10.01

Positively transform 1-9C plasmid, intending to get pSB3T5 plasmid backbone.

One 5ml culture of LB medium and antibiotic (Tetracycline, 50ng/ml) was inoculated with a single positive colony from a LB agar plate. Cultures were grown in tubes for 14 hrs at 37°C with shaking at 70 rpm.

10.02

Miniprep of the fresh culture containing 1-9C plasmid.

Digest 1-9C plasmid and T7p + RBS + CI plasmid with XbaI and PstI
Double digestion system:

1.5μL	XbaI
1.5μL	PstI
2μL	10×M buffer
10μL	1-9C plasmid (5μL for T7p + RBS + CI plasmid)
5μL	ddH_2O (5μL for T7p + RBS + CI plasmid)
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20μL	Total

Electrophoresis the digested samples, and extract the 1-9C plasmid backbone and T7p + RBS + CI insert.

10.03

Ligate the T7p + RBS + CI insert to 1-9C plasmid backbone.
System:

3μL	insert
1μL	T7p vector
1μL	10× Ligase buffer
1μL	Ligase
4μL	ddH_2O
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10μL	Total

Transform the ligation product, and plate on agar plate.

10.04

Pick 3 colonies for each RBS, 18 colonies in total, incubated in LB medium with Tetracycline, 50ng/ml for 14 hrs.

Miniprep these 18 culture samples.

Digest the plasmids with XbaI and PstI to detect the successful ligation.
Double digestion system:

1.5μL	XbaI
1.5μL	PstI
2μL	10×M buffer
10μL	sample plasmid
5μL	ddH_2O (5μL for T7p + RBS + CI plasmid)
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20μL	Total

Electrophoresis the digested samples detect the successful ligation. The result showed that all the colonies did come from successful ligation.