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## Preface

## How to Use This Guide

Purpose of This Guide	This guide provides procedures for conducting allelic discrimination assays using the Applied Biosystems 7900HT Fast Real-Time PCR System (7900HT Fast System).
Audience	This guide is intended for principal investigators and laboratory staff who conduct allelic discrimination assays using the 7900HT Fast System.
Assumptions	This guide assumes that you have:
	<ul> <li>Familiarity with Microsoft Windows<sup>®</sup> XP operating system.</li> <li>Knowledge of general techniques for handling DNA samples and preparing them for PCR.</li> <li>A general understanding of hard drives and data storage, file transfers, and copying and pasting.</li> <li>Networking experience, if you plan to integrate the 7900HT Fast System into your existing laboratory data flow</li> </ul>
Text Conventions	<ul> <li>This guide uses the following conventions:</li> <li>Bold indicates user action. For example: Type 0, then press Enter for each of the remaining fields.</li> <li><i>Italic</i> text indicates new or important words and is also used for emphasis. For example: Before analyzing, <i>always</i> prepare fresh matrix.</li> <li>A right arrow bracket (&gt;) separates successive commands you select from a drop-down or shortcut menu. For example: Select File &gt; Open.</li> </ul>
User Attention Words	The following user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below: <b>Note</b> – Provides information that may be of interest or help but is not critical to the use of the product.

**IMPORTANT!** – Provides 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.

**Safety** Follow specific safety practices when using this instrument. For safety guidelines, refer to the "Safety and EMC Compliance" section in the *Applied Biosystems 7900HT Fast Real-Time PCR System Site Preparation Guide* (PN 4351923).

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- 1. Go to www.appliedbiosystems.com, click Support, then click MSDS Search.
- **2.** In the Keyword Search field, enter the chemical name, product name, MSDS 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
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- 4. To have a copy of the document sent by fax or e-mail:
  - a. Select the Fax or Email checkbox beneath the document title.
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  - c. Enter the required information.
  - d. Click View/Deliver Selected Documents Now.

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## How to Obtain More Information

Related Documentation	For more information about using the 7900HT Fast System and performing assays, refer to:
	• Applied Biosystems 7900HT Fast Real-Time PCR System Allelic Discrimination Getting Started Guide (PN 4364015)
	• Applied Biosystems 7900HT Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide (PN 4364014)
	• Applied Biosystems 7900HT Fast Real-Time PCR System Plus-Minus Getting Started Guide (PN 4364017)
	<ul> <li>Applied Biosystems 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C<sub>T</sub> Getting Started Guide (PN 4364016)</li> </ul>
	• Sequence Detection Systems Software version 2.3 Online Help (SDS Online Help)
	• Applied Biosystems 7900HT Fast Real-Time PCR System Maintenance and Troubleshooting Guide (PN 4365542)
	• Applied Biosystems 7900HT Fast Real-Time PCR System Site Preparation Guide (PN 4351923)
	• ABI PRISM <sup>®</sup> 6100 Nucleic Acid PrepStation Users Guide (PN 4326242)
	Assays-by-Design Service for SNP Assays Protocol (PN 4334431)
	• DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs Protocol (PN 4343586)
	<ul> <li>NucPrep<sup>®</sup> Chemistry Protocol: Isolation of Genomic DNA from Animal and Plant Tissue (PN 4333959)</li> </ul>
	• Primer Express <sup>®</sup> Software v3.0 Getting Started Guide (PN 4362460)
	• Real-Time PCR Systems Chemistry Guide (PN 4348358)
	• TaqMan <sup>®</sup> Low Density Array Getting Started Guide (PN 4319399)
	• TaqMan <sup>®</sup> SNP Genotyping Assays Protocol (PN 4332856)
	TaqMan <sup>®</sup> Universal PCR Master Mix Protocol (PN 4304449)
	• TransPrep Chemistry Protocol: Purification of gDNA from Filtrates Obtained After the Isolation of RNA from Homogenized Animal or Plant Tissue Samples (PN 4326965)
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

## How to Obtain Support

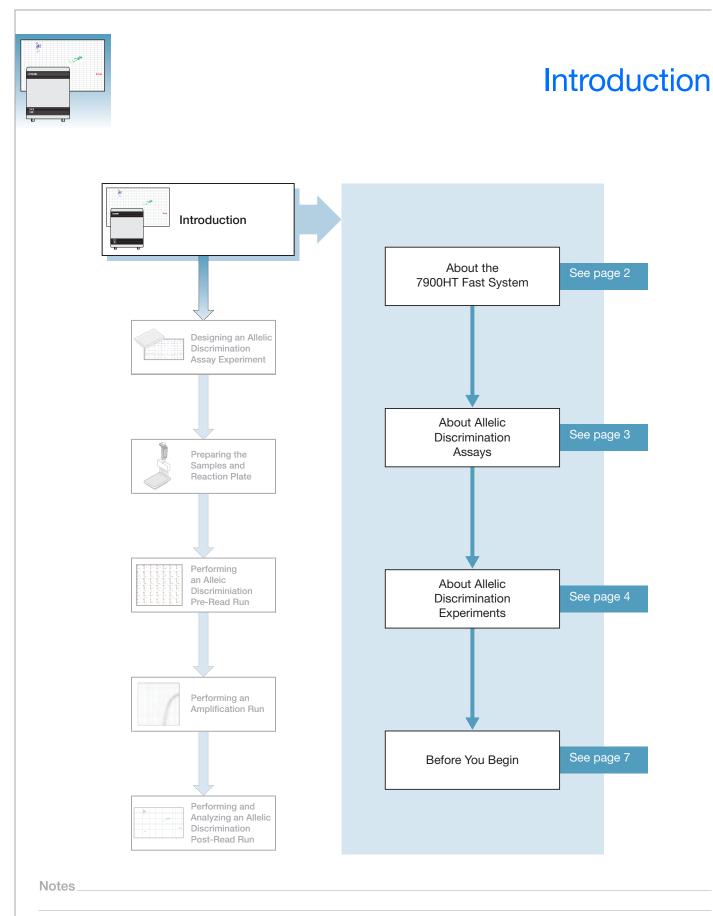
To contact Applied Biosystems Technical Support from North America by telephone, call **1.800.762.4001**.

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:

- Obtain worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities
- 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

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## About the 7900HT Fast System

SystemThe Applied Biosystems 7900HT Fast Real-Time PCR System (7900HT Fast System)Descriptionuses fluorescent-based PCR chemistries to provide:

- Quantitative detection of nucleic acid sequences using real-time analysis
- Qualitative detection of nucleic acid sequences using end-point and dissociation-curve analysis

For more information on the 7900HT Fast System, refer to the *Sequence Detection Systems Software version 2.3 Online Help (SDS Online Help)* and the *Applied Biosystems 7900HT Fast Real-Time PCR System Maintenance and Troubleshooting Guide* (PN 4365542).

**Note:** To access the *SDS Online Help*, select **Help > SDS Online Help** from the SDS software menu bar.

#### Supported Assays and Consumables

You can perform several assay types on the 7900HT Fast System using reactions plates in the 96-well, 384-well, or TaqMan<sup>®</sup> Low Density Array format. This guide describes the allelic discrimination assay.

	Reaction Plate and System Block Options			
Assay Type	Fast 96-well	Standard 96-well	Standard 384-well	TaqMan <sup>®</sup> Low Density Array (TLDA)
Standard curve (AQ)	Yes	Yes	Yes	No
Comparative C <sub>T</sub> (RQ)	Yes	Yes	Yes	Yes
Allelic discrimination	No	Yes	Yes	No
Plus/minus	No	Yes	Yes	No

#### Allelic Discrimination Assay Configuration

Allelic discrimination assays can be run on the 7900HT Fast System using standard 96well and standard 384-well reaction plates with standard reagents and standard protocols.

**IMPORTANT!** Allelic discrimination assays are not supported using Fast reaction plates, Fast reagents, Fast protocols, or the TaqMan<sup>®</sup> Low Density Array.

**IMPORTANT!** Be sure you use standard reaction plates on the 7900HT Fast System with a standard block. Fast 96-well reaction plates do not fit into the standard 96-well block and standard 96-well reaction plates do not fit into the Fast 96-well block.



## **About Allelic Discrimination Assays**

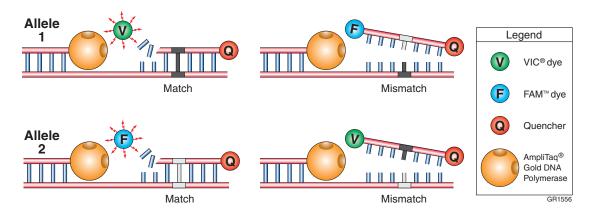
**Definition** An allelic discrimination assay is a multiplexed (more than one primer/probe pair per reaction) end-point (data is collected at the end of the PCR process) assay that detects variants of a single nucleic acid sequence. The presence of two primer/probe pairs in each reaction allows genotyping of the two possible variants at the single-nucleic polymorphism (SNP) site in a target template sequence. The actual quantity of target sequence is not determined.

For each sample in an allelic discrimination assay, a unique pair of fluorescent dye detectors is used, for example, two TaqMan<sup>®</sup> MGB (minor groove binder) probes that target an SNP site (Afonina *et al.*, 1997; Kutyavin *et al.*, 1997). One fluorescent dye detector is a perfect match to the wild type (allele 1) and the other fluorescent dye detector is a perfect match to the mutation (allele 2).

The allelic discrimination assay classifies unknown samples as:

- Homozygotes (samples having only allele 1 or allele 2)
- Heterozygotes (samples having both allele 1 and allele 2)

The allelic discrimination assay measures the change in fluorescence of the dyes associated with the probes. The figure below illustrates results from matches and mismatches between target and probe sequences in TaqMan<sup>®</sup> SNP Genotyping Assays (Livak *et al.*, 1995).



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

A substantial increase in	Indicates	
VIC <sup>®</sup> dye fluorescence only	Homozygosity for allele 1	
FAM <sup>™</sup> dye fluorescence only	Homozygosity for allele 2	
Both fluorescence signals	Heterozygosity allele 1-allele 2	



#### Terms Used in Allelic Discrimination Analysis

No template control (NTC)	A sample that does not contain template. Shows background signal and is used as the negative control. Provides a means of measuring contamination that might give a false positive signal (Kwok and Higuchi, 1989).	
Nucleic acid target (also called "target template" or "target")	Nucleotide sequence that you want to genotype.	
Unknown sample (U; also called "sample of interest")	The sample for which you want to determine the genotype of a specific target	
Passive reference	A dye that provides an internal fluorescence reference to which the reporter dye signal can be normalized during data analysis. Normalization is necessary to correct for fluorescence fluctuations caused by changes in concentration or in volume.	
Reporter dye	The dye attached to the 5' end of a TaqMan <sup>®</sup> probe. Provides a fluorescence signal that indicates specific amplification.	
Normalized reporter (Rn)	The ratio of the fluorescence emission intensity of the reporter dye to the fluorescence emission intensity of the passive reference dye.	

## **About Allelic Discrimination Experiments**

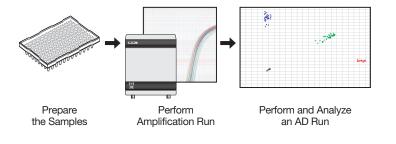
Allelic Discrimination Experiment Workflow After you design an allelic discrimination experiment and prepare the DNA samples, you need to perform:

• An amplification run using a Standard Curve (AQ) plate document to generate real-time PCR data. The real-time PCR data can be used to analyze and troubleshoot the PCR data for the allelic discrimination assay, if needed.

**Note:** The amplification run can be performed either on the 7900HT Fast System or offline using any thermal cycler.

• An allelic discrimination run using an Allelic Discrimination (AD) plate document. The SDS software analyzes the data, then you assign allele calls (automatically or manually).

The following figure illustrates the complete process:





1

Sample A sample experiment representing a typical allelic discrimination experiment is provided in Appendix A on page 73. You can use the summarized procedures of the sample experiment in Appendix A to familiarize yourself with the entire allelic discrimination assay workflow.

References to the sample experiment are provided in Chapter 2 through Chapter 6, where applicable.

#### Required User-Supplied Materials

#### **Chemistry/Reagents**

Item	Source			
Any of the following DNA isolation and purification chemistry systems:				
ABI PRISM <sup>®</sup> 6100 Nucleic Acid PrepStation	Applied Biosystems (PN 6100-01)			
<ul> <li>ABI PRISM<sup>®</sup> TransPrep System (purification of gDNA after isolation of RNA from animal and plant tissue)</li> </ul>	Applied Biosystems web site			
<ul> <li>BloodPrep<sup>™</sup> Chemistry (genomic DNA from fresh or frozen blood)</li> </ul>	Applied Biosystems (PN 4346860)			
<ul> <li>NucPrep<sup>®</sup> Chemistry (DNA from animal and plant tissue)</li> </ul>	Applied Biosystems (PN 4340274)			
TaqMan <sup>®</sup> reagents appropriate for your probes and pr	rimers:			
For TaqMan <sup>®</sup> SNP Genotyping Assays, Custom TaqMan <sup>®</sup> SNP Genotyping Assays, and custom probes/primer design with Primer Express <sup>®</sup> Software, use either:				
<ul> <li>TaqMan<sup>®</sup> Universal PCR Master Mix, No AmpErase<sup>®</sup> UNG, 200 reactions</li> </ul>	Applied Biosystems (PN 4324018)			
TaqMan <sup>®</sup> Universal PCR Master Mix	Applied Biosystems (PN 4304437)			
For TaqMan <sup>®</sup> Pre-Developed Assay Reagents for Allelic Discrimination (TaqMan <sup>®</sup> PDARs for Allelic Discrimination) use:				
TaqMan <sup>®</sup> Universal PCR Master Mix	Applied Biosystems (PN 4304437)			
Labeled primers and probes from one of the following sources:				
TaqMan <sup>®</sup> SNP Genotyping Assays (predesigned primers and probes)	Applied Biosystems web site			
TaqMan <sup>®</sup> PDARs for Allelic Discrimination	Applied Biosystems web site			
<ul> <li>Custom TaqMan<sup>®</sup> SNP Genotyping Assays (predesigned primers and probes)</li> </ul>	Applied Biosystems web site			
<ul> <li>Primer Express<sup>®</sup> Software (custom-designed primers and probes)</li> </ul>	Contact your Applied Biosystems sales representative.			

Notes

Allelic Discrimination Assay Getting Started Guide for the 7900HT Fast System



#### **Reaction Plates and Covers**

**IMPORTANT!** Do not use MicroAmp<sup>®</sup> caps (domed) or Optical Tubes with the 7900HT Fast System. You can use Optical Caps (PN 4323032) *only* on the standard 96-well plates with the 7900HT Fast System.

Item	Source
Standard 96-well reaction plates	
<ul> <li>MicroAmp<sup>®</sup> 96-Well Optical Reaction Plate with Barcode (code 128), 20 plates</li> </ul>	Applied Biosystems (PN 4306737)
<ul> <li>MicroAmp<sup>®</sup> 96-Well Optical Reaction Plate with Barcode (code 128), 25-Pack, 500 plates Includes 25 of PN 4306737, MicroAmp<sup>®</sup> 96-Well Optical Reaction Plates with Barcode</li> </ul>	Applied Biosystems (PN 4326659)
<ul> <li>MicroAmp<sup>®</sup> 96-Well Optical Reaction Plate with Barcode (code 128) and ABI PRISM<sup>®</sup> Optical Adhesive Covers, 100 plates/100 covers</li> <li>Includes 100 ABI PRISM<sup>®</sup> Optical Adhesive Covers (PN 4311971) and 5 of PN 4306737, MicroAmp<sup>®</sup> 96-Well Optical Reaction Plates with Barcode</li> </ul>	Applied Biosystems (PN 4314320)
<ul> <li>MicroAmp<sup>®</sup> Splash Free Support Base for 96- Well Reaction Plates, 10 bases</li> </ul>	Applied Biosystems (PN 4312063)
Standard 384-well reaction plates	
<ul> <li>384-Well Clear Optical Reaction Plate with Barcode (code 128), 50 plates</li> </ul>	Applied Biosystems (PN 4309849)
<ul> <li>384-Well Clear Optical Reaction Plate with Barcode (code 128), 10-Pack, 500 plates</li> <li>Includes 10 of PN 4309849, 384-Well Clear</li> <li>Optical Reaction Plates with Barcode</li> </ul>	Applied Biosystems (PN 4326270)
Optical adhesive covers	
<ul> <li>ABI PRISM<sup>®</sup> Optical Adhesive Cover Starter Kit, 20 covers</li> <li>Includes 20 ABI PRISM<sup>®</sup> Optical Adhesive Covers, an Applicator, and an ABI PRISM<sup>®</sup> Optical Cover Compression Pad.</li> </ul>	Applied Biosystems (PN 4313663)
ABI PRISM <sup>®</sup> Optical Adhesive Covers, 100 covers	Applied Biosystems (PN 4311971)
ABI PRISM <sup>®</sup> Optical Adhesive Covers, 25 covers	Applied Biosystems (PN 4360954)
<ul> <li>Optical Caps, 8 Caps/Strip, 2400 Caps/300 Strips</li> </ul>	Applied Biosystems (PN 4323032)



#### Other Consumables and Equipment

Item	Source	
Centrifuge with adapter for standard 96-well or standard 384-well reaction plates	Major laboratory supplier (MLS)	
Gloves	MLS	
Microcentrifuge	MLS	
Microcentrifuge tubes, sterile 1.5-mL	MLS	
Nuclease-free water	MLS	
Pipette tips, with filter plugs	MLS	
Pipettors, positive-displacement	MLS	
Tris-EDTA (TE) Buffer, pH 8.0	MLS	
Vortexer	MLS	

## **Before You Begin**

Background and Pure Dye Calibrations	Check that background and pure dye calibrations have been performed regularly to ensure optimal performance of the 7900HT Fast System. For more information about background and pure dye calibrations, refer to the <i>Sequence Detection Systems</i> <i>version 2.3 Software Online Help (SDS Online Help)</i> and the <i>Applied Biosystems</i> 7900HT Fast Real-Time PCR System Maintenance and Troubleshooting Guide (PN 4365542).
Accessing the SDS Online Help	Some steps in this chapter refer you to the <i>SDS Online Help</i> for more information. To access the <i>SDS Online Help</i> , select <b>Help &gt; SDS Online Help</b> from the SDS software menu bar.
Automation Options	The 7900HT Fast System can run prepared reaction plates individually or in groups using the Automation Accessory with the Zymark <sup>®</sup> Twister Microplate Handler. If you are not using the Automation Accessory, you must run reaction plates individually.
	For clarity, this chapter only illustrates running an individual reaction plate. For information on running multiple reaction plates, see Appendix B on page 83. For information on automated operation of the 7900HT Fast System using the Automation Accessory, see the <i>SDS Online Help</i> .

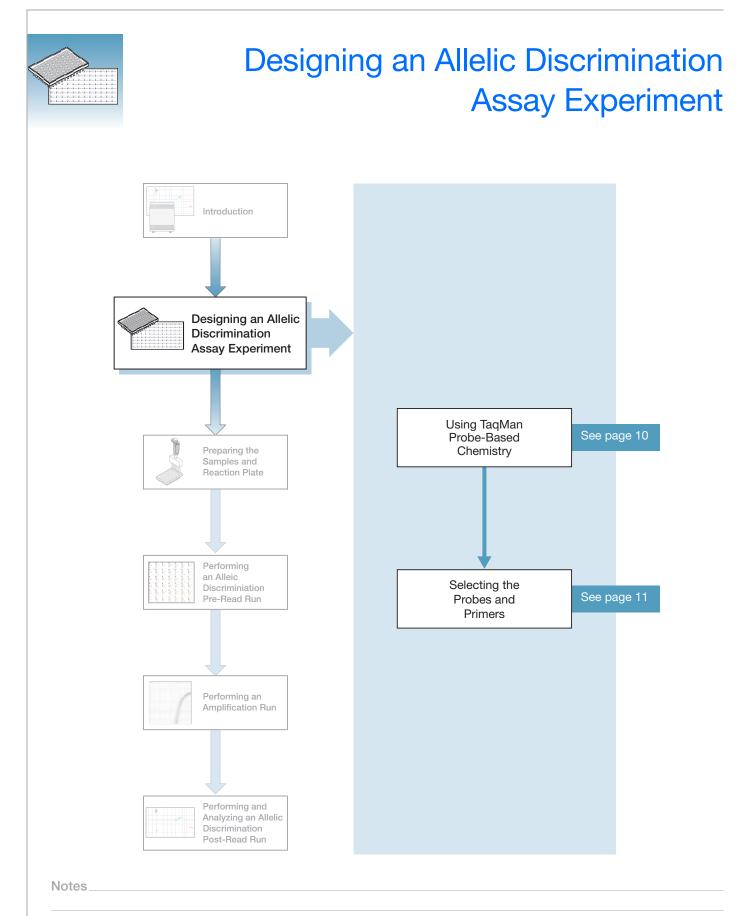
Notes

1



Chapter 1 Introduction Before You Begin

2





## Using TaqMan Probe-Based Chemistry

About the Allelic discrimination assays use the fluorogenic 5' nuclease chemistry (also known as TaqMan<sup>®</sup> probe-based chemistry).

**IMPORTANT!** The SYBR<sup>®</sup> Green I dye chemistry and Fast chemistry are not supported for allelic discrimination assays.

Description		Process		
TaqMan probe-based chemistry uses a fluorogenic probe to detect specific PCR product as it accumulates during PCR cycles (Lee <i>et al.,</i> 1993).		Step 1: Polymerization A reporter (R) and a quencher (Q) are attached to the 5' and 3' ends of a TaqMan probe.	Step 2: Strand Displacement When both dyes are attached to the probe, reporter dye emission is quenched.	
		Step 3: Cleavage During each extension cycle, the Applied Biosystems hot-start DNA polymerase system cleaves the reporter dye from the probe.	Step 4: Polymerization Completed After being separated from the quencher, the reporter dye emits its characteristic fluorescence.	
Two Types of TaqMan ProbesApplied Biosystems offers two types of TaqMan probes: <ul><li>TaqMan probes with TAMRA<sup>™</sup> dye as a quencher</li><li>TaqMan<sup>®</sup> MGB (minor groove binder) probes with non-fluorescent quencher              (NFQ)</li><li>For more information about TaqMan probe-based chemistry, refer to the <i>Real-Time PCR</i>              Systems Chemistry Guide (PN 4348358).</li></ul>				
Chemistry Kits for Allelic				

Allelic Discrimination Assays (PN 4324018)

• TaqMan<sup>®</sup> Universal PCR Master Mix (PN 4304437, contains AmpErase<sup>®</sup> UNG)



### **Selecting the Probes and Primers**

Each allelic discrimination primer/probe set contains two probes, one probe for allele 1 and one probe for allele 2. Applied Biosystems provides three options for selecting primers and probes:

- TaqMan<sup>®</sup> SNP Genotyping Assays Provide biologically informative, fully validated, or predesigned TaqMan<sup>®</sup> MGB-probe-based assays for genotyping single nucleotide polymorphisms (SNPs). For information on available TaqMan<sup>®</sup> SNP Genotyping Assays:
  - a. Go to http://www.appliedbiosystems.com.
  - **b.** Click the **myScience** tab at the top of the page to go to the myScience Genomic Products page.
  - c. In the Genotyping section, click the TaqMan<sup>®</sup> SNP Genotyping Assays Search option.
  - d. Use specific filtering criteria to search for the assay of interest.
- **2.** Custom TaqMan<sup>®</sup> SNP Genotyping Assays Designs, synthesizes, formulates, and delivers quality-controlled primer and probe sets. Use this service if the assay you need is not currently available. For ordering information:
  - a. Go to http://www.appliedbiosystems.com.
  - **b.** Click the **myScience** tab at the top of the page to go to the myScience Genomic Products page.
  - **c.** In the Genotyping section, click the Custom TaqMan<sup>®</sup> SNP Genotyping Assays **Info** option.
- **3.** TaqMan<sup>®</sup> Pre-Developed Assay Reagents for Allelic Discrimination (TaqMan<sup>®</sup> PDARs for Allelic Discrimination) Provide optimized assays for the discrimination of specific alleles. For ordering information:
  - a. Go to http://www.appliedbiosystems.com.
  - b. In the Search section, select All Sections, type PDAR, then click Go.
  - c. In the Search Results section, click TaqMan<sup>®</sup> Pre-Developed Assay Reagents for Allelic Discrimination.
  - d. Click the Ordering Information tab.



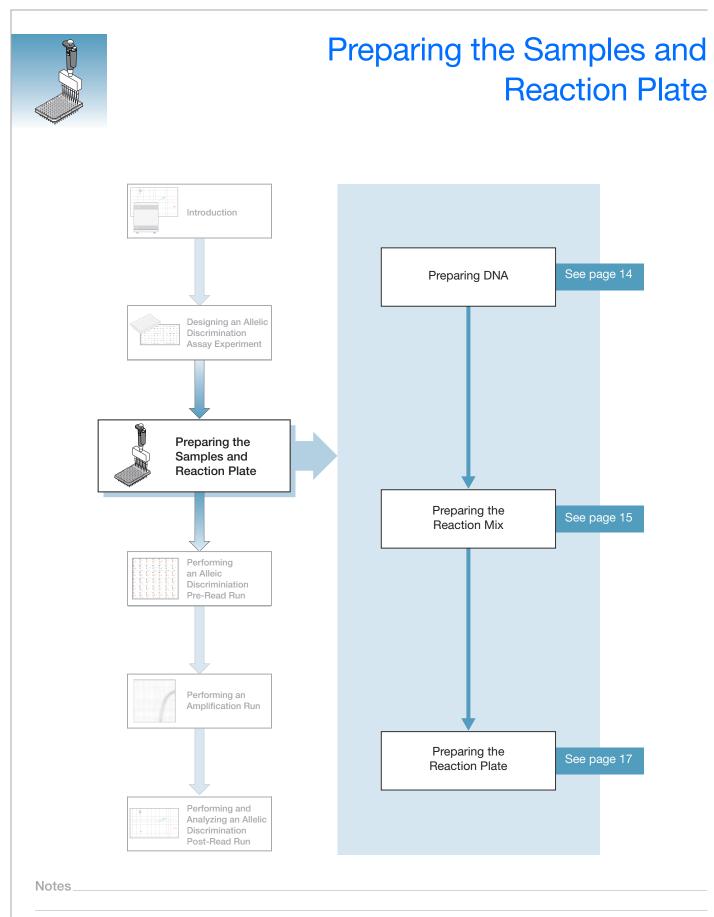
**4. Primer Express**<sup>®</sup> **Software** – Helps you design primers and probes for your own assays. For more information about using this software, refer to the *Primer Express*<sup>®</sup> *Software v3.0 Getting Started Guide* (PN 4362460).

Applied Biosystems provides Assay Design Guidelines, which have been developed specifically for quantification assays (pertinent to the amplification step in allelic discrimination assays). When used in their entirety, these steps provide a rapid and reliable system for assay design and optimization. For information about the Assay Design Guidelines, refer to the *Real-Time PCR Systems Chemistry Guide* (PN 4348358).

#### Sample Experiment

The objective of the sample experiment was to determine the genotype of 92 individuals at the SNP site of a desired target sequence.

The primers and probes were ordered from TaqMan<sup>®</sup> SNP Genotyping Assays (AB Assay ID C 2984390 10). The probe for allele 1 (AL-1) was labeled with VIC<sup>®</sup> dye; the probe for allele 2 (AL-2) was labeled with FAM<sup>™</sup> dye.





## **Preparing DNA**

Systems and Chemistries for DNA Isolation Applied Biosystems supplies several instrument systems and chemistries for DNA isolation from a variety of starting materials, such as blood, tissue, cell cultures, and plant material.

System	Part Number
ABI PRISM <sup>®</sup> 6100 Nucleic Acid PrepStation	6100-01
ABI PRISM <sup>®</sup> TransPrep System (purification of gDNA after isolation of RNA from animal and plant tissue)	Applied Biosystems web site
BloodPrep <sup>™</sup> Chemistry (genomic DNA from fresh or frozen blood)	4346860
NucPrep <sup>®</sup> Chemistry (DNA from animal and plant tissue)	4340274

For more information, refer to:

- ABI PRISM<sup>®</sup> 6100 Nucleic Acid PrepStation Users Guide (PN 4326242)
- TransPrep Chemistry Protocol: Purification of gDNA from Filtrates Obtained After the Isolation of RNA from Homogenized Animal or Plant Tissue Samples (PN 4326965)
- DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs Protocol (using BloodPrep<sup>™</sup> Chemistry, PN 4343586)
- NucPrep<sup>®</sup> Chemistry Protocol: Isolation of Genomic DNA from Animal and Plant Tissue (PN 4333959)

**Quality of DNA** Ensure that the DNA you use for allelic discrimination assay experiments:

- Is extracted from the raw material you are testing with an optimized protocol
- Does not contain PCR inhibitors
- Has an  $A_{260/280}$  ratio greater than 1.7
- Is intact as visualized by gel electrophoresis
- Has not been heated above 60 °C, which can cause degradation

#### Sample Experiment

In the sample experiment, genomic DNA was isolated from blood using:

- A BloodPrep<sup>™</sup> Chemistry Kit
- The recommended template for TaqMan<sup>®</sup> SNP Genotyping Assays: purified genomic DNA (1 to 20 ng). The final concentration of genomic DNA for all samples in the sample experiment was 10 ng/μL.



## **Preparing the Reaction Mix**

**Reagents and** Allelic discrimination assays can be run on the 7900HT Fast System using standard reagents and standard protocols.

**IMPORTANT!** Allelic discrimination assays are not supported using Fast reagents or Fast protocols.

#### **Custom-Designed Assays**

If you use the Primer Express<sup>®</sup> Software to design probes and primers for your SNP genotyping assay, follow instructions in the *TaqMan*<sup>®</sup> Universal PCR Master Mix Protocol (PN 4304449) and the Real-Time PCR Systems Chemistry Guide (PN 4348358) to optimize primer and probe concentrations.

If you obtain your assay from the Custom TaqMan<sup>®</sup> SNP Genotyping Assays, follow instructions in the protocol for Custom TaqMan<sup>®</sup> SNP Genotyping Assays: *Assays-by-Design Service for SNP Assays Protocol* (PN 4334431).

### TaqMan PDARs for Allelic Discrimination

TaqMan PDARs for Allelic Discrimination require only three components:

- Genomic DNA sample
- Allelic Discrimination Assay Mix (10X), specific for each polymorphism
- TaqMan<sup>®</sup> Universal PCR Master Mix (2×)

**Note:** Allele 1 and 2 control DNA is included with each assay, allowing each homozygote signal to be generated on each run.

For instructions on how to use TaqMan PDARs for Allelic Discrimination, refer to the *Pre-Developed TaqMan® Assay Reagents Allelic Discrimination Protocol* (PN 4312214).

#### TaqMan SNP Genotyping Assays

The allelic discrimination reaction mix contains:

- SNP Genotyping Assay Mix (20×)
- Master mix:
  - TaqMan® Universal PCR Master Mix, No AmpErase® UNG, or
  - TaqMan<sup>®</sup> Universal PCR Master Mix

Notes\_

Allelic Discrimination Assay Getting Started Guide for the 7900HT Fast System



The reagents, volumes, and final concentrations provided below are for wet DNA samples and are excerpted from the *TaqMan*<sup>®</sup> *SNP Genotyping Assays Protocol* (PN 4332856).

**Note:** If you are using dried-down DNA samples, refer to the *TaqMan*<sup>®</sup> *SNP Genotyping Assays Protocol* (PN 4332856) for instructions on preparing the reaction mix.

## Preparing the Reaction Mix

**1.** Calculate the number of reactions to be performed for each assay.

**2.** Calculate the volume of components needed for all wells on the reaction plate:

	Volume (μl	/reaction)
Component	Standard 96-Well Reaction Plate	Standard 384-Well Reaction Plate
<ul> <li>TaqMan<sup>®</sup> Universal PCR Master Mix (2×), No AmpErase<sup>®</sup> UNG, <i>or</i></li> <li>TaqMan<sup>®</sup> Universal PCR Master Mix (2×)</li> </ul>	12.50	2.50
SNP Genotyping Assay Mix (20×)	1.25	0.25
Total	13.75	2.75

Note: Prepare extra volume to account for pipetting losses.

**3.** Swirl the bottle of master mix gently to resuspend.

**CAUTION** CHEMICAL HAZARD. TaqMan<sup>®</sup> Universal PCR Master Mix (2×), No AmpErase<sup>®</sup> 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.

**CAUTION** CHEMICAL HAZARD. TaqMan<sup>®</sup> Universal PCR Master Mix (2×) 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

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

WARNING CHEMICAL HAZARD. SNP Genotyping Assay Mix (20×) 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.



3

**5.** Pipette the volumes required for all wells on the reaction plate (plus extra volume to account for pipetting losses) of master mix and SNP Genotyping Assay Mix (20×) into a microcentrifuge tube. Cap the tube.

#### Sample Experiment

For the sample experiment:

- The reaction mix was prepared with TaqMan<sup>®</sup> Universal PCR Master Mix (2×), No AmpErase<sup>®</sup> UNG.
- Volumes were calculated for a standard 384-well reaction plate. Enough reaction mix was prepared for 4 NTCs and 92 samples/unknowns, plus 10% extra to account for pipetting losses.

Component	Volume (μL) for One Reaction	Volume (µL) for 106 Reactionsª
TaqMan <sup>®</sup> Universal PCR Master Mix (2X), No AmpErase <sup>®</sup> UNG	2.5	265.0
SNP Genotyping Assay Mix (20×)	0.25	26.5
Total	2.75	291.5
a. Extra volume was included to account for pipe	tting losses.	·

### **Preparing the Reaction Plate**

Reaction Plate Formats	Allelic discrimination assays can be run on the 7900HT Fast System using standard 96- well and standard 384-well reaction plates.
	<b>IMPORTANT!</b> Allelic discrimination assays are not supported using Fast reaction plates or the TaqMan <sup>®</sup> Low Density Array.
Reaction Plate Components	<ul> <li>A reaction plate for an allelic discrimination assay contains the following:</li> <li>Reaction mix</li> <li>No Template Controls (NTCs)</li> <li>Samples: Unknown genomic DNA</li> <li>Nuclease-free water</li> <li>Controls (optional): Known genomic DNA</li> <li>The recommended final reaction volumes are:</li> <li>25 μL for a standard 96-well reaction plate</li> <li>5 μL for a standard 384-well reaction plate</li> </ul>



Preparing the Reaction Plate

- **1.** Invert the reaction mix tube prepared in the previous section to mix.
- 2. Centrifuge the tube briefly to spin down the contents and to eliminate air bubbles.
  - **3.** Pipette the appropriate reaction mix volume into each well of the reaction plate:
    - 13.75  $\mu$ L per well for a standard 96-well reaction plate
    - + 2.75  $\mu$ L per well for a standard 384-well reaction plate
  - **4.** Dilute 1 to 20 ng of each sample into nuclease-free water for a total sample volume of:
    - 11.25 µL per well for a standard 96-well reaction plate
    - $2.25 \,\mu\text{L}$  per well for a standard 384-well reaction plate

#### Sample Experiment

For the sample experiment, the recommended template for TaqMan<sup>®</sup> SNP Genotyping Assays was used: purified genomic DNA (1 to 20 ng). The final concentration of genomic DNA for all samples in the sample experiment was 10 ng/ $\mu$ L.

**5.** Pipette the following volumes of NTC, sample, and (if using) control into the appropriate wells of your reaction plate:

	Volume (μL/reaction)			
Component	Standard 96-Well Reaction Plate	Standard 384-Well Reaction Plate		
NTC: Nuclease-free water or TE (Tris-EDTA) buffer	11.25	2.25		
Sample: Unknown genomic DNA	11.25	2.25		
<i>Optional</i> Control: Known genomic DNA	11.25	2.25		

**Note:** Include NTCs on each reaction plate for both manual evaluation and optimal auto-calling of allelic discrimination assay data. If available, known genomic DNA controls may be included and used to corroborate assay performance.

**IMPORTANT!** Use a calibrated, positive-displacement pipettor to minimize contamination and error. Change tips between samples to prevent cross-contamination.

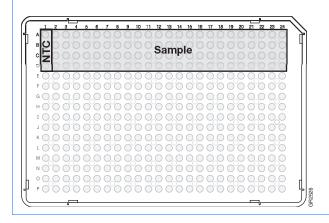


#### Sample Experiment

To set up the reaction plate for the sample experiment:

- $2.75 \,\mu\text{L}$  of reaction mix were pipetted into each well of a standard 384-well reaction plate.
- 2.25  $\mu L$  of NTC or sample/unknown were added to the wells, as shown below.

To Prepare	We added	To wells
NTC	$2.25\mu L$ of nuclease-free water or TE buffer	A1, B1, C1, and D1
Sample or Unknown	2.25 $\mu L$ of diluted purified genomic DNA	A2 to A24
		B2 to B24
		C2 to C24
		D2 to D24



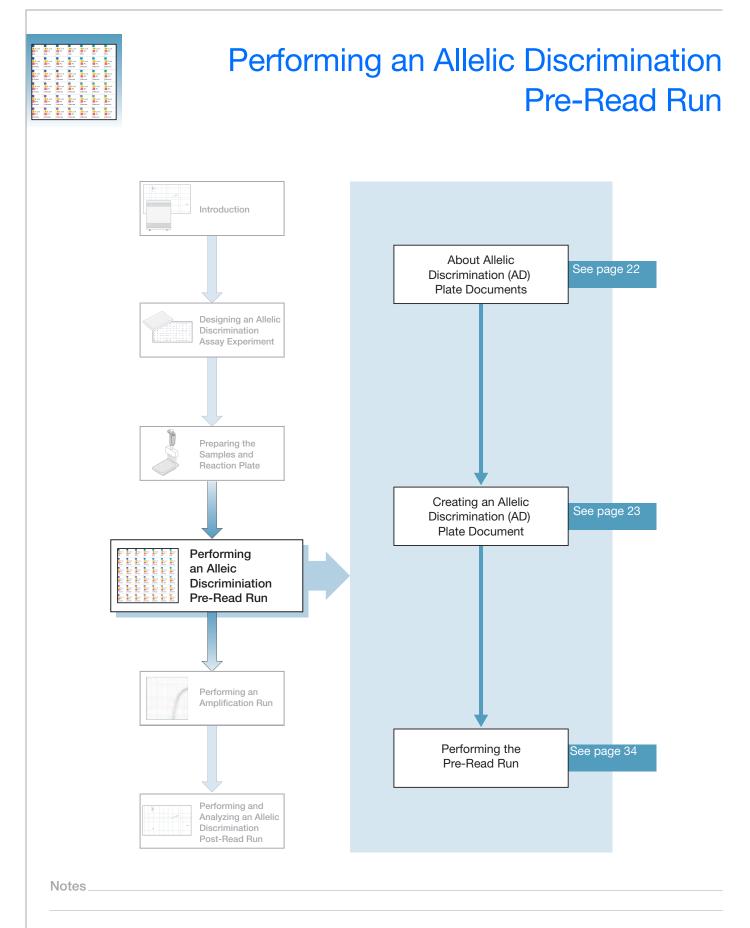
**6.** Cover the reaction plate with an optical adhesive cover or Optical Caps.

**IMPORTANT!** Do not use MicroAmp<sup>®</sup> caps (domed) or Optical tubes with the 7900HT Fast System. You can use Optical Caps (PN 4323032) *only* on the standard 96-well plates with the 7900HT Fast System.

7. Keep the reaction plate on ice until loading in the 7900HT Fast System.



Chapter 3 Preparing the Samples and Reaction Plate Preparing the Reaction Plate





## About Allelic Discrimination (AD) Plate Documents

An Allelic Discrimination (AD) plate document is an SDS software document that stores data collected from an allelic discrimination assay run for a single reaction plate (standard 96-well or standard 384-well reaction plate). Allelic Discrimination (AD) plate documents also store other information about the run, including sample names and detectors.

## Plate Document<br/>ParametersWhen you create an Allelic Discrimination (AD) plate document, you define specific<br/>parameters for each allelic discrimination assay reaction plate:

- **Detectors** A virtual representation in the SDS software of a TaqMan<sup>®</sup> probe and primer set and associated fluorescent dye that detects a single target nucleic acid sequence.
- Markers A set of two detectors that discriminate between different alleles of a common locus. Allele 1 is detected by one detector (for example, FAM<sup>™</sup> dye), and allele 2 is detected by the second detector (for example, VIC<sup>®</sup> dye).
- **Tasks** A setting that you apply to the markers in a well of a plate document, which determines the way the SDS software uses the data collected from the well during analysis. Allelic Discrimination (AD) plate document markers use two types of tasks:

Task	Symbol	Apply to markers of wells that contain
Unknown	0	PCR reagents for the amplification of target sequences
No Template Control (NTC)	N	no target template

Options for<br/>Creating a Plate<br/>DocumentThis section describes how to create a plate document using the New Plate Document<br/>Wizard. For information about other ways to create plate documents, see the SDS Online<br/>Help.

# Reaction Plate<br/>OptionsThe allelic discrimination assay can be run on either a standard 96-well reaction plate or<br/>a standard 384-well reaction plate. For clarity, this chapter only illustrates running a<br/>standard 384-well reaction plate.



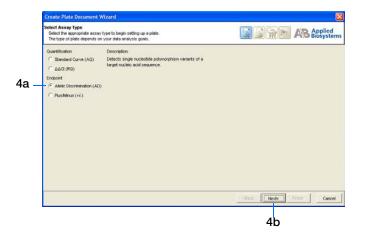
## **Creating an Allelic Discrimination (AD) Plate Document**

- If it is not already running, double-click on the desktop (or select Start > All Programs > Applied Biosystems > SDS 2.3 > SDS 2.3) to start the SDS software.
- 2. If your System Administrator has enabled the login option, the Login dialog box appears. Enter your User Name and Password, then click OK.

**Note:** If the login option is not enabled, no Login dialog box appears. Skip to step 3 below.

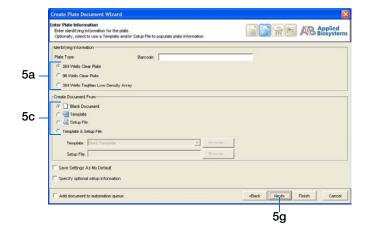
- **3.** In the SDS software menu bar, click *(or select File > New Plate Wizard)* to open the Create Plate Document Wizard.
- **4.** Select the assay type:
  - a. Select Allelic Discrimination (AD).
  - b. Click Next>.

Login		X
User Name:		
	OK Cancel	



- **5.** Enter the plate information:
  - a. Select a Plate Type: **384 Wells Clear Plate** or **96 Wells Clear Plate**.
  - **b.** *Optional.* In the Barcode field, scan or enter the barcode for the reaction plate.

**Note:** The Automation Accessory must be installed in order to use barcodes. A barcode is required if you are adding the plate document to the automation queue. See step f on page 24.





**c.** In the Create Document From pane, select your preferred source.

Note: You can create a plate document from scratch (select **Blank Document**) or you can create a plate document from a template and/or an existing plate document (select **Template**, **Setup File**, or **Template** & **Setup File**). For more information, see the *SDS Online Help*.

- d. *Optional.* Check Save Settings As My Default. Check this option if you want the SDS software to automatically apply the current page settings every time you use the Create Plate Document Wizard.
- e. *Optional.* Check **Specify optional setup information**. Check this option if you want to enter a name, study, or comments for the plate document.
- f. *Optional.* Check Add document to automation queue. Check this option if you want to add the finished plate document to the Automation Controller software plate queue.

**Note:** This checkbox is only enabled if you are connected to an Automation Accessory. For more information, see Appendix B on page 83.

**Note:** This checkbox appears on subsequent pages of the Create Plate Document Wizard. You only need to select this option on one page to enable it.

g. Click Next>.



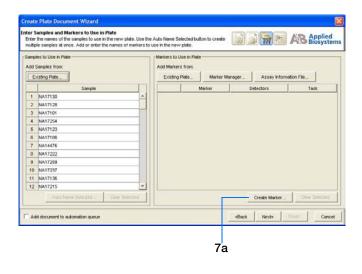
- **6.** Enter the samples to use in the plate:
  - **a.** Double-click in the Sample field to activate the cursor.
  - **b.** Type a sample name (for example, **NTC**), then press **Enter**. The sample row is numbered and another row appears.
  - **c.** Repeat steps a through b for your remaining samples.

**Note:** You may also copy sample names from an existing plate document (click **Existing Plate**) or enter multiple sample names at once (click **Auto-Name Selected**). For more information, see the *SDS Online Help*.

	Create Plate Document Wizard				×
	Enter Samples and Detectors to Use in Plate Enter the names of the samples to use in the new plate. Use th multiple samples at once. Add or enter the names of detectors		on to create	🖬 🖻 🗛	oplied osystems
	Samples to Use in Plate Add Samples from:	Add Detectors from:			
	Existing Plate	Existing Plate	Detector Manager or Reporter	Assay Information File Task Oty	(unit)
6a -					
	Auto Neme Selected Over Selected		_Cre	the Detector	electeu
	C Add document to automation queue		<back< th=""><th>Nexts</th><th>Cancel</th></back<>	Nexts	Cancel
Add	ples to Use in Plate Samples from: Existing Plate				
	Sample				
1	NTC		6b a	nd 6c	
2	NA17204		-002		
*					

- **7.** Enter markers to use in the plate:
  - **a.** Click **Create Marker** to open the Marker Information dialog box.

Note: You can also add existing markers from other sources (click Existing Plate, Marker Manager, or Assay Information File). For more information, see the *SDS Online Help*.



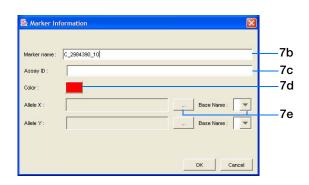


b. Enter a Marker name (for example, C\_2984390\_10).

**Note:** The marker name must be unique and it should reflect the locus or polymorphism it targets.

- c. Leave the Assay ID field blank.
- d. Click the Color box to open the Marker Color dialog box, select a color to represent the marker, then click **OK**.
- e. Click the Allele X Browse button ()) to open the Detector Manager.
- **8.** Enter detectors to use in the plate:
  - **a.** Click **Create Detector** to open the Add Detector dialog box.

Note: You may also add detectors from other sources (click Existing Plate, Detector Manager, or Assay Information File). For more information, see the *SDS Online Help*.



amples to Use in Plate		Detectors to Use in Pla Add Detectors from Existing Plate		tor Manager	Assay Informatio	m File
Sample		Dete	tor	Reporter	Task	Gty (unit)
1 NTC				40 - C		
2 NA17204						
3 NA17101	100					
4 NA17254						
5 NA17123						
5 NA17123 6 NA17106						
and the second second second						
6 NA17106						
6 NA17106 7 NA14476						
5 NA17106 7 NA14476 8 NA17222						
6 NA17106 7 NA14476 8 NA17222 9 NA17259						
6 NA17106 7 NA14476 8 NA17222 9 NA17259 10 NA17237						
6 4417106 7 4414476 9 4437222 9 4437229 10 4437239 10 4437237 11 441736 12 4437215					nate Delector	Ciew Selected

8a

b. Enter a Name for the detector (for example, AL-1).

**Note:** The name of the detector must be unique and it should reflect the target locus of the assay. Do not use the same name for multiple detectors. The SDS software does not distinguish between detectors of the same name, even if they use a different dye set.

Add Detector			
Name:	AL-1	8b	
Group:	Default 🗾 -	—8c	
Description:	Allele 1 for Marker C_2984390_10	8d	
AIF Assay ID:		—8e	
Reporter:	vic 💌	—8f	
Quencher:	Non Fluorescent		
Color:	<b>—</b>	8g	
Notes:	-	—8h	
Created:	May 26, 2005 8:15:51 AM		
Last Modified:	May 26, 2005 8:15:51 AM		
	OK Cancel		8i



**c.** *Optional.* In the Group field, enter or select a group for the detector.

**Note:** A detector group is an optional feature of the SDS software designed to help you organize your detectors.

- **d.** *Optional.* In the Description field, enter a brief description of the assay (up to 32 characters).
- e. Leave the AIF Assay ID field blank.

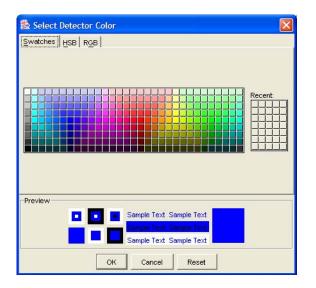
**Note:** For more information on Assay Information Files (AIF), see the *SDS Online Help*.

f. From the Reporter/Quencher drop-down lists, select the appropriate dyes for the detector.

**Note:** For TaqMan<sup>®</sup> probes, select **TAMRA** as the quencher; for TaqMan<sup>®</sup> MGB probes select **None** as the quencher.

**Note:** If you are using a custom dye not manufactured by Applied Biosystems, you must create and run a pure dye plate for the dye before applying it to a detector. For more information, see the *SDS Online Help*.

**g.** Click the **Color** box to open the Select Detector Color dialog box, select a color to represent the detector, then click **OK**.





- **h.** *Optional*. In the Notes field, enter any additional comments for the detector (up to 200 characters).
- i. Click **OK**. The software saves the new detector and displays it in the Create Plate Document Wizard.
- j. Repeat steps 8a through 8e for Allele Y, then skip to step 8k.

**IMPORTANT!** The detector for Allele X and the detector for Allele Y should have different reporter dyes (for example,  $VIC^{\mathbb{R}}$  dye for detector AL-1 and FAM<sup>TM</sup> dye for detector AL-2).

50 5	es to Use in Plate Semples from visting Plate	Add	ctors to Use in Pla Detectors from: Scisting Plate		ictor Manager	Assay Inform	tion File
	Sample		Dete	tor	Reporter	Task	City (unit)
1	NTC	1	AL-1		VIC	Unknown	0.0
2	NA17204	2	AL-2	_	FAM	Unknown	0.0
3	NA17101						
4	NA17254						
5	NA17123						
б	NA17106						
7	NA14476						
8	NA17222						
9	NA17259						
10	NA17237						
11	NA17136						
12	NA17215 *						
						Innate Detector	Clear Selected

k. Click Next>.

#### Sample Experiment

In the sample experiment, there were two detectors: AL-1 and AL-2.

For your own experiment, enter the appropriate number of detectors. Use detector names that represent the detectors in your experiment.

47 47	i.	F	P.	P.	١.	ŀ
Last Pl	1	Ŀ	2	Let Pl	1	ł
1.0	and the second	t.	-	1.0	a an en heren	ł
1 ani 41	1.0	i.	1.1	1 - 4 (*)	1 mil	ł
1.0	1	Ŀ	1	1.0	1	ł
4.10	1.	Ŀ	1	1	1.1	ł

- I. *Optional*. Select a base (A, C, G, or T) from the Allele X and Allele Y Base Name drop-down lists.
- **m.** Click **OK**. The Marker Information dialog box closes and the new marker is displayed in the Create Plate Document Wizard.
- **9.** Repeat step 7 to create markers for any remaining assays on the plate.
- 10. Click Next>.

Assay ID : Color : Allele X : AL-1 Allele X : AL-2 Base Name :  Allele Y : AL-2 Base Name : Base Name	Assay D: Color: Allele X: AL-1 Allele X: AL-1 Allele Y: AL-2 Base Name: Color Color Color Allele Y: AL-2 Base Name: Color	Aarker Information					X	
Assay ID: Color: Allele X: Allele X: Allele Y: Allele Y: Alle Y: All Y:	Assay D : Color : Allele X : Allele X : Allele Y : Alle Y : Allele Y : Allele Y : Allele Y : Alle Y : Allele Y : Alle Y : Alle Y : Allele Y : Alle Y : All							
Assay ID: Color: Allele X: Allele X: Allele Y: Allele Y: Alle Y: All Y:	Assay D : Color : Allele X : Allele X : Allele Y : Alle Y : Allele Y : Allele Y : Allele Y : Alle Y : Allele Y : Alle Y : Alle Y : Allele Y : Alle Y : All	Marker name : C 2984390 10						
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Nilele Y :       AL-2       Base Name :       Image: Concel       Base Name :       Bas	Allele Y : AL-2 Base Name : OK Cancel OK Cancel	Allele X : AL-1				Base Name :	<b>_</b>	
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#### Sample Experiment

In the sample experiment, only one marker was used: C\_2984390\_10. The detectors assigned to it were AL-1 and AL-2.

For your own experiment, enter the appropriate number of markers with the appropriate detectors assigned. Use marker and detector names that represent the markers and detectors in your experiment.



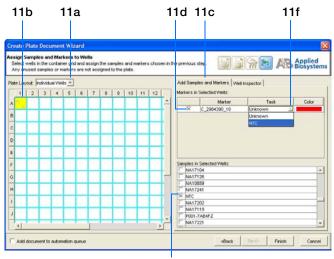
- **11.** Assign samples and markers to the wells:
  - a. From the Plate Layout drop-down list, select Individual Wells.

**IMPORTANT!** If you change the plate layout *after* assigning samples or markers to the wells, all assignments are cleared from the Plate Grid.

**b.** In the Plate Grid, select the well(s) containing the first sample.

**Note:** To select more than one well at a time, hold down the **ctrl** key or **shift** key while selecting the wells.

- c. Select the Add Samples and Markers tab.
- **d.** In the Markers in Selected Wells pane, check the appropriate marker for the selected well(s).
- e. In the Samples in Selected Wells pane, check the appropriate sample for the selected well(s).
- f. Click in the Task field and select a task for the marker from the drop-down list: Unknown or NTC.
- **g.** Repeat steps 11b through 11f for the remaining samples, then go on to step 12.



11e



- **12.** Verify the sample setup information for each well:
  - a. Select the Well Inspector tab.
  - **b.** Select the desired well(s) in the Plate Grid.

**Note:** To select more than one well at a time, hold down the **ctrl** key or **shift** key while selecting the wells.

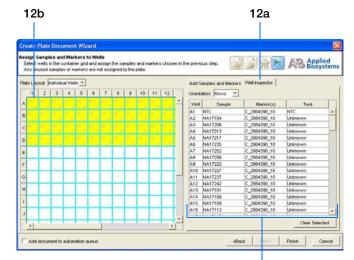
**c.** View the sample setup information for the selected well(s) in the Well Inspector table.

**Note:** If you need to correct the sample setup information for a well, select the well in the Well Inspector table, click **Clear Selected**, then assign the correct setup information.

**13.** Click **Finish**. The Create Plate Document Wizard closes and the new plate document is displayed.

**Note:** If your experiment does not use all the wells in a reaction plate, do not omit the wells from use at this time. You can omit unused wells after the run. For information about omitting unused wells, see the *SDS Online Help*.

**Note:** If necessary, you can change the sample setup information (sample name, detector, task) *after* a run is complete.



12c. The sample setup information for the selected well(s) is displayed here.



### Sample Experiment

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**14.** Save the new plate document:

**IMPORTANT!** Before the plate document can be run, you must save it as an SDS 7900HT Document (\*.sds). If you close the plate document without first saving it, your information will be lost.

a. Select File > Save to open the Save As dialog box.

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- **b.** In the Save in field, navigate to and select a directory for the new plate document.
- c. In the File name field, either:
  - Enter a file name for the plate document, *or*
  - Enter or scan the barcode number for the reaction plate

**Note:** The SDS software does not require that the plate document name match the barcode of the corresponding reaction plate.

- d. From the Files of type drop-down list, select **SDS 7900HT Document (\*.sds)**.
- e. Click Save. The software saves the plate document to the specified directory.

		14b
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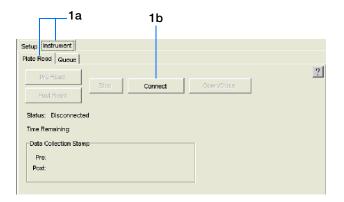


# Performing the Pre-Read Run

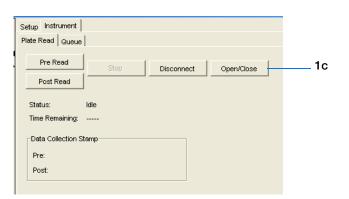
### About the Pre-Read Run

A pre-read run records the background fluorescence of each well of the allelic discrimination plate before performing PCR amplification (see Chapter 5). Then, during the post-read run (see Chapter 6), the pre-read fluorescence is subtracted from the post-read fluorescence to ensure that fluorescence due only to amplification is recorded.

- **1.** To run an individual plate:
  - a. In the Allelic Discrimination (AD) plate document, select the Instrument > Plate Read tabs.
  - **b.** Click **Connect** to connect the plate document to the instrument.



**c.** Click **Open/Close**. The instrument tray rotates to the OUT position.



Standard 384-well reaction plate



**d.** Place the prepared reaction plate into the instrument tray as shown.

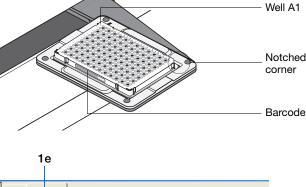
**IMPORTANT!** For the standard 384-well and standard 96-well reaction plates, the A1 position is located in the top-left side of the instrument.

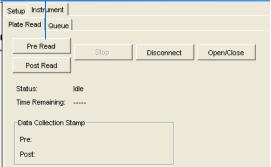
e. Click **Pre Read**. The instrument tray rotates to the IN position.

During the pre-read run, the instrument collects one fluorescence scan per well.

As the instrument performs the run, it displays status information in the Plate Read tab.

- **2.** After the pre-read run is finished:
  - A message indicates whether or not the run was successful. Click **OK** to close the dialog box. The instrument tray rotates to the OUT position.
  - The date and time of completion is recorded in the Date Collection Stamp pane and the Pre Read button is disabled.





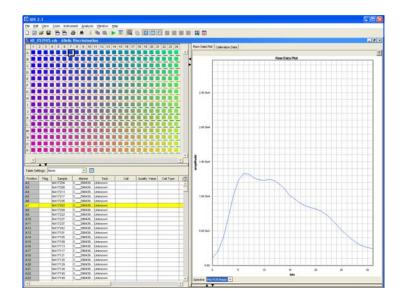
)		
The run completed successfully.		
OK		-2
	The run completed successfully.	



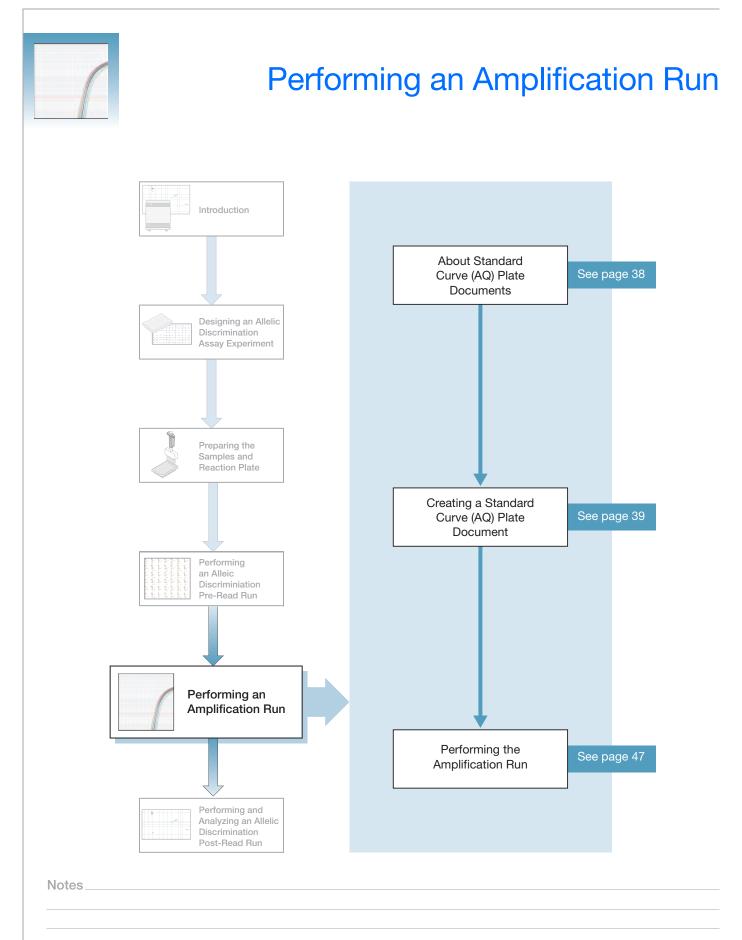
Notes\_



- **3.** *Optional*. Review the fluorescence data generated during the pre-read run:
  - a. Click 🔛 (Hide/Show System Raw Data Pane).
  - **b.** In the Plate Grid, select the well(s) you want to view.
  - c. Select the **Raw Data Plot** tab, then select **Pre PCR Read** from the Spectra drop-down list to view the pre-read run data.



4. Select File > Close. All raw data generated during the pre-read run and any changes to the plate document settings are saved to the file that you specified in step 14 on page 32.





# About Standard Curve (AQ) Plate Documents

Using Standard Curve (AQ) Plate Documents for Amplification	You create and use Standard Curve (AQ) plate documents to store real-time data for allelic discrimination assays. Because the Standard Curve (AQ) plate document is used only to amplify target sequences (not to quantify the PCR data), you do not need a standard curve for the plate.
Benefits of Real-Time Amplification	Because the allelic discrimination assay is an end-point assay, you can amplify the target sequences offline using any thermal cycler. However, using the 7900HT Fast System to amplify the target sequences provides real-time PCR data. When you perform allele-calling (described in "Assigning Calls" on page 62), you can study the amplification plots if you observe questionable calls or do not observe data for a well.
Options for Creating a Plate Document	This section describes how to create a plate document using the New Plate Document Wizard. For information about other ways to create plate documents, see the <i>SDS Online Help</i> .
Reaction Plate Options	The allelic discrimination assay can be run on either a standard 96-well reaction plate or a standard 384-well reaction plate. For clarity, this chapter only illustrates running a standard 384-well reaction plate.



# Creating a Standard Curve (AQ) Plate Document

- **1.** Double-click **o** on the desktop (or select **Start** > All Programs > Applied Biosystems > SDS 2.3 > SDS 2.3) to start the SDS software.
- **2.** If your System Administrator has enabled the login option, the Login dialog box appears. Enter your User Name and Password, then click OK.

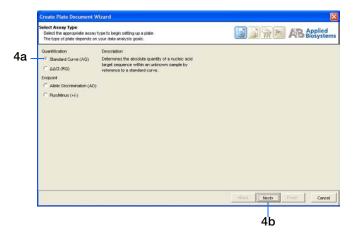
Note: If the login option is not enabled, no Login dialog box appears. Skip to step 3 below.

- **3.** In the SDS software menu bar, click (or select File > New Plate Wizard) to open the Create Plate Document Wizard.
- **4.** Select the assay type:
  - a. Select Standard Curve (AQ).

Note: A standard curve is not necessary for a non-quantitation amplification run.

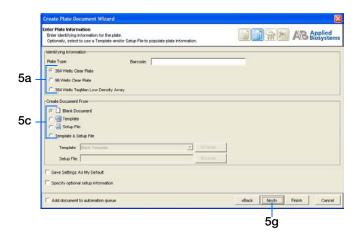
b. Click Next>.





- **5.** Enter the plate information:
  - a. Select a Plate Type: 384 Wells Clear Plate or 96 Wells Clear Plate.
  - **b.** *Optional.* In the Barcode field, scan or enter the barcode for the reaction plate.

Note: The Automation Accessory must be installed in order to use barcodes. A barcode is required if you are adding the plate document to the automation queue. See step f on page 40.



Notes



**c.** In the Create Document From pane, select your preferred source.

Note: You can create a plate document from scratch (select **Blank Document**) or you can create a plate document from a template and/or an existing plate document (select **Template**, **Setup File**, or **Template** & **Setup File**). For more information, see the *SDS Online Help*.

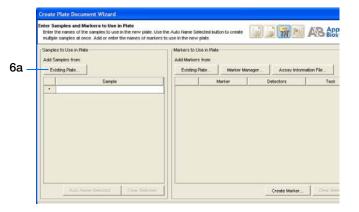
- d. *Optional.* Check Save Settings As My Default. Check this option if you want the SDS software to automatically apply the current page settings every time you use the Create Plate Document Wizard.
- e. *Optional.* Check **Specify optional setup information**. Check this option if you want to enter a name, study, or comments for the plate document.
- f. *Optional*. Check Add document to automation queue. Check this option if you want to add the finished plate document to the Automation Controller software plate queue.

**Note:** This checkbox is only enabled if you are connected to an Automation Accessory. For more information, see Appendix B on page 83.

**Note:** This checkbox appears on subsequent pages of the Create Plate Document Wizard. You only need to select this option on one page to enable it.

g. Click Next>.

- **6.** Import the sample names from the Allelic Discrimination (AD) plate document you created in Chapter 4 (see step 6 on page 25).
  - a. Click Existing Plate.

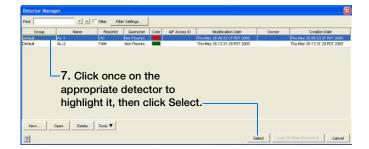


- **b.** In the Open dialog box, navigate to and select the Standard Curve (AQ) plate document.
- **c.** Click **Open**. The sample names appear in the Create Plate Document Wizard.

					6b			
Open							×	
Look in:	SD:	S Document	s		<u> </u>			
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7. In the Detector Manager, locate the appropriate detector from the Allelic Discrimination (AD) plate document you created in Chapter 4 (see step 8 on page 26), click once on the detector to highlight it, then click Select.



- **8.** Repeat step 7 for any remaining detectors.
- 9. Click Next>.

	tes to Use in Plate Samples from: visting Plate		Add D	etectors from: sting Plate		ctor Manager	Assay Inform	ation File	
	Sample			Detecto	×	Reporter	Task	Qty (unit	4
1	NTC	-	1	AL-1		VIC	Unknown	0.0	
2	NA17204		2	AL-2		FAM	Unknown	0.0	_
3	NA17101	-							
4	NA17254								
5	NA17123								
б	NA17106								
7	NA14476								
8	NA17222								
	NA17259								
9									
9	NA17237								
	NA17237 NA17136								
10	NA17136								

#### **Sample Experiment**

In the sample experiment, there were two detectors: AL-1 and AL-2.

For your own experiment, enter the appropriate number of detectors. Use detector names that represent the detectors in your experiment.



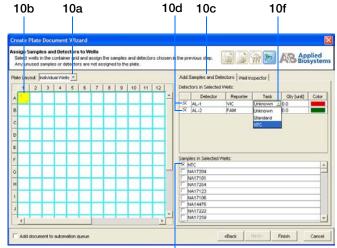
- **10.** Assign samples and detectors to the wells, using the same assignments you used for the Allelic Discrimination (AD) plate document (see step 11 on page 30):
  - a. From the Plate Layout drop-down list, select Individual Wells.

**IMPORTANT!** If you change the plate layout *after* assigning samples or detectors to the wells, all assignments are cleared from the Plate Grid.

**b.** In the Plate Grid, select the well(s) containing the first sample.

**Note:** To select more than one well at a time, hold down the **ctrl** key or **shift** key while selecting the wells.

- c. Select the Add Samples and Detectors tab.
- **d.** In the Detectors in Selected Wells pane, check the appropriate detector(s) for the selected well(s).
- e. In the Samples in Selected Wells pane, check the appropriate sample for the selected well(s).
- f. Click in the Task field and select a task for the detector from the drop-down list: Unknown or NTC. Do not select Standard.
- **g.** Repeat steps 10b through 10d for the remaining samples.



10e



- **11.** *Optional.* Verify the sample setup information for each well:
  - a. Select the Well Inspector tab.
  - **b.** Select the desired well(s) in the Plate Grid.

**Note:** To select more than one well at a time, hold down the **ctrl** key or **shift** key while selecting the wells.

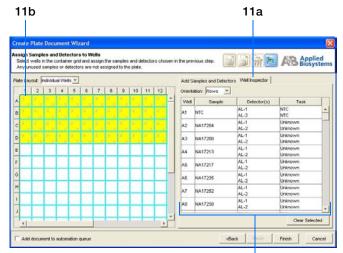
**c.** View the sample setup information for the selected well(s) in the Well Inspector table.

**Note:** If you need to correct the sample setup information for a well, select the well in the Well Inspector table, click **Clear Selected**, then assign the correct setup information.

**12.** Click **Finish**. The Create Plate Document Wizard closes and the new plate document is displayed.

**Note:** If your experiment does not use all the wells in a reaction plate, do not omit the wells from use at this time. You can omit unused wells after the run. For information about omitting unused wells, see the *SDS Online Help*.

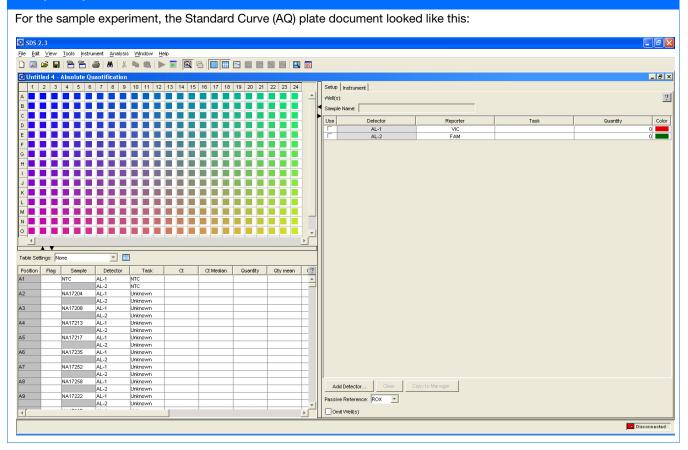
**Note:** If necessary, you can change the sample setup information (sample name, detector, task) after a run is complete.



11c. The sample setup information for the selected wells is displayed here.



#### Sample Experiment



Notes

Allelic Discrimination Assay Getting Started Guide for the 7900HT Fast System



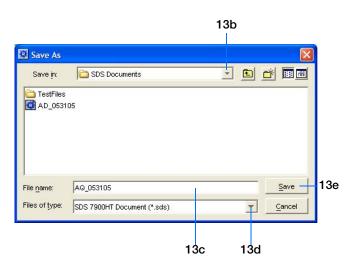
**13.** Save the new plate document:

**IMPORTANT!** Before the plate document can be run, you must save it as an SDS 7900HT Document (\*.sds). If you close the plate document without first saving it, your information will be lost.

- a. Select File > Save to open the Save As dialog box.
- **b.** In the Save in field, navigate to and select a directory for the new plate document.
- c. In the File name field, either:
  - Enter a file name for the plate document, *or*
  - Enter or scan the barcode number for the reaction plate

**Note:** The SDS software does not require that the plate document name match the barcode of the corresponding reaction plate.

- d. From the Files of type drop-down list, select **SDS 7900HT Document (\*.sds)**.
- e. Click Save. The software saves the plate document to the specified directory.





## Performing the Amplification Run

- **1.** In the Standard Curve (AQ) plate document you just created, select the **Setup** tab.
- Verify that the Passive Reference setting is ROX dye. (ROX<sup>™</sup> dye is the default.)

**Note:** If your experiment does not use all the wells in a reaction plate, do not omit the wells from use at this time. You can omit unused wells after the run. For information about omitting unused wells, see the *SDS Online Help*.

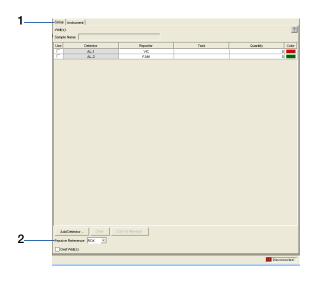
**Note:** If necessary, you can change the sample setup information (sample name, detector, task) after a run is complete.

- **3.** Select the **Instrument > Thermal Cycler** tabs.
- 4. Select a Mode: Standard or 9600 Emulation.

**Note:** When Standard mode is selected, the SDS software uses the 9700 thermal cycler ramp rate in the 7900HT Fast System for standard PCR reactions. When the 9600 Emulation mode is selected, the SDS software reduces the 7900HT Fast System ramp rate to match that of the 9600 thermal cycler in the ABI PRISM<sup>®</sup> 7700 Sequence Detection System.

**5.** Enter a Sample Volume.

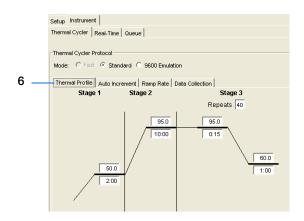
**Note:** The default sample volumes are 50  $\mu$ L for a standard 96-well reaction plate and 20  $\mu$ L for a standard 384-well reaction plate.







6. Select the Thermal Profile tab.

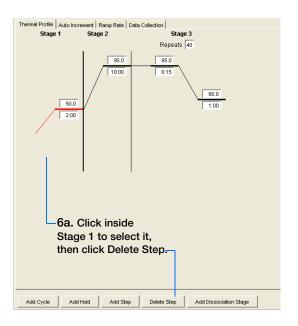


• If you are using TaqMan<sup>®</sup> Universal PCR Master Mix (PN 4304437, which contains AmpErase<sup>®</sup> UNG), accept the default times and temperatures (shown at right).

	Times and Te	emperatures			
Initial	Steps	PCR (Each of 40 cycles)			
AmpErase <sup>®</sup> UNG Activation	AmpliTaq Gold <sup>®</sup> DNA Polymerase Activation	Melt	Anneal/ Extend		
HOLD	HOLD	CYC	CLE		
2 min @ 50 °C	10 min @ 95 °C	15 sec @ 95 °C	1 min @ 60 °C		

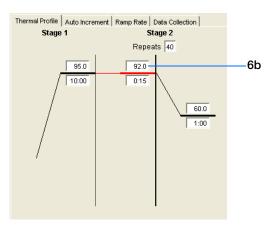
 If you are using TaqMan<sup>®</sup> Universal PCR Master Mix, No AmpErase<sup>®</sup> UNG (PN 4324018):

**a.** Delete the default Stage 1 by clicking inside the stage to select it (the ramp line turns red), then clicking **Delete Step**.





**b.** Change the temperature for the new Stage 2 from 95.0 to 92.0 by double-clicking inside the top box, then typing **92.0**.



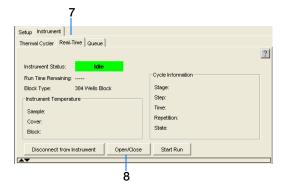
**c.** Accept the remaining default times and temperatures.

Times	and Temperature	S
Initial Steps	PCR (Each c	of 40 cycles)
AmpliTaq Gold <sup>®</sup> DNA Polymerase Activation	Melt	Anneal/Extend
HOLD	CYC	CLE
10 min @ 95 °C	15 sec @ 92 °C	1 min @ 60 °C

#### **Sample Experiment**

For the sample experiment, the following parameters were selected for the amplification run:

- Mode: Standard.
- Sample Volume: 5 μL (This is the recommended volume for TaqMan<sup>®</sup> SNP Genotyping Assays run on a standard 384well reaction plate.)
- Thermal Profile: The thermal profile was changed per steps 6a through 6c above for TaqMan<sup>®</sup> Universal PCR Master Mix, No AmpErase<sup>®</sup> UNG.
- 7. Select the Real-Time tab.
- **8.** Click **Open/Close**. The instrument tray rotates to the OUT position.



Notes



**9.** Place the prepared reaction plate into the instrument tray as shown.

**IMPORTANT!** For standard 384-well and standard 96-well reaction plates, the A1 position is located in the top-left side of the instrument.

**10.** Click **Start Run**. The instrument tray rotates to the IN position and the instrument performs the amplification run.

As the instrument performs the amplification run, it displays real-time status information in the Real-Time tab and records the fluorescence resulting from cleavage of TaqMan<sup>®</sup> probes in the presence of the target sequences.

After the run, the status values and buttons are grayed-out, the Analysis button is enabled (>), and a message indicates whether or not the run is successful.

**11.** Select **File** > **Save**. All data generated during the run are saved to the plate document.

**Note:** If necessary, the amplification run data from the Standard Curve (AQ) plate document can be analyzed later to help troubleshoot the allelic discrimination assay results. For more information, see Appendix C on page 89.

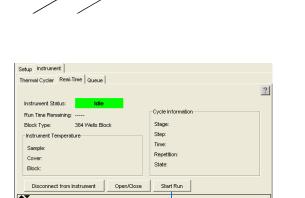
**12.** Close the plate document, then proceed to Chapter 6 on page 51 to complete the allelic discrimination assay workflow.

Well A1

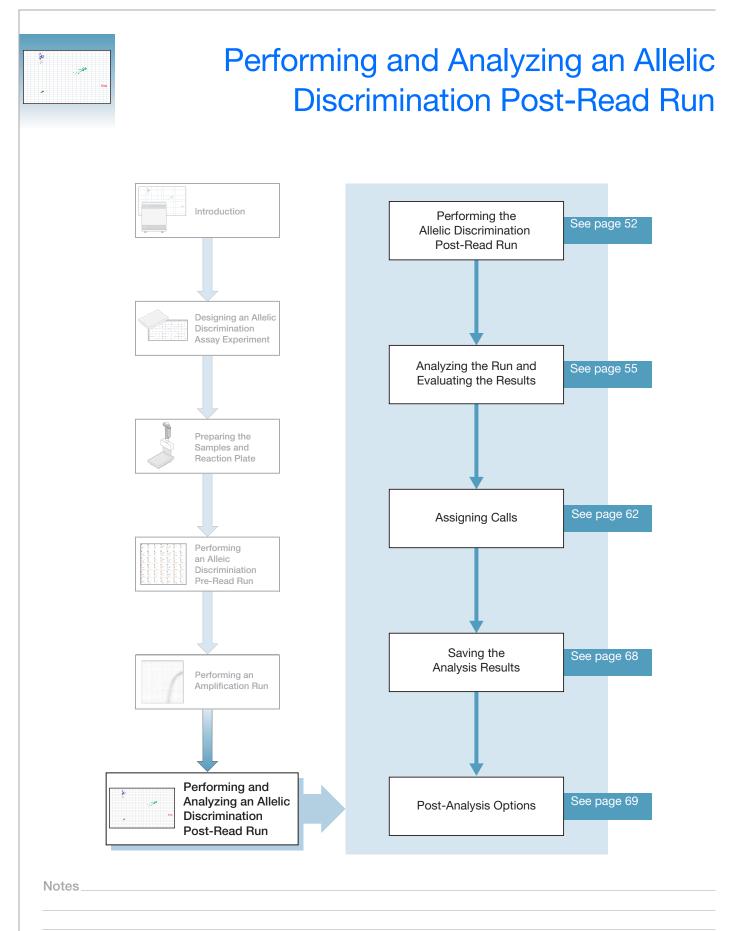
corner

Barcode

Standard 384-well reaction plate



10



# Performing the Allelic Discrimination Post-Read Run

### About the Post-Read Run

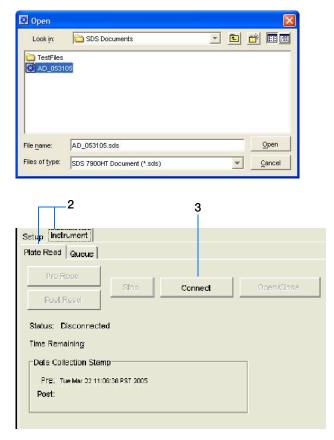
A post-read run records the fluorescence of each well of the allelic discrimination plate after performing PCR amplification (see Chapter 5). During the postread run, the pre-read background fluorescence (see Chapter 4) is subtracted from the post-read fluorescence to ensure that fluorescence due only to amplification is recorded.

### **Reaction Plate Options**

The plus/minus assay can be run on both standard 96well and standard 384-well reaction plates. For clarity, this chapter only illustrates the running of a standard 96-well reaction plate.

#### To perform the post-read run:

- Select File > Open, browse to open the previously saved pre-read plate document, then click Open. The allelic discrimination plate document opens in the main SDS software window.
- In the Allelic Discrimination (AD) plate document, select the Instrument > Plate Read tabs.
- **3.** Click **Connect** to connect the plate document to the instrument.





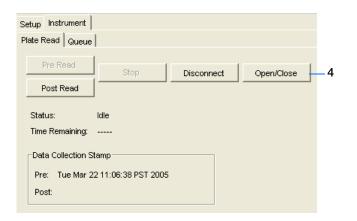
**4.** Click **Open/Close**. The instrument tray rotates to the OUT position.

**5.** Place the prepared reaction plate into the

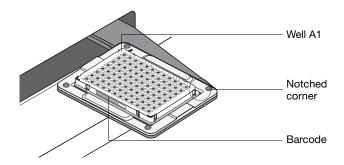
**IMPORTANT!** For the standard 384-well and

standard 96-well reaction plates, the A1 position is located in the top-left side of the instrument.

instrument tray as shown.



Standard 384-well reaction plate

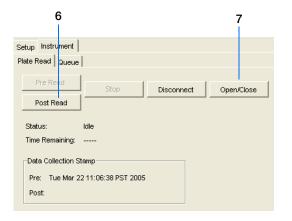


**6.** Click **Post Read**. The instrument tray rotates to the IN position and the instrument performs the run.

As the instrument performs the run, it displays status information in the Plate Read tab.

After the run, the status values and buttons are grayed-out, the Analysis button is enabled (>), and a message indicates whether or not the run is successful.

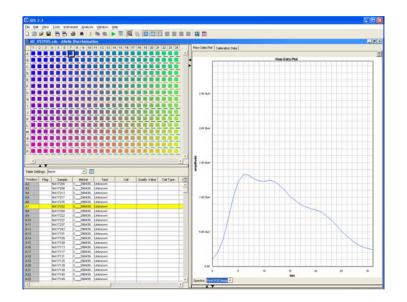
**7.** When the run is complete, click **Open/Close** to eject the reaction plate.



Notes



- **8.** *Optional*. Review the fluorescence data generated during the post-read run:
  - a. Click 🔛 (Hide/Show System Raw Data Pane).
  - **b.** In the Plate Grid, select the well(s) you want to view.
  - c. Select the **Raw Data Plot** tab, then select **Post PCR Read** from the Spectra drop-down list to view the post-read run data.
- **9.** Select **File** > **Close**. All raw data generated during the post-read run and any changes to the plate document settings are saved to the file that you specified in step 1 on page 52.





# Analyzing the Run and Evaluating the Results

### Analyzing the Run

 In the Allelic Discrimination (AD) plate document, click (or select Analysis > Analyze). The SDS software analyzes the run data.

#### Tip: Reanalyzing Data

Once you click the Analyze button, it becomes disabled. To reanalyze the data, you can:

- Reenable the Analyze button by changing the setup information in the plate document (for example, removing a well or omitting a marker assignment), then click (or select Analysis > Analyze) again.
- Change analysis settings in the Analysis Settings dialog box, then click OK. The SDS software automatically reanalyzes the data when you click OK. (For information on using the Analysis Settings dialog box, see "Assigning Calls" on page 62.)

**Note:** In order for the SDS software to automatically reanalyze the data via the Analysis Settings dialog box, you must have already analyzed the data once by clicking the Analyze button.

**2.** Select the **Results** tab, then proceed to "Evaluating the Results" below.

### **Evaluating the Results**

To evaluate the analysis results, you can use the:

- Allelic Discrimination Plot (page 57)
- Results Grid (page 60)
- Results Table (page 61)

**Note:** The results displays are actively synchronized. For example, selecting a well in the Results Grid also selects the corresponding well in the Results Table and Allelic Discrimination Plot.

After evaluating the initial analysis results:

• If you are satisfied with the initial analysis results, proceed to "Saving the Analysis Results" on page 68.

Notes.



• If you are not satisfied with the initial analysis results, you can assign calls (see page 62) or scrutinize the allele calls.

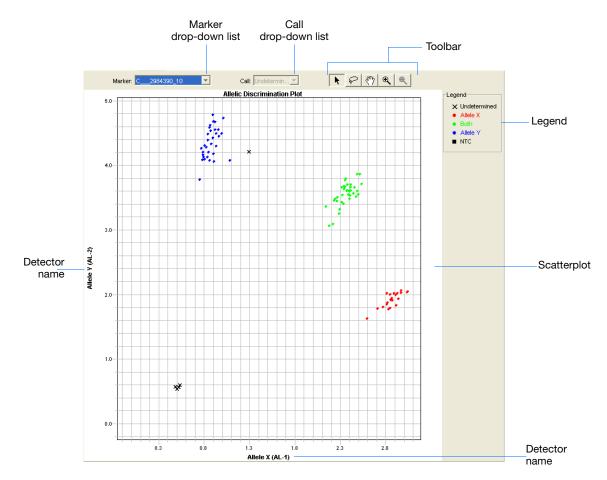
Note: For information on scrutinizing the allele calls, see the SDS Online Help.



#### Using the Allelic Discrimination Plot

The SDS software graphs the results of allelic discrimination runs on a scatterplot (the Allelic Discrimination Plot) that contrasts reporter dye fluorescence. After signal normalization and multicomponent analysis, the SDS software graphs the normalized data from each well as a single datapoint on the scatterplot.

The figure below illustrates the components of the Allelic Discrimination Plot.



- Marker drop-down list Determines the marker data that the SDS software displays in the scatterplot.
- Call drop-down list When a datapoint is selected, this menu allows you to assign an allele call to the datapoint within the scatterplot.
- Toolbar Contains the following tools for manipulating the scatterplot:

lcon	Description
k	<ul><li>Selects:</li><li>Individual datapoints by clicking, or</li><li>Groups of datapoints by clicking and dragging a box across a group of datapoints.</li></ul>
P	Selects groups of datapoints by encircling them with the tool.

Notes\_

lcon	Description
<b>N</b>	Repositions the view within the scatterplot by clicking and dragging the screen.
Đ	Zooms in on the scatterplot by: <ul> <li>Clicking the mouse button within the scatterplot, or</li> <li>Clicking and dragging a section of the scatterplot to view.</li> </ul>
୍	Zooms out on the scatterplot by clicking the mouse button within the scatterplot.

- Scatterplot A scatterplot of datapoints from the run (also called the Allelic Discrimination Plot).
- Legend An explanation of the symbols in the scatterplot.
- **Detector names** The names you assigned to the detectors are displayed on the axes of the scatterplot.

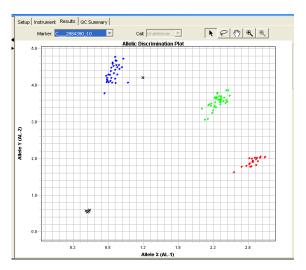
**Note:** You can adjust the appearance of the Allelic Discrimination Plot or the datapoints it contains using the Display Settings dialog box (**View > Display Setting**).

**Note:** For more information on the Allelic Discrimination Plot tools or the Display Settings dialog box, see the *SDS Online Help*.

#### To evaluate results using the Allelic Discrimination Plot:

In the Results tab, select a marker from the Marker drop-down list.

- If the auto caller is enabled:
  - Alleles are identified for the selected marker and displayed in the Allelic Discrimination Plot:

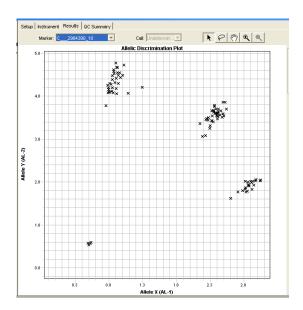




- The samples are grouped as follows:

Samples Containing	Are Grouped In	Symbol
Allele X	Lower right corner of the plot	•
Allele Y	Upper left corner of the plot	•
Both (Allele X and Allele Y – heterozygote)	Approximately midway between the Allele X and Allele Y groups	•
No Template Control (NTC)	Bottom left corner of the plot (Not shown in the example.)	
Undetermined	Anywhere on plot	×

• If the auto caller is not enabled, crossmarks (× – Undetermined) representing the selected marker are displayed in the Allelic Discrimination Plot.





#### Using the Results Grid The Results Grid displays the assay-specific setup and analysis properties for the plate document in a well format corresponding to the type of reaction plate used for the run. The following parameters can be displayed for each well in the plate document:

- Well color
- Flag
- Sample
- Marker Color
- Marker
- Number of markers
- Call: Allele Y, Allele X, Both, Undetermined. The call symbols are as follows:

Wells Containing	Symbol		
Allele X	•		
Allele Y	•		
Both (Allele X and Allele Y – heterozygote)	•		
NTC or Undetermined	×		

**Note:** You can configure which parameters are displayed in the Results Grid by selecting **View > Display Setting**, then selecting **Results Grid** in the Display Settings dialog box. For more information, see the *SDS Online Help*.

To evaluate results using the Results Grid:

1. Right-click inside the Plate Grid, then select **Result View**.

Note: The default view for the Plate Grid is the Setup View.

- 2. If desired, select View > Grid Zoom to zoom in on individual wells.
- **3.** Pass the cursor over a well to see the information for that well.

	1	2	3	4	
A	NTC C2984390_10 X 1	NA17204 0 C2984390_10	NA17208 0 C2984390_10	NA17213 C2984390_10	NA I
в	NA17201 C2984390_10	NA17205 0 C2984390_10	NA17210 C2984390_10	NA17214 C2984390_10	NA L
с	Sample Name NA17202 Markers: C298430C298439 1		NA17211 C2084390_10	NA17215 0 C2984390_10	NA L
D	NA17203 0 C2984390_10 (	NA17207 0 C2984390_10	NA17212 0 C2984390_10  1	NA17216 02984390_10	NA 1



Using the Results Table

#### The Results table displays the assay-specific setup and analysis properties for the plate document in a table format. The following parameters can be displayed for each well in the plate document:

- Well position
- Flag
- Sample
- Marker
- Task: Unknown, NTC
- Call: Allele Y, Allele X, Both, NTC, Undetermined
- Quality Value
- Call Type
- Allele X R<sub>n</sub>
- Allele Y R<sub>n</sub>
- Passive Reference R<sub>n</sub>

**Note:** See Appendix C for an explanation of terms used in quantitation analysis.

• Flags: HMD (the well has missing data), FOS (fluorescence is off-scale), LME (large mean squared error), EW (the well is empty), BPR (bad passive reference)

Note: You can configure which parameters are displayed in the Results Table by double-clicking on a column header, by selecting a user-defined Table Settings profile from the drop-down list, or by clicking (Table Settings) to open the Table Settings dialog box. For more information, see the *SDS Online Help*.



Position	Flag	Sample	Marker	Task	Call	Quality Value	Call Type	Allele X Rn	?
A1		NTC	C2984390_10	NTC	NTC	0.88	Automatic	4.99E-1	
A2		NA17204	C2984390_10	Unknown	Both	99.76	Automatic	2.3	
A3		NA17208	C2984390_10	Unknown	AL-1	99.76	Automatic	2.84	
A4		NA17213	C2984390_10	Unknown	Both	99.82	Automatic	2.34	
A5		NA17217	C2984390_10	Unknown	AL-1	99.56	Automatic	2.8	
A6		NA17235	C2984390_10	Unknown	Both	99.33	Automatic	2.34	
A7		NA17252	C2984390_10	Unknown	AL-2	99.53	Automatic	9.03E-1	
A8		NA17258	C2984390_10	Unknown	Both	99.47	Automatic	2.34	
A9		NA17222	C2984390_10	Unknown	AL-1	99.49	Automatic	2.96	1
A10		NA17227	C2984390_10	Unknown	AL-2	99.17	Automatic	8.83E-1	
A11		NA17237	C2984390_10	Unknown	Both	99.14	Automatic	2.47	
A12		NA17242	C2984390_10	Unknown	AL-2	99.65	Automatic	8.5E-1	
A13		NA17101	C2984390_10	Unknown	AL-2	99.73	Automatic	8.47E-1	
A14		NA17105	C2984390_10	Unknown	AL-1	99.86	Automatic	2.9	
A15		NA17109	C2984390_10	Unknown	AL-2	99.96	Automatic	8.84E-1	
A16		NA17113	C2984390_10	Unknown	AL-2	99.9	Automatic	7.9E-1	
A17		NA17117	C2984390_10	Unknown	AL-1	99.67	Automatic	2.95	
A18		NA17121	C2984390_10	Unknown	AL-2	99.84	Automatic	8.58E-1	-

# **Assigning Calls**

**Note:** Assigning calls is optional. If you are satisfied with the initial analysis results (page 55), you do not need to assign calls.

The analysis of SNP (or genotyping) data involves the automatic or manual "calling" of sample data for each marker. The "calls" are data labels assigned to individual samples to reflect their genomic content.

You can call sample data:

- Automatically, using the auto caller (see page 62)
- Manually, using the toolbar and scatterplot (see page 65)

### Assigning Calls Automatically



- **2.** Set the marker analysis settings:
  - a. Select the Marker tab.
  - **b.** Select **All Markers** from the Marker dropdown list.

**Note:** You can also select individual markers from the Marker drop-down list.

- c. Select the Auto caller enabled check box to activate automatic analysis.
- d. If you expect the analyzed data to consist of only two clusters, select the 2-cluster calling enabled check box.
- e. In the Quality Value field, enter a percentage value to apply as the quality interval for auto-calling samples. (The greater the value, the more stringent the allele calling.)
- f. Click **Apply** to save your settings in the Marker tab.
- **3.** Optional. Assign flags:
  - a. Select the Plate tab.
  - **b.** Assign flags as desired.

**Note:** When you assign flags, you can flag conditions and omit wells when certain criteria are met (for example, when a well has missing data). For more information, see Appendix D on page 99.

- **c.** Click **Apply** to save your settings in the Plate tab.
- **4.** Click **OK** to close the Analysis Settings dialog box. The SDS software automatically reanalyzes the data using your new analysis settings.

**Note:** In order for the SDS software to automatically reanalyze the data via the Analysis Settings dialog box, you must have already analyzed the data once by clicking the Analyze button (see step 1 on page 55).



3a

late Marker   Flag Condition and Omit Settings				
Flag Condition and Omit Plates When	Flag	Condition	Flag Conditi	Omit
Fluorescence is off-scale (FOS)				
A well has missing data (HMD)	2			Ē
_arge mean squared error is (LME)	2	>	1000	Г
A well is empty (EW)	<u> </u>			-
Plate quality is (PQ)	2	>	61	
Bad passive reference signal (BPR)	2	<	500	-
The distance between cluster and NTCs is (DCN)	2	<=	1	
Number of clusters is (NOC)	2			
Percentage of outliers is (POU)	2	>	10	
Hardy Weinberg value is (HW)	2	<	0.5	
The number of samples is (SNS)	2	<=	2	
SNP quality is (SQ)	2	<	0.8	Г

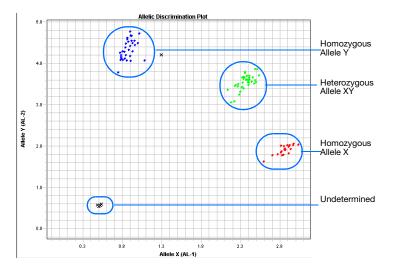
3c

Notes.



- **5.** Select the **Results** tab to display the Allelic Discrimination Plot.
- **6.** Select a marker from the Marker drop-down list. For the selected marker:
  - Alleles are identified in the Allelic Discrimination Plot
  - Samples are grouped as follows:

Samples Containing	Are Grouped In	Symbol
Allele X	Lower right corner of the plot	•
Allele Y	Upper left corner of the plot	•
Both (Allele X and Allele Y – heterozygote)	Approximately midway between the Allele X and Allele Y groups	•
No Template Control (NTC)	Bottom left corner of the plot (Not shown in the example.)	
Undetermined	Anywhere on plot	×

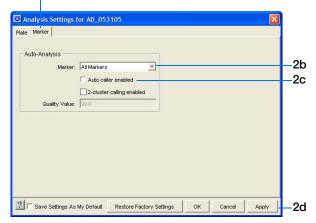




## Assigning Calls Manually

- In the Allelic Discrimination (AD) plate document, click (or select Analysis > Analysis Settings) to open the Analysis Settings dialog box.
- **2.** Set the marker analysis settings:
  - a. Select the Marker tab.
  - **b.** Select **All Markers** from the **Marker** dropdown list.
  - **c.** Deselect the **Auto caller enabled** check box.
  - d. Click Apply to save your settings in the Marker tab.

#### 2a



#### 3a

Analysis Settings for AD\_053105 Plate Marker Flag Condition and Omit Settings Flag Condition and Omit Plates When Flag ... Condition Flag Conditi... Omit Fluorescence is off-scale (FOS) বিবি A well has missing data (HMD) Large mean squared error is (LME) A well is empty (EW) 1000 Plate quality is (PQ) 61 V Bad passive reference signal (BPR) 500 The distance between cluster and NTCs is (DCN) Number of clusters is (NOC) বিববিব Percentage of outliers is (POU) 10 Hardy Weinberg value is (HW) 0.5 The number of samples is (SNS V SNP quality is (SQ) 0.8 ? 🔽 Save Settings As My Default Restore Factory Settings OK Cancel Apply 3c

- **3.** Optional. Assign flags:
  - a. Select the Plate tab.
  - **b.** Assign flags as desired.

**Note:** When you assign flags, you can flag conditions and omit wells when certain criteria are met (for example, when a well has missing data). For more information, see Appendix D on page 99.

**c.** Click **Apply** to save your settings in the Plate tab.

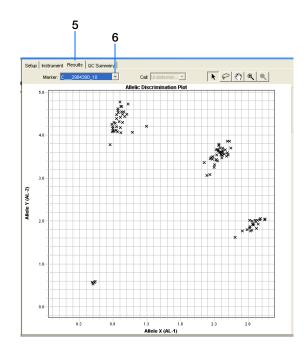
6



**4.** Click **OK** to close the Analysis Settings dialog box. The SDS software automatically reanalyzes the data using your new analysis settings.

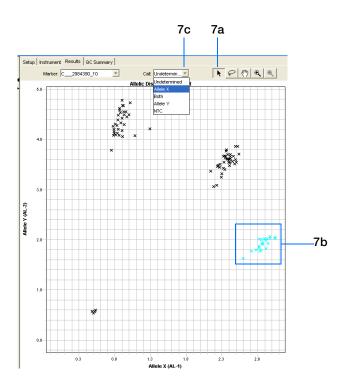
**Note:** In order for the SDS software to automatically reanalyze the data via the Analysis Settings dialog box, you must have already analyzed the data once by clicking the Analyze button (see step 1 on page 55).

- **5.** Select the **Results** tab to display the Allelic Discrimination Plot.
- 6. Select a marker from the Marker drop-down list. Crossmarks (× – Undetermined) representing the selected marker are displayed in the Allelic Discrimination Plot.





- 7. To assign calls:
  - **a.** Click the **k** selection tool.
  - **b.** Click-drag a box around the allele data points in the lower-right of the plot.
  - c. In the Call drop-down list, select Allele X.
  - **d.** Click-drag a box around the allele data points in the upper-left of the plot.
  - e. In the Call drop-down list, select Allele Y.
  - f. Click-drag a box around the allele data points in the center of the plot.
  - g. In the Call drop-down list, select Both.
  - **h.** Click-drag a box around any allele data points that are not included in any of the grouped data points.
  - i. In the Call drop-down list, select **Undetermined**.
- **8.** If you are assigning calls for multiple markers, select a different marker from the Marker drop-down list and repeat step 7.



Notes

6

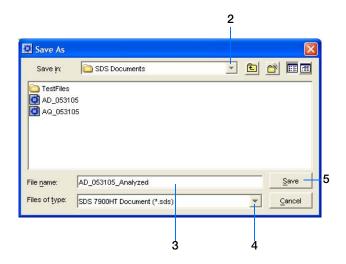
## Saving the Analysis Results

**IMPORTANT!** Although the SDS software saves any changes made to the appearance of a plate document and to the analysis settings, it does not save the calls made during the analysis.

- Select File > Save As to open the Save As dialog box.
- **2.** In the Save in field, navigate to and select a directory for the analyzed plate document.
- **3.** In the File name field, either:
  - Enter a file name for the plate document, *or*
  - Enter or scan the barcode number for the reaction plate

**Note:** The SDS software does not require that the plate document name match the barcode of the corresponding reaction plate.

- 4. From the Files of type drop-down list, select **SDS 7900HT Document (\*.sds)**.
- **5.** Click **Save**. The software saves the plate document to the specified directory.



## **Post-Analysis Options**

The following options are available after the analysis:

- Exporting data as text files (below)
- Exporting data as graphic files (page 70)
- Printing a data report (page 72)

## **Exporting Data as Text Files**

You can export raw or analyzed data from plate documents into tab-delimited text files (\*.txt). The text files can then be imported into spreadsheet software, such as Microsoft<sup>®</sup> Excel software.

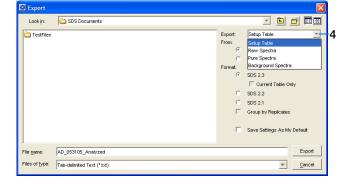
- **1.** Open the plate document from which you wish to export data.
- 2. Select File > Export to open the Export dialog box.
- **3.** In the Look in field, navigate to and select a directory for the new file.
- **4.** From the Export drop-down list, select the data type to export.

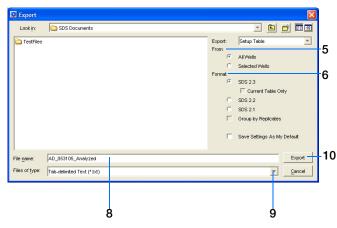
**Note:** For more information about the data type options, see the *SDS Online Help*.

**5.** Select to export data from **All Wells** or a select group of wells (**Selected Wells**).

**Note:** To use the Selected Wells option, you must select a subset of wells in the plate document before opening the Export dialog box.

- **6.** From the Format options, select the appropriate software version.
- **7.** *Optional.* Check **Save Settings As My Default**. Check this option if you want the SDS software to automatically apply the current settings every time you export data as a text file.









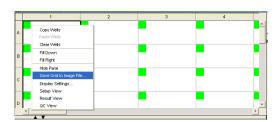
- **8.** In the File name field, type a name for the new file.
- **9.** From the Files of type drop-down list, select **Tab-delimited Text (\*.txt)**.
- **10.** Click **Export**. The software exports the new file to the specified directory.

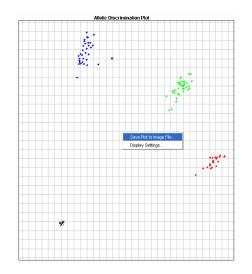
## **Exporting Data as Graphic Files**

You can export the Plate Grid and plot images from plate documents as Joint Photographic Experts Group (JPEG) graphic files (\*.jpeg). The JPEG files can then be viewed in most common word processing, spreadsheet, and HTML-based software.

- **1.** Open the plate document from which you wish to export data.
- **2.** To export the:
  - Plate Grid, right-click in the Plate Grid, then select **Save Grid to Image File**. The Save dialog box appears.
  - Plot, right-click in the plot, then select **Save Plot to Image File**. The Save dialog box appears.

**Note:** If desired, adjust the plot dimensions (length and width) as you want them to appear in the exported file. The exported file retains the dimensions of the original screen element.







- **3.** Complete the Save dialog box:
  - **a.** In the Save in field, navigate to and select a directory for the new file.
  - **b.** In the File name field, type a name for the new file.
  - c. From the Files of type drop-down list, select **JPEG File**.
  - **d.** Click **Save**. The software saves the new file to the specified directory.

		3a
Save		
Save in:	SDS Documents	• 🗈 🖻 🔳
🛅 TestFiles		
File <u>n</u> ame:	AD_053105_Analyzed_GridImage	<u>Save</u>
Files of type:	JPEG File	<u>Cancel</u>

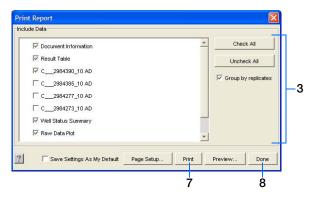


## Printing a Data Report

You can use the SDS software to print a report of the analyzed data containing individual or multiple elements of the plate document.

**Note:** For more information on printing reports, see the *SDS Online Help*.

- **1.** Open the plate document from which you wish to print a report.
- **3.** In the Include Data pane, select the plate document element(s) to print.
- **4.** *Optional.* To format the display of the report and how the report is printed, click **Page Setup**.
- **5.** *Optional.* Check **Save Settings As My Default**. Check this option if you want the SDS software to automatically apply the current settings every time you print a report.
- **6.** *Optional.* Click **Preview** to view the report before printing it.
- **7.** Click **Print** to open the Print dialog box and print the report.
- **8.** When the report has finished printing, click **Done** to close the Print Report dialog box.



## Sample Experiment

## About the Sample Experiment

**Overview** To better illustrate how to design, perform, and analyze allelic discrimination experiments, this appendix guides you through a sample experiment. The sample experiment represents a typical allelic discrimination experiment setup that you can use as a quick-start procedure to familiarize yourself with the allelic discrimination workflow. Detailed steps in the allelic discrimination workflow are described in the preceding chapters of this guide. Also in the preceding chapters are Sample Experiment boxes, which provide details for some of the related steps in the sample experiment.

**Description** The objective of the sample experiment was to determine the genotype of 92 individuals at the SNP site of a desired target sequence.

The experiment used multiplex PCR. The primers and probes were ordered from TaqMan<sup>®</sup> SNP Genotyping Assays (AB Assay ID C 2984390 10).

Reactions were set up for PCR using the TaqMan<sup>®</sup> Universal PCR Master Mix (2×), No AmpErase<sup>®</sup> UNG, and appropriate primers and probes.

The sample experiment data and results were generated using a 7900HT Fast System by performing:

- An amplification run using a Standard Curve (AQ) plate document to generate real-time PCR data. The real-time PCR data could then be used to analyze and troubleshoot the PCR data for the allelic discrimination assay, if needed.
- An allelic discrimination run using an Allelic Discrimination (AD) plate document. The SDS software analyzed the data, then we assigned allele calls (automatically or manually).

Notes.

Α

## Sample Allelic Discrimination Experiment Procedure

- 1. Design the experiment (for more detail, see Chapter 2 on page 9):
  - a. Order the appropriate master mix. The sample experiment used TaqMan<sup>®</sup> Universal PCR Master Mix, No AmpErase<sup>®</sup> UNG.
  - **b.** Select and order the probes and primers.
- **2.** Extract the DNA from samples (for more detail, see "Preparing DNA" on page 14).

The sample DNA for the sample experiment was extracted using the BloodPrep<sup>TM</sup> Chemistry Kit (PN 4346860) to obtain a final concentration of 10 ng/ $\mu$ L of DNA for each sample.

**3.** Prepare the reaction mix (for more detail, see "Preparing the Reaction Mix" on page 15).

The sample experiment was run on a standard 384-well reaction plate. As shown below, enough reaction mix was prepared for 4 NTCs and 92 samples/unknowns, plus 10% extra to account for pipetting losses.

	Volume (μL)						
Component	One Reaction	106 Reactions <sup>‡</sup>					
TaqMan <sup>®</sup> Universal PCR Master Mix (2×), No AmpErase <sup>®</sup> UNG	2.50	265					
SNP Genotyping Assay Mix (20X)	0.25	26.5					
Total	2.75	291.5					

‡ Extra volume was included to account for pipetting losses.

**CAUTION** CHEMICAL HAZARD. TaqMan<sup>®</sup> Universal PCR Master Mix (2×), No AmpErase<sup>®</sup> UNG, may cause eye and skin irritation Exposure may cause discomfort if

irritation. Exposure may cause discomfort if swallowed or inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**CAUTION** CHEMICAL HAZARD. **TaqMan®** Universal PCR Master Mix (2×) 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.

WARNING CHEMICAL HAZARD. SNP Genotyping Assay Mix (20×) contains

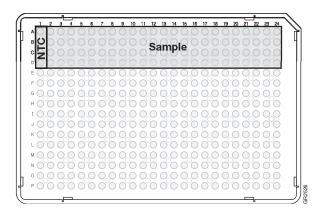
**Genotyping Assay Mix (20×)** contains **formamide**. Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and

- **4.** Prepare the reaction plate (for more detail, see "Preparing the Reaction Plate" on page 17). A reaction plate for an allelic discrimination assay contains the following:
  - Reaction mix
  - No Template Controls (NTCs)
  - Unknown genomic DNA samples
  - Known genomic DNA controls (optional, not included in the sample experiment)

For the sample experiment, a standard 384-well reaction plate was used. 2.75  $\mu$ L of reaction mix were pipetted into each well. 2.25  $\mu$ L of the following solutions were pipetted into the indicated wells:

To wells	We added
A1, B1, C1, and D1	Nuclease-free water or
(NTC, No Template Control)	TE (Tris-EDTA) buffer
A2 through A24	Diluted purified
B2 through B24	genomic DNA
C2 through C24	
D2 through D24	
(Sample or Unknown)	

**5.** Create a Standard Curve (AQ) plate document for amplifying samples (for more detail, see "Creating a Standard Curve (AQ) Plate Document" on page 39):



Notes\_

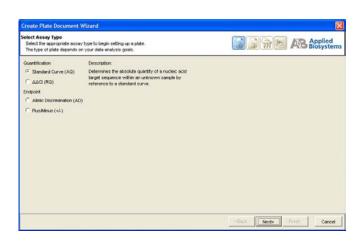
Α

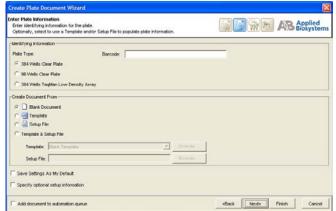
- a. Select File > New Plate Wizard.
- b. For the assay type, select Standard Curve (AQ), then click Next.

**Note:** A standard curve is not necessary for a non-quantitation amplification run.

c. Select 96 Wells Clear Plate or 384 Wells Clear Plate, select Blank Document, then click Next.

d. Enter the samples and detectors to use in the plate, then click Next.





amples to Use in Plate dd Samples from Existing Plate	Add Detectors from: Existing Plate	etector Manager	Assay Information	on File
Sample	Detector	Reporter	Task	Gty [unit]

- e. Assign detectors, samples, and tasks to the wells.
- Create Plate Document Witzerd
   Image: Second Sec
- f. Select the Well Inspector tab to verify the sample setup information for each well, then click Finish.

lote	Loy	out [	ndividi	Jal We	is <u>*</u>									Add S	amples and Detecto	ra Well Inspector		
	1	2	3	4	5	6	7	8	9	10	11	12		Orienti	stion: Rows -			
٨													-	Well	Sample	Detector(s)	Task	T
в														A1	NTC	AL-1 AL-2	NTC NTC	-
с														A2	NA17204	AL-1 AL-2	Unknown Unknown	1
D														A3	NA17208	AL-1 AL-2	Unknown	
E													H	A4	NA17213	AL-1 AL-2	Unknown	
F						1								AS	NA17217	AL-1 AL-2	Unknown	
G													Н	A6	NA17235	AL-1 AL-2	Unknown	
н						1								A7	NA17252	AL-1 AL-2	Unknown Unknown	
1									-					Aß	NA17258	AL-1 AL-2	Unknown	

- g. Save the new plate document.
- **6.** Perform the amplification run (for more detail, see "Performing the Amplification Run" on page 47):
  - a. In the Standard Curve (AQ) plate document, select the Instrument > Thermal Cycler tabs.
  - b. For the Mode, select **Standard** or **9600 Emulation**.
  - **c.** Enter a Sample Volume.

d. Select the **Thermal Profile** tab; accept the default stages (shown at right) or modify the thermal profile as needed.

	Times and Te	emperatures				
Initial	Steps	PCR (Each of 40 cycles)				
AmpErase <sup>®</sup> UNG Activation	AmpliTaq Gold <sup>®</sup> DNA Polymerase Activation	Melt	Anneal/ Extend			
HOLD	HOLD	CYC	CLE			
2 min @ 50 °C	10 min @ 95 °C	15 sec @ 95 °C	1 min @ 60 °C			

For the sample experiment (using TaqMan<sup>®</sup> Universal PCR Master Mix, No AmpErase<sup>®</sup> UNG), the thermal profile was modified as shown at right.

Times and Temperatures									
Initial Steps	PCR (Each c	of 40 cycles)							
AmpliTaq Gold <sup>®</sup> DNA Polymerase Activation	Melt	Anneal/Extend							
HOLD	D CYCLE								
10 min @ 95 °C	15 sec @ 92 °C	1 min @ 60 °C							

- e. Select the **Real-Time** tab, click **Open/Close**, then load the reaction plate into the instrument
- f. Click Start Run. As the instrument performs the amplification run, it displays real-time status information in the Real-Time tab.
- g. When the amplification run is complete, select File > Save, then close the plate document.
- Create an Allelic Discrimination (AD) plate document (for more detail, see "Creating an Allelic Discrimination (AD) Plate Document" on page 23):

AB Biosystem

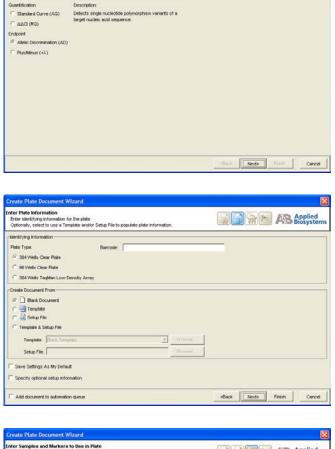
- a. Select File > New Plate Wizard.
- b. For the assay type, select Allelic Discrimination (AD), then click Next.

c. Select 96 Wells Clear Plate or 384 Wells Clear Plate, select Blank Document, then click Next.

**d.** Import the sample names from the Standard Curve (AQ) plate document you just created.

Notes.

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ielect Assey Type Select the appropriate assay type to begin setting up a plate The type of plate depends on your data analysis goals.

td Samples from Existing Plate	Add Markers for Edisting Plate.	e' j	ger Assay inform	ation File
Sample		Marker	Detectora	Task
1 NA17130				
2 NA17128				
3 NA17101				
4 NA17254				
5 NA17123				
6 NA17106				
6 NA17106 7 NA14476				
7 NA14476				
7 NA14476 8 NA17222				
7 NA14476 8 NA17222 9 NA17259				

- e. Enter the markers to use in the plate, then click Next.
- es and Markers to Use in Plate arres of the samples to use in the ne relea at once. Add or enter the name AB Applied Biosystem v plate. Use the Auto Name Selected by Samples to Use in P Markers t Add Sample's from Add Markers from Existing Plate... Existing Plate. Attev 1 NA17130 1 NA17130 2 NA17130 3 NA17101 4 NA17254 5 NA17101 6 NA17254 5 NA17123 6 NA17229 9 NA17259 10 NA17237 11 NA17136 12 NA17215 Create Marker «Back Next» Add document to automation queue Cancel
- f. Assign markers, samples, and tasks to the wells.

	e Layou	e la	dividi.	al Wei	ls_*									Add Sample	es and Markers Vie	Inspector	
	1	2	3	4	5	6	7	8	9	10	11	12		Markers in S	Selected Wells:		
A													-		Marker	Task	Color
	-	-	-	-	-	-			-	-	-	-	-11	18	C_2904390_10	Unknown	
B		-					-									Unknown	
c													t I			NTC	
D													t I				
E													t				
F		-												Samples in 1	Selected Wells:		
	-	-	-		-		-		-	-		-	-8	NA171			
Ġ		_		-							. L		11	T NA171			
	1	-	-	-	-	1.	-		-	-			100	NA108			
1				1.1										T NA172	41		
H				1					-					IX NTC			
E .								-						NA172			
HII														1 266,1711	19		
1 1 7	-						-					_		F P001-7	A 6 4 6 7		

**g.** Select the Well Inspector tab to verify the sample setup information for each well, then click **Finish**.

late Li	ayo	ie 🖥	ndividi.	al We	49 *									Adds	amples and Marker	Wel Inspector		
1	1	2	3	4	5	6	7	8	9	10	11	12			ation: Rows *			
										1			1	Well	Sample	Marker(s)	Task	
-	+	-	-	-	-	-	-	-	1-	+	-	-	<b>t</b> )	A1	NTC	C 2984390 10	NTC	-
3	-									÷.,	п.,		1 - I	A2	NA17104	C 2984390 10	Unknown	-
													t (	A3	NA17208	C_2984390_10	Unknown	
-													1	A4	NA17213	C_2984390_10	Unknown	-
													T (	AS	NA17217	C_2984390_10	Unknown	
-													15	AG	NA17235	C_2984390_10	Unknown	_
	Т				1		1			1.0			T	A7	NA17252	C_2984390_10	Unknown	
-	4	_				_			_	-	_	-	4.1	A8	NA17258	C_2984390_10	Unknown	
e i				100			1							A9	NA17222	C_2984390_10	Unknown	
-	-	-	_	-	-		-	-	-	-	-	-	-3	A10	NA17227	C_2904390_10	Unknown	
													115	A11	NA17237	C_2904390_10	Unknown	
-	+	-		-	-	-		-	-		-	-	-	A12	NA17242	C_2984390_10	Unknown	
H														A13	NA17101	C_2984390_10	Unknown	
	+	-	-			-	-	-	-	-	-	-	till	A14	NA17105	C_2984390_10	Unknown	
1				1.1		-			-					A15	NA17109	C_2984390_10	Unknown	
	-	-	-	-	-	-	-	-	-	-	-	-	1	A16	NA17113	C 2904390 10	Unknown	

**h.** Save the new plate document.

Α

- Perform the allelic discrimination run (for more detail, see "Performing the Allelic Discrimination Post-Read Run" on page 52):
  - a. In the Allelic Discrimination (AD) plate document, select the Instrument > Plate Read tabs.
  - **b.** Click **Open/Close**, then load the reaction plate into the instrument.
  - **c.** Click **Post Read**. As the instrument performs the run, it displays status information in the Plate Read tab.
- **9.** Analyze and evaluate the allelic discrimination run (for more detail, see "Analyzing the Run and Evaluating the Results" on page 55):
  - a. Click (or select Analysis > Analyze). The SDS software analyzes the run data.
  - b. Select the Results tab.
  - **c.** View the analyzed run data in the Plate Grid, Results Table, and Allelic Discrimination Plot.
- **10.** If desired, assign calls automatically or manually (for more detail, see "Assigning Calls" on page 62).
- **11.** Save the plate document (for more detail, see "Saving the Analysis Results" on page 68).
- **12.** If desired, export or print the results data (for more detail, see "Post-Analysis Options" on page 69).

Appendix A Sample Experiment About the Sample Experiment

## **SDS Automation Controller Software**

## **Overview**

The Sequence Detection Systems (SDS) Automation Controller Software v2.3 provides an interface between the Zymark<sup>®</sup> Twister Microplate Handler, the 7900HT Fast System, the fixed-position bar code reader, and the plate documents created in main Sequence Detection Systems Software v2.3.

This Automation Controller software controls and coordinates the action of the 7900HT instrument and the automation module. It initiates and controls the sequence detection run and acquires data during the run.

This option allows for plates to be run as part of a group or batch allowing for highthroughput unattended operation. For more information about the Automation Controller, refer to the *SDS Online Help*.

## Using the Automation Controller Software

**Note:** Before launching the Automation Controller software, you must close the main Sequence Detection Systems Software v2.3 application. Failure to do so will result in an instrument connection failure.

**1.** Double click the Automation Controller shortcut icon. An initialization pop-up will appear displaying the status of instrument connection and other verifications. If you encounter an error, refer to the *SDS Online Help*.

Initializing SDS Automation Console
Before connecting to the instrument, the system must perform the following initialization steps:
1. Connecting to Instrument: 🛛 🗹
2: Verifying Barcode Reader: 🛛 🗹
3. Verifying Robot Connection: 📃
Overall Initialization Status: Pass

Notes

В

**2.** If you have already created and saved the desired plates (sent them to the queue) in the main SDS application, then the plates will appear in the Plate Queue tab. For more information about creating plate documents and sending them to the queue, refer to "Creating a Standard Curve (AQ) Plate Document" on page 39 or "Creating an Allelic Discrimination (AD) Plate Document" on page 23.

le Edit Instrument Tools Help			
2 4 4 7 7 3 8	生效		
Instrument Overview	Plate Queue Run Statu	is Processed Plates	
Status: Connected	1 Plates Currently Queu	ed	
Elock Type: 394 Wells Block	# Barcode	Plate Name	Run Typ
Serial Number: 201491 Laser Power:	1 384N14X6TI	D:\Applied Biosystems\SDS Documents\LinkBied 1.sds	Plote Read
Batch Status Queued Rates: 1 Processed Pitcher: 0 Pitces with Errors: Batch Log: Currently Running Rote Run Time Remaining: Barcodei Barcodei Run Type:			
		Save List Add Plates Vern Plate In an rgd contain the plates for this batch 12 Fistack 3 Fistack 4 Filestack plates after run	nove Selected
	Enable Batch Settings	How 🖓 E-mail notifications	Batch Settings
		(1994)	en/Close Door Start Batc

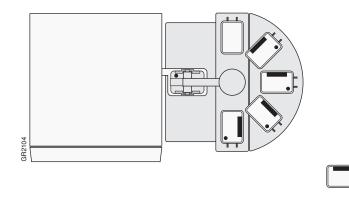
- **3.** You can add plates by clicking **Add Plates** or (or click **2**).
  - **a.** At the Open dialog box, navigate to the location where the plate files are located.

😼 Oper	]			×
Look in:	SDS Documents	<b>_</b>	E	
				-
File name:	Γ			Open
Files of type:	SDS 7900HT Documents (*.sds)		*	Cancel

**b.** Use the barcode reader to scan the Plate IDs. Note that you can automatically scan the next plate without having to select Add Plates again. You can also save the list of queued plates, by clicking **Save List**.

**Note:** To remove a plate, click on the plate name then click **Remove**. To remove all plates, click **Remove All**.

**4.** Place the plates in the input stack of the Zymark<sup>®</sup> Twister Microplate Handler. The order of the plates may vary from the order they were scanned in (the Handler's fixed barcode reader will verify the location of each plate). The software matches the plate document and the method before it starts the run. Orient the plates inside the stacks so that the well A1 of each plate corresponds to the locations shown in the figure below.



**5.** Click into the boxes next to each stack location where the plates are located.

Automation Robot Settings	
Select the stacks that contain the plates for this batch	
🔽 Stack 1 🥅 Stack 2 🥅 Stack 3 🗍 Stack 4 🗍	Restack plates after run

- 6. Select Tools > Batch Settings to specify the desired output settings.
  - **a.** In the Batch Settings dialog box, at the Auto-Analysis Workflow tab, click into a box to select that export option. You can also change the export directory by clicking the Browse button (located in the Export Destination box) and specifying a new location for the file data to be exported to. Note that after analysis exported data will be saved as \*.txt files.

6a				
Batch Settings				X
Auto-Analysi; Workflow	E-mail Notifications			
🔽 Automati ally export da	ata after analysis			?
Data to Export				
🔽 Background Spectra	🔽 Clipped	🔽 Multicomponent	🔽 Pure Spectra	
🔽 Raw Spectra	🔽 Results Table	🔽 Setup Table	🔽 Dissociation Curv	e
Export Destination				
Filename Format: AssayN	Name_PlateBarcode_Plate	Name_PlateType_DataType	Change Format.	
Export Directory C:\App	lied Biosystems\SDS Docu	Browse Reset to	Default	
			Car	ncel
			6b	

**b.** Click on **Change Format** to assign attributes to be included in the export file name.

**c.** At the Filename dialog box, click into a field box (under the Include column) to deselect an attribute.

Select Auto-/	t the a 4 nalys	sis workflow	at should be put together to create the exported file name during
	clude 지 지 지	to Include in Export File Name Attribute Name Assay Name Plate Barcode Plate Name Plate Name Plate Type Data Type ove Up Move Down	Preview of Export File Name The preview shows the order of attribute names. These name placeholders would actually be replaced by the associated attribute data from the plate file Attribute Order Preview: AssayName_PlateBarcode_PlateName_PlateType
			OKCancel

d. Click OK.

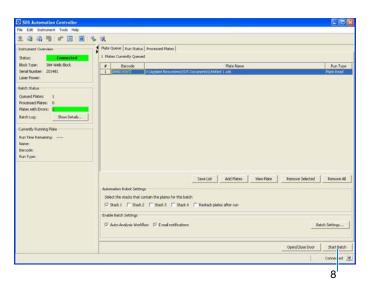
\_

e. Back at the Batch Settings dialog box, click on the **Email Notifications** tab to specify desired email notification options. For more information on email settings, see your network administrator.

6e	
Batch Settings	×
Auto-Analysis Workflow E-mail Notifications	
✓ E-mail notificationsE-mail Settings	?
Select the events that should trigger email alerts	
🖙 Instrument Error 🔽 Run Started 🖵 Run Completed 🖵 Batch started 🖓 Batch Completed	
OKCance	1

- f. Click OK.
- 7. Verify that the plate adapter and the output stack of the Handler are empty.

8. Click Start Batch.

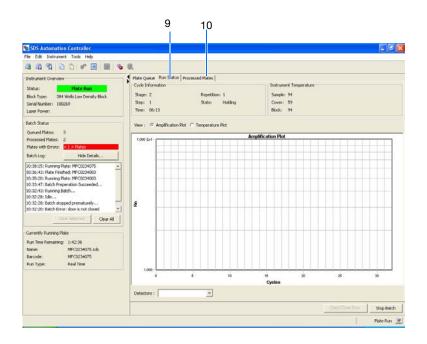


**Note:** Note that if the plate type does not match the plate block installed on the instrument, the Automation Controller will not start. You can assign plate types by selecting **Tools > Options** and selecting the desired plate format. The handler loads the plates and the instrument starts the run.

**Note:** To stop the run, click **Stop Batch**. You will see a warning message asking you to verify stopping the run.

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**9.** Click on the **Run Status** tab to view the plate run status. Note that once a plate is being processed, it will not appear in the Processed Plates tab or be listed in the Queued Plates section of the Batch Status box. The plate information will be available under the Currently Running Plate section.



**Note:** For standard curve (AQ) and ddCt (RQ) plates, a view of the amplification and temperature plot will be available and can be selected by clicking on the radial button.

**10.** Click on **Processed Plates** to view information about the processed plates.

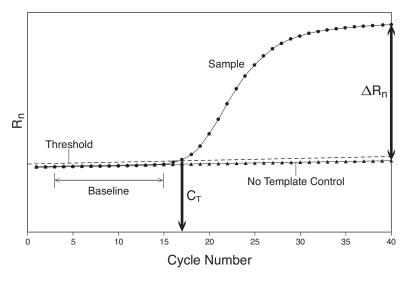
## Analyzing and Viewing Amplification Data

## **Terms Used in Quantitation Analysis**

The following are terms commonly used in quantitation analysis.

Term	Definition
Baseline	A line fit to fluorescence intensity values during the initial cycles of PCR, in which there is little change in fluorescence signal.
Threshold cycle (C <sub>T</sub> )	The fractional cycle number at which the fluorescence intensity exceeds the threshold intensity.
Passive reference	A dye that provides an internal fluorescence reference to which the reporter dye signal can be normalized during data analysis. Normalization is necessary to correct for fluorescence fluctuations caused by changes in concentration or volume.
Reporter dye	The dye attached to the 5' end of a TaqMan <sup>®</sup> probe. The dye provides a signal that indicates specific amplification.
Normalized reporter (R <sub>n</sub> )	The ratio of the fluorescence intensity of the reporter dye signal to the fluorescence intensity of the passive reference dye signal.
Delta R <sub>n</sub> (∆R <sub>n</sub> )	The magnitude of the signal generated by a set of PCR conditions. ( $\Delta R_n = R_n - \text{baseline})$

The figure below is a representative DNA amplification plot and includes some of the terms defined above.



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## Analyzing the Amplification Data

Before you can analyze the amplification data from the standard curve (AQ) plate document you generated in Chapter 5, you must specify parameter values (baseline and threshold settings or  $C_T$ ) for the analysis.

Unless you have already determined the optimal baseline and threshold settings ( $C_T$ ) for your experiment, use the automatic baseline and threshold feature of the SDS software (Automatic  $C_T$ ) to analyze the amplification run. If the baseline and threshold are called correctly for each well, you can proceed to view the results (see "Reviewing the Automatic  $C_T$  Results" on page 91). Otherwise, you must manually set the baseline and threshold (see "Reviewing the Automatic  $C_T$  Results" on page 91).

For more information about determining  $C_T$ , refer to the *Real-Time PCR Systems Chemistry Guide* (PN 4348358). For more information about manually adjusting  $C_T$ , refer to the *SDS Online Help*.

Starting the Analysis

- **1.** Open the AQ plate document you want to analyze.
- 2. Click 🗾 (or select Analysis > Analysis Settings). The Analysis Settings dialog box opens.

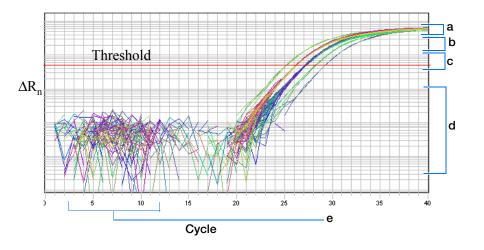
etector All Dete	otoro	-		
Ct Analysis	CLUIS			
Automatic Ct				
C Manual Ct				
Threshold				
<ul> <li>Automatic</li> </ul>	Baseline			
C		 End(Cycle): 15		

- **3.** In the Detector drop-down list of the Detector tab, select **All Detectors**.
- **4.** In the Ct Analysis window, select **Automatic Ct**. The SDS software will automatically generate baseline values for each well and threshold values for each detector.
- 5. Click **OK** to apply any changes and close the Analysis Settings dialog box.
- **6.** Click ► (or select **Analysis** > **Analyze**). The Results and QC Summary tabs are available after analysis completes.

## Reviewing the<br/>Automatic C<br/>ResultsClick the Results tab to view results of the amplification run for each well. The SDS<br/>software Automatic C<br/>T analysis process calculates baseline and threshold values for a<br/>detector based on the assumption that the data exhibits a "typical" amplification curve.

A typical amplification curve has a:

- Plateau phase (a)
- Linear phase (b)
- Exponential (geometric) phase (c)
- Background (d)
- Baseline (e)



**IMPORTANT!** Experimental error (such as contamination, pipetting errors, etc.) can produce atypical amplification curves that can result in incorrect baseline and threshold value calculations by the SDS software. Therefore, Applied Biosystems recommends you examine the amplification plot and review the assigned baseline and threshold parameter values for each well after analysis completes, and if necessary manually adjust the baseline and threshold. For more information, see the *SDS Online Help*.

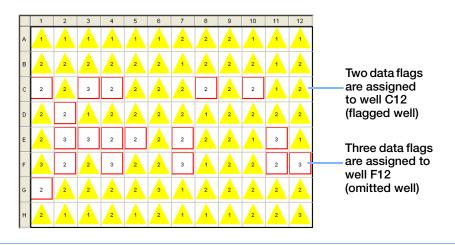
## **Viewing the Amplification Data**

The following options are available for further determination of the analysis results:

- Using the Plate Grid Results View (below)
- Using the Raw Data Plot (page 93)
- Using the Multicomponent Data Plot (page 93)
- Using the Amplification Plot Views (page 94)

#### Using the Plate Grid Results View

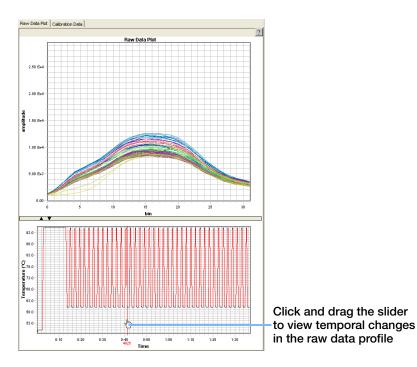
Before analysis, the plate grid displays the setup information for each well, including: detector and sample information. After analysis, the plate grid displays the category and number of data flags generated for each well of the plate, as illustrated below.



**Note:** A list of the data flags generated for the plate document is also displayed in both the QC Summary tab and Results Table. See the *SDS Online Help* for more information about viewing and configuring the plate data flags.

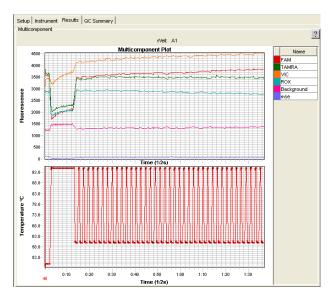
#### Using the Raw Data Plot

The Raw Data Plot (click 🔛) displays the fluorescence spectra and temperature profile of selected wells. The vertical slider in the Temperature plot allows you to see the spectra for each run cycle by dragging it with the pointer.



#### Using the Multicomponent Data Plot

The Multicomponent Data Plot (click  $\square$ ) displays the complete spectral contribution of each dye in a selected well over the duration of the PCR run.



Note: Only one well can be displayed at a time.

Notes

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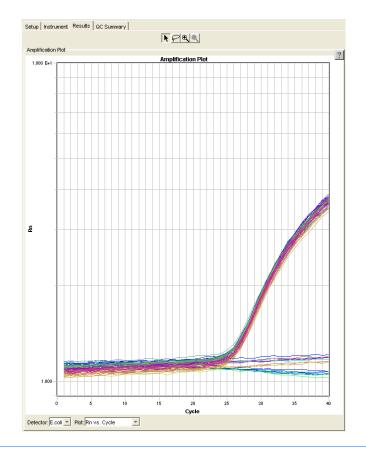
Using the<br/>Amplification Plot<br/>ViewsThe Amplification Plot views allow you to review the post-run amplification results for<br/>selected wells from the plate grid.<br/>Several different views are available from the Plot drop-down list:

- **R**<sub>n</sub> vs. Cycle (below)
- $\Delta \mathbf{R}_{n}$  vs. Cycle (page 95)
- C<sub>T</sub> vs. Well Position (page 96)

**Note:** For more information on using the Amplification Plot views to evaluate amplification results, see the *SDS Online Help*.

#### R<sub>n</sub> vs. Cycle (linear scale)

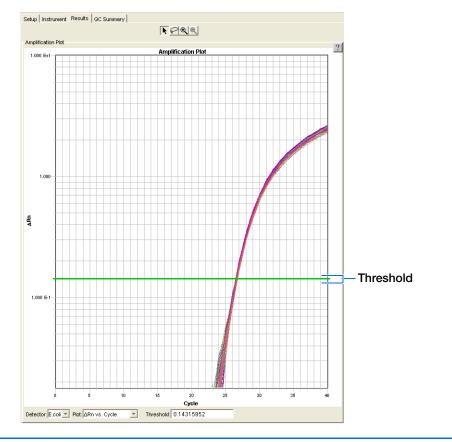
This plot displays normalized reporter dye fluorescence signal (as  $R_n$ ) in linear scale as a function of run cycle. You can use this plot to identify and examine irregular amplification.



**Note:** For more information about  $R_n$ , refer to the *Real-Time PCR Systems Chemistry Guide* (PN 4348358).

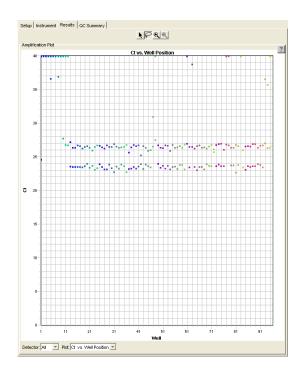
#### $\Delta R_n$ vs.Cycle (log scale)

This plot displays the reporter dye fluorescence signal (as  $\Delta R_n$ ) in log scale as a function of run cycle, and is the default view after analysis completes. You can use this plot to identify and examine irregular amplification and to manually set the threshold and baseline parameters for the run.



**IMPORTANT!** Applied Biosystems recommends verifying that the baseline and threshold were called correctly for each well. If necessary, adjust the values manually as described in the *SDS Online Help*.

#### Ct vs. Well Position Plot



This plot displays threshold run cycle  $(C_T)$  as a function of well position. You can use this plot to locate outliers from detector data sets. See "Omitting Samples" below.

# **Omitting Samples** Experimental error (such as contamination, pipetting errors, etc.) can produce atypical amplification data for some wells. These wells typically produce $C_T$ values that differ significantly from the average. If included in the baseline and threshold value calculations, these outlying data (outliers) can result in erroneous threshold run cycle $(C_T)$ measurements.

To ensure precision, carefully review your data set for outliers. You can remove outliers manually using the Ct vs. Well Position plot and the plate grid. For more information about omitting wells, refer to the *SDS Online Help*.

**Note:** You will need to re-analyze the data each time you remove samples from the data set.

#### Adjusting Plot Settings

Click or select **View > Display Setting** to open the Display Settings dialog box and adjust the selected plot settings.

Display Settings				
Select Pane/View Results Grid Background Plot Rew Data Plot New Port	Results Grid	view as default once analysis h Attribute	Show	
Multicomp. Data Plot         Amplification Plot         CT vs. Well Position         Standard Curve Plot         Dissociation Curve	1 2 3 4 5	Well Color Flag Detector Color Detector Sample	য য য য	+
	Move	Up Move Down		
? Save as default for future documents		Apply	Ok C	Cancel

**Note:** The adjustable settings depend on which plot you are viewing. Refer to the *SDS Online Help* for more information.

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Appendix C Analyzing and Viewing Amplification Data Viewing the Amplification Data

## Flags and Filtering for Allelic Discrimination (AD) Plate Documents

## **Overview**

In Sequence Detection Systems (SDS) Software v2.3, you can automatically flag and filter results data to meet specified criteria. Flags and filters are assay-specific and are assigned at either the system level or are user-configured.

- System level flags are not user-configurable. These flags are displayed in the Results Table and the QC Summary tab.
- User-configured flags are set/defined by the user. These flags are also displayed in the Results Table and QC Summary tab.

For more information about flags and filters, see the SDS Online Help.

Using the Analysis Settings Dialog Box to Assign Flags You assign flags when you configure the analysis settings for an individual plate document or study. When you open the Analysis Settings dialog box for your current plate document, a list of the flags applicable to that plate document appears in the Plate tab.

- 1. Click 🗾 (or select Analysis > Analysis Settings) to open the Analysis Settings dialog box.
- **2.** Select the **Plate** tab. A list of all applicable flags for the open plate document is displayed.

For more information about assigning flags, see the SDS Online Help.

**Plate Tab** The contents of the Plate tab are described on page 100.

Flag Condition and Omit Settings	11		[	
Flag Condition and Omit Plates When	Flag	Condition	Flag Conditi	Omit
Fluorescence is off-scale (FOS)	V			
A well has missing data (HMD)	V		-	
Large mean squared error is (LME)	V	>	1000	
A well is empty (EW)	V			
Plate quality is (PQ)	V	>	61	
Bad passive reference signal (BPR)	<u>र</u>	<	500	
The distance between cluster and NTCs is (DCN)		<=	1	
Number of clusters is (NOC)				
Percentage of outliers is (POU)		>	10	
Hardy Weinberg value is (HW)	V I	<	0.5	
The number of samples is (SNS)	<b>N</b>	<=	2	
SNP quality is (SQ)	V	<	0.8	

No.	Column	Description
1	Flag Condition and Omit Plates When	Name of the flag setting.
2	Flag Condition	Indicates whether or not to flag a condition. If the checkbox is checked, the selected condition will be flagged.
3	Condition	Allows you to select conditions for the flag from a drop-down list. The drop-down list contains conditions such as <, >, =, etc.
		<b>Note:</b> The Condition drop-down lists vary per flag. If conditions are not applicable to a flag, no drop-down list is available.
4	Flag Condition Value	Allows you to enter a numeric range or threshold for a flag. If you specify a value outside the allowed range/threshold, an error message is displayed.
		<b>Note:</b> If ranges/thresholds are not applicable to a flag, you will not be able to enter a value.
5	Omit	Determines whether or not to omit a well if the flag condition is met. If the checkbox is checked, the well will be omitted if the condition is met.
		<b>Note:</b> Well omission behavior is different depending on whether they are omitted manually or by the flag and filter settings. Wells omitted here will contain result values that were generated until the wells were omitted. You will be able to view the flag data/condition for omitted wells. However, when wells are omitted manually, results data for these wells are lost.

## **Viewing Flags**

In the plate document, you can view flags in the:

• QC View of the Plate Grid

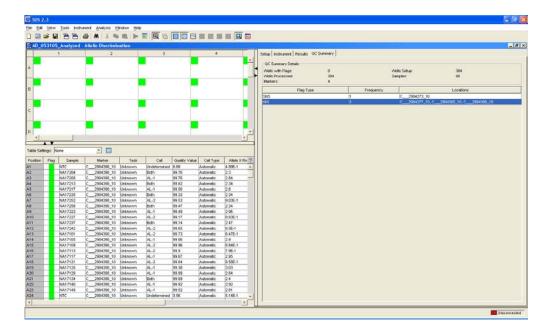
For Allelic Discrimination (AD) plate documents, the Plate Grid automatically switches to QC View after analysis. (To zoom in on the flags, click **View > Grid Zoom**.) Only flags are displayed in the QC view; all other display items (sample name, detector color, etc.) are turned off.

• QC Summary Tab

The QC Summary tab provides an overview of the plate document's quality.

• Results Table

View flags, filter the results based on flags, and sort the table by flags.



Flag lcons The following flag icons are used in the Results Table and QC view of the Plate Grid:

lcon	Description
	Passing well (no flags are assigned)
3	Flagged well
	Well omitted by the algorithm
$\boxtimes$	Well omitted manually
	<b>Note:</b> In the Results Table, the Flag column is blank for manually omitted wells.

Appendix D Flags and Filtering for Allelic Discrimination (AD) Plate Documents *Viewing Flags* 

## References

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Lee, L. G., Connell, C. R., and Block, W. 1993. Allelic discrimination by nick-translation PCR with fluorogenic probes. *Nucleic Acids Res.* 21:3761–3766.

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References

## Index

#### **Numerics**

7900HT Fast Real-Time PCR System 2

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