Ribosponge Lab Notebook: July

July 1, 2014: The well-plate was retrieved. Planktonic bacteria in LB were rinsed out and the biofilm bacteria were stained with crystal violet (described in Methods). The plate was left to dry overnight. (JS, CM)

July 2, 2014: The dye was dissolved in 30% acetic acid and transferred to a clear-bottomed well plate. A_{550} was recorded with a plate reader.

Well	ZK1056	ELS-30	Background average	ZK1056 minus background	ELS-30 minus background
1	0.183	0.091			
2	0.178	0.115			
3	0.170	0.105			
4	0.169	0.101			
5	0.213	0.122			
6	1.868 (outlier)	0.147			
Average	0.183 (1-5)	0.114 (1-6)	0.033	**0.150**	**0.081**

ZK1056 (A_{550} = 0.150) had approximately a two-fold increase in biofilm formation over ELS-30 (A_{550} = 0.081), which suggests the strain, growth conditions, and assay are working as intended. (JS, CM)

July 9, 2014: To determine whether well-plates treated for tissue culture (TC) or nonbinding (NB) wells would provide better growth conditions, biofilm assays were started for both types of wells. (PT, CG)

July 10, 2014: Another set of cultures was started about 24 hours later so a second time point could be assessed for the tissue-culture treated well-plates. A 5mL culture of ELS-16 was started and grown overnight. (PT, MG)

July 11, 2014: The cultures in the well-plates which had been growing statically for about 48 hours and 24 hours, we rinsed of planktonic bacteria and stained with crystal violet. They were left to dry overnight. The culture of ELS-16 was recovered and its Litmus28i_I716104 plasmid was purified using the QIAprep Spin Miniprep Kit. (PT, MG)

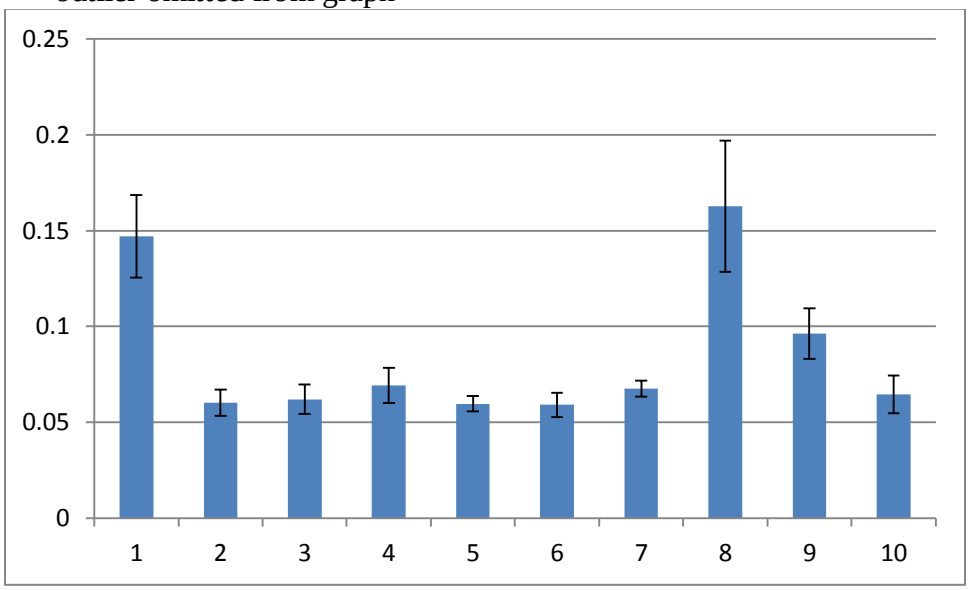
#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1		7/1/2014 7:25:40 PM	36.4	ng/µl	0.728	0.442	1.65	0.77	DNA	50

July 12: The stained wells were dissolved in 80% ethanol/ 20% acetone. The A_{550} of the solution was taken:

A_{550}

TC (1)	TC (2)	TC (3)	TC (4)	TC (5)	TC (6)	TC (7)	NB (8)	NB (9)	NB (10)
48 hr	48 hr	48 hr	24 hr	24 hr	24 hr	0 hr	22 hr	22 hr	0 hr
ZK1056	**ELS-30**	**ELS-34**	**ZK1056**	**ELS-30**	**ELS-34**	**empty**	**ZK1056**	**ELS-30**	**empty**
0.551*	0.058	0.073	0.085	0.058	0.074	0.068	0.217	0.082	0.06
0.171	0.061	0.057	0.062	0.056	0.056	0.065	0.135	0.115	0.072
0.118	0.057	0.055	0.064	0.057	0.061	0.067	0.155	0.097	0.053
0.138	0.077	0.056	0.066	0.068	0.055	0.071	0.123	0.098	0.06
0.15	0.058	0.072	0.063	0.058	0.057	0.066	0.166	0.112	0.059
0.133	0.057	0.06	0.064	0.06	0.054	0.076	0.144	0.091	0.061
0.172	0.056	0.055	0.067	0.063	0.056	0.063	0.199	0.098	0.084
0.578*	0.059	0.068	0.083	0.058	0.06	0.065	1.83*	0.077	0.068

* = outlier omitted from graph



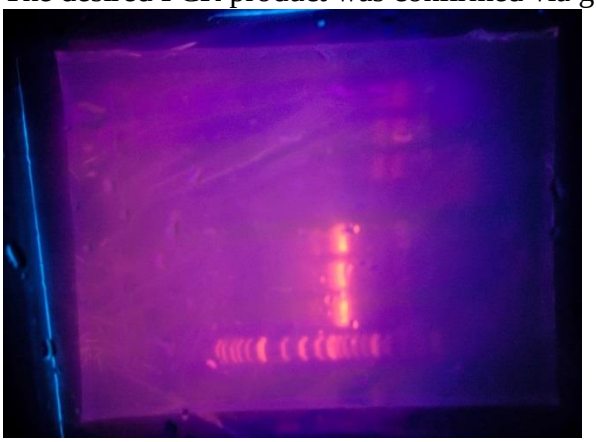
These data suggest that a greater differentiation between biofilm forming and non-biofilm forming strains is achieved with well-plates with non-binding surfaces after ~24 hours. The non-binding well-plates were used for all subsequent biofilm assays (MG, CM)

July 28, 2014: The merRNA construct (housed in a pUC57 vector) was retrieved and resuspended in ultrapure water. This plasmid was transformed into JM109 cells via heat-shock. (JS) Additionally, the merRNA fragment was amplified via PCR using the Phusion High-Fidelity PCR Master Mix with HF Buffer. The primers used were:

5- TCG AGC TGC AGG GAA ACA CAG AAA A -3

5- TCT ATG GAT CCA ACG CAT GAG AAA GCC C -3

The desired PCR product was confirmed via gel electrophoresis. (BF)



The remaining PCR reactions were purified using the QIAquick PCR Purification Kit (MG).

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	merRNA full, #1	Barnum Lab	7/29/2014 1:04:09 AM	61.4	ng/µl	1.229	0.657	1.87	2.02	DNA	50
2	merRNA full, #1	Barnum Lab	7/29/2014 1:05:25 AM	61.7	ng/µl	1.234	0.668	1.85	2.01	DNA	50
3	merRNA full, #2	Barnum Lab	7/29/2014 1:06:27 AM	55.8	ng/µl	1.117	0.586	1.91	2.18	DNA	50

July 29, 2014: The JM109 cells transformed with merRNA-pUC57 were retrieved and stored at 4°C. The merRNA PCR fragment and Litmus28i_I716104 vector were digested with EcoRI-HF and BamHI-HF. The vector was dephosphorylated with Antarctic phosphatase. Both reactions were purified using the QIAquick PCR Purification Kit. Both fragments were ligated using T4 DNA Ligase and transformed into JM109 cells via heat-shock. Cells were plated on ampicillin media and grown overnight. (CG, PT)

July 30, 2014: The plate was retrieved. No colonies were observed. The digest, purification, ligation, and transformation steps were repeated with a larger quantity of DNA. In case cell quality was the issue, starter cultures of JM109 and ZK1056 cells were grown overnight such that TSS competent cells could be made. (CM, PC)

July 31, 2014: The plate was retrieved. Again, no colonies were observed, even when left for a full 24 hours. The JM109 and ZK1056 overnight cultures were used to seed 50 mL cultures which were grown to OD_{600}= 0.4, at which point they were rendered competent with TSS buffer. A full 1 ug of each DNA component was digested this time. Additionally, more of each fragment was used in the ligation reaction. JM109 cells were transformed with this ligation reaction and plated under ampicillin selection. (PT, CM)