eTA cloning vector

Cat. No. PU-ETA-050 Size: 50 reactions

Store at -20℃

Cat. No: **PU-ETA-050**
Concentration: 25 ng/ ul
Quantity: 20 ul x 5 tubes
PC DNA: control DNA, 600 bp, 10 ng/ul

Description: eTA vector is designed to directly clone PCR products with a overhang dA residue at 3' end produced by Taq DNA polymerase.

➢ Application

1. Clone PCR products.
2. Blue/white screen for recombinants.
3. Confirm the white clone with unique restriction endonuclease Hind III
4. Sequence the insert with universal primer M13 forward or M13 reverse primer.
5. High efficiency of cloning up to 5 kb insert.

➢ Notes

1. For Blue-white selection, spread the mixture evenly onto a plate containing 50 mg/ml ampicillin plus 100 mM IPTG 10 μl and 50 mg/ml X-gal 20 μl . After overnight growth at 37℃, pick up the white colonies for checking clones with insert DNA.
2. Screen for clones with inserted PCR product
   Colony PCR is a easy and reliable method to screen for clones with inserted PCR products up to 1.5 kb. For larger insert, we recommend *Hind* III digestion to screen the positive clones. For small insert (<1.5 kb), the positivel rate should be over 80%. For larger fragments, the insert rate is 30-50%. Amplicon sizes of colony PCR will be 150 bp larger than the insert DNA .

➢ Quality Control

1. After ligation with control DNA (600 bp), the ratio of white colonies was over 90%.
2. Over 85% of white colonies contained inserted DNA after *Hind* III digestion.
3. Sequencing was done to confirm the existence of T overhang.

➢ Multiple Cloning Site of eTA vector

M13 forward

```
361 TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGAATT GTAATACGAC TCACTATAGG
    AAAGGGTCAG TGCTGCAACA TTTTGCTGCC GGTCACTTAA CATTGTGCTG AGTGATATCC
```

*created by ligation

Hind III

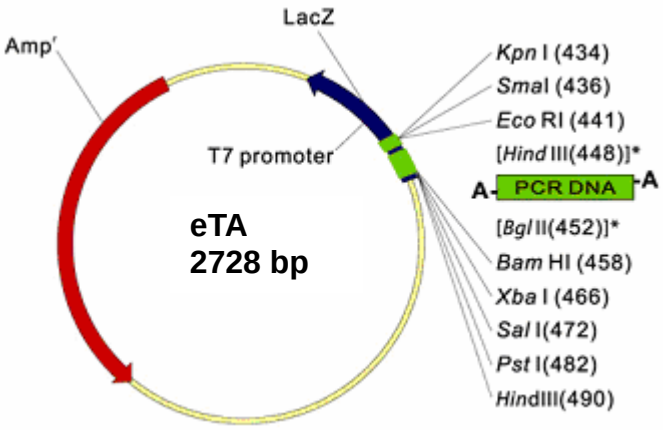
***Kpn* I *Sma* I *Eco*R I** **insert DNA** ***Bam*H I *Xba* I**

```
421 GCGAGCTCGG TACCCGGGCG AATTCCAAGC T-T -A GATCTGGATCCCCTCTAG
    CGCTCGAGCC ATGGGCCCGC TTAAGGTTCG A A- T-CTAGACCTAGGGGAGATC
```

***Sal* I *Pst* I *Hind* III**

```
471 AGTCGACCTGC AGGCATGCAA GCTTGGCGTA ATCATGGTCA TAGCTGTTTC CTGTGTGAAA
    TCAGCTGGACG TCCGTACGTT CGAACCGCAT TAGTACCAGT ATCGACAAAG GACACACTTT
```

M13 reverse



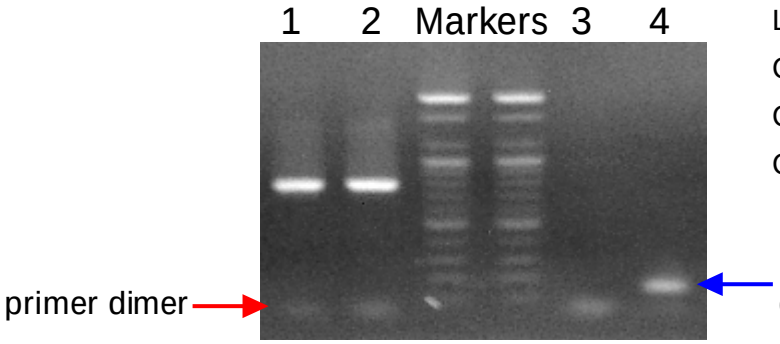
➢ Screen for clones with inserted PCR product

Colony PCR is a easy and reliable method to screen for clones with insert PCR products up to 1.5 kb. For small insert (<1.5 kb), the positive rate (clones with insert PCR product) should be over 80%. For larger insert, we recommend *Hind* III digestion to screen the inserted clones and the positive rate should be 30-50%. Amplicon sizes of colony PCR will be 150 bp larger than the insert DNA .

Example QC report

➢ Colony PCR

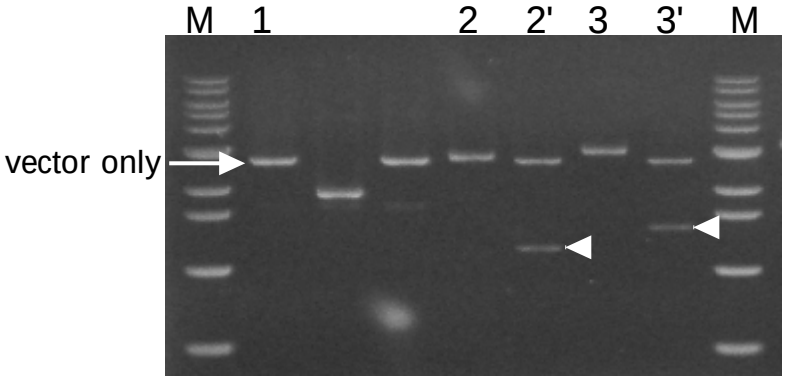
After transformation, the clones with insert gene were indentified by colony PCR using M13 primer pair (PU-UNP-04M). Amplicon sizes of colony PCR will be 150 bp larger than the inserted DNA . The 100 bp DNA Ladder (BN-D-1030) consists of 13 double strand DNA fragments ranging in sizes from 100 to 1,000 bp in 100 bp increments, and additional fragments of 1,200, 1,600, 2,000 bp.



Left: result of colony PCR from four clones
Clone#1 and #2: with insert DNA.
Clone#3: clone without eTA vector
Clone#4: clone with empty eTA vector

➢ *Hin*d III digestion

Digestion with *Hin*d III will cut the insert DNA (arrowhead) from the vector (arrow).



1: vetor/Hind III
2: TA clones of 1.2 kb insert
2': cut with *Hin*d III
3: TA clone of 1.5 kb insert
3': cut with *Hin*d III
Marker sizes (BN-D-1040):
500 bp, 1, 1.6, 2, 3, 4, 5, 6, 8, 10 kb

Quality control Protocol

1. Preparation of reaction mixture (Control DNA)

Item	Concentration	Volume (ul)
Ligation High	2X	5
eTA vector	25 ng/ul	2
PC DNA	10 ng/ul	3
		10 ul

2. perform ligation at 16 ℃ for 2 hr
3. take 2 μl of ligation mixtures to perform transformation reaction.
4. Check insert by colony PCR with M13 primer pair or *Hind* III digestion**

* It is possible to perform ligation reaction without purifying the PCR product. However, we recommend a purification process to ensure a higher insert rate, especially for insert DNA larger than 1.5 kb.
** Digestion with *Hin*d III will cut the insert DNA from the vector.

Preparation of PCR cocktail	1x	10x
	concentration volume (ul)	volume
water	19.5	195
M13 Primer Pair	10 uM 0.5	5
total volume	20 ul	200

↓

＊ Aliquot PCR cocktail to AccuPower PCR PreMix tube, vortex to dissolve the premix pellet and briefly spin it down to collect the liquid.

↓

＊ Add single colony to reaction mix by picking the colony with either a sterile loop or pipette tip (do not use wood) and twirling this in the liquid in the tube.

PCR program

initial denature	95 ℃ 5 min	
denature	95 ℃ 30 sec	35 cycles
annealing	55 ℃ 30 sec	
extention	72 ℃ 1 min/kb	

➢ Quality Control

1. After ligation with control DNA (600 bp), the ratio of white colonies was over 90%.
2. Over 85% of white colonies contained inserted DNA after *Hind* III digestion.
3. Sequencing was done to confirm the existence of T overhang.

eTA Vector sequence reference points:

Base pairs 2728

Multiple cloning region 434-490

T7 promoter 402-439

lac Z gene 511-149

Ampr gene 2528-1671

M13 universal primer 359-375

M13 reverse primer 528-507

The foreign DNA can be inserted at base 452(*)and a T-residue has been added to both 3'-ends. The added T is not included in this sequence.

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1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA
61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG
121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC
181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC
241 ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT
301 TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT
361 TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGAATT GTAATACGAC TCACTATAGG
421 GCGAGCTCGG TACCCGGGCG AATTCCAAGC T*GATCTGGATCCCCTCTAG AGTCGACCTGC
481 AGGCATGCAA GCTTGGCGTA ATCATGGTCA TAGCTGTTTC CTGTGTGAAA TTGTTATCCG
541 CTCACAATTC CACACAACAT ACGAGCCGGA AGCATAAAGT GTAAAGCCTG GGGTGCCTAA
601 TGAGTGAGCT AACTCACATT AATTGCGTTG CGCTCACTGC CCGCTTTCCA GTCGGGAAAC
661 CTGTCGTGCC AGCTGCATTA ATGAATCGGC CAACGCGCGG GGAGAGGCGG TTTGCGTATT
721 GGGCGCTCTT CCGCTTCCTC GCTCACTGAC TCGCTGCGCT CGGTCGTTCG GCTGCGGCGA
781 GCGGTATCAG CTCACTCAAA GGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA
841 GGAAAGAACA TGTGAGCAAA AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG
901 CTGGCGTTTT TCCATAGGCT CCGCCCCCCT GACGAGCATC ACAAAAATCG ACGCTCAAGT
961 CAGAGGTGGC GAAACCCGAC AGGACTATAA AGATACCAGG CGTTTCCCCC TGGAAGCTCC
1021 CTCGTGCGCT CTCCTGTTCC GACCCTGCCG CTTACCGGAT ACCTGTCCGC CTTTCTCCCT
1081 TCGGGAAGCG TGGCGCTTTC TCATAGCTCA CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC
1141 GTTCGCTCCA AGCTGGGCTG TGTGCACGAA CCCCCCGTTC AGCCCGACCG CTGCGCCTTA
1201 TCCGGTAACT ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA
1261 GCCACTGGTA ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG
1321 TGGTGGCCTA ACTACGGCTA CACTAGAAGG ACAGTATTTG GTATCTGCGC TCTGCTGAAG
1381 CCAGTTACCT TCGGAAAAAG AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT
1441 AGCGGTGGTT TTTTTGTTTG CAAGCAGCAG ATTACGCGCA GAAAAAAAGG ATCTCAAGAA
1501 GATCCTTTGA TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG
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1561 ATTTTGGTCA TGAGATTATC AAAAAGGATC TTCACCTAGA TCCTTTTAAA TTAAAAATGA
1621 AGTTTTAAAT CAATCTAAAG TATATATGAG TAAACTTGGT CTGACAGTTA CCAATGCTTA
1681 ATCAGTGAGG CACCTATCTC AGCGATCTGT CTATTTCGTT CATCCATAGT TGCCTGACTC
1741 CCCGTCGTGT AGATAACTAC GATACGGGAG GGCTTACCAT CTGGCCCCAG TGCTGCAATG
1801 ATACCGCGAG ACCCACGCTC ACCGGCTCCA GATTTATCAG CAATAAACCA GCCAGCCGGA
1861 AGGGCCGAGC GCAGAAGTGG TCCTGCAACT TTATCCGCCT CCATCCAGTC TATTAATTGT
1921 TGCCGGGAAG CTAGAGTAAG TAGTTCGCCA GTTAATAGTT TGCGCAACGT TGTTGCCATT
1981 GCTACAGGCA TCGTGGTGTC ACGCTCGTCG TTTGGTATGG CTTCATTCAG CTCCGGTTCC
2041 CAACGATCAA GGCGAGTTAC ATGATCCCCC ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC
2101 GGTCCTCCGA TCGTTGTCAG AAGTAAGTTG GCCGCAGTGT TATCACTCAT GGTTATGGCA
2161 GCACTGCATA ATTCTCTTAC TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG
2221 TACTCAACCA AGTCATTCTG AGAATAGTGT ATGCGGCGAC CGAGTTGCTC TTGCCCGGCG
2281 TCAATACGGG ATAATACCGC GCCACATAGC AGAACTTTAA AAGTGCTCAT CATTGGAAAA
2341 CGTTCTTCGG GGCGAAAACT CTCAAGGATC TTACCGCTGT TGAGATCCAG TTCGATGTAA
2401 CCCACTCGTG CACCCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA
2461 GCAAAAACAG GAAGGCAAAA TGCCGCAAAA AAGGGAATAA GGGCGACACG GAAATGTTGA
2521 ATACTCATAC TCTTCCTTTT TCAATATTAT TGAAGCATTT ATCAGGGTTA TTGTCTCATG
2581 AGCGGATACA TATTTGAATG TATTTAGAAA AATAAACAAA TAGGGGTTCC GCGCACATTT
2641 CCCCGAAAAG TGCCACCTGA CGTCTAAGAA ACCATTATTA TCATGACATT AACCTATAAA
2701 AATAGGCGTA TCACGAGGCC CTTTCGTC