

August 25, 2014

Transformation of TR6 into BL15 Gold

- 4 μ l of TR6
- 50 μ l of cells
- Plate 400 μ l and 100 μ l on LB + Cam
- Incubate at 37 °C overnight
- iGEM Transformation Protocol:
<http://parts.igem.org/Help:Protocols/Transformation>

Overnights of superoxide generators with J23100 promoter: KR, KRCO, SNCO

August 26, 2014

Photobleaching:

- Spin KillerRed, Codon Optimized KillerRed and Codon Optimized Supernova down
- Re-suspend in 3 ml PBS
- 1 hour photobleaching
- Control in 37 °C incubator for 1 hour
- Make two serial dilutions of cells (10^{-1} and 10^{-2}) into water
- Plate 10 μ l of cells on LB + Cam plates
- Incubate overnight at 37 °C

Estrogen Biosensor Testing

- Stock solutions of estrogen (17β -Estradiol) and synthetic estrogen (17α -Estradiol)
 - Concentration: 10 mg/ml
- Treat 1 ml samples of the estrogen receptor sensor and the control (ER DEAD) with 1 μ l of the respective stock solutions

August 27, 2014

Results from previous day:

- Plates from superoxide generators appear to show decrease
- Not consistent, repeated photobleaching

Sample	Colonies from Photobleaching	Colonies from Control
KillerRed	>400 (~492)	>400 (~532)
KillerRed Codon Optimized	>400	29 *inconsistency
Supernova Codon Optimized	36	>200

- No red fluorescence detected from the estrogen biosensor
- Data on excel file

Photobleaching:

- Used cells from the previous day re-suspended in 3 ml PBS
- 1 hour photobleaching
- Control in 37 °C incubator for 1 hour
- Make two serial dilutions of cells (10^{-1} and 10^{-2}) into water
- Plate 10 μ l of cells on LB + Cam plates
- Incubate overnight at 37 °C

Estrogen Biosensor Testing

- Stock solutions of estrogen (new 17 β -Estradiol)
 - Concentration: 10 mg/ml
- Treat 1 ml samples of the estrogen receptor sensor (ER WT) and the control (ER DEAD) with 1 μ l of the respective stock solutions
- Shake at 28 °C overnight

August 28, 2014

Results from previous day:

- Plates from superoxide generators appear to show decrease
- Not consistent, repeated photobleaching

Sample	Colonies from Photobleaching	Colonies from Control
KillerRed	~116	Confluent (~600)
KillerRed Codon Optimized	Confluent (TMTC) ¹	~ 380
Supernova Codon Optimized	~580	Confluent (TMTC) ¹

1. TMTC = Too Many To Count

- Repeat photobleaching experiment and plating
- No red fluorescence from estrogen receptor sensor
- Superoxide generator cultures in the fridge
- Overnight cultures of WT d15 Intein + T7 RNA Polymerase + RFP and Dead d15, are growing in the 37 °C room

August 30, 2014

Sample	Number of Colonies
KR Control	~476
KR Photobleach	~604
KRCO Control	~804
KRCO (1) Photobleach	~672
KRCO (2) Photobleach	~928
SNCO Control	~368
SNCO Photobleach	15

- FAP intein tested in TECAN
 - Kinetic cycle: 1.5 hours, 5 min intervals
 - Shaking: 5 sec, orbital mode, 2 mm amplitude
 - Fluorescence intensity: RFP and dye