# High Throughput Processing of DNA Samples on FTA Paper for PCR Analysis

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FTA paper (Whatman Inc., Clifton, NJ) is a paper specially treated to bind and protect from degradation nucleic acids from blood, plant and animal tissue extracts and other sources. For analysis, a small disc is punched from the FTA paper containing the DNA sample of interest, washed, dried and used for polymerase chain reaction (PCR). We wished to take advantage of the simplicity and speed of DNA isolation on the FTA paper and ease of storage that does not require freezing. For DNA isolation from plants, leaves are crushed on the FTA paper and the extract is allowed to soak into the paper and dry (1). Lacking, however, was an expedient and reliable procedure for processing moderate numbers of samples (up to several hundred) for analysis. One of the bottlenecks in the workflow is the FTA paper disc washing. For high throughput applications in diagnostic labs, fully robotized stations handle disc punching, washing and PCR. On the other end of the spectrum, a small number of discs can be placed in microcentrifuge tubes and the washing liquid added and removed manually using a pipettor (1).

An improved method of disc washing was described by Lange et al. (2) who used a 96-well PCR plate modified by piercing a small hole in the bottom of each well. The holes were small enough so that 50 μl of the washing liquid was retained in the well by surface tension, yet large enough for the liquid to be pulled through and removed by centrifugation. Following washing and drying, the discs were transferred to a new PCR plate. However, a centrifuge capable of accommodating 96-well plates may not be available in all laboratories, and the time required for acceleration and deceleration of the centrifuge greatly prolongs the entire washing process.

We improved the procedure by using negative pressure (vacuum) instead of centrifugation to remove the washing liquid from the modified 96-well PCR plate. We constructed a vacuum manifold using materials readily available in the lab (Fig. 1A). The removal of the washing liquid is very fast (several seconds) and because of the minimal cost of the equipment, each researcher can have his/her own manifold at arm's reach on the bench. Additionally, drying of discs after the final wash is greatly accelerated using the vacuum manifold.

Further improvement in sample processing speed was achieved in disc punching. We use a modified 1/16" diameter scrapbook hole puncher (Fiskars Brands, Inc., Wausau, WI) available in most craft supply stores, from which the confetti trap was removed and a truncated 1 ml pipette tip was attached (Fig. 1B) to funnel the punched disc directly into desired position of the washing plate.

Our protocol (Table 1) proved reliable and sufficiently fast to process up to several hundred samples per day. A single person can punch, wash and dry 192 discs (two 96-well plates) in less than 2 hours. Using the FTA paper it is thus possible to isolate and analyze several thousands of DNA samples in the course of few weeks. We currently use this protocol for genotypic analysis of T-DNA insertion mutants from the SALK collection (3) (Fig. 2) and for genetic mapping in *Arabidopsis*. One limitation of all commonly available hole punches of the current design is their insufficient reach. We circumvent this shortcoming by cutting a slit in the FTA paper card that allows the central samples to be punched also. Since the buildup of static charge on dry discs can hinder transfer of washed discs into the new PCR plate, we prefer to transfer discs immediately after the final vacuum drying step, before they get overly dry.

## Acknowledgments

We thank Ms. Linda Danhof who suggested the use of a scrapbook hole punch.

**Table 1**. Sample protocol for analysis of plant DNA samples. See manufacturer's instructions for DNA isolation and disc washing recommendations for other specimens (http://www.whatman.com)

| Step | Operation |
|---|---|
| 1 | Isolate DNA on FTA paper as recommended for the given source. |
| 2 | Punch 1/16" diameter discs containing DNA from the samples of interest and deposit into the 96-well washing plate. |
| 3 | Wash the discs twice with 50 μl FTA wash buffer (Whatman) and twice with $TE^{-1}$ buffer (10 mM Tris pH 8, 0.1 mM EDTA), 5 min each. Apply vacuum to remove the washing liquid after each wash. |
| 4 | Continue to apply vacuum for additional 10 min to accelerate drying. |
| 5 | Transfer discs by placing a new PCR plate on top of the washing plate, aligning the openings of both plates. Secure the assembly with rubber bands and turn upside down to transfer the discs into the new plate. Note that the sequence of either rows or columns will be reversed because of this transfer. Washed discs may be stored dry at room temperature for up to several days. |
| 6 | Add 10 μl or more PCR master mix, perform PCR, and analyze the products on a gel. |

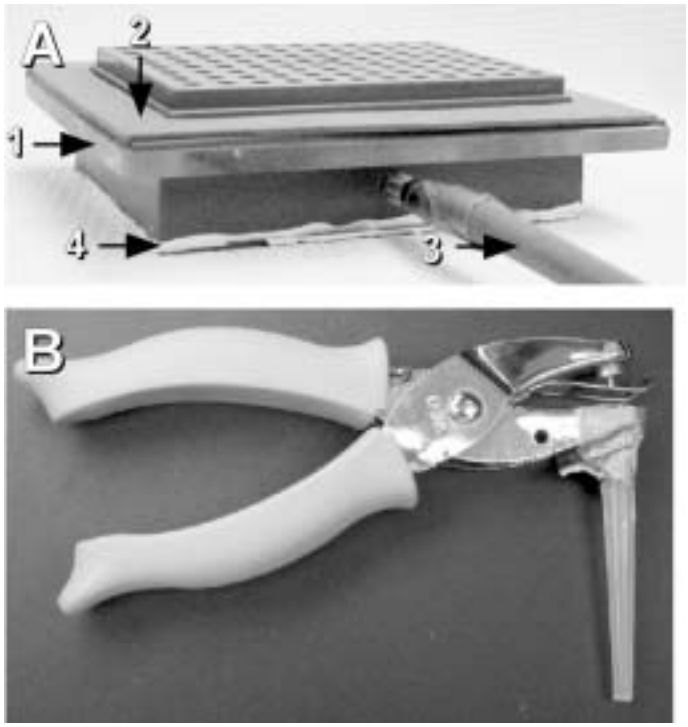
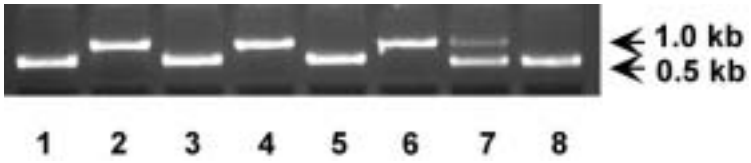



**Figure 1.** Hardware for FTA Paper Disc Punching and Washing.
(A) The vacuum manifold. An acrylic skirt frame (1) with a rubber foam layer on top (2) is mounted on a pipette tip box (Dot Scientific, Burton, MI; UltraGuard 10μl Self Sealing tips, cat # UGA104-96RS) with a vacuum hose attached (3). The junction between the box and the acrylic frame, as well as the bottom lid of the box (4) is sealed with a silicone sealant. The vacuum seal is created between the rubber foam gasket on the manifold and the skirt of the 96-well washing plate (not shown), modified from Falcon® PCR plates (BD Biosciences, Bedford, MA; ref. # 352135) by punching a hole in each well using a 25G needle or a sewing pin of similar diameter.
(B) Disc hole punch adapted as described in the text.

**Figure 2.** Identification of T-DNA Insertional Mutants of *Arabidopsis*.
FTA paper discs with DNA samples from *Arabidopsis thaliana* T-DNA insertion line SALK_10009 (obtained from Arabidopsis Biological Resource Center, Columbus, OH) were analyzed via PCR in 10 μl reactions containing 0.4U Ex-Taq DNA polymerase (Panvera, Madison, WI), 1x Ex-Taq reaction buffer, 0.20 mM each dATP, dCTP, dGTP, dTTP, 0.25 μM each T-DNA left border primer LBb1 (5'-GCGTGGACCGCTTGCTGCAACT-3'), and the forward (5'-AGAGCTAGGCCCATGTCCAGG-3') and reverse (5'-TCAGCTTGCTCAGAAACTCCGA-3') gene-specific primers flanking the T-DNA insertion site. Amplification conditions: 96°C 1 min; 32 cycles of 94°C (30 s), 65°C (15 s), 72°C (1 min); final extension at 72°C for 4 min. 75% of the reaction volume was loaded on 1.5% agarose gel. Wild-type plants yield ~1kb product of amplification with gene-specific primers (lanes 2, 4, and 6). Plants homozygous for T-DNA insertion yield ~0.5 kb product of amplification between LBb1 primer and one of the gene-specific primers (lanes 1, 3, 5, and 8). The presence of both bands (lane 7) indicates a heterozygous plant.




## References

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