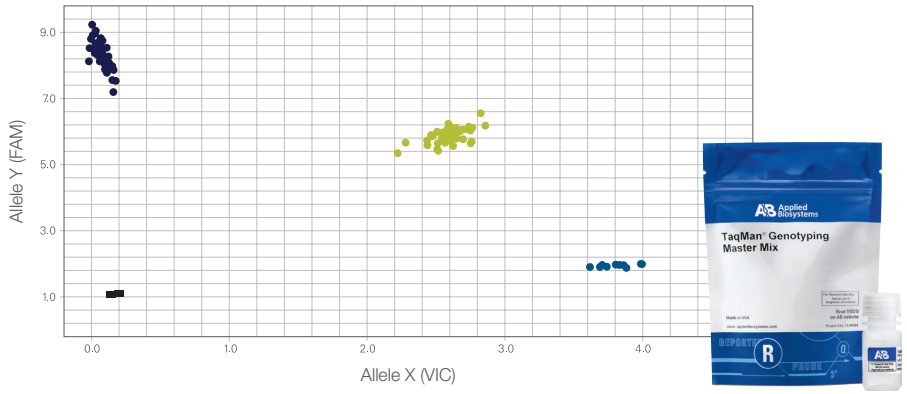
TaqMan Genotyping Master Mix

Real-time PCR master mix tailored for SNP genotyping studies

Tailored for unrivaled cluster resolution for unambiguous single-nucleotide polymorphism (SNP) allelic discrimination, the Applied Biosystems™ TaqMan™ Genotyping Master Mix is optimized for genotyping applications, including:

- Candidate gene studies
- Drug target validation
- Disease association studies
- Population genetics
- Linkage mapping
- Agricultural applications
- Copy number variation analysis



Introduction

TaqMan Genotyping Master Mix is designed to deliver reliable, cost-effective SNP detection for accurate and reproducible allelic discrimination. The master mix optimizes the preferential binding of the allele-specific probe, providing exceptional separation and clustering of alleles and consistently strong fluorescent signals. Powered with the highly purified Applied Biosystems™ AmpliTaq Gold™ DNA Polymerase, UP (Ultra Pure), TaqMan Genotyping Master Mix can replace Applied Biosystems™ TaqMan™ Universal PCR Master Mix in existing SNP genotyping protocols using the same reaction setup and thermal cycling conditions.

Benefits

- Specifically formulated for endpoint fluorescent detection of SNPs and insertions/deletions
- Discrete clusters and high call rates for accurate and reproducible allelic discrimination
- Reliable discrimination of SNPs in difficult targets
- Excellent room-temperature stability for flexible pre- and post-PCR setup and analysis
- Universal thermal cycling conditions for consistent results
- Validated for use with Applied Biosystems™ TaqMan™ SNP Genotyping Assays, TaqMan™ Copy Number Assays, and TaqMan™ Mutation Detection Assays

Optimized formulation for exceptional performance

TaqMan Genotyping Master Mix is a convenient 2X mix for TaqMan probe–based genotyping reactions. It includes the following components:

- AmpliTaq Gold DNA Polymerase, UP (Ultra Pure), a highly purified DNA polymerase. This hot-start enzyme is inactive at room temperature, so reactions can be set up on the benchtop. The enzyme is activated during thermal cycling.
- Optimized components including buffer and dNTPs for consistent, reliable genotypes
- Passive internal reference based on proprietary ROX™ dye for precise data analysis

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Setting a new standard for allelic discrimination

For clear genotyping results, each allele-specific TaqMan™ probe must yield bright and consistent fluorescent signals to provide discrete clusters that are widely separated, indicating excellent specificity. The performance of TaqMan Genotyping Master Mix was tested using 3 ng samples of human genomic DNA (gDNA) and a validated SNP assay to genotype dbSNP rs2293052 in the gene *NOS1*. The resulting cluster plot (Figure 1) shows strong fluorescent signals for each allele and clear separation between the three clusters—easily discriminating the two homozygous and one heterozygous genotypes. In a comparison against five commercially available mixes, TaqMan Genotyping Master Mix shows the highest average call rate (Figure 2). Tight, well-separated clusters for each genotype provide exceptional call rates and, most importantly, accurate and efficient SNP analysis.

Consistent performance—even with difficult templates

TaqMan Genotyping Master Mix offers unambiguous allelic discrimination even for the most challenging assays. For example, GC-rich targets can present amplification challenges that reduce SNP detection because of persistent secondary structure. Human gDNA samples were genotyped for a SNP in a GC-rich region using a TaqMan SNP Assay to genotype dbSNP rs12214 in the cathepsin D gene. As shown in Figure 3, TaqMan Genotyping Master Mix yields brighter fluorescent signals, tighter clusters, and more accurate allele calling compared to a mix from supplier "S". These data demonstrate that TaqMan Genotyping Master Mix provides higher call rates for reliable SNP genotyping in difficult targets, eliminating the need to retest uncalled samples.

Copy number variation applications

Copy number variation is an important polymorphism in the human genome that can be associated with certain genomic disorders as well as some simple genetic and complex diseases. TaqMan Genotyping Master Mix, used with TaqMan Copy Number Assays, provides relative quantitation of an experimental gene compared to a reference gene in a duplex PCR. Between 1 and 3 copies of *CYP2D6, the* gene for a drug-metabolizing enzyme, were detected for 92 human gDNA samples when the samples were amplified using TaqMan Genotyping Master Mix (Figure 4).

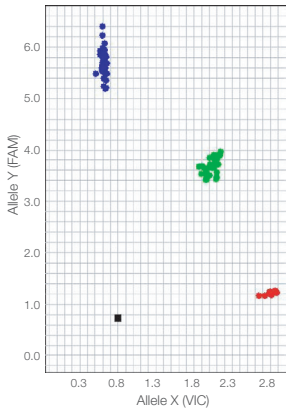

Figure 1. TaqMan Genotyping Master Mix provides bright fluorescence signals for discrete, well-separated allelic clusters. Cluster plot of 94 gDNA samples and two no-template controls genotyped using Applied Biosystems™ TaqMan™ SNP Genotyping Assay C__15969983_10, with PCR performed on the Applied Biosystems™ GeneAmp™ PCR System 9700 and allelic discrimination on the 7900HT Fast Real-Time PCR System.

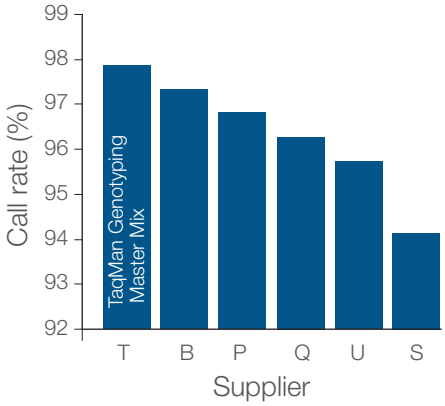

Figure 2. TaqMan Genotyping Master Mix provides the highest SNP call rates, outperforming other master mixes. Average call rates for 94 gDNA samples and two no-template controls genotyped using Applied Biosystems™ TaqMan™ SNP Genotyping Assay C__27102425_10, with PCR performed on the GeneAmp PCR System 9700 and allelic discrimination on the 7900HT Fast Real-Time PCR System.

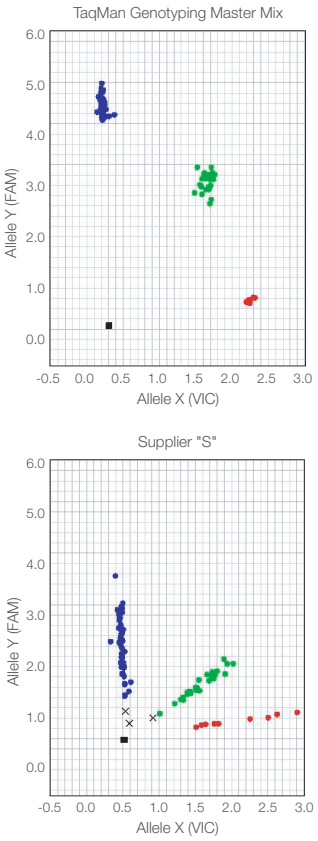

Figure 3. Consistent, reliable SNP detection in a GC-rich region using TaqMan Genotyping Master Mix. Genotyping assays were compared using TaqMan Genotyping Master Mix and a PCR master mix from supplier "S" on a set of 94 human gDNA samples (3 ng) and two no-template controls, using Applied Biosystems™ TaqMan™ SNP Assay C__12050942_10. PCR was performed on the GeneAmp PCR System 9700 and allelic discrimination on the 7900HT Fast Real-Time PCR System.

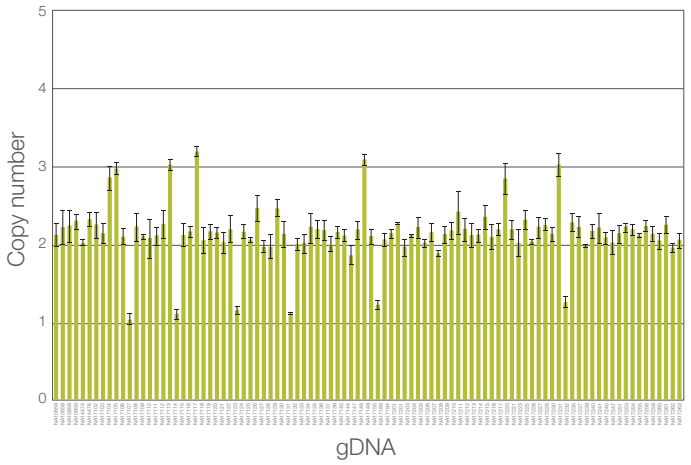

Figure 4. TaqMan Genotyping Master Mix is used for amplification in TaqMan Copy Number Assays. TaqMan Genotyping Master Mix is used with a TaqMan Copy Number Assay designed to target the *CYP2D6* gene, determining the copy number for this target in 92 gDNA samples. The RNase P reference gene is present in two copies per diploid genome or one copy per haploid genome.

Somatic mutation detection applications

TaqMan Mutation Detection Assays can detect somatic mutations in genes that are associated with cancer from different sample types, such as cell lines, formalin-fixed, paraffin-embedded tissue samples, and fresh frozen tissue samples. TaqMan Genotyping Master Mix, combined with TaqMan Mutation Detection Assays, which use competitive allele-specific Applied Biosystems™ TaqMan™ PCR (castPCR™) technology, can help detect rare amounts of mutated DNA in a sample that contains large amounts of normal, wild type DNA.

Pre- and post-PCR stability

Benchtop stability of real-time PCR mixes provides the flexibility to perform experiments over multiple days. To demonstrate the stability of TaqMan Genotyping Master Mix, both pre- and post-PCR storage conditions were tested to determine the effects on genotyping data. To evaluate pre-PCR stability, reactions were set up at room temperature (24°C), stored in the dark for up to three days, thermal-cycled for PCR, and read for endpoint fluorescence to assign alleles. To assess post-PCR stability, PCR was conducted immediately after reaction setup, but reactions were left on the bench for up to three days before measuring endpoint fluorescence for allelic discrimination. Even after three days at room temperature, either before or after PCR, TaqMan Genotyping Master Mix yielded tight clusters and reproducible results (Figure 5). The excellent benchtop stability of TaqMan Genotyping Master Mix gives ample flexibility for experimental setup and sample processing.

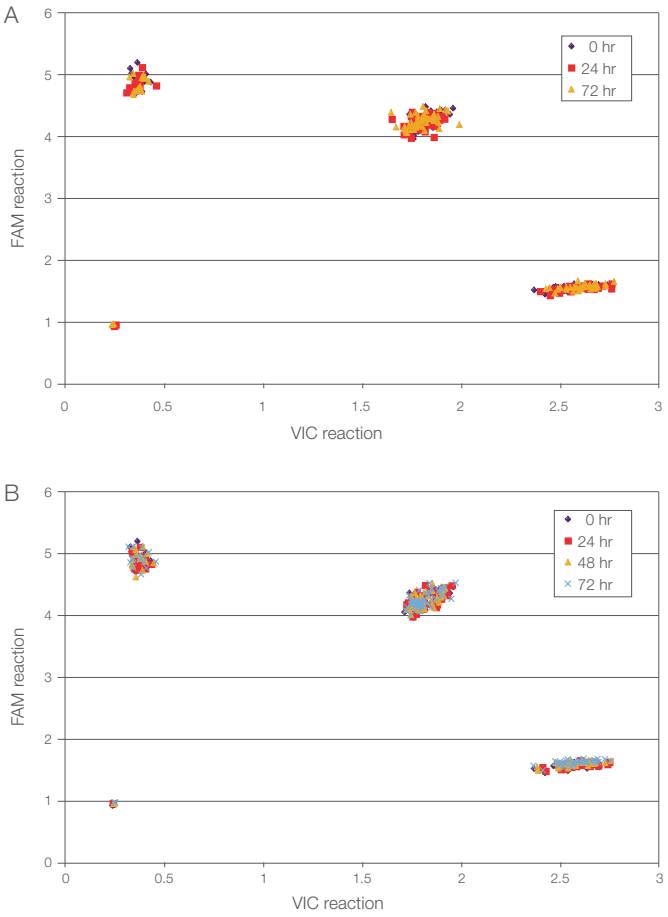

Figure 5. TaqMan Genotyping Master Mix provides pre- and post-PCR stability for up to 3 days. Genotyping reactions were set up using 94 gDNA samples and two no-template controls with TaqMan Genotyping Master Mix and TaqMan SNP Assay C___2188620_10. Reactions were left on the bench either **(A)** before or **(B)** after thermal cycling for the indicated amounts of time. PCR was conducted on the GeneAmp PCR System 9700 and allelic discrimination on the 7900HT Fast Real-Time PCR System.

Conclusion

TaqMan Genotyping Master Mix:

- Demonstrates extremely reliable allelic discrimination for SNP genotyping, with discrete clusters for high call rates even with challenging targets
- Provides reliable quantitation of DNA copy number when used with TaqMan Copy Number Assays
- Offers robust benchtop stability at room temperature, pre- and post-PCR, and consistent results across multiple instruments over multiple days to meet all throughput needs
- Complements TaqMan Mutation Detection Assays to provide high specificity and sensitivity for mutant allele detection

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Instruments and assays compatible with TaqMan Genotyping Master Mix (standard thermal cycling mode)

Instruments and assays
QuantStudio 3/5/6/7/12 Real-Time PCR Systems
Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems
Applied Biosystems™ 7000, 7300, 7500, 7500 Fast, and 7900HT Fast Real-Time PCR Systems
Applied Biosystems™ Veriti™ Thermal Cyclers
GeneAmp PCR System 9700
Applied Biosystems™ 9800 Fast Thermal Cycler
TaqMan SNP Genotyping Assays
TaqMan Drug Metabolism Genotyping Assays
TaqMan Copy Number Assays
TaqMan Mutation Detection Assays
Applied Biosystems™ Custom TaqMan™ SNP Genotyping Assays
21 CFR Part 11 compliance module

Ordering information

Product	Unit size	Reactions*	Cat. No.
TaqMan Genotyping Master Mix			
Mini pack	1 mL tube	40	4371353
1-pack	10 mL bottle	400	4371355
2-pack	2 x 10 mL bottles	800	4381656
Single bulk pack	50 mL bottle	2,000	4371357
Multi-bulk pack	2 x 50 mL bottles	4,000	4381657
Quick Reference Card	1 card	—	4371130
Protocol	1 protocol	—	4371131

* Assumes 50 µL reaction volume; consult protocol for other recommended reaction volumes.

Find out more at **thermofisher.com/taqmanmm**

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