

Preparation of Isolated DNA from FTA® Cards

FTA Cards provide a cost-effective room temperature method for collecting, shipping, archiving and processing nucleic acids from a wide variety of biological samples. FTA Cards inactivate pathogens for user safety and facilitate rapid purification of nucleic acids. Purification of nucleic acids on FTA Cards takes less than 30 minutes per sample when performed manually. With automation, time and labor is reduced and throughput is increased.

The following automated wash procedure has been developed to meet high throughput demands, facilitate handling and standardize DNA sample purification. The process requires no operator intervention after the setup is complete and takes about one hour for the purification of 96 samples.

The method produces high quality DNA for PCR and other downstream applications.



DNA ISOLATION PROCEDURE OVERVIEW USING AN AUTOMATED LIQUID HANDLER*

- **Remove** 2.0 mm discs from desired samples on FTA Cards and place each disc into a separate 200 μ L well of a PCR plate.
- **Dispense** 180 μ L of FTA Purification Reagent into each well.
- **Incubate** for 5 minutes at room temperature.
- **Wash** discs with 10 mix cycles of 100 μ L each of FTA Purification Reagent.
- **Aspirate and discard** 180 μ L of spent reagent from each well. Make sure that the discs remain in the well after each wash.
- **Repeat** steps 2–5 two more times with FTA Purification Reagent (total of 3 washes). Make sure that the discs remain in the well after each wash.
- **Dispense** 180 μ L of TE⁻¹ buffer (10 mM, Tris-HCl, 0.1 mM EDTA, pH 8.0) into each well.
- **Incubate** for 5 minutes at room temperature.
- **Wash** mix with 10 mix cycles (100 μ L) of TE⁻¹ buffer.
- **Aspirate and discard** 180 μ L of spent buffer from each well. Make sure that the discs remain in the well after each wash.
- **Repeat** washes with TE⁻¹ Buffer (total of 2 washes).
- **Air dry** discs at 50° C for 15 minutes, or at room temperature for 1 hour.
- **Sample** discs are ready for addition of PCR master mix.

*For a detailed automation protocol please call our Technical Services Department at 1-800-WHATMAN

Preparation of Isolated DNA from FTA® Cards Using an Automated Liquid Handling System

RESULTS

Figure 1. Amplification of Large Target DNA from Blood Samples on FTA Cards

This presents a typical example of high quality DNA produced using the automated wash protocol to process FTA sample discs. The successful amplification of a large, single locus target illustrates the superior quality of the isolated DNA template and demonstrates that the automated wash procedure does not damage genomic DNA.

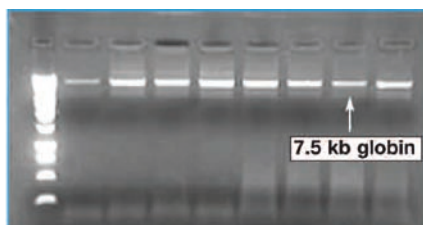


Figure 1. Amplification of Large Target DNA from Blood Samples on FTA Cards.

DNA on 2.0 mm FTA discs amplified in 50 µL of PCR master mix and electrophoresed on an 0.8% agarose gel containing ethidium bromide

Figure 2. Purification of DNA from Multiple Sample Types on FTA Cards

DNA from one individual's blood and buccal cells stored on FTA Cards was washed simultaneously on a liquid handler. This demonstrates that a clean reproducible signal is obtained from both sample types. The ability to process multiple sample types from different sources on the same automated platform greatly reduces labor and time.

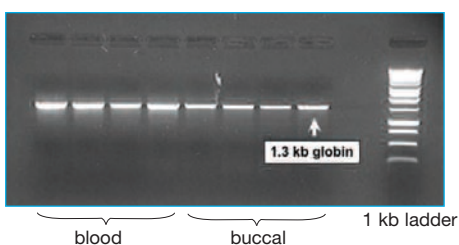
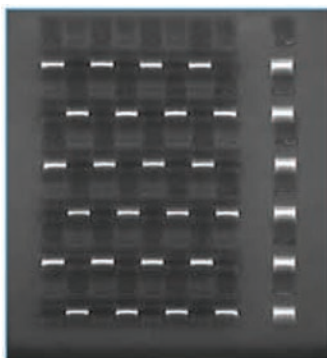


Figure 2. Purification of DNA from Multiple Sample Types on FTA Cards.

DNA on 2.0 mm FTA discs amplified in 25 µL of PCR master mix and electrophoresed on an 0.8% agarose gel containing ethidium bromide

Figure 3. No Cross-Contamination Between Reaction Wells

This demonstrates that washing of FTA sample discs using this automated procedure does not result in cross-contamination between adjacent wells. Unstained and bloodstained discs were placed in the same microplate in an alternating pattern. All discs were washed and prepared for PCR as described. No cross-contamination was observed between adjacent wells and the amplified bands were highly reproducible.



Amplification of G3PDH from washed, bloodstained and unstained FTA sample discs

Figure 3. No Cross-Contamination Between Reaction Wells.

DNA on 2.0 mm FTA discs amplified in 25 µL of PCR master mix and electrophoresed on an 0.8% agarose gel containing ethidium bromide

CONCLUSION

Automated processing of DNA on FTA Cards provides a reproducible and efficient high throughput method for producing high quality DNA for PCR and other downstream applications.

RELATED PUBLICATIONS

- Belgrader and Marino, 1997. Automated Sample Processing Using Robotics for Genetic Typing of STR Polymorphisms by Capillary Electrophoresis. *Lab. Robotics and Automation* 9:3-7
- Belgrader et al., 1995. Automated DNA Purification and Amplification from Bloodstain Cards using a Robotic Workstation. *BioTechniques* 19:427-432
- Williams et al., 1996. Automation of in situ Sample Preparation for PCR Using the FTA DNA Collection System and the Rosys Laboratory Workstation. *Advanced Forensic Haemogenetics*.

Whatman Quality

Whatman is a global leader in separations technology and is known in the scientific community for providing innovative products and solutions. Our instinct for simplification accelerates the rate of discovery, reduces costs and saves time. For more information, visit www.whatman.com

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