Activity: Design Your Own BioBrick

Introduction:

Polymerase chain reaction (PCR) requires two flanking primers surrounding the DNA template selected for amplification. Primer sequences must anneal to template DNA so that the DNA polymerase may begin replication. With an optimal length of 18-22 base pairs, primers are designed using the Wallace Rule, which states that the melting temperature of the primer can be determined by adding 4 degrees for every C or G in the primer sequence, adding 2 degrees for every A or T, and subtracting two degrees. Ultimately, the melting temperature should be above 55 degrees, usually between 55 and 60 degrees Celsius. The 3' nucleotide of the primer should ideally be a C or G so that the primer is more likely to anneal at this last base pair (because C and G bind with 3 hydrogen bonds, rather than with only 2 like A and T).

Objective: To develop primers for a specific gene, both by hand and by using either NCBI Primer Blast or Primer3. Students should become familiar with and feel comfortable using these powerful tools.

Procedure:

1. Choose a gene to introduce into your BioBrick. Look up the specific gene sequence on NCBI Blast: http://www.ncbi.nlm.nih.gov/gene. (Note: You may use the same gene previously selected for Activity 3: Design You Own BioBrick).

2. Analyze the beginning and ending sequences of the gene to determine the best possible primers.

3. Use NCBI Primer Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) or Primer3 (http://frodo.wi.mit.edu/) to design primers on the computer. Compare the results to the primers determined by hand!