204 B

Basics of Biotechnology

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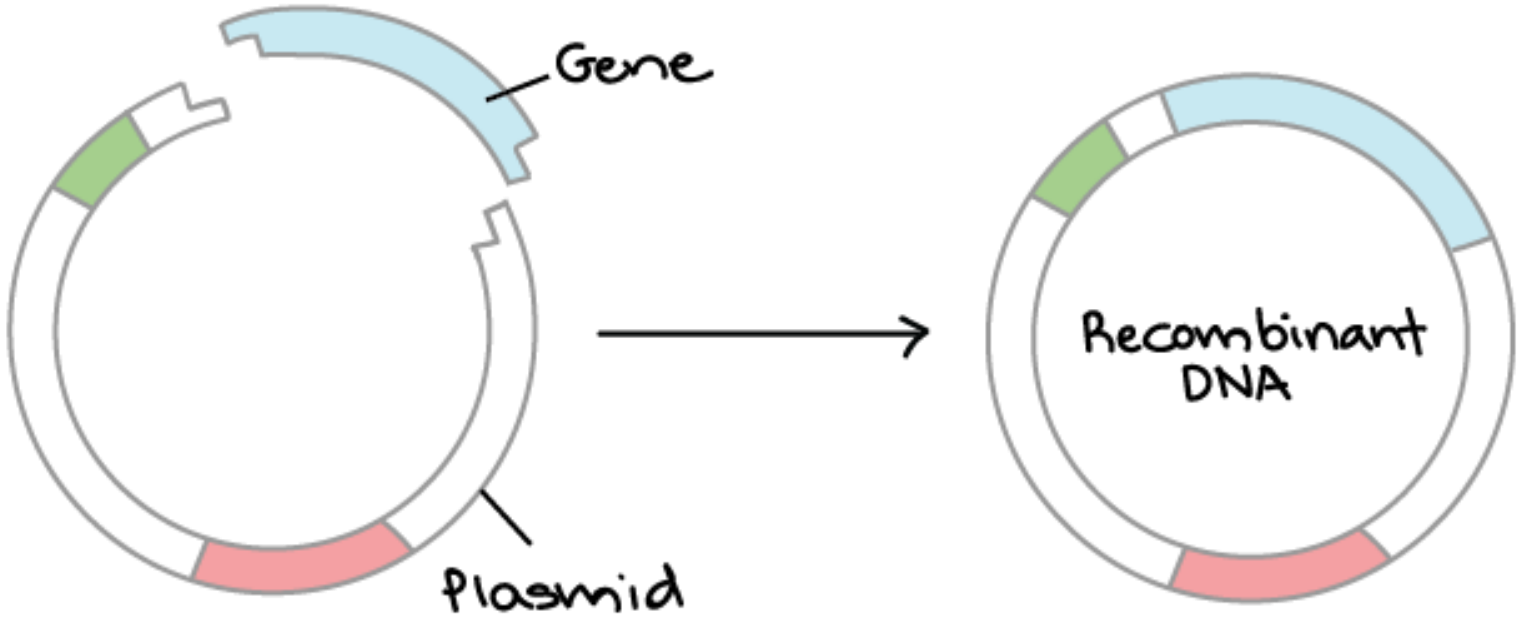
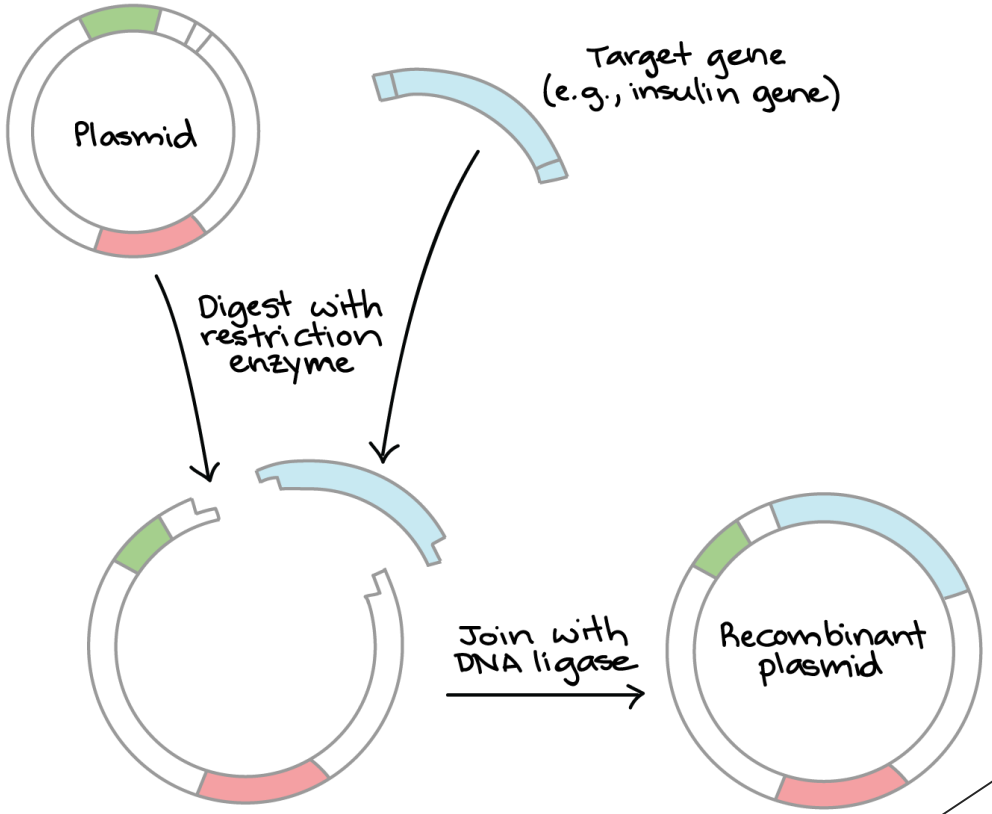
DNA cloning

- DNA cloning is a molecular biology technique that makes many identical copies of a piece of DNA, such as a gene.
- In a typical cloning experiment, a target gene is inserted into a circular piece of DNA called a plasmid.
- The plasmid is introduced into bacteria via a process called transformation, and bacteria carrying the plasmid are selected using antibiotics.
- Bacteria with the correct plasmid are used to make more plasmid DNA or, in some cases, induced to express the gene and make protein.

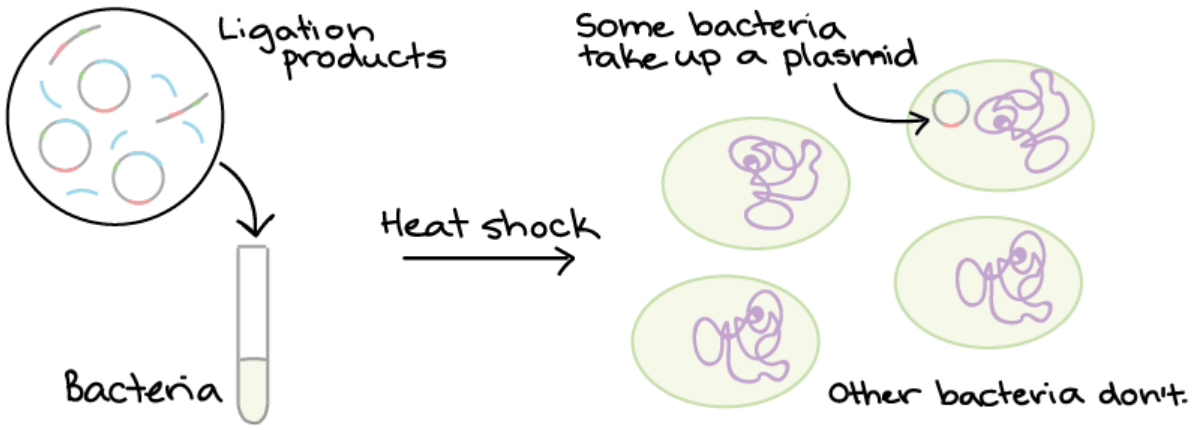

Gene
Plasmid
Recombinant DNA

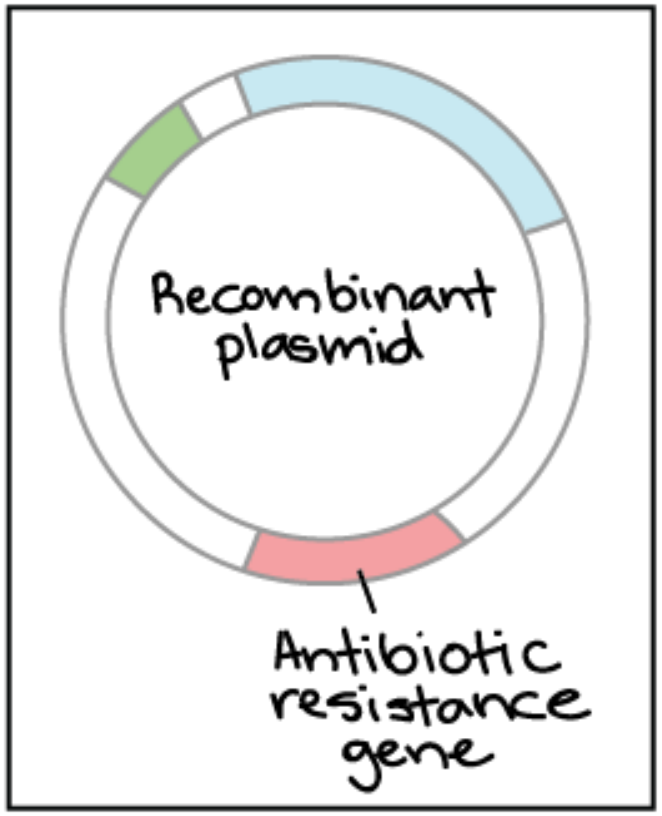
Steps of DNA cloning

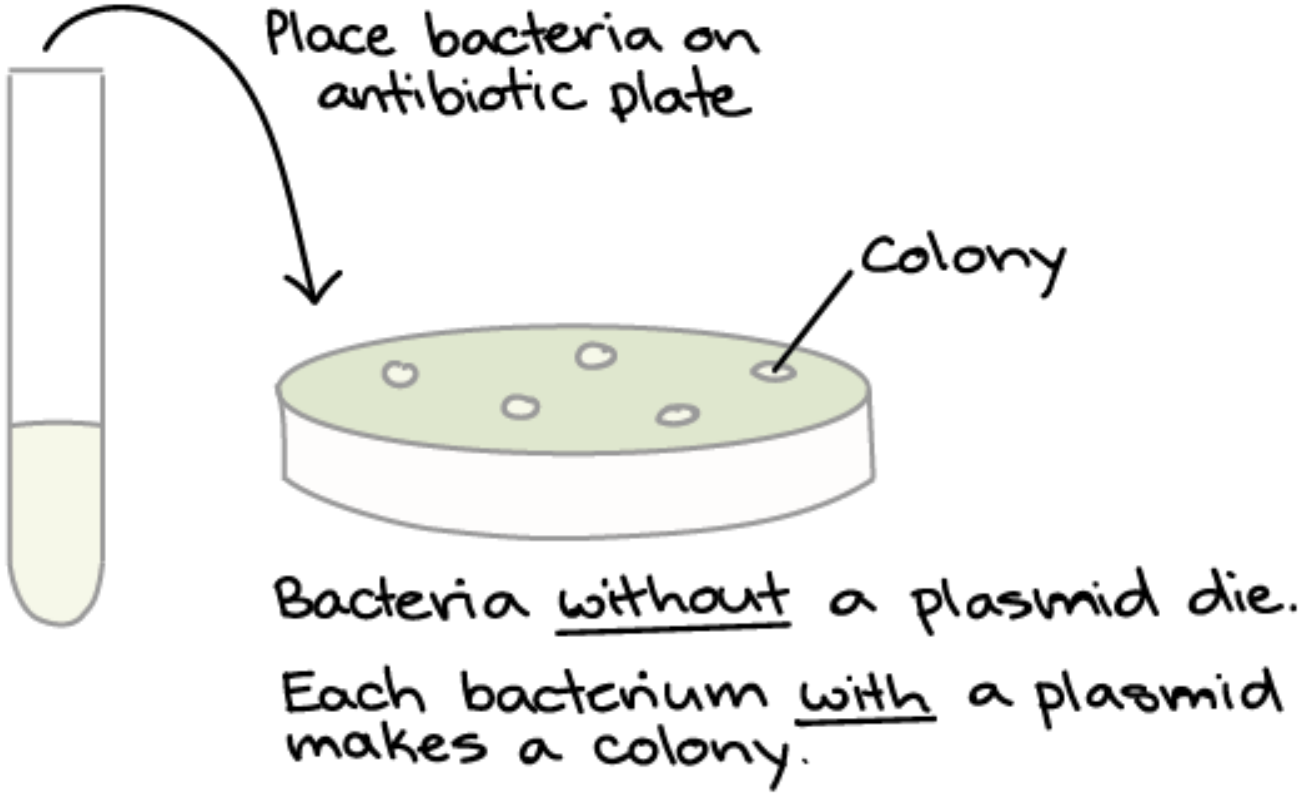
- **1. Cutting and pasting DNA**



2. Bacterial transformation and selection



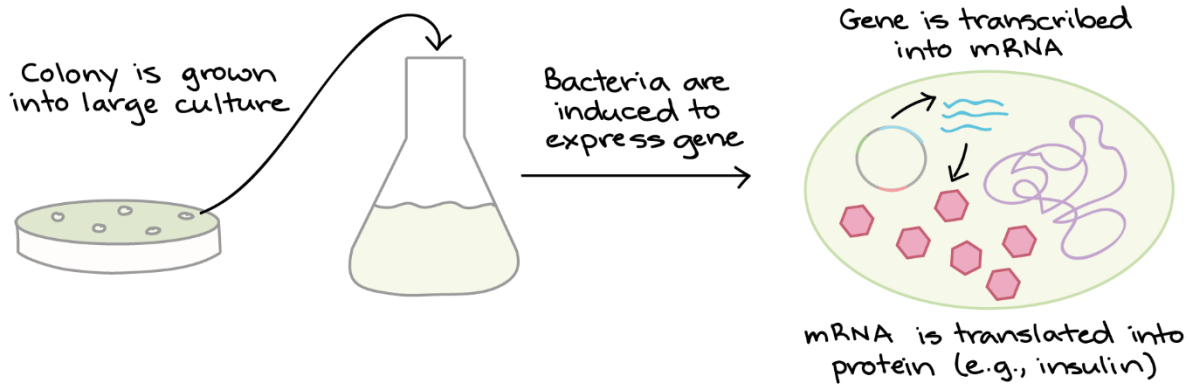




Bacteria <u>without</u> a plasmid die.

Each bacterium <u>with</u> a plasmid makes a colony.

3. Protein production



Uses of DNA cloning

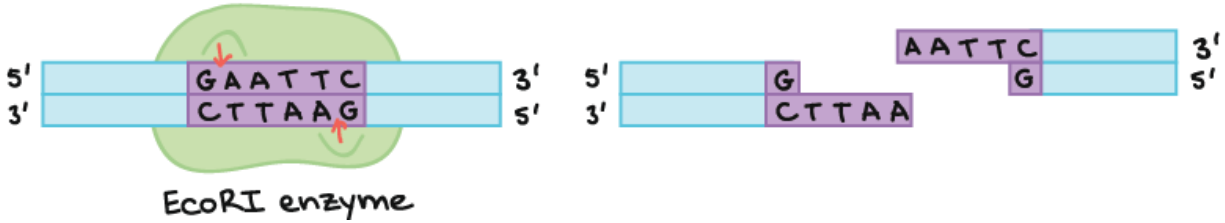
- Biopharmaceuticals
- Gene therapy
- Gene analysis

Restriction enzymes & DNA ligase

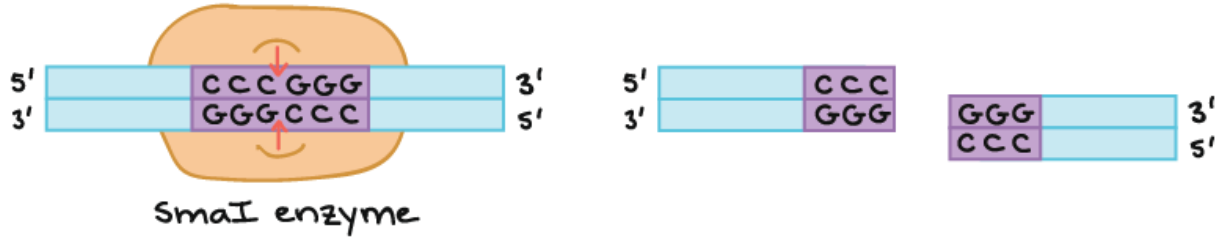
- Restriction enzymes are DNA-cutting enzymes. Each enzyme recognizes one or a few target sequences and cuts DNA at or near those sequences.
- Many restriction enzymes make staggered cuts, producing ends with single-stranded DNA overhangs. However, some produce blunt ends.
- DNA ligase is a DNA-joining enzyme. If two pieces of DNA have matching ends, ligase can link them to form a single, unbroken molecule of DNA.
- In DNA cloning, restriction enzymes and DNA ligase are used to insert genes and other pieces of DNA into plasmids.

- As an example of how a restriction enzyme recognizes and cuts at a DNA sequence, let's consider EcoRI, a common restriction enzyme used in labs. EcoRI cuts at the following site:

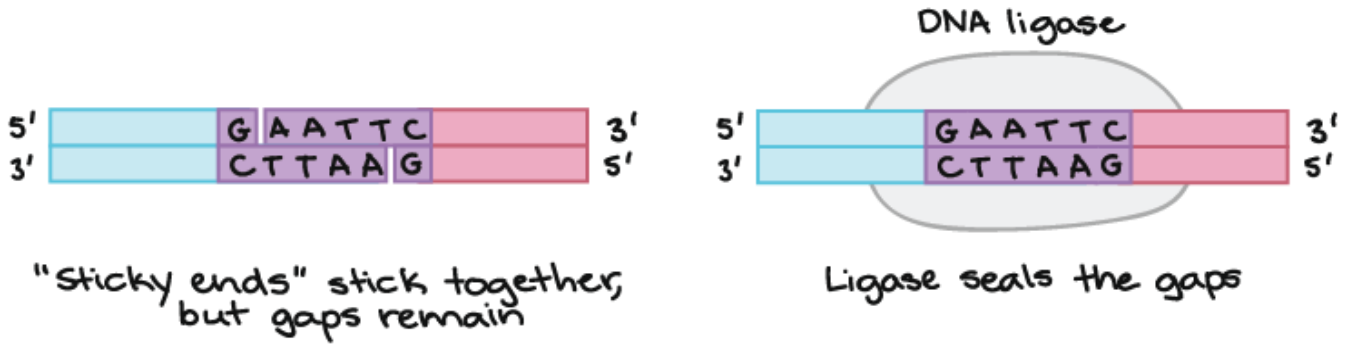
- When EcoRI recognizes and cuts this site, it always does so in a very specific pattern that produces ends with single-stranded DNA “overhangs”:



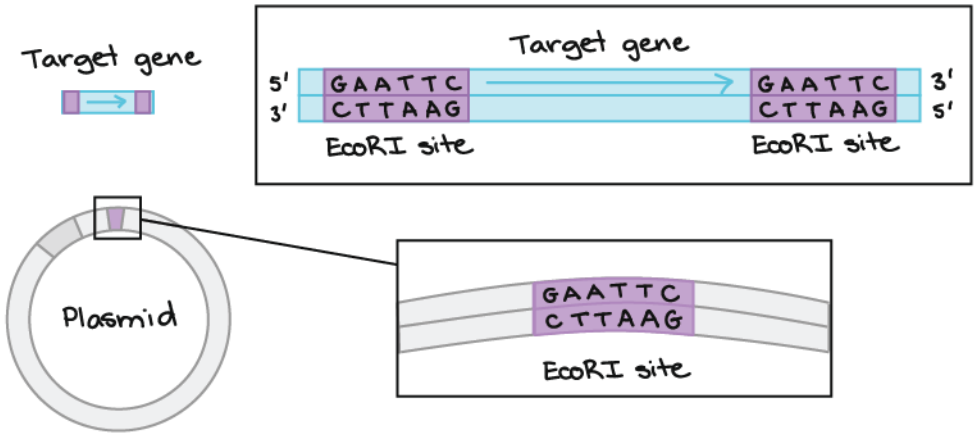
- Not all restriction enzymes produce sticky ends. Some are “blunt cutters,” which cut straight down the middle of a target sequence and leave no overhang. The restriction enzyme SmaI is an example of a blunt cutter:

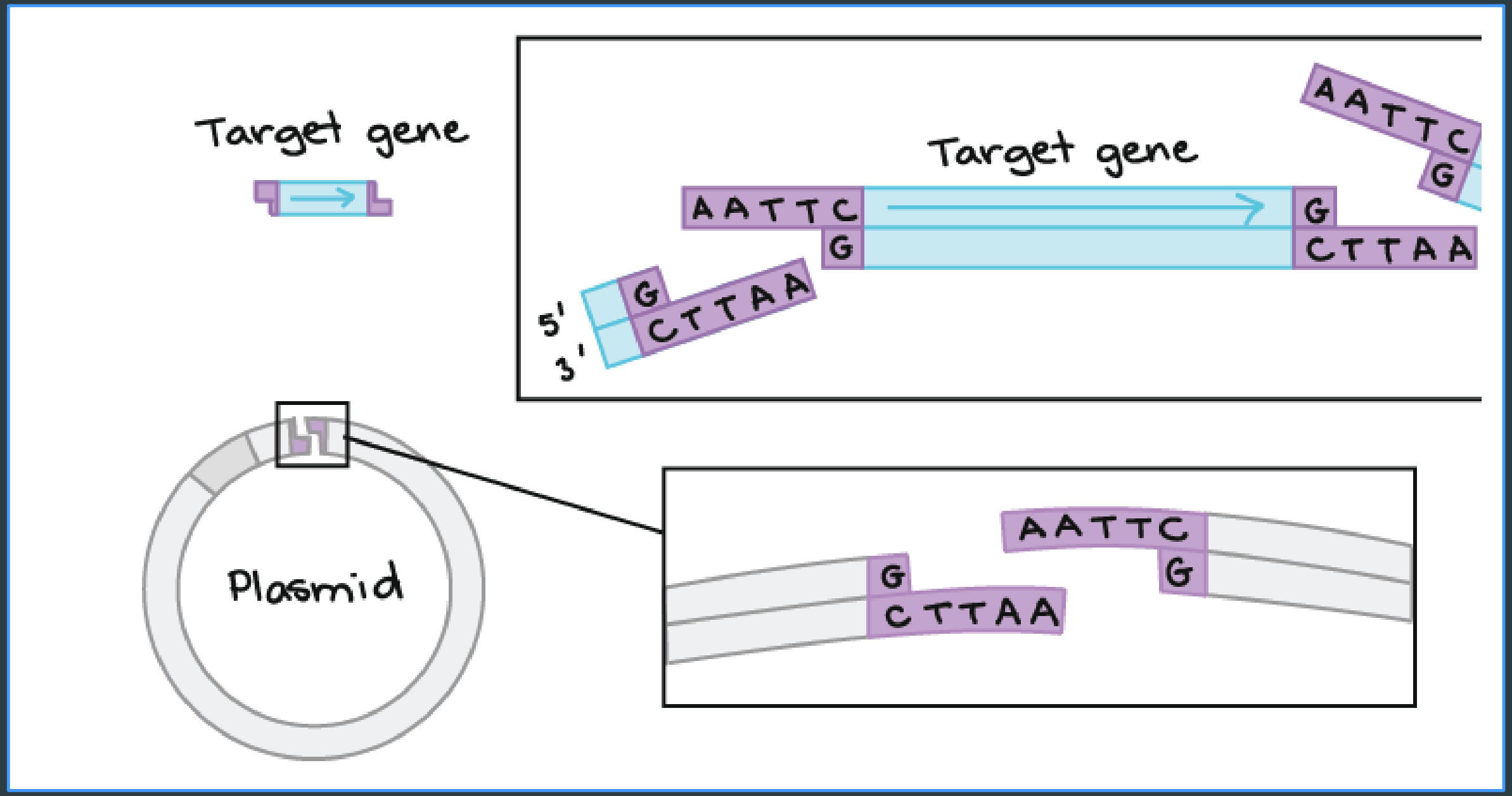


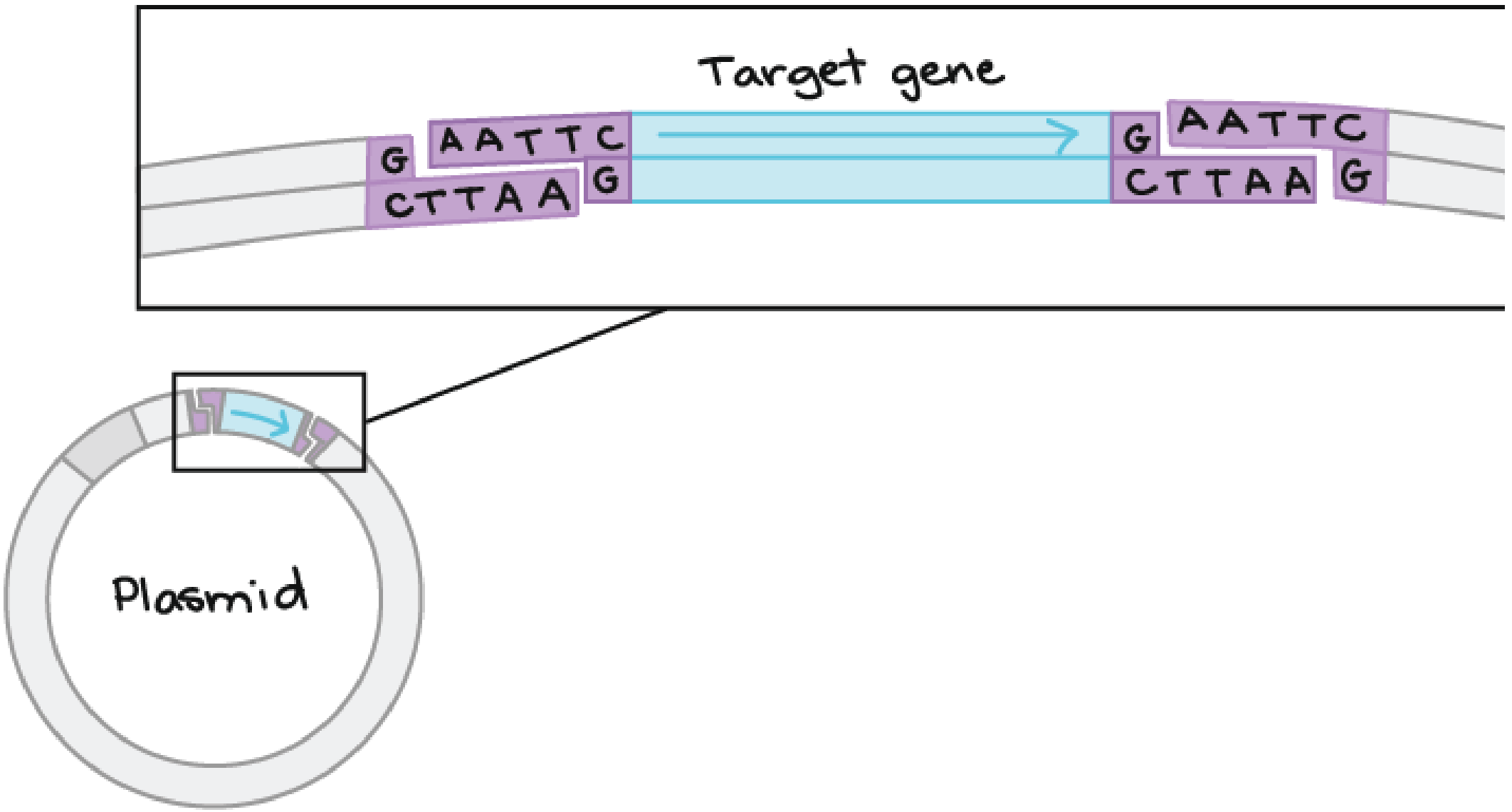
DNA ligase

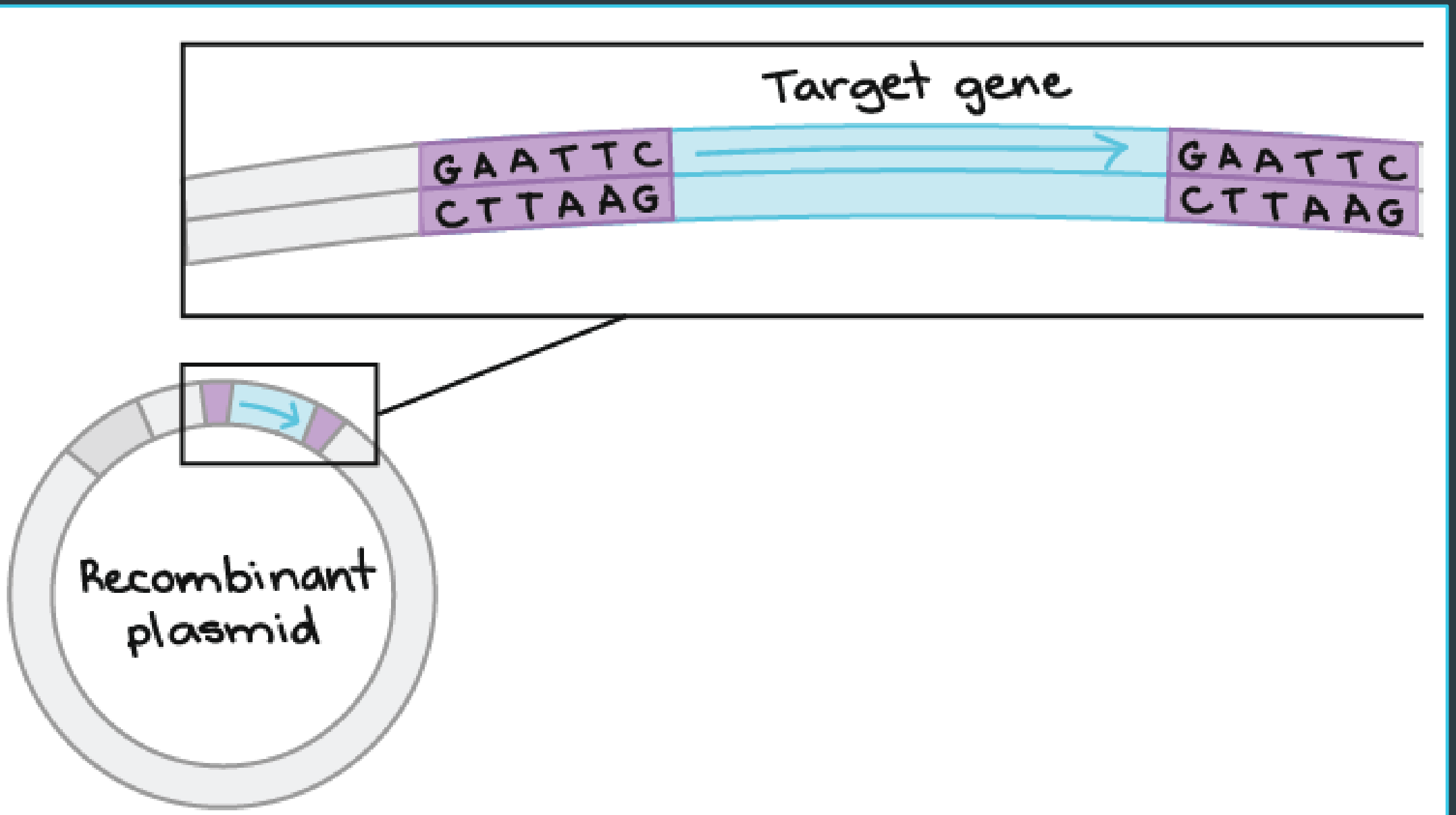

5′
3′
G AATTC
CTTAA G
3′
5′
"Sticky ends" stick together, but gaps remain
DNA ligase
5′
3′
GAATTC
CTTAAG
3′
5′
Ligase seals the gaps

Example: Building a recombinant plasmid




Target gene
Target gene
AATTC
G
AATTC
G
CTTAA
5'
3'
G
CTTAA
Plasmid
AATTC
G
G
CTTAA


Target gene
G
AATTC
CTTAA
G
G
AATTC
CTTAA
G
Plasmid


Target gene
GAATTC
CTTAAG
GAATTC
CTTAAG
Recombinant plasmid

Restriction digests and ligations involve many molecules of DNA