

Exercise walk-through

5.11

Recombinant DNA technology and drug production (statements 8.17–8.19)

Pearson Edexcel · International A-Level · Biology

Four stages, one purpose

Stage	Where	Yield
Glycolysis	cytoplasm	2 ATP + 2 reduced NAD
Link reaction	matrix	CO ₂ + reduced NAD
Krebs cycle	matrix	ATP + reduced NAD + reduced FAD
Oxidative phosphorylation	cristae	most ATP; O ₂ to water

Recombinant DNA: cut, paste, select

Cut : the same restriction enzyme opens plasmid and gene



Paste : complementary sticky ends sealed by DNA ligase



Transform : heat shock carries plasmids into bacteria



Select : antibiotic marker - only transformants survive

Check yourself

Your turn

Why is the human insulin gene made from mRNA before inserting into bacteria?

- ☐ A. bacteria cannot splice introns, and mature mRNA has none
- ☐ B. mRNA is more stable than DNA
- ☐ C. mRNA is double-stranded and easier to cut
- ☐ D. restriction enzymes only cut RNA

Exercises 2.1·2.3

2.1 Name the enzyme that cuts DNA at a recognition sequence and the enzyme that joins fragments. [2]

2.2 State why the same restriction enzyme is used on the plasmid and the human gene. [2]

2.3 Name the vector most commonly used to transform bacteria. [1]

Exercises 2.1·2.3

2.1 Name the enzyme that cuts DNA at a recognition sequence and the enzyme that joins fragments. [2]

2.1 Restriction enzyme; DNA ligase.

2.2 State why the same restriction enzyme is used on the plasmid and the human gene. [2]

2.2 So both DNAs are cut with the same sticky ends, which are complementary and can be ligated together.

2.3 Name the vector most commonly used to transform bacteria. [1]

2.3 A plasmid.

Exercises 3.1·3.2

3.1 Describe how human insulin is produced by genetically modified bacteria, from isolating the gene to harvesting the product, naming the enzymes used at each step. [6]

3.2 A plasmid carries genes for ampicillin resistance and for tetracycline resistance. The human gene is inserted into the tetracycline gene. Bacteria are plated on agar containing each antibiotic. Explain which colonies carry the recombinant plasmid and how you would identify them from the two plates. [5]

Exercises 3.1-3.2

3.1 Describe how human insulin is produced by genetically modified bacteria, from isolating the gene to harvesting the product, naming the enzymes used at each step. [6]

3.1 Isolate mRNA for insulin from human pancreatic cells and use reverse transcriptase to make complementary DNA without introns; cut the cDNA and a plasmid with the same restriction enzyme; join with DNA ligase to make the recombinant plasmid; transform bacteria and select on antibiotic medium using the marker gene; grow the selected bacteria in a fermenter; they transcribe and translate the human gene and the insulin is extracted and purified.

3.2 A plasmid carries genes for ampicillin resistance and for tetracycline resistance. The human gene is inserted into the tetracycline gene. Bacteria are plated on agar containing each antibiotic. Explain which colonies carry the recombinant plasmid and how you would identify them from the two plates. [5]

3.2 All colonies growing on the ampicillin plate carry a plasmid (its amp^R gene protects them). Colonies that grow on ampicillin but NOT on tetracycline carry the recombinant plasmid - inserting the human gene into the tet^R gene destroyed it. Replica-plate the amp survivors onto tetracycline medium to spot which fail to grow.

Exercise 3.3

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3.3 Advantages: the protein folds and is modified as in a human (bacteria cannot add sugar groups); a dairy goat produces large amounts continuously in milk, harvested simply by milking. Risk: the inserted gene could escape into wild populations or the animal could suffer welfare problems; ethical concerns about engineering animals.