

AllBio V3 Cloning Kit

Please read the user manual carefully before use.

Cat. No. ABTGMBV301

Storage

AllBio T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Descriptions

AllBio V3 Cloning Vector provides dual-*EcoR* I and dual-*Not* I enzyme digestion sites. It is designed for cloning and sequencing *Taq* amplified PCR products. The cloned insert can be released from a single enzyme digestion.

- 5 minutes fast ligation of *Taq*-amplified PCR products.
- Ampicillin resistance gene for selection.
- Easy blue/white selection.
- T7 promoter, SP6 promoter, M13 forward and M13 reverse primers for sequencing.
- T7 promoter, SP6 promoter for *in vitro* transcription.
- AllBio T1 phage resistant chemically competent cell, high transformation efficiency (>10⁹ cfu/μg pUC19 DNA) and fast growing.

Kit Contents

Component	10 rxns
AllBio V3 Cloning Vector (10 ng/μl)	10 μl
Control Template (5 ng/μl)	5 μl
Control Primers (10 μM)	5 μl
M13 Forward Primer (10 μM)	50 μl
M13 Reverse Primer (10 μM)	50 μl
AllBio T1 Phage Resistant Chemically Competent Cell (Option) -70°C Cat. ABTGCD501-10	5x100 μl

Preparation of PCR Products

1. Primer requirement: primer cannot be phosphorylated
 2. Enzyme choice: *Taq* DNA polymerases
 3. Reaction conditions: in order to ensure the integrity of amplification products, 5-10 minutes of post-extension step is required.
- After amplification reaction, use agarose gel electrophoresis to verify the quality and quantity of PCR product

Setting Up the Cloning Reaction System

Add following components into a microcentrifuge tube.

PCR products-----0.5-4 μl (can be increased or reduced based on PCR product yield, not more than 4 μl)

AllBio V3 Cloning Vector-----1 μl

Gently mix well, incubate at room temperature (20°C-37°C) for 5 minutes, and then place the tube on the ice.

1. Optimal amount of insert
Molar ratio of vector to insert = 1:7 (1 kb, ~20 ng; 2 kb, ~40 ng)
2. Optimal volume of vector: 1 μl (10 ng)
3. Optimal reaction volume: 3~5 μl
4. Optimal incubation time
 - (1) 0.1~1 kb (including 1 kb): 5~10 minutes
 - (2) 1~2 kb (including 2 kb): 10~15 minutes
 - (3) 2~3 kb (including 3 kb): 15~20 minutes
 - (4) ≥3 kb: 20~30 minutes

Use the maximum incubation time if the insert is gel purified

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5. Optimal incubation temperature: for most PCR inserts, the optimal temperature is about 25°C; for some PCR inserts, optimal results can be achieved with higher temperature (up to 37°C).

Transformation

1. Add 5 µl of ligation products into 50 µl of AllBio T1 Chemically Competent Cell (ligation product should be added immediately after thawing the cells on ice) and mix by tapping gently. Incubate on ice for 20-30 minutes.
2. Heat shock at 42°C water bath for exactly 30 seconds, and place on ice for 2 minutes.
3. Add 250 µl of room temperature SOC or LB medium. Incubate at 37°C for 1 hour at 200 rpm.
4. Mix 8 µl of IPTG (500 mM) with 40 µl of X-gal (20 mg/ml), spread the mixture on a selective plate, and incubate the plate at 37°C for 30 minutes.
5. After the IPTG and X-gal is soaked, spread 200 µl transformants on the plate and incubate overnight (to obtain more colonies, centrifuge cells vial at 4000 rpm for 1 minute, discard a portion of supernatant and keep 100-150 µl of it. Gently tapping to suspend the cells, plate all the cells and incubate overnight).

Identification of Positive Clones and Sequencing

Identify Positive Clones by PCR

1. Pick several white colonies and place them in 10 µl sterile water, mix by vortexing.
2. Add 1 µl mixture into 25 µl reaction volume. Identify positive clones with M13 forward primer and reverse primer.

3. PCR reaction conditions

94°C	10min	} 30 cycles
94°C	30 sec	
55°C	30 sec	
72°C	x min*	
72°C	5-10 min	

* (depends on the insert size and PCR enzymes)

The PCR product size from vector self-ligation is 253 bp.

Analyzing positive Clones by restriction enzyme digestion

Inoculate positive clones on LB/Amp^r or LB/Kan^r liquid medium, grow at 37°C for 6 hours at 200 rpm. Isolate plasmid DNA by plasmid MiniPrep Kit. Analyze colonies by restriction enzyme digestion with proper restriction endonuclease.

Sequencing

Analyze the sequence by sequencing with M13 F, M13 R and T7 promoter.

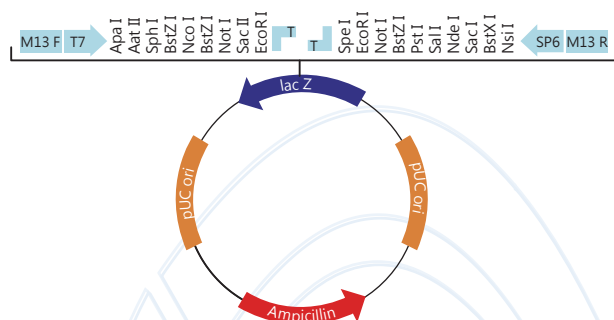
PCR for control insert (700 bp)

Component	Volume	Final Concentration
Control Template	1 µl	0.1 ng/µl
Control Primers (10 µM)	1 µl	0.2 µM
P Easy Taq PCR SuperMix	25 µl	1X
ddH ₂ O	Variable	-
Total volume	50 µl	-

Thermal cycling conditions for control insert

94°C	2~5min	} 30 cycles
94°C	30 sec	
55°C	30 sec	
72°C	1 min	
72°C	10 min	

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AllBio V3
(3039 bp)

- Lacoperon sequence: bases 2860-3020,190-419
- Multiple cloning site: bases 10-152
- SP6 priming site: bases 163-182
- M13 reverse priming site: bases 200-216
- LacZ start codon: base 204
- Lac operator: bases 224-240
- pUC origin: bases 543-1216
- Ampicillin resistance ORF (c): bases 1361-2221
- f1 origin: bases 2421-2858
- M13 forward priming site: bases 3000-3016
- T7 promoter priming site: bases 3023-3
- (c) = complementary strand

