Cat. # 6121

For Research Use

TaKaRa

3'-Full RACE Core Set

Product Manual

v201210Da

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I. Description

3'-Full RACE Core Set (20 reactions)

RNA can be analyzed by cloning and sequencing after target region was amplified through RT-PCR. However, in essence, there are many difficulties in obtaining full-length cDNA clones from mRNA. RACE (rapid-amplification of cDNA Ends) method is effective to overcome these difficulties because mRNA is reverse-transcribed with a gene-specific primer and cDNA is modified at the 3' and 5' end with homo-oligonucleotide synthesized with this procedure.

TaKaRa 3'-Full RACE Core Set is a core set designed to specifically amplify the region which contains 3'-end of target mRNA utilizing 3'RACE procedure. The supplied Oligo dT-3 sites Adaptor Primer is specially designed for efficient cDNA synthesis from 3'-end of polyA$^+$ mRNA. Also as the supplied 3 sites Adaptor Primer contains restriction sites of *Bam*H I, *Kpn* I, and *Xba* I within its sequence, cloning after RT-PCR can be easily achieved. In this case, it is recommended to add the sequence (which is shown as below) at the 5' side of the upstream specific primer.

Xba I *Kpn* I *Bam*H I

5'-CTGATCTAGAGGTACCGGATCC-3'

This set is optimized for the use with *TaKaRa Taq*, or *TaKaRa Ex Taq*, *TaKaRa LA Taq*.

＊ U.S. Patent 5,436,149 for LA Technology is owned by TAKARA BIO INC.

II. Components

1.	AMV Reverse Transcriptase XL ＊1 (originated from Avian MyeloblastosisBacillus caldtenax)	(5 U/μl)	20 μl
2.	RNase Inhibitor	(40 U/μl)	10 μl
3.	Oligo dT-3 sites Adaptor Primer ＊2	(2.5 μM)	20 μl
4.	RNase Free dH_2O		500 μl
5.	3sites Adaptor Primer ＊2	(20 μM)	20 μl
6.	10X RNA PCR Buffer (100 mM Tris-HCl (pH8.3), 500 mM KCl)		40 μl
7.	dNTP Mixture	(ea. 10 mM)	40 μl
8.	$MgCl_2$	(25 mM)	80 μl
9.	Control F-1-3sites Adaptor Primer ＊2 (upstream primer for Positive Control RNA)	(20 μM)	10 μl
10.	Positive Control RNA ＊3 (Transcribed polyA$^+$ mRNA of pSPTet3 plasmid)	(2 x 10^5 copies/μl)	10 μl

＊ 1: Manufactured by Life Sciences, Co.

＊ 2 : Primer Sequences

- Oligo dT-3sites Adaptor Primer :
  This primer was designed originally by Takara to have dT region and the restriction sites of *Bam*H I, *Kpn* I, and *Xba* I.
- 3sites Adaptor Primer :
  5'-CTGATCTAGAGGTACCGGATCC-3'
- Control F-1-3sites Adaptor Primer :
  5'-CTGATCTAGAGGTACCGGATCCATATCGCCGACATCACCGATG-3'

＊ 3 : Positive Control RNA

Supplied control RNA is *in vitro* transcribed RNA using SP6 RNA polymerase from plasmid pSPTet3 inserted with DNA fragment (approximately 1.4 kb) having tetracycline resistant gene, originated from pBR322, in the downstream of SP6 promoter.

This control RNA is a polyA$^+$ mRNA containing 30 bases of polyA$^+$ at the tail. When full-length double-stranded cDNA is synthesized from this control RNA, tetracycline resistant plasmid is obtained by inserting this cDNA.

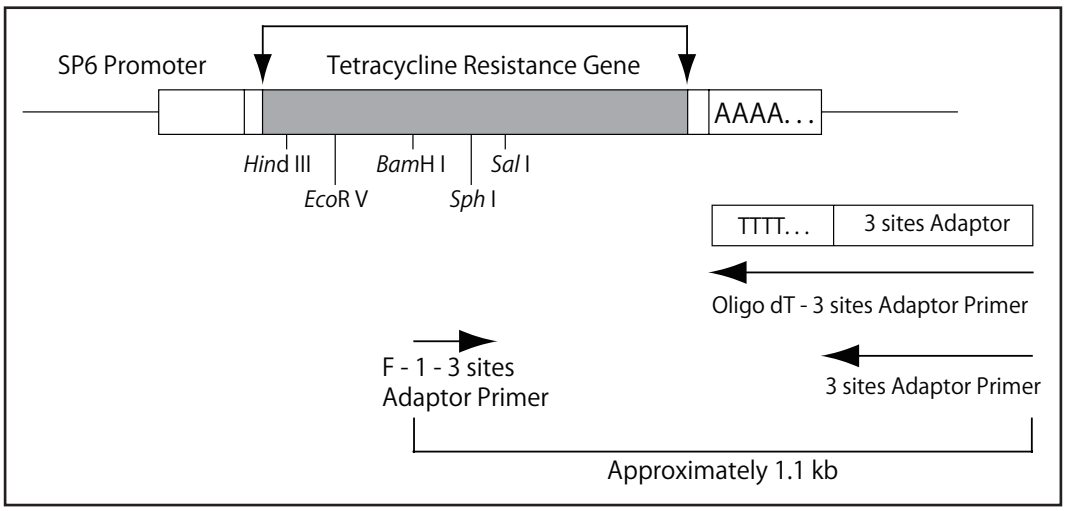


Fig. 1 Amplified DNA fragment using control RNA and control primers

III. Reagents not supplied in the Set

1. Upstream primer specific to the known region
2. DNA polymerase for PCR

IV. Equipment required

1. Authorized thermal cycler
2. Microcentrifuge tube (made of polypropylene)
3. Agarose gel electrophoresis appratus
4. Microcentrifuge
5. Micropipets and pipette tips (autoclaved)

V. Storage

-20℃

VI. Principle

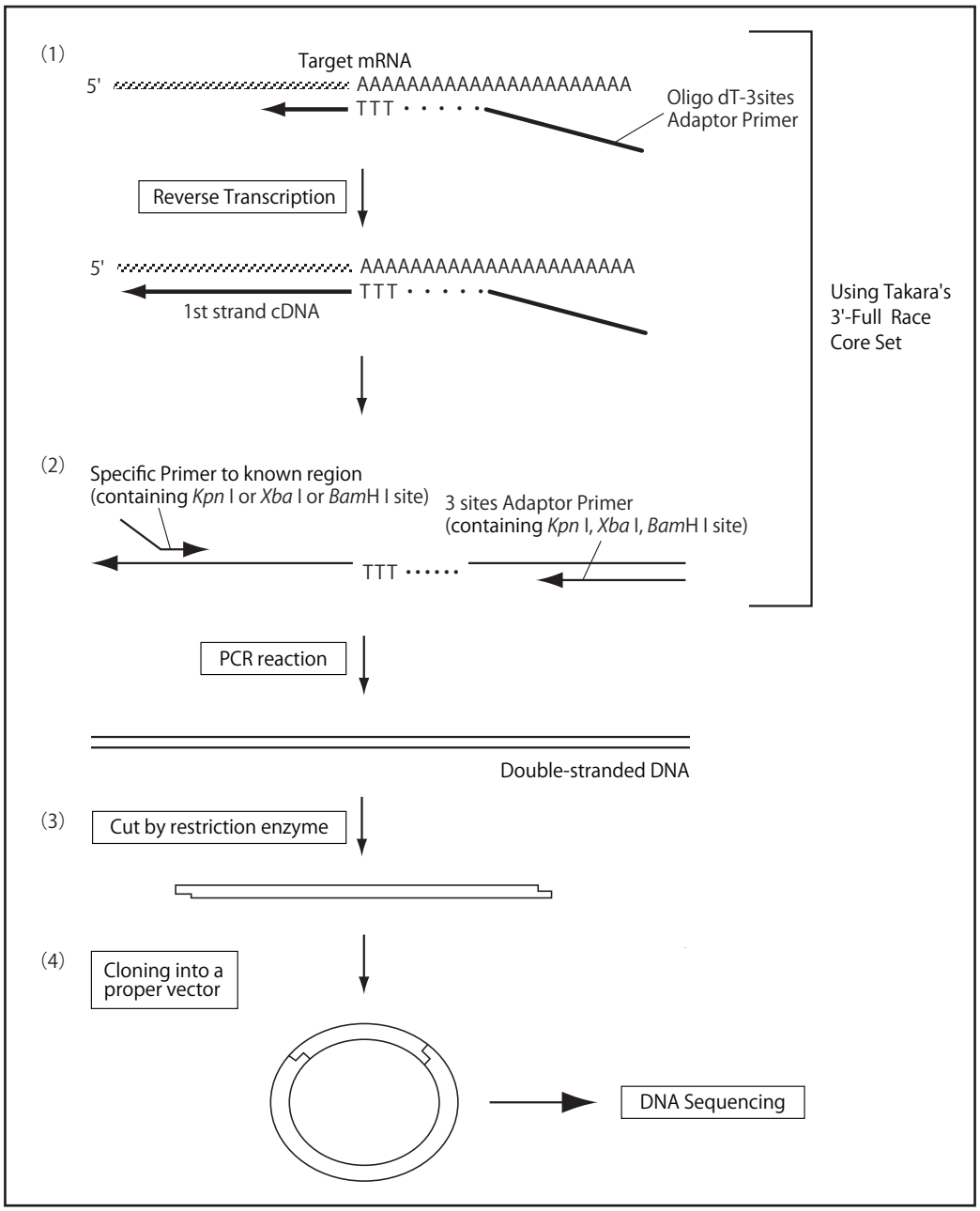


Fig.2 Schematic diagram of 3'RACE using Takara 3'RACE Core Set

(1) Synthesize 1st strand cDNA by reverse transcription of target mRNA with Oligo dT-3 sites Adaptor Primer.
(2) Perform PCR with a gene-specific primer (not supplied) and 3 sites Adaptor Primer.
(3) Cut PCR products with an appropriate restriction enzyme.
(4) Clone the obtained DNA fragments into a proper vector and perform DNA sequencing.

VII. Preparation of RNA sample

TaKaRa 3'-Full RACE Core Set is designed to perform cDNA synthesis from 3'end of mRNA. The purity of RNA sample will affect the yield of cDNA synthesis. So it is essential to inhibit the activity of RNase in the cells and also to prevent the contamination of RNase derived from equipments and solutions used.
Extra precautions should be taken during the sample preparation; put on clean disposable gloves, dedicate a table to exclusive use for RNA preparation, and avoid unnecessary talks during the operations to prevent the contamination of RNase from operators' sweat or saliva.

A. Equipment

Disposable plastic equipments shall be used. In case using glass tools, treat the glass tools with DEPC (diethylpyrocarbonate) prior to use.

(1) Treat glass tools with 0.1% DEPC solution at 37℃, 12 hours.
(2) Autoclave at 120℃, 30 min., to remove DEPC.
It is recommended to prepare all the equipments as the exclusive use for RNA preparation.

B. Reagent

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

C. Preparation method

The RNA isolation kits such as RNAiso Plus (Cat. #9108/9109) also can be used for isolating high purity of total RNA. The purified RNA sample should be dissolved in sterilized distilled water or sterilized TE buffer at process of RNA isolation lastly.

D. RNA Sample Amount

Approximately 1 μg of total RNA is appropriate per one reaction.

VIII. Note

1) Enzymes, such as RTase and RNase Inhibitor, shall 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 buffer can lead to pipetting errors.

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

3) It is recommended to store Positive Control RNA by dispensing into several tubes in a required small amount. Avoid repeated freeze-thaw cycles to prevent degradation of RNA. It is advisable to store at -70 to -80℃ when deep freezer is available.

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

IX. Protocol

1. General Protocol

A. Reverse Transcription

1. Prepare the reaction mixture in a tube by combining the reagents in the proportions shown.

Reagents	Volume	Final concentration
10X RNA PCR Buffer	2 μl	1 X
$MgCl_2$ (25 mM)	4 μl	5 mM
dNTP Mixture (ea. 10 mM)	2 μl	1 mM
AMV Reverse Transcriptase XL (5 U/μl)	1 μl	0.25 U/μl
RNase Inhibitor (40 U/μl)	0.5 μl	1 U/μl
Oligo dT-3sites Adaptor Primer (2.5 μM)	1 μl	0.125 μM
Positive Control RNA (2 x 10^5 copies/μl)	1 μl	
or Experimental Sample ＊1 [total RNA : 1 μg/μl]		
RNase Free dH_2O	8.5 μl	
Total volume	20 μl per sample	

＊1 : When using low expression sample RNA, the volume can be increased up to 9.5 μl.

2. Overlay mineral oil (50 - 100 μl) to avoid the evaporation of the reaction mixture. (Some thermal cyclers do not require mineral oil.)

3. Place all tubes in a Thermal Cycler and set the parameters by the following condition.

30℃	10 min.	1 cycle
50℃	15 - 30 min.	
95℃	5 min.	
5℃	5 min.	

B. PCR reaction

This set is optimized for the use with *TaKaRa Taq* (Cat. #R001A), or *TaKaRa Ex Taq* (Cat. #RR001A), *TaKaRa LA Taq* (Cat. #RR002A), With *TaKaRa Taq* (Cat. #R001A), enough efficiency can be obtained. When amplifying long target cDNA or when higher efficiency should be required, the use of *TaKaRa Ex Taq* or *TaKaRa LA Taq* is recommended for better results.

1. Prepare reaction mixture by combining the following reagents.

◆ In case of using *TaKaRa Taq* (Cat. #R001A)

Reagents	Volume	Final conc. in PCR reaction (per 50 μl mixture)
10X PCR Buffer (Mg^{2+} free) (supplied with *TaKaRa Taq*)	4 μl	0.8 X
$MgCl_2$ (25 mM)	3 μl	2.5 mM
TaKaRa Taq (5 U/μl)	0.25 μl	0.025 U/μl
Upstream PCR Primer (20 μM) (Control F-1 3sites Adaptor Primer for Control RNA)	0.5 μl	0.2 μM
3 sites Adaptor Primer(20 μM)	0.5 μl	0.2 μM
RT reactant	10 μl	
Sterilized distilled water	31.75 μl	
Total volume	50 μl	

Note : No need to add further dNTP Mixture in PCR reaction when using *TaKaRa Taq*.

◆ In case of using *TaKaRa Ex Taq* (Cat. #RR001A) or *TaKaRa LA Taq* (Cat. #RR002M)

Reagents	Volume	Final conc. in PCR reaction (per 50 μl mixture)
10X *Ex Taq* Buffer (Mg^{2+} free) ＊1 or 10X LA PCR Buffer (Mg^{2+} free)＊1	5 μl	1X
dNTP Mixture (2.5 mM)	8 μl	0.6 mM
$MgCl_2$ (25 mM)	3 μl	2.5 mM
TaKaRa Ex Taq (5 U/μl) or *TaKaRa LA Taq* (5 U/μl)	0.25 μl	0.025 U/μl
Upstream PCR Primer (20 μM) ＊2	0.5 μl	0.2 μM
3sites Adaptor Primer (20 μM)	0.5 μl	0.2 μM
Obtained RT reactant	10 μl	
Sterilized distilled water	25.75 μl	
Total volume	50 μl per sample	

＊1 : When 10X *Ex Taq* Buffer (Mg^{2+} free) is used, add 4 μl of 25 mM $MgCl_2$.
When 10X LA PCR Buffer II (Mg^{2+} free) is used, add 5 μl of 25 mM $MgCl_2$. In these case, decrease the volume of sterilized distilled water to adjust the reaction volume to 50 μl.

＊2 : Control F-1-3 sites Adaptor Primer is used for Positive Control RNA.

2. Place the tubes in a Thermal Cycler and perform ampification under the following condition ＊2.

94℃	30 sec.	┐
55℃	30 sec.	├ 30 cycles
72℃	2 min.	┘

＊2 : Conditions for PCR
1) Annealing Temperature :
When using control RNA , annealing is performed at 55℃. The optimal temperature needs to be determined empirically by testing temperatures within the range of 37 - 65℃ depending on used RNA samples.
2) Extension time:
The length of the target sequence will affect the required extension time. Typically, *TaKaRa Taq*, *TaKaRa Ex Taq*, and *TaKaRa LA Taq* extend DNA at 1 kb per 1 - 2 min. at 72℃.

3. After the amplification is completed, apply 5 - 10 μl of the reactant for agarose gel electrophoresis to verify the amplified DNA fragments. ＊3

＊3 : Approximately 1.1 kb of amplified fragment can be confirmed when using Positive Control RNA.

2. Application example

Cloning of 3'-region of Human Transferrin Receptor mRNA

total RNA originated from HL60 was amplified through 3'RACE method using mRNA of human transferrin receptor as a target.

a) Reverse Transcription

1. Prepare the reaction mixture in a tube by combining the reagents in the proportions shown as below.

Reagents	Volume	
10X RNA PCR Buffer	2 μl	
$MgCl_2$ (25 mM)	4 μl	
dNTP Mixture (ea. 10 mM)	2 μl	
RNase Inhibitor (40 U/μl)	0.5 μl	
AMV Reverse Transcriptase XL (5 U/μl)	1 μl	
Oligo dT 3sites Adaptor Primer (2.5 μM)	1 μl	
total RNA (originated from HL60, 1 μg/μl)	1 μl	
RNase Free dH_2O	8.5 μl	
Total volume	20 μl	per sample

2. Place the tubes in a Thermal Cycler and perform the reaction by the following condition.

30℃	10 min.	┐
50℃	30 min.	├ 1 cycle
95℃	5 min.	┘
5℃	5 min.	

b) PCR

1. Prepare reaction mixture for PCR by combining the following reagents.

Reagents	Volume	
10X LA PCR Buffer II (Mg^{2+} plus) (Supplied in TaKaRa LA PCR Kit Ver.2)	5 μl	
25 mM $MgCl_2$	5 μl	
dNTP Mixture (ea. 2.5 mM)	8 μl	
TaKaRa LA Taq (5 U/μl)	0.25 μl	
Upstream Specific Primer (20 μM)	0.5 μl	
3 sites Adaptor Primer (20 μM)	0.5 μl	
Obtained RT reactant	10 μl	
Sterilized distilled water	20.75 μl	
Total volume	20 μl	per sample

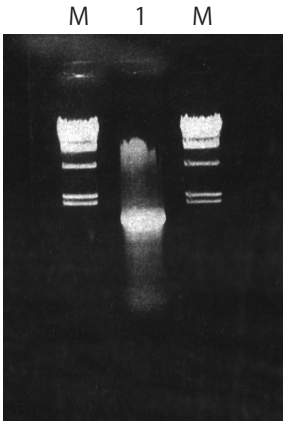
＊ The primer has the sequence containing *Bam*H I site at 5'-termini

2. Place the tubes in a Thermal Cycler and perform PCR under the following condition.

94℃ 30 sec.
55℃ 30 sec.] 30 cycles
72℃ 5 min.

c) Result

1% Agarose gel electrophoresis
(Applied volume : 8 μl/lane)



←

Lane
M : λ *Hin*d III digest
1 : 3' RACE PCR products
(Approx. 1.5 kp)

d) Recovery of PCR products

PCR products were purified with NucleoSpin® Gel and PCR Clean-up (Cat. #740609.10/.50/.250).

e) Cut by restrictin enzyme

PCR products were cut by *Bam*H I (Cat. #1010) to be subcloned into pUC118 DNA digested with *Bam*H I, dephosphorylated with alkaline phosphatase (Cat. #3321).

Prepare the reaction mixture by combining the followings.
Incubate at 30℃ for 1 hour.

PCR products (Amplified DNA (0.3 μg/μl)	2 μl
*Bam*H I (10 U/μl)	1 μl
10X Buffer K (Supplied with *Bam*H I)	5 μl
Sterilized distilled water	42 μl
Total volume	50 μl

f) Ligation

Using DNA Ligation Kit Ver.2.1 (Cat. #6022), DNA fragment (Approx. 1.5 kp) digested with *Bam*H I was ligated into pUC118 DNA digested with *Bam*H I, dephosphorylated with alkaline phosphatase (Cat. #3321).

Prepare the reaction mixture by combining the followings.
Incubate at 16℃ for 1 hour.

DNA fragment digested with *Bam*H I	6.5 μl (200 ng)
pUC118 DNA digested with *Bam*H I, dephosphorylated with alkaline phosphatase	0.5 μl (50 ng)
Enzyme Solution I [Supplied in DNA Ligation Kit Ver.2.1(Cat. #6022)]	7 μl
Total volume	14 μl

g) Transformation

The ligation reaction solution was transformed into *E. coli* JM109 Competent Cells (Cat. #9052). The reactant was plated on L-plate containing ampicilin, IPTG, and X-Gal. The medium was cultured at 37℃ for 18 hours. The white colonies were picked up.

The colonies which was confirmed to have the inserts were cultured on L-medium and the plasmid was collected. Through sequence analysis, it is confirmed that the target fragment was inserted in the plasmid.

X. Related products

TaKaRa Taq™ (Cat. #R001A/B/C)
TaKaRa Ex Taq™ (Cat. #RR001A/B/C)
TaKaRa Taq™ Hot Start Version (Cat. #R007A/B)
TaKaRa Ex Taq™ Hot Start Version (Cat. #RR006A/B)
TaKaRa LA Taq™ (Cat. #RR002A/RR002M)
TaKaRa LA Taq™ Hot Start Version (Cat. #RR042A/B)
AMV Reverse Transcriptase XL for RT-PCR (Cat. #2630A)
Recombinant RNase Inhibitor (Cat. #2313A/B)

XI. References

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