Application Note

Direct PCR from blood preserved on Whatman FTA and 903 Cards using Thermo Scientific Phusion Blood Direct PCR Kit

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Key words

- Whatman FTA Elute Cards
- Whatman FTA Gene Cards
- Whatman 903 Cards
- Blood
- Direct PCR
- Heparin
- EDTA
- Na Citrate
- Human
- Mouse
- Pig
- Cat
- Dog
- Bovine

Introduction

Thermo Scientific Phusion Blood Direct PCR Kit enables DNA amplification directly from blood samples stored on Whatman FTA® and 903® Cards. No prior DNA extraction or purification steps are needed. When combined with Thermo Scientific Piko Thermal Cycler and Piko UTW (ultra-thin walled) reaction vessels. PCR products can be amplified in as little as 30 minutes.

Abstract

Paper cards are a popular method of storing blood due to their ease of use and long term stability at room temperature (1, 2). However, these cards often contain various chemicals that may inhibit enzymatic reactions, and thus special extraction buffers and/or protocols are generally recommended before such samples can be used in PCR. Phusion® Blood DNA Polymerase is a unique, engineered hot start enzyme based on fusion protein technology (3). This polymerase contains a dsDNA binding domain which allows efficient amplification of DNA even in the presence of a wide range of inhibitors, including those found in blood and in storage cards. Phusion Blood Direct PCR Kit contains a complete set of reagents optimized to perform in the presence of blood regardless of the storage method or anticoagulant used. Here we present protocols for the robust amplification of genomic DNA from blood dried onto Whatman 903, FTA Elute and FTA Gene Cards.

Table 1. Reaction conditions for PCR

Component	25 µL reaction	50 µL reaction	Final conc.
H_2O	Add to 25 µL	Add to 50 µL	
2x Phusion Blood PCR Buffer	12.5 µL	25 µL	1x
Primer F (Forward)	x µL	x µL	0.5 µM
Primer R (Reverse)	x µL	x µL	0.5 µM
Phusion Blood DNA Polymerase	0.5 µL	1 µL	
903/FTA Card	1 mm punch	1 mm punch	
Optional components for reaction optimization*			
50 mM $MgCl_2$	0.75 µL	1.5 µL	
50 mM EDTA	0.6 - 1.25 µL	1.25 - 2.5 µL	
DMSO	1.25 µL	2.5 µL	5%

* See Phusion Blood Direct PCR Kit manual for more instructions related to optional components.

Table 2. Cycling protocols

	2-step protocol		3-step protocol		
Cycle step	Temp.	Time	Temp.	Time	Cycles
Lysis of cells	98°C	5 min	98°C	5 min	1
Denaturation Annealing* Extension**	98°C - 72°C	1 s - 15-30 s /kb	98°C x°C 72°C	1 s 5 s 15-30 s/kb	35-40
Final extension	72°C 4°C	1 min hold	72°C 4°C	1 min hold	1

* 2-step protocol is applicable when primer Tm values are at least 69-72°C as calculated with Thermo Scientific' Tm calculator. As a basic rule, for primers > 20 nt, anneal at Tm +3°C of the lower Tm primer. For primers ≤ 20 nt use an annealing temperature equal to the Tm of the lower Tm primer.

** The recommended extension time is 15 s for amplicons ≤ 1 kb, and 30 s/kb for amplicons > 1 kb.

Materials and methods

- Thermo Scientific Phusion Blood Direct PCR Kit
- Thermo Scientific Piko Thermal Cycler
- Thermo Scientific Piko UTW tubes or plates
- Primers: (Forward and Reverse)

506 bp fragment of human Cathepsin K gene

F:GAGAATCGCTTGAACCCGGGAGGTGT AGGT 30 nt Tm 78.1°C

R:CCTGCTGATGCCTGGCCTCTTTCTTC TTTG 30 nt Tm 78.1°C

1020 bp fragment of human glutathione peroxidase 3 gene

F:CATCAGCCCGTCTAGGAACCCAGTCAT CAG 30 nt Tm 77.6°C

R:CTCCTTCATCCCGCTACACCACGCATA CAC 30 nt Tm 77.9°C

3.8 kb fragment of human beta-globin gene

F:GCACTGGCTTAGGAGTTGGACT 22 nt Tm 65.9°C

R:ACAGACACCCAGGCCTACTTG 21 nt Tm 65.6°C

7.5 kb fragment of human beta-globin gene

F:GCACTGGCTTAGGAG TTGGACTTCAA ACC
29 nt Tm 73.9°C

R:CAACTGCTGAAAGAGATGCGGTGGG
25 nt Tm 75.1°C

237 bp fragment of human SOX21 gene 5' region (control primers of Phusion Blood Direct PCR Kit)

F:AGCCCTTGGGGASTTGAATTGCTG
24 nt Tm 73.5°C

R:GCACTCCAGAGGACAGCRGTGTCAATA
27 nt Tm 72.2°C/75.3°C (R=A/G)

Sample preparation

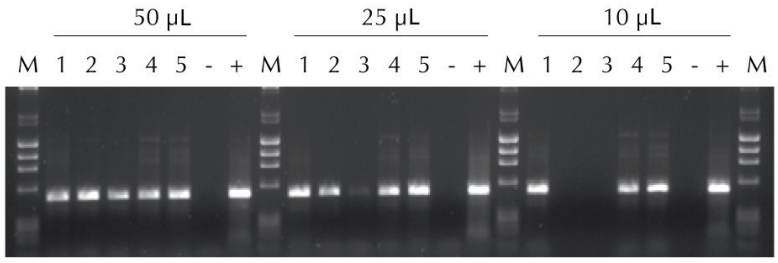
Fresh blood or blood preserved with heparin (1.4 IU/mL), K_2EDTA (1.8 mg/mL), or Na Citrate (109 mM) was applied onto Whatman 903 Cards, FTA Elute Cards, or FTA Gene Cards and dried as per the manufacturer's instructions. For direct PCR, a 1 mm disc was punched out of the sample in the card and used in the following PCR reaction volumes:

- Whatman 903: 10-50 µL
- Whatman FTA Elute Card: 25-50 µL
- Whatman FTA Gene Card: 50 µL

If larger punches or smaller reaction volumes were used, punches were washed with 20 µL of H_2O at 50°C for 3 minutes. After removing the H_2O, PCR components were added directly to the rinsed punch.

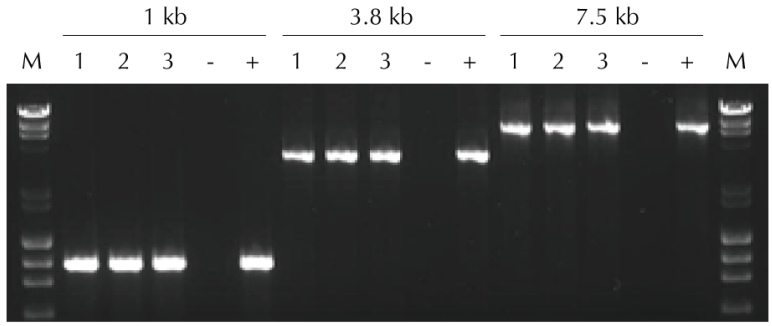
Results

With Phusion Blood Direct PCR Kit, it is possible to amplify DNA directly from blood stored on various filter cards. We have seen that the various types of storage cards show differing levels of inhibition when used in Direct PCR (Figure 1). 903 Cards showed almost no inhibition, and a 1 mm punch could be used with reaction volumes as low as 10 µL. FTA Elute Cards inhibited direct PCR reactions slightly; a 1 mm disc in a 25-50 µL reaction worked well, but when placed in a 10 µL reaction, the PCR was totally inhibited. FTA Gene Cards showed the greatest level of inhibition. Without any pre-treatments, a 1 mm punch of FTA Gene Card worked well only in a 50 µL reaction volume. For smaller reaction volumes, a very simple washing protocol was enough to remove inhibitors from both FTA Elute and FTA Gene Cards. After washing the card punch for 3 minutes with H_2O, it could be used for direct PCR with Phusion Blood Direct PCR Kit at all reaction volumes tested (Figure 1). Direct PCR from blood dried onto cards is also applicable to long amplicons. Punches from 903 Cards and rinsed punches from FTA Elute and FTA Gene Cards (all 1 mm in



1 = 903 Card, 2 = FTA Elute Card, 3 = FTA Gene Card, 4 = FTA Elute Card, washed, 5 = FTA Gene Card, washed, M = Size Marker, - Negative control, + Positive control (purified human genomic DNA).

Figure 1. Direct amplification of a 500 bp genomic DNA fragment from human blood treated with heparin and preserved on various cards. Reactions were performed from 1 mm punches either rinsed or placed directly into PCR reactions of 50, 25 or 10 µL in volume. A 2-step PCR protocol described in Materials and Methods was used. Using Piko® Thermal Cycler and Piko UTW® reaction vessels, the total PCR protocol time was 30 minutes.



1 = 903 Card, 2 = FTA Gene Card, 3 = FTA Elute Card, M = Size Marker, - Negative control, + Positive control (purified human genomic DNA).

Figure 2. Direct PCR of 1 kb, 3.8 kb and 7.5 kb gDNA amplicons from human blood treated with EDTA and preserved on various cards. Reactions were performed from 1 mm punches in 50 µl reactions (FTA Gene Card punches were rinsed as described in Materials and Methods for 7.5 kb fragment). A 2-step protocol was used for 1 kb and 7,5 kb fragments and a 3-step protocol for 3.8 kb amplicon. Using Piko Thermal Cycler and UTW reaction vessels the total PCR protocol times were 30 min for 1 kb, 1 h 35 min for 3.8 kb, and 2 h 33 min for 7.5 kb fragment.

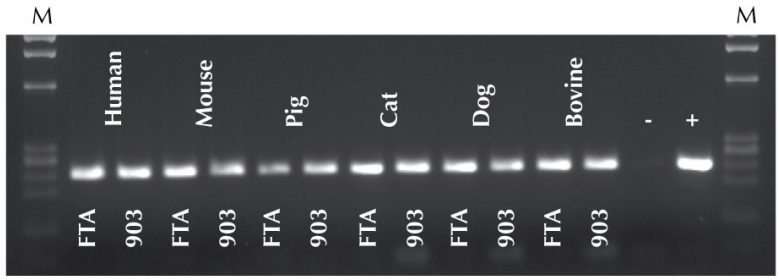


Figure 3. Direct PCR performed on blood from several mammalian species treated with EDTA and preserved on 903 and FTA Gene Cards. Reactions were performed from 1 mm punches using the universal control primers included in the Phusion Blood Direct PCR Kit and 20 µL reaction volume (FTA Gene Cards were rinsed). The 2-step PCR protocol time was 30 minutes. Piko Thermal Cycler and UTW reaction vessels were used in PCR. M Size Marker, - Negative control, + Positive control (purified human genomic DNA).

diameter) were used in 50 µL reaction volumes with primers specific for 1 kb, 3.8 kb and 7.5 kb amplicons. In all cases, PCR worked well (Figure 2). The Phusion Blood Direct PCR Kit is compatible with blood from variety of species. A highly conserved 237 bp region upstream of the SOX21 gene (4) was successfully amplified from blood of a number of vertebrate species dried onto 903 and FTA Gene Cards (Figure 3).

Discussion

Amplification of DNA directly from storage cards commonly used for preserving blood samples is particularly challenging due to PCR inhibitors present in both blood and the storage cards themselves. With conventional DNA polymerases, a thorough washing protocol is required to remove the contaminants from the punch discs before PCR (5). We show here that Phusion Blood Direct PCR Kit allows amplification of DNA directly from blood stored on cards such as Whatman 903, FTA Elute and FTA Gene Cards with no or very little pretreatment. Direct PCR from a 1 mm punch worked well for all the cards tested in 50 µL PCR reaction volume. In smaller reaction volumes, 903 Cards could be used directly whereas FTA Cards were applicable after a brief washing step. When combined with the fast Piko Thermal Cycler and Piko UTW reaction vessels, total protocol times can be significantly reduced while maintaining good product yields with no DNA isolation or purification steps.

References

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The Thermo Scientific Direct PCR approach allows for amplification of DNA directly from various starting materials such as blood, mouse ear and tail tissues, plants, and FFPE tissue samples. For more information about the Direct PCR products and protocols, please visit

www.thermoscientific.com/directpcr