CYCV04-CHANGYU pBLUE-T Cloning Kit

Contents	20 rxns (CYCV0401)	60 rxns (CYCV0402)	20 rxns (CYCV0403)	60 rxns (CYCV0404)
pBLUE-T Vector (30 ng/μl)	20 μl	60 μl	20 μl	60 μl
1000 bp Control (30 ng/μl)	5 μl	5 μl	5 μl	5 μl
10× PEG Enhancer	50 μl	150 μl	50 μl	150 μl
2× Quick Ligation Buffer	-	-	100 μl	300 μl
10× Ligation Buffer	-	-	40 μl	120 μl
T4 DNA Ligase (5 U/μl)	-	-	20 μl (100 U)	60 μl (300 U)

Note: CYCV0401 / CYCV0402 contain the vector, control insert and PEG Enhancer; CYCV0403 / CYCV0404 also contain the ligation buffers and T4 DNA Ligase.

Note: Store at -20 °C. The vector can be frozen and thawed at least 10 times without loss of performance.

Description

Many thermostable DNA polymerases, such as *Taq* and *Tth* DNA polymerase, add a single 3'-A overhang to PCR products. Such products are conveniently cloned into a vector carrying a single 3'-T overhang. pBLUE-T Vector is derived from pBlueScript II SK(+): a suitable sequence was introduced at the EcoR I site, and digestion with Xcm I directly leaves an unpaired 3'-T at each end, which gives higher recombination efficiency.

Two EcoR I sites flank the insertion site, so inserts can be checked by an inexpensive EcoR I single digestion. The vector has no Nde I or Nco I site, which makes it convenient for cloning genes that contain these sites.

The ligation system gives low background (blue colonies below 10%) and high recombination (more than 90% of white colonies carry an insert), and supports quick ligation. The full vector sequence corresponds to pBlueScript II SK(+) except for small differences at the cloning site; technical data are given at the end of this manual.

❖ Procedure

1. Preparation. Whether the PCR product needs purification depends on its quality: very clean products can be ligated directly, but products amplified from a plasmid template must be purified, because the template plasmid can also give white colonies. PCR products can be separated by agarose gel electrophoresis and recovered with a gel extraction kit (fragments above 70 bp are recovered well). Products of *Taq*, *Tth*, AmpliTaq and KlenTaq DNA polymerases carry a 3'-A overhang and can be cloned directly. Mixtures of *Taq* with proofreading enzymes (*Pfu*, *Pwo*, *Tli*, Deep Vent) may give 3'-A ends. Products of polymerases with 3'→5' exonuclease activity (high-fidelity enzymes) are blunt-ended and must be A-tailed first.

2. Standard ligation. In a standard 10 µl reaction, add 1 µl (30 ng) vector, X µl PCR product, 1 µl 10× Ligation Buffer and 0.5-1 µl (2.5-5 Weiss units) T4 DNA Ligase, and make up with water. Exact quantification of the PCR product is usually unnecessary: a PCR product : vector molar ratio of 2:1 to 10:1 gives good results, 3:1 is recommended.

10× Ligation Buffer (thaw and mix well before use)	1 µl
10× PEG Enhancer	1 µl
pBLUE-T Vector	1 µl
Purified PCR product (or 1 µl 1000 bp Control)	X µl
Sterile water	Y µl
T4 DNA Ligase (5 Weiss units/µl), added last	0.5-1 µl
Final Volume	10 µl

Ligate overnight at 16 °C (standard 2.5 Weiss units per 10 µl) for the most transformants; 1 hour at 16 °C with 5 Weiss units per 10 µl meets the needs of most experiments. A PCR machine can be used. Cool on ice, then transform or store at -20 °C.

3. Quick ligation. Set up the following reaction:

2× Quick Ligation Buffer	5 µl
pBLUE-T Vector	1 µl
Purified PCR product (or 1 µl 1000 bp Control)	X µl
Sterile water	Y µl
T4 DNA Ligase (5 Weiss units/µl), added last	1 µl
Final Volume	10 µl

The 2× Quick Ligation Buffer contains all components optimized for quick ligation. Ligate at 16 °C for 30 minutes (up to 2 hours for long inserts); 5-10 minutes at room temperature (22-25 °C) also meets the needs of most experiments. Cool on ice, then transform or store at -20 °C.

4. Transformation. Thaw 100 µl competent cells on ice and resuspend by flicking. Add 4-5 µl of the ligation (up to all of it, as long as it is no more than 1/10 of the cell volume), mix gently and keep on ice for 30 minutes. Heat-shock at 42 °C for 90 seconds, then ice for 2-3 minutes. Add 500 µl LB or SOC medium without antibiotics and shake at 37 °C, 150 rpm for 60 minutes.

5. Plating. Spread 200 µl on an **ampicillin** plate pre-spread with 16 µl of 50 mg/ml IPTG and 40 µl of 20 mg/ml X-gal. Adjust the volume to the ligation and competent-cell efficiency. Leftover culture can be kept at 4 °C and plated the next day if needed. Keep the plate upright at 37 °C for 1 hour to absorb excess liquid, then invert and incubate overnight.

Note: 5-minute room-temperature transformation: add 4-5 µl ligation to 100 µl thawed competent cells, mix gently and leave at room temperature for 5 minutes, add 500 µl LB or SOC and shake at 37 °C, 150 rpm for 60 minutes, then plate as above.

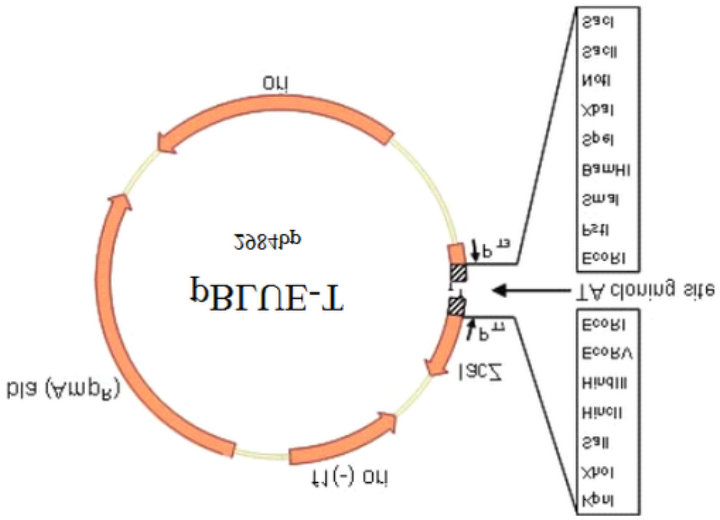
Screening and identification

Blue/white screening: an insert in pBLUE-T disrupts the lacZ coding sequence and the activity of the β-galactosidase α-fragment, so recombinant clones are white on X-gal/IPTG plates while non-recombinant clones are blue. If the insert does not shift the lacZ reading frame or is very small, recombinant colonies may be pale blue or show a pale blue centre with a white rim ("fish-eye"). Pick white or pale blue colonies into liquid medium containing ampicillin and grow overnight at 37 °C.

Identification: (a) extract plasmid and check the insert by EcoR I single digestion or other suitable enzymes on an agarose gel; (b) colony PCR (see Molecular Cloning, 3rd edition, or contact us); (c) sequencing with T3 and T7 promoter primers or other suitable primers.

How much PCR product to use? A PCR product : vector molar ratio of 2:1 to 10:1 (3:1 recommended) gives good results. Insert (ng) = [vector (ng) × insert size (kb) ÷ vector size (kb)] × molar ratio (insert : vector). Example: 30 ng vector (2.984 kb), a 500 bp insert and a 5:1 ratio need [30 × 0.5 ÷ 2.984] × 5 ≈ 25.1 ng of insert.

Map of pBLUE-T and promoter / multiple cloning site sequence



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