

Gene Technology



What is Gene Technology?

- **Gene technology** is a broad field which includes analysis of DNA as well as **genetic engineering** and other forms of genetic modification.
- Genetic engineering refers the artificial manipulation of genes: adding or subtracting genes, or changing the way genes work.
- Organisms with artificially altered DNA are referred to as **genetically modified organisms (GMOs)**.
- Gene technologies have great potential to benefit humanity through:
 - increasing crop production
 - increasing livestock production
 - preventing and fighting disease
 - reducing pollution and waste
 - producing new products
 - detecting and preventing crime



Why Gene Technology?

- Despite potential benefits, gene technology is highly controversial.
- Some people feel very strongly that safety concerns associated with the technology have not been adequately addressed.

- Environmentally friendly
- Could improve the sustainability of crop and livestock production
- Could potentially benefit the health of many
- More predictable and directed than selective breeding



- Who owns and regulates the GMOs?
- Third world economies are at risk of exploitation
- Biological risks have not been adequately addressed
- Animal ethics issues
- The costs of errors

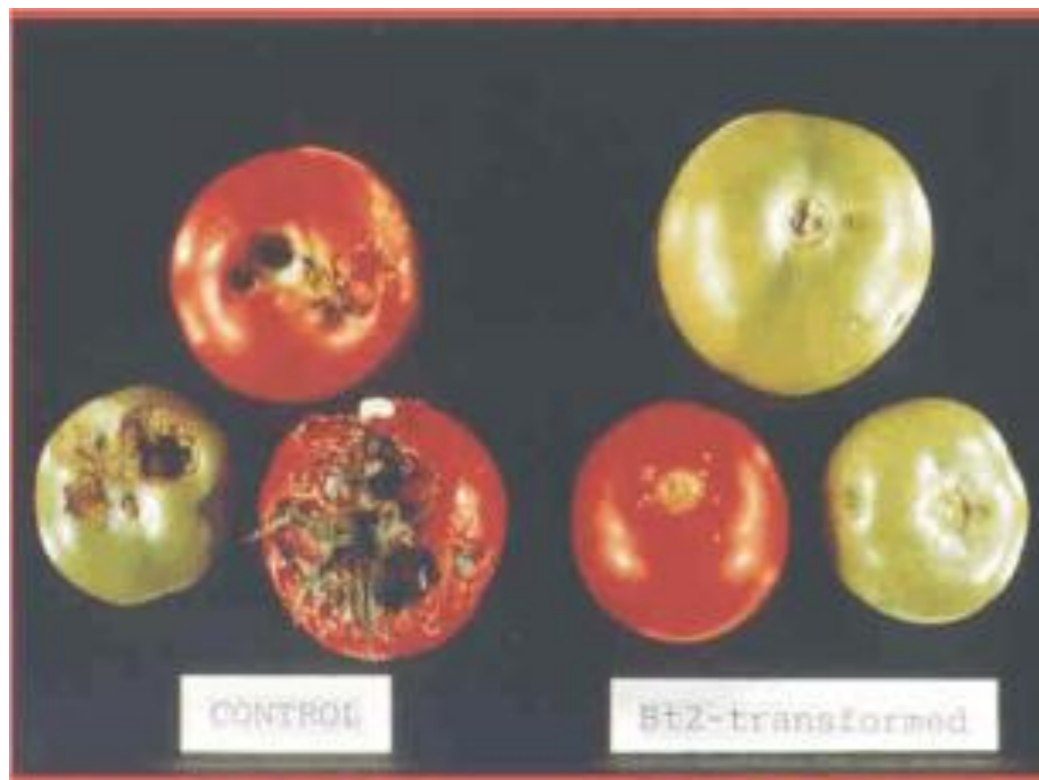
What is “**genetic engineering**” good for?

One of the most notable accomplishments of genetic engineering is the production of human **insulin** from bacteria, replacing the often troublesome and limited source from cattle and pigs.

In other words... genetically engineering the bacteria with the gene for HUMAN insulin so that the bacteria make the protein for us.

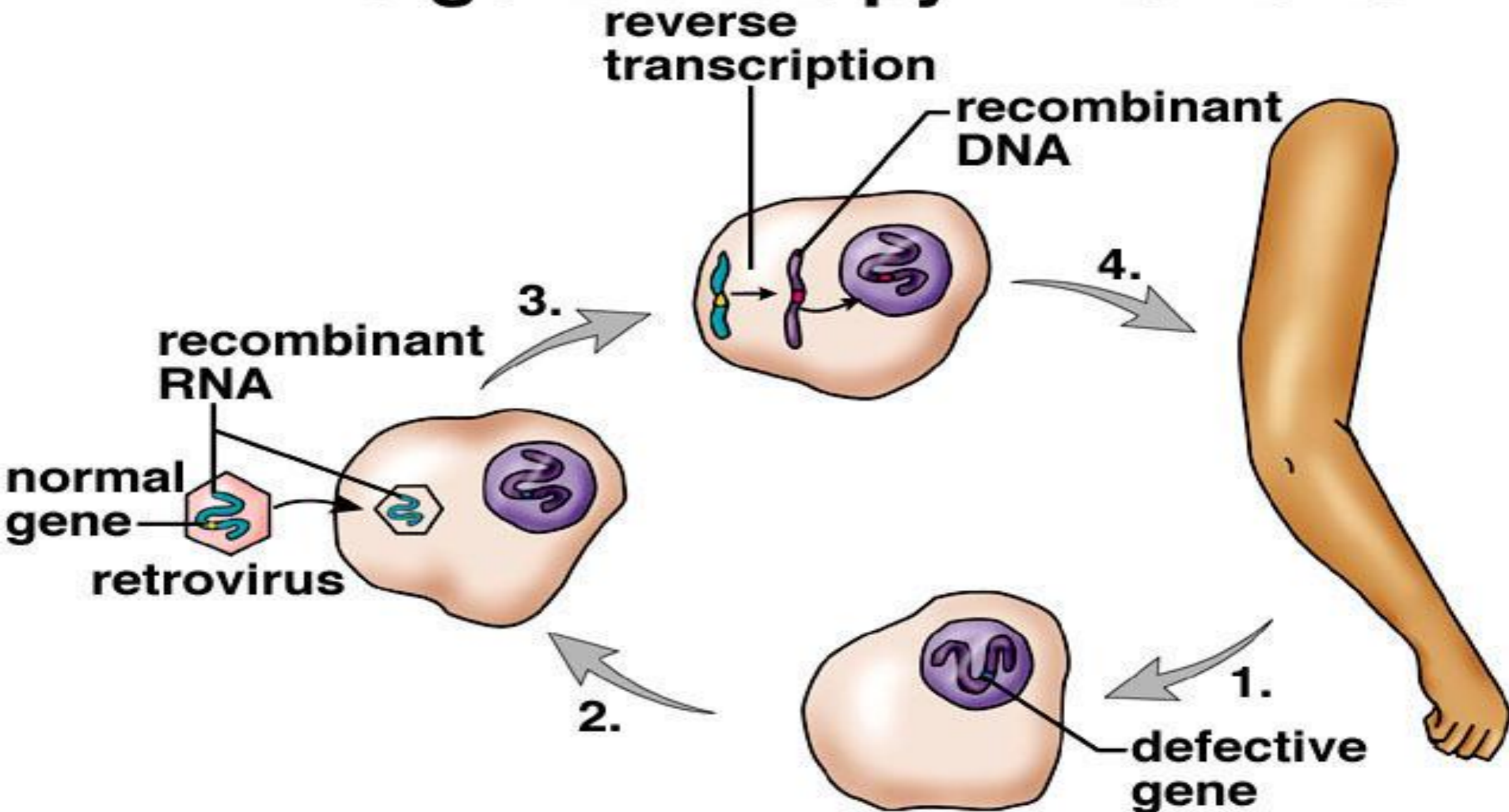


Genetically modified crop plants could **increase food production** or allow utilization of marginal lands such as those with high salt content.



Inserting normal genes into the bodies' cells of an organism to correct a genetic defect is called gene therapy

Ex viro gene therapy in humans



More controversial is **eugenic engineering**, the insertion of genes into a normal individual to influence a particular trait ("**designer babies**")



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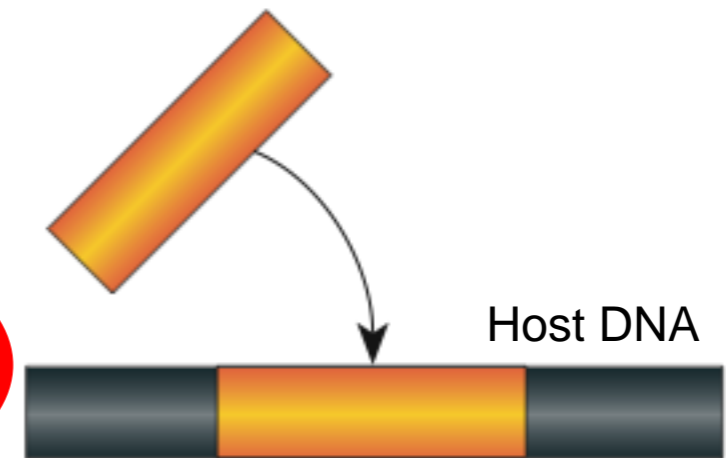


Producing GMOs

- **GMOs** may be created by modifying their DNA in one of three ways:

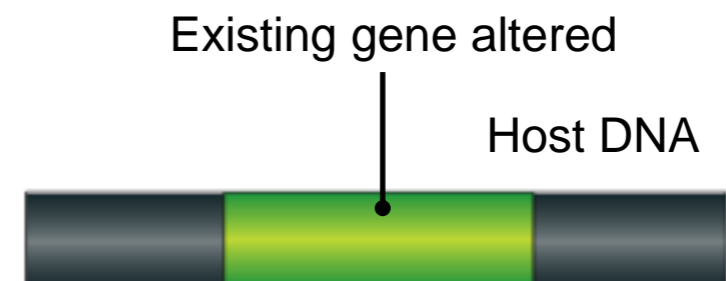
Adding a Foreign Gene

A foreign gene is added which will enable the GMO to carry out a new genetic program. Organisms altered in this way are referred to as **transgenic**.



Alter an Existing Gene

An existing gene already present in the organism may be altered to make it express at a higher level (e.g. growth hormone) or in a different way (in tissue that would not normally express it). This method is also used for **gene therapy**.



Delete or 'Turn Off' a Gene

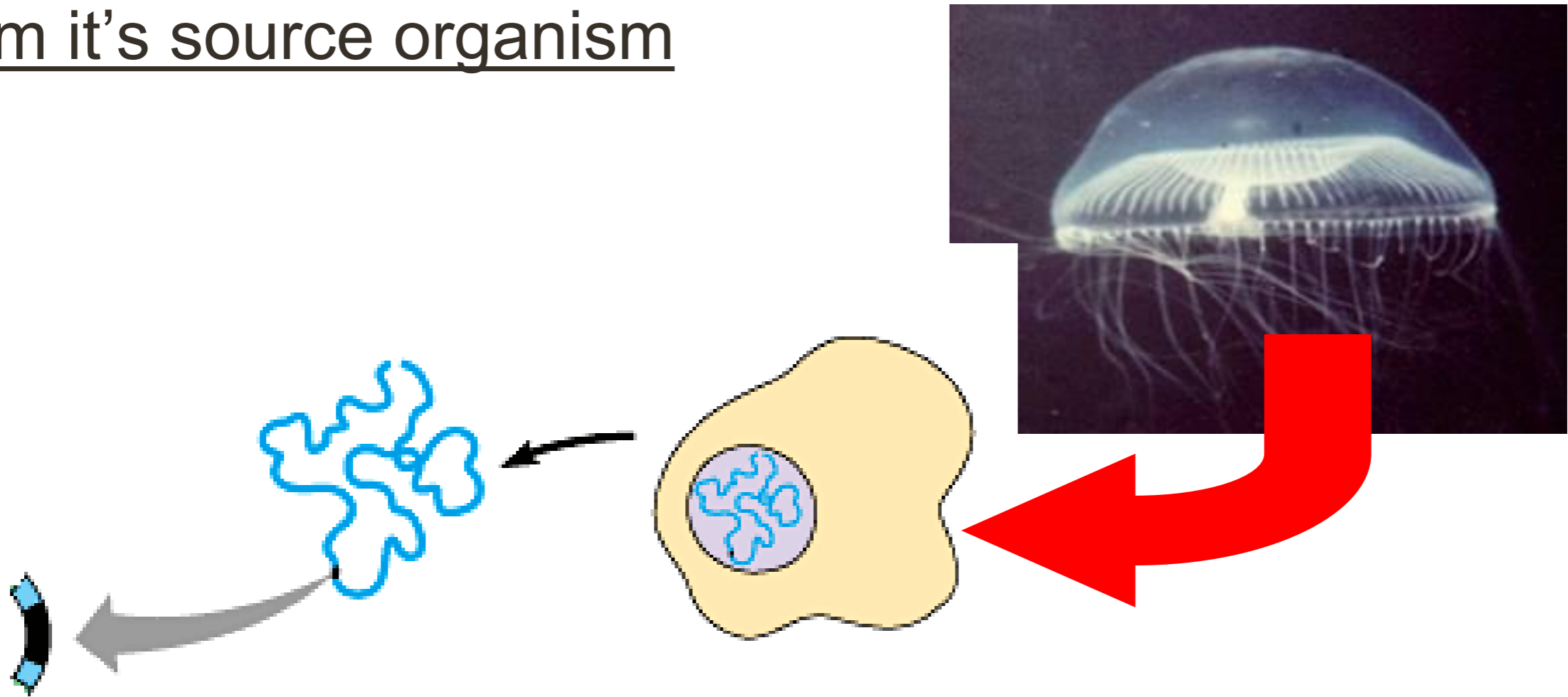
An existing gene may be deleted or deactivated to prevent the expression of a trait (e.g. the deactivation of the ripening gene in tomatoes).



Adding a Gene

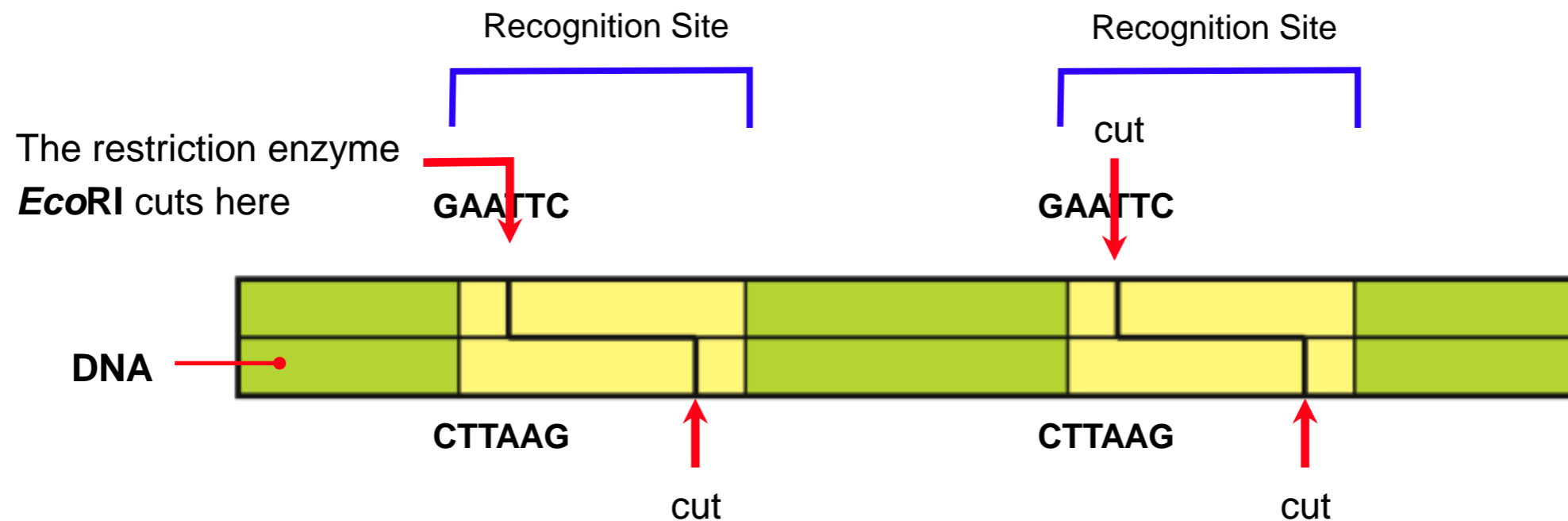
Step 1

- Use a restriction enzyme to cut out the gene of interest from its source organism



Review: Restriction Enzymes

- **Restriction enzymes** are one of the essential tools of genetic engineering. Purified forms of these naturally occurring **bacterial enzymes** are used as “**molecular scissors**”, allowing genetic engineers to cut up DNA in a controlled way.
- Restriction enzymes are used to cut DNA molecules at very precise sequences of 4 to 8 base pairs called **restriction sites** (see below).
- By using over **400** restriction enzymes recognizing about **100** recognition sites, genetic engineers are able to isolate and sequence DNA, and manipulate individual genes derived from any type of organism.



Review: Restriction Sites

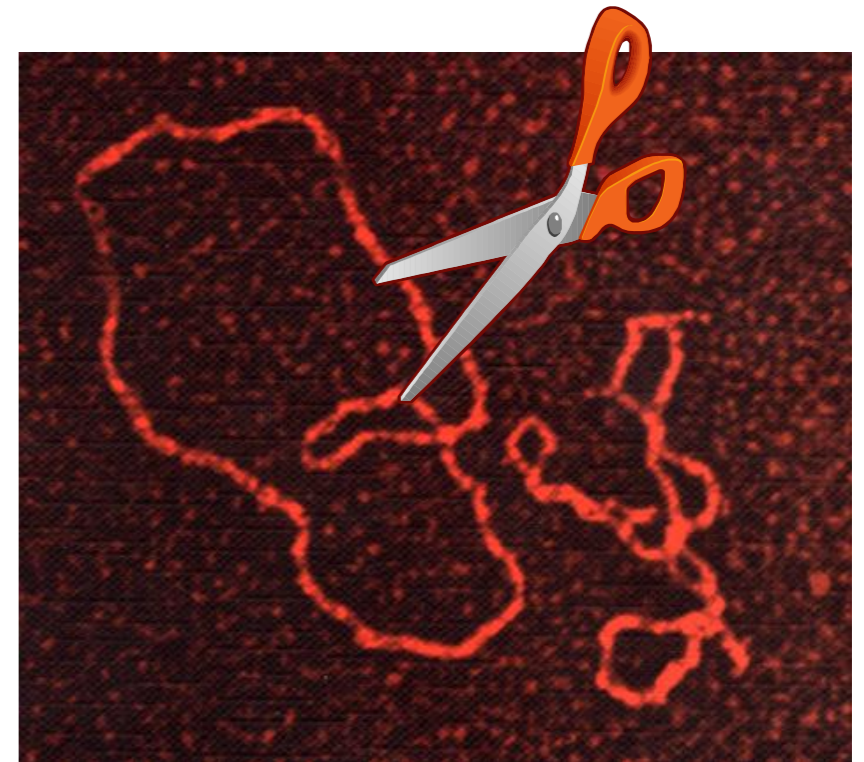
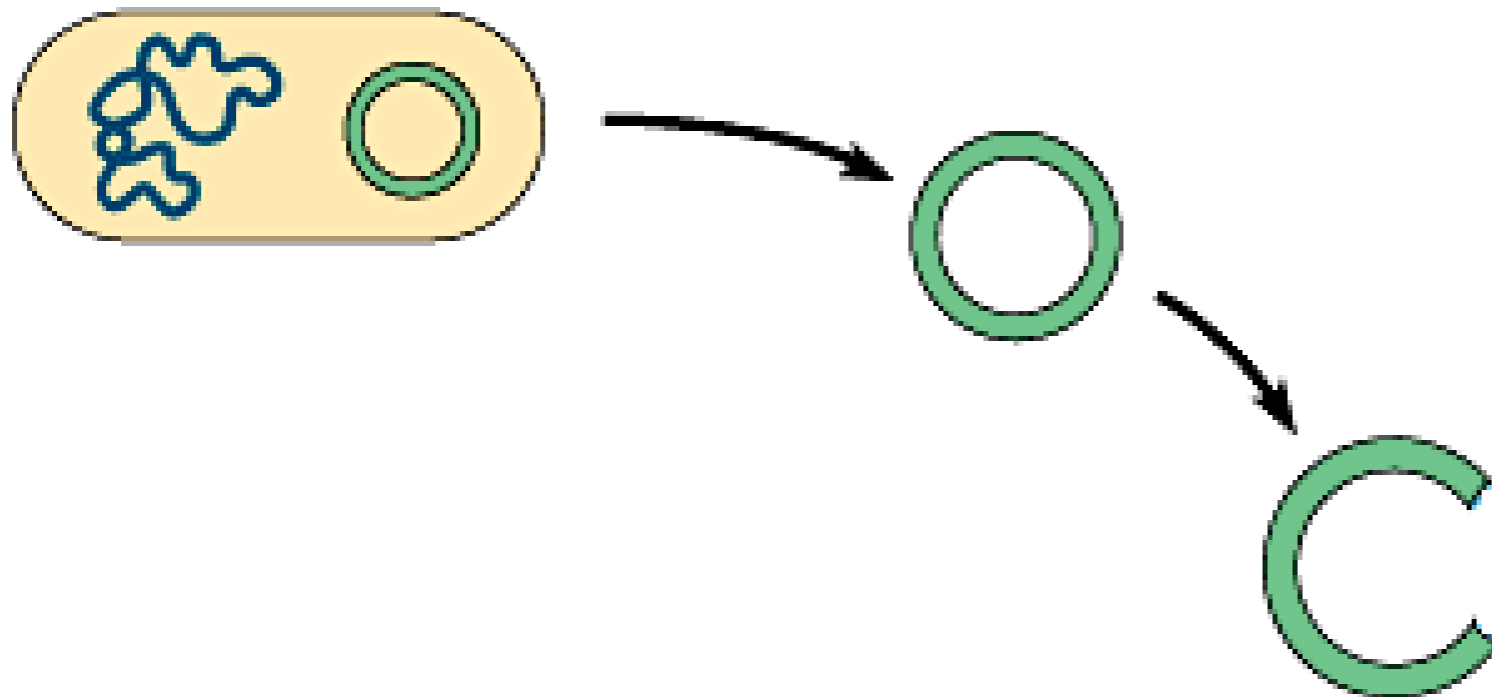
- Restriction enzymes are **named** according to the **bacterial species** they were first isolated from, followed by a **number** to distinguish different enzymes isolated from the same organism.
- e.g. **BamHI** was isolated from the bacteria *Bacillus amyloliquefaciens* strain H.
- A restriction enzyme cuts the double-stranded DNA molecule at its specific **restriction site**:

| Enzyme | Source | Recognition Sites |
|----------------|-------------------------------------|-------------------|
| <i>EcoRI</i> | <i>Escherichia coli</i> RY13 | GAATTC |
| <i>BamHI</i> | <i>Bacillus amyloliquefaciens</i> H | GGATCC |
| <i>HaeIII</i> | <i>Haemophilus aegyptius</i> | GGCC |
| <i>HindIII</i> | <i>Haemophilus influenzae</i> Rd | AAGCTT |
| <i>HpaI</i> | <i>Haemophilus parainfluenzae</i> | GTTAAC |
| <i>HpaII</i> | <i>Haemophilus parainfluenzae</i> | CCGG |
| <i>MboI</i> | <i>Moraxella bovis</i> | GATC |
| <i>NotI</i> | <i>Nocardia otitidis-caviarum</i> | GCGGCCGC |
| <i>TaqI</i> | <i>Thermus aquaticus</i> | TCGA |

Adding a Gene

Step 2

- Use **THE SAME** restriction enzyme to cut open the DNA strand of the organism being given the gene
- Must be the same enzyme so the “sticky ends” are complementary

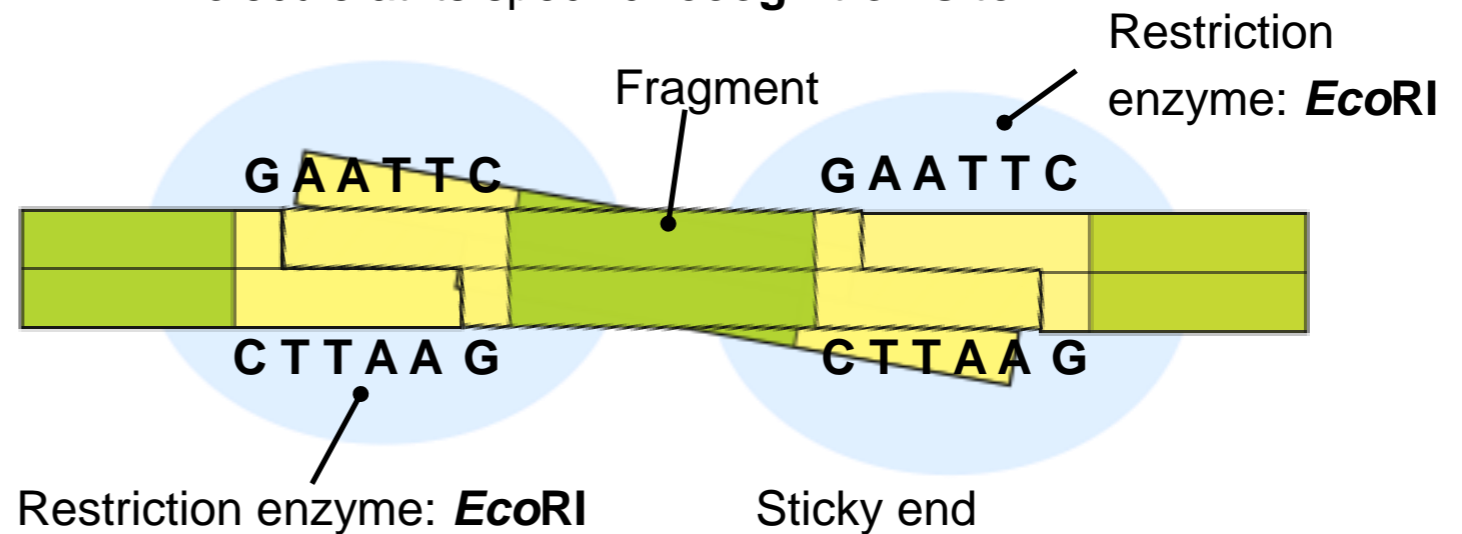


Review: Sticky Ends

It is possible to use **restriction enzymes** that cut leaving an overhang; a so-called “**sticky end**”.

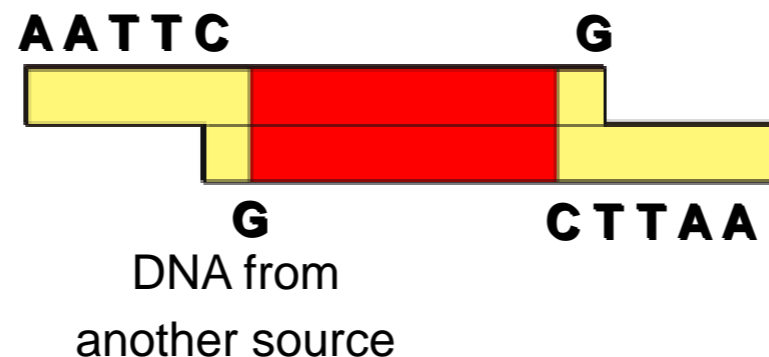
DNA cut in such a way produces ends which may only be joined to **other sticky ends** with a *complementary base sequence*.

1 A **restriction enzyme** cuts the double-stranded DNA molecule at its specific **recognition site**



2 The cuts produce a DNA fragment with two “**sticky**” ends

The two different fragments cut by the same restriction enzyme have identical sticky ends and are able to join together

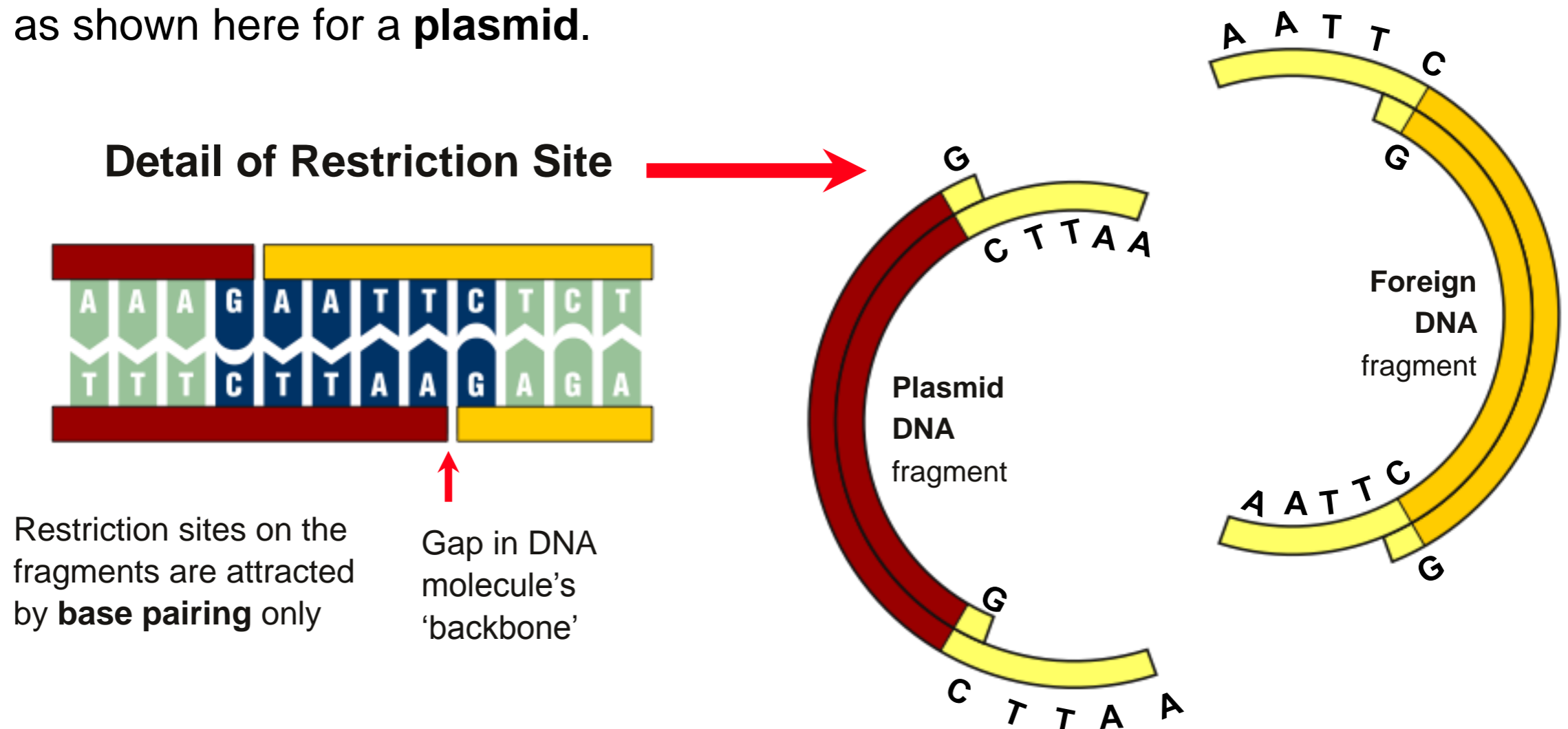


3 When two fragments of DNA cut by the same restriction enzyme come together, they can join by base-pairing

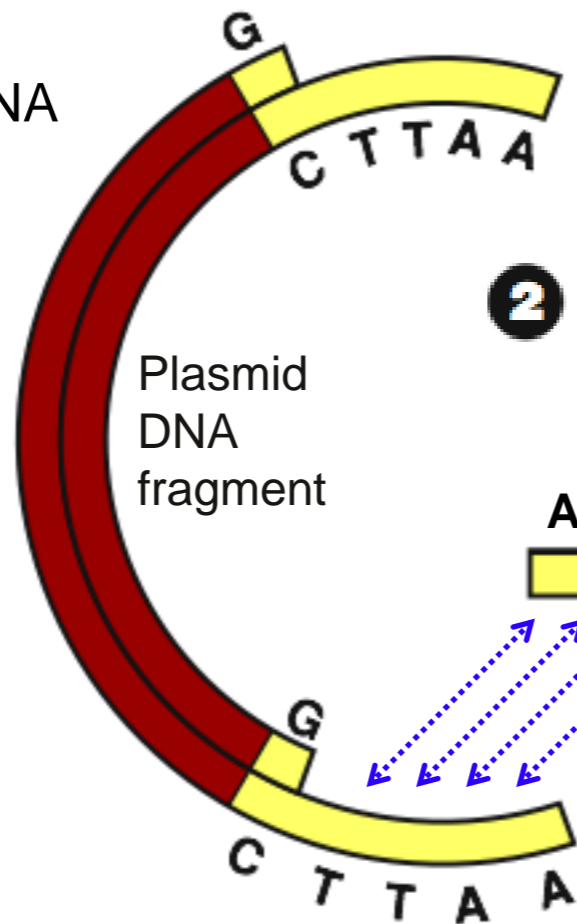
Adding a Gene

Step 3: Anneal

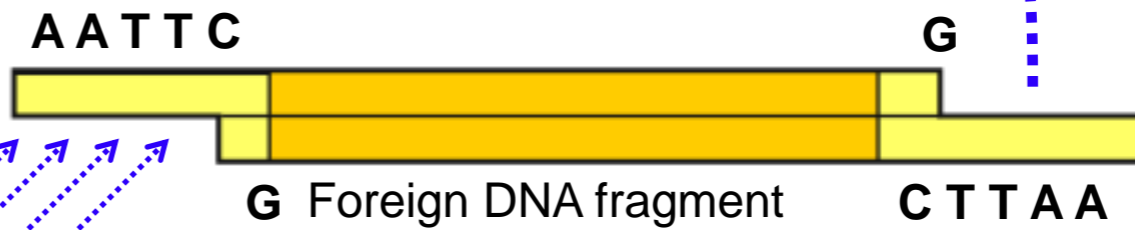
- When the two matching “sticky ends” come together, they join by base pairing. This process is called **annealing**.
- This can allow DNA fragments from a different source, perhaps a plasmid, to be joined to the DNA fragment.
- The joined fragments will usually form either a linear molecule or a circular one, as shown here for a **plasmid**.



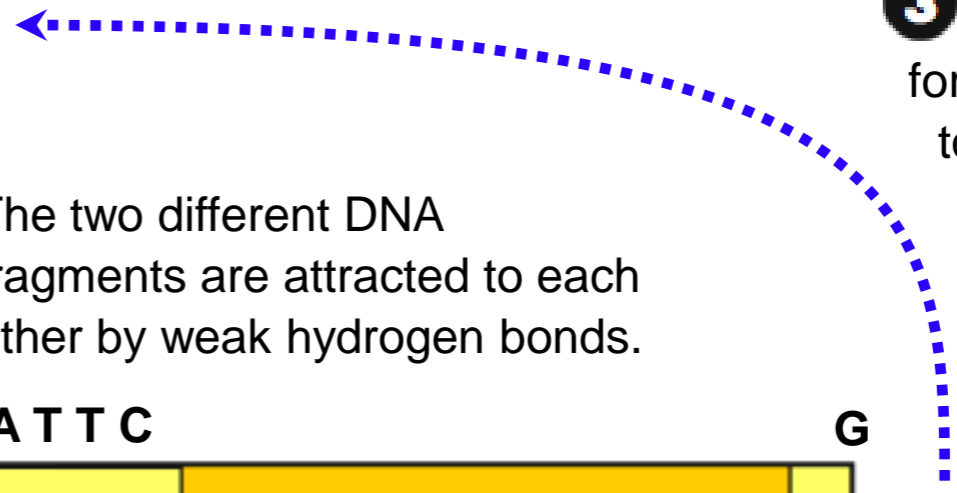
1 Two pieces of DNA are cut using the same restriction enzyme.



2 The two different DNA fragments are attracted to each other by weak hydrogen bonds.



3 This other end of the foreign DNA is attracted to the remaining sticky end of the plasmid.



Adding a Gene

Step 4: Ligation

- DNA fragments produced using restriction enzymes may be reassembled by a process called **ligation**.
- Pieces of DNA are joined together using the enzyme **DNA ligase**.

