

Direct PCR from Dried Blood without DNA Extraction Using the FailSafe™ PCR System

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The FailSafe™ PCR System enabled consistent and reproducible PCR amplification of six different gene targets directly from dried blood without prior nucleic acid extraction of the genomic DNA template.

Introduction

Dried blood spots collected on Specimen Collection Cards, commonly called “Guthrie cards” (Figure 1), have been used widely as valuable resources for genetic studies, such as, determination of hereditary diseases like phenylketonuria and congenital hypothyroidism. The ease of storage and transportation of these cards with blood spots also lends them to a variety of large field studies, especially in remote areas. For example, over 15,000 Guthrie cards with dried blood spots were collected during a pre-natal HIV transmission study in South Africa.¹

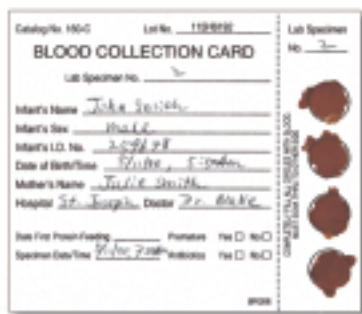


Figure 1. An example of a Guthrie card.

Polymerase chain reaction (PCR) is commonly used when evaluating Guthrie card blood spots. Unfortunately, amplification of DNA from dried Guthrie card blood spots presents considerable technical difficulties due to the presence of natural PCR inhibitors, e.g., protein, heavy metals, heme, and heme degradation products,² and the fact that the amount of genetic material is limited. A number of specialized protocols have been developed to effectively recover DNA from Guthrie card blood spots,^{3,4} although they still prove to be time consuming, cumbersome, and expensive, especially for high throughput studies.

In a previous report,⁵ we demonstrated that the FailSafe PCR System enabled direct PCR amplification using whole

Gene Region amplified	PCR Cycling conditions	Expected size of PCR product
<i>apoE</i>	94°C 5 minutes 95°C 30 seconds 60°C 30 seconds 72°C 1 minute	268 bp
CFTR (cystic fibrosis transmembrane conductance regulator) exon 11	94°C 2 minutes 94°C 10 seconds 53°C 10 seconds 74°C 10 seconds 74°C 5 minutes	233 bp
Human microsatellites: DXS6789 DX7132 GATA31E08 GATA175D03	94°C 2 minutes 94°C 1 minute 55°C 1 minute 72°C 1 minute 74°C 4 minutes	118 - 150 bp 283 - 299 bp 226 - 254 bp 170 - 186 bp

Table 1. PCR cycling parameters and expected product size from each of the six primer pairs tested. Each 50 µl FailSafe PCR reaction contained 25 µl of the appropriate FailSafe PCR 2X PreMix, 50 pmoles of each of the respective primers and 2.5 U of FailSafe PCR Enzyme Mix. One nanogram of purified human genomic DNA was used to identify the optimal FailSafe 2X PreMix for each primer pair.

blood that had been preserved under various conditions, without prior purification of the DNA template. This article reports a fast and easy way to obtain consistent, reproducible PCR amplification directly from dried blood spots collected on Guthrie cards or glass slides, without the need for DNA extraction.

Materials and Methods

Identifying optimal PCR conditions

Primer pairs for six different gene targets were tested independently with each dried blood sample. First, the optimal PCR reaction conditions for each primer pair were identified using the FailSafe PCR PreMix Selection Kit and purified human genomic DNA as a template. The appropriate optimal FailSafe PCR PreMix was then used for all subsequent amplifications with each primer pair using dried

blood samples. For example, FailSafe PCR PreMix J was identified as optimal for the *apoE* gene amplification (Figure 2). Similarly, FailSafe PreMix C was chosen for amplification of CFTR exon 11, and PreMix G was chosen for amplification of the four human microsatellite markers (DXS6789, DXS7132, GATA31E08, and GATA175D03). The primer pairs, PCR amplification conditions, and PCR product sizes for the 6 primer pairs are presented in Table 1.

Methods for processing dried blood samples

Fifty microliters of freshly drawn whole blood was spotted onto Guthrie cards and glass slides. Blood spots were allowed to dry for at least 48 hours before use. Three different methods were used to process the dried blood samples for use in PCR (Figure 3).

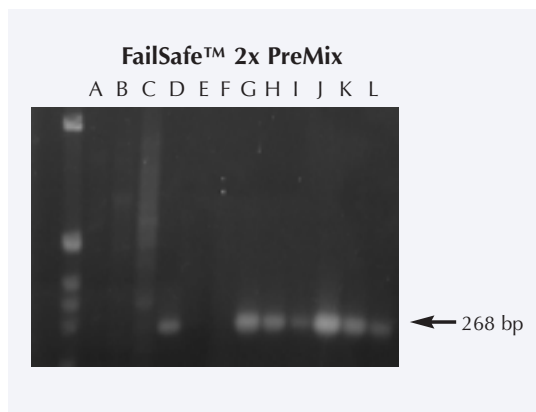


Figure 2. Identification of the optimal FailSafe 2X PreMix for amplification of the *apoE* gene. The *apoE* gene was amplified from 1 ng of purified human genomic DNA using all 12 FailSafe PCR 2X PreMixes (A-L) contained in the FailSafe PreMix Selection Kit. Amplification conditions were as described in Table 1. PCR products were electrophoresed on a 2% agarose gel and visualized with SyberGold™ (Molecular Probes). FailSafe 2X PreMix J produced optimal results for this template/primer pair.

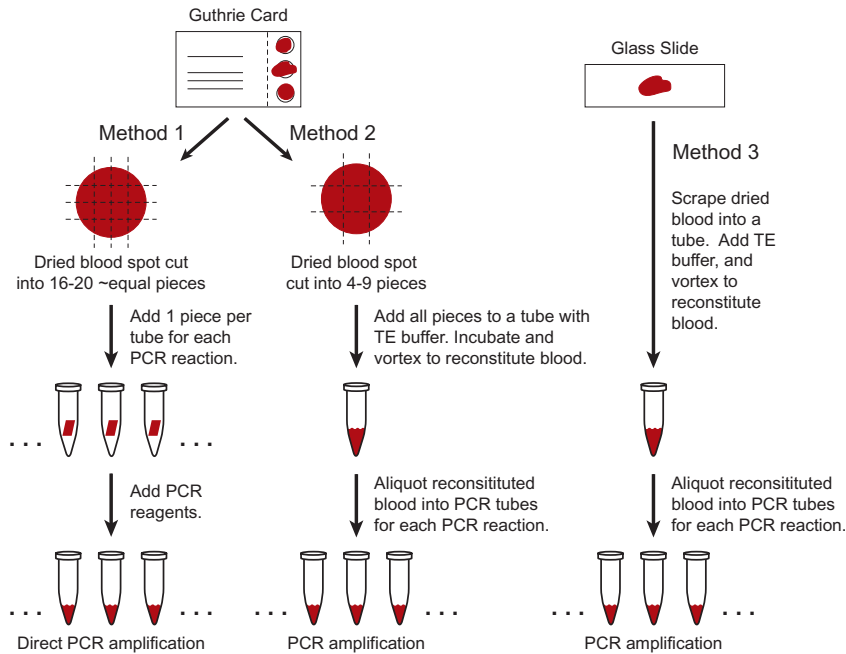


Figure 3. Summary of the three dried blood sample methods used for direct PCR.

In Method 1, the sample used for FailSafe PCR was a piece of a dried blood spot on a Guthrie card. Thus, a Guthrie card circle, completely filled with 50 μ l of dried blood, was cut into 20 pieces of similar size (each piece > 2mm x 2mm), and stored in a sterile tube at room temperature until used. A single piece of the cut up Guthrie card, corresponding to about 2.5 μ l of whole blood, was used for analysis by FailSafe PCR.

In Method 2, the sample used for FailSafe PCR consisted of reconstituted blood samples obtained by elution of dried blood spots from Guthrie cards. Guthrie card blood spots were cut into approximately 10 pieces and combined in a sterile 1.5 ml tube. Seventy-five microliters of TE buffer (10 mM Tris-HCl, pH 7.5; 1 mM EDTA) was added, and the solution was incubated at room temperature for 10 minutes, and then vortexed vigorously. The reconstituted blood solutions were stored at 4°C until used. One microliter of the resulting reconstituted blood, corresponding to about 0.67 μ l of the original whole blood sample, was used for analysis by FailSafe PCR. In another experiment using reconstituted blood samples prepared according to Method 2, Guthrie cards were prepared for two individuals using spots containing only 5 μ l of blood. Each dried blood spot was then eluted with 10 μ l of TE buffer and FailSafe PCR of human microsatellite marker GATA31E08 was performed using 1 μ l of the resulting reconstituted

blood solution, which corresponds to 0.5 μ l of the original whole blood sample.

In Method 3, the sample used for FailSafe PCR was reconstituted blood from dried spots on glass slides. Thus, 50 μ l samples of fresh blood were dried on a glass slide for 72 hours at room temperature. Then, dried blood samples were individually scraped into a sterile microcentrifuge tube, reconstituted with 75 μ l of TE buffer, vortexed vigorously, and incubated at room temperature for 10 minutes. The reconstituted blood solutions were stored at 4°C until used. One microliter of the resulting reconstituted blood was used for analysis by FailSafe PCR, which corresponds to about 0.67 μ l of the original whole blood sample.

FailSafe PCR

FailSafe PCR was performed using the amounts and kinds of samples as

described in the section above entitled "Methods for processing dried blood samples," and other reaction components and conditions as described in Table 1 for each of the six primer pairs. Prior to PCR, the reaction tubes containing Guthrie card pieces were incubated for 5 minutes at room temperature and vortexed. Then, PCR reactions were incubated using the cycling conditions described in Table 1 and analyzed on 2% agarose gels visualized with SyberGold™ (Molecular Probes).

Results

Figure 4 shows PCR results obtained using samples processed according to Method 1. Using dried blood spot pieces from Guthrie cards for three different individuals, all amplifications were successful with all six primer pairs tested. These amplifications were successfully repeated three times to confirm the consistency and reliability of this direct PCR method.

FailSafe PCR amplifications were also successful with all six primer pairs and all reconstituted blood samples tested using samples prepared by Method 2. As an example, the *apoE* amplification from reconstituted dried blood from Guthrie cards is shown in Figure 5, Lane 4. PCR amplifications using each of the six different primer pairs were successfully repeated three times, confirming the consistency and reproducibility of PCR using samples prepared using this method. Fibers from the Guthrie cards that were present in the reconstituted blood samples did not appear to inhibit FailSafe PCR amplification.

As observed with the other two methods, FailSafe PCR was also successful with all six primer pairs using reconstituted blood samples prepared according to Method 3 from dried blood spots on glass slides. As an example, the *apoE* amplification from reconstituted blood from glass slides is

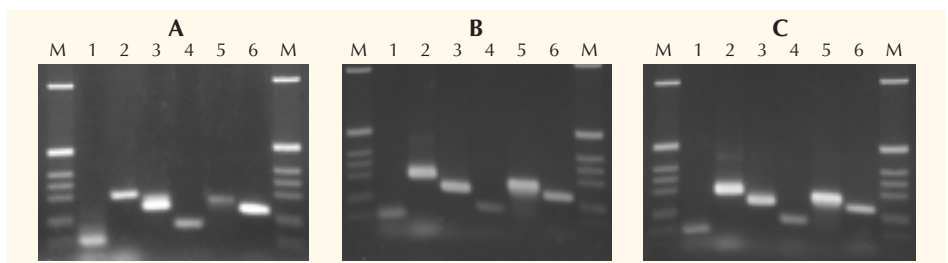


Figure 4. Direct PCR amplification of dried blood from cut up Guthrie card pieces using six different primer pairs and blood from three different individuals. Six different PCR amplifications were carried out using Guthrie card pieces from the dried blood of three individuals (A,B,C). M, DNA size standards; Lane 1, Human microsatellite DXS6789; Lane 2, Human microsatellite DXS 7132; Lane 3, Human microsatellite GATA31E08; Lane 4, Human microsatellite GATA175D03; Lane 5, *apoE*; Lane 6, CFTR exon 11. PCR products were electrophoresed on a 2% agarose gel and visualized with SyberGold™ staining.

shown in Figure 5, Lane 5. The six different amplifications were also successfully repeated three times.

Figure 5 summarizes the direct PCR amplification results obtained with the *apoE* primer pair using all three dried blood preparation methods.

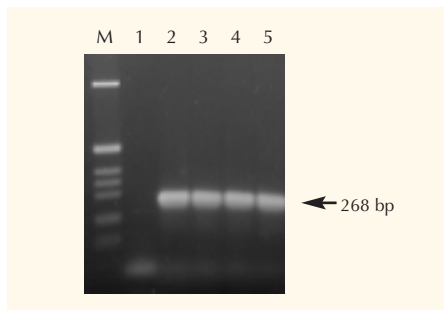


Figure 5. Amplification of the *apoE* gene by direct PCR of dried blood samples prepared by three different methods. M, DNA size standards; Lane 1, Negative control; Lane 2, Positive control using 1 ng of purified human genomic DNA; Lane 3, Method 1; Lane 4, Method 2; Lane 5, Method 3.

Conclusion

The FailSafe PCR System permits consistent and reproducible direct amplification of genomic DNA targets in samples of dried whole blood on Guthrie cards or glass slides without any prior DNA extraction. Thus, the FailSafe PCR System provides a simple, economical and sensitive method for analysis of DNA templates in blood samples, both for small numbers of samples or for high throughput applications.

References

1. Briggar, R. J., et al. (1997) *J. Acqui. Immune Defic. Syndr. Hum. Retrovirol.* **14**, 368.
2. Makowski, G. S., et al. (1996) *Ann. Clin. Lab Sci.* **26**, 458.
3. Polski, J. M., et al. (1998) *Mol. Pathol.* **51**, 215.
4. Iovannisci, D. M., et al. (2000) *EPICENTRE Forum* **7**(1), 6.
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FailSafe™ PCR PreMix Selection Kit
FS99060 60 Units

Contents:

60 units FailSafe™ PCR Enzyme Mix and 12 FailSafe™ PCR 2X PreMixes.

See the center insert for additional information on the FailSafe PCR System.

High Fidelity PCR Amplification of DNA from 20 Kb to >40 Kb Using the MasterAmp™ Extra-Long PCR Kit

The FailSafe™ PCR System is ideal for consistent and accurate amplification of any template up to about 20 Kb, whatever its sequence and without need for “hot start” techniques. However, for sequences up to >40 Kb, the MasterAmp™ Extra-Long PCR Kit enables consistent and accurate amplification (see Figure below for lambda DNA regions). “Hot start” techniques are typically not required when using the MasterAmp Extra-Long Kit.

The MasterPure™ Extra-Long DNA Polymerase contained in the kit combines MasterAmp™ Taq DNA Polymerase with a proprietary 3' → 5' proofreading enzyme to achieve PCR fidelity at least three times better than Taq DNA Polymerase alone. The kit

includes MasterAmp Extra-Long DNA Polymerase and nine different Extra-Long PCR 2X PreMixes for convenient and fast PCR set-up. The nine Extra-Long PCR PreMixes each contain buffer, dNTPS and differing amounts of both Mg²⁺ and MasterAmp™ PCR Enhancer (with betaine*). Once the optimal PreMix is identified for a particular template/primer combination, consistent amplification of the template will be achieved using the same PreMix.

* Patents issued and pending.

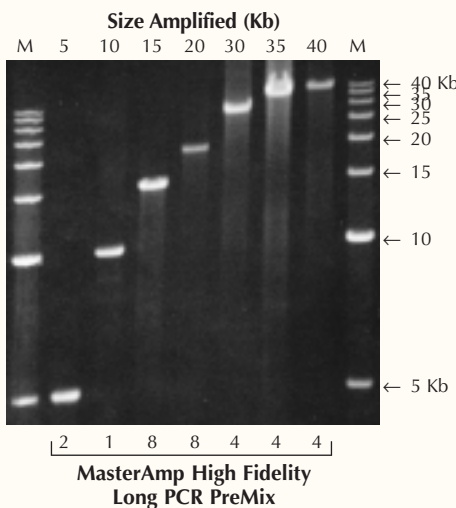


Figure 1. Amplification of 5, 10, 15, 20, 30, 35, and 40 Kb sequences from lambda DNA. One nanogram of lambda DNA was used to amplify 5, 10, 15, 20, 30, 35, and 40 Kb sequences. Lane M, 5 Kb DNA ladder. Results were analyzed on a 0.5% agarose gel run at 30 V for 20 hours.

MasterAmp™ Extra-Long PCR Kit

MHF9220 50 Reactions

Contents:

- MasterAmp™ Extra-Long PCR PreMixes 1-9
- MasterAmp™ Extra-Long DNA Polymerase Mix
- Control Lambda DNA/Primers

Individual Extra-Long PCR 2X PreMixes

- MasterAmp™ Extra-Long PCR 2X PreMix 1**
MHF925A 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 2**
MHF925B 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 3**
MHF925C 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 4**
MHF925D 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 5**
MHF925E 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 6**
MHF925F 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 7**
MHF925G 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 8**
MHF925H 5 ml
- MasterAmp™ Extra-Long PCR 2X PreMix 9**
MHF925I 5 ml

MasterAmp™ Extra-Long DNA Polymerase Mix

- QU92125 125 U
- QU92500 500 U
- QU9201K 1,000 U