

Lab Diary for Supercoilometer

Date: Monday, 12 September 2011 10:00

Topic: PCR to clone out the tyrT promoter

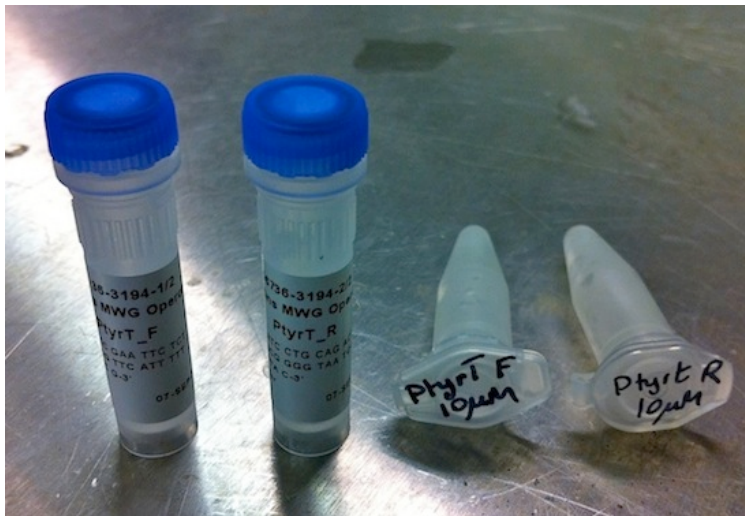
1) Set up the PCR reaction mixture below to clone out the tyrT promoter from the E. coli genome.

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 μ M

Reagent	Volume
Distilled H ₂ O	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 faint 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 μ l sample and measured the DNA concentration using NanoDrop.

Concentration: 10.0 ng/ μ l

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.