SEED Academy, Spring 2011
Synthetic Biology Module

Homework #3
Due March 3, 2011

1) **PCR**. If you need a refresher, watch this video about PCR (http://www.sumanasinc.com/webcontent/animations/content/pcr.html).

   a) Calculate the theoretical yields (# of strands of target DNA per strand of template DNA) of 100% efficient PCR for:

      a.i. 1 Cycle

      a.ii. 5 Cycles

      a.iii. 25 Cycles

      a.iv. n Cycles

   b) For a 50 μL, 100% efficient 25-cycle generic 1000-basepair sequence amplification…

      b.i. If your template is 10,000 basepairs at a concentration of 10 ng/μL, what volume is required to have an initial concentration of template of 50 pM? (Hint: Assume each base has a mass of 600 Daltons)

      b.ii. How many deoxynucleotide triphosphates (dNTPs, i.e. A, C, G, T) are required to complete the amplification?

      b.iii. If you want the concentration of dNTPs to decrease by less than 10% over the course of the reaction, what is the minimum required concentration of dNTPs (μM) to start?

b.iv. Complete the recipe for this PCR reaction below. Your polymerase stock is 2 Units/µL and you wish to add 1 Unit. Your primer stocks are 50 µM and you desire 0.5 µM final concentration. The buffer is 10X concentration. The dNTPs stock solution is 10 mM and you want a final concentration of 100 µM.

Template	____ µL
Buffer	____ µL
Polymerase	____ µL
dNTPs	____ µL
Primers	____ µL
Water	____ µL
Total	_50_ µL

c) List the three phases of a PCR cycle, what happens in each phase, and the relative temperature (hot, medium, cool) for each phase.

d) Design the forward and reverse primers (20 base pairs long) you would use to PCR amplify this DNA sequence from your favorite organism. Assume that you want to amplify *the entire* sequence shown here (i.e. your primers should start at the very edges of the sequence below).

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5′ – ATTAGTCTAGAAATTCGCGACGTAGTCAGCA - 3′
3′ – TAATCAGATCTTTAAGCGCTGCATCAGTCGT - 5′
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2) **More on your Final Project**
At this point we have had a good bit of discussion about the "abstraction hierarchy" in synthetic biology, particularly focusing on *devices*. Now it's time to think about your project in terms of the abstraction hierarchy. Last week we asked you to think about *how* your system would work. The natural next question is, "What devices are necessary to achieve those functions?" You should keep notes on your answers to this question, leading into the final project! For the 2-3 ideas you considered in depth last week, complete the following tasks:

   a) *Device List*. Very simple: list (or, if easier, describe) the devices your system would need in order to function. Try to think of at least two devices for each system (hint: the easier it is to think about these devices, the more likely it is that your system is plausible). For example, with the bacterial photography system, two devices would be a 1. Light sensor and 2. Color changer.

   b) *Choosing a Chassis Organism.* We want you to think more deeply about the organism in which you would like to work. What would be the capabilities of your "perfect" organism? Can withstand boiling temperatures? Can grow without oxygen? Consider where and in what conditions the organisms that have the desired capabilities live, what their nutritional requirements are, etc. The idea is that you think about and determine the organism that is best suited for your needs. (If you don't think you need fancy capabilities, consider well-understood organisms like E. coli or yeast!) Write down 1-3 necessary qualities for your organism and 1-3 potentially suitable organisms.

   c) *Real World Biological Interactions with System Interfaces*. From (a) you have a few device lists. Look into how nature and/or bioengineers have solved the problem of designing similar devices. (Hint: as a first pass, Google and Wikipedia are your BFFs). For example, for the bacterial photography system, you could look up ways that natural organisms sense light. Try to lean toward single-cell-scale solutions rather than multicellular ones (e.g. "light-sensitive protein" rather than "eyes"). For each of your devices, write down 2-3 facts describing the biology related to your device's function.

   d) Go to the course wiki (http://openwetware.org/wiki/SEED/2012) and write down the password.

Resources: Google and other search engines, the library, molecular biology textbooks, **TAs, and Instructors!**

Please do not hesitate to email instructors! We can help you with questions on any part of the homework (although we won't feed you the answers of course). Even if you don't have specific questions, we can also provide useful feedback on the design questions and help steer you in the right direction.