5.11 Recombinant DNA technology and drug production

Name: _____ Class: _____ Date: _____ **Total: 21 marks**

KEY WORDS restriction enzyme • sticky end • DNA ligase • plasmid • marker gene

Objective

Practise statements 8.17-8.19: producing recombinant DNA with restriction enzymes, ligase and plasmid vectors, transforming host cells, selecting with marker genes, and making human proteins in GM organisms.

1 Key knowledge and worked examples

■ The recombinant DNA toolkit

Restriction enzyme : cuts DNA at one specific recognition sequence, often staggered, leaving **sticky ends** . **DNA ligase** : seals the sugar-phosphate backbone between sticky ends. **Plasmid** : the vector - a small circular DNA that bacteria copy and share. **Marker gene** : antibiotic resistance or fluorescence, to find the cells that took the plasmid in.

■ The insulin recipe

Isolate the human mRNA for insulin (from pancreatic cells); reverse transcriptase makes a DNA copy without introns - bacteria cannot splice; cut the copy and the plasmid with the SAME restriction enzyme so their sticky ends match; ligate into the plasmid; transform bacteria (heat shock or electroporation); plate on antibiotic medium - only transformants carrying the marker survive; grow in a fermenter and harvest the insulin. This replaces extraction from pigs - safer, scalable, no rejection risk.

■ Worked example: the marker-plate logic

Plate on ampicillin: survivors carry the plasmid (with its amp-resistance gene). To find which ALSO carry the insulin gene, the gene is inserted inside a second marker (e.g. tetracycline resistance): colonies that survive ampicillin but NOT tetracycline carry the insert, because the insertion broke the tet gene.

2 Practice

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]

3 Exam-style questions

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]

3.3 Human growth hormone can be produced in the milk of GM goats. Give two advantages and one risk of producing a human protein in a GM animal rather than in bacteria. [5]

Solutions

Original-task marking: M1 method, A1 accuracy, B1 reasoning.

2.1 Restriction enzyme; DNA ligase.

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

2.3 A plasmid.

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

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.