

Automated DNA Purification from Mawi iSWAB Tubes

Purify human genomic DNA from buccal samples collected in Mawi DNA Technologies iSWAB tubes using the Maxwell® RSC Blood DNA Kit with the Maxwell® RSC Instrument.

Kit: Maxwell® RSC Blood DNA Kit (Cat.# AS1400)

Analyses: Absorbance, Fluorescent DNA-binding dye, qPCR

Sample Type: Human buccal samples in iSWAB tube

Input: 200µl

Materials Required:

- Maxwell® RSC Blood DNA Kit (Cat.# AS1400)
- Maxwell® RSC Instrument (Cat.# AS4500) or Maxwell® RSC 48 Instrument (Cat.# AS8500)
- iSWAB tube, Mawi DNA Technologies (e.g. Cat.# ISWAB-DSC)
- iClean flocked swab (included with iSWAB Collection Kit)
- Heat block set to 56°C

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM419, available at:

www.promega.com/protocols

or contact Technical Services at:
techserv@promega.com

Protocol:

1. Transfer 200µl of iSWAB solution into a 1.5ml tube.
2. Add 30µl of Proteinase K Solution.
3. Add 300µl of Lysis Buffer.
4. Vortex for 10 seconds.
5. Incubate at 56°C for 20 minutes.
6. Meanwhile, prepare cartridges as indicated in the technical manual (TM419).
 - a. Add 50µl of Elution Buffer to each Elution Tube.
7. Transfer all volume into well #1 of the Maxwell® cartridge.
8. Select the Maxwell® RSC Blood DNA run method, place the prepared deck tray in the Maxwell® RSC Instrument, and start the method.

Results:

Human genomic DNA can be purified from buccal samples collected with Mawi iSWAB tubes. The purified human DNA is amplifiable and intact, as measured by the ProNex® DNA QC Assay.

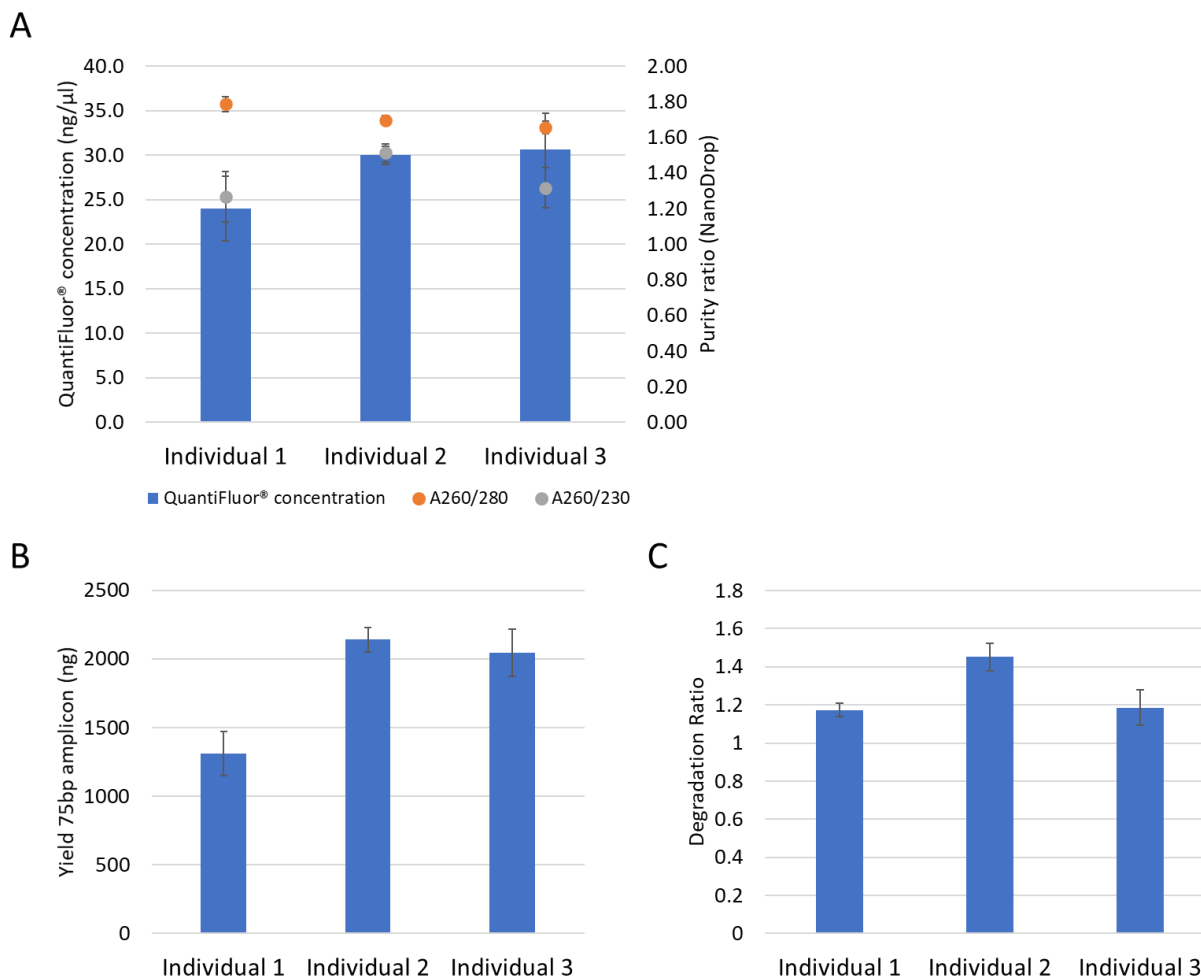


Figure 1. Analyses of DNA purified from human buccal samples in iSWAB-DNA tubes. Human buccal samples were collected from three individuals (1 tube per cheek swab—2 tubes total), according to iSWAB Discovery product instructions, and incubated overnight at room temperature. Samples from each individual were pooled together prior to purification. **A. Concentration and purity ratios.** DNA concentration (blue bars) was measured with the Quantifluor® ONE dsDNA System (Cat.# E4871) using K562 DNA (Cat.# E4931) as a standard on the Quantus™ Fluorometer (Cat.# E6150). Purity ratios were measured with a NanoDrop™ 8000 instrument (orange circles, A260/280; gray circles, A260/230). Mean ± standard deviation is shown for each measurement, n=3 purifications per individual. **B,C. Amplifiable human DNA yield and quality.** 2μl of each DNA sample was amplified in duplicate with the ProNex® DNA QC Assay BioRad CFX96™ (Cat.# NG1004) on the Bio-Rad CFX96 Touch Real-Time PCR Detection System. Yield (B) was calculated by multiplying the concentration of the 75bp amplicon by the elution volume. Degradation ratio (C) was calculated by dividing the concentration of the 75bp amplicon by the concentration of the 300bp amplicon ([75bp]/[300bp]). This ratio measures the integrity of the DNA. The ratios measured for each sample are close to 1, which indicates that the purified DNA is intact.