## Class 3: Polymerase Chain Reaction

## **Reading assignment**

Dale From Genes to Genomes Chapter 4

## Classroom activity (time limit: 30 minutes)

1. Given a DNA sequence you will want to design a set of primers to produce a particular PCR product. What aspects of the structure of that primer are likely to be important?

2. Some PCR reactions done using genomic DNA as the template result in DNA fragments that are not from the region targeted. Why would that happen? What measures could be taken to reduce the generation of those products?

3. Predict the effect on PCR efficiency as the annealing temperature is raised 10° below the Tm to 10° above it. Why do you think you would see that result?

BIOL 426/626: Approaches to Molecular Biology

4. What effect do you expect if the time for the extension step is reduced to below 1 minute per 1000 nucleotides extended? What about when it is extended to more than 1 minute per 1000 nucleotides?

5. What is the possible contamination resource of RT-PCR? How do you reduce it?

6. Advantages of real-time PCR vs. traditional PCR