Automated genomic DNA extraction from large volume whole blood

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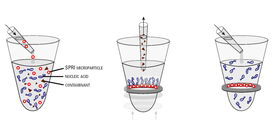
Introduction

Whole blood samples are critical for disease prediction and diagnosis. Obtaining intact, high quality, and concentrated genomic DNA (gDNA) from whole blood is the critical step for downstream research applications such as qPCR, microarray analysis, and next generation sequencing (NGS). The term biobank refers to a large collection of tissue samples such as blood and serum that are collected for research purposes. In order to have access to large amounts of human samples and data, an increasing number of researchers are looking to access available samples through biobanks.

Here we present an automated, high-quality, research-ready DNA extraction method from large volume blood. GenFind V3 DNA Isolation kit automated on the KingFisher Duo Prime system provides scalable and consistent system for gDNA extraction from 2 mL of whole blood. GenFind V3 DNA Isolation Kit uses SPRI (Solid Phase Reverse Immobilization) paramagnetic bead technology to isolate genomic DNA from fresh or frozen whole blood and serum containing Citrate, EDTA or Heparin anticoagulants. The combined automation and chemistry platform processing 6 samples within 2 hours (if KingFisher Presto system is preferred, 24 samples can be processed within 2 hours). A comparison between manual extraction and automated extraction was done with multiple donors. Quality metrics for gDNA yield, purity and integrity were accessed by NanoDrop and gDNA TapeStation. The gDNA yield from 2 mL whole blood was between 30-80 µg. Recovered gDNA exhibited high purity with A260/A280 ratios is about 1.8 and A260/A230 ratios is grater than 1.6. DNA integrity as measured by DNA integrity number (DIN) was above 9.0. The extract gDNA using GenFind V3 and KingFisher Duo Prime system was suitable for downstream applications such as PCR, Microarray and NGS.

Why trust Beckman extraction

SPRI Technology



GenFind V3 Workflow

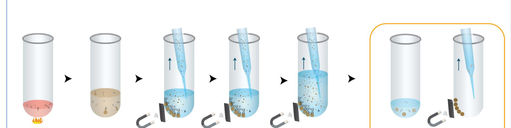
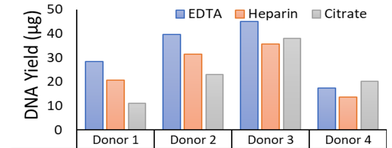


Figure 1. AMPure XP chemistry is based on SPRI paramagnetic bead technology, where the nucleic acid is immobilized on beads, contaminants washed away, then eluted. GenFind V3 DNA Isolation Kit uses SPRI paramagnetic bead technology to isolate genomic DNA from fresh or frozen whole blood and serum.

Genomic DNA manual extraction from 2 mL blood using GenFind V3

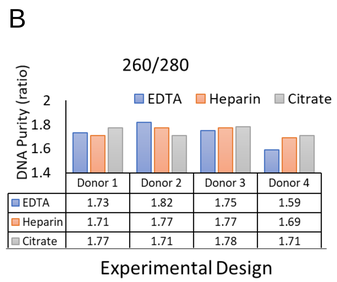
A



	Donor 1	Donor 2	Donor 3	Donor 4
EDTA	28.368	39.74	45	17.408
Heparin	20.668	31.42	35.692	13.668
Citrate	11.04	23.128	38.104	20.296

Experimental Design

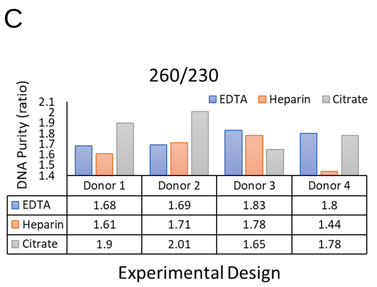
B



	Donor 1	Donor 2	Donor 3	Donor 4
EDTA	1.73	1.82	1.75	1.59
Heparin	1.71	1.77	1.77	1.69
Citrate	1.77	1.71	1.78	1.71

Experimental Design

C



	Donor 1	Donor 2	Donor 3	Donor 4
EDTA	1.68	1.69	1.83	1.8
Heparin	1.61	1.71	1.78	1.44
Citrate	1.9	2.01	1.65	1.78

Experimental Design

Figure 2. Genomic DNA was extracted from 2 mL frozen whole blood. Total 4 different donors and three different blood collection tubes were used in the manual extraction demonstration. (A) DNA yield was measured by NanoDrop (Thermo Fisher Scientific). (B and C) DNA purity was accessed by NanoDrop (Thermo Fisher Scientific). 260/280 is about 1.8 and 260/230 is greater than 1.6 for all tested donors with three blood collection tubes: Citrate, EDTA or Heparin.

Automated genomic DNA extraction from 2 mL blood using GenFind V3

A

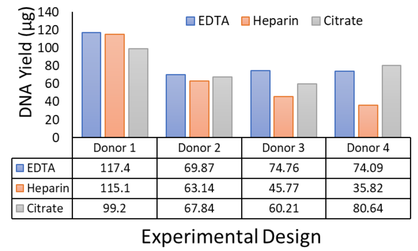
B

Figure 3 KingFisher Duo Prime system. (A) The platform was used in GenFind V3 demonstration. (B) The magnetic head used for GenFind V3.

Table 1. KingFisher Duo Prime DNA isolation set-up

DNA purification step	Plate row	Reagent	Volume (µl)	Automation parameters: Mixing time/Mixing speed/Pause time	Collect count/Time [s]	Loop	
Lysis	Plate 1-A	Blood Lysis buffer LBB	2000 1750	3 min/Fast/ 5 min/ Slow/ 25 min	NA	1	Heating 42°C
Bind	Plate 1-A	Lysate Bind buffer BBB	3750 1250	2 min/ Fast/ 8 min	5/ 30 s	2	
Wash WBB-1	Plate 1-B	Wash buffer WBB	4000	1.5 min/Half/ 2 min/Bottom/ 1.5 min/Fast/3 min	5/ 30 s	1	
Wash WBB-2	Plate 1-C	Wash buffer WBB	4000	1.5 min/Half/ 2 min/Bottom/ 1.5 min/Fast/3 min	5/ 30 s	1	
Wash WBC-1	Plate 1-D	Wash buffer WBC	5000	1.5 min/Half/ 2 min/Bottom/ 1.5 min/Fast/3 min	5/ 30 s	1	
Wash WBC-2	Plate 2-A	Wash buffer WBC	5000	1.5 min/Half/ 2 min/Bottom/ 1.5 min/Fast/3 min	5/ 30 s	1	
Dry	Plate 2-A	NA	NA	1 min			
Elution	Plate 2-B	Nuclease Free Water	400	30 s/ Slow/ 1 min	5/ 30 s	1	

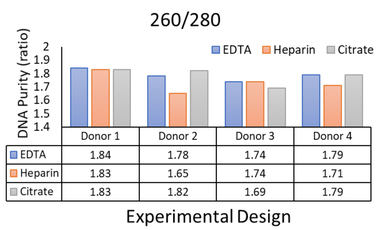
A



	Donor 1	Donor 2	Donor 3	Donor 4
EDTA	117.4	69.87	74.76	74.09
Heparin	115.1	63.14	45.77	35.82
Citrate	99.2	67.84	60.21	80.64

Experimental Design

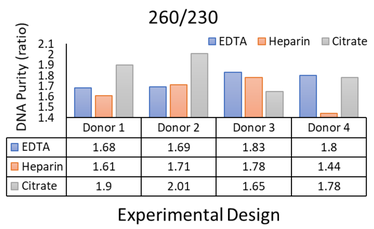
B



	Donor 1	Donor 2	Donor 3	Donor 4
EDTA	1.84	1.78	1.74	1.79
Heparin	1.83	1.65	1.74	1.71
Citrate	1.83	1.82	1.69	1.79

Experimental Design

C



	Donor 1	Donor 2	Donor 3	Donor 4
EDTA	1.68	1.69	1.83	1.8
Heparin	1.61	1.71	1.78	1.44
Citrate	1.9	2.01	1.65	1.78

Experimental Design

Figure 4. Genomic DNA was extracted from 2 mL frozen whole blood using KingFisher Duo System. Same cohort of samples were used in the automation extraction demonstration. (A) DNA yield was measured by NanoDrop (Thermo Fisher Scientific). The yield was higher when using automated extraction method than manual extraction. (B and C) DNA purity was accessed by NanoDrop (Thermo Fisher Scientific). 260/280 is about 1.8 and 260/230 is greater than 1.6 for all tested donors with three blood collection tubes: Citrate, EDTA or Heparin.

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