Blend Taq® / Blend Taq® -Plus-

<Blend Taq®>	BTQ-101	250 U	200 reactions
<Blend Taq® -Plus->	BTQ-201	250 U	200 reactions

Store at -20°C

Contents

CAUSION
All reagents in this kit are intended for research purposes. Do not use for diagnosis or clinical purposes. Please observe general laboratory precaution and utilize safety while using this kit.

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[1] Introduction

Description

Blend Taq® and Blend Taq® -Plus- are highly efficient Taq-based DNA polymerases developed based on the Barns' method.[1)] This method uses a DNA polymerase w lacked 3'→5' exonuclease (proofreading) activity (e.g., Taq DNA polymerase) a small amount of an archaeal DNA polymerase with proofreading activity. Because the proofreading activity repairs misincorporated nucleotide bases causing the termination of the polymerase reaction, PCR with a 'mixed' enzyme solution enables highly efficient amplification.

The enzyme solution of Blend Taq® -Plus- contains anti-Taq DNA polymerase antibodies that inhibit polymerase activity, allowing for Hot Start PCR.

Blend Taq® and Blend Taq® -Plus- generate dA overhang-ended PCR products. Therefore, the PCR products can be cloned using a standard TA-cloning method.

Features

-This enzyme is effective for the amplification of various targets from small template amounts. The elongation ability of this enzyme is much greater than that of the normal Taq DNA polymerase.

-Hot Start technology using anti-Taq DNA polymerase antibodies results in highly efficient amplification. <Blend Taq® (Code No. BTQ-101) does not use hot start>

-The PCR error ratio of this enzyme is approximately 3-4 times less than that of Taq DNA polymerase.

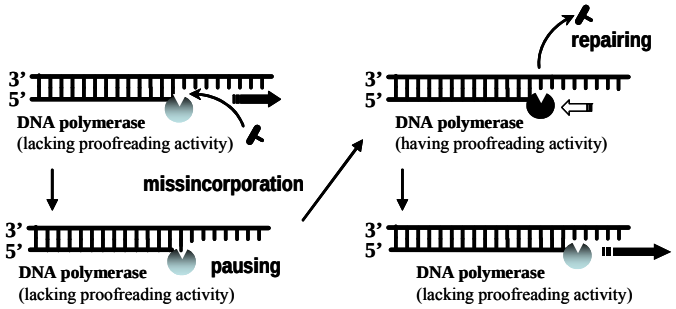

Fig. 1. The principles of the Barns' technology.

[2] Components

The provided reagents include the following components for 200 reactions:

<Blend Taq®>	Blend Taq® (2.5 U/µl)	100 µl
	10x PCR Buffer for Blend Taq®	1.0 ml
	2 mM dNTPs	1.0 ml
<Blend Taq® -Plus->	Blend Taq® -Plus- (2.5 U/µl)*	100 µl
	10x PCR Buffer for Blend Taq®	1.0 ml
	2 mM dNTPs	1.0 ml

*This enzyme solution contains anti-Taq DNA polymerase antibodies.

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[3] Quality Testing

Quality check was performed by amplifying the human β-globin gene (17.5 kb).

[4] Primer Design

PCR primers should be designed according to general guidelines. For the amplification of a long target (≥ 6 kb), the melting temperature (Tm) of the primers should be set over 70 °C.

[5] Cloning of PCR products

The PCR products of Blend Taq® and Blend Taq® -Plus- can be cloned according to a standard TA-cloning method.

[6] Protocol

1. Standard reaction setup

The following procedures are designed to be used with the components provided in this kit. Before preparing the reaction mixture, all components should be completely thawed, except for the enzyme solution.

Component	Volume	Final Concentration
10x Buffer	5 µl	1 x
2 mM dNTPs*	5 µl	0.2 mM each
10 pmol/µl Primer #1	1 µl	0.2 µM
10 pmol/µl Primer #2	1 µl	0.2 µM
Template DNA	X µl	Genomic DNA 10-1000 ng/50 µl Plasmid DNA 1-50 ng/50 µl cDNA ≤200 ng（RNA equiv.)/50 µl *E. coli* cells (small amount)
PCR grade water	Y µl	
Blend Taq® (2.5 U/µl) or Blend Taq® -Plus (2.5 U/µl)	0.5 µl	1.25 U / 50 µl
Total reaction volume	50 µl	

* Do not use dNTPs from other kits or companies.

Notes:

-Thin-wall tubes are recommended for PCR use. A total reaction volume of 50 µl is recommended.

-For amplification of a long target (≥ 10 kb), the final concentration of dNTPs should be 0.3-0.4 mM.

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2. Cycling conditions

The following cycling steps are recommended.

(1) Cycling conditions for < 6kb targets.

< 3-step cycle >		
Pre-denaturation	94 °C, 2 min	
Denaturation	94 °C, 30 sec.	25-35 cycles
Annealing	Tm-[5-10] °C*, 30 sec.	
Extension	72 °C, 1 min/kb	

*Tm value of the primer minus 5°C-10°C

(2) Cycling conditions for ≥ 6kb products.

< 2-step cycle >		
Pre-denaturation	94 °C, 2 min	
Denaturation	94 °C, 30 sec.	25-35 cycles
Extension	68 °C, 1 min/kb	

Note: If the Tm value of the primer is under 73 °C, the 3-step cycle is recommended.

Notes:

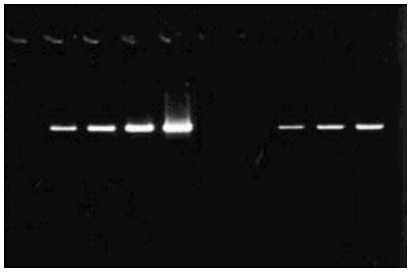
-Extension time should be set at 1min per 1 kb of target length.
-For colony-direct PCR, the pre-denaturation step should be set for 4 min.

[7] Examples

Example 1. Comparison of the sensitivity of PCR with a Taq-based PCR enzyme.

Blend Taq® -Plus- Taq-based PCR enzyme (company A)

1 2 3 4 5 1 2 3 4 5



Template: Human Genomic DNA
Target: β-globin 3.6 kb

Lane 1: 0 ng human genomic DNA
Lane 2: 5 ng human genomic DNA
Lane 3: 10 ng human genomic DNA
Lane 4: 20 ng human genomic DNA
Lane 5: 40 ng human genomic DNA

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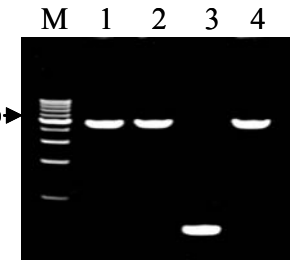
Example 2. Colony-direct PCR using *E. coli* cells.



Template: Small amount of *E. coli* cells (JM109) from colonies
Insert: Transferrin receptor 4.5 kb
Vector: pBluescript II
PCR cycling condition:

94 ℃ 4 min.
98 ℃ 30 sec. ←
55 ℃ 30 sec.] 30 cycles
72 ℃ 4.5 min.

Lane 1: Insert (+)
Lane 2: Insert (+)
Lane 3: Insert (-)
Lane 4: Insert (+)

F primer: TCGAGGTCGACGGTATCGAT (20mer GC=55%)
R primer: CGCTCTAGAACTAGTGGATC (20mer GC=50%)

*The primers are designed on the vector.

[8] Troubleshooting

Symptoms	Cause	Solution
No PCR product / low yield	Cycling conditions are not optimal	Lower annealing temperature increments to a maximum of Tm-5 °C.
		Increase the number of cycles by 2-5 cycles.
	Primers are not good	Check the design and/or quality of primers.
	Too much *E. coli* cells (Colony direct PCR)	Decrease the amount of *E. coli* cells from colonies.
Smearing / extra band(s)	Cycling conditions are not optimal	Decrease the number of cycles by 2-5 cycles.

[9] Related products

Product name	Package	Code No.
Highly efficient cDNA synthesis kit **ReverTra Ace® -α-**	100 rxns	FSK-101
Highly efficient reverse transcriptase **ReverTra Ace®**	10,000 U	TRT-101
RNase inhibitor (Recombinant type)	2,500 U	SIN-201
High Speed PCR enzyme **KOD Dash**	200 U x1	LDP-101
High fidelity PCR enzyme **KOD -Plus-**	200 U x1	KOD-201
Highly reliable PCR enzyme **KOD FX**	200 U x1	KFX-101

[10] References

1) Barns WM, *Proc. Natl. Acad. Sci.* USA, 91: 2216-2220 (1994)

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