

Recombinant DNA technology

Improving Wild strains of microorganisms

1. Mutagenesis

Exposure to UV light and other forms of radiation or mutagenic chemicals results in mutations which may produce an improved strain of micro-organism.

2 Recombinant DNA technology

plant/animal genes transferred to microbes to make desired animal/plant protein.

Two key enzymes in Recombinant DNA technology

1. Endonuclease

Same endonuclease is used to cut open the plasmid and cut the gene out of the chromosome to produce COMPLEMENTARY sticky ends.

2. Ligase

Seals genes into plasmid.

Vector

A vector is a DNA molecule used to carry foreign genetic information into another cell.

Types of Vectors

1. Plasmids

2. Artificial chromosomes

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

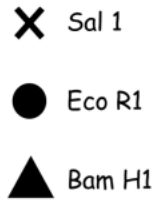
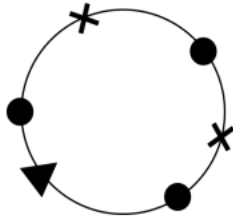
Bacteria vs Yeast Plasmids

In bacteria the protein cannot fold the polypeptide properly so often the protein is inactive.

Yeast cells avoid this problem as they can fold the polypeptide correctly and the protein in active.

Restriction Endonuclease Puzzles

Circle Plasmid Endonuclease Puzzles



Rule

Number of restriction sites = Number of fragments produced

Worked Example

Sal I = 2 restriction sites = 2 DNA fragments

Sal I + Eco R1 = 4 restriction sites = 4 DNA fragments

Linear DNA Endonuclease Puzzles



Rule

Number of restriction sites = Number of fragments produced PLUS ONE

Name of enzyme	Shape
Eco R1	Triangle
Bam HI	Square
Sal I	Circle

Worked Example

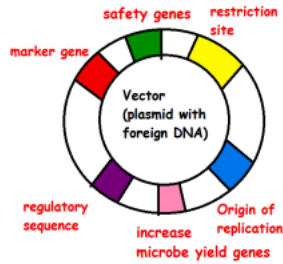
Sal I = 1 circle restriction site = 2 DNA fragments

Sal I + Eco R1 = 3 restriction sites (1 circle & 2 triangle) = 4 DNA fragments

Genes on Vector

Genes on Vectors

1. Selective marker gene (Antibiotic resistance)
2. Regulatory sequence
3. Restriction site
4. ORI sequence
5. Safety genes



Restriction Site

Contain target sequences of DNA where specific restriction endonucleases cut .

ORI sequence

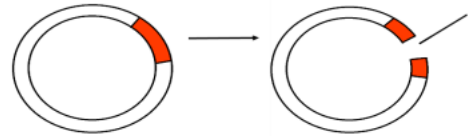
Self replication of plasmid/ artificial chromosome.

Regulatory sequences

Controls gene expression (turn genes ON or OFF).

Safety genes

Introducing genes to prevent microbes surviving in external environment



Selectable marker (Antibiotic Resistance)

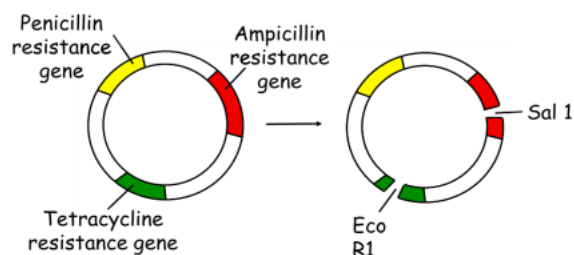
Expose bacteria to selectable marker (antibiotics)

Only transformed bacteria/those with plasmid survive/grow as they have antibiotic resistance.

Interrupting genes on a vector

Restriction sites can often cut through genes on a vector, interrupting the gene expression.

Restriction enzymes Eco R1 and Sal 1 have interrupted the Ampicillin and tetracycline resistance genes which result in these genes coming inactive.



Penicillin is unaffected therefore the resistance gene will still be expressed.

Genes on Vector

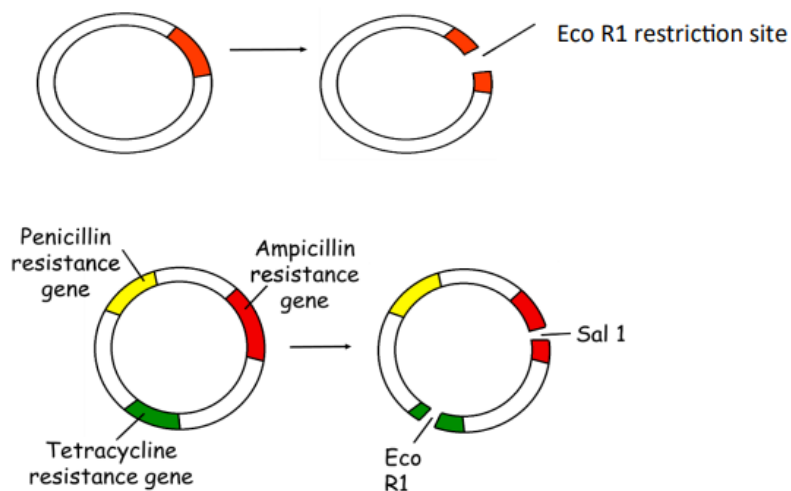
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