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09/05/17

AIM: PREPARATION OF LB ~~PLATES~~ LIQUID MEDIUM

MATERIALS: (i) LB powder
(ii) Flasks
(iii) Weighing machine
(iv) Cotton plug

PROTOCOL:

(i) Take a 1 L autoclave bottle
(ii) Add 25g LB broth powder
(iii) Add 1000 ml ultrapure water
(iv) Mix it well, powder will not dissolve
(v) Replace the cap to the bottle but leave it slightly loose for pressure equalization to occur. Place a piece of autoclave ~~top~~ tape on the top or Al foil.
(vi) Autoclave (121°C, 20 minutes)
(vii) Cool to RT and use. (Do not tighten cap until cool)

PROTOCOL:

(i) Prepare 1L of LB liquid (25g broth powder per 1L) in 2000ml Erlenmeyer flask.

(ii) Add 15g bacteriological agar (1.5%)

(iii) Swirl to mix.

(iv) Add a fresh piece of aluminium foil to cover the top, add a fresh piece of autoclave tape.

(v) Autoclave (121°C, 15 minutes)

(vi) Let it cool down to 50°C. Media should still be liquid.

(vii) Add antibiotics if necessary and mix.

(viii) Set the plates and label.

(ix) Pour the media into the plates (Open the lid of plate, remove the foil. Pour just enough LB agar into the plate to cover the bottom, replace the lid)

(x) Work quickly so that it doesn't solidify. Plates should be stored in cool places upside down.

Amount of water : 100 mL (make it to 100mL not water 100mL)

LB broth : 2.5g

Agar : 1.5g

2) In Flask II (500 mL flask)

Amount of water : 150 mL (make it to 150m

(ii) Incubate @ 37°C for 2 hours

(iii) Spread plate 100 µl of the culture on LA plates in laminar hood.

10/05/17

AIM: PREPARING SOB (Super Optimal Broth) (25mL) pH-7.5

~~PROCEDURE~~ MATERIALS:

(i) 0.125g of ~~KOAC pH-7~~ yeast extract (0.1506g)
(ii) ~~80~~ 0.5g of ~~$CaCl_2 \cdot 2H_2O$~~ Tryptone (1.00g)
(iii) 0.0146g of NaCl ~~(0.0094)~~ (0.00291g)
(iv) 0.00465g of KCl (0.00746g)
(v) 0.06g of $MgSO_4$ (0.1207g)

20/05/17 100mL SOB

	Req.	Amt. put
(i) Yeast extract	0.5g	0.5003g
(ii) Tryptone	2g	1.99g
(iii) NaCl	0.0586g	0.059g
(iv) KCl	0.0186g	0.0198
(v) $MgSO_4 \cdot 7H_2O$	0.492g	0.4881g

Autoclave at 122°C

AIM: PREPARING CCMB80 Buffer (25mL)

(i) 10mM KOAC pH 7.0 - 0.024g
(ii) 80mM $CaCl_2 \cdot 2H_2O$ (11.8g/L) - 0.294g
(iii) 20mM $MnCl_2 \cdot 4H_2O$ - 0.1g
(iv) 10mM $MgCl_2 \cdot 6H_2O$ - 0.05g
(v) 10% glycerol - 2.5mL
(vi) Water - 25mL

Filter sterlization of the buffer
Fill the syringe with the buffer
↓
~~Put the filter on the tip of syringe~~
↓
~~Push the piston and~~ collect filtrate in another falcon.

50mL Ultra Comp. Cells
Prep. of SOB & CCMB80
Efficiency test of comp. cells

12/05/17 – 13/05/17

Ultra-Competent cells : iGEM

(i) Innoculate 5ml of SOB medium with 20µl vial of seed stock and grow at 20°C OD600nm of 0.3.

(20°C helps in making the cells competent by changing the membrane flexibility)

(ii) Let it grow for 16 hours
↓
Room temperature
↓
Aim for lower OD.

(iii) Pre-chill as many flat-bottom centrifuges as you can in an ice bucket.

(iv) Transfer the culture to flat-bottom centrifuge. weigh and balance the tubes on a scale (As close as possible $\Delta w = 1g$)

(v) Centrifuge at 3000g at 4°C for 10 min in a flat centrifuge bottle

(vi) Decant supernatant into waste receptable, bleach before pouring down the drain.

(vii) Gently resuspend in $\frac{80}{250}$ ml = 320µl of ice cold CCMB80 buffer.

(Tip: Add 160µl first resuspend and

- After pipetting, there will still be some residual cells stuck to the bottom. Swirl bottles gently to resuspend these remaining cells -

(viii) Incubate on ice for 20 minutes.

(ix) Centrifuge again at 3000 g at 4°C for 10m Decant supernatant into waste receptacle and bleach before pouring down the drain.

(x) Resuspend cell pellet in 40µl of ice cold CCMB80 buffer
If using multiple flat bottom centrifuge bottle, combine the cells post-resuspension.

(xi) Use Nanodrop to measure OD of a mixture of 160µl of LB and 40µl of the resuspended cells.

(xii) Use a mixture of 160µl of LB and 40µl CCMB80 buffer as the blank.

Prepare for aliquoting

- Make labels for aliquots. Use these to label storage ~~vials~~ microcentrifuge tubes.
- Pre-chill on dry ice.

(xv) Aliquot into chilled 1.5 ml microcentrifuge tubes (Flash freezed in liquid nitrogen)

(xvi) Store at −80°C indefinitely.

* Aliquot 260 µl in each eppi. (for 5 reactions at a time) 10 µl is extra for pepetting errores. This saves eppis and efforts.

On 12/05/17, when we performed the experiment we ran out of blank (SOB) and the OD was very low after 16 hours. We took the OD readings in plate reader. We should have done it in standard spectro-photometer.

OD Measurement in plate reader

9 10 11 12

* Odd number samples (SA1, SA3..) - E.coli DH5α
Even number samples (SA2, SA4..) - E.coli MG1655

OD should be around 0.3.
We incubated again at 20°C for 2 more hours and took OD measurements.

OD MEASUREMENT (NO blank) - Mistake

	1	2	3	4	5	6	7	8	9	10	11	12
H	SA1	SA2	SA3	SA4	SA5	SA6	SA7	SA8	SA9	SA10		

OD had reduced.

We didnot proceed further because we didnot have any more blank.

14/5/17 Evening 6 pm

- Pre inoculation from the LA plate.
- Took 2ml SOB in 15ml falcon tube.
- Using sterile 200µl pipette tips picked up one colony each of DH5α and MG1655.
- Transferred the colony into the falcon with SOB.
- Incubated the culture at 37°C @ 180 rpm for 12 hours.

15/5/17 Morning 6:00 am
Stored the culture in 4°C fridge.
Prepared 150 ml of SOB in the evening.

16/5/17 1:00 am (Night)

: Inoculated the main culture

: 3 samples for DH5α and 2 for MG1655

SOB: 2X 5ml + 8ml + 7ml = 25ml SOB
for MG1655

Inoculate with 100 µl (20×5µl) culture

3 X 5ml + 8ml + 7ml = 30ml SOB
for DH5α

Inoculate with (20×6 µl) = 120µl
DH5α

- Grow @ 20°c @ 250rpm for 16 hrs

16/5/17 10:30 am

Measured O.D

Using spectrophotometer using 600 nm.

Time	Duration since inoculation	MG1655	DH5α
10.53 am	~ 10 hours	0.515	0.055
11.57 am	11 hours		0.102
1.05 pm	12 hours		0.132
2.05 pm	13 hrs		0.14
3.10 pm	14 hrs		0.203
4:00 pm	15 hrs		0.212
4:30pm	16 hrs		0.222

Since the O.D of MG1655 > 0.3, we diluted it 1:1 with SOB.

(When you dilute culture to get appropriate O.D always let it grow for 2 hours

(i) Always balance the eppis by putting them in the exact opposite slots.

(ii) Put the hinge side towards outside so that pellet forms on that side and its easier for mixing.

18/05/17 12:30 pm – 4:00 pm

AIM: To check the ~~competence~~ efficiency of competent cells.

PROCEDURE:

Clean the working area with 70% ethanol.

↓

Thaw competent cells on ice. Label one 1.5ml microcentrifuge tubes for each transformation and then pre-chill by placing it on ice.

↓

Spin down the DNA tubes from the competent Cell test kit to collect DNA into the bottom of each tube prior to use.

A quick spin (20-30 secs) at 8000-10000 rpm. (50µl of DNA in each tube in kit)

↓

Pipet 1µl of DNA into each microcentrifuge tube.

↓

Pipet 50µl of competent cells into each tube. Flick the tube gently with your finger to mix.

↓

Incubate on ice for 30 minutes.

↓

Set the thermomixer at 42°C.

↓

After 30 minutes ice incubation, heat shock the cells at 42°C for ~~600~~ 60 seconds.

Immed

Add

Pipe

Incu

C

CALCULA

Efficienc
of

Amount

Expected
for 10µl -

~~at 37°C~~ overnight (approx. 16 hours)

↓

Count the number of colonies on a light field or a dark background.

CALCULATING COMPETENT CELL EFFICIENCY

Efficiency (in cfu/μg) = [colonies on plate (cfu) / Amount of DNA plated (ng)] × 1000 (ng/μg)

Amount of DNA plated = Volume of DNA added (1μl)
× conc. of DNA (refer to vial, convert to ng/μl)
× [volume plated (50μl) / total reaction volume (1000 μl)]

Expected Result: Efficiency - $1.5 \times 10^8 - 6 \times 10^8$ cfu/μg
for 10μl - 280-360 colonies ; 50μl - 500+-1000+ DNA

TROUBLESHOOT:

(i) MG1655 (50µl) was ~~plated~~ spreaded on a LB plate to check if its viable.

→ We got colonies.

→ Cells are viable.

(ii) We did a second transformation test.

↓

Used 100pg/~~mg~~ml plasmids from the competent cells checking kit.

↓

Transformed culture was centrifuged at 3000G and ~~added~~ 100µl of LB

NOTES ON TRANSFORMATION (COMPETENCE EFFICIENCY)

→ Always keep the buffer and your sample on ice (even while pipetting if possible)

→ Add cells to the plasmid rather vise-versa because plasmid is a small amount (1μL).

It doesnot get properly mixed if add on a bigger volume.

→ Add 950μl of LB instead of 250μl and incubate for 2 hours at 37°C, 250rpm.

→ Plate 50μl of the culture on one side of the plate and then centrifuge and decant and resuspend the pellet in some amount of LB (say 50μL) and plate it on the other half.

In this way you plate all the cells that you have or are transformed.

Kept it in the incubator at 37°C at 220 rpm.

Prepared CCMB80 Buffer and filter sterlised it.

Preparation of CCMB80 Buffer (250 mL) pH-7

(i) 10mM KOAc (10ml of a 1M stock/L)

M.W - 98.15 g/mol

$$10 \times 10^{-3} = \frac{x}{98.15} \times \frac{1000}{250}$$

$$\Rightarrow x = \frac{10 \times 10^{-3}}{0.04075}$$

$$= \boxed{0.245\ g}$$

(ii) 80 mM $CaCl_2 \cdot 2H_2O$ (11.8 g/L)

M.W - 147 g/mol

$$80 \times 10^{-3} = \frac{x}{147} \times \frac{1000}{250}$$

$$\Rightarrow x = \frac{80 \times 10^{-3} \times 147 \times 250}{1000}$$

(iii) 20mM $MnCl_2 \cdot 4H_2O$ (4 g/L)

M.W – 197.91 g/mol

$$20 \times 10^{-3} = \frac{x}{197.91} \times \frac{1000}{250}$$

$$\Rightarrow \frac{20 \times 10^{-3} \times 197.91}{4} = x$$

$$\Rightarrow x = \boxed{0.989\,g \simeq 1\,g.}$$

(iv) 10mM $MgCl_2 \cdot 6H_2O$ (2g/L)

M.W – 203.31 g/mol

$$10 \times 10^{-3} = \frac{x}{203.31} \times \frac{1000}{250}$$

$$\Rightarrow \frac{10 \times 10^{-3} \times 203.31}{4} = x$$

$$\Rightarrow x = \boxed{0.508\,g}$$

(V) 10% glycerol – 25 ml

Preparation of SOB pH. 7.5 (150ml)

(i) LB powder

For 250ml ⟶ 6.25 g LB

For 150ml ⟶ $\frac{6.25}{250} \times 150$

$= 3.75\,g$

$$= 0.0279\ g \qquad \frac{}{1000}$$

(iii) 20mM of $MgSO_4 \cdot 7H_2O$

Amt. of $MgSO_4 \cdot 7H_2O = 246.46 \times 20 \times 10^{-3} \times \frac{150}{1000}$

$= 0.739\ g$

After 16 hours of incubation
OD was 0.227

↓

4ml of culture was taken in a 50ml falcon.

↓

Resuspend in 1.28 ml of CCMB80 buffer
~~2060µl~~ (2 times 640 µl)

↓

Incubate for 20 min on ice

↓

Got no pellet :c

Wt. of $MgCl_2$ for 80mM for 500 ml sol^n

$$= \frac{80 \times 95.211 \times 500}{1000} \times 10^{-3}$$

$$= 3.8084 g$$

(ii) Put secondary culture at 37°C.

(iii) Measure O.D after an hour.

(iv) Take the culture and put it in two falcons equally (5ml & 5ml).

(v) Put these in tubes at 0°C in ice for 10mins.

(vi) Centrifuge it for 10mins at 4°C and 4100 rpm.

(vii) Decant the tubes and 3ml (in each tube) of $MgCl_2$ - $CaCl_2$ soln and (resuspend using 1ml pipette gently)

(viii) Then incubate the cells (the tube) in ice for 45 mins.

(ix) Centrifuge the tubes for 10 mins at 4°C and 4100 rpm.

(xii) Add 80% glycerol (0.2ml) in each tube.
(xiii) Fill the microcentrifuge tube with 500µl of culture in each tube.

25/05/17 Making 80% glycerol stock

→ Made 20ml of 80% glycerol stock
16ml of glycerol
4ml of water

Took 5ml saturated MG1655 culture in a 15ml falcon.

↓

Added 5ml 80% glycerol solution

↓

Aliquoted 1ml in 1.5ml eppis.

↓

BACTERIAL GROWTH CURVE (24 hours) 23

Strains : DH5α and MG1655

Medium : SOB

Temp : 20°C Shaking: 250 rpm

2 flasks were autoclaved with cotton plug and kept for growing the cultures.

↓

Took 50ml SOB in both

↓

8:10 pm { Inoculated with 200µl of DH5α and 250µl of MG1655 respectively

↓

Took O.D measurements in spectrophotometer after every 1 hour

TIME	Optical Density DH5α	MG1655
8:15 pm	0.031	0.008
8:35 pm	0.019	=
9:10 pm	0.048	0.045
10:10 pm	0.053	0.048
11:10 pm	0.050	0.075
12:10 am	0.050	0.098
1:10 am	0.055	0.145
2:10 am	0.060	0.205
3:10 am	0.066	0.311
4:10 am	0.073	0.523
5:10 am	0.077	0.726

6:10 pm	0.090	1.068
7:10 am	0.101	1.536
8:10 am	0.122	2.148
9:10 am	0.144	2.796
10:10 am	0.159	3.134
11:10 am	0.176	3.920
12:10 pm	0.211	5.36
1:10 pm	0.257	5.120
2:10 pm	0.258	5.080
3:10 pm	0.319	6.280
4:10 pm	0.417	10.5
5:10 pm	0.457	8
6:10 pm	0.539	6.26

COMPETENT CELLS STOCK (DH5α)

Primary inoculation :

* Took 2ml of autoclaved LB in 15ml falcon (×2)

↓

Inoculated with 50µl of satured culture of DH5α

↓

Incubated overnight at 37°C, 250 rpm

↓

Stored them in 4°C

Secondary inoculation :

Trans

Cen

Transferred the culture to ice

↓

Centrifuged it at 3000 g, 4°C for 10 mins

↓ → Discard supernatant

Resuspend in 9ml ice cold CCMB80

↓

Incubate on ice for 20 mins

↓

~~Incubate~~ Centrifuge @ 3000g 4°C for 10mins

↓ → Discard supernatant

Resuspend in 1.2ml ice cold CCMB80 buffer

↓

Measure OD of cell suspension

50µl cells + 950 µl LB Blank: 50µl CCMB80 + 950µl LB

$D_f = 20$

Measured OD = 0.097

O.D of cell suspension = 0.097 X 20

= 1.94

↓

Dilute the cell suspension 1:0.5

1.15 ml cells + 0.575 CCMB80 buffer

Final O.D = 1.29

↓

Aliquote 50µl 10 nos

260 µl 6 nos

↓

Incubated on ice for 20 mins

↓

~~Flash~~ stored at -80°C

SOB Preparation (250 ml)

(i) LB powder - 6.25g

(ii) 2.5mM of KCl - $2.5 \times 10^{-3} \times 74.55 \times \frac{250}{1000}$

$= 0.046\,g$

(iii) 20mM of $MgSO_4 \cdot 7H_2O$ - $.20 \times 10^{-3} \times 246.46 \times \frac{250}{1000}$

$= 1.23\,g$

Efficiency test

Sample - DH5α comp cells

+ve control - MG1655 comp cells

-ve control - DH5α without plasmid

Took 50µl comp cells in 2 eppis

-ve control

↓

1µl milli Q

↓

Add 50µl comp cells

↓

Incubate on ice for 30 mins

RFP transformation

↓

1µl 50pg/µl RFP plasmid

↓

Add 50µl comp cells

Heat shock @ 42°C 60secs in water bath

↓

Add 950µl LB and incubate @ 37°C for 2 hours

↓

Plated 50µl on LA - cam plates

↓

Grow @ 37°C overnight

↓

Count the number of colonies

	No. of colonies	Efficiency
	285	1.14×10^8
- ve control	0	
+ ve control	0	

TROUBLESHOOT (Transformation with RFP plasmid again)

DH5α (J)	DH5α (Ar)	MG1655 (J)
50µl	50µl	50µl

* Same protocol of efficiency test

No. of colonies = 319 (J) Efficiency = 1.59×10^8

No. of colonies = 100 (Ar) Efficiency = 0.5×10^8

06/06/17

Preparation of 10X PBS (100mL)

- 1.37 M NaCl – 8g
- 27mM KCl – 0.2g
- 43 mM $Na_2HPO_4 \cdot 7H_2O$ – 1.15g
- 14 mM KH_2PO_4 – 0.2g

Set pH to 7.3

Dilution to 1X PBS (40mL)

→ Take 4mL 10X PBS
→ Add 36mL distilled water
→ mix thoroughly

Preparation of fluorescein standard

→ Spin down the tubes 3000 rpm, 30 secs

→ Resuspend in 1mL 1X PBS (2X fluores

→ Take 500µL and dilute with 500µL of 1XPBS (1X fluorescein)

Molar mass of Fluorescein = 332.31

Required weight = $\frac{332.31 \times 100 \times 10^{-3} \times 1}{1000}$

= 0.0332 (100 mM)

Insoluble in 1ml PBS

50mL 1mM Fluorescein

$\frac{332.1 \times 10^{-3} \times 50}{1000}$

= 0.0167 g

5mM Flourescein 10ml

Required wt = $\frac{332.1 \times 5 \times 10^{-3} \times 10}{1000}$

= 17 mg

* We used 1M NaOH

0.4g in 10ml of distilled water.

Fluorescein was not dissolving in PBS because the one in our lab was not a sodium salt as we have in the

Plate used for measurement:
Corning 3686, 96 well plate, half area, flat bottom
Blank plate

Arrangement in the plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	CA 1	CA 2	CA 3	CA 4	CA 5	CA 6	CA 7	CA 8	CA 9	CA 10	CA 11	CA 12 (NaOH)
B	CA 1	CA 2	CA 3	CA 4	CA 5	CA 6	CA 7	CA 8	CA 9	CA 10	CA 11	CA 12 (PBS)
C	CA 1	CA 2	CA 3	CA 4	CA 5	CA 6	CA 7	CA 8	CA 9	CA 10	CA 11	CA 12 (PBS)
D	1	2	3	4	5	6	7	8	9	10	11	12 (PBS)
E	1	2	3	4	5	6	7	8	9	10	11	12 (PBS)
F	1	2	3	4	5	6	7	8	9	10	11	12 (PBS)
G	1	2	3	4	5	6	7	8	9	10	11	12 (PBS)

CA 1-A-1 – Fluorescein with NaOH (1M) (100 µL)
D1-1 – Sodium Fluorescein with PBS (100 µL) (1X)
D2-2 – serial dilution (1:1)
– D-11-11
D12 – PBS (100 µL)

E, F, G are replicates.

OD Measurements made (Corning - costar -96 wells - Transparent plate Full area - Flat bottom)

	1	2	3	4	5	6	7	8	9	10	11	12
A	120µL H_2O	200µL Ludox	200µL H_2O									240µL H_2O
B	120µL H_2O	200µL Ludox	200µL H_2O									240µL H_2O
C	120µL H_2O	200µL Ludox	200µL H_2O									240µL H_2O
D	120µL H_2O	200µL Ludox	200µL H_2O									240µL H_2O

28/06/17

Plasmid constructs for Hijack Module
To be sequenced by IDT -

(1) RBS + Arac gene with SSRA tag + double terminator
Well 4G Kit Plate-4

RBS - BBa_B0030
AraC - BBa_C0080
SSRA - BBa_M0052
Double terminator - BBa_B0014

(2) DnaA dependent promoter (PolA) + RBS
PolA promoter : BBa_K847210
RBS : BBa_B0030
* GC content was low
Could not be synthesised

(3) RBS + LacI with SsrA + double terminator
RBS : BBa_B0032
LacI : BBa_C0012
SsrA : BBa_M0052
Double terminator : BBa_B0014

→ Petri plates (LA + Cam)
→ 42°C water bath with floating foam tube rack
→ Ice and ice bucket
→ Spreader

PROCEDURE:

Resuspend DNA in selected wells with 10µl of autoclaved milliQ water.

Pipette up and down several times.

↓ Resuspension will be red in colour.

→ Petri plates (LA + Cam)

→ 42°C water bath with floating foam tube rack

→ Ice and ice bucket

→ Spreader

PROCEDURE:

Close the tubes and incubate on ice for 30mins.

↓

Heat shock tubes at 42°C for 60 secs.

↓

Incubate on ice for 5 mins

↓

Pipette 950 µl of LB to each transformation

↓

Incubate at 37°C for 2 hours, 250rpm

↓

Pipette 100µl of each transformation onto petri dishes and spread with sterilized

(on the side wall)

↓

Pipette 50µl of compcells in a 1.5ml tube and pipette up and down properly to mix the plasmid well

↓

Pipette 1µl of control DNA into 1.5ml tube. (10pg/µL RFP plasmid)

↓

Pipette 50µl of compcells and mix well.

↓

Close the tubes and incubate on ice for 30mins.

↓

Heat shock tubes at 42°C for 60 secs.

↓

Incubate on ice for 5 mins

↓

Pipette 950 µl of LB to each transformation

↓

Incubate at 37°C for 2 hours, 250rpm

↓

Pipette 100µl of each transformation onto petri dishes and spread with sterilized spreader.

↓

Resuspend the cells in 100 µl and pipette the whole thing onto a petri plate and spread it using sterilised spreader.

↓

Incubate transformations overnight (14-18 hours) at 37°C

(Repeat) U

1st Innoculation

Took 15ml falcons (x 16)

↓

Added 2ml LB to each of them

↓

Added 1.42µl of chloramphenicol to each of them

↓

Labeled them (1.T.D.1, 2 T.D.1, 1 T.D.2, and wrapped them with Alu-foil 2 T.D.2...)

↓

Picked up colonies using 200µl pipette tips (2 colonies for each device)

↓

Innoculated in 2ml LB + cam

↓

Kept all the falcons in the incubator at 37°C, 250 rpm overnight.

2nd Innoculation

Took 50ml falcons (x 16) and labeled them

↓

fed

↓

11:00 am Took samples for 0 hr time point

↓ (1.5ml each in eppi's)

Put them on ice and then at 4°C

Black Pl
Corning

Plate 1

1

A +ve

B u

Took all the eppis in an ice bucket for the Fluorescence and O.D measurements in the plate reader.

OUTLINE SKETCH OF THE PLATE READER

Fluorescence Measurement

Black Plate

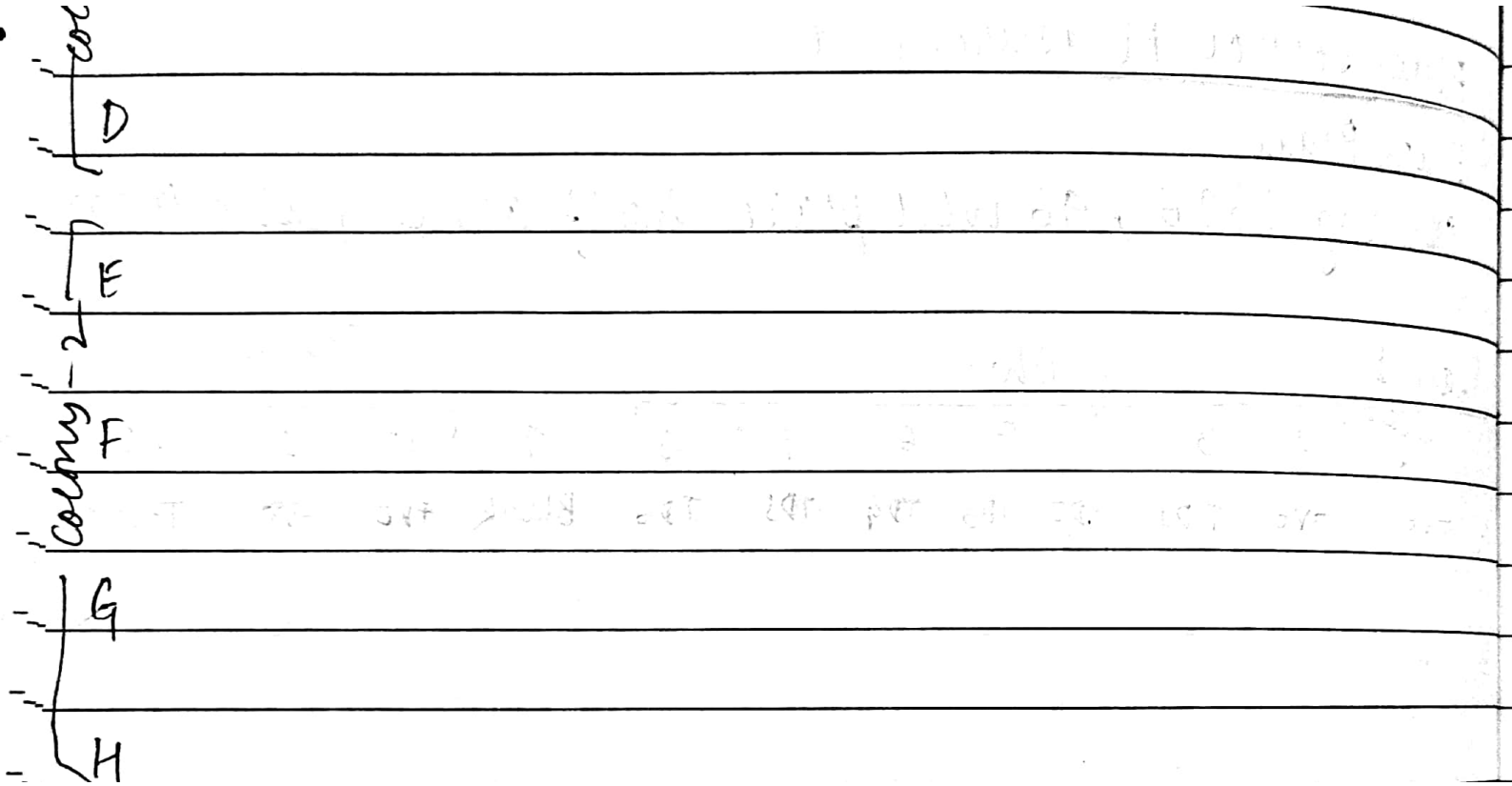
Corning 3686, 96 well plate, half area, flat bottom

Plate 1 — 0hrs — 2hrs

	1	2	3	4	5	6	7	8	9	10	11	12
A	+ve	-ve	T·D1	T·D2	TD3	TD4	TD5	TD6	Blank	+ve	-ve	T·D·1
B	"	"										
C												
D												
E												
F												
G												
H												

colony 1

colony 2



O.D Measurement:

Transparent plate

Corning, 96 well plate, full area, flat bottom

Plate 1 — 0hrs

	1	2	3	4	5	6	7	8	9	10 (2hrs)	11	12
A	+ve	-ve	T·1	T·2	T·3	T·4	T·5	T·6	Blank	+ve	-ve	T·1
B												
C												
D												
E												
F												
G												
H												

Colony 1 (A–D), Colony 2 (E–H)

Plate 2

	1	2	3 (2hrs)	4	5	6	7 (Colony 1)	8 (Colony 2)	9 (4hrs)	10	11	12
A	T·2	T·3	T·4	T·5	T·6	Blank	+ve	+ve	T·1	T·2	T·3	T·4
B							+ve	+ve				
C							+ve	+ve				
D							+ve	+ve				
E							-ve	-ve				
F							-ve	-ve				
G							-ve	-ve				
H												

Colony 1 (A–D), Colony 2 (E–G)

D

E

F

G

H

Plotted all the graphs in Excel.

Fluorescence Vs Time

OD Vs Time

3/07/17

MINIPREP PLASMID EXTRACTION

Extraction of plasmid from GFP colonies (BBa-K1390) PSB1C3 backbone.

PROTOCOL:

Inoculation of transformed colony for miniprep.

Take 2ml LB-Cam (25 µg/ml) in a 15ml falcon

↓

Pick one GFP transformed colony into the falcon with 10 µl tip

↓

Incubate at 37°C, 220 rpm for 18hrs

Miniprep plasmid extraction

Pipette 1ml culture into 2 1.5ml eppys each

↓

Centrifuge at 8000 rpm (6800 rcf) for 3 mins at 25°C

↓ → Discard supernatant

Resuspend pellet in 125 µl P1 buffer

↓ → Add RNAse to P1 buffer, write open date

Pool them together into a 1.5ml eppy

(lysis) ↓

Add 250 µl P2 buffer and mix well by inverting the tubes (Not more than 5 mins)

↓ (4 to 6 times)

Put date on the bottle when opened

↓

Add 350µl N_3 buffer and mix by inverting the tubes 4 to 6 times

↓

Centrifuge at 17900 rcf or 13000 rpm at 25°C for 10 mins

↓ → Discard pellet

Pipette 800 µl supernatant into the column

↓

Centrifuge at 13000 rpm or 17900 rcf for 60 sec

↓ → Discard flow through

Add 750µl PB buffer to the column

↓

Centrifuge at 13000 rpm for 60 secs

↓ → Discard flow through

Add 750 µl of PE buffer to the bed

↓

Centrifuge at 13000 rpm for 60 secs

↓ → Discard flow through

Centrifuge

↓

Transfer the column into 1.5ml eppy

↓

Temp: 37°C Add 50µl EB and let it stand for 60 secs

↓

Centrifuge at 13,000 rpm for 60 secs

↓

store at -20°C, label

to disable the nuclease to degrade DNA.

(2) P2 buffer : Lysis buffer
(200mM NaOH and SDS)
↓ ↓
Base Soap
Destroys cell membrane - SDS
NaOH denatures DNA
} High pH

(3) N3 buffer : Neutralization buffer
Guanidine hydrochloride + acetic acid
Renatures DNA : neutral pH
Precipitates as - SDS salts

(4) PE
Useful to wash out other components
(has buffer + salt + ethanol)
Prevents DNA from dissociating from column while you wash away other contamination.

Nanodroop Conc. of GFP+ssRA(BBa_K1390~~EdG0833~~ 08) — 120.3 ~~pg~~ ng/μL

(6) EB: Elution Buffer
contains 10mM tris at pH 8-9
→ DNA is soluble in tris buffer.
(usable again)

07/07/17

Pipetteted 1mL culture into 2 eppis
↓
Centrifuged at 6800 rcf - 3 mins 25°C
↓ → Discard supernatant
Pellet resuspension in 125μL P1 buffer with RNAase
↓
Pooled both samples together
↓
Added 250μL P_2 buffer mixed well waited for 2mins (Blue colour appeared)
↓
Added N3 buffer and mixed well (Waited 2mins) (Turned whitish)
↓
Centrifuged at 17900 rcf, 10mins, 25°C
↓ → Discard pellet
Pipette 800μL supernatant into column
↓
Centrifuged at 17900, 1min
↓
Added PE buffer (750μL) to the bed } X2
↓
Centrifuged
↓
~~Add PB buffer (500μL) to the column~~ } X2
↓
Centrifuged
↓ Added 50μL EB kept at 37°C for 1.5 mins centrifuged.

Accidentally skipped PB buffer addition. Did after PE buffer ✱

PREPARATION OF MEDIA

YPD Medium (For 1000mL)

(i) Peptone - 20g
(ii) Yeast extract - 10g
(iii) Water - 950mL

Autoclave

Add 40% (w/v) glucose solution 50mL
(filter sterilized)

(For 200mL)

(i) Peptone - 4g
(ii) Yeast extract - 2g
(iii) Water - 190mL
(iv) Glucose - 10mL (4g glucose in distilled water
Filter sterilized)

YP Agar Plates (For 500mL)

(i) Yeast extract - 5g
(ii) Peptone - 10g

For 100 ml (approx - 5 plates)

(i) Yeast extract - 1g
(ii) Peptone - 2g
(iii) Agar - 2g
(iv) Water - 95 mL
(v) Glucose - 5mL (filter sterlized)
(2g glucose in 5mL of distilled water)

Primary Inoculum (5:30 pm)

(i) Took 1mL milliQ water in an eppi and heated it at 40°C in water bath.
(ii) Put a pinch full of baker's yeast (Saccharomyces cerevisiae) in it
(iii) Dissolved it using vortex mixer and kept it at 4°C.
(iv) Took 5mL of YPD media in a sterile falcon (X2)
(v) Took 100µL of the yeast culture and inoculated it in 5mL YPD (X2)
(vi) Kept the falcons at 30°C, 180 rpm overnight

Mistake

We added 50mL of glucose to 190mL of the media instead of 10mL.
We had an extra flask with 190mL YPD (glucose unadded) and used it. We added the right amount of glucose to it.

Secondary inoculation 8:30 am

(i) In 2, 500ml flasks with 247.5ml media we inoculated 2.5ml of primary.

(ii) ~~Kept it inside the incubator at 30°C, 180 rpm.~~

(iii) Took the 0th hour reading immediately after inoculation after shaking it well (collected 1ml in an eppi)

(iii) Kept the flasks in the inbator at 30°C, 180 rpm.

(iv) Took readings after every one hour. (collected 1ml of the sample in eppis and stored at 4°C)

11:20 am

* We did a third inoculation in 200ml of media with right amount of glucose.

Repeated the same steps as above with it.

Methylene Blue soln – 0.4 % (W/V)

0.04g – methylene blue powder
10mL – water (distilled)
Filter sterilisation

Trypan Blue solution 0.1 % (W/V)

0.01g – Trypan blue powder
10mL – distilled water
Filter sterilisation

TAKING MEASUREMENTS

(i) Resuspending Culture

Centrifuge the aliquot of each time point (1mL) at 1500 rpm (or 2000g) for 2mins to pellet cells

↓

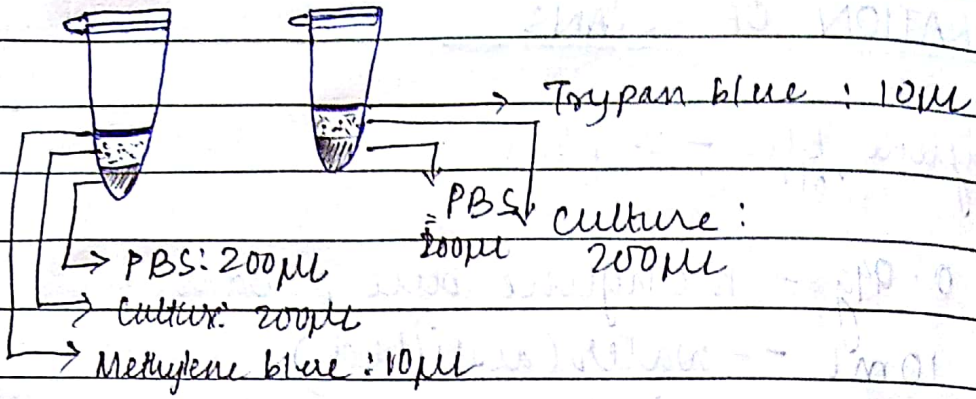
~~Its sterile tm~~

Remove the supernatant and resuspend cells in 1mL PBS

(ii) Staining

(i) Make Triplicates of 200µL PBS + 200µL culture

(ii) Add ~~10µL~~ of methylene blue to one



(iii) Vortex gently and let it sit for 3-5 mins at room temp

(iv) Load and visualise

(iii) Hemocytometer : Loading

Clean hemocytometer surface and coverslip with 70% ethanol

↓

Place coverslip on hemocytometer

↓

Take 10µl of sample and load it on hemocytometer

(a) Invert the tube several times to resuspend the cells

(b) Pipette 10µl of sample at once ~~in the corners~~

(c) Add 10µl in the V-shaped groove in hemocytometer

(v) COUNTING CELLS

(a) Count cells only from images taken, and not directly from hemocytometer

RESULT:

Live cells were not getting stained with methylene blue.

~~Dye~~ Dyes were not getting dissolved in water so we dissolved it in ethanol.

4% w/v of methylene blue and Safrin ~~was used~~ from lab were used for staining.

PFA Fixing

Spin down the growth cell sample 1500 rpm for 2 mins

↓

Resuspend in PBS; (PBS wash)

↓

Spin down again @. 1500 rpm, 2mins

↓

Resuspend in 4% PFA, 200µL

↓

Incubate on ice for 90 mins

↓

Spin down @ 1500 rpm, 2mins

↓

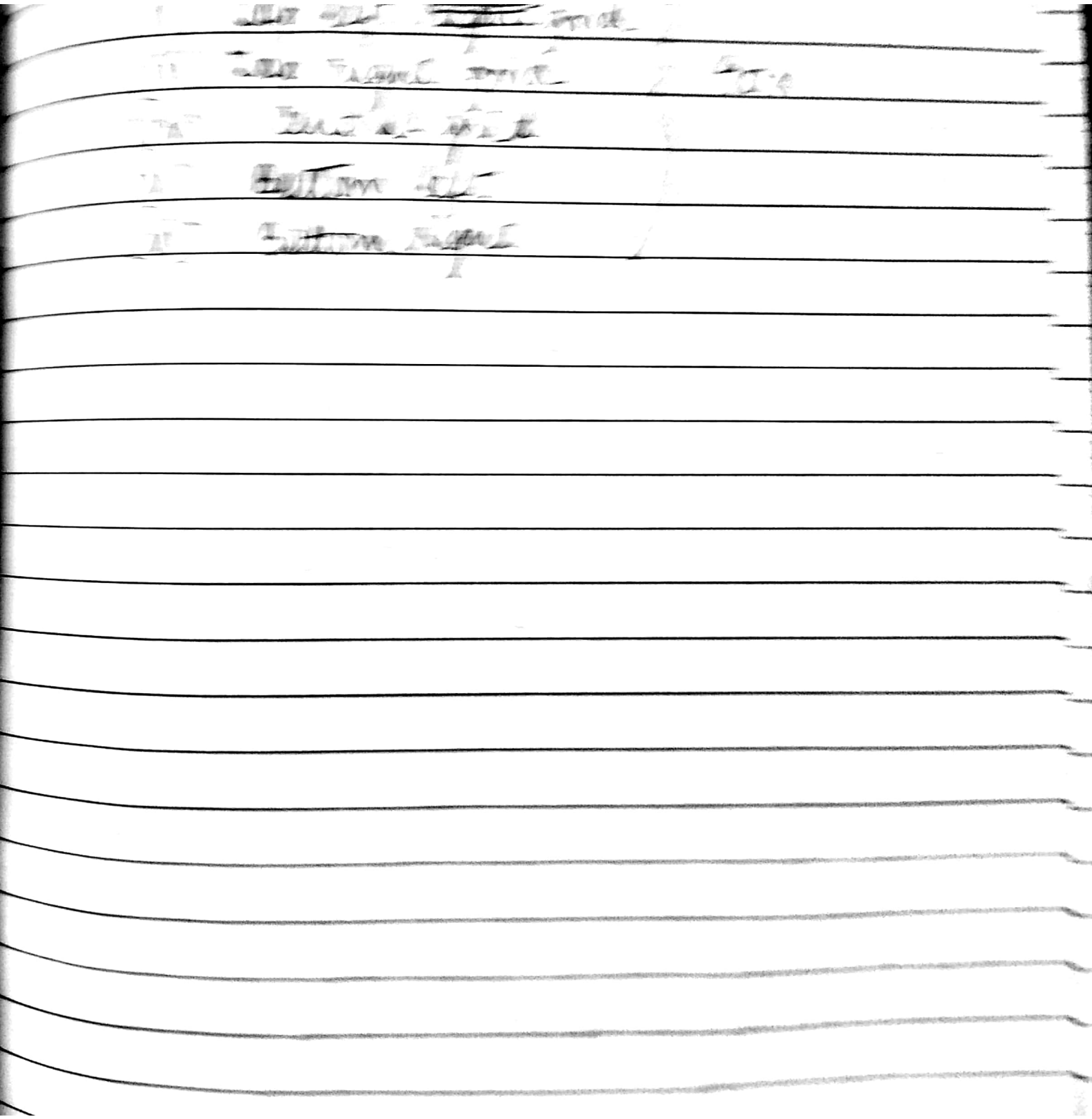
Resuspend YC and YA cultures
in 800µL PBS
and YB in 1mL PBS

↓

Make duplicates of 200µL PBS + 200µL cultu

↓

Add 10µL of methyl Blue to one
replicate
and 10µL of Safranin to other
replicate



Enzyme Master Mix for Plasmid backbone (25µL, 5 rxns)

→ 5µL NEB Buffer 2
→ 0.5µL BSA
→ 0.5µL EcoRI-HF
→ 0.5µL PstI
→ 0.5µL DpnI (used to digest any template DNA from production)
→ 18µL dH_2O

Enzyme Master Mix for Part A (25µL, 5 rxns)

→ 5µL NEB Buffer 2
→ 0.5µL BSA
→ 0.5µL EcoRI-HF
→ 0.5µL SpeI
→ 18.5µL dH_2O

Enzyme Master Mix for Part B (25µL, 5 rxns)

→ 5µL NEB Buffer 2
→ 0.5µL BSA
→ 0.5µL XbaI
→ 0.5µL PstI
→ 18.5µL dH_2O

→ Add

→ Add

Diges

→ Add

→ Add

Diges

→ Add 4

→ Add

Dige

Ligation

→ Add 2µ

Add equ

Add equ

Add 1µ

Add

Digest Plasmid Backbone

→ Add 4µl linearized plasmid backbone (25ng/µl for 100ng total)
→ Add 4µl of Enzyme Master Mix

Digest Part A

→ Add 4µl Part A (25ng/µl for 100ng total)
→ Add 4µl of Enzyme Master Mix

Digest Part B

→ Add 4µl Part B (25ng/µl for 100ng total)
→ Add 4µl of Enzyme Master Mix

Digest all three reactions at 37°C for 30 minutes and heat kill at 80°C for 20 mins.

Ligation

→ Add 2µl of digested Plasmid Backbone (25ng)
↓
Add equimolar amount of Part A (< 3µl)
↓
Add equimolar amount of Part B (< 3µl)
↓
Add 1µl T4 DNA ligase buffer

↓

Transform with 1-2μl of product

↓

50 µl of DH5α cells (glycerol stock)

↓

Incubated at 37°C, 230rpm

03/08/17

HYBRID PROMOTER CONSTRUCT

(PlacIAra)

Conc. by nanodrop measurement = 39.3 ng/µl

Digestion

Backbone (PSB1C3)	Part (Hybrid promoter)
Conc. = 25 ng/µl	Conc = 39.3 ng/µl
25 ng backbone = 1 µl	25 ng part = 0.64 µl
Master mix = 4 µl	Master mix = 4 µl
dH_2O = 3 µl	dH_2O = 3.36 µl
8 µl	8 µl

→ Digest each at 37°C / 30 min

→ Heat kill at 80°C / 20 min
(Denaturation of enzyme)

Ligation

Add 2 µl of digested plasmid backbone
(= 6.25 ng)

↓

↓

Add 1µl T4 DNA ligase buffer

↓

Add 0.5 µl T4 DNA ligase

↓

Add water upto 10µl
= 4.5µl

↓

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

↓

Transform with 1-2µl of product

React

Part	Control
Digested backbone = 2µl	2µl
Part = 2µl	~~2µl~~
T4 DNA ligase buffer = 1µl	1µl
T4 DNA ligase = 0.5µl	0.5µl
Water = 4.5µl	6.5µl
10µl	10µl

3/8/17

TRANSFORMATION:

Took 50 µl of comp. cells in ~~one~~ two epp.

↓

Added 1µl of the construct & control & mixed well

↓

↓

30 min ice incubation

↓

45 sec at 42°C waterbath

↓

5 min ice incubation

↓

Added 200µl of LB

↓

2½ hours at 37°C, 220 rpm

↓

Plating on Cam plates (1A)

18 hrs incubation at 37°C, 220 rpm

↓

4/2/17 O.D - 0.137

Comp. cell Preparation ↓

Transfer to 50ml falcon on ice

↓

Centrifuge at 3000 rcf at 4°C for 10 mins

↓

Resuspend in 2ml CCMB80

↓

OD measurement

↓

Sample 950µl SOB + 50µl cells

Blank 950µl SOB + 50µl CCMB80

↓

OD of suspension = $\frac{0.039}{0.78} \times 20$

= 0.78

↓

Dilute the cell suspension to a final OD of 1-1.5

↓ Centrifuged at 3000 rcf, 4°C, 10min

~~Dilution~~ — ~~removed supernatant and~~ diluted in 1ml of CCMB80

Final OD - 0.78×2

= 1.54

↓

Aliquot 50µl of the cells into eppi

↓

20 mins ice incubation

↓

Store in -80°C

TRANSFORMATION 9/8/17

Took 50µl of competent cells

↓

Added 1µl of 100 ng/µl of RFP plasmid (Comp. test kit)

↓

Ice incubation for 30 mins

↓

Heat shock - 42°C - 60 secs

↓

Ice incubation for 5 mins

↓

Added 200µl of LB

↓

Incubation at 37°C, 220 rpm, 2 hours

↓

Plated the pellet in LB-cam plates

T7 BACTERIOPHAGE EXPERIMENTS

12/7/17

Serial dilution and concentration estimation of T7 lytic phage

Clean the working area with EtOH

↓

wear gloves and other protective equipments

↓

Keep the flame of the burner on near working area
(work within 8cm of the flame)

↓

Dilution of the stock is as follows
10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}

Measure out 900µl milli-Q water use $MgSO_4$ in labelled eppis
(1-10^{-1}, 2-10^{-2}, 3-10^{-3}, 4-10^{-4}, 5-10^{-5})

↓

Pipette out 100µl phage lysate into 1eppi

↓ mix well

Pipette out 100µl from eppi 1 to 2

↓ mix well

Pipette out 100µl from eppi 2 to 3

↓ mix well

Repeat dilutions till 5

↓

Grow E.coli till an 0.0 0.3-0.7
(expo. phase)

Add 200 µl of cell culture

↓

Add 100 µl of different phage dilutions

(make 2 replicates for each dilutions)

vortex ↓ wait for 4-11 mins @ 55°C

Mix and pour on LB plate and spread uniformly.

↓

Incubate overnight @ 37°C

↓

Count the number of plaque

Dilution	# of plaque
10^{-2}	uncountable
10^{-3}	uncountable
10^{-4}	360, 200

$$Pfu_c = \frac{\#\ plaque}{dilution \times volume}$$

$$= \frac{360}{10^{4} \times 0.1}$$

$$= 3.6 \times 10^{7}$$

$$Pfu_c = \frac{200}{10^{-4} \times 0.1} = 2 \times 10^{7}$$

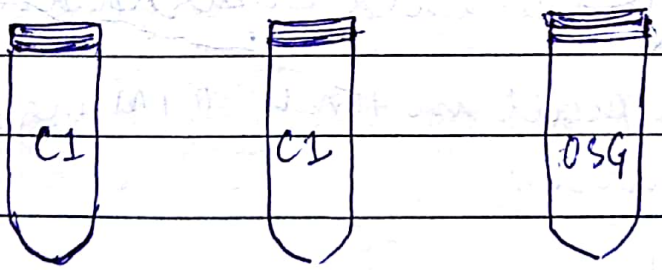
13/7/17

HOST DYNAMICS AND PHAGE KINETICS

grow E.coli to an O.D = 0.2

↓

Inoculate 9mL LB with 1mL of O.D = 0.2 culture



Label the eppis as shown

↓

Measure O.D of C1 ~~and~~ every 15 mins.

↓

Calculate vol. of phage for MOI = 0.0001

$$V_{phage} = \frac{0.0001 \times 0.2 \times 10 \times 4.5 \times 10^8}{2.8 \times 10^6}$$

$\approx$ 240µl

↓

Add 240µl phage lysate to C2 and OSG

↓ Store OSG in ice

Transfer C2 to incubator and check OD every 15 mins.

Centrifuge OSG tube @ 10000 rpm for 10mins at 4°C

↓ → Discard supernatant

Dissolve the pellet in 10ml 0.1M $MgSO_4$ solution

↓

Centrifuge @ 10000 rpm 4°C 10 min

↓ → Discard supernatant

Dissolve the pellet in 10ml 0.1M $MgSO_4$

Centrifuge @ 10000 rpm 4°C 10mins

Dissolve pellet in 10 ml LB and immediately transfer to 90ml LB
Total volume = 100 ml

↓

Incubate @ 37°C in water bath

↓

Take samples every 5 mins starting @ t = 0 till t = 45 mins

↓

Time (t)	0	5	10	15	20	25	30	35	40	45
Dilutions (i)	10^{-2}	10^{-2}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}
(ii)	10^{-3}	10^{-3}	10^{-4}	10^{-4}	10^{-4}	10^{-5}	10^{-5}	10^{-5}	10^{-6}	10^{-6}
(iii)	10^{-4}	10^{-4}	10^{-5}	10^{-5}	10^{-5}	10^{-6}	10^{-6}	10^{-6}	10^{-7}	10^{-7}

↓

Count plaques on each plate and back calculate phage concentration

07/08/17

TROUBLESHOOT FOR 3A Assembly

Gel preparation 100mL Agarose gel

(i) 1g of Agarose
(ii) 100mL TAE → cook for 2mins in microwave
(iii) Few drops of EtBr

Casted on a tray and put a comb.

Gel running

Remove the comb

↓

Put the gel tray on gel electrophoresis machine

↓

2μl of ~~DNA ladder~~ reconstituted DNA was added to the 1st well

↓

3 drops of loading dye were added to parafilm strip (1μl each)

↓

3μl of hybrid promoter construct
3μl of RBS + AmilCP construct
4μl of RBS + CJ Blue construct
were mixed with loading dye and added to well 2, 3, 4 respectively.

↓

It was run at 80V for 1 hour

~~Hybrid Promot~~

RESULT- Did not find a band in the image.

REPEAT - Plasmid construct &

Digestion

PSB1C3 backbone

conc = 25ng/µL

25ng backbone = 1µL

Mastermix = 4µL

dH_2O = 3µL

8µL

Part (Hybrid Promoter)

conc. = 25ng/µL

Part = 1µL

Mastermix = 4µL

dH_2O = 3µL

8µL

↓

Digest at 37°C for 30mins

Heat kill at 80°C for 20 mins

Preparation of enzyme mastermix

PSB1C3 backbone (2 Rxns)

10µL

NEB Buffer 3.1	–	2µL
EcoRI-HF	–	0.2µL
Pst I	–	0.2µL
Dpn I	–	0.2µL
dH_2O	–	7.4µL
		10µL

ECOR I-HF	-	0.2µl
Pst I	-	0.2µl
dH_2O	-	7.6µl
		10µl

To check the digested product

Gel Preparation (50ml)

0.5g Agarose
50ml TAE
Heat for 2 mins in oven
Few drops of EtBr

↓

Casted the gel on a tray and put the comb.

↓

After it got solidified, took out the comb

↓

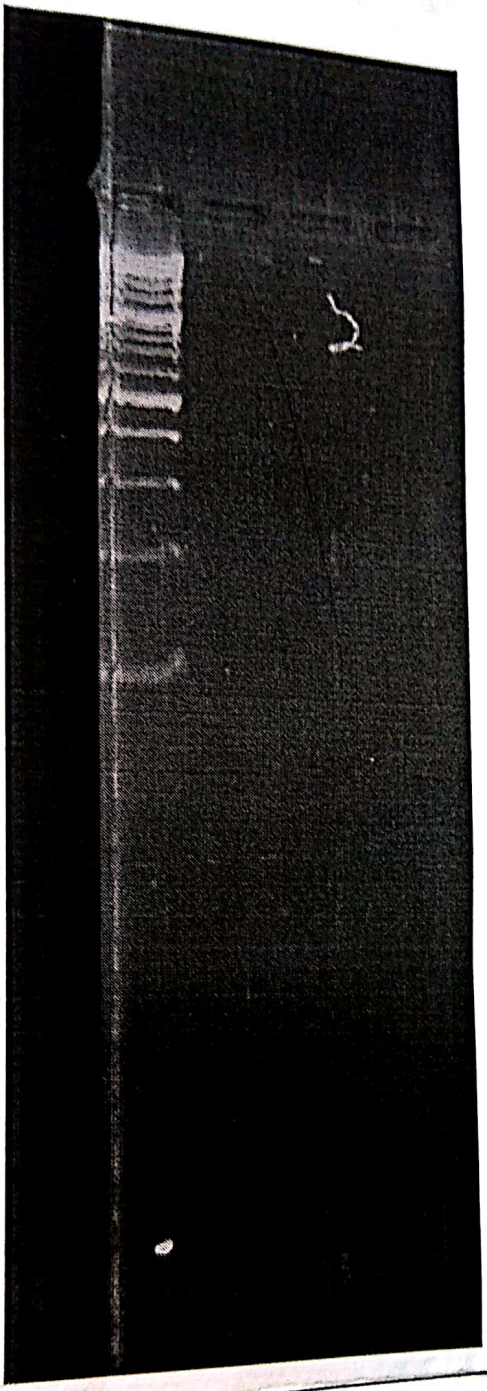
Put the tray on gel electrophoresis machine

↓

~~Ran the gel at 110V for 40 mins~~

3 wells were loaded with

(i) Reconstituted 2log DNA ladder - 2µl
(ii) 4µl (3+1) of PSB1C3 digested backbone
(iii) 4µl (3+1) of hybrid promoter



Usually RF cloning has two PCR ~~cycles~~ reactions
Since we have a megaprimer, we will start with secondary reaction.

Ratio of insect to plasmid:

A molar insert : plasmid ratio ≥ 20

Conc. of hybrid promoter (insert) : 39.4 ng/µl

Conc. of the plasmid (AmilCP gene in PSB1C3) : 52.6 ng/µl

Molar mass of hybrid promoter = 50.4×10^3 g/mole

Molar mass of AmilCP plasmid = 8.43×10^5 g/mole

If we take 100ng of the plasmid

Amount of plasmid = 0.798 µl ≈ 0.8 µl

Moles of plasmid = $\frac{100 \times 10^{-9}}{8.43 \times 10^5}$

$= \frac{100 \times 10^{-9}}{8.43 \times 10^5} \times \frac{1000}{0.8 \times 10^{-6}}$

$= 0.741 \times 10^{-5}$ g/mol

$= 11.86 \times 10^{-14}$ moles

Moles of insert = $11.86 \times 10^{-14} \times 20$

We need $20 \times 0.741 \times 10^{-5}$ g of insert

$20 \times 0.741 \times 10^{-5}$ = moles of insert

$20 \times 11.86 \times 10^{-14}$ = moles of insert

Moles of insert = 237.24×10^{-14}

Weight of insert = $\frac{237.24}{} \times 10^{-14} \times 50.4 \times 10^{3}$ g/mol

= 119.57 ng

≈ 120 ng

Amount of insert (in µl) = $\frac{120 \text{ ng}}{40 \text{ ng/µl}}$

= 3 µl

PCR Mix (20µl)

	Test	Control
Plasmid (62.6 ng/µl)	1.6 µl	1.6 µl
Mega primer:	3 µl	milli Q (3 µl)
PCR Buffer (5X)	4 µl	4 µl
10mM dNTP mix:	0.4 µl	0.4 µl
Phusion: (2U/µl)	0.2 µl	0.2 µl
Milli Q:	10.8 µl	10.8 µl
	20 µl	20 µl

↓

Mix all components well.

2-step Thermal cycler conditions

Denature @ 98°C for 30secs - 1X

↓

Denature @ 98°C for 8 secs — 15X

↓

Extension @ 72°C 30sec/Kb

Plasmid around ~ 2.0Kb

@ ~~70 secs~~ 60 secs

↓

Final extension @ 72°C for 5mins - 1X

↓

Add 20units of DpnI to the reaction mixture

↓

Incubate for 2hrs at 37°C

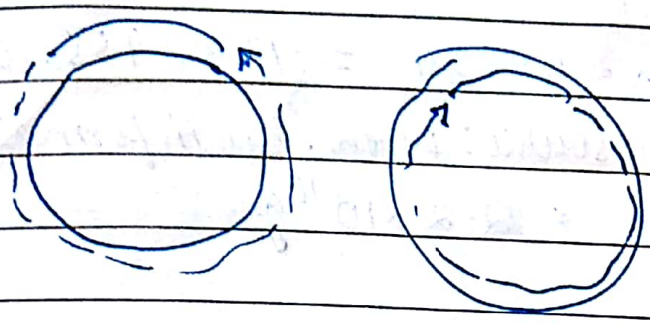
↓

Heat kill @ 80°C for 20 mins

↓

Transform into ultra-comp cells

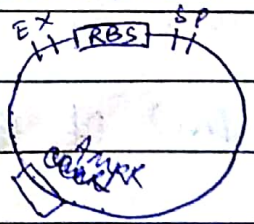
Size of insert - 163
Size of parent plasmid - 2740
Size of PSB1C3 backbone - 2070
Size of desired plasmid - 2233



Amil CP gene in the parent plasmid is much bigger than the insert.
Thus, the insert would not loop like it should. It would be in strain ~~insert~~ instead.
So we'll use a backbone with a small insert like RBS or promoter

We are going to run 2 PCR reactions.
One with template plasmid : BBa_B0034

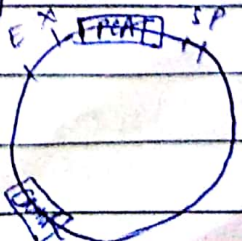
RBS + pSB1A3



Size of RBS - 12 bp
Total size of plasmid - 2167 bp
Other with template plasmid : BBa_I14033

Promoter + PSB1C3

Size of Promoter - 38 bp



Total size of plasmid - 2108 bp

Approx. MW of nucleic acids

MW of dsDNA = (# nucleotides × 607.4) + 157.9

(Source: Thermo Fischer Scientific)

MW of BBa_B0034 = 1316393.7 Da

MW of BBa_B0034 = 1338955.5 Da

(Source: www.bioinformatics.org)

$= 2.2 \times 10^{-18}$ g

Molar mass = $2.2 \times 10^{-18} \times 6.022 \times 10^{23}$ g/mol

$= 13.24 \times 10^{5}$ g/mol.

MW of BBa_I14033 = 1280557.1 Da

(Approx. by formula)

By calculator = 1302479.30 Da

$= 2.16 \times 10^{-18}$ g

Molar mass = 2.16×10^{-18} g × 6.022×10^{23} g/mol

$= 13 \times 10^{5}$ g/mol

MW of Hyd. Promoter = 9916.1 Da

(Approx)

MW (Calculator) = 10039.47 Da

$= 1.67 \times 10^{-19}$ g

Molar mass = $1.67 \times 10^{-19} \times 6.022 \times 10^{23}$

$= 10.06 \times 10^{4}$ g/mol

Amount

Conc:

Volume

Moles

Moles of Hyd pr

for

wt. of pro

Conc. of

Vol.

P

Plasmid

Insert

Buffer (10x

Pfx Platinum polymer

10mM dNTP

50mM Mg

MilliQ

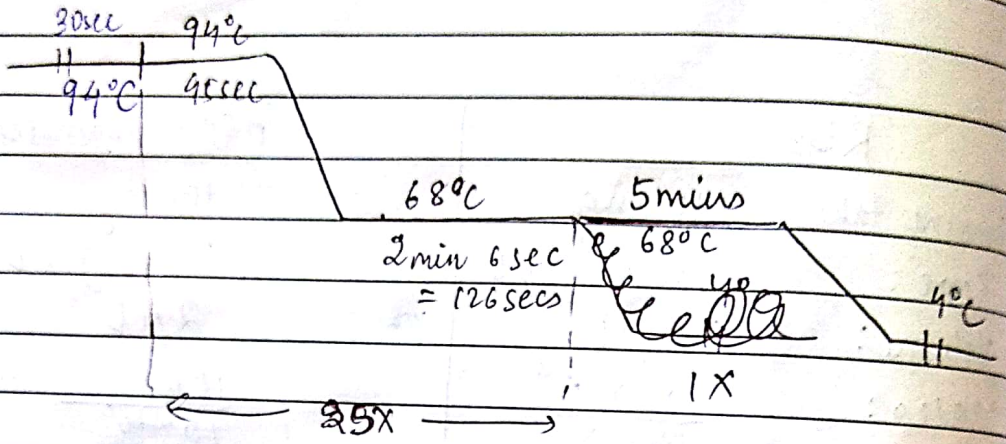
Conc. of RBS plasmid = 50.1 ng/μL
Conc. of pCat plasmid = 47.8 ng/μL

RBS	pCAT promoter
Amount taken: 100 ng	100 ng
Conc: 50.1 ng/μl	47.8 ng/μl
Volume: 2 μl	2 μl
Moles: $\frac{100 \times 10^{-9}}{13.24 \times 10^5}$	$\frac{100 \times 10^{-9}}{10 \times 10^4} = 15.4 \times 10^{-13}$
$= 7.55 \times 10^{-14}$	
Moles of Hyd promoter: $20 \times 7.55 \times 10^{-14}$	Moles of Hybrid promoter: $20 \times 15.4 \times 10^{-13}$
for 20:1 ratio $= 151 \times 10^{-14}$	$= 15.4 \times 10^{-13}$
Wt. of promoter = 151 ng	Wt. of promoter: 155 ng
Conc. of promoter = 40 ng/μl	40 ng/μl
Vol. of promoter = 4 μl	4 μl

PCR Mix (50μL)

	RBS	pCAT	Control
Plasmid	2 μL	2 μl	2 μl
Insert	4 μl	4 μl	–
Buffer (10X)	5 μl	5 μl	5 μl
Pfx Platinum polymerase	1 μl	1 μl	1 μl
10mM dNTP mix	1.5 μl	1.5 μl	1.5 μl
50mM $MgSO_4$	1 μl	1 μl	1 μl
Milli Q	35.5 μl	25.5 μl	34.5 μl

2-Step PCR Tm = 72°C



↓

20 units of Dpn I (1µl) (stock = 20000 U/ml)

↓

2hrs 37°C

↓

~~80°C / 20min inactivation~~

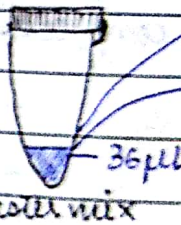
Mastermix for 20µl reactions (x 2)

10X Pfx Buffer	–	4µl
10mM dNTP	–	1.2µl
50mM $MgSO_4$	–	0.8µl
Megaprimer	–	8µl
~~Template~~ Pfx Polymerase	–	1.6µl
Milli Q water	–	20.4µl
		36µl

Reactio

10X Pfx Buf
10mM dNTP
50mM Mgs
Megaprime
Template D
Pfx polym
Distilled

We a

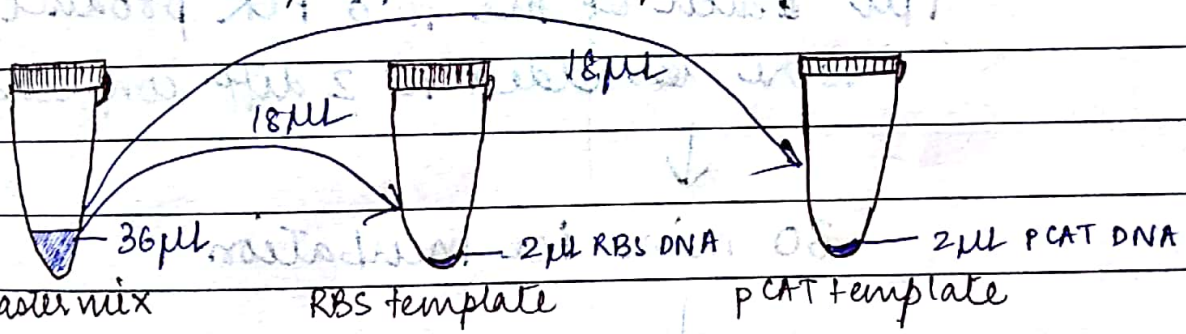


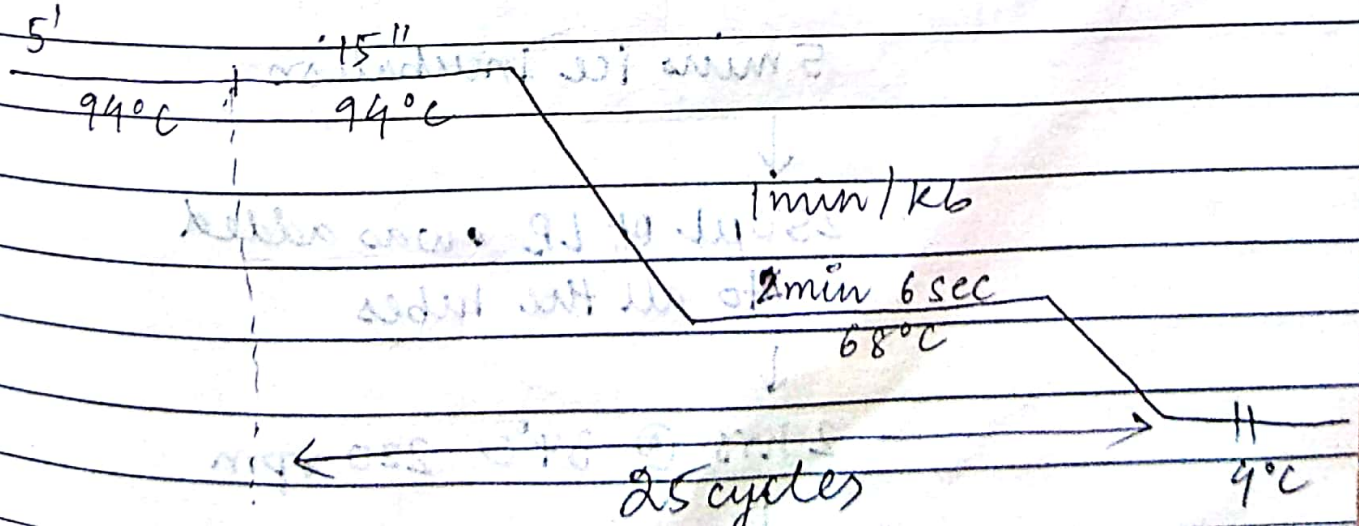
5'
94°C

Reactions

	pCAT	RBS	Final conc
10X Pfx Buffer	1 µl	1 µl	1X
10mM dNTP	0.3 µl	0.3 µl	0.3 mM
50mM $MgSO_4$	0.2 µl	0.2 µl	1 mM
Megaprimer	4 µl	4 µl	
Template DNA	2 µl	2 µl	
Pfx polymerase	0.4 µl	0.4 µl	1 U
Distilled water	2.1 µl	2.1 µl	

We added 18µl of the mastermix to 2µl each of RBS template DNA and pCAT promoter template DNA.





↓

2 hrs at 37°C

↓

80°C / 20 min heat inactivation

↓

Transformation

4 comp cells stock were ~~brought~~ taken

↓ (2 MG1655, 2 DH5α)

1 µl each of the two PCR product were added to 2 diff. comp cells

↓

30 mins ice incubation

↓

1 min @ 42°C water bath

↓

5 mins ice incubation

↓

250 µl of LB was added to all the tubes

↓

2 hrs @ 37°C 220 rpm

↓

Remove 200µl of LB

↓

Resuspend pellet in the remaining LB

↓

Plate the held amount in respective antibiotic plates (LA)

RBS template – Amp plate

pCAT template – Cam plate

Result –

	No. of colonies
RBS –	3 (MG1655)
pCAT –	3 (DH5α)

23/08/17

Antibiotic stock Preparation

① Ampicillin
Stock : 100 mg/ml in 50% ethanol
Working: 100 µg/ml
Colour code: ORANGE
Line code: I

② Chloramphenicol
Stock: 35mg/ml in 100% ethanol
Working: 35 µg/ml
Colour code: GREEN
Line code: II

③ Kanamycin
Stock: 35 mg/ml in distilled water
Working: 35µg/ml
Colour code: Red
Line code: III

④ Tetracycline
Stock: 15 mg/ml in 50% ethanol
Working: 15 µg/ml
Colour code: Yellow
Line code: IIII

NOTE: Light sensitive, Wrap with Aluminium

Amount of distilled water = 5 mL

Stock (1mL x 10) Tubes wrapped with Al foil
Amount of Retracycline powder = 150 mg (15x10)
Amount of 100% ethanol = 5 mL
Amount of distilled water = 5mL

26/08/17

Preparation of SOB (250mL)

LB powder - 6.25g
2.5mM of KCl — 0.0466g
20mM of $MgSO_4$ = 1.232g

M.W. 246.5g/mol

$\frac{20 \times 10^{-3} \times 250 \times 246.5}{1000}$

CCMB80 buffer (100mL)

① 10mM KOAc M.W = 98.15g/mol

$\frac{10 \times 10^{-3} \times 100}{1000} \times 98.15$

= 0.098g

② 80mM $CaCl_2 \cdot 2H_2O$ M.W = 147 g/mol

$\frac{80 \times 10^{-3} \times 100}{(1000)} \times 147 = 1.176$ g

(3) 20mM $MnCl_2 \cdot H_2O$ MW = 197.91 g/mol

$= \frac{20 \times 10^{-3} \times 100}{1000} \times 197.91 = 0.395g$

(4) 10mM $MgCl_2 \cdot 6H_2O$ M.W = 203.31 g/mol

$\frac{10 \times 10^{-3} \times 100}{1000} \times 203.31$

$= 0.203g$

(5) 10% V/V Glycerol

$\frac{100 \times 10}{100} = 10mL$

Adjust pH to 6.5

Filter sterilize and store at 4°C

29/08/17

Inoculation of SOB (100mL) with 1ml of MG1655 seed stock for comp: cell preparation.

29/08/17

Transformation of the PCR product (Hydpm) did not work.

No transformants.

The competent cells were not competent.

Inoculation

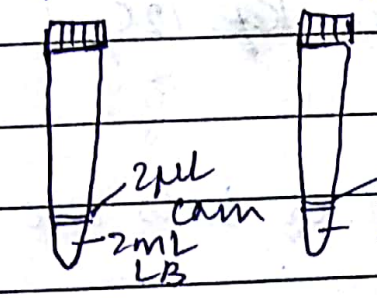
Take 15ml falcon x 2

↓

Pour 2ml LB in each

↓

Put 2μl Ampicillin in one
and
2μl Chloramphenicol in other



↓

Vortex the two falcons

↓

Pick a colony from pCAT temp plate and inoculate with a 2μl tip

↓

Pick a colony from RBS temp plate with 2μl tip inoculate

↓

Incubate @ 37°C
with 220 rpm
for 18 hours

Plasmid Extraction

Pipette 1ml culture into 1.5mL eppindorf x 2

↓

Centrifuge at 8000rpm (6800rcf) for
3 mins at 25°C

↓ → Discard supernatant

Resuspend pellet in 125µL P1 buffer

↓

Pool them together into one tube

↓

Add 250µL P2 buffer and mix well
by inverting the tubes 4-6 times
(Not more than 5 mins) No vortex

↓

Add 350µL N3 buffer & mix by
inverting the tubes 4-6 times

↓

Centrifuge @ 13000 rpm (17900rcf) at
25°C for 10mins

↓ → Discard pellet

↓

Pipette 800µL supernatant into QIAprep
column & centrifuge @ 13000rpm/60sec

Wash QIA prep spin column by adding 0.5 ml buffer PB and centrifuge for 30-60 secs.

↓ Discard the flow-through

Centrifuge at 13000 rpm for 60 secs

↓ Discard flow through

Add 750 µl PE buffer & centifuge 13000 rpm for 1 min

↓

Centrifuge / 13000 rpm / 60 secs

↓ Discard flowthrough

Centrifuge / 13000 rpm / 60 secs

↓

Transfer column in 1.5 mL eppy

↓

Add 50 µl buffer EB at the center of each column

↓

Stand for 5 min @ 37°C

Nanodrop measurement

Yield

93.6 ng/µL - Hyb - pr - Amp 260/280 = 2.01

81.4 ng/µL - Hyb - pr - cam 260/280 = 1.98

Media Preparation

LA (200ml)

LB Powder - 5g

Agar - 3g

Dimilled water - upto 200ml

For 100 ml

LB powder - 2.5g

Agar - 1.5g

Water - upto 100ml

Antibiotic stock (Kanamycin) - 50mg/ml

Amount of Kanamycin - 250mg

Amount of MilliQ water - 5ml

- Filter sterilized with 0.2µm filter
- Aliquoted in 5 eff microcentrifuge tubes

1.5% Gel Preparation

Take 0.75 g Agarose ~~was taken~~

↓

Add 50 ml 1X TAE

↓

Heat

↓

Add EtBr

↓

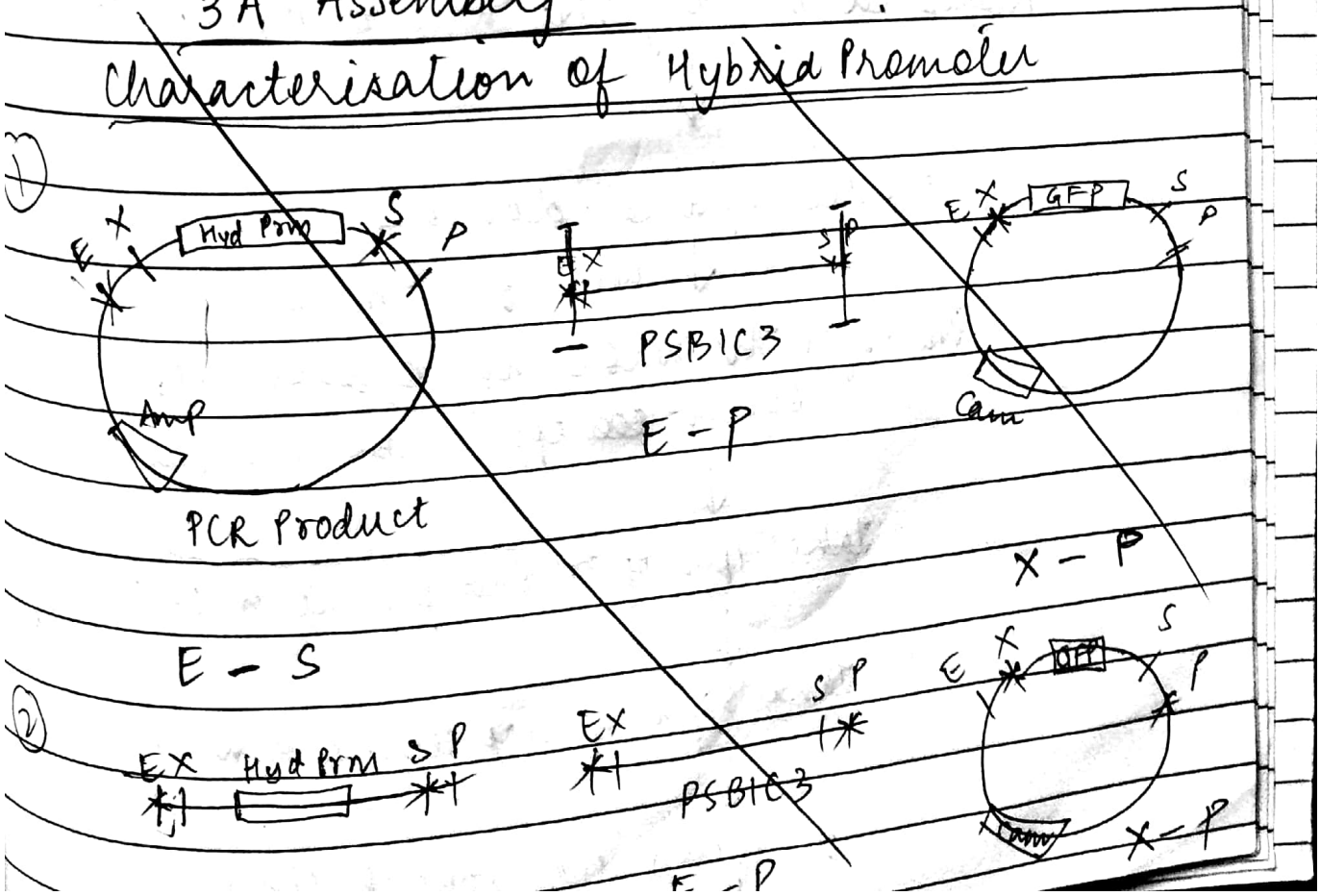
Pour on the tray

↓

Put the comb

3A Assembly

Characterisation of Hybrid Promoter



NEB Buffer

EcoRI	0.2 µl
PstI	0.2 µl
DpnI	0.2 µl
dH_2O	7.4 µl

Enzyme master mix for promoter

2/9/17 ~~NEB Buffer~~

TRANSFORMATION OF RBS (BBa_B0030) into MG1655 comp cells

RBS - BBa_B0030

Kit Plate - 4 , 4G

Resuspend the dry DNA with 10 µl autoclaved milliQ water

↓

Pipette up and down

↓ wait for 5 mins

Transfer the whole amount into 600 µl eppy tube and label

↓

Take 1 µl of it and pipette in another eppy

↓

Take 50 µl of comp cells and mix well

↓

↓

Heat shock @ 42°C, 1min

↓

Ice incubation - 5mins

↓

Add 250µL LB and ~~incubate~~ incubate @ 37°C for 2hrs

↓

Plate on Cam plates (100µL)

Result:

No. of colonies: 1

04/9/17

Gel running for PCR product (Hybrid Promoter)

Digestion

	P_{Hyd} Amp	P_{Hyd} Cam	RBS Elowitz	pCAT
	P_{Hyd} Amp	RBS Elowitz	P_{Hyd} cam	pCAT
RE (XbaI)	1 µl	1 µl	1µl	1µl
DNA (1µg)	10.6µl	20 µl	12.2µl	2 µl
10X cut Smart NEB Buffer	5µl	5µl	5µl	5µl
Total Reaction Volume	50µl	50µl	50 µl	50µl
Incubation time	1hr 20min ↓ ↓	1hr 20min ↓ ↓	1hr 20min ↓ ↓	1hr 20min ↓ ↓
Incubation Temp	37°C 65°C	37°C 65°C	37°C 65°C	37°C 65°C

Plasmid	concentration
P_{Hyd} Amp	93.6 ng/µl
RBS Elowitz	50.1 ng/µl
P_{Hyd} cam	81.4 ng/µl
pCAT.	47.8 ng/µl

93.6×10^{-3} µg → 1 µl

1µg → $\frac{1}{93.6} \times 10^3$

= 10.68 µl

50.1×10^{-3} µg → 1 µl

1 µg → $\frac{1}{50.1} \times 10^3$

= 20µl

81.4×10^{-3} µg → 1µL

1µg → $\frac{1}{81.4} \times 10^3$

= 12.2µL

47.8×10^{-3} µg → 1µL

1µg → $\frac{1}{47.8} \times 10^3$

= 21µL

Make 1.5% Agarose gel
(0.75g Agarose in 50mL water)

↓

Heat and dissolve

↓

Add a drop of EtBr (~1µL)

↓

Pour into the tray

↓

Put the comb

↓

Let it solidify

↓

Remove comb,
Run gel in gel electrophoresis

80V, 1hr

↓

Image get under UV

Result- No different betw" the size of different plasmids

1.5%. couldn't give good resolution.

2M NaCl — 2.92 g

Tris-HCl (2M) 10 mL — 2.42

pH - 7.9 (Use fuming HCl to adjust pH)

$MgCl_2 \cdot 6H_2O$ (1M) ~~10 mL~~ 3 mL — ~~2.42~~ ~~2.03~~ 0.1604 g

BSA (1000 µg/mL) (10X) - 4mL

NaCl - Mol. wt - 58.44 g/mol

$$\frac{x}{58.44} \times \frac{1000}{25} = 2$$

$$\Rightarrow x = \frac{2}{40} \times 58.44$$

$$= 2.92 g$$

Tris Base - Mol. wt = 121.14 g/mol

$$\frac{x}{121.14} \times \frac{1000}{10} = 2$$

$$\Rightarrow x = \frac{2}{100} \times 121.14$$

$$= 2.42 g$$

$MgCl_2 \cdot 6H_2O$ - Mol. wt = 203.3 g/mol

Inoculation of colonies in 2ml LB

↓

Incubation for 15hrs @ 37°C
220 rpm

↓

Pipette 1ml culture into 1.5ml eppi x 2

↓

@ 13000 rpm @ 25°C / 10mins

↓ → Discard pellet

Resuspend RNA pellet in 500µl supernatant mix @ RNA prep column & centrifuge @ 13000 rpm / 60secs

↓ → Discard flow through

Wash RNA prep spin column by adding 0.5ml buffer RB and centrifuge for 30-60secs

↓ → Discard flow through

Centrifuge @ 13000 rpm for 60secs

↓ → Discard flow through

Transfer column in 1.5ml eppy

↓

Add 50µl buffer EB at the centre of each column

↓

Stand for 5min @ 37°C (But we did for 1hr)

↓

Centrifuge @ 13000 rpm / 60sec

↓

store at -20°C

RBS Elowitz (50µL)	66.6	1.94	2.05
pCat (50µL)	63.7 ~~47.8~~	~~1.83~~	1.2 ~~3.00~~
Hyd-Pr-Amp (25µL)	~~7.09~~	1.71 ~~1.67~~	0.76 ~~0.90~~
Hyd-Pr-Cam (25µL)	112.8	1.9	1.81
RBS-Aarti CB0030) (50µL)	73.8	1.76	0.95

7/9/17 **Plans**

→ Miniprep Hyd-Pr-Amp
→ Transform PCR product again
→ Miniprep multiple colonies from the same
→ ~~Digest with~~ Double digestion Hyd-Pr-Amp, Cam, RBS El., pCAT
Run on 2% gel

ORF cloning repeat with Opn 1 control

Colony PCR

[redacted]
PSB1 5)

NEB buffer 3.1 (1X)
100mM NaCl
50mM Tris-HCl
10mM $MgCl_2$
100 µg/mL BSA

NEB buffer 2.1 (1X)
50mM NaCl
10mM Tris-HCl
10mM $MgCl_2$
100µg/mL BSA

pH for both buffers are 7.9 @ 25°C

Enzyme Efficiency Table

Enzyme	Buffer 2.1	Buffer 3.1
EcoRI	100*	50
XbaI	100	75
SpeI	100	25
PstI	75	100

Buffer conversion (2.1 → 3.1)

NaCl: $\frac{50mM \times 2 + x\,mM \times 1}{20} = \frac{100\,mM \times 2}{20}$

$x = 100\,mM$ NaCl for 1X

Tris HCl: $\frac{10\,mM \times 2 + y\,mM \times 1}{20} = \frac{60\,mM \times 2}{20}$

Step-1

(1) Backbone digestion (10µl)

Enzyme mastermix

(i) 2µl NEB buffer 3.1
(ii) 0.2µl FtoR I
(iii) 0.2µl Pst I
(iv) 0.2µl Dpn I
(v) 7.4µl dH_2O

4µl of above mastermix
+
4µl of backbone (PSIT3)

Incubate 37°C / 30 mins - (Incubator)
Heat kill 80°C / 20 mins - (Thermomixer)
(Put the thermomixer at 80°C prior to the experiment)

Step-2

(2) Part A (RBS - BBa_B0030)

(i) SpeI digestion in Buffer 2.1

(a) 2µl NEB Buffer 2.1 (manually made in the Lab)

(b) 0.2µl SpeI

4µl of master mix
+
200ng of DNA (Conc. of miniprepped RBS - 73.8 ng/µL)
(3µl of BBa-B0030)
+
1µl dH_2O
[73.8 × 3 = 221.4ng]

Incubate 37°C / 30 mins
Heat kill 80°C / 20mins

Step-1 and Step-2(i) can happen at the same time.
Pipette all the components and incubate together.

(ii) ECORI digestion in buffer 3.1

(a) 8µl digested product
(b) 0.8µl Buffer 2.1
(c) 0.8µl Buffer converter
(d) 5.1µl dH_2O
(e) 0.2µl ECORI

15µl

Incubate 37°C / 30mins
Heat kill 80°C / 20mins

4μl master mix (2μl + 2μl dH_2O)

↓

Incubate 37°C / 30min

Heat kill 80°C / 20min

This can also be done with step-1

(a) 2μl NEB buffer 3.1

(b) 0.2μl XbaI

(c) 0.2μl PstI

(d) 7.6μl dH_2O

100 ng of DNA (4μL) (GFP conc^n - 120.3 ng/μl)

+

4μl master mix (2μl + 2μl dH_2O)

↓

Incubate 37°C / 30min

Heat kill 80°C / 20min

This can also be done with step-1

↓

Filter Sterilise and Heat inactivate at 80°C

Aliquot into 1.2ml microcentrifuge tubes

Preparation of Buffer 2.1 (10X)

1X

50mM NaCl
10mM Tris-HCl
10mM $MgCl_2$
100µg/ml BSA
} pH - 7.9

16/09/17 - 17/09/17

Part A (6X) & C (For Zakhiya, & Jyotirish and Aarti)

(a) 6µL NEB Buffer 2.1 (manually made)
(b) 0.6µL SpeI
(c) 7.8 x 3 = 23.4 µL dH_2O.

Ligation Mastermix (13 reactn)

T4 Ligase buffer : 13µL
T4 Ligase : 6.5µL
Milli Q H_2O : 6.5µL
(Ran out of T4 ligase)

For 3A ligation :

4µL RBS + 2µL GFP + 2µL PSB1T3 Digests

↓

16°C overnight

↓

80°C / 20 mins

100 µl of ligation product

↓

50 µl of competent cells

↓

Ice incubation for 30 mins

↓

Heat shock at 42°C

↓

5 mins ice incubation

↓

Incubate at 37°C for 1 hour

↓

Plating Test plates

① -ve control

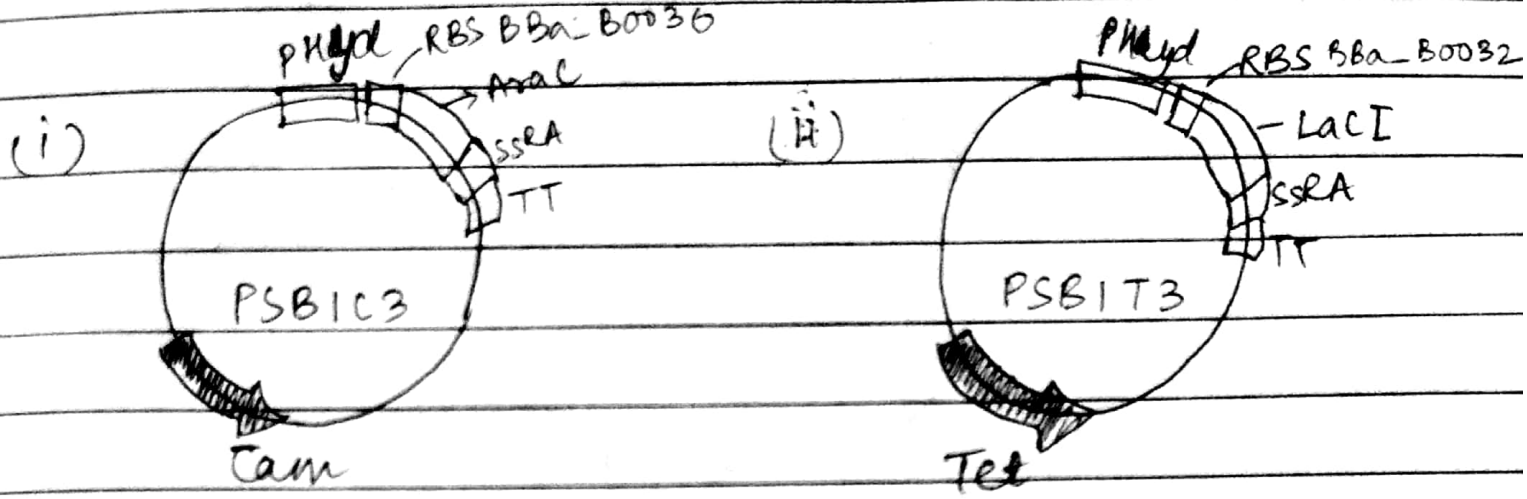
② ligation product

↓

No colonies were found.

Constructs: (i) pHyd (g-block) + AraC (g-block) + PSB1C3

(ii) pHyd (g-block) + LacI (g-block) + PSB1T3



Digestion

① 5x enzyme mastermix for plasmid backbone (100ng, 5 rxns) 25µL

(i)	NEB Buffer 3.1	5µL
(ii)	EcoRI	0.5µL
(iii)	PstI	0.5µL
(iv)	DpnI	0.5µL
(v)	dH_2O	18.5µL
		25µL

4µL of mastermix
+ 4µL (100ng) of plasmid backbone (PSB1C3 and PSB1T3)

↓

Incubate 37°C / 30min
Heat inactivate 80°C/20min

NEB Buffer 2.1 (From Lab) 2 µL

SpeI 0.2 µL

dH_2O 7.8 µL

10 µL

↓

3 µL of master mix

1 µL of dH_2O

+

150 ng pHyd – 4 µL

↓

Incubate 37°C / 30min

Heat inactivate 80°C / 20mins

(ii) EcoRI digestion

(a) 8 µL digested product

(b) 0.8 µL Buffer 2.1

(c) 0.8 µL Buffer converter

(d) 5.6 µL dH_2O. (Should have added 6µL)

(e) 0.8 µL EcoRI (Should have added 0.2µL. By mistake added 0.8

16 µL

Part B

Mastermix -

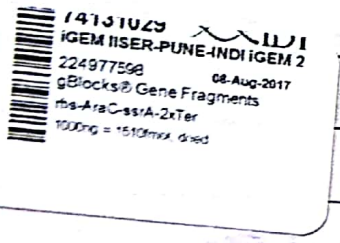
(a) NEB Buffer 3.1 - 2μL
(b) XbaI - 0.2μL
(c) PstI - 0.2μL
(d) dH_2O - 7.6μL

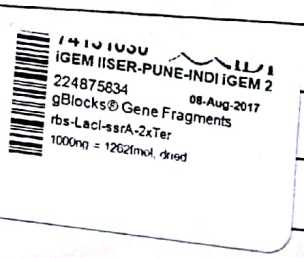
10μL

100ng DNA (5μL)
+
4μL mastermix

Incubate 37°C / 30min
Heat 80°C / 20min,

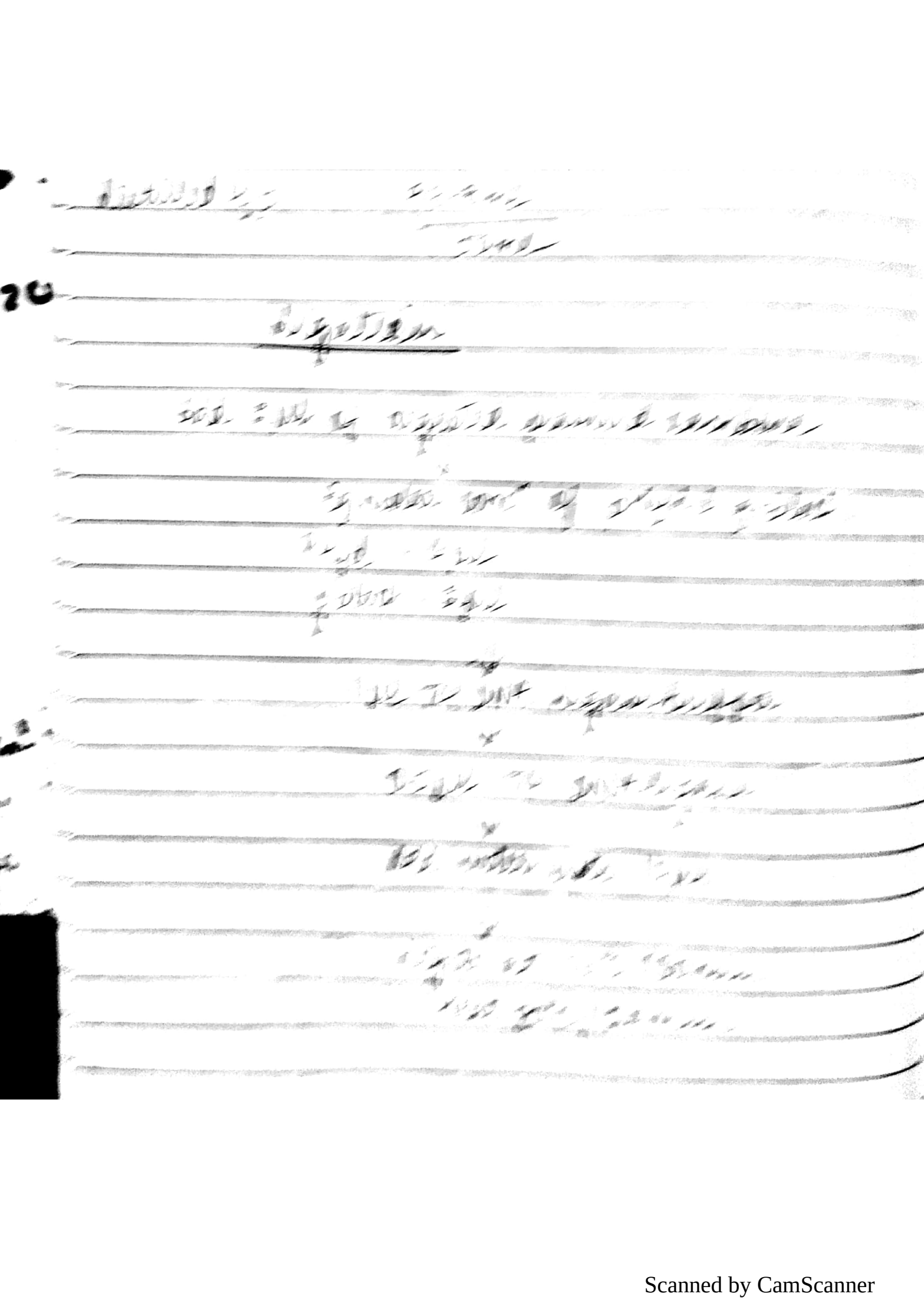
Resuspension of gblocks





1000ng
↓
added 40μL TE
↓
Final conc^n - 25ng/μL

1000ng
↓
added 40μL TE
↓
Final conc^n - 25ng/μL



Ligation Mastermix : 4 rkns
T4 ligase buffer : 4μl
T4 DNA ligase : 2μl
dH_2O : 2μl

3A ligation :
2μl Mastermix + 4μl Promoter +
2μl gblock + 2μl backbone

28/9/17
Gel electrophoresis of double digested product (14/9/17)

2% gel : 1g agarose + 50 ml TAE
heat 1:30 mins
↓
Add ~1 μl EtBr
↓
Pour gel & put comb
↓
Let it solidify
↓
Run in TAE buffer

Column 2 → Elowitz RBS 5μl + 1 μl dye
Column 3 → pHyb Amp 5 μl + 1 μl dye
Column 4 → 2log ladder 2μl
Column 5 → pHyb CAM 5 μl + 1 μl dye
Column 6 → pCAT 5μl + 1μl dye
80V / 1 hr.

28/9/17

RESULT OF GEL -

Didn't see anything on any lane
Can not think of a possible reason
for this result.

29/9/17

Transformation of ligated product

Take 1 µl of ligated product

↓

Add 50 µl of comp cells

↓

30 min incubation (30 mins)

↓

Heat shock @ 42°C / 1 min

↓

30 min incubation 5 mins

↓

Add 250 µl LB

↓

Incubate @ 37°C / 2 hrs

↓

Plating

Amp-L - Amp

Lac I - Tet

29/9/17

Result - No colonies were formed

30/9/17

TROUBLESHOOT -

Transformation of Amp product

↓

No colonies

Re-ligation

4 µl of digested promoter
2 µl of digest gblock (dra C)
2 µl of digested backbone
2 µl of ligation master mix
---
10 µl

Result: No colonies

23/10/17 Part A 3A Assembly

(1) Backbone digestion (10μl) PSB1T3
- (i) 1μl NEB buffer 3.1
- (ii) 0.2μl EcoRI
- (iii) 0.2μl PstI
- (iv) 0.2μl DpnI
- (v) 4μl PSB1T3 (100ng)
- (vi) 4.4μl dH_2O

(2) Part A (RBS-BBa-B0030) – (10μL)
- (i) 1μl Cut smart Buffer (NEB)
- (ii) 0.2μl EcoRI
- (ii) 0.2μl SpeI
- (iii) 2μl RBS (Conc. 73.8 ng/μl)
- (iv) 6.6μl dH_2O

(3) Part B (GFP with ssRA tag) BBa-K139...
- (i) 1μl NEB Buffer 2.1
- (ii) 0.2μl XbaI
- (iii) 0.2μl PstI
- (iv) 1μl GFP
- (v) dH_2O

Keep energizing at 37°C / 4 hours

Heat kill - 20 mins

Ligation

Molar wt. of all constructs -

Source: Thermofischer

Mol. wt. in daltons = (# nucleotides X 607.4) + 157.9 Da

1 Da = 1.67×10^{-24} g

$A_0 = 6.023 \times 10^{23}$ molecules/mol

(1) GFP (2823 bp.)

Mol. mass = 1724864.5 g/mol

(2) RBS (2085 bp.)

Mol. mass = 1273984.5 g/mol

(3) Backbone (PSB1T3) - B# of b.p = 2461

Molar mass = 1503451.2 g/mol

1 M = 1503451.2

x M = 100×10^{-9} g

$\Rightarrow x\ M = \frac{100 \times 10^{-9}}{1503451.2}$

$= 6.65 \times 10^{-14}$ M

GFP

IM = 1724864.5 g

$6.65 \times 10^{-14} \times 1724864.5\ g$

$= 1.14 \times 10^{-7}\ g$

$= 114\ ng$

$1\ \mu l$

RBS

IM 1273984.5

$6.65 \times 10^{-14} - ?$

$6.65 \times 10^{-14} \times 1273984.5$

$= 0.084 \times 10^{-7}$

$= 8.4\ ng$

Ligation

(i) 2 µl digested backbone
(ii) 2 µl Part A digest
(iii) 2 µl Part B digest
(iv) 1 µl T4 DNA ligase buffer
(v) 0.5 µl T4 DNA ligase
(vi) 2.5 µl dH_2O

80°/ 20 mins

4/10/17 - Transformation

1µl ligated product without heat kill

↓

Add 50µl comp cells

↓

9ice incubation for 30mins

↓

Heat shock at 42°C / 1min

↓

9ice incubation / 5 mins

↓

Add 250µl SOC

↓

Incubate at 37°C / 2hours

↓

~~Plate~~ Centrifuge at 3000g/5mins

↓

Resuspend in 100µl SOC

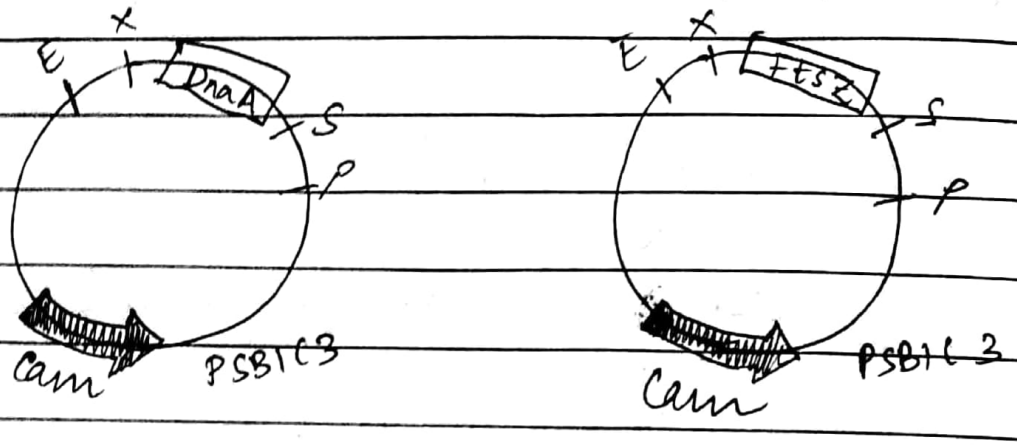
↓

~~Plate~~ on Tet plate

Result: No. of colonies: 44

Neg. control No. of colonies = 0

FtsZ - 1197 bp MW - 731462.8 g/mol



25 ng/µL

PSB1C3 - 2070 bp MW - 1264820.3 g/mol

Digestion

Component	10 µL reaction
DNA (DnaA & FtsZ ~~& PSB1C3~~)	4 µgL (100 ng)
10X NEB Buffer 3.1	1 µgL
EcoRI	0.2 µL
PstI	0.2 µL
dH_2O	4.6 µL

↓

37°C / 4 hours

↓

80°C / 20 mins

DNA (PSB1C3)	4 µL
10X NEB Buffer 3.1	1 µL
EcoRI	0.2 µL
PstI	

→ 37°C / 4 hours

Ligation

Add T4 DNA ligase at the end.

Component	10μL Reaction
10X T4 DNA ligase Buffer	1μL
Vector DNA (pDNA) (PSB1C3)	2μL
Insert DNA (DNAA)	4.2μL
T4 DNA ligase	0.5μL
dH_2O	2.3μL
	10μL

10X T4 DNA Ligase Buffer	1μL
Vector DNA (T1sZ) (PSB1C3)	2μL
Insert DNA (PSB1C3) (T1sZ)	3.5μL
T4 DNA ligase	0.5μL
dH_2O	3μL
↓ 16°C / overnight	10μL

We want 20ng of Vector DNA

100ng ⟶ 10μL

20ng ⟶ 2μL

1M - 1264820.3 g/mol

x ⟶ 20×10^{-9} g

xM ⟶ $\frac{20 \times 10^{-9}}{1264820.3}$

$= 1.58 \times 10^{-14}$ M

∴ Molarity of insert = $3 \times 1.58 \times 10^{-14}$ M

$= 4.74 \times 10^{-14}$ M

DnaA

1M = 895807.8 g

x g — 4.74×10^{-14} M

$895807.8 \times 4.74 \times 10^{-14}$

= 42.4 ng

Vol. of DnaA = 4.24 µL

Wt. of FtsZ required

731462.8 g — 1M

x — 4.74×10^{-14} M

$x = 3467133.6 \times 10^{-14}$ g

= 34.6 ng

Vol. of FtsZ ~ 3.5 µL

7/10/17

Transformation

2 Transformations directly after ligation (DnaA and FtsZ) - Arani

Incubated in SOC for 4-5 hours at 37°C.

3 Transformations after heat inactivation (DnaA, FtsZ, control)

Incubated in SOC for 2 hours at 37°C

Plated on lan plates.

Result -

	No. of colonies
FtsZ (Aarti) -	60
DnaA (Aarti) -	0
Backbone control -	0

Miniprep of 6 colonies for the clones from 3A Assembly (~~[illegible]~~ RBS (BBa.B0030) + GFP + PSB1T3) [SSRA]

Nanodrop :

	Concn (μg/mL)	260:280
Colony 1	96.5	1.97
Colony 2	116.7	1.71
Colony 3	109.3	1.83
Colony 4	78.1	1.93
* Colony 5	107.4	0.94
Colony 6	105	1.92

Gel running for confirmation

- Make 1% Agarose Gel

7/10

Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7
2 log ladder	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Colony 6
2 μl	5+1 μl	5+1 μl	5+1 μl	5+1 μl	5+1 μl	5+1 μl

8/10 The circular plasmid was cut with PstI

Digestion mixture (2 sam)

PstI - 1.2 μl

NEB Buffer 3.1 - 12 μl

dH_2O - ~~[illegible]~~ 8 μl

20 μl

+

[illegible] μl of DNA

7/10

Glycerol stock Preparation

Take 1ml of saturated culture (in SOB) of each colony

↓

Add 1ml of 80% glycerol (6ml 100% glycerol + 1.5ml dH_2O)

↓

Aliquot in 12 eppis (2 replicates for each colony)

↓

Flash freeze in Liq. N_2

↓

Store at -80°C

8/10

Inoculation for Miniprep (FtsZ + PSB1C3)

Took 5 falcons (15ml)

↓

Add 2ml SOB in each falcon

↓

Add 2µl Cam in each

↓

Pick 5 colonies and inoculate in respective falcons

↓

Incubate at 37°C / overnight
220° rpm

↓

Miniprep (Avani)

Nanodrop measurements (FtsZ ligated product)

	Conc^{n} (µg/mL)	260/280
Colony 1	55.4	1.994
Colony 2	36.2	1.786
Colony 3	52.2	1.96
Colony 4	54.5	2.007
Colony 5	50.34	1.98

Troubleshoot (2A)

Transformation

1µl of DnaA, Backbone, RFP plasmid in to 600µl eppi.

↓

50µl comp cells

↓

30 mins ice incubation

↓

1min heat shock (42°C)

↓

5 mins ice incubation

↓

Add 250 µl SOC into all the eppi's

↓

Incubation at 37°C / 220rpm / 3 hours
↓plate on Cam plates

Confirmation of the Construct with Gel

Digestion mixture (5 rxns) - FtsZ + PSB1C3
(Single cut)

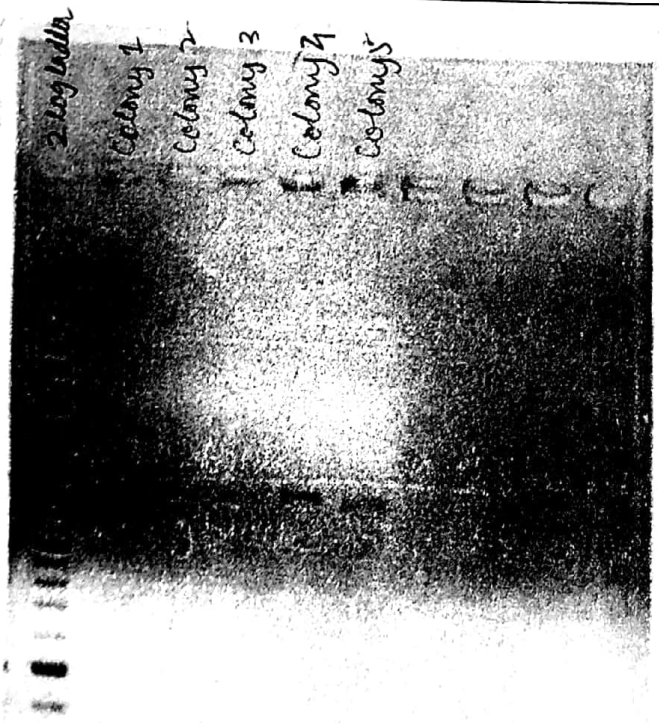
NEB Buffer 3.1 - 5µL
Pst I - 0.5µL
dH_2O - 19.5µL

↓

4µl master mix
+
4µl DNA (Colony 1, Colony 2... Colony 5)

digested

The DNA were run on a 0.8% gel.



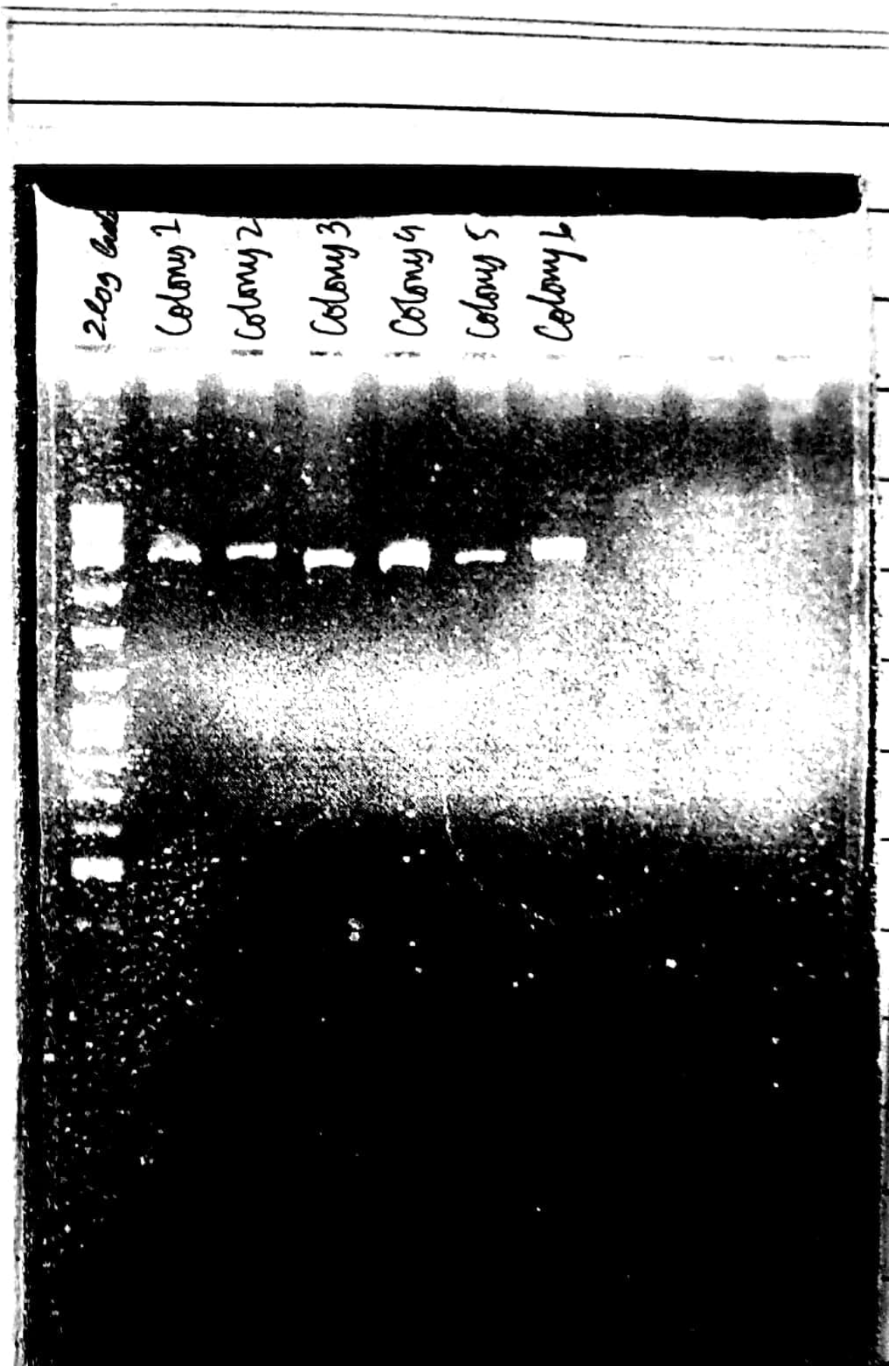
Size of PSB1C3- 2070

Size of FtsZ - 1197

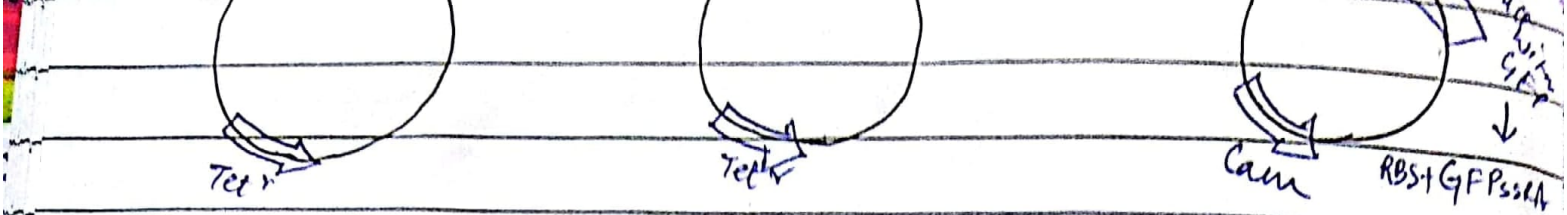
Gel image of the

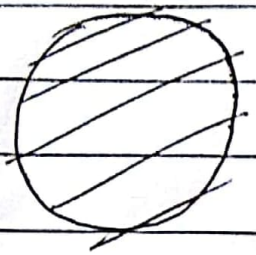
of 3A construct (RBS+GFP+PSB1T3)
a 0.8%.

2-Log DNA Ladder visualized by ethidium bromide staining on a 1.0% TBE agarose gel. Mass values are for 1 µg/lane.



Suggested Load:
10 µl/ gel lane



(d) 

Digestion of Part A (P Hyd) Conc^n: 343 ng/µl

pHyd -	2 µl
EcoRI -	0.2 µl
Spe I -	0.2 µl
10x Cutsmart -	1 µl
dH_2O -	6.6 µl
	10 µl

↓

Digest at 37°C for 3½ hours

↓

Heat inactivate @ 80°C for 20 mins

Part B

LacI gblock -	25 ng/µl
Arac gblock -	25 ng/µl
RBS - GFPssRA (colony 1) -	96.5 ng/µl

Digestion reac^n

	LacI	Arac	RBS+GFP
Part	4 µl	4 µl	2 µl
XbaI	0.2 µl	0.2 µl	0.2 µl
PstI	0.2 µl	0.2 µl	0.2 µl
B Buffer 3.1	1 µl	1 µl	1 µl
dH_2O	4.6 µl	4.6 µl	6.6 µl
	10 µl	10 µl	10 µl

↓

Digest at 37°C for 3½ hours
Heat inactivate at 80°C for 20 mins

Backbone Digest conc^n - 25ng/µL

	Tet^r	Cam^r
Part	4µL	4µL
EcoRI	0.2µL	0.2µL
Pst I	0.2µL	0.2µL
Dpn I	0.2µL	0.2µL
NEB Buffer 3.1	1µL	1µL
dH_2O	4.4µL	4.4µL
	10µL	10µL

↓

Digest @ 37°C for 3½ hours (water bath)

↓

Heat inactivate at 80°C for 20 mins (Thermomixer)

Ligation

It is done using 1:1 molar ratio of the backbone and parts.

Part	Molar mass (g/mol)	BP
PSB1T3	150345.2	2461
PSB1C3	126482.0.3	2070
pHyd (PlacI/Ara)	50.4×10^3	163
LacI gblock	78.4×10^4	1283
Arac gblock	1.5×10^4	1072

↓

Digest at 37°C for 3½ hours
Heat inactivate at 80°C for 20 mins

Backbone Digest Concⁿ - 25ng/μl

Part	Tet²	Cam²
EcoR I	4μl	4μl
Pst I	0.2μl	0.2μl
Dpn I	0.2μl	0.2μl
NEB Buffer 3.1	0.2μl	0.2μl
dH₂O	1μl	1μl
	4.4μl	4.4μl
	10μl	10μl

↓

Digest @ 37°C for 3½ hours (water bath)

↓

Heat inactivate at 80°C for 20 mins (Thermomixer)

Ligation

It is done using 1:1 molar ratio of the backbone and parts.

Part	Molar mass (g/mol)	BP
PSB1T3	150345.2	2461
PSB1C3	126482.3	2070
pHyd (Plasmid)	50.4×10^3	163

and 1.58×10^{-14} M of PSB1C3

Ligation reaction

	LacI (TetI) (in µL)	AraC (TetI) (in µL)	GFP ssrA (in µL)
T4 ligase	0.5	0.5	0.5
10X Buffer (T4 ligase)	1	1	1
Vector digest	2	2	2
Part digest	1	1	0.4
PHyd	0.2	0.2	0.2
dH_2O	5.3	5.3	5.9
	10µL	10µL	10µL

↓ ~~Ligate @ 16°C / overnight~~

↓

Transformation

Religation of DnaA

T4 ligase	0.5µL
T4 ligase Buffer (10X)	1 µL
PSB1C3	2 µL
DnaA	4.2µL
dH_2O	2.3 µL
	10µL

Transformed all the above ligated products along with FtsZ + PSB1C3, Snehal's construct — PHyd Promoter + GFP + PET15B (Amp),

(iii) Hyd Promoter + RBS + GFPssRA (cam) – 2

(iv) FtsZ – 0

(v) DnaA – 0

(vi) Snehal (GFP) – 129

Troubleshoot:

Retransformation of AraC, LacI, FtsZ & DnaA constructs.

* Grown in SOB and not SOC.

Characterization of Hyd Promoter + RBS + GFPssRA (cam)

To check for the right construct

Picked up the two colonies

↓ 2ml

Grew on SOB + 2µl cam overnight

↓

Used 1ml for miniprep and 1ml for induction of IPTG and Arabinose

Induction of promoter with 0.7% Arabinose and 1mM IPTG

Measured OD after 30 mins (0.5 mL culture + 0.5 mL SOB)

Colony 1 - 0.126

Colony 2 - 0.167

Actual OD

Colony 1 - $0.126 \times 2 = 0.252$

Colony 2 - $0.167 \times 2 = 0.334$

After 20 minutes

Induced with 1mM IPTG and 0.7% Arabinose

① Control 1

Comp cells in SOB

10 µL comp cells (MG1655) in 1mL of SOB

② Uninduced culture - Control 2

0.75mL

③ Control - 3 - SOB (Blank)

④ Induced culture -

0.75mL

Arabinose stock - 20%

Amount to be added = x

$C_1 = 20\%$, $V_1 = ?$ $C_2 = 0.7\%$, $V_2 = 750\,\mu L$

$20 \times x = 0.7 \times (0.75 + x)$

$= \frac{0.7 \times (0.75+x)}{20} = 0.0262 + 0.035x$

$= 26.2\,\mu L$ $= 27.2\,\mu L$

$\Rightarrow x = 0.75 \times 10^{-3}$ mL

$= 0.75$ µL

Induced with 27.2µL of Arabinose (0.7%) and (0.75mL culture)

~~0.75~~ 0.75µL of IPTG (1mM).

Grew the culture for 1hour 30 mins.
Pipetteted 100µL in the black Corning half plate. ~~plate~~ Checked fluorescence.

Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank (SOB)	UI1	UI2	I1	I2	-ve control comp cells						
B	Blank	UI1	UI2	I1	I2	CC						
C	Blank	UT1	UI2	I1	I2	CC						

UI1 - Uninduced colony 1

I1 - Induced colony 1

No fluorescence

Grew for one hour more.
No fluorescence.

BSA - Bovine serum albumin

Mol. wt = 66.5 KDa

It is used to stabilize some restriction enzyme during digestion of DNA and prevents adhesion of the enzyme to reaction tubes, pipet tips. (Does crowding)

NEB Buffer 1.1 (1X)

10mM Bis-Tris-Propane-HCl
10mM $MgCl_2$
100µg/ml BSA
pH 7.0 @ 25°C

NEB Buffer 2.1 (1X)

50mM NaCl
10mM Tris-HCl
10mM $MgCl_2$
100µg/ml BSA
pH 7.9 @ 25°C

NEB Buffer 3.1 (1X)

100mM NaCl
50mM Tris-HCl
10mM $MgCl_2$
100µg/ml BSA
pH 7.9 @ 25°C

Cut Smart Buffer

50mM Potassium Acetate
20mM Tris-acetate
10mM Mg-acetate
100µg/ml BSA
pH 7.9 @ 25°C

Gel

Ara 1 (cut) | Ara 1 (uncut) | Ara 2 (cut) | Ara 2 (uncut) | 2log ladder | LacI 1 cut | LacI 2 uncut | LacI 2 cut | LacI 2 uncut

Delayed in imaging.

- DNA diffused.

Gel for 3A part (HP + RBS + GFP ssRA)

Double cut ~~get~~ DNA (ECORI & ~~S~~PstI)

	HP+RBS+GFP (C1)	HP+RBS+GFP (C2)
DNA	2 µl	2 µl
NEB Buffer 3.1 (10X)	1 µl	1 µl
EcoRI	0.2 µl	0.2 µl

Lane 1 - Colony 1
Lane 2 - 2 log ladder
Lane 3 - Colony 2
Lane 4 - Colony 1

Troubleshoot for Rf-cloning

Took 2µl of PCR product (both Pcat & RBS)
↓
Ran it of 0.8% gel

Result - could not visualise

PpnI digestion

	HP(cam)	HP(Amp)	Pcat	RBS (Amp)
DNA or PCR product	3µl	3µl	2µl	2µl
PpnI	0.2µl	0.2µl	0.2µl	0.2µl
Cut Smart(10x) Buffer	1µl	1µl	1µl	1µl
dH_2O	5.8µl	5.8µl	6.8µl	6.8µl
	10µl	10µl	10µl	10µl

↓
@ 37°C 2½ hours (WB)

Inoculating device Colonies	15
Hemocytometry collaboration	24
Secondary inoculation,	26
Preperation of Staine	27
PFA fixing	30
Part resuspension and transformd.	31
Transformation of resuspended brick	36
IISC Collaboration picture count	32-35
Mini Prep plasmid extraction	37
3A assembly Gj blue	40
Transformation of Ligated Product	41
Plating, Kanamycin plates	42 43
Tris HCl	43
(Chareece) Nanodrop	45
Resuspension [3 wells] 5-20b 5-20d 33f	45
Nanodrop (Zalenja + JS)	
Backbone digestion, RBS digestion (JS)	46
3A assembly	47

26/5/17 * Preparation of Ultra Competent Cells.
3/6/17

Keep cells always in ice, keeping them outside drastically reduces competency.

Inoculate 10 mL SOB with 40 μL of Saturated MG1655 inoculum (2:00 am)

grow a single colony in 2 mL LB/SOB @ 37°C overnight for inoculum

↓

Incubate @ 20°C 250 rpm overnight

↓

Checked OD @ 11 am (9 Hrs post inoculation)

OD = 0.308

↓ Cooled it on ice

Centrifuge @ 3000 g 4°C for 10 min

↳ Discard supernatant.

Resuspend it in 3 mL ice cold CCMB80

↓ incubate on ice 20 min

Centrifuge @ 3000 g 4°C for 10 min

↳ Discard Supernatant.

* Immerse the stand fully in ice. Keep on ice. Make sure eppi is not changing above ice in the stand.

Dissolve the pellet in 400 μL ice cold CCMB80

↓

Measure OD of the cell suspension

50 μL cells + 950 μL SOB

Blank : 50 μL CCMB80 + 950 μL SOB.

Dilution factor = 20.

Measured OD = ~~0.348~~ 0.377

OD of cell Suspension = ~~0.37~~ ~~0.39~~ 0.377 X 20 = 7.54

↓

Diluted the cell suspension 1:6 } Final O.D b/w 1 – 1.5
350µL cells + 1750µL CCMB80

Final OD = 1.008

↓

Aliquot 50µL in 1.5mL eppis

↓

Incubate on ice for 20min

↓

Flash freeze using liquid N_2 [Should've done this]

↓

Store @ -80°C in cryo box
(Labelled CC on cap, MG1655, date 3/6/17
on the side of eppi)

26/5/17
06/17

* Transformation for efficiency test

Take 50µL competent cells in 1.5mL eppi

↓

Add 1 mL 100pg/µL plasmid from kit

↓

Incubate on ice for 30 min

↓

60 sec heat shock in Water bath @42C

↓

Incubate on ice for 5 min

↓

Add 950µL LB and mix gently

↓

Plate 50μL on half of a LA - Chloramphenicol plate, pellet down the culture resuspend in 50μL LB and plate it on the other half. {Sohit Kim} {twice}

↓

Incubate @ 37°C overnight

↓

Count the no. of colonies

- Arani ⇒ 25 colonies
- Jyothish ⇒ 337 colonies
- Zakhuyi ⇒ 53 colonies

04/06/17.

Charni (Glycerol stock) = 5 colonies

Zakhuyi (growth culture) = 106 colonies

Efficiency Calculation:

26/5/17

Total Volume of culture = 1000μL

Volume plated = 50μL

Transformation efficiency ⇒ CFU / μg DNA.

$$T.E = \frac{\text{no. of colonies} \times \text{volume of culture} \times 10^6 \ (\text{ng} \to \mu g)}{\text{Conc. of plasmid} \times \text{Volume of plasmid} \times \text{Volume plated}}$$

$$TE = \frac{53 \times 1000 \times 10^6}{} \quad 1.06 \times 10^7$$

TE ≡ # Cfu / µg plasmid Plasmid conc. µg/µL

Transformation Efficiency = # colonies × Vol of culture (µL) × dilution factor / (conc. plasmid × Vol of plasmid × volume plated)

unit ⇒ cfu / µg of DNA

29/5

04/06/17

Efficiency = $\frac{106 \times 10^6}{5} = 2.12 \times 10^7$

Error: The cells were left @ −20°C for ~~about~~ 520 hrs.

29/5/17 * Bacterial growth Curve

Autoclaved 2 conical flasks

↓

Took 50mL SOB in both

↓

Inoculated one with 200µL DH5α } 8:10 pm
and the other with 200µL MG1655

↓

Took OD measurements and left the cultures for incubation @ 20°C and 250 rpm

Time	DH5α : OD	MG1655 : OD
8:15 pm	0.031 (Bad quvette)	0.008
8:35 pm	0.019	
9:10 pm	0.048	0.045
10:10 pm	0.053	0.048
11:10 pm	0.050	0.075
12:10 am	0.050	0.098
1:10 am	0.055	0.145
2:10 am	0.060	0.205
3:10 am	0.066	0.311
4:10 am	0.073	0.523
5:10 am	0.077	0.726
6:10 am	0.090	1.068
7:10 am	0.101	1.536
8:10 am	0.122	2.148
9:10 am	0.144	2.796

10:10 am	0.159	3.134
11:10pm	0.176	3.920
12:10pm	0.211	5.36
1:10pm	0.257	5.120
2:10pm	0.258	5.080
3:10pm	0.319	6.280
4:10pm	0.417	10.50.
5:10pm	0.457	8.00
6:10 pm	0.539	6.26
7:10pm	0.613	6.52
8:10pm.	0.772.	7.52

5|6|17 (C,J,Ad,Z) MEASUREMENT KIT

(measurement of fluorescence)

Required: CamLA plates, Biobricks, DH5α cells,

Procedure:

Bio bricks available as dry DNA ⇐ 1) TRANSFORMATION, DH5α cells, Cam resistance.

⇒ Resuspended each plasmid in 10μl distilled water (diH_2O) (used Milli Q water)*

→ [Kit plate 6 – 20B, 20D, 20F, 20H, 20J, 20L, 20N, 20P)

↓

→ Meanwhile, Labelled 8 epes as 20B, ...,} along with biobrick name.

↓

Thaw ⇐ Thawed Competent Cells in ice for atleast 8-10 mins. {DH5α, x5 (260μl in 1.5 epis), time: Stored in -80°C}.

↓

Wait for 5mins after Resuspension for the DNA ito be dissolved properly

↓

After 5mins, Set 10µL on the pipette and pipeted out the DNA soln from the well and transferred into Corresponding epis (Labelled)

↓

1) Labelled 8 more epis, 2) Added 1 µL of DNA soln into the corresponding epis

3) Added 50µL of DH5α Cells into each epi.

↓

Kept it on ice incubation for 30mins (Kept water bath for heating till 42°C)

↓

After 30min ice incubation gave a brief heat shock at 42°C for 60sec (Also heat shocked the 5X DH5α Stock for -ve Control)

↓

Immediately transferred the epis into ice and incubated in ice for 5mins.

↓

added 950µL of LB into each of the epis (9 epis)

↓

incubated at 37°C, 250 rpm for 2hrs.

06/06/2017. Preperation of Phosphate buffer.

PBS Buffer.

Materials req: NaCl, KCl, Na_2HPO_4, KH_2PO_4.
Scott Bottle, pH meter, Weighing

METHODOLOGY:-

1. Measure the following on a paper boat on weighing machine [100ml, 10X]
   1. NaCl (58.44 g/mol) 8g
   2. KCl (74.55 g/mol) 0.2g
   3. Na_2HPO_4 (141.96 g/mol) 1.44g
   4. KH_2PO_4 (136.09 g/mol) 0.24g.
2. dissolve in 80ml and adjust pH to 7.4
3. Make the solution upto 100mL.

08/06/2017 LB Medium

Aim: Preperation of LB medium

Materials: HIMEDIA LURIA BERTANI BROTH (M1245 - 500G)
Erlenmeyer conical flask, glassware.
100mL 200mL 150mL
2.5g 5g 3.75g.

PROTOCOL:

1. In 1L flask add 5g of LB.
2. dissolve it in distilled water.
3. Make the mark till 200.
4. Make & insert a cotton plug, cover it with Al foil and give it for autoclaving.

03/06/2017 SOB [Super Optimal Broth]

Aim : Prepare 100mL SOB.

Materials req : i) 0.25g yeast extract ii) 1g tryptone iii) 0.0291 g NaCl (iv) 0.0094g KCl v) 0.1207g $MgSO_4$

PROCEDURE : 1) Add double the amount of above mentioned ingrediants to a beaker. (pH to 7.5)

↓

Make up the volume till 100mL

↓

Magnetic bead stirring.

↓

transfer to Scott bottle

↓

Autoclave (Store at RT)

03/06/17 LA MEDIUM (PLATE) 200 mL

Aim : Preperation of LA medium for plates. [1.5% Agarose, 10-12 plates 250mL]

Materials : LB Broth (M1245-500G), plastic plates (8cm diameter), Agar Agar (RM026-500G)

Protocol : [200mL]

1. Take 5g broth and dissolve in 200mL H_2O.
2. Add 3g bacterial agar, swirl it.
3. Cover with cotton plug and alufoil.
4. Autoclave (121°c, 15min)
5. Cool it to 50°c. [barely hot on wrist]
6. Add antibiotic if need (Chloramphenicol)

7. Labe
8. → Pour (inside wood?) Cover
9. Stor

-100mL-

LB : 0.5g

Agar : 1.5g

H_2O : 100ml (make upto)

06/06/17.

* PBS
* dilu

Te

* Preparin
  > Spin
  > Resu
  > Tak

7. Label the plates.
8. → Pour around 20 mL, enough to create a covering layer at the bottom of the plate. (while mixed)
9. Close plates upside down. [work quickly]

	-100mL-	-150 ml (500mL) (bulk)-
LB:	2.5g	3.75g
Agar:	1.5g	2.25g
H_2O (Shake upto)	100ml	150ml

26/06/17. Measurement Track.

* PBS prepared. 10x
* dilute to 1x PBS (40mL)

Took 4 mL 10x PBS
added ↓ 36mL distilled water.
Mix ↓ thoroughly.

* Preparing fluorescein standard
  > Spin down the tubes (3000 rpm, 30 sec)
  ↓
  > Resuspend in 1mL 1x PBS (2x fluorescein)
  ↓
  > Take 500µL ~~of~~ & dilute with 500µL of 1x PBS (1x fluorescein) (50mM)

(I) Preparing our own 100 mM fluorescein (1mL)

Molar mass of fluorescein = 376.275 gm

weight req = $\frac{\text{molar mass} \times \text{Conc} \times \text{Vol}}{1000}$

$= \frac{376.275 \times 100 \times 10^{-3} \times 1}{1000}$

$= 0.0376275$

$= 0.03763$ gm.

Dissolve in 1 mL 1x PBS.

Molar mass of fluorescein = 332.31

Weight req = $332.31 \times 100 \times 10^{-3} \times 1$

$= 0.033231$ 100mM.

(Insoluble)

50 mL 1 mM Fluorescein

Mass = $\frac{332.1 \times 10^{-3} \times 50}{1000}$

$= 16.605 \times 10^{-3}$ g

$= 0.0167$ g

Plate used : Corning 3686, 96 well half area

x 5mM

Nees

= 17 mg

Blank = 1ml Cam +1ml LB . [500µl CAM(LB)+1ml H_2O]

	BD	AD
12:30pm -ve C	1.906	0.751
12:38pm +ve C		0.712
TD1 C		0.707
TD2 C		0.648
TD3 C		0.653
TD4 C		0.626
TD5 C		0.668
TD6 C		0.787
-ve J		0.675 (0.666)
+ve J		0.678
TD1 J		0.850
TD2 J		0.687
TD3 J		0.745
TD4 J		0.658
TD5 J		0.807
TD6 J		0.740.

So $5mL \times 25 \times 10^{-3}\ mg/mL = x\ mL \times 35\ mg/mL$

$x = \frac{25}{7} = 3.57\ \mu L$ of 35 mg/mL conc.

For 100 mL LB $\Rightarrow 100 \times 25 \times 10^{-3} = x \times 35\ mg/mL$

$x = 3.57 \times 20$

$= 71.4\ \mu L$ of 35 mg/mL conc.

For 220 mL $\Rightarrow 220\ mL \times 25 \times 10^{-3}\ mg/mL = x \times 35\ mg/mL$

$$x = \frac{3.14 \times 25 \times 10^{-2}}{35}$$

$= 15.70 \times 10^{-2}$,

$= 157.0 \times 10^{-3}\ mg$

$= 157\ \mu L$

Take 5 mL in 15 mL falcon and pick a (repeat for all 8 devices) colony into it using a 1 mL tip

↓

Incubate @ 37°c, 220 rpm, 16–18 Hrs.

(Incubate @ 11:20 pm)

9:6:17 Started checking OD for cultures @ 11 am

↓

found dilutions using excel sheet

↓

Inoculated 12mL LB with appropriate amount of inoculum

↓

Took Zero time point @ 2:00 pm

↓

Took 2Hrs time point @ 4:00pm

→ Cancelled

Cancel the expmnt due to the suspection of contamination as cultures were open.

6:17 → Colony inoculation of devices.

Took 5mL of LB-Cam on 16:15mL falcons

↓

Picked a colony into it using 10µl tip

↓

Inoculated overnight @ 37°C 220 rpm.

6:17 checked OD and inoculated LB-cam to get a final OD 0.02

↓

Started incubation @ 11:00 am and took zero time point

2 Hrs @

4 Hrs

6 Hrs @

Learned

@ 11 am

t

preparate

0 pm

1:00pm

due

rlamination

ices

15mL falcon

10µL tip

220 rpm.

18-cam

1:00 am

↓

2 Hrs @ 1 pm
4 Hrs @ 3:00pm
6 Hrs @ 5:00pm

} Aliquotted 1.5mL from each culture and stored on ice @ 4°C.

↓

Measured OD & fluorescence on plate readers.

Unsuccessful Collaboration

Aim :

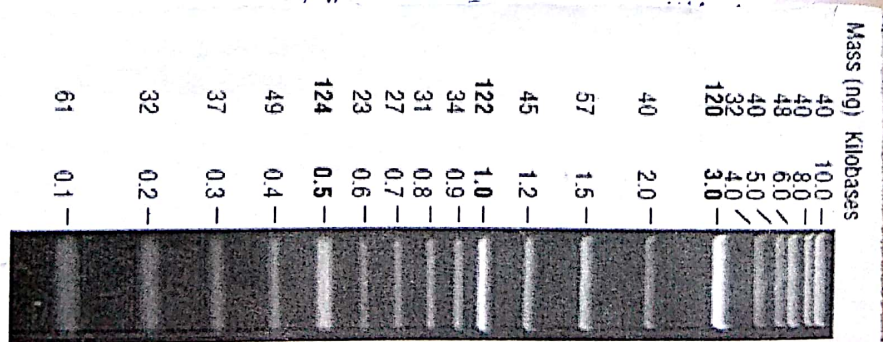
Preparation of media

YPD Media (for 200mL)

(i) Peptone – 4g
(ii) Yeast extract – 2g
(iii) Glucose – 10mL
(iv) Water – 190 mL (4g glucose in distilled water filter sterilized)

YP agar plate : (approx – 5plates)

(i) Yeast extract – 1g
(ii) Peptone – 2g
(iii) Agar – 2g
(iv) Water – 95 mL
(v) Glucose – 5mL (filter sterilized)



Collaboration IISc.

Primary inoculum. (5:30pm)

(i) Took 1ml boiling water in eppi @ 42°C.

↓

(ii) a 1/4th eppi Bakers yeast (Saccharomyces cerevisiae)

↓

(iii) dissolve using Vortex. mixer → store @ 4°C.

↓

(iv) 5mL of YPD media in a sterile falcon (x2)

↓

(v) Took 100µl of the yeast Culture and inoculate in 5mL (YPD) X2

↓

Store @ 30°C and 180 rpm overnight

Error: We added 50mL of glucose to 190ml of the media instead of 10mL

We had an extra flask with 190mL YPD (glucose unadded) we added the right amount of glucose to it and used it.

But the primary inoculation was still done in the excess glucose media.

Can cause death due to osmotic pressure.

9/7/17 Secondary inoculation

1. added 2.5 ml of pinnoculum to each flasks labelled YA and YB.

↓

Swirled and took out 1 ml OD reading @ 8:30 am

↓

i.e. kept the flasks in the incubator @ 30°C, 180 rpm.

↓

* did not pellet them down. took readings after every one hour (collected 1 ml of the sample in eppis and stored @ 4°C)

11:30 am.

We did a 3rd inoculation in 200ml of media with the correct amnt of glucose

2. Making glucose solution

Eg of glucose in 20 ml water

[5 for agar, 10 for cultures]

→ added 20 ml glucose to the culture [cotton plug was wet]

→ Took out 2 ml.

→ added 2 ml of primary inoculum

→ Swirled it and took out 1 ml OD reading. (11:20 am)

PREPERAT

TAKING

1) Str. PFA fixing for Hemocytometry.

Spun down the growth cell samples

↓

Resuspend in PBS (PBS wash)

↓

Spun down.

↓

Resuspend in 4% PFA.

↓

Crystal violet } no dead cells
Safranin }

incubate for 90 min

↓

Blue dye } dead cells,

add dye. [ ]

↓

Vortex and incubate for 5 min

↓

★ YC / YA in 800µl.

Spun down.

↓

★ YB in 1ml.

Resuspend in PBS [

Pictures are taken in the order

- 10x - whole grid 
- Top left grid
- Top right grid
- Central grid
- bottom left grid
- bottom right grid

} 40x

10/7/17 *

Part resuspension and transformation

Part name – BBa_K592011

Description – Cj Blue, green chromoprotein. (Cam)

Length - 702

Well 4-21/2K.

Resuspension:

→ The well ~~was~~ content was resuspended in 10 µl milliq water.

→ transfered it to epi and is stored in -20°.

RBS – BBa_B0034. Ampicillin

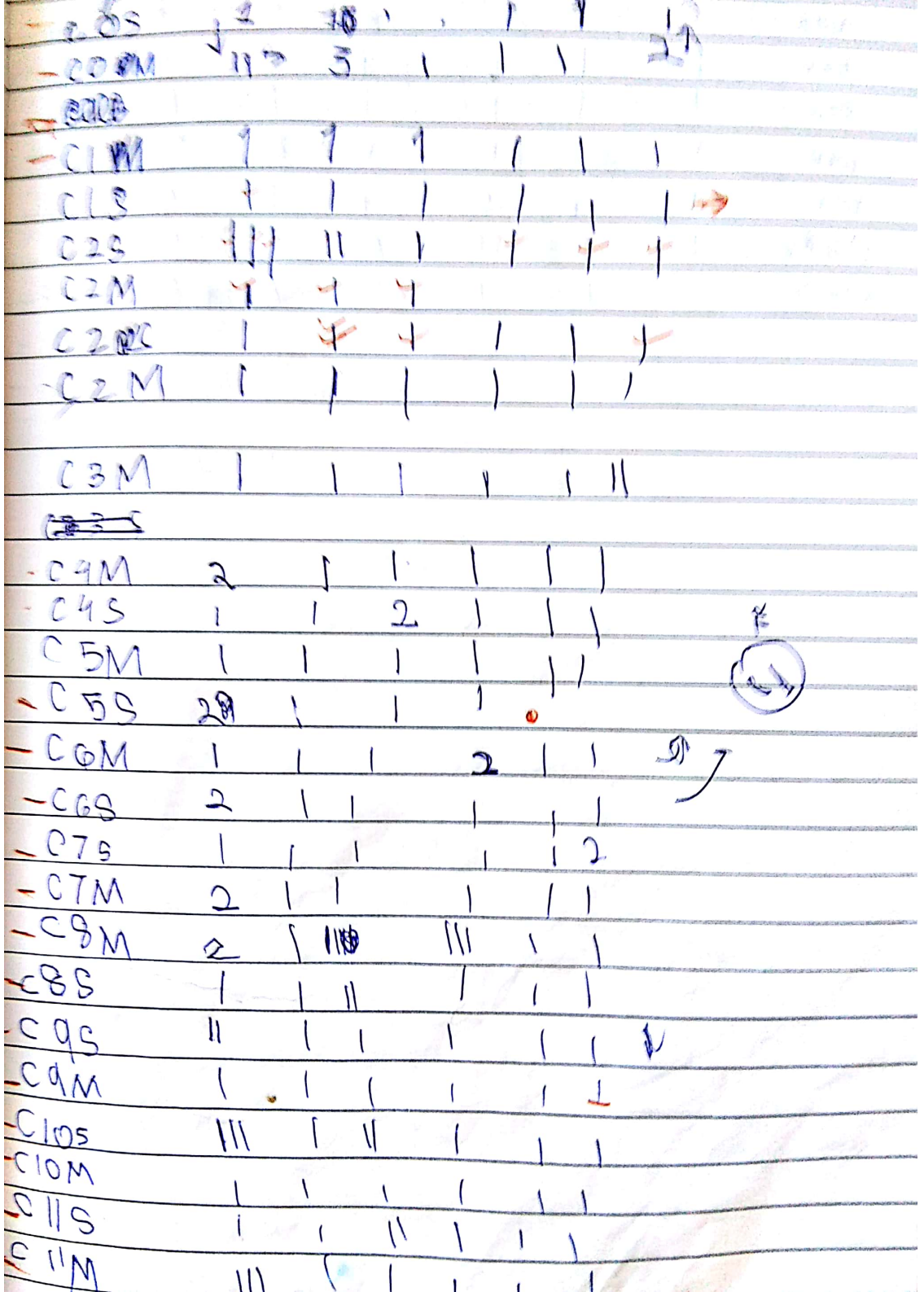
Back bone – Kannamycin / tetracycline

IISC Collaboration Radioscopic Imag

Sample	10x	TL	TR	C	BL	BR	
B10V	1	1	1	1	1	1	
B10S	II	1	1	1	II	1	14
B11V	1	1	1	1	II	1	20
B11S	II	1	1	II	1	II	26
B12 V	1	1	1	1	1	1	31
B12 S	II	1	1	1	1	1	43
B13V	1	1	IIII	1	1	1	51
B13S	1	1	1	1	1	1	57
B14V	1	1	1	1	1	1	63
B14S	1	1	1	1	1	1	69
B15 V	II	1	1	1	1	1	76
B15S	1	1	1	1	1	1	82
B16V	1	1	1	1	1	1	88
B16S	1	1	1	1	1	1	94
B0 V	1	1	1	1	1	1	100
B0S	1	1	1	1	1	1	107
B1V	1	1	1	1	1	1	113
B1S	1	1	1	1	1	1	119
B2V	1	1	1	1	1	1	126
B2S	1	1	1	1	1	1	132
B3 V	1	1	1	1	1	1	139
B3S	1	1	1	1	1	1	144
B4V	1	1	1	1	1	1	150
B4S	1	1	1	1	1	1	156
B5V	1	1	1	1	1	1	162
B5S	1	1	1	1	1	1	168
B6V	1	1	1	1	1	1	174
B6S	1	1	1	1	1	1	180

Clockwise and Co

Sample	10x
C0S	1
C0M	11
C1M	1
C1S	1
C2S	111
C2M	1
C2S	1
C2M	1
C3M	1
C3S	
C4M	2
C4S	1
C5M	1
C5S	2
C6M	1
C6S	2
C7S	1
C7M	2
C8M	2
C8S	1
C9S	11
C9M	1
C10S	III
C10M	1
C11S	1
C11M	III



B7V						
B7S						
B8V						
B8S						
B9V						
B9S						
C16 8V						
C16 S						

Zah

Samy

- C125
- C13
- C13
- C14
- C1
- C15
- C15
- C3

12/07/17 → Pouring YPA plates.

11/07/17. Transformation of Resuspended DNA

Part name BBa_K592011 : cjBlue.

↓

Pipette 1 µL plasmid into an eppe

↓

Add 50µL competent cells and mix well

↓

Incubate on ice for 30min

↓

60 sec heat shock @ 42°C in waterbath

↓

Incubate on ice for 5min

↓ add 200 µL LB

Incubate @ 37°C 220 rpm for 2 hrs

↓

Pellet and Plate 200µL on antibiotic plates.

↓

Grow them @ 37°C overnight

↓

Transfer the plates to 4°C.

23/7/17. Inoculate for miniprep (cjBlue)

Take 5ml LB - Cam in 15 ml falcon.

↓

Inoculate with single colony

↓

Incubate @ 37°C 220 rpm for 18 hrs.

24/7/17 ·

Take

Centrif

24/8/17 · Miniprep plasmid extraction (cj Blue).

Take 1.5 mL each of culture in 2 eppis

↓

Centrifuge @ 6800 rcf for 3 min

↳ Discard Supernatant.

Resuspend the pellets in 125 µL of P1 each.

and pool the replicates together.

↓

Add 250 µL P2, mix by inverting

let it stand for 4 min

↓

Add 350 µL N3 buffer, mix. Observe

↓

Centrifuge @ 17900 rcf for 10 mins.

↳ Discard pellet

Load 800 µL Supernatant in the column

↓

Centrifuge @ 17900 rcf, 60 seconds

↳ Discard flow through

Add 500 µL PB, Spin @ 17900 rcf for 60 sec

↳ Discard flow through.

Add 750 µL PE, Centrifuge @ 17900 rcf 60 sec

↳ Discard flow through.

↓

Keep the column in a new sterile eppi

↓

Incubate @ 37°C for 5 min after

adding 50 µL EB to the column.

↓

Centrifuge @ 17900 rcf for 60 sec.

↓

Measure the yield and store @ -20°c.

Gj Blue conc 56.6, 1.85.

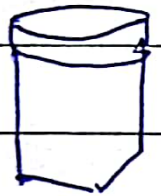
> H_2O.

@7.4μL

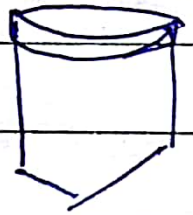
> Enzyme master mix for Cj Blue.

- 2μL NEB Buffer 3.1
- 0.2μL XbaI
- 0.2μL PstI
- 7.6μL dH_2O

Back bone digest | RBS digest | Cj Blue digest



- 4μL M.mix
- 4μL pSB1K3(25ng/μL)



- 4μL M.mix
- 2μL RBS
- 2μL dH_2O

- 4μL M.mix
- 2μL Cj Blue
- 2μL dH_2O.

Digest @ 37°c for 30mins

↓

Heat kill @ 80°c for 20min

↓

↓

Add 0.5µL T4 DNA ligase

↓

Add 2.5µL dH_2O (final volume 10µL)

↓

ligate 16°c / 30min, heat kill 80°c / 20min

↓

Store @ -20°c.

3/8/17. * Transformation of Cells with ligated.
(DH5α) product.

Take 2µL of ligated product in 1.5mL eppi.

↓

Add 50µL DH5α competent cells

↓

Incubate on ice for 30 min

↓

60 sec heat Shock @ 42°C

↓

Incubate on ice for 5 min

↓

Add 250µL LB and mix gently

↓

Incubate @ 37° of 2Hrs.

↓

Plate 50µL on a plate. (control on J, half plate)

↓

Incubate over night @ 37°c.

42

↓

Count colonies = 0.

7/8/17 Confirmation gel for ligated product ①

14/8/17 Plating the pellets

The remaining inoculum in the epi was pelleted down and plated (50µL).

Colonies = 95 - 105 (15 big)

Kanamycin Plates.

→ Pour new Kanamycin plates with 35 µg/ml

$C_1V_1 = C_2V_2$

$50 mg/mL \times 2 \mu L = 35 \mu g/mL \times 200 mL$

$2 \mu L = \frac{7000 \mu g \times mL}{50 mg}$

$= 140 \times 10^{-3} mL$

$= 140 \mu L.$

$35 \mu g/mL \times 2 \mu L = 35 \mu g/mL \times 200$

$x \mu L = \frac{\mu g/mL \times 200}{mg/mL}$

$= 2 \times 10^{-3} mL$

$= 2 \mu L$

3/8/17

→ New kanna plates (35 µg/ml) and test with MG1655 Comp Cells.

Result - no growth in 2 plates but in 1.

not reliable result.

4/8/17 - Tris HCl

→ Make Tris HCl (diluted with fuming HCl)

10 ml → 2.42 g tris base in Milli Q.

* fuming HCl not available hence used 1 N HCl.

* attaining pH at given conc for given Volume

13/07/17 CHARCHE NANODROP

Pramod	P1	71.8 ng/µL	260/280 = 1.96
Sandeep	G1	46.4 ng/µL	260/280 = 2.00
MP		104.1 ng/µL	260/280 = 1.82
			260/230 = 1.02

14/07/17

RESUSPENSION 1) TG PURPLE (J & Z)
2) double terminators

1) TG complete TG purple (Cam) ~~[illegible]~~ 96a 5-20b

2) Double terminator (Cam) 96a 2-28

3) Comp AmiSCP (Cam) 96 5-20d.

16/07/17

Zakhija Jyothish Nanodrops.

1. RFP	111.1	1.73
2. White Amil CP	113.0	1.74
3. Blue Amil CP	121.0	1.80.

→ Making tetr and Amp plates 5 each.
→ LA given autoclaved & unused.

was not possible.

4/9/17 Make Tris HCl

Tris base - 2.42g X2

Fuming HCl - 5-6 ml

Milli Q - Rest.

pH obtained - 7.91.

8/9/17

Assist Jagathish in Comp Cell preperation in CA Lab.

13/09/17 CHARVEE NANODROP

Receiver	R1	71.8 ng/μL	260/280 = 1.96
Sender	S1	46.4 ng/μL	260/280 = 2.00
REP		184.1 ng/μL	260/280 = 1.72
			260/230 = 1.08

14/09/17

RESUSPENSION 1) TS PURPLE (J & Z)

2) double Terminator

1.) ~~TS~~ competile TS Purple - ~~B2a~~ Bba-5_20b (Cam)

2.) Double terminator (Cam) Bba_3_3f

3) Comp AmilCP (cam) ~~150~~ 5_20d.

46

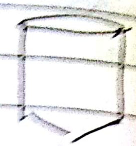
4/04/23 (?)

Backbone digestion (10µL total, 2 reactions)

Enzyme Master mix (PSB1A3)

- 2µL NEB buffer 3.1
- 0.2 µL Eco RI
- 0.2 µL Pst I
- 0.2 µL Dpn I
- 7.4µL dH_2O

} 4µL backbone → 4µL master mix



Incubate 37°C / 30min
Heat kill 80°C / 20min

* Part A (BBa_B0034) digestion : 2 step

Step 1: SpeI digestion in Buffer 2.1

- 2µL NEB buffer 2.1
- 0.2µL SpeI
- 7.8µL dH_2O

} 250ng DNA in 4µL → 4µL master mix

Incubate 37°C / 30min
Heat kill 80°C / 20min

Step 2: Eco RI digestion in buffer 3.1

- Take 4µL digested product
- 0.5µL buffer 2.1
- 0.5 µL buffer Conversion
- 5.1 µL dH_2O
- 0.2µL EcoRI

} 15µL mixture

Incubate 37°C / 30min
Heat kill 80°C / 20min

* Part B (Cj Blue) digestion

Enzyme master mix
- 7.6µL dH_2O
- 2µL NEB buffer 3.1
- 0.2µL XbaI
- 0.2µL PstI

} 250ng DNA in 4µL, 4µL master mix

Buffer Conversion 2.1 → 3.1 (TS.)

* Prepared 2M NaCl. - 25mL
  2M Tris HCl - 30mL pH 7.91
  1 M $MgCl_2 \cdot 6H_2O$ = 3mL
  100µg/mL BSA - 5mL

* NaCl : M.W = 58.44 g/mol
  $x/58.44 \times 1000/25 = 2$
  $x = 2.922g$.

* Tris : MW = 121.14 g/mol
  $x/121.14 \times 1000/36 = 2$.
  $x = 7.2684g$

* $MgCl_2 \cdot 6H_2O$: MW = 203.3 g/mol
  $x/203.3 \times 1000/3 = 1$.
  $x = 0.61g$.

* 3A assembly : promoter RBS + G'blue + backbone.

48

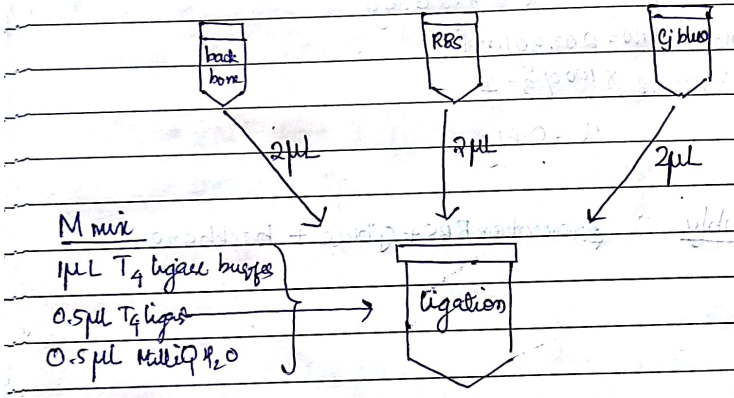
16/17/09/2017.

Ligation Master mix – 12 reactions.

T4 ligase buffer – 14 µL
T4 ligase – 7 µL
Milli Q – 7 µL

For 3A ligation 2 µL master mix + 2 µL RBS + 2 µL Cj Blue + 2 µL backbone.
8 + 2 = 5 µL

• 3A assembly



17th/09/17

Ligation was done for (10 Hrs) @ 16°C.
@ 10:30am Shifted to 80°C for 20min.

*

Transfor

Transforming an

MG1655 Cells u
ligation pr

Pelleted the

Resuspended
antibiotic

Incuba

[a plate was left in
hood since the plug
popped in the oven]

Transforming and plating ligation products.

MG1655 Cells were transformed with 1 µL ligation products. (Transformation as in pg 41)

↓

Pelleted the cells @ 3000g 10 minutes
→ Supernatent discarded

↓

Resuspended in 25µL LB and plated it on antibiotic selection plate.

↓

Incubate @ 37°c for grow the overnight

[1 plate was left in hood since the plug popped in the oven]

Colonies : 4

1/10/17. Inoculation of Gblue - RBS colonies.

1. Inoculated 2 colonies in 2ml LB in a 15ml falcon with 2µL tetr antibiotic.

2/10/17 - Miniprep. [Protocol as in page 37 (24/7/17)]
- (Video shooting)

3/10/17 3A assembly pCAT/G13 promoter + Gblue RBS (PSB1A3)

- pCAT + RBS-Gblue + pSB1A3
- G13 + RBS-Gblue + pSB1A3.

Master mix for backbone. (2X)

- 2µL NAB buffer 3.1
- 0.2µL EcoRI
- 0.2 pst I
- 0.2 DPN I
- 7.4 µL dH_2O.

4µL master mix → Incubate 37°/30min
4µL backbone. Heat killed 80°C (20min)
(per reaction 4µL)
MM

M.mix for promoter : Step 1

- Buffer 2 + BSA
- 2µL NEB buffer 2
- 0.2µL SpeI
- 0.2µL BSA
- 7.6µL dH_2O.

- Buffer 2.1
- 2µL NEB buffer 2.1
- 0.2µL SpeI
- 7.8µL dH_2O.

4

Step 2 : ECoRI in buffer 3.1

4µL buffer 2.1
4µL buffer converter
31µL dH_2O
1µL ECoRI

8µL digest → digest @ 37°C/30min
8µL m.mix Heat kill @ 80°C/20min

M.mix for chromophore digestion

- 4µL NEB buffer 3.1
- 0.4µL XbaI
- 0.4µL pst I
- 15.2 µl dH_2O

4µl DNA
4µl Mmix →

Incubate @ 37°C/30min
Heat kill @ 80°C/20min

Single pot ligations

Promoter digest 2µL → Chromophore 2µl → Backbone 2µL →

4µL per set ←
- 1µl T4 ligase buffer
- 0.5µl T4 DNA ligase
- 2.5µl dH_2O

Incubate @ 16°c for 30min
Heat kill @ 80°c for 20min.

3/10/17 Transfor...
7pm
Transfor...
plating ...
colon...

Trouble ...
...

5/10/17 Transf...
ECoRI
Quenching ...
Colo...

6/10/17 Gel ...
nano...
- 0.5 g aga...
- 50ml 1X TAE
- One drop ...
set the ...

Digestion ...
Gel run...
→ ...

Master m...
- 6µl Buff...
- 0.5µL PC...
- 19.5µL d...
↓
5µL MM...

3/10/17 Transformation and plating.
7pm

Transformation as in (pg 41)
plating was done on tetra plates.
colonies – no colonies (0)
both in -ve and + plates.
Trouble shoot: might be due to 2.1 buffer instead of 2.0.

5/10/17 Transformation and plating.
ECORI & SpeI was digested in buffer 3.1
Overnight digestion.
Colonies = 0.

6/10/17 Gel running for RBS CJ blue miniprepped
nano drop = 36 ng/μL.

- 0.5 g agarose.
- 50ml 1xTAE
- one drop EtBr.

set the gel for half an hour.

Digestion of CJBlue RBS with EcoRI for
Gel running: 1% Gel.

~ DLS.

Master mix for ECORI

- 6μL Buffer 2.1 × 2 12 μL
- 0.6μL ECORI × 2 1.2 μL
- 19.5μL dH2O. (vortex → spin down) 46.8μL

↓

5μL MMix + 5μL D. construct

↓

- Incubate @ 37°C waterbath for 30 min
- heat kill @ 80°C 20min.
- Spin down @ 7000 ref. 5min. (RT)
- ↓

Mix 5µL product with 1µL 6X loading buffer and load into respective wells.

↓

Run the gel along with 2 log DNA ladder. @ 80V for 1 hr.

↓

Image it.

9.10/17. Transformation: for 2A product.

- PL-pSB1C3
- B13-pSB1C3.

→ Water bath was already in use hence heat shock got delayed by 1.15 hrs.

→ Colonies = 0

10/10/17 Inoculation

TsPurple, Amil CP, P CAT.

– 2ml – SOB with CAM. (colon

11/10/12

PL

2

In

spe

13.10.12.

- 0.5g
-
-
- Mak

4 Gel Ru

- 0.5g Ag
- 50ml
- 10µL

TROUBLE SHOO

→ Use a di

→ Elam

11/10/12 Innoculation

PL - Chloramphenicol 2A assembly

2 colonies

Innoculation for Competent Cell preperation.

13.10.12. SOB preperation. (250ml)

- ~~0.5g Agar~~ 6.25g LB.
- 1.288g $MgSO_4 \cdot 7H_2O$
- 0.0465g KCl (purified)
- Make upto 250ml

Gel Running:

- 0.5g Agarose
- 50ml 1X TAE.
- 10µL EtBr.

TROUBLE SHOOTING.

→ Use a diff recovery medium

→ Plasm DNA ref / qpm prblm eliminated

Comp Amid CP.

conc.	52.3 ng/µL
A260	1.045
A280	0.655
260/280	1.59
260/230	6.72.

pCAT

Conc.	67.6 ng/µL
A260	1.353
A280	0.813
260/280	1.66
260/230	0.83.

Gel Running and digestion:

EcoRI → PstI (* confirm)

3 constructs · [pLet cAmid CP, Comp Amid CP, & purple pCAT]

⇒ digestion for the 3 constructs:-

Master Mix:

Buffer	3.1	4µL
~~EcoRI~~ PstI		0.4µL
dH_2O		15.6µL.

Add 5µL mastermix + 5µL construct.

digest 1/2 hour @ 37°C. [water bath]

Heat inactive @ 80°C 20 min.

gel loading:

1μL loading buffer + 5μL digested product

ladder(2μL) | PLofLA.CP | pCAT | AmilCP | ts purple

1μL 6× loading buffer + 5μL digested product

* 3A Assembly:

→ Composite ts purple.

- 0.2μL XbaI
- 0.2μL Pst I
- 2μL Buffer 3.1
- 7.6μL dH_2O

10μL M.mix → 7μL ts.purple + 3μL dH_2O

37°C/30m 80°C/20m

→ Backbone pSB1T3

- 0.2μL EcoRI
- 0.2μL Pst I
- 0.2μL DPNI
- 2μL Buffer 3.1
- 7.4μL dH_2O.

10μL Mmix → 5μL pCAT + 10μL pSB1T3

37°C/30m 80°C/20m

→ pCAT digestion

- 0.2μL EcoRI.
- 0.2μL SpeI
- 2μL NEBA buffer 2.0
- 2μL BSA
- 5.6μL dH_2O.

10μL Mix → 5μL pCAT + 5μL dH_2O.

37°C 80°C/20m

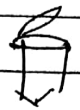
→ composite

- 0.2μ
- 0.2μ
- 2μL
- 7.6μ

→ composite anneal

- 0.2 µl XbaI
- 0.2 µl PstI
- 2 µl buffer 1
- 7.6 µl dH_2O

} 10 µl Mmix → 5 µl bmilcp + 5 µl dH_2O.



37°C/30min
80°C/20mn

2 mL LB + 100 µL MG1655 Comp cells (15 mL falcon)

2 mL LB + 2 µL tetracyclin + 1 B.AmilCP colony (9)

2 mL LB + 2 µL Cam + RFP colony

On plate mark the picked B.AmilCP colony as 9.

2A - PSB1T3 + G13 / PL-PLeft

Backbone digestion

- EcoRI - 0.2 µL
- Pst I - 0.2 µL
- Dpn I - 0.2 µL
- buffer 3.1 - 2 µL
- dH_2O - 7.4 µL
- PSB1T3 - 10 µL

→ 37°C/30 min, 80°C/20 min

Promoter digestion

- EcoRI - 0.2 µL
- Pst I - 0.2 µL
- buffer 3.1 - 2 µL
- dH_2O - 7.6 µL

5 µL → • 5 µL G13 → (10 µL) 37°C/30 min, 80°C/20 min → 2 µL pSB1T3 digest + 6 µL

5 µL → • 5 µL pl-pleft → (10 µL) 37°C/30 min, 80°C/20 min → 2 µL pSB1T3 digest + 6 µL

→ 16°C/30 min, 80°C/20 min

To autoclave :-

LA : 100 mL , Eppi : 50 nos , Water bath, refill

20/10/17 -

Transformation of 2A product of 8/10

→ 2A product → G13 PSB1C3, 8/10/17.

21/10/17 -

1) Inoculation of G13 PSB1C3 transformed (8/10/17 - 20/10/17) product. (4 colonies

2) Inoculation of PCAT AmilCP-PSB1T3,

22/10/17

- G13, PL PLEFT digestion

- 0.2 µL EcoRI
- 0.2 µL SpeI
- 2 µL buffer 2.0
- 2 µL BSA
- 5.6 µL dH_2O.

5µL + 5µL MM

↓ ↓

5µL 5µ

22/10/17

→ Mini prep.

→ Transformat

→ 1) Eff

- 10
- 5
- 10

→ 2) P

→ 3) PL

→ Nano drop

Con: 56.7 ng/µl

A260

A 280

260/280

260/230

→ Nano Drop

Conc

A 260

A 280

260/280

260/230

22/10/17

→ Mini prep of G13 PSB1C3 and pCAT Amil CP (PSB1T3)

→ Transformation

→ 1) Efficiency transformation
- 10pg/µl
- 50pg/µl
- 100pg/µl

→ 2) PL-PSb1C3 (8.10.17)

→ 3) PL-PLEFT (16.9.17)

→ Nano drop G13 PSB1C3 (22/10/17 mini prep)

Conc: 56.7 ng/µl

A260	1.122
A280	0.569
260/280	1.97
260/230	2.03

→ Nano Drop (pCAT Amil CP PSB1T3 miniprep 22/10/17)

Conc	95.3 ng/µl
A260	1.907
A280	0.984
260/280	1.94
260/230	2.05

• 3.9 × 2 = 7.8 µL dH_2O.

4 µL Plasmid + 4 µL Enzyme Master Mix.

Incubate @ 37°C for 30 min

↓

Heat kill @ 80°C for 20 min.

↓

Load in the GEL.

↓

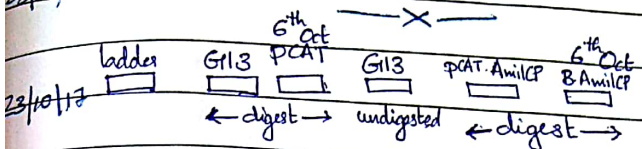
Run the gel @ 80 V, for 1 Hour.

↓

Image the gel using gel doc.

Cast Gel — 1.05g Agarose, 50ml 1x Tae 10µl Et Br)

22/10/17



23/10/17 plating.

23/10/17

Aarti Run EcoRI digest of G113 & Pcat AmilCP of 22/10/17.

- PL-pLeft digestion

- 0.2 µL EcoRI	- 0.2 µL EcoRI
- 0.2 µL Pst I	- 0.2 µL Pst I
- 1 µL NEB buff 3.1	- 1 µL NEB buffer 3.1
- 2.6 µL dH_2O	- 2.6 µL dH_2O
- 6 µL PL-pLeft	- 6 µL pLeft

Digestion for Hijack Module.

1) AraC
2) LacI
3) pHyB
4)

Do in incubat tim
IPTG
0.5 ... Nz

1) cast gel
2) Inoculate pCAT AmilCP psB1T3
① (2ml LB tet R)
3) Inoculate ~~pCAT G13~~ pCAT tspurple psB1T3
① (2ml LB tet R)
4) Inoculate cell.
① (1 ml LB CAM)
5) Inoculate pL psB1C3
in ① colony (2ml LB Cm)
② (2ml LB CAM)

• pCAT tspurple psB1 T3 (25/10/17) (4V) miniprep..
• ~~psB1C3 backbone~~
•

1:40 2:00 3:30 4:: 8:00→6:00
To do
12:30 ← 12:30 — 10:30
✗ 2A : pCAT. tspurple psB1T3
✓ ~~2A : G13 AmilCP psB1C3~~
• 2x 2A : G13 tspurple psB1C3
~~G13 AmilCP psB1T3~~
- 0.2 µL EcoRI
- 0.2 µL pst1
- 1 µL 3.1
- 5 µL ~~G13~~ pCAT tsp
- 3.6 µL dH₂O

10/17 Tran...
1) pL-Pleft Am...
2) pleft psB1C...
3) G13 ts purple
4) pCAT Tspurp...
5) G13 ts purp...
6) pCAT. Amil C...
7) pL pleft ...
8) G13 AmilC...
9) pL pleft

...lating (J + ...
Inoculation of ...
Replenishing ...

...17.
Inocculation
Mini prep (J...
Digestion E & P
• ~~pCAT~~ G13 . tspurp...

• ~~pCAT~~ G13 . tspurp...

• pCAT . tspurp...

2 µg ladder

24/10/17 Transformation.

1) PL Pleft Amil CP (2A) pSB1C3
2) pleft pSB1C3
3) G13 ts purple pSB1T3 (22/10)
4) pCAT Tspurple pSB1T3
5) G13 ts purple pSB1C3 (2A)(23)
6) pCAT. Amil CP pSB1C3 (23/10)
7) pl pleft pSB1C3.
8) G13 Amil CP pSB1A3 (3/10).
9) pL pleft Amil CP pSB1T3 (3A) 23/10.

m) plating (J + Z) (7pm)
Inoculation of 2 constructs (J)
Replenishing consumables.

25/10/17.

Inoculation of rest.
Mini prep (J)
Digestion E & P

- ~~pCAT~~ G13 · tspurple · pSB1T3 (1) → 5µL
  Mmix → 5µL
  } 37°C/60min, 80°C/20min
- ~~pCAT~~ G13 · tspurple · pSB1T3 (2) → 5µL
  Mmix → 5µL
  } "
- pCAT · tspurple pSB1T3 → 5µL
  Mmix → 5µL
  } "

Ligation

Master mix: 1 µL T4 ligase
2 µL T4 ligase buffer
5 µL dH_2O

i) G13-tspurple-tet^r①[E&P] → 2µL
pSBIC3 [E&P] → 1µL
T4 Mmix → 2µL
} 16°C/4Hrs

ii) G13-tspurple-tet^r②[E&P] → 2µL
pSBIC3 [E&P] → 1µL
T4 Mmix → 2µL
} 〃

iii) pCAT-tspurple-Tet^r [E&P] → 2µL
pSBIC3 [E&P] → 1µL
T4 Mmix → 2µL
} 〃

iv) G13 [E&P] (22nd-Oct) → 2µL
Composite AmilCP [E&P] (23rd Oct) → 1µL
T4 Mmix → 2µL
} 2.5
〃 0.5

iv) v) (ccdB:
T4 ligase - 05 - 0.25 µL (directly in small epi)
T_4 ligase buffer, 0.5µL
Insert (digested) = 3.25µL
pSB1C3 (digested) = 1µL.

6am (Inoculation)
1) pCAT Amil
2) Inoculate
3) Inoculate
4) Inoculate

26/10/17
9:30am Grep
products.

2pm gel
reactio
• 0.2
• 2µL
• 7.8µL

6am (Inoculation for characterisation, plate reader
1) pCAT dmil(P pSB1T3 [2ml LB-tet R) ④
2) Inoculate +ggfpSB1T3 (2ml LB-tet R) ⑤
3) Inoculate eff (Dep coupl/w) [1ml LB cam] ⑥
4) Inoculate pL pSB1C3- (2ml LB cam). ⑦

°C/4-Hrs

26/10/17
9:30am Gel running Transformation of ligated products.

2pm gel running:

9 reactions
- 0.2 EcoRI × 4 = 0.8 } 5µl per reaction.
- 2µl 3.1 Buffer × 4 = 8 }
- 7.8µl dH_2O × 4 = 31.2 }

2.5
" 0.5

idly in
Small epis)