Biotechnology: Principles and Processes

Standard difficulty · 20 questions · 40 marks · Time: 42 minutes

Name: _____ Date: _____ Score: ____ / 40

Show your working for Sections B and C. Answers are on the last page — try every question first!

Section A — Choose the correct answer — 8 × 1 = 8 marks

1. The first recombinant DNA was made in 1972 by:
(a) Kary Mullis **(b)** Paul Berg
(c) Watson and Crick **(d)** Stanley Cohen and Herbert Boyer

2. Which enzyme recognises 5′-CTGCAG-3′?
(a) PstI **(b)** PvuI **(c)** BglII **(d)** SmaI

3. Which of these is a palindromic sequence (it reads the same 5′→3′ on both strands)?
(a) 5′-GCTGGG-3′ **(b)** 5′-AGATAT-3′ **(c)** 5′-CAATTG-3′ **(d)** 5′-TAGAGG-3′

4. PCR starts with 5 DNA molecules. How many copies are there after 3 cycles?
(a) 40 **(b)** 80 **(c)** 30 **(d)** 20

5. An enzyme cuts a circular plasmid at 1 site. The number of fragments formed is:
(a) 3 **(b)** 2 **(c)** 1 **(d)** 0

6. A restriction enzyme has an 8-base recognition site. In 1,114,112 bp of random DNA, it is expected to cut about how many times?
(a) 17 **(b)** 68 **(c)** 136 **(d)** 34

7. Fragments of 2,500, 3,000, 4,000, 5,000 bp are separated by agarose gel electrophoresis. Which moves least from the wells?
(a) 2,500 bp **(b)** 4,000 bp **(c)** 3,000 bp **(d)** 5,000 bp

8. Assertion (A): XbaI (5′-T↓CTAGA-3′) leaves sticky ends.
Reason (R): Restriction enzymes belong to a class of enzymes called nucleases.
(a) Both A and R are true, and R is the correct explanation of A.
(b) Both A and R are true, but R is not the correct explanation of A.
(c) A is true, but R is false.
(d) A is false, but R is true.

Section B — Short answer — 8 × 2 = 16 marks

9. What is genetic engineering?

10. SacI recognises 5′-GAGCTC-3′ and cuts between T and C on each strand. Write the other strand, show where the cuts are, and say what kind of ends are formed.

11. What does each part of the name EcoRI stand for?

12. How many times will NcoI (5′-CCATGG-3′) cut this linear DNA, and how many fragments will form? 5′-TTCCCATGGCATTGTCCATGGTTTCGCCATGGAACTT-3′

13. A linear DNA of 5,300 bp is cut at 1,300 bp and 1,700 bp from its left end. Give the sizes of the fragments and the one that moves farthest in a gel.

14. How many copies of a DNA segment are made from 2 template molecules after 27 PCR cycles? Why is Taq polymerase used?

15. A gene is inserted at the PvuI site of pBR322, which lies in its ampicillin-resistance gene. On which of ampicillin and tetracycline plates will cells that took up no plasmid grow?

16. Explain (a) agarose and (b) a stirred-tank bioreactor.

Section C — Word problems — 4 × 4 = 16 marks

17. Explain the steps in recombinant DNA technology.

18. A circular plasmid of 5,700 bp is cut at positions 1,900 and 5,400 (counted from a fixed point on the circle). How many fragments form, and what are their sizes? In what order (nearest the wells first) will they appear in a gel? How is a band recovered?

19. Describe the three steps of a PCR cycle. Starting from 10 DNA molecules, how many copies are there after 20 cycles? Why is a thermostable polymerase essential?

20. Describe the steps in making recombinant DNA and putting it into E. coli DH5α, using BamHI (5′-GGATCC-3′) to cut the DNA and pBR322, whose BamHI site lies in the tetracycline-resistance gene. How are recombinants picked out?

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Answer key

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Q	Answer	Working
1	**(d) Stanley Cohen and Herbert Boyer**	—
2	**(a) PstI**	—
3	**(c) 5′-CAATTG-3′**	the other strand of CAATTG, read 5′→3′, is CAATTG
4	**(a) 40**	each cycle doubles the DNA: 5×2^3
5	**(c) 1**	a circle cut k times gives k pieces
6	**(a) 17**	a given 8-base sequence occurs once in 4^8 = 65,536 bp
7	**(d) 5,000 bp**	DNA is negatively charged and moves to the anode; smaller fragments move farther through the gel
8	**(b) Both A and R are true, but R is not the correct explanation of A.**	A is true: XbaI leaves sticky ends with a 5′ overhang CTAG. R is true but does not explain A.
9	**Techniques that alter the chemistry of genetic material (DNA and RNA), introduce it into a host organism and so change the host's phenotype.**	—
10	**3′-CTCGAG-5′; top 5′-GAGCT↓C-3′; sticky ends with a 3′ overhang AGCT**	the site is palindromic, so both strands are cut at the same place in the 5′→3′ reading
11	**E: genus Escherichia; co: species coli; R: strain RY13; I: the first enzyme isolated from this strain**	first letter: genus; next two letters: species; then the strain, if any; the Roman numeral gives the order of isolation
12	**3 times; 4 fragments**	look for the recognition sequence; a line cut k times gives k + 1 pieces
13	**1,300 bp, 400 bp, 3,600 bp; the 400 bp fragment**	1,700 − 1,300 = 400; 5,300 − 1,700 = 3,600
14	**2×2^{27} = 268,435,456; it comes from Thermus aquaticus and stays active at the high temperatures used to denature DNA**	each cycle doubles the DNA
15	**They grow on neither antibiotic**	insertional inactivation of a resistance gene
16	**(a) A natural polymer from seaweed used as the matrix in gel electrophoresis; (b) a large vessel with a stirrer that mixes contents and supplies oxygen evenly**	—
17	**1. Isolation of DNA: cells are broken with enzymes (lysozyme, cellulase, chitinase), RNA and protein removed with RNase and protease, and DNA precipitated with chilled ethanol. 2. Cutting DNA at specific sites with restriction enzymes, checked by gel**	—

Q	Answer	Working
	electrophoresis. 3. Amplifying the gene of interest by PCR. 4. Ligating the gene into a vector with DNA ligase. 5. Inserting the recombinant DNA into a host. 6. Obtaining the foreign gene product, in bioreactors, followed by downstream processing.	
18	**2 fragments: 2,200 bp, 3,500 bp. From the wells: 3,500, 2,200 bp (smallest moves farthest). The band is seen with ethidium bromide under UV, cut out of the gel and extracted (elution)**	a circle cut k times gives k pieces; one piece wraps past the fixed point
19	**Denaturation: heating separates the two strands. Annealing: two primers bind to the ends of the target on the single strands. Extension: Taq polymerase adds nucleotides from the primers, copying each strand. After 20 cycles: $10 \times 2^{20} = 10{,}485{,}760$ copies. Taq polymerase from Thermus aquaticus survives the high temperature of denaturation, so it need not be added again each cycle**	—
20	**Isolate DNA (break cells with lysozyme, remove RNA and protein with RNase and protease, precipitate DNA with chilled ethanol). Cut the gene and the plasmid with BamHI, which leaves matching ends at GGATCC. Join them with DNA ligase. Make E. coli competent with Ca^{2+} ions and give a heat shock (ice, 42 °C, ice) so it takes up the plasmid. Grow on ampicillin: only transformants survive. Recombinants have lost tetracycline resistance by insertional inactivation, so they fail to grow on tetracycline (replica plating)**	—

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