8.4 GENE TECHNOLOGIES

Recombinant DNA technology

- Complementary sticky ends between plasmid and DNA fragment
- Restriction endonucleases cut plasmid to allow insertion of DNA fragment
- Ligases join the plasmid and DNA
- Vector transports DNA fragment into host cell e.g. plasmid
- Need to add promoter and terminator regions to the DNA fragment
- Plasmid now has recombinant DNA
- Plasmid is introduced into host cells
- Requires calcium ions and temperature changes
- Cells become transformed
- Marker genes are used to identify which cells have taken up the plasmid
- Cells are cultured, gene product is collected

Amplification of the DNA fragment or gene

- Transforming cells and culturing them is an *in vivo* way of amplifying DNA fragments
- Polymerase chain reaction (PCR) is a way of amplifying DNA fragments *in vitro*
- Temperature changes
- Requires the DNA fragment, DNA polymerase, nucleotides, primers and a thermocycler

Transfer of DNA fragments between organisms

- Production of fragments of DNA
  - Convert mRNA to cDNA by reverse transcriptase
  - Restriction enzymes to cut DNA at specific positions
  - Gene machine to create the gene fragment

Genetic fingerprinting

- DNA probes are short, single-stranded DNA sections with a label attached
  - Hybridisation with DNA
  - Can be used to identify specific alleles, and so can be used to screen for genetic conditions and for personalised medicine
  - Radioactive or fluorescent labels
- Gel electrophoresis
  - Separates fragments by size – smallest ones travel furthest
- Variable number tandem repeats (VNTRs)
  - Unique to each person
  - Medical diagnosis e.g Huntington's disease
  - Forensic science
  - Can determine genetic relationships

AQA

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