

TaqMan™ Genotyper Software

GETTING STARTED GUIDE

For v1.7 or later

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Revision	Date	Description
C	29 November 2022	For TaqMan™ Genotyper Software v1.7 or later. • The statement covering Limited Use Label Licenses was removed. • The list of compatible real-time PCR systems and software was updated (“Compatible real-time PCR systems” on page 11). • The information about the experiment type was updated to indicate that it can be changed after importing the experiments (“Enter the study properties” on page 37). • The experiment criteria for OpenArray™ Plates were updated (“Experiment criteria” on page 14). • The instructions to install the software were updated to remove the instructions to log in. The default installation does not have security settings enabled (“Install the software” on page 17). • Instructions were added to enable the security settings (“(Optional) Enable system security” on page 18). • The information about the user name and password when security settings are enabled was updated. The minimum and maximum number of characters can be configured. The minimum length of 8 characters and the maximum length of 32 characters are the default settings (“User names” on page 22 and “Passwords” on page 22). • Information was added about the number of passwords that cannot be reused when the security settings are enabled (“Passwords” on page 22). • The information about password expiration was updated to include options for configuration (“Password expiration” on page 22). • The information about a session timeout was updated (“Session timeout” on page 23). • The instructions to set up the genotyping preferences were updated. The default instrument type is not a setting (“Configure genotyping preferences” on page 33). • The instructions to enter study properties were updated. The instrument is not selected (“Enter the study properties” on page 37). • The call settings were updated to include multiplate analysis, normalize cluster, and an assay ID search (“Call setting descriptions” on page 40). • The call settings and control identifiers were updated to include an assay ID search (“Call setting descriptions” on page 40 and “Add control identifiers” on page 43). • The option to view the trace in the scatter plot for real-time experiments was added (“View the data in the scatter plot” on page 66). • The option to select samples to view in the scatter plot was added (“View the data in the scatter plot” on page 66). • The option to adjust the number of cycles with the Show Cycle slider in the scatter plot was added (“View the data in the scatter plot” on page 66). • A workflow was provided in order to customize and save analysis settings (“Set the analysis settings” on page 38). • The information about changing the cycle number was updated to include the Show Cycle slider in the scatter plot (“Change the cycle number and baseline normalization” on page 126). • The option to export the QC summary was added (“Export the analysis data” on page 101). • The instructions to export the analysis data were updated to include a preview before the files are exported (“Export the analysis data” on page 101). • Internet Explorer™ was removed as the example of a browser.
B	7 January 2020	• Updated for general style and formatting. • Updated list of compatible real-time PCR systems and system software. • Added network configuration and security and password security information.

Revision	Date	Description
A	1 April 2010	New document for the TaqMan™ Genotyper Software.

The information in this guide is subject to change without notice.

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This chapter contains important information about using the Applied Biosystems™ TaqMan™ Genotyper Software.

This version of the document is for TaqMan™ Genotyper Software v1.7.

About the TaqMan™ Genotyper Software

The TaqMan™ Genotyper Software is a standalone software application. You can use the software to analyze raw data from genotyping experiments that were created on an Applied Biosystems™ real-time PCR system.

Features

Use the TaqMan™ Genotyper Software to perform the following tasks:

- Create a study.
 - Import multiple experiments into a single study.
 - Import assay information files (TXT or XML) to update assay information.
 - Set the analysis settings.
 - Import Supplementary Sample Information (SSI) files to update sample information.
 - Import reference panel files to add reference samples to a study.
- Generate a study template, then use the study template to create new studies. The software analyzes the data according to the analysis settings that were defined in the study template.
- Analyze the study data using one of two call methods.
 - Autocalling—The software algorithm is used to call the data points.
 - Classification Scheme—You define the cluster boundaries that are used to call the data points.
- View the study results. For example, view a summary of the Quality Control (QC) statistics at the study level, assay level, experiment level, and sample level.

- Export the following data:
 - Analysis results
 - Analysis settings
 - Audit trails
- Transfer studies from one TaqMan™ Genotyper Software application to another.
- Ensure data security. The security feature allows you to perform the following tasks:
 - Set security parameters to manage users (set up user accounts and assign user roles).
 - Track changes to the data.

Compatible real-time PCR systems

The TaqMan™ Genotyper Software can import genotyping experiments that have been created on one of the real-time PCR systems listed below.

Applied Biosystems™ real-time PCR system	Experiment file extension	System software
ViiA™ 7 Real-Time PCR System	EDS	ViiA™ 7 Software v1.0 and v1.1
7900HT Fast Real-Time PCR System	SDS	SDS Software v2.1, v2.2.2 (Enterprise), v2.3 (Non-Enterprise), and v2.4 (Enterprise and Non-Enterprise)
7500 Fast Real-Time PCR System	EDS	7500 Software v2.0 and v2.3
	SDS	SDS Software v1.4 and v1.5
7500 Real-Time PCR Instrument	EDS	7500 Software v2.0 and v2.3
	SDS	SDS Software v1.4 and v1.5
StepOnePlus™ Real-Time PCR System	EDS	StepOne™ Software v2.0, v2.1, v2.2, and v2.3
QuantStudio™ 12K Flex Real-Time PCR System Includes the OpenArray™ Plate format.	EDS	QuantStudio™ 12K Flex Software v1.0 or later
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems	EDS	QuantStudio™ 6 and 7 Flex Software v1.0 QuantStudio™ Real-Time PCR Software v1.0 or later
QuantStudio™ 3 and 5 Real-Time PCR Systems	EDS	QuantStudio™ Design and Analysis Software v1.2 or later
QuantStudio™ 1 Real-Time PCR System	EDS	QuantStudio™ Design and Analysis Software v1.5 or later

(continued)

Applied Biosystems™ real-time PCR system	Experiment file extension	System software
QuantStudio™ 1 Plus Real-Time PCR System	EDS	v1.0 or later (instrument) QuantStudio™ Design and Analysis SC Software v1.0 or later
QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems	EDS	QuantStudio™ Design and Analysis Software v2 or later
QuantStudio™ 5 Dx Real-Time PCR System	EDS	QuantStudio™ 5 Dx Software v1.0 or later Dionni™ Software v3.0 or later
QuantStudio™ 7 Pro Dx Real-Time PCR System	EDS	v1.0 or later (instrument) Dionni™ Software v3.0 or later

Instrument calibration

IMPORTANT! The TaqMan™ Genotyper Software performs optimally when analyzing data generated from properly maintained and calibrated real-time PCR instruments. We strongly recommends that you perform regular calibration and maintenance procedures on your real-time PCR instrument.

Screen captures in this guide

Most of the screen captures in this guide depict studies that contain SDS experiment files created on the 7900HT Fast Real-Time PCR System. Unless otherwise noted, however, the procedures are applicable to all of the experiment files listed in the table above.

Overview of studies

In the TaqMan™ Genotyper Software, a *study* is a collection of genotyping experiments.

Note: In the TaqMan™ Genotyper Software, an *experiment* contains genotyping data from a single reaction plate. The reaction plate must be from one of the Applied Biosystems™ real-time PCR systems (see “Compatible real-time PCR systems” on page 11).

A study allows you to overlay and analyze raw data from multiple genotyping experiments together. Within the study, you can observe the statistical distribution of the data over all of the experiments. Studies can be especially useful when individual experiments have only a small number of samples on the reaction plate because clustering relies on a reasonably large number of samples.

Recommended number of studies

The table below lists the recommended number of studies that you can open concurrently in the TaqMan™ Genotyper Software.

Study size	Number of studies	
	Recommended	Maximum
Small (~3000 data points per study)	6	8
Large (~60,000 data points per study)	3	5

Overview of experiments

In the TaqMan™ Genotyper Software, an *experiment* contains genotyping data from a single reaction plate. The reaction plate must be from one of the Applied Biosystems™ real-time PCR systems (see “Compatible real-time PCR systems” on page 11).

Compatible genotyping experiments

You can import the following types of genotyping experiments into a TaqMan™ Genotyper Software study:

Genotyping experiment	Data collection
Endpoint	At the end of the run (post-read)
Endpoint + pre-PCR Read	- At the beginning of the run (pre-read) <i>and</i> - At the end of the run (post-read)
Real-time	During all cycles of the run (real-time read) Note: Only real-time PCR experiments with between 20 and 60 cycles are allowed.

IMPORTANT! The TaqMan™ Genotyper Software supports only those experiments that meet all of the criteria specified (see “Experiment criteria” on page 14).

Recommended number of experiments

The recommended number of experiment files that you can import into the TaqMan™ Genotyper Software depends on your real-time PCR system:

Real-time PCR system	Recommended number of experiment files
QuantStudio™ 12K Flex Real-Time PCR System with TaqMan™ OpenArray™ Plates	100
All other systems	400 ^[1]

^[1] This number applies to both 96-well and 384-well reaction plates.

Experiment criteria

IMPORTANT! Each genotyping experiment that you import into a single study must meet the criteria listed in the table below.

Real-time PCR system	Criteria
All compatible real-time PCR systems (see “Compatible real-time PCR systems” on page 11).	The experiment must have a unique experiment name.
	The experiment must have been performed on the same instrument type.
	The experiment must not include more than one data collection point per run stage.
	The experiment must be the same experiment type: Endpoint, Endpoint + pre-PCR read, or Real-time. Note: In the TaqMan™ Genotyper Software, the experiment type names refer to the times at which data collection occurs (see “Compatible genotyping experiments” on page 13). Your instrument software may use different names for these experiment types.
	The experiment must have a sample ID assigned to each well. The TaqMan™ Genotyper Software ignores any well that does not have a sample ID assigned. Note: You cannot edit sample IDs in the TaqMan™ Genotyper Software. Ensure that the sample IDs are correctly assigned in the experiment file before you import the file into the TaqMan™ Genotyper Software.
	The experiment must have an assay ID assigned to each well. The TaqMan™ Genotyper Software ignores any well that does not have an assay ID assigned. Note: You cannot edit assay IDs in the TaqMan™ Genotyper Software. Ensure that the assay IDs are correctly assigned in the experiment file or assay information file before you import the file into the TaqMan™ Genotyper Software.

(continued)

Real-time PCR system	Criteria
All compatible real-time PCR systems (see “Compatible real-time PCR systems” on page 11).	If a user creates a study from a study template, the experiment cannot include an assay ID that is not already in the study template.
	If you want to analyze data for the same assay across multiple experiments, that assay must have <i>exactly</i> the same assay ID in each of the experiment files.
	(For Endpoint and Endpoint + pre-PCR read experiments) The experiment must have a marker (assay) name assigned to each well. The software ignores any well that does not have a marker name assigned. The software uses the marker name from the experiment file as the assay ID, and ignores the assay ID that is listed in the experiment file.
7900HT Fast Real-Time PCR System and 7500 Real-Time PCR System	For real-time experiments (AQ), the software assigns the assay ID based on the original experiment file, as follows: - The software ignores the assay ID that is listed in the experiment file, and instead uses the FAM™ dye detector name from the experiment file as the assay ID. - The software ignores any well that does not have FAM™ dye and VIC™ dye detectors assigned.
7500 Fast Real-Time PCR System, StepOnePlus™ Real-Time PCR System, and ViiA™ 7 Real-Time PCR System	The experiment must be a genotyping experiment (AD).
7500 Fast Real-Time PCR System, QuantStudio™ 12K Flex Real-Time PCR System with TaqMan™ OpenArray™ Plates, StepOnePlus™ Real-Time PCR System, and ViiA™ 7 Real-Time PCR System	The software assigns the assay ID based on the original experiment file, as follows: - For genotyping (with or without amplification) or AD experiments, the software uses the marker name or SNP assay ID/name, depending on the instrument software. - For AQ experiments, the software uses the FAM™ dye detector name. The software ignores any well that does not have one of these names assigned to it in the original experiment file.

For more information

For information about setting up genotyping experiments in the real-time PCR system software, see the user documentation for your system.

About the field error icon

When you enter information in the software fields, the field error icon might be displayed (⚠️).

The field error icon indicates that a field is not correctly completed. Mouse-over the icon for information on how to correctly complete the field.

① Example of information associated with the field error icon.

Network and password security requirements

Network configuration and security

The network configuration and security settings of your laboratory or facility (such as firewalls, anti-virus software, network passwords) are the sole responsibility of your facility administrator, IT, and security personnel. This product does not provide any network or security configuration files, utilities, or instructions.

If external or network drives are connected to the software, it is the responsibility of your IT personnel to ensure that such drives are configured and secured correctly to prevent data corruption or loss. It is the responsibility of your facility administrator, IT, and security personnel to prevent the use of any unsecured ports (such as USB, Ethernet) and ensure that the system security is maintained.

Password security

Thermo Fisher Scientific strongly recommends that you maintain unique passwords for all accounts in use on this product. All passwords should be reset upon first sign in to the product. Change passwords according to your organization's password policy.

It is the sole responsibility of your IT personnel to develop and enforce secure use of passwords.



Manage the software

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Required user role

To perform the procedures in this chapter, you must have the Administrator user role.

Note: For more information on user roles, see “User roles and tasks” on page 20.

Install or uninstall the software

Install the software

Computer specifications

- Intel™ CPU, 2.4 GHz
- 2 GB of RAM
- Screen resolution: 1280 × 1024 pixels
- 20 GB of disk space
- Microsoft™ Windows™ 10 Professional operating system
- Adobe Reader™ software v9.0 or later installed

IMPORTANT! In order to install the software, you must have Computer Administrator privileges in the Windows™ 10 Professional operating system.

Install the software

1. Download the TaqMan™ Genotyper Software from the Thermo Fisher Scientific website.
2. Follow the prompts to install the software.
3. When the installation completes, double-click  or open TaqMan™ Genotyper Software from the Windows™ menu.

The software is installed without the system security enabled. Login is not required. To enable system security, see “(Optional) Enable system security” on page 18.

(Optional) Enable system security

For information about the system security, see “Manage user accounts and system settings” on page 19.

1. Click **Tools** ▶ **Security** ▶ **Settings**.
The **Security Settings** dialog box is displayed.
2. In the **Security Settings** dialog box, click the **System** tab.
3. Select the **Enable System Security** checkbox, then click **Apply Settings**.
4. In the **Save** dialog box, click **OK**.
5. Close the **Security Settings** dialog box.

Uninstall the software

All data are removed when the software is uninstalled

IMPORTANT! When you uninstall the TaqMan™ Genotyper Software, the InstallShield Wizard permanently removes all data stored in the software installation folder and all its subfolders, including:

- User account information
- Studies
- Study templates
- Reference panels

Saving data before an uninstall

Before you uninstall the software, Applied Biosystems™ recommends that you save data as follows:

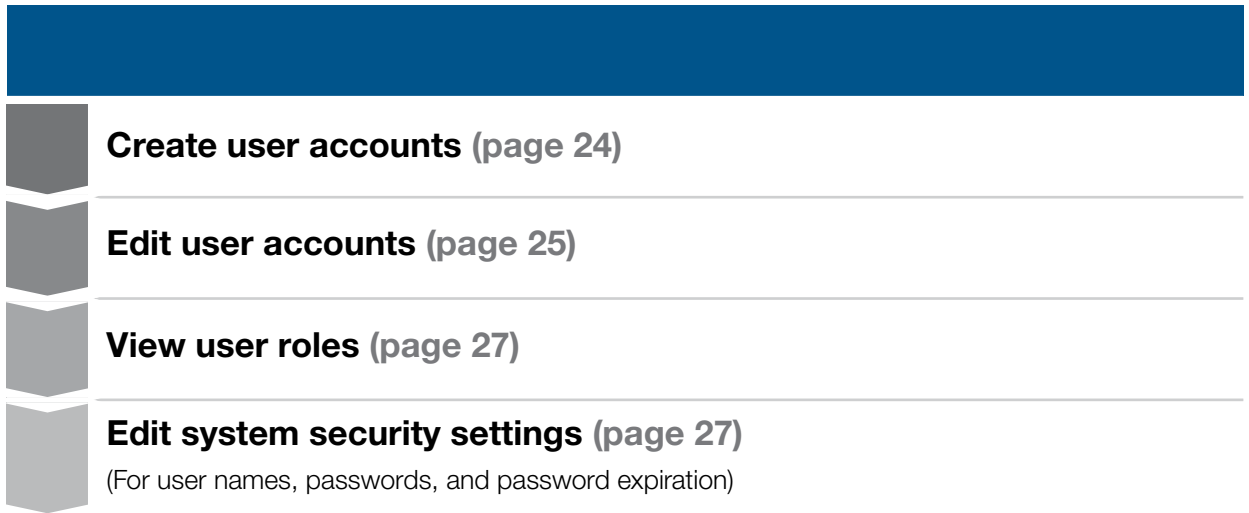
Data type	Backup method
Studies	Keep study data by transferring studies out. See “Transfer a study” on page 112.
Study templates	By default, study templates and reference panels are saved as files in the software installation/user data folder. Copy the user data folder (or selected files in the folder) to a backup device or to another computer. Note: When you select the system preferences, you may choose to save study templates and reference panels to a location other than the software installation/user data folder. See “(Optional) Configure the system preferences” on page 31.
Reference panels	

Uninstall the software

Uninstall the software according to your operating system.

Manage user accounts and system settings

Workflow



Software security specifications

This section describes the TaqMan™ Genotyper Software security specifications for:

- User roles and tasks (see page 20)
- User names (see page 22)
- Passwords (see page 22)
- Password expiration (see page 22)
- Login attempts (see page 23)
- Session timeout (see page 23)

User roles and tasks

The tasks each user role is allowed to perform are listed in the table below. Tasks that are not listed in the table can be performed by all user roles (for example, all users can view data).

IMPORTANT! The user roles and tasks are set in the software and cannot be changed.

Task	User role		
	Administrator	Scientist	Technician
Assay (Library)			
Edit assays	✓	✓	—
Delete an assay	✓	✓	—
Delete all assays	✓	✓	—
Import an assay information file (TXT or XML)	✓	✓	—
Export			
Edit the Export Legend	✓	✓	—
Export data (results, classification schemes, audit trails)	✓	✓	✓
Sample (Library)			
Edit samples	✓	✓	✓
Delete a sample	✓	✓	✓
Delete all samples	✓	✓	✓
Import a Supplementary Sample Information (SSI) file	✓	✓	✓
Reference panels			
Delete a reference panel	✓	✓	—
Delete all reference panels	✓	✓	—
Import a reference panel	✓	✓	—
Generate a reference panel file	✓	✓	—
Tag/Un-tag samples as reference samples	✓	✓	—
Study			
Create a study	✓	✓	—
Create a study template from a study	✓	✓	—
Create a study from a study template	✓	✓	✓

(continued)

Task	User role		
	Administrator	Scientist	Technician
Open a study	✓	✓	✓
Edit the study properties	✓	✓	✓
Save a study	✓	✓	✓
Delete a study	✓	✓	—
Add experiments to a study	✓	✓	✓
Remove experiments from a study	✓	✓	✓
Transfer a study out	✓	✓	✓
Transfer a study in	✓	✓	✓
Analysis			
Edit analysis settings (call settings, control identifiers, QC settings) at the study and assay levels	✓	✓	—
Select and edit classification schemes	✓	✓	—
Change the ranges for the scatter plot (Set Plot View)	✓	✓	—
Analyze and reanalyze the data (that is, click the Analyze button)	✓	✓	✓
Omit and un-omit data points/wells	✓	✓	✓
Perform manual calls	✓	✓	✓
Enter comments for data points/wells	✓	✓	✓
Preferences			
Configure system preferences			
IMPORTANT! Exceptions are noted in the two rows below.	✓	✓	✓
Select whether or not to override study templates	✓	✓	—
Select whether or not to include failed samples in exported data	✓	✓	—
User Account Management			
Create user accounts	✓	—	—

(continued)

Task	User role		
	Administrator	Scientist	Technician
Edit user accounts (this includes resetting passwords, changing user roles, and suspending or inactivating user accounts)	✓	—	—
Log into a user's session after it has been locked due to too many failed login attempts (six failed login attempts within 30 minutes)	✓	—	—
Log into a user's session after it has timed out (no activity after 30 minutes) Note: All users are allowed to log into their own sessions after a timeout.	✓	—	—

User names

User names are configurable. The user name has a minimum length and a maximum length (see “Edit system security settings” on page 27). The default length is between 8 and 32 characters.

A user name can include spaces, as long as the spaces are not leading, trailing, or multiple consecutive spaces.

Note: Once a user name has been saved in the software, it cannot be changed. If you edit a user account, the **User Name** field is disabled and cannot be edited (see “Edit user accounts” on page 25).

Passwords

Passwords are configurable. The password has a minimum length and a maximum length (see “Edit system security settings” on page 27). The default length is between 8 and 32 characters.

The number of previous passwords that cannot be reused is also configurable. The default is 24 passwords.

A password has the following requirements:

- A password must include at least one letter.
- A password can include spaces.
- A password cannot be the user's first or last name.

Password expiration

The password expiration is configurable (see “Edit system security settings” on page 27).

The password can be set to expire, and the length of time for expiry can be configured. The default setting is for the password to expire after 60 days.

The user can be sent a notification before password expiration. The length of time for the notification to be sent can be configured. The default setting is for a notification to be sent 10 days before expiration.

Failed login attempts

A user's account will be suspended if six failed login attempts are made within 30 minutes. An Administrator must reactivate the suspended account in order for that user to continue using the account (see "Edit user accounts" on page 25).

Note: The allowed number of failed login attempts is set in the software and cannot be changed.

Session timeout

The settings can be configured for the software to time out after 30 minutes of inactivity ("Edit system security settings" on page 27).

Note: The period of activity is 30 minutes and it cannot be changed.

After a timeout, the same user or an Administrator must log in.

Manage user accounts

You can perform the following user account management tasks in the software:

- Create user accounts (see page 24)
- Edit user accounts (see page 25)
- View user roles (see page 27)

Create user accounts

1. In the menu bar, select **Tools** ▶ **Security** ▶ **Settings**.
2. In the **Security Settings** window, select the **Users** tab, then click **Create** to open the **Create User Account** dialog box.

The screenshot shows the 'Create User Account' dialog box. It has a title bar with a close button (X). Below the title bar is a message: 'User name is required.' with a blue information icon. The dialog contains several fields and controls, numbered 1 through 7:

- 1: * User Name: (text field)
- 2: * Password: (password field) and * Re-Enter Password: (password field)
- 3: * First Name: (text field) and * Last Name: (text field)
- 4: Other optional information (Phone: (text field), Email: (text field), and Comments: (text area))
- 5: * User Role: (drop-down list, currently showing 'Technician')
- 6: ☒ Password Expires at First Login (checkbox)
- 7: * Status: (drop-down list, currently showing 'ACTIVE')

At the bottom right are 'Save' and 'Cancel' buttons.

Figure 1 Create User Account dialog box

- 1 **User Name** field
 - 2 Password fields
 - 3 **First Name** and **Last Name** fields
 - 4 Other optional information
 - 5 **User Role** drop-down list
 - 6 **Password Expires at First Login** checkbox
 - 7 **Status** checkbox
3. Enter a user name.
Follow the security specifications (see “User names” on page 22).
 4. Enter a password, then re-enter the password.
Follow the security specifications (see “Passwords” on page 22).
 5. Enter the user’s first and last names.
 6. Select a user role.
 - **Administrator**
 - **Scientist**
 - **Technician**

Note: For a list of functions that each user role is allowed to perform, see “User roles and tasks” on page 20.

7. Select or deselect the **Password Expires at First Login** checkbox.
When selected, users must change their password at initial login.
8. From the **Status** drop-down list, select **ACTIVE**.
9. *(Optional)* Complete the remaining fields as needed.
10. Click **Save**.
The new user is listed in the **Security Settings** window.

Edit user accounts

Use the edit function to change user information as needed, including the following items:

- Passwords
- User roles
- Account status

1. In the menu bar, select **Tools** ▶ **Security** ▶ **Settings**.
2. In the **Security Settings** window, select the **Users** tab, select the account of interest, then click **Edit** to open the **Edit User Account** dialog box.

The screenshot shows the 'Edit User Account' dialog box. It contains the following fields and controls:

- 1** points to the *** User Name** field, which is disabled and contains 'User Name Example'.
- 2** points to the *** Password** and *** Re-Enter Password** fields, both masked with dots.
- 3** points to the *** First Name** (containing 'Example') and *** Last Name** (containing 'Example') fields, along with a small **MI** (Middle Initial) field.
- 4** points to the optional information section, including **Phone**, **Email**, and a large **Comments** text area.
- 5** points to the *** User Role** drop-down list, which is currently set to 'Technician'.
- 6** points to the **✓ Password Expires at First Login** checkbox, which is checked.
- 7** points to the *** Status** drop-down list, which is currently set to 'ACTIVE'.

At the bottom right, there are **Save** and **Cancel** buttons.

Figure 2 Edit User Account dialog box

- ① **User Name** field (cannot be edited)
 - ② Password fields
 - ③ **First Name** and **Last Name** fields
 - ④ Other optional information
 - ⑤ **User Role** drop-down list
 - ⑥ **Password Expires at First Login** checkbox
 - ⑦ **Status** checkbox
3. To change a user's password, enter a new password, then re-enter the new password to confirm it. Follow the security specifications (see "Passwords" on page 22).
 4. To change a user role, select an option from the **User Role** drop-down list.
 - **Administrator**
 - **Scientist**
 - **Technician**

For a list of functions that each user role is allowed to perform, see "User roles and tasks" on page 20.

Note: At least one administrator is required. The administrator role for the only user with this role cannot be changed.

5. To change a user's account status, select an option from the **Status** drop-down list.

- **ACTIVE**
- **INACTIVE**
- **SUSPENDED**

Note: The software automatically sets the **SUSPENDED** option when a user is locked out of the software because of too many failed login attempts. The **SUSPENDED** option can also be manually selected.

6. To change any of the remaining user information, enter new information or select new options as needed.

Note: The **User Name** field is disabled and cannot be edited.

7. Click **Save** to return to the **Security Settings** window.

View user roles

1. In the menu bar, select **Tools ▶ Security ▶ Settings**.

2. In the **Security Settings** window, select the **Roles** tab.

The following information is displayed:

- **Role**
- **Description**
- **Last Modified**
- **# of Users**

Security Settings ×

Users Roles System			
Role	Description	Last Modified	# of Users
Administrator		2010-02-25 02:10:01	1
Technician		2010-02-25 02:16:03	2
Scientist		2010-02-25 02:10:53	0

(Optional) Edit system security settings

You can edit the system security settings for user names, user passwords, and password expiration (see “Edit system security settings” on page 27).

Edit system security settings

1. In the menu bar, select **Tools ▶ Security ▶ Settings**.

2. In the **Security Settings** window, select the **System** tab.

3. Enter new user name settings.

The length of the user name is edited (see “User names” on page 22).

4. Enter new password settings.

The length of the password and reuse policy are edited (see “Passwords” on page 22).

5. Enter new password expiration settings.

Expiration, length of time until expiration, and notifications are edited (see “Password expiration” on page 22).

6. To revert to the previously saved changes, click **Revert Settings**.

IMPORTANT! The **Revert Settings** button only applies if you have made changes, but have not applied them yet. After clicking **Apply Settings** (step 7), you cannot revert to the previous settings.

7. To save the changes, click **Apply Settings**.

The screenshot shows the 'Security Settings' dialog box with the 'System' tab selected. At the top, there are tabs for 'Users', 'Roles', and 'System'. Below the tabs, there is a checkbox labeled 'Enable System Security' which is checked. The main content area is divided into four sections: 'User Names', 'User Passwords', 'Password Expiration', and 'Session Timeout'. Each section contains specific configuration options and values. At the bottom right, there are two buttons: 'Revert Settings' and 'Apply Settings'.

Security Settings ×

Users Roles **System**

☒ **Enable System Security**

User Names

The length of user names must be between and characters.
Space characters not allowed : trailing, leading and multiple consecutive spaces.

User Passwords

The length of user passwords must be between and characters. Space characters allowed.
User may not reuse the previous passwords.

Password Expiration

☒ Passwords expire automatically after days.
☒ Notify the user days before expiration.

Session Timeout

☒ Enable session timeout after 30 minutes. (Requires restart of the software)

Revert Settings **Apply Settings**

Figure 3 System tab

3

Log in and configure preferences

■ Required user role	29
■ Log in	29
■ Change your password	30
■ About failed login attempts and session timeout	31
■ (Optional) Configure the system preferences	31

Required user role

You might not be able to perform all of the procedures in this chapter. The table below indicates the user roles that are allowed to perform each procedure in this chapter if the system security is enabled.

Note: For more information on user roles, see “User roles and tasks” on page 20.

Function	User role		
	Administrator	Scientist	Technician
Log in to the software (see page 29)	✓	✓	✓
Change your password (see page 30)	✓	✓	✓
(Optional) Configure the system preferences (see page 31) Note: Users with the Technician role are not allowed to configure the Allow study templates to be overwritten or Don't export samples if call rate failed checkboxes (see page 31).	✓	✓	✓

Log in

Log in is only required if the system security is enabled.

1. Double-click the software icon  or select use the Windows™ start menu.
The software is the **Applied Biosystems** folder in the Windows™ start menu.
2. In the **Login** dialog box, enter the user name and password provided by the administrator.
3. If prompted to change your password, click **OK**, then change your password.
See “Change your password” on page 30.

4. Enter your new password.
5. Click **Login** to open the software.

Change your password

When to change your password

- You might be required to change your initial password, if the administrator has set your password to pre-expire.
- You can change your password at any time.
- You might need to change your password periodically if the administrator has configured the software for password expiration. The length of time depends on the password requirements set by the administrator.

(Optional) Change your password

1. In the menu bar, select **Tools ▸ Security ▸ Change Password**.
2. In the **Change Password** dialog box, enter your old password.
3. Enter a new password, then enter the new password again to confirm it.
4. Click **OK**.

Note:

- If the password fields are not completed correctly, the **OK** button is inactive.
 - The  icon indicates that a field is not correctly completed. For more information, see “About the field error icon” on page 16.
-

Change Password ×

 New Password 1 must be not the same as Last name.

New Password 1 must contain at least 8 total characters.

New Password 1 must contain at least 1 alpha characters.

Old Password:

New Password:

Confirm Password:

 ①



① Field error icon

5. At the **Password has been changed successfully** prompt, click **OK**.

About failed login attempts and session timeout

Failed login attempts

Your user account will be suspended if you make six failed login attempts within 30 minutes. An Administrator must reactivate your account in order for you to continue using the account. (If you have Administrator privileges, for procedures on reactivating an account, see “Edit user accounts” on page 25.)

Session timeout

Your session will timeout if there is no activity after 30 minutes. You must log in again to continue using the software. An Administrator can also log in to your session after a timeout.

(Optional) Configure the system preferences

Configure general system preferences

1. In the menu bar, select **Tools ▶ Preferences**.
The **Preferences** dialog box is displayed.
2. Select the **General** tab.

3. To change the default home location for all files, browse to and select a destination folder, then click **Select Folder**.

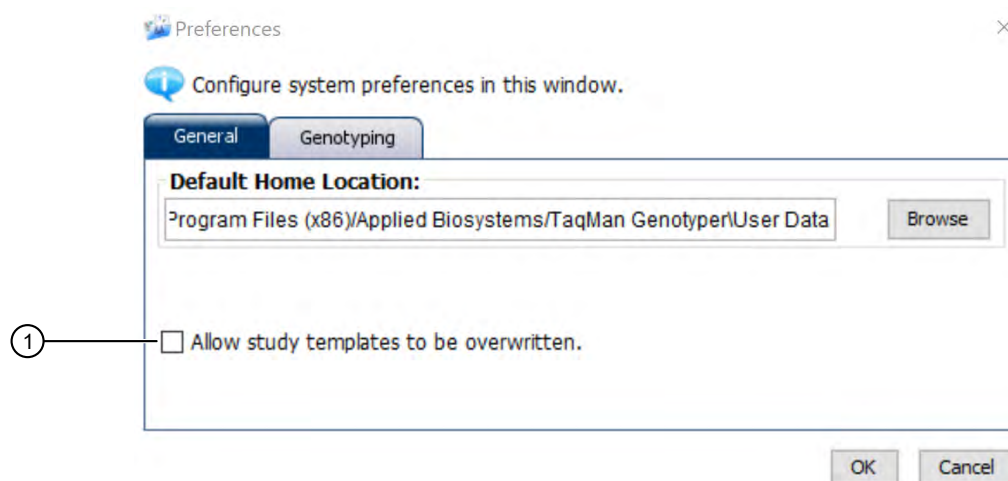
Note: The default home location is the location that the software uses to open and/or save study template, reference panel, assay information, SSI, and export files. The default home location is also the location the software uses to transfer studies in and out.

Note: For the *current* user session, your most recent open/save/transfer location overrides the default home location. That is, if you set the default home location in the **Preferences** dialog box, but later open/save/transfer from or to a new location, the software subsequently uses the new location during the current user session. After logging out and logging back in, the software reverts to the default home location that you set in the **General** tab.

4. Select or deselect the **Allow study templates to be overwritten** checkbox.
 - Selected—The software will overwrite an existing study template if you generate a new study template using the same name and save location as the existing study template. The software will prompt you to confirm the overwrite.
 - Deselected—The software will not overwrite an existing study template if you generate a new study template using the same name and save location as the existing study template. You will receive an error message stating that the operation cannot be performed. (If you want to replace the existing study template with your new study template, you must first manually delete the existing study template file.)

Note: If you have the Technician user role, you are not allowed to configure the **Allow study templates to be overwritten** checkbox.

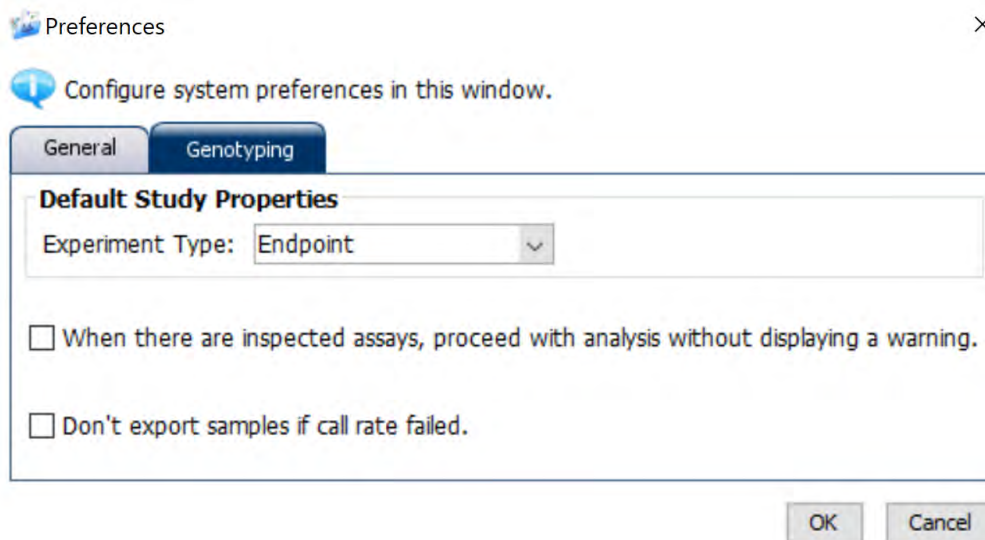
5. Click **OK** to save all changes.



- ① **Allow study templates to be overwritten** checkbox

Configure genotyping preferences

1. In the menu bar, select **Tools ▶ Preferences**.
The **Preferences** dialog box is displayed.
2. Select the **Genotyping** tab.



3. Select an experiment type from the **Experiment Type** dropdown list.
 - **Endpoint**
 - **Real-time**
 - **Endpoint + pre-PCR read**
4. Select or deselect the **When there are inspected assays, proceed with analysis without displaying a warning** checkbox.
 - Selected—The software will not display a warning prompt before you reanalyze inspected assays.
 - Deselected—The software will display a warning prompt before you reanalyze inspected assays. That is, if a user marked an assay as *Inspected* in the **Results** screen, then you later attempt to analyze the data, a warning prompt is displayed. The warning prompt states that the *Inspected* status will be cleared if the assay results change because of the analysis.
5. Select or deselect the **Don't export samples if call rate failed** checkbox.
 - Selected—The software will not export data for any samples that fail the sample call rate (that is, the samples do not meet the QC thresholds set by the user).
 - Deselected—The software will export data for all samples, including data for those samples that fail the sample call rate.

Note: If you have the Technician user role, you are not allowed to configure the **Don't export samples if call rate failed** checkbox.

6. Click **OK** to save all changes.

4

Create a study

■ Required user role	35
■ Workflow	36
■ Create a study and enter properties	36
■ Set the analysis settings	38
■ Import experiments	47

Required user role

You may not be able to perform all of the procedures in this chapter. The table below indicates the user roles that are allowed to perform each procedure in this chapter.

Note: For more information on user roles, see “User roles and tasks” on page 20.

Function	User role		
	Administrator	Scientist	Technician
Create a study (see page 36) Note: Technicians can create a study using the Create Study from Template function. See page 115.	✓	✓	—
Enter the study properties (see page 37)	✓	✓	✓
Set the analysis settings (see page 38)	✓	✓	—
Import experiments (see page 47)	✓	✓	✓

Workflow

“Create a study and enter properties” on page 36

“Create a study” on page 36



“Enter the study properties” on page 37



“Set the analysis settings” on page 38

“Set the call settings” on page 38



“Add control identifiers” on page 42



“Set the QC settings” on page 44



“Import experiments” on page 47

“Import experiments” on page 47



“View the imported data” on page 49

Create a study and enter properties

Create a study

In the toolbar on the **Home** screen, click  **Create Study**.

The **Study Properties** screen is displayed.

Creating studies from study templates

Note: You can also create a study from a study template ( **Create Study from Template**). See Chapter 8, “Create a study from a study template”.

IMPORTANT! If you have the Technician user role, you must create studies from a study template. You cannot create studies using the  button.

Enter the study properties

1. In the **Workflow Menu** pane, select **Setup ▶ Properties** to open the **Study Properties** screen. The **Study Properties** screen is the default view.

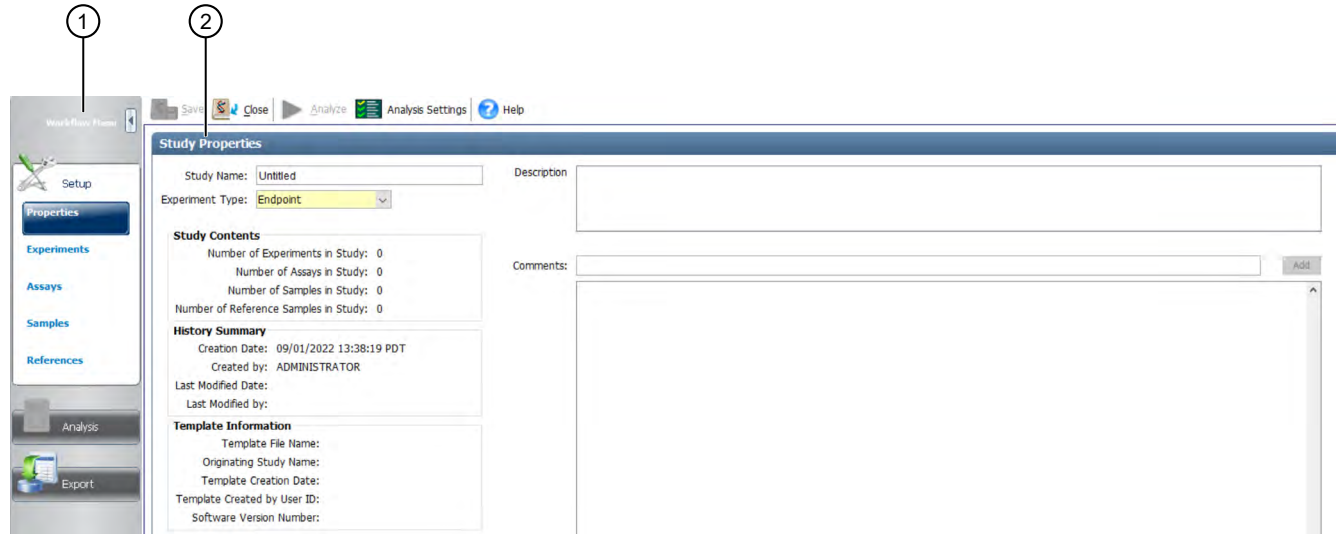


Figure 4 Properties screen

- ① Workflow Menu pane
- ② Study Properties screen

2. In the **Study Name** field, enter a study name.
3. In the **Experiment Type** dropdown list, select the genotyping experiment type.
 - **Endpoint**
 - **Real-time**
 - **Endpoint + pre-PCR read**

IMPORTANT! A study can support only one type of experiment. The experiment type can be changed after an experiment is imported. This allows for all of the experiments in the study to be the same type.

4. (Optional) Enter a description of the study.
5. (Optional) In the **Comments** field, enter comments about the study, then click **Add**.
The software records the comments, your user name, and the date/time you added the comments.

IMPORTANT! The **Comments** field allows you to enter detailed information about the study (for example, observations about the data, reasons why you made specific decisions, etc.). After you click Add, the comment is permanently recorded in the study (that is, the comment cannot be modified or removed) and the comment is included in any audit trails that are exported for the study (see “Export an audit trail” on page 107).

Note: You can enter comments at any time. You may prefer to enter comments after viewing and analyzing the data. See “(Optional) Enter comments for the study” on page 98.

Set the analysis settings

Set the analysis settings that you want the TaqMan™ Genotyper Software to use as the default when analyzing the study.

Note: The analysis settings apply only to the current study.

- Set the call settings (see page 38).
- Add control identifiers (see page 42).
- Set the QC settings (see page 44).

The following steps can be used to customize and save the analysis settings:

1. Set up a study with the applicable analysis settings (see “Create a study and enter properties” on page 36).
2. Generate a template from the study (LAT file; see “Generate a study template” on page 117) .
3. Create a new study from a template, selecting the new template that was generated (see “Create a study from a template and enter properties” on page 118).

The analysis settings from the template are applied to the new study.

Statistical implications

If you change the analysis settings, this may have statistical implications on data analysis. For more information, see “Impact of analysis settings” on page 143.

Set the call settings

About the call settings

The call settings determine the following items:

- The method the software uses to call data points.
- Whether or not reference samples or positive controls will be used to bias the genotype calls.
- The information to extract from the data files.

Set the call settings

1. In the toolbar, click  **Analysis Settings** to open the **Analysis Settings** dialog box.

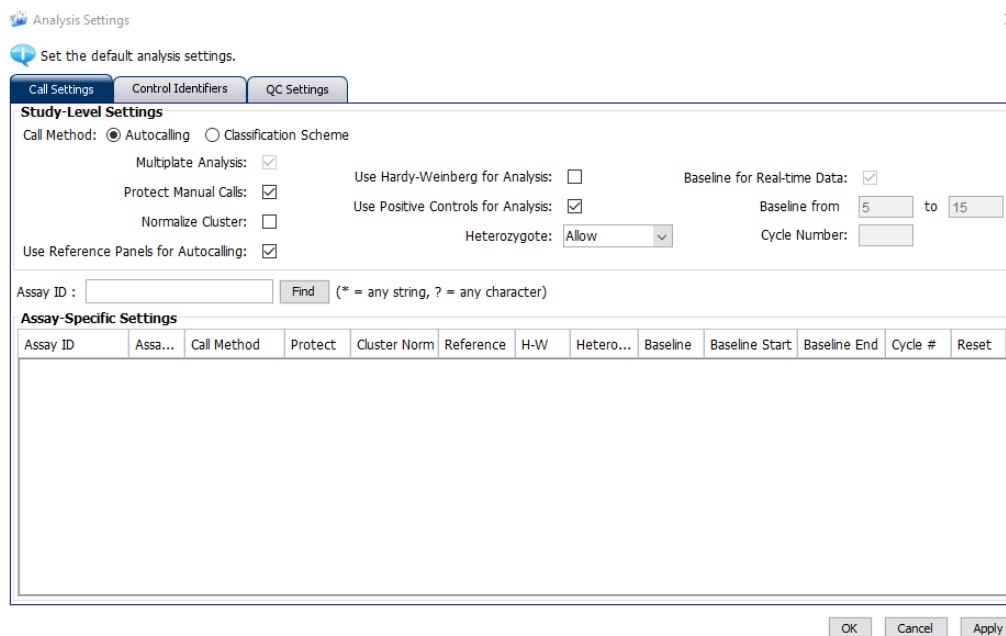


Figure 5 Analysis Settings dialog box, Call Settings tab

2. Select the **Call Settings** tab.
3. In the **Study-Level Settings** pane, accept the defaults, or select new values appropriate for your laboratory.
See “Call setting descriptions” on page 40.

Note: The Study-Level Settings apply to all assays in the study.

4. (Optional) Enter an assay ID in the **Assay ID** field, the click **Find** to find a specific assay within the study.
5. In the **Assay-Specific Settings** pane, accept the defaults, or select new values appropriate for your laboratory.
See “Call setting descriptions” on page 40.

Note: By default, the Assay-Specific Settings are the same as the Study-Level Settings. If you modify the Assay-Specific Settings, your changes apply only to the selected assay (and override the Study-Level Settings for the selected assay). If needed, click the **Reset** symbol to reset the assay’s settings to the Study-Level Settings.

6. Click **Apply** to save the changes.
7. Click **OK** to close the **Analysis Settings** dialog box.

Call setting descriptions

Settings		Description
Study-level	Assay-specific	
Call Method	Call Method	Select one of the following items: • Autocalling—the software algorithm is used to call the data points. • Classification Scheme—you define the cluster boundaries that are used to call the data points.
Multiplate Analysis	Multiplate Analysis	When selected, the software normalizes the data for all plates, enabling data comparison across plates.
Protect Manual Calls	Protect	When selected, the software protects all manual calls. That is, when the software analyzes the data, it will not modify any data points that have been manually called.
Normalize Cluster	Cluster Norm	<i>(Autocalling of OpenArray™ projects only)</i> When selected, the software normalizes the data for all plates per assay, enabling improved data comparison across plates. The normalization option is available as a global setting. If desired, you can individually enable or disable cluster normalization for specific assays by selecting the appropriate entries in the Normalize Cluster column of the settings table. IMPORTANT! The cluster normalization option assumes that data point distribution in your genotype clusters are approximately similar across the arrays or plates that you are normalizing. IMPORTANT! The cluster normalization feature is designed specifically to optimize the analysis of OpenArray™ experiments, where genotype clusters typically consist of a large number of samples. Before using cluster normalization, confirm that the clusters generated from your experiments consist of at least 20 samples in the largest cluster.
Use Reference Panels for Autocalling	Reference	<i>This option is enabled only if Autocalling is selected as the call method.</i> When selected, the software uses reference sample data (imported from a reference panel file) to bias the calls of Unknown data points. For more information on reference panel files, see “(Optional) Generate and import a reference panel file” on page 59.

(continued)

Settings		Description
Study-level	Assay-specific	
Use Hardy-Weinberg for Analysis	H-W	<i>This option is enabled only if Autocalling is selected as the call method.</i> When selected, the software uses the Hardy-Weinberg equilibrium statistics to bias the calls of data points. IMPORTANT! Using the Hardy-Weinberg equilibrium to influence calls can lead to incorrect genotypes. You should only enable this feature if your sample population was selected following Hardy-Weinberg assumptions. For more information, see “Hardy-Weinberg checkbox” on page 143.
Use Positive Controls for Analysis	NA	<i>This option is enabled only if Autocalling is selected as the call method.</i> When selected, the software uses positive control data to bias the calls of Unknown data points. Note: You cannot manually call positive controls. For more information on positive controls, see “Add control identifiers” on page 42.
Heterozygote	Heterozygotes	<i>This option is enabled only if Autocalling is selected as the call method.</i> Select one of the following from the drop-down list: • Allow—The autocaller will make heterozygote genotype calls. • Disallow—The autocaller will not make any heterozygote genotype calls. • Disallow in Males—For samples from males, the autocaller will not make any heterozygote genotype calls. Note: In order for the autocaller to perform the Disallow in Males function, your samples must be labeled as <i>male</i> or <i>female</i> in the Gender column of the Samples screen.

(continued)

Settings		Description
Study-level	Assay-specific	
Baseline for Real-time Data	Baseline	<i>This option is enabled only for the Real-time experiment types.</i> When selected, the software subtracts the baseline fluorescent signal at early PCR cycles from the final fluorescent signal. The final fluorescent signal is generated at the default or user-selected cycle number (see Cycle # below). For more information, see Appendix C, “Optimizing scatter plot data (for Real-time experiment types)”. Note: This option is disabled for Real-time experiment types if the study already contains wells that were imported using current settings.
Cycle Number	Cycle #	<i>This option is enabled only for the Real-time experiment types.</i> By default, the software uses the fluorescent signal extracted from the final cycle in the protocol to create genotyping scatter plots from Real-time experiment files. You can change the cycle number that the software uses. The cycle number you enter must be between cycle number 20 and cycle number 60. If you leave the Cycle Number field empty (blank), the software interprets that as the final cycle. For more information, see Appendix C, “Optimizing scatter plot data (for Real-time experiment types)”. If the Show Cycle slider in the scatter plot was adjusted, the cycle number is also updated in the Analysis Settings dialog box. It is updated for the assay in the Assay-Specific Settings pane. Note: This option is disabled for real-time experiment types if the study already contains wells that were imported using current settings.

Add control identifiers

About control identifiers

Control identifiers identify the samples that the software uses as controls when analyzing a study. You can identify samples as:

- **NTC** (no template control)—The well does not contain any template. The software automatically identifies any well in the study that has a sample ID of **NTC** as a no template control.

IMPORTANT! We strongly recommend that you run two NTC wells with every assay in a study.

- **Negative Controls**—The well does not contain known template. The well is set up to *not* display amplification signal. (For example, the well may contain a non-target template, include an inhibitor, etc.)

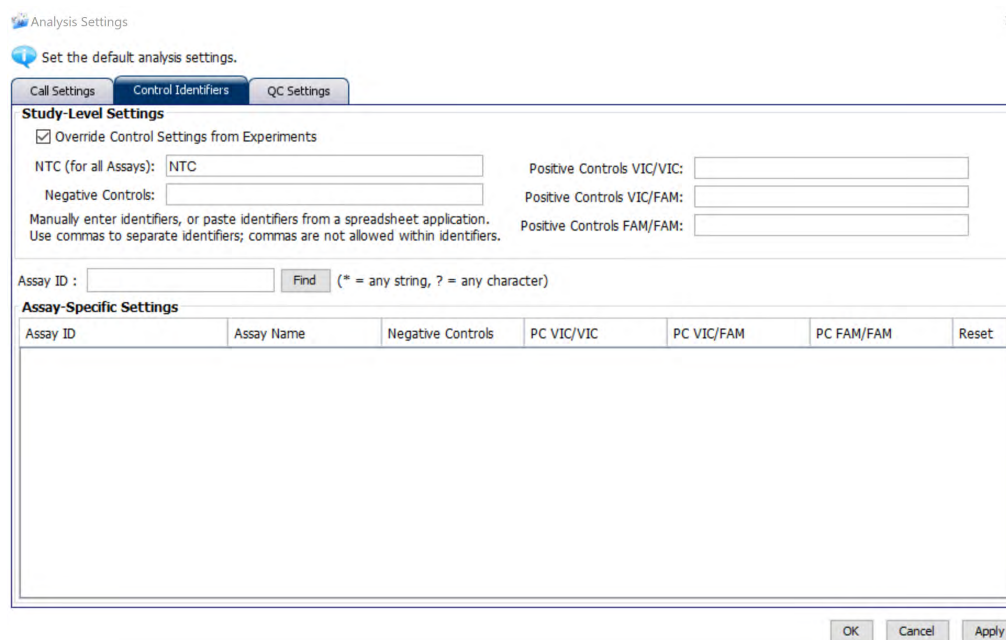
- **Positive Controls**—The well contains known template to generate a specific genotype call for an assay. You can specify positive controls for VIC™ dye/VIC™ dye, VIC™ dye/FAM™ dye, or FAM™ dye/FAM™ dye.

Positive controls are not called by the software and cannot be manually called. In the **Results** screen, each positive control is identified as the genotype that was assigned to it in the **Control Identifiers** tab. This allows the software to preferentially use the positive control data points to bias the calls of Unknown data points. We recommend that you carefully review the coordinates of positive control data points and omit these data points if they are not located in an expected position in the scatter plot.

Note: Positive controls are similar to reference samples in that the software can use both to bias the calls of Unknown data points. However, a positive control represents a well that physically contains known template and is included in one of the experiments in the current study. A reference sample can represent any well from any experiment in any study. For more information about reference samples, see “(Optional) Generate and import a reference panel file” on page 59.

Add control identifiers

1. In the toolbar, click  **Analysis Settings** to open the **Analysis Settings** dialog box.
2. Select the **Control Identifiers** tab.



Analysis Settings

Set the default analysis settings.

Call Settings Control Identifiers QC Settings

Study-Level Settings

☒ Override Control Settings from Experiments

NTC (for all Assays): Positive Controls VIC/VIC:

Negative Controls: Positive Controls VIC/FAM:

Manually enter identifiers, or paste identifiers from a spreadsheet application. Use commas to separate identifiers; commas are not allowed within identifiers. Positive Controls FAM/FAM:

Assay ID : Find (* = any string, ? = any character)

Assay-Specific Settings

Assay ID	Assay Name	Negative Controls	PC VIC/VIC	PC VIC/FAM	PC FAM/FAM	Reset

OK Cancel Apply

Figure 6 Analysis Settings dialog box, Control Identifiers tab

3. Select or deselect the **Override Control Settings from Experiments** checkbox.
 - **Selected**—The software uses the control identifiers that you set in the **Control Identifiers** tab (step 4 and step 6), and overrides any control identifiers (tasks) that may have been set in the original experiment file. If a sample ID does not match a control identifier, the software assumes the sample is an Unknown.
 - **Deselected**—The software uses the control identifiers (tasks) that were set in the original experiment file.
4. In the **Study-Level Settings** pane, enter the sample IDs to use as controls for all assays in the study. Use a comma to separate multiple control identifiers.

Note: The Study-Level Settings apply to all assays in the study.

5. (Optional) Enter an assay ID in the **Assay ID** field, then click **Find** to find a specific assay within the study.
6. In the **Assay-Specific Settings** pane, enter the sample IDs to use as controls for individual assays in the study. Use a comma to separate multiple control identifiers.

Note: By default, the Assay-Specific Settings are the same as the Study-Level Settings. If you modify the Assay-Specific Settings, your changes apply only to the selected assay (and override the Study-Level Settings for the selected assay). If needed, click the **Reset** symbol to reset the assay's settings to the Study-Level Settings.

7. Click **Apply** to save the changes.
8. Click **OK** to close the **Analysis Settings** dialog box.

Set the QC settings

About the QC settings

The QC settings are a collection of criteria that the software uses to analyze data in a study. You select the flags (criteria) that you want the software to use, and if applicable, enter a condition and threshold value appropriate for your laboratory.

There are two categories of criteria that the software uses to analyze data. The first category looks at concordance of data points, either to an expected call or to a replicate call. The second category looks at the data to meet a specified condition and threshold value. In either case, if the resulting data violates a criterion, a flag is generated.

By default, all flags are enabled in the software.

Set the QC settings

1. In the toolbar, click  **Analysis Settings** to open the **Analysis Settings** dialog box.
2. Select the **QC Settings** tab.
3. In the **Well-Level QC Flags** pane:
 - a. Select the flags that the software uses to analyze the study at the well level.

- b. For each flag, select condition and threshold values that are appropriate for your laboratory. See “Flag descriptions” on page 45.
4. In the **Study-Level QC Flags** pane:
 - a. Select the flags that the software uses to analyze all assays in the study.
 - b. For each flag, select condition and threshold values that are appropriate for your laboratory. See “Flag descriptions” on page 45.
5. Click **OK** to save the changes and close the **Analysis Settings** dialog box.

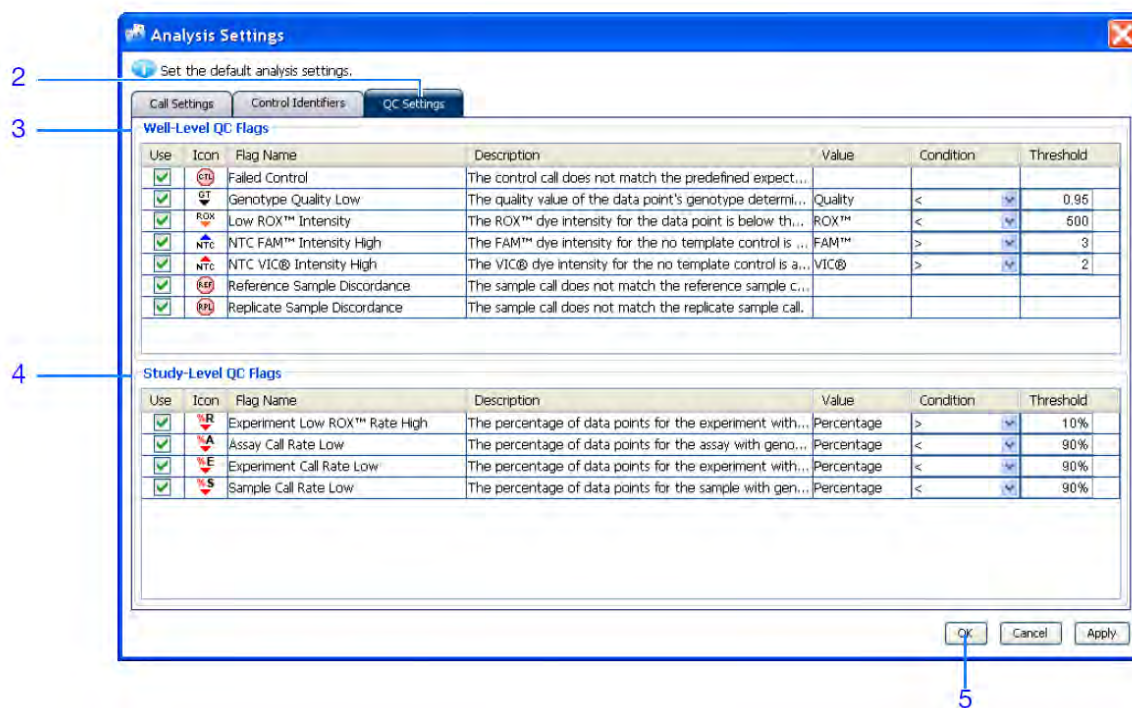


Figure 7 Analysis Settings dialog box, QC Settings tab (the numbers called out in the screen capture refer to the step numbers in this procedure)

Flag descriptions

Table 1 Well-level QC flags

Flag	Description
Failed Control	A Failed Control flag can be raised for any data point that is identified as a control: NTC, Negative Control, or Positive Control. If the user-assigned control identifier (or <i>task</i>) for a data point is inconsistent with the call that would be assigned by the software algorithm to an Unknown with the same FAM™ dye and VIC™ dye intensities, a flag is raised.

Table 1 Well-level QC flags (continued)

Flag	Description
Genotype Quality Low	A Genotype Quality Low flag can be raised for any data point that is identified or tasked as an Unknown. If the quality value assigned by the software algorithm for a data point is below the threshold, a flag will be raised.
Low ROX™ Intensity	A Low ROX™ Intensity flag can be raised for any data point. If the ROX™ dye intensity determined by the software for a data point is below the threshold, a flag will be raised.
NTC FAM™ Intensity High	An NTC FAM™ Intensity High flag can be raised for any data point that is identified or tasked as an NTC. If the FAM™ dye signal intensity for a data point tasked as NTC is greater than the threshold, a flag will be raised.
NTC VIC™ Intensity High	An NTC VIC™ Intensity High flag can be raised for any data point that is identified or tasked as an NTC. If the VIC™ dye signal intensity for a data point tasked as NTC is greater than the threshold, a flag will be raised.
Reference Sample Discordance	A Reference Sample Discordance flag can be raised for any data point that is identified or tasked as an Unknown. If the software algorithm-assigned genotype for a data point is discordant with the genotype of a reference sample data point that has exactly the same sample/assay identification, a flag will be raised.
Replicate Sample Discordance	A Replicate Sample Discordance flag can be raised for any data point that is identified or tasked as an Unknown. If the software algorithm-assigned genotype for a data point is discordant with the genotype of a replicate sample data point that has exactly the same sample/assay identification, a flag will be raised. A flag will be raised for all data points that have the sample/assay identification, because the software cannot know which data point has the correct genotype.

Table 2 Study-level QC flags

Flag	Description
Experiment Low ROX™ Rate High	An Experiment Low ROX™ Rate High flag can be raised for any experiment. If the percentage of data points in an experiment with a low ROX™ dye intensity flag is greater than the threshold, a flag will be raised.
Assay Call Rate Low	An Assay Call Rate Low flag can be raised for any assay. If the percentage of Unknown data points with a genotype call for an assay is less than the threshold, a flag will be raised.

Table 2 Study-level QC flags (continued)

Flag	Description
Experiment Call Rate Low	An Experiment Call Rate Low flag can be raised for any experiment. If the percentage of Unknown data points in an experiment with a genotype call is less than the threshold, a flag will be raised.
Sample Call Rate Low	A Sample Call Rate Low flag can be raised for any sample identified or tasked as an Unknown. If the percentage of assays with a genotype call for an Unknown sample is less than the threshold, a flag will be raised.

Import experiments

This section provides step-by-step procedures to complete the following tasks:

- Import experiments into the study (see page 47)
- View the imported data (see page 49)

Recommended number of experiments

The recommended number of experiment files that you can import into the TaqMan™ Genotyper Software depends on your real-time PCR system:

Real-time PCR system	Recommended number of experiment files
QuantStudio™ 12K Flex Real-Time PCR System with TaqMan™ OpenArray™ Plates	100
All other systems	400 ^[1]

^[1] This number applies to both 96-well and 384-well reaction plates.

Experiment criteria

IMPORTANT! The TaqMan™ Genotyper Software supports only those experiments that meet the specified criteria (see “Experiment criteria” on page 14).

Import experiments

1. In the **Workflow Menu** pane, select **Setup ► Experiments** to open the **Experiments** screen.
2. Click **Import**.
3. In the **Import** dialog box, browse to and select the experiment of interest.

To import more than one experiment at a time, press the Ctrl key or Shift key when you select the experiments. Be sure that all experiments meet the specified criteria (see “Experiment criteria” on page 14).

4. Click **Import**.

Note: The software automatically analyzes the study whenever you import one or more experiments.

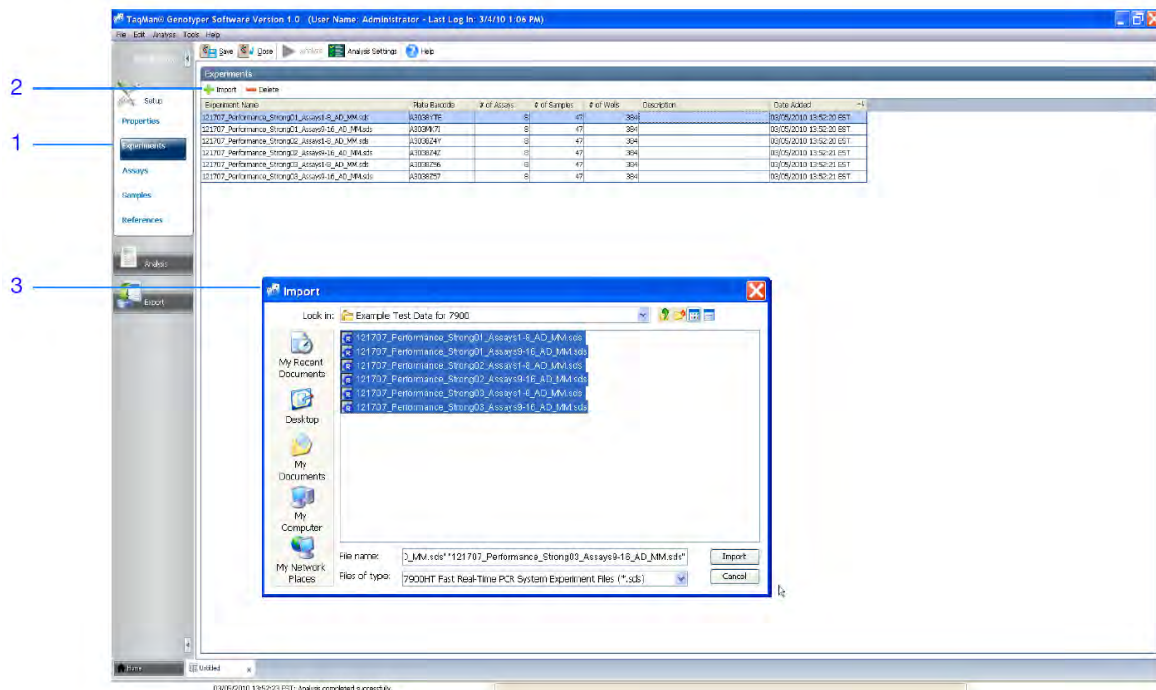


Figure 8 Experiments screen (the numbers called out in the screen capture refer to the step numbers in this procedure)

View the imported data

View the imported data in the **Experiments** screen to verify that the data is correct. For example, verify that the correct number of wells, assays, and samples were imported for each experiment file.

If an experiment file contained incorrect information, you can delete the experiment from the study. See “(Optional) Delete an experiment” on page 49.

Note: You cannot edit the experiment information in the TaqMan™ Genotyper Software.

(Optional) Delete an experiment

IMPORTANT! Deleting an experiment does not remove its associated assays or samples from the TaqMan™ Genotyper Software. If you want to remove the assays and/or samples, you must manually delete them. See “(Optional) Delete an assay” on page 55 and “(Optional) Delete a sample” on page 58.

1. In the **Experiments** screen, select the experiment to delete.
To delete more than one experiment at a time, press the Ctrl key or Shift key when you select the experiments.
2. Click **Delete**, then click **Yes** to confirm.

Note: The software automatically analyzes the study whenever you delete one or more experiments.



Import assay, sample, and reference sample information

■ Required user role	50
■ Workflow	51
■ (Optional) Import assay information	52
■ (Optional) Import sample information	55
■ (Optional) Generate and import a reference panel file	59

Required user role

You may not be able to perform all of the procedures in this chapter. The table below indicates the user roles that are allowed to perform each procedure in this chapter.

Note: For more information on user roles, see “User roles and tasks” on page 20.

Function	User role		
	Administrator	Scientist	Technician
(Optional) Import assay information (see page 52)	✓	✓	—
(Optional) Import sample information (see page 55)	✓	✓	✓
(Optional) Generate and import a reference panel file (see page 59)	✓	✓	—

Workflow



(Optional) Import assay information

This section provides step-by-step procedures to complete the following tasks:

- Import an assay information file (see page 52)
- View the imported assay information (see page 54)
- (Optional) Edit the assay information using the **Edit Assay** dialog box (see page 54)
- (Optional) Edit the assay information in the assay information file (see page 54)
- (Optional) Delete an assay (see page 55)

About assay information files

Download the assay information file for your TaqMan™ assay order from thermofisher.com/taqmanfiles. The assay information file contains reference information about your order and technical details of all assays in the shipment.

You can import an assay information file into the TaqMan™ Genotyper Software to add supplementary assay information to a study. There are three types of assay information files, but the TaqMan™ Genotyper Software supports only TXT and XML files, HTML files cannot be imported.

IMPORTANT! The assay information file must include an assay ID (in the **Assay ID** column) for each assay listed in the file. The software matches the assay IDs in the assay information file with the existing assay IDs in the study.

IMPORTANT! When you import an assay information file, information from the file populates the corresponding columns in the **Assays** screen. All data in the **Assays** screen are replaced for all assays that are identified in the assay information file. If the assay information file does not contain information for an assay, the existing data in the **Assays** screen is left as is.

For more information on assay information files, see Appendix A, “Assay information files”.

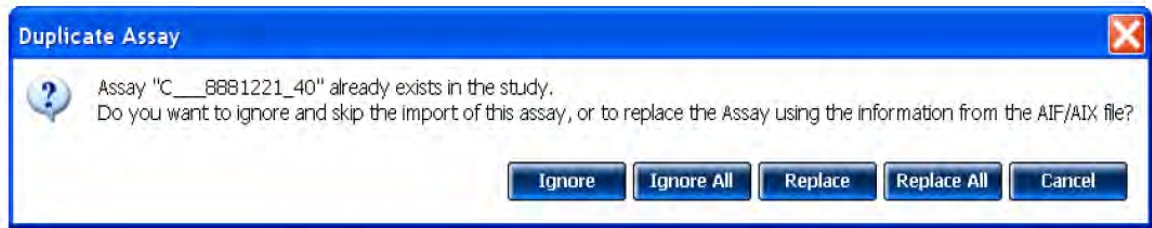
Field size limits in the Assays screen

There are size limits for all of the **Assays** screen fields that are populated by importing an assay information file. If the corresponding fields in the assay information file exceed these size limits, the software displays an error message at import and you will not be able to import the file.

Import an assay information file

1. In the **Workflow Menu** pane, select **Setup ▶ Assays** to open the **Assays** screen.
2. Click **Import**.
3. In the **Import** dialog box, browse to and select the assay information file of interest (TXT or XML), then click **Import**.

4. If the assay information file contains information for an assay ID that is already in the study, the software displays the following message:



5. Click the appropriate option:

Option	Description
Ignore or Ignore All	Ignores any new assay information from the assay information file and keeps the assay information that already exists in the study.
Replace or Replace All	Replaces the assay information that already exists in the study with any new assay information from the assay information file.
Cancel	Cancels the import.

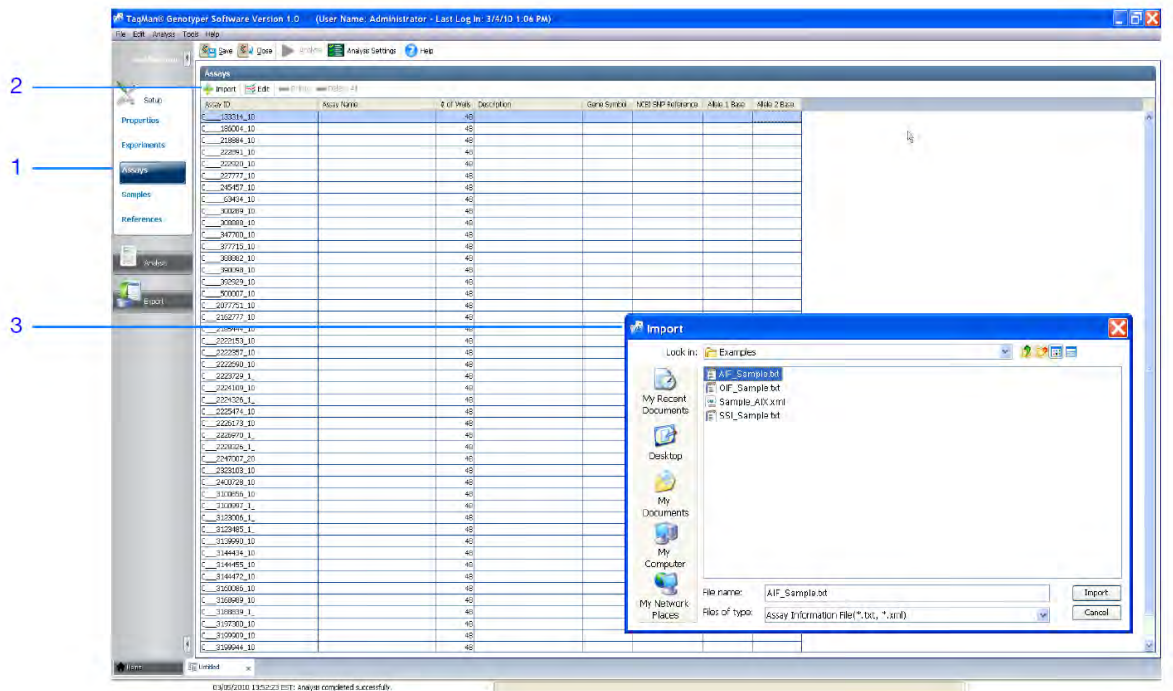


Figure 9 Assays screen (the numbers called out in the screen capture refer to the step numbers in this procedure)

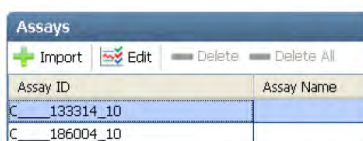
View the imported assay information

View the imported assay information in the **Assays** screen. If the assay information file contained incorrect information:

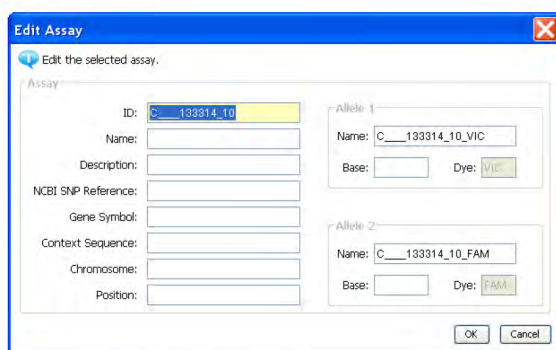
- You can use the **Edit Assay** dialog box to edit the assay information directly in the TaqMan™ Genotyper Software (see page 54).
- You can edit the assay information in the assay information file, then re-import the file into the TaqMan™ Genotyper Software (see page 54).
- You can import a different assay information file to replace the incorrect information.
- You may be able to delete individual assays from the TaqMan™ Genotyper Software (see page 55).

(Optional) Edit the assay using the Edit Assay dialog box

1. In the **Assays** screen, select the assay of interest, then click **Edit**.



2. In the **Edit Assay** dialog box, edit the assay information as needed, then click **OK**.



(Optional) Edit and re-import the assay information files

1. Edit the assay information file (see page 122).
2. Import the assay information file into the TaqMan™ Genotyper Software (see page 52).

(Optional) Delete an assay

IMPORTANT! You cannot delete an assay if there are any wells associated with the assay. All experimental data must be deleted for the assay before the assay can be deleted from a study.

1. In the **Assays** screen, select the assay to delete.
To delete more than one assay at a time, press the Ctrl key or Shift key when you select the assays.
2. Click **Delete**, then click **Yes** to confirm.

(Optional) Import sample information

This section provides step-by-step procedures to complete the following tasks:

- Import an SSI file (see page 56)
- View the imported sample information (see page 57)
- (Optional) Edit the sample information using the **Edit Sample** dialog box (see page 58)
- (Optional) Edit the sample information in the SSI file (see page 58)
- (Optional) Delete a sample (see page 58)

Overview of SSI files

A Supplementary Sample Information (SSI) file is a user-created text (TXT) file. The SSI file contains detailed supplementary information about your samples (for example, gender and population). Import an SSI file into the TaqMan™ Genotyper Software to add the supplementary sample information to a study.

IMPORTANT! The SSI file must include a sample ID (in the **Sample ID** column) for each sample listed in the SSI file. The software matches the sample IDs in the SSI file with the existing sample IDs in the study.

IMPORTANT! When you import an SSI file, information from the SSI file populates the corresponding columns in the **Samples** screen. All data in the **Samples** screen are replaced for all samples that are identified in the SSI file. If the SSI file does not contain information for a sample, the existing data in the **Samples** screen is left as is.

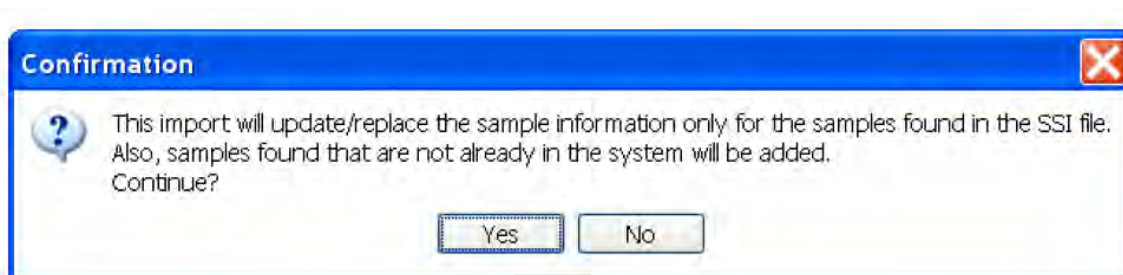
For more information on SSI files, see Appendix B, “Supplementary sample information (SSI) files”.

Field size limits in the Samples screen

There are size limits for all of the **Samples** screen fields that are populated by importing an SSI file. If the corresponding fields in the SSI file exceed these size limits, the software displays an error message at import and you will not be able to import the file.

Import an SSI file

1. In the **Workflow Menu** pane, select **Setup ▶ Samples** to open the **Samples** screen.
2. Click **Import**.
3. Perform one of the following actions at the confirmation prompt.
 - Click **Yes** to continue.
 - Click **No** if you do *not* want to replace the existing sample information.
Clicking **No** cancels the import.



4. In the **Import** dialog box, browse to and select the SSI file of interest, then click **Import**.

Note: The software automatically analyzes the study whenever you import an SSI file. If the SSI file lists any assays in the **Non-Consent Assay IDs** column, the software excludes these assays from the analysis.

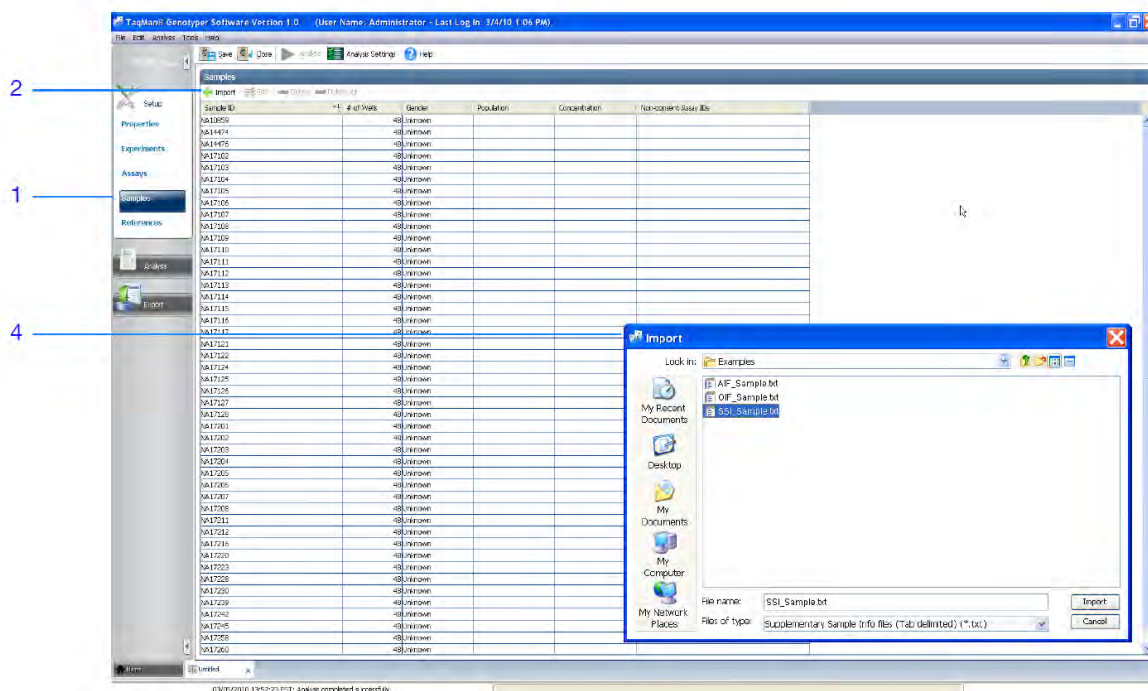


Figure 10 Samples screen (the numbers called out in the screen capture refer to the step numbers in this procedure)

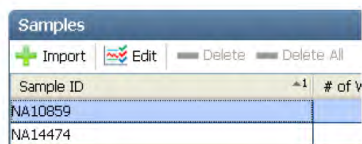
View the imported sample information

View the imported sample information in the **Samples** screen. If the SSI file contained incorrect information:

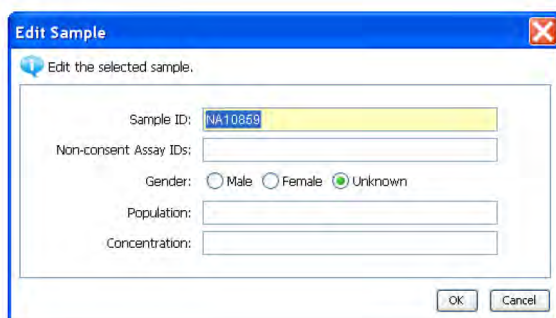
- You can use the **Edit Sample** dialog box to edit the sample information directly in the TaqMan™ Genotyper Software (see page 58).
- You can edit the sample information in the SSI file, then re-import the SSI file into the TaqMan™ Genotyper Software (see page 58).
- You can import another SSI file to replace the incorrect information.
- You may be able to delete individual samples from the TaqMan™ Genotyper Software (see page 58).

(Optional) Edit the sample using the Edit Sample dialog box

1. On the **Samples** screen, select the sample of interest, then click **Edit**.



2. In the **Edit Sample** dialog box, edit the sample information as needed, then click **OK**.



(Optional) Edit and re-import the SSI file

1. Open the SSI file of interest, using a spreadsheet application, for example, Microsoft™ Excel™ software.
2. Edit the sample information in the SSI file as needed.
You can add or change information in any of the existing cells, but be sure to follow all parameters (see “Create an SSI file” on page 124).
3. Save the SSI file as a TXT file, then close the file.
4. Import the SSI file into the TaqMan™ Genotyper Software (see “(Optional) Import sample information” on page 55).

(Optional) Delete a sample

IMPORTANT! You cannot delete a sample if there are any wells associated with the sample. All experimental data must be deleted for the sample before the sample can be deleted from a study.

1. In the **Samples** screen, select the sample to delete.
To delete more than one sample at a time, press the Ctrl key or Shift key when you select the samples.
2. Click **Delete**, then click **Yes** to confirm.

Note: The software automatically analyzes the study whenever you delete one or more samples.

(Optional) Generate and import a reference panel file

This section provides step-by-step procedures to complete the following tasks:

- Generate a reference panel file (see [page 59](#))
- Import the reference panel file (see [page 62](#))
- (Optional) Delete a reference panel file (see [page 63](#))

Overview of reference panel files and reference samples

A *reference panel file* is a user-generated TaqMan™ Genotyper Software file that contains reference samples.

Reference samples are data points in an experiment that you select to be representative of the clusters for an individual assay. You can identify, collect, and store reference samples for multiple assays in a reference panel file for use in current or future studies. After you import a reference panel file into a study, the software uses the reference samples to bias the calls of Unknown data points. The reference samples cannot be modified and are not calculated in the call rates.

Note: Reference samples are similar to positive controls in that the software can use both to bias the calls of Unknown data points. However, a positive control represents a well that physically contains known template and is included in one of the experiments in the current study. A reference sample can represent any well from any experiment in any study. For more information on reference samples, see (“(Optional) Generate and import a reference panel file” on [page 59](#)).

Generate a reference panel file

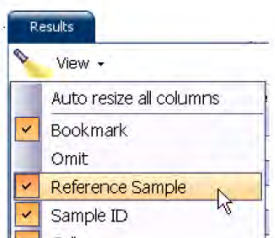
Before you can import a reference panel file into a study, you must first generate the file in the TaqMan™ Genotyper Software.

1. In the TaqMan™ Genotyper Software, open a study that contains the data points that you want to use as reference samples.

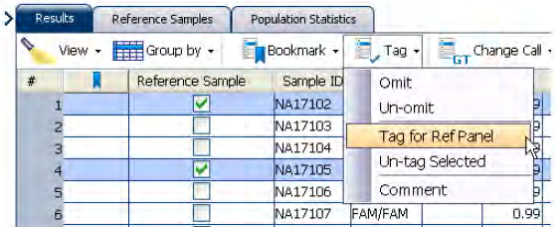
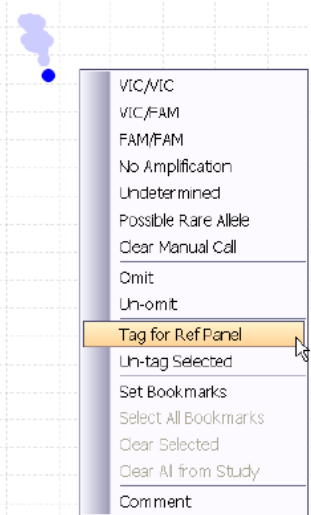
Note: You must select the data points at the assay level. The software doesn't allow you to select data points at the study level (that is, if you select a data point for one assay, it is not selected for all assays in the study.)

2. In the **Workflow Menu** pane, select **Analysis ▶ Results** to open the **Results** screen.
3. Select the **Results** tab.

4. From **View** drop-down list, select **Reference Sample** to display the **Reference Sample** column in the **Results** table.



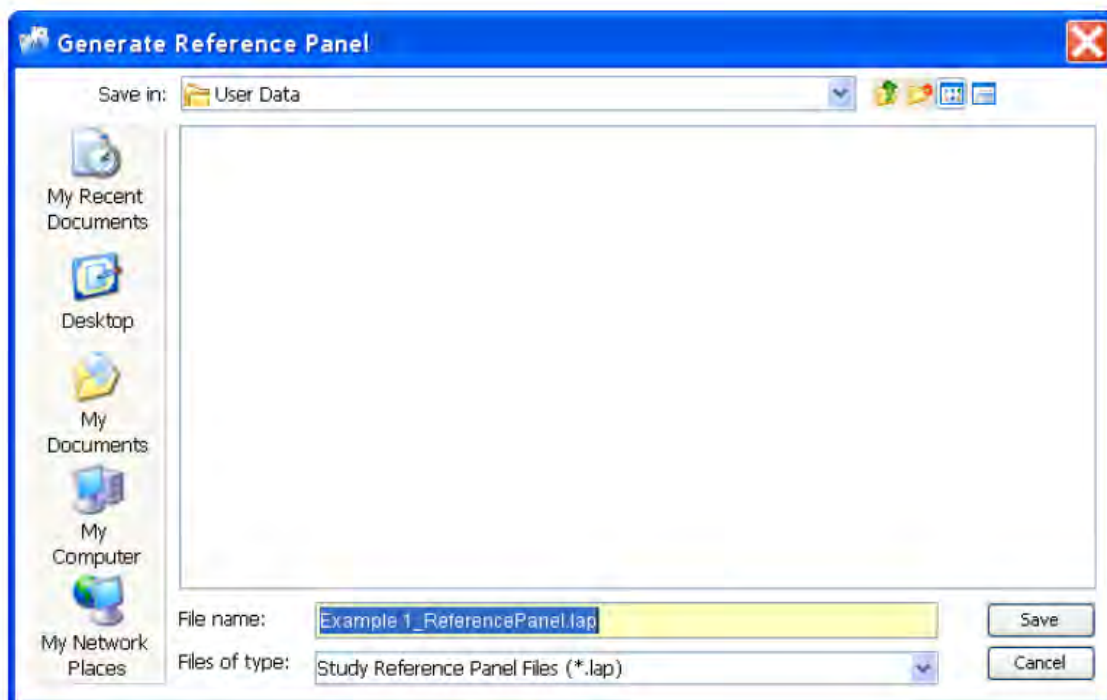
5. In the **Results** table or the scatter plot, select the samples that you want to use as reference samples.

Option	Action
Results table	1. Select a sample in the Results table. To select more than one sample at a time, press the Ctrl key or Shift key. 2. From the Tag drop-down list, select Tag for Ref Panel.  Note: To remove a reference sample tag from a sample, you can either: 1) Deselect the checkbox in the Reference Sample column; or 2) Select the sample, then select Un-tag Selected from the Tag drop-down list.
Scatter plot	1. Select a sample by drawing a box around the data point in the scatter plot. 2. Right-click in the scatter plot, then select Tag for Ref Panel from the list.  Note: To remove a reference sample tag from a sample, draw a box around the data point, right-click in the scatter plot, then select Un-tag Selected from the list.

In the **Reference Sample** column in the **Results** table, checkmarks appear next to the selected samples.

6. Save the study.
7. From the menu bar, select **File ▶ Generate Reference Panel**.

8. In the **Generate Reference Panel** dialog box, browse to and select a save location, enter a name for the reference panel file (or accept the default name), then click **Save**.



Import the reference panel file

1. In the TaqMan™ Genotyper Software, open a study that you want to import the reference panel file into.
2. In the **Workflow Menu** pane, select **Setup ▶ References** to open the **References** screen.
3. Click **Import**.
4. In the **Import** dialog box, browse to and select the reference panel file of interest, then click **Import**.

Note: The software automatically analyzes the study whenever you import a reference panel file.

5. To view the imported reference samples for each assay:
 - a. From the **Workflow Menu** pane, select **Analysis ▶ Results**.

- b. In the **Results** screen, select the **Reference Samples** tab. For more information, see “View the data in the References Samples table” on page 73.

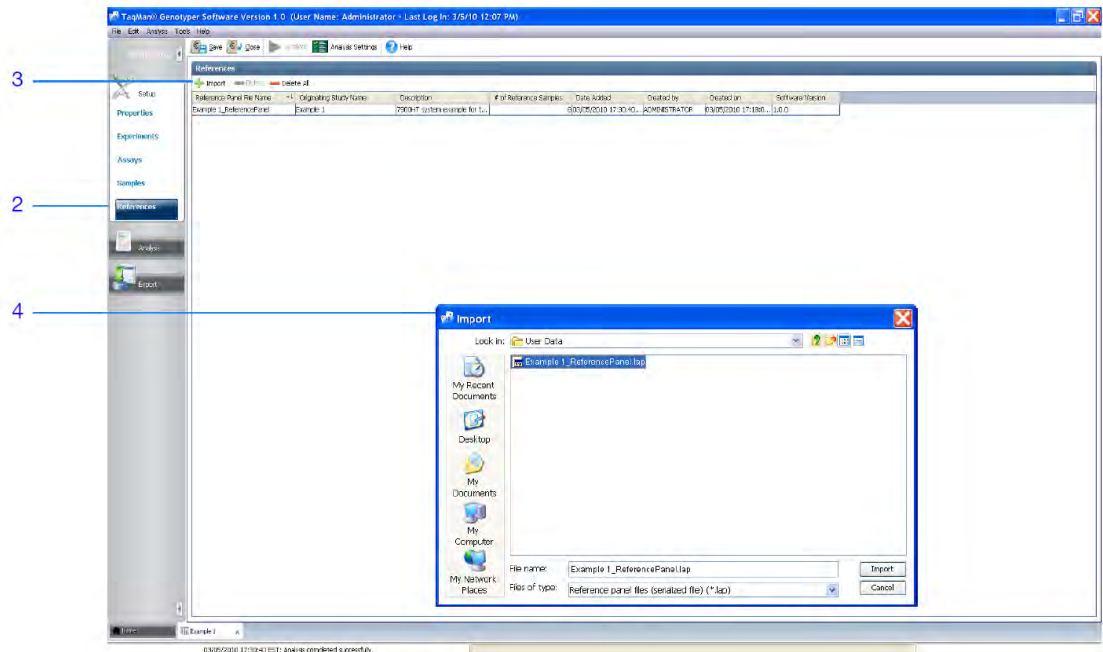


Figure 11 References screen (the numbers called out in the screen capture refer to the step numbers in this procedure)

(Optional) Delete a reference panel file

1. In the **References** screen, select the reference panel file to delete.
To delete more than one file at a time, press the Ctrl key or Shift key when you select the files.
2. Click **Delete**, then click **Yes** to confirm.

Note: The software automatically analyzes the study whenever you delete a reference panel file.

6

View and analyze the data

■ Required user role	64
■ Workflow	65
■ Analyze the data using Autocalling	66
■ Analyze the data using Classification Schemes	75
■ View the data in the Quality Control screen	81
■ (Optional) Enter comments for the study	98

Required user role

You may not be able to perform all of the procedures in this chapter. The table below indicates the user roles that are allowed to perform each procedure in this chapter.

Note: For more information on user roles, see “User roles and tasks” on page 20.

Function	User role		
	Administrator	Scientist	Technician
Analyze the data using Autocalling (see page 66)	✓	✓	—
Analyze the data using Classification Schemes (see page 75)	✓	✓	—
View the data in the Quality Control screen (see page 81)	✓	✓	✓
(Optional) Enter comments for the study (see page 98)	✓	✓	✓

Workflow

“Analyze the data using Autocalling” on page 66

“Select the call method” on page 66



“View the data in the scatter plot” on page 66



“Review the calls and perform manual calling” on page 68



“View and modify the data in the Results table” on page 70



“View the data in the References Samples table” on page 73



“View the data in the Population Statistics table” on page 74



“Save the study” on page 75



“Analyze the data using Classification Schemes” on page 75

“Select the call method” on page 75



“View the data in the scatter plot” on page 76



“Modify the classification boundaries” on page 78



“Save the study” on page 81



“View the data in the Quality Control screen” on page 81

“View the data in the Flag Summary tab” on page 82



“View the data in the Assays tab” on page 85



“View the data in the Experiments tab” on page 88



“View the data in the Samples tab” on page 95



“(Optional) Enter comments for the study” on page 98

Analyze the data using Autocalling

This section provides step-by-step procedures for analyzing the study data when you select **Autocalling** as the call method.

- Select the call method (see page 66)
- View data in the scatter plot (see page 66)
- Review the genotype calls and (if needed) perform manual calling (see page 68)
- View and modify data in the **Results** table (see page 70)
- View data in the **Reference Samples** table (see page 73)
- View data in the **Population Statistics** table (see page 74)
- Save the study (see page 75)

Note: If you are using **Classification Scheme** as the call method, see page 75.

Select the call method

If your default call method is not set to **Autocalling**:

1. In the **Workflow Menu** pane, select **Analysis ▶ Results**.
2. In the toolbar, click  **Analysis Settings**.
3. In the **Analysis Settings** dialog box, select the **Call Settings** tab.
4. In the **Study-Level Settings** pane, select **Autocalling** as the call method, then click **OK**.

Note: The software automatically analyzes the study whenever you edit the analysis settings.

View the data in the scatter plot

1. In the **Workflow Menu** pane, select **Analysis ▶ Results**.
2. In the **Assays** table, select the assay of interest.
The software displays the data points for the selected assay in the scatter plot and in the **Results** table.

3. In the **Results** table, select a sample or multiple samples.

Use control + click or shift + click to select multiple samples.

The data points associated with the selected samples are displayed in color. The non-selected data points are displayed in gray.

4. In the scatter plot, use the **View** drop-down list to select the items to display in the plot.

- **Data Points**
- **References** (reference samples)
- **Omitted Wells**
- **Legend**
- **Trace** (real-time experiments only)

IMPORTANT! The option to view the trace is not available for studies created in earlier versions of the software. Create a new study in TaqMan™ Genotyper Software v1.7 and reimport the results files in order to view the trace. See Chapter 4, “Create a study”.

5. Use the **Show Cycle** slider to adjust the number of cycles, then click  **Analyze**.

The data points are displayed in a faded color until the data are reanalyzed.

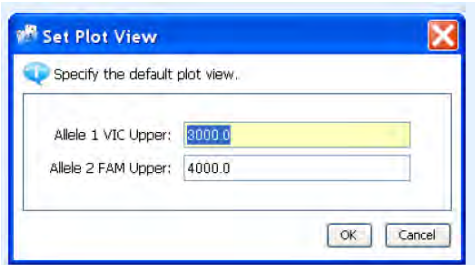
The default number of cycles is from the data files. If the data files contain different numbers of cycles, the default is the lowest number of cycles.

The data are reanalyzed for the number of cycles that is selected.

6. Use the scatter plot tools as needed.

Tool	Description
 (Select Data Points)	Select data points in the plot. Click and drag a box around an individual data point or a group of data points. When you select data points in the scatter plot, the corresponding samples are selected in the Results table.
 (Pan)	Reposition the plot.
 (Zoom In)	Enlarge an area of the plot to view. Click  , then draw a box around the data points you want to view more closely. To zoom back out, click  (Zoom Out to Show ALL Data Points) (described below).
 (Zoom Out to Show ALL Data Points)	Show all data points in the plot.
 (Select All)	Select all data points in the plot.

(continued)

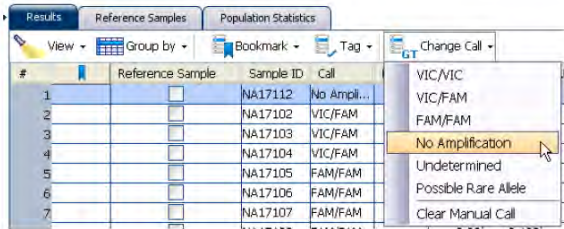
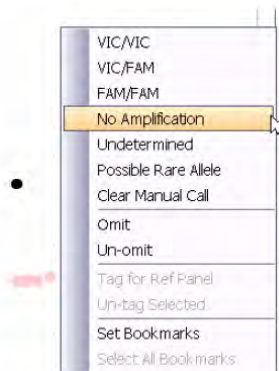
Tool	Description
 (Set Plot View)	Change the ranges for the scatter plot: - Click  (Set Plot View). - In the Set Plot View dialog box, enter a value for the: - Allele 1 upper boundary (VIC™ dye) - Allele 2 upper boundary (FAM™ dye) - Click OK.  Note: In order to display all data points, the software may override the maximum range that you set.
 (Save as Image File)	Save the plot as an image file: JPG, PDF, PNG, or SVG.
< or >	Expand or collapse the plot.

Review the calls and perform manual calling

- In the scatter plot, select **View Legend** from the **View** drop-down list. The legend appears at the bottom of the scatter plot.



2. Review the genotype calls.
3. If any of the calls are incorrect, perform manual calling in the **Results** table or the scatter plot:

Option	Action
Results table	1. Select the sample in the Results table. 2. From Change Call drop-down list, select the appropriate call.  3. Click  Analyze to view the new results. Note: To remove a manual call, select the sample, then select Clear Manual Call from the Change Call drop-down list.
Scatter plot	1. Click and drag a box around the data point. 2. Right-click in the scatter plot, then select the appropriate call from the list.  3. Click  Analyze to view the new results. Note: To remove the manual call, draw a box around the data point, right-click in the scatter plot, then select Clear Manual Call from the list.

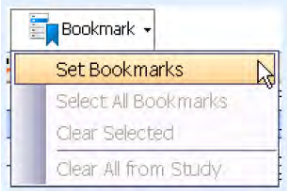
For all manually called wells/data points, in the **Results** table:

- Checkmarks appear in the **Manual** column
- N/A appears in the **Quality** column

#	Reference Sample	Sample ID	Call	Manual	Quality	VI
1	☐	NA17112	VIC/FAM	☒	N/A	
2	☐	NA17102	VIC/FAM		0.99	

View and modify the data in the Results table

1. Click the **Results** tab.
The **Results** tab is the default view.
2. From the **View** drop-down list for the **Results** table, select all items so that all columns are displayed in the **Results** table.
3. Use the **Results** table tools as needed:

Tool	Description
Mouse/cursor	Select wells. To select: • An individual well, select the well in the Results table. • More than one well at a time, press the Ctrl key or Shift key when you select the wells in the Results table. • All wells at the same time, select any well, then press Ctrl+A. When you select wells in the Results table, the corresponding data points are selected in the scatter plot. All of the other data points are displayed in gray.
 Group by drop-down list	Select how to group the samples in the Results table. For example, if you select Experiment Name, the samples are grouped according the experiment that they belong to. To collapse or expand the groups, select Collapse All or Expand All in the Group by drop-down list, or click the +/- icon in the Results table next to each group.
 Bookmark drop-down list	Set a bookmark for a well, clear a bookmark from a well, or select all bookmarks in the study.  The bookmarks persist in the Results and Quality Control screens, so you can easily find bookmarked wells.

(continued)

Tool	Description
 Tag drop-down list	Omit/un-omit a well from the analysis. After you omit or un-omit a well, click  Analyze to reanalyze the study. For omitted wells, the software: - Does not display calls or tasks in the Results table (the Call and Task columns are empty/blank). - Does not include the omitted wells in the analysis. For un-omitted wells, the software: - Reassigns the tasks based on the settings in the Analysis Settings dialog box (Control Identifiers tab). (For control wells) Assigns the call as <i>N/A</i>. (For non-control wells) Assigns the call as <i>Undetermined</i>.
	Tag/Un-tag a well as a reference sample (for generating a reference panel file). For more information on reference samples, see “(Optional) Generate and import a reference panel file” on page 59.
	Enter comments about a well.
 Change Call drop-down list	Perform manual calling. See “Review the calls and perform manual calling” on page 68.
< or >	Expand or collapse the Results table.

4. Use the **Results** table columns as needed:

Column	Description
 (Bookmark)	Determine whether or not the well has been bookmarked. See the Bookmark drop-down list in step 3.
Omit	Omit/Un-omit a well from the analysis. See the Tag drop-down list in step 3.
Reference Sample	Tag/Un-tag a well as a reference sample (for generating a reference panel file). For more information on reference samples, see “(Optional) Generate and import a reference panel file” on page 59.
Sample ID	View the ID (a unique name or number) of the sample.
Call	View the genotype call assigned to the well.
Manual	Determine whether or not the sample has been manually called. See “Review the calls and perform manual calling” on page 68.

(continued)

Column	Description
Quality	View the estimated quality value of the call made by the autocaller algorithm. The algorithm outputs a quality value for each data point with the following properties: - The quality value is a number between 0 and 1. - The quality value is always 0 for <i>Invalid</i> data points. - The quality value is always 1 for <i>No Amplification</i> data points. - For FAM™ dye/FAM™ dye, FAM™ dye/VIC™ dye, and VIC™ dye/VIC™ dye calls, the Quality Value is a higher value for calls more likely to be correct and a lower value for calls more likely to be incorrect. The quality value is <i>N/A</i> if a well is manually called, is a control, or is called using the Classification Scheme call method.
VIC™	View the normalized fluorescent value of the VIC™ dye. QuantStudio™ 12K Flex Real-Time PCR System with TaqMan™ OpenArray™ Plates fluorescent values are not normalized by the ROX™ passive reference dye.
FAM™	View the normalized fluorescent value of the FAM™ dye. QuantStudio™ 12K Flex Real-Time PCR System with TaqMan™ OpenArray™ Plates fluorescent values are not normalized by the ROX™ passive reference dye.
ROX™	View the fluorescent value of the ROX™ dye. FAM™ dye and VIC™ dye fluorescent values are normalized by the ROX™ passive reference dye. QuantStudio™ 12K Flex Real-Time PCR System with TaqMan™ OpenArray™ Plates fluorescent values are not normalized by the ROX™ passive reference dye. Note: ROX™ dye is in the TaqMan™ master mix used in TaqMan™ assays.
Task	View the task assigned to the well. A task is the function that a sample performs: - Unknown - No template control (NTC) (control identifier) - Positive control (control identifier) for VIC™ dye/VIC™ dye, FAM™ dye/FAM™ dye, or VIC™ dye/FAM™ dye - Negative control (control identifier) For more information on control identifiers, see “About control identifiers” on page 42.
Gender	View the gender assigned to the sample: - Unknown - Female - Male If no gender is assigned to a sample, the Gender column displays <i>Unknown</i> for that sample.

(continued)

Column	Description
Population	View the population assigned to the sample. If no population is assigned to a sample, the Population column is empty (blank) for that sample.
Well	View the location of the well in the reaction plate. For example, P18 indicates that the sample is located in row P, column 18.
Experiment Name	View the name of the experiment file that the well belongs to.
Plate Barcode	View the name or barcode of the reaction plate in which the experiment was run.
Comments	View any comments made by a user about the well. To enter comments, use the Tag drop-down list (see step 3).

5. (Optional) Click  **Analyze** to view the new results.

View the data in the References Samples table

- Click the **References Samples** tab.
- Use the table tools as needed:

Tool	Description
 View drop-down list	Select the columns to display in the Reference Samples table.
 Group by drop-down list	Select how to group the samples in the Reference Samples table. For example, if you select Experiment Name , the samples are grouped according to the experiment that they belong to. To collapse or expand the groups, select Collapse All or Expand All in the Group by drop-down list, or click the +/- icon in the Reference Samples table next to each group.
 Tag drop-down list	Tag/Un-tag a well as a reference sample (for generating a reference panel file). For more information on reference samples, see “(Optional) Generate and import a reference panel file” on page 59.
< or >	Expand or collapse the Reference Samples table.

- Use the table columns as needed:

Column	Description
Sample ID	View the ID (a unique name or number) of the sample.
Reference Sample	Tag/Un-tag a well as a reference sample (for generating a reference panel file). For more information on reference samples, see “(Optional) Generate and import a reference panel file” on page 59.
Call	View the genotype call assigned to the well.
VIC™	View the normalized fluorescence value of the VIC™ dye.
FAM™	View the normalized fluorescence value of the FAM™ dye

(continued)

Column	Description
Well	View the location of the well in the reaction plate. For example, P18 indicates that the sample is located in row P, column 18.
Experiment Name	View the name of the experiment file that the well belongs to.
Plate Barcode	View the name or barcode of the reaction plate in which the experiment was run.
Reference Panel File Name	View the name of the file that contains the reference panel.
Originating Study Name	View the name of the study that the reference panel file was created from.

View the data in the Population Statistics table

1. Click the **Population Statistics** tab.
2. Use the table columns as needed:

Column	Description
Population	View the population name(s) assigned to the samples in the study. By default, the population of Unknown samples is named <i>All</i> . You can edit the population of some or all of the samples in a study using the Edit Sample dialog box (see “(Optional) Edit the sample using the Edit Sample dialog box” on page 58). Each population name that you assign appears in the Population Statistics tab.
Allele 1 Freq	View the frequency of allele 1 determined for each population in the study.
Allele 2 Freq	View the frequency of allele 2 determined for each population in the study.
1/1 Freq	View the frequency of genotype 1/1 determined for each population in the study.
1/2 Freq	View the frequency of genotype 1/2 determined for each population in the study.
2/2 Freq	View the frequency of genotype 2/2 determined for each population in the study.
Chi-Squared	View the Chi-Squared value calculated for each population in the study. The calculated Chi-Squared value is used to determine if the experimental data is in Hardy-Weinberg equilibrium based on the observed and expected number of genotype calls, assuming 1 degree of freedom.
P-Value	View the P-value calculated for each population in the study. The calculated P-value is the probability of the differences in observed and expected genotype calls accounted for by chance alone. Note: Applied Biosystems™ recommends that you review Hardy-Weinberg equilibrium fundamentals for application of this P-value.

Save the study

1. (Optional) Click  **Analyze** to view the new results.
2. (Optional) Select the **Inspected** checkbox next to the assay. The software marks the assay as inspected.

Note: If you do not reanalyze the study, the Inspected status persists between users and between TaqMan™ Genotyper Software applications. If you do reanalyze the study, the Inspected status will be cleared if the assay results change because of the analysis.

3. Click  **Save**.

Analyze the data using Classification Schemes

This section provides step-by-step procedures for analyzing the study data when you select **Classification Schemes** as the call method.

- Select the call method (see page 75)
- View data in the scatter plot (see page 76)
- Modify the classification boundaries (see page 78)
- Save the study (see page 81)

Note: If you are using **Autocalling** as the call method, see page 66.

About classification schemes and genotype calls

The classification schemes broadly divide the scatter plot surface into regions where samples from each genotype are expected. If you select the correct boundaries, then most data points are expected to lie in the center of their appropriate genotype region. Some data points that are on the edge of these regions may be misclassified by chance because of the positioning of the boundaries.

You should carefully review data points that are on the edge of each genotype region. Advanced users may be able to determine the true genotype of these data points. Alternatively, you may choose to label these data points as outliers, or omit them from the analysis.

Select the call method

If your default call method is not set to **Classification Scheme**:

1. In the **Workflow Menu** pane, select **Analysis ▶ Results**.
2. In the toolbar, click  **Analysis Settings**.
3. In the **Analysis Settings** dialog box, select the **Call Settings** tab.

4. In the **Study-Level Settings** pane, select **Classification Scheme** as the call method, then click **OK**.

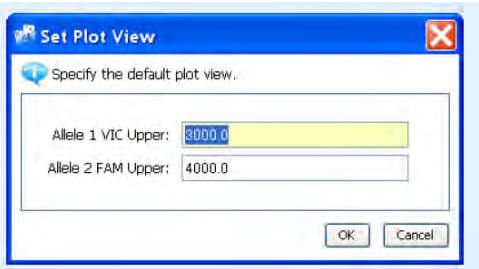
Note: The software automatically analyzes the study whenever you edit the analysis settings.

View the data in the scatter plot

1. In the **Workflow Menu** pane, select **Analysis ▶ Results**.
2. In the **Assays** table, select the assay of interest. The software displays the data points for the selected assay in the scatter plot.
3. In the scatter plot, use the **View** drop-down list to select the items to display in the plot:
 - **Data Points**
 - **References** (reference samples)
 - **Omitted Wells**
 - **Legend**
 - **Classification Scheme**
4. Use the scatter plot tools as needed.

Tool	Description
 (Select Data Points)	Select data points in the plot. Click and drag a box around an individual data point or a group of data points. When you select data points in the scatter plot, the corresponding samples are selected in the Results table.
 (Pan)	Reposition the plot.
 (Zoom In)	Enlarge an area of the plot to view. Click  , then draw a box around the data points you want to view more closely. To zoom back out, click  (Zoom Out to Show ALL Data Points) (described below).
 (Zoom Out to Show ALL Data Points)	Show all data points in the plot.
 (Select All)	Select all data points in the plot.

(continued)

Tool	Description
 (Set Plot View)	Change the ranges for the scatter plot: 1. Click  (Set Plot View). 2. In the Set Plot View dialog box, enter a value for the: • Allele 1 upper boundary (VIC™ dye) • Allele 2 upper boundary (FAM™ dye) 3. Click OK.  Note: In order to display all data points, the software may override the maximum range that you set.
 (Edit Classification Boundaries)	Move the boundary lines for each classification region in the current scheme: • Click and drag a boundary line to adjust a classification region. • Click and drag the crosshairs to resize the ellipse. Note: The boundary lines should not intersect outside the NO AMP region (ellipse).
 (Undo)	Undo the previous edit to the classification boundaries (see above).
 (Select New Scheme)	Open the Select New Scheme dialog box, then select from a palette of classification schemes. A classification scheme allows you to divide the plot into regions, where each region represents a specific call: • Homozygous for Allele 1 (FAM™ dye/FAM™ dye) • Heterozygous for Allele 1 and Allele 2 (FAM™ dye/VIC™ dye) • Homozygous for Allele 2 (VIC™ dye/VIC™ dye) • No amplification • Undetermined (white, unlabeled region(s) within a scheme) • Possible Rare Allele (PRA) Note: An invalid region is anywhere outside of the entire “fan” shape of the scheme. There are five schemes available in the Select New Scheme dialog box (see “Modify the classification boundaries” on page 78). Each scheme is a different combination of the regions listed above.

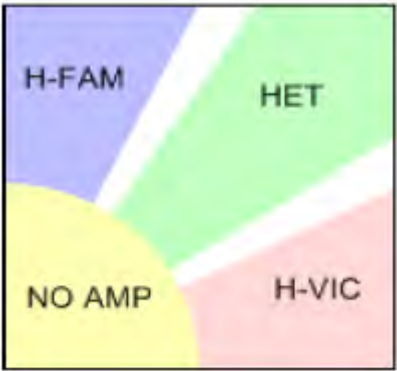
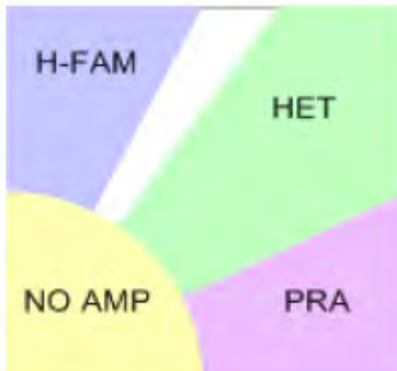
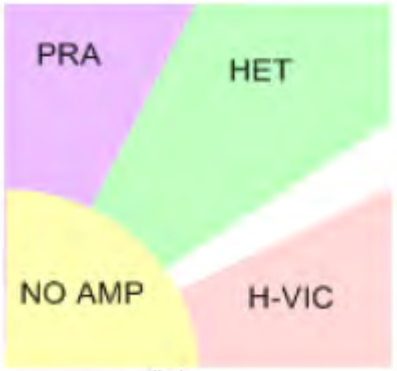
(continued)

Tool	Description
 (Save as Image File)	Save the plot as an image file: JPG, PDF, PNG, or SVG.
< or >	Expand or collapse the plot.

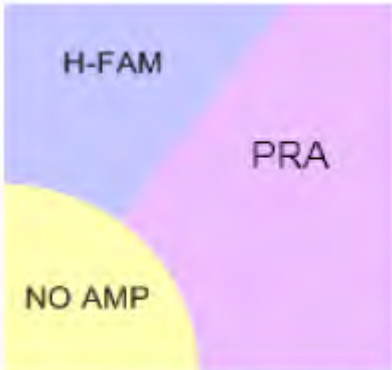
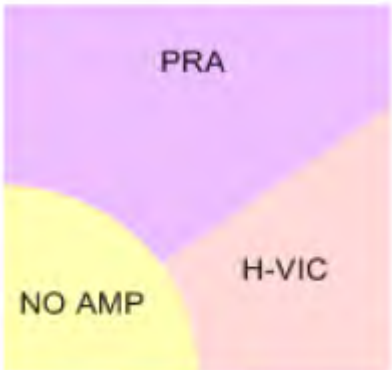
Modify the classification boundaries

1. Select a classification scheme:
 - a. In the scatter plot, click  **(Select New Scheme)** to open the **Select New Scheme** dialog box.

b. Select an appropriate scheme, then click **OK**. Each scheme is described in the table below.

Scheme	Regions defined
Standard Diploid Genotypes  The diagram shows a square divided into five regions: H-FAM (blue, top-left), HET (green, top-right), NO AMP (yellow, bottom-left), H-VIC (red, bottom-right), and a white unlabeled region (bottom-center).	Five regions are defined: • Homozygous FAM™ dye/FAM™ dye • Heterozygous FAM™ dye/VIC™ dye • Homozygous VIC™ dye/VIC™ dye • No amplification • Undetermined (white, unlabeled regions) Note: The software assigns this scheme as the default.
Rare VIC™ Allele  The diagram shows a square divided into five regions: H-FAM (blue, top-left), HET (green, top-right), NO AMP (yellow, bottom-left), PRA (purple, bottom-right), and a white unlabeled region (bottom-center).	Five regions are defined: • Homozygous FAM™ dye/FAM™ dye • Heterozygous FAM™ dye/VIC™ dye • Possible Rare Allele (PRA) • No amplification • Undetermined (white, unlabeled region)
Rare FAM™ Allele  The diagram shows a square divided into five regions: PRA (purple, top-left), HET (green, top-right), NO AMP (yellow, bottom-left), H-VIC (red, bottom-right), and a white unlabeled region (bottom-center).	Five regions are defined: • Possible Rare Allele (PRA) • Heterozygous FAM™ dye/VIC™ dye • Homozygous VIC™ dye/VIC™ dye • No amplification • Undetermined (white, unlabeled region)

(continued)

	Scheme	Regions defined
Extremely Rare VIC™ Allele		Three regions are defined: • Homozygous FAM™ dye/FAM™ dye • Possible Rare Allele (PRA) • No amplification
Extremely Rare FAM™ Allele		Three regions are defined: • Possible Rare Allele (PRA) • Homozygous VIC™ dye/VIC™ dye • No amplification

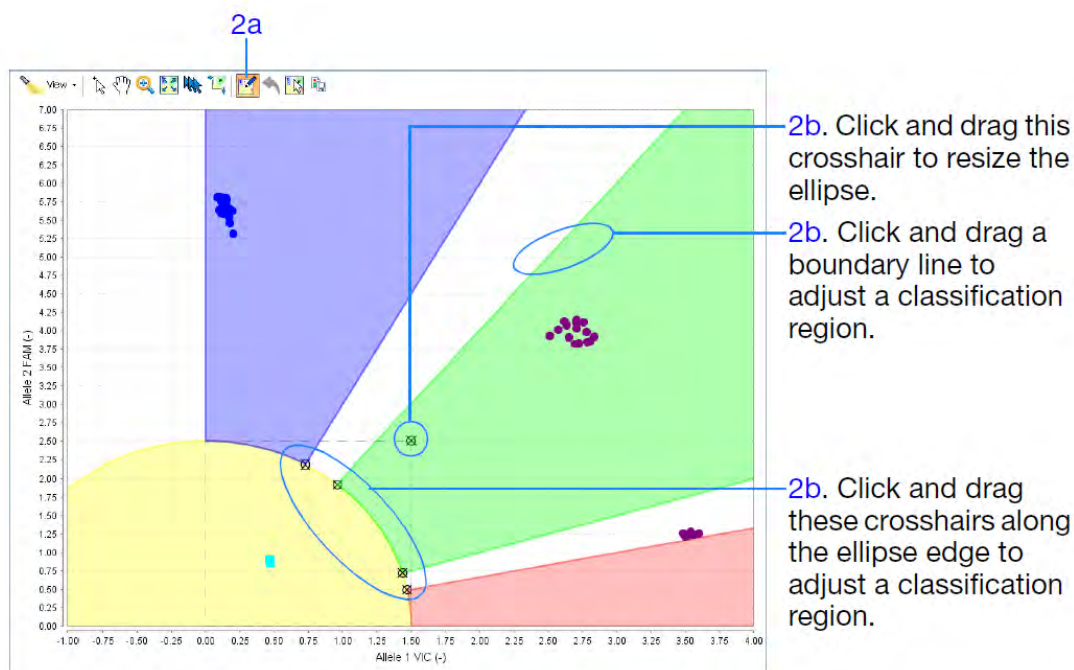
c. Click  **Analyze** to view the new results.

2. Edit the classification boundaries to adjust the classification regions for the current scheme:

a. Click  **(Edit Classification Boundaries)**.

- b. Click and drag the boundary lines for each region you want to adjust.

Note: The boundary lines should not intersect outside the NO AMP region (ellipse).



- c. Click  **Analyze** to view the new results.

Save the study

1. (Optional) Click  **Analyze** to view the new results.
2. (Optional) Select the **Inspected** checkbox next to the assay. The software marks the assay as inspected.

Note: If you do not reanalyze the study, the Inspected status persists between users and between TaqMan™ Genotyper Software applications. If you do reanalyze the study, the Inspected status will be cleared if the assay results change because of the analysis.

3. Click  **Save**.

View the data in the Quality Control screen

This section provides step-by-step procedures for viewing the data in the **Quality Control** screen.

- **Flag Summary** tab (see page 82)
- **Assays** tab (see page 85)
- **Experiments** tab (see page 88)
- **Samples** tab (see page 95)

View the data in the Flag Summary tab

View the data in the **Flag Summary** tab to identify the number of failures throughout the study.

View the data

1. In the **Workflow Menu** pane, select **Analysis ► Quality Control**, then select the **Flag Summary** tab.
2. In the **Well-Level QC Flags** pane, view any flags generated by the analysis, then perform the recommended action:

QC Flag	Description	Action
Failed Control	A Failed Control flag can be raised for any data point that is identified as a control: NTC, Negative Control, or Positive Control. If the user-assigned control identifier (or task) for a data point is inconsistent with the call that would be assigned by the software algorithm to an Unknown with the same FAM™ dye and VIC™ dye intensities, a flag is raised.	Review the flagged data point. You can omit the well or ignore the flag if it appears to be inappropriate. Note: The Failed Control flag can be very important if you selected to use positive controls for analysis and bias the genotype calls. See “Add control identifiers” on page 42.
Genotype Quality Low	A Genotype Quality Low flag can be raised for any data point that is identified or tasked as an Unknown. If the quality value assigned by the software algorithm for a data point is below the threshold, a flag will be raised.	Review the flagged data point and determine if the data point is an outlier or located in acceptable coordinates. You can manually assign a call or modify the quality value threshold to include the data point.
Low ROX™ Intensity	A Low ROX™ Intensity flag can be raised for any data point. If the ROX™ dye intensity determined by the software for a data point is below the threshold, a flag will be raised.	No action should be taken for these data points. If the ROX™ dye intensity is below the default threshold, the data point does not meet the minimum conditions for assigning a call.
NTC FAM™ Intensity High	An NTC FAM™ Intensity High flag can be raised for any data point that is identified or tasked as an NTC. If the FAM™ dye signal intensity for a data point tasked as NTC is greater than the threshold, a flag will be raised.	Review the flagged data point and determine if the high signal is acceptable or not. You can omit the well, raise the threshold to remove the flag, or ignore the flag. Note: If the coordinates of the NTC data point are located next to Unknown data points, this could indicate experiment cross-contamination, or an amplifying NTC. Track this for further troubleshooting.

(continued)

QC Flag	Description	Action
NTC VIC™ Intensity High	An NTC VIC™ Intensity High flag can be raised for any data point that is identified or tasked as an NTC. If the VIC™ dye signal intensity for a data point tasked as NTC is greater than the threshold, a flag will be raised.	Review the flagged data point and determine if the high signal is acceptable or not. You can omit the well, raise the threshold to remove the flag, or ignore the flag. Note: If the coordinates of the NTC data point are located next to Unknown data points, this could indicate experiment cross-contamination, or an amplifying NTC. Track this for further troubleshooting.
Reference Sample Discordance	A Reference Sample Discordance flag can be raised for any data point that is identified or tasked as an Unknown. If the software algorithm-assigned genotype for a data point is discordant with the genotype of a reference sample data point that has exactly the same sample/assay identification, a flag will be raised.	Review the flagged data point and determine if the coordinates are located in an acceptable location. You can manually assign a different call, omit the well, or ignore the flag.
Replicate Sample Discordance	A Replicate Sample Discordance flag can be raised for any data point that is identified or tasked as an Unknown. If the software algorithm-assigned genotype for a data point is discordant with the genotype of a replicate sample data point that has exactly the same sample/assay identification, a flag will be raised. A flag will be raised for all data points that have the sample/assay identification, because the software cannot know which data point has the correct genotype.	Review the flagged data point and determine which of the replicate data points are assigned the correct genotype. You can manually assign a different call, omit the well, or ignore the flag.

3. In the **Study-Level QC Flags** pane, view any flags generated by the analysis, then perform the recommended action:

QC Flag	Description	Action
Experiment Low ROX™ Rate High	An Experiment Low ROX™ Rate High flag can be raised for any experiment. If the percentage of data points in an experiment with a low ROX™ dye intensity flag is greater than the threshold, a flag will be raised.	Review the flagged experiment and determine if the issue is isolated to certain wells of the experiment or throughout. You can remove the experiment from the study and perform the experiment again or ignore the flag.
Assay Call Rate Low	An Assay Call Rate Low flag can be raised for any assay. If the percentage of Unknown data points with a genotype call for an assay is less than the threshold, a flag will be raised.	Review the flagged assay and determine if the issue is isolated to a few data points or throughout. You can omit the data for the assay, perform the experiment with the assay again, or ignore the flag.
Experiment Call Rate Low	An Experiment Call Rate Low flag can be raised for any experiment. If the percentage of Unknown data points with a genotype call in an experiment is less than the threshold, a flag will be raised.	Review the flagged experiment and determine if the issue is isolated to certain wells of the experiment or throughout. You can remove the experiment from the study, perform the experiment again, or ignore the flag.
Sample Call Rate Low	A Sample Call Rate Low flag can be raised for any sample identified or tasked as an Unknown. If the percentage of assays with a genotype call for Unknown samples is less than the threshold, a flag will be raised.	Review the flagged sample and determine if the issue is isolated to a few data points or throughout. You can omit the data for the sample, perform the experiment with the sample again, or ignore the flag.

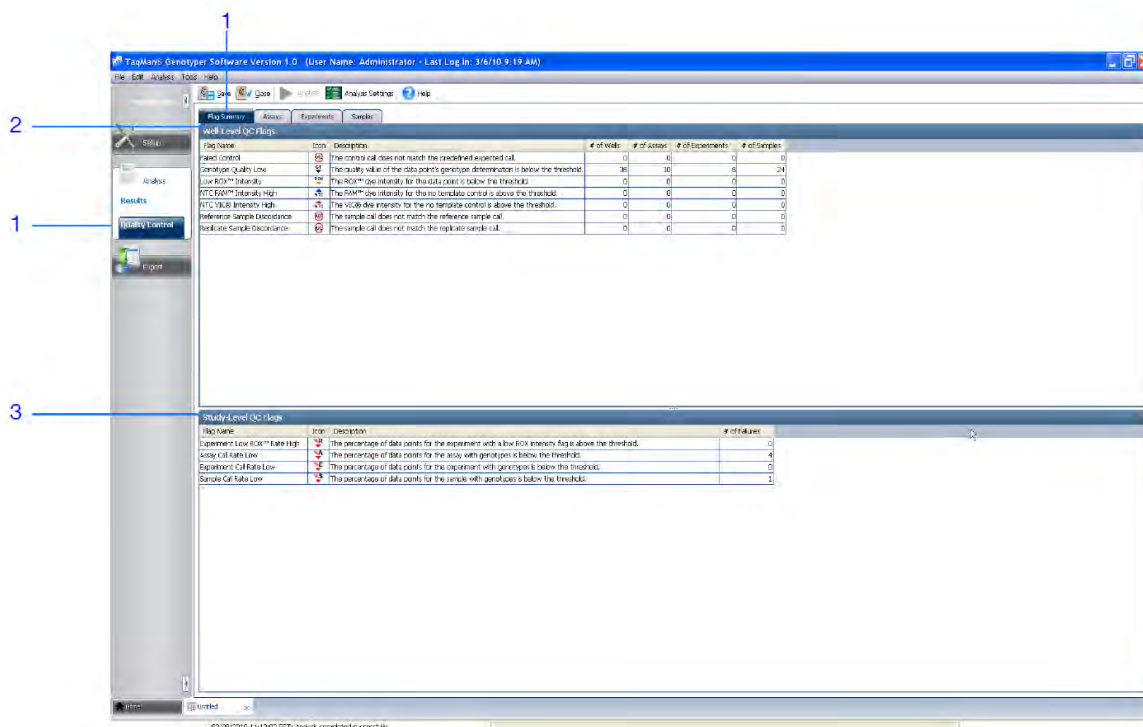


Figure 12 Quality Control screen, Flag Summary tab (the numbers called out in the screen capture refer to the step numbers in this procedure)

View the data in the Assays tab

About the Assays tab

The **Assays** tab displays the:

- **Assays** table—For each assay in the study, an overview of the pass/fail status of each QC setting (flag).
- Scatter plot—A graphical representation of the genotype calls for each data point in the selected assay.
- **Results** table—Detailed QC information about all samples for which the selected assay was run.

About the assay call rate

The assay call rate is the percentage of successful calls for each assay in the study. Successful calls are: FAM™ dye/FAM™ dye, VIC™ dye/VIC™ dye, and FAM™ dye/VIC™ dye.

The software calculates the assay call rate over all samples for which that assay was run. The software:

- Does not include NTC, Negative Control, Positive Control, or reference sample wells.
- Considers invalid, undetermined, and PRA calls as no-calls.
- Recalculates the call rates whenever the data is reanalyzed. The data can be reanalyzed automatically (for example, when you import or delete an experiment) or manually (when you click  **Analyze**).

Note: If you manually change a call, the software does not recalculate the call rates until you click  **Analyze**.

View the data in the Assays tab

1. In the **Workflow Menu** pane, select **Analysis** ▶ **Quality Control**, then select the **Assays** tab.
2. In the **Assays** table, look for any assays that have a:
 - Low Assay Call Rate flag (🔴 appears in the  column).
 - Value in a flag column; the value indicates the number of wells with the flag

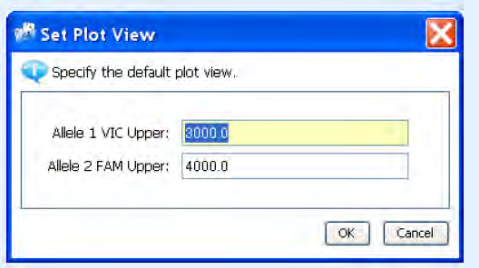
In the example below, the selected assay has a failing Assay Call Rate (58.7%) and 12 wells with the Genotype Quality Low flag (🔴).

Flag Summary											
Assays											
#	Assay Name	Assay ID	Assay Call Rate	🔴	ROX	NTC	NTC	NTC	QT	CTL	REF
1		C_133314_10	100%	🟢	0	0	0	0	0	0	0
2		C_186004_10	100%	🟢	0	0	0	0	0	0	0
3		C_218884_10	97.8%	🟢	0	0	0	0	0	0	0
4		C_222891_10	100%	🟢	0	0	0	0	0	0	0
5		C_222920_10	100%	🟢	0	0	0	0	0	0	0
6		C_227777_10	58.7%	🔴	0	0	0	12	0	0	0
7		C_245457_10	100%	🟢	0	0	0	0	0	0	0
8		C_63434_10	100%	🟢	0	0	0	0	0	0	0

3. In the **Assays** table, select an assay. The software displays the data for the selected assay in the scatter plot and in the **Results** table.
4. Use the **View** drop-down list to select the items to display in the scatter plot:
 - **Omitted Wells**
 - **Wells with Flags**
 - **Wells without Flags**
 - **Legend**
 - **Classification Scheme** (This option is available only if you selected **Classification Scheme** as the call method.)

Legend				
■ NTC (2)	● VIC/VIC (12)	● VIC/FAM (11)	● FAM/FAM (4)	
● No Amp (7)	● Undet (12)	● PFA (0)	× Invalid (0)	× Omitted (0)
■ Squares for Controls (2)		○ No Fill for Flags (12)		

5. Use the scatter plot tools as needed.

Tool	Description
 (Select Data Points)	Select data points in the plot. Click and drag a box around an individual data point or a group of data points. When you select data points in the scatter plot, the corresponding samples are selected in the Results table.
 (Pan)	Reposition the plot.
 (Zoom In)	Enlarge an area of the plot to view. Click  , then draw a box around the data points you want to view more closely. To zoom back out, click  (Zoom Out to Show ALL Data Points) (described below).
 (Zoom Out to Show ALL Data Points)	Show all data points in the plot.
 (Select All)	Select all data points in the plot.
 (Set Plot View)	Change the ranges for the scatter plot: - Click  (Set Plot View). - In the Set Plot View dialog box, enter a value for the: - Allele 1 upper boundary (VIC™ dye) - Allele 2 upper boundary (FAM™ dye) - Click OK.  Note: In order to display all data points, the software may override the maximum range that you set.
 (Save as Image File)	Save the plot as an image file: JPG, PDF, PNG, or SVG.
< or >	Expand or collapse the plot.

6. To view the data in the **Results Table**:

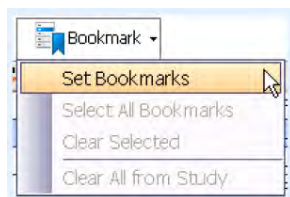
- From the **View** drop-down list, select all items so that all columns are displayed in the **Results** table.
- Look in the flag columns to see the wells with flags.

7. (Optional) Bookmark any wells of interest:

- Select the well to bookmark.

- b. From the **Bookmark** drop-down list, select **Set Bookmarks**.

The bookmarks persist in the **Results** and **Quality Control** screens, so you can easily find the bookmarked well.



8. (Optional) Modify the data or reset the analysis settings (see “Analyze the data using Autocalling” on page 66 and “Analyze the data using Classification Schemes” on page 75 or “Set the analysis settings” on page 38).

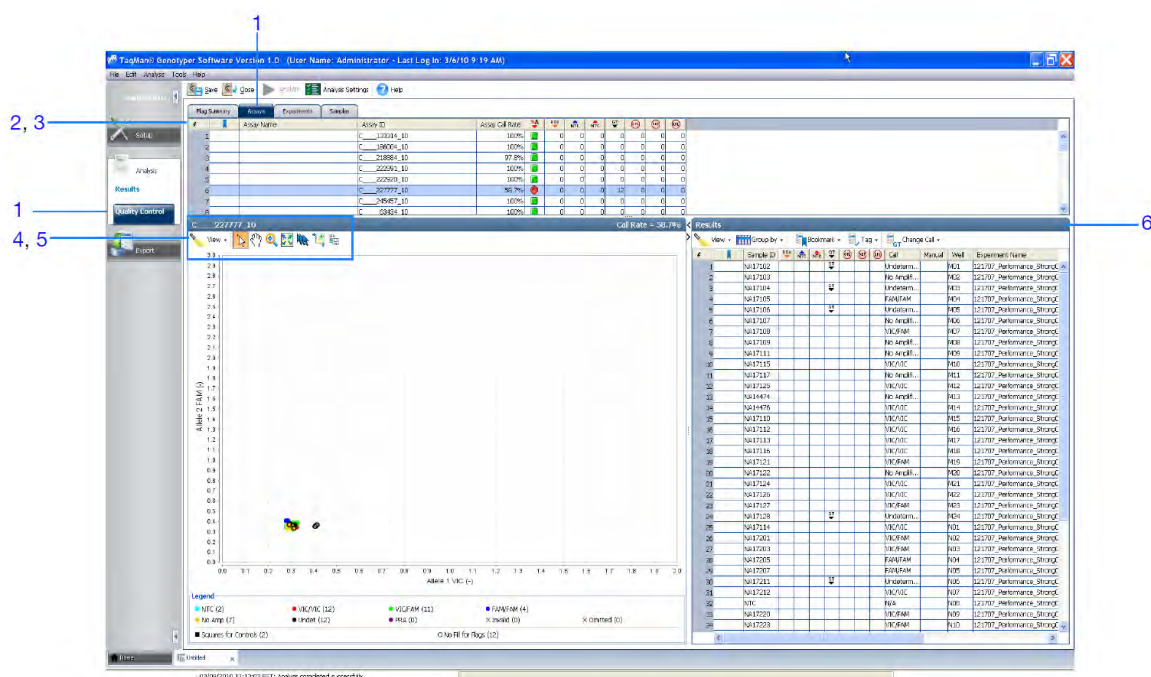


Figure 13 Quality Control screen, Assays tab (the numbers called out in the screen capture refer to the step numbers in this procedure)

View the data in the Experiments tab

About the Experiments tab

The **Experiments** tab displays the:

- **Experiments** table—For each experiment in the study, an overview of the pass/fail status of each QC setting (flag).
- Plate layout—An illustration of the reaction plate for the selected experiment.
- **Results** table—Detailed QC information about all samples/assays in the selected experiment.

About the experiment call rate

The experiment call rate is the percentage of successful calls for each experiment in the study. Successful calls are: FAM™ dye/FAM™ dye, VIC™ dye/VIC™ dye, and FAM™ dye/VIC™ dye.

The software calculates the experiment call rate over all wells on the reaction plate (regardless of assay or sample). The software:

- Does not include NTC, Negative Control, Positive Control, or reference sample wells.
- Considers invalid, undetermined, and PRA calls as no-calls.
- Recalculates the call rates whenever the data is reanalyzed. The data can be reanalyzed automatically (for example, when you import or delete an experiment) or manually (when you click  **Analyze**).

Note: If you manually change a call, the software does not recalculate the call rates until you click  **Analyze**.

View the data in the Experiments tab

1. In the **Workflow Menu** pane, select **Analysis** ▶ **Quality Control**, then select the **Experiments** tab.
2. In the **Experiments** table, look for any experiments that have a:
 - Low Experiment Call Rate flag (🔴 appears in the  column)
 - Value in a flag column; the value indicates the number of wells with the flag

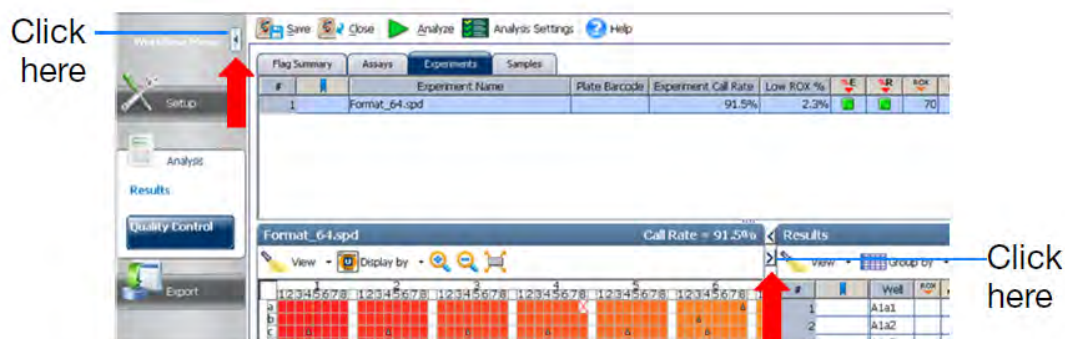
In the example below, the selected experiment has a passing Experiment Call Rate (94.6%) and 12 wells with the Genotype Quality Low flag ().

Flag Summary		Assays	Experiments	Samples														
#		Experiment Name	Plate Barcode	Experiment Call Rate	Low ROX %	🔴E	🔴R	ROX	NTC	NTC	GT	OTL	RET	RTL				
1		121707_Performance_Strong01_Assays1-...	A3038YTE	94.6%	0%	🟢	🟢	0	0	0	12	0	0	0				
2		121707_Performance_Strong01_Assays9-...	A303MK7I	96.7%	0%	🟢	🟢	0	0	0	7	0	0	0				
3		121707_Performance_Strong02_Assays1-...	A3038Z4Y	96.5%	0%	🟢	🟢	0	0	0	5	0	0	0				
4		121707_Performance_Strong02_Assays9-...	A3038Z4Z	98.9%	0%	🟢	🟢	0	0	0	2	0	0	0				
5		121707_Performance_Strong03_Assays1-...	A3038Z56	96.7%	0%	🟢	🟢	0	0	0	9	0	0	0				
6		121707_Performance_Strong03_Assays9-...	A3038Z57	99.7%	0%	🟢	🟢	0	0	0	1	0	0	0				

3. In the **Experiments** table, select an experiment. The software displays the data for the selected experiment in the plate layout and in the **Results** table.

4. To view the data in the plate layout:

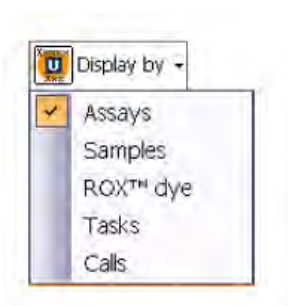
- a. (Recommended for TaqMan™ OpenArray™ Plates) Click the expand buttons to expand the plate layout view.

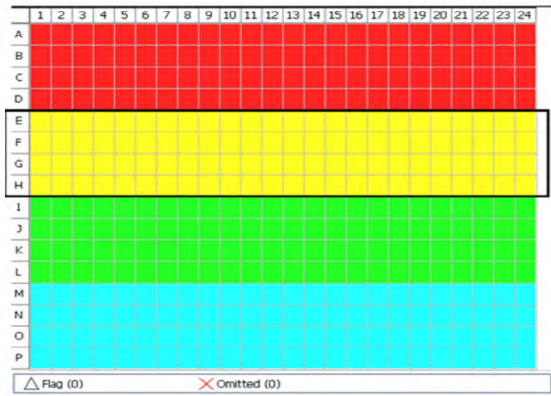
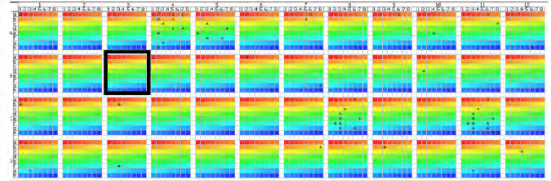
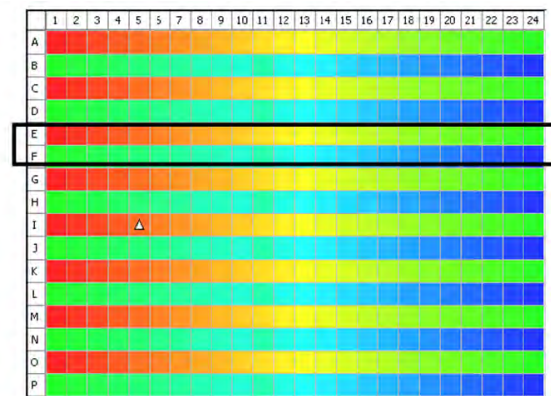


- b. Use the **View** drop-down list to select the items to display in the plate layout:

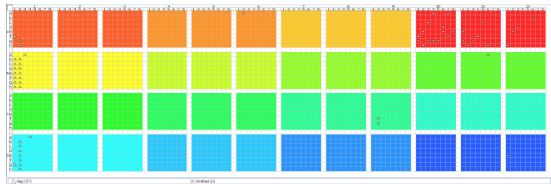
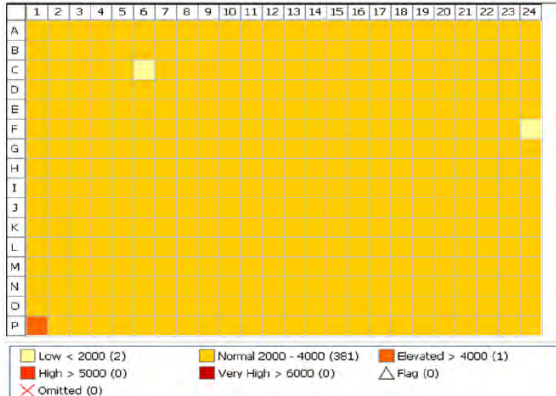
- **Omitted Wells**
- **Wells with Flags**
- **Wells without Flags**
- **Legend**

- c. One at a time, select the options as needed in the **Display by** drop-down list. Each option is described in the table below.

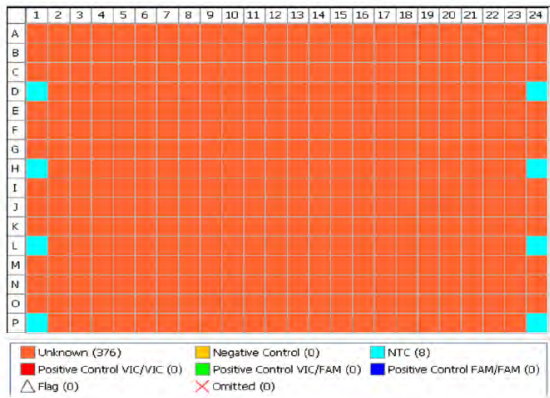


Option	Description	Plate layout examples
Assays	Displays the location of each assay in the reaction plate. Each color represents a different assay.	7900HT Fast Real-Time PCR System <i>In this example, four assays were run in a 384-well reaction plate. The boxed area represents one assay.</i> 
		TaqMan™ OpenArray™ Plates <i>In this example, each subarray contains 64 assays. The boxed area represents one assay.</i> 
Samples	Displays the location of each sample in the reaction plate. Each color represents a different sample.	7900HT Fast Real-Time PCR System <i>In this example, 96 samples (replicated four times) were run in a 384-well reaction plate. The boxed area represents one sample set.</i> 

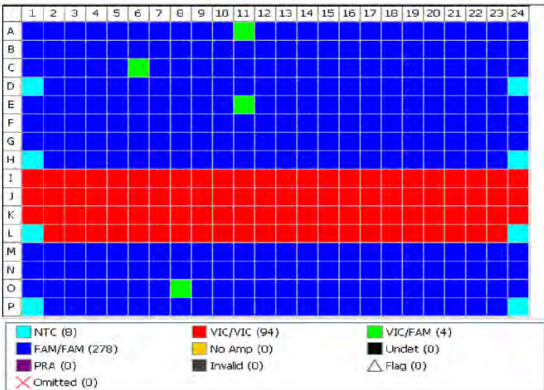
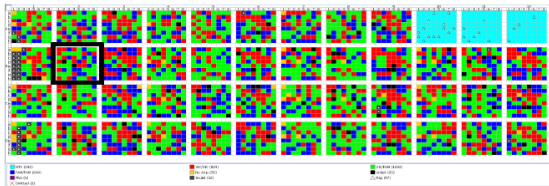
(continued)

Option	Description	Plate layout examples
Samples	Displays the location of each sample in the reaction plate. Each color represents a different sample.	TaqMan™ OpenArray™ Plates <i>In this example, 48 samples were run in a TaqMan™ OpenArray™ Plate.</i> 
ROX™ dye	Displays the ROX™ dye levels in the reaction plate. Each color represents a range, from Low to Very High.	7900HT Fast Real-Time PCR System <i>In this example, the ROX™ dye levels are uniform across the reaction plate, indicating that there were no experimental problems.</i> Note: Only three wells (C6, F24, and P1) deviate slightly.  TaqMan™ OpenArray™ Plates <i>In this example, the ROX™ dye levels range from low to normal. Wells with low values could indicate sample loading issues.</i> 

(continued)

Option	Description	Plate layout examples
Tasks	Displays the tasks/control identifiers assigned each well in the reaction plate. Each color represents a different task/control identifier.	7900HT Fast Real-Time PCR System <i>In this example, eight NTCs were run in the reaction plate; all other wells contain Unknown samples.</i> 
		TaqMan™ OpenArray™ Plates <i>In this example, three NTCs were run in the TaqMan™ OpenArray™ Plate; all other through-holes contain Unknown samples.</i> 

(continued)

Option	Description	Plate layout examples
Calls	Displays the genotype calls assigned each well in the reaction plate. Each color represents a different call.	7900HT Fast Real-Time PCR System <i>In this example, the genotype calls were:</i> - Mostly homozygous FAM™ dye genotypes - Some homozygous VIC™ dye genotypes - Very few heterozygous FAM™ dye/VIC™ dye genotypes 
		TaqMan™ OpenArray™ Plates <i>In this example, each subarray represents the genotype calls for one sample across 64 assays. The boxed area represents one assay.</i> 

5. Use the plate layout tools as needed:

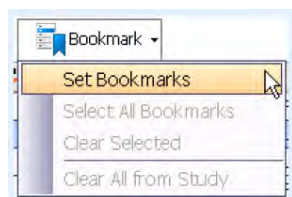
Tool	Description
 (Zoom In)	Enlarge an area of the plate layout to view.
 (Zoom Out)	Decrease an area of the plate layout to view.
 (Fit to Size)	Fit the entire reaction plate illustration into the plate layout view.
< or >	Expand or collapse the plate layout.

6. To view the data in the **Results** table:

- From the **View** drop-down list, select all items so that all columns are displayed in the **Results** table.
- Look in the flag columns to see the wells with flags.

7. (Optional) Bookmark any wells of interest:
 - a. Select the well to bookmark.
 - b. From the **Bookmark** drop-down list, select **Set Bookmarks**.

The bookmarks persist in the **Results** and **Quality Control** screens, so you can easily find the bookmarked well.



8. (Optional) Modify the data or reset the analysis settings (see “Analyze the data using Autocalling” on page 66 and “Analyze the data using Classification Schemes” on page 75 or “Set the analysis settings” on page 38).

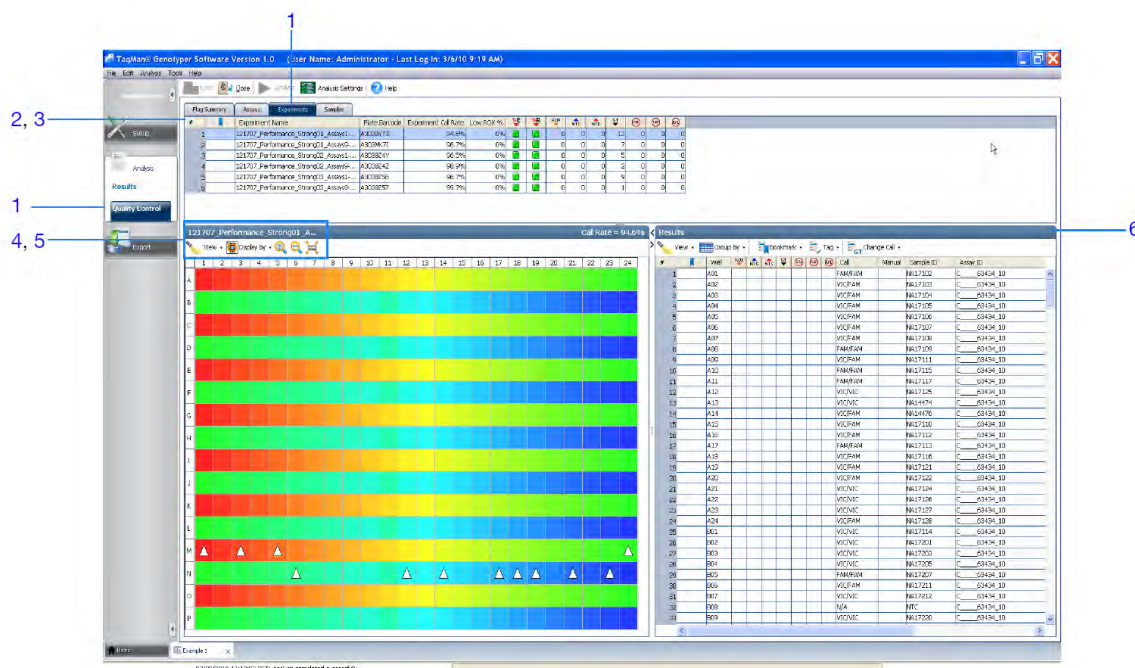


Figure 14 Quality Control screen, Experiments tab (the numbers called out in the screen capture refer to the step numbers in this procedure)

View the data in the Samples tab

About the Samples tab

The **Samples** tab displays the:

- **Samples** table—For each sample in the study, an overview of the pass/fail status of each QC setting (flag).
- **Results** table—Detailed QC information about all assays that were run for the selected sample.

About the sample call rate

The sample call rate is the percentage of successful calls for each sample in the study. Successful calls are: FAM™ dye/FAM™ dye, VIC™ dye/VIC™ dye, and FAM™ dye/VIC™ dye.

The software calculates the sample call rate over all assays run on that sample. The software:

- Does not include NTC or reference sample wells.
- Does include Negative Controls and Positive Control wells.
- Considers invalid, undetermined, and PRA calls as no-calls.
- Recalculates the call rates whenever the data is reanalyzed. The data can be reanalyzed automatically (for example, when you import or delete an experiment) or manually (when you click  **Analyze**).

Note: If you manually change a call, the software does not recalculate the call rates until you click  **Analyze**.

View the data in the Samples tab

1. In the **Workflow Menu** pane, select **Analysis** ▶ **Quality Control**, then select the **Samples** tab.
2. In the **Samples** table, look for any samples that have a:
 - Low Sample Call Rate flag ( appears in the  column)
 - Value in a flag column; the value indicates the number of wells with the flag

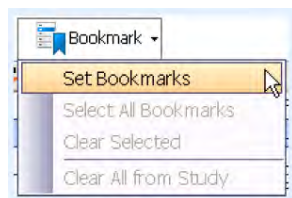
In the example below, the selected sample has a failing Sample Call Rate (87.5%) and 6 wells with the Genotype Quality Low flag ().

Flag Summary											
Assays											
Experiments											
Samples											
#	Sample ID	Sample Call Rate									
1	NA10859	97.9%		0	0	0	1	0	0	0	0
2	NA14474	97.9%		0	0	0	0	0	0	0	0
3	NA14476	95.8%		0	0	0	2	0	0	0	0
4	NA17102	97.9%		0	0	0	1	0	0	0	0
5	NA17103	97.9%		0	0	0	0	0	0	0	0
6	NA17104	97.9%		0	0	0	1	0	0	0	0
7	NA17105	100%		0	0	0	0	0	0	0	0
8	NA17106	87.5%		0	0	0	6	0	0	0	0
9	NA17107	95.8%		0	0	0	0	0	0	0	0

3. In the **Samples** table, select a sample. The software displays the data for the selected sample in the **Results** table.
4. To view the data in the **Results** table:
 - a. From the **View** drop-down list, select all items so that all columns are displayed in the **Results** table.
 - b. Look in the flag columns to see the wells with flags.
5. (Optional) Bookmark any wells of interest:
 - a. Select the well to bookmark.

- b. From the **Bookmark** drop-down list, select **Set Bookmarks**.

The bookmarks persist in the **Results** and **Quality Control** screens, so you can easily find the bookmarked well.



6. (Optional) Modify the data or reset the analysis settings (see “Analyze the data using Autocalling” on page 66 and “Analyze the data using Classification Schemes” on page 75 or “Set the analysis settings” on page 38).

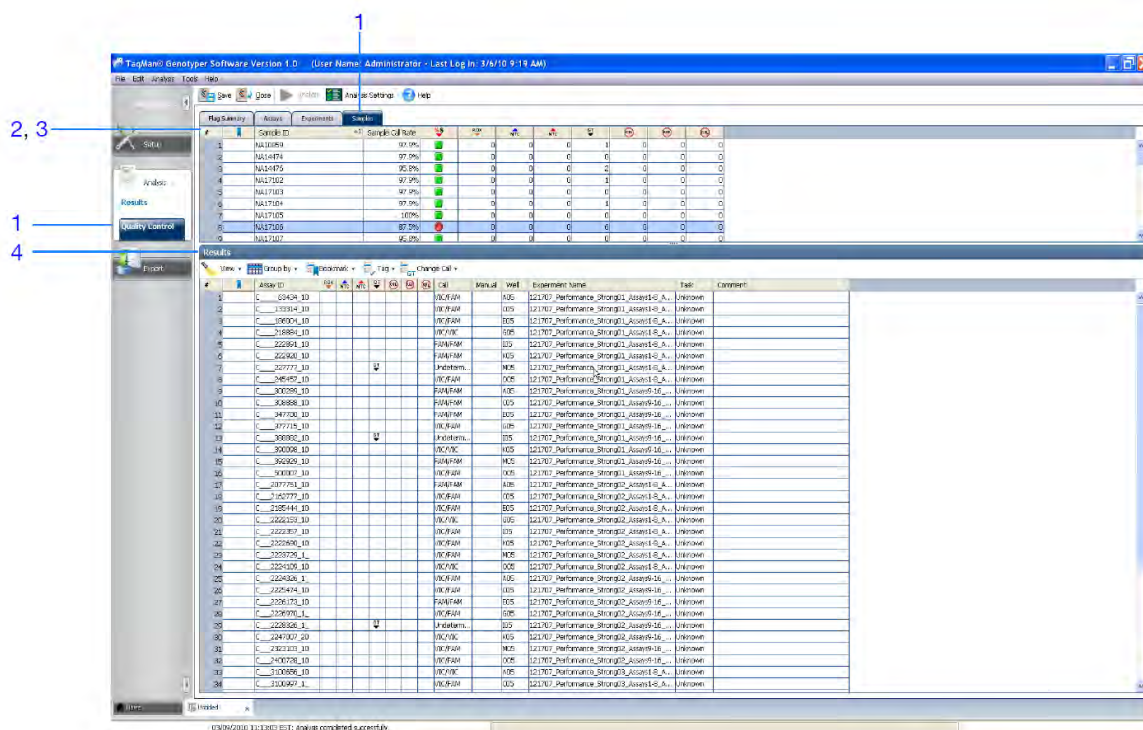


Figure 15 Quality Control screen, Samples tab (the numbers called out in the screen capture refer to the step numbers in this procedure)

(Optional) Enter comments for the study

In the **Properties** screen, enter comments for the study.

IMPORTANT! The **Comments** field allows you to enter detailed information about the study (for example, observations about the data, reasons why you made specific decisions, etc.). After you click **Add**, the comment is permanently recorded in the study (that is, the comment cannot be modified or removed) and the comment is included in any audit trails that are exported for the study (see “Export an audit trail” on page 107).

Enter comments

1. In the **Workflow Menu** pane, select **Setup ▶ Properties**.
2. In the **Comments** field, enter comments about the study, then click **Add**. The software records the comment, your user name, and the date/time you added the comment.

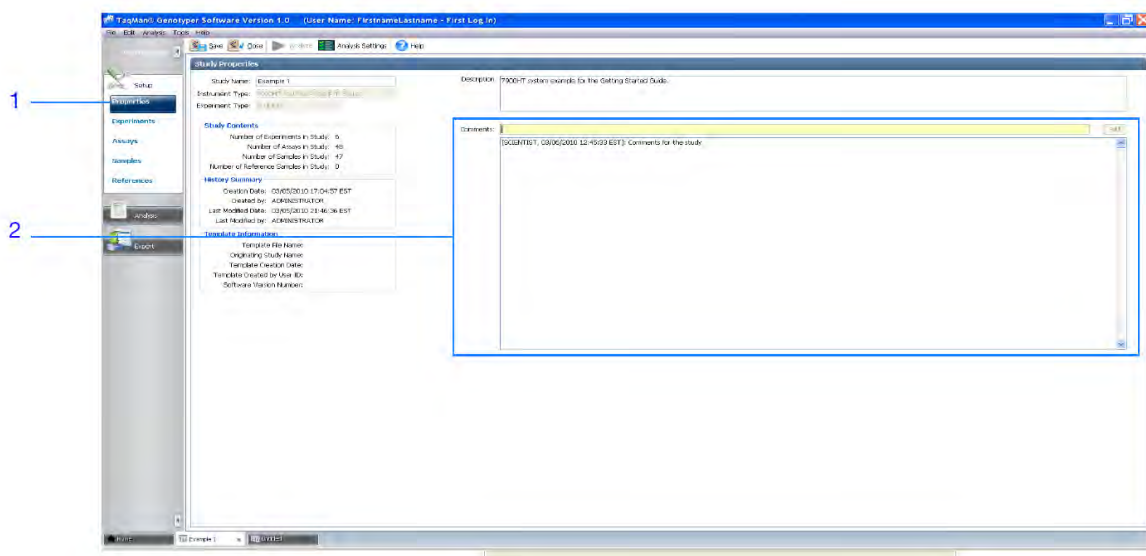


Figure 16 Properties screen, Comments field (the numbers called out in the screen capture refer to the step numbers in this procedure)

7

Export and transfer data

- Required user role 99
- Workflow 100
- (Optional) Modify the legend for exported data 101
- Export analysis data 101
- Export an audit trail 107
- Transfer a study 112

Required user role

You may not be able to perform all of the procedures in this chapter. The table below indicates the user roles that are allowed to perform each procedure in this chapter.

Note: For more information on user roles, see “User roles and tasks” on page 20.

Function	User role		
	Administrator	Scientist	Technician
(Optional) Modify the legend for exported data (see page 101)	✓	✓	—
Export analysis data (see page 101)	✓	✓	✓
Export an audit trail (see page 107)	✓	✓	✓
Transfer a study (see page 112)	✓	✓	✓

Workflow

Export workflow

“(Optional) Modify the legend for exported data” on page 101



“Export analysis data” on page 101

“Export the analysis data” on page 101



“Open the exported file” on page 103



“Read the exported file” on page 104



“Export an audit trail” on page 107

“Export the audit trail” on page 108



“Open the exported audit trail” on page 109



“Read the audit trail” on page 110

Transfer workflow

“Transfer a study” on page 112

“Transfer out” on page 112



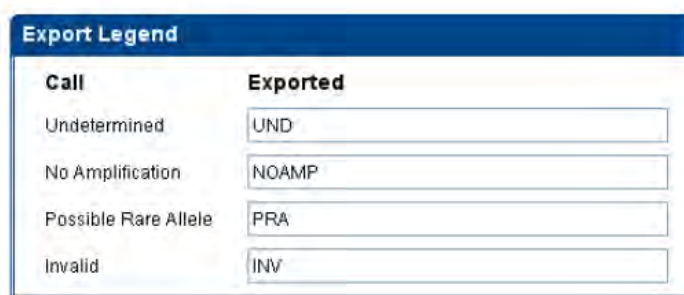
“Transfer in” on page 114

(Optional) Modify the legend for exported data

Modify the legend

In the **Export** screen, determine how the calls will be labeled in exported files:

1. In the **Workflow Menu** pane, select **Export ▶ Analysis Data**.
2. In the **Export Legend** pane, enter new labels for one or more of the following calls: **Undetermined, No Amplification, Possible Rare Allele, Invalid**.



Call	Exported
Undetermined	UND
No Amplification	NOAMP
Possible Rare Allele	PRA
Invalid	INV

Export analysis data

This section provides step-by-step procedures to complete the following tasks:

- Export the analysis data (see page 101)
- Open the exported file (see page 103)
- Read the exported file (see page 104)

About the analysis data

You can export the following types of analysis data:

- Analysis Results (Basic)
- Analysis Results (Advanced)
- Analysis Settings

For detailed information about the exported file contents, see “Read the exported file” on page 104.

Export the analysis data

1. In the **Workflow Menu** pane, select **Export ▶ Analysis Data**.
2. Select the type of data to export (you can all of the options at the same time):
 - **Analysis Results**
 - **Analysis Settings**
 - **QC Summary**

The **QC Summary** includes quality control data from the following items:

- QC flags, including well-level QC flags and study-level QC flags
- Assays
- Experiments
- Samples

3. If you selected **Analysis Results** in step 2, select **Basic** or **Advanced**.

For a description of the data that is exported, see “Read the exported file” on page 104.

4. Select to export the data in one file or in separate files:

Option	Description
One File	All data types you selected in step 2 (Analysis Results and/or Analysis Settings) are exported together in one file.
Separate Files	All data types you selected in step 2 (Analysis Results and/or Analysis Settings) are exported separately in different files.

5. For each type of data that you selected in step 2, enter information for the file destination:

- **File Name**—Accept the default file name, or enter a new name. The default format for the file name is:
study name_yyyymmdd_hhmmss_file type
where:
 - *study name* is the name of the current study
 - *yyymmdd* and *hhmmss* are the current date and time
 - *file type* is the type of data exported: Results or Settings
- **File Location**—Accept the default location for the exported file, or click **Browse** and select another location.
- **File Type**—Select the type of file to export:
 - CSV—Exports the data as a comma-separated-value file, where values are delimited by commas. You can open the CSV file with a spreadsheet application (such as Microsoft™ Excel™ Software).
 - TXT—Exports the data as a tab-delimited file, where values are delimited by tabs. You can open the TXT file with a spreadsheet application (such as Microsoft™ Excel™ Software).
 - *(Advanced Analysis Results only)* XML—Exports the data as an XML file. You can open the XML file with an Internet browser.

6. Select/deselect **Open file(s) when export is complete**. If selected, the software automatically opens the file after export.

7. Click **Export preview**.

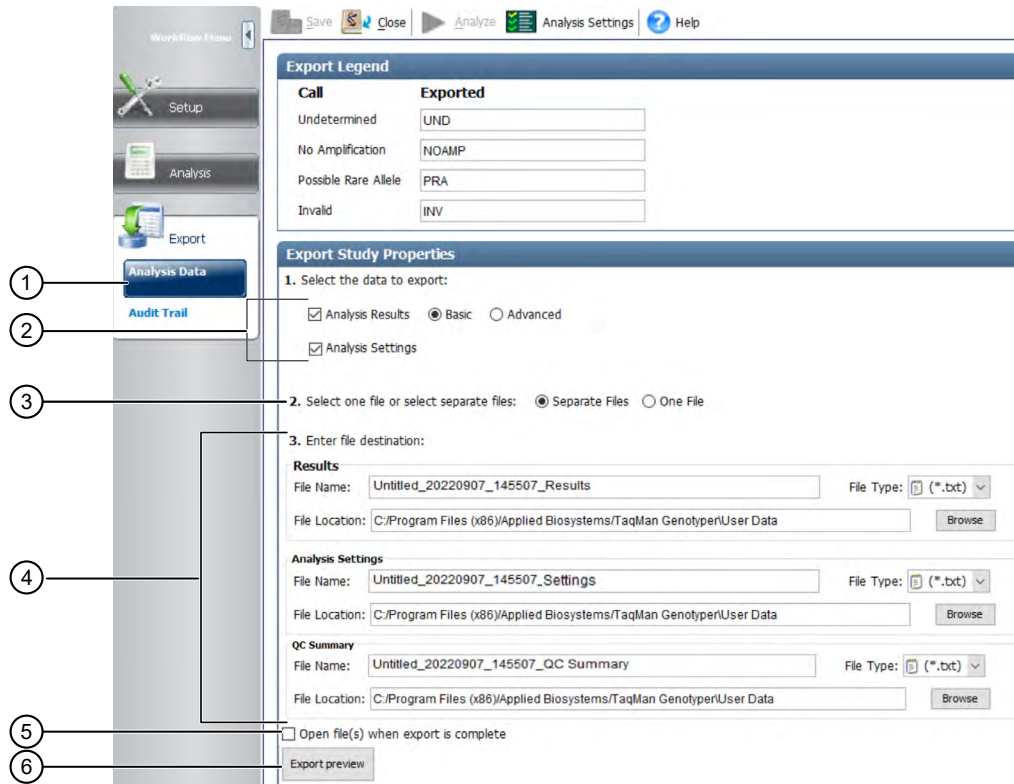


Figure 17 Analysis Data screen (the numbers called out in the screen capture refer to the step numbers in this procedure)

- 1 Analysis Data
- 2 Type of data to export
- 3 File destination
- 4 Open file(s) when export is complete checkbox
- 5 Export preview button

The **Export Preview** dialog box opens.

8. In the **Export Preview** dialog box, in the **Select Contents** field, select the contents for the report.
9. Click **Start Export**.

Open the exported file

1. Locate the exported file (CSV, TXT, or XML file).
2. Right-click the file, then select **Open With** ► **Microsoft™ Office Excel™** to view the contents as a spreadsheet in the Microsoft™ Excel™ software.
3. Use the AutoFit function in the Excel™ software to make the data easier to read:
 - a. Click the upper-left corner of the spreadsheet to select all cells.

b. Select **Format** ► **Column** ► **AutoFit Selection**.

Click here

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	# Exported By : GUEST														
2	# Export Date : 02/09/2010 11:50:58 EST														
3	# Study Name : Test 012510														
4	# Experiment Type : Endpoint														
5	# Instrument Type : 7900HT Fast Real-Time PCR System														
6	# Software Version Number : 1.0.0														
7	# Creation Date : 01/25/2010 10:53:26 EST														
8	# Created By : GUEST														
9	# Last Modified Date : 02/09/2010 11:50:08 EST														
10	# Last Modified By : GUEST														
11	# Template File Name														
12	# Template Originating Study Name														
13	# Template Creation Date														
14	# Template Created By User ID														
15	# Template Software Version Number														
16															
17															
18	Assay Name	Assay ID	Gene Symbol	NCBI SNP Reference	Sample ID	Cell	Manual	Quality	V/C(Rn)	I/FAM(Rn)	ROX	Task	Gender	Population	Wt

Read the exported file

Examples of the exported files are shown below.

- Analysis Results (Basic) (see page 104).
- Analysis Results (Advanced) (see page 105).
- Analysis Settings (see page 106).

Analysis Results (Basic)

The Analysis Results (Basic) exported file contains:

- Study information—General information about the study. For example, the date/time the study was exported, the user who created the study, etc.
- Sample/well information—General information about each sample in the study. For example, the Assay ID, Sample ID, genotype call, etc.

Study information

Sample information

Figure 18 Analysis Data (Basic)—Example of an exported file

Analysis Results (Advanced)

The Analysis Results (Advanced) exported file contains:

- **Study information**—General information about the study. For example, the date/time the study was exported, the user who created the study, etc.
- **Sample/well information**—Detailed information about each sample in the study. For example, the Assay ID, Sample ID, genotype call, etc.

Study information

Sample information

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	# Exported By:	GUEST													
2	# Export Date:	02/09/2010 11:50:58 EST													
3	# Study Name:	Test 012510													
4	# Experiment Type:	Endpoint													
5	# Instrument Type:	7900HT Fast Real-Time PCR System													
6	# Software Version Number:	1.0.0													
7	# Creation Date:	01/25/2010 10:53:26 EST													
8	# Created By:	GUEST													
9	# Last Modified Date:	02/09/2010 11:50:08 EST													
10	# Last Modified By:	GUEST													
11	# Template File Name:														
12	# Template Originating Study Name:														
13	# Template Creation Date:														
14	# Template Created By User ID:														
15	# Template Software Version Number:														
16															
17															
18	Assay Name	Assay ID	Gene Symbol	NCBI/SNP Reference	Sample ID	Call	Manual	Quality	VIC(Rn)	FAM(Rn)	POX	Task	Gender	Population Well	Experiment Name
19		C_183434_10			NA17102	UND	FALSE	N/A	1.697	4.88	2.498.81	Unknown	Unknown		A01_121707_Performance
20		C_133314_10			NA17102	PRA	FALSE	N/A	0.171	5.659	1.661.29	Unknown	Unknown		C01_121707_Performance
21		C_180004_10			NA17102	YIC/VIC	FALSE	N/A	3.287	0.859	2.084.08	Unknown	Unknown		E01_121707_Performance
22		C_218884_10			NA17102	NDAMP	FALSE	N/A	0.755	0.825	1.905.45	Unknown	Unknown		G01_121707_Performance
23		C_222891_10			NA17102	YIC/FAM	FALSE	N/A	1.436	2.837	1.923.52	Unknown	Unknown		I01_121707_Performance
24		C_222800_10			NA17102	YIC/FAM	FALSE	N/A	2.392	4.311	2.086.62	Unknown	Unknown		K01_121707_Performance
25		C_245457_10			NA17102	NDAMP	FALSE	N/A	0.313	0.333	2.534.89	Unknown	Unknown		MD1_121707_Performance
26		C_245457_10			NA17102	YIC/FAM	FALSE	N/A	2.875	4.879	2.500.10	Unknown	Unknown		OD1_121707_Performance
27		C_300089_10			NA17102	NDAMP	FALSE	N/A	1.132	0.773	2.436.75	Unknown	Unknown		A01_121707_Performance
28		C_308888_10			NA17102	UND	FALSE	N/A	2.339	6.821	2.009.30	Unknown	Unknown		C01_121707_Performance
29		C_347700_10			NA17102	FAM/FAM	FALSE	N/A	0.646	4.941	1.963.31	Unknown	Unknown		E01_121707_Performance
30		C_377715_10			NA17102	FAM/FAM	FALSE	N/A	0.804	4.551	2.017.67	Unknown	Unknown		G01_121707_Performance
31		C_398882_10			NA17102	NDAMP	FALSE	N/A	0.511	0.761	2.219.71	Unknown	Unknown		I01_121707_Performance
32		C_390096_10			NA17102	UND	FALSE	N/A	3.088	1.356	2.839.08	Unknown	Unknown		K01_121707_Performance
33		C_392479_10			NA17102	FAM/FAM	FALSE	N/A	0.191	4.639	2.329.78	Unknown	Unknown		MD1_121707_Performance
34		C_300007_10			NA17102	UND	FALSE	N/A	2.04	4.152	2.850.03	Unknown	Unknown		OD1_121707_Performance
35		C_207751_10			NA17102	FAM/FAM	FALSE	N/A	0.025	3.453	1.832.58	Unknown	Unknown		A01_121707_Performance
36		C_218777_10			NA17102	FAM/FAM	FALSE	N/A	0.339	7.02	1.466.64	Unknown	Unknown		C01_121707_Performance
37		C_218544_10			NA17102	FAM/FAM	FALSE	N/A	1.963	7.164	1.193.79	Unknown	Unknown		E01_121707_Performance
38		C_2222153_10			NA17102	YIC/VIC	FALSE	N/A	2.785	0.891	1.887.44	Unknown	Unknown		G01_121707_Performance
39		C_2222557_10			NA17102	NDAMP	FALSE	N/A	0.487	0.607	1.869.69	Unknown	Unknown		I01_121707_Performance
40		C_2222980_10			NA17102	YIC/FAM	FALSE	N/A	3.102	1.967	2.170.83	Unknown	Unknown		K01_121707_Performance
41		C_2223729_1			NA17102	INV	FALSE	N/A	-0.171	7.574	1.694.84	Unknown	Unknown		MD1_121707_Performance
42		C_223400_10			NA17102	NDAMP	FALSE	N/A	0.759	0.751	3.569.59	Unknown	Unknown		OD1_121707_Performance
43	# # # # # Test 012510_1002009_115008_Res/														

Figure 19 Analysis Data (Advanced)—Example of an exported file

Analysis Settings

The Analysis Settings exported file contains:

- Study information—General information about the study. For example, the date/time the study was exported, the user who created the study, etc.
- Call settings—The call settings for the study, as set in the **Analysis Settings** dialog box.
- Cluster boundary parameters—The cluster boundary parameters if any of the assays were analyzed with the **Classification Scheme** call method.

Figure 20 Analysis Settings—Example of an exported file

About the audit trail

An audit trail records changes that have been made to a study. Each time you save a study, the software adds a new section to the audit trail to record the changes made for that save. For a description of the types of changes that are recorded, see “Read the audit trail” on page 110.

Export the audit trail

1. In the **Workflow Menu** pane, select **Export ▶ Audit Trail**.
2. Enter information for the file destination:
 - **File Name**—Accept the default file name, or enter a new name. The default format for the file name is:
`study_name_yyyyymmdd_hhmmss_Audit`
where:
 - `study_name` is the name of the current study
 - `yyyymmdd` and `hhmmss` are the current date and time
 - **File Location**—Accept the default location for the exported file, or click **Browse** and select another location.
 - **File Type**—Select the type of file to export:
 - CSV—Exports the data as a comma-separated-value file, where values are delimited by commas. You can open the CSV file with a spreadsheet application (such as Microsoft[™] Excel[™] Software).
 - TXT—Exports the data as a tab-delimited file, where values are delimited by tabs. You can open the TXT file with a spreadsheet application (such as Microsoft[™] Excel[™] Software).
3. Choose the date range for the audit trail.
4. Select/deselect **Open file(s) when export is complete**.

- Click **Export**. If prompted, analyze and save the study to proceed with the export.

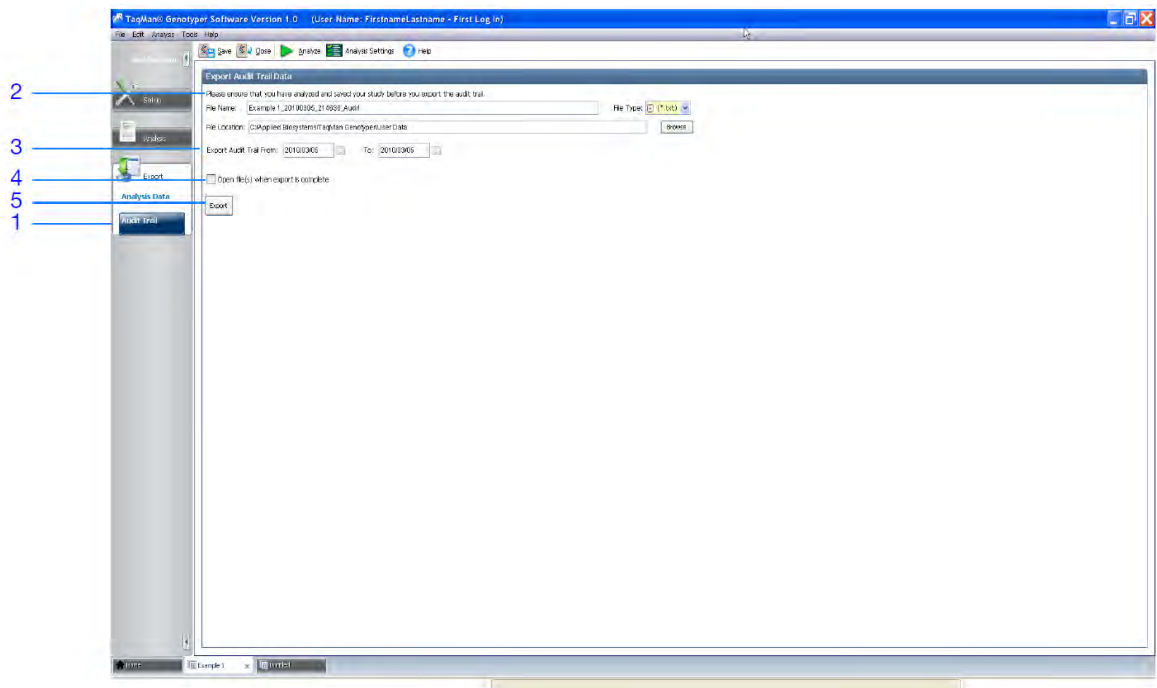


Figure 21 Audit Trail screen (the numbers called out in the screen capture refer to the step numbers in this procedure)

Open the exported audit trail

- Locate the exported audit trail (CSV or TXT file).
- Right-click the file, then select **Open With ▶ Microsoft™ Office Excel™** to view the contents as a spreadsheet in the Microsoft™ Excel™ software.
- Use the AutoFit function in the Excel™ software to make the data easier to read:
 - Click the upper-left corner of the spreadsheet to select all cells.
 - Select **Format ▶ Column ▶ AutoFit Selection**.

Click here

	A	B	C	D	E	F	G	H	I	J
1	# Exported By :	GUEST								
2	# Export Date :	02/08/2010 15:25:47 EST								
3	# Study Name :	Test 012510								
4	# Experiment Type :	Endpoint								
5	# Instrument Type :	7900HT Fast Real-Time PCR System								
6	# Software Version Number :	1.0.0								
7	# Creation Date :	01/25/2010 10:53:28 EST								
8	# Created By :	GUEST								
9	# Last Modified Date :	02/08/2010 15:25:42 EST								
10	# Last Modified By :	GUEST								
11	# Template File Name :									
12	# Template Originating Study Name :									
13	# Template Creation Date :									
14	# Template Created By User ID :									
15	# Template Software Version Number :									
16										
17	Date	User Name	User Full Name							
18	Mon Jan 25 11:14:11 EST 2010	GUEST	GUEST							

Read the audit trail

The audit trail contains:

- Study information—General information about the study. For example, the date/time the study was exported, the user who created the study, etc.
- Save information—Headings and recorded information for each time the study is saved. For descriptions of the headings and recorded information, see “Headings for each save” on page 111.

Study information	1	# Exported By	GUEST								
	2	# Export Date	02/09/2010 13:48:41 EST								
	3	# Study Name	Test 012510								
	4	# Experiment Type	Endpoint								
	5	# Instrument Type	7900HT Fast Real-Time PCR System								
	6	# Software Version Number	1.0.0								
	7	# Creation Date	01/25/2010 10:53:26 EST								
	8	# Created By	GUEST								
	9	# Last Modified Date	02/04/2010 11:50:08 EST								
	10	# Last Modified By	GUEST								
	11	# Template File Name									
	12	# Template Originating Study Name									
	13	# Template Creation Date									
	14	# Template Created By User ID									
	15	# Template Software Version Number									
Save #1	17	Date	Mon Feb 08 15:25:48 EST 2010	User Name	GUEST	User Full Name	GUEST	Headings for Save #1			
	19	Record Name		Record Type	UPDATE	Action	UPDATE				
	20			Study Assays							
	21			Attribute	Old Value	New Value					
	22	C_133314_10		Is Inspected	TRUE	FALSE					
	23										
	24	C_180004_10		Is Inspected	TRUE	FALSE					
	25										
	26	C_218884_10		Is Inspected	TRUE	FALSE					
	27										
	28	Record Name		Record Type	UPDATE	Action	UPDATE				
	29			Study Assays							
	30			Attribute	Old Value	New Value					
	31	GTCallSettings Assay C_2077751_10		Protect	TRUE	FALSE					
	32										
	33	GTCallSettings Assay C_2162777_10		Protect	TRUE	FALSE					
	34										
	35	GTCallSettings Assay C_2165444_10		Protect	TRUE	FALSE					
	36										
	37	GTCallSettings Assay C_2222153_10		Protect	TRUE	FALSE					
	38										
	39	GTCallSettings Assay C_2222357_10		Protect	TRUE	FALSE					
	40										
	41	GTCallSettings Assay C_2222690_10		Protect	TRUE	FALSE					
	42										
	43	Bookmarks		TRUE	FALSE						
	44										
	45										
	46										
	47										
	48										
	49										
	50										
Save #2	150	Date	Mon Jan 25 11:14:11 EST 2010	User Name	GUEST	User Full Name	GUEST	Headings for Save #2			
	152	Record Name		Record Type	INSERT	Action	INSERT				
	153			Study Config							
	154			Attribute	Old Value	New Value					
	155			Experiment Type		Endpoint					
	156			Instrument Type		7900HT Fast Real-Time PCR System					
	157			Comments		()					
	158			Description							
	159			Study Name		Test 012510					
	160			User		GUEST					
	162	Record Name		Record Type	INSERT	Action	INSERT				
	163			Study Assays							
	164			Attribute	Old Value	New Value					
	165	C_2077751_10									
	166			NCBI SNP Reference							
	167			Allele1 Base							
	168			Allele1 Name		C_2077751_10_VIC					
	169			Allele2 Base							
	170			Allele2 Name		C_2077751_10_FAM					
	171			Chromosome							
	172			Contest Sequence							
	173			Description							
	174			Gene Symbol							
	175			Is Inspected		FALSE					
	176			Name							
	177			# of Wells		48					
	178			Position							
	179										
	180										
	181										
	182										
	183										
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Figure 22 Audit trail—Example of an exported file

Headings for each save

Each time you save a study, the software adds a new section to the audit trail to record the changes made for that save. In the exported audit trail, each save is separated by an empty row and includes the following headings:

Save heading	Description
Date	The date/time the audit record was saved
User Name	The user ID for the user who saved the study
User Full Name	The full name of the user who saved the study

Recorded information for each save

After each save, the software records the information listed below.

Record ^[1]	Description
Record Name	(Reserved) The name of the save record
Record Type	The type of information recorded: - Study.Config— The study properties, experiment list, and comments added to the study (Comment History field) - Study.Assays— Assay properties - Study.Sample— Sample properties - Study.AnalysisSetting— Analysis settings - Study.Export— The Export Legend - Study.QCStats— The QC results displayed in the Quality Control screen Note: The record types exported in the audit trail vary per save, depending on the changes made to the data. For example, if no changes are made to the Export Legend, the Study.Export record type does not appear in the audit trail.
Action	The type of change made to the record type: - INSERT— The record is created after the previous save. - UPDATE— The record contains changes since the previous save. - DELETE— The record has been deleted after the previous save.
Attribute: Old Value and New Value	The attributes listed for each save vary, depending on the changes made to the study. The audit trail records the old value and the new value for each attribute. See “About attributes” on page 112.

^[1] The software automatically records the Allele 1 Base as VIC™ dye and the Allele 2 Base as FAM™ dye (that is, the dyes are not read from the assay information file).

About attributes

- For a newly created study, the full set of attributes are listed under all record types, and the Action is INSERT.
- For subsequent changes, only attributes that were changed since the last save are added to the audit trail. In the example above, when the study was saved at 16:29:40 only the description was changed; as a result, the audit trail has only one attribute (Description) in the Study.Config record. The Action is UPDATE.
- The DELETE Action is used when a user deletes all assays or samples. The Study.Assays or Study.Sample attribute is recorded as DELETE.
- Some attributes include a dot notation to separate them into multiple levels. For example, the Study.Assays record type contains up to 100 assays, and each assay has a set of attributes (such as Allele 1 Base). Under Study.Assays, **C__11214910_20.Alele1 Base** refers to the Allele1 base attribute of assay C__11214910_20.

Transfer a study

The Transfer Study function allows you to move a study for backup or sharing purposes. You can:

- Transfer out a study (see page 112).
- Transfer in a study (see page 114).

Where studies are stored

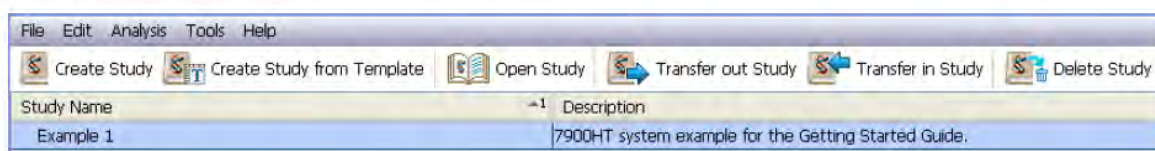
The software stores studies as internal files in the application workspace. These internal files are not accessible to users. The only way to access a study created in another TaqMan™ Genotyper Software application is to transfer it in. Conversely, the only way another user can access a study created in your TaqMan™ Genotyper Software application is for you to transfer the study out.

Modifying studies outside of the TaqMan™ Genotyper Software

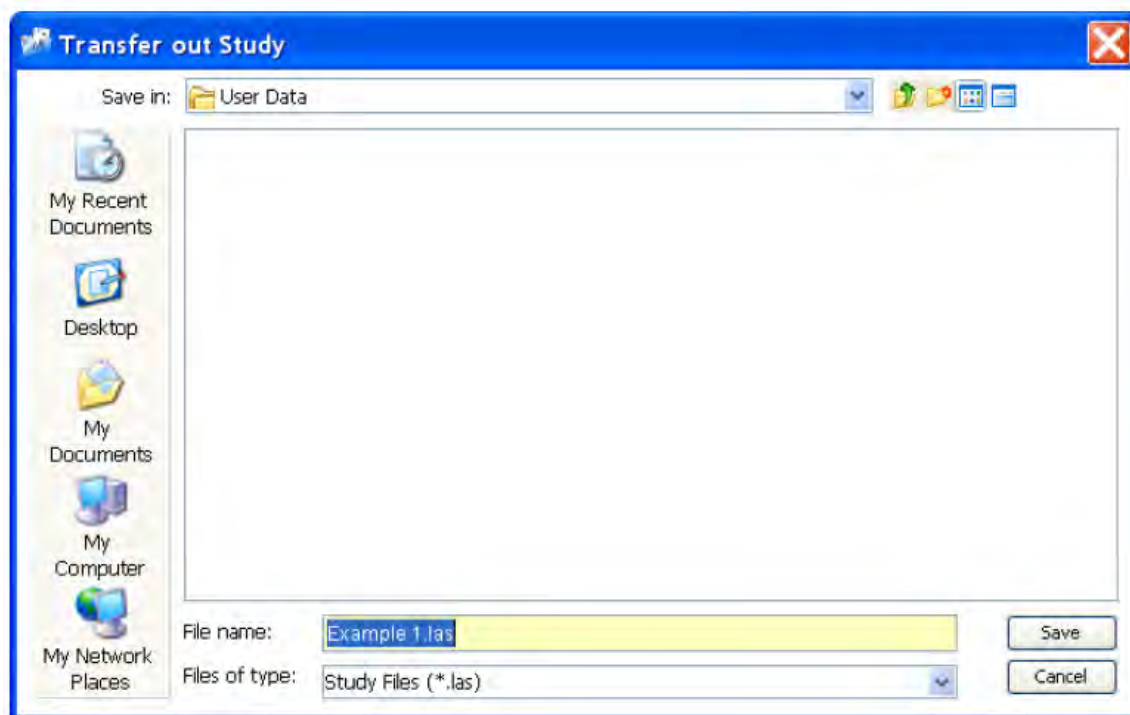
If you modify a study outside of the TaqMan™ Genotyper Software (that is, in another software program), you will not be able to open the study in the TaqMan™ Genotyper Software. The software will display an error message stating that the study is corrupt.

Transfer out

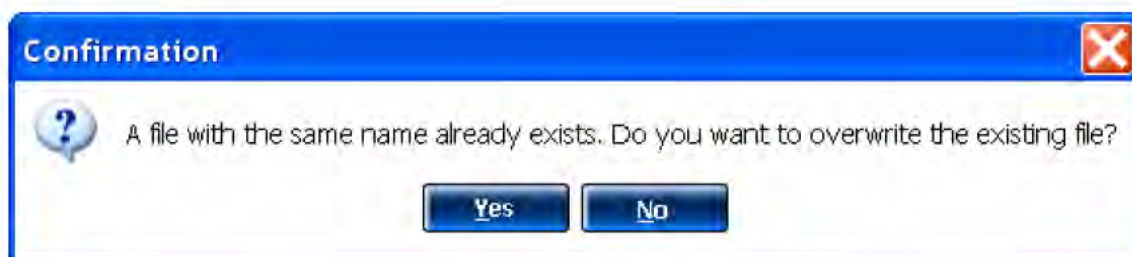
1. In the **Home** screen, select the study of interest, then click  **Transfer out Study**.



2. Browse to a save location, enter a name for the study file (LAS), then click **Save**.



3. If an existing study file (LAS) has the same name as the study you are transferring out, you will receive the following prompt:



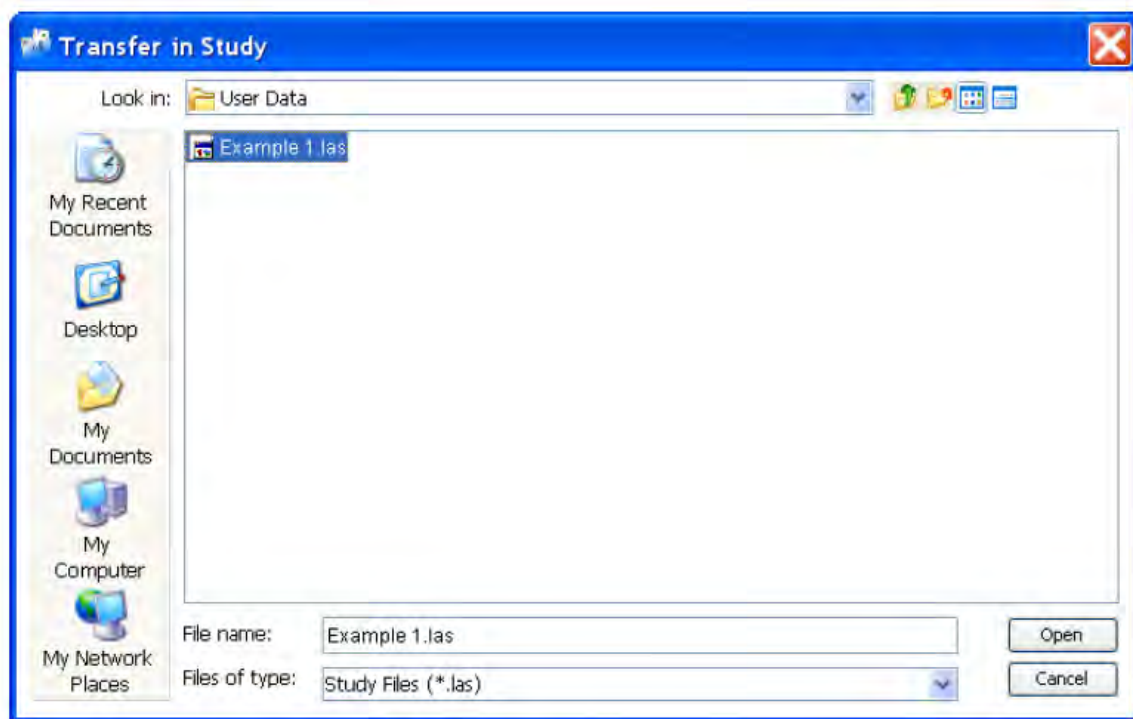
- Click **Yes** to overwrite the existing study file.

IMPORTANT! If you click **Yes**, all information in the existing study file (LAS) will be lost.

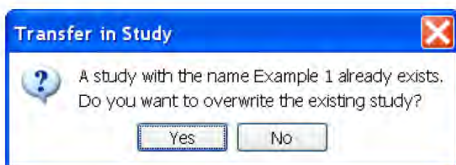
- Click **No** to cancel the transfer.

Transfer in

1. In the **Home** screen, click  **Transfer in Study**.
2. Browse to and select the study file (LAS) of interest, then click **Open**.



3. If an existing study has the same name as the study file you are transferring in, you will receive the following prompt:



- Click **Yes** to overwrite the existing study.

IMPORTANT! If you click **Yes**, all information in the existing study will be lost.

- Click **No** to cancel the transfer.



Create a study from a study template

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■ Workflow	116
■ Generate a study template	116
■ Create a study from a template and enter properties	118
■ Import experiments	119
■ (Optional) Import assay, sample, and reference sample information	120

Required user role

You may not be able to perform all of the procedures in this chapter. The table below indicates the user roles that are allowed to perform each procedure in this chapter.

Note: For more information on user roles, see “User roles and tasks” on page 20.

Function	User role		
	Administrator	Scientist	Technician
Generate a study template (see page 116)	✓	✓	—
Create a study from a study template (see page 118)	✓	✓	✓
Enter the study properties (see page 119)	✓	✓	✓
Import experiments (see page 119)	✓	✓	✓
(Optional) Import assay, sample, and reference sample information (see page 120)	✓	✓	✓ ^[1]

^[1] The Technician can import sample information, but cannot import assay or reference sample information.

Workflow

“Generate a study template” on page 116



“Create a study from a template and enter properties” on page 118



“Import experiments” on page 119



“(Optional) Import assay, sample, and reference sample information” on page 120

Generate a study template

Required user role

Only the Administrator and Scientist user roles are allowed to generate study templates. A typical workflow looks like this:

- The Scientist creates a study for the purpose of generating a study template. The Scientist enters assay information and sets analysis criteria in the study, then generates the study template.
- The Technician uses the study template that the Scientist created to create a study. The Technician cannot change the assay information or analysis criteria.

About study templates

You can use study templates to create studies that have predefined analysis criteria and assay information. A study template contains:

Category	Contents
Analysis settings (as set in the Analysis Settings dialog box)	Call settings
	Control identifiers
	QC settings
All assay information	All assay information included in the study (for example, Assay IDs, allele names, etc.)
Traceability information	The user name of the person who generated the study template
	The date and time the study template was last saved
	The name of the study that the study template was created from ^[1]
	The software version number

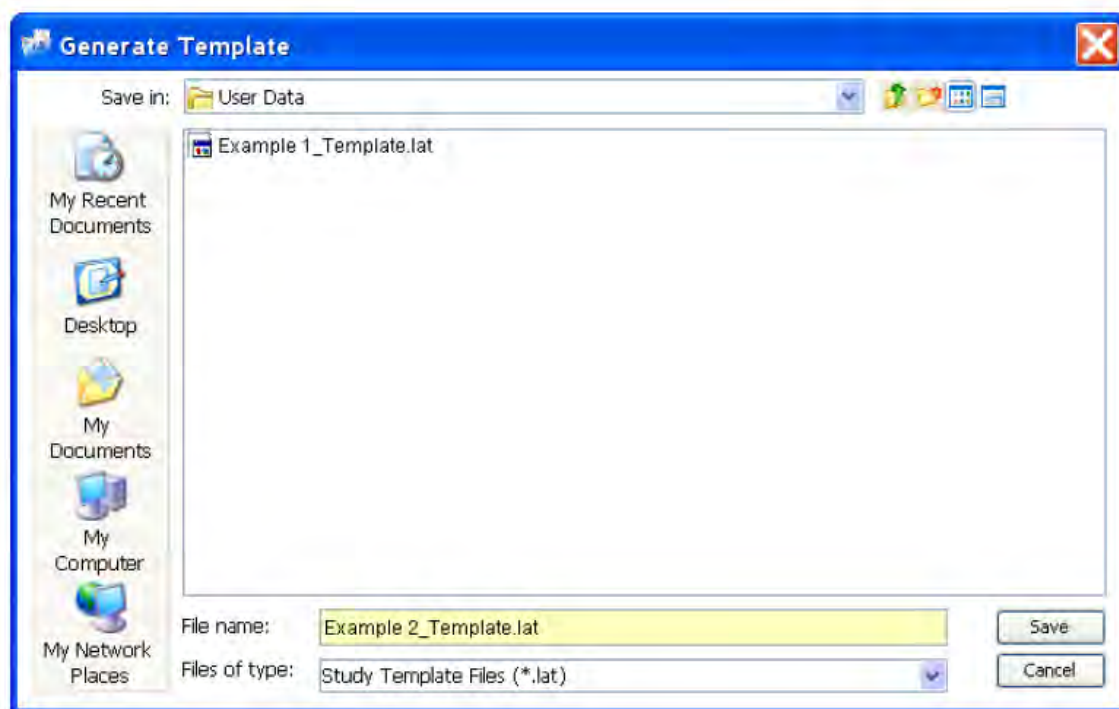
^[1] The study name is displayed in the software and is also exported in the audit trail.

Generate a study template

1. Create a study according to the procedures in Chapter 4, “Create a study”.
2. In the toolbar, click  **Analysis Settings** to open the **Analysis Settings** dialog box. Be sure that the analysis settings are correctly set up.
3. In the **Workflow Menu** pane, select **Setup ▶ Assays** to view the **Assays** screen. Be sure that all assays that will be used in future studies using this study template are present.

IMPORTANT! You will not be able to add any assays to a study if the assays are not in the study template used to create the study.

4. Analyze the study according to the procedures in Chapter 6, “View and analyze the data” until you are satisfied with the study results.
5. In the menu bar, select **File ▶ Generate Template** to open the **Generate Template** dialog box.
6. Browse to and select a save location, enter a name for the study template (or accept the default name), then click **Save**.



About modifying existing study templates

You cannot modify existing study templates. To make changes, generate a new study template:

1. Open the original study (the study from which the study template was created).
- OR

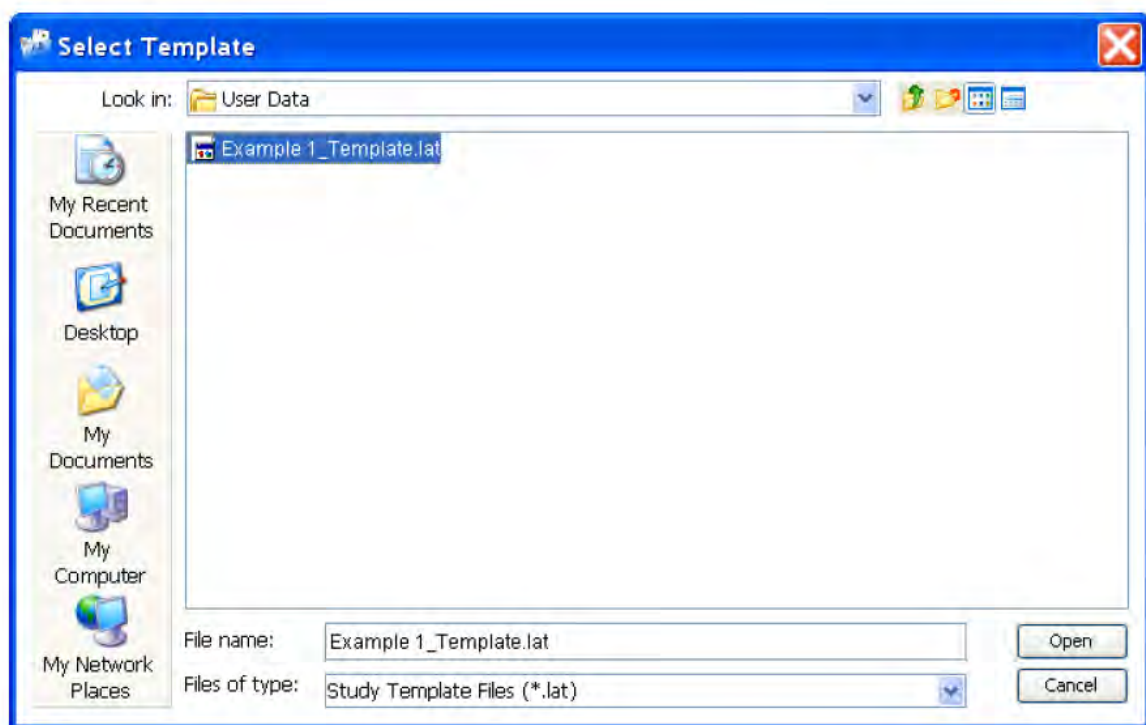
Create a new study using the study template.

2. Change the study as desired, then save the study.
3. Generate a new study template (see “Generate a study template” on page 117).

Create a study from a template and enter properties

Create a study from a study template

1. In the toolbar on the **Home** screen, click  **Create Study from Template**.
2. In the **Select Template** dialog box, browse to and select the study template file (LAT) of interest, then click **Open**.



Creating studies without a study template

If you have the Administrator or Scientist user role, you can also create a study without using a study template (). For procedures, see Chapter 4, “Create a study”.

IMPORTANT! If you have the Technician user role, you must create studies from a study template. You cannot create studies using the  button.

Enter the study properties

Enter study properties to identify the study:

1. In the **Workflow Menu** pane, select **Setup ▶ Properties** to open the **Study Properties** screen. (The **Study Properties** screen is the default view.)
2. Enter a study name.
3. (Optional) Enter a description of the study.
4. (Optional) In the **Comments** field, enter comments for the study, then click **Add**. The software records the comments, your user name, and the date/time you added the comments.

IMPORTANT! The **Comments** field allows you to enter detailed information about the study (for example, observations about the data, reasons why you made specific decisions, etc.). After you click **Add**, the comment is permanently recorded in the study (that is, the comment cannot be modified or removed) and the comment is included in any audit trails that are exported for the study (see “Export an audit trail” on page 107).

Note: You can enter comments at any time. You may prefer to enter comments after viewing the data. See “(Optional) Enter comments for the study” on page 98.

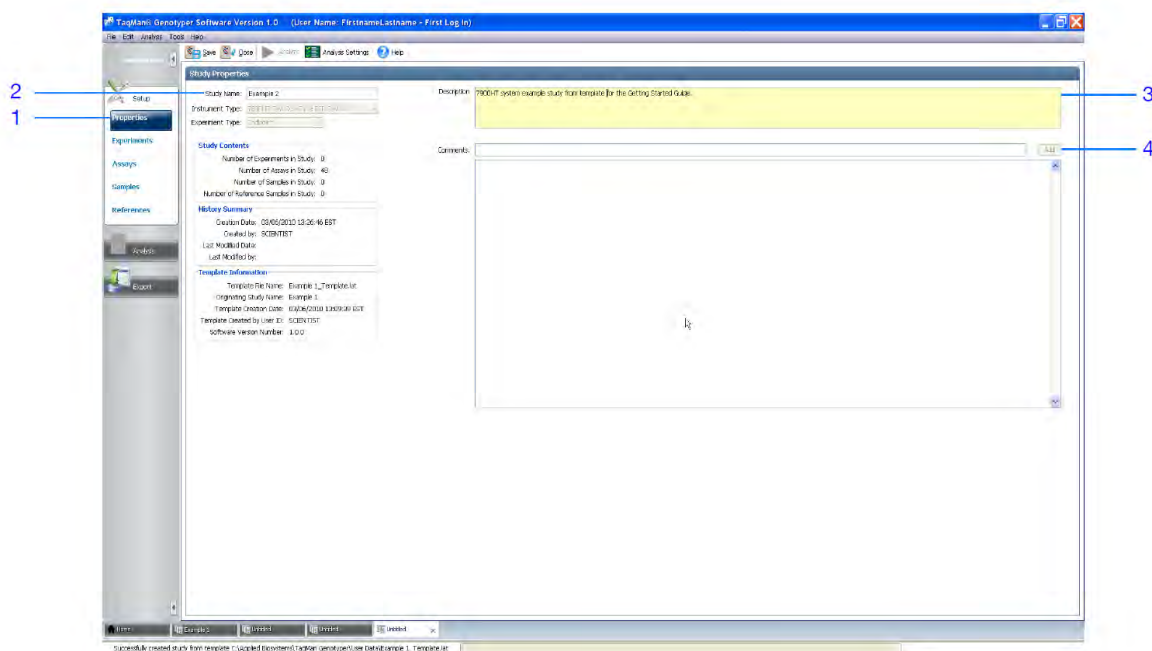


Figure 23 Properties screen (the numbers called out in the screen capture refer to the step numbers in this procedure)

Import experiments

Import experiments into the study (see “Import experiments” on page 47).

(Optional) Import assay, sample, and reference sample information

Import the following into the study:

- Assay information (see [page 52](#)).
- Sample information (see [page 55](#)).
- Reference panel files (containing reference sample information). See [page 59](#).



Assay information files

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■ Editing assay information files	122

About assay information files

Download assay information files for the assays you are using from thermofisher.com/taqmanfiles. The assay information file contains reference information about your order and technical details of all assays in the shipment. See details about assay information files in *Understanding Your Shipment* (Pub. No. MAN0017153).

File types

The assay information may be provided as a TXT file and/or as both XML and HTML files, depending on the product line and order date. You can use the:

- HTML file as a reference; open HTML files in a Web browser.
- XML and TXT files for electronic data importation and manipulation.

Importing into the TaqMan™ Genotyper Software

You can use the assay information file to import assay information into the TaqMan™ Genotyper Software. The TaqMan™ Genotyper Software supports only TXT and XML files, HTML files cannot be imported.

For information on importing assay information into the TaqMan™ Genotyper Software, see “(Optional) Import assay information” on page 52.

Example assay information files

For your reference, the TaqMan™ Genotyper Software includes the following example assay information files:

- AIF_Sample.txt
- OIF_Sample.txt
- Sample_AIX.xml

The default location is:

C:\Applied Biosystems\TaqMan Genotyper\Examples\<file name>

Editing assay information files

Before importing the assay information file into the TaqMan™ Genotyper Software, you can:

- Edit the existing columns.
- Add an Assay Name column and/or a Description column to the assay information file.

Note: This procedure is for editing assay information files *outside* of the TaqMan™ Genotyper Software. For information on editing assay information within the software, see “(Optional) Edit the assay using the Edit Assay dialog box” on page 54.

Columns that are imported into the software

The table below describes the columns of the assay information file that are imported into the TaqMan™ Genotyper Software.

Column name	Description	Example
Assay ID	An alphanumeric string that identifies the assay	C__7586657__20
Reporter 1 Dye	The reporter dye label for the reporter 1 probe	N/A
Reporter 2 Dye	The reporter dye label for the reporter 2 probe	N/A
Context Sequence	The 25-nucleotide sequences surrounding the target SNP or other polymorphism	5' – TCC TCA GAG GGA TCA CTT GTG ACA T[C /T] GGT AGC ATG GAG AAG GT A GGT GGA G–3'
Gene Symbol	The Entrez Gene symbol for the gene	ABCB1
NCBI SNP Reference	The NCBI identification number that corresponds to the SNP	rs1045642

Open the assay information file

Open the file with the appropriate program:

Option	Description
TXT and XML files	Select Open with Excel™ .
HTML files	Select a browser option.

Edit the assay information file

1. Open the TXT or XML file for the assay information file of interest (see “Open the assay information file” on page 122).

IMPORTANT! You can edit only TXT and XML assay information files. You cannot edit HTML files.



2. Edit the information in any of the existing cells, as needed. For the columns that are imported into the TaqMan™ Genotyper Software, follow the editing constraints described below.

Column name	Editing constraints
Assay ID	The assay ID must be 1 to 20 characters in length.
Reporter 1 Dye	Enter up to 8 characters for the reporter dye label.
Reporter 2 Dye	Enter up to 8 characters for the reporter dye label.
Context Sequence	• Enter up to 80 characters for the context sequence. • Bracket the target SNP or other polymorphism: TCCTCAGAGGGATCACTTGTGACAT[C/T]GGTAGCATGGAGA AGGTAGGTGGAG The TaqMan™ Genotyper Software reads the context sequence, then extracts the Allele 1 base and Allele 2 base. In the example above, the Allele 1 base is C and the Allele 2 base is T.
Gene Symbol	Enter up to 80 characters for the gene symbol.
NCBI SNP Reference	Enter up to 20 characters for the reference number.

3. (Optional) Add a new column labeled **Assay Name** anywhere in the spreadsheet, then enter a name for the assay. You can enter up to 128 characters in the **Assay Name** field. When the assay information file is imported into the software, the assay name is displayed in the **Setup ▶ Assays** screen and in the **Analysis ▶ Results** screen.

Note: Assay names are optional because the TaqMan™ Genotyper Software uses the assay ID, and not the assay name, to correlate assay information when you import experiments into a study. However, you may find it easier to recognize the assay names rather than the assay IDs when you view the data in the software.

4. (Optional) Add a new column labeled **Description** anywhere in the spreadsheet, then enter a description of the assay. You can enter up to 1024 characters in the **Description** field. When the assay information file is imported into the software, the description is displayed in the **Setup ▶ Assays** screen and in the **Analysis ▶ Results** screen.

Note: A description can be any information about the assay that you want to display in the software. If an RS number is not available for the assay when data is exported, the description is used in place of the RS number in the export files.

5. Save the assay information file as a TXT or XML file, then close the file.



Supplementary sample information (SSI) files

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■ Create an SSI file	124

About SSI files

SSI files are text (TXT) files that contain information about one or more samples in a study. You create SSI files in a spreadsheet application, then import the files into the TaqMan™ Genotyper Software to update the sample information for a study.

The TaqMan™ Genotyper Software includes an example SSI file (`SSI_Sample.txt`) that you can use as a starting point for creating your own SSI files. The default location is:

`C:\Applied Biosystems\TaqMan Genotyper\Examples\SSI_Sample.txt`

Create an SSI file

1. Open a spreadsheet application (such as Microsoft™ Excel™ Software).
2. In each row, enter the following information for each sample. The sample ID (column A, **Sample ID**) is required; all other information is optional.

Column:	A (required)	B	C	D	E
Heading:	Sample ID	Gender	Population	Non-Consent Assay IDs ^[1]	Concentration ^[2]
Examples:	Samp1	F	African-American	a1, a4	0.5 ng/μL
	Samp3	F	Caucasian	a4	0.5 ng/μL
	Samp4	M	Japanese		0.5 ng/μL

^[1] Assay IDs for any assays you want to exclude from the sample.

^[2] Sample concentration.

You can list the samples in any order, but you must follow these parameters:

- Enter one sample per row.
 - Enter the column headings exactly as shown, including uppercase and lowercase letters:
 - **Sample ID**
 - **Gender**
 - **Population**
 - **Non-Consent Assay IDs**
 - **Concentration**
 - Enter a sample ID in the **Sample ID** column. In order for the software to correctly correlate sample information in the SSI file with sample information in the imported experiment files, the sample IDs in the SSI file must exactly match the sample IDs in the experiment file, including uppercase and lowercase letters.
 - For any assay that you want to exclude from the sample, enter the assay ID in the **Non-Consent Assay IDs** column; separate multiple assay IDs by commas. All assay IDs must exactly match the assay IDs in the experiment file. If you do not include information in the **Non-Consent Assay IDs** column, all assays are included for that sample.
3. Save the spreadsheet as a text (TXT) file.



Optimizing scatter plot data (for Real-time experiment types)

- Recommended for advanced users 126
- Change the cycle number and baseline normalization 126

Recommended for advanced users

IMPORTANT! We recommend that only advanced users optimize the scatter plot data.

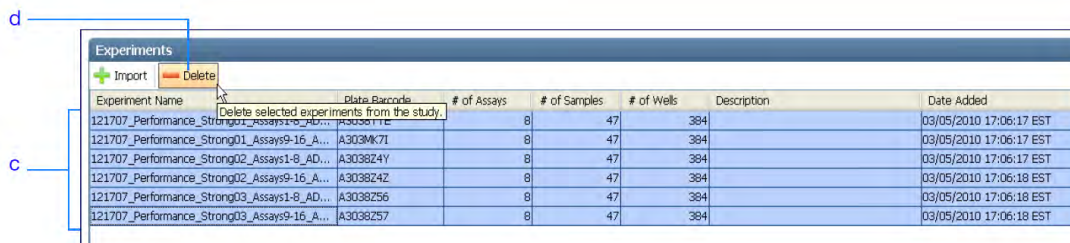
Change the cycle number and baseline normalization

IMPORTANT! The TaqMan™ Genotyper Software does not retain real-time data for the real-time experiment types. The assay parameters are applied only when the experiment file is loaded into a study. You must first delete the experiments that contain the assays to be changed, change the settings in the **Analysis Settings** dialog box, then add the experiments back into the study.

1. Delete the experiments from the study:
 - a. Open the study of interest.
 - b. In the **Workflow Menu** pane, select **Setup ▶ Experiments**.
 - c. In the **Experiments** screen, select the experiments in the study that contain the assays to be changed (press the Ctrl key or Shift key when you select the experiments).

Note: If needed, you can delete all of the experiments in the study.

- d. Click **Delete**, then click **Yes** to confirm.



2. In the toolbar, click **Analysis Settings** to open the **Analysis Settings** dialog box.



3. Select the **Call Settings** tab.
4. Select or deselect the **Baseline for Real-time Data** checkbox.

Note: To apply the change to all assays in the study, select/deselect the baseline in the **Study-Level Settings** pane. To apply the change to individual assays, select/deselect the baseline in the **Assay-Specific Settings** pane.

5. In the **Cycle Number** field, change the cycle number used to generate the endpoint R_n plots. The cycle number you enter must be between cycle number 20 and cycle number 60. If you leave the **Cycle Number** field empty (blank), the software interprets that as the final cycle.

Note: To apply the change to all assays in the study, change the cycle number in the **Study-Level Settings** pane. To apply the change to individual assays, change the cycle number for the applicable rows in the table in the **Assay-Specific Settings** pane.

The **Show Cycle** slider in the scatter plot is updated to correspond to the number of cycles set in the **Analysis Settings** dialog box.

If the **Show Cycle** slider in the scatter plot was adjusted, the cycle number is also updated in the **Analysis Settings** dialog box. It is updated for the assay in the **Assay-Specific Settings** pane.

6. Click **OK** to save the changes and close the **Analysis Settings** dialog box.
7. Add the deleted experiments back to the study (see “Import experiments” on page 47).

Analysis Settings

Set the default analysis settings.

Call Settings | Control Identifiers | QC Settings

Study-Level Settings

Call Method: ☒ Autocalling ☐ Classification Scheme

Multipate Analysis: ☒ Use Hardy-Weinberg for Analysis: ☐ Baseline for Real-time Data: ☒

Protect Manual Calls: ☒ Use Positive Controls for Analysis: ☒ Baseline from: 5 to 15

Normalize Cluster: ☐ Heterozygote: Allow Cycle Number:

Use Reference Panels for Autocalling: ☒

Assay ID: Find (* = any string, ? = any character)

Assay-Specific Settings

Assay ID	Assa...	Call Method	Protect	Cluster Norm	Reference	H-W	Hetero...	Baseline	Baseline Start	Baseline End	Cycle #	Reset
----------	---------	-------------	---------	--------------	-----------	-----	-----------	----------	----------------	--------------	---------	-------

OK Cancel Apply

Figure 24 Analysis Settings dialog box, Call Settings tab



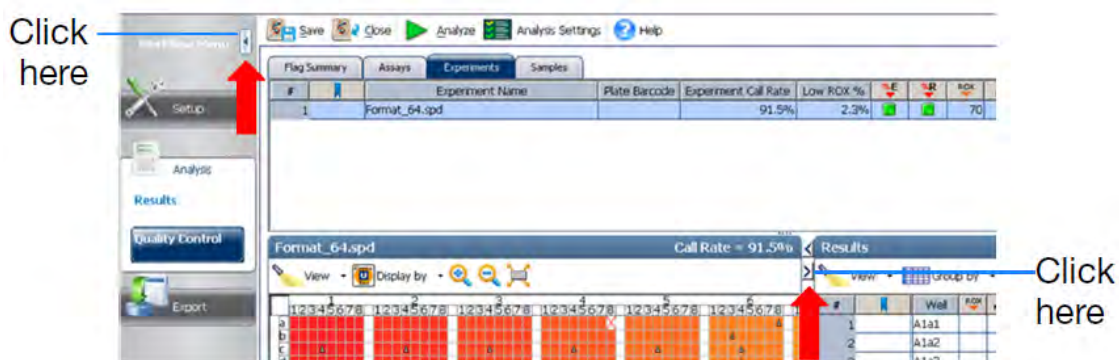
Troubleshooting genotyping data

■ How to view data for troubleshooting purposes	128
■ Troubleshooting table	133
■ Risks	135
■ Impact of analysis settings	143

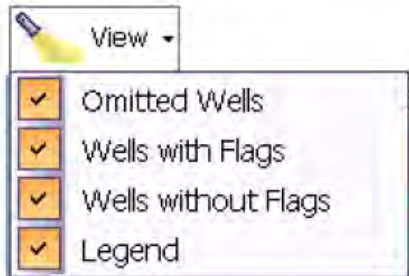
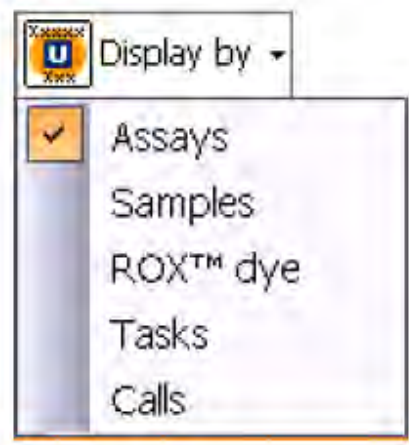
How to view data for troubleshooting purposes

View the data in the Quality Control screen

1. In the **Workflow Menu** pane, select **Analysis** ▶ **Quality Control**, to open the **Quality Control** screen.
2. Select the **Flag Summary** tab to identify the number of failures throughout the study.
3. View the call rates in the **Assays**, **Experiments**, and **Samples** tabs.
4. View the plate layout:
 - a. Select the **Experiments** tab, then select an experiment. The software displays the data for the selected experiment in the plate layout and in the **Results** table.
 - b. (Recommended for TaqMan™ OpenArray™ Plates) Click the expand buttons to expand the plate layout view.



c. Use the **View** and **Display by** drop-down lists to select the items to display.

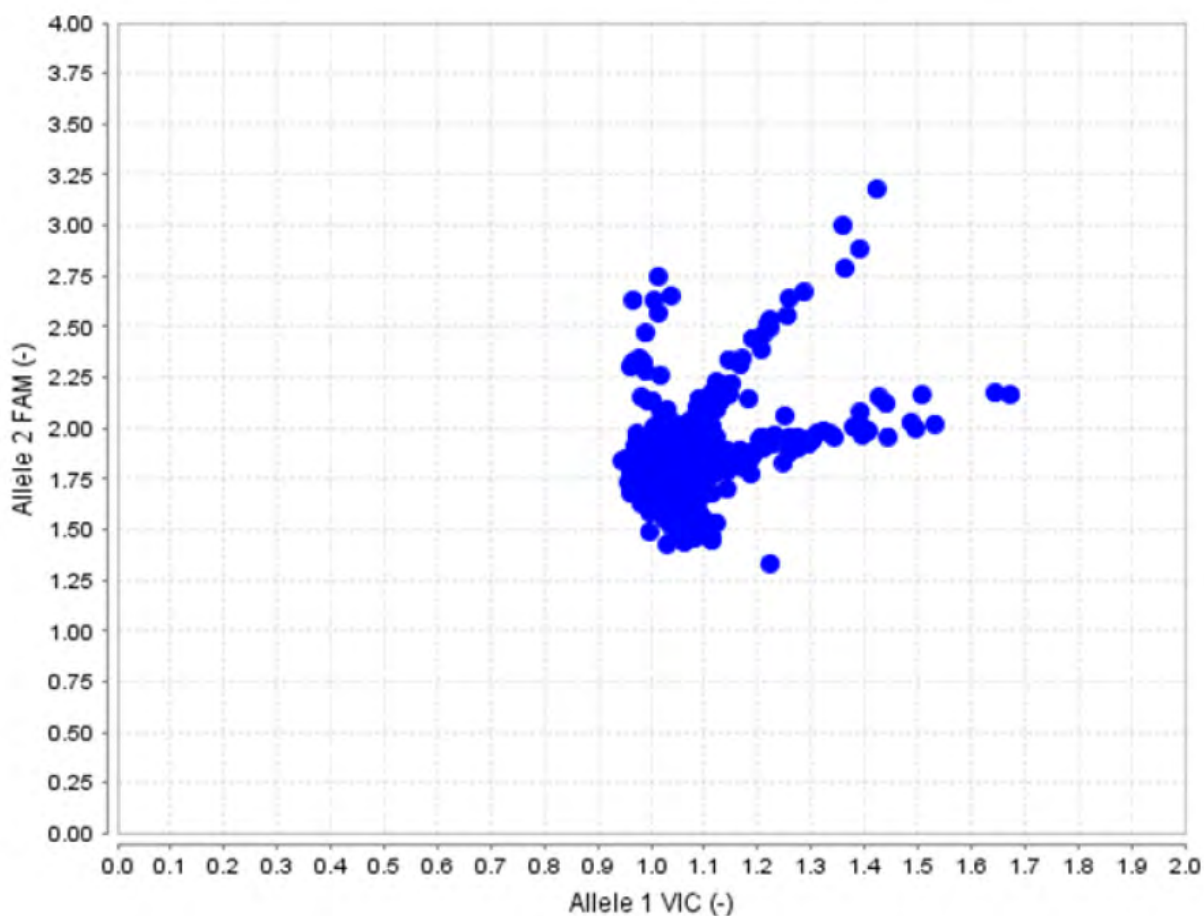
Menu	Options	Illustration
View	• Omitted Wells • Wells with Flags • Wells without Flags • Legend	
Display by	• Assays • Samples • ROX™ dye • Tasks • Calls Note: The Display by drop-down list options can be selected only one at a time.	

Example 1: 7900HT Fast Real-Time PCR System

Problem

In this example, none of the experiments in the study included no template controls (NTCs) on the reaction plate. In the absence of NTCs as reference points, the TaqMan™ Genotyper Software algorithm bases its genotype calls on the VIC™ dye and FAM™ dye values and assigns a FAM™ dye/FAM™ dye call to all samples, rather than *Undetermined* or *No Amplification*.

In the scatter plot below, you can see that most samples do not amplify, and all samples are assigned the same genotype.

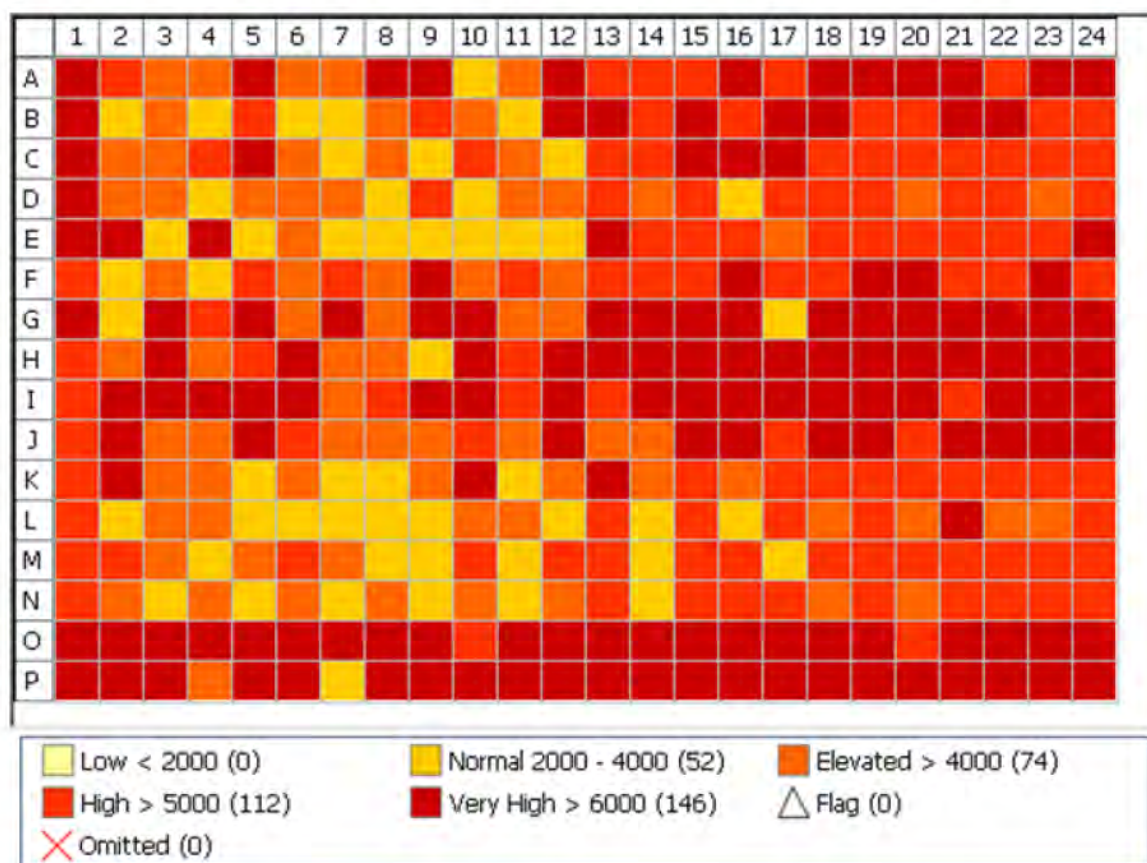


Use the Quality Control screen to determine the cause

1. In the **Quality Control** screen, select the **Experiments** tab to view the plate layout.
2. From the **View** drop-down list, select **Legend**.
3. From the **Display by** drop-down list, select **ROX™ dye**.

In a good experiment, the ROX™ dye level should be uniform throughout the reaction plate. In this example, the ROX™ dye level shows high variability across the reaction plate. This indicates experimental problems, such as:

- Pipetting errors
- Evaporation
- Poor quality of the DNA samples (the samples likely contain PCR inhibitors)
- Incorrect quantitation (consider quantitating using RNase P chemistry)



Example 2: TaqMan™ OpenArray™ Plates

Problem

The study in this example contains three TaqMan™ OpenArray™ Plates. One of the samples on one of the plates has a low call rate.

Use the Quality Control screen to determine the cause

1. In the **Quality Control** screen, select the **Assays** and **Samples** tabs to view the assay and sample call rates. The sample circled in red below (sample NA17245) has a low call rate of 60.9%.

Flag Summary											
Assays											
Experiments											
Samples											
#	Sample ID	Sample Call Rate	S	ROX	NTC	NTC	ST	CTL	AD	RF	
38	NA17220	93.8%	0	0	0	0	11	0	0	0	42
39	NA17223	93.8%	0	0	0	0	10	0	0	0	39
40	NA17228	93.2%	0	0	0	0	11	0	0	0	30
41	NA17230	96.9%	0	0	0	0	5	0	0	0	27
42	NA17239	96.9%	0	0	0	0	4	0	0	0	30
43	NA17242	94.5%	0	0	0	0	7	0	0	0	33
44	NA17245	60.9%	64	0	0	0	74	0	0	0	192
45	NA17256	91.1%	0	0	0	0	17	0	0	0	45
46	NA17260	90.1%	0	0	0	0	17	0	0	0	54
47	NTC	N/A	N/A	0	138	37	5	5	0	0	12

2. For each experiment in the study, view the ROX™ dye display in the plate layout to look for potential plate loading errors:
 - a. Select the **Experiments** tab, then select an experiment.
 - b. From the **View** drop-down list, select **Legend**.
 - c. From the **Display by** drop-down list, select **ROX™ dye**.

The ROX™ dye display shows that several subarrays were only partially loaded or not loaded at all on the plate (per the Legend, light yellow indicates a low ROX™ dye level).

- d. Move the cursor over the subarrays with low ROX™ dye levels to identify the sample ID with the low call rate. Sample NA17245 (circled in red below) was not loaded on this plate, which explains the low call rate.

Note: Low ROX™ dye levels in the TaqMan™ OpenArray™ plate subarrays are consistent with unloaded subarrays revealed in the ImageJ software ROX™ dye images.





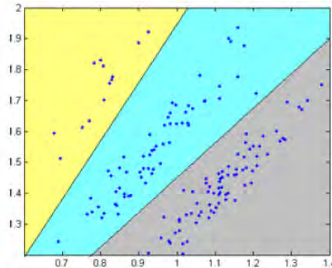
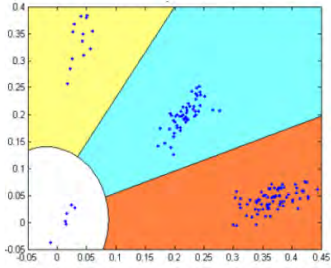
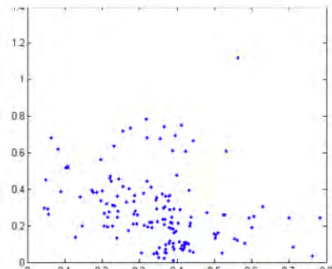
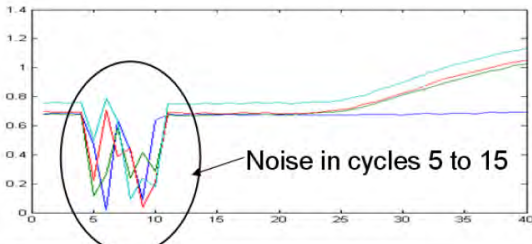
For more information

For more information on the **Quality Control** screen, see “View the data in the Quality Control screen” on page 81.

Troubleshooting table

Observation	Possible cause	Recommended action
The genotypes of replicate samples are discordant The NTCs or negative controls have genotype calls	The wells may have been contaminated with sample from a neighboring well	1. Select Analysis ▶ Quality Control ▶ Experiments to view the plate layout. 2. Compare the genotypes of neighboring samples in the plate to the discordant samples, NTCs, or negative controls. 3. If the neighboring samples carry the “suspicious” genotype, contamination is likely. You should repeat the experiment.
<i>For Real-time experiments</i> Incorrect genotype calls when the data is baselined (that is, Baseline is selected in the Analysis Settings dialog box)	The data from the region selected for the baseline has spurious noise	Select Analysis ▶ Results, then review the data points in question in the scatter plot. After reviewing, you may need to: • Deselect the Baseline checkbox in the Analysis Settings dialog box, then reanalyze the data. • Review the amplification curve in the instrument software.

(continued)

Observation	Possible cause	Recommended action
For Real-time experiments Incorrect genotype calls when the data is baselined (that is, Baseline is selected in the Analysis Settings dialog box)	As shown below, selecting Baseline typically improves the data:  Not baselined  Baselined However, when noise is present in cycles 5 to 15, it may be better to deselect Baseline. In the example below, Baseline is selected, but the three clusters are indistinguishable. When the amplification curves are viewed in the instrument software, spurious noise can be seen in cycles 5 to 15. Note: The baseline region (cycles 5 to 15) cannot be changed. If there is significant noise in this area, the only option is to turn baselining off (deselect Baseline).  Baselined  Noise in cycles 5 to 15 Amplification curves viewed in the original experiment file (instrument software)	
The ROX™ dye value is small	Less sample may have been loaded into the well, which indicates a pipetting error.	Follow good pipetting practices.
The ROX™ dye value is high	There was likely a concentration of ROX™ dye in the well due to evaporation, especially around the edges of the plate.	• Check the volumes in each well. • Check the seal on the reaction plate.



Risks

All data are removed when the software is uninstalled

IMPORTANT! When you uninstall the TaqMan™ Genotyper Software, the InstallShield Wizard permanently removes all data stored in the software installation folder and all its subfolders, including:

- User account information
- Studies
- Study templates
- Reference panels

Saving data before an uninstall

Before you uninstall the software, Applied Biosystems™ recommends that you save data as follows:

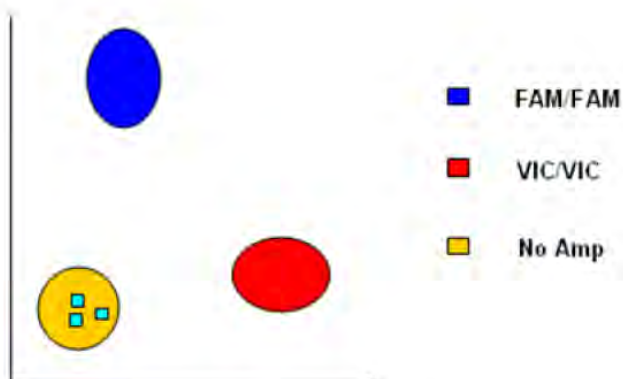
Data type	Backup method
Studies	Keep study data by transferring studies out. See “Transfer a study” on page 112.
Study templates	By default, study templates and reference panels are saved as files in the software installation/user data folder. Copy the user data folder (or selected files in the folder) to a backup device or to another computer. Note: When you select the system preferences, you may choose to save study templates and reference panels to a location other than the software installation/user data folder. See “(Optional) Configure the system preferences” on page 31.
Reference panels	

Y chromosome-specific SNP targets

Assays that detect Y chromosome-specific SNP targets amplify only male samples. Because there is only one copy of the Y chromosome, there is no heterozygote cluster. Female samples lack the Y chromosome and will not amplify; the female samples will cluster around the NTC. See “Heterozygote menu for targets on the X and Y chromosomes” on page 144.

Sample gender information is crucial for the algorithm to correctly call the Y chromosome-specific SNP genotypes. If needed, you can change the gender information in the **Setup ▶ Samples** screen, using the **Edit Sample** dialog box. See “(Optional) Edit the sample using the Edit Sample dialog box” on page 58.

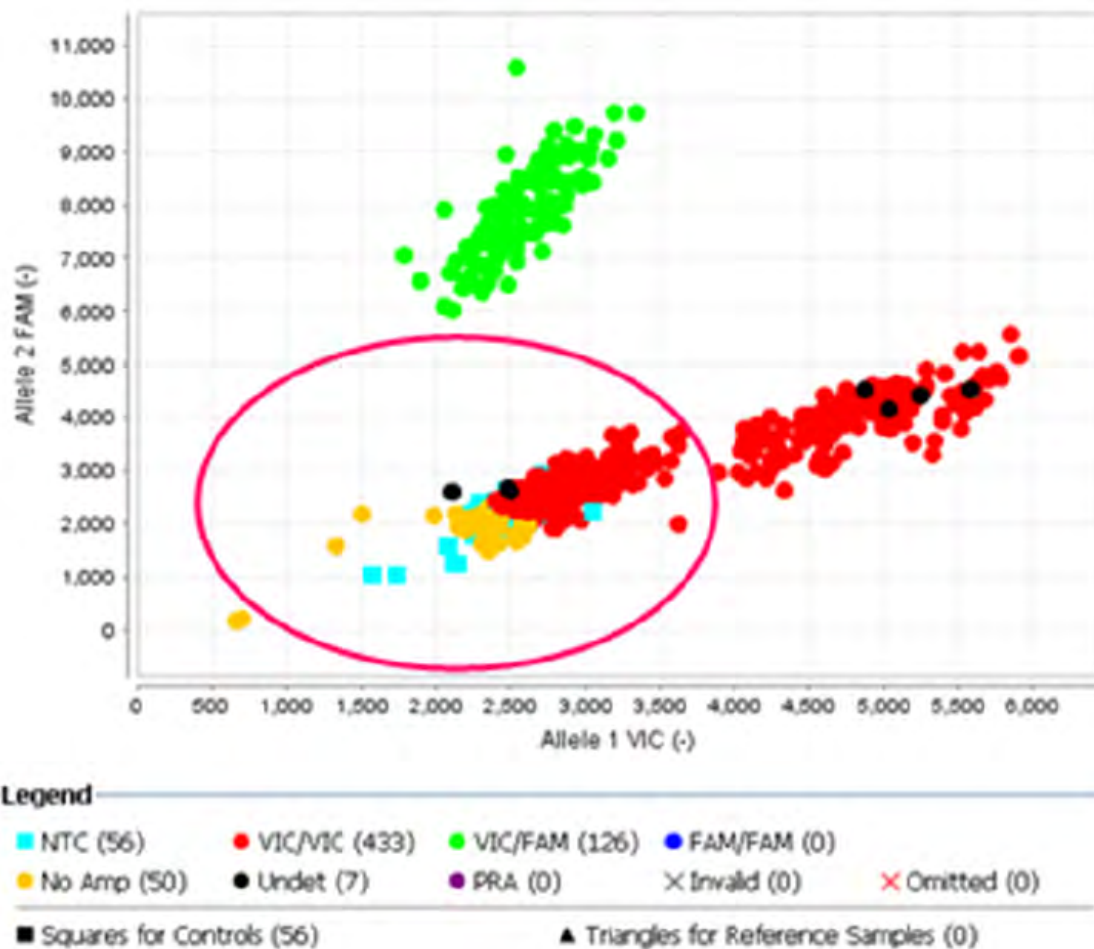
The figure below shows an ideal scatter plot for a Y chromosome-specific assay. All female samples are called as *No Amp*.



What to do if the software assigns an incorrect genotype to female samples

Even when sample gender information is assigned, the TaqMan™ Genotyper Software may occasionally assign an incorrect genotype to female samples.

In the example below, the default analysis settings were applied to a Y chromosome-specific SNP assay. The circled data points around the NTCs are female samples. This assay generates some non-specific amplification in female samples, and the slope of the resulting cluster is similar to the VIC™ dye/VIC™ dye cluster. In addition, the gap between the two clusters is small. The software algorithm interprets the No Amp cluster and the VIC™ dye/VIC™ dye cluster as one cluster and calls most of the female samples as VIC™ dye/VIC™ dye.



To correct the genotype calls:

1. In the toolbar, click **Analysis Settings** to open the **Analysis Settings** dialog box.
2. Select the **Call Settings** tab.
3. Change the default analysis settings for the Y chromosome-specific SNP assay:
 - a. In the **Assay-Specific Settings** pane, select the assay.
 - b. Deselect the **Reference** checkbox.
 - c. Deselect the **H-W** (Hardy-Weinberg) checkbox.
 - d. From the **Heterozygote** drop-down list, select **Disallow in Males**.

e. Click **Apply** to save the changes and reanalyze the assay.

Call Settings Control Identifiers QC Settings

Study-Level Settings

Call Method: ☒ Autocalling ☐ Classification Scheme

Protect Manual Calls: ☒ Use Hardy-Weinberg for Analysis: ☒ Baseline for Real-time Data: ☐

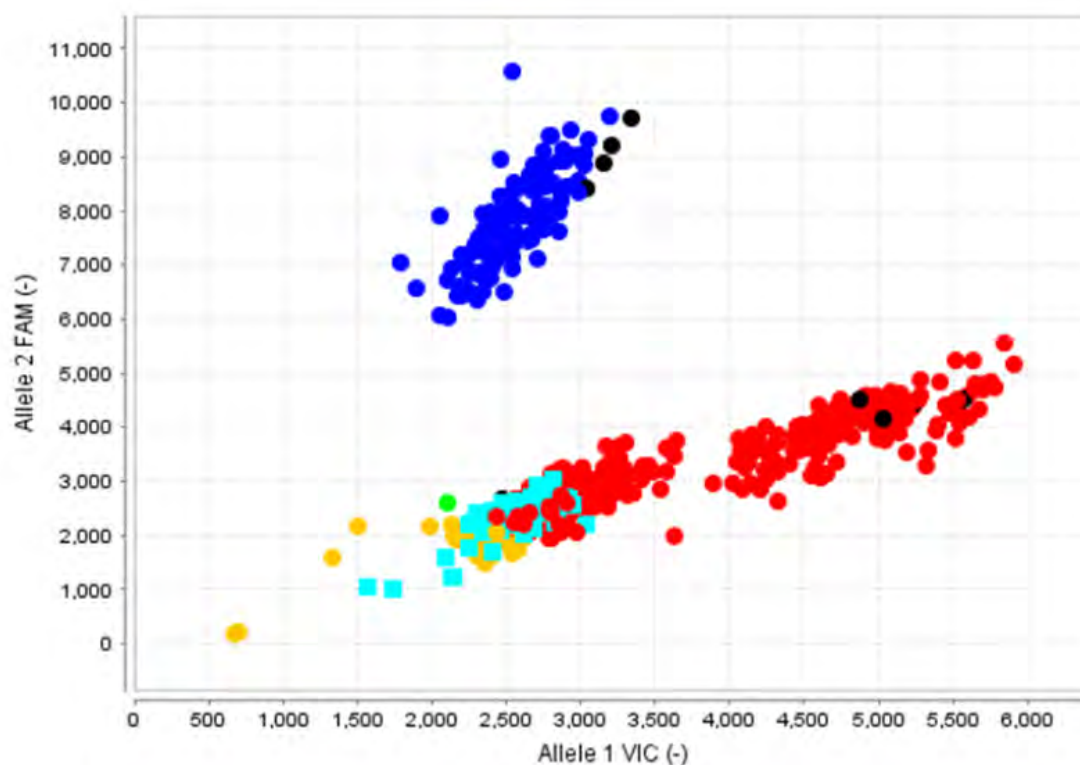
Use Reference Panels for Autocalling: ☒ Use Positive Controls for Analysis: ☒ Cycle Number:

Heterozygote:

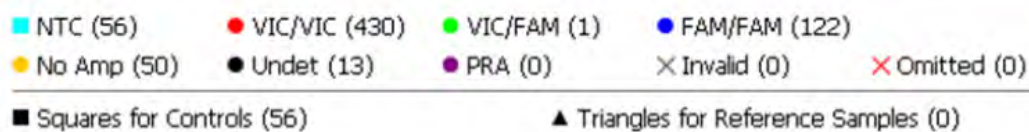
Assay-Specific Settings

Assay ID	Assay Name	Call Method	Protect	Reference	H-W	Heterozygotes	Baseline	Cycle #	Reset
C_2259382_10		Autocalling	☒	☐	☐	Disallow in Males	☒		
C_7558868_10		Autocalling	☒	☒	☒	Allow	☒		
C_3095552_1		Autocalling	☒	☒	☒	Allow	☒		

As shown below, the two male clusters are now correctly called, and there is no heterozygote cluster.

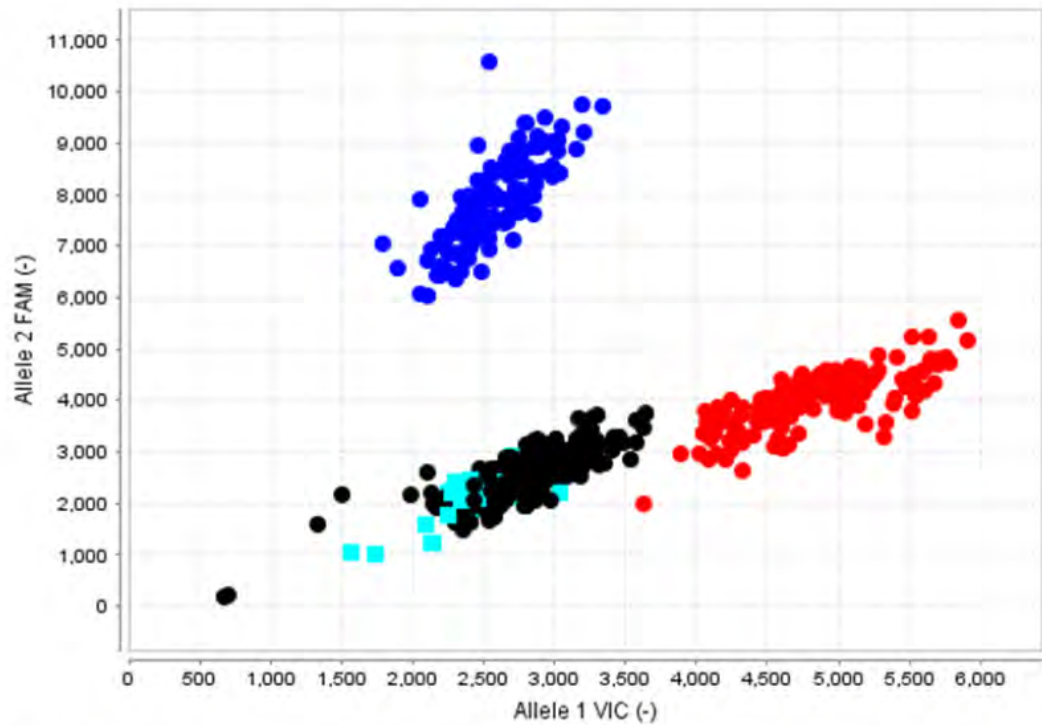


Legend



4. Manually change the calls for the female samples around the NTC to *Undetermined* or *No Amplification*. You can either:

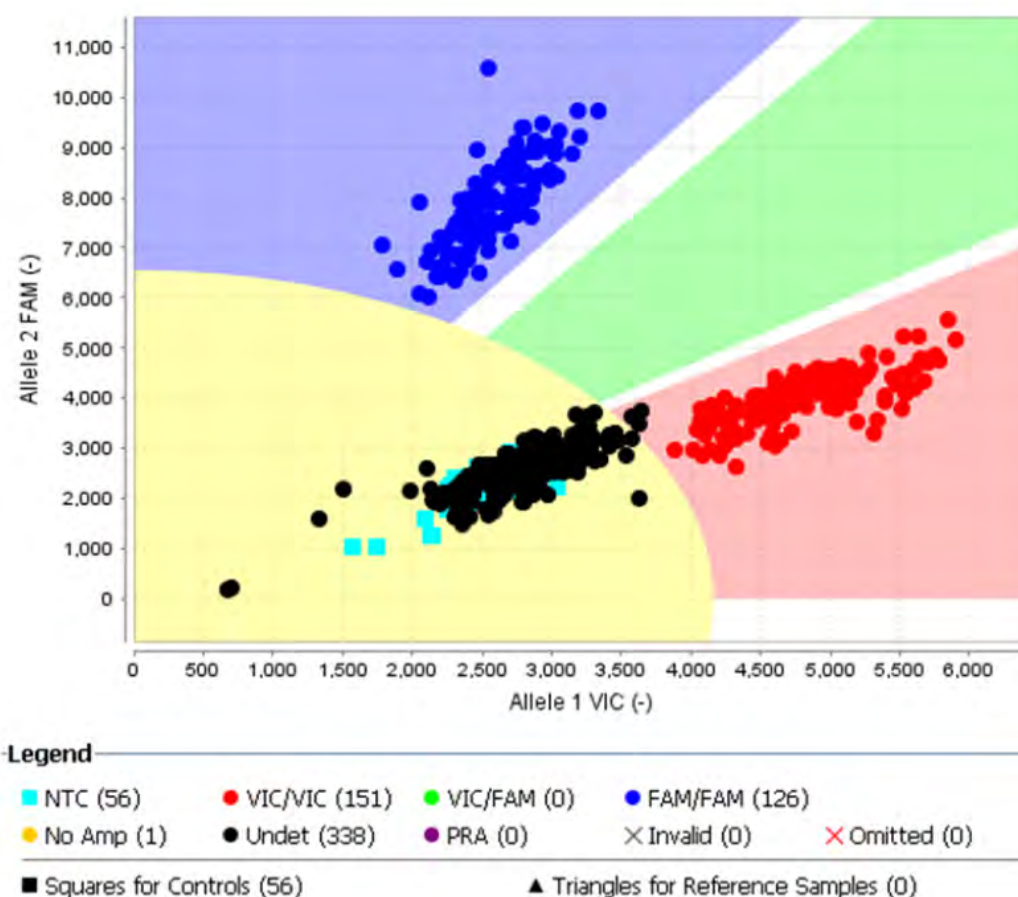
- Change the calls in the **Results** table (see “Review the calls and perform manual calling” on page 68). In the scatter plot shown below, the calls for the female samples were changed to *Undetermined*.



Legend

■ NTC (56)	● VIC/VIC (152)	● VIC/FAM (0)	● FAM/FAM (126)
● No Amp (0)	● Undet (338)	● PRA (0)	× Invalid (0)
■ Squares for Controls (56)	▲ Triangles for Reference Samples (0)	×	×
		×	×

- Apply a classification scheme, then expand the yellow No Amplification region to include the female samples. See “Analyze the data using Classification Schemes” on page 75.



Sample IDs for no template controls

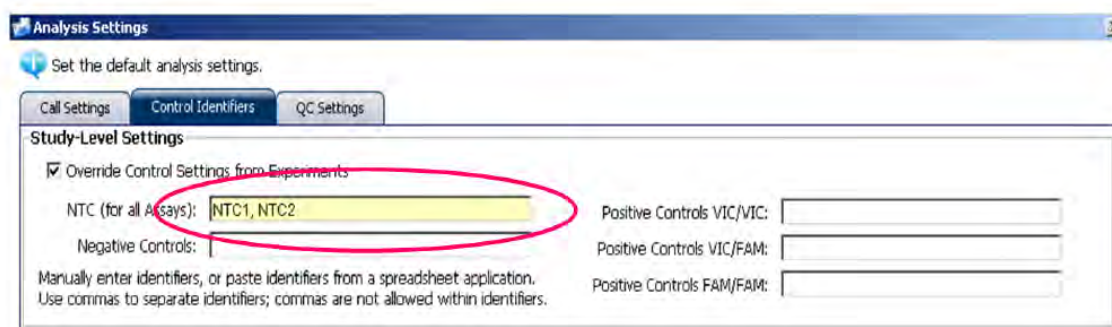
The software automatically recognizes sample IDs of *NTC* as no template controls. If you use any other sample ID for a no template control, you must assign the sample ID with the NTC control identifier.

For example, on a reaction plate with multiple no template controls, you may have sample IDs of *NTC1*, *NTC2*, etc. If these sample IDs are not assigned with the NTC control identifier, the software will not recognize them and will not display them as NTCs in the **Results** and **Quality Control** screens.

To assign the NTC control identifier:

1. In the toolbar, click  **Analysis Settings** to open the **Analysis Settings** dialog box.
2. Select the **Control Identifiers** tab.

3. In the NTC field, enter the sample IDs to use as controls. Use a comma to separate multiple control identifiers.



4. Click **Apply** to save the changes.

For more information on control identifiers, see “Add control identifiers” on page 42.

Data set size

Applied Biosystems™ conducted a study to estimate the effect of data set size and cluster diversity on genotype call accuracy. Based on this study:

- There is no noticeable decrease in call accuracy when the number of data points within a cluster is small.
- There is a decrease in call accuracy when the overall number of data points in all clusters together is small. For this reason, the software algorithm implements a set-size-dependent Quality value cap. The cap is approximately 95% for set size 10 (that is, Quality values will not exceed 95% unless there are at least 10 non-NTC data points).

Plate-to-plate variation

The TaqMan™ Genotyper Software can combine data from multiple experiments (reaction plates) from the same instrument type and analyze them together. The ability to combine data from multiple experiments:

- Is crucial for data sets with a low number of data points (such as data from the TaqMan™ Array Cards) and for assays that are likely to detect rare alleles.
- Allows you to rapidly scan large amounts of data produced by high throughput systems (such as the QuantStudio™ 12K Flex Real-Time PCR System with TaqMan™ OpenArray™ Plates).

Some amount of plate-to-plate variation is observed in the heterozygous and homozygous clusters for the same assay between plates and runs. The software algorithm has a limited ability to account for this plate-to-plate variation. Applied Biosystems™ recommends that you review the results to determine if the plate-to-plate variation was too high for the algorithm to call the combined data appropriately.

Assay IDs and case-sensitivity

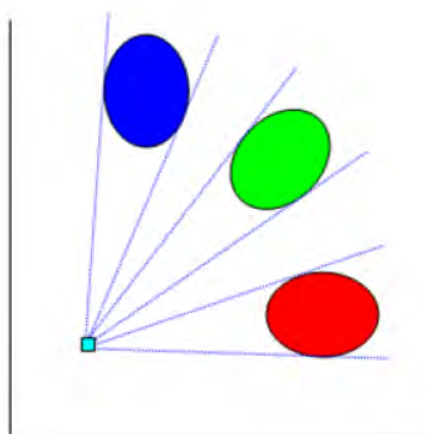
The TaqMan™ Genotyper Software does not differentiate uppercase and lowercase letters in assay IDs. If you have two different assays with the same name, but one includes uppercase letters and the other assay includes lowercase letters, the software interprets the two assays as the same assay. For example, the software recognizes all three assay IDs below as the same assay:

- ABC1a
- Abc1a
- abc1A

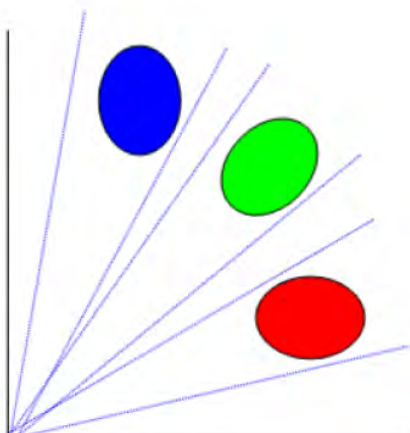
Running reaction plates without no template controls

If you run a reaction plate without a no template control (NTC), the genotype call accuracy may be negatively affected.

As shown in the scatter plot below, the TaqMan™ Genotyper Software algorithm uses the NTC as a starting point for the angles of the other data points.



In the absence of an NTC, the algorithm uses the origin of the scatter plot as a starting point for the angles of all data points. This may result in less accurate genotype calling.



Impact of analysis settings

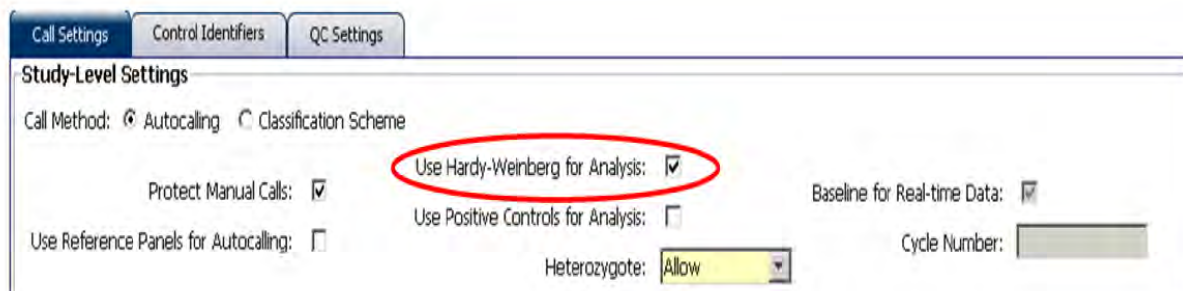
This section describes the impact of the following analysis settings in the **Analysis Settings** dialog box:

- Hardy-Weinberg checkbox (see page 143)
- Heterozygote drop-down list, for haploid targets (see page 144)
- Heterozygote drop-down list, for targets on the X and Y chromosomes (see page 144)
- Positive Controls fields (see page 145)
- Negative Controls fields (see page 147)

Note: To open the **Analysis Settings** dialog box, click  **Analysis Settings** in the toolbar.

Hardy-Weinberg checkbox

For single- and double-cluster targets expected to follow the Hardy-Weinberg equilibrium, select **Use Hardy-Weinberg for Analysis** to favor Hardy-Weinberg compliant genotype distributions over other distributions and reduce the risk of incorrect calls.



Note: X and Y chromosome-specific SNP targets do not follow the Hardy-Weinberg distribution. SNP targets located in the pseudo-autosomal region of the X and Y chromosomes behave like autosomal SNP targets and follow the Hardy-Weinberg distribution.

Heterozygote menu for haploid targets

SNP targets in haploid genomes (for example, mitochondria) cannot be heterozygous because there is only one copy of the chromosome. For SNP targets in haploid genomes, select **Disallow** from the **Heterozygote** drop-down list to improve the accuracy of genotype calls.

The screenshot shows the 'Study-Level Settings' window with three tabs: 'Call Settings', 'Control Identifiers', and 'QC Settings'. The 'Call Settings' tab is active. It contains the following settings:

- Call Method: ☒ Autocalling ☐ Classification Scheme
- Protect Manual Calls: ☒
- Use Reference Panels for Autocalling: ☐
- Use Hardy-Weinberg for Analysis: ☒
- Use Positive Controls for Analysis: ☐
- Baseline for Real-time Data: ☒
- Heterozygote: **Disallow** (circled in red)
- Cycle Number:

Note: If you select **Disallow** for other types of targets, the genotype call accuracy may be negatively impacted.

Heterozygote menu for targets on the X and Y chromosomes

SNP targets on the:

- X chromosome cannot be heterozygous in males because males carry only one copy of the X chromosome.
- Y chromosome cannot be heterozygous in males because there is only one copy of the Y chromosome.

Y chromosome-specific assays will not amplify female samples because females do not carry the Y chromosome.

Note: SNP targets located in the pseudo-autosomal region of the X and Y chromosomes behave like autosomal SNP targets and will follow the Hardy-Weinberg distribution.

For SNP targets on the X and Y chromosomes, select **Disallow in Males** from the **Heterozygote** drop-down list to improve the accuracy of the genotype calls.

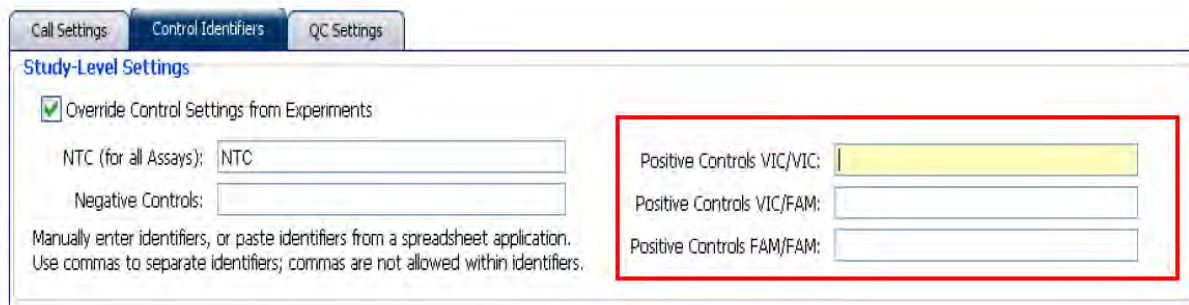
The screenshot shows the 'Study-Level Settings' window with three tabs: 'Call Settings', 'Control Identifiers', and 'QC Settings'. The 'Call Settings' tab is active. It contains the following settings:

- Call Method: ☒ Autocalling ☐ Classification Scheme
- Protect Manual Calls: ☒
- Use Reference Panels for Autocalling: ☐
- Use Hardy-Weinberg for Analysis: ☒
- Use Positive Controls for Analysis: ☐
- Baseline for Real-time Data: ☒
- Heterozygote: **Disallow in M...** (circled in red)
- Cycle Number:

Note: If you select **Disallow in Males** for other types of targets, the genotype call accuracy may be negatively impacted.

Positive controls fields

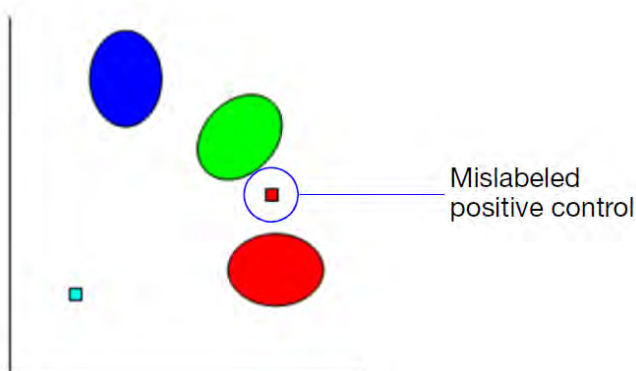
When you identify a sample ID as a positive control (or reference sample), the TaqMan™ Genotyper Software uses the positive control data to bias the calls of the Unknown data points. If you assign an incorrect genotype to the positive control, the genotype call accuracy may be negatively impacted.



The software validates positive controls to some extent because the software weighs the location of other data points against the location of the positive control. If the genotype assigned to the positive control is likely incorrect, the software overrides the control's impact on the calls of the Unknown data points. See "Example 1" on page 145, "Example 2" on page 146, and "Example 3" on page 146.

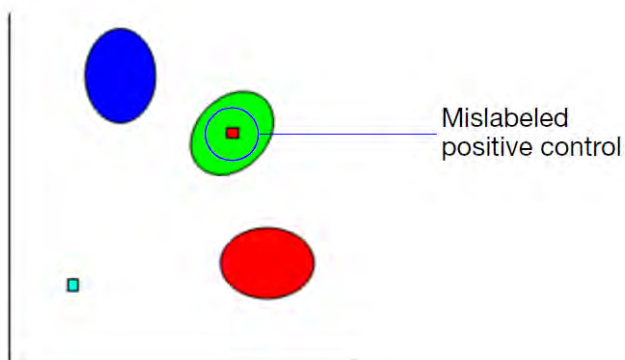
Example 1

In Example 1, the positive control is VIC™ dye/FAM™ dye, but it has been incorrectly labeled as VIC™ dye/VIC™ dye. In the scatter plot, this positive control shows an ambiguous location between the heterozygous cluster and the homozygous VIC™ dye/VIC™ dye cluster. If there is a mislabeled positive control in this location, the genotype call accuracy will be negatively impacted.



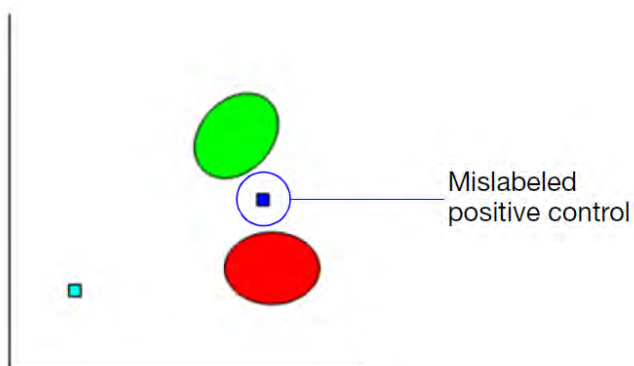
Example 2

In Example 2, the positive control is VIC™ dye/FAM™ dye, but it has been incorrectly labeled as VIC™ dye/VIC™ dye. In contrast to Example 1, the mislabeled positive control is clearly located within the heterozygous cluster (see “Example 1” on page 145). The control genotype is likely incorrect, and the software overrides the control’s impact on the calls of the Unknown data points.



Example 3

Example 3 is a special scenario with fewer than three clusters. In Example 3, the positive control is VIC™ dye/FAM™ dye, but it has been incorrectly labeled as FAM™ dye/FAM™ dye. Because there are fewer than three clusters, the positive control heavily influences the genotype calls; in this instance, the entire heterozygous cluster may be called as FAM™ dye/FAM™ dye.



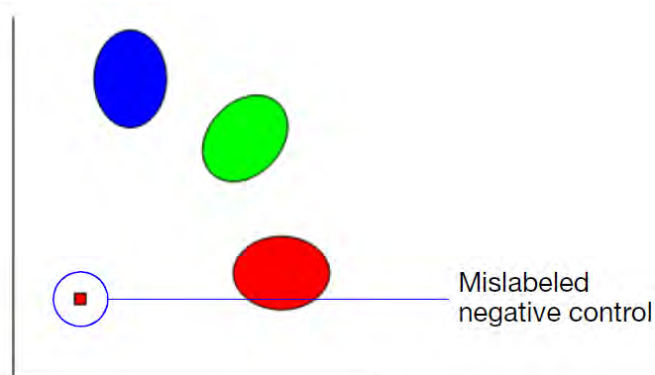
Negative controls field

When you identify a sample ID as a negative control, the TaqMan™ Genotyper Software can correct for nonspecific amplification patterns. If you assign an incorrect sample ID to the negative control, the genotype call accuracy may be negatively impacted.

The software validates negative controls to some extent because the software weighs the location of other data points against the location of the negative control. If the genotype assigned to the negative control is likely incorrect, the software overrides the control's impact on the Unknown data points. See "Example" on page 147.

Example

In this example, the negative control has been incorrectly labeled as VIC™ dye/VIC™ dye. In the scatter plot, the negative control is clearly separated from all three clusters. The control genotype is likely incorrect, and the software overrides the control's impact on the calls of the Unknown data points.





Documentation and support

Related documentation

Document	Pub. No.
<i>TaqMan™ Genotyper Software Quick Reference</i>	4448638

Customer and technical support

Visit thermofisher.com/support for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
 - Product FAQs
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- Order and web support
- Product documentation
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

