July 28, 2014

Ligation
Purpose: To ligate the T7 promoter and RFP into the pSB3K3 vector as part of the biosensor construction

Table 1. Ligation Reaction of T7 Promoter and RFP into vector

Reagent	**Amount (µl)**
T7 Promoter DNA	2
RFP DNA	3
pSB3K3 vector DNA	3
Ligation Buffer	1
Ligase Enzyme	1
Total Volume	10

Table 2. Ligation Reaction of T7 Promoter and RFP into vector

Reagent	**Amount (µl)**
T7 Promoter DNA	1
RFP DNA	4
pSB3K3 vector DNA	3
Ligation Buffer	1
Ligase Enzyme	1
Total Volume	10

Table 3. Ligation Reaction of T7 Promoter and RFP into vector

Reagent	**Amount (µl)**
T7 Promoter DNA	1
RFP DNA	3
pSB3K3 vector DNA	4
Ligation Buffer	1
Ligase Enzyme	1
Total Volume	10

Protocol: http://www.thermoscientificbio.com/uploadedfiles/resources/el001-product-information.pdf

Transformation into MACH Cells

- 50 µl of cells for each transformation
- Plate 400 µl transformants on LB + Kan plates
- Incubate at 37 °C overnight

iGEM Transformation Protocol: http://parts.igem.org/Help:Protocols/Transformation

Overnight

- Cultures of superoxide generators and RFP
- Cultures of Interlab Plasmids and real E0240 + J23115 promoter

July 29, 2014

Minipreps for real J23115 promoter (+ E0240)

Screen colonies on plates containing 1:3:4 and 1:4:3 ratios of T7 promoter and RFP
Purpose: To determine whether the colonies contained the desired product

- Pick and suspend colony in 50 µl of water

Table 1. PCR Protocol to Screen Colonies

Reagent	**Volume (µl) for single reaction**	**Volume (µl) for master mix**
Water	8	56
2X PCR Buffer	10	70
Primer 1 (10 uM)	0.5	3.5
Primer 2 (10 uM)	0.5	3.5
Colony	1	-
	Total: 20	

- Aliquot out 19 µl master mix into each PCR tube
- Add 1 µl of colony resuspended in water

Table 2. PCR Conditions for Screening

Temperature (°C)	**Time**
95	2 minutes
95	20 seconds
50	30 seconds
68	20 seconds
68	5 minute
4	Hold

- Repeat for 30 cycles

Table 3. Order of Colonies Screened

Tube	1	2	3	4	5	6	7
Sample	TR1	TR2	TR3	TR4	TR5	TR6	TR7

- Run 3 µl of each reaction on 1 % agarose gel
- Gel Photo: 072914

Overnight

Purpose: To indicate whether there is green fluorescence to determine if ligation was successful

- Cultures of TR4, TR5, TR6, TR7 and pSB3K3 as a control

July 30, 2014

T7_RFP cells do not show green fluorescence when compared to pSB3K3 control

Minipreps of T7_RFP 4, 5, 6, 7 to send out for sequencing

Co-Transformation of T7 Promoter + RFP and ER Intein Sensor into MACH cells

- 50 µl of cells for each DNA sample

Table 1. Ratio of T7 Promoter + RFP to ER Intein Sensor DNA

DNA	**Volume (µl)**	**Volume (µl)**	**Volume (µl)**
T7 Promoter + RFP	2	4	4
ER Intein	2	2	4

- Plate 250 µl transformants on LB + Kan (spread plated with 15 µl Cam)
- Plate 250 µl on LB + Cam (spread plated with 9 µl Kan)
- Incubate at 37 °C overnight

iGEM Transformation Protocol: http://parts.igem.org/Help:Protocols/Transformation

July 31, 2014

Overnight cultures for transformants containing sensor

August 1, 2014

Testing the Estrogen Biosensor

- Dilute 4 ml overnight cultures into 20 ml LB
- Make 10X estrogen stock solution of 10 mg estrogen in 1 ml of ethanol
- Make serial dilutions from 10X stock solution to create 10^{-1}, 10^{-2} and 10^{-3} solutions of estrogen in ethanol
    - The second time, serial dilutions were made in water
- Add 2 ml of diluted cell culture into each tube
- Treat each sample with 200 µl of respective estrogen serial dilution

Plate Diagram:

0 (treated)	0.001 (treated)	0.01 (treated)	0.1(treated)	1 (treated)
0 (untreated control)	0.001 (untreated control)	0.01 (untreated control)	0.1 (untreated control)	1 (untreated control)

(treated with estrogen in water, rows E and F of plate)

0.001 (untreated)	0.001 (untreated)	0.001 (treated)	0.1 (treated)
0.001 (untreated)	0.001 (treated)	0.1 (untreated)	0.1 (treated)