

6/16/17

Pouring Plates:

• already have the agar made

Agar cooled

put in ~~the~~ microwave for 2:30, check to make sure it isn't boiling over

• can put the aluminium from the autoclave in the microwave

have to let agar cool before we pour

Pouring w/ Sterile technique

6/19/17

→ Poured plates removed from -20°C last Friday; too frozen to use

- Monday, too much liquid on plates, need to remake agar

Troubleshoot attempt → fill up glass casserole pan w/ shallow layer of water
try to remelt agar and let solidify again ...

Nope X

6/20/17

→ Remake agar solution 3 autoclave

→ create stock solution of antibiotic (kanamycin)

- write date on newly opened antibiotic bottles

50 milligram/ml

5 ml stock solution

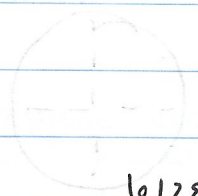
• 25 g into 5 ml stock vial (DIW)

filter sterilize → write that filter sterilized on label

2.5 mL / 250 mL

chlor
.250 g in 10 ml EtOH
● 250 g in 250 ml
Strep
.5 g in 10 ml D.W

612717



612817

Poured LB plates - 16
observed E.coli (DH-5α) plated on 612717 -
no isolates observed

6/29

Making chloramphenicol Stock Solution (25mg/ml)

.25g chloramphenicol

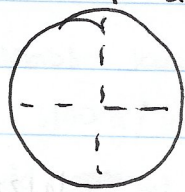
10 ml ethanol

Working concentration is 25ug/ml

↳ add 1ml stock sol'n to 1L ^{LB} agar → .25 ml Fu .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 OD₆₀₀ Reference point

- add 100 μ L LUDex into wells A1, B1, C1, D1 of 96 well plate
 - add 1 mL LUDex into cuvette
- add 100 μ L of H₂O into wells A2, B2, C2, D2
 - add 1 mL H₂O into cuvette
- measure the absorbance of all samples at 600 nm

	Spectropho.		Microplate Reader	
	H ₂ O	LUDX	H ₂ O	LUDX
Trial 1				
Trial 2				
Trial 3				
Trial 4				

71117

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

71317

F1111F

Poured more plates w/ LB and chloramphenicol

Poured 100 ml of phosphate buffer into 100 ml

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: 890/20

Trial 4:

Excitation: 400/30

Emission: 508/30

Interlab

measurements on the reference spectrophotometer

1 mL Ludox into a cuvette

1 mL H₂O into another cuvette

Ref. Spec.

	H ₂ O	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 bridge.

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

2050.0

0.5H

1150.0

0

1.00T

2050.0

0

5.00T

1150.0

0

8.00T

1250.0

0

1.00T

10
6/18/17

making 250 ml of Luria brom

~~9.25 g~~ 6.25 LB

250 ml of DI water

Dilution of 100 mM Calcium Chloride to 30 mM:

$$M_1 V_1 = M_2 V_2$$

$$(100 \text{ mM}) V_1 = 10 \text{ mL} (30 \text{ mM})$$

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

6/11/17

Competent cell making

→ cells only @ .21 OD₆₀₀; not enough, need to be @ least .5

→ put cells in incubator

Interlab

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

6/12/17

Interlab cont'd

	OD ₆₀₀
20A x2	.0320
20B x2	.0222
20D x2	.0450
20F x2	.0022

OD₆₀₀ of pre-competent cells
.59 .647

↳ can proceed to next step

~~Ande~~ ~~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

→ Eluate 100 μl tube

#13/17
7/13/17

- OD₆₀₀ readings for last 4 test device plates

20J - .0850

20L - .0166

20N - .0692

20P - .0693

0.020	20A
0.030	20B
0.055	20C
0.070	20D
0.090	20E

7/14/17

Asked Prateep about shaking incubator - they said we can't use

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

Checking cell competency:

Transformed competent cells from freezer stock

w/

7/16/17

Interlab

Checking overnight cultures CID600

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

F11011F

	Dilutions			
	(mL)	(mL)	(mL)	(mL)
	1 LB	2 LB	1 LB	2 LB
20B	0.502	9.498	5.83	4.17
20D	0.496	9.504	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
		5220.0		1258.0

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

Files saved to flash drive)

trial 1 (Excitation 400/30 Emission 500/20)

trial 2 (Excitation 360/140 Emission 530/20)

trial 3 (Excitation 485/20 Emission Plug)

trial 4 (Excitation 400/30 Emission 500/20)

~~AAAAA~~

~~Handwritten scribbles and numbers, possibly a table or list, heavily obscured by heavy black ink strokes.~~

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	0.006	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

Sul amp in Smc Luria Broth
Sul chlor in Smc Luria Broth
Sul kanamycin in Smc Luria Broth
Sul tetracycline in Smc Luria Broth

7/18/17

Miniprep on plasmid cultures: PET, pAcy, pCDF, pCOLA
*pellet didn't form in 1st step centrifugation

7/20/17

- More LB media; ~~make~~ leave in incubator for tomorrow's competency

7/21/17

- Create TSS buffer \rightarrow w/ $MgCl_2$ this time; filter sterilize in biosafety cabinet

9:00 am - Took out overnight culture \rightarrow dilute 1/100 ~~3 ml in 27 ml LB~~

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 - \emptyset growth?

NEG2 - plate covered by bacterial growth

7/24

Competency Test #2

A1

↓
C2

\emptyset

only N1 in LB w/o chlor showed growth

7/25 Troubleshooting

SOC Media Recipe

1000 mL

1) 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

↓ 0.1 g / 10 ml

add 2.5 ml / 250 ml

0.5 g / 10 ml

add
250 ~~x~~ / 250 ml

~~1.25 g / 250 ml~~

7/26/17

Transformation w/ pBLU

↳ plasmid w/ ampicillin resistant gene

→ 3 cell lines

→ negative control = plate on LB. BL21-1
BL21-2
DH5 α

2x Plates
LB + Amp

- ① BL21-#1
Bath
- ② BL21-#2
- ③ DH5 α

→ 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	∅ ↓	BL21-1 ⊖	↓ growth growth growth
BL21-1-B			
BL21-2-A			
BL21-2-B			
DH5 α -A			
DH5 α -B			

Neg = LB only

antibodies of streptococcus

→ Problem: microbiol - 32049
microbiol - A1009

heat shock degradation? - 739
not long enough - 7909
(microbiol)

↳ attempt diff protocol, modify New England Labs

streptococcus x oi + also x oi + strep ind x0001

7-28-17

Taking concentration of miniprep plasmids

w/ nanodrop w/ EB Buffer

PAeye	conc. 1.8 ng/ul	260/280: 3.31	260/230: 0.81
PCDF	conc. 1.4 ng/ul	260/280: 3.52	260/230: 0.46
PET	conc. 2.2 ng/ul	260/280: 4.46	260/230: 1.41
P6LA	conc. 1.6 ng/ul	260/280: -46.37	260/230: 1.73
w/ H ₂ O			

PAeye	conc. 11.8 ng/ul	260/280: 1.68	260/230: 0.66
PCDF	conc. 1.4 ng/ul	260/280: 2.24	260/230: 0.53
PET	conc. 41.6 ng/ul	260/280: 1.59	260/230: 0.64
P6LA	conc. 2.6 ng/ul	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 on LB.

BL21-A

BL21-B

DH5α

→ 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		BL21-1 ⊖		
BL21-1-B		BL21-2 ⊖		
BL21-2-A		DH5α ⊖		
BL21-2-B		BL21-1 Neg		growth
DH5α-A		BL21-2 Neg		growth
DH5α-B		BL5α Neg		growth

Neg = LB only

samples 100 of individual 600

→ Problem: microbio - 52049
microbio - 43009

heat (shock) duration? - 739
not long enough
(microbio 52049) microbio 739 - 7009

↳ attempt diff protocol, modify New England Labs

individual 101 + 2100 x 100 + 1000 ind 20001

7-28-17

Taking concentration of miniprep plasmids

w/ nanodrop w/ EB Buffer

PAeye	conc.	1.8 ng/ul	260/280: 3.31	260/230: 0.81
PCDF	conc.	1.4 ng/ul	260/280: 3.52	260/230: 0.46
PIET	conc.	2.2 ng/ul	260/280: 4.46	260/230: 1.41
P6LA	conc.	1.6 ng/ul	260/280: -46.37	260/230: 1.73
w/ H ₂ O				

PAeye	conc.	11.8 ng/ul	260/280: 1.68	260/230: 0.66
PCDF	conc.	1.4 ng/ul	260/280: 1.24	260/230: 0.53
PIET	conc.	41.6 ng/ul	260/280: 1.59	260/230: 0.64
P6LA	conc.	2.6 ng/ul	260/280: 1.89	260/230: 1.21

add antibiotic to cell culture

pAcyc - Chloram

pCOLA - Kanamycin

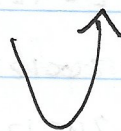
pET - Ampicillin (Carbenicillin)

pCDF - Streptomycin (Spectinomycin)

1000x 10ml broth + 100 μ cells + 10 μ antibiotic

Overnight cultures \rightarrow need enough growth to not see-through suspension

8/1/17



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

713117

Cloning

Conc. of vector: $100\text{ng}/\mu\text{L}$ (

$5\mu\text{L}$ of vector

Choose resuspension vol.

$50\mu\text{L}$ res. $200\text{ng}/\mu\text{L}$

$5\mu\text{L}$ of resuspended g-blocks

$10\mu\text{L}$ (buffer, enzymes, etc)

$7\mu\text{L}$ H₂O, 2 buffer, $\frac{1}{2}(\times 2)$ enzymes (Pst I, EcoRI)

digest separately

→ $5\mu\text{L}$ H₂O instead of g-blocks or vector
 $12\mu\text{L}$ total (Puc)

left on in 37°C H₂O bath

$0.5\mu\text{L}$ dephosphorase? into Puc leave for 30min

column purify?

Use Qiagquick PCR Purification Microcentrifuge
and Vacuum protocol

Once genes are ligated need to be stored in -20°C

3 min

- 2ul vector
- 6ul insert
- 2ul buffer
- 1ul ligase
- 9ul H₂O

Conc. of vector & ligase
 Conc. of vector & ligase
 Conc. of vector & ligase
 Conc. of vector & ligase

Final reaction buffer (15ul) (x2) enzyme (1.5ul) (x2) (15ul) (x2)

15ul total (2ul) instead of 2 blocks of vector
 digest separately

left on in 8°C the pan

0.2ul endonuclease into 2ul leave for 30min

column buffer?

see Molecular PCR Purification Microcentrifuge
 can vacuum protocol

1000

812117

Cloning w/ pSB1T3

100 ng 1 clone

25 ng μ L = 4 μ L

Purification

1 spin column for each tube (vector + genes)

1) add 5 volumes of PB buffer to 1 volume reaction mixture

(so 100 μ L of PB to 20 μ L sol.)

2) pour sample into column and centrifuge 60s

3) discard flow through and centrifuge 60s

4) add .75 mL PE buffer to column and discard flow through

F11510

5) Centrifuge for 1 min

6) Place column in a clean tube

7) to elute DNA add 50 μ l of EB

the membrane and centrifuge
let sit 1 min

8/3

Miniprep

check nanodrop []'s of plasmid DNA

	Conc.	260/280	260/230
pAsgc	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 cult

PDC + ARO9

+ PUC + PUC

100 μ l

5 ml LB

50 μ l ampicillin (10 mg/ml) + colony + 5 ml sec

Student Government
↳ travel funds for giving presentations @ scientific meeting
\$500

8/8

• Miniprep on PUC + A209 and PUC + POC

8116117

Transformed A2010 and ADH in pSB1T3 into DH5 α cells

Miniprep Concentrations

	260/280%	260/230%	[conc]
↳ PDC (A)	1.92	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 H₂O
- 200 ml 5X M9 salts^{XX}
- ~~1M~~ 2 ml 1M MgSO₄
- 20 ml 20% solution of appropriate C source (glucose)
- 0.1 ml 1M CaCl₂
- (to 980 ml) sterile deionized H₂O
- * 100 microgram/ml for 500 ml

XX for M9 salt:

(per liter)

dissolve the following in sterile DI H₂O

- 64 g Na₂HPO₄ · 7 H₂O
- 15 g KH₂PO₄
- 2.5 g NaCl
- 5.0 g NH₄Cl

- Salt solution into 200 ml aliquots

8/26/17

Made miniprep overnight solutions

pET + 8 ~~µl~~^{µl} Amp in 10 mL SOC

pAcyo + 15 µl Chlor in 10 mL SOC

PLIDF + 4 µl Spred in 10 mL SOC

PCOLA + 4 µl Kara in 10 mL SOC

8/27/17

- miniprep

Incubate 3 min @ lysis step

pCDF	<u>conc</u> 71.9 ng/µl	<u>260/280</u> - 1.62	<u>260/230</u> - .65
pacyc	181.7 ng/µl	1.83	1.75
PET	142.0 ng/µl	1.88	2.08
PCOLA	210.3 ng/µl	1.84	1.59

8129117

Digestion and ligation into of g-blocks DNA
into Duet vectors

8 reaction tubes:

4 vectors: pAcyc, pET, pCDF, pCOLA

4 inserts: AR010, AR09, ADH1, PIDC

Digestion of vectors:

in a microcentrifuge tube combine:

12 μ L H₂O (purified)

5 μ L vector

2 μ L buffer (2.1)

$\frac{1}{2}$ μ L EcoRI

$\frac{1}{2}$ μ L Pst I

Digestion of inserts

12 μ L H₂O (purified)

5 μ L insert

2 μ L buffer (2.1)

$\frac{1}{2}$ μ L EcoRI

$\frac{1}{2}$ μ L Pst I

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

F11P518

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

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

Ligation

if possible ligate each insert into each vector

for example

 pCola - 1 tube
Aro10 → Paacv - 1 tube
 → PET - 1 tube
 ↓ pCDF - 1 tube

Ligation mastermix for each tube

9ul H₂O

1ul insert

2ul vector

2ul ligase buffer

1ul ligase

leave ligation overnight

8/30 minimal media

- ✓ 250 ml DIW
- ✓ 7.5 g Agar

Autoclave

(ma salts)

- 239 ml DIW ✓
- 6.4 g Na_2HPO_4 ✓
- 1.5 g KH_2PO_4 ✓
- 0.25 g Nacl ✓
- 0.5 g NH_4Cl ✓

8/31

9/1/17

make TAE buffer for gels.

Prepare stock of EDTA.

46.17

93.05g EDTA

Dissolve 400 ml deionized water

adjust pH 8. NaOH

500 ml

Minimal media

ma salts

- 250 DIW ✓
- 7.5 g agar ✓

+

- 6.4 g Na_2PO_4 ✓
- 1.5 g KH_2PO_4 ✓
- 0.25 g Nacl ✓
- 0.5 g NH_4Cl ✓
- 339 ml DIW ✓

combine when cooled
after autoclaving
60 min

Glucose (20%) = 10 ml

1 M MgSO_4 = 1 ml

1 M CaCl_2 = 1 ml

filter sterilize (x10)
(.246 g + 1 ml DIW)
2.46 g + 10 ml

Tryptophan

100 mg/ml → 500 ml → .05 g Trp

8/29/17

Transformed Duet clones into BLZ1 cells
used pUC19 as positive control

8/30/17

Transformations failed except for + control
-asked Dr. Zersah - do not transform clones into
BLZ1

9/14/17

Transformed Duet clones into DH5 α
cells

Paired plates

9/15/17

Some transformations into DH5 α were successful
:

9/16/17

	Conc	260/280	260/230
ADH-pacyc	52.9 ng/ml	2.03	2.13
AzC9-pCDF	130.3 ng/ml	1.85	2.02
AzC9-pacyc	185.5 ng/ml	1.82	1.60
AzC10-pacyc	85.7 ng/ml	1.75	1.11
AzC10-pCDF	784. ng/ml	1.86	2.10
AzC10-pCDFD	203.7 ng/ml	1.22	1.09
ADH-pCDF	286.8 ng/ml	1.77	1.26
PDC-pCDF C	56.5 ng/ml	1.88	1.91
PDC-pCDF F	97.6 ng/ml	1.85	1.71

9/13/17

	Conc. ng/ml	260/280	260/230
ARO9 - PACYC	6.3	2.53	0.99
ARO9 - PCDF	45.5	1.84	1.41
ADH - PCDF	38.2	1.83	1.65
PDC - PCDF	134.9	1.91	2.20
ARO10 - PCDF X2	11.4	1.84	0.97
ARO10 - PET	10.2	1.69	0.68
ADH - PET	11.6	1.75	1.10
ARO10 - PACYC	9.6	2.00	1.13
ARO10 - PET	56.7	1.87	1.70
ARO10 - 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 antibiotics stock solutions

chloramphenicol 25 mg/ml

25 mg/ml

~~250 ml~~ → 2.5×10^{-4}

250 ml

~~$25 \text{ mg/ml} (250 \text{ ml}) = 6.25 \times 10^3 \text{ mg} = 6.25 \text{ g}$~~

$25 \text{ mg/ml} (10 \text{ ml}) = 250 \text{ mg} = .25 \text{ g}$

Kanamycin 50 mg/ml

$50 \text{ mg/ml} (10 \text{ ml}) = 500 \text{ mg} = .5 \text{ g}$

Streptomycin

$50 \text{ mg/ml} (10 \text{ ml}) = .5 \text{ g}$

9/14/16

Transformations into 1DHSd mat worked:

AR09 → PET

ADH → pCOLA

AR010 → PET

didn't work:

PDC → pCOLA

ADH → PET

AR09 → pCOLA

AR010 → pCOLA

Transformations into BLZ1 mat worked:

1 AR09 → pAcyc -

2 ADL4 → pAcyc -

3 ADH → pCIDF }
ADH → pCIDF }

4 AR010 → pAcyc -

ADH → pAcyc -

AR010 → pAcyc -

AR09 → pAcyc -

5 AR09 → pCIDF x2

6 AR010 → pCIDF

Didn't work:

PCR PCR

ADH PCR

ADH PCR

Grew overnight cultures of ~~ADH~~ ADH + PET, ADH + ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

ADH + PCR

9/15/16

Miniprep on the overnight cultures of AR09-pET, AR2010-pET, and AIDH-pCOLA

9/16/16

Quantifying the miniprep of AR09-pET, AR2010-pET and AIDH-pCOLA

	Cone:	260/280	260/230
AIDH-pCOLA	154.7 ng/ul	1.89	2.08
AR09-pET	584.0 ng/ul	1.26	2.19
AR2010-pET	233.5 ng/ul	1.90	2.06

Transforming AIDH-pCOLA, AR09-pET, AR2010-pET into BL21 cells

9/17/16

From yesterday's transformations into BL21
ADH+pcOLA did not work
AR210+PET and AR209+PET and the 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 we ready to run as soon as
we can successfully run a test ladder.

Made SOC Media and LB Ager

9/20/17

Check OD of diluted cells > 0.5
followed cell competency procedure
spun down cultures + resuspended pellet
in TSS

9121117

CHM 341

Digestion of the previous ligations:

A209 → pCDF

ADH → pacyc

A209 → pacyc

PDC → pacyc

ADH → pCDF

A2010 → pacyc

PDC → pCDF

A2010 → pCDF

PDC → pCOLA

ADH → pCOLA

ADH → pET

for gel electrophoresis

9/25/17

Blank = LB + chlor

F1151P

Interlab

OD₆₀₀ overnight cultures

21B 0.1777

21B x 2 0.1796

21D 0.16411

x 2 0.1501

21F 0.1273

0.1288

21H 0.3418

0.1334

21J 0.2027

0.1590

21L 0.1567

0.1434

21N 0.1698

0.1480

21P 0.1751

0.1595

Dilutions

	Vol of OC	LB		Vol OC	LB
Z1B	1.16	8.84	Z1B x 2	1.11	8.89
Z1D	1.27	8.73	Z1D x 2	1.33	8.67
Z1F	1.57	8.43	Z1F x 2	1.55	8.45
Z1H	0.59	9.41	Z1H x 2	1.50	8.50
Z1J	0.99	9.01	Z1J x 2	1.25	8.75
Z1L	1.28	8.72	Z1L x 2	1.39	8.61
Z1N	1.17	8.83	Z1N x 2	1.35	8.65
Z1P	1.14	8.86	Z1P x 2	1.26	8.74

Absorbance

	2hr 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	0.0445	.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
21E	.1648	.1707	.7114	.7507
21Ex2	.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	.4597	.4583 4583	1.2309	1.2310

9/26

Digested

→ Acl 9, Acl 10, AD41
vector

·PSB backbone

disc

Disc 270

Acid, 10/10, AD 11

vector

help program

9/30/17

Transformations

AR09 + pC1DF - ADH + PET
 - pUC19 Shrep + amp
~~AR09 + pC1DF~~ and shrep plate
 ADH + pC1DF - AR010 + PET
 - pUC19
 AR09 + pacyc - ADH + pC1DF ~ chlor + shrep
 - AR010 + pC1DF + chlor plate
 AR010 + pacyc - ADH + pC1DF
 - AR09 + pC1DF
 ADH + pacyc - AR010 + pC1DF

10/1/17

Checked transformations - none were successful

Repeating cell competency procedure w/ overnight cultures

Absorbance for cell competency

ADH + pC1DF	0.4360
AR09 + pC1DF	0.3310
AR010 + pC1DF	0.3325
ADH + pacyc	0.6068
AR09 + pacyc	0.5601
AR010 + pacyc	0.4342

Cels

DLadda

ADH PET

ADH ~~PEPF~~ PCDF

ADH PCyC

ADH PCOLA

Ar09 PCyC

PDC PCDF

Ar09 PCDF

Ar010 PCyC

PDC PCyC

PDC PCOLA

Ar010 PCDF

Absorbance for cell comp.

ADH + pC1F + ARC10 + pET	0.4656
ARC9 + pET + ARC10 + pC1F	0.0945
ARC9 + pET + ADH + pC1F	0.4921
ARC10 (pC1F) + ADH (pET)	0.1445
ADH (paxc) + ARC10 (pET)	0.5662
ARC10 (pET) + ARC9 (pC1F)	0.5160

10/10/17

Used sybr safe ~~g~~ in gels.

ran 1 kb ladder

Success glow under UV light.