June 16, 2014

Digestion of Plasmids
Purpose: To create non-functional red fluorescent protein encoding plasmid for use as a control

Table 1. Single Digestion of mRFP Plasmid

Reagent	**Amount (µl)**
10X FastDigest Buffer	2
Plasmid DNA	16
Restriction Enzyme HindIII	2
Total Volume	20

Protocol: http://www.thermoscientificbio.com/uploadedFiles/Resources/k069-product-information.pdf

Ligation of plasmid back together
Purpose: To close the cut mRFP plasmid, creating a non-functional plasmid encoding for a truncated protein that will not fluoresce

Table 2. Ligation Reaction for RFP Plasmid

Reagent	**Amount (µl)**
Plasmid DNA	8
Ligation Buffer (Thermo L)	1
Ligase Enzyme (Ligase)	1
Total Volume	10

- Ligate for 10 minutes at room temperature
- Put on ice until ready for transformation

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

Transformation of RFP Plasmid into MACH Cells
Purpose: To create control cells that don't fluoresce to use as blank when measuring fluorescence using TECAN

- 50 µl of competent MACH cells into each ligation reaction
- 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 J23101 + E0240, J23115 + E0240, and pSB3K3 in MACH and Top 10 Cells for Interlab study (6 cultures total)

June 17, 2014

TECAN

Top 10 and MACH Cell Cultures and Interlab plasmids:

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

Overnight:

- Culture for MACH cells containing HindII cut mRFP plasmid
- Cultures of fluorescence proteins containing cells (MACH and Top 10 Cells)

June 18, 2014

Mini Preps of MACH Cells Containing mRFP cut with HindIII

Table 1. Concentrations of Plasmid DNA Isolated

Sample	Concentration (ng/µl)
mRFP cut with HindIII	207.3

Protocol for GeneJet Plasmid Miniprep:
http://www.thermoscientificbio.com/uploadedfiles/resources/k0502-product-information.pdf

Transformation of RFP Plasmid into Top10 Cells
Purpose: To create control cells that don't fluoresce to use as blank when measuring fluorescence using TECAN

- 1 µl DNA for 50 µl cells
- Plate 400 µl transformants on LB + Amp plates
- Incubate at 37 °C overnight

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

TECAN
MACH Cell Cultures Expressing Fluorescent Proteins:

- 1/10 dilutions of cell samples: 5 ml LB, 500 µl cells
- Start at ~10:30 AM
- Time points:
  - 1:00 PM - induced cells with 1 mM iptg
  - 2:00 PM
  - 3:00 PM
  - 4:30 PM
- See Table 2 (June 3, 2014) for parameters
- Note: Data on Dropbox

Overnight:

- Cultures for 4L-RFP, 3K-YFP, YFP + RFP to be mini-prepped

June 19, 2014

Mini Preps of Top10 Cells Containing 4L-RFP and 3K-YFP

Table 1. Concentrations of Plasmid DNA Isolated

Sample	Concentration (ng/µl)
4L-RFP	283.0
3K-YFP	64.7

Protocol for GeneJet Plasmid Miniprep:
http://www.thermoscientificbio.com/uploadedfiles/resources/k0502-product-information.pdf

DNA sent out for sequencing

Table 2. Sequencing Codes and their Corresponding Plasmid and Primer

Sequencing Code	Plasmid	Primer (F/R)
iGEM100	J23101 + E	F
iGEM101	J23101 + E	R
iGEM102	J23115 + E	F
iGEM103	J23115 + E	R
iGEM104	pSB3K3	F
iGEM105	pSB3K3	R

Transformation of pSB3K3 and WT Lac Plasmid into MACH Cells
Purpose: To be used in cloning

- 5 µl DNA for 50 µl cells
- Plate 400 µl transformants on LB + Kan (pSB3K3) and LB + CAM (WT Lac) plates
- Incubate at 37 °C overnight

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