LAB 6
POP BEAD CLONING
Modeling Recombinant DNA Construction

This lab was modified from a lab written by Elmer Kelmer, a biology teacher at Parkway South High School. No lab outline is necessary.

OBJECTIVES

After working through this activity, students should be able to

1. Differentiate between restriction enzymes and restriction sites
2. Model the process of recombinant DNA Technology
3. Describe how restriction enzymes can be used to help create recombinant DNA
4. Show how fragments of DNA from a digested plasmid appear after gel electrophoresis

BACKGROUND

Agricultural biotechnology is a collection of scientific techniques, including genetic engineering, used to create, improve or modify plants, animals and microorganisms. Using conventional techniques, such as selective breeding, scientists have been working to improve plants and animals for human benefit for hundreds of years. Modern techniques now enable scientists to move genes (and therefore, desirable traits) in ways they could not before—and with greater ease and precision.

Through the use of agricultural biotechnology, we can improve how foods are grown to provide a more abundant food supply and to reduce the use of chemicals such as pesticides and herbicides. This makes modern agriculture more environmentally friendly. In the future, biotechnology will provide fruits, vegetables and grains with better nutrition and improved taste. Through genetic engineering, we can insert genes into bacteria to produce a plentiful supply of any desired protein or biological molecule. The bacteria reproduce making more and more little "molecular factories," and the products they produce can be harvested, purified and provided to people who need them. Plasmids can be removed from bacteria and reinserted into another bacterium used to transfer the gene into the genome of a plant. This makes plasmids the perfect vehicle for putting new genes into other organisms. Recombinant DNA consists of a combination of DNA from two or more different organisms.

This lab teaches the process of constructing a recombinant plasmid for genetic engineering. The gene being transferred is a mutated *aroA* gene, whose protein is 5-enolpyruvoyl shikimate-3-phosphate synthase (EPSPS). EPSPS is an enzyme required in the biochemical pathway in plants that synthesizes essential aromatic amino acids phenylalanine, tyrosin and tryptophan. The recombinant plasmid would then be transferred into plant tissue to instill glyphosate resistant. Glyphosate is a popular herbicide that inhibits EPSPS and is sold by Monsanto under the name Roundup. Bacteria resistant to glyphosate were discovered to have a mutated *aroA* gene whose ESPS was not inhibited by glyphosate. Monsanto removed the *aroA* gene from this strain of bacteria and successfully cloned it and transferred it into soybeans, canola and wheat (currently in field trials), allowing farmers to spray these crops with Roundup to kill weeds without

killing the crop. This lab exercise will help you understand the process of constructing such a recombinant plasmid.

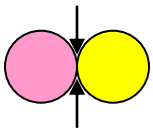
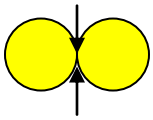
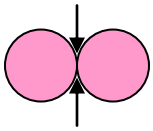
MATERIALS
In your bead kit, you should have one linear set of beads in the following order:

Hole end of 13 White, 2 pink, 1 white, 1 pink, 1 yellow, 2 white, 2 red, 2 yellow, 4 red, 2 white, 2 yellow, 2 white, 1 pink, 1 yellow, 7 white, **knob end** of 7th white bead

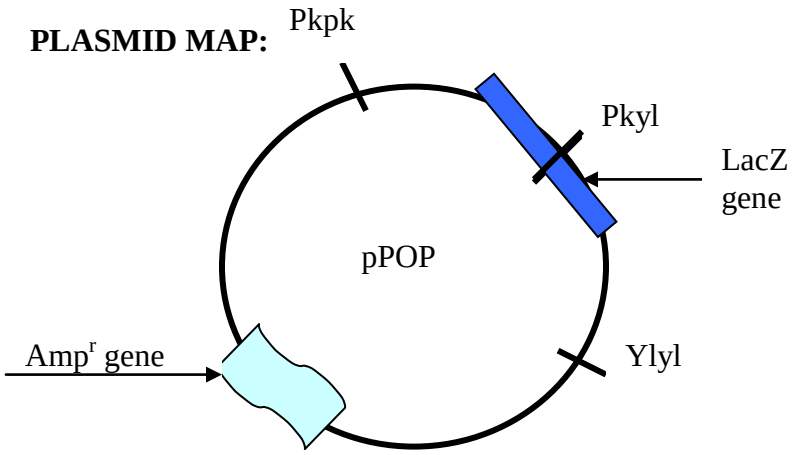
There should also be one circular set of beads in the following order, starting after the twisted white bead:

Hole end of 5 orange, 2 pink, 1 orange, 3 blue, 1 pink, 1 yellow, 3 blue, 1 orange, 2 yellow, 2 orange, 1 twisted white bead- **knob end of twisted white bead .**

KEY:

Restriction Enzyme	Restriction site
PkyI	 Pink-yellow
YlyI	 Yellow-yellow
Pkpk	 Pink-pink

PLASMID MAP:



(Reading clockwise)
Pkpk → PkyI = 750 bp
PkyI → YlyI = 850 bp
YlyI → Pkpk = 1400 bp

KEY:
White = genomic DNA bps

Orange = plasmid DNA bps

Pink, yellow = nucleotide base pairs (bp) within restriction sites

Twisted = The ampicillin resistance gene (amp^r); This gene's product allows survival on ampicillin in the growth media.

Blue = The lacZ gene; when X-gal is in the growth media, the bacteria produce a blue waste product

Red = The mutated *aroA* gene - makes plants Roundup® ready

PROCEDURE

1. Draw the plasmid map below (see previous page). Be sure to write the distances in bp for each fragment on the map.

2. Is it possible for a restriction site to be within a gene? Would the gene be functional?

3. Orient the pPOP bead plasmid as it is shown in the map that you drew. Imagine that your hands are the restriction enzyme called Ylyl . What will that enzyme do if it interacts with pPOP? Have Ylyl cut the DNA. Lay your now linear plasmid so that the end with the hole is to the left. Draw the map of the resulting DNA fragment. (NOTE: even though a restriction site has been cut, the site should still be shown on both ends on your map.)

4. Re-circularize pPOP. What is the base pair size of pPOP according to the map?

5. How many genes are present on pPOP? What are their names?

6. How many restriction sites are present on pPOP?

7. If you add the restriction enzyme Pkpk, to this plasmid (your hands) what happens? Lay the plasmid with the hole end to the left. Draw a map of the resulting DNA fragment.

8. Re-circularize pPOP. Add the restriction enzyme Pkyl and lay the plasmid with the hole end to the left. Draw a map of the resulting DNA fragment.

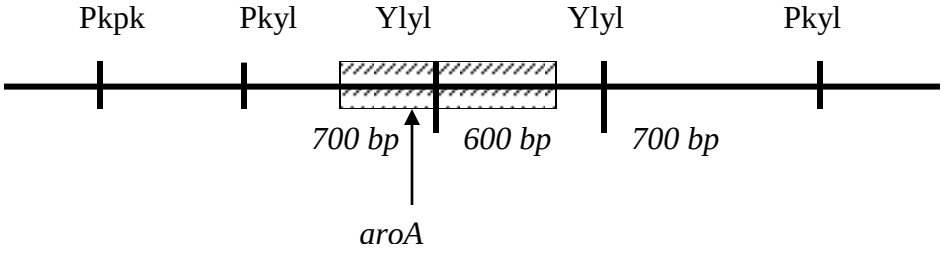
9. Re-circularize pPOP. Spread the genomic DNA chromosome on the bench with the hole end to the left.

10. How many genes are present and what are their names?

11. How many restriction sites are present?

12. Draw the map for this strand of DNA.

13. Check your map; it should look like this:



14. Examine the models of pPOP and the genomic DNA. Choose ONE restriction enzyme that will a) cut the plasmid in ONE location, AND b) cut the genomic DNA in two places, removing the whole *aroA* gene. Cut pPOP and then cut out the *aroA* gene from the genomic DNA with this restriction enzyme (your hands).

15. What is the size in base pairs of the fragment that contains the *aroA* gene?

16. Ligate (reattach) the ends of the plasmid with the complimentary ends of the *aroA* gene fragment, i.e., re-circularize the plasmid with the *aroA* insert DNA.

17. Draw a complete map of your recombinant plasmid, below. The base pair sizes must be written on the map.

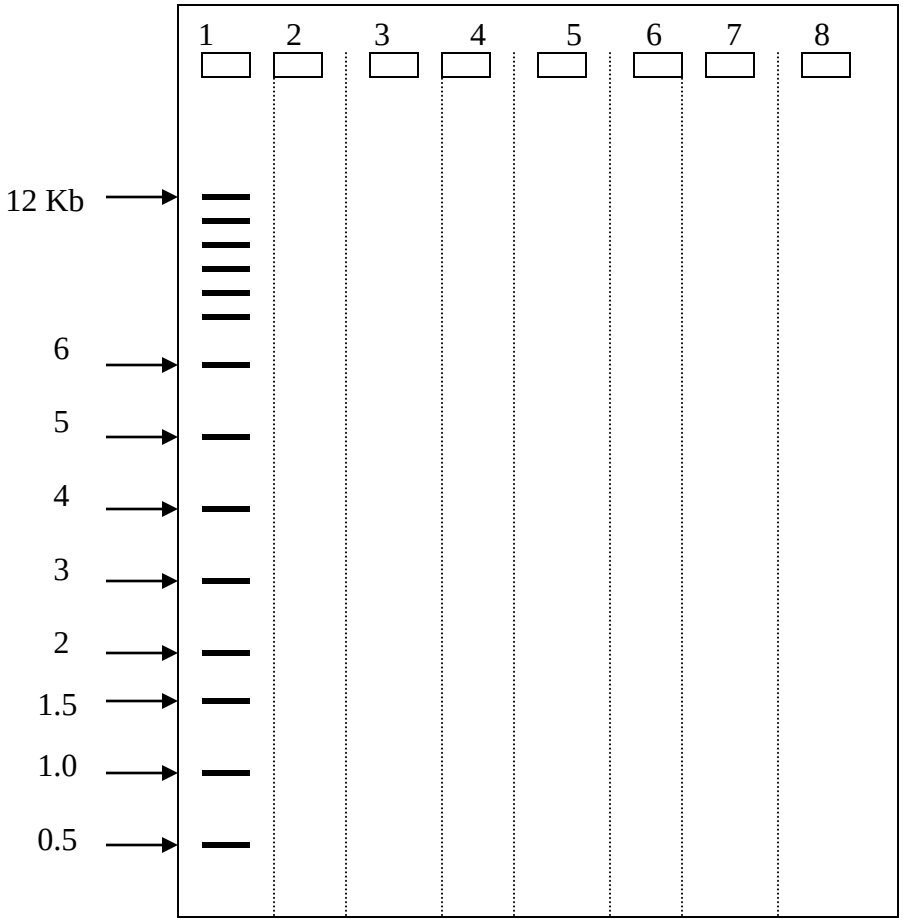
18. Will the *lacZ* gene be functional in the recombinant plasmid? Explain your answer.

19. Will the amp^r gene be functional in the recombinant plasmid? Explain.

20. What is the bp size of the recombinant plasmid.

21. If the recombinant plasmid were cut with the following enzymes and combinations of enzymes, draw the way the fragments would appear on the finished gel after electrophoresis and staining. Use the map of the recombinant plasmid, rather than the pop beads, to determine the size of all the resulting fragments in each digestion.

1. Kb ladder
2. Pkpk
3. Pkyl
4. Ylyl
5. Pkpk + Pkyl
6. Pkpk + Ylyl
7. Pkyl + Ylyl
8. Uncut recombinant plasmid



Sources:

Clark, David P., and Lonnie D. Russell. *Molecular Biology Made Simple and Fun* 2nd Edition.. Vienna, IL Cache River Press, 2000.