Mole®

DNA extraction from filter spots with dried blood

“ 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:
By using the GeneMole® instrument high quality DNA can be extracted from one single dried blood spot. Automated extraction on the GeneMole® requires minimal hands-on time.

Introduction

For blood collection and storage the use of filter papers is an attractive alternative to the use of large-volume tubes for many reasons. The establishment of a bio-bank for genetic epidemiology usually requires storage of anti-coagulated blood at -70°C. As an alternative for this, the conservation of blood samples on filter paper allows for storage of a large number of samples within a small space at room temperature.

Here it has been evaluated whether DNA isolated from dried blood spots using the GeneMole® instrument is suitable for downstream analyses such as TaqMan® genotyping.

Methods

The filter spots used were collected about 4 years ago and have been stored at room temperature.

A punched piece of filter paper (diameter about 2mm) was placed in 100 µl H_2O, vortexed and incubated at room temperature overnight. The solution from the overnight incubation was transferred into a new vessel and used as input in the GeneMole® extraction. Extraction was performed according to the protocols given in the MoleStrips™ DNA Blood kit. The GeneMole® elution volume was specified as 100 µl.

Factor V Leiden (F5, R506Q, rs6025) genotypes in all samples were determined using a custom 5´-nuclease assays (TaqMan®). Primer and probes were designed and manufactured using Applied Biosystems “Assay-by-Design” custom service (Applied Biosystems, Vienna, Austria).

PCR was performed in a Primus 96 plus thermal cycler (MWG Biotech AG, Penzberg, Germany) using a total volume of 15 µl containing 7.5 µl TaqMan® Genotyping MasterMix (Applied Biosystems, 4371355), 0.38 µl 40x Assay-mix (Applied Biosystems, 4331349), 4.12 µl H2O and 3 µl GeneMole® extracted DNA. Reactions were overlaid with 15 µl mineral oil. Cycling parameters were: 10 min at 94°C for primary denaturation, followed by 40 cycles of 20 sec. at 92°C and 1 min at 60°C.

Fluorescence was measured in a lambda Fluoro 320 Plus plate reader (MWG Biotech AG, Penzberg, Germany) using excitation/emission filters of 485/530 nm for FAM-labeled probes and 530/572 nm for VIC-labeled probes. The data was exported into Excel format and depicted and analyzed as a scatter plot.

Results

To test whether DNA extracted from dried blood spots using the Gene**Mole**® instrument is suitable for genotyping, factor V Leiden (rs6025) genotypes of 12 independent dried blood spot samples were determined using a TaqMan® genotyping assay.

The TaqMan® assay is a PCR based method and utilizes the Taq DNA polymerase's 5'nuclease activity to identify SNP's. The assay requires forward and reverse PCR primers that will amplify the region containing the SNP.

Allele discrimination is achieved using allele-specific probes that hybridize to the SNP site. The allele-specific probes have a fluorophore linked to their 5'end and a quencher molecule at their 3'end. During the PCR amplification step, if the allele specific probe is complementary to the SNP allele, the probe will bind to the target DNA strand and get degraded by the 5' nuclease activity of the Taq polymerase as it extends the DNA from the PCR primers. The degradation of the probe results in the separation of the fluorophore from the quencher molecule, generating a detectable signal. If the allele-specific probe is not perfectly complementary it will not bind as efficiently and the nuclease will be prevented from acting on the probe.

In the experiment shown here the genotypes are determined by a red (VIC) / green (FAM) relative fluorescence ratio. The scatter plot in figure 2 shows that 6 samples show high VIC dye fluorescence and therefore do not contain any mutation whereas the other 6 samples show both VIC dye and FAM dye fluorescence and therefore are heterozygous for the tested SNP.

From figure 2 we can also see that by using Gene**Mole**® extracted DNA, the TaqMan® SNP genotyping assay produced well defined clusters clearly separating the alleles from each other.

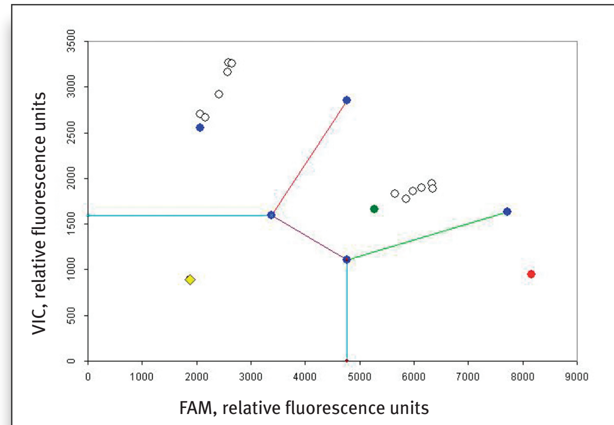

Figure 2: Scatter plot generated from TaqMan® SNP genotyping assay (SNP rs6025) of 12 different dried blood spots. The DNA used in the assay was extracted on the Gene**Mole**® instrument. Coloured circles indicate control samples with known genotypes. Blue: RR genotype; green: RQ: genotype; red: QQ genotype; yellow: no target control.

Conclusion

The DNA extracted from dried blood on the Gene**Mole**® instrument is suitable for TaqMan® genotyping methods. Well defined and tight clusters clearly separating the alleles from each other were obtained by using the Gene**Mole**® extracted DNA in the TaqMan® SNP genotyping assay. Compared to manual methods DNA extraction on the Gene**Mole**® is faster and required minimal hands-on time.

Acknowledgement

The data presented here was kindly provided by Univ. -Doz. Dr. Wilfried Renner at Labor Renner, Austria, www.labor-renner.at

Reference

TaqMan® SNP Genotyping assay Protocol from Applied Biosystems Rev.B 06/2004.

"Simplify When Possible"

Ordering Information

Product	Content	Product Number
Gene**Mole**®	Instrument	MGI10-000-000
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.