Lethbridge iGEM Collegiate 2013 Notebook (July)

July 9 2013

Restriction of K542003 and K542005 for assembly

K542003 (Upstream Part)	K542005 (Downstream Part)
16ul water	16ul water
0.5ul BSA	0.5ul BSA
2.5ul NEB Buffer 2	2.5ul NEB Buffer 2
5ul upstream part	5ul downstream part
0.5ul SpeI	0.5ul XbaI
0.5ul PstI	0.5ul PstI

-mixtures made

-incubated at 37C for 1 hour

-heat killed at 80C for 20 mins

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 Usptream part, 1ul 6x Loading dye, 4ul TAE

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

Sizes:

K542003 – 978bp

K542005 – 1755bp

pSB1C3 – 2070

July 10 2013

Restrict K542005 for assembly with X and P

K542005
16ul water
0.5ul BSA
2.5ul NEB Buffer 2
5ul upstream part
0.5ul XbaI
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 K542005, 1ul 6x Loading dye, 4ul TAE

July 11 2013

Ligation of K542003 and K542005
Followed iGEM 2011 book 1 page 11 protocol x3

Transformation of Ligation

Followed iGEM 2011 protocol

July 12 2013

Restriction of New and Old K542005 and K542003 samples

K542003 (Upstream Part)	K542005 (Downstream Part)
16ul water	16ul water
0.5ul BSA	0.5ul BSA
2.5ul NEB Buffer 2	2.5ul NEB Buffer 2
5ul upstream part	5ul downstream part
0.5ul SpeI	0.5ul XbaI
0.5ul PstI	0.5ul PstI

-mixtures made

-incubated at 37C for 1 hour

-heat killed at 80C for 20 mins

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

Gel 1 (right)

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

July 14 2013

Miniprep and confirmation of K542005 and K542003 assembly

Followed Qiagen kit miniprep protocol

Ligation A	Ligation B	Ligation C
16ul water	16ul water	16ul water
0.5ul BSA	0.5ul BSA	0.5ul BSA
2.5ul NEB Buffer 2	2.5ul NEB Buffer 2	2.5ul NEB Buffer 2
5ul upstream part	5ul upstream part	5ul downstream part
0.5ul EcoRI	0.5ul EcoRI	0.5ul EcoRI
0.5ul PstI	0.5ul PstI	0.5ul PstI

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

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

Lane 2 - 1ul Ligation A cut, 1ul 6x Loading dye, 4ul TAE

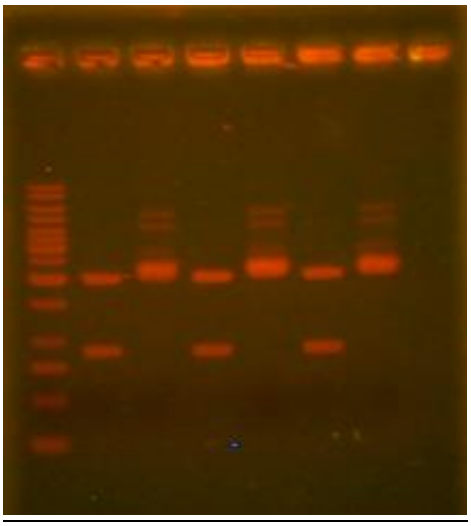
Lane 3 - 1ul Ligation A uncut, 1ul 6x Loading dye, 4ul TAE

Lane 4 - 1ul Ligation B cut, 1ul 6x Loading dye, 4ul TAE

Lane 5 - 1ul Ligation B uncut, 1ul 6x Loading dye, 4ul TAE

Lane 6 - 1ul Ligation C cut, 1ul 6x Loading dye, 4ul TAE

Lane 7 - 1ul Ligation C uncut, 1ul 6x Loading dye, 4ul TAE



Looks as though the restrictions used for the ligations were incomplete, no successful assemblies

July 15 2013

Agarose gel of all restricted and unrestricted samples

July 20 2013

Receipt of CFP-PK401-YFP synthesis order

Received in pUC57 with Kan resistance (4ug lyophilized DNA)

Resuspended in 40ul h20

Resuspension transformed

- 2ul DNA added to 20ul cells
- 30 minutes on ice
- 45 seconds in 42C waterbath
- 5 minutes on ice
- Added 400ul of SOC media
- Tube placed in shaker at 37C for 1 hour
- 100ul and 200ul of mixture plated and placed at 37C overnight.

July 23 2013

Re-run restriction of K542005 and K542003 for eLumazine assembly

K542003 (Upstream Part)	K542005 (Downstream Part)
16ul water	16ul water
0.5ul BSA	0.5ul BSA
2.5ul NEB Buffer 2	2.5ul NEB Buffer 2
5ul upstream part	5ul downstream part
0.5ul SpeI	0.5ul XbaI
0.5ul PstI	0.5ul PstI

Putting the CFP-PK401-YFP into pSB1C3

Picked two colonies from each of the 200ul (PK401 2A and 2B) and 100ul (PK401 1A and 1B) transformation plates.

Restrictions of PK401 and destination plasmid

CFP-PK401-YFP (pUC57)	J04450 (pSB1C3)
16ul water	16ul water
0.5ul BSA	0.5ul BSA
2.5ul NEB Buffer 2	2.5ul NEB Buffer 2
5ul upstream part	5ul downstream part
0.5ul EcoRI	0.5ul EcoRI
0.5ul PstI	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 PK401 2A, 1ul 6x Loading dye, 4ul TAE

Lane 3 - 1ul PK401 2B, 1ul 6x Loading dye, 4ul TAE

Lane 4 - 1ul PK401 1A, 1ul 6x Loading dye, 4ul TAE

Lane 5 - 1ul PK401 1B, 1ul 6x Loading dye, 4ul TAE

Lane 6 - 1ul J04450, 1ul 6x Loading dye, 4ul TAE

Lane 7 - 1ul K542005, 1ul 6x Loading dye, 4ul TAE

Lane 8 - 1ul K542003, 1ul 6x Loading dye, 4ul TAE

K542005 and K542003 / PK401 and pSB1C3 ligations

Attempt to take restrictions and do a shotgun ligation of inserts (PK401 -2A, 2B, 1A, 1B/ K542005) and backbones (J04450/K542003)

Ligation Mixtures:

11.5ul H2O	11.5ul H2O
4ul PK401 restricted with E and P	4ul K542005 restricted with X and P
4ul J04450 restricted with E and P	4ul K542003 restricted with S and P
2ul T4 Ligase buffer	2ul T4 Ligase buffer
0.5ul T4 Ligase	0.5ul T4 Ligase

Incubated at Room temperature for 2 hours

Transformed using protocol on July 20th 2013

July 25 2013

Liquid cultures of ligations started

Found the glycerol stock of K542006

- K542006 is K542003 and K542005 in the same vector

Restriction of J04500-eLum and K542006 for assembly

J04500-eLum (upstream)	K542006 (Downstream)
16ul water	16ul water
0.5ul BSA	0.5ul BSA
2.5ul NEB Buffer 2	2.5ul NEB Buffer 2
5ul upstream part	5ul downstream part
0.5ul SpeI	0.5ul XbaI
0.5ul PstI	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 K542006 (X and P), 1ul 6x loading dye, 4ul TAE

Lane 3 - 1ul J04500-eLum (S and P), 1ul 6x loading dye, 4ul TAE

Lane 4 - 2ul K542006 (X and P), 2ul 6x loading dye, 8ul TAE

Lane 5 - 2ul J04500-eLum (S and P), 2ul 6x loading dye, 8ul TAE

July 26 2013

Miniprep of July 23 ligations and transformations of PK401 in pSB1C3

Followed Biobasic miniprep protocol

Restricted minipreps

PK401 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 kB Generuler Ladder, 1ul 6x loading dye, 4ul TAE

Lane 2 - 1ul PK401 1A, 1ul 6x loading dye, 4ul TAE

Lane 3 - 1ul PK401 1B, 1ul 6x loading dye, 4ul TAE

Lane 4 - 1ul PK401 2A, 1ul 6x loading dye, 4ul TAE

Lane 5 - 1ul PK401 2B, 1ul 6x loading dye, 4ul TAE