TOYOBO

Instruction manual TArget Clone 0811 A4164K

TArget Clone/ TArget Clone -Plus-

<Target Clone> TAK-101 10 reactions
<Target Clone –Plus-> TAK-201 10 reactions
Store at -20 °C

Contents

CAUTION
All reagents in this kit are intended for research purposes. Do not use for diagnosis or clinical purposes. Please observe general laboratory precautions and follow safety guidelines while using this kit.

COSMO BIO CO., LTD.
Inspiration for Life Science
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[1] Introduction

Description

TArget Clone and TArget Clone -Plus- are high efficient TA cloning kits.

TArget Clone [Code No. TAK-101] can be applied to the TA cloning of PCR products amplified using Taq DNA polymerase, Blend Taq [Code No. BTQ-101], Blend Taq -Plus- [Code No. BTQ-201] or KOD Dash [Code No. LDP-101]. Almost all PCR products having dA overhangs at the 3'end are applicable.

TArget Clone -Plus- [Code No. TAK-201] can be applied to the TA cloning of blunt-end PCR products amplified using KOD -Plus- [Code No. KOD-201] or KOD FX [Code No. KFX-101]. TArget Clone -Plus- contains the 10 x A-attachment mix. This reagent is a mixture of anti-KOD DNA polymerase antibody[1)] specific to KOD 3'→5' exonuclease activity (proof-reading activity) and Taq DNA polymerase, which exhibits terminal transferase activity. The 10 x A-attachment mix allows blunt end PCR products to acquire overhanging dA at the 3'-ends.

Product Name	Applicable for PCR products amplified with
TArget Clone	Taq DNA polymerase, Blend Taq, Blend Taq -Plus-, KOD Dash <PCR products having overhanging dA at the 3'-ends>
TArget Clone -Plus-	KOD -Plus-, KOD FX

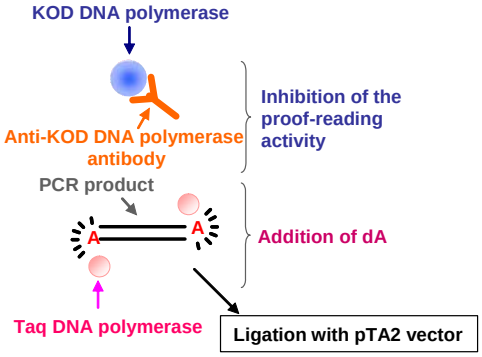
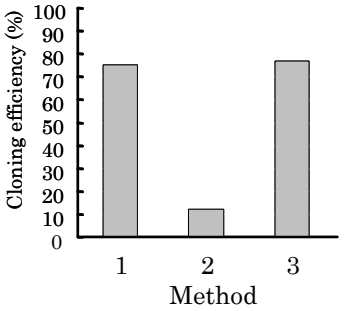


Fig. 1 Principle of 10x A-attachment mix



1: PCR products of Taq DNA polymerase
2: PCR products of KOD -Plus-
3: PCR products of KOD -Plus-
1, 2: TArget Clone
3: TArget Clone -Plus-

Fig. 2 Comparison of efficiency of each product

Features

[TArget Clone & TArget Clone -Plus-]
- Ligation step can be completed in 5 min.
- PCR products can be used without purification.
-The vector generates blue *E. coli* colonies on X-gal/(IPTG) plates in the case of no inserts.

[TArget Clone -Plus-]
- Realizes TA cloning of blunt-end products from KOD -Plus- and KOD FX.
- Addition of dA to the 3'-ends of PCR product can be competed in 10 min at 60 °C.

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[2] Components

The kit includes the following reagents, which can be used for 10 reactions. All reagents should be stored at -20 °C.

[TArget Clone]

pTA2 Vector（50ng/µl）	10 µl
2x Ligation Buffer	50 µl
T4 DNA Ligase	10 µl

[TArget Clone -Plus-]

pTA2 Vector（50ng/µl）	10 µl
2x Ligation Buffer	50 µl
T4 DNA Ligase	10 µl
10x A-attachment Mix	10 µl

Caution

-All reagents should be centrifuged briefly prior to use because of their small volume.
-Although the TA2 Vector is stable against freezing and thawing for at least 10cycles this reagent should not be repeatedly frozen and thawed many times.

[3] Protocol

1. Protocol for PCR products having 3' overhanging dA at the 3' ends

PCR products of the following PCR enzymes are available:
Taq DNA polymerase, Tth DNA polymerase, Blend Taq [Code No. BTQ-101], Blend Taq -Plus- [Code No. BTQ-201], and KOD Dash [Code No. LDP-101]

(1) Prepare the following reagents.

Distilled water	（3-X） µl
2x Ligation Buffer	5 µl
pTA2 Vector（50ng/µl）	1 µl
PCR product*	X µl
T4 DNA Ligase	1 µl
Total Volume	10 µl

*Unpurified and purified PCR products can be used.

Notes

-The molar ratio of pTA Vector:PCR product should be set 1: >3. The minimum volume of the PCR product can be circulated the following formulation;
X (µl) = 50*Y / Z
[Y: size of PCR product (kb), Z: concentration of PCR products (ng/µl)]

-The last extension step in a PCR cycle should be prolonged up to 5-10 min in order to ensure the addition of dA at the 3' end of PCR products.

-PCR products should be used immediately after the PCR cycles. The PCR products can be stored at -20 °C.

-PCR products should be analyzed on an agarose gel prior to use. If extra bands or primer dimers were observed, the target DNA should be purified using kits such as MagExtractor -PCR & Gel clean up-[Code No. NPK-601].

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(2) Incubate at room temperature (15-25 °C) for 5-30 min.

Notes

Small PCR products (≤ 2 kb) can be cloned efficiently into the T vector in 5 min. The reaction efficiency can be increased by prolonging the reaction time up to 30 min at room temperature. The reaction can be performed at 4 °C overnight.

(3) Transform *E. coli* using the reacted solution.

2. Protocol for blunt end PCR products of KOD -Plus- and KOD FX

(1) Transfer 9 µl of PCR products* to a fresh tube.

*PCR products from KOD-Plus- [Code No. KOD-201] or KOD FX [Code No. KFX-101] should be used without purification.

Notes

-The A-attachment reaction should be performed just prior to the ligation reaction

-PCR products should be analyzed on an agarose gel prior to use. If extra bands or primer dimers were observed, the target DNA should be purified using kits such as MagExtractor –PCR & Gel clean up-[Code No. NPK-601]. 10 x A-attachment mix does not contain buffer components, dNTPs, or magnesium, etc. When using purified PCR products and prior to adding 10x A-attachment mix, PCR buffer, dNTPs, and (magnesium**) must be added to the purified DNA solution to create a 1x PCR reaction solution.

**If magnesium is present in the PCR buffer, the addition of magnesium is not necessary.

(2) Add 1 µl of 10 x A-attachment mix and mix well.

(3) Incubate at 60°C for 10 minutes.

Notes

This reaction can be prolonged up to 30 minutes. In most cases, efficiency can be reached in 10 minutes, but maximum efficiency is achieved in 30 minutes.

(4) Prepare the following reagents.

Distilled water	(3-X) µl
2x Ligation Buffer	5 µl
pTA2 Vector (50ng/µl)	1 µl
dA-attached PCR products	X µl
T4 DNA Ligase	1 µl
Total Volume	10 µl

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Notes

-The molar ratio of pTA Vector:PCR product should be set 1: >3. The minimum volume of the PCR product can be circulated the following formulation;

X (μl) = 50Y / Z

[Y: size of PCR product (kb), Z: concentration of PCR products (ng/μl)]

(5) Incubate at room temperature (15-25 °C) for 5-30 min.

Notes

Small PCR products (≤ 2 kb) can be cloned efficiently into the T vector in 5 min. The reaction efficiency can be increased by prolonging the reaction time up to 30 min at room temperature. The reaction can be performed at 4 °C overnight.

(6) Transform *E. coli* using the reacted solution***.

***Up to 10 μl of the ligation mixture can be used for transformation of 100 μl of competent *E. coli* cells

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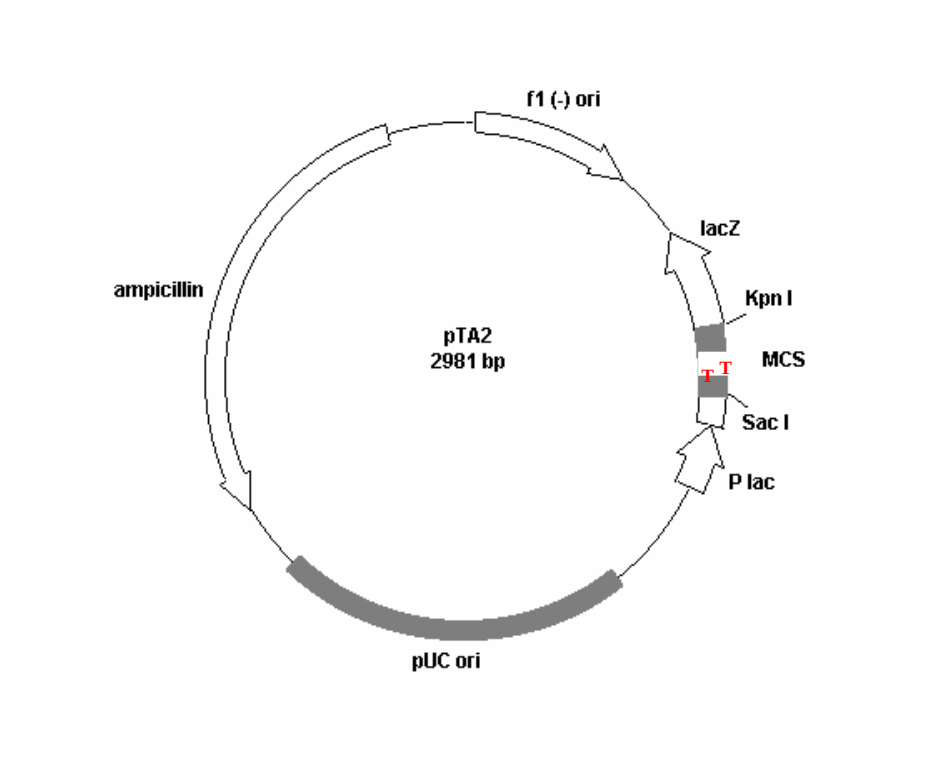
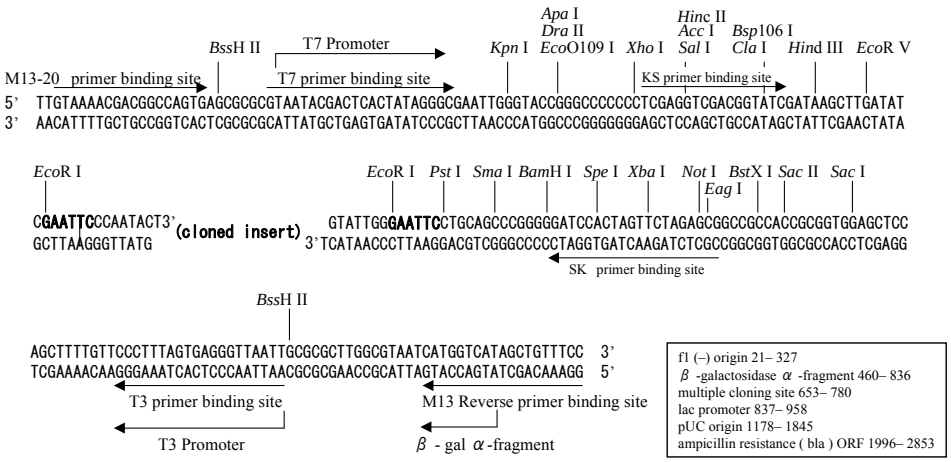
[4] Vector information

1. Map of pTA2 Vector



*The vector generates blue *E. coli* colonies on X-gal/(IPTG) plates in case of no inserts.

2. DNA sequence around the multi-cloning site of pTA2 Vector.



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3. Sequence of pTA2 Vector (2981 bp)

```
   1  CTGACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG
  51  CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC
 101  TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCTTTCCC CGTCAAGCTC
 151  TAAATCGGGG GCTCCCTTTA GGGTTCCGAT TTAGTGCTTT ACGGCACCTC
 201  GACCCCAAAA AACTTGATTA GGGTGATGGT TCACGTAGTG GGCCATCGCC
 251  CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG TTCTTTAATA
 301  GTGGACTCTT GTTCCAAACT GGAACAACAC TCAACCCTAT CTCGGTCTAT
 351  TCTTTTGATT TATAAGGGAT TTTGCCGATT TCGGCCTATT GGTTAAAAAA
 401  TGAGCTGATT TAACAAAAAT TTAACGCGAA TTTTAACAAA ATATTAACGC
 451  TTACAATTTC CATTCGCCAT TCAGGCTGCG CAACTGTTGG GAAGGGCGAT
 501  CGGTGCGGGC CTCTTCGCTA TTACGCCAGC TGGCGAAAGG GGGATGTGCT
 551  GCAAGGCGAT TAAGTTGGGT AACGCCAGGG TTTTCCCAGT CACGACGTTG
 601  TAAAACGACG GCCAGTGAGC GCGCGTAATA CGACTCACTA TAGGGCGAAT
 651  TGGGTACCGG GCCCCCCCTC GAGGTCGACG GTATCGATAA GCTTGATATC
 701  GAATTCCCAA TAC*GTATTGGGAATTCCTGC AGCCCGGGGG ATCCACTAGT
 751  TCTAGAGCGG CCGCCACCGC GGTGGAGCTC CAGCTTTTGT TCCCTTTAGT
 801  GAGGGTTAAT TGCGCGCTTG GCGTAATCAT GGTCATAGCT GTTTCCTGTG
 851  TGAAATTGTT ATCCGCTCAC AATTCCACAC AACATACGAG CCGGAAGCAT
 901  AAAGTGTAAA GCCTGGGGTG CCTAATGAGT GAGCTAACTC ACATTAATTG
 951  CGTTGCGCTC ACTGCCCGCT TTCCAGTCGG GAAACCTGTC GTGCCAGCTG
1001  CATTAATGAA TCGGCCAACG CGCGGGGAGA GGCGGTTTGC GTATTGGGCG
1051  CTCTTCCGCT TCCTCGCTCA CTGACTCGCT GCGCTCGGTC GTTCGGCTGC
1101  GGCGAGCGGT ATCAGCTCAC TCAAAGGCGG TAATACGGTT ATCCACAGAA
1151  TCAGGGGATA ACGCAGGAAA GAACATGTGA GCAAAAGGCC AGCAAAAGGC
1201  CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC
1251  CCCCTGACGA GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC
1301  CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT
1351  GCGCTCTCCT GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCTTTC
1401  TCCCTTCGGG AAGCGTGGCG CTTTCTCATA GCTCACGCTG TAGGTATCTC
1451  AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC
1501  CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA
1551  ACCCGGTAAG ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG
1601  ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG
1651  GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC TGCGCTCTGC
1701  TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA
1751  CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC
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1801  GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT
1851  CTGACGCTCA GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA
1901  TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA AATGAAGTTT
1951  TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT
2001  GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT TCGTTCATCC
2051  ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT
2101  ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACCGG
2151  CTCCAGATTT ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA
2201  AGTGGTCCTG CAACTTTATC CGCCTCCATC CAGTCTATTA ATTGTTGCCG
2251  GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA TAGTTTGCGC AACGTTGTTG
2301  CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG TATGGCTTCA
2351  TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT
2401  GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA
2451  AGTTGGCCGC AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT
2501  CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG GTGAGTACTC
2551  AACCAAGTCA TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTTGCC
2601  CGGCGTCAAT ACGGGATAAT ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG
2651  CTCATCATTG GAAAACGTTC TTCGGGGCGA AAACTCTCAA GGATCTTACC
2701  GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC AACTGATCTT
2751  CAGCATCTTT TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG
2801  CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT GTTGAATACT
2851  CATACTCTTC CTTTTTCAAT ATTATTGAAG CATTTATCAG GGTTATTGTC
2901  TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG
2951  GTTCCGCGCA  CATTTCCCCG  AAAAGTGCCA  C                  .
```

“ ∗ ”shows the site having dT at the 3’ ends

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4. Frequency and position of restriction sites

The following table showing the cutting sites in the pTA2 Vector by restriction enzymes having 1-10 cutting sites in the vector.

Restriction enzyme	Frequency	Position
*Acc*65I	1	653
*Acc*I	1	674
*Af*lIII	1	1173
*Ahd*I	1	2061
*Alw*44I	2	1487, 2733
*Alw*I	10	739, 740, 1740, 1814, 1826, 1911, 1924, 2388, 2691, 2709
*Alw*NI	1	1584
*Apa*I	1	659
*Apo*I	3	417, 428, 701
*Ava*I	2	668, 733
*Ava*II	2	2204, 2426
*Bam*HI	1	739
*Ban*I	4	193, 653, 917, 2014
*Ban*II	3	159, 659, 775
*Bci*VI	2	1382, 2909
*Bfa*I	6	81, 746, 752, 1668, 1921, 2256
*Bgl*I	2	466, 2180
*Bpm*I	2	778, 2151
*Bsa*AI	1	232
*Bsa*HI	1	2602
*Bsa*I	1	2133
*Bsa*JI	6	575, 733, 734, 767, 912, 1333
*Bsa*WI	3	1379, 1526, 2357
*Bse*MII	4	1448, 1857, 2023, 2563
*Bsi*EI	6	497, 758, 1086, 1510, 2433, 2582
*Bsi*HKAI	4	775, 1487, 2648, 2733
*Bsl*I	8	9, 335, 664, 1015, 1189, 1207, 1373, 1652
*Bsm*AI	2	2133, 2898
*Bsp*120I	1	659
*Bsp*1286I	6	159, 659, 775, 1487, 2648, 2733
*Bsp*HI	2	1893, 2901
*Bsp*LU11I	1	1173
*Bsr*BI	5	88, 755, 863, 1104, 2905

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*Bsr*DI	2	2120,	2302			
*Bss*HII	2	619,	812			
*Bss*SI	2	1346,	2730			
*Bst*F5I	4	542,	2046,	2227,	2514	
*Bst*XI	1	764				
*Cfr*10I	2	129,	2146			
*Cla*I	1	683				
*Csp*6I	2	654,	2545			
*Dde*I	4	1448,	1857,	2023,	2563	
*Dra*I	3	1930,	1949,	2641		
*Dra*III	1	232				
*Drd*I	2	275,	1275			
*Dsa*I	1	767				
*Eae*I	4	609,	758,	1012,	2454	
*Ear*I	3	511,	1051,	2855		
*Ec*l136II	1	775				
*Eco*52I	1	758				
*Eco*57I	2	1700,	2748			
*Eco*0109I	1	659				
*Eco*RI	2	701,	721,			
*Eco*RII	5	575,	912,	1200,	1321,	1334
*Eco*RV	1	695				
*Fau*I	5	33,	87,	505,	965,	1022
*Fok*I	4	542,	2046,	2227,	2514	
*Fsp*I	2	477,	2286			
*Hae*II	4	75,	83,	1047,	1417	
*Hga*I	4	3,	1275,	1853,	2603	
*Hin*cII	1	674				
*Hin*dIII	1	689				
*Hin*fI	8	282,	304,	632,	1008,	1073
		1148,	1544,	2061		
*Hph*I	6	222,	1917,	2144,	2540,	2766
		2781				
*Kpn*I	1	653				
*Mae*II	8	123,	233,	276,	288,	595
		1876,	2292,	2665		
*Mbo*II	8	103,	512,	1052,	1823,	1914
		2669,	2747,	2856		
*Ms*I	4	765,	2314,	2473,	2832	
*Msp*A1I	6	527,	767,	995,	1513,	1758
		2699				
*Mva*I	5	575,	912,	1200,	1321,	1334
*Nae*I	1	129				
*Nci*I	6	657,	733,	734,	1552,	2248
		2599				
*Ngo*MIV	1	129				

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*Nla*III	8	828, 1174, 1894, 2385, 2395 2473, 2509, 2902
*Not*I	1	757
*Nsp*I	1	1173
*Ple*I	6	282, 304, 632, 1073, 1544 2061
*Psp*1406I	2	2291, 2664
*Pst*I	1	727
*Pvu*I	2	497, 2433
*Pvu*II	2	527, 995
*Rsa*I	2	654, 2545
*Sac*I	1	775
*Sac*II	1	767
*Sal*I	1	674
*Sap*I	1	1050
*Sau*96I	8	240, 507, 659, 660, 2108 2187, 2204, 2426
*Sca*I	1	2544
*Sch*I	6	282, 304, 632, 1073, 1544 2061
*Sfa*NI	4	1261, 2313, 2523, 2753
*Sfc*I	6	11, 638, 727, 1438, 1629 2307
*Sma*I	1	733
*Sml*I	5	668, 1279, 1541, 1818, 2686
*Spe*I	1	745
*Ssp*I	2	440, 2868
*Taa*I	8	258, 483, 678, 1135, 1206 1676, 1989, 2504
*Tai*I	8	123, 233, 276, 288, 595 1876, 2292, 2665
*Taq*I	7	199, 669, 675, 684, 699 1273, 2717
*Tat*I	1	2544
*Tfi*I	2	1008, 1148
*Tsp*45I	4	57, 589, 2323, 2534
*Tsp*RI	10	613, 960, 1069, 1575, 1588 1859, 2008, 2113, 2460, 2487
*Vsp*I	3	943, 1002, 2237
*Xba*I	1	751
*Xho*I	1	668
*Xho*II	7	739, 1814, 1825, 1911, 1923 2691, 2708
*Xma*I	1	733
*Xmn*I	1	2661

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Restriction enzymes have no restriction site

*Aat*II	*Acc*III	*Af*/II	*Age*I	*Asc*I
*Bbe*I	*Bbs*I	*Bbv*CI	*Bc*/I	*Bg*/II
Bl/nI	*Bpu*10I	*Bpu*1102I	*Bsa*BI	*Bse*RI
*Bsg*I	*Bsi*WI	*Bsm*BI	*Bsm*FI	*Bsm*I
*Bsp*MI	*Bsr*GI	*Bst*1107I	*Bst*API	*Bst*BI
*Bst*EII	*Bsu*36I	*Eco*47III	*Eco*72I	*Eco*NI
*Ehe*I	*Fse*I	*Hpa*I	*Kas*I	*Mlu*I
*Msc*I	*Mun*I	*Nar*I	*Nco*I	*Nde*I
*Nhe*I	*Nru*I	*Nsi*I	*Pac*I	*Pme*I
*Ppu*10I	*Ppu*MI	*Psh*AI	*Rsr*II	*San*DI
*Sbf*I	*Sex*AI	*Sfi*I	*Sgf*I	*Sgr*AI
*Sna*BI	*Sph*I	S*rf*I	*Stu*I	*Sty*I
*Swa*I	*Tth*111I	*Van*91I	*Xcm*I	

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[5] Reagents

1. LB medium (1L)

10 g bacto-tryptone
5 g bacto-yeast extract
10 g NaCl
Dissolve in 1 L water
Adjust pH to 7.0 with 1N NaOH
Autoclave
Store at room temperature

2. LB/Ampicilin plate

10 g bacto-triptone
5 g bacto-yeast extract
10 g NaCl
Dissolve in 1 L water
Adjust pH to 7.0 with 1N NaOH
15 g Bacto agar
Autoclave
Add ampicillin solution (final concentration 100 µg/ml) at 50 °C
Dispense the medium into sterilized petri dishes
Store at 4 °C

3. 100 mM IPTS (10 ml)

0.24 g IPTG
Dissolve in 10 ml distilled water
Filter with a 0.22 µm filter
Store at -20 °C

4. 4% X-gal (10 ml)

0.4 g X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside)
Dissolve in 10 ml N,N-dimethyl-formamide
Store at -20 °C

5. LB/Ampicillin/IPTG/X-gal plate

LB/Ampicillin plate
Apply 20 µl 100 mM IPTG and 20 µl 4% X-gal, and spread

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[6] Example

Example 1. TA cloning of a 500 bp-PCR product amplified with KOD- Plus- and Blend Taq.

The human β-globin gene (0.5 kb) was amplified using KOD -Plus- [Code No. KOD-201] or Blend Taq [BTQ-101]. The unpurified PCR products were cloned into the pTA2 vectors using TArget Clone or TArget Clone -Plus- according to each instruction manual. Subsequently, DH5α competent cells were transformed using the ligation products and cultured on the LB/Amp/X-gal plate overnight at 37 °C.

As shown Fig. 1, PCR products from Blend Taq were successfully cloned into the pTA2 Vector using both kits. PCR products from KOD -Plus- were cloned efficiently into pTA2 Vector using TArget Clone -Plus-.

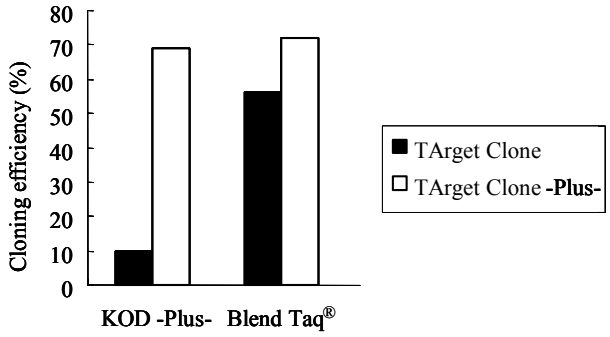

Fig. 1 Comparison of cloning efficiency

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[7] Troubleshooting

Symptom	Cause	Solution
No transformants	Low efficiency of competent cells	Use the competent cells having the efficiency 10^8 cfu/μg-pBR322.
	Excess concentration of the antibiotics in the agarose culture plates	The optimal concentration of ampicillin is 50-100 μg/ml.
Excess transformants	Deterioration of amplicillin	Ampicillin plates can be stored at 4 °C for 1 month. The optimal concentration of the ampicillin is 50-100 mg/ml.
Few white colonies	Insufficient addition of dA at the 3' ends of PCR products	<<TArget Clone>> -Use a DNA polymerase that exhibits terminal transferase activity. -Prolong the last extension step of the PCR cycle. -Store the PCR products at -20 °C. <<TArget Clone -Plus->> TArget Clone is corresponds to the KOD series enzymes. In the case of other high fidelity enzymes, the PCR products should be purified and dA added according to the protocol on p3. PCR products can be purified by DNA fragment purification kit such as MagExtractor -PCR & Gel Clean up- [Code No. NPK-601].
	Degradation of dT of pTA Vector.	Do not leave pTA Vector at room temperature and/or freeze and thaw too many times.
	Inefficient ligation	Prolong the ligation time up to 2 hr (room temperature) or overnight (4 °C).
	The ligation temperature is too high	The ligation reaction should be performed ≤ 25 °C. Higher temperature than 25 °C decrease the cloning efficiency.
	The concentration of the PCR products is too low.	-Use concentrated PCR products. The optimal molar ratio of pTA2: PCR products is 1: ≥3. -Decrease pTA Vector decrement up to 1/2 -1/5.
	PCR buffer inhibits the ligation reaction	Purify the PCR products prior to use. A certain PCR buffer may inhibit ligation reaction. PCR products can be purified by DNA fragment purification kit such as MagExtractor -PCR & Gel Clean up- [Code No. NPK-601].

Few white colonies	Excessive irradiation of UV when excising the DNA band from agarose gel.	Excessive irradiation of UV light to the PCR products decreases the cloning efficiency. Use longer wavelength UV, when excising the DNA bands from an agarose gel.
	Lac Z is slightly expressing.	Check the pale blue colonies.
Excessive white colonies	Low concentration of IPTG and X-gal	Use appropriate concentration of IPTG and/or X-gal.
No insert	The concentration of the PCR products is too low.	Use concentrated PCR products. The optimal molar ratio of pTA2: PCR products is 1: ≥3.
	PCR products contain primer dimers	Purify the PCR products.

[8] References

1) Mizuguchi H, Nakatsuji M, Fujiwara S, Takagi M and Imanaka T, *J Biochem*., 126: 762-8 (1999)

[9] Related products

Product name	Package	Code No.
10x A-attachment mix	25 reactions	TAK-301
Ligation high Ver.2	750 µl (100 reactions)	LGK-201

10 x A-attachment mix is a reagent comprising anti-KOD DNA polymerase antibody specific to KOD 3'→5' exonuclease activity (proof-reading activity), as well as Taq DNA polymerase, which exhibits terminal transferase activity. PCR products from KOD -Plus- [Code No. KOD-201] and KOD FX [Code No. KFX-101] possess blunt ends due to 3'→ 5' exonuclease activity of the KOD DNA polymerase. The 10 x A-attachment mix allows for PCR products to acquire overhanging dA at the 3'-ends. Products with 3'-dA overhangs can be directly cloned into arbitrary T-vectors using ligation reagents, such as Ligation high Ver.2 [Code No. LGK-201].

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