Bi 1x, Spring 2010

Week 3, Session 1

- PCR

PCR

In today's lab, you will use PCR to amplify a region of the 16S ribosomal RNA gene in the genomic samples you extracted in week 2.

Materials:

- Sterile, ultrapure PCR-grade water
- 16S forward primer ("F")
- 16S reverse primer ("R")
- 2X PCR Master Mix
- Your purified bacterial DNA from last week
- PCR tubes

Special handling notes:

- All components of the PCR reaction must be kept on ice at all times.
- Use only filtered pipet tips and change tips every time to avoid contamination.
- Never mix your reactions by vortexing; only mix by pipetting up and down or flicking the tube gently.

About the master mix:

A PCR master mix is a concentrated solution of DNA polymerase, dNTPs (deoxynucleotide triphosphates: dATP, dTTP, dCTP, dGTP), and ions or additives—all the components required for PCR except DNA template and primers. Master mixes are more convenient than using separate components. The master mix you will be using today is Invitrogen Supermix II, which is optimized for amplification of genomic DNA.

Protocol:

Prepare your three PCR reactions on ice in the provided **200 µl PCR reaction tubes** following the instructions in the table given below.

> *Note: The first reaction lacks the PCR primers, the second reaction contains both primers but lacks template DNA, and the third reaction contains all of the components necessary for successful PCR. The first two reactions are controls that are typically used in a PCR experiment to ensure that amplification depends on both PCR primers and DNA template (i.e., that there has been no contamination of these reagents).*

Make sure that all solutions are completely thawed before using them. Add the components **in the order given, starting with the water**. Pipet each component directly **into the water** and mix by pipetting up and down.

	Reaction		
Reagent	**"No primer control"**	**"No template control"**	**Normal**
PCR-grade H2O	calc	calc	calc
2X PCR master mix	10.0 µl	10.0 µl	10.0 µl
Bacterial DNA template	0.5 ng (calc)	--	0.5 ng (calc)
16S forward primer	--	0.8 µl	0.8 µl
16S reverse primer	--	0.8 µl	0.8 µl
Total volume:	20 µl	20 µl	20 µl

Note: You will have to calculate the volume of template and water to add to your reactions. Since you have to add a very small amount of template, you should first dilute it so you can pipette accurately. For example, if your template concentration was 10 ng/µl, you should make a 1:100 dilution (using PCR grade water) and then use 5 µl of that dilution in your PCR reaction.

When your reactions are ready, keep them on ice until your TAs instruct you to place them in the thermal cycler. Make sure that your tubes are clearly labeled with your initials. When all reactions are ready, the TAs will start the thermal cycler. The details of the PCR program are given below. The reactions will be collected later on when the cycle has finished and given to you at the beginning of Week #5.

PCR Program

Step	**Time**	**Temperature**	**Cycles**
Initial denaturation	6m 30s	95ºC	1X
Denaturation	20s	95ºC	32X
Annealing	30s	56ºC	
Extension	1m 10s	68ºC	
Final extension	10m	68ºC	1X