Promega

Product Application

Automated Purification of DNA from Fingernail Clippings

Purify amplifiable human DNA from fingernail clippings using the Maxwell® FSC DNA IQ Casework Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® FSC DNA IQ Casework Kit (Cat.# AS1550)
Casework Extraction Kit (Cat.# DC6745)

Analyses: Absorbance, qPCR

Sample Type(s): Human fingernail clippings

Input: 20mg, cut into pieces

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 TM499, available at:
www.promega.com/protocols

or contact Technical Services at:
techserv@promega.com

Materials Required:

- 1.5ml Click-Fit™ tube (Cat.# V4745) or similar
- Heat block set to 56°C
- Maxwell® RSC Instrument (Cat.# AS4500) or Maxwell® RSC 48 Instrument (Cat.# AS8500)
- Instrument method for the Maxwell® FSC DNA IQ Casework Kit on the Maxwell® RSC Instrument (obtain by emailing Promega Technical Services)

Protocol:

1. Cut fingernail clippings into pieces and add 20mg of fingernail to a 1.5ml Click-Fit™ tube.
2. Prepare the Casework Extraction cocktail, as described in Table 1, immediately prior to use. Volumes can be scaled up for multiple reactions.

Table 1. Preparation of the Casework Extraction cocktail

Reagent	Volume per Sample (µl)
Casework Extraction Buffer	386
Proteinase K (MC5008)	10
1-thioglycerol	4

3. Add 400µl of the Casework Extraction cocktail to 20mg of fingernail sample. Vortex to mix.
4. Incubate sample at 56°C for 2 hours.
5. Add an additional 10µl of Proteinase K to each tube. Vortex sample. Continue to incubate sample at 56°C for an additional 2 hours.
6. Centrifuge sample at maximum speed in a microcentrifuge (>16,000 x *g*) for 2 minutes. Transfer supernatant to a fresh tube.
7. Add 200µl of Lysis Buffer (A826) to the sample. Vortex for 10 seconds.
8. Prepare Maxwell® FSC DNA IQ Casework cartridges according to the Technical Manual (TM499). Place a plunger in well #8. Add 50µl of Elution Buffer to the elution tube.
9. Add the entire volume of the lysate to well #1 of the Maxwell® FSC DNA IQ Casework cartridge.
10. Purify on a Maxwell® RSC Instrument using the Maxwell® FSC DNA IQ Casework method.

Results:

DNA was purified from the nail trimmings of six individuals using the above protocol. DNA was quantified using absorbance (Fig. 1) and human DNA was quantified and assessed for degradation using qPCR (Fig. 2). Amplifiable DNA was purified from all samples, though the amount of DNA purified is highly variable between samples. As expected, some samples also exhibit some DNA degradation.

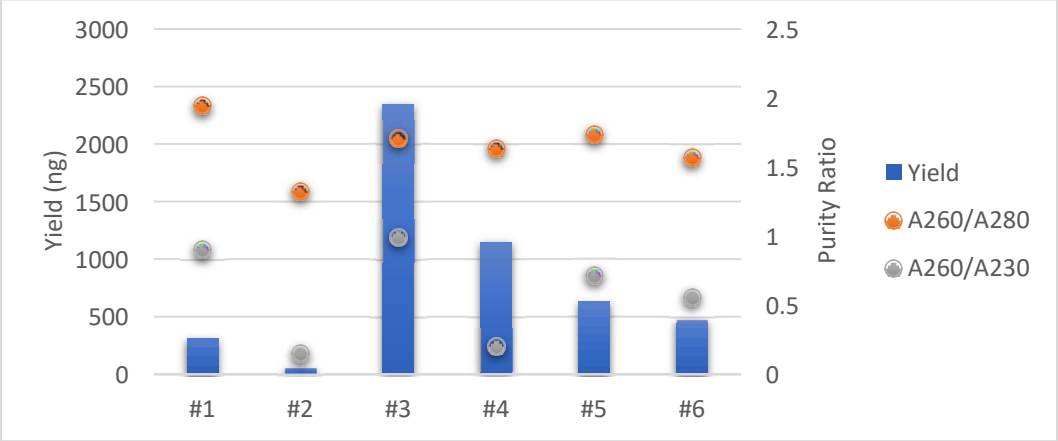

Figure 1. Spectrophotometric analysis of sample yield and purity ratios for eluates. Samples from 6 individuals were pre-processed using the DNA IQ™ Casework Extraction kit and then purified using the Maxwell® FSC DNA IQ Casework Kit on the Maxwell® RSC Instrument. Absorbance at 230, 260, and 280nm was measured on the NanoDrop™ One. Yield was calculated based on sample absorbance at 260nm and eluate volume (blue bars). Absorbance ratios, A260/A280 and A260/A230, are also plotted with orange and gray points, respectively (n = 1).

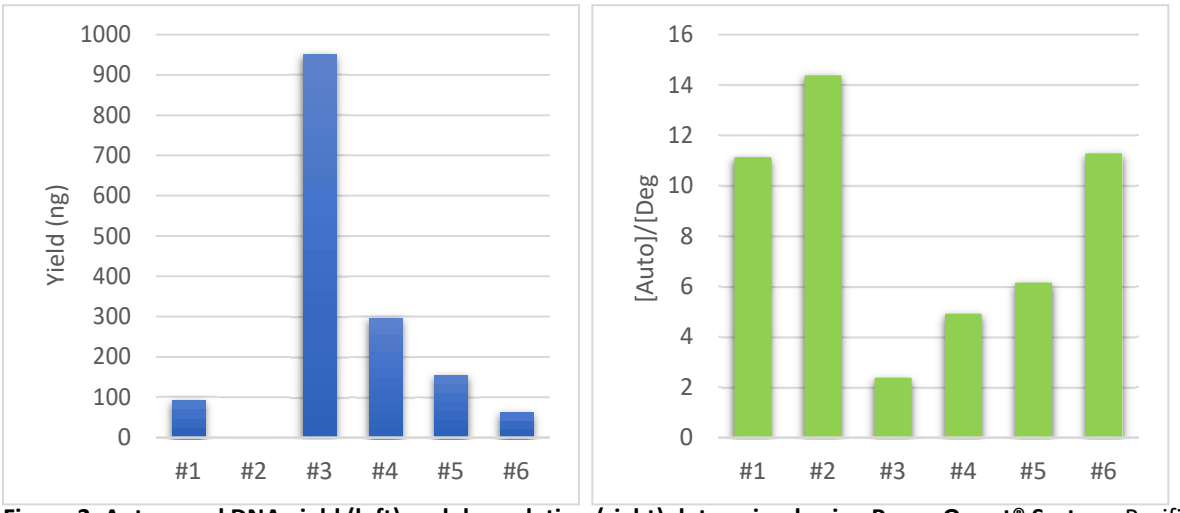

Figure 2. Autosomal DNA yield (left) and degradation (right) determined using PowerQuant® System. Purified sample DNA was amplified using the PowerQuant® System (Cat.# PQ5002) on an ABI 7500 Fast Real-Time PCR Instrument according to the Technical Manual. (Left) Sample concentration was determined based on amplification of an autosomal target relative to the standard curve (R^2 = 1.000) prepared with human genomic DNA, and yield was calculated based on the eluate volume (n = 1). (Right) Degradation of the sample DNA was assessed using the relative amplification of the autosomal qPCR target and the degradation qPCR target for each sample (relative to their respective standard curves, R^2 > 0.99). Higher [Auto]/[Deg] ratios indicate more degraded DNA.