Ribosponge Lab Notebook: October

October 1, 2014: A new culture of JM109 with BBa_J04450 in pSB1C3 was grown overnight. (CM)

October 2, 2014: A miniprep was performed on the culture from the previous day. The purified plasmid was digested with EcoRI-HF and PstI, dephosphorylated, ligated with the merRNA fragment (without the promoter/terminators), and transformed into JM109. (CM)

October 3, 2014: Many colonies were seen. Several were used to seed overnight cultures. (CM)

October 4, 2014: DNA was purified from the overnight culture via minipreps. The purified DNA was used as a template in a PCR reaction using the following primers:
5- GCT AAT TGA ATT CGC GGC CGC TTC TAG AG -3

5- TCG AGC CTG CAG CGG CCG CTA CTA GTA -3

The PCR reaction was run on an agarose gel. Bands at ~500 bp were observed for all, suggesting that the desired merRNA fragment was successfully inserted.

Four of the plasmids were prepped for sequencing using the same primers for the reaction.

October 6, 2014: The sequencing materials were dropped off and sequenced by Eton Bio. Results indicated that the merRNA was successfully inserted with no mismatches in the sequence. (CM)

October 10, 2014: The merRNA in pSB1C3 plasmid (BBa_K1427000) was hand-delivered to iGEM HQ. (CM)

October 11, 2014: BBa_J04450 in pSB1C3 and the merRNA (with the promoter/terminators) were digested with EcoRI-HF and PstI. The vector was dephosphorylated with Antarctic Phosphatase, ligated with the merRNA fragment, and transformed into JM109 cells. (CM)

October 12, 2014: Colonies were observed. Several were used to grow liquid cultures overnight. (CM)

October 13, 2014: The plasmids in the overnight cultures were purified via minipreps. Three were readied for sequencing. (CM)

October 15, 2014: The samples were dropped off for sequencing with Eton Bio. Results confirmed the desired sequence. (CM)

October 17, 2014: This plasmid (BBa_K1427001) was hand-delivered to iGEM HQ. (CG)