Week 4: PCR of SNX ligated DNA

I. Introduction:

A PCR reaction is performed to evaluate the success of the SNX linker DNA ligation to the scallop genomic DNA. If the SNX linker DNA was successfully attached to the genomic DNA, then multiple PCR products in the range of 200 to 1000 bps should be seen when the SNX forward primer is used. Additionally, the PCR reactions will amplify SNX linker attached genomic DNA for the subsequent subtractive hybridizations. Appropriate controls should include double stranded SNX linker DNA, REN/MBN/CIP scallop genomic DNA and water. The following week a small sample is subjected to electrophoresis on a 2% TBE agarose gel to verify the ligation.

II. Materials:

1. REN/MBN/CIP SNX ligated scallop DNA
2. NEB 10X Thermopol buffer w/o MgSO₄
3. 100 mM MgSO₄
4. 2 U/µL NEB Vent (exo-) DNA polymerase
5. dNTP mixture: 2 mM each dNTP
6. 10 µM SNX forward primer
7. 5 µM ds SNX linker DNA
8. molecular biology grade sterile water
9. 200 µL PCR tubes
10. National Labnet Co Model C-120 mini centrifuge and adapters
11. Eppendorf Mastercycler gradient thermocycler
12. Microliter digital pipets and tips
13. Horizontal gel electrophoresis apparatus and power supply
14. 10,000 GelStar stain, Cambrex Bio Science Rockland, Inc.
15. 1X TBE buffer
16. Agarose; FMC BioProducts SeaKem LE agarose
17. 10X Sample dilutor
18. Sterile 1.5 mL microfuge tubes
19. NEB 100 bp step ladder
20. Fotodyne photodocumentation system

III. Methods:

1. Thaw and place on ice your REN/MBN/CIP SNX ligated scallop DNA.

2. A PCR master mix has been prepared as follows:

   NEB 10X Thermopol buffer: 5.0 µL
   100 mM MgSO4: 1.0 µL
   10 µM SNX forward primer: 4.0 µL
   2 U/µL NEB Vent (exo-) DNA polymerase: 0.5 µL
   2 mM dNTPs: 5.0 µL
   sterile water: 32.5 µL
   48.0 µL

3. Into four separate four PCR tubes place 48.0 µL of the PCR master mix. To these tubes add 2 µL of REN/MBN/CIP SNX ligated scallop DNA, 2 µL of ds SNX linker DNA, 2 µL of REN/MBN/CIP scallop DNA, and 2 µL of water, respectively. Mix gently and spin down briefly with the nanofuge.

4. Perform the PCR reaction in the following manner:
96°C for 5 minutes followed by 40 cycles of:

   45 seconds at 96°C
   1 minute at 60°C
   1 minute at 72°C

   This is then followed by a 72°C extension for 7 minutes and storage in the freezer until the following week.
5. The following week prepare a 2% TBE agarose gel with an 8-tooth comb as previously described.

6. To separate labeled microfuge tubes add 9 µL of each PCR sample and to each add 1 µL of the 10X sample dilutor. Mix gently and spin down briefly in the nanofuge. These four mixtures will be used in the next step and subjected to the electrophoresis. Keep the remaining PCR mixtures on ice.

7. To lanes 2-5 add 10 µL of the REN/MBN/CIP SNX ligated scallop DNA PCR mixture, 10 µL of the ds SNX linker DNA PCR mixture, 10 µL of the REN/MBN/CIP scallop DNA PCR mixture, 10 µL of the water control PCR mixture, respectively. To lane 7 add 3 µL of the NEB 100 bp step ladder.

8. Run the electrophoresis at 125 V for approximately 1 h 15 min.

9. After the electrophoresis is completed, remove the gel and examine with the Fotodyne photodocumentation system.

10. Comment on the results for each PCR reaction.

11. Retain and freeze the REN/MBN/CIP SNX ligated scallop DNA PCR mixture for next week’s hybridization reactions.

IV. Points for discussion:

Estimate the concentrations in the 50 µL PCR mixture for the REN/MBN/CIP SNX ligated scallop DNA, the REN/MBN/CIP scallop DNA and the ds SNX linker DNA. Explain how the gel results support that the SNX linker DNA blunt end ligation was successful or not. Finally, calculate the volume of REN/MBN/CIP SNX ligated scallop DNA PCR mixture would be required for 100 ng of DNA for the hybridization step.

V. References: