Restriction Digestion Protocol - Introduction

Function I: Recognition

A restriction enzyme recognizes a pattern of bases (usually 4-8) in DNA and binds there. For Type II enzymes cleavage activity usually requires magnesium, does not require energy from ATP and is inside the recognition site. Usually the recognition is symmetrical, in a palindrome

Palindromes we like:

Able was I ere I saw Elba (why is this ascribed to Napolean Bonaparte?)

A man, a plan, a canal, Panama

Race Car.

With DNA the palindrome can either be like a mirror on one strand (GTAATG) , or using the opposite strand (inverted repeat) for the palindrome, where it reads the same forward and back if you use the complementary strand:

5′ …NNNNG|AATT CNNNN … 3′
3′ …NNNNC TTAA|GNNNN … 5′

Note that this is the recognition site for Eco RI, one of the enzymes that we will use.
What was two double-stranded chains now becomes 4, with ends that look like this:
5′ …NNNNG3′ 5′AATTCNNNN … 3′
3′ …NNNNCTTAA5′ 3′GNNNN … 5′
Because these have overhands, the ends are called ‘sticky’. This enabled the first DNA cloning, by matching up sticky ends.

The recognition site for Hind III, the other restriction enzyme that we will use is
5′ …NNNNA|AGCT TNNNN … 3′
3′ …NNNNT TCGA|ANNNN … 5′

Function II: Digestion

The restriction enzyme hydrolyzes (adds a hydro= water) one of the phosphodiester backbone bonds – so the chain is broken.

The digestion can be either asymmetrical (shown above) or symmetrical

5′ ….CCC|GGG … 3′
3′ ….GGG|CCC … 5′

This gives ‘blunt’ ends instead of sticky ones.

Enzymes are proteins, and they need to be in a good environment in order to keep their shape and have both recognition and digestion occur properly. The reaction conditions are described in terms of the solution, or buffer, they should be in.

Eco RI Reaction Conditions:

1X Buffer

100mM Tris-HCl
50mM NaCl
10mM $MgCl_2$
0.025% Triton X-100
pH 7.5

Most active at 37C.

If there is too much glycerol in the reaction you get ‘star’ activity – the enzyme will cut at sequences a little different than the recognition sequence. Since the storage buffer (what the enzyme is in) has 50% glycerol, you have to be sure that you don’t add more than $1/10^{th}$ volume of enzyme in total (at most 5 microliters in a 50 ul reaction).

One Unit of enzyme will digest 1 microgram of DNA in 1 hour if the reaction is performed at 37C in a volume of 50ul.

The Hind III restriction enzyme has the following buffer components:
1X Buffer

10mM Tris-HCl
50mM NaCl
10mM $MgCl_2$
1mM Dithiothreitol
pH 7.9

Most active at 37C. Use 1 unit to digest 1 ug of DNA in one hour.

Star activity may happen if the glycerol concentration is more than 5%. Since the enzyme Storage buffer has 50% glycerol, you may not use more than 5 units of the enzyme in 50ul of a reaction.

Performing the Digestion

Preheat the heating block to 37C.

You will digest 5 ug of the plant DNA with either Eco RI or HindIII. In your ice bucket you have 4 tubes:

1. You have a concentration of DNA on your sample tube.
2. You have a tube with 10X buffer – make sure it is thawed and mixed before you use it.
3. You have a tube with some water in it.
4. You have a tube with some enzyme in it, at a concentration of 10 units per microliter.

A. Calculate the amount of each that you need to add to a restriction digestion in order digest 5ug (5000ng) of DNA with 5 units of enzyme.

For example if my DNA is at 190 ng/ul, I would need: 5000ng/190ng/ul = 26 ul of my sample.
My sample needs:

B. Calculate the amount of of 10X Reaction buffer that you will need for a 50ul digestion.

For example if I were to do a 100ul reaction, I would need: 50ul (1X) = x ul (10X) or
50ul(1X)/10X = 5ul
My sample needs 50 (1)/10 =

C. Calculate the volume of enzyme you will need to add if you use 1 unit per microgram of DNA, or 5 units total

For example, if the enzyme concentration is 1 Unit/ul and I need 5 Units, then I would need:
5 Units/1 Unit/ul = 5 ul
My sample needs:

D. Calculate the amount of water you need to add in order for the total volume to be 100ul.

For example, using the amounts I have in parts A, B and C I would need:
50 ul (total) – 26ul (DNA) – 5ul (10X) – 5 ul (enzyme) = 14 ul of water.
My sample needs:

Now set up your digestion in a 1.5 ml Eppendorf tube:
Always use this order:

1. Water
2. 10X buffer
3. DNA

Mix carefully by pipetting up and down.

4. Enzyme

Mix carefully by pipetting up and down.

Spin very briefly in the small centrifuge to collect all of the sample in the bottom of the tube.

Place in the pre-warmed heating block.

Note the start time.

This reaction needs at least one hour, and this is an enzyme that can go longer (up to 8 hours) without the enzyme degrading. Since these samples have some remaining carbohydrate, it is reasonable to give them some extra time. At the end of the day, Note the end time, then add 5 ul of 7.5M Ammonium acetate and 100 ul of 100% ethanol to each tube and mix by inverting a few times.

We will centrifuge them tomorrow, resuspend the DNA in 25 ul of TE buffer, then load 5 ul of each sample in a lane of an agarose gel.

Questions:

Can I use the same buffer for both Eco RI and Hind III? What leads you to this conclusion?

If I have a sequence of DNA, what sorts of tools could I use to figure out the sizes of fragments that I will get (pattern-recognition)?:

Will the average length of my DNA get bigger, smaller or stay the same after the digestion?

Do the EcoRI and Hind III sticky ends fit together?

Will the EcoRI and Hind III fragments be identical?