Bi 1x, Spring 2010

Week 5

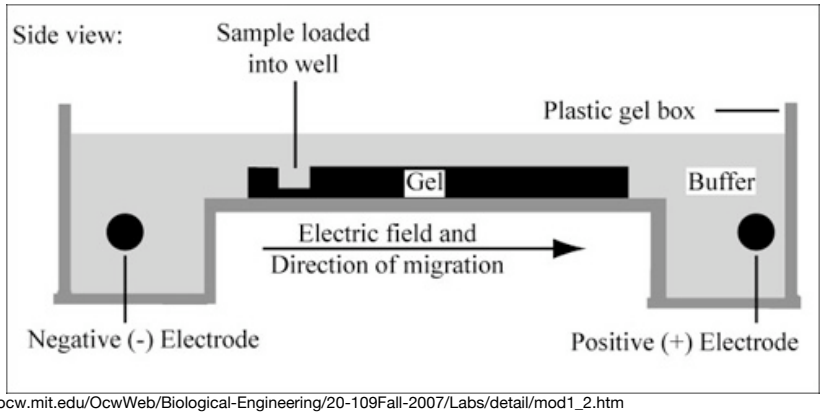
- Agarose gel electrophoresis

Materials

- Your digestion reactions from last week
- 6X DNA loading dye
- 1 kb and 100 bp ladders (pre-stained)
- HindIII digested lambda DNA (pre-stained)
- pZE21-lacZ DNA (pre-stained)
- Your PCR reactions

Background

Agarose gel electrophoresis is a powerful separation method frequently used to analyze DNA fragments generated by restriction enzymes. The agarose gel consists of microscopic pores that act as a molecular sieve. Samples of DNA are loaded into wells made in the gel during casting, as shown in the figure below.

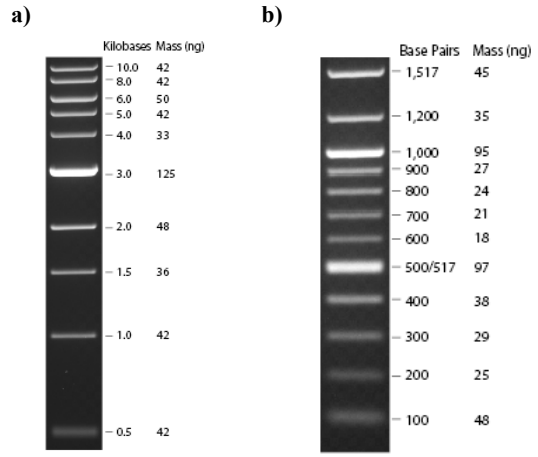


http://ocw.mit.edu/OcwWeb/Biological-Engineering/20-109Fall-2007/Labs/detail/mod1_2.htm

Since DNA has a strong negative charge at neutral pH, it migrates through the gel towards the positive electrode during electrophoresis. The DNA molecules are separated in the gel according to their size and shape. Linear DNA molecules are separated according to their size. The smaller the linear fragment, the faster it migrates. If the size of two fragments are similar or identical, they will migrate together in the gel. If DNA is cleaved many times, the wide range of fragments produced will appear as a smear after electrophoresis.

Circular DNAs such as plasmids are supercoiled. Supercoiled DNA has a more compact and entangled shape (like a twisted rubber band) than its corresponding non-supercoiled forms (linear, nicked and relaxed circles). When supercoiled DNA is cleaved by a restriction enzyme just once it unravels to its linear form. If supercoiled DNA is nicked (a phosphate bond is broken anywhere in the molecule, in either strand) it completely unravels to form a circle. Under the electrophoresis conditions being used in this experiment, supercoiled DNA migrates faster than its linear form and linear DNA migrates faster than its nicked circular form. (EDVOTEK EDVO-kit #102 Manual)

In order to determine the size of your DNA from the migration distance, you will use "DNA ladders," which are molecular weight standards that consist of DNA fragments of known sizes. The ladders you will use today are shown below:



DNA Ladders
a) Example of 1 kb DNA ladder on a 0.8% agarose gel, stained with EtBr.
b) Example of the 100bp DNA ladder on a 1.3% agarose gel, stained with EtBr.

Protocol:

You will run the following samples on a 1% TAE-Agarose gel (prepared by your TAs):

a. 100 bp DNA ladder
b. **EcoRI/HindIII lambda DNA double digest (from session 1)**
c. HindIII digest of lambda DNA (as a reference)
d. **pZE21-LacZ – HindIII single digest (from session 1)**
e. **pZE21-LacZ – KpnI/HindIII double digest (from session 1)**
f. Undigested pZE21-LacZ (as a reference)
g. PCR product
h. PCR no template control
i. PCR no primer control
j. 1 kb DNA ladder

1. **Mix 10 µl of each of your restriction digests with 2 µl of 6X DNA loading dye. Mix by pipetting up and down.**
    a. *DNA loading dye consists of glycerol—which will help your solutions sink into the wells of the agarose gel—and reference dyes that help you determine how far your samples have migrated.*
2. **Mix 10 µl of each of your PCR reactions with 2 µl of 6x loading dye. Mix by pipetting up and down.**
3. **Load your samples on the gel in the order listed above. Be sure to write it in your notebook also!**
    a. For samples that are supplied to you pre-stained (ladders, references), load 5 µl.
    b. For your own restriction digests from session 1, load 10 µl of sample/dye mixture.
    c. To load the gel, place your pipette beneath the buffer surface layer, part-way into your target well. Very slowly depress the pipette plunger to load your sample. As you depress the plunger, you will notice the sample fall into the well. Do not rush! This may cause the sample to flow out of the well.
    d. *Loading samples onto agarose gels is tricky! Ask your TA to demonstrate how to load a gel before attempting to do it yourself.*