Whatman®

Application Note

Techniques and Technical Insight to Optimize Use of Whatman Products

Cross-Contamination Study: Carryover Does Not Occur During Punching and Processing of FTA® or CloneSaver™ Cards

Introduction

FTA Cards provide a proven effective method for collecting, shipping, archiving and processing nucleic acids from a wide variety of biological sources. Samples applied to FTA Cards are used in a wide variety of applications such as genetic screening, pathogen detection and human identification studies. CloneSaver Cards utilize the same FTA Technology and are designed for use with plasmid and BAC samples. In order to analyze nucleic acids stored on FTA Cards, a disc is removed from the card using a coring device, such as a Harris Micro Punch™. This procedure is easy, convenient and reproducible. To demonstrate that there is no cross-contamination between samples during punching and processing, a study was performed using sample discs removed with the Harris Micro Punch according to published instructions*.

A. Collection and Processing of Blood Samples on FTA Cards

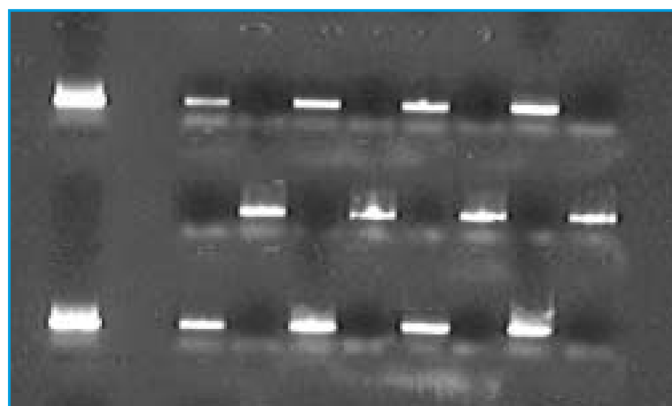
Method

Fingerstick whole blood was applied to an FTA Card and allowed to dry for one hour at room temperature. A sample disc was taken from a bloodstained portion of the card, using a 1.2mm Harris Micro Punch, and placed in a PCR amplification tube. A "blank" disc was then removed from a virgin (clean) portion of the card and placed into a different PCR amplification tube. This process was repeated for a total of 24 sample discs, 12 bloodstained discs and 12 "blank" discs. Although a punch cleaning procedure between samples is recommended, the Micro Punch was not cleaned in this experiment to create an "extreme condition", i.e., one most likely to result in carryover. All discs were then washed three times with FTA Purification Reagent for 5 minutes each, and rinsed twice with TE^{-1} buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) for 5 minutes as per standard FTA protocol. PCR amplifications were performed for G3PDH globin for all 24 discs. The PCR products were electrophoresed on a 0.8% agarose gel using standard methods.

*Harris Micro Punch Package Inserts: 1.2mm Punch–Whatman Catalog Number WB100005; 2.0mm Punch–Whatman Catalog Number WB100007.

Results

Figure 1. PCR Amplification of DNA from Blood Stained and Blank Discs Taken from FTA Cards



+control 10ng/µL DNA

alternate bloodstained and blank discs

Figure 1 consists of 24 separate PCR gels of alternate bloodstained and blank 1.2mm FTA discs. The gels from the FTA discs containing blood exhibit abundant amplification product while no amplification product is seen in the gels from the blank FTA discs. No cross-contamination or carryover was observed even though the recommended punch cleaning procedure was not implemented between blood samples and blank discs.

Harris Micro Punches are recommended for precise punching of FTA Cards and CloneSaver Cards.

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B. Collection and Processing of Plasmid Samples on CloneSaver™ Cards

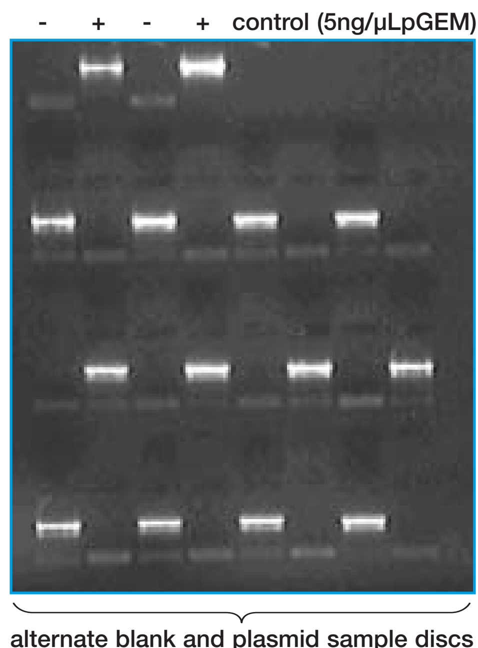
Method

5µL aliquots of a bacterial suspension containing the plasmid pGEM with an OD600 of 2.1 were applied to different sample areas on a CloneSaver Card. The Card was then stored at room temperature for 8 weeks prior to analysis. Using a 2.0mm Harris Micro Punch™, alternate plasmid and blank sample discs were taken from the CloneSaver Card. A recommended "cleaning punch" was taken between samples from a clean area of the card. Plasmid and blank discs were washed with two quick rinses of 200µL of TE^{-1} buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). Following the wash process, 5µL of TE^{-1} buffer was added to the discs and incubated for 10 minutes at room temperature to remove plasmid DNA bound to the discs. 1µL of eluate was added to each PCR amplification reaction. All samples were amplified for a 1.8kb target using SP6 and T7 primers. PCR products were electrophoresed on a 0.8% agarose gel.

Results

Figure 2. PCR Amplification of DNA Eluted from Plasmid and Blank Disc Taken from CloneSaver Cards

- + - + control (5ng/µLpGEM)



alternate blank and plasmid sample discs

Figure 2 consists of 24 separate PCR gels of alternating plasmid and blank punches taken with a cleaning punch between samples. The high sensitivity of the assay is able to detect any carryover between samples at the femptoliter level. Eluate from discs containing plasmid DNA generated strong bands while no amplification product was observed using eluates from blank discs.

Conclusion

There is no evidence of cross-contamination or carryover from one FTA or CloneSaver sample disc to another when using the Harris Micro Punch and taking a cleaning punch between samples. In fact, punches of blood samples on FTA Cards taken under "extreme conditions", i.e., with no cleaning punch between samples, exhibited no carryover to the blank discs. A cleaning procedure between samples such as taking a blank punch, using a blast of compressed air or rinsing the punch tip with ethanol is recommended to provide protection against sample carryover. We expect that the same observations would be seen using equivalent manual punches and commercially available automated punching instruments.

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