Application Note 51714 FTA technology

# Reproducible collection of buccal cells for DNA analysis using the Whatman™ EasiCollect™ System

EasiCollect is a novel development in the process of collecting buccal cell samples for genetic analysis. The EasiCollect system allows the uniform collection and application of cells to the surface of an FTA™ Card for the capture of DNA.

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

The method produces high quality DNA for PCR and other downstream applications such as STR and SNP genotyping.

Buccal cell collection, in the past, has been a notoriously difficult process. This has mainly been due to the differences in the ability of various individuals to shed cells. The reliability of most methods of buccal cell collection has always been a problem. There have also been issues with the ability of the cells to be deposited on a matrix in a uniform manner for reproducible sampling. Sampling an area of a matrix has occasionally missed deposits of cells. This has led to resampling, reprocessing and reamplification of samples costing time and money. There has clearly been a need for a more reliable way to collect and process buccal cell samples for genetic analysis.

EasiCollect eliminates the difficulties of buccal cell collection. The novel design has been engineered for maximal cell collection and uniform application to FTA, a leading DNA collection and storage matrix (Fig 1). An optimized foam collection surface has been specifically selected for its ability to capture the largest number of cells to overcome individual differences. The pressure clips molded into the EasiCollect device ensures a uniform and constant pressure for the reproducible transfer of cells to FTA. The FTA Card chosen for the device is compatible with manual or automated punching systems which encompass all degrees of laboratory throughput.

**Fig 1.** The EasiCollect system combines an optimized foam collection surface with the FTA card for collection of buccal cells and preservation of DNA.

Whatman

| Advantages | benefits |
|---|---|
| Novel foam composition | Large numbers of cells captured for analysis |
| Consistent cell transfer | No need to resample |
| Uniform cell distribution | Confidence of amplifiable DNA in each punch |
| FTA inside | Superior room temperature storage and rapid DNA preparation |
| Automation friendly FTA Card | Fits high-throughput punching devices for maximum throughput |


**Fig 2.** EasiCollect method

## Easy steps to buccal cell collection (Fig 2)

1. Collect cells by rubbing each cheek for 15 s
2. Peel off the protective film exposing the FTA Card
3. Snap the foam head in place for 10 s to transfer the cells to the FTA Card
4. Bend device to lift the foam from the FTA Card. Remove card, dry and process

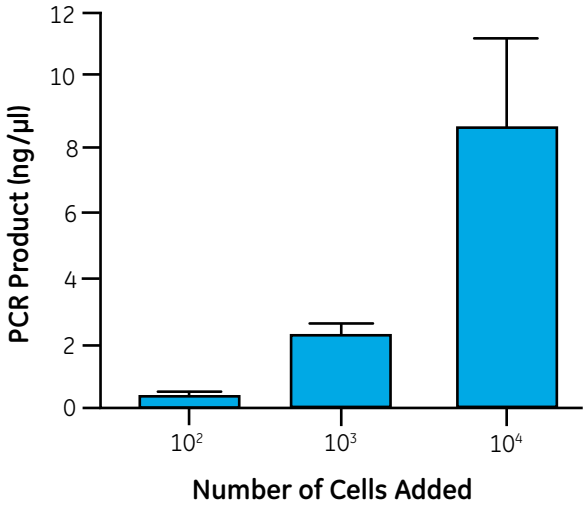



**Fig 3.** THP-1 cells applied with EasiCollect

## Foam capture and cellular transfer of THP-1 cells

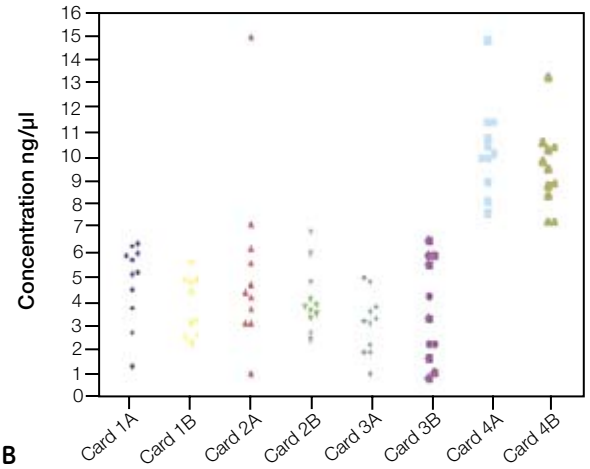
In order to test the ability of EasiCollect to transfer cells to the FTA Cards, varying numbers of cultured THP-1 cells were applied to the foam applicator of the EasiCollect device and transferred to the FTA Card. Quantitiation of a β-globin PCR fragment end-point analysis was used to determine the yield of DNA present on an FTA disc; the yield of PCR amplicon is approximately proportional to the concentration of DNA on the disc. Cells were applied to the EasiCollect foam applicator as 100 µl containing $10^2$, $10^3$ and $10^4$ cells and subsequently transferred to the FTA matrix by closing the device and allowing the foam head to be held in place for 10 s.

Figure 2 shows a yield of the PCR product obtained from each dilution of cultured cells transferred from the EasiCollect device to the FTA Card. As few as 100 cells could be detected after application to the FTA Card demonstrating excellent transfer and detection of cells in sufficient quantities to support amplification of DNA. A 2.0 mm disc was punched from the FTA Card for PCR.

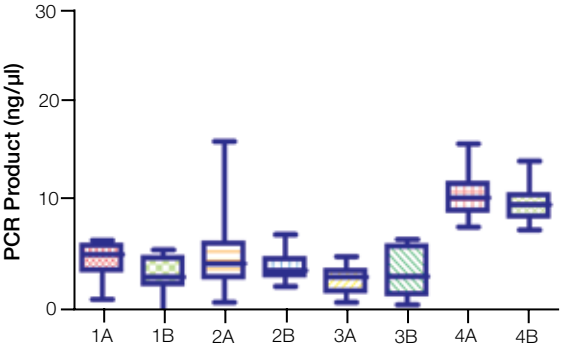


EasiCollect™


4A




4B




4C

**Fig 4.** Distribution of cells applied to FTA Cards using EasiCollect

## Buccal cell distribution using EasiCollect

In order to examine the distribution and uniformity of cells applied using the EasiCollect device, a map of the sample area was prepared. Discs (2 mm) were taken from the card in a specific pattern (Fig 4A) to cover the sample area including the periphery. Each disc was amplified for a 268 bp portion of the β-globin gene and quantitated using an Experion™ Automated Electrophoresis System (Bio-Rad) by running 1K chips following the manufacturer's instructions (Fig 4B).

Analysis of 88 discs demonstrated amplifiable DNA in all but one disc. This represents a 99% success rate from all regions of the sample area. The Box and Whiskers Plot in Figure 4C shows a very tight distribution of DNA in duplicate samples from four individuals.

## STR analysis

Buccal cell samples were taken for STR analysis. Two 1.2 mm punches were collected from central locations of each FTA card from four separate individuals. All discs were processed following standard protocols. STR analysis was performed using the Promega PowerPlex 16™ system following manufacturer's instructions. PCR was carried out using an Applied Biosystems 7900HT thermocycler and PCR products were separated on an Applied Biosystem's PRISM™ 310 Genetic Analyzer. Analysis of products was carried out using GeneMapper 3.2™ software.

Analysis of STR profiles demonstrate excellent peak shape, height and baseline to baseline determination. All alleles were called accurately compared to the kit standards (Fig 5).

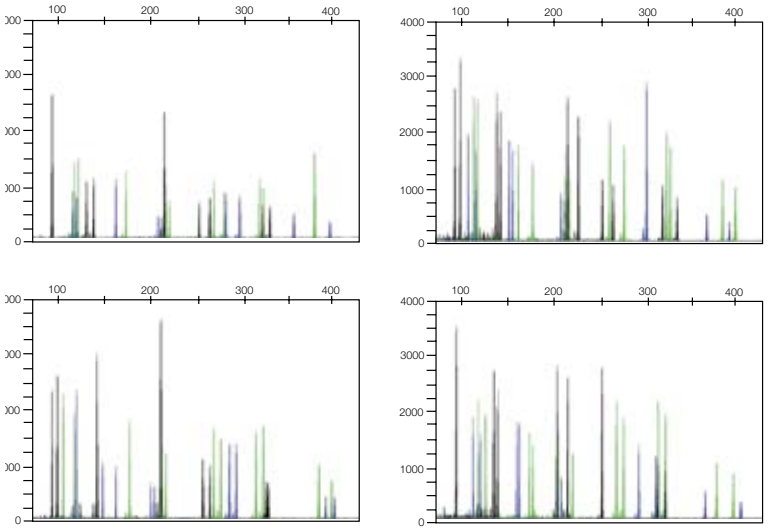
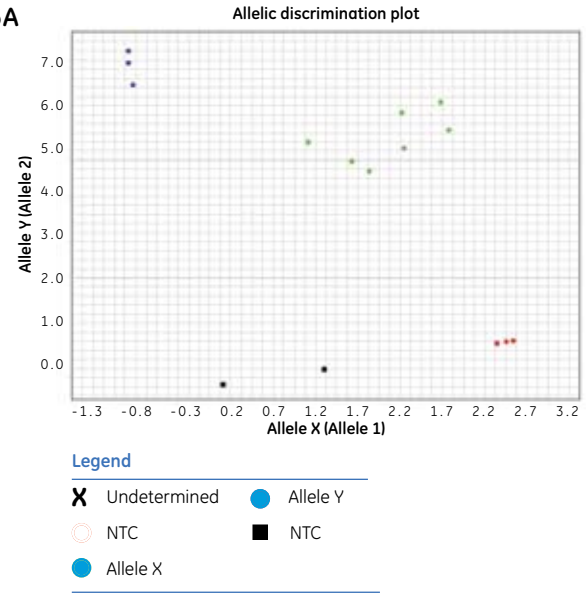



**Fig 5.** STR analysis of 4 individuals



EasiCollect™

6A




6B

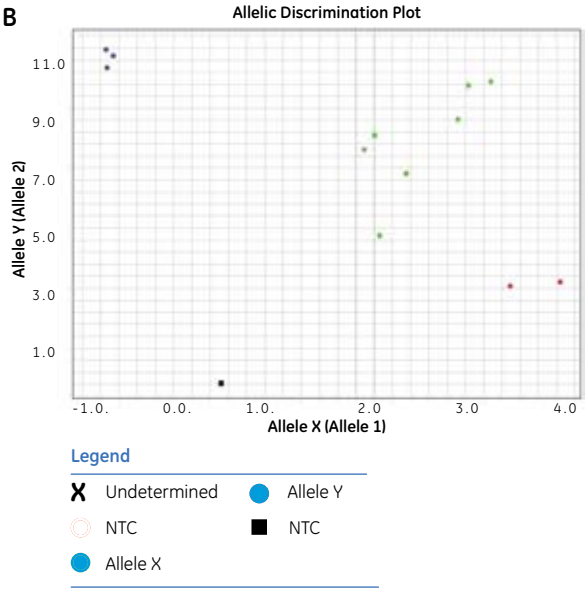



**Fig 6.** Allelic discrimination SNP genotyping

## Allele discrimination analysis

The plot in Fig 6. shows a typical example of mutation detection from high quality DNA captured by FTA Cards using the EasiCollect system. FTA sample discs (3.0 mm) were prepared from buccal cell spots collected with EasiCollect. The discs were used as template for end-point PCR using TaqMan™ probes for CYP2C9*2 (A) and CYP2C19*2 (B) to discriminate between two alleles of single nucleotide polymorphisms (SNPs). In this example the individual was heterozygous for the alleles.

## Conclusions

EasiCollect is a simple to use device to reproducibly collect buccal cells for consistent transfer to FTA cards. EasiCollect represents a major step forward in the collection of DNA for genetic analysis in forensics, human identification, SNP genotyping, pharmacogenomics and for future developments. Coupled with the ability to preserve DNA on FTA Cards, the EasiCollect system is a valuable tool for the collection and storage of DNA.

## Ordering information

| Catalog number | Description | Quantity/pack |
|---|---|---|
| WB120462 | EasiCollect | 50 |

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