

Mole®

DNA extraction from human blood

“GeneMole® is a robust benchtop instrument that provides automated extraction of high quality nucleic acid. With reduced hands on time, automation allows time to focus on other operations as well as limits exposure to chemicals and infectious agents. GeneMole® can process 1-16 samples in one run and all the reagents required are available as pre-filled disposable MoleStrips™.”

Introduction

Blood accounts for 7% of the human body weight. In vertebrates blood is composed of blood cells suspended in blood plasma. The DNA containing white blood cells makes up approximately 1% of blood. A white blood cell count (WBC) in a healthy individual is usually within the range of 4-11 mill white blood cells/ml.

Here we show that the GeneMole® instrument can be used to extract high quality DNA from an input of 100 or 200 µl whole human blood. GeneMole® processes 1-8 samples in less than 30 minutes and 9-16 samples in less than an hour. The extracted DNA is suitable for sensitive downstream assays such as PCR and sequencing.

Methods

Genomic DNA was extracted from 100 µl or 200 µl blood according to the protocol given in the MoleStrips™ DNA Blood Kit. MoleStrips™, tips and tubes were loaded onto the freestanding work tray according to the chosen number of samples (1-16). The blood samples were added to the sample tubes, the work tray placed into the GeneMole® and the “DNA Blood” protocol selected from the drop-down menu on the instruments built in touch screen.

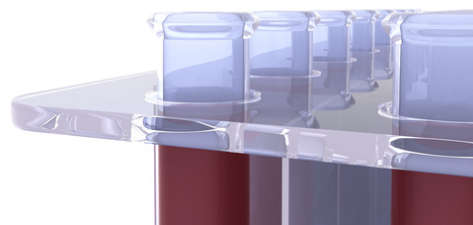


Figure 1
The GeneMole® instrument can extract DNA directly from 16 whole blood samples in less than 1 hour.

Results

DNA extracted from 16 samples of 200 µl whole blood on the GeneMole® instrument was analysed by 1% agarose gel electrophoresis. The GeneMole® elution volume was specified as 200 µl. High molecular weight DNA (>20kb) was consistently extracted from all 16 samples and the measured total DNA yield was similar (~ 4 µg) for all samples within the run (Figure 2).

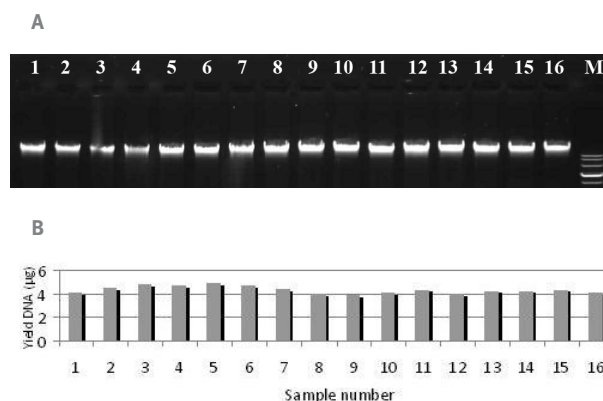


Figure 2
A) Aliquots (10 µl) of each eluate analysed by 1% agarose gel electrophoresis (stained with SYBR safe from Invitrogen). B) Typical DNA yield obtained for 16 sample blood run (%CV = 6.9).

Several full 16 sample blood runs were performed and different parameters measured. See Table 1 for a summary of key parameters.

Table 1: Key GeneMole® parameters (input of 200 µl whole blood).

Key parameter	GeneMole® Protocol
Protocol duration 8 samples	28 minutes
Protocol duration 16 samples	56 minutes
Average yield	
100 µl elution volume	3.4 ± 0.2 µg
200 µl elution volume	4.4 ± 0.2 µg
Average concentration	
100 µl elution volume	34.4 ± 1.8 µg/ml
200 µl elution volume	22.1 ± 1.0 µg/ml
Average OD 260:280	
100 µl elution volume	1.82 ± 0.05%
200 µl elution volume	1.73 ± 0.04%
Sample compatibility	EDTA, citrate and ACD

A cross contamination test was performed to confirm the absence of contamination between samples placed next to each other in the GeneMole® instrument. A 200 µl blood sample was added to tubes 1, 3, 5 and 7 and 200 µl water was added to sample tubes 2, 4, 6 and 8. A real-time PCR assay was performed using 2,5 µl of extracted DNA and primers amplifying a 295 bp fragment for the human ACTB gene. Figure 3 clearly shows no detectable amplification products in the negative control samples.

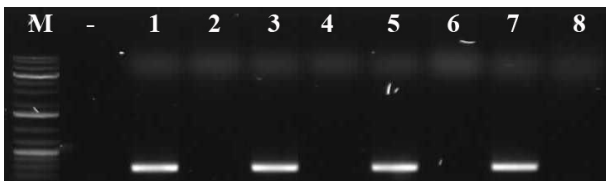


Figure 3
Agarose gel (1%) showing real-time PCR products (10 µl) of the cross contamination test. Amplicon length was 295 bp.

Also, a 4 orders in magnitude serial dilution of the same real-time experiment was set up. These results shown that there is no PCR inhibitors in the extrated DNA (Figure 4).

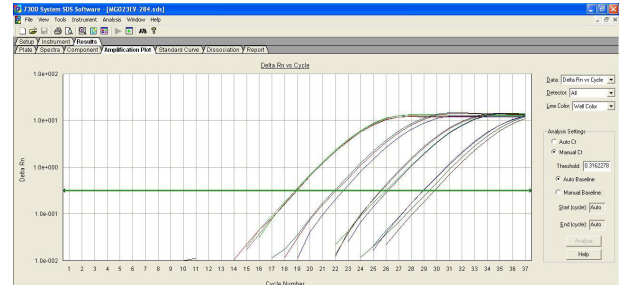


Figure 4
The logarithmic amplification plot from real-time PCR. The dilution series was 4 orders of magnitude (equivalent: 1 µl, 0.1 µl, 0.01 µl and 0.001 µl). The dCt value indicated that no PCR inhibitors were present in the sample.

Comment

As a general rule for optimal PCR experiements we recommend to use <10% template (max 2.5 µl template in a 25 µl PCR reaction) when the GeneMole® elution volume is specified as 200 µl. Similarly we recommend to use <6% template (max 1.5 µl in a 25 µl PCR reaction) when the GeneMole® elution volume is specified as 100 µl.

Please note that patients with conical infections, leukaemia or similar might have an abnormal amount of white blood cells. Please reduce the amount of blood input (dilute blood in PBS) for these samples. For DNA extraction from blood samples of other animals please refer to www.molegenetics.com.

Conclusion

GeneMole® offers a robust and fully automated procedure for DNA extraction from whole blood. The extracted DNA is of high quality and suitable for sensitive down stream applications.

“Simplify When Possible”

Ordering Information

Product

GeneMole®

MoleStrips™ DNA Blood

MoleStrips™ DNA Blood Convenience Kit¹

Content

Instrument

64 Reagent Strips

32 Reagent Strips

Product Number

MG10-000-000

MGK20-100-102

MGK20-100-101

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