Lethbridge iGEM Collegiate 2013 Notebook (September)

September 1 2013

Miniprep of overnight cultures

We mini-prepped the overnight cultures of J04500- E-lumazine
Followed BioBasic Protocol from BioBasics miniprep kit.

September 2 2013

Confirmation of RFP for delivery of new construct synthesis

We needed to send pSB1C3 in for delivery of our new PK401 construct. Had an old prep of J04450 that needed to be confirmed.

J04450 in pSB1C3
16ul water
0.5ul BSA
2.5ul NEB Buffer 2
5ul upstream part
0.5ul EcoRI
0.5ul PstI

Confirmation gel: 1% 20ml agarose gel (TAE)– ran @ 135v for 30 mins in 1x TAE

Lane 1 - 1ul Ladder, 1ul 6x loading dye, 4ul TAE

Lane 2 - 1ul J04450 (E and P), 1ul 6x loading dye, 4ul TAE

Lane 3 - 1ul J04450 uncut, 1ul 6x loading dye, 4ul TAE

No bands present in lanes 2 and 3. The preps are no good.

Received sequencing data for our PK401-pSB1C3 plasmid

The previous insertion of PK401 into pSB1C3 was confirmed as unsuccessful when we received the sequencing results. We will begin assembly again.

Testing for expression of E-lumazine synthase

1 uL of overnight culture containing LB culture and J04500-E-Lumazine in pSB1K3 plasmid in E.coli into 99 uL of fresh LB media containing 100 uL of Kanamycin antibiotic.

Time (24 hour clock)	OD_{600}
16:07	0
17:07	0.025
18:07	0.457
18:40	0.392
19:09	0.407
19:50	0.419
20:40	0.440
21:30	0.549
22:05	0.614 (100 uL of IPTG was added, sample 1 taken)
23:08	1.262 (sample 2 taken)
23:55	1.355
1:05	1.438

Confirmation gel: 1% 20ml agarose gel (TAE) – ran @ 100v for 30 mins in 1x TAE

Lane 1 – 1ul Ladder, 1ul 6x Loading dye, 4ul TAE

Lane 2 - 1ul Pseudoknot PCR to isolate pseudoknot, 1ul 6x Loading dye, 4ul TAE

Lane 3 - 1ul RFP, 1ul 6x Loading dye, 4ul TAE

September 3 2013

Retrying confirmation of RFP for delivery of new construct synthesis

We needed to send pSB1C3 in for delivery of our new PK401 construct. Tried 3 other J04450 that were found in storage in the -20c freezer.

J04450 in pSB1C3 (x3)
16ul water
0.5ul BSA
2.5ul NEB Buffer 2
5ul upstream part
0.5ul EcoRI
0.5ul PstI

Confirmation gel: 1% 20ml agarose gel (TAE)– ran @ 135v for 30 mins in 1x TAE

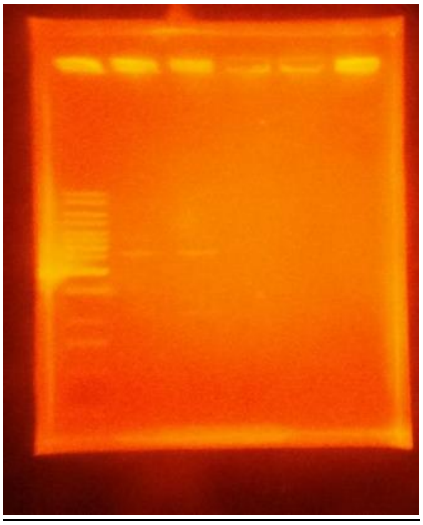
Lane 1 - 1ul 1kb Ladder, 1ul 6x loading dye, 4ul TAE

Lane 2 - 1ul J04450 A (E and P), 1ul 6x loading dye, 4ul TAE

Lane 3 - 1ul J04450 B (E and P), 1ul 6x loading dye, 4ul TAE

Lane 4 - 1ul J04450 C (E and P), 1ul 6x loading dye, 4ul TAE

Correct bands are present in lanes 2 and 3 indicating that these preps both contain J04450. Prep A was sent as the backbone for our new construct.



Induction timecourse of PK401 and control cultures.

OD600						
Time (hrs)	PK401	K542006	ECFP	EYFP	K542001	K542000
0	0.127	0.148	0.167	0.160	0.146	0.169
1	0.282	0.373	0.296	0.247	0.354	0.380
2	0.697	0.819	0.8	0.503	0.777	0.761
2.5* added 1mM IPTG	0.943	0.984	1.055	0.661	0.964	0.959
3.5	1.23	1.19	1.345	0	1.171	1.131
4.5	1.372	1.291	1.497	0	1.276	1.214

September 5 2013

Running a tris-tricine gel for E-lumazine expression

Ran a tris-tricine gel in order to try and see expression of Elumazine as SDS PAGE did not have good enough resolution.

DEFINE SAMPLES

Lane	Content
1	Standard
2	S1
3	S2
4	S3
5	S4

Transformed PK401 CFP-YFP-1 in pUC57 Kan+. Need to transfer it into pSB1C3 and send it for sequencing ASAP.

New primers were resuspended in milliqH_2O at 100uMol then aliquoted to 10uMol

Ran 2 PCR's under 8 different conditions

Component	Volume (uL)	MM
Milliq H_2O	3.77	30.16
10 X t4 Pfu buffer	0.5	4
10 um Primer 1	0.1	0.8
10 um Primer 2	0.1	0.8
DNA	0.4	3.2
Pfu polymerase	0.1	0.8
10 um dntps	0.1	0.8

Taq buffer and polymerase were used with primers 7 and 8.

Thermocycler conditions

Step	Temperature (°C)	Time (seconds)
Initial Denaturation	95	180
30 cycles	95	30
"	X	30
"	68	200
Final extension	68	5
Hold	4	∞

X values for thermocycler conditions in table found below

LABEL THIS

Tube	Temperature (°C)
T1	45.0
T2	45.5
T3	46.9
T4	49.0
T5	51.4
T6	53.8
T7	56.2
T8	58.6
P1	49.0
P2	51.4
P3	53.8
P4	56.2
P5	58.6
P6	60.9
P7	63.1
P8	64.5

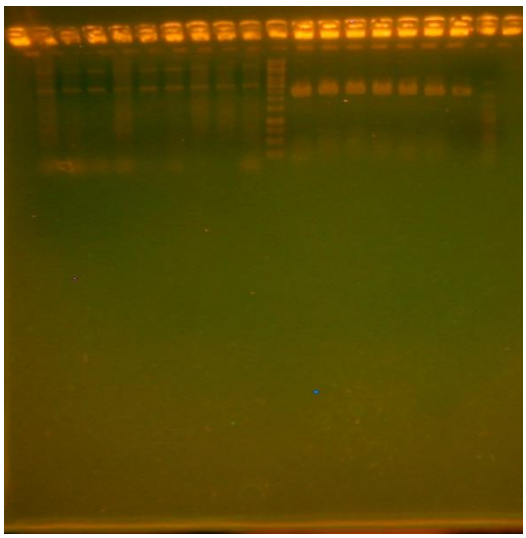
September 23rd 2013

Made an unbalanced dNTP's mix for the error prone PCR of PK401

Component	Volume (uL)
dATP	20
dGTP	20
dTTP	100
dCTP	100
milliqH2O	260

Ran an error prone PCR on PK401

	PCR conditions								
Component	1	2	3	4	5	6	7	8	9
milliqH20	19.35	19.1	18.8	18.9	18.7	18.1	19.8	17.4	18.7
5X go Taq flexi buffer	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
dGTP (2mM)	0.5	0.5	0.5	1.5	2.5	2.0	-	2.5	-
Unbalanced dNTP's (10X)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	-	-
dNTP's 10mM	-	-	-	-	-	-	-	0.5	0.5
iGEM 2013 primer 7	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
iGEM 2013 primer 8	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Template DNA	1	1	1	1	1	1	1	1	1
Taq Flexi polymeraze	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
MnCl2	-	0.290	0.6	0.8	1.14	0.9	-	-	1.14
MgCl2	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8



Lane	Contents
1	Error prone PCR condition 1
2	Error prone PCR condition 2
3	Error prone PCR condition 3
4	Error prone PCR condition 4
5	Error prone PCR condition 5
6	Error prone PCR condition 6
7	Error prone PCR condition 7
8	Error prone PCR condition 8
9	Error prone PCR condition 9
10	1 KB ladder
11	J04500-Elum-B0015 colony A
12	J04500-Elum-B0015 colony B
13	J04500-Elum-B0015 colony C
14	J04500-Elum-B0015 colony D
15	J04500-Elum-B0015 colony E
16	J04500-Elum-B0015 colony F
17	B0015 in pSB1C3
18	100 bp ladder

September 25, 2013

Sequencing of J04500-Elum-B0015

Component	Volume (uL)
DNA	1.0
Primer	2.5
Milliq H_2O	2.5