

June 9, 2014

Interlab Measurement Study:

Identified fluorescing colonies using Typhoon FLA 9000

Table 1. Fluorescence in Cells Containing the J23115 Promoter

	Fluorescence (Y/N)
Plate 1	N
Plate 2	Y
Plate 3	N

Table 2. Fluorescence in Cells Containing the J23101 Promoter

	Fluorescence (Y/N)
Plate 1	Y
Plate 2	N
Plate 3	Y

Transformation of Top 10 Cells with Fluorescent Proteins

- 50 µl of cells for each transformation
- Plated 400 µl transformants on LB + AMP plates
- Incubate in 37 °C incubator overnight

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

- Overnight cultures of fluorescent cells containing J23115 and J23101 promoters at 37 °C
- Streaked fresh LB + AMP plates with MACH cell colonies containing each fluorescent protein (FP).

June 10, 2014

- Due to mistake in antibiotic added, we had to re-do overnight cultures of fluorescent cells containing J23115 and J23101 promoters at 37 °C
- Overnight cultures of Top 10 cells expressing fluorescent proteins at 37 °C

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Interlab Measurement Study: Mini Preps (GeneJet Plasmid Mini-Prep Kit)

Table 1. Concentrations of Plasmid DNA Isolated

Sample	Concentration (ng/ μ l)
E0240 + J23115	197.8
E0240 + J23101	216.9

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

Transformation

- 1 μ l of plasmid DNA into Top 10 Cells
- Plasmids: BBa_J23101 + BBa_E0240, BBa_J23115 + BBa_E0240
- Plate 400 μ l transformants on LB + CAM plates
- Incubate at 37 °C overnight

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

- Slight modification: allowed to recover for 2.5 hours

Fluorescent Protein Analysis:

TECAN

Top 10 Cell Cultures:

- 1/10 dilutions of cell samples: 5 ml LB, 500 μ l cells
- Samples: BFPH, eGFP, YFP, OFP, RFP, SN
- Start at ~10:30 AM
- Time points:
 - 1:00PM - induced cells with 1mM iptg
 - 2:00 PM
 - 3:00 PM - Dropped BFP sample, continued with remainder
 - 4:00 PM
 - 5:00 PM
- See Table 2 (June 3, 2014 – Week One) for parameters

Fluorescent Protein Analysis:

Overnight

Overnight Cultures of Top 10 Cells expressing fluorescent proteins

June 12, 2014

Fluorescent Protein Analysis:

TECAN

Top 10 Cell Cultures:

- 1/10 dilutions of cell samples: 5 ml LB, 500 μ l cells
- Duplicates of each sample
- Samples: BFPH, eGFP, YFP, OFP, RFP
- Start at ~10:45 AM
- Time points:
 - 1:00PM - induced cells with 1mM IPTG
 - 2:00 PM
 - 3:00 PM - accidentally didn't start shaker after taking sample
 - 4:00 PM
 - 5:00 PM
- See Table 2 (June 3, 2014) for parameters

Transformation

- 1 μ l of plasmid DNA into 50 μ l Top 10 Cells
- Plasmids: pSB3K3, 4L-RFP, 3K-YFP, YFP + RFP
- Plate 400 μ l transformants on LB + Kan plates
- Incubate at 37 °C overnight
- iGEM Transformation Protocol:
<http://parts.igem.org/Help:Protocols/Transformation>

Overnight:

Overnight cultures of Top 10 cells and MACH cells containing fluorescent proteins

Overnight cultures of Top 10 cells containing BBa_J23101 + BBa_E0240 and BBa_J23115 + BBa_E0240 plasmids

June 13, 2014

TECAN

Top 10 and MACH Cell Cultures (for fluorescent protein analysis) and Interlab plasmids:

- 1/10 dilutions of cell samples:
 - 5 ml LB, 500 μ l cells
- Duplicates of each sample
- Tested singles for the Interlab samples
- Plate 1:
 - Samples: BFPH, eGFP, YFP, OFP, RFP
 - Interlab: BBa_J23101 + BBa_E0240
- Plate 2:
 - Samples: BFPH, eGFP, YFP, OFP, RFP
 - Interlab: BBa_J231015 + BBa_E0240
- Start at ~10:00 AM
- Time points:
 - 12:00PM - induced cells with 1mM IPTG
 - 1:00 PM
 - 2:00 PM
 - 3:00 PM
 - 4:00 PM
- See Table 2 (June 3, 2014- Week One) for parameters