*Plant Molecular Biology Reporter* **18:** 61–64, 2000


*Protocols*

# A Rapid Method for High Throughput DNA Extraction from Plant Material for PCR Amplification

EVE DILWORTH and JUERG E. FREY*
*Swiss Federal Research Station for Fruit-Growing, Viticulture and Horticulture; CH-8820 Wädenswil; Switzerland*

**Abstract.** A rapid and reliable method is described for high throughput extraction of DNA from plant material using glass beads in a flat-bottomed microtitre plate. This procedure is quick, inexpensive, and allows up to 96 samples to be processed in parallel. PCR products produced by the recovered DNA are consistently equivalent to those produced through traditional extraction methods.

## Introduction

Marker assisted plant breeding requires the screening of a large number of progeny to identify resistant offspring. We are investigating apple tree (*Malus domestica* Borkh.) resistance to fungal diseases using molecular markers for scab (*Venturia inaequalis* (Cooke.) G. Wint) and powdery mildew (*Podosphaera leucotricha* (Ell. & Ev.) Salm). Identification of resistant plants by symptom assessment can often lead to misclassification. Furthermore, phenotypic assessment of powdery mildew resistance is inconsistent in young trees (Seglias et al., 1997), making it necessary for the trees to be maintained for several years before reliable classification is possible. A reliable molecular method is important. Although primers for the detection of the mildew resistance (Pl1) have been formulated (Markussen et al., 1995), we found that 60% of the time required for sample processing, from leaf collection to PCR results, was used for DNA extraction. This represented a serious bottleneck, but here we describe a new extraction method based on a 96-well microtitre plate. This reduces the extraction time while keeping the cost low and avoiding the use of hazardous chemicals.

## Materials and Methods

Extraction buffer[1] (prepared with high purity Milli-Q A10 system water, autoclaving not required):

10 mM Tris-HCL pH 8.0
1 mM EDTA

*Author for correspondence. e-mail: Juerg.Frey@faw.admin.ch; fax: 00 41 17 80 63 41; ph: 00 41 17 83 63 32.
[1] Modified from Kawaski 1990.

0.5% Tween 20
50 mg/ml Qiagen Proteinase K
Plant material: Freeze-dried young apple leaves
Technical equipment: Eppendorf Comfort Thermomixer

*DNA extraction*

1. Using the tip of a standard Pasteur pipette, cut two circular sections of leaf material (1-2 mm diameter). Transfer them to the well of a flat-bottomed microtitre plate (Polylabo SA) using a sterile wooden toothpick.
2. Add three glass beads (3 mm diameter) and 100 µl extraction buffer to each well used.
3. Cover the microtitre plate using 96-well plastic plate covers (Polylabo SA) or Scotch Tape pads (Qiagen)[2].
4. Using an Eppendorf comfort thermomixer, incubate the microtitre plate at 65°C for 10 min with intermittent shaking (1500 rpm, 1 min alternate intervals of shaking and resting).
5. Centrifuge the plate briefly at 1350 rpm.
6. Repeat steps 4 and 5 twice[3].

*PCR amplification*

The samples were used directly for PCR with AT20 primers (Markussen et al. 1995). The total PCR reaction volume was 12.5 µl and contained 0.4 µl of extraction product, 10 mM Tris HCL pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatine, 0.2 mM each dATP, dCTP, dGTP, dTTP, 0.2µM each primer AT20forward and AT20reverse, and 0.5 units *Taq* Polymerase (Ampligene). Amplifications were carried out in a Perkin Elmer GeneAmp PCR System 9600. The initial step of 95°C for 5 min was followed by 36 cycles of 94°C for 30 s, 62°C for 1 min and 72°C for 2 min and 1 cycle of 10 min at 72°C. PCR products were electrophoresed on 1% agarose gels, either at 70 volts with a 15 lane gel for 50 min or at 150 volts in a 32 lane gel for 105 min.

## Results and Discussion

This method was routinely used to screen apple leaves for the mildew resistance gene PI1 using AT20 primers (Markussen et al., 1995). Two primer sets AL07s and M18 (Gianfranceschi et al., 1999) for identifying the scab resistance Vf gene also worked well with DNA obtained with this extraction method. Previously, DNA was extracted following the method of Koller et al. (1999) that uses Qiagen Lysis buffers and Chloroform:Isoamylalcohol. The results from PCR amplification from DNA extracted using these two methods were of equivalent quality (Figure 1).

Standardising the amount of plant material used for extraction and the amount of homogenate used in PCR is important as too much can inhibit the PCR reaction. This inhibition is most likely due to a higher concentration of plant

[2] Important to avoid contamination between samples.
[3] Results obtained after only one repetition but greatly improved after two repetitions.

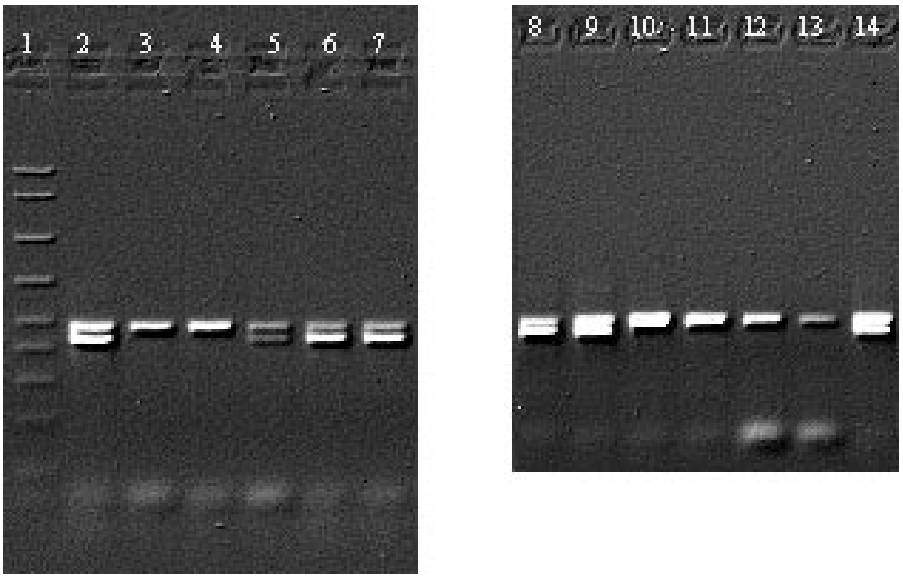


*Figure 1*. Comparison of PCR amplifications from two DNA extraction methods. All samples were amplified with AT20 primers that identify PL1. The lower 450 bp fragment is only present in mildew-resistant plants. The upper 500 bp fragment occurs in susceptible and resistant plants. Lanes 2-7: PCR product from DNA extracted using the method of Koller et al. (1999); Lanes 8-14: PCR from DNA extracted using the new method; Lane 1: AmpliSize Molecular Ruler 50-2000 bp Ladder (BioRAD).

proteins and secondary components. For the initial extraction, two leaf sections should be taken using the tip of a Pasteur pipette, as described. If four or more are added or the sections are cut inaccurately, the success rate of subsequent PCR reactions is reduced. Using a fluorometer, the DNA was quantified from the extraction mixture of a number of samples, each containing between 0.5-2.0 ng/µl DNA. After test PCR reactions with varying amounts of extraction mix, from 0.25 µl to 3 µl, it was found that 0.4 µl produced the best results. PCR was inhibited if 1-3 µl were added and only weak banding patterns could be seen if smaller amounts (0.25-0.3 µl) were used.

Denaturation of Proteinase K in the extraction buffer is essential since this also inhibits PCR. We found that a five minute 95°C initial PCR step was sufficient to inactivate this enzyme. Six-hundred-and-fifty plants were analysed. First round PCR from 73% of the plants produced clear results, 25% required repetition PCR or quick extraction and PCR. For only 2% it was necessary to return to the previous extraction method.

Although high-tech equipment is currently available for DNA extraction via grinding, the technique described is a simple and inexpensive alternative. The material costs for our extraction method were six to seven fold cheaper than the materials for the previous method, reducing the cost per sample to $0.14.

Furthermore, lengthy and hazardous chloroform precipitation is not required. DNA extraction time was reduced by at least 50% compared to the previous extraction method, making it possible to process 96 samples in 2 h. The DNA recovered is stable and of sufficient quality for excellent PCR results.

**Acknowledgements**

We would like to thank Dr. B. Koller for supplying PCR primers and Dr. T. Adams, Dr. C. Gessler and Dr. M. Kellerhals for advice and critical reading of the manuscript.

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