JUNE 10, 2014

Making LB media (Protocol taken from Addgene)

1. Prepare liquid LB. For example, to make 400mL of LB:
Weigh out the following into a 500mL glass bottle
- 4g NaCl
- 4g Tryptone
- 2g Yeast Extract
- and (dH_2O) to **400mL**

> ***Note:*** *If your lab has pre-mixed LB agar powder, use the suggested amount, instead of the other dry ingredients above.*

Loosely close the cap on the bottle (do NOT close all the way or the bottle may explode!) and then loosely cover the entire top of the bottle with aluminum foil. Autoclave and allow to cool to room temperature. Now screw on the top of the bottle and store the LB at room temperature.
2. When ready to grow your culture, add liquid LB to a tube or flask and add the appropriate antibiotic to the correct concentration (see table below).

> ***Note:*** *If you intend to do a mini-prep you will usually want to start 2mL in a falcon tube, but for larger preps you might want to use as much as a liter of LB in a 2L Erlenmeyer flask.*

3. Using a sterile pipette tip or toothpick, select a single colony from your LB agar plate.
4. Drop the tip or toothpick into the liquid LB + antibiotic and swirl.
5. Loosely cover the culture with sterile aluminum foil or a cap that is not air tight.
6. Incubate bacterial culture at 37°C for 12-18hr in a shacking incubator.

> ***Note:*** *Some plasmids or strains require growth at 30°C. If so, you will likely need to grow for a longer time to get the correct density of bacteria since they will grow more slowly at lower temperatures.*

7. After incubation, check for growth, which is characterized by a cloudy haze in the media.

> ***Note:*** *Some protocols require bacteria to be in the log phase of growth. Check the instructions for your specific protocol and conduct an OD600 to measure the density of your culture if needed.*

> ***Note:****A good negative control is LB media + antibiotic without any bacteria inoculated. You should see no growth in this culture after overnight incubation.*

Making agar plates (Protocol taken from Addgene)

1. Weigh out the following into a 1L Erlenmeyer flask:
- 5g NaCl
- 5g Tryptone

- 2.5g Yeast Extract
- 7.5g Agar
- and (dH_2O) to 500mL

Note: *If your lab has pre-mixed LB agar powder, use the suggested amount instead of the other dry ingredients above.*

2. Swirl to mix - the contents do not have to be completely in solution, but any powder left on the sides of the flask will caramelize on the glass during autoclaving.
3. Cover the top of the flask with aluminum foil and label with autoclave tape.
4. Autoclave on the liquid setting for 20 minutes or according to your autoclave's specifications.
5. After removing the solution from the autoclave, allow the agar solution to cool to 55°C.

Note: *This can be done by placing the flask in a 55°C oven or water bath, as this will hold the temperature and it can be left unattended for some time.*

6. When pouring plates, keep your bench area sterile by working near a flame or bunsen burner.
7. Add the appropriate amount of desired antibiotic to the solution (500µL if you are using a 1,000x antibiotic stock) and swirl to mix.
8. Pour ~20mL of LB agar per 10cm polystyrene Petri dish.

Note: *Pour slowly from the flask into the center of the petri dish. When the agar has spread to cover about 2/3 of the dish stop pouring and the agar should spread to cover the entire plate. You may need to tilt the plate slightly to get the agar to spread out completely. If you pour in too much, the plate will be fine, but it will reduce the number of plates you can make per batch.*

Note: *If bubbles are introduced during the pouring, these can be removed by quickly passing the flame of an inverted bunsen burner over the surface of the plate. Be careful, if you leave the flame too long it will melt the petri dish. Also be careful not to burn yourself.*

9. Place the lids on the plates and allow them to cool for 30-60 minutes (until solidified) then invert the plates. Let sit for several more hours or overnight.
10. Label the bottom of plates with antibiotic and date and store in plastic bags or sealed with parafilm at 4°C.

Making antibiotic stocks (Endy group wiki on OpenWetWare)

Ampicillin

Stocks & Usage

- Stock Concentration - 50mg/ml in H_2O
- Aliquots - 100µl and 500µl
- Working Concentration - ~~50µg/ml~~100ug/mL

Preparation of stock solution

- Ampicillin is kept in the 4C fridge in Barnum 200. It is light sensitive.
- Weigh 500mg of ampicillin in a small weigh boat
- Add 10ml of ultrapure water to a 15mL falcon tube.
- Add the ampicillin to the falcon tube
- Mix/vortex so all the ampicillin goes into solution.
- Filter sterilize the solution into 1.5mL aliquots inside 2.0mL centrifuge tubes.
- Store at -20C.

Kanamycin

Stocks & Usage

- Stock Concentration - 10mg/ml in H_2O
- Aliquots - 200µl and 1ml
- Working Concentration - 20µg/ml

Preparation of stock solution

- Kanamycin is kept in the 4C fridge in Barnum 200. It is light sensitive.
- Weigh 100mg of kanamycin in a small weigh boat
- Add 10ml of ultrapure water to a 15mL falcon tube.
- Add the kanamycin to the falcon tube
- Mix/vortex so all the kanamycin goes into solution.
- Filter sterilize the solution into 1.5mL aliquots inside 2.0mL centrifuge tubes.
- Store at -20C.

Chloramphenicol

- Stock concentration - 34mg/ml in 100% Ethanol
- Aliquots - 1ml
- Working concentration = 25µ/ml (Stringent), 170µ/ml (relaxed)

Preparation of 80ml stock solution

- Chloramphenicol is kept at room temperature. It is near our other chemicals
- Weight 340mg of chloramphenicol sulfate into a small weight boat.
- Add 10ml 100% EtOH to the chloramphenicol
    - 95% EtOH probably works just fine, but I haven't tried it.
- Mix/vortex vigorously so all the chloramphenicol goes into solution.
- Aliquot into eppendorfs.
    - N.B. There is no need to filter sterilize, as it is in EtOH.
- Store at -20C.

JUNE 11, 2014

Received ZK1056 cells from the Kolter Lab at Harvard
(strain is described in [this publication](#)**)**

- Plate is stored in 4C in fridge in Barnum 200.
- ZK1056 cells grow forming biofilms using LB media.

JUNE 13, 2014

Retrieve plates containing strains shipped by Endy lab to Nair lab

Strain information

NUMBER	NAME	STRAIN	RESISTANCE	PLASMID
ELS-16	Litmus28i_I716104	XL1-Blue	Ampicillin	ColE1, M13
ELS-28	Litmus28i	XL1-Blue	Ampicillin	ColE1
ELS-30	Rp437	MG1655	Streptomycin	
ELS-34	Rp437, F+	MG1655	Streptomycin, Tetracycline	
ELS-43	HpdO	RP437, F+	Kanamycin	p15A

- Table from document

Plates shipping history:

Fedex Tracking #: 770271765169

6/12/2014 - Thursday		
3:00 pm	Delivered	MEDFORD, MA
8:28 am	On FedEx vehicle for delivery	SOUTH BOSTON, MA
7:23 am	At local FedEx facility	SOUTH BOSTON, MA
6:27 am	At destination sort facility	EAST BOSTON, MA
4:02 am	Departed FedEx location	MEMPHIS, TN
1:53 am	Arrived at FedEx location	MEMPHIS, TN
6/11/2014 - Wednesday		
8:41 pm	Left FedEx origin facility	MENLO PARK, CA
7:38 pm	Picked up	MENLO PARK, CA
4:19 pm	Shipment information sent to FedEx	

- Plates were received and signed for by M. RICCI
- Placed in Prof. Nair's box and left overnight. Then retrieved by Todd C. on morning of Friday June 13 and placed in 4C fridge.

Retrieve Streptomycin and Tetracycline stocks from Nair lab

- Tetracycline 10mg/mL stock → dilute 1000x for working conc of 10ug/mL
- Streptomycin 50mg/mL stock → dilute 1000x for working conc of 10ug/mL
-

Remake ampicillin stock

Ampicillin

Stocks & Usage

- Stock Concentration - 50mg/ml in H_2O
- Aliquots - 100µl and 500µl
- Working Concentration - ~~50µg/ml~~100ug/mL

Preparation of stock solution

- Ampicillin is kept in the 4C fridge in Barnum 200. It is light sensitive.
- Weigh 500mg of ampicillin in a small weigh boat
- Add 10ml of ultrapure water to a 15mL falcon tube.
- Add the ampicillin to the falcon tube
- Mix/vortex so all the ampicillin goes into solution.
- Filter sterilize the solution into 1.5mL aliquots inside 2.0mL centrifuge tubes.
- Store at -20C.

Grow liquid cultures of strains

NUMBER	NAME	RESISTANCE	Antibitic conc.	Culture
ELS-16	Litmus28i_I716104	Ampicillin	100ug/mL	5mL LB + 10uL 50mg/mL amp stock
ELS-28	Litmus28i	Ampicillin	100ug/mL	5mL LB + 10uL 50mg/mL amp stock
ELS-30	Rp437	Streptomycin	50ug/mL	5mL LB + 5uL 50mg/mL strep stock
ELS-34	Rp437, F+	Streptomycin, Tetracycline	50ug/mL, 10ug/mL	5mL LB + 5uL 50mg/mL strep stock 5mL LB + 5uL 10mg/mL tetr stock
ELS-43	HpdO	Kanamycin	50ug/mL	5mL LB + 25uL 10mg/mL kan stock
ZK1056		None		5mL LB

- Grow in incubator with shaking at 37C
- Started at 8pm

Autoclave

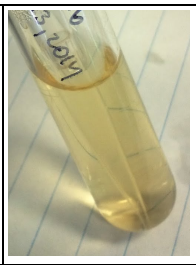
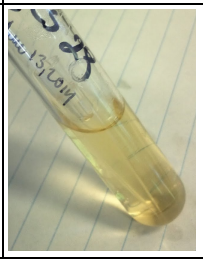
- 4x 250mL LB agar plate mix in 1000mL flasks. Remove and store at RT on bench.
- 2x 250mL LB agar plate mix in 500mL media bottles. Remove and store at RT on bench.
- 1x 175mL LB agar plate mix in 500mL media bottles. Remove and store at RT on bench.

JUNE 14, 2014

Cultures results from June 13, 2014

NUMBER	NAME	RESISTANCE	Final antibitic conc.	Culture	Growing?
ELS-16	Litmus28i_I716104	Ampicillin	100ug/mL	5mL LB + 10uL 50mg/mL amp stock	Left in incubator for longer
ELS-28	Litmus28i	Ampicillin	100ug/mL	5mL LB + 10uL 50mg/mL amp stock	Left in incubator for longer
ELS-30	Rp437	Streptomycin	50ug/mL	5mL LB + 5uL 50mg/mL strep stock	Yes
ELS-34	Rp437, F+	Streptomycin, Tetracycline	50ug/mL, 10ug/mL	5mL LB + 5uL 50mg/mL strep stock 5mL LB + 5uL 10mg/mL tetr stock	Left in incubator for longer
ELS-43	HpdO	Kanamycin	50ug/mL	5mL LB + 25uL 10mg/mL kan stock	Yes
ZK1056		None		5mL LB	Yes

- Cultures which grew are highlighted in green. Glycerol stocks were setup of these by adding 500ul of culture to 500uL sterile 50% glycerol v/v in DI water (from house line). Sotred in -80C
- Cultures which did not grow (clear LB) are in white. They were left in the incubator

ELS-16		**ELS-34**	
ELS-28		**ELS-43**	
ELS-30		**ZK1056**	

Cultures cells (Attempt 2)

NUMBER	NAME	RESISTANCE	Antibitic conc.	Culture
ELS-16	Litmus28i_I716104	Ampicillin	100ug/mL	5mL LB + 10uL 50mg/mL amp stock
ELS-28	Litmus28i	Ampicillin	100ug/mL	5mL LB + 10uL 50mg/mL amp stock
ELS-30	Rp437	Streptomycin	50ug/mL	5mL LB + 5uL 50mg/mL strep stock
ELS-34	Rp437, F+	Streptomycin, Tetracycline	50ug/mL, 10ug/mL	5mL LB + 5uL 50mg/mL strep stock 5mL LB + 5uL 10mg/mL tetr stock
ELS-43	HpdO	Kanamycin	50ug/mL	5mL LB + 25uL 10mg/mL kan stock
ZK1056		None		5mL LB

-
- Attempt 2 at culturing these cells. 5mL LB stocks with requisite antibiotics were remade as per table
- Michaela, peter, Mike, and Connor restarted cultures and placed them in incubator at 37C overnight starting 5pm on 6/14/14
- To be retrieved by Mike Zalesne and stored at 4C by noon June 15, 2014

Pour LB Agar plates

- 4x 250mL agar plate mix in 1L erlenmeyer flasks were microwaved and then kept liquid at 55C+ via water bath

- **Specifications for each plate**

	Volume agar	Stock used	Final concentration
Ampicillin	250mL LB agar	500uL 50mg/mL stock	100ug/mL
Kanamycin	250mL LB agar	1250uL 10mg/mL stock	50ug/mL
Streptomycin	250mL LB agar	250uL 50mg/mL stock	50ug/mL
Streptomycin, Tetracycline	250mL LB agar	250uL 10mg/mL stock, 250uL 50mg/mL stoc	50ug/mL, 10ug/mL

- Plates were stored in 4C fridge

Autoclave solid waste from biohazard bin

- Biological waste was autoclaved using left autoclave and disposed of in trash

JUNE 15, 2014

Cultures results from June 14, 2014

NUMBER	NAME	STRAIN	RESISTANCE	PLASMID	Culture	Growing?
ELS-16	Litmus28i_I716104	XL1-Blue	Ampicillin	ColE1, M13	5mL LB + 10uL 50mg/mL amp stock	Yes
ELS-28	Litmus28i	XL1-Blue	Ampicillin	ColE1	5mL LB + 10uL 50mg/mL amp stock	Yes
ELS-30	Rp437	MG1655	Streptomycin		5mL LB + 5uL 50mg/mL strep stock	Yes
ELS-34	Rp437, F+	MG1655	Streptomycin, Tetracycline		5mL LB + 5uL 50mg/mL strep stock 5mL LB + 5uL 10mg/mL tetr stock	Yes
ELS-43	HpdO	RP437, F+	Kanamycin	p15A	5mL LB + 25uL 10mg/mL kan stock	Yes
ZK1056			None		5mL LB	Yes

- Collected cultures from 37C incubator shaker at 10am.
- All cultures registered growth!

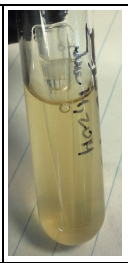
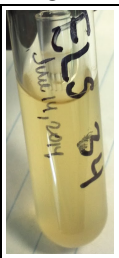
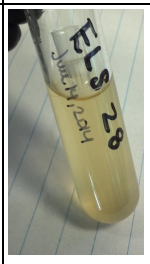
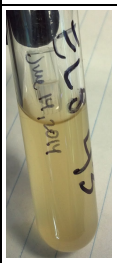
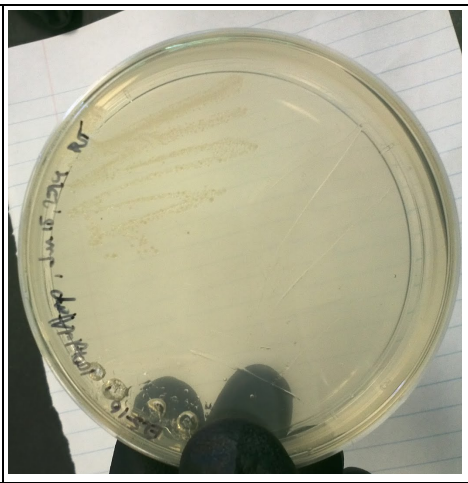
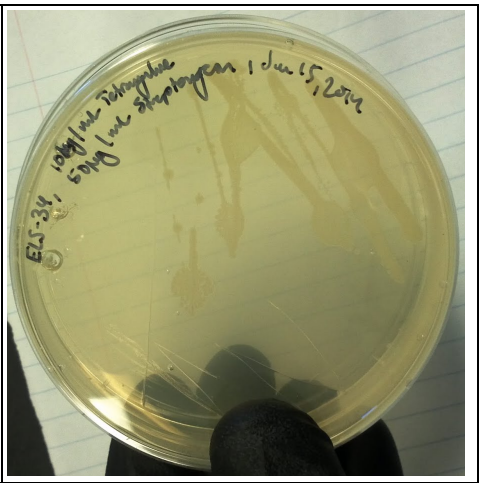
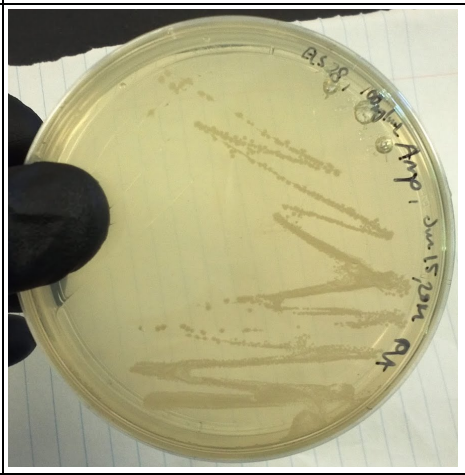
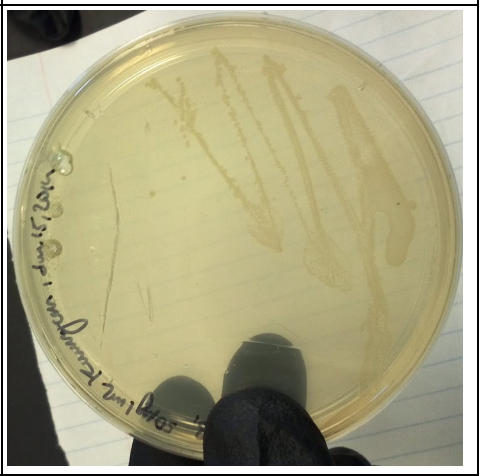
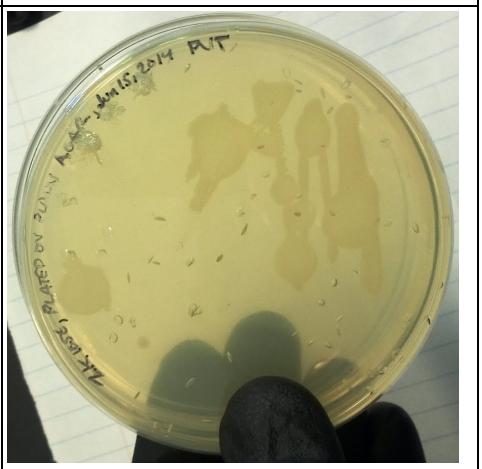
ELS-16		**ELS-34**	
ELS-28		**ELS-43**	
ELS-30		**ZK1056**	

Plate cultures from June 14, 2015 on agar plates with appropriate selection marker

JUNE 16, 2014

Agar plate results from June 14, 2015

ELS-16		ELS-34	
ELS-28		ELS-43	
ELS-30		ZK1056	

JUNE 20, 2014

Cultures cells (From plates of June 16)

NUMBER	NAME	RESISTANCE	Antibitic conc.	Culture
ELS-16	Litmus28i_I716104	Ampicillin	100ug/mL	5mL LB + 10uL 50mg/mL amp stock
ELS-28	Litmus28i	Ampicillin	100ug/mL	5mL LB + 10uL 50mg/mL amp stock
ELS-30	Rp437	Streptomycin	50ug/mL	5mL LB + 5uL 50mg/mL strep stock
ELS-34	Rp437, F+	Streptomycin, Tetracycline	50ug/mL, 10ug/mL	5mL LB + 5uL 50mg/mL strep stock 5mL LB + 5uL 10mg/mL tetr stock
ELS-43	HpdO	Kanamycin	50ug/mL	5mL LB + 25uL 10mg/mL kan stock
ZK1056		None		5mL LB

- **cells were place in incubator at midnight June 20**
- **will retrieve by 2pm on June 21st**
- **cells will be used to make glycerol stocks**
- **cells will be used as starter culture for transformable cells**

JUNE 21, 2014

Glycerol stocks made, liquid cultures started

Glycerol stocks:

500 uL of 50% glycerol and 500 uL liquid cell cultures of all six strains were added to tubes and placed in the

-80C freezer.

Liquid cultures:

250 mL flasks were filled with 100 mL LB broth and appropriate volume of antibiotics (the volume for a 5 mL culture 20X). 1 mL of each starter culture was added to the appropriate flask. Cultures were left overnight in the 37C shaking incubator.

JUNE 22, 2014

Liquid cultures moved to fridge

All six liquid cultures were moved from the incubator to the fridge.
OD_{600} of ELS-28: 0.875.

The other cultures were not measured due to time limitations, but appeared to have comparable cell densities.

This OD_{600} suggests late log phase growth. Though not ideal, these cells can likely still be rendered chemically competent.

JUNE 24, 2014

Inoculated LB + ampicillin plates with ELS-16 and ELS-28 cells from glycerol stocks.

Streak plates directly from glycerol stocks of ELS-16 and ELS-28.
Replaced stocks immediately in -80c freezer.
Plates were put in incubator at 6:15 PM.
Incubator was turned on.

JUNE 25, 2014

Plates taken out of the fridge.

Took out the plates and parafilmed them. Took pictures, and into the fridge.

Pictures: Google won't let me upload. Will manually scale down the images and reupload when I get a chance.

https://s3.amazonaws.com/pushbullet-uploads/ujwxi4UdjFs-pFf1Q9FVtD57MqEW3d6KK2YBE4d33yQA/IMG_20140625_140816.jpg

https://s3.amazonaws.com/pushbullet-uploads/ujwxi4UdjFs-zHj80oOHNWARaU2vIAsNsvGULmmMA2Er/IMG_20140625_140758.jpg

https://s3.amazonaws.com/pushbullet-uploads/ujwxi4UdjFs-3XYt9Yrjkyttaqhs1QIkPMEiCFqCXHb8/IMG_20140625_140838.jpg

JUNE 29, 2014

Competent cells made

Strain	Starter culture volume	Added to __ LB	with antibiotics	OD600 after 6 hours
ELS-30	250 uL	25 mL	25 uL Strep	1.190
ELS-34	250 uL	25 mL	25 uL Strep, 25 uL Tet	0.790
ZK1056	250 uL	25 mL	None	1.210

Cells were incubated in the 37C shaker for ~ 6 hours and rendered competent in accordance with protocols(in the Protocols [Antibody] folder). 10 mL of 0.1 M CaCl2 was used for the first resuspension, and 5 mL 85% 0.1 M CaCl2/ 15% (50% glycerol) for the second).

300 uL of cells were aliquoted into 15 1.5 mL tubes and frozen in the -80C freezer.
Abbreviations on the tubes are:
ELS-30 = **30**
ELS-34 = **34**
ZK1056 = **ZK**

Biofilm assay started

Mixtures:

	Culture volume	LB volume
ZK1056 (biofilm-forming)	6 uL	594 uL
RP437 (control)	6 uL	594 uL

100 uL of each mixture was aliquoted into six wells on a flat-bottom 96 well plate.
The plate was covered in foil and left to incubate at 37C for ~ 24 hours.

JUNE 30, 2014

Biofilm assay completed

To complete the assay, the following solutions were made:

0.1% crystal violet
- -667 uL crystal violet solution (3g in 1L solution)
- -1333 uL water

30% acetic acid
- -450 uL glacial acetic acid
- -1050 uL water

96-well plate removed after ~24 hours at 37C.
Planktonic bacteria in the wells were washed by submersion in water.
125 uL of 0.1% crystal violet was added to each well and left to stain for 10 minutes.
Excess crystal violet was removed with several rinsing steps and allowed to air dry.
200 uL of 30% acetic acid was added to each well.
125 uL of solution from each well was pipetted into a clean 96-well clear flat-bottom plate.
The plate was read by the plate reader in Barnum 116.

A550 readings

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

JULY 9, 2014

Biofilm assay started

Strain	Culture volume	LB volume
ZK1056 (biofilm-forming)	8 uL	792 uL
RP437 (ELS-30, control)	8 uL	792 uL
RP437 F+ (ELS-34, control)	8 uL	792 uL

100 uL of each 1:100 cell culture was added to eight wells in a curved-bottom 96-well culture plate.

The cells began static incubation at 37C at ~8PM.

July 11, 2014

Biofilm assay results.

TC	TC	TC	TC	TC	TC	TC	NB	NB	NB	TS	TS
48h	48h	48h	24	24	24	N/A	22h	22h	N/A	N/A	N/A
ZK1056	**ELS-30**	**ELS-34**	**ZK1056**	**ELS-30**	**ELS-34**	**polystyr**	**ZK1056**	**ELS-30**	**polystyr**	**empty**	**empty**
0.551	0.058	0.073	0.085	0.058	0.074	0.068	0.217	0.082	0.06	0.038	0.04
0.171	0.061	0.057	0.062	0.056	0.056	0.065	0.135	0.115	0.072	0.038	0.042
0.118	0.057	0.055	0.064	0.057	0.061	0.067	0.155	0.097	0.053	0.04	0.038
0.138	0.077	0.056	0.066	0.068	0.055	0.071	0.123	0.098	0.06	0.039	0.038
0.15	0.058	0.072	0.063	0.058	0.057	0.066	0.166	0.112	0.059	0.038	0.038
0.133	0.057	0.06	0.064	0.06	0.054	0.076	0.144	0.091	0.061	0.038	0.038
0.172	0.056	0.055	0.067	0.063	0.056	0.063	0.199	0.098	0.084	0.038	0.038
0.578	0.059	0.068	0.083	0.058	0.06	0.065	1.83	0.077	0.068	0.038	0.042

TC = Tissue culture treated plate

NB = Non-binding surface plate

TS

Miniprep of ELS-16 (Litmus28i_I716104)

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1		7/11/2014 7:25:40 PM	36.4	ng/µl	0.728	0.442	1.65	0.77	DNA	50
2		7/11/2014 7:27:59 PM	16.5	ng/µl	0.329	0.189	1.75	1.49	DNA	50
3		7/11/2014 7:29:21 PM	18.6	ng/µl	0.372	0.215	1.73	1.23	DNA	50

July 28, 2014

Transform genscript pUC57 vector into JM109

Grow cultures overnight

pour lb amp plates

pcr the merRNA, full fragment with promoters and terminators
pcr purify
nanodrop

#	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

July 30, 2014

Start culture of ELS-16 (Litmus28i_I716104) from glycerol stock on ampicillin lb plate

August 1, 2014

liquid culture from glycerol stock of ELS16 was miniprep'd
eluted in 25uL + 20uL of ultrapure h2o

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	LITMUS28i+T7RNAP	Grad, Biology	8/1/2014 3:21:16 PM	39.5	ng/µl	0.791	0.424	1.86	1.57	DNA	50

Started new liquid cultures (1-4) from plate that connor made from glycerold stock
started new plate from liquid culture that

August 2, 2014

A

August 3, 2014

A

August 4, 2014

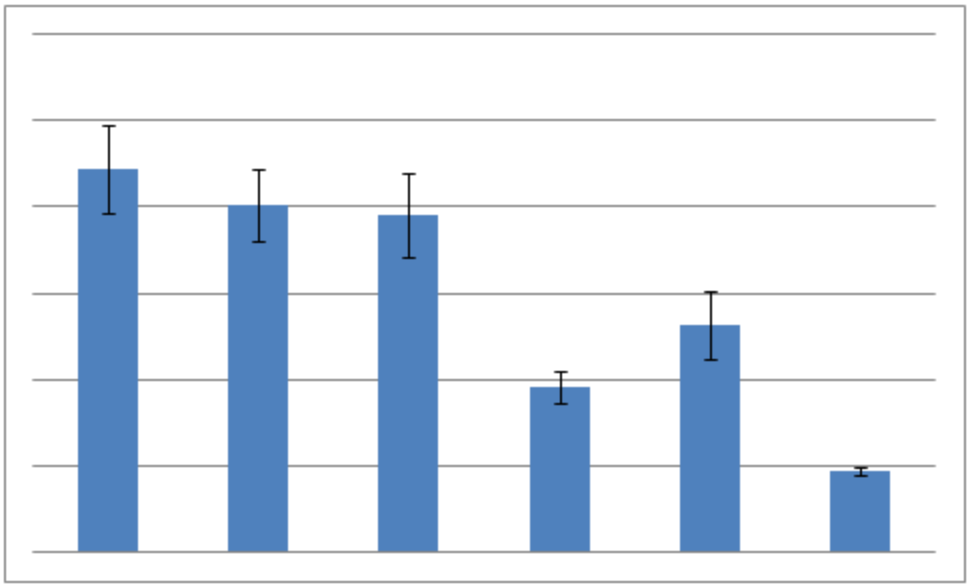
#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	M1	Barnum Lab	8/4/2014 1:01:43 PM	35.7	ng/µl	0.714	0.38	1.88	1.85	DNA	50
2	M2	Barnum Lab	8/4/2014 1:02:28 PM	39.6	ng/µl	0.792	0.445	1.78	1.25	DNA	50
3	M3	Barnum Lab	8/4/2014 1:03:09 PM	35.9	ng/µl	0.719	0.389	1.85	1.61	DNA	50
4	M4	Barnum Lab	8/4/2014 1:03:41 PM	56	ng/µl	1.119	0.656	1.7	1.05	DNA	50
5	M4	Barnum Lab	8/4/2014 1:05:15 PM	36.1	ng/µl	0.722	0.391	1.85	1.92	DNA	50
6	M5	Barnum Lab	8/4/2014 1:06:04 PM	40.8	ng/µl	0.815	0.461	1.77	1.26	DNA	50
7	M6	Barnum Lab	8/4/2014 1:06:40 PM	51.9	ng/µl	1.038	0.585	1.77	1.29	DNA	50
8	M6	Barnum Lab	8/4/2014 1:07:44 PM	43.5	ng/µl	0.87	0.474	1.84	1.75	DNA	50
9	I1	Barnum Lab	8/4/2014	25	ng/µl	0.501	0.274	1.83	1.15	DNA	50

			1:08:32 PM								
10	I2	Barnum Lab	8/4/2014 1:09:27 PM	24.5	ng/µl	0.491	0.275	1.79	1.03	DNA	50
11	I3	Barnum Lab	8/4/2014 1:10:20 PM	29.4	ng/µl	0.589	0.349	1.69	0.88	DNA	50
12	I4	Barnum Lab	8/4/2014 1:11:24 PM	24.3	ng/µl	0.486	0.279	1.74	0.9	DNA	50
13	I5	Barnum Lab	8/4/2014 1:12:30 PM	35	ng/µl	0.701	0.421	1.66	0.86	DNA	50
14	I6	Barnum Lab	8/4/2014 1:13:36 PM	22.3	ng/µl	0.446	0.25	1.78	1.07	DNA	50

August 13, 2014

Biofilm assay plate was processed according to O'Toole protocol
75uL of each well was taken and spectra obtained at 600nm to find how many bacteria are in suspension
Plate was left to dry overnight

1	2	3	4	5	0	1	2	3	4	5	0
0.263	0.199	0.215	0.08	0.099	0.045	0.201	0.186	0.213	0.095	0.099	0.049
0.245	0.174	0.229	0.078	0.116	0.042	0.23	0.221	0.185	0.109	0.119	0.048
0.243	0.2	0.212	0.099	0.132	0.045	0.219	0.214	0.221	0.086	0.127	0.045
0.267	0.208	0.178	0.094	0.158	0.047	0.226	0.218	0.193	0.096	0.125	0.05
0.252	0.204	0.231	0.093	0.139	0.047	0.205	0.212	0.179	0.086	0.132	0.05
0.221	0.204	0.206	0.105	0.142	0.045	0.206	0.226	0.194	0.095	0.145	0.044
0.199	0.178	0.167	0.102	0.152	0.047	0.191	0.147	0.15	0.105	0.117	0.047
0.189	0.214	0.177	0.107	0.171	0.213	0.195	0.215	0.169	0.1	0.13	0.048



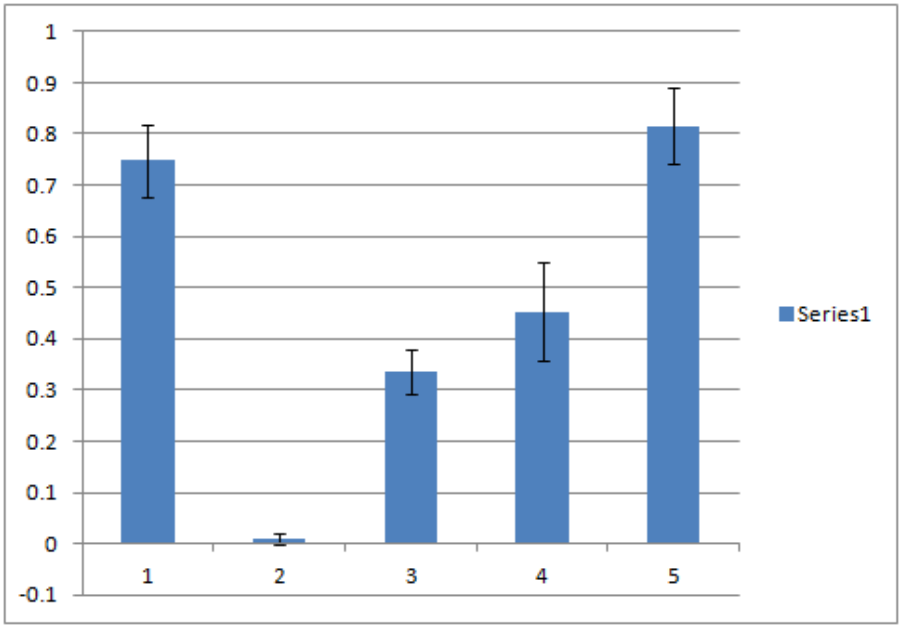
August 14, 2014

Biofilm assay completed. Dissolved in 330 uL 80% EtOH/20% acetone. 125 ul transferred to new plate. A550 taken.

Raw data:

1	2	3	4	5	0	1	2	3	4	5	0
0.988	0.068	0.396	0.638	0.911	0.054	0.903	0.075	0.493	0.682	0.969	0.106
0.86	0.082	0.296	0.677	0.759	0.051	0.757	0.073	0.43	0.579	0.961	0.059
0.863	0.087	0.397	0.48	0.904	0.057	0.853	0.085	0.389	0.552	0.97	0.069
0.808	0.065	0.399	0.536	0.944	0.058	0.804	0.061	0.411	0.533	0.89	0.053
0.759	0.064	0.448	0.428	0.85	0.057	0.81	0.063	0.389	0.443	0.913	0.054
0.779	0.064	0.393	0.455	0.899	0.057	0.745	0.065	0.401	0.409	0.836	0.055
0.726	0.053	0.36	0.508	0.831	0.048	0.741	0.059	0.342	0.373	0.809	0.056
0.77	0.057	0.39	0.471	0.804	0.048	0.752	0.054	0.387	0.419	0.737	0.054

Average A550 of all 16 wells for each sample minus the average blank A550.



Glycerol stocks made. Litmus 28i +T7 RNAP transformed into JM109. Second biofilm assay completed from 1:50 dilutions. Incubated at 37C.