IGEM-ZJUT 2018 WetLab recorder：Yankang

Time：7 月 30 日

1、Experimental purpose: pTargetF linearized PCR product (pTF ori-) eliminate template; pTF ori- and p15A purification; one-step cloning

2、Material

pTargetF linearized PCR product; p15A ori PCR product; ClonExpress II One Step Cloning Kit(Vazyme); Q.Cut DpnI (Takara); MiniBEST DNA Fragment Purification Kit (TaKaRa)

3、Experimental procedure

Eliminate the template:

(1) Add 1ul of DpnI and 5ul of Buffer to 50ul of PCR product;

(2) Incubate for 2 hours at 37 ℃ in a metal bath.

Purification of pTF ori- and p15A:

Purification step according to the MiniBEST DNA Fragment Purification Kit instructions.

The concentration determination results were: pTF ori-(380 ng/ul); p15A (242 ng/ul)

One-step cloning

(1) The purified product of pTF ori- is diluted 7 times, and the purified product of p15A is diluted 8 times；

(2) Formulate the following system:

	experimental group	Control group1	Control group2
Diluted pTF ori-	1 ul	1ul	0
Diluted p15A	1 ul	0	1ul
5x CE Ⅱ Buffer	4 ul	4ul	4ul
Exnase Ⅱ	2 ul	2ul	2ul
ddH2O	12 ul	13ul	13ul

(3) Mix and react at 37 ℃ for 30 min；

(4) Store at -20 ℃

Transformation

(1) pTF-p15A one-step cloning product, pTF ori- one-step cloning control group, E. coli DH5α competent cells were pre-cooled on ice for 5 min;

(2) Take 10 ul of the one-step clone product in 1.5 ml EP tube, add 50ul of competent state, place on ice for 30min;

(3) Heated at 42 ℃ metal bath for 90s (precise timing), immediately placed on ice for 2min;

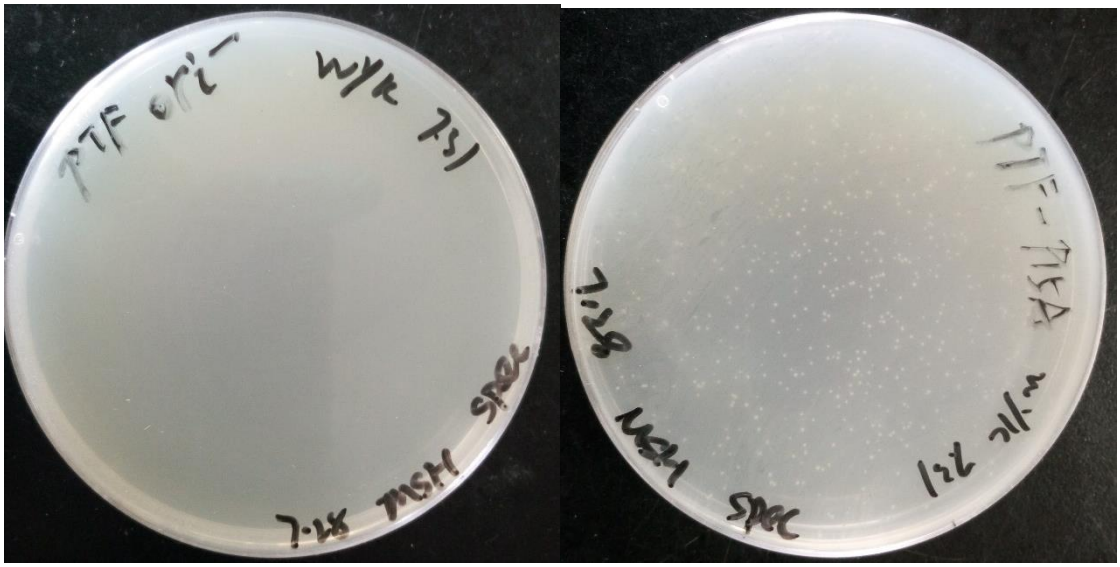
(4) Take LB medium preheated at 37 ℃, add 1 ml per tube, and incubate at 37 ℃ for 1 h;

(5)Centrifuge at 4000 rpm for 5 min, discard 800 ul of supernatant, and resuspend the cells with the remaining liquid;

(6) A 50 ul bacterial suspension was applied to a spectinomycin (spec) plate and cultured overnight in a 37 ℃ incubator.

Time：7 月 30 日

4、Results



pTF-p15A pTF ori$^-$

The negative control grew aseptically, indicating that there was no PTarget vector plasmid with no dereplicon linearization in the one-step cloned product. The number of colonies in the experimental group was significantly higher than that in the negative control group (negative control aseptic drop), and the one-step cloning linkage was likely to be successful. Next, colony PCR will be performed to further verify whether the pTF-p15A recombinant plasmid has been successfully introduced into the transformant.