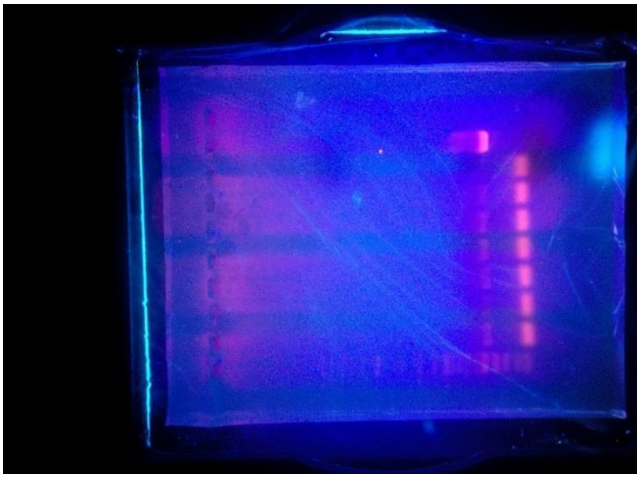
Ribosponge Lab Notebook: August

August 1, 2014: The plate of JM109 cells transformed with the merRNA+ Litmus28i_I716104 was retrieved. Colonies were seen. Although it is not known why this cloning procedure worked, all subsequent transformations were carried out via TSS cells. Eight colonies were picked and resuspended in water. This mixture was used a template for a single colony PCR reaction to confirm merRNA insertion.



PCR suggests that the merRNA fragment was inserted into all colonies.

The cell suspensions were also used to seed larger liquid cultures. (PT)

August 2, 2014: The cultures of JM109 with merRNA+ Litmus28i_I716104 were removed from the shaking incubator and left benchtop. (PT)

August 6, 2014: A miniprep was performed on the JM109 merRNA+ Litmus28i_I716104 cultures using the QIAprep Spin Miniprep Kit. (BF) This plasmid was then transformed into ZK1056 cells. Additionally, Litmus28i_I716104 was transformed into ZK1056 (CM, PC)

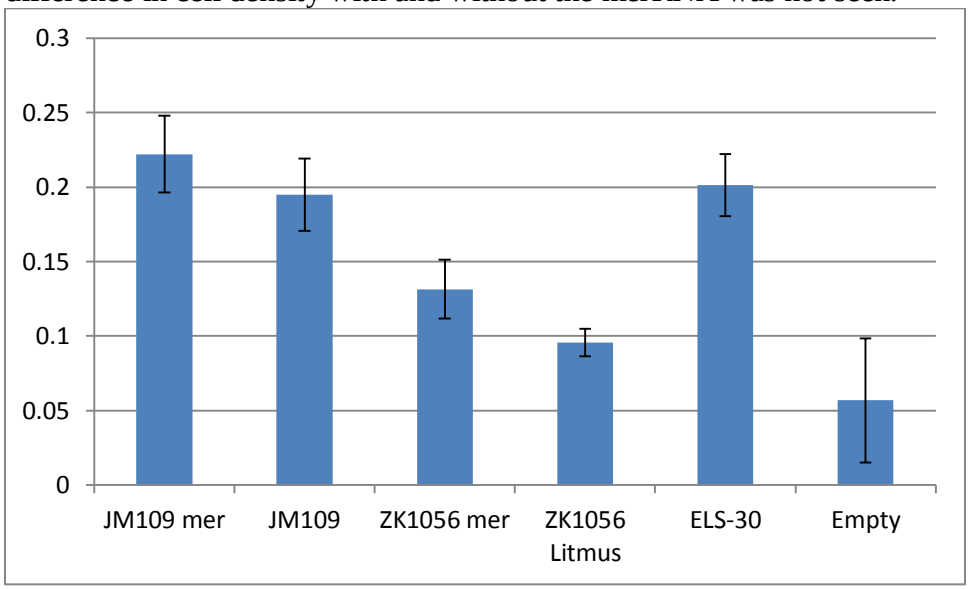
August 7, 2014: The transformations from the previous day were recovered. Colonies were observed. A single colony of each was then used to seed a liquid culture of ZK1056 which was grown overnight. (PT)

August 8, 2014: The ZK1056 cells with merRNA+ Litmus28i_I716104 were removed from the incubator and left bench top. (PC)

August 11, 2014: Liquid cultures of JM109 and ZK1056, both with and without the merRNA, were started. A non-biofilming forming strain (ELS-30) was also started (PC, CM)

August 12, 2014: Cultures for a biofilm assay were started in a 96-well plate using 1:100 dilutions of these five strains. The cultures were incubated at 30°C. (PT, CM)

August 13, 2014: After ~ 24 hours of static incubation, the planktonic bacteria were removed, transferred to a clear-bottom plate, and the A_{600} was found with a plate reader. A substantial difference in cell density with and without the merRNA was not seen.



The cells in a biofilm were stained with crystal violet, rinsed, and left to dry overnight. (PT, CM)

August 14, 2014: The stained well were dissolved and the A_{550} was read. Data are in the Results section. A second biofilm assay was started at 37°C. (CM, PT)

August 15, 2014: After 24 hours, the cultures were removed, rinsed of planktonic bacteria, and stained with crystal violet. (CM, PT)

August 16, 2014: The crystal violet was dissolved and the A_{550} was recorded. Data is in the Results section. Lastly, a biofilm assay at 25°C was started. (CM, PT)

August 17, 2014: Planktonic bacteria were rinsed out and the wells were stained with crystal violet. (CM, PT, PC)

August 18, 2014: The stained wells were dissolved and the A_{550} was recorded. Data are in the Results section. (CM, PT)

August 31, 2014: The merRNA sequence was amplified with the following primers containing the Biobrick prefix and suffix:
merRNA only:
5- AAT TAA TTG AAT TCG CGG CCG CTT CTA GAG CAA GGC TCG GGA GAC TTA CCT C -3

5- AAT ATA TTC TGC AGC GGC CGC TAC TAG TAT CGC CAA AAA AAG TGC TAG CGA G -3

Composite part (merRNA+promoter+terminators):
5- AAT TAA TTG AAT TCG CGG CCG CTT CTA GAG GGA AAC ACA GAA AAA AGC CCG CAC -3

5- AAT ATA TTC TGC AGC GGC CGC TAC TAG TAA ACG CAT GAG AAA GCC CCC GG -3

The PCR products were purified with the QIAquick PCR Purification Kit (MG, CM)