InkBiology

ANSWER BOOK

Recombinant DNA technology: isolating, inserting and expressing a gene

Gene regulation, genomics and biotechnology · Unit B17

AQA 3.8.4.1 · OCR A 6.1.3 · Edexcel A Topic 2, Topic 6 · CAIE 19.1, 19.2

5 questions · 18 marks

NAVY EDITION

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SECTION B · Standard, 3 to 5 marks

B1 **B1** a restriction endonuclease cuts only at its own recognition sequence, and an offset cut through the two strands leaves each fragment with a short single-stranded overhang, a sticky end. **B1** using one enzyme on both means every fragment carries the same overhang, so the exposed bases of the gene are complementary to those of the cut plasmid. **B1** the overhangs pair by hydrogen bonding between complementary bases, which holds the pieces together but leaves the sugar-phosphate backbones unjoined. **B1** DNA ligase catalyses the formation of phosphodiester bonds along both strands, which makes the join permanent. [4]

B2 **M1** the number of molecules doubles each cycle, so the number at the end is the starting number multiplied by 2 raised to the number of cycles. **M1** $5 \times 2^{20} = 5 \times 1\,048\,576$. **A1** 5 242 880 molecules. **A1** $20 \times 90 = 1800$ seconds, which is 30 minutes. [4]

B3 **B1** after transformation the culture holds three populations: cells with no plasmid, cells with a self-closed plasmid and cells with the recombinant plasmid. They look identical. **B1** a marker gene gives an observable difference, so the very small proportion of cells that took up the plasmid wanted can be found without testing every colony. **B1** a plasmid that closed on itself has an intact fluorescent protein gene, so it makes the protein and its colony glows under ultraviolet light. **B1** a plasmid carrying the insert has that gene interrupted in the middle, so no functional protein is made and the colonies wanted are the ones that do not glow. [4]

B4 **B1** 95 °C, at which the hydrogen bonds between paired bases break and the two strands separate. **B1** 50 to 65 °C, at which the primers bind to their complementary sequences at each end of the target region. **B1** 72 °C, which is the optimum for Taq polymerase as it extends each primer to build the new strand. [3]

B5 **B1** every nucleotide carries a phosphate group and phosphate groups are negatively charged, so every fragment is attracted towards the positive electrode. **B1** charge does not separate the fragments, because a longer fragment carries proportionately more of it. **B1** the agarose gel is a mesh, and a short fragment threads through it more easily than a long one, so in a given time the short fragments travel further. [3]