Study Guide — BI Problem 6: Molecular Biology (Pretest)

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

Vocabulary to know cold

Term	Definition
Recombinant DNA	A single DNA molecule made by joining DNA from two different sources.
Restriction enzyme	A protein that cuts DNA at a specific recognition sequence.
DNA ligase	An enzyme that seals (pastes) DNA fragments together by forming phosphodiester bonds.
Plasmid	A small, circular, self-replicating DNA molecule used as a cloning vector in bacteria.
Transformation	The uptake of foreign DNA, such as a plasmid, by a bacterial cell.
PCR	Polymerase chain reaction; a method that makes millions of copies of a targeted DNA segment.
Gel electrophoresis	A technique that separates DNA fragments by size using an electric field through a gel.
DNA ladder	A mixture of DNA fragments of known sizes used to estimate the size of sample bands.
No-template control (NTC)	A PCR reaction with all reagents but no DNA; used to detect contamination.
Selectable marker	A gene (often antibiotic resistance) that lets researchers identify cells that took up a plasmid.
Biohazard waste	Waste containing biological material that must be disposed of in a labeled biohazard container.

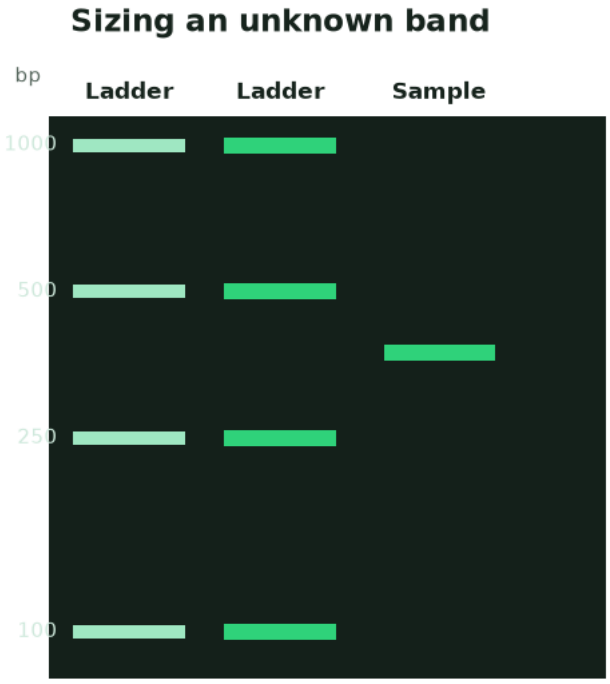
Worked examples (follow the reasoning)

Example 1. You want to insert a human gene into a plasmid. List the basic order of steps in making the recombinant plasmid.

- Cut both the human DNA and the plasmid with the SAME restriction enzyme so they have matching ends.
- Mix the cut fragments so the complementary ends can pair up.
- Add DNA ligase to seal the gene into the plasmid, forming recombinant DNA.
- Transform the recombinant plasmid into bacteria.
- Grow on antibiotic plates so only transformed cells survive.

Answer: Cut with restriction enzyme, anneal matching ends, ligate, transform, then select with antibiotic.

Example 2. A gel has a ladder with bands at 1000, 500, 250, and 100 bp. An unknown sample band sits halfway between the 500 and 250 markers. About how large is it?



- Find the two ladder bands the unknown sits between: 500 bp and 250 bp.
- A band positioned about midway corresponds to a size between 250 and 500 bp.
- Estimate roughly 375 bp.

Answer: About 375 base pairs.

Practice questions (with reasoning)

1. Which enzyme would you add to seal a gene into a cut plasmid?

A. Restriction enzyme
B. DNA ligase
C. Helicase
D. Amylase

Answer: B. DNA ligase joins the fragments together.

2. A band appears in your no-template control lane. The most likely explanation is:

A. The PCR worked perfectly
B. Contamination of reagents with DNA
C. The ladder was loaded twice
D. Too little buffer was used

Answer: B. Any band in the NTC indicates DNA contamination because no template was added.

3. Enzymes used in molecular biology should be kept on ice during use and 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.