DRUG DEVELOPMENT - INSULIN PRODUCTION:

Research about 2 other uses of the techniques used in biotechnology eg: stem cells, disease resistance in crops, drug development, human identification and forensic analysis

- Type 1 diabetes, is a condition that is caused by the deficiency of the protein hormone insulin which is produced in the pancreas.
- In order to maintain blood glucose levels and sufficient stores of glycogen and fats, insulin injections are given to sufferers regularly.
- In the past, insulin was extracted from pigs and sheep, but this was extremely ineffective as it was time consuming, expensive and the resulting product was not identical to human insulin (this led to problems such as allergic response and risks of contracting diseases).

THE PROCESS:

Cutting DNA into fragments:

- Using several restriction enzymes the human genome of a normal individual (doesn't suffer from diabetes) is cut into various sized fragments.
- The lengths of the fragments depend on the relative positions of the recognition sites.

Sorting of DNA fragments:

- DNA fragments can be sorted according to size (length) using the process of Electrophoresis.
- DNA fragments that are small move quickly and further in the gel than fragments that are longer and move much slower. Fragments of same size move at same rate.

Finding required gene:

- Probes are used to locate specific DNA segments (insulin gene).
- Probes are made of a single stranded DNA or RNA with a complementary base sequence in one of the strands of the target DNA (insulin gene).
- In order for this to occur the DNA (insulin gene) must be denatured, as the probe needs to attach to the bases of one strand.
- Probes are labelled with wither a fluorescent or a radioactive marker, so we are able to identify the target DNA (insulin gene).

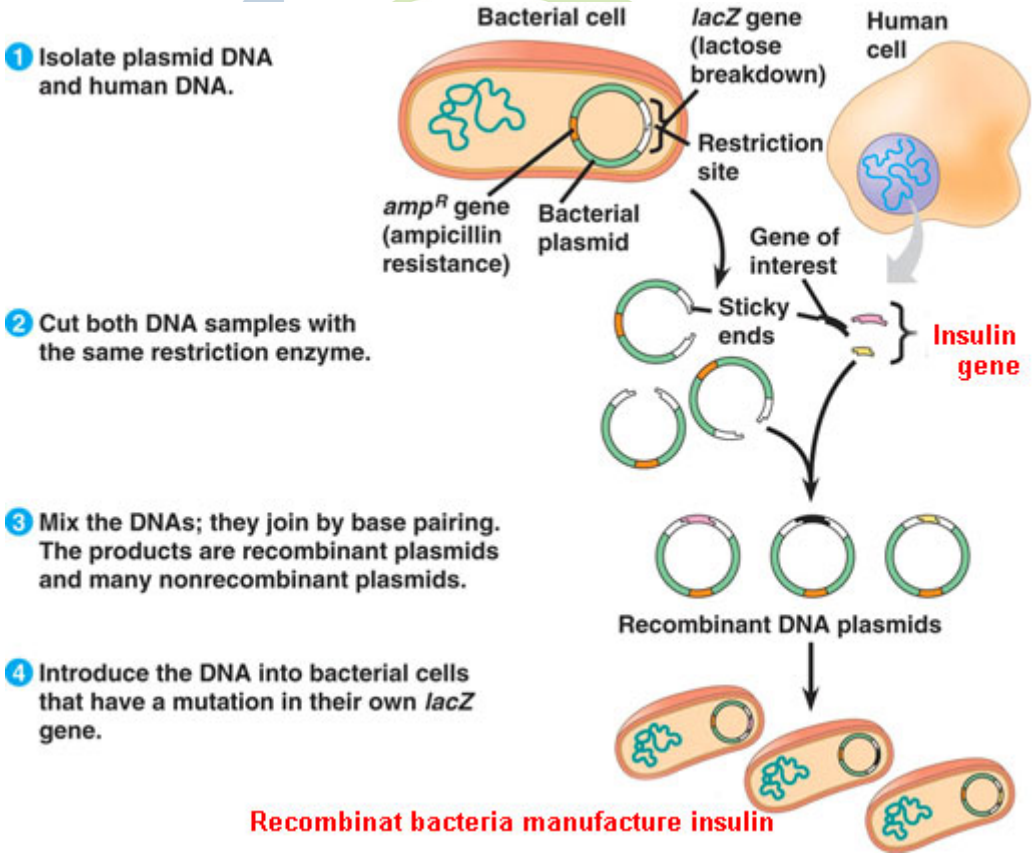
OR

Reverse Transcription:

- If the product of the gene (insulin) is known then you would know where to find lots of mRNA for this protein (pancreas).
- Thus the mRNA is extracted from the specialised cell from the pancreas which produces insulin.
- By reverse transcription, a copy DNA can be formed, for this process to occur, the enzyme reverse transcriptase is needed and is only made by retroviruses like the HIV virus. Also free nucleotides are needed.
- A complementary DNA strand is built upon the mRNA template.
- Then amplify the gene coding for insulin production using PCR.

THEN

- Use the same restriction enzyme that attaches to the human genome to cut a section out of the vector in this case plasmids (small circular pieces of DNA found most commonly in bacteria).
- The donor DNA (insulin gene) fragments are prepared using the same cutting enzyme.
- The donor DNA (insulin gene) fragments and the plasmids are mixed and their sticky ends pair.
- Some of the plasmids join with the donor DNA, using Ligase to make joins permanent meanwhile some plasmids just glue themselves back together.
- Plasmids thus contain the donor DNA (insulin gene) are then selected from the mixture. These plasmids can then be reintroduced into a bacterial cell using either an electric pulse or heat shock.
- The bacterial cells which accept and contain the plasmid that was modified are said to be genetically transformed.
- Genetic markers are used to differentiate between the transformed bacteria and those that haven't. The transformed bacteria are collected and left to reproduce, and thus there is a mass production of insulin.



DNA PROFILING:

DNA profiling involves electrophoresis of non coding DNA regions, especially hyper variable regions (describes regions of chromosomal DNA in which great variation exists in unrelated individuals, often due to the difference in number of repeats of short sequences) called short tandem repeats. STRs are where sequences of just two to five base pairs are repeated. The number of repeats varies from individual to individual (except identical twins that have the same DNA and thus the same STRs).

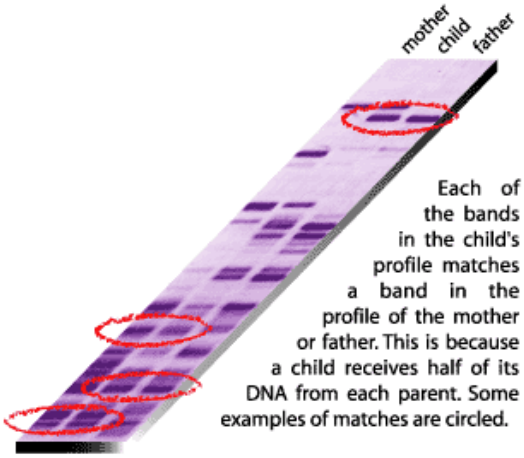
Eg:

ATCTTCTAACACATGACCGATCATGCATGCATGCATGCATGCATGCATGCATGCATGCATGCATGTTCCATGATAGCACAT

At each of the STR locus, one individual is either homozygous or heterozygous, and so can have a maximum of just two different alleles. Alleles are inherited in Mendelian fashion. Thus DNA profiles are commonly used in paternity tests. As the offspring inherits one allele from mother and the other from the father.

Example:

	Mother	**Father**	**Child**
Number of CATG repeats within STR	7, 11	15, 3	11, 3



Each of the bands in the child's profile matches a band in the profile of the mother or father. This is because a child receives half of its DNA from each parent. Some examples of matches are circled.

DNA profiling involves the use of nine STRs from different human chromosomes. These nine STR's are chosen by their qualities for example, easily producible, robust, highly informative and have low mutation rates. A tenth marker (not a STR) is used to identify gender (Amel gene locus, present on both X and Y, where X has less base pairs than the Y). Multiple copies of the STRs are made using PCR

Then they are separated using gel electrophoresis and exposed to various coloured fluorescent dyes, which allows them to be seen. Thus DNA profiling is also used for forensic purposes as if the

The result can be shown as the actual banding patterns from the gel electrophoresis or shown in Graph form, where horizontal axis at the top identifies the size of the various STR alleles. Meanwhile the vertical axis is the number of base pairs. Since the STR's of each individual vary the graph has peaks with different heights (as number of base pairs varies).

This process is mainly used for forensic purposes as if the same banding patterns obtained from gel electrophoresis for the nine STR's and gender marker of a suspect is the same as the one found in sample taken crime scene, it can inferred that the suspect is indeed guilty.

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"TSFX is fantastic, I don't know how I would have made it through Year 12 without them."

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