

Full Length Research Paper

Optimization of FTA technology for large scale plant DNA isolation for use in marker assisted selection

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Accepted 6 February, 2006

Conventional methods for DNA acquisition and storage require expensive reagents and equipments. Experimental fields located in remote areas and large sample size presents greater challenge to developing country institutions constrained financially. FTA™ technology uses a single format utilizing basic tools found in laboratory. In this study, FTA™ cards were used to collect over 3000 samples from a field located 130 km and used to screen progeny plants using PCR-marker-assisted selection. PCR product yields and quality are sufficient for reliable scoring, distinguishing heterozygous from homozygous plants using ABI 3730 sequencer. Results indicate that this method is faster, easier and in-expensive.

Key words: FTA™, large-scale, DNA sampling, field set up, marker assisted selection.

INTRODUCTION

Plant DNA sample collection, isolation, purification and storage are pre-requisite to any downstream DNA molecular application. Currently, most convention methods for plant DNA require liquid nitrogen for collection of fresh tissue material, and grinding of both fresh material and lyophilized tissue. Lyophilisation of fresh tissue is also often use. Transportation of the fresh tissue samples to the laboratory requires cold temperatures to prevent DNA degradation. DNA isolation and purification is then performed using various methods including commercial DNA extraction kits, CTAB (Saghai-Marrof et al., 1984), phenol/chloroform, sodium dodecyl sulphate (SDS), microwave treatment of DNA (Saini et al., 1999) among others. Upon DNA extraction and purification, there is need for +4°C to -20°C fridges/freezer for storage. In addition, specialized equipments such as centrifuges, lyophilizer, fume hood, and special disposal for hazardous chemicals are required. All these requirements contribute greatly to the overall expense, which may be quite limiting to a simple laboratory in the

developing countries. More importantly, such methods become a big challenge when handling very large numbers of samples, and when the samples are to be collected from a field that is located in a remote area far from the laboratory.

A different method for collecting, and storing biological material using a solid matrix was first described for blood samples for diagnostics (Guthrie and Susi, 1963), and later for PCR for medical and forensic application (Nelson et al., 1990; Carducci et al., 1992). Today, a number of collection papers are available from different manufactures including IsoCode card (Schleicher and Schull, Dassel, Germany), FTA™ cards, and Generation Capture System (Biozym Diagnostik GmbH, Hessisch-Oldendorf, Germany).

Flinders Technology Associates (FTA™) is a simple technology that reduces the steps of DNA collection, transportation, purification and storage and consequently, reducing the cost and time required to process a DNA to the final step of purified DNA ready for down stream application. FTA™ classic card (Whatman Inc., Clifton, NJ) is a whatman paper that has been impregnated with a patented chemical formulation that lyses cells, captures and immobilizes nucleic acids in the paper matrix, in addition to having denaturing, chelating and free radical trap all geared towards preventing nucleic acids damage (<http://www.whatman.com>). The chemicals are non-organic and safe to use. Application of the plant DNA is

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done using very simple method of pounding the leaf material directly onto the FTA paper applying moderate pounding/pressure with a blunt object such as a pestle (Lange et al., 1998; Lin et al., 2000) and stapler (Drescher and Graner, 2002). This method is very simple and applicable to field conditions that are far from the laboratory in remote areas, and also for large number of samples. Storage is carried out at room temperature for long period. Genomic DNA stored on FTA Cards at room temperature for over 14 years has been successfully amplified by PCR. In contrast, genomic DNA stored at room temperature on non-FTA Cards for over six months did not amplify. Sample integrity is optimized when FTA cards are stored in a multi-barrier pouch with a desiccant Packet. FTA Cards offer a compact room-temperature storage system that reduces the need for precious freezer space. Another advantage of FTA is that it can be scaled up for high throughput (Stanislav and David, 2005; Belgrader et al., 1997). Whatman has automated the purification procedure of DNA stored on FTA Cards using Beckman Coulter Biomek 2000 liquid.

Molecular breeding often requires sampling tens of thousands of individual plants for DNA to be used in PCR-marker assisted selection. FTA technology has been used for a number of plant species ranging from simple to complex genomes including mono and dicots (Lin et al., 2000), as well as large and highly repetitive genome (Drescher and Graner, 2002). In addition, the nucleic acids on FTA can be used for a number of downstream applications including PCR, RAPD, AFLP, RT-PCR among others. FTA has been used in a wide range of fields including animal identification, molecular biology (conservation and genotyping, diversity, MAS), forensics, food and agriculture, testing drug discovery, transgenic, transfusion medicine, plasmid screening, diagnostics, STR analysis, pharmacogenomics, and genomics (Moscoso et al., 2004; Drescher and Graner, 2002; Becker et al., 2004; Halbert et al., 2004; Hsiao et al., 1998).

In the present study, FTA was used to collect over 3000 individual maize plants from an experimental field in a remote area.

MATERIALS AND METHODS

DNA collection

The experimental field was located in a remote place with high temperatures of 30°C approximately 130 km from the laboratory. There were 3,508 BC1F1 maize progeny plants of crosses between QPM and non-QPM inbred lines. All the plants were tagged individually before sampling. DNA collection was done on 3 month old plants using the whatman FTA card. One FTA classic card measures 7.5 x 13 cm, and they were labelled prior to the day of sampling. Briefly, the second or third leaf was excised from the plant, wrapped round the FTA paper strip, and placed in a small polythene bag. A pair of pliers was used to press the leaf sample extract on to the FTA paper until both sides of the FTA were soaked. Ethanol (70%) was used to clean pliers in between

samples to prevent cross contamination, in cases where the polythene bag had cut exposing the pliers to the plant tissue. The FTA card was then hanged on the drying line using a paper clip for air drying for 2 to 5 h, and later stored in an air tight plastic container. For QPM and non-QPM inbred lines, one month old plants were used as DNA source for both FTA and CTAB. Briefly, a young leaf was excised from the plant, placed in falcon tube and immediately dropped in liquid nitrogen. DNA was immediately extracted from the plant tissue using modified CTAB method (Doyle and Doyle, 1990). DNA samples were ran in 0.8% agarose gel for quality check and stored at -20°C until used for PCR.

FTA processing for PCR

Processing of FTA for PCR was performed as described by the manufacturer, with modification. Briefly, 2 FTA discs measuring 1.2 mm each were punched from the FTA disc using 1.2 mm Harris Micro punch (whatman, Inc. US), and placed in a 96-well or 384-well PCR plate containing 50 µl and 35 µl of FTA wash solution, respectively. The PCR plate was incubated at room temperature for 15 min with shaking, and the solution removed with pipettor. FTA purification reagent (50 µl) was used twice, followed by one rinse with 100 µl of double distilled water for 5 min, and once with 50 µl absolute ethanol for 5 min. We also checked the use of double distilled water instead of TE buffer (10 mM Tris-HCL, 0.1 mM EDTA, pH 8). The FTA discs were dried in an oven for 15 min at 56 °C and ready for PCR amplification. Washing was carried out directly in the PCR tubes/plates to minimize the tedious step of transferring discs from wash tubes/plate to new PCR tubes/plate. For extracted genomic DNA, 5 µl was used as template.

PCR amplification and product analysis

Amplification reaction contained 20 µl of PCR mix (1X Reddymix, 3 mM MgCl₂, 1.25 U Taq, 0.2 mM dNTPs, 40 pM each primer) and 2 FTA disc or 5 µl of CTAB extracted gDNA. PCR amplification was carried out in iCycler thermocycle from Bio-Rad using a profile of initial denaturation for 2 min at 94°C, and 40 cycles of denaturation for 1 min at 94°C, annealing temperature of 62°C for 2 min, extension for 2 min at 72

We compared three different methods for PCR product analysis using agarose gel electrophoresis (2-4% normal agarose, 4% metaphor agarose at a ratio of 2:1 metaphor: saekem), denaturing polyacrylamide gel electrophoresis (PAGE) and ABI Prism sequencer (ABI Prism 377 DNA sequencer, and ABI Prism 3730 capillary sequencer). PCR products of the progeny were analysed using ABI3730 sequencer as the polymorphism between parents and progeny was very low (less than 10 bp) and difficult to resolve using the metaphor agarose. Briefly, 12 µl of 500LIZ size standard was mixed with 1 ml of HiDi formamide, 9 µl of this mix added to 1 µl of PCR product and sent for ABI 3730 analysis.

RESULTS AND DISCUSSION

For FTA technology, plant sample application is the step with greatest limitation as it is labour intensive, especially for a crop like maize that has very tough leaf tissue, and a lot of time is spent on one sample. Previous work show that amount of plant material applied on FTA is critical and enough sap has to be pressed until it is observed on the other side of the FTA (Drescher and Graner, 2002). To optimize on this, we wrapped plant tissue round the FTA strip, so that both sides of the paper were in direct

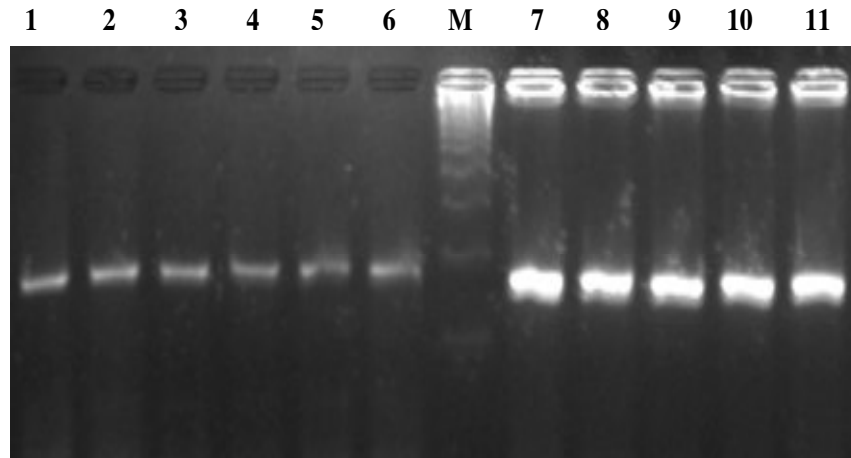


Figure 1. Comparing FTA discs and CTAB extracted DNA samples amplified by primer umc1066 on 4% metaphor agarose. M denotes 50 bp DNA ladder. Lanes 1-6 contains FTA discs and lanes 7-11 CTAB extracted gDNA of maize inbred lines

contact with leaf tissue, increasing the amount of sap applied. The time taken per sample was greatly reduced when using pliers instead of stapler or pestle, while the amount of sap applied was also increased. One person could perform an average of 100 samples per day. The age of the plant at the point of sampling was also critical with older plants (over three months) being more difficult to sample compared to young plants (about one month old) increasing the time required per sample. Weather and time of the day were also critical, with sampling early in morning giving better results than later in the day when temperatures were very high causing the plants to loose moisture. Use of pliers was also more suited for field set up as requirement for a table for pounding was not necessary.

When punching discs, care must be taken to prevent cross contamination because the dry FTA discs have static charge and tend to jump out of the well. This was solved by applying FTA purification reagent into the wells before the disc punching. To further reduce costs, double distilled water was used instead of TE buffer and the amount of FTA purification reagent was also reduced from 200 μ l to 50 μ l. Cost cutting was further achieved by using a single PCR tub/plate for cleaning as well as for PCR reaction. Substituting FTA purification reagent with other wash solutions has been shown to result to both lower product yield and complete PCR failures (Drescher and Graner, 2002). Washing of FTA removes the stabilizing agents of FTA and processed discs were immediately used without storage. Storage of purified FTA discs up to 4 months has been shown to significantly reduce the product yield (Drescher and Graner, 2002).

We used 40 PCR cycles to counteract the effect of relatively low concentrations of DNA template in FTA compared to extracted DNA. PCR product yield was sufficient for reliable scoring using SSR markers in marker assisted backcrossing. The CTAB extracted DNA

had more amplification (Figure 1, lanes 7-11) compared to FTA (Figure 1, lanes 1-6). Complex genomes have been used previously for the detection of single copy genes with success. Another advantage of FTA discs is their use as template up to four times using different primers on successive PCRs (Del Rio et al., 1996).

Plant breeders are currently utilizing molecular marker technique for backcrossing programs such as marker-assisted -selection (MAS) to select for presence of target genes (foreground selection) and may be limited financially or may not wish to invest in expensive laboratory equipment, and the procedure described here may offer practical alternative. Furthermore, transport of samples from long distant greenhouse or experimental fields to the laboratory can be carried it without the need for chilling and storage can also be performed at room temperature without need for freezers. Handling of large samples by this procedure can be further enhanced by high throughput potential using robots (Stanislav and David, 2005).

PCR product analysis was compared using agarose gel electrophoresis, PAGE, and ABI Prism sequencer. Agarose gel electrophoresis showed very good quality bands for both normal agarose (data not shown) and metaphor agarose (Figure 1). However, for SSR markers showing polymorphism of less than 10 bp, resolution was not achieved by this method. PAGE and ABI Prism 377 DNA sequencer also showed very nice bands and resolution was also achieved (data not shown). ABI Prism 3730 capillary sequencer showed very good bands with good resolution (Figure 2).

In conclusion, our investigation along with previous work by others showed a simple, procedure that greatly reduces the time and cost required to handle DNA sampling particularly for large sample sizes and for field conditions.

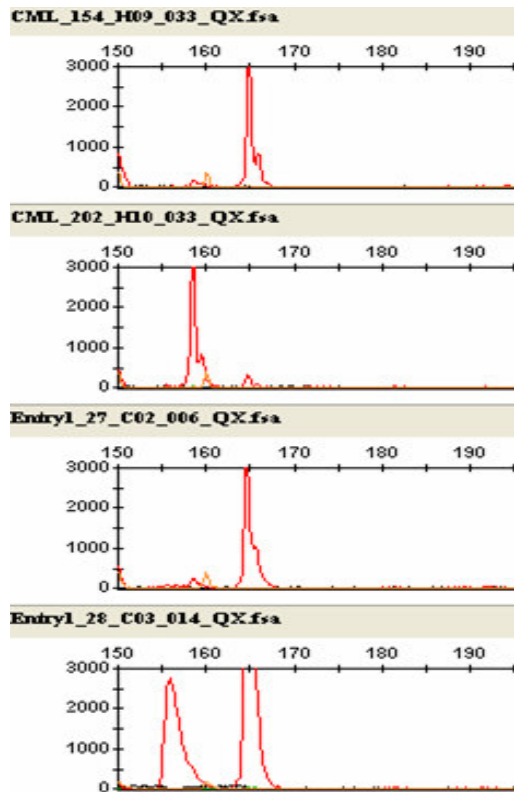


Figure 2. PCR products amplified by primer phi057 analysed using ABI 3730 capillary sequencer. Electropherograms from top to bottom are for parent inbred lines CML154 and CML202, BC1F1 progeny plants 27 and 28.

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