Ribosponge Lab Notebook: June

June 10, 2014: Several bottles of LB broth and LB agar were made and autoclaved. Agar was spiked with antibiotic to achieve an appropriate working concentrations and poured to make plates. (Amp: 100 ug/mL, Kan: 20 ug/mL, Cam: 25 ug/mL, Strep: 10 ug/mL, Tet: 10 ug/mL). (PT, CG)

June 11, 2014: Plated ZK1056 cells kindly provided by the Kotler Lab at Harvard were retrieved and stored at 4°C. (PT, CG)

June 13, 2014: Agar plates containing strains kindly supplied by the Endy Lab were retrieved and stored at 4°C.

Number	Name	Resistance
ELS-16	Litmus28i_I716104	Ampicillin
ELS-28	Litmus28i	Ampicillin
ELS-30	Rp437	Streptomycin
ELS-34	Rp437, F+	Streptomycin, Tetracycline
ELS-43	HpdO	Kanamycin

5 mL cultures with the appropriate antibiotic were started from single colonies of these five Endy Lab strains, along with the ZK1056. Cells were grown for ~16 hours at 37°C while shaking. (PT, CG)

June 14, 2014: Bacterial cultures from the previous day were checked. Only ELS-30, ELS-43, and ZK1056 successfully grew. Bacterial stocks were made for these three with 500 uL cell culture and 500 uL 50% glycerol and stored at -80°C. 5mL cultures for all cells were restarted and allowed to grow overnight. (MZ, CM, MG)

June 15, 2014: Cultures were checked. All strains grew to a high density. Glycerol stocks were made for ELS-16, ELS-28, and ELS-34 and stored at -80°C (CM).

June 16, 2014: The five Endy Lab strains plus ZK1056 were streaked onto the appropriate plate from frozen stock to confirm cell viability. Plates were incubated at 37°C overnight. (PT)

June 17, 2014: Plates were retrieved. Growth was registered for all strains. Plates were stored at 4°C. (PT,CM)

June 23, 2014: Frozen JM109 cells kindly provided by the Fuhrman Lab at Tufts were retrieved and stored at -80°C. (PT)

June 24, 2014: 5mL cultures of JM109 and ZK1056 were started from the frozen stock and grown overnight at 37°C while shaking. (CM)

June 25, 2014: The overnight JM109 and ZK1056 cultures were used to seed 100 mL cultures. These were grown for approximately 6 hours until $OD_{600} \sim 0.850$. Cells were rendered chemically competent via resuspension in $CaCl_2$ + glycerol. To test effectiveness of these cells, a heat-shock transformation was performed with the pET15b vector. Cells were plated on ampicillin agar plates and incubated overnight at 37°C. The remaining competent cell aliquots were stored at -80°C. (CM, PC, CG)

June 26, 2014: Plates from the previous day's transformations were retrieved after about 16 hours. Colonies were registered for both JM109 and ZK1056, confirming effectiveness of the competent cells. (BF)

June 29, 2014: 5 mL cultures of ZK1056 and ELS-30 were started from frozen stocks and grown overnight. ZK1056 is known to form biofilms, whereas ELS-30 (the control) does not. (CM)

June 30, 2014: A 1:100 dilutions of the overnight cultures were made and used to start a biofilm assay in a 96-well plate (described in Methods). The cultures were incubated at 37°C for about 21 hours. (CM, MG)