

# 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 things here are usually stated and almost never used: that a gel separates by the **logarithm** of the size, and that PCR doubles the DNA every cycle. Part 3 makes the first one do some work, and Part 4 finds out what the second one leaves out.

## Part 1 • Digesting a plasmid

The plasmid is **2,738 bp** and circular. Run each enzyme in the tool and record what it does. One of them will do nothing at all — record that too.

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

**1. Explain what a restriction enzyme does, and why its recognition sequence reads the same on both strands.**

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**2. State the general rule relating the number of cuts to the number of fragments, for a circular molecule and for a linear one, and explain the difference.**

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**Part 2 · The check that costs nothing**

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Record the fragment lengths from the EcoRI digest, then add them up.

| Fragment   | Length (bp) | How you would get this from the cut positions |
|------------|-------------|-----------------------------------------------|
| Fragment 1 |             |                                               |
| Fragment 2 |             |                                               |
| Fragment 3 |             |                                               |
| Total      |             |                                               |

**3. State the check you should always perform on a set of fragment lengths, and explain what a failed check would tell you.**

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**4. One enzyme in Part 1 produced no cuts. Explain why that is a result worth reporting rather than a failed experiment.**

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Part 3 · Sizing an unknown band

Switch to **Measure it**. Use each calibration in turn and record what it says about the three unknown bands. Then compare both against 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. State the  $r^2$  of each calibration, and explain what  $r^2$  is telling you about which model to trust.

6. One of the two calibrations returns an impossible answer for one band. Identify it, and explain why an impossible answer is more informative than a merely inaccurate one.

7. A gel shows three bands. Explain why you cannot conclude that the digest produced exactly three fragments, and state how you might tell.

## Part 4 · What PCR actually produces

Open **Copy it** and step through the cycles. The third column is the number of strands that are **exactly** the length you wanted.

| 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.**

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**9. “PCR doubles the amount of your product every cycle from the start.” Evaluate that statement using two rows of your table.**

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## Part 5 · Getting a gene into a cell

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A million bacterial cells are mixed with a plasmid carrying an antibiotic resistance gene, then split between two plates.

| 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 that low success rate is not a problem in practice.**

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**11. For each technique in this topic, state in one line the question it answers. Restriction enzymes; gel electrophoresis; PCR; transformation; sequencing.**

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## What the model simplifies

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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.