Lab Diary for Stress Lights 2.0

Date: Friday, 19 August 2011 16:00
Topic: Transformation of competent cells with mNark, RBS, YFP.LVA & Terminator

Organism: TOP *E Coli*
Labelling codes: E for digestion and M for ligation mixtures.

Build an improved version of oximeter based on the 2009 UCL iGEM submission using the mNark promoter and GFP

1) Carried out transformation of competent cell stocks separately with RBS (2M), YFP.LVA (6E) and Terminator (23L) DNA from iGEM Kit Plate 1 (spring 2011 distribution kit)
Carried out transformation of competent cell stock with mNark mini-prep sample form UCL iGEM 2009 storage box

2) Plated the cells for mNAKR, RBS, YFP.LVA and terminator on 3 separate ampicillin plates and placed them inside the incubator for overnight growth at 37°C.

Date: Saturday, 20 August 2011 18:00
Topic: Set up overnight cultures

1) Set up overnight cultures from a single colony from each of the transformed cell plates in selective LB medium. Left the cultures to grow overnight inside the shaker.

Date: Sunday, 21 August 2011 11:00
Topic: Restriction digestion of individual parts & ligation

1) Mini-prepped the overnight cultures into a single eppendorf tube of 60 µl volume each separately and measured concentration using NanoDrop:

Sample	First	Second	Mean
mNARK/RBS	99.3	98.5	**98.90**
mNARK (2)	84.1	84.2	**84.15**
RBS (1)	29.4	31.4	**30.40**
RBS (2)	25.8	28.4	**27.10**
YFP.LVA (1)	38.8	41.0	**39.90**
YFP.LVA (2)	32.4	33.3	**32.85**
TER (1)	29.0	36.9	**32.95**
TER (2)	33.2	29.9	**31.55**

Error: The spectrophotometer was blanked with water and not elution buffer.

Remeasured DNA concentration of mini-prep samples (2) using elution buffer as blank:

Sample	First	Second	Mean
mNARK (2)	85.2	87.0	**86.10**
RBS (2)	64.7	50.1	**57.40**
YFP.LVA (2)	34.0	36.5	**35.25**
TER (2)	33.1	41.9	**37.50**

Concentration appears to be higher rather and so we should have sufficient DNA for our digestion mixtures.

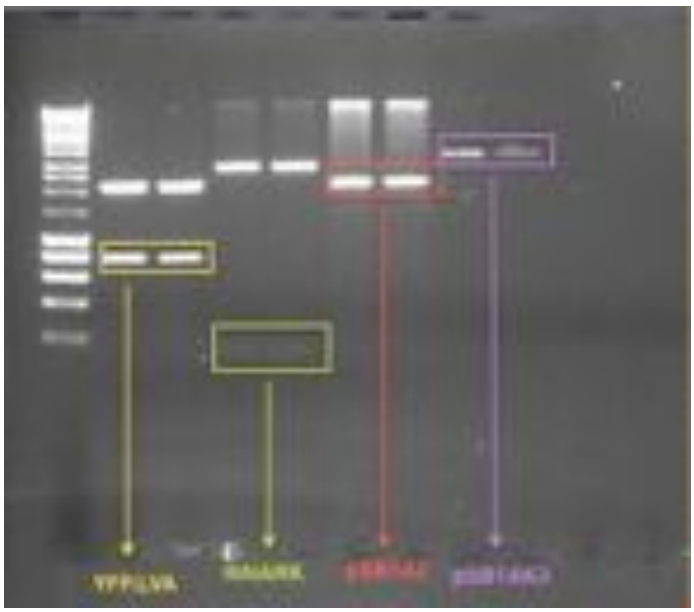
2) Set up restriction digestion with mini-prep samples (1):

Sample	E1	E2	E3	E4
mNARK	5.0			
RBS		17.0		
YFP.LVA			13.0	
TER				15.0
EcoRI	1.0		1.0	
XbaI		1.0		1.0
SpeI	1.0		1.0	
PstI		1.0		1.0
Enzyme Buffer	5.0 (4)	5.0 (3)	5.0 (4)	5.0 (3)
BSA	0.5	0.5	0.5	0.5
Water	37.5	25.5	29.5	27.5
TOTAL	**50.0**	**50.0**	**50.0**	**50.0**

Pipetting PstI was difficult.

Also digested iGEM linearised plasmid backbones pSB1K3 (E5) and pSB1T3 (E6) with EcoRI and PstI

3) Performed gel electrophoresis with E1, 2, 3 & 4



4) Set up the following ligation mixtures:

Sample	M1	M2
mNARK (E1)	2.0	
RBS (E2)	2.0	
YFP.LVA (E3)		2.0
Terminator (E4)		2.0
pSB1T3 (E6)		2.0
pSB1K3 (E5)	2.0	
Ligase	1.0	1.0
Ligase buffer	2.0	2.0
Water	11.0	11.0
TOTAL	**20.0**	**20.0**

5) Transformed separate stocks of competent E coli with ligation mixtures
Plated cells with mNark/RBS on kanamycin plate
Plated cells with YFP.LVA/Terminator on tetracycline plate

Date: Monday, 22 August 2011 18:00
Topic: Set up overnight cultures of semi-composite parts

1) Set up overnight cultures from a single colony from each of the transformed cell plates in selective LB medium. Left the cultures to grow overnight inside the shaker.

Date: Tuesday, 23 August 2011 11:00
Topic: Mini-prep, digestion and final ligation

1) Mini-prepped the overnight cultures into 2 separate eppendorf tubes of 60 µl volume each and measured concentration using NanoDrop:

Sample	Concentration
mNark/RBS/pSB1K3	57.8
YFP.LVA/TER/pSB1T3	118.7

2) Set up restriction digestion with mini-prep samples:

Sample	E7	E8	E9
mNark/RBS/pSB1K3	9.0		
YFP.LVA/TER/pSB1T3		5.0	
pSB1C3 (linearised)			7.5
EcoRI	1.0		1.0
XbaI		1.0	
SpeI	1.0		
PstI		1.0	1.0
Enzyme Buffer	5.0 (4)	5.0 (3)	5.0 (3)
BSA	0.5	0.5	0.5
Water	38.5	42.5	40.0
TOTAL	**50.0**	**50.0**	**50.0**

3) Performed gel electrophoresis with E7, 8 & 9. Gel bands confirmed the semi-composite parts.

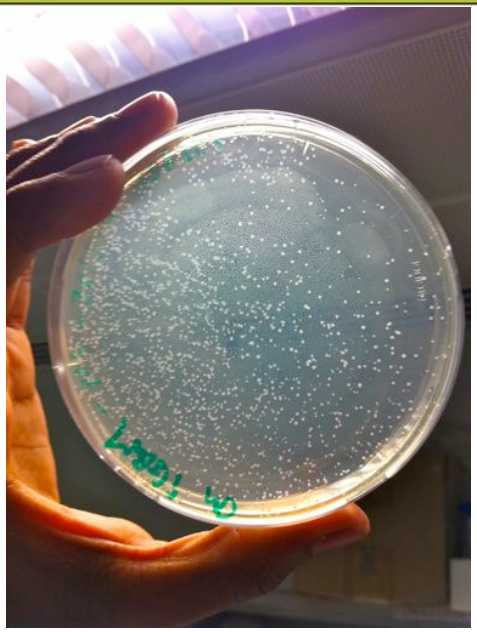
4) Set up the following ligation mixtures:

Sample	M3
mNARK/RBS (E7)	2.5
YFP.LVA/Ter (E8)	2.5
pSB1C3 (E9)	2.0
Ligase	1.0
Ligase buffer	2.0
Water	10.0
TOTAL	**20.0**

5) Transformed a stock of competent E coli with the ligation mixture
Plated cells with final construct on chloramphenicol plate

Date: Wednesday, 24 August 2011 18:00
Topic: Set up overnight cultures of final construct

1) Set up overnight cultures from a single colony from the chloramphenicol plate in selective LB medium. Left the culture to grow overnight inside the shaker.



Date: Thursday, 25 August 2011 11:00
Topic: Mini-prep, gel and sequencing

1) Set up glycerol stock from the overnight culture and stored it at -80°C

2) Mini-prepped the overnight culture into 50 µl volume and measured concentration using nano-drop.

DNA concentration - 284 ng/µl

3) Digested 5 µl of the mini-prep sample with EcoRI and PstI. Ran the digestion mixture on agarose gel by electrophoreses. The bands confirmed the size of the final construct and plasmid backbone.

4) Sent 10 µl of mini-prep sample for sequencing to Wolfson Institute for Biomedical Research for sequencing of new improved device

Date: Friday, 26 August 2011 14:00
Topic: Check sequencing result

The sequence for forward strand:

GCCTTACGGCCTTACGTATAAAATAGGCGTATCACGAGGCAGAATTTCAGATAAAAAAAATCC
TTAGCTTTCGCTAAGGATGATTTCTGGAATTCGCGGCCGCTTCTAGAGGTATTGATAAATATCA
ATGATAGATAAAGTTATCTTATCGTTTGATTTACATCAAATTGCCTTTAGCTACAGACACTAAGG
TGGCAGACTACTAGAGAAAGAGGAGAAATACTAGATGGTGAGCAAGGGCGAGGAGCTGTTCA
CCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGT
GTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACC
ACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTGCAAT
GCTTCGCCCGCTACCCCGACCACATGAAGCTGCACGACTTCTTCAAGTCCGCCATGCCCGAA
GGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGA
GGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAG

GAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATAT
CATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGG
ACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGT
GCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGA
AGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGG
ACGAGCTGTACAAGTAATAATACTAGAGCCATGCATCAAATAAAACGAAAGGCTCAGTCGAAA
GACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTTCTCTACTAAAAGTCACACTGG
CTCACCTCGGTGGGCCTTTTCTGCGTTTATAATACTAGTAGCGCCGCTGCAGTCGCCAAAAGG
GCCAGTGTTCACCACCTGCCTGTTTCCTGAAACCGATAGATACCTCCGTATGCAAGCTTCTTG
CCTCACTGAACTCGCTGTGCGC

The sequence for reverse strand:

TATTACCCCTAATCTTGTTGCACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCCTGCAT
AACGCGAAGTAATCTTTTCGGTTTTAAAGAAAAAGGGCAGGGTGGTGACACCTTGCCCTTTTT
TGCCGGACTGCAGCGGCCGCTACTAGTATATAAACGCAGAAAGGCCCACCCGAAGGTGAGCC
AGTGTGACTCTAGTAGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAGGCCCAGTCTTT
CGACTGAGCCTTTCGTTTTATTTGATGCCTGGCTCTAGTATTATTACTTGTACAGCTCGTCCAT
GCCGAGAGTGATCCCGGCGGCGGTCACGAACTCCAGCAGGACCATGTGATCGCGCTTCTCG
TTGGGGTCTTTGCTCAGGGCGGACTGGTAGCTCAGGTAGTGGTTGTCGGGCAGCAGCACGG
GGCCGTCGCCGATGGGGGTGTTCTGCTGGTAGTGGTCGGCGAGCTGCACGCTGCCGTCCTC
GATGTTGTGGCGGATCTTGAAGTTCACCTTGATGCCGTTCTTCTGCTTGTCGGCCATGATATA
GACGTTGTGGCTGTTGTAGTTGTACTCCAGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAA
GTCGATGCCCTTCAGCTCGATGCGGTTCACCAGGGTGTCGCCCTCGAACTTCACCTCGGCGC
GGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGCGCTCCTGGACGTAGCCTTCGGGC
ATGGCGGACTTGAAGAAGTCGTGCAGCTTCATGTGGTCGGGGTAGCGGGCGAAGCATTGCAG
GCCGTAGCCGAAGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCA
TATGAACTTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGA
ACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATTGGGCACCACCCCCGGTGAACA
GCTCCTCGCCCCTTGCTCACCAATCTAGTATTTCCTCCTCCTTTCCTCTAGTAGTTCTGCCACC
TTAGTGTCTGTAGCTAAGGTCTAATTTTGATTGTTTACATTCCAACGATTAAGAATACCTTTATC
TATTCATTGATAATTTGATCATACCCTCCTAGAAGCGTCGGCGAATTCCTGAATTTCCATTTCG
TTTAGCAGCCTAAGGCAATTGTTTGTATGCTGGAAGATTTCGTGCCCCTCGCGCGGGGATTAC
GAC

The sequencing data was analysed with ‘ApE’ software and all the parts for the construct are present.
Therefore it could be concluded that the new device was assembled correctly.