Asia Hepato Gene Co.
Product Datasheet

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pM-NT TA Cloning kit

10 Reactions

Application:

1. PCR product cloning
2. Protein Expression in E. coli

Kit Components:

	Item	Concentration	Amount	Storage
1	pM-NT TA vector	· 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℃
2	10x Ligation Buffer A	0.4M Tris-HCl, 0.1mM $MgCl_2$, 0.1M DTT, 5mM ATP	10μl	-20℃
3	10x Ligation Buffer B	Enhance ligation efficiency	10μl	-20℃
4	Ligase	50mM KCl, 20mM Tris-HCl.(pH7.5).0.1mM EDTA, 1mM DTT, 50% glycerol	5μl	-20℃
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℃
6	T7 Forward primer	10μM/μl in 1×TE buffer, pH8.0 ,Tm 50℃	50μl	-20℃
		Sequence: 5'-TAATACGACTCACTATAGGG-3'		
7	T7 Reverse primer	10μM/μl in 1×TE buffer, pH8.0, Tm 50℃	50μl	-20℃
		Sequence: 5'-ATCCGGATATAGTTCCTCCTTTC-3'		
8	DH5α competent cell	Transformation efficiency >$3x10^8$ cfu/μg DNA	100μl×10	-80℃
9	pUC19 Control DNA	10^{-4} μg/μl	5μl	-20℃

PCR Primer Design:

Forward primer	• 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	• include a stop codon or • design the reverse PCR primer to hybridize downstream of the native stop codon.

High Yield PCR Product:

94℃→	94℃→	X℃→	72℃→	72℃→	25℃
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

pM-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℃) for 5-20 minutes will suffice for PCR product <3kb.
4. If PCR product >3kb, incubate at 4℃ 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℃ for 10 minutes
4. 42℃ 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℃ 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,
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.

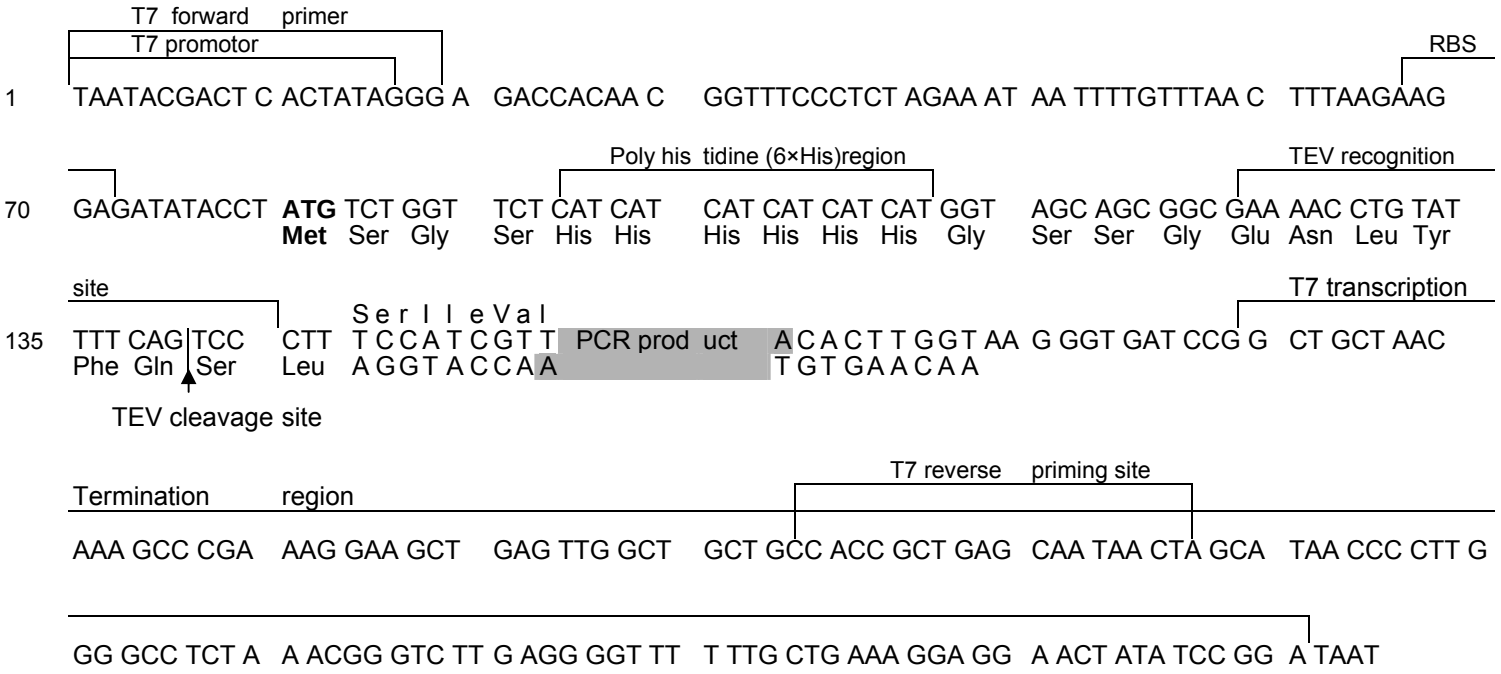
Long-Term Storage:

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

Protein Expression Test:

1. Transform pM-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 into1ml 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

pM-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