AP BIOLOGY · UNIT 6 · TOPIC 6.8

Biotechnology

Learning objective 6.8.A Explain the use of genetic engineering techniques in analyzing or manipulating DNA.

Name: Date: Period:

Use the tool **Cut, Copy, Measure**. Parts 1 and 2 use **Cut it**, Part 3 uses **Measure it**, and Parts 4 and 5 use **Copy it**. Two facts are tested here: that a gel separates fragments by the **logarithm** of their size, and that PCR doubles the DNA every cycle. Part 3 uses the first fact to size unknown bands, and Part 4 tests what the second fact leaves out.

Part 1 · Digesting a plasmid

The plasmid is **2,738 bp** and circular. Press EcoRI, BamHI and HindIII in turn, and record the result for each enzyme, including any enzyme that does not cut.

Enzyme	Recognition site	Cuts	Fragments (circular)	Fragments if linear
EcoRI				
BamHI				
HindIII				

1. (a) Describe what a restriction enzyme does.

(b) Explain how a recognition sequence that reads the same on both strands allows one enzyme to cut both strands.

2. (a) Describe the relationship between the number of cuts and the number of fragments for a circular molecule and for a linear molecule.

(b) Explain why the two relationships are different.

Part 2 · The check that costs nothing

Press EcoRI. Record the length of each fragment, and how it can be calculated from the cut positions on the plasmid map. Then add the lengths.

Fragment	Length (bp)	How it is calculated from the cut positions
Fragment 1		
Fragment 2		
Fragment 3		
Total		

3. (a) Describe the check that should be performed on any set of fragment lengths from a digest.

(b) Explain what a failed check would indicate.

4. Two enzymes in Part 1 produced no cuts. Explain why this result provides information about the plasmid rather than indicating a failed experiment.

Part 3 · Sizing an unknown band

Switch to **Measure it**. Press Plot against log(size) and Plot against size in turn, and record the size each calibration gives for the three unknown bands. Then compare both with the true sizes.

Band	Distance (mm)	Size from log(size) line	Size from size line	True size
Band A	33.4			
Band B	41.2			
Band C	52.9			

5. (a) Identify the r^2 value of each calibration.

(b) Explain what the r^2 values indicate about which calibration should be used to size the unknown bands.

6. One of the two calibrations gives an impossible size for one band.
(a) Identify the calibration and the band.

(b) Explain why an impossible result is stronger evidence that a model is wrong than an inaccurate result is.

7. A gel shows three bands from a digest.
(a) Explain why the digest could have produced more than three fragments.

(b) Describe how the gel could indicate that one band contains more than one fragment.

Part 4 · What PCR actually produces

Open **Copy it** and press each cycle button in the table, from 1 cycle to 30 cycles. The third column is the number of strands that are **exactly** the target length.

Cycle	Total strands	Exactly the target length	% that is product
1			
2			
3			
4			
6			
10			
20			
30			

8. Explain what determines which region of DNA is amplified by PCR.

9. A student claims that “PCR doubles the amount of the target product every cycle, starting from the first cycle.” Using two rows of the Part 4 table, evaluate the student’s claim.

Part 5 · Getting a gene into a cell

Researchers mix bacterial cells with a plasmid that carries an antibiotic resistance gene. They spread one million of the cells on a plate with no antibiotic and one million on a plate with the antibiotic. Use the Putting a gene into a cell panel of Copy it to complete the table.

Plate	What grows	Colonies	Is the plate any use?
No antibiotic			
With antibiotic			

10. Transformation succeeds in about 1 cell in 10,000. Explain why this low success rate is not a problem when the cells are grown on the plate with the antibiotic.

11. For each of these techniques, identify the question that the technique answers.
(a) Restriction enzymes:

(b) Gel electrophoresis:

(c) PCR:

(d) Bacterial transformation:

(e) DNA sequencing:

What the model simplifies

The plasmid sequence was built for this tool rather than taken from a real plasmid, so that the number of recognition sites would be known in advance and the search would have something definite to find. The searching, the cut positions and the fragment lengths are all genuinely computed from that sequence.

Restriction enzymes are shown as cutting at a point. Most cut the two strands at different places, leaving short single-stranded overhangs (“sticky ends”), and that offset is exactly what makes it possible to join a fragment into a plasmid cut with the same enzyme.

The gel model uses one straight line through log(size) across the whole range. Real gels depart from it at both extremes: very large fragments stop separating, and very small ones run off the end. The calibration also depends on the gel's concentration and the voltage, so a ladder must be run on the same gel as the samples.

The PCR simulation counts single strands, which is why the exact-length strand first appears at cycle 2 here and the familiar figure is cycle 3. That figure counts double-stranded molecules, which need one more cycle. It also assumes every strand is copied in every cycle, and it ignores the plateau: reagents run out, so the doubling cannot continue indefinitely.

The transformation efficiency is a plausible figure rather than a measured one; real efficiencies vary enormously with the method and the cells. The structure of the argument, that selection makes a low success rate irrelevant, does not depend on the exact number.