CYCV16-CHANGYU pTOPO-Blunt Kit

Contents	20 rxns (CYCV1601)	80 rxns (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 stored at -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 polymerases 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 *Vaccinia* 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 transfers the incised vector DNA strand to the DNA fragment to be cloned with free 5'-OH termini. This transferring reaction is rapid and reproducible. The cloning vector pTOPO-Blunt included in this kit is a high-copy-number plasmid engineered to tolerate mildly 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
ddH_2O	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 have been tested to give satisfactory results. Excessive

amounts of DNA inserts e.g. >300ng will significantly 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 temperature between 15-30°C.

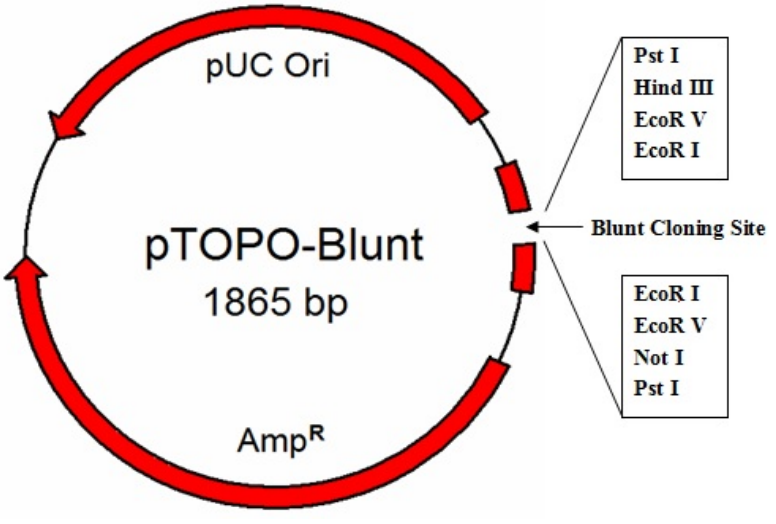
   Recent R&D data indicate incubation at this step produces no detectable benefits. In any case, do not let the incubation go beyond 5 minutes. Extended incubation for larger inserts up to 5 kb is unnecessary and may introduce background.

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. Follow the competent cell manufacturer's instructions to complete the transformation.

Map of pTOPO-Blunt：

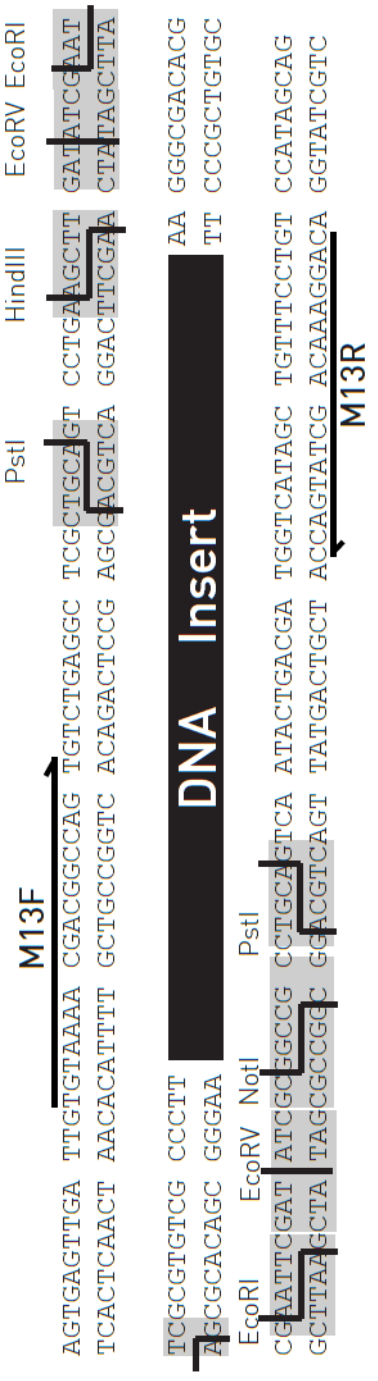


❖ **Sequencing Primer：**

M13F: TGTAAAACGACGGCCAGT

M13R: CAGGAAACAGCTATGACC

❖ **MCS information:**



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