TaqMan Drug Metabolism Genotyping Assays for basic and clinical research

- Polymorphisms targeted in 221 genes encoding drug metabolism enzymes and associated transport proteins
- Versatility to detect SNPs, insertion/deletions (indels), and multinucleotide polymorphisms (MNPs)
- Inventoried assays with proven performance for quick turnaround
- Performance tested on 180 unique DNA samples from four human populations
- Aligned with allele nomenclature from public allele nomenclature sites

Introduction

Thermo Fisher Scientific offers 2,700 unique Applied Biosystems™ TaqMan™ Drug Metabolism Genotyping Assays for detecting polymorphisms in 221 genes that code for various drug metabolism enzymes (DMEs) and associated transport proteins. Polymorphisms associated with these genes may influence the rate of drug metabolism within individuals, thus potentially affecting drug efficacy and the occurrence of side effects. The complex nature of these genes had limited research because few technologies and products could effectively characterize these polymorphisms. All of the assays in this collection target potentially causative polymorphisms, including those within regulatory elements, coding regions, and associated splice junctions.

TaqMan Drug Metabolism Genotyping Assays were developed using advanced bioinformatics and wet lab stringency. The assays were designed with information from several public SNP databases, including recognized public allele nomenclature sites. All assays passed performance tests involving 180 unique DNA samples from four different populations. These assays can be used with all other Applied Biosystems™ TaqMan™ SNP Genotyping Assays and real-time PCR systems.

Genes relevant for drug metabolism

Genes that code for DMEs are important targets for study in both drug development and clinical research, as they influence individual drug (or xenobiotic) response.

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Some examples of these important genes include:

- ***CYP2D6*:** One of the genes within the cytochrome P450 family, it encodes an enzyme believed to be responsible for metabolizing 20–25% of antiarrhythmics, antidepressants, antipsychotics, beta-blockers, and analgesics.
- ***NAT1* and *NAT2* (N-acetyltransferase):** Enzymes from these genes metabolize a variety of carcinogenic compounds in addition to isoniazid, which is the first-line treatment for tuberculosis.
- ***MDR1* (also known as *ABCB1* and P-glycoprotein 1):** A multidrug resistance gene that is representative of the drug transport genes. Their encoded enzymes are involved in the transport of metabolites across the cellular membrane.

For a complete list of genes, visit **thermofisher.com/taqmandme**

Identifying and mapping polymorphisms

Polymorphisms within the DME collection include single-nucleotide polymorphisms (SNPs), insertion/deletions (indels), and multinucleotide polymorphisms (MNPs). These polymorphisms were identified from three sources: public data, including dbSNP, HGMD, and public allele nomenclature sites; collaborations within the pharmaceutical industry; and SNP databases proprietary to Thermo Fisher. Initially, all polymorphisms were identified for the 221 genes, and the set was then filtered to include only polymorphisms within regulatory elements, coding regions, and splice junctions. Public allele nomenclature sites were used to help assign common "allele" names to specific polymorphisms in the DME collection. Available common allele names are included in a free downloadable file that contains the polymorphism and refSNP number from dbSNP (if available).

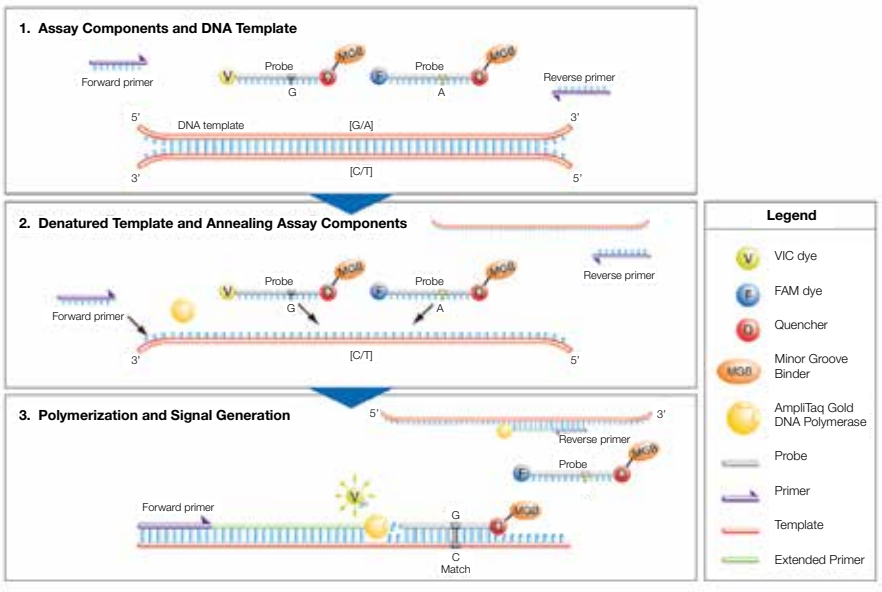

Figure 1. TaqMan Genotyping Assay steps. (1) The four TaqMan Genotyping Assay components and the target DNA template with the SNP (in brackets). (2) The denatured DNA target and annealing of the assay components. (3) Signal generation leading to specific allele detection.

Choosing TaqMan Genotyping Assay technology

TaqMan™ allelic discrimination technology was chosen for our TaqMan™ Genotyping Assays because it is robust, highly reproducible, and easy to use (Figure 1). Each assay contains two allele-specific probes and a primer pair to detect the specific SNP target. Both the probes and primers uniquely align within the genome, enabling the TaqMan Genotyping Assay technology to provide unmatched specificity. It is this specificity that allows these assays to detect targets residing in highly homologous gene families that may include pseudogenes. All assay designs are analyzed against the genome, using BLAST, for unique alignment.

All assays in this collection are run under the same instrument conditions, eliminating the need to optimize assay performance. Thermal cycling parameters unique for these assays involve 50 cycles with a 90-second extension time. All other instrument parameters are identical to those used for our TaqMan SNP Genotyping Assays.

Proven performance

Each assay within this collection is initially run in duplicate using 3 ng samples of DNA from 45 African-Americans and 45 Caucasians. If the assay demonstrates good performance, it is run with an additional 45 Chinese and 45 Japanese DNA samples (Figures 2 and 3). All assays are evaluated for reproducibility, strong signal intensity, and robust genotype calls.

Product flexibility

TaqMan Drug Metabolism Genotyping Assays can be ordered and used separately or as a set. Simply select the number of assays required to meet your project needs—one assay, or all assays available for one gene or a collection of genes. Individual assays can be ordered online at **thermofisher.com/taqmandme**. You can also find predefined TaqMan Drug Metabolism Genotyping Assay Sets for PharmaADME core markers and other specific research areas, at **thermofisher.com/dmesets**.

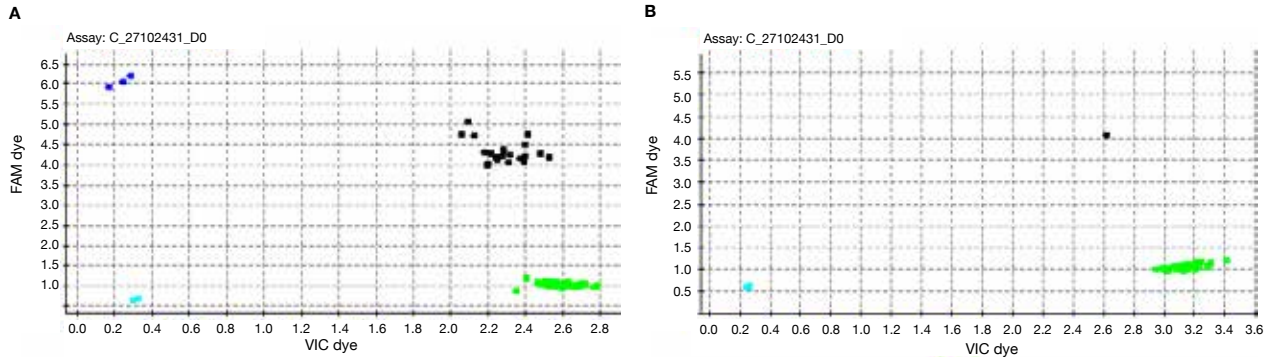

Figure 2. Allelic discrimination plots for the C__27102431_D0 assay run on (A) 45 African-American and Caucasian gDNA samples each, and (B) 45 Chinese and Japanese gDNA samples each. C__27102431_D0 targets the *CYP2D6*4* g.1846G>A polymorphism, which encodes an mRNA splicing defect that results in a nonfunctional CYP2D6 protein. If an individual carries two nonfunctional *CYP2D6* alleles, they will have the poor metabolizer (PM) phenotype and the metabolism of numerous drugs will be impacted.

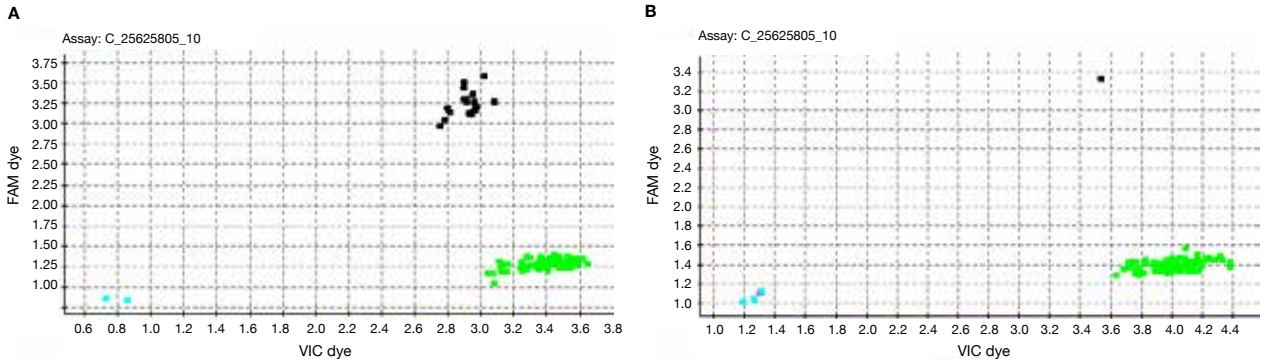

Figure 3. Allelic discrimination plots for the C__25625805_10 assay run on (A) 45 African-American and Caucasian gDNA samples each, and (B) 45 Chinese and Japanese gDNA samples each. C__25625805_10 targets the *CYP2C9*2* c.430C>T, g.3608C>T polymorphism, which encodes a missense mutation (R144C) in the CYP2C9 protein. Common genetic variations in *CYP2C9* and *VKORC1* genes significantly impact an individual's senstivity to warfarin and dosage requirements. The major allele C of the *CYP2C9*2* g.3608C>T SNP is associated with warfarin insensitivity, whereas the minor allele T is associated with decreased enzyme activity and sensitivity to warfarin.

DME Assay Index

A DME Assay Index is also available with all drug metabolism assays. This file lists each assay along with context sequence, location on the NCBI assembly, the refSNP number (from dbSNP), and the common allele nomenclature mapped from a public allele nomenclature site, when available.

Additionally, the gene name (and various aliases), SNP type, amino acid change, and observed minor allele frequencies are provided in this file.

The complete DME Assay Index can be downloaded at **thermofisher.com/taqmandme**, and is also included on the CD that ships with each assay order.

Quick delivery, convenient format

For fast delivery, all assays in this collection have been manufactured and placed into inventory and are ready to ship at ambient temperature. Like other TaqMan SNP Genotyping Assays, these single-tube products consist of two allele-specific TaqMan™ MGB probes (labeled with either VIC™ or 6-FAM™ dye) and two gene-specific primers. Additionally, all products are formulated and packaged in the small-scale size: a 20X, single-tube formulation, supporting 750 reactions at 5 µL per reaction.

Reliable real-time PCR platforms

Thermo Fisher offers a suite of superior Applied Biosystems™ instrument platforms for processing and analyzing TaqMan SNP Genotyping Assays. These instruments, which meet all throughput needs and budgets, include the ProFlex™ PCR System, SimpliAmp™ and Veriti™ Thermal Cyclers, the StepOne™, StepOnePlus™, 7500, and 7500 Fast real-time PCR systems, as well as the QuantStudio™ 3, 5, 6, 7, and 12K Flex real-time PCR systems. Following PCR amplification, an endpoint read can be performed on any Applied Biosystems real-time PCR system. All of these dependable instruments offer the advanced multicolor detection capabilities required for highly accurate and reproducible allelic discrimination assays.

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Features and benefits at a glance

Feature	Benefit
Target 2,700 high-value polymorphisms located in either the regulatory elements or coding regions of 221 drug metabolism genes	Ability to study polymorphisms thought to be associated with metabolism rates for specific drugs, and polymorphisms that have been difficult to study
Assays can be selected individually	Choose the assays that meet your project needs; you are not locked into a fixed set, making it easier to perform follow-up studies as the project evolves
Can detect multiple types of polymorphisms	TaqMan Assay chemistry allows you to study SNPs, indels, and MNPs all on one platform
This assay collection uses an enhanced development pipeline to produce assays that can be used on existing real-time PCR platforms	Seamless integration of new assays with currently available assays; all assays have been shown to perform well (Note: 50 cycles and 90-second extension times needed—all other conditions identical)
Advanced bioinformatics to map public allele information to specific polymorphisms and physical genome locations	Helps save time and effort in identifying the assays you are looking for—no need to design your own
Advanced bioinformatics to overcome homology issues and enable accurate detection of specific polymorphisms	Confidence in studying the right SNP to generate the correct result

Ordering information

Product	Size	Cat. No.
TaqMan Drug Metabolism Genotyping Assays	Small-scale	4362691
Includes CD with protocol, Assay Information File (AIF), DME Assay Index, and troubleshooting guide		

TaqMan Drug Metabolism Genotyping Assays are available through the website at thermofisher.com/taqmandme

For more information and full terms of the TaqMan™ Assays qPCR Guarantee, visit **thermofisher.com/taqmanguarantee**

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