GENE TECH

genetic engineering

- aim: remove a gene from 1 organism & transfer it into another. So gene is expressed in new host.
- Recombinant DNA (rDNA) => DNA made by joining pieces from 2 or more different sources.

gene transfer overview

1. gene of interest cut from a chromosome & isolated (restriction enzymes)
4. Multiple copies of gene are made by PCR
2. gene inserted into vector...
   - vector inserts gene into new cell
3. cells that have the new gene are identified & cloned.

- tools:
  - → enzymes (restriction endonucleases, ligase, reverse transcriptase)
  - → vectors (plasmid / virus)
  - → gene of interest
  - → host

1 restriction enzymes

- naturally produced by bacteria → restriction endonucleases
  - ↳ natural function → break down DNA of invading viruses
- Each binds to a specific target site on DNA & cuts
  - ↳ specific sequence of bases
- many restriction sites are palindromic
- cut to give sticky / blunt ends
- sticky ends → short lengths of unpaired bases
  - → can easily form H-bonds w/ complementary sequences of bases cut w/ same restriction enzyme.
- gel electrophoresis & gene probes used to find gene of interest
- PCR used to make copies

2 vectors

- Getting plasmids into host cell:
  - → A gene is removed from DNA, using a restriction enzyme.
  - → Plasmids extracted from bacterial cells (centrifuge), & cut using same restriction enzyme
  - → isolated gene & cut plasmid have complementary sticky ends. ∴ join by H-bonds
  - → DNA ligase used to form phosphodiester bonds. rDNA formed.
  - → bacteria put into ↑[Ca²⁺], cooled, then heat shock. take up plasmid w/ gene → transformed.

of plasmids

- exist naturally in bacteria ∴ easily taken up.
- small ∴ easy to use.
- double stranded ∴ pro & eukaryotic genes can be used
- replicate independently within bacteria
- can be transferred between diff species.
- of ARTIFICIAL PLASMIDS
  - → low Mr ∴ readily taken up
  - → several target sites for diff restriction enzymes in short length of DNA
    - → polylinker
  - → one or more marker genes ∴ cells taken up plasmid can be identified.

3 identifying bacteria w/ rDNA

- antibiotic resistance
  - ↳ gene inserted @ point for resistance then grown on agar.
- GFP (Green fluorescent protein)
  - ↳ enzymes that make this protein inserted onto plasmid.
  - ↳ UV ∴ to identify.
- GUS (enzyme β-glucuronidase)
  - ↳ if cell contains this enzyme, when incubated w/ specific colourless substrates, can transform into coloured.
- DNA polymerase in bacteria copies plasmids,
- bacteria divide by binary fission,
- each daughter cell have copies ∴ transcribe & translate gene.

insulin production

- instead of cutting out gene, insulin mRNA extracted.
- mRNA incubated w/ enzyme reverse transcriptase → cDNA
  - ↳ make single stranded DNA
- cut w/ restriction enzyme & inserted into plasmid.

promoters

- if want gene in bacterium to be expressed, must insert promoter.
- insulin gene inserted next to β-galactosidase gene so they shared a promoter.
- promoter switched on the gene when lactose present & glucose absent
- promoter allows RNA polymerase to bind to DNA & recognise which is template strand.

for mRNA to bind

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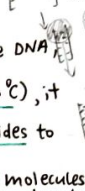
gel electrophoresis

- for proteins / DNA
- place mixture into wells cut into agarose gel & apply electric field.
- movement depends on:
  - net charge
  - size
  - composition of gel.
- PROTEINS.
  - used to separate polypeptides produced by diff alleles of many genes.
  - in sickle cell, a variant of β-globin has amino acid with non-polar R ∴ can be separated & compare.
- DNA (−)
  - fragments move to anode (+) due to phosphate group.

polymerase chain reaction

(4)

- what is added:
  - DNA sample, primers (NOT promoters), nucleotides, Taq polymerase, buffer.
- PCR = method for rapid production of a very large number of copies of a particular fragment of DNA.
- PROCESS:
  - DNA heated briefly (90°C) to denature DNA which separates the double helix.
  - Primer DNA is added after cooling (65°C), it attaches to start of DNA strand.
  - DNA polymerase uses free nucleotides to synthesise complementary strands.
  - gene copied & forms part of 2 DNA molecules
    - heating then denatures DNA, starts a new cycle of copying.
- not destroyed by denaturation step ∴ does not have to be replaced.
- high optimum temp ∴ efficient. ∵ elongation temp does not have to be dropped below annealing process.



bioinformatics

↳ collection, processing & analysis of biological information and data using computer software.

- for comparison
- for understanding genes & proteins
- development of vaccines.

gene tech & medicine

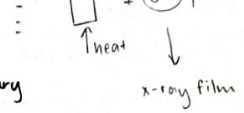
bacteria:
- few ethical problems
- no need to collect blood from many donors.
- simple nutritional requirements
- not much space required.

bacteria:
- bacteria do not modify proteins in same way as eukaryotes.

- large scale production
- protein identical to human protein

genetic profiling

- region of DNA that is known to vary between different people is chosen.
- they have VNTRs. ····AGGAGGAGG···· ····AGGAGG···· (→ diff length in diff ppl)
- DNA cut using restriction enzymes known to cleave it close to VNTR regions.
- use electrophoresis to separate
- to make fragments visible:
  - transferred onto absorbent paper
  - paper heated to separate DNA molecules
  - probes added, they have complementary base sequences to VNTRs.
  - probes radioactive so when paper placed on x-ray film, film goes dark @ matching positions w/ VNTR.
  - OR use probes w/ fluorescent stain.



microarrays

- short lengths of single stranded DNA are attached to a piece of plastic.
- compare genes in 2 species
- detect which genes are being expressed at any specific time.
- DNA collected, cut into fragments & denatured = single strand.
- each labelled w/ diff colour flourescent tags
- mixed together & hybridise w/ probes
- UV ∴, if spot mixture of both colours, they have DNA w/ exactly same base sequence.
- mRNA from 2 types of cells collected.
- reverse transcriptase used to convert mRNA to cDNA
- cDNA labelled w/ flourescent tags & allowed to hybridise w/ probes.
- spots that flouresce indicate genes being transcribed in the cell.
- intensity of light emitted by each spot indicates the level of activity of each gene.

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genetic screening

- check for the presence of a particular allele.
- screened for faulty alleles (cancer) / in embryo
- 'designer baby'
- → treat & reverse genetic disease in humans
  - → carry out genetic tests to see if ppl are carriers
  - → carry out " see if fetus has genetic disease.
  - → identify specific genes (eg breast cancer)
- ETHICS
  - → if affects ethnic groups, avoid discrimination
  - → if fetus has condition, some have therapeutic abortion.
  - → some embryos 'chosen' (preselection)
- provides info about increased risk of people having genetic conditions.
- → prepare for late onset genetic conditions
- → identify whether embryos will develop genetic condition.
- → identify fetuses that needs treatment
- → allow parents to prepare for child's treatment.
- → early diagnosis
- → allows couple who are carriers to make decisions about starting a family.

gene therapy

- → cure disorders by inserting normal alleles into cells.
- → common vectors → viruses
  - → liposomes
- → side effects → leukaemia ∵ genes inserted randomly by retrovirus.

cystic fibrosis

- → abnormally thick mucus
- → prone to bacterial infections
- → ducts easily blocked.
- → due to recessive allele of gene that codes for CFTR.

CFTR

- → sits in cell surface membrane & allow Cl^- to pass out of cells
- → water moves out & dilutes mucus.
- → In CF, most common = CFTR missing 1 amino acid.
  - ↳ ∴ cell does not place faulty CFTR in csm
- → CF caused by single gene ∴ good candidate
- → Problems:
  - → short lifespan of nose lining cells (aerosol)
  - → virus caused side effects (virus vector)
- → alternative:
  - → PTC124 drug - allow translation of protein even w/ stop codon
    - ✓ just 1 pill/day
  - → DNA inserted directly (naked DNA)
    - ↳ CF(?) usually for muscle / heart disorders.

somatic & germ cell gene therapy

- → insert allele into germ cells
  - ↳ cells involved in sexual reproduction.
- → illegal in humans
  - ↳ ALL cells of child would be produced from engineered zygote.

herbicide resistant crops

- → Glyphosate inhibits enzyme involved in synthesis of 3 amino acids.
- → normally plant would die,
- → microorganisms have versions of the enzyme that are unaffected
- → transferred into crop plants.
- → GM plant will become an agricultural weed
- → pollen will transfer to wild relatives, producing hybrid offspring that are invasive weeds
- → herbicide-resistant weeds will evolve because so much of same herbicide used.

insect resistant crops

- → detrimental effects
  - → evolution of resistance by the insect pests
  - → damaging effect on other species of insects
  - → transfer of added gene to other species.
- → less pesticide used ∴ ↓ risk to non-target species.
- → Bt toxin lethal to insects but harmless to other animals.
- → taken from Bacillus thuringiensis
- → ∴ crops w/ Bt toxin gene produce their own insecticides.
- → insect populations can evolve resistance to toxins.
- → ↑ no. of crop plants containing genes for Bt toxin accelerates evolution.
- → GM crop seed ##, its cost may remove any advantage.
- → if ↑ GM grown, danger of losing biodiversity.

golden rice

- → produce variety of rice that contains carotene in endosperm.
- → genes for carotene production extracted from maize & bacterium.
- → genes inserted into bacteria which can naturally infect plants.
- → bacteria mixed w/ rice embryos.
- → CONTROVERSIES
  - → GR wrong way to solve vit A deficiency
  - → help out of poverty instead.

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GM animals

- growth hormone regulating gene & promoter injected into fertilised egg of Atlanti salmon
- ∴ can produce growth hormone throughout the year.
- safe ∴ GM salmon do not compete w/ wild salmon & has no significant effects on environment
- Female Gmo sterile.

Social implications of GM

- improved, cheaper medicines
- improved food supplies
- improved nutritional quality of foods
- cleaner environment
- improved treatment of genetic diseases.

- unexpected reductions in crop yields due to ecological disturbance.
- farmers made dependent on specific varieties, needing fresh seed annually & expensive fertilisers
- reduced natural biodiversity ∴ reduced possibility of new varieties arising
- reduced effectiveness of antibiotics as more bacteria became resistant
  - ↳ antibiotic-resistance genes could be accidentally transferred into pathogenic organism.
- herbicide that can now be used on crop will leave toxic residues.