Protocol for the construct of *Ptms* in pSB1C3

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I. PCR of *Ptms* from two single strand oligonucleotides.

Designed primers:

Primer name	Primer sequence
Forward Primer	GCTTCTAGAGCATGAAGTCTCCTTGAAATCAGAAGATATTTAGGATATATTTTTCT ATGGAT TCTAGA: XbaI cutting site.
Reverse Primer	GTTTCTTCCTGCAGCGGCCGCTACTAGTACAATATCCCTTTTATCCATAGAAAAAT ATATCCTAAATATCT CTGCAG: PstI cutting site ACTAGT: SpeI cutting site

PCR set-up:

Reagents	*Ptms*
ddH_2O	11.1uL
Phusion® HF Buffer (5X)	4uL
Phusion® polymerase	0.4uL
Forward primer (100uM)	2uL
Reverse primer (100uM)	2uL
dNTP	0.5uL
Reaction volume	20uL

PCR program:

Steps	Temperature (Celsius)	Duration (minute : second)
1.Denaturation	95	01:00
2.Annealing	95-n n is the times of run cylces.	00:30 Repeat for 70 times.
3.Extension	72	00:40
7.Storage	8	infinite

II. PCR clean-up.

1. Standard procedures for phenol chloroform.

III. Digestion of secondary PCR product and pSB1C3.

Digestion set-up:

Reagents	*Ptms*	pSB1C3
ddH_2O	Add to 20uL	Add to 20uL
10 X NEB No.4 digestion buffer	2uL	2uL

PCR product	1ug	-
pSB1C3	-	1ug
EcoRI – HF	0.5uL	0.5uL
PstI – HF	0.5uL	0.5uL
Reaction volume	20uL	20uL

Incubate under 37 degree Celsius for 2 hours.

IV. Gel purification of digested pSB1C3.

1. Prepare a 40mL 0.8% agarose gel with CyberSafe as the stain.
2. Load the digested pSB1C3.
3. Run the gel under 130 V for 60 minutes.
4. Cut the bands of pSB1C3 backbone (nearly 2000bp).
5. Purify them by Favorgen gel purification Kit.

V. Ligation of digested secondary PCR product and pSB1C3.

Ligation set-up:

Reagent	Volume
ddH_2O	Add to 10uL
10X Invitrogen ligase buffer	1uL
Invitrogen T4 ligase	0.5uL
Insert (Digested *Ptms*)	100ng
Backbone (Digested pSB1C3)	50ng

Incubate under 37 degree Celsius for 2 hours.

VI. Transformation.

1. Add 10uL of the ligation product in (vii) to 100uL of *E. coli DH10* β competent cells.
2. Put the mixture in 1 on ice for 10 minutes.
3. Heat shock the mixture for 90 seconds under 42 degree Celsius.
4. Chill the mixture on ice for 2 minutes.
5. Add 1mL LB into the mixture.
6. Incubate the mixture in 5 for 1 hour under 37 degree Celsius.
7. Centrifuge the mixture for 90 seconds under 13200 rpm.
8. Remove 1mL supernatant.
9. Resuspend the pellet.
10. Transfer the resuspende solution onto a 25ug/mL chloramphenicol LB plate.
11. Spread the plate.
12. Incubate for overnight.

VII. Plasmid extraction of *Ptms* in pSB1C3.

1. Use the Favorgen plasmid extraction mini-Kit to extract the intended plasmid.