PrimeSTAR® GXL (Cat.# R050A/B)

Application: Use of PrimeSTAR® GXL to Amplify Plant Genomic DNA

Plant DNA samples may contain PCR inhibitors such as polyphenols, tannins, polysaccharides, or excess nucleic acids. As a result, some PCR polymerases do not amplify plant DNA samples efficiently.

Genomic DNA was extracted from *Arabidopsis thaliana*, tomato, or spinach leaf tissue samples. The samples were prepared using Takara Bio Plant DNA Isolation Reagent (Cat.# 9194), which uses a simple benzyl chloride extraction method. Two PCR enzymes were used for PCR amplification of target genes: *TaKaRa Ex Taq*® Hot Start Version (Cat.# RR006A) or the high-fidelity enzyme PrimeSTAR® GXL DNA Polymerase (Cat.# R050A).

Methods

Leaf tissue (10- to 20-mg samples each) was collected from young leaves of *Arabidopsis*, young leaves of tomato, or mature leaves of spinach. To prepare DNA, Takara Bio Plant DNA Isolation Reagent was used according to the product User Manual. No additional purification steps were performed.

For PCR, 2 µl of genomic DNA was used as template in a 25-µl reaction according to the recommended conditions for each enzyme. Reactions were analyzed by agarose gel electrophoresis (see Figure 1). The amount of template used per reaction ranged from 2 µl of undiluted DNA sample (lane 1) to 2 µl of a 40-fold dilution (lane 6).

Target genes:

– Arabidopsis *MERI5B* gene encoding xyloglucan: xyloglucosyl transferase (approx. 1.0 kb)
– Tomato *XET* gene encoding xyloglucan endotransglycosylase (approx. 0.6 kb)
– Spinach *coxI* gene encoding Cytochrome c oxidase subunit 1 (approx. 0.5 kb)

Results

Amplification products are shown in Figure 1. Lanes correspond to the template used for each PCR reaction (2 µl per 25-µl reaction): Lane 1, undiluted DNA sample; lane 2, 2-fold dilution; lane 3, 5-fold dilution; lane 3, 10-fold solution; lane 5, 20-fold dilution; lane 6, 40-fold dilution. M, 250 bp DNA Ladder.

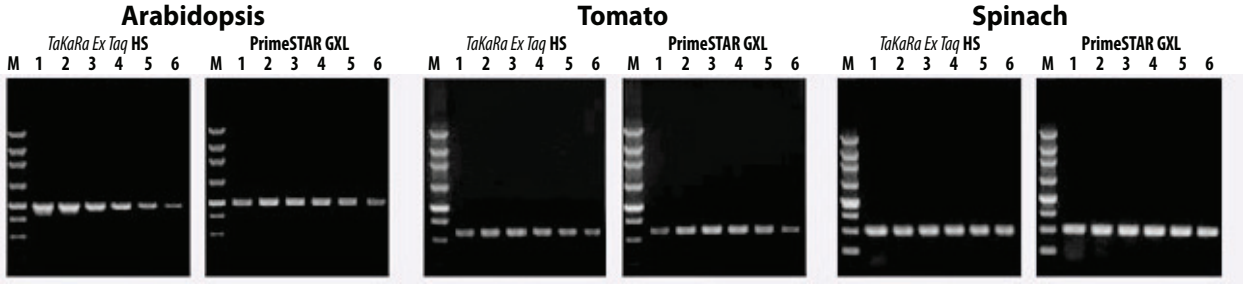

Figure 1. Amplification of gene targets from genomic DNA extracted from *Arabidopsis*, tomato, or spinach leaf using either *TaKaRa Ex Taq* Hot Start Version or PrimeSTAR GXL DNA Polymerase.

Conclusions

Efficient amplification from plant genomic DNA template was observed with both TaKaRa *Ex Taq* Hot Start Version and PrimeSTAR GXL DNA Polymerase. This is particularly notable for PrimeSTAR GXL, as most high-fidelity PCR enzymes do not amplify efficiently in the presence of excess nucleic acids and/or endogenous PCR inhibitors commonly found in plants such as polyphenols or tannins. For applications requiring accurate amplification such as cloning and expression, PrimeSTAR GXL is recommended.