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SYMPOSIUM

Maximizing Deoxyribonucleic Acid Yield from Dried Blood Spots

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Abstract

Background:

One source of deoxyribonucleic acid (DNA) for genetic studies is the utilization of dried blood spots stored on paper cards (Guthrie cards) collected shortly after birth. These cards represent an important source of material for epidemiologic and population-based genetic studies. Extraction of DNA from these cards can lead to variable amounts of recovered DNA. We report here results of our efforts to maximize yield from this valuable, but nonrenewable, resource.

Method:

Commercial methods of DNA extraction from blood cards were used, and protocol modifications were introduced that enhanced DNA yield.

Results:

Use of a commercial solvent prior to DNA extraction steps gave greater yields than extraction without the solvent. Modification of the elution step by use of prewarmed extraction buffer and a soaking step at an elevated temperature increased yield by 6- to 10-fold.

Conclusions:

The modified DNA extraction method yielded as much as 660 ng of DNA from a single 5-mm-diameter punch of a blood spot card. The DNA performed well in downstream, polymerase chain reaction-based applications.

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Introduction

Obtaining sufficient numbers of samples for meaningful data analyses of genetic studies can be challenging. "Guthrie cards," paper cards with dried blood collected from all newborns for neonatal screening as mandated by state laws,[1] have long been recognized as a valuable resource for population-based and epidemiological studies.[2–5] Deoxyribonucleic acid (DNA) contained in the blood spots is quite stable and is useful decades after collection.[4,6] Even ribonucleic acid, which is far less stable than DNA in solution, appears to be stable on the dried cards.[7,8] Efficient extraction of DNA from Guthrie cards is desirable to fully utilize this valuable resource.

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Abbreviations: (DNA) deoxyribonucleic acid, (HLA) human leukocyte antigen, (PCR) polymerase chain reaction, (SNPs) single nucleotide polymorphisms, (WGA) whole genome amplification

Keywords: blood spot, DNA sample preparation, Guthrie card, HLA genotyping

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A number of commercial methods exist for this purpose, and they can have variable yields. We aimed to generate population-based human leukocyte antigen (HLA) genotyping data from a set of 1000 Guthrie cards from infants reported as African American. Our HLA "linear array" genotyping assay protocol uses a relatively large amount of genomic DNA (60 ng per assay) compared to methods for genotyping of single nucleotide polymorphisms (SNPs), which generally require small amounts (~2 ng) per genotype assay. Six to eight linear array assays are required for full eight-locus HLA genotyping; thus, to follow the protocol, a minimum of 360 ng of DNA must be extracted from each blood spot. We evaluated use of a commercial solvent, developed after the initial DNA preparations were made from these blood cards, for its ability to increase yield. In addition, we introduced a warm incubation step prior to eluting the DNA, a technique used successfully to increase yields in DNA preparations from whole blood. We report here the results of these simple modifications of commercial protocols that have, in our hands, increased DNA yields sufficiently to allow complete eight-locus HLA genotyping with linear array technology on most of these samples.

Materials and Methods

Materials

These experiments were performed on Guthrie cards from the State of California, Genetic Disease Branch. We obtained 4000 cards, 1000 from each of four self-reported ethnic groups, from babies born in California in 1998. Samples were used with permission from the state and with approval of the Institutional Review Board from Children's Hospital and Research Center Oakland for population genetic studies.

DNA Extraction

Spotted blood was removed from the cards with a paper punch. Initial DNA extractions were performed with QIAGEN blood extraction kits (QIAGEN, Valencia, CA) in a 96-well block extractor per the manufacturer's instructions. Second-round DNA extractions were performed using the GenSolve reagent (GenVault, Carlsbad, CA), followed by QIAamp blood kit purification in individual sample format, with the modification that the elution step was performed with buffer preheated to 70°C, followed by incubation of the spin columns in a 70°C oven for 10 minutes prior to spinning. A flow diagram of the modified procedure can be seen in **Figure 1**.

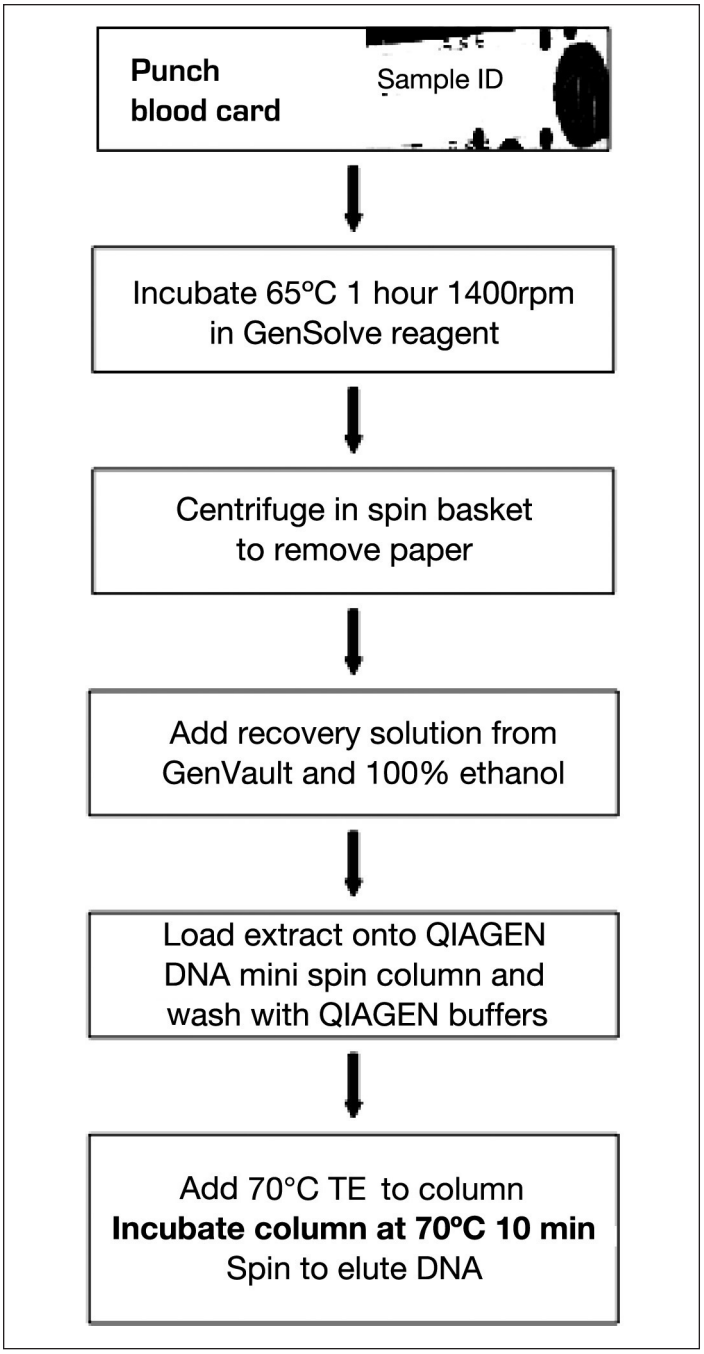

Figure 1. An overview of modified DNA extraction. The method combines use of the GenSolve reagent and protocol to extract blood from paper (GenVault, Carlsbad, CA) with QIAGEN DNA purification technology (QIAGEN Inc., Valencia, CA), with a modified elution step. Spin baskets are from Promega Corporation (Madison, WI).

Yield Evaluation for Heated Elution

Two 5-mm punches were taken from each of three blood cards and prepared using the GenSolve reagent with 1 hour of shaking at 65°C. One-half of each sample was eluted according to the manufacturer's

instructions by adding 200 µl of room temperature elution buffer (TE; 10 m*M* Tris, pH 8.0, 0.1 m*M* EDTA) (Teknova, Hollister, CA) to DNA bound to the spin column and spinning immediately. The other half was eluted by adding TE that had been prewarmed to 70°C and placing the spin columns into a 70°C oven for 10 minutes prior to spinning the column.

Linear Array HLA Genotyping

The downstream application for these blood spot DNA preparations was HLA genotyping by the "linear array" method. This method involves amplification of one or more polymorphic exons from an HLA locus followed by hybridization of the polymerase chain reaction (PCR) product to a series of oligonucleotide probes immobilized on a backed nylon membrane, as described elsewhere.[9] Our laboratory standard amount of input genomic DNA for the linear array assays is 60 ng.

Results

Initial DNA extractions were performed on 4000 Guthrie cards using a 96-well block extractor and standard commercial protocols. DNA samples were utilized successfully in a high-sensitivity kinetic PCR assay to genotype two SNPs from the hereditary hemochromatosis gene (data not shown). At least half of the DNA was available for use in the African American HLA genotyping study. Limited amounts of DNA and lack of access to appropriate technology to quantify such small amounts of DNA necessitated empirical determination of starting quantities of template DNA for use in the linear array reactions. Results were generally readable; however, probe intensities were generally faint and varied among samples. An example of primary data produced from the initial blood spot DNA preparations can be seen in **Figure 2A**. Based on later NanoDrop (Thermo Scientific, Wilmington, DE) spectrophotometer measurements, we estimated that input DNA amounts for the original blood spot DNA preparations were likely as low as 1 to 4 ng per assay. Genotyping analysis of three of the eight classical HLA loci (DRB1, DQA1, and DQB1) required the use of essentially all the remaining DNA from the first round of purification.

Continuation of the project required extraction of residual blood on the 1000 Guthrie cards from African American infants. Because the amount of residual blood varied, the amount of starting material for the extractions varied as well. We investigated commercial technologies for the extraction of DNA from paper cards and tested whether modification of the initial steps in our DNA extraction protocol, using a solvent developed for that purpose (GenSolve, GenVault Carlsbad, CA), might improve DNA yields. Nine samples were preincubated with the GenSolve reagent by heated shaking at 65°C, and nine samples were prepared with the reagents and extraction protocol included with the QIAGEN extraction kit. We note here that these preparations differed from the initial preparations in that they were performed in individual tubes rather than in the 96-well block extractor, which itself may have led to improved DNA yields. After the DNA was extracted from the paper, subsequent DNA purification steps were performed on both sets of samples with the QIAGEN QIAamp blood kit according to the manufacturer's instructions, with the modification that the elution step was performed at 70°C (**Figure 1**). As measured by NanoDrop spectrophotometry, the average concentration of the nine preparations soaked in the GenSolve reagent was 12.77 ng/µl, while the average concentration of the samples that were extracted with the QIAGEN kit alone was 3.81 ng/µl (data not shown). With an elution volume of 200 µl, the total yield of DNA, although variable among preparations, was as much as 2000 ng, as measured by NanoDrop spectrophotometry. Based on those pilot data, second-round DNA preparations were performed with the GenSolve reagent, and resulting concentrations were measured by NanoDrop spectrophotometry. DNA concentrations were normalized to 10 ng/µl, and 6 µl was used in each linear array assay. We note here that the concentrations of DNA produced in these preparations were near the low end of the range for measurement by the NanoDrop instrument. We subsequently discovered, using PicoGreen technology (Molecular Probes, Eugene, OR), that our NanoDrop measurements were approximately 6- to 10-fold too high. Thus, the starting material for these assays was not 60 ng, but more likely 6 to 10 ng. Even so, the increase in the amount of input DNA and the normalization of the DNA concentrations combined to produce more intense signals that were more uniform among samples, creating data that were much easier to interpret. An example of these data can be seen in **Figure 2B**. For comparison, **Figure 2C** shows typical data from the same HLA genotyping assay, using 60 ng DNA purified from whole blood as the starting material. Assays from blood card DNA prepared by the modified, warm elution method (**Figure 2B**) were more similar to the standard (**Figure 2C**) than assays performed on the initial set of DNA preparations (**Figure 2A**).

To test whether our modification of the elution step of the DNA extraction protocol actually increased DNA

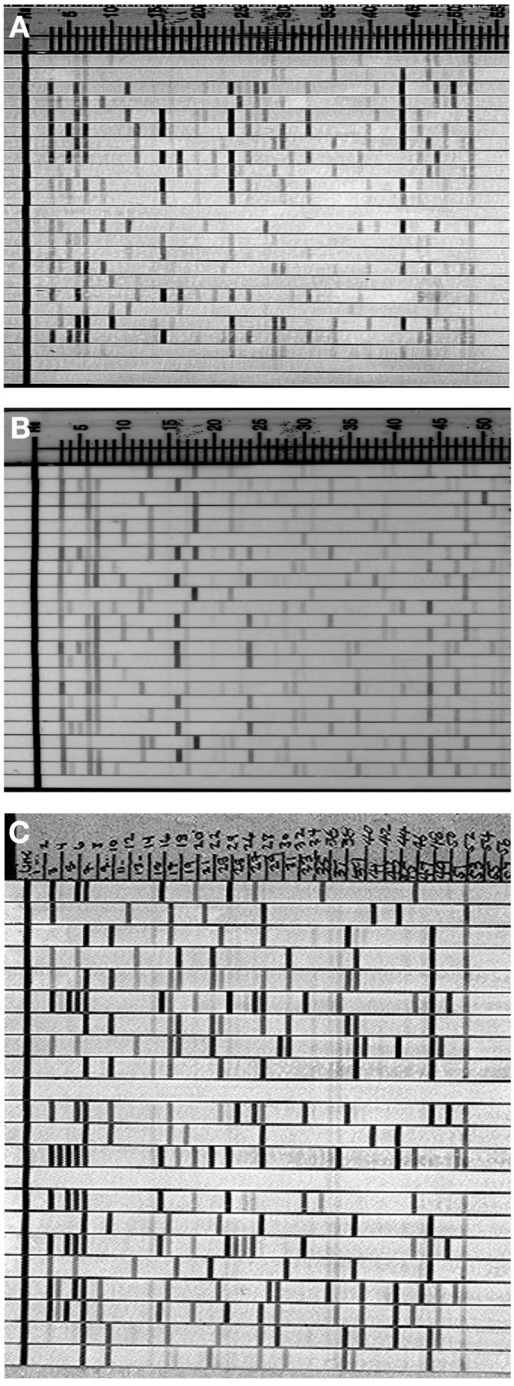

Figure 2. Examples of linear arrays from our DQA1/DQB1 genotyping assay performed with different starting template DNA as follows: **(A)** Guthrie card DNA from initial preparations using the QIAGEN 96-well block extractor and standard QIAGEN protocols. Note the variability of signals among the different strips. **(B)** Guthrie card DNA from our second round of purifications, incorporating the GenSolve reagent and the warm elution process. Note the relative uniform signals among the different strips. **(C)** DNA purified from whole blood and normalized with 60 ng total DNA used for each assay. In B and C, blank strips represent negative (no template) controls.

yield, DNA was prepared from two 5-mm punches of three different blood spots, with half of each sample eluted at room temperature without soaking and half eluted with the modified warm soaking method (see Methods). Results of this experiment are represented graphically in **Figure 3**. Although the sample-to-sample variation was great among the three samples (total yield as measured by PicoGreen technology ranged from 66 to 660 ng DNA), data clearly show that samples with high-temperature elution gave consistently higher yields than samples eluted at room temperature using either method of measurement. In addition, these data confirmed our suspicion that measurements done by NanoDrop spectrophotometry gave artificially high readings compared to measurements done with PicoGreen technology. Sample 3 gave the highest yield, with 3200 ng total as measured by NanoDrop spectrophotometry; 660 ng total when measured by PicoGreen technology, which is a more conservative, and probably more accurate, number than the 3200-ng yield as measured by NanoDrop spectrophotometry. In our experiments, NanoDrop measurements appeared to overestimate the DNA concentration by approximately 6- to 10-fold, probably due to the fact that our concentrations were near the lower limit of detection by the NanoDrop instrument. The PicoGreen system was developed for use in a much lower range of concentration than the NanoDrop instrument. These yields came from a single 5-mm punch of a Guthrie card. Typical blood spots on a card are large enough to allow three to five 5-mm punches.

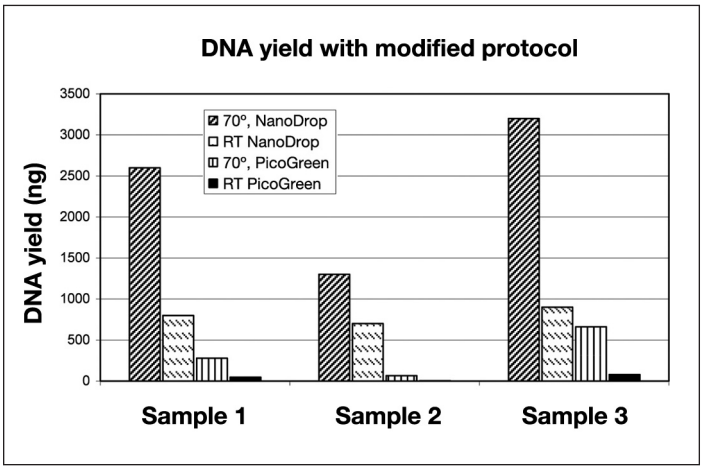

Figure 3. Total DNA yield from direct comparison of DNA extraction protocols varying only by the elution step. Data are shown for both NanoDrop and PicoGreen measurements and show that despite variation in total yield among samples, samples eluted with the modified protocol have greater yield than the same samples eluted at room temperature (RT) without the added soaking step.

Discussion

Guthrie cards are generated on newborns throughout the United States for state-mandated newborn screening programs. Leftover blood on these cards represents a valuable resource for genetic studies. Each card contains a finite amount of blood, and because the researchers are blinded to the individual identities of the subjects from whom the blood was taken, this resource in nonrenewable. Thus, maximizing the DNA yield from the card can be essential to allow studies such as our survey of HLA genotypes in African Americans born in California. Whole genome amplification (WGA) is one possible method that can be used to increase the amount of DNA for limited samples; however, our experience with WGA samples has been that the amplification process is not uniform and leads to drop-down or dropout of certain HLA alleles in our genotyping system. HLA genotyping results are more uniform and accurate when generated from genomic DNA that has not been subjected to the WGA process; thus, extracting the maximum possible amount of DNA from the cards is crucial to our downstream application.

During the course of this study, we improved the efficiency of our original DNA extraction method in two ways: (1) modification of the initial steps of the protocol to include the GenSolve reagent and (2) modification of the DNA elution step to use prewarmed elution buffer and include a 70°C soaking step before spinning. With these modifications, we were able to get as much as a 660-ng total yield from a single 5-mm punch, enough to do complete, eight-locus HLA genotyping from the primary DNA sample.

Conclusion

We reported here results of simple modifications of commercial protocols that have significantly increased DNA yield in our hands. Use of a commercial solvent to extract blood from the paper and modification of the elution process by including a 70°C incubation step combined to increase yields of DNA sufficiently to allow complete HLA genotyping for most of these samples. Data show that simple modifications of commercial extraction protocols can help maximize the yield from Guthrie cards.

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Disclosure:

The authors are employed at Children's Hospital Oakland Research Institute, the research arm of Children's Hospital and Research Center Oakland, and have no affiliation with any company whose products were utilized for this research.

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