

July 14, 2014

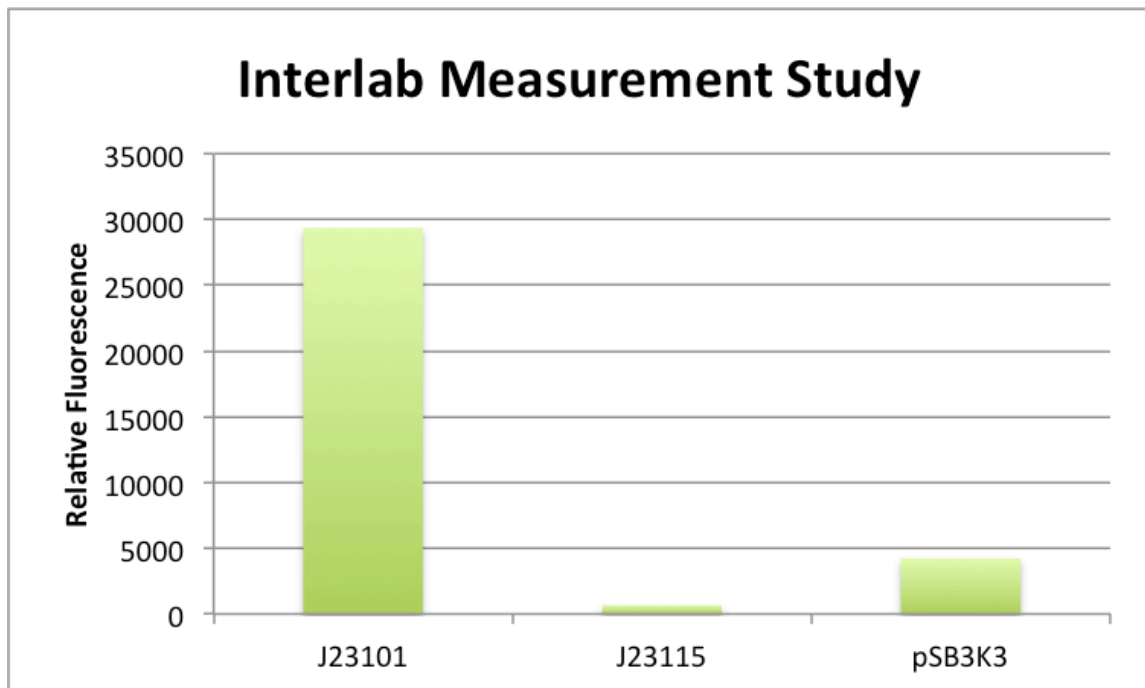
Overnight cultures for Interlab Measurement Study plasmids

Streaked out pSB3K3 on Kan plates

July 15, 2014

Interlab Measurement Study

- Used TECAN to measure fluorescence from overnight cultures of J23101, J23115, pSB3K3
 - Blank: LB broth
 - Emission/Excitation = 501/511 nm



- Overnight cultures for mRFP cut with HindIII (control), J23101, J23115 and pSB3K3
- Spread plate MACH cells with RFP for photobleaching on Amp plates
 - 300 μ l and 400 μ l of culture on different plates
- Streaked out mRFP cut with HindIII on Amp plate for control sample when measuring fluorescence

July 16, 2014

Interlab Study

- Used TECAN to measure fluorescence from overnight cultures of J23101, J23115, pSB3K3
 - Blank: Top10 cells containing mRFP cut with HindIII
 - Emission/Excitation = 501/511 nm

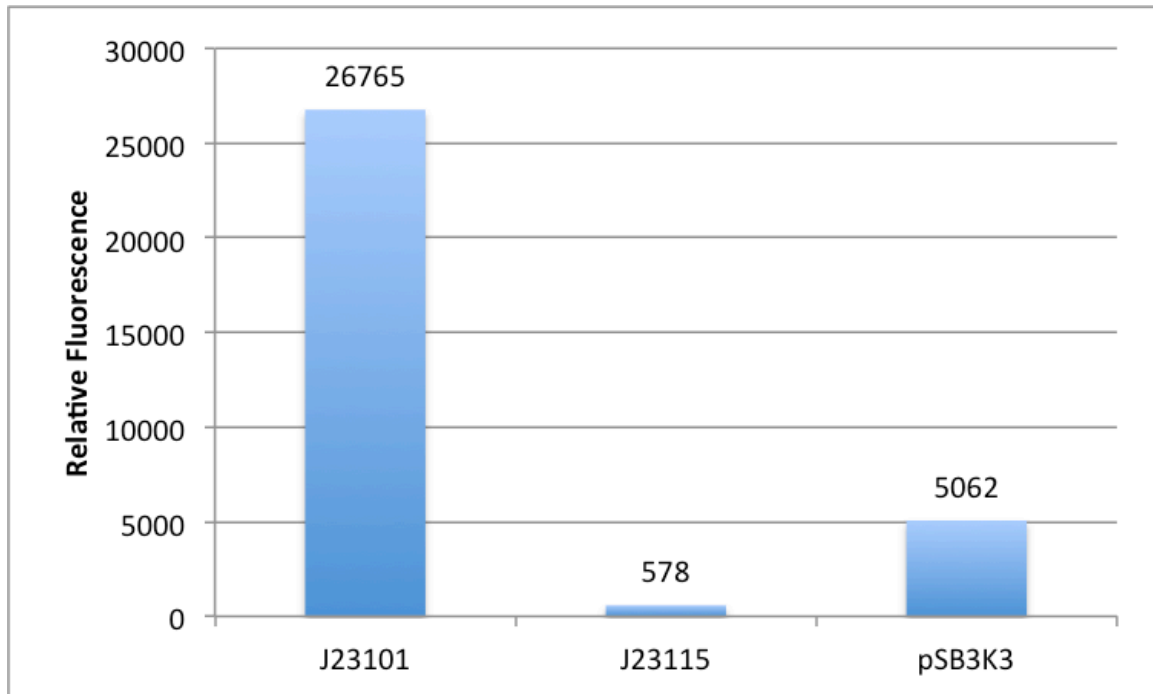


Table 1. Protocol for Digestion of WT Lac Plasmid

Reagent	Amount (μ l)
10X FastDigest Buffer	2
Water	6
Plasmid DNA	8
Restriction Enzyme PstI	2
Restriction Enzyme SpeI	2
Total Volume	20

Table 2. Protocol for Digestion of Killer Red and Supernova Plasmids

Reagent	Amount (μ l)
10X FastDigest Buffer	2
Water	6
Plasmid DNA	8
Restriction Enzyme PstI	2
Restriction Enzyme XbaI	2
Total Volume	20

- Digest at 37 °C for 30 - 45 minutes
- Gel Photo (Name: 062514c)
 - All fluorescent proteins and Killer Red (prefix/suffix) showed bands
 - None for Killer Red (RBS) or Super Nova

Protocol: <http://www.thermoscientificbio.com/uploadedFiles/Resources/fast-digestion-dna.pdf>

Ligation of Superoxide Generators into WT Lac and BioBrick Vectors

Table 3. Ligation Reaction of Killer Red and Supernova (codon optimized) and Original Killer Red into WT Lac Vector

Reagent	Amount (μ l)
Vector DNA	4
Insert DNA	4
Ligation Buffer	1
T4 Ligase Enzyme	1
Total Volume	10

- Ligate for 10 minutes at room temperature

Transformation into MACH Cells

- 50 μ l cells for each ligation reaction
- Plate 400 μ l transformants on LB + CAM plates
- Incubate at 37 °C overnight

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

July 17, 2014

Screened for fluorescent colonies

- RFP containing cells
- WT Lac with Killer Red, WT Lac with codon optimized Killer Red, WT Lac with codon optimized Supernova

Overnight cultures for the original Killer Red, codon optimized Killer Red and codon optimized Supernova

July 18, 2014

Minipreps for Superoxide generators

Protocol from GeneJet Plasmid Miniprep kit:

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

Sent out for sequencing with forward and reverse primers (see sequencing results doc)