CYCV08-CHANGYU pZERO-Blunt Cloning Kit

Contents	20 rxns (CYCV0801)	40 rxns (CYCV0802)
pZERO-Blunt Vector (40 ng/μl)	20 μl	40 μl
900 bp Control (40 ng/μl)	5 μl	5 μl
2× Quick Ligation Buffer	100 μl	200 μl
T4 DNA Ligase (5 U/μl)	20 μl	40 μl
Forward Sequencing Primer (10 μM)	50 μl	100 μl
Reverse Sequencing Primer (10 μM)	50 μl	100 μl

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

Description

This zero-background blunt-end quick ligation kit is a lethal-gene positive-selection cloning system for efficient cloning of blunt-end PCR products and any other blunt-ended DNA fragments, phosphorylated or not. Ligation of the positive-selection vector with an insert takes only 5 minutes and gives more than 95% positive recombinant clones. Blunt PCR products from proofreading polymerases (such as *Pfu*) can be ligated directly, and the ligation can be transformed directly into common *E. coli* strains.

pZERO-Blunt Vector carries a lethal gene that contains the multiple cloning site. When an insert is ligated in, the lethal gene is no longer expressed correctly and cells carrying the recombinant grow normally; self-ligated empty vector expresses the lethal gene and its host cells do not survive ("zero" background). No blue/white screening is needed.

For convenient restriction mapping and insert manipulation, the multiple cloning site is flanked by a Bgl II site on each side. The vector also carries a T7 promoter for in vivo or in vitro transcription and insert sequencing.

❖ Procedure

1. Preparation. Recover blunt PCR products or blunt restriction fragments from an agarose gel, cutting the band under long-wave UV or visible light to avoid DNA damage, which causes ligation failure. A gel extraction kit recovers fragments above 70 bp well.

2. Quick ligation. In a standard 10 μl reaction, add 1 μl (40 ng) vector, X μl PCR product, 5 μl 2× Quick Ligation Buffer and 0.9 μl T4 DNA Ligase, and make up with sterile water. Exact quantification of the PCR product is usually unnecessary: a molar ratio of 1:1 to 10:1 (insert : vector) gives good results, 3:1 is recommended (see the formula below).

2× Quick Ligation Buffer	5 μl
pZERO-Blunt Vector	1 μl
Purified PCR product (or 1 μl 900 bp Control)	X μl
Sterile water	Y μl
T4 DNA Ligase (5 Weiss units/μl), added last	0.9 μl
Final Volume	10 μl

Ligate at 22 °C for 5-10 minutes (a PCR machine can be used). Inserts longer than 3 kb can be ligated for up to 30 minutes. Do not ligate for more than 30 minutes, as this may reduce the number of transformants. Cool on ice, then transform or store at -20 °C.

3. 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.

4. Spread 200 µl on LB agar containing **100 µg/ml ampicillin**. 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. Keep the plate upright at 37 °C for 1 hour to absorb excess liquid, then invert and incubate overnight.

Screening and identification

1. Routine check: grow colonies overnight at 37 °C with shaking in 1-5 ml LB containing 100 µg/ml ampicillin, keep a stock, extract plasmid and check the insert by PCR or restriction digestion.

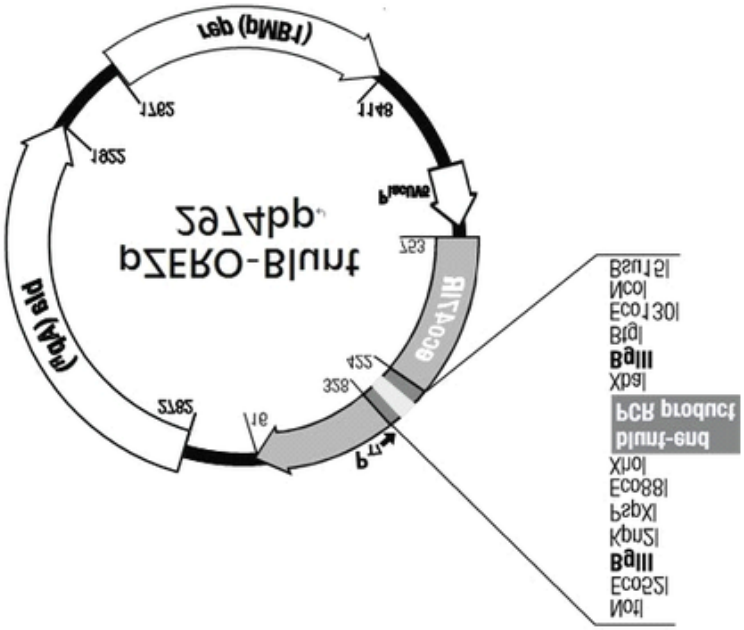
2. Quick check: colony PCR (for example with the CYCV05 Universal T-Vector Colony PCR Kit).

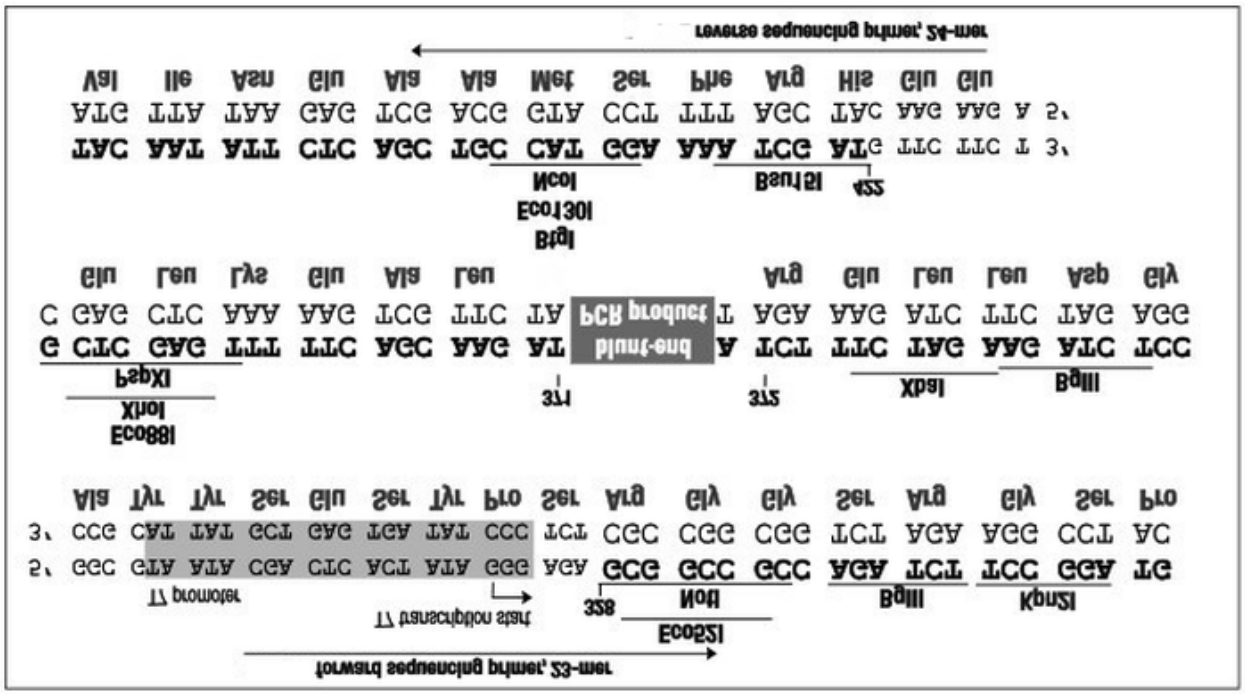
3. Sequencing: use the kit primers (also suitable for colony PCR) or a T7 promoter primer.

Kit primer	Sequence (5' to 3')
Forward sequencing primer (23-mer)	CGACTCACTATAGGGAGAGCGGC
Reverse sequencing primer (24-mer)	AAGAACATCGATTTTCCATGGCAG

How much PCR product to use? A PCR product : vector molar ratio of 1:1 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 (2.974 kb), a 500 bp insert and a 3:1 ratio need [40 × 0.5 ÷ 2.974] × 3 ≈ 20.2 ng of insert.

Map of pZERO-Blunt and cloning site sequence





This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.