Application note
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Comparison of custom designed KASP and TaqMan genotyping assays for a rare genetic variant identified through resequencing GWAS loci

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Abstract

Through their unbiased approach, genome wide association studies (GWAS) have uncovered many new disease risk genes associated with complex disorders. However, identifying the functional variant directly involved in a disease is more complicated. Direct genotyping in replication case and control samples is often required to confirm a variant's association with a given disease. It is therefore important to have a reliable genotyping assay available. This note compares the effectiveness of two genotyping technologies, TaqMan™ and KASP™, for successful design of custom genotyping assays for a novel rare variant suggested to be associated with Alzheimer's disease.

Introduction

Nearly all complex diseases have a genetic component which influences an individual's disease risk. GWAS have completely changed the discovery of disease risk genes in complex disorders, allowing an unbiased approach to discovering disease-associated genetic loci (Manolio, 2010). By comparing common single nucleotide polymorphisms (SNPs) in case and control subjects, GWAS have uncovered new genes that associate with disease. This in turn has revealed new disease pathways and mechanisms.
However, the common associated GWAS SNP is rarely the disease-causing variant (Schaub *et al.*, 2012). GWAS SNPs represent genetic areas of high linkage disequilibrium which contain many SNPs that each need to be investigated for a functional role in the disease process, further complicating the identification of the actual causative variants (Schaub *et al.*, 2012). Additionally, it is thought that both common and rare variants will be responsible for GWAS associations (Bodmer & Bonilla, 2008) and complete knowledge of variation within the gene region identified by GWAS will be needed to uncover functional alleles. It has therefore become increasingly important to identify and directly genotype rare variants.

A next-generation sequencing (NGS) study was undertaken to uncover rare variants surrounding the gene loci associated with the complex neurodegenerative dementia Alzheimer's disease. Following functional annotation with *in silico* databases, several variants were prioritised for direct genotyping in case and control samples to gather further support for their potential role as the functional variant responsible for the GWAS signal.

The aim of this study was to test the effectiveness of two genotyping technologies, TaqMan and KASP, for successful design of custom genotyping assays for one of the rare deleterious missense coding variants identified in the NGS study.

The custom KASP assay successfully produced distinct genotyping clusters, and identified the Sanger-validated, heterozygous positive control sample.

Materials and methods

Assay design

The SNP of interest is a C to T change with a NGS minor allele frequency (MAF) of 2%. The SNP and 500 nucleotide base pairs (bp) sequence surrounding the SNP was prepared for submission as per the Custom TaqMan Assays Design and Ordering Guide. The sequence was annotated with known variants using 1000 genomes browser and repetitive sequence was masked using RepeatMasker before being submitted to Life Technologies Custom TaqMan Assay Design tool. The SNP passed the assay design stage and a custom non-human assay was ordered.

For the KASP assay, the SNP of interest and 100 bp surrounding sequence was annotated as for the TaqMan assay and submitted to LGC via their SNP submission template. The KASP assay was then designed using the Kraken™ software system (LGC) and validated with LGC's assay validation service. The custom KASP assay failed LGC's initial genotyping QC on 48 human samples, so a Sanger sequence-verified, heterozygous positive control sample was used to verify and optimise the assay in-house by LGC (http://www.lgcgenomics.com/kasp).

Custom genotyping

Samples were obtained from the Nottingham DNA bank and consent was obtained for all samples. DNA was extracted from control Caucasian individuals of European descent using a standard phenol-chloroform method. Two sample plates of 182 DNA samples were first genotyped using the TaqMan custom design assay and then using the custom KASP assay. Additionally, four no-template controls and a Sanger sequence-verified, heterozygous positive control were included on each reaction plate.

All TaqMan genotyping reactions were carried out in total volumes of 10 µL containing 1X TaqMan Universal PCR Master Mix, No Amperase UNG, 1X Custom SNP Genotyping Assay and 20 ng DNA. Reaction plates were cycled on a MX3000P (Agilent) at the following conditions: 50°C for 2 min, 95°C for 10 min, then 55 cycles of 95°C for 15 sec, 60°C for 10 min with a fluorescence read point at the end of the 60°C step.

KASP genotyping reactions were also carried out in 10 µL total volumes containing 1X KASP Master mix, 1X Custom KASP Assay and 20 ng DNA. Plates were cycled on a Veriti 96-Well Fast Thermal Cycler (Applied Biosystems) at the following conditions: 94°C for 15 min, then 10 cycles of 94°C for 20 sec, 61°C for 1 min (dropping by 0.6°C per cycle), then 26 cycles of 94°C for 20 sec, 55°C for 1 min followed by 9 cycles of 94°C for 20 sec and 57°C for 1 min. Fluorescent readings were taken pre- and post-cycling on a MX3000P (Agilent).

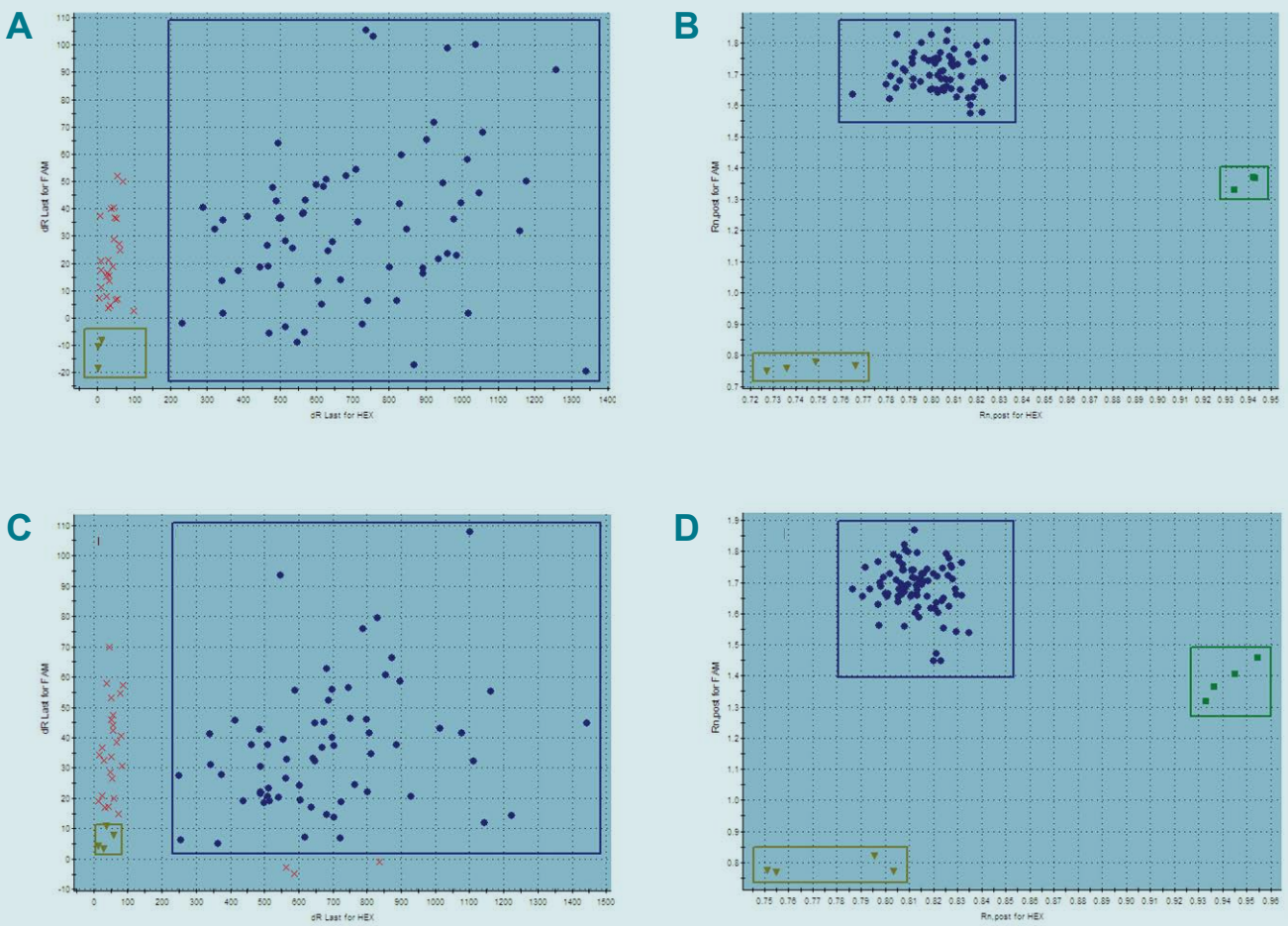

Figure 1. End point plots comparing the genotyping data for sample plate 1 using (A) TaqMan and (B) KASP and sample plate 2 (C) TaqMan and (D) KASP. Numerous fails were reported for the TaqMan assays (red crosses) and the positive control was not correctly called on either of the plates. The yellow triangles relate to the four no-template control samples that were run alongside the DNA samples.

Results

The TaqMan assay failed to successfully genotype the Sanger-validated, heterozygous positive control sample which was included on both sample plates (Figure 1A and 1C). In addition, numerous samples failed to successfully amplify and were not assigned a genotype with the TaqMan assay. The same sample plates that were genotyped using the custom KASP assay successfully produced distinct genotyping clusters, and identified the Sanger-validated, heterozygous positive control sample in the same cluster as several other heterozygous samples on each plate (green squares, Figure 1B and 1D).

Conclusions

Direct genotyping of rare variants is an important follow up to perform on the genetic loci identified in GWAS studies. Many of the associated rare variants identified in a resequencing study will be novel. It is therefore essential to use a direct genotyping method which produces reliable genotyping from custom sequence submissions.

Despite passing initial design checks in the TaqMan Assay Design process, the TaqMan assay was unable to amplify known heterozygous samples. Additionally, numerous samples failed to amplify indicating that the assay was not working well for all DNA samples.

The custom KASP assay did successfully pass the LGC assay validation as a known positive control sample could be run alongside the 48 human DNA samples used to validate the assay in-house. Although the assay is not specifically designed to run on an MX3000P (Agilent), the assay worked well on this machine and tight genotyping clusters were produced (Figure 1).

References

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Key words: KASP, GWAS, TaqMan, Alzheimer's disease, Mx3000P, genotyping.