

Recombinant DNA:

=> DNA made artificially joining together pieces of DNA from two or more different species/organism.

2) Genetic engineering

The deliberate manipulation

of genetic material to modify

specific characteristics of an

organism and that this may

involve transferring a gene into

an organism so that it is expressed.

~~genes to be transferred into an~~

Explain how gene ~~that~~ can be transferred into
into ~~an organism~~ is synthesized by ~~and~~

⇒) ~~extracting extracted from the~~
DNA of a donor organism

⇒) by using enzyme restriction
endonucleases (details)

⇒) synthesised from the mRNA of
a donor organism by
reverse transcriptase enzyme.

↳ makes ~~cDNA~~ from mRNA.

⇒) synthesised chemically from
nucleotides.

Roles of restriction endonucleases:

- ⇒ Cuts DNA at specific site or base sequence.
- ⇒ Sequence recognised by the enzyme is usually palindromic so sticky ends are produced; they are cut in staggered fashion.
- ⇒ Enzyme derived from bacteria which are used by bacteria to destroy viral DNA.

Roles of DNA ligases

⇒ joins sugar-phosphate backbone by forming phosphodiester bond between gene and vector.

Roles of Reverse transcriptase:

⇒ makes cDNA from mRNA

Roles of DNA polymerase

⇒ Makes double-stranded DNA from ssDNA/cDNA.

- Roles of Plasmid \rightarrow (so can be taken up by cells/bacteria)
- \Rightarrow Small circles of DNA
 - \Rightarrow easy to extract from bacteria.
 - \Rightarrow , contains restriction sites
 - \Rightarrow Can multiply independently.
 - \Rightarrow Circular so increased stability. ~~Add a vector~~

Why promoter is added in a organism as well the desired gene?

- # to allow binding of RNA polymerase and transcription factors.
- # to switch on gene so the gene is expressed by transcription.

⇒ at right time and in
correct tissue.

⇒ in eukaryotes, precise position
of promoter is important

regulation in bacteria is not
so complex as in eukaryotes

and for protein synthesis

part of transcription and
post-transcriptional modification

is required in eukaryotes

and involves many steps

Crene marker

- => identification of the transformed cells
in recombinant.
- => Easy to detect
- => No Bad effect observed in GM organism (Use of fluorescent as marker in gene technology)
- 2) marker gene linked to gene for protein. => emits bright light when exposed to UV light.
- => with promoter => add marker gene to the vector
- => more economical & fine grained => easy to identify transformed bacteria
- => gene of interest added close to marker gene
- => easy identify recombinant DNA and transgenic organisms
- 2) no known risk.

Principle of genetic engineering

⇒ The deliberate manipulation of DNA to modify specific characteristics of an organism.

↓
⇒ The gene (that will be added) is cut from the species using restriction enzyme.

⇒ Plasmid Vector is cut by restriction enzyme.

⇒ Combine the gene with plasmid by DNA ligase.

⇒ Introduce the recombinant plasmid

into the host cell (Bacteria)

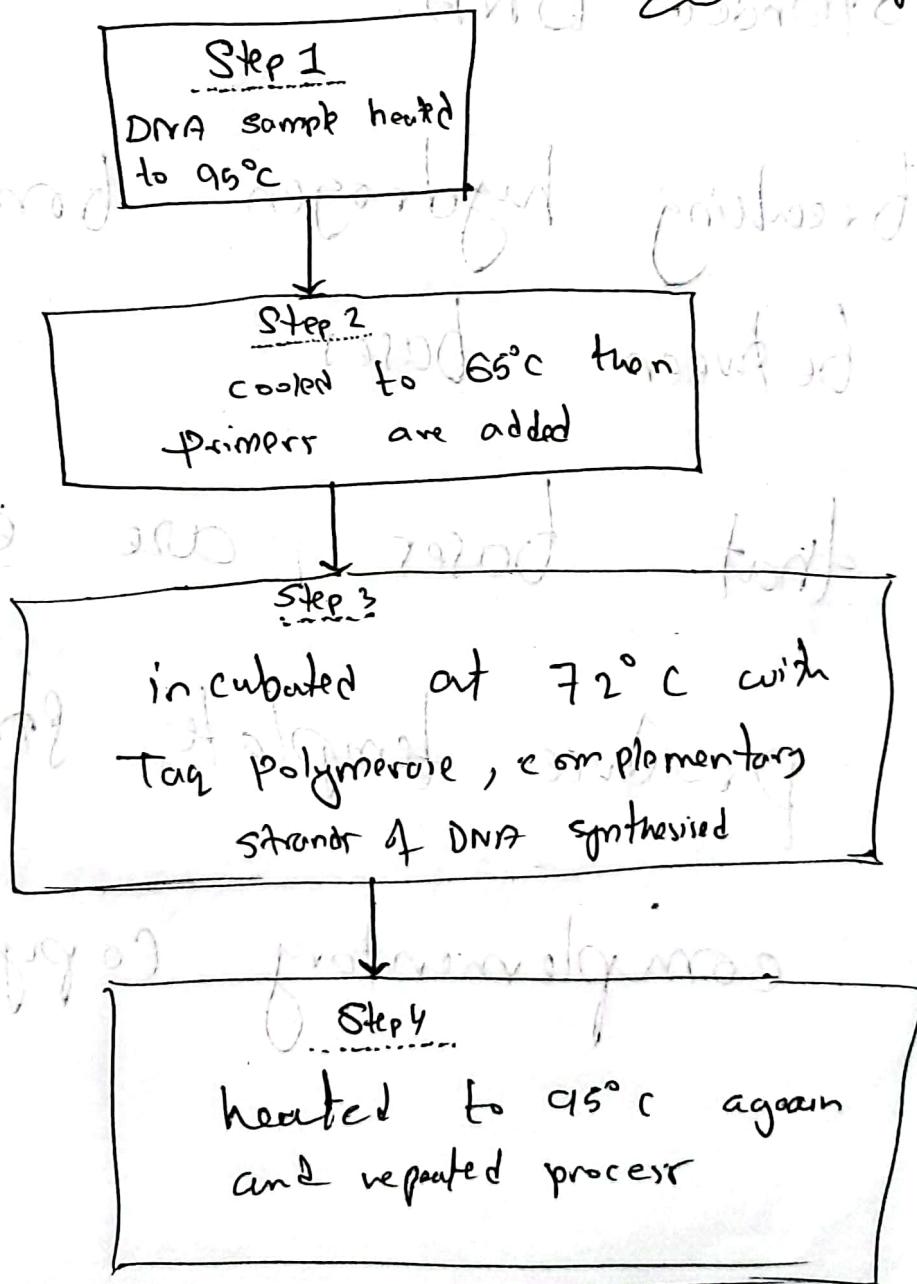
- ⇒ The host cell divide dues to increase number of recombinant plasmid.
- ⇒ for transcription, specific promoters used with marker gene.

Gene editing Crispr/Cas9

- =) A form of genetic engineering
- =) Involving the insertion, deletion, or replacement of DNA at specific sites in the genome
- =) Using Crispr/Cas9 system

Polymerase Chain Reaction

PCR is used to produce large amount of DNA from a very small original sample. (Used to clone and amplify DNA)



Step 1 : (heated at 95°C) denatured

=> To separate the two strands of DNA → making single stranded DNA

=> by breaking hydrogen bonds

between bases

=> So that bases are exposed

=> To produce template strand

for complementary copying

Step 2: Primers added

- ⇒ Primers bind to ssDNA by complementary base pairing.
- ⇒ attach close to the specific section of DNA.
- ⇒ DNA Polymerase only attaches to double-stranded DNA.
- ⇒ Primers reduce re-annealing of separated strands at 60-65°C (they are specific).

Role of Tag polymerase

→ makes of bond

⇒ Synthesis of complementary strand

DNA strands

strands of each other

⇒ by using free dNTPs

⇒ Tag Polymerase is heat AND (

AND) stable so works at high

temperature of 70°C

⇒ So does not need to be added in again for each cycle.

⇒ process is efficient more and faster than

normal
DNA polymerase

same of different A.M. for
DNA polymerase
moves in either side
for short time
but if you stop it
it moves in opposite
directions now
it moves in
the same direction
but it moves in
different ways above
the DNA

Gel electrophoresis

- ⇒ DNA fragmented by restriction enzyme
- ⇒ loaded into wells in agar gel.
- ⇒ at negative end or cathode e,
- ⇒ Electrolyte has buffer.
- ⇒ DC current applied
- ⇒ Phosphate group of DNA is negative charge
- ⇒ we charged DNA attracted to anode, positive elec

- ⇒ Short pierces / smaller mass
more faster.
- ⇒ Southern blotting occurs
- ⇒ probes or fluorescent dye added
- ⇒ UV lights are used to see patterns of stripes
- ⇒ increased by PCR (Desired gene)

Outline of microarray uses

- => Obtain mRNA/DNA from the cell or tissue sample or produce by transcription.
- => Reverse transcription of mRNA to produce cDNA
- => Denature into single-stranded DNA.
- => Cut DNA into small fragments.
- => add fluorescent to label cDNA.

- ⇒ Microarray has ss DNA probes
- ⇒ each from known gene
- ⇒ cDNA hybridises to complementary probe / ss DNA on microarray
- ⇒ Laser used to detect fluorescence which shows the expressed gene.
- ⇒ intensity of fluorescence shows level of gene expression.
- ⇒ high intensity emitted light indicates many mRNA molecules were present in the sample.

Data bases

Bioinformatics: Database computer software analysis of biological sequences

Amino acid sequences

and DNA sequences.

Database provides information about nucleotide sequences of genes and genomes

* amino acid sequences of proteins and protein structures.

Benefits:-) Data stored in large amount of
for amino acid sequencer or
nucleotide sequences

-) can share database information
or sequence anywhere online

2) can compare multiple sequences

-) rapid and faster process.

-) can count nucleotide differences

-) Cheaper

-) less labour in terms of time

-) less cost in exchange of speed

Genetic technology applied to medicine

• diagnosis

• treatment

~~Recombinant insulin~~

Adequate

Advantages of using recombinant insulin:

⇒ Identical to human insulin

⇒ More rapid response

⇒ No immune response / side effects

⇒ no ethical or moral issues

⇒ Cheaper to produce in large volume

- ⇒ Unt uncontaminated
- ⇒ Good for people who have developed tolerance to animal insulin

Advantages of Using recombinant FSH :

- ⇒ Large or unlimited supply
- ⇒ no risk of infection.
- ⇒ no ethical issues
- ⇒ less chance of rejection by immune responses
- ⇒ cheap and quick to produce
- ⇒ no need to wait for donor.

Advantages of using recombinant adenosine deaminase:

- ⇒ no ethical and moral issues
- ⇒ no immune response → no risk of side effects in
- ⇒ no infection
- ⇒ cheap and ~~easy~~ to make to produce in large ~~volume~~ volume

Deficiency

Deficiency of adenosine deaminase (ADA) due to mutation on 1st gene ADA on chromosome 20.

→ Causal (SCID) Severe combined immunodeficiency.

→ The enzyme ~~is~~ catalyse the deamination of adenine and deoxyadenine, toxic to T-lymphocytes.

Genetic Screening: Testing an embryo, fetus or adult to find out whether a particular allele is present.

BRCA1 & BRCA2

Advantage for BRCA1 & BRCA2

- ⇒ enables early treatment
- ⇒ prevents early death
- ⇒ removes worry (not present)
- ⇒ planning a family
- ⇒ Early diagnosis cheaper than later treatment.

Advantage for Huntington's disease:

- ⇒ women can choose not to have children (if present)
- ⇒ If embryo has the allele can choose abortion.
- ⇒ Select unaffected, IVF embryo to implant.
- ⇒ remove worry.

Cystic fibrosis:

- When CFTR gene does not code for transport protein,
- ⇒ No channels for Cl^- ions
 - ⇒ Cl^- ions don't move out
 - ⇒ Less water leaves cell.
 - ⇒ Mucus on cell surface membrane stays thick.
 - ⇒ So mucus ~~excess~~ accumulates.
 - ⇒ difficulty in breathing, more infections

Advantages

No present:

- ⇒ removes worry
- ⇒ woman ~~she~~ can have children
- ⇒ planning family.

Present

- ⇒ early treatments of CF symptoms
- ⇒ reduces frequency of CF population

Ethical issues of genetic screening

- ⇒ embryos might be destroyed as viable embryo discarded
- ⇒ contrary to religious beliefs.
- ⇒ People with faulty allele who otherwise could not have children can now do so, may not develop disease
- ⇒ ~~social~~ reduced cost as long term early diagnosis cheaper
- ⇒ removes worry.

Gene therapy

- Involves the addition of normal correct allele to human or animal
- can cure or reduce symptoms of genetic disease
- ⇒ with only recessive allele not dominant
- =) such as
 - 1) Haemophilia (due to lack of F8)
 - 2) LCA (inherited eye disease)
 - 3) CF
 - 4) Sickle cell anaemia
(NOT Huntington)

Outline how SCID can be treated by gene therapy:

- ⇒ obtain normal ADA allele
- ⇒ insert allele into virus vector
- ⇒ remove stem cells
- ⇒ insert the virus with allele into stem cell.
- ⇒ return stem cells to body.

Vector: liposome

LCA

↳ autosomal recessive eye disease

→ treated with AAV adenovirus vector.

⇒ normal dominant allele is injected into retina

Blood of 2700 mice tested

Social and ethical considerations of gene therapy

=> new gene must not insert in wrong place

=> must not cause cancer

=> must not cause infection

=> must not cause immune response.

~~Genes~~

Genetically modified organisms in agriculture

GM crop - herbicide

- ⇒ More food production
- ⇒ more income for farmers
- ⇒ can kill weed and increase yield.

GM insect resistant crops

- ⇒ less insecticide needs to be used
- ⇒ less money spent
- ⇒ more food production / more yield
more income.
- ⇒ cheaper to consumer
- 2) ~~increases~~ Increase the quality of crops.

Ethical issues

- ⇒ resistance to — may be transferred to wild plants.
- ⇒ may kill useful insects.
- ⇒ decrease in biodiversity.
- ⇒ insects may become resistant to toxins.
- ⇒ toxic to human.
- ⇒ GM seeds are expensive.

Social implications of using GM organisms in food production

advantage:

- ⇒ increase in yield
- ⇒ improved quality
- ⇒ Insect resistant crops so less money spent on pesticide.

disadvantage

- ⇒ consumer resistance to GM crops.
- ⇒ expensive.
- ⇒ may be unsafe for humans.

G.M Salmon

=) advantage:

- =) G.M salmon may grow faster
- =) less stressful on G.M salmon
- =) risk of infection with injections

drawbacks via G.M for food safety

cross breeding

harmful to environment
genetic engineering

genetic drift at gene transfer
mutation at target

genetic stability, unpredictable

genetic instability, unpredictable

genetic instability, unpredictable