I. Introduction

Microsatellite loci are highly informative genetic markers useful for population genetic studies, linkage mapping and parentage determination. Methods to identify novel microsatellite loci typically use subtractive hybridization to enrich small insert genomic libraries for repeat sequences. Part of the process in the present protocol is attachment of an oligonucleotide linker, named SNX for its built-in restriction sites (Stu I, Nhe I and Xmn I), to the genomic DNA fragments so that repeat containing sequences can be amplified by PCR with subsequent insertion into plasmids for cloning. We will be using a protocol that attaches a blunt end linker that allows a combination of restriction enzymes to digest the genomic DNA into fragments in the 200-1000 bp range. This protocol is species independent as the ligation of the linker DNA is independent of the restriction enzymes used to digest the genomic DNA. This protocol also improves the recovery of sequences after subtractive hybridization and results in representative small-insert libraries with a high proportion of positive clones.

This is the first lab of a semester long project to construct microsatellite libraries for bay scallops. In this first week you will be given a solution of DNA that already has been isolated from bay scallops. With this solution you will determine its concentration and purity and subject it to digestion overnight with restriction endonuclease enzymes (RENs). The REN digestion mixture, containing ~5 µg of genomic DNA, will have four different RENs: three blunt end cutters (Alu I, Hae III, Rsa I) and one staggered end cutter (Nhe I). These enzymes and the sequences that they recognize are shown:

Alu I (from Arthrobacter luteus)  
5’…AG\textsubscript{v} CT…3’
3’…TC\textsubscript{A} GA…5’

Hae III (from Haemophilus aegyptius)  
5’…GG\textsubscript{v} CC…3’
3’…CC\textsubscript{A} GG…5’
Rsa I (from *Rhodopseudomonas sphaeroides*)

5’…GT\textsuperscript{\textbullet}AC…3’

3’…CA\textsuperscript{\textbullet}TG…5’

Nhe I (from *Neisseria mucosa heidelbergensis*)

5’…G\textsuperscript{\textbullet}CTAGC…3’

3’…CGGCC\textsuperscript{\textbullet}G…5’

The blunt ends that are generated are required for attaching the SNX linker DNA whose function is fully described in the Microsatellite Protocol. The Nhe I REN is required so that when Nhe I is used again later to cut the attached SNX linker DNA to generate overhand DNA to allow insertion into a plasmid, no genomic DNA will be cut. The incubation takes place overnight in a 37°C water bath. The following day heat-denature the RENs by placing the digestion mixture into a 65°C water bath.

II. Materials

1. Scallop DNA provided by the instructor
2. T.E buffer (10 mM Tris, 0.10 mM EDTA, pH 8.0)
3. 10X New England BioLabs (NEB) Buffer #2
4. NEB 100X BSA solution
5. NEB 10 U/µL Hae III
6. NEB 10 U/µL Rsa I
7. NEB 8 U/ µL Alu I
8. NEB 5U/ µL Nhe I
9. Beckman DU 640 spectrophotometer and quartz micro-cuvettes
10. 37°C and 65°C water baths.
11. Zymo Research Clean and Concentration kit.

III. Methods:

1. Make a 1:4 dilution of the scallop DNA with T.E buffer such that the final volume is 80 µL.
2. Using the Beckman spectrophotometer and T.E as the blank, obtain A_{260} and A_{280} values. Calculate the DNA concentration for the original, undiluted scallop solution and the A_{260}/A_{280}. The DNA concentration is calculated as follows:

\[
[DNA] \text{ in ng/µL} = A_{260} \times 50 \text{ ng/µL} \times \text{dilution factor}
\]

Consult with your instructor to see if the scallop concentration is sufficient for the REN digestion step; the minimum DNA concentration is 62.5 ng/µL to give ~5 µL of genomic scallop DNA. If a concentration step is required consult with your instructor and use the Zymo Research Clean and Concentration kit.

3. Pipet the following reagents into a clean, sterile, labeled 1.5 mL microfuge tube.

- Genomic scallop DNA: x µL to give ~5 µg genomic DNA
- NEB Buffer #2: 10.0 µL
- NEB 100X BSA solution: 1.0 µL
- 10 U/µL Hae III: 2.0 µL
- 10 U/µL Rsa I: 2.0 µL
- 8 U/µL Alu I: 2.0 µL
- 5U/Nhe I: 2.0 µL
- mol bio grade H_2O: y µL
- Total volume: 100.0 µL

4. Mix gently by finger flicking and place in a 37°C water bath overnight.

5. The next day, note the time removed and place the REN digestion mixture into a 65°C water bath for 20 min. to inactivate the RENs.

6. Store the samples at -80°C until needed.

IV. Points for Discussion:

What is the DNA concentration of your undiluted scallop DNA solution? What is the A_{260}/A_{280} value? Is this a relatively pure preparation? How much DNA, in µg, was used in your digestion? How many total Units of each REN was in the digestion reaction? How long was the digestion?

V. References: