CYCV11-CHANGYU pBLUE-T Simple Cloning Kit

Contents	20 rxns (CYCV1101)	60 rxns (CYCV1102)	20 rxns (CYCV1103)	60 rxns (CYCV1104)
pBLUE-T Simple 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: CYCV1101 / CYCV1102 contain the vector, control insert and PEG Enhancer; CYCV1103 / CYCV1104 also contain the ligation buffers and T4 DNA Ligase.

Note: Store at -20 °C. Stable for 6 months.

Description

pBLUE-T Simple Vector is a dedicated vector for highly efficient TA cloning of PCR products. It is derived from pBlueScript II SK(+). Unlike conventional T-vectors made by EcoR V digestion followed by T-tailing, Xcm I sites were introduced into the original multiple cloning site, so that Xcm I digestion directly leaves an unpaired 3'-T at each end, giving higher recombination efficiency.

Simple vector: the multiple cloning sites of pBlueScript II SK(+) have been removed, so suitable restriction sites should be introduced on the PCR primers. Digestion at the primer-introduced sites is then not affected by other cloning sites on the vector, which greatly improves digestion efficiency and subcloning success.

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.

Note: M13 universal primers are recommended for sequencing (see the map 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. 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 Simple 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 (2.5 Weiss units per 10 µl is sufficient) for the most transformants. Because the PEG Enhancer raises ligation efficiency several-fold, 30 minutes at 16 °C with 5 Weiss units per 10 µl meets the needs of most experiments. 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 Simple 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 22 °C for 5-10 minutes (10 minutes recommended; up to 30 minutes for long inserts), or at 16 °C for 30 minutes. Cool on ice, then transform or store at -20 °C.

4. Transformation. Thaw 50-100 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. If few clones are expected, centrifuge (4,000 rpm, 2 minutes), remove part of the medium and plate the resuspended cells. 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.

Screening and identification

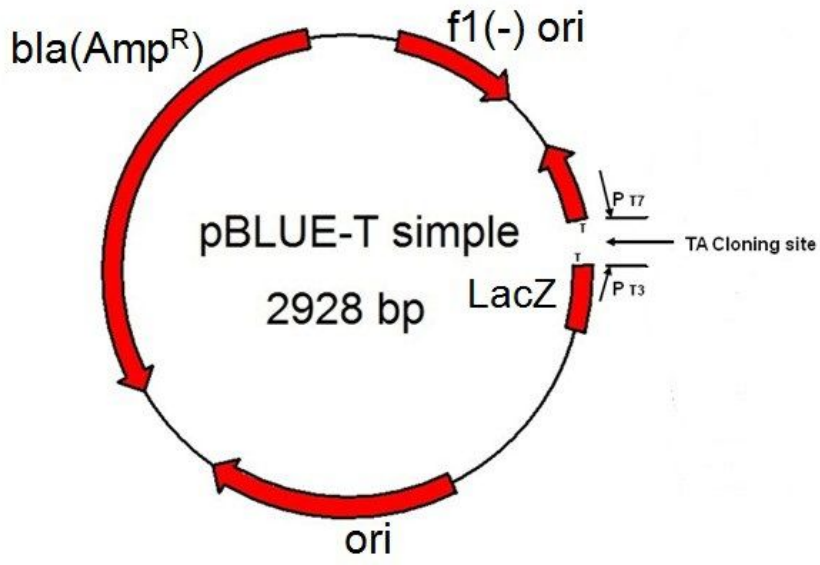
Blue/white screening: an insert in pBLUE-T Simple disrupts the lacZ coding sequence and the activity of the β-galactosidase α-fragment, so recombinant clones are white on X-gal/IPTG plates while non-recombinant clones are blue. If the insert does not shift the lacZ reading frame or is very small, recombinant 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 M13 universal primers or T3/T7 promoter primers.

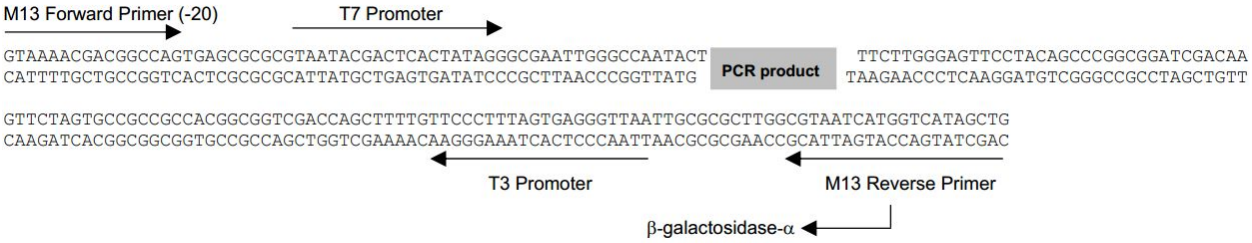
How much PCR product to use? A PCR product : vector molar ratio of 2:1 to 10:1 (3:1 recommended) gives good results. Insert (ng) = [vector (ng) × insert size (kb) ÷ vector size (kb)] × molar

ratio (insert : vector). Example: 40 ng vector (2.928 kb), a 1000 bp insert and a 3:1 ratio need [40 × 1.0 ÷ 2.928] × 3 ≈ 41.0 ng of insert.

Map of pBLUE-T Simple



Promoter and cloning site sequence



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