Study Guide — BI Problem 6: Molecular Biology (Mid-Unit Test)

Biomedical Innovations · Problem 6 — Molecular Biology · Modeled on the PLTW Guided Review format

Vocabulary to know cold

Term	Definition
Restriction enzyme	A protein that recognizes a specific DNA sequence and cuts the double helix at that site.
Recognition sequence	The short, often palindromic DNA sequence where a restriction enzyme binds and cuts (e.g., GAATTC for
Sticky (cohesive) ends	Single-stranded overhangs left by a staggered cut; they base-pair with complementary overhangs.
Blunt ends	Cut ends with no overhang, produced by enzymes that cut both strands at the same position.
DNA ligase	Enzyme that joins DNA fragments by forming phosphodiester bonds in the backbone.
Plasmid vector	A circular DNA carrier used to move a gene of interest into a host cell.
Competent cells	Bacteria treated so they can take up foreign DNA from their surroundings.
Heat shock	A brief temperature spike that helps competent cells take up plasmid DNA.
Selectable marker	A gene, often antibiotic resistance, that lets only transformed cells grow on a selective plate.
Restriction digest	A reaction in which restriction enzyme(s) cut DNA into defined fragments.
Buffer	A solution that holds the pH and salt conditions an enzyme needs to function.
Loading dye	A dense, colored solution added to samples so they sink into gel wells and their progress is visible.

Worked examples (follow the reasoning)

Example 1. A 6000 bp circular plasmid has restriction sites for an enzyme at positions 0, 2000, and 4500. How many fragments result and what are their sizes?

- A circular molecule cut at 3 sites yields 3 fragments (cuts = fragments for a circle).
- Fragment 1: from 0 to 2000 = 2000 bp.
- Fragment 2: from 2000 to 4500 = 2500 bp.
- Fragment 3: from 4500 back around to 0 (6000) = 1500 bp.
- Check: 2000 + 2500 + 1500 = 6000 bp total.

Answer: Three fragments: 2000 bp, 2500 bp, and 1500 bp.

Example 2. You digest a plasmid and the insert with the same enzyme, ligate, transform, and plate on ampicillin. No colonies grow. Give one likely reason and a fix.

- If no colonies appear, transformation or selection failed at some step.
- A common cause is that the heat-shock step was skipped or cells were not competent, so no plasmid entered the cells.
- Another cause is that ligase was inactive (left warm/old), so no recombinant plasmid formed.
- Fix: use fresh competent cells, include the proper heat-shock, and use ligase stored at -20 C with fresh buffer; include a positive control plasmid.

Answer: Likely failed transformation or inactive ligase; use competent cells with correct heat shock and active ligase, plus a positive control.

Practice questions (with reasoning)

1. Two DNA fragments cut with the same enzyme join easily because they share:

A. The same length
B. Complementary sticky ends
C. The same color
D. Identical genes

Answer: B. Matching overhangs from the same enzyme base-pair, allowing ligation.

2. A circular plasmid is cut once by a restriction enzyme. On a gel this appears as:

A. No bands
B. A single linear band
C. Three bands
D. A smear only

Answer: B. One cut in a circle produces one linear fragment, seen as a single band.

3. Transformed bacteria are selected by growing them on plates containing antibiotic because:

A. Antibiotic feeds the bacteria
B. Only plasmid-containing cells carry the resistance gene and survive
C. Antibiotic colors the colonies
D. It speeds up ligation

Answer: B. The resistance marker on the plasmid lets only transformed cells grow.

4. Enzymes should be removed from the freezer, kept on ice, and used promptly because:

A. Cold breaks them
B. Warmth denatures them and lowers activity
C. Light cuts the DNA
D. Ice is required to read the gel

Answer: B. Heat denatures the protein enzyme, so it is kept cold to preserve activity.