June 23, 2014

Estrogen Receptor Intein Construction: PCR
Purpose: To synthesize the estrogen receptor intein

Table 1. PCR Protocol to Synthesize Estrogen Receptor

Reagent	**Volume (µl)**	**Volume (µl)**
Water	12.4	5.4
5X HF PCR Buffer	4	4
dNTPs	0.4	0.4
Primer 1 (10 uM Nterm DNA)	1	5
Primer 2 (10 uM Cterm DNA)	1	5
Phusion Taq enzyme	0.4	0.4
	Total: 20.4	~20.4

Table 2. PCR Conditions for First Round

Temperature (°C)	**Time**
98	2 minutes
98	10 seconds
60	15 seconds
72	1.5 minutes
72	1 minute
4	Hold

- Repeat for 35 cycles
- Take 2.5 µl DNA from this PCR reaction for the next PCR

Table 3. PCR Protocol to Synthesize Estrogen Receptor

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (10 uM Nterm DNA)	2.5
Primer 2 (10 uM Cterm DNA)	2.5
DNA	2.5
Phusion Taq enzyme	0.5
	Total: 50

- Same Conditions
- Take 2 - 3 µl DNA and run in 1 % agarose gel

Results: No bands present

Overnight:

- Cultures for Top10 Cells expressing fluorescent proteins

June 24, 2014

PCR to Synthesize Estrogen Responsive Intein

- Repeat protocol from previous day (tables 1, 2, and 3 from June 23, 2014)

PCR to Prepare Fluorescent Protein DNA for Submission to iGEM Registry

Table 1. Protocol for PCR Cocktail

Reagent	**Volume (µl)**
Water	155
5X HF PCR Buffer	50
dNTPs	5
Primer 1 (Prefix)	12.5
Primer 2 (Suffix)	12.5
Phusion Taq enzyme	2.5
	Total: 237.5

- Add 47.5 µl of PCR Cocktail to each tube (5 tubes)
- Add 2.5 µl of DNA to corresponding tube
- PCR Conditions in Table 2. (June 23, 2014)

Overnight:

- Streak out MACH cells with fluorescent proteins
- Culture of MACH cells containing WT Lac

June 25, 2014

PCR for RFP with His tag

Table 1. Protocol for Single PCR Reaction

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (10 uM Prefix)	2.5
Primer 2 (10 uM Suffix)	2.5
DNA	2.5
Phusion Taq enzyme	0.5
	Total: 50

- PCR Conditions in Table 2. (June 23, 2014)

PCR for Killer Red and Super Nova with BioBricks Prefix and Suffix

Table 2. Protocol for Single PCR Reaction

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (10 uM Prefix)	2.5
Primer 2 (10 uM Suffix)	2.5
DNA (Killer Red or Super Nova respectively)	2.5
Phusion Taq enzyme	0.5
	Total: 50

- PCR Conditions in Table 2. (June 23, 2014)

PCR for Killer Red and Super Nova with Ribosome Binding Site

Table 2. Protocol for Single PCR Reaction

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (10 uM XbaPreRBS)	2.5
Primer 2 (10 uM PstSuf6His)	2.5
DNA (Killer Red or Super Nova respectively)	2.5
Phusion Taq enzyme	0.5
	Total: 50

- PCR Conditions in Table 2. (June 23, 2014)

Miniprep of WT Lac Plasmid
Protocol for GeneJet Plasmid Miniprep:
http://www.thermoscientificbio.com/uploadedfiles/resources/k0502-product-information.pdf
Yield = 225.5 ng/µl

Digestion of Wild Type Lac Vector and Fluorescent Proteins for Ligation

Table 3. Protocol for Digestion of WT Lac Plasmid

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

- Digest at 37 °C for 30 - 45 minutes
- Gel Photo (Name: 062514b)

Table 4. Protocol for Digestion of Fluorescent Proteins, Killer Red and Super Nova Plasmids

Reagent	**Amount (µl)**
10X FastDigest Buffer	2
Plasmid DNA	14
Restriction Enzyme XbaI	2
Restriction Enzyme SpeI	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 Fluorescent Proteins into WT Lac and BioBrick Vectors

Table 5. Ligation Reaction of Fluorescent Proteins

Reagent	**Amount (µl)**
Vector DNA (WT Lac or BioBrick Vector)	2
Insert DNA (Fluorescent Proteins)	6
Ligation Buffer	1
T4 Ligase Enzyme	1
Total Volume	10

- Ligate for 10 minutes at room temperature

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

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

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

June 26, 2014

Overnight:

- Cultures of the transformants (FP's in BBKR and WT Lac Backbones)