Application Information

Genetic Analysis

Validation of the Agencourt® Genfind™ Blood and Serum Genomic DNA Isolation Kit for PCR Detection and Genotyping

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Abstract

Life science researchers working with whole blood face many challenges in sample preparation. Blood is a viscous substance rich in protein, lipids and other cellular materials that require removal in order to purify genomic DNA (gDNA). Traditional methods for isolating gDNA from blood typically use fixed starting volumes and can be labor intensive. We present a scalable, 96-well automation friendly method that utilizes SPRI® technology for effectively isolating genomic DNA from whole blood (fresh or frozen) and serum.

Introduction

Isolation and purification of nucleic acids is a crucial step in basic research, molecular diagnostics and pharmacogenomic applications. Whole blood is commonly used for the extraction of genomic DNA in clinical research settings. Several methods can be used to extract DNA from blood, but many of the existing techniques are not easily adapted to automation platforms. Today's lab environments have an increasing need for high throughput and efficient methods for extracting gDNA from whole blood with minimal manual intervention.

Solid Phase Reversible Immobilization (SPRI) is a patented, high performance nucleic acid purification technology invented at the Whitehead Institute (Hawkins et al., *NAR* 1995, 23:22). It's magnetic bead-based protocols do not require filtration or centrifugation steps, which makes Agencourt Genfind exceptionally suited for automated applications. Target nucleic acids are immobilized onto paramagnetic microparticles in the presence of specific buffer conditions. Contaminants are removed by washing the beads. Purified nucleic acids are eluted off the beads into aqueous buffers that are compatible with downstream PCR*-based applications.

Materials and Methods

Genomic DNA Isolation

The Agencourt Genfind Blood and Serum DNA Isolation Kit was used to isolate genomic DNA from fresh or frozen horse or human blood preserved with different anticoagulants, using starting volumes of 50 – 400 µL. For competitor kit testing, the manufacturers' protocols (for purifying 200 µL blood) were followed (scaling up if necessary) with elution into 200 µL of the recommended elution buffer.

Amplification

PCR reactions were performed using 1 µL purified template, 5 µL AccuPrime Supermix I (Invitrogen), and 0.2 µL 20 µM primer mix in a 10 µL reaction volume with a T_m of 60°C according to the manufacturer's instructions. The primers used were specific for transferrin (horse samples) or ARF1 (human samples).

Restriction Digestion

Approximately 0.3 µg of genomic DNA purified with the Agencourt Genfind Blood and Serum DNA Isolation Kit was digested using increasing amounts of *Alu* I (New England Biolabs), from 0.02 – 2 U, for 2 hours at 37°C. The reaction volume was 20 µL for each concentration. Each digest was run on a 0.8% E-Gel (Invitrogen).

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DNA Stability Tests

Genomic DNA was prepared in duplicate from citrate-stabilized horse blood with the Agencourt® Genfind™ Blood and Serum DNA Isolation Kit. After storage at -20°C for 5 days, the DNA was thawed and run on a 0.8% E-Gel. The DNA was used as template for a PCR reaction with horse transferrin primers. The DNA was then split into two equal portions. One portion was stored at -20°C and the other at 4°C. Every week for 21 weeks, the samples were run on a 0.8% E-Gel and used as template for PCR to analyze the stability of samples stored at 4°C or -20°C.

Pulsed-field Gel Electrophoresis

Approximately 1 µg of genomic DNA prepared with the Agencourt Genfind Blood and Serum DNA Isolation Kit was run on a 1% agarose pulse-field gel for 11 hours at a gradient of 6 V/cm with an included angle of 120°, an initial switch time of 1 second and a final switch time of 6 seconds. The gel was visualized with ethidium bromide.

Results

Isolation of Genomic DNA from Human Blood

The Agencourt Genfind Blood and Serum DNA Isolation Kit extracts genomic DNA of high purity from human blood preserved with heparin (Figure 1A). DNA isolated with the Agencourt Genfind kit was used as a template in PCR amplification. The resulting products correspond to the expected gene size. No inhibition of the reaction was observed, indicating efficient removal of contaminants and PCR inhibitors (Figure 1B).

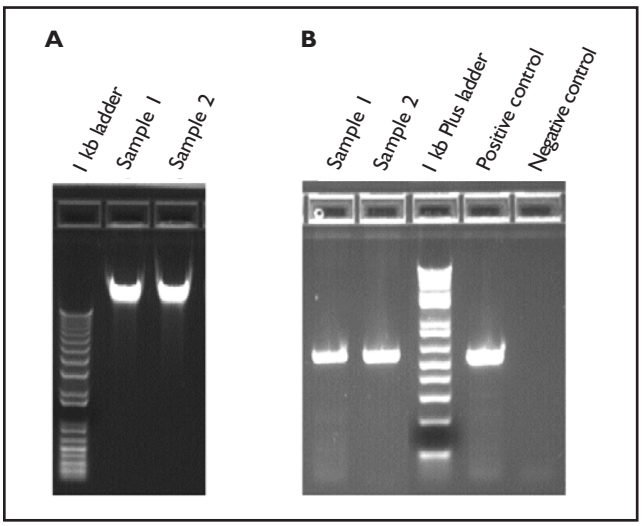

Figure 1. A) 20 µL of genomic DNA isolated from 200 µL human blood preserved with heparin was loaded onto the gel. The average DNA yield from 200 µL blood was 9.6 µg. B) PCR of genomic DNA using ARF1 primers. 1 µL of 200 µL total eluent from Figure 1A was used as template in a 10 µL PCR with human ADP ribosylation factor 1 (ARF1) primers. The expected product size is 543 bp.

Comparison of Different Anticoagulants

Since most methods of analyzing genomic DNA involve PCR amplification of selected regions, the efficiency of PCR from SPRI®-purified genomic DNA was examined. Performance of the Agencourt Genfind kit was analyzed using horse blood preserved with the most commonly used anticoagulants (Heparin, EDTA, and citrate – Figure 2A). Using Agencourt Genfind, DNA was isolated and amplified from blood containing these anticoagulants. No inhibition of PCR was detected (Figure 2B). In contrast, some studies found that DNA isolated using alternate methods from blood samples with heparin could not be amplified by PCR (Smythe et al., *BMC Infectious Diseases*, 2002, 2:13; Yokota et al., *Journal of Clinical Laboratory Analysis*, 1999, 13: 133 – 140). These results indicate that the SPRI purification method is efficient in removing residual

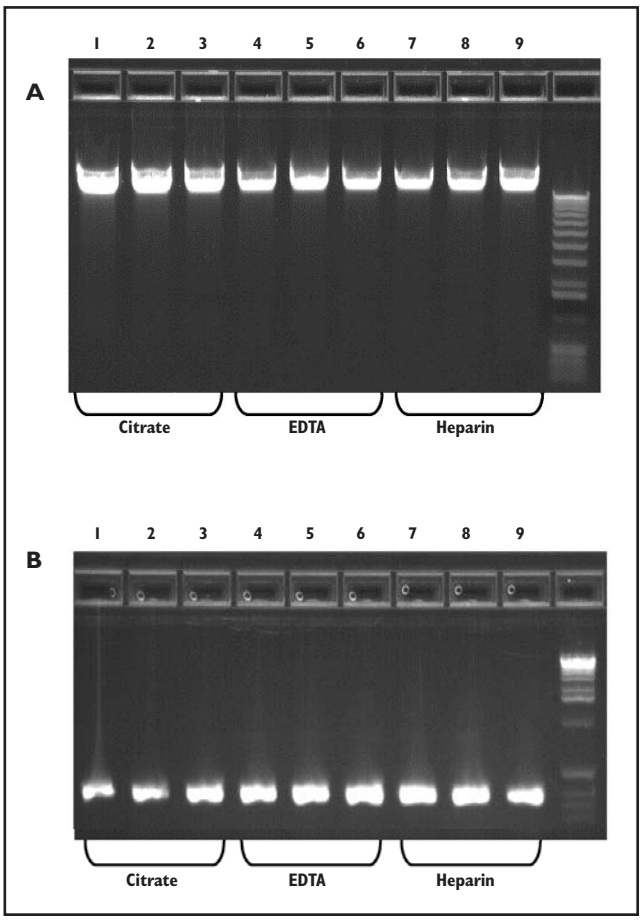

Figure 2. The Agencourt Genfind kit was used to isolate genomic DNA from whole horse blood. A) Genomic DNA isolated from 200 µL horse blood preserved with citrate (lanes 1–3), EDTA (lanes 4 – 6), or heparin (lanes 7 – 9), stored at 4°C for less than two weeks. B) PCR of genomic DNA using transferrin primers. The last lane contains a 1 kb ladder.

heparin or other molecules, which may act as PCR inhibitors.

Also included in the evaluation was a comparison of fresh and frozen blood samples. All samples were tested in the 0.2 mL and the 0.4 mL kit format. The results of this comparison are summarized in Figure 3. For the 0.2 mL protocol in a 96-well microplate, the yield ranged from 6 to 14 µg of genomic DNA (Figure 3A). Isolation of DNA from 0.4 mL blood in 2 mL microcentrifuge tubes resulted in 14 – 24 µg of genomic DNA (Figure 3B). With both formats, blood samples with heparin or citrate as anticoagulant generated a slightly higher yield than blood samples preserved with EDTA.

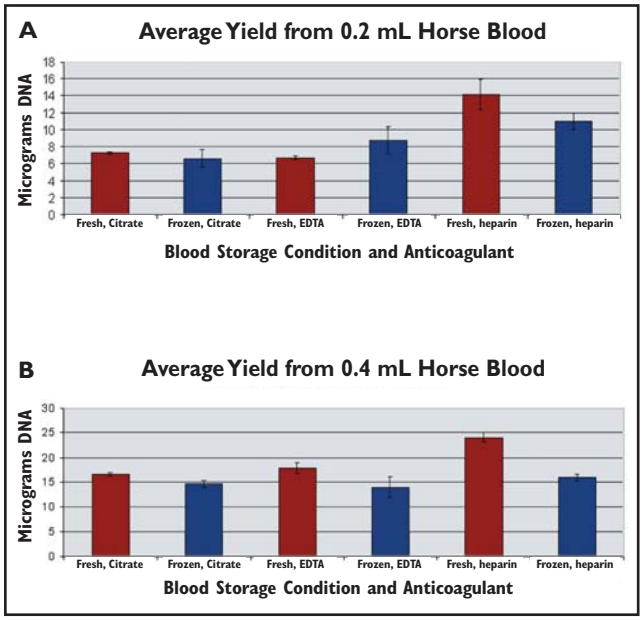

Figure 3. Average yields of genomic DNA isolated from horse blood with Agencourt Genfind. Genomic DNA was isolated from fresh or frozen horse blood preserved with citrate, EDTA, or heparin. Results shown are the average from three preparations with standard deviations. A) 0.2 mL blood prep. B) 0.4 mL blood prep.

Downstream Applications

Pulsed-field Gel Electrophoresis

Equal amounts of DNA purified from fresh horse blood with the Agencourt Genfind Blood and Serum DNA Isolation Kit were run on a pulse-field agarose gel (Figure 4). The average fragment size of the recovered DNA was approximately 40 kb, with no visible degradation of the products. The tight bands on the pulsed-field gel indicate that the genomic DNA is left intact during purification.

Restriction Digest

Library construction, Southern blot analysis, and other applications require digestion of genomic DNA

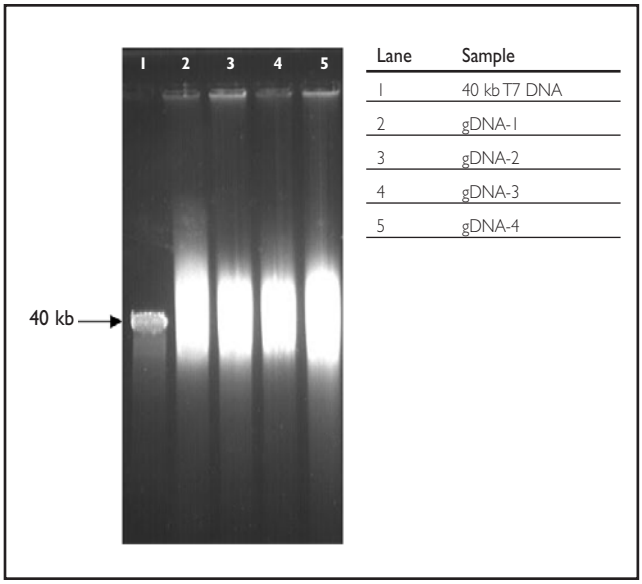

Figure 4. Approximately 1 µg of genomic DNA prepared with the Agencourt Genfind kit was run on a 1% agarose pulsed-field gel and was visualized with ethidium bromide.

with restriction enzymes. In order to test for the presence of residual contaminants that might inhibit restriction enzymes, a restriction digest of Agencourt Genfind-purified genomic DNA was performed. *Alu* I was chosen to digest the genomic DNA into smaller fragments. Uncut DNA was run on the same gel as a control. The DNA was cut to completion with as little as 0.1 U of enzyme (Figure 5). Therefore, restriction digestion of Agencourt Genfind-purified genomic DNA with *Alu* I is not inhibited, indicating no residual contaminants. Restriction digests of genomic DNA with other restriction enzymes gave similar results (data not shown).

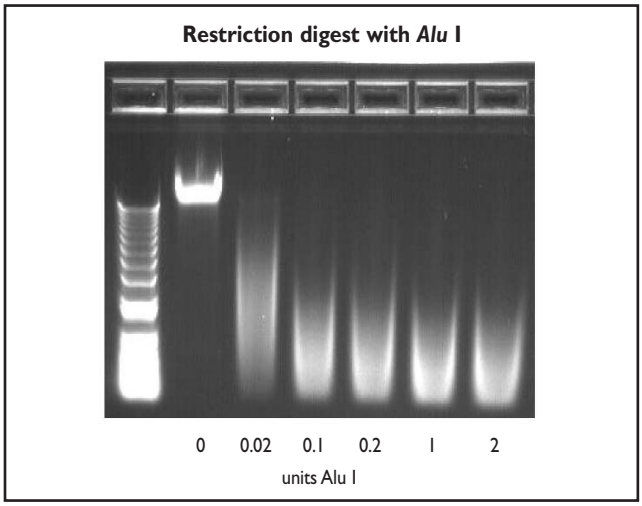

Figure 5. Restriction digest of genomic DNA purified with Agencourt Genfind. 0.3 µg genomic DNA purified with Agencourt Genfind was digested with different amounts of *Alu* I (NEB) for 2 hours at 37°C. The digest was run on a 0.8% agarose E-Gel (Invitrogen).

Comparison with Other DNA Purification Kits

The Agencourt® Genfind™ Blood and Serum DNA Isolation Kit was compared to genomic DNA purification kits from other vendors by isolating gDNA from 200 µL horse blood using the different methods. The yields were determined by using a Quant-iT PicoGreen assay (Invitrogen). The results are summarized in Figure 6. The yields with the Agencourt Genfind kit were 2 – 5 times higher using 200 µL starting material than with any of the other kits tested.

Stability of Purified DNA

Genomic DNA purified with the Agencourt Genfind Blood and Serum DNA Isolation Kit was stored at 4°C and -20°C for extended periods of time to evaluate the stability of the isolated DNA. The DNA was analyzed weekly by gel electrophoresis and by PCR amplification. The DNA showed no signs of degradation after storage at -20°C for 21 weeks or at 4°C for 20 weeks (Figure 7A). PCR amplification of the stored DNA also did not change after extended storage times (Figure 7B).

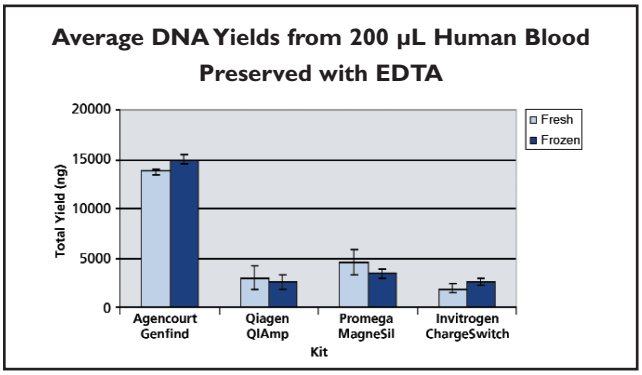

Figure 6. Comparison of gDNA yields from 200 µL of fresh human blood preserved with EDTA using SPRI and competitor kits. Results shown are the average and standard deviation from three preparations. All isolations were performed according to the manufacturers' protocol. DNA was quantified using the Quant-iT PicoGreen ds DNA Assay kit (Invitrogen).

Conclusion

The Agencourt Genfind Blood and Serum DNA Isolation Kit delivered superior results in recovering gDNA from whole fresh and frozen blood. In comparison to other commercially available kits, Agencourt Genfind provided significant gains in sample scalability, recovery, and PCR amplification. As a result, this SPRI®-based method is ideal for obtaining increased DNA recovery over competitive methods.

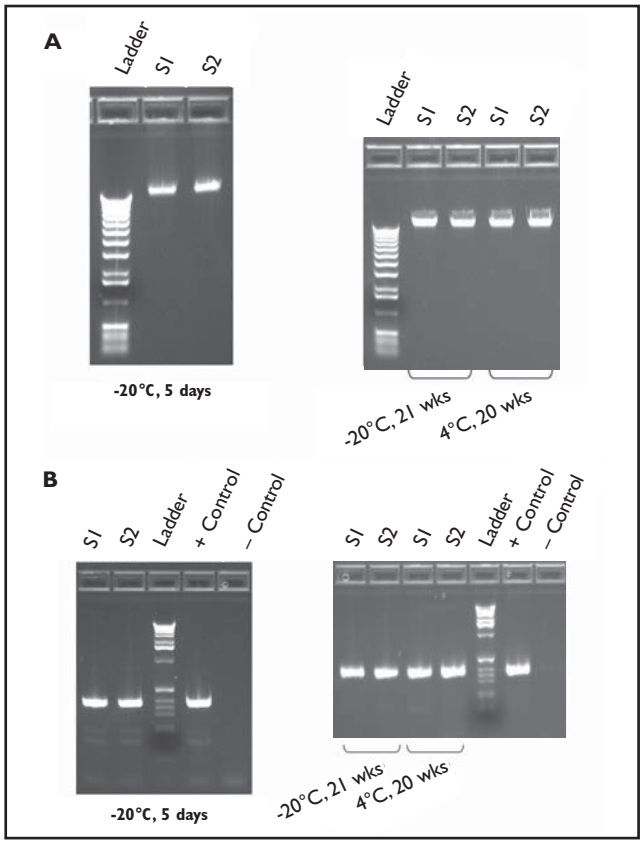

Figure 7. Stability of genomic DNA purified with Agencourt Genfind. A) Genomic DNA purified with Agencourt Genfind from horse blood was run on a 0.8% agarose E-Gel (Invitrogen) after storage at -20°C for 5 days or 21 weeks, or at 4°C for 20 weeks. B) The samples in part A were used as template for PCR with horse transferrin primers.

** The PCR process is covered by patents owned by Roche Molecular Systems, Inc., and F. Hoffman-La Roche, Ltd.*
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