Bi 1X, Spring 2011

Week 3

- **Session 2: PCR**

In this session, 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
- Filter tips

Special handling notes:

- All components of the PCR reaction must be kept **on ice** at all times.
- Use only **filter tips** and change tips every time to avoid contamination.
- **ONLY** mix your reactions 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 AccuPrime Supermix II, which is optimized for amplification of genomic DNA templates 200bp – 4kbp in size.

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 H_2O	11.5 µL	10.5 µL	9.5 µL
2X PCR master mix	12.5 µL	12.5 µL	12.5 µL
Bacterial DNA template	1 µL	--	1 µL
16S forward primer	--	1 µL	1 µL
16S reverse primer	--	1 µL	1 µL
Total volume:	25 µL	25 µL	25 µL

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 next week.

PCR Program

Step	**Temperature**	**Time**	**Cycles**
Initial denaturation	95°C	5 min	1X
Denaturation	95°C	20 sec	32X
Annealing	56°C	30 sec	
Extension	68°C	1 min 10 sec	
Final extension	68°C	10 min	

After cycling, the reaction will be maintained at 4°C and stored at -20°C by the TAs.

Question: The extension time (except the final extension) is set up following the "1 minute per kbp of PCR product" protocol. Can you figure out the length of the PCR product from the above PCR program?