Genetic Engineering

- The intentional alteration of a genome of an organism by substituting or introducing new genetic material into the genome.
- One example of genetic engineering in use is the production of insulin for diabetics.

Diabetes

- Diabetes is a disease caused by a deficiency in the production of insulin. Approximately 5% of all deaths are caused by diabetes.
- Type 1 diabetes results when the body fails to produce insulin, it is an autoimmune disorder that requires people with it to be given insulin.
- Type 2 diabetes results when the body doesn’t produce enough insulin, or cannot use the insulin produced. The main risk factors for type 2 diabetes are a genetic predisposition and being overweight.

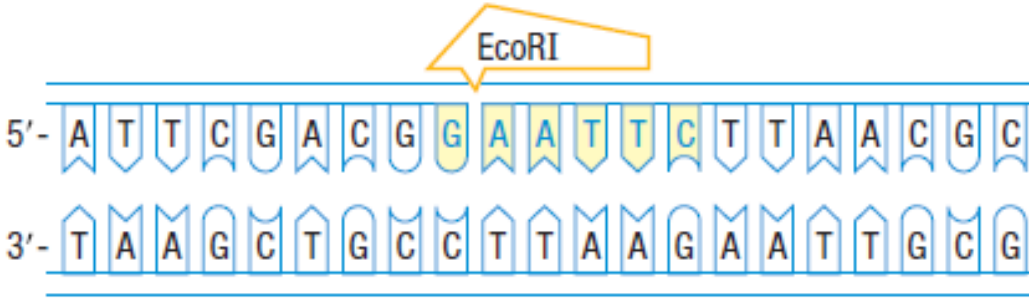
- Insulin was discovered by two researchers at the University of Toronto in 1922.
- Insulin was initially collected from the pancreases of pigs and cows. Some people unfortunately had an allergic reaction to this insulin.
- Scientists attempted to mass-produce human insulin. They discovered that they could insert the human insulin gene into bacteria, and the bacteria would make human insulin.

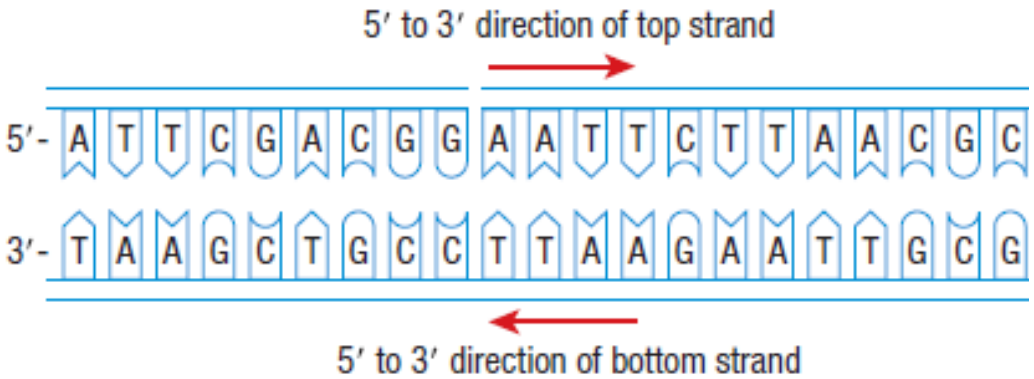
- Bacteria are useful tools for genetic engineers because they reproduce quickly, are easy to keep alive, and contain plasmids.
- The bacteria *Escherichia coli (E. coli)* are used to produce biosynthetic human insulin.
- The human gene that codes for insulin is inserted into an E. coli plasmid.
- The E. coli transcribes and translates the piece of human DNA to make the human protein insulin, which is then harvested from the bacteria.
- The DNA that contains genes from two species is called recombinant DNA.

Restriction enzymes

- The first step in genetic recombination is to cut a DNA fragment that contains the desired gene.
- Scientists used restriction enzymes (also called restriction endonucleases) which occur naturally in bacteria.
- A restriction enzyme acts like molecular scissors, cutting a DNA molecule at specific locations.

- The restriction enzymes cut the DNA molecule, creating pieces known as restriction fragments.
- Each restriction enzyme recognizes a specific sequence of nucleotides on a DNA strand, called the recognition site.
- For example, the enzyme EcoRI binds at a recognition site with the base-pair sequence 5’-GAATTC-3’, and then cuts the bond in the DNA backbone between the G and the A.





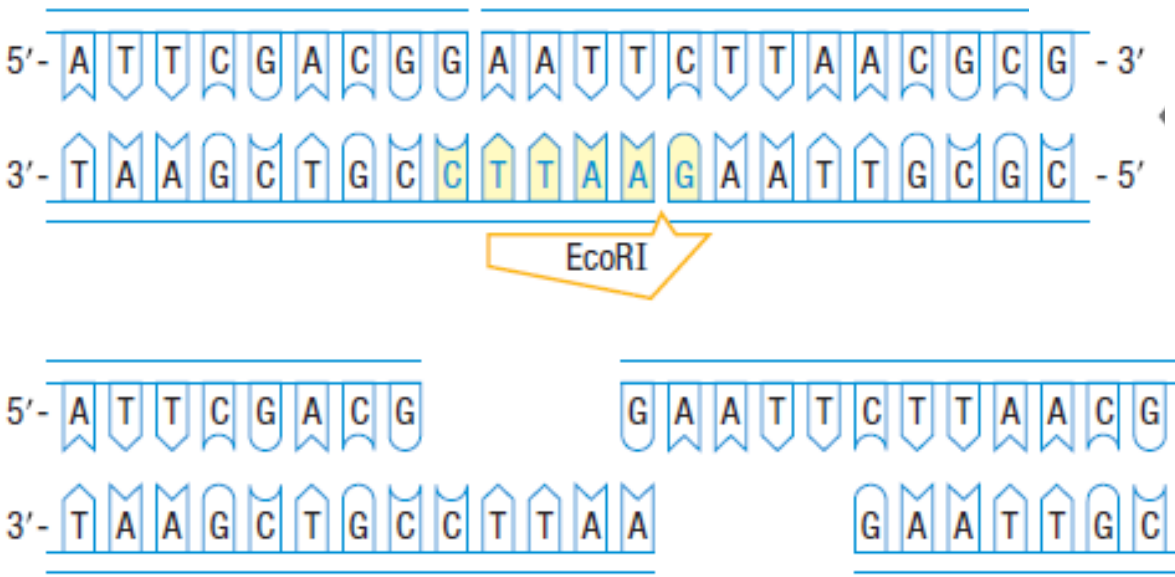
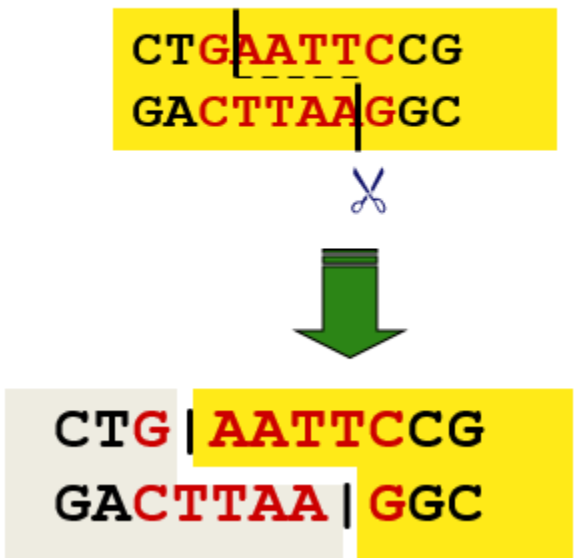
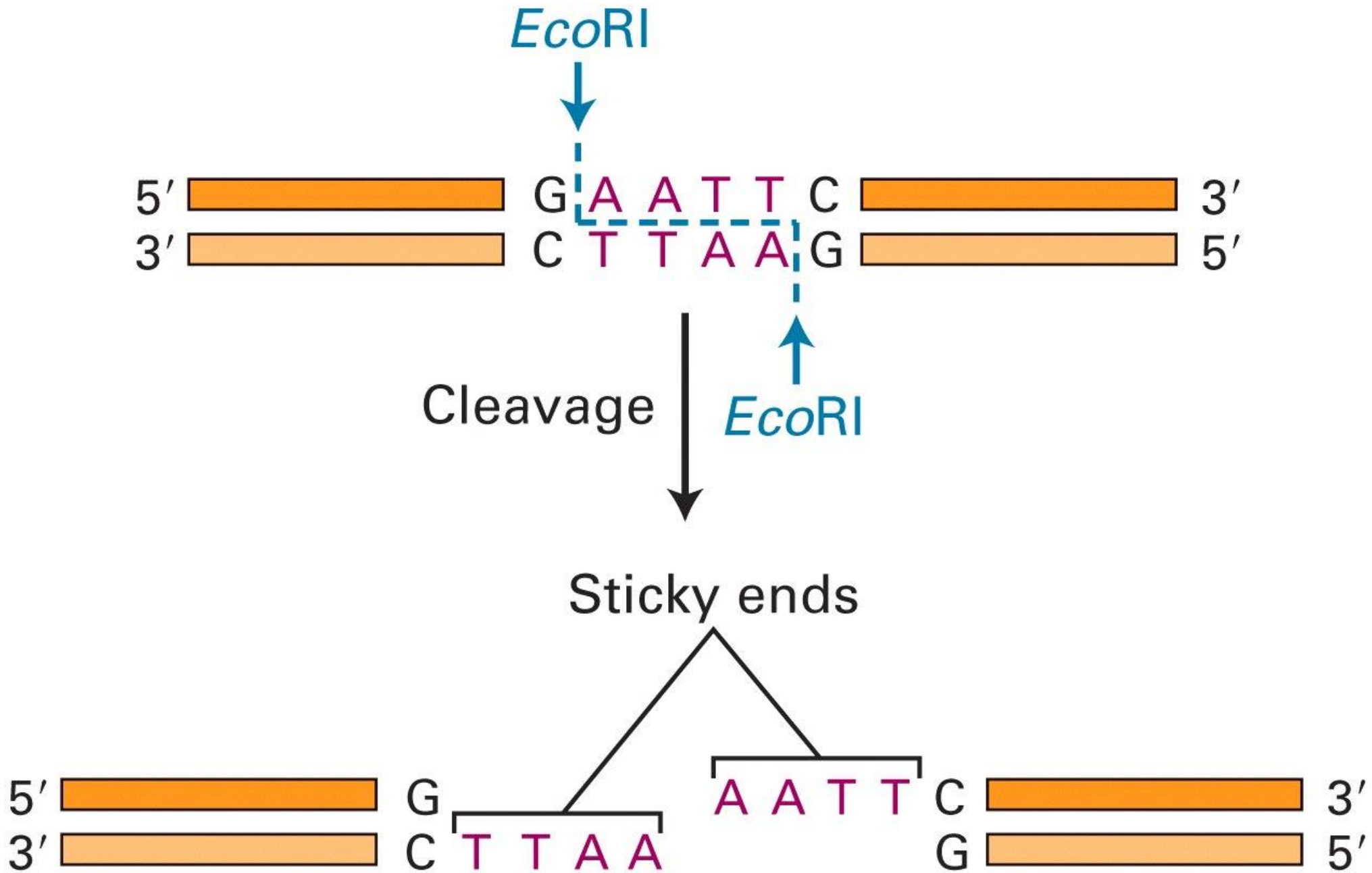


Table 1 Examples of Restriction Enzymes

Enzyme name	Recognition site	End type	Outcome after restriction enzyme digestion
EcoRI	5′-GAATTC-3′ 3′-CTTAAG-5′	sticky	5′-G AATTC-3′ 3′-CTTAA G-5′
XhoI	5′-CTCGAG-3′ 3′-GAGCTC-5′	sticky	5′-G TCGAG-3′ 3′-GAGCT C-5′
HindIII	5′-AAGCTT-3′ 3′-TTCGAA-5′	sticky	5′-A TCGAC-3′ 3′-CAGGT G-5′
SmaI	5′-ACCCGGGT-3′ 3′-TGGGCCCA-5′	blunt	5′-ACCC GGGT-3′ 3′-TGGG CCCA-5′



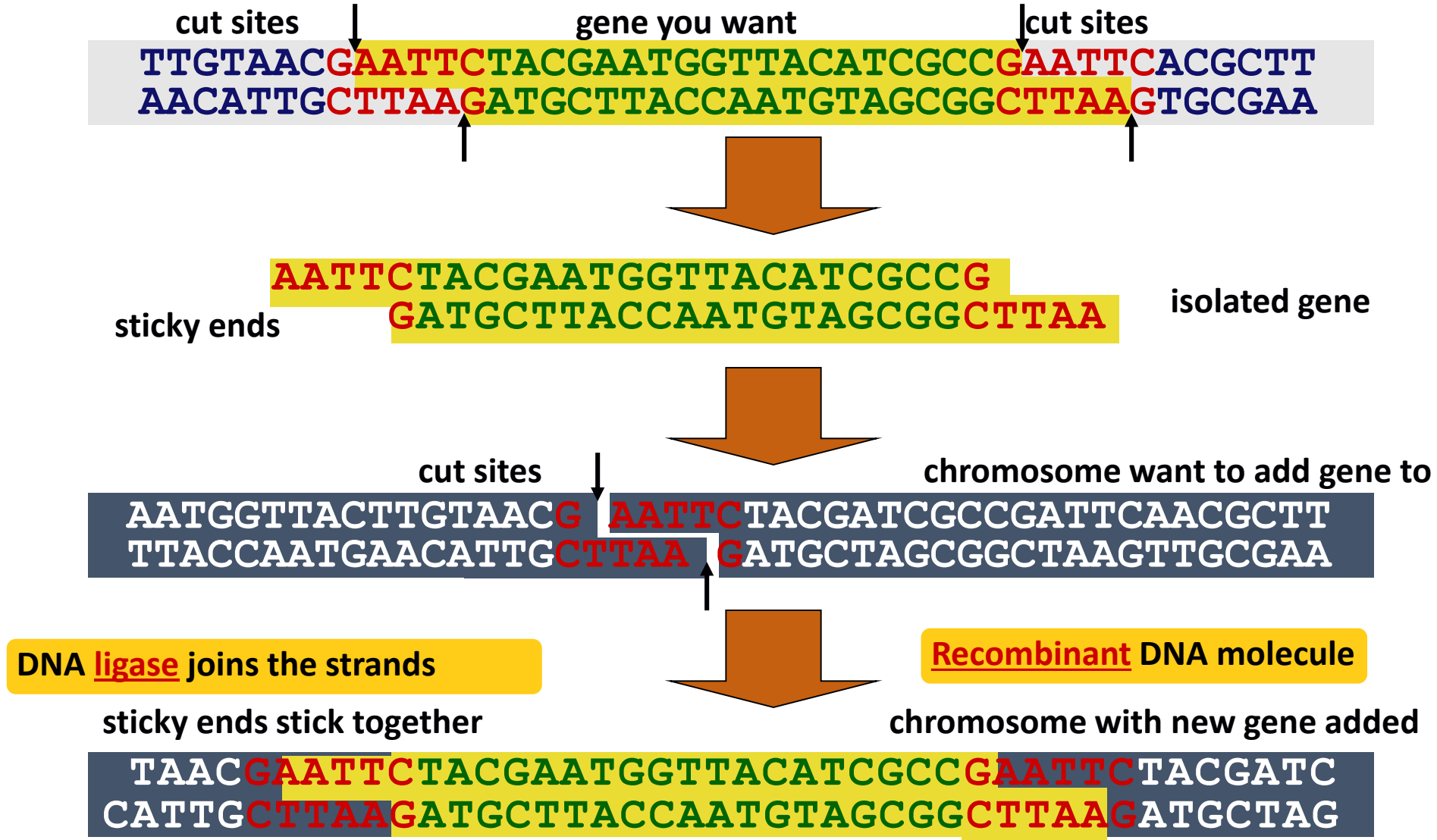

EcoRI
5′
3′
G A A T T C
C T T A A G
3′
5′
Cleavage
EcoRI
Sticky ends
5′
3′
G
C T T A A
A A T T C
G
3′
5′

- The recognition site on the DNA is a palindrome when you look at both strands.
- These cuts allow the DNA molecule to be easily separated, and create “sticky ends” or overhangs.
- Some restriction enzymes cut straight across the strand creating blunt ends.
- Restriction enzymes that create sticky ends are easier to use, because the ends can easily be joined to other pieces of DNA cut with the same restriction enzyme.

DNA Ligase

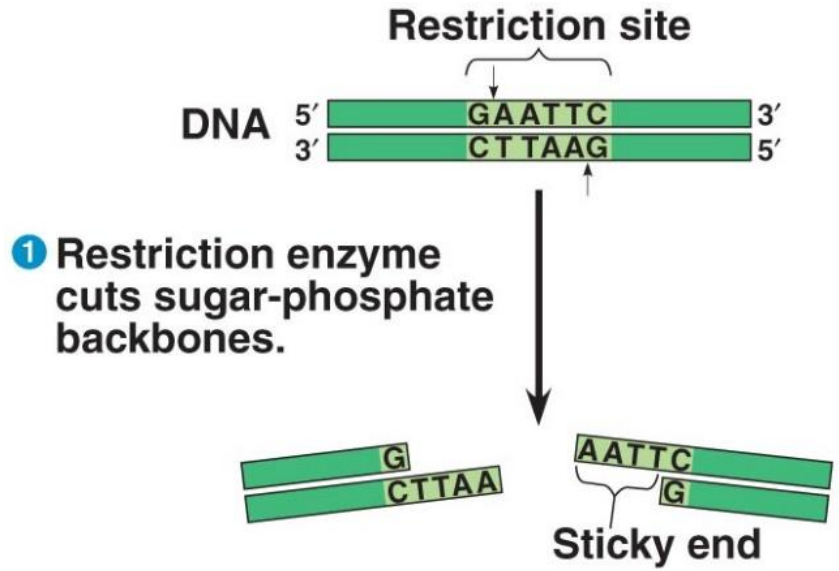
- DNA ligase is the enzyme that is used to join the strands of DNA back together after they have been cut by restriction enzymes.
- DNA ligase rejoins the DNA backbone.

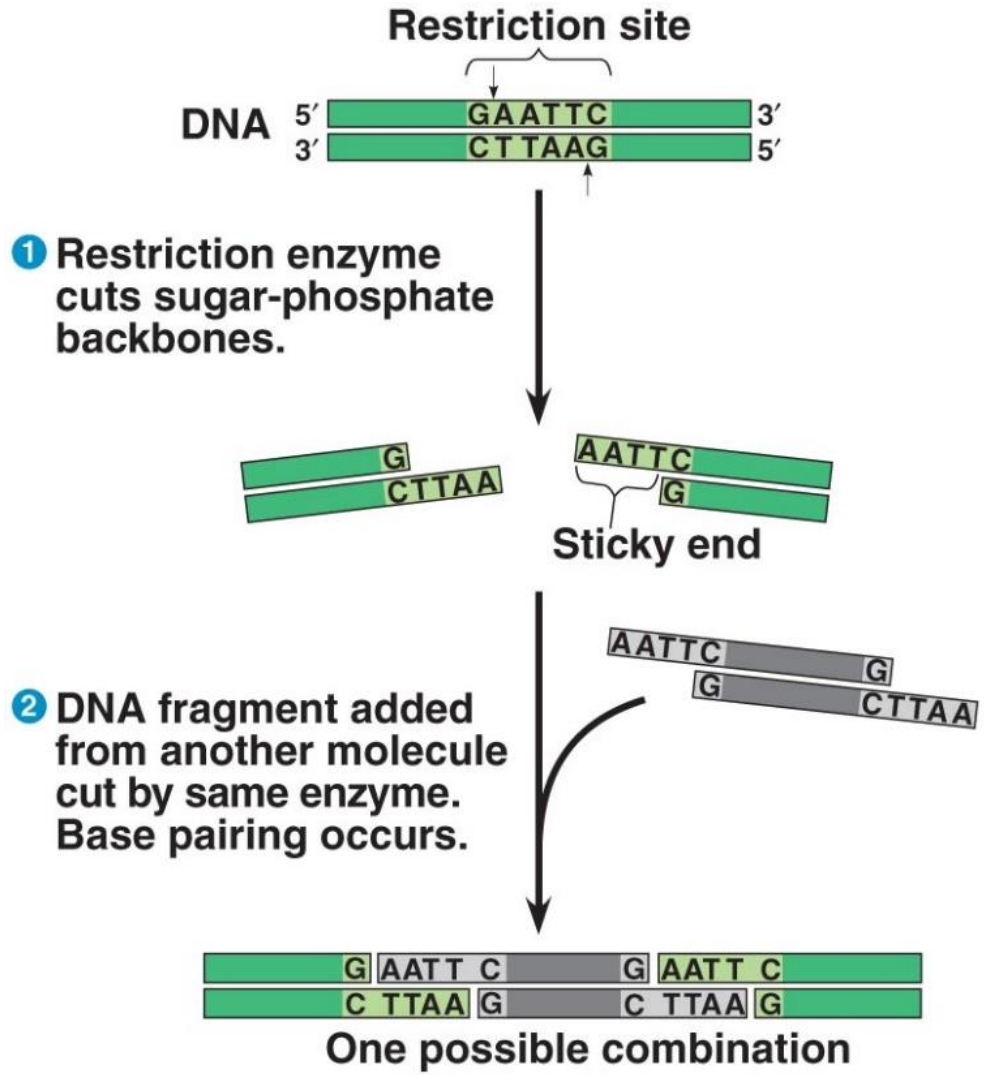
Sticky ends help glue genes together



Plasmids

- Plasmids – (as you will recall) – are small circular pieces of DNA found in bacteria, that replicate independently of the chromosomal DNA.
- Plasmids often contain genes that code for proteins that provide resistance to antibiotics (such as ampicillin)
- A cell that is able to take up foreign DNA is called a competent cell.
- A plasmid that has been designed to be a vehicle for transferring foreign genetic materials is called a vector.
- Plasmids have a copy number – the number of plasmids that will be found in the bacterium.

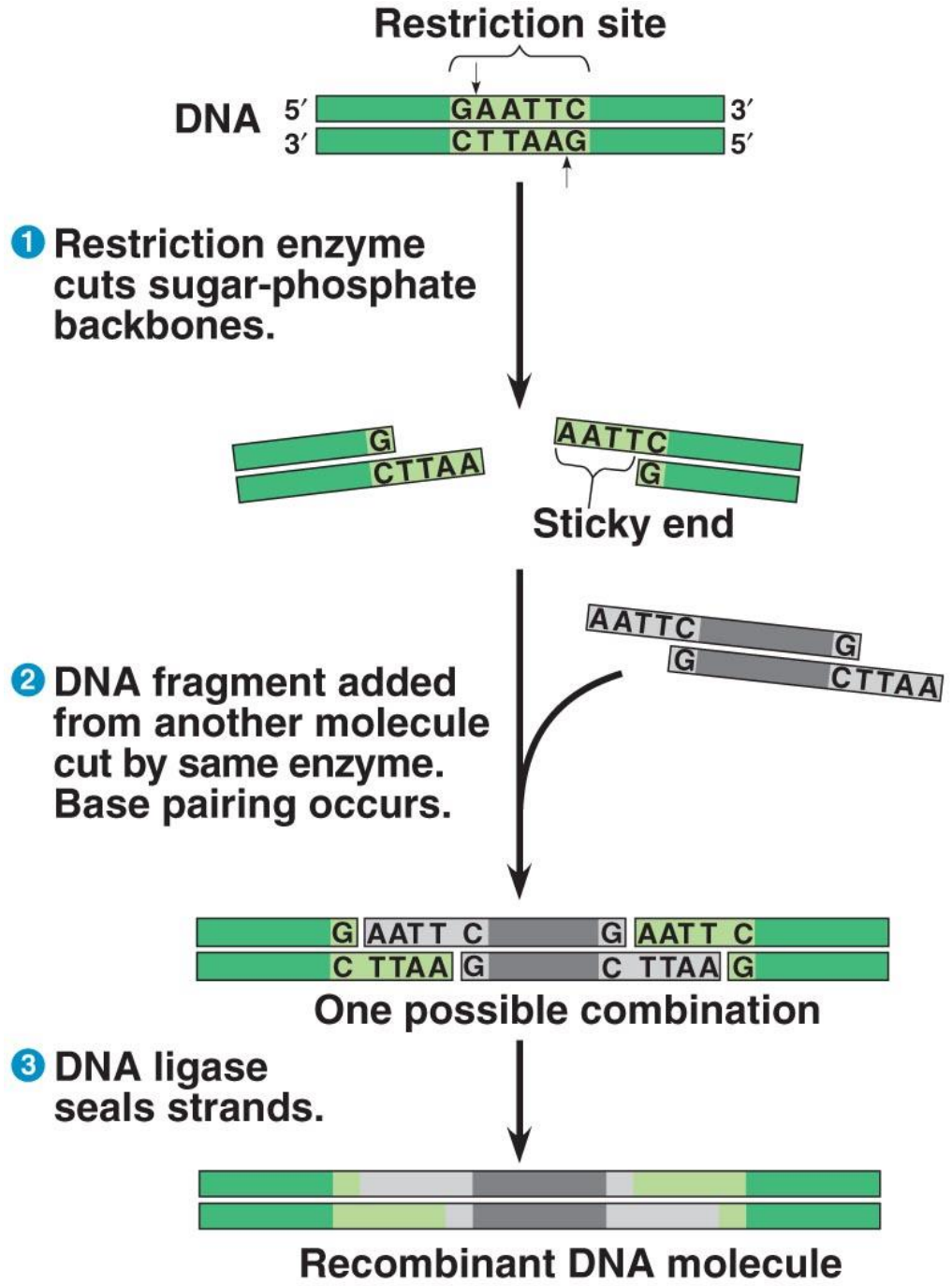


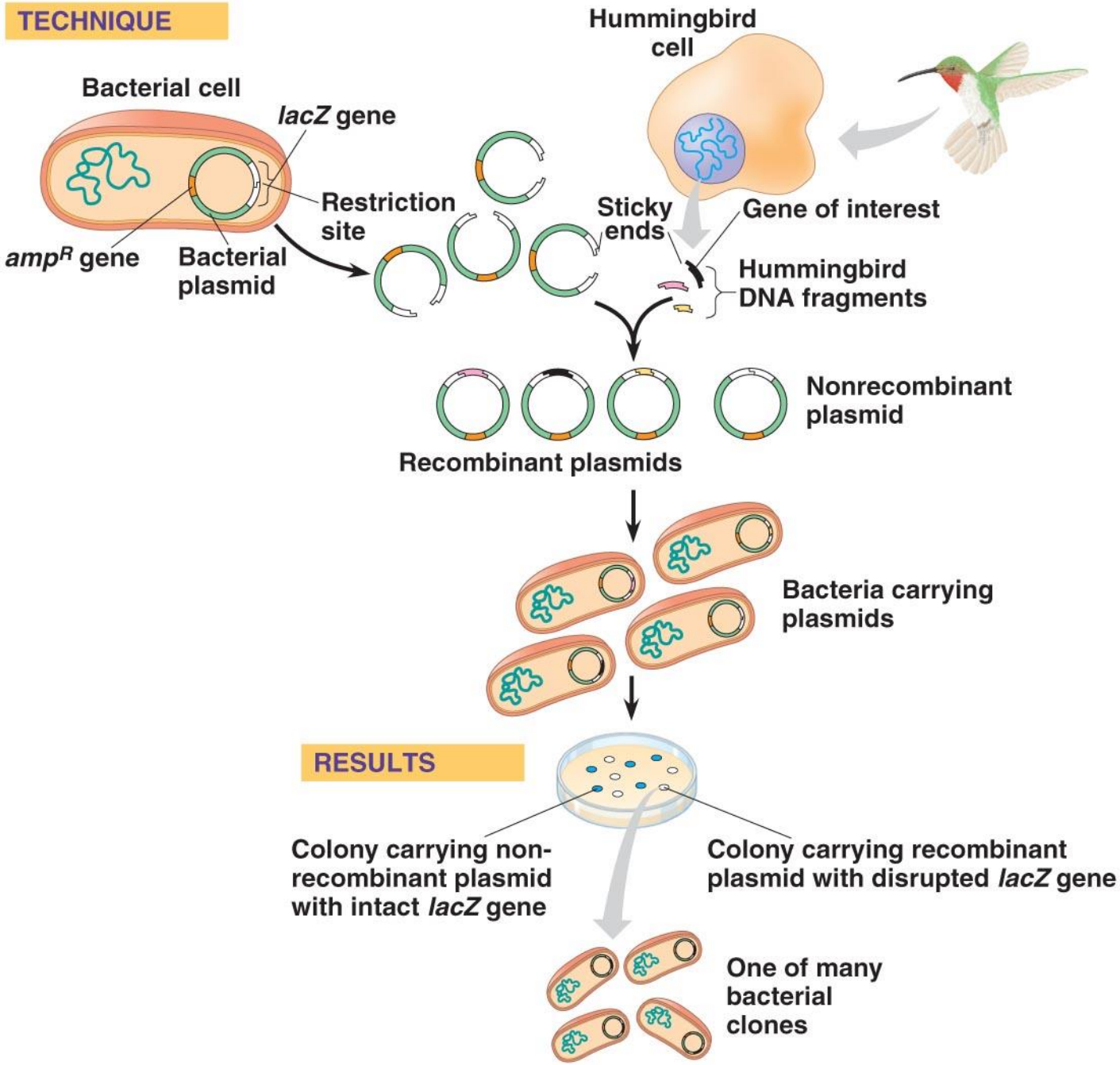


1. Restriction enzyme cuts the backbones.

2. DNA fragment added from another molecule cut by the same enzyme. Base pairing occurs.

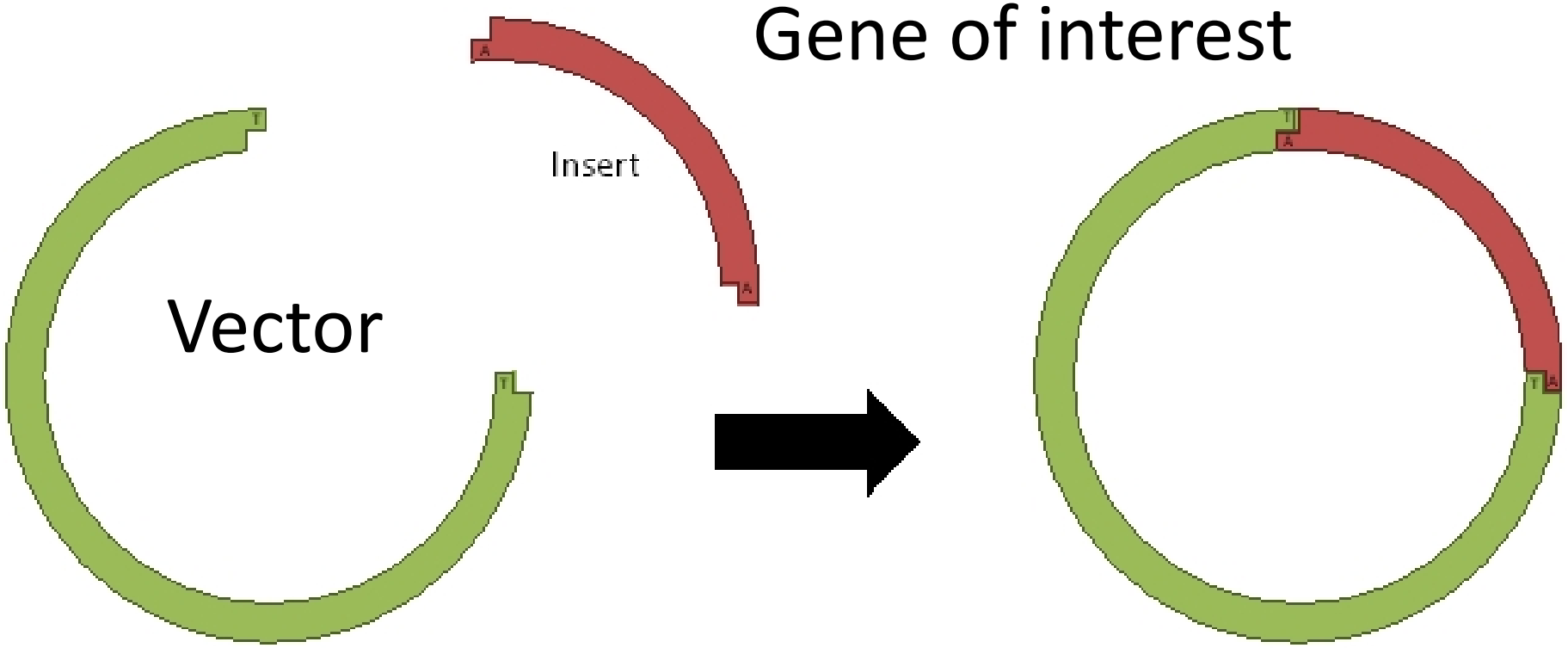
3. DNA ligase seals strands.





- If a fragment containing a target gene is cut with the same restriction enzyme as a plasmid, the plasmid and foreign fragment can be placed in the same solution and will stick together because of complimentary sticky ends.
- The resulting new circular piece of DNA that carries the foreign gene is called recombinant DNA.
- The plasmid can be introduced to a host cell, where it replicates and the gene can be synthesized to a protein.

Gene of interest



Recombinant plasmid

Transformation

- Using specific conditions, plasmids can enter bacterial cells. This process is called transformation.
- One way to make bacteria take up the plasmid is to place the bacteria in a solution of calcium chloride and recombinant plasmids in an ice water bath.
- As the bacteria cool, the calcium ions stabilize the phospholipid bilayer.
- The solution is quickly heated and re-cooled, and the sudden change disrupts the membrane allowing the plasmid to enter.

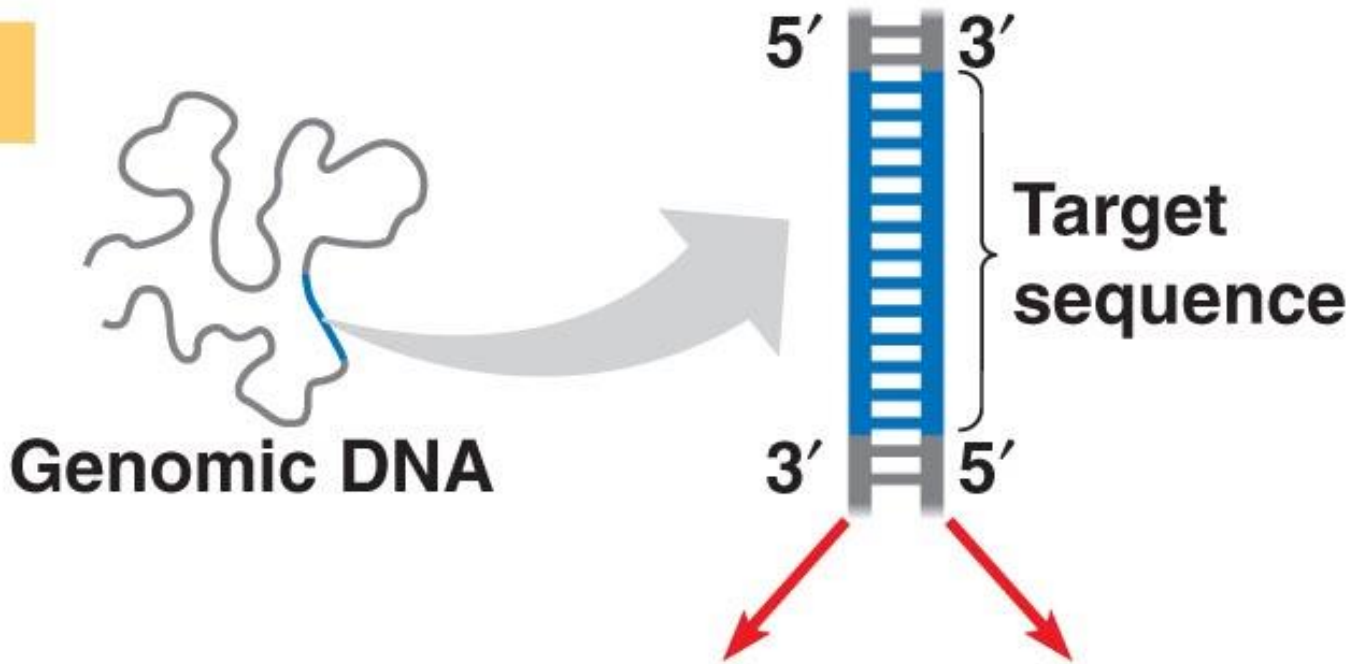
PCR Polymerase Chain Reaction

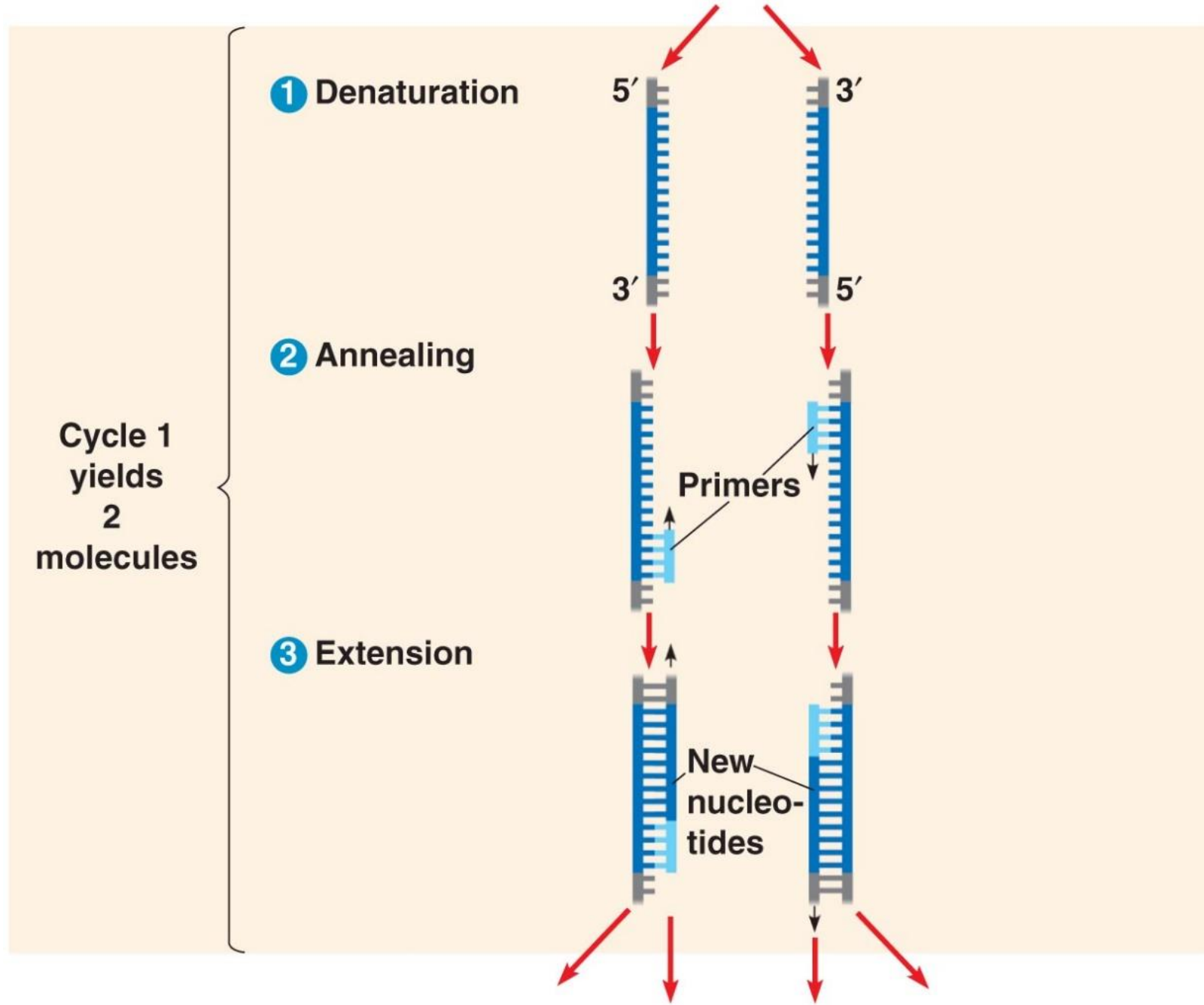
- This a powerful process that can be used to amplify the number of copies of DNA from one biological sample in a very short time.
- PCR replicates a specific section of a chromosome.
- In PCR a small test tube is filled with the target DNA, DNA primers that match the target, and free nucleotides.
- The first step is to denature or separate a double-stranded DNA into single strands by heating it to 95°C for a short period.

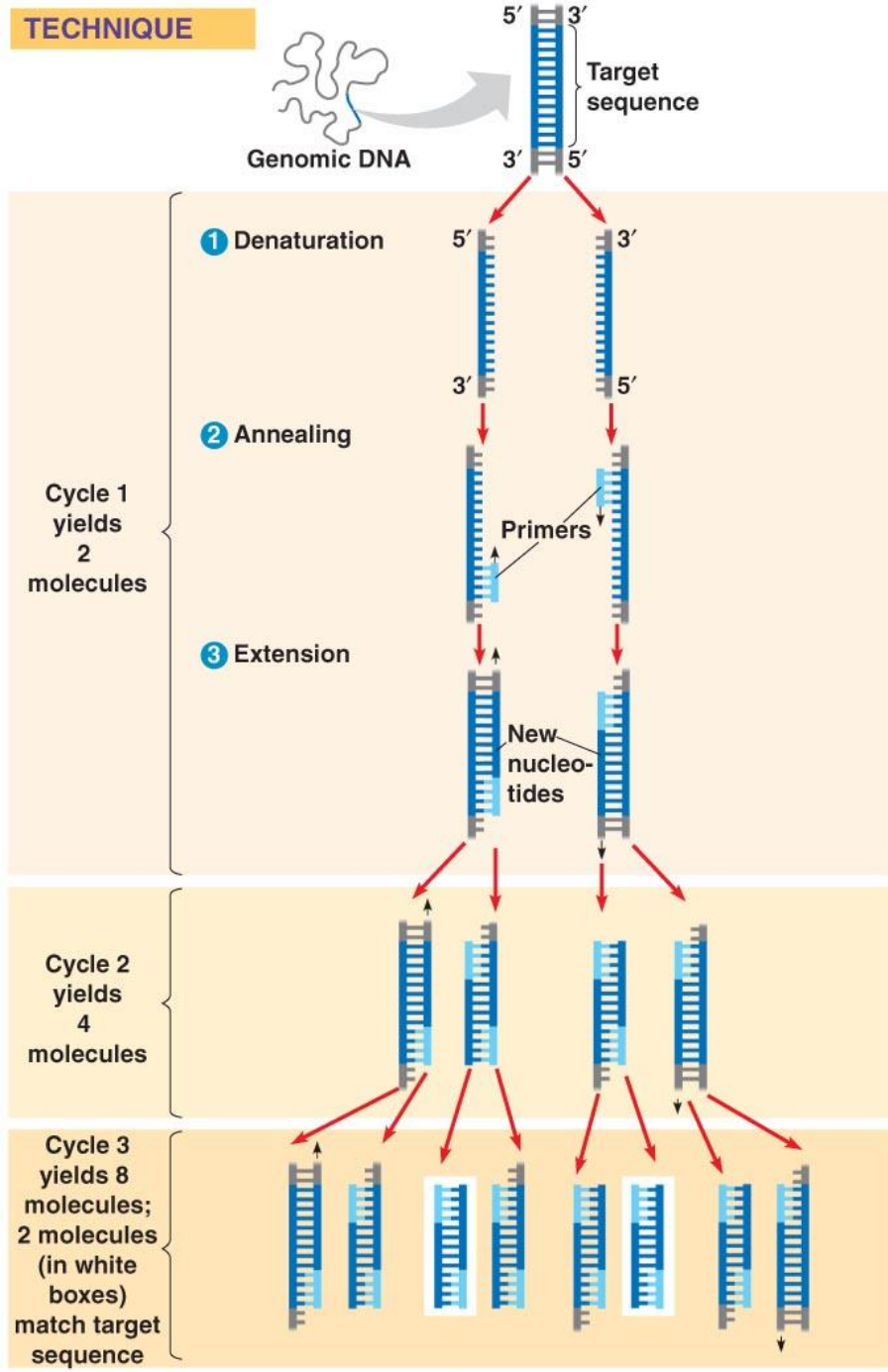
- The second step is to reduce the temperature back down to 50 - 65°C which allows the DNA primers to bind to the single stranded DNA.
- There are two primers, each complimentary to one end of the targeted DNA fragment.
- The next step is to elongate the DNA, making new strands, this occurs at 72°C using a specific enzyme called Taq polymerase.

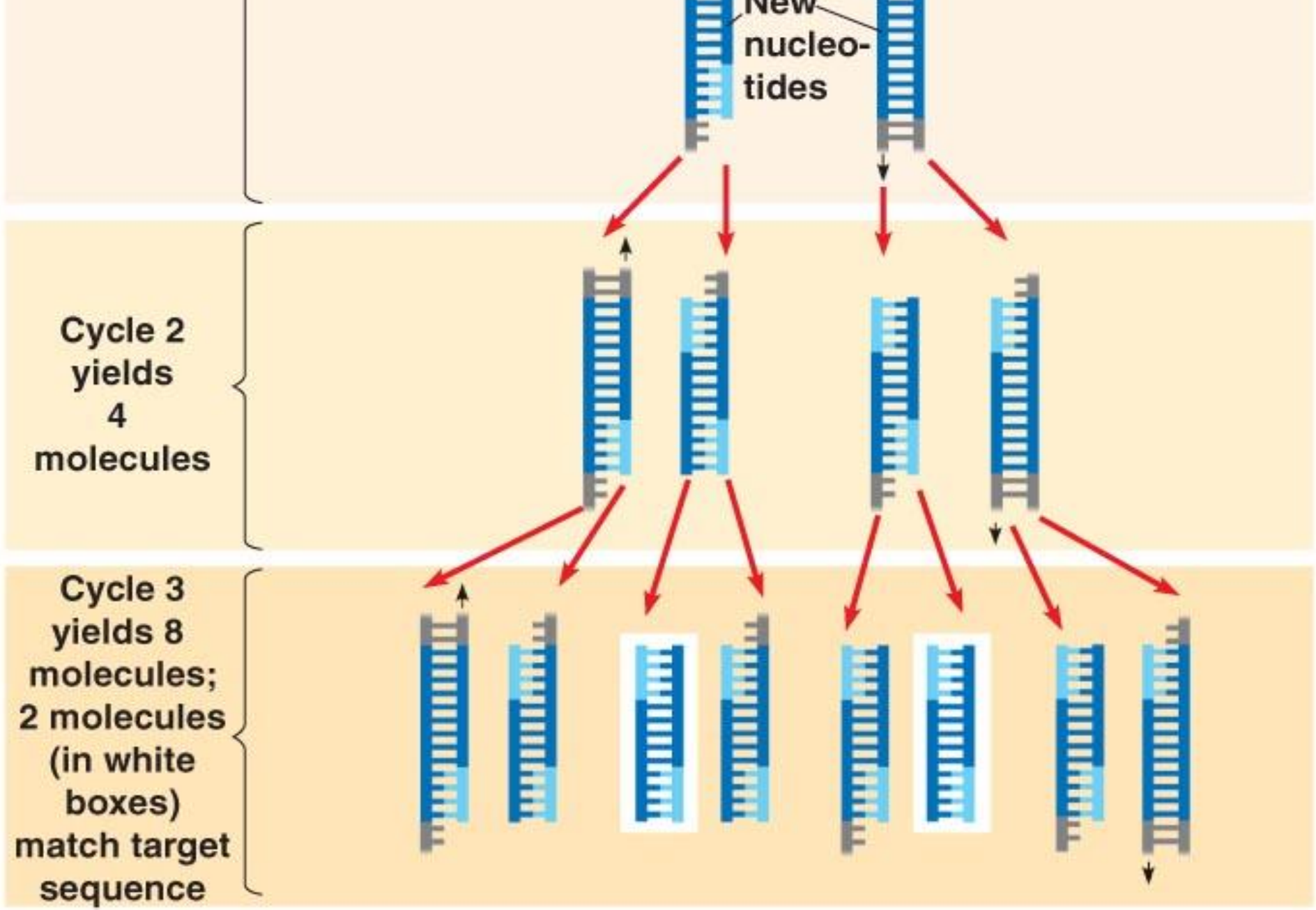
- Taq polymerase comes from a bacterium that is found in hot springs, so the enzyme works in high temperatures.
- After the two new double stranded sequences have been created, the cycle is repeated to separate the strands again, and synthesize four new strands.
- The cycles are repeated over and over, increasing the amount of the target DNA exponentially.

TECHNIQUE









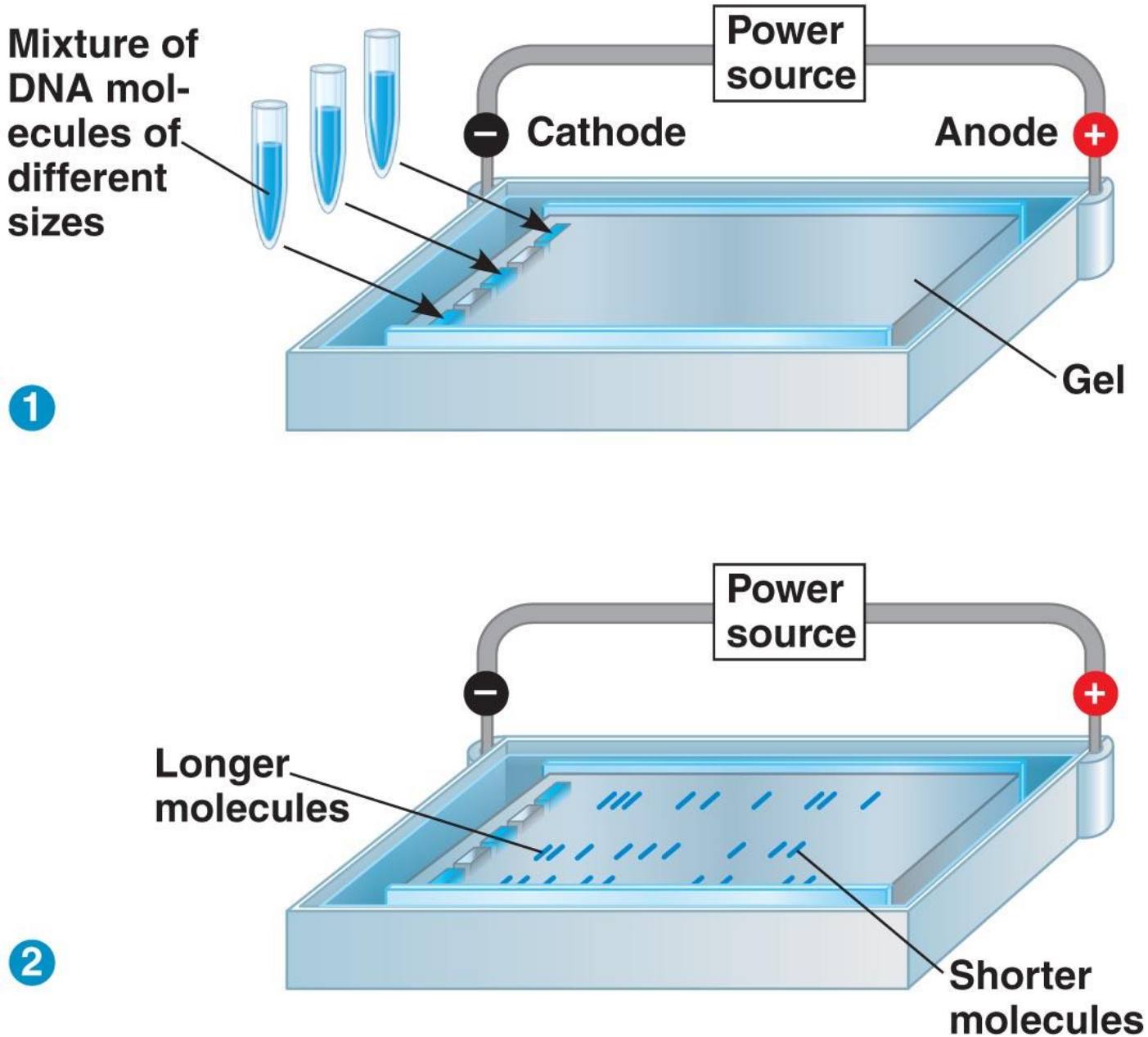
PCR

- PCR is used in cases of forensics to identify a suspect, by making an amplified amount of DNA found at a crime scene.

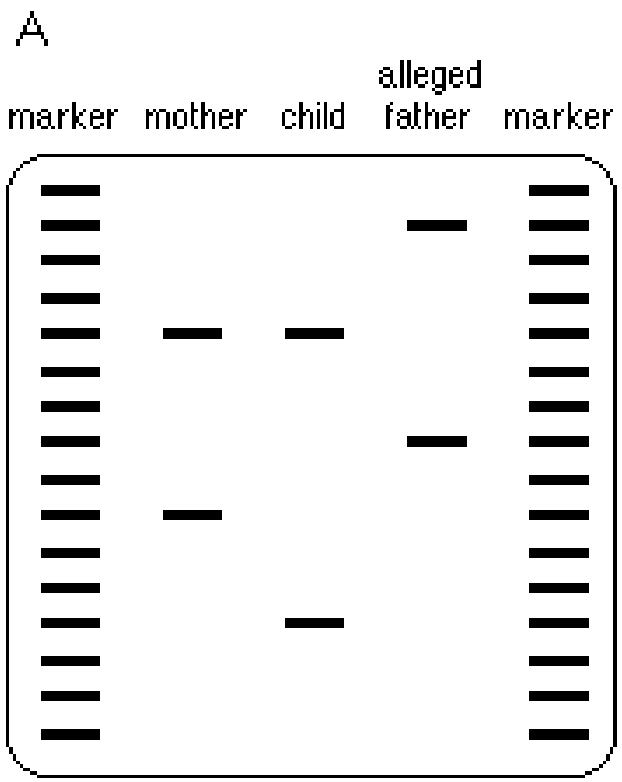
Gel Electrophoresis

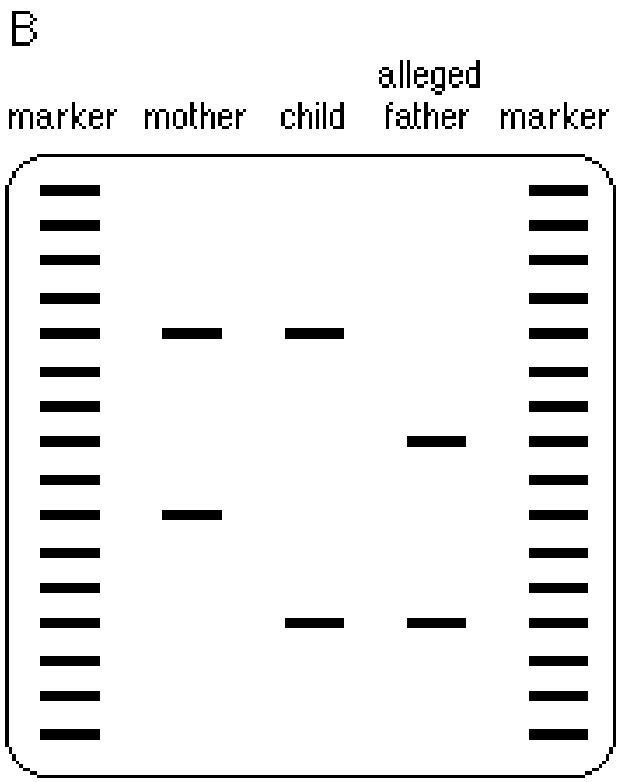
- Gel electrophoresis is a process that uses the physical and chemical properties of DNA to separate fragments.
- The DNA fragments are separated by size as they move through a gel called agarose.
- Negatively charged DNA fragments travel through the gel as electricity is passed through the gel.
- The fragments move away from the negative terminal toward the positive terminal, with the smallest fragments moving the furthest.

TECHNIQUE



- As well as the sample DNA to be run on the gel, one well contains sample markers. Pieces of DNA of known length.
- These markers act as a ruler allowing the size of the fragments to be identified.
- After the DNA is run on the gel it is dyed with a special dye that attaches to DNA – often ethidium bromide.



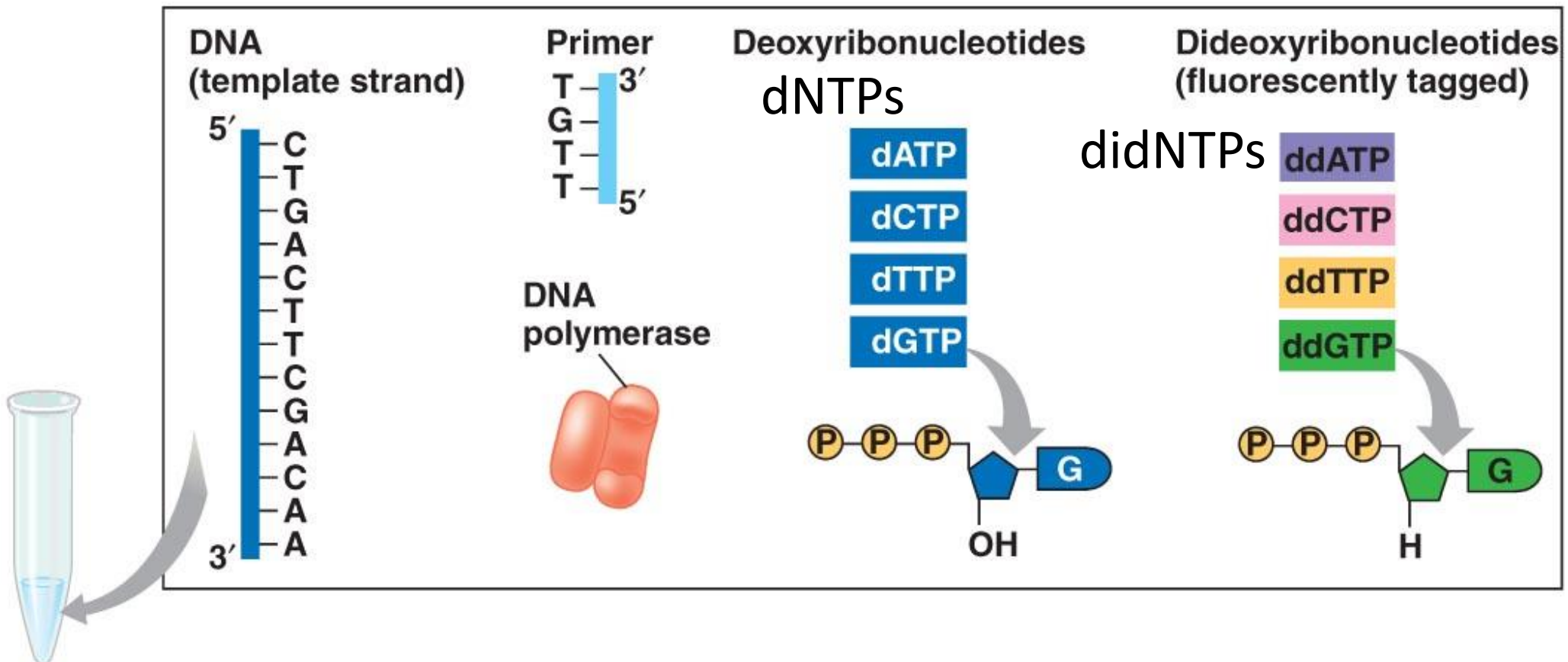


- DNA sequences can be compared to identify paternity, or a suspect at a crime scene.

Dideoxy DNA Sequencing

- DNA sequencing is a process that allows researchers to identify the exact nucleotide sequence for a fragment of DNA.
- Dideoxy sequencing relies on special terminator nucleotides, when they are added to a chain, they prevent binding of the next nucleotide.

TECHNIQUE



- For this process four test tubes are set up, each with many copies of the normal nucleotides (A, T, C, G), multiple copies of the DNA to be sequenced, DNA polymerase, and a DNA primer.
- Into each of the four tubes, only ONE of the labelled dideoxy nucleotides is added.
- The DNA polymerase is allowed to synthesize multiple strands of DNA in each of the four tubes.

- Then the tubes are separated by gel electrophoresis, and the sequence of DNA can be read.