Problem Set 4. BioBrick Creation: Mining nature for new parts

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The power of the parts registry is limited by the number of unique and non-interfering parts. Your mission is, in teams of four, to find a promoter-regulator pair from nature that is not contained in the BioBricks database.

Problem 1. The first stage is to locate a suitable part to grab from nature. Your constraints are as follows: the part may not be in the parts database already, it should be in one of the strains in the strains list (URL below), and it should be independent of promoters that are present in *e. coli*. You can use BLAST to search for the sequence within the parts database, and in standard.

a. Find the promoter, and write the name, genbank number and organism.

b. Write the strain number from the strains database.

c. Write the sequence, plus 50 bases on either side of the sequence, and an annotation of the features on the promoter.

Problem 2. Find the protein that regulates the promoter region.

a. Find the protein, and write the name and genbank number.

b. Write the sequence, plus 50 bases on either side of the sequence.

Problem 3. Design primers to PCR both the promoter region and the regulatory protein. Your PCR primers should have BioBrick ends (). When designing your primers, you need to have the biobrick ends, and a region that is homologous to the sequences you are cloning. The regions need to be in the range of 18-20 bases, such that they end with a G or a C, and the melting temperature T should range between 55 and 60 degrees. Further, each pair of homologous regions Ts should be within a degree.

a. Write the sequences for the forward and reverse promoter for the promoter region, and their melting temperatures.

b. Write the sequences for the forward and reverse promoter for the regulator region. And their melting temperatures.

c. Verify that the primers do not self-anneal (nothing to write).

d. Write the PCR mix for your two PCR reactions.

e. Write the protocol for the PCR program for your PCR reactions. Remember that the annealing temperature should be 5 degrees below the melting temperature, and extension time is 1 minute per 1000 bases.

f. Send an excel file to the TA's, in each row, the name of each primer and the sequence, your primers should be labelled with bbp, the initials for your team, , date, and 3-4 letters of the gene name and F or R for forward or reverse e.g. bbp-jg0915plac.F.

Problem 4. Enter the part into the parts database, insert the sequence, the original source of the sequence, relevant literature on how it works, how it was discovered. On the sequence, make annotations regarding the features of the sequence from the literature (operator sites, -10/-35 positions, transcription start). Include a printout of your BioBrick data sheet from the registry.