Premix Ex Taq™ DNA Polymerase Hot Start (Cat.# RR030A)

Application: Amplification of GC-rich DNA Fragments using *TaKaRa LA Taq*® or *Ex Taq*® DNA Polymerase

When amplifying template DNA that is GC-rich, conventional *Taq* DNA Polymerase often does not perform well. The superior amplification efficiency of *TaKaRa LA Taq*® and *TaKaRa Ex Taq*® allows amplification of difficult DNA targets, such as those with high GC content.

This application example illustrates the ability of these enzymes to amplify a 3 kb fragment that has 70% GC content.

Methods:

Template: VIII-5-pUC118 (a ~3 kb GC-rich fragment inserted into the *Hin*d III site of pUC118)

Amplification size: ~3 kb

Primers: M3-30 (an extended M3 Primer)
5′-CAGTCACGACGTTGTAAAACGACGGCCAGT-3′
RV-32 (an extended RV Primer)
5′-GATAACAATTTCACACAGGAAACAGCTATGAC-3′
(10 pmol of each primer was used per 50 µl PCR reaction)

dNTP mixture: 7-deaza-dGTP was used instead of dGTP

PCR conditions:

94°C, 1 min.
↓
98°C, 20 sec.
68°C, 5 min. } 30 cycles

Thermal cycler: TaKaRa PCR Thermal Cycler 480*

Enzymes: *TaKaRa LA Taq*® – LA PCR Kit Ver. 2.1 (Cat.# RR013A)
TaKaRa Ex Taq® – *Premix Ex Taq*™ DNA Polymerase Hot Start Version (Cat.# RR030A)

Results:

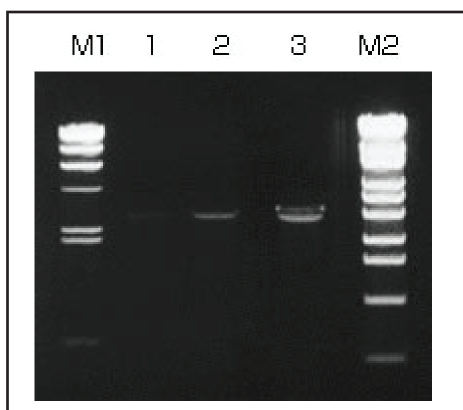

Figure 1. Amplification of a GC-rich fragment using various DNA polymerases. PCR products were amplified using either conventional *Taq* (lane 1), *Ex Taq*® (lane 2), or *LA Taq*® (lane 3) and were run on a 1% L03 agarose gel (8 µl PCR reaction per lane). Lanes M1 and M2 contain molecular weight markers (λ-*Hin*d III digest and λ-*Eco*T14 digest, respectively).

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

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Using 7-deaza-dGTP instead of dGTP in the PCR reaction can improve amplification of GC-rich templates. In addition, further optimization using a mixture of dGTP and 7-deaza-dGTP (or dITP) can also improve amplification efficiency (Figure 2).

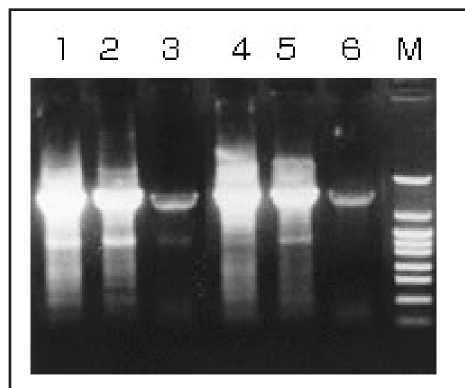

Figure 2. Amplifications of a GC-rich fragment using various ratios of dGTP and 7-deaza-dGTP or dITP. PCR products were amplified using either *LA Taq*® (lanes 1–3) or *Ex Taq*® (lanes 4–6) and were run on a 1% L03 agarose gel (10 µl). The PCR reactions contained various combinations of dGTP, dITP, and 7-deaza-dGTP as indicated in the table above. Lane M contains a pHY molecular weight marker.

Lane 1: dGTP:dITP = 3:1
Lane 2: dGTP 7-deaza-dGTP = 3:1
Lane 3: 7-deaza-dGTP alone

Lane 4: dGTP:dITP = 3:1
Lane 5: dGTP:7-deaza-dGTP = 3:1
Lane 6: 7-deaza-dGTP alone

Conclusions:

A GC-rich fragment that was not efficiently amplified using conventional *Taq* could be successfully amplified with both *TaKaRa Ex Taq*® and *TaKaRa LA Taq*® DNA polymerases.

Amplification of GC-rich target sequences can be improved by replacing dGTP with dITP or 7-deaza-dGTP. Depending on fragment to be amplified, the optimal ratio of dGTP to 7-deaza-dGTP (or dITP) may vary. Therefore, we recommend evaluating various ratios to determine the ideal conditions.