Ribosponge Lab Notebook: September

September 1, 2014: A PCR reaction was run to amplify pSB1C3 from the linearized vector using the following primers:
5- GCC GCT GCA GTC CGG CAA AAA A -3
5- ATG AAT TCC AGA AAT CAT CCT TAG CG -3

The reaction was purified with the QIAquick PCR Purification Kit.

The purified pSB1C3 PCR reaction and the merRNA PCR products from August 31 were digested with EcoRI-HF and PstI. The vector was dephosphorylated with Antarctic Phosphatase. Both reactions were purified, ligated together with T4 DNA Ligase, and transformed into TSS competent JM109 cells. (CM, CG)

September 2, 2014: The transformed bacteria from the previous day were recovered. No colonies were observed. (CM)

September 10, 2014: The PCR reaction first done on September 1 was repeated with a longer extension time. Again, the vector and merRNA insert were digested with EcoRI-HF and PstI, purified, ligated, and transformed into TSS competent JM109. (CM)

September 11, 2014: Again, no colonies were seen. (CG)

September 15, 2014: Thinking that the linearized vector may not be a good template, BBa_J04450 from the 2014 Distribution Plate was transformed into JM109. (PC, CM)

September 16, 2014: The plate of transformed cell was recovered. Many colonies were seen. A single colony was used to start a liquid culture which was grown overnight. (PC, CM)

September 17, 2014: A miniprep was performed on the cells containing BBa_J04450 in pSB1C3. (MG)

September 22, 2014: The PCR to amplify pSB1C3 was repeated using BBa_J04450 in pSB1C3 as the template. (CM)

September 23, 2014: The pSB1C3 PCR product was purified, digested with EcoRI-HF and PstI, ligated with the merRNA fragment, and transformed into JM109. (PC, CM)

September 24, 2014: No colonies were seen. (CM)