The technique PCR

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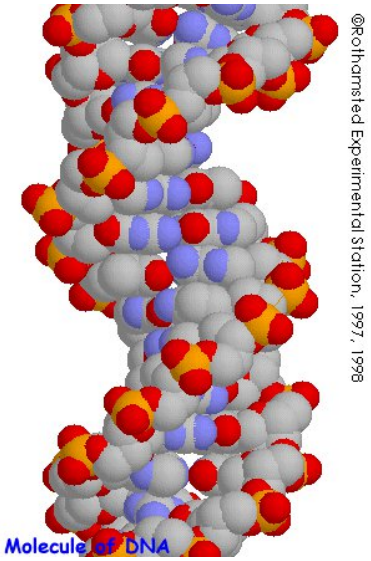
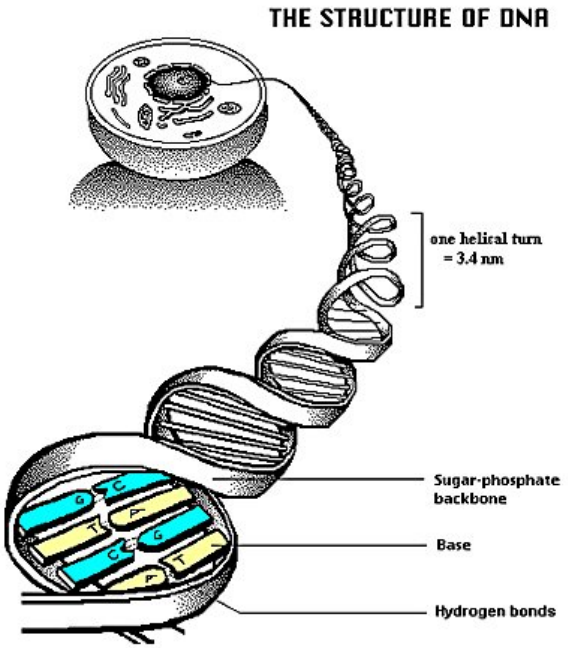
The structure of DNA

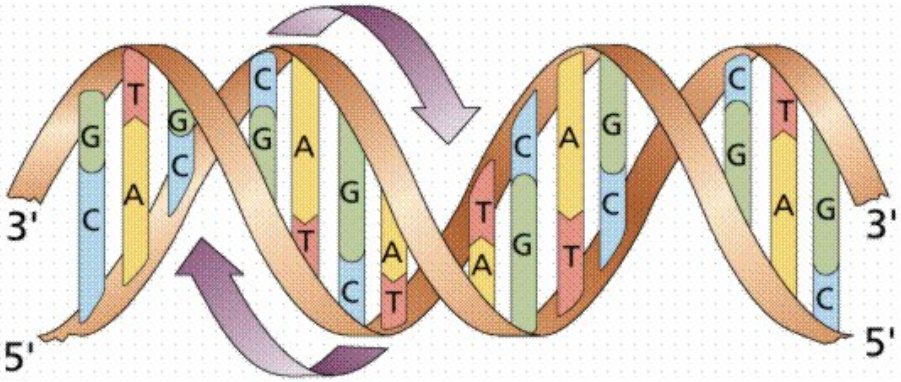


Double Helix

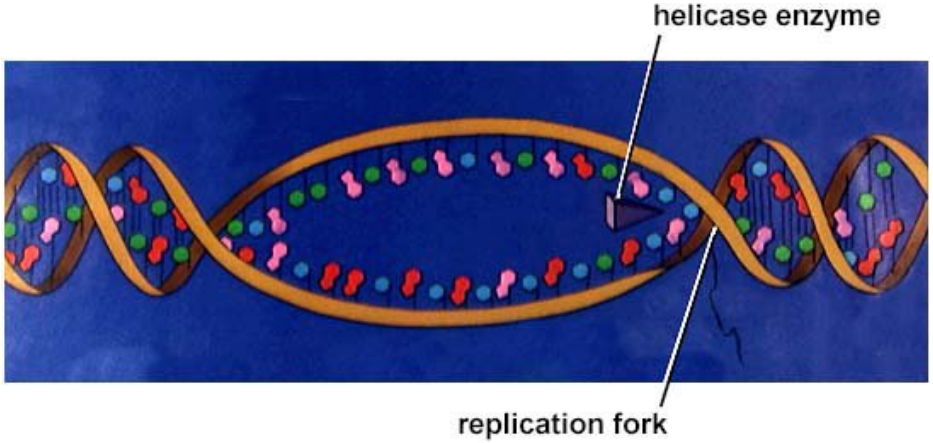


Complementary Base Pairing

The structure of DNA



Antiparallel Strands



Unzipping

The Problem

How do we identify and detect a specific sequence in a genome?

Problem:

How do we identify and detect a specific sequence in a genome?

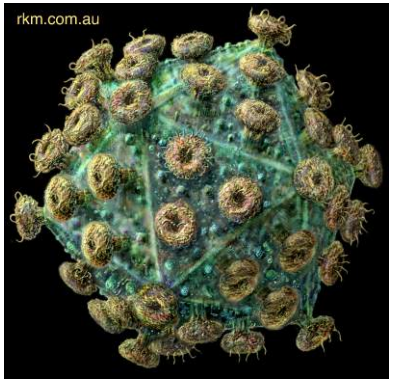
- TWO BIG ISSUES:
  - **There are a LOT of other sequences in a genome that we're not interested in detecting. (SPECIFICITY)**
  - **The amount of DNA in samples we're interested in is VERY small. (AMPLIFICATION)**

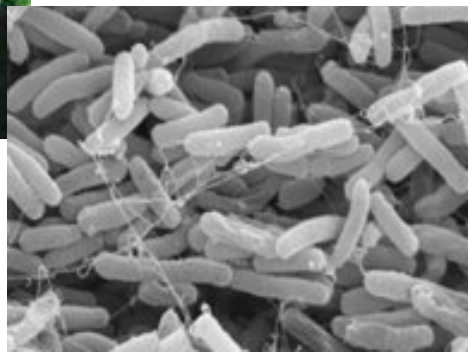
The Problem:

Specificity

How do we identify and detect a specific sequence in a genome?

- Pine: 68 billion bp
- Corn: 5.0 billion bp
- Soybean: 1.1 billion bp
- Human: 3.4 billion bp
- Housefly: 900 million bp
- Rice: 400 million bp
- E. coli: 4.6 million bp
- HIV: 9.7 thousand bp





Just How Big Is 3.4 Billion?

- The human genome is 3.4 B bp
- If the bases were written in standard 10-point type, on a tape measure...
- ...The tape would stretch for 5,366 MILES!
- **Identifying a 500bp sequence in a genome would be like finding a section of this tape measure only 4 feet long!**

How many molecules do we need to be able to see them?

- To be visible on an agarose gel, need around 10 ng DNA for fluorescent stain (or around 25ng for FastBlast).
- For a 500-bp product band, weighing 660 g/mol.bp, therefore need 10e-9 / (500*660) = 3.03e-14 moles.
- Avogadro's number = 6.02e23.
- Therefore need 1.8e10 copies!

- In other words, to "see" a single "gene", the DNA in a sample of 100 cells would have to be multiplied 180 million times!!!!!

The Problem:

Specificity

Amplfication

SPECIFICITY

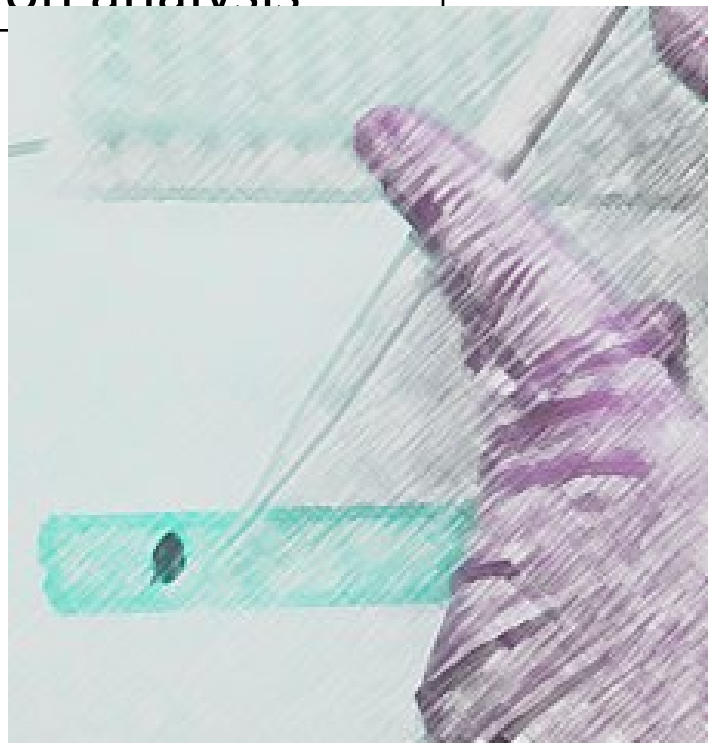
AMPLIFICATION

- How do we identify and detect a specific sequence in a genome?
- TWO BIG ISSUES:
  - There are a LOT of other sequences in a genome that we're not interested in detecting.
  - The amount of DNA in samples we're interested in is VERY small.

PCR solves BOTH of these issues!!!

So what's PCR used for?

- Forensic DNA detection
- Identifying transgenic plants
- Detection and quantification of viral infection
- Cloning
- Detection of ancient DNA
- Gene expression analysis



PCR History

The Invention

In what has been called by some the greatest achievement of modern molecular biology, **Kary B. Mullis** developed the **polymerase chain reaction** (PCR) in 1983. PCR allows the rapid synthesis of designated fragments of **DNA**. Using the technique, over one billion copies can be synthesized in a matter of hours.

PCR is valuable to scientists by assisting **gene mapping**, the study of gene functions, cell identification, and to forensic scientists in criminal identification. Cetus Corporation, Mullis' employer at the time of his discovery, was the first to commercialize the PCR process. In 1991, Cetus sold the PCR patent to Hoffman-La Roche for a price of $300 million. It is currently an indispensable tool for molecular biologists and the development of genetic engineering.

Mr. PCR: Kary B. Mullis

(1944 -)
The inventor of the DNA synthesis process known as the Polymerase Chain Reaction (PCR). The process is an invaluable tool to today's molecular biologists and biotechnology corporations.

Mullis, born in Lenoir, North Carolina, attended the University of Georgia Tech for his undergraduate work in chemistry, and then obtained a Ph. D. in biochemistry from Cal Berkeley.

In 1983, working for Cetus Corporation, Mullis developed the Polymerase Chain Reaction, a technique for the rapid synthesis of a DNA sequence. The simple process involved heating a vial containing the DNA fragment to split the two strands of the DNA molecule, adding oligonucleotide primers to bring about reproduction, and finally using polymerase to replicate the DNA strands. Each cycle doubles the amount of DNA, so multiple cycles increase the amount of DNA exponentially, creating huge numbers of copies of the DNA fragment.
Mullis left Cetus in 1986. For his development of PCR, he was co-awarded the Nobel Prize in chemistry in 1993.

The Invention of PCR

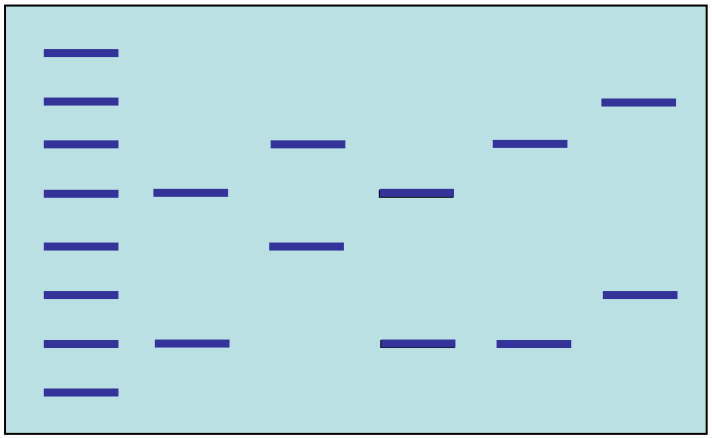
The process, which Dr. Mullis conceptualized in 1983, is hailed as one of the monumental scientific techniques of the twentieth century. A method of amplifying DNA, PCR multiplies a single, microscopic strand of the genetic material billions of times within hours. Mullis explains:

"It was a chemical procedure that would make the structures of the molecules of our genes as easy to see as billboards in the desert and as easy to manipulate as Tinkertoys....It would find infectious diseases by detecting the genes of pathogens that were difficult or impossible to culture....The field of molecular paleobiology would blossom because of P.C.R. Its practitioners would inquire into the specifics of evolution from the DNA in ancient specimens....And when DNA was finally found on other planets, it would be P.C.R. that would tell us whether we had been there before."

PCR

Visualizing Results

The final result of the traditional PCR procedure is a gel with a series of bands:



Bands can be compared against each other, and to known size-standards, to determine the presence or absence of a specific amplification product.