DNA genotek

DNA from Oragene®/saliva samples† is compatible with TaqMan® SNP genotyping

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DNA collected with the Oragene® self-collection kit works well with TaqMan® SNP genotyping assays. SNPs in the thymidylate synthetase and apolipoprotein E genes were reliably detected.

Introduction

Single-nucleotide polymorphisms (SNPs) are abundant, and are estimated to occur at 1 out of every 1,000 bases in the human genome[1]. In addition to diagnostic applications, SNPs are useful as markers in population genetics and evolutionary studies[2]. The TaqMan 5' nuclease assay is a widely-used SNP genotyping technology from Applied Biosystems. The purpose of this study was to evaluate the compatibility of Oragene self-collection kits with TaqMan SNP genotyping assays.

Materials and methods

DNA collection

Saliva was collected from 25 donors using Oragene kits. DNA was purified from 200 µL aliquots of Oragene/saliva samples using the prepIT™•L2P protocol[3]. Purified DNA was redissolved in 200 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). DNA was quantified using a fluorimeter and SYBR® Green I dye (Molecular Probes) according to the F/D Protocol[4].

SNP genotyping

Validated TaqMan SNP genotyping assays were obtained from Applied Biosystems. The assays are described in Table 1. The probes were labeled with FAM or VIC dye at the 5' end, and a minor-groove binder and non-fluorescent quencher at the 3' end.

The reaction components for the allelic discrimination reactions were set up according to Table 2. Although it is recommended to use 1 to 20 ng of DNA per reaction[5], 1 µL of DNA was added to each 25 µL PCR reaction without adjusting the concentrations. SNP genotyping reactions were performed on a Rotor-Gene 3000™ real-time quantitative thermal cycler (Corbett Research) using the cycling conditions in Table 3.

Gene		TaqMan Assay ID	Ref SNP ID
Thymidylate synthetase (TYMS)	C/G	C___1637541_1_	rs2298581
Apolipoprotein E (APOE)	A/G	C___3084818_10	rs760136

***Table 1**: TaqMan probes and primers.*

Component	Volume (µL)
2× TaqMan Universal PCR Master Mix	12.5
20× TaqMan SNP Genotyping Assay Mix	1.25
DNA (varying concentrations)	1.0
H_2O	10.25
Total volume	**25.0**

***Table 2**: Reaction components.*

Step	Temperature	Time	Cycles
1	95°C	10 minutes	1
2	92°C	15 seconds	45
3	60°C	1 minute	45

***Table 3**: Cycling conditions.*

† Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER.

Results

DNA quantification

The purified DNA from 25 samples had a median concentration of 19.1 ng/µL and ranged from 1.2 to 53.4 ng/µL. Consequently, a number of the genotyping reactions used more than the 20 ng of DNA template recommended by the manufacturer.

Allelic discrimination plots

Figure 1 shows the allelic discrimination plot for the thymidylate synthetase assay and Figure 2 shows the plot for the apolipoprotein E assay. The plots are presented as the signal (average fluorescence between cycles 41 and 45) minus the background (average fluorescence between cycles 21 and 25).

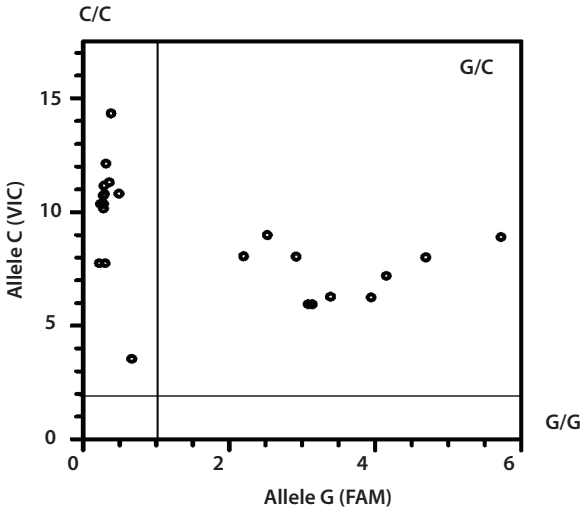

***Figure 1**: Allelic discrimination plot for the thymidylate synthetase assay.*

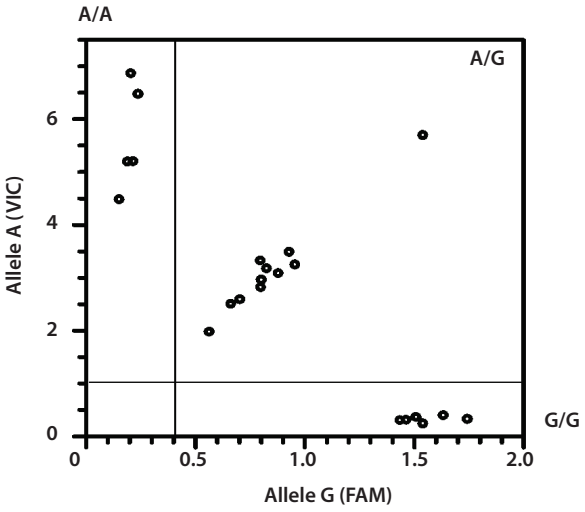

***Figure 2**: Allelic discrimination plot for the apolipoprotein E assay.*

Discussion and conclusions

TaqMan assays are widely used for SNP genotyping. As with other molecular genetic techniques, the quality and purity of DNA is important for reliable results. Our findings indicate that DNA samples collected with Oragene kits and purified using prepIT•L2P are suitable for allelic discrimination using TaqMan probes. Clear discrimination between different genotypes is evident in Figures 1 and 2.

Although it is recommended to use 1 to 20 ng of DNA per reaction, this study used DNA amounts ranging from 1.2 to 53.4 ng and all 25 samples gave clear, interpretable results. It was possible to perform the reactions without first determining the amount of DNA. Thus, the step of adjusting the DNA concentrations could be avoided. In summary, DNA collected with Oragene is suitable for SNP genotyping with TaqMan assays.

References

1 Sachidanandam, R., et al. (2001). A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature*. 409, 928–933.

2 Schork, N. J., Cardon, L. R. and Xu, X. (1998). The future of genetic epidemiology. *Trends Genet*. 14, 266–272.

3 Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.

4 DNA quantification using the Fluorescence/DNase (F/D) assay. Replaced with DNA quantification using SYBR Green I dye and a micro-plate reader. DNA Genotek. PD-PR-075.

5 June 2004. TaqMan SNP genotyping assays – protocol. Applied Biosystems. Part number 4332856, Rev. B.

Oragene®•DNA is not available for sale in the United States.

Oragene®•DISCOVER is for research use only, not for use in diagnostic procedures.

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