Exeter iGEM Lab Book

Welcome to our lab book. The following pages contain a chronological description of everything we did in the lab – starting on Day 1. Different days contain short descriptions of our experiments and notes, as well as references to the appropriate protocols.

We were not adept at keeping a concise lab book during the first few weeks of iGEM – the descriptions get more organised and detailed as the weeks progress. The first part of our lab book relates to the Interlab Study, and the later parts to our actual project, Ribonostics.

All protocols can be found at the bottom of the page,and there will links throughout. If you have any questions you can email the team or individual members emails can be found on our team page.

04/06/15

- Interlab Study - hydrating DNA, transformation into competent DH5α cells, overnight cultures, miniprep.

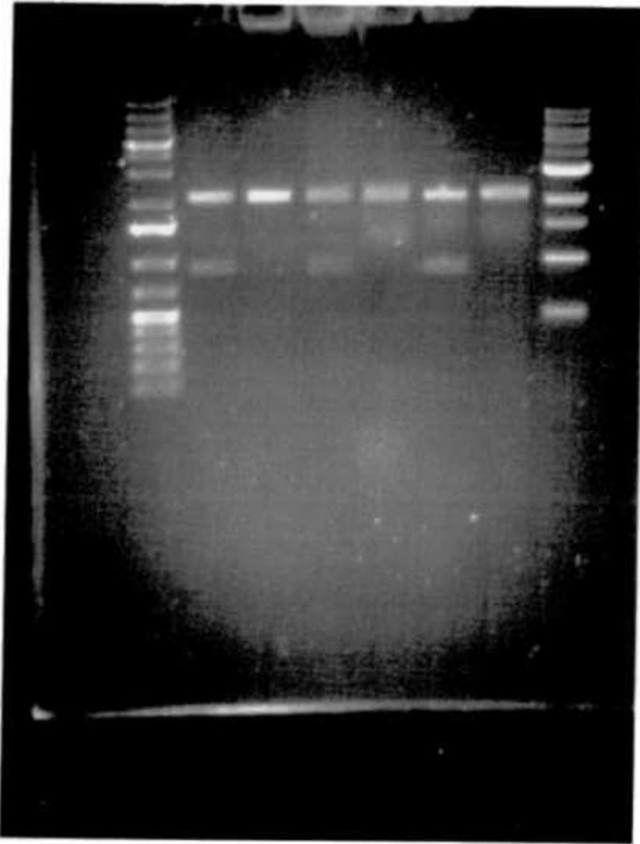
	BioBrick construct DNA concentration (μg/mL)	
Construct	I13504	J23108
Jasmine, Joe, Emilia (Star)	264.0	18.8
Amy, Yemi, Dan (Sharkisha)	314.0	23.6
Bradley, Georgina (Bob)	19.3	260.0

05/06/15

Digestion of our insert and backbone.

Results of our transformations from our plates.

Construct	Antibiotic	Colony number
Star	Cam	0
	Cam	32
	Amp	16
	Amp	0
Sharkisha	Cam	29
	Cam	6
	Amp	0
	Amp	15
Bob	Cam	36
	Cam	50
	Amp	0
	Amp	7



08/06/15

Diagnostic digestion of previous ligations:

- II3504 + J23108 = iGEMILS08A
- II3504 + J23108 = iGEMILS08B

Used EcoRI and SpeI during our digestion.

09/06/15

- Diagnostic digestion of previous ligations.
- Planned to miniprep iGEMILS08A AND iGEMILS08B, **BUT** we realised that we were using the wrong BioBricks all along - J23111, J23109, and J23108, **INSTEAD** of J23101, J23106 and J23117.
- Resuspending DNA from Kit 1 (BioBricks J23101, J23106, J23117).
- Hydrating DNA from the kit.
- Poured plates necessary for our experiments.
- Chloramphenicol plates – 450 µl chloramphenicol needed for bottle of LB broth of 450 mL.
- Chloramphenicol plates poured out on the lab bench, not inside a flow hood. This can be done as they contain antibiotic – never do this with just LB broth (if it is just LB broth, use flow hood and aseptic technique).
- Transformation (E. coli with J23101, J23106 and J23117).

- Plates containing E. coli with plasmids J23101, J23106 and J23117, put in the 37°C static incubator (2 each – one with 20 µl of culture, one with 200 µl of culture).
- Take out at 9 am on 10/06/15.

10/06/15

- Plates taken out at 9 am and put in the cold room until 3 pm.
- After 3 pm, they were taken out and overnight cultures were made. Transformation plates used:
  J23117: 200 µl
  J23101: 200 µl
  J23106: 200 µl
- Making overnight cultures.

11/06/15

- Miniprep J23106A, B, C, J23117 A, B (C has been discarded due to possible contamination), J23101A, B, C.
- Miniprep and ligation.

Names of newly ligated plasmids:

- J23101 + I13504 = IGEMILS101
- J23106 + I13504 = IGEMILS106
- J23117 + I13504 = IGEMILS117

- Making agarose gel for electrophoresis.
- Making competent cells.
- Qubit.

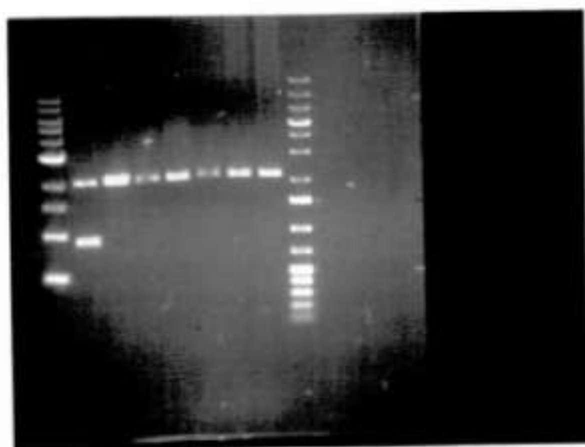
Qubit readings

Plasmid	**Concentration of DNA (ng/mL)**	**Stock concentration (ng/µl)**
J23101A	-	49.60
J23101B	-	2.54
J23101C	-	13.40
J23106A	1.360	154.00
J23106B	0.492	83.80
J23107C	0.118	87.20
J23117A	0.110	44.20
J23117B	0.189	72.80
I13504	0.962	103.00

Plasmid	**Volume needed for digest (µl)**
J23101A	5.00
J23101B	98.40
J23101C	18.60
J23106A	1.60

J23106B	3.00
J23107C	2.90
J23117A	5.70
J23117B	3.40
I13504	2.40

Plasmid	Water to be added (µl)
101A	11.0
106A	14.4
106B	13.0
106C	13.1
117A	10.3
117B	12.6
I13504	13.5



Gel electrophoresis of plasmids

From left to right :

1. ladder 1
2. I13504
3. 101A
4. 117A
5. 117B
6. 106A
7. 106B
8. 106C
9. ladder 2

- Agarose gel electrophoresis.

12/06/15

- Autoclaving.

15/06/15

- Checked if colonies have been transformed (should glow green) – ILS101, ILS106, ILS117.
- Overnight cultures of ILS101, ILS106, ILS117 (6 mL LB, 6 µl CAM, culture).
- Glycerol stocks.
- Pour CAM plates.
- Miniprep ILS101.
- overnight cultures 106, 117 and 108.
- Diagnostic digestion.

16/06/15

- Miniprep.
- Digest.
- Re-plating.
- Glycerol stocks.

Instructions for the Qubit

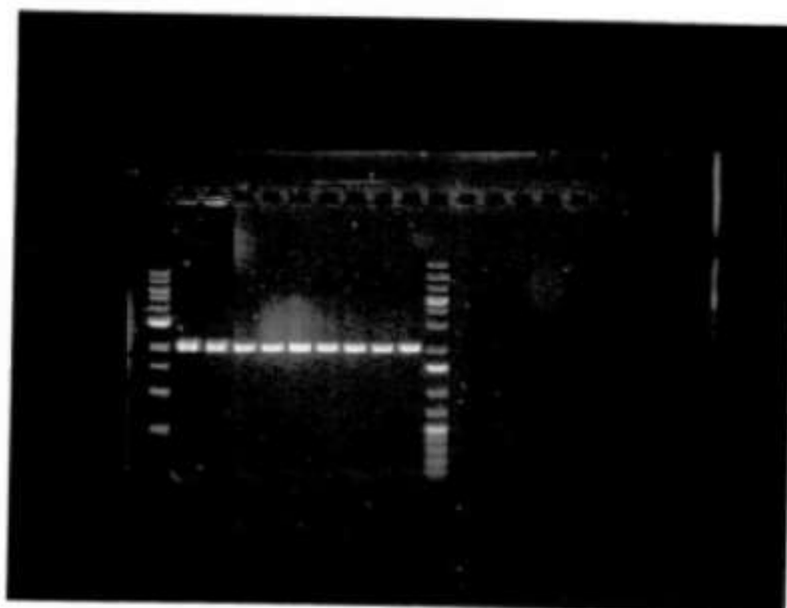
1. Go to the cupboard near the fridge at the end of the lab, take the Qubit kit out of the blue tray
2. From the fridge, get the Qubit BR standards 1+1. They are kept together in a little plastic bag. Not HS ❼ do not use any bottle with HS!
3. Follow standard Qubit protocol.

Qubit results

Plasmid	Concentration (ng/mL)
ILS101A	Too low
ILS101B	3.14
ILS101C	2.66
ILS101D	2.89
ILS101E	3.13
ILS106A	2.84
ILS106B	2.15
ILS106C	0.90
ILS106D	3.83
ILS106E	4.28
ILS117A	2.92
ILS117B	0.98
ILS117C	Too low
ILS117D	1.24
ILS117E	1.12

Control digest

	X1 (µl)	X10 (µl)
H_2O	6.2	62
Buffer	1.0	10
EcoRI	0.4	4
PstI	0.4	4
temp	2.0	-
total	10.0	8



Left to Right:

1. 1kb ladder
2. 101B
3. 101C
4. 101D
5. 101E
6. 106A
7. 106B
8. 106D
9. 106E
10. 117A
11. 1kb ruler and ladder.

22/06/15

- Interlab study failed again.
- Start again with miniprepped J23101, J23106, J23117 and I13504.
- Do a standard digestion and a traditional digestion (1 µl of each enzyme, 1 µg of DNA, 2 µl green buffer, gel extraction – 1% agarose gel – run the whole sample).

Qubit readings

Plasmid	**Volume needed for digest (µl)**	
	250 ng	**1000 ng**
J23101A	5.00	20.0
J23101B	98.40	393.6
J23101C	18.60	74.4
J23106A	1.60	6.4
J23106B	3.00	12.0
J23106C	2.90	11.6
J23117A	5.70	22.6
J23117B	3.40	13.6
I13504	2.40	9.6

Digestion mix

	Volume for digest (µl)		**Water**	
	250 ng	1000 ng	250 ng	1000 ng
J23101A	5.00	16.0	11.0	-

J23106A	1.60	6.4	14.4	9.6
J23106B	3.00	12.0	13.0	4.0
J23106C	2.90	11.6	13.1	4.4
J23117A	5.70	16.0	10.3	-
J23117B	3.40	13.6	12.6	2.4
I13504	2.40	9.6	13.6	6.4

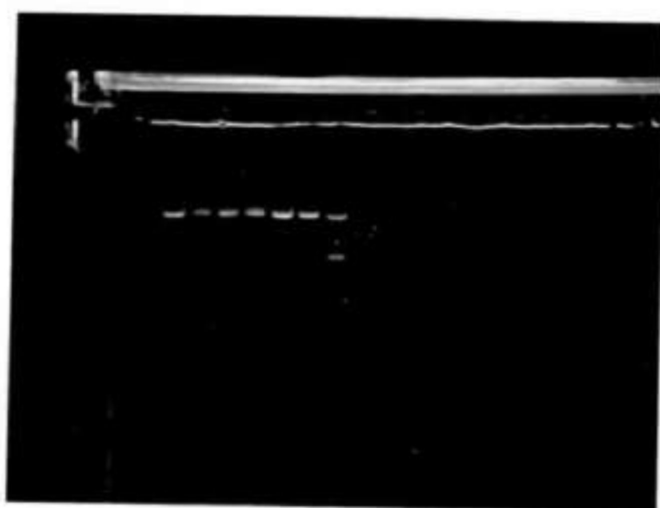
- Enzyme 1 – 1 µl.
- Enzyme 2 – 1 µl.
- Buffer 1 – 2 µl.
- Gel extraction.

Gel extraction

J1A	50 mg
J6A	50 mg
J6B	30 mg
J6C	50 mg
J7A	50 mg
J7B	50 mg
IA	75 mg

Qubit readings from gel extraction

Plasmid	**Concentration (ng/mL)**
J1A	Too low
J6A	2.30
J6B	5.58
J6C	Too low
J7A	7.24
J7B	3.96
IA	1.58



Left to Right:

1. 1 kb ladder
2. J1A
3. J6A
4. J6B
5. J6C
6. J7A
7. J7B
8. IA

23/06/15

- Preparing competent E. coli.

Solutions required:

- TF-1 (200 mL).
- Potassium chloride or rubidium chloride. For 1L = 7.4 g/L, for 200 mL = 1.48 g/L.
- 1M potassium acetate pH 7.5 (9.81 g in 100 mL). For 1L = 30 mL, for 200 mL = 6 mL.
- Calcium chloride dihydrate. For 1 L = 1.5 g/L, for 200 mL = 6.3 g.
- Glycerol. For 1 L = 150 g/L, for 200 mL = 30g/L.
- Make up the above to a final volume of 950 mL, adjust to pH 6.4 with acetic acid (app. 5 mL), dispense into 10 x 95 mL aliquots, autoclave, allow to cool and then add 5 mL (per 95 mL) of 1M manganese chloride tetrahydrate filter sterilised stock (19.8 g in 100 mL).
- TF-2 (200 mL).

24/06/15

Ligation calculations:

For insert

$$\frac{\text{[illegible]} \times \text{[illegible]}}{\text{[illegible]}} \times \text{[illegible]} \frac{\text{[illegible]}}{\text{[illegible]}} = \text{[illegible]}$$

Example:

$$\frac{25\,\text{[illegible]} \times 0.875\,\text{[illegible]}}{2.105\,\text{[illegible]}} \times \text{[illegible]} \frac{1}{1} = 10.4\,\text{[illegible]}$$

Then:

$$\frac{\text{[illegible]}}{\text{[illegible]}}$$

Example

$$\frac{10.4}{1.58} = 6.58$$

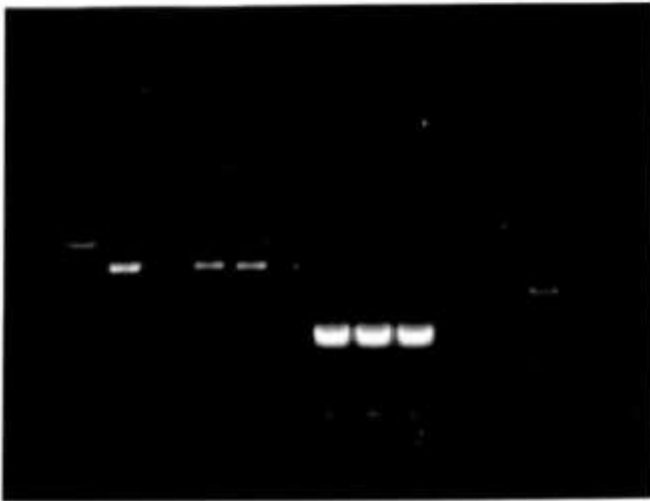
- 6.58 – use maximum volume of insert because it is the limiting factor.

For vector

$\frac{250}{\text{[illegible]}}$ = μg/mL ❼ convert to ng/μL (move decimal by 1 point)

1. Digestion of miniprepped DNA for diagnostic tests
2. Gel electrophoresis of the above
3. Ligation mix

	J1A (μl)	J6B (μl)	J7A (μl)
J	1.9	1.9	1.9
I	6.6	6.6	6.6
T4	0.6	0.5	0.5
T4B	1.0	1.0	1.0



Controls for the next experiments:

- TOP10 – no DNA, no ampicillin, no chloramphenicol.
- TOP10 + donor DNA – ampicillin, no chloramphenicol.
- TOP10 + lec. DNA – no ampicillin, chloramphenicol.
- TOP10 + ligation mix – no ampicillin, chloramphenicol.

25/06/15

Continuation of experiments from yesterday:

- Nothing grew on the antibiotic plates – only on the empty plate ➐ competent cells are fine.
- Transformation of J1, J6 and J7 with competent cells.
- Mini protocol for the water bath – press the S button, hold it, turn knob to adjust temperature. Release S and press it again to confirm. Wait for it to change.

Plating out:

- Transformed J1, J6, J7 – on chloramphenicol.
- Digested JN1, JN6, JN7, IN – on chloramphenicol.
- Competent cells – 1 chloramphenicol, 1 empty.

26/06/15

J1, J6, J7 – lots of colonies but no obviously glowing ones.

Monday:

- Overnight cultures of J1, J6, J7.

Tuesday:

- Miniprep J1, J6, J7 and send for sequencing.

29/06/15

- Made overnight cultures of J1, J6, J7.
- Picked two colonies from each plate, so have overnight cultures of J1, J6 and J7 (x2).

30/06/15

- Making solid agar.
- Miniprepped J1, J6 and J7, did Qubit and sent for sequencing.

Qubit readings

Plasmid	Concentration (ng/mL)
J1A	188
J1B	178
J6A	75
J6B	108
J7A	Too high
J7B	Too high

03/08/15

- gBlock preparation.
- gBlock construction.

Transformed:

1. Prepared 6 aliquots of 50 µl of NEB DH5alpha competent cells.
2. Added 2 µl of correct DNA into each aliquot (devices J23101, J23106, J23117) and NEB positive control #N2611A.
3. Place of ice for 20 mins, followed by heat shock at 42°C for 2 mins.
4. Leave on ice for 4 mins.
5. Add 950 µl of SOC media.
6. Shake incubate at 37°C for 1 hour.
7. Pellet cells at 10 000 rpm for 5 mins.
8. Remove 700 µl of supernatant and resuspend.
9. Plate all 300 µl in each tube onto a Cam plate, except from the NEB positive control which gets plated onto an Amp plate.
10. Incubate overnight at 37°C.

Other tasks:

- Made 2x 500 mL solid agar.
- Made chloramphenicol antibiotic: 35 mg/ml of Cam – powder from the fridge. We made 25 mL so 35 x 25 = 875 mg of CAM powder added into 25 mL of ethanol. Has to be in ethanol because Cam does not dissolve in water.
- Made Cam plates – 100 µl Cam for every 100 mL of agar, so we were using 300 mL – needed 300 µl of CAM.

04/08/15

- Checked fluorescence of cells – had red fluorescence because the plasmid reformed.
- Parafilmed the plates and put them in the cold room.
- Later picked colonies and prepared overnight cultures. Only picked the largest non-red colonies to make overnight cultures with.

Non-Interlab tasks:

- Standard transformation on iGEM plate 4 position 4B. Rehydrated DNA was transferred to a 1.5 mL Eppendorf tube – used 2 x 2 µl, one set of 2 µl into 50 µl of DH5α and then another set.
- Remainder of DNA put in the freezer.
- After transformation plated out onto fresh Cam plates. Overnight incubation at 37°C.
- This was done to get more pSB1C3 backbone.

05/08/15

- Followed standard miniprep protocol for the Interlab constructs.
- Can repeat elution step to increase yield, for plasmids/cosmids >20 kb program elution buffer at 70C. discard column and store at -20°C.
- Did this for: 5 non-red colonies for each device (1-3), 1 red colony from each device (1-3) and 1 colony from iGEM positive control.

Non-Interlab work:

- Made overnight cultures for backbone pSB1C3 work.
- Followed standard protocol.
- Did this for 5 colonies from plate 1 and 5 colonies from plate 2.

06/08/15

Labwork:

- Miniprepped (using standard protocol) all 10 of our cultures.
- Made new agar as ones made earlier in the week in the week failed due to putting in 0.75 g agar; when we needed 5.7 g – did not set.
- 3 of these miniprepped cultures were used for the digest, also 2 cultures from failed interlab.

Protocol for past digestion of different of DNA:

1. Combine the following reaction components at room temperature in this order: nuclease free water (15 µl), 10X FastDigest green buffer (2 µl), DNA (2 µl), FastDigest enzyme (1 µl).
2. Mix gently and spin down.
3. Incubate at 37°C for 5 minutes.
4. If the fast digest green buffer was used, load an aliquot of the reaction mixture directly on a gel.

Interlab Study

- Using backbones made by Jasmine and Georgina (J+G) and also the backbone made by Bradley and Joe (J+B), from failed ILS attempt.
- gBlock construction protocol followed again on ILS gBlocks.
- Transformation protocol followed for the ILS gBlocks.

Made more Cam plates:

- Continuing with one of each plasmid (J+G's plasmid is pSB1C3 prepared on way, J+B's plasmid is also pSB1C3 prepared another way).
- Plating out: plasmid J/G at 50 ng x 3, plasmid J/G at 100 g x 3, plasmid J/B at 50 ng x 3, plasmid J/B at 100 ng x 3.

07/08/15

- Yesterday these were plated out: plasmid J/G at 50 ng x 3, plasmid J/G at 100 g x 3, plasmid J/B at 50 ng x 3, plasmid J/B at 100 ng x 3.
- These are plasmids prepped one way by J/G and another way by J/B.
- Interlab gBlocks were put into two differently prepared plasmids at two different concentrations.
- They do not glow green and there is limited growth (1-20 colonies).
- These will be sent for sequencing – put 10 µl of miniprepped sample into tubes, add labels and put in the send box.
- Did a typical digest DNA gel – same gel electrophoresis protocol we have always used.
- Ran the usual 2 ladders and 3 samples: I1C, I2A and D1.

10/08/15

- Started the ILS mostly from scratch again today.
- Digestion of ILS gBlocks: I1C, I2A, D1, D2 and D3.
- Ran a DNA gel on the backbones we were going to use to check if they had been cut properly. This wasn't evident, so we digested backbone provided by iGEM so we could compare the two gels.
- Ran both gels using the usual 1Kb ladder and generuler 1Kb plus ladder.
- The iGEM backbone was correct (according to the gel run) so the enzymes were working, from this point on we will be using iGEM provided backbone.

11/08/15

- Digested more iGEM pSB1C3 to get more backbone to use for ILS.
- gBlock construction of ILS constructs.
- Transformation of ILS constructs.
- Plating the iGEM ILS negative and positive control, NEB positive control, D1, D2 and D3.

12/08/15

- gBlock preparation of our toehold switches.
- gBlock construction of our toehold switches.
- There was some technical difficulties with the gBlock construction, meaning we had to re-work the concentrations and continue the construction. However more Toeholds have been ordered, if they fail to work.
- Transformation of ILS constructs.
- Transformation of Toehold switches.

13/08/15

- Prepared overnight cultures of our ILS constructs and the one Toehold set that worked (Zeus D2 J23100).
- Ran TECAN on our cultures of ILS constructs. Sequencing showed that the gBlock had gone in - TECAN showed that there was fluorescence of all 3 devices.

14/08/15

- Miniprepped the Toehold that had worked (Zeus D2 J23100) and then sent off for sequencing to see whether our Toehold had been successfully transformed.
- Made glycerol stocks of all ILS constructs.
- Made some more competent DH5α cells.
- Made 3 litres of LB broth for next week's experiments.

17/08/15

- Making 1 Litre of agar for more plates when we need them.
- Made 40% glycerol.
- Set up protocols on our FLUOstar microplate reader.
- Transformation of DNA 106C to check the competency of the homemade DH5α cells (used LB instead of SOC media).
- Plated out 106C three times.
- Overnight cultures of the ILS constructs and controls from glycerol stocks.

18/08/15

- Overnight cultures from yesterday worked so were prepared for fluorescence readings on the FLUOstar Omega microplate reader.
- Used a Spectrophotometer to measure the optical density of the overnight cultures, containing 900μl LB broth and 100μl of overnight culture.
- In a 2 ml tube containing 1 ml LB broth and varying volumes of of culture to get achieve optical density that equals 0.1. Used a pipetting robot to load 200μl total of LB broth and CAM mix and sample into the each well of the microplate.
- The microplate was loaded into the FLUOstar and set to measure OD and fluorescence over a 24 hour time period.

19/08/15

- Used Qubit to measure the DNA concentration of our ILS constructs.

- Used Qubit to measure the DNA concentration of two old toehold switches (Zeus T7 and Green T7).
- gBlock preparation of two of the new toehold switches that arrived (Zeus J23100 and Green J23100).
- gBlock construction of two of the new toehold switches that arrived (Zeus J23100 and Green J23100).
- Transformation of the two old and two new toehold switches, except used 950μl of LB broth rather than SOC media as there was none available to use.
- Plated out all four toehold switch transformations on CAM plates as well as the NEB positive control onto an AMP plate.

Prepared LB broth and CAM mix for microplate readings tomorrow:

- In a 250 ml flask; 100 ml of LB broth and 100μl of CAM was mixed.
- This was then aliquoted into forty 2 ml tubes (each containing 1 ml of the mix) and twelve 2 ml tubes (each containing 1.5 ml of the mix).
- Prepared 16 overnight cultures of ILS constructs (x3 of each D1, D2, D3, I20270 and R0040) plus one containing just DH5α cells.
- Overnight cultures were prepared from glycerol stocks of the ILS constructs and their repeats.

20/08/15

- Overnight culture FC.
- FLUOstar - 15 samples and 5 blanks, for 24 hours at 37℃ (gain = 1342).
- Robot pipetter to set up up microplates - one in FLUOstar and one in TECAN.
- gBlock construction of two new and two old TH's (ZJ, GJ, ZT7 and GT7).
- TECAN - 15 samples at 37℃for 6 hours (gain = 56).
- Transform two new TH's and two old TH's (ZJ, GJ, ZT7 and GT7).
- Plate all TH's two old and two new (ZJ, GJ, ZT7 and GT7).
- Overnight cultures of all ILS constructs.
- Overnight cultures of two TH's that worked after yesterday's transformation (ZJ and GJ).
- Made 3 ampicillin agar plates with a final antibiotic concentration of 0.001% i.e. used 40μl AMP in 40 ml of LB agar.

21/08/15

- Made glycerol stocks of our three further characterisation parts (eforRed, aeBlue and amajLime).
- Glycerol stocks of TH's that worked, i.e. GJ and ZJ.
- Miniprepped ILS positive and negative control so we would have the DNA ready for further use.
- Qubit ILS positive and negative control to determine the concentration of DNA for future reference.
- Miniprepped the TH's that worked so they could be sent for sequencing.
- Qubit all TH's to determine concentration of DNA to see how efficiently they were prepared.
- Qubit all further characterisation parts (eforRed, aeBlue and amajlime).
- TECAN - continued readings from same plate as yesterday, taking readings at t=22, t=23 and t=24.

24/08/15

- First cell free kit testing on two of the FC's - aeBlue and eforRed as these had enough DNA to meet the S30 cell free kit requirements.
- Overnight culture of all ILS constructs ready for FACS tomorrow.
- Overnight culture of all TH's from the transformation on thursday ready for glycerol stocks.
- Overnight cultures of FC's ready for miniprep so we can obtain higher levels of DNA to test final FC - amajlime.

25/08/15

- Miniprepped ILS constructs.
- Qubit of ILS constructs.
- FACS and Imagestream to measure and picture fluorescence of the ILS constructs.
- FLUOstar used to measure absorbance of the FC's from protein extraction.
- Protein extraction of FC's.
- Miniprepped TH's and sent for sequencing.
- Qubit TH's.
- Glycerol stocks of GT7 and ZT7.
- Miniprepped FC's and sent for sequencing.
- Qubit FC's.
- Agarose gel electrophoresis - did a digest of TH's.
- Protein SDS PAGE gel ran with FC samples loaded.
- Overnight cultures of ILS.
- Streak plate of all FC's.

26/08/15

- FLUOstar to measure ILS constructs cell free.
- Overnight cultures of FC's plus a DH5α control.
- FLUOstar used to measure absorbance of the FC's from protein extraction.

27/08/15

- Cell free set up of ILS constructs, trying to determine why it wasn't working - discovered luciferin was missing in the luciferase control.

28/08/15

- Imagestream to obtain images of our ILS constructs.

01/09/15

- Cell free testing of ILS constructs as a GFP control.
- Overnight cultures of TH's GT7A, GT7B, ZT7A, ZT7B, GJA and GJB.
- Protein extraction of FC's.

- Protein SDS PAGE gel ran with FC samples loaded.
- Miniprepped FC's.

02/09/15

- Miniprepped GJA, GJB, GT7A, GT7B, ZT7A and ZT7B.
- Sent ZJA, GJA, GT7B, ZT7B, and D2A for sequencing.
- Agarose gel electrophoresis - did a diagnostic digest of TH's.

07/09/15

- Q5 mutagenesis of ZJ and GJ.
- Protein SDS PAGE gel ran with FC samples loaded and BSA dilution series to check concentrations of the chromoprotein.
- gBlock preparation of GT7.
- gBlock construction of GT7 and ZT7.
- Transformation and plating of GT7 and ZT7.
- Transformation and plating of Q5 mutagenesis (GJ and ZJ).

08/09/15

- Q5 mutagenesis then plating of GJ and ZJ, the ones that failed yesterday.
- Made two bottles of solid agar - for use next week.
- Made 4 bottles of LB broth.
- Made 27 CAM plates.
- Overnight cultures of all TH's and Q5's.
- Rehydrated Green RNA for initial testing.

Cell free testing of GJ with Green RNA:

- At many different ratios; 0:1, 1:1, 10:1, 50:1, 500:1, 20,000:1 and then ILS D1 positive control.
- Measured fluorescence on FLUOstar plate reader and seems to have not worked therefore we will try again tomorrow with more measurements.

09/09/15

- Miniprepped and sequenced GT7 and ZT7.
- Miniprepped and sequenced the Q5's that worked.
- Cell free testing of GJ with Green RNA at many different ratios; 0:1, 20:1, 500:1, 20,000:1, 50,000:1 and then ILS D1 positive control. Measured fluorescence on FLUOstar plate reader and seems to have worked!
- Made glycerol stocks of GT7, ZT7, G-Xbai, G-Suffix and Z-stop.
- Overnight cultures of chromoproteins.
- Overnight cultures of Q5's - Zeus switch, Green suffix A and B, Zeus stem A, B, C, D, and E.

10/09/15

- Cell free testing of chromoproteins - running overnight on the FLUOstar microplate reader.
- Protein extraction and SDS PAGE gel ran with the three chromoprotein samples and BSA dilution series to check concentrations of the chromoprotein.
- Miniprepped Q5's from yesterday.
- Agarose gel electrophoresis - did a diagnostic digest of Q5's.

11/09/15

- Analysed cell free chromoprotein data that was run overnight.
- Standard curve of chromoproteins (eforRed, aeBlue and amajLime) using dilutions of 0, 0.1, 0.25, 0.5, 1 and 1.5. We worked out max dilutions using the concentrations we obtained from analysing the protein extraction on SDS PAGE yesterday.
- Q5 mutagenesis of Zeus Stop to add prefix and suffix, Green suffix to add prefix, and finally Green suffix to add Xbai site.
- Transformation and plating of Q5 mutagenesis constructs made today.

14/09/15

- Miniprepped Q5's - GJ, GJB, GSU + X, GSU + X2, GSU + X3 and GSU + prefix.
- Sent Q5's for sequencing.
- Transformation and plating of Q5 mutagenesis constructs.
- Made glycerol stocks of GJ, GJB, GSU + X, GSU + X2, GSU + X3 and GSU + prefix.

15/09/15

- Cell free testing of GJ toehold in 0:1, 20:1, 500:1, 20,00:1 and 50,000:1 ratios against wrong Green trigger and wrong Zeus trigger.
- Optical limit of chromoproteins to determine the concentration at which you can see expression of the chromoprotein with the naked eye.
- Qubit of GT7 toeholds.
- Overnight cultures of GJ, GJB, GSU + X, GSU + prefix, ZJ stop, ZJ minus stop, GSU + prefix minus psti site.

16/09/15

- Miniprep GJ, GJB, GSU + X, GSU + prefix, ZJ stop, ZJ minus stop, GSU + prefix minus psti site.
- Qubit GJ, GJB, GSU + X, GSU + prefix, ZJ stop, ZJ minus stop, GSU + prefix minus psti site.
- Cell free testing of GJ toehold in 0:1, 5:1, 50:1, 100:1, 200:1 and 400:1 ratios with correct Green trigger against wrong Green trigger and Zeus trigger.

17/09/15

- Miniprep of GT7 minus psti, ZJA, ZJ minus stop and GJ.
- Qubit of GT7 minus psti, ZJA, ZJ minus stop and GJ.
- Visual limit of chromoproteins.

- Cell free testing of GT7 - 0:1, 5:1, 50:1, 100:1, 200:1 and 400:1 with Green trigger against wrong Green trigger.