Week of 10/2

Project: Lab Notebook
Authors: Catherine Dunaway
Dates: 2017-10-02 to 2017-10-08

MONDAY, 10/2/17

People present Albert
Lab Silberg, Keck 201
Goal Plate Shewanella and E. coli cultures for fluorescence standard curve

E. coli, Shewanella fluorescence standard curve

1. Plated 100 uL of 5000x dilution of E. coli and Shewanella stock
    a. E. coli went on Kan25 plate; Shewanella went on plain LB plate
2. E. coli plate in 37C incubator and Shewanella in 30C incubator at 5:35 pm
3. Take out @ 9:00 am tomorrow.

TUESDAY, 10/3/17

People present Anna
Lab Silberg, Keck 201
Goal Repeat failed GOlden Gate Assemblies

According to sequencing results, we need to repeat cassette assemblies for:

1. ChrB
2. LacI
3. cys + PgntK
4. Pcon + mCherry (for comparing with PgntK)
5. nemA
6. ChrR6 cassettes 1 and 2 for BioBricks

LacI, ChrB, cys

Performed Gooden Gate Assemblies following protocol 1

Repeated Golden Gate Assemblies

	A	B	C	D	E	F
1	**LacI**		**ChrB**		**cys + PgntK**	
2	Reagent	Volume (µL)	Reagent	Volume (uL)	Reagent	Volume (µL)
3	p438	0.56	p438	0.4	p438 (10x dil)	0.56
4	p523	0.2	Con L4 (505)	0.2	501 (LS)	0.25
5	p459	0.2	ChrB	0.2	PgntK	0.29
6	LacI	0.5	Con RE (520)	0.2	cysPUWA	2.76
7	p463	0.21			530	0.22
8	p514	0.2				
9						
10	BsaI	0.5	BsaI	0.5	BsaI	0.5
11	T4 DNA Ligase	0.5	T4 DNA Ligase	0.5	T4 DNA Ligase	0.5
12	T4 Buffer	1.5	T4 Buffer	1.5	T4 Buffer	1.5
13	10X BSA	1.5	10X BSA	1.5	10X BSA	1.5
14	H20	8.92	H20	9.79	H20	6.71

E. coli, Shewanella fluorescence standard curve

1. Took out E. coli, Shewanella plates @ 9:15 am

WEDNESDAY, 10/4/17

People present Albert
Lab Silberg, Keck 201
Goal Run overnight TECAN measurement for fluorescence standard curve

E. coli, Shewanella fluorescence curve

1. Set up 96-well plate:
    a. 12 wells ea. for E. coli and Shewanella
    b. 200 uL m9 media for each well
    c. picked one colony into each well
2. Put plate in TECAN to read
    a. 24 hrs, 30C, shaking
    b. measure OD, GFP + RFP fluorescence

THURSDAY, 10/5/17

1. obtained data from TECAN
2. No wells had bacteria growing
    a. possible problems: didn't pick colonies properly
    b. m9 wasn't prepared correctly

FRIDAY, 10/6/17

People present Anna
Lab Silberg, Keck 201
Goal Repeat failed Golden Gate Assemblies

Golden Gate Assembly

Perform Golden Gate Assemblies following protocol 1

Repeated Golden Gate Assemblies 2

	A	B	C	D	E	F	G	H
1	**Pcon**		**nemA**		**ChrR6 c1**		**ChrR6 c2**	
2	Reagent	Volume (uL)	Reagent	Volume (uL)	Reagent	Volume (uL)	Reagent	Volume (uL)
3	438	0.56	438	0.56	438	0.56	438	0.56
4	501 (conLS)	0.25	p502 (ConL1)	0.2	501 (conLS)	0.25	522 (OpconL2)	0.24
5	Pcon(476)	0.2	Pcon(476)	0.2	476 (Pcon)	0.2	457 (RBS)	0.26
6	RBS(458)	0.2	nemA (50fmol/ul, need 40 fmol)	1.25	RBS(458)	0.2	453 (yfp)	0.2
7	454 (mCherry)	0.2	p531 (OpconR2)	0.2	ChrR6	0.38	463 (terminator)	0.21
8	terminator (463)	0.21	RBS(458)	0.2	531 (OpconL2)	0.2	520 (conRE)	0.2
9	conR1 (511)	0.2						
10								
11	BsaI	0.5	BsaI	0.5	BsaI	0.5	BsaI	0.5
12	T4 DNA Ligase	0.5	T4 DNA Ligase	0.5	T4 DNA Ligase	0.5	T4 DNA Ligase	0.5
13	T4 Buffer	1.5	T4 Buffer	1.5	T4 Buffer	1.5	T4 Buffer	1.5
14	BSA + PEG --- in "E. coli toolkit" box	1.5	BSA + PEG --- in "E. coli toolkit" box	1.5	BSA + PEG --- in "E. coli toolkit" box	1.5	BSA + PEG --- in "E. coli toolkit" box	1.5
15	H20	9.18	H20	8.39	H20	9.77	H20	9.89

Pchr + ChrR6 cassette PCR

Perform PCR to eliminate illegal cut site in ChrR6 cassette following protocol 2

ChrR6 cassette PCR

	A	B
1	**Component**	**Volume (uL)**
2	Phusion GC buffer	10
3	dNTPs	1
4	Fwd Primer	2.5
5	Rev Primer	2.5
6	Phusion DNA polymerase	0.5
7	template DNA	
8	H2O	33.5
9	**TOTAL VOLUME**	50

Table3: Chr6 test pcr thermocycling conditions

	A	B	C	D	E	F	G	H
1	**Step #**	1	2	3	4	5	6	7
2	**Temp**	98C	98C	65 C	72C	GO TO Step 2	72C	4C
3	**Time**	0:30	0:10	0:30	0:24	35X	5:00	hold

Made liquid cultures of nemA, cys, LacI in SOB + Amp100

SATURDAY, 10/7/17

People present Anna
Lab Silberg, Keck 201
Goal Transform the assemblies

Assembly transformations

Transformed assemblies made on 10/6 into HS competent E. Coli EW11 following protocol 5.

Running pcr on a gel

Ran digested ChrR6 on a gel. Band observed around 900 kb. Gel purified the band. Concentration of the purified sample was 19.0 ng/uL

SUNDAY, 10/8/17

Colonies were obseerved for all assemblies except ChrB. Shyam suggested that toxicity might be an issue since we now have a high-copy vector. We will repeat ChrB assembly with both 439 and 440 (medium and low copy)

Week of 10/09

Project: Lab Notebook
Authors: Catherine Dunaway
Dates: 2017-10-09 to 2017-10-15

MONDAY, 10/9/17

People present Anna
Lab Silberg, Keck 201
Goal Miniprep and test digest the cassettes, digest BioBrick backbone

E. coli, Shewanella fluorescence standard curve

1. Picked 4 colonies each of Shewanella and E. coli + cultured in 4 mL of plain m9; made one negative control of m9 with no culture
2. E. coli went in 37C shaking incubator, Shewanella went in 30C shaking incubator; in @ 4:20 pm
3. Will take out at 8:20 pm

Ch134 bacterial transformation plated at 5:00 pm

Cassettes miniprep

Performed miniprep following Qiagen kit protocol

Cassettes Miniprep

	A	B
1	**sample**	**concentration**
2	nemA Pcon	302.9
3	nemA Pchr	74.3
4	ChrR6 C1	204.1
5	cys	136.8
6	LacI	199.2
7	Pcon + mCherry	164.2

ChrR6 cassette and backbone (23O) digestion

Performed digest of ChrR6 cassette and BioBrick backbone for BioBrick assembly

BioBrick Backbone Digestion

	A	B	C
1		**ChrR6 cassette**	**backbone (23O)**
2	**DNA (ul for ~1 ug)**	15.00	2.70
3	**CutSmart 10X Buffer (ul)**	5	5
4	**XbaI-HF (ul)**	2.5	2.5
5	**SpeI-HF (ul)**	2.5	2.5
6	**H2O (ul)**	25.00	37.30
7	**Total**	50	50

Cassettes test digest

Performed test digest following NEB protocol (optimizing restriction endonucleases)

Cassettes Test Digest

	A	B	C	D	E	F	G
1	**sample**	**concentration (ng/uL)**	**uL of DNA for 0.1ug (1000ng)**	**Enzyme (uL)**	**3.1 NEB CutSMart Buffer (ul)**	**dH2O (uL)**	**Total Rxn Volume (uL)**
2	nemA Pcon	302.9	3.30	1	5	40.70	50
3	nemA Pchr	74.3	13.46	1	5	30.54	50
4	ChrR6 C1	204.1	4.90	1	5	39.10	50
5	cys	136.8	7.31	1	5	36.69	50
6	LacI	199.2	5.02	1	5	38.98	50
7	Pcon + mCherry	164.2	6.09	1	5	37.91	50

Test digest was successful for all cassettes

TUESDAY, 10/10/17

People present Anna, Catherine
Lab Silberg, Keck 201
Goal Prepare samplles for sequencing, miniprep ChrB, gel purify digested backbone, ligate ChrR6 cassette BioBrick.

Samples for sequencing

Samples for Sequencing

	A	B	C	D	E	F	G	H
1	**Sample**	**ng/ul**	**volume of sample for 500 ng (ul)**	**volume of primers (ul)**	**volume of water (ul)**	**primer ID**	**name on order**	**# on order**
2	nemA Pcon fwd	302.9	1.7	2.5	10.8	C54	nAconF	1
3	nemA Pcon rev	302.9	1.7	2.5	10.8	C55	nAconR	2
4	nemA Pchr fwd	74.3	6.7	2.5	5.8	C54	nAchrF	3
5	nemA Pchr rev	74.3	6.7	2.5	5.8	C55	nAchrR	4
6	ChrR6 C1 fwd	204.1	2.4	2.5	10.1	C54	chrR61F	5
7	ChrR6 C1 rev	204.1	2.4	2.5	10.1	C55	chrR61R	6
8	cys fwd	136.8	3.7	2.5	8.8	C54	cysF	7
9	LacI fwd	199.2	2.5	2.5	10.0	C54	lacIF	8
10	LacI rev	199.2	2.5	2.5	10.0	C55	lacIR	9
11	Pcon + mCherry fwd	164.2	3.0	2.5	9.5	C54	PconF	10
12	Pcon + mCherry rev	164.2	3.0	2.5	9.5	C55	PconR	11
13	cys rev	136.8	3.7	2.5	8.8	C55	cysR	12
14	chrB fwd	88.9	5.6	2.5	6.9	C54	chrBF	13
15	chrB rev	88.9	5.6	2.5	6.9	C55	chrBR	14

ChrRB Miniprep

miniprepped the sample follwing Quiagen kit protocol

ChrB miniprep

	A	B
1	**sample**	**ng/ul**
2	chrB	88.9

BioBrick backbone (230) gel purification
Gel purified the backbone following Zymo kit protocol

ChrR6 cassette BioBrick Ligation

Ligate insert and backbone into biobrick

ChrR6 cassette BioBrick Ligation

	A	B
1	insert	5 uL
2	backbone	4 uL
3	T4 buffer	2 uL
4	T4 Ligase	1 uL
5	water	8 uL

Ligate at 16C/30 min
Heat kill 80C/20 min

E. coli, Shewanella fluorescence standard curve

1. Took out E. coli cultures at 10:00 am. Left Shewanella in incubator to grow longer—media in culture tubes was yellowish, but still clear.

THURSDAY, 10/12/17

Golden Gate Assembly with end-ligation for Chromium Circuit

Performed Golden Gate assembly following protocol 1

Chromium Circuit Golden Gate Assem…

	A	B	C	D
1	**Chromium circuit**		**Chromium circuit**	
2	**Reagent**	**Volume (µL)**	**Reagent**	**Volume (µL)**
3	561	0.31	551	0.33
4	539	0.21	539	0.21
5	KS cassette	0.72	KS cassette	0.72
6	chrR6 cassette	0.32	chrR6 cassette	0.32
7	yfp cassette	0.52	yfp cassette	0.52
8	LacI cassette	0.42	LacI cassette	0.42
9	ChrB cassette	0.93	ChrB cassette	0.93
10	543	0.38	543	0.38
11				
12	BsmbI	0.5	BsmbI	0.5
13	T4 DNA Ligase	0.5	T4 DNA Ligase	0.5
14	T4 Buffer	1.5	T4 Buffer	1.5
15	10X BSA	1.5	10X BSA	1.5
16	H20	6.98	H20	6.96

Thermocycling conditions for end-ligation:

End-On-Ligation Golden Gate Assembly

	A	B	C	D
1		Step	Temp	Time
2		Initial Digestion (opt.)	37°C	10 min
3	Repeat25×	Digestion	37°C	1.5 min
4		Annealing & Ligation	16°C	3 min
5		Storage	16°C	∞

cys + sbp + PgntK multigene golden gate assem…

	A	B
1	**Chromium circuit**	
2	**Reagent**	**Volume (µL)**
3	552	0.56
4	540	0.33
5	PgntK + cys	1.14
6	sbp cassette	0.2
7	546	0.31
8		
9		
10	BsmbI	0.5
11	T4 DNA Ligase	0.5
12	T4 Buffer	1.5
13	10X BSA	1.5
14	H20	8.25

FRIDAY, 10/13/17

Cys + PgntK + sbp Golden Gate Assembly with 559 (Chl resistant)

Cys + PgntK + sbp assembly 559

	A	B
1	**Chromium circuit**	
2	Reagent	Volume (µL)
3	559	0.69
4	540	0.33
5	PgntK + cys	1.14
6	sbp cassette	0.2
7	546	0.31
8		
9	BsmbI	0.5
10	T4 DNA Ligase	0.5
11	T4 Buffer	1.5
12	10X BSA	1.5
13	H20	8.12

SATURDAY, 10/14/17

Goals: Clean and concentrate cys multigene assemblies (552 and 559 vectors). Transform cys multigenes.

Procedure

- Used the Zymo Clean and Concentrator Kit to clean the Golden Gate assembly products of the 552 and 559 cys multigene assemblies

SUNDAY, 10/15/17

Goals: make liquid cultures of cys multigenes (552 and 559 vectors) and 551 vector in the morning; miniprep in the evening. Test digest cys multigenes.

Make Liquid Cultures

- Made 4 ml liquid cultures of cys multigenes (552 and 559 vectors) and 551 vector by picking single colonies and putting in SOB media with appropriate antibiotic
- Put liquid cultures in 37C shaking incubator at 8am

Miniprep Liquid Cultures of Cys Multigenes

- Retrieved liquid culutures at 8:30pm
    - both cys cultures were turbid, the 551 vector culture was not
- Miniprepped the cys cultures according to Protocol 9
- Prepared another liquid culture of the 551 vector by picking a new colony
    - will check this liquid culture tomorrow

Test Digestion of cys multigenes

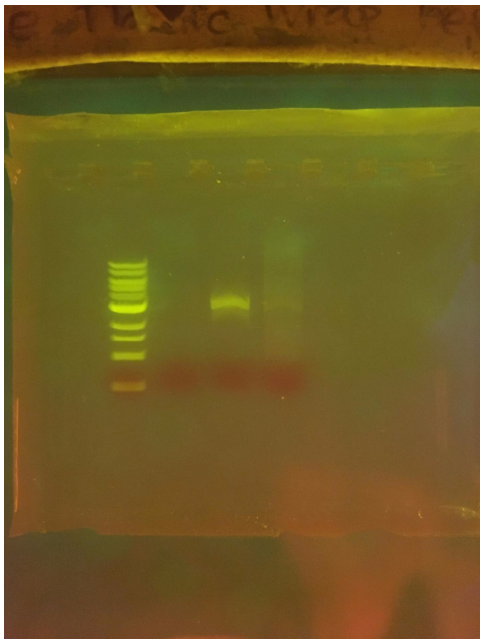
- Set up (3) test digest reactions according to the table below
    - digested both cys multigenes with BsaI + Cutsmart buffer (incubate at 37C for 1 hour)
    - digested the 559 vector (control) with BsmbI + 3.1 buffer (incubate at 55C for 15 min)
- Prepared an 0.8% agarose gel according to Protocol 7

- Added 6X loading dye to a final concentration of 1X and loaded samples and a 1kb ladder into gel
- Performed gel electrophoresis according to Protocol 7
    - Results and virtual digest shown in images below

Test Digestion Reaction Set Up

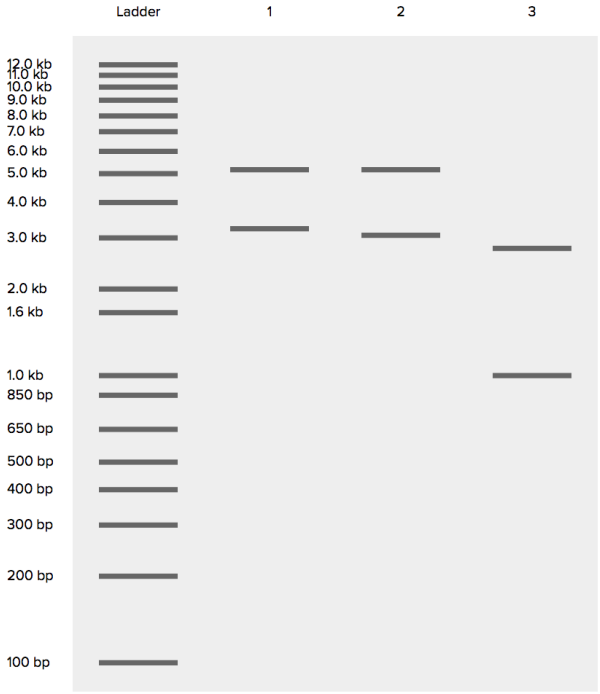
	A	B	C	D	E	F	G
1	**sample**	**ng/ul**	**uL of DNA for 0.1ug (1000ng)**	**Enzyme (uL)**	**Buffer (ul)**	**dH2O (uL)**	**Total Rxn Volume (uL)**
2	cys multigene (552 vector)	51.7	19.3	1.0	5.0	24.7	50.0
3	cys multigene (559 vector)	126.6	7.9	1.0	5.0	36.1	50.0
4	559 vector (control)	135	7.4	1.0	5	36.6	50.0

cys multigene digest result (1 kb ladder, cys 552, cys 559, 559 vector control)



Virtual Digest of cys multigenes

Ladder	Life 1 kb Plus
1	Pgntk-cysPUWA-sbp multigene (552 vector) - BsaI
2	PgntK-cysPUWA-sbp multigene (559 vector) - BsaI
3	pSPB559 ChlR pSC101 - BsmBI



Week of 10/16

Project: Lab Notebook
Authors: Catherine Dunaway
Dates: 2017-10-16 to 2017-10-19

MONDAY, 10/16/17

Test Digest 561

Test Digestion Reaction Set Up

	A	B	C	D	E	F	G
1	**sample**	**ng/ul**	**uL of DNA for 0.1ug (1000ng)**	**Enzyme (uL)**	**Buffer (ul)**	**dH2O (uL)**	**Total Rxn Volume (uL)**
2	561 multigene vector (ChrR6) 1	112.7	8.87	1.0	5.0	35.1	50.0
3	561 multigene vector (ChrR6) 2	145.4	6.88	1.0	5.0	37.1	50.0

ChrR6 BioBrick transformation

TUESDAY, 10/17/17

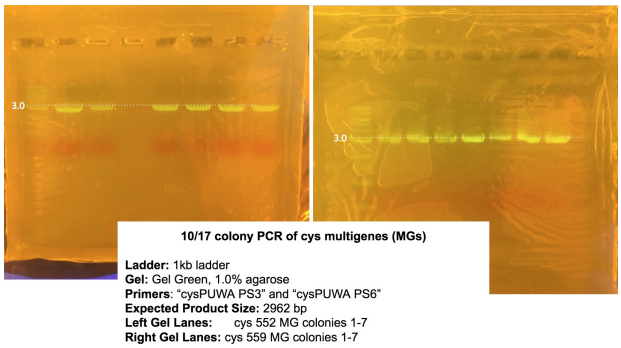
Goals: make Kanamycin 50 ug/ml LB agar plates and perform colony PCR on the cys multigenes remediation circuit multigenes

Procedure:
Made plates according to Protocol 8

Colony PCR:

- Performed colony PCR on 7 white colonies from each of the following plate transformations according to Protocol 3:
  - cys 552 multigene
  - cys 559 multigene
    - Primers used "cysPUWA PS3" and "cysPUWA PS6"; product size=2962bp; annealing temperature 64C; extension time=90 seconds

cys multigene colony pcrs



Double Digest of Pgntk part plasmid

	A	B
1		**pgntK part plasmid (69 ng/uL)**
2	**DNA (ul for ~1 ug)**	14.49
3	**CutSmart 10X Buffer (ul)**	5
4	**EcoRI-HF (ul)**	2.5
5	**PstI-HF (ul)**	2.5
6	**H2O (ul)**	25.51
7	**Total**	50

THURSDAY, 10/19/17

Potassium Chromate 1,5-Diphenylcarbazide Assay (DPC)

- We followed Protocol 10 to measure the chromate reduction of liquid cultures using a DPC assay.
    - Retrieved (12) previously prepared liquid cultures as detailed in table below (6 were transformed with Pcon-chrR6 and 6 were transformed with Pcon-nemA cassettes)
    - We diluted all of the cultures with SOB so that they had the OD of 0.4
        - We also prepared a standard curve by preparing SOB samples with chromate concentrations from 0-140 uM. We also measured the Abs540 of these samples after mixing with the DPC coloring solution (see Protocol 10)
    - We added varying amounts of potassium chromate to each sample to generate the concentrations in column C in the table below
    - We measured the Abs540 at time 0 hours and time 12 hours. We also measured the OD600 at 12 hours. These values can be found in the table below.

DPC assay, 0-100 uM chromate

	A	B	C	D	E	F
1	**Sample**	**OD600 @ time 0**	**K2CrO4 (uM)**	**Absorbance of supernatant at 540 nm w/ DPC @ time 0 (avg of 3 readings)**	**OD600 @ time 12 hours**	**Absorbance of supernatant at 540 nm w/ DPC @ time 12 hours (avg of 3 readings)**
2	chrR6 1	0.4	0	0.0030	5.9290	0.1834
3	chrR6 2		20	0.0024	5.7560	0.1823
4	chrR6 3		40	0.0084	6.0460	0.1789
5	chrR6 4		60	0.0085	6.4270	0.1895
6	chrR6 5		80	0.0154	5.9710	0.1898
7	chrR6 6		100	0.0334	5.5140	0.1961
8	nemA 1	0.4	0	0.0043	5.6900	0.1781
9	nemA 2		20	0.0028	5.0750	0.1802
10	nemA 3		40	0.0061	4.8050	0.1835
11	nemA 4		60	0.0081	4.9710	0.1858
12	nemA 5		80	0.0134	5.1070	0.1794
13	nemA 6		100	0.0144	5.6370	0.1879

Week of 10/23

Project: Lab Notebook
Authors: Catherine Dunaway
Dates: 2017-10-22 to 2017-10-27

SUNDAY, 10/22/17

MONDAY, 10/23/17

E. coli, Shewanella fluorescence standard curve; second try

1. Picked 1 colony each of Shewanella and E. coli + cultured in 5 mL of plain m9
2. E. coli went in 37C shaking incubator, Shewanella went in 30C shaking incubator; in @ 3:30 pm
3. Will take out at 8:30 am

Tuesday Update: nothing grew; possible problems: something wrong with m9, colonies on plate were too old/not picked properly

cys cassette pcr to remove illegal BB cut sites

	A	B	C	D
1	**Component**	**cys 552 (77.5 ng/ul)**	**cys 559 (501.4 ng/ul)**	**MM (2.3 reactions)**
2	H20	33.80	33.80	77.74
3	5X Phusion HF or GC Buffer (uL)	10.00	10.00	23
4	10 mM dNTPs (ul)	1.00	1.00	2.3
5	10 µM forward primer (ul)	2.50	2.50	5.75
6	10 µM reverse primer (ul)	2.50	2.50	5.75
7	template DNA (ul for ~10 ng)	0.20	0.20	---
8	phusion polymerase (ul)	0.50	0.50	1.15

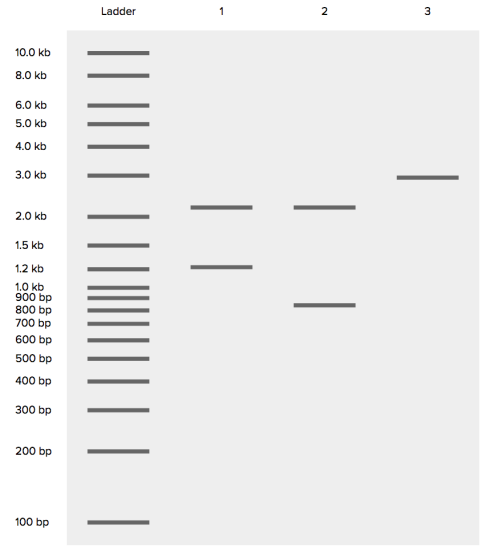
Double Digest of cys CDS for biobrick

	A	B
1		**cys CDS**
2	**DNA (ul for ~1 ug)**	18.52
3	**CutSmart 10X Buffer (ul)**	5
4	**EcoRI-HF (ul)**	2.5
5	**PstI-HF (ul)**	2.5
6	**H2O (ul)**	21.48
7	**Total**	50
8	**[AFTER 1 HOUR AT 37 C]**	
9	BsrDI 2x enzyme	

Virtual NotI digest

Highlighted Fragment –

Ladder NEB 2-Log
1 LacI cassette assembly - NotI
2 Chr6 Cassette Assembly - NotI
3 pSPB439 sfgfp (1-6r1) - NotI



Excised the 4kb band and gel purified it. Nanodropped the gel purified band and got 4.5 ng/ul

TUESDAY, 10/24/17

gel purified the ~5kb band from the cys MG PCR. Digested gel purified PCR product with X & S. Ligated digest with 23O plasmid.

double digest of cys MG PCR produc

	A	B	C
1		**cys MG (2.7 ng/ul)**	**NOTE**
2	**DNA (ul for ~1 ug)**	19.0	<--- concentration was very low, so used full volume. Corresponds to 51.3 ng.
3	**CutSmart 10X Buffer (ul)**	5	
4	**Xbal (ul) (10 units)**	0.5	
5	**SpeI-HF (ul) (ul) (10 units)**	0.5	
6	**H2O (ul)**	25.00	
7	**Total**	50	
8	INCUBATE AT 37C/15 MINUTES, INACTIVATE AT 80C/20 MINUTES		

Ligation of cys cds and cys MG

	A	B	C
1		**cys MG (use E/P digested backbone)**	**cys CDS (use X/S digested backbone "23O"**
2	**DNA**	6.5	6.5
3	**Digested Plasmid Backbone (uL)***	2	2
4	**T4 DNA ligase Buffer**	1	1
5	**T4 DNA ligase**	0.5	0.5
6	**H2O (ul)**	0	0
7	**Total**	10	10
8	LIGATE AT 16C/30 MINUTES, INACTIVATE AT 80C/20 MINUTES		

Pgntk +mcherry assembly

	A	B
1	**Reagent**	**Volume (uL)**
2	438	0.56
3	501 (conLS)	0.25
4	PgntK	0.76
5	RBS(458)	0.2
6	454 (mCherry)	0.2
7	terminator (463)	0.21
8	conR1 (511)	0.2
9	BsaI	0.5
10	T4 DNA Ligase	0.5
11	T4 Buffer	1.5
12	BSA + PEG --- in "E. coli toolkit" box	1.5
13	H2O	8.62

Transformations 10/24

	A	B	C	D
1	**Name**	**Antibiotic Resistance**	**Bacteria**	**Plating Notes**
2	cys CDS biobrick	Chl	MG1655	400 ul, remaining transformation recovery in fridge
3	cys multigene biobrick	Chl		
4	Pgntk + mcherry	Amp		
5	Pcon + mcherry	Amp		
6	cys MG 559	Chl		
7	cys MG 559 + chrR6 (Pcon)	Chl & Amp		
8	cys MG 559 + nemA (Pcon)	Chl & Amp		
9	KS cassette	Amp	DH10B	200 ul on plate1, 800 ul on plate2

put plates in ~1am on 10/25

WEDNESDAY, 10/25/17

Remediation/KS Multigene Test Digests

	A	B	C	D	E	F	G	H
1	**sample**	**ng/ul**	**uL of DNA for 500 ng**	**EcorI**	**PstI**	**Buffer (ul)**	**dH2O (uL)**	**Total Rxn Volume (uL)**
2	MG green chl	259.20	1.93	1.25	1.25	2.50	18.07	25.0
3	MG green Kan	222.90	2.24	1.25	1.25	2.50	17.76	25.0
4	MG yellow Chl	70.20	7.12	1.25	1.25	2.50	12.88	25.0
5	MG yellow Kan	25.70	19.46	1.25	1.25	2.50	0.54	25.0
6	MG white Chl	109.00	4.59	1.25	1.25	2.50	15.41	25.0
7	MG white Chl	64.50	7.75	1.25	1.25	2.50	12.25	25.0
8	MG white KAn	99.20	5.04	1.25	1.25	2.50	14.96	25.0
9	MG white Kan	85.90	5.82	1.25	1.25	2.50	14.18	25.0

Timecourse Test of IPTG Induction of Kill Switch & Pcon vs. Pgntk Timecourse

1. Followed Protocol 11 to test the IPTG induction of our "kill switch" cassette in MG1655 E coli (Ptrc promoter + mcherry construct)
    a. tested 0mM, 0.01, 0.1 and 1.0 mM induction of IPTG over 14 hours in TECAN plate reader
    b. initial OD measurements in Table below; diluted samples to 0.05 in M9 media (clear media)
    c. Prepared standard curve with M9 media
    d. Took fluoresence excitation/emission measurements at 587/610nm and OD measurements at Abs700
2. In the same 96-well plate used to do the IPTG timecourse, we added samples of Pcon+mcherry and Pgntk+mcherry transformed MG1655 as well as un-transformed MG1655 bacteria (control)
    a. initial OD measurements in Table below; diluted samples to 0.05 in M9 media (clear media)
    b. Took fluoresence excitation/emission measurements at 587/610nm and OD measurements at Abs700

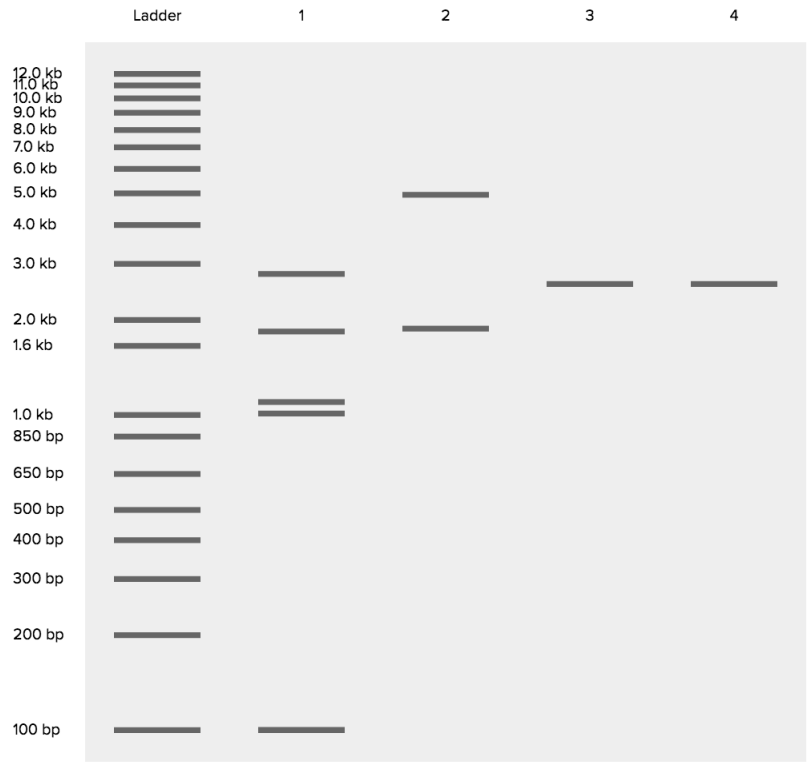
c. 14 hour timecourse

KS induction w/ IPTG; Comparing Pcon and Pgntk		
	A	B
1	sample	initial OD600
2	KS (SOB)	0.236
3	Pcon (SOB)	1.567
4	MG1655 (SOB)	5.663
5	KS (LB)	0.200
6	Pcon(LB)	2.705
7	PgntK (LB)	2.600
8	MG1655 (LB)	2.937

THURSDAY, 10/26/17

Remediation/KS Multigene Virtual Digest

Ladder Life 1 kb Plus
1 reduction/kill switch multigene 561 - EcoRI PstI
2 reduction/kill switch multigene 561 - SpeI XbaI
3 pSPB561 ChlR CloDF13 - EcoRI PstI
4 pSPB561 ChlR CloDF13 - SpeI XbaI



Test Digest Pgntk cassettes w/ Bsmbl

	A	B
1		**ng/ul**
2	Pgntk + mcherry A	181.7
3	Pgntk + mcherry B	348.1

Potassium Chromate 1,5-Diphenylcarbazide Assay (DPC) TAKE 2

- We followed Protocol 10 to measure the chromate reduction of liquid cultures using a DPC assay with concentrations from 0-300 uM chromate
    - Retrieved (10) previously prepared liquid cultures as detailed in table below (All were either in SOB or LB)
    - We diluted all of the cultures with M9 media so that they had an OD of 0.05
        - We also prepared a standard curve by preparing M9 samples with chromate concentrations from 0-300 uM. We also measured the Abs540 of these samples after mixing with the DPC coloring solution (see Protocol 10)
    - We prepared a serial dilution and added the same volume of varying concentrations of chromate to the samples to generate samples ranging from 0-300 uM chromate for each of the culture types
    - We measured the Abs540 at time 0 hours and time 12 hours. We also measured the OD600 at 12 hours.

DPC assay, 0-300 uM chromate

	A	B
1	**Culture**	**Initial OD600**
2	chrR6 (Pcon) A	5.055
3	chrR6 (Pcon) B	0.721
4	nemA (Pcon) A	5.164
5	nemA (Pcon) B	1.541
6	chrR6 + cys A	3.355
7	chrR6 + cys B	2.883
8	nemA + cys A	3.574
9	nemA + cys B	1.822
10	MG1655 A	5.404
11	MG1655 B	2.565

The cultures highlighted in green were cotransformed with a reductase cassette (either Pcon+chrR6 or Pcon+nemA) and the cysPUWA multigene cassette

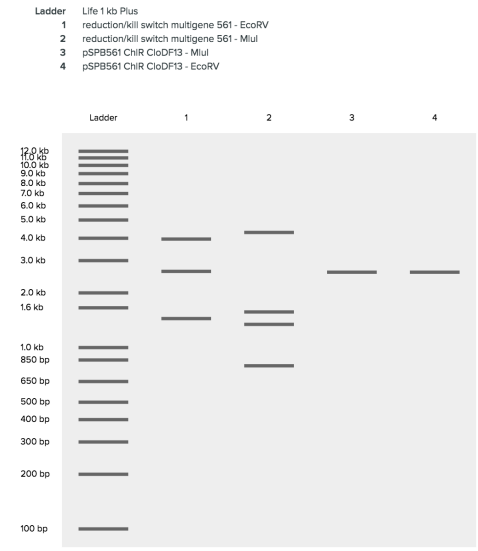
FRIDAY, 10/27/17

Test Digest Rxn set up for remediation/KS MC

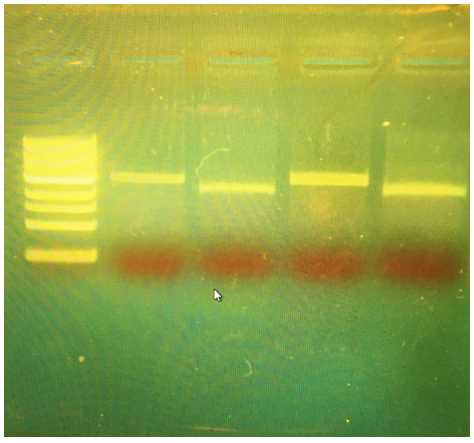
	A	B	C	D	E
1		**MG #9, EcoRV**	**MG #9, MluI-HF**	**MG #10, EcoRV**	**MG #10, MluI-HF**
2	**water**	33.36	33.36	33.36	33.36
3	**enzyme* (20000U/ul)**	1	1	1	1
4	**buffer**	5	5	5	5
5	**DNA**	10.64	10.64	10.64	10.64

37C for 1 hour

Virtual Digest remediation/KS MG



Test Digest Results



lane 1=1kb ladder
lane 2=MG #9, EcoRV
lane3=MG #9 MluI-HF
lane4=MG #10 EcoRV
lane5=MG #10 MluI-HF

Week of 10/30

Project: Lab Notebook
Authors: Anna Guseva
Dates: 2017-10-30 to 2017-10-31

MONDAY, 10/30/17

Goals: Set up the chromate OD assay to test the cys multigene as compared to MG1655 E. coli. Also, transform Pgntk+mcherry today so that tomorrow we can do a promoter fluorescence test to compare Pgntk and Pcon.

Procedure for Chromate OD Assay

1. Perform a serial dilution to prepare potassium chromate in LB samples
    a. Will add 20 ul of each solution to 180 ul of liquid culture to generate final concentrations of 0 uM, 5, 10, 20, 40, 80, 100 and 160 uM. Therefore, we prepared chromate samples with concentrations of 0, 250, 500, 1000, 2000, 4000, 5000 and 8000 uM.
2. Dilute all of the liquid cultures with LB to OD600=0.05 (See Table below)
3. Add 180 uL of each of the cultures to the appropriate well in a 96-well plate according to the layout below
    a. (Add 180 ul of only LB media to the wells in the 11th and 12th columns as a blank)
4. Add 20uL of the appropriate chromate solution to each of the wells according to the plate layout below
5. Take OD600 measurements at times: 0, 3, 6, 9 and 12 hours.

chromate OD assay set up

	A	B	C	D	E
1	**Liquid Culture**	**1:10 Dilution OD600**	**Undiluted OD600**	**desired final OD**	**amount of culture to add to 1.5 mL media to reach desired final OD**
2	MG1655 colony 1	0.3877	3.877	0.05	19.60
3	MG1655 colony 2	0.3502	3.502	0.05	21.73
4	MG1655 colony 3	0.4476	4.476	0.05	16.95
5	cys multigene (MG) colony 1	0.5165	5.165	0.05	14.66
6	cys MG colony 2	0.4402	4.402	0.05	17.23
7	cys MG colony 3	0.5039	5.039	0.05	15.03

96 Well plate layout for chromate OD test

	96 well plate format											
uM CrVI	1	2	3	4	5	6	7	8	9	10	11	12
0	MG1655 colony 1	MG1655 colony 2	MG1655 colony 3	cys multigene colony 1	cys multigene colony 2	cys multigene colony 3	[EMPTY]	[EMPTY]	[EMPTY]	[EMPTY]	LB [blank]	LB [blank]
5												
10												
20												
40												
80												
100												
160												

Pgntk Transformation

Followed Protocol 5 to transform MG1655 bacteria with Pgntk+mcherry construct via heat shock. Plated the recovery on Amp100 plates and put into the 37C incubator. Will check the plates in about 12 hours to pick red-colored colonies.

TUESDAY, 10/31/17

Goals: Set up the promoter fluorescence test.

Procedure for Promoter Fluorescence Test

1. Dilute all of the liquid cultures to OD600=0.05 (See Table below) using the appropriate media (either M9 or LB, see color-code in table below)
2. Add 180 uL of each of the cultures to the appropriate well in a 96-well plate according to the layout below
    a. (Add 180 ul of only media to the wells in the 11th and 12th columns as blanks)
3. Take fluorescence (exictation/emission = 587/610) measurements at times: 0, 3, 6, 9 and 12 hours.

Promoter Fluorescence Assay Set Up, part 2

	A	B	C	D	E
1	**Liquid Culture**	**1:10 Dilution OD600**	**Undiluted OD600**	**desired final OD**	**amount of culture to add to 1 mL media to reach desired final OD**
2	Pcon+mcherry colony 1	0.55	5.5	0.05	9.17
3	Pcon+mcherry colony 2	0.5632	5.632	0.05	8.96
4	Pcon+mcherry colony 3	0.3816	3.816	0.05	13.28
5	Pcon+mcherry colony 4	0.4628	4.628	0.05	10.92
6	Pcon+mcherry colony 5	0.4809	4.809	0.05	10.51
7	Pcon+mcherry colony 6	0.5531	5.531	0.05	9.12
8	Pgntk+mcherry (A) colony 1	0.6179	6.179	0.05	8.16
9	Pgntk+mcherry (A) colony 2	0.564	5.64	0.05	8.94
10	Pgntk+mcherry (A) colony 3	0.6669	6.669	0.05	7.55
11	Pgntk+mcherry (A) colony 4	0.3864	3.864	0.05	13.11
12	Pgntk+mcherry (A) colony 5	0.3444	3.444	0.05	14.73
13	Pgntk+mcherry (A) colony 6	0.4643	4.643	0.05	10.89
14	Pgntk+mcherry (B) colony 1	0.7086	7.086	0.05	7.11
15	Pgntk+mcherry (B) colony 2	0.5416	5.416	0.05	9.32
16	Pgntk+mcherry (B) colony 3	0.6288	6.288	0.05	8.02
17	Pgntk+mcherry (B) colony 4	0.4776	4.776	0.05	10.58
18	Pgntk+mcherry (B) colony 5	0.4081	4.081	0.05	12.40
19	Pgntk+mcherry (B) colony 6	0.3394	3.394	0.05	14.95

cultures made w/ LB are highlighted in yellow; cultures made w/ M9 are highlighted in pink

96 well plate layout for promoter fluorescence reporter test

96 well plate format

	1	2	3	4	5	6	7	8	9	10	11	12
A	Pgntk+mcherry (A) colony 1	Pgntk+mcherry (A) colony 2	Pgntk+mcherry (A) colony 3	Pgntk+mcherry (B) colony 1	Pgntk+mcherry (B) colony 2	Pgntk+mcherry (B) colony 3	Pcon+mcherry colony 1	Pcon+mcherry colony 2	Pcon+mcherry colony 3	[EMPTY]	LB [blank]	M9 [blank]
B												
C												
D												
E	Pgntk+mcherry (A) colony 4	Pgntk+mcherry (A) colony 5	Pgntk+mcherry (A) colony 6	Pgntk+mcherry (B) colony 4	Pgntk+mcherry (B) colony 5	Pgntk+mcherry (B) colony 6	Pcon+mcherry colony 4	Pcon+mcherry colony 5	Pcon+mcherry colony 6			
F												
G												
H												