Cat. # RR013A

For Research Use

TaKaRa

TaKaRa LA PCR™ Kit Ver. 2.1

Product Manual

v201305Da

Table of Contents

I. Description

LA PCR Kit Ver.2.1 is designed for long and accurate PCR amplification using LA (long and accurate) PCR technology. Using *TaKaRa LA Taq* in conjunction with the optimized reaction buffer (10X LA PCR Buffer II) enables the accurate amplification of long PCR products up to 48 kb using λ DNA or up to 30 kb using genomic DNA as a template.

LA PCR Kit Ver.2.1 includes all of the reagents required for LA PCR and provides efficient amplification, high fidelity, and long PCR product yield. In addition, by using the supplied 2X GC Buffer I, II, this kit can be used to amplify GC rich templates.

II. Components

(50 reactions, 50 μl)

(1) *TaKaRa LA Taq*＊1 (5 units/μl) ..... 125 units
(2) dNTP Mixture＊2 (2.5 mM each.) ..... 400 μl
(3) 10X LA PCR Buffer II (25 mM Mg^{2+} plus) ..... 250 μl
(4) 10X LA PCR Buffer II (Mg^{2+} free) ..... 250 μl
(5) $MgCl_2$ (25 mM) ..... 500 μl
(6) Control Template ..... 10 μl
(100 ng/μl genomic DNA derived from HL60)
(7) Control Primer LA3＊4 (10 μM) ..... 10 μl
(8) Control Primer LA4＊4 (10 μM) ..... 10 μl
(9) λ-*Hin*d III digested MW Marker ＊5 (100 ng/μl) ..... 20 μl
(10) 2X GC Buffer I ＊3 (5 mM Mg^{2+} plus) ..... 1.25 ml
(11) 2X GC Buffer II ＊3 (5 mM Mg^{2+} plus) ..... 1.25 ml
(12) Control Primer GC1 ＊4 (10 μM) ..... 10 μl
(13) Control Primer GC2 ＊4 (10 μM) ..... 10 μl

＊1: *TaKaRa LA Taq*

- Concentration: 5 units/μl
- Form: Supplied in 20 mM Tris-HCl (pH8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, 0.5% Nonidet P-40, 50% Glycerol
- Unit definition: One unit is the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble products in 30 min. at 74℃, pH9.3, with activated soluble salmon sperm DNA as the template-primer.
- Reaction mixture for unit definition:

25 mM	TAPS (pH 9.3 at 25℃)
50 mM	KCl
2 mM	$MgCl_2$
0.1 mM	DTT
200 μM	each dATP・dGTP・dCTP
100 μM	[^{3}H]-dTTP
0.25 mg/ml	activated salmon sperm DNA

- Purity: Nicking, endonuclease, and exonuclease activity were not detected after incubation of 0.6 μg of supercoiled pBR322 DNA, 0.6 μg of λ DNA, or 0.6 μg of λ-*Hin*d III digest with 10 units of this enzyme for 1 hour at 74℃.

＊2: dNTP Mixture is ready for use in PCR reactions without dilution.

・Concentration : 2.5 mM of each dNTP
・Form : Dissolved in water (sodium salts), pH7 - 9
・Purity : ≧ 98% for each dNTP

＊3: GC Buffers

When a template may have complex secondary structure or high GC content, use 2X GC Buffer I first. If amplification is not successful, improved results may be obtained using 2X GC Buffer II.

＊4: Primer sequences

Control Primer LA3:
5′-ACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGA-3′
Control Primer LA4:
5′-TGCACCTGCTCTGTGATTATGACTATCCCACAGTC-3′
Control Primer LA3 and LA4 will result in a 17.5 kb amplified DNA fragment from the Control Template.

Control Primer GC1: 5′-GGGAGGGGACCGGGGAACAGAG-3′
Control Primer GC2: 5′-GAACAGTCCGTCACTTCACGTG-3′
Control Primer GC1 and GC2 will result a 1,255 bp amplified DNA fragment with high GC content from the Control Template.

＊5: λ-*Hin*d III digest MW Marker
The range of size marker: 23,130; 9,416; 6,557; 4,361; 2,322; 2,027; 564; 125 bp.

III. Materials Required but not Provided

1. Reagents

- Agarose gel
  e.g., SeaKem® Gold Agarose (Lonza)
- DNA staining reagent
  e.g., GelStar® Nucleic Acid Stain (Lonza), SYBR® Green Nucleic Acid Stain (Lonza), or Ethidium Bromide
  Note : When using GelStar® or SYBR® Green I for staining a gel, a filter designated for use with GelStar® or SYBR® Green I should be used.
- 6X Loading Buffer, containing 36% Glycerol and 0.05% Xylene Cyanol, 0.05% Bromophenol Blue, 30 mM EDTA.
- Sterile distilled water
- Upstream and downstream primers

2. Materials

- PCR Instrument
  e.g., TaKaRa PCR Thermal Cycler Dice Gradient/Standard (Cat. #TP600/TP650)*
- Microcentrifuge tubes (polypropylene)
- Agarose gel electrophoresis apparatus
- Microcentrifuge
- Micropipettes and pipette tips (autoclaved)

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

IV. Storage -20℃

V. Protocol

A. Amplification of long DNA fragments

This kit contains Control Primer LA3 and LA4 to amplify a 17.5 kb fragment from the Control Template (genomic DNA from HL60).

1) Prepare the reaction mixture in PCR tubes by combining the following reagents in a total volume of 50 μl.

[Using 10X LA PCR Buffer II (Mg^{2+} plus)]

Component		Final concentration
TaKaRa LA Taq	0.5 μl	2.5 units/50 μl
10X LA PCR Buffer II (Mg^{2+} plus)	5 μl	[1X]
dNTP Mixture	8 μl	each 400 μM
Template＊1	< 1 μg	
Primer 1＊2	10 - 50 pmol	0.2 - 1.0 μM
Primer 2＊2	10 - 50 pmol	0.2 - 1.0 μM
Sterile distilled water	up to 50 μl	

[Using 10X LA PCR Buffer II (Mg^{2+} free)]

Component		Final concentration
TaKaRa LA Taq	0.5 μl	2.5 units/50 μl
10X LA PCR Buffer II (Mg^{2+} free)	5 μl	[1X]
25 mM $MgCl_2$	5 μl	2.5 mM
dNTP Mixture	8 μl	each 400 μM
Template ＊1	< 1 μg	
Primer 1 ＊2	10 - 50 pmol	0.2 - 1.0 μM
Primer 2 ＊2	10 - 50 pmol	0.2 - 1.0 μM
Sterile distilled water	up to 50 μl	

＊1 : For the positive control experiment, use 2 μl (200 ng) of Control Template.
＊2 : For amplification of the Control Template, use 1 μl (final conc. 0.2 μM) of Control Primer LA3 and LA4.

2) Use the following conditions for PCR amplification.

98℃ 10 sec. ＊3 (denaturation)
68℃ 30 sec. - 1 min./kb ＊4 (annealing and extension)] 30 cycles

＊3 : Denaturation conditions vary depending on the thermal cycler and tubes used for PCR. 5 - 10 sec. at 98℃ or 20 - 30 sec. at 94℃ is recommended.
＊4 : The annealing and extension time varies depending on the amplicon size. The recommended time setting is 30 sec. - 1 min. per 1 kb. For the positive control reaction using Control Primer LA3 and LA4, the optimal time is 15 min.

3) Use 5 - 10 μl of PCR reaction solution for analysis by agarose gel electrophoresis and verify PCR amplification products.

B. Amplification of DNA fragments with high GC content

This kit contains Control Primer GC1 and GC2 that amplify a 1,255 bp portion of the Control Template (genomic DNA from HL60) that has a GC content of 65%.

1) Prepare the reaction mixture in PCR tubes by combining the following reagents in a total volume of 50 μl.

Component		Final concentration
TaKaRa LA Taq	0.5 μl	2.5 units/50 μl
2X GC Buffer I or 2X GC Buffer II＊1	25 μl	[1X]
dNTP Mixture	8 μl	each 400 μM
Template＊2	< 1 μg	
Primer 1＊3	10 - 50 pmol	0.2 - 1.0 μM
Primer 2＊3	10 - 50 pmol	0.2 - 1.0 μM
Sterilized distilled water	up to 50 μl	

＊1 : Use 2X GC Buffer I first. If DNA amplification is not obtained, amplification may be improved by using 2X GC Buffer II.
＊2 : For the positive control experiment, use 2 μl (200 ng) of Control Template.
＊3 : For amplification of the Control Template, use 1 μl (final conc. 0.2 μM) of Control Primer GC1 and GC2.

2) Use the following conditions for PCR amplification.

94℃ 1 min.
94℃ 30 sec. ＊4
60℃ 30 sec.] 30 cycles
72℃ 1 min./kb ＊5
72℃ 5 min.

＊4 : Denaturation conditions vary depending on the thermal cycler and tubes used for PCR. 5 - 10 sec. at 98℃ or 20 - 30 sec. at 94℃ is recommended.
＊5 : The extention time varies depending on the amplicon size.
The recommended time setting is 1 min. per 1 kb. For the positive control reaction using Control Primer GC1 and GC2, the optimum time is 2 min.

3) Use 5 - 10 μl of PCR reaction solution for analysis by agarose gel electrophoresis and verify PCR amplification products.

C. Electrophoresis

1) Combine 5 - 10 μl of PCR reaction solution and 1/5 volume of 6X Loading Buffer.
2) Pipet the samples into the wells of an agarose gel, and run the gel. The running conditions will vary according to size of amplified DNA.
3) After electrophoresis, stain gel by soaking in Ethidium Bromide solution (0.5 μg/ml) for 20 - 30 min., or SYBR Green I (Lonza).
4) Verify the bands of amplified DNA under UV illumination.

For the positive control experiment, a 17.5 kb amplification product should be obtained when the Control Primer LA3 and LA4 are used, and a 1,225 bp amplification product should be obtained when the Control Primer GC1 and GC2 are used.

VI. Experimental Examples

1) Amplification from a human genomic DNA template

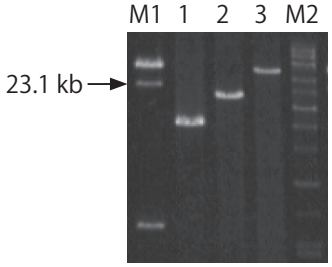
・Target : β-globin cluster region (17.5 kb, 21.5 kb); TPA gene region (27 kb)

・Reaction mixture :

Component	Volume	Final concentration
10X LA PCR Buffer II (Mg^{2+} plus)	5 μl	[1X]
dNTP Mixture	8 μl	each 400 μM
Sense Primer (20 μM)	0.5 μl	0.2 μM
Antisense Primer (20 μM)	0.5 μl	0.2 μM
TaKaRa LA Taq	0.5 μl	2.5 units/50 μl
Human genomic DNA (500 ng/μl)	1 μl	500 ng/50 μl
Sterile distilled water	34.5 μl	
total	50 μl	

・PCR conditions :

94℃ 1 min.
↓
98℃ 10 sec.
68℃ 15 min. (17.5 kb, 21.5 kb) or 20 min. (27 kb)] 30 cycles
↓
72℃ 10 min.

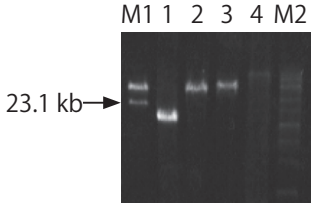


Lane : PCR products size
1 : 17.5 kb (β-globin cluster)
2 : 21.5 kb (β-globin cluster)
3 : 27 kb (TPA gene)
M1 : λ-*Hin*d III digest
M2 : High Molecular Weight DNA Marker (Life Technologies)

0.4% Agarose gel electrophoresis
Volume analyzed : 5 μl

2) Amplification from *E. coli* genomic DNA template (>18 kb)

・ Template DNA : 100 ng/50 μl
・ Reaction conditions (composition of reaction mixture and PCR conditions) : same as example 1)



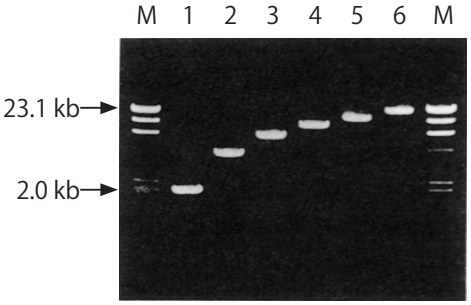
Lane : PCR products size
1 : 20 kb
2 : 28 kb
3 : 30 kb
4 : 38 kb
M1 : λ-*Hin*d III digest
M2 : High Molecular Weight DNA Marker (Life Technologies)

0.4% Agarose gel electrophoresis
Volume analyzed : 5 μl

3) Amplification from *E. coli* genomic DNA template (≦ 18 kb)

- The composition of reaction mixture is the same as example 2)
- PCR conditions :

94℃ 1 min.
↓
98℃ 10 sec. ┐
68℃ 3 min. (2 kb, 4 kb), or ┐ 30 cycles
5 min. (6 kb, 8 kb), or ┘
15 min. (10 kb, 18 kb) ┘
↓
72℃ 10 min.



Lane : PCR products size
1 : 2 kb
2 : 4 kb
3 : 6 kb
4 : 8 kb
5 : 10 kb
6 : 18 kb
M : λ-*Hin*d III digest

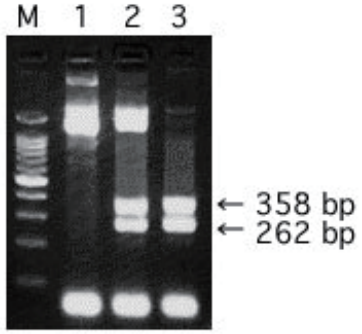
0.4% Agarose gel electrophoresis
Volume analyzed : 5 μl

4) Amplification of Huntington's disease gene (high GC content)

The amplification performances were compared for the conventional LA PCR Buffer and GC Buffer I, II.

- Target: Huntington's disease (HD) gene IT 15 CAG repeat region
  262 bp-GC content: 73%
  358 bp-GC content: 71.5%
- PCR conditions :

94℃ 1 min.
94℃ 30 sec. ┐
60℃ 30 sec. ┘ 30 cycles
72℃ 1 min. ┘
72℃ 5 min.



Lane : Buffer
1 : 10X LA PCR Buffer
2 : 2X GC Buffer I
3 : 2X GC Buffer II
M : 100 bp DNA Ladder

3% Agarose gel electrophoresis
(NuSieve 3 : 1 Agarose gel (Lonza))
Volume analyzed : 5 μl

VII. Note

1) Mix the kit components well by pipetting or inverting the tubes up and down after thawing completely at room temperature to 37℃. Avoid mixing vigorously or vortexing. When mixing 10X LA PCR Buffer and *TaKaRa LA Taq*, mix carefully and gently to avoid producing bubbles or inactivating enzymes. Each component should be kept on ice until used.
2) Mix the reaction mixture gently by pipetting before starting reactions. Do not vortex.
3) Highly specific primers should be used to obtain long PCR products. Use 25 - 30 -mer primers for high specificity.
4) Highly pure template DNA should be used. For amplification of long fragments, use is 4 - 5 times the amount of template DNA than that used for general PCR.

VIII. Q & A

1. PCR optimization

The optimal reaction conditions vary according to amplicon size, reaction volumes, and type of thermal cycler used.

1) Number of cycles
Set the cycle number to 25 - 35 cycles by considering the quantity or complexity of template DNA and the length of amplified DNA fragments. Too few cycles may not generate enough amplified product, while over cycling may produce a diffuse smear upon electrophoresis.

2) Initial denaturation
94℃ for 1 - 2 min. is recommended even for genomic DNA templates.

3) Denaturation conditions
When using thin-wall PCR tubes, use either a 98℃ for 10 sec. or 94℃ for 20 sec for denaturation. When using normal PCR tubes, 94℃ for 30 sec. or 98℃ for 20 sec is recommended for denaturation. A denaturation time that is too short or a denaturation temperature that is too low may cause either diffuse smearing upon electrophoresis or poor amplification efficiency. A denaturation time that is too long or a denaturation temperature that is too high may not generate product.

4) Conditions for Annealing and Extension
Determine the optimum annealing temperature experimentally by varying temperatures in 2℃ increments over a range of 55 - 68℃. As *TaKaRa LA Taq* shows sufficient activity at 60 - 68℃, Shuttle PCR (two step PCR) can be conducted by setting the annealing-extension temperature within this range. For combined annealing/extension at 68℃ (two step PCR), the recommended time setting is 30 sec. to 1 min. per 1 kb. When the temperature is set below 68℃, additional time will be required. An annealing temperature that is too high may not generate amplification products, while a temperature that is too low enhances non-specific reactions. An extension time that is too short generates no amplification products or primarily non-specific, short products; extension times that are too long cause diffusely smeared electrophoresis bands.

2. Primer design

Specificity of primers is very important for the generation of long DNA amplification products. For example, if they are highly specific, even 20-mer primers can generate DNA fragments greater than 20 kb.
If possible, design primers according to the following criteria.

1) The primer length should be 25 to 30 bases.
2) The difference between the optimum annealing temperature of paired primers should be within 2 - 3℃.
3) Choose primers whose GC content is around 40 - 60%.
4) Avoid primer sequences which form hairpin loops or primer-dimers, especially at the 3' end.
5) Avoid primers containing inosine.

3. Primer concentration

The optimal concentration will range from 0.1 to 1.0 μM. At lower than optimum concentration, amplification yield may be poor; at higher concentrations, non-specific reactions may outperform primer-specific amplification.
In practice, primer concentrations can be determined depending on the characteristics and amounts of template DNA: low primer concentrations are recommended either for highly complex DNA such as human genomic DNA, or when using high concentrations of template DNA. High primer concentrations are preferred for low complexity templates such as plasmid DNA, or when there is a limiting amount of template DNA.

4. Enzyme amount

It is recommended that 2.5 units of *TaKaRa LA Taq* be used in a 50 μl reaction, but the amount may be changed to optimize reaction performance. The following factors should be taken into consideration: quantity or complexity of template DNA and the length of amplified DNA fragment. Excess enzyme may allow non-specific reactions to occur and result in a diffuse smear upon electrophoresis. When the enzyme concentration is too low, the efficiency of amplification may be diminished.

5. Template DNA

When amplifying DNA fragments longer than 10 kb by PCR, the preparation of intact (free of nicks), highly pure template DNA through repeated extraction/purification is essential. Samples that are prepared by only lysing cells with either heat treatment or protease digestion are not appropriate for LA PCR.

Use the following protocol to prepare human genomic DNA and *E. coli* genomic DNA for LA PCR.

1) Protocol for the preparation of human genomic DNA

1. Harvest HL60 cells (from 15 petri dishes or about 1.5 x 10^9 cells) and wash twice with 0.7% NaCl.
2. Suspend cells with 15 ml of 10 mM Tris-HCl (pH8.3) in a polycarbonate centrifuge tube.
3. Add 150 μl of 10 mg/ml Proteinase K and 150 μl of 10% SDS to cell suspension for lysis.
4. Incubate the mixture at 60℃ for one hour and subsequently at 37℃ for an additional 16 hours.
5. Add 15 ml of phenol saturated with 1 M Tris-HCl (pH8.0), into the tube and mix gently for 15 min. with repeated inversions of the tube.
6. Centrifuge at 9,000 rpm (approx. 6,000X *g*) at room temperature for 10 min.
7. Transfer the aqueous phase to a new tube.
8. Add 15 ml of phenol/chloroform/isoamylalcohol (25 : 24 : 1, saturated with TE buffer, pH8.0) into the tube and gently mix by inversion for 15 min.
9. Centrifuge at 9,000 rpm (approx. 6,000X *g*) at room temperature for 10 min.
10. Transfer the aqueous phase to a new tube.
11. Add 15 ml of chloroform/isoamylalcohol (24 : 1) to the tube and gently mix by inversion for 15 min.
12. Centrifuge at 9,000 rpm (approx. 6,000X *g*) at room temperature for 10 min.
13. Transfer the aqueous phase to a new tube.
14. Add 1.5 ml of 3 M Sodium Acetate (pH5.2) and 30 ml of 99.5% ethanol and mix gently.
15. Collect DNA around a thin glass stick and wash with 80% ethanol.
16. Dry DNA.
17. Dissolve DNA in 10 ml of TE buffer by letting it stand at 4℃ overnight.
18. Add 100 μl of 10 mg/ml RNase A into the DNA solution and incubate at 37℃ for 1 hour.
19. Add 10 ml of phenol/chloroform/isoamylalcohol (25 : 24 : 1) and mix gently for 5 min.
20. Centrifuge at 9,000 rpm (approx. 6,000X *g*) at room temperature for 10 min.
21. Transfer the aqueous phase into a new tube.
22. Add 10 ml of chloroform/isoamylalcohol (24 : 1) and mix gently for 5 min.
23. Centrifuge at 9,000 rpm (approx. 6,000X *g*) at room temperature for 10 min.
24. Transfer the aqueous phase into a new tube.
25. Add 1 ml of 3 M Sodium Acetate (pH5.2) and 20 ml of 99.5% ethanol to the tube and mix gently.
26. Collect DNA around a thin glass stick.
27. Wash DNA with 80% ethanol.
28. Dry DNA.
29. Dissolve DNA in 2 ml of TE buffer and bring DNA concentration to 0.5 mg/ml.

2) Protocol for the preparation of *E. coli* genomic DNA

1. Grow *E. coli* overnight in two 1-liter flasks containing 200 ml of L-broth.
2. Harvest cells and suspend in 40 ml of 50 mM Tris-HCl (pH8.0) containing 50 mM EDTA.
3. Freeze cells at -80℃ for 30 min.
4. Add 4 ml of 10 mg/ml lysozyme solution in 0.25 M Tris-HCl (pH8.0) to frozen cells and then thaw at room temperature with occasional mixing.
5. Leave thawed cells on ice for 45 min.
6. Add 8 ml of STEP (0.5% SDS, 50 mM Tris-HCl (pH8.0), 0.4 M EDTA and 1 mg/ml Proteinase K) and incubate at 50℃ for one hour.
7. Add 45 ml of TE (10 mM Tris-HCl (pH8.3) and 1 mM EDTA).
8. Then add 96 ml of phenol/chloroform/isoamylalcohol (25 : 24 : 1, saturated with TE buffer pH8.0) into the tube and gently mix by repeated inversion for 5 min.
9. Centrifuge at 5,000 rpm (approx. 4,000X *g*) at room temperature for 15 min.
10. Transfer the aqueous phase to a new tube.
11. Add 96 ml of chloroform/isoamylalcohol (24 : 1) into the tube and gently mix by inversion for 5 min.
12. Centrifuge at 5,000 rpm (approx. 4,000X *g*) at room temperature for 15 min.
13. Transfer the aqueous phase to a new tube.
14. Add 9 ml of 3 M Sodium Acetate (pH5.2) and 225 ml of 99.5% ethanol and mix gently.
15. Collect DNA around a thin glass stick and wash it with 80% ethanol.
16. Dry DNA.
17. Dissolve DNA in 20 ml of TE buffer by letting it stand at 4℃ overnight.
18. Add 10 μl of 10 mg/ml RNase A into the DNA solution and incubate at 37℃ for 30 min.
19. Add 20 ml of phenol/chloroform/isoamylalcohol (25 : 24 : 1, saturated with TE buffer, pH8.0) and mix gently by inversion for 5 min.
20. Centrifuge at 10,000 rpm (approx. 7,500X *g*) at room temperature for 15 min.
21. Transfer the aqueous phase into a new tube.
22. Add 20 ml of chloroform/isoamylalcohol (24 : 1) and mix gently for 5 min.
23. Centrifuge at 10,000 rpm (approx. 7,500X *g*) at room temperature for 10 min.
24. Transfer the aqueous phase into a new tube.
25. Add 2 ml of 3 M Sodium Acetate (pH5.2) and 50 ml of 99.5% ethanol into the tube and mix gently.
26. Collect DNA around a thin glass stick.
27. Wash DNA with 80% ethanol.
28. Dry DNA.
29. Dissolve DNA in 20 ml of TE buffer by letting it stand at 4℃ overnight. (DNA concentration will be around 0.1 mg/ml.)

Human and *E. coli g*enomic DNA prepared using the above protocols are available for use as control templates in LA PCR.

LA PCR Genome DNA Set (20 reactions) .....Cat. #9060
(includes both human genomic DNA and *E. coli* genomic DNA)

Recommended DNA amount in LA PCR
- human genomic DNA: 0.1 - 1 μg / 50 μl PCR
- *E. coli* genomic DNA: 10 - 100 ng / 50 μl PCR
- λ phage DNA: 0.5 - 2.5 ng / 50 μl PCR

6. Is it possible to use λ phage particles directly for LA PCR ?

Lysates from approximately 10^6 - 10^7 PFU obtained by heating at 99℃ for 10 min. can be used to amplify DNA fragments in around 8 kb.

7. Is it possible to use cell lysates obtained by either heat treatment (98℃ for 2 min.) or protease digestion from either mammalian cells or *E. coli* cells directly for LA PCR amplification ?

- For *E. coli*, it is possible. Lysates obtained by heat-treatment alone can be used to amplify DNA fragments around 10 kb.
  (∗*E. coli* cells cultured in L-broth medium at 37℃ overnight. 2 μl of the cell culture used for 50 μl.)
- In case of human cells (cultured), the extent of purification significantly affects the length of extension by PCR amplification. Templates prepared by heat treatment alone have an amplification limit of several hundred bases, while combined treatment with heat and protease may generate fragments 1 - 2 kb. For longer length amplifications, it is recommended to use purified DNA prepared according to the standard protocol described in 5.

8. Amplified products are entirely smeared upon electrophoresis.

Possible Causes	Comments and suggestions
Too much enzyme	Reduce the enzyme amount in 0.5 units steps.
Denaturation time too short	Increase the denaturation time by increments of 5 sec.
Denaturation temperature too low	Raise the denaturation temperature by 0.5℃ intervals.
dNTP concentration too low	Increase the dNTP concentration in by increments of 50 μM.
Extension time too long	Shorten the extension time by decrements of 1 min.
Too many PCR cycles	Reduce the number of cycles in steps of 2 cycles.
Too much template	Reduce the template amount by decrements of 20%.

9. Multiple, nonspecific amplified products upon electrophoresis

Possible Causes	Comments and suggestions
Primer concentration too high	Decrease the primer concentration in steps of 0.1 μM.
Poor primer design	Enhance the specificity of primers by changing the complementary region of the template.
Too much enzyme	Reduce the enzyme amount in 0.5 units steps.
Too many PCR cycles	Reduce the number of cycles in steps of 2 cycles.
Annealing temperature too low	Raise the annealing temperature by 2℃ intervals.
Nonspecific annealing of primers	Use Hot Start method to avoid. This phenomenon occurs during heating from room temperature to the denaturation temperature (94 - 98℃).
Extension time too short	Increase the extension time by increments of 1 min.
Poor denaturation	Raise the denaturation temperature by 0.5℃ intervals and extend the time by increments of 5 sec.
Too much template	Reduce the template amount by decrements of 20%.

10. Do PCR products amplified with *TaKaRa LA Taq* have A overhangs? Is it possible to use the products for TA cloning?

Yes, PCR products have an A overhang and can be used for TA cloning. The efficiency will be lowered in cases where long PCR products (> 5 kb) are cloned into a T-Vector

11. What type of agarose gel should be used for the electrophoresis to analyze long DNA fragments generated by LA PCR ?

0.4% SeaKem® Gold Agarose (Lonza) is recommended.

IX. References

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X. Related Products

TaKaRa LA Taq® (Cat. #RR002A/B)
TaKaRa LA Taq® Hot Start Version (Cat. #RR042A/B)
TaKaRa LA Taq® with GC Buffer (Cat. #RR02AG/BG)
Taq Antibody (Cat. #9002A/B)
LA PCR Genome DNA Set (Cat. #9060)
TaKaRa PCR Thermal Cycler Dice™ Gradient/Standard (Cat. #TP600/TP650)*

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

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[P1] PCR Notice
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