9:03 a.m.

(pLL1074)

- Received pLO1297 plasmid miniprep from Marybeth.

- Preparing LB Broth:
  - Put 25.04g Miller LB Broth into clean 1L Erlenmeyer flask.
  - About 1L of Milli-Q water was added
  - Split media into two 1L Erlenmeyer flasks, about 500mL each.
  - Sterilized LB media using the "Ben" autoclave, set to cycle #3 liquid
    - 1 hr purge time
    - 15 min sterilization at 121.0°C
    - Code is 9003!
  - Left at room temperature to cool @ 11:03 pm

- Electroporation of E. coli → Invitrogen, 10 pg/mL
  - Obtained pUC19 (vector control plasmid) and two 50μL vials of Top Ten electrocompetent E. coli (Invitrogen) from Marybeth
    - Transfer from -80°C straight to ice
  - Transferred 40 μL of E. coli suspension into labeled tubes, one for pLO1297 and one for pUC19.
  - Added 2 μL of appropriate plasmid DNA to each tube.
  - Incubated for 30 seconds on ice.
  - Transferred the mixture to appropriate electroporation cuvette kept on ice.
  - Electroporation apparatus was set to deliver pulse of 25 μF, 3.5kV, and 200 Ω resistance
    - Used Bio-Rad Gene Pulser Cuvette, 0.2cm electrode gap.
    - No arcing occurred in either of the pulses.
  - 1 mL of LB media (room temp) was added to each of the electroporation cuvettes
  - Cells were transferred to labeled, sterile 17 x 100 mm polypropylene tubes.
  - Cultures were incubated in the rotator @ 11:54 am. in the 37°C room.
  - Retrieved culture at 1:00 pm
  - For each culture, a plate was streaked (3 quadrants), and a plate was spread w/ 200 μL cell suspension.
  - Plates, once suspension was absorbed, was inverted and stored at 37°C
  - Remaining culture was stored in 4°C refridgerator.

1:20 pm Mary Knowles

Continued on Page 2

Read and Understood By

Isabel M. Burgess 4/25/17
Signed Date

Signed Date

MATERIALS
- prepped colonies
- LB + amp stock

PROCEDURE
- 4 tubes inoculated:
  - 2 ml of media
  - one colony

PLATE	OBSERVATIONS	INOCULATED?
ECOLI + PLC1297 SPREAD	too many colonies to count! May have to repeat w/ diluted sample.	1
ECOLI + PLC1297 STREAK	Little growth from starting point of streak. colonies too small to be useful?	1
ECOLI + PVC19 SPREAD	Great growth! Almost too dense to get colonies, but not so dense that we can't.	4
ECOLI + PVC19 STREAK	Little growth along edges + start of streak, but large enough colonies to be of use.	1

-Plates moved to 4°C teaching fridge.
- Tubes inoculated in 37°C room until Thursday. on shaker.

Continued on Page 3

Read and Understood By

AH 4/26/17
Signed Date Signed Date

12:29 pm 4/27/17

- E.c Freezing Samples from yesterday!
  - E.coli + pLC1297 streak solution does not look turbid; left in the 37°C shaker in the additional growth; doubtful if it will occur.
  - Made 1mL transferred 1mL of the spread (E. coli + pLC1297) suspension to a cryovial and the remainder to another
  - Transferred 1mL of the other cultures to their labeled, respective cryovials.
  - Cryovials were stored in our box in T1's shelf on the -80°C downstairs.
  - Remaining cell suspensions were put in the 4°C fridge.

12:51 pm Mwe[illegible]

Continued on Page

Read and Understood By

Signed Date Signed Date

PROJECT Confirmation of E. coli transformation by PCR

Notebook No. X

Continued from Page

MCL 2:39pm 5/10/17

- Incubation/Growth of X transformed E. coli cultures
  - Thawed a vial of E. coli + pLO1297 spread culture and a vial of E. coli + pUC19 spread culture.
  - Put into 5mL of LB + Amp media and incubated with aeration (shaker) in the 37°C room.
- Moved plates (LB+Amp & LB+Tet) to the 4°C room.

3:16pm Myungtaeglee

Continued on Page

Read and Understood By

Signed | Date | Signed | Date

PROJECT Mini Prep of E. Coli Transformation

Notebook No. 1

Continued from Page

DJ & IB 10:45am 5/11/17

- Centrifuging E. Coli Cultures (turbid) @ 3000 rpm for 10 minutes
- Pelleted pLO1297 & pUC19 retrieved and supernatant pipetted off.
- Mini prep of pLO1297 & pUC19 (using Qiagen Mini prep Kit)
  - Resuspended ea. pellet w/ 250 µL of P1 (RNase added)
  - Added 250 µL of P2 (Lysis Buffer) to ea. resuspension and inverted 4-6 times. (~2 minutes)
  - Centrifuging lysed samples @ 13000 rpm for 10 minutes.
  - Transfered 800µL of pLO1297 & 800µL of pUC19 supernatant to QIAprep 2.0 spin columns
  - Centrifuged @ 13,300 rpm for 45 seconds (Discarded Flow Through)
  - Added 500µL of PB (Binding Buffer) to ea. spin column
  - Centrifuged @ 13,300 rpm for 45 seconds. (Discarded Flow Through)
  - Added 750µL of PE (Wash Buffer) to ea. spin column
  - Centrifuged @ 13,300 rpm for 45 seconds (Discarded Flow Through)
  - Centrifuges @ 13,300 rpm for 60 seconds to remove residual wash buffer (Discarded Flow Through)
  - Added 50µL of DI H_2O to ea. spin column after transfering ea. spin column to a clean 1.5 mL microcentrifuge tube.
- Samples were labelled, dated and placed in -80°C in Lynd lab.

12:05 PM

R Joo

Isabel Burgess

Continued on Page

Read and Understood By

Signed | Date | Signed | Date

HJ 10:00 AM 5/16/17

- ~~Inoculation~~ Growth of transformed E. Coli a vial of
  - Thawed a vial of pLO12a7+ E.Coli ~~Spread~~ & pUC19 + E.Coli spread 2
  - Put into 5mL of LB (no AMP) Media
- Recovery of E. Coli cells & Inoculation / Growth of Recovered Cells
  - Centrifuged ea. Falcon tube @ 3500rpm for 10 minutes x2
  - 5mL of LB+ Amp media placed in 2 new labelled Falcon Tubes
  - pellet of E.coli pLO12a7 spread & pellet from E. Coli + pUC19 spread & ea. resuspended with 1mL of LB+AMP.
  - Resuspended E.Coli + pLO12a7 spread culture and E.coli pUC19 spread & Culture ea. transfered to 5mL LB+ Amp Media.
  - Aerated via shaker in 37°C room overnight

12:30 PM Hyejoo Jo

Continued on Page

Read and Understood By

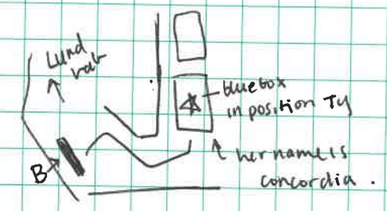
Signed _____ Date _____ Signed _____ Date _____

AJH 11:53AM 5/18/17

WORKING IN STERILE CONDITIONS
- WIPE BENCH W/ 70% ETOH
- TURN FLAME ON

- Suspensions removed from incubation @ 37°C
- 1 mL of suspensions transferred into cryovials (5 labelled pLO12a97 + 5 labelled pUC19 E.coli) any excess suspension placed back in 37°C jar
- cryovials placed in Ty's box @ -80°C

12:21 - AH



Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

MCL 5/19/17 2:12 pm

- Checked lab supplies & updated notebook
- PCR primers are not here yet, but had ordered and designed pLOI297 fwd primer and AdhII reverse primers from pLOI297 sequence.
- Established a primer list for lab.

2:22 pm Margot Longjohn

HJ 5/25/17 1:06 PM

- Diluted Primers from 100 µM to 10 µM
  - 1 µL of Primer mixed w/ 9 µL of milli-Q H_2O in DNAase free 1 mL eppendorf tubes.
- NanoDrop of DNA
  - pLOI247 concentration = 37.465 ng/µL
  - pUC19 concentration = 193.446 ng/µL
  - pLL1074 concentration = 75.5 ng/µL
- PCR
  - pLOI297 concentration used as ctl.
  - pLOI297 pre-transformed (pLL1074) diluted 1:2 (water)
  - pUC19 concentration diluted 1:4 (water)

	PLL1074	pUC19	WATER
TAB	①	②	③	④

- 50 µl rxn

FWD -	1 µL
REV -	1 µL
DNA	1 µL
OneTaq	25 µL
water	22 µL

- 35 cycles

Method:	STEP	TEMP	TIME
OneTaq Quick-Load 2x Master Mix w/ Std. Buffer.	Initial Denaturation	94°C	30 sec
	35 cycles	94°C	30 sec
		45-68°C	45 sec
		68°C	3 min
	Final Extension	68°C	5 min
	Hold	7°C	∞

Continued on Page

Signed (Dan) 2:30 pm Date

Signed (Alice) 5/25/17 Date

~~Culture of~~ PLV

Gel

0.3
- ~~1~~ g of agarose added to flask with ~~100~~ 30 mL TAE. Swirl to suspend.
- heat for 1 minute until clear
- Let cool to room temp & pour into tray to set.

- 1kb ladder used (10x)
- GelRed = dye for gel (3 µl)
- 14 µl product + 6 µl of loading dye.

Run @ ~~100~~ 120 V for ~~40~~ 45 min. cranked to V for last few minutes.

LANES							
1	2	3	4	5	6	7	8
- Ladder (1kb)	- pLOI247 undigested	- pUC19 undigested	- pUC19 EcoRI digest	- pUC19 BamHI digest	- pLOI247 EcoRI digest	- pLOI247 BamHI digest	- Ladder (1kb)

Culture
- media
  - LB + Amp.
    - tryptone (10g/L)
    - yeast extract (5g/L)
    - NaCl (5g/L)
  - Glucose (100g/L) or Lactose (100g/L) or xylose (80 g/L)
  - autoclaved @ 121°C for 15 min in distilled water.
- double concentration of glucose (200g/L) in water autoclaved for 45 min
- 50:50 mix of LB+Amp and glucose (50 mL each)

Restriction Digest: @ ~~37°C~~ 12:30 pm 7/24/17

- H_2O 7 µl
- Cutsmart 1 µl
- pUC19 or pLOI247 1 µl
- BamHI or EcoRI 1 µl @ 37°C for 1 hour. Margot Longjohn

Continued on Page

Signed Alice Date

Signed 7/24/17 Date

10
PROJECT _____

Notebook No. _____
Continued from Page 9

~~Preparing plates~~
take LB media + agar and pour into empty plates to cool.
+ amp.

To Do List
- make new plates
- digest product
- grow up new cultures
- run gel
- prepare cultures in ethanol media

~~Ethanol Producing Culture~~ @ 2:10pm 7/24/17

- 0.500 mL of pUC19 is added to 2.50 mL of LB+Amp and 3.00 mL of 20 g/L Glucose in dH_2O.
- 0.500 mL of pLOI2247 is added to 2.50 mL of LB+Amp and 3.00 mL of 200 g/L Glucose in dH_2O.
- Left to shake overnight in 37°C room

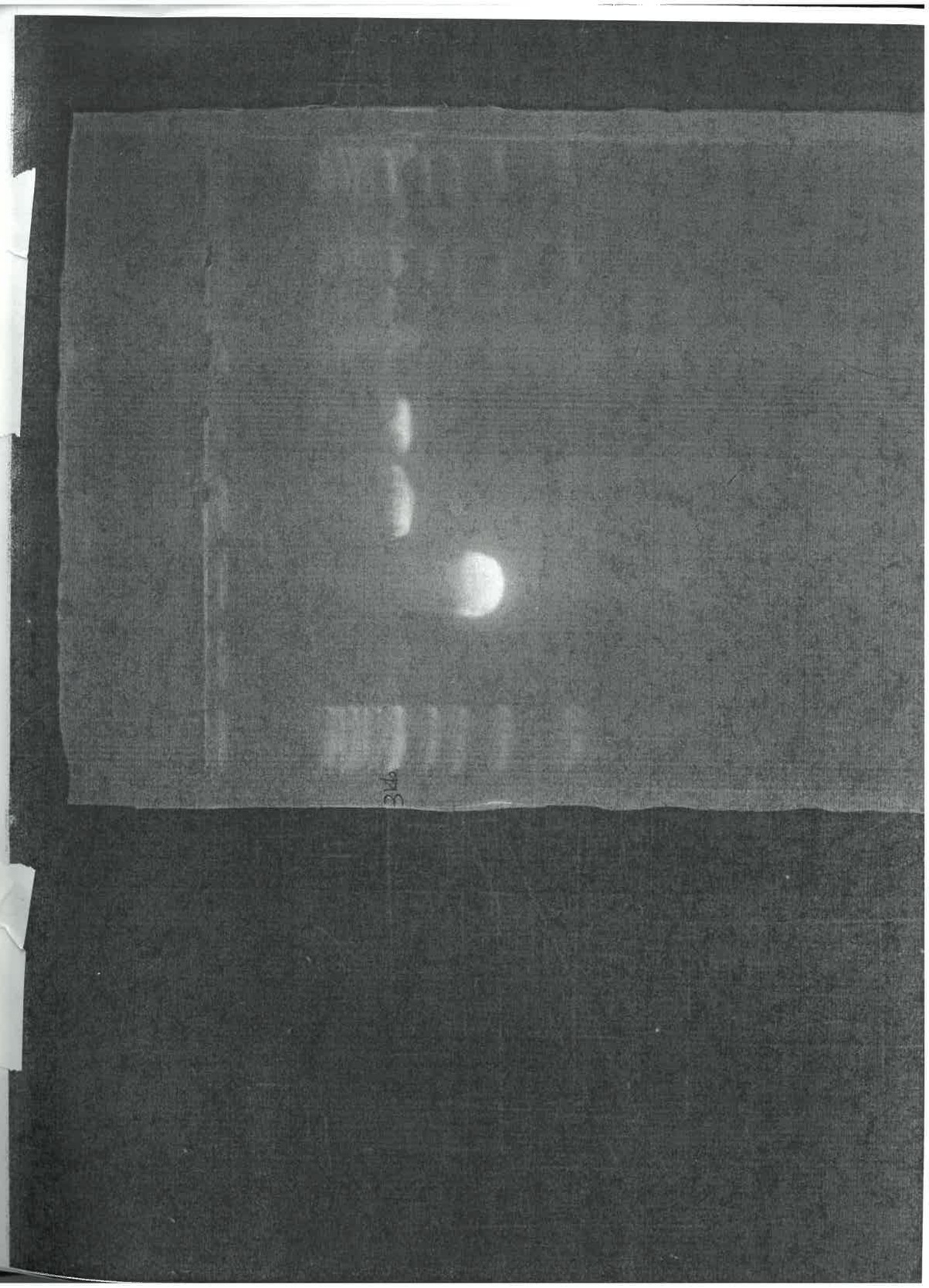
Plasmid Confirmation / Gel Visualization

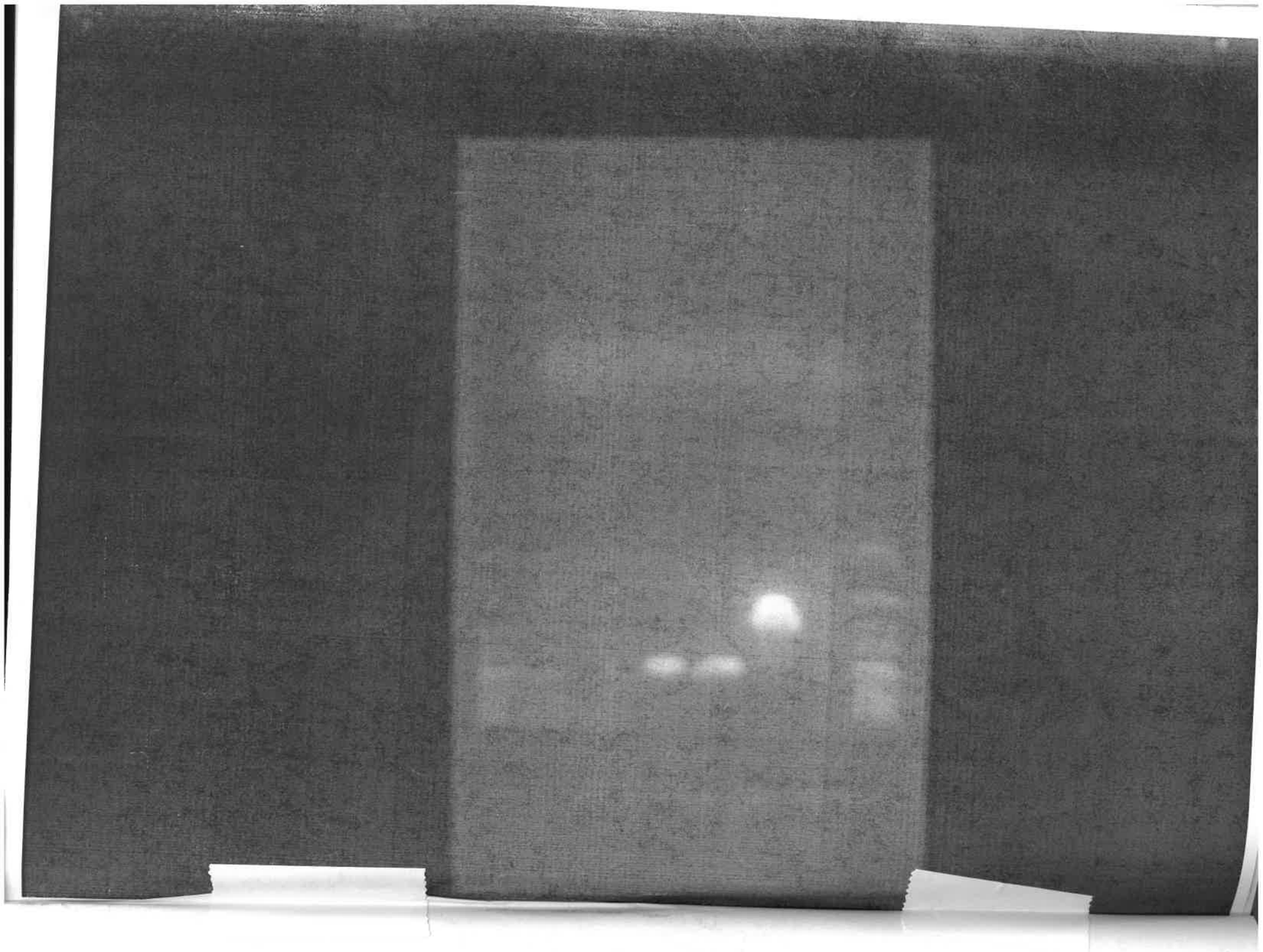
- ~~Go~~ Go to centrifuge room (1st room before autoclave room on the left)
- Place gel on UV desk, place shield over UV light, place switch on "High"
- To take picture, place picture mount on top of the UV desk
- Turn camera on
- Make sure the picture mount display and camera are both on and connected via a black cable.
- Take picture.
- Unplug black cable.
- Plug in white cable from computer
- Export Image.

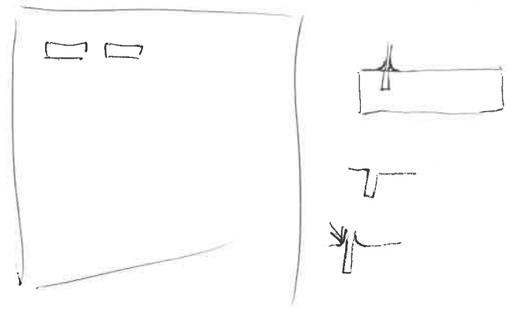
Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____







typical digest:

H_2O	6 µl
Cutsmart (10x)	1 µl
plasmid	1 µl
Bam HI	1 µl
Eco RI	1 µl
	10 µl

← 0.1 mL of pUC19 added to 2.90 mL of LB+Amp 3.00 mL of 20 g/L of Glucose + dH_2O.
repeat w/ tetra/LB.
incubated @ 37°C overnight.
plates incubated @ 37°C overnight.

← LB+Amp Plate streaked w/ pLOI2a7 using inoculating loop and Thawed pLOI2a7 stock used to make overnight cultures.

Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

MCL 7/5/17 8:10 a.m.

- Lab Meeting Notes:
  - B. subtillis - BOT as type strain
    - 9 g/L EtOH from 20 g/L glucose in one study
  - Geobacillus stearothermophilus
    - Got ATCC #, strain

- To do:
  - Look at theoretical maximum yield
  - Redo transformation

8:44 a.m.

Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

HPJ 8/9/17 8:35AM

- Electroporation of E. Coli
  - Invitrogen TopTEN One Shot electrocompetent cells acquired from Tyler Morgbeth (2 50 µL vials). pUC19 obtained from Morgbeth (1 10 µL vial)
  - 40 µL of E. Coli added to each labelled tube, one for pUC19 & one for pUC1074
  - 2 µL of ea. plasmid (pUC19 & pUC1074) added to ea. tube of cells
  - Incubate on ice for 30 seconds
  - Cell-plasmid solution transferred to 2 mm electroporation cuvettes, pre-chilled on ice.
  - Electroporation performed w/ electroporation apparatus set to 25 µF, 2.5 kV, 200 Ω resistance, 2 mm gap.
    i.e. (Pre-Set Protocols → Bacterial → 25 µF, 2.5 kV, 2 mm)
  - Cells pulsed, no arc observed
  - 1 mL of SOC added to ea. cuvette.
    - transferred the cells to Falcon culture tubes & placed in 37°C for 30 min to recover @ 9:40 AM
  - Cells (cultures) retrieved from 37°C room @ 9:40 AM
  - For each culture, one LB+Amp plate was streaked and one LB-Amp plate was spread w/ 200 µL of culture. After culture had absorbed, plates were placed in 37°C room, remaining culture was placed in 4°C room.

9:55 AM HPJ

Continued on Page

Read and Understood By

HPJ 8/9/17 _____ _____

Signed Date Signed Date

HDJJ 8/10/17 9:00 am

- Retrieved plates from 37°C Room
  - pUC19 streak, had 13 pickable colonies
  - pLL1074 streak, colonies were too small (24 more hours of growth)
  - pUC19 & pLL1074 200 µL spreads were too dense
- 50 µL & 10 µL (diluted in 100 µL LB) were spread for pUC19 & pLL1074 on LB+Amp plates.
- pUC19 colony was picked & placed in 4 mL LB+Amp + 1 mL 20% Glucose.
  - left in shak 37°C to grow overnight
- All plates placed in 37°C room overnight

Continued on Page

Procedure

- Retrieve samples from 4°C aliquot 1 mL of growth into mct.
- separate out growth vs no growth ^ centrifuge the vials with growth for 1 minute @ top speed 15000 rpm
- prepare standards (see table on pg 16) [Std 1x, 2x, 8x, Glu, EtOH, media]

glucose 20g/L : 0.2043g – 0.0074g in 10 mL
glucose 200g/L : 2.0184g – 0.0672g in 10mL

- add 20 µl of 10% H_2SO_4 to denature any lingering protein in filter tubes
- add 400 µl of each sample into filter tubes.
- centrifuge for 1 min @ 15000 rpm
- transfer all flow through (420 µl) into HPLC tubes.
- go get Dan to help with software.

pos

next pg for table of samples. →

calc:

100g/L = density (~~mg~~ g/mL) x
~~0.1g/mL~~ = 0.789 g/mL · X mL
100 g/L
10 x 0.127 mL = X
for 10 mL
Stock → 1.27 mL ethanol in water (10 mL)
for 10 ~~mL~~ → 0.12~~7~~ mL ethanol in water (10 mL)

for 10 mL glucose 200 g/L = ~~200~~ g 2 g / 10 mL H_2O
20 g/L = 0.2 g / 10 mL H_2O

Continued on Page

PROJECT HPLC

Notebook No. 1

Continued from Page 15

HPLC Vial #	Vial name	#1 Injection vol 40 ul	#2 Injection vol 40 ul
97	1		
98	2		
99	3		
100	4		
101	5		
102	6		
103	7		
104	8		
105	20 GL		— (stds not reinjected)
106	200 GL		—
107	10 EtoH		—
108	100 EtoH		
109	Std 1x		
110	Std 2x		
111	Std 8x		
112	~~Std 4x~~		

median

AJH 10:39

Continued on Page

Read and Understood By

Alice Hinn — Signed — 8/15/17 — Date

Signed — Date

PROJECT HPLC results

Notebook No.

Continued from Page

		height		Injection volume	Ret (min)
EtoH	100 g/L	422951	~~uV~~ uV	40 uL	
etoh	10 g/L	54781	uV	40 uL	21.5
Std 1X	(50 mM)	13500	uV		21.6
Std 2x	(25 mM)	6800	uV		
std 8x	(6.3 mM)	1642	uV		

Samples	
1	2ole
2	139
3	184
4	230
5	545
6	0 — didn't grow
7	220
8	255
M	27 — median

Glucose	20 g/L	40520	4 uL	9.46
Gluc	200 g/L	433738	4 uL	9.435

sample	
1	269082
2	27 5033
3	27 3093
4	25 3321
5	267028
6	281417
7	269156
8	262923
M	0

4 uL

Continued from Page

Read and Understood By

Signed — Date — Signed — Date

HDS 8:50am 9/13/17

Preparing LB + Glucose Liquid Media

- 12.5g of LB Broth was added to 500mL of DI H_2O in a 500mL bottle (x2) → Add 5g Glucose for 10g/L solution.
- bottles were autoclaved
- 1 bottle was mixed w/ 50mg ampicillin (carbenicillin)
- 1 bottle was mixed w/ 5mg tetracycline.
- stored in 4°C Fridge

Preparing LB + Glucose Solid Media

- 14g of LB+Agar was added to 350mL of DI H_2O in a 500 mL bottle (x2)
- Add 7g Glucose for 2% solution
- Bottles were autoclaved.
- 1 bottle was mixed w/ 0.35mg ampicillin (carbenicillin)
- 1 bottle was mixed w/ 3.5mg tetracycline
- Plates were poured after bottles cooled.

HJ. 9/14/17 4:00pm

Continued on Page

Read and Understood By

Signed Jennifer Jew Date 9/14/17 Signed _____ Date _____

HDS 9/18/17 2:40PM

-Electroporation of E. Coli

- Acquired Invitrogen TopTEN OneShot electrocompetent E. coli (2x 50μL vials)
- pUC19 from ;GEM -80°C shelf

Transferred

- 40μL of E.coli added to ea. labelled microcentrifuge tube (1 for ea. plasmid: pLL1074 & pUC19)
- Added 2μL of appropriate DNA plasmid to ea. tube
  - incubated for 30 seconds on ice.
  - Transferred mixture to appropriate electroporation cuvette kept on ice
  - Electroporation apparatus was set to deliver pulse @ 25mF, 2.5kV, and 200Ω resistance
    - Used Bio-Rad Gene Pulser (@ 0.1cm electrode gap)
    - No arching occurred.
  - 1mL SOC (@ room temp) added to ea. cuvette
  - Cuvette solution transferred to labelled, sterile 17x100mm polypropylene tube (culture tube)
  - Cultures were incubated @ 37°C for 45 minutes in rotator. @ 3:11pm

- Cultures retreived from 37°C rotator @ 4:55PM
  - 10μL of ea. culture was spread in 50μL of LB+ Amp + Glucose liquid media on a LB+ Amp+ Glucose plate.
  - Plates placed in 37°C shelf over night @ 4:10pm.

Continued on Page

Read and Understood By

Signed Jennifer Jew Date 9/18/17 Signed _____ Date _____

HDS 7/18/17 4:00PM

- Antibiotic Stock Solutions
  - Ampicillin (Carbenicillin) 50mg/mL stock
    - ~250mg of Carbenicillin
    - Weigh
    - Transfer carbenicillin to sterile orange cap tube (15mL)
    - Add DI H_2O till 5mL mark
    - Vortex solution
    - Filter sterilize solution w/ 10mL syringe & syringe filter into another sterile orange cap tube (15mL)
    - Label & store in 4°C fridge

- Tetracycline 5mg/mL stock in DMSO
  - weigh 25mg of tetracycline
  - Transfer tetracycline to sterile orange cap tube (15mL)
  - Add ~~DI H_2O till 5mL mark~~ DMSO till 5mL mark
  - Vortex solution
  - Filter sterilize w/ 10mL syringe & syringe filter into another sterile orange cap tube (15mL)
  - Label & store in 4°C fridge

HDS 9/18/17 5:00pm

Continued on Page

Read and Understood By

Signed 9/18/17 Date

Signed Date

HDS 9/19/17

- pLL1074
  - 90mL : 50mL cell: LB dilution had too many colonies
  - Innoculation loop used to gather approximately 2cm² of colonies
  - Colonies were resuspended in 100mL of LB+ Ampt Gluc liquid media
  - An LB + Ampt 2% Gluc plate was then streaked from the resuspension.
  - Plate placed in 37°C shelf overnight. @ 10:15 AM

Future Suggestions:
① Dilute 10mL : 1mL & serial dilutions (2x)

- pUC19
  - Plate showed many pickable colonies,
  - pUC19 may be more dilute than pLL1074 plasmid elution.

Both pLL1074 & pUC19 original spread plates were kept at Room. Temp. till next day

- Tetracycline Plating
  - 700µL of 5mg/mL Tetracycline in DMSO stock added to 350 mL of LB+ Agar + 2% Glucose solid media.
  - LB media mixed vigorously
  - Plates poured w/ approximately 25mL each in sterile hood.
  - Plates stored in 4°C after drying.

- Control Plates
  - TopTEN Electrocomp E. coli streaked w/o media or transformation onto LB+ Ampt 2% Glucose & LB + Tetra + 2% Glucose plates.
  - Plates placed in 37°C shelf overnight @ 11:18 AM

HDS 11:18 9/19/17

Continued on Page

Read and Understood By

HDS 9/19/17

Signed Date

Signed Date

HDJ 9/20/17 1:00PM

- pLL1074 ~~streak~~ - resuspension streak plate was retrieved from 37°C @ 11:15AM by Mary Deffa
- pUC19 Spread Plate
  - 10 colonies were picked
  - ~~10 each~~ colonies were placed in 2mL LB+Amp+10g/L Glucose each
  - ~~10 colonies were placed in 2mL LB+Amp+~~
- pLL1074 streak plate
  - 10 colonies were picked
  - each colony was placed in 2mL LB+Amp+10g/L Glucose each.
- All cultures placed in 37°C rotatory shaker @ 1:52PM & left overnight to grow.

Control Plates:

- Tetra plate w/ untransformed E.coli showed no growth.
- Amp plate w/ untransformed E.coli showed growth (negative control failed)
  - inoculation loop of E.coli was resuspended in 100µL of LB, Amp+Gluc & streaked on an LB+Amp+Gluc plate.
  - Plate placed in 37°C @ 2:00PM, overnight

HDJ 9/20/17 2:00PM

Continued on Page

HDJ 9/21/17 8:30AM

- Innoculations
  - All 10 pLL1074 tubes showed growth
  - All 10 pUC19 tubes showed growth
- Control Amp Plate (Negative Control)
  - Showed no growth

HDJ 9/21/17 9:00AM

Isolating / ~~Fluo~~ P. Fluorescens

HDJ 9/22/17 8:30AM

Continued on Page

HDJ 8:42AM 9/22/17

- Retrieve samples from 4°C (10x plate & 10x pCL1074)
  - aliquot 1mL of ea. tube into labelled microcentrifuge tube
  - centrifuge

pCL1074 : #1 x 1-10

pUC19 : #2 x 1-10

Standards:

200g/L	Gluc	1x
20g/L	Gluc	2x
100g/L	EtOH	8x
10g/L	EtOH	

(Type & Glucose Protocol)

~~APPROVED~~

HDJ 10:30AM 9/22/17

Continued on Page

Read and Understood By

Signed _____ Date 9/22/17

Signed _____ Date _____

HDJ 10:30AM 9/22/17

- P. Fluorescens streaks retrieved from 30°C room
  - 6 colonies picked & isolated in 2mL LB in labelled culture tubes
  - 6 cultures placed on shaker in 30°C room overnight @ 11:00 AM.

HDJ 11:00AM

HPLC Run Protocol

HDJ 2:40PM 9/23/17

- P. Fluorescens cultures retrieved from 30°C
  - Aliquoted into 500mL aliquots.
  - ea. aliquot was transfered into a 1.0mL cryovial w/ 500µL of 50% Glycerol
  - ~~18~~ 8 cryovials were placed in the -80°C shelf. (last i.e. 4th vial for ea. culture was discarded b/c was less than 500mL)

HDJ 3:10PM 9/23/17

Continued on Page

Read and Understood By

Signed _____ Date 9/22/17

Signed _____ Date 9/23/17

HDJ 9/22/17 11:00AM

Protocol:

A: Click Empower Logo
↓
Username + Pwd
↓
Click [OK]
↓
Project: Reference Fermentation or log: Ref. Ferm
Chrom. Syst: Sys B_acid ChromSyst:
↓
Click [OK]
↓
File → New Sample Set → Using Sample Set Method
↓
"Ref Ferm_Template Method" (bottom of list)
↓
(Before Opening Door)
Click on [View Acquisition] (make sure it is checked)
↓
~~Lock for~~
See if Other Samples are running
(via graph @ Bottom Left Corner)
to see time left till next injection
↓
look for Empty Rack (load samples)
by toggling through racks into racks DON'T PUT RACKS IN YET
↓
Set up Sample Table. Injection Volume: 40 uL
↓
Check for bubbles
↓
Put in Racks (if time)
↓
Hit PLAY
(Always Name Run)

HDJ 11:53 AM 9/22/17

Tube #		
1	49	~~pHL074~~ pUC19 :1
2		
3		
4		
5		
6		
7		
8		
9		
10	58	10
11	59	pHL074 :1
12		
13		
14		
15		
16		
17		
18		
19		
20	68	10
	69	1x
	70	2x
	71	8x
	72	200g/L Gluc
	73	20g/L Gluc
	74	100g/L EtOH
	75	10g/L EtOH

Continued on Page

Empower
↓
Login → ~~Advanced~~ Advanced
↓
Pro Interface
↓
~~Process~~
Ref_Ferm → Sys results. "iGEM pHL074 Test"
↓
Right Click → Alter Sample Set
↓
Process
↓
Use spec. process method
↓
"RefFerm. 99-2
~~Result Set~~ Result Set
↓
Switch from UV to RI channel
↓
Click the [button] button
↓
Click the [button] Next Injection button

Peak Relabeling:

1. [button] - Manual ID peaks button
2. Drag Glucose peak or peak of interest on to curve reading riding
3. File → Save All * Make Sure to Update Result List & Grab Most recent Result Set

Export Data
~~Results~~ → Result Sets → Right Click → Globe → File → Copy Table → Paste in Excel
"View As Peaks" ↓ Peaks
"Highlights All"

Continued on Page

Standard | Injection Volume | Ret (min)

Sample #	Vial	Ret (min)	Peak Height (μV)
pUC19	1		
	2		
	3		
	4		
	5		
	6		
	7		
	8		
	9		
	10		
pUC19 + EtOH	11		
	12		
	13		
	14		
	15		
	16		
	17		
	18		
	19		
	20		

Gel [illegible]: 9.5 min

EtOH
No EtOH
No EtOH
EtOH
No EtOH
NO EtOH
EtOH
EtOH
No EtOH
No EtOH

↑
Last of Plasmid

Reasons: ① Replicon issue?
② Antibiotic Resistance Marker?

Goals:
- Design Plasmid: Replicon, Antib. resistance Marker, iGEM requirements

Continued on Page

Read and Understood By

Signed | Date | Signed | Date

Continued on Page

Read and Understood By

Signed | Date | Signed | Date

HDS 9/29/17 1:26 Pm

- pMQ72 cloning vector obtained from George O'Toole group. in LB stab in E. coli carry strain.
  - vial w/ LB stab placed in 37°C for overnight culture @ 1:26 Pm

---

HDS 10/2/17 4:00 Pm.

- Gentamycin Antibiotic Stock
  - 50mg Gentamycin weighed and transferred into DI H_2O (5mL)
  - 10mg/mL stock
    - ~~[illegible]~~ µL for 10µg/mL
    - 10 ~~[illegible]~~ µL for 100µg/mL

- Innoculation of Colony from Stab
  - 10µL of Gentamycin stock added to 2mL of LB (no antibiotic) x2 (2 tubes prepared) in labelled tubes.
  - Stab retreived from 37°C shelf @ 4:00 Pm 10/2/17
  - Innoculation loop used to scrape colonies and deposite into ea. tube.
  - tubes placed in 37°C shaker over night @ 4:40 pm

Mini-Prep
- Retrieved tubes from 37°C @ 9:00 Am 10/3/17
  - Froze 2 aliquots of 250µL of culture ea. w/ 250µL 50% Glycerol in -80°C
  - Aliquoted 1.75 mL into 2mL micro centrifuge tubes
  - Spin @ 8000 rpm for 3 min
  - Discard supernatant
  - Resuspend pellets in 250µL of P1
  - Add 250µL of P2 to ea. tube & invert 4-6 times look for blue (Lyse Blue) indicating proper lysing
  - Add 350µL of Buffer N3 to ea. tube & invert till clear
  - Transfer to spin columns, centrifuge @ max speed for 10 min

Using Qiagen spin MiniPrep Kit

FAILED

Continued on Page

Read and Understood By

Signed | Date | Signed | Date

Mini Prep.

HDS 10/4/17 8:00 Am

- Retrieve ~~P. Fluorescens~~ cultures of pMQ72 in E. coli from 37°C
  - ✓ Transfer 2mL of ea. culture to a 2mL microcentrifuge tube.
  - ✓ Centrifuge @ 8000rpm for 3 min @ 25°C (room temp)
  - ✓ Resuspend pellet in 250 µL Buffer P1 & transfer to another microcentrifuge tube
  - ✓ Add 250 µL Buffer P2 & mix by inverting 4-6 times (until solution Blue) NO MORE THAN 5 Minutes before next step
  - ✓ Add 350 µL Buffer N3 & mix by inverting (until CLEAR)
  - ✓ Centrifuge for 10 min @ 13,000 rpm (or max rpm)
  - ✓ Transfer 800 µL of SUPERNATANT to QIAprep 2.0 spin column
  - ✓ Centrifuge for ~~30~~ 60s, discard flow through
  - ✓ Wash spin column w/ 0.75mL (500µL) Buffer ~~PE~~ PB
  - ✓ Centrifuge for 60s, discard flow through
  - ✓ Wash spin column w/ 750µL Buffer ~~PE~~
  - ✓ Centrifuge for 60s, discard Flow through
  - ✓ Centrifuge 1 min (Remove residual Wash Buffer)
  - ✓ Place spin column in Clean, Labelled 1.5mL · microf
  - ✓ Add 50 µL EB @ center of spin column, let stand for 1 min
  - ✓ ~~spin~~ Centrifuge 1 min.

Nanodrop: #1 260/280: Abs 1.86 [DNA]: 304.7 ng/µL
#2 260/280: Abs: 1.87 [DNA]: 2963 ng/µL

Restriction Enzyme Digest (NcoI, SpuMJ, BsgI, NdeI, PstI, SmaBI SacI, NcoI, StuI)

- Reagents added in listed quantities in following orders

DI H_2O	5 µL		
10x CutSmart	4 µL	45 µL	
DNA (pMQ72)	30 µL		
(Enzyme)	1 µL		

(Master) ⇒

DI H_2O •	33.4 µL	40 µL
10x CutSmart •	4 µL	
pMQ72 DNA •	1.6 µL	
Restriction Enzyme NcoI-HF •	1 µL	

- Mix Solution by pipetting
- Leave Rxn in 37°C for 45 min. @ 9:24 Am

Continued on Page

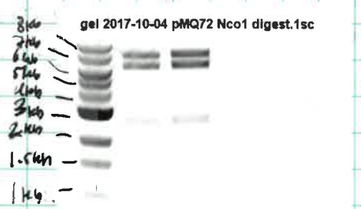
Read and Understood By

Signed | Date | Signed | Date

- Gel Prep:
  - Wet Gel holder & well
  - Fit Gel holder into well for pouring.
  - Prepare Gel by adding 50mL of TAE buffer x Flask
  - Add 1% Agar (0.5g) into TAE buffer
  - Heat in Microwave till clear (~1:30)
    occassionally stopping heating to swirl Flask
  - ~~Pour~~ Add 0.5µL of EtBr (1µL per 100mL)
  - Swirl, & pour w/o making bubbles into gel holder.
  - let gel solidify

- Running Gel:
  - Vortex loading buffer (15% Glycerol)
  - Add 6mL Loading buffer to eg. sample (3µL per 20µL sample)
  - Add 10µL of DNA ladder to left most well
  - Add 20µL of eg. sample into subsequent wells

PL 1 2 3



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Read and Understood By

Signed Date Signed Date

Prep.

RXN #1
- Primer #1: XDB44
- Primer #2: XDB45
- Template: pMQ72 DNA
- amplicon size: 3.1kb
- annealing temp: 65°C

50µL RXN.

Q5 Mastermix 2x	25µL
10µM XDB44	2.5µL
10µM XDB45	2.5µL
pMQ72 DNA	1µL (30ng/µL, 1µL=30ng)
DI H_2O	19µL

98°C 0:30
35x [98°C 0:10 / 65°C 0:50 / 72°C 1:50]
72°C 2:00
4°C ∞

RXN #2
- Primer #1 XDB47
- Primer #2 XDB48
- Template: pLOI297/pLL1074 DNA
- amplicon size: 1.7kb
- annealing temp: 67°C

50µL RXN

Q5 Mastermix 2x	25µL
10µM XDB47	2.5µL
10µM XDB48	2.5µL
pLL1074 DNA	1µL (8.9ng/µL×1µL=8.9ng)
DI H_2O	19µL

98°C 0:30
35x [98°C 0:10 / 67°C 0:30 / 72°C 0:51]
72°C 2:00
4°C ∞

RXN #3
- Primer #1 XDB49
- Primer #2 XDB50
- Template pLL1074 DNA
- amplicon size 1kb
- annealing temp 66°C

50µL RXN

Q5 Mastermix 2x	25µL
10µM XDB49	2.5µL
10µM XDB50	2.5µL
pLL1074 DNA	1µL (8.9ng/µL×1µL=8.9ng)
DI H_2O	19µL

98°C 0:30
35x [98°C 0:10 / 66°C 0:30 / 72°C 0:30]
72°C 2:00
4°C ∞

RXN #4
- Primer #1 XDB52
- Primer #2 XDB53
- Template pMQ72 DNA
- amplicon size 1.5kb

annealing temp. 72°C

50µL RXN

Q5 master mix 2x	25µL
10µM XDB52	2.5µL
10µM XDB53	2.5µL
pMQ72 DNA	1µL (30ng/µL×1µL=30ng)
DI H_2O	19µL

Continued on Page 34

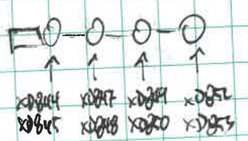
Read and Understood By

Signed Date Signed Date

	98°C	0:30
35x	98°C	0:10
	72°C	0:30
	72°C	0:45
	72°C	2:00
	4°C	∞

HDJ 10/6/17 3:00 PM

- Running PCRs.
  - Running ea. Rxn (A) Ea. of the 4 diff. annealing temps. (16 Rxn in total)
  - dilute Primers by 10x... → 50 µL Primer + 450 µL H_2O.
  - added ea. Rxn to PCR tube.



(Time Saver Qualified) HDJ 10/5/17 9:30AM.

- ~~Digest~~ Obtain EcoRV-HF & Cutsmart Buffer from -20°C fridge.

DI H_2O	33.4 µL	40 µL
10x Cutsmart	4 µL	
pMQ72 DNA	1.6 µL	
EcoRV-HF	1 µL	

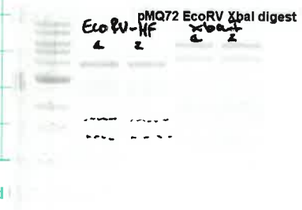
(Time Saver Qualified)

- Obtain ~~Nsi-HF~~ XbaI & Cut Smart Buffer from -20°C fridge

DI H_2O	33.4 µL	40 µL
10x CutSmart	4 µL	
pMQ72 DNA	1.6 µL	
EcoRV-HF	1 µL	

- All samples placed in *37°C for incubation @ 10:07 AM
- Gel Poured
  - 50mL of TAE buffer was poured in a Conical Flask
  - 0.5g (1%) Agar was added to buffe
  - Flask was microwaved for 1min 30sec, till buffe was clear & agar had dissolved.
  - Flask was cooled w/ water briefly & 0.5 mL of EtBr was added, mix by gentle swirling
  - Note: EXTRA CAUTION WHEN USING EtBr!!
  - Poured gel into gel holder. @ 10:14 AM
- Incubated digest samples retrieved @ 10:35 AM
- 6 mL loading Dye added to ea. sample, mix by stirring w/ pipette tip
- Samples & DNA ladder loaded in gel @ 10:45 AM
- Gel run @ 10:51 AM, set for 30 min.

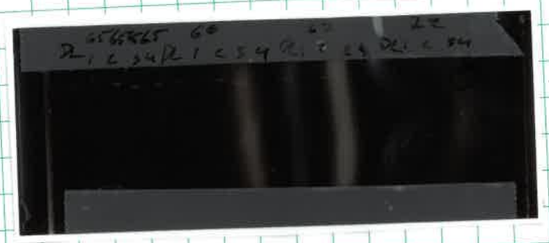
XbaI 2	1
XbaI 4	1
EcoRV-HF 2	1
EcoRV-HF 4	1
DL	1



HDJ 10/9/17 4:30 pm

- Gel Pouring
  - 100 mL TAE buffer + 1g Agar melted in Erlenmeyer
  - microwave for ~ 1:50
  - 1 µL EtBr added
  - Gel left to set @ 3:14 pm.
  - 2x 20 comb

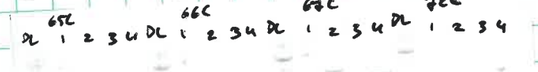
- PCR Rxns retrieved @ 4:40 pm.
  @15 minutes



DL	
72°4	
72°3	
72°2	
72°1	
67°4	
67°3	
67°2	
67°1	
DL	
66°4	
66°3	
66°2	
66°1	
DL	
65°4	
65°3	
65°2	
65°1	
DL	

- DNA Present
- @ 72°C, only 72°C annealing rxns showed bands.

@ 45 minutes. pMQ72 & pLL1074 PCR



DL 1 2 3 4 DL 1 2 3 4 DL 1 2 3 4 DL 1 2 3 4
65C 66C 68C 72C
2kb
1.5kb
1kb

* #'s refer to Rxn #, corresponding to PCR protocol on pg. 33.

- Samples loaded on gel @ 5:30 pm

Continued on Page

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Signed Date Signed Date

Loops, should have done this before (el) HDJ 10/10/17 8:00 am

- Gel prepped PCR products, kept @ 4°C overnight
- Zymo DNA Clean & Concentration kit used on Rxns. 2, 3, & 4 @ 67°C
  - Following Protocol:

Buffer Preparation

✓ *Before starting*: Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **DNA Wash Buffer** concentrate. Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA Wash Buffer** concentrate.

✓ DNA Wash Buffer included with D4001S and D4001T is supplied ready-to-use and does not require the addition of ethanol prior to use.

Protocol

All centrifugation steps should be performed between 10,000 - 16,000 x g.

1. In a 1.5 ml microcentrifuge tube, add 2-7 volumes of **DNA Binding Buffer** to each volume of DNA sample (see table below). Mix briefly by vortexing. ✓

Application	DNA Binding Buffer : Sample	Example
Plasmid, genomic DNA (>2 kb)	2 : 1	200 µl : 100 µl
PCR product, DNA fragment	5 : 1	500 µl : 100 µl
ssDNA[1] (e.g. cDNA, M13 phage)	7 : 1	700 µl : 100 µl

For efficient recovery of genomic or large DNA (> 20 kb to > 200 kb), use the **Genomic DNA Clean & Concentrator™ (Cat. Nos. D4010, D4011)**

2. Transfer mixture to a provided **Zymo-Spin™ Column**[2] in a **Collection Tube**. ✓
3. Centrifuge for 30 seconds. Discard the flow-through. ✓
4. Add 200 µl **DNA Wash Buffer** to the column. Centrifuge for 30 seconds. Repeat the wash step. ✓
5. Add ≥ 6 µl **DNA Elution Buffer**[3] or water[4] directly to the column matrix and incubate at room temperature for one minute. ✓ Transfer the column to a 1.5 ml microcentrifuge tube and centrifuge for 30 seconds to elute the DNA.

Ultra-pure DNA is now ready for use.

For **Assistance** please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com

Notes:

[1] For ssDNA purification see **Appendix A** on page 5.

[2] The sample capacity of the column is 800 µl. Therefore, it may be necessary to load and spin a column multiple times if a sample has a volume larger than 800 µl.

[3] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA

[4] Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH is >6.0. Waiting 1 minute prior to elution may improve the yield of larger (> 6 kb) DNA. For even larger DNA (> 10 kb), the total yield may be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**

DNA: 48 mL DNA : Buffer ⇒ 1:5
Buffer: 240 mL (kept)
* (3) 67 was spilled.

(3) 66 : 38 mL DNA : 190 mL Buffer
(4) 72 : 38 mL DNA : 190 mL Buffer

66(3): 260/280: 1.85 [DNA] = 309.9 ng/µL
67(2): 260/280: 1.86 [DNA] = 380 ng/µL
67(3): 260/280: 1.85 [DNA] = 482.3 ng/µL
67(4): 260/280: 1.87 [DNA] = 208.8 ng/µL
72(4): 260/280: 1.87 [DNA] = 38 ng/µL

- Nanodropped PCR products.

- Gradient PCR of Rxns

Prep 50 µL Rxns

Primer #1	XDB44
Primer #2	XDB45
Template	pMQ72 DNA
amplicon size	3.0kb
annealing temp	60°C - 75°C (2° increments)

25 Master Mix 2x	25 µL
10 µM XDB44	2.5 µL
10 µM XDB45	2.5 µL
pMQ72 DNA	1 µL (50 ng/µL → 3 ng)
DI H_2O	19 µL

72	98°C	0:30
72.1	98°C	0:10
69.4	60-75°C	0:30
65.9	72°C	1:30
63.5	72°C	7:00
61.4	4°C	∞
60		

35x

@ 10:06 AM

Continued on Page

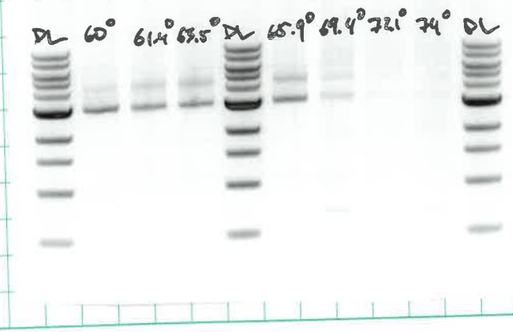
Read and Understood By

Signed Date Signed Date

- 66(3) → 4.5 µL + 40.5 µL H_2O = 30.5 ng/µL
  67(2) → 4.5 µL + 40.5 µL H_2O = 38.0 ng/µL
  67(3) → 4.5 µL + 40.5 µL H_2O = 48.2 ng/µL
  67(4) → 4.5 µL + 40.5 µL H_2O = 80.9 ng/µL
  72(4) → 4.5 µL + 40.5 µL H_2O = 38.1 ng/µL
- After Dilution, all samples were stored in -80°C

- Gel of Gradient PCR
  - Gel set @ 1:10 PM w/ 10 wells.
  - PCR products retrieved @ 1:30 PM, 2 µL diluted in 18 µL H_2O ⇒ 10x dilution.
  - Gel run @ 120V @ 2:00 PM for 45 min

Gradient PCR of RXN 4 (XDBUN & XDB45-QMQ72)



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Read and Understood By

Signed Date Signed Date

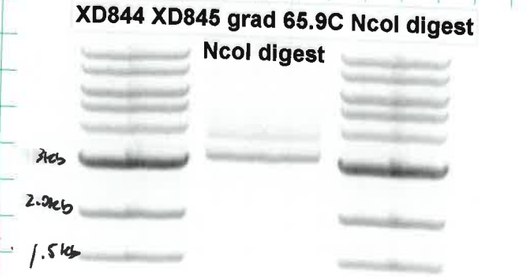
HDJ 10/10/17

- PCR Reaction for 65.9°C, used for NcoI-HF digest @ 4:00 PM
  - 3 µL 65.9°C PCR product used in digest for 30 µL digest

Protocol:

DI H_2O	23 µL
10x CutSmart	3 µL
65.9°C DNA	3 µL
NcoI-HF	1 µL
	30 µL

- 10x CutSmart Vortexed before usage
- Solution mixed by stirring w/ pipette tip
- Reaction left in 37°C till gel solidified
  - by @ 4:44 PM → taken out @ 5:37 PM
- Gel @ 110V for 1 hr @ 5:47 PM



DNA CLEAN & Concentrate of ~~PCR~~ Gradient PCR product @ 60°C

- PCR Product @ 60°C obtained
  - Protocol on p57 followed: 60 µL 48 µL DNA : 240 µL Buffer

60°C(1) : 260/280 = 1.85, [DNA] = 595.3 ng/µL

1.5 µL + 18.5 µL H_2O = 39.53 ng/µL

Continued on Page

Read and Understood By

Signed Date Signed Date

HDJ 10/18/17 12:50 pm

- 60(1), 67(2), 66(3), & 72(4) Fragments retrieved from the -80°C
- 6 blocks diluted to 12.5 ng/mL by 20 µL H_2O.
- 3 RXNs setup by following Protocol

Gibson Assembly Protocol

1. Setup the following reaction mix on ice

(Don't incubate)

		Volume (uL) Gibson (+)	Gibson (-)	Positive Control
Fragments	XD851 (gblock)	2.4	2.4	10 µL
	XD846 (gblock)	2.1	2.1	Pos. Ctrl.
	60(1)	✓2.4	2.4	
	67(2)	✓1.4	1.4	
	66(3)	✓1.0	1.0	
	72(4)	✓1.2	1.2	
Non-Fragments	Gibson Master Mix (2x)	✓10.0	0	10 µL
	H20	0	10	0
Total Vol		20.515	20.5	20 µL

2. Incubate @ 50 °C for 1 hour

3. Place reaction mixture on ice or in -20 °C after incubation

* Note: Gibson (-) only for Gel "DO NOT INCUBATE"

- RXNs were setup in PCR tubes.
- Incubate in Thermocycler, ie: PCR machine.
  - 50°C Forever → Step Step → 50°C 1:00 → 4°C Forever.
  - File → USERS → DAN → GIBSON → Pause (60 min)
- Incubation @ 1:38 pm.

- RXNs retrieved from 4°C thermocycler @ 4:50 pm.
- 10µL of Gibson (+) & (+)-control aliquoted & -80°C
- 10µL of Gibson (-), 10µL (+)-control, & 20µL Gibson (-) stained w/ DNA binding buffer & ran on gel

Continued on Page

120 V
- @ 15 min @ 5:30 pm

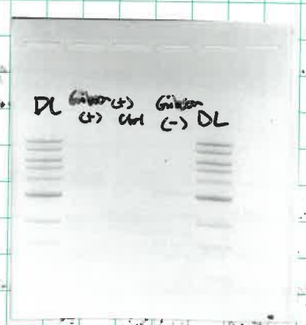
HDJ 10/12/17 Re: Gibson

pMQ72-DBa Gibson @ 15 min, 120V

DL
Gibson (-)
(+)
Gibson (+)
DL

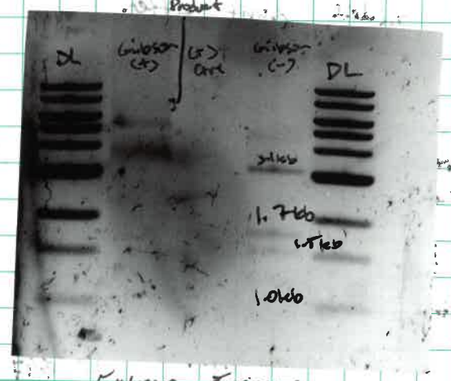
pMQ72-3Ba Gibson @ 75 min, 120V

Over exposed



Under Exposed

Desired Product: 7786 bp.



Extreme Exposure

Continued on Page

HDJ 7:00 PM 10/13/17

Preparing LB+ Glucose Plates
- Gentamycin plates made w/ Gentamycin Stock
  - 14g of LB+ Agar added to 350 mL DI H_2O in 500mL Bottle
  - Add 7g Glucose to bottle for 2% wt Solution
  - Autoclave
  - Add 35mg (100mg/mL), i.e. 3.5mL of 10mg/mL Gentamycin Stock.
  - Pour plates.

- Transformation of E. coli (Electrocompetent) by Electroporation
  - Retrieve Invitrogen TopTEN Electrocompetent E. coli from -80°C (3 vials)
  - Retrieve Gibson (+) & (+) control from -80°C
  - Make Gibson (-) mixture according to Gibson protocol on pg. 40.
  - Transfer 40μL of E. coli to ea. labelled microcentrifuge tube on ice.
  - Add 2μL of ea. DNA mixture (Gibson (+), (+) ctrl, Gibson (-))
  - Incubate on ice for 30 sec
  - Transfer mixture to labelled prechilled 0.1cm gap electroporation cuvette.
  - Electroporation apparatus set to pulse @ 25μF, 2.5kV, 200Ω
    - Bio Rad Gene Pulser @ Gibson (+) & (+) ctrl
  - 950μL SOC media added right after.
  - Culture transferred to LB media for recovery in 37°C for 45 minutes. @ 3:30 PM
  - Cultures retrieved from 37°C @ 4:38 PM
  - 100μL plated in ~~streak~~ spread for ea. culture. @ 5:25 PM
  - ~~100x & 10,000x dilutions plated by taking 10μL and diluting in 990μL of LB.~~
    - Gibson (+) & Gibson (-) → LB + Gent + 2% Gluc.
    - For (+) ctrl → LB + Amp + 2% Gluc

HDJ 5:30 PM 10/13/17

Continued on Page

Read and Understood By

Signed 10/13/17 Date Signed Date

HDJ 3:00 PM 10/13/17

- Retrieve NEB 5-α Competent E. coli (2 vials) 50μL ea.
  - Thaw tube on ice for 10 min
  - Add 2μL of ea. DNA (Gibson (+) & (+) ctrl) to cell mixture.
  - Mix by flicking 4-5 times
  - Place mixture on ice for 30 min.
  - Heat Shocke @ 42°C for 30 sec.
  - Pipette 950μL SOC into ea. tube
  - Place in 37°C for 60min,
  - Cultures retrieved from 37°C @ 5:45 PM
  - 100μL NEB 5-α (+) ctrl plated on LB Amp + 2% Gluc
  - 100μL NEB 5-α Gibson (+) plated on LB+ Gent + 2% Gluc
  - 10μL Gibson (+) diluted w/ 990μL LB → 100× Gibson (+)
  - 50μL 100× Gibson (+) plated on LB+ Gent + 2% Gluc.
  - 10μL 10× Gibson (+) diluted w/ 90μL LB → 100× Gibson (+)
  - 50μL 100× Gibson (+) plated on LB+ Gent + 2% Gluc
  - ~~40μL 100× Gibson (+) diluted w/ 90μL LB → 1000× Gibson (+)~~
  - ~~50μL 1000× Gibson (+) plated on LB+ Gent + 2% Gluc~~

- Plates placed in 37°C @ 5:50 PM

Continued on Page

Read and Understood By

Signed Date Signed Date

10/14/17 11:30pm

- Plates Retrieved from 37°C shelf @ 11:30 pm:

Electroporation	Colony #
(+) Ctrl	1000+
Gibson (-)	100+
Gibson (+)	~50

Chemical (NEB 5-α)	
(+) Ctrl	200+
Gibson (+) 1x	
Gibson (+) 10x	
Gibson (+) 100x	

100mL 2000g/L glucose + 1900mL LB + 20mL Gent Stock

- Electroporated colony inoculation
  - 8 colonies picked & inoculated in 2mL LB + Gent + 10g/L ea. for pMQ72-BBa.
  - 8 colonies picked & inoculated in 2mL LB + Gent + 10g/L ea. for pMQ72-BBa

- 1x, 10x, 100x returned to 37°C
- Cultures placed in 37°C @ 12:30 pm

Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

10/16/17 4:50pm

- Miniprep of Gibson (+) Culture colonies
  - Total Cultures started from 37°C @ 9:50pm.
  - QIAprep Spin Miniprep Kit used. (courtesy Cynthia Lab)
  - 1mL of ea. culture added to a labelled mct (1.5mL) ✓
  - centrifuged @ 8000 rpm for 3 min ✓
  - Supernatant Saved. ✓
  - Pellet resuspended w/ 250µL Buffer P1 ✓
  - 250µL Buffer P2 added, mixed by inverting 4-6 times. ✓
  - Add 350µL Buffer N3, mix by inverting 4-6x, until colorless. ✓
  - Centrifuge for 10 min @ max rpm. ✓
  - Apply 800µL Supernatant to QIAprep 2.0 spin column. ✓
  - Centrifuge 60 60s. ✓
  - Discard Flow through ✓
  - Wash w/ 500µL Buffer PB ✓
  - Centrifuge 60s. ✓
  - Wash w/ 750µL Buffer PE ✓
  - Centrifuge 60s. ✓
  - Discard Flow through. ✓
  - Centrifuge 60s, remove residual PE. ✓
  - Place spin column in New labelled 1.5mL mct. ✓
  - Elute DNA w/ 50µL DI H_2O, sit 1min. ✓
  - Centrifuge 1min.

	pMQ72-BBa	[DNA] (ng/mL)	Gibson (-)	260/280	[DNA] (ng/mL)
1	260/280: 1.85	186.7	1	1.86	116.5
2	260/280: 1.85	94.0	2	1.84	175.1
3	260/280: 1.84	106.3	3	1.82	110.6
4	260/280: 1.88	168.5 (Epoch)	4	1.88	104.4
5	260/280: 1.91	105.9	5	1.88 GB	167.2
6	260/280: 1.87	64.6	6	1.79	147.8
7	260/280: 1.88	121.9	7	1.85	110.2
8	260/280: 1.93	71.4	8	1.77	121.7

- 0.5mL of ea. pMQ72-BBa culture added to 0.5mL 50% Glycerol & put in −80°C.

Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

HPJ 10/16/17 6:05pm

- Digest of pMQ72-B3a w/ AlfII, EcoRV-HF, & MscI

↳ Digest:

DI H_2O	23 units	
10x CutSmart	3 µL	= 30 µL
pMQ72-B3a Prep	3 µL	
Enzyme	1 µL	

	1	2	3	4	5	6	7	8
MscI								
EcoRV-HF								
AlfII	·	·	·	·	·	·		

↳ Placed in 37°C @ 7:30 PM

↳ Taken out @ 8:75 PM

Continued on Page _____

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

HPJ 10/16/17 7:30pm

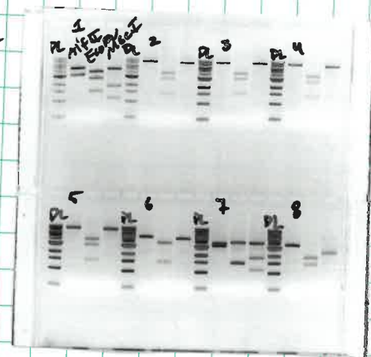
- 100mL gel cast w/ 8% 16-lane combs.
↳ 100mL TAE buffer + 1g Agar dissolved in microwave (~1:50min)
↳ 1µL EtBr added & gel poured into mold @ ~7:40 PM

Gel Setup:

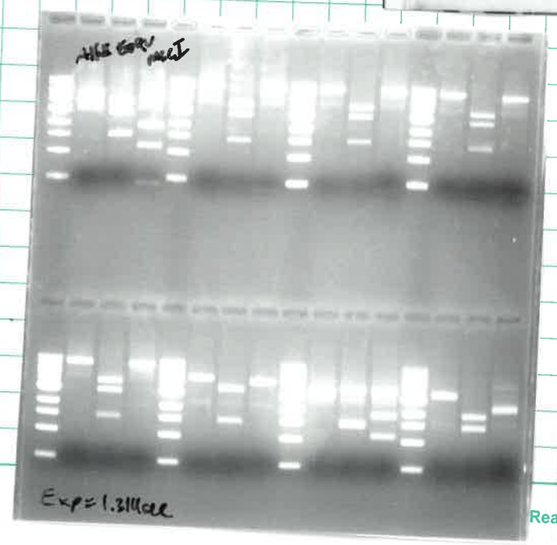
	DI	AlfII (1)	EcoRV MscI (3) (1)	DI	AlfII EcoRV MscI (2)	AlfII EcoRV MscI	AlfII EcoRV MscI	AlfII EcoRV MscI
µL	5	5	5	2	6	6	6	7	7	7	8	8	8

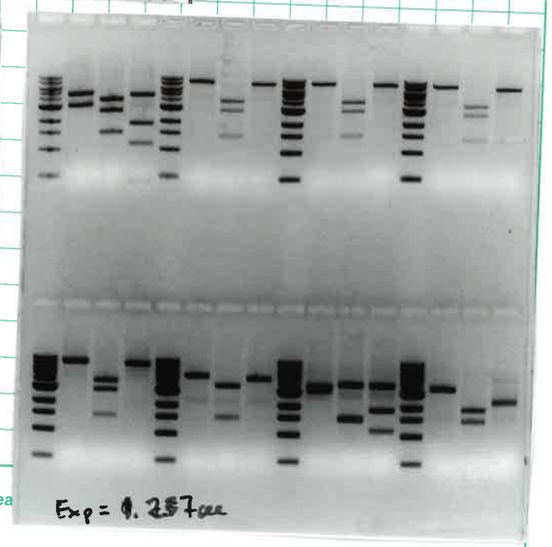
↳ 3µL / 30µL solution ⇒ 9.5µL loading dye added to ea.
↳ Gel set to run @ 8:40 PM @ 175 V for 45 min.

pMQ72-B3a AlfII, EcoRV, MscI digest →



Expected Bands:
Exp-0.7(7500 AlfII: 3.1kb, 4.7kb
EcoRV: 1.5kb, 2.6kb, 3.7kb
MscI: 420bp, 1.1kb, 1.8kb, 4.4kb





Rea

Signed _____ Date _____ Signed _____ Date _____

HPJ 10/17/17 9:00AM

- Retrieve Supernatant of Colony 1 from 4°C.
  - Aliquot 1ml into microcentrifuge tubes & centrifuge dwn.
  - Prep. standards
    (200 g/L Gluc, 100 g/L Gluc, 100 g/L EtOH, 10 g/L EtOH)
    - add 20 µL 4% H_2SO_4 (supernatant)
    - add 400 µL of ~~filtrate~~ to filter tubes (Spin-X)
    - centrifuge @ 15000 rpm for 1 min
    - Transfer Flow through (420 µL) to HPLC tube
- ~~Load HPLC~~
- Retrieve Fermentation HPLC standards (1x, 2x, 8x) retrieved From Shaker's & fridge (4°C.

25 µL pMQ72-SBa 1

200 g/L Glucose	32
100 g/L Glucose	16
100 g/L EtOH	8
10 g/L EtOH	1
	2
	8

Continued on Page

Read and Understood By

Signed Date Signed Date

HPJ 10/17/17 10:00AM

- Retrieve Gibson (+) & Gibson (-) from -80°C
  - Retrieve CutSmart & DpnI enzyme from Shaker.
  - Thaw and Vortex CutSmart

Follow:

DI H_2O	~~79~~ 86 µL
CutSmart 10X	10 µL
Gibson (+)	~~10~~ 3 µL
DpnI	1 µL
	100 µL

DI H_2O	79 µL ✓
CutSmart 10X	10 µL ✓
Gibson (-)	10 µL ✓
DpnI	1 µL ✓
	100 µL

- Digests Placed in 37°C @ 11:20 AM for 1 hour.
- Digest Placed on ice @ 12:37 PM

~~- Transformation of E. coli w/ DpnI digest (Electroporation)~~
~~- Retrieve 1 50 µL vial of Mix N Go Shot Electrocompetent E. coli from -80°C~~
~~- Prechill 2x 1mm electroporation cuvettes~~

Continued on Page

Read and Understood By

Signed Date Signed Date

PROJECT DNA Clean & Concentration of DpnI digest

Notebook No.
Continued from Page

HDJ 10/17/17 1:00PM

- Use ZYMOGEN DNA Clean & Concentrate 5 kit
- in 1.5 mL ~~__~~ mct add 100 µL of DpnI digest & 200 µL DNA binding buffer (~~2~~ 1:2 DNA: Binding) — refer to pg 57 — AJ
- Vortex mixture
- Transfer mixture to a Zymo Clean Spin Column
- Centrifuge 30 s
- Discard Flow Through
- Add 200 µL DNA Wash Buffer.
- Centrifuge 30s
- Add 200 µL DNA Wash Buffer
- Centrifuge 30s
- discard Flow Through
- Add 10 µL DI H_2O, incubate 1 min
- Transfer Spin column to clean 1.5 mL mct
- Spin 30s.

	260/280	[DNA]
Gibson (+) DpnI	2.11	3.2 ng/µL
Gibson (-) DpnI	1.40	9.9 ng/µL

Continued on Page

Read and Understood By

Signed Date Signed Date

PROJECT Transformation of E. coli w/ Gibson(+)/(-) DpnI (Electroporation)

Notebook No.
Continued from Page

HDJ AJ 10/17/17 1:30PM

- Electroporation — Invitrogen
  - Retrieve 1 vial 20 µL One Shot TOP10 Electrocompetent E. coli
  - Use Clean & ~~Concentrate~~ Concentrated Gibson (+) DpnI & Gibson (-) DpnI
  - Add 10 µL of E. coli into 1.5 mL mct (2x)
  - Add 2 µL of Gibson (+) DpnI ~~or~~ & Gibson (-) DpnI ea.
  - Incubate for 30 sec on ice
  - Transfer mixture to labelled, prechilled 1mm gap Electroporation cuvette
  - Electroporation apparatus set to pulse @ 25 µF, 2.5 kV, 200Ω
    - BioRad Gene Pulser
    - Gibson (-) DpnI $\frac{\text{Circuit}}{\text{[illegible]}}$ arced @ 2.5 kV
  - 950 µL SOC media added immediately after.
  - ~~Culture transferred to 15 mL media~~
  - Cultures transferred to culture tubes & incubated @ 37°C for recovery (45 minutes) @ 1:52pm & @ 2:05pm @ 2:41pm
  - Cultures removed from 37°C @ 2:43pm @ 3:24pm
  - 100 µL plated in spread for ea. culture on ~~LB+~~ LB+ Gent + 2% Gluc. plate. @ 4:40 pm

* ~~40µ~~ 1 µL Gibson (-) DpnI into 9 µL DI H_2O for 10x dilution.
- Transform 2 µL 10x dilution Gibson (-) DpnI into 10 µL E. coli.

HDJ 10/17/17 3:30 PM

Innoculation of ~~E.coli~~ Cultures from E.coli + (Gibson (+)) Electroporation plate
- ~~St~~ LB + Gent + 20g/L Glucose media prepared ~~[illegible]~~
  - 1900 µL LB + 100 µL 200g/L Glucose + 20 µL Gentamycin Stock (10mg/mL)

added to a Culture Tube (push cap)
- 16 colonies picked & innoculated in 2 mL LB + 100 µg/mL Gent + 10g/L Glucose
- Cultures placed in 37°C @ 6:45PM

* New Media is actually 10g/L Glucose.

Continued on Page

Read and Understood By

Signed Date Signed Date

10/18/17 2:45pm.

- 16 colonies (pMQ72-BBa (1a-16a) retrieved from 37°C @ 2:45pm.
- ✓ 1mL of ea. aliquoted to appropriately labelled 1.5mL mict ✓
- ✓ Centrifuged @ 8000rpm for 3min. ✓
- ✓ Supernatant Saved in 1.5mL mict (Separate & Labelled) ✓
- ✓ pellet resuspended in 250μL P1 buffer. ✓
- ✓ Add 250 μL P2 buffer, mix by inverting (4-6x), should turn blue
- ✓ Add 350 μL ~~Bad~~ N3 buffer, mix by inverting (4-6x), should turn clear white
- ✓ Centrifuge @ 13000rpm for 10min.
- ✓ Apply 800μL supernatant to QIAprep 2.0 spin column
- ✓ Centrifuge 60s, discard Flow through
- ✓ Wash w/ 500μL PB buffer
- ✓ Centrifuge 60s, discard Flow through
- ✓ Wash w/ 750μL PE buffer
- ✓ Centrifuge 60s, discard Flow through
- ✓ Centrifuge 60s, to remove residual buffer.
- ✓ Place QIAprep 2.0 spin column in clean, labelled 1.5mL mict
- ✓ Extract w/ 50μL H_2O, incubate 1min
- ✓ Spin for 60s.

Sample	260/280	[DNA] (ng/μL)
1a	1.76	~~1887~~
2a	1.78	205.2
3a	1.87	216.1
4a	1.87	143.0
5a	1.88	197.7
6a	1.79	54.6
7a	1.87	88.4
8a	1.87	119.9
9a	1.72	70.1
10a	1.89	~~146.8~~
11a	1.87	141.1
12a	1.82	196.6
13a	1.89	173.2
14a	1.87	85.6
15a	1.87	185.6
16a	1.71	227.3

- All cultures ~~cryo~~ transfered 0.5mL into cryovial w/ 0.5mL of 50% glycerol

Continued on Page

Read and Understood By

Signed Date Signed Date

10/18/17 4:30pm

- Digest of 1a-16a pMQ72-BBa w/ AflII, EcoRV, MscI

Digest

DI H_2O	23μL
10x Cutsmart	3μL
pMQ72-BBa DNA	3μL
Enzyme:	1μL
total	30μL

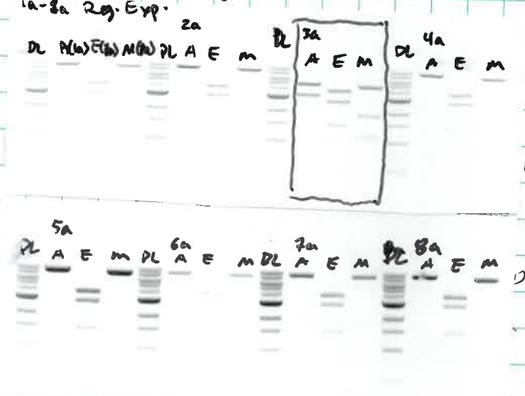
Expected Bands:
AflII: 3.1kb, 4.7kb
EcoRV: 1.5kb, 2.6kb, 7.7kb
MscI: 420Bp, 1kb, 1.8kb, 4.4kb

A = AflII
E = EcoRV-HF
M = MscI

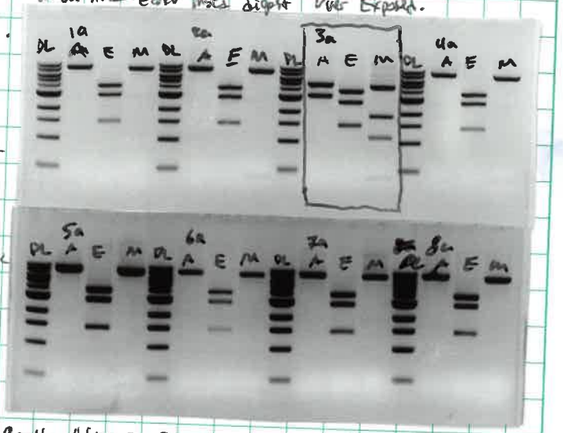
- Placed in 37°C @ 5:30pm
- retrieved from 37°C @ 7:00pm

- 4.5μL loading dye added to ea.
- Gel run @ 7:40pm

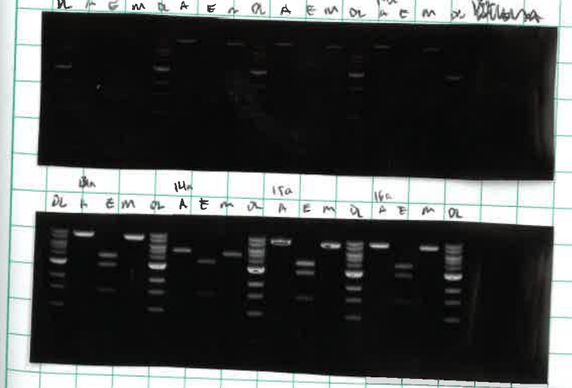
1a-8a Reg. Exp.



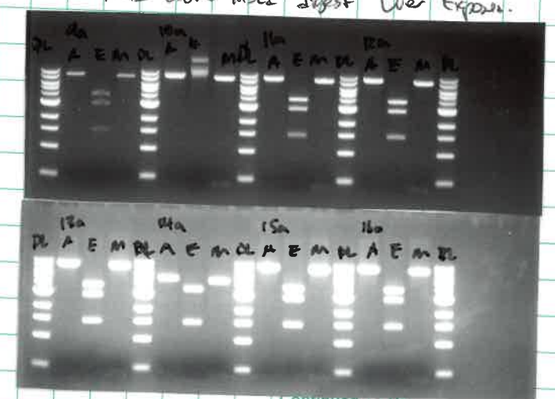
1a-8a AflII EcoRV MscI digest Over Exposed.



9a-16a EcoRV MscI digest Regular Exposure



9a-16a AflII EcoRV MscI digest Over Exposed.



* Colony 3a showed all proper bands, indicating ~~Astos~~ Gibson (x), successful transformation.

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Read and Understood By

Signed Date Signed Date

10/18/17 HDJ 7:40pm

- 1mL of pMQ72, pMQ72_BBa Col1, & LB+Gent+ 20g/L media centrifuged aliquoted & centrifuged 1min @ 15000 rpm.
  - 400µL of supernatant applied to SpinX HPLC ~~col~~ column
  - 20µL of H_2SO_4 added.
  - Centrifuged for 1min @ 15000 rpm.
  - Transfer ~~flow~~ flow through to HPLC vial
  - Prepare 20g/L Glucose, 2g/L glucose, 100g/L EtOH, 10g/L EtOH, 1x, 2x, 8x in similar fashion
  - ~~1x~~ ~~2x, 8x~~
  - Stored in 4°C @ 8:15 pm.

Continued on Page

Read and Understood By

Signed | Date | Signed | Date

HDJ 10/18/17 6:00pm

- ~~Plates~~ pMQ72-BBa Col1 DpnI, Gibson(-) DpnI, Gibson(-) 10x DpnI plates retrieved from 37°C @ 2:45pm.
  - 20 colonies found on pMQ72-BBa Col1 DpnI
  - 0 colonies on either Gibson(-) DpnI or 10x Gibson(-) DpnI.
  - All 20 colonies picked into 10mL of LB+Gent+ 20g/L Glucose.

Media: 1800µL LB+ 200µL 200g/L Glucose + 20µL 100mg/mL Gentamycin.

- Culture placed at 37°C rotator @ 7:00pm

HDJ 10/19/17

HPLC prep of pMQ72-BBa Col1 DpnI colonies. /Mini prep of pMQ72-BBa Col1 Colonies.

- Colonies retrieved from 37°C @
  - 1mL aliquoted to labelled 1.5mL mct
  - Spin @ 8000 rpm for 3min.
  - SAVE Supernatant for HPLC in 1.5mL mct (Separate & labelled)
  - Pellet Resuspended in 250µL P1 buffer.
  - Add 250µL P2 buffer mix by inverting (4-6x), should turn blue
  - Add 350µL N3 buffer, mix by inverting (4-6x), should turn colorless
  - Centrifuge @ 13,000 rpm, for 10min.
  - Apply 800µL supernatant to QIAprep 2.0 spin column
  - Centrifuge 60s, discard flow through
  - Wash w/ 500µL PB buffer
  - Centrifuge 60s, discard Flow through
  - Wash w/ 750µL PE buffer
  - Centrifuge 60s, discard Flow through
  - Centrifuge 60s, discard flow through, to remove residual PE buffer.
  - Place QIAprep 2.0 spin column in clean, labelled 1.5mL mct
  - Extract w/ 50µL Hyclone H_2O, incubate 1min.
  - Spin 60s.

Nanodrop →

Continued on Page

Read and Understood By

Signed | Date | Signed | Date

PROJECT Nanodrop of Miniprep's pMQ72-BBa Col DpnI

Notebook No. _____

Continued from Page _____

HDJ 10/19/17 10:00AM

Sample	260/280	[DNA] (ng/μL)	Sample	260/280	[DNA] (ng/mL)
1	1.83	86.3	11	1.76	99.6
2	1.87	74.3	12	1.73	120.1
3	1.84	60.3	13	1.74	297.9
4	1.91	52.7	14	1.91	60.4
5	1.82	100.1	15	1.85	183.2
6	1.71	148.7	16	1.61	184.7
7	1.72	180.5	17	1.63	126.4
8	1.73	102.4	18	1.75	385.7
9	1.89	64.4	19	1.68	297.3
10	1.67	181.2	20	1.76	101.5

Continued on Page

Read and Understood By

Signed Date Signed Date

PROJECT HPLC Prep of pMQ72-BBa Col DpnI colonies.

Notebook No. _____

Continued from Page _____

HDJ 10/19/17 ~~4:00~~ 2:00pm.

- 1 mL of supernatant centrifuged @ 15,000 for 1 min.
  - 400 μL aliquoted to a Spin-X HPLC Spin column.
  - 20 μL H_2SO_4 added.
  - Spin @ 15,000 for 1 min

HDJ 10/20/17 2:54pm.

Vial #	Sample	Inj. 1 Vol 40 μL	Vial #	Sample
49	1X		101	DpnI 15
50	2X		102	16
51	0X		103	17
52	100 g/L EtOH		104	18
53	10 g/L EtOH		105	19
54	20 g/L Glucose		106	20
55	2 g/L Glucose		107	
56	pMQ72		108	
57	pMQ72-BBa Col		109	
58	Blank		110	
59	DpnI 1			
60				
61				
62				
63				
64				
65				
66				
67				
68				
69				
70				
71				
72	4			

Continued on Page

Read and Understood By

Signed Date Signed Date

HDJ 10/20/17
EJS 4:00pm

- Prep P. fluorescens cultures from P. Fluorescens 1
- -80°C stocks
  - 8 tubes labelled.
  - 2mL LB media added to ea. tube,
  - inoculation loop scratched into of -80°C stock & inoculated culture.
  - Tubes 5-8 placed in 37°C @ 4:30 pm
  - Tubes 1-4 placed in 30°C @ 4:30 pm

* Grew in 30°C only, 1/4 cultures showed growth in 37°C while 4/4 cultures showed growth in 30°C

- Cells taken out of 30°C & 37°C @ 6:20 pm.
- Cells placed in 4°C @ 6:40 pm.

Preparing P. Fluorescens Competent cells.

HDJ 10/23/17
1:00 pm

- 300mM Sucrose solution provided by O'Toole Group (5.13g Sucrose in 50mL H_2O)
  - ✓ 8.0mL of overnight culture, retrieved from 4°C, ~~store~~ were aliquoted into 8x 1.5mL tubes
  - ✓ Cells centrifuged for 2min @ 16,000 x g, discard supernatant
  - ✓ Resuspend cells w/ 1mL of 300mM sucrose each, resuspend by pipetting up & down
  - ✓ Centrifuge for 2min @ 16,000 x g, discard supernatant
  - Resuspend cells w/ 1mL of 300mM sucrose ea., resuspend by pipetting up & down
  - Centrifuge for 2min @ 16,000 x g, discard supernatant
  - Resuspend final in 100µL 300mM Sucrose.
  - 3 vials used for transformation, the rest placed in -80°C.

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20 x 35/10
70/10

Liquid media

HDJ 10/20/17
PJS 5:00pm

- Liquid LB + 20g/L Glucose 350
  - ~~[illegible]~~ g of LB to ~~[illegible]~~mL of DI H_2O
  - in a 500mL bottle.
  - Add 7g of Glucose
  - Autoclave Bottle., bottle finished autoclaving @ 6:05pm
  - Add. 350 µL of Concentrated Stock.

5

100mg/mL
350, 100µL/mL

- 100mg/mL concentrated Gentamycin Concentrated Stock
  - 500mg of Gentamycin into 5mL of H_2O
  - Syringe filter into 15mL centrifuge tubes.

Continued on Page

HDJ 10/23/17
2:00pm

- 3 vials of P. Fluorescens (Electrocompetent)
  - 2 µL of pMQ72, pMQ72-BBa, and pMQ72-BBa DpnI added to ea. vial respectively. ~~1:100~~ in 5 µL
  - mixed & incubated in ice for 30 sec
  - Pulsed @ ~~2.5kV~~ 2.5kV, 200 Ω, 25 µF, 2mm
  - ~~Recovered~~ Recover for 1 hour @ 30°C in 900 µL SOC @ 6:00am
  - Plate recovered cells (100 µL) on LB + Gent + Gluc plates.
  - Incubate overnight @ 30°C

- No growth on either plate.

Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

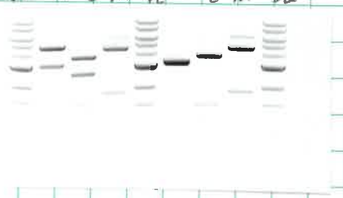
HDJ 10/23/17
2:30pm

- Miniprepped DNA of DpnI + pMQ72-BBa Col I + 20 ↑ pMQ72-BBa Col Sa. retrieved from -20°C.
  - digests setup as follows:

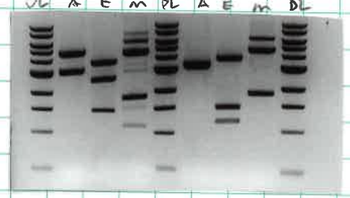
DI H_2O	23 µL	— Master Stocks of Enzyme diluted by 5 times
10× CutSmart	3 µL	
pMQ72-BBa DpnI DNA	3 µL	
Enzyme	1 µL	
	30 µL	

  - Reactions placed in 37°C @ 4:46pm.
  - Reactions retrieved @ 6:00pm
  - All RXNS. treated w/ 0.5 µL of DNA binding buffer.
  - Gel run @ 130 V, 45 min. w/ 1kb ladder, Gel ran @ 6:30pm

pMQ72-BBa Col I DpnI 13↑15 Regular Exposure.
DL 13 A E m PL 15 A E m DL



pMQ72-BBa Col. I DpnI 13↑15 Over Exposed.
DL 13 A E m PL 15 A E m DL

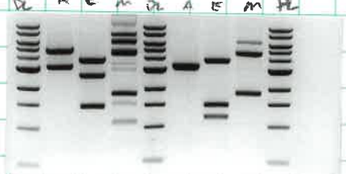


A = AflII
E = EcoRV-HF
M = MseI

Expected Bands
AflII: 7.1kb, 4.7kb
EcoRV: 5.5kb, 2.6kb, 3.7kb
MseI: 470bp, 1.1kb, 1.8kb, 4.4kb

pMQ72-BBa Col I DpnI 18↑19 Regular Exposure
DL 18 A E m PL 19 A E m DL

pMQ72-BBa Col. I DpnI 18↑19 Over Exposed.
DL 18 A E m PL 19 A E m DL



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Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

MCL 3:10pm 6/23/17

- pSB1C3-BBa_J04450 in 20M kit plate 4 - well 4B.
  - Resuspended w/ Hydrae MB-Grade water, 10μL.
  - 2μL of resuspended plasmid was added to 40μL One-Shot TopTen electrocompetent E. coli.
  - Electroporated @ 25kV, 25μF, 200Ω
  - Recovery w/ 450μL SOC medium for 40 mins at 37°C, on rotation.
  - 200μL of the transformed bacterial suspension were spread on an LB plate that had been spread w/ 10μL of 25mg/mL chloramphenicol in DMSO.
  - Added 1.2mL of SOC medium to remaining bacterial suspension, then added 20μL of chloramphenicol to the 2mL of SOC medium
    → Note: this is 10x the recommended chloramphenicol dosage.

6:03p.m.

Continued on Page

KBJ 10/24/17
AJ 1:00pm.

- AB+Agar media retrieved from 55°C @ 1:15pm.
  - for Chloramphenicol add 33.3mL of concentrated Chloramphenicol stock (25mg/mL) to get 25μg/mL
  - for Gentamycin 70mL of concentrated Gentamycin stock (100mg/mL) to get 20μg/mL

Continued on Page

MLL 1:37 pm 10/24/17

- No colony growth observed on the plate of broth transformation inoculants, we suspect that higher dosage Chloramphenicol led to cell death.
- Transformed OneShot Top Ten E. coli w/ pSB1C3_BBa_J04450 by electroporation
  - 40 µL E. coli + 2µL Plasmid
  - 2.5kV, 25 µF, 200 Ω on electroporator. 1 mm cuvette
  - Placed on gentle rotation @ 37°C for 1 hr
  - Two transformations by electroporation were done. Spread 200 µL of each electroporated/transformed E. coli on LB + 25 µg/mL Chloramphenicol plates.
  - Plates stored in 37°C
  - Two tubes were filled w/ 200 µL SOC media + 5 mL LB media for a total of 6 µL
    - 6 µL of 25 mg/mL chloramphenicol stock solution was added to each tube for final concentration of 25 µg/mL.
  - The two tubes were placed in 37°C on rotation.

3:46 p.m.

- No Growth HDJ 4:00pm 10/25/17

Read and Understood By

Signed Date Signed Date

HDJ 10/24/17

- Retrieve 3 vials of competent cells from 4°C AJ 1:30pm
  - ~~Add~~ Retrieve vials of miniprep dpnI for pMa7z-BBa_J3, pMa7z-BBa_J04450 DpnI 13, pMa7z-BBa_J04450 DpnI 18 from -20°C
  - Add 3µL of DNA to ea. labelled vial of competent cells.
  - Incubate on ice for 30 seconds
  - Transfer to pre-chilled 1mm gap electroporation cuvette.
  - Pulse @ ~~2.5kV~~ 2.5kV, 200Ω, 25µF on BioRad Gene Pulser
  - Diluted & Extracted w/ 900µL SOC outgrowth media into labelled culture tubes
  - Cultures placed in 37°C shaker for 1hr recovery @ 7:15pm

For pMa7z HDJ 5pm 10/24/17

- retrieve pMa7z from -80°C @ 3:00pm. 3:30pm
  - retrieve vial of competent cells from 4°C @ 3:30pm
  - Add 3µL of DNA to the vial.
  - Incubate on ice for 30 seconds
  - Transfer to pre-chilled 1mm gap electroporation cuvette.
  - Pulse @ 2.5kV, 200Ω, 25µF on BioRad Gene Pulser
  - Extract w/ 900µL SOC
  - Recover 1hour @ 37°C

- 100µL of ea. culture spread on a LB + Gm20 + 2% Glucose plate. and placed in 37°C @ 5:00pm for overnight growth.

- 2 remaining vials of P. Fluorescens competent cells placed in -80°C. @ 5:07pm.

HDJ

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HDJ 10/24/17

- Regrowth of pMQ72-BBa Col I. from Stock - glycerol stock into 3x 6mL LB+ Cm+ IPTG + 20g/L Gluc.
  - placed in 37°C

HDJ

Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

HDJ 10/25/17
12:30PM

- Dummy Cuvette Check
  - 50µL of Invitrogen Electrocompetent cells transferred to a 1mm cuvette.

Transformation of E. coli w/ pSB1C3 BBa_J04450

- pSB1C3-BBa_J04450 in 2017 kit plate 4 - well 4C3
  - 2µL of resuspended plasmid added to 40µL One-Shot Top10 electro-competent E. coli
  - Electroporated @ 2.5kV, 75µF, 200Ω, 1mm gap.
  - Relayed w/ 450µL SOC @ 37°C @ for 1hr.
  - 100µL plated on LB+ Cm25 and incubated @ 37°C @ overnight

HDJ.

HDJ 10/26/17
9:00AM.

- No growth visible @ 9:00AM.
- Growth of colonies visible @ 11:00AM
- Red colonies observed, left @ 37°C for more growth.
- * Note: Let E. coli grow at least ~~16-18~~ 20 hours before picking.

Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

HDJ 10/25/17
EB 2:30pm

- Retrieve Qiagen Miniprep Kit

√ Aliquot 2x 6mL overnight cultures into 2mL ea. in 2mL mcf.
√ Centrifuge 3 min @ 8000 rpm, discard supernatant
√ Resuspend pellet in 250μL P1 Buffer.
√ Add 250 μL P2 Buffer, mix by inverting 4-6 times.
√ Add 350 μL of N3 Buffer, mix by inverting 4-6 times
√ Centrifuge @ 13,000 rpm for 10 minutes.
√ Apply 800 μL of supernatant to QIAprep 2.0 spin column
√ Spin 60s, discard supernatant Flow through
√ Add 500 μL PB Buffer,
√ Spin 60s, discard supernatant Flow through
√ Add 770μL PE Buffer
√ Spin 60s, discard supernatant Flow through
√ Spin 60s, discard Flow through
√ Move Spin columns to clean, labelled 1.5mL mcf
√ Add 50μL H_2O to center of column, incubate 1min
PE H_2O
√ Spin 60s.

- Nanodrop

Sample	260/280	[DNA] (ng/μL)
1	1.91	157.3
2	1.90	141.2
3	1.87	94.0
4	1.88	135.7
5	1.71	173.7
6	1.87	185.7

HDJ

Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

HDJ 10/25/17
EB 4:15pm

- Make master mixes of EcoRI & PstI

EcoRI	2μL
$DI H_2O$	23μL
Cutsmart	3μL

→ 27μL rxn + 3μL DNA

PstI	2μL
$DI H_2O$	23μL
Cutsmart	3μL

→ 27μL rxn + 3μL DNA

- 13.5μL of ea. master mix added to (1.5mL mcf for ea. rxn.
- 3μL of miniprepped DNA added to ea. 27μL Gluth or EcoRI & PstI. rxns. Incubated @ 37°C @ 4:15 PM

- Gel Prepped for Gel Purification, ie 50mL TAE + 0.5g Agarose + Syrbr Safe Dye (1μL dye : 10mL Buffer)

Sample	260/280	[DNA] (ng/μL)	Sample	260/280	[DNA] (ng/μL)
3.0kb #1	1.71	10.0	4.8kb #1	1.99	19.3
3.0kb #2	1.84	8.7	4.8kb #2	2.00	19.1
3.0kb #3	1.74	13.8	4.8kb #3	2.02	19.7
3.0kb #4	1.85	6.2	4.8kb #4	2.19	8.7
3.0kb #5	1.96	7.9	4.8kb #5	1.70	7.1
3.0kb #6	2.08	10.0	4.8kb #6	1.96	15.1

Gel Purification - use Zymoclean Gel DNA recovery kit.
√ Excise DNA band from gel w/ band remover, place in 1.5mL mcf
√ Add 3 volumes of ADB to ea. volume of agarose excised.
√ Incubate @ 37°C for ~~10~~ 5-20 min, until gel slice dissolved.
√ Transfer melted agarose solution to Zymo-Spin™ Column in a Collection Tube
√ Centrifuge 60s, discard flow-through. @ 13,000 rpm
√ Add 200μL DNA wash Buffer
√ Spin 30s, discard Flow-through @ 13,000 rpm.
√ Repeat Wash step.
√ Add 6μL H_2O to column, sit 1min.
√ Place column in clean, labelled 1.5mL mcf
√ Spin 60s. @ 13,000 rpm

For Nanodrop, See ~~pg. 77~~ Above.

Continued on Page

HDJ

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

- 2 vials (100µL) ea. of competent P. Fluorescens retrieved from -80°C.
  - aliquoted to 50µL in 1.5mL mcf.
  - 2µL DNA added to ea. 1.5mL mcf.
  - Incubated for 30 seconds.
  - Transferred to Prechilled 2mm gap electroporation cuvette.
  - Pulse @ 2.5kV, 200Ω, 25µF.
  - Recover w/ 950µL SOC in 30°C for 1 hour. @ 5:30Pm
- 100µL plated on a LB+ 6mM 2O + 2% Glucose Plate ½ incubated @ 30°C overnight @ 6:45Pm.

HDJ.

HDJ 10/26/17 9:00Am

- No visible Growth @ 9:00Am
- Colonies visible @ 11:00Am.
- Note, allow at least 17-18 hours of Growth for ~~E.coli~~ P. Fluorescens.
- Colonies left to grow longer in 30°C.
- colonies looked clear.

HDJ

Continued on Page

Read and Understood By

Signed Date Signed Date

HDJ 10/25/17 5:30Pm.

- 16 tubes prepped.
  - 2mL LB added to ea. tube via Serological pipette.
  - Innoculation loop scratched P. Fluorescens Frozen stock from -80°C
  - Loop swirled in LB
  - Tubes incubated in 30°C overnight on shaker @ 6:00Pm.

HDJ

- No Growth visible @ 9:00Am
- Growth in 10/16 tubes observed @ 11:00Am.
- Allow for at least 19 - 20 hours of growth.
- Cultures retrieved from 30°C @ 5:00Pm.

HDS

HDJ 10/26/17 8:30 Pm.

- Cultures ~~split into~~ transferred to 2.0 mL mcf.
  - cells centrifuged for 2min @ 16,000×g, discard supernatant
  - cells resuspended w/ 1mL 300mM Sucrose. Resuspend by gentle pipetting up & down
  - cells centrifuged for 2min @ 16,000×g discard supernatant
  - resuspend cells w/ 1mL of 300mM Sucrose
  - Centrifuge 2min @ 16,000 x g, discard supernatant
  - Resuspend w/ 100µL 300mM Sucrose
  - Store electrocompetent cells in -80°C.

HDJ

Continued on Page

Read and Understood By

Signed Date Signed Date

PROJECT Electroporation of pSB1C3_BBa_I746107, ~~Nanodrop of Gel Purified DNA~~ pSB1C3_BBa_K1450001, pSB1C3_BBa_J04550, pSB3C5_BBa_J04550

Notebook No.
Continued from Page

~~HDJ 10/25/17~~
HDJ 10/26/17
10:30PM.

- pSB1C3-BBa-I746107 ~~resuspended in~~ found on Plate 4, well 1A, resuspended in 10µL HyClone H_2O.
- pSB1C3_BBa-K1450001, found on Plate 4, well 1C, resuspended in 10µL HyClone H_2O.
- pSB1C3_BBa-J04550, retrieved from −20°C, ~~as~~ resuspended in 10µL HyClone H_2O.
- pSB3C5_BBa_J04550, found in Plate 4, well 4B, resuspended in 12µL HyClone H_2O.

∟ ~~6x~~ 1mm Electroporation cuvettes prechilled.
∟ 5 vials of Invitrogen, OneShot, TopTEN electrocompetent e.coli retrieved from −80°C
∟ pUC19, positive control retrieved from iGEM −80°C
∟ 40µL E.coli mixed w/ 2µL DNA
∟ ~~mixed~~ Transfered to Electroporation cuvette. pSB1C3_BBa_K1450001 Arced.
∟ Incubated 30 seconds
∟ Pulsed on BioRad Gene Pulser: 2.5kV, 200Ω, 25µF
∟ Recovered in 950µL SOC media and @ 37°C shaking for 1hr @ 11:20AM.

∟ 100µL of pSB1C3 BBa I746107, pSB1C3 BBa_J04550, pSB3C5 BBa-J04550 plated on LB+ Cm 20 + 2% Glucose.
∟ 100µL of pUC19 plated on LB+ Amp+ 2% Glucose.
∟ plates incubated @ 37°C overnight @ 12:40PM.

HDJ

Continued on Page

Read and Understood By

Signed _______ Date _______ Signed _______ Date _______

PROJECT Innoculating pMQ72, pMQ72_BBa C01I & pMQ72-BBa DpnI 18 Fluorescent Colonies.

Notebook No.
Continued from Page

HDJ 10/26/17
5:00PM.

- Plates retrieved from 30°C @ 5:00PM.
  ∟ 100s of colonies found on ea. plate.
∟ ~~8~~ 6 colonies of ~~pMQ~~ pMQ72 innoculated.
∟ 12 colonies of pMQ72-BBa C01I innoculated.
∟ 12 colonies of pMQ72-BBa DpnI 18 innoculated.
- All colonies innoculated in 2mL LB + Gm20 + 20g/L Glucose.
∟ Cultures incubated @ 30°C @ 6:30PM. overnight

HDJ

HDJ 10/27/17
5:00PM.

- No visible Growth.
  ∟ Plates left @ Room temp overnight.
  ∟ Colonies repicked. (10 ea. for pMQ72-BBa C01I & pMQ72-BBa DpnI(8))
  ∟ 8 colonies repicked for pMQ72.
  ∟ Colonies picked in 2mL LB + Gm20 + 20g/L gluc.
  ∟ Optimal Growth for picking seems b/w 36hr & 48hr.
- Cultures incubated @ 30°C overnight @ 5:30PM

Continued on Page

Read and Understood By

Signed _______ Date _______ Signed _______ Date _______

HDJ 10/26/17
@5:00pm.

- 6 colonies picked from pSB1C3-BBa-J04550 on CAT cm 25 plate.
  - innoculated in 2mL of LB + Cm25.
    (2mL LB + 2uL Cm25 concentrated stock)
  - Cultures incubated @ ~~37°C~~ @ 6:15pm. overnight

---

pSB1C3-BBa-J04550 miniprep HDJ 10/26/17
@ 6:20pm.

- 300uL H_2O used to resuspend remaining, unpicked colonies on plate.
- 8000rpm for 3min
- ~~Apply~~ Discard Supernatant
- Resuspend pellet in 250uL P1 buffer.
- Add 250uL P2 Buffer, mix by inverting 4-6 times.
- Add 350uL N3 Buffer, mix by inverting 4-6 times.
- Centrifuge for 10min @ 13,000rpm.
- Apply 800uL supernatant to QiaPrep 2.0 spin column.
- Spin @ 13,000rpm for 1min, discard flow through
- Add 500uL PB buffer
- Spin @ 13,000 rpm for 1min, discard flow through
- Add 750uL PE buffer.
- Spin @ 13,000 rpm for 1min, discard flow through.
- Spin @ 13,000rpm again for 1min, discard flow through.
- Transfer column to clean, labelled 1.5mL tube
- Add 50uL H_2O to column, incubate 1min.
- Spin @ 13,000 rpm for 1min to elute DNA.

- Nanodrop.

	260/280:	[DNA] (ng/uL)
pSB1C3-J04550	1.63	13.0

- DNA stored in -20°C

HDJ

Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

HDJ 10/26/17
@ 4:50pm.

- ~~Isolation~~
  - 96-well plate for submission taken from iGEM distribution kit
  - 2.68 uL of Miniprep #3 of (944.0 ng/uL) added to well A1 to dry ~ 250 ng of DNA.
  - left w/ lid on in laminar flow hood overnight to dry down.

HDJ

Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

PROJECT Miniprep of pSB1C3_JOU500 Colonies.

Notebook No. _____

Continued from Page _____

HDJ 10/27/17
4:00pm

- Cultures retrieved from 37°C @ 4:00pm.

- ea. culture was transferred to a 7.0 mL vial
- Centrifuged 3min @ 8,000rpm.
- Supernatant ~~Solute~~ is discarded
- pellet ~~resuspended~~ in 250uL P1 buffer.
- 250uL P2 buffer added, mix by inverting 4-6 times.
- 350uL N3 buffer added, mix by inverting 4-6 time.
- Spin 10min @ 13,000rpm.
- 800uL applied to a QIAprep 2.0 spin column
- Spin 1min @ 13,000rpm, discard flow thru.
- Add 500uL PB buffer.
- Spin 1min @ 13,000 rpm, discard flow thru.
- Add 750uL PE buffer. to wash.
- Spin 1min @ 13,000 rpm, discard flow thru.
- ~~Repeat wash step~~ Spin 1min @ 13,000 rpm, discard flow through
- Transfer spin columns to new, labelled 1.5 mL vial.
- Add 50uL Hyclone H_2O to center of ea. column.
- incubate 1min
- Spin 1min @ 13,000 rpm to elute DNA.

- Nanodrop.

Sample	260/280	[DNA] (ng/uL)
JOU500 1	1.70	85.5
JOU500 2	1.64	179.0
JOU500 3	1.65	152.7
JOU500 4	1.81	80.4
JOU500 5	1.72	113.0
JOU500 6	1.66	146.0

- DNA placed in. -20°C.

HDJ

Continued on Page

Read and Understood By

Signed Date Signed Date

PROJECT Miniprep of pMQ77, pMQ77_BBa_C01I, pMQ77-BBa_P0118

Notebook No. _____

Continued from Page _____

HDJ 10/2
4:00pm

- Cultures obtained from 30°C @ 4:00pm.
  - no visible growth after 20 hours.

- None - performed.

HDJ

Continued on Page

Read and Understood By

Signed Date Signed Date

PROJECT ~~Innoculating~~ Colonies of pSB1C3-JOU550

Notebook No. _____

Continued from Page _____

HDJ 10/27/17

5 530 pm.

- Plates for pSB1C3-J746107, pSB1C3-JOU550, pSB3C5-JOU550 retrieved from 37°C @ 5:00pm.
- Colonies exhibited growth.
- plates were wrapped & placed @ 4°C.

HDJ

Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____

PROJECT _____

Notebook No. _____

Continued from Page _____

Continued on Page

Read and Understood By

Signed _____ Date _____ Signed _____ Date _____