Restriction mapping exercise

Overview

The plan is to provide a short activity for A level students, similar to the DNA profiling option offered to KS4 (GCSE) students. This restriction mapping exercise enables them to utilise and hone valuable practical skills using common molecular biology equipment and requires logic and reasoning skills to interpret the results. It fits with the syllabus, covering restriction enzyme use, agarose gel electrophoresis and providing an interesting link to the preliminary work done on the HGP, which can be discussed during the pedagogical uses of this new practical activity.

Practically, 2 enzymes will be needed, preferably ones that cut one of the existing plasmids two and three times. Ideally the enzyme sites will fall into sites of interest, verifying that they are likely to be present (for example, the *araC* gene, *rfp* gene and beta-lactamase gene). One of the enzymes used in the DNA profiling is useful in this respect; *PstI* cuts pARA-R twice, once in the *rfp* gene and once in the beta-lactamase gene. I have browsed all of the enzymes that cut the pARA-R plasmid three times. *Dra*I is the cheapest (£209 per 10,000 Units) and will cut both of the *Pst*I fragments of pARA-R. It cuts within the beta-lactamase and *araC* genes. It will also cut in the same restriction buffer as *PstI*; cutsmart. The location of the restriction sites is shown in Figure 1.

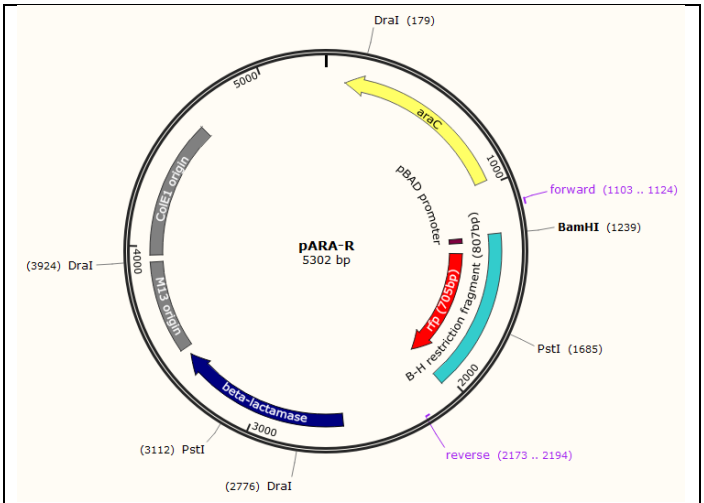

Figure 1. A plasmid map of pARA-R, showing the location of the *Bam*HI, *Pst*I and *Dra*I restriction sites.

Expected results

The size of the expected restriction fragments from single and double digests is given in Table 1 and an illustration is shown in Figure 2. From these results it should be possible to create a simple restriction map of the plasmid.

Restriction enzyme(s) used	Expected DNA fragment sizes (bp)
*Bam*HI	5302
*Pst*I	1427 + 3875
*Dra*I	2597 + 1148 + 1557
*Pst*I + *Dra*I	1557+ 1506 + 1091 + 336 + 812

Table 1. Size of the expected restriction fragments from digestion of the pARA-R plasmid.

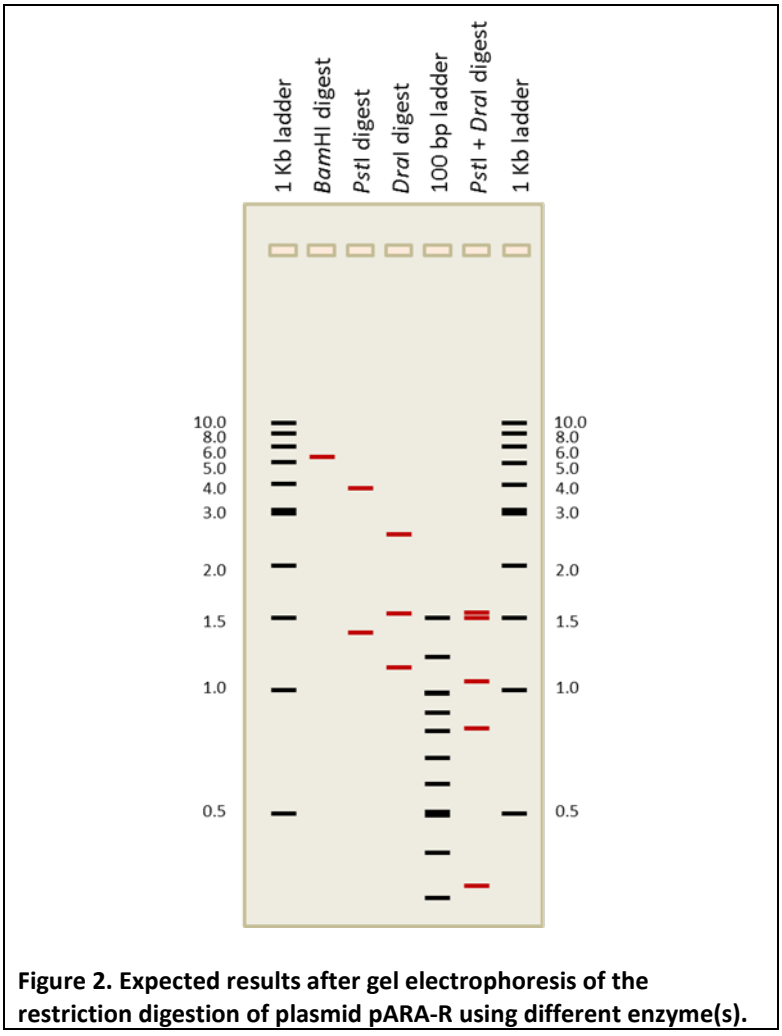

Figure 2. Expected results after gel electrophoresis of the restriction digestion of plasmid pARA-R using different enzyme(s).

Observation and interpretation

The expected conclusions that students can draw from the results using their skills of observation and interpretation are:

- Lane 1 (undigested plasmid): Undigested plasmid will be present in circular form, or multiple circular forms and provides a comparison to see that the restriction digestions are working.
- Lane 2 (plasmid digested with *Bam*HI): A single band appears, so *Bam*HI must have a single recognition sequence and cut the plasmid circle of DNA once, producing one linear piece of DNA, allowing the size of the plasmid to be determined more accurately than use of circular DNA (at 5302 bp). If the restriction digestion is incomplete, multiple forms of the plasmid may appear, which would confuse the size determination, but which could be used to discuss enzyme reactions, their rates and confounding factors.
- Lane 3 (plasmid digested with *Pst*I): 2 bands appear, so *Pst*I must have two recognition sequences within the circular plasmid DNA, producing two linear pieces of DNA after restriction digestion (at 1427 bp and 3875 bp). If the restriction digestion is incomplete, *Pst*I may only cut at one of the recognition sequences, producing a band at 5302 bp, or multiple forms of uncut circular plasmid could be present.
- Lane 4 (plasmid digested with *Dra*I): 3 bands appear, so *Dra*I must have three recognition sequences within the circular plasmid DNA, producing three linear pieces of DNA after restriction digestion (at 2597 bp, 1148 bp and 1557 bp). If the restriction digestion is

incomplete, *Dra*I may only cut at one of the recognition sequences, producing a band at 5302 bp. It may cut at two of the recognition sequences, producing bands of 3745 bp, 4154 bp or 2705 bp, or multiple forms of uncut circular plasmid could be present.

- Lane 5 (plasmid double-digested with *Pst*I and *Dra*I): 4 bands appear, however you'd expect 5 bands if *Pst*I cuts the circular plasmid twice and *Dra*I cuts it three times. The band sizes are; 1557 bp, 1506 bp, 1091 bp, 336 bp and 812bp. It is noticeable that one of the bands is thicker than the others. This is because 2 similarly sizes bands have not been completely separated by gel electrophoresis (the bands of 1557 bp and 1506 bp).

Building up a restriction map

In order to build up a restriction map of the plasmid, showing the order of the restriction sites *Pst*I and *Dra*I, students need to use their logic and reasoning skills to understand these steps:

- Immediately it can be seen that the 1557 bp band from the plasmid digested with *Dra*I is still present, whilst the 2597 bp and 1148 bp bands have both been cut, each producing 2 smaller linear DNA fragments. So, on the restriction map there must be a DNA fragment cut by *Dra*I either end that is 1557 bp long (Figure 3a).
- The 1557 bp fragment in the double digest combines with the 1506 bp and 812 bp fragments from the double digest to make the 3875 bp fragment from the *Pst*I digest. Therefore the 1506 bp and 812 bp fragments must flank the 1557 bp fragment and have a *Pst*I site either end (Figure 3b).
- The fragment from the *Dra*I restriction digest that includes the 812 bp fragment from the double digest is 1148 bp long, also including the 336 bp fragment from the double digest. This means that the 336 bp fragment from the double digest must be next to the 812 bp fragment and have a *Dra*I restriction site at the end of it (Figure 3c).
- There are then 2 ways to complete the restriction map. Either by recognising that the 2597 bp *Dra*I restriction fragment containing the 1506 bp fragment from the double digest, also contains the 1091 bp fragment (Figure 3d), **or** that the 1427 bp *Pst*I restriction fragment containing the 336 bp fragment from the double digest, also contains the 1091 bp fragment (not shown).

Producing a plasmid restriction map that is a mirror image of the one shown (Figure 3d) is also completely correct. Students sometimes find this confusing.

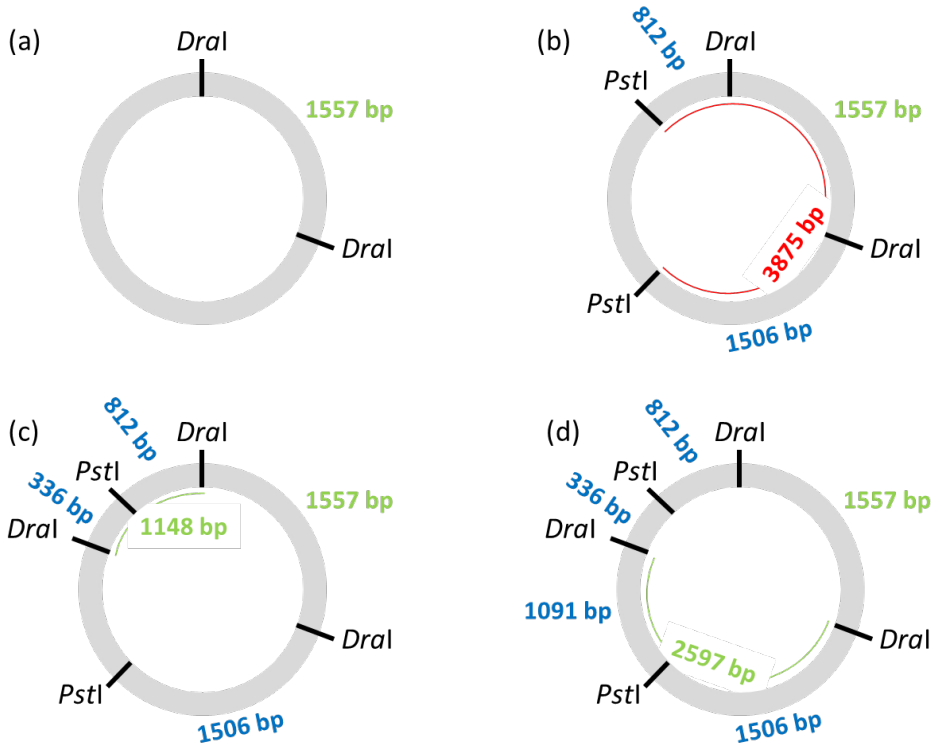

Figure 3. The steps in drawing a restriction map of a plasmid (not drawn to scale). The fragment sizes are colour-coded, so that green represents fragment sizes from digestion with *Dra*I, red from digestion with *Pst*I, and blue from double digestion with *Dra*I and *Pst*I. (a) The plasmid must contain a 1557 bp DNA fragment flanked by *Dra*I restriction sites. (b) Either side of the 1557 bp fragment there must be 1506 bp and 812 bp fragments, flanked by *Pst*I restriction sites. (c) The 336 bp fragment created by double digest must fit next to the 812 bp fragment and have a *Dra*I site next to it. (d) The remaining fragment size must be 1091 bp.

Adding to the restriction map

Additional data can then be provided to show how the restriction map continues to be built up. This mimics the process used in the Human Genome Mapping project. In this exercise we will add data on where the *Bam*HI restriction site is.

Expected results

To locate the *Bam*HI restriction site on the existing plasmid map, students would need data on band sizes, determined by gel electrophoresis, after restriction digestion with *Bam*HI + *Pst*I, *Bam*HI + *Dra*I, *Bam*HI + *Pst*I + *Dra*I (see Table 2).

Restriction enzymes used	Expected DNA fragment sizes (bp)
*Bam*HI + *Pst*I	1427 + 446 + 3429
*Bam*HI + *Dra*I	1148 + 1537 + 1060 + 1557
*Bam*HI + *Pst*I + *Dra*I	446 + 1060 + 1557 + 1091 + 336 + 812

Table 2. Size of the restriction fragments after digestion of the pARA-R plasmid.

Students could either be supplied with this data, or complete the additional lab work. The expected results are shown in Figure 4.

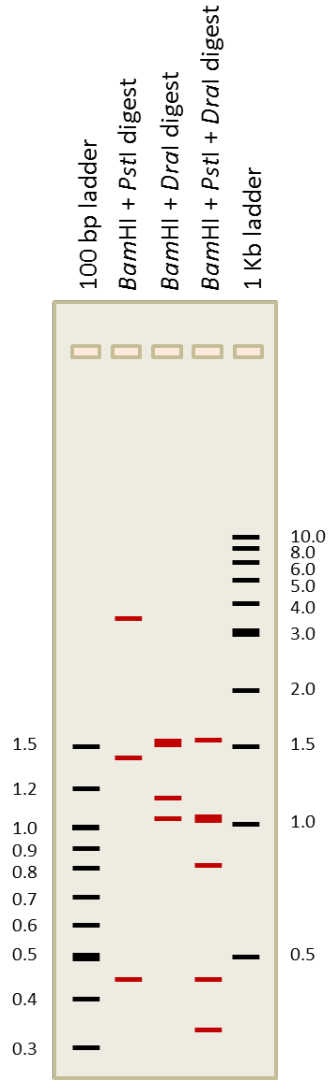

Figure 4. Expected results after gel electrophoresis of the restriction digestion of plasmid pARA-R using different enzyme(s) in combination with *Bam*HI.

Observation and interpretation

When observing and interpreting these results, students should note that:

- Lane 1 (plasmid double-digested with *Bam*HI and *Pst*I): 3 bands appear as expected if *Bam*HI cuts the plasmid once and *Pst*I cuts the plasmid twice. The band sizes are; 3429 bp, 1427 bp and 446 bp.
- Lane 2 (plasmid double-digested with *Bam*HI and *Dra*I): 3 bands appear, where 4 would be expected if *Bam*HI cuts the plasmid once and *Dra*I cuts the plasmid 3 times. It is noticeable that one of the bands is thicker than the others, suggesting that there are 2 bands of similar size that have not been separated by gel electrophoresis. This is indeed the case. The band sizes are; 1557 bp, 1537 bp, 1148 bp and 1060 bp. The 1557 bp and 1537 bp bands are not sufficiently different in size to separate on this gel.
- Lane 3 (plasmid triple-digested with *Bam*HI, *Pst*I and *Dra*I): 5 bands appear, however 6 would be expected if the plasmid is cut once by *Bam*HI, twice by *Pst*I and three times by

*Dra*I. Again this is due to two fragments of very similar size not being separated by gel electrophoresis. The band sizes are; 1557 bp, 1091 bp, 1060 bp, 812 bp, 446 bp and 336 bp. The 1091 bp and 1060 bp bands are not sufficiently different in size to separate on this gel.

Adding to the restriction map

To integrate this information with the current restriction map that the students have they need to use their logic and reasoning skills, to comprehend that:

- The fragment sizes produced from these restriction digests confirm previous results that *Bam*HI cuts the plasmid once, *Pst*I cuts the plasmid twice and *Dra*I cuts the plasmid three times.
- Comparison between results for the *Pst*I, *Dra*I double digest, and the *Bam*HI, *Pst*I, *Dra*I triple digest, show that *Bam*HI cuts within the 1506 bp fragment of the *Pst*I, *Dra*I double digest (Figure 5a).
- Comparison of the *Dra*I restriction digest and *Dra*I, *Bam*HI double digest, reveals that the 1557 bp and 1148 bp *Dra*I digest fragments remain after double digestion with *Dra*I and *Bam*HI. This means that the 2597 bp *Dra*I fragment must have been cut by *Bam*HI to produce the 1537 bp and 1060 bp fragments by double digest (Figure 5b).
- There is only one way that the 1537 bp and 1060 bp fragments could be arranged within the 2597 bp *Dra*I fragment, that places the *Bam*HI site within the 1506 bp *Dra*I, *Pst*I fragment (Figure 5c).
- Alternatively the placement of the *Bam*HI site within the 1506 bp *Dra*I, *Pst*I fragment could be determined using the *Pst*I restriction digest and *Pst*I, *Bam*HI double digest. In this case it is the 3875 bp *Pst*I fragment that is cut by *Bam*HI, producing 3429 bp and 446 bp bands (observed by comparison of the *Pst*I restriction digest and *Pst*I, *Bam*HI double digest). There is only one way that the 3429 bp and 446 bp bands could be arranged within the 3875 bp *Pst*I fragment, that places the *Bam*HI site within the 1506 bp *Dra*I, *Pst*I fragment (Figure 5d).

Whilst the restriction map of the plasmid can be a mirror image of the one shown in Figure 5d, the fragment lengths and order must be preserved.

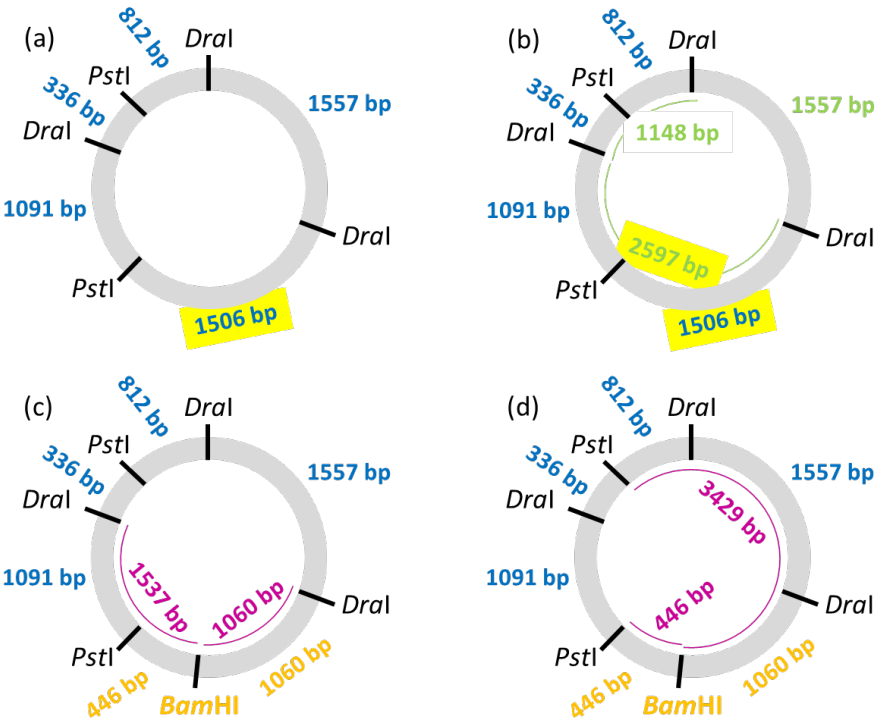

Figure 5. The steps in adding a *Bam*HI restriction site to the existing restriction map of a plasmid (not drawn to scale). The fragment sizes are colour-coded, so that green represents fragment sizes from a restriction digestion with a single enzyme, blue from the *Pst*I, *Dra*I double digest, purple from double digests including *Bam*HI and orange from the *Bam*HI, *Pst*I, *Dra*I triple digest. (a) The *Bam*HI restriction site is within the 1506 bp fragment of the PstI, DraI double digest. (b) The *Bam*HI restriction site is within the 2597 bp fragment of the *Dra*I digest. (c) There is only one way to fit the *Bam*HI site on the restriction map using both of these observations. (d) The *Bam*HI site could also be fitted on the restriction map using data from the *Dra*I digest.

Classroom delivery

This exercise can be performed using practical work, or as a written exercise, giving the students either the figures of electrophoresis gels, or tables of fragment sizes for them to use their skills in observation, interpretation, logic and reasoning to construct a restriction map of the plasmid.

It is a practical example of how restriction mapping works, which was one of the first steps in mapping the Human Genome. It allowed sequenced fragments to be fitted together in the correct order (unlike shotgun sequencing, which sequenced DNA fragments randomly and then used the increased computing power available to find overlaps). This makes it a relevant, as well as hopefully interesting and informative, contribution to teaching and learning about the Human Genome Project.