ThyA fitness module

-ThyA codes for Thymidylate synthase

-Cloned downstream of promoter+ RBS, gene facilitated growth in E.coli without thymine

Previous work with ThyA in Davidson College

-Building J100135 plasmid with promoter+ C-Dog (S0528)+ thyA

-Grew cells with S05028 in LB Amp and performed miniprep according to the “Zippy Miniprep” protocol

-ThyA J100132 had BsaI sites and sticky ends built in, so designed primers to match sticky ends

-Transformed JM109 (wild type) competent cells, and grew JM109s on LB Amp plates overnight

-Transformed ThyA- cells with ThyA containing J100135 (promoter and RBS)

-Grew ThyA cells with and without Thymine

-Built J100065 (promoter + Riboswitch D + thyA)

-Attempted to amplify promoter, riboswitch and the plasmid, but there were errors in PCR, so she was not able to

Results

-Control- 10 fold difference between thyA- cells with thymine than without

-J100135 construct had same result as thyA-cells with thymine

Our objective

-Make J100065 workable

-Use ThyA fitness module in our programmed evolution of using the theophylline ligand

Sunday 05/25/14

Davidson College

-Growing part # J100136 (T5+riboswitchD+thyA); two samples

-Growing positive control J100135 on ThyA- cells with thyA gene insert, Kan resistant

-Growing thyA-cells, kan resistant