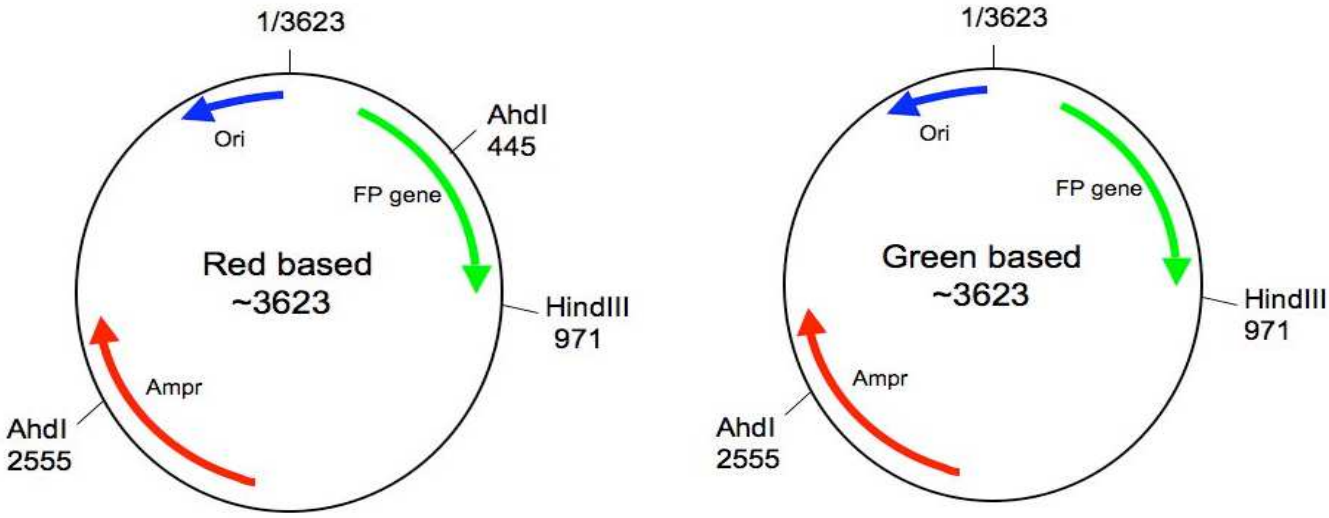
Name_____

FP plasmid digestion lab

Background: There are 2 main naturally occurring fluorescent proteins (FP's): red or green. The green fluorescent protein comes originally from jellyfish, and the red fluorescent protein comes originally from corals. Making small changes in the DNA sequence of these original proteins formed all the varieties or color generated by the FP plasmids. Using restriction enzymes you can identify if the engineered protein in your digest was made from either the original red or green protein.



Purpose: To determine which naturally occurring fluorescent protein was used to make the various FP plasmids.

Important laboratory technique reminders:

a. Keep reagents on ice at all times. The reagents and enzymes are temperature sensitive
b. Use sterile technique. DNA is easily destroyed by contaminant enzymes. Contaminants can also inhibit restriction enzyme performance.
c. Pipette slowly and carefully. Small pipetting errors can have significant impact on the results.

Procedure:

Part 1 Digest prediction

1. Use the restriction maps of FP plasmids above to predict the size of fragments that will be made by the digests of HindIII and AhdI (each enzyme will be used separately). Record this information in the table on your worksheet.

Part 2 Setting up the digests

1. Label 3--1.5ml reaction tubes (1-3) Keep tubes on ice.

2. Add reagents listed in the table below in the following order. Water, buffer, Plasmid DNA, Enzyme. Pipette each new reagent directly into the bottom of the tube. You are working with very small volumes. Watch your pipette tips to make sure that each reagent is added. Make sure to change tips every time!! Always add enzymes last.

Reaction table FP digest

Tubes	Sterile H2O	10X buffer	FP plasmid DNA	Enzyme	Total rxn volume
1	13ul	2ul buffer 4	5ul	None	20ul
2	11ul	2ul buffer 2	5ul	2ul HindIII	20ul
3	11ul	2ul buffer 4	5ul	2ul AhdI	20ul

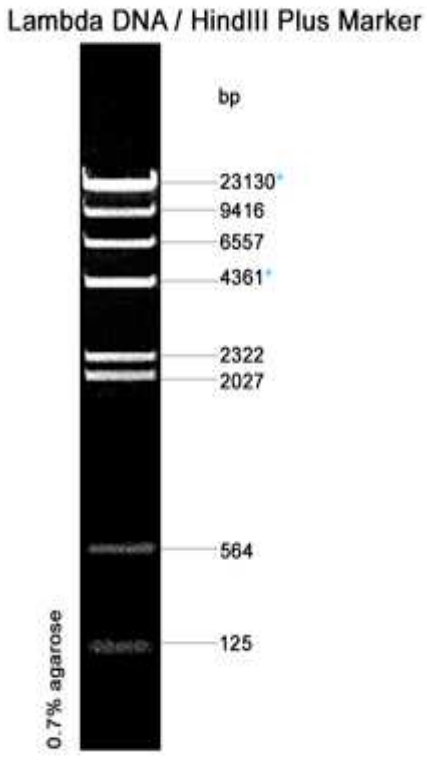
3. Tightly cap all tubes. Give tubes a quick spin (1-2 sec) in the microfuge to pool reagents.

4. Place tubes in a float and incubate in the water bath at 37C for ~ 40 min

5. Store tubes at 4C (refrigerator) overnight, or -20 C (freezer) for longer storage as directed by your teacher until they are used for electrophoresis.

Part 3 Running the electrophoresis gel:

1. Set up gel box with wells at the negative (black) electrode. Use 0.5X TBE as running buffer
2. Each gel will hold samples for 2 groups
3. Add 3ul of loading dye to each of the digest tubes mix and quick spin to pool.
4. Load all of digest sample (23ul) left to right in lanes 1-3 or 5-7. Record each lane on your worksheet.
5. Load 10 ul of DNA marker (lambda HindIII) in lane 4.
6. Have your partner group load their samples.
7. Record the location of all samples in gel sample tracking table on your worksheet. Run gel at 130-170v.
8. When the dye is ½- ¾ down the gel turn off the power and remove your gel and place in a large weigh boat and give to your teacher for staining. Be sure to label your weigh boat as directed by your teacher.

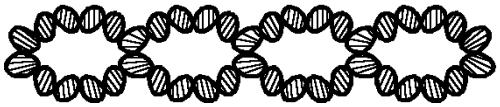


Part 4 Analysis of digests by gel electrophoresis

1. Obtain the picture of your gel and label each lane with its contents directly on the picture. (See note below on interpretation of the undigested plasmid sample)
2. Label each identifiable band in the DNA marker directly on the picture with the molecular weight (number of base pairs). Use the diagram of the Lambda HindIII marker to label lane 4.
3. Measure the distance in mm from the well to the leading edge of each band of the DNA marker.
4. Using semi log graph paper create a standard curve by making a graph of the distance traveled (x axis) and base pair size (y axis) of each band and draw a best-fit line.
5. Using the standard curve line estimate the size of each of the fragments of the plasmid digests. Compare these answers to the estimates made in part 1.
6. Answer all questions on your worksheet

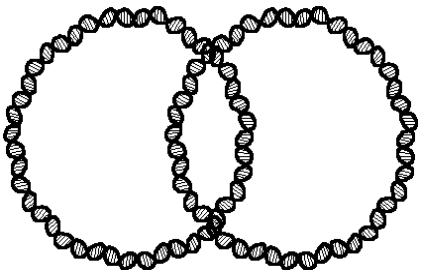
NOTE:

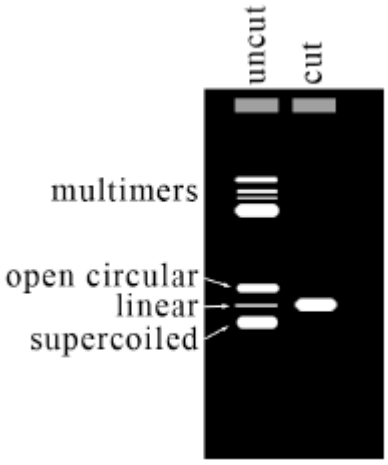
In the electrophorsis of the FP plasmids, it is likely that two or three bands will appear in the undigested plasmid lanes. This is because plasmids isolated from cells exist in several forms. One form of plasmid is called "**supercoiled**." You can visualize this form by thinking of a circular piece of plastic tubing that is twisted. This twisting or supercoiling results in a very compact molecule; one that will move through the gel very quickly for its size.



A second plasmid form is called a "**nicked-circle**" or an "open-circle." Often a plasmid will experience a break in one of the covalent bonds located in its sugar-phosphate backbone along one of the two nucleotide strands. Repeated freezing and thawing of the plasmid or other rough treatment can cause the break. This will cause the supercoiled plasmid to unwind. This circular plasmid form will not move through the agarose gel as easily as the supercoiled form; although it is the *same* size, in terms of base pairs, it will be located closer to the well that the supercoiled form.

The last plasmid form we are likely to see is called the "**multimer**." When bacteria replicate plasmids, the plasmids are often replicated so fast that they end up in linked together like links in a chain. If two plasmids are linked, the multimer will be twice as large as a single plasmid and will migrate very slowly through the gel. Starting closest to the well, you might observe a multimer, followed by a nicked-circle band and, finally, a fast traveling supercoiled band.





The image on the left shows the possible bands that might appear on your gel in the undigested (no enzyme) sample of the plasmids.