Bi 1X, Spring 2012

Week 4

- **Session 1: Restriction enzyme digestion**

Restriction enzymes are enzymes that bind to specific DNA sequences and cleave ("digest") the DNA at or next to the binding site. You will become familiar with restriction enzymes during this and next week's lab sessions on both bulk and single molecule scales.

Note: This document describes bulk restriction digests only.

Materials:

- PCR tubes
- Sterile, ultrapure water
- pZE21-lacZ plasmid DNA
- 10X NEB Buffer #2
- 10X EcoRI Buffer
- 10X BSA Solution
- HindIII predigested lambda phage DNA
- *EcoRI, HindIII, and KpnI restriction enzymes will be supplied by your TAs*

Background:

Most useful restriction enzymes recognize 4-8 base pair restriction sites. These sites are symmetric, inverted repeats called palindromes. Shown below are the restriction sites of the three enzymes you will be using today: EcoRI, HindIII, and KpnI. Notice how the 5' to 3' sequence is identical on the top and bottom strands.

Some enzymes, like KpnI, produce sequences with 3' overhangs upon cleavage. Others, like EcoRI and HindIII, produce 5' overhanging ends. Additional enzymes can produce blunt sequences. It is also important to remember that restriction sequences are not necessarily unique to an enzyme—multiple enzymes often have the same recognition sequence. To look up the recognition sequences of different enzymes, you can consult the New England Biolabs (NEB) REBASE database (http://rebase.neb.com/rebase/rebase.html).

Restriction enzymes are generally supplied as a given number of units. These units correspond to a metric of enzymatic activity, as specified by the manufacturer. Today, you will be using enzymes from NEB, which uses the following definition for a "unit":

> One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Lastly, restriction enzymes, like all enzymes, have certain optimal conditions for that must be met for full activity. Different restriction enzymes have different condition requirements—salt, metal, detergent, and additive concentrations can all have drastic effects on activity (though many enzymes are perfectly happy in generic buffers). One common additive (which you will be using today) is acetylated bovine serum albumin (BSA). BSA levels and other reaction conditions are usually optimized by the manufacturers, who supply specific buffers with each enzyme.

Today, you will familiarize yourself with restriction enzymes by digesting genomic DNA from the bacteriophage lambda (discussed in class and this week's homework) as well as a plasmid DNA (pZE21-lacZ). A restriction map of this plasmid has been supplied at the end of this document. You will run your samples on an agarose gel next week to analyze the results of your digestions.

Protocol:

Today you will perform the following restriction digests (per pair):

1. No enzyme control of the plasmid pZE21-lacZ
2. HindIII single digest of the plasmid pZE21-lacZ
3. KpnI/HindIII double digest of the plasmid pZE21-lacZ
4. EcoRI digest of HindIII predigested lambda phage DNA

Set the reactions up as listed in the table in 200 µL PCR tubes. Pipetting in the order the reagents are listed, starting with the water.

Note: You will have to calculate the volume of reagents to add to your reactions to either match the amount of DNA as listed or make sure the final concentration of buffers to be 1X. For example, for a total volume of 30 µL reaction, you should use 3 µL of BSA (10X) stock provided in your digestion reaction. ***Be sure to write down the volume of reagents used in table format in your notebook.***

No Enzyme Control of pZE21-lacZ plasmid

Reagent:	**Amount:**
Sterile water	calc.
Plasmid (pZE21-lacZ) DNA (100 ng/µl)	300 ng (calc.)
NEB Buffer #2 (10X)	calc.
BSA (10X)	calc.
Total:	30 µL

HindIII Single Digest of pZE21-lacZ plasmid

Reagent:	**Amount:**
Sterile water	calc.
Plasmid (pZE21-LacZ) DNA (100 ng/µl)	300 ng (calc.)
NEB Buffer #2 (10X)	calc.
BSA (10X)	calc.
HindIII (10 units/µL) *	1 µL
Total:	30 µL

HindIII/KpnI Double Digest of pZE21-lacZ plasmid

Reagent:	**Amount:**
Sterile water	calc.
Plasmid (pZE21-LacZ) DNA (100 ng/µl)	300 ng (calc.)
NEB Buffer #2 (10X)	calc.
BSA (10X)	calc.
HindIII (10 units/µL) *	1 µL
KpnI (10 units/µL)*	1 µL
Total:	30 µL

EcoRI Digest of HindIII digested lambda DNA

Reagent:	**Amount:**
Sterile water	calc.
HindIII predigested lambda DNA (0.5 µg/µL)	1.5 µg (calc.)
NEB EcoRI Buffer (10X)	calc.
BSA (10X)	calc.
EcoRI (20 units/µL) *	1 µL
Total:	30 µL

*Restriction enzymes are supplied in a viscous, glycerol containing solution. Your TAs will pass around the enzymes **on ice** to be added after you set up **all of the reactions** except adding the enzymes. **Avoid touching the bottom of the enzyme tube with your hand to prevent denaturation. Pipette with care!**

After you have assembled your reactions, you should **mix** them, **spin** them down briefly, and **place** them at 37ºC for the remainder of the lab session (~3 hours). Your TAs will freeze them afterwards for use the following week.

Appendix: Gene map of plasmid pZE21-lacZ

Key:

- *Promoter* – RNA polymerase binding site, transcriptional regulator
- *Origin of Replication* – site where plasmid replication begins for division, regulates *copy number*
- *Restriction Sites* – sequence-specific enzymatic DNA cleavage sites, leaves *sticky ends* for proper insert ligation
- *Kanamycin* – encodes gene for Kanamycin (fungal) antibiotic resistance, imparts severe selective advantage in proper media
- *Non-descript DNA* – contain other restriction sites for gene insertion