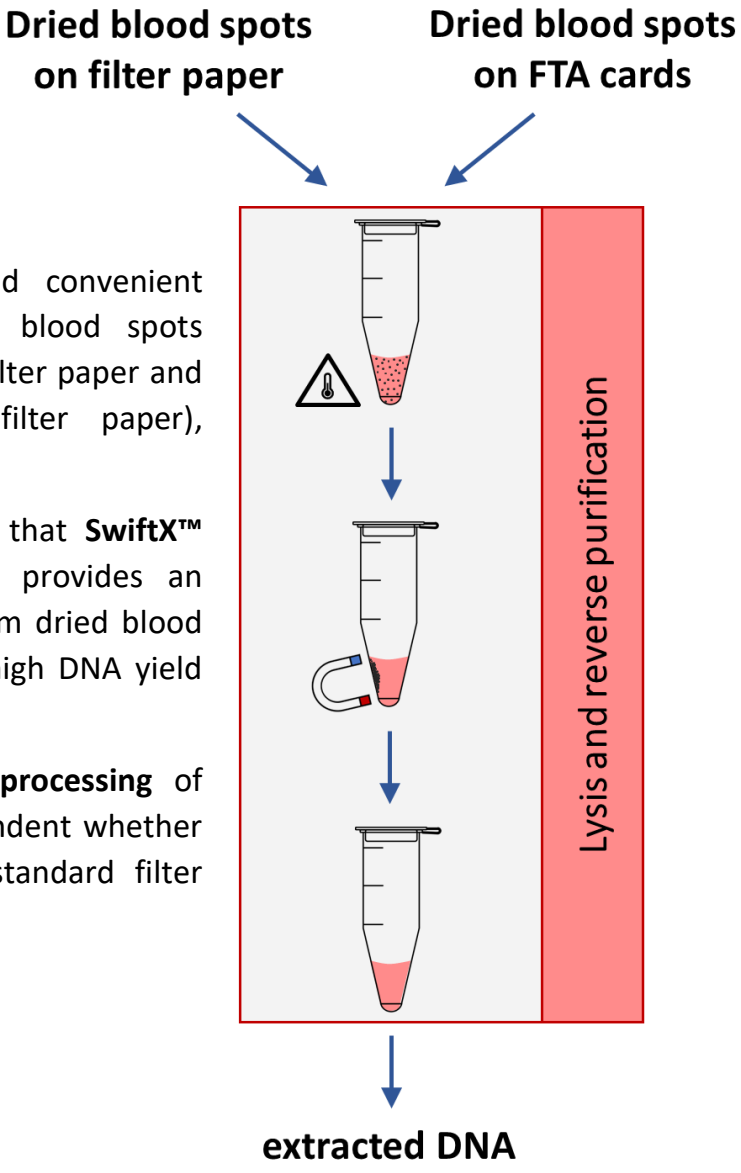
Extraction of DNA from dried blood spots on standard filter paper and FTA cards

Related product

This application note is related to **SwiftX™ DNA** (cat.no. SXD-25).



Summary

Several protocols for rapid and convenient extraction of DNA from dried blood spots deposited on standard cellulose filter paper and on FTA cards (impregnated filter paper), respectively, have been validated.

The collected data demonstrate that **SwiftX™ DNA** applied using **Protocol 1** provides an excellent way to extract DNA from dried blood spots. The procedure combines high DNA yield with a short processing time.

SwiftX™ DNA enables routine processing of dried blood spot samples independent whether they have been deposited on standard filter paper or on FTA cards.

Introduction

Blood is a valuable sample specimen widely used in human as well as veterinary diagnostics. If capillary blood is utilized, it either needs to be processed immediately or needs to be stored for later processing. The latter option requires the sample to be preserved until testing, which is often achieved by spotting the blood sample onto a filter paper and drying it (Katakura et al. (1997) Parasitol Res 83:241).

If the filter paper is not chemically treated, the dried blood spot (DBS) should be stored frozen to avoid sample degradation over time. Alternatively, the blood can be spotted on so-called FTA cards, invented by Flinders Technology Associates. These cards are manufactured by Whatman and are available from numerous vendors (e.g. Qiagen QIAcard FTA classic). FTA cards consist of filter paper that is impregnated with a proprietary mix of additives, which leads to lysis of cells and prevents degradation of released nucleic acids by nucleases. Conveniently, samples spotted and dried on FTA cards do not require frozen storage (Smith & Burgoyne (2004) BMC Ecol 4:4, Picard-Meyer et al. (2006) 140:174).

However, both types of DBS storage come with challenges for the DNA extraction procedure. Cells in DBS on standard filter paper are coagulated and stick strongly to the paper support, which requires a thorough approach to release the genetic material. DNA from FTA cards, in contrast, can be released rather easily, but the detergents in the paper are highly inhibitory to most downstream analysis methods as it contains SDS.

This application note describes easy and rapid procedures based on SwiftX™ DNA for extraction of genomic DNA from blood spots dried on both standard filter paper as well as FTA cards, respectively.

Experimental details

The following sample types and extraction methods have been tested:

1. **Capillary blood spotted and dried on filter paper, stored at -20°C**
    a. ***Rapid extraction protocol:*** SwiftX™ DNA using a modified Protocol 1
        - 2x2mm dried blood sample placed in a microtube
        - Lysis mixture (180µL Buffer DL, 10µL Proteinase K, 30µL Beads A) added
        - incubated for 10 minutes at 60°C *(can be ommitted – see Results)*
        - Incubated for 10 minutes at 95°C
        - Sample briefly cooled down and placed in a magnetic rack for 1 minute
        - 170µL supernatant transferred to a new tube
    b. ***Reference extraction protocol:*** Qiagen QIAamp DNA Mini Kit
        - 2x2mm dried blood sample placed in a microtube
        - 200µL Buffer ATL added
        - 20µL Proteinase K added
        - 200µL Buffer AL added and mixed
        - Incubated 10 minutes at 60°C
        - 200µL ethanol added and mixed

- Binding mixture loaded onto spin column
- Column centrifuged for 1 minute at 8000xg
- Column transferred to new tube
- 500µL Buffer AW1 added
- Column centrifuged for 1 minute at 8000xg
- Column transferred to new tube
- 500µL Buffer AW2 added
- Column centrifuged for 1 minute at 12000xg
- Column transferred to new tube
- Column centrifuged for 1 minute at 12000xg
- Column transferred to new tube
- 200µL Buffer AE added
- Incubated for 1 minute at room temperature
- Column centrifuged for 1 minute at 8000xg

2. **<u>Capillary blood spotted and dried on FTA cards, stored at ambient temperature</u>**
   a. ***Rapid extraction protocol:*** SwiftX™ DNA using Protocol 1
      - 2x2mm dried blood sample placed in a microtube
      - Lysis mixture (180µL Buffer DL, 30µL Beads A, 10µL Proteinase K) added *(Proteinase K can be omitted – see Results)*
      - Incubated for 10 minutes at 95°C
      - Sample briefly cooled down and placed in a magnetic rack for 1 minute
      - 170µL supernatant transferred to a new tube

   b. ***Reference extraction protocol 1:*** Qiagen QIAamp DNA Mini Kit
      - 2x2mm dried blood sample placed in a microtube
      - See protocol detailed in section 1.b for detailed workflow

   c. ***Reference extraction protocol 2:*** Qiagen Fast Elution protocol for FTA cards
      - 2x2mm dried blood sample placed in a microtube
      - 500µL Buffer TE pH 9 added
      - Sample vortexed for 5 seconds
      - Buffer TE discarded
      - 500µL Buffer TE pH 9 added
      - Sample vortexed for 5 seconds
      - Buffer TE discarded
      - 100µL Buffer TE pH 9 added
      - Incubated 30 minutes at 95°C while shaken with 1000rpm
      - Cooled down
      - Supernatant transferred to new tube

Quantification of the extracted genomic DNA was performed with an in-house real-time PCR assay for quantitative determination of human DNA using Thermo Maxima Probe Mastermix and Qiagen Quantifast Probe Mastermix.

For separation of the magnetic particles, the **magnetic rack (cat.no. MAG-12, MAG-4)** can be used, which is available from Xpedite Diagnostics.

Results

Extraction of genomic DNA from dried blood spots (DBS)

Figure 1 shows the results for extraction of genomic DNA from six blood spots dried on standard filter paper. The dried blood spots have been cut into equal pieces of 2x2 mm size and have then been processed as described in the section “Experimental Details”. The PCR analyses of all extracted samples were performed using 1µL or 5µL of DNA, respectively.

The data (Figure 1, left diagram) revealed that the rapid extraction using SwiftX DNA Protocol 1 and Proteinase K resulted in an at least 2-fold higher recovery of DNA from the dried blood spots compared to the reference extraction method. Furthermore, the variation in DNA recovery was equal or lower with SwiftX DNA extraction compared to conventional spin-column purification.

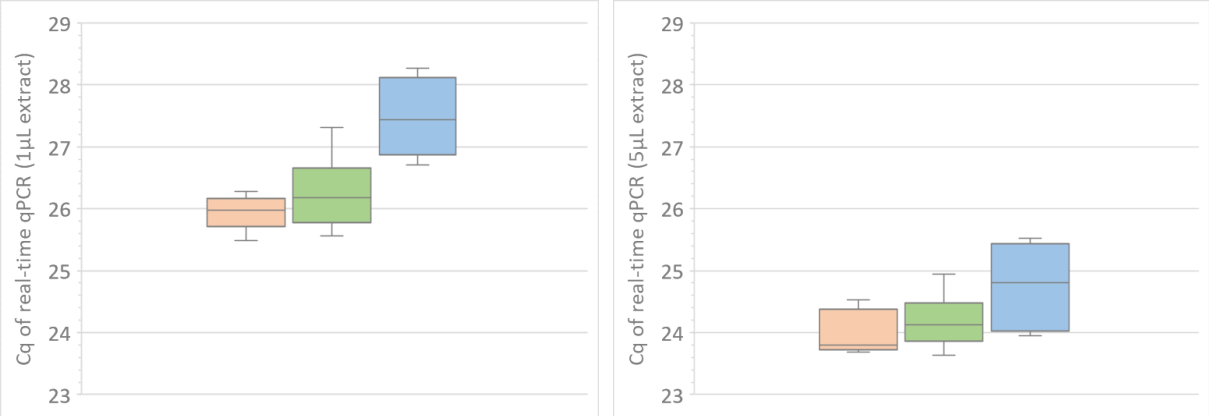


Figure 1. Box plots of real-time PCR quantification of DNA from dried blood spots extracted with different methods. **Left:** 1µL DNA extract used in a 20µL PCR reaction. **Right:** 5µL DNA extract per 20µL PCR reaction. ***Red:*** SwiftX DNA Prot. 1 + Proteinase K + 95°C heating only; ***Green:*** SwiftX DNA Prot. 1 + Proteinase K + 60°C/95°C heating; ***Blue:*** QIAamp DNA Mini Kit.

It should be mentioned here that the DNA extracted from DBS with SwiftX DNA Protocol 1 showed a greenish color. However, even when a high volume of extracted DNA was subjected to real-time PCR analysis (5µL or 25% (v/v)), the Cq values of the SwiftX-extracted nucleic acids remained lower than the Cq values of nucleic acids retrieved from the reference method (Figure 1, right diagram). This shows that despite of the semi-pure nature of SwiftX-extracted DNA samples, the remaining substances did not lead to significant inhibition of the PCR reactions.

Furthermore, it can be concluded that there is no separate incubation for Proteinase K activation required for efficient DNA recovery. In fact, we observed that incubation of the sample directly at 95°C without prior incubation at 60°C resulted in a slightly higher DNA yield and a slightly lower variation in DNA recovery.

Extraction of genomic DNA from dried blood spots on FTA paper cards (DBS-FTA)

Figure 2 summarizes the results for extraction of genomic DNA from blood spots dried on FTA cards. Six blood spots have been cut into equal pieces of 2x2 mm size and have then been processed as described in the section "Experimental Details". We compared SwiftX rapid extraction – with and without the use of Proteinase K – with two different reference extraction methods: the QIAamp DNA Mini kit encompassing a full DNA purification process and a protocol recommended by Qiagen for rapid heat-based elution of DNA from FTA paper material, respectively. The PCR analyses of all extracted samples were performed using 2µL of DNA for the PCR analysis.

The results revealed that the standard spin-column kit recovered less than 10% of the amount of DNA compared to all other methods as judged by the difference in Cq values. In contrast, the DNA recovery of the two SwiftX protocols was essentially identical to the heat elution protocol recommended by Qiagen - even though the Qiagen protocol is much longer and more cumbersome to perform since it involves repeated wash steps and a 30-minute heat elution step.

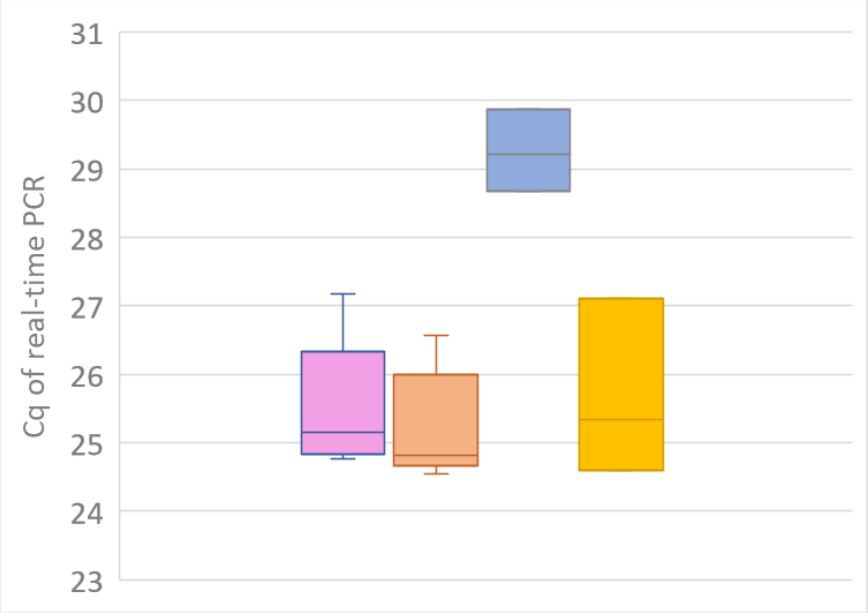


Figure 2. Box plot of real-time PCR quantification of DNA from blood samples on FTA paper extracted with different methods (2µL DNA extract used in a 20µL PCR reaction). ***Purple:*** SwiftX DNA Protocol 1; ***Red:*** SwiftX DNA Prot. 1 + Proteinase K; ***Blue:*** QIAamp DNA Mini Kit; ***Yellow:*** Heat-elution protocol recommended by Qiagen.

There was no significant difference between the two SwiftX protocols in the amount of recovered DNA. The addition of Proteinase K did not increase the DNA level, which can be explained by the fact that cells within the material spotted onto FTA cards are readily lysed and their DNA "just" needs to be eluted.

We noticed that the SwiftX extracts had a clear red coloration, which is apparently originating from hemoglobin of lysed red blood cells on the FTA filter paper sample. However, this did not hamper the amplification results achieved with the SwiftX-extracted DNA. Based on that, it is evident that the washing steps of the Qiagen protocol are not essential for the quality of the

eluted DNA if the elution process is not performed as rigorous as instructed by Qiagen (30 minutes heating at 95°C with continuous shaking at 1000 rpm).

Discussion

The data presented here demonstrate the excellent sensitivity of SwiftX™ DNA for the extraction of genomic DNA from dried blood spots. Beyond the extraction of genomic DNA, the process is also applicable to rapid and convenient extraction of DNA from parasites and bacteria in dried blood spot samples.

SwiftX™ DNA achieves highly sensitive DNA extraction with an advanced convenience and processing time compared to conventional DNA extraction (as exemplified by the QIAamp Mini DNA Kit) and to an existing fast heat elution protocol as illustrated by Figure 3.

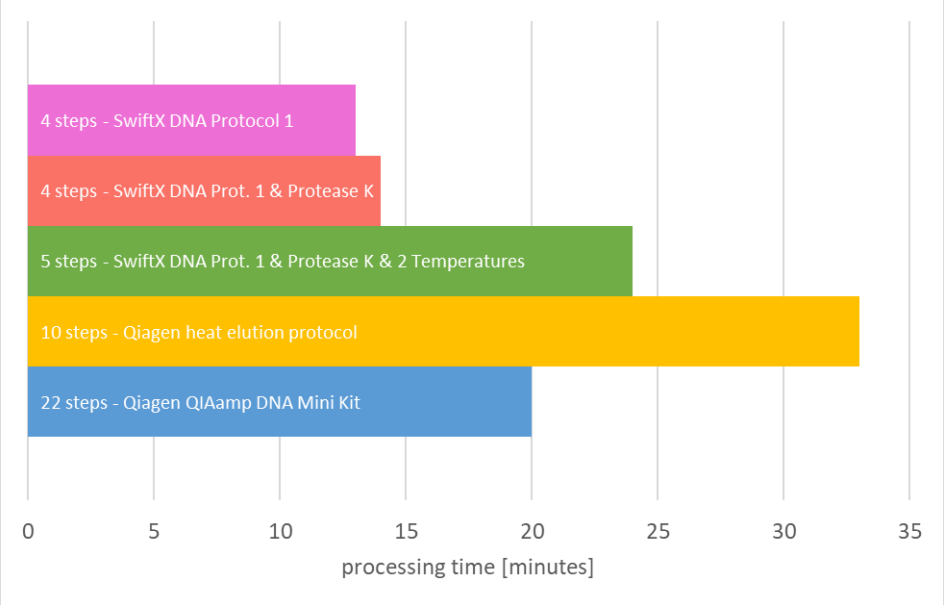


Figure 3. Comparison of protocol steps and processing time per sample for the different DNA extraction protocols tested in this work.

For rapid extraction of DNA from **dried blood spots on standard filter paper**, we recommend performing **Protocol 1** of the SwiftX DNA handbook **supplemented with 10µL Proteinase K** (20 mg/mL). A 60°C incubation is not required.

For rapid extraction of DNA from **dried blood spots on FTA cards**, we recommend performing **Protocol 1** of the SwiftX DNA handbook.