TaqMan® Drug Metabolism Genotyping Assays

Protocol

Applied Biosystems

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NOTICE TO PURCHASER

TaqMan® probes are covered by U.S. patents and foreign counterparts and patents pending licensed to Applied Biosystems.

Part Number 4362038 Rev. B
09/2010

Contents

Preface

This preface contains:

Safety

Safety Alert Words

Seven safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word–**IMPORTANT, CAUTION, WARNING, DANGER**–implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

WARNING **CHEMICAL HAZARD**. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page iv.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.

3. Find the document of interest, right-click the document title, then select any of the following:
    - **Open** – To view the document
    - **Print Target** – To print the document
    - **Save Target As** – To download a PDF version of the document to a destination that you choose
4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical Waste Hazard

CHEMICAL WASTE HAZARD. Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.

- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Biological Hazard Safety

BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eye wear, clothing, and gloves. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **http://bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).

Additional information about biohazard guidelines is available at: **http://www.cdc.gov**

How to Obtain Support

To contact Applied Biosystems Technical Support from North America by telephone, call **1.800.899.5858**.

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training

- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Product Overview

Product Description

TaqMan® Drug Metabolism Genotyping Assays are a comprehensive collection of assays that are optimized for genotyping single nucleotide polymorphisms (SNPs), insertions and deletions (indels), and multi-nucleotide polymorphisms (MNPs) in drug metabolism related genes. The assay uses the 5′ nuclease chemistry for amplifying and detecting specific polymorphisms in purified genomic DNA samples. Each assay allows researchers to genotype individuals for a single polymorphism. All assays were developed using Applied Biosystems bioinformatics assay design process, and genomic information from public SNP databases and public genome assemblies.

Available Products

To view the available TaqMan Drug Metabolism Genotyping Assays (PN 4362691), go to:

http://www.appliedbiosystems.com

For more information about ordering TaqMan Drug Metabolism Genotyping Assays, see "Ordering Assays" on page 26.

TaqMan® Drug Metabolism Genotyping Assay Products

Type	Scale	Concen-tration	Number of Reactions		Part Number
			25-µL Reaction 96-Well	5-µL Reaction 384-Well	
Inventoried	Small	20X	150	750	4362691

Product Properties

All TaqMan Drug Metabolism Genotyping Assays:

- Are designed and optimized to work with TaqMan® Universal PCR Master Mix using the same thermal cycling conditions. This facilitates a simple workflow for all throughput requirements and in any drug metabolism genotyping study.
- Require only three components:
  - 3 to 20 ng of purified genomic DNA sample per well, with all wells in a given study having the same amount of DNA
  - 20× Drug Metabolism Genotyping Assay Mix (specific for each polymorphism)
  - 2× TaqMan Universal PCR Master Mix (with or without AmpErase® UNG)
- Require only one PCR amplification step and an endpoint reading to obtain results.

Assay Contents

Each TaqMan Drug Metabolism Genotyping Assay shipment consists of:

- One tube containing 20× Drug Metabolism Genotyping Assay Mix.
- A CD-ROM containing the assay information file (AIF), the DME Assay Index, and PDF versions of the protocol and product insert.

About the Drug Metabolism Genotyping Assay Mix

The Drug Metabolism Genotyping Assay Mix contains:

- Sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest
- Two TaqMan® MGB probes:
  - One probe labeled with VIC® dye detects the Allele 1 sequence
  - One probe labeled with FAM™ dye detects the Allele 2 sequence

Context Sequence Representation

All reporter dye information for all TaqMan Drug Metabolism Genotyping Assays is represented as follows.

If the context sequence is XXXXX[A/B]XXXXX... then the:

- A allele *always* represents the VIC dye.
- B allele *always* represents the FAM dye.

For example, the clip in Table 1 would result in the following allele-to-dye association:

Line 1: VIC® dye associated with the G allele, FAM™ dye associated with the A allele

Line 2: VIC =T, FAM = C

Line 3: VIC = G, FAM = A

Line 4: VIC = T, FAM = C

Table 1 Section from an assay information file

Context Sequence	Probe Design
GAGGGGAGGCGGCTGCCCACCCTTC[G/A]ACACTATTACCCATCGGTCACTTGT	Reverse
GATACTGCTTTTCCTATTAACCCAT[T/C]AGTGATGGGGTCAGAAGGCTGAGGC	Forward
TTCCACAAGTTCTCAAAGCAACTAT[G/A]TTCATAACTTAATCTCTCTTTTTTT	Reverse
ATTGACATCTGTATAAACCGTGTGA[T/C]GGCAGTGATTTAGTAACTTTTTGTC	Forward

About the Assay Information File

The assay information file (AIF) consists of:

- Genomic information about the polymorphism, including gene name, chromosomal location, context sequence (with nucleotide variation in square brackets), and allele frequency (if available)
- Information about the packaging of each assay tube, including the location of the tube in the plate rack and the 2-D bar code on the bottom of the tube.

About the DME Assay Index

The DME (Drug Metabolism Enzyme) Assay Index consists of additional information about the polymorphism, including:

- The gene to which the polymorphism location is mapped
- Allele nomenclature (from public allele nomenclature sites, when available)
- Polymorphism (for example, A/G)
- Amino acid change (if applicable)
- Phenotype (from OMIM and/or Pharm GKB)

About TaqMan Drug Metabolism Genotyping Assays

Assay Components

Each TaqMan drug metabolism genotyping assay consists of a single tube containing:

- Two primers for amplifying the polymorphism of interest
- Two TaqMan MGB probes for detecting alleles

About the Probes

Each TaqMan MGB probe contains:

- A reporter dye at the 5′ end of each probe
  - VIC® dye is linked to the 5′ end of the Allele 1 probe.
  - FAM™ dye is linked to the 5′ end of the Allele 2 probe.
- A minor groove binder (MGB)

  This modification increases the melting temperature (T_m) for a given probe length (Afonina *et al.*, 1997; Kutyavin *et al.*, 1997), which allows the design of shorter probes. This results in greater differences in T_m values between matched and mismatched probes, which produces more robust allelic discrimination.
- A nonfluorescent quencher (NFQ) at the 3′ end of the probe

  Because the quencher does not fluoresce, Applied Biosystems sequence detection systems can measure reporter dye contributions with greater sensitivity.

5′ Nuclease Assay

During PCR:

- Each TaqMan MGB probe anneals specifically to its complementary sequence between the forward and reverse primer sites.
- When the oligonucleotide probe is intact, the proximity of the reporter dye to the quencher dye results in quenching of the reporter fluorescence primarily by Förster-type energy transfer (FRET; Förster, 1948; Lakowicz, 1983).
- AmpliTaq Gold® DNA polymerase extends the primers bound to the genomic DNA template.
- AmpliTaq Gold DNA polymerase cleaves only probes that are hybridized to the target.
- Cleavage separates the reporter dye from the quencher dye, which results in increased fluorescence by the reporter.

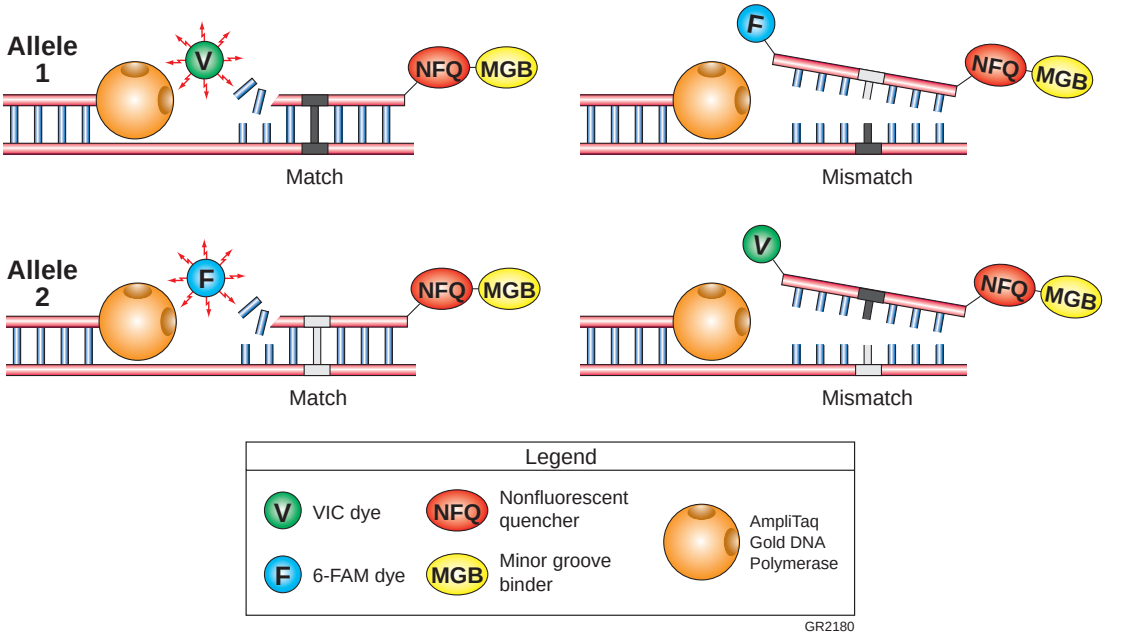
- The increase in fluorescence signal occurs when probes that have hybridized to the complementary sequence are cleaved. Thus, the fluorescence signal generated by PCR amplification indicates which alleles are present in the sample.

Mismatches Between Probe and Target Sequences

Even single nucleotide mismatches between a probe and the target sequence reduce the efficiency of probe hybridization, which in turn reduces the amount of reporter dye cleaved from a quenched probe. Furthermore, AmpliTaq® Gold DNA polymerase is more likely to displace a mismatched probe without cleaving it. Each of these factors minimizes the production of nonspecific fluorescence signals.

Drug Metabolism Genotyping Assay Results

The figure below shows results from matches and mismatches between target and probe sequences in TaqMan Drug Metabolism Genotyping Assays (Livak *et al.*, 1995).



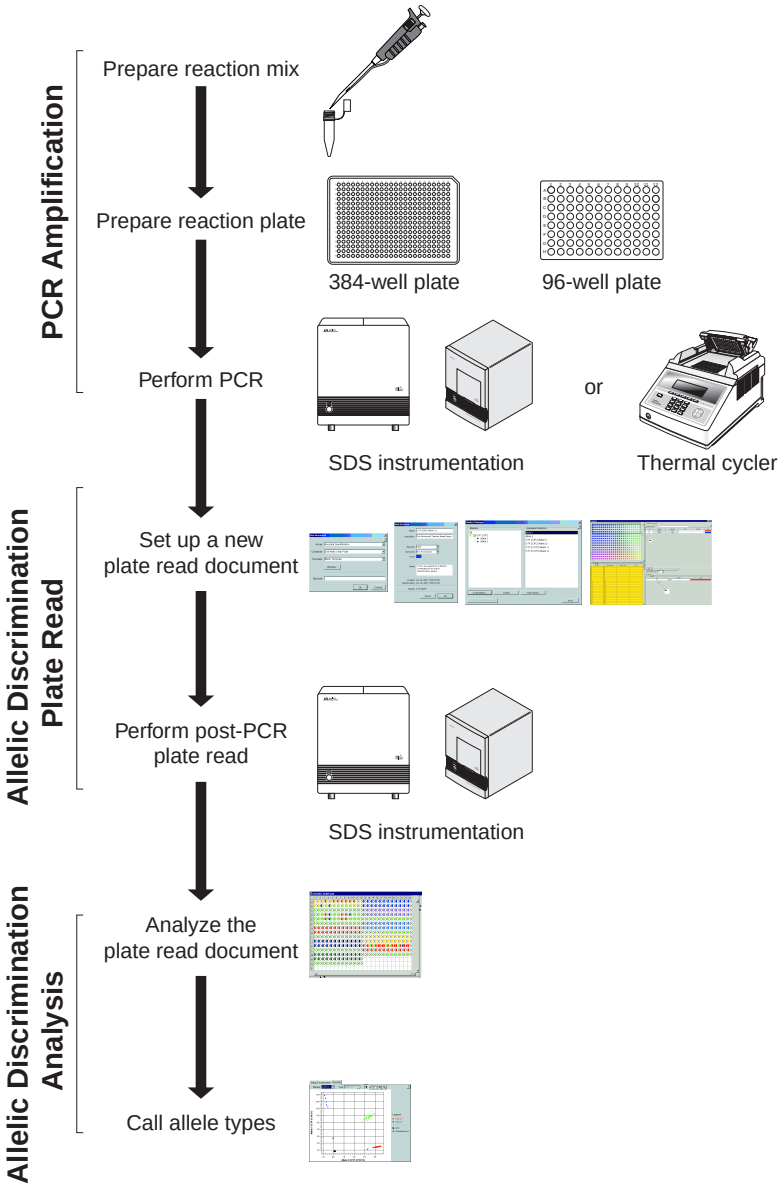
The table below shows the correlation between fluorescence signals and sequences in a sample.

A substantial increase in…	Indicates…
VIC® dye fluorescence only	Homozygosity for Allele 1
FAM™ dye fluorescence only	Homozygosity for Allele 2
Both VIC and FAM fluorescence	Allele 1-Allele 2 heterozygosity

Assay Workflow

The figure below shows an overview of the procedure for using TaqMan Drug Metabolism Genotyping Assays.

Appendix B Ordering Assays and Equipment on page 26
summarizes the materials needed to perform the assay.



PCR Amplification

Overview

During the first step of a TaqMan® Drug Metabolism Genotyping assay, AmpliTaq Gold DNA polymerase from the TaqMan Universal PCR Master Mix, No AmpErase UNG (see note), amplifies target DNA using sequence-specific primers. TaqMan MGB probes from the Drug Metabolism Genotyping Assay Mix provide a fluorescence readout of the amplification of each allele.

Note: Alternatively, TaqMan Universal PCR Master Mix, containing AmpErase UNG, can be used. (See Appendix C PCR Laboratory Practices on page 34.) Only Master Mix without UNG is referred to in this protocol.

General Process

PCR amplification requires that you:

1. Prepare an optical reaction plate containing the following for each assay (see page 13):
   - No Template Controls (NTCs) (at least two are strongly recommended; see note below)
   - Genomic DNA with known genotype at SNP of interest (optional controls)
   - Genomic DNA samples with unknown genotype at SNP of interest

   IMPORTANT! Applied Biosystems strongly recommends using at least two NTCs per assay to:

   - Orient the VIC® and/or FAM™ clusters to an origin.
   - Enhance the detection of genomic DNA contamination on a given set of plates.
2. Prepare the reaction mix, as described on page 11
3. Perform PCR, as described on page 15

See Appendix C PCR Laboratory Practices on page 34 for general instructions on avoiding false positive amplifications.

Reagent Preparation Guidelines

- Keep all TaqMan Drug Metabolism Genotyping Assays protected from light, in the freezer, until you are ready to use them. Excessive exposure to light may affect the fluorescent probes.
- Minimize freeze-thaw cycles.

- Prior to use:
  - Thoroughly mix the TaqMan Universal PCR Master Mix, No AmpErase UNG, by swirling the bottle.
  - Resuspend the assay mix by vortexing, then centrifuge the tube briefly.
  - After thawing frozen genomic DNA samples, resuspend the samples by vortexing, then centrifuge the tubes briefly.
- Prepare the reaction mix for each assay before transferring it to the optical reaction plate for thermal cycling.
- Mix reagents thoroughly after adding the reaction mix to the genomic DNA samples to avoid stratification of the reagents and/or air bubbles in the well. Stratification can lead to "stringy" clusters (see "Troubleshooting" on page 17).

Recommended Template

The recommended template for TaqMan Drug Metabolism Genotyping Assays is purified genomic DNA (3 to 20 ng). Quantify genomic DNA using the TaqMan® RNase P Detection Reagents Kit (PN 4316831) and the TaqMan® DNA Template Reagents Kit (PN 401970).

Quantifying Genomic DNA

Applied Biosystems recommends quantifying the amount of genomic DNA in samples before using TaqMan Drug Metabolism Genotyping Assays.

Genomic DNA (gDNA) should be quantitated by a reliable method such as UV/VIS spectrophotometry (A260/A280 measurement) (Haque *et al.* 2003) or real-time quantification by RNase P.

If you use the RNase P method, generate a standard curve using the DNA template standards in the TaqMan® DNA Template Reagents Kit (PN 401970) and the RNase P gene primers and probe provided in the TaqMan RNase P Detection Reagents Kit (PN 4316831).

Note: Refer to the appropriate instrument user guide for details on performing and analyzing real-time runs using RNase P.

Methods for Adding DNA

TaqMan®Drug Metabolism Genotyping Assays allow you to use either wet or dried-down DNA. If your experiment requires multiple plates that use the same gDNA, or if you plan to use the same gDNA in several experiments, it is convenient to dry-down the gDNA in the plates, which are then ready for use at any time. Both methods are described below.

To create a plate with wet DNA:

1. Dilute each purified genomic DNA sample with DNase-free water to deliver a final DNA mass in the range of 3 to 20 ng per reaction well.

   IMPORTANT! All wells belonging to the same drug metabolism genotyping assay must contain the same amount of sample or control.

If you are preparing a…	Then the volume of DNA sample and DNase-free water per reaction should be…
384-well reaction plate	2.25 µL
96-well reaction plate	11.25 µL

   Note: Multiple drug metabolism genotyping assays may be run on one reaction plate, but must be analyzed separately.

2. Into each well of the 96-well or 384-well optical reaction plate, pipette one control or sample aliquot of the volume (indicated in step 1) appropriate for the plate type.

To create a plate with dried-down DNA:

1. Pipet one control or sample (3 to 20 ng of purified genomic DNA) into each well of a 96-well or 384-well optical reaction plate.

   All wells belonging to the same drug metabolism genotyping assay must contain the same amount of sample or control.

To create a plate with dried-down DNA: ***(continued)***

2.	Dry down the samples completely by evaporation at room temperature in a dark, amplicon-free location. (Cover the plate with a lint-free tissue while drying.) **Note:** Multiple drug metabolism genotyping assays may be run on one reaction plate, but must be analyzed separately.

Preparing the Reaction Mix

The reaction mix contains TaqMan Drug Metabolism Genotyping Assay Mix, TaqMan Universal PCR Master Mix, No AmpErase UNG, and DNase-free water. The recommended final reaction volume per well is 5 µL for a 384-well plate and 25 µL for a 96-well plate.

WARNING **CHEMICAL HAZARD. Drug Metabolism Genotyping Assay Mix** contains **formamide**. Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

CAUTION **CHEMICAL HAZARD. TaqMan 2× Universal PCR Master Mix, No AmpErase UNG** may cause eye and skin irritation. Exposure may cause discomfort if swallowed or inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To prepare the reaction mix:

1.	Calculate the number of reactions to be performed for each assay. **Note:** Include at least two NTCs and, if available, at least one known genomic DNA control on each plate for optimal analysis and troubleshooting capabilities.

To prepare the reaction mix: ***(continued)***

2. Calculate the total volume of each component needed for each assay, using the table below. Be sure to choose the appropriate DNA delivery method for your experiment.

Component	Wet DNA Method		Dry-Down DNA Method	
	Volume (µL)/Well			
	384-well plate	96-well plate	384-well plate	96-well plate
2× TaqMan Universal PCR Master Mix, No AmpErase UNG	2.50	12.50	2.50	12.50
20× Drug Metabolism Genotyping Assay Mix	0.25	1.25	0.25	1.25
DNase-free water	(none)	(none)	2.25	11.25
Total Volume per Well	**2.75**	**13.75**	**5.00**	**25.00**

Note: In your calculations, include some extra reactions to compensate for the volume loss that occurs during pipetting.

3. Gently swirl the bottle of 2× TaqMan Universal PCR Master Mix, No AmpErase UNG (abbreviated as "UMM" in subsequent steps). Ensure the 2× UMM is well mixed before use.

4. Vortex and centrifuge the 20× Drug Metabolism Genotyping Assay Mix briefly.

5. Pipette the required total volumes of 2× UMM and 20× Drug Metabolism Genotyping Assay Mix into a sterile test tube.

To prepare the reaction mix: ***(continued)***

6.	Flick and invert the tube(s) to mix.
7.	Centrifuge the tube(s) briefly to spin down the contents and to eliminate any air bubbles from the solution.

Preparing the DNA Reaction Plate

For each assay and on each reaction plate run controls to ensure optimal analysis and troubleshooting capabilities of TaqMan® Drug Metabolism Genotyping Assays.

- Two No Template Controls (NTCs, DNase-free water) per assay (strongly recommended)
- Known genomic DNA controls (optional)

To prepare the DNA reaction plate:

1. Into each well of your DNA reaction plate pipette the reaction mix indicated below.

Plate Type	Volume of Reaction Mix (µL)/Well	
	Wet DNA Delivery Method	Dry-Down DNA Method
384-well	2.75	5
96-well	13.75	25

   IMPORTANT! Be sure that no cross-contamination occurs from well to well during pipetting.

2. Inspect all the wells for uniformity of volume, and note which wells do not appear to contain the proper volume. (See "Troubleshooting" on page 17.)
3. Seal the plate with an optical adhesive cover (required if using the ABI PRISM® 7900HT Real-Time PCR System) or with optical caps.
4. Vortex the plate to mix the wells.

To prepare the DNA reaction plate: ***(continued)***

5.	Centrifuge the plate briefly to spin down the contents and eliminate and air bubbles.

Selecting a Thermal Cycler

This PCR protocol has been tested using GeneAmp® PCR System 9700 and the ABI PRISM® 7900HT Real-Time PCR System thermal cyclers for PCR amplification. Other Applied Biosystems instruments than can be used for PCR amplification are shown in the table below.

Instrument	Notes
ABI PRISM 7000 SDS	These systems allow real-time analysis of PCR, which is helpful for troubleshooting. If using a Sequence Detection System for PCR amplification, perform the endpoint plate read separately.
ABI PRISM 7900HT Real-Time PCR System	
Applied Biosystems 7300 Real-Time PCR System	
Applied Biosystems 7500 Real-Time PCR System	
Applied Biosystems 7500 Fast Real-Time PCR System	TaqMan Drug Metabolism Genotyping Assays can be performed on a 7500 Fast System using *standard* reagents and *standard* cycling protocols. **Note:** TaqMan Drug Metabolism Genotyping Assays are not supported using Fast reagents and protocols.

IMPORTANT! Because of differences in ramp rates and thermal accuracy, you may need to adjust the settings if you use thermal cyclers other than those indicated above.

IMPORTANT! Use of thermal cyclers from manufacturers other than Applied Biosystems is not supported by Applied Biosystems.

Performing PCR

Note: The TaqMan® Drug Metabolism Genotyping Assays use a 90 second extension time and 50 cycles.

These conditions are chosen for optimal performance because the average amplicon size of TaqMan Drug Metabolism Genotyping Assays is longer than the average amplicon size of most TaqMan SNP Genotyping Assays.

To perform PCR:

1. Specify the thermal cycling conditions.

   IMPORTANT! These conditions are optimized for use only with TaqMan Drug Metabolism Genotyping Assays on the instruments specified on page 14.

AmpliTaq Gold Enzyme Activation	PCR (50 Cycles)	
HOLD	Denature	Anneal/Extend
10 min at 95 °C	15 sec at 92 °C	90 sec at 60 °C

   Note: Refer to the appropriate instrument user guide for help with programming your thermal cycler.

2. Specify the reaction volume according to the table below.

Plate Type	Reaction Volume (µL)/Well
384-well	5
96-well	25

3. Load the reaction plate into the thermal cycler, then start the run.

Allelic Discrimination Plate Read and Analysis

Overview

After PCR amplification, you perform an endpoint plate read using an Applied Biosystems Sequence Detection System. The Sequence Detection System (SDS) Software uses the fluorescence measurements made during the plate read to plot fluorescence (Rn) values based on the signals from each well. The plotted fluorescence signals indicate which alleles are in each sample.

Refer to the allelic discrimination section of the appropriate instrument user guide for instructions on how to use the system software to perform the plate read and analysis.

Analysis Workflow

Analyzing data for SNP genotyping requires that you:

- Create and set up an allelic discrimination plate read document
- Perform an allelic discrimination plate read on an SDS instrument
- Analyze the plate read document
- Make manual allele calls or reviewing automatic allele calls
- Convert allele calls to genotypes

Troubleshooting

Observation	Possible Cause	Recommended Action
NTCs generated fluorescence signals that cluster with genomic DNA samples rather than close to the origin.	DNA contamination of the NTC wells and also other wells may have occurred.	Test your reagents for the presence of contaminating nucleic acid.
NTCs generated high fluorescence signal but did not cluster with DNA samples.	Some assays have high NTC fluorescence.	Measure Rn-NTC values for each cluster. If clusters are well separated from NTCs, make allele calls as usual.
Distinct FAM™ or VIC® homozygote clusters or heterozygote clusters were not observed.	Reporter dyes were not appropriately assigned.	1. Verify that reporter dyes are assigned to the correct allele. 2. Reanalyze the plate read.
	Quencher dye was improperly selected.	
A sample did not cluster with one specific allele type.	The sample may contain: • More or less DNA than other samples • A rare allelic variation or sequence duplication • Mixtures of multiple alleles from contamination	1. Recheck the DNA concentrations of the samples. 2. Retest the samples to verify true DNA outlier versus one-time PCR artifact. 3. Test the sample using a different SNP genotyping assay.
	Inaccurate reagent delivery or evaporation occurred.	Check all wells for uniformity in volume, noting which wells do not appear to contain the proper volume. Redo any reactions that did not contain the proper volume.
	Air bubbles are present in the wells.	Check each well for bubbles, then redo any reactions that contained bubbles.
Unknown genomic samples did not generate fluorescence signals.	The sample may: • Contain no DNA • Contain PCR inhibitors	1. Recheck the DNA concentrations of the samples using the procedure in "Quantifying Genomic DNA" on page 9. 2. Retest the sample to verify. 3. Test the sample using a different SNP genotyping assay.

Observation	Possible Cause	Recommended Action
Clusters appear "stringy", that is in long stringy clusters on the three vectors from the NTCs. The Rn of the points varies widely, and the cluster may string all the way back to the NTCs.	• Inefficient mixing of reagents. • DNA samples are not of equal concentration due to – Poor or inaccurate DNA quantitation – Poor DNA quality • Poor performing thermal cycler.	• Mix the contents of each well after adding the reaction mix to the DNA samples (especially to wet DNA) by briefly vortexing the reaction plate and then centrifuging the plate prior to thermal cycling. • All the DNA samples on the plate should be of equivalent concentration, within the recommended range of 3 to 20 ng. If 3 ng of DNA is selected, then all samples on the plate should be at 3 ng. Do not mix 3-ng samples with 20-ng samples on the same plate. In addition, low DNA quantity (<1 ng/well) can cause poor amplification results. • Make sure DNA samples do not contain varying levels of DNA inhibitors. • Ensure that all wells of the thermal cycler are performing equally and calibrate if necessary.
All samples cluster with the NTCs	PCR amplification did not occur due to one of the following: • One or more of the reaction components was not added. • AmpliTaq Gold DNA polymerase was not activated efficiently. • The annealing temperature on the thermal cycler was too high or too low for the primers and/or probes. • Genomic DNA was impure or of insufficient quantity. • Presence of a PCR inhibitor in the reaction.	• Make sure the DNA, TaqMan Drug Metabolism Genotyping Assay and UMM were added to the reaction plates. • Make sure AmpliTaq Gold DNA polymerase was activated by implementing the initial 10 minute cycle at 95 °C. • Make sure the thermal cycler is set to the correct annealing and extension temperatures. Ensure that thermal cyclers are calibrated and maintained regularly. • Make sure that 3 to 20 ng of high-quality DNA sample was added to each well. • Impure DNA samples, as well as other inhibitors such as high concentrations of EDTA, can inhibit the PCR process.

Observation	Possible Cause	Recommended Action
Some samples cluster with the NTCs.	The samples that did not amplify may have a known null allele (for example GSTM1 or GSTT1 genes).	• Check the literature for the possibility of a null genotype. • Try amplifying the gene with another set of PCR primers. If another primer set within the gene also does not amplify, it suggests the presence of a null genotype.
	The samples that did not amplify may have a a rare sequence variation under one of the primers that prevented amplification.	Sequence the affected samples to look for a rare mutation.
Many scattered points, many of which are at very high Rn values	Empty or very low volume wells are on the plates. The 7900HT reads a small amount of FAM™, VIC®, and ROX™ signal in the plastic plate and produces an artificial Rn for an empty well that is plotted like all other points.	Check robotic systems and pipettors for delivery failures. Empty or low volume wells can result from robotic pipetting and/or delivery failures. These can be caused by a clogged pipette tip, a loose pipette tip, or an air bubble. Empty wells contain very low or no spectral signal.
Scattered points creating “cloudy” or diffuse clusters.	Plate contains sample DNA of varying concentrations.	• Requantitate sample DNA plate. • Ensure accurate DNA delivery to each well of the plate.

Bibliography

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Appendix A Assay Information

This appendix describes the assay information that you receive with the shipment of a TaqMan® Drug Metabolism Genotyping Assay.

About the Assay Information File (AIF)

With each TaqMan Drug Metabolism Genotyping Assay order, you receive assay information on a CD-ROM. The assay information file (AIF):

- Is identical to other AIFs for TaqMan Genotyping Assay products.
- Includes the number from the bar code on the plate in which the assays were shipped.
- Is provided in tab-delimited format.
- Includes 55 data fields; this information includes 55 columns and 2 rows (see Table 2 on page 22).
- Is most easily read when opened in Excel or a similar spreadsheet program.

You can use the AIF to:

- Identify which assay is included in each tube
- Locate each tube in the assay rack
- Provide the 2-D bar code for each tube
- Determine assay IDs
- Identify the chromosome location of the SNP
- View the minor allele frequencies in Caucasian, African American, Japanese, and Chinese populations, when available
- View the context sequence

For LIMS Users

The AIF on your CD-ROM is named so that your LIMS system automatically uploads it. The AIF file name has the format: DME__SNP_xx_yy.txt, where:

- DME-SNP refers to the TaqMan Drug Metabolism Genotyping Assays
- xx is the order number (up to 10 characters)
- yy is the plate ID number (up to 10 characters)

Determining Tube Contents

To determine the contents of each assay tube, match the Assay ID on the tube label with values in the Assay ID and Well Loc columns in the AIF. The Well Loc column indicates the plate rack position for the assay in the corresponding row of the AIF.

AIF Columns

Table 2 describes the columns in the AIF. The columns that have information important for Drug Metabolism Genotyping Assays are: Order Number, Assay ID, Context Sequence, Gene Symbol, and Minor Allele Frequencies.

Note: Because the information in the AIF varies by product line, all the fields are not populated for the Drug Metabolism Genotyping Assays. In Table 2, the Example column will say "Blank" for those cases.

Table 2 Contents of the assay information file

Column	Example	Description
Customer Name	Company XYZ	The name of your organization or institution
Order Number	123456789	Your sales order number
Ship Date	07-Jul-2005	The date that the product is packaged for shipment
Delivery Number	See packing slip	A unique number for shipment, see packing slip for details
Part Number	4362691	Product number used for ordering the assay
Product Type	Drug Metabolism Genotyping Assays	The type of product, which is indicated by the part number
Assay ID	DME-SNP: C_XXXXXXXXX_10	A unique identifier for the assay
Lot Number	41263	A unique identifier for the manufacturing batch
Plate Type	96-position tube rack v1	96-position tube rack v1
Plate ID	1234567	1D barcode ID of the plate in which the assay is shipped
Vial Type	10-digit bar coded tube	10-digit 2D bar coded tube

Table 2 Contents of the assay information file *(continued)*

Column	Example	Description
Vial ID	0004696076	A unique 2D barcode of the assay tube
Well Location	B02	The well location of the assay in the associated bar-coded plate
Assay Mix Conc.	20X	The concentration of the assay mix (primers and probe(s))
Forward Primer Name	Blank	N/A for Drug Metabolism Genotyping Assays
Forward Primer Seq.	Blank	N/A for Drug Metabolism Genotyping Assays
Forward Primer Conc.	18	The concentration (in µM) of the forward primer
Reverse Primer Name	Blank	N/A for Drug Metabolism Genotyping Assays
Reverse Primer Seq.	Blank	N/A for Drug Metabolism Genotyping Assays
Reverse Primer Conc.	18	The concentration (in µM) of the reverse primer
Reporter 1 Name	Blank	N/A for Drug Metabolism Genotyping Assays
Reporter 1 Dye	VIC®	The dye label for reporter 1
Reporter 1 Sequence	Blank	The nucleotide sequence of reporter 1
Reporter 1 Conc.	8	The concentration (in µM) of reporter 1
Reporter 1 Quencher	NFQ	A quencher used for reporter 1 of the assay
Reporter 2 Name	Blank	N/A for Drug Metabolism Genotyping Assays
Reporter 2 Dye	FAM™	The dye label for reporter 2
Reporter 2 Sequence	Blank	N/A for Drug Metabolism Genotyping Assays

Table 2 Contents of the assay information file *(continued)*

Column	Example	Description
Reporter 2 Conc.	8	The concentration (in μM) of reporter 2
Reporter 2 Quencher	NFQ	A quencher used for reporter 2 of the assay
Context Sequence	DME-SNP: ...NNNNNNNNN[A1/A2]NNNNNNNNN... (25 nucleotides on both sides of the SNP site) As an example: CTCCTCTGACACTGTCGCTTCTCCA[T/C]GGCATTAGATTTTCAGTCCTGCTCA	The nucleotide sequence surrounding the probe. The SNP is in brackets [allele 1_VIC labeled/allele 2_FAM labeled]. In this example, the SNP [T/C] can be read as T = allele 1 is VIC labeled, C = Allele 2 is FAM labeled
Design Strand	Forward	For DME-SNP sequences: • Forward – the probe binds to the same strand as the forward primer • Reverse – the probe binds to the same strand as the reverse primer.
Category	Chromosome 9	Chromosome location of SNP
Category ID	Chr9	Chromosome location of SNP
Group	D9S1776-D9S1682	Microsatellite markers associated with the SNP
Group ID	D9S1776	Microsatellite markers associated with the SNP
Gene Symbol	C5	LocusLink symbol for associated gene
Gene Name	Complement component 5	LocusLink gene name
Chromosome	9	Chromosome on which gene/SNP is found
Species	Homo_sapiens	Genome against which the assay was designed
Target Exons	Blank	N/A for Drug Metabolism Genotyping Assays

Table 2 Contents of the assay information file *(continued)*

Column	Example	Description
NCBI Gene Reference	NM_001735	NCBI transcript ID that is detected by the assay
NCBI SNP Reference	rs17611	Reference ID from NCBI-dbSNP database
Medline Reference	2274082	PubMed references for gene
Celera ID	hCV11720402	Unique assay ID in Celera Discovery System (CDS)
Cytogenetic Band	9q34	Chromosomal band location of gene (if not available, then chromosome number is given)
SNP Type	Mis-sense Mutation	Type of SNP (based on Celera Assembly); Acceptor Splice Site, Donor Splice Site, Intergenic/Unknown, Intron, Mis-sense Mutation, Nonsense Mutation, Putative UTR 5', Silent Mutation, UTR 3', UTR 5'
Minor Allele Freq - Caucasian	0.48	As calculated by SNP genotyping at Applied Biosystems - Caucasian
Minor Allele Freq - African-American	0.14	As calculated by SNP genotyping at Applied Biosystems - African-American
Minor Allele Freq - Japanese	0.24	As calculated by SNP genotyping at Applied Biosystems - Japanese
Minor Allele Freq - Chinese	0.36	As calculated by SNP genotyping at Applied Biosystems - Chinese
Celera Build Assembly Number	R27	Version of the Celera assembly from which the coordinate position is obtained
Location on Celera Assembly	97035650	Nucleotide location on the Celera human genome assembly (as referenced)

Appendix B Ordering Assays and Equipment

Ordering Assays

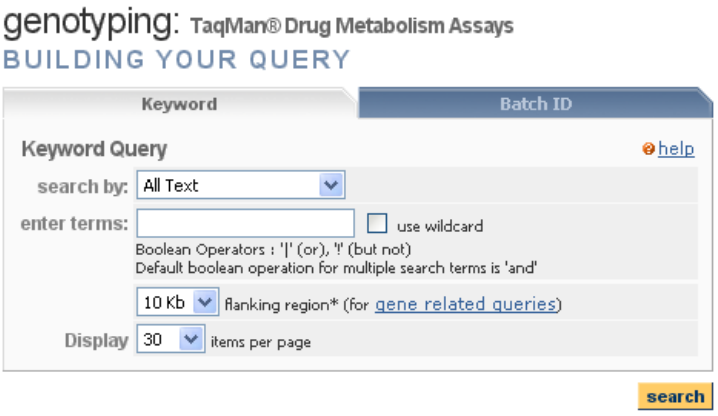
There are four ways to locate and order TaqMan® Drug Metabolism Genotyping Assays:

- The TaqMan Drug Metabolism Assays search (page 26)
- Using the DME Assay Index file as input to a TaqMan Drug Metabolism Assays search (page 28)
- The TaqMan SNP Genotyping Assay search (page 29)
- Quick Order (page 29)

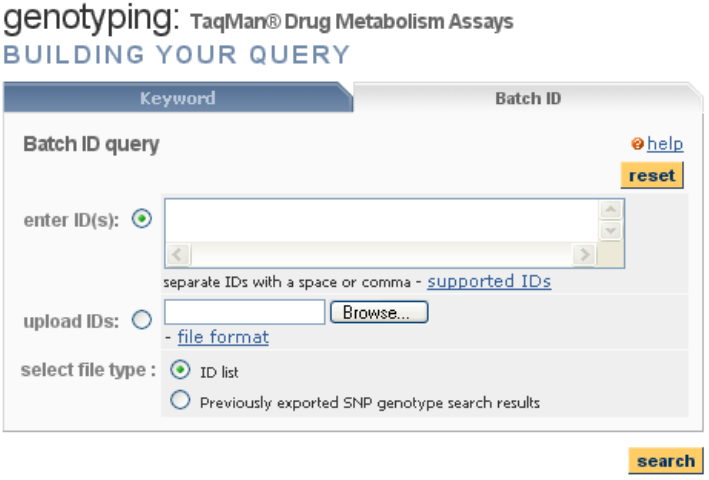
To order using the TaqMan Drug Metabolism Genotyping Assays search:

1.	Go to **http://myscience.appliedbiosystems.com**.
2.	Click **Search** to the right of the TaqMan Drug Metabolism Assays listing.

To order using the TaqMan Drug Metabolism Genotyping Assays search: ***(continued)***

3.	In the **search by** drop-down list, select the identification category by which to search for the assay of interest.  **Note:** Each TaqMan Drug Metabolism Genotyping Assay is named using a unique assay identifier containing up to 14 digits (for example, C_12345678_20). For easy identification, common allele nomenclature references and other public identifiers are also included when available. **Note:** If you want to search using an allele name, select **Allele Nomenclature** in the **search by** list. Otherwise, if your allele name contains an asterisk (such as CYP2B6*1B), the asterisk is interpreted as a wild card, and you may receive multiple search results.
4.	Enter search term(s), then click **search**. In the search results you can view the mapping information, rs information, public identifiers, context sequence, minor allele frequencies, and more.
5.	Select the assays you want to purchase, add them to your shopping cart, then proceed to checkout.

To order using the DME Assay Index file to identify your assay of interest:

1.	Copy the Assay ID of interest from the assay index file.
2.	Go to **http://myscience.appliedbiosystems.com**
3.	Click **Search** to the right of the TaqMan® Drug Metabolism Assays listing.
4.	Select the **Batch ID** tab. 
5.	Paste the assay ID into the **enter ID(s)** field, then click **search**. In the search results you can view the mapping information, rs information, public identifiers, context sequence, minor allele frequencies, and more.
6.	Select the assays you want to purchase, add them to your shopping cart, then proceed to checkout.

To order using the TaqMan SNP Genotyping Assays search, which identifies all types of genotyping assays, including Drug Metabolism Genotyping Assays:

1.	Go to **http://myscience.appliedbiosystems.com**.
2.	Click **Search** to the right of the TaqMan® SNP Genotyping Assays listing.
3.	In the **search by** drop-down list, select the identification category by which to search for the assay of interest.
4.	Enter search term(s) and click **search**. In the search results you can view the mapping information, rs information, public identifiers, context sequence, minor allele frequencies, and more. All types of genotyping assays for your gene are identified. TaqMan® Drug Metabolism Genotyping Assays are indicated by this symbol:  .
5.	Select the assays you want to purchase, add them to your shopping cart, then proceed to checkout.

If you have previously ordered TaqMan Drug Metabolism Genotyping Assays and know exactly what you want, use Quick Order.

To order using Quick Order:

1.	Go to **http://myscience.appliedbiosystems.com**.
2.	Click **Home** in the blue bar at the top of the page.
3.	Under Shopping, in the Shortcuts area, click **Quick Order**.
4.	Log in, if necessary, then enter the assay ID(s) and the quantity.
5.	Click **Add to Basket**, then proceed to checkout.

Storage and Stability

- Store the assay mixes at –15 to –25 °C.
- Minimize freeze-thaw cycles.

If you expect to freeze-thaw the assay mixes more than three times, you should consider sub-aliquoting the assay mixes to minimize the number of freeze-thaw cycles.

IMPORTANT! Protect all TaqMan Drug Metabolism Genotyping Assays from direct exposure to light. Excessive exposure to light may affect the fluorescent probes.

Materials Not Included In Assays

The following tables include materials for using the TaqMan Drug Metabolism Genotyping Assays. Unless otherwise noted, the listed items are available from major laboratory suppliers (MLSs).

Instruments	Source
ABI PRISM® 7900HT Sequence Detection System	Contact your local Applied Biosystems sales office. **Note:** TaqMan® Drug Metabolism Genotyping Assays have been tested by Applied Biosystems using the 9700 thermal cycler and 7900HT systems.
ABI PRISM® 7000 Sequence Detection System	
GeneAmp® PCR System 9700 thermal cycler	
Applied Biosystems 7300 Real-Time PCR System	
Applied Biosystems 7500 Real-Time PCR System	
Applied Biosystems 7500 Fast Real-Time PCR System	

Consumables & Other Materials	Source
ABI PRISM® 96-Well Optical Reaction Plate With Barcode (code 128)	Applied Biosystems (PN 4326659)
ABI PRISM® 384-Well Clear Optical Reaction Plate With Barcode (code 128)	Applied Biosystems (PN 4309849)
ABI PRISM® Optical Adhesive Covers	Applied Biosystems (PN 4311971)
ABI PRISM® Optical Adhesive Cover Starter Kit	Applied Biosystems (PN 4313663)

Consumables & Other Materials	Source
ABI PRISM® Optical Caps, 8 caps/strip	Applied Biosystems (PN 4323032)
ABI PRISM® Cap Installing Tool	Applied Biosystems (PN 4330015)
MicroAmp® Multi Removal Tool	Applied Biosystems (PN 4313950)
TaqMan® DNA Template Reagents	Applied Biosystems (PN 401970)
TaqMan® RNase P Detection Reagents Kit	Applied Biosystems (PN 4316831)
TaqMan® RNase P Control Reagents	Applied Biosystems (PN 4316884)
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 200 reactions	Applied Biosystems (PN 4324018)
TaqMan® Universal PCR Master Mix, 200 reactions	Applied Biosystems (PN 4304437)
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 2000 reactions	Applied Biosystems (PN 4326614)
TaqMan® Universal PCR Master Mix, 2000 reactions	Applied Biosystems (PN 4326708)
10-Pack, TaqMan® Universal PCR Master Mix, No AmpErase® UNG	Applied Biosystems (PN 4324020)
10-Pack, TaqMan® Universal PCR Master Mix	Applied Biosystems (PN 4305719)
Accessories for tubes of assay mixes:	

Consumables & Other Materials	Source
• Decapper for single caps (PN 54000) • Decapper for eight caps (PN 54001)	Micronic BV* PO Box 604 8200 AP Lelystad Netherlands Telephone: +031.320.277090 Fax: +0031.320.277088 **www.micronic.com**
• Solid caps for 0.7-mL/1.4-mL tubes, 10 capmats (for 96 wells) per case (PN 4463)	Apogent* Technologies, Inc. 30 Penhallow St. Portsmouth NH 03801 USA Telephone: +1.800.327.9970 +1.603.433.6131 Fax: +1.603.431.0860 **www.apogent.com**
Centrifuge with plate adapter	MLS
DNase-free, sterile-filtered water	MLS
Disposable gloves	MLS
Microcentrifuge	MLS
Microsoft Excel® or equivalent spreadsheet software	Software suppliers
Pipette tips, aerosol-resistant	MLS
Pipettors: • Positive-displacement • Air-displacement • Multichannel • Robotic pipetting station	MLS
Polypropylene tubes	MLS

Consumables & Other Materials	Source
TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0, made using DNase-free, sterile-filtered water)	MLS

* Other vendors supply similar products

Appendix C PCR Laboratory Practices

Introduction

PCR assays require special laboratory practices to avoid false positive amplifications (Kwok and Higuchi, 1989). The high throughput and repetition of these assays can lead to amplification of a single DNA molecule (Saiki *et al.*, 1985; Mullis and Faloona, 1987).

General PCR Practices

- Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves when preparing samples for PCR amplification.
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas, dedicated equipment, and supplies for:
  - Genomic DNA sample preparation
  - PCR setup
  - PCR amplification
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Try not to splash or spray PCR samples.
- Do not open sealed reaction plates.
- Keep reactions and components sealed as much as possible.
- Use positive-displacement pipettes or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with freshly diluted 10% bleach solution.
- When an open reaction plate is used, use TaqMan Universal Master Mix containing AmpErase UNG (uracil-N-glycosylase) to minimize contamination risk.

Note: AmpErase uracil-N-glycosylase (UNG) is a 26-kDa recombinant enzyme encoded by the *Escherichia coli* uracil-N-glycolase gene. This gene has been inserted into an *E. coli* host to direct expression of the native form of the enzyme (Kwok and Higuchi, 1989).

UNG acts on single- and double-stranded dU-containing DNA. It acts by hydrolyzing uracil-glycosidic bonds at dU-containing sites. The enzyme causes the release of uracil, thereby creating and alkali-sensitive apyridimic site in the DNA. The enzyme has no activity on RNA or dT-containing DNA (Longo *et al.* 1990).

For 5′ nuclease activities, AmpErase UNG treatment can prevent the reamplification of carryover PCR products. When dUTP replaces dTTP in PCR amplification, AmpErase UNG treatment can remove up to 200,000 copies of amplicon per 50 µL reaction.

Worldwide Sales and Support

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at **www.appliedbiosystems.com**.

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Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

7/2010

Applied Biosystems

Part Number 4362038 Rev. B