June 30, 2014

PCR to Clone ER Intein into T7 RNA Polymerase

- 4 Reactions

Table 1. Protocol for Single PCR Reaction

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (T7Ov491R)	2.5
DNA (1/100 T7 RNA Polymerase)	2.5
Phusion Taq enzyme	0.5
	Total: 50

Table 2. Protocol for Single PCR Reaction

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERintF)	2.5
Primer 2 (OvERintR)	2.5
DNA (1/100 ER Intein)	2.5
Phusion Taq enzyme	0.5
	Total: 50

Table 3. Protocol for Single PCR Reaction

Reagent	**Volume (µl)**
Water	31

5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERdeadF)	2.5
Primer 2 (OvERdeadR)	2.5
DNA (1/100 ER Intein)	2.5
Phusion Taq enzyme	0.5
	Total: 50

Table 4. Protocol for Single PCR Reaction

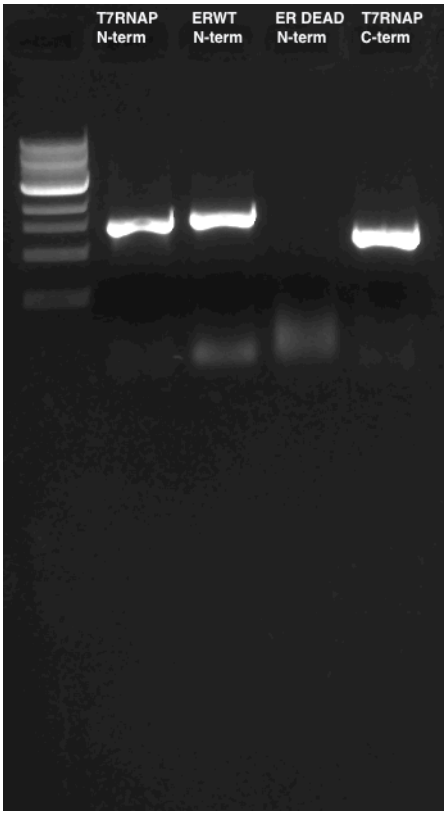
Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7Ov492F)	2.5
Primer 2 (PstT7R)	2.5
DNA (1/100 T7 ER Intein)	2.5
Phusion Taq enzyme	0.5
	Total: 50

Table 5. PCR Conditions

Temperature (°C)	**Time**
98	2 minutes
98	10 seconds
60	15 seconds
72	100 seconds
72	1 minute
4	Hold

- Run 2 µl of product in 1 % agarose gel
    - Check for expected bands

Gel Photo:



Wrong primers for ER Intein DEAD reaction

July 1, 2014

Repeated ER Intein DEAD PCR reaction with correct primers

Table 1. Protocol for Single PCR Reaction to Clone ER Intein into T7 RNA Polymerase

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
DNA (T7 N-term with overhang)	2.5
DNA (ER Intein with overhang)	2.5
DNA (T7 C-term with overhang)	2.5
Phusion Taq enzyme	0.5
	Total: 50

Table 2. PCR Conditions to Clone ER Intein into T7 RNA Polymerase

Temperature (°C)	**Time**
98	2 minutes
98	10 seconds
60	15 seconds
72	4 minutes
72	5 minute
4	Hold

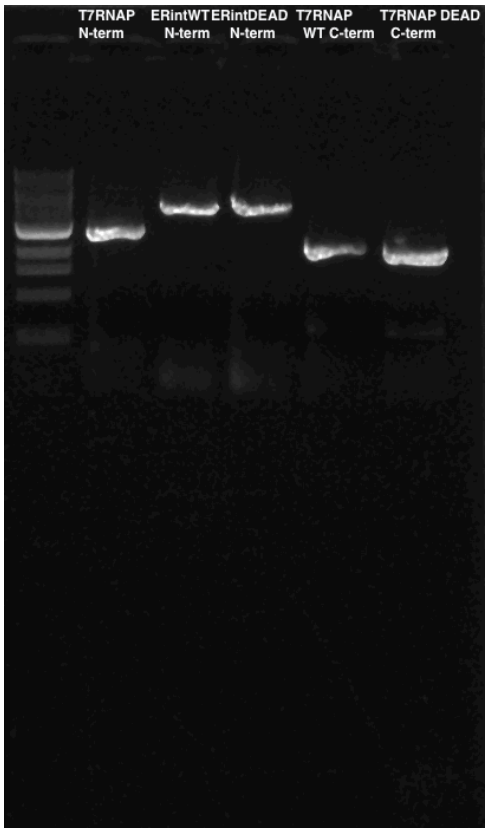
- Run 2 µl of product in 1 % agarose gel

Table 3. Protocol for Single PCR Reaction to Clone ER Intein into T7 RNA Polymerase with outside primers

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (T7PstR)	2.5
DNA (PCR Reaction from Table 1.)	2.5
Phusion Taq enzyme	0.5
	Total: 50

- Run 2 µl of product in 1 % agarose gel
    - Bands at expected product size

Gel Photo:



July 2, 2014

Repeated PCR to synthesize T7 RNA Polymerase + ER Intein in two parts

Table 1. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase N-Terminus and ER Intein

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (OvERintR)	2.5
DNA (T7 N-Term)	1.25
DNA (ER Intein)	1.25
Phusion Taq enzyme	0.5
	Total: 50

- Repeat reaction using DNA diluted 1/10

Table 2. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase C-Terminus and ER Intein

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERintF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 C-Term)	1.0
DNA (ER Intein)	1.5
Phusion Taq enzyme	0.5
	Total: 50

Table 3. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase N-Terminus and ER Intein DEAD

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (OvERintR)	2.5
DNA (T7 N-Term)	1.0
DNA (ER Intein DEAD)	1.75
Phusion Taq enzyme	0.5
	Total: 50

- Repeat reaction using DNA diluted 1/10

Table 4. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase C-Terminus and ER Intein DEAD

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERintF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 C-Term)	0.75
DNA (ER Intein DEAD)	1.75
Phusion Taq enzyme	0.5
	Total: 50

- Run in 1 % agarose gel (Gel Photo)
- Gel purify product

Table 5. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase with ER Intein

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 N-Term + ER Intein)	2.0
DNA (T7 C-Term)	0.5
Phusion Taq enzyme	0.5
	Total: 50

Table 6. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase with ER Intein

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 C-Term + ER Intein)	2.0
DNA (T7 N-Term)	0.5
Phusion Taq enzyme	0.5
	Total: 50

Table 7. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase with ER Intein DEAD

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERintF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 N-Term + ER Intein DEAD)	2.0
DNA (T7 C-Term)	0.5
Phusion Taq enzyme	0.5
	Total: 50

Table 8. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase with ER Intein DEAD

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERintF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 N-Term + ER Intein)	2.0
DNA (T7 C-Term)	0.5
Phusion Taq enzyme	0.5
	Total: 50

- PCR conditions same as in Table 5. June 30, 2014
- Run in 1 % agarose gel (Gel Photo)

Table 9. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase with ER Intein

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERintF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 N-Term)	0.9
DNA (ER Intein)	0.9
DNA (T7 C-Term)	0.7
Phusion Taq enzyme	0.5
	Total: 50

Table 10. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase with ER Intein DEAD

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (OvERintF)	2.5
Primer 2 (T7PstR)	2.5
DNA (T7 N-Term)	0.7
DNA (ER Intein DEAD)	1.1
DNA (T7 C-Term)	0.7
Phusion Taq enzyme	0.5
	Total: 50

- PCR conditions same as in Table 5. June 30, 2014

- Run in 1 % agarose gel (Gel Photo)

July 3, 2014

Results from yesterday's PCR: no product

Table 1. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase N-terminus with ER Intein (half)

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (NcoOvR)	2.5
DNA (T7 N-Term)	1.25
DNA (ER Intein)	1.25
Phusion Taq enzyme	0.5
	Total: 50

Table 2. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase C-Terminus with ER Intein (half)

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (NcoOvF)	2.5
Primer 2 (PstT7R)	2.5
DNA (T7 C-Term)	1
DNA (ER Intein)	1.5
Phusion Taq enzyme	0.5
	Total: 50

Table 3. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase N-terminus with ER Intein DEAD (half)

Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (T7XbaF)	2.5
Primer 2 (NcoOvR)	2.5
DNA (T7 N-Term)	1.25
DNA (ER Intein DEAD)	1.25
Phusion Taq enzyme	0.5
	Total: 50

Table 4. Protocol for Single PCR Reaction to Synthesize T7 RNA Polymerase C-Terminus with ER Intein DEAD (half)

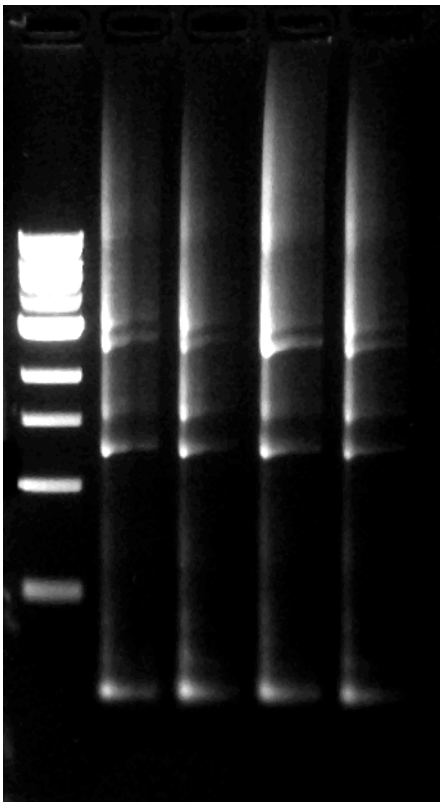
Reagent	**Volume (µl)**
Water	31
5X HF PCR Buffer	10
dNTPs	1
Primer 1 (NcoOvF)	2.5
Primer 2 (PstT7R)	2.5
DNA (T7 C-Term)	0.75
DNA (ER Intein DEAD)	1.75
Phusion Taq enzyme	0.5
	Total: 50

Table 5. PCR Conditions to Clone ER Intein into T7 RNA Polymerase

Temperature (°C)	Time
98	2 minutes
98	10 seconds
60	15 seconds
72	2.5 minutes
72	5 minutes
4	Hold

- Repeat cycle 35 times
- Run 2 µl in 1 % agarose gel
- Smearing shows amplification of other products

Gel Photo:



- Repeated initial PCR reaction used to synthesize the T7 RNAP and ER Sensor at one time

  Gel Photo:

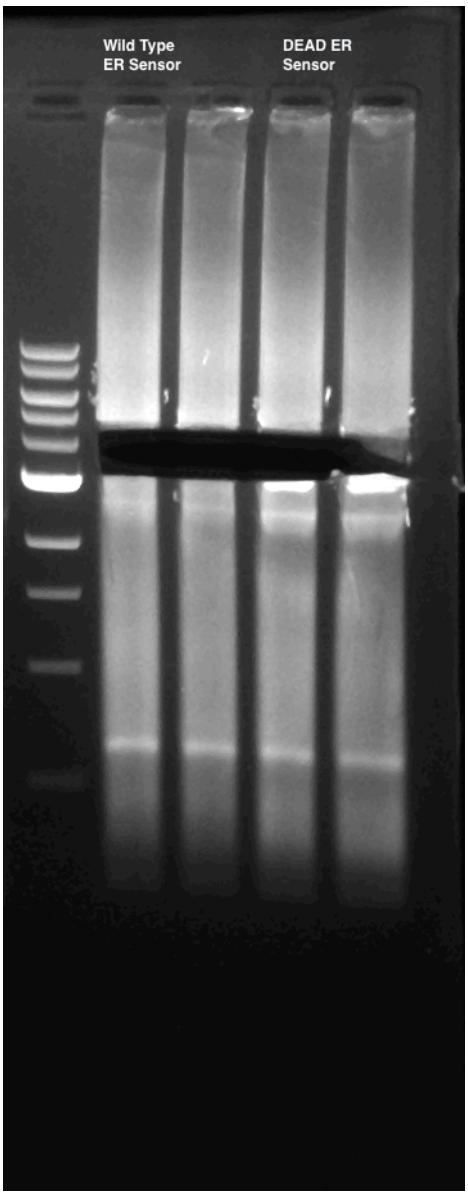


Table 6. Ligation Reaction of T7 RNA Polymerase + ER Intein into BioBrick Vector

Reagent	**Amount (µl)**
Vector DNA	2
T7 N-terminus DNA	3
T7 C-terminus DNA	3
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 T7 RNA Polymerase + ER Intein into MACH Cells

Purpose: To clone T7 RNA Polymerase + ER Intein

- 50 µl of competent MACH cells into 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