

Mole®

DNA extraction from saliva samples collected with the Oragene®•DNA self collection kit

“ GeneMole® is a benchtop instrument for automated nucleic acid purification. Automation reduces the risk of injuries caused by repetitive pipetting, limits exposure to chemicals and infectious agents, and gives you more time to focus on other operations. GeneMole® can process 1-16 samples in one run and all the reagents required are available as pre-filled disposable MoleStrips™. ”



Figure 1:
The Oragene®•DNA
Self-Collection Kit,
tube format.

Introduction

Saliva is an excellent source of DNA for genetics testing. Cells are constantly released from the cheek and end up in a person's saliva. In addition sample collection is quick, easy and non-invasive. Saliva samples collected with the Oragene®•DNA Self-Collection kit are stable for years at room temperature and expensive shipment cost associated with blood collection is therefore avoided. Thousands of clinicians, researchers and investigators worldwide are using Oragene®•DNA for their saliva sample collection.

Here automated DNA extraction from saliva samples collected with Oragene system has been evaluated on the GeneMole® instrument.

Methods

Saliva samples were collected according to the manufacturer's instructions using the tube format (OG-300) of the Oragene®•DNA Self-Collection Kit. The samples were incubated at 50 °C for 2 h prior to DNA extraction (or incubated at 50 °C overnight if it was more convenient).

Genomic DNA was extracted from 200 µl samples according to the instructions given in the MoleStrips™ DNA Blood Kit.

The elution volume was specified as 100 µl.

Quality and yield was measured on the NanoDrop 1000 Spectrophotometer from Thermo Scientific. Agarose gels were stained with SYBR Safe DNA gel stain from Invitrogen. For real-time PCR the QuantiTect SYBR Green PCR Kits from Qiagen was used. Beta-actin primers were from Sigma, and data was generated on the iCycler from Bio Rad. Cycling parameters were: 95°C for 15 min, followed by 35 cycles of 15 sec at 95 °C, 30 sec at 55 °C and 30 sec at 72 °C.

Results

Aliquots of 200 µl from one donor were added to 8 sample tubes and placed in the worktray along with pipetting tips, elution tubes and MoleStrips™ DNA Blood. From the touch screen the protocol "DNA Blood 8" and an elution volume of "100 µl" were selected.

Aliquots of the eluted DNA were analysed on agarose gel as shown in Figure 2.

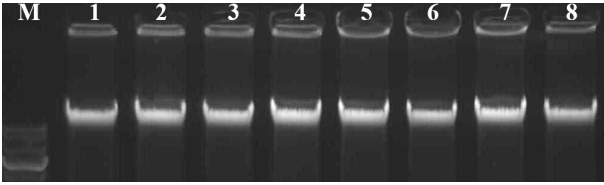


Figure 2: 1% Agarose gel analysis of aliquots (10 µl) of DNA extracted from 200 µl Oragene®•DNA saliva samples on the GeneMole® instrument. M: GeneRuler™ 1 kb DNA Ladder Plus (largest band is 20000 bp).

The quality and yield obtained was measured by using a NanoDrop Spectrophotometer (Figure 3). The mean yield obtained was 59.4 ng/µl (%CV = 3.7) and A260/A280 ratios ranged between 1.82 to 1.87.

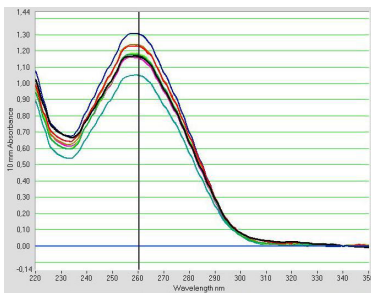


Figure 3: Nanodrop analysis of the samples shown in figure 2.

A GeneMole® extraction was also set up with saliva samples collected from 3 different donors. The saliva samples were incubated at 50 °C for 2 hours and extraction was performed on parallel samples of 200 µl as described above. Figure 3 shows an agarose gel with DNA extracted from the three different saliva donors. NanoDrop data gave mean DNA yield values of 60, 55 and 81 ng/ul for donor 1, 2 and 3 respectively.

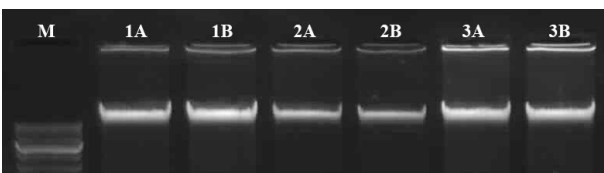


Figure 3: 1% Agarose gel analysis of aliquots (10 µl) of DNA extracted from parallel samples of 200µl Oragene®•DNA saliva from 3 different donors. M: GeneRuler™ 1 kb DNA Ladder Plus.

A PCR experiment with primers amplifying the beta-actin gene was set up with the genomic DNA extracted from the three different donors as template. Real-time experiments using serial diluted templates over 4 order of magnitude indicate the absence of PCR inhibitors in the extracted DNA. The standard curve with a slope of -3,451 indicates a PCR efficiency of 94.9% (Figure 4).

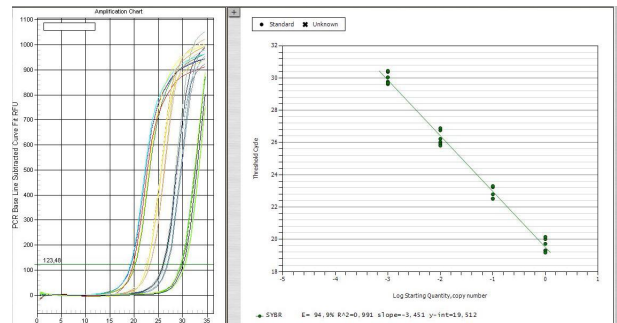


Figure 4: Real-time PCR analysis for the diluted templates (equivalent: 1 µl, 0.1 µl, 0.01 µl and 0.001 µl) extracted from three different patients.

Conclusion

The Oragene® •DNA Self-Collection kit from DNA Genetek is fully compatible with automated DNA purification on the GeneMole® instrument.

With the GeneMole® DNA from 16 saliva samples can automatically be extracted in less than 1 hour with excellent yields, and just a few minutes hands on time.

The eluted DNA is of high molecular weight and the beta-actin gene was amplified successfully from the purified DNA samples indicating that DNA is free of inhibitors and suitable for PCR analysis.

Acknowledgement

Oragene® •DNA Self-Collection kits were kindly provided by Jon Chatburn and Kerry Hicks from DNA Genetek INC. The picture of the OG-300 was provided by the product manager for Oragene® •DNA, Cecilia Becerril.

“Simplify When Possible”

Ordering Information

Product

GeneMole®

MoleStrips™ DNA Blood

MoleStrips™ DNA Blood

Content

Instrument

Convenience Kit¹

64 Reagent Strips

Prod. No.

MG10-000

MG10-101

MG10-102

¹ The convenience kit contains 32 Reagent Strips and all the required tips, tubes and caps.