Cat. # 6122

For Research Use

TaKaRa

5'-Full RACE Core Set

Product Manual

v201210Da

Table of Contents

Description:

5'-Full RACE Core Set (10 reactions)

RNA can be analyzed by cloning and sequencing after the target region is amplified through RT-PCR. However, there are many difficulties in obtaining full-length cDNA clones from mRNA. The RACE (Rapid-Amplification of cDNA Ends) method is effective in overcoming these difficulties. TaKaRa 5'-Full RACE Core Set is a core set designed to perform the 5'-RACE procedure. This set achieves amplification of an unknown 5'-end region of mRNA utilizing inverse PCR and allows efficient 5'-RACE. This set is optimized for the use with *TaKaRa Taq* (Cat.#R001), *TaKaRa Ex Taq* (Cat.#RR001), or *TakaRa LA Taq* (Cat. #RR002)*. All the reagents required for cDNA synthesis from RNA and for the subsequent
circularization of the obtained cDNA are supplied in this set.

Kit components:

1. AMV Reverse Transcriptase XL (5 units/μl)*1 ..... 10 μl
   (originated from Avian Myeloblastosis Virus)
2. RNase Inhibitor (40 units/μl) ..... 10 μl
3. 10X RT Buffer (containing dNTP Mix)*2 ..... 15 μl
4. RNase Free dH_2O ..... 1 ml
5. RNase H (60 units/μl) ..... 10 μl
6. 5X Hybrid RNA Degradation Buffer ..... 150 μl
7. T4 RNA Ligase (40 units/μl) ..... 10 μl
8. 5X RNA (ssDNA) Ligation Buffer*3 ..... 80 μl
9. 40% PEG#6000 ..... 200 μl
10. Positive Control RT-Primer (200 pmol/μl)*4 ..... 10 μl
11. Positive Control 1st Primer Pair (ea. 20 pmol/μl)*4 ..... 10 μl
12. Positive Control 2nd Primer Pair (ea.20 pmol/μl)*4 ..... 10 μl
13. Positive Control RNA (10 ng/μl) ..... 10 μl

＊1 Manufactured by Life Sciences, Co.
＊2 When thawing, if the solution is cloudy, keep it at room temperature until it becomes clear. It must be used after clear.
＊3 Though the solution is colored, it is no problem for the use.
＊4 Primer Sequences

・Positive Control RT-Primer:
5'-(P)AAAATGACCCAG-3'

・Positive Control 1st Primer pair:
S1: 5'-AGCGCTTGTTTCGGCGTGGGTATGGTG-3'
A1: 5'-CTGGCGATGCTGTCGGAATGGACGATA-3'

・Positive Control 2nd Primer pair:
S2: 5'-ACCTACTACTGGGCTGCTTCCTAATGC-3'
A2: 5'-TAGATTTCATACACGGTGCCTGACTGC-3'

Storage:

-20℃

Reagents not supplied in the kit:

1. DNA polymerase for PCR
   TaKaRa Taq (Cat.#R001A/B/C)
   TaKaRa Ex Taq (Cat.#RR001A/B/C)
   TaKaRa LA Taq (Cat.#RR002)
2. 5' end-phosphorylated RT-Primer (for reverse transcription)
3. 1st PCR Primer pair (for 1st PCR)
4. 2nd PCR Primer pair (for 2nd PCR)
5. Mineral Oil (if necessary)
6. Agarose gel
   ex. NuSieve™ 3:1 Agarose (Lonza)

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)

Principle

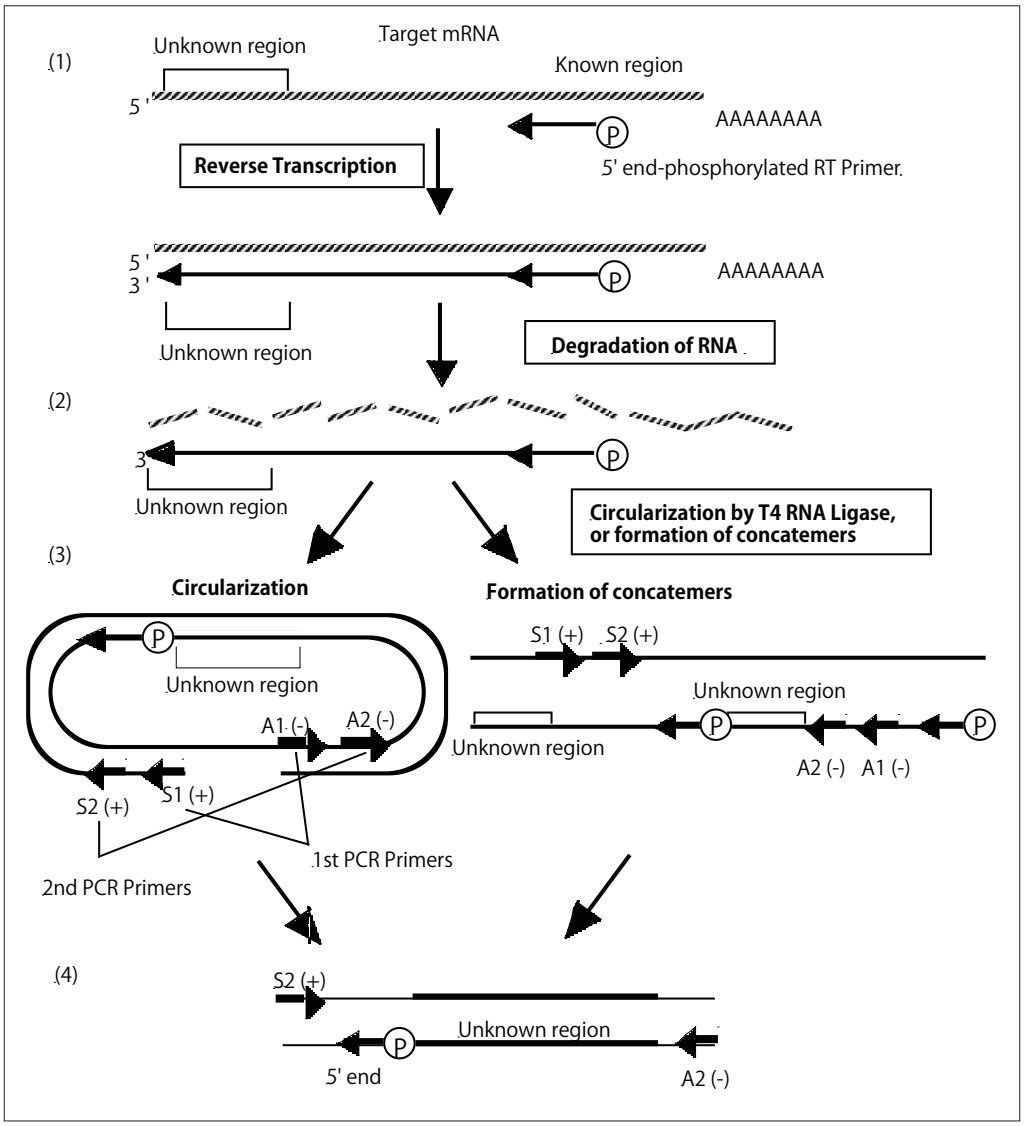


Fig.1 Schematic diagram of 5'RACE using Takara 5'-Full RACE Core Set

(1) Synthesize 1st strand cDNA by reverse transcription from target mRNA using 5' end-phosphorylated RT Primer which is specific to the target RNA.

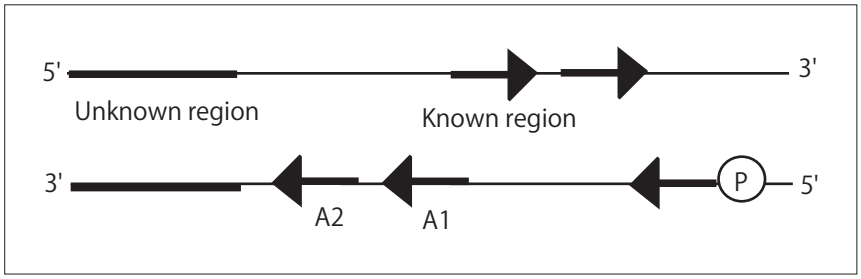
(2) Degradation of hybrid DNA-RNA to separate RNA by treatment with RNase H.

(3) Circularization of single-strand cDNA or formation of concatemers by RNA Ligase.

(4) DNA Amplification by PCR.

If possible, please design RT Primer at near the part of 5'- end. When the generated template DNA is used in PCR step, if PCR product less than 1 kbp, good reaction efficiency will be expected. If PCR products exceeding 1 kb, reaction efficiency may decrease.

Guidelines for Designing Primer:



A. RT-Primer
RT-Primer should be 12-15 nucleotides long with a 5' phosphorylated end for the subsequent ligation. It is advisable to phosphorylate the primer during its synthesis.

B. PCR Primers
The distances between S1 and S2, and between A1 and A2 do not have a substantial influence on results.

The following points should be considered in designing primers;

(1) Primers should be approximately 20 nucleotides long.

(2) Primers should have a balanced G/C and A/T content and avoid to be partially rich in G/C or A/T content. Especially 3'-end side of primers should not have high content of A/T.

(3) Primers that will not form secondary structure should be designed.

(4) Primers for 1st PCR and 2nd PCR that will not form primer dimers each other. Especially 3 or 4 nucleotides from 3'-end should not be complementary to the corresponding sequences.

Preparation of RNA sample:

TaKaRa 5'-full RACE Core Set is designed to perform the reverse transcription from mRNA to cDNA and subsequent amplification. 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 with 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 speaking during the operations to prevent RNase contamination from operators' sweat or saliva.

A. Equipment
Disposable plastic equipment should 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, should be prepared with heat sterilized glass equipment (180℃ , 60 min.), or if possible, using glass equipment treated with DEPC as described above. Reagents and distilled water should be exclusively used for RNA preparation.

C. Preparation method
RNA simple purification methods can yield enough amount of RNA for reverse transcription and subsequent PCR. However, it is recommended to use highly purified RNA obtained by GTC(Guanidine thiocyanate) method, etc.

Note:

(1) For reverse transcription, master mix of reagents (containing RNase Free sterilized distilled water, buffers, etc.) for all samples can be prepared first, then aliquoted to individual tubes. Using such a mixture will allow accurate reagent dispensing: minimize reagents pipetting losses, and avoid repeat dispensing of the each reagent. This helps to minimize variation of the data among the experiments.

(2) Enzymes, such as RTase 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 buffer 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
Optimum PCR conditions vary depending on the thermal cycler used. It is recommended to perform a control experiment to determine the optimal conditions prior to using a sample.

Protocol:

1. General Protocol

A. 1st Strand cDNA synthesis

1. Prepare the reaction mixture in a tube by combining the following reagents to a total volume of 15 μl.

polyA$^+$ mRNA or Total RNA	0.5-5 μl
10X RT Buffer	1.5 μl
RNase Inhibitor (40 units/μl)	0.5 μl
AMV Reverse Transcriptase XL(5 units/μl)	1 μl
5' end-phosphorylated RT-Primer* (200 pmol/μl)	1 μl
RNase Free d_2O	up to 15 μl

*RT-Primer
i) It should be phosphorylated at 5' end for the subsequent ligation.
(It is advisable to phosphorylate when synthesizing the primer.)
ii)The primer of 12-15 mers is recommended.

2. Place all tubes in a Thermal Cycler and amplify.*

＊（1）For some thermal cyclers, a mineral oil overlay (approx.50 μl) is required to avoid evaporation of the reaction mixture.
（2）The reaction conditions should be optimized for the used thermal cycler.

30℃ 10 min.
↓
50℃ 30 - 60 min.
↓
80℃ 2 min.
↓
4℃

B. Degradation of Hybrid RNA

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

1st strand cDNA solution obtained at A.	15 μl
5X Hybrid RNA Degradation Buffer	15 μl
dH_2O	45 μl
Total	75 μl

2. Add 1 μl of RNase H and incubate at 30℃ for 1hour.
3. After incubation, perform ethanol precipitation.

C. Ligation reaction

1. Prepare the reaction mixture by combining the following reagents to a total volume of 40 μl.

5X RNA (ssDNA) Ligation Buffer	8 μl
40% PEG #6000	20 μl
dH_2O	up to 40 μl

2. Add the above mixture into the single-stranded cDNA which has been collected by ethanol precipitation at B. Mix well.
3. Add 1 μl of T4 RNA Ligase and incubate at 15℃ for overnight (15-18 hours).
4. Store at -20℃ until starting the next reaction.

D. PCR reaction

This set is optimized for the use with *TaKaRa Taq* (Cat. #R001), or *TaKaRa Ex Taq* (Cat. #RR001), or *TaKaRa LA Taq* (Cat. #RR002) for PCR reaction. *TaKaRa Taq* (Cat. #R001) can be used with good efficiency. However, when amplifying long target DNA or when higher efficiency is required, the use of *TaKaRa Ex Taq* or LA PCR Kit Ver.2 is recommended for better results.

◆ In case of using *TaKaRa LA Taq* (Cat.#RR002)

Dilute the ligation reaction (obtained at C) 10 times with TE Buffer and use it as template in PCR reaction.

The pattern of the amplified product may vary with the dilution ratio. Consider the dilution ratio for the ligation reaction product according to each sample.

(1) 1st PCR reaction

1. Prepare the reaction mixture to a total volume of 50 μl by combining the following reagents.

Template	1 μl
10X LA PCR Buffer II (Mg^{2+} free)	5 μl
25 mM $MgCl_2$	5 μl
dNTP Mixture (2.5 mM)	8 μl
1st PCR S1 Primer (20 pmol/μl)	0.5 μl
1st PCR A1 Primer (20 pmol/μl)	0.5 μl
TaKaRa LA Taq (5 units/μl)	0.5 μl
Sterilized distilled water	up to 50 μl

2. Place the tubes in a Thermal Cycler and amplify*.

＊ 1) For some thermal cyclers, a mineral oil overlay (approx.50 μl) is required to avoid evaporation of the reaction mixture.

2) The reaction conditions should be optimized for the used thermal cycler.

94℃	3 min.	
↓		
94℃	30 sec.	
50-65℃	30 sec.	25 cycles
68-72℃	0.5-5 min.	

(2) 2nd PCR reaction

1. Prepare the reaction mixture to a total volume of 50 μl by combining the following reagents.

1st PCR reactant*	1 μl
10x LA PCR Buffer II (Mg^{2+} free)	5 μl
25 mM $MgCl_2$	5 μl
dNTP Mixture (2.5 mM)	8 μl
2nd PCR S2 Primer (20 pmol/μl)	0.5 μl
2nd PCR A2 Primer (20 pmol/μl)	0.5 μl
TaKaRa LA Taq (5 units/μl)	0.5 μl
Sterilized distilled water	up to 50 μl

＊ To assure a clear PCR product, it is recommended to perform 2nd PCR using 3 dilutions (no dilution, 10 fold, and 100 fold) of the 1st PCR reaction mix.

2.Place the tubes in a Thermal Cycler and amplify*.

＊ 1) For some thermal cyclers, a mineral oil overlay (approx.50 μl) is required to avoid evaporation of the reaction mixture.

2) The reaction conditions should be optimized for the used thermal cycler.

94℃	30 sec.	25-30 cycles
50-65℃	30 sec.	
68-72℃	0.5-5 min.	

3. After the amplification is completed, apply a portion of the reaction for agarose gel electrophoresis and analyze the amplified fragment.

2. Control Experiment

A. 1st Strand cDNA synthesis

1. Prepare the reaction mixture in a tube by combining the following reagents to a total volume of 15 μl.

Positive Control RNA	1 μl
10X RT Buffer	1.5 μl
RNase Inhibitor (40 units/μl)	0.5 μl
AMV Reverse Transcriptase XL(5 units/μl)	1 μl
Positive Control RT-Primer	1 μl
RNase Free dH_2O	up to 15 μl

2. Place all tubes in a Thermal Cycler and amplify.*

*1) For some thermal cyclers, a mineral oil overlay (approx.50 μl) is required to avoid evaporation of the reaction mixture.

2) The reaction conditions should be optimized for the used thermal cycler.

30℃ 10 min.
↓
50℃ 30 min.
↓
80℃ 2 min.
↓
4℃

B. Degradation of Hybrid RNA

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

1st strand cDNA mixture obtained at A	15 μl
5X Hybrid RNA Degradation Buffer	15 μl
dH_2O	45 μl
Total	75 μl

2. Add 1 μl of RNase H and incubate at 30℃ for 1hour.
3. After incubation, perform ethanol precipitation.

C. Ligation reaction

1. Prepare the reaction mixture by combining the following reagents to a total volume of 40 μl.

5X RNA (ssDNA) Ligation Buffer	8 μl
40% PEG #6000	20 μl
dH_2O	up to 40 μl

2. Add the above mixture into the single-stranded cDNA which has been collected by ethanol precipitation at B. Mix well.
3. Add 1 μl of T4 RNA Ligase and incubate at 15℃ for overnight(15-18hours).
4. Store at -20℃ until starting the next reaction.

D. PCR reaction

◆ Using *TaKaRa LA Taq* (Cat. #RR002)

(1) 1st PCR reaction

Dilute the ligation reaction (obtained at C) 10 times with TE Buffer and use it as template in PCR reaction.

1. Prepare the reaction mixture to a total volume of 50 μl by combining the following reagents.

Template	1 μl
10X LA PCR Buffer II (Mg^{2+} free)	5 μl
25 mM $MgCl_2$	5 μl
dNTP Mixture (2.5 mM)	8 μl
Positive Control 1st Primer pair	1 μl
TaKaRa LA Taq (5 units/μl)	0.5 μl
Sterilized distilled water	up to 50 μl

2. Place the tubes in a Thermal Cycler and amplify*.

*1) For some thermal cyclers, a mineral oil overlay (approx.50 μl) is required to avoid evaporation of the reaction mixture.

2) The reaction conditions should be optimized for the used thermal cycler.

Example
94℃ 3 min.
↓
94℃ , 30 sec.
55℃ , 30 sec.
72℃ , 30 sec.
] 25 cycles

(2) 2nd PCR reaction

1. Prepare the reaction mixture to a total volume of 50 μl by combining the following reagents.

1st PCR reactant	1 μl
10X LA PCR Buffer II (Mg^{2+} free)	5 μl
25 mM $MgCl_2$	5 μl
dNTP Mixture (2.5 mM)	8 μl
Positive Control 2nd Primer pair	1 μl
TaKaRa LA Taq (5 units/μl)	0.5 μl
Sterilized distilled water	up to 50 μl

2. Place the tubes in a Thermal Cycler and amplify*.

＊1) For some thermal cyclers, a mineral oil overlay (approx.50 μl) is required to avoid evaporation of the reaction mixture.

2) The reaction conditions should be optimized for the used thermal cycler.

94℃ 30 sec.
55℃ 30 sec.
72℃ 30 sec.
] 27 cycles

3. After the amplification is completed, use a portion of the reaction for agarose gel electrophoresis and verify the amplified fragments.
The 310 bp* of DNA fragments are obtained.
(*The fragment size may vary slightly according to the reactions.)

3. Application example

Cloning of 5'-region of Human β-actin mRNA

The 5'-region of human β-actin mRNA was amplified through 5'RACE method of polyA$^+$ mRNA of HeLa cell.

[Primer Sequence]

RT primer: 5'-(P)CAGGGCAGTGAT-3'
S1 primer: 5'-GATGGCCACGGCTGCTTCCA-3'
S2 primer: 5'-CTCTTCCAGCCTTCCTTCCT-3'
A1 primer: 5'-GGTTGGCCTTGGGGTTCAGG-3'
A2 primer: 5'-TCTCCATGTCGTCCCAGTTG-3'

A. 1st Strand cDNA Synthesis

1. Prepare the reaction mixture in a tube by combining the following reagents to a total volume of 15 μl.

HeLa cell polyA$^+$ RNA	0.5 μg
10X RT Buffer	1.5 μl
RNase Inhibitor (40 units/μl)	0.5 μl
AMV Reverse Transcriptase XL (5 units/μl)	1 μl
RT Primer (200 pmol/μl)	1 μl
RNase Free distilled H_2O	up to 15 μl

2. Place the tubes in a Thermal Cycler and amplify.
   *1) For some thermal cyclers, a mineral oil overlay (approx.50 μl) is required to avoid evaporation of the reaction mixture.
   2) The reaction conditions should be optimized for the used thermal cycler.

30℃ , 10 min.
↓
50℃ 30 min.
↓
80℃ , 2 min.
↓
4℃

B. Degradation of Hybrid RNA

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

1st Strand cDNA solution	15 μl
5X Hybrid RNA Degradation Buffer	15 μl
RNase Free distilled H_2O	45 μl
Total volume	75 μl

2. Add 1 μl of RNase H and incubate at 30℃ for 1 hour.
3. After incubation, perform ethanol precipitation.

C. Ligation reaction

1. Prepare the reaction mixture by combining the following reagents to a total volume of 40 μl.

5X RNA (ssDNA) Ligation Buffer	8 μl
40% PEG #6000	20 μl
RNase Free distilled H_2O	up to 40 μl

2. Add the above mixture into the single-stranded cDNA which has been collected by ethanol precipitation at B. Mix well.
3. Add 1 μl of T4 RNA Ligase and incubate at 15℃ for overnight (15-18 hours).

D. PCR reaction

◆ Using *TaKaRa LA Taq* (Cat.#RR002)

(1) 1st PCR reaction

Dilute the ligation reaction (obtained at C) 10 times with TE Buffer and use it as template in PCR reaction.

1. Prepare the reaction mixture to have the total volume of 50 μl by combining the following reagents.

Template	1 μl
10X LA PCR Buffer II (Mg^{2+} free)	5 μl
25 mM $MgCl_2$	5 μl
dNTP Mixture (2.5 mM)	8 μl
S1 Primer (20 pmol/μl)	0.5 μl
A1 Primer (20 pmol/μl)	0.5 μl
TaKaRa LA Taq (5 units/μl)	0.5 μl
Sterilized distilled water	up to 50 μl

2. Place the tubes in a Thermal Cycler and amplify*.

*1) For some thermal cyclers, a mineral oil overlay (approx.50 μl) is required to avoid evaporation of the reaction mixture.

2) The reaction conditions should be optimized for the used thermal cycler.

94℃	3 min.	
↓		
94℃	30 sec.	⎤
65℃	30 sec.	⎥ 25 cycles
68℃	30 sec.	⎦

(2) 2nd PCR reaction

1. Prepare the reaction mixture to a total volume of 50 μl by combining the following reagents.

1st PCR reactant	1 μl
10X LA PCR Buffer II (Mg^{2+} free)	5 μl
25 mM $MgCl_2$	5 μl
dNTP Mixture (2.5 mM)	8 μl
S2 Primer (20 pmol/μl)	0.5 μl
A2 Primer (20 pmol/μl)	0.5 μl
TaKaRa LA Taq (5 units/μl)	0.5 μl
Sterilized distilled water	up to 50 μl

2. Place the tubes in a Thermal Cycler and amplify*.

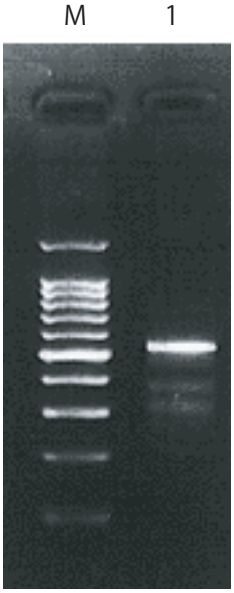
*1) For some thermal cyclers, a mineral oil overlay (approx.50 μl) is required to avoid evaporation of the reaction mixture.

2) The reaction conditions should be optimized for the used thermal cycler.

94℃	30 sec.	⎤
65℃	30 sec.	⎥ 27 cycles
68℃	30 sec.	⎦

E. Result

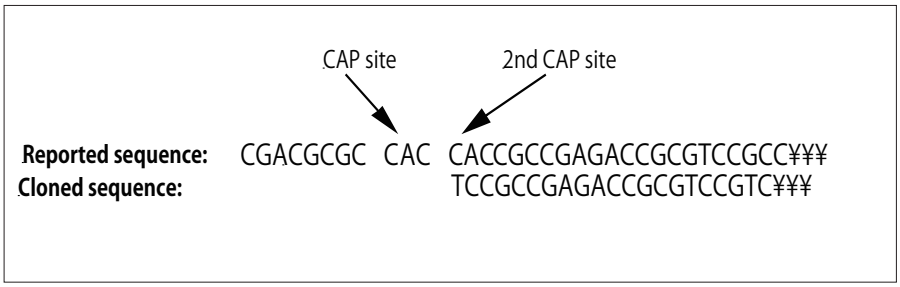
The PCR reactant was applied to agarose gel electrophoresis.
(Applied volume: 5 μl/lane)



Lane
M: 100 bp ladder
1: β -actin 5'-RACE fragment

(2% Nusieve™ 3:1 Agarose (Lonza))

The sequence of the amplified fragment was analyzed after subcloned by TA cloning.



The fragment was confirmed to contain the sequence to β-actin mRNA CAP site as reported.

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Related Products

TaKaRa Taq™ .....(Cat.#R001)
TaKaRa Ex Taq™ .....(Cat.#RR001)
TaKaRa LA Taq™ .....(Cat.#RR002)
TaKaRa LA PCR ™ Kit Ver.2.1 .....(Cat.#RR013)
TaKaRa Taq™ Hot Start Version .....(Cat.#R007A/B)
TaKaRa Ex Taq ™ Hot Version .....(Cat.#RR006A/B)
TaKaRa LA Taq ™ Hot Start Version .....(Cat.#RR042A/B)
Premix *Taq* ™ (*TaKaRa Taq*™ Version) .....(Cat.#R004)
Premix *Taq* ™ (*Ex Taq*™ Version) .....(Cat.#RR003)
One Shot LA PCR™ Mix .....(Cat.#RR004)
Reverse Transcriptase XL(AMV) for RT-PCR Kit .....(Cat.#2630A)
Ribonuclease Inhibitor .....(Cat.#2311A/B)
T4 RNA Ligase .....(Cat.#2050)

References

1) Frohman, M. A., Dush, M. K., Martin, G. R. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 8998-9002.
2) Maruyama, I.N., Rakow, T.L., Maruyama, H.I. (1995) *Nucleic Acid Research,* **23**, 3796-3797.

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