BECKMAN COULTER Life Sciences

Isolation of DNA from Buccal Swabs using DNAdvance Kit

Please reference the current DNAdvance protocol for product information (Part Number A48706, A48705).

Researchers working on human genome sequencing may use this protocol.

Purpose

Buccal swabs are a non-invasive way to collect human DNA samples. Different swab types recommend varying collection methods, and Puritan polyester tipped applicators (PN 25-806-2PD) recommend swabbing the inside of the cheek with two different swabs. This protocol increases the volume of lysis buffer so that these two swabs from a single individual can be run in a single well and have the swab heads covered by lysis buffer. Subsequent steps have been modified to retain optimal buffer ratios. This protocol was also tested with a single Copan FLOQSwab (PN 50E011E01) to ensure that both brands can be run using the same protocol.

Materials Used

Material	Part Number	Supplier
1.2mL 96-well plate	AB1127	ThermoFisher Scientific
100% Ethanol (Molecular Grade)	AB00138	AmericanBio
Nuclease-free water (Molecular Grade)	AM9932	ThermoFisher Scientific
7 Bar Magnet for 96-Well Plate	771MWZM-1ALT	V&P Scientific
DNAdvance Kit	A48706, A48705	Beckman Coulter
Polyester tipped applicators	25-806-2PD	Puritan
FLOQSwab	50E011E01	Copan

Protocol

1. **Lysis**
   a. Make Lysis Buffer master mix with **441 µL Lysis Buffer, 16 µL Proteinase K,** and **343 µL nuclease free water** per sample.
   b. Cut **1-2 buccal swab heads** and put into a single well in a deep well plate. Add **800 µL** of **Lysis Buffer master mix** to plate.
   c. Seal plate and **incubate** at **60˚C** for **15 minutes**.
   d. *Optional* spin to remove condensation before unsealing plate.
2. **Bind**
   a. **Transfer** the lysate to a new well, leaving the buccal swab heads behind. The volume will be **~500 µL**.
   b. Add **150 µL Bind 1**.
   c. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
   d. Vortex to fully resuspend **Bind 2 Buffer**.
   e. Add **255 µL Bind 2 Buffer**.
   f. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.

(continued on reverse)

g. Incubate at room temperature for 5 minutes.
h. Place plate on **magnet** for **4 minutes** or until solution clears.
i. Remove and discard the supernatant without disrupting the beads.

3. **Wash (x 3)**
   a. Add **340 µL 70% ethanol**.
   b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
   c. Place on the **magnet** for **3 minutes**.
   d. Remove and discard the supernatant without disrupting the beads.
   e. **Repeat** ethanol wash (steps 3a-3d) for a total of **3 ethanol washes**.
4. **Elution**
   a. Allow beads to air dry (~1 min)
   b. Add **60 µL Elution Buffer**.
   c. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
   d. Place on **magnet** for **3 minutes**.
   e. Remove and **Save** the supernatant without disrupting the beads.

Sample	Conc. (ng/µL)	260/280	total ng
Copan Donor 1	7.855	1.6	471.3
Copan Donor 2	24.91	1.6	1494.6
Copan Donor 3	18.36	1.7	1101.6
Puritan Donor 1	17.99	1.7	1079.4
Puritan Donor 2	52.46	1.6	3147.6
Puritan Donor 3	31.23	1.6	1873.8

Table 1. Yield of DNA from Individual Replicates.

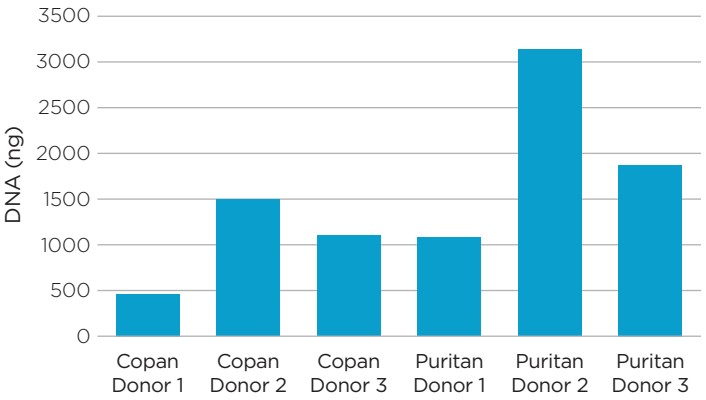

Figure 1. Yield of DNA from 1 copan FLOQSwab or 2 puritan polyester swabs.

Example Data

Puritan polyester swabs were wet on the tongue and then run ten times on each cheek. This method was followed for both swabs in the puritan package. Since puritan recommends two swabs per person, both swabs were run in a single well for this protocol. Copan FLOQSwabs were sampled using the same method, but only using the single swab provided. The protocol was run as described above.

In order to run both puritan swabs in a single well, the volume of Lysis Buffer was increased to completely cover both swab heads. If the swab heads are not covered by the Lysis Buffer, DNA yields are lower and less consistent. Subsequent reagents were scaled up to work with the larger volumes of Lysis Buffer.

Two puritan polyester swabs resulted in greater than 1 µg of DNA with concentrations ranging from ~20-50 ng/µL. A single FLOQSwab resulted in slightly less yield, with concentrations from ~10 – 25 ng/µL and total yield of greater than 450 ng. Both swab types worked well with this protocol; however, copan swabs can also be run with lower amounts of lysis and subsequent buffers. Little protein contamination was seen, with 260/280 values of 1.6-1.7.

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