Designing the Promoter Construct for Reporter Plasmid

There was a number of difficulties in designing a promoter for our reporter plasmid. First and foremost, we needed to determine what was the best way to determine if the virus had infected the cell prior to seeing symptoms. We decided to utilize the proteins that the virus naturally makes upon infection. Upon infection, the virus generates both CI and Cro proteins which in turn determines whether the cell becomes lytic or lysogenic. The decision to go lysogenic or lytic is determined by a number of other proteins and conditions, but these are the final two proteins involved in the decision. Thus, we decided to use the right Promoter of Bacteriophage lambda. Here we met another two problems. The first was that we know that CI and Cro bind to the operators of the right promoter but they will also form a complex with the left promoter. We were not sure to what extent this would affect our promoter, and thus, decided to leave off the left promoter believing that the protein complexes will still bind. The other problem that we faced was that the Prm (the left promoter of the "Right Promoter," there are two promoters for each of the "left" and "right" promoters) did not have a ribosome binding site for which to start translation and translation naturally started right after the +1 site. The Pr did have a ribosome binding site. The issue with the ribosome binding site is the primary reason for so many constructs. The other reason was to compare the different approaches to synthetic biology using the iGEM approach with the prefix and suffix, and using a more traditional approach.

KEY:
Turquoise: -35 sites  
Bright Green: -10 sites  
Pink: +1 sites  
Dark Green: Translational STOP codon frequently found in E. coli.  
Yellow: Shine Dalgarno Sequence (Beginning of Ribosome Binding Site - RBS)  
Colored/Underlined & Italized: Restriction sites  
Bold & Underlined: Operators

Promoter Construct with Cro RBS and iGEM Restriction Sites

This promoter contains the iGEM prefix (EcoRI, NotI, XbaI) and suffix (SpeI, NotI, PstI). It also contains the original RBS of the Pr, which has been copied over to the Prm. A ribosome binding site was added to the Prm because it was felt that in order to express the fluorescent proteins, a RBS was needed. The translational stop codon was added six basepairs after the +1 site so that translation would not start at the +1 like it would do naturally in the lambda genome.

Promoter Construct with iGEM Restriction Sites only

This promoter is an exact replicate of the first promoter, but without the ribosome binding sites. The reason for this is because iGEM provides ribosome binding sites as a part of its DNA registry and most of the reporter genes already have RBS built into the parts.

Promoter Construct with Cro RBS and BspHI & NcoI Restriction Sites

This promoter takes a more traditional approach to synthetic biology. It contains the STOP codon that all of the previous promoters contain as well as a new RBS. This RBS was modified from the original Pr RBS so that it would work with the two new restriction sites (BspHI and NcoI - from left to right respectively). The restriction sites were designed around the two commercial reporter genes that we had on hand: AcGFp and DsRed. Both have NcoI sites right at the start of the sequence for the gene. The BspHI is compatible with NcoI and it allows for easy screening once the plasmid is constructed.

Promoter Construct with New RBS and BspHI & NcoI Restriction Sites

This promoter is exactly the same as the third promoter, but for one difference. The RBS has been modified to be AT rich, verses the previous RBS which has a number of GC basepairs. This should insure better expression of our reporter genes.