Lethbridge iGEM Collegiate 2013 Notebook (August)

August 7 2013

Overexpression and S30 extract of PK401 and control cultures

- 50ml cultures with PK401 in pSB1C3 and a control plasmid (n-terminal arginine tag in pSB1C3) grown overnight with 1mM IPTG
- Cultures pelleted for 10 minutes at 5000g
- 7ml Buffer A added per gram of cell pellet (3.22ml Buffer A added)
- Cells resuspended in Buffer A by stirring on ice
- Lysozyme added to final concentration of 1mg/ml (3.2 mg) and continue stirring
- Sodium deoxycholate added to 12.5mg/g of cells (5.75 mg) and continued stirring
- A few crystals of DNase added
- Mixture stirred in 4C fridge for 2 hours
- Spun at 3000g for 30 minutes at 4˚C
- Spun at 30,000g for 45 minutes at 4˚C

Rough FRET analysis of S30 Lysates

S30 extracts from PK401 overexpression cultures stimulated @ 424nm and 510nm
Measure emission from 500-600nm

Time course expression analysis of PK401

- 50 ml cultures started from 5ml overnight uninduced cultures (2 PK401 cultures and 1 control – n-terminal arginine tag)
- 2ml Samples taken at each hour and OD600 taken
- 1 ml of sample suspended in 50ul 8M urea
- Left over culture pelleted at 5000g and kept in -20c freezer

OD600			
Time (hrs)	PK401-A	PK401-B	N-term Tag
0	0.141	0.15	0.164
1	0.305	0.314	0.40
2 * added 1mM IPTG	0.759	0.705	0.813
3	1.015	1.023	1.048
4	1.2	1.185	1.177
5	1.374	1.317	1.288

August 12 2013

SDS gel of Timecourse Overexpresssion

2 SDS Gels

12% Resolving Gel

5% Stacking Gel

The gel did not give an indication of expression levels. Construct must not be correct.

August 15 2013

Ran a PCR in order to isolate the pseudoknot

Component Volume

Rxn Components	Volume (uL)
Milliq h20	37.7
10x pfu buffer.	5
10um igem 2013 primer 1.	1
10 um igem 2013 primer 2.	1
Synthesized pseudoknot construct DNA.	4
pfu polymerase	1
10 um dntps.	1

Thermocycler Conditions

Step	Temperature (°C)	Time (seconds)
Initial Denaturation	95	30
30 cycles	95	30
“	65	30
“	68	60
Final extension	68	300
Hold	4	∞

August 29 2013

Overnight cultures of J04450 and J04650

We put glycerol stocks of J04450 and J04650 in LB media overnight (5 uL LB + 5 uL chloramphenicol)

August 30 2013

Minipreps of J04450 and J04650

We mini-prepped the overnight cultures of J04450 and J04650.

Followed BioBasic Protocol from BioBasics miniprep kit.

August 31 2013

PCR of J04450 and J04650

Ran a PCR in order to isolate the pseudoknot from the 2013 iGEM synthesized construct

Reaction components	Volume (uL)
milliq H_2O	37.7
10X Pfu buffer	5
10 um primer 1 (iGEM-2013-1 PK forward)	1
10 um primer 2 (iGEM-2013-1 PK reverse)	1
PK construct DNA	4
Pfu polymerase	1
10 um dntps	1

Also growing BBA_J04500-eLumazine overnight (5 uL LB + 5 uL chloramphenicol)

Thermocycler Conditions

Step	Temperature (°C)	Time (seconds)
Initial Denaturation	95	30
30 cycles	95	30
“	65	30
“	68	60
Final extension	68	300
Hold	4	∞