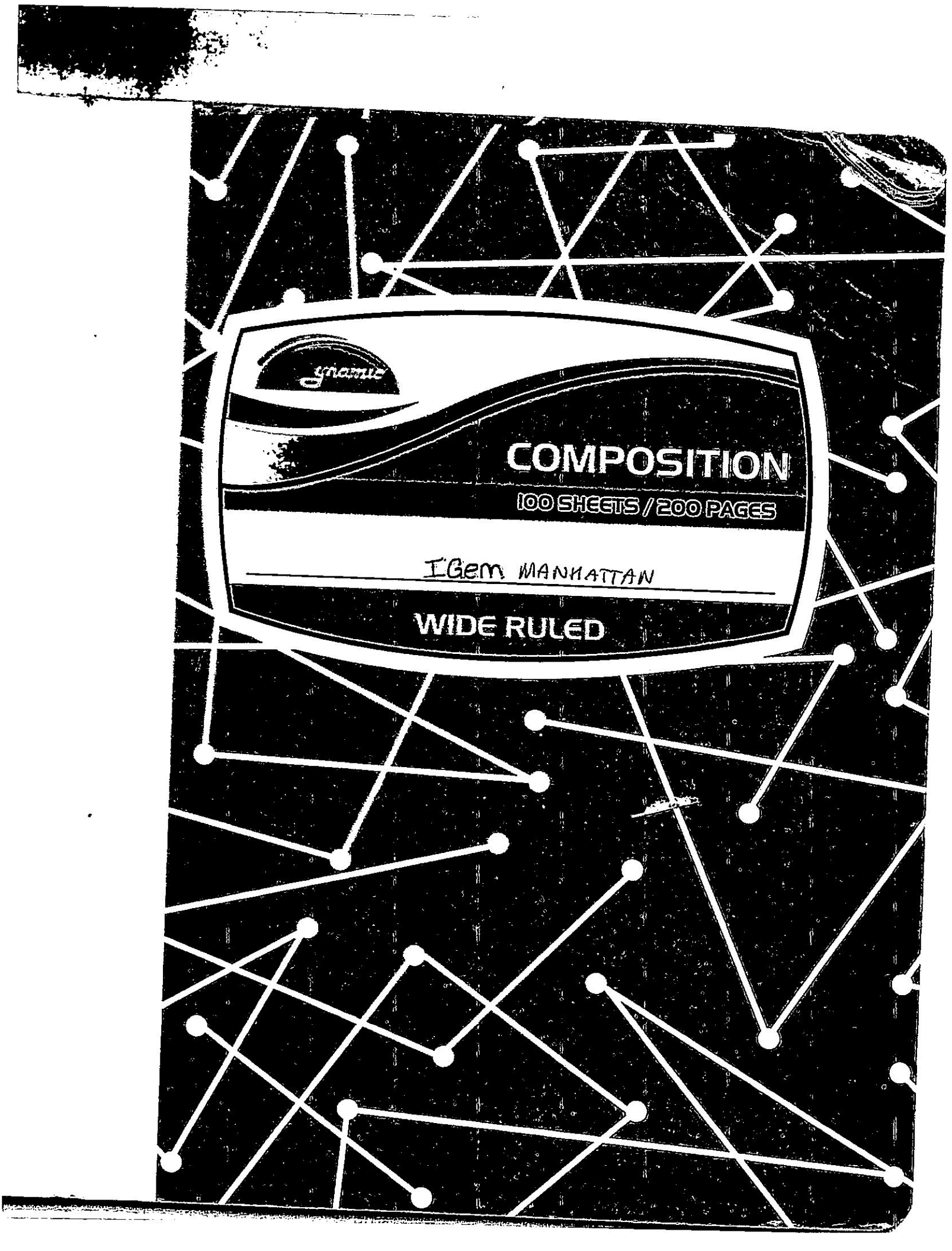

gnome
COMPOSITION
100 SHEETS / 200 PAGES
IGem MANHATTAN
WIDE RULED

~~Procedure for making~~ 5/30/17

1. Streak out frozen glycerol stock of bacterial cells (TOP10, DH5α, etc.) onto an LB plate (no antibiotics since these cells don't have a plasmid in them). Work sterile. Grow plate overnight at 37°C.

5/30/17

1. Autoclave
   - 1 L LB
   - 1 L of 100 mM $CaCl_2$
   - 1 L of 100 mM $MgCl_2$
   - 100 mL of 85 mM $CaCl_2$, 15% glycerol v/v
   - 4 centrifuge bottles + caps
   - Lots of microfuge tubes
2. Chill overnight at 4°C
   - 100 mM $CaCl_2$
   - 100 mM $MgCl_2$
   - 85 mM $CaCl_2$, 15% glycerol v/v
   - Centrifuge rotor
3. Prepare starter culture of cells
   - Select a single colony of E. coli from fresh LB plate and inoculate a 10 mL starter culture of LB.

Grow culture @ 37° C in shaker overnight.

Procedure I for making competant cells 5/3/17

1. Inoculate 1 L of LB media w/ 10 mL starter culture and grow @ 37°C shaker. Measure the OD_{600} every hour, then every 15-20 mins when the OD gets above 0.2
2. When the OD_{600} reaches 0.35-0.4 put the cells on ice immediately. Chill the culture for 20-30 mins, swirling occasionally to ensure even cooling. Place centrifuge bottles on ice at this time
3. Spin #1: Pour 500 mL into ice cold centrifuge bottles. Harvest the cells by centrifugation @ 3000 g (~4000 rpm in the Beckman JA-10 rotor) for 20 ~~15~~ mins. at 4° C
4. Decant the supernatant and gently resuspend each pellet in about 200 mL of ice cold $MgCl_2$. Make sure to prepare a blank bottle as a balance.

5. Spin #2: Harvest the cells by centrifugation at 2000 g (~3000 rpm in the Beckman JA-10 rotor) for ~~15~~ 20 mins. at 4°C
6. Decant the supernatant and resuspend the pellet in about 100 mL of ice cold $CaCl_2$. Keep this suspension on ice for at least 20 mins. Start putting 1.5 mL microfuge tubes on ice if not already chilled.
7. Spin #3: Harvest the cells by centrifugation at 2000 g (~3000 rpm in the Beckman JA-10 rotor) for ~~15~~ 20 mins at 4°C. Chill a 50 mL conical tube on ice.
8. Decant the supernatant and resuspend the pellet in ~25 mL of ice cold 85 mM $CaCl_2$, 15% glycerol. Transfer the suspension to the 50 mL conical tube
9. Spin #4: Harvest the cells by centrifugation at 1000 g (~2100 rpm

in the Beckman GH-3.8 rotor for 15 20 mins at 4°C.

10. Decant the supernatant and resuspend the pellet in 1 mL of ice cold 85 mM $CaCl_2$, 15% glycerol. The final OD_{600} of the suspended cells should be ~ 200-250.

11. Aliquot 50 µL into sterile 1.5 mL microfuge tubes and snap freeze with liquid nitrogen. Store frozen cells in the -80°C freezer.

Testing for efficiency:

06/1/17

Results:

No - no cells

low - no cells

medium - no cells

high - 10 cells (red)

$$\text{efficiency (cfu/ug)} = \left[\frac{\text{colonies on plate (cfu)}}{\text{amount of DNA plated (ng)} \times \frac{1000}{\text{ng/ug}}}\right]$$

(cfu: colony forming units)

high - 100 pg/mL of DNA ⇒ total of 100 pg in transformation

10 pg of DNA was plated

$$10\text{ pg}\left(\frac{1\text{ ng}}{1000\text{ pg}}\right) = .01\text{ ng}$$

$$\text{efficiency} = \left(\frac{10\text{ cfu}}{.01\text{ ng} \times 1000\left(\frac{\text{ng}}{\text{ug}}\right)}\right) = 1.0 \times 10^6$$

ideal efficiency: 1.5×10^8 to 6×10^8

Buffers.

10 mM KOAc per (10 ml of a 1M stock)

Making of SOB (.5 L)

- 2.5 g yeast extract
- 10 g tryptone
- .292 g NaCl — .2929 g
- ~~.18~~ .093 g KCl — .0928
- 1.2 g $MgSO_4$ — 1.22

SOB (1 L) (PH of 7.5)

- 5 g yeast extract
- 20 g tryptone
- 0.584 g NaCl
- 0.186 g KCl
- 2.4 g $MgSO_4$

↳ Autoclaved the SOB

154 g

Procs
1)
2)
3)
4)
5)
6)
7)
8)
9)
10)
11)

06/07/17

Sample A

absorbance = .045
.052
.105
.156

Sample B

absorbance = 0.044
.107
.160
.317

Procedure:

1) Pick single colony of cells from SOB streaked plate.
2) Inoculate 2mL SOB → grow O/N @ RT
3) Inoculate 250mL SOB w/ 1mL of O/N culture.
4) Grow until OD_{600} = 0.3 (RT)
5) Fill an ice bucket half way and pre-chill tubes.
6) Transfer cells to centrifuge bottles (flat bottom)
7) Centrifuge @ 3000 x g @ 4°C for 10 min
   * ↳ (2060 rpm on our centrifuge)
8) Remove liquid and then resuspend cells gently in 80mL of ice cold CCMB80 buffer.
9) Incubate on ice for 20 min.
10) Centrifuge again @ 3000 x g (2060 Rpm) @ 4°C for 10 min.
11) Remove liquid and resuspend pellet in 10mL of ice cold CCMB80.

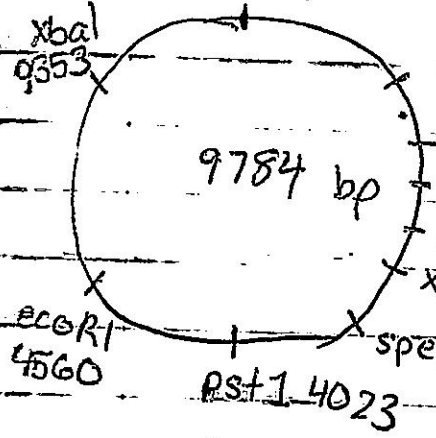
pH of LB = 7.55

Orange cells = .15

Black cells = .317

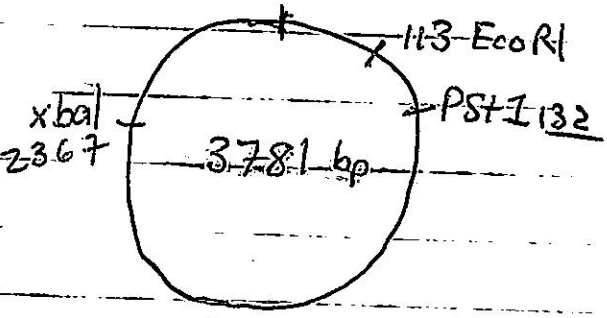
06/13/17

5E8



4560

1B6



No spe1

5E8

- 1 µL DNA
- 1 µL of each enzyme
- 2.5 µL buffer
- enough H_2O to make the entire mixture 25 µL

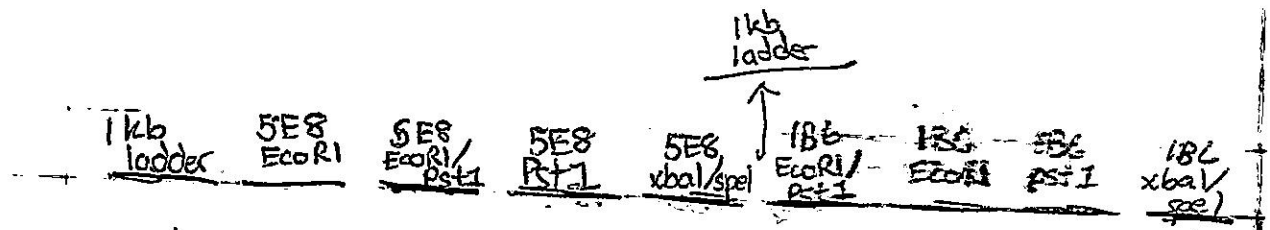
1B6

- 3 µL DNA
- 1 µL each enzyme
- 2.5 µL buffer
- enough water (H_2O) to make entire mixture 25 µL

Four Reactions

1. EcoR1 / Pst1 → Buffer O
2. EcoR1 → EcoR1 buffer
3. Pst1 → Buffer O
4. xbal / Spe1 → tango Buffer.

Time the tubes were put in incubator = 12:50 @ 37°C



Plates 1, 2, 3 → pSB1C3

plasmid backbones: pSB1C3, pSB1A3, pSB1K3, pSB1T3

RFP gene: BBa_j04450 in pSB1C3
↳ Plate 1 → well 230

Plate 1+

RFP: BBa_j04450

Specified Resistance	Plasmid Backbone	Plate	Well
chloramphenicol	pSB1C3	1	23C
ampicillin	pSB1A3	4	6H → single error
kanamycin	pSB1K3	4	6B
tetracycline	pSB1T3	4	5B → single error

Date: 06/20/17

- Used sputter machine to sputter gold onto a glass slide until it was 20.1 nm thick.

- Made two .1 M solutions of $Cl_2Ni \cdot 6H_2O$ (200 mL) and $NaBH_4$ (200 mL)
- Put membrane in DI water and sonicated water.
- Put polycarbonate membrane (7-10 microns) through in the u-tube and then filled each of the sides w/ each of the solutions simultaneously.
- Waited for reaction for 15 min.

Then repeated procedure but w/ anodized aluminum oxide 60 microns thick membrane.
And waited 15 min.

06/22/17

- Made .01 M solution (200 mL) of $MgCl_2 \cdot 6H_2O$, and .01 M solution (200 mL) of $NaBH_4$
- Grew nanowires with .01 M solutions and another membrane w/ .1 M solutions.
- Membrane w/ .01 M solutions ran for 30 min and conductivity was good .24 [ohms]
- Dissolved the polycarbonate membrane (.01 M 30 min) with *methylene chloride, and centrifuged at 4000 rpm for 7 min and discarded methylene chloride and repeated two times
- *Methylene chloride was discarded into waste beaker after centrifuge

06/28/17

Digest with EcoR1 / Pst1

① #2 DNA
② #7 DNA
} (100 ng) in separate tubes (100 ng).

Digest w/ EcoR1 / Pst1

③ pCDF duet plasmid (1100 ng total)

④ linearized pSBIC3 (100 ng total).

Digest w/ EcoR1/Pst1 (#2 DNA / #7 DNA)

1/2

- 10 µL DNA
- 2.5 µL buffer O
- 0.5 µL enzyme 1
- 0.5 µL enzyme 2
- 11.5 µL H_2O

} total of 25 µL in rxn.

last

Digest w/ EcoR1/ Pst1

③ pCDF duet plasmid (1100 ng)

- 2 µL of plasmid
- 0.5 µL of enzyme 1
- 0.5 µL of enzyme 2.
- 12.5 µL of buffer O
- 9.5 µL water

} total of 25 µL in rxn

Digest w/ EcoR1 / pst1

④ linearized pSB1C3 (100 ng total)

- 4 µL of pSB1C3
- 0.5 µL of enzyme 1
- 0.5 µL of enzyme 2 ← Last
- 6.25 µL of buffer 0

} total of 25 µL in rxn

- 13.75 µL H_2O

↳ PstI → not sensitive to heat inactivation
therefore: cleanup kit

General DNA cleanup from enzymatic reactions protocol

1. Adjust the volume of the reaction mixture to 200 µL w/ water, nuclease-free or TE buffer (not included).
2. Add 100 µL of Binding Buffer. Mix thoroughly by pipetting.
3. Add 300 µL of ethanol (96-100%) and mix by pipetting.
4. Transfer the mixture to the DNA Purification Micro Column preassembled w/ a collection tube. Centrifuge the column for 30-60 seconds at 14,000× g. Discard the flow through. Place the DNA Purification Micro Column back into the collection tube.

5. Add 700 µL of Wash Buffer (supplemented w/ ethanol) to the DNA Purification Micro Column and centrifuge for 30-60 seconds at 14,000 × g. Discard the flow-through and place the purification column back into the collection tube.

6. Repeat step 5

7. Centrifuge the empty DNA Purification Micro Column for an additional 1 minute at 14,000 × g to completely remove residual Wash Buffer

Note: This step is essential to avoid residual ethanol in the purified DNA soln. The presence of ethanol in the DNA sample may inhibit downstream enzymatic rxns.

8. Transfer the DNA Purification Micro Column into a clean 1.5 mL microcentrifuge tube

9. Add 10 µL of water to the center of the DNA Purification Micro Column membrane. Centrifuge for 1 minute at 14,000 × g to elute DNA.

10. Discard the purification column and store the purified DNA @ -20°C.

Electrophoresis

PCR
EcoRI/pst1
whole sample.

ladder.
5 µL

7/7/17

- Digest of insert fragments

1) iGEM_2017_2
   iGEM_2017_7
   } Both @ 10 ng/mL
   - we want to digest 200 ng total in a final volume of 25 µL

2) 200 ng × 1 µL / 10 ng = 20 µL of each fragment needed per rxn

3) Buffer (Buffer 2) is 10x concentration:
   25 µL final volume → 25/10 = 2.5 µL needed in rxn

4) 0.5 µL of each enzyme needed → 0.5 µL PstI
   0.5 µL EcoR1-HF
   1.0 µL Total

5) H_2O added to make the final total volume equal to 25 µL

RXN #1	RXN #2
20 µL iGEM_2017_2	20 µL iGEM_2017_7
2.5 µL Buffer 2	2.5 µL Buffer 2
1.5 µL H_2O	1.5 µL H_2O
0.5 µL PstI	0.5 µL PstI
0.5 µL EcoR I-HF	0.5 µL EcoR I-HF

6) Incubate at 37 °C for 60 min.
7) Incubate at 80 °C for 20 min.

PCDF-duet plasmid digest

- digest a total of 1000 ng in a final volume of 20 µL
  - PCDF-duet @ 150 ng/µL

1) $1000\ ng \times \frac{1\ \mu L}{150\ ng} = 6.7\ \mu L$ per rxn
2) Buffer (Buffer 2) is 10x concentration:
   - 20 µL final volume → $20/10 = 2.0\ \mu L$ needed
3) 0.5 µL of each enzyme: 0.5 µL PstI
   0.5 µL EcoR I-HF
   1.0 µL Total
4) H_2O added to make the final volume equal to 20 µL

RXN

6.7 uL pCDF-duet

2.0 uL Buffer 2

10.3 uL H_2O

0.5 uL Pst1

0.5 uL EcoR1-HF

5). Incubate for 60 min @ 37°C

6) Incubate for 20 min @ 80°C

7) Run DNA on gel for electrophoresis separation (45-60 min)

07/11/17.

- Followed Procedure 1 for making competant cells. with BL21 and DH5α

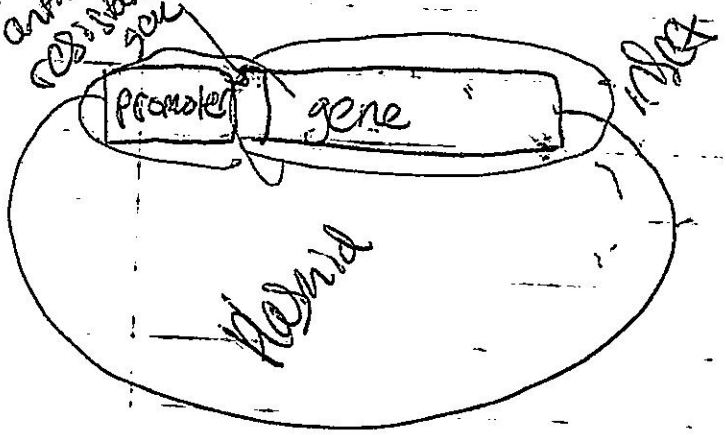
Steps done for:

BL21
1 3 5 7 9
2 4 6 8

DHJα
1 3 5 7 9
2 4 6 8

Microfuge Tubes
BL21 = X DHJα = O

(Marker antibiotic resistance gene) lac operon system



Competent Cell Transformation with Expression Vectors

07/13/17

50 ~~30~~ µL DNA in each

2 ~~3~~ µL from wells.

Ice for 30 min.
In incubator for 45 sec @ 42°C
Ice for 2 min
put in 500 µL LB.
then in incubator (37°) for 1 hr.
then put contents from microfuge tubes into chloramphenicol

1 = BBa_K1316012 Plate 5 - 7E
2 = ~~BBa~~ BBa_K608002 Plate 1 - 30
3 = BBa_I20270 Plate 7 - 21B
4 = BBa_R0040 Plate 7 - 21D
5 = BBa_J364000 Plate 7 - 21F
6 = BBa_J364001 Plate 7 - 21H
7 = BBa_J364002 Plate 7 - 21J
8 = BBa_J364003 Plate 7 - ~~21M~~ 21L
9 = BBa_J364004 Plate 7 - 21N
10 = BBa_J364005 Plate 7 - 21P

Kit

7/18/17

took 6uL from DNA and put it into seperate tubes.

Master → Digest

~~Rxn Super mix~~

25 uL Total

2.5 uL buffer x 6 = 15 uL → 15uL buffer

6 uL DNA x 6 = 36 uL DNA + 1uL PstI + 1uL EcoRI

16.5 uL H_2O x 6 = 99 uL H_2O → 97 uL H_2O

25

114 uL / 6

→ 19uL of master mix in each

loaded

Incubate at 37°C for 1 hour

made agarose gel. (1%)

↳ [50mL TBE with 0.5g agarose DNA.]

"SYBR Safe"

- add red dye to gel (5 uL)

~~Glowing dye~~

Loading Gel (~~Purple~~)

3 mL glycerol

25 ~~mg~~ mg bromophenol

dH_2O to 10 mL

- 5uL of dye in DNA.

adder	D2 1	D2 2	D2 3	D2 4	D2 5	D2 6	D7 1	D7 2	D7 3	D7 4
									D7 5	D7 6

Chosen tube: D7-3

↳ Used 1 uL, 2 uL, 3uL for the transformation procedure from 07/13/17.

- Transferred cells from Spec ~~(Dr. Bryan and Jewett)~~ D2 and B2 ~~into~~ plates into culture tubes 5mL LB and 5uL of spec

7/19/17

↳ DNA extration using GeneJET Plasmid Miniprep Kit.

from D2 and B2
↳ Cut with EcoRI / PstI
↳ RAN gel for 1:20 and 1:100

Dilution: 2.14

7/20/17

↳ Absorbance of LB/Spec w/ BL21 cells/ PCDF-iGem-7#3 spec 1. was .575

~~After 20 min absorbance:~~

Started w/ OD of .75

① ↳ took out 1 mL out of LB/spec w/ BL21 cells PCDF-iGem-7#3 and labeled a microfuge tube (X) negative, put it in iGem box.

② ↳ Meas Put 40 µL of 1M IPTG in flask w/ rest of LB/spec w/ BL21 cells

③ ↳ We let it incubate @ 37°C for 1 hr.

④ Took 1mL from step 3. Spun down cells and put tube in Igem box.
⑤ Repeated steps 3-4 for after 2hrs.

- Used Thermo Scientific Gene Jet Gel extraction kit. to extract DNA

1 - OD_{260} → .037 Å 37 ng/μl
2 - OD_{260} → .045 Å 45 ng/μl

Ligation

Ratio:
1: 40 ng vector
3 58.4 ng insert

1 μL vector DNA
6 μL insert (iGEM_X)
1 μL T4 DNA Ligase Buffer.
1 μL H_2O
1 μL T4 DNA ligase
10 μL total.

RT 30 min → Transform into competent cells

4) Killed the ligase (@ 65°C for 10 min)

~~(1) Isolate DNA from BL21~~

(2)

- Redo [illegible]
- Make 400 µl +Kan of Culture
- Make LB Spec plates
- Auto clave Big Flats
- Make 2L of LB Media
  (split into 4 500 ml Bottles)

07/25/17

- 2 500 mL of LB w/ spec ~~in them~~ and iGem-7 PCDF and 1 1000 mL of LB w/ spec iGem-7 PCDF were incubated until an absorbance of

Gel electrophoresis: w/ PCDF duet E/P

Comparison of cut vs uncut DNA

ladder — PCDF duet E/P — control

- incubated another PCDF duet E/P at 80 °C.

EcoR1/PST1

Weight of DNA gel slice: .05g

- Follow protocol A. DNA extraction from the gel using centrifuge from Thermo Scientific GeneJET GEL Extraction Kit. until step 9.

Procedure:

1. Excise gel slice containing DNA fragment. ~~with~~ Record weight of the gel slice. .05g
2. Add 1:1 volume of Binding Buffer. 100µL
3. Incubate gel mixture @ 50-60° C for 10 min. Vortex gel mixture.

recover one of plasmid

4. Transfer up to 800 µL of gel solution to the GeneJET purification column. Centrifuge for 1 min. Discard flow-through.

5. Add 700 µL of Wash Buffer to the column. Spin for 1 min. Discard flow through.

6. Centrifuge again for 1 min and Discard flow through.

7. Add 15 µL of H_2O ↗ wait 1 min. Centrifuge for 3 min at 8000 rpm and collect in microfuge tube.

8. Repeat w/ 10 µL of H_2O → wait 1 min.

9. Discard the GeneJET purification column and store DNA at -20°C.

4 sets of ×

① Negative
② Control PCDF 40 ng/µL
③ Purified DNA from Gel
④ Heated @ 80°C.

1 uL of Control
1 uL of treated
4 uL of Purified Gel.

Transformation to Competant Cells
Iced for 20 min.
Incubate for 45 sec @ 42°C
Ice for 2 min.
put in 500 uL LB.
Incubate (37°C) for 1 hr.
Then put contents from microfuge tubes onto spec. plates.

wait 1 min

7/26/17

T Ligation w/ insert 2, 8, 9, and a negative control.

1 40 ng/μL vector
58.4 ng/μL insert
1 μL vector DNA
6 μL insert (iGem_7)
1 μL T4 DNA Ligase Buffer
1 μL H_2O
1 μL T4 DNA ligase
Total: 10 μL

⇨ Transformation to competent cells:

Ice for 20 min
Incubate for 45 sec @ 42°C
Ice for 2 min
put in 500 μl of LB
Incubate (37°C) for 1 hour
Then put contents from microfuge tubes onto spec plates.

50mL

7/27/17

↳ STS-PAGE on iGem-7 pCOF w/ IPTG and iGEM-7 Neg-

↳ Put 100 µL of STS-PAGE buffer (blue buffer) in each tube and boil @ 95°C for 30 min.

Making Gold Anode

50 mM _____ and 50 mM

.1 M Sodium borohydride
$NaBH_4$
MW: 37.83 g/mol

$\left(\frac{.1\ mol}{L}\right)(.05\ L) = .005\ mol$

$\left(\frac{37.83\ g}{mol}\right)(.005\ mol) = .189\ g$

.189 g $NaBH_4$

.05 M tetrachloroauric (III) acid trihydrate
$AuCl_4H * 3H_2O$
MW: 393.83 g/mol

$\left(\frac{.05\ mol}{L}\right)(.05\ L) = .0025\ mol$

$\left(393.83\ \frac{g}{mol}\right)(.0025\ mol)$

= .984 g
$HAuCl_4 * 3H_2O$

.005 mM $NaBH_4$

.0189 g $NaBH_4$
50 mL

.0025 mM $HAuCl_4 * 3H_2O$

.0984 g
50 mL

- Set up u-tube w/ _____ membrane
and .01 M ~~and .005 M~~ $NaBH_4$ solution
and .005 M $HAuCl_4 * 3H_2O$

Test

02 DNA

1: ~~.041~~ .036

2: ~~.023~~ .020

3: ~~.024~~ .043

4: ~~.046~~ .040

5: ~~.054~~ .030

6: .015

7: .020

8: .012

9: .032

10: .020

Mastermix
28 mL H2O
30mL buffer 2
1mL RST+ ECoRI
60 mL total

60mL total
30 mL buffer
30 mL
1 mL enzyme
1 mL enzyme
28 mL H2O

10 → 25mL = 250mL → 12

12 x 25 = 300mL total

12 x 20 240

- Extraction DNA from cells from plate 9. using the RapidPURE Plasmid Mini Kit Protocol

1. Prepare Wash Solution by adding 100% ethanol
2. Spin 1.5 mL of overnight culture, discard supernatant.
3. Resuspend cell pellet in 50 µL Pre-Lysis Buffer
4. Add 100 µL Alkaline Lysis Solution, invert to mix.
5. Incubate @ room temp. 3 mins.
6. Add 100 µL Neutralizing Sol'n + incubate 1 min. @ room temp. mixing or vortexing gently several times.
7. Microfuge 2 mins. @ room temp.
8. Transfer supernatant (cleared lysate) to new tube.
9. Add 250 µL Rapid PURE Mini Salt Sol'n to cleared lysate.
10. Transfer mixture to assembled RapidPURE Turbo cartridge.

11. Spin 30 sec. @ ≤14000 x g.
12. Add 350 µL prepared Wash Sol'n
13. Spin 30 sec. @ ≤14000 x g
14. Discard a flow through
15. Spin 2 mins.
16. Insert Turbo Filter into a clean recovery tube
17. Add 100 µL water or TE to filter membrane
18. Incubate 5 mins. @ room temp.
19. Spin 1 min. to collect DNA.

~~Egem 2~~
WT = .94

Egem 7 = .82

- Cells for PCDF_ Igem-7 and PCDF-iGem-2 (W6) were grown in LB/spec @ 30°C and samples were taken every hour.

Making gold nanowires

$HAuCl_4 \cdot 3H_2O$ .005 M (100 mL)

$NaBH_4$

.0189 g $NaBH_4$ in 100 mL H_2O

Igem 9

#8: 0.103

#6: 0.136

#3: 0.098

#1: 0.075

#2: 0.130

#6: 0.097

#7: 0.155

#4: 0.137

#5

Igem 2

~~#3~~: 0.095

iGem gel = left → top
Brian gel = right → bottom

black dot. ✓ 10% SDS

8/2/17

STS - PAGE

- took ~~igem-plate 2 UtPBKA~~, ~~and atubes~~ and iGem-7 samples w/o IPTG and added the blue buffer (100 μL)
- heated for 20 min @ 92°C
- Spin them down.

~~Gelorder~~

Gel order for STS - PAGE (Expression)

ladder	iGem_7	iGem_7 w/ IPTG 1 hr	iGem_7 w/ IPTG 2 hr	iGem_7 w/ IPTG 3 hr	iGem_7 w/ IPTG final	iGem_2 wt	iGem_2 w/ IPTG 1 hr	iGem_2 w/ IPTG 2 hr	iGem 2 w/ IPTG 3 hr	iGem_2 w/ IPTG final

① Western Blot

② Coomassie Stain

100 V
Time: 1:30
Amp: 1.00A
WAT: 300W

ladde

IPTG end hourly samples w/ IPTG

Digest for DNA extractions from plate - 9

Master Mix

2.5 µL buffer × 9 = 22.5 µL →	22.5 µL buff
6 µL DNA × 9 = 54 µL DNA	1 µL PstI
	1 µL EcoRI
16.5 µL H_2O × 9 = 148.5 µL H_2O	146.5 µL H_2O
	171 µL

9 = 19 µL of master mix in each

Gem 2 w/ 3 hr

- Incubate @ 37°C for 1 hr.
- make agarose gel (17.)
  - 50 mL TBE
  - 0.5 g agarose DNA
- add red dye (SUber safe) to gel (5 µL)
- 5 µL of dye in each tube.

Gel electrophoresis (digest)

ladder	iGem-9 #1	iGem-9 #2	iGem-9 #3	iGem-9 #4	iGem-9 #5	iGem-9 #6	iGem-9 #7	iGem-9 #8	iGem 2 #3

08/03/17

Run Samples for Coomassie on 12% gel
- dilute .005 M $HAuCl_4$ by half
- and do @ equal concentrations.
- if it still contamination dilute $NaBH_4$ by half

$NaBH_4$	$HAuCl_4 \cdot 3H_2O$
.005 M.	.005 M.
MW: 37.83 g/mol	MW: 393.83 g/mol.
$\left(\frac{.005\ mol}{L}\right)\left(.1L\right) = .0005\ mol$	$\left(\frac{.005 mol}{L}\right)\left(.1L\right) = .0005$ mol
$\frac{37.83 g}{mol}\left(.0005 mol\right)$	$\frac{393.83 g}{mol}\left(.0005 mol\right)$
$= .0189$.	$= .196$

ladder

Try: $NaBH_4$ .005 M and $HAuCl_4$ .0025 M

List of things to do 08/15/17

1. Running SDS Page for verification of test expression for iGem-7 / iGem-2
2. Same gel for expression #7
3. Pick cells from plate #9, and let grow overnight.
4. Re-do #8, #9.
5. Glycerol stock of #7, #2

SDS PAGE

ladder	iGem-2 Wt	iGem2 w/ IPTG 1hr	iGem 2 Wt 2hr	iGem2 3hr	iGem 2 final	iGem7	iGem7 w/ IPTG 1hr	iGem7 IPTG 2hr	iGem7 IPTG 3hr	iGem7 final

Making of Au nanowires:

$NaBH_4$
.0025 M

.00945 g in 100 mL H_2O

$HAuCl_4 \cdot 3H_2O$
.0025 M

Di

08/16/17

- Isolating DNA from plates heat 9, - Cut 8, Cut ⊖.

Ran out of pre-lysis buffer:
Following has pre-lysis buffer and followed RapidPURE Plasmid Mini kit protocol:
Heat 9: 2-6
Cut 8: 1-6

Used different neutralization solution for heat 9-6

Control Digest: PCDF iGem 2 #3

Digest for DNA extraction from Plates Heat 9, cut 8, cut ⊖

2.5 µL buffer × 19	=	47.5 µL buffer
6 µL DNA × 19	=	114 µL DNA
16.5 µL H_2O × 19	=	313.5 µL H_2O

- Incubate @ 37°C for 1 hour.

47.5 µL buffer
1 µL PstI
1 µL EcoRI
311.5 µL H_2O
361 µL
19
= 19 µL of master mix in each

08/17/17

- Gel electrophoresis for DNA from heat 9, cut 8, cut ⊖ plates

~~005 HA~~ Gel 1

ladder	control	control	cut 8 1	cut 8 1	cut 8 2	cut 8 2	cut 8 3	cut 8 4	cut 8 5	
cut 8 5	cut 8 6	Heat 9 1	Heat 9 2	Heat 9 3						

Gel 2

ladder	control	Heat 9 4	Heat 9 5	Heat 9 6	cut ⊖ 1	cut ⊖ 2	cut ⊖ 3	cut ⊖ 4	cut ⊖ 5	
cut ⊖ 6										

Heat 9 - 4 looks like it worked.

Au nanowires

.005 M $HAuCl_4$ w/ .0025 M $NaBH_4$

200 nm membrane

- 8 and 9

cut 8
5

cut E
5

08/../17

- Ligation of PCDF-Duet naked -

Digests

PCDF - 15 µL total

① 10 µL DNA
1.5 µL Buffer 2
last { 1 µL PstI
1 µL EcoRI
1.5 µL H_2O.

iGem 8

Same

iGem 9 - 25 µL total

20 µL DNA
2.5 µL Buffer 2
1 µL PstI
1 µL EcoRI
.5 µL H_2O

- Incubate for 2hr

iGem 8/PCDF - 35µL total

10 PCDF, 20 iGem 8 } 30 µL DNA
3.5 µL buffer
1µL PstI
1 µL EcoRI

PCDF iGem-#
vector: insert.

Ligation: 1:3

Total = (10 µL)

✓ 1 µL PCDF
✓✓ 7 µL insert iGem-#
✓ 1 µL buffer
✓ 1 µL ligase
last

neg. control

✓ 7 µL H_2O
✓ 1 µL PCDF
✓ 1 µL buffer
✓ 1 µL ligase → last

Reminder

- Run Gel for expressions

Control & total 10uL.

- ✓ - 3.5 mL PCDF
- ✓ - 1 mL buffer
- ✓ - 1 uL ligase
- ✓ - 4.5 H_2O

Ligation for PCDF/i

- ✓ 8 uL of tube w/ PCDF & item 8
- ✓ 1 uL buffer
- ✓ 1 uL ligase.

Digest 09/05/17

PCDF - 20 uL total

10 uL DNA
2 uL Buffer 2
1 uL PstI
1 uL EcoRI
6 uL H_2O

@ 37°C for 1 hr.
then 80°C for 20 min.

agarose Gel

Control - 20uL

6uL DNA
2 uL Buffer 2
1uL PstI
1 uL EcoRI
10 uL H_2O

0.196 A IGEM2

0.102 A IGEM7

- 3 times → 3 tubes 09/21/17

- combine @ step 5

DNA extraction from gel using centrifuge for PCOF/ecoR1/Pst1

Digest of 8 & 9: (total = 15 uL)

10 uL DNA

1.5 uL Buffer 2

Last { 1 uL Pst1
{ 1 uL EcoR1

1.5 uL H_2O

borax

Gold nanowires 09/27/17

.0025 M $HAuCl_4$ + .005 M $NaBH_4$
↳ good results

.0025 M $HAuCl_4$ | .05 M $NaBH_4$
↳ too high [$NaBH_4$]

.0025 M $HAuCl_4$ | .0025 M $NaBH_4$

9/28/17

.0025 M $HAuCl_4$ .005 M $NaBH_4$
↳ .0189 g

10/2/17

1) EcoRI XSTI Digest of Igem 9 and PCI 3
   ↳ Successful (picture of gel SAVED)
2) B/21 transformation using Igem #9 Sample 1 and last two - PCI 3

Protocol followed Standard

7/13/17

Except 1ul of DNA was used (higher concentrated sample)

10/2/17

1) Boost XSTE Digest of Item 9 and PCl 3
↳ Successful (picture of Gel SAVED ↳ last two PCl 3

2) B/2 transformation using Item #9 Sample 1

Protocol followed SAME as 7/13/17

Except 1ul of DNA was used (higher concentrated sample)

10/4/17

- Cell culture : 500 mL of (LB & cells) 50mL
  450 uL of spec
  Absorbance = .301
- Waited 45 min then saw absorbance.
  Absorbance = .905
- Save 1 mL of it
- record time of putting in 500 uL IPTG.
  ↓
  12:55 p.m.

tube.

BioBricks

* Digest linearized pSB1C3 as outlined on iGEM page:

Master Mix:
- 5 uL NEB 2
- 5 uL BSA (2 mg/mL stock)
- 0.5 uL EcoR1-HF (NEB)
- 0.5 uL PstI (NEB)
- 0.5 uL DpnI (Thermo)
- 13.5 uL H_2O

25 uL total.

Digest:
- 4 uL PSB1C3 (linearized) (25 ng/uL, 100 ug total)
- + 4 uL enzyme mix

8 uL total (12.5 ng/uL)

37° 45 min
80° 20 min

DT #2, 8, 9, 7 digested w/ PstI / EcoRI
#2 @ 8 ng/uL.
7, 8, 9 @ 6.6 ng/uL

Ligation: (Transform 2 uL)
Red colonies no good!

25 ng of #2 #7, 8, 9
3.1 uL 3.8 uL

Ligation #2
- ✓ 2 uL vector
- ✓ 3.1 uL insert
- ✓ 1 uL Buffer
- ✓ 0.5 uL Ligase
- ✓ 3.4 uL water

10 uL.

- 2 uL pSB1C3 (25 ug total)
- 25 ng total insert
- 1 uL T4 Buffer
- 0.5 uL T4 ligase
- H_2O to 10 uL

16°C 30 min, 80°C 20 min

for MtrCAB

10/9/17.

DNA extraction from GEL following protocal A from THERMO SCIENTIFIC GeneJET & a few steps were changed. we followed the exact procedure from 07/25/17.

OD260 Absorbance: .642

95 uL H_2O