

TaqMan® SNP Genotyping Assays

TaqMan® SNP Genotyping Assays from Life Technologies provide a highly flexible technology for detection of polymorphisms within any genome. TaqMan® Assays have the simplest workflow available and are the quickest way to generate genotyping data. Based on powerful TaqMan® probe and primer chemistry and designs, and coupled to dependable Applied Biosystems® instruments and software, these made-to-order assays produce high-confidence results. TaqMan® Assays are ideal for genotyping applications including screening, association, candidate region, candidate gene, and fine-mapping studies.

Content-rich marker selection tools simplify study design and help you select from a library of human and mouse assays. This library includes over 4.5 million genome-wide human assays (of which 3.5 million are HapMap SNP-based assays, 160,000 are validated assays, and over 70,000 are coding region assays) and 10,000 mouse assays. We also offer 2,700 inventoried drug metabolism genotyping assays. Additionally, with Custom TaqMan® SNP Genotyping Assays you can confidentially submit target SNP sequences for any genome to create your own assays. Let TaqMan® SNP Genotyping Assays accelerate the pace of your discovery by eliminating time-consuming experimental design and optimization.

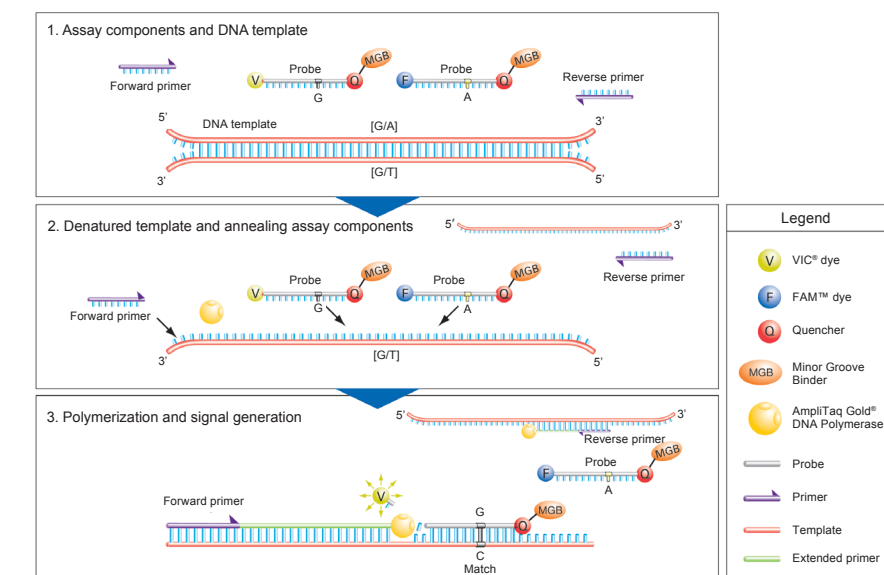


Figure 1. Allelic discrimination is achieved by the selective annealing of TaqMan® MGB probes.

Powerful, proven chemistry

Whether your genotyping studies require targeted detection of essential SNPs, or the flexibility to choose SNPs for mapping, TaqMan® SNP Genotyping Assays are the technology of choice. Proven TaqMan® probes, which incorporate minor groove binder (MGB) technology at the 3' end, deliver superior allelic discrimination. The MGB molecule binds to the minor groove of the DNA helix, improving hybridization-based assays by stabilizing the MGB probe-template complex. This increased binding stability permits the use of probes as short as 13 bases for improved mismatch discrimination and greater flexibility when

designing assays for difficult or variable sequences. In addition to SNP detection, TaqMan® probes can be designed to detect multiple nucleotide polymorphisms (MNPs) and insertion/deletions (indels).

Detection is achieved with proven 5' nuclease chemistry by means of exonuclease cleavage of an allele-specific 5' dye label, which generates the permanent assay signal (Figure 1). All MGB probes include a nonfluorescent quencher (NFQ) that virtually eliminates the background fluorescence associated with traditional quenchers and provides a greater signal-to-noise ratio for superior assay sensitivity.

TaqMan® SNP Genotyping Assays collection

TaqMan® SNP Genotyping Assays are the world's largest collection of single-tube, ready-to-use SNP assays available. The TaqMan® SNP Genotyping Assays library consists of two collections of human assays and one of mouse assays, and can be supplemented with assays designed using our Custom TaqMan® SNP Genotyping Assays Service.

Over 4.5 million human SNP genotyping assays

This assay group contains over 4.5 million genome-wide SNPs, providing unprecedented marker coverage. Included in this collection are 160,000 validated assays that have approximately 10 kb spacing across gene regions. These assays were subjected to an extensive minor allele frequency test in 2–4 ethnic populations (45 individual samples per ethnic group) and, as a result, offer the highest success rate. Also included are over 70,000 assays for the detection of nonsynonymous SNPs in coding regions, including many putative functional SNPs. Visit www.lifetechnologies.com/taqmansnp for more information.

Over 10,000 mouse SNP genotyping assays

The Mouse TaqMan® Pre-designed SNP Genotyping Assays collection consists of over 10,000 assays, and can be supplemented with assays designed using our Custom TaqMan® SNP Genotyping Assays Service.

TaqMan® Drug Metabolism Genotyping Assays

The collection of TaqMan® Drug Metabolism Genotyping Assays includes 2,700 assays that target high-value polymorphisms in 221 drug metabolism genes. These assays have proven performance in four different ethnic population samples, consisting of 45 individuals each. To enable easy identification, these assays have been mapped to the common public allele nomenclature websites where possible. Visit www.lifetechnologies.com/taqmandme for more information.

All TaqMan® SNP Genotyping Assays are generated using next-generation algorithms from the Life Technologies bioinformatics pipeline. For all pre-designed assays, bioinformatics evaluation of target SNP sequences includes the masking of adjacent SNPs and ambiguous bases so that assay

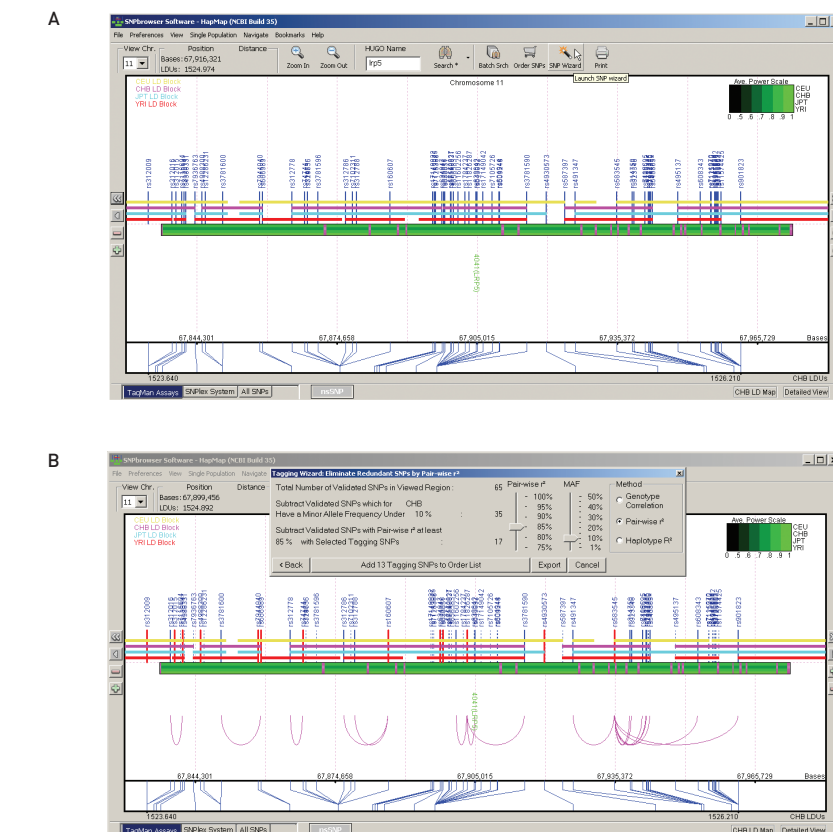


Figure 2. Identifying optimal subsets of SNPs with the SNP Wizard. (A) Search and visualize your gene of interest, and then activate the SNP Wizard. **(B)** Choose a tagging SNP method, and set parameters to reduce the number of SNPs needed to represent common haplotypes. A summary panel appears that displays algorithm results and allows you to easily adjust your parameter selection. Selected tagging SNPs are shown in red on the graphical map.

design and subsequent performance is not affected by the poor quality of the underlying sequence. Lastly, the assay designs are aligned to the human genome using BLAST to ensure that each assay binds uniquely to the intended polymorphism. As the Custom TaqMan® SNP Genotyping Assay Service is confidential and secure, you simply perform your own bioinformatics analysis prior to submitting your sequence for assay design.

Free marker selection tools

SNPbrowser™ Software for human SNPs

SNPbrowser™ Software for human SNPs simplifies study design by facilitating easy and intuitive selection of the optimal SNP assay set for each project. You can download SNPbrowser™ Software for free at www.lifetechnologies.com/snpbrowser.

SNPbrowser™ Software provides a physical map view of the human genome. Two different genotype data sources are incorporated into the software to generate unique linkage disequilibrium (LD) maps and haplotype block information. These two sources are the public HapMap Project and the >20 million genotypes generated during the development of the 160,000 TaqMan® Validated SNP Genotyping Assays.

The genotyping data also facilitate the use of tagging SNP methods, which allow more affordable study design by reducing the number of SNPs while conserving study statistical power (Figure 2).

SNPbrowser™ Software allows you to optimize study design based on LD patterns, minor allele frequency requirements, total

number of cases and controls, and statistical power. Finally, the software includes a SNP density tool that prioritizes the selection of validated SNPs while supplementing with additional SNPs to fulfill the marker density requirements for your study.

When you have identified your SNPs of interest, order TaqMan® SNP Genotyping Assays by directly uploading Assay IDs from the software to our website at www.lifetechnologies.com/taqmansnp.

Mouse SNPbrowser™ Software

Mouse SNPbrowser™ Software enables efficient and easy selection of appropriate Mouse TaqMan® SNP Genotyping Assays to discriminate between strains of interest, especially for genotype/phenotype mapping and strain verification. It visualizes informative SNPs that distinguish two selected mouse strains at a user-definable resolution. The collection consists of ~10,000 mouse SNPs from 44 of the most common strains.

Mouse SNPbrowser™ Software supports genetic monitoring, genetic mapping, and speed congenics applications by offering a variety of tools to search, view, and export SNP information, as well as purchase assays. The software displays the mouse chromosome map so you can view SNP location and obtain National Center for Biotechnology Information (NCBI) data for SNPs of interest. The software allows SNP selection for two applications: genetic monitoring and genetic mapping.

For genetic monitoring, the software selects optimal SNPs to distinguish between any number of strains. SNPs can be selected for

each chromosome or across the genome. For genetic mapping, the software segments each chromosome into evenly sized blocks based on a user-specified mapping resolution, and searches for distinguishing SNPs in each block to generate evenly spaced SNP coverage throughout the genome. Mouse SNPbrowser™ Software also features a SNP Wizard to assist in defining search criteria. A help text file is provided within the tool to familiarize you with the extensive functionality offered. You can download Mouse SNPbrowser™ Software for free at www.lifetechnologies.com/mousesnpbrowser.

Custom assay service for any possible SNP

Custom TaqMan® SNP Genotyping Assays can be developed for any SNP in any organism. This service can generate assays for the detection of SNPs, MNPs, or indels of up to 6 bases.

Custom TaqMan® SNP Genotyping Assays provide you with a complete service that includes secure and confidential ordering, assay design and manufacturing, and quality-control testing for synthesis accuracy and formulation completeness. Additionally, custom human assays are subjected to a functional test on 20 unique DNA samples.

Use the free Custom TaqMan® Assay Design Tool to input and submit your sequence for assay design. This easy-to-use online resource lets you quickly submit your sequence information and start the ordering process securely and confidentially. Access the Custom TaqMan® Assay Design Tool at www.lifetechnologies.com/snpcaadt.

Quality design and manufacturing

Probes and primers used in TaqMan® SNP Genotyping Assays are designed using our rigorous bioinformatics pipeline. This proprietary group of algorithms has generated millions of TaqMan® Assay designs by utilizing heuristic design rules deduced from both manufacturing and assay performance data. All assays are designed to perform under universal reaction conditions, as calculated probe and primer melting temperatures are consistent and include contributions from associated probe conjugates (i.e., dyes and MGB).

After manufacturing, assay components undergo extensive laboratory testing at our state-of-the-art manufacturing facility. Quality-control testing includes mass spectrometry for sequence verification and formulation assessments of probe and primer concentrations. Additionally, all human SNP genotyping assays are functionally tested to ensure allelic discrimination.

Simple workflow for quick results

TaqMan® SNP Genotyping Assays constitute the simplest SNP genotyping technology available. We deliver your ready-to-use SNP genotyping assay at ambient temperature in a convenient, single-tube format. The rest is easy. Just combine the assay with TaqMan® Genotyping Master Mix or TaqMan® Universal PCR Master Mix and your purified DNA sample (Figure 3). There is no need to optimize probe, primer, salt concentrations, or temperature because all assays use universal reagent concentrations and thermal cycling conditions.

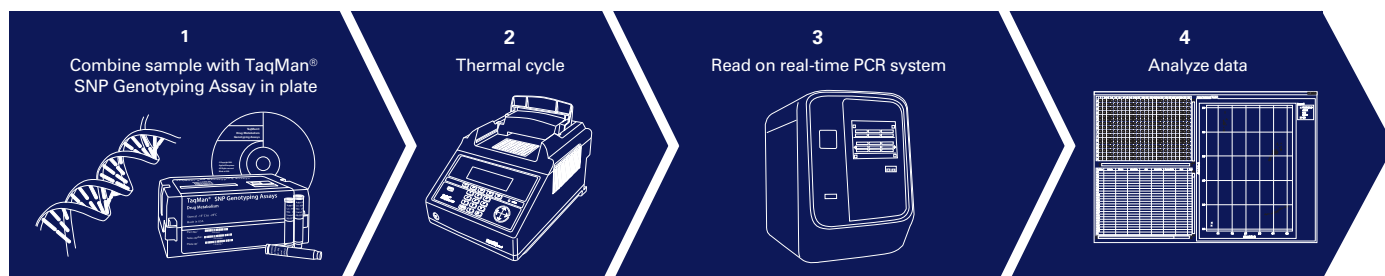


Figure 3. A simple workflow and reliable instruments combine to generate fast, high-confidence results.

After generating an endpoint read using a thermal cycler or real-time PCR instrument, no transfers, washes, or additional reagents are required, and the plate remains sealed; just read the plate and analyze the genotypes (Figure 4). This reduces the chance of contamination, sample mix-up, and sample loss. The simplicity of the chemistry allows you to easily automate the reaction for massively parallel genotyping studies, readily increasing the number of assays, number of samples, or both. Additionally, the analysis software allows you to auto-call genotypes, minimizing manual intervention.

Reliable real-time PCR platforms

A suite of superior Applied Biosystems® instrument platforms is available for processing and analyzing TaqMan® SNP Genotyping Assays (Table 1). These instruments, which meet all throughput needs and budgets, include the GeneAmp® PCR System 9700 and Veriti® Thermal Cyclers for endpoint PCR, 7500, 7500 Fast, 7900HT Fast, ViiA™ 7 (Figure 5), StepOne®, and StepOnePlus® Real-Time PCR Systems, and the high-throughput QuantStudio™ 12K Flex Real-Time PCR System (Figure 5). 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.

Data analysis software

The sophisticated SDS software package provided with all Applied Biosystems® real-time PCR systems facilitates experimental setup, data collection, and assay performance analysis. The SDS software uses an advanced multicomponent algorithm to calculate the distinct signal contribution of each allele of a marker from the fluorescence measurements of each sample well during the assay plate read. The multicomponent data collected from the plate read are stored as SDS files, ready for genotype determination by the SDS software or optional TaqMan® Genotyper Software.

TaqMan® Genotyper Software is a great resource for fast and accurate genotype calling. It is a free SNP genotyping data analysis tool for use with TaqMan® SNP Genotyping Assays performed in 48-, 96-,

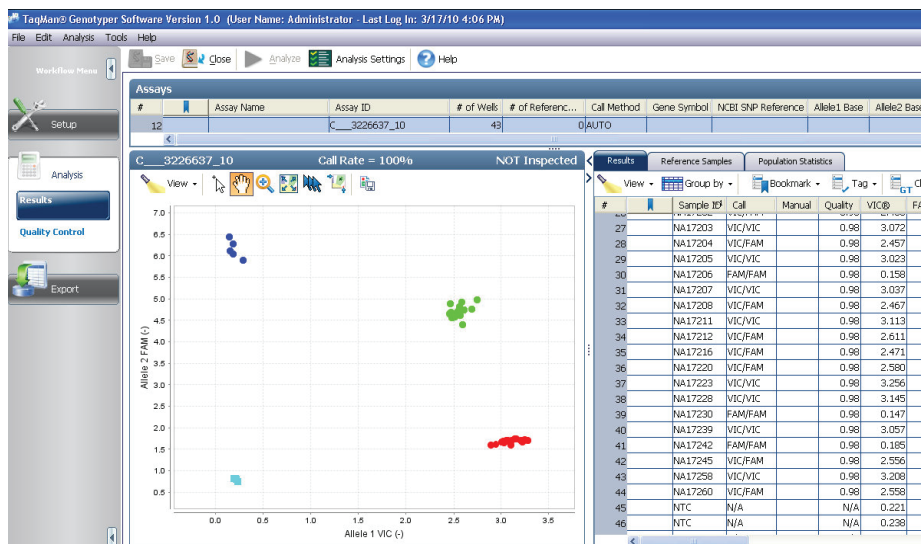


Figure 4. TaqMan® Genotyper Software automatically determines sample genotypes and displays data.

Table 1. Applied Biosystems® instrument capacities.

Instrument	Capacity
GeneAmp® PCR System 9700 Thermal Cycler	60-, 96-, Dual 96-, or Dual 384-well blocks
Veriti® Thermal Cycler	60-, 96- (standard or Fast), or 384-well block
7500/7500 Fast Real-Time PCR System	96-well block (standard or Fast)
7900HT Fast Real-Time PCR System	96- and 384-well blocks (standard or Fast)
ViiA™ 7 Real-Time PCR System	96- and 384-well blocks (standard or Fast)
StepOne® Real-Time PCR System	48-well block (standard or Fast)
StepOnePlus® Real-Time PCR System	96-well block (standard or Fast)
QuantStudio™ 12K Flex Real-Time PCR System	96-well (standard or Fast), 384-well, TaqMan® Array Card, and OpenArray® plate blocks



Figure 5. The flexible ViiA™ 7 Real-Time PCR System (left) and the QuantStudio™ 12K Flex Real-Time PCR System (right), which offers the highest throughput of all Applied Biosystems® real-time PCR instruments.

or 384-well microtiter plates or on TaqMan® OpenArray® Plates. It has a state-of-the-art genotype-calling algorithm, an intuitive user interface, and enhanced study-based analysis features. The software enables multi-plate data analysis for high-throughput workflows and improved accuracy in genotype calling; versatile export features and comprehensive quality-control features facilitate streamlining of the entire workflow. TaqMan® Genotyper Software can be downloaded at www.lifetechnologies.com/taqmangenotyper.

Simple ordering

Selecting and ordering TaqMan® SNP Genotyping Assays is as simple as “point and click.” Use SNPbrowser™ Software to select the most informative SNPs for your genotyping studies. As you identify SNPs of interest, simply upload your selected TaqMan® SNP Genotyping Assays to our online ordering tool.

Our new online ordering tool (Figure 6) enables you to search, select, and order from our catalog of over 4.5 million made-to-order predesigned TaqMan® SNP Genotyping Assays. You can search for SNPs using any of several criteria: National Center for Biotechnology Information (NCBI) gene ID, NCBI SNP reference ID (rs#), or gene symbol. You can further refine your search by using SNP type (i.e., intragenic, 5' or 3' UTR, chromosome, etc.).

Our Custom TaqMan® SNP Genotyping Assays supply you with SNPs that are not available from our predesigned assay collection, including those from any nonhuman organism. This service designs assays for all possible SNP, MNP, and indel targets but without the up-front bioinformatic preparation used for the predesigned made-to-order assays. Our complementary Custom TaqMan® Assay Design Tool conveniently formats your target sequence for submission to our manufacturing facilities. To order custom assays, simply prepare your target sequence according to the Design and Ordering Guide, and upload your submission file at www.lifetechnologies.com/snpcadt.

The screenshot displays the TaqMan SNP Assay search and order tool interface. On the left, there are filter panels for 'Narrow Your Results' (Allele Freq. Known), 'Species' (Human selected), 'Assay Type' (Functionality Tested, Validated), and 'SNP Type' (Intragenic, Transition Substitution, Intron, Transversion Substitution). The main search area shows a search bar with 'C_60512706_20' and a 'View Assay on Map' link. Below the search bar, a table lists search results for SNP ID rs34545365, Gene BRCA1, Location Chr.17: 41251811, SNP Type Silent Mutation, Assay Type Functionality Tested, and Made To Order | Cat. # 4351379. A price of (USD) 318.00 and an 'add to cart' button are visible. The 'Product Details' section shows SNP ID rs34545365, Assay Type Functionality Tested, NCBI dbSNP Submissions 1, Location Chr.17:41251811 on NCBI Build 37, Set Membership C/T, Context Sequence [VIC/FAM] CCTTACCCAATTCAATGTAGACAGA[C/T]GTCTTTGAGGTTGTATCCGCT GCT, Polymorphism C/T, Transition Substitution, and Literature Links BRCA1 PubMed Links. The 'Gene Details' table is as follows:

Gene	Gene Name	SNP Type	Transcript Location	Transcript Accession	Protein ID
BRCA1	breast cancer 1, early onset	Silent Mutation		NM_007294.3	NP_009225.1
		Transition Substitution			
		Silent Mutation		NM_007297.3	NP_009228.2
		Transition Substitution			
		Silent Mutation		NM_007298.3	NP_009229.2
		Transition Substitution			
BRCA1	breast cancer 1, early onset	Silent Mutation		NM_007299.3	NP_009230.2
		Transition Substitution			
BRCA1	breast cancer 1, early onset	Silent Mutation		NM_007300.3	NP_009231.2
		Transition Substitution			

Figure 6. Our new TaqMan® Assay search and order tool makes online ordering easy. For convenient online ordering and multiple search options for all our genotyping assays, including keyword, batch, and location searches, visit www.lifetechnologies.com/taqmansnp.

Ordering information

	Human assays (Cat. No.)	Nonhuman assays (Cat. No.)	Number of SNPs	No. of 5 μ L reactions (384-well plate)	No. of 25 μ L reactions (96-well plate)	Assay mix formulation	Assay type
Predesigned TaqMan[®] SNP Genotyping Assays for Human and Mouse							
Small-scale	4351379	4351384*	>4.5 million	1,500	300	40X	Made-to-Order
Medium-scale	4351376	4351382*	>4.5 million	5,000	1,000	40X	Made-to-Order
Large-scale	4351374	4351380*	>4.5 million	12,000	2,400	80X	Made-to-Order

Custom TaqMan[®] SNP Genotyping Assays

Small-scale	4331349	4332077	∞	1,500	300	40X	Made-to-Order
Medium-scale	4332072	4332075	∞	5,000	1,000	40X	Made-to-Order
Large-scale	4332073	4332076	∞	12,000	2,400	80X	Made-to-Order

TaqMan[®] Drug Metabolism Genotyping Assays

Small-scale	4362691	NA	2,700	750	150	20X	Inventoried
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All assays are quality-control tested using a mass spectrometer to verify sequence and yield. All assays have a VIC[®] dye-labeled probe, a FAM[™] dye-labeled probe, and two target-specific primers. All assays, excluding Custom TaqMan[®] SNP Genotyping Assays, undergo bioinformatics evaluation of target SNP sequences.

Functional testing against 20 unique genomic DNA samples is performed on all custom and predesigned made-to-order human TaqMan[®] SNP Genotyping Assays. Validation testing against four populations (45 samples/population) was performed on all 160,000 validated TaqMan[®] SNP Genotyping Assays, and all TaqMan[®] Drug Metabolism Genotyping Assays.

* Over 10,000 mouse assays available.



For more information and full terms of the TaqMan[®] Assays QPCR Guarantee, visit www.lifetechnologies.com/taqmanguarantee.

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