MyTaq™ Plant-PCR Kit

A Powerful Tool for Plant Genome Analysis

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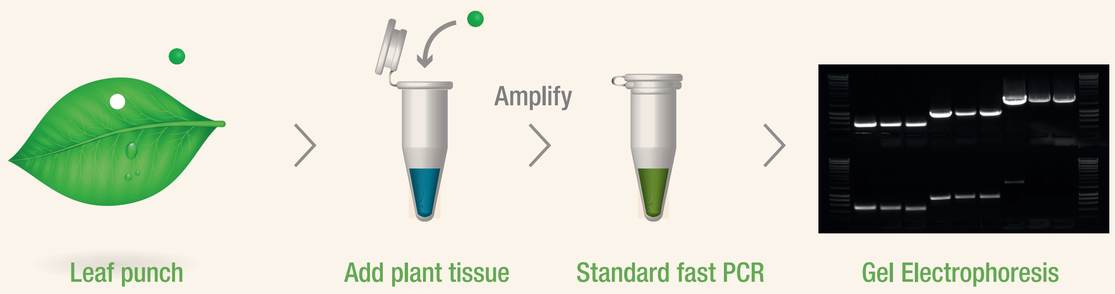
Introduction

Direct amplification of DNA from plant leaf samples is a fast and convenient technique which avoids the need for laborious and time consuming nucleic acid extractions prior to PCR. However, obtaining consistent and satisfactory results can be challenging, given the wide diversity in plant physiology and cell wall structure.

In order to be amplified, genomic DNA needs to be accessible after efficient cell lysis, a process hampered by the plant cell wall. The composition and structure of the cell wall varies between plant species and is the main physical challenge to successful PCR. Once the cell has been lysed, not only is the DNA available for amplification, but also all the metabolites and cell constituents are released and can pose inhibition problems to downstream processes, a secondary chemical challenge to successful PCR.

We present a solution (MyTaq Plant-PCR Kit) for overcoming both physical and chemical challenges, which allows amplification of DNA from plant leaf samples added directly to the PCR mixture, without the need for pre-purification of genomic DNA.



Robust Direct PCR

The MyTaq Plant-PCR Kit directly amplifies DNA from both fresh (Fig. 1a, 1b, 1c) and dry (Fig. 1d) leaf samples from various species demonstrating the robustness of the PCR chemistry. This ability to amplify DNA from a variety of plants is an advantage for assay development as it reduces lengthy optimisation steps. All experiments, here and below, were performed on a Mastercycler epgradient S PCR machine (Eppendorf UK).

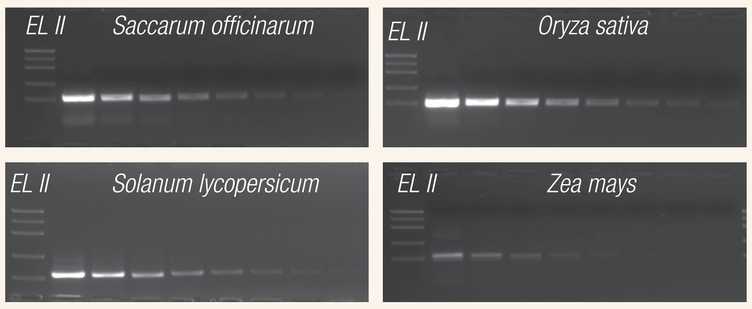


Figure 1a: Direct amplification from fresh plant samples: Amplification of a 0.5 kb fragment was carried out directly from leaf punches (Ø1.2 mm) from *Saccharum officinarum, Oryza sativa, Solanum lycopersicum* and *Zea mays*. The following PCR cycling conditions were used: 95°C – 3 min; followed by 40 cycles of 95°C -15 s; 58°C– 15 s and 72°C – 45 s. A two-fold serial dilution of the PCR product was run on a 1% agarose gel, (0.5 µg/mL EtBr staining). Mw marker EasyLadder II *(ELII)* (Bioline Reagents Ltd).

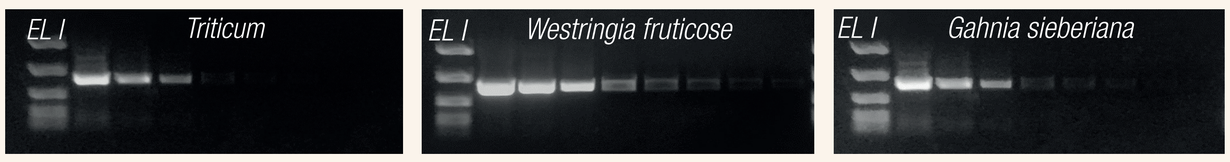


Figure 1b: Direct amplification from fresh plant samples: Amplification of a 0.7 kb fragment was carried out directly from leaf punches (Ø1.2 mm) from *Triticum, Westringia fruticose* and *Gahnia sieberiana*. The following PCR cycling conditions were used: 95°C – 3 min; followed by 40 cycles of 95°C -15 s; 51°C – 30 s and 72°C – 45 s. A two-fold serial dilution of the PCR product was run on a 1% agarose gel, (0.1 µg/mL EtBr staining). Mw marker EasyLadder I *(EL I)* (Bioline Reagents Ltd).

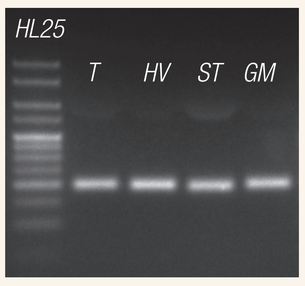


Figure 1c

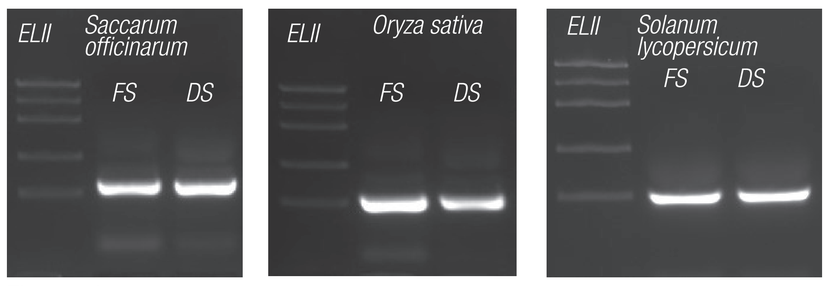


Figure 1d

Figure 1c: Direct amplification from fresh plant samples: Amplification of a 92 bp fragment was carried out directly from leaf punches (Ø1.2 mm) from *Triticum (T), Hordeum vulgare (HV), Solanum tuberosum (ST)* and *Glycine max (GM)*. The following PCR cycling conditions were used: 95°C – 3 min; followed by 40 cycles of 95°C -15 s; 60°C– 15 s and 72°C – 45 s. 20 µL of the PCR reaction was run on a 1% agarose gel, (0.5x Sybr Safe, ThermoFisher, staining). Mw marker HyperLadder™ 25 bp *(HL 25)* (Bioline Reagents Ltd).

Figure 1d: Amplification from dried samples: Amplification of a 0.5 kb fragment from fresh *(FS)* and dried *(DS)* leaf punches (Ø1.2 mm) from *Saccharum officinarum, Oryza sativa* and *Solanum lycopersicum*. Dry samples were pre-treated with 100 µL of extraction buffer (1.25% w/v SDS) and incubated at 95°C for 5 minutes. 1 µL of this extract was then used as template in a 50 µL PCR reaction. The following PCR cycling conditions were used: 95°C – 3 min; followed by 40 cycles of 95°C -15 s; 58°C – 15 s and 72°C – 45 s. 20 µL of the PCR reaction was run on a 1% agarose gel, (0.5 µg/mL EtBr staining). Mw marker EasyLadder II *(EL II)* (Bioline Reagents Ltd).

Tolerance to PCR Inhibition

Tolerance to PCR inhibitors is critical to the performance of a direct PCR mix. An overloading experiment was carried out to test the resistance of the MyTaq Plant-PCR Kit and no effects on the yield or specificity of the PCR were observed.

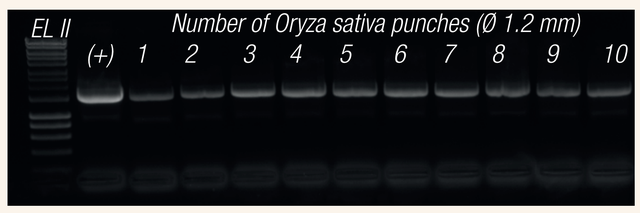


Figure 2: Leaf punch overloading: Amplification of a 0.5 kb fragment from *Oryza sativa* was carried out in the presence of up to 10 leaf punches (Ø1.2 mm). Purified DNA *(+)* from *Oryza sativa* was used as a positive control. The following PCR cycling conditions were used: 95°C – 3 min; followed by 40 cycles of 95°C -15 s; 58°C – 15 s and 72°C – 45 s. 20 µL of the PCR reaction was run on a 1% agarose gel, (0.5 µg/mL EtBr staining). Mw marker EasyLadder II *(EL II)* (Bioline Reagents Ltd).

Speed

The compatibility of MyTaq Plant-PCR chemistry with fast cycling protocols was tested. The results (Fig. 3) demonstrate that the MyTaq Plant-PCR Kit is perfectly suited to fast conditions allowing 1 kb amplification in just 20 minutes.

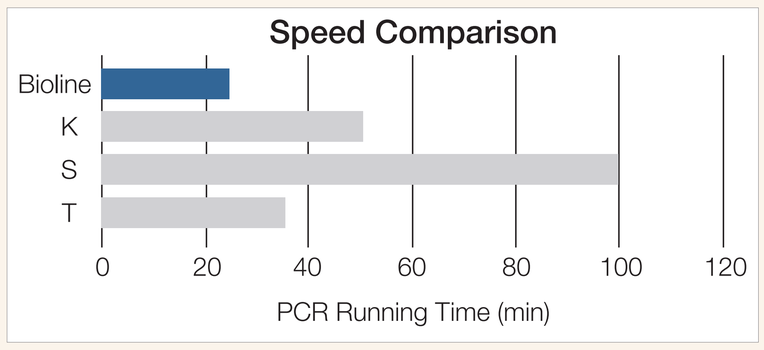


Figure 3: Fast amplification of a 1 kb fragment from *Oryza sativa*: Comparison of PCR running times as given by Mastercycler epgradient S thermocycler (Eppendorf) for MyTaq Plant-PCR Kit and suppliers S, K and T. The PCR cycling conditions used for each kit followed the manufacturers recommendations.

Multiplexing

The multiplexing ability of MyTaq Plant-PCR Kit was used to screen several potato cultivars for H1-conferred potato cyst nematode (PCN) resistance (Fig. 4). Clear and distinct bands were observed, allowing clear genotyping of all the screened cultivars.

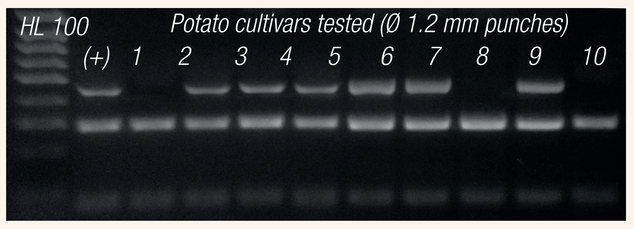


Figure 4: Multiplex amplification: Screening of 10 potato cultivars for potato cyst nematode (PCN) resistance was carried out with a 3-plex assay targeting 57R (452 bp), BCH (290 bp) and PhyB (80 bp) regions of the genome [1]. The following PCR cycling conditions were used: 95°C – 3 min; followed by 40 cycles of 95°C -15 s; 55°C – 15 s and 72°C – 15 s. 20 µL of the PCR reaction was run on a 1% agarose gel (0.5 µg/mL EtBr staining). 3 bands for 57R, BCH and PhyB indicate resistant cultivars, while 2 bands for BCH and PhyB indicate non-resistant cultivars. Mw marker HyperLadder 100 *(HL 100)* (Bioline Reagents Ltd).

Compatibility with Sanger Sequencing Chemistry

In order to speed up the DNA analysis workflow, the interaction of MyTaq Plant-PCR Kit with Sanger sequencing chemistry was investigated (Fig 5). PCR products with different content of MyTaq Plant-PCR Kit buffer were sequenced and no significant difference in results was observed.

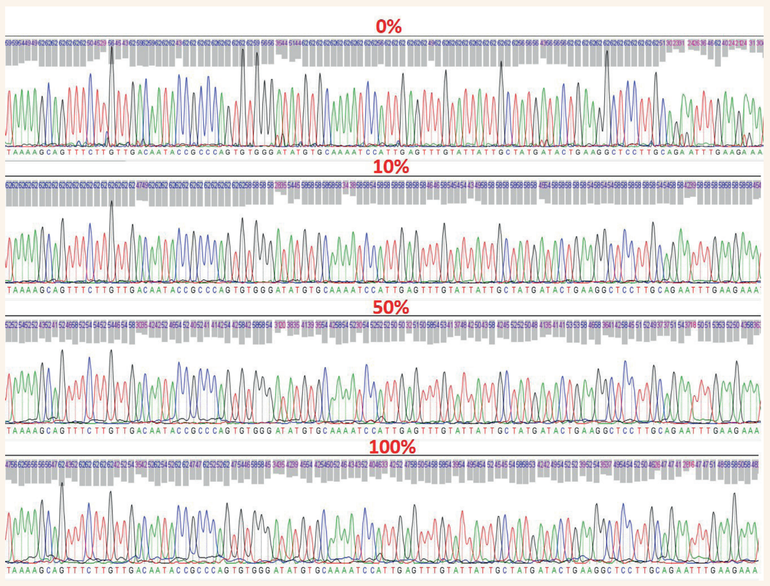


Figure 5: Compatibility with Sanger sequencing chemistry: PCR products artificially contaminated with various amounts of MyTaq Plant-PCR Kit (0 to 100%) were sequenced and the quality of the resulting data was compared.

Conclusion

MyTaq Plant-PCR Kit is a fast and robust solution for direct amplification of DNA from a variety of plant leaf samples. It produces specific and consistent results without the need for complicated purification steps, making it a powerful tool for plant genome analysis.

References:

[1] Schultz, L., Cogan, . N. O. I., McLean, K., Dale, M. F. B., Bryan, . G. J., Forster, . J. W. and Slater, A. T. Evaluation and implementation of a potential diagnostic molecular marker for H1-conferred potato cyst nematode resistance in potato (Solanum tuberosum L.). Plant Breeding, 131: 315–321. doi:10.1111/j.1439-0523.2012.01949.x (2012).

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