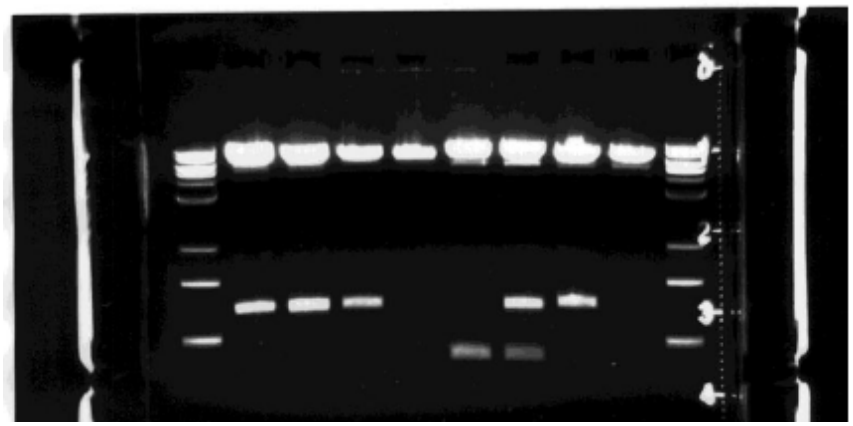
Bi 10 Prelab 5 (25 points)

Any questions regarding this prelab should be addressed to David Miller (drmiller@caltech.edu)

1. (4 points) Draw a gel containing the products of an EcoR1 digestion of a plasmid containing one copy of GFP (lane 2), two copies of GFP (lane 3), and three copies of GFP (lane 4).

2. (1 point) Which lane is the most important in a gel?

3. (14 points) The gel below, found in your lab manual on page 38, is an example of restriction digests of constructs created via the cloning steps you carried out in lab. Based on the gel and the vector map in the appendix of the lab manual, state how many copies of the GFP gene each miniprep has and in which orientation the GFP gene(s) are inserted. In your explanation for each miniprep, please sketch out the vector map and clearly state the sizes of the fragments produced from EcoRI and NcoI digestion. Which of the colonies should appear green? If you wanted to express the MBP-GFP fusion protein (with one copy of GFP), which miniprep DNA would you use?



Gel imaged provided by Sunny Chun, 2007

Loading order (left to right):

Lane 1: 1 kb DNA ladder
Lane 2: miniprep 1 digested with EcoRI

Lane 3: miniprep 2 digested with EcoRI
Lane 4: miniprep 3 digested with EcoRI
Lane 5: miniprep 4 digested with EcoRI
Lane 6: miniprep 1 digested with NcoI
Lane 7: miniprep 2 digested with NcoI
Lane 8: miniprep 3 digested with NcoI
Lane 9: miniprep 4 digested with NcoI
Lane 10: 1 kb DNA ladder

4. (6 points) Huntington's disease is a trinucleotide repeat disorder—40 or more repeats of the CAG codon (which codes for the amino acid glutamine) causes full penetrance of the disease as well as early onset. Describe a complete experiment that you can perform to test whether an individual will be susceptible to fully penetrant Huntington's disease.

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