

Mole®

DNA extraction from buccal swabs

“ 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: Buccal swabs are an effective, non-invasive source of DNA. Automated DNA extraction from buccal swabs can be achieved by using the GeneMole® instrument.

Introduction

DNA collection usually requires blood extraction, which is painful for the donor and can limit the number of donors that are willing to participate in an epidemiological study or a clinical trial. Buccal swabs, containing buccal epithelial cells and white blood cells found in the mouth are an effective, non-invasive source of DNA.

Here it has been evaluated whether DNA extracted from buccal swabs using the GeneMole® instrument is suitable for downstream analysis such as TaqMan® genotyping.

Methods

Samples were collected using buccal swab brushes and stored dry for up to 2 weeks at room temperature. Swabs were placed head down in tubes containing 200 µl H₂O and twisted/stirred manually for about 10 seconds to release cells. 100 µl of this solution was 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 buccal swabs using the GeneMole® instrument is suitable for genotyping, factor V Leiden (rs6025) genotypes of 8 independent swab 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 4 samples show high VIC dye fluorescence and therefore do not contain any mutation whereas the other 4 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 GeneMole® extracted DNA, the TaqMan® SNP genotyping assay produced well defined clusters clearly separating the alleles from each other.

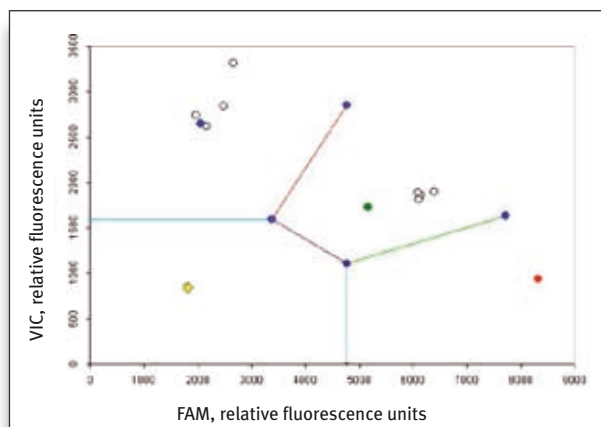


Figure 2: Scatter plot generated from TaqMan® SNP genotyping assay (SNP rs6025) of 8 different buccal swab samples. The DNA used in the assay was extracted on the GeneMole® instrument. Coloured circles indicate control samples with known genotypes. Blue: RR genotype; green: RQ genotype; red: QQ genotype; yellow: no target control.

Conclusion

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

Acknowledgement

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Reference

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

“Simplify When Possible”

Ordering Information

| Product | Content | Prod. No. |
|-----------------------|------------------------------|-----------|
| GeneMole® | Instrument | MG10-000 |
| MoleStrips™ DNA Blood | Convenience Kit ¹ | MG10-101 |
| MoleStrips™ DNA Blood | 64 Reagent Strips | MG10-102 |

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