Cat. # R110A

For Research Use

TaKaRa

TaKaRa EpiTaq™ HS (for bisulfite-treated DNA)

Product Manual

v1111Da

Table of Contents

I. Description

TaKaRa EpiTaq HS (for bisulfite-treated DNA) is a DNA polymerase optimized for PCR amplifications using bisulfite-treated DNA containing uracil as template. PCR reactions with bisulfite-treated DNA often pose particular technical challenges for conventional enzymes. TaKaRa EpiTaq HS and the reagents contained in this kit, however, allow adjustment of magnesium and dNTP concentrations to achieve optimal amplification efficiencies and specificities, facilitating excellent amplifications even for targets that previously were impossible to amplify.

TaKaRa EpiTaq HS is also a hot-start PCR enzyme with an anti-*Taq* antibody. Prior to high-temperature denaturation, the anti-*Taq* antibody suppresses polymerase activity, thereby preventing non-specific amplifications as a result of mispriming or primer dimer formation before starting PCR. This allows the use of conventional PCR conditions without requiring a special denaturation step.

II. Components

(1)	TaKaRa EpiTaq HS (5 U/μl)	250 U
(2)	10X EpiTaq PCR Buffer (Mg^{2+} free)	1 ml
(3)	dNTP Mixture (2.5 mM each)	1.2 ml
(4)	25 mM $MgCl_2$	1.2 ml

III. Storage -20℃

IV. Composition of PCR Mixture

	Volume	Final Concentration
TaKaRa EpiTaq HS (5 U/μl)	0.25 μl	1.25 U / 50 μl
10X EpiTaq PCR Buffer (Mg^{2+} free)	5 μl	1X
25 mM $MgCl_2$	5 μl	2.5 mM
dNTP Mixture (2.5 mM each)	6 μl	0.3 mM
Template	< 100 ng	
Primer 1	20 pmol	0.4 μM
Primer 2	20 pmol	0.4 μM
Sterilized distilled water	to 50 μl	

For a first trial, use the reaction mixture described above. If this reaction mixture fails to result in desired levels of product amplification or results in the appearance of extra bands, adjusting the concentration of $MgCl_2$, dNTP mixture, or primer may improve results. For details, see section X. Troubleshooting.

PCR reaction mixtures can be prepared at room temperature. Each component, however, should be kept on ice.

V. PCR Conditions

98℃	10 sec. ＊1	30 - 40 cycles
55℃	30 sec.	
72℃	30 sec. (~ 500 bp) or 1 min. (500 bp ~ 1 kb)＊2	

＊1: Select denaturation conditions compatible with the PCR thermal cycler and tubes used for the reaction. In general, treat at 98℃ for 5 - 10 sec. or at 94℃ for 20 - 30 sec.

＊2: Set extension time according to the amplification size. In general, use 30 sec. for an amplification products of up to 500 bp, and 1 min. for amplification products ranging from 500 bp to 1 kb. As a general guideline, use 1 min. per 1 kb when the product size exceeds 1 kb.

If template DNA was damaged during the bisulfite treatment process, amplification efficiency will decrease for larger products.

VI. Electrophoresis of Amplification Products

After the reaction is complete, add 6X Loading Buffer (supplied with TaKaRa DNA molecular weight markers) to an aliquot of the reaction mixture (5 - 10 μl) in a ratio of 1:5 by volume, and analyze using agarose gel electrophoresis. After electrophoresis, stain the gel using 1.0 μg/ml of ethidium bromide for 20 - 30 min. Visualize DNA bands under UV irradiation.

VII. PCR Products

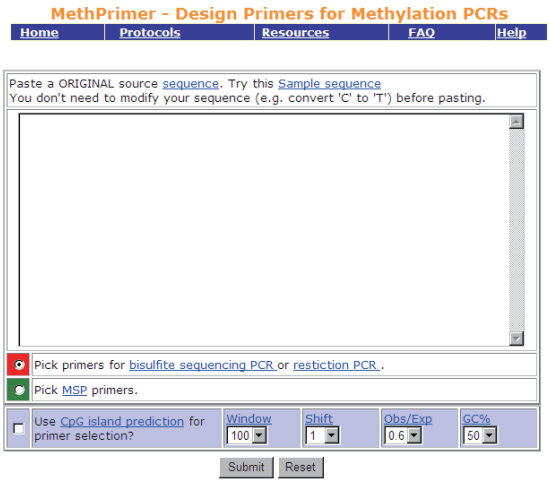
The majority of PCR products amplified using TaKaRa EpiTaq HS have a single A nucleotide added at 3' termini, allowing the PCR products to be cloned directly into T-vectors such as pMD20 (Cat. #3270) or pMD19 (Simple) (Cat. #3271).

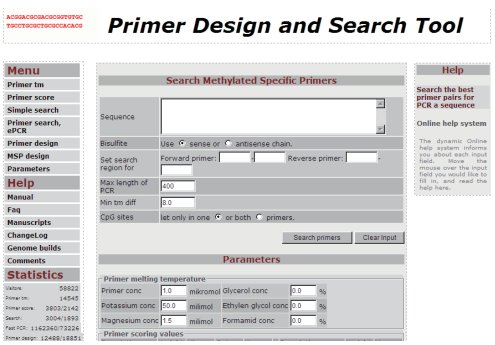
These PCR products may also be used for blunt-end cloning after blunting and phosphorylation.

VIII. Primer Design

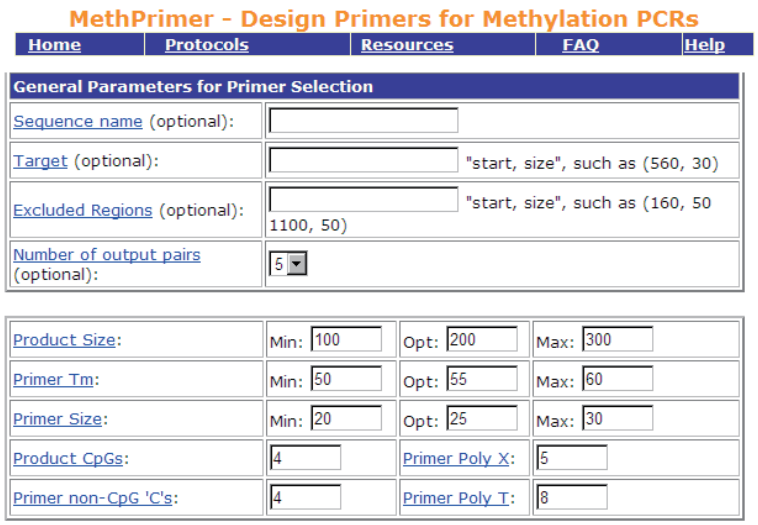
When designing primers for bisulfite-treated DNA, the use of a primer design tool specifically created for this purpose is recommended. The following design tools are available online free of charge. (For specific operating procedures, please refer to each web application.)
To ensure successful amplification with bisulfite-treated DNA as template, an amplification product length of no more than 300 bp is recommended. At Takara Bio, amplifications of up to approximately 1 kb have been achieved. (See section IX. Experimental Examples.)

MethPrimer
http://www.urogene.org/methprimer/index1.html





BiSearch
http://bisearch.enzim.hu/?m=msp



IX. Experimental Examples

PCR amplification was performed with bisulfite-treated HeLa genomic DNA as template.

[Method]

Bisulfite treatment: MethylEasy™ *Xceed* Rapid DNA Bisulfite Modification Kit (Cat. #ME002)
Target: CpG island region upstream of *CDH1*, *MLH1*, or *BRCA1* genes
Amplification size: 153 bp (*CDH1*), 297 bp (*CDH1*), 136 bp (*MLH1*), 292 bp (*MLH1*), 613 bp (*BRCA1*), 1,017 bp (*BRCA1*)

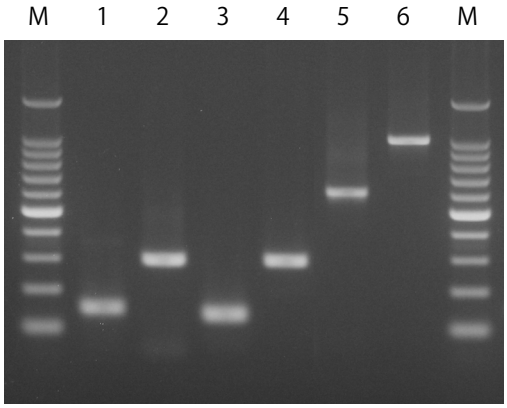
<Reaction mixture composition>

	Volume	Final Concentration
Bisulfite-treated HeLa genomic DNA (50 ng/μl)	2 μl	100 ng / 50 μl
10X EpiTaq PCR Buffer (Mg^{2+} free)	5 μl	1 ×
25 mM $MgCl_2$	5 μl	2.5 mM
dNTP Mixture (2.5 mM each)	6 μl	0.3 mM
Sense Primer (20 μM)	1 μl	0.4 μM
Antisense Primer (20 μM)	1 μl	0.4 μM
TaKaRa EpiTaq HS (5 U/μl)	0.25 μl	1.25 U / 50 μl
Sterile distilled water	to 50 μl	

<PCR Conditions>

98℃ 10 sec.
55℃ 30 sec.
72℃ [30 sec. for product <500 bp,
1 min. for products ≧ 500 bp]
] 40 cycles

[Result]



Lane:	Size:	Gene:
1:	153 bp	(*CDH1*)
2:	297 bp	(*CDH1*)
3:	136 bp	(*MLH1*)
4:	292 bp	(*MLH1*)
5:	613 bp	(*BRCA1*)
6:	1,017 bp	(*BRCA1*)

M: 100 bp DNA Ladder

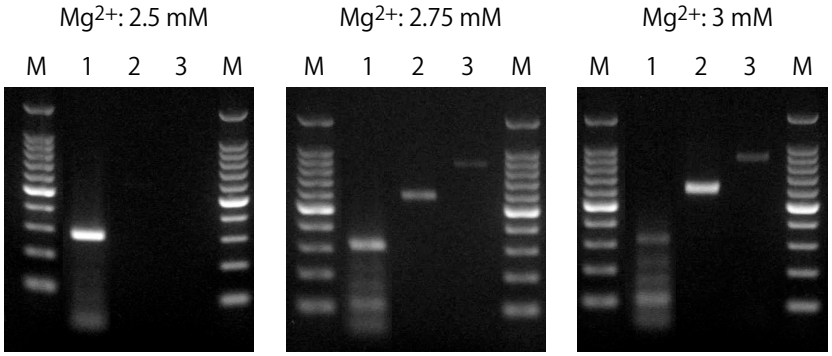
Agarose: Agarose L03 (Cat. #5003)
Gel concentration: 2%
Stain: Ethidium bromide

Figure 1. PCR with bisulfite-treated HeLa genomic DNA as template

X. Troubleshooting

Event	Possible causes	Action
No amplification or poor amplification efficiency	Mg^{2+}concentration	Raise the Mg^{2+} concentration; try 2.5 - 3 mM. (See Figure 2)
	dNTP concentration	Lower the dNTP concentration; try 0.2 - 0.3 mM
	Annealing temperature	Try lowering temperature from the default of 55℃. Lower by 2℃ per trial.
	Extension time	Increase the extension time from 30 sec. to 1 min. or from 1 to 2 min.
	Primer concentration	Raise the primer concentration; try 0.4 - 1 μM.
	Template fragmentation occurred during bisulfite treatment of DNA	Re-prepare bisulfite-treated template DNA.
Electrophoresis analysis shows smeared band(s) or extra band(s)	Mg^{2+}concentration	Lower the Mg^{2+} concentration; try 2 - 2.5 mM
	dNTP concentration	Raise the dNTP concentration; try 0.3 - 0.4 mM.
	Annealing temperature	Try raising temperature from the default of 55℃. Raise by 2℃ per trial.
	Primer concentration	Lower the primer concentration; try 0.2 - 0.4 μM.

＊ Lowering the Mg^{2+} concentration increases specificity, while raising the Mg^{2+} concentration improves amplification efficiency and extension. Raising the dNTP concentration increases specificity, while lowering the dNTP concentration improves amplification efficiency and extension.



Lane:	Size:	Gene:
1:	316 bp	(*CDKN2*)
2:	607 bp	(*MLH1*)
3:	877 bp	(*MLH1*)

M:100 bp DNA Ladder

Agarose:	Agarose L03
Gel concentration:	2%
Stain:	Ethidium bromide

Figure 2. Improvements achieved by altering Mg^{2+} concentration

XI. Related Product

MethylEasy™ *Xceed* Rapid DNA Bisulphite Modification Kit (Cat. #ME002)

NOTICE TO PURCHASER : LIMITED LICENSE

[L15] Hot Start PCR
Licensed under U.S. Patent No. 5,338,671 and 5,587,287 and corresponding patents in other countries.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

Takara products may not be resold or transferred, modified for resale or transfer, or used to manufacture commercial products without written approval from TAKARA BIO INC.

If you require licenses for other use, please contact us by phone at +81 77 543 7247 or from our website at www.takara-bio.com.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product web page. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.