TaqMan® SNP Genotyping Assays

Protocol

Applied Biosystems

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Contents

Preface

This preface covers:

Safety

Safety Alert Words

Four 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.

Definitions

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

– 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.

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

– 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

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 (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page vi.)
- 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 in 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

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field of the MSDS Search page:
   a. Type in the chemical name, part number, or other information that you expect to appear in the MSDS of interest.
   b. Select the language of your choice.
   c. Click **Search**.
3. To view, download, or print the document of interest:

a. Right-click the document title.

b. Select:

- **Open** – To view the document
- **Save Target As** – To download a PDF version of the document to a destination that you choose
- **Print Target** – To print the document

4. To have a copy of an MSDS sent by fax or e-mail, in the Search Results page:

a. Select **Fax** or **Email** below the document title.

b. Click **RETRIEVE DOCUMENTS** at the end of the document list.

c. Enter the required information.

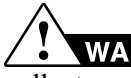
d. Click **View/Deliver Selected Documents Now**.

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical Waste Hazards

HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.

CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.



CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

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.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological Hazard Safety

BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, 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 equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All

work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- 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**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

http://www.cdc.gov

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, go to **http://www.appliedbiosystems.com**, then click the link for **Support**. (See "How to Obtain Support" below).

How to Obtain Support

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® SNP Genotyping Assays provide the largest collection of ready-to-use human single nucleotide polymorphisms (SNP) assays available. All assays were designed using our powerful bioinformatics pipeline and software, as well as genomic information from Celera Genomics and public databases:

- TaqMan® Validated SNP Genotyping Assays: ~ 160,000 gene-centric assays. These highly tested, top performing assays where validated by genotype and subsequent minor allele frequency (MAF) analysis on 2 or 4 ethnic group populations (45 individual samples per ethnic group). Inventoried for fast availability.
- TaqMan® Coding SNP Genotyping Assays: ~ 30,000 assays for the detection of informative and putative functional, nonsynonymous cSNPs in gene-coding regions. Inventoried assays, which are functionally tested to assure quality performance.
- TaqMan® Pre-Designed SNP Genotyping Assays: > 3 million genome-wide assays including assays to > 2.5 million HapMan SNPs, as well as to ~ 30,000 high value nonsynonymous cSNPs (including known disease mutations and SNPs in protein domains associated with drug binding regions). Made to order assays, available in multiple scales, are manufactured and functionally tested upon ordering.

TaqMan SNP Genotyping Assays provide optimized assays for genotyping SNPs. The products use the 5´ nuclease assay for amplifying and detecting specific SNP alleles in purified genomic DNA samples. Each assay allows researchers to genotype individuals for a specific SNP.

Available Products

To view the available TaqMan SNP Genotyping Assays (PNs 4331183, 4351374, 4351376, 4351379), go to:

http://www.appliedbiosystems.com

For more information about ordering TaqMan SNP Genotyping Assays, see "Ordering TaqMan SNP Genotyping Assays" on page 33.

Other TaqMan® Genotyping Assays:

TaqMan® Drug Metabolism Genotyping Assays (PN 4362691) are a comprehensive collection of assays that are optimized for genotying single nucleotide polymorphisms (SNPs), insertions and deletions (indels), and multi-bucleic polymorphisms (MNPs) in drug metabolism related genes. For more information about these assays and to view the available product list, please visit our Web site.

Custom TaqMan® SNP Genotyping Assays Service is available when a product for a SNP of interest is not found on the Applied Biosystems site. This assay development service that designs, synthesizes, formulates, and delivers analytically quality-controlled primer and probe sets for genotyping assays based on sequence information you submit. Assays to SNPs, as well as to indels and MNPs up to 6 bases in length, for both human and non-human targets, can be designed. To learn more about this service and how to place an order, visit the Applied Biosystems site or contact your Applied Biosystems representative.

TaqMan® SNP Genotyping Assay Products

Type	Scale	Concen-tration	Number of Reactions		Part Number
			25-µL Reaction 96-Well	5-mL Reaction 384-Well	
Inventoried	Small	20X	150	750	4331183
Made to order	Small	40X	300	1,500	4351379
Made to order	Medium	40X	1,000	5,000	4351376
Made to order	Large	80X	2,400	12,000	4351374

Product Properties

All TaqMan SNP Genotyping Assays:

- Are designed and optimized to work with TaqMan® Universal PCR Master Mix (with or without AmpErase® UNG) using the same thermal cycling conditions.
- Require only three components:
  - 1 to 20 ng of purified gDNA sample per well
  - 20×, 40×, or 80× SNP Genotyping Assay (depending on product and assay scale)
  - TaqMan® Universal PCR Master Mix (with or without AmpErase® UNG)
- Require only one PCR amplification step and an endpoint reading to obtain results.

SNP Genotyping Assay Contents

The 20×, 40×, or 80× SNP Genotyping Assay 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

Shipment Contents

Each TaqMan SNP Genotyping Assay shipment consists of:

- One tube for each assay ordered, containing the TaqMan SNP Genotyping Assay. Each tube is identified with a label on the side and a 2-D bar code on the bottom.
- Printed copy of the Data Sheet, which includes information about each assay ordered.
- Printed copy of the product insert, which includes an overview of the assays and PCR amplification protocols.
- CD-ROM containing:
  - A pdf file of the *TaqMan® SNP Genotyping Assays Protocol* (PN 4332856)
  - A pdf file of the product insert
  - The assay information file (AIF)
  - A text file titled “Understanding Your Assay Information File” (PN 4343831)

About the Assay Information File

The assay information file (AIF) contains information about each TaqMan SNP Genotyping Assay in your order, and genomic information about the SNP, including chromosomal location, allele frequency (for validated assays), context sequence, and reporter dye-SNP allele associations.

For more information about the AIF, see "Assay Information in the AIF" on page 27.

SNP Genotyping Assay Storage Recommendations

Storage and Stability

- Store the SNP Genotyping Assays at –15 to –25 °C in the dark.

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

- Do not perform more than 10 freeze-thaw cycles. If you expect to freeze-thaw the SNP Genotyping Assays more than three times, consider sub-aliquoting the SNP Genotyping Assays to minimize the number of freeze-thaw cycles.
- Applied Biosystems recommends diluting the 40× and 80× SNP Genotyping Assays to a 20× working stock; see "Diluting SNP Genotyping Assays" on page 5 below.
- It may be possible to store an assay for longer than a year. For long-term storage, dilute assays to a 20× stock for best stability.

Diluting SNP Genotyping Assays

Applied Biosystems recommends that you dilute the SNP Genotyping Assays to a 20× working stock, then aliquot for routine use. By having aliquots, you can minimize freeze-thaw cycles and protect the SNP Genotyping Assays from exposure to light.

WARNING CHEMICAL HAZARD. SNP Genotyping Assay (<2% 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.

To dilute SNP Genotyping Assays:

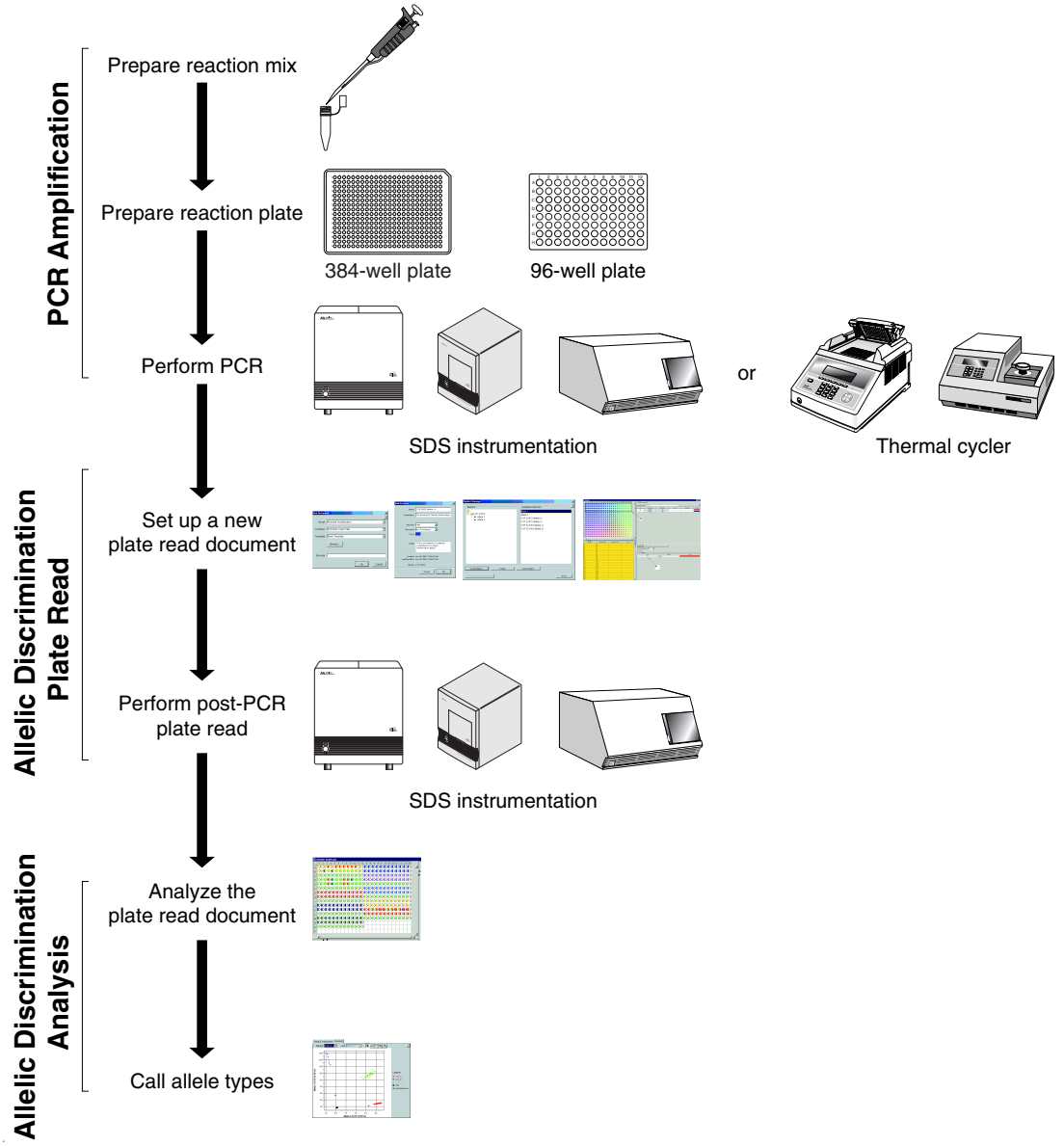
1.	Dilute 40× or 80× SNP Genotyping Assay to a 20× working stock with 1× TE buffer. **Note:** The 1× TE buffer should be 10 mM Tris-HCl, 1 mM EDTA, pH 8.0, and made using DNase-free, sterile-filtered water.
2.	Vortex, then centrifuge the mixture.

To dilute SNP Genotyping Assays: *(continued)*

3.	Store multiple aliquots of the SNP Genotyping Assays at –15 to –25 °C.

Workflow

The following diagram provides a simplified overview of the procedure for using TaqMan SNP Genotyping Assays.



Sample Preparation

Recommended Template

The recommended template for TaqMan SNP Genotyping Assays is purified genomic DNA (1 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 SNP Genotyping Assays. Generate a standard curve using the DNA template standards provided 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 detailed instructions on performing and analyzing runs.

CAUTION **CHEMICAL HAZARD. TaqMan Universal PCR Master Mix** 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 quantify genomic DNA:

1.	Create and set up a sequence detector plate document.
2.	Prepare the reaction plate using the following components: • 2× TaqMan Universal PCR Master Mix, No AmpErase UNG • 20× Primer and TaqMan Probe (FAM) dye mix • DNA standard template or genomic DNA sample • DNase-free, sterile-filtered water **Note:** Use at least three replicates of each standard or sample, and use all five DNA standards to ensure an accurate standard curve is generated. The range of known copy number should bracket anticipated copy numbers of the unknown samples on the same plate.

To quantify genomic DNA: ***(continued)***

3. Run the plate on an ABI PRISM® Sequence Detection System or Real-Time PCR System using the following thermal cycling conditions:

	AmpliTaq Gold Enzyme Activation	**PCR**	
	HOLD	CYCLE (40 cycles)	
		Denature	Anneal/ Extend
Time	10 min	15 sec	1 min
Temp	95 °C	92 °C	60 °C

4. Generate a standard curve to quantify the amount of DNA in each sample.

Methods for Adding DNA

There are two methods for adding genomic DNA to the reaction. Decide which method to use based on the experimental design.

The two methods are described in the table below.

Method	Method Description	Experimental Uses
Wet DNA delivery	1. SNP reaction mix is aliquoted to an optical reaction plate. 2. Genomic DNA is delivered to the final reaction mix. **Note:** In this method, the liquid used to resuspend the DNA is used as a component of the final reaction.	Many different DNA templates tested on a limited number of SNP targets
DNA predelivery and dry-down	1. Genomic DNA is delivered to the bottom surface of an optical reaction plate. 2. The DNA sample is dried down completely by evaporation. 3. The SNP reaction mix is added, and the DNA disperses in the final reaction mix.	• DNA concentration requires large sample volumes (2 to 5 µL) to run the assay *or* • Limited number of DNA templates tested repeatedly on different SNP targets *or* • A large number of DNA samples are prepared in plates, dried down, and stored before use

To prepare a plate with wet DNA:

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

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

If you prepare a…	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 assays canbe run on one reaction plate, but they 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 appropriate for the plate type (indicated in step 1).

To prepare a plate with dried-down DNA:

1. Into each well of a 96-well or 384-well optical reaction plate, pipette one control or sample aliquot (1 to 20 ng of DNA).

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

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.

   IMPORTANT! If you use gDNA, do not accelerate drying by heating the plate. Heating the plate may cause problems with gDNA recovery.

   Note: Multiple assays can be run on one reaction plate, but must be analyzed separately.

PCR Amplification

Overview

During the first step of a TaqMan SNP Genotyping Assay experiment, AmpliTaq Gold® DNA polymerase from the TaqMan Universal PCR Master Mix, No AmpErase UNG, amplifies target DNA using sequence-specific primers. TaqMan MGB probes from the SNP Genotyping Assay provide a fluorescence signal for the amplification of each allele.

Note: Applied Biosystems recommends using TaqMan Universal PCR Master Mix, No AmpErase UNG, which is the master mix referred to in this protocol. However, you can use TaqMan Universal PCR Master Mix, which contains AmpErase UNG. For more information on UNG, see "PCR Laboratory Practices" on page 45.

General Process

PCR amplification requires that you:

- Prepare the reaction mix (page 14).
- Add the reaction mix to the prepared DNA reaction plate (page 16).
- Perform PCR (page 18).

See "PCR Laboratory Practices" on page 45 for general instructions on avoiding false positive amplifications.

Assay Setup Guidelines

To ensure optimal analysis and troubleshooting of the TaqMan SNP Genotyping Assays, prepare an optical reaction plate containing the following for each assay:

- DNA samples with unknown genotype at the SNP of interest.
- *(*Strongly recommended) Two No Template Controls (NTCs); use DNase-free water. Applied Biosystems strongly recommends using at least two NTCs per assay to:
  - Orient the VIC-dye and/or FAM-dye clusters to an origin.
  - Enable the detection of DNA contamination on a given set of plates.
- (Optional) DNA controls with known genotype at the SNP of interest.

Reagent and Sample Preparation Guidelines

- Keep all TaqMan SNP 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.
- To dilute 40× or 80× SNP Genotyping Assays to a 20× working stock solution, see "Diluting SNP Genotyping Assays" on page 5.
- Before using the assay reagents:
  - Thoroughly mix the TaqMan Universal PCR Master Mix, No AmpErase UNG, by swirling the bottle.
  - Resuspend the SNP Genotyping Assay by vortexing, then centrifuge the tube briefly.
  - (For wet DNA only) After thawing frozen 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.
- After adding the reagents to the DNA samples, mix thoroughly to avoid stratification of the reagents and/or air bubbles in the well. Stratification can lead to "stringy" clusters (see page 22 in the "Troubleshooting" section).

Preparing the Reaction Mix

The reaction mix is made from 20×, 40×, or 80× SNP Genotyping Assay, 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.

Note: For instructions on diluting the 40× or 80× SNP Genotyping Assay to a 20× working stock solution, see "Diluting SNP Genotyping Assays" on page 5.

WARNING **CHEMICAL HAZARD. SNP Genotyping Assay (<2% 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® Universal PCR Master Mix (2×), 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. Applied Biosystems recommends including: • At least two NTCs on each plate. • If available, at least one known DNA control on each plate.

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	
	µL/well			
	384-well plate	96-well plate	384-well plate	96-well plate
TaqMan Universal PCR Master Mix (2×), No AmpErase UNG	2.50	12.50	2.50	12.50
20× working stock of SNP Genotyping Assay	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 TaqMan Universal PCR Master Mix (2×), No AmpErase UNG (abbreviated as "UMM" in subsequent steps). Ensure the UMM is well mixed before use.

4. Vortex and centrifuge the 20× SNP Genotyping Assay briefly.

5. Pipette the required total volumes of UMM and 20× SNP Genotyping Assay into a sterile microcentrifuge tube.

6. Cap the tube and invert several times to mix.

To prepare the reaction mix *(continued)*:

7.	Centrifuge the tube briefly to spin down the contents and to eliminate any air bubbles from the reaction mix.

Adding the Reaction Mix

To add the reaction mix to the prepared DNA reaction plate :

1. Into each well of your DNA reaction plate (as prepared per the procedures on page 10), pipette the reaction mix as 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 page 21 in the "Troubleshooting" section.)
3. Seal the plate with the appropriate cover. (See page 36 for a list of covers compatible with your instrument.)
4. Vortex the plate to mix the wells.
5. Centrifuge the plate briefly to spin down the contents and eliminate any air bubbles.

Selecting a Thermal Cycler

Applied Biosystems instruments that can be used for PCR amplification are shown in the table below. See page 36 for a list of reaction plates and covers that can be used on each instrument.

Instrument Type	Instrument Name
Thermal Cycler	Applied Biosystems 9800 Fast Thermal Cycler, using the 9700/9600 emulation mode **Note:** TaqMan SNP Genotyping Assays can be performed on a 9800 Fast Thermal Cycler using *standard* reagents and *standard* cycling protocols. TaqMan SNP Genotyping Assays are not supported using Fast reagents or Fast protocols.
	GeneAmp® PCR System 9700
Real-Time PCR System These instruments allow real-time analysis of PCR, which is helpful for troubleshooting. If using a Real-Time PCR System for PCR amplification, perform the endpoint plate read separately.	Applied Biosystems 7900HT Fast Real-Time PCR System‡ **Note:** TaqMan SNP Genotyping Assays can be performed on a 7900HT Fast System using *standard* reagents and *standard* cycling protocols. TaqMan SNP Genotyping Assays are not supported using Fast reagents or Fast protocols.
	Applied Biosystems 7500 Fast Real-Time PCR System **Note:** TaqMan SNP Genotyping Assays can be performed on a 7500 Fast System using *standard* reagents and *standard* cycling protocols. TaqMan SNP Genotyping Assays are not supported using Fast reagents or Fast protocols.
	Applied Biosystems 7500 Real-Time PCR System
	Applied Biosystems 7300 Real-Time PCR System
	ABI PRISM® 7000 Sequence Detection System

‡ *Applied Biosystems 7900HT Fast Real-Time PCR System* refers to all 7900HT instruments, regardless of which sample block is used (Fast or standard)..

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

To perform PCR:

1. Specify the thermal cycling conditions.

   IMPORTANT! These conditions are optimized for use only with TaqMan SNP Genotyping Assays on the instruments specified on page 35.

Standard Protocol		
AmpliTaq Gold Enzyme Activation	**PCR (40 Cycles)**	
HOLD	Denature	Anneal/Extend
10 min at 95 °C	15 sec at 92 °C	1 min at 60 °C
Alternate Protocol‡		
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, perform an endpoint plate read using an Applied Biosystems Real-Time PCR 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.

General Process

Performing a plate read and analyzing the data from TaqMan SNP Genotyping Assays requires that you:

- Create and set up an allelic discrimination plate read document in the SDS software.
- Perform an allelic discrimination plate read on an Applied Biosystems Real-Time PCR System.
- Analyze the plate read document.
- Make manual allele calls or review automatic allele calls.
- Convert allele calls to genotypes.

For More Information

For information about analyzing your data, refer to the following documents:

- 7900HT Fast System
  - *Applied Biosystems 7900HT Fast Real-Time PCR System Allelic Discrimination Getting Started Guide* (PN 4364015)
  - *Sequence Detection Systems Software Online Help for the Applied Biosystems 7900HT Fast Real-Time PCR System*
- 7300/7500/7500 Fast Systems
  - *Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Allelic Discrimination Getting Started Guide* (PN 4347822)
  - *Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Online Help*
- 7000 System – *ABI PRISM® 7000 Sequence Detection System User Guide* (PN 4330228)

Troubleshooting

Troubleshooting Table

Observation	Possible Cause	Recommended Action
NTCs generated fluorescence signals that cluster with 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-dye 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.	

Observation	Possible Cause	Recommended Action
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 DNA 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 to see if the sample itself is contaminated.
	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 during the PCR.	Remove air bubbles by swinging, tapping, or briefly centrifuging the reaction plate. For future assays, use a pipetting technique that does not form air bubbles. See "Avoiding Pipetting Errors" on page 25.
	A second SNP exists under the probe. A second SNP exists under the primer.	Conventionally not an issue with validated or pre-designed assays. However, polymorphisms underlying probe or primer sequences that were unknown when the assay design was made and/or were not present in tested populations at high enough MAF, may cause extra clusters. These may be detected by BLAST analysis to current NCBI dbSNP sequences or by sequence analysis.
Unknown DNA samples did not generate fluorescence signals.	The sample may: • Not contain DNA • Contain PCR inhibitors	1. Recheck the DNA concentrations of the samples using one of the methods listed in "Quantifying Genomic DNA" on page 8. 2. Retest the sample to verify the result. 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. • ROX™ dye signal varies across plate	• Prepare the reaction mix first (page 14), then add the reaction mix to the prepared DNA reaction plate (page 16). • 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.
	DNA samples are not of equal concentration due to: • Poor or inaccurate DNA quantitation • Poor DNA quality	• All the DNA samples on the plate should be of equivalent concentration, within the recommended range of 1 to 20 ng. If 1 ng of DNA is selected, then all samples on the plate should be at 1 ng. Do not mix 1-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. • Look at the ROX™ dye signal to ensure uniformity.
	Poor thermal cycler performance.	Ensure that all wells of the thermal cycler are performing equally, then calibrate if necessary.
	Incorrect reagents were used (for example, master mix, assay, or water).	Repeat the experiment, making sure to use the correct reagents. (The reagents are listed on page 15).

Observation	Possible Cause	Recommended Action
All samples cluster with the NTCs (PCR amplification did not occur)	One or more of the reaction components was not added.	Make sure the DNA, Custom TaqMan SNP Genotyping Assay, and UMM were added to the reaction plates.
	AmpliTaq Gold DNA polymerase was not activated efficiently.	Make sure AmpliTaq Gold DNA polymerase was activated by implementing the initial 10-minute cycle at 95 °C.
	The annealing temperature on the thermal cycler was too high or too low for the primers and/or probes.	Make sure the thermal cycler is set to the correct annealing and extension temperatures and times. Ensure that thermal cyclers are calibrated and maintained regularly.
	DNA was impure or of insufficient quantity.	Make sure that 1 to 20 ng of high-quality DNA sample was added to each well.
	A PCR inhibitor is present in the reaction. Impure DNA samples, as well as other inhibitors such as high concentrations of EDTA, can inhibit the PCR process.	Make sure you follow correct laboratory practices when preparing your samples. See"PCR Laboratory Practices" on page 45.
Some samples cluster with the NTCs.	The samples that did not amplify may have a known null allele (for example, GSTM1 or GSTT1 genes are known to be absent in a significant percentage of the population).	• 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 may have evaporated.	Repeat the experiment, making sure to: • Use the correct reagents. (The reagents are listed on page 15). • Use the recommended volumes: 5 µL for 384-well reaction plates and 25 µL for 96-well reaction plates.
	The samples may be missing DNA template.	
	The samples may be missing a reagent (for example, master mix, SNP Genotyping Assay, or water).	
	DNA was impure or of insufficient quantity.	Make sure that 1 to 20 ng of high-quality DNA sample was added to each well.

Observation	Possible Cause	Recommended Action
Samples appear in a single cluster	The primer and probe sequences target non-specific sequences.	Conventionally not an issue with validated or predesigned assays. In rare instances, a duplicated region of the genome that is represented in the genomic assembly as a single location produces this result for a predesigned assay. These may be detected by BLAST analysis of the assay content sequence to the NCBI nr database sequences.
	The minor allele frequency (MAF) is low.	Check the MAF for the SNP. You may need a larger sample size to see the minor allele.
	The SNP is a pseudo-SNP, non-polymorphic SNP, or non-informative SNP for the population.	• Verify that it is a SNP. • Check for a population-specific MAF.
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, so that each well contains the same amount of DNA (for example, all samples are at 1 ng).
	Incorrect reagents were used (for example, master mix, assay, or water).	Repeat the experiment, making sure to use the correct reagents. (The reagents are listed on page 15).
	Varied amounts of reagent were dispensed to the plate.	Check the ROX™ dye level across the plate to verify this cause. For future assays, use a pipetting technique that ensures uniform amounts of reagent are dispensed to the plate. See “Avoiding Pipetting Errors” on page 25.

Avoiding Pipetting Errors

- Improve pipetting precision, as follows:
  - Calibrate and service the pipettors regularly.
  - Pipette larger volumes.
  - Reduce the number of pipetting steps whenever possible.
  - Increase the consistency of the pipetting method
  - Consult the manufacturer about the correct method of dispensing liquid volumes accurately from the pipettor. For example, some pipettors are designed to deliver the designated volume at the first plunger stop, so "blowing out" the residue may cause error.
- Use master reaction mixes. Applied Biosystems highly recommends using a master reaction mix, as follows:
  a. Mix all common components (including the same template) to a set of reactions together.

     Note: When you make each master reaction mix, add 5 to 10% additional volume to compensate for pipetting losses.

  b. Dispense the mix to the replicate wells of the plate.

Appendix A: Assay Information in the AIF

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

About the Assay Information File (AIF)

With each TaqMan SNP Genotyping Assay order, you receive a CD-ROM on which is an assay information file (AIF). The AIF:

- Is identical in format to other AIFs for TaqMan®-based assays.
- Includes the number from the bar code on the box 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 on page 28).
- Is most easily read when opened in Microsoft® Excel software or a similar spreadsheet program.

You can use the AIF to:

- Identify which assay is included in each assay tube.
- Associate the 2-D bar code on each assay tube with the Vial ID.
- Determine assay IDs.
- Determine the SNP content sequences.
- Determine reporter dye-SNP allele associations.

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: AoD_SNP_xx_yy.txt or TQMN_SNP_xx_yy.txt, where:

- AoD_SNP or TQMN_SNP refers to the TaqMan SNP 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.

AIF Columns

The table below describes the columns in the AIF.

Note: Because the information in the AIF varies by product line, all the fields are not filled for the Custom TaqMan SNP Genotyping Assays. In the table below, the Example column indicates "Blank" for those cases.

Contents of the assay information file *:*

Column	Example	Description
Customer Name	University of X	The name of your organization or institution
Order Number	185185185	Your sales order number
Ship Date	15-JAN-06	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	4351379	Product number used for ordering the assay
Product Type	TaqMan® SNP Genotyping Assay Service	The type of product, which is indicated by the part number (part numbers are listed on page 33)
Assay ID	C__12345678_10	A unique identifier for the assay
Lot Number	654321	A unique identifier for the manufacturing batch
Plate Type	96-position tube rack v1	The type of container in which the assay is shipped
Plate ID	1234567	Barcode ID of the box in which the assay is shipped
Vial Type	10-digit barcoded tube	The type of tube containing the assay
Vial ID	0004696076	A unique 2-D barcode of the assay tube
Well Location	B02	The well location of the assay in the associated barcoded plate
Assay Mix Conc.	40×	The concentration of the SNP Genotyping Assay [primers and probe(s)]

Contents of the assay information file *(continued):*

Column	Example	Description
Forward Primer Name	Blank	Not available for Taqman Genotyping Assays
Forward Primer Seq.	Blank	Not available for Taqman Genotyping Assays
Forward Primer Conc.	36	The concentration (in μM) of the forward primer
Reverse Primer Name	Blank	Not available for Taqman Genotyping Assays
Reverse Primer Seq.	Blank	Not available for Taqman Genotyping Assays
Reverse Primer Conc.	36	The concentration (in μM) of the reverse primer
Reporter 1 Name	Blank	Not available for Taqman Genotyping Assays
Reporter 1 Dye	VIC®	The dye label for reporter 1
Reporter 1 Sequence	Blank	Not available for Taqman Genotyping Assays
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	Not available for Taqman Genotyping Assays
Reporter 2 Dye	FAM™	The dye label for reporter 2
Reporter 2 Sequence	Blank	Not available for Taqman Genotyping Assays
Reporter 2 Conc.	8	The concentration (in μM) of reporter 2
Reporter 2 Quencher	NFQ	A quencher used for reporter 2 of the assay

Contents of the assay information file *(continued):*

Column	Example	Description
Context Sequence	...AGCTA[T/G]CAGTC...	The nucleotide sequence surrounding the SNP site. SNP alleles are in brackets. The order of the alleles corresponds to the association with reporter dyes, where [Allele1 = VIC/Allele2 = FAM].
Design Strand	Forward	• Forward – The probe binds to the forward strand • Reverse – The probe binds to the reverse strand
Category	Chromosome 12	Chromosome location of the SNP
Category ID	Chr12	Chromosome location of the SNP
Group	D12S345-D12S1663	Microsatellite markers associated with the SNP
Group ID	D12S345	Microsatellite markers associated with the SNP
Gene Symbol	IRAK4	LocusLink symbol for the associated gene
Gene Name	interleukin-1 receptor associated kinase4	LocusLink gene name
Chromosome	12	Chromosome on which gene/SNP is found
Species	Homo sapiens	Organism for which the assay was designed
Target Exons	Blank	Not available for Taqman Genotyping Assays
NCBI Gene Reference	NM_016123.1	NCBI transcript ID that is detected by the assay
NCBI SNP Reference	rs46532	Reference ID from the NCBI-dbSNP database
Medline Reference	Blank	Not available for Taqman Genotyping Assays
Celera ID	hCV12345678	Unique transcript or SNP ID in the Celera Discovery System (CDS)

Contents of the assay information file *(continued):*

Column	Example	Description
Cytogenetic Band	12q12f	Chromosomal band location of the gene (if not available, then chromosome number is indicated)
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	0.28	As calculated by SNP genotyping at Applied Biosystems - Caucasian; only for validated assays
Minor Allele Freq - African-American	0.34	As calculated by SNP genotyping at Applied Biosystems - African-American; only for validated assays
Minor Allele Freq - Japanese	0.24	As calculated by SNP genotyping at Applied Biosystems - Japanese; only for validated assays
Minor Allele Freq - Chinese	0.25	As calculated by SNP genotyping at Applied Biosystems - Chinese; only for validated assays
Celera Build Assembly Number	R27	Version of the Celera assembly from which the coordinate position is obtained
Location on Celera Assembly	42982742	Nucleotide location on the Celera human genome assembly (as referenced)
NCBI Assembly Number	35	Version of the NCBI assembly from which the coordinate position is obtained
Location on NCBI Assembly	42466593	Nucleotide location on the NCBI human genome assembly (as referenced)

Appendix B: Ordering TaqMan SNP Genotyping Assays

Assay Part Numbers

Use the TaqMan® SNP Genotyping Assays part numbers that correspond to the type of assay and the number of reactions you require.

Type	Scale	Concen-tration	Number of Reactions		Part Number
			25-µL Reaction 96-Well	5-mL Reaction 384-Well	
Inventoried	Small	20X	150	750	4331183
Made to order	Small	40X	300	1,500	4351379
Made to order	Medium	40X	1,000	5,000	4351376
Made to order	Large	80X	2,400	12,000	4351374

SNPbrowser™ Software

This free software tool simplifies association study design by enabling easy and intuitive selection of the optimal set of SNPs. It includes visualization of a physical map, a linkage disequilibrium map, and putative haplotype block information obtained from the analysis of over 3 million SNPs genotyped by either the International HapMap Project or Applied Biosystems in four major populations. SNPs are selected by a variety of workflows following navigation to the genomic region of interest by gene, SNP, or location searches, including the use of sophisticated Taq SNP selection and SNPs by density selection tools. This software allows for easy ordering of SNP genotyping assays through the Applied Biosystems store.

To learn more about and obtain SNPbrowser™ Software, visit:

http://www.allsnps.com/snpbrowser

At the site, register for a serial number and download the SNPbrowser Software.

Applied Biosystems Web Site

To order assays from the Applied Biosystems store:

1.	Go to **http://www.appliedbiosystems.com**. Under Assays & Arrays, click **SNP Genotyping Assays**. Then under Real Time PCR-based Assays click the **TaqMan SNP Geotyping Assays** link to reach the TaqMan SNP Genotyping Assays product page.
2.	Select **Search**, then enter your search parameters. You can search by: • Gene name • SNP ID • Assay ID • Assay type • SNP type • Minor Allele Frequency thresholds in selected populations
3.	Select **Search**.
4.	From the search results list, which provides annotation for assays and SNPs, select assays to add to your shopping basket. **Note:** You must be logged on to add assays to your shopping basket.
5.	View your shopping basket to select assay scales and complete your order.

Appendix C: User-Supplied Materials and Equipment

The following tables list materials and equipment required for using the TaqMan® SNP Genotyping Assays.

These items are not supplied with the TaqMan SNP Genotyping Assays. Unless otherwise noted, the listed items are available from major laboratory suppliers (MLSs).

Instruments

Instrument	Source
Thermal Cyclers	
Applied Biosystems 9800 Fast Thermal Cycler	Contact your local Applied Biosystems sales office.
GeneAmp® PCR System 9700	
Real-Time PCR Systems	
Applied Biosystems 7900HT Fast Real-Time PCR System‡	Contact your local Applied Biosystems sales office.
Applied Biosystems 7500 Fast Real-Time PCR System	
Applied Biosystems 7500 Real-Time PCR System	
Applied Biosystems 7300 Real-Time PCR System	
ABI PRISM® 7000 Sequence Detection System	

‡ *Applied Biosystems 7900HT Fast Real-Time PCR System* refers to all 7900HT instruments, regardless of the sample block used (Fast or standard).

Reaction Plates and Covers

The table below lists the reaction plates and covers you can use to perform Taqman SNP Genotyping Assays and the instruments on which each reaction plate and cover can be used.

Reaction Plate and Cover	Applied Biosystems Part Number	Compatible Instrument
96-Well Plates		
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 20 plates	4306737	• 9700 instrument • 7900HT Fast instrument, standard blocks • 7500 instrument • 7300 instrument • 7000 instrument
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 500 plates	4326659	
MicroAmp™ Optical 96-Well Reaction Plates and Optical Caps, 20 plates/2400 caps	403012	
MicroAmp™ Optical 96-Well Reaction Plate with Barcode and Optical Adhesive Films, 100 plates/100 covers	4314320	
MicroAmp™ Optical 96-Well Reaction Plate, 10 plates	N8010560	
MicroAmp™ Optical 96-Well Reaction Plate, 500 plates	4316813	
MicroAmp™ Fast Optical 96-Well Thermal Cycling Plate with Barcode, 20 plates	4346906	• 9800 instrument • 7900HT Fast instrument • 7500 Fast instrument
MicroAmp™ Fast Optical 96-Well Thermal Cycling Plate with Barcode, 200 plates	4366932	
384-Well Plates		
MicroAmp™ Optical 384-Well Reaction Plate with Barcode, 50 plates	4309849	• 9700 instrument • 7900HT Fast instrument, standard blocks
MicroAmp™ Optical 384-Well Reaction Plate with Barcode, 500 plates	4326270	
MicroAmp™ Optical 384-Well Reaction Plate, No Barcode, 1000 plates	4343370	
MicroAmp™ Optical 384-Well Reaction Plate with Barcode, 1000 plates	4343814	

Reaction Plate and Cover	Applied Biosystems Part Number	Compatible Instrument
Covers		
MicroAmp™ Optical Adhesive Film Kit, 20 covers	4313663	• 9800 instrument • 9700 instrument • 7900HT Fast instrument • 7500/7500 Fast instrument • 7300 instrument • 7000 instrument
MicroAmp™ Optical Adhesive Film, 100 covers	4311971	
MicroAmp™ Optical Adhesive Film, 25 films	4360954	
MicroAmp™ Optical 8-Cap Strip, 8 caps/strip, 300 strips	4323032	• 9800 instrument • 9700 instrument • 7900HT Fast instrument, standard blocks • 7500 instrument • 7300 instrument
MicroAmp™ Clear Adhesive Film, 100 films	4306311	• 9800 instrument • 9700 instrument
MicroAmp™ Optical 8-Tube Strip (0.2 mL), 125 strips	4316567	• 9700 instrument • 7500 instrument • 7300 instrument

Reagents

Reagent	Source
Dilution of 40× or 80× SNP Genotyping Assay	
TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0, made using DNase-free, sterile-filtered water)	MLS (Major Laboratory Supplier)
Sample Preparation: gDNA Quantification	
TaqMan® DNA Template Reagents Kit	Applied Biosystems (PN 401970)
TaqMan® RNase P Detection Reagents Kit	Applied Biosystems (PN 4316831)
PCR Amplification	
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)
Various Procedures	
DNase-free, sterile-filtered water	MLS

Other Equipment and Consumables

Item	Source
Centrifuge with plate adapter	MLS
Microcentrifuge	MLS
Vortexer	MLS
2-D barcode reader	Consumables suppliers
MicroAmp™ Cap Installing Tool	Applied Biosystems (PN 4330015)
MicroAmp™ Multi Removal Tool	Applied Biosystems (PN 4313950)
Polypropylene tubes	MLS
Pipettors: • Positive-displacement • Air-displacement • Multichannel • Robotic pipetting station	MLS
Pipette tips, aerosol-resistant	MLS
Disposable gloves	MLS

Documents

Document	Applied Biosystems Part Number
ABI PRISM® 7000 Sequence Detection System User Guide	4330228
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Allelic Discrimination Getting Started Guide	4347822
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Online Help	NA
Applied Biosystems 7900HT Fast Real-Time PCR System Allelic Discrimination Getting Started Guide	4364015
Custom TaqMan® Genomic Assays Protocol: Submission Guidelines	4367671
Real-Time PCR Systems Chemistry Guide	4348358
Sequence Detection Systems Software Online Help for the Applied Biosystems 7900HT Fast Real-Time PCR System	NA
TaqMan® Universal PCR Master Mix Protocol	4304449

Software

Software	Source
SDS Software v1.2.3 or later for the 7000 System	Contact your local Applied Biosystems sales office
SDS Software v1.3.1 or later for the 7300/7500/7500 Fast Systems	
SDS Software v2.2.2 or later for the 7900HT Fast System	
SNPbrowser™ Software (available at **http://www.allsnps.com/snpbrowser**)	Free from Applied Biosystems
Microsoft® Excel software or equivalent spreadsheet software	Software suppliers

Appendix D: Chemistry Overview

Assay Components

Each TaqMan® SNP Genotyping Assay consists of a single tube containing:

- Two primers for amplifying the polymorphic sequence of interest
- Two TaqMan® MGB probes for distinguishing between the two 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) at the 3′ end of each probe. 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. Shorter probes result in greater differences in T_m values between matched and mismatched probes, producing robust allelic discrimination.

  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.
- A nonfluorescent quencher (NFQ) at the 3′ end of each probe. Because the quencher does not fluoresce, Applied Biosystems Real-Time PCR Systems can measure reporter dye contributions with greater sensitivity than with TAMRA® dye quencher-based probes.

5′ Nuclease Assay

During PCR, the following steps occur:

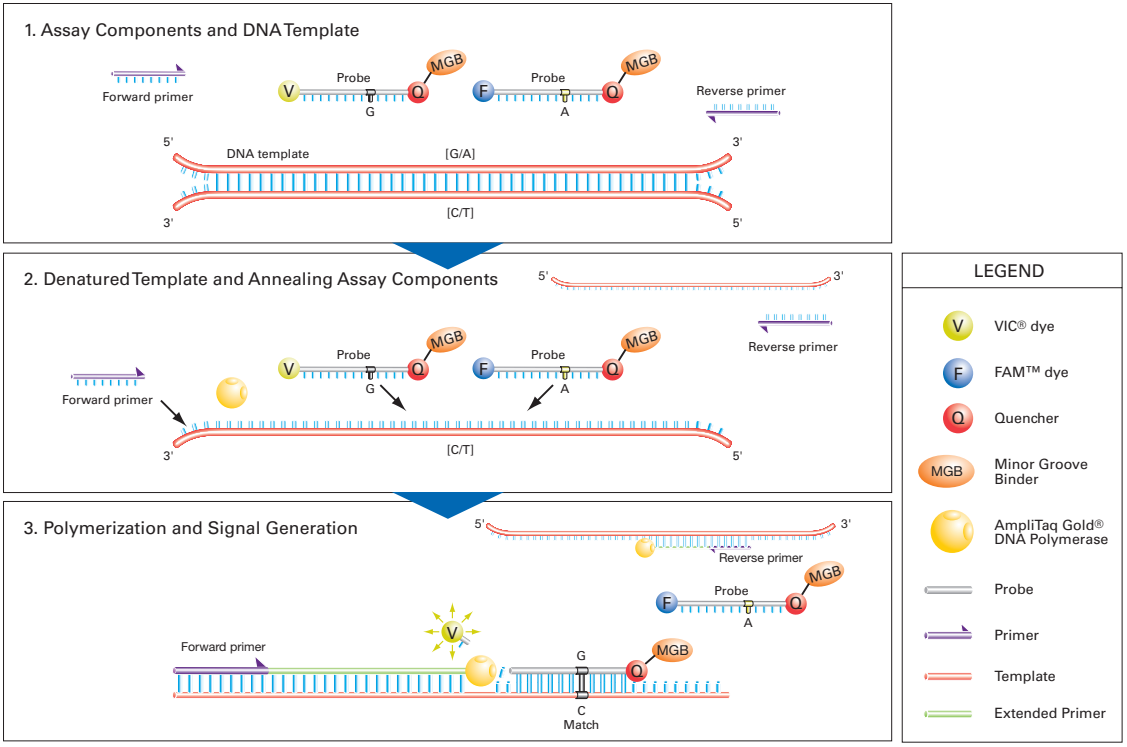
1. Each TaqMan MGB probe anneals specifically to its complementary sequence between the forward and reverse primer sites.

2. 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).
3. AmpliTaq Gold® DNA polymerase extends the primers bound to the template DNA.
4. AmpliTaq Gold DNA polymerase cleaves only probes that are hybridized to the target.
5. Cleavage separates the reporter dye from the quencher dye, which results in increased fluorescence by the reporter.
6. 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.

   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-dye fluorescence	Allele 1-Allele 2 heterozygosity

The figure on page 43 illustrates the 5′ nuclease assay process.



5′ Nuclease assay process

Appendix E: 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:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes and reaction plates 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.
- If you must open a sealed reaction plate, use TaqMan® Universal PCR Master Mix containing AmpErase® UNG (uracil-N-glycosylase) to minimize the risk of carryover contamination.

About AmpErase UNG

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 an 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.

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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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