Bio 181 Internet exercise week #~~5~~
Due ~~Wednesday, March 21st~~

Hot Start PCR using Promega's *Taq*Beads

Visit & read: **http://www.promega.com/pnotes/60/6079_02/6079_02_core.pdf**

Answer the following questions about the *Taq*Bead PCR system.

1. Why use a "hot start" PCR technique?

2. In the Promega system, how is the polymerase kept sequestered from the other PCR reaction components at lower temperatures?

3. What is a hairpin loop, and how can it allow a single primer molecule prime itself? (Draw a picture and label 5' and 3' ends.)

4. What are (amplified) primer-dimers, and how do they form? (Draw a picture and label 5' and 3' ends.)

5. "Additional considerations": Name three factors to consider when designing PCR primers.

6. Magnesium ion concentrations can have a big impact on PCR success. Free [Mg] is affected by the concentrations of the other PCR reactants, and the best concentration must often be determined experimentally.
   A. Too little free [Mg] causes:
   B. Too much free [Mg] causes:

PCR Part 2: *Pfu* DNA polymerase

Visit:
http://www.promega.com/pnotes/68/7381_07/7381_07_core.pdf
Read: Abstract, Introduction, Fidelity through Table 2, Cloning, Application, Summary (**skip** figure2, table3, Performance, figure3, figure4)

7. The DNA polymerase isolated from *Pyrococcus furiosis* possesses what nuclease activity that is missing from *Taq* DNA polymerase?

8. What is the main advantage of *Pfu* over *Taq* in PCR?

9. Does *Pfu* have terminal transferase-like activity as *Taq* does?

10. Why does Promega recommend using hot start PCR techniques when working with *Pfu*?