

LA PCR Genome DNA Set (Cat.# 9060) and *TaKaRa LA Taq*[®] (Cat. # RR002A)

Application: Long Range PCR of Human and *E. coli* genomic targets using the Genome DNA Set for LA PCR and *TaKaRa LA Taq*[®] DNA Polymerase

The LA PCR Genome DNA Set (Cat.# 9060) includes highly purified genomic DNA from human and *E. coli* and appropriate primers for amplifying large targets. These templates and primers are intended for use as controls for long range PCR (also referred to as Long and Accurate (LA) PCR), and provide a useful method for optimizing PCR conditions. Here, human and *E. coli* genomic DNA were amplified with the appropriate primers from this set using *TaKaRa LA Taq*[®] DNA polymerase (Cat. # RR002A).

Methods

Component	Amount	Final Concentration
DNA (LA PCR Genome DNA Set)	1 μ l	Human Genome: 500 ng; <i>E. coli</i> Genome: 100 ng
10X LA PCR Buffer II (Mg ²⁺ Free)	5 μ l	1X
25 mM MgCl ₂	5 μ l	2.5 mM
dNTP Mixture	8 μ l	400 μ M each
Primer #1 (LA PCR Genome DNA Set)	1 μ l	0.2 μ M
Primer #2 (LA PCR Genome DNA Set)	1 μ l	0.2 μ M
<i>TaKaRa LA Taq</i>	0.5 μ l	2.5 U/50 μ l
Sterile distilled water	up to 50 μ l	

The reaction mixture was mixed gently by pipetting before starting the PCR reaction. PCR was performed using a TaKaRa PCR Thermal Cycler MP* was performed with the following cycling conditions:

94°C, 1 min. x1 cycle
98°C, 10 sec. }
68°C, 15 min. } 30 cycles
72°C, 10 min. x1 cycle

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

Results

The PCR products were analyzed by electrophoresis on a 0.6% SeaKem Gold Agarose gel. A total of 8 μ l of the human genomic DNA amplification reaction and 4 μ l of the *E. coli* genomic DNA amplification reaction were analyzed.

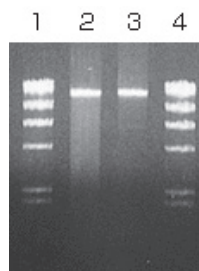


Figure 1. PCR products obtained using the LA PCR Genome Set. *TaKaRa LA Taq* DNA Polymerase was used to amplify products from either a 17.5 kb human genomic target (lane 2) or a 20 kb *E. coli* genomic target (lane 3). Lanes 1 and 4 contain molecular weight markers (λ -Hind III digest).

Conclusions

Large (>15 kb) products were amplified efficiently from both human and *E. coli* genomic templates (from the LA PCR Genome DNA Set) using *LA Taq* DNA polymerase.