Cat. # RR023A

For Research Use

TaKaRa

*Bca*BEST™ RNA PCR Kit Ver.1.1

Product Manual

v202108Da

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I. Description

PCR (Polymerase Chain Reaction) is a simple and powerful method that allows *in vitro* amplification of DNA fragments through a succession of three incubation steps at different temperatures. In principle, PCR can be used to amplify DNA segments, but not directly amplify RNA. However, synthesis of cDNA from RNA using reverse transcriptase enables PCR to be applied to RNA analysis. The method, has been used for structural analysis of RNA, efficient cDNA cloning, analysis of gene expression at the RNA level, etc.
The *Bca*BEST RNA PCR Kit is designed to perform reverse transcription of RNA to cDNA using *Bca*BEST Polymerase and subsequent PCR amplification using *Bca-Optimized*™ *Taq* all in a single tube. *Bca*BEST Polymerase is isolated from *Bacillus caldotenax* and has both DNA polymerase and reverse transcriptase activity. *Bca-Optimized Taq* has been designed by Takara Bio based on LA Technology. Including these two specially designed enzymes, this kit contains all the necessary reagents for reverse transcription and PCR.
This kit is very useful for synthesizing cDNA from RNA that has complex secondary structure because the optimal reaction temperature for *Bca*BEST Polymerase is 65℃, higher than conventional reverse transcriptases.

II. Components (100 reactions＊1)

No.	Component	Concentration	Volume
1.	*Bca*BEST Polymerase	(22 units/μl)	50 μl
2.	RNase Inhibitor	(40 units/μl)	25 μl
3.	Random 9 mers＊2	(50 μM)	50 μl
4.	Oligo dT Primer＊2	(50 μM)	50 μl
5.	RNase Free dH_2O		1 ml
6.	*Bca-Optimized Taq*	(5 units/μl)	25 μl
7.	2X *Bca* 1st Buffer		1.25 ml x 2
8.	5X *Bca* 2nd Buffer		800 μl
9.	dNTP Mixture	(ea. 10 mM)	50 μl
10.	$MgSO_4$	(25 mM)	500 μl
11.	Control F-1 primer＊2 (upstream sense primer for Positive Control RNA)	(20 μM)	25 μl
12.	Control R-1 primer＊2 (downstream antisense primer for Positive Control RNA)	(20 μM)	25 μl
13.	Positive Control RNA＊3	(2 x 10^5 copies/μl)	25 μl

＊1 The total reaction volume; RT reaction 10 μl, PCR 50 μl
＊2 Guidelines for primer design

Primer	Sequence
Random 9 mers	5'-NNNNNNNNN-3'
Oligo dT Primer	The original Oligo dT primer designed by Takara Bio. This primer does not include the region complementary to M13 Primer M4, and is different from Oligo dT Adaptor Primer which is supplied in TaKaRa RNA PCR™ Kit (AMV) Ver.3.0 (Cat. #RR019A/B) and TaKaRa RNA LA PCR™ Kit (AMV) Ver.1.1 (Cat .#RR012A).
Control F-1 primer	5'-CTGCTCGCTTCGCTACTTGGA-3'
Control R-1 primer	5'-CGGCACCTGTCCTACGAGTTG-3'

＊3 Positive Control RNA

The supplied control RNA is RNA transcribed *in vitro* using SP6 RNA Polymerase from the plasmid pSPTet3 containing tetracycline resistance gene from pBR322 down-stream of SP6 promoter. (insert size: approximately 1.4 kb)

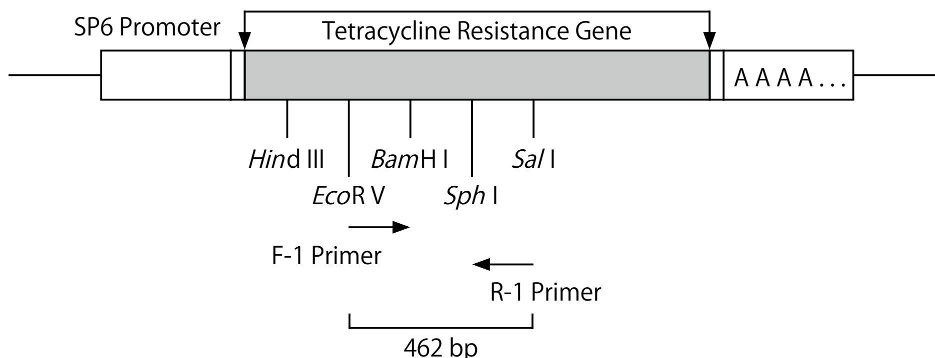


Figure. 1 Amplified DNA fragment using Positive Control RNA and control primers

III. Materials Required but not Provided

- Agarose gel
  e.g., Agarose L03「TAKARA」(Cat. #5003/5003B)
  PrimeGel™ Agarose LE 1-20K GAT (Cat. #5801A)
  PrimeGel Agarose PCR-Sieve (Cat. #5810A)
- Authorized thermal cyclers (with temperature gradient function)
  e.g., TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)*
  TaKaRa PCR Thermal Cycler Dice *Touch* (Cat. #TP350)*
- Agarose gel electrophoresis apparatus
- Microcentrifuge
- Micropipettes and pipette tips (autoclaved)

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

IV. Storage -20℃

V. Principle

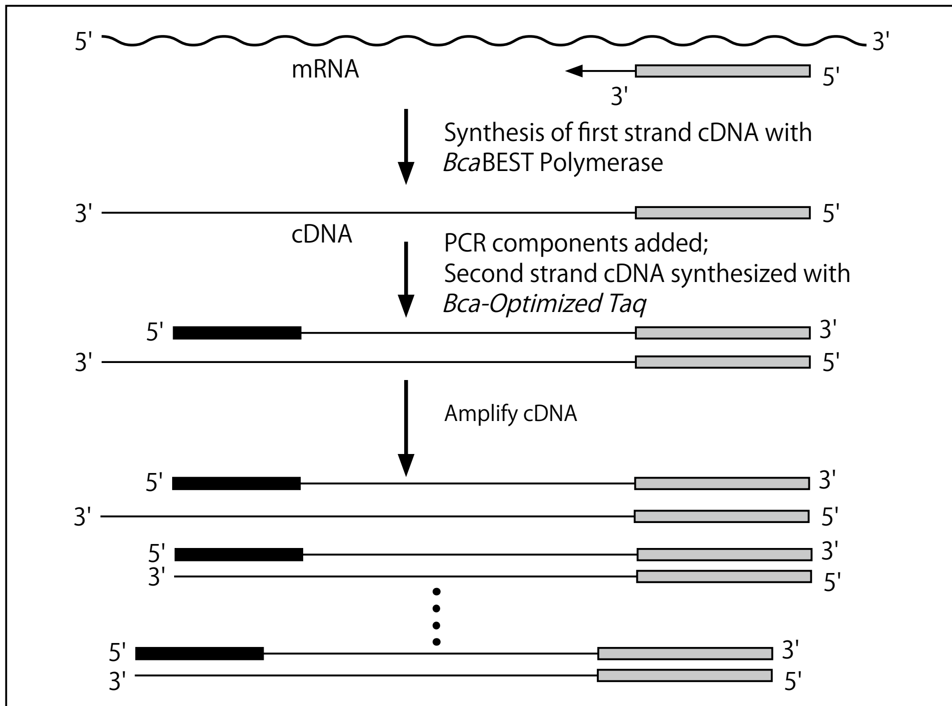


Figure 2. Schematic diagram of RNA PCR

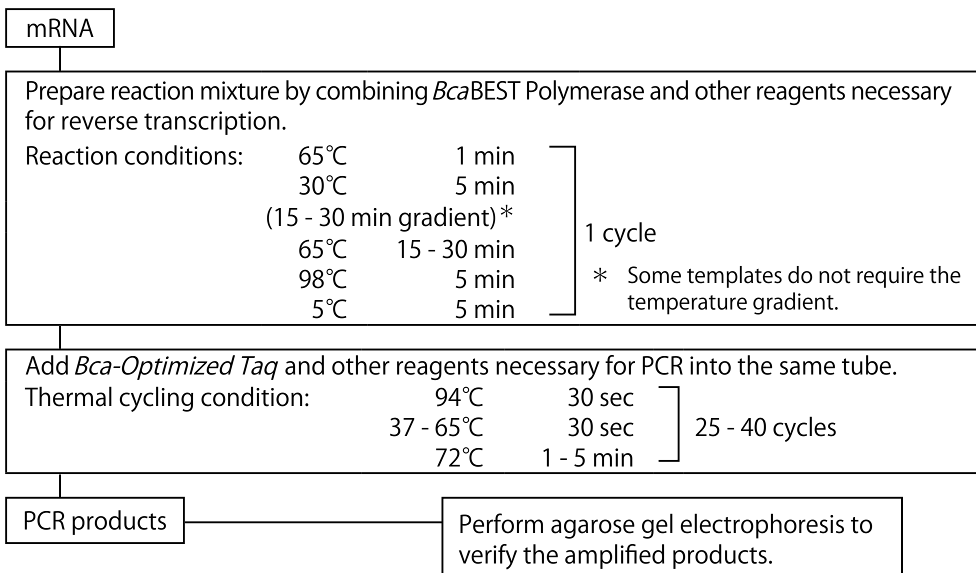


Figure 3. Flow chart of RNA PCR

This kit allows reverse transcription from RNA to cDNA using *Bca*BEST Polymerase and subsequent amplification in the same tube utilizing *Bca-Optimized Taq*.
Random 9 mers, Oligo dT Primer, or a specific downstream primer that acts as an anti-sense primer in PCR can be used for cDNA synthesis.

VI. Features

Template RNA	For all samples; especially effective for mRNA with high GC content or with a complex structure
Amplified Size	up to 5 kb
Reverse Transcriptase	*Bca*BEST Polymerase (Optimal reaction temperature: 65℃)
DNA Polymerase	*Bca-Optimized Taq*
RNase Inhibitor	Supplied in the kit
Primer for 1st strand cDNA synthesis	Random 9 mers, Oligo dT Primer or Specific downstream PCR primer
Protocol	Single tube reaction (RTase is heat inactivated prior to PCR)

VII. Preparation of RNA Sample

This kit is designed to perform the reverse transcription of RNA to cDNA and subsequent amplification.
The purity of the RNA sample will affect the yield of cDNA. Therefore, it is essential to inhibit RNase activity in the cells and also to prevent contamination by RNases derived from equipment and solutions used.
Extra precautions should be taken during sample preparation; wear clean disposable gloves, dedicate a work surface exclusively for RNA preparation, and avoid unnecessary talking during the procedure to prevent the contamination of RNase from operators' sweat or saliva.

A. Equipment

Disposable plastic equipment should be used. If using glass tools, treat the tools with DEPC (diethylpyrocarbonate) prior to use.

(1) Treat glass tools with 0.1% DEPC solution at 37℃ for 12 hours.
(2) Autoclave at 120℃ for 30min. to remove DEPC.

It is recommended to prepare all equipment exclusively for RNA preparation.

B. Reagent

Reagents for RNA preparation, including sterile purified water, should be prepared with heat sterilized glass tools (180℃, 60 min), or if possible tools treated with 0.1% DEPC solution and autoclaved. Reagents and sterile purified water should be exclusively used for RNA preparation.

C. Preparation method of RNA sample

Simple purification methods can yield sufficient RNA for reverse transcription and subsequent PCR. However, it is recommended to use highly purified RNA obtained by GTC (Guanidine thiocyanate) method, etc.
For extraction from tissue and cells, NucleoSpin RNA (Cat. #740955.10) or RNAiso Plus (Cat. #9108/9109) is recommended.

D. RNA Sample Amount

Approximately 500 ng of total RNA should be used per reaction.

VIII. Precautions

Read these precautions before use and follow them when using this product.

1) For both reverse transcription and PCR amplification, prepare a master mix of reagents (containing RNase-free dH_2O, buffers, dNTP Mixture, $MgSO_4$, etc) first, then aliquot to individual tubes. Using such a mixture will allow accurate dispensing of reagents, minimize reagents loss due to pipetting, and avoid repeated dispensing of the each reagent. This helps to minimize variation among experiments.

2) Enzymes such as RTase (*Bca*BEST Polymerase), *Bca-Optimized Taq*, and RNase Inhibitor should be mixed gently by pipetting. Avoid generating bubbles. Gently spin down the solution prior to mixing. Pipette enzymes carefully and slowly as the viscosity of the 50% glycerol in the enzyme solution can lead to pipetting errors.

3) Keep enzymes at -20℃ until just before use and return into the freezer promptly after use.

4) Use new disposable pipette tips to avoid contamination between samples.

5) PCR conditions
Optimal PCR conditions vary depending on the thermal cycler used. It is recommended to perform a control experiment to determine the appropriate conditions prior to using a sample.

6) Primer Selection
The primer for reverse transcription should be either Random 9 mers, Oligo dT Primer, or a specific downstream PCR primer. For short mRNAs with no hairpin structures, any one of the above three primers can be used.

[General guideline of the primer selection]

<u>Random 9 mers :</u>
Use for transcription of long RNAs or for RNAs with hairpin structures. Also can be used to reverse transcribe all RNA (rRNA, mRNA and tRNA). Any PCR primers work well in PCR for cDNA synthesized with Random 9 mers.

<u>Specific downstream PCR primer (anti-sense primer in PCR) :</u>
Use for the target RNAs for which the sequence is already determined.

<u>Oligo dT Primer :</u>
Use only for mRNAs with polyA tails (Note: prokaryotic RNA, eukaryotic rRNA and tRNA, and some eukaryotic mRNA do not have polyA tails)
This primer was designed originally by Takara Bio for efficient cDNA synthesis.

IX. Protocol

Typical RT-PCR example

A. Reverse Transcription

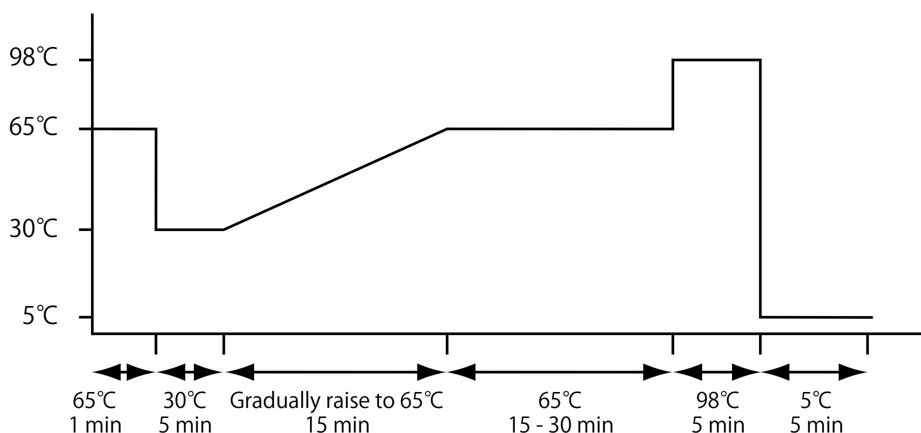
1. Prepare the reaction mixture in a tube shown below. The primer for a cDNA synthesis should be either Random 9 mers, Oligo dT Primer, or a specific downstream PCR primer (Control R-1 primer for the control experiment). See "VIII-6) Primer Selection" for selecting the primer to use.

Reagent	Amount	Final conc.
2X *Bca* 1st Buffer	5 μl	1X
25 mM $MgSO_4$	2 μl	5 mM
dNTP Mixture (10 mM each)	0.5 μl	0.5 mM
RNase Inhibitor (40 units/μl)	0.25 μl	1 unit/μl
*Bca*BEST Polymerase (22 units/μl)	0.5 μl	1.1 units/μl
Oligo dT Primer or Random 9 mers or Specific downstream PCR primer	0.5 μl	
Positive Control RNA or Experimental Sample*1	0.5 μl	[1 x 10^5 copies] or [≦ 500 ng total RNA]
RNase Free dH_2O	0.75 μl	
Total	10 μl per sample	

*1 When analyzing low expression RNAs, the volume can be increased up to 1.25 μl.

2. Place all tubes in a thermal cycler and perform the reaction with the program as below*2.

*2 For the Positive Control RNA, the temperature gradient is performed over 15 min, and incubation time at 65℃ is 15 min.



B. PCR

Two kinds of reaction mixtures are available for this kit, A and B.
Method A is used for general reactions. If an amplification is not successful with method A method, B method is recommended.

1. Prepare the reaction mixture shown below. (**A method**)

Reagent	Amount	Final conc. in PCR (per 50 μl mixture)
$MgSO_4$ (25 mM)	3 μl	2.5 mM
5X *Bca* 2nd Buffer	8 μl	1X
Bca-Optimized Taq	0.25 μl	1.25 units/50 μl
Upstream sense PCR Primer (20 μM) (F-1 Primer for Control RNA)	0.5 μl	0.2 μM
Downstream antisense PCR Primer (20 μM)* (R-1 Primer for Control RNA)	0.5 μl	0.2 μM
Sterile purified water	27.75 μl	
Total	40 μl per sample	

B method

In case that an amplified fragment cannot be obtained with the method above (A), it is recommended to perform PCR again by preparing a reaction mixture with the following composition. This reaction mixture is especially effective for amplification of templates with complex structure.

Reagent	Amount	Final conc. in PCR (per 50 μl mixture)
$MgSO_4$ (25 mM)	3 μl	2.5 mM
2X *Bca* 1st Buffer	20 μl	1X
Bca-Optimized Taq	0.25 μl	1.25 units/50 μl
Upstream sense PCR Primer (20 μM) (F-1 Primer for Control RNA)	0.5 μl	0.2 μM
Downstream antisense PCR Primer (20 μM)* (R-1 Primer for Control RNA)	0.5 μl	0.2 μM
Sterile purified water	15.75 μl	
Total	40 μl per sample	

* When the downstream PCR primer is used for reverse transcription, add 0.5 μl of sterile purified water instead of downstream primer.

2. Add 40 μl of the above mixture into the tube containing the cDNA solution obtained at step A.
3. Spin for approximately 10 seconds with a microcentrifuge.
4. Place the tubes in a Thermal Cycler and perform amplification under the optimal condition.

General PCR condition

94℃	30 sec	25 - 30 cycles
37 - 65℃	30 sec	
72℃	1 - 10 min	
72℃	5 min	

PCR condition for Positive Control RNA

94℃	30 sec	28 cycles
60℃	30 sec	
72℃	1 min	

5. After the amplification is complete, use 5 - 10 μl of the reaction for agarose gel electrophoresis to verify the amplified DNA fragments. The PCR amplified product can frozen until subsequent analysis.

【 Amplified DNA size when using the supplied Positive Control RNA 】

Primer for reverse transcription	PCR Primers	Amplified fragment
Oligo dT Primer	F-1, R-1	462 bp
Random 9 mers	F-1, R-1	462 bp
R-1 Primer	F-1, R-1	462 bp

PCR Conditions

1) Annealing Temperature:
60℃ is optimal for amplification of control RNA. It may be necessary to lower or raise the annealing temperature for RNA samples. (The optimal temperature need to be determined empirically by testing temperatures within the range of 37℃ to 65℃).
2) Extension time:
The length of the target sequence will affect the required extension time. Typically, *Bca-Optimize Taq* has an extension rate of 1 - 2 min/1 kb at 72℃.
3) Cycle Number:
When small volume of cDNA is used, 30 - 50 cycles are required for PCR amplification.

X. References

1) Kawasaki, E. S. and Wang, A. M. PCR Technology (Erlich, H. A. ed.). *Stockton Press*. (1989) 89-97.
2) Lynas, C., Cook, S. D., Laycock, K. A., Bradfield, J. W. B., and Maitland, N. J. *J Pathology*. (1989) **157**: 285-289.
3) Frohman, M. A., Dush, M. K., and Martin, G. R. *Proc. Natl Acad Sci USA*. (1988) **85**: 8998-9002.

XI. Related Products

TaKaRa PCR Thermal Cycler Dice™ *Touch* (Cat. #TP350)＊
TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)＊
NucleoSpin RNA (Cat. #740955.10/.50/.250)
RNAiso Plus (Cat. #9108/9109)
RNase-OFF® (Cat. #9037)
Agarose L03「TAKARA」(Cat. #5003/5003B)

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

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