CYCV15-CHANGYU pTOPO-TA Simple Kit

Contents	20 rxns (CYCV1501)	80 rxns (CYCV1502)
pTOPO-T Simple 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 TA Simple Topoisomerase Cloning Kit is designed for fast cloning of DNA fragments up to 10 kb generated by *Taq* DNA polymerase which introduces single 3'-A overhang to the end of the DNA product. DNA fragments generated by restriction digestion or obtained by mechanical shearing can also be effectively cloned after end polishing using *Taq* DNA polymerase. 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-TA Simple included in this kit is a high copy number plasmids engineered to tolerate mildly toxic genes. Regions flanking the cloning site of pTOPO-TA Simple vectors do not contain any common restriction sites which eliminates possible redundancy of restriction sites.

❖ 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-T Simple Vector	1μl
10 × Enhancer	1μl
ddH_2O	Xμl
Final Volume	10μl

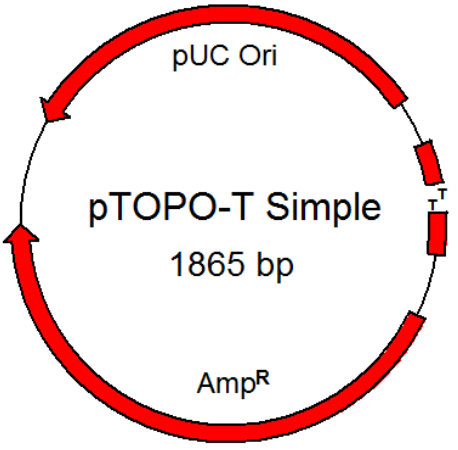
DNA fragments MUST NOT be 5'-phosphorylated. TA-ended DNA fragment is preferred. Highest cloning efficiency was observed from PCR fragments generated by *Taq* DNA polymerase or fragments tailored by Taq polymerase. 10-150 ng of

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-T Simple:**

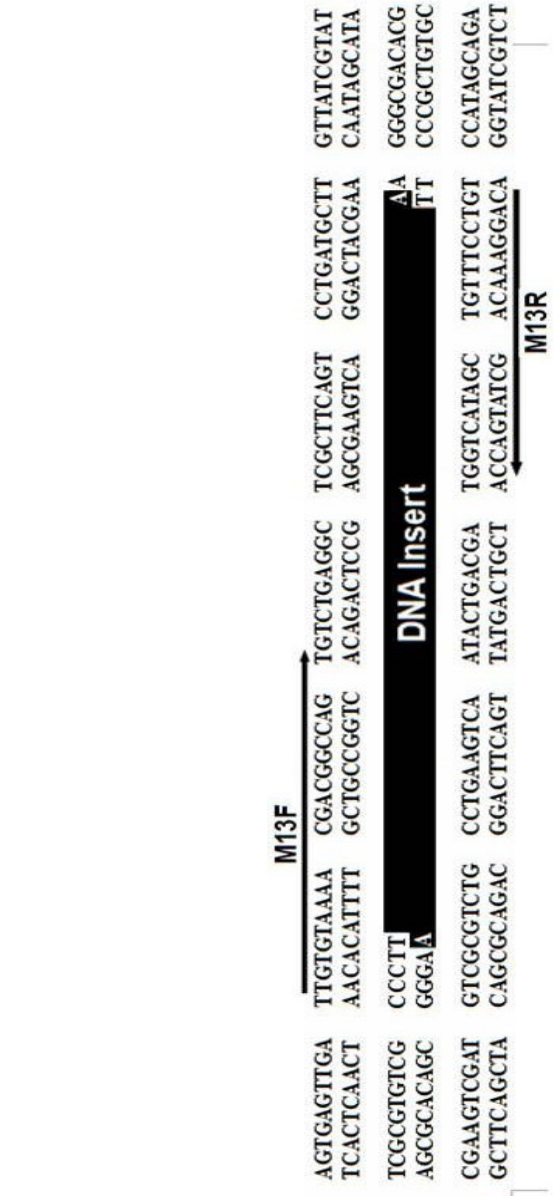


❖ **Sequencing Primer:**

M13F: TGTAAAACGACGGCCAGT

M13R: CAGGAAACAGCTATGACC

❖ **Cloning site information:**



This product is furnished for LABORATORY RESEARCH USE ONLY.
Not for diagnostic or therapeutic use.