Bi 1X, Spring 2013

Week 3

- **Background: 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. In the next few weeks, you will become familiar with restriction enzymes on both bulk and single molecule scales.

Note: This document describes bulk restriction digests only.

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.

EcoRI: **HindIII:** **KpnI:**

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. Your digests used 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 used eariler) 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.

Earlier this week, you familiarized 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 today to analyze the results of your digestions.

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