BECKMAN COULTER
Life Sciences

DNA extraction from up to 2 mL blood using GenFind V3

This method is applicable for scientists who want to extract DNA from up to 2 mL fresh or frozen whole blood.

Please reference the current GenFind V3 protocol (Part number: C34880 or C34881) for product information

Purpose

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. 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 a high-quality, research-ready DNA extraction method from large volume whole blood by using GenFind V3.

Material Used

Material	Part Number	Supplier
GenFind V3	C34880 or C34881	Beckman Coulter
Proteinase K	C34821or C34827	Beckman Coulter
Lysis (LBB)	C34822	Beckman Coulter
Bind (BBB)	C34823	Beckman Coulter
Wash (WBB)	C34825	Beckman Coulter
Wash (WBC)	C34826	Beckman Coulter
Reaction Plate 10 mL 24-well plate	P-DW-10ML-24-C-S	Axygen
24-well magnet ring plate	A000440	Alpaqua Magnum FLX®24
Nuclease-Free Water	AM9932	Ambion
Ethanol	AB-00138	American Bioanalytical

Protocol

1. **Preparation/Lysis**
    a. Thaw the frozen samples at **room temperature** or **37°C**
    b. Invert the blood collection tubes several times to mix well
    c. Transfer **2 mL of** blood to 10 mL 24-well plate
    d. Add **300 µL PK** to the plate
    e. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    f. Add **1.75 mL LBB** to the plate
    g. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    h. Incubate the plate for **40 minutes** at **37°C**

2. **Bind**
    a. Vortex the bottle of BBB to fully resuspend the beads
    b. Add **1.25 mL** of **BBB** to the plate
    c. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    d. **Incubate** the plate for **10 minutes** at **room temperature**
    e. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    f. **Incubate** the plate for **10 minutes** at **room temperature**
    g. Place the plate on a **magnet** for **15 min** (or until supernatant is clear)
    h. Remove and discard the supernatant without disrupting the beads
    i. Remove the plate from the magnet

3. **WBB Wash**
    a. Add **4 mL** of **WBB** to the plate
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. Place the plate on a **magnet** for **10 minutes** (or the supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads
    e. Remove the plate from the magnet
    f. Repeat step 3a-e for a total of **2 washes**

4. **WBC Wash**
    a. Add **5 mL** of **Wash buffer WBC** to the plate
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. Place the plate on a **magnet** for **10 minutes** (or the supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads
    e. Remove the plate from the magnet
    f. Repeat step 4a-e for a total of **2 washes**
    g. Air dry the samples on the magnet for **3 minutes**

5. **Elute**
    a. Add **400 µL** of **nuclease free water** to the plate
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. **Incubate** for **5 minutes** at **room temperature**
    d. Place the plate on a **magnet** for **4 minutes** (or until supernatant is clear)
    e. Remove and **save** the supernatant without disrupting the beads

Results

We demonstrated genomic DNA extraction from 2 mL frozen whole blood. DNA was isolated from healthy blood donors (n = 4) using three different blood collection tubes (EDTA, Heparin and Citrate). DNA yield (A) and purity (B and C) was accessed by NanoDrop (Thermo Fisher Scientific).
The average DNA yield is about 32 µg, 25 µg and 23 µg for EDTA, Heparin and Citrate collection tube, respectively. For donors 1-3 the A260/A280 averages 1.76 and the A260/A230 ratios are greater than 1.6. Donor 4 seems like it may be an outlier with an average A260/280 ratio of 1.66 and an A260/A230 ratio ranging from 1.0 to 1.8.

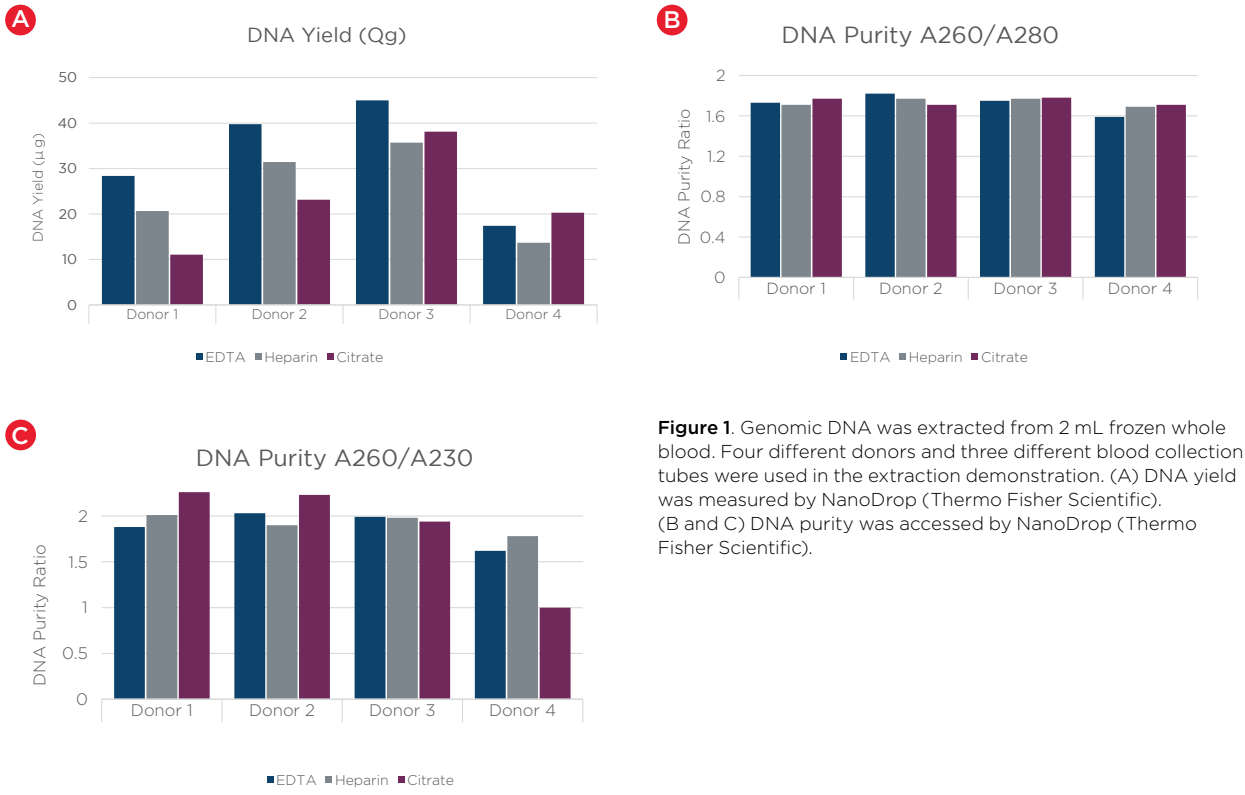

Figure 1. Genomic DNA was extracted from 2 mL frozen whole blood. Four different donors and three different blood collection tubes were used in the extraction demonstration. (A) DNA yield was measured by NanoDrop (Thermo Fisher Scientific). (B and C) DNA purity was accessed by NanoDrop (Thermo Fisher Scientific).

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AAG-5589SP07.19