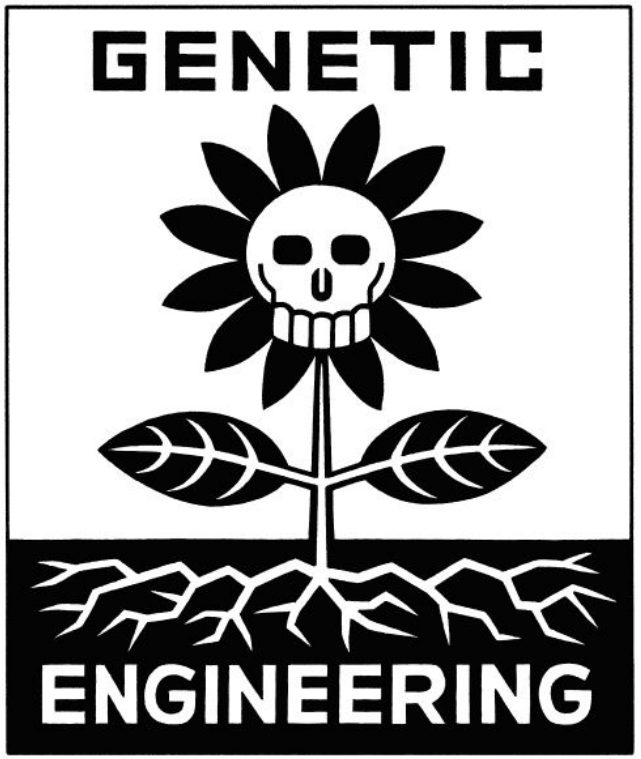
G11 Genetic Engineering



The essence of genetic engineering

Learning objective

- explain the essence of genetic engineering

Success criteria

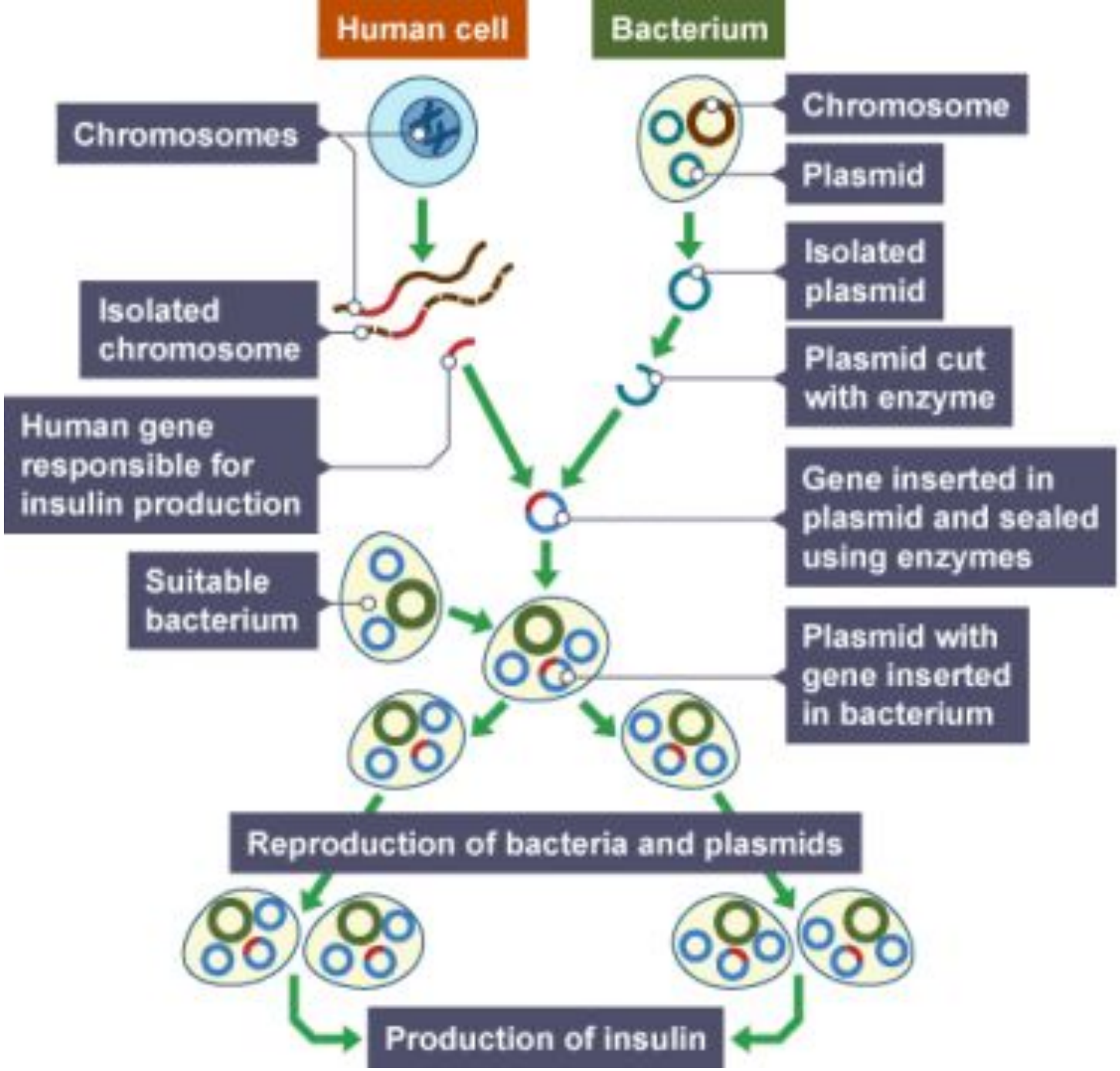
1.Gives the concept of genetic engineering.
2. Describes the stages of genetic engineering.
3. Explains the importance of genetic engineering

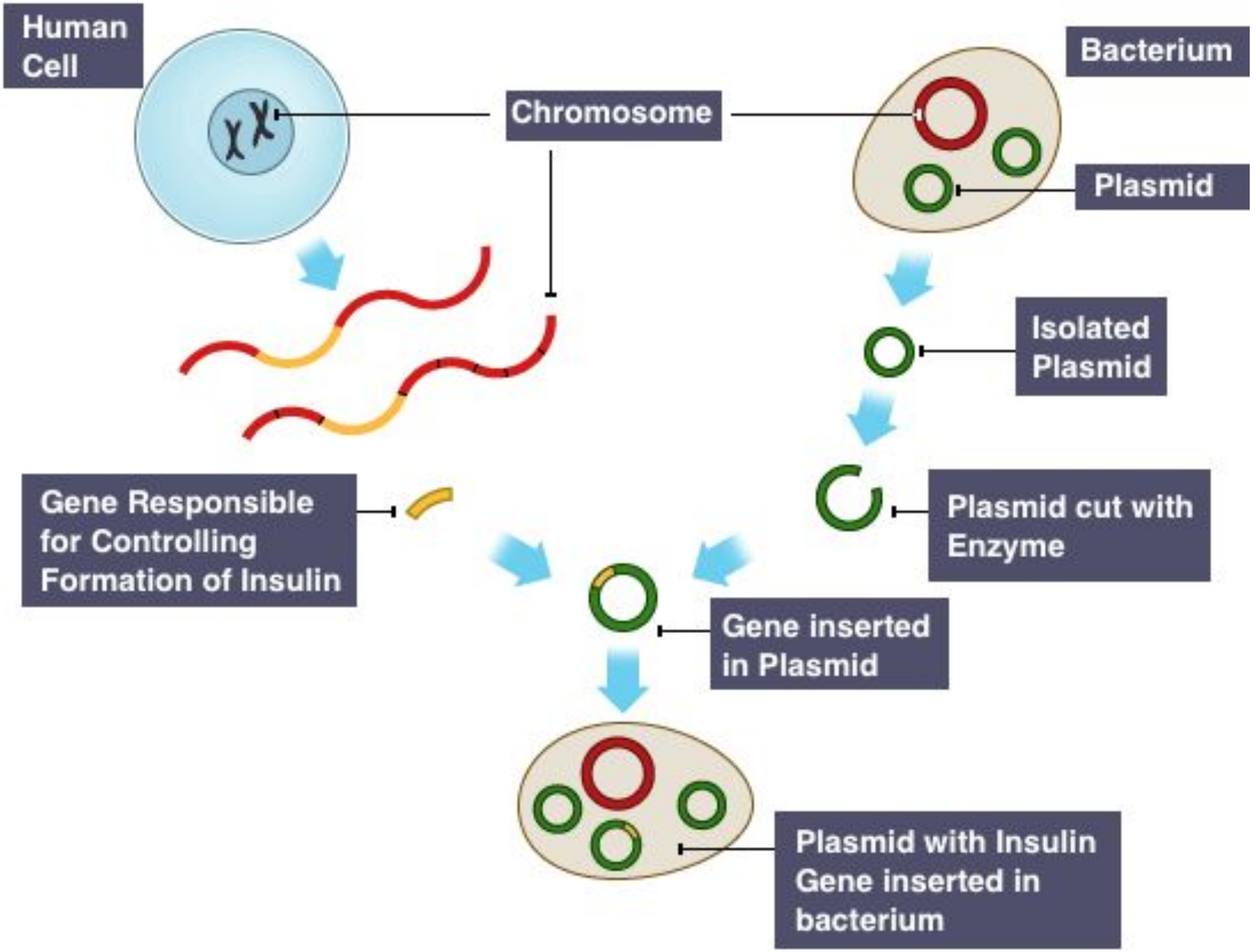
Terminology

- Restriction enzyme, DNA ligase, DNA polymerase, reverse transcriptase
- Genetic engineering
- Recombinant DNA
- Insulin
- Vector, plasmid
- Base pairing, sticky ends, DNA stand,
- Host cell, transformed, mRNA, complementary DNA – cDNA

Production of GMOs is a multistage process which can be summarized as follows:

1. **identification** of the gene interest;
2. **isolation** of the gene of interest;
3. **amplifying** the gene to produce many copies;
4. associating the gene with an appropriate promoter and poly A sequence and insertion into plasmids;
5. multiplying the plasmid in bacteria and recovering the cloned **construct** for injection;
6. **transference** of the construct into the recipient tissue, usually fertilized eggs;
7. **integration** of gene into recipient genome;
8. **expression** of gene in recipient genome; and
9. inheritance of gene through further generations.


Human cell
Bacterium
Chromosomes
Chromosome
Plasmid
Isolated chromosome
Isolated plasmid
Plasmid cut with enzyme
Human gene responsible for insulin production
Gene inserted in plasmid and sealed using enzymes
Suitable bacterium
Plasmid with gene inserted in bacterium
Reproduction of bacteria and plasmids
Production of insulin

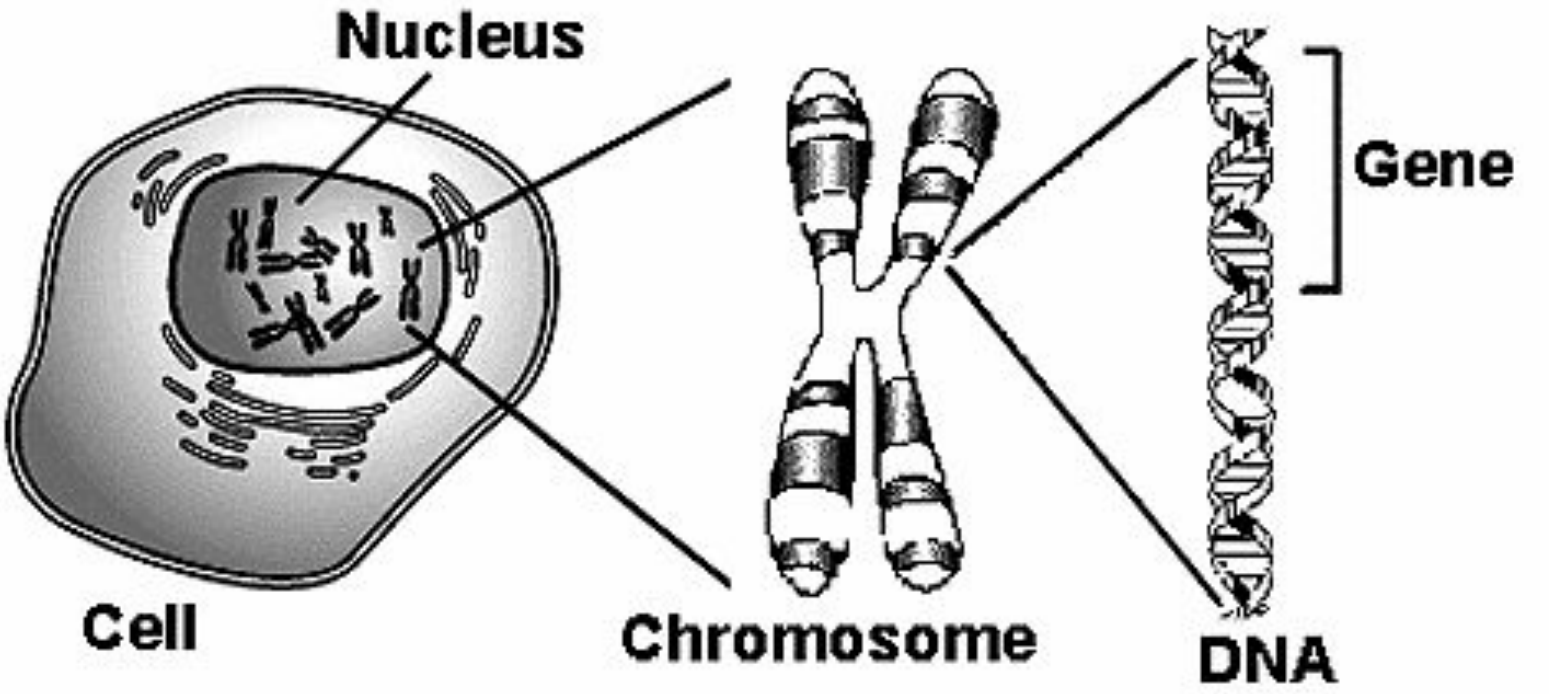

Human Cell
Chromosome
Bacterium
Plasmid
Isolated Plasmid
Plasmid cut with Enzyme
Gene Responsible for Controlling Formation of Insulin
Gene inserted in Plasmid
Plasmid with Insulin Gene inserted in bacterium

You can extract and produce human insulin in bacteria:

1. Get a **human chromosome** containing the insulin gene
2. Use a **restriction enzyme** to cut the insulin gene out
3. Use the same **restriction enzyme** to cut the **plasmid** out from the **bacterium**
4. Mix the **plasmid** and **DNA fragment** with the enzyme **DNA ligase** to produce **recombinant DNA**
5. Mix the plasmid with **e-coli** (bacteria)
6. Open the pores of the bacteria, by applying **temporary heat** or an **electric shock** to allow **plasmid** to enter
7. The bacteria can grow in huge numbers in a fermenter

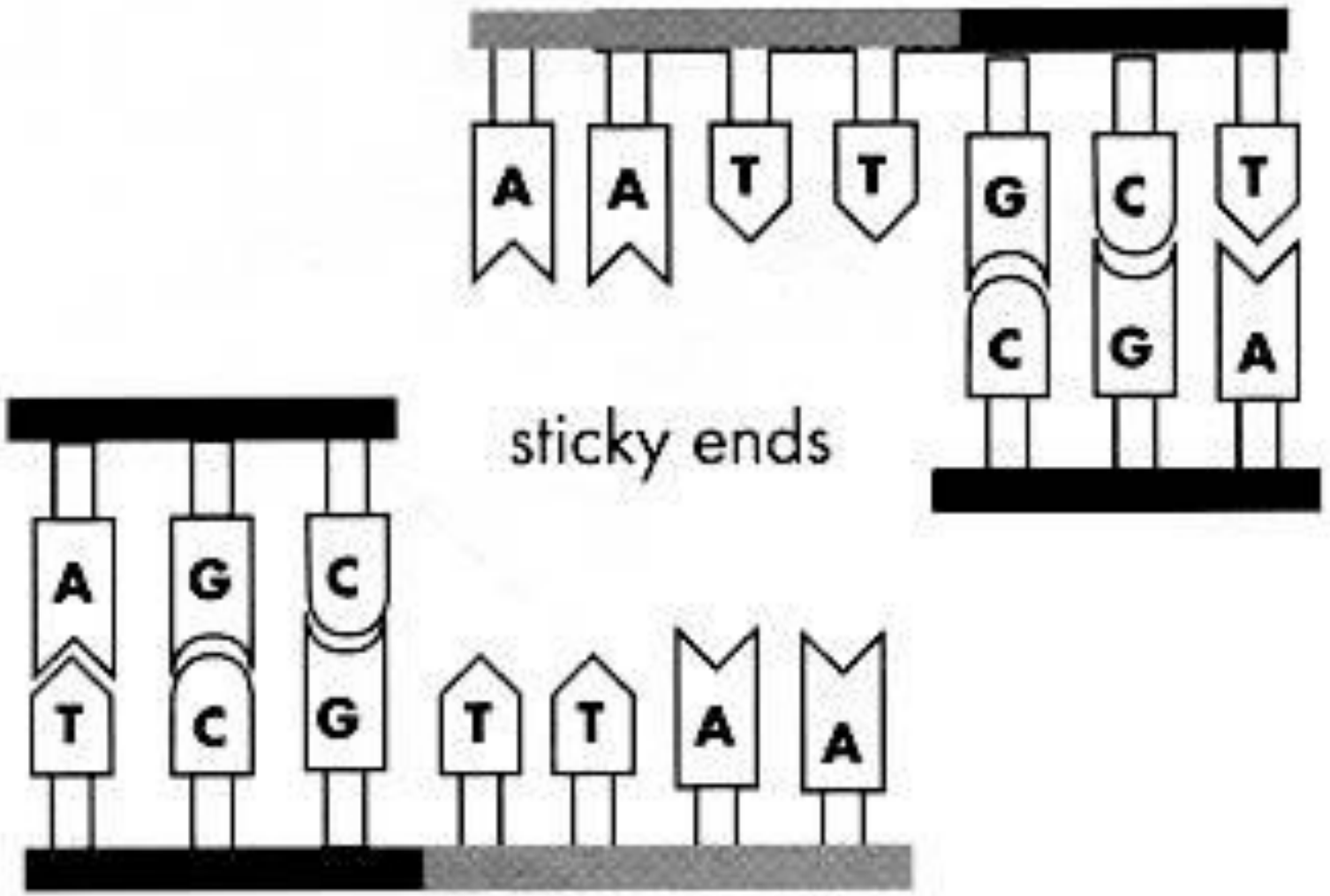
Restriction - Cutting up the DNA

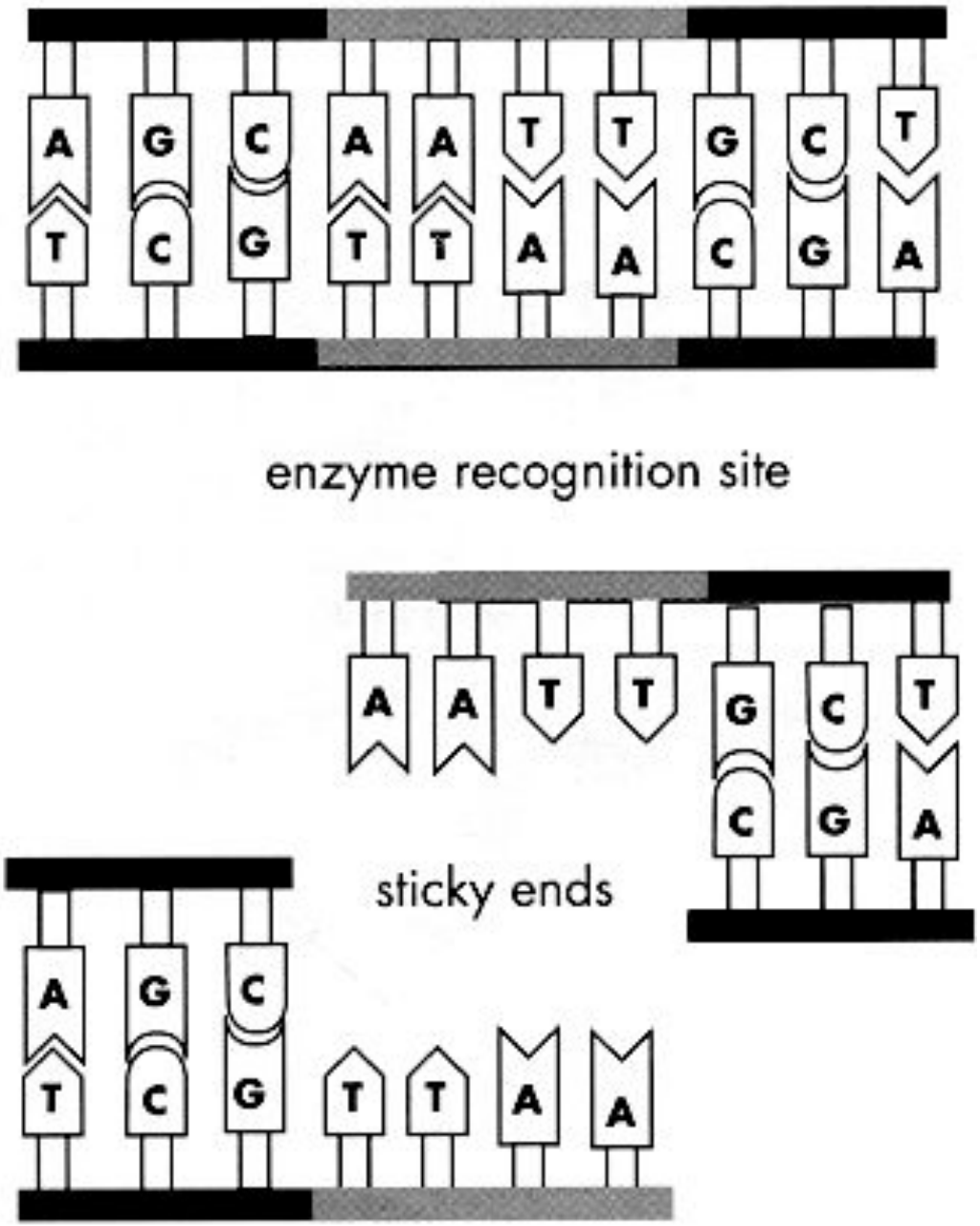
- **We need to isolate the gene that is required from the DNA.**



- Enzymes can be used that cut the DNA strand isolating the gene. These enzymes are called **restriction endonucleases.**

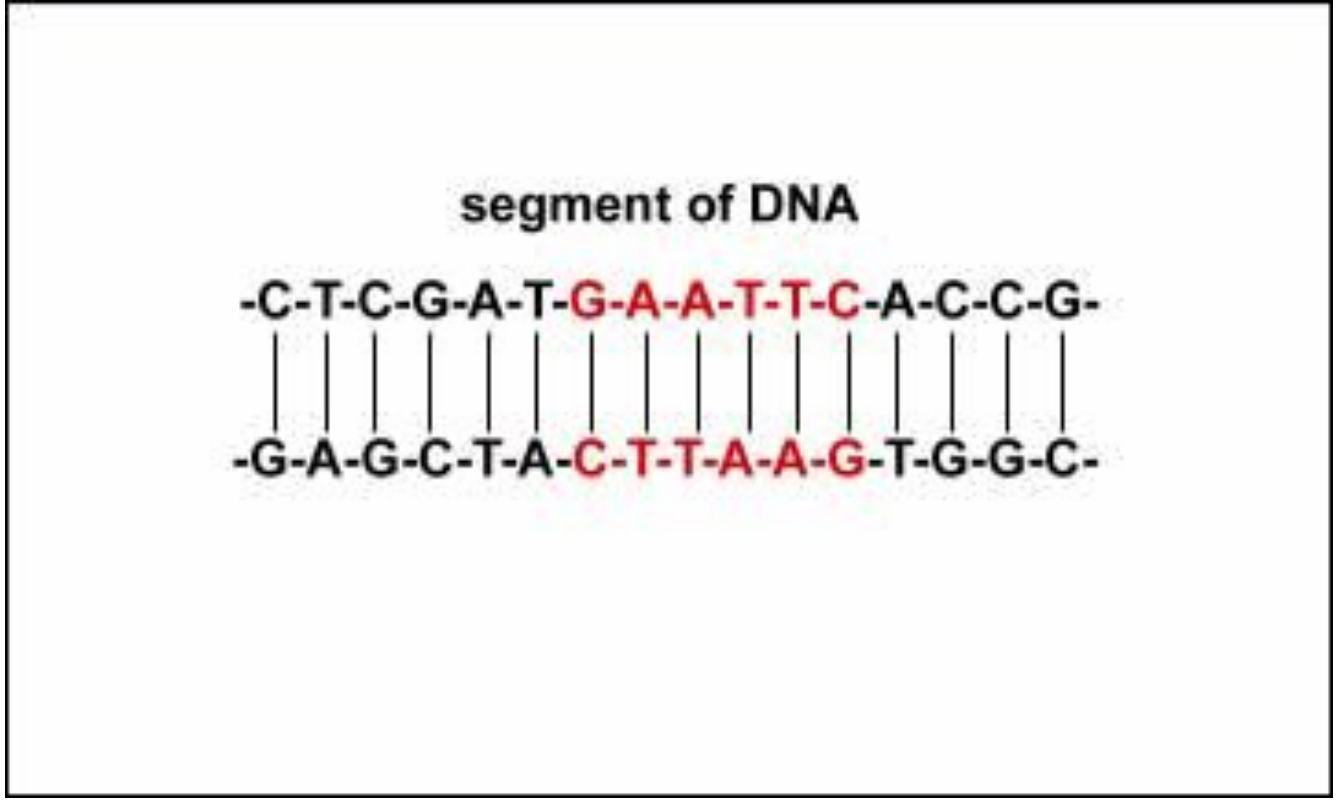
Restriction endonucleases cut DNA at specific base sequences (eg) AATT





Cutting DNA strands

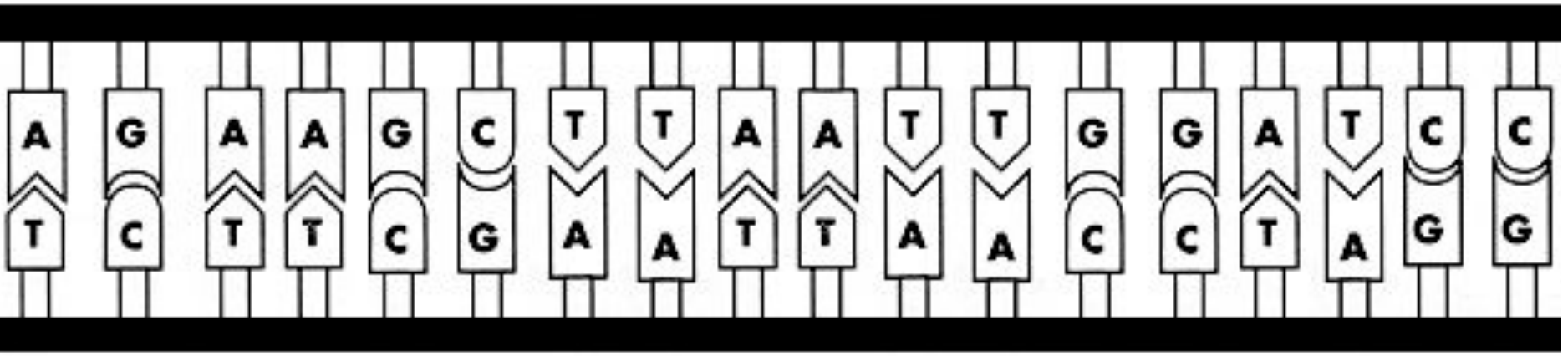
The enzyme cuts the DNA backbone twice, therefore, the site "reads" the same way backwards as forwards--a palindrome.
(eg) Hannah or race car.

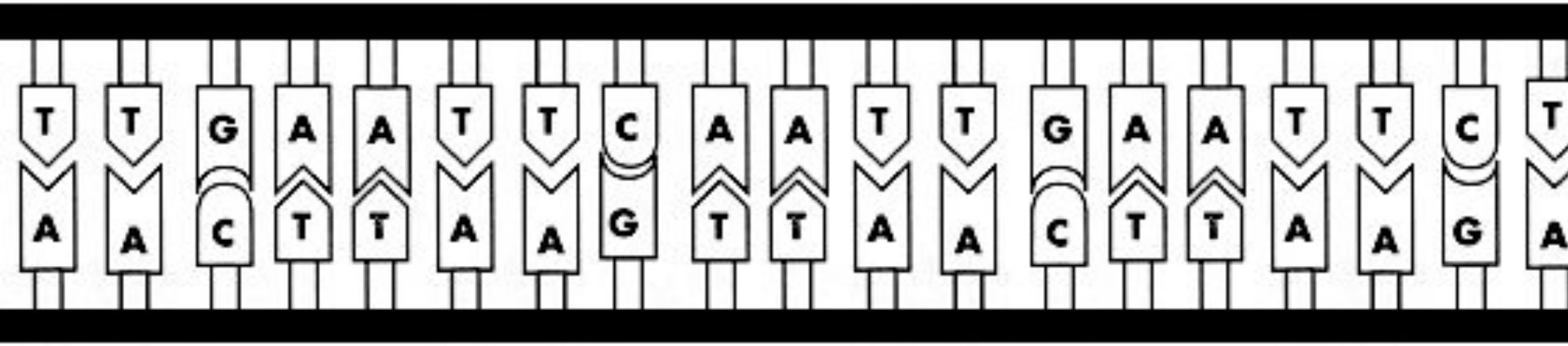


- Different restriction enzymes cut the DNA at different points (these enzymes are found naturally in bacteria).

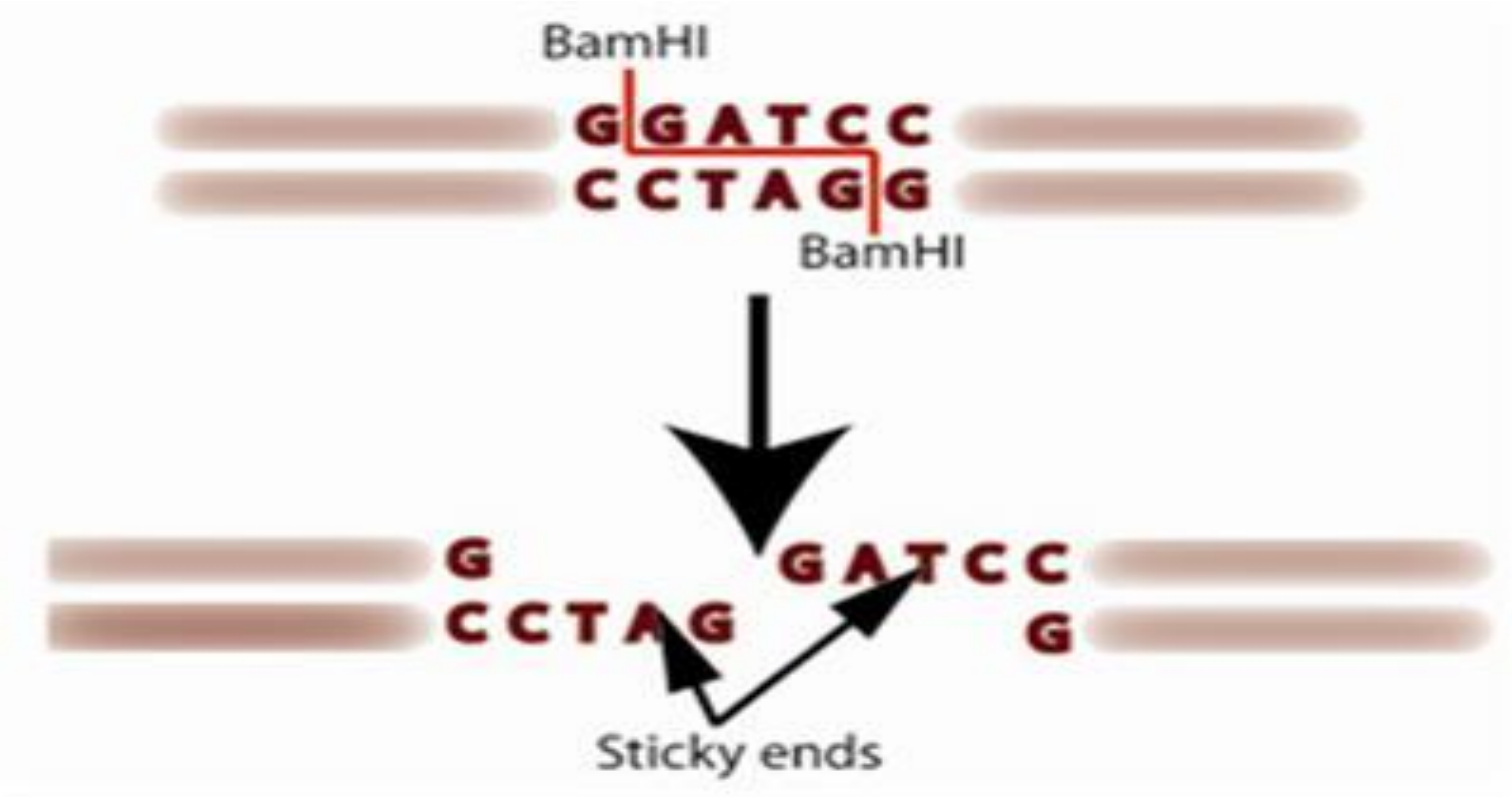
Different restriction enzymes produce different sticky ends

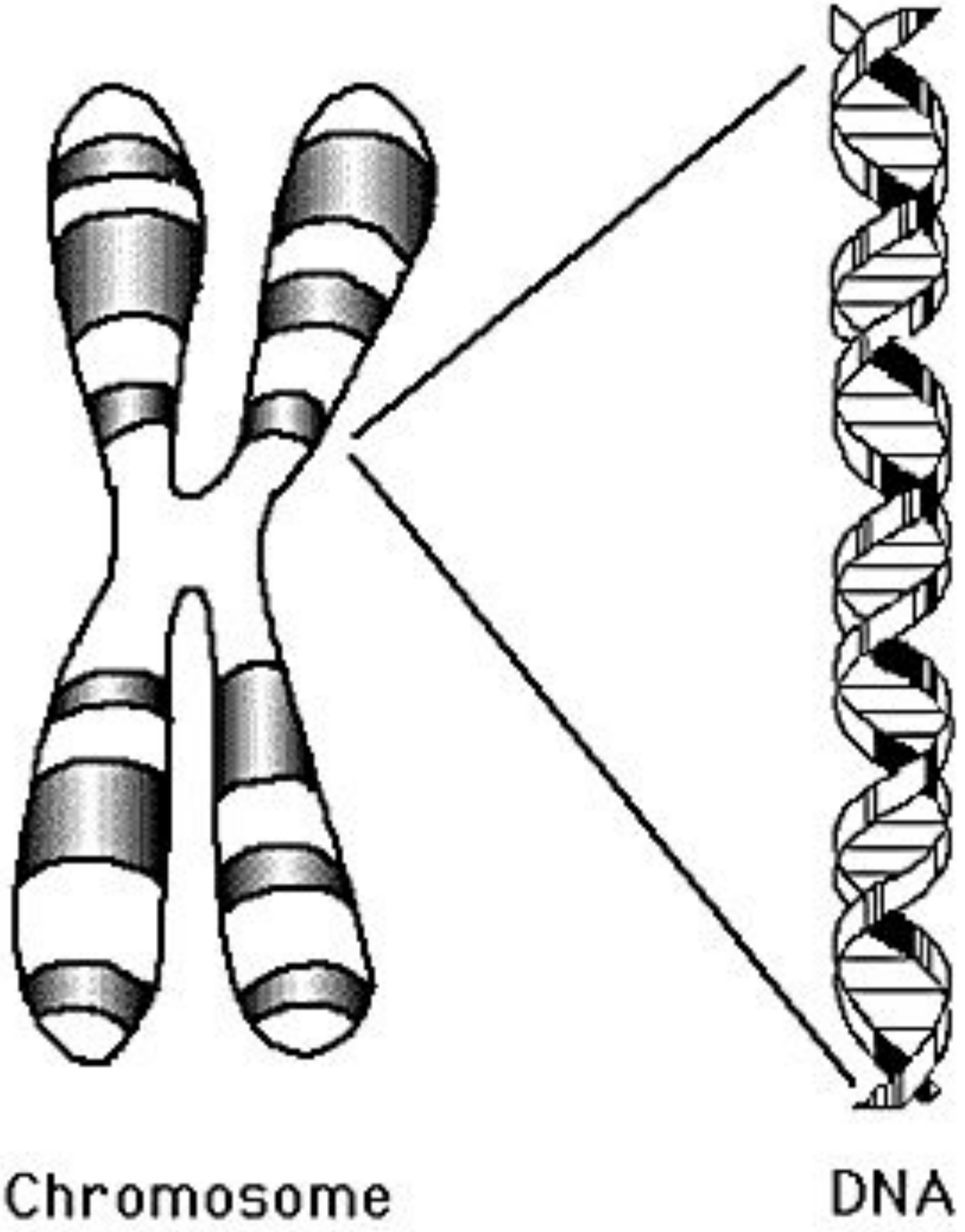
Enzyme	Bacterial origin	Recognition site
EcoRI	*E. coli*	G|AATTC CTTAA|G
HindIII	*H. influenzae*	A|AGCTT TTCGA|A
Bam H1	*B. amyloliquefaciens*	G|GATCC CCTAG|G


A G A A G C T T A A T T G G A T C C
T C T T C G A A T T A A C C T A G G


T T G A A T T C A A T T G A A T T C T
A A C T T A A G T T A A C T T A A G A

- These tails are called sticky ends –easily join with other DNA molecules which have the complimentary bases.

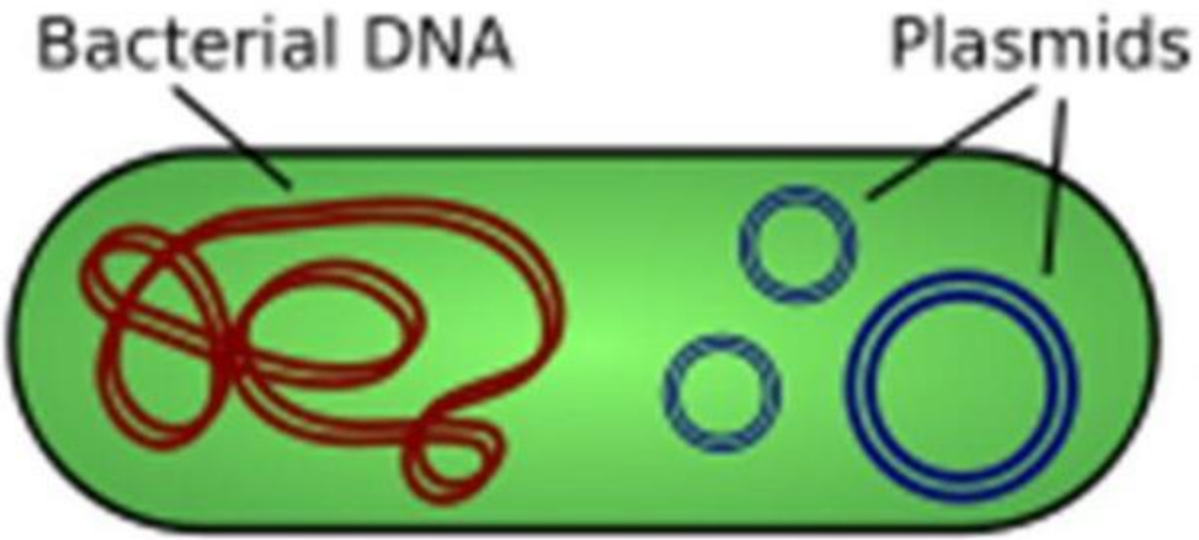




You will need to cut the DNA twice, either side of the

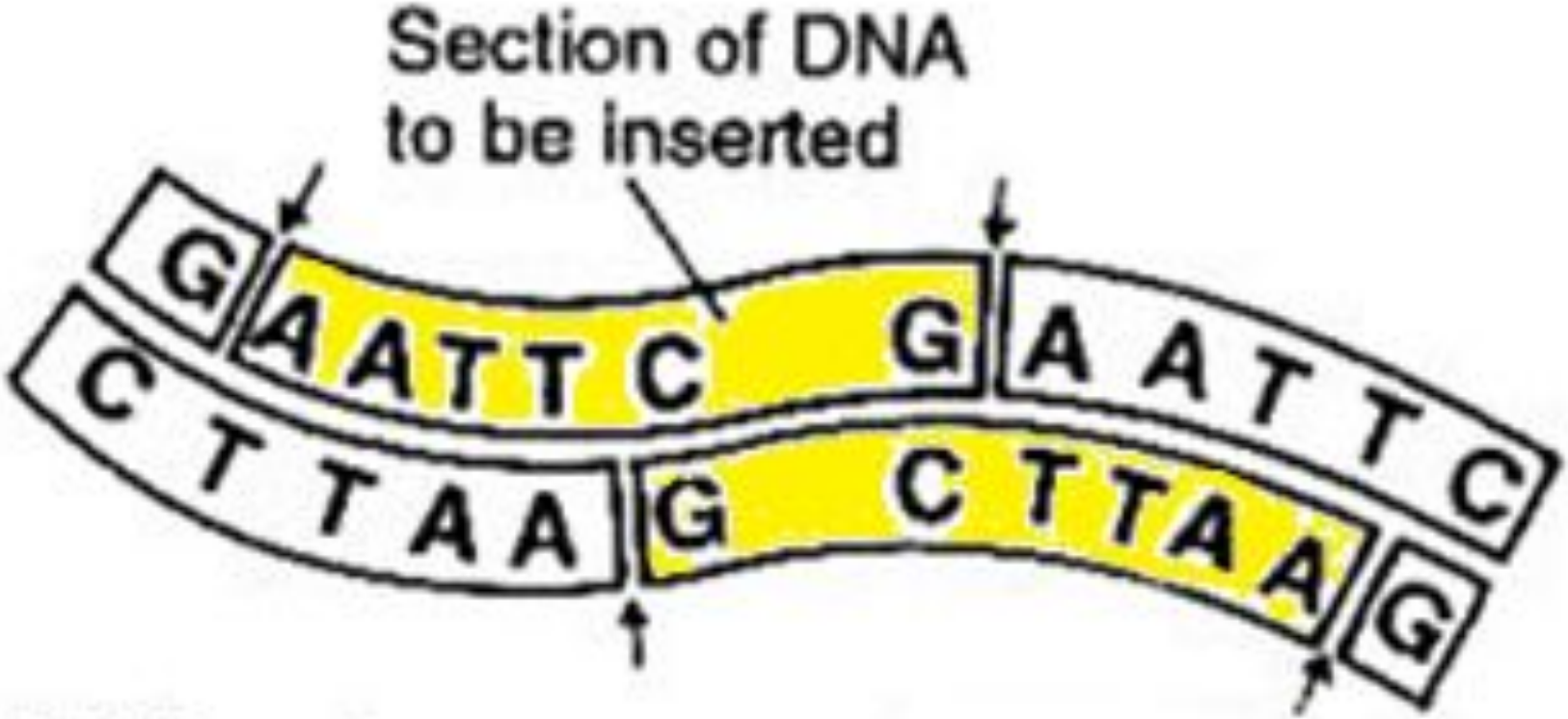
Using restriction enzymes you can cut out the gene. But then what are you going to do with it?

Inserting the isolated gene into a plasmid.



Ligation – the gene is inserted into a vector.

- The isolated gene is inserted into a vector. The vector is a piece of DNA that can take the gene into the chosen organism.



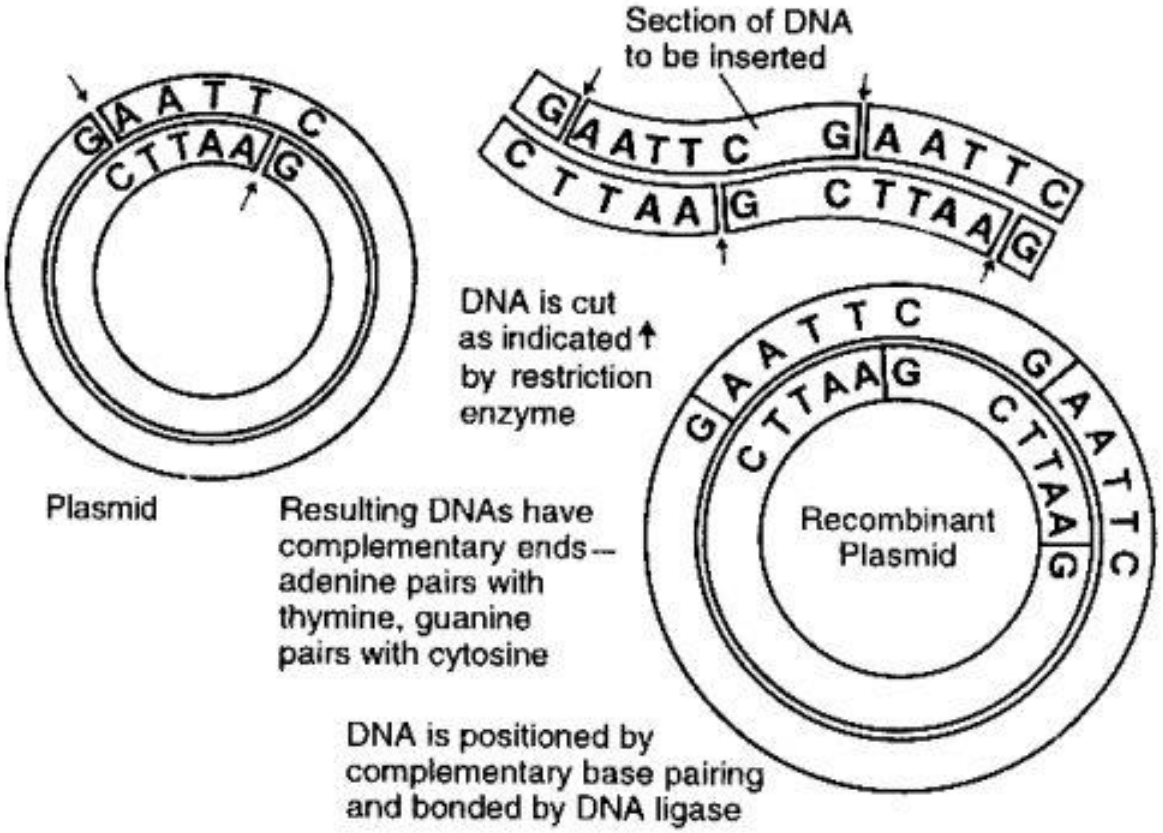
DNA is cut
as indicated ↑
by restriction
enzyme

Plasmid

- The **same** restriction enzymes used to cut out the gene is used to cut open the plasmid.

- Once DNA and the plasmid have been cut the enzyme is denatured to stop it cutting DNA.

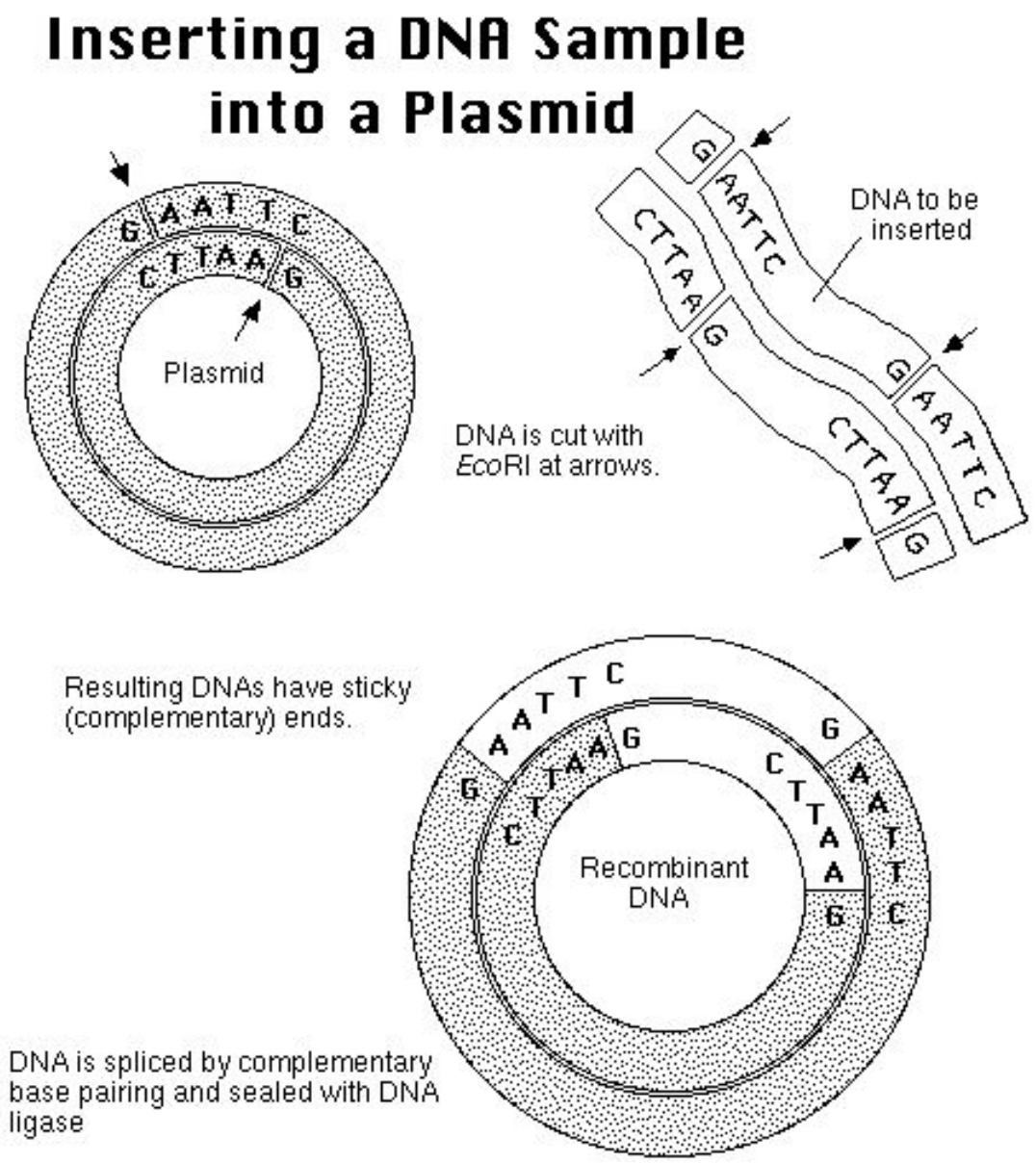
- The broken plasmid has sticky ends that are complimentary to the donor gene.
- The donor gene will easily combine with the complimentary sticky ends of the plasmid.

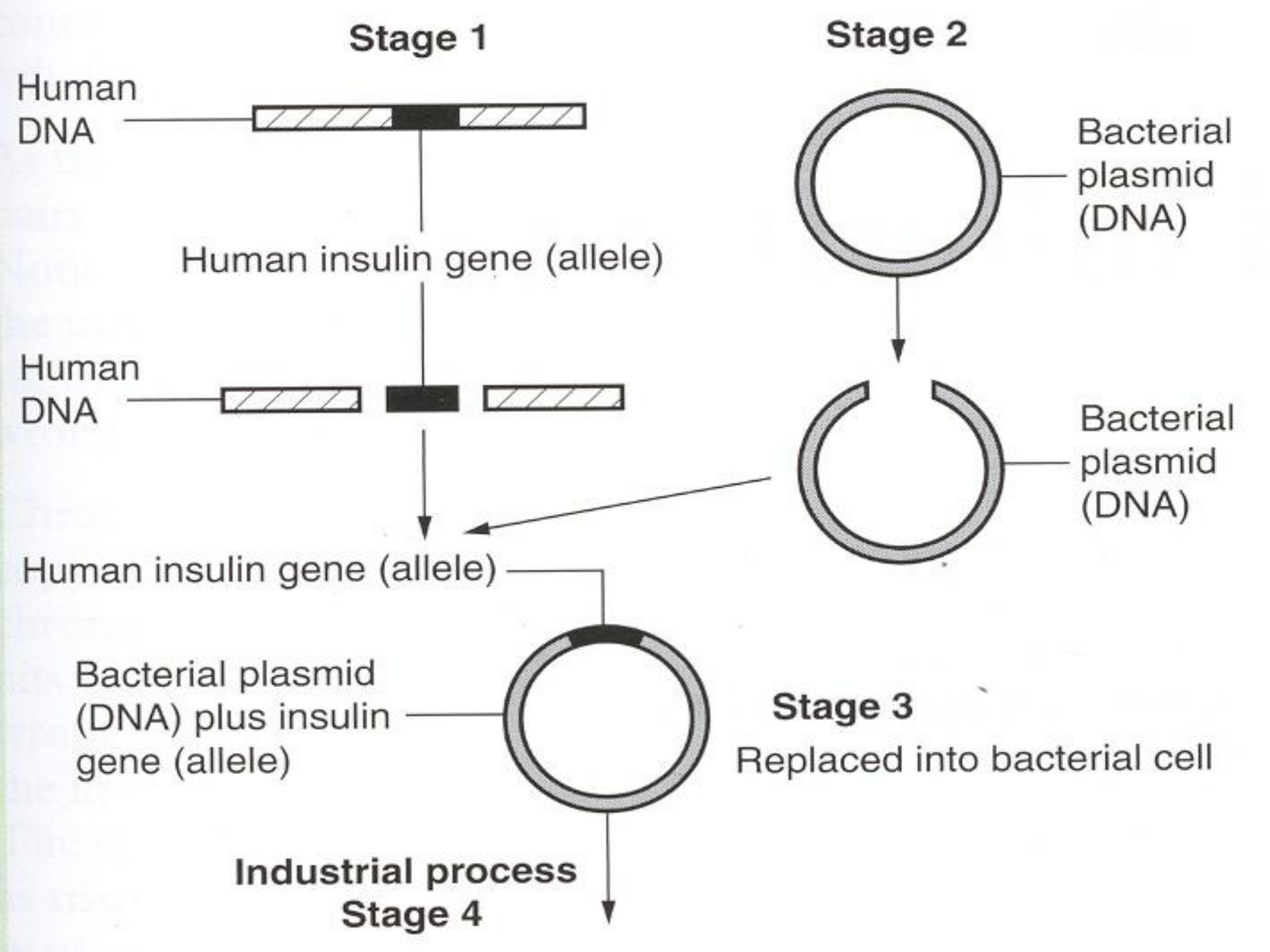


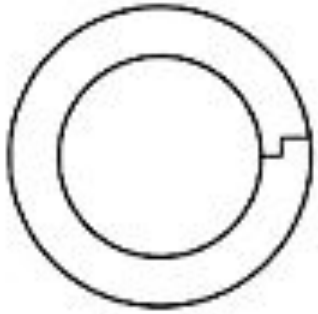
- The gene is inserted into the plasmid loop using the enzyme **ligase**.

Recombinant DNA plasmid

- Ligase catalyses the ligation reaction that joins two backbones of DNA together.
- The new DNA is called **recombinant DNA.**

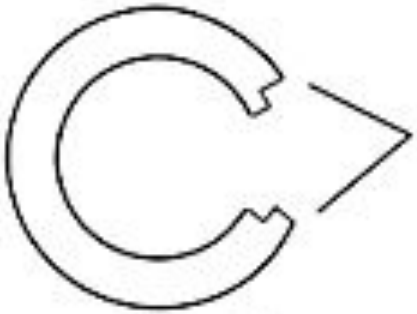



Stage 1
Stage 2
Human DNA
Bacterial plasmid (DNA)
Human insulin gene (allele)
Human DNA
Bacterial plasmid (DNA)
Human insulin gene (allele)
Bacterial plasmid (DNA) plus insulin gene (allele)
Stage 3
Replaced into bacterial cell
Industrial process
Stage 4



enzyme recognition site

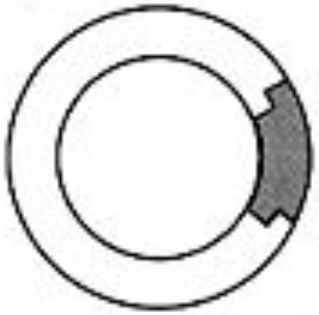
plasmid



sticky ends

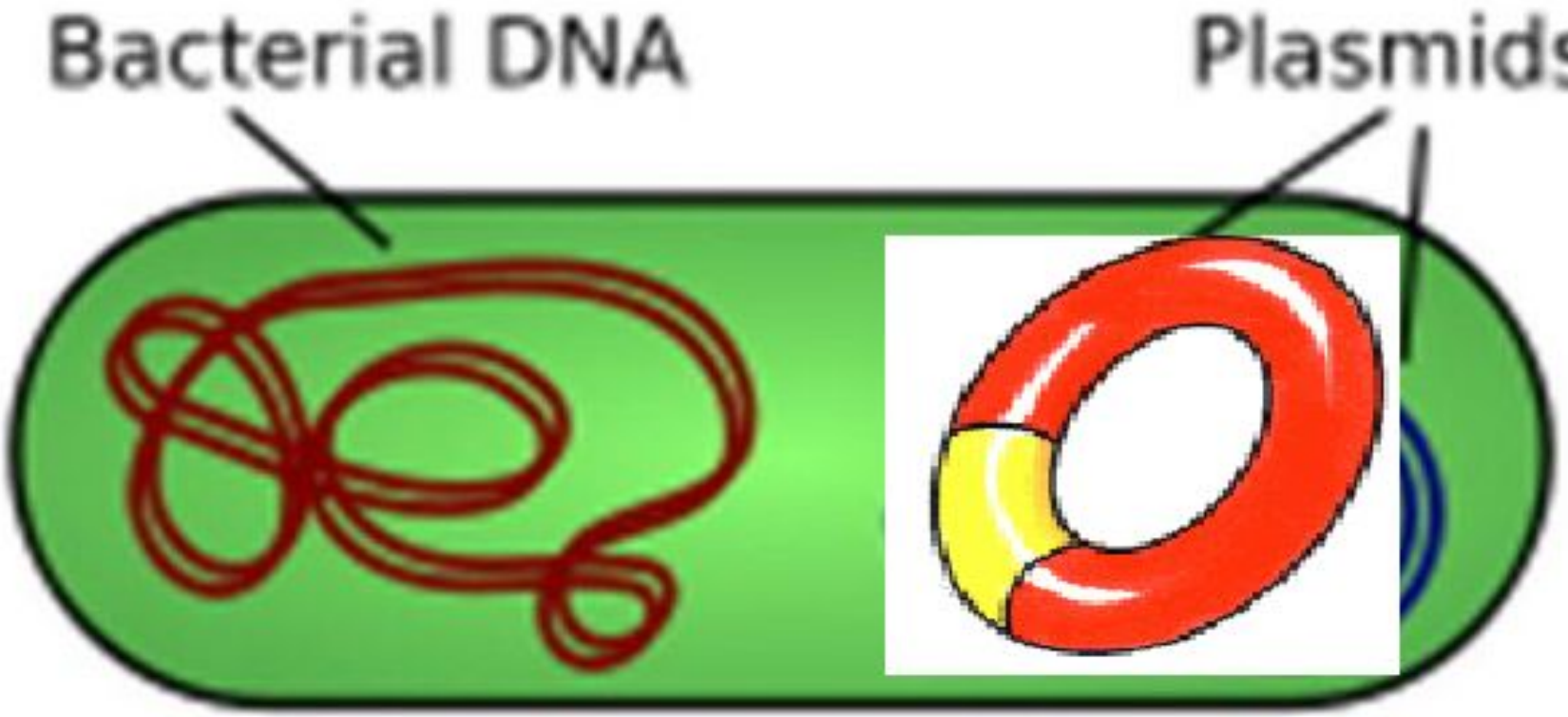
cut plasmid

foreign DNA



recombinant plasmid

Creating recombinant plasmids



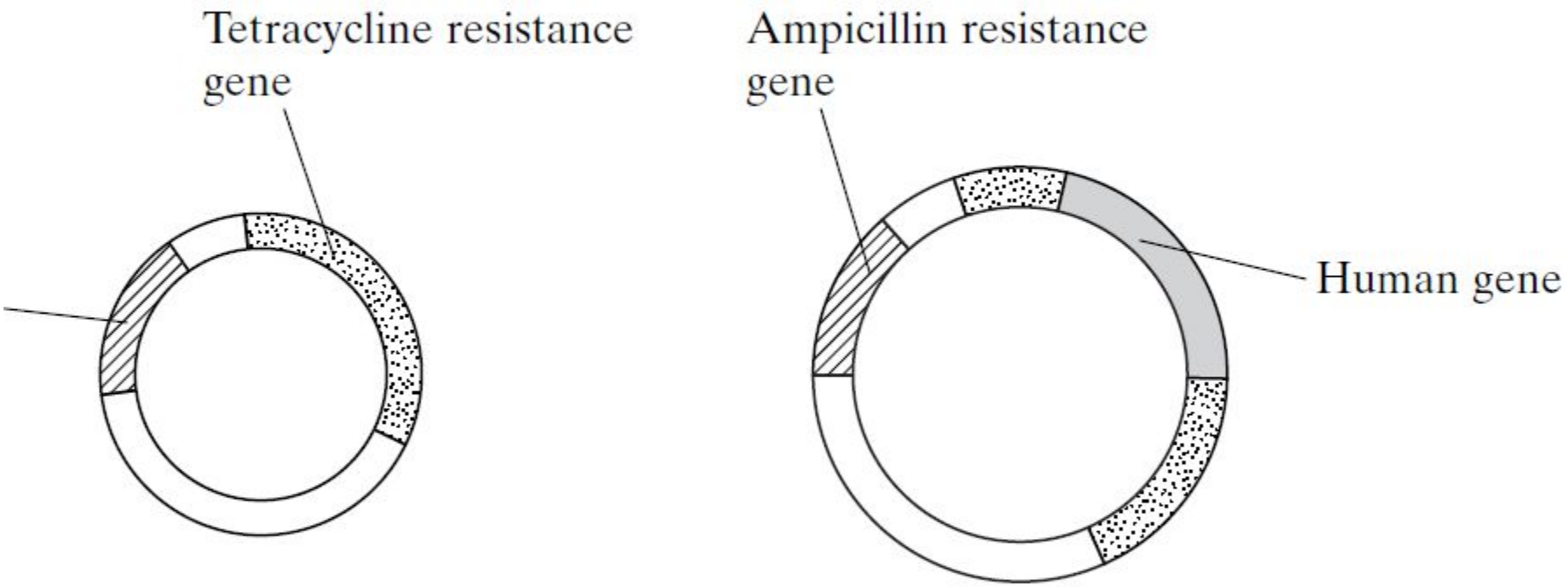
Transformation:

- Plasmids containing the donar gene must now be transferred into the microbe. Those bacteria that do contain plasmids with recombinant DNA are said to have undergone **transformation**.

- Transformation is not very efficient. You now need to identify and isolate those bacteria that have been transformed.

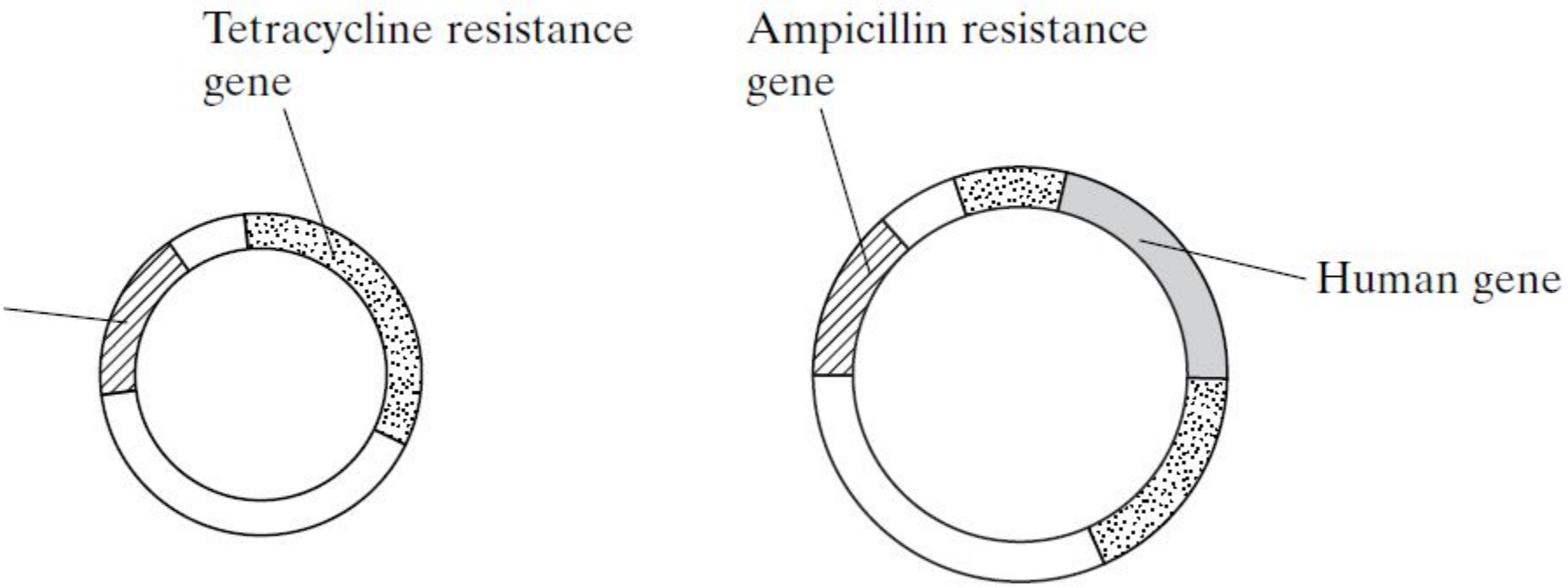
Selection - Use a marker gene

- The plasmid contains two genes for anti biotic resistance

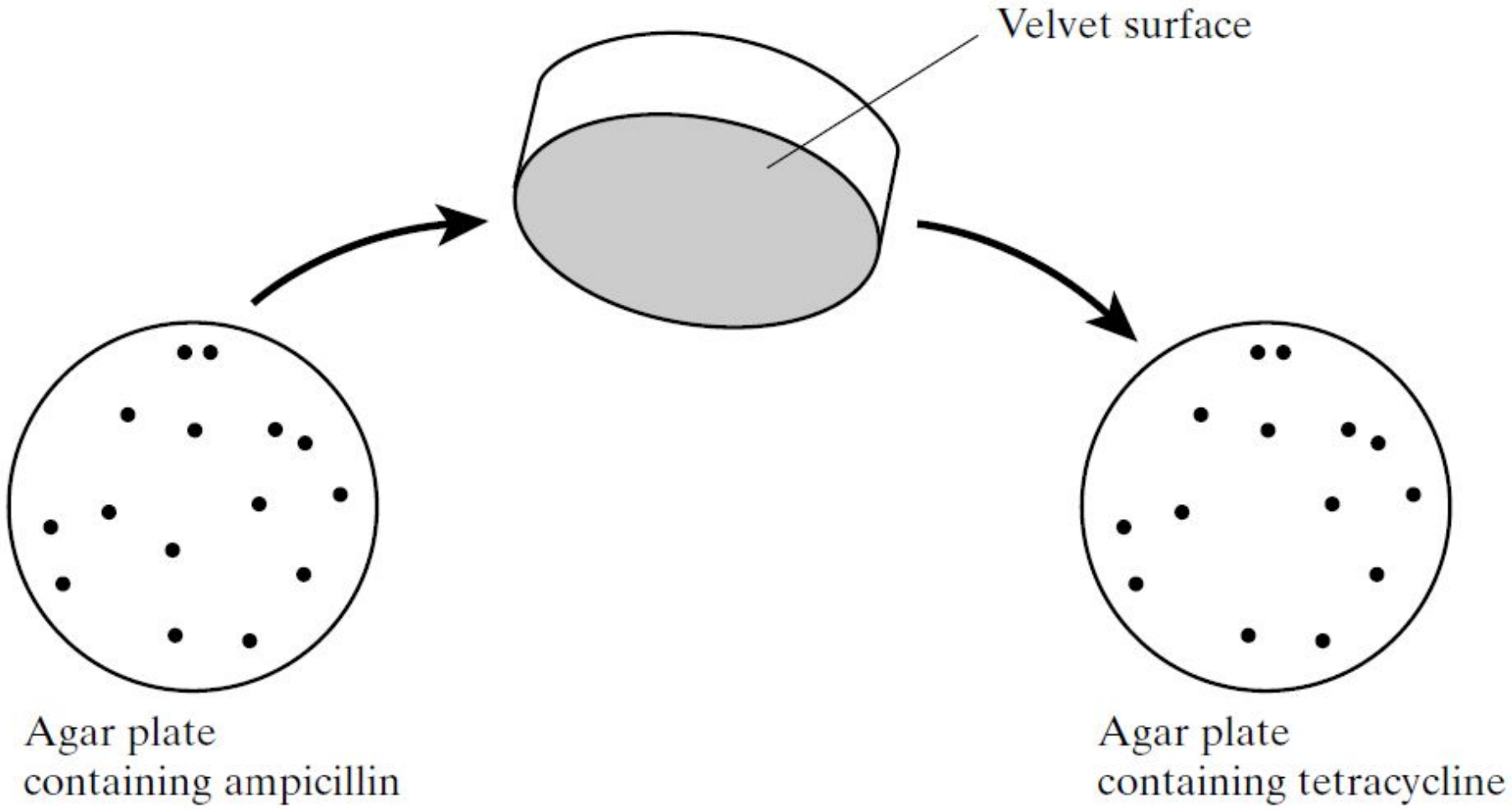


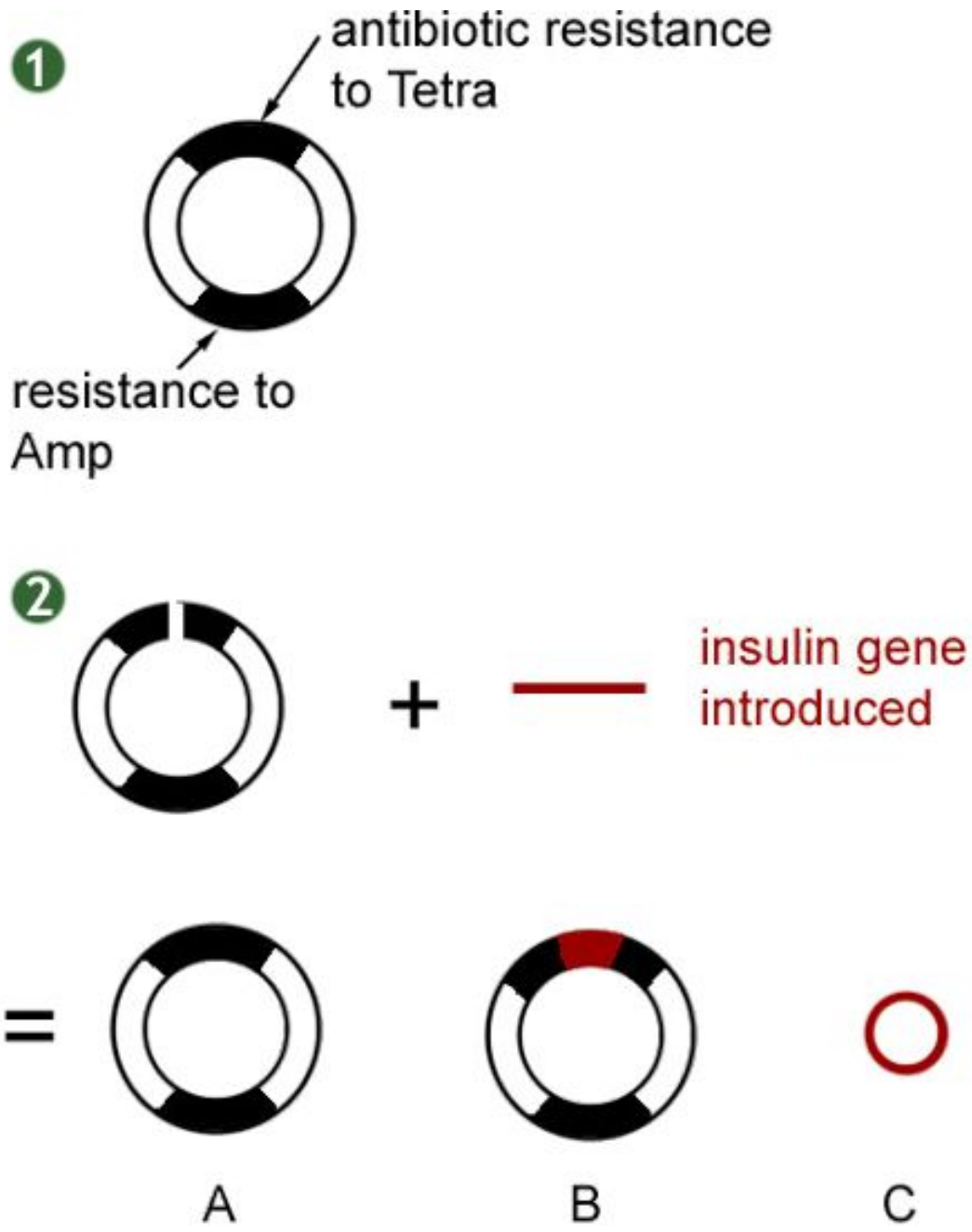
Selection - Use a marker gene

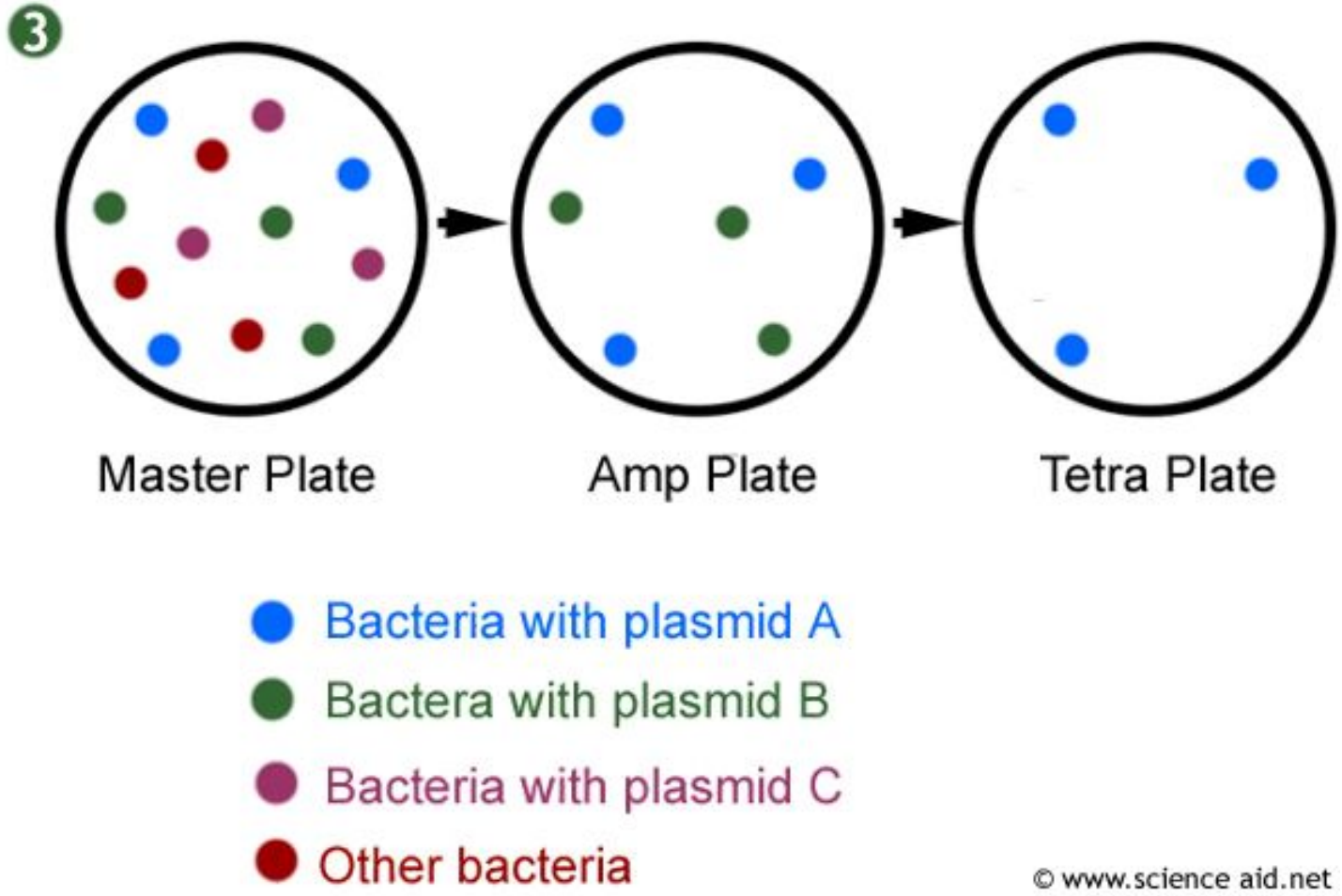
- One is broken by the inserted gene.



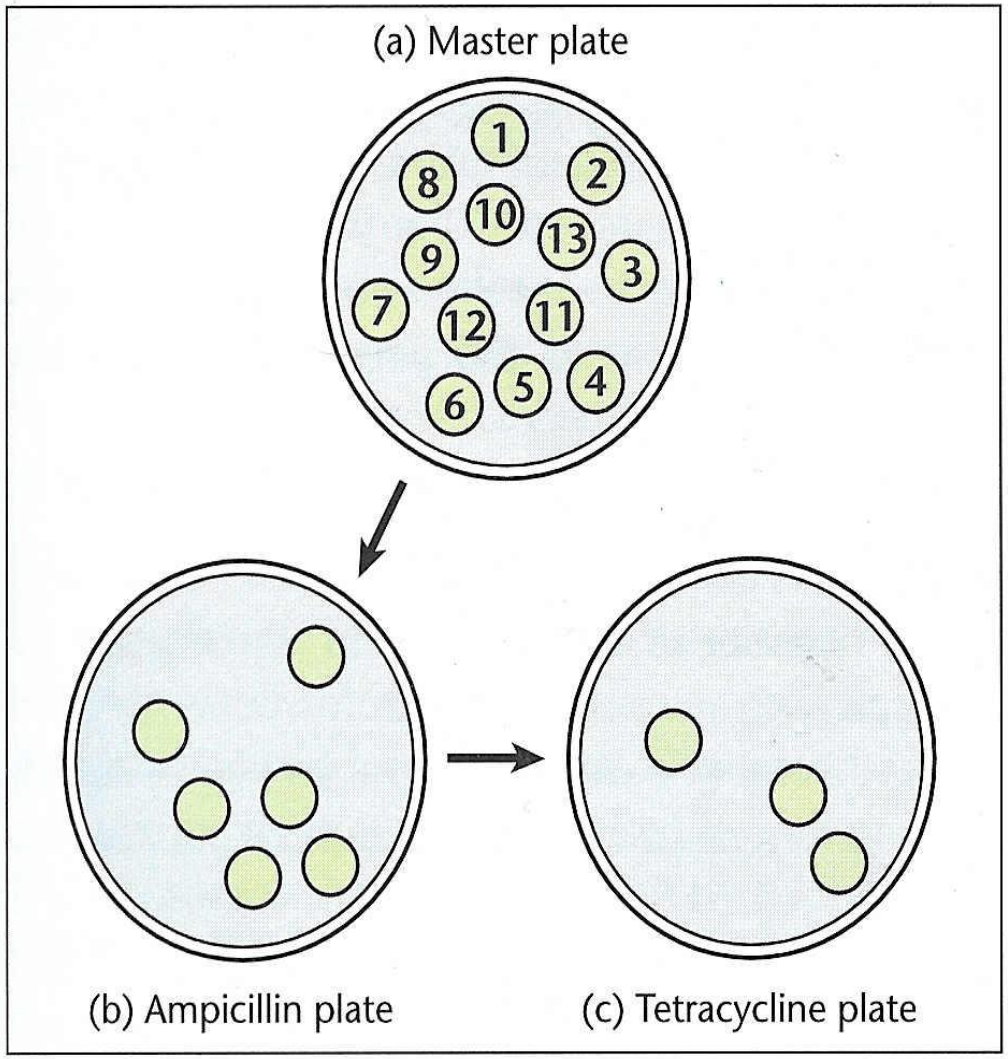
- The plasmids are taken up by the bacteria and replica plating is used to identify the bacteria with the recombinant plasmid.






3
Master Plate
Amp Plate
Tetra Plate
Bacteria with plasmid A
Bactera with plasmid B
Bacteria with plasmid C
Other bacteria
© www.science aid.net

The bacteria are grown on culture plates, where they form visible colonies:



They can be transferred to identical positions on plates containing ampicillin and then tetracycline. The bacteria with the 'new' gene will be able to grow on ampicillin, but not tetracycline. The required transformed bacteria can be identified, ready to be grown on a large scale

Culturing

- **Replica plating**

- The transformed bacteria are then cultured on an industrial scale. The useful product is extracted.
- (The vector can be transferred by micropipette or by a virus to inject the DNA into another organism)

In vivo gene cloning -

- These methods of gene cloning are called *in vivo* as the gene fragment is transferred to a host cell using a vector. The gene is cloned within a living organism.

Advantages

- The production of useful organisms with new features.

Disadvantages

- Inserted genes may have unexpected harmful effects.