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A2 Biology OCR

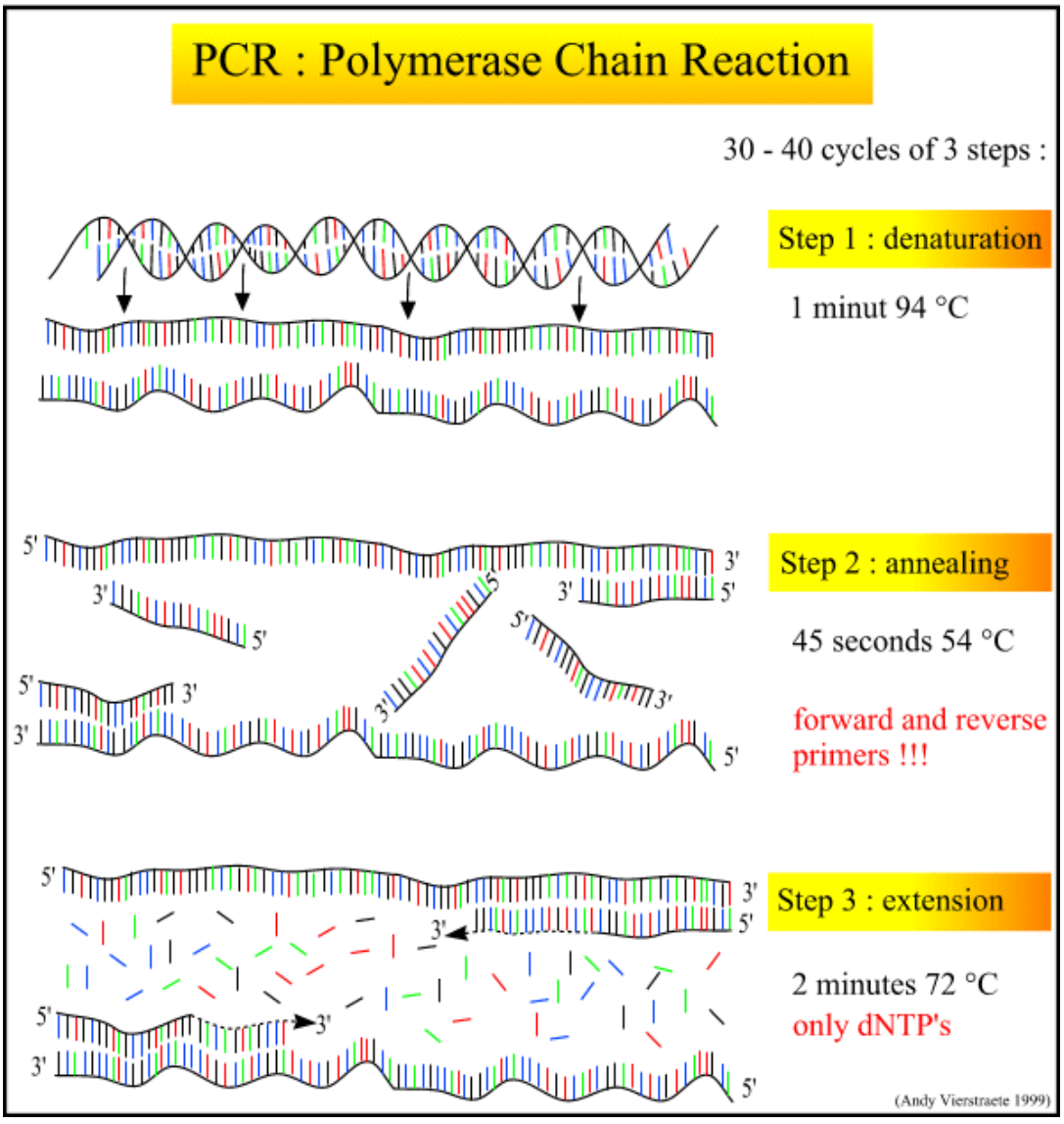
Unit F215: Control, genomes and environment

Module 2.3 Genomes and gene technologies

Notes & Questions

Outline how the polymerase chain reaction (PCR) can be used to make multiple copies of DNA fragments.

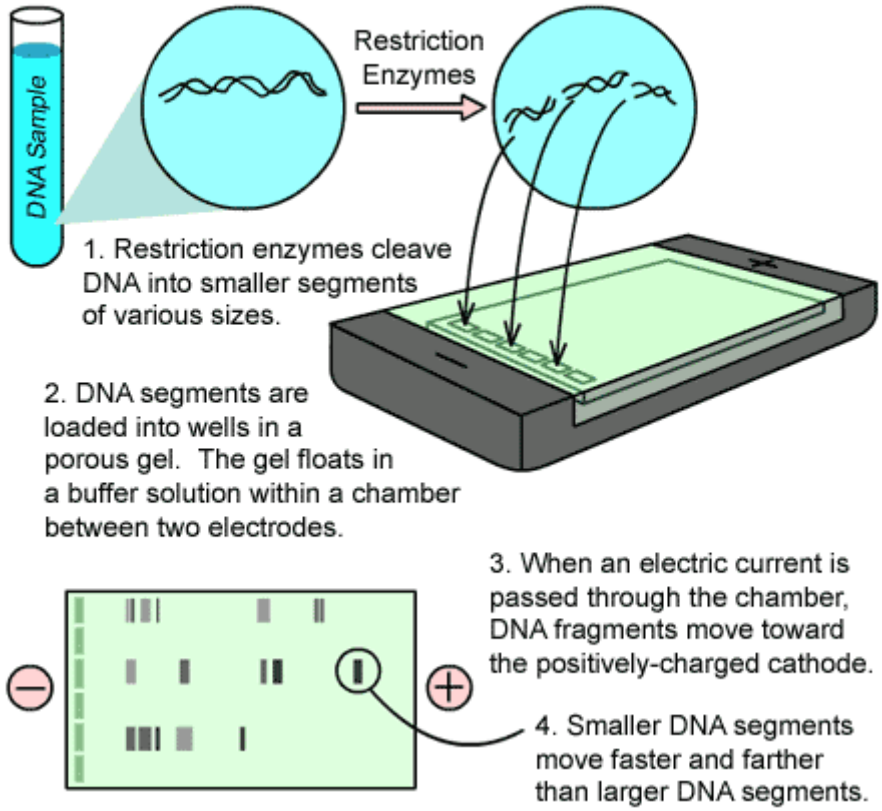
- Artificial DNA replication
- Generates multiple copies of a DNA sample
- Can only copy pieces of DNA a few 100 bases long
- Needs a primer (single stranded DNA 10-20 bases long) to allow DNA polymerase to bind



- **PCR steps**
  - Mix DNA sample, free DNA nucleotides, DNA primers (one for each strand) and DNA Polymerase (Taq polymerase)
  - Heat components to **95ºC** (1 minute)
    - Breaks the hydrogen bonds between complementary bases on each strand
    - Makes the DNA single stranded
  - Cool to **55ºC** (45 seconds)
    - Primers anneal to the 3' end
    - Taq Polymerase can now bind to primers
  - Heat to **72ºC** (2 minutes)
    - Optimum temperature for Taq Polynmerase
    - A new strand of DNA is synthesised from each template strand.

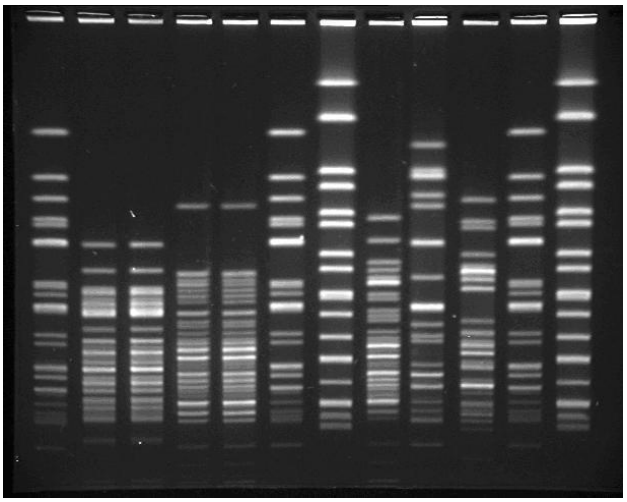
Outline how DNA fragments can be separated by size using electrophoresis.

Figure S-2: Gel Electrophoresis



- **Gel Electrophoresis**
- Separates DNA fragments according to their size
- Accurate to 1 base difference in length

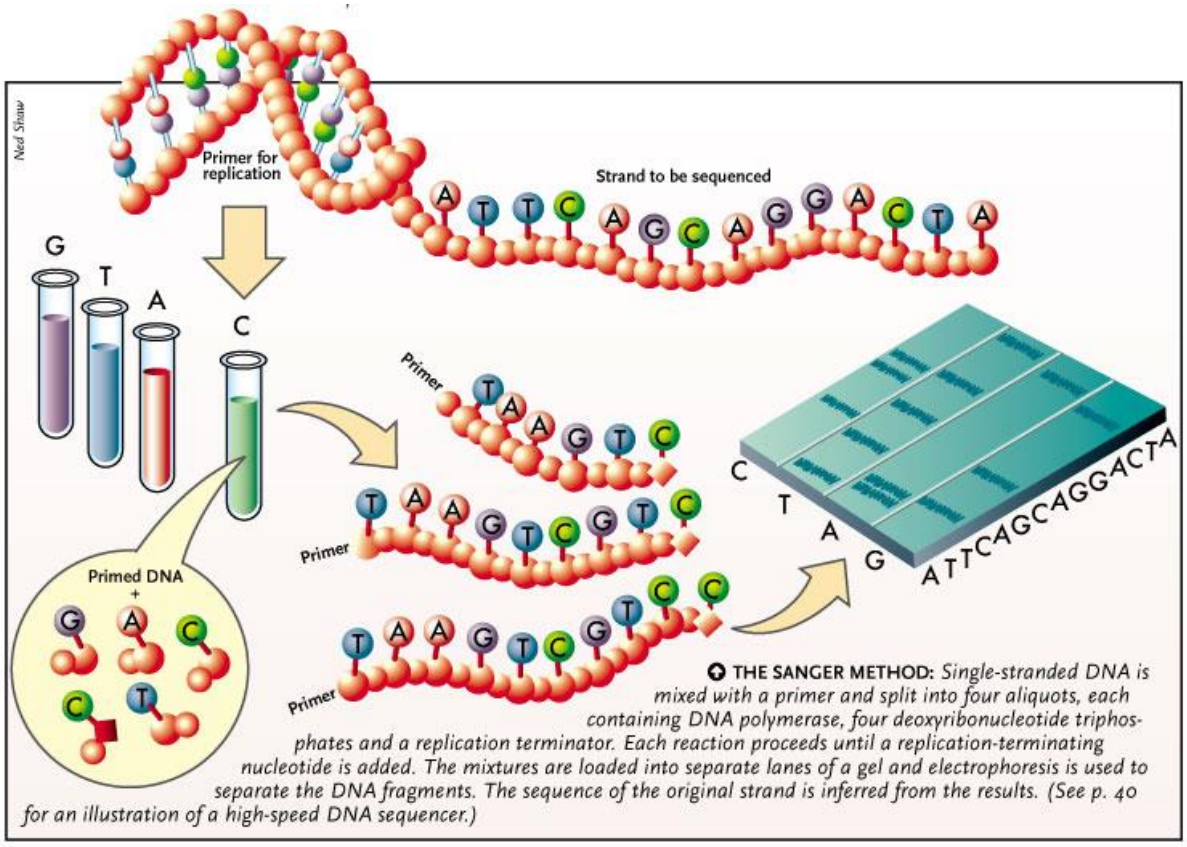
- Formation of the gel plate
    - Agarose powder is dissolved and poured into a gel mould.
    - A comb is inserted into the gel and the gel is left to set.
- Setting up the tank
    - The gel once set is placed into the electrophoresis tank
    - A buffer solution is added completely covering the gel plate
    - The buffer solution provides free electrons which can produced a current when attached to the electrodes
- Treating and loading the DNA
    - DNA sample is treated with restriction enzymes to cut it into fragments
    - DNA is mixed with a dye and placed into wells in the agar gel plate at the end with the cathode (Negative end)
- Electrophoresis process
    - The DNA is negatively charged due to the phosphate groups and so when current flows it will be pulled to the anode (positive electrode)
    - Shorter pieces will travel further up the gel and therefore faster in the given time.
- Results – Southern Blotting
    - The DNA is invisible and so when being amplified in PCR radioactively tagged nucleotide should have been used.
    - Lift the gel plate from the tank and drain off the buffer solution
    - Compress the gel plate against a nylon cloth over night
    - The DNA should lift off the gel and transfer to the nylon cloth
    - Expose the cloth to photographic film and the DNA bands should become visible.



Outline the steps involved in sequencing the genome of an organism.

- This technology uses both PCR and gel electrophoresis
- The reaction mixture contains
    - Taq Polymerase
    - DNA primers
    - DNA sample strand
    - DNA nucleotides
    - DNA nucleotides with fluorescence markers (These are also chain terminating nucleotide)

- The process is set off like normal PCR
- As it runs through the cycles the DNA strand is separated and free nucleotides are added to synthesis a new strand
- The Taq Polymerase can attach a normal free or a chain terminating fluorescent tagged DNA nucleotuide
- It is random which binds in each cycle and with enough cycles DNA fragments will be synthesised for every possible length of DNA.



THE SANGER METHOD: *Single-stranded DNA is mixed with a primer and split into four aliquots, each containing DNA polymerase, four deoxyribonucleotide triphosphates and a replication terminator. Each reaction proceeds until a replication-terminating nucleotide is added. The mixtures are loaded into separate lanes of a gel and electrophoresis is used to separate the DNA fragments. The sequence of the original strand is inferred from the results. (See p. 40 for an illustration of a high-speed DNA sequencer.)*

- If the DNA is run through gel electrophoresis, these fragments all separate out according to length
- If the fragments are allowed to run off the gel plate.
- They break a laser beam and the colour of the fluorescent marker is recorded.
- The first fragment of DNA off the gel will be 1 base in length and represent the first base in the sequence
- The base is identified by the colour that breaks the laser, for example;
    - Red = adenine
    - Blue = Thymine
    - Green = Cytosine
    - Yellow = Guanine
- The next off the gel is two bases long and the colour identifies the second base
- This continues until the whole DNA sample has been sequenced.

- **The genome – the entire set of human genes**
- Sequencing the genome
- First the genome is mapped using microsatellites (sequences 3 -4 bases long)
- The shot gun approach – the genome is sheared into smaller fragments, each around 100,00o bases long
- These fragments are inserted into Bacterial Artificial Chromosomes (BACs)
- BACs are inserted into E.coli and cultured
- DNA is then extracted, one BAC at a time
- It is cut into even smaller fragments using restriction enzymes
- The base sequence is worked out as previously described
- The fragments are resembled by computers using the microsatellites and overlapping regions

Outline how gene sequencing allows for genome-wide comparisons between individuals and between species.

- Identifies genes essential for life
- Highlights evolutionary relationships
- Model effects of mutations on genes
- Highlight what genes cause pathogenic effects
- Identification of genetic disorders

Define the term *recombinant*.

- DNA from 2 or more different sources joined together
- The organism that receives the gene is referred to as the recipient and is known as being transgenic.

Explain that genetic engineering involves the extraction of genes from one organism, or the manufacture of genes, in order to place them in another organism (often of a different species) such that the receiving organism expresses the gene product.

- **Obtaining the gene of interest**
- **Method 1**
  - It can be located using probes
  - Cut out of the genome using restriction enzymes
  - Leaving sticky ends
- **Method 2**
  - If the DNA sequence is known the gene can be sequenced
  - Sticky ends are added which will be complementary to the ones formed when the vector is cut
- **Method 3**
  - Isolate mRNA from the transcribed gene
  - Use the enzyme reverse transcriptase
  - Makes single stranded DNA copy of the mRNA strand
  - DNA polymerase with make it double stranded
  - Sticky ends are added which will be complementary to the ones formed when the vector is cut

- **Cutting the Vector**
  - Use restriction enzyme to cut the vector
  - Sticky ends form

- **Placing the gene into a vector**
  - The complementary sticky ends line up
  - Hydrogen bonds form between the tow sticky ends
  - A with T, and C with G
  - DNA ligase seals the sugar-Phosphate backbone

- **Inserting the vector into the recipient cell**
- **Method 1**
  - Ca^{2+} are used along with Heat Shock (cooling to freezing and heating to 40^{o}C rapidly)
- **Method 2**
  - Electroporation
- **Method 3**
  - Microinjection
- **Method 4**
  - Viral transfer
- **Method 5**
  - Liposomes
- **Method 6**
  - Ti Plasmids

Describe how sections of DNA containing a desired gene can be extracted from a donor organism using restriction enzymes.

- Restriction enzymes (endonucleases) are isolated from Bacteria
- They cut DNA at specific palindromic sequences of between 4 – 6 bases long
- They hydrolyse the sugar-phosphate back bone of the DNA molecule.
- They can leave blunt or more often sticky ends
- Sticky ends are free unpaired DNA bases
- Restriction enzymes can cut isolated genes from genomes and also cut bacterial plasmids.
- If the same restriction enzyme is used to cut the plasmid and the gene from the genome then the sticky ends will be complementary.

Explain how isolated DNA fragments can be placed in plasmids, with reference to the role of ligase.

- DNA ligase seals the nicks in the DNA backbone
- Joining sugar-phosphate backbone.
- They seal the recombinant DNA.
- Usually the gene and the cut plasmid have complementary sticky ends and so they are lined up and bonded through hydrogen bonds.

Other Enzymes used in genetic engineering

- **Reverse transcriptase**
  - Produces single stranded DNA
  - From mRNA of a transcribed gene
- **DNA Polymerase / Taq Polymerase**
  - Synthesises a double stranded DNA molecule from a single stranded DNA molecule
  - Used in PCR
  - High optimum temperature

Describe how DNA probes can be used to identify fragments containing specific sequences.

- DNA probes are short single stranded pieces of DNA (50 -80 bases long)
- They are complementary base sequence to the piece of DNA under investigation
- Used to locate
  - A desired gene for genetic engineering
  - Genes for comparisons between genomes
  - Identify the presence or absence of alleles
- The probe is labelled either with
  - P^{32} radioactive groups and visible when exposed to photographic film
  - Fluorescent markers and are visible under UV light

State other vectors into which fragments of DNA may be incorporated.

- Vectors include
  - Yeast Chromosomes
  - Bacterial Plasmids
  - Viral genomes

Explain how plasmids may be taken up by bacterial cells in order to produce a transgenic microorganism that can express a desired gene product.

- **Method 1**
  - Ca^{2+} are used along with Heat Shock (cooling to freezing and heating to $40^{o}C$ rapidly)
- **Method 2**
  - Electroporation
- **Method 3**
  - Microinjection
- **Method 4**
  - Viral transfer
- **Method 5**
  - Liposomes
- **Method 6**
  - Ti Plasmids

Outline how genetic markers in plasmids can be used to identify the bacteria that have taken up a recombinant plasmid.

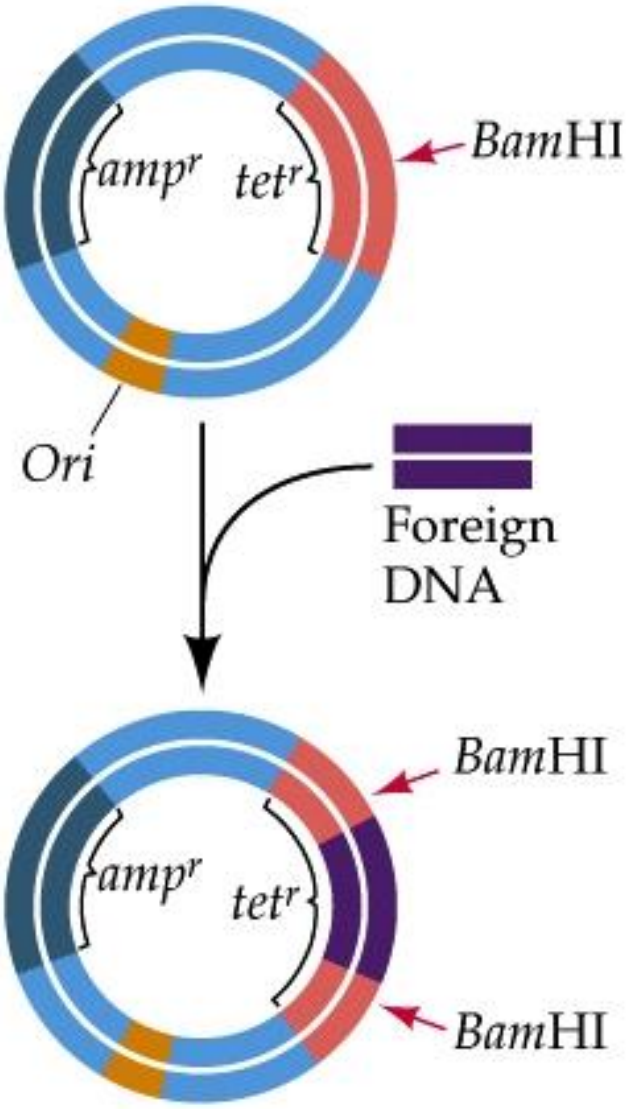
- Genetic markers are sequences of DNA that are associated with particular traits
- In the case of insulin production the genetic markers are two genes for antibiotic resistance
- The two antibiotic resistance genes in this case are;
  - Tetracycline resistance
  - Ampicillin resistance

- These genetic markers allow us to identify if the E.coli has;
  - Taken up the plasmid at all
- If it has taken up a plasmid has it taken up
  - just the plasmid without the insulin gene
  - The recombinant plasmid with the insulin gene (what we are after)

- The restriction site that leaves sticky ends for the insulin gene is in the middle of the tetracycline gene.

- The results would look like this

	Agar plate with tetracycline	Agar plate with Ampicillin
No plasmid	X	X
Plasmid but no insulin gene	✓	✓
Recombinant gene	X	✓



Outline the process involved in the genetic engineering of bacteria to produce human insulin.

1 identify / find, gene (for insulin) / length of DNA coding for insulin;

2 obtain / isolate / extract,
gene / length of DNA (for insulin); obtain / isolate / extract,
mRNA (for insulin);

3 restriction enzyme / named e.g.; reverse transcriptase;

4 cut plasmid; cut plasmid;

5 use same restriction enzyme; use restriction enzyme / named e.g.;

6 ref to, complementary ends / sticky ends / described;

7 insert, gene / AW, into plasmid;

8 recombinant DNA;

9 plasmid uptake by bacteria;

10 identify those bacteria that have taken up the plasmid;

11 provide with, raw materials / nutrients;

Producing Insulin in a bioreactor

12 fermenter / bioreactor;

13 bacteria produce insulin;

14 extract and purify / downstream processing;

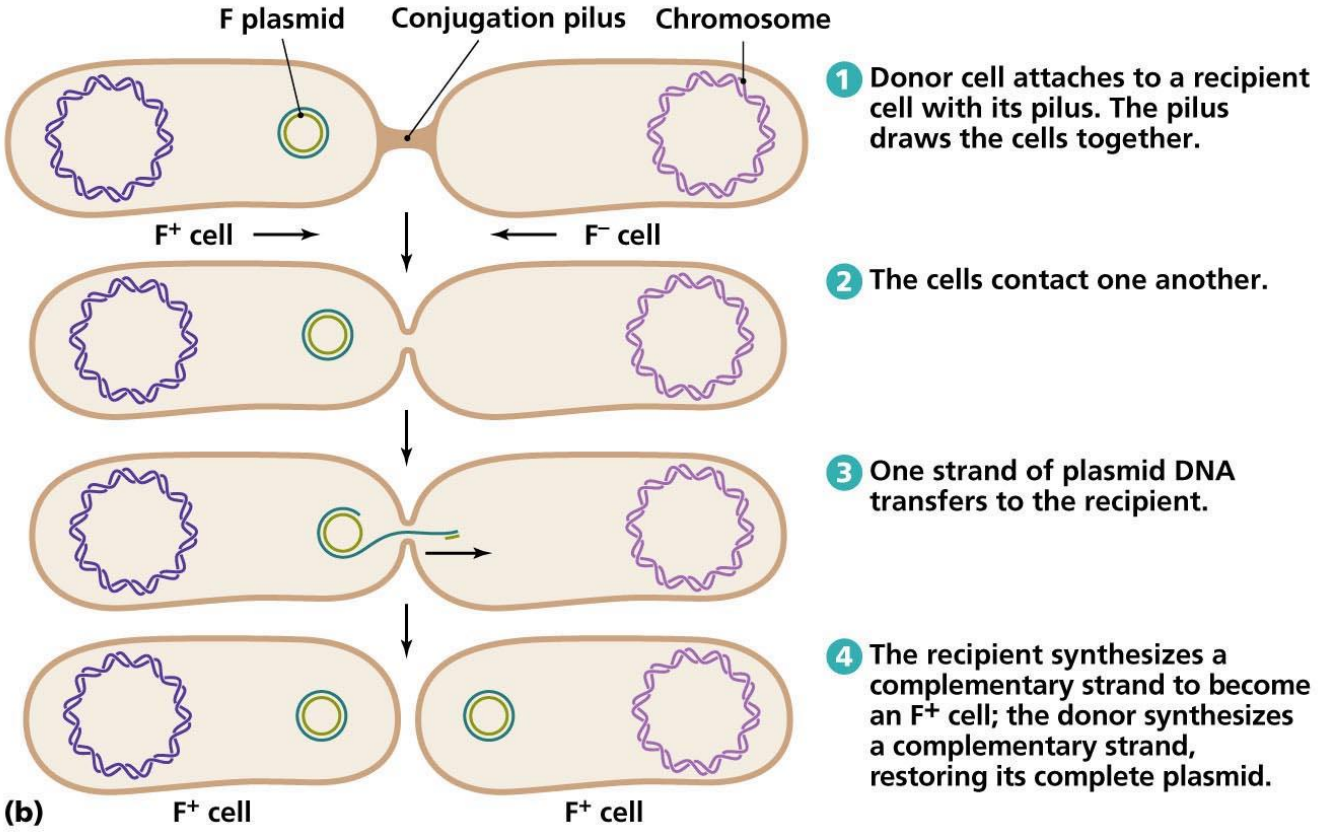
15 AVP; **e.g.**. detail of uptake by bacteria
method of identifying those that took up plasmid
PCR
ligase *7 max*

16 advantage 1; e.g. more reliable supply

17 advantage 2; greater / faster, production
overcomes ethical problem described
less risk of disease
less risk of, rejection / side effects
human insulin so more effective

Describe the advantage to microorganisms of the capacity to take up plasmid DNA from the environment.

- **Advantages**
- Plasmid DNA usually codes for antibiotic resistance
- Transferring the plasmid between individuals of the same and even different species increased the chances of survival



Outline the process involved in the genetic engineering of *Golden Rice*™.

- **Vitamin A (Retinol)**
- Obtained from animal sources
- Vegetarians can acquire beta-carotene (a photosynthetic pigment and precursor to Vitamin A) which is converted to vitamin A in the human gut.

- **Rice (*Oryza Sativa*)**
- Contains beta-carotene in the green parts of the plant
- However, the edible endosperm does not contain any beta-carotene as the genes here have been switched off.

- **Issue**
- As rice is the staple for most people in the developing world there is a lot of problems with lack of vitamin A in their diet
- 500,000 people go blind / year
- 1 – 2 million die / year
- 120 million are affected in some way / year

- **Golden Rice™**
- Genetically engineer beta-carotene into the endosperm

- **1992 – 2000 Switzerland – beta-carotene accumulated into the endosperm**
- Most of the genes for the necessary enzymes are present already in the rice endosperm

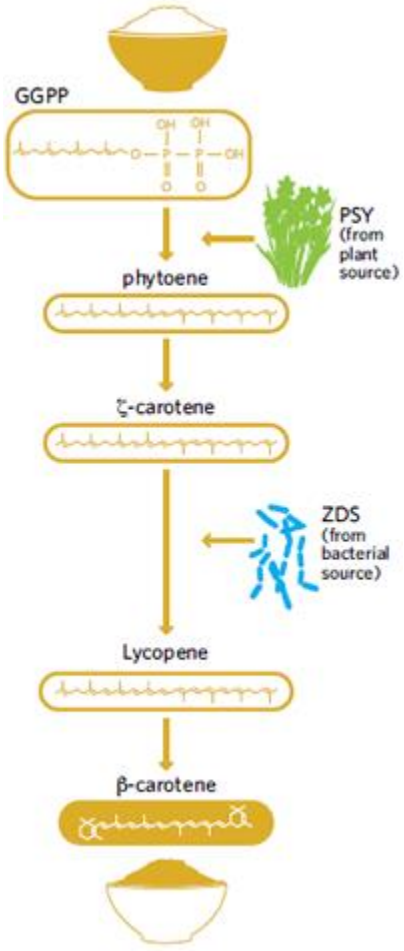
- Only 2 added
  - **Phytoene synthetase (from Daffodils)**
  - Converts a precursor molecule into phytoene

  - **Crt 1 (from bacteria - *Erwinia uredovora)***
  - Converts phytoene into lycopene

- Not enough beta-cartoene in endosperm

- **2004 Louisiana USA**
- Cross breeding produces varieties with 3 – 4 more beta-carotene than original

- **2005 Syngenta UK**
- **Golden Rice 2™**
- 20 times the beta-carotene of the original



Outline how animals can be genetically engineered for xenotransplantation.

- **Allotransplantation**
  - Transplanting cells, tissues or organs between individuals of the same species
  - The organ is recognised as not 'self' and so can trigger an immune response = rejection of the cell / tissue / organ
- **Xenotransplantation**
  - Transplantation of cells, tissues or organs between animals of different species
  -
  - **Pigs & Humans**
  - **2003**
    - Pigs engineered to lack alpha-1,3-transferase which triggers rejections in humans
  - **2006**
    - E5'N Human nucleotidase enzyme) is engineered into pig cells which decreases the immune response

  - **Problems using pigs**
  - Physiological
    - Different organ size
    - Pigs lifespan is shorter = 15 years
    - Pigs core body temperature = 39^{o}C

  - Ethical/medical
    - Animal welfare
    - Religious beliefs
    - Disease transfer

Explain the term *gene therapy.*

- **Gene therapy**
  - Therapeutic technique where the functional allele of a particular gene is placed in cells of an individual lacking functioning alleles of that particular gene.
  - Can be used to treat recessive conditions but not dominant ones such as Huntington's disease.

- **Methods of gene therapy**
  - Placing a functional allele of the gene
  - Using interference RNA which can bind to mRNA and silence the effect of the gene
  - Killing specific cells by adding genes so the cells express proteins (cell surface proteins) which make the cell vulnerable to attack from the immune system. Cancers can be treated in this manner

Explain the differences between somatic cell gene therapy and germ line cell gene therapy.

- **Somatic Cell Gene Therapy**
  - Body cells = specialised
  - All contain a full chromosome set but most are turned off

- **Germline Cell Gene Therapy**
  - Engineering a gene into a sperm, egg or zygote cell
  - All future cells also have trait

Somatic cell Gene Therapy	Germline cell Gene Therapy
Delivery into the recipient cell is **tricky** as cell needs to be removed, treated and replaced = Ex-vivo Therapy	Straight forward delivery as techniques are not cell specific
Treatment is short lived and needs to repeated regularly	Modifications are found in future generations
Difficulty getting allele into the genome, viruses trigger immune response, Liposomes are not efficient	Unethical to engineer embryos
Modifications are limited to the patient	All cells have modification and any mitosis results in daughter cells having the trait as well

Discuss the ethical concerns raised by the genetic manipulation of animals (including humans), plants and microorganisms.

	Benefits	Risks
Microorganisms	* Produce human proteins e.g. Insulin / HGH	* Containment / escape * Mutations = pathogens * Antibiotic resistance
Plants	* Golden rice * Pest resistance * Weedkiller/pesticide resistance	* Less genetic variation * Toxic varieties and allergic reactions * Features passed on to weeds
Animals	* Increase milk/meat yeild * Xenotransplantation * Pharmaceuticals in milk	* Animal welfare * Religious beliefs
Humans	* Gene Therapy	* Unpredictable effects * Germline gene therapy is making decisions for furture generations * Designer babies

Questions

1. One product manufactured using microorganisms is insulin. The process involves genetically engineering bacteria to synthesise human insulin.

(i) Describe how the isolated human insulin gene is inserted into a bacteria plasmid.

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[4]

(ii) Suggest **two** ways in which the bacteria which take up the modified plasmids can be identified.

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[2]

[Total 6 marks]

2. Suggest **one** reason why it is considered preferable to use genetically engineered sources of human insulin rather than insulin obtained from pigs.

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[Total 1 mark]

3

Recombinant DNA technology, using restriction enzymes, enables bacteria such as *Escherichia coli* to produce human proteins.

(i) Explain what is meant by a *restriction enzyme*.

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[3]

(ii) Outline the formation of recombinant DNA.

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[4]

[Total 7 marks]

4

In this question, one mark is available for the quality of the use and organisation of scientific terms.

People who have one form of diabetes are unable to make insulin. In order to control blood sugar concentration, these people need to receive insulin. Originally, insulin was obtained from animals, such as pigs. Now, bacteria are transformed by genetic engineering to make proteins, such as insulin. This is the source of the majority of insulin now used by diabetics.

Describe how genetic engineering has been used to produce human insulin **and** the advantages of obtaining insulin in this way.

[8]

Quality of Written Communication [1]

[Total 9 marks]

5

The DNA target sites of four restriction enzymes are shown in the table below. The points at which the strands of DNA are cut are shown by arrows and lines.

restriction enzyme	target site
Sau3AI	↓G – A – T – C – – C – T – A – G ↑
BamHI	– G ↓ G – A – T – C – C – – C – C – T – A – G ↑ G –
HinfI	– G ↓ A – N – T – C – – C – T – N – A ↑ G – 'N/N' may be any complementary base pair

With reference to the information above,

(i) describe the characteristics of a restriction enzyme's target site;

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[2]

(ii) explain whether or not a piece of DNA cut by **Sau3AI** could join with one cut by **BamHI**;

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[3]

(iii) show on the figure below the result of exposing this piece of DNA to **HinfI.**

–G – A – T – T – C – A –G – A – A – T – T – T – C – G– A – A – T – C –

– C – T – A – A –G – T – C – T – T – A – A – A – G – C – T – T – A – G –

[1]

[Total 6 marks]

6

In this question, one mark is available for the quality of use and organisation of scientific terms.

Describe the roles of restriction enzymes and other enzymes in genetic engineering.

[8]

Quality of Written Communication [1]

5.2.3

[Total 9 marks]

7

Babies born with severe combined immune deficiency (SCID) have no defence against common infections and quickly become ill when the protection from maternal antibodies is lost.

SCID is caused by a mutant allele of the gene coding for an enzyme, adenosine deaminase (ADA).

Gene therapy for SCID has been carried out using the procedure shown in the figure below.

bone marrow cells removed from baby with SCID
↓
stem cells of immune system isolated
↓
stem cells infected with harmless genetically engineered virus containing the normal, dominant, allele for ADA
↓
stem cells take up normal allele for ADA
↓
stem cells transfused back into baby
↓
immune system develops T and B lymphocytes

(i) Describe how the DNA of the harmless virus referred to above can be genetically engineered to carry the normal allele of the human gene for ADA.

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[4]

(ii) Explain why it is easier to perform gene therapy when the normal allele is the dominant allele of the gene concerned.

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[2]

[Total 6 marks]