Pre-Developed TaqMan[®] Assay Reagents

Allelic Discrimination

Protocol



© Copyright 2000-2005, 2010 Applied Biosystems. All rights reserved.

For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice. Applied Biosystems assumes no responsibility for any errors that may appear in this document. This document is believed to be complete and accurate at the time of publication. In no event shall Applied Biosystems be liable for incidental, special, multiple, or consequential damages in connection with or arising from the use of this document.

FOR PRE-DEVELOPED TAQMAN® ASSAY REAGENTS FOR ALLELIC DISCRIMINATION

NOTICE TO PURCHASER: LIMITED LICENSE

The use of uracil-N-glycosylase for carryover prevention is licensed by Invitrogen Corporation under U.S. patents and foreign equivalents for research purposes only. No right for use in other applications, including the diagnosis of disease in humans, animals, or plants under any patents owned by Invitrogen Corporation are covered by the purchase of this product.

ABI PRISM, Applied Biosystems, MicroAmp, and VIC are registered trademarks and AB (Design), and FAM are trademarks of Applied Biosystems or its subsidiaries in the U.S. and/or certain other countries.

AmpErase, AmpliTaq Gold, GeneAmp, and TaqMan are registered trademarks of Roche Molecular Systems, Inc.

All other trademarks are the sole property of their respective owners.

Part Number 4312214 Rev. D 9/2010

Contents

Preface

Safetyv
Safety Alert Words v
Chemical Hazard Warningv
Chemical Safety Guidelines vi
About MSDSs vi
Obtaining MSDSs vi
Chemical Waste Hazard vii
Chemical Waste Safety Guidelines vii
Waste Disposal
Biological Hazard Safety viii
How to Obtain Support ix

Using the Pre-Developed TaqMan[®] Assay Reagents – Allelic Discrimination

About TaqMan PDARs for AD	. 1
Product Overview	. 1
About This Protocol	. 1
Allelic Discrimination Using the 5' Nuclease Assay	. 2
AD Assay Mix Features	. 2
Modified TaqMan Probes	. 2
5' Nuclease Assay	. 2
Preventing Contamination	. 4
Overview	. 4
About AmpErase UNG	. 4
General PCR Practices	. 4
Materials and Equipment	. 5
Assay Contents	. 5
Storage and Stability	. 5
Required Materials and Equipment Not Included	. 6

Allelic Discrimination Assay Overview	. 7
PCR Amplification	. 8
PCR Amplification Overview	. 8
Recommended Template	. 8
Reagent Preparation Guidelines	. 8
Assay Setup Recommendations	. 8
Preparing the Reaction Mix	. 9
Preparing the Reaction Plate	11
Selecting a Thermal Cycler	12
Performing PCR	12
Endpoint Plate Read	13
Endpoint Plate Read Overview	13
Calibrating the 7700 System	13
SDS Software	13
Creating a New Plate Read File	13
Setting Up a Plate Read File	15
Loading the Reaction Plate	17
Running a Plate Read	17
Plate Read Analysis	19
Plate Read Analysis Overview	19
Setting Up the Plate Read Analysis	19
Confirming the SDS Software Configuration	23
Analyzing a Plate Read	24
Calling Allele Types	24
Analyzing Allele Types	30
Troubleshooting	31
SDS Software Features for TagMan PDARs for AD	32
Menu Items	32
New Plate Read Setup View	35
Old Plate Read Setup View	35
Plate Read Analysis View	36
Allelic Discrimination Window	36
References	37

Preface

This preface contains:
Safetyv
How to Obtain Support

Safety

Safety Alert Words	Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word– IMPORTANT, CAUTION, WARNING, DANGER –implies a particular level of observation or action, as defined below:
	IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.
	CAUTION – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
	WARNING – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
	DANGER – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.
Chemical Hazard Warning	WARNING CHEMICAL HAZARD . Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

Chemical Safety	To minimize the hazards of chemicals:
Guidelines	 Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page vi.) Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
	• Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
	• Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
	• Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.
About MSDSs	Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to <i>new</i> customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.
	Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.
Obtaining MSDSs	You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.
	To obtain MSDSs:
	1. Go to https://docs.appliedbiosystems.com/msdssearch.html
	2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click Search .

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

Chemical Waste Hazard

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

To minimize the hazards of chemical waste:

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

- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.
- **Waste Disposal** If potentially hazardous waste is generated when you operate the instrument, you must:
 - Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
 - Ensure the health and safety of all personnel in your laboratory.
 - Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

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

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

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

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

How to Obtain Support

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

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

At the Support page, you can:

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

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

Using the Pre-Developed TaqMan[®] Assay Reagents – Allelic Discrimination

About TaqMan PDARs for AD

Pre-Developed TaqMan[®] Assay Reagents for Allelic Discrimination Product Overview (TaqMan[®] PDARs for AD) are optimized assays for the discrimination of specific alleles. Each TagMan PDAR for AD allows researchers to genotype individuals for specific alleles. TagMan PDARs for AD are easy to perform because the method requires only three components: • Genomic DNA sample • 10X Allelic Discrimination Assay Mix (specific for each polymorphism) • 2X TaqMan[®] Universal PCR Master Mix Allele 1 and Allele 2 Controls are also provided with each assay. About This This protocol describes how to: Protocol • Prepare reaction plates for PCR on an ABI PRISM[®] 7700 Sequence Detection System (SDS), GeneAmp[®] PCR System 9700, or GeneAmp[®] PCR System 9600. • Perform an endpoint plate read on the 7700 SDS only. • Analyze endpoint plate reads and call allele types using ABI PRISM[®] SDS software v. 1.7 or later.

Allelic Discrimination Using the 5' Nuclease Assay

AD Assay Mix Features	 The Allelic Discrimination Assay Mix (AD Assay Mix) contains: Sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest Two modified TaqMan probes: One probe matches the Allele 1 sequence. One probe matches the Allele 2 sequence.
Modified TaqMan Probes	 Each modified TaqMan probe contains: A reporter dye at the 5'-end of each probe: VIC[®] dye is linked to the 5'-end of the Allele 1 probe. FAM[™] dye is linked to the 5'-end of the Allele 2 probe. A nonfluorescent quencher at the 3'-end of the probe: Because the quencher does not fluoresce, the 7700 SDS can measure reporter dye contributions more accurately. A minor groove binder: This modification increases the melting temperature (T_m) without increasing probe length (Afonina <i>etpal.</i>, 1997; Kutyavin <i>etpal.</i>, 1997), permitting the design of shorter probes. This modification results in greater differences in T_m values between matched and mismatched probes, producing more accurate allelic discrimination.
5´ Nuclease Assay	During PCR, each probe anneals specifically to complementary sequences between the forward and reverse primer sites. AmpliTaq Gold [®] DNA polymerase can cleave only probes that hybridize to the target. Cleavage separates the reporter dye from the quencher dye, increasing fluorescence by the reporter. Thus, the fluorescence signal(s) generated by PCR amplification indicate(s) the sequences that are present in the sample. Mismatches Between Probe and Target Sequences Mismatches between a probe and target reduce the efficiency of probe hybridization. Furthermore, AmpliTaq Gold DNA polymerase is more likely to displace the mismatched probe rather than cleave it to release reporter dye.

The next figure illustrates results from matches and mismatches between target and probe sequences in TaqMan PDARs for AD assays (Livak et al., 1995; Livak et al., 1999).



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

A substantial increase in	Indicates
VIC dye fluorescence only	Homozygosity for Allele 1
FAM dye fluorescence only	Homozygosity for Allele 2
Both fluorescent signals	Heterozygosity

Preventing Contamination

Overview	PCR assays require special laboratory practices to avoid false positive amplifications (Kwok and Higuchi, 1989). The high throughput and repetition of these assays can lead to amplification of a single DNA molecule (Saiki et al., 1985; Mullis and Faloona, 1987).	
About AmpErase UNG	AmpErase [®] uracil-N-glycosylase (UNG) is a pure, nuclease-free, 26-kDa recombinant enzyme encoded by the <i>Escherichia coli</i> uracil-N-glycosylase gene. This gene has been inserted into an <i>E. coli</i> host to direct expression of the native form of the enzyme (Kwok and Higuchi, 1989).	
	UNG acts on single- and double-stranded dU-containing DNA by hydrolyzing uracil-glycosidic bonds at dU-containing DNA sites. The enzyme causes the release of uracil, thereby creating an alkali- sensitive apyrimidic site in the DNA. The enzyme has no activity on RNA or dT-containing DNA (Longo et al., 1990).	
General PCR	Please follow these recommended procedures:	
Practices	• Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves when preparing samples for PCR amplification.	
	• Change gloves whenever you suspect that they are contaminated.	
	• Maintain separate areas, dedicated equipment, and supplies for:	
	 Sample preparation 	
	– PCR setup	
	- PCR amplification	
	 Never bring amplified PCR products into the PCR setup area. Open and close all sample tubes carefully. Try not to splash or 	
	spray PCR samples.	
	• Keep reactions and components capped as much as possible.	
	• Use positive-displacement pipettes or aerosol-resistant pipette tips.	
	• Periodically clean lab benches and equipment with 10% bleach solution.	

Materials and Equipment

Assay Contents

This product contains sufficient reagent to perform 400 PCR reactions of 25 μ L each. This product also contains sufficient control reagents to perform 16 control reactions for each allele.

Item	Description
Allelic Discrimination (AD) Assay Mix	 10× mixture for 400 reactions^a Forward and reverse primers flanking a specific polymorphic sequence VIC dye-labeled probe that binds Allele 1 FAM dye-labeled probe that binds Allele 2
Allele 1 Control (AL1)	5X solution for 40 reactions ^a Template with the AL1 sequence
Allele 2 Control (AL2)	5X solution for 40 reactions ^a Template with the AL2 sequence
TaqMan [®] Universal PCR Master Mix	 2× solution for 400 reactions^a AmpliTaq[®] Gold DNA polymerase AmpErase[®] UNG dNTPs Passive reference Optimized buffer

a. Reaction size is 25 μ L.

Visit our web site for a list of available assays (see the "How to Obtain Support" on page ix).

Storage and
StabilityAll components of the TaqMan PDARs for AD should be stored at
2 to 8 °C.

IMPORTANT! Do not expose the AD Assay Mix to direct light. Excessive exposure to light damages the fluorescent probes.

Required Materials and Equipment Not Included

Instruments	Source	
ABI PRISM [®] 7700 Sequence Detection System	Contact your local Applied Biosystems	
GeneAmp [®] PCR System 9700 thermal cycler	sales office.	
GeneAmp [®] PCR System 9600 thermal cycler	_	
Software		
Sequence Detector Software v.1.7		

Materials	Source
ABI PRISM [®] Sequence Detection Systems Software v. 1.7 Upgrade Kit	Applied Biosystems PN 4313011ª
MicroAmp [®] Optical 96-Well Reaction Plates and Optical Caps	Applied Biosystems PN 403012
Sequence Detection Systems Spectral Calibration Kit	Applied Biosystems PN 4305822
ABI PRISM [®] 7700 Sequence Detection System User Bulletin: Generating New Spectra Components ^b	Applied Biosystems PN 4306234
Centrifuge with 96-well plate adapter	Major Laboratory Supplier (MLS)
DNase-free water	MLS
Disposable gloves	MLS
Microcentrifuge	MLS
Pipette tips, aerosol-resistant	MLS
Pipettors, positive-displacement, air-displacement	MLS
Polypropylene tubes	MLS
TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)	MLS

a. Required to run allelic discrimination assays using TaqMan PDARs for AD. Contact Applied Biosystems technical support to obtain this version.

b. Necessary only for 7700 instruments not calibrated with the VIC dye. See "Calibrating the 7700 System" on page 13 for more information.

Allelic Discrimination Assay Overview



PCR Amplification

PCR Amplification	In this step, you:Prepare the reaction mix
Overview	 Prepare an optical 96-well reaction plate containing: No Template Controls Allele 1 Controls Allele 2 Controls Genomic DNA samples Perform PCR
Recommended Template	The recommended template for TaqMan PDARs for AD assays is genomic DNA (2 to 20 ng/ μ L).
Reagent Preparation Guidelines	 The following guidelines ensure optimal PCR performance: Keep all TaqMan PDAR mixes protected from light, in the refrigerator, until you are ready to use them. Excessive exposure to light damages the fluorescent probes. Before using TaqMan PDARs for AD, resuspend the 10X AD Assay Mix by vortexing and briefly centrifuge the tube. Resuspend the 2X TaqMan Universal PCR Master Mix by swirling the bottle. Prior to use, thaw any frozen genomic DNA samples by placing them on ice. When thawed, resuspend the samples by vortexing and then briefly centrifuge the tubes. Prepare the reaction mix for each assay in a separate microcentrifuge tube before transferring it to the reaction plate for thermal cycling and fluorescence analysis.
Assay Setup Recommenda- tions	 Follow Applied Biosystems recommendations for designing assay setup to ensure optimal performance of TaqMan PDARs for AD. Run controls for each assay for a high confidence level. Four No Template Controls (NTCs) Four Allele 1 Controls (AL1) Four Allele 2 Controls (AL2) Run one assay per plate for high throughput.

The figure below shows an example of the placement of control and sample reactions.

NTC	NTC	NTC	NTC	AL1	AL1	AL1	AL1	AL2	AL2	AL2	AL2
A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
UNKN											
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
UNKN											
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
UNKN											
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UNKN											
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
UNKN											
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
UNKN											
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
UNKN											
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Preparing the Reaction Mix

The reaction mix for TaqMan PDARs for AD assays contains TaqMan Universal PCR Master Mix, AD Assay Mix, and DNase-free water.

CAUTION CHEMICAL HAZARD. TaqMan Universal PCR Master Mix may cause eye and skin irritation. Exposure may cause discomfort if swallowed or inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To prepare the reaction mix:

1.	Calculate the number of reactions to be performed for each assay.
	Note: Include at least 4 NTCs, 4 AL1 Controls, and 4 AL2 Controls on each plate for a high confidence level for allele calls.

To prepare the reaction mix: (continued)

Component	Volume (μL) per Reaction
TaqMan Universal PCR Master Mix (2×)	12.5
Allelic Discrimination Assay Mix (10X)	2.5
DNase-free water	5.0
Total	20.0
Gently swirl the bottle of 2× TaqMan U	Universal PCI
Vortex and briefly centrifuge the 10× A Pipette the required volumes of 2× Tac PCR Master Mix and 10X AD Assay M	AD Assay Miz Man Univers Aix into a ster
Vortex and briefly centrifuge the 10× A Pipette the required volumes of 2× Tac PCR Master Mix and 10X AD Assay M tube.	AD Assay Mix Man Univers Aix into a ster Then use
Waster Wix to resuspend. Vortex and briefly centrifuge the 10× A Pipette the required volumes of 2× Tag PCR Master Mix and 10X AD Assay M tube. If the assay contains 1–75 samples and controls	AD Assay Miz Man Univers Itx into a ster Then use 1.5-mL tube
Waster Wix to resuspend. Vortex and briefly centrifuge the 10× A Pipette the required volumes of 2× Taq PCR Master Mix and 10X AD Assay M tube. If the assay contains 1–75 samples and controls 75–100 samples and controls	AD Assay Miz Man Univers fix into a ster Then use 1.5-mL tube 2.0-mL tube
Waster Wix to resuspend. Vortex and briefly centrifuge the 10× A Pipette the required volumes of 2× Tag PCR Master Mix and 10X AD Assay M tube. If the assay contains 1–75 samples and controls 75–100 samples and controls 100–500 samples and controls	AD Assay Mix Man Univers Mix into a ster Then use 1.5-mL tube 2.0-mL tube 5.0-mL tube
Waster Wix to resuspend. Vortex and briefly centrifuge the 10× A Pipette the required volumes of 2× Taq PCR Master Mix and 10X AD Assay N tube. If the assay contains 1–75 samples and controls 75–100 samples and controls 100–500 samples and controls Invert the tube(s) to mix.	AD Assay Miz Man Univers fix into a ster Then use 1.5-mL tube 2.0-mL tube 5.0-mL tube

Preparing the Reaction Plate

Follow the assay setup recommendations on page 8 for preparing the reaction plate.

To prepare the reaction plate:

1. Pipette one control or sample into each well of a MicroAmp Optical 96-Well Reaction Plate.

Note: You may run multiple allelic discrimination assays on one reaction plate.

	Sample Type	Component	Volume (µL) per Well	Well Position ^a				
	No Template Control (NTC)	1× TE buffer	5	A1–A4				
	Allele 1 Control (AL1)	5× AL1 control	5	A5–A8				
	Allele 2 Control (AL2)	5× AL2 control	5	A9-A12				
	Unknown (UNKN)	Genomic DNA (2 to 20 ng/µL)	5	B1–H12				
	a. Recommende Note: Use a ca minimize conta	ed placement for ru alibrated, positive amination and err	inning one assa -displacement :or.	ay per plate.				
2.	Invert the reaction mix tube(s) prepared on page 9 to remix.							
3.	Briefly centrifuge the tube(s) to spin down the contents and to eliminate air bubbles.							
4.	Pipette 20 μ L of reaction mix into each well. Be sure that no cross-contamination occurs from well to well.							
5.	Cap the plate v	with MicroAmp®	Optical Caps.					
6.	Centrifuge the eliminate any a	plate to spin dow air bubbles.	n the contents	s and to				

Selecting a Thermal Cycler Because the data acquired during PCR amplification is not necessary for analysis, any of the following instruments can be used for PCR amplification:

• ABI PRISM 7700 Sequence Detection System

Note: Use of the 7700 Sequence Detection system allows for real-time analysis of PCR, which is helpful for troubleshooting.

- GeneAmp PCR System 9700 thermal cycler
- GeneAmp PCR System 9600 thermal cycler

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

Performing PCR To perform PCR:

1

	Ducanona	the the model	arvaling	aanditiana
	Program	ine mermai	cycnng	conditions
•	1 I O SI MIII	the therman	e, enns	contaitiono.

IMPORTANT! These conditions are optimized for use only with TaqMan PDARs for AD because of the modified TaqMan probes.

	Initial	Steps	Each	of 50 Cycles
Stage	Hold	Hold Hold		Anneal/Extend
Temp (°C)	50	95	92	60
Time	2 min	10 min	15 sec	90 sec

Note: The 2-minute, 50 °C step is required for optimal AmpErase UNG enzyme activity. The 10-minute, 95 °C step is required to activate the AmpliTaq Gold DNA Polymerase.

2.	Set the reaction volume to $25 \ \mu L$.
3.	Load the reaction plate into the thermal cycler.
4.	Begin thermal cycling.

Endpoint Plate Read

Endpoint Plate	In this s	tep, you use the 7700 SDS and SDS software v. 1.7 or later to:					
Read Overview	• Create and set up a plate read file						
	• Ru	n an endpoint plate read					
Calibrating the 7700 System	Before using TaqMan PDARs for AD, the 7700 System should be calibrated for the VIC dye using the Sequence Detection Systems Spectral Calibration Kit. This kit provides the standards needed to calibrate the instrument for use with products containing TaqMar VIC dyes. For more information, refer to <i>ABI PRISM 7700 Sequen</i> <i>Detection System User Bulletin: Generating New Spectra</i> <i>Components</i> .						
SDS Software	Allelic discrimination assays using TaqMan PDARs for AD requires installation of SDS software v. 1.7 or later.						
	If you d Applied	o not have the latest version, you can receive it by contacting l Biosystems technical support (see the "Preface" on page v).					
Creating a New Plate Bead File	To crea	te a new plate read file:					
	1.	Launch the SDS software.					
	2.	Close the untitled window that opens.					

To create a new plate read file: (continued)

- 3. Create a new plate read file.
 - a. From the File menu, select New Plate.
 - b. In the **New Plate** dialog box, select the following options:

Plate Type	Allelic Discrimination
Plate Format	Standard Plate
Run	Plate Read

c. Click **OK** to open the plate read window.

Sample Type : Not In Us Sample Name : Replicate :		nple Type: Not In Use nple Name: Replicate:							Comm	ent:	iser im ir		_ Iype pop· men
Sho	w Analysi	s	-		_	-	_	-					
		2	5	4	5	6	/	8	9	10	-11	12	Sho
•													Anal
в													butte
С													
D													
E													
F													
6													

Setting Up a Plate Read File

In this procedure, the plate read file is configured with the appropriate sample types, reporter dyes, quenchers, and assay setup.

To set up a plate read file:

	1.	From the Sample Type pop-up menu, select Sample Type Setup to open the Sample Type Setup dialog box.
		Sample Type Setup
		Acronym Name Color Reporter IPC+ Internal Positive IPC- Internal Positive AL1 Allel 1 VIC ‡ AL2 Allel 2 FAM< ‡ NTC No Template Control NAC No Amplification UNKN Unknown Reference Quencher
		RUX TAMRA \$
	2.	 Complete the Sample Type Setup dialog box. For Allele 1 (AL1), select VIC for the reporter dye. For Allele 2 (AL2), select FAM for the reporter dye. Uncheck the box next to Quencher. Click OK.
-	3.	 Assign labels to the No Template Control wells (NTC). a. Select the wells representing NTC by clicking them. b. From the Sample Type pop-up menu, select NTC - No Template Control.
	4.	 Assign labels to the control wells with Allele 1 Control (AL1). a. Select the wells representing AL1 by clicking them. b. From the Sample Type pop-up menu, select AL1-Allele 1.

	Assign labels to the control wells with Allele 2 Control (AL2).											
	a. Select the wells representing AL2 by clicking the										nem	
	b. F	From 1 L2-A	the S Allelo	amp e 2 .	le Ty	pe p	op-uj	p me	nu, s	select	t	
6.	Assig (UNK	n labe N).	els to	the	samp	ole w	ells v	with	unkr	nown	gen	otyj
	a. S	elect lickir	the v ng the	wells em.	repr	resen	ting	UNK	IN sa	ampl	es by	/
	b. F	rom t J NK N	the S N-Un	amp I kno	le Ty wn .	pe p	op-uj	p me	nu, s	select	t	
	All we	ells in	ı use	shou	ıld no	ow b	e lab	eled	with	the	samp	ole
	type.											
					7	untitle	ed 2		7			
		C	L _ ∐nkno⊶	n								
	Sample Ty Sample Na	/pe:[UNKN	I - UIIKIIOW		<u> </u>				770 Star	O Allelic ndard Plat	Discrimiı te	nation
	Sample Ty Sample Na Replic	/pe: <u>UNKN</u> me: ate:							770 Star Comm	O Allelic ndard Plat nent:	Discrimii te	nation
	Sample Ty Sample Na Replic	/pe: UNKN me: ate:	- UNKNOW						770 Star Comm	O Allelic ndard Plat nent:	Discrimiı te	nation
	Sample Ty Sample Na Replic	/pe: UNKN me:ate:	- Onknow						770 Star Comm	O Allelic Idard Plat	Discrimit te	nation
	Sample Ty Sample Na Replic	rpe : UNKN me :	3 NTC A3	4 NTC A4	5 AL1 AS	6 AL1 A6	7 AL1 A7	8 AL1 A8	770 Star Comm 9 AL2 A9	0 Allelic ndard Plat nent : 10 AL2 A10	Discrimin te 11 AL2 A11	12 AL2 A12
	Sample Ty Sample Na Replic Show Ana 1 A NTC A1	rpe : UNKN me :	3 NTC A3	4 NTC A4	5 AL1 A5	6 AL1 A6	7 AL1 A7	8 AL1 A8 UNKN	9 AL2 A9 UNKN	0 Allelic hdard Plat hent: 10 AL2 A10 UNKN	Discrimin te 11 AL2 A11 UNKN	12 AL2 AL2 UNKN
	Sample T: Sample Na Replic	rpe : UNKN me : ate : lysis 2 NTC A2 UNKN B2	3 NTC A3 UNKN B3	4 NTC A4 UNKN B4	5 AL1 A5 UNKN B5	6 AL1 A6 UNKN B6	7 AL1 A7 UNKN B7	8 AL1 A8 UNKN BS	770 Star Comm 9 AL2 A9 UNKN B9	0 Allelic ndard Plat hent: 10 AL2 A10 UNKN B10	Discrimit le 11 AL2 A11 UNKN B11	12 AL2 A12 UNKN B12
	Sample Na Sample Na Replic	IVER INTERIOR INTERIORI INTERIO INTERIO INTERIO INTERIORI INTERIO INTERIORI INTERIORI	3 NTC A3 UNKN B3 UNKN C3	4 NTC A4 UNKN B4 UNKN C4	S AL1 AL1 UNKN B5 UNKN C5	6 AL1 A6 UNKN B6 UNKN C6	7 AL1 A7 UNKN B7 UNKN C7	8 AL1 AS UNKN BS UNKN CS	770 Star Comm 9 AL2 A9 UNKN B9 UNKN C9	O Allelic ndard Plat sent : 10 AL2 AL2 AL2 AL2 BI0 UNKN BI0	11 AL2 AL1 UNKN B11 UNKN C11	12 AL2 AL2 A12 UNKM B12 UNKM C12
	Sample T; Sample Na Replic Show Ana 1 A ATC A D UNKK D UNKK D D	IVEN INTERACTION INTERACTIONI INTERACTICA INTERACTIC	3 NTC A3 UNKN B3 UNKN C3 UNKN D3	4 NTC A4 UNKN B4 UNKN C4 UNKN D4	5 AL1 A5 UNKN B5 UNKN C5 UNKN D5	6 AL1 AG UNKN BG UNKN CG UNKN DG	7 AL1 A7 UNKN B7 UNKN C7 UNKN D7	8 AL1 AS UNKN BS UNKN CS UNKN DS	770 Star Comm 9 AL2 A9 UNKN B9 UNKN C9 UNKN D9	O Allelic ndard Plat sent : 10 AL2 A10 UNKN B10 UNKN C10 UNKN D10	11 AL2 A11 UNKN B11 UNKN C11 UNKN D11	12 AL2 AL2 A12 UNKN B12 UNKN C12 UNKN D12
	Sample T; Sample Na Replic Show Ana 1 A NTC A NTC A D UNKK D UNKK D UNKK C C C C	ysis ysis Vysis Vysis Vysis Vikkn	3 NTC A3 UNKN B3 UNKN C3 UNKN D3 UNKN E5	4 NTC A4 UNKN B4 UNKN C4 UNKN D4 UNKN D4	S AL1 A5 UNKN B5 UNKN C5 UNKN D5 UNKN UNKN	6 AL1 A6 UNKN B6 UNKN C6 UNKN D6 UNKN E6	7 AL1 A7 UNKN B7 UNKN C7 UNKN D7 UNKN E7	8 AL1 AS UNKN BS UNKN CS UNKN DS UNKN ES	770 Star Comm 9 AL2 A9 UNKN B9 UNKN D9 UNKN D9 UNKN E9	O Allelic hdard Plat hent : 10 AL2 A10 UNKN B10 UNKN C10 UNKN D10 UNKN D10	Discrimit te 11 AL2 A11 UNKCN B11 UNKCN D11 UNKCN D11 UNKCN	12 AL2 AL2 AL2 AL2 AL2 AL2 AL2 AL2 UNKN D12 UNKN D12 UNKN D12 UNKN D12 UNKN D12
	Sample T; Sample Ra Replic Show Ana 1 NTC A 1 NTC A 1 NTC A 1 NTC C C C C UNEN D UNEN E E	IVIEN IN INTERIOR INTERIORI INTERI	3 NTC A3 UNKN B3 UNKN D3 UNKN B3 UNKN UNKN UNKN	4 NTC A4 UNKN B4 UNKN C4 UNKN D4 UNKN	S ALI AS UNKN BS UNKN DS UNKN UNKN UNKN	6 AL1 AG UNKN B6 UNKN C6 UNKN D6 UNKN	7 AL1 AZ1 UNKN B7 UNKN D7 UNKN E7	8 AL1 AS UNKN BS UNKN DS UNKN ES UNKN	770 Star Comm 9 AL2 A9 UNKN UNKN UNKN D9 UNKN E9 UNKN	O Allelic hdard Plat hent: 10 AL2 AL2 AL2 AL2 UNKN UNKN UNKN UNKN UNKN UNKN UNKN UNKN UNKN	Disorimit te 11 AL2 A11 UNKN B11 UNKN D11 UNKN E11 UNKN	12 AL2 AL2 AL2 UNKN B12 UNKN C12 UNKN D12 UNKN E12 UNKN E12 UNKN
	Sample T: Sample T: Sample Re Plic Show Ana 1 NTC A 1 NTC A 1 NTC C C C C C C C C C C C C C C C C C C	INKN FEELUNKN	3 NTC A3 UNKN C3 UNKN D3 UNKN E3 UNKN F5	4 NTC A4 UNKN B4 UNKN C4 UNKN D4 UNKN F4 UNKN F4	S AL1 AS UNKN BS UNKN DS UNKN ES UNKN FS	6 AL1 A6 UNKN B6 UNKN D6 UNKN E6 UNKN F6	7 AL1 A7 UNKN B7 UNKN D7 UNKN E7 UNKN F7	8 AL1 A8 UNKN B8 UNKN C8 UNKN E8 UNKN F8	9 AL2 A9 UNKN B9 UNKN C9 UNKN F9 UNKN F9	O Allelic dard Plat sent : 10 AL2 A10 UNKN B10 UNKN D10 UNKN E10 UNKN E10	Disorimit te 11 AL2 A11 UNKN B11 UNKN D11 UNKN E11 UNKN E11 UNKN	12 AL2 AL2 A12 UNKK B12 UNKK C12 UNKK E12 UNKK F12 UNKK
	Sample T: Sample Replic Show Ana 1 A AT B B B C C C C1 UNEC D UNEC E E UNEC F F F F F F F G UNEC	IVIEN INTERNATIONAL INTERNATIONALIZIA INTERNATIONALI INTERNATIONAL	3 NTC A3 UNKN B5 UNKN C3 UNKN C3 UNKN F3 UNKN F3	4 NTC A4 UNKN B4 UNKN C4 UNKN C4 UNKN F4 UNKN F4 UNKN 64	5 AL1 AS UNKN CS UNKN CS UNKN CS UNKN F5 UNKN F5	6 AL1 A6 UNKN C6 UNKN D6 UNKN F6 UNKN F6	7 AL1 A7 UNKN UNKN	B AL1 AS UNKN UNKN CS UNKN DS UNKN FS UNKN SS	9 AL2 A9 UNKN C9 UNKN C9 UNKN C9 UNKN F9 UNKN F9 UNKN F9	O Allelic dard Plat sent :	Discrimit te 11 AL2 A11 UNKN C11 UNKN C11 UNKN F11 UNKN F11 UNKN F11	12 AL2 AL2 AL2 AL2 AL2 UNKN B12 UNKN D12 UNKK D12 UNKK E12 UNKK E12 UNKK E12 UNKK G12
	Sample T; Sample Ra Replic Show Ana 1 Replic Show Ana 9 UNEN B B B B B B B C C C C C C C C C C C C	UNKN total 2 NTC NTC UNKN	3 NTC A3 UNKN B3 UNKN C3 UNKN D3 UNKN F3 UNKN F3 UNKN H3	4 NTC DARN D4 UNKN C4 UNKN C4 UNKN E4 UNKN F4 UNKN H4	S AL1 AS UNKN BS UNKN CS UNKN ES UNKN F5 UNKN H5	6 AL1 ABC UNKN CCS UNKN ECS UNKN FCS UNKN FCS UNKN HCS	7 AL1 A7 UNKN B7 UNKN C7 UNKN E7 UNKN F7 UNKN H7	8 AL1 BS UNKN CS UNKN CS UNKN FS UNKN HS	9 AL2 A9 UNKN B9 UNKN C9 UNKN E9 UNKN F9 UNKN F9 UNKN H9	0 Allelic dard Plat sent : 10 AL2 AL2 AL2 AL2 AL2 AL2 AL2 AL2 AL2 AL2	Discrimit te 11 AL2 A11 AL2 A11 UNKN C11 UNKN C11 UNKN F11 UNKN F11 UNKN H11	12 12 4L2 4L2 4L2 12 11 12 12 11 12 12 12 12 12 12 12 12
	Sample T; Sample Ra Replic Show Ana 1 NTC A NTC A NTC NTC A NTC A NTC A NTC A NTC NTC A NTC NTC A NTC NTC NTC NTC NTC NTC NTC NTC NTC NTC	UNKN Igsis 2 Igsis UNKN UNKN <	3 NTC AS UNICH B3 UNICH C3 UNICH D3 UNICH E3 UNICH H3 UNICH H3	4 NTC A4 UNKN C4 UNKN D4 UNKN E4 UNKN F4 UNKN H4	S AL1 AS UNICH BS UNICH CS UNICH DS UNICH UNICN UNICN UNICN UNICN UNICN UNICN UNICN UNICN UNICN	6 AL1 A6 UNKN D6 UNKN C6 UNKN F6 UNKN H6	Z AT A7 UNKN B7 UNKN C7 UNKN UNKN F7 UNKN H7	B ALI AB UNKN BB UNKN CS UNKN DB UNKN FS UNKN HS	770 Star Comm 9 4.2 A9 UNKN C9 UNKN C9 UNKN E9 UNKN E9 UNKN F9 UNKN F9 UNKN F9 UNKN F9	O Allelic ddard Plat eent : AL2 A10 UNKN B10 UNKN D10 UNKN F10 UNKN F10 UNKN H10	Discrimin e 11 A12 A11 UNKA C11 UNKA E11 UNKA	12 12 4L2 4L2 4L2 12 12 12 12 12 12 12 12 12 12 12 12 12

To set up a plate read file: (continued)

Loading the Reaction Plate

To load the reaction plate:

1.	Place the reaction plate into the sample compartment.
2.	Pull the heated cover forward, and secure it in place by tightening the knob.
3.	Turn the knob clockwise to lower and secure the heated cover over the sample plate.
	IMPORTANT! When securing the heated cover in place, be careful not to overtighten the knob. Damage to the instrument could occur.

Running a Plate Read The 7700 System can detect and measure the fluorescence signals generated from the PCR amplification during an endpoint plate read.

To run a plate read:



To run a plate read: (continued)

4.	Remove the reaction plate from the instrument.
	IMPORTANT! To avoid PCR contamination with amplified product, do not remove the caps from the plate.
5.	Discard the reaction plate after analyzing the plate read, when you are confident that the plate read was successful.

Plate Read Analysis

Plate Read
Analysis
OverviewAfter the plate read is completed, SDS software calculates the
fluorescence measurements made during the plate read and plots Rn
values based on the signals from each well. Using the software, you
can determine which alleles are present in each sample.

In this step you use SDS software v. 1.7 or later to:

- Analyze a plate read
- Call allele types manually

Setting Up the Plate Read Analysis **Note:** You can open completed endpoint plate read files at a later time for analysis.

To set up the plate read analysis:

1.	Launch the SDS software and close the untitled window that opens.
2.	From the File menu, select Open Plate.

Sar San	nple Type nple Name Replicate	:: UNKN :: ::	- Unknow	n	:	untitle	2d 2		770 Star Comm	0 Allelic ndard Plat nent:	Disor imir e	ation	Sample Type pop up menu
Sł	iow Analys	is	3	4	5	6	7	8	9	10	11	12	Show
٨	NTC A1	NTC A2	NTC A3	NTC A4	AL1 A5	AL1 A6	AL1 A7	AL1 A8	AL2 A9	AL2 A10	AL2 A11	AL2 A12	button
в	UNKN B1	UNKN B2	UNKN B3	UNKN B4	UNKN B5	UNKN B6	UNKN B7	UNKN B8	UNKN B9	UNKN B10	UNKN B11	UNKN B12	
c	UNKN C1	UNKN C2	UNKN C3	UNKN C4	UNKN C5	UNKN C6	UNKN C7	UNKN C8	UNKN C9	UNKN C10	UNKN C11	UNKN C12	
	UNKN D1	UNKN D2	UNKN D3	UNKN D4	UNKN D5	UNKN D6	UNKN D7	UNKN D8	UNKN D9	UNKN D10	UNKN D11	UNKN D12	
-	UNKN E1	UNKN E2	UNKN E3	UNKN E4	UNKN E5	UNKN E6	UNKN E7	UNKN ES	UNKN E9	UNKN E10	UNKN E11	UNKN E12	
- -	UNKN F1	UNKN F2	UNKN F3	UNKN F4	UNKN F5	UNKN F6	UNKN F7	UNKN F8	UNKN F9	UNKN F10	UNKN F11	UNKN F12	
	UNKN G1	UNKN G2	UNKN G3	UNKN G4	UNKN G5	UNKN G6	UNKN G7	UNKN G8	UNKN G9	UNKN G10	UNKN G11	UNKN G12	
Ŭ H	UNKN H1	UNKN H2	UNKN H3	UNKN H4	UNKN H5	UNKN H6	UNKN H7	UNKN HS	UNKN H9	UNKN H10	UNKN H11	UNKN H12	
	If the plate contains									Tł	nen	•	2
		0	ne as	ssay				Go to	o step	o 5.			
		Μ	Multiple assays					Follow the actions below to label wells from all but one assay as Not In Use .					to label say as

To set up the plate read analysis: (continued)

To set up the plate read analysis: (continued)









To set up the plate read analysis: (continued)

Confirming the SDS Software Configuration

Perform this step to confirm that the software uses spectral compensation for endpoint plate reads. Once confirmed, this step is not required for subsequent plate read analyses.

To confirm the SDS software configuration:

1.	Select Instrument > Diagnostics > Advanced Options.						
	Advanced Options						
	Yiever						
	Display mse in Multicomponent View						
	Display best fit in Raw Spectra View						
	Analysis: Spectra Components						
	Use background in "Spectra Components" folder						
	Use pure spectra in "Spectra Components" folder						
	Miscellaneous						
	Set 7700 Exposure Time 25						
	Use Spectral Compensation for Real Time						
	Cancel OK						
2.	Select the Use Spectral Compensation for Endpoint and click OK.						
3.	When the warning message opens, click OK .						
	Please quit and re-launch the application in order to use your new settings.						
	ОК						
	Note: You do not need to quit and relaunch the SDS software at this time.						

Analyzing a Plate To analyze a plate read: Read



Calling Allele Accurate allelic discrimination using TaqMan PDARs for AD requires making manual allele calls.

To call allele types:

1.	Using the Dye pop-up menu at the top of the Allelic Discrimination window, change the view to Dye Components .
2.	Zoom out until all crossmarks are visible in the graph.
3.	Click the zoom out magnifying glass tool to select it. Θ

4.	Click the zoom out magnifying glass on the graph to zoom out.							
	Note: Select the marks with the lasso tool to confirm that all appropriate wells have been selected.							
	Allelic Discrimination Call Underscription Call Call Underscription Call Call Underscription Call Call Call Underscription Call Ligned Call Call Call Call Ligned Call Call Call Call Ligned Call Call Call Call Call Call Call Call Call Call Call Call Call Call Call Call Call Call Call <t< th=""></t<>							
	1 2 3 4 5 6 7 10 11 12 21 Selected marks 6 1 1 1 1 1 1 12 1 1 12 1 1 12 1							
5.	If allele types were automatically called, designate the crossmarks as Undetermined by using the Call pop-up menu.							
	IMPORTANT! Manual allele calling is required.							
6.	Crop and zoom the crossmarks. a. Click the zoom in magnifying glass tool to select it.							
	b. Click and drag the zoom in magnifying glass on the graph to crop and magnify all marks.							
	Call Alter 1 2 Call Alter 1 2							







	10.	Select clusters and call allele types (step 8 and step 9) until									
		all calls are made.									
		Allelic Discrimination									
		▶ P ℓ Dye Components Φ ♥ Graph									
		A 10.00									
		■ 0 1/2 ■ No DNA ■ 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2									
		0.36 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.46 Allele 1									
		V Well Sample AL2 Rn AL1 Rn TAM Rn NTCm T1m T2m T1n T2n Call Vell Sample AL2 Rn AL1 Rn TAM Rn NTCm T1m T2m T1n T2n Call									
		A1 NTC 4.0606 0.4877 1.0000 9.96-01 -0.005 0.019 0.000 0.000 No Amp A2 NTC 3.8921 0.5156 1.0000 1.0E+00 0.005 -0.012 0.000 No Amp									
		A3 NTC 4.1088 0.4955 1.0000 9.7E-01 -0.003 0.028 0.000 0.000 No Amp A4 NTC 3.7731 0.5062 1.0000 1.0E+00 0.003 -0.035 0.000 0.000 No Amp									
		▽ Tray									
		M No. Amp. INo. Amp. INo. Amp. II 1 1 1 2 2 2 2 B 2 2 2 2 2 2 2 1 and 2 1									
		D I and 2 I an									
		6 <u>1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1</u>									
11.		Print the Allelic Discrimination window and export the									
		results.									
	12.	Analyze any additional assays on the plate.									
		a. Close the Allelic Discrimination window to activate									
		the plate read window.									
		h Click Show Satur to raturn to the Satur view									
		b. Chek show setup to return to the setup view.									
		c. Use the Sample Type pop-up menu to label assay									
		wells NTC, AL1, AL2, or UNKN and wells from									
		other assays as Not In Use.									
		d. Click Show Analysis.									
		e. Repeat "Analyzing a Plate Read" on page 24 and									
		"Calling Allele Types" on page 24.									
		Note: You can analyze only one assay at a time.									
	1										

Analyzing Allele Types

Review the results to confirm allele types.

To analyze allele types:



Troubleshooting

Observation	Possible Cause	Suggested Action
Wells in the Tray section of the graph remained	Some samples are plotted beyond the	1. Zoom out with the "-" magnifying glass tool until all crossmarks are visible.
uncalled after all crossmarks were typed.	range of the graph.	Confirm that all marks are visible in the graph by selecting all crossmarks in the graph with the lasso tool.
		The selected marks are highlighted in the Tray section of the Allelic Discrimination window.
		3. Designate all crossmarks as undetermined, and proceed with "Calling Allele Types" on page 24.
NTCs generated fluorescence signals.	PCR contamination may have occurred.	Test your buffers for the presence of nucleic acid.
Distinct clusters were not observed.	Reporter dyes were not appropriately assigned.	1. Confirm the options in the Sample Type Setup dialog box (see "Setting Up a Plate Read File" on page 15).
	Quencher was selected.	2. Reanalyze the plate read.
A sample did not cluster with one specific allele	The sample may: Contain more or less 	1. Check the DNA concentrations of the samples.
type.	DNA than other	2. Retest the sample to confirm.
	 Contain a rare allelic variation or sequence duplication 	3. Test the sample using a different TaqMan PDARs for AD assay.
UNKN samples did not	The sample may:	1. Check the DNA concentrations of the samples
signals.	Contain no DNA Contain PCP	2. Retest the sample to confirm.
	inhibitors	3. Test the sample using a different TagMan
	Be homozygous for a rare allelic variation	PDARs for AD assay.

SDS Software Features for TaqMan PDARs for AD

Menu Items The table below describes the commands used to perform allelic discrimination assays using TaqMan PDARs for AD.

Menu	Command	Function		
File	New Plate	Create a new plate read file.		
New Plate %N Open Plate %O Close %W	Open Plate	Open an existing plate read file.		
Save #S Save As	Close	Close the plate read file.		
Export Import Page Setup	Save	Save the plate read file.		
Print %P Quit %Q	Save As	Save the plate read file with another name.		
	Export	Export data and results (tab delimited).		
	MultiComponent	Export post-read multicomponent fluorescence data.		
	Results	Export allele calls and Rn, m, and n values.		
	Experimental Report	Export run and sample information.		
	Page Setup	Set up orientation, scale, and printer for printing.		
	Print	Print the Allelic Discrimination window or the Experiment Report.		
	Quit	Quit the SDS software.		

Table 1 SDS Software Commands for AD Assays

Menu	Command	Function		
Edit	Cut	Cut selected wells.		
Edit Can't Undo 発Z Cut 発X Copy 発C	Сору	Copy selected wells from the Setup view only.		
Paste 35V Clear Select All %A Preferences	Paste	Paste cut or copied wells from the Setup view only.		
	Clear	Clear wells.		
	Select All	Select all wells.		
	Preferences	Set up New Document Defaults and Document Settings.		
Setup	Sample Type Palette	Show sample types and dye information.		
Setup Thermal Cycler Conditions Sample Type Palette		Label wells with sample types after sample types are set.		

Table 1 SDS Software Commands for AD Assays (continued)

Menu	Command	Function				
Analysis Analysis Display	Allele Settings	Set how calls appear in the Tray section of the Allelic Discrimination window.				
Statistics Settings % Allele Settings	-	Default settings are shown below.				
Allelic Discrimination % Amplification Plot % Multicomponent % Raw Spectra % Standard Curve % Analyze %		Allele Call Settings				
		Allele 1/2: 1 and 2 Cancel OK				
	Allelic Discrimination	Open the Allelic Discrimination window after analyzing a plate read.				
	Analyze	Analyze a plate read from the Analysis view.				
Window Window	Toggle Setup/Analysis	Switch between the Setup view and the Analysis view.				
Experiment Report Event Log		Equivalent to Show Setup and Show Analysis buttons.				
	Experiment Report	View the Experiment Report, which contains run and sample information.				

Table 1 SDS Software Commands for AD Assays (continued)

New Plate Read Setup View

From this view you can:

- Set up sample types and reporter dyes
- Assign sample types for each well

			ſ			untitle	ed 2						Sample
Sar Sar	nple Type nple Name Replicate	e: [Not In	Use		*				770 Star Comm	D Allelic Idard Plat ent:	Diserimiı te	nation	Type pop-up menu
S	now Analys	is											Show
A	1	2	3	4	5	6	7	8	9	10	11	12	Analysis button
c													
D													
E													
F													
6													
н													

Old Plate Read Setup View

From this view you can:

- Confirm Sample Type Setup
- Designate wells as Not In Use, if necessary

] untitled 2										Sample		
Sa Sai	mple Type: UNKN - Unknown 2 7700 Alleli Standard Pl Replicate: Comment:								0 Allelic ndard Pla nent:	Disorimii te	nation	Type pop-u menu	
s	how Analys	sis											Show
	1	2	3	4	5	6	7	8	9	10	11	12	Δnalvsis
	NTC A1	NTC A2	NTC A3	NTC A4	AL1 AS	AL1 A6	AL1 A7	AL1 AS	AL2 A9	AL2 A10	AL2 A11	AL2 A12	button
в	UNKN B1	UNKN B2	UNKN B3	UNKN B4	UNIKN BS	UNKN B6	UNKN B7	UNKN BS	UNKN B9	UNKN B10	UNKN B11	UNKN B12	
с	UNKN C1	UNKN C2	UNKN C3	UNKN C4	UNKN C5	UNKN C6	UNKN C7	UNKN C8	UNKN C9	UNKN C10	UNKN C11	UNKN C12	
D	UNKN D1	UNKN D2	UNKN D3	UNKN D4	UNKN D5	UNKN D6	UNKN D7	UNKN D8	UNKN D9	UNKN D10	UNKN D11	UNKN D12	
E	UNKN E1	UNKN E2	UNKN E3	UNKN E4	UNKN E5	UNKN E6	UNKN E7	UNKN E8	UNKN E9	UNKN E10	UNKN E11	UNKN E12	
F	UNKN F1	UNKN F2	UNKN F3	UNKN F4	UNKN F5	UNKN F6	UNKN F7	UNKN F8	UNKN F9	UNKN F10	UNKN F11	UNKN F12	
6	UNKN G1	UNKN G2	UNKN G3	UNKN G4	UNKN G5	UNKN G6	UNKN 67	UNKN G8	UNKN 69	UNKN G10	UNKN G11	UNKN G12	
н	UNKN H1	UNKN H2	UNKN H3	UNKN H4	UNKN H5	UNKN H6	UNKN H7	UNKN H8	UNKN H9	UNKN H10	UNKN H1 1	UNKN H12	

Plate Read Analysis View

From this view you can:

- Perform an endpoint plate read
- Analyze the plate read file



Allelic Discrimination Window

This window appears after analyzing the endpoint plate read and selecting Allelic Discrimination from the Analysis menu. From this window you can:

- Zoom in and zoom out of the graph to view crossmarks from each reaction
- Call allele types
- Print the window



References

Afonina, I., Zivarts, M., Kutyavin, I., et al. 1997. Efficient priming of PCR with short oligonucleotides conjugated to a minor groove binder. *Nucleic Acids Res.* 25:2657–2660.

Kutyavin, I.V., Lukhtanov, E.A., Gamper, H.B., and Meyer, R.B. 1997. Oligonucleotides with conjugated dihydropyrroloindole tripeptides: base composition and backbone effects on hybridization. *Nucleic Acids Res.* 25:3718–3723.

Kwok, S. and Higuchi, R. 1989. Avoiding false positives with PCR. *Nature* 339:237–238.

Livak, K.J., Flood, S.J.A., Marmaro, J., and Mullah, K.B., inventors; Perkin-Elmer Corporation (Foster City, CA), assignee. 2 Mar. 1999. Hybridization assay using self-quenching fluorescence probe. United States patent 5,876,930.

Livak, K.J., Marmaro, J., and Todd, J.A. 1995. Towards fully automated genome-wide polymorphism screening [letter]. *Nat. Genet.* 9:341–342.

Longo, M.C., Berninger, M.S., and Hartley, J.L. 1990. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene* 93:125–128.

Mullis, K.B. and Faloona, F.A. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods Enzymol.* 155:335–350.

Saiki, R.K., Scharf, S., Faloona, F., et al. 1985. Enzymatic amplification of β -globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230:1350–1354.

Headquarters

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

Worldwide Sales and Support

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

www.appliedbiosystems.com



Applied Biosystems is committed to providing the world's leading technology and information for life scientists.

Printed in USA, 9/2010 Part Number 4312214 Rev. D