Application note 51781 FTA technology

Application of FTA-based technology for sample collection, transport, purification, and storage of PCR-ready plant DNA

The availability of PCR-based methods in plant molecular research has increased dramatically. Applications of these methods alone, or in combination with other tools, range from marker-assisted selection and molecular mapping, to variety identification and phylogeny research, genomics, and chip technology. PCR is also being used routinely in the detection of Genetically Modified (GM) organisms. Screening can be done at various stages starting with callus cells, then small seedlings, and finally the propagated plants. When screening many samples, a fast and easy DNA preparation method could be beneficial. Here we describe a simple, matrix-based DNA purification procedure using the Whatman™ FTA™ Card technology.

Fig 1. FTA Classic Card with applied leaf tissue samples.

Collection of plant DNA on FTA cards is a simple procedure that can be done even in the field. Plant tissue is physically crushed on the card and the DNA binds to the matrix (Fig 1.). The chemical coating on the FTA Card protects the DNA from degradation and allows the cards to be stored at room temperature for extended periods of time. To prepare the sample for PCR, the matrix is washed with two nontoxic aqueous buffers. The DNA remains bound to the matrix throughout purification and a small disk (1.2 or 2 mm in diameter) of the matrix provides enough template for PCR analysis.

The following examples describe the use of FTA Cards in several PCR-based applications. Potato leaf tissue was sampled on FTA Card for the detection of GM DNA sequences. Soybean DNA was amplified with SSR primers for a potential application in variety identification. We also demonstrate the reuse of FTA Card disks in more than one PCR amplification. To test the overall quality of the stored DNA in addition to specific PCR samples, we also used primers for a high copy number chloroplast gene, *rbc*L, and the chloroplast *trnL* intron sequence, as well as for detection of low copy nuclear genes *Rca* and *Rhg4*. The collected DNA samples were tested immediately or after storage for several mo at room temperature. Comparison data with competitive DNA purification methods is also provided.

Sample application: Press plant tissue onto the card or apply homogenate. Allow to dry completely.

Disk removal: Punch a disk out of FTA matrix impregnated with plant material.

FTA purification/reagent washes: Place the disk in PCR tube and wash twice with FTA purification reagent. Discard used reagent after each wash.

TE-^{1} rinses: Wash twice with TE-^{1} buffer (10 mM Tris, 0.1 mM EDTA pH 8.0) and discard used buffer after each wash.

Drying step: Dry disk in PCR tube.

Direct to PCR: Add PCR master mix directly to the disk and amplify.

Fig 2. FTA plant protocol overview

Detection of GM potato lines from DNA stored on the FTA Cards

6 mo storage

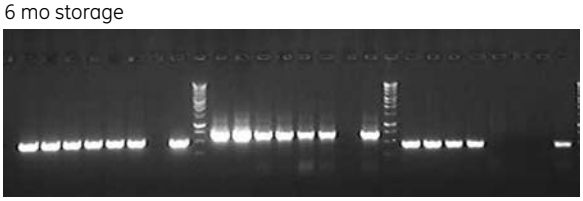


Fig 4. Leaf tissue from three potato selections (R,N=transgenic lines; At=a commercial nontransgenic variety) was pressed on the FTA Card and stored at room temperature. One 2 mm disk from the card was used per 50 µl PCR reaction. Three genes were amplified: a high copy number organelle gene *(rbcL)*, a low copy number nuclear gene *(Rca)* and the Bt-Cry3A transgene.

10 mo storage

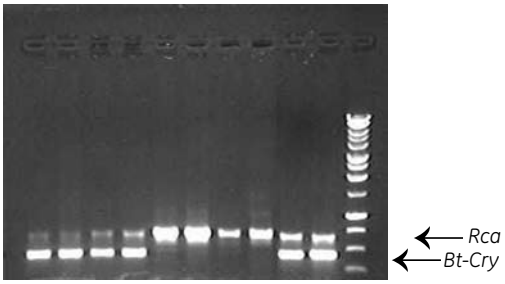


Fig 5. Five potato selections were resampled on FTA Cards (R, N, Y = transgenic; A, At = nontransgenic) and stored at room temperature. PCR amplification mixture contains two sets of primers–one to detect a 439 bp fragment within the synthetic *Bt-Cry3A* gene (1790 bp in size) and one to amplify a 700 bp fragment of a low-copy number nuclear gene *(Rca)*, M = 1 kb marker.

PCR amplification of DNA from several plant species stored up to 11 mo on FTA Cards

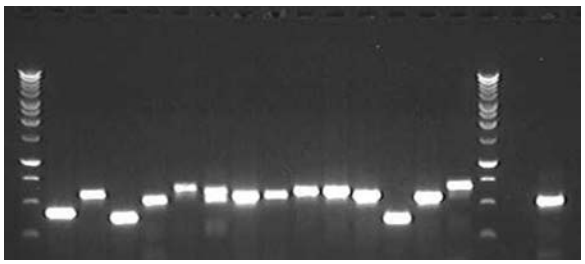


Fig 3. Amplification with universal primers for the intron sequence between the chloroplast tRNA gene trnL (UAA) 5′ exon and trnL (UAA) 3′ exon. Key to plant species: AL = alfalfa, AR = Arabidopsis, BR = Brassica, CR = corn, CT = cotton, CU = cucumber, PT = potato,P2 = diploid potato, RC = rice, RY = ryegrass, SY = soybean, SP = spinach, TM = tomato, WH = wheat, M = 1-kb marker.

Amplification with universal primers for the intron sequence between the chloroplast tRNA gene *trn*L (UAA) 5′ exon and *trn*L (UAA) 3′ exon.

Plant DNA was collected on the FTA Card by placing the leaf over the card, overlaying it with plastic film and crushing the tissue with a hard object (porcelain pestle or tack hammer). One 2 mm disk from the sample matrix was used in a 50 µl PCR reaction. Fig 3. shows that FTA Cards can be successfully used to collect, store, purify and analyze DNA from a wide variety of plant species.

The quality of plant DNA stored on the FTA Card for several mo supports amplification of low copy number genes, including transgenes.

Use of FTA Cards for SSR amplification

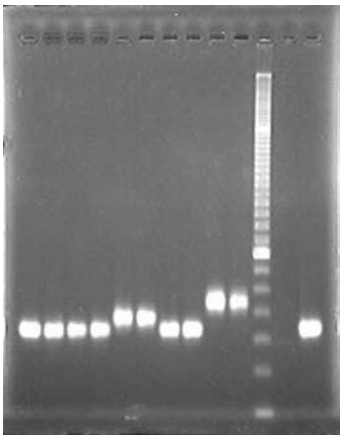


Fig 6. Leaf samples from 3 wk old plants of 5 soybean selections were applied to the FTA Card. One 2 mm disk was used in a 50 µl PCR reaction. 10 µl of the PCR reaction were loaded in duplicate on 3% Metaphor agarose gel (BioWhittaker Molecular Applications Inc.). M = 20 bp marker.

PCR amplification of SATT309, a soybean SSR marker closely linked to soybean cyst nematode resistance.

Fig 6. shows that DNA collected and purified on the FTA Card can be successfully used in marker-assisted selection and genotype analysis.

Reuse of FTA disks for PCR amplification

First PCR *(trnL)*

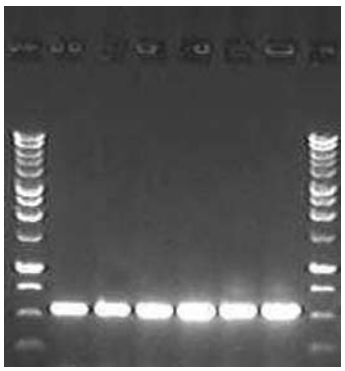


Fig 7. FTA disks with DNA from three different soybean varieties were first used to amplify a noncoding chloroplast DNA sequence.

Second PCR *(Rhg4)*

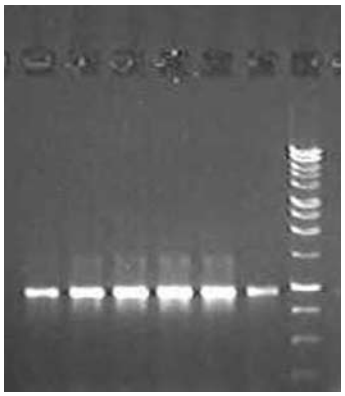


Fig 8. Afterwards, the FTA disks were removed from the PCR reaction mixture, washed twice with TE-^{1} buffer, dried and used in a second PCR reaction to amplify a low copy number *(Rhg4)* locus.

DNA samples stored on FTA Cards can be successfully reused for amplification after a simple washing procedure.

Performance of DNA stored on the FTA Cards compared to DNA stored on a card-based purification system from Company G

D 1

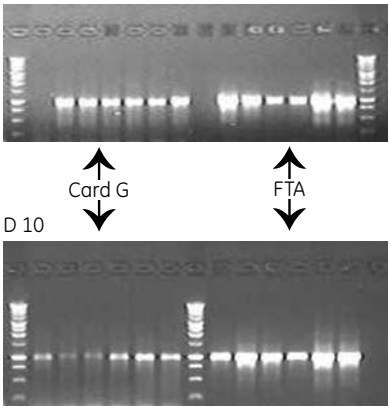
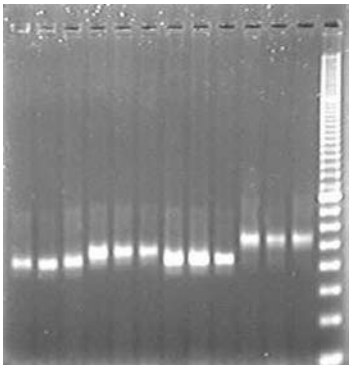


Fig 9. Amplification of *Rhg4* locus.Leaf samples from 3 wk old plants of three soybean varieties were applied to the FTA Card and a competitor's card (Card G). Quality of the DNA was tested by PCR amplification on D 1 (top gel) and after 10-d storage at room temperature (lower gel). One 2 mm disk of FTA Card and one 3 mm disk from Card G were used in a 50 µl PCR reaction. Samples run in duplicate. M=1 kb marker.

FTA



Card G

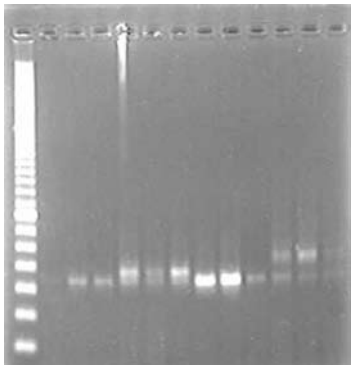
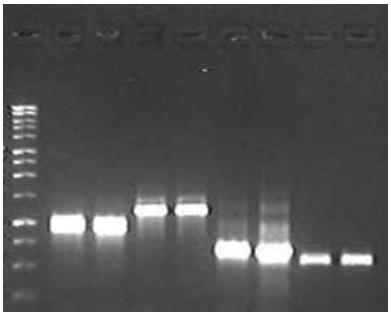


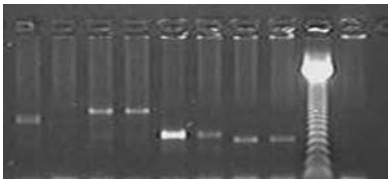
Fig 10. Amplification of SSR marker cards stored at room temperature for 3 mo. Disks from cards with DNA samples from four different soybean varieties were used in PCR amplification of SATT309, a soybean SSR marker closely linked to soybean cyst nematode resistance. Samples were tested in triplicate and run on 3% Metaphor agarose gel. M = 20 bp marker.

Quality of PCR amplification of soybean DNA stored on FTA Card remained high during storage at room temperature while amplification from DNA stored on the competitor's card was less consistent.

PCR amplification from samples purified on the FTA Cards compared to a chemical DNA extraction method



FTA



Kit S

Fig 11. Leaf tissue from four species (SY=soybean, AL=alfalfa, PT=potato, RC=rice) was sampled on the FTA Card according to the protocol and 2 mm disk used in a 25 µl PCR reaction (top gel). For comparison, DNA extract was obtained from a 6 mm leaf disk using Kit S. A small aliquot was used in a 20 µl PCR reaction (lower gel). Amplification of a low copy number gene *(Rca)* was performed on the day of purification. M1 = 1 kb marker, M2 = 123 bp marker

PCR amplification of a low-copy number gene was successfully achieved using the DNA prepared on the FTA Cards. The DNA extract obtained with Kit S did not provide the sensitivity required for the analysis.

FTA Card performance summary

- FTA Cards are a convenient tool for simple preparation of PCR-ready DNA from various plant species. The solid phase format enables out-of-lab collection and simplifies DNA storage and shipping.
- DNA stored and purified on the FTA Card can be successfully used in various PCR-based research protocols such as marker-based selection, varietal identification, phylogeny analysis, and amplification of low-copy number loci, such as transgenes.
- The purification protocol uses only nontoxic aqueous solutions and is done at room temperature. There is no need to freeze-dry or chemically disrupt the tissue.

Product information

FTA Classic Card

Four sample areas for leaf press or plant homogenate application (up to 25 µl per sample area). Convenient for multiple applications of the same specimen or collection of multiple plant samples on one card. Different samples can be processed independently.

Indicating FTA Classic Card

Same as FTA Classic Card with a color indicator that changes from pink to white when sample is applied. Recommended for use with clear samples such as cultured cells or callus.

FTA Mini Card

Two sample areas for leaf press or plant homogenate application (up to 25 µl per sample area). Convenient for protocols that require different locations for testing and archiving samples. Different samples can be processed independently.

Indicating FTA Mini Card

Same as FTA Mini Card with a color indicator that changes from pink to white when sample is applied. Recommended for use with clear samples such as cultured cells or callus.

FTA Micro Card

One sample area for leaf press or plant homogenate application (up to 25 µl per sample area). Recommended when only one sample is needed.

Indicating FTA Micro Card

Same as FTA Micro Card with a color indicator that changes from pink to white when sample is applied. Recommended for use with clear samples such as cultured cells or callus.

FTA Gene Card

An FTA Card encased in a chipboard frame. Three sample areas for application of up to 10 µl plant homogenate per sample area. Can be utilized in many automatic dispensing/pipetting systems when used with the FTA Gene Card Tray (WB100030).

CloneSaver™ card

FTA Technology incorporated into a 96-well format for high throughput applications. Includes a color indicator that changes from pink to white when sample is applied. Designed for storage, archiving, and retrieval of plasmid and BAC DNA.

Accessories

FTA Purification Reagent

- For purification of nucleic acids stored on FTA Cards.
- Ensures superior quality DNA for PCR or RFLP analysis.
- Removes PCR inhibitors such as chlorophyll and other potential contaminants.
- Nontoxic, hypoallergenic aqueous solution.

FTA Gene Card Tray

- Holds two FTA Gene cards for use in automatic dispensing/ pipetting systems.
- Tray footprint conforms to SBS standards.

Harris Micro Punches™ and Uni-Core Punches (1.2 mm or 2.0 mm) and Cutting Mat

- Recommended for the precise punching of FTA Cards. No sample carryover when recommended procedures are used.
- Micro punch tips provide up to 2000 punches. Polished steel tip is case hardened and can be sterilized.
- Disposable Uni-Core punches last for up to 300 punches.
- 1.2 mm Punch recommended for use with FTA Cards containing whole blood and samples with high DNA content.
- 2.0 mm Punch recommended for use with FTA Cards containing plant samples, buccal cells, plasmids, and other samples with lower DNA content.

Multi-Barrier pouches

Large Pouch

- For transporting or storing FTA Classic Cards.
- Seven laminated layers that protect the card from exposure to gas or liquid contamination.
- Tamper-evident seal maintains sample security.
- Outer paper surface for labeling or writing.

Small Pouch

- Same construction in a smaller size for storing FTA Gene Cards, Mini Cards or Micro Cards.

CloneSaver Resealable Pouch

- Same construction plus a zip-lock resealable closure for easy, repeated access to CloneSaver or FTA Cards.

Storage Desiccant packets

- Ensures FTA Cards remain dry during transport or storage.
- Changes from blue to pink to indicate absorption of moisture

Ordering information

Catalog number	Description	Qty/pack
WB120205	FTA Classic Card	100
WB120206	Indicating FTA Classic Card	100
WB120055	FTA Mini Card	100
WB120056	Indicating FTA Mini Card	100
WB120210	FTA Micro Card	100
WB120211	Indicating FTA Micro Card	100
WB120208	FTA Gene Card	100
WB120028	CloneSaver Card	10
WB120204	FTA Purification Reagent	500 ml
WB100030	FTA Gene Card Tray	20
WB100005	Harris Micro Punch 1.2 mm (with Mat)	1
WB100006	Replacement Tip 1.2 mm	1
WB100007	Harris Micro Punch 2.0 mm (with Mat)	1
WB100008	Replacement Tip 2.0 mm	1
WB100020	Replacement Cutting Mat	1
WB100028	Harris Uni-Core 1.25 mm punch	4
WB100029	Harris Uni-Core 2.0 mm punch	4
WB100036	Multi-barrier pouch, small	100
WB100037	Multi-barrier pouch, large	100
WB100024	CloneSaver Resealable Multi-Barrier Pouch	50
WB100003	Desiccant Packets (1 g)	1000

For research use only. Not for use in diagnostic procedures.

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