

# TArget Clone/ TArget Clone -Plus-

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

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## 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.

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**JAPAN**  
TOYOBO CO., LTD.  
Tel(81)-6-6348-3888  
www.toyobo.co.jp/e/bio  
tech\_osaka@toyobo.jp

**CHINA**  
TOYOBO Bio-Technology, CO., LTD.  
Tel(86)-21-58794900.4140

**FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.**

## [1] Introduction

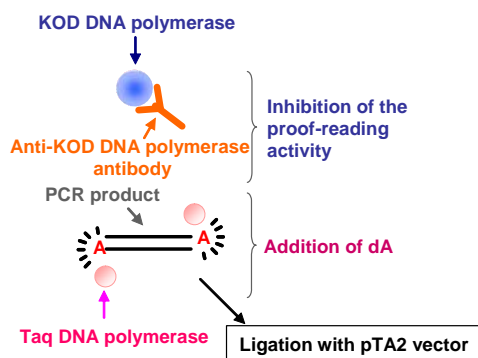
### Description

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

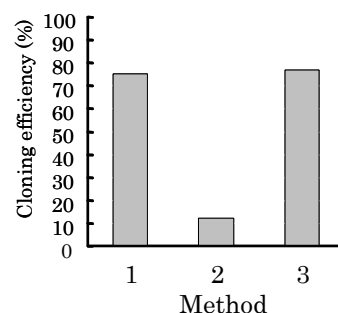
TARget Clone [Code No. TAK-101] can be applied to the TA cloning of PCR products amplified using Taq DNA polymerase, Blend Taq [Code No. BTQ-101], Blend Taq -Plus- [Code No. BTQ-201] or KOD Dash [Code No. LDP-101]. Almost all PCR products having dA overhangs at the 3' end are applicable.

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<sup>1)</sup> 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 completed in 10 min at 60 °C.

## [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]		[Target Clone -Plus-]	
pTA2 Vector (50ng/μl)	10 μl	pTA2 Vector (50ng/μl)	10 μl
2x Ligation Buffer	50 μl	2x Ligation Buffer	50 μl
T4 DNA Ligase	10 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
<hr/>	
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;  

$$X (\mu\text{l}) = 50 * Y / Z$$
[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].

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

**Notes**

Small PCR products ( $\leq 2$  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  $\mu$ l 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  $\mu$ l 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) $\mu$ l
2x Ligation Buffer	5 $\mu$ l
pTA2 Vector (50ng/ $\mu$ l)	1 $\mu$ l
dA-attached PCR products	X $\mu$ l
T4 DNA Ligase	1 $\mu$ l
<hr/>	
Total Volume	10 $\mu$ l

**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;

$$X (\mu\text{l}) = 50Y / Z$$

[Y: size of PCR product (kb), Z: concentration of PCR products (ng/ $\mu\text{l}$ )]

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

**Notes**

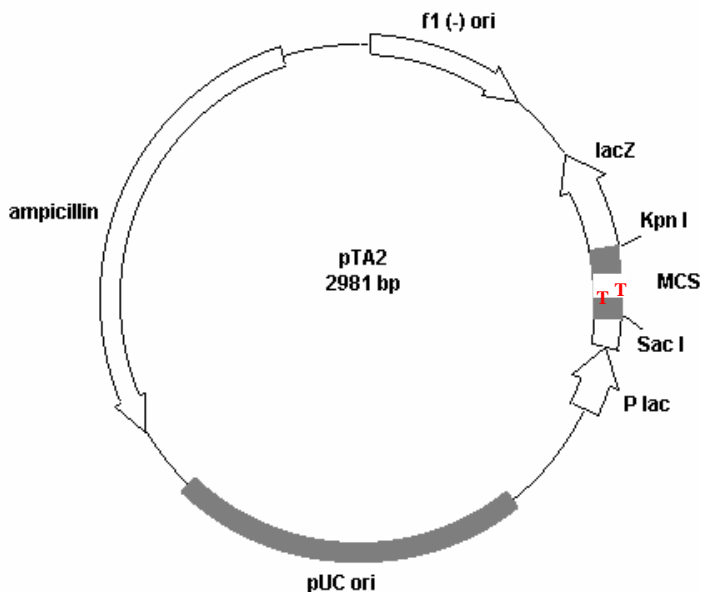
Small PCR products ( $\leq 2$  kb) can be cloned efficiently into the T vector in 5 min. The reaction efficiency can be increased by prolonging the reaction time up to 30 min at room temperature. The reaction can be performed at 4 °C overnight.

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

\*\*\*Up to 10  $\mu\text{l}$  of the ligation mixture can be used for transformation of 100  $\mu\text{l}$  of competent *E. coli* cells

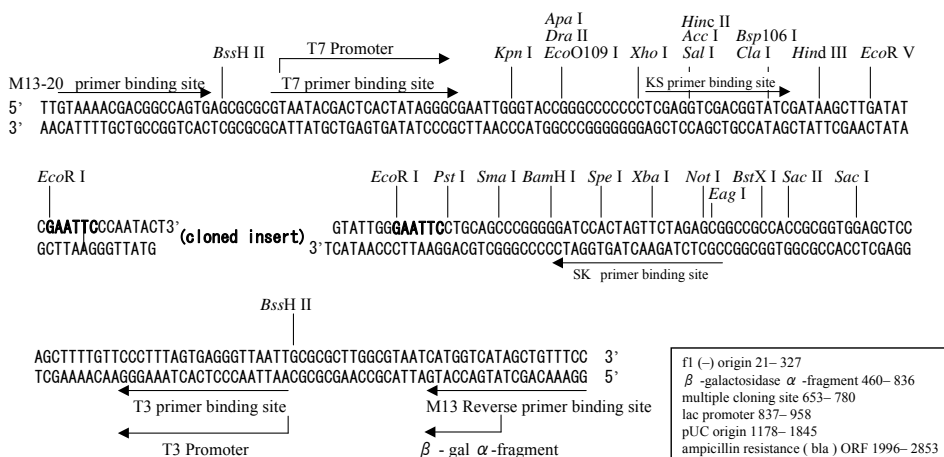
[4] Vector information

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 CTCCTTTTCG
101 TTTCTTCCCT TCCTTTCTCG CCACGTTTCG CGGCTTTCCC CGTCAAGCTC
151 TAAATCGGGG GCTCCCTTTA GGGTTCCGAT TAGTGCTTT ACGGCACCTC
201 GACCCCAAAA AACTTGATTA GGGTGATGGT TCACGTAGTG GGCCATCGCC
251 CTGATAGACG GTTTTTTCGCC CTTTGACGTT GGAGTCCACG TTCTTTAATA
301 GTGGACTCTT GTTCCAAACT GGAACAACAC TCAACCCTAT CTCGGTCTAT
351 TCTTTTGATT TATAAGGGAT TTTGCCGATT TCGGCCTATT GGTTAAAAAA
401 TGAGCTGATT TAACAAAAAT TTAACGCGAA TTTTAACAAA ATATTAACGC
451 TTACAATTTT CATTTCGCCAT TCAGGCTGCG CAACTGTTGG GAAGGGCGAT
501 CGGTGCGGGC CTCTTCGCTA TTACGCCAGC TGGCGAAAGG GGGATGTGCT
551 GCAAGGCGAT TAAGTTGGGT AACGCCAGGG TTTTCCCAGT CACGACGTTG
601 TAAAACGACG GCCAGTGAGC GCGCGTAATA CGACTACTA TAGGGCGAAT
651 TGGGTACCGG GCCCCCCCTC GAGGTCGACG GTATCGATAA GCTTGATATC
701 GAATTCCTAA TAC*GTATTGGGAATTCCTGC AGCCCGGGG ATCCACTAGT
751 TCTAGAGCGG CCGCCACCGC GGTGGAGCTC CAGCTTTTGT TCCCTTTAGT
801 GAGGGTTAAT TGCGCGCTTG GCGTAATCAT GGTATAGCT GTTTCCTGTG
851 TGAAATTGTT ATCCGCTCAC AATTCCACAC AACATACGAG CCGGAAGCAT
901 AAAGTGTAAT GCCTGGGGTG CCTAATGAGT GAGCTAACTC ACATTAATTG
951 CGTTGCGCTC ACTGCCCGCT TTCCAGTCGG GAAACCTGTC GTGCCAGCTG
1001 CATTAATGAA TCGGCCAACG CGCGGGGAGA GGCGGTTTGC GTATTGGGCG
1051 CTCTTCCGCT TCCTCGCTCA CTGACTCGCT GCGCTCGGTC GTTCGGCTGC
1101 GCGGAGCGGT ATCAGCTCAC TCAAAGGCGG TAATACGGTT ATCCACAGAA
1151 TCAGGGGATA ACGCAGGAAA GAACATGTGA GCAAAAGGCC AGCAAAAGGC
1201 CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCAT AGGCTCCGCC
1251 CCCCTGACGA GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC
1301 CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCTGGAA GCTCCCTCGT
1351 GCGCTCTCCT GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCTTTT
1401 TCCCTTCGGG AAGCGTGGCG CTTTCTCATA GCTCACGCTG TAGGTATCTC
1451 AGTTCGGTGT AGGTCGTTTC CTCCAAGCTG GGCTGTGTGC ACGAACCCCC
1501 CGTTCAGCCC GACCCTGCG CTTATCCGG TAACTATCGT CTTGAGTCCA
1551 ACCCGGTAAG ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGAACAGG
1601 ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG
1651 GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC TCGCTCTGCT
1701 TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA
1751 CAAACCACCG CTGTTAGCGG TGTTTTTTTT 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 AGTGGTCTCG CAACTTTATC CGCCTCCATC CAGTCTATTA ATTGTTGCCG
2251 GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA TAGTTTGC GC AACGTTGTTG
2301 CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG TATGGCTTCA
2351 TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT
2401 GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA
2451 AGTTGGCCGC AGTGTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT
2501 CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG GTGAGTACTC
2551 AACCAAGTCA TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTTGCC
2601 CGGCGTCAAT ACGGATAAT ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG
2651 CTCATCATTTG GAAAACGTTT TCCGGGGCGA AACTCTCAA GGATCTTACC
2701 GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC AACTGATCTT
2751 CAGCATCTTT TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG
2801 CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT GTTGAATACT
2851 CATACTCTTC CTTTTTCAAT ATTATTGAAG CATTTATCAG GGTATTGTGTC
2901 TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG
2951 GTCCGCGCA CATTCCCCG AAAAGTGCCA C .

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“\*” shows the site having dT at the 3' ends



#### 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
<i>Acc65I</i>	1	653
<i>AccI</i>	1	674
<i>AflIII</i>	1	1173
<i>AhdI</i>	1	2061
<i>A/II44I</i>	2	1487, 2733
<i>A/II</i>	10	739, 740, 1740, 1814, 1826, 1911, 1924, 2388, 2691, 2709
<i>A/III</i>	1	1584
<i>Apal</i>	1	659
<i>ApoI</i>	3	417, 428, 701
<i>AvaI</i>	2	668, 733
<i>AvaII</i>	2	2204, 2426
<i>BamHI</i>	1	739
<i>BanI</i>	4	193, 653, 917, 2014
<i>BanII</i>	3	159, 659, 775
<i>BcIV</i>	2	1382, 2909
<i>BfaI</i>	6	81, 746, 752, 1668, 1921, 2256
<i>BglI</i>	2	466, 2180
<i>BpmI</i>	2	778, 2151
<i>BsaAI</i>	1	232
<i>BsaHI</i>	1	2602
<i>Bsal</i>	1	2133
<i>BsaJI</i>	6	575, 733, 734, 767, 912, 1333
<i>BsaNI</i>	3	1379, 1526, 2357
<i>BsaMII</i>	4	1448, 1857, 2023, 2563
<i>BsIEI</i>	6	497, 758, 1086, 1510, 2433, 2582
<i>BsIHKAI</i>	4	775, 1487, 2648, 2733
<i>BsII</i>	8	9, 335, 664, 1015, 1189, 1207, 1373, 1652
<i>BsmAI</i>	2	2133, 2898
<i>Bsp120I</i>	1	659
<i>Bsp1286I</i>	6	159, 659, 775, 1487, 2648, 2733
<i>BspHI</i>	2	1893, 2901
<i>BspLU11I</i>	1	1173
<i>BsrBI</i>	5	88, 755, 863, 1104, 2905



<i>BsrDI</i>	2	2120,	2302			
<i>BssHII</i>	2	619,	812			
<i>BssSI</i>	2	1346,	2730			
<i>BstFI</i>	4	542,	2046,	2227,	2514	
<i>BstXI</i>	1	764				
<i>Cfr10I</i>	2	129,	2146			
<i>ClaI</i>	1	683				
<i>Csp6I</i>	2	654,	2545			
<i>DdeI</i>	4	1448,	1857,	2023,	2563	
<i>DraI</i>	3	1930,	1949,	2641		
<i>DraIII</i>	1	232				
<i>DrdI</i>	2	275,	1275			
<i>DsaI</i>	1	767				
<i>EaeI</i>	4	609,	758,	1012,	2454	
<i>EarI</i>	3	511,	1051,	2855		
<i>Eco136II</i>	1	775				
<i>Eco52I</i>	1	758				
<i>Eco57I</i>	2	1700,	2748			
<i>EcoO109I</i>	1	659				
<i>EcoRI</i>	2	701,	721,			
<i>EcoRII</i>	5	575,	912,	1200,	1321,	1334
<i>EcoRV</i>	1	695				
<i>FauI</i>	5	33,	87,	505,	965,	1022
<i>FokI</i>	4	542,	2046,	2227,	2514	
<i>FspI</i>	2	477,	2286			
<i>HaeII</i>	4	75,	83,	1047,	1417	
<i>HgaI</i>	4	3,	1275,	1853,	2603	
<i>HincII</i>	1	674				
<i>HindIII</i>	1	689				
<i>HinfI</i>	8	282,	304,	632,	1008,	1073
		1148,	1544,	2061		
<i>HphI</i>	6	222,	1917,	2144,	2540,	2766
		2781				
<i>KpnI</i>	1	653				
<i>MaeII</i>	8	123,	233,	276,	288,	595
		1876,	2292,	2665		
<i>MboII</i>	8	103,	512,	1052,	1823,	1914
		2669,	2747,	2856		
<i>MsiI</i>	4	765,	2314,	2473,	2832	
<i>MspAII</i>	6	527,	767,	995,	1513,	1758
		2699				
<i>MvaI</i>	5	575,	912,	1200,	1321,	1334
<i>NaeI</i>	1	129				
<i>NciI</i>	6	657,	733,	734,	1552,	2248
		2599				
<i>NgoMIV</i>	1	129				

**JAPAN**  
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 Tel(81)-6-6348-3888  
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 tech\_osaka@toyobo.jp

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<i>NlaIII</i>	8	828, 2473,	1174, 2509,	1894, 2902	2385,	2395
<i>NotI</i>	1	757				
<i>NspI</i>	1	1173				
<i>PleI</i>	6	282, 2061	304,	632,	1073,	1544
<i>PspI406I</i>	2	2291,	2664			
<i>PstI</i>	1	727				
<i>PvuI</i>	2	497,	2433			
<i>PvuII</i>	2	527,	995			
<i>RsaI</i>	2	654,	2545			
<i>SacI</i>	1	775				
<i>SacII</i>	1	767				
<i>SaI</i>	1	674				
<i>SapI</i>	1	1050				
<i>Sau86I</i>	8	240, 2187,	507, 2204,	659, 2426	660,	2108
<i>Scal</i>	1	2544				
<i>SchI</i>	6	282, 2061	304,	632,	1073,	1544
<i>SfaNI</i>	4	1261,	2313,	2523,	2753	
<i>SfcI</i>	6	11, 2307	638,	727,	1438,	1629
<i>SmaI</i>	1	733				
<i>SmlI</i>	5	668,	1279,	1541,	1818,	2686
<i>SpeI</i>	1	745				
<i>SspI</i>	2	440,	2868			
<i>TaaI</i>	8	258, 1676,	483, 1989,	678, 2504	1135,	1206
<i>TaiI</i>	8	123, 1876,	233, 2292,	276, 2665	288,	595
<i>TaqI</i>	7	199, 1273,	669, 2717	675,	684,	699
<i>TatI</i>	1	2544				
<i>TfiI</i>	2	1008,	1148			
<i>Tsp45I</i>	4	57,	589,	2323,	2534	
<i>TspRI</i>	10	613, 1859,	960, 2008,	1069, 2113,	1575, 2460,	1588 2487
<i>VspI</i>	3	943,	1002,	2237		
<i>XbaI</i>	1	751				
<i>XhoI</i>	1	668				
<i>XhoII</i>	7	739, 2691,	1814, 2708	1825,	1911,	1923
<i>XmaI</i>	1	733				
<i>XmnI</i>	1	2661				

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Restriction enzymes have no restriction site

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<i>Aat</i> II	<i>Acc</i> II	<i>Afl</i> II	<i>Age</i> I	<i>Asc</i> I
<i>Bbe</i> I	<i>Bbs</i> I	<i>Bbv</i> CI	<i>Bcl</i> I	<i>Bgl</i> II
<i>Bln</i> I	<i>Bpu</i> I01	<i>Bpu</i> I102I	<i>Bsa</i> BI	<i>Bse</i> RI
<i>Bsg</i> I	<i>Bs</i> NI	<i>Bsn</i> BI	<i>Bsr</i> FI	<i>Bsm</i> I
<i>Bsp</i> MI	<i>Bsr</i> GI	<i>Bst</i> I107I	<i>Bst</i> API	<i>Bst</i> BI
<i>Bst</i> EII	<i>Bsu</i> 36I	<i>Eco</i> 47III	<i>Eco</i> 72I	<i>Eco</i> NI
<i>Ehe</i> I	<i>Fse</i> I	<i>Hpa</i> I	<i>Kas</i> I	<i>Mlu</i> I
<i>Msc</i> I	<i>Mun</i> I	<i>Nar</i> I	<i>Nco</i> I	<i>Nde</i> I
<i>Nhe</i> I	<i>Nru</i> I	<i>Nsi</i> I	<i>Pac</i> I	<i>Pme</i> I
<i>Ppu</i> I01	<i>Ppu</i> MI	<i>Psh</i> AI	<i>Rsr</i> II	<i>Sar</i> DI
<i>Sbf</i> I	<i>Sex</i> AI	<i>Sfi</i> I	<i>Sgf</i> I	<i>Sgr</i> AI
<i>Sna</i> BI	<i>Sph</i> I	<i>Srf</i> I	<i>Stu</i> I	<i>Sty</i> I
<i>Swa</i> I	<i>Tth</i> I11I	<i>Var</i> 91I	<i>Xcm</i> I	

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**JAPAN**

TOYOBO CO., LTD.  
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[www.toyobo.co.jp/e/bio](http://www.toyobo.co.jp/e/bio)  
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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/Ampicillin plate**

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  
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

## [6] Example

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

The human  $\beta$ -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, DH5 $\alpha$  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-.

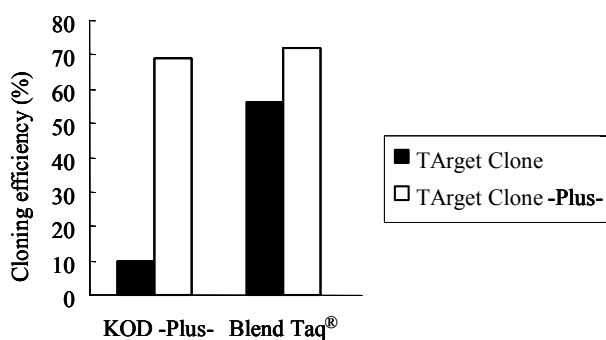


Fig. 1 Comparison of cloning efficiency

## [7] Troubleshooting

Symptom	Cause	Solution
No transformants	Low efficiency of competent cells	Use the competent cells having the efficiency $10^8$ cfu/ $\mu$ g-pBR322.
	Excess concentration of the antibiotics in the agarose culture plates	The optimal concentration of ampicillin is 50-100 $\mu$ g/ml.
Excess transformants	Deterioration of ampicillin	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 $\leq$ 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: $\geq$ 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: $\geq 3$ .
	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.
<b>10x A-attachment mix</b>	25 reactions	TAK-301
<b>Ligation high Ver.2</b>	750 $\mu$ l (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].