TaKaRa LA Taq® DNA Polymerase (Cat.# RR002A)

Application: Examples of Long-Range PCR with *TaKaRa LA Taq* DNA Polymerase

TaKaRa LA Taq DNA Polymerase (Cat. # RR002A) is optimized for long-range PCR. The following Application Note shows examples of long-range PCR with *E. coli* genomic DNA or human genomic DNA as template using *LA Taq* DNA Polymerase.

Example 1: Amplification of *E. coli* products up to 38 kb in length

Methods:

100 ng of *E. coli* genomic DNA was used as the template in a 50 µl reaction. *TaKaRa LA Taq* DNA polymerase (Cat. # RR002A) was used for amplification using the recommended conditions. After PCR, 5 µl of the reaction mixture was used for electrophoresis on a 0.4% agarose gel.

Results:

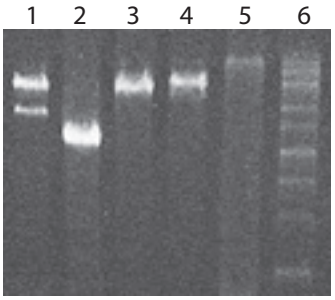

Figure 1. Products amplified from *E. coli* genomic DNA using *TaKaRa LA Taq* DNA Polymerase. Amplified product sizes are 20 kb (lane 2), 28 kb (lane 3), 30 kb (lane 4), and 38 kb (lane 5). Molecular weight markers: Lane 1, HindIII-digested lambda DNA; lane 6, High Molecular Weight DNA Markers (BRL).

Example 2: Amplification of *E. coli* products up to 18 kb in length

Methods:

PCR was performed using a using TaKaRa PCR Thermal Cycler (Cat. # TP480)* with the following conditions:

94°C	1 min.	
↓		
98°C	20 sec.	] 30 cycles
68°C	3 min. (for 2 and 4 kb products)	
	5 min. (for 6 and 8 kb products)	
	15 min. (for 10 and 18 kb products)	
↓		
72°C	10 min.	

After PCR, 5 µl of the reaction mixture was used for electrophoresis on a 0.4% agarose gel.

* TaKaRa PCR Thermal Cycler is not available in all geographic locations. Check for availability in your region.

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Results:

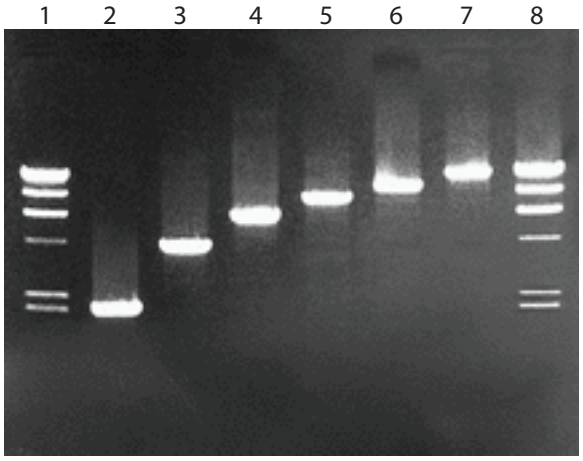

Figure 2. Products amplified using *TaKaRa LA Taq* from *E. coli* genomic DNA. Amplified product sizes are 2 kb (lane 2), 4 kb (lane 3), 6 kb (lane 4), 8 kb (lane 5), 10 kb (lane 6), and 18 kb (lane 7). Lanes 1 and 8 contain HindIII-digested lambda DNA as a molecular weight marker.

Example 3: Long-range PCR using human genomic DNA

The TPA gene region and the β-globin cluster region were amplified from human genomic DNA using *TaKaRa LA Taq* DNA polymerase according to the conditions in Table 1.

Table 1. Reaction conditions

Component	Volume	Final concentration
Human genomic DNA (500 ng/µl)	1 µl	500 ng per 50 µl reaction
10x LA PCR Buffer II (Mg^{2+} Plus)	5 µl	1X
dNTPs	8 µl	400 µM each
Sense Primer (20 pmol/µl)	0.5 µl	0.2 µM
Antisense Primer (20 pmol/µl)	0.5 µl	0.2 µM
TaKaRa LA Taq	0.5 µl	2.5 U per 50 µl reaction
Sterile distilled water	34.5 µl	

After the 50 µl reactions were prepared, each reaction was overlayed with an equal volume of mineral oil. PCR was performed using a using TaKaRa PCR Thermal Cycler (Cat. # TP480)* with the following conditions:

94°C 1 min
↓
98°C 20 sec
68°C 20 min] 14 cycles
↓
98°C 20 sec
68°C 20 min + 15 sec/cycle] 16 cycles
↓
72°C 10 min

After PCR, 5 µl of the reaction mixture was used for electrophoresis on a 0.4% agarose gel.

* TaKaRa PCR Thermal Cycler is not available in all geographic locations. Check for availability in your region.

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Results:

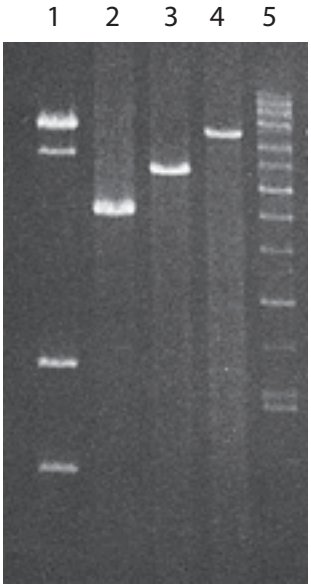

Figure 3. Products amplified by *TaKaRa LA Taq* from human genomic DNA. *LA Taq* was used to amplify 17.5 kb and 21.5 kb fragments of the β-globin cluster region (lanes 2 and 3, respectively) and a 27 kb portion of the TPA gene region (lane 4). Molecular weight markers: Lane 1, HindIII-digested lambda DNA; lane 5, High Molecular Weight DNA Markers (BRL).

Conclusion:

Amplification of *E. coli* products up to 38 kb in length or human products up to 18 kb in length was achieved using *TaKaRa LA Taq* DNA Polymerase.