CYCV16-CHANGYU pTOPO-Blunt Kit

Contents	20 apps (CYCV1601)	80 apps (CYCV1602)
pTOPO-Blunt Vector(30ng/μl)	20 μl	80 μl
1000bp Control（30ng/μl）	5 μl	5 μl
10 × Enhancer	20 μl	80 μl

Note: All reagents, when store in -20 °C, are stable for 12 months.

❖ Description

The Zero Background Blunt Topoisomerase Cloning Kit is designed for fast cloning of blunt ended DNA fragments up to 10 kb generated by high fidelity DNA polymerase such as KOD, Pfu and Phusion. DNA fragments obtained by restriction digestion or mechanical shearing can also be cloned after end polishing to become blunt ended. It utilizes DNA strand transfer activity of Viccinia virus topoisomerase I. Vaccinia virus DNA topoisomerase I forms a 3'-phosphoryl intermediate with the plasmid vector containing cleavage recognition motif of 5'CCCTT↓. Covalently bound topoisomerase I then transfer the incised vector DNA strand to the DNA fragment to be cloned with free 5'-OH terminuses. This transferring reaction is rapid and reproducible. The cloning vector pTOPO-Blunt included in this kit are high copy number plasmids engineered to tolerate mild toxic genes. Regions flanking the cloning site of pTOPO-Blunt vectors have multiple common restriction sites for release of the cloned fragment by single or double restriction digestion.

❖ Procedure

1. Set up the topoisomerase cloning reaction by mixing the reagents in the order shown.

DNA Fragment or 1μl 1000bp control	0.5-8μl
pTOPO-Blunt Vector	1μl
10 × Enhancer	1μl
diH2O	Xμl
Final Volume	10μl

DNA fragments MUST NOT be 5' phosphorylated. Blunt ended DNA fragment is preferred. Highest cloning efficiency was observed from PCR fragments generated by high fidelity enzymes such as KOD, Phusion, Pfu etc. 10-150 ng of DNA fragments ranging from 100 bp to 5000 bp has been tested to give satisfactory

results. Excess amount of DNA inserts e.g. ›300ng, will reduce cloning efficiency. Refer to the following table.

Fragment size (bp)	Optimal amount (ng)
100-1000	10-40
1000-2000	40-80
2000-5000	80-150

2. Mix the reaction gently and incubate for 5 minutes at room temperatures between 15-30°C.

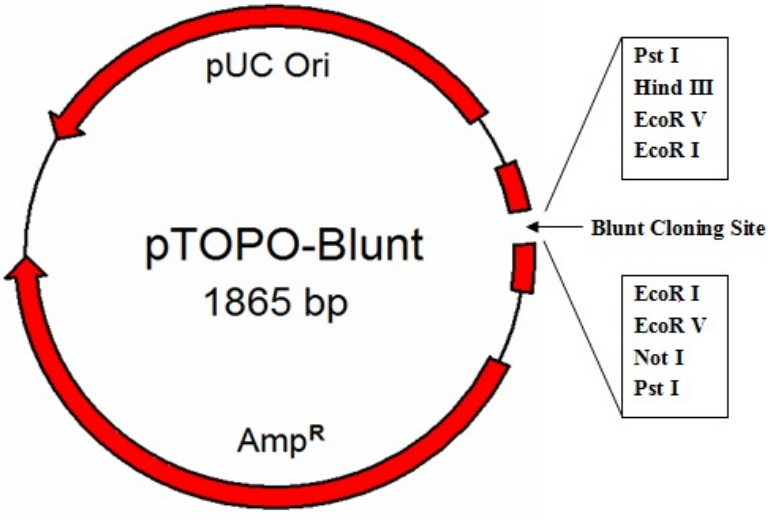
   Recent R&D data indicate incubation at this step produce no detectable benefits. In any case, do not let the incubation go beyond 5 minutes. Extended incubation for larger inserts up to 5 kb is unnecessary and may introduce background.

3. Add 5 µl of the cloning reaction into 50 µl chemically competent E. coli and mix gently. Do not mix by pipetting up and down.

4. Incubate on ice for 2-30 minutes.

5. Following the instruction of competent cells to complete transformation.

Map of pTOPO-Blunt：

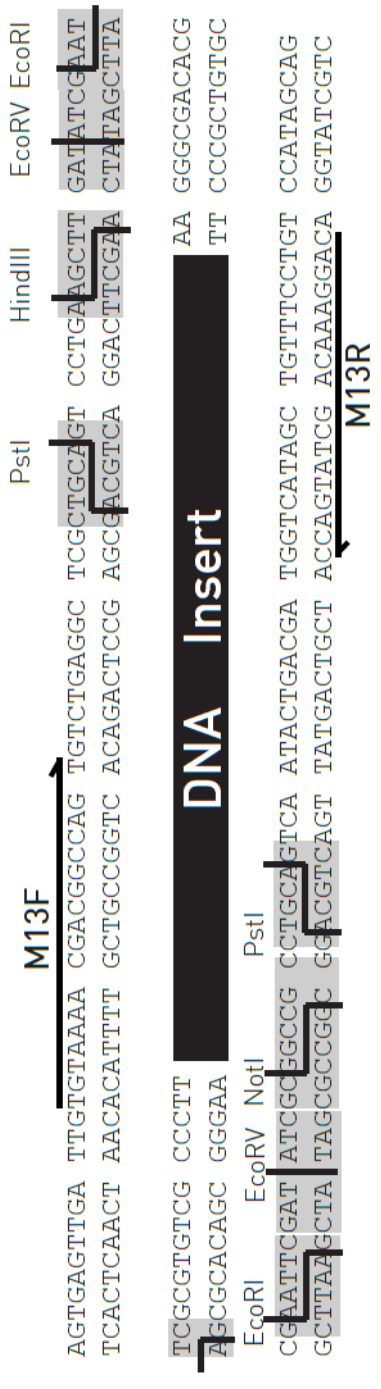


❖ **Sequencing Primer：**

M13F: TGTAAAACGACGGCCAGT

M13R: CAGGAAACAGCTATGACC

❖ **MCS information:**



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