

August 4, 2014

Overnight cultures for sensor, cells only (containing BFP as control) and cells containing RFP

- Grow at 37 °C

Overnight cultures for Interlab plasmids (using superoxide generators as control)

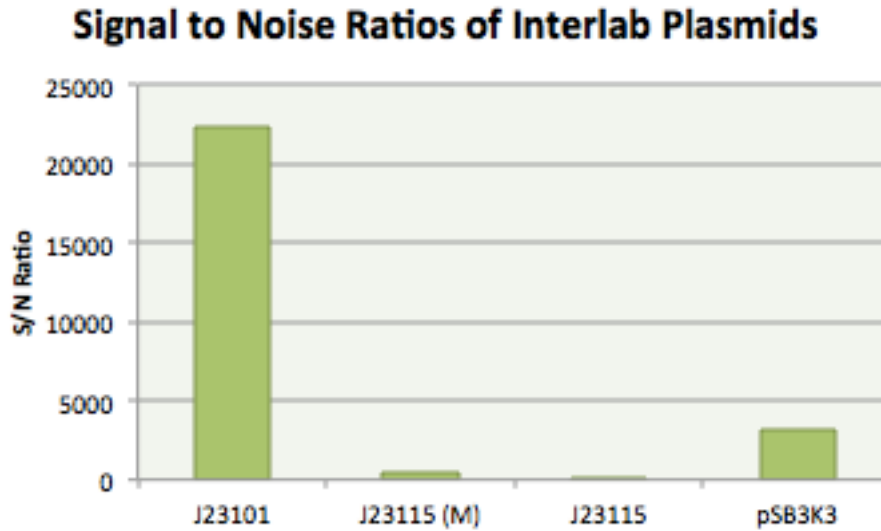
Overnight cultures for fluorescent proteins (in Top10 cells)

August 5, 2014

Table 1. Measured Fluorescence from Interlab Plasmids

Sample	Average (RFU)	OD	Standard Deviation
Control (Top10 Cells containing KillerRed plasmid)	118	0.455366661	2
J23101 + E0240	44849.66667	0.474000007	826.1018904
J23115(M) + E0240	1134.333333	0.46843333	11.06044002
J23115 + E0240	398.3333333	0.518433332	11.15048579
pSB3K3	6484.66667	0.507166664	109.9878781

Figure 1. Interlab Measurement Study Data



Estrogen Biosensor

- Diluted 4 ml overnight cultures of sensor and controls in 20 ml LB with Cam and Kan
- Grow at 30 °C during the day (~ 6-7 hours)
- Made 1 mg/ml stock solution of estrogen in DMSO
 - 10 mg 17- α estradiol (synthetic) and 17- β estradiol (real) in 1 ml DMSO
 - Dilute 100 μ l of 10 mg/ml stock solution 900 μ l DMSO to make up 1 mg/ml stock solutions
 - Make serial dilutions of 1 mg/ml stock solutions into water
 - Create 10⁻², 10⁻³ and 10⁻⁴ mg/ml solutions of both synthetic and real estradiol

- Treat samples (grown at 30 °C during the day) with different concentrations of estradiol solutions
- Grow overnight at 30 °C

Fluorescent Proteins

- Problems with yellow fluorescent protein
- Retransformed Top10 Cells with fluorescent proteins in WT Lac Plasmid (Cam resistance)

Transformation:

- 1 µl DNA for 50 µl cells
- Plate 400 µl transformants on LB + Cam
- Incubate at 37 °C overnight
- iGEM Transformation Protocol:
<http://parts.igem.org/Help:Protocols/Transformation>

Superoxide Generators

Overnight Cultures for KR, KR_CO, SN_CO, mRFP, and real J23115 + E0240 (as control)

August 6, 2014

Measured fluorescence from sensor

- Grew during the day at 30 °C

Measured KillerRed left in refrigerator overnight

Put Superoxide Generator overnight cultures in refrigerator

Transformants grew

- Overnight cultures for transformed Top10 cells (with fluorescent proteins)

Overnight cultures for transformants containing FP's in J23115 promoter (4 replicates, to screen)

Overnight cultures for estrogen intein sensor and BFP control cells

August 7, 2014

TECAN

Purpose: To screen for fluorescent protein expression with J23115 promoter

Results: Cultures 2 and 4 for BFP, none for GFP, 2 and 3 for OFP, both for YFP and RFP

Minipreps for cultures with expression

Protocol from GeneJet Plasmid Miniprep kit:

<http://www.thermoscientificbio.com/uploadedfiles/resources/k0502-product-information.pdf>

Top 10 Cell Cultures:

- 1/10 dilutions of cell samples: 5 ml LB, 500 µl cells
- Samples: BFPH, eGFP, YFP, OFP, RFP, SN
- Start at ~11:45 AM
- Time points:
 - 2:00PM
 - 3:00 PM
 - 4:00 PM
- See Table 2 (June 3, 2014) for parameters
- Note: Data on Dropbox

Streak plated positive 115+FPs

Overnights of five 115+GFP colonies from plate to find positive and restarted one 115+RFP as control

August 8, 2014

TECAN

Purpose: To screen for GFP expression with J23115 promoter

Results: Cultures 1 and 3 expressed GFP

When compared to Interlab plasmids (using same gain of 93) the expression was similar.

Minipreps for cultures with expression

Protocol from GeneJet Plasmid Miniprep kit:

<http://www.thermoscientificbio.com/uploadedfiles/resources/k0502-product-information.pdf>

Co-Transformation of T7-RFP and J23115-T7RNAP (non-intein control) into MACH cells

- 4 μ l of T7-RFP and 4 μ l of 115-T7RNAP
- 50 μ l of cells for each DNA sample
- Plate 400 μ l on LB + Cam (spread plated with 9 μ l Kan)
- Incubate at 37 °C overnight

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