Gene Technology

Exploring DNA

- Scientists can not explore and manipulate DNA
  - Copying DNA in a laboratory – the polymerase chain reaction
  - Use DNA to reveal its owner's identity – DNA profiling and mapping DNA by finding where every A,T, C and G is – Human Genome Project
  - Cutting and pasting genes to make new organisms – gene transfer
  - Cloning cells and animals

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

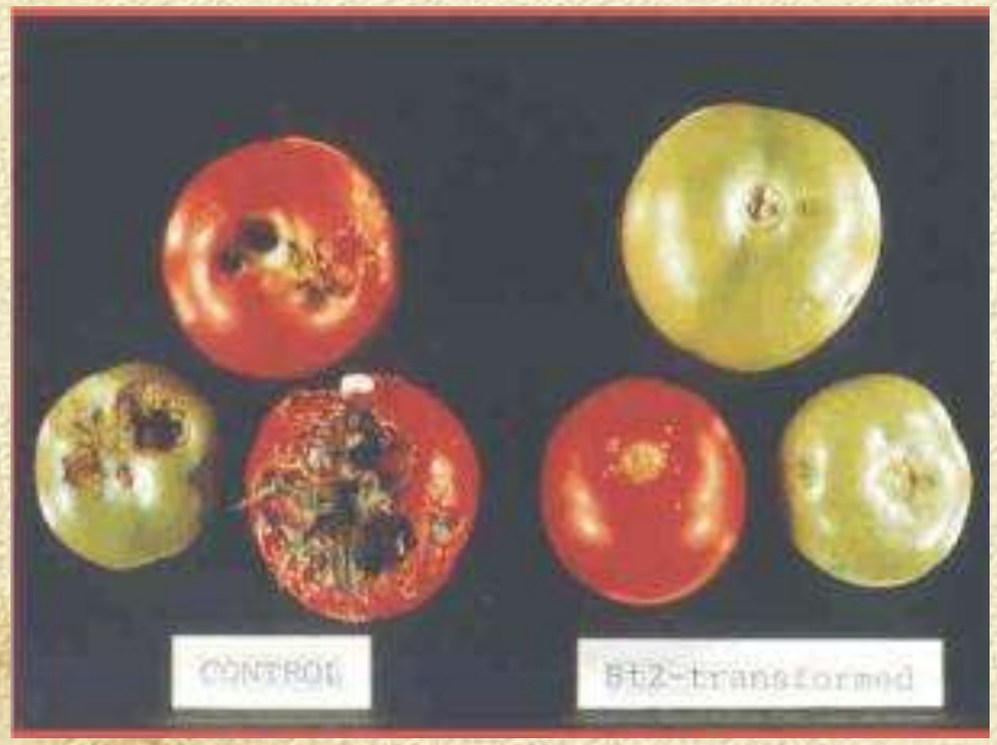
☒ Animal ethics issues

☒ The costs of errors

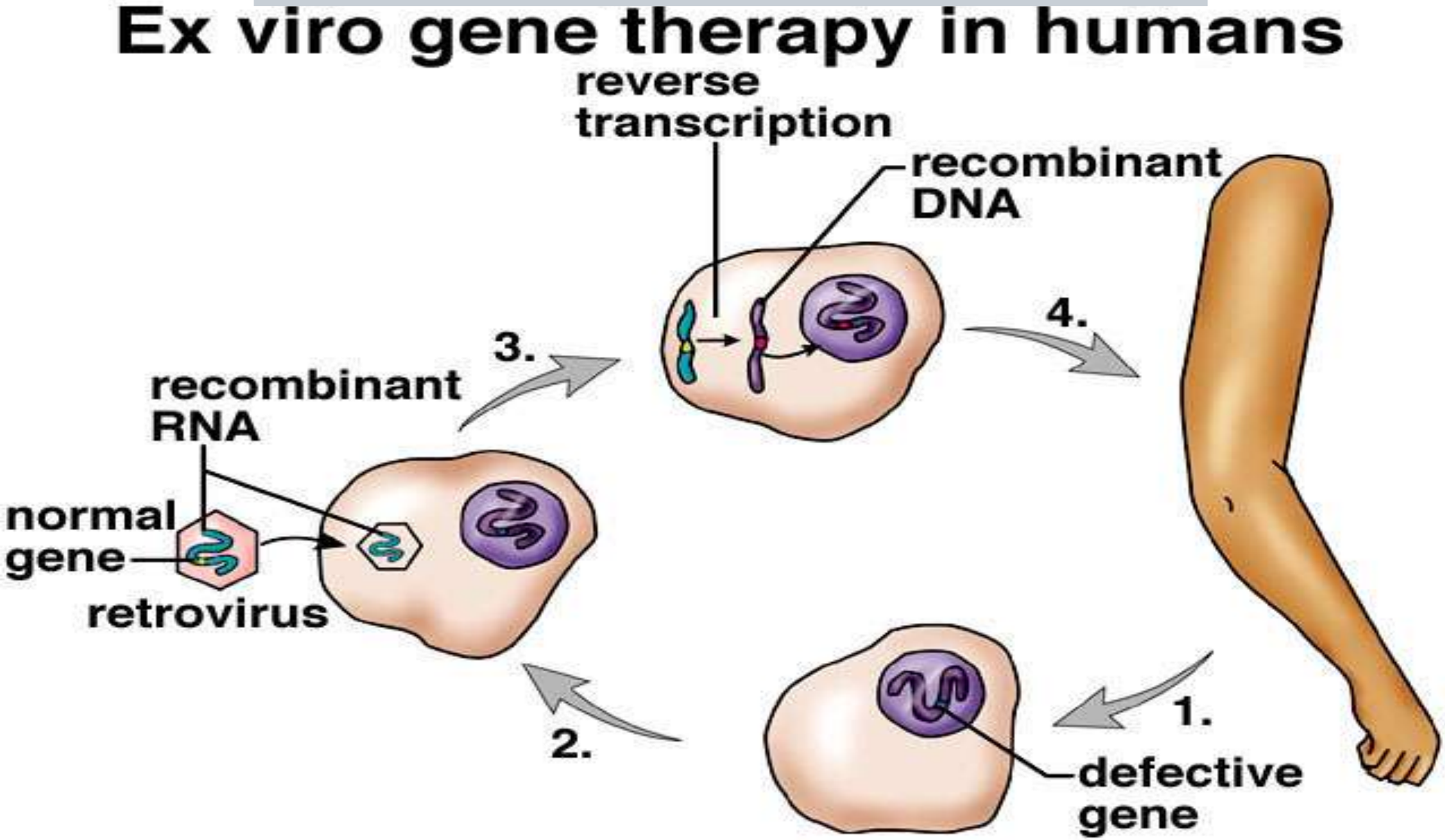
What is “**genetic engineering**” good for?

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.

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**



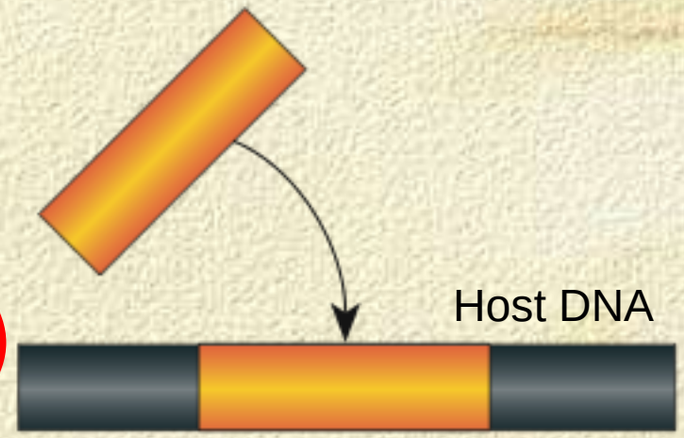
More controversial is **eugenic engineering**, the insertion of genes into a normal individual to influence a particular trait ("**designer babies**")

Producing GMOs

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

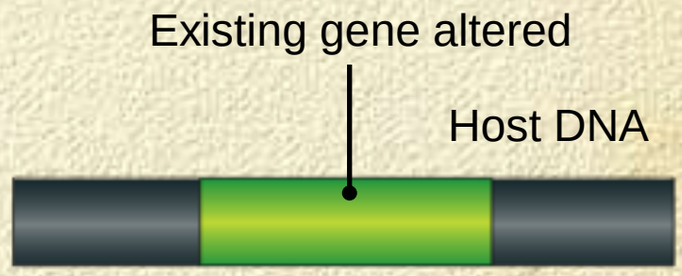
Adding a Foreign Gene

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**.

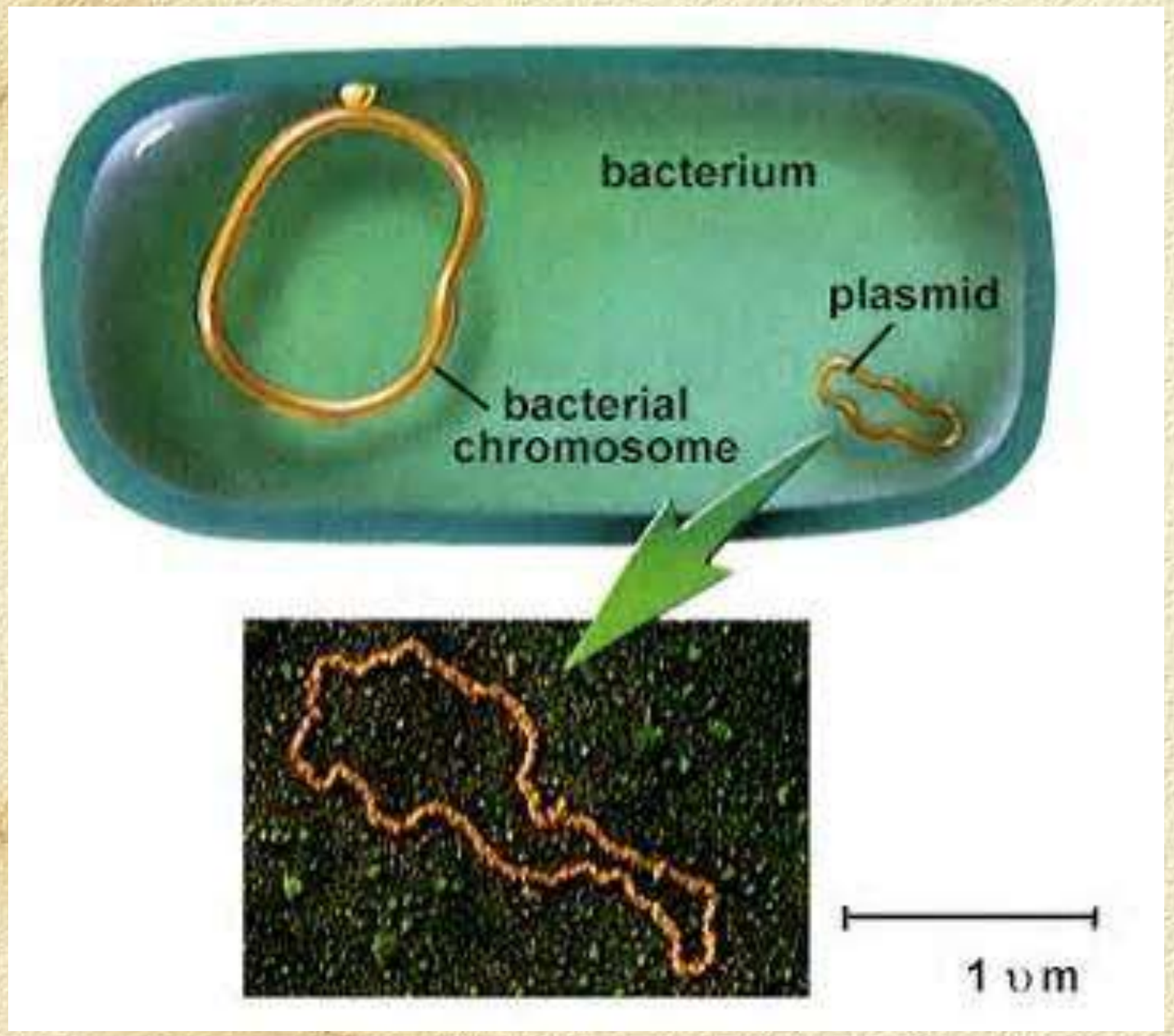


Delete or 'Turn Off' a Gene

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).

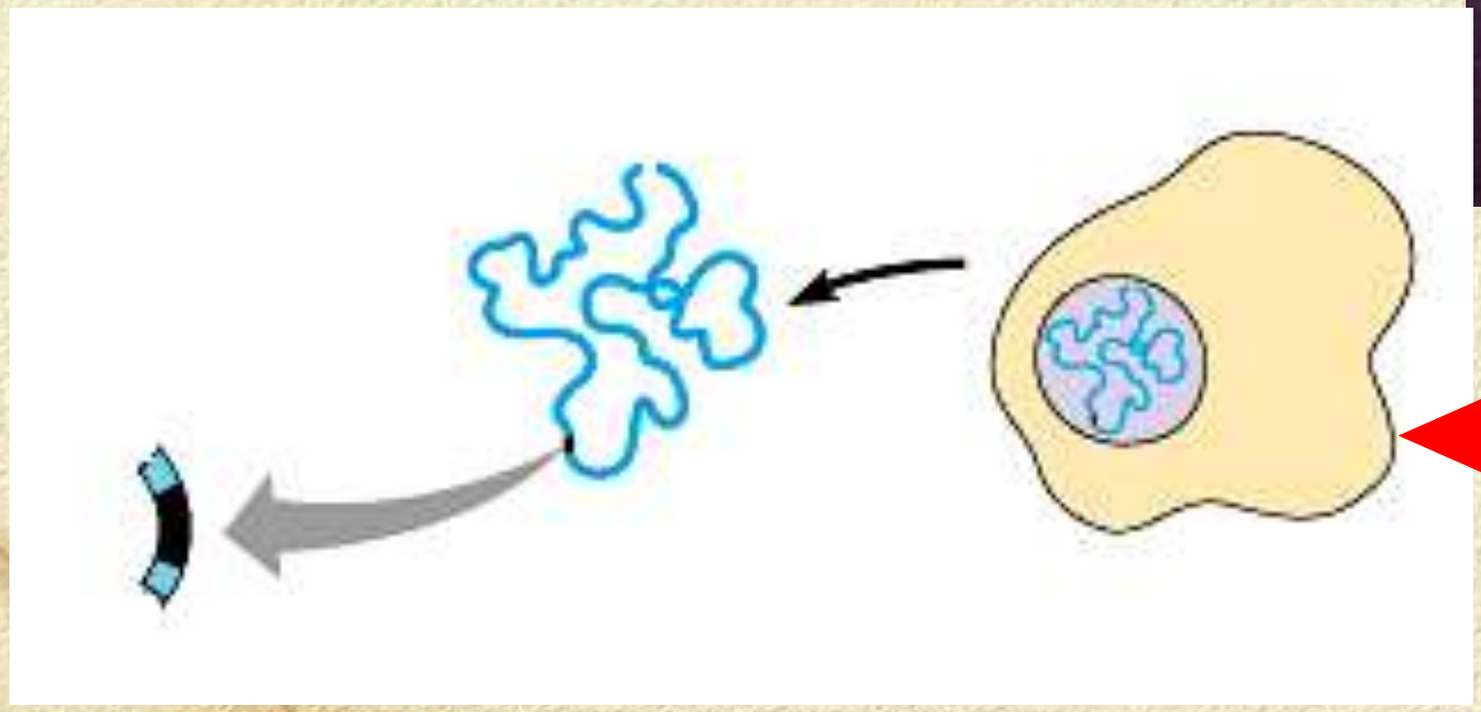
ADDING GENES AND RESTRICTION ENZYMES

Adding A Gene



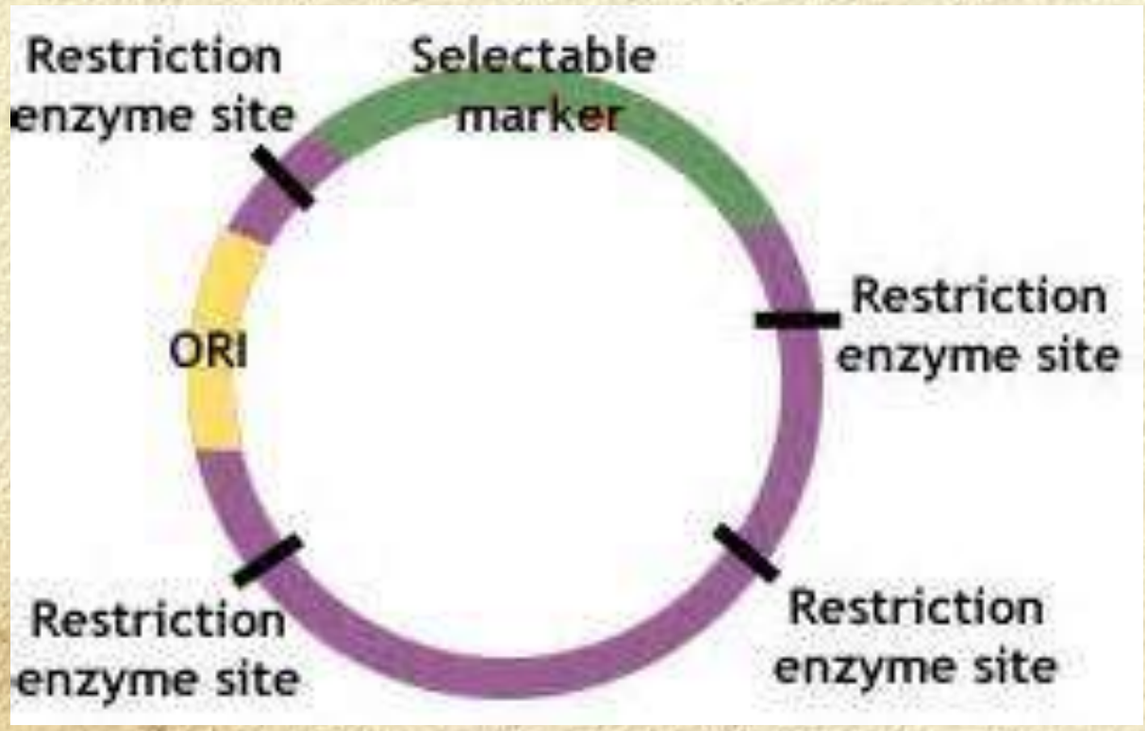
Adding a Gene
Step 1

- Use a restriction enzyme to cut out the gene of interest from it's 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..



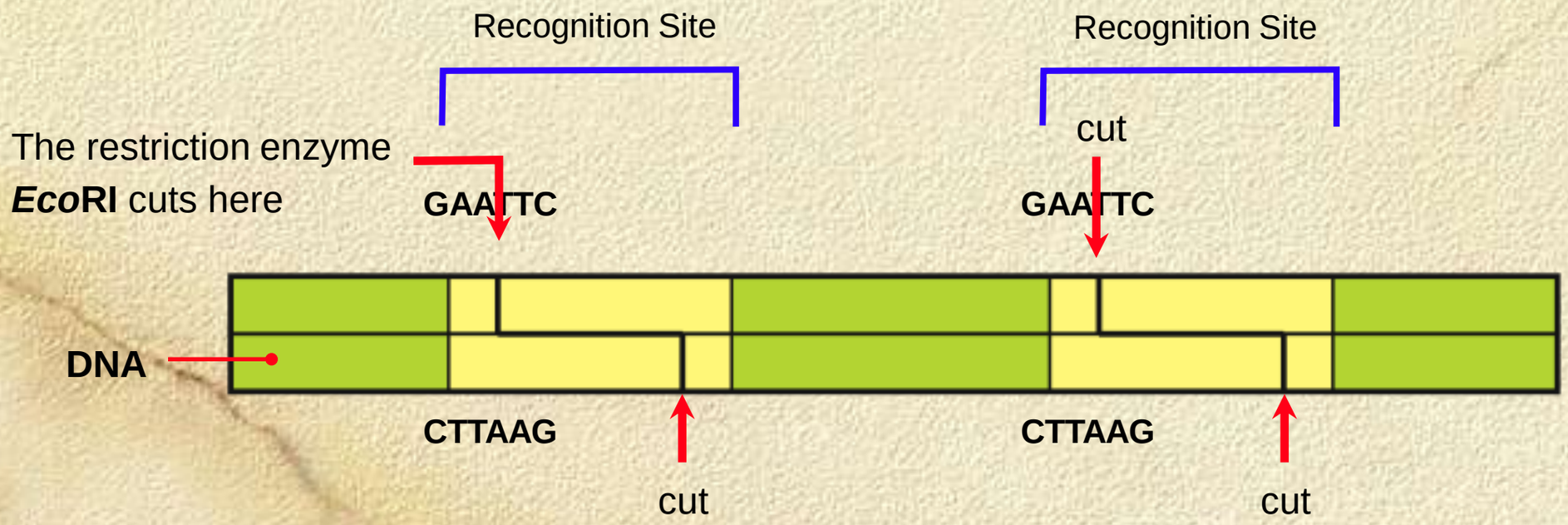
Review: Restriction Sites

- 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. ***Bam*HI** 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
***Eco*RI**	*Escherichia coli* RY13	**GAATTC**
***Bam*HI**	*Bacillus amyloliquefaciens* H	**GGATCC**
***Hae*III**	*Haemophilus aegyptius*	**GGCC**
***Hind*III**	*Haemophilus influenzae* Rd	**AAGCTT**
***Hpa*I**	*Haemophilus parainfluenzae*	**GTTAAC**
***Hpa*II**	*Haemophilus parainfluenzae*	**CCGG**
***Mbo*I**	*Moraxella bovis*	**GATC**
***Not*I**	*Norcardia otitidis-caviarum*	**GCGGCCGC**
***Taq*I**	*Thermus aquaticus*	**TCGA**

Review: Restriction Enzymes

- Restriction enzymes are used to cut DNA molecules at very precise sequences of 4 to 6 base pairs called **restriction sites** (see below)that is a palindrome.
- A DNA palindrome is a sequence in which the “top” strand read from 5' to 3' is the same as the “bottom” strand read from 5' to 3'.
  - For example,
    - 5' GAATTC 3'
    - 3' CTTAAG 5'

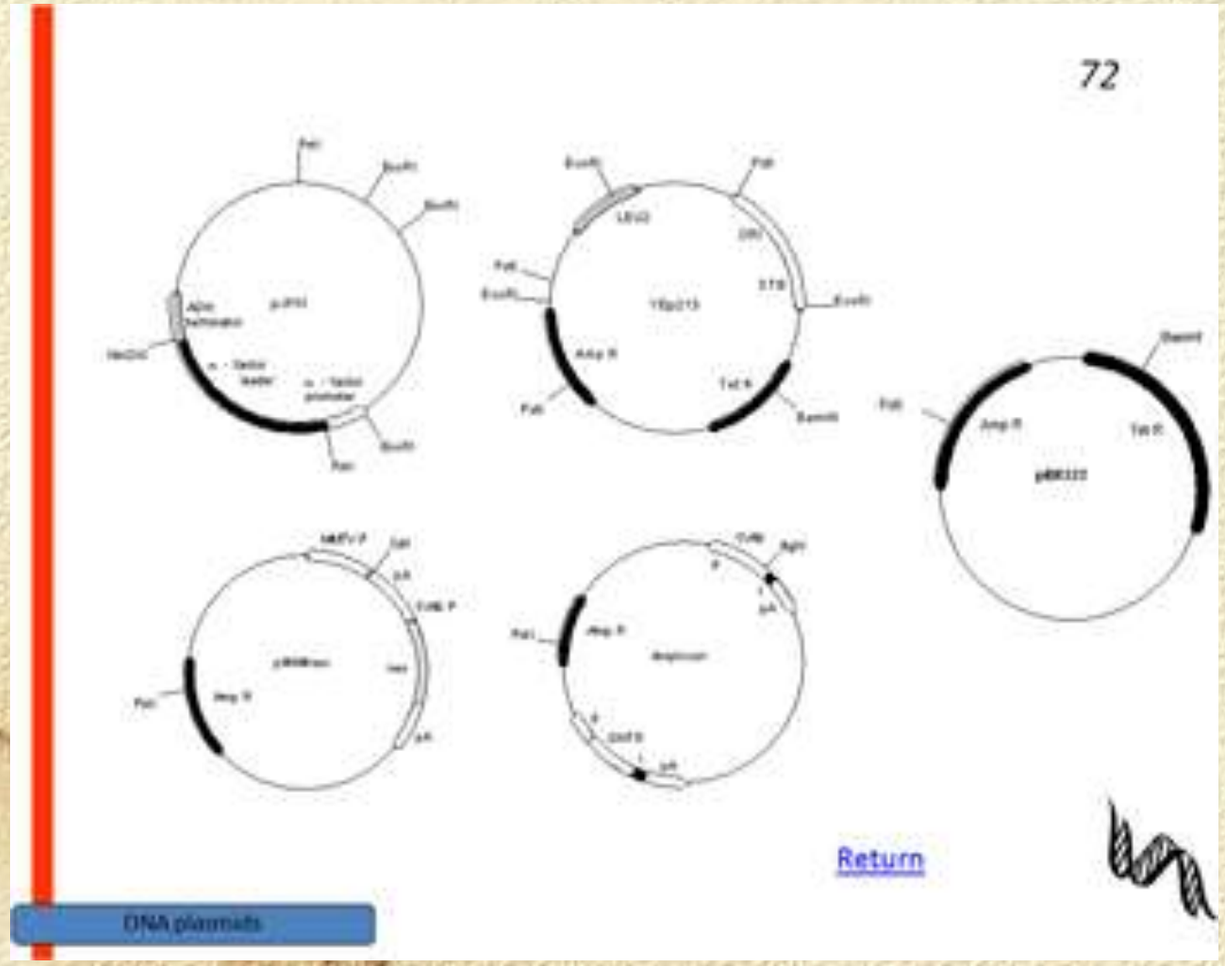


Sticky and Blunt

The end of the DNA can either be blunt or stick

Restriction Sites

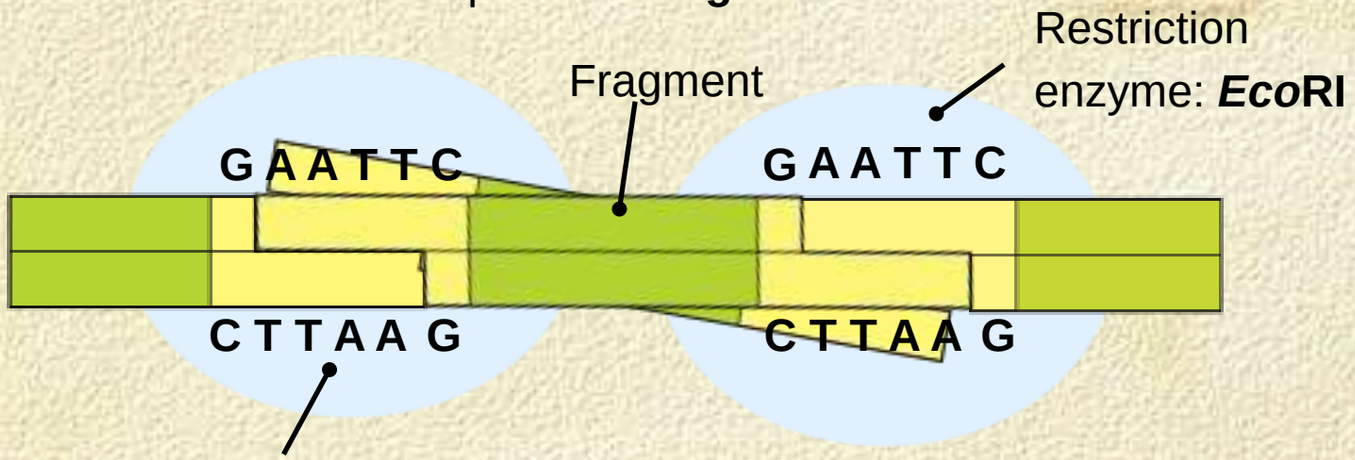
- Before cutting DNA scientists figure out the restriction map, showing the locations of cleavage sites for many different enzymes.
- These maps are used like road maps to the DNA molecule



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

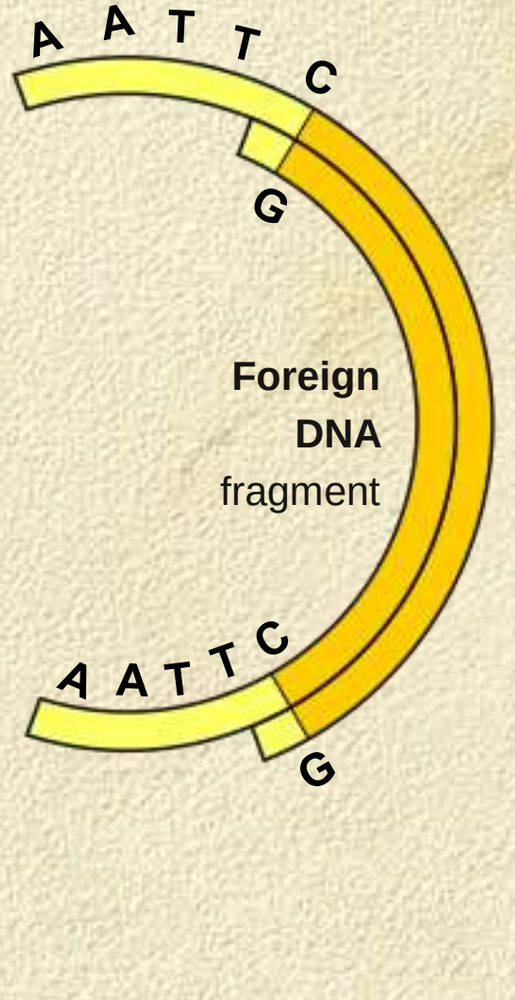
Adding a Gene
Step 3: Anneal

- 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**.

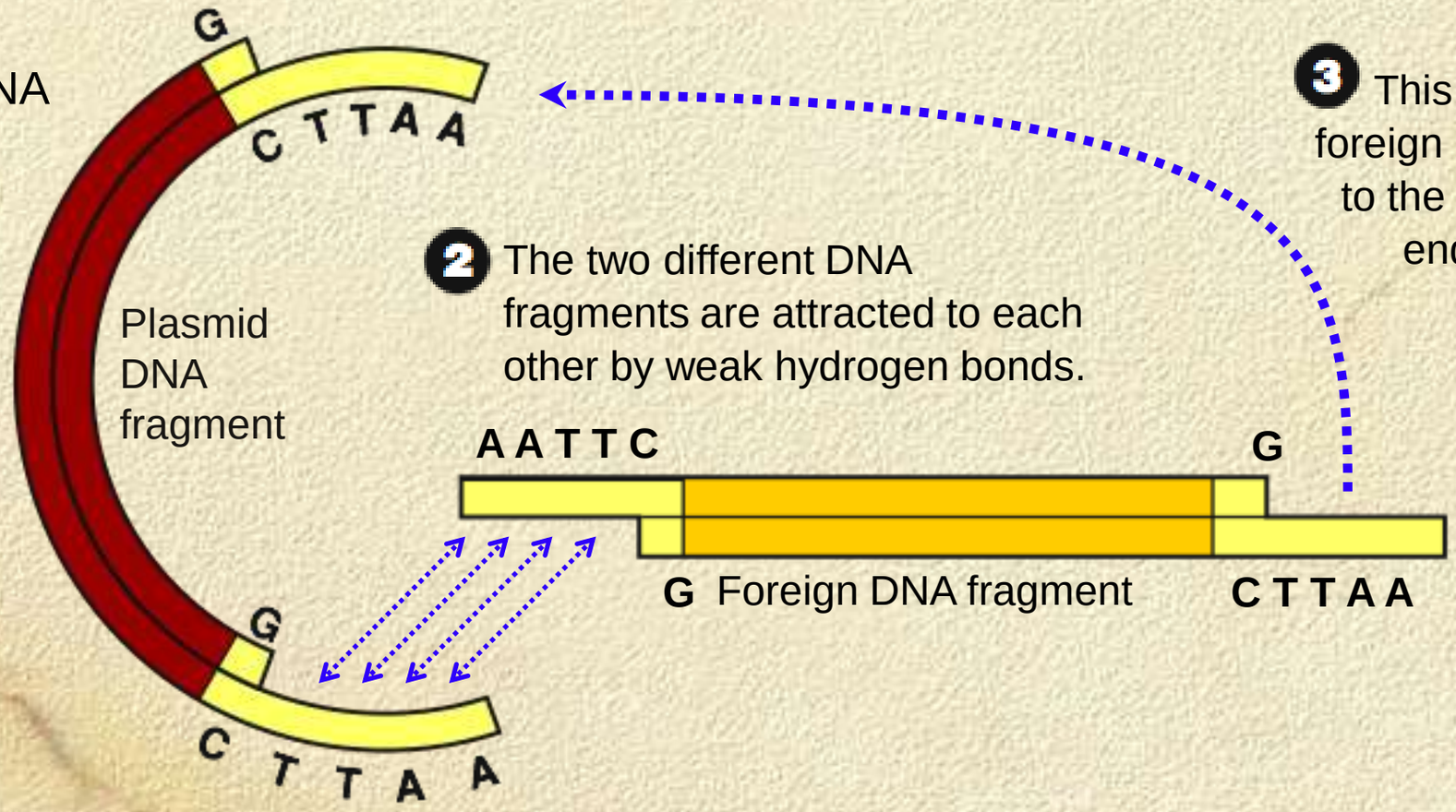
Detail of Restriction Site

Restriction sites on the fragments are attracted by **base pairing** only

Gap in DNA molecule’s ‘backbone’



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



Adding a Gene
Step 4: Ligation

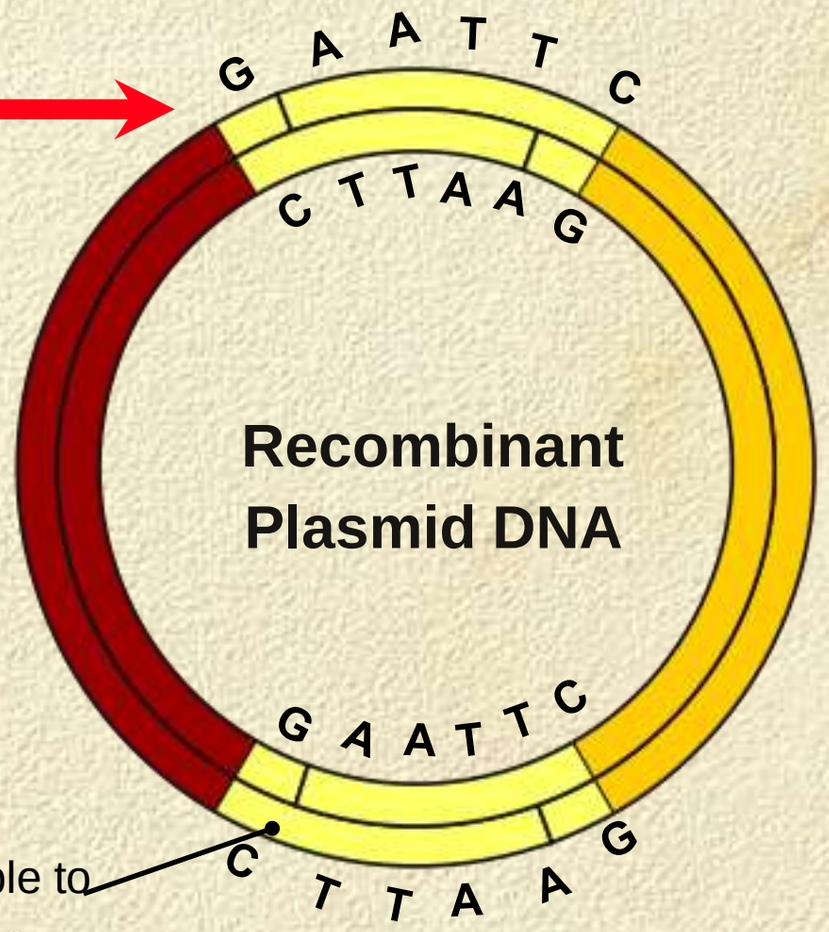
- DNA fragments produced using restriction enzymes may be reassembled by a process called **ligation**.
- Pieces of DNA are joined together using the enzyme **DNA ligase**.

Detail of Restriction Site

Fragments linked permanently by **DNA ligase**

No break in DNA molecule

DNA ligase



The fragments are able to join together under the influence of DNA ligase.