

Bi1x Spring 2012
Assignment #2
Due: Friday May 4th

This problem set is composed of two parts, one about the gels/digests and one about the Dicty project. The gel images were emailed to you, and the Dicty movies are on the server in the folder Bi1X2012/Dicty.

Gels/Digests:

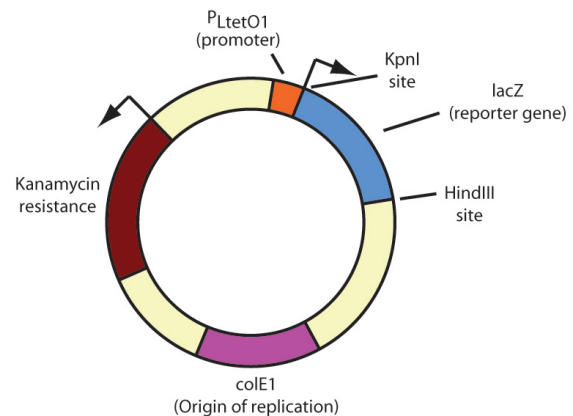
Download your gel and open it in an image analysis program. I would suggest Fiji, <http://fiji.sc/wiki/index.php/Fiji>. Anything with the ability to change the contrast of the gel will do though. Contrast control is important for seeing very faint bands, or distinguishing between the ladder bands.

As a reference, the 1kbp and 100 bp ladders we used can be found here:
http://www.neb.com/nebecomm/productfiles/779/images/N3232_fig1_v1_000034.gif.
http://www.neb.com/nebecomm/productfiles/778/images/N3231_fig1_v1_000034.gif.

The site map for the lambda genome can be found here:
http://www.neb.com/nebecomm/tech_reference/restriction_enzymes/sites/Lambda_sites.pdf.

To the right is the vector map for pZE21-lacZ, the plasmid we digested. The total length of the plasmid is 5298 bp. The HindIII site is defined as position 1, and the KpnI site is at position 2222.

Use these references and your gel to answer the following questions. (note: some gels turned out better than others. If there is a serious problem with your gel, you can try and use another group's gel, but you must make a note of this, and explain why you aren't using your own gel.)

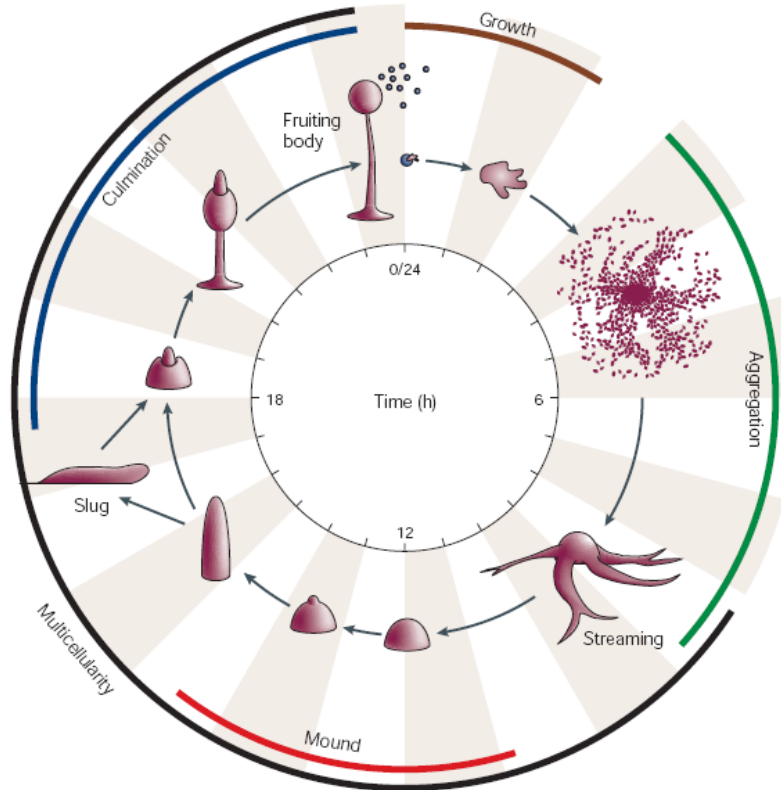


1. Using the ladder as a guide, label all the major bands in your digestion with their approximate base pair size (turn in the labeled image).
2. Compare the single and double digests bands to the vector map above. Do the sizes make sense based on the map above?
3. Now compare the undigested plasmid to the two digests. What is odd about this, and how can you explain it? (hint: consider the structure of the digested vs. undigested DNA, and how gel electrophoresis work to discriminate based on size.)
4. Using the site map for the lambda genome, calculate the size of all the fragments you should have gotten with your double digestion.

- Try and match all the fragments from the previous part to bands on your gel. Its ok if you cannot find some, but do the best you can. You can either do this directly on the gel, or mark the bands on the gel with A, B, C, etc. and assign the labels values.

Dicty:

- Identify the main stages of Dicty development in your movie. How long does it spend in every stage? Compare your results with the graph below.



- How does Dicty interact with the bacterial cells? What is the mean number of bacterial cells that got endocytosed by Dicty cells over 30 minutes?
- Compare your results with the movies of different bacteria. Do you notice any preference for a given bacteria?
- Which part of the cell will be the hardest to 'digest'? (Hint: one of the techniques used to distinguish groups of bacteria is Gram staining. Check which bacterial species are Gram positive and which are Gram negative. What is the difference between them?)
- Do you see a direction which the majority of the cells prefers? To answer this question you may use ImageJ, record the starting and end point for 10 different cells and illustrate absolute translation over the duration of your movie as a vector.