muninn

Protein Purification Protocol

Connor

1) Perform all steps on ice

2) Resuspend cell pellet in 30ml Binding Buffer (20mM Tris-HCl, 500mM NaCl, 1mM EDTA, pH 8.0)

3) Sonicate cell suspension for 10 mins (100 amplitude; 1 second on, 2 seconds off) Put tube in beaker with ice
   a) Take sample of raw lysate for later SDS-PAGE - Store at 4°C

4) Clarify lysate via centrifugation (16,000rpm, 4°C, 20 mins in a Beckham Coulter Optima TM L-100 XP Ultrafuge)
   a) Take sample of cell pellet for later SDS-PAGE - Store at 4°C
   b) Sample supernatant.

5) Filter supernatant via syringe method using a 0.22µm filtercup.

6) Equilibrate StrepTrap HP 5mL column (GE Healthcare, UK) with binding buffer
   a) Keep waste flow through for later SDS-PAGE - Store at 4°C

7) Load filtered supernatant onto equilibrated column

8) Elute Sepharose-bound protein using a gradient of binding buffer and elution buffer (20mM Tris-HCl, 500mM NaCl, 1mM EDTA, 2.5mM desthiobiotin, pH 8.0)

9) Clean column using dd-H_2O and regenerate with 0.5M NaOH then store in 20% ethanol at 4°C.

10) Perform an SDS-PAGE on clarified lysate, cell pellet, flow-through and purified fractions determined via UV peaks in elution profile.

Escherichia Coli Transformation Protocol

Connor

1) Defrost 50 µl of chemically competent E. Coli
2) Add plasmid DNA
   - 2 µl if sequence verified plasmid
   - 5 µl for ligations.
3) Incubate on ice for 30 minutes
4) Heat Shock at 42°C for 1 minute
5) Incubate on ice for 2 minutes
6) Add 200 µl of LB.
7) Incubate at 37°C for 1 hour
8) Plate entire contents onto LB plate with ∝
9) Incubate plates at 37°C overnight

Agarose Gel Electrophoresis Protocol

Karen

1) Measure 0.5g of agarose
2) Mix agarose powder with 50ml 0.5XTBE in a 250ml microwavable flask
3) Microwave for 1 minute in 30 second intervals, stop and swirl, until the agarose is completely dissolved (do not overboil the solution as some buffer will evaporate and thus alter the final percentage of agarose in the gel)
4) Allow the agarose solution to cool to about 50°C (When you can comfortable keep your hand on the flask for 10 seconds)
5) Add 5µl of 10,000x Gel Red and gently mix to prevent formation of bubbles.
6) Pour the agarose into a gel tray with the well comb in place
7) Let sit for 20-30 minutes until completely solidified
8) Once solidified, place the agarose gel into the gel box
9) Fill gel box with 0.5xTBE until the gel is covered
10) Add 2µl of loading buffer to each of your DNA samples
11) Carefully load a molecular weight ladder into the first lane of the gel
12) Carefully load your samples into the additional wells of the gel
13) Run the gel at 100V for 1-1.5 hours (until the dye line is approximately 75-80% of the way down the gel)
14) Turn off power, disconnect the electrodes from the power source and carefully remove the gel from the gel box
15) Visualise your DNA fragments using any device that has UV light

22/07/19

1) $MgCl_2$ - 0.1 M
   - Measure 9.5211 g
   - Add 80ml of dH_2O
   - Top up to 100ml
   - Take 10ml and add 90ml dH_2O = 0.1 M
   - Autoclave

2) $CaCl_2$ - 0.1M
   - Measure 11.1g $CaCl_2$
   - Add to 80ml of dH_2O
   - Top up to 100ml
   - Take 10ml and add 90ml dH_2O = 0.1 M
   - Autoclave.

3) 100ml LB broth in a 250 conical flask
   - To be autoclaved

GENE CONSTRUCTS - IDT

- Glycine activator 944bp
  1000ng => 1715 fmol

- PEPD
  1000ng => 967 fmol

- Eicosane 1547 bp
  1000ng => 1046 fmol

- GCUR TF
  500ng => 1094 fmol

- ChaC
  1000ng => 1773 fmol

- GCUA TF
  1000ng => 1426 fmol.

- Eicosanol RFP
  1000ng => 1709 fmol

- Nitroxoline
  1000ng => 1708 fmol.

23/07/19

Matt

G-Block Suspension

1) Centrifuge the tube for 3-5 seconds at a minimum 3000×g to pellet the material to the bottom of the tube

2) Add the following volume of TE buffer to get a final concentration of DNA:
   - Glycine detector - 100µl
   - PepD gene - 100µl
   - Eicosane → 1-Eicosanol Forward - 100µl
   - Nitroxoline RFP biosensor - 100µl
   - GCVR TF - 50µl
   - ChaC Gene - 100µl
   - GCVA TF - 100µl
   - Eicosanol RFP - 100µl

3) Briefly vortex and centrifuge.

Notes

- Plated out E.Coli K12 (MG1655) grown O/N in LB. (Plated on LB agar at 37°C)
- Need to inoculate 10ml of LB.

23/7/19

Connor

Chemically Competent Cells Protocol

Amount of Substance

Solution	Concentration
$MgCl_2$	0.1 M
$CaCl_2$	0.1 M
Glycerol	100%

Procedure

1. Inoculate a single colony of appropriate cells into 10ml LB in a sterilin. Add antibiotic if needed, and culture O/N at 37°C, 200 rpm
2. Inoculate 100ml LB with 1ml O/N culture
3. Incubate at 37°C, 200rpm until OD_{600} = 0.3 - 0.6 (~2 hours)
4. Transfer to 2x 50ml Falcon tubes and leave on ice for 30 mins.
5. Centrifuge at 4000 x g, 5mins, 4°C
6. Re-Suspend pellet gently in 2.5ml ice cold 0.1M $MgCl_2$
7. Incubate on ice for 30 mins
8. Centrifuge at 4000 x g, 5mins, 4°C
9. Re-Suspend pellet gently in 2.5ml ice cold 0.1M $MgCl_2$
10. Incubate on ice for 30 mins
11. Centrifuge at 4000 x g, 5mins, 4°C
12. Re-Suspend pellet gently in 2ml ice cold 0.1M $CaCl_2$ / Glycerol Solution (1.7ml 0.1M $CaCl_2$, 0.3ml 100% Glycerol)
13. Aliquot (50µl) and flash freeze in liquid nitrogen
14. Store in -80°C freezer.

24/07/19

Calculations of amounts of DNA in fmol for the reaction (MoClo)

Concentration of DNA is in ng/µl = each one = 10ng/µl DNA
Must convert to fmol / µl. Need to use DNA amount of 20 fmol

- Glycine activator = 10 ng/µl = 17.15 fmol/µl
  ↳ 10 fmol / 17.15 = 0.58

- PepD = 10ng/µl = 9.67 fmol/µl
  ↳ 10 fmol / 9.67 = 1.03 µl

- Eicosane = 10ng/µl = 10.46 fmol/µl
  ↳ 10 fmol / 10.46 = 0.96 µl

- GCUR TF = 10ng/µl = 21.88 fmol/µl
  ↳ 10 fmol / 21.88 = 0.46 µl

- Cha C = 10ng/µl = 17.53 fmol/µl
  ↳ 10 fmol / 17.53 = 0.57 µl

- GCUA TF = 10ng/µl = 14.26 fmol/µl
  ↳ 10 fmol / 14.26 = 0.7 µl

- Eicosanol RFP = 10ng/µl = 17.09 fmol/µl
  ↳ 10 fmol / 17.09 = 0.59 µl

- Nitroxiline = 10ng/µl = 17.08 fmol/µl
  ↳ 10 fmol / 17.08 = 0.59 µl

DVK - AF = 2203bp
= 111.21 fmol

$C_1V_1 = C_2V_2$
= 10 µl

$111.21 \times V1 = 10.21 \times 1$

0.09 µl + 6.91 EB

Level 1

DNA parts - add µl (calculate - 1 µl plasmid + DNA (µl))

10x ligase buffer - 1 µl

Ligase at 20 U/µl - 1 µl

BsaI at 10µ/µl - 1 µl

ddH_2O - Make to 20 µl

glycine activator = ddH_2O (µl) 15.42

PepD = 14.97

Eicosane = 15.04

GCVR TF = 15.54

ChaC = 15.43

GCVA TF = 15.3

Eicosanol RFP = 15.41

Nitroxoline = 15.41

DNA ligase = 3.77 ng/µl = 260/280 = 0.66

Testing Composite cells

Incubate on ice at → 1) 14:53 → take out at 15:23
2) 14:57 → take out at 15:27

↳ Transform with DUK AE SpT RFP Bools

Take 1 out at 4:28pm
Take 2 out at 4:32pm

25/7/19

Transformations

1) Glycine Activator - bad
2) Pep D - bad
3) Eicosane - bad
4) GCVR TF - bad
5) Cha C - good
6) GCVA TF - good
7) Eicosanol RFP - good
8) Nitroxoline - good

26/7/19

1) Pep D - bad
2) Eicosane - bad
3) GCVR TF - bad

1/8/19

Karen

* Kanamycin LB plates poured

* Gel electrophoresis - 1% agarose gel

(1g agarose + 100ml of 1xTBE 0.5g + 50ml (1xTBE)
1g + 50ml (0.5xTBE))

Gel ① (10 wells)

Well	1	2	3	4	5	6	7	8	9	10
Sample	Ladder	- Ve control	+ Ve control	Glycine Activator	Pep D	Eicosane	GCVR	Redo Pep D	Ladder	—

- 2 µl of sample + 2 µl of loading buffer (Gel loading dye purple (6x))
- 10 µl of ladder (1Kb)
- 100v, 1 hour ⇒ only band on +Ve control.

Gel ② (10 wells)

Well	1	2	3	4	5	6	7	8
Sample	Ladder	+ Ve control	- Ve control	ChaC	GCVA	Eicosanol RFP	Nitroxoline	Ladder

Worked

- 2 µl Sample + 2 µl Loading buffer
- 10 µl of Ladder (1kb)
- 100v, 50 mins (out of time)

2/8/19

Calculations for MoClo

To do calculations
- Glycine activator
- Pep D
- Eicosane
- GCUR TF
- Cha C

MoClo transformations

- Make 10fmol/µl of DVK-AF stock
  - ↳ 2203bp/111.21 fmol
    0.09 µl of plasmid and resuspend in 0.91 EB
  - ↳ 10fmol/µl
  - ↳ xS= 0.45 µl of plasmid + 4.55 µl EB. Enough for 5 reactions.

- Glycine activator
  - ↳ 1 µl of DVK-AF 10fmol/µl stock (10fmol)
  - ↳ 0.63µl of glycine activator stock (11fmol)
  } 20 fmol DNA
  - ↳ 1 µl 10× ligase buffer
  - ↳ 1 µl ligase at 20U/µl
  - ↳ 1 µl BsaI at 10U/µl (add last)
  - ↳ 15.37 ddH_2O

- Pep D
  - ↳ 1 µl DVK-AF
  - ↳ 1.14 µl Pep D
  - ↳ 14.86µl ddH_2O

- Eicosane
  - ↳ 1µl DVK-AF
  - ↳ 1.06µl Eicosane
  - ↳ 14.94µl ddH_2O

- GCUR TF
  - ↳ 1µl DVK-AF plasmid
  - ↳ 0.51 µl GCUR
  - ↳ 15.49µl ddH_2O

- ChaC
  - ↳ 1 µl DVK-AF plasmid
  - ↳ 0.63 µl ChaC
  - ↳ 15.37µl ddH_2O

6/8/19

Karen

QIAprep Spin Miniprep Kit

1. Pellet 1-5ml bacterial overnight culture >8000rpm, 3 min, RTP
2. Resuspend pelleted bacterial cells in 250µl Buffer P1, transfer to microcentrifuge tube
3. Add 250µl Buffer P2 and mix thoroughly by inverting tube 4-6 times until clear
4. Add 350µl Buffer N3, mix immediately and thoroughly by inverting tube 4-6 times
5. Centrifuge 10 min, 13000 rpm table top microcentrifuge
6. Apply 800µl supernatant (step5) in the QIAprep 2.0 spin column by pipetting. Centrifuge 30-60s, discard flow through.
7. Wash QIAprep spin column by adding 0.5ml Buffer PB, centrifuge 30-60s, discard flow through
8. Wash QIAprep spin column by adding 0.75ml Buffer PE, centrifuge 30-60s, discard flow through
9. Centrifuge 1 min to remove residual wash buffer.
10. Place QIAprep 2.0 column in clean 1.5ml microcentrifuge tube. To elute DNA add 50µl Buffer EB or water to column, let stand for 1 min, centrifuge for 1 min

Overnight culture of E. coli DH5α DVK-AF

Glycerol stocks made.

7/8/19

Sequencing GCUA, eicosanol, nitroxoline

TS00533602 - GCUA (F)

TS00533603 - GCUA (R)

TS00533604 - Eicosanol (F)

TS00533605 - Eicosanol (R)

TS00533606 - Nitroxoline (F)

TS00533607 - Nitroxoline (R)

MoClo repeat (Level 1) (doubled amount of insert)

DNA Parts

1. Glycine activator
2. PepD
3. Eicosane
4. GCUR
5. ChaC

To be repeated.

6. GCUA
7. Eicosanol
8. Nitroxoline

Plasmid DVK-AF nanodrop (2203bp)

1. 23.6 ng/µl
2. 23.4 ng/µl ≈ 23.6 ng/µl → 16.51 fmol
3. 23.8 ng/µl

=> 0.61 µl of plasmid for 10fmol

1) Glycine Activator
- 0.61 µl DVK-AF
- 1.16 µl insert
- 1 µl 10x Ligase buffer
- 1 µl Ligase at 20U/µl
- 1 µl BsaI at 10U/µl
- 15.23 µl ddH_2O

2) PepD
- 0.61 µl DVK-AF
- 2.06 µl insert
- 1 µl 10x Ligase buffer
- 1 µl Ligase at 20U/µl
- 1 µl BsaI at 10U/µl
- 14.33 µl ddH_2O

3) Eicosane
- 0.61 µl DVK-AF
- 1.92 µl insert
- 1 µl 10x Ligase buffer
- 1 µl Ligase at 20U/µl
- 1 µl BsaI at 10U/µl
- 14.47 µl ddH_2O

4) GCUR-TF
- 0.61 µl DVK-AF
- 0.92 µl insert
- 1 µl 10x Ligase buffer
- 1 µl Ligase at 20U/µl
- 1 µl BsaI at 10U/µl
- 15.47 µl ddH_2O

5) ChaC
- 0.61 µl DVK-AF
- 1.14 µl insert
- 1 µl 10x Ligase buffer
- 1 µl Ligase at 20U/µl
- 1 µl BsaI at 10U/µl
- 15.25 µl ddH_2O

6) GCVA
- 0.61 µl DVK-AF
- 1.4 µl insert
- 1 µl 10x Ligase buffer
- 1 µl Ligase at 20U/µl
- 1 µl BsaI at 10U/µl
- 14.99 µl ddH_2O

7) Eicosanol
- 0.61 µl DVK-AF
- 1.18 µl insert
- 1 µl 10x Ligase buffer
- 1 µl Ligase at 20U/µl
- 1 µl BsaI at 10U/µl
- 15.21 µl ddH_2O

8) Nitroxoline
- 0.61 µl DVK-AF
- 1.18 µl insert
- 1 µl 10x Ligase buffer
- 1 µl Ligase at 20U/µl
- 1 µl BsaI at 10U/µl
- 15.21 µl ddH_2O

8/8/19

Gel electrophoresis of all new MoClo assemblies

Well	1	2	3	4	5	6	7	8	9	10	11	12
Sample	Ladder	- Ve TG Buffer	+ Ve DVK-AF	Glycine Activator	Pep D	Eicosane	GCVR	ChaC	GCVA	Eic-RFP	Nitrox.	Ladder

Transformations -
Transformed E. Coli DH5α with the following assembled plasmids:
↳ glycine activator
↳ Pep D
↳ Eicosane
↳ GCVR
↳ ChaC
+ 1 control

Re plated K12

9/8/19

MoClo assembly of Glycine activator and Eicosane

- 2 µl loading buffer (6x) + 2µl sample
- 8 µl ladder

Well	1	2	3	4	5	6
Sample	Ladder	-Ve	+Ve	Gly	Eic	Ladder

14/8/19

Aims - Take out + Check transformations
- Miniprep ChaC + glyA (glyA didn't work)
- Send miniprep for sequencing.

Transformations

None worked :(

Miniprep

Overnight of ChaC worked. GlyA didn't

Miniprep ChaC

ChaC(F) - TS00533669

ChaC(R) - TS00533670

Transformation

↳ PepD, Eicosane, GCVR - used 5µl instead of 2µl

15/8/19

Aims - MoClo of PepD, Eicosane and glycine activator
- Transform bacteria
- Plate Kanamycin plates.

Transformations

↳ GlyA, PepD, Eicosane, GCVR => none worked

↳ Jasmines M6 + 0974

=> Freeze Shock 30 mins. Take out at 15:43

- How long Shock lasts - glutathione 1.5mmol/L

16/8/19

Gel electrophoresis

Well	1	2	3	4	5	6	7	8	9	10	11	12
Sample	Ladder	-Ve	+Ve	Glycine Activator	PepD	Eicosane	GCVR	ChaC	Cas1	Cas2	Cas3	Ladder

20/8/19

Aims - MoClo of Glycine activator, PepD, Eicosane, GCUR + ChaC
- Ratio of plasmid : G block

Glycine Activator

1:1 ratio = 0.61 µl DUK-AF
0.58 µl insert
15.81 µl ddH_2O

1:2 ratio = 0.61µl DUK-AF
1.16µl insert
15.23µl ddH_2O

1:3 ratio = 0.61 µl DUK-AF
2.32 µl insert
14.07 µl ddH_2O

1:4 ratio = 0.61 µl DUK-AF
3.48µl insert
12.91 µl ddH_2O

1:5 ratio = 0.61 µl DUK-AF
4.64 µl insert
11.75 µl ddH_2O

2:1 ratio = 1.22 µl DUK-AF
0.58 µl insert
15.20µl ddH_2O

Pep D

1:1 ratio = 0.61 µl DUK-AF
1.03 µl insert
15.36 µl ddH_2O

1:2 ratio = 0.61 µl DUKAF
2.06 µl insert
14.33 µl ddH_2O

1:3 ratio = 0.61 µl DUK-AF
3.09 µl insert
13.30 µl ddH_2O

1:4 ratio = 0.61 µl DUK-AF
4.12 µl insert
12.27 µl ddH_2O

1:5 ratio = 0.61 µl DUK-AF
5.15 µl insert
11.24 µl ddH_2O

2:1 ratio = 1.22 µl DUK-AF
1.03 µl insert
14.75 µl ddH_2O

GCUR

1:1 ratio = 0.61µl DUK-AF
0.46µl insert
15.93 µl ddH_2O

1:2 ratio = 0.61 µl DUK-AF
0.92µl insert
15.47 µl ddH_2O

1:3 ratio = 0.61 µl DUK-AF
1.38µl insert
15.01 µl ddH_2O

1:4 ratio = 0.61µl DUK-AF
1.84µl insert
14.55 µl ddH_2O

1:5 ratio = 0.61 µl DUK-AF
2.30µl insert
14.09µl ddH_2O

2:1 ratio = 1.22 µl DUK-AF
0.46 µl insert
15.32 µl ddH_2O

ChaC

1:1 ratio = 0.61 µl DUK-AF
0.57 µl insert
15.82 µl ddH_2O

1:2 ratio = 0.61 µl DUK-AF
1.14 µl insert
15.25 µl ddH_2O

1:3 ratio = 0.61 µl DUK-AF
1.71 µl insert
14.68 µl ddH_2O

1:4 ratio = 0.61 µl DUK-AF
2.28 µl insert
14.11 µl ddH_2O

1:5 ratio = 0.61 µl DUK-AF
2.85 µl insert
13.54 µl ddH_2O

2:1 ratio = 1.22 µl DUK-AF
0.57 µl insert
15.21 µl ddH_2O

Eicosane (LadA)

1:1 ratio = 0.61 µl DUK-AF → 21.2 ng/µl run out. used other & changed amount
0.96 µl insert
15.43 µl ddH_2O

1:2 ratio = 0.61 µl DUK-AF
1.92 µl insert
14.47 µl ddH_2O

1:3 ratio = 0.61 µl DUK-AF
2.88 µl insert
13.51 µl ddH_2O

1:4 ratio = 0.61 µl DUK-AF
3.84 µl insert
12.55 µl ddH_2O

1:5 ratio = 0.61 µl DUK-AF
4.80 µl insert
11.59 µl ddH_2O

2:1 ratio = 1.22 µl DUK-AF
0.96 µl insert
14.82 µl ddH_2O

PCR

Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Sample	Ladder	-Ve	+Ve	GA 1	GA 2	GA 3	GA 4	GA 5	PepD 1	PepD 2	PepD 3	PepD 4	PepD 5	GCUR 1	GCUR 2	GCUR 3	GCUR 4	GCUR 5	ChaC 1	ChaC 2	ChaC 3	ChaC 4	ChaC 5	Ladder

20/8/19

Alice + Emily.

Miniprep Cas repeat (suspended in T6)

1% gel ran - 100V
- 80min + extra 30 mins (0.7% agarose next time)

4μl sample
2μl Loading dye

Nanodrop result of miniprep
35.18ng/μl
55.77ng/μl

Transforming BL21DE3 E.coli

→ 50μl of chemically competent E.coli was defrosted

→ 2μl plasmid DNA was added

→ This was incubated on ice for 30 mins

→ This was then heat shocked at 42°C for 1min

→ The mixture was incubated on ice for another 2 mins

→ 200μl of LB was added

→ This was incubated at 37°C for 1 hour

→ The contents was plated onto an LB plate with Chloramphenicol

→ Plates were incubated overnight at 37°C

21/8/19

→ Ran gels from yesterday - bands present

→ Transformations of 'Gly-active', ChaC, Pep D, GCUR - none worked

→ Transformed E. coli with DUK-AF
↳ worked.

22/8/19

→ Overnight culture of DUK-AF

21/8/19

Alice

Protein expression testing - overnight culture

Day 1

1) Aliquot 10ml Liquid LB into 50ml falcon tube

2) Add antibiotic (Chloramphenicol 10 µl)

3) Take a single colony of transferred e-coli (BL21 DE3) Cas13a- add to the falcon tube.

4) Loosely cap

5) Incubate at 37°C with Shaking.

Day 2 - 22/8/19

6) Subculture cells 1/100 dilution in 10ml Liquid LB in a Sterilin (100 µl overnight culture in 10ml LB)

7) Incubate at 37°C with Shaking until OD_{600} is between 0.4 - 0.6 (monitored hourly)

Start - 9:10am

10:00 OD_{600} = 0.035
11:00 OD_{600} = 0.071
12:00 OD_{600} = 0.179
13:00 OD_{600} = 0.476 - Final measure.

8) Remove 500 µl into an eppendorf and centrifuge at 13,000 rpm for 2 mins. Discard Supernatant and label cells as 'time = 0' control

9) Split the culture into 2 Sterilins - 2.5ml each with 3 µl IPTG added.

10) Incubate at 37°C with Shaking for 3-4 hours

Start - 13:30
End - 16:45

11) Remove 500 µl of each sample into a 1.5ml eppendorf respectively.
- Centrifuge Samples at 13,000rpm for 2 mins.
- discard the Supernatant
- Label and refridgerate.

Day 3 - 23/8/19

SDS-PAGE prepared by Connor (acrylamide at 12%)

12) Add 50µl of SDS-PAGE Loading buffer to each pellet and thoroughly re-suspend them - Include the 'time=0' pellet

13) Seal the eppendorf tubes. Incubate samples at 100°C on a heatblock for 10 mins. (This solubilises the pellet)

14) Vortex every 2 mins to mix
- Careful of pressure build up
- open and close gently before vortexing.

Centrifuge samples at 13,000rpm for 2 mins

15) Prepare SDS-PAGE with appropriate acrylamide % (15% recommended)

16) Assemble the gel in the gel-running tank with a buffer dam on the other side of the gel holder

17) Remove the comb from the gel

18) Add 1× SDS-PAGE running buffer to the internal reservoir
- make sure there are no air bubbles in the lanes
- check that the reservoir doesn't leak.

19) Fill external reservoir to the line with 1× SDS-PAGE running buffer.

20) Load 5-10 µl of protein ladder into the first well.

21) Load 15µl of each sample into separate wells.

Well # 1 = Ladder
2 = 'time=0'
3 = control
4 = With IPTG

22) Run the gel at 200 volts for 40 mins or until blue dye starts to leak from the bottom of the gel

23) Stain the gel with Coomassie blue to see the protein bands

First SDS-PAGE was unsuccessful due to glass breakage and the gel being ripped.

A second attempt was made with Annie's guidance.

24) Add 0.5g Coomassie brilliant blue r-250 to a clean 500ml duran bottle, this is for the coomassie stain.

25) Add 200ml ethanol to the duran and mix

26) Carefully add 250ml of acetic acid to the duran and mix

27) add 50ml distilled water to the duran and mix.

28) Make destain by adding 1L ethanol and 500ml acetic acid to a 10L carboy, filling to 5L with distilled water and mixing.

Both stains were available were pre-available in the lab for general use.

23/8/19

Alice

Gel Staining

1) Carefully remove the SDS-PAGE from the glass and place it in a plastic tray (dd-water makes this easier)

2) Rinse the gel with dd-water to remove excess buffer

3) Cover the gel in buffer - it should be fully submerged.

4) Place a cracked lid on the plastic tray and microwave for 40 seconds
Immediately cover and take to the fume hood.

5) Open in the fume hood for 5 mins, then re-cover and leave to stain for ~1 hour.

6) Remove the stain (pour into original bottle), and rinse the gel with dd-water.

7) Repeat with destain, but decant the used destain into a carboy with an activated charcoal filter.

8) Image gel using ✗
If bands are faint, repeat stain/destain as necessary.

23/8/19

→ Miniprep DUIC-AF bacteria
  ↳ 2× Jasmine, 2× iGEM

→ Make agar plates - minimal media different concentrations of glycine, cysteine and tryptophan.

Minimal media agar plates

1. 400ml of dH_2O, 22.56g minimal media (M9 salts) and 4g agar

2. Make 10mM/L stock solutions of glycine, cysteine + tryptophan
   → Glycine (75.07g/mol) = 0.0751g in 100ml dH_2O
   → Cysteine (121.16g/mol) = 0.12116g in 100ml dH_2O
   → Tryptophan (204.23g/mol) = 0.20423 in 100ml dH_2O

27/08/19

Emily

Eicosanol Stock Solution

$10\,cm^3$ Stock Solution at 50mM
$= 0.01\,dm^3$
$5 \times 10^{-5}\,mol\,dm^{-3}$

$C = 5 \times 10^{-5}\,mol\,dm^{-3}$
$V = 0.01\,dm^3$
$n = 5 \times 10^{-7}$ moles
$Mr = 298.62$
$m = 0.14931\,g$

$n = CV$
$n = 5 \times 10^{-5} \times 0.01$
$n = 5 \times 10^{-7}$

$m = n \times Mr$
$m = 5 \times 10^{-7} \times 298.62$
$m = 1.4931 \times 10^{-4}$

$Mr_{eicosanol} = 298.62$

mass used = 0.14931 g eicosanol

To make stock 0.14931g eicosanol was dissolved in a minimal amount of acetone and diluted appropriately with ddH_2O.

28/08/19

Minimal acetone was too much ∴ ethanol and DMSO were tried and Ethyl Ether was found to be the most effective.

27/08/19

→ MoClo transformation of GlyA, ChaC, PepD, Eic, GCVR
→ Gel electrophoresis of MoClo
→ Transformation

1:1, 1:2, 1:3 } ratio plasmid : Gblock

DVK-AF = 91.84 ng/mL → 62.24 fmol
→ Stock of sonic = 10 fmol (8.04 mL in 41.96)

GlyA

1:1 ratio – 0.58 µL insert
constant in all ratios:
- 1.00 µL DVK-AF
- 1.00 µL ligase buffer
- 1.00 µL ligase
- 1.00 µL BsaI

15.42 µL ddH_2O

1:2 ratio – 1.16 µL insert
14.84 µL ddH_2O

1:3 ratio – 1.74 µL insert
14.26 µL ddH_2O

PepD

1:1 ratio – 1.03 µL insert
constant in all ratios:
- 1.00 µL DVK-AF
- 1.00 µL ligase
- 1.00 µL L Buffer
- 1.00 µL BsaI

14.97 µL $dd H_2O$

1:2 ratio – 2.06 µL insert
13.94 µL ddH_2O

1:3 ratio – 3.09 µL insert
12.91 µL ddH_2O

Eicosane

1:1 ratio – 0.96 µL insert
constant in all ratios:
- 1.00 µL DVK-AF
- 1.00 µL ligase
- 1.00 µL L Buffer
- 1.00 µL BsaI

15.04 µL ddH_2O

1:2 ratio – 1.92 µL insert
14.08 µL ddH_2O

1:3 ratio – 2.88 µL insert
13.12 µL ddH_2O

GCVR-TF

1:1 ratio – 0.46 µL insert
constant in all ratios:
- 1.00 µL DVK-AF
- 1.00 µL ligase
- 1.00 µL ligase buffer
- 1.00 µL BsaI

15.54 µL ddH_2O

1:2 ratio – 0.92 µL insert
15.08 µL ddH_2O

1:3 ratio – 1.38 µL insert
14.62 µL ddH_2O

ChaC

1:1 ratio – 0.57 µL insert
constant in all ratios:
- 1.00 µL DVK-AF
- 1.00 µL ligase
- 1.00 µL ligase buffer
- 1.00 µL BsaI

15.43 µL ddH_2O

1:2 ratio – 1.14 µL insert
14.86 µL ddH_2O

1:3 ratio – 1.71 insert
14.29 µL ddH_2O

Gel electrophoresis of MoClo

150ml of 0.5 x TBE + 2.25 g agarose

Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Sample	Ladder	- Ve	+Ve	GA 1	GA 2	GA 5	PepD 1	PepD 2	PepD 3	Eic 1	Eic 2	Eic 3	GCUR 1	GCUR 2	GCUR 3	ChaC 1	ChaC 2	ChaC 3	Ladder

28/08/19

→ Test the effects of Diethyl ether on the competence of E.coli DH5α
→ Plate Eic-RFP DH5α bacteria
→ Transformations

Stock solutions – 50%, 10ml
- 20% (0.2ml diethyl ether + 0.8ml water)
- 1% (10µl in 190µl water)
- 0.1% (20µl in 180 µl water).

Final Con(%)	Diethylether(µl)	LB (µl)	E.coli (µl)
1.000	10 (20%)	188	2
0.500	5 (20%)	193	2
0.100	1 (20%)	197	2
0.050	10 (1%)	188	2
0.010	2 (1%)	196	2
0.005	10 (0.1%)	188	2
0.001	2 (0.1%)	196	2
10.000	20 (100%)	178	2
5.000	10 (100%)	188	2

Grow for 16hrs – 180rpm, 37°C, OD600

28/8/19

Transformation

Bacteria = DH5α E. coli

↳ Gly A 1:1, Gly A 1:2, Pep D 1:1, Pep D 1:2, Eic 1:1, Eic 1:2, GCUR 1:1, GCUR 1:2, ChaC 1:1, ChaC 1:2.

1. Add 5μl of plasmid to chemically competent bacteria + put on ice for 30 mins
2. Heat Shock for 1min at 42°C
3. Place on ice for 1min, then add 200μl LB
4. Incubate at 37°C for 1 hour
5. Plate on Kanamycin plate overnight.

29/8/19

Results

- Transformations that worked:
  - → Glycine activator 1:1
  - → Pep D 1:2
  - → Eic 1:1
- Transformations that didn't work:
  - → ChaC
  - → GCUR

To do

- Make overnight cultures of Gly A, Pep D + Eic
- Minimal media
- Transform ChaC + GCUR
  - ↳ Use 1:1, 1:2 again.

30/8/19

- Transformations
  → ChaC and GCUR maybe worked

- Overnight
  → Eic and Pep D worked
  → Glycine Activator not grown. Have to transform again.

Today

- Miniprep Pep D + Eic

Eic 1

80.16 ng/μl 1.88 260/280

80.67 ng/μl 1.90 260/280

82.18 ng/μl 1.92 260/280.

Eic 2

70.10 ng/μl 1.89 260/280

71.10 ng/μl 1.91 260/280

70.72 ng/μl 1.92 260/280

Pep 1

105 ng/μl 1.89 260/280

107 ng/μl 1.89 260/280

107.11 ng/μl 1.89 260/280

Pep 2

100.66 1.89 260/280

100.99 1.91 260/280

101.03 1.92 260/280.

2/9/19

To do List

- Make/autoclave 100mL LB + 400mL LB Agar
- Plate reader assay (16h in plate reader)
- Overnight incubation of diethyl ether
- Inoculate 10µl LB overnight of DH5α
- Send DNA ~~x~~ ~~x~~ sequence (PepD + Eic)

16h Plate reader assay

Final Con (%)	Diethylether (µl)	LB (µL)	E. coli (µL)
1.000	10 (20%)	178	2
0.500	5 (20%)	188	2
0.100	1 (20%)	188	2
0.050	10 (1%)	193	2
0.010	2 (1%)	197	2
0.005	10 (0.1%)	188	2
0.001	2 (0.1%)	196	2
10.000	20 (100%)	188	2
5.000	10 (100%)	196	2

3x control (LB + E.coli - ~~x~~) . (CA) - control A

Stocks

- 20% - 0.2ml of 100%, 0.8mL H_2O
- 1% - 10µl of 20%, 190µL H_2O
- 0.1% - 20µL of 1%, 180µL H_2O.

Sent for sequencing

TS00942498 - Eic F①

TS00942499 - Eic F②

TS00942500 - Pep F①

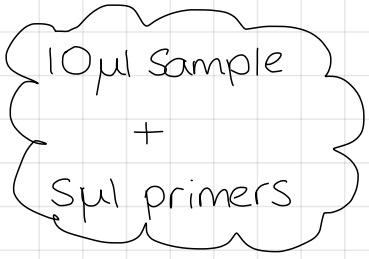
TS00942501 - Pep F②

TS00942502 - Eic R①

TS00942503 - Eic R②

TS00942504 - Pep R①

TS00942505 - Pep R②



16hr incubation.

Final volume = 600µl

Final Con (%)	Diethylether (µl)	LB (µl)	E. coli (µl)
1.000	30 (20%)	534	6
0.500	15 (20%)	564	6
0.100	3 (20%)	564	6
0.050	30 (1%)	579	6
0.010	6 (1%)	591	6
0.005	30 (0.1%)	564	6
0.001	6 (0.1%)	588	6
10.000	60 (100%)	564	6
5.000	30 (100%)	588	6