BECKMAN COULTER Life Sciences

DNA extraction from Zeesan Saliva DNA sample collection kit using the GenFind V3 reagent kit

This method is applicable for scientists who want to extract DNA from Zeesan Saliva DNA sample collection kit (SAL-2000L). Please reference the current GenFind V3 protocol (Part number: C34880 or C34881) for product information.

Purpose

Saliva contains buccal epithelial cells and white blood cells which provide rich genetic data. Due to easy access and its non-invasive character, saliva is an alternative to blood collection. Here we present a high-quality DNA extraction method from saliva using the GenFind V3 reagent kit.

Material Used

Material	Part Number	Supplier
GenFind V3 Reagent Kit	C34880 or C34881	Beckman Coulter Life Sciences
Proteinase K	C34821or C34827	Beckman Coulter Life Sciences
Lysis (LBB)	C34822	Beckman Coulter Life Sciences
Bind (BBB)	C34823	Beckman Coulter Life Sciences
96 Square Deep-Well Plate, Polypropylene 2 mL	609681	Beckman Coulter Life Sciences
Magnetic Separation Plate for 96 Deep-well 7 Magnetic Bars	VP-771MWZM-1-ALT	V&P
Nuclease-Free Water	AM9932	Ambion
Ethanol	AB-00138	American Bioanalytical

Protocol

1. **Preparation/Lysis**
    a. Collect saliva according to Zeensan saliva collection kit instructions.
    b. Keep saliva at **room temperature** before DNA extraction
    c. Transfer **300 µL** of saliva to each well of a 2 mL 96-well plate
    d. Add **700 µL LBB** to each sample well of the plate
    e. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    f. Add **45 µL PK** to to each sample well
    g. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    h. Incubate the plate for **60 minutes** at **55°C**

2. **Bind**
    a. Vortex the bottle of BBB to fully resuspend the beads
    b. Add **450 µL** of **BBB** to each sample well
    c. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    d. **Incubate** the plate for **5 minutes** at **room temperature**
    e. Place the plate on a **magnet** for **10 min** (or until supernatant is clear)
    f. Remove and discard the supernatant without disrupting the beads
    g. Remove the plate from the magnet

3. **WBB Wash**
    h. Add **1.2 mL** of **WBB** to each sample well
    i. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    j. Place the plate on a **magnet** for **10 minutes** (or the supernatant is clear)
    k. Remove and discard the supernatant without disrupting the beads
    l. Remove the plate from the magnet
    m. Repeat step 3a-e for a total of **2 washes**

4. **WBC Wash**
    n. Add **1.6 mL** of **Wash buffer WBC** to each sample well
    o. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    p. Place the plate on a **magnet** for **10 minutes** (or until the supernatant is clear)
    q. Remove and discard the supernatant without disrupting the beads
    r. Remove the plate from the magnet
    s. Repeat step 4a-e for a total of **2 washes**
    t. Air dry the samples on the magnet for **3 minutes**

5. **Elute**
    a. Add **150 µL** of **Nuclease-free water** to each sample well
    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

Genomic DNA (gDNA) was isolated from three healthy donors using Zeesan Saliva DNA sample collection kit. DNA yield was measured by Quant-iT™ PicoGreen™ dsDNA Assay (Thermo Fisher Scientific). An average of 10.3 µg of gDNA was extracted from 300 µL saliva (Fig. 1A). Purity was assessed by NanoDrop (Thermo Fisher Scientific). The average 260/280 is 1.9 and the average 260/230 is greater than 2.0 (Fig. 1B). DNA integrity was assessed using Agilent gDNA ScreenTape Assay. The DNA integrity number (DIN) scores averaged 8.2 and DNA peak size was larger than 55K indicating the gDNA extracted from saliva sample is of high quality (Figure 1C and D).

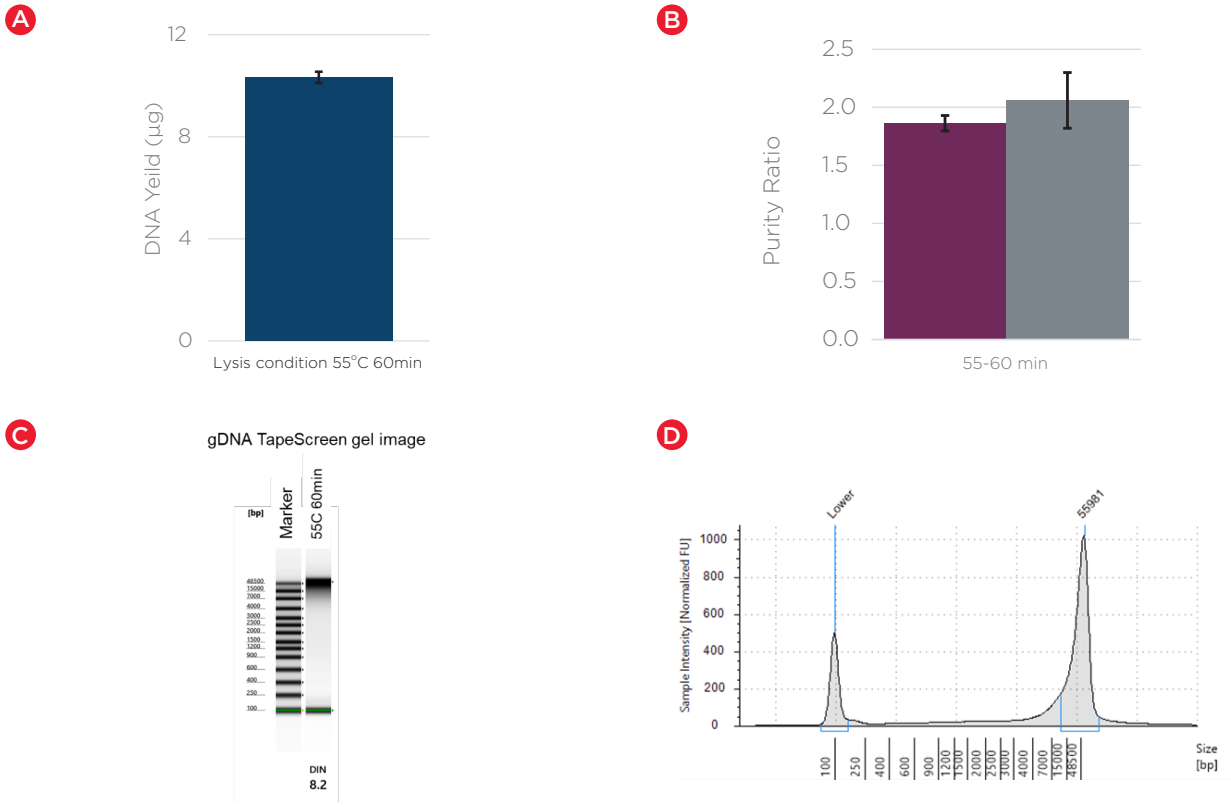

Figure 1. Genomic DNA extraction from 300 µL saliva. DNA was isolated from 3 healthy donors. (A) DNA yield was measured by Quant-iT™ PicoGreen™ dsDNA Assay (Thermo Fisher Scientific). The error bar represents the standard deviation of three donor replicates. (B) Purity was assessed by NanoDrop (Thermo Fisher Scientific); the average 260/280 is about 1.9 and 260/230 is greater than 2.0. The error bar represents the standard deviation of three donor replicates. (C and D) DNA integrity was analyzed by using Agilent gDNA ScreenTape Assay.

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2023-GBL-EN-102632-v2