Concentrating again, he reviewed his idea. You heat a double strand of DNA to make the strands separate. When the strands cool, you add the polymerase enzyme, a mixture of the bases (A, T, C, and G), along with DNA *primers* (which start or "prime" the polymerase enzyme reaction). The polymerase builds a complementary strand of DNA for each original strand, so you have two identical copies of double-stranded DNA. You have doubled the number of DNA sections. What if you heated these two sections and repeated the process? And then did it again?

Kary knew that the products of such a "loop" grow exponentially, like a chain letter. In this case, they would grow by the power of two: 2, 4, 8, 16, 32, 64, and so on. Two to the power of ten was...1024!

Kary stopped the car to check his calculations. By heating and cooling the DNA and polymerase in a similar "loop" process, he could get ever larger numbers of the same DNA fragment. By the time Kary reached his cabin, he was calling this process the *polymerase chain reaction* or PCR. His only concern was that someone might have done it already.

Back at work, he researched all the experiments done with DNA polymerase. No chain reaction. He told his friends and colleagues about it. "That's nice," they seemed to think. "Another of Kary's nutty ideas." He planned a simple experiment in a single test tube to tell him whether or not the idea could work. The result: it worked!



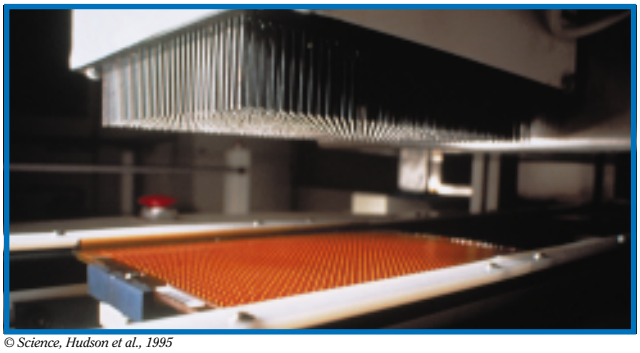
Since then, the uses of PCR have grown almost as fast as the chain reaction. The *Human Genome Project* would not be possible without the ability to make millions of low-cost copies of a DNA section within hours. For example, scientists need many copies of a chromosome to study and to construct maps. (See pages 10 and 11.) In addition, once a gene is found, scientists make copies or "clones" for gene libraries so researchers around the world can use them. In other research areas, scientists now can take a tiny DNA fragment from a mummy or a drop of blood at a crime scene and make enough copies to analyze. PCR is now so essential to scientific research that his off-the-wall idea earned Kary Mullis the Nobel Prize in Chemistry in 1993. Like many great discoveries, PCR now seems so simple and obvious that many a scientist must be saying, "Why didn't I think of that!"■

Careers Galore

People from many fields are involved in the *Human Genome Project*. Find out more about what people do in these fields. Do any of these seem like future careers for you?

- Molecular genetics
- Classical genetics
- Computers
- Robotics
- Chemistry
- Optical physics
- Cell biology
- Medicine/physiology
- Mathematics
- Ethics
- Genetic counseling
- Photo imaging

The "Genomatron," a super-duper PCR machine that made possible the most complete physical map of the entire human genome in 1995. This detail shows a 1,536 head pipette that dispenses DNA to be tested.



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Genetic Linkage Map from page 11

green yellow orange red

Physical Map with Markers from page 11

Answers from page 19: #3) Marker B. #4) Yes.