Application Note

Thermo Scientific Phire Plant Direct PCR Kit: Plant Genotyping And Transgene Detection Without DNA Purification

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Abstract

Thermo Scientific™ Phire™ Plant Direct PCR Kit enables DNA amplification directly from various plant tissues. Here we present simple and robust protocols for plant genotyping and transgene detection without the need to purify the DNA before PCR. This saves both time and cost. In this application note the Thermo Scientific™ Piko™ Thermal Cycler and UTW PCR vessels were used to obtain optimal results in the minimal amount of time.

Introduction

PCR-based target DNA detection has many applications in plant research, such as plant genotype analysis and verification of RNAi transgenes. PCR from plant tissues traditionally involves an initial DNA isolation step which may require expensive or toxic reagents, and is time consuming with an increased risk of cross contamination[1,2]. However, using the Phire Plant Direct PCR Kit the DNA target can be detected easily, without prior DNA extraction.

Here we demonstrate that the presence of a transgene (or an RNAi vector) in gerbera plant individuals can be detected easily both from plant leaves and petal tissue without prior DNA extraction. In another model we present an example of a derived cleaved amplified polymorphic sequence analysis (dCAPS)[3] performed directly from *Arabidopsis* plant leaves.

Materials and Methods

- Phire Plant Direct PCR Kit
- SspI
- 24-well Piko Thermal Cycler
- Piko PCR Plates
- Gerbera and *Arabidopsis* tissues

Gerbera Leaves And Petal Tissues

Direct protocol: A 0.50 mm punch was cut from a plant leaf using the Harris Uni-Core™ (included in the kit). The samples were placed directly into 20 µL PCR reactions. Reactions were run using a 24-well Piko Thermal Cycler and Piko PCR Plates. The resulting PCR products were analyzed on agarose gels.

Arabidopsis Leaves

Direct protocol: A 0.50 mm punch was cut from a plant leaf using the Harris Uni-Core™. The samples were placed directly into 50 µL PCR reactions. Reactions were run using a 24-well Piko Thermal Cycler and Piko PCR Plates.

Restriction digestion: After PCR, the reactions were spun down. The restriction digestion reactions were performed with SspI directly in the supernatants, and the resulting fragments were analyzed on agarose gel. *Note: When amplifying DNA directly from plant tissues, the PCR products include plant and PCR derived components that may interfere with the restriction digestion enzyme.* Therefore, it may be necessary to either dilute (such as 1:2 or 1:3 in water) or purify the PCR product before the subsequent digestion.

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Table 1. Reaction conditions for PCR.

Components	20 µL Reaction	50 µL Reaction	Final Conc.
H_2O	add to 20 µL	add to 50 µL	
2x Phire Plant PCR Buffer	10 µL	25 µL	1x
primer A	x µL	x µL	0.5 µM
primer B	x µL	x µL	0.5 µM
Phire Hot Start II DNA Polymerase	0.4 µL	1 µL	
Plant tissue	0.5 mm punch	0.5 mm punch	

Table 2. Cycling conditions.

	3-step protocol		
Cycle Step	Temp.	Time	Cycles
Initial denaturation	98 °C	5 minute	1
Denaturation Annealing Extension	98 °C X °C 72 °C	5 s - 20 s	40
Final extension	72 °C 4 °C	1 minute hold	1

Results and Discussion

In this application note we describe the performance of the Phire Plant Direct PCR Kit in two target DNA detection approaches. First we demonstrated that an RNAi vector can be easily detected from gerbera plants without any template purification (Figure 1). Twelve gerbera individuals were tested by simply placing a 0.50 mm punch of leave or petal tissue into a PCR reaction. Five individuals gave a positive signal with RNAi transgene-specific primers indicating the presence of the RNAi vector.

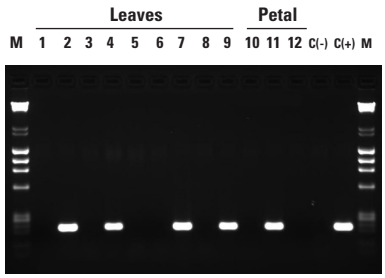

Figure 1. Detection of RNAi vector in gerbera plants using the Thermo Scientific Direct PCR approach. Punches (0.50 mm) from plant leaves or petal tissues were placed directly in 20 µL PCR reactions, and 210 bp products were amplified using transgene-specific primers. The results indicate that plant individuals corresponding to samples 2, 4, 7, 9 and 11 contain the RNAi transgene. The obtained results were confirmed by conventional analysis of CTAB DNA extraction followed by PCR (data not shown).

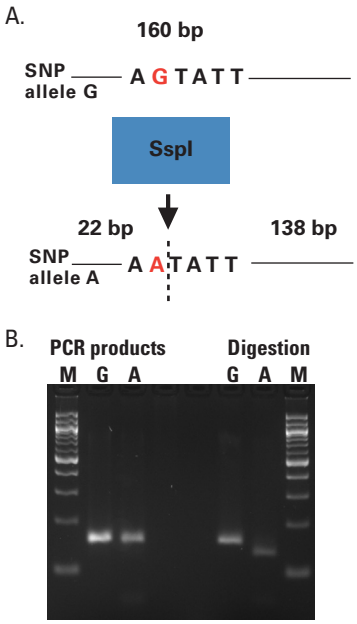

Figure 2. Genotyping of *Arabidopsis* plant individuals with dCAPS technique directly from leave punches. Punches (0.50 mm) from plant leaves were placed directly in 50 µL PCR reactions, and 160 PCR products containing the SNP site (G or A allele) were amplified with primers introducing a unique SspI site to the A-allele. The unpurified PCR products were digested with SspI restriction enzyme. The resulting fragments were analyzed on 3% agarose gel. M, size marker; A and G correspond to the SNP alleles of each sample. The obtained results were confirmed by conventional analysis of DNA extraction followed by PCR and restriction digestion.

The results were confirmed with a conventional DNA extraction method using hexadecyltrimethylammonium bromide (CTAB; data not shown). The sample material was also tested with the control primer set supplied with the kit to ensure the reagents and samples were in good condition (data not shown).

Next we tested the suitability of the Phire Plant Direct PCR Kit to dCAPS genotyping. In dCAPS genotyping assays, single nucleotide polymorphisms (SNPs) are identified by SNP allele specific restriction endonuclease digestion. The restriction site within the SNP of interest is either introduced or destroyed by PCR using a primer with one or more mismatches to the target DNA. After PCR, the PCR products are digested and the resulting fragments revealing the genotype of each individual are analyzed on agarose gel.

In this example the SNP site of interest in the *Arabidopsis* genome was amplified from plant leaf punches with the Phire Plant Direct PCR Kit. The forward primer contained one mismatch at the 3'-end creating an *SspI* specific restriction site in the target DNA which included the SNP of interest (A-allele) but not in the other allele of that particular SNP (Figure 2). Note that even though the 3'-5'-exonuclease activity of the Phire Hot Start II DNA Polymerase is moderate, it was necessary to use one 3'-terminal phosphorothioate (**PTO**) modification in the 3'-end of forward primer. The PCR product was digested with SspI directly in the PCR reaction mix. Agarose gel analysis revealed high yields of digested product. This result confirmed that no DNA purification is required prior to dCAPS genotyping when using the Phire Plant Direct PCR Kit.

Acknowledgements

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References

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2. C.S. Kim, C.H. Lee, et al., A simple and rapid method for isolation of high quality genomic DNA from fruit trees and conifers using PVP. *Nucleic Acids Res.* 25(5), 1085-1086 (1997).
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