Cat. # R040A

For Research Use

TaKaRa

PrimeSTAR® HS (Premix)

Product Manual

v201804Da

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I. Description

PrimeSTAR HS (Premix) is an optimized mixture composed of PrimeSTAR HS DNA Polymerase, which is a high-fidelity DNA polymerase developed by TaKaRa Bio Inc, reaction buffer and dNTP mixture as 2-fold concentration. As this product offers quick preparation of reaction mixture and reduction of contamination risk, it is also useful for high-throughput application. PrimeSTAR HS DNA Polymerase has a matchless proof reading activity due to very strong 3'-5' exonuclease activity, and besides its amplification efficiency is higher than that of *Taq* DNA Polymerase. Furthermore an antibody-mediated hot start formulation prevents false initiation events during the reaction assembly due to mispriming and primer digestion. When used with Takara Bio's optimized reaction buffer, PrimeSTAR HS achieves the high fidelity, high sensitivity, and high specificity required for applications such as DNA amplification from cDNA library.

II. Components (for 100 reactions)

PrimeSTAR HS (Premix)	500 μl x 5	
Content :	PrimeSTAR HS DNA Polymerase	1.25 U/25 μl
	dNTP Mixture	2X conc. ; 0.4 mM each
	PrimeSTAR Buffer	2X conc. ; including 2 mM Mg^{2+}

III. Storage

-20℃ for long-term storage. 4℃ for short-term storage (up to 3 months).

Note : If used frequently, store at 4℃ ; repeated freezing and thawing will decrease its activity. Gently mix well before use and centrifuge briefly.

IV. Features

A : Accuracy

Mutation frequency of PrimeSTAR HS was investigated by actually analyzing large number of bases of sequence data.

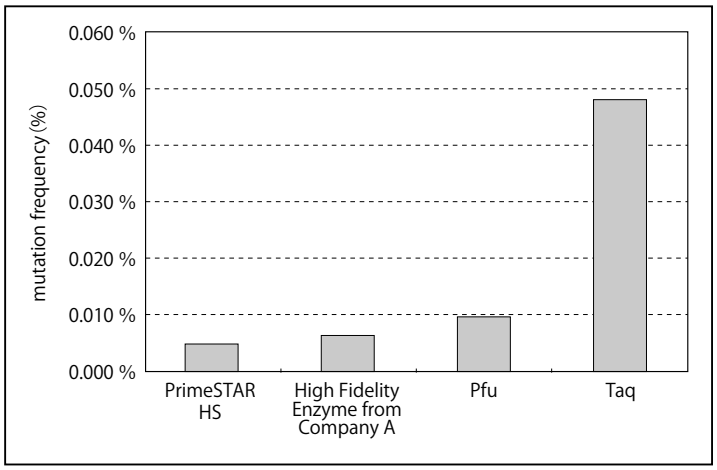
[Method]

Eight arbitrarily selected GC-rich regions were amplified with PrimeSTAR HS and other enzymes, using the *Thermus thermophilus* HB8 genomic DNA as a template. Each PCR product (approx. 500 bp each) was cloned into a suitable plasmid. Multiple clones were picked up per region respectively, and were subjected to sequence analysis.

[Result]

Sequence analysis of DNA fragments amplified using PrimeSTAR HS demonstrated only 12 mismatched bases per 249,941 total bases. This is higher fidelity than an alternative high fidelity enzyme from Company A, and 10X higher fidelity than *Taq* DNA polymerase.

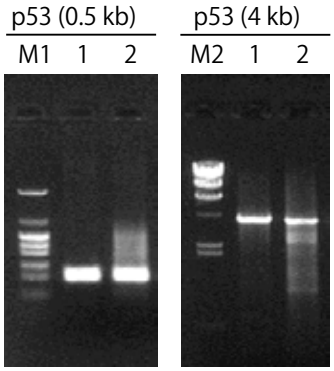
Fidelity Comparison with competitors



The above method is the most realistic method to investigate the mutation frequency. Based on the above sequence analysis results, it is strongly recommended to use PrimeSTAR HS DNA Polymerase for the PCR amplification that requires the extreme accuracy.

B : High Priming efficiency - Short Annealing Time

PrimeSTAR HS DNA Polymerase possesses extremely high priming efficiency. Thus by using a short annealing time, only 5 or 15 seconds, highly specific amplification can be achieved. Refer to "VI. PCR Conditions" for determining reaction conditions.



M1 : pHY Marker
M2 : λ-*Hin*d III digest
1 : Annealing at 55℃ for 5 sec
2 : Annealing at 55℃ for 30 sec

Template : Human genomic DNA 100 ng
50 μl PCR reaction
3-step PCR Method, 30 cycles

C : Amplification ability using genomic DNA as a template

Amplification of various DNA fragment sizes, using human genomic and *E. coli* genomic DNA as the template was confirmed.

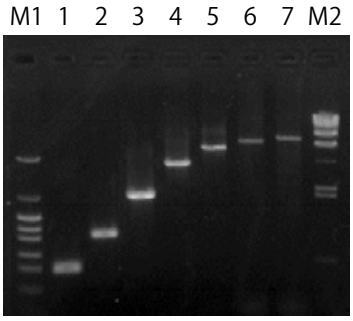
Template : Human genomic DNA [50 ng/50 μl PCR reaction]
Thermal cycler : TaKaRa PCR Thermal Cycler Dice™
Thermocycling conditions :

0.5 - 6 kb : 3-step PCR Method

98℃	10 sec	30 cycles
60℃	5 sec	
72℃	1 min/kb	

7.5 - 8.5 kb : 2-step PCR Method

98℃	10 sec	30 cycles
68℃	8 min	

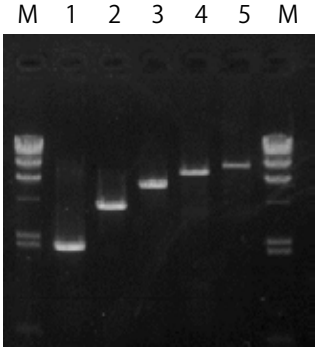


M1 : pHY Marker
1 : 0.5 kb
2 : 1 kb
3 : 2 kb
4 : 4 kb
5 : 6 kb
6 : 7.5 kb
7 : 8.5 kb
M2 : λ-*Hin*d III digest

Template : *E.coli* genomic DNA [100 pg/50 μl PCR reaction]
Thermal cycler : TaKaRa PCR Thermal Cycler Dice
Thermocycling conditions :

3-step PCR Method

98℃	10 sec	30 cycles
60℃	5 sec	
72℃	1 min/kb	



M : λ-*Hin*d III digest
1 : 2 kb
2 : 4 kb
3 : 6 kb
4 : 8 kb
5 : 10 kb

V. General Composition of PCR Reaction Mixture

Reagents	Volume	Final conc.
PrimeSTAR HS (Premix)	25 μl	1X
Primer 1	10 - 15 pmol	0.2 - 0.3 μM
Primer 2	10 - 15 pmol	0.2 - 0.3 μM
Template	< 200 ng	
Sterile purified water	up to 50 μl	
Total	50 μl	

VI. PCR Conditions

(A) 3-step PCR Method

98℃	10 sec	⎫
55℃	5 sec or 15 sec	⎬ 30 cycles
72℃	1 min/kb	⎭

(B) 2-step PCR Method

98℃	10 sec	⎫
68℃	1 min/kb	⎭ 30 cycles

Takara Bio recommends first trying the 3-step PCR with PrimeSTAR HS (Premix).

・Denaturation conditions : 98℃, 5 - 10 sec.
Alternatively, if a lower denaturation temperature 94℃ is used, set a denaturation time to be 10 - 15 sec must also be used.

・Annealing temperature : Initially, try at 55℃ (optimization may be required.)

・Annealing time : Annealing time is dependent upon primer Tm values. Calculate primer Tm values using the following formula (＊);

When $T_m \geqq 55℃$ Set it for 5 sec.
When $T_m < 55℃$ Set it for 15 sec.

(＊) Tm value (℃) ＝ [(the number of A and T) x 2] + [(the number of G and C) x 4] - 5

The above Tm value formula is valid for primers whose lengths are ≦ 25 mer. For primers longer than 25 mer, an annealing time of 5 sec should be used.

[Important Note]
Because the priming efficiency of PrimeSTAR HS is extremely high, an annealing time of 5 sec or 15 sec should be adopted. Longer annealing times may cause smearing of PCR products.

Please try the 2-step PCR when smeared DNA products are observed in following agarose gel electrophoresis of amplified DNA that has been obtained through the 3-step PCR or when using the primers with Tm values ≧ 70℃. Please refer to "VII. Optimization of parameters, IX. Troubleshooting" for further PCR condition.

VII. Optimization of Parameters

In order to obtain the best PCR results, it is important to optimize your PrimeSTAR HS reaction parameters to fully utilize the enzyme's properties and advantages.

1) Template DNA

Recommended template DNA amounts (assuming a 50 μl reaction) :

Human genomic DNA :	5 ng - 200 ng (< 200 ng)
E.coli genomic DNA :	100 pg - 100 ng
cDNA library :	1 ng - 200 ng
λ DNA :	10 pg - 10 ng
Plasmid DNA :	10 pg - 1 ng

Avoid using excess amounts of template DNA , which can lower enzyme reactivity.

No usable for template containing Uracil, such as Bisulphite-treated DNA.

2) Primer and PCR condition

The use of commercially available primer design software, such as OLIGO.
Primer Analysis Software (Molecular Biology Insights) is recommended for obtaining appropriate primer sequences that follow general primer design guidelines yet are tailored specifically for your template DNA.

Guidelines for Primer Design :

a) Primer length :
For general amplification of DNA fragments, 20 - 25 mer primers are suitable. Exact PCR conditions should be determined by referring to "VI. PCR conditions".

b) Modified bases :
Never use primers containing inosine (I) with PrimeSTAR HS (Premix)

c) Degenerate primers :
Degenerate primers may be used with PrimeSTAR HS (Premix)

3) Annealing conditions

Annealing conditions should be determined by referring to "VI. PCR conditions". When the amplified PCR product is of low yield, refer to the following troubleshooting :

< Smearing and/or extra bands appear on agarose gels. >

i) Shorten the annealing time. For example, decrease time from 15 sec to 5 sec.
ii) If the annealing time is already 5 sec, then raise the annealing temperature to 58 - 65℃.
iii) Try 2-step PCR.

< Target product is not amplified. >

i) Extend the annealing time. For example, increase time from 5 sec to 15 sec.
ii) Lower the annealing temperature to 50 - 53℃.

VIII. Electrophoresis, Cloning, and Sequencing of Amplified Products

1) Electrophoresis of the amplified product

TAE Buffer is recommended for agarose gel electrophoresis of amplified products that are obtained using PrimeSTAR HS (Premix).

Note : Use of TBE Buffer may result in DNA band patterns which are enlarged at the gel bottom.

2) Cloning

Most PCR products amplified with PrimeSTAR HS (Premix) have blunt-end termini. Accordingly they can directly be cloned into blunt-end vectors (if necessary, phosphorylate before cloning), but are not clonable into T-vectors. Mighty Cloning Reagent Set (Blunt End) (Cat. #6027) is recommended for cloning into a blunt-end vector.

3) Restriction enzyme reaction

Prior to performing restriction enzyme digestion of amplified PCR products, remove all traces of PrimeSTAR HS (Premix) from the reaction mix by phenol/ chloroform extraction. Particularly for 3'-protruding restriction enzymes, such as *Pst* I, the 3'-protruding termini produced by these enzymes may be deleted during digestion by 3' → 5' exonuclease activity of residues of PrimeSTAR HS (Premix).

4) Direct sequencing

It is recommmended to perform phenol/chloroform extraction of PCR products prior to direct sequencing and ensure inactivation of 3' → 5' exonuclease activity.

IX. Troubleshooting

1) Problem : No amplified product, or low amplification efficiency.

Remedy :

i) Extension time : Use on extension time > 1 min/kb.
ii) Annealing time : 15 sec.
iii) Annealing temperature : Lower temperature in decrements of 2℃. Alternatively, perform cycling using the 3-step PCR Method.
iv) Purity and quality of template DNA : Use an appropriate amount of template DNA; refer to "VII. Optimization of Parameters". Use a more highly purified template DNA.
v) Primer concentration : Test a final primer concentration in the range of 0.2 - 0.5 μM.

2) Problem : Extra bands appear or DNA smearing is observed during agarose gel electrophoresis.

Remedy :

i) Annealing time : 5 sec.
ii) Annealing temperature : Raise the temperature in increments of 2℃. Alternatively, perform cycling using the 2-step PCR Method.
iii) Extension time : 1 min/kb. Avoid excessive extension times.
iv) Template DNA : Use an appropriate amount of template DNA. Avoid excessive amounts of template DNA.
v) Cycle number : 25 - 30 cycles.
vi) Primer concentration : Determine the optimal concentration that lies within a range of 0.2 - 0.3 μM (final conc.).

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Thermal Cycler Dice is a trademark of Takara Bio Inc.