**Bi 1x, Spring 2010**

**Week 4**

* **Session 2**
	+ Restriction enzyme digestion

**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 week’s lab sessions on both bulk and single molecule scales.

*Note:* *This document describes bulk restriction digests only.*

**Materials:**

* Sterile, ultrapure water
* pZE21-lacZ plasmid DNA
* 10X NEB2 Buffer
* 10X EcoRI Buffer
* 10X BSA Solution
* HindIII digested lambda phage DNA
* *EcoRI, HindIII, and KpnI restriction enzymes will be supplied by your TAs when you are ready to use them.*

**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:**:::Downloads:EcoR-I-cutsite_1_v1_000025.gif | **HindIII::::Downloads:Hind-III-cutsite_1_v1_000019.gif** | **KpnI::::Downloads:Kpn-I-cutsite_1_v1_000021.gif** |

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 your prelab) as well as plasmid DNA. A restriction map of this plasmid has been supplied at the end of this document. You will run your samples on an agarose gel tomorrow to analyze the results of your digestions.

**Protocol:**

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

1. HindIII single digest of the plasmid pZE21-LacZ
2. KpnI/HindIII double digest of the plasmid pZE21-lacZ
3. EcoRI digest of HindIII digested (predigested) lambda phage DNA

**Set the reactions up as follows, pipetting in the order the reagents are listed.**

*HindIII Single Digest of pZE21-lacZ plasmid*

|  |  |
| --- | --- |
| **Reagent:** | **Amount:** |
| **Sterile water** | **19.5 µl** |
| **Plasmid (pZE21-LacZ) DNA (86 ng/µl)** | **3.5 µl** |
| **NEB Buffer 2 (10X)**  | **3 µl** |
| **BSA (10X)** | **3 µl** |
| **HindIII (10 units/ul) \*** | **1 ul** |

Total Volume: 30 ul

*HindIII/KpnI Double Digest of pZE21-lacZ plasmid*

|  |  |
| --- | --- |
| **Reagent:** | **Amount:** |
| **Sterile water** | **18.5 µl** |
| **Plasmid (pZE21-LacZ) DNA (86 ng/µl)** | **3.5 µl** |
| **NEB Buffer 2 (10X)**  | **3 µl** |
| **BSA (10X)** | **3 µl** |
| **KpnI (10 units/ul) \*** | **1 ul** |
| **HindIII (10 units/ul) \*** | **1 ul** |

Total Volume: 30 ul

*EcoRI Digest of HindIII digested lambda DNA*

|  |  |
| --- | --- |
| **Reagent:** | **Amount:** |
| **Sterile water** | **18.5 µl** |
| **HindIII digested lambda DNA (0.5 µg/µl)** | **3 µl** |
| **NEB EcoRI buffer (10X)**  | **3 µl** |
| **BSA (10X)** | **3 µl** |
| **EcoRI (10 units/ul) \*** | **2 ul** |

Total Volume: 30 ul

\* Restriction enzymes are supplied in a viscous, glycerol containing solution. 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 (~2 hours). Your TAs will freeze them afterwards for use during the next lab session.**