Date: 8/3/14 People in lab: Emily Puleo, Joshua Heath

Title: Chemical Transformation

Start Time: 5 pm

Purpose: Prepare backbone (chlor resistance with promoter and RBS)

Protocol: LTM Ed. 2 Chemical Transformations

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
2014 1-30	BBa_K608002	Plate 1 2014 kit well 30	1
2014 3-3F	BBa_B0015	Plate 3 2014 kit well 3F	1
8/3 CT1	20 uL	2014 1-30	1
8/3 CT2	200 uL	2014 1-30	1
8/3 CT3	20 uL	2014 3-3F	1
8/3 CT4	200 uL	2014 3-3F	1

Results: no growth

Notes:

Stop Time: 8 pm

Next: Try again with different comp cells

Date: 8/5/14 People in lab: Emily Puleo

Title: Chemical Transformation

Start Time: 4 pm

Purpose: Transform Pro + RBS and TT **Protocol:** LTM Ed. 2 Chemical Transformations

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
8/5 CT1	20 uL	2014 1-30	1
8/5 CT2	200 uL	2014 1-30	1
8/5 CT3	20 uL	2014 3-3F	1
8/5 CT4	200 uL	2014 3-3F	1

Results:

Notes:

Stop Time: 7:15 pm

Next:

- Ladder PCR1 PCR2 PCR3 PCR4 PCR5 PCR6

Date: 8/6/14 People in lab: Joshua Heath

Title: Digestion

Start Time: 1:20 pm

Purpose: Separate hmp fragment from plasmid

Protocol: LTM Ed. 2 Digestions

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
8/6 D1	hmp in elution buffer	7/30 MP3	1
8/6 D2	hmp in elution buffer	7/30 MP4	1

Results:

Notes:

Stop Time: 3:25 pm

Next:

Date: 8/6/14 People in lab: Joshua Heath

Title: Innoculation

Start Time: 2:50 pm

Purpose: To increase the amount of plasmid to work with

Protocol: LTM Ed. 2 Innoculation

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
8/6 BC1	---	8/5 CT1	1
8/6 BC2	---	8/5 CT2	1
8/6 BC3	---	8/5 CT3	1
8/6 BC4	---	8/5 CT4	1

Results:

Notes:

Stop Time: 3:35 pm

Next: Miniprep

Title: Miniprep and Nanodrop

Start Time: 11:25 am

Purpose: To purify plasmid DNA and measure purity

Protocol: LTM Ed. 2 Miniprep and Nanodrop

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
8/7 MP1	ng/uL = 57.8; 260/280 = 1.98; 260/230 = 2.04	8/6 BC1	1
8/7 MP2	ng/uL = 54.2; 260/280 = 2.01; 260/230 = 2.1	8/6 BC2	1
8/7 MP3	ng/uL = 69.7; 260/230 = 2.04; 260/230 = 2.09	8/6 BC3	1
8/7 MP4	ng/uL = 61.8; 260/280 = 2.06; 260/230 = 2.12	8/6 BC4	1

Results:

Notes:

Stop Time: 2:15 pm

Next: Re-innoculate to try for better concentrations

Date: 8/8/14 People in lab: Emily Puleo, Joshua Heath

Title: Inoculation

Start Time: 2 pm

Purpose: Innoculate pro+RBS and TT

Protocol: LTM Ed. 2 Innoculation

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
8/8 BC1	pro+RBS	8/5 CT1	1
8/8 BC2	pro+RBS	8/5 CT2	1
8/8 BC3	TT	8/5 CT3	1
8/8 BC4	TT	8/5 CT4	1

Results:

Notes:

Stop Time: 2:30 pm

Next: Miniprep

Date: 8/9/14 People in lab: Emily Puleo, Joshua Heath

Title: Miniprep and Nanodrop

Start Time: 10:15 am

Purpose: Purify plasmid DNA and measure purity

Protocol: LTM Ed. 2 Miniprep and Nanodrop

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
8/9 MP1	ng/uL = 52.1; 260/280 = 2.1; 260/230 = 2.05	8/8 BC1	1
8/9 MP2	ng/uL = 58.7; 260/280 = 2.11; 260/230 = 2.07	8/8 BC2	1
8/9 MP3	ng/uL = 58.1; 260/280 = 2.1; 260/230 = 2.05	8/8 BC3	1
8/9 MP4	ng/uL = 51.4; 260/280 = 2.05; 260/230 = 1.98	8/8 BC4	1

Results:

Notes: When 8/8 BC4 was being taken out of the incubator, the lid came off

Stop Time: 12:30 pm

Next:

Date: 8/11/14 People in lab: Emily Puleo, Joshua Heath

Title: Gel Extraction of hmp

Start Time: 1 pm

Purpose: Isolate hmp from vector

Protocol: LTM Ed. 2 Gel Extraction

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
8/11 GE1	hmp	8/6 D1	1
8/11 GE2	hmp	8/6 D2	1

Results:

Notes:

Stop Time: 1:45 pm

Next: Ligate with pro+rbs part

Date: 8/11/14 People in lab: Emily Puleo, Joshua Heath

Title: Digest pro+RBS and TT

Start Time: 1:45 pm

Purpose: Prep for ligation

Protocol: LTM Ed. 2 Digest

Exceptions: For pro+RBS = 15.5 uL DNA, 5 uL milliQ, 1 uL speI and pstI; for TT = 1 uL xbal and pstI

Products:

Sample Label	Description	Source Label	Quantity
8/11 D1	pro+RBS digested with S and P	8/9 MP1	1
8/11 D2	pro+RBS digested with S and P	8/9 MP2	1
8/11 D3	TT digested with X and P	8/9 MP3	1
8/11 D4	TT digested with X and P	8/9 MP4	1

Results:

Notes:

Stop Time: 3:45 pm

Next: Nanodrop

Date: 8/11/14 People in lab: Emily Puleo, Joshua Heath

Title: Nanodrop

Start Time: 3:55 pm

Purpose: Measure the purity and concentration of the samples

Protocol: LTM Ed. 2 Nanodrop

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
---	ng/uL = 17.5; 260/280 = 11.16; 260/230 = 0.11	8/11 GE1	1
---	ng/uL = 30.9; 260/280 = 9.79; 260/280 = 0.6	8/11 GE2	1

Results:

Notes:

Stop Time: 4:05 pm

Next: Ligation

Date: 8/12/14 People in lab: Joshua Heath

Title: Ligation

Start Time: 3:50 pm

Purpose: LIgate the pro+RBS and hmp parts

Protocol: LTM Ed. 2 Ligation

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
8/12 L1	pro+RBS in chlor resistant backbone plus hmp	8/11 GE1 and D2	1

Results:

Notes:

Stop Time: 4:10 pm

Next: Chemical Transformation

Date: 8/13/14 People in lab: Emily Puleo

Title: Gel extraction and ligation of hmp in pro+RBS backbone and gel extraction of 8/11 D2 and D3

Start Time: 12:30 pm

Purpose:

Protocol: LTM Ed. 2 Gel Extraction nad transformation

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
8/13 GE1	Pro+RBS	8/11 D2	1
8/13 GE2	TT	8/11 D3	1
8/13 L1	Pro+RBS and hmp	8/11 GE1 and D1	1

Results:

Notes:

Stop Time: 4 pm

Next: Chemical Transformation

Date: 8/14/14 People in lab: Emily Puleo

Title: Chemical Transformation of 8/13 L1

Start Time: 1 pm

Purpose: Check ligation success and prepare for miniprep

Protocol: LTM Ed. 2 Chemical Transformation

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
8/14 CT1	20 uL	8/13 L1	1
8/14 CT2	200 uL	8/13 L1	1

Results:

Notes:

Stop Time: 3:15 pm

Next: Innoculation

Date: 8/16/14 People in lab: Kira Buckowing

Title: Genomic Prep and PCR of PAO1

Start Time: 10 am

Purpose: To obtain usable components needed for nosZ

Protocol: LTM Ed. 2 PCR; DNA, RNA and protein purification kit manual

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
GP1 8/16	Genomic prep of PAO1 cells reconstituted from a dried up plate	---	1
PAO1 Temp 8/16	PCR of resuspended PAO1 cells to check for usability	---	1

Results:

Notes: Incubation for Genomic Prep ~ 3hrs, optional RNA clean up step followed

Stop Time: 3 pm

Next: Gel electrophoresis of PCR to test, PCR of GP1 to check viability

Date: 8/18/14 People in lab: Emily Puleo, Kelsey Crossen

Title: Ligation of hmp and pro+RBS

Start Time: 1:35 pm

Purpose: To ligate together hmp and pro+RBS parts for transformation into competent cells

Protocol: LTM Ed. 2 Ligation

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
L1 8/18	ligated product	8/13 GE1; 8/11 GE2	1

Results:

Notes:

Stop Time: 1:50 pm

Next: Chemical Transformation

Date: 8/19/14 People in lab: Emily Puleo, Kelsey Crossen

Title: Chemical Transformation

Start Time: 3:45 pm

Purpose: To transform ligated product into comp cells

Protocol: LTM Ed. 2 Chemical Transformation

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
CT1 8/19	20 uL	L1 8/18	1
CT2 8/19	200 uL	L1 8/18	1
CT3 8/19	20 uL	L1 8/18	1
CT4 8/19	200 uL	L1 8/8	1

Results: (8/20) no growth present, only misleading air bubbles that looked like colonies; retry with differences in vector/insert ratio or new buffer

Notes:

Stop Time: 6:14 pm

Next: Start liquid cultures from colonies that grow

Date: 8/20/14 People in lab: Kira Buckowing

Title: PCR of GP1 8/16

Start Time: 10:20 am

Purpose: To test the sample to see if the procedure was successful

Protocol: LTM Ed. 2 PCR

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
GP1 PCR 8/20	PCR of Genomic Prep	GP1 8/16	1

Results:

Notes:

Stop Time: 2 pm

Next: Gel electrophoresis

Date: 8/25/14 People in lab: Kira Buckowing

Title: Cel Electrophoresis of GP1 PCR 8/20

Start Time: 8:30 am

Purpose: To finish testing to see if the genomic prep worked as expected

Protocol: LTM Ed. 2 Gels

Exceptions:

Products:

Well	1	2	3	4	5	6	7	8	
-	Ladder	-	GP1 PCR	-	C11 Orange	-	C11 Black	-	C11 Black

Products:

Results:

Notes: iGEM sample was run with others for Dr. W (advisor) lab to test primers that were not properly stored

Stop Time: 10:10 am

Next: PCR of GP1 with nosZ primers after positive result

Date: 8/27/14 People in lab: Kelsey Crossen

Title: Transformation of hmp and pro+RBS backbone and RFP in chlor resistant backbone to test comp cells

Start Time: 2 pm

Purpose: To test if comp cells are working by running a control with the target reaction for the project

Protocol: LTM Ed. 2 Chemical Transformation

Exceptions: 1.5 uL RFP and 1.5 uL Ligation product used and run in the same transformation

Products:

Sample Label	Description	Source Label	Quantity
CT1 8/27	20 uL	L1 8/18 and DH5 alpha 2013 DJW	1
CT2 8/27	200 uL	L1 8/18 and DH5 alpha 2013 DJW	1
CT3 8/27	20 uL	RFP in chlor bb stock 12/18/13	1
CT4 8/27	200 uL	RFP in chlor bb stock 12/18/13	1

Results: (8/29) No growth - comp cells are deemed bad

Notes: DH5 alpha cells used from bottom shelf of -80 C freezer

Stop Time: 5 pm

N**ext:** Comp cells need to be remade

Date: 8/31/14 People in lab: Kira Buckowing

Title: PCR of GP1 and PAO1 Temp and Gel to check products

Start Time: 10:45 am

Purpose: To get a positive result to nosZ

Protocol: LTM Ed. 2 PCR and Gels

Exceptions: PCR 1 and 3 = 2 uL DNA, 2.5 uL primers; PCR 2 and 4 = 7.5 uL DNA, 2.5 uL primers

Products:

Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
-	Ladder	-	PCR 1	-	PCR2	-	PCR 3	-	PCR4

Products:

Results:

Notes: The band looks to be the wrong size, but its the first band that has ever shown up for something with nosZ

Stop Time:3:45 pm

Next: Troubleshooting - Test primers again with controls

Date: 8/31/14 People in lab: Kira Buckowing

Title: innoculation of DH5 Alpha stock

Start Time: 4 pm

Purpose: Prep for making comp cells

Protocol: LTM Ed. 2 Innoculation

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
DH5 alphs stock 8/31	DH5 alpha cells in LB broth	---	1

Results:

Notes: Stored in fridge after incubation period

Stop Time: 4:05 pm

Next: Competent cell creation

Date: 8/31/14 People in lab: Kira Buckowing

Title: PCR and Gel of GP1 and PAO1 Temp with controls

Start Time: 4:30 pm

Purpose: Testing primers

Protocol: LTM Ed. 2 PCR and Gels

Exceptions: 1 and 2 - 2 uL DNA with old primers; 3 and 4 = 7.5 uL DNA with old primers; 5 and 6 = 2 uL DNA with new primers; 7 and 8 = 7.5 uL with new primers

Products:

Well	1	2	3	4	5	6	7	8	9	10
-	Ladder	PCR1	PCR2	PCR3	PCR4	PCR5	PCR6	PCR7	PCR8	Ladder

Products:

Results: Bands are still too short, by about 1000 base pairs

Notes: Annealing temp or primers may be the issue

Stop Time: 9 pm

Next: More troubleshooting