Cat. # RR039Q

For Research Use

TaKaRa

Premix Ex Taq™
(Perfect Real Time)

Product Manual

v201311Da

Table of Contents

I. Description

Premix Ex Taq (Perfect Real Time) is designed for real-time PCR (qPCR) detection with TaqMan®＊1 probes. This product is also suitable for fast PCR. *Premix Ex Taq* allows accurate target quantification and detection over a broad dynamic range and makes it possible to obtain highly reproducible and reliable real-time PCR results.
The product is supplied premixed at a 2X concentration to facilitate easy preparation of reaction mixtures. The combination of *TaKaRa Ex Taq* HS, a hot start PCR enzyme that uses an anti-*Taq* antibody, and a buffer optimized for real-time PCR suppresses non-specific amplification and allows high amplification efficiency and high detection sensitivity in real-time PCR analyses.

Compatible real-time PCR instruments:

- ABI PRISM® 7000/7700＊2, Applied Biosystems® 7300/7500 Real-Time PCR System, 7500 Fast Real-Time PCR System (Life Technologies)
- LightCycler® (Roche Diagnostics) ＊3
- Smart Cycler® System/Smart Cycler® II System (Cepheid) ＊4
- Mx3000P™ (Agilent Technologies)
- Thermal Cycler Dice Real Time System *II* (TaKaRa Bio) ＊5
- Thermal Cycler Dice Real Time System *Lite* (TaKaRa Bio) ＊5

＊ 1 : TaqMan® is a registered trademark of Roche Molecular Systems Inc.
＊ 2 : ABI PRISM® is a registered trademark of Applera Corporation.
＊ 3 : LightCycler® is a registered trademark of Roche Diagnostics.
＊ 4 : Smart Cycler® is a registered trademark of Cepheid.
＊ 5 : Not available in all geographic locations. Check for availability in your area.

II. Principle

This product employs *TaKaRa Ex Taq* HS for hot start PCR. PCR products are detected with TaqMan® probes using real time monitoring.

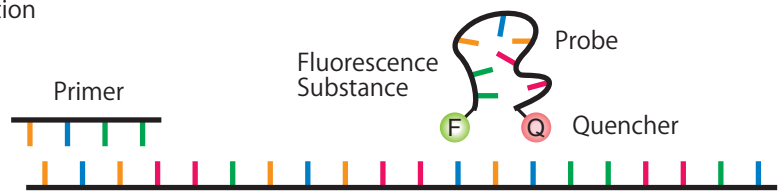
1) PCR

PCR (Polymerase Chain Reaction) is a simple and powerful method to amplify a small amount of target DNA by cycling at three different temperatures; double-stranded target DNA is heat denatured (denaturation step), two primers complementary to the target segment are annealed at low temperature (annealing step), and the annealed primers are then extended at an intermediate temperature (extension step) with thermostable DNA polymerase. As the target copy number doubles each cycle, DNA fragments can be amplified up to 10^6 -fold in a short period.
Because this reagent utilizes a hot start thermostable enzyme (*TaKaRa Ex Taq* HS), non-specific amplification due to mispriming prior to cycling or primer-dimer formation is minimized. As a result, highly specific and sensitive detection of target DNA is achieved.

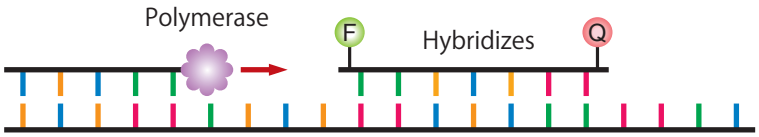
2) Fluorescence detection : TaqMan® Probe method

The TaqMan® method is based on a combination of TaqMan® Technology and a real-time PCR instrument. Oligonucleotides modified with fluorescence substance (e.g. FAM) at the 5' -end and with quencher (e.g. TAMRA) at the 3' -end are added to the reaction system. During the annealing phase of PCR, the TaqMan probe specifically hybridizes with the template DNA; however, fluorescence of the fluorophore is inhibited by the presence of the nearby quencher. During the extension phase of the PCR, the 5'-3' exonuclease activity of *Taq* DNA polymerase degrades the TaqMan probe that is hybridized to the template. This results in a release of quencher suppression and fluorescence emission. The fluorescence intensity correlates with the amount of amplified product.

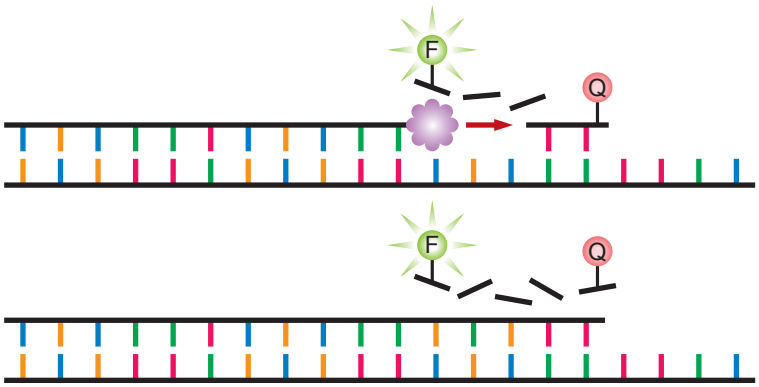
1) Heat denaturation



2) Primer annealing/Probe hybridization



3) Extension



III. Components [for 40 reactions x 50 μl PCR]

Premix Ex Taq (Perfect Real Time) (2X conc.) ＊1	1.0 ml
ROX Reference Dye (50X conc.) ＊2	40 μl
ROX Reference Dye II (50X conc.) ＊2	40 μl

＊1 : Contains *TaKaRa Ex Taq* HS, dNTP Mixture, and Mg^{2+}.

＊2 : ROX Reference Dye/Dye II is used for normalization of fluorescence intensity by background subtraction. For the ABI PRISM® 7000/7700 and Applied Biosystems® 7300 Real-Time PCR System, the use of ROX Reference Dye (50X) is recommended. For the Applied Biosystems® 7500 Real-Time PCR System, 7500 Fast Real-Time PCR System, and Mx3000P™, the use of ROX Reference Dye II is recommended. ROX reference dye is not required for the Smart Cycler®, LightCycler®, or Thermal Cycler Dice Real Time System *II* and *Lite*.

Reagents and Instruments Required but Not Supplied in this product

1. Thermal Cycler for real-time PCR (refer to page 3 for compatible instruments)
2. Reaction tubes or plates for real time PCR
3. PCR primers
4. TaqMan® probe for detection
5. Sterile distilled water
6. Micropipettes and micropipette tips (sterile, with filter)

IV. Storage

Store at 4℃ (stable for up to 6 months).

＊ Protect from light; be careful not to introduce contamination.

1. This product is shipped frozen at -20℃ .
2. This product may be frozen at -20℃ for long-term storage. Once thawed, it should be stored at 4℃ and used within 6 months.

V. Features

1) Quick and accurate detection and quantification of target gene using real-time PCR.
2) Easy-to-use 2X premix reduces pipetting steps.
3) High amplification efficiency and high detection sensitivity.

VI. Precautions for Use

Read through the following precautions prior to starting the protocol.

1) Prior to use, make sure the reagent is thoroughly mixed by gently inverting the tube several times without generating bubbles; bubbles may impair reactivity. Do not vortex.
   When stored at -20℃, *Premix Ex Taq* (2X) may precipitate. To dissolve the precipitant completely, place at room temperature (below 30℃) briefly, then invert the tube several times. The presence of precipitate is indicative of poorly mixed reagent. Make sure reagent is evenly mixed before use.

2) Place the reagent on ice immediately after it has thawed.

3) This product is not supplied with TaqMan® primers and probes.

4) Use new disposable tips to minimize potential cross-contamination between samples when preparing reaction mixtures or dispensing aliquots.

5) *TaKaRa Ex Taq* HS in this premix is a hot start PCR enzyme that contains an anti-*Taq* antibody that inhibits polymerase activity. Do not perform the pre-PCR incubation (5 - 15 min. at 95℃) that is required for other companies' chemically modified hot start PCR enzymes. Prolonged denaturation may inactivate the enzyme, affecting amplification efficiency and quantification accuracy.

   For the initial denaturation step, incubation at 95℃ for 30 sec. is generally sufficient.

VII. Protocol

A) General Overview of PCR Conditions

Initial denaturation

Step	Temperature	Time	Detection	Comment
Initial denaturation	95℃	30 sec.	Off	In most cases, initial denaturation at 95℃ for 30 sec. is sufficient even for difficult to denature templates such as circular plasmids and genomic DNAs. This procedure may be extended to 1 - 2 min. at 95℃ depending on template condition. Prolonged denaturation may inactivate the enzyme. Therefore, do not perform denaturation for more than 2 min.

Shuttle PCR (2 step PCR) number of cycles: 30 - 45 cycles

Step	Temperature	Time	Detection	Comment
Denaturation	95℃	3 - 5 sec.	Off	In general, real-time PCR amplification products do not exceed 300 bp. Therefore, denaturation at 95℃ for about 3 - 5 sec. is usually sufficient.
Annealing/ extension	56 - 64℃	20 - 30 sec. (31, 34 sec.)＊	On	Please try the Standard Protocol (shuttle PCR, 2-step PCR) first. When optimizing reaction conditions, evaluate results using an annealing/extension temperature between 56℃ and 64℃. If poor reactivity is observed, increasing incubation time for this step may improve results.

＊: Some Life Technologies instruments do not allow a detection-step setting of less than 30 sec.
ABI PRISM® 7700 allows a setting of 30 sec. or longer.
ABI PRISM® 7000 and Applied Biosystems® 7300 allow a setting of 31 sec. or longer.
Applied Biosystems® 7500 allows a setting of 34 sec. or longer.

B) Protocol for ABI PRISM® 7000/7700 or Applied Biosystems® 7300/7500/7500 Fast Real-Time PCR System

1. Prepare PCR reaction mixture.

   < per reaction >

Reagents	Volume	Volume	Final conc.
Premix Ex Taq (2X)	10 μl	25 μl	1X
PCR Forward Primer (10 μM)	0.4 μl	1 μl	0.2 μM ＊1
PCR Reverse Primer (10 μM)	0.4 μl	1 μl	0.2 μM ＊1
TaqMan® probe	0.8 μl	2 μl	＊2
ROX Reference Dye or Dye II (50X) ＊3	0.4 μl	1 μl	1X
template	2 μl	4 μl	＊4
dH_2O	6 μl	16 μl	
Total	20 μl ＊5	50 μl ＊5	

＊1 : The final concentration of primers should be 0.2 μM for most reactions. If this does not work, determine the optimal concentrations within the range 0.1 - 1.0 μM.

＊2 : The probe concentration will differ depending on the specific real-time PCR instrument and the type of fluorescent label. The amount necessary should be determined by referring to the operation manual of instrument and the product insert supplied with probe.

＊3 : The ROX Reference Dye/Dye II is intended for normalization of fluorescent signal intensities among wells when used with real-time PCR instruments that have this option. For the ABI PRISM® 7000/7700 and Applied Biosystems® 7300 Real-Time PCR System, ROX Reference Dye (50X) is recommended. For the Applied Biosystems® 7500 Real-Time PCR System and 7500 Fast Real-Time PCR System, ROX Reference Dye II is recommended.

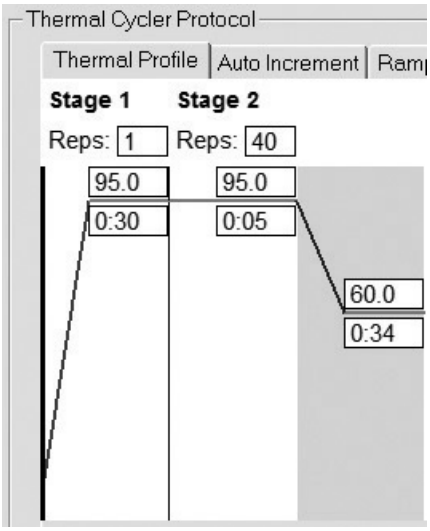
＊4 : The final template concentration varies depending on the copy number of target DNA present in the template solution. The optimal amount should be determined by preparing a dilution series. DNA templates should be used in amounts less than 100 ng per 20 μl reaction. When the cDNA (reverse transcription solution) is used as a template, it should be added in a volume that is less than 10% of the total volume of the PCR reaction mixture.

＊5 : Adjust according to the recommended volume for each apparatus.

2. Start the reaction.

   The shuttle PCR standard protocol is recommended. Try this protocol first, then optimize the reaction conditions if needed. (Refer to "General Overview of PCR Conditions" on page 6.)

[ABI PRISM® 7000/7700, 7300/7500 Real-Time PCR System]



Shuttle PCR Standard Protocol

Stage 1 : Initial denaturation
Reps : 1
95℃ 30 sec.
Stage 2 : PCR
Reps : 40
95℃ 5 sec.
60℃ 30 sec. (31sec., 34sec.) ＊

＊: 30 sec. with the ABI PRISM® 7700, 31 sec. with the ABI PRISM® 7000 and Applied Biosystems® 7300 Real-Time PCR System, and 34 sec. with the 7500 Real-Time PCR System.

[7500 Fast Real-Time PCR System]

Shuttle PCR Standard Protocol

Holding Stage
Reps：1
95℃ 30 sec.
Cycling Stage
Number of Cycles : 40
95℃ 3 sec.
60℃ 30 sec.

Note : This product combines the high performance of *TaKaRa Ex Taq* HS, which is an enzyme for hot start PCR that uses a *Taq* antibody. Initial denaturation prior to PCR should be at 95℃ for 30 sec. There is no need for incubation at 95℃ for (5 -) 15 min. as the initial denaturation, such as required for chemically modified *Taq* polymerases. Excessive heat treatment can decrease enzyme activity, and the amplification efficiency and accuracy in quantification can also be affected.

3. After reaction completion, verify the amplification curves. Establish the standard curve when absolute quantification is performed.
   Refer to the instruction manual for the real-time PCR instrument used for specific analysis methods.

C) Protocol for LightCycler® Systems

1. Prepare the following PCR reaction mixture.

<per reaction>

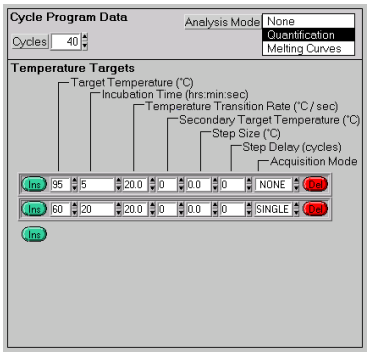
Reagents	Volume	Final conc.
Premix Ex Taq (2X)	10 μl	1X
PCR Forward Primer (10 μM)	0.4 μl	0.2 μM＊1
PCR Reverse Primer (10 μM)	0.4 μl	0.2 μM＊1
TaqMan® probe	0.8 μl	＊2
template (<100 ng)	2 μl	＊3
dH_2O	6.4 μl	
Total	20 μl	

＊1 : The final concentration of primers should be 0.2 μM for most reactions. If this does not work, determine the optimal concentrations within the range 0.1 - 1.0 μM.

＊2 : The probe concentration will differ depending on the specific real-time PCR instrument and the type of fluorescent label. The amount necessary should be determined by referring to the operation manual of instrument and the product insert supplied with probe.

＊3 : The final template concentration varies depending on the copy number of target DNA present in the template solution. The optimal amount should be determined by preparing a dilution series. DNA templates should be used in amounts less than 100 ng. When the cDNA (reverse transcription solution) is used as a template, it should be added in a volume that is less than 10% of the total volume of the PCR reaction mixture.

2. After gently centrifuging LightCycler® capillaries, place the capillaries in the LightCycler instrument and start the reaction.
The shuttle PCR standard protocol is recommended. Try this protocol first, then optimize the reaction condition if needed. (Refer to "General Overview of PCR Conditions".)



Shuttle PCR Standard Protocol
Stage 1 : Initial denaturation
Reps : 1
95℃ 30 sec. 20℃/sec.
Stage 2 : PCR
Reps : 40
95℃ 5 sec. 20℃/sec.
60℃ 20 sec. 20℃/sec.

Note : This product combines the high performance of *TaKaRa Ex Taq* HS, which is an enzyme for hot start PCR that uses a *Taq* antibody. Initial denaturation prior to PCR should be at 95℃ for 30 sec. There is no need for incubation at 95℃ for (5 -) 15 min. as the initial denaturation, such as required for chemically modified *Taq* polymerases. Excessive heat treatment can decrease enzyme activity, and the amplification efficiency and accuracy in quantification can also be affected.

3. After reaction completion, verify the amplification curves. Establish the standard curve when absolute quantification is performed.
   Refer to the instruction manual for the real-time PCR instrument used for specific analysis methods.

D) Protocol for the Smart Cycler® II System

1. Prepare the following PCR reaction mixture.

<per reaction>

Reagents	Volume	Final conc.
Premix Ex Taq (2X)	12.5 μl	1X
PCR Forward Primer (10 μM)	0.5 μl	0.2 μM＊1
PCR Reverse Primer (10 μM)	0.5 μl	0.2 μM＊1
TaqMan® probe	1 μl	＊2
template (<100 ng)	2 μl	＊3
dH_2O	8.5 μl	
Total	25 μl	

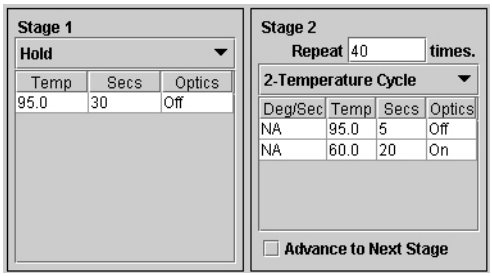
＊1 : The final concentration of primers should be 0.2 μM for most reactions. If this does not work, determine the optimal concentrations within the range 0.1 - 1.0 μM.

＊2 : The probe concentration will differ depending on the specific real-time PCR instrument and the type of fluorescent label. The amount necessary should be determined by referring to the operation manual of instrument and the product insert supplied with probe. In general, for the Smart Cycler® System, the final probe concentration should be between 0.1 and 0.5 μM.

＊3 : The final template concentration varies depending on the copy number of target DNA present in the template solution. The optimal amount should be determined by preparing a dilution series. DNA templates should be used in amounts less than 100 ng. When the cDNA (reverse transcription solution) is used as a template, it should be added in a volume that is less than 10% of the total volume of the PCR reaction mixture.

2. Gently centrifuge the reaction tubes using the centrifuge exclusively for Smart Cycler®. Load the reaction tubes in the Smart Cycler® II System and start the reaction.
   The shuttle PCR standard protocol is recommended. Try this protocol first, then optimize the reaction condition if needed. (Refer to "General Overview of PCR Conditions".)

Stage 1 : Initial Denaturation Stage 2 : PCR reaction



Shuttle PCR Standard Protocol

Stage 1 : Initial denaturation
Hold
95℃ 30 sec.
Stage 2 : PCR
Repeat : 40
95℃ 5 sec.
60℃ 20 sec.

Note : This product combines the high performance of *TaKaRa Ex Taq* HS, which is an enzyme for hot start PCR that uses a *Taq* antibody. Initial denaturation prior to PCR should be at 95℃ for 30 sec. There is no need for incubation at 95℃ for (5 -) 15 min. as the initial denaturation, such as required for chemically modified *Taq* polymerases. Excessive heat treatment can decrease enzyme activity, and the amplification efficiency and accuracy in quantification can also be affected.

3. After reaction completion, verify the amplification curves. Establish a standard curve when absolute quantification is done.
   Refer to the instruction manual for the real-time PCR instrument used for specific analysis methods.

E) Protocol for the Mx3000P™ System

1. Prepare the following PCR reaction mixture.

<per reaction>

Reagents	Volume	Final conc.
Premix Ex Taq (2X)	12.5 μl	1X
PCR Forward Primer (10 μM)	0.5 μl	0.2 μM＊1
PCR Reverse Primer (10 μM)	0.5 μl	0.2 μM＊1
TaqMan® probe	1 μl	＊2
ROX Reference Dye II (50X)＊3	0.5 μl	1X
template (<100 ng)	2 μl	＊4
dH_2O	8.0 μl	
Total	25 μl	

＊ 1 : The final concentration of primers should be 0.2 μM for most reactions. If this does not work, determine the optimal concentrations within the range 0.1 - 1.0 μM.

＊ 2 : The probe concentration will differ depending on the specific real-time PCR instrument and the type of fluorescent label. The amount necessary should be determined by referring to the operation manual of instrument and the product insert supplied with probe.

＊ 3 : For Mx3000P™, ROX Reference Dye II is recommended.

＊ 4 : The final template concentration varies depending on the copy number of target DNA present in the template solution. The optimal amount should be determined by preparing a dilution series. DNA templates should be used in amounts less than 100 ng. When the cDNA (reverse transcription solution) is used as a template, it should be added in a volume that is less than 10% of the total volume of the PCR reaction mixture.

2. Start the reaction.

   The shuttle PCR standard protocol is recommended. Try this protocol first, then optimize the reaction conditions if needed. (Refer to "General Overview of PCR Conditions".)

   Shuttle PCR Standard Protocol

   Stage 1 : Initial denaturation
   Reps : 1
   95℃ 30 sec.
   Stage 2 : PCR
   Reps : 40
   95℃ 5 sec.
   60℃ 20 sec.

Note : This product combines the high performance of *TaKaRa Ex Taq* HS, which is an enzyme for hot start PCR that uses a Taq antibody. Initial denaturation prior to PCR should be at 95℃ for 30 sec. There is no need for incubation at 95℃ for (5 -) 15 min. as the initial denaturation, such as required for chemically modified Taq polymerases. Excessive heat treatment can decrease enzyme activity, and the amplification efficiency and accuracy in quantification can also be affected.

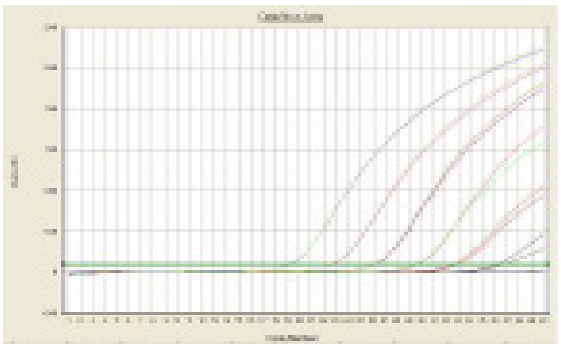
3. After reaction completion, verify the amplification curves. Establish a standard curve when absolute quantification is done.
   Refer to the instruction manual for the real-time PCR instrument used for specific analysis methods.

VIII. Application Example

Applied Biosystems® 7500 Fast Real-Time PCR System

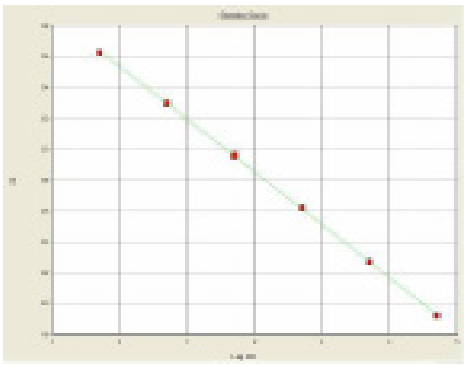
Target : Mouse Gapd
Primer/Probe : TaqMan® Gene Expression Assays (Life Technologies)
Template : cDNA serial dilution (corresponding to total RNA 500 fg - 50 ng)

Amplification curve



95℃ 30 sec.
↓
95℃ 3 sec. ┐
60℃ 25 sec. ┘ 45 cycle

Standard curve



R^2 = 0.9998
Slope = -3.417

IX. Related Products

Premix Ex Taq™ (Probe qPCR) (Cat. #RR390A/B)
PrimeScript™ RT reagent Kit (Perfect Real Time) (Cat. #RR037A/B)
PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Cat. #RR047A/B)
PrimeScript™ RT Master Mix (Perfect Real Time) (Cat. #RR036A/B)
One Step PrimeScript™ RT-PCR Kit (Perfect Real Time) (Cat. #RR064A/B)
SYBR® *Premix Ex Taq* ™ II (Tli RNaseH Plus) (Cat. #RR820A/B)
SYBR® *Premix Ex Taq* ™ (Tli RNaseH Plus) (Cat. #RR420A/B)
One Step SYBR® PrimeScript™ RT-PCR Kit II (Perfect Real Time) (Cat. #RR086A)
Thermal Cycler Dice™ Real Time System *II* (Cat. #TP900/TP960)＊
Thermal Cycler Dice™ Real Time System *Lite* (Cat. #TP700/TP760)＊

＊: Not available in all geographic locations. Check for availability in your area.

NOTICE TO PURCHASER: LIMITED LICENSE

[P7] PCR Notice
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[L15] Hot Start PCR
Licensed under U.S. Patent No. 5.338,671 and 5,587,287, and corresponding patents in other countries.

[L52] Rox Reference Dye (Research Field)
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[M57] LA Technology
This product is covered by the claims 6-16 outside the U.S. corresponding to the expired U.S. Patent No. 5,436,149.

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.

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