CYCV21-CHANGYU pTOPO-TA/Blunt Kit

Contents	20 rxns (CYCV2101)	80 rxns (CYCV2102)
pTOPO-TA/Blunt Vector (30 ng/μl)	40 μl	160 μl
1000 bp Control (30 ng/μl)	5 μl	5 μl
10× Enhancer	20 μl	80 μl

Note: Reaction counts are for the standard 10 μl system. With a 5 μl reaction (all components halved), CYCV2101 gives 40 reactions and CYCV2102 gives 160 reactions.

Note: All reagents, when stored at -20 °C, are stable for at least 12 months. Shipped with ice packs; 1-2 days at ambient temperature during transit does not affect performance. Competent cells are not included.

Description

The Zero Background pTOPO-TA/Blunt Cloning Kit uses topoisomerase instead of T4 DNA ligase. Topoisomerase joins DNA fragments to the vector within seconds to minutes with close to 100% efficiency, and the kit is designed for any PCR product, whether it carries 3'-A overhangs or blunt ends. No ligase is required.

The vector is smaller than 2 kb (1865 bp). A smaller TOPO vector accepts larger inserts more efficiently, and the recombinant plasmid is more than 2 kb smaller than with conventional vectors, which raises transformation efficiency and the number of transformants. Inserts of up to 10 kb can be cloned; for a 5 kb insert, at least 8 of 10 picked colonies may contain the insert.

The vector is ampicillin-resistant, so recovery after transformation takes only 0-10 minutes instead of about 1 hour for kanamycin vectors. Zero-background (lethal-gene) selection means that vector without an insert does not give colonies, so blue/white screening and colony PCR screening are not needed; in most cases a randomly picked colony carries the insert.

Note: Sequence only with the M13F/M13R primers given in this manual. Do not use M13(-47) / M13(-48) primers.

❖ Procedure

1. Preparation. Use normally designed PCR primers; no changes are required, but the primers **must not** be phosphorylated. Any PCR product can be ligated directly, whether it has A-ends or blunt ends. Gel purification of the PCR product is recommended to avoid later problems. If the product shows only the target band, without non-specific bands or primer dimers, you can also try ligating it directly. PCR products amplified from a plasmid template are best purified, because the template plasmid can also give colonies.

2. Set up a 10 μl reaction at room temperature (a 0.2 ml PCR tube in a PCR machine is recommended for temperature control):

Component	**10 μl reaction**	**5 μl reaction**
Purified PCR product, or control insert	0.5-5 μl (control: 1 μl)	0.25-2.5 μl (control: 0.5 μl)
pTOPO-TA/Blunt Vector	2 μl	1 μl
10× Enhancer	1 μl	0.5 μl
Sterile water	X μl	X μl
Final Volume	10 μl	5 μl

Mix gently by pipetting or by flicking the tube, then spin briefly to collect the liquid. Set up this step at room temperature (25-37 °C), **not on ice**. For a 5 μl reaction, halve every component; the number of reactions doubles.

Recommended insert amounts (10 µl reaction). Too much insert reduces the number of transformants:

Insert size (bp)	Recommended amount (ng)
100-1000	10-40
1000-2000	40-80
2000-5000	80-180

3. Incubate for 5 minutes at 37 °C (or at room temperature, 25-37 °C), preferably in a PCR machine. For long or difficult inserts, extend the incubation to 10-15 minutes at 37 °C; this can markedly increase the number of transformants.

4. Place the reaction on ice and proceed directly to transformation.

Transformation

1. Take the competent cells from -80 °C and thaw them on ice (about 1-3 minutes).

2. Immediately add 5 µl of the reaction (up to the whole 10 µl, as long as it is no more than 1/10 of the cell volume). Mix by flicking the tube gently; do not pipette up and down. Keep on ice for 5 minutes.

3. Heat-shock at 42 °C for 60 seconds, then return to ice for 2-3 minutes without shaking.

4. Recovery (optional): add 500 µl LB or SOC medium without antibiotics and shake at 37 °C, 200 rpm for 10-20 minutes. The medium (pre-warmed to 22-37 °C) can be added directly to the 1.5 ml tube, which is then fixed horizontally in the shaker. With commercial competent cells and inserts up to 2 kb, 10-15 minutes gives enough transformants; with low-efficiency (home-made) cells, few transformants or long inserts, extend recovery to 30-60 minutes. If transformants are plentiful, recovery can be skipped and the cells plated directly after heat shock, so the whole process from ligation to plating takes about 15 minutes.

5. Spread 100-200 µl on LB agar containing **100 µg/ml ampicillin** and incubate overnight.

Note: This vector is ampicillin-resistant. Do not use kanamycin plates.

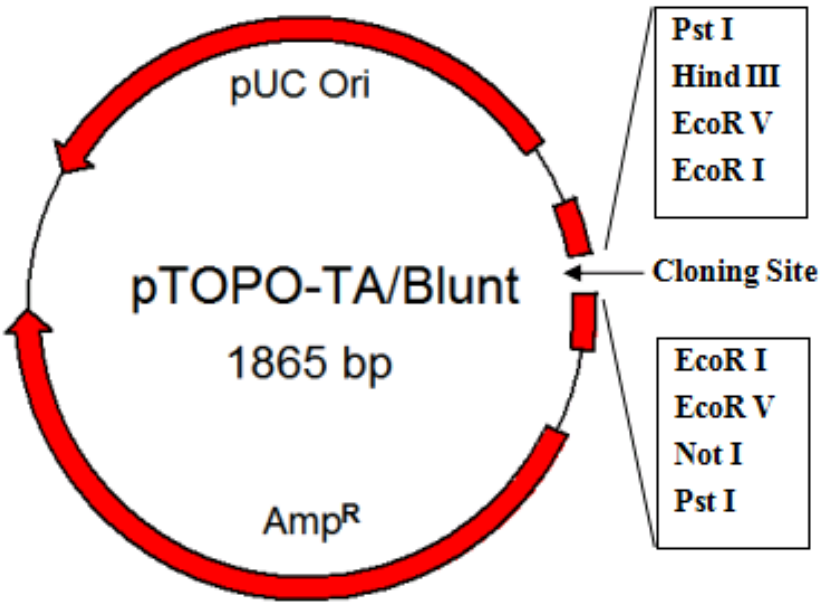
Screening and identification

Bacteria carrying vector without an insert are killed by the lethal gene, so false positives are very rare: normally growing colonies (not contaminants, and not unusually few) almost always contain an insert. For inserts up to 2-3 kb we recommend sequencing 1-2 colonies directly instead of colony PCR.

1. Colony PCR on TOPO vectors can give false negatives (no band, or a band of unexpected size, although an insert is present). A positive colony PCR result can be trusted; a negative or unexpected result should be checked by sequencing, plasmid size on a gel, or restriction digestion.

2. For larger inserts, plasmid size on an agarose gel already identifies recombinant clones. Inserts can also be checked by EcoR I or EcoR V single digestion to release the insert, or other suitable enzymes, followed by agarose gel electrophoresis.

Map of pTOPO-TA/Blunt

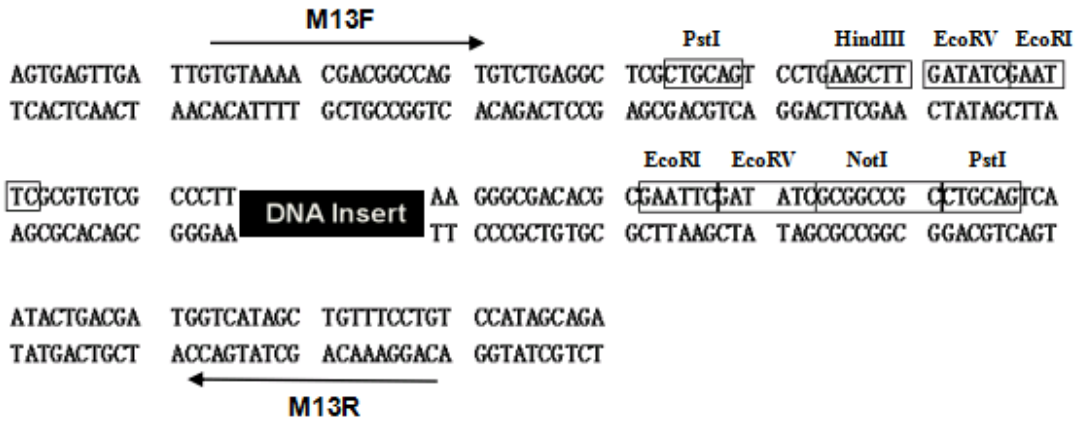


Sequencing primers

Primer	Sequence (5' to 3')
M13F	TGTAAAACGACGGCCAGT
M13R	CAGGAAACAGCTATGACC

Note: Do not use M13(-47) / M13(-48) primers with this vector. Several different "M13 universal primer" sequences exist, and some synthesis providers' default M13 primers differ from the ones above - check the sequence before ordering.

MCS information



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