BECKMAN COULTER Life Sciences

DNA extraction from Oragene® Dx OGD-600 Saliva collection tubes using DNAdvance

Please reference current DNAdvance Instructions for use and product information (C42213, A48705, or A48706)

This method is applicable for scientists who want to extract DNA from Oragene Dx OGD-600 Saliva collection tubes

Purpose

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

Materials Used

Material	Part Number	Supplier
Oragene Dx OGD-600	OGD-600	DNA Genotek
2 ml 96-well plate	609681	Beckman Coulter
7 Bar Magnet for 96-Well Plate	771MWZM-1ALT	V&P Scientific
100% Ethanol (Molecular Grade)	AB00138	AmericanBio
Nuclease-free water (Molecular Grade)	AB02123	AmericanBio
Pre-Bind (PBBA)	C42179, C42198, or C42204	Beckman Coulter Life Sciences
Bind (BBE)	C42193, C43199, or C42205	Beckman Coulter Life Sciences
Elution (EBA)	C42195, C42201, or C42207	Beckman Coulter Life Sciences

Protocol

1. **Sample Preparation**
    a. Collect and store saliva sample according to the manufacturer's instructions
2. **Lysis**
    a. **Incubate** the tubes for **1 hour** at **50°C**
    b. Transfer **500 µL** of **sample** to 2 mL 96-well plate
3. **Bind**
    a. Add **200 µL** of **PBBA** to the plate
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. Vortex the bottle of **BBE** to fully resuspend the beads
    d. Add **340 µL** of **BBE** to the plate
    e. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    f. **Incubate** the plate for **1 minute** at **room temperature**
    g. Place the plate on a **magnet** for **8 minutes** (or until the supernatant is clear)
    h. Remove and **discard** the supernatant without disrupting the beads
    i. Remove the plate from the magnet

4. **Wash**
    a. Add **700 µL** of **70% Ethanol** to plate
    b. **Mix** by pipetting up and down 20 times, or until thoroughly mixed
    c. Place the plate on a **magnet** for **2 minutes** (or until the 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-3.e for a total of **3 washes**

5. **Elute**
    a. Add **50 µL** of **EBA** to plate
    b. **Mix** by pipetting up and down **10 times,** or until thoroughly mixed
    c. Place the plate on a **magnet** for **3 minutes** (or until the supernatant is clear)
    d. Remove and **Save** the supernatant without disrupting the beads

Example Data

Genomic DNA (gDNA) was isolated from 82 healthy donors using the Oragen Dx OGD-600 sample collection tube. DNA yield was measured on a NanoDrop (ThermoFisher Scientific). The average yield varied from about 0.9 µg to 24 µg and averaged 8 µg; the wide range in yields can most likely be attributed to donor variability in buccal cells and variability in following manufacturer's instructions. The different yields can be seen below in figure 1. The average yield was 8 µgs. The average A260/A280 ratio was 1.7 (data not shown).

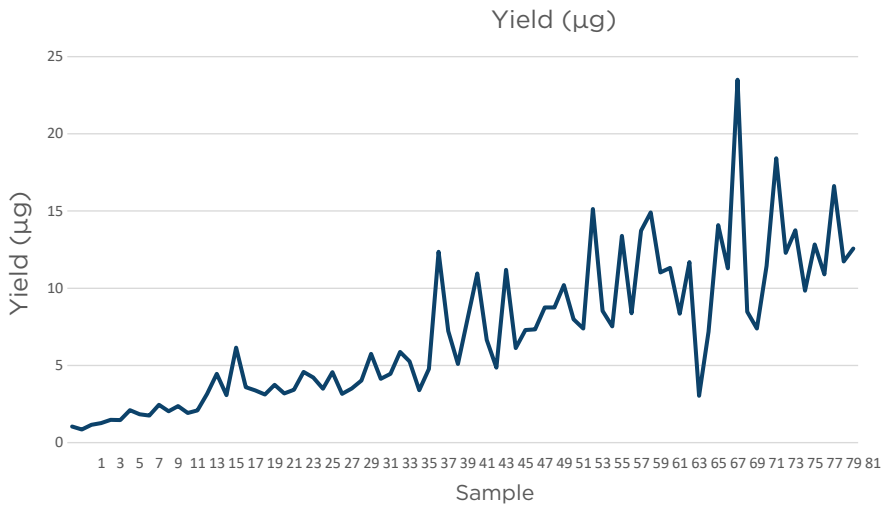

Figure 1. The variable DNA yields collected from 82 donors as assessed on a NanoDrop (ThermoFisher Scientific).

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