Study Guide — BI Problem 6: Molecular Biology (End-of-Unit Cumulative Test)

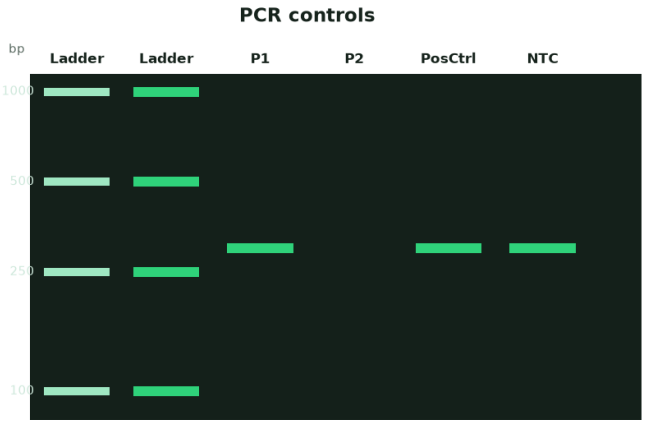
Biomedical Innovations · Problem 6 — Molecular Biology · Modeled on the PLTW Guided Review format

Vocabulary to know cold

Term	Definition
Recombinant DNA	DNA formed by joining segments from two different sources into one molecule.
Restriction enzyme	Protein that cuts DNA at a specific recognition sequence, leaving sticky or blunt ends.
DNA ligase	Enzyme that seals DNA fragments together via phosphodiester bonds.
Plasmid	Small circular DNA used as a cloning vector; often carries a selectable marker.
Transformation	Uptake of foreign DNA (a plasmid) by a competent bacterial cell.
PCR	Amplification method using denature, anneal, and extend steps to copy a target sequence.
qPCR / standard curve	Real-time PCR; a standard curve relates known DNA amounts to signal so unknowns can be quantified.
Gel electrophoresis	Size-based separation of DNA fragments in a gel under an electric field.
DNA ladder	Known-size fragments used to estimate sample band sizes.
No-template control (NTC)	Reaction with no DNA template; a band signals contamination.
Positive control	A sample known to give a result; confirms reagents and equipment worked.
Selectable marker	A gene (often antibiotic resistance) that identifies transformed cells.
Sensitivity	Proportion of true positives a test correctly detects (spiral, Problem 5).
Specimen identifiers	At least two unique patient identifiers required on a labeled sample (spiral, Problem 1).

Worked examples (follow the reasoning)

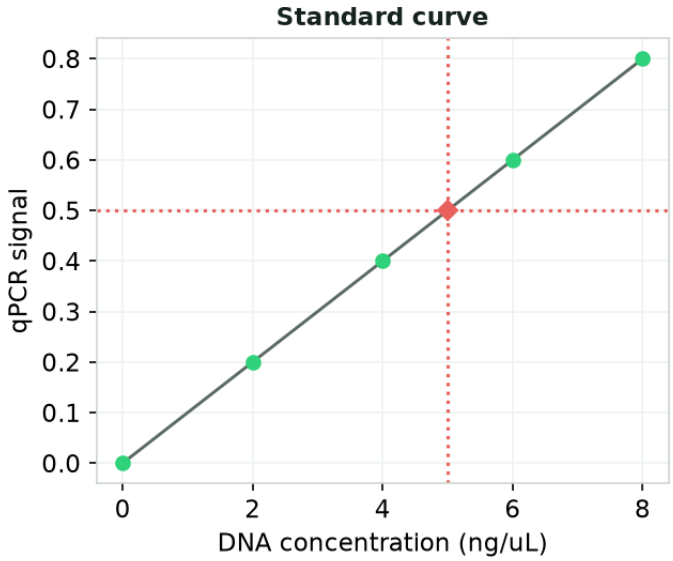
Example 1. Interpret a PCR gel with controls: Patient 1 = 300 bp band, Patient 2 = no band, Positive control = 300 bp, NTC = 300 bp. What is your conclusion?



- Check the positive control: it shows a band, so the assay can detect target.
- Check the NTC: it shows a band, but it should be blank. This means the reagents are contaminated.
- Because the NTC failed, the patient results cannot be trusted, even P1's apparent positive.
- Action: discard the run, decontaminate, use fresh reagents/filter tips, and repeat.

Answer: The run is invalid: the NTC band shows contamination, so patient results must be repeated with clean reagents.

Example 2. Using a qPCR standard curve where signal = 0.1 x concentration (ng/uL), an unknown gives a signal of 0.50. Find the concentration.



- The line is signal = 0.1 x concentration.
- Set 0.50 = 0.1 x concentration.
- Solve: concentration = 0.50 / 0.1 = 5.
- Read the curve at signal 0.50 to confirm it lands at 5 ng/uL.

Answer: About 5 ng/uL.

Example 3. A 6000 bp circular plasmid is cut by an enzyme at positions 0, 1500, and 4000. List the fragment sizes.

- Three cuts in a circle give three fragments.
- 0 to 1500 = 1500 bp.
- 1500 to 4000 = 2500 bp.
- 4000 back to 6000/0 = 2000 bp.
- Check: 1500 + 2500 + 2000 = 6000 bp.

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

Practice questions (with reasoning)

1. A band appears in the no-template control. The PCR results should be:

A. Accepted as valid
B. Rejected because reagents are contaminated
C. Doubled in concentration
D. Loaded again without changes

Answer: B. An NTC band means contamination, invalidating the run.

2. The correct order of one PCR cycle is:

A. Anneal, extend, denature
B. Denature, anneal, extend
C. Extend, denature, anneal
D. Anneal, denature, extend

Answer: B. Denaturation separates strands, primers anneal, then polymerase extends.

3. Bacteria grow on an ampicillin plate only if they:

A. Are dead
B. Carry the plasmid's antibiotic-resistance gene
C. Lost the plasmid
D. Were never transformed

Answer: B. The selectable marker on the plasmid confers survival on the antibiotic.

4. SPIRAL (Problem 5): A test with high sensitivity is best at:

A. Ruling out disease in healthy people
B. Correctly detecting people who truly have the disease
C. Lowering reagent cost
D. Speeding up centrifugation

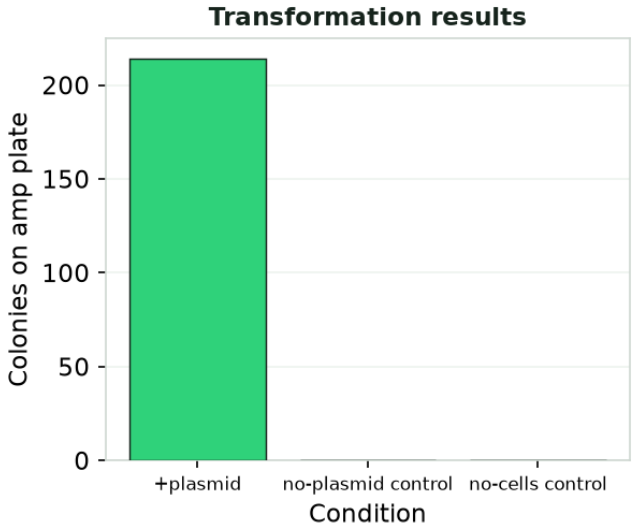
Answer: B. Sensitivity is the true-positive rate, so high sensitivity detects true cases well.

5. Enzymes for cloning and PCR are stored long-term at:

A. 37 C
B. -20 C
C. Room temperature
D. 100 C

Answer: B. -20 C preserves enzyme activity for storage; keep on ice during use.

6. The bar graph shows colony counts from a transformation experiment plated on ampicillin. Based on the controls, what does the result for the +plasmid cells indicate?



A. The experiment failed because the controls grew
B. The transformation worked: only cells that took up the resistance plasmid grew, and clean controls show no contamination
C. Ampicillin was inactive
D. The cells were dead

Answer: B. Colonies only on the +plasmid plate, with both controls at zero, show successful uptake of the resistance plasmid and no contamination.