

Unit 2 Metabolism & Survival

Key Area 7: Genetic control of metabolism

Wild strains of micro-organisms can be improved by Mutagenesis or Recombinant DNA technology.

Mutagenesis

Mutagenesis is the process of inducing mutations.

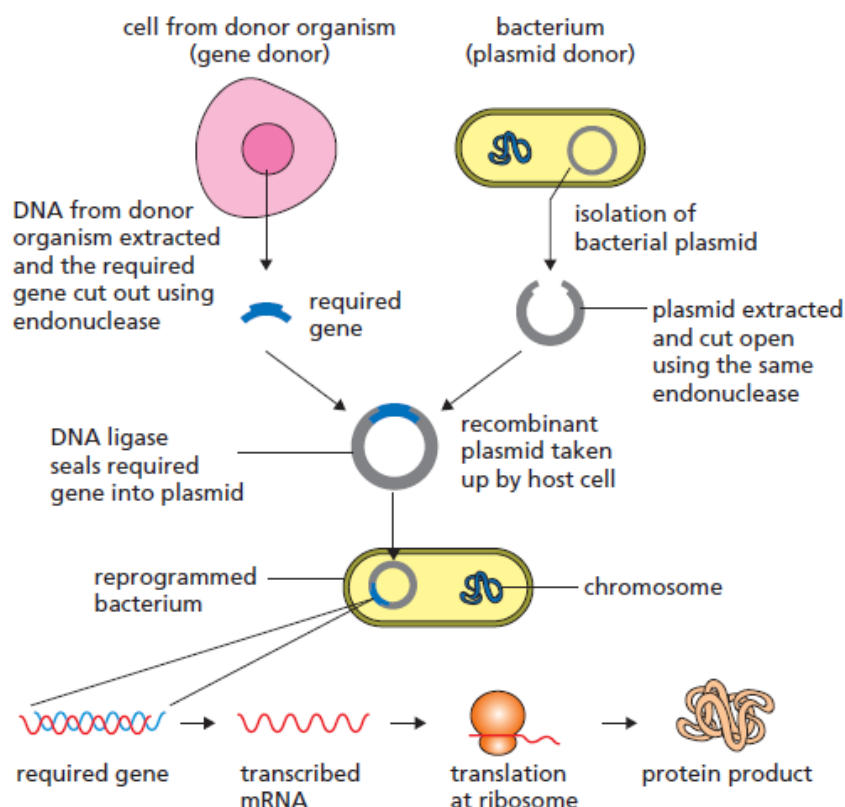
Exposure to **UV light** and other forms of **Radiation** (e.g. X-rays or Gamma rays) or

Chemicals (e.g Mustard gas) results in mutations. **Some of these mutations produce an improved strain of micro-organism.**

Recombinant DNA Technology

Recombinant DNA technology involves the use of **recombinant plasmids** and **artificial chromosomes** as **vectors**.

A vector is a DNA molecule used to carry foreign genetic information (DNA) from a donor organism into a host cell.

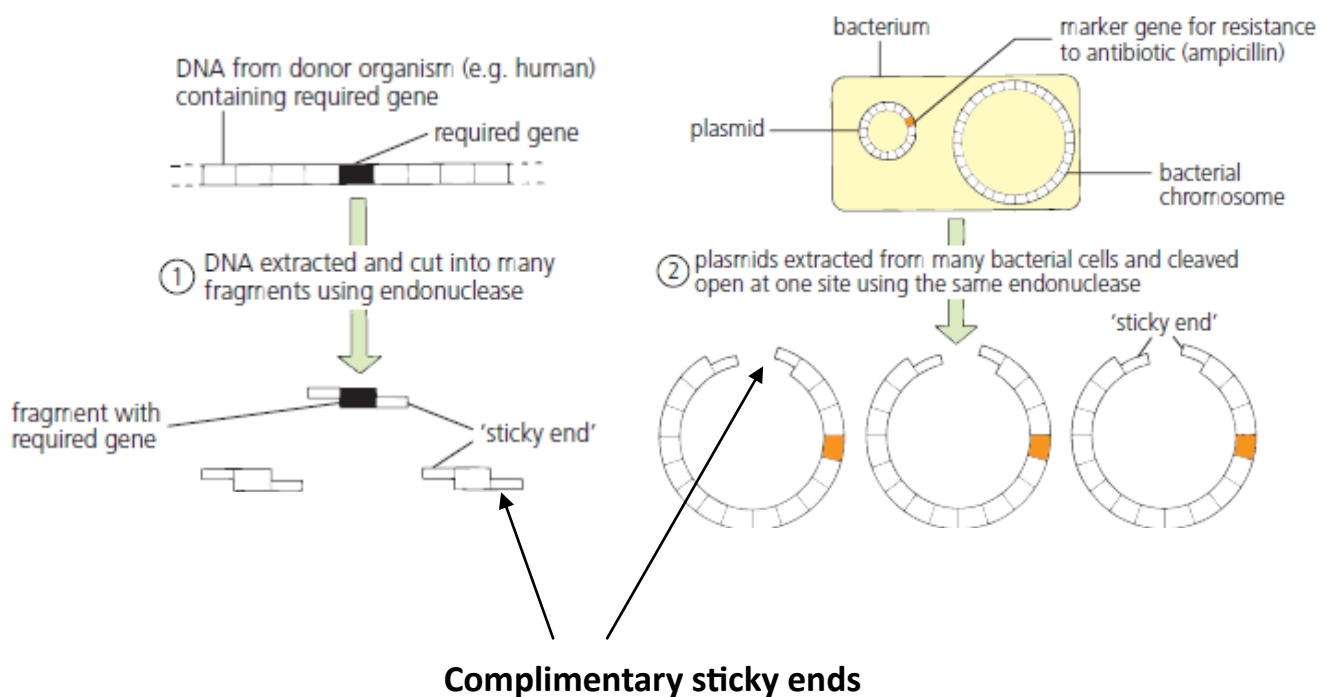


Artificial chromosomes are preferable to plasmids as vectors when **larger fragments** of foreign DNA are required to be inserted.

Restriction Endonuclease Enzymes

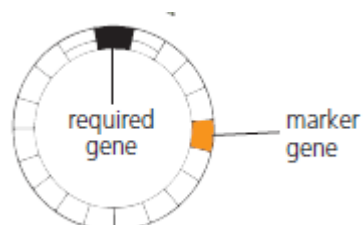
Restriction endonucleases **cut open plasmids** and **specific genes out of chromosomes** leaving **sticky ends**.

Complimentary sticky ends are produced when the **same restriction endonuclease** is used to **cut open the plasmid** and the **gene from the chromosome**.

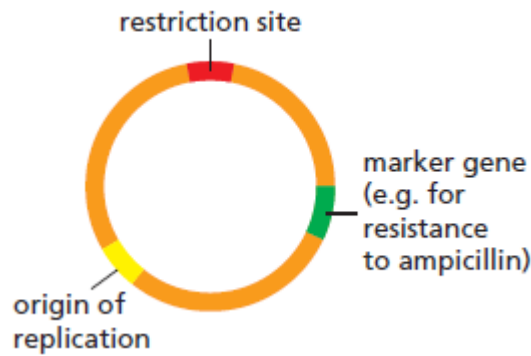


Ligase

Ligase is the enzyme used to **seal the gene into the plasmid**.



Recombinant plasmids and artificial chromosomes contain **restriction sites**, **regulatory sequences**, **an origin of replication** and **selectable markers**.



Restriction sites contain **target sequences of DNA** where **specific restriction endonucleases** cut.

E.g.

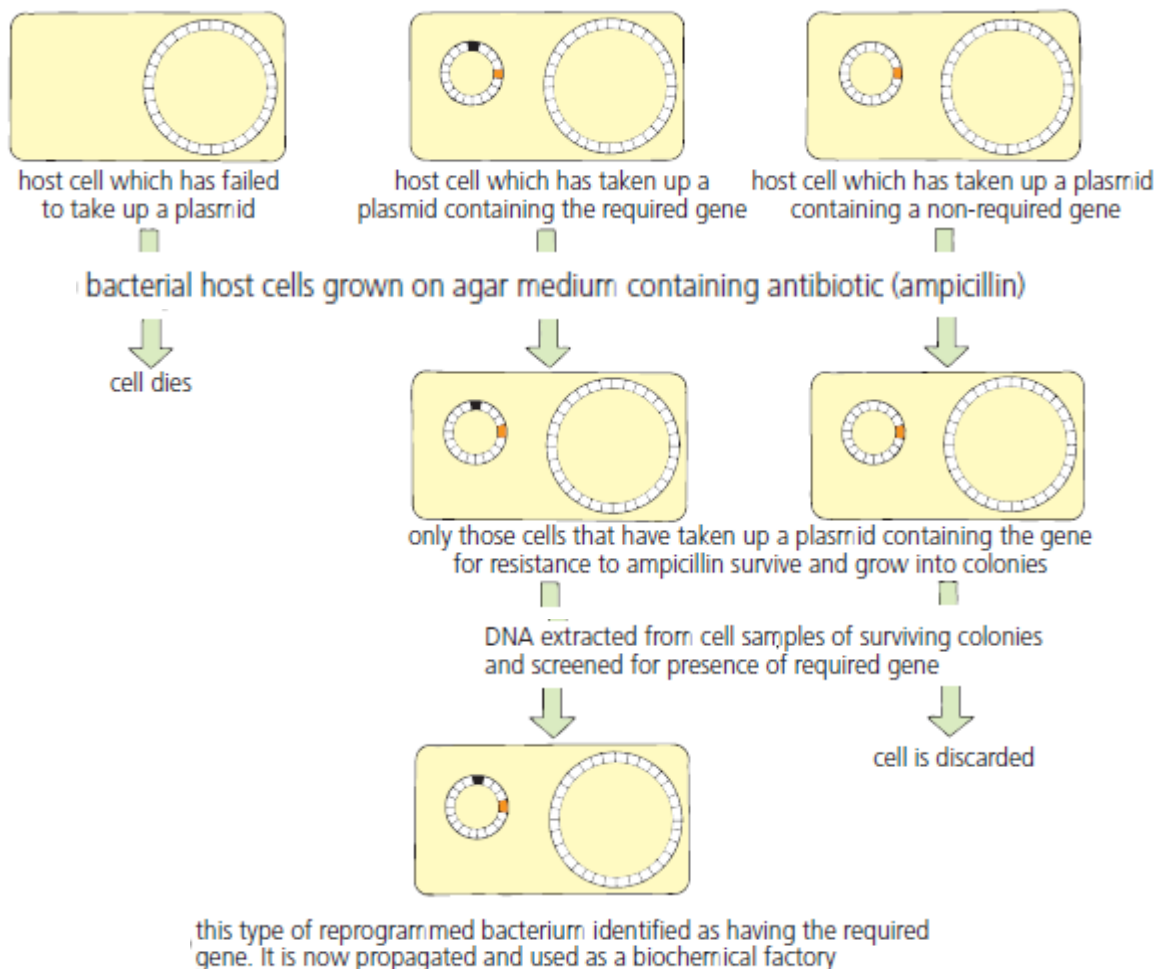
Enzyme	Recognition sequence on DNA	Position on DNA cut by enzyme	Result
1	GGCC CCGG		<p>two 'blunt' ends</p>
2	GATC CTAG		<p>two 'sticky' ends</p>
3	CTGCAG GACGTC		<p>two 'sticky' ends</p>

Regulatory sequences control gene expression.

Origin of Replication allows **self-replication** of the **plasmid/artificial chromosome**.

Selectable Marker Genes

Selectable markers such as **antibiotic resistance genes** protect the micro-organism from a selective agent (antibiotic) that would normally kill it or prevent it growing.



Selectable marker genes present in the vector ensure that only micro-organisms that have taken up the vector grow in the presence of the selective agent (antibiotic).

i.e

To identify bacterial cells which have taken up the recombinant plasmid, the cells are grown in a nutrient medium containing an antibiotic. **Only bacterial cells which contain the recombinant plasmid survive** (due to the presence of the marker gene/antibiotic resistance gene). **Bacterial cells which have not taken up the recombinant plasmid do not have resistance to the antibiotic and so are killed.**

Safety precautions

As a safety mechanism, **genes are often introduced** that **prevent the survival of the micro-organism in an external environment.**

Limitations of using Prokaryotes in Recombinant DNA technology

Plant or animal (eukaryotic) DNA expressed in bacteria (prokaryotes) may result in **polypeptides being incorrectly folded** (since they are not capable of post-translational modification).

Recombinant Yeast Cells (which are eukaryotic) may be used instead to produce active forms of the protein which are inactive in bacteria.