Hands-on DNA: Bacterial Evolution – Restriction Enzyme Activity

Students work in groups of 4, each group should have…

- Set of four different paper DNA sequences
- Pencil/pen
- Scissors

Take a DNA sequence each.

Find the EcoRI restriction sites in your sequence (GAATTC) and draw a box around them on both strands.

The four different sequences have different numbers of restriction sites (see information below).

*Make sure students draw a box that includes both DNA strands, not just the top 'sense' strand. If they read the bases on the lower strand from right to left they will also read as '*GAATTC*'.*

Use scissors to cut the DNA at the appropriate place – between G and A on the top strand, between the two strands, and between A and G on the bottom strand to produce "sticky ends".

The scissors represent the action of the restriction enzyme, which cuts the sugar-phosphate backbone at the restriction site (and nowhere else) on both strands of the DNA. This generates double-stranded DNA fragments.

Students may find it easier to cut in the right place if they first draw a line between G and A then a line between A and G on bottom strand then draw a horizontal line between the two strands joining their first two lines together.

Count the number of bases in the top strand of each of your fragments and record the results on your worksheet. The total of all your fragments should be 200bp.

This demonstrates that in a given length of DNA (all four DNA sequences are 200bp long) the number and location of restriction sites will determine the number of fragments and their individual sizes.

Prompt the students to compare the four sequences in their group – do they have the same number of restriction sites? Are the same numbers of fragments generated? Are the fragments the same size? Why not?

How might this technique be useful? DNA fingerprinting, paternity testing etc.

DNA Sequence Information

Sequence	Number of restriction sites	Number of fragments	Fragment sizes (bp)
1	3	4	12, 29, 47, 112
2	2	3	29, 144, 27
3	3	4	10, 63, 33, 94
4	1	2	79, 121

Learning Objectives and Additional Information

- There are many different restriction enzymes, each with a different recognition site.
- DNA is double-stranded and the restriction enzyme site has the same sequence when read forwards in the 'sense' strand and in the reverse direction on the 'anti-sense' strand. These sites are often described as 'palindromic' i.e. they read the same forwards as backwards (on different strands!).
- EcoRI is a common restriction enzyme which cuts close to one end of the restriction site (G^AATTC), when both strands are cut it generates overhanging 'sticky ends'.

Optional Extension Activity: Gel Electrophoresis

Students will need:

- Their 'digested' DNA sequences from the restriction enzyme activity
- DNA size marker and lane marker cards

Either immediately after the digest exercise **or** as part of the explanation of gel electrophoresis later in the workshop, students can simulate gel electrophoresis of their DNA fragments in the following way:

Either using the wall of the lab (or on flip charts or benches if this is not practical) students should arrange their DNA size marker cards in order (largest at the top, smallest at the bottom as a DNA ladder would run on a gel) and arrange their fragments in size order in relation to the marker cards. Students should be prompted to keep each set of fragments separate, in its own 'lane' and not to mix fragments from different sequences together. Using the lane marker cards (numbered 1-4) should help with this.

Or by placing the DNA and lane marker cards on the floor, students can recreate gel electrophoresis in a large space in the lab, each holding a DNA fragment and moving an appropriate distance through the 'gel'.

Learning objectives:

- DNA fragments move different distances through the gel, depending on their size – smaller fragments move faster and therefore further than large fragments.
- DNA moves from negative to positive electrode due to its overall negative charge.
- The pattern of fragments obtained when different DNA is digested with the same enzyme will be different if the sequences of the original DNA were different – this is the basis for techniques such as DNA fingerprinting (for solving crimes) and paternity testing.