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Applied Biosystems High Resolution Melt Module for ViiA™ 7 Software v1.1

Getting Started Guide

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Contents

About This Guide

Purpose

This guide is designed to help you quickly learn to perform High Resolution Melt experiments with ViiA™ 7 Software.

This guide provides step-by-step procedures for:

- Calibrating an Applied Biosystems ViiA™ 7 Real-Time PCR System for High Resolution Melt experiments
- Performing High Resolution Melt (HRM) experiments: Designing the experiment, preparing the reactions, running the reactions, and reviewing and analyzing the HRM data using ViiA™ 7 Software

Prerequisites

This guide assumes that you have working knowledge of the:

- Microsoft® Windows® XP operating system
- Software for your ViiA™ 7 Real-Time PCR System
- General techniques for handling DNA samples and preparing them for PCR

How to use this guide

This guide functions as both a tutorial and a guide for performing an HRM experiment. It contains:

- Instructions specific to the example experiment data file provided in the ViiA™ 7 Software
- Tips for running your own experiments

Note: First-time users of the ViiA™ 7 Real-Time PCR System, please read *Getting Started with ViiA™ 7 Experiments*. It is the first booklet of the *Applied Biosystems ViiA™ 7 Real-Time PCR System Getting Started Guides* (Part no. 4441434).

Safety conventions used in this document

Safety alert words

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

Definitions

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

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.

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard symbols that are affixed to Applied Biosystems instruments* (see "Safety symbols" on page 39).

Examples

The following examples show the use of safety alert words:

IMPORTANT! You must create a separate sample entry spreadsheet for each 384-well plate.

CAUTION! Do not install on the computer additional software other than antivirus software. Changes to the configured software could void the instrument warranty and cause the system to be nonoperational.

WARNING! Physical Injury Hazard. Using the instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument

DANGER! ELECTRICAL HAZARD. Failure to ground the instrument properly can lead to an electrical shock. Ground the instrument according to the provided instructions.

Safety information

Safety labels on instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Applied Biosystems instruments in combination with the safety symbols described in the preceding section.

Hazard symbol	English	Français
	**CAUTION!** Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**ATTENTION!** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**ATTENTION!** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
	**WARNING!** Hot lamp.	**AVERTISSEMENT!** Lampe brûlante.
	WARNING! Hot. Do not remove lamp until **15** min after disconnecting supply.	**AVERTISSEMENT!** Lampe brûlante, après avoir déconnecté le câble d'alimentation de l'appareil, attendre environ **15** minutes avant d'effectuer un remplacement de la lampe.
	WARNING! Hot. Replace lamp with an Applied Biosystems lamp.	**AVERTISSEMENT!** Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems.
	CAUTION! Hot surface.	**ATTENTION!** Surface brûlante.
	**DANGER!** High voltage.	**DANGER!** Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems qualified service personnel.	**AVERTISSEMENT!** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de chez Applied Biosystems.
	**CAUTION!** Moving parts. Crush/ pinch hazard.	**ATTENTION!** Pièces en mouvement, risque de pincement et/ou d'écrasement.

How to obtain support

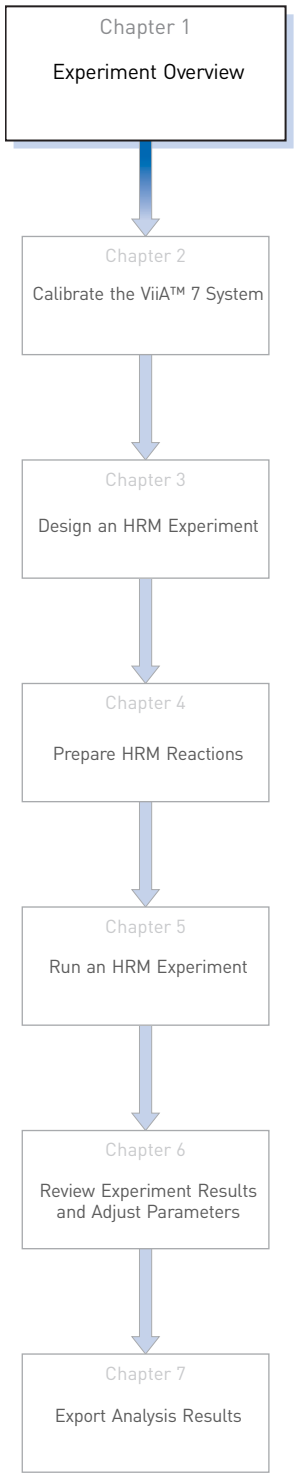
For the latest services and support information for all locations, go to **www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, certificates of analysis, and other related documents
- Order Safety Data Sheets (SDSs) for any chemicals supplied by Applied Biosystems or Ambion (available to you free 24 hours a day).
- 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 Life Technologies Technical Support and Sales facilities.

1 Experiment Overview



About HRM experiments

High Resolution Melt (HRM) analysis is a new, post-PCR analysis method used for identifying genetic variation in nucleic acid sequences. Simple and fast, this method is based on PCR melt (dissociation) curve techniques and is enabled by the recent availability of improved double-stranded DNA (dsDNA)–binding dyes along with next-generation real-time PCR instrumentation and analysis software. HRM analysis can discriminate DNA sequences based on their composition, length, GC content, or strand complementarity.

The ViiA™ 7 System consists of the ViiA™ 7 Instrument, ViiA™ 7 Software, computer, and associated devices.

The ViiA™ 7 System can perform:

- **Mutation scanning experiments** – Screen DNA samples for new single-base changes, insertions/deletions, or other unknown mutations.
- **Methylation studies** – Determine the percentage of methylated DNA in unknown samples.
- **Genotyping experiments** – Determine the genotype of a DNA sample.

For all types of experiments, the ViiA™ 7 Software compares the melt curves of the unknown samples to the melt curves of the other unknown samples and to the melt curves of the positive controls and assigns each unknown a variant call. If the unknown matches a positive control the variant call is the name of the control. If the unknown matches to another unknown, but not to a positive control, the variant call is "variant X", where X is a number.

The type of sample used as the positive controls depends on the type of experiment:

- **Mutation scanning experiments** – One or more samples with the wild type sequence. The variant call is either "wild type" or "variant X".
- **Methylation studies** – Methylated DNA standards that contain from 0% to 100% methylated DNA. The variant calls are the % of methylation.
- **Genotyping experiments** – Three samples: one homozygous for Allele 1, one homozygous for Allele 2, and one heterozygous for both alleles (Allele 1 and Allele 2). The variant calls are the genotypes.

  Note: The example experiment shows a basic genotyping experiment. For information on mutation and methylation experiments, see the *Applied Biosystems High Resolution Melting Getting Started Guide* (Part no. 4393102), *Perform an HRM Methylation Study Quick Reference Card* (Part no.4457856), and *Perform an HRM Mutation Scanning Experiment Quick Reference Card* (Part no. 4457855).

HRM experiment workflow

The HRM experiment workflow is straightforward; most of the work lies in the design of the PCR primers, reagents, and reaction conditions. For more information see, "Design an HRM Experiment" on page 23.

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About the HRM example experiment

To illustrate how to perform HRM experiments, the software installs an example genotyping experiment file to lead you through the process of designing, preparing, running, and analyzing an HRM experiment. The example experiment allows you to quickly familiarize yourself with the process of performing a High Resolution Melt experiment on the ViiA™ 7 system.

To view the example experiment in the ViiA™ 7 Software: **Open ▸ experiments ▸ examples ▸ ViiA7_384-Well_High_Resolution_Melt_ Example.eds**

The example experiment is a very basic genotyping experiment and it is intended for instructional purposes only. The experiment has these characteristics:

- It is comprised of 3 samples, Homo 1 (Allele A), Homo 2 (Allele G), and Hetero (Allele A/G).
- The three samples each use 96 wells of the 384-well plate. One well in each of the three samples is marked as a positive control.
- The remaining 96 wells in the example experiment plate are empty.
- There are no negative controls.

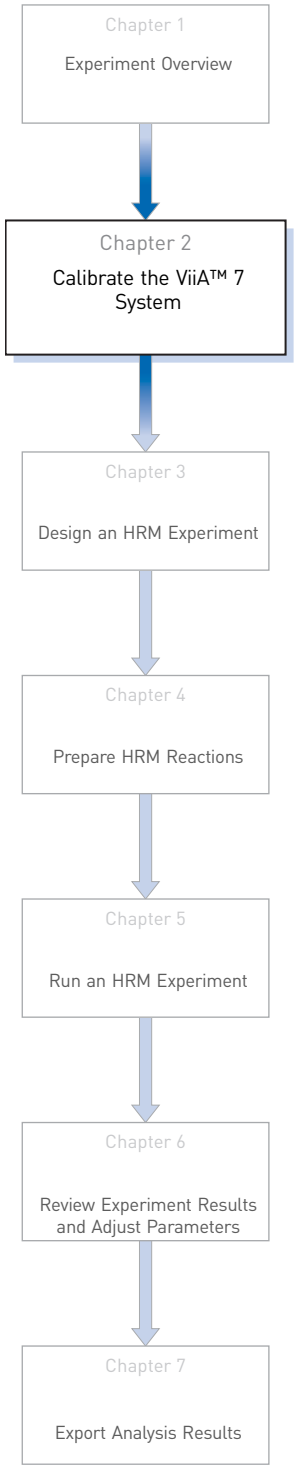
Perform the example experiment with the MeltDoctor™ HRM Positive Control Kit.

Tips for running your own HRM experiment

This guide contains instructions specific to the HRM example experiment. It also functions as a guide for your own experiments; tips for running your own experiments are provided at various points.

Note: When you create your own HRM experiments, you may wish to keep the example file open on another tab in the software and use it as a reference.

2 Calibrate the ViiA™ 7 System



About HRM calibration

This chapter describes how to calibrate an Applied Biosystems ViiA™ 7 Real-Time PCR System for either the MeltDoctor™ HRM Dye or for an alternative HRM dye.

During HRM calibration, the Applied Biosystems ViiA™ 7 Real-Time PCR System performs a PCR amplification of the template in the HRM calibration consumable and a melt curve analysis of the amplified PCR product.

The procedures in this chapter use MeltDoctor™ HRM Dye to perform the calibration. If you use a different HRM dye, you must perform a separate dye calibration. Follow the calibration workflow in this chapter, but substitute your HRM dye of choice for the MeltDoctor™ HRM Dye. For component volumes for non-MeltDoctor™ HRM Dye, refer to the manufacturer's instructions.

You should optimize your reactions for any non-MeltDoctor™ HRM Dye that you choose, because each dye interacts uniquely with all other reaction components, affecting the HRM sensitivity of the analysis.

Prepare a MeltDoctor™ HRM calibration plate

Note: The 96-well standard plate is not available as a ready-to-use calibration plate. If you are using a 96-well 0.2-mL block, you will need to prepare your own HRM calibration plate. See "Prepare a 96-well 0.2 mL HRM calibration plate" on page 75.

IMPORTANT! Before you can perform an HRM calibration on your ViiA™ 7 System, the region of interest, background, and uniformity calibrations must be current. For more information, see *Applied Biosystems ViiA™ 7 Real-Time PCR System User Guide* (Part no. 4442661).

Required materials for HRM calibration

- Centrifuge
- Powder-free gloves
- Safety goggles
- Applied Biosystems MeltDoctor™ HRM Calibration Plate, 384-Well (Part no. 4425559)

  or
- Applied Biosystems MeltDoctor™ HRM Calibration Plate, 96-Well Fast (Part no. 4425618)

Prepare the MeltDoctor™ HRM calibration plate

This procedure is for preparing a MeltDoctor™ HRM Calibration Plate. These plates are ready-to-use, and they contain all the components required for pure dye and HRM calibration.

1. Remove the MeltDoctor™ HRM Calibration Plate from the freezer, then allow it to thaw. Use it as soon as it is thawed. Do not leave the plate at room temperature.
2. Spin the plate briefly to collect liquid at the bottom of the wells.
3. Verify that the liquid in each of the wells of the MeltDoctor™ HRM Calibration Plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

Perform the HRM calibration

1. In the ViiA™ 7 Instrument Console, select your instrument from the list of instruments on the network, then click **Add to My Instruments**.

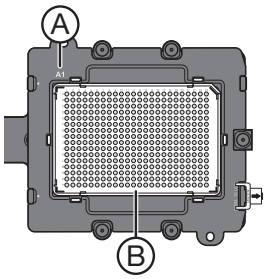
   Note: You must add an instrument to your list before you can manage it.

2. Double-click the instrument icon to open the **Instrument Manager**.

3. Select the type of calibration:
   - For MeltDoctor™ HRM Calibration Plate:
     Maintenance ▸ HRM ▸ MeltDoctor™ HRM Calibration
   - For a custom HRM calibration plate:
     Maintenance ▸ HRM ▸ Non-MeltDoctor™ HRM Calibration

4. Click **Start Calibration**.

5. Follow the instructions of the ViiA™ 7 Software. When the instrument drawer opens, load the HRM Calibration Plate. Ensure that the plate is properly aligned in the holder.
   - (**A**) Load 96/384-well plates with A1 position at the top-left corner of the plate adapter.
   - (**B**) Load plates with the bar code facing the front of the instrument.

   IMPORTANT! Loading/unloading of plates should be carried out by operators who have been warned of the moving parts hazard and have been adequately trained.

6. Start the calibration:
   a. In the Setup tab, select **Check the box when the HRM calibration plate has been loaded**, then click **Next**.
   b. In the Run tab, click **START RUN** to start the calibration.

   IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the ViiA 7 ™ Instrument is in operation.

   Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach temperature.

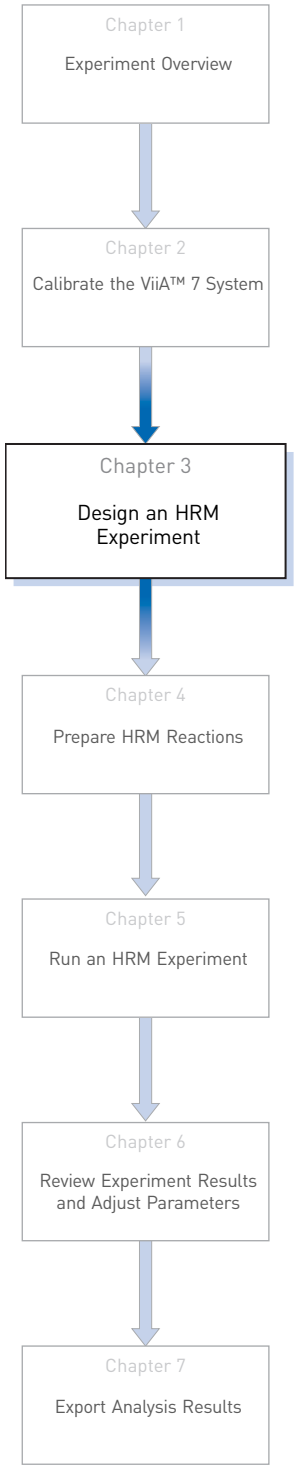
7. When the run is complete, click **Next**, then verify the status of the calibration.
   a. Review the Derivative Melt Curve plot for a single sharp peak.
   b. Review the Plate Layout tab to see that all wells are free of flags.
   c. After you inspect all HRM images, click **Next**, then remove and discard the plate when the instrument ejects the instrument tray.

IMPORTANT! If the ViiA™ 7 Instrument does not eject the plate, remove the plate as explained in "ViiA™ 7 Instrument: Instrument does not eject the plate" on page 84.

WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the plate can be heated to 100°C. Before removing the plate, wait until it reaches room temperature.

8. In the HRM Calibration screen, click **Finish** to complete the calibration, then click **Yes** when prompted to save the results.

3 Design an HRM Experiment



Design and order the primers

Note: If you are using the MeltDoctor™ HRM Positive Control Kit to run the example experiment, you do not need to design primers because the kit contains primers designed to amplify the alleles in the positive control DNA.

Using Primer Express® Software v3.0 or later, design the primers to amplify the sequence of interest. HPLC-purified primers are recommended for best performance, although desalted primers are usable in some cases.

We recommend using these guidelines when designing primers:

Design attribute	Design guidelines
Amplicon	Length is 60 to 250 basepairs (longer amplicons may require optimization)
Primer length	~20 bases each
Tm	58 °C to 62 °C (Optimal Tm is 60 °C)
% GC content	30–80% GC content in each primer
3′ end	No more than 2 G+C residues in the last 5 nucleotides at the 3′ end
Repeating oligonucleotides	Avoid consecutive identical nucleotides. If you are unable to avoid consecutive identical nucleotides, make sure that each primer contains fewer than 4 consecutive Gs.

To order primers, go to www.appliedbiosystems.com, then log into the Applied Biosystems Store if you have an account; register if you are a new user. For more information, see "How to order custom primers" on page 70. For a list of HRM reagents, see "Ordering Information" on page 69.

Plan to use controls

Include controls for each target sequence in your HRM experiment.

- At least one negative control
- At least one positive control to represent each expected variant (for genotyping experiments)

  Run 3-5 replicates for each expected variant to improve your results. Running multiple positive controls allows you to more effectively define the natural spread or variation within different samples of the same sequence, or within replicates of the same genotype
- At least one wild type control (for mutation scanning experiments).

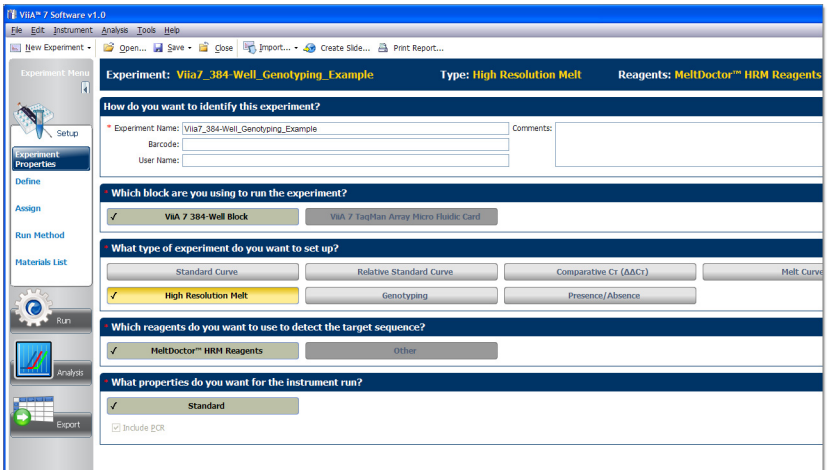
  Run up to 5 replicates for each wild type control to improve your results. Running multiple wild type controls allows you to more effectively define the natural spread or variation within the normal population.

Define the experiment properties

The table below lists the experiment properties used in the example experiment. For your own experiment, enter properties as appropriate.

To create a new experiment, access the ViiA™ 7 Software: **New Experiment ▸ Experiment Setup**. Enter:

Field	Entry
Experiment Name	ViiA7_384_Well_Genotyping (Using a different name than the example experiment to avoid saving over it)
Barcode	Leave field empty
User Name	Example User
Comments	Genotyping example
Block	384-Well Block
Experiment Type	High Resolution Melt
Reagents	MeltDoctor™ HRM Reagents
Instrument Run properties (Ramp speed)	Standard
PCR checkbox	Check to perform PCR on the ViiA™ 7 Instrument



Define targets, samples, and controls

These are the definitions used in the HRM example experiment. For your own HRM experiment, define targets, samples and controls as appropriate.

Click **Define** to access the Define screen. Enter:

1. Targets

Target Name	Reporter	Quencher	Color
HRM	MELTDOCTOR	None	

2. Samples

Sample Name	Color
Homo 1 (Allele A/A)	
Homo 2 (Allele G/G)	
Hetero (Allele A/G)	

3. Define Controls

Sample Name	Color
Hetero	
Homo 1	
Homo 2	

Tips for defining controls in your own HRM experiment

If you are creating your own HRM experiment, include controls for each target sequence in your HRM experiment:

- At least one negative control
- At least one positive control to represent each expected genotype (for genotyping experiments)

Run 3-5 replicates for each expected variant to improve your results. Running multiple positive controls allows you to more effectively define the natural spread or variation within different samples of the same sequence, or within replicates of the same genotype.

If you wish to use a passive reference dye, select it from the drop-down menu at the bottom of the Define screen.

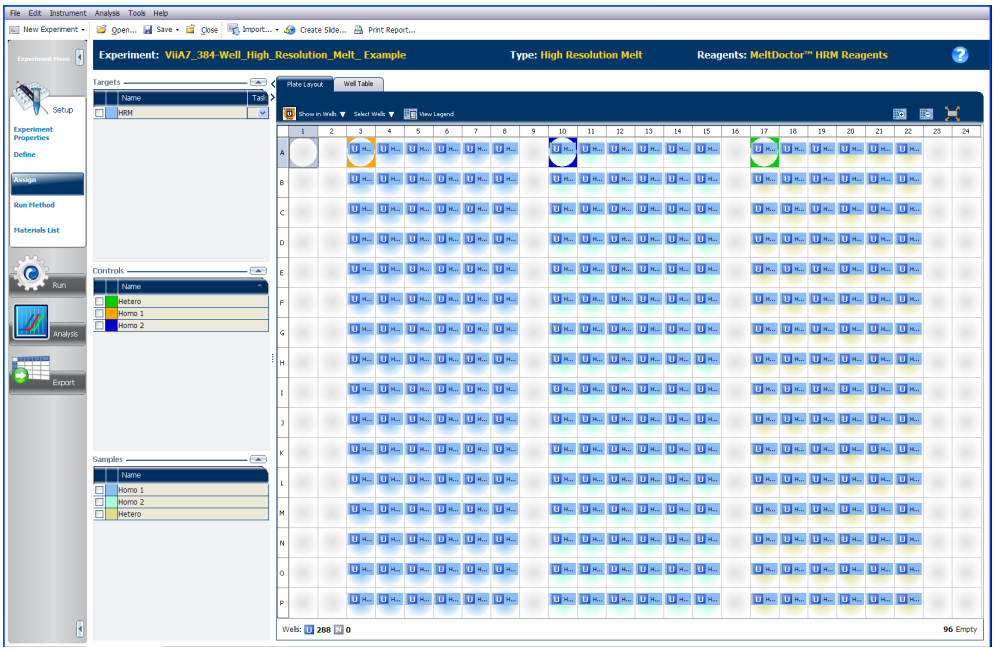
Assign samples and controls

These are the samples and controls as they are assigned in the HRM example experiment. For your own HRM experiment, assign samples and controls as appropriate.

Assign samples

1. Select columns 3-8 in the Plate Layout and click in the checkbox for Sample Homo 1.

2. Repeat for columns 10-15 with Sample Homo 2 and for columns 17-22 with sample Hetero.



Assign controls

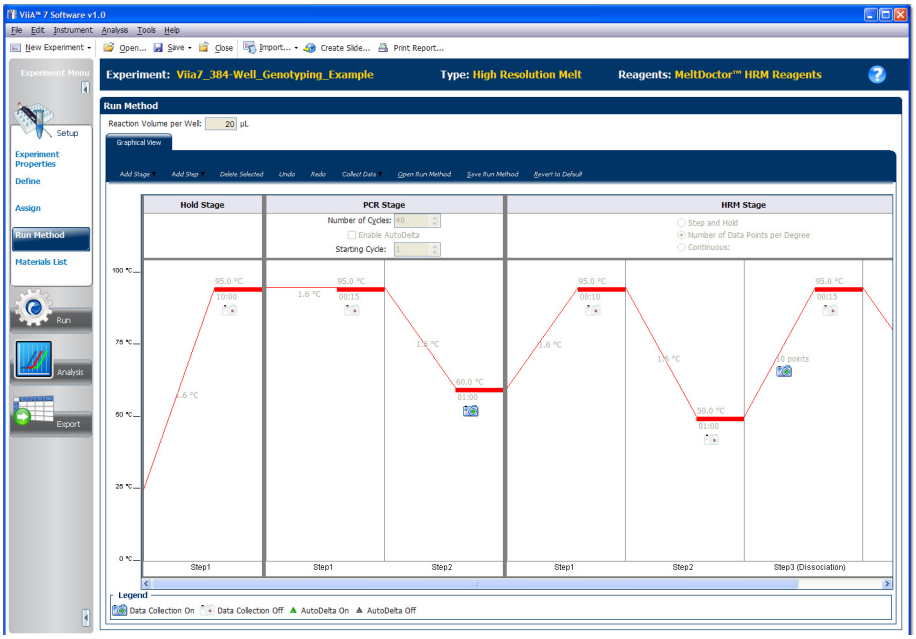
1. Select the top-left corner well of the 96 wells selected for sample Homo 1 and click the checkbox for Control Homo 1 to assign it as a control.

2. Repeat for Homo 2 sample wells with Control Homo 2 and Hetero sample wells with Control Hetero.

Set up the run method

This is the default run method. It has been optimized for use with the MeltDoctor™ HRM Reagents.

1. Click **Run Method** to access the Run Method screen.



The default settings are:

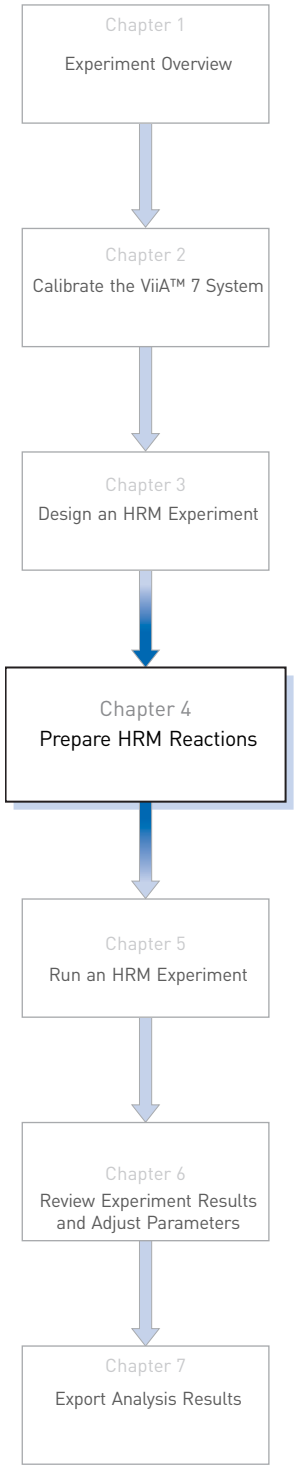
Stage	Step	Temp	Time
Holding	Enzyme activation	95 °C	10 min
Cycling (40 cycles)	Denature	95 °C	15 sec
	Anneal/extend	60 °C	1 min
Melt curve/dissociation	Denature	95 °C	10 sec
	Anneal	60 °C	1 min
	High resolution melting	95 °C	15 sec
	Anneal	60 °C	15 sec

Note that the Reaction Volume per Well is 20 µL.

If you wish to change the settings for your own experiment, see **ViiA™ 7 Software Help** (click ? or press **F1**).

2. If you have modified the settings, **Save** the file.

4 Prepare HRM Reactions



Required materials

Basic materials for all HRM experiments

You need the following basic materials, plus additional materials for either the example experiment or for your own experiment.

- Microcentrifuge tubes
- MicroAmp® Optical 96/384-Well Reaction Plate
- MicroAmp® Optical Adhesive Film
- Deionized water
- Pipettors and pipette tips
- Vortexer
- Centrifuge

Additional materials for example experiment

In addition to the basic materials you need:

- MeltDoctor™ HRM Positive Control Kit (Part no. 4410126), components from the kit:
  - MeltDoctor™ HRM Primer Mix (20×)
  - MeltDoctor™ HRM Allele A DNA (20×)
  - MeltDoctor™ HRM Allele G DNA (20×)
  - MeltDoctor™ HRM Allele A/G DNA (20×)
- MeltDoctor™ HRM Master Mix (Part no. 4415440)

Additional materials for your own HRM experiments

In addition to the basic materials you need:

- Forward and reverse primers (5 µM each)
- DNA samples
- MeltDoctor™ HRM Master Mix

Note: If you use HRM reagents from another manufacturer you must first perform calibration using those dyes. See "Prepare a custom HRM calibration plate" on page 76.

Prepare the HRM reactions for the example experiment

This procedure describes how to prepare reactions for the HRM example genotyping experiment. For your own HRM experiment, see "Tips for preparing the reactions for your own HRM experiment" below.

Prepare the reactions for each replicate group separately, then transfer the reactions to a reaction plate appropriate for your instrument.

1. Prepare the reactions using the Positive Control Kit in separate appropriately sized, labeled tubes:

Components	384-well reaction plate	
	Volume for one 20-µL reaction	**Volume for three 20-µL replicates plus 10% excess**
MeltDoctor™ HRM Master Mix	10 µL	33.0 µL
One type of allele DNA: • MeltDoctor™ HRM Allele A DNA (20X) • MeltDoctor™ HRM Allele G DNA (20X) • MeltDoctor™ HRM Allele A/G DNA (20X)	1 µL	3.3 µL
MeltDoctor™ HRM Primer Mix (20X)	1 µL	3.3 µL
Deionized water	8 µL	26.4 µL
Total volume	**20 µL**	**66 µL**

IMPORTANT! Include excess volume in your calculations to compensate for the loss that occurs during reagent transfers. Applied Biosystems recommends an excess volume of at least 10%.

2. Vortex the reactions to mix, then spin the tubes briefly.

3. Prepare a reaction plate appropriate for your instrument:
   a. Pipet each reaction replicate into the appropriate wells of the optical reaction plate.
   b. Seal the reaction plate with optical adhesive film, then spin the reaction plate.
   c. Confirm that the liquid is at the bottom of the wells in the reaction plate.

Note: If you plan to wait more than 24 hours before running the plate, store the plate at 4°C. Allow the plate to warm to room temperature, then spin the plate briefly before running it.

Note: For detailed information on preparing a reaction plate,see *Prepare Reactions*, in *Getting Started with ViiA™ 7 Experiments*. It is the first booklet of the *Applied Biosystems ViiA™ 7 Real-Time PCR System Getting Started Guides* (Part no. 4441434).

Tips for preparing the reactions for your own HRM experiment

When you perform your own HRM genotyping experiment, you may wish to include negative controls. If you are performing PCR on the ViiA™ 7 Instrument, negative controls will tell you if you have contamination in your samples.

1. Follow the same procedure for creating positive controls as in "Prepare the HRM reactions for the example experiment", but replace Primer mix with your forward and reverse primers and the HRM Alleles with your DNA samples.

2. In addition to the positive control reactions, also prepare negative control reactions in an appropriately sized, labeled tube. [

Components	Volume for one 20-µL reaction	Volume for three 20-µL replicates plus 10% excess
MeltDoctor™ HRM Master Mix	10 µL	33.0 µL
MeltDoctor™ HRM Primer Mix (20X)	1 µL	3.3 µL
Deionized water	9 µL	29.7 µL
Total volume	**20 µL**	**66.00 µL**

IMPORTANT! Include excess volume in your calculations to compensate for the loss that occurs during reagent transfers. We recommend an excess volume of at least 10%.

3. Vortex the reactions to mix, then spin the tubes briefly.

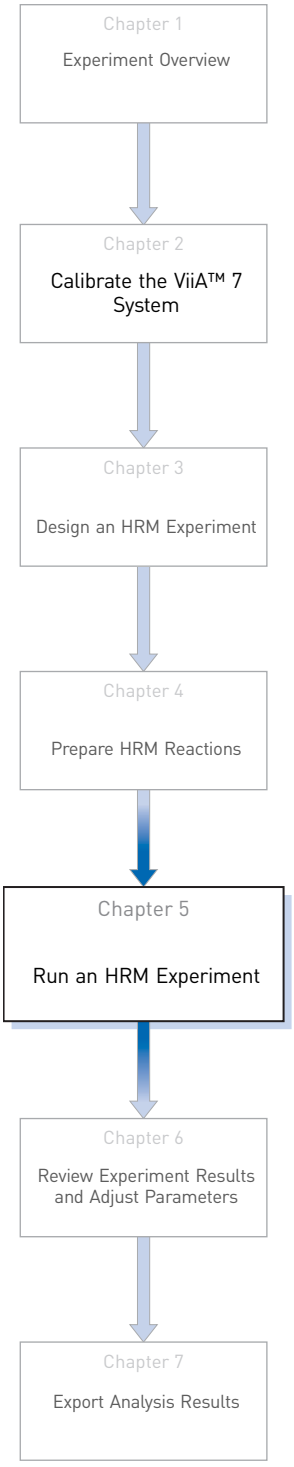
4. Prepare a reaction plate appropriate for your instrument:
   a. Pipet the negative controls, positive controls, and your samples into the appropriate wells of the optical reaction plate.
   b. Seal the reaction plate with optical adhesive film, then spin the reaction plate.
   c. Confirm that the liquid is at the bottom of the wells in the reaction plate.

Note: If you plan to wait more than 24 hours before running the plate, store the plate at 4°C. Allow the plate to warm to room temperature, then spin the plate briefly before running it.

Note: For detailed information on preparing a reaction plate, see *Prepare Reactions*, in *Getting Started with ViiA™ 7 Experiments*. It is the first booklet of the *Applied Biosystems ViiA™ 7 Real-Time PCR System Getting Started Guides* (Part no. 4441434).

Note: For information about using the MeltDoctor™ HRM Reagent Kit to optimize your reactions, see "Optimizing the reaction conditions" on page 78.

5 Run an HRM Experiment



Prepare for the run

Open the HRM experiment

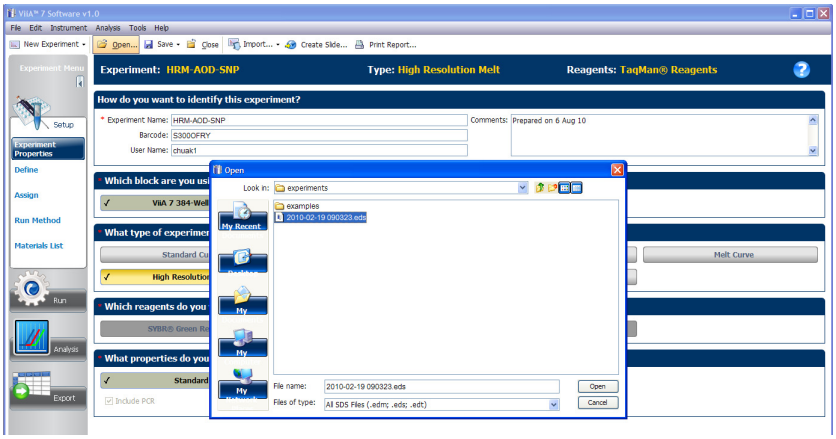
From the Home screen, open one of the following:

- The example experiment:

  Open ▸ experiments ▸ examples ▸ ViiA7_384-Well_High_Resolution_Melt_Example.eds

- Your experiment:

  Open ▸ experiments ▸ <your HRM experiment.eds>



Load the reaction plate Into the instrument

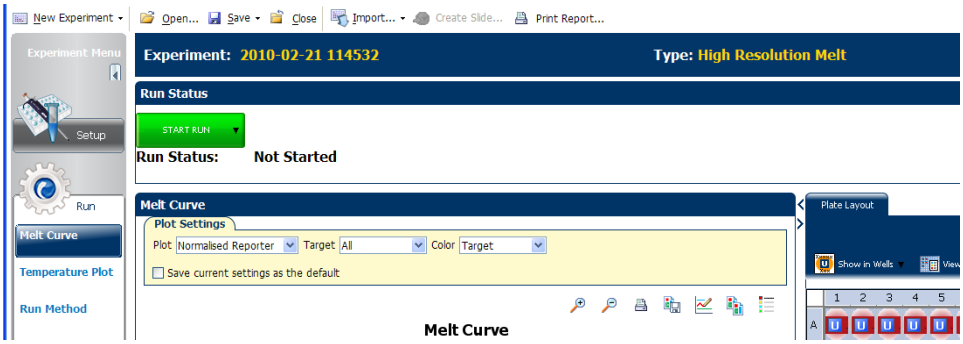
IMPORTANT! Loading/unloading of plates should be carried out by operators that have been warned of the moving parts hazard and have been adequately trained.

1. In the Home screen of the ViiA™ 7 Software, click **Instrument Console**.
2. Select your instrument from the list of instruments.
3. If your instrument is in the "On the Network" group, add it to **My Instrument**.
4. Click **Open Door** in the Instrument Console tool bar.
5. Load your prepared reaction plate.
6. Close the door, either from the touchscreen or the software.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the temperature of the sample block(s) can exceed 100°C. If the instrument has been used recently, keep your hands away until the sample block(s) reaches room temperature.

Start the run

Once the reaction plate is loaded, start the run by selecting **Start Run** in the Melt Curve window.



Monitor the run

You can view the progress of the run in real-time as described below. During the run, periodically view all three plots available from the ViiA™ 7 Software for potential problems.

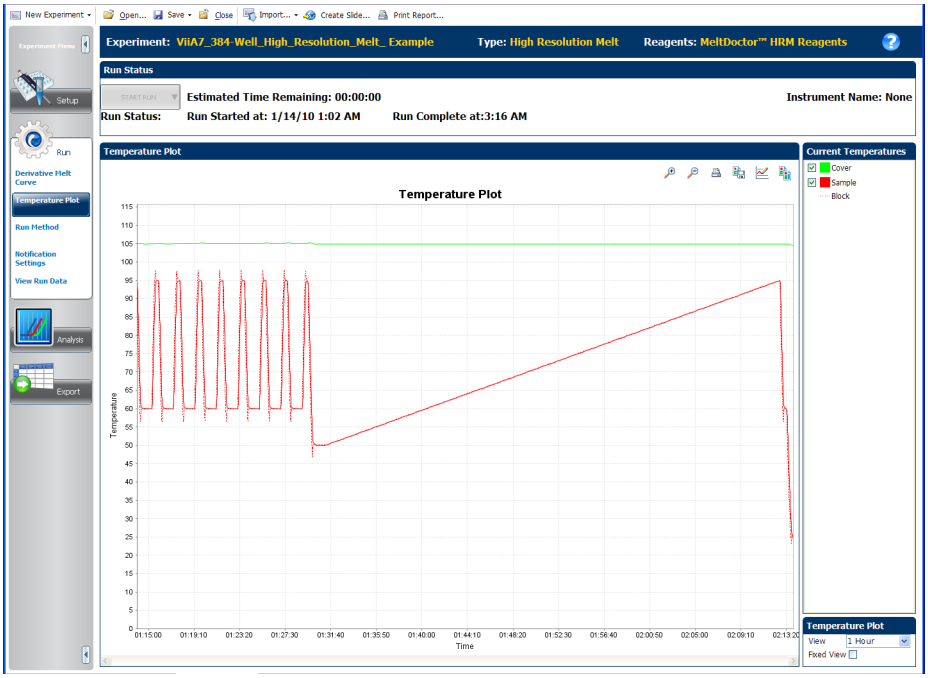
To...	Action
View temperature data for the run in real-time	Select **Temperature**
View progress of the run in the Run Method screen	Select **Run Method**
Enable/disable the Notification Settings	Select or deselect **Notification Settings**
Stop the run	1. In the ViiA™ 7 Software, click **STOP RUN**. 2. In the Stop Run dialog, click one of the following: • **Stop Immediately** to stop the run immediately. • **Stop after Current Cycle/Hold** to stop the run after the current cycle or hold. • **Cancel** to continue the run.

About the Temperature Plot screen

During a run, the Temperature Plot screen displays the temperatures of the sample block(s), the heated cover, and samples (calculated) in real-time.

To...	Action
Add/remove temperature plots	Select **Cover** or **Sample Block** to view the presence of the associated data in the plot.
Change the time displayed by plot	Click the **Plot Properties** icon. Select the amount of time to display in the plot.
Display a fixed time window during the instrument run If the entire plot does not fit in the screen, the screen is not updated as the run progresses. For example, if you select 10 minutes from the View drop-down menu, the plot will show data for 10 minutes. If the run lasts more than 10 minutes: • The plot updates as the run progresses with Fixed View deselected. • The plot updates as the run progresses with Fixed View deselected	Select **Fixed View**.

The figure below shows the Temperature Plot screen as it appears during the example experiment.



The Temperature Plot screen can be useful for identifying hardware failures. When monitoring the Temperature Plot screen, observe the Sample and Block plots for abnormal behavior.

- In general, the Sample and Block plots should mirror each other approximately. A significant deviation of the plots may indicate a problem.
- The Cover plot should maintain the constant temperature specified in the method. A departure from the constant temperature may indicate a problem.

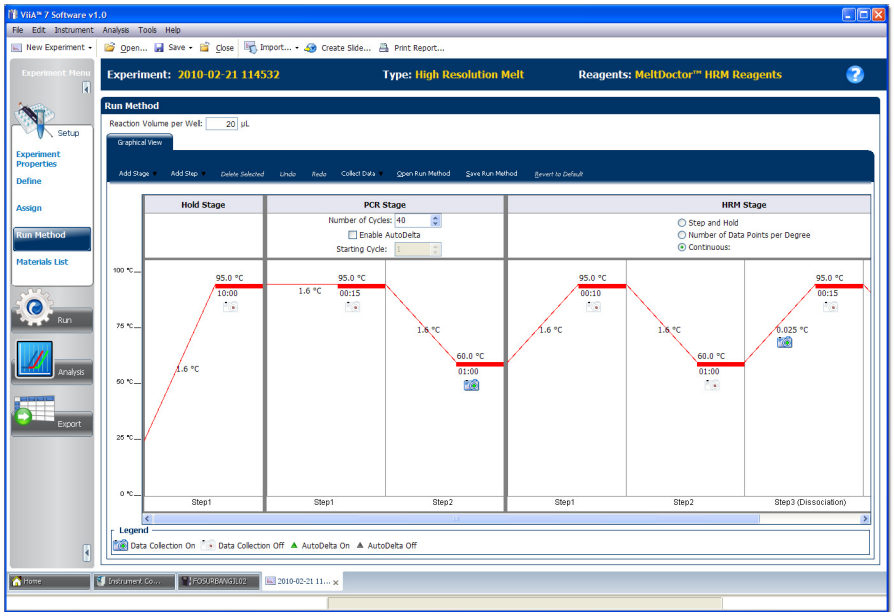
If you notice an abnormal temperature plot, troubleshoot the error as explained in the ViiA™ 7 Software Help (click ? or press F1).

Note: The Sample temperature displayed in the Current Temperatures group is an estimated value.

About the Run Method screen

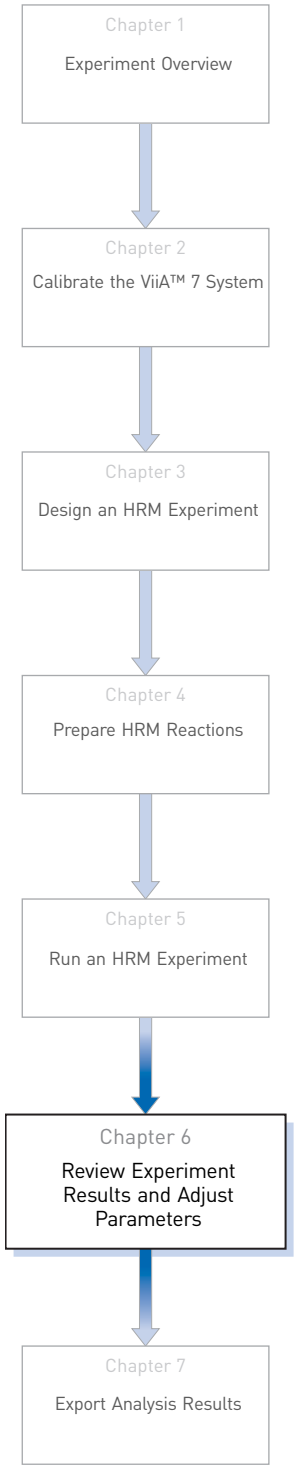
The Run Method screen displays the run method selected for the run in progress. The software updates the Run Status field throughout the run. The figure below shows the Run Method screen as it appears in the example experiment.

To...	Action
Change the number of cycles	In the Adjust # of Cycles field, enter the number of cycles to apply to the Cycling Stage.
Add a Hold stage to the end of the run	Select **Add Holding Stage to End**.
Apply your changes	Click **Send to Instrument**.



If an alert appears, click the error for more information and troubleshoot the problem as explained in the ViiA™ 7 Software Help (click or press F1).

6 Review Experiment Results and Adjust Parameters



How to evaluate the results

Review of the results occurs in three steps:

1. Perform an initial review of the High Resolution Melt Plots (see page 41), the Plate Layout (see page 45), and the Well Table (see page 47) to evaluate the genotype calls made by the ViiA™ 7 Software.

2. Perform a thorough review of the QC Summary (see page 50) to evaluate the samples that triggered QC flags. Review the raw data (see page 51) and amplification data (see page 54) for the samples that exhibit abnormal amplification.

3. If necessary, define the analysis settings (see page 58) or modify the calls manually (see page 62).

After evaluating the results, you can publish the results as explained in "Publish the data" on page 64.

View the experiment results

If this is an active experiment, select **Analyze** from the Experiment menu. If you are viewing a saved experiment, open the experiment file.

- To view the HRM example experiment:
  Open ▸ experiments ▸ examples ▸ ViiA7_384-Well_High_Resolution_Melt_Example.eds.
- To view your own experiment:
  Open ▸ experiments ▸ <your experiment.eds>

Review the High Resolution Melt Plots

Perform an initial review of the experiment results in the High Resolution Melt Plots.

The High Resolution Melt Plots are:

- Raw Melt Curves
- Derivative Melt Curves
- Aligned Melt Curves
- Difference Plot

View the plots

1. From the Experiment Menu pane, select **Analysis ▸ High Resolution Melt Plots**.

   Note: If no data are displayed, click Analyze.

2. If you wish to examine a certain well or set of wells, click the **Plate Layout** tab, then select a well or set of wells.

You can view up to four plots simultaneously. From the Experiment Menu, select **Analysis ▸ Multiple Plots View**.

- To display four plots, select Show plots in a 2x2 matrix.
- To display two plots in rows, select Show plots in two rows. To display two plots in columns, select **Show plots in two columns**.
- To display a specific plot, select the plot from the drop-down menu above each plot display.

Analysis guidelines for High Resolution Melt plots

Confirm that all controls have the correct genotype.

1. If using positive controls, confirm the calls for the positive controls:
   a. From the well table, select the wells containing a positive control to highlight the corresponding melt curve in the analysis plots.
   b. Confirm that the color of the line corresponds to the correct genotype.
   c. Repeat steps a and b for the wells containing the other positive controls.

2. Screen the negative controls to ensure that samples failed to amplify:
   a. From the well table, select the wells containing a negative control to highlight the corresponding melt curve in the analysis plots.
   b. Check that the selected wells in the well table are negative controls, and not unknown samples.

      Samples that grouped with the negative controls may:
      - Contain no DNA
      - Contain PCR inhibitors
      - Be homozygous for a sequence deletion

3. Confirm the results of the samples that did not group tightly or are grouped with negative controls by retesting them.

4. If you select to run replicate reactions, carefully review your data set for curves that do not align tightly with the other samples in the group (outliers) to ensure the accuracy of the genotype calls. If outliers are present, confirm the results of the associated samples by retesting them.

5. Look for how many different variant groups (different colors) are displayed. If you see more than you were expecting, you may have sample contamination or may need to modify the analysis settings.

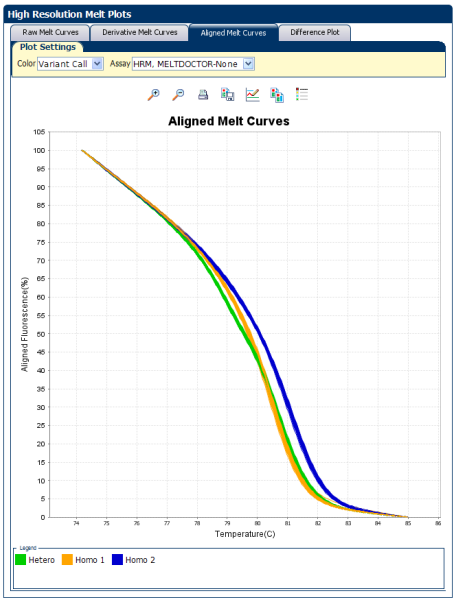
About melt curves

The melt profile of a PCR product depends on its GC content, length, sequence, and heterozygosity. High resolution melt analysis calls variants or genotypes based on the differences in the shape of the melt curves and the differences in the Tm values.

The **Aligned Melt Curves** plot displays the melt curves as % melt (0-100%) over temperature. The melt curves are aligned to the same fluorescence level using the Pre- and Post-melt regions.

Note the following in the screen shot of the example experiment results:

- Hetero has a different curve shape compared to Homo 1 and Homo 2. The shape of the melt curve is an indicator of heteroduplex formation.
- Homo 1 and Homo 2 are distinguished from each other based on the difference in Tm values.



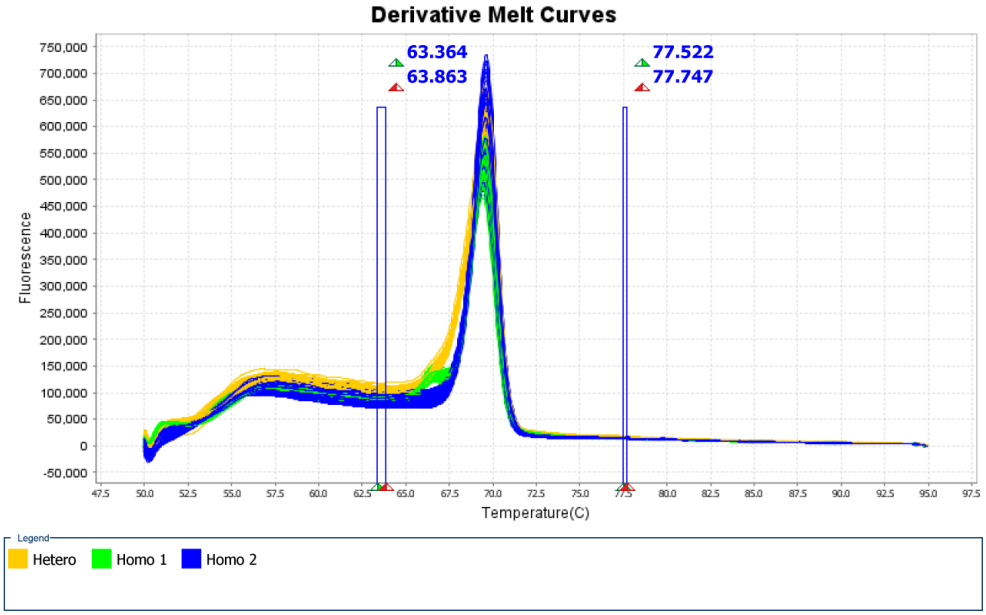
About the Pre- and Post-melt regions

In the **Derivative Melt Curves** plot and the **Raw Melt Curves** plot, there are two pairs of vertical lines before and after the data peak. These lines define the Pre- and Post-melt regions used to scale the data in the Aligned Melt Curves and Difference Plot.

- Pre-melt region: The pair of lines to the left of the peak indicate the Pre-melt Start and Stop temperatures when every amplicon is double-stranded.

  Fluorescence data from the Pre-melt region corresponds to 100% fluorescence in the Aligned Melt Curves Plot.
- Active melt region: The data peak indicates the active melt region of the plot. Data from the active melt region are used to plot the Aligned Melt Curves Plot.
- Post-melt region: The set of lines to the right of the peak indicate the Post-melt Start and Stop temperatures when every amplicon is single-stranded.

  Fluorescence data from the Post-melt region correspond to 0% fluorescence in the Aligned Melt Curves Plot.



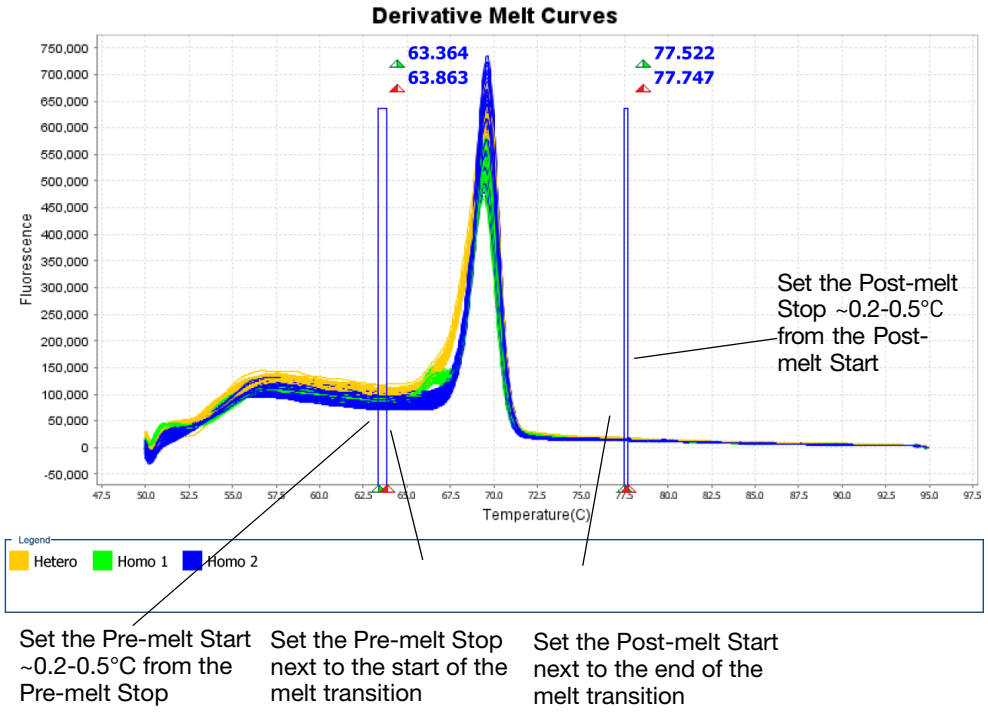
Review and adjust the Pre- and Post-melt regions

When you analyze an HRM experiment, the software calculates the Pre- and Post-melt regions using default settings. You can review and adjust the Pre- and Post-melt regions to optimize your separation and variant calls. For most experiments, set the Pre- and Post-melt regions as close as possible to the melt transition region.

1. In the Data pane, select the **Derivative Melt Curves** tab.
2. Click in the Plate Layout and press **Ctrl+A** to select all wells.
3. Set the Pre-melt region:
   a. Click and drag the Pre-melt Stop temperature line (red arrow on the left) adjacent to the start of the melt transition region.

b. Click and drag the Pre-melt Start temperature line (green arrow on the left) approximately 0.2 to 0.5°C from the Pre-melt Stop temperature line.

Note: The Pre-melt region should be within a flat area where there are no large spikes or slopes in the fluorescence levels.



4. Set the Post-melt region:

   a. Click and drag the Post-melt Start temperature line (green arrow on the right) adjacent to the end of the melt transition region.

   b. Click and drag the Post-melt Stop temperature line (red arrow on the right) approximately 0.2-0.5°C from the Post-melt Start temperature line.

      Note: The Post-melt region should be within a flat area where there are no large spikes or slopes in the fluorescent levels.

5. Click Analyze.

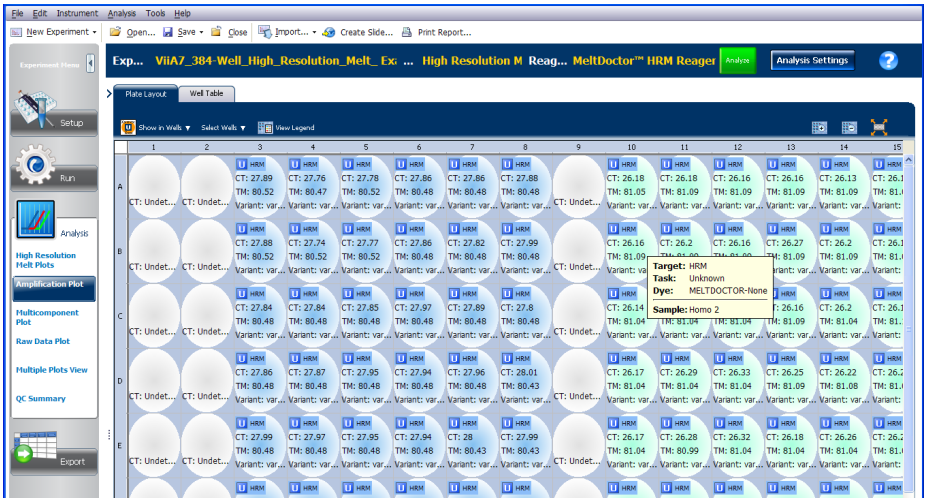
   The software reanalyzes the data using the new Pre- and Post-melt regions. The colors of the melt curves change to reflect the new results.

   For information on saving these settings, see "Apply custom Pre- and Post-melt settings to an assay" on page 60.

View the plate layout

Review the experiment results in the plate layout. The plate layout displays the assay-specific setup and analysis properties for the experiment in a well format corresponding to the type of reaction plate used for the run.

1. Click the ‹ icon to maximize the plate layout and hide the plots.
2. Click Show in Wells, then select or deselect a parameter that you want the wells to display.
3. Repeat step 2 until the plate layout contains all of the desired parameters.



Parameter	Description
Sample Color	The color of the sample applied to the well.
Target	The nucleic acid sequence in the plate layout that you want to amplify and detect.
Control Color	The color assigned to the control samples in the plate layout.
TM	The temperature at which 50% of the DNA is double-stranded and 50% of the DNA is dissociated into single-stranded DNA.
Control Value	The name of the variant in the well.
Flags	The number of QC flags the well triggered.

Example experiment plate layout results

For the example experiment, confirm that the ViiA™ 7 Software called:

- 95 samples as Homo 1 (homozygous), plus one control (Variant: Control-Homo 1)
- 95 samples as Homo 2 (homozygous), plus one control (Variant: Control-Homo 2)
- 95 samples as Hetero (heterozygous), plus one control (Variant: Control-Hetero)

If necessary, click the  icon to zoom in and read the contents of a well.

Confirm that no wells of the reaction plate triggered QC flag(s).

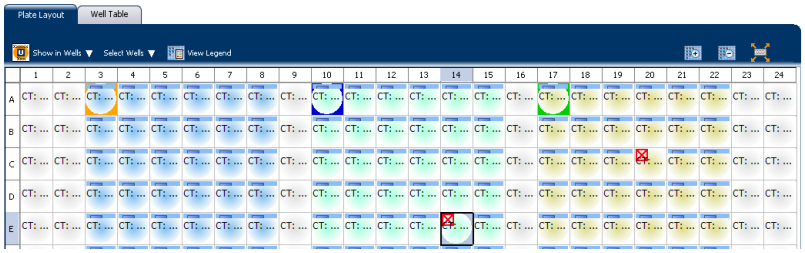
Analysis guidelines for the plate layout view

When you analyze your own experiment:

- You may wish to omit wells with outliers.

  The plate layout displays  in the top-left corner of wells omitted by the user; and it displays  in the corner of wells omitted by the QC flag settings.

  To omit wells, select wells with the mouse and right-click. Select **Omit**



- Note the location of any samples that triggers QC flags (). Understanding the position of errors can aid in diagnosing any failures that may occur.
- You can select the entire reaction plate, areas of the reaction plate, or specific wells:
  - Click the upper-left corner of the reaction plate to select all wells.
  - Left-click the mouse and drag across an area to select it.
  - Select Sample, Target, or Task from the Select Items menu in the View Plate tab. Then select the sample, target, or task name from the second Select Items menu to select wells of a specific type using the well-selection tool.
- You can adjust the plate layout:
  - Use the  (Zoom In),  (Zoom Out), and  (Fit All) buttons to increase or decrease the wells shown.
  - Use the arrow tabs to expand the plate layout to cover the entire screen.

View the well table

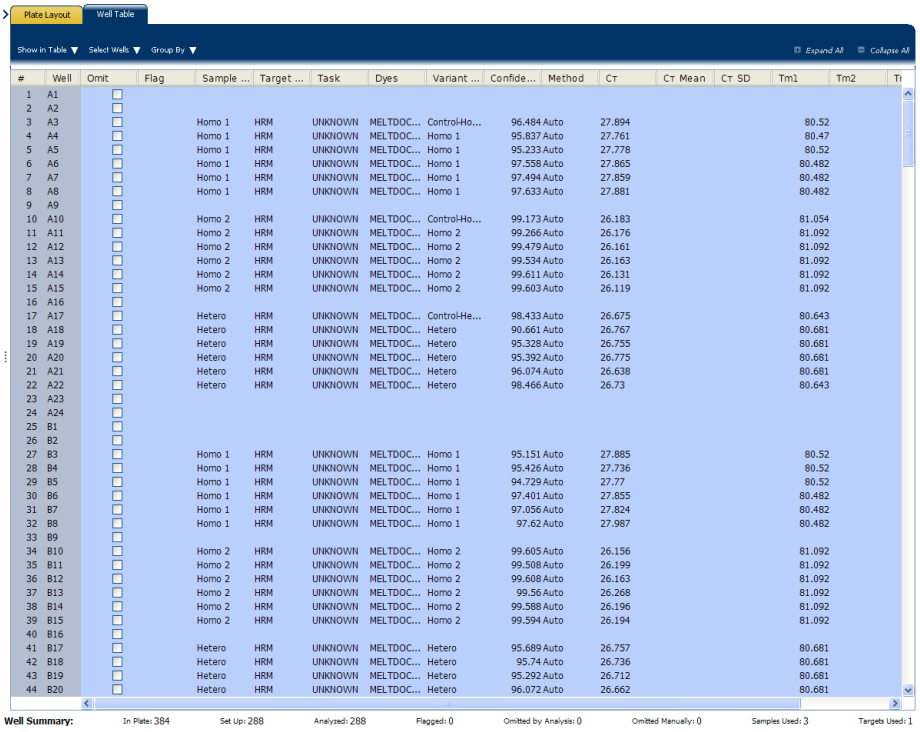
Review the details of the experiment results in the well table and identify flagged wells. The well table displays the assay-specific setup and analysis properties for the experiment in a table format.

Example experiment well data

If you are running the example experiment, confirm that no wells of the reaction plate triggered QC flags (1).

Review the well table

1. Select the Well Table tab.



#	Well	Omit	Flag	Sample ...	Target ...	Task	Dyes	Variant ...	Confide...	Method	Ct	Ct Mean	Ct SD	Tm1	Tm2
1	A1														
2	A2														
3	A3			Homo 1	HRM	UNKNOWN	MELTDOC...	Control-Ho...	96.484	Auto	27.894			80.52	
4	A4			Homo 1	HRM	UNKNOWN	MELTDOC...	Homo 1	95.837	Auto	27.761			80.47	
5	A5			Homo 1	HRM	UNKNOWN	MELTDOC...	Homo 1	95.233	Auto	27.778			80.52	
6	A6			Homo 1	HRM	UNKNOWN	MELTDOC...	Homo 1	97.558	Auto	27.865			80.482	
7	A7			Homo 1	HRM	UNKNOWN	MELTDOC...	Homo 1	97.494	Auto	27.859			80.482	
8	A8			Homo 1	HRM	UNKNOWN	MELTDOC...	Homo 1	97.633	Auto	27.881			80.482	
9	A9														
10	A10			Homo 2	HRM	UNKNOWN	MELTDOC...	Control-Ho...	99.173	Auto	26.183			81.054	
11	A11			Homo 2	HRM	UNKNOWN	MELTDOC...	Homo 2	99.266	Auto	26.176			81.092	
12	A12			Homo 2	HRM	UNKNOWN	MELTDOC...	Homo 2	99.479	Auto	26.161			81.092	
13	A13			Homo 2	HRM	UNKNOWN	MELTDOC...	Homo 2	99.534	Auto	26.163			81.092	
14	A14			Homo 2	HRM	UNKNOWN	MELTDOC...	Homo 2	99.611	Auto	26.131			81.092	
15	A15			Homo 2	HRM	UNKNOWN	MELTDOC...	Homo 2	99.603	Auto	26.119			81.092	
16	A16														
17	A17			Hetero	HRM	UNKNOWN	MELTDOC...	Control-He...	98.433	Auto	26.675			80.643	
18	A18			Hetero	HRM	UNKNOWN	MELTDOC...	Hetero	90.661	Auto	26.767			80.681	
19	A19			Hetero	HRM	UNKNOWN	MELTDOC...	Hetero	95.328	Auto	26.755			80.681	
20	A20			Hetero	HRM	UNKNOWN	MELTDOC...	Hetero	95.392	Auto	26.775			80.681	
21	A21			Hetero	HRM	UNKNOWN	MELTDOC...	Hetero	96.074	Auto	26.638			80.681	
22	A22			Hetero	HRM	UNKNOWN	MELTDOC...	Hetero	98.466	Auto	26.73			80.643	
23	A23														
24	A24														
25	B1														
26	B2														
27	B3			Homo 1	HRM	UNKNOWN	MELTDOC...	Homo 1	95.151	Auto	27.885			80.52	
28	B4			Homo 1	HRM	UNKNOWN	MELTDOC...	Homo 1	95.426	Auto	27.736			80.52	
29	B5			Homo 1	HRM	UNKNOWN	MELTDOC...	Homo 1	94.729	Auto	27.77			80.52	
30	B6			Homo 1	HRM	UNKNOWN	MELTDOC...	Homo 1	97.401	Auto	27.855			80.482	
31	B7			Homo 1	HRM	UNKNOWN	MELTDOC...	Homo 1	97.056	Auto	27.824			80.482	
32	B8			Homo 1	HRM	UNKNOWN	MELTDOC...	Homo 1	97.62	Auto	27.987			80.482	
33	B9														
34	B10			Homo 2	HRM	UNKNOWN	MELTDOC...	Homo 2	99.605	Auto	26.156			81.092	
35	B11			Homo 2	HRM	UNKNOWN	MELTDOC...	Homo 2	99.508	Auto	26.199			81.092	
36	B12			Homo 2	HRM	UNKNOWN	MELTDOC...	Homo 2	99.608	Auto	26.163			81.092	
37	B13			Homo 2	HRM	UNKNOWN	MELTDOC...	Homo 2	99.56	Auto	26.268			81.092	
38	B14			Homo 2	HRM	UNKNOWN	MELTDOC...	Homo 2	99.588	Auto	26.196			81.092	
39	B15			Homo 2	HRM	UNKNOWN	MELTDOC...	Homo 2	99.594	Auto	26.194			81.092	
40	B16														
41	B17			Hetero	HRM	UNKNOWN	MELTDOC...	Hetero	95.689	Auto	26.757			80.681	
42	B18			Hetero	HRM	UNKNOWN	MELTDOC...	Hetero	95.74	Auto	26.736			80.681	
43	B19			Hetero	HRM	UNKNOWN	MELTDOC...	Hetero	95.292	Auto	26.712			80.681	
44	B20			Hetero	HRM	UNKNOWN	MELTDOC...	Hetero	96.072	Auto	26.662			80.681	

2. Click the Flag column header to sort the data so that the wells that triggered flags appear at the top of the table.

3. Confirm the integrity of the controls:
   a. From the Group By menu, select Task to organize the table rows by their function on the reaction plate.
   b. Confirm that each of the controls do not display flags (1).
   c. Click the – icons to collapse the negative and positive controls.

4. Click > beside the Plate Layout tab to display the Well Table and the plots simultaneously.

The table below shows the information in the Well Table view.

Column	Description
Well	The position of the well on the reaction plate.
Omit	A check mark indicates that the well has been removed from the analysis.
Flag	A  indicates that the well triggered the number of flags listed inside the symbol.
Sample Name	The name of the sample.
Target Name	The name of the test assay evaluated in the well.
Task	The task assigned to the well (Unknown, Negative Control, or Positive Control).
Dyes	Reporter dyes in wells.
Variant Call	Call for the sample in well. Can be assigned by software (Auto) or manually. See "Manually set the number of variants" on page 60.
Confidence (%)	The quality value calculated for the genotype call.
Method	The method used to assign the call to the sample (Auto if assigned by the ViiA™ 7 Software, or Manual if applied by a user).
C_T (Cycle Threshold)	The PCR cycle number at which the fluorescence crosses the threshold in the amplification plot.
C_TMean	The arithmetic average of the PCR cycle numbers at which the fluorescence crosses the threshold in the amplification plot for all selected samples.
C_T SD	The standard deviation of the C_T Mean.
TM1	Melting temperature at which half of the DNA has dissociated into single strands.
TM2	The secondary melting temperature.
TM3	The tertiary melting temperature.
Comments	Comments that have been added to the sample descriptions.

Analysis guidelines for the well table view

When you analyze your own experiment:

1. If you are using positive controls, confirm the integrity of the positive controls:
   a. From the Group By menu, select Variant Call to organize the table rows by their function on the reaction plate. Then select the positive control rows.
   b. Confirm that the positive controls do not display flag(s).
   c. Repeat steps a and b for each positive control.
2. Review the data for the Unknown samples. For each row that displays a flag, note the data and the flag(s) triggered by the associated well.
3. Select areas of the table or wells of a specified type by:
   - Clicking and dragging across the rows you want to select.
   - Selecting Sample, Target, or Task from the Select Wells menu, then selecting the sample, target, or task name from the submenu to select specific wells.

4. Group the rows of the plate layout by selecting an option from the Group By menu. You can then collapse or expand the lists either by clicking the +/- icon next to individual lists, or by clicking Collapse All or Expand All.

5. Omit a well from the analysis by selecting the Omit check box for that well. To include the well in the analysis, deselect the Omit check box.

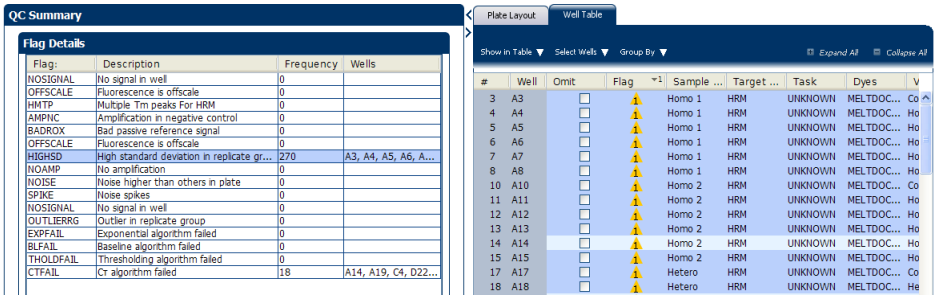
   Note: You must reanalyze the experiment each time you omit or include a well.

View the QC Summary

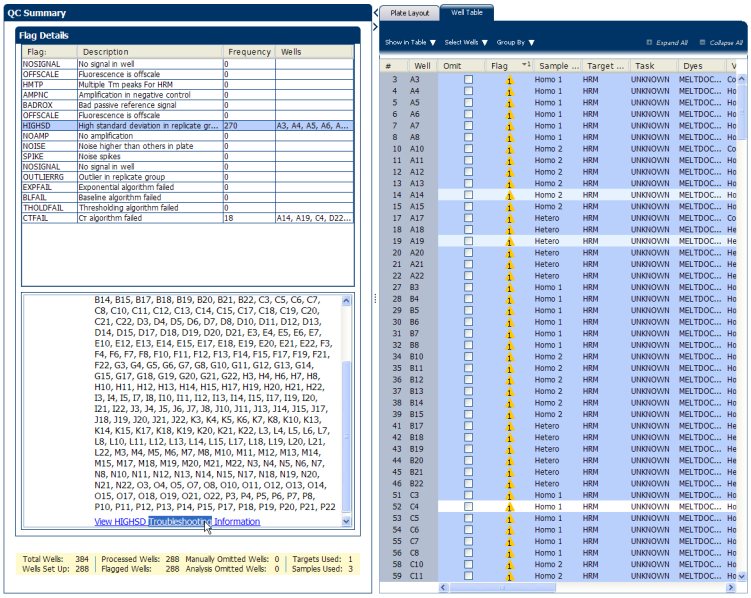
Review the summary of the QC flags triggered by the experiment data and troubleshoot the flags. The QC summary displays a frequency and location of all QC flags. If a flag does not appear in the experiment, its frequency is 0. If the frequency is not 0, that flag appears at the well position listed in the location column. Clicking a flag displays the flag details, including a list of all flagged wells.

Review the QC Summary

1. In the Experiment Menu, select **QC Summary**.



2. In the Flag Details table, look in the Frequency and Wells columns to determine which flags appear in the experiment.



3. In the Flag Details table, check each flag with a frequency >0 to display detailed information about the flag.

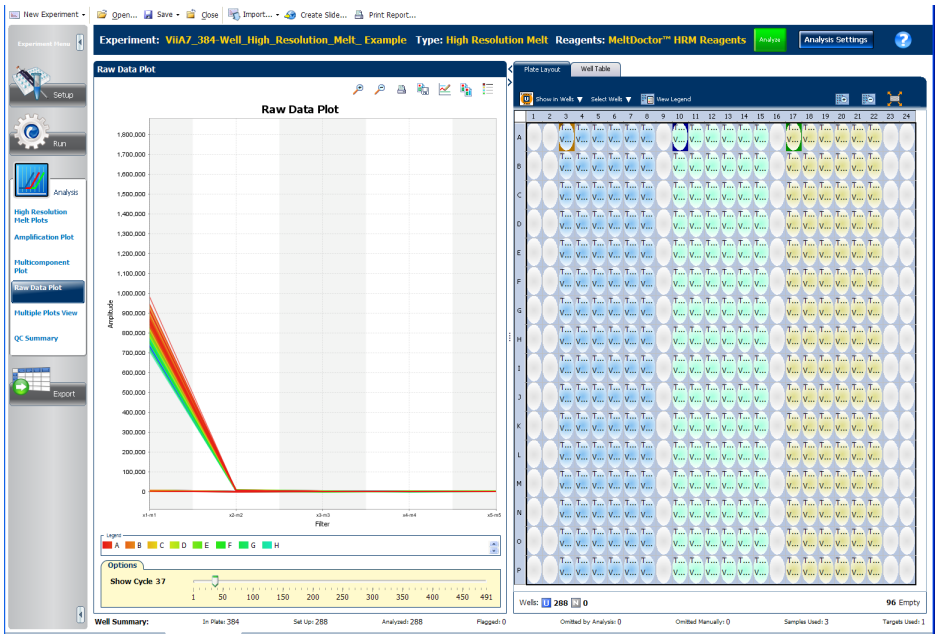
4. (*Optional*) For those flags with frequency >0, click the troubleshooting link to view information on correcting the flag.

View the Raw Data Plot

The Raw Data Plot displays the amplitude of the raw fluorescence collected in each of the filters during the run cycle indicated by the Show Cycle slider. The plot displays the raw spectra for the wells selected in the plate layout or the well table.

Review the Raw Data Plot screen for a stable increase in signal (no abrupt changes or dips) from the appropriate filter.

1. In the navigation pane, select **Raw Data Plot**.



2. In the well table, select the wells that you want to inspect.

   Note: The legend displays the color code for each row of the reaction plate.

3. Drag the Show Cycle slider to view temporal changes in each filter of the raw data profile. (There are 40 PCR amplification cycles in the default Run Method. After 40 cycles there is only one filter being used in the default protocol). The filters are:

Filter set	Color	Filter wavelength (nm)‡		Supported dyes
		Excitation	Emission	
x1-m1	Blue	470±15	520±15	FAM™ and SYBR® Green dyes
x2-m2	Green	520±10	558±12	VIC®, JOE™, TET™, and HEX™ dyes
x3-m3	Yellow	549.5±10	586.5±10	TAMRA™ and NED™ dyes
x4-m4	Orange	580±10	623±14	ROX™ dye
x5-m5	Red	640±10	682±14	LIZ™ dye
x6-m6	Deep red	662±10	711±12	None§

‡ The central wavelengths are the optimized wavelengths.
§ No Applied Biosystems supported dye currently available.

View the Multicomponent Plot

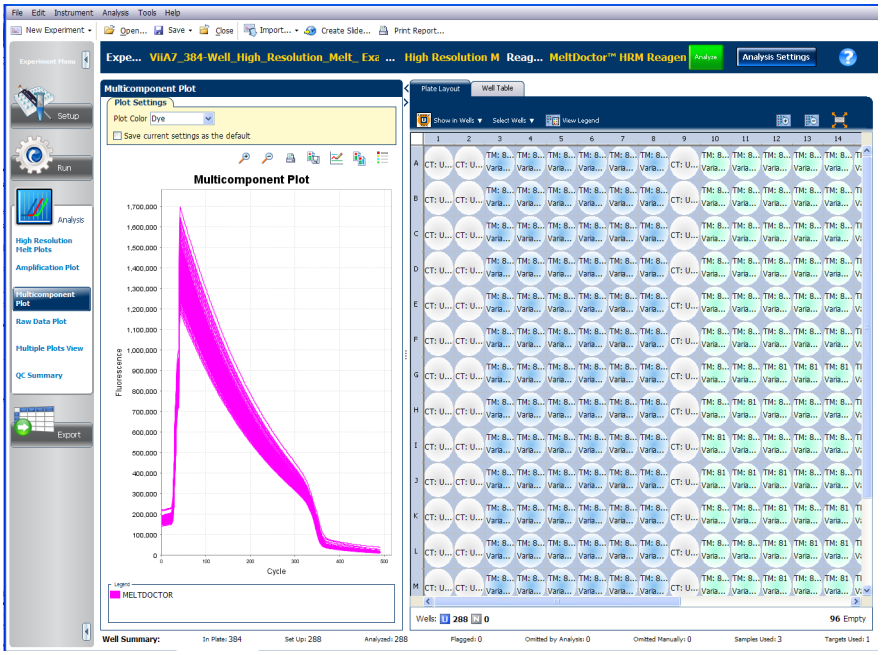
The Multicomponent Plot displays the complete spectral contribution of each dye in a selected well over the duration of the PCR run.

Review the Multicomponent Plot for:

- Any dye you have included as a passive reference
- Mediator™ HRM dye or your custom reporter dye
- Spikes, dips, and/or sudden changes
- Amplification in the negative control wells

Review the Multicomponent plot

1. In the navigation column, select Multicomponent Plot.



2. Select one unknown well in the plate layout to display the corresponding data in the Multicomponent Plot.

   Note: If you select multiple wells, the Multicomponent Plot screen displays the data for all selected wells simultaneously.

3. From the Plot Color drop-down menu, select Dye.

4. If the Legend is not displayed, click  Show a legend for the plot.

5. Check the dye signals in the Amplification Plot. The signals should increase throughout the PCR, indicating normal amplification.

6. If you have included one, check the Passive Reference dye signal. It should remain constant throughout the PCR process.

   In the HRM example experiment, there is no Passive Reference dye.

7. Select the negative control wells one at time and check for amplification. If amplification has taken place, there may be contamination in your plate wells.

   In the HRM example experiment, the negative control wells are empty.

Analysis guidelines for Multicomponent Plot

When reviewing the Multicomponent Plot look for:

- **Passive reference** – The passive reference dye fluorescence level should remain relatively constant throughout the PCR process.
- **Reporter dye** – The reporter dye fluorescence level should display a flat region corresponding to the baseline, followed by a rapid rise in fluorescence as the amplification proceeds.
- **Any irregularities in the signal** – There should not be any spikes, dips, and/or sudden changes in the fluorescent signal.
- **Negative control wells** – There should not be any amplification in the negative control wells.

View the Amplification Plot

If you collected real-time data for your experiment, review the amplification data to further understand the flags triggered by the experiment data.

The Amplification Plot screen displays amplification of all samples in the selected wells. Use the amplification plots to confirm the results of the experiment:

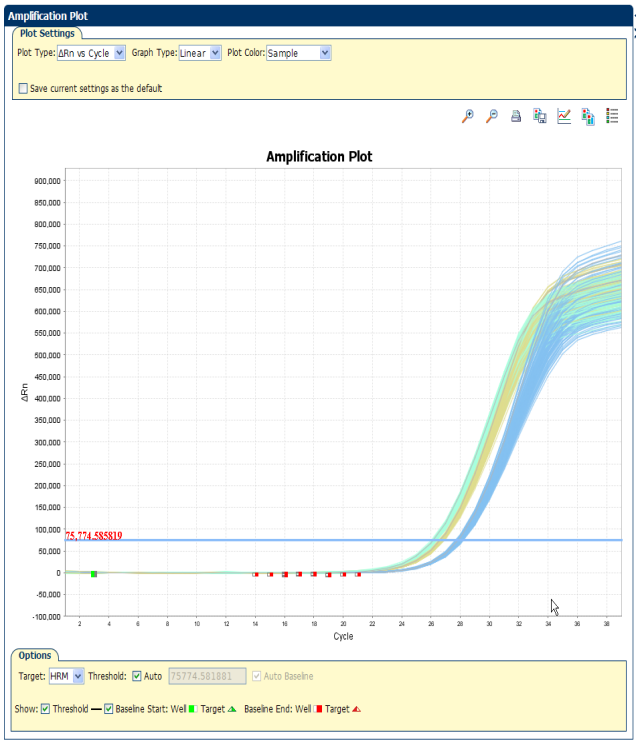
- **ΔRn vs Cycle** – This plot displays ΔR_n as a function of cycle number. You can use this plot to identify and examine irregular amplification and to view threshold and baseline values for the run.
- **Rn vs Cycle** – This plot displays Rn as a function of cycle number. You can use this plot to identify and examine irregular amplification.
- **C_T vs Well** – This plot displays C_T as a function of well position. You can use this plot to locate outlying amplification (outliers)

Each amplification plot can be viewed as a linear or log10 graph.

Review the results

1. In the Experiment Menu, select Amplification Plot.
2. In the Amplification Plot:
   a. From the Plot Type drop-down menu, select ΔRn vs Cycle.
   b. From the Plot Color drop-down menu, select Sample.
   c. If the Legend is not displayed, click Show a legend for the plot.

3. View the baseline values:
   a. From the Graph Type drop-down menu, select **Linear**.
   b. Select **Baseline Start:** to show the start cycle and end cycle.



4. Verify that the baseline is set correctly: The end cycle should be set a few cycles before the cycle number where significant fluorescent signal is detected.

   In the example experiment, the baseline is set correctly.

5. View the threshold values:
   a. From the Graph Type drop-down menu, select **Log**.
   b. Select **Threshold** to show the threshold.
   c. Verify that the threshold is set correctly.

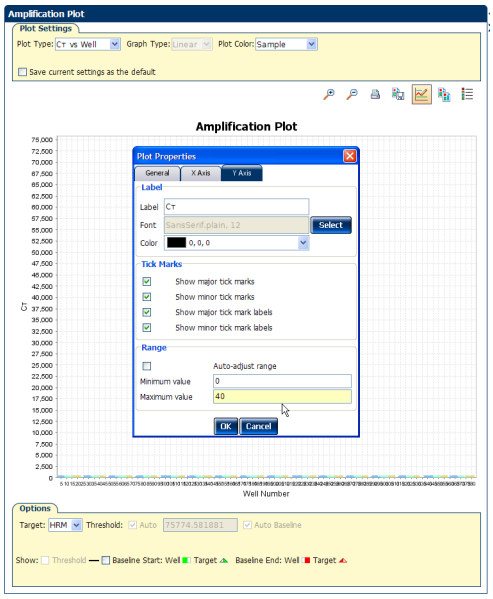
      In the example experiment, the threshold is in the exponential phase.

6. Repeat above steps for all targets.

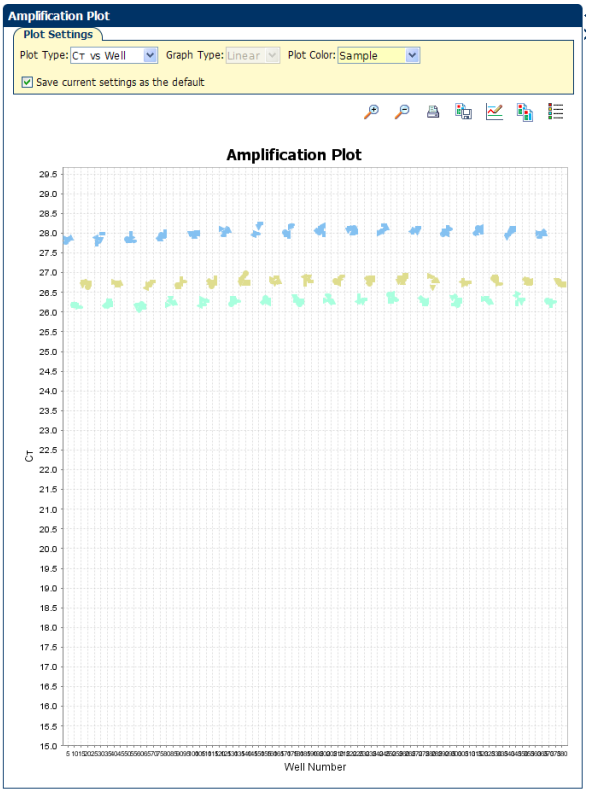
7. From the Plot Type drop-down menu, select **CT vs Well** and from the Plot Color drop-down menu, select **Sample**.

8. If the Y axis scale is set too high, you will need to change the plot properties, so that the Y axis goes to 40.

   Click on **Plot Properties** icon , select **Y axis** tab, change Y axis to **40**, and **Save**.



9. Confirm that the replicate wells have achieved similar amplification.



Analysis guidelines for Amplification Plot

When you analyze the Amplification Plot, look for:

- **Outliers**
- **A typical amplification plot** – The ViiA™ 7 Software automatically calculates baseline and threshold values based on the assumption that the data exhibit a *typical* amplification plot. A typical amplification plot has four distinct sections:
  - Plateau phase
  - Linear phase
  - Exponential (geometric phase)
  - Baseline

For information on troubleshooting issues with amplification, see "Troubleshooting HRM Experiments" on page 79.

Modify the Analysis Settings

If you are dissatisfied with how the ViiA™ 7 Software is calling genotypes or the wells which are flagged, review and adjust the analysis settings and/or calls as needed.

Use the example experiment data to review and adjust the analysis settings to learn how the C_T, flag, and call settings contribute to the analysis of the genotyping data.

Adjust the C_T settings

The C_T Settings are available only for experiments that include amplification data.

HRM analysis does not use C_T data. The main use of C_T data is to confirm that amplification proceeded correctly in all samples.

1. Select **Analysis** ▸ **Analysis Settings** ▸ **C_T Settings** tab.

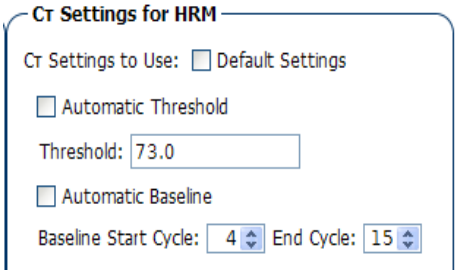
   Note: The Relative Threshold algorithm has no user-defined settings. Use Baseline Threshold if you wish to adjust parameters.

2. Select a target in the **Select a Target** list. (HRM is the only target in the example experiment.)

3. In the **C_T Settings for HRM** section, uncheck **Default Settings.**

4. Uncheck **Automatic Threshold**, then enter a new threshold value.

5. Uncheck **Automatic Baseline**, then enter new baseline values.



6. Repeat steps 1-5 for any other targets for which you wish to modify C_T settings.

7. Click **Apply Analysis Settings** to analyze the data using the new settings.

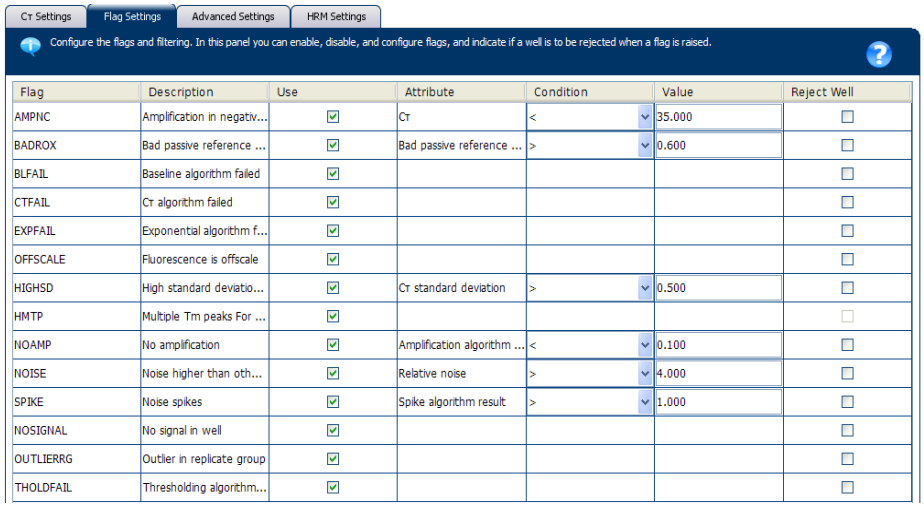
Adjust the flag settings

1. Select the **Flag Settings** tab.

2. In the Use column, select the check box of each flag that you want to enable

3. Adjust the value(s) for the enabled flags as needed.

4. If you want an enabled QC flag to automatically omit wells that test positive for the condition it defines, select the **Reject Well** check box for the flag.



Flag	Description	Use	Attribute	Condition	Value	Reject Well
AMPNC	Amplification in negativ...	☑	Cт	<	35.000	☐
BADROX	Bad passive reference ...	☑	Bad passive reference ...	>	0.600	☐
BLFAIL	Baseline algorithm failed	☑				☐
CTFAIL	Cт algorithm failed	☑				☐
EXPFAIL	Exponential algorithm f...	☑				☐
OFFSCALE	Fluorescence is offscale	☑				☐
HIGHSD	High standard deviatio...	☑	Cт standard deviation	>	0.500	☐
HMTP	Multiple Tm peaks For ...	☑				☐
NOAMP	No amplification	☑	Amplification algorithm ...	<	0.100	☐
NOISE	Noise higher than oth...	☑	Relative noise	>	4.000	☐
SPIKE	Noise spikes	☑	Spike algorithm result	>	1.000	☐
NOSIGNAL	No signal in well	☑				☐
OUTLIERRG	Outlier in replicate group	☑				☐
THOLDFAIL	Thresholding algorithm...	☑				☐

5. Click **Apply Analysis Settings** to analyze the data using the new settings.

Adjust Pre- and Post-melt call settings to automatic or manual

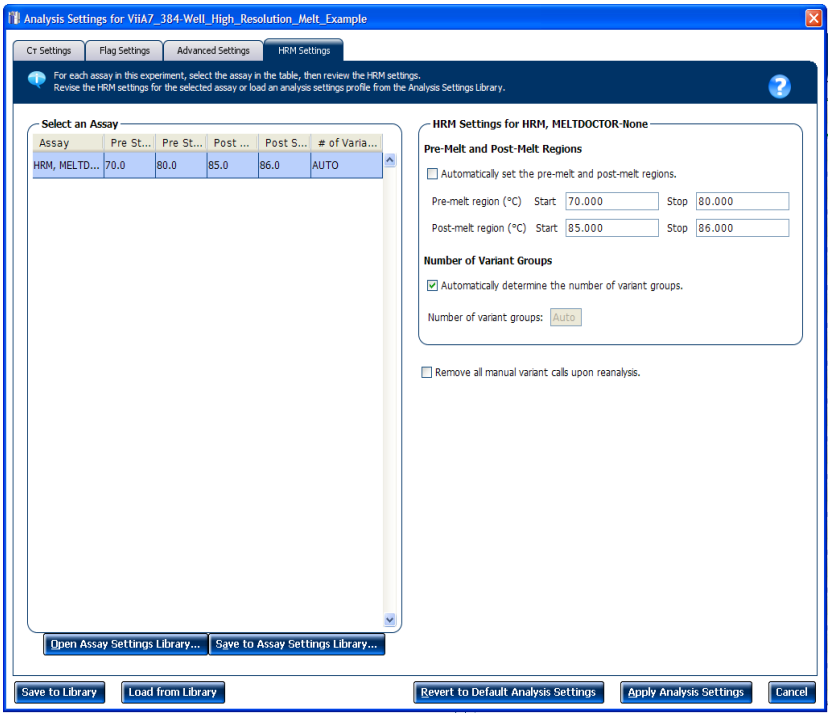
Assays can use either the default Pre- and Post-melt settings or settings that are manually assigned.

You can individually assign manual or automatic settings to different assays.

1. Select **Analysis** ▸ **Analysis Settings** ▸ **HRM Setting**s tab.

2. Adjust the call assay settings:
   a. If you want to set the Pre- and Post-melt regions to specific values, uncheck **Automatically set the Pre-melt and Post-melt regions**.

b. Enter new settings for Pre-melt Start and Stop temperatures and for Post-melt Start and Stop temperatures.



c. Click **Apply Analysis Settings** to analyze the data using the new settings.

Apply custom Pre- and Post-melt settings to an assay

If you have manually adjusted the Pre- and Post-melt settings, you can uncheck Automatic Calling to apply these settings to any future analysis of a selected assay.

1. Select **Analysis** ▸ **Analysis Settings** ▸ **HRM Settings** tab.
2. Uncheck **Automatically set the Pre-melt and Post-melt regions** in the **HRM Settings** pane.
3. Click **Apply Analysis Settings**.
4. Save the experiment

   The software will use the Pre-and Post-melt settings you entered for any future analysis performed on this assay.

Manually set the number of variants

You can adjust the sensitivity of the software algorithm by manually setting the number of expected variants.

1. Select **Analysis** ▸ **Analysis Settings** ▸ **HRM Settings** tab.

2. Uncheck **Automatically determine the number of variant groups** in the **HRM Settings** pane.

3. Enter the number of variant groups you wish the ViiA™ 7 Software to use when analyzing.

4. Click **Apply Analysis Settings** to have the software reanalyze your file. Click **Save to Assay Settings Library...** to save your settings.

Perform manual calls

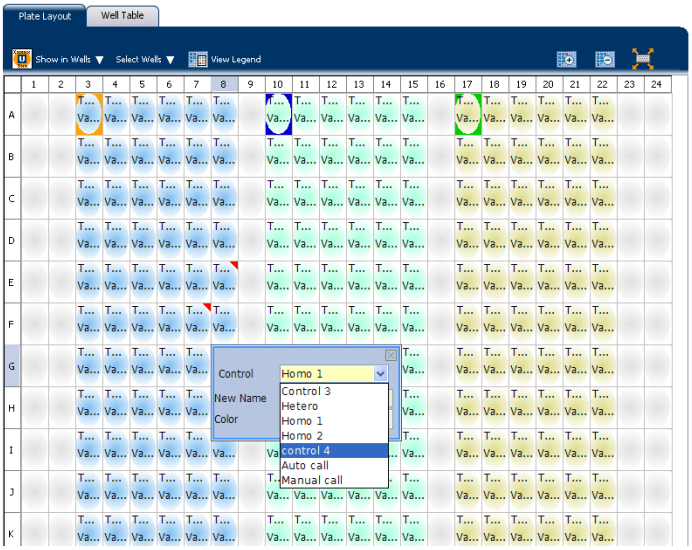
Perform manual calls when you want to manually assign a sample to a variant group.

Perform manual calls in the plate layout

1. In the **Plate Layout** tab, double-click on a well in the plate view.
2. From the **Manual Call** dialog box, you can assign the sample to:
   - **An existing variant call** - Click **Select**, select the appropriate call from the drop-down menu, then click **OK**.
   - **A new variant call** - Click **New**, enter a name for the new call, select a color, then click **OK**.

     In the **Plate Layout** tab, the upper right corner of the sample well is marked with a red triangle.

     In the **Well Table** tab, in the Method column, *Manual* appears next to the selected sample.



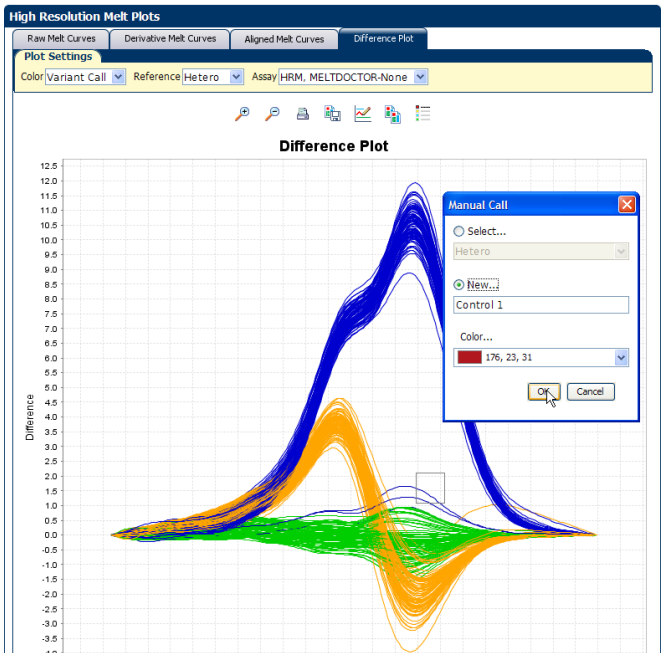
3. Repeat steps 1 and 2 to assign more manual calls.
4. Click **Analyze**. The software reanalyzes the data using the manual calls.

Change selected manual calls to auto calls in the plate layout

1. Double-click on the well to be assigned as an auto call.
2. Right-click in the selection box and select **Auto Call**.
3. Repeat steps 1 and 2 to change other manual calls to auto calls.
4. Click **Analyze**. The software reanalyzes the data.

Perform manual calls in the HRM plots

1. In the **Difference Plot** or **Aligned Melt Curves** plot, click and drag with the mouse to select samples to be called manually. Right-click on the selection box and select **Manual Call** from the menu.



2. From the **Manual Call** dialog box, you can assign the sample to:
   - **An existing variant call** - Click **Select**, select the appropriate call from the drop-down menu, then click **OK**.
   - **A new variant call** - Click **New**, enter a name for the new call, select a color, then click **OK**.

     In the **Plate Layout** tab, the upper right corner of each sample well selected is marked with a red triangle.

     In the **Well Layout** tab, in the Method column, *Manual* appears next to the selected sample.

3. Click **Analyze**. The software reanalyzes the data

Change selected manual calls to auto calls in the HRM plots

1. In the **Difference Plot** or **Aligned Melt Curves** plot, click and drag to select the wells you wish to Auto call.

2. Right-click in the selection box and select **Auto Call**.

3. Repeat steps 1 and 2 to change other manual calls to auto calls.

4. Click **Analyze**. The software reanalyzes the data using the auto call.

Change all manual calls to auto calls

You can quickly change all manual calls to auto calls:

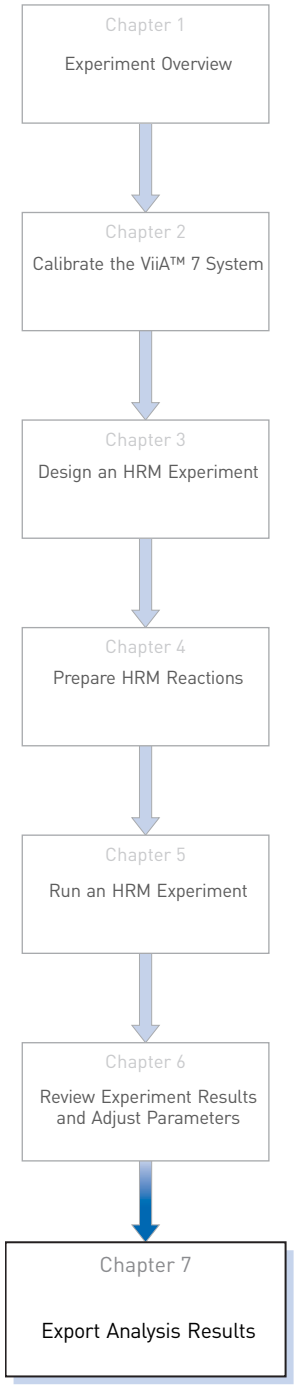
1. Click **Analysis** ▸ **Analysis Settings** ▸ **HRM Settings** tab.
2. Check **Remove all manual variant calls upon reanalysis**.
3. Click **Apply Analysis Settings**.
   The software removes all manual calls and reanalyzes the data using auto calls.

Publish the data

You can publish the experiment data in several ways:

- Save the plot as an image file
- Print the plot
- Print the plate layout
- Create slides
- Print a report
- Export data (see "How to export analysis results" on page 66)

7 Export Analysis Results



How to export analysis results

This procedure shows how to export the HRM example experiment to a .txt file. For information on advanced export, see Appendix D, "ViiA™ 7 Software Reference" on page 85.

1. Open the HRM example experiment file. **Open ▸ experiments ▸ examples <ViiA7_384-Well_High_Resolution_Melt_ Example.eds>**.

2. In the Experiment Menu, click  **Export.**

Note: If you want the data to be exported automatically after analysis, select the **Auto Export** checkbox during experiment setup or before running the experiment. Auto Export is unchecked for the example experiment.

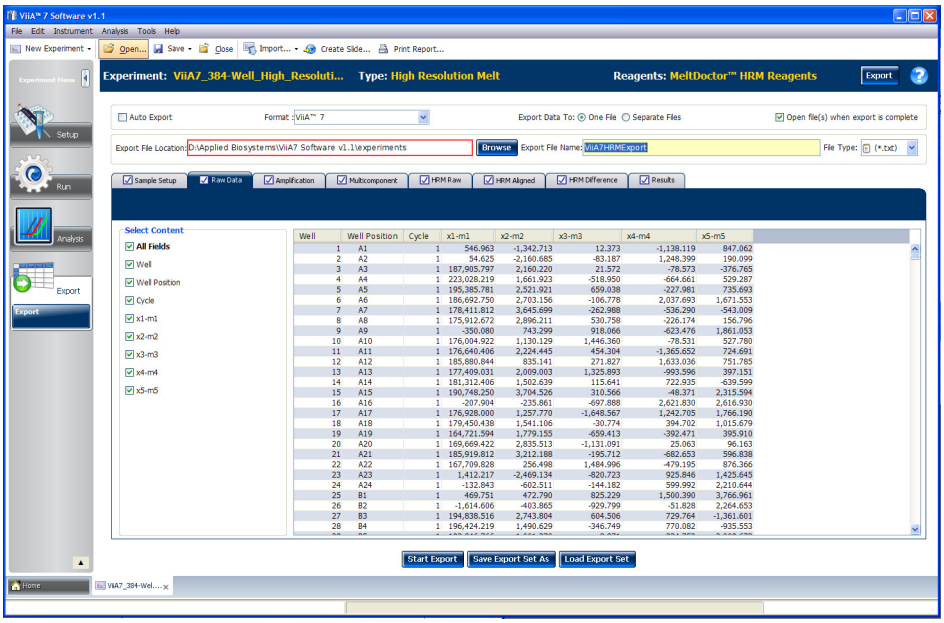
3. Select **Via 7 Format**.

4. Complete the Export dialog box as shown below:

Field or Selection	Entry
Select Data to export/ Select Content	All Fields
Export Data To	One File
Export File Name	ViiA7HRMExport
File Type	*.txt
Export file Location	D:\Applied Biosystems\ViiA7 Software v1.1 \experiments is the default location. You can save to the location of your choice.

Note: By default, all of the experiment data will be exported. This file can be quite large. If you wish, you can uncheck tabs that contain data that you wish to exclude from the file.

Your export screen should look like this:



Your exported file should look like this when opened in Notepad:

```
* Block Type = 384-Well Block
* Chemistry = MELT_DOCTOR
* Experiment File Name = C:\Applied Biosystems\ViiA7 Software v1.1\experiments\examples\ViiA7_384-Well_High_Resolution_Melt_Example.eds
* Experiment Name = ViiA7_384-Well_High_Resolution_Melt_Example
* Experiment Run End Time = 2010-01-14 03:16:16 AM PST
* Experiment Type = High Resolution Melt
* Instrument Name = NA
* Instrument Serial Number = 278880018
* Instrument Type = ViiA 7
* Number of Cluster for target HRM = Auto
* Passive Reference =
* Quantification Cycle Method = Ct
* Signal Smoothing On = false
* Stage/ Cycle where Analysis is performed = Stage 2, Step 2

[Sample Setup]
Well    Well Position   Sample Name Sample Color    Target Name Target Color    Task    Reporter    Quencher    Comments
1       A1
2       A2
3       A3      Homo 1  "RGB(132,193,241)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
4       A4      Homo 1  "RGB(132,193,241)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
5       A5      Homo 1  "RGB(132,193,241)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
6       A6      Homo 1  "RGB(132,193,241)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
7       A7      Homo 1  "RGB(132,193,241)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
8       A8      Homo 1  "RGB(132,193,241)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
9       A9
10      A10     Homo 2  "RGB(168,255,222)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
11      A11     Homo 2  "RGB(168,255,222)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
12      A12     Homo 2  "RGB(168,255,222)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
13      A13     Homo 2  "RGB(168,255,222)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
14      A14     Homo 2  "RGB(168,255,222)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
15      A15     Homo 2  "RGB(168,255,222)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
16      A16
17      A17     Hetero  "RGB(223,221,142)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
18      A18     Hetero  "RGB(223,221,142)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
19      A19     Hetero  "RGB(223,221,142)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
20      A20     Hetero  "RGB(223,221,142)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
21      A21     Hetero  "RGB(223,221,142)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
22      A22     Hetero  "RGB(223,221,142)"  HRM "RGB(139,189,249)"  UNKNOWN MELTDOCTOR  None
```

A Ordering Information

How to order

You can order materials accessories from the Applied Biosystems by:

- Accessing the Applied Biosystems store from the ViiA™ 7 Software.
- Ordering directly from the Applied Biosystems store over the internet.

You can order materials accessories directly from the Applied Biosystems store over the internet.

Note: Product availability and pricing may vary according to your region or country. Online ordering through the Applied Biosystems Store is not available in all countries. Contact your local Applied Biosystems representative for help.

- Confirm that your computer has an Internet connection.
- We recommend the following browsers and Adobe® Acrobat® Reader versions to use the Applied Biosystems web site:

Operating system	Microsoft® Internet Explorer	Apple® Safari	Mozilla® Firefox	Adobe® Acrobat® Reader
Microsoft® Windows®	v6.x or later	Macintosh® application only	v2.x or later	v4.0 or later
Macintosh®	None‡	v2.0.4 or later		

‡ Browser not available for this platform.

Note: Make sure that cookies and Java Script are turned on for the web site to function correctly.

How to order from the ViiA™ 7 Software

1. To find your assay on the Applied Biosystems Store, complete the Find Assay pane in the ViiA™ 7 Software:
   a. Enter a gene name in the Enter Gene Name field, then click **Find Assay**.
   b. In the Find Assay Results dialog box, select your assay.
   c. Click **Apply Assay Selection**. The selected assay gets added to your shopping list.
2. Check that the Experiment Shopping List contains the desired materials, other than the assay selected in the previous step, and that the quantities are correct, then click Order Materials in List.

3. In the Order Materials - Login dialog box, enter your user name and password for the Applied Biosystems Online Store, then click Log In and Submit.

   Note: If you do not have an account with the Applied Biosystems Online Store, click **Register Now** to create an account.

   When you are connected to the Applied Biosystems Store, follow the prompts to complete your order.

How to order HRM products from the Applied Biosystems website

1. Go to **www.appliedbiosystems.com**
2. In the search field, either enter the part number of the product you are interested in or enter search terms (HRM, ViiA 7, and so forth). Alternatively, go to **Products** ▸ **Real-Time PCR** and browse by category.

   Note: For a list of part numbers see, "Materials and equipment for HRM calibration and HRM experiments" on page 71.
3. Select the desired components and complete the order as instructed.

How to order custom primers

1. Go to **www.appliedbiosystems.com**, then log into the Applied Biosystems Store if you have an account; register if you are a new user.
2. Below the **Gene Expression** heading, click **Custom Primers and Probes** ▸ **Custom Unlabeled Primers** ▸ **Sequence Detection Primers**.
3. In the Ordering Information tab, select the check box next to the quantity of primers to order, then click **Configure**.
4. Follow the instructions on the web page to configure the primers:
   a. Select purification and formulation options.
   b. Enter or upload the primer names and sequences.
   c. Review the oligos to order.

      Note: If any of the oligos are invalid, follow the instructions on the web page to edit the sequence information.
5. Click **Add to Basket**.
6. Follow the link to your Shopping Basket, then follow the instructions on the web page to place your order.

Materials and equipment for HRM calibration and HRM experiments

MeltDoctor™ HRM reagents

Item	Applied Biosystems Part Number
MeltDoctor™ HRM Calibration Plate, Fast 96-Well	4425618
MeltDoctor™ HRM Calibration Plate, 384-Well	4425559
MeltDoctor™ HRM Calibration Standard (20X), 1 mL	4425562
MeltDoctor™ HRM Master Mix, 5 mL bottle	4415440
MeltDoctor™ HRM Master Mix, 5 × 5 mL bottle	4415452
MeltDoctor™ HRM Master Mix, 10 × 5 mL bottle	4415450
MeltDoctor™ HRM Master Mix, 50 mL bottle	4409535
MeltDoctor™ HRM Positive Control Kit: • MeltDoctor™ HRM Allele A DNA (20X), 150 µL • MeltDoctor™ HRM Allele G DNA (20X), 150 µL • MeltDoctor™ HRM Allele A/G DNA (20X), 150 µL • MeltDoctor™ HRM Primer Mix (20X), 500 µL	4410126
MeltDoctor™ HRM Reagent Kit: • AmpliTaq Gold® 360 DNA Polymerase • AmpliTaq Gold® 360 Buffer • 360 GC Enhancer • GeneAmp® dNTP Blend • MeltDoctor™ HRM Dye (20X)	4425557
AmpliTaq Gold® 360 Master Mix, 1 mL	4398876
AmpliTaq Gold® 360 Master Mix, 5 mL	4398881
AmpliTaq Gold® 360 Master Mix, 10 x 5 mL	4398901
AmpliTaq Gold® 360 Master Mix, 50 mL	4398886

Equipment and software

Item	Source
Applied Biosystems ViiA™ 7 Software	Part no. 4397808
Primer Express® Software v3.0 or later	Applied Biosystems
Centrifuge with plate adapters	Major laboratory suppliers (MLS)
Lab equipment	MLS
Microcentrifuge	MLS
Microcentrifuge tubes	MLS

Item	Source
Pipettors and pipette tips	MLS
Vortexer	MLS

Supplies

Item	Source
Appropriate reaction plate for your instrument: • MicroAmp® Optical 384-Well Reaction Plate with Barcode, 50 plates • MicroAmp® Optical 384-Well Reaction Plate with Barcode, 500 plates • MicroAmp® Optical 384-Well Reaction Plate with Barcode, 1000 plates • MicroAmp® Optical 96-Well Reaction Plate with Barcode, 0.1 mL, 20 plates • MicroAmp® Optical 96-Well Reaction Plate with Barcode, 0.1 mL, 200 plates • MicroAmp® Optical 96-Well Reaction Plate with Barcode, 0.2 mL • MicroAmp® Optical 96-Well Reaction Plate , 0.2 mL	Applied Biosystems • Part no. 4309849 • Part no. 4326270 • Part no. 4343814 • Part no. 4346906 • Part no. 4366932 • Part no. 4306737 • Part no. N8010560
MicroAmp® Optical Adhesive Film: • 25 covers • 100 covers	Applied Biosystems • Part no. 4360954 • Part no. 4311971
Microcentrifuge tubes	MLS
Pipettors and pipette tips	MLS

B Supplemental Information and Procedures

This appendix contains supplemental information and procedures for preparing and running HRM reactions and for using the ViiA™ 7 Software.

HRM dyes and MeltDoctor™

The melt profile of a PCR product is best obtained with high-resolution melt dyes (HRM dyes). HRM dyes are double-stranded DNA(dsDNA)-binding dyes that have high fluorescence when bound to dsDNA and low fluorescence in the unbound state. HRM analysis uses dsDNA-binding dyes that are brighter than those previously used, and they do not inhibit PCR at high-dye concentrations. With traditional dyes (for example, SYBR® Green I dye), only limited concentrations of the dye can be used before the dye inhibits the PCR.

Of all the dyes that are pre-installed in the Dye Library, only the MeltDoctor™ HRM Dye is valid for HRM.

Custom HRM dyes

This getting started guide describes procedures for calibrating your instrument and performing HRM experiments using the MeltDoctor™ HRM Dye.

If you choose to use a different HRM dye, calibrate your instrument for that dye. Follow the procedures provided, but substitute for the MeltDoctor™ HRM Dye with your HRM dye and prepare your own calibration plate. See "Prepare a custom HRM calibration plate" on page 76.

You should also optimize your reactions for the HRM dye that you choose, because each dye interacts uniquely with all other reaction components.

Prepare a 96-well 0.2 mL HRM calibration plate

This procedure is for preparing a 96-well 0.2 mL HRM calibration plate using the MeltDoctor™ HRM Master Mix and MeltDoctor™ HRM Calibration Standard.

IMPORTANT! The HRM calibration plate should be prepared fresh and used immediately. It is important to perform the custom dye calibration and HRM calibration on the same day that the HRM calibration plate is prepared.

Note: If you are using the MeltDoctor™ HRM Reagent Kit instead of the MeltDoctor™ HRM Master Mix, use the same component volumes in the HRM calibration plate that you are using in your HRM reactions.

Required materials

- MeltDoctor™ HRM Master Mix
- MeltDoctor™ HRM Calibration Standard
- MicroAmp® Optical Adhesive Film
- Deionized water
- 96-well 0.2 mL reaction plate

Prepare the 96-well 0.2 mL HRM calibration plate

1. Add the required volumes of each component to an appropriately sized tube:

Component	Volume (µL)	
	1 reaction	110 reactions (includes 10% excess)
MeltDoctor™ HRM Master Mix	10	1100
MeltDoctor™ HRM Calibration Standard (20X)	1	110
Deionized water	9	990
Total volume	**20**	**2200**

2. Cap the tube, then vortex to mix.
3. Spin the tube briefly.
4. Pipet the HRM calibration reactions to each well of an appropriate reaction plate for your instrument.

IMPORTANT! Accurate pipetting is required for proper calibration.

5. Inspect the plate to make sure all wells contain liquid.

   IMPORTANT! Empty wells may cause the calibration to fail.

6. Seal the reaction plate with optical adhesive film, then spin the reaction plate.

7. Verify that the liquid in each of the wells of the HRM calibration plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

Prepare a custom HRM calibration plate

This procedure is for preparation of a custom HRM calibration plate.

IMPORTANT! A custom dye must fluoresce within the 520 to 650 nm spectral range measured by the ViiA™ 7 System.

You will need to perform a custom dye calibration before you can use the custom dye in the HRM calibration plate.

For information on calibrating a custom dye, see Appendix C, Creating Custom Calibration Plates and Array Cards in the *Applied Biosystems ViiA™ 7 Real-Time PCR System User Guide* (Part no. 4442661).

For component volumes, refer to the dye manufacturer's instructions.

IMPORTANT! The HRM calibration plate should be prepared fresh and used immediately. It is important to perform the custom dye calibration and HRM calibration on the same day that the HRM calibration plate is prepared.

Required materials

- AmpliTaq Gold® 360 Master Mix, 1 mL (Part no. 4398876) or your master mix of choice
- Your custom dye
- MeltDoctor™ HRM Calibration Standard
- MicroAmp® Optical Adhesive Film
- Deionized water
- Appropriate reaction plate

Prepare the custom HRM calibration plate

1. Add the required volumes of each component to an appropriately sized tube.

Components	Volume (µL)		
	1 reaction	110 reactions (96-well) (includes 10% excess)	425 reactions (384-well) (includes 10% excess)
AmpliTaq Gold® 360 Master Mix	10	1100	4250
Custom dye (20x) (typical dye concentration 0.1 µM	1	110	425
MeltDoctor™ HRM Calibration Standard (20X)	1	110	425
Deionized water	8	880	3400
Total volume	**20**	**2200**	**8500**

2. Cap the tube, then vortex to mix.
3. Spin the tube briefly.
4. Pipet the HRM calibration reactions to each well of an appropriate reaction plate for your instrument.

IMPORTANT! Accurate pipetting is required for proper calibration.

5. Inspect the plate to make sure all wells contain liquid.

IMPORTANT! Empty wells may cause the calibration to fail.

6. Seal the reaction plate with optical adhesive film, then spin the reaction plate.
7. Verify that the liquid in each of the wells of the HRM calibration plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

Prepare the DNA templates

1. Purify all the DNA samples in an HRM experiment using the same method. Watch out for salt carryover because it will subtly change the thermodynamics of the DNA melting transition.
2. Perform agarose gel electrophoresis and spectrophotometry to make sure the DNA template is intact and is not contaminated with other DNAs, RNAs, proteins, or organic chemicals. Proteins and organic chemicals may inhibit the PCR amplification, and contaminating DNAs and RNAs may result in sub-optimal PCR performance or increased change of non-specific amplification.

3. Determine the quantity of DNA using spectrophotometry. If too little DNA template is added to the reaction, the fluorescence signal may not be sufficient for successful HRM analysis. If too much DNA template is added to the reaction, the PCR may be inhibited.

4. (*Optional*) Dilute the DNA to 20 ng/µL.

Optimizing the reaction conditions

If you want to optimize the reaction conditions, use the MeltDoctor™ HRM Reagent Kit.

For more information on optimizing your HRM reactions, refer to *A Guide to High Resolution Melting (HRM) Analysis* (Stock number O-081740 0509).

Recommended reaction component volumes using the MeltDoctor™ HRM Reagent Kit

Components	Volume for one 20-µL reaction	Final concentration	Acceptable concentration range
AmpliTaq Gold® 360 Buffer, 10×	2 µL	1×	1×
25 mM Magnesium Chloride	1.6 µL	2 mM	1.5 to 3.5 mM
GeneAmp® dNTP Blend, 10 mM	0.4 µL	200 µM each	100 to 300 µM each
Primer 1 (5 µM)	1.2 µL	0.3 µM	0.2 to 0.5 µM
Primer 2 (5 µM)	1.2 µL	0.3 µM	0.2 to 0.5 µM
MeltDoctor™ HRM Dye (20X)	1.0 µL	1X	0.5X to 2X
AmpliTaq Gold® 360 DNA Polymerase (5 U/µL)	0.4 µL	0.1 U/µL	0.05 to 0.15 U/µL
Human gDNA (20 ng/µL)	1 µL	1 ng/µL	10 pg/µL to 10 ng/µL
Deionized water	11.2 µL		
Total volume	**20 µL**		

C Troubleshooting HRM Experiments

Problems with HRM experiments are usually evidenced by abnormal amplification plots or by abnormal HRM curves.

For more guidance on troubleshooting, refer to:

- Applied Biosystems Real-Time PCR Troubleshooting Tool: **www.appliedbiosystems.com/troubleshoot**
- *Applied Biosystems Guide to High Resolution Melting (HRM) Analysis* (Stock number O-081740-0509)

Late amplification: C_T value >30 for a majority of samples

The amplification reaction may not reach the plateau phase. HRM resolution may be affected by the lower increase in fluorescence.

Possible causes	Recommended action
Poor DNA quality.	Re-extract the DNA.
Amount of DNA added to the HRM reactions is too low.	Perform PCR optimization, and increase sample input or increase the number of amplification cycles.

Some late amplification: C_T value >30 for some samples

Sample outliers with C_T values that are greater than those for the replicates also have a Tm shift in the HRM curve. The resulting Tm shift may affect the variant call.

Possible causes	Recommended action
Reaction volume for the outlier is visibly greater than or less than the reaction volume for the replicates.	Repeat the HRM reactions, and make sure that you add the correct volumes to each well. Also, after you seal the plate, spin the plate briefly.
Amount of DNA added to the HRM reactions is too low.	Repeat the HRM reactions with more DNA in each reaction.
PCR inhibition.	If the amplification curve also has a low slope and all replicates for a sample are affected, see page 81 to troubleshoot PCR inhibition in your HRM reactions.

PCR inhibition: Amplification curve with low slope and C_T values higher than expected

The amplification curve has a low slope and the amplification reaction may not reach the plateau phase. HRM resolution may be affected by the lower increase in fluorescence.

Possible causes	Recommended action
DNA sample contains contaminants that inhibit PCR.	Dilute the samples 1:10 or 1:100, then repeat the HRM reactions.
Incorrect salt concentration.	Perform a $MgCl_2$ titration to find the optimal salt concentration for each reaction.
Reaction does not contain sufficient enzyme.	Optimize the reaction using the MeltDoctor™ HRM Reagent Kit. You can add up to 0.15 U/µL AmpliTaq Gold® 360 DNA Polymerase to each reaction.
Reaction does not contain sufficient primer.	Optimize the reaction using the MeltDoctor™ HRM Reagent Kit. You can add up to 0.5 µM of each primer to each reaction.
Amplicon is greater than 200 bp.	Increase the extension timeS during the amplification reaction.
Primers are amplifying multiple targets.	Perform a BLAST search to ensure primer specificity. If the primers are not specific, design new primers.
	Reduce the number of amplification cycles.

Nonspecific amplification: Decreased PCR efficiency and multiple amplicons

Decreased PCR efficiency and multiple amplicons may affect the melting behavior of the true target amplicons.

Possible causes	Recommended action
Incorrect salt concentration.	Perform a $MgCl_2$ titration to find the optimal salt concentration for each reaction.
Primers are amplifying multiple targets.	Perform a BLAST search to ensure primer specificity. If the primers are not specific, design new primers.
	Reduce the number of amplification cycles.
	After PCR amplification, consider running some of the PCR product on a gel to make sure that it contains a single band.

Replicates are widely spread: Sample replicates show a wide spread in HRM curves

A wide spread within a population leads to difficulties in assessing true sequence differences, particularly between two different homozygous populations.

Possible causes	Recommended action
Population spread	Use multiple controls for HRM analysis to help you define the population spread.
Incorrect salt concentration.	Perform a $MgCl_2$ titration to find the optimal salt concentration for each reaction.
DNA starting concentrations vary widely between samples.	Make sure that the starting DNA concentrations are similar for the samples that you are testing.
Low PCR efficiencies.	Ensure efficient PCR.

Multiple melt regions: Complex melt curves with multiple melting regions

Complex melt curves are difficult to interpret. If the amplicon is too long, the melt curve may have multiple melt regions because of the regional sequence context of the amplicon.

Possible causes	Recommended action
The amplicon contains more than one SNP (genotyping experiments only).	Sequence the PCR product to confirm whether the amplicon contains more than 1 SNP. If the sequencing reveals more SNPs, redesign the primers so that the amplicon contains only 1 SNP.
The amplicon is too long.	Redesign the primers to reduce the amplicon size.

More than three different variant calls (HRM genotyping experiments only)

If the target contains unknown SNPs, multiple heterozygous and homozygous amplicons can be produced. If the amplicon is too long, the melt curve may have multiple melt regions even without a SNP because of the regional sequence context of the amplicon.

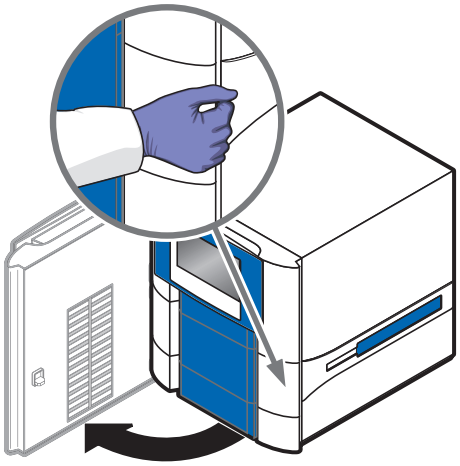
Possible causes	Recommended action
The amplicon contains more than 1 SNP.	Sequence the PCR product to confirm whether the amplicon contains more than 1 SNP. If the sequencing reveals more SNPs, redesign the primers so that the amplicon contains only 1 SNP.
The amplicon is too long.	Redesign the primers to reduce the amplicon size.

Messy HRM curves: Diagonal wavy curves below heterozygous clusters

HRM data from negative controls and unamplified samples skew the Pre- and Post-melt curve settings and interfere with the variant calls.

Possible cause	Recommended action
Negative controls and unamplified samples are included in the HRM analysis.	Omit negative controls and unamplified samples from the HRM analysis. Refer to the *Applied Biosystems High Resolution Melting Software Help*.

ViiA™ 7 Instrument: Instrument does not eject the plate

Possible cause	Recommended action
The adhesive cover may have adhered the plate to the heated cover within the instrument.	Power 1. Power off the ViiA™ 7 Instrument. 2. Wait for 15 minutes, then power on the ViiA™ 7 Instrument and eject the plate. 3. If the plate does not eject, power off and unplug the cord of the ViiA™ 7 Instrument, then open the access door.  4. While wearing powder-free gloves, reach into the ViiA™ 7 Instrument and remove the plate from the heated cover, then close the access door. 5. Perform a background calibration to confirm that the sample block has not been contaminated.

ViiA™7 Instrument: Instrument malfunction

Possible cause	Recommended action
Multiple possible causes.	Contact a local Life Technologies Field Service Office.

D ViiA™ 7 Software Reference

This appendix covers:

ViiA™ 7 Software command line application

The ViiA™ 7 Software includes a command line application that allows you to generate and export batches of experiment files from an MS DOS prompt or a batch file. The application is intended for advanced users (such as laboratory administrators) who choose to create or export experiments using a scripting language.

Note: After you use the command line application to generate experiment files, validate the contents of the files by opening them in the ViiA™ 7 Software.

Running the command line application

Running the application from a command prompt

1. In the desktop, select **Start ▸ Run**.
2. In the Run dialog box, enter **cmd** in the Open field, then click **OK**.
3. In the DOS prompt, change to the installation directory and enter the command:
   a. Enter **cd D:\applied biosystems\ViiA7**, then press **Enter**.
   b. Enter **cmdlineutil.exe**, followed by **-expgen** or **-export**, then all applicable parameters and arguments. See "Command syntax and arguments" on page 87 for a complete list of command line parameters.

Viewing the command line help

The command line application includes a help function that provides the information in this chapter. To view help for:

- The entire application, enter **cmdlineutil.exe –help**
- A particular function, enter **cmdlineutil.exe –expgen -help** to view the file generation help, or **cmdlineutil.exe –export -help** to view the file export help.

Supporting files for experiment creation

The file generation function (`cmdlineutil.exe -expgen`) can use the files shown below. The command does not require all input files.

- **Assay information file** (.AIF or .AIX) is a tab-delimited or XML data file that is shipped on a CD with each TaqMan® assay order. The file, which contains data that describes the assay, can be imported by the ViiA™ 7 Software and used to set up related experiments.
- **Barcode file** (.TXT) is a user-created, tab-delimited text file that contains the barcodes of all consumables for which you want to create experiment files. See "Barcode file format" on page 95 for the barcode file specification.
- **Sample file** (.TXT) is a user-created, tab-delimited text file that describes the samples to be applied to the experiment files. See "Sample file format" on page 93 for the sample file specification.
- **Setup file** (.TXT) is a user-created, tab-delimited text file that describes the layout of a consumable for an experiment to be run on the HRM software. The file defines how the information from the assay information file, the barcode file, and the sample file are applied to a single generated experiment. See "Plate setup file format" on page 91 for the setup file specification.

Command syntax and arguments

Batch file creation

The command used to create batches of files uses the following syntax:

```
cmdlineutil.exe -expgen [ parameters ]
```

The following table lists the acceptable parameters that can be included in any order. See "Examples" on page 89 for an example of the experiment creation command.

Parameter	Description
`-a <filepath>`	(*Optional*) Specifies the path and name (<*filepath*>) of the assay information file (.AIF or .AIX) that the software uses to create new experiment files. Example:`-a "D:\assayfiles\assayfile.aif"`
`-b <filepath>`	(*Optional*) Specifies the path and name (<*filepath*>) of the barcode file that the software uses to create new files. If the `-b` parameter is not used, then the software creates the number of experiment specified by the `-n` parameter. Example:`-b "D:\barcodefiles\barcodefile.txt"`
`-c <string>`	(*Optional*) When the `-f` parameter is included, specifies the alphanumeric string that the software includes in the file names of the new experiments. If no value is supplied, "custom" is used as the default value. Example:`-c "Batch001_"`
`-f <option>`	(*Optional*) Specifies the convention that the software uses to name the new files. The convention can consist of all or some of the following interchangeable arguments, in any order: • Custom Name Field – The alphanumeric string specified by the `-c` parameter. • ID – The barcode of the plate specified in the barcode file specified by the `-b` parameter. Example:`-f "Custom Name Field_ID"` If the `-f` parameter is used without arguments, then the software names files according to the following convention: `"Custom Name Field_ID"`
`-l <dirpath>`	(*Required*) Specifies the path of the directory (<*dirpath*>) to which the software saves the new files. Example:`-l "D:\Applied Biosystems\ViiA7 Software v1.1\experiments"`
`-m <filepath>`	(*Optional*) Specifies the path and name (<*filepath*>) of the sample file that the software uses to create new files. Example:`-m "D:\samplefiles\samplefile.txt"`
`-n <integer>`	(*Optional*) If the `-b` parameter is not included, specifies number of experiments (<*integer*>) that the software will create. If no value is supplied, the software creates 25 experiments by default. Example:`-n 31`
`-s <filepath>`	(*Optional*) Specifies the path and name (<*filepath*>) of the setup file that the software uses to create new files. Example:`-s "D:\setupfiles\setupfile.txt"`
`-t <filepath>`	(*Required*) Specifies the path and name (<*filepath*>) of the ViiA™ 7 Software template file that the software uses to create new files. Example:`-t "D:\Applied Biosystems\ViiA7 Software v1.1 \experiments\templatefile.edt"`
`-v`	(*Optional*) Configures the software to operate in verbose mode, where the software displays each operation as it is performed.

Results exportation

The command used to export the results from experiment files uses the following syntax:

```
cmdlineutil.exe -export [ parameters ]
```

The following table lists the acceptable parameters that can be included in any order. See "Examples" on page 89 for and examples of the experiment export command.

Parameter	Description
`-e <dirpath>`	(*Required*) Specifies the path to the directory (<*dirpath*>) that contains the experiment files (.EDS) for which the software exports data. Example:`-e "D:\Applied Biosystems\ViiA7 Software v1.1\experiments\"`
`-f <option>`	(*Required*) Specifies the format of the exported data (see page 96 for the export file specifications): • ViiA7 – Exports data in a format compatible with the HRM software.
`-l <path>`	(*Optional*) Specifies the path (<*path*>) of the directory to which the software saves the exported files. Example:`-l "D:\exports\"`
`-s <option>`	(*Optional*) Specifies the data spanning option (<*option*>) that determines how the software exports data from multiple experiments: • single – Exports data for all experiments into one contiguous data file. • multiple – Exports data for each experiment to a separate data file. Example:`-s "multiple"`
`-x <filepath>`	(*Required*) Specifies the file format of the exported file: • ViiA 7 export format: txt, xls, or xlsx

Examples

Batch file creation

The following example uses all parameters described in "Command syntax and arguments" on page 87 (required and optional) to generate a set of experiment files.

```
cmdlineutil.exe -expgen -t "D:\Applied Biosystems\ViiA7 Software
  v1.1\experiments\templates\hrm_experiment.edt" -a "D:\Applied
  Biosystems\ViiA7 Software v1.1\experiments\examples\AIF\AIF_820629.txt"
  -s "D:\Applied Biosystems\ViiA7 Software v1.1\experiments\examples\Plate
  Setup Files\SDS_820629.txt" -m "D:\Applied Biosystems\ViiA7 Software
  v1.1\experiments\examples\SampleNames\SampleFileNames.txt"
  -c "alloptionsused" -f "Plate Barcode_Custom Name Field_Plate Type"
  -b "C:\ViiA7\barcodes - v12.txt" -l "C:\ViiA7\Experiment"
```

For this example, the command line application:

- Imports assay definitions from the `AIF_820629.txt` assay information file.
- Imports sample names from the `SampleFileNames.txt` sample file.
- Generates an experiment for each barcode in the `barcodes - v12.txt` barcode file, where each new experiment uses the settings found in the standard_curve.edt template file and the `SDS_820629.txt` setup file.

Note: The setup file links the information from the `AIF_820629.txt` and `SampleFileNames.txt` to each new experiment file.

- Saves all generated files using the following naming convention:

  <*barcode*>_alloptionsused_96/384Well
- Saves all generated files to:

  `C:\ViiA7\Experiment\`<*date/time*>

Note: The command line application automatically creates a time-stamped folder at the export location for each batch operation. For example, the folder created for files generated on April 7, 2010 at 12:48:35 would be: `2010-04-07 124835`

Import formats and file specifications

The ViiA™ 7 Software supports several import file formats that can be used to automate the creation of experiments and the importation of assay and sample data. The files can be used with the command line application (see page 86) or the ViiA™ 7 Software application programming interface (API) to integrate the HRM software into a laboratory information management system (LIMS). For a detailed explanation of the API, or for information on integrating the HRM software into a laboratory workflow, see the *Applied Biosystems High Resolution Melting Software Robotics User Guide* (Part no. 4442663).

Note: The file specifications listed in this appendix are subject to change. For updated information, review the ViiA™ 7 Software Release Notes found at: D:\AppliedBiosystems\ViiA7\release-notes.html.

About the import file formats

File format	Description	See...
Plate setup file (.TXT)	A user-created, tab-delimited text file that describes the layout of a consumable for an experiment to be run on the HRM software. The file defines the arrangement of assays and samples on the consumable, and provides other experiment data, such as the thermal profile and data collection settings.	page 91
Sample file (.TXT)	A user-created, tab-delimited text file containing sample data that can be imported into the ViiA™ 7 Software for use in related experiments.	page 93
Assay information file (.AIF or .AIX)	A tab-delimited or XML data file that is shipped on a CD with each TaqMan® assay ordered from Applied Biosystems. The file, which contains data describing the assay, can be imported into the ViiA™ 7 Software for use in related experiments.	page 86
Barcode file (.TXT)	A user-created, text file containing the barcodes of consumables for which you want to create experiment files using the command line utility.	page 95

Conventions

The following conventions are used in the rest of this section:

- `normal` – Normal text must be entered exactly as it appears.
- <*italic*> – Italicized text between brackets must be substituted with custom values.
- `[required text]` – Text appearing between brackets is required information. All information inside the brackets must be present for the ViiA™ 7 Software to import it.
- `{optional text}` – Text appearing between braces is optional.
- Unless noted otherwise, separate all fields in a row using a tab character (U+0009).
- Unless noted otherwise, end all rows using a carriage-return character (U+000D).

Plate setup file format

You can use plate setup files to automatically populate setup information into an open experiment in the ViiA™ 7 Software or into new experiments created by the command line application (see page 86). A plate setup file is a tab-delimited ASCII text file (.TXT) that contains data that describes the location experiment data information. The files can be created manually using a text processor or generated automatically by third-party applications.

IMPORTANT! To guarantee a successful importation of the plate setup file into a experiment, the file must contain all the elements described in the following section and in the order that they appear.

File structure

Plate setup file consists of a header, which specifies the instrument model for which the experiment is designed and the and the and a sample setup section.

Section	Description	See...
Plate setup file header	Defines the instrument model for which the experiment is designed and the dye used as the passive reference.	page 91
Plate setup file body	Defines the contents of a 96/384-well plate or array card, including target, SNP assay, sample, and task assignments.	page 95

Plate setup file header

The plate setup file begins with a header that consists of two lines. Each line starts with an asterisk (*) and ends with a carriage return in the following pattern:

```
* <field name> = <field value>
```

The header must contain the lines shown in the following table.

Field	Description	Valid Values
Instrument Type	The model of HRM software for which the experiment is designed.	ViiA 7
Passive Reference	The dye that the experiment will use as a passive reference.	• The name of a dye in the Dye Library of the ViiA™ 7 Software‡, or • <*blank*> if the consumable does not contain a passive reference.

‡ Custom dyes are allowed as long as they are in Dye Library.

Note: The ViiA™ 7 Software automatically removes any leading and trailing whitespace around the field name and field value.

Example:

```
* Instrument Type = ViiA 7
* Passive Reference = ROX
```

Plate setup file body

The body of a plate setup file contains either target information, which can be imported into all experiments except genotyping, or SNP assay information, which can be imported into only genotyping experiments. The body consists of three required elements (the header, the column header, and the body) that describe the contents of a 96/384-well plate or array card. The sample setup column header and body can appear in any order.

IMPORTANT! Observe the following guidelines when creating a plate setup file:

- Do not insert blank lines between the sample setup header and the column header.
- Do not use illegal characters, including backslash (\), tab, asterisk (*), hard return, soft return, brackets([or]), or comma (,).

Sample Setup Header

The header contains the label that defines the beginning of the sample setup data.

Example:

```
[Sample Setup]
```

Sample Setup Column Header

The column header contains the headings that define the positions of the data columns in the sample setup body. The headings are separated by tab characters. See "Plate setup data columns" on page 93 for a list of the data column headers.

Example:

```
Well Sample Name Sample Color  Biogroup Name Biogroup Color Target Name…
```

Sample Setup Body

Contains the sample setup data where each row defines the contents of a single well on the consumable, including the: well contents (sample, target, or SNP assay added to the well), task assignments, and comments. If a well contains multiple assays (multiplex PCR), the data for the additional assays are defined on separate lines by repeating the well designation. See "Plate setup data columns" on page 93 for a list of the data column headers.

Note: The sample setup data rows can occur in any order.

Example:

```
Well Sample Name Sample Color  Biogroup Name Biogroup Color Target Name…
1    Liver  cDNA "RGB(25,0,0)"
2    Liver  cDNA "RGB(25,0,0)"
3    Liver  cDNA "RGB(25,0,0)"
4    Heart  cDNA "RGB(0,25,0)"
5    Heart  cDNA "RGB(0,25,0)"
…
```

Plate setup data columns

The following table lists the headings and columns that are present in the plate setup file body. to all experiment types followed by the columns that are specific to genotyping experiments and non-genotyping experiments.

	Column name	Description	Valid values
All experiments	Well	The number of the well on the consumable, where the well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.	<*Positive integer (1 to 96/384)*>§
	Sample Name	The name of the sample contained by the associated well.	<*100-character string*>
	Sample Color	(*Optional*) The RGB color of the associated sample.	"RGB(<*r*>,<*g*>,<*b*>)"#
	Biogroup Name	(*Optional*) The name of the associated biological group.	<*100-character string*>
	Biogroup Color	(*Optional*) The RGB color of the biological group.	"RGB(<*r*>,<*g*>,<*b*>)"#
	Comments	(*Optional*) Additional text that describes the well.	"<*1024-character string*>"
All except genotyping	Target Name	The name of the target detected or amplified by the assay in the associated well.	<*100-character string*>‡‡
	Target Color	(*Optional*) The RGB color of the target.	"RGB(<*r*>,<*g*>,<*b*>)"#
	Task	The task assignment of the target assay at the well.‡	<UNKNOWN | STANDARD | NTC | ENDOGENOUS | IPC | BlockedIPC>
	Reporter	The reporter dye used by the associated target assay.	<*dye name*>‡‡§§
	Quencher	The quencher dye used by the associated target assay.	<*dye name*>§§
	Quantity	(*Optional*) The quantity of standard present in the given well expressed as a float or integer. If the associated well is not assigned the STANDARD task, then the field is blank.	<*float or Integer*>

‡ See the appropriate *Applied Biosystems ViiA™ 7 Real-Time PCR System Getting Started Guide* (Part no. 4441434) to determine the tasks applicable to your experiment.
§ Cannot be blank.
# Contains (r)ed, (b)lue, and (g)reen color values between 0 to 255. The field must be set within double quotes with no spaces between the values.
‡‡ Can be empty if the Task field is empty. Otherwise, the field must contain a value.
§§ The dye must already exist in the ViiA™ 7 Software Dye Library. The dye name must be 100 characters or less.

Sample file format

The ViiA™ 7 Software can import sample files to populate sample information into an open experiment. A sample file is a tab-delimited ASCII text file (.TXT) that contains sample/well designations and custom sample properties. The files can be created manually using a text processor or generated automatically by third-party applications.

IMPORTANT! To guarantee a successful importation, the file must contain all the elements described in the following section and in the order that they appear.

Note: The command line application (see page 86) does not import sample files. If you are using the application to create experiments, use plate setup files to import sample information into the new experiments (see "Plate setup file format" on page 91).

File structure

Sample file header row

The sample file begins with an optional header row that contains column headers for well number ("Well"), sample name ("Sample Name"), and optional custom properties names. The order of the columns is important and cannot be changed.

Sample file body

A body of rows, which contains the sample data, follows the optional header row. Each body row defines the sample information for a single well on the consumable, including the: well number, sample name, and any applicable custom fields. The body can contain data for a subset of wells on the consumable, so the rows for empty wells can be omitted from the file. The sample body rows can occur in any order.

Note: The sample rows can occur in any order.

Column name	Description	Valid values
Well	The number of the well on the consumable, where the well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.	*<Positive integer (1 to 96/384)>*
Sample Name	The name of the sample contained by the associated well.	*<100-character string>*
Custom1... Custom6	(*Optional*) Additional text that describes the sample in the well.	*<1024-character string>*

Example file

```
Well Sample Name Custom1 Custom2 Custom3 Custom4 Custom5 Custom6
21   Sample      1test1  test2   test3   test4   test5   test6
22   Sample      2test1  test2   test3   test4   test5   test6
23   Sample      3test1  test2   test3   test4   test5   test6
1    Sample      5test1  test2   test3   test4   test5   test6
2    Sample      6test1  test2   test3   test4   test5   test6
3    Sample      7test1  test2   test3   test4   test5   test6
4    Sample      8test1  test2   test3   test4   test5   test6
…
```

Barcode file format

The ViiA™ 7 Software command line application can import barcode files to populate experiment files (EDS) it generates with barcode information. A barcode file is a tab-delimited ASCII text file (.TXT) that contains a list of barcodes. The files can be created manually using a text processor or generated automatically by third-party applications.

IMPORTANT! To guarantee a successful importation, the file must contain all the elements described in the following section and in the order that they appear.

File structure

The barcode file contains a list of barcodes, where each line defines a single barcode terminated by a carriage return. The barcodes can occur in any order and cannot contain starting or trailing white space.

Note: The ViiA™ 7 Software command line application does not validate the barcodes.

Example file

```
HA996346102
IB894812348
DD834814679
EK209825848
AF092387348
FF225676243
…
```

Export formats and file specifications

This appendix describes the export formats supported by the ViiA™ 7 Software. The information provided in this appendix is intended for users who want to integrate the ViiA™ 7 Software with third-party applications, including downstream analysis software and laboratory information management system (LIMS) tools.

Note: The file specifications listed in this appendix are subject to change. For updated information, review the ViiA™ 7 Software Release Notes found at: D:\AppliedBiosystems\ViiA7\release-notes.html.

Export formats

The ViiA™ 7 Software can export setup and results data from experiment files (EDS) in several file formats that allow further downstream analysis. The export formats feature standardized data structures and markup to maximize accessibility by downstream applications.

The ViiA™ 7 Software supports the following export formats for HRM:

File format	Description	See...
ViiA™ 7 export file	A ViiA™ 7-formatted text file that contains setup and/or results data exported from an experiment file (EDS).	page 97

Export formats and the ViiA™ 7 Software API

The export formats can be used in combination with the ViiA™ 7 Software application programming interface (API) to integrate the HRM software into a laboratory information management system (LIMS) workflow. For a detailed explanation of the software API, or for information on integrating the HRM software, see the *Applied Biosystems High Resolution Melting Software Robotics User Guide* (Part no. 4442663).

ViiA™ 7 export format

The ViiA™ 7 Software can export setup and results data from experiment files (EDS) to tab-delimited text files (TXT) in a native HRM software export format. Data exported in the ViiA™ 7 export format can be opened by common spreadsheet applications, such as Microsoft Excel, or imported by laboratory information management system (LIMS) applications or databases that have been configured to parse the file format.

File structure

The following table shows the data structure common to data exported in the ViiA™ 7 export format, regardless of experiment type. Each row represents one or more lines of data in the exported file that correspond to a common functional group. Because the the ViiA™ 7 export format allows the user to customize and/or omit columns. The columns and orders described below are the default configuration: all columns in their natural order. Actual files may contain fewer columns if the user modified the configuration.

Section	Description	See...
File header	Describes the qualities of the ViiA™ 7 Instrument used to run the experiment and several general experiment properties, such as the date/time of the run and the dye used as the passive reference.	page 98
Sample setup data	Describes the configuration of samples on the experiment consumable, including sample location, target or SNP assay properties, and task assignments.	page 99
Raw data	Contains the raw data collected by the ViiA™ 7 Instrument during the experiment run.	page 99
Amplification data	Contains the normalized to the passive reference dye data collected during the cycling stage of PCR amplification, which the ViiA™ 7 Software uses to generate the amplification plot. **Note:** Not applicable for presence/absence, genotyping, or melt curve experiments that are run without a PCR (cycling) stage.	page 100
Multicomponent data	Contains the spectral data used by the ViiA™ 7 Software to generate the multicomponent plot that displays the contribution of each dye over the duration of the PCR run.	page 101
Results data	Contains the normalized to the passive reference dye, processed, and analyzed application-specific data generated by the ViiA™ 7 Software.	page 101

File header

The plate setup file begins with a header that describes the qualities of the ViiA™ 7 Instrument used to run the experiment and several other general experiment properties. Each line starts with an asterisk (*) and ends with a carriage return in the following pattern:

```
* <field name> = <field value>
```

Note: The ViiA™ 7 Software automatically removes any leading and trailing whitespace around the field name and field value.

The header contains the lines listed in the following table.

Field	Description	Output
Block Type	The model of the sample block installed in the ViiA™ 7 Instrument at the time the experiment was run.	`96/384-well` or `array card`
Calibration Expired	Indicates whether the calibration of the ViiA™ 7 Instrument was current at the time that the experiment was run.	`Yes` or `No`
Chemistry	The chemistry of the experiment.	<*100-character string*>
Experiment File Name	The path to the experiment file on the local computer hard drive.	<*filepath*>
Experiment Name	The name of experiment entered into the Experiment Name field.	<*100-character string*>
Experiment Run End Time	The date and time that the ViiA™ 7 Instrument finished running the experiment.	<*date and time*>
Experiment Type	The type of chemistry application for which the experiment is designed.	`Standard Curve`, `Presence/Absence`, `Relative Standard Curve`, or `DDCt Quantification`
Instrument Type	The model of the ViiA™ 7 Instrument that ran the experiment.	`ViiA 7`
Passive Reference	The dye used as a passive reference (or blank if the consumable did not contain one).	<*100-character string*>
Signal Smoothing On	Indicates whether smoothing is turned on for the experiment.	`true` or `false`
Stage\Cycle where Analysis is performed	The stage and cycle during the thermal cycling protocol when the ViiA™ 7 Instrument collected data.	Stage <*integer*>, Step <*integer*>
Calibration Date	The date and time that the current background, ROI, uniformity, or pure dye calibration was performed and when it will expire.	<*date and time*>
Calibration Expiration Date		<*date and time*>
Instrument serial number	The serial number of the ViiA™ 7 Instrument that ran the experiment.	<*100-character string*>
Quantification cycle method	The method of quantification for the associated experiment.	<*100-character string*>

Sample setup data

When selected as an export option, the ViiA™ 7 Software exports sample setup data after the file header. The sample setup data describes the sample configuration on the experiment consumable, including positions, sample names, task assignments, assay information, and color coding.

The data consists of a column header followed by the sample data fields, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the ViiA™ 7 Software lists the data for each additional assay on separate rows, repeating the well number and sample information. The data included in the sample setup data export varies depending on experiment type.

Raw data

The ViiA™ 7 Software can export the unprocessed raw data (R) collected by the ViiA™ 7 Instrument during the experiment run. The raw data consists of fluorescence readings collected by the ViiA™ 7 Instrument that have not been normalized to the passive reference.

The section begins with a column header followed by the raw data, where each row contains the data for a single well separated by tab characters. Each line of raw data consists of readings sorted by bin, where each bin represents an excitation/emission filter pair that was selected during experiment setup. The bins are named for the corresponding filter combination according to the following convention:

<excitation filter name>-<emission filter name>

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1 to 96/384)‡
Cycle	The cycle of the run during which the ViiA™ 7 Instrument recorded the fluorescence.	Integer
<Bin #>	The raw fluorescence for the well measured by the ViiA™ 7 Instrument for the associated bin at the designated cycle.	Float

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

HRM Raw

The following table describes the raw data exported from high resolution melt curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1 to 96/384)‡
Reading	1-based index of the reading	Integer
Temperature	Temperature in C	Float
Fluorescence	Fluorescence value	Float
Derivative	Value of the fluorescence curve derivative for this reading point	Float

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Amplification data

The ViiA™ 7 Software can export the processed amplification data used to generate the amplification plot of a real-time PCR experiment. The amplification data (R_n) are the raw fluorescence readings collected by the ViiA™ 7 Instrument normalized to the fluorescence from the passive reference. If available, the exported amplification data also exports the baseline-compensated normalized fluorescence data (ΔR_n) calculated by the ViiA™ 7 Software.

The section begins with a column header followed by the amplification data, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the ViiA™ 7 Software lists the data for each additional assay on separate rows, repeating the well number and sample information.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1 to 96/384)‡
Cycle	The cycle of the run during which the ViiA™ 7 Instrument recorded the fluorescence.	Integer
Target Name	Genotyping experiments – The name of the SNP assay assigned to the well and the allele name.	*<SNP assay name>-<allele name>*
	All other experiments – The name of the target assigned to the well.	Name of the target
Rn	The raw fluorescence for the associated well normalized to the fluorescence of the passive reference dye (reporter signal / passive reference signal).	Float
Delta Rn	The baseline compensated R_n value for the associated well	Float

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Multicomponent data

The ViiA™ 7 Software can export the data used to generate the multicomponent plot of a real-time PCR experiment. The multicomponent data tracks the raw fluorescence of all reporter dyes present on the reaction consumable throughout the duration of the experiment run.

The section begins with a column header followed by the multicomponent data, where each row contains the data for a single well separated by tab characters. The multicomponent data contains a dye column for each dye present on the reaction consumable, including reporter dyes, quencher dyes (except non-fluorescent dyes), and the passive reference.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1 to 96/384)‡
Cycle	The cycle of the run during which the ViiA™ 7 Instrument recorded the fluorescence data.	Integer
<*Dye name*>	The raw fluorescence for the designated dye measured by the ViiA™ 7 Instrument at the specified well and cycle.	Float

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Results data

The ViiA™ 7 Software can export the results data from an analyzed experiment file. The format and content of the results data depends on the experiment type and the analysis settings.

The section begins with a column header followed by the results data, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the ViiA™ 7 Software lists the data for each additional assay on separate rows, repeating the well number and sample information.

This section describes the following results data formats:

Biological Replicate Results

The following table describes the results data exported from high resolution melt curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Biogroup Name	The name of the biological replicate group.	100-character string
Target Name	The name of the target assay assigned to the well.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN` or `NTC`
RQ	The relative quantity calculated for the replicate wells of the target/sample combination.	Float
RQ Min	The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.	Float
RQ Max	The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.	Float
Ct Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Delta Ct Mean	The average ΔC_T of the replicate wells for the specified target/sample combination.	Float
Delta Ct SD	The standard deviation of the ΔC_T for the replicate well. Depending on the analysis settings, this column may by replaced with "Delta Ct SE" (the standard error of the ΔC_T).	Float
Delta Delta Ct	The $\Delta\Delta C_T$ value of the replicate wells for the specified target/sample combination.	Float

Technical Replicate Results

The following table describes the results data exported from high resolution melt curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Sample Name	The name of the sample contained by the well.	100-character string
Target Name	The name of the target assay assigned to the well.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN` or `NTC`
RQ	The relative quantity calculated for the replicate wells of the target/sample combination.	Float
RQ Min	The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.	Float

Column	Description	Output
RQ Max	The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.	Float
Ct Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Delta Ct Mean	The average ΔC_T of the replicate wells for the specified target/sample combination.	Float
Delta Ct SD	The standard deviation of the ΔC_T for the replicate well. Depending on the analysis settings, this column may by replaced with "Delta Ct SE" (the standard error of the ΔC_T).	Float
Delta Delta Ct	The $\Delta\Delta C_T$ value of the replicate wells for the specified target/sample combination.	Float

HRM

The following table describes the results data exported from high resolution melt curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1 to 96/384)‡
Sample Name	The name of the sample contained by the well.	100-character string
Target Name	The name of the target assay assigned to the well.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN` or `NTC`
Reporter	The reporter dye that labels the probe for the target assay.	100-character string
Quencher	The quencher dye that labels the probe for the target assay.	100-character string
Variant Calls	The variant call assigned to the sample at the specified well.	`Hetero`, `Homo 1`, or `Homo 2`
Confidence Value	The calculated confidence of the automatic variant call.	Float (1 to 100)
CT	The calculated threshold cycle (C_T) for the target at the specified well.	Float
Ct Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Ct SD	The standard deviation of the average C_T of the replicate wells for the specified target.	Float
Number of Flags	The number of quality flags generated by the sample during the analysis.	Integer
Tm	Melting point	Float
Tm1... Tm3	The first, second, and third melting temperatures (T_m) calculated in degrees Celsius.	Float
Comments	Additional text that describes the well	1024-character string
Custom1... Custom6	The contents of the custom text fields found in the Results table of the experiment.	1024-character string (per field)
If analysis flags are present, results data is present in additional columns named for the associated flags.		`true` or `false`

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Technical Analysis Result (study)

The following table describes the results data exported from high resolution melt curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Sample Name	The name of the sample contained by the well.	100-character string
Target Name	The name of the target assay assigned to the well.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN` or `NTC`
Omitted	Indicates whether the well was omitted from the analysis (true) or included (false).	`true` or `false`
RQ	The relative quantity calculated for the replicate wells of the target/sample combination.	Float
RQ Min	The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.	Float
RQ Max	The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.	Float
Ct Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Delta Ct Mean	The average ΔC_T of the replicate wells for the specified target/sample combination.	Float
Delta Ct SD	The standard deviation of the ΔC_T for the replicate well. Depending on the analysis settings, this column may by replaced with "Delta Ct SE" (the standard error of the ΔC_T).	Float
Delta Delta Ct	The $\Delta\Delta C_T$ value of the replicate wells for the specified target/sample combination.	Float

BioGroup Analysis Results (study)

The following table describes the results data exported from high resolution melt curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Biogroup Name	The name of the biological replicate group.	100-character string
Target	The name of the target assay assigned to the well.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN` or `NTC`
Omitted	Indicates whether the well was omitted from the analysis (true) or included (false).	`true` or `false`
# Tech Replicates	The number of technical replicates in the associated biological replicate group.	Integer

Column	Description	Output
RQ	The relative quantity calculated for the replicate wells of the target/sample combination.	Float
RQ Min	The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.	Float
RQ Max	The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.	Float
Ct Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Delta Ct Mean	The average ΔC_T of the replicate wells for the specified target/sample combination.	Float
Delta Ct SD	The standard deviation of the ΔC_T for the replicate well. Depending on the analysis settings, this column may by replaced with "Delta Ct SE" (the standard error of the ΔC_T).	Float
Delta Delta Ct	The $\Delta\Delta C_T$ value of the replicate wells for the specified target/sample combination.	Float

HRM Difference

The following table describes the results data exported from high resolution melt curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1 to 96/384)‡
Reading	1-based index of the reading	Integer
Temperature	Temperature in C	Float
Difference	HRM difference value	Float

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

E Safety and EMC Compliance Information

Safety conventions used in this document

Safety alert words

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

Definitions

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

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, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

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.

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard symbols that are affixed to Applied Biosystems instruments* (see "Safety symbols" on page 110).

Examples

The following examples show the use of safety alert words:

IMPORTANT! You must create a separate sample entry spreadsheet for each 96-well plate.

CAUTION! Do not install on the computer additional software other than antivirus software. Changes to the configured software could void the instrument warranty and cause the system to be nonoperational.

WARNING! Physical Injury Hazard. Using the instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument

DANGER! ELECTRICAL HAZARD. Failure to ground the instrument properly can lead to an electrical shock. Ground the instrument according to the provided instructions.

Symbols on instruments

Electrical symbols on instruments

The following table describes the electrical symbols that may be displayed on Applied Biosystems instruments.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.

Safety symbols

The following table describes the safety symbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or with text that explains the relevant hazard. These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description	Français
	Caution, risk of danger. Consult the user documentation for addtional information and instructions, and proceed with appropriate caution.	**ATTENTION!** Lire les fiches techniques de sûreté et d'utilisation de matériels avant toute manipulation de produits. Procéder délicatement.
	Caution, risk of electric shock.	**ATTENTION!** Risque de choc électrique.
	Caution, hot surface.	**ATTENTION!** Surface brûlante.
 	Caution, moving parts.	**ATTENTION!** Pièces en mouvement, risque de pincement et/ou d'écrasement.
	Caution, laser radiation.	**ATTENTION!** Radiation Laser.
	Caution, ultraviolet light.	**ATTENTION!** Emission ultra violet.

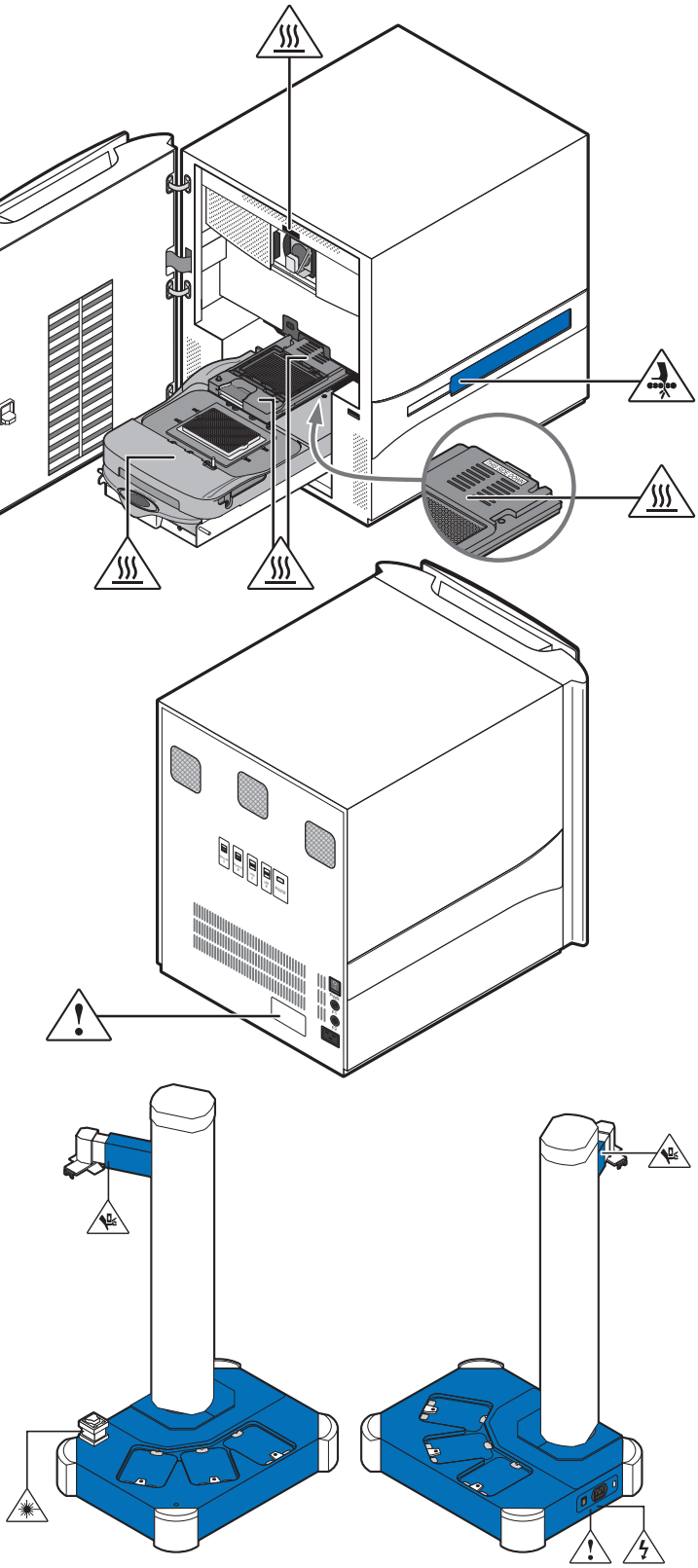
Environmental symbols on instruments

The following symbol applies to all Applied Biosystems electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your local Applied Biosystems Customer Service office for equipment pick-up and recycling. See **www.appliedbiosystems.com** for a list of customer service offices in the European Union.

Locations of safety labels on instruments

The ViiA™ 7 Instrument contains warnings at the locations shown below:



General instrument safety

WARNING! Physical Injury Hazard. Use this product only as specified in this document. Using the instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

Moving and lifting the instrument

CAUTION! Physical Injury Hazard. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and lifting stand-alone computers and monitors

WARNING! Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs). See "Obtain an SDS" on page 118.

Cleaning or decontaminating the instrument

CAUTION! Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Physical hazard safety

Ultraviolet light

WARNING! ULTRAVIOLET LIGHT HAZARD. Looking directly at a UV light source can cause serious eye damage. Never look directly at a UV light source and always prevent others from UV exposure. Follow the manufacturer's recommendations for appropriate protective eyewear and clothing.

Moving parts

WARNING! PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Electrical safety

DANGER! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the ViiA™ 7 Instrument without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

WARNING! FIRE HAZARD. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.

WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

DANGER! ELECTRICAL HAZARD. Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

DANGER! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

DANGER! ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage rating

The instrument has an installation (overvoltage) category of II, and is classified as portable equipment.

Bar code scanner laser safety

Laser classification

The bar code scanners included with the ViiA™ 7 Instrument are categorized as Class 2 (II) lasers.

Laser safety requirements

Class 2 (II) lasers are low-power, visible-light lasers that can damage the eyes. Never look directly into the laser beam. The scanner is designed to prevent human access to harmful levels of laser light during normal operation, user maintenance, or during prescribed service operations.

WARNING! LASER HAZARD. Class 2 (II) lasers can cause damage to eyes. Avoid looking into a Class 2 (II) laser beam or pointing a Class 2 (II) laser beam into another person's eyes.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

WARNING! MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

U.S. and Canadian safety standards

The instrument has been tested to and complies with standard:

UL 61010-1:2nd Edition/CSA C22.2 No. 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

UL 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

Canadian EMC standard

This instrument has been tested to and complies with standard:

ICES-001, Issue 3: "Industrial, Scientific, and Medical Radio Frequency Generators."
Cet appareil numerique de la classe B est conforme a la norme NMB-001 du Canada.

European safety and EMC standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 2006/95/EC). This instrument has been tested to and complies with standards:

EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."

EN 61010-2-010:2003, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

EN 61010-2-081:2002+A1:2003, "Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes."

EMC

The ViiA™ 7 Real-Time PCR System meets European requirements for emission and immunity (EMC Directive 2004/108/EC).

EN 61326-1:2006 "Electrical equipment for measurement, control and laboratory use-Part 1 General EMC requirements." (Group 1, Class B)

Australia and New Zealand EMC standards

This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

Chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "Obtain an SDS" on page 118.)
- 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 SDS.
- 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 SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

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

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

Obtain an SDS

The Safety Data Sheet (SDS) for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain an SDS:

1. Go to **www3.appliedbiosystems.com/sup/msds/search.htm**
2. In the Keyword Search field of the SDS Search page:
   a. Type in the chemical name, part number, or other information that you expect to appear in the SDS of interest.
   b. Select the language of your choice.
   c. Click **Search**.

   A list of relevant SDS documents appears.
3. To view, download, or print the document of interest:
4. Right-click the document title.
5. Select:
   - **Open** – To view the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose
   - **Print Target** – To print the document
6. To have a copy of an SDS sent by email, send your request to: **SDS_Inquiry_CCRM@appliedbiosystems.com**
7. To have a copy of an SDS sent by fax to the US or Canada, send your fax request to 650-442-2252.
8. To have a copy of an SDS sent by fax to a region outside of the US or Canada, scroll to the bottom of your SDS Search results web page and click **regional office**.

From the Country/Region drop-down menu, select your location.

The contact phone number and the fax request number for your region appear.

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

Chemical waste safety

Chemical waste hazard

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

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

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

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) 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 SDS.
- 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 SDS.
- Handle chemical wastes in a fume hood.
- After emptying a 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

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (**www.cdc.gov/biosafety/publications/index.htm**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/ nara/cfr/waisidx_01/ 29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

In the EU:

Check local guidelines and legislation on biohazard and biosafety precautions and refer to the best practices published in the World Health Organisation (WHO) Laboratory Biosafety Manual, third edition.

http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Related documentation

The following related documents are shipped with the ViiA™ 7 System:

Document	Purpose and audience
Applied Biosystems ViiA™ 7 Real-Time PCR System Quick Reference Guide (PN 4448987)	Explains how to perform genotyping and gene expression experiments using the ViiA™ 7 System. Intended for laboratory staff and principal investigators who perform experiments using the ViiA™ 7 System.
Applied Biosystems ViiA™ 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security (PN 4442661)	Explains how to calibrate, maintain, network, and secure the ViiA™ 7 System. Intended for laboratory staff and principal investigators who maintain the ViiA™ 7 System.
Applied Biosystems ViiA™ 7 Real-Time PCR System Getting Started Guide (PN 4441434)	Explains how to perform experiments on the ViiA™ 7 System. The guide functions as both a: • Tutorial, using example experiment data provided with the ViiA™ 7 Software. • Guide for your own experiments. Intended for laboratory staff and principal investigators who perform experiments using the ViiA™ 7 System.
Applied Biosystems ViiA™ 7 Real-Time PCR System Robotics User Guide (PN 4442663) (Only ships if you have ordered the robot)	Explains how to integrate a robotic plate handler with the ViiA™ 7 System. Intended for engineering personnel who are responsible for integrating a robotic plate handler with the ViiA™ 7 System.

Portable document format (PDF) versions of the Quick Reference Guide, User Guide, and Getting Started Guide are available on the ViiA™ 7 Software CD.

Note: To open the user documentation included on the *Applied Biosystems ViiA™ 7* Real-Time PCR Instrument Software CD, use the Adobe® Acrobat® Reader® software available from **www.adobe.com**

Obtaining information from the Help system

The ViiA™ 7 System has a Help system that describes how to use each feature of the user interface. Access the Help system by doing one of the following:

- Click in the toolbar of the ViiA™ 7 Software window.
- Select **Help** ▸ **ViiA 7 Software Help**.
- Press **F1**.

You can use the Help system to find topics of interest by:

- Reviewing the table of contents
- Searching for a specific topic

Obtaining support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Life Technologies Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

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applied biosystems™
by life technologies™

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone 760.603.7200
www.lifetechnologies.com

Technical Resources and Support
For the latest technical resources and support information for all locations, please refer to our Web site at
www.appliedbiosystems.com