17/6

(Spencer, Laura, Henry, Richard, Janelle)

(p3) Transformation of iGEM plasmids in E. coli *(XLIB)*

Retrieved 7 plasmids from iGEM plate 4 with 10uL water. Added 1uL of each plasmid to 80uL CaCl2 competent XLIB cells then incubated for 2hr on ice. Transformed cells by heat shock at 37degC for 2 min. Incubated in 300uL LB at 37degC for 90min. Poured 1uL/mL antibiotic (chloramphenicol, kanamycin, ampicillin) in liquid LB agar, pour in plates to set. Plated 50uL of plasmid/cell solution onto antibiotic-supplemented LB agar plates.

1.1 (p5) Gel Electrophoresis of pUCiGEM1 and pUCiGEM2 digests

Note: pUCiGEM1 was digested with Nco1 and Sal1 and pUCiGEM2 was opened by Nco1 and Sal1 by Spencer previously.

Electrophoresis in agarose gel at 80V for 40 min. Smallest fragments were cut from gel and purified.

1.1 (p7) Ligation to produce pUC-PG1.

Ligated pUCiGEM1 insert with pUCiGEM2 vector. Incubated at 4degC overnight.

(p9) Fragment isolation from ordered genes.

Restriction digest as follows. Incubated at 37degC overnight.

Table 1. Plasmid digests

Plasmid	REs	Buffer	Exp. Fragment
pUCiGEM3	BglII / Sal1	D	eCFP (0.72 kbp)
pUCiGEM4	BglII / Xho1	D	yCFP (0.72 kbp)
pUCiGEM5	BamH1 / Xho1	B	NPTII (0.8 kbp)
pUCiGEM6	BamH1 / Xho1	B	AmiGFP (0.7 kbp)
pUCiGEM7	BamH1 / Xho1	B	AmiCP (0.67 kbp)
pUCiGEM8	BamH1 / Xho1	B	HSLYZ (0.4 kbp)

18/6

(Spencer, Matt, Henry, Janelle)

1.1 (pg11) Gel Electrophoresis of Plasmid digests from pg9

Electrophoresis in agarose gel at 80V for 45 min for each of the 6 digested plasmids from pg9. Smallest fragments of each lane were cut from gel and cleaned via Wizard SV Gel and PCR clean up system (see protocol in lab book). Isolated fragments were placed in freezer.

1.1 (pg13) Transformation of XLIB E. Coli with Ligated plasmid 1.1 (from pg7)
Removed ligation product and control from fridge and left at RT for 30 min. Added 80 uL of CaCl2 competent cells on ice. Left on ice for 2 hrs. Heatshock at 37degC for 2 min. Added 300 uL LB and incubated at 37degC for 90 min. Centrifuged to pellet, removed supernatant and resuspended in 100uL. Plated on Ampicillin supp. agar. Incubated at 37degC overnight.

19/6
(Spencer, Matt, Henry, Janelle)

(pg2) Repeat of Chloramphenicol Transformation (from pg2)
One colony was transferred from each of the 3 repeated plates to chloramphenicol supplemented LB and left on bench.

1.1 (pg13) Culturing of Transformed cells with 1.1
Some colonies were observed for the control plate (a ligated plasmid without insert) therefore two colonies were taken from the transformed cells and cultured in 2 mL of LB supplemented with ampicillin.

(pg15) Miniprep and digestion of iGEM Plasmids from E. coli
Pelleted all (10 mL) of liquid culture for low copy number plasmids (pSB1A3 and pSB1K3 observed pink colour) and pelleted 1.5 mL of liquid culture for high copy numbers (pSB4A5 and pSB4K5). Plasmids purified then digested using 0.25 uL of Xho1 and Spe1 each. Incubated at 37degC for 3.5 hrs.
Separated fragments by gel electrophoresis for approximately 60 minutes.

The gel did not show expected fragments - particularly for pSBIK3 showed 2 ~1000kb bands. This may possibly be due to using a dead restriction enzyme. Repeated restriction enzyme digest with NotI instead of SpeI/XhoI. Furthermore miniprepped 2 more colonies of pSBIK3.
*SpeI/XhoI digest unexpected fragments as pSB1K3 plasmid actually has 2 XhoI sites which give fragments of 1026bp, 1031bp, 1216bp - as observed - so plasmids are correct.

WEEK 2

22/6
(Spencer, Matt, Janelle)

2.1 (p. 21) Restriction digest of pUCiGEM5
Prepared mastermix with 2uL of 10x Buffer B, 0.5uL SpeI, 0.5uL XbaI and 7uL of water. Added 10uL of mastermix to 10uL of pUCiGEM5 to cut out insert. Incubated at 37degC for 30min.

(p. 21) Gel electrophoresis of iGEM plasmids and inserts

Separated digested fragments of XhoI/SpeI cut iGEM plasmids, NcoI/HindIII cut pET16, BglII/SalI and NcoI/HindIII cut pUC-PG1, the NotI digest for the chloramphenicol-resistance iGEM plasmids, XbaI/SpeI cut pUC-iGEM5.
pSB4K5 fragment may not have run due to obstructions in gel.

(p. 23) Gel cleaning and ligation
Relevant insert/vector bands cut out from gel then collected in tubes in **Table 2**:

Table 2

Tube	Sample	REs
1	psBIC3	XbaI/SpeI
2	psB3C5	XbaI/SpeI
3	psBIA3	XbaI/SpeI
4	psB4A5	XbaI/SpeI
5	pUC-iGEM5	XbaI/SpeI
6	pUC-PG1	BglII/SalI
7	pUC-pG1	NcoI/HindIII
8	pET16	NcoI/HindIII
9	psB4C5	XbaI/SpeI
10	psB4K5	XbaI/SpeI
11	psB4K3	XbaI/SpeI

DNA extracted from gel.

Ligation reactions set up with insert and vectors shown in Column 2 and 3 (**Table 3**). Incubated at RT for 50 minutes.

Table 3.

Expt	Insert	Vector	Resistance
2.3a	XbaI/SpeI pUC-iGEM5	XbaI/SpeI pSBIC3	chloramphenicol
2.3a C	XbaI/SpeI pUC-iGEM5	XbaI/SpeI pSBIC3	
2.3b	XbaI/SpeI pUC-iGEM5	XbaI/SpeI pSB3C5	

2.3b C	-	XbaI/SpeI pSB3C5	
1.2a	BglII/SalI eCFP	BglII/SalI pUC-PG1	ampicillin
1.2b	BglII/XhoI eYFP	glII/SalI pUC-PG1	
1.2 C	-	BglII/SalI pUC-PG1	
2.1	NcoI/HindIII pUC-PG1	NcoI/HindIII pET-16	
2.1 C1	-	NcoI/HindIII pET-16	
2.1 C2	NcoI/HindIII pUC-PG1	-	

Poured 1uL/mL ampicillin-supplemented agar plates.

(p. 25) Transformation of E. coli *with ligation products*
E. coli transformed with ligation products as per protocol on page 13. Ligation products added to 200uL CaCl2 comptentent cells on ice, then heat shocked for 2 min. Cells rested on ice again for 5 minutes before incubation on shaker at 37degC for 30 min. Cells spun down at 8000rpm for 1 min then plated out on ampicillin/chloramphenicol-supplemented LB plates and incubated at 37degC overnight.

23/6

(Spencer, Ellen, Janelle)

(p. 23) Transformation of E. coli *with ligation products from* (**Table 3**)
The appropriate antibiotic-supplemented liquid LB was inoculated with a single colony for each replicate. The 2.3a and 2.3b colonies were small so were left at 37degC.

Table 4.

Experiment	Colonies	Inoculate LB vol	Replicates
2.3a	~240	1.5mL	1
2.3a C	3	-	-
2.3b	>800	7.5mL	2 (2.3b(1), 2.3b(2))

2.3b C	~18	-	-
1.2a	~400	1.5 mL	2 (1.2a(1), 1.2a(2))
1.2b	~320	1.5 mL	2 (1.2b(1), 1.2b(2))
1.2 C	~30	-	-
2.1	~18	7.5 mL	4 (2.1a, 2.1b, 2.1c, 2.1d)
2.1 C1	~3	-	-
2.1 C2	~11	-	-

24/6

(Spencer, Ellen, Janelle)

(*p. 25*) *Ligation product plasmid purification*
Transformed ligation products from previous day were spun down and the pellet resuspended. 10uL of each culture was used to purify plasmid DNA. Lysate was centrifuged for an additional ten minutes because a proper pellet didn't form due to insufficient mixing after addition of neutralisation solution.

(p. 29) Restriction digest of purified ligation products 2.3a, 2.3b, 1.2a, 1.2b, 2.1

Restriction enzyme experiments are shown below. The digests were added and incubated at 37degC for 2 hours.

Table 5

Experiment	Plasmid	Digest	Expected fragments (kb)
1.2a	pUC-PG2	BglII/SalI	0.73 + 6
	pUC-PG2	BamH1/Xho1	0.72 + 6(collect)
1.2b	pUC-PG3	BglII/SalI	6.7 (linear)
	pUC-PG3	BamHI/Xho1	0.72 + 6(collect)
2.3a	pUC-PG6	XbaI/Spe1	1.82 + 2.2

2.3b	pUC-PG7	Xba1/Spe1	1.82 + 2.8
2.1	pET-CRE1	Xba1/Spe1	5.4 + 3.6(collect)

(p. 29) Gel electrophoresis of ligation plasmids 2.3a, 2.3b, 1.2, 2.1
1g/100mL agarose gel with 10uL SYBRSafe DNA stain made and used. Electrophoresis of restriction digest treated plasmids was run in agarose gel at 80V for 60 min. All experiments were as expected except for 2.1d which was discarded. Replicates were consolidated into same containers.

The 6kb fragments from 1.2a(2) and 1.2b(2), and the 3.6kb fragment from 2.1c were cut from the gel and cleaned as outlined on p5. Fragments were then dried in speedVAC for 5 minutes on low.

(p. 31) Ligation of plasmid segments from 1.2a, 1.2b and 2.1
Ligation experiments were run with the vectors and inserts shown in **Table 6**. The ligations were conducted by adding 1uL 10x ligation buffer, 2uL vector and 1uL ligase followed by the addition of 6uL of insert (or RNAse free water for controls).

Table 6

Experiment	**Vector**	**Insert**
1.3a	pUC-PG2(BamH1/Xho1)	eYFP(BglII/SalI)
1.3a control	pUC-PG2(BamH1/Xho1)	none (water)
1.3b	pUC-PG3(BamH1/Xho1)	eCFP(BglII/SalI)
1.3b control	pUC-PG3(BamH1/Xho1)	none (water)
2.2c	pSB1A3(XbaI/Spe1)	pET-CRE1(Xba1/Spe1) 3.6kb fragment
2.2c control	pSB1A3(XbaI/Spe1)	none (water)
2.2d	pSB4A5(Xba1/Spe1)	pET-CRE1(Xba1/Spe1) 3.6kb fragment
2.2d control	pSB4A5(Xba1/Spe1)	none (water)

(p. 31) 2.1, 2.3a, 2.3b. Transformation of pET-CRE1, pSBIC-PG6, pSB3C-PG7 into E. coli
Prepared 2 x 1.5mL suspensions of BL21 *E. coli* in LB. Pelleted and resuspended in 15% glycerol, 0.1M CaCl2. Added 200uL cells to 1uL plasmid (pET-CRE1, pSBIC-PG6, pSB3C-PG7). Incubated for 30min on ice, heat shock for 2min in 37degC waterbath, return to ice, add 200uL LB-Ab, incubate for 1.5hr at 37degC on shaker.

(p. 31) 2.2a/b RE digest of pET-CRE1 controls

Prepared Mastermix of 6uL 10x Buffer B, 30uL pET-CRE1, 22uL water and 2uL of StuI. To 40uL of this, added 2uL of NheI, then split this solution into 2 x 20uL.

25/6

(Spencer, Ellen, Janelle)

(p. 33) 2.1, 2.3a, 2.3b. Transformation of pET-CRE1, pSBIC-PG6, pSB3C-PG7 into E. coli

pSBIC-PG6 transformation yielded no colonies, therefore repeated transformation with 2 hour incubation with plasmid.

pSB3C-PG7 transformation yielded ~25 colonies (varying sizes) and pET-CRE1 transformation yielded ~90 colonies. 5mL overnight cultures of one colony from each plate were prepared to make glycerol stocks for storage.

(p. 33) 2.2a/b Gel electrophoresis, ligation and transformation of pET-CRE1 controls

Ran gel for ~40 minutes.

Cleaned gel via protocol on page 5. Religation of linearised plasmid from 2.2a and ligation of 2.2b 260bp and large fragments to give pET-CRE2 and pET-CRE3 respectively.

For 2.2a, added 6uL H2O as no insert (no control). For 2.2b, added 2uL insert and 4uL water as not enough insert. Incubated for 1 hour at room temperature.

Transformed into *E. coli* with 200uL LB-Amp.

(p. 33) Transformation of 1.3a, 1.3b, 2.2c, 2.2d ligation products

Added 200uL competent cells to each. Incubated for 25 minutes of ice, 2 minute heat shock at 37degC, returned to ice then added 300uL of LB-Amp. Incubated for 90 minutes at 37degC.

26/6

(Spencer, Janelle)

(p. 35) Plates from 25/6.

Table 7

Experiment	Plasmid	Number of colonies
2.2a	pET-CRE2	3
2.2b	pET-CRE3	0 (0 in control)
2.2c	pSB1A-CRE1	14 (0 in control)
2.2d	pSB4A-CRE1	~140 (1 red, 1 red colony in control)
2.3a	pSB1C-PG6	~80 colonies (various sizes)
1.3a	pUC-PG4	>200 colonies (various sizes) (0 in control)
1.3b	pUC-PG5	>200 colonies (vairous sizes) (0 in control)

Repeated 2.2b pET-CRE1 digest and ligation.
Prepared Mastermix: 4uL 10x Biffer B, 20uL pET-CRE1, 2uL StuI, 2uL NheI, 12uL water. Split into 30uL sample to collect 270bp fragment and 10uL sample to dephosphorylate and collect large fragment.

(p. 37) 2.4 Cotransformation of BL21 E. coli *with pET-CRE1 and PSBIC-PG6/PSB3C-PG7.*
Pelleted ~5mL overnight culture of pET-CRE1 transformed BL21 in LB-Amp. Resuspended in 200uL 15% glycerol, 0.1M CaCl2 for transformation with PSBIC-PG6/PSB3C-PG7. Plated on ampicillin and chloramphenicol-supplemented agar.

(p. 39) 2.1, 2.3a, 2.3b Preparation of pET-CRE1, pSB3C-PG7 and PSB1C-PG6 glycerol stocks
Combined 300uL sterile 80% (v/v) glycerol and 500uL miniprep culture of cells, froze in liquid nitrogen and stores at -80degC in freezer box SWA-4.

27/7
(Spencer, Janelle)

(p. 35) 2.2b Repeat transformation of pET-CRE3
Digest fragments separated by gel electrophoresis. 270bp fragment and dephosphorylated vector backbone cut out and purified. Fragments ligated and incubated for 2 nights at 4degC.

(p. 35) 2.2a, c,d Transformation of CRE1 plasmids
Purified plasmids, transformed into *E. coli* and plated on ampicillin-supplemented agar. Checked plasmids by digestion with SpeI and XbaI. Digest incubated for 2 nights.

(p. 37) 2.4 Cotransformation of BL21 E. coli *with pET-CRE1 and PSBIC-PG6/PSB3C-PG7.*
pSB3C-PG7/pET-CRE1 transformation yielded 2 colonies. Both colonies inoculated in LB.
pSB1C-PG6/pET-CRE1 transformation yielded no colonies.
Poured 1uL/mL ampicillin/chloramphenicol supplemented agar plates.
Both co-transformations repeated. Plates incubated for 2 nights at room temperature.

(p. 41) 2.4 Test of pET-CRE1/pSB3C-PG7 transformed BL21 for blue-light induced kanamycin resistance & RFP synthesis
Poured 1uL/mL ampicillin/chloramphenicol and ampicillin/chloramphenicol/kanamycin supplemented agar plates; half also added 8uL 0.1mM IPTG.
Plated and stored transformed BL21 in gel-viewing box under following conditions (**Table 8**).
Plates kept in dark wrapped in aluminium foil and placed above plates in blue light, wrapped in cling wrap and placed above blue light source on plate (so plates do not heat up) for 2 nights.

Table 8

	Dark		**Blue**	
Antibiotic	**+IPTG**	**-IPTG**	**+IPTG**	**-IPTG**

Amp/Chlor	red	white	red/white	white
Amp/Chlor/Kan	no growth	no growth	(red)/white	no growth

29/6
(Spencer, Laura, Janelle)

30/6
(Spencer, Laura, Janelle)