

Automated DNA Purification from Saliva with the AutoGenFlex

M. Miyake¹, B. French¹, and P. Lem²

¹AutoGen, Holliston, MA, USA

²DNA Genotek, Ottawa, ON, Canada

The AutoGenFlex is a high-throughput instrument that can extract high molecular weight genomic DNA from saliva samples collected with Oragene™. From a 0.5-ml sample aliquot, the average DNA yield was 15.3 µg and the $A_{260/280}$ ratio was 1.87.

Introduction

The AutoGenFlex (AutoGen) is an automated instrument that can isolate up to 40 samples of genomic DNA from whole blood, cultured cells, and human and animal tissue in a 4–6 hour period. For saliva specimens, the AutoGenFlex can process sample volumes up to 0.5 mL. The purified DNA is suitable for PCR, sequencing, and other molecular biology applications (ref. 1).

Due to its non-invasiveness and simplicity of use, the Oragene DNA Self-Collection Kit enables the convenient collection of samples from large numbers of subjects. The Oragene kit collects 2 mL of whole saliva, which mixes with 2 mL of Oragene DNA-preserving solution inside the kit (4 mL total sample volume). The purpose of this study was to assess the DNA yield and quality of Oragene samples purified with the AutoGenFlex.



Figure 1 . AutoGenFlex instrument.

Materials and Methods

Sample collection and pre-treatment

Saliva samples were collected from 10 donors using Oragene kits. The samples were incubated overnight at 50°C and then stored at room temperature.

Protocol overview

An aliquot of 0.5 mL from each Oragene sample was transferred into Sample Tube Units (AutoGen) and loaded onto the AutoGenFlex. The instrument processed the Oragene samples using the Tissue DNA protocol and reagents from the AutoGenFlex Tissue DNA Extraction Kit.

In brief, the automated protocol started with the addition of Deproteinizing Solution B to each sample. Next, the samples were centrifuged to pellet the precipitated proteins and the aqueous phase was transferred into corresponding DNA Tube Units, containing DNA Precipitation Solution A. The DNA Tube Units were gently agitated and then centrifuged to pellet the extracted DNA. The DNA was washed twice using DNA Wash Solution A, and the DNA Tube Units were placed on the heater to remove any residual alcohol. After heating, Resuspension Solution A (0.5 mL) was added to each sample and the DNA Tube Units were gently agitated. To ensure complete DNA rehydration, the extracted DNA was incubated for 1 hour at 55°C while shaking gently, followed by an overnight incubation at 4°C.

DNA quantification

The extracted DNA was quantified using a UV/Vis spectrophotometer that measured the absorbance of each sample at 260 nm, 280 nm, and 320 nm (background). The $A_{260/280}$ ratio was calculated by subtracting the background at 320 nm from the absorbance at 260 nm and 280 nm, and dividing the adjusted values.

Agarose gel analysis

To determine the molecular weight of the extracted DNA, 800 ng from each sample was loaded onto a 0.5% TAE agarose gel, containing ethidium bromide (0.5 µg/mL), and run for 1.5 hours at 80 V. A 48.5 kb marker was used as a size reference.

Results

From the 0.5-mL aliquots, the average DNA yield from the 10 samples was 15.3 µg, with a range from 8.2 to 36.0 µg. The average $A_{260/280}$ ratio was 1.87, with a range from 1.80 to 1.98. Figure 2 shows that the extracted DNA had molecular weights of 48.5 kb or greater.

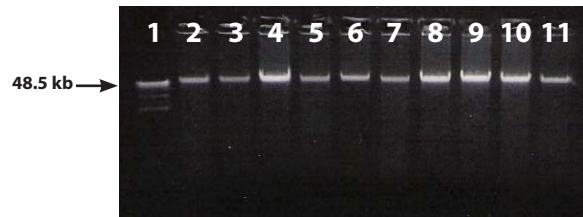


Figure 2. Agarose gel electrophoresis of DNA extracted with the AutoGenFlex. Lane 1 contains a 48.5 kb marker, and Lanes #2-11 contain purified DNA from Donors #1-10, respectively.

Discussion and Conclusions

The AutoGenFlex is capable of extracting high molecular weight genomic DNA from saliva samples collected with Oragene. The average DNA yield from a 0.5 mL aliquot was 15.3 μg . The average $A_{260/280}$ ratio was 1.87 and this indicated that the AutoGenFlex was effective at removing protein contaminants.

The extraction took place in an automated fashion, thus saving time and labor compared to manual processing. Overall, the AutoGenFlex represents an automated method of extracting DNA from Oragene samples, and its high-throughput capabilities may be particularly useful for processing large numbers of samples.

References

1. AutoGen website: <http://www.autogen.com>