

Mole®

DNA extraction for genetic testing of familial hypercholesterolemia

“ 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™. ”

Introduction

Cholesterol is transported in the circulatory system within lipoprotein particles. One important lipoprotein particle is low density lipoprotein (LDL). High blood cholesterol concentration is associated with increased risk of coronary heart disease. LDL receptors located in the membrane of human cells capture the LDL particles and remove them from the blood stream (Figure 1A).

Familial hypercholesterolemia (FH) is a result of defective LDL receptors caused by mutations in the LDL receptor gene (Figure 1B). With defective LDL receptors fewer LDL particles are removed from the blood and abnormally high blood cholesterol levels are generated. Without treatment 50 % of affected males will experience a heart attack before they reach the age of 50 whereas 60% of affected women will experience the same before the age of 60.

Medication in combination with restricted diet is effective treatments for individuals with FH. When the cholesterol levels are reduced with treatment the risk for coronary heart disease will be reduced. Therefore it is very important that individuals with FH are diagnosed and placed on preventive treatment.

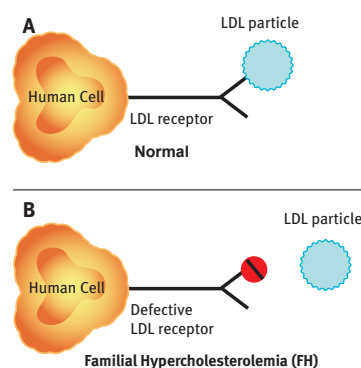


Figure 1: Familial hypercholesterolemia (FH) is caused by defective LDL-receptor.

Here the GeneMole® instrument is used to extract DNA for genetic testing of FH.

Methods

Genomic DNA was extracted from 200 µl EDTA blood according to the protocol given in the MoleStrips™ DNA Blood Kit. The GeneMole® elution volume was specified as 200 µl. Of the eluted DNA, 0.5 µl was used as input in the PCR reaction. The amplified PCR product was sequenced on an ABI 3730 DNA analyser.

Results

With an input of 200 µl blood and the elution volume specified as 200 µl, GeneMole® gave an average DNA concentration of 22 µg/ml and a A260/A280 value > 1.8. The GeneMole® extracted DNA was PCR amplified and sequenced as described under Methods. Figure 2 shows data obtained after sequencing of the GeneMole® extracted DNA.

Figure 2A shows parts of the LDL receptor sequence from a healthy individual, whereas figure 2B shows parts of the LDL receptor sequence from an individual with FH. In the sequence shown here the affected individual has a G to A mutation. This mutation changes the amino acid tryptophane to a stop codon which results in a truncated protein and FH. A zoomed in view of the relevant positions in the two sequences can be seen in figure 3. Figure 3A shows the normal sequence whereas figure 3B shows the aforementioned mutation in an individual affected with FH.

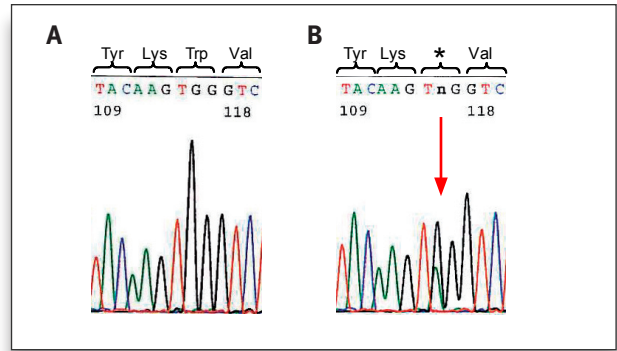


Figure 3: LDL receptor sequence in an unaffected individual A) and in an individual with FH B).

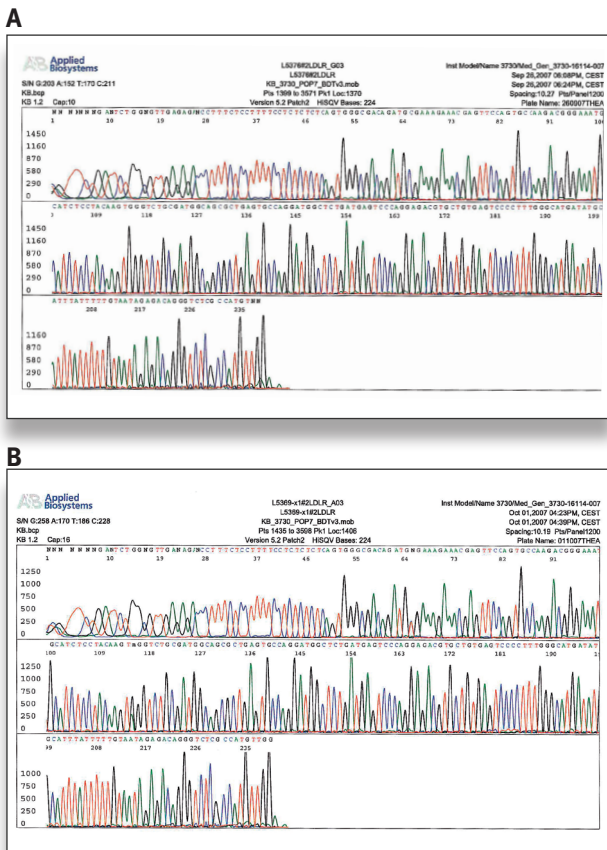


Figure 2: Sequence data obtained from DNA extracted with the GeneMole® instrument. (A) The DNA sequence from a normal healthy individual. (B) The DNA sequence from an individual with FH.

Conclusion

GeneMole® offers an automated solution for DNA extraction with the yield and purity required for applications such as PCR amplification and sequencing.

Genomic DNA extracted with the GeneMole® is free of PCR inhibitors and the PCR products are suitable for sequencing.

DNA extraction with the GeneMole® is affordable and gives you more time to focus on other tasks.

Acknowledgement

The data presented in this Application Note was kindly provided by Tove Skodje and Nina Tranum at the Department of Medical Genetics, Rikshospitalet University Hospital, Oslo, Norway.



“Simplify When Possible”

Ordering Information

Product	Content	Product Number
GeneMole®	Instrument	MG10-000-000
MoleStrips™ DNA Blood	64 Reagent Strips	MGK20-100-102
MoleStrips™ DNA Blood Convenience Kit ¹	32 Reagent Strips	MGK20-100-101

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