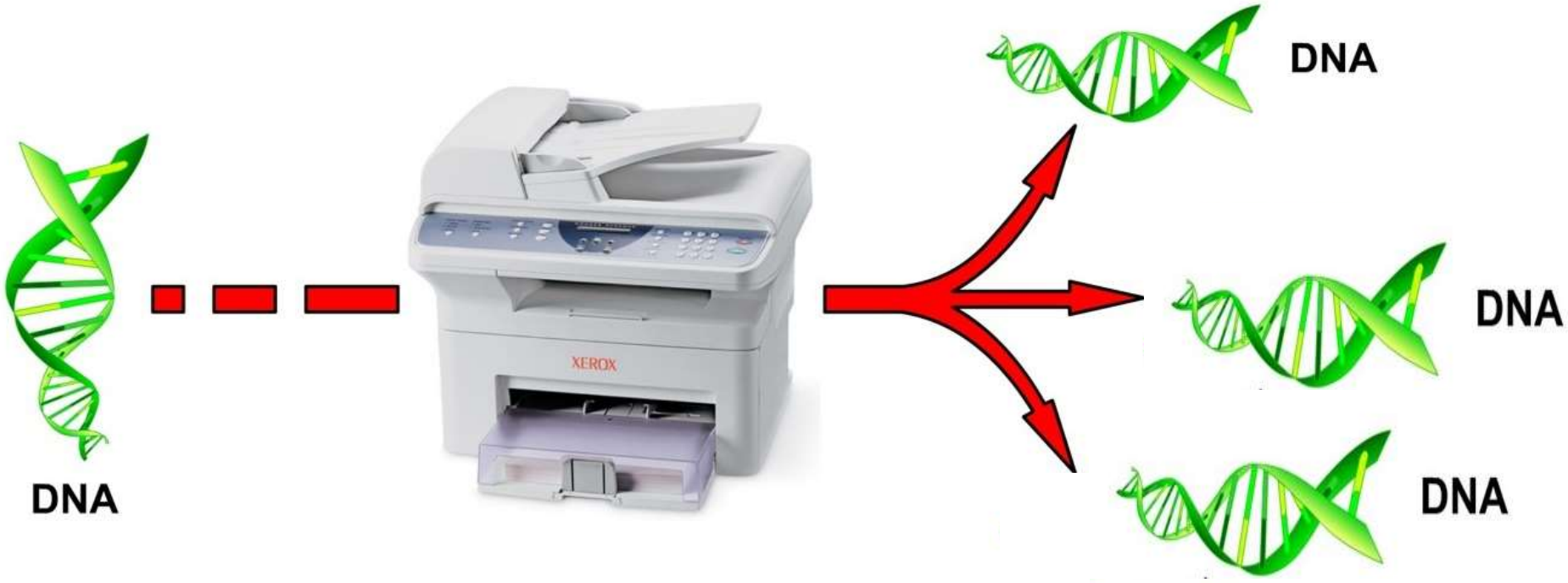
Polymerase chain reaction (PCR)

Polymerase Chain Reaction (PCR)=DNA Photocopier



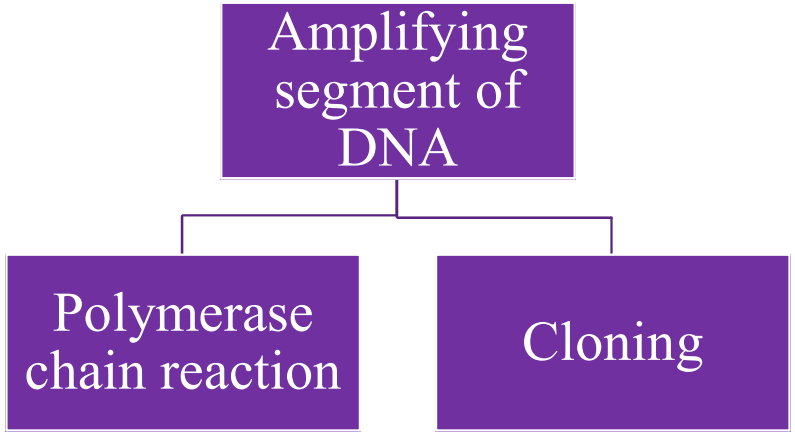
DNA amplification:

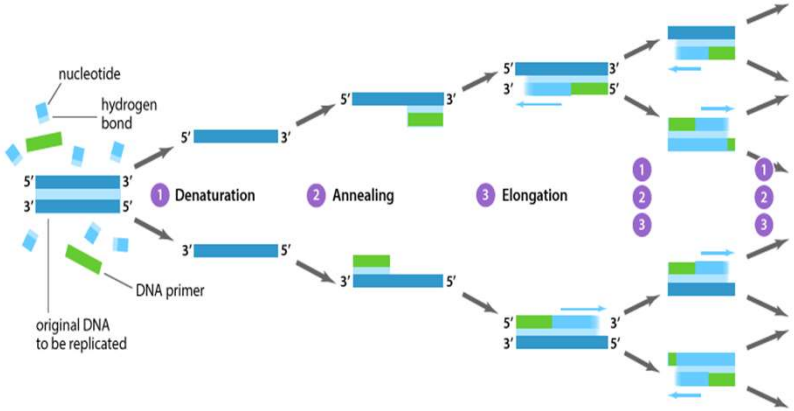
- In a crime scene, a sample of DNA was found, however **amount of DNA was not enough to** be analyzed.

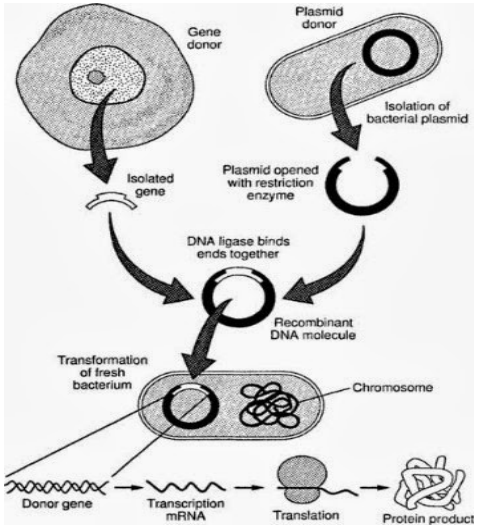
- After DNA extraction, the scientist want to study a specific **part of a gene** to do sequencing.

- How scientist solve these problem ?

- The solution is to do **amplification of parts of DNA**!!
- Mainly there are two methods:





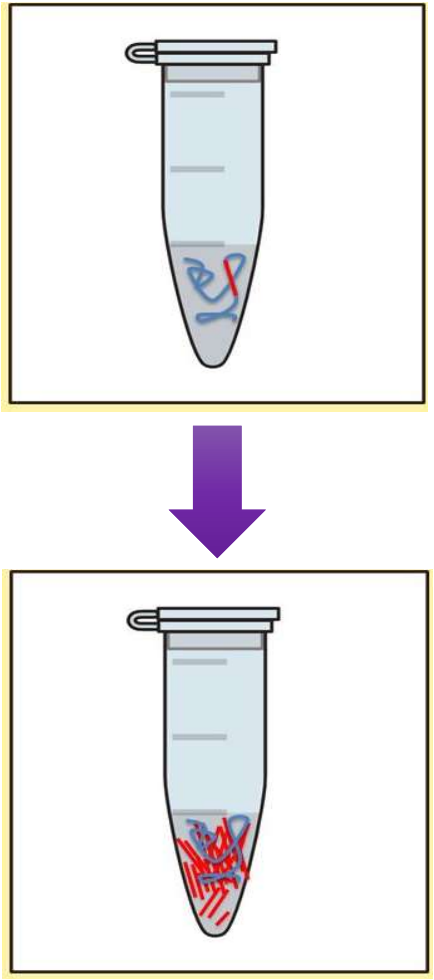


PCR-Polymerase Chain Reaction:

- PCR is a means to amplify a particular piece of DNA .

  ➔ **Amplify=** making numerous copies of a segment of DNA.

- PCR can make billions of copies of a **target sequence of DNA** in short time.



- It is a laboratory version of DNA Replication in cells.

  ➔ The laboratory version is commonly called "in vitro" since it occurs in a test tube while "in vivo" signifies occurring in a living cell.

- So…

➔ How the amplification will be done?

➔ How you will determine your target sequence?

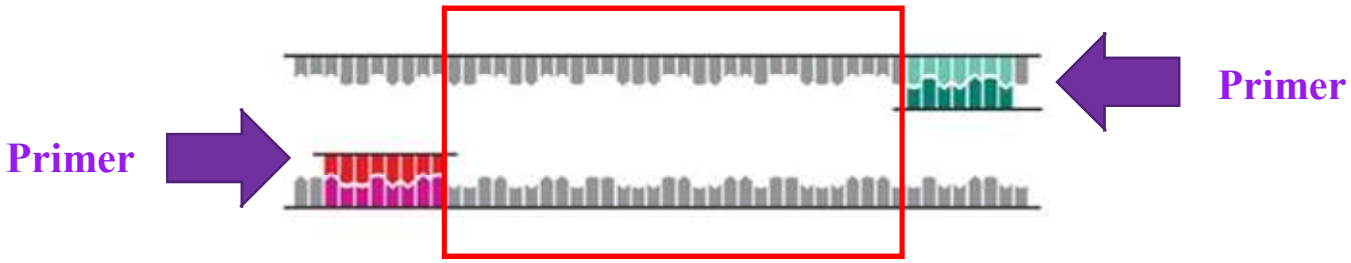
➔ How the amplification will be specific for certain segment?

You must to understand these questions

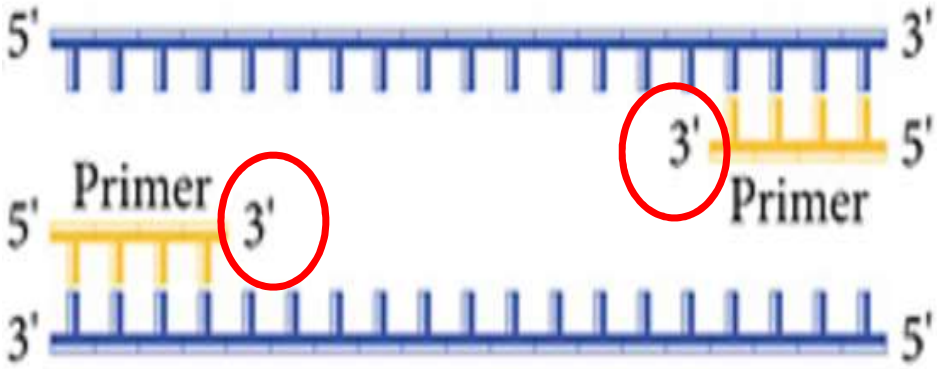
Amplification of a specific target sequence:

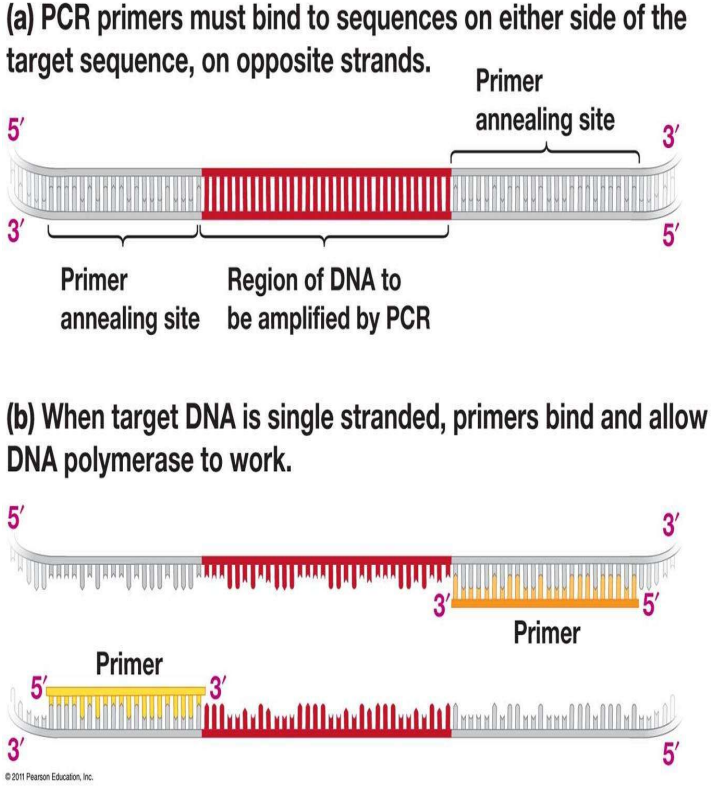
- PCR **does not copy all of the DNA** in the sample. It copies only a very specific sequence of genetic code from a template DNA, targeted by PCR primers.

- It does require the knowledge of some DNA sequence information which flanks the fragment of DNA to be amplified (**target DNA**).

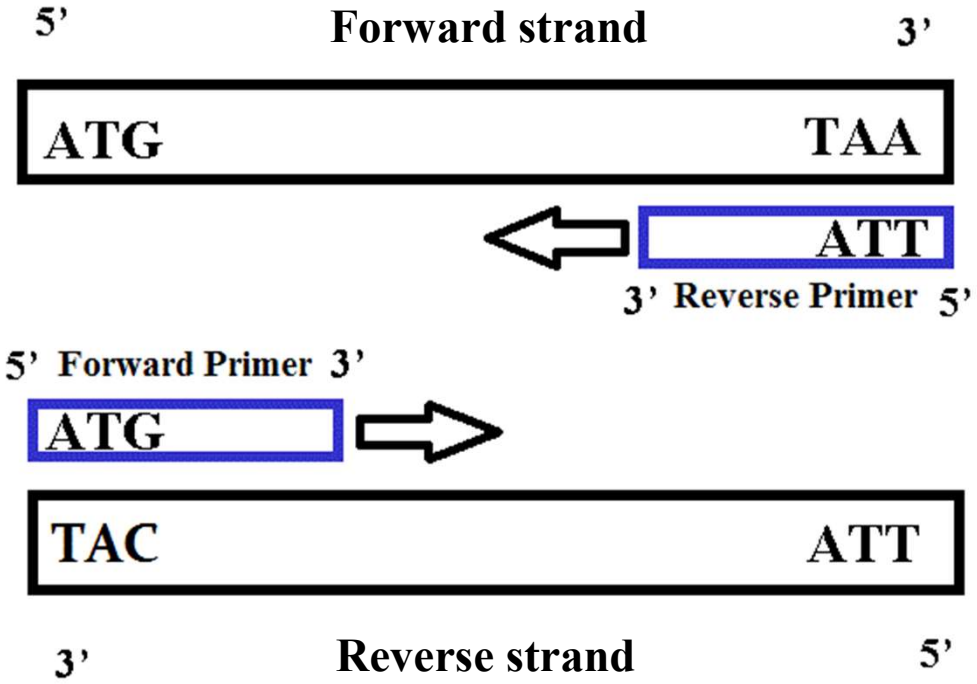


- From this information two synthetic oligonucleotide primers may be chemically synthesised each complementary to a stretch of DNA to the 3' side of the target DNA, one oligonucleotide for each of the two DNA strands (DNA polymerase can add a nucleotide only onto a **preexisting 3'-OH group**).





Why we need two primers ?



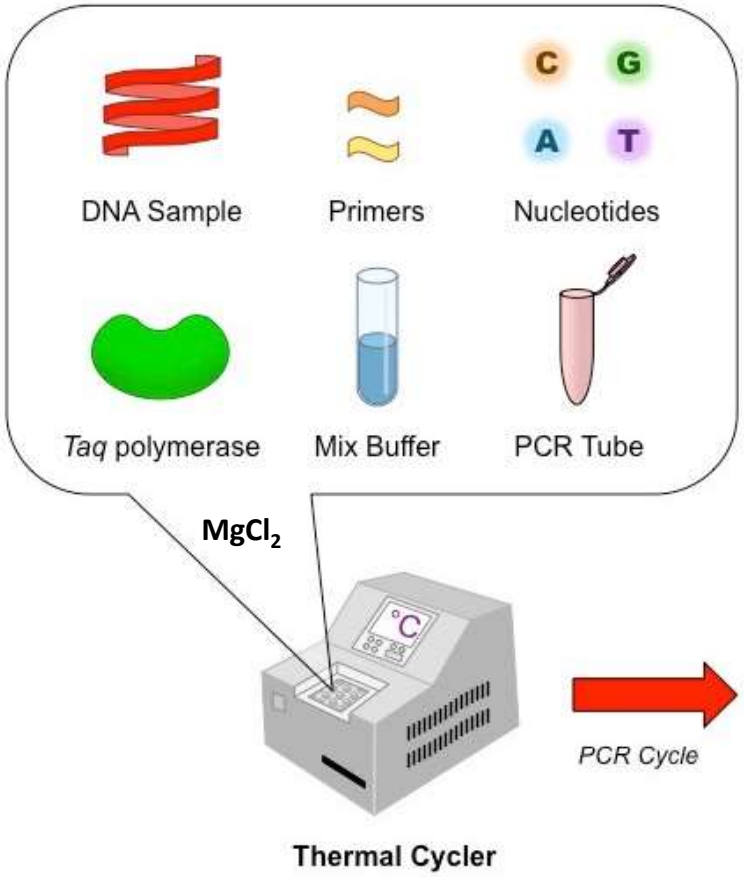
- In a PCR reaction you need **two primers** to amplify the target sequence:

➔**One called:** Forward primer, which have the same sequence of forward DNA strand and bind to the complementary reverse strand.

➔**The second called:** Reverse primer, which have the same sequence of reverse DNA strand and bind to the complementary forward strand.

*If there is only one primer, only one strand of the double stranded DNA will be amplified in the PCR reaction.

Components of PCR



Additional reagents may included