2.5 mL / 250 mL

6127117

Chlor
.250 g in 10 ml EtOH
.250 g in 250 ml
Strep
.5 g in 10 ml D.W

6128117

Poured LB plates - 16

observed E. coli (DH-5α) plated on 6127117 -

no isolates observed

6/29

Making chloramphenicol stock solution (25mg/ml)

.25g chloramphenicol

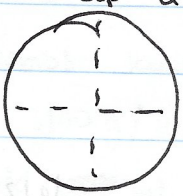
10 mL ethanol

Working concentration is 25 µg/ml

↳ add 1ml stock soln to 1L LB agar → .25 ml to .25L

Streaking e.coli for colony isolates

only pick up a little bit of E. coli

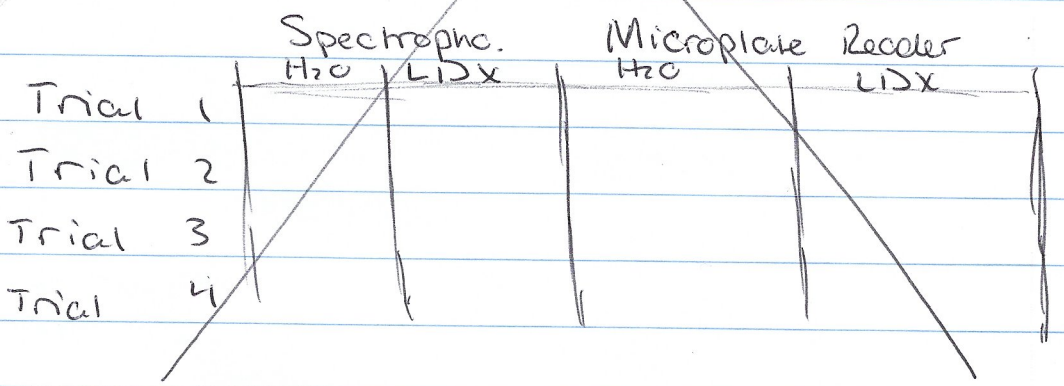


6/30/17

Interlab Measurement Study: Day 1

Calibrating OD_{600} Reference point

- add 100 uL LUDOX into wells A1, B1, C1, D1 of 96 well plate
  - add 1 mL LUDOX into cuvette
- add 100 uL of H_2O into wells A2, B2, C2, D2
  - add 1 mL H_2O into cuvette
- measure the absorbance of all samples at 600 nm



7/1/17

Plated 100 uL of transformed cells onto plates containing chloramphenicol - left plates to incubate in the lab over the weekend - 48 hours

7/3/17

Poured more plates w/ LB and chloramphenicol

Mixed PBS buffer for Interlab Study

8g NaCl

0.2g KCl

1.44g Na_2HPO_4

0.24g KH_2PO_4

adjust pH to 7.4

Trial 1:

Excitation: 360/40

Emission: 530/20

Trial 2:

Excitation: 485/20

Emission: Plug

Trial 3:

Excitation: 460/40

Emission: 590/20

Trial 4:

Excitation: 400/30

Emission: 508/30

7/5/17

Interlab

measurements on the reference spectrophotometer

1mL Ludox into a cuvette

1mL H_2O into another cuvette

Ref Spec.

	H_2O	Ludox
Trial 1	0	0.0211
Trial 2	0	0.0205
Trial 3	0	0.0219
Trial 4	0	0.0234

7/7/17

Cells plated on 7/3 seem to be doing well, put them in the fridge.

Plated the other cells J-P, and + and incubated overnight @ 37°C

7/8/17

Took cells out of incubator, most looked good, except +, which we left on the counter until Monday

IC

6/8/17

making 250 mL of Luria broth

~~9.25 g~~ 6.25 LB

250 mL of DI water

Dilution of 100mM Calcium Chloride to 30mM:

$M_1V_1 = M_2V_2$

$(100mM)V_1 = 10mL\,(30mM)$

$V_1 = \frac{300\,mL \cdot mM}{100\,mM} = 3mL$

6/11/17

Competent cell making

→ cells only @ .21 OD_{600}; not enough, need to be @ least .5

→ put cells in incubator

Interlab

→ inoculated cells into LB + chloram. → shake → incubate

6/12/17

Interlab con't'd

	OD_{600}
20A x2	.0320
20B x2	.0222
20D x2	.0450
20F x2	.0022

OD_{600} of pre-competent cells

.59 .647

↳ can proceed to next step

~~Back to LB media~~

→ Incubate on ice 10 min

~~OD before dilution~~

→ Centrifuge 2 falcon tubes (~25 ml each) @ 3000 rpm 10 min

→ Add $CaCl_2$ (in TSE buffer)

10% of initial volume → 2-5 ml / tube

→ resuspend + vortex

→ Eliquot 100 μl tube

~~7/13/17~~

7/13/17

- OD_{600} readings for last 4 test device plates

20J - .0850

20L - .0166

20N - .0692

20P - .0693

7/14/17

Asked Pratap about shaking incubator - may said we can't use

Before starting miniprep: need to grow 1-5mL of overnight cultures in LB and correct antibiotic

Checking cell competency:

Transformed competent cells from freezerstock w/

7/16/17

Interlab

Checking overnight cultures OD600

	1	2
20 B	0.3983	0.0342
20 D	0.4034	0.0683
20 F	0.3569	0.0031
20 H	0.3798	0.0344
20 J	0.3643	0.0652
20 L	0.3558	0.0015
20 N	0.3736	0.0613
20 P	0.3751	0.0552

using a blank of LB + chlor

According to excel sheet target volume is 10, according to protocols 12mL, going w/ excel sheet

Dilutions

	1 (mL)	LB	2 (mL)	LB
20B	0.502	9.498	5.83	4.17
20D	0.496	9.50	2.93	7.07
20F	0.56	9.44	*	
20H	0.53	9.47	5.81	4.19
20J	0.55	9.45	3.07	6.93
20L	0.56	9.44	*	
20N	0.54	9.46	3.26	6.74
20P	0.53	9.47	3.62	6.38

the 2nd trial of 20F and 20L both had absorbances of the overnight cultures lower than 0.02, so the entire culture volume was incubated

T: 0 hr

Filesaved to flash drive)

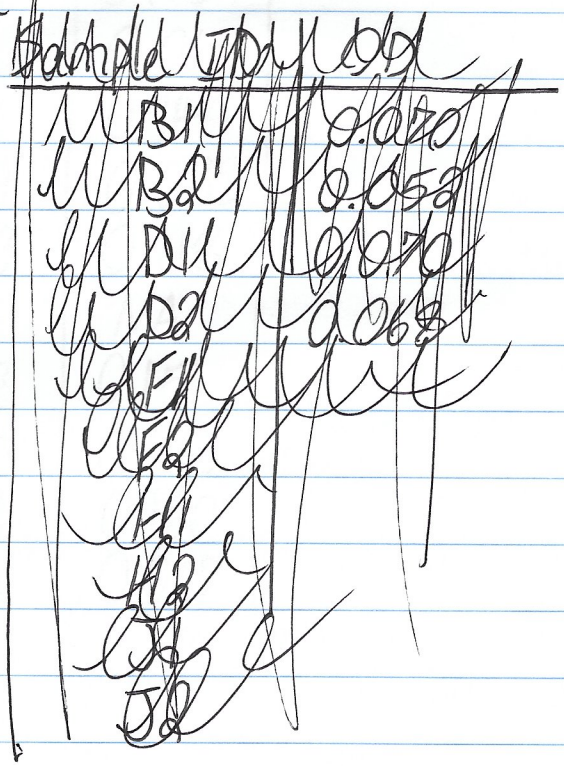
trial 1 (Excitation 400/30 Emission 508/20)

trial 2 (Excitation 360/40 Emission 530/20)

trial 3 (Excitation 485/20 Emission Plug)

trial 4 (Excitation 400/30 Emission 508/20)

[redacted]



OD Readings

ID	2-hr	4-hr	6-hr
B1	0.070	0.089	0.139
B2	0.052	0.005	0.033
D1	0.070	0.103	0.168
D2	0.068	0.065	0.096
F1	0.024	-0.013	0.048
F2	0.906	NA	NA
H1	0.072	0.079	0.118
H2	0.050	-0.005	0.010
J1	0.083	0.094	0.144
J2	0.064	0.044	0.088
L1	0.058	0.063	0.118
L2	0.004	NA	NA
N1	0.080	0.088	0.151
N2	0.059	0.035	0.065
P1	0.089	0.108	0.160
P2	0.068	0.026	0.067

FL: 2 hr
Saved on flashdrive
Trial 5 (Excitation 360/40 Emission 530/20
Trial 6 (Excitation 460/40 Emission 590/20
Trial 7 (Excitation 400/30 Emission 508/20

FL: 4 hr
Saved on flashdrive
Trial 8 (Excitation 360/40 Emission 530/20)
Trial 9 (Excitation 460/40 Emission 590/20)
Trial 10 (Excitation 400/30 Emission 508/20)

FL: 6 hr
Saved on flashdrive
Trial 11 "
Trial 12 "
Trial 13 "

5mL amp in 5mL Luria Broth
5mL chlor in 5mL Luria Broth
5mL kanamycin in 5mL Luria Broth
5mL tetracycline in 5mL Luria Broth

7/18/17

Miniprep on plasmid cultures: pET, pAcyc, pCDF, pCOLA

*pellet didn't form in 1st step centrifugion

7/20/17

- More LB media; [redacted] lean in incubator for tomorrow's competency

7/21/17

- Create TSS buffer → w/ $MgCl_2$ this time; filter sterilize in biosafety cabinet
9:00 am - Took out overnight culture → dilute 1/100 [redacted]

Don't want to make too many tubes .... 3 ml cells + 27 ml LB

7/23

Took BL21 "competent" cells out of incubator

↳ from competency check procedure

Count colonies:

A1 - 10 pg

A2 - 10 pg

B1 - 50 pg

B2 - 50 pg

C1 - 100 pg

C2 - 100 pg

NEG1 - @ growth? ∅

NEG2 - plate covered by bacterial growth

7/24

Competency Test #2

A1 ∅ only N1 on LB w/o chlor showed growth

↓

C2

7/25 Troubleshooting

SOC Media Recipe

1000 mL

i) Add the following to 900 ml DIW

20 g (Bactone) Tryptone

5 g (Bactone) Yeast Extract

2 ml NaCl (5M)

10 ml $MgCl_2$ (1M)

2.5 ml KCl (1M)

10 ml $MgSO_4$ (1M)

20 ml glucose (1M)

→ 500 ml ; split b/w 2x 500 ml flasks

Ampicillin + ~~Kanamycin~~ Streptomycin stock solution

10 mg/mL

↓ .1 g/10 ml

add 2.5 ml/250 ml

.5 g/10 ml

↳ add 250 x/250 ml

7/26/17

Transformation w/ pBLU

↳ plasmid w/ ampicillin resistant gene

→ 3 cell lines

2x plates LB+Amp

① BL21 #1 Beth

② BL21 #2

③ DH5α

→ negative control = plate BL21-1 BL21-2 DH5α on LB.

→ plate cell lines w/o plasmid pBLU on LB+Amp

Follow New England protocol

Total: 12 plates

✗ Incubator still warming up when put in for 1hr

↳ add ~30 of incubation?

⊖ = no plasmid DNA

7/27-28

Results of incubation

BL21-1-A	∅	BL21-1 ⊖	∅
BL21-1B		BL21-2 ⊖	
BL21 2-A		DH5α ⊖	
BL21 2-B		BL21-1 Neg	growth
DH5α-A		BL21-2 Neg	growth
DH5α-B		DH5α Neg	growth

Neg = LB only

→ Problem:
heat shock/transformation?
not long enough

↳ attempt diff protocol, modify New England Labs

7·28·17

Taking concentration of miniprepped plasmids

w/ nanodrop w/ EB Buffer

pAcyc	conc.	1.8 ng/μL	260/280: 3.31	260/230: 0.81
pCDF	conc.	1.4 ng/μL	260/280: 3.52	260/230: 0.46
pET	conc.	2.2 ng/μL	260/280: 4.46	260/230: 1.41
pGLA	conc.	1.6 ng/μL	260/280: -46.37	260/230: 1.73

w/ H_2O

pAcyc	conc.	11.8 ng/μL	260/280: 1.68	260/230: 0.66
pCDF	conc.	1.4 ng/μL	260/280: 2.24	260/230: 0.53
pET	conc.	41.6 ng/μL	260/280: 1.59	260/230: 0.64
pGLA	conc.	2.6 ng/μL	260/280: 1.89	260/230: 1.21

7/26/17

Transformation w/ pBLU

↳ plasmid w/ ampicillin resistant

→ 3 cell lines

2x plates LB + Amp

① BL21 #1 Batch

② BL21 #2

③ DH5α

→ negative control = plate BL21-1, BL21-2, DH5α on LB.

→ plate cell lines w/o plasmid pBLU on LB + Amp

Follow New England protocol

Total: 12 plates

* Incubator still warming up when put in for 1hr

↳ add ~30 of incubation?

7/27-28

Results of incubation

⊖ = no plasmid DNA

BL21-1-A	∅
BL21-1B	∅
BL21 2-A	∅
BL21 2-B	∅
DH5α-A	∅
DH5α-B	∅

BL21-1 ⊖	∅
BL21-2 ⊖	∅
DH5α ⊖	∅
BL21-1 Neg	growth
BL21-2 Neg	growth
BL21 α Neg	growth

Neg = LB only

→ Problem:

heat shock not long enough

↳ attempt diff protocol, modify New England Labs

7-28-17

Taking concentration of miniprepped plasmids

w/ nanodrop w/ EB Buffer

pAcyc	conc.	1.8 ng/μL	260/280: 3.31	260/230: 0.81
pCDF	conc.	1.4 ng/μL	260/280: 3.52	260/230: 0.46
pET	conc.	2.2 ng/μL	260/280: 4.46	260/230: 1.41
pGLA	conc.	1.6 ng/μL	260/280: -46.37	260/230: 1.73

w/ H_2O

pAcyc	conc.	11.8 ng/μL	260/280: 1.68	260/230: 0.66
pCDF	conc.	1.4 ng/μL	260/280: 2.24	260/230: 0.53
pET	conc.	41.6 ng/μL	260/280: 1.59	260/230: 0.64
pGLA	conc.	2.6 ng/μL	260/280: 1.89	260/230: 1.21

add antibiotic to cell culture

pACYC - Chloram

pCOLA - Kanamycin

pET - Ampicillin (Carbenicillin)

pCDF - Streptomycin (Spectenomycin)

1000X 10ml broth + 100 λ cells + 10 λ antibiotic

Overnight cultures → need enough growth to not see-through suspension

8/1/17

* once genes are hydrated, need to be stored in −20°C *

7/31/17

Cloning

Conc. of vector: 100ng/uL (

5uL of vector

choose resuspension vol.

50uL rcs. 20ng/uL

5uL of resuspended g-blocks

10uL (buffer, enzymes, H2O)

7uL H2O, 2 buffer, 1/2 (x2) enzymes (Pst1, EcoR1)

digest seperately

+5uL H2O instead of g-blocks or vector

12uL total H2O (PUC)

left ~~at~~ in 37°C H2O bath

0.5uL dephosphorolase? into PUC leave for 30min

column purify?

Use Qiaquick PCR Purification Microcentrifuge and Vacuum protocol

2uL vector

6uL insert

2uL buffer

1uL ligase

9uL H_2O

8/2/17

Cloning w/ pSB1T3
1 ng 1 clone
25 ng/mL = 4 mL

Purification
1 spin column for each tube (vector + genes)
1) add 5 volumes of PB buffer to 1 volume reaction mixture
(so 100 mL of PB to 20 mL sol.)
2) pour sample into column and centrifuge 60s
3) discard flow through centrifuge 60s
4) add .75 mL PE buffer to column and discard flow through

5) Centrifuge for 1 min

6) Place column in a clean tube

7) to elute DNA add 50ul of EB to the membrane and centrifuge let sit 1 min

8/3

Miniprep

↳ check nanodrop [ ]'s of plasmid DNA

	[Conc.]	260/280	260/230
pAroG	- 12.8 ng/ml	1.93	1.56
pCDF	- 27.2 ng/ml	1.90	1.70
PET	- 15.4 ng/ml	2.0	1.44
pCOLA	- 4.8 ng/ml	2.04	4.5

8/4 Transformation

1000 mg/ml of ampicillin to overnight cell cultu

PDC + PUC

AROG + PUC

1000X

50x ampicillin (10 mg/ml) + colony + 5 ml SOC

5 ml LB

Student Government
↳ travel funds for girls presentations @ scientific meeting
~$500

8/8

- Miniprep on PUC + AB09 and PUC + POC

8/16/17

Transformed ARO 10 and ADH in pSB1T3 into DH5α cells

Miniprep concentrations

			[conc]
↳ PDC (A)	260/280: 1.92	260/230: 2.07	45.8 ng/ml
ARO9 (A)	2.00	0.88	12.2 ng/ml
PDC (B)	~~26.6~~ 1.92	1.79	26.6 ng/ml
ARO9 (B)	2.29	1.92	5.1 ng/ml

*** 1X add to nanodrop reader ***

8/22/17

— M9 Minimal Medium recipe

(per liter)

- 750 ml sterile H_2O
- 200 ml 5x M9 salts [xx]
- ~~1 M~~ 2 ml 1M $MgSO_4$
- 20 ml 20% solution of appropriate C source (glucose)
- 0.1 ml 1M $CaCl_2$
- (to 980 ml) sterile deionized H_2O

* 100 micrograms/ml for 500 ml

xx for M9 salt: (per liter)

dissolve the following in sterile DI H_2O

- 64 g $Na_2HPO_4 \cdot 7H_2O$
- 15 g KH_2PO_4
- 2.5 g $NaCl$
- 5.0 g NH_4Cl

— Salt solution into 200 ml aliquots

8/26/17

Make miniprep overnight solutions

pET + 8 uL Amp in 10mL SOC

pAcyc + 1 uL Chlor in 10mL SOC

pLIDF + 4 uL Strep in 10mL SOC

pCOLA + 4 uL Kana in 10mL SOC

8/27/17

- Miniprep

Incubate 3 min @ lysis step

	Conc =	260/280 -	260/230 -
pCOLF	71.9 ng/ul	1.62	.65
pacyc	181.7 ng/ul	1.83	1.75
PET	142.0 ng/ul	1.88	2.08
pCOLA	210.3 ng/ul	1.84	1.59

8/29/17

Digestion and Ligation ~~into~~ of g-blocks DNA into Duet vectors

8 reaction tubes:
4 vectors: pACYC, pET, pCDF, pCOLA
4 inserts: ARO10, ARO9, AtDH1, PDC

Digestion of vectors:
in a microcentrifuge tube combine:
12 uL H_2O (purified)
5 uL vector
2 uL buffer (2.1)
1/2 uL EcoR1
1/2 uL Pst 1

Digestion of inserts
12 uL H_2O (purified)
5 uL insert
2 uL buffer (2.1)
1/2 uL EcoR1
1/2 uL Pst 1

Heat all 8 tubes for 30 min in 37°C water bath

after heating, add 0.5uL phosphatase into the tubes w/ the vectors
heat for 30 min in 37°C water bath

column purify all solutions using procedure in group - Qiaprep columns are the same as Qiaspin

Ligation
if possible ligate each insert into each vector
for example

Aro1C → pCola - 1 tube
→ Pacyc - 1 tube
→ pET - 1 tube
→ pCDF - 1 tube

Ligation mastermix for each tube
9uL H2O
6uL insert
2uL vector
2uL ligase buffer
1uL ligase

leave ligation overnight

8/30 minimal media

- 250 ml DIW
- 7.5 g Agar

autoclave

(mg salts)

- 239 ml DIW
- 6.4 g Na_2HPO_4
- 1.5 g KH_2PO_4
- .25 g NaCl
- .5 g NH_4Cl

~~8/31~~
9/1/17

Make TAE buffer for gels.

Prepare stock of EDTA. 46.17

93.05 g EDTA

Dissolve 400 ml deionized water

adjust pH 8. NaOH

500 ml

Minimal media

250 DIW
7.5 g agar

+

mg salts

- 6.4 g Na_2PO_4
- 1.5 g KH_2PO_4
- .25 g NaCl
- .5 g NH_4Cl
- 239 ml DIW

Combine when cooled after autoclaving 20 min

Glucose (20%) = 10 ml

1 M $MgSO_4$ = 1 ml

1 M $CaCl_2$ = .1 ml

filter sterilize (.246 g + 1 ml DIW) x10

2.46 g + 10 ml

Tryptophan

100 mg/ml → 500 ml → .05 g Trp

8/29/17

Transformed Duet clones into BL21 cells
used pUC19 as positive control

8/30/17

Transformations failed except for + control
-asked Dr. Reisch - do not transform clones into
BL21

9/4/17

Transformed Duet clones into DH5α cells

Paured plates

9/5/17

Some transformations into DH5α were successful:

9/6/17

	Conc	260/280	260/230
ADH-pacyc	52.9 ng/uL	2.03	2.13
AR09-pCDF	130.3 ng/uL	1.85	2.02
AR09-pacyc	185.5 ng/uL	1.82	1.60
AR01C-pacyc	85.7 ng/uL	1.75	1.11
AR01C-pCDF	784. ng/uL	1.86	2.10
AR01C-pCDFD	203.7 ng/uL	1.72	1.09
ADH-pCDF	286.8 ng/uL	1.77	1.26
PDC-pCDF C	56.5 ng/uL	1.88	1.91
PDC-pCDFF	97.6 ng/uL	1.85	1.71

9/13/17

	Conc. ng/μL	260/280	260/230
AR09 - PACYC	6.3	2.53	0.99
AR09 - PCDF	45.5	1.84	1.41
ADH - PCDF	38.2	1.83	1.65
PDC - PCDF	134.9	1.91	2.20
AR010 - PCDF x2	11.4	1.84	0.97
AR010 - PET	10.2	1.69	0.68
ADH - PET	11.6	1.75	1.10
AR010 - PACYC	9.6	2.00	1.13
AR010 - PET	56.7	1.87	1.70
AR010 - PCDF	7.6	1.99	1.02
ADH1 - PCDF	77.8	1.88	1.86
ADH1 - PACYC	12.2	1.81	0.98
ADH1 - PET x2	49.2	1.87	1.86

9/14/16

mg → g

more antibiotic stock solutions

chloramphenicol

25mg/ml

~~250ul~~ → ~~2.5×10^{-4}~~

250ml

~~25mg/ml (250ml) = 6.25×10^{3} mg = 6.25g~~

25mg/ml (10ml) = 250mg = .25g

Kanamycin 50mg/ml

50mg/ml (10ml) = 500mg = .5g

Streptomycin

50mg/ml (10ml) = .5g

9/14/16

Transformations into DH5α mat worked:

ARO9→pETT

ADH→pCOLA

ARO10→pETT

didn't work:

PDC→pCOLA

ADH→pETT

ARO9→pCOLA

ARO10→pCOLA

Transformations into BL21 mat worked:

1 ARO9→pACYC -

2 ADH→pACYC -

3 ADH→pCDF }
ADH→pCDF }

4 ARO10→pACYC -

ADH→pACYC -

ARO10→pACYC -

ARO9→pACYC -

5 ARO9→pCDF x2

6 ARO10→pCDF

Didn't work:

pDC+pCDF

~~AD1C+pCDF~~

DD1+pCDF

Grew overnight cultures of ~~AD1~~ AD09+pET, AD01 AD1+pCOLA

9/15/16

Miniprep on the overnight cultures of A2C9 pET, A2C10-pET, and A1H-pCOLA

9/16/16

Quantifying the minipreps of A2C9-pET, A2C10-pET and A1H-pCOLA

	Conc:	260/280	260/230
A1H1-pCOLA	154.7 ng/μL	1.89	2.08
A2C9-pET	584.0 ng/μL	1.86	2.19
A2C10-pET	233.5 ng/μL	1.90	2.06

Transforming A1H1-pCOLA, A2C9-pET, A2C10-pET into BL21 cells

9/17/16

From yesterday's transformations into BL21

A1D4+pCOLA did not work

AR010+PET and AR09+PET and me control did work

Gel Electrophoresis:

after making another gel and testing the GE apparatus w/ the smaller 1000 bp ladder we still don't see any bands. We may need a new electrode, which cannot run at optimum voltage. Plasmids have been digested and ligated and are ready to run as soon as we can successfully run a test ladder.

Made SOC Media and LB Agar

9/20/17

Check OD of diluted cells >0.5

followed cell competency procedure

spun down cultures + resuspended pellet in TSS

9/21/17

Digestion of the previous ligations:

A2C9 + pCDF

ADH + pacyc

A2C9 + pacyc

PDC + Pacyc

ADH + pCDF

A2C1C + Pacyc

PDC + pCDF

A2C1C + pCDF

PDC + pCOLA

ADH + pCOLA

ADH + pET

for gel electrophoresis

9/25/17 Blank = LB + chlor

Interlab

OD_{600} overnight cultures

21B	0.1727	21B x2	0.1796
21D	0.16121	x2	0.1501
21F	0.1273		0.1288
21H	0.3418		0.1334
21J	0.2027		0.1596
21L	0.1567		0.1434
21N	0.1698		0.1486
21P	0.1751		0.1595

Dilutions

	Vol of QC	LB		Vol QC	LB
21B	1.16	8.84	21B×2	1.11	8.89
21D	1.22	8.78	21D×2	1.33	8.67
21F	1.57	8.42	21F×2	1.55	8.44
21H	0.59	9.41	21H×2	1.50	8.50
21J	0.99	9.01	21J×2	1.25	8.74
21L	1.28	8.72	21L×2	1.39	8.61
21N	1.17	8.82	21N×2	1.35	8.65
21P	1.14	8.85	21P×2	1.26	8.75

Absorbance

	0hr			2hr	
B1	.0508	.0546		0.3803	.3744
B1x2	.0477	.0485		.3184	.3130
D1	.0557	.0566		.4275	.4285
D1x2	.0529	.0535		.3873	.3836
F1	.0517	.0521		.1301	.1301
F1x2	.0480	.0477		.1434	.1437
H1	.0457	.0462		.3531	.3486
H1x2	.0849	.0860		.4036	.4077
J1	.0537	.0550		.3698	.3741
J1x2	.0506	.0518		.3709	.3626
L1	.0427	.0429		.2528	.2455
L1x2	.0457	.0471		.2470	.2412
N1	.0602	.0606		.3380	.3372
N1x2	.0648	.0656		.3754	.3584
P1	[illegible]	.0445	.0446	.3235	.3159
P1x2	.0409	.0408		.2922	.2865

	4hr		6hr	
21B	~~.4652~~ .4652	.4458	1.1136	1.1121
21Bx2	.4710	.4652	1.1699	1.1715
21D	.5221	.5101	1.2623	1.2602
21Dx2	.5059	.5146	1.3110	1.3093
21F	.1648	.1707	.7114	.7507
21Fx2	.1727	.1676	.6790	.6741
21H	.4271	.4273	1.0997	1.1028
21Hx2	.5214	.5072	1.2792	1.2838
21J	.4734	.4737	1.2798	1.2783
21Jx2	.4666	.4613	1.2702	1.2677
21L	.3847	.3698	1.0632	1.0670
21Lx2	.4030	.4003	1.0638	1.0638
21N	.4936	.4928	1.2543	1.2551
21Nx2	.5413	.5392	1.3578	1.3559
21P	.4705	.4728	1.2703	1.2667
21Px2	.4507	~~.4554~~ .4583	1.2809	1.2810

9/26

Digested (Ao9, Ao10, AD41

Vector

·PSB backbone

9/30/17

Transformations

AR09 + pCIDF	- ADH + PET pUC19	Strep + amp
~~AR010 + pCIDF~~		and strep plate
	- AR010 + PET	
ADH + pCIDF	- pUC19	
AR09 + pACYC	- ADH + pCIDF - AR010 + pCIDF	↳ chlor + strep + chlor plate
AR010 + pACYC	- ADH + pCIDF - AR09 + pCIDF	
ADH + pACYC	- AR09 + pCIDF - AR010 + pCIDF	

10/1/17

Checked transformations - none were successful

Redoing cell competency procedure w/ overnight cultures

Absorbance for cell competency	
ADH + pCIDF	0.4360
AR09 + pCIDF	0.3316
AR010 + pCIDF	0.3325
ADH + pACYC	0.6068
AR09 + pACYC	0.5661
AR010 + pACYC	0.4342

Cols

① Ladder

ADH PET

ADH ~~PEPF~~ PCDF

ADH Pcype

ADH PCoLa

Aro9 PaCyC

PDC PCDF

Aro9 PCDF

Aro10 PacyC

PDC PacyC

PDC Pcola

Aro10 PCDF

10/5/17

Absorbance for cell comp.

ADH + pCDF + ARC1C + pET	0.4656
ARC9 + pET + ARC1C + pCDF	0.0945
ARC9 + pET + ADH + pCDF	0.4921
ARC1C (pCDF) + ADH (pET)	0.1445
ADH (pacyc) + ARC1C (pET)	0.5662
ARC1C (pET) + ARC9 (pCDF)	0.5160

10/10/17

Used Sybr Safe in gels.

ran 1 kb latter

Success glow under UV light.