June 3, 2014

MiniPreps of MACH Cells Expressing Fluorescence
Purpose: To isolate plasmid DNA from MACH cells

Table 1. Concentrations of Plasmid DNA Isolated

Sample	Concentration (ng/µl)
BFPH	66.6
eGFP	128.9
YFP	51.1
OFP	80.0
RFP	120.8
SuperNova	128.1

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

TECAN
Purpose: To measure fluorescence from MACH Cells

Cell Cultures of MACH Cells for Fluorescence:

- 3 ml LB and 300 µl cell culture at 10:45 AM
- Time point 1: 12:45 PM
- Time point 2: 4:45 PM

- In Row D: 50 µL cell culture (at time point 1)

- Used the following parameters in Table 2 to observe the gain and brightness of the following fluorescent proteins:

Table 2. Parameters Used to Analyze Fluorescent Proteins using TECAN

Fluorescent Protein Sample	Excitation Wavelength (nm)	Emission Wavelength (nm)
BFPH	399	456
eGFP	488	509
YFP	514	527
OFP	548	562
RFP	584	607
SuperNova	579	610

Interlab Measurement Study:

Transformation of MACH Cells with BBa_J23101, BBa_J23115 and BBa_E0240 Plasmids

- 5 µl of plasmid DNA into 50 µl thawed ultra-competent cells
- Plasmids: BBa_J23101, BBa_J23115, BBa_E0240
- Plate 400 µl transformants on LB + CAM plates
- Incubate at 37 °C overnight

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

June 4, 2014

TECAN

Purpose: To measure fluorescence from MACH cells

Cell Cultures:

- 100 µl cell culture diluted in 1 ml LB, in triplicate for each sample
- Samples: BFPH, eGFP, YFP, OFP, RFP
- Start at 11:22 AM
- End: 5:30 PM
- Time points:
    - 11:50 AM
    - 12:20 PM
    - 12:50 PM
    - 1:20 PM
    - 1:50 PM
    - 2:20 PM
    - 5:30 PM
- See Table 2 (June 3, 2014) for TECAN parameters

Overnight at 37 °C:
Overnight Cultures of BBa_J23101, BBa_J23115, BBa_E0240, each in 5 ml LB + CAM

June 5, 2014

Interlab Measurement Study: Mini Preps of MACH Cells

Table 1. Concentrations of Plasmid DNA Isolated

Sample	Concentration (ng/µl)
E0240	365.2
J23101	472.7
J23115	238.2

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

Restriction Enzyme Digestion

- Amounts used for each restriction enzyme digestion reaction are shown below in Tables 2 - 4.
- Double digestion of plasmids containing promoters (J23101 and J23115) used as test

Table 2. Double Digestion of J23101 and J23115 Plasmids

Reagent	Amount (µl)
10X FastDigest Buffer	2
Plasmid DNA	2
Restriction Enzyme Xbal	1
Restriction Enzyme SpeI	1
Water (to bring up to volume)	12
Total Volume	20

Table 3. Single Digestion of J23101 and J23115 Plasmids

Reagent	**Amount (µl)**
10X FastDigest Buffer	2
Plasmid DNA	8
Restriction Enzyme SpeI	2
Water (to bring up to volume)	8
Total Volume	20

Table 4. Single Digestion of E0240 Plasmid

Reagent	**Amount (µl)**
10X FastDigest Buffer	2
Plasmid DNA	8
Restriction Enzyme Xbal	2
Restriction Enzyme SpeI	2
Water (to bring up to volume)	6
Total Volume	20

- Digest at 37 °C for 30 - 45 minutes
    - Start - 11:45 AM
    - End - 12:30 PM

Restriction Enzyme Digest Protocol:
http://www.thermoscientificbio.com/uploadedFiles/Resources/fast-digestion-dna.pdf

Agarose Gel Electrophoresis

- 1 % agarose gel made of:
    - 50 ml 0.5X TAE
    - 0.5 g agarose
    - 2.5 µl ethidium bromide
- Run at 73 V
    - Expected size of insert (from E0240) - 884 bp
    - Expected size of vector backbone (J23101 and J23115) - 2105 bp
    - Start - 12:40 PM
    - End - 1:40 PM

Isolated insert and vector backbone (GeneJet Gel Extraction Kit)

- 250 - 750 µl binding buffer (depending on mass of gel cut-out)
- Incubated at 42 °C until gel melted

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

Ligation

- Ligate GFP (E0240 insert) into vector backbone (J23101 or J23115)

Table 5. Ligation Reaction of E0240 insert and J23101 or J23115 vector

Reagent	**Amount (µl)**
Promoter DNA (J23101 or J23115)	1
Insert DNA (E0240)	7
Ligation Buffer	1
Ligase Enzyme	1
Total Volume	10

Table 6. Ligation Control Sample Reaction

Reagent	**Amount (µl)**
Promoter DNA (J23101)	1
Water	8
Ligation Buffer	1
Total Volume	10

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

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

Transformation

- 50 µl of cells for each transformation
- Plated 400 µl transformants on LB + CAM plates
- Incubated at 37 °C overnight

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

June 6, 2014

Interlab Measurement Study: Picked Fluorescent Colonies

- Picked fluorescing colonies identified using Typhoon FLA 9000
- Streaked on LB + CAM plates
- Placed overnight in incubator at 37 °C