

Highly Efficient Recovery of DNA from Dried Blood Using the MasterPure™ Complete DNA and RNA Purification Kit

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Introduction

Archival bloods collected on Guthrie card filter paper serve as a valuable resource for retrospective genetic studies. Although the amount of blood sample obtained in this manner is limited, it is a convenient method for the collection and long term storage of material suitable for post amplification molecular genetic assays. Very large numbers of Guthrie cards may be housed with minimal storage requirements, and these specimens will be suitable for analysis even when held for many years. These features make Guthrie cards an attractive method of specimen collection for large scale monitoring programs, as would be implemented at the state or national level,^{1,2} and may be the preferred method of blood specimen collection and transport in rural or isolated areas.

A significant advantage of dried blood spots is that extraction of the genetic material from the spot may be delayed indefinitely with minimal decay of the DNA, until the need for specimen analysis arises. For example, our laboratory is currently evaluating candidate genes involved in birth defects from archival bloods originally collected as part of a state wide program to monitor phenylketonuria. The blood spots we obtain for these studies are typically 10-20 years old.

Despite the advantages that Guthrie cards may offer, the efficient recovery of genetic material from dried blood may be problematic. Numerous PCR inhibitors have been reported to be especially troublesome for dried blood,³ further complicating sample preparation from specimens containing limited amounts of genetic material. This has prompted the development of specialized DNA extraction protocols for use with Guthrie cards.⁴ The availability of a kit suitable for the preparation of DNA from small amounts of dried blood would be invaluable to investigators working with specimens that are restricted in quantity and non-replenishable due to their archival nature. Such a kit would find utility in the analysis of forensic specimens as well.

Recently, Epicentre introduced the MasterPure™ Complete DNA and RNA Purification Kit for the purification of RNA or DNA from a variety of tissue sources. This kit is based on the salt precipitation method described by Miller *et al.*,⁵ and includes a reagent that promotes the efficient recovery of small quantities of genetic material, substantially improving the sensitivity of the precipitation step.⁶ Until now, however, its use with dried blood has not been investigated. We present here a protocol employing the

MasterPure Complete Kit to extract blood stored on Guthrie cards, and demonstrate the recovery of DNA from archival blood spots approximately 10 years old. Furthermore, we demonstrate the recovery of sufficient material for numerous amplification reactions from a spot containing as little as 1 μ l of blood.

Methods

MasterPure Complete Protocol for Blood Spots

Whole blood (15 μ l) was spotted to Guthrie card filter paper and allowed to dry overnight to several days. The EDTA preserved blood was stored 2 to 30 days prior to spotting. Two additional archival samples approximately 10 years old were also tested. Punches were taken from archival spots which were collected directly from a heel prick and contained approximately 6-8 μ l of blood.

A protocol for the extraction of DNA from blood spotted on Guthrie cards is outlined in Table 1.

Table 1. Extraction protocol.

1. Dilute 1 μ l of 50 μ g/ μ l Proteinase K into 300 μ l of Tissue and Cell Lysis Solution for each sample. Apply 300 μ l of this mix to each microcentrifuge tube containing a Guthrie card punch.
2. Incubate 30 minutes at 65°C, inverting occasionally. Alternatively, incubate at 55°C overnight.
3. Add 160 μ l of MPC Protein Precipitation Reagent to 300 μ l of lysed sample and vortex vigorously for 10 seconds. Chill on ice for \geq 30 minutes.
4. Pellet the debris by centrifugation for 30 minutes at \geq 10,000 x g in a microcentrifuge.
5. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
6. Add 600 μ l of isopropanol to the recovered supernatant. Invert the tube several times. Freeze at -20°C, one hour to overnight.
7. Pellet the DNA by centrifugation at 4°C for 30 minutes at \geq 10,000 x g in a microcentrifuge.
8. Carefully pour off the isopropanol without dislodging the DNA pellet, and wash with 70% ethanol.
9. Dry pellet and resuspend in 10-20 μ l of TE buffer, depending on the initial size of the punch.

PCR Amplification

PCR reactions were performed on a PTC200 thermal cycler (MJ Research) in 0.2 ml thin walled tubes. A 1.1 kb sequence containing the entire coding region of human N-acetyltransferase-2 was amplified using the following conditions. PCR reactions contained 0.1 μ M of the forward primer (5'-TCTAGCATGAATCACTCTGC-3') and reverse primer (5'-GGAACAAATTGGACTTGG-3'), 3 μ l of 10 X Taq PCR buffer, 3 mM MgCl₂ (final concentration), 200 μ M each dNTP, and 0.75U of Taq DNA Polymerase (chemically modified; requires heat activation), in a total volume of 30 μ l. Cycling conditions consisted of an enzyme activation step at 95°C for 15 minutes, then three cycles of 98°C for 15 seconds, 56°C for 1 minute, and 72°C for 1 minute, followed by 33 cycles of 95°C for 30 seconds, 56°C for 1 minute, and 72°C for 1 minute, with a final extension at 72°C for 5 minutes. Negative controls included sterile water rather than DNA template. Amplification products were resolved on 1.5% agarose gels and stained with ethidium bromide.

Results and Discussion

Initially a prolonged incubation of the blood spot in the presence of proteinase K was performed at 55°C overnight. The specimens extracted consisted of two archival blood spots, both approximately 10 years old, as well as two additional bloods, 2 days and 1 month old, respectively. Each archival blood spot extracted contained approximately 6-8 μ l of blood; each fresh blood spot contained approximately 15 μ l of blood. The blood volumes contained on these punches are estimates derived by comparing the size of the punch with the standard 1/2 inch diameter Guthrie card collection area, which holds approximately 50 μ l of blood. Following the extraction procedure, the dried DNA pellets were resuspended in 20 μ l of TE buffer, and 1 μ l of each sample was employed in a 30 μ l PCR reaction. Samples (5 μ l) of each were then subjected to agarose gel electrophoresis.

The results shown in Figure 1 illustrate the efficient amplification of a 1.1kb fragment containing the entire coding region of the human N-acetyltransferase-2 gene in all blood samples extracted by this method. The results obtained for archival blood spots (lanes 1 and 2) are essentially equivalent to those obtained for freshly spotted blood (lanes 3-6) as well as from fresh liquid blood (lane 7, PCR positive control) suggesting that minimal degradation results from long term storage of blood spots. Small differences in the amount of PCR product that may be observed between samples can be attributed to differences in the sizes of the blood spots that were extracted.

Reduced Proteinase K Digestion Time Using Quantified Blood Spots

In order to determine a lower limit for the amount of dried blood that could be extracted using this kit, measured amounts of blood were spotted to Guthrie cards and

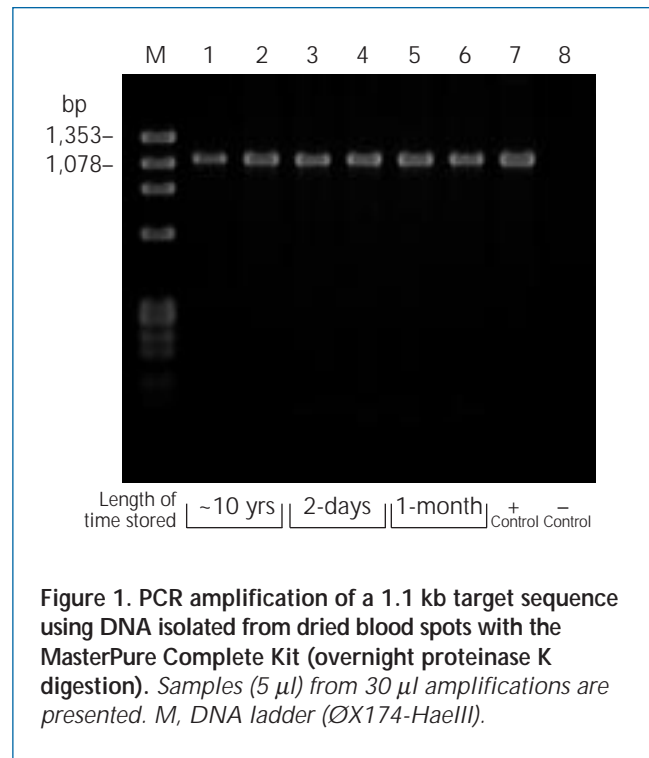


Figure 1. PCR amplification of a 1.1 kb target sequence using DNA isolated from dried blood spots with the MasterPure Complete Kit (overnight proteinase K digestion). Samples (5 μ l) from 30 μ l amplifications are presented. M, DNA ladder (\emptyset X174-HaeIII).

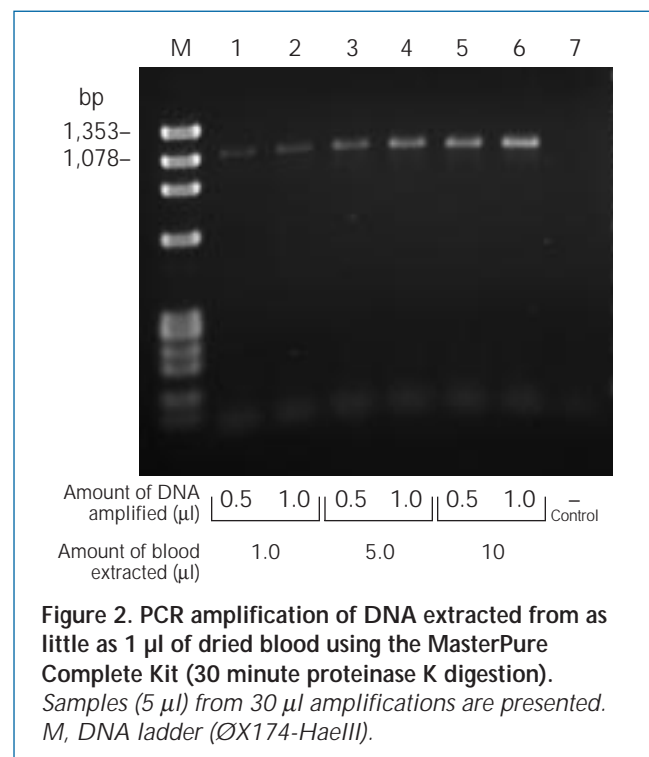


Figure 2. PCR amplification of DNA extracted from as little as 1 μ l of dried blood using the MasterPure Complete Kit (30 minute proteinase K digestion). Samples (5 μ l) from 30 μ l amplifications are presented. M, DNA ladder (\emptyset X174-HaeIII).

punches containing the entire blood spot were extracted. The volume of blood spotted ranged from 1 to 10 μ l. The proteinase K digestion step was also modified (65°C for 30 min) in order to reduce preparation time. The resulting DNA pellets were resuspended in 10 μ l of TE buffer; 0.5 and 1.0 μ l aliquots were subsequently subjected to amplification. The results shown in Figure 2 demonstrate that as little as 1 μ l of blood (lanes 1 and 2) spotted to a

continued

Guthrie card can be extracted using this kit to provide enough material for a minimum of 10-20 amplification reactions. Furthermore, lanes 3-6 suggest quantitative recovery of the DNA, since the larger the volume of blood spotted, the greater the amount of PCR product that is produced when all other parameters of the extraction and amplification reactions remain constant.

Conclusion

The MasterPure Complete DNA and RNA Purification Kit provides a simple and efficient method of purifying small quantities of DNA from dried blood specimens. With this methodology, as little as 1 µl of dried blood can be extracted, generating enough genomic DNA to perform a minimum of 10-20 amplifications. This offers a significant advantage over other extraction protocols, which consume the entire Guthrie card collection spot (containing approximately 50 µl of blood) in a single amplification reaction. Thus, use of the MasterPure Complete Kit will allow investigators to make efficient use of archival dried blood specimens, which are limited in quantity and non-replenishable.

References

1. Dezateux, C. (1998) *Br. Med. Bull.* **54**, 877.
2. Caggana, M. *et al.* (1998) *Hum. Mutat.* **11**, 404.
3. Makowski, G.S. *et al.* (1996) *Ann. Clin. Lab. Sci.* **26**, 458.
4. Makowski, G.S. *et al.* (1998) *Ann. Clin. Lab. Sci.* **28**, 254.
5. Miller, S.A. *et al.* (1988) *Nucleic Acids Res.* **16**, 1215.
6. Watson, J. (1999) *Epicentre Forum* **6**, 6.

MasterPure Complete DNA and RNA Purification Kit

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MC85200-F71	200 Purifications

*Limit 2 per customer

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Recover Intact DNA 50 kb to >2 Mb in Length

GELase™ Agarose Gel-Digesting Preparation

GELase Agarose Gel-Digesting Preparation is a unique enzyme solution developed at EPICENTRE for quantitative recovery of intact DNA from low melting point (LMP) agarose gels following electrophoresis in TAE, TBE, MOPS, or phosphate buffers. Excised gel bands can be digested in the above-mentioned buffers, or for higher activity, GELase Buffer may be added to or exchanged with those buffers.

Applications:

Recover high molecular weight nucleic acids from low melting point (LMP) agarose gels for use in:

- Preparation of YAC, BAC, cosmid, and plasmid vectors
- Subcloning from YACs, BACs, and cosmids
- Microinjection
- Size selection of genomic DNA for subsequent cloning
- Restriction mapping
- PCR

Benefits:

- Gentle procedure — purify multi-megabase DNA that is intact and biologically active
- Recoveries of DNA consistently approach 100%
- Protocol requires minimal hands-on time
- High activity—GELase Preparation is more active than other gel-digesting enzymes*
- Cost effective—GELase is priced well below spin column or other gel-digesting methods*

GELase™ Agarose Gel-Digesting Preparation

1 U/ul

G09050-F71	50 U
G09100-F71	100 U
G09200-F71	200 U

0.2 U/ul

G31050-F71	50 U
G31100-F71	100 U
G31200-F71	200 U

Both Concentrations Include GELase™ 50X Reaction Buffer

*One unit of GELase Preparation is equivalent to approximately three units of other gel-digesting enzymes.

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References

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