

Amplification of human genomic DNA from blood on FTA™ with GenomiPhi™

Key Words: DNA Amplification • FTA Room temperature • FTA Purification Reagent • WGA • GenomiPhi • DNA

Whatman™ FTA is an excellent DNA storage and preparation medium for samples to be amplified by strand displacement amplification.

Whatman FTA products are powerful tools that simplify the collection, shipment, storage and purification of nucleic acids from a wide variety of sources. Animal, plant or microbe cellular samples are applied directly onto an FTA Card, activating chemicals in the card that lyse the cells, denature proteins and immobilize the nucleic acids (Fig 1.). After drying, nucleic acids are protected from enzymatic, microbial, oxidative, and free-radical damage. The samples can be stored for years at room temperature before analysis (17 yr to date for blood on FTA Cards).

For analysis or use of the stored DNA, a small disk is cut from the sample area on FTA Cards. Washing with FTA Purification Reagent removes contaminants. DNA immobilized in the washed disk can then be amplified by PCR, cut by restriction endonucleases or used in other ways. For some applications, however, larger amounts of DNA are needed than the disk—or even the whole original sample—contains. They may also require more sections of the sequence than even multiplex PCR can supply. These applications have in the past required isolation of DNA from larger samples. Whole genome amplification (WGA) has been developed to supply essentially unlimited quantities of DNA from small samples.

The GenomiPhi DNA Amplification Kit uses Phi29 DNA polymerase and a multiple strand displacement mechanism for

whole genome amplification. It produces microgram quantities of high molecular weight copies from nanogram amounts of linear template DNA. A small input sample preserved on FTA Cards can provide enough amplified DNA for multiple SNP analyses and other downstream applications. (See www.gelifesciences.com/genomiphi for details.) This application note shows an example of GenomiPhi amplification of human genomic DNA stored and purified on FTA Cards.



Fig 1. FTA Blood Stain Card.

Methods

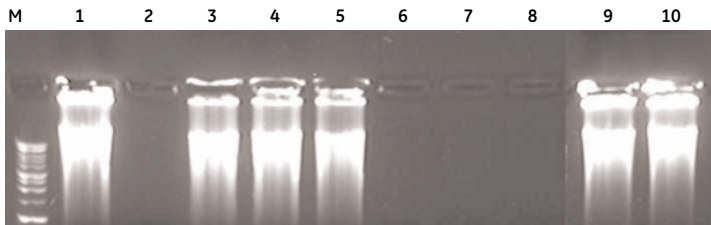
Following standard FTA protocols, fresh human blood from a finger-stick was spotted onto an FTA Card and allowed to dry. The sample was then stored in the dark at room temperature for 5 wk. Disks of 1.2 or 2.0 mm diameter were cut from the bloodstained areas with a Harris Micro Punch™. Control disks were cut from unused areas of the card. All disks were washed three times for 5 min with 200 μ l FTA Purification Reagent and twice for 5 min with 200 μ l TE⁻¹ (10 mM Tris, 0.1 mM EDTA, pH 8.0).

Amplification followed the standard GenomiPhi protocol. Each washed disk was added to 9 μ l GenomiPhi sample buffer. Control disks received 20 ng purified human genomic DNA before amplification. Samples were incubated 3 min at 95°C and 3 min at 4°C. 10 μ l GenomiPhi Reaction Buffer containing 1 μ l polymerase was added to each tube. The tubes were briefly mixed by finger vortexing and incubated overnight (about 16 h) at 30°C, then 10 min at 65°C and were finally stored at 4°C. A 4 μ l sample was removed from each tube for electrophoresis in 0.6% agarose containing ethidium bromide.



Recommended protocol

- 1) Preserve sample normally on FTA Cards. Store in a dark, dry place at room temperature.
- 2) Punch disks (1.2 mm for blood) and transfer to tubes.
- 3) Wash disks 3 x 5 min in 200 µl FTA Reagent.
- 4) Rinse disks 2 x 5 min in 200 µl TE⁻¹. Disks should be colorless.
- 5) Dry disks 1 h at room temperature (optional).
- 6) Add 10 µl (or 9 µl for wet disks) GenomiPhi sample buffer.
- 7) Incubate 3 min at 95°C and chill on ice.
- 8) Centrifuge briefly if necessary to collect condensate.
- 9) Add 10 µl GenomiPhi Reaction Mix containing 1 µl Phi29 polymerase and mix.
- 10) Incubate overnight (or about 16 h) at 30°C.
- 11) Incubate 10 min at 65°C and store at 4°C.



- Lane M:** 1 kb DNA ladder
Lane 1: Positive control DNA (no FTA)
Lane 2: Negative control (no DNA or FTA)
Lanes 3-5: Amplifying 1.2 mm sample disks
Lanes 6-8: Amplifying 2.0 mm sample disks
Lane 9: Amplifying 1.2 mm control (unspotted) disk plus positive control DNA
Lane 10: Amplifying 2.0 mm control disk plus positive control DNA

Fig 2. Electrophoresis gel of amplified human genomic DNA from blood on FTA

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Results

The figure shows successful GenomiPhi amplification of human genomic DNA immobilized on 1.2 mm disks cut from FTA containing human blood (lanes 3–5). The 2.0 mm disks did not give successful amplification in this experiment (lanes 6–8). Added DNA was still amplified in the presence of washed FTA control disks of either size (lanes 9 and 10).

The amplification of human DNA from blood stored on FTA also succeeded with smaller (1.2 mm) but not larger (2.0 mm) disks in two additional experiments (data not shown). However, GenomiPhi did amplify DNA on larger disks of buccal samples, while washed disks did not inhibit amplification of control DNA. It appears that excess DNA template on the larger blood sample disks may inhibit the process.

Conclusions

Human blood DNA preserved on FTA Cards is a suitable sample for whole genome amplification by GenomiPhi. This approach combines the FTA benefits of collecting small original samples and storing them safely and inexpensively at room temperature with the WGA benefits of easily amplifying large enough quantities of DNA for the most demanding applications.

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Phi 29 DNA Polymerase: Phi 29 DNA polymerase and its use for DNA synthesis is covered by US patent numbers 5,854,033, 5,198,543, 5,576,204 and 5,001,050.

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