Lab Diary for Supercoilometer

Date: Monday, 12 September 2011 10:00
Topic:PCR to clone out the tyrT promoter

1) Set up the PCR reaction mixture below to clone out the tyrT promoter from the E. coli genome.

For template, a stock of competent TOP10 E coli cell was taken and vortexed to lyse the cell as much as possible.
The primer concentration used for PCR were 10 µM

Reagent	Volume
Distilled H_2O	32.5
5 x HF buffer	10.0
dNTP	1.0
Forward primer	2.5
Reverse primer	2.5
Template	1.0
Phusion polymerase	0.5
TOTAL	**50**

Temperature	Time	Cycles
98 °C	30s	x 1
98 °C	10s	x 30
60 °C	30s	
72 °C	15s	
72 °C	10m	x 1
4 °C	Hold	

2) Ran the PCR product on a gel and fain band was observed confirming the PCR success

Date: Tuesday, 13 September 2011 10:00
Topic:PCR purification and transformation with CFP

1) PCR purified the TyrT promoter cloned from the E. coli genome into 50 µl sample and measured the DNA concentration using NanoDrop.
Concentration: 10.0 ng/µl

2) Transformed a stock of Top10 E. coli cell with the CFP expression cassette

(RBS+eCFP.LVA+Ter) and plated the cell on ampicillin plate.

Date: Wednesday, 14 September 2011 18:00
Topic: Overnight culture of CFP

1) Set up overnight culture of the CFP expression plasmid cells in 2 ml selective LB medium

Date: Thursday, 15 September 2011 10:00
Topic: Digestion and ligation

1) Setup the following digestions for TyrT promoter

Sample	**F2**	**F3**
TyrT	20.0	20.0
RBS+CFP+TER		
EcoRI	1.0	1.0
XbaI		
SpeI	1.0	
PstI		1.0
Enzyme Buffer	5.0	5.0
BSA	0.5	0.5
Water	22.5	22.5
TOTAL	**50.0**	**50.0**

2) Setup the following ligation:

Sample	**M18**
TyrT (F3)	6.0
pSB1C3	2.0
Ligase	1.0
Ligase buffer	2.0
Water	9.0
TOTAL	**20.0**

3) Transformed a stock of competent TOP10 E. coli cell with the ligation mixture and plated it on chloramphenicol plate.

Date: Friday, 16 September 2011 18:00
Topic: Overnight culture of tyrT BioBrick

1) Set up overnight culture of the TyrT promoter BioBrick containing cells in 2 ml selective LB medium

Date: Saturday, 17 September 2011 10:56
Topic: Mini-prep of tyrT promoter

1) Mini-prepped the cell sample to extract the pSB1C3 plasmid containing the cloned TyrT promoter. Stored the sample in the freezer at - 80 °C

2) This project was stopped here to time constraints. The supercoilometer device will be finished at a later time by the future UCL iGEM team.