CYCV22-CHANGYU pTOPO-TA/Blunt Simple Kit

Contents	20 rxns (CYCV2201)	80 rxns (CYCV2202)
pTOPO-TA/Blunt Simple 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), CYCV2201 gives 40 reactions and CYCV2202 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 Simple 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.

Simple vector: there are no multiple cloning sites flanking the insertion site. When needed, introduce suitable restriction sites on the PCR primers. Digestion at these primer-introduced sites is then not affected by other cloning sites on the vector, which improves digestion efficiency and subcloning success.

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 Simple 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.

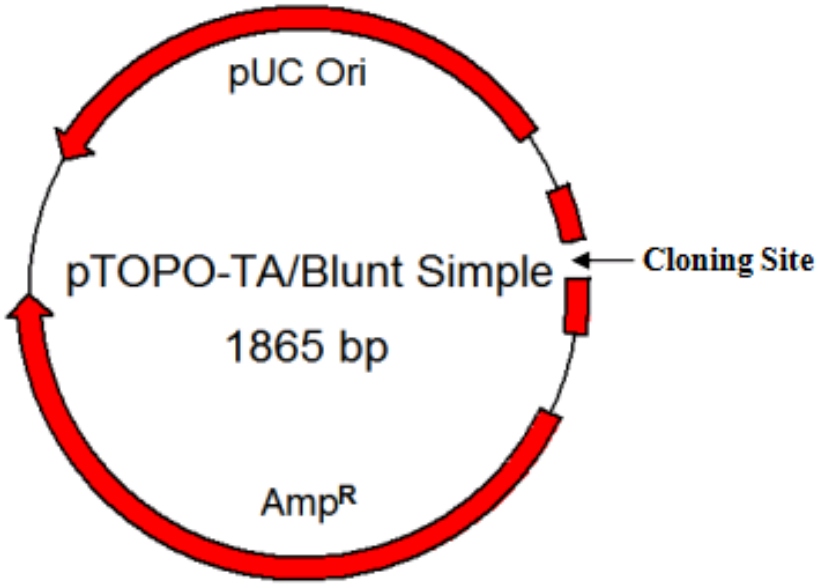
Screening and identification

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 restriction sites on the vector backbone such as Apa I / Bgl I / BsaH I, or sites introduced on the PCR primers, followed by agarose gel electrophoresis.

Map of pTOPO-TA/Blunt Simple

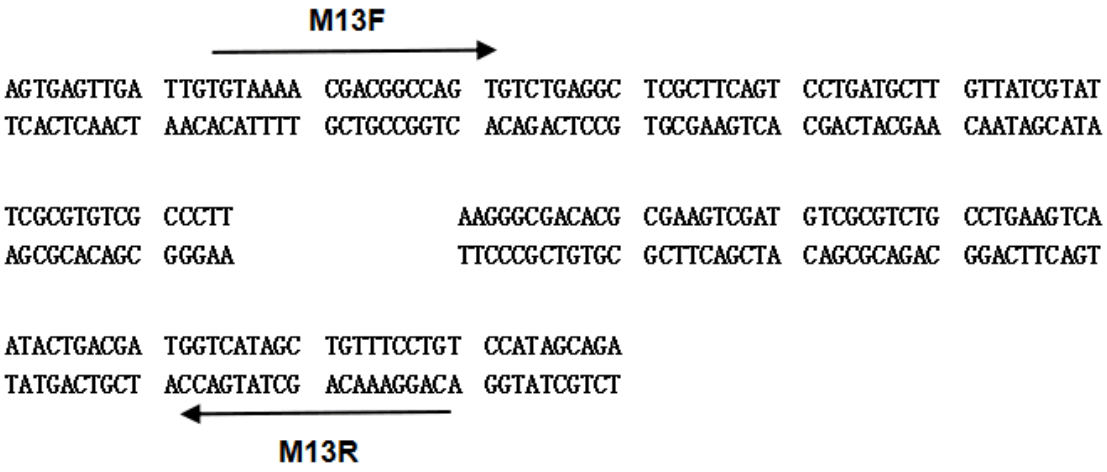


Sequencing primers

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.

Cloning site sequence



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