GenScript Quick T-A Cloning Kit

Technical Manual No. 0224 **Version 20061030**

I. DESCRIPTION

Quick T-A Cloning Kit is designed for convenient cloning for PCR products with 3'-A overhang. Linear blunt plasmid was added one thymine base at its 3'-end following *Eco*RV digestion. It includes special quick ligation buffer. Reaction can be incubated for 30 minutes at 16℃. *Lac*Z report gene in the vector allows the blue-white screening based the principle of α-complement. Any sequencing primers used for the pUC57 are suitable for the sequencing of the pUC57-T vector.

II. KIT COMPONENTS

The kit (SD0228) contains the following components:

pUC57-T Vector (50 ng/μl)	20 μl
Control Insert (50 ng/μl)	10 μl
2× Quick Ligation Buffer	200 μl
T4 DNA Ligase (5 Weiss units/μl)	20 μl
X-Gal/ IPTG premix	1,000 μl

III. VECTOR DESCRIPTION

pUC57-T vector is designed for convenient cloning for PCR products with 3'-A overhang. Linear blunt plasmid was added one thymine base at its 3'-end following *Eco*RV digestion. Fig. 1 is the map and restriction sites of pUC57-T Vector.

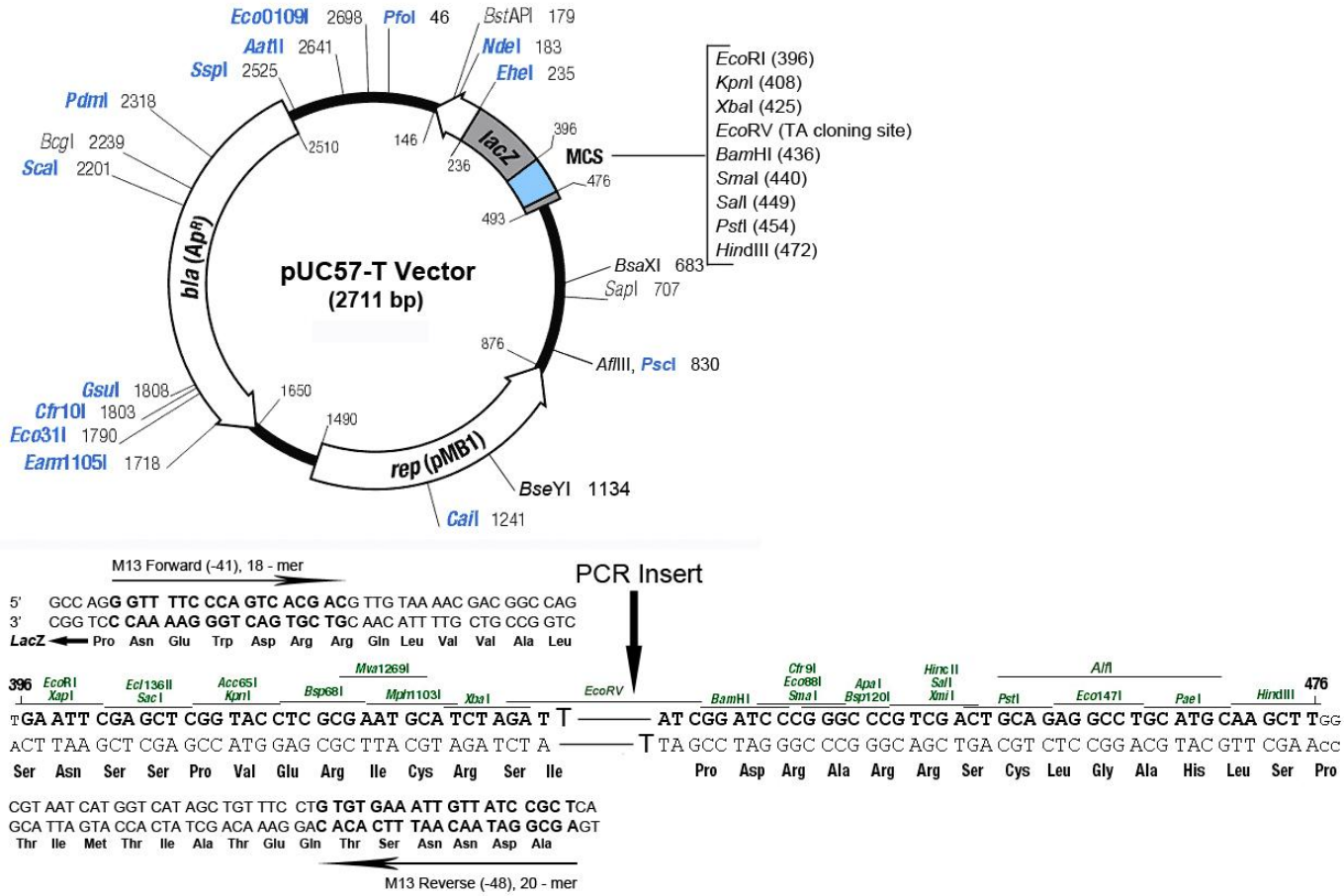

Fig. 1 Map of pUC57-T Vector

Sequencing Primers:

Forward primer: M13 Forward (-41), DA0004
Reverse primer: M13 Reverse (-48), DA0006.

IV. STORAGE

Store at -20℃.

V. QUALITY CONTROL

Each lot of pUC57-T Vector is tested with PCR Cloning and Screening. Results showed that the recombinant efficiency of the white colonies is above 85%.

VI. TA CLONING PROCEDURE

A. Ligation

1. Set up your ligation system as the following

pUC57-T Vector (50 ng/μl)	1 μl
Control Insert* (50 ng/μl)	1 μl
ddH_2O	up to 5 μl

2. Add 5 μl of 2×Quick Ligation Buffer and mix.

3. Add 1 µl of T4 DNA Ligase and mix thoroughly.
4. Centrifuge briefly and incubate at 16℃ for 30 minutes.
5. Chill on ice (Do not heat inactive).

* Control insert is a size of 500 bp PCR fragment amplified by *Taq* DNA polymerase. The optimal mol ratio of insert and vector is 3:1 to 6:1.

B. Transformation

1. Briefly centrifuge the ligation reaction.
2. Add 2 µl of the ligation reaction to 50 µl competent cell.
3. Incubate on the ice for 30 minutes.
4. Heat-shock at 42℃ for 2 minutes.
5. Chill on ice for 3 minutes.
6. Add 1 ml of LB to the tube, and then shake it at 200 rpm for 1 hour.
7. Plate 50 µl of X-Gal/ IPTG Premix on the LB/Amp plates, dry it before use.
8. Plate an appropriate amount of cells on the plates.
9. Incubate the plates at 37℃ and grow overnight.

C. PCR Screening

Pick white colonies to do PCR Screening with M13 Forward (-41) (DA0004) and M13 Reverse (-48) (DA0006). The following picture (Fig. 2) is our screening result when control insert of 500 bp is cloned into pUC57-T vector.

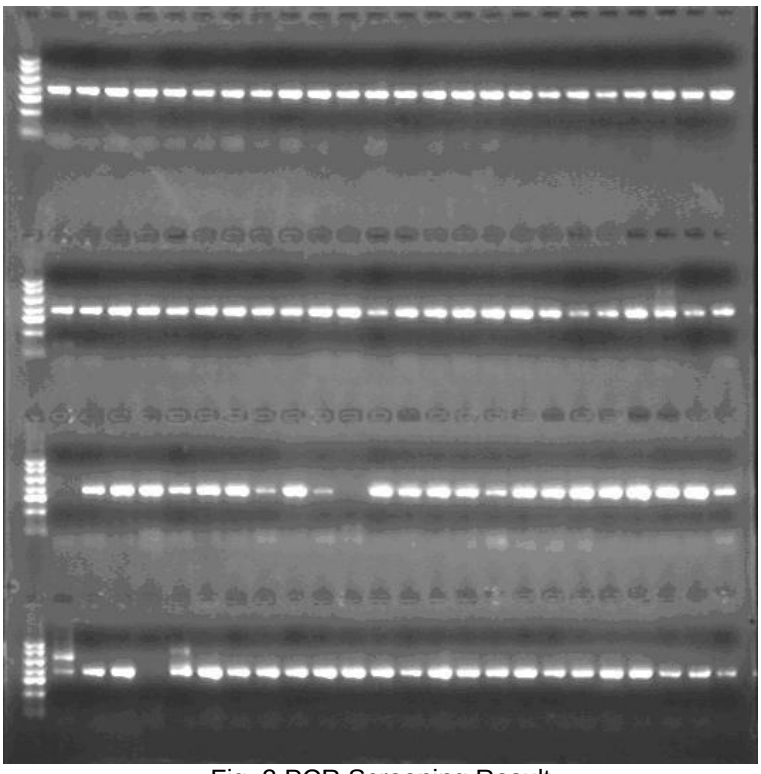

Fig. 2 PCR Screening Result

D. Sequencing

Sequencing the positive colonies with the following primers:

Forward primer DA0004: M13 Forward (-41) (GGTTTTCCCAGTCACGAC)
Reverse primer DA0006: M13 Reverse (-48) (AGCGGATAACAATTTCACAC)

Fig. 3 and Fig.4 show the additional T base at 5'-end and 3'-end sequence of insert.

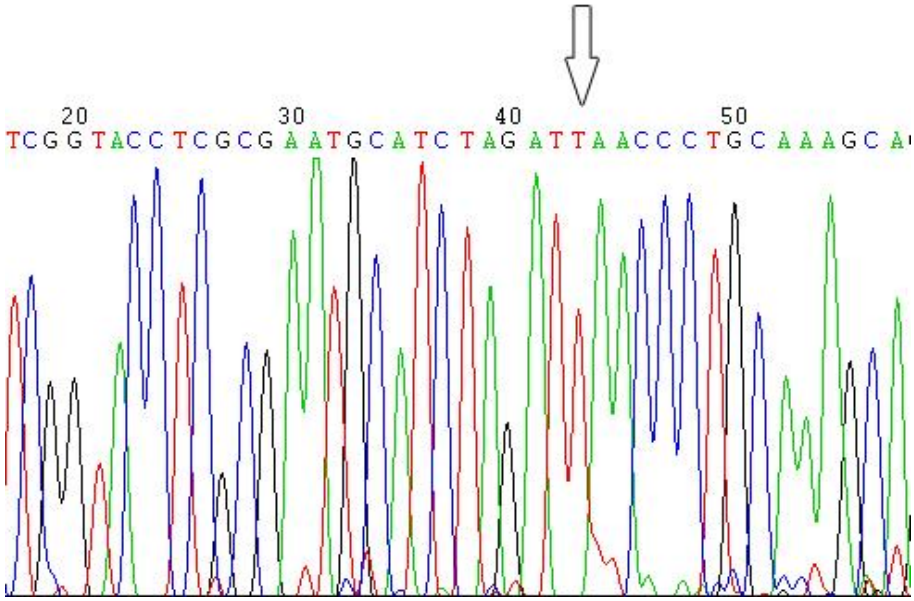

Fig. 3 5'-end sequencing result of the cloned PCR product

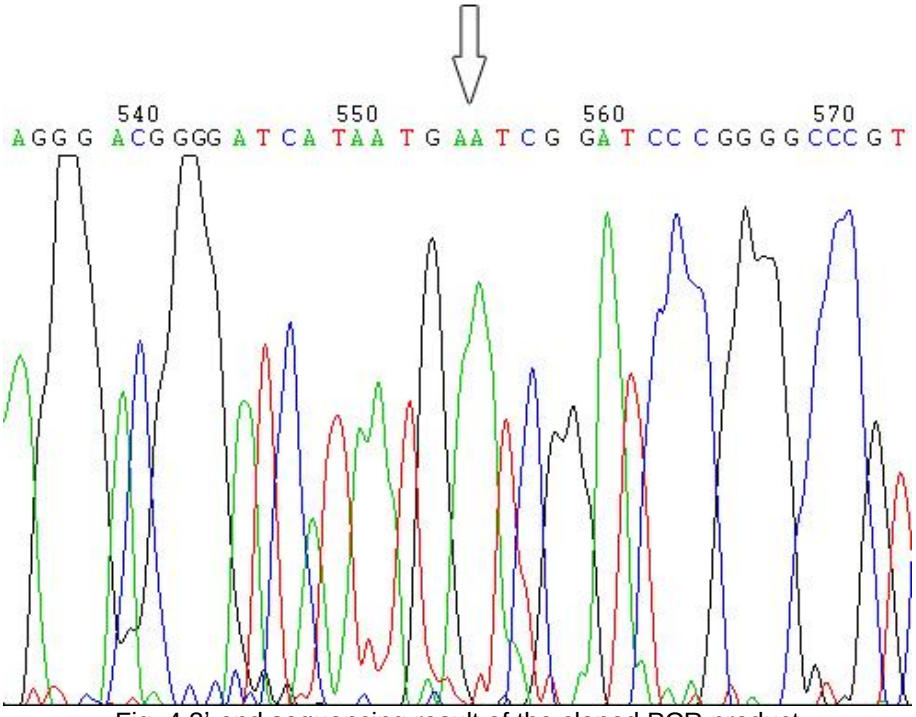

Fig. 4 3'-end sequencing result of the cloned PCR product

VII. TROUBLE SHOOTING

Problem	Probable Causes	Solution
Low colonies	Low efficiency competent cell	Use high efficiency competent cell (1×10^8 cfu/μg). Use a supercoiled plasmid with known concentration as a control to test the efficiency of your competent cell. Transform total volume of 10 μl ligation reaction into the competent cell.
	Uncorrect ligation reaction	Take notice of the concentration of quick ligation buffer is 2×.
Low white colonies with control insert	Improper mol ratio of insert and vector	The optimal mol ratio of insert and vector is 3:1 to 6:1
	Low activity of T4 DNA ligase	Store at -20℃ after use
	Low performance of ligation buffer	Avoid multiple freeze-thaw cycles.
	Unpurified PCR products	Gel-purified the PCR products is the best method. Avoid long time of exposure to the UV light.
No colonies	PCR product with no 3'-A overhang	Ensure the fragments are amplified with the polymerase like *Taq* DNA polymerase. If the fragment with blunt end, add 3'-A with *Taq* DNA polymerase and dATP.

VIII. ORDER INFORMATION

Quick T-A Cloning Kit: Cat. No. SD0228

For Research Use Only.

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