**RECOMBINANT DNA** (pages 199 – 206)

1. What are synthetic hormones?

Man made / artificial hormones that are chemically identical to natural occurring hormones.

1. Define recombinant DNA.

DNA that has been formed artificially by combining genes of two different species.

1. State the type of enzyme used to cut DNA. Restriction enzymes
2. Define recognition site.

A specific sequence of bases that is recognised and then cut by a restriction enzyme.

1. Distinguish between sticky ends and blunt ends, draw a diagram to aid in your response.

Sticky ends have unpaired nucleotides. Blunt ends do not – all bases are bonded with their complimentary base pair.



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7. The table on page 26 shows some restriction enzymes, the bacteria in which they were discovered and from which they were named, and the base sequence recognition sites on DNA where the enzymes cut the DNA, which are marked by arrows.

Refer to this table and answer the following questions in the spaces provided:

a. What is the abbreviation for the restriction enzyme from E. coli? Eco Rl

2.What is the base sequence recognition site for the restriction enzyme Eco R1?

G/AATTC

CTTAA/G.

3.From which bacteria was the Bam Hl restriction enzyme first isolated/discovered?

*Bacillus amy/oliquefaciens H*

4.What is the base sequence recognition site for Hind 111?

A/ A G C T T

T T C G A/ A

*5.*What is the abbreviation for the restriction enzyme from *Thermus aquaticus?*

Taq 1

6.What is the base sequence recognition site for Taq 1?

T /C G A

A G C /T

7.Hae 111 has the base sequence recognition site GGCC. Is cleavage at this site likely to produce "sticky ends" or "blunt ends" on the DNA?

blunt

8.Which other restriction enzyme in the table will cut DNA to produce "blunt ends"?

Alu 1

9.Which restriction enzymes in the table will cut DNA to produce "sticky ends"?

BamHl, Eco Rl, Hind 111, Sail, Taq 1

10.What is the common property of the base sequences of most restriction enzyme recognition sites?

palindrome

k. Which of the following base sequences on DNA are likely to be restriction enzyme recognition sites? B&D



1. State what a vector is and identify a vector used in recombinant technologies.

In genetic engineering, a vector is agent that is being used to carry/transfer DNA into another cell eg bacterial plasmids

1. State what a plasmid is and where it is found.

A plasmid is circular DNA found in bacteria.

1. State what a host cell is and identify host cells used in recombinant technologies.

The cell which houses the vector eg bacteria or yeast

1. Which DNA fragment (A, B, C) could be inserted into the plasmid below? Explain your answer.



B only. Plasmid complimentary to bases exposed at sticky end of DNA fragment

1. Scientists want to produce the protein made by gene X in large quantities. To do this they decide to clone gene X using bacterial plasmids.
2. Name the group of enzymes that would be used to cut gene and the plasmid. Restriction enzyme
3. Name the enzyme used to paste the gene into the plasmid. DNA ligase
4. Explain why this technique is called recombinant DNA technology.

It combines DNA from two different organisms.

1. State two practical applications of this technology to human health.

Formation of hormones and vaccines

1. The action of cutting DNA is illustrated below. Describe the procedure.
	1. Restriction enzyme recognizes the recognition site (specific sequence of bases) on the DNA strand.
	2. This forms sticky ends – exposed unpaired nucleotide bases overhang at each end as shown in the diagram.
2. Explain why ligation can be considered the reverse of the restriction enzyme process.

Restriction enzymes cut DNA and ligation sticks the DNA back together.

1. An image detailing ligation is displayed to the right. \

Describe how to create a recombinant DNA plasmid.

* 1. The DNA plasmid of the bacteria is cut using

 the same restriction enzyme.

* 1. This forms sticky ends.
	2. Complimentary base pairs will form between the sticky ends of the plasmid

and inserted gene due to hydrogen bonds forming.

* 1. DNA ligase will adhere the human gene into

the plasmid at the sticky ends by reforming

the bonds between the sugars in the DNA

backbone – ligat

1. Using the image below, in detail, describe the steps involved in producing insulin using recombinant DNA.
	1. Human gene for insulin is isolated from the DNA and cut using a

restriction enzyme. This creates isolated gene with sticky ends.

* 1. Plasmid from the bacteria is also cut using same restriction

enzyme creating complimentary stick ends.

* 1. Ligation occurs where DNA ligase joins the human insulin gene

into the plasmid. The complimentary sticky ends form hydrogen

bonds between the exposed nucleotides. DNA ligase then bonds

sugar backbone.

* 1. The recombinant DNA plasmid is placed back inside the bacterium.
	2. The bacterium is cultured and multiplies/proliferates therefore

multiple bacterium with the insulin gene are produced.

* 1. Human insulin created by the bacteria is filtered/harvested and purified.
	2. Human insulin injected or pumped to treat Type I diabetes

**Modelling Recombinant DNA**

Aim: To simulate the process of creating a recombinant insulin plasmid.

Materials

* Scissors - Tape - Insulin gene and plasmid

Method

1. Cut the plasmid and join the pieces 1 to 4 together to form a loop with the base letters outwards.
2. Cut the DNA sequence containing the insulin gene.
3. ****You are given the following three restriction enzymes
* **Eco R1** which has the recognition site:
* **Sal 1** which has the recognition site:
* **Bam H1** which has the recognition site:
1. Choose the best enzyme to use to make the recombinant plasmid. This enzyme must cut BOTH the plasmid in ONE place and cut the DNA sequence near the start and end of the insulin gene.
2. Using the enzyme, find the recognition site on the plasmid and cut between the first two bases as usual, then along between the bases. Then open the plasmid out into one strip.
3. Now use the same enzyme to cut the insulin gene from the DNA sequence.
4. Now join the insulin gene into the plasmid by matching up the bases and tape in place. You have now created a model of an insulin gene plasmid!

Discussion

1. Which restriction enzyme did you choose and why?
2. Describe what the tape in this model represented.

DNA ligase

1. Explain why sticky ends need to be formed for recombination to occur.

Exposed nucleotides allow for hydrogen bonds to form with complimentary bases.

1. Describe the remaining steps in order for insulin to be utilised for treatment.

The recombinant plasmid (vector) needs to be placed into a host cell eg bacteria. The bacteria then needs to be cultured. The insulin produced would then be harvested and purified.

Learning Objectives:

*Gene therapy can be used to treat a range of diseases, including diabetes mellitus (SHE 3.2)*

* Define the term vector, give examples and outline the properties that a vector must have in order to be suitable for use as a vector in biotechnology
* Describe (using an example) the process of gene therapy to replace defective genes including how genes are inserted using vectors
* Explain how gene therapy can be used to treat patients with diseases including diabetes mellitus

**Gene Therapy** (pages 213 – 214)

* 1. State the aim of gene therapy.

To treat genetic abnormality by identifying faulty genes and inserting healthy ones.

* 1. Identify three single gene diseases in which gene therapy research is focusing.

Cystic fibrosis, sickle cell anaemia, muscular dystrophy – potential for type 1 diabetes

* 1. Identify the vector used in gene therapy and explain why this vector is appropriate.

Virus/Viral phage. Used as viruses can easily transfer genetic material into a cell.

Identify the six steps involved in gene therapy.

* 1. Obtain normal functioning human gene
	2. Remove gene using restriction enzymes
	3. Amplify using PCR (explored in Unit 4)
	4. Select a vector to carry gene (usually a virus) and insert gene into vector
	5. Vector is introduced into a sample of patients cells, and then cultured (to amplify correct gene)
	6. Cells are then transferred back into patient
	7. Explain the process of how gene therapy could be used to treat diabetes type 1.

Type 1 diabetes is caused by an autoimmune disease that targets and destroys beta cells in the islets of Langerhans in the pancreas. Gene therapy is used to reprogram the alpha cells to produce insulin as alpha cells are not damaged. In order to achieve this, the gene for insulin is introduced into a vector. The vector is when used to transfer genetic material into the alpha cells. These cells are able to use protein synthesis to produce insulin.

* 1. Discuss if gene therapy would be useful in the treatment of diabetes type 2.

Diabetes type 2 is caused by the inability of cells to respond to insulin. There is therefore insulin being produced. Gene therapy is less likely to be used to treat diabetes type 2.

**Complete Chapter 8 Review Questions 1-6, 10, 11, 13-16, 19-24 on page 229 of your textbook.**