

iGEM 2016: Team Pittsburgh
Week 19 Lab Notebook

Wednesday, September 28

LacZ Mutagenesis (Maddie)

Thursday, September 29

DpnI Digest of LacZ Mutagenesis (Maya)

PCR Amplification of PT3-GFP (Maya)

Gel Purification of LacZ and T3amps, gel check of PT3-T3 (Maya)

PT3-GFP Amp. Digest with Xba, Pst (Maya)

Ligation of PT7-G (Maya)

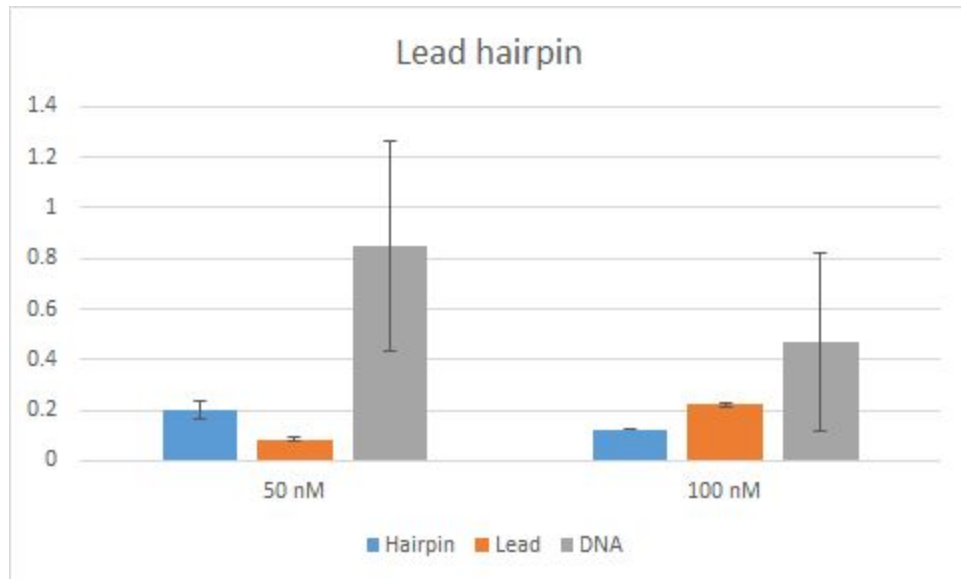
Modeling (Maya/Adam)

Cell-free reaction lead hairpin at 50 and 100 nM (Claire)

	Sol'n A	45.6	
	B	34.2	
	RNase x19	5.7	
	sub	5.7	
	switch @ 55.6	8.8 8.54	=475 ng
	H ₂ O	2.59 2.85	
	mg	0.6	
		10.8/rxn	
M14-15	Triggers 50 nM hp		1.2 μ L 500 nM hp
M16-17	50 nM hp + Pb	1 μ L hp @ 1 μ M 1 μ L Pb @ 40 μ M	\Rightarrow 1.2 μ L
M18-19	50 nM DNA		1.2 μ L 500 nM DNA
M20-21	100 nM hp		1.2 μ L 1 μ M hp
M22-23	100 nM hp + Pb	1 μ L hp @ 2 μ M 1 μ L Pb @ 40 μ M	\Rightarrow 1.2 μ L
<i>a little more (Cheryl)</i>	100 nM DNA		1.2 μ L 1 μ M DNA
M24 & A24			

Condition	hairpin				lead				DNA			
	1	2	average	st dev	1	2	average	st dev	1	2	average	st dev
50 nM	0.2266	0.1725	0.19955	0.038254	0.089	0.0772	0.0831	0.008344	1.1455	0.5585	0.852	0.415072
100 nM	0.1223	0.1225	0.1224	0.000141	0.2249	0.2175	0.2212	0.005233	0.7182	0.2237	0.47095	0.349664





Gel analysis of cleavage (Praneeth)

Friday, September 30

PT7-RBS to T3 (Maya)

Send out PT3-T3 for sequencing (Maya)