



Asia Hepato Gene Co. Product Datasheet

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pBE-NT TA Cloning kit

10 Reactions

Application:

1. PCR product cloning
2. Protein Expression in E. coli
3. Multiple cloning region

Kit Components:

	Item	Concentration	Amount	Storage
1	pBE-NT TA vector	<ul style="list-style-type: none"> • Size: 3,000bp • 50ng/μl • Protein expression in E. coli • Ampicillin resistance, IPTG induction • N-terminal Polyhistidine (6×His) tag for detection and purification 	25μl	-20°C
2	10x Ligation Buffer A	0.4M Tris-HCl, 0.1mM MgCl ₂ , 0.1M DTT, 5mM ATP	10μl	-20°C
3	10x Ligation Buffer B	Enhance ligation efficiency	10μl	-20°C
4	Ligase	50mM KCl, 20mM Tris-HCl.(pH7.5).0.1mM EDTA, 1mM DTT, 50% glycerol	5μl	-20°C
5	SOC Medium	2% Tryptone, 0.5% Yeast Extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl ₂ , 10 mM MgSO ₄ , 20 mM glucose.	1.5ml	25°C
6	T7 Forward primer	10μM/μl in 1×TE buffer, pH8.0 ,Tm 50°C Sequence: 5'-TAATACGACTCACTATAGGG-3'	50μl	-20°C
7	T7 Reverse primer	10μM/μl in 1×TE buffer, pH8.0, Tm 50°C Sequence: 5'-ATCCGGATATAGTTCCTCCTTTC-3'	50μl	-20°C
8	DH5α competent cell	Transformation efficiency >3x10 ⁸ cfu/μg DNA	100μl×10	-80°C
9	pUC19 Control DNA	10 ⁻⁴ μg/μl	5μl	-20°C

PCR Primer Design:

Forward primer	<ul style="list-style-type: none"> • For protein expression place the gene of interest in frame, the first 3 nt with or without initiation ATG codon • If you don't need N-terminal tag and the protein can't be purified by tag. A second RBS (e.g. AGGAGA) 6-10 base pairs 5'of the initiation ATG codon of your protein
Reverse primer	<ul style="list-style-type: none"> • include a stop codon or • design the reverse primer to hybridize downstream of the native stop codon.

For research use only, not for diagnostic or therapeutic use.

High Yield PCR Product:

94°C → 94°C → X°C → 72°C → 72°C → 25°C
5min 30sec 30sec 30sec 7min end
30 cycles

Load 16µl PCR product to run 1-2% small wells agarose gel, target gene should be clearly visible on the gel

Ligation:

1. Add as following and carefully flick the tube 5-6 times to mix. Do not vortex.
* PCR product contain Ampicillin resistance template must be purified by agarose gel to remove Ampicillin resistance template

pBE-NT TA vector	2.5µl	2.5µl	2.5µl	2.5µl
PCR product <3kb	1µl	2µl	3µl	4µl
10x Ligation Buffer A	1µl	1µl	1µl	1µl
10x Ligation Buffer B	1µl	1µl	1µl	1µl
Ligase	0.5µl	0.5µl	0.5µl	0.5µl
dH2O	4µl	3µl	2µl	1µl
total	10µl	10µl	10µl	10µl

2. The optimal ligation efficiency molar ratio vector : insert = 1 : 3.
Low yield and long PCR product need more.
3. Centrifuge briefly and incubate at room temperature (25°C) for 5-20 minutes will suffice for PCR product <3kb.
4. If PCR product >3kb, incubate at 4°C overnight

Transformation:

1. Thaw DH5α competent cell at room temperature before use
2. Add 108µl competent cell into 10µl ligation mixture and mix gently.
3. On ice or 4°C for 10 minutes
4. 42°C 45 seconds
5. Add 80µl SOC Medium
6. Spread 168µl cells onto 20-50µg/ml Ampicillin plate. Excessive antibiotic significantly decreased the transformation efficiency
7. 37°C overnight

Analyzing Positive Clones:

1. Pick 12 colonies or more and culture them overnight in LB containing 20-50 µg/ml ampicillin.
2. Isolate plasmid DNA.
3. Analyze the plasmids by restriction analysis, sequencing, or
4. PCR use **target gene forward primer** and **T7 Reverse primer** (add 0.5µl per 25µl PCR reaction) to confirm the presence and correct orientation of the insert.

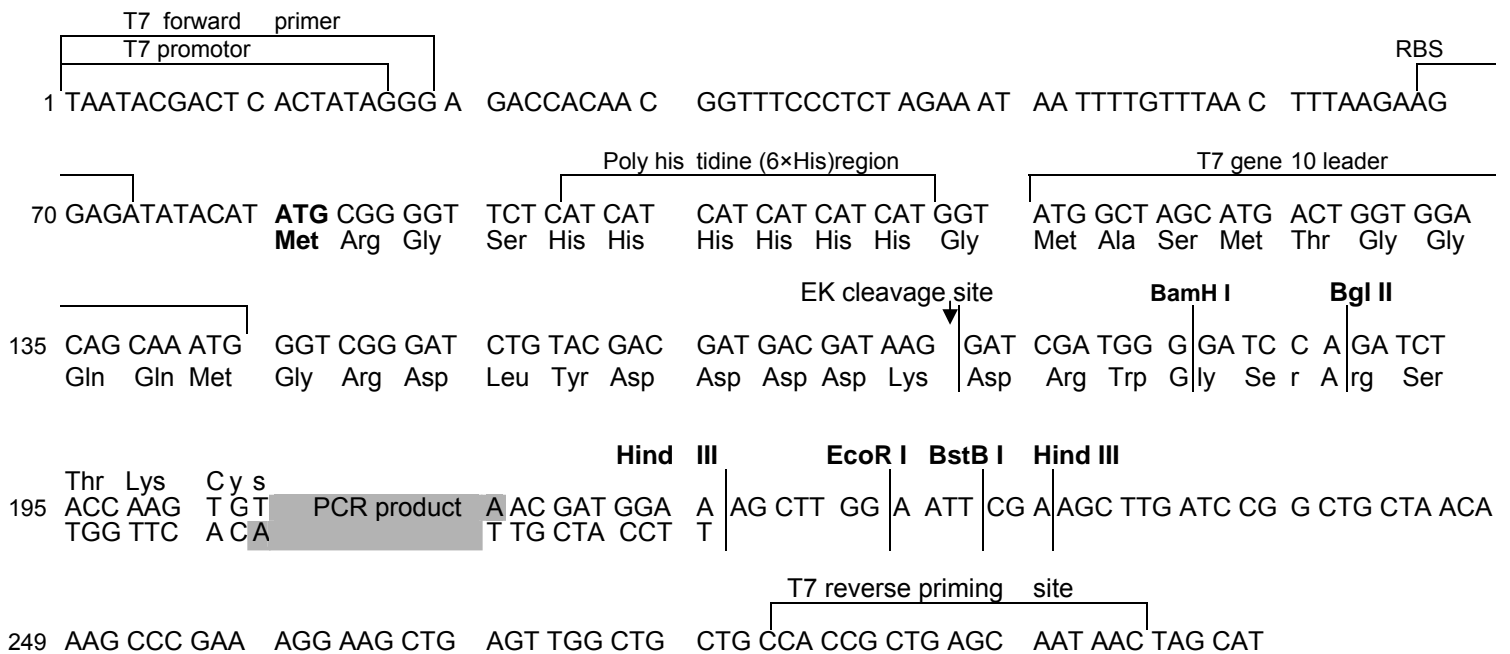
Long-Term Storage:

Keep the correct clone in 20-30% glycerol and store at -80°C.

Protein Expression Test:

1. Transform pBE-NT TA expression construct into E. coli BL21(DE3), or BL21(DE3)pLysS
2. Select 1 colony and culture in 1ml 2YT medium containing 20 µg/ml ampicillin, pick 4 colonies. Shaking at 37°C until the OD600 reaches 0.4-0.6.
3. Add 1µl 0.5M IPTG into 1ml 2YT culture, Keep 1 colony without IPTG.
4. Shaking at 37°C for 3 hours
5. Pour the culture into 1.5ml eppendorf, centrifuge at maximum speed for 1 minute, and discard supernatant.
6. Resuspend each cell pellet in 100 µl of 1X SDS-PAGE sample buffer.
7. Boil 5 minutes and centrifuge briefly.
8. Load 5-10 µl of each sample on a polyacrylamide gel and electrophorese. Save your samples by storing them at -20°C.
9. Gel staining with coomassie dye

pBE-NT TA vector cloning site:



The phenotypes of protein toxicity in molecular biology studies

- Toxic protein cloning problems
 - No colony in cloning
 - Fewer colonies than those in regular cloning experiments
 - Small percentage of positive clones
 - Wrong orientation
 - Mutations leading to defective products or no expression
- Toxic protein expression problems
 - No expression
 - Low yield
 - Defective proteins
 - The expression seems inconsistent or unpredictable.
- Toxic protein host cell growth rate and cell density problems
 - Cell grows significantly slower to reach its normal density before or after induction.
- Transformation efficiency problems
 - The transformation efficiency of the vector containing a toxic protein is lower than the control