pBM16K Topo Cloning Kit

User's Instruction

Description

pBM16K Topo Cloning Kit utilizes the inherent biological activity of DNA topoisomerase I. Topoisomerase can cleave and rejoin supercoiled DNA ends and therefore can be used to insert DNA fragments into vectors. It is not only suitable for cloning blunt-end PCR products which are amplified by high-fidelity DNA polymerases such as Pfu, sPfu, KOD, Xerox, Phusion, Q5, and SuperGold™, but also can be used to clone PCR products with an extra "A" nucleotide at the 3'end which are amplified by DNA polymerases such as Taq, Taq plus, Tth and klenTaq. The pBM16K vector in the kit is linearized. Positive clones can be identified by colony PCR with primers M13F and M13R.

Kit Contents

	20 preps
1. pBM16K Vector (20 ng/μl)	20 μl
2. 10 × Topo cloning buffer	20 μl
3. Control Insert LacZα	5 μl
4. M13F Primer (add 60 μl ddH_2O before use)	0.1 OD
5. M13R Primer (add 60 μl ddH_2O before use)	0.1 OD

	60 preps
1. pBM16K Vector (20 ng/μl)	20 μl × 3
2. 10 × Topo cloning buffer	20 μl × 3
3. Control Insert LacZα	5 μl
4. M13F Primer (add 60 μl ddH_2O before use)	0.1 OD × 3
5. M13R Primer (add 60 μl ddH_2O before use)	0.1 OD × 3

Features

- The ligation reaction takes only 5 min.
- Suitable for both blunt-end and A-tailed PCR products.
- The vector adopts a new preparation process with zero background and no need for blue-white spot screening.

- There are Sma I and EcoR V restriction sites on both sides of the cloning site, which is suitable for mono-restriction endonuclease analysis.
- The vector carries kanamycin resistance gene.

Protocol

Setting up ligation reaction

1. Set up ligation reaction as the following table (take 0.2 ml PCR tube as an example):

Reagent	Volume
DNA fragment	0.5-8 μl
pBM16K Vector	1 μl
10 × Topo cloning buffer	1 μl
RNase-Free Water	Up to 10 μl

2. Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin.

Temperature for ligation reaction (Recommended)

1. Room temperature (20℃-30℃) for 5-15 min

   a) We recommend using PCR thermocycler to set the temperature at 25℃. If the result of electrophoresis shows that there is no primer dimer or nonspecific bands after amplification, then you can take 1-3 μl PCR amplification product directly for cloning without purification.

2. Keep PCR tubes on ice. The amount of DNA used for ligation depends on the length. Please check the following table to make sure you add optimal volume.

DNA Fragment Length (bp)	Optimal Volume (ng)
100-1,000	20-50
1,000-2,000	50-100
2,000-5,000	100-200

Positive control reaction

1. Take 1 μl control insert LacZα (1 kb) for cloning and transform into competent E. coli cells with alpha complementary function (such as DH5α, TOP10, Mach1-T1, etc.).The bacterial liquid is coated on the LB Kan plate containing IPTG and X-gal. Check the plates on the next day. The blue colony is positive, indicating that there is fragment insertion, and the white colony is the empty vector.
2. Keep PCR tubes on ice. The amount of DNA used for ligation depends on the length. Please check the following table to make sure you add optimal volume.

Transformation (using 100 μl frozen competent cells as example)

1. Thaw a vial of frozen competent cells on ice. Tap tube gently to ensure that the cells are suspended.
2. Transfer 5 μl of reaction mixture into competent cells, mix gently to ensure even distribution of the DNA solution (flicking the tube, not vortex). Leave the tube on ice for 20-30 minutes.
3. Heat-shock at 42℃ for 30 sec, and immediately place them directly on ice for 2 min.
4. Add 500 μl of room temperature SOC/LB medium to the cells and then incubate at 37℃ for 60 min while shacking at 200 rpm.
5. Centrifuge at 4,000 rpm for 1 min. Discard the supernatant fluid. Tap tube gently to ensure that the cells are suspend with the remaining liquid (100-200 μl).
6. Spread 50 μl of cells on the selection plate and incubate overnight at 37℃.

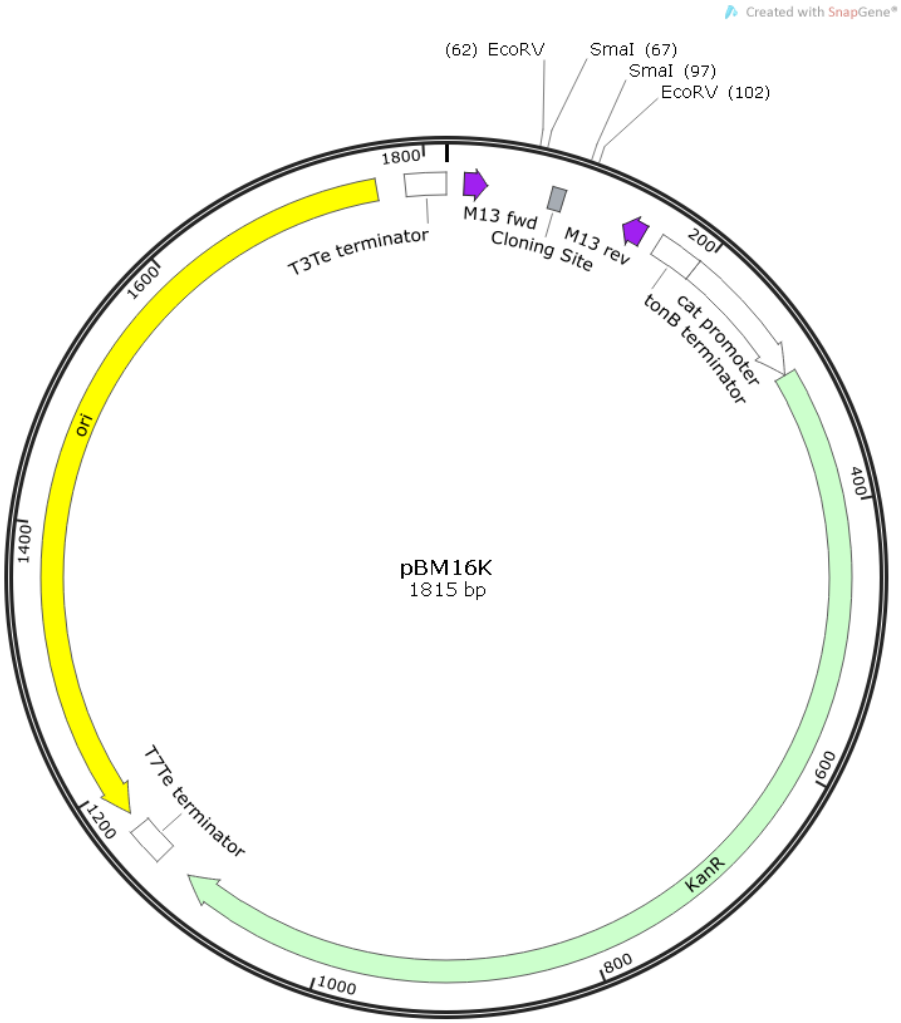
Analysis of positive clones

1. Analyzing by PCR: Pick single colony into 10 μl of ddH_2O. Mix by vortexing or pipetting up and down. Add 1 μl of mixture into 25 μl of PCR system. Identify the positive clones by M13F and M13R primers.
2. Analyzing by restriction enzyme digestion: Pick single colonies and culture them overnight in LB medium containing the appropriate selection antibiotic. Isolate plasmid DNA by SiMax™ Plasmid DNA Miniprep. Analyze the plasmids by restriction enzyme digestion.
3. Sequencing: Perform sequence analysis.

Vector Information

Cloning site

Vector map



pBM16K sequence landmarks

- M13F primer binding site:14-30
- M13R primer binding site:135-151
- Kanamycin resistance ORF:299-1114
- pUC ori:1177-1764

Sequence

>pBM16K

AGTAGTTGATTGTGTAAAACGACGGCCAGTGTCTGAGGCTCGCTTCAGTCCTGATGC
TTGATATCCCGGGCGTGTCGCCCTT$$$AAGGGCGACACGCCCGGGATATCGCGTCT
GCCTGAAGTCAATACTGACGATGGTCATAGCTGTTTCCTGTCCATAGCAGAAAGTCAA
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CATAATGAAATAAGATCACTACCGGGCGTATTTTTTGAGTTATCGAGATTTTCAGGAGC
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