2-log DNA ~~do~~ ladder 0.1-10 Kb

Mass (ng)	Kilobases
40	10.0
40	8.0
48	6.0
40	5.0
32	4.0
120	**3.0**
40	2.0
57	1.5
45	1.2
122	**1.0**
34	0.9
31	0.8
27	0.7
23	0.6
124	**0.5**
49	0.4
37	0.3
32	0.2
61	0.1

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20151012

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9-May-17 (written over "Apr")
Tuesday

-1-

1. Preparation of LA-plate (1.5 % Agarose)

(10-12 plates) 250ml

Materials: L.B broth (HIMEDIA : M1245-500G), Plastic plates (8cm, diameter), Agar agar (RM026-500G)

Protocol for 200 ml

- ✓ Take 5g broth powder and dissolve in 200 ml H_2O
- ✓ Add 3g bacteriological agar. Swirl it
- ✓ Cover with the closed cotton plug with aluminium foil
- ✓ Autoclave (121°C, 15 min)
- Cool it to 50°C
- Add antibiotic (if needed) : Not for this one
- Label the plates
- Pour enough to cover the bottom of the plate (20 ml)
- work quickly
- Store plates upside down

	– 100 ml –	– 150 ml – (500mL conical flask)
✓ LB :	2.5 g	3.75 g
✓ Agar :	1.5 g	2.25 g
✓ H_2O :	100 ml	make upto 150 ml

Pouring the plates : Took the 100 ml media inside the fume hood Poured 5 plates (approx : 20 ml each)
Kept the plates overnight in the hood

Scanned by CamScanner

10% Acetic acid = $\frac{12.8 \times 40}{1000}$ = ~~0.512 mL~~

Filter sterilization of CCMB 80 { Prepared by Avani

Fill the Syringe with CCMB80 (inside hood)

↓

Fix the 0.2 micron filter on the syringe nozzle.

↓

Gently press the pistol and filter the media into a sterile falcon.

↓

Store @ 4°C.

* Seed stock inoculation (Do in hood)

✓ Take 2ml sterile SOB into 15 ml falcons

↓

✓ Pick a single colony with sterile 200 µl pipette tip

↓

✓ Pipette up and down and drop the tip into SOB.

↓

✓ Incubate @ 37°c overnight.

May/17

* Preparation of competent Cells.

- Clean the working area.

Take 5 ml each of SOB media in 2 ~~15~~ 50 ml falcons

↓ inoculate 20 µl of culture

~~Start~~ Incubate @ 20°C till an OD 0.3 600 nm.

-20°C -22-

Efficiency Calculation -22-

CCMB 80 Buffer -16-

Glycerol stock Standard Protocol -19-

Transformation Protocol -21-

CaCl2 Method competent cells -17-

Seed Stock Preparation iGEM -5-

Preparing Ultra competent cells -19-

Measuring Competence -4-

Miniprep Plasmid extraction -37-

↓

Transfer 1 ml culture to 1.5 ml eppis (prechilled)

↓

Centrifuge at 4°C 3000g for 10 minutes

↓

Resuspend in 320 μl ice cold CCMB80

↓

Incubate on ice for 20 minutes.

↓

Centrifuge 4°C 3000g 10 minutes

↓

Resuspend in 40 μl ice cold CCMB80

↓

Measure OD : 200 μl ~~SOC~~ LB 50 μl cells. (160 μl LB : 40 μl)

↓

Dilute the remaining to OD 1.0 – 1.5

↓

Incubate on ice for 20 mins

↓

Store at -80°C

* Measurement of competence

Thaw competent cells on ice.

↓

Pre chill 1.5 mL eppis

↓

Spin down competence test kit 8000-10000 rpm 20-30 sec

↓

Pipet 1 μL DNA into eppis

↓

Pipet 50 μl competent cells. (flick gently to mix)

↓

Incubate on ice for 30 minutes

↓ Set thermo mixer to 42°C 300 rpm

Heat shock for 45 seconds. : Use waterbath 42°C

↓

Immediately incubate on ice for 5 minutes

↓

Add 950 μL SOC, incubate 37°C for 1 hour 200-300 rpm (2Hrs is better 250rpm)

↓

Plate 100 μL from each ~~plate~~ tube on LA - chloramphenicol plates

↓

Count number of colonies.

13/May/17

Seed stock preparation

15 % glycerol. Final volume 1 mL

$\frac{x}{1} \times 100 = 15$ $x = 150\,\mu l$ +

850 μl cells

Aliquot 50 μl and store @ -80°C

OD - Measurements : 96 well plates: Plate reader.

Plate layout :

	1	2	3	4	5	6	7	8	9	10	11	12
G										Blanc	DH5α	K12
H	DH5α	K12	DH5α	K12	DH5α	K12	J DH5α	J K12				

Scanned by CamScanner

> So Incubated the culture again at 20°C for 2 more hours and checked the OD again

Plate layout:

1 2 3 4 5 6 7 8 9 10 11 12

H

> O.D was still observed to be much lesser (< 1/3rd of required) than required value
> So we didnt proceed any further with the protocols of preparing competent cells.

* Preparation of SOB media (150 ml)

✓ 0.75 g	yeast extract	0.5% w/v
✓ 3.0 g	Tryptone	2% w/v
✓ 0.088 g	NaCl	10 mM NaCl
✓ 0.028 g	KCl	2.5 mM KCl
✓ 0.739 g	$MgSO_4 \cdot 7H_2O$	20 mM $MgSO_4 \cdot 7H_2O$

> Auto claved the media and stored @ 4°C

14/May/17

* Pre inoculation from plate.

> Took 2 mL SOB in two 15 mL falcons inside the hood
> Using a ~~sterile~~ sterile 200 µL pipette tip, picked up a single colony from the plate
> Transferred the colony into the falcon with SOB and labelled it
> Incubated the falcons at 37°C @ 180 rpm

* Inoculating the main culture.

3 samples for DH5α and ~~one~~ 2 for MG1655

MG1655 : 2×5 mL + 8 mL + 7 mL = 25 mL SOB

Inoculate with 100 μL seedstock MG1655

DH5α : 3×5 mL + 8 mL + 7 mL = 30 mL SOB

Inoculate 120 μL DH5α seedstock.

120 μl : 30 ml →

> Meaure the above mentioned volumes of SOB into 250 mL scott bottles.

> Inoculate with the above mentioned volumes of the pre inoculum or seed stock.

> Grow @ 20°C @ 280 rpm for 16 Hrs.

[Inoculated and started incubation @ 12.52 a.m]

> Measure O.D periodically.

1.5 ml for O.D measurement.

Hrs post inoculation	O.D of the culture	
10 Hrs	0.515	MG1655
10 Hrs	0.055	DH5α

Scanned by CamScanner

Labelled 15 eppis as MG1655 and kept in ice

↓

Pipetted 1 mL of liquid culture into each eppi

↓

Centrifuged @ 3000 g @ 4°C for 10 minutes

↓

Discard ← [Discarded the supernatant in liquid discard and added EtOH.]

↓

Resuspended the pellet in 160 μl ice cold CCMB80 To make a suspension, then add 160 μL more of ice cold CCMB80.

↓

Incubate in ice for 20 minutes

↓

Centrifuge @ 3000g @ 4°C for 10 minutes

↓

[Discard the supernatant in liquid waste]

↓

Resuspend the pellet in 40 μl ice cold CCMB80 (15 samples)

↓

Pool all the samples together into 1.5 mL eppi

8 × 40 = 320 μl in one eppi

7 × 40 = 280 μl in another one

↓

Take 150 μl of cells into an eppi add 650 μL LB from each eppi

↓

Pool them together for O.D measurement

300 μl

So

Al

Fl

St

[La

* Blank correction done

* OD for MG1655 J : 1.355

MG1655 A : 1.111

Dilution factor = $\frac{800}{150}$

= 5.33

So optical density for the competent cells in CCMB80

OD for MG1655 J : 7.22215

MG1655 A : 5.92163

So diluted the cells with CCMB 80 as follows

150 μl cells + 650 μl ice cold CCMB80 × 2 replica

Stored one replica as it is

↓

Aliquoted the other replica as 50 μL aliquotes in 1.5 mL sterile eppis

↓

Flash frozen the samples in liquid nitrogen

↓

Stored in −80°C fridge in cryobox.

[labelled as : Competent cells : on the lid

MG1655, JyotiRish, 16/5/17 : On the side]

Scanned by CamScanner

* Preparation and storing of seed stocks.

> Inoculated a single colony in 2 ml SOB in a sterile 15 mL falcon

↓

Incubated at 37°C for 12 Hrs

↓

Transferred the culture to 4°C fridge

↓

150 μl glycerol + 850 μl DH5α
150 μl glycerol + 850 μl MG1655 } All pipeting done inside hood

↓

Made 1 ml aliquotes each of DH5α and MG1655

↓

Froze the aliquotes in liquid nitrogen

↓

Stored them @ -80°C in igem Cryobox

[Labelled as : Seed stock : on the lid
MG1655 , 16/5/17 on side
DH5α , 16/5/17 on side]

* Chloramphenicol : LA plates

> Made LA and autoclaved — 100 mL — 150 mL

> Cooled it down to room temp. and added antibiotic Chloramphenicol (35 mg/mL) — 100 μL — 150 μL

> Poured 12 plates and labelled them as LA - Chlm - 17/5/17

PLATE preparation | Seed Stock preparation GEM -5- | Testing competence -4-

18/5/17

*

Scanned by CamScanner

8/5/19

* Checking competency of cells.

Spin the efficiency kit after thoughing it in ice @ 9000 rpm for 30 sec.

↓

Thaw competent cells on ice (Stored @ -80°C)

↓

Transfer everything into laminar hood

↓

Pipetted 1 µL of plasmid DNA into 50 µL competent cell aliquot

↓

Incubate for 20 minutes on ice

↓

Heat shocked the cells @ 42°C for 45 sec

↓ quickly.

Incubate on ice for five mins.

↓

Added 950 µL LB to the eppi

↓

Incubated @ 37°C 250 rpm for 1 Hour

↓

Plated 50µL and 10µL of the competent cells on chloramphenicol plates

↓

Incubate overnight (16 Hrs) @ 37°C

↓

Got zero colonies in the plate after transformation

SOB - 20°C -28-
Efficiency Calculation -2?
CCMB 80 Buffer -1?
Glycerol stock Standard Protocol -19-
Transformation Protocol -21-
$CaCl_2$ Method competent cells -1?
Preparing Ultra competent cells -19-
Miniprep Plasmid extraction -37-

Scanned by CamScanner

19/5/17

* Pre-inoculation

Took 2 mL SOB in 15 mL falcon

↓

Using a 200μL pipette tip, picked a colony from the plate

↓

Pipetted into the media and released ~~discarded~~ the tip inside the falcon

↓

Grew it overnight @ 37°C (12 Hrs)

22/5/17

* Growth Curve and Comp cells Inoculation

Took SOB (25 mL) in 4 conical flasks.

↓

23/5/17

Inoculated with 100 μL of pre inoculum { DH5α : 12:00 ; MG1655 : 5:00 }

↓

Incubated overnight @ 20°C

↓

Measured OD at 1 Hr intervals

Time post inoculation	DH5α	MG1655
9:30 am	0.39	0.04
11:15 am	0.57	0.08
12: noon	0.82	0.10
1:00 pm	0.93	0.18
1:30 pm		0.21

Scanned by CamScanner

2:00pm	0.26
2:38pm	0.33
3:05pm	0.37
~~4:05pm~~	0.37

* Competent cells preparation

Measured OD of culture (0.4) Avani

↓

Pipetted 1 mL into 6 chilled eppis

↓

Centrifuged @ 3000g 4°C for 10 mins

↓ Discarded supernatant

Resuspended the cells in CCMB 80 : 320 μL/each

↓ incubate on ice 20 mins

Centrifuged @ 3000g 4°C for 10 mins

↓ Discarded supernatant

Resuspended in 40 μL ~~50~~ CCMB 80

↓

Pooled it together in an eppi

> OD measurement

50 μL cells + 950 μL LB ⇒ 1000 μL total

Dilution factor = $\frac{1000}{50}$ = 20

Measured OD = 0.383

OD of cells = 0.383 × 20

= 7.66

Efficiency Calculation -22-
CCMB 80 Buffer -16-
Glycerol stock Standard Protocol -19-
Transformation Protocol -21-
$CaCl_2$ Method competent cells -17-
Preparing Ultra competent cells -19-
Miniprep Plasmid extraction -37-

Scanned by CamScanner

> Dilution of cells.

diluted 1:7

100µL cells + 600µL CCMB80

Final OD of cells = $\frac{7.66}{7}$

= 1.094

Aliquote 50µL in labelled eppis
[labelled CC on cap, MG1655; 23/5/17 on side]

↓

Incubate on ice for 20 minutes

↓

Flash freeze using liquid nitrogen

↓

Store at -80°C, in iGEM 2017 Cryobox.

24/5/17

* Testing the efficiency of comp. Cells.

Though a vial of comp. Cells on ice

↓

Added 1µL 100pg/µL plasmid

↓

Ice incubation for 30 minutes

↓

Heat shock @ 42°C for 60 seconds.

Add
In
Pl
Pel
Re
Inc
*
To
Pi

↓

Added 250 µL of LB media } ~~Use 950 µL for~~ 250µL CCMB80 seperates out

↓

Incubated @ 37°C for 1 hour 280 rpm.

↓

Plated 50µL into LA - Chloramphenicol plate

↓

Pelleted the remaining cells

↓

Resuspended in 50µL LB and plated on the same plate

↓

Incubated overnight (28°C since another experiment is going on).

15/17

* Inoculating Glycerol stock.

Took 2 mL LB in 15 mL falcon

↓

Pipetted glycerol stock into LB

↓

Incubated @ 37°C overnight (4:30 pm)

Scanned by CamScanner

CCMB 80 Buffer -16-

A PLATE Preparation

* Preparing CCMB 80 (250 mL)

(i) 10 mM KOAc M.W = 98.15 g/mol

$\frac{10\times10^{-3}\times 250}{1000} \times 98.15 = 0.245\ g$

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

$\frac{80\times10^{-3}\times 250}{1000} \times 147 = 2.94\ g$

25

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

$\frac{20\times10^{-3}\times 250}{1000} \times 197.91 = 0.989\ g \approx 1 g$

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

$\frac{10\times10^{-3}\times 250}{1000} \times 203.31 = 0.508\ g$

Stock solution DEM -5-

(v) 10% v/v glycerol

$\frac{250\times10}{100} = 25\ mL$

> Adjust pH to ~~7~~ 6.5 ; We adjusted it to 6.92

> Filter sterilized and stored @ 4°C } See page -3-
> Use 2 - 0.2 µ filter

Making competence -4-

* Competent cells : $CaCl_2$ method

Solution I : $MgCl_2 - CaCl_2$ solution
80 mM $MgCl_2$ 20 mM $CaCl_2$

Solution II : $CaCl_2$ solution
0.1 M $CaCl_2$ solution

25/5/17

$CaCl_2$ protocol : Competent cells.

Take 1% of culture in 10 mL LB

$\frac{1}{100} \times 10 = 100 \mu L$

↓

Inoculate 10 mL LB with 100µL primary inoculum.

↓ 250rpm

Incubate @ 37°C till an OD 0.6 - 0.8 (200rpm)

↓ Take 10 mL in 15mL falcon

Centrifuge @ 4100 rpm 4°C for 10 mins.

↓ → Discard supernatant

Resuspend in 6 mL $MgCl_2 - CaCl_2$ solution (80mM) (20mM)

↓

Centrifuge @ 4100 rpm 4°C for 10 mins

↓ → Discard supernatant

Resuspend in 0.1 M $CaCl_2$ solution : 400µL

↓

Add 80µL 80% glycerol solution

SUB - 20°C
Efficiency Calculation
Glycerol stock Standard Protocol
Transformation Protocol
$CaCl_2$ Method competent
Preparation Ultra competent cells
Miniprep Plasmid extraction

↓

Aliquot 50μL in 1.5mL eppis

↓

Flash freeze in liquid nitrogen

↓

Store at -80°C cryobox.

* Competent cells :

> Took the primary inoculum out at 11:20

↓

Inoculated 10mL LB with 100μL primary

↓

Incubated @ 37°C for ——— Hrs 220rpm

↓

Checked O.D of culture @ 2:30 pm.

MG1655 : 1.252

DH5 α : 0.612

Since OD crossed the required limit and reagents were not ready, we abandoned the experiment and instead grew them till saturation

*grow a single colony in 2mL LB/SOB @ 37°C

Immerse the stand fully in ice.

CCMB 80 Buffer -16-

PLATE [illegible]paration

[illegible]Cl₂ method [illegible]petent cells

[illegible]d Stock Preparation GEM -5-

[illegible]ing [illegible]mpetence -4-

* Preparation of glycerol stock.

Took 5 mL saturated MG1655 culture in a 15 mL falcon
↓
Added 5 mL 80% glycerol solution
↓
Aliquoted 1 mL in 1.5 mL eppis
↓
Flash freezing was done with liquid N_2
↓
Stored in -80°C inside cryobox.

11:20

2d/5/17

* Preparation of Ultracompetent Cells.

(Keep the cells always in ice, keeping them outside drastically reduces competency)

(* grow a single colony in 5 mL LB/SOB @ 37°C overnight for inoculum)

Inoculated 10 mL SOB with 40 µL of
→ Saturated MG1655 inoculum (2:00 am)
↓
Incubated @ 20°C 250 rpm overnight
↓
Checked OD @ 11 am (9 Hrs post inoculation)
OD = 0.399
↓ Cooled it on ice
Centrifuge @ 3000 g 4°C for 10 minutes
↓ Discard supernatant
Resuspend it in 3 mL ice cold CCMB80
↓ Incubate on ice 20 mins.
Centrifuge @ 3000 g 4°C for 10 mins.

(Keep on ice. Make sure eppi is not hanging above ice in the stand)

(* Immerse the stand fully in ice.)

↓ Discard supernatant

Dissolve the pellet in 400µL icecold CCMB80

↓

Measure OD of the cell suspension

50µL cells + 950µL SOB. Blank: 50µL CCMB80 + 950µL SOB

Dilution factor = 20

Measured OD = 0.348

OD of cell suspension = 0.348×20
= 6.96

↓

Diluted the cell suspension 1:5
350µL cells + 1400µL CCMB80 } Final O.D b/w 1-1.5

Final OD = 1.392

↓

~~Aliquot~~ Aliquot 50µL in 1.5 mL eppis

↓

Incubate on ice for 20mins.

↓

Flash freeze using liquid N_2

↓

Store @ -80°C in cryo box
(Labelled CC on cap, MG1655; 26/5/17 on the side of eppi)

Store the cell suspension in ice throughout all these steps

Take plasmid first and then add comp. cells. (Proper mixing) *

CCMB80 Buffer -16-

PLATE preparation

Glycerol stock Standard Protocol -19-

$CaCl_2$ method competent cells -17-

SOB preparation

Glycerol Stock Preparation GEM -5-

Preparing Ultracompetent cells -19-

Measuring competence -4-

60 second heatshock in water bath @ 42°c

↓

Incubate on ice for 5 minutes

↓

Add 950 µL LB and mix gently

↓

O.D

- 1.5

Incubate @ 37°C for 2 Hrs.

↓

Plate 50 µL on half of a LA - cloramphenicol plate, pellet down the culture resuspend in 50 µL LB and plate it on the other half.

↓

Incubate @ 37°c overnight.

↓

Count the number of colonies.

Avani ⇒ 25 colonies

Zakiya ⇒ 53 colonies

Jyothish ⇒ 337 Colonies.

17

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Efficiency calculation

Total volume of culture = 1000 μL
Volume plated = 50 μL

Transformation efficiency ⇒ cfu / μg DNA.

$$T.E = \frac{\text{no of colonies} \times \text{volume of culture}}{\text{conc. of plasmid} \times \text{volume of plasmid} \times \text{volume plated}} \times 10^6 \text{ (Pg→μg conv.)}$$

$$T.E_J = \frac{337 \times 1000 \times 10^6}{100\ \text{Pg}/\mu L \times 1\ \mu L \times 50}$$

$$= \underline{\underline{6.74 \times 10^7}}\ \text{cfu}/\mu g \text{ of DNA}$$

~~iGEM T.E = cfu × D_f × 10^5~~
~~= 337 × 20 × 10^5~~
~~= 6.64 × 10^8~~

Wrong!! No explanation for 10^5 factor

30/5/17

TE in # cfu/μg plasmid Plasmid conc. Pg/μL

$$\text{Transformation Efficiency} = \frac{\#\ \text{Colonies} \times \text{Vol. of culture } (\mu L) \times 10^6\ (\text{Pg}\to\mu g)}{\text{conc. plasmid} \times \text{vol. of plasmid} \times \text{volume plated}}$$

Unit ⇒ cfu / μg of DNA

29/5/17

* Bacterial

Auto

Tool

Inc

an

Toc

Time
8:15 p
8:35 p
9:10 p
10:10 p
11:10 p
12:10
1:10
2:10
3:10
4:10
5:10
6:10
7:10
8:10
9:10 a

Scanned by CamScanner

Bacterial growth curve
SOB - 20°C
-23-

29/5/17

* Bacterial growth curve

Autoclaved 2 conical flasks
↓
Took 50 mL SOB in both
↓
Inoculated one with 200µL DH5α and the other with 200µL MG1655 } 8:10 pm
↓
Took OD measurements and left the culture for incubation @ 20°C and 250 rpm

Time	DH5α : OD	MG1655 : OD
8:15 pm	0.031 x bad cuvette	0.008
8:35 pm	0.049	
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 / 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

30/5/17

A.
(g→µg conv.)
e plated
ng !!
explanation
5 factor
6 (Pg→µg)
me plated

Efficiency calculation

Total volume of culture = 1000µL
Volume plated = 50µL

Transformation efficiency ⇒ cfu / µg DNA.

$$T.E = \frac{\text{no. of colonies} \times \text{volume of culture}}{\text{conc. of plasmid} \times \text{volume of plasmid} \times \text{volume plated}} \times 10^{6} \; (\text{Pg} \to \mu\text{g conv.})$$

$$T.E_J = \frac{337 \times 1000 \times 10^{6}}{100\ \text{Pg}/\mu L \times 1\,\mu L \times 50}$$

$= 6.74 \times 10^{7}$ cfu/µg of DNA

~~iGEM T.E = cfu × D_f × 10^5~~
~~= 337 × 20 × 10^5~~
~~= 6.64×10^8~~

Wrong!! No explanation for 10^5 factor

30/5/17

TE in # cfu/µg plasmid Plasmid conc. Pg/µL

$$\text{Transformation Efficiency} = \frac{\#\,\text{Colonies} \times \text{Vol. of culture}\,(\mu L) \times 10^{6}\;(\text{Pg} \to \mu g)}{\text{conc. plasmid} \times \text{vol. of plasmid} \times \text{volume plated}}$$

Unit ⇒ cfu/µg of DNA

29/5/

* Bacte

A

Tim
8:1
8:2
9:1
10:1
11:1
12:
1:
2:
3:
4:
5:
6:
7:
8:1
9:1
10:1

Scanned by CamScanner

29/5/17

* Bacterial growth curve

Autoclaved 2 conical flasks

↓

Took 50 mL SOB in both

↓

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

↓

Took OD measurements and left the culture for incubation

@ 20°C and 250 rpm

Time	DH5α:OD	MG1655:OD
8:15 pm	0.031x bad cuvette	0.008
8:35 pm	0.049	
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	0.078	0.528
5:10 am	0.077	0.726 / 0.726
6:10 am	0.090	1.068
7:10 am	0.101	1.536
8:10 am	0.122	2.148
9:10am	0.144	2.796
	0.159	3.134

30/5/17

Bacterial growth curve - 20°C -23-

Miniprep Plasmid extraction

11:10 am	0.176	3.920
12:10 pm	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 / 10.50
5:10pm	0.457	8.00
6:10pm	0.539	6.26
7:10 pm	0.613	6.52
8:10 pm	0.772	7.52

29/5/17

* Primary inoculation

> Took 2 mL sterile ~~SOB~~ LB in 15 mL falcon.
> Inoculated it with 50μL of saturated culture of DH5α.
> Repeated the steps for MG1655
> Incubated overnight @ 37°C, 250rpm 12 Hrs (3:30 am → 3:30 pm)
> Stored the falcons @ 4°C

30/5/17

* Secondary inoculation for comp. cells.

> Took 35 ml SOB each in 4 conical flasks
> Inoculated 2 of them with 140μL of DH5α each (10:00 pm)
> Incubate overnight @ 20°C 250rpm

SOB -20°C -23-
...16-
...19-
...21-
...17-
...5-
...19-

Checked OD @ 5:00pm

$OD = 0.160 + 0.025$

$= 0.185$

↓

Transfer the culture to ice

↓

Centrifuge at 3000g 4°C for 10 minutes

↳ Discard supernatant

Resuspend in 9 mL ice cold CCMB 80

↓

* Incubate on ice for 20 minutes

↓

Centrifuge @ 3000g 4°C for 10 mins

↳ Discard supernatant

Resuspend in 1.2 mL ice cold CCMB80

↓

Measure OD of cell suspension.

50 μL cells + 950 μL LB

Blank : 50 μL CCMB80 + 950 μL LB

$D_f = 20$

Measured OD = 0.083

OD of cell suspension = 0.083 × 20

= 1.66

↓

Dilute the cell suspension 1 : 0.5

... cells + 0.575 mL CCMB 80

Miniprep Plasmid extraction -37-

Final OD = 1.10

↓

Aliquot 50 μL 10 nos

260 μL 4 nos

↓

Incubate on ice for 20 mins

↓

Flash freeze in liquid N_2

↓

Store in -80°C (cc, DH5α, J, 31/5/17)

1/6/17

* Efficiency test { -ve : DH5α ; Sample : DH5α ; +ve : MG1655 }

> Took 50μL comp cells each in 2 eppis

-ve control	RFP - transformation
↓	↓
1 μL milli Q	1 μL 50pg/μL RFP plasmid
↓	↓
Add 50μL comp cells	Add 50 μL comp cells
↓	↓

Incubate on ice for 30 minutes

↓

Heat shock @ 42°C 60sec in waterbath

↓

Add 950 μL LB and incubate @ 37°C for 2 Hrs

↓

Plated 50μL on LA - Cam plates

↓

Scanned by CamScanner

Grow @ 37°C overnight

↓

Count the number of colonies

no of colonies: 285

Efficiency: 1.14×10^8 cfu/µg of DNA

1/6/17

* Transformation with RFP: Troubleshoot

DH5α (J)	DH5α (Ar)	MG1655 (J)
50µL	50µL	50µL

↓

Add 1µL 100pg/µL plasmid, mix well

↓

Incubate on ice 35 mins

↓

Heat shock @ 44°C, 45 sec in waterbath

↓

Incubate on ice for 5 mins

↓

Add 950µL LB and incubate @ 37°C for 2 Hrs.

↓

Plate 20µL on LA-Cam plate and grow overnight @ 37°C

No of colonies in plate = 285

Scanned by CamScanner

Transformation efficiency $= \frac{285 \times 1000 \times 10^6}{50 \times 1 \times 50}$

$= 1.14 \times 10^8$ cfu/µg of DNA

6.6.17

Measurement Track

* Preparing ~~10x~~ PBS (100 mL)

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

} Set pH to 7.3

* Diluting to 1x PBS (40 mL)

> Took 4 mL 10x PBS
> Added 36 mL distilled water
> Mix thoroughly.

* Preparing fluorescein standard

> Spin down the tubes 3000 rpm, 30 sec

↓

> Resuspend in 1 mL 1x PBS (2x fluorescein)

↓

> Take 500 µL and dilute with 500 µL of 1x PBS (1x fluorescein) (50 mM)

Bacterial growth curve SOB -20°C -23-
Effi Ca
CCMB 80 Buffer -16-
PLATE Preparation
Glycerol stock Standard Protocol -19-
Transformation Protocol -21-
$CaCl_2$ method competent cells -17-
Preparing competent cells -19-
Plat

Weight required $= \frac{\text{molar mass} \times \text{conc.} \times \text{vol}}{1000}$

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

$= 0.0376275$

$= 0.03763$ gm

Dissolve in ~ 1 mL 1x PBS.

Molar mass of fluorescein = 332.31

Weight required $= \frac{332.31 \times 100 \times 10^{-3} \times 1}{1000}$

$= 0.033231$ 100 mM

Insoluble

50 mL 1 mM Flourescein

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

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

$= 0.0167$ g

Scanned by CamScanner

2.6.17 Tuesday

× 5 mM Flourescein solution 10 mL

$$\text{Weight} = \frac{332.1 \times 5\times10^{-3}\times 10}{1000}$$

$\approx$ 17 mg

- Corning - Costar - 96 wells - Full area - Flat bottom

OD Measurement and pathlength correction

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

- Fluorescence measurement

	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
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
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
D	L 1	L 2	L 3	L 4	L 5	L 6	L 7	L 8	L 9	L 10	L 11	L 12
E	L 1	L 2	L 3	L 4	L 5	L 6	L 7	L 8	L 9	L 10	L 11	L 12
F	L 1	L 2	L 3	L 4	L 5	L 6	L 7	L 8	L 9	L 10	L 11	L 12
G	L 1	L 2	L 3	L 4	L 5	L 6	L 7	L 8	L 9	L 10	L 11	L 12

(repeat f

Measurements were made at medium high and ~~the~~ high ranges.

λ Excitation - 501 nm

λ Emission - ~~50~~511 nm

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

$x = \frac{25}{7}$

$= 3.57\,\mu L$ of 35 mg/mL Cam

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

$= 3.57 \times 20$

$= 71.4\,\mu L$ of 35 mg/mL Cam

For 40 mL $\rightarrow 8 \times 3.57$

$= 28.56\,\mu L$

$\approx 28.6\,\mu L$ of 35 mg/mL Cam

$$\begin{array}{r} 357 \\ \times\ 8 \\ \hline 28.56 \end{array}$$

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

↓

Incubate @ 37°C, 220 rpm, 16-18 Hrs

(Incubated @ 11:20 pm).

9.6.17

Started checking OD for cultures @ 11 am

↓

Found dilutions using excel sheet

↓

Inoculated 12 mL LB with appropriate amount of inoculum

↓

Took zero time point @ 2:00 pm

↓

Took 2 Hrs time point @ 4:00 pm

→ Cultures were open: Cancelled the experiment.

17

> Colony inoculation of devices

Took 5 mL LB-Cam in 16 : 15mL falcons

↓

Picked a colony into it using 10µL tip

↓

Incubated overnight @ 37°C 220 rpm

13.6.17

Checked OD and inoculated LB-Cam to get a final OD 0.02

↓

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

TP		1
0	A	+ve
Colony 1	B	+ve
	C	+ve
	D	+ve
	E	+ve
Colony 2	F	+ve
	G	+ve
	H	+ve

4 Hrs time point @ 3:00pm ⎫ from each
6 Hrs time point @ 5:00pm ⎭ culture, and
Stored on ice 4°C.

↓

Measured OD and fluorescence on platereader.

Time point - 0 hrs (columns 1–8); Time point - 2 hrs (columns 10–12)

TP		1	2	3	4	5	6	7	8	9	10	11	12
0	A	+ve	-ve	TD1	TD2	TD3	TD4	TD5	TD6	Blank	+ve	-ve	TD1
Colony 1	B	-ve	-ve	TD1	TD2	TD3	TD4	TD5	TD6	Blank	+ve	-ve	TD1
	C	+ve	-ve	TD1	TD2	TD3	TD4	TD5	TD6	Blank	+ve	-ve	TD1
	D	+ve	-ve	TD1	TD2	TD3	TD4	TD5	TD6	Blank	+ve	-ve	TD1
	E	+ve	-ve ✗	TD1	TD2	TD3	TD4	TD5	TD6	Blank	+ve	-ve	TD1
Colony 2	F	+ve	-ve	TD1	TD2	TD3	TD4	TD5	TD6	Blank	+ve	-ve	TD1
	G	+ve	-ve	TD1	TD2	TD3	TD4	TD5	TD6	Blank	+ve	-ve	TD1
	H	+ve	-ve	TD1	TD2	TD3	TD4	TD5	TD6	Blank	+ve	-ve	TD1

	Time pt - 2hrs						Time pt - 6hrs					
	1	2	3	4	5	6	7	8	9	10	11	12
A	TD2	TD3	TD4	TD5	TD6	Blank	+ve	-ve	TD1	TD2	TD3	TD4
B	TD2	TD3	TD4	TD5	TD6	Blank	+ve	-ve	TD1	TD2	TD3	TD4
C	TD2	TD3	TD4	TD5	"	"	"	"	"	"	"	"
D	TD2	TD3	TD4	TD5	"	"	"	"	"	"	"	"
E	TD2	TD3	"	"	"	"	"	"	"	"	"	"
F	TD2	TD3	"	"	"	"	"	"	"	"	"	"
[illegible]	[illegible]	TD3	"	"	"	"	"	"	"	"	"	"
[illegible]	TD2	TD3	"	"	"	"	"	"	"	"	"	"

Columns = 1 (rows A–D)
Columns = 2 (rows E–F)

	1	2	3	4	5	6	7	8	9	10	11	12
A	T.D5	TD6	B	?	N	TD1	2	3	4	5	6	
B	T.D5											
C												
D												
E												
F												
G												
H												

6hrs (TD1–6)

OD measurement plate-2

	1	2	3	4	5	6	7	8	9	10	11	12
A	TD2	TD3	4	5	6	2	3	4	5	6	+	+
	col 1					Col-2					col2 col1 ; 4Hrs	
B	"	"										"
C	"	"									"	
D	"	"									"	
E	-Ve	1	2	3	4	-Ve	1	2	3	4	Blank	Blank
	col 1					col 2						
F												
G												
H												

Scanned by CamScanner

Bacterial growth curve SOB -20°C -23-
Effi Ca
CCMB 80 Buffer -16-
LA PLATE Preparation
Glycerol stock Standard Protocol -19-
Transformation Protocol -21-
$CaCl_2$ method competent cells -17-
SOB preparation
...ed Stock Preparation GEM -5-
...reparing ...tracompetent cells -19-
...sing ...petence -4-

28.6.17

Transformation of parts.

Promoter : pBla : 3_2L : ~~Ampicillin~~ Chloramphenicol
RBS : : 4_1~~0~~N : Ampicillin
AmilCP : : 1_19E : Chloramphenicol
RFP : : 3_11N : Chloramphenicol

↓

Resuspend in 10 μL milliQ H_2O

↓

Store @ -20°C in eppis.

↓

Pipette 1 μL plasmid into an eppi

↓

Add 50μL competent cells and mix well

↓

Incubate on ice for 30 minutes

↓

60 sec heat shock @ 42°C in waterbath

↓

Incubate on ice for 5 minutes

↓ Add 950μL LB

Incubate @ 37°C 220rpm for 2 Hrs

↓

Plate 200μL on antibiotic plates

↓

Grow them @ 37°C overnight.

↓

Transfer the plates to 4°C

Scanned by CamScanner

29.6.17

- Inoculating transformed colony for miniprep.

Took 2 mL LB-Cam (25 µg/mL) in a 15 mL falcon

↓

Picked one RFP transformed colony into the falcon with 10 µL tip

↓

Incubated @ 37°C 220 rpm for 18 Hrs

30.6.17

Miniprep plasmid extraction [RFP BBa_E1010

Pipette 1 mL culture into 2, 1.5 mL eppis each

↓

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

↓ → Discard supernatant

Resuspend the pellet in 125 µL P1 buffer (resuspension)

↓

Pool them together into a 1.5 mL eppi

Date on bottle only opened.

↓

RNAase A Add 250 µL P2 (Lysis) buffer and mix well by inverting the tubes (3 min)

↓

Scanned by CamScanner

Centrifuge @ 17900 rcf for 60 sec

↳ Discard flow through

Add 500 μL PB buffer into the column

↓

Centrifuge @ 17900 rcf for 60 sec

↳ Discard flow through

Add 750 μL PE buffer to the bed

↓

Centrifuge @ 17900 rcf for 60 sec

↳ Discard flow through

Centrifuge @ 17900 rcf for 60 sec

↓

Transfer the column into 1.5 mL eppi

↓

Temp: 37°C Add 50 μL EB and let it stand for 1 min

Incubation with EB 1 min

↓

Centrifuge @ 17900 rcf for 60 sec.

↓

Store @ -20°C; Labelled as

BBa_E1010, RFP, 3_11N

5/7/17 Gel running and nanodrop for plasmid

Scanned by CamScanner

Add 5_ mL 1x TAE

↓

[icro]wave for 2 min

↓

gel tray, once cooled

µL cyber safe and put the comb

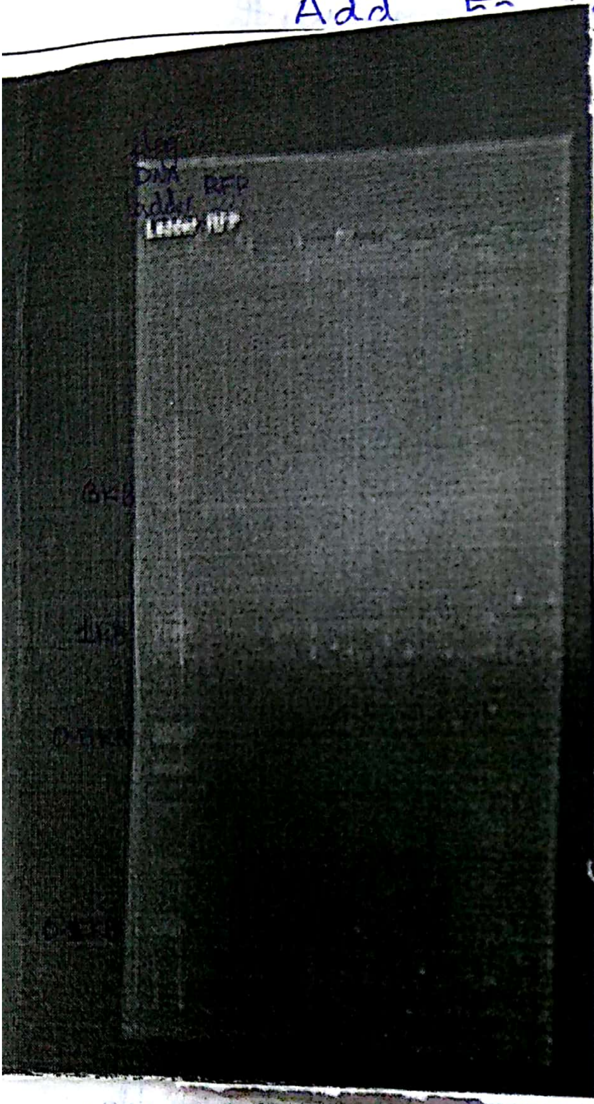
↓

is solidified transfer

to the gel runner

↓

gel as shown below



↓

80 V for 1 hour.

↓

Take the gel image using gel doc.

* Nano drop

Set blank using 1 µL EB-buffer

↓

Load 1 µL plasmid and check the reading

Scanned by CamScanner

* Miniprep for Promoter, RBS, Amil CP.

> Inoculated 2 mL LB Cam with Amil CP transformed cells

> Inoculated 2 mL LB Amp each with pBla and RBS transformed cells

↓

Incubated @ 37°C, 220 rpm Overnight

↓

9/7/17

Follow the miniprep protocol. Page : 37

↓

Pellet down 1 mL bacterial culture {6800 rcf, 3 min}

↓

Resuspended the pellet in 250μL P1

↓

Added 250μL P2 and mixed by inverting (3 minutes)

↓

Added N3 buffer and mixed thoroughly.

↓

Spin @ 17900 rcf for 10 minutes

↓ → Discard pellet

Pipette 800μL of supernatant into column

↓

Spin @ 17900 rcf for 60 seconds

↓

* Dont repeat: Reloaded the flow through to the column — Avoid this step

↓

Spin @ 17900 rcf for 60 sec.

Steps got interchanged while doing the experiment

9/7/17

*

Scanned by CamScanner

Steps got interchanged while doing the experim[ent]

~~Discard flow through~~ Add 500 μL PB buffer

↓

Spin @ 17900 rcf for 60 seconds

↓ Discard flowthrough

Add 750 μL PE buffer

↓

Spin @ 17900 rcf for 60 seconds

↓ Discard flowthrough & 17900 rcf 60 sec again

Place the column in a new eppi and add 50 μL EB, incubate @ 37°C for 5 min

↓

Spin it down @ 17900 rcf for 60 sec

↓

Measure conc using nano drop

~~pBla Promoter:~~

RBS : 42.2 ng/μL 260/280 : 1.89

↓

Store @ -20°C in labelled eppis

9/7/17

* Miniprep for promoter, RBS, Amil CP : Repeat

Inoculate 5 mL LB with a single colony

pBLA promoter : Chloramphenicol

RBS B0034 : Ampicillin

Amil CP : Chloramphenicol

↓

10/7/17

↓

Pipette 1 mL each of eachculture in 1·5 mL eppis

↓

Pellet down the cells : centrifuge 6800 rcf for 3 mins.

↓ ↳ Discard supernatant

Resuspend the pellet in 125 μL P1

↓

Pool the respective replicates together

↓

Add 250 μL P2 buffer, mix by inverting,
Let it stand for 3 mins.

↓

Add 350 μL N_3 buffer, mix by inverting.

↓

Centrifuge @ 17900 rcf for 10 mins.

↓ ↳ Discard pellet

Pipette 800 μL supernatant into the column

↓

Centrifuge @ 17900 rcf, 60 seconds

↓ ↳ Discard flowthrough

Add 500 μL PB, spin @ 17900 rcf 60 sec.

↓ ↳ Discard flowthrough

Add 750 μL PE, spin @ 17900 rcf 60 sec

↓ ↳ Discard flowthrough

Spin the eppi @ 17900 rcf 60 sec.

↓ ↳ Discard flow through

Transfer the column to a new sterile eppi

↓

Add 50 μL EB, incubate @ 37°C for 5 mins.

11/7/17

* Transf

Locati

Resus

A li

Follo

Ad

In

60

5

A

↺ 3000 rcf 4°C 10 min Pe

Scanned by CamScanner

Store the eppis @ -20°C

11/7/17

* Transformation of p(CAT) promoter BBa_I 14033

Location of p(CAT) BBa_I14033 : 3_13D

↓

Resuspend in 10 µL milli Q H_2O

↓

Aliquot in 1.5 mL eppi with proper label.

Store @ -20°C in cryobox

Follow transformation protocol : Page : 21

Add 1 µL plasmid to 50 µL comp cells

↓

Incubate on ice for 30 minutes

↓

60 sec heat shock on water bath

↓

5 min ice incubation.

↓

Add 250 µL LB and incubate

@ 37°C 220 rpm 2 Hrs

↓

3000 rcf 4°C 10 min Pellet down and plate pellets on Cam-LA plate.

Scanned by CamScanner

↓

Incubate overnight @ 37°C

# of colonies =

12/7/17

* Serial dilution and conc. estimation of T7 lytic phage

Clean the working area with EtOH

↓

Wear gloves and other protective equipments

↓

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

The stock has to be diluted as follows

10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}

Measure out 900µL Milli-Q H_2O (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 eppi 1

↓ mix well

Pipette out 100µL from eppi 1 to 2

↓ mix well

Pipette out 100µL from eppi 2 to 3

Keep in water bath @ 55°C

Pf

Pfu

Scanned by CamScanner

Clean the working area with EtOH

↓

Wear gloves and other protective equipments

↓

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

The stock has to be diluted as follows
10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}

Measure out 900µL Milli-Q H_2O (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 eppi 1

↓ mix well

Pipette out 100µL from eppi 1 to 2

↓ mix well

Pipette out 100µL from eppi 2 to 3

Scanned by CamScanner

↓ mix well

Repeat dilutions till 5.

↓

Grow E-coli till an OD 0.3 - 0.7 (exponential phase)

OD of cell culture = 0.274

↓

Keep in water bath @ 55°C:

- Take 3 mL soft agar in 15mL falcon

↓

- Add 200μL of cell culture

↓

- Add 100μL of different phage dilutions (make 2 replicates for each dilution)

vortex ↓ wait for 4-11 mins @ 55°C

Mix well 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

$$Pfu_{R} = \frac{\#\ plaque}{dilution \times volume} = \frac{360}{10^{-4} \times 0.1} = 3.6 \times 10^{7}$$

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

Scanned by CamScanner

Grow E-coli till an OD 0.3 - 0.7
(exponential phase)

OD of cell culture = 0.274

↓

Keep in water bath @ 55°C:

Take 3 mL soft agar in 15mL falcon

↓

Add 200μL of cell culture

↓

Add 100μL of different phage dilutions
(make 2 replicates for each dilution)

vortex ↓ wait for 4-11 mins @ 55°C Attach

Mix well 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

$$Pfu_{1} = \frac{\#\ plaque}{dilution \times volume} = \frac{360}{10^{-4} \times 0.1}$$

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

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

Scanned by CamScanner

$Pfu_{avg} = 2.8 \times 10^7$ phages/mL

OD of E coli $1\ OD = 4.8 \times 10^8$ cells/mL

Multiplicity of infection $MOI = \frac{V_{phage} \times 2.8 \times 10^7}{10\,mL \times 0.2 \times 4.8 \times 10^8} = 1$

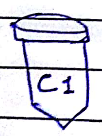
inoculum $V_{phage} = \frac{10 \times 0.2 \times 4.8 \times 10^8}{2.8 \times 10^7}$

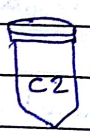
$= \frac{96}{2.8}$

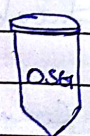
13/7/17 * Host dynamics and phage kinetics

Grow E. Coli to an OD = 0.2

Inoculate 9mL LB with 1 mL of OD = 0.2 culture







Label the eppis as shown.

Measure the OD of C1 every 15 mins.

Time   OD

→ See next page for readings

↓

Transfer all three tubes to ice

↓

Calculate volume of phage for MOI = 0.0001

$$V_{phage} = \frac{0.0001 \times 0.2 \times 10 \times 4.8 \times 10^8}{2.8 \times 10^6}$$

$\approx$ 240 μL

↓

Add 240 μL phage lysate to C2 and OSG

↓ Store OSG in ice.

Transfer C1 and C2 to incubator and check their OD every 15 minutes

time	OD C1	OD C2
0	0.025	0.025
19	0.054	0.054
39	0.074	0.074
59	0.132	0.132
74	0.206	0.206
84	0.26	0.265
103	0.353	0.373
119	0.484	0.491
134	0.765	0.261
153	1	0.037
168	3.27	0.039
188	2.9 / 2.8	0.024
206		0.024
221	3	0.024

23/7/17

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

↓ Discard supernatant

~~Discard~~ Dissolve the pellet in 10 mL 0.1 M $MgSO_4$ solution

↓

Centrifuge @ 10000 rpm 4 °C 10 min

↓ Discard supernatant

24/7/17

Dissolve the pellet in 10 mL 0.1 M $MgSO_4$

Centrifuge @ 10000 rpm 4 °C 10 min

↓ Discard supernatant

Dissolve the pellet in 10 mL LB and immedictly transfer to 90 mL LB

total volume = 100 mL

↓

Incubate @ 37 °C on water bath

↓

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

	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 in each plate and back-calculate phage conc.

23/4/17 • Inoculation for miniprep p(CAT)

Take ~~8~~ 5 mL LB-cam in 15 mL falcon

↓

Inoculate with a single colony

↓

Incubate @ 37°C 220 rpm for 18 Hrs.

24/4/17 • Miniprep plasmid extraction p(CAT)

Take 1.5 mL each of culture in 2 eppis

↓

Centrifuge @ 6800 rcf for 3 minutes

↓ → Discard supernatant

Resuspend the pellets in 125 µL P1 each and pool the replicates together

↓

Add 250 µL P2, mix by inverting
Let it stand for 4 mins.

↓

Add 350 µL N3 buffer, mix by inverting

↓

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 flowthrough

Add 750µL PE, centrifuge @ 17900 rcf 60sec.

↓ Discard flowthrough

Spin @ 17900 rcf for 60sec.

↓ Discard flowthrough

Keep the column in a new sterile eppi

↓

Incubate @ 37°C for 5 min after adding 50µL EB to the column

↓

Centrifuge @ 17900 rcf for 60sec.

↓

Measure the yield and store @ -20°C

p(CAT) conc : 47.8 260/280 : 1.97

* Gel running for p(CAT), RBS, Anil CP : Post EcoRI digestion

Use NEB buffer 3.1 for EcoRI

> Enzyme master mix preparation (25µL 5 reactions)

- 5µL NEB buffer 3.1
- ~~0.5µL BSA~~
- 0.5µL EcoRI
- 19.5µL d H_2O

Take 4µL plasmid DNA in an eppi

↓

Add 4µL enzyme master mix

2kb DNA ladder   p(CAT)   R

3Kb

2Kb

2/8/17

* 3A

> Ev

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↓

80 V for 1 Hour.

↓

using gel doc.

plasmids.

Concentration (ng/µL)	260/280
47.8	1.97
62.6	1.93
104.5	1.93
50.1	2.03
56.6	1.85
120.3	1.73

Amil CP, RBS

for backbone (2x reactions)

buffer 2.1

Scanned by CamScanner

↓

Incubate @ 37°C for 30 min

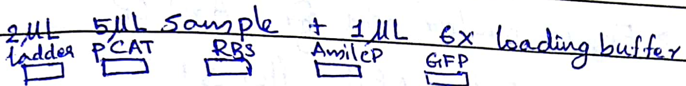
↓

Heat kill @ 80°C for 20 min

↓

Load in the gel as shown below

2 µL ladder, 5 µL sample + 1 µL 6x loading buffer



↓

Run the gel @ 80 V for 1 Hour.

↓

Image the gel using gel doc.

* Nano drop for the plasmids.

Construct	Concentration (ng/µL)	260/280
p(CAT)	47.8	1.97
Amil CP	62.6	1.93
RFP	104.5	1.93
RBS	50.1	2.03
cjBlue	56.6	1.85
GFP	120.3	1.73

* 3A assembly : Amil CP, RBS
  - > Enzyme master mix for backbone (2x reactions)
    - 2 µL NEB buffer 2.1
    - 0.[illegible] µL EcoRI — 0.2 µL
    - 0.[illegible] µL Pst I — 0.2 µL
    - 0.[illegible] µL DPNI — 0.2 µL
    - [illegible] µL H_2O — 7.4 µL

> Enzyme master mix for RBS (2x reactions)

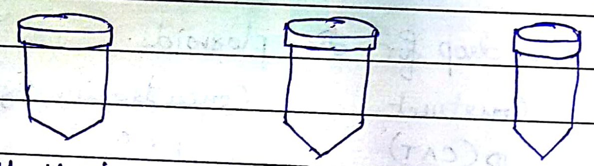
- 3 µL NEB buffer 3.1
- 0.2 µL EcoRI
- 0.2 µL Spe I
- 7.6 µL d H_2O

> Enzyme master mix for Amil CP

- 2 µL NEB buffer 3.1
- 0.2 µL Xba I
- 0.2 µL Pst I
- 7.6 µL d H_2O

3/8/17

*



Back bone digest	RBS digest	Amil CP digest
• 4 µL M.mix	• 4 µL M.mix	• 4 µL M.mix
• 4 µL pSB1K3 (25ng/µl)	• 2 µL RBS	• 2 µL Amil CP
	• 2 µL d H_2O	• 2 µL d H_2O

Digest @ 37°C for 30 mins

↓

Heat kill @ 80°C for 20 mins

↓

Take 2 µL of digested backbone in an eppi

↓

Add 2 µL digested RBS in it

↓

Add 2 µL digested Amil CP

Scanned by CamScanner

↓

Add 1 μL T4 DNA ligase buffer

↓

Add 0.5 μL T4 DNA ligase

↓

Add 2.5 μL dH_2O (final volume 10 μL)

↓

Ligate 16°C/30 min, heat kill 80°C/20 min

↓

Store @ -20°C

3/8/17

* Transformation of cells with ligated product

Take 2 μL of ligated product in 1.5 mL eppi

↓

Add 50 μL DH5α competent cells

↓

Incubate on ice for 30 minutes

↓

60 sec heat shock @ 42°C

↓

Incubate on ice for 5 minutes

↓

Add 250 μL LB and mix gently

↓

Incubate @ 37°C for 2 Hrs

↓

Plate 50 μL on half plate

Plate the control on other half.

ix

CP

0

Confirmation of Duplicated product

[gel photo label] 2 log DNA ladder | Hybrid Promoter | AmilCP | Cj blue

...arose gel (Page 39)

...instead of cybersafe)

...shown below

3 µL RBS+ AmilCP [ ] | 4 µL RBS+ Cj Blue [ ] } + 1 µL 6X loading dye.

...V for 60 minutes

... was taken using gel doc

...served between 2 Kb

...kb bands.

9/8/17

Re

Pellet plating : Transformation on 3-8-17

Confirmatory gel for ligated product

Make 50 mL 1% Agarose gel (Page 39)
(Used EtBr instead of cybersafe)

↓

Loaded the gel as shown below

2 μL 2log DNA ladder	3 μL Hybrid promoter	3 μL RBS+ AmilCP	4 μL RBS+ Cj Blue

} + 1 μL 6X loading dye.

9/8/17

↓

Ran the gel @ 80V for 60 minutes

↓

Image of the gel was taken using gel doc

↓

A band is observed between 2 Kb and 3 Kb bands.

Pellet plating : Transformation on 3-8-17

Ins

Gro

Replatin

Tak

Inocul

Incub

Grow overnight @ 37°C

↓

Colonies were observed on plate: on 9.8.17

Primary inoculation for comp. cell preparation

Took 4 mL SOB in 15 mL falcon

↓

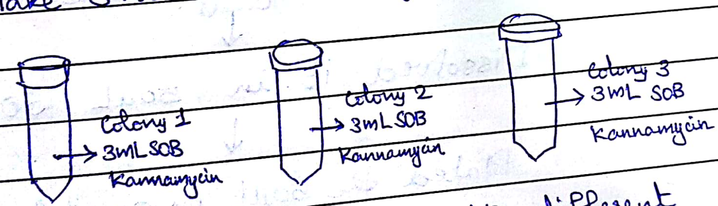
Inoculate with a single colony from DH5α master plate

↓

Grow @ 20°C 220rpm overnight

Replating RBS+AmilCP colonies

Take 3 mL SOB-Kannamycin in 3 falcons (15 mL)



Inoculate different falcons with different colonies

↓

Incubate @ 37°C 220 rpm 3 Hrs

↓

Plate 100μL from each falcon on an LA- Kannamycin plate

↓

Incubate @ 37°C overnight.

Scanned by CamScanner

↓

Count the number of colonies in each plate

plate 1 : 0

plate 2 : 0

plate 3 : 0

- Comp. Cell preparation (8.8.17 continued)

No growth was seen in primary after 20Hrs post inoculation. So transferred it to 37°C 220 rpm to grow till saturation

(Cells not growing, Abandoned the experiment)

(Transferred the culture to 37°C 220 rpm incubator)

10/8/17

Replating RBS+AmilCP colonies (Plate from 8.8.17)

Picked a colony from the main plate

↓

Dissolved it in 50μL SOB

↓

Plated the 50μL on an LA-Kannamycin plate

↓

Incubated at 37°C overnight

11/8/17

Took the plate from the incubator

It had enough colonies, stored it @ 4°C.

16/8/17

17/8/17

C

18/8/17

Com

Scanned by CamScanner

16/8/17 Made 100 mL LB, 200 mL SOB

17/8/17 Colony inoculation for miniprep

Took 4 mL each of LB-Kannamycin in 3 15mL falcons

↓

Picked 3 different colonies and inoculated in 3 mL media

↓

Incubated @ 37°C 220 rpm 18 Hrs.

↓

No growth in the media (OD 600 = 0)
Abandoned the experiment } 18/8/17

Primary inoculation for comp. cells

Took 4 mL plain SOB in 15mL falcon

↓

Inoculated it with 10μL saturated culture of DH5α - cells

↓

Incubated @ 20°C 220 rpm

18/8/17 Comp cell preparation

Inoculated 100 mL SOB with 400μL primary culture in a sterile flask

↓

Incubated @ 20°C 250 rpm 18 Hrs

Scanned by CamScanner

19/8/17 Checked OD of the cell culture

OD = 1.12

Take 50 mL each on 2 falcons.

↓ Cool on ice

Centrifuge @ 3000g 4°C for 10 mins.

↓ discard supernatant

Resuspend the pellet in 15 mL CCMB80 each

↓

Incubate on ice for 20 mins

↓

Centrifuge @ 3000g 4°C for 10 mins.

↓ Discard supernatant

Dissolve the pellet in 2 mL CCMB 80 each and pool them together

Postponed to 23rd Aug

Measure OD : 50 µL cells + 950 µL SDB Df = 20

Measured OD =

OD of cell suspension = 20 x

=

↓

Dilute the cell suspension 1 :

µL cells + µL CCMB 80

Final OD =

↓

Aliquot 50 µL in 1.5 mL eppis

↓

Incubate on ice for 20 mins

Postponed to 28rd Aug

20/8/17

21/8/17

• F

* F

* Color

2

Scanned by CamScanner

↓

Flash freeze the eppis and store @ -80°C.

(Postponed to 23rd Aug)

Took 100 mL fresh SOB in ~~a flask~~ BOD bottle

↓

Inoculated with 400μL DH5α culture (OD = 1.12)

↓

Incubated @ 20°C 250 rpm 18 Hrs

↓

20/8/17

Measured OD of culture @ 17.30 Hrs post inoculation

OD = 1.235

21/8/17

- Prepared 100 mL SOB

* Primary inoculation for ~~[illegible]~~ DH5α comp cells.

2 mL SOB was taken in 15mL falcon

↓

Inoculated it with 1 colony from DH5α master plate

↓

Grew it overnight @ 20°C 250 rpm

* Colony inoculation for miniprep

2 mL LB was taken in a 15mL falcon

↓

Stock conc. of Kannamycin = 50 mg/mL
Working conc. of Kanamycin = 35 μg/mL

So 2 mL × 35 μg/mL = x μL × 50 mg/mL

x = 1.4 μL

↓

Inoculated the media with a single colony

↓

Incubated @ 37°C 220 rpm overnight

↓

22/8/17

No growth seen in the tubes.

* Secondary Inoculation for comp cells

Inoculated 100 mL SOB with 200 μL primary culture of OD 1.23

↓

Incubated @ 18°C 250 rpm 13 Hrs

↓

23/8/17

Checked OD against SOB blank

OD = 0.817

↓

Scanned by CamScanner

measured OD = 0.350 D_f = 20

OD of cell suspension = 7.0

Dilute 1 : 4   (culture: CCMB 80)

Volume of suspension = 4 mL

Volume of CCMB 80 to add = 16 mL

Final OD = $\frac{7}{5}$ = 1.4

Made 25 1x Aliquotes (50µL / tube)

and 75 5x Aliquotes (260µL / tube)

↓

Flash freezing was done using liquid N_2

↓

Stored all the eppis @ -80°C

* Efficiency transformation

1µL 50 ng/µL ~~RPS~~ RFP construct was used

↓

Standard transformation protocol (Page: 21)

↓

Add 250µL LB, ~~incute~~ incubate @ 37°C 2 Hrs

↓

Plate on chloramphenicol plate

Negative control is also made, where no template DNA was added

↓

Count the number of colonies

mary

t

Scanned by CamScanner

\# of colonies = ~~Non specific~~ No growth

Efficiency = 0

* RBS (B0034) + Amil CP transformation

1 µL of ligated product was taken (Page : 51, 2/8/17)

↓

Transformation was performed (Page 21)

↓

250µL LB was added and incubated @ 37°C 220rpm 2 Hrs

↓

Plated 50µL on a Kanamycin plate on half, and pellet in other half

↓

Incubated at 37°C overnight

↓

?7/8/17 Count the number of colonies

↓

Uncountable number of colonies {Non specific growth}

?/8/17 Comp cell preparation

Inoculated 100 mL SOB with 1 mL seed stock

↓

Incubated @ 18°C 250 rpm

↓

Flask got opened inside the incubator

Abandoned the experiment

30/8/17

Measure OD of cell suspension 50μL cells + 950μL LB

OD = 0.416   Df = 20

OD of cell suspension = 0.416 × 20
= 8.32

Dilute with CCMB 80 1:5

Volume of suspension = 4 mL

Add CCMB 80 = 20 mL

Incubate on ice for 20 minutes

Aliquote 260 μL / eppi (5x reactions)

Flash freeze using liquid N_2

↓

Scanned by CamScanner

31/Aug/2017

Efficiency test transformation

Took 1 µL RFP construct (100 pg/µL) in a 1.5 mL eppi

↓

Added 50 µL of competent cells

↓

Incubated on ice for 20 minutes (Short incubation than usual refer page: 21)

↓

Heat shock @ 42°C for 60 seconds

↓

Incubate on ice for 5 minutes

↓

Add 250 µL SOC and incubate at 37°C 220 rpm for 2 Hrs

↓

Plate 50 µL of the cells in a chloramphenicol selection plate

↓

Incubate @ 37°C overnight

↓

[illegible]/Sep/2017

Count the number of colonies

colonies = 860

Transformation Efficiency = $\frac{860 \times 300 \times 10^6}{100 \times 1 \times 50}$ cfu/µg DNA

= 5.16×10^7 cfu/µg of DNA

1/Sep/2017

* 3A

NEB b

100 m

50 m

10 m

100 µ

pH for

Enzym

Enzy

EcoR

Xba

Spe

Pst

Conv

NaCl : 50

Tris.HCl : 10

So I need of stock 1M NaCl

1/Sep/2017

* 3A assembly (Amil CP + RBS (B0034) + pSB1T3)

NEB buffer 3.1 (1x)	NEB buffer 2.1 (1x)
100 mM NaCl	50 mM NaCl
50 mM Tris-HCl	10 mM Tris-HCl
10 mM $MgCl_2$	10 mM $MgCl_2$
100 µg/mL BSA	100µg/mL BSA

21) pH for both buffers are 7.9 @ 25°C

Enzyme Efficiency table.

Enzyme	Buffer 2.1	Buffer 3.1
EcoRI	100 *	50
Xba I	100	75
Spe I	100	25
Pst I	75	100

Converting buffer 2.1 to 3.1

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

$x = 100$ mM NaCl for 1X

Tris.HCl: $\frac{10\,mM \times 2 + y\,mM \times 1}{20} = \frac{50\,mM \times 2}{20}$

$y = 80$ mM ~~NaCl~~ Tris.HCl for 1X

So I need to make a buffer conversion solution of stock concentration 10X ~~containing~~ constituting 1M NaCl and 0.8 M Tris HCl.

chloramphenicol

µg DNA

of DNA

* Back bone digestion (10 µL total, 2 reactions)

Enzyme master mix

- 2 µL NEB buffer 3.1
- 0.2 µL EcoRI
- 0.2 µL PstI
- 0.2 µL DpnI
- 7.4 µL d H_2O

4 µL backbone → 4 µL master mix

Incubate 37°C / 30 min

Heat kill 80°C / 20 min

* Part A (RBS B0034) digestion : 2 step

Step 1 : SpeI digestion in Buffer 2.1

- 2 µL NEB buffer 2.1
- 0.2 µL SpeI
- 7.8 µL d H_2O

200 ng DNA ~~with~~ in 4 µL → 4 µL master mix

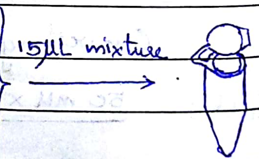
Incubate 37°C / 30 min

Heat kill 80°C / 20 min

Step 2 : EcoRI digestion in buffer 3.1

- Take 8 µL digested product
- 0.8 µL buffer 2.1
- 0.8 µL buffer converter
- 6.~~4~~1 µL d H_2O
- 0.2 µL EcoRI

15 µL mixture →



Incubate 37°C / 30 min

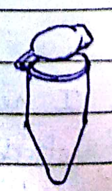
Heat kill 80°C / 20 min

- Part B (AmilCP / ~~RBS~~ RFP) digestion

Enzyme master mix

- 2 µL NEB buffer 3.1
- 0.2 µL XbaI
- 0.2 µL ~~SpeI~~ PstI
- 7.6 µL d H_2O

100 ng DNA in 4 µL → 4 µL master mix



* Prepared

* NaCl

- Tris

- $MgCl_2$

5th Sept 2017

- Prepared
- Prepared Kanam
- Stored
- Kept overni

* Prepared 2 M NaCl - 25 mL
  2 M Tris-HCl - 30 mL pH : 7.91
  1 M $MgCl_2 \cdot 6H_2O$ - 3 mL
  1000 µg/mL BSA - 5 mL

* NaCl : M.W = 58.44 g/mol

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

$$x = 2.922 \text{ g}$$

• Tris : MW = 121.14 g/mol

$$\frac{x}{121.14} \times \frac{1000}{30} = 2$$

$$x = 7.2684 \text{ g}$$

• $MgCl_2 \cdot 6H_2O$: MW = 203.3 g/mol

$$\frac{x}{203.3} \times \frac{1000}{3} = 1$$

$$x = 0.61 \text{ g}$$

5-Sept-2017

• Prepared 4 LA plates
• Prepared 6 LA plates with 50 µg/mL of Kanamycin.
• Stored the plates @ 4°C in 4°C room.
• Kept 2 non antibiotic plates in the hood overnight to check for contamination

Incubated overnight @ 37°C

* Inoculating with glycerol stock

Inoculated 2 mL sterile LB with glycerol stock of cells

Scanned by CamScanner

* Inoculating with glycerol stock

Inoculated 2 mL sterile LB with glycerol stock of cells

Scanned by CamScanner

O/N culture

Incubated @ 37°C 170 rpm and checked OD

$OD_{1 Hr\ 50 min}$ = 0.162

$OD_{2 Hr\ 15 min}$ = 0.412

Aliquoted 45 mL each in 2 falcons

Followed the Ultra comp. cell preparation Protocol (Refer page: 58)

Measured OD of 50µL cell suspension + 950µL SOB

$D_f = 20$

OD = 0.402

OD of cell suspension = 20 × 0.402
= 8.04

Dilute the cell suspension 1:5

4 mL cells + 20 mL CCMB 80

Final OD = 1.34

Scanned by CamScanner

$$\text{Transformation efficiency} = \frac{55 \times 300 \times 10^{6}}{10 \times 1 \times 50}$$

$$= 3.3 \times 10^{7} \text{ cfu}/\mu\text{g DN}$$

>Lig

Scanned by CamScanner

AmilCP : 2 µL + 2 µL dH_2O : 50 ng/µL

RFP : 1 µL + 3 µL dH_2O : 100 ng/µL

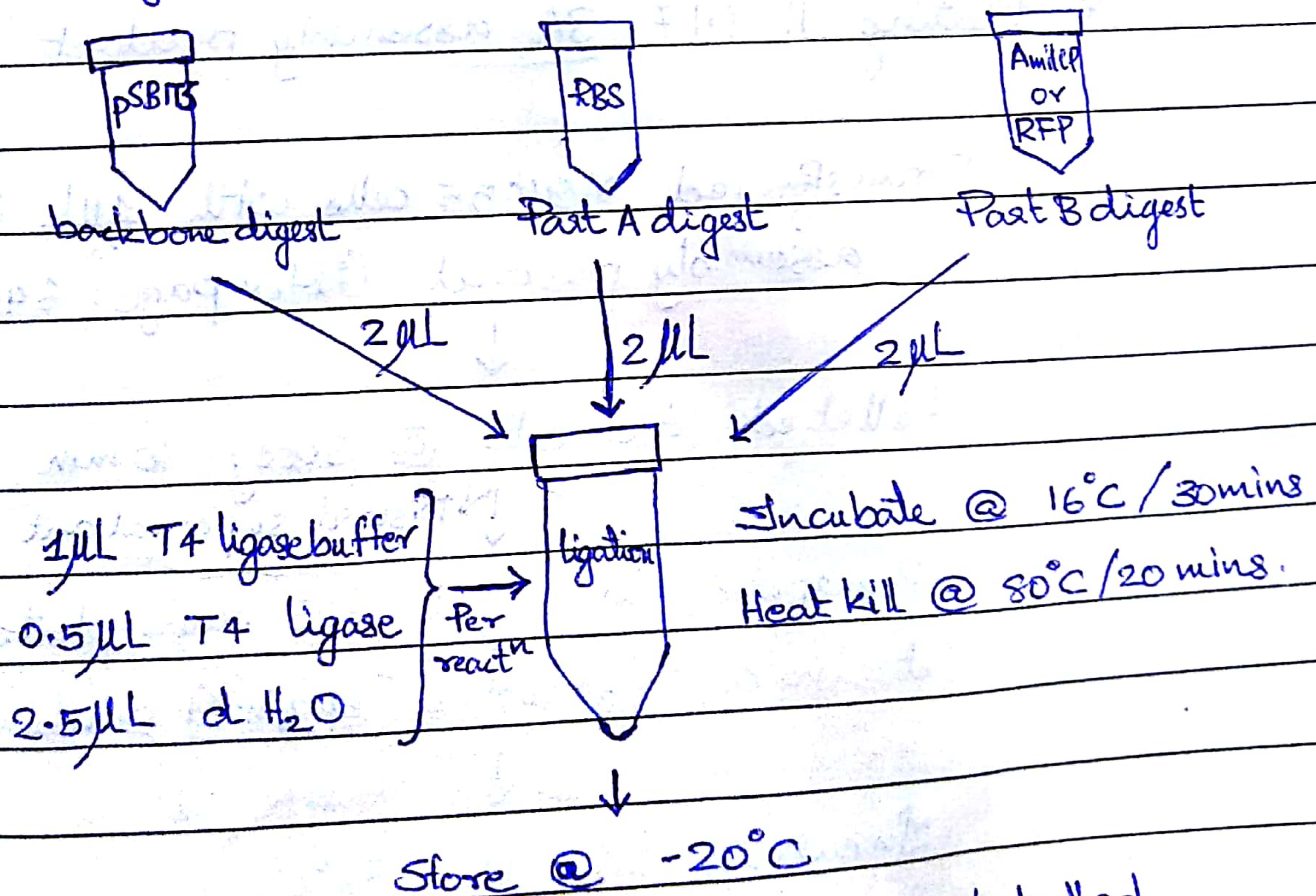
↓

Digested @ 37°C in water bath

↓

Heat kill @ 80°C in thermomixer.

> Ligation reaction



↓

Store @ -20°C

* All digested and ligated products are labelled along with the enzymes used and date (11.9.17) and stored in biobricks box in -20°C.

Scanned by CamScanner

* Plating 2.8.17 3A assembly product

Transformed MG1655 with 3A product
see page: 21 for protocol.
↓
Pelletted the cells @ 3000g 10 mins.
↓ → Discard supernatant
Resuspend the pellet in 50μL SOB
↓
Plate 25μL in 33μg/mL Kanamycin (Magenta)
plate and rest in 50μg/mL " plate
↓ Incubate @ 37°C O/N

12. Sept. 2017

Count the number of colonies
> No colonies found in selection plates

* Plating 11.9.17 3A assembly product

Transformed MG1655 cells with 1μL 3A
assembly product (Refer page: 64)
↓
Pelleted the cells @ 3000g 10 min
↓ → Discard supernatant
Resuspend in 25μL SOB and plate
it in a tetracyclin (15μg/mL) selection plate
↓
Incubate O/N @ 37°C

13th Sept. 2017

Pick

14th Sept

* Prep

Buff

Stock sol

For 20

NaCl :

Tris HC

Scanned by CamScanner

13th Sept '17

white colonies in RBS+AmilCP = 235

Blue colonies in RBS + AmilCP = 20

colonies in RBS + RFP = 296

Picked 3 colony each from each category

RBS + RFP plate : colony 1, 2, 3

RBS + Amil CP Blue : Colony 4, 5, 6

RBS + Amil CP white : Colony 1, 2, 3

↓

Inoculated in 2 mL LB-tetracyclin

↓

Incubated @ 37°C 150 rpm overnight
(220 rpm is optimum, Incubator not available)

14th Sept

* Preparation of buffer 2.1

Buffer 2.1 10X = 500 mM NaCl
100 mM Tris HCl
100 mM $MgCl_2$
1000 μg/mL BSA

Stock solutions : 2M NaCl
2M Tris HCl
1 M $MgCl_2$

For 20 mL 10X buffer :

NaCl : $2 \times 10^3 \times x = 500 \times 20$

$x = 5$ ml

Scanned by CamScanner

$MgCl_2$: $1 \times 10^3 \times x = 100 \times 20$

$x = 2$ mL

↓

Adjust the pH to 7.9

↓

Filter sterilize and aliquot 1 mL in 1.5 mL sterile eppis

* Preparing glycerol stock for the 3×3 Inoculated cultures

Take 100 µL of bacterial culture

↓

Add 100 µL 80% glycerol to it

↓

Label with construct, colony type, backbone and date, flash freeze and store @ -80°C

* Miniprep for plasmid extraction

Inoculum 1, 4 of RBS+AmilCP and Inoculum 1 of RBS+ RFP was chosen for miniprep

↓

Followed Quiagen miniprep protocol (Refer page: 37)

↓

Total culture volume per construct = 1 mL

Total P1 used per construct = 250 µL

↓

Eluted using EB buffer 50 µL

Stored @ -20°C with labels

15th Sept-2017

16th Sept-20

Scanned by CamScanner

14-Sept-2017
15

* Digestion gel running

• Single digest mastermix.

NEB buffer 3.1 : 5µL
EcoRI : 0.5µL
d H_2O : 19.5µL

Took 4µL DNA with 4µL M.mix

Incubated @ 37°C for 30mins

Heat inactivated @ 80°C

-Sept-2017

* 3A assembly : Promoter + Chromophore + pSB1A3

Concentration of chromophore DNA

Part	conc. ng/µL	260/280
RFP	111.1	1.73
W.AmilCP	113.0	1.74
B.AmilCP	121.0	1.80

Digestions were done using the usual procedure.
(see page: 66)

Volume of constructs used are as follows

pSB1A3	8µL	: 25ng/µL	(E.P digest)
pLeft :	4µL	: 25 ng/µL	(E.S digest)
pL :	4µL	: 25ng/µL	
G13	4µL	: 25 ng/µL	

pL pLeft : 4 μL : 25 ng/μL } (E.S digest)
p(CAT) : 2 μL + 2μL dH_2O : 47.8 ng/μL }

RFP, W.AmilCP, B. Amil CP : 1μL + 3μL dH_2O : 121.0 ng/μL (X.P digest)
(1.3 μL + 2.7 for 5 rctns) AmilCP.Blue

↓

Digest @ 37°C in waterbath 30 min

Heat kill @ 80°C in thermomixer. 20 min

* 2A digestion for part submission.

pSB1C3	4 μL	(E.P digestion)
pLeft	1μL + 3μL dH_2O	
pL	1μL + 3μL dH_2O	
G13	1μL + 3μL dH_2O	
pL pLeft	1μL + 3 μL dH_2O	

Digested using backbone digestion mix @ 37°C for 30 min.

Heat kill @ 80°C for 20 min.

* Ligation mastermix - 13 reactions

T4 ligase buffer : 13 μL
T4 ligase : 6.5 μL
MilliQ H_2O : 6.5 μL

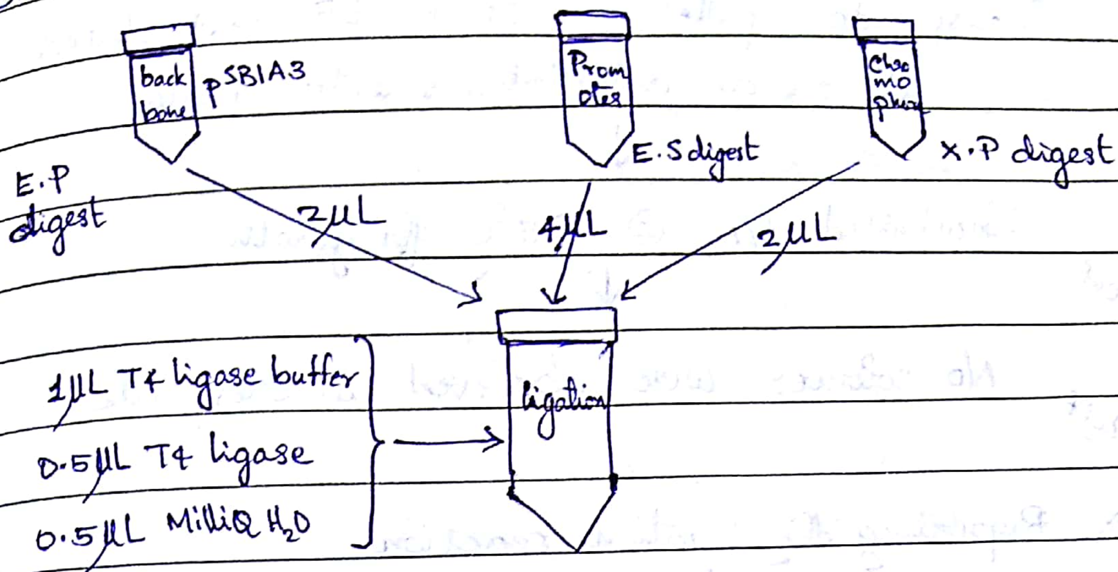
For 3A ligation : 2μL Mmix + 4μL promoter + 2μL chromophore + 2 μL backbone

For 2A ligation : 2μL Mmix + 4μL promoter + 2μL backbone + 2 μL milliQ H_2O

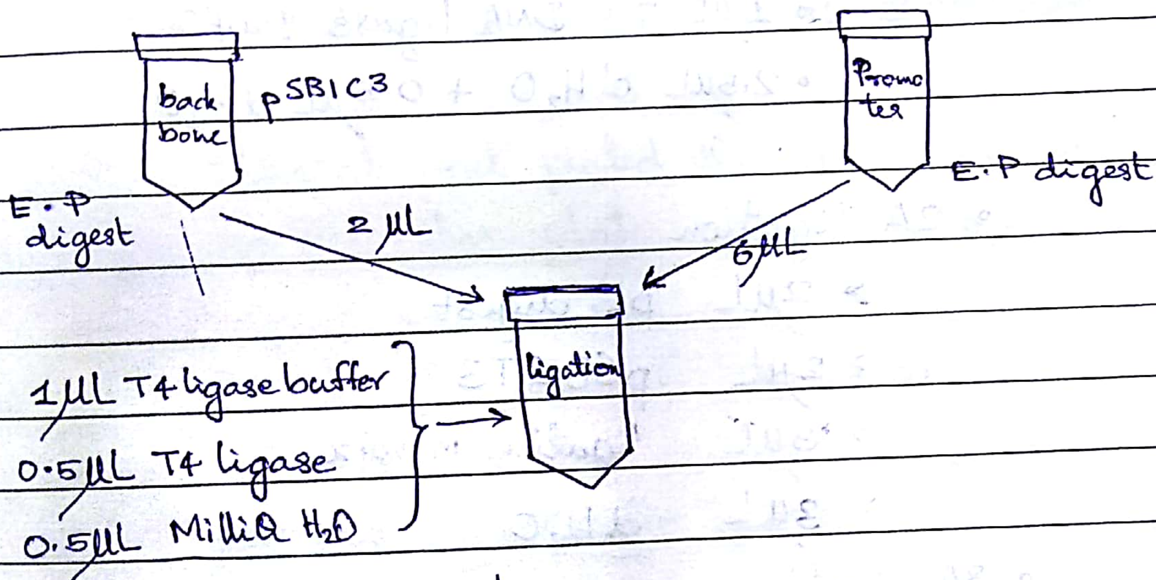
• 3A - as
E.P digest
1μL
0.5μL
0.5μL
• 2A -
E.P dig
1μL
0.5
0.5
17th Sept-2017
*

Scanned by CamScanner

• 3A - assembly :-



• 2A - assembly.



↓

ligations were done overnight (10 Hrs) @ 16°C

17th Sept-2017

* Transforming and plating ligation products

MG1655 cells were transformed with 1µL ligation product (Refer page: 75-77)
(Refer page: 64 for transformation protocol)

↓

Pelleted the cells @ 3000 g 10 minutes

Scanned by CamScanner

↓ Discard supernatant

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

↓

Incubated O/N @ 37°C for growth

↓

18th Sept. 2014

No colonies were observed in the plate.

22nd Sept - 2014

* Repeating the ligation reaction

- Ligation Master mix for 2 reactions
  - 1μL T4 DNA ligase
  - 1μL T4 DNA ligase buffer
  - 2.5μL dH_2O + 0.5μL dH_2O

- 2A ligation
  > 2μL pL digest
  > 2μL pSB1T3
  > 3μL ligation M.mix
  > 3μL dH_2O
- 3A ligation
  > 2μL pCAT digest
  > 2μL ~~8 Amil CP digest~~ RFP digest [Amil CP digest over]
  > 2μL pSB1A3 digest
  > 3μL ligation M.mix
  > 1μL dH_2O.

* ligation was done @ 16°C for 30 minutes

↓

Heat kill @ 80°C for 20 minutes

Scanned by CamScanner

* Transforming and plating ligation products

MG1655 comp cells were transformed using 1μL of following constructs

- PL - pSB1C3 (2A, 16th Sept.)
- PL - pSB1T3 (2A, 22nd Sept.)
- pCAT.RFP. pSB1A3 (3A, 22nd sept)
- composite AmilCP - pSB1C3 (Resuspended 5_20D. 2015)
- Composite ts purple - pSB1C3 (Resuspended 5_20B. 2015)

↓

Incubated @ 37°C for 2Hrs @ 220rpm

↓

Pelleted and plated the transformants in respective plates with -ve controls also

↓

Incubated O/N @ 37° for growth

23rd-Sept-2017

↓

Observed the plate for growth

pL - pSB1C3 = 0

pL - pSB1T3 = 0

pCAT.RFP = 0

Amil CP = 1

ts purple = 9

27th-Sept-2017

* Microscopic Imaging of AmilCP cells

Took 10μL of liquid culture of cells

↓

Smeared it on the slide, put a coverslip

Scanned by CamScanner

Observed through microscope and took pictures

Images were not clear, and chromophore not visible.

Heat fixing was done to observe the cells in non-motile phase

Observed under 100x magnification

Images were taken using a mobile camera

The cells were seen as greenish cells.

* Gel running for part size confirmation

1% Agarose - EtBr gel was casted

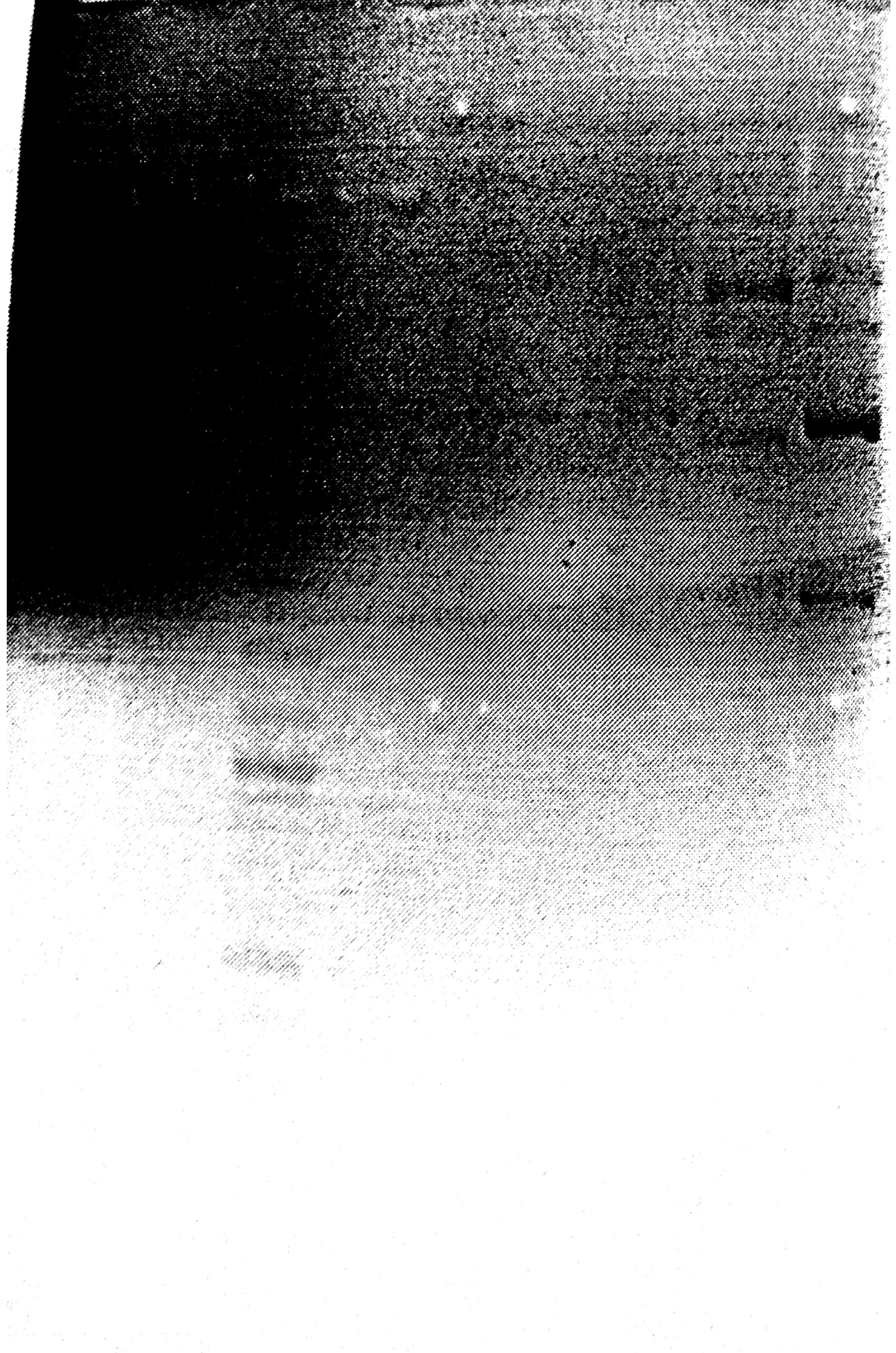
DNA was loaded as shown below

ladder	W·AmilCP	B·AmilCP	RFP	W·AmilCP	B·AmilCP

(W·AmilCP, B·AmilCP, RFP: EcoRI digested)

Bands were observed slightly above 3Kb for both W and B amilCP but W.AmilCP was missing in digested product. B-AmilCP without digestion had many bands ranging from 5Kb to 10 Kb. New miniprep might be required for a pure product extraction

Scanned by CamScanner



Scanned by CamScanner

3rd Oct-20

- 3A - assembly with pCAT promoter : Troubleshoot and G13 promoter from g-block

Following constructs are to be assembled through 3A

> p(CAT) + RBS.AmilCP + pSB1A3
  - using buffer 2.1 .
  - using buffer 2 + BSA .
  - using heat inactivated 2.1 X

> G13 + RBS. AmilCP + pSB1A3 .

Conc. of g-block : 1.65 fmoles/ng

Conc. of plasmids : —

pSB1A3 : 2157 bp

G13-gblock : 980 bp

Use 4µL pSB1A3 : 100ng

Use 2µL G13+ 2µL dH_2O : 50ng ~~x2~~

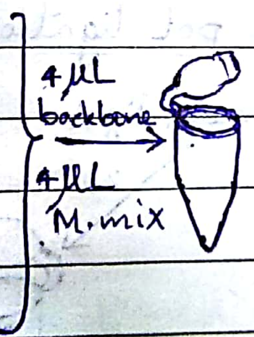
Since 2 step use 4µL G13 : 100ng

Use 2µL B.AmilCP+ 2µL dH_2O : 100ng.

Use buffer 2 with BSA.

M. mix for backbone pSB1A3 ; pSB1A3

- 2µL NEB buffer 3.1
- 0.2µL EcoRI
- 0.2µL PstI
- 0.2µL DpnI
- 7.4µL dH_2O



Incubate 37°/30min

Heat kill @ 80°C/20min

M. mix for Promoter : Step 1

- Buffer 2 + BSA
  - 2µL NEBbuffer 2.
  - 0.2µL Spe I

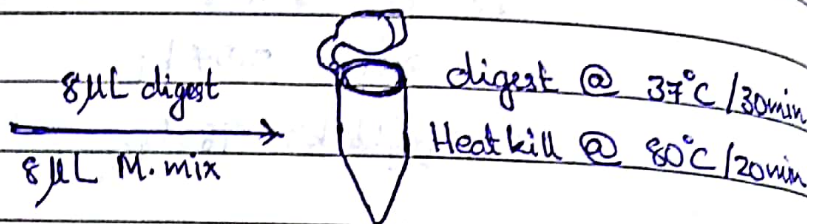
- Buffer 2.1
  - 2µL NEB buffer 2.1
  - 0.2µL SpeI

- 0.2 µL BSA
- 7.6 µL dH_2O

- 7.8 µL dH_2O

Step 2: EcoRI in buffer 3.1

~~4 µL buffer 2.1~~	4 µL buffer 2.1
~~4 µL buffer converter~~	4 µL buffer converter
~~24.8 µL buffer dH_2O~~	31 µL dH_2O
	1 µL EcoRI



M.mix for chromophore digestion

- 4 µL NEB buffer 3.1
- 0.4 µL XbaI
- 0.4 µL PstI
- 15.2 µL dH_2O

4 µL DNA → 4 µL M mix

Incubate @ 37°C /30

Heat kill @ 80°C /20

> Single pot ligation

Promoter digest 2 µL, Chromophor 2 µL, Backbone 2 µL

4 µL per rctn ←

- 1 µL T4 ligase b
- 0.5 µL T4 DNA li
- 2.5 µL dH_2O

Incubate @ 16°C for 30mins

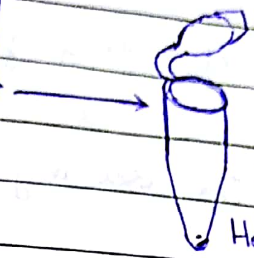
Heat kill @ 80°C for 30 mins

4-10-2017

* Promoter p(CCAT) [Part A] digestion using buffer 3.1

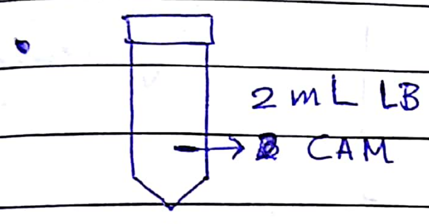
Digest promoter with EcoRI and SpeI using buffer 3.1 as ~~below~~ below

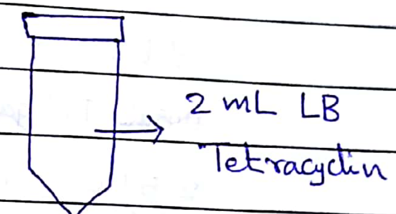
1 µL buffer 2.1
0.2 µL EcoRI
0.2 µL SpeI
3.6 µL dH_2O
5 µL pCAT



Incubate @ 37°C O/N
Heat kill @ 80°C 20 minutes

* Inoculation for mini-prep p(CAT) and RBS.AmilCP (blue)





Picked p(CAT) colony

Inoculate 100µL B.AmilCP colony 6

↓

Incubate O/N @ 37°C 150 rpm

* Transformation of 3A-products

MG1655 cells were transformed with the 3A products from 3-10-17
(For transformation protocol refer page: 64)

↓

Pelleted the cells @ 3000g 10 minutes
↓ Discard supernatant

Scanned by CamScanner

↓

Resuspended the pellet in 25μL LB and plated on 100μg/mL Ampicillin selection plate

↓

Incubated O/N @ 37°C for growth

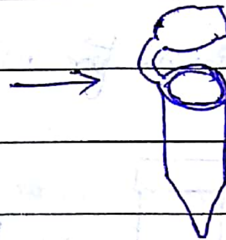
↓

No colony growth seen on plate.

6th-Oct-2017

* 3A-assembly revised : pCAT. AmilCP

2μL pSB1A3 digest
2μL pCAT digest
2μL B.AmilCP digest
1μL T4 DNA ligase
0.5μL T4 ligase buffer
2.5μL d H_2O

→ 

ligate @ 16°C for 1 hour
Heat kill @ 80°C 30 minutes

* Transforming 3A-product

1μL of following products were used for transformation (Refer page : 64 for protocol)

- p(CAT) + RBS.AmilCP + pSB1A3; not heat inactivated
- p(CAT) + RBS.AmilCP + pSB1A3; after heat inactivation

↓

250μL SOC was added for 2 Hr incubation @ 37°C @ 150 rpm

↓

Pellet down the cells @ 3000g for 10 minutes

Scanned by CamScanner

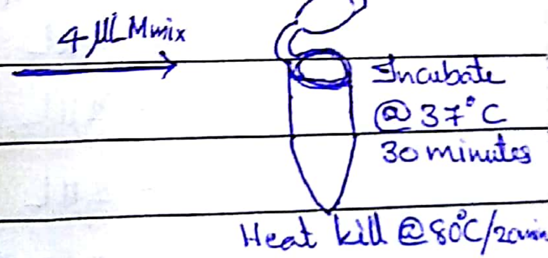
... supernatant
Resuspend the pellet in 25 µL SOC and plate on 100µg/mL Ampicillin LA-plate
↓
Incubate O/N @ 37°C for growth.
↓
colonies : efficiency plate = 10
colonies : pCAT.AmilCP (NHI) = 0
Colonies : pCAT.AmilCP = 0

$$\text{Efficiency} = \frac{\#\text{colonies} \times \text{vol plated} \times 10^6}{\text{Vol. culture} \times \text{conc. DNA} \times \text{vol DNA}}$$

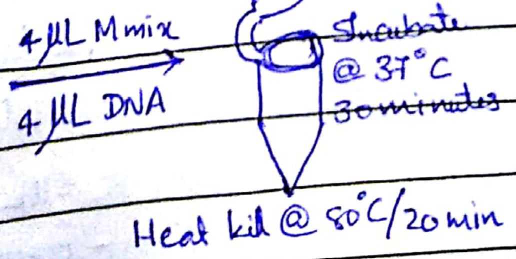
$$= \frac{10 \times 300 \times 10^6}{300 \times 10 \times 1}$$

$$= 10^6 \text{ cfu}/\mu g \text{ DNA}$$

* 2A-assembly strategy
- G-block digestion master mix
  - 0.2 µL EcoRI
  - 0.2 µL Pst I
  - 2 µL buffer 3.1
  - 7.6 µL dH_2O



- Backbone digestion mastermix
  - 0.2 µL EcoRI
  - 0.2 µL Pst I
  - 0.2 µL DPnI
  - 2 µL buffer 3.1
  - 7.4 µL dH_2O



Scanned by CamScanner

Length of g-blocks

GI13 : 980 bases pairs
PL : 864 bases pairs
PLeft : 850 bases pairs
PL-pLeft : 939 bases pairs

pSB1C3 = 2070 bases pairs

Insert : Back bone = 3:1 molarity
since molarity ∝ $\frac{1}{\text{molar mass}}$ and insert has half the molar mass of backbone

for 4µL 25 ng/µL back bone, take 6µL 25ng/µL

ie is ~~[crossed out]~~ $\frac{25\times4}{2070} \times \frac{900\times3}{25} \approx 5-6\mu L$

* 3A : Assembly : pCAT + AmilCP + pSB1A3
  - pCAT digestion : 1 : Spe I
    - 4µL pCAT
    - 1µL buffer 2.0
    - 1µL BSA
    - 0.2µL Spe I
    - 3.8 µL dH_2O

    } Digest @ 37°C ~~30min~~ 1 Hr
    Heat kill @ 80°C /20min

  digestion : 2 : EcoRI
    - 1µL buffer converter
    - 1µL buffer 2.0
    - 1µL BSA
    - 0.2µL EcoRI

    } Digest @ 37°C 1 Hr
    Heat kill @ 80°C / 20 min

-6.8 µ
• Amil C
• pSB
Li

Scanned by CamScanner

- AmilCP digestion
  - 2 µL B. AmilCP
  - 1 µL Buffer 3.1
  - 0.2 µL XbaI
  - 0.2 µL Pst I
  - 6.6 µL d H_2O

  Digest @ 37°C 1 Hr
  Heat kill @ 80°C/20min

- pSB1A3 digestion
  - 4 µL pSB1A3
  - 1 µL Buffer 3.1
  - 0.2 µL EcoRI
  - 0.2 µL Pst I
  - ~~[illegible] µL~~
  - 0.2 µL DpnI
  - 4.4 µL d H_2O

  Digest @ 37°C 1 Hr
  Heat kill @ 80°C/20min

- Ligation of parts
  - ~~[illegible]~~
  - 2.5 µL pSB1A3
  - 2.5 µL AmilCP
  - 2.5 µL pCAT
  - 0.5 µL T4
  - 1 µL T4 ligase buffer

Scanned by CamScanner

Confirmation of 3A products

50µL MG1655 competent cells (30-8-17) were transformed with the following plasmids
- pCAT-AmilCP : 5-10-17
- RFP : Efficiency test kit : 10pg/µL

↓

Transformation was done using standard protocol (Refer page: 64)

↓

Transformed cells were incubated @ 37°C 150rpm in 250 µL SOC.

↓

Pelleted the cells @ 3000 g for 10 mins

↓

Resuspended the pellet in 25µL SOC and plated it on Amp. selection plate

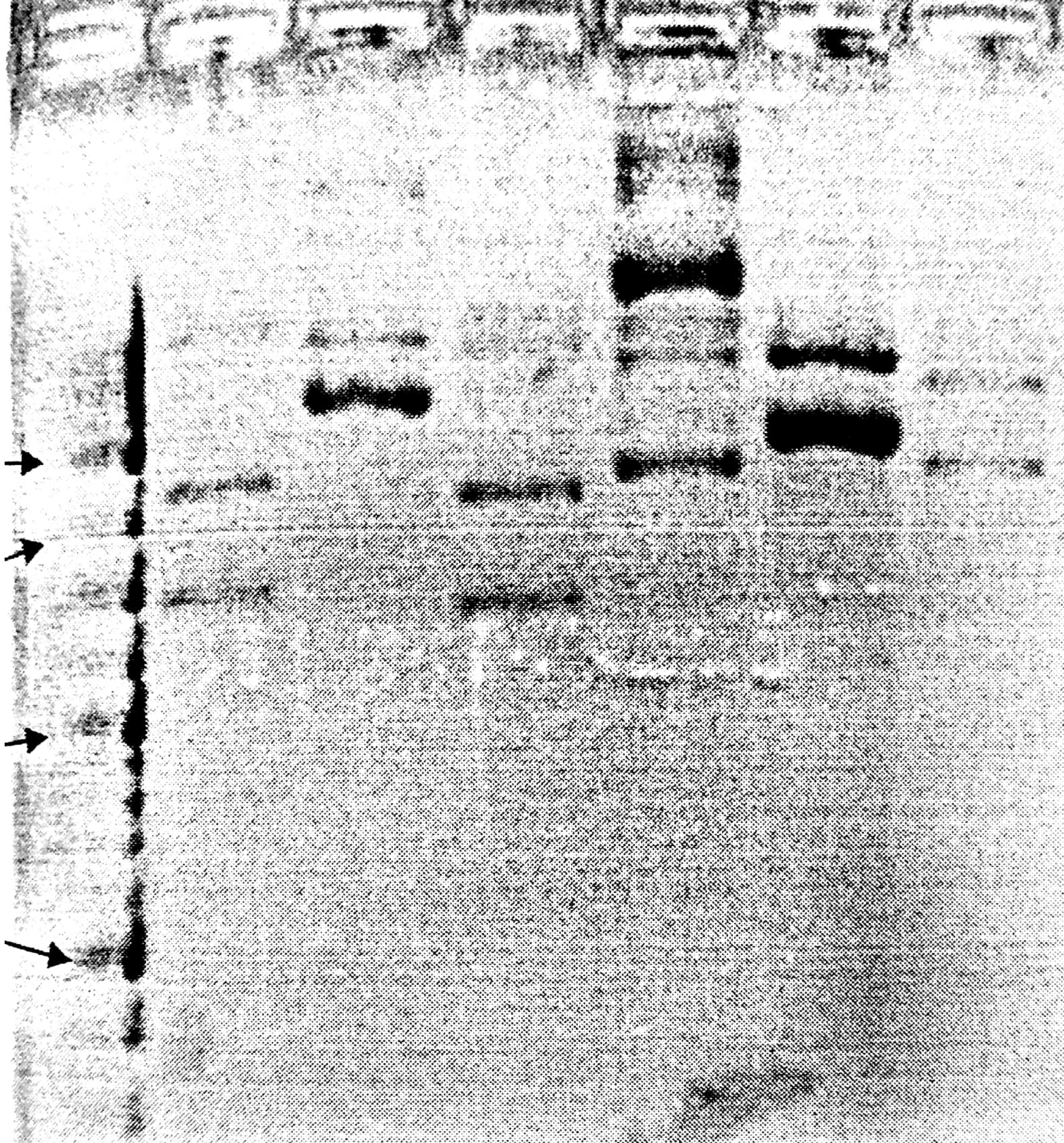
↓

Incubated O/N @ 37°C for growth

* Ligations modifications
  > Since the first ligation mix was spun @ R.T for 1 min, I was worried some non-specific ligation might have happened, so started another ligation in the eppi labelled 3A product 1Hr ligation and 1Hr ligation was aliquoted in eppis labelled as 1Hr S (Spun) 3A and 1Hr NS (Not spun) 3A.

3Kb
2Kb
1Kb
0.5Kb

Scanned by CamScanner



Scanned by CamScanner

Prepar...

Samples were loaded as follows.

2 log DNA ladder	Sender	Receiver	PCAT	ACP 14-9	A.CP 5-10	cj blue
2 µL	5 µL + 1 µL 6xLB	5 µL + 1 µL 6xLB	5 µL + 1 µL 6xLB	5 µL + 1 µL 6xLB	5 µL + 1 µL 6xLB	5 µL + 1 µL 6x LB

Ran the gel @ 80°V for 1 Hour

Imaged the gel using life technologies gel imager

* Nanodrop for pCAT and B. AmilCP

Construct	Conc. (ng/µL)	260/280
pCAT	13.0	1.92
B. AmilCP	109.2	1.84

Oct 2017

Transforming 3A-products

Transformed MG1655 comp cells with 1 µL 3A-product

Added 250 µL SOC and incubated @ 37°C 150 rpm for 8 Hours

Scanned by CamScanner

8th-Oct-20

* 2A-assembly.

- Backbone digestion
  - 0.2 μL EcoRI
  - 0.2 μL Pst I
  - 0.2 μL DPnI
  - 2 μL NEB buffer 3.1
  - 7.4 μL d H_2O
  - 10 μL pSB1C3

  } Incubate @ 37°C/4Hrs
  Heat kill @ 80°C/20 mins

- Insert digestion
  - 0.2 μL EcoRI
  - 0.2 μL Pst I
  - 1 μL NEB buffer 3.1
  - 2.6 μL d H_2O
  - 6 μL G13/PL

  } Incubate @ 37°C/4Hrs
  Heat kill @ 80°C/20 mins

> Ligation of products
  - 2 μL insert digest
  - 2 μL pSB1C3
  - 0.5 μL T4 DNA ligase
  - 1 μL T4 buffer
  - 4.5 μL d H_2O

  } ligate @ 16°C/16 Hrs

9th-Oct-2017

Transformation of 2A product

Transformed 50 μL MG1655 with 1 μL 2A-product
(Refer page: 64 for protocol)
↓
Added 250 μL SOC and incubated @ 37°C
150 rpm for 2 hours

Pelletted d
Resuspend
plated o
Incubat
No co

10-10-17 * Inoculat
Picke
Inc

11-Oct-2017 * Minip
Pelle
Resu
Add

Accidentally spun @ 6800 rcf instead of 17900 rcf } Add
Tra
C

Scanned by CamScanner

Pelletted the cells @ 3000g for 10 minutes.
↓ ↳ Discard supernatant
Resuspended the pellet in 25 µL SOC and plated on chloramphenicol selection plate
↓
Incubated overnight @ 37°C
↓
No colonies were observed in the plate.

10.-17
* Inoculation for miniprep : tspurple, AmilCP, pCAT
Picked one colony each into SOB - Cam
↓
Incubated O/N @ 37°C 150 rpm

-ct. 2017
* Miniprep plasmid isolation
Pelletted 1 mL culture @ 6800 rcf for 3 mins
↓ ↳ Discard supernatant
Resuspended the pellet in 250 µL P1
↓
Added 250 µL P2, mixed by inverting waited for 3 minutes
↓
Added 350 µL N3 buffer, mixed well and centrifuged @ 6800 rcf 10 minutes
↓
Transfered 800 µL supernatant to the column.
↓
Centrifuged @ 6800 rcf for 60 seconds

-ntally
@
-rcf
-d of
rcf

Scanned by CamScanner

Had to spin @ 17900 rcf accidentally used excort

Added 500 µL PB buffer and centrifuged @ 6800 rcf for 60 seconds
↓ Discard flow through
Added 750 µL PE buffer and centrifuged @ 6800 rcf for 60 seconds
↓ Discard flow through
Spun the column again @ 6800 rcf 60 seconds
↓
Added 50 µL EB, Incubated @ room temperature for 5 minutes
↓
Centrifuge @ 17900 rcf, after transferring the column to a fresh labelled eppi. 60 sec.
↓
Store it @ -20°C indefinitely.

* Inoculation for miniprep
2A assembly product pLeft - pSB1C3 was inoculated in 2 mL LB-cam
↓
Incubated O/N @ 37°C @ 150 rpm.

* Primary inoculation for competent cells
Took 2 mL SOB in a 15 mL falcon
↓
Inoculated with 100 µL MG1655 glycerol stock
↓
Incubated O/N @ 37°C @ 150 rpm

Scanned by CamScanner

Allow it to dry on a glass slide

↓

Add 2 μL mountant [20 mM Tris pH:8, 0.5% N-propyl gallate, 90% glycerol]

* $CaCl_2$ Method storage V/s CCMB80 storage

- $CaCl_2$
  - 80μL 80% glycerol in 480μL solution

$$\text{Glycerol \%} = \frac{80 \times 80}{480} = 13.33$$

- CCMB80
  - Create cell suspension in CCMB80

Glycerol % = 10%

16th Oct-2017

* Cell fixation protocol

Pellet 200μL cells @ 13000 rpm, 25°C

↓

i) Resuspend in 200μL 1x pBS
ii) Pellet @ 13000 rpm, 3 min @ 25°C
} 2x

↓

Resuspend in 100μL 4% PFA

↓

Keep @ Room temperature for 30 mins.

↓

2x PBS washes to remove PFA

↓

Resuspend in 100 μL d H_2O.

Scanned by CamScanner

↓

Place 5μL on a slide

↓

Smear it and air dry

↓

Add 5μL mountant

↓

Put a cover slip and seal with nail polish.

* Competent cell preparation

Inoculated 100 mL SOB with 100μL MG1655 glycerol stock.

↓

Incubated @ ~~18~~ 18°C, 200 rpm for 7 Hrs

↓ OD = 0.339

Aliquoted 45 mL in 2 50 mL falcon

↓

Followed the ultra competent cell preparation protocol (Refer page: 58)

↓

Measured OD of 50μL cells + 950μL SOB

OD = 0.360

OD of cell suspension = OD × 20 = 7.2

↓

Dilute the cell suspension 1 : 5 (8 mL)

Final OD = $\frac{7.2}{6}$ = 1.2

Scanned by CamScanner

Aliquoted 260 μL in 600μL eppis.

↓

Incubate on ice for 20 minutes

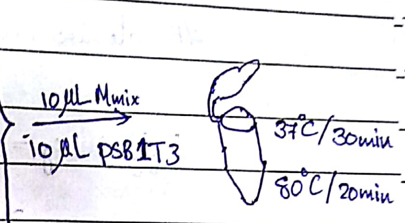
↓

Flash freeze in liquid nitrogen and store @ -80°C.

* 3A- assembly

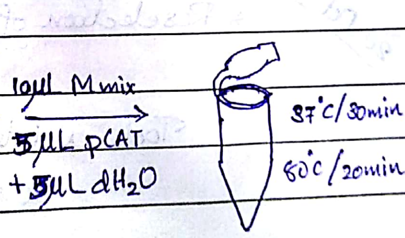
- Backbone pSB1T3
  - 0.2μL EcoRI
  - 0.2μL Pst I
  - 0.2μL Dpn I
  - 2 μL buffer 3.1
  - 7.4μL dH_2O

10μL Mmix → 10μL pSB1T3



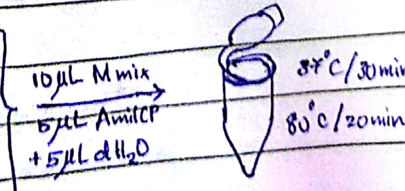
- pCAT digestion
  - 0.2μL EcoRI
  - 0.2μL Spe I
  - 2 μL buffer 2.0
  - 2μL BSA
  - 5.6μL dH_2O

10μL Mmix → 5μL pCAT + 5μL dH_2O



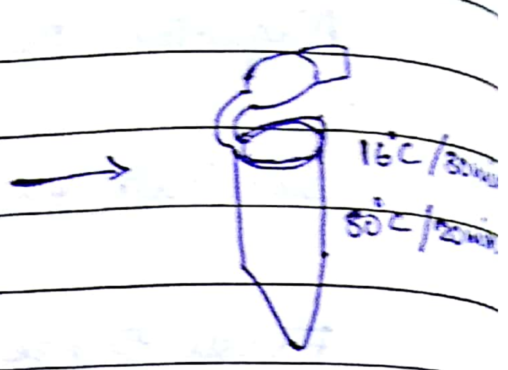
- composite AmilCP digestion
  - 0.2 μL xbaI
  - 0.2μL Pst I
  - 2 μL buffer 3.1
  - 7.6 μL dH_2O

10μL Mmix → ~~5μL~~ AmilCP + 5μL dH_2O



Scanned by CamScanner

- Ligation of digested constructs
  - 0.5 μL T4 DNA ligase
  - 1 μL T4 ligase buffer
  - 2.5 μL pCAT
  - 2.5 μL Amil CP
  - 2.5 μL pSB1T3
  - 1 μL dH_2O



* Efficiency calculation

\# colonies on plate = 61

$$\text{Efficiency} = \frac{\# \times 300 \times 10^6}{100 \times 1 \times 10}$$

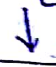
$$= 61 \times 3 \times 10^5$$

$$= 1.83 \times 10^7 \text{ cfu}/\mu g \text{ of DNA}$$

20-Oct-2017

* Reselection of pCAT-AmilCP colonies

Took a tetracyclin plate and divided it into four quadrants



Streaked 4 different colonies into the four quadrants

Incubated O/N @ 37°C 220rpm

Scanned by CamScanner

* Transformation of G-13_pSB1C3

Transformed 50µL MG1655 cells with 1µL G-13_pSB1C3 2A product (8th oct) (Protocol : Refer page : 64)

Added 250µL SOC, Incubated @ 37°C 220rpm for 5 hours for growth

Pelleted @ 3000g for 10minutes in minirotor

Resuspended the pellet in 25µL SOC and plated it on a chloramphenicol selection plate

Incubated O/N @ 37°C for growth

Count the number of colonies

# of colonies = 47

9-Oct-2015

* Preparation of microscopy slides

Took 300µL O/N inoculated culture

Pelleted down 13000 rpm for 3minutes

Give 1X PBS washes : 200µL 2X

~~Add 200µL~~ Pellet @ 13000rpm for 3 mins

Scanned by CamScanner

↓

~~Resuspend~~ Pellet the cells @ 13000 rpm

↓

Give 1x PBS buffer washes 2x

↓

Pellet @ 13000 rpm for 3 mins.

↓

Resuspend in 1 mL milliQ H_2O

↓

Smear 5μL on a slide and air dry it

↓

Add 5μL mountant media, put coverslip and seal with nail polish.

Oct-2017

* Efficiency test transformations

- Transformed cells with 1μL of 10 pg/μL, 50 pg/μL and 100 pg/μL efficiency test constructs

↓

Plated the pellet after 2Hr 37°C incubation on CAM plate and incubated O/N @ 37°C

2A

* ~~2A~~

* Lig

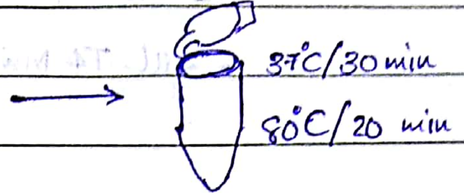
~~2A~~

- 3A

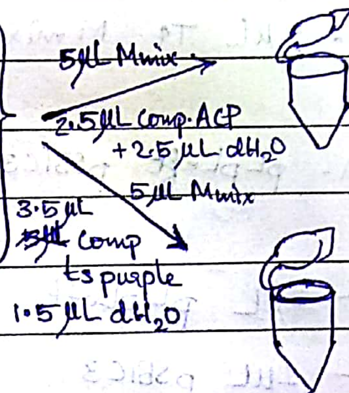
In our box
16th Oct

* ~~3A~~ 2A digestions

- E&P digestion ~~with~~ pCAT. AmilCP. pSB1T3
  - 0.2 µL EcoRI
  - 0.2 µL Pst I
  - 2 µL buffer 3.1
  - 7.6 µL dH_2O
  - ~~10~~ 2.5 µL pCAT. AmilCP + 7.5 dH_2O miniprep 22nd Oct



- E&X digestion comp. AmilCP, comp. ts purple (15.10.17)
  - 0.2 µL EcoRI
  - 0.2 µL XbaI
  - 2 µL buffer 3.1
  - 7.6 µL dH_2O



* Ligations

~~3A ligations~~ * Master mix for 1x reactions (10 µL)

- 1 µL T4 DNA ligase buf
- 0.5 µL T4 ligase
- 2.5 µL dH_2O

} Split 2 µL, 2µL for 5µL ligation

- 3A ligations
  - 1 µL G13 [E&S] <22.10.17> – 1 µL pL-pLeft [E&S]
  - 1 µL comp ts purple [X&P] – 1 µL comp AmilCP [X&P]
  - 1 µL pSB1T3 [E&P] – 1 µL pSB1T3 [E&P]
  - 2 µL Mmix – 2 µL Mmix

In our box 16th Oct

ligations to do

16°C / 2-6 Hrs

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- 2A ligations

- 2µL G13 [E&S] 22.10.17
- 1µL comp ts purple [E&X]
- 2µL T4 Mmix

- 2µL pL-pLeft [E&S] 22.10
- 1µL comp. AmilCP [E&X]
- 2µL T4 Mmix

ligations

16°C / 2-6 Hrs

- 2A : pSB1T3 to pSB1C3 conversion
  - 2µL pCAT.AmilCP [E&P]
  - 1µL pSB1C3 [E&P]
  - 2µL T4 M mix

- 2A- pLpLeft pSB1C3, pLeft pSB1C3

  - 2µL pLpLeft
  - 1µL pSB1C3
  - 2µL T4 Mmix

  - 2µL pLeft
  - 1µL pSB1C3
  - 2µL T4 Mmix

- 3A ligation pCAT, ts purple, pSB1T3
  - 1µL pCAT
  - 1µL ts purple
  - 1µL pSB1T3
  - 2µL T4 Mmix

24th-Oct-2017

* Transformation of ligated products

50µL MG1655 cells were transformed with 1µL ligated product (Protocol : page 64)
↓
Pelletted the cells @ 3000g for 10 minutes post 5 hours of incubati...

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↓

Incubated the plates @ 37°C for 12 Hrs

↓

Growth is seen in all plates

25th-Oct-2017

* <u>Inoculation for miniprep</u>

Picked colonies from plate and inoculated in 2mL LB with respective antibiotic as shown below.

pCAT-tspurple-pSB1T3	Tetracyclin
pCAT-Amil CP-pSB1C3	Chloramphenicol
pLeft-pSB1C3	"
pL-pLeft-pSB1C3	"
G13-tspurple-pSB1C3	"
pL-pLeft-AmilCP-pSB1C3	"
G13-tspurple-pSB1T3 ①	Tetracyclin
G13-tspurple-pSB1T3 - ②	Tetracyclin

↓

Incubated @ 37°C for 12 Hrs.

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