BECKMAN COULTER
Life Sciences

DNA extraction FTA cards or filter paper blood spots using GenFind V3

Method is applicable for scientists extracting DNA from blood spots on filter paper or FTA cards.

Please reference current GenFind V3 protocol for product information (Part Number: C34880, C34881)

Purpose

Blood spots on filter paper or FTA cards are used for ease of transport and storage of blood for later DNA isolated from the spots. Due to the absorbance of the paper, the isolation of DNA requires slight modification to the protocol. This method provides instruction for additional lysis buffer volumes and optimized lysis conditions for extraction of DNA.

Materials Used

Material	Part Number	Supplier
Nuclease-free water (Molecular Grade)	W4502	Sigma-Aldrich
2 mL 96-well plate	609681	Beckman Coulter
7 Bar Magnet for 96-Well Plate	771MWZM-1ALT	V&P Scientific
Whatman® 903 Protein saver card	WHA10534612	Sigma-Aldrich
100% Ethanol (Molecular Grade)	AB00138	AmericanBio

Protocol

1. **Sample Preparation**
    a. Remove blood spots by cutting or punching out the spot on filter paper and put in 2mL 96-well plate
        i. Up to **1 x 0.5 inch** circle containing **50 µL** of blood used for data shown below
2. **Lysis**
    a. Add **500 µL** of **Lysis (LBB)** to plate
    b. Add **30 µL** of Proteinase K
    c. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    d. **Incubate** the plate for **1 hour** at **room temperature**
3. **Bind**
    a. Vortex to fully resuspend the beads in bottle of **Bind (BBB)**
    b. **Move** the lysate to a new well avoiding the filter paper
        i. Note: Use a pipette tip to maneuver it to the side of the well and the filter paper should stick to the side of the well when the lysate is removed.
    c. Add **300 µL** of **Bind (BBB)** to the plate
    d. **Incubate** the plate for **5 minutes** at **room temperature**
    e. Place the plate on a **magnet** for **15 minutes**
    f. Remove and discard the supernatant without disrupting the beads
    g. Remove the plate from the magnet

4. **Wash**
    a. Add **800 µL** of **Wash (WBB)** to 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 until supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads
    e. Remove the plate from the magnet
    f. Repeat steps 4.a-4.e for a total of **2 washes**
    g. Add **1600 µL** of **Wash (WBC)** to plate
    h. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    i. Place the plate on a **magnet** for **6 minutes** (or until supernatant is clear)
    j. Remove and discard the supernatant without disrupting the beads
    k. Remove the plate from the magnet
    l. Repeat steps 4.g-4.k for a total of **2 washes**

5. **Elute**
    a. Add **40 µL** of **nuclease free water** to plate
    b. **Incubate** the plate for **2 minutes** at **room temperature**
    c. Place the plate on a **magnet** for **2 minutes** (or until supernatant is clear)
    d. Remove and **Save** the supernatant without disrupting the beads

Example Data

Genomic DNA (gDNA) was extracted from dried blood spots on Whatman® 903 Protein saver card. The dried blood spots were made with 50 µL of blood and kept at room temperature (23°C) for 34 days. Genomic DNA was extracted from the dried blood spots on the Whatman® 903 Protein using the protocol described above. The DNA concentration was evaluated using Quant-iT DNA assay kit (Thermo Fisher Scientific). DNA yield from 5 replicates averaged at 82 ng ± 38 ng with an average concentration of 2.05 ng/µL; similar to results previously published using a more labor intensive method (Saavedra-Matiz et. al., 2013). To test for the carryover of PCR inhibitors, a qPCR was performed using a primer set (forward primer 5´-ggacttcgagcaagagatgg-3´ and reverse primer 5´-agcactgtgttggcgtacag-3´) designed to span Exon 4 and 5 of the beta (β)-actin gene (ActB) to produce 327 base pair amplicons. The amplification plot of the 5 samples can be seen in the figure below. Samples 2 and 4 have a higher concentration of DNA, which corresponds to their amplification plot.

Sample	Yield (ng)
1	74.6
2	111.9
3	67.2
4	134.4
5	23.6

Table 1. The yield of DNA extracted from 5 replicate dried blood spots as measured by using Quant-iT DNA assay kit.

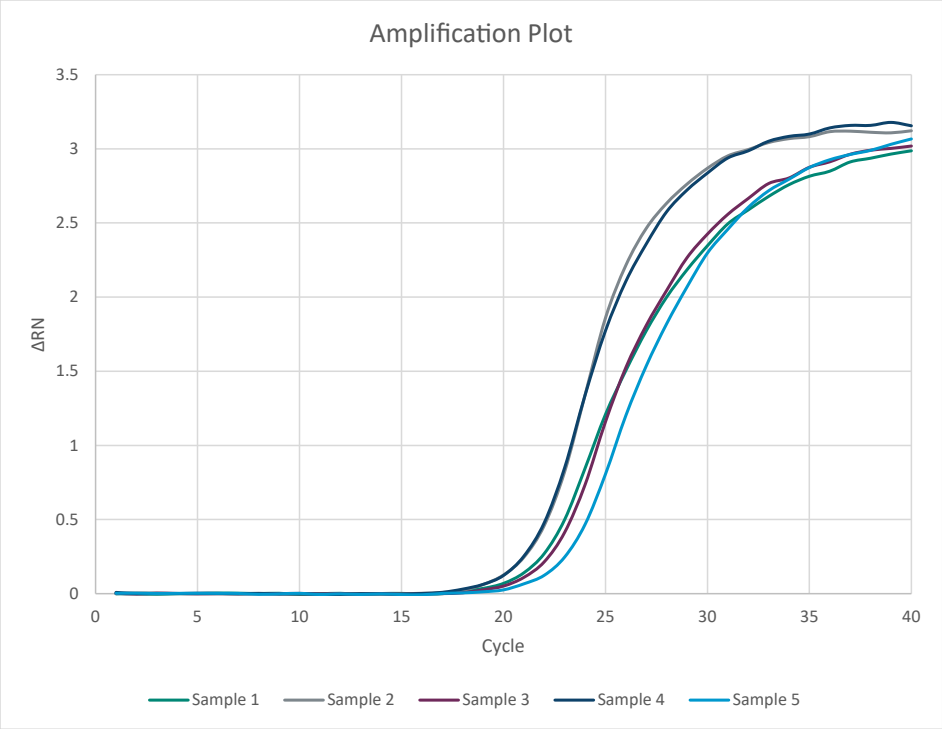

Figure. The amplification plot of the 5 samples. An estimated 20ng of DNA was used and the Ct values averaged at 19.7 with a standard deviation of 0.75 and a negative control had a Ct value of 34.9, indicating that PCR inhibitors removal from the sample during the extraction process.

References

Saavedra-Matiz, C.A., Isabell, J.T., Biski, C.K., Duva, S.J., Sweeney, M.L, Parker, A.L., Young, A.J., DiAntonio, L.L., Krein, L.M., Nichols, M.J., Caggana, M. Cost-effective and scalable DNA extraction method from dried blood spots. Clinical Chemistry. 2013. 59(7): 1045-51.

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