www.toyobo.co.jp/e/bio

Instruction manual for KOD FX Neo 1103

F1100K

KOD FX Neo

KFX-201 200 U 200 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 safety while using this kit.

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

KOD FX Neo is based on the DNA polymerase from the hyperthermophilic Archaeon *Thermococcus kodakaraensis* KOD1[1) 2)]. KOD FX Neo provides greater efficiency and elongation capabilities than conventional PCR enzymes. In particular, KOD FX Neo shows greater amplification success from crude specimens.

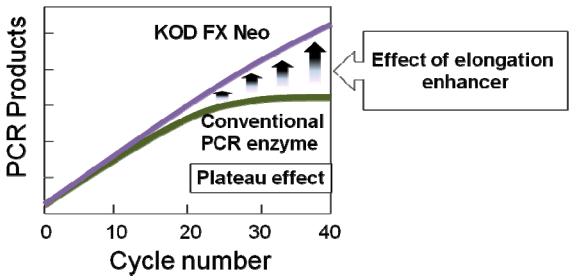
KOD FX Neo is the improved version of the previous KOD FX (Code No. KFX-101). This product contains a unique "elongation enhancer" that suppresses the "plateau effect", enabling greater elongation rates and capabilities.

The KOD FX Neo enzyme solution contains two types of anti-KOD DNA polymerase antibodies that inhibit the polymerase and 3'→5' exonuclease activities, thus allowing for Hot Start PCR[3)]. KOD FX Neo generates blunt-end PCR products because of its 3'→5' exonuclease (proof-reading) activity.

Features

-This enzyme is effective for amplification from crude samples (*e.g.* biological samples, foodstuffs, soil extract, etc). Various samples or lysates can be used directly as templates.

-"Elongation enhancer" enables greater amplification efficiency and elongation capability (up to 40 kb from human genomic DNA) compared to conventional PCR. This enzyme is useful for amplifying difficult targets, such as high G/C, A/T, and/or long targets.



-Various microorganisms (e.g. yeast, fungus, gram-positive bacteria) can be directly used as templates for PCR.

-The PCR error ratio of KOD FX Neo is about 10 times less than that of Taq DNA polymerase and is equal to the previous version (KOD FX).

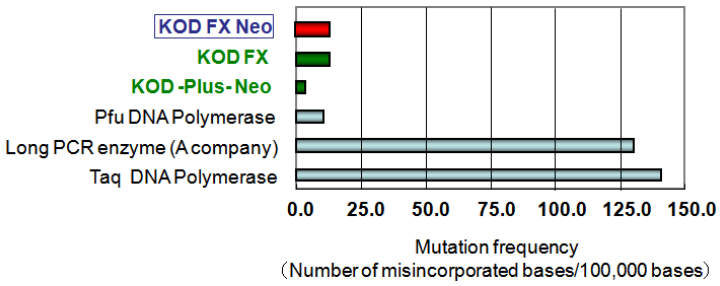


Table 1. Comparison of the mutation frequency of each PCR enzyme.

Fidelity was measured as the mutation frequency by sequencing the PCR product. After cloning the PCR product (5.7 kb of the human β-globin region), about 96 clones were selected and sequenced.

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

[2] Components

KOD FX Neo (1.0 U/µl) *	200 µl × 1
2× PCR Buffer for KOD FX Neo**	1.7 ml × 3
2 mM dNTPs	1 ml × 2

*The enzyme solution contains anti-KOD DNA polymerase antibodies that neutralize the polymerase and 3'→5' exonuclease activities.

** 2× PCR Buffer for KOD FX Neo is a liquid (not frozen) when stored at -20°C. Although it does freeze below -20°C, the quality is not affected.

[3] Quality Testing

Quality testing is performed by amplification of the human β-globin gene (32 kb).

[4] Primer Design

-Primers should be 22–35 bases with a melting temperature (T_m) over 63°C.

-Optimal GC content of primers is 45–60%. The ideal GC contents of the 5' half and the 3' half are 60–70% and 45–50%, respectively.

-The priming efficiency of primers can be promoted by anchoring the 3'end of primers with G or C.

-Primers should be designed so as not to generate intermolecular secondary structures or primer dimers.

-Primers for long target amplification should be 25–35 bases with Tm over 65°C.

-Primers containing inosine cannot be used.

-The Tm of primers should be calculated using the Nearest Neighbor method. The Tm values in this manual were calculated using this method with the following parameters.

Na^+ concentration: 50 mM

Oligonucleotide concentration: 0.5 µM

[5] Cloning of PCR products

-KOD FX Neo generates blunt-end PCR products because of its 3'→5' exonuclease (proof-reading) activity. Therefore, PCR products can be cloned using blunt-end cloning methods.

-PCR products of KOD FX Neo should be purified prior to restriction enzyme treatments in cloning steps. The 3'→5' exonuclease activity of KOD DNA polymerase remains at the end of the PCR reaction.

-The dedicated TA cloning kit "TArget clone™ -Plus- (Code No. TAK-201)" is recommended for the cloning of blunt end PCR products produced by KOD DNA polymerase (see [11] Related product).

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

[6] Protocol

1. Standard reaction setup

The following protocol is designed for use with the components provided in this kit. Before preparing the mixture, all components should be completely thawed, except for the enzyme solution.

Component	Volume	Final Concentration
PCR grade water	X µl	1×
2x PCR buffer for KOD FX Neo	25 µl	1×
2mM dNTPs*	10 µl	0.4 mM each
10pmol /µl Primer #1	1.5 µl	0.15–0.3 µM
10pmol /µl Primer #2	1.5 µl	0.15–0.3 µM
Template DNA	Y µl	Genomic DNA ≤200 ng / 50 µl Plasmid DNA ≤50 ng / 50 µl cDNA ≤200 ng（RNA equiv.) / 50 µl Crude sample ≤0.5–2 µl/50 µl (see [7])
KOD FX Neo (1.0U/µl)	1 µl	1.0 U / 50 µl
Total reaction volume	50 µl	

* Do not use dNTPs from other kits or companies.

Notes:

-Optimal primer concentration is 0.3 µM. In the case of long targets (≥10 kb), a reduced primer concentration (0.15 µM) may give more effective amplification.

-For PCR reactions, thin-wall tubes are recommended. Reaction setup to a total reaction volume of 50 µl is also recommended.

-Crude samples (e.g., cultured animal cell suspension) should be added up to 2 µl for each 50-µl reaction.

2. Cycling conditions

Two-step cycle conditions can be easily used for amplification using KOD FX Neo with primers ≥20 mer and Tm ≥68°C [recommended cycle]. If the Tm value of the primer is under 68°C, the 3-step cycle condition is effective. For trouble shooting of poor amplification (no band, smear etc) or long target amplification (>10 kb), the step-down cycle is recommended. The extension time should be set at 1 min./kb when crude samples are used as templates.

<Recommended cycle>

< 2-step cycle >	For purified DNA /cDNA	For crude samples	
Pre-denaturation :	94°C, 2 min.	94°C, 2 min.	
Denaturation :	98°C, 10 sec.	98°C, 10 sec.	25–45 cycles
Extension :	68°C, 30 sec. /kb	68°C, 1 min. /kb	

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

<Other cycles>

< 3-step cycle >	For purified DNA /cDNA	For crude samples	
Pre-denaturation :	94°C, 2 min.	94°C, 2 min.	
Denaturation :	98°C, 10 sec.	98°C, 10 sec.	↰ 25–45 cycles
Annealing	Tm °C*, 30 sec.	Tm °C, 30 sec.	
Extension :	68°C,30 sec. /kb	68°C, 1 min. /kb	

*Tm value of the primer.

< Step-down cycle >	For purified DNA /cDNA	For crude samples	
Pre-denaturation :	94°C, 2 min.	94°C, 2 min.	
Denaturation :	98°C, 10 sec.	98°C, 10 sec.	↰ 5 cycles
Extension :	74°C, 30 sec. /kb	74°C, 1 min. /kb	
Denaturation :	98°C, 10 sec.	98°C, 10 sec.	↰ 5 cycles
Extension :	72°C, 30 sec. /kb	72°C, 1 min. /kb	
Denaturation :	98°C, 10 sec.	98°C, 10 sec.	↰ 5 cycles
Extension :	70°C, 30 sec. /kb	70°C, 1 min. /kb	
Denaturation :	98°C, 10 sec.	98°C, 10 sec.	↰ 15–30 cycles
Extension :	68°C, 30 sec. /kb	68°C, 1 min. /kb	
Extension :	68°C, 7 min.	68°C, 7 min.	

Notes:
-For amplification from crude samples, the extension time should be 1 min./kb.
-For amplification from a low copy DNA or for longer targets (> 10 kb), longer extension times may enhance the efficiency.
-Poor amplification may be improved by changing the denaturation step to 94°C, 15 sec.

[7] Templates

a. Purified DNA or cDNA

Appropriate template amounts for 50 µl reaction are summarized in the following table.

		Approved range	Recommended
Genomic DNA	Eukaryotic DNA	5–200 ng	50 ng
	Prokaryotic DNA	0.1–100 ng	10 ng
Plasmid DNA		10 pg–50 ng	1 ng
cDNA		< 200 ng (RNA equiv.)	50 ng (RNA equiv.)
Lambda phage DNA		0.01–10 ng	1 ng

-Contaminating RNA in cDNA or genomic DNA inhibits the PCR reaction by chelating Mg^{2+}. PCR should be performed using template DNA containing <200 ng RNA.

-Quality of template DNA should be checked by electrophoresis. The length and purity of template DNA affects amplification results.

-Templates containing uracil cannot be used for amplification.

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

b. Tissues and cells

When adding biological samples directly to the PCR reaction solution, the following samples can be applied to 50 µl reaction.

Sample	Appropriate template amount	Remarks
E. coli	Picked small amount of cells from colonies	
Yeast	Picked small amount of cells from colonies	
Fungus	Picked small amount of cells from colonies	When reproducibility is not good, suspended cells in TE buffer should be added (2–5 µl)
Cultured cells	10^1–10^5 cells/2 µl medium or PBS	
Whole Blood	1–2 µl	
Nail	1 × 1 mm	As the concentration of extracted DNA is low, 35–40 cycles are needed.
Hair root	1–2 cm	
Leaf	2 × 2 mm	
Milled rice	0.5 × 0.5 mm	
Mouse tail	1 × 1 mm	On an agarose gel assay, a portion of amplicon may remain in the slots.

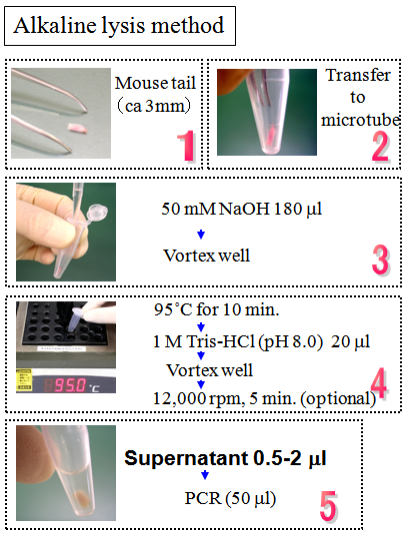
*In the case of the direct amplification from animal tissues, such as mouse tail, a portion of the amplification product may remain in the gel slot on an agarose gel assay. Adding 10 µl of 20 mg/ml proteinase K to 50 µl PCR products prior to the electrophoresis is effective to dissociate the aggregates.

c. Lysate

To make the lysate for PCR, the following methods are recommended. The lysates can be stored at 4°C for several weeks. For long term storage, the lysates should be stored at -20°C.

<Alkaline lysis method>

The following “Alkaline lysis method” is recommended for rapid preparation of mouse tails or nail lysates suitable for amplification with KOD FX Neo.



*Mouse tail cannot be dissolved completely.

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

[96-well PCR plate protocol by alkaline lysis method]

The following protocol is useful for the preparation of lysates from a large number of mouse tail samples.

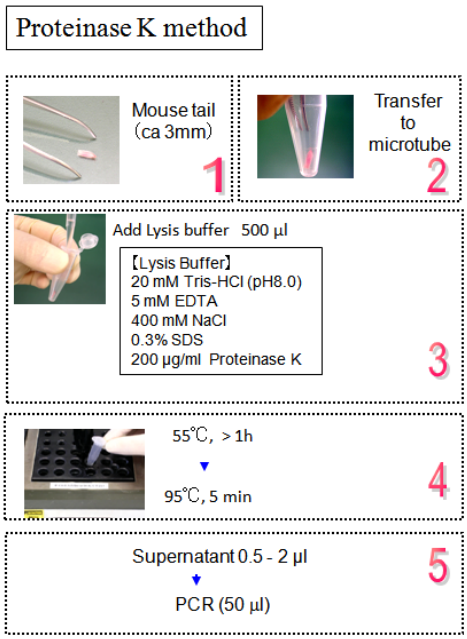
1. Transfer mouse tails (ca 3mm) to a 96-well PCR plate.
2. Add 180 µl of 50mM NaOH and vortex.
3. Spin down.
4. Incubate at 95°C for 10 min using thermal cycler.
5. Add 20 µl of 1M Tris-HCl (pH 8.0) and vortex.
6. Spin down.

Supernatant 0.5–2 µl → PCR (50 µl)

<Proteinase K method>

The following "Proteinase K method" is recommended for an efficient preparation of mouse tails or nail lysates suitable for amplification with KOD FX Neo. This protocol can also be applied to the following samples.

Mouse tail: 3 mm
Nail: 3 mm
Leaf: 3 × 3 mm
A rice grain



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

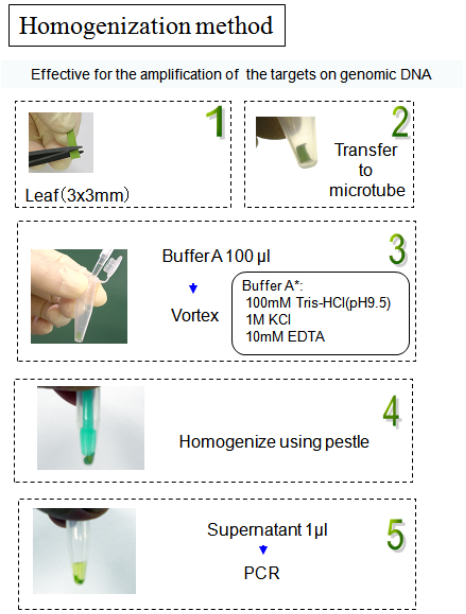
<One step method>

The following “One-step method” is recommended for the rapid preparation of a plant tissue lysate suitable for amplification with KOD FX Neo.

*BioTechniques, 19: 394 (1995)

<Homogenization method>

The following “Homogenization method” is recommended for an effective preparation of a plant tissue lysate suitable for amplification with KOD FX Neo. This method is effective for the amplification of genomic DNA targets.



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

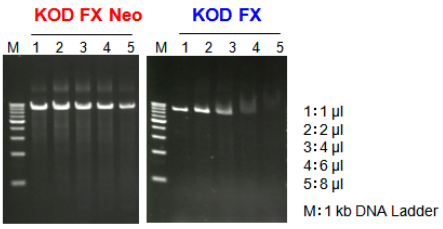
[8] Examples

Example 1. Amplification from crude samples

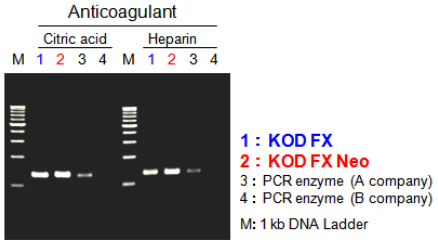
a. Whole blood samples

The human β-globin gene (8.5 kb) was amplified using KOD FX Neo and KOD FX (previous version) with various amounts of blood (EDTA) specimens as templates. PCR was performed using 50 µl reaction solution contains 1–8 µl whole blood samples according to the standard protocol with 30 cycles. As a result, KOD FX Neo successfully amplified the targets using 1–8 µl whole blood specimens.

Next, the human β-globin gene (1.3 kb) was amplified using various PCR enzymes with whole blood specimens containing citric acid and heparin as anticoagulants. KOD FX Neo amplified the targets efficiently from both whole blood samples.



Template : Whole blood (EDTA) (1~8 µl)
Target: Human β-globin 8.5 kb
Total reaction volume: 50 µl

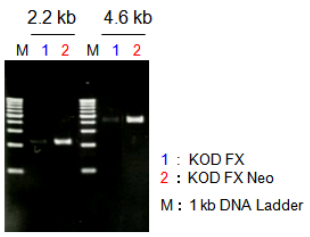


Template : Whole blood 5 µl
Target : Human β-globin 1.3 kb

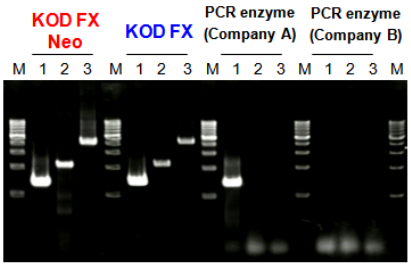
b. Leaf

Two targets (2.2 and 4.6 kb) were amplified using lysates from tobacco leaves. Each PCR reaction was performed according to the recommended conditions with 35 cycles. KOD FX Neo showed greater amplification from lysates prepared by the "one-step method".

Various targets were then directly amplified using small pieces of tobacco leaves (2 × 2 mm). KOD FX Neo successfully amplified DNA using these templates.



Template: Lysate* [Tobacco] 1 µl
* One-step method
Target : ribulose-1,5-bisphosphate carboxylase / oxygenease large subunit N-methyltransferase gene (rbcmtT)



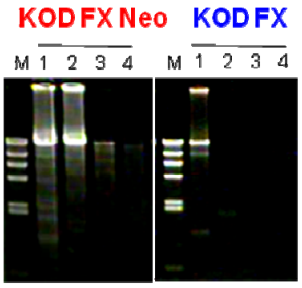
Template : Tobacco leaf (2 x 2 mm)
Target:
1 : ribulose-1,5-bisphosphate carboxylase / oxygenease large subunit gene (rbcL) 1.3 kb
2 : rbcmtT 2.2 kb
3 : rbcmtT 4.6 kb
M: 1 kb DNA Ladder

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

Example 2. Elongation capability

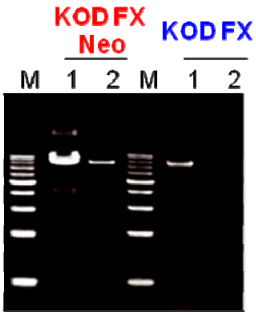
The efficiency for long targets was compared between KOD FX Neo and KOD FX (previous version) using human genomic DNA as templates. The amplification was performed according to the standard protocol with a step-down cycle. KOD FX Neo successfully amplified targets of up to 40 kb.



Template : Human genomic DNA 200 ng
1 : Human tissue-type plasminogen activator (tPA) 24 kb
2 : Human β-globin 32 kb
3 : Human β-globin 40 kb
4 : Human β-globin 50 kb
M : λ/*Hin*dIII digest

Example 3. Amplification efficiency and sensitivity

The human β-globin gene (8.5 kb) was amplified using the standard protocol with 40 cycles. KOD FX Neo showed higher sensitivity than KOD FX (previous version). KOD FX Neo can suppress the “plateau effect” on PCR after 20 cycles due to the “elongation enhancer”. Therefore, KOD FX Neo shows greater sensitivity than conventional reagents in the range of 30–45 cycles during PCR.



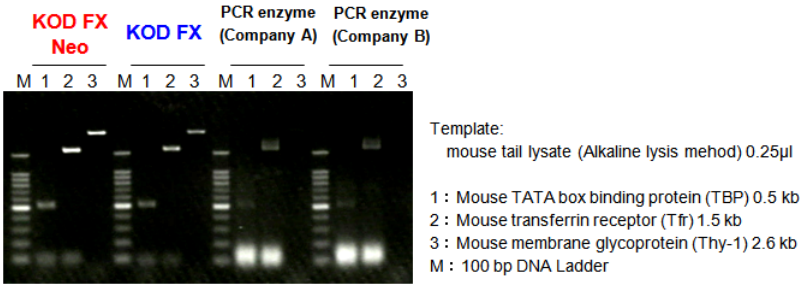
Template: Human DNA
Target : Human β-globin 8.5 kb
1 : 0.1 ng
2 : 0.01 ng
M : 1 kb DNA Ladder

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

Example 4. Amplification from mouse tail lysates

The amplification efficiency was then compared between various PCR enzymes using mouse tail lysates as templates. KOD FX Neo showed greater amplification efficiency than the other enzymes.

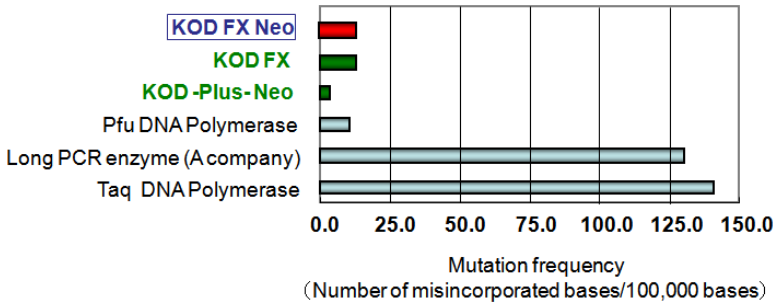


Example 5. PCR error ratio

The error ratio of various PCR enzymes were compared by determining the sequences of the amplicons from human β-globin gene. The amplicons were cloned into the vector using TArget clone™ -Plus- (Code No. TAK-201) and the sequences were determined.

The result indicates that the error ratio of KOD FX Neo is equal to that of KOD FX (previous version) and approximately 10 times lower than rTaq DNA polymerase.

Table. Comparison of the mutation frequency of each PCR enzyme.



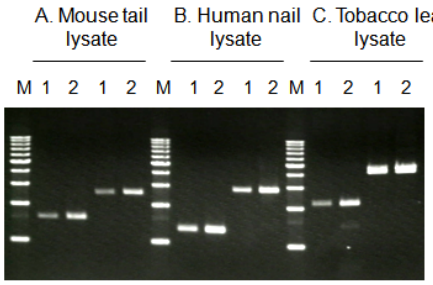
Fidelity was measured as a mutation frequency by sequencing the PCR product. After cloning, the PCR product (5.7 kb of the human β-globin region), about 96 clones were selected and sequenced.

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

Example 6　Extension time for crude samples

Various targets were amplified using crude lysates with two cycling conditions (extension time: 30 sec./kb or 60 sec./kb). As a result, longer extension conditions gave greater amplification.



1：30 sec./kb
2：60 sec./kb

A. Mouse tail lysate (Alkaline lysis method)
Targets : Mouse TBP 0.5 kb
Mouse Tfr 1.5 kb

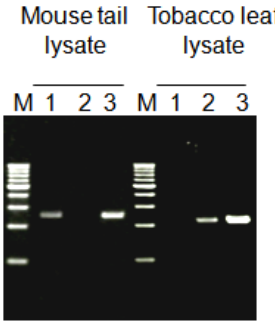
B. Human nail lysate (Alkaline lysis method)
Targets : Human β-globin 1.3 kb
Human β-globin 2.8 kb

C:Tabacco leaf lysate (One-step method)
Targets : rbcmtT 2.2 kb
rbcmtT 4.6 kb

M：1 kb DNA Ladder

Example 7　Preparation methods of crude lysates

Amplification efficiency was compared using various lysates from mouse tail and tobacco leaf lysates. For mouse tail lysates, the alkaline lysis method and proteinase K method gave superior results. For plant lysates, the one-step and proteinase k methods showed superior results.



Targets: Mouse Thy-1 2.6 kb
:Tobacco rbcmtT 2.2 kb

1: Alkaline lysis method
2: One-step method
3: Proteinase K method

M：1 kb DNA Ladder

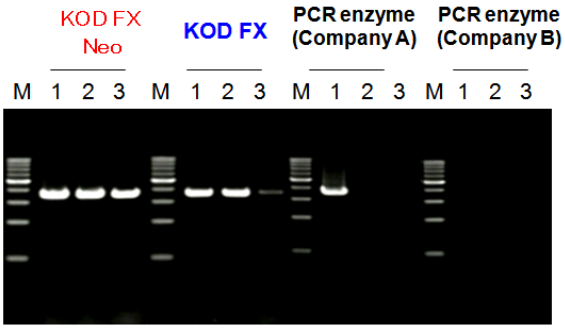
JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

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

Application data: Amplification using soil specimens.

Inhibitory effect of humic acid was compared between various PCR enzymes. Humic acid is the major organic constituent of soil or compost. It is produced by biodegradation of dead organic matters. The humic acid can be hardly separated from DNA on purification and inhibits PCR.

The results indicate that KOD FX Neo can amplify targets under high concentrations of humic acid.

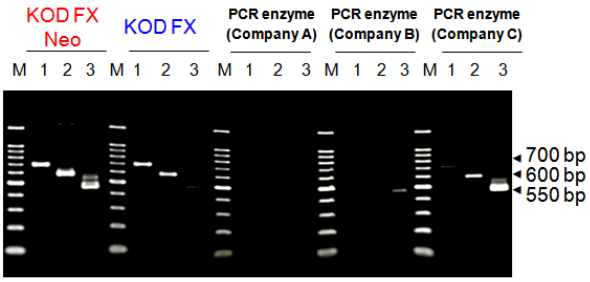


M: 1kb Ladder Marker
1: Humic acid* 0 µl
2: Humic acid* 2 µl
3: Humic acid* 4 µl

Template: 10ng Human genomic DNA
Target: Human β-globin gene 3.6kb

*The solution of humic acid (OD280=1) was used as a inhibitor.

rDNA was amplified from roughly prepared extracts from 100 mg compost specimens using the alkaline method [see p5] using various PCR enzymes. In this experiment, three primer sets for prokaryote, Bacillus sp. and high G/C gram-negative bacteria were used. The results indicate that KOD FX Neo is the only PCR reagent can amplify all targets.



1 Prokaryote derived rDNA(700 bp)
2 *Bacillus sp.* derived rDNA (600 bp)
3 High G/C gram-positive bacteria derived rDNA (550 bp)

1 Fwd ATTAGATACCCTDGTAGTCC
Rev TACCTTGTTACGACTT

2 Fwd AGGGTCATTGGAAACTGGG
Rev CGTGTTGTAGCCCAGGTCATA

3 Fwd GGCCTTCGGGTTGTAAACC
Rev CTTTGAGTTTTAGCCTTGCGGC

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

[9] Troubleshooting

Symptom	Cause	Solution
No PCR product / low yield	Cycling condition is not suitable.	Increase the extension time up to 60 sec./kb.
		Increase the number of cycles by 2–5 cycles.
		Use a 3-step cycle instead of a 2-step cycle. Lower annealing temperature in the 3-step cycling decrements up to Tm-5–10°C.
		Use a step-down cycle. (This solution is effective for long targets over 10 kb)
	Template DNA is not good in quality and /or quantity.	Increase the amount of template DNA.
		Decrease the amount of template DNA to reduce the contaminated PCR inhibitors.
		Use purified templates.
		Decrease the amount of cDNA to reduce the inhibition by contaminated RNA.
		Degrade or eliminate RNA in the DNA sample.
	Primer is not good.	Decrease the primer concentration from 0.3 mM to 0.15 µM. (This solution may be effective for the amplification of targets over 10 kb)
		Use fresh primers.
		Redesign primers.
	Enzyme concentration is low.	Increase enzyme concentration to 1.5–2.0 U/ 50 µl.
Smearing / Extra band	Cycling conditions are not suitable.	Change from 3-step cycling to 2-step cycling.
		Change from 2-step cycling to step-down cycling.
		Decrease the number of cycles by 2–5 cycles.
	Too much template DNA.	Reduce the amount of template DNA.
	Quality of primers is not sufficient.	Use fresh primers.
		Redesign primers. (Longer primers may eliminate smearing or extra bands)
	Too much enzyme	Reduce Enzyme to 0.5–0.8U/ 50µl reaction
Poor TA cloning efficiency	PCR products have blunt-ends.	Clone the PCR products according to general blunt-end cloning guide lines. Use TArget Clone™ -Plus- (Code No. TAK-201) [see related products]

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

[10] References

1) Takagi M, Nishioka M, Kakihara H, Kitabayashi M, Inoue H, Kawakami B, Oka M, and Imanaka T., *Appl Environ Microbiol.*, 63: 4504-10 (1997)

2) Hashimoto H, Nishioka M, Fujiwara S, Takagi M, Imanaka T, Inoue T and Kai Y, *J Mol Biol.*, 306: 469-77 (2001)

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

[11] Related products

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

TArget Clone -Plus- is a highly efficient TA cloning kit. The kit can be applied to the TA cloning of blunt-ended PCR products amplified using KOD -Plus- [Code No. KOD-201], KOD -Plus- Neo [Code No. KOD-401], KOD FX [Code No. KFX-101] or KOD FX Neo [Code No. KFX-201]. The kit contains pTA2 Vector, 2x Ligation Buffer, T4 DNA Ligase, and 10× A-attachment Mix.

10× 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], KOD -Plus- Neo [Code No. KOD-401], KOD FX [Code No. KFX-101] and KOD FX Neo [Code No. KFX-201]. possess blunt ends due to 3'→5' exonuclease activity of the KOD DNA polymerase. The 10× 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].

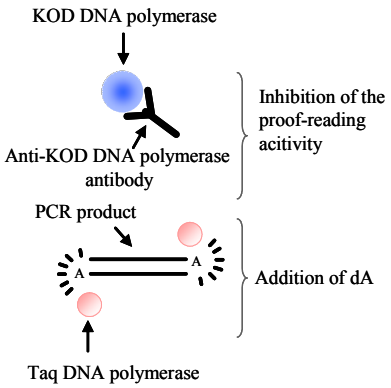


Figure. Principle of the 10× A-attachment mix

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

NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the United States: 5,079,352, 5,789,224, 5,618,711, 6,127,155 and claims outside the United States corresponding to US Patent No. 4,889,818. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim (such as the patented 5' Nuclease Process claims in US Patent Nos. 5,210,015 and 5,487,972), no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

The use of betaine in DNA polymerase reactions is covered by intellectual property, including U.S. Patent No. 6,270,962 and related patents issued or pending in other countries ("I.P."), exclusively licensed to EPICENTRE Technologies Corporation, 726 Post Road, Madison, WI 53713, USA ("EPICENTRE"). The right to use betaine in conjunction with KOD DNA Polymerase in PCR is sublicensed by EPICENTRE to TOYOBO Co., Ltd. solely for research purposes. The purchase of this product conveys to the buyer a limited, non-exclusive, non-transferable right under the I.P. to use the purchased product containing betaine and KOD DNA polymerase in PCR solely for research purposes. No rights are granted to resell, repackage, or further sublicense. No other license is granted to the buyer, whether expressly, by implication, by estoppel or otherwise

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