

DNA Extraction from FTA® Cards Using the GenSolve DNA Recovery Kit

Easy capture and long-term room temperature storage of DNA by FTA combined with easy elution with GenSolve

FTA® Technology is a fast and easy way to collect DNA from a variety of biological samples for rapid DNA preparation and for room temperature storage. Blood, buccal cells, plants, bacteria and many other sample types are applied to FTA Cards where the cells are instantly lysed and DNA is captured in the matrix (Figure 1).

FTA Cards are coated with a proprietary chemical formulation that denatures proteins including damaging nucleases. FTA Cards also denature viral coat proteins and inhibit the growth of bacterial and fungal micro-organisms which makes the samples safe to handle and allows the samples to be classified as non-biohazardous.

Due to the chemical formula on FTA Cards captured DNA is protected from all forms of DNA damage that can cause degradation. DNA samples can be stored at room temperature and DNA from blood samples applied to FTA Cards has been stable for over 17 years and counting.

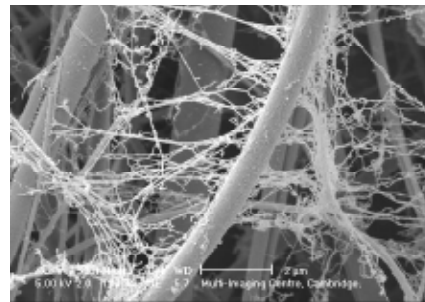


Figure 1. 1 Double stranded DNA captured in the fibers of the FTA matrix

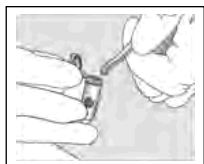
Advantages of FTA:

- Protection from DNA degradation
- DNA stored over 17 years at room temperature
- Simple sample collection procedures, no special training required
- Bactericidal and Virucidal for safe handling
- No need to classify samples as biohazard
- Cells lysed on contact with FTA and DNA bound to matrix
- Chemistry versatile for a variety of sample types; one product for many samples





Step 1: Apply sample to FTA. Dry thoroughly



Step 2: Punch a 6 mm disc and place in microfuge tube



Step 3: Add 620 μ L of GenSolve Recovery Solution A and protease. Incubate 1 hr at 65°C with shaking



Step 4: Prepare a 2mL tube with 20 μ L of GenSolve Recovery Solution B. Transfer disc and elution solution to a spin basket. Centrifuge to collect DNA.

Concentrate and purify DNA by bind-wash- elute method

Step 5: Purify DNA.

Figure 2. Overview of FTA and GenSolve process

GenSolve

GenSolve is a robust kit for extracting double-stranded DNA from FTA Cards. Existing methods of extracting DNA from the matrix include alkaline conditions, and high heat, both of which result in the recovery of single-stranded DNA; restriction digest or organic extractions which are labor intensive but result in double stranded DNA.

Many genetic analyses today require high quality double-stranded DNA as input material for accurate results. The GenSolve Kit components allow researchers who have archived blood spots on FTA Cards to recover double-stranded DNA at a quality and quantity suitable for the newer assays.

Overview of GenSolve Protocol

The GenSolve procedure (Figure 2) consists of incubating 6 mm FTA discs at 65°C with a protease combined with a proprietary high pH solution and 1% lithium dodecyl sulfate. The incubation releases DNA, proteins and cellular debris from the matrix in a highly efficient manner. After centrifugation the eluate can be purified by a standard bind-wash-elute method for DNA isolation and concentration. The entire process from punch to purified double stranded DNA takes less than 1.5 hours.

GenSolve Performance

Yield of DNA:

GenSolve recovers a significant amount of the DNA trapped within the matrix when whole blood is spotted to the FTA Cards. Yields are between 50 – 360 ng from 10 μ L of whole blood with an average of 132 ng (Table 1 and Figure 3). Yield varies depending on the white blood cell count of the initial sample and the normal variability between donors.

Table 1

Yield of DNA from 6 mm Discs of FTA Cards spotted with Whole Blood

Typical results of double stranded DNA* purified from 10 μ L of blood spotted on FTA Cards	
Average yield	132 ng
Range	50 – 360 ng**
Overall CV	11%
*DNA quantified by PicoGreen (Invitrogen)	
** DNA yield dependent on white blood cell count	

Reproducibility:

GenSolve is reproducible with an average CV of 11%. Over 90 discs containing 10 μ L of whole blood from 30 unique samples were processed by the GenSolve method in triplicate. The % CV was calculated from the standard deviation for each triplicate.

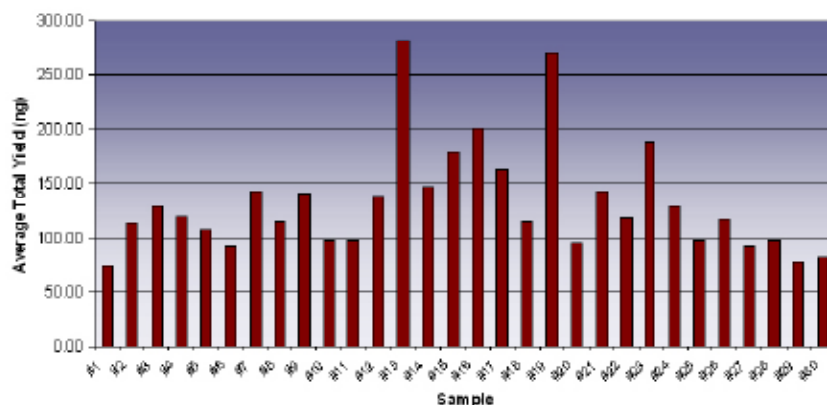


Figure 3. Yield of DNA recovered from 30 unique samples on FTA Cards. The average DNA recovered was 132 ng per 6 mm disc. The yield ranged between 75 and 280 ng. Double stranded DNA was quantitated by PicoGreen.

High Molecular Weight DNA:

Double stranded DNA recovered from FTA Cards via the GenSolve method is approximately 35 kb and is intact. Figure 4 shows a 0.8% agarose gel of 6 unique samples recovered from FTA using GenSolve and purified using a QIAmp kit (Qiagen). High molecular weight DNA is recovered from the FTA Cards.

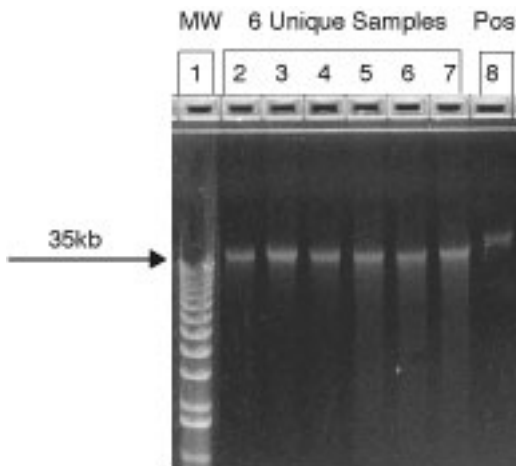


Figure 4. High molecular weight DNA recovered from FTA Cards using GenSolve. Lane 1 is a MW ladder; Lanes 2 – 7 are 6 unique samples; Lane 8 is positive control human DNA (Promega).

PCR Amplification of DNA Recovered from FTA Cards:

DNA recovered from FTA Cards using GenSolve and purified with a QIAmp kit can serve as template for PCR amplifications. Both real-time and end-point PCR can be accomplished with the purified DNA. Figure 5 shows a 1.2% agarose gel of a 558 bp amelogenin amplicon from 9 unique samples on FTA Cards.

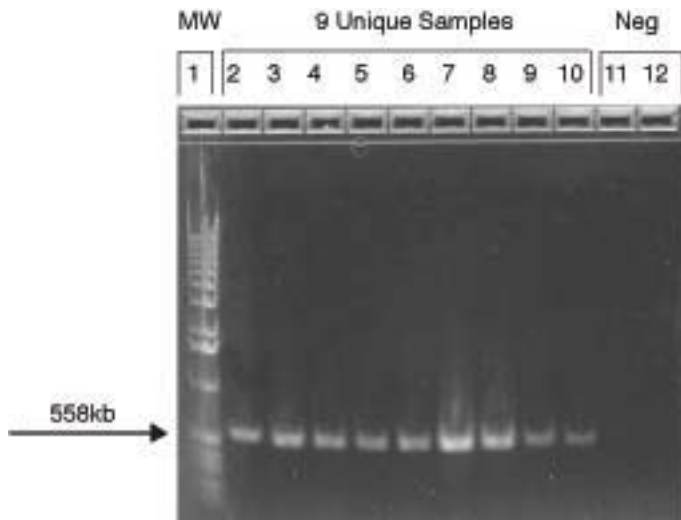


Figure 5. PCR amplification of a 558 bp fragment of amelogenin. Lane 1 is a MW ladder; Lanes 2 – 10 are 9 unique samples; Lanes 11 & 12 are negative controls.

SNP Genotyping:

Allelic Discrimination SNP Genotyping:

DNA recovered from FTA Cards with GenSolve and purified with QIAmp was used in a TaqMan allelic discrimination assay amplifying the dbSNP ID rs3818 polymorphism. Figure 6 shows the data demonstrating both homozygous and heterozygous samples.

Affymetrix SNP Genotyping:

DNA was isolated from FTA Cards with GenSolve. DNA was also isolated from the same whole blood sample as applied to FTA Cards by a QIAmp kit following manufacturer's directions. Approximately 250 ng of each preparation of DNA was digested with Xba I restriction enzyme followed by ligation with Affymetrix Xba I linkers. The fragments were amplified by PCR followed by fragmentation and end-labeling. The fragments were hybridized to the GeneChip Human Mapping 10K Array XBA 142 2.0. Table 2 shows that the average call rate was 97% for both FTA/GenSolve and QIAmp purified DNA with 100% concordance between the two samples. This demonstrates the quality of the DNA purified with FTA and GenSolve from whole blood.

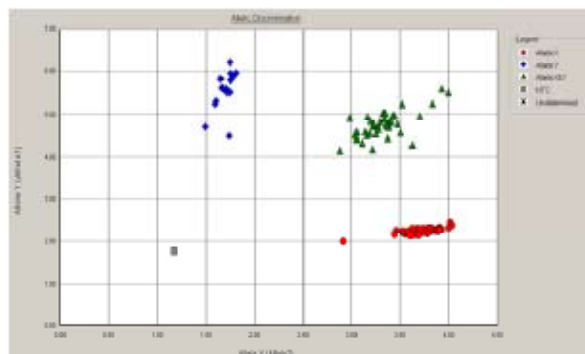


Figure 6. Allelic discrimination SNP assay DNA from 30 unique samples were amplified in a TaqMan assay for dbSNP ID rs3818

Table 2

Affymetrix GeneChip Mapping 10K Assay Results

	GenSolve	QIAmp
Average Call Rate	97%	97%
% Concordant Calls		
Between Replicates	100%	100%
% Concordant Calls		
Between Sample Types	100%	

Illumina SNP Genotyping:

DNA purified from FTA Cards using GenSolve was genotyped by the BeadLab platform using the GoldenGate Assay. DNA, at a mean concentration of 62.8 ng/ μ L, was analyzed using a single OPA (Oligo Pool All) Linkage Array. This OPA is the first oligo pool in the four-OPA Linkage Panel and includes 1,516 loci.

Thirty-eight unique samples were used in the assay and 5 chosen to run in duplicate to examine reproducibility. Two samples were dropped from the analysis due to low call frequency and a total of 41 samples were carried through to completion. A total of 62,156 calls were attempted, of those 61,818 were successful for an overall call rate of 99.46% (Table 3).

Table 3
Illumina GoldenGate Assay Results

Parameter	Number Successful	Number Attempted	Success Rate
Sample success rate	41	43	95.35%
Genotypes (Call Rate)	61,818	62,156	99.46%
Reproducibility	13,296	13,296	100%

Conclusions:

- FTA preserves and protects DNA for downstream applications.
- GenSolve is an efficient means to extract double-stranded DNA from FTA Cards
- DNA extracted using GenSolve is comparable to DNA extracted using traditional methods
- DNA extracted from FTA using GenSolve is of high quality with a MW of at least 35kb
- DNA extracted with GenSolve can be used in a variety of downstream applications such as PCR and SNP genotyping

Ordering Information:

Catalog Number Description

WB100050 GenSolve Kit, 100 recoveries

WB100040 Harris Uni-Core Punch, 6 mm

Whatman is a leader in separations technologies for basic and biomedical research. Whatman offers products for the collection and capture of nucleic acids for a wide variety of molecular testing in the fields of forensics, biomedical, agricultural and basic research. For more information, visit the company's website at <http://www.whatman.com>.

GE is Imagination at Work — a diversified technology, media and financial services company focused on solving some of the world's toughest problems. With products and services ranging from aircraft engines, power generation, water processing and security technology to medical imaging, business and consumer financing, media content and advanced materials, GE serves customers in more than 100 countries and employs more than 300,000 people worldwide. For more information, visit the company's Web site at <http://www.ge.com>.

Whatman and FTA are registered trademarks of Whatman Inc, part of GE Healthcare

QIAamp is a registered trademark of QIAGEN GmbH

PicoGreen is a registered trademark of Invitrogen, Inc.

GeneChip is a registered trademark of Affymetrix, Inc.

Illumina and GoldenGate are registered trademarks of Illumina.

Data courtesy of GenVault, Inc

Illumina GoldenGate Assay data first presented in Pub. No. 370-2007-032 15Dec07

Whatman Inc.

200 Park Avenue, Suite 210

Florham Park, NJ 07932 USA

Tel: 1-800-WHATMAN (US and Canada)

Fax: 1-973-245-8329

Email: info@whatman.com

www.whatman.com

51728 6/08

