Agilent MassCode PCR Research Solution

Software Familiarization Guide

Revision A, August 2011

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Agilent Technologies

Notices

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Software Revision

This guide applies to the Agilent G9200AA MassCode PCR Software version A.01.00 or higher until superseded.

If you have comments about this guide, please send an e-mail to masscode.support@agilent.com.

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In This Guide...

This guide takes you through a series of exercises to familiarize you with the operation and data analysis capabilities of the MassCode PCR software.

Chapter 1 Exercises for Basic Users

The exercises in this chapter are performed in the software's Basic mode. The Basic mode is appropriate for users who intend to use Agilent-supplied target panels and LC/MS methods and have no or little experience with LC/MS technology.

Chapter 2 Exercises for Advanced Users

The exercises in this chapter are performed in the software's Advanced mode. The Advanced mode is appropriate for users who may want to customize their own target panels and/or have used LC/MS technology before.

Before you start...

Where to find more information

- The *Agilent MassCode PCR Research Solution Quick Start Guide* provides an overview of the solution.
- If you are setting up the system yourself, the *Agilent MassCode PCR Research Solution Installation Guide* covers modification of the LC/MS instrument and installation of the MassCode PCR software. Install the software before you start the exercises in this guide.
- The software's online help system provides in-depth information on the operation of the MassCode PCR program and can be displayed in the following ways:
  - Click **Help > Contents** from the menu bar.
  - Press [F1] to get more information about the currently displayed screen or dialog box.
- Agilent Tech Support can be reached by phone or email.
  - *Email:* masscode.support@agilent.com or click **Help > Email Feedback** in the software.
  - *Phone:* From the US and Canada call 800-227-9770 (select options 3-4-3). For Agilent's worldwide sales and support center telephone numbers, go to www.agilent.com/chem/contactus.

How to use this guide

The exercises in the *Software Familiarization Guide* are intended to familiarize you with the processes for working with experiments, including defining the plate setup, running an experiment, and analyzing experiment results. The data analysis exercises use the sample experiment file that comes pre-loaded with the MassCode PCR program.

The exercises in chapter 1 are performed in the program's Basic mode. The exercises in chapter 2 are performed in the Advanced mode. Select the set of exercises that is most appropriate for your needs and experience level.

We recommend reading this guide and performing the exercises before you begin your own experiment.

Contents

Chapter 1
Exercises for Basic Users

The exercises in this chapter are performed in the software's Basic mode. The Basic mode is appropriate for users who intend to use Agilent-supplied target panels and LC/MS methods and have no or little experience with LC/MS technology.

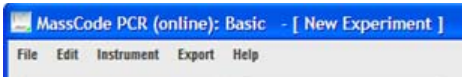
Exercise 1. Launch the online program and open the help system

The tasks in this exercise show you how to launch the MassCode PCR program, switch to Basic mode, and explore the contents of the software's help system.

Task 1. Launch the program and switch to Basic mode

Task 1 shows you how to open the online program and set the program to Basic mode.

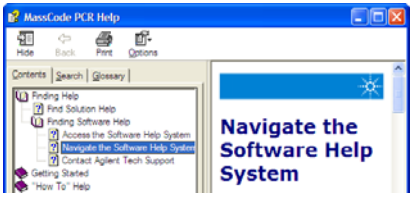
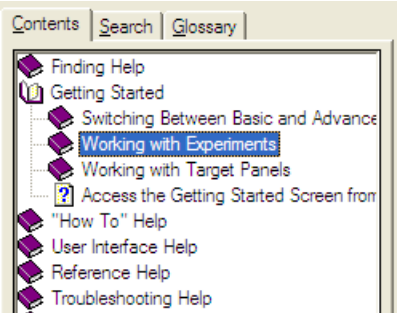
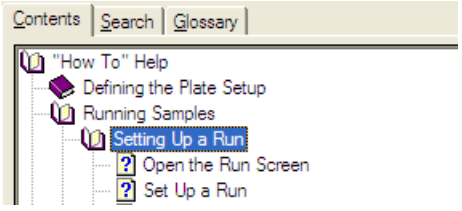
Task 1. Launch the program and switch to Basic mode

Steps	Detailed Instructions	Screen Images
1 Open the online MassCode PCR program. The online program is used to connect to the LC/MS.	• Double-click the MassCode PCR Online program icon on your desktop. The program opens to the Getting Started screen.	
2 Check that you are in the Basic mode by examining the title bar of the program window.	• If the title bar lists **Basic** you are in Basic mode. • If the title bar lists **Advanced**, click **File > Switch to Basic** and check the title bar again.	

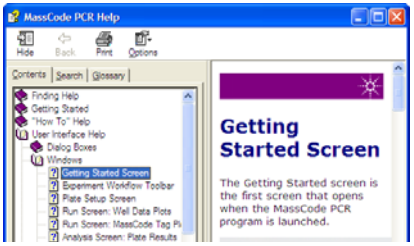
Task 2. Use the help system

Task 2 shows you how to open and navigate the software help system.

Task 2. Use the help system

Steps	Detailed Instructions	Screen Images
1 Open the help system.	• Click **Help > Contents**. The MassCode PCR Help window opens to the topic *Navigate the Software Help System*.	
2 Locate and read the help topic *Working with Experiments*.	**a** In the table of contents on the left side of the window, double-click the book called *Getting Started*. The book expands to show the contents of the chapter. **b** Click on the sub-book *Working with Experiments*. The contents of the *Working with Experiments* help topic opens on the right side of the Help window. **c** Read the topic to learn about MassCode PCR experiments and the experiment workflow.	
3 Locate the How-To help topics.	**a** Double-click the book called *"How To" Help*. The book expands to show the contents of the chapter. **b** Double-click any of the sub-books to view the topics contained within. **c** Click on any individual topic to read its contents.	
4 Close the help system.	• Click the X in the upper right corner of the MassCode PCR Help window.	

Task 2. Use the help system

Steps	Detailed Instructions	Screen Images
5 Open the help topic for the currently open screen of the software (the Getting Started screen).	• On the keyboard, press [F1]. The help window opens with the topic for the Getting Started screen displayed on the right side. From any screen or dialog box in the program, you can press [F1] to open the *User Interface Help* topic pertaining to that particular screen or dialog box.	
6 Close the help system.	• Click the X in the upper right corner of the MassCode PCR Help window.	

Exercise 2. Start a new experiment

The tasks in this exercise show you how to define the plate setup for a new experiment plate, set up a run, and save the experiment settings in the Basic mode.

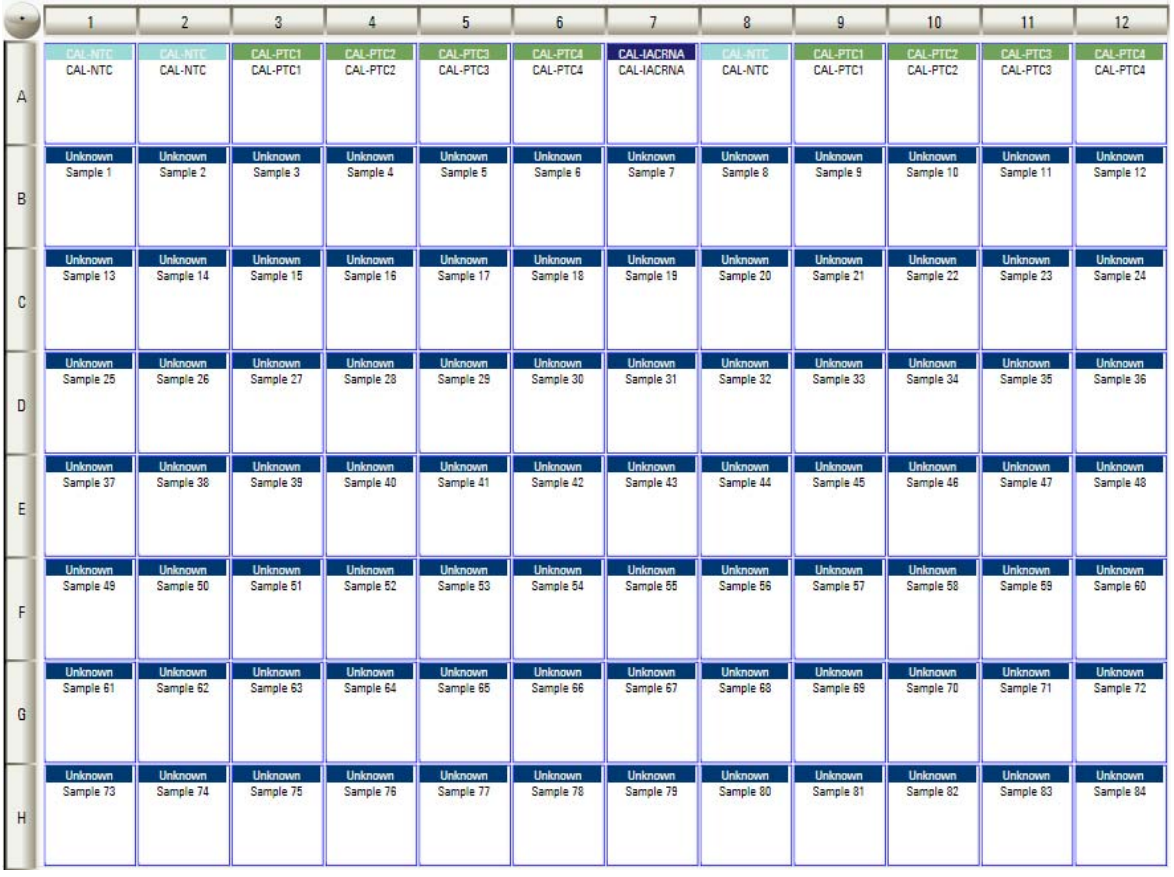


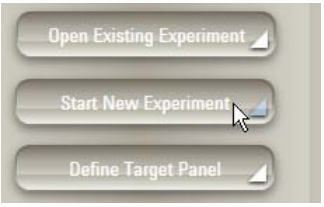
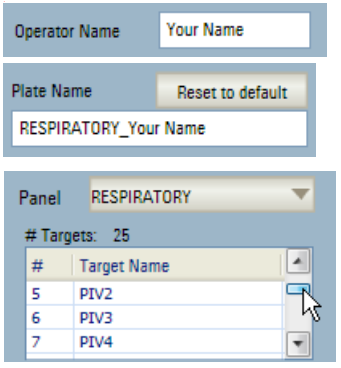
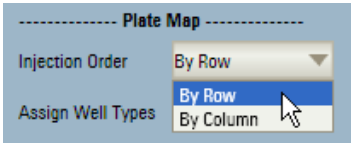
Figure 1 Completed Plate Setup

Task 1. Define the plate setup

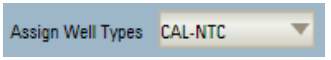
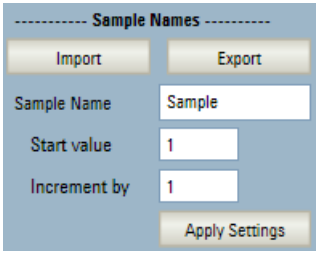
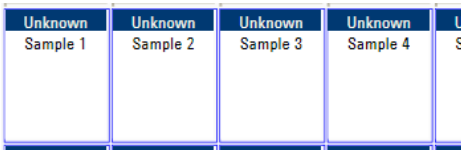
Task 1 walks you through the process of defining the plate setup. The steps cover assigning the operator name and plate name, selecting the panel and injection order, assigning the well types, and assigning sample names.

The goal is to set up a plate that mimics the one used in the sample experiment that comes pre-loaded with the MassCode PCR program. The plate setup you create in this task is shown in Figure 1 on page 11.

Task 1. Define the plate setup

Steps	Detailed Instructions	Screen Images
1 Open a new experiment.	• Click **Start New Experiment** on the Getting Started screen. The program opens to a blank plate setup.	
2 Enter the operator name and select the target panel. • **Operator Name:** Your name • **Panel:** Respiratory (The respiratory panel is the default target panel.)	**a** Type your name into the **Operator Name** field on the left side of the screen. Once you remove the cursor from the field, the operator name is added to the default plate name. **b** In the **Panel** drop-down list, check that RESPIRATORY is selected. To see the targets included in the panel, you can scroll through the list (see image on right).	
3 Set the injection order to *By Row*. *By Row* is the default injection order.	• In the **Injection Order** drop-down list, check that **By Row** is selected. This selection indicates to the software to process the wells of the plate from left to right during the run, starting with row A.	

Task 1. Define the plate setup

Steps	Detailed Instructions	Screen Images
4 Assign the calibrator wells. • **Wells A1, A2 and A8:** CAL-NTC well type • **Wells A3 and A9:** CAL-PTC1 well type • **Wells A4 and A10:** CAL-PTC2 well type • **Wells A5 and A11:** CAL-PTC3 well type • **Wells A6 and A12:** CAL-PTC4 well type • **Well A7:** CAL-IACRNA well type	**a** Select the group of wells that are to be assigned to the same well type. • To select multiple wells, press [Ctrl] while clicking on individual wells. • To select/deselect all wells on the plate, click the circle in the upper left corner of the plate image. **b** In the **Assign Well Types** drop-down list, select the desired well type.	   **Important:** A total of 12 calibrator wells is required for proper data analysis.
5 Assign the remaining wells to the Unknown well type.	**a** With the cursor, click and drag across the block of wells from B-01 to H-12 to select all remaining wells. **b** In the **Assign Well Types** drop-down list, select **Unknown**.	
6 Assign sample names to the Unknown wells. At the completion of this step, refer to Figure 1. At this point, your plate setup should be identical to that shown in the figure.	**a** Assign the core sample name. • With the Unknown wells selected, type "Sample" followed by a space into the **Sample Name** field. The name "Sample" appears in each Unknown well. **b** Add incremental numbers to the sample names. • In the **Start value** field, leave the default value of 1. • In the **Increment by** field, leave the default value of 1. • Click **Apply Settings**. An incremental number is added to the end of each sample name.	  Because the injection order is *By Row*, the numbering is assigned horizontally.

Task 2. Set up a run

Task 2 shows you how to set up a run. In Basic mode, the only run settings that can be changed are the name and location of the data file that is created when a run is started. The data file is a folder (with extension *.D*) that contains all the settings and data for an experiment.

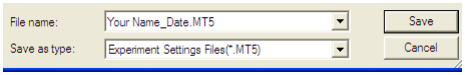
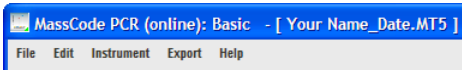
Task 2. Set up a run

Steps	Detailed Instructions	Screen Images
1 Navigate to the Run view.	• In the workflow toolbar, click **Run**.	
2 Change the data file name. • **Data File Name:** Sample_your name.D. Data file names must end in ".D" and are limited to 40 legal characters.	• In the **Data File Name** field, type "Sample_" followed by your name. End the name with ".D".	

Task 3. Save the experiment settings

Task 3 shows you how to save the experiment settings to a separate Experiment Settings file. The Experiment Settings file (file extension MT5) contains the complete settings for an experiment: the plate setup, the run settings, and the experiment notes.

Task 3. Save the experiment settings

Steps	Detailed Instructions	Screen Images
• Save the Experiment Settings file. Note that if you want to open a saved Experiment Settings file, you can do so from the Getting Started screen (click **File > Open Experiment Settings File**).	**a** Click **File > Save Experiment Settings As**. The **Save As** dialog box opens. **b** In the **File name** field, type your name and today's date separated with an underscore. **c** Click **Save**.	 After the experiment settings have been saved, the name of the MT5 file appears in the title bar. 

Exercise 3. Open an existing experiment

The tasks in this exercise show you how to open an existing (post-run) experiment, save a copy of the experiment, and print out a copy of the *Results Interpretation Tables* from the software's help system.

For this exercise and the remaining exercises, we will work with the sample experiment that comes pre-loaded with the MassCode PCR program. You are instructed to perform the exercises in the offline program, but these exercises can also be performed in the online program. To proceed in the online program, simply skip step 1 in Task 1 below (do not close ChemStation).

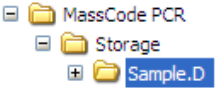
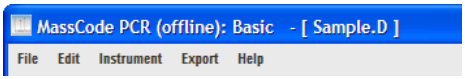
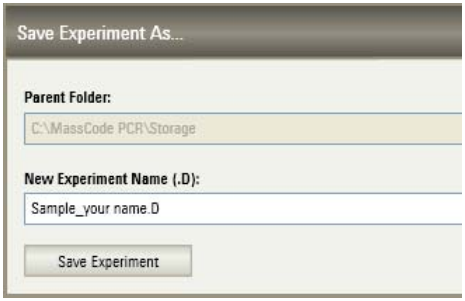
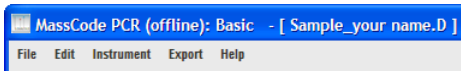
Task 1. Open an existing experiment and save a copy

Task 1 walks you through closing the online program, opening the offline program, opening the sample experiment, and creating a copy of that experiment.

Task 1. Open the sample experiment and save a copy

Steps	Detailed Instructions	Screen Images
1 Close the online program and open the offline program. Skip this step and proceed to step 2 if you prefer to perform this exercise and all remaining exercises in the online program.	**a** Click **File > Exit** to close the experiment and the program. **b** Double-click the MassCode PCR Offline program icon on your desktop. The program opens to the Getting Started screen.	

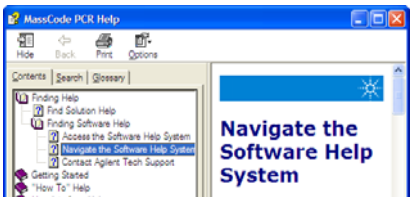
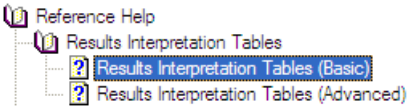
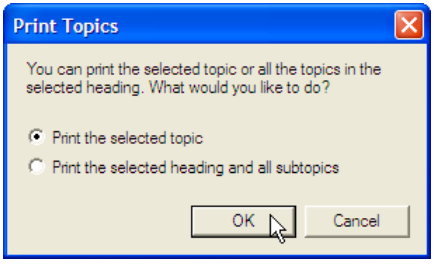
Task 1. Open the sample experiment and save a copy

Steps	Detailed Instructions	Screen Images
2 Open the sample experiment.	**a** Click **Open Existing Experiment**. The **Browse for Folder** dialog box opens. **b** Browse to the data file for the sample experiment: C:\MassCode PCR\Storage\Sample.D. **c** With the Sample.D folder selected, click **OK** in the dialog box. The experiment opens to the Report view. **d** Check the title bar to verify that you are in Basic mode.	  
3 Save a copy of the experiment.	**a** Click **File > Save Experiment As**. The **Save Experiment As** dialog box opens. **b** Next to the **Parent Folder** field, click **Browse**. The **Browse For Folder** dialog box opens. **c** Browse to the folder C:\MassCode PCR\Storage. Select the folder and click **OK**. **d** In the **New Experiment Name** field, type "Sample_" followed by your name. End the experiment name with ".D". **e** Click **Save Experiment**. The dialog box closes.	  After you have saved the experiment, the title bar indicates that you are working in the copy of the experiment that you created.

Task 2. Print the Results Interpretation Tables – Basic

Task 2 walks you through opening and printing the help system topic that contains the results interpretation tables for basic users.

Task 2. Print the Results Interpretation Tables – Basic

Steps	Detailed Instructions	Screen Images
1 Open the MassCode PCR help system.	• Click **Help > Contents**. The MassCode PCR Help window opens to the topic *Navigate the Software Help System*.	
2 Locate the help topic *Results Interpretation Tables (Basic)* in the *Reference Help* chapter.	**a** Double-click the *Reference Help* book. **b** Double-click the sub-book *Results Interpretation Tables*. **c** Click the topic *Results Interpretation Tables (Basic)*.	
3 Print out the topic. Keep this printout at hand. We will refer to it as we complete the exercises for analyzing the results of the sample experiment.	**a** Click the Print icon at the top of the Help window. **b** In the **Print Topics** dialog box, select the option **Print the selected topic** and click **OK**. **c** Close the Help window.	

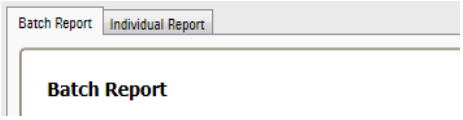
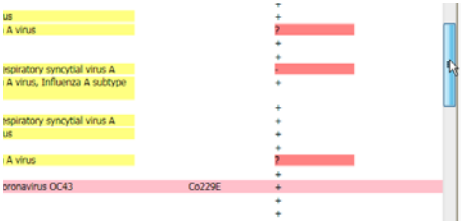
Exercise 4. Analyze the experiment results in the Report view

The tasks in this exercise show you how to view the results of the experiment using the Report view of the program and edit the color code scheme that is used to depict calls on the report. Two tabs are available for the Report view: *Batch Report* and *Individual Report*. By default, the program opens the sample experiment to the batch report when in Basic mode.

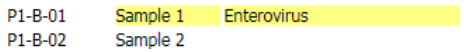
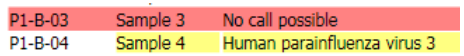
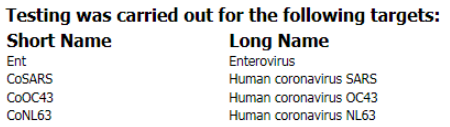
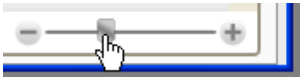
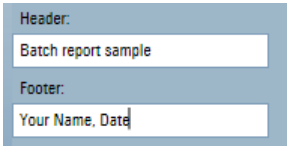
Task 1. Review the experiment results in the batch report and export to Excel

Task 1 demonstrates the features of the batch report. The task also shows you how to export the report to a text file.

Task 2. Review the experiment results in the batch report and export to Excel

Steps	Detailed Instructions	Screen Images
1 If you are not already in the Report view, navigate to that screen.	• In the workflow toolbar, click **Analysis** then **Report**.	
2 Review the summary information at the top of the batch report.	You are viewing the batch report when the **Batch Report** tab is selected on the Report screen.	
3 Review the contents of the batch report.	• Use the scroll bar on the right side of the report to scroll through the report. Each row summarizes the results for a single well.	 The color coding of the rows indicates the call in that well. The color code key is on the left side of the screen under **Call Output**.

Task 2. Review the experiment results in the batch report and export to Excel

Steps	Detailed Instructions	Screen Images
4 Compare the batch report results for wells B-01 and B-02. In both wells, the internal control target was detected (the **Internal Control** column has a +).	• Well B-01 is called as positive because the Enterovirus target was detected. • Well B-02 was called as negative because no targets, other than the internal control target (IAC), were detected.	
5 Compare the results in wells B-03 and B-04. In both wells, the internal control target was not positively detected (the **Internal Control** column has a –).	• Well B-03 is called as NC (no call possible) because no targets (including the IAC) were successfully amplified. • Well B-04 is positive because, although the IAC target is indeterminate, another RNA target (human parainfluenza virus 3) was positive. Refer to your printout of the results interpretation table for more information.	
6 Review the information at the bottom of the batch report.	• Scroll to the bottom of the report. The long and short names of the targets in the target panel are listed.	
7 Zoom in and out on the report.	**a** In the lower right corner of the report, click and drag on the zoom scroll bar and note the change in magnification. **b** Select a magnification level that you prefer for ease of viewing.	
8 Add a header and footer. The header and footer appear on the printed report.	**a** In the **Header** field on the left side of the screen, type "Batch report sample". **b** In the **Footer** field, type your name and the date.	

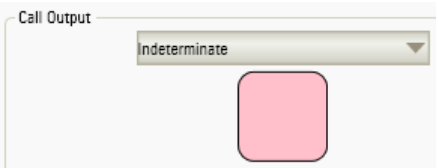
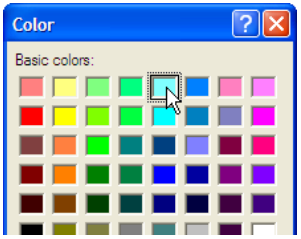
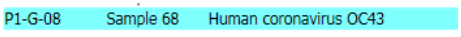
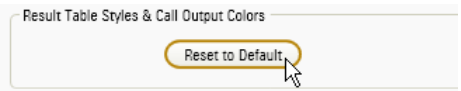
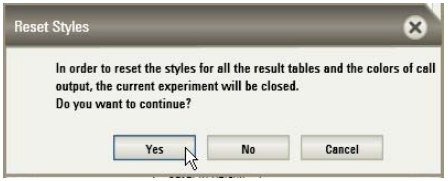
Task 2. Review the experiment results in the batch report and export to Excel

Steps	Detailed Instructions	Screen Images
9 Export the batch report to a text file. This file can be opened in Microsoft® Excel as a tab-delimited text file.	**a** Click **Export > Export Report to File**. The **Save As** dialog box opens prompting you to choose a location and file name for the text file. The default location is the experiment data folder. **b** In the **File name** field, type "Batch report sample data". **c** Click **Save**. You can view the contents of this file in Excel if desired.	

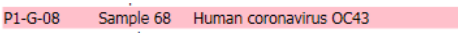
Task 2. Change the color code and reset back to default

Task 2 shows you how to change the color code scheme for a call and then reset the color code back to the default setting.

Task 2. Change the color code and reset back to default

Steps	Detailed Instructions	Screen Images
1 Change the color code for indeterminate calls from pink to aqua.	**a** Click **Edit > Preferences** to open the **Preferences** dialog box. **b** Click the **ColorCode/Table Styles** tab. **c** Under **Call Output**, select **Indeterminate**. **a** Click **Edit Color**. The **Color** dialog box opens. **b** Click on the aqua square and click **OK** to close the **Color** dialog box. **c** Click **OK** to close the **Preferences** dialog box.	 
2 View your changes on the batch report.	The row for well G-08, which has an indeterminate call, is now aqua.	
3 Reset the indeterminate color code back to the default setting. In order to reset the color codes, the program needs to close the experiment.	**a** Click **Edit > Preferences** to open the **Preferences** dialog box. **b** Click the **ColorCode/Table Styles** tab. **c** Click **Reset to Default**. The **Reset Styles** dialog box opens notifying you that the experiment needs to be closed to continue. **d** Click **Yes** in this dialog box. **e** If prompted to save changes to the open experiment, click **Yes**. The experiment closes and the Getting Started screen opens. **f** Click **OK** on the **Preferences** dialog box to close the dialog box.	 

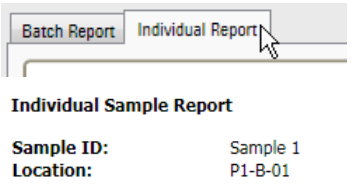
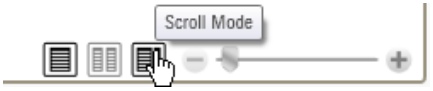
Task 2. Change the color code and reset back to default

Steps	Detailed Instructions	Screen Images
4 Re-open your copy of the sample experiment and verify that the color code for indeterminate calls has returned to the default pink color.	**a** Click **Open Existing Experiment**. The **Browse for Folder** dialog box opens. **b** Browse to your copy of the sample experiment (*Sample_your name.D*) that you saved earlier. **c** Click **OK**. The experiment opens to the batch report. **d** Examine the color code in well G-08. The indeterminate call has returned to the pink color code.	

Task 3. Review the experiment results in the individual report

Task 3 demonstrates the features of the individual report and describes options for viewing the report and finding information.

Task 3. Review the experiment results in the individual report

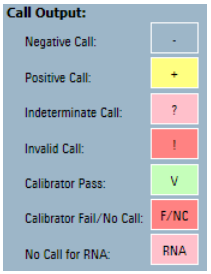
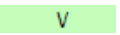
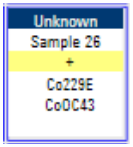
Steps	Detailed Instructions	Screen Images
1 Navigate to the individual report and review page 1.	• Click the **Individual Report** tab at the top of the report window. Page 1 of the individual report is for well B-01, the first Unknown well on the plate. In Basic mode, the individual report does not include the calibrators.	
2 Review the individual report for well G-08.	• You can click through page by page using the forward arrow at the bottom center of the report, or use the Find tool in the bottom left corner. The sample in this well is positive for one target (human coronavirus OC43) and indeterminate for another (human coronavirus 229E).	
3 Switch the view to Scroll Mode.	• Click the Scroll Mode button near the bottom right corner of the report. You can now use the scroll bar on the right side of the report to scroll through the list of wells.	

Exercise 5. Analyze the experiment results in the Plate Results view

The task in this exercise shows you how the data in the Plate Results view are displayed. The task also shows you how to copy the plate results image to another application.

Task 1. Review the results in the Plate Results view

Task 1. Review the results in the Plate Results view

Steps	Detailed Instructions	Screen Images
1 Navigate to the Plate Results view.	• Below the workflow toolbar, click **Plate Results**.	
2 Review the color code key on the left side of the screen.	• Unknown wells can be called as positive (+), negative (–), indeterminate (?), no call for all targets (NC), or no call for RNA targets only (RNA). • Calibrator wells can be called as passed (V) or failed (F).	
3 Examine the top row of wells containing the calibrator reactions.	All wells in this row are marked with a green “V” which means all the calibrator reactions passed and are valid.	
4 Examine the Unknown wells in rows B through H.	The wells called as positive are color coded yellow and the positive targets are listed in the well. In samples with a co-infection (e.g. Sample 26) both positive targets are listed.	

Task 1. Review the results in the Plate Results view

Steps	Detailed Instructions	Screen Images
5 Copy the plate results image into another program.	**a** Click **Export > Export Chart to Clipboard**. The image is on the clipboard. **b** Open Microsoft Word or Powerpoint. **c** Click **Edit > Paste**. The image of the plate results is pasted into the file. **d** Close the application without saving changes.	

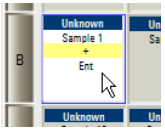
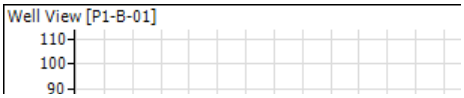
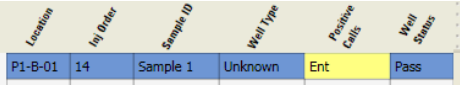
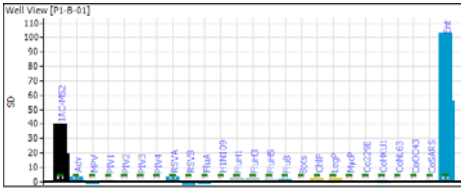
Exercise 6. Analyze the experiment results in the Batch Results view

The tasks in this exercise show you how to view the results of the experiment using the Batch Results view of the program. The Batch Results can be displayed for a single well (called the well view) or a single target (called the target view).

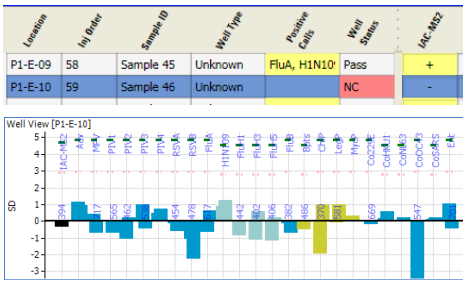
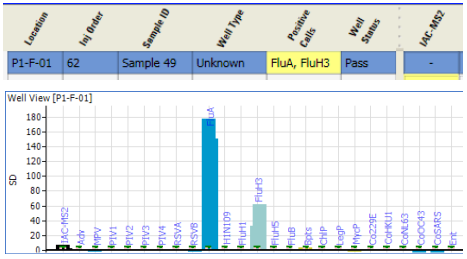
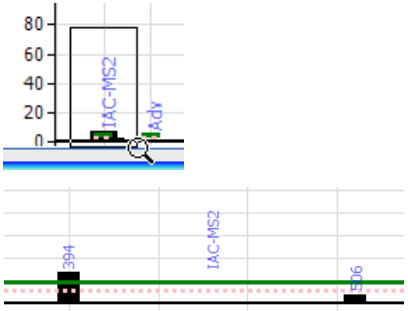
Task 1. Review the results in the Batch Results Well View

Task 1 shows you how to examine the Batch Results for individual wells.

Task 1. Review the results in the Batch Results Well View

Steps	Detailed Instructions	Screen Images
1 Display the Batch Results Well View for well B-01. The well view displays the results for all targets within a single well.	**a** While still on the Plate Results screen, click directly on well B-01 (Sample 1). **b** In the workflow toolbar, click **Batch Results** to navigate to the Batch Results view. The Batch Results Well View for well B-01 is displayed in the graph on the top half of the screen. Note that the selected well is indicated in the upper left corner of the graph.	  
2 Review the Batch Results table and graph for well B-01. The table appears on the bottom half of the Batch Results view. The graph appears on the top half.	The Batch Results table indicates that well B-01 is positive for the Ent target and that the IAC-MS2 target was detected. The graph indicates that IAC-MS2 and Ent are the only targets in which the SD over Avg of both ions for each target passes the green cutoff value. (The green line is the cutoff for positive calls; see your printout of the Results Interpretation Tables for details.)	 

Task 1. Review the results in the Batch Results Well View

Steps	Detailed Instructions	Screen Images
3 Display the Batch Results for well E-10.	• In the Batch Results table, click the row corresponding to well E-10. You may need to scroll down to find this row.	
4 Review the Batch Results table and graph for well E-10.	In the table, the IAC-MS2 target is listed as negative (and no other targets are detected), resulting in a well status of NC (no call possible for any target.) In the graph, none of the ions pass the pink cutoff value. (The pink line is the cutoff for indeterminate calls; see your printout of the Results Interpretation Tables for details).	
5 Display the Batch Results for well F-01.	• In the Batch Results table, click the row corresponding to well F-01.	
6 Review the Batch Results table and graph for well F-01.	In the table, the IAC-MS2 target is listed as negative but the well status is *Pass* (indicating that target calls are valid). In the graph, note that both ions for the FluA target and both ions for the FluH3 target exceed the green line, resulting in a positive call for those targets.	
7 Zoom in on the IAC-MS2 target on the graph.	**a** Right click on the graph near the IAC-MS2 target and drag across the area you want to zoom in on. **b** Note that one ion (394) crosses the green cutoff line but the other ion (506) is below the pink line, resulting in a negative call for the IAC-MS2 target in this well. **c** Double-click anywhere on the graph to zoom out again.	

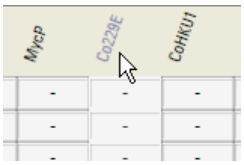
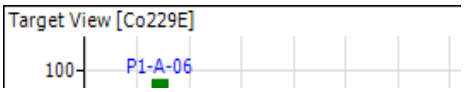
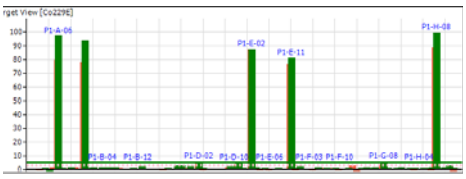
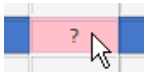
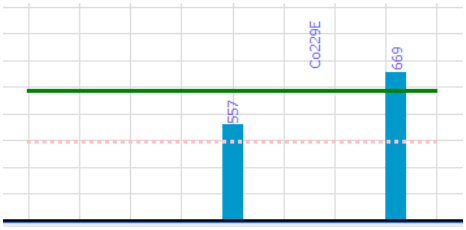
Task 1. Review the results in the Batch Results Well View

Steps	Detailed Instructions	Screen Images
8 Compare the results in wells E-10 and F-01. Refer to your printout of the Results Interpretation Tables.	Although the IAC-MS2 target was not detected in either well, only well E-10 received a NC call. The difference is that well E-10 had no positive targets (potentially indicating that the cDNA synthesis or PCR failed) while well F-01 was positive for FluA and FluH3 (indicating that the cDNA synthesis and PCR were successful even though IAC-MS2 was not detected).	

Task 2. Review the results in the Batch Results Target View

Task 2 shows you how to examine the Batch Results for an individual target.

Task 2. Review the results in the Batch Results Target View

Steps	Detailed Instructions	Screen Images
1 Display the Batch Results in target view for the Co229E target.	**a** Locate the column for the Co229E target in the Batch Results table. You may have to scroll over to see this column. **b** Click on the Co229E target name in the column heading. The Batch Results for target Co229E are displayed, as indicated in the upper left corner of the graph.	 
2 Review the Batch Results graph for target Co229E.	In the graph, the results for target Co229E are displayed for each well. You can quickly identify the wells in which this target was detected (specifically, wells A-06, A-12, E-02, E-11 and H-08).	
3 Display the Batch Results for the Co229E target in the indeterminate well (well G-08). To be called indeterminate, a target must have both ions between the pink and green cutoff lines or one ion between the pink and green lines and one ion above the green line.	**a** In the Batch Results table, scroll down to locate the row for well G-08. The Co229E column has a pink "?" in this row. **b** Click directly on the "?" in the Co229E column of the table. The graph displays the Co229E results specifically for the selected well.	  Note that one ion (669) is above the green cutoff line and the other ion (557) is between the pink and green cutoff lines, resulting in the indeterminate call.

Chapter 2
Exercises for Advanced Users

The exercises in this chapter are performed in the software's Advanced mode. The Advanced mode is appropriate for users who may want to customize their own target panels and/or have used LC/MS technology before.

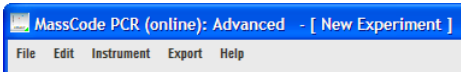
Exercise 1. Launch the online program and open the help system

The tasks in this exercise show you how to launch the MassCode PCR program, switch to Advanced mode, and explore the contents of the software's help system.

Task 1. Launch the program and switch to Advanced mode

Task 1 shows you how to open the online program and set the program to Advanced mode.

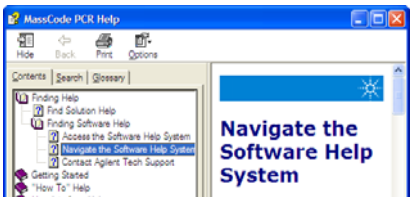
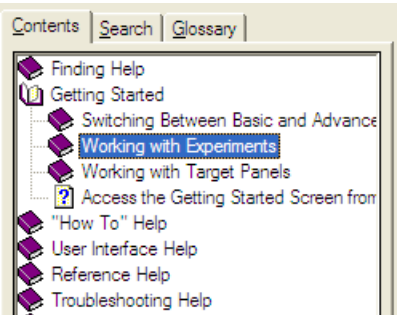
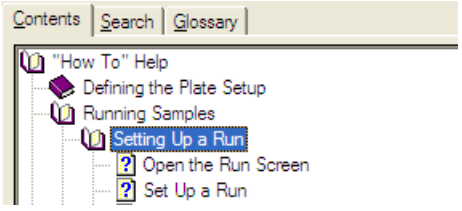
Task 1. Launch the program and switch to Advanced mode

Steps	Detailed Instructions	Screen Images
1 Open the online MassCode PCR program. The online program is used to connect to the LC/MS.	• Double-click the MassCode PCR Online program icon on your desktop. The program opens to the Getting Started screen.	
2 Check that you are in the Advanced mode by examining the title bar of the program window.	• If the title bar lists **Advanced** you are in Advanced mode. • If the title bar lists **Basic**, click **File > Switch to Advanced** and check the title bar again.	

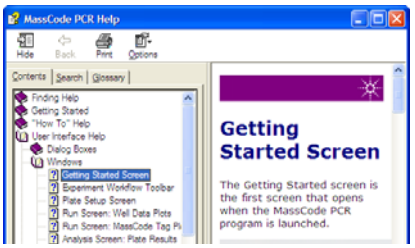
Task 2. Use the help system

Task 2 shows you how to open and navigate the software help system.

Task 2. Use the help system

Steps	Detailed Instructions	Screen Images
1 Open the help system for the software.	• Click **Help > Contents**. The MassCode PCR Help window opens to the topic *Navigate the Software Help System*.	
2 Locate and read the help topic *Working with Experiments*.	**a** In the table of contents on the left side of the window, double-click the book called *Getting Started*. The book expands to show the contents of the chapter. **b** Click on the sub-book *Working with Experiments*. The contents of the *Working with Experiments* help topic opens on the right side of the Help window. **c** Read the topic to learn about MassCode PCR experiments and the experiment workflow.	
3 Locate the How-To help topics.	**a** Double-click the book called *"How To" Help*. The book expands to show the contents of the chapter. **b** Double-click any of the sub-books to view the topics contained within. **c** Click on any individual topic to read its contents.	
4 Close the help system.	• Click the X in the upper right corner of the MassCode PCR Help window.	

Task 2. Use the help system

Steps	Detailed Instructions	Screen Images
5 Open the help topic for the currently open screen of the software (the Getting Started screen).	• On the keyboard, press [F1]. The help window opens with the topic for the Getting Started screen displayed on the right side. From any screen or dialog box in the program, you can press [F1] to open the *User Interface Help* topic pertaining to that particular screen or dialog box.	
6 Close the help system.	• Click the X in the upper right corner of the MassCode PCR Help window.	

Exercise 2. Start a new experiment

The tasks in this exercise show you how to define the plate setup for a new experiment plate, set up the run parameters that are available in the Advanced mode, and save the experiment settings.

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL-NTC CAL-NTC	CAL-NTC CAL-NTC	CAL-PTC1 CAL-PTC1	CAL-PTC2 CAL-PTC2	CAL-PTC3 CAL-PTC3	CAL-PTC4 CAL-PTC4	CAL-IACRNA CAL-IACRNA	CAL-NTC CAL-NTC	CAL-PTC1 CAL-PTC1	CAL-PTC2 CAL-PTC2	CAL-PTC3 CAL-PTC3	CAL-PTC4 CAL-PTC4
B	Unknown Sample 1	Unknown Sample 2	Unknown Sample 3	Unknown Sample 4	Unknown Sample 5	Unknown Sample 6	Unknown Sample 7	Unknown Sample 8	Unknown Sample 9	Unknown Sample 10	Unknown Sample 11	Unknown Sample 12
C	Unknown Sample 13	Unknown Sample 14	Unknown Sample 15	Unknown Sample 16	Unknown Sample 17	Unknown Sample 18	Unknown Sample 19	Unknown Sample 20	Unknown Sample 21	Unknown Sample 22	Unknown Sample 23	Unknown Sample 24
D	Unknown Sample 25	Unknown Sample 26	Unknown Sample 27	Unknown Sample 28	Unknown Sample 29	Unknown Sample 30	Unknown Sample 31	Unknown Sample 32	Unknown Sample 33	Unknown Sample 34	Unknown Sample 35	Unknown Sample 36
E	Unknown Sample 37	Unknown Sample 38	Unknown Sample 39	Unknown Sample 40	Unknown Sample 41	Unknown Sample 42	Unknown Sample 43	Unknown Sample 44	Unknown Sample 45	Unknown Sample 46	Unknown Sample 47	Unknown Sample 48
F	Unknown Sample 49	Unknown Sample 50	Unknown Sample 51	Unknown Sample 52	Unknown Sample 53	Unknown Sample 54	Unknown Sample 55	Unknown Sample 56	Unknown Sample 57	Unknown Sample 58	Unknown Sample 59	Unknown Sample 60
G	Unknown Sample 61	Unknown Sample 62	Unknown Sample 63	Unknown Sample 64	Unknown Sample 65	Unknown Sample 66	Unknown Sample 67	Unknown Sample 68	Unknown Sample 69	Unknown Sample 70	Unknown Sample 71	Unknown Sample 72
H	Unknown Sample 73	Unknown Sample 74	Unknown Sample 75	Unknown Sample 76	Unknown Sample 77	Unknown Sample 78	Unknown Sample 79	Unknown Sample 80	Unknown Sample 81	Unknown Sample 82	Unknown Sample 83	Unknown Sample 84

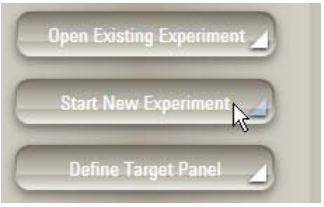
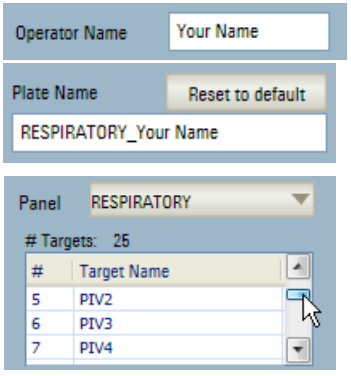
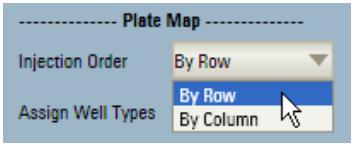
Figure 2 Completed Plate Setup

Task 1. Define the plate setup

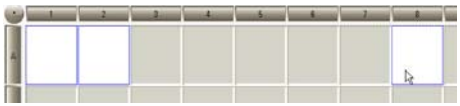
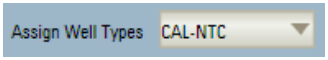
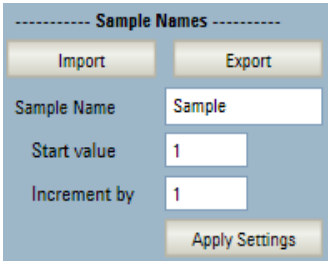
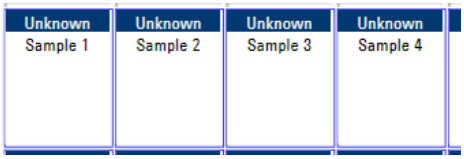
Task 1 walks you through the process of defining the plate setup. The steps cover assigning the operator name and plate name, selecting the panel and injection order, assigning the well types, and assigning sample names.

The goal is to set up a plate that mimics the one used in the sample experiment that comes pre-loaded with the MassCode PCR program. The plate setup you create in this task is shown in Figure 2.

Task 1. Define the plate setup

Steps	Detailed Instructions	Screen Images
1 Open a new experiment.	• Click **Start New Experiment** on the Getting Started screen. The program opens to a blank plate setup.	
2 Enter the operator name and select the target panel. • **Operator Name:** Your name • **Panel:** Respiratory (The respiratory panel is the default target panel.)	**a** Type your name into the **Operator Name** field on the left side of the screen. Once you remove the cursor from the field, the operator name is added to the default plate name. **b** In the **Panel** drop-down list, check that RESPIRATORY is selected. To see the targets included in the panel, you can scroll through the list (see image on right).	
3 Set the injection order to *By Row*. *By Row* is the default injection order.	• In the **Injection Order** drop-down list, check that **By Row** is selected. This selection indicates to the software to process the wells of the plate from left to right during the run, starting with row A.	

Task 1. Define the plate setup

Steps	Detailed Instructions	Screen Images
4 Assign the calibrator wells. • **Wells A1, A2 and A8:** CAL-NTC well type • **Wells A3 and A9:** CAL-PTC1 well type • **Wells A4 and A10:** CAL-PTC2 well type • **Wells A5 and A11:** CAL-PTC3 well type • **Wells A6 and A12:** CAL-PTC4 well type • **Well A7:** CAL-IACRNA well type	**a** Select the group of wells that are to be assigned to the same well type. • To select multiple wells, press [Ctrl] while clicking on individual wells. • To select/deselect all wells on the plate, click the circle in the upper left corner of the plate image. **b** In the **Assign Well Types** drop-down list, select the desired well type.	   **Important:**A total of 12 calibrator wells is required for proper data analysis.
5 Assign the remaining wells to the Unknown well type.	**a** With the cursor, click and drag across the block of wells from B-01 to H-12 to select all remaining wells. **b** In the **Assign Well Types** drop-down list, select **Unknown**.	
6 Assign sample names to the Unknown wells. At the completion of this step, refer to Figure 2. At this point, your plate setup should be identical to that shown in the figure.	**a** Assign the core sample name. • With the Unknown wells selected, type "Sample" followed by a space into the **Sample Name** field. The name "Sample" appears in each Unknown well. **b** Add incremental numbers to the sample names. • In the **Start value** field, leave the default value of 1. • In the **Increment by** field, leave the default value of 1. • Click **Apply Settings**. An incremental number is added to the end of each sample name.	  Because the injection order is *by row*, the numbering is assigned horizontally.

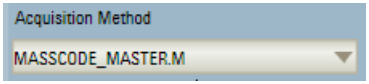
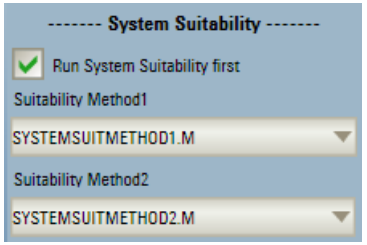
Task 2. Set up a run

Task 2 shows you how to set up the run. The task includes designating a name and location for the data file that is created when a run is started. The data file is a folder (with extension *.D*) that contains all the settings and data for an experiment.

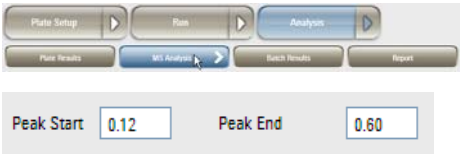
Task 2 also shows you how to select an acquisition method and the methods for the system suitability test. We will not change the default selections for these methods, but you can see where those selections are made if you do need to change them when running an actual experiment.

This task also includes a step demonstrating where you can change the peak start and peak end values.

Task 2. Set up a run

Steps	Detailed Instructions	Screen Images
1 Navigate to the Run view.	• In the workflow toolbar, click **Run**.	
2 Change the data file name. • **Data File Name:** Sample_your name.D. Data file names must end in ".D" and are limited to 40 legal characters.	• In the **Data File Name** field, type "Sample_" followed by your name. End the name with ".D".	
3 Select methods (if not using the respiratory panel). For the respiratory panel, the default methods (shown in the image on the right) are appropriate.	• In the **Acquisition Method** drop-down list, select the method appropriate for running an experiment with your panel. (For information on creating methods, see the online help chapter *Reference Help > MassCode Methods*.) • In the **Suitability Method1** and **Suitability Method2** drop-down lists, select the methods you want to use to perform the system suitability test.	 

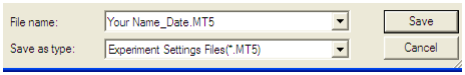
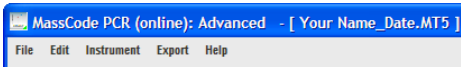
Task 2. Set up a run

Steps	Detailed Instructions	Screen Images
4 View peak start and peak end times. Changing these settings may be necessary to make sure the peaks for each well are within the start and end times.	• In the workflow toolbar, click **Analysis** and then click **MS Analysis**. The MS Analysis screen opens. The **Peak Start** and **Peak End** fields are displayed on the left side of the screen. If you needed to change the default values, you would enter new values into the fields and click **Set As Default**.	 Note that the table and graph on the MS Analysis screen are blank because the experiment has not been run and does not contain any data.

Task 3. Save the experiment settings

Task 3 shows you how to save the experiment settings to a separate Experiment Settings file. The Experiment Settings file (file extension MT5) contains the complete settings for an experiment: the plate setup, the run settings, the peak start and peak end values, and the experiment notes.

Task 3. Save the experiment settings

Steps	Detailed Instructions	Screen Images
• Save the Experiment Settings file. Note that if you want to open a saved Experiment Settings file, you can do so from the Getting Started screen (click **File > Open Experiment Settings File**).	**a** Click **File > Save Experiment Settings As**. The **Save As** dialog box opens. **b** In the **File name** field, type your name and today's date separated with an underscore. **c** Click **Save**.	 After the experiment settings have been saved, the name of the MT5 file appears in the title bar. 

NOTE After a run, the LC and MS go into standby automatically. If you do not want the instrument to go into standby automatically, refer to the instructions in the software help system ("How To" Help > Running Samples > Working with ChemStation).

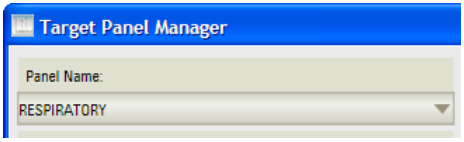
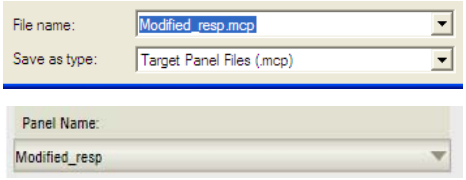
Exercise 3. Create and delete a target panel

The tasks in this exercise show you how to create a new target panel, modify the target library, and delete a target panel.

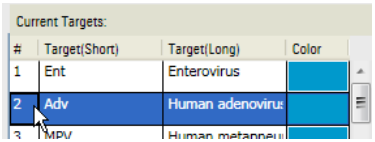
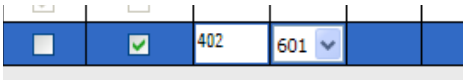
Task 1. Modify a target panel

Task 1 shows you how to create a new target panel by modifying an existing panel.

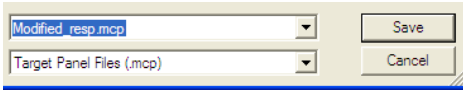
Task 1. Modify a target panel

Steps	Detailed Instructions	Screen Images
1 Open the Target Panel Manager for the respiratory panel.	**a** Click **File > Target Panel Manager**. The **Target Panel Manager** dialog box opens. **b** In the panel name drop-down list, select RESPIRATORY.	
2 Save a copy of the respiratory target panel and name it *Modified_resp*. Target panel files have file extension MCP.	**a** In the Target Panel Manager, click **Save As**. The **Save As** dialog box opens. **b** In the **File name** field, type the name "Modified_resp.mcp". **c** Click **Save**. The **Save As** dialog box closes. **d** Check that **Modified_resp** is now selected in the **Panel Name** drop-down list.	

Task 1. Modify a target panel

Steps	Detailed Instructions	Screen Images
3 Remove the Adv target from the Modified_resp panel and save.	**a** In the **Current Targets** list, click the Adv target to select it. **b** Click the **<<** button to move this target out of the **Current Targets** list and into the **Available Targets** list. **c** Click **Save As**. The **Save As** dialog box opens. **d** Check that *Modified_resp.mcp* is selected in the **File name** field and click **Save**. **e** When asked if you want to replace the existing panel of that name, click **Yes**.	
4 Create a new target in the Target Library Manager. • **Short target name:** Adv2 • **Long target name:** New human adenovirus • **MassCode tags:** • 402 and 601	**a** In the Target Panel Manager, click **Target Library Manager**. The **Target Library Manager** dialog box opens. **b** Click **Add Target**. A new row is added to the bottom of the target table. **c** In the new row, click the cell in the **Target(Short)** column and type *Adv2*. **d** Click the cell in the **Target(Long)** column and type *New human adenovirus*. **e** In the **DNA Type** column, mark the check box to indicate that the target is for a species with a DNA genome. **f** Double-click in the **Grp 1** cell and expand the drop-down list. Select *402* **g** Double-click in the **Grp 2** cell and expand the drop-down list. Select *601*. **h** Click **OK**. When asked if you want to save your changes, click **Yes**. The Target Library Manager closes and you are returned to the Target Panel Manager.	 

Task 1. Modify a target panel

Steps	Detailed Instructions	Screen Images
5 Add the Adv2 target to the Modified_resp panel and close the Target Panel Manager.	**a** Select the Adv2 target in the **Available Targets** list. **b** Click the **>>** button to add the target to the Modified_resp panel. **c** Click **Save As**. **d** In the **Save As** dialog box, confirm that *Modified_resp.mcp* is selected in the **File Name** field then click **Save.** **e** Click **OK** to close the Target Panel Manager.	

Task 2. Delete a target panel

Task 2 shows you how to delete the target panel that we created in the previous task.

Task 2. Delete a target panel

Steps	Detailed Instructions	Screen Images
1 Open the Target Panel Manager for the Modified_resp panel that we created in task 1.	**a** Click **File > Target Panel Manager**. The **Target Panel Manager** dialog box opens. **b** In the panel name drop-down list, select **Modified_resp**.	
2 Delete the Modified_resp panel.	**a** Click **Delete Panel**. The **Attention** dialog box opens asking you to confirm that you want the panel deleted. **b** Click **Yes**. **c** Click **OK** to close the Target Panel Manager.	

Exercise 4. Open an existing experiment

The tasks in this exercise show you how to open an existing (post-run) experiment, save a copy of the experiment, and print out a copy of the *Results Interpretation Tables* from the software's help system.

For this exercise and the remaining exercises, we will work with the sample experiment that comes pre-loaded with the MassCode PCR program. You are instructed to perform the exercises in the offline program, but these exercises can also be performed in the online program. To proceed in the online program, simply skip step 1 in Task 1 below (do not close ChemStation).

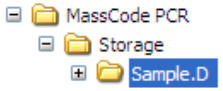
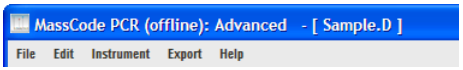
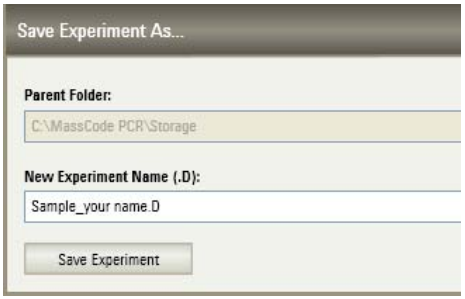
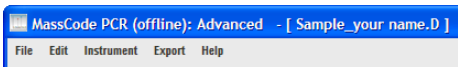
Task 1. Open the sample experiment and save a copy

Task 1 walks you through closing the online program, opening the offline program, opening the sample experiment, and creating a copy of that experiment.

Task 1. Open the sample experiment and save a copy

Steps	Detailed Instructions	Screen Images
1 Close the online program and open the offline program. Skip this step and proceed to step 2 if you prefer to perform this exercise and all remaining exercises in the online program.	**a** Click **File > Exit** to close the experiment and the program. **b** Double-click the MassCode PCR Offline program icon on your desktop. The program opens to the Getting Started screen.	

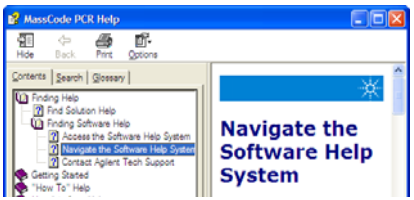
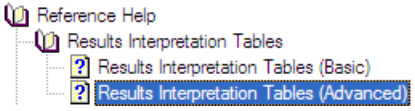
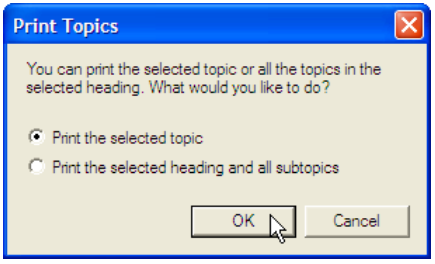
Task 1. Open the sample experiment and save a copy

Steps	Detailed Instructions	Screen Images
2 Open the sample experiment.	**a** Click **Open Existing Experiment**. The **Browse for Folder** dialog box opens. **b** Browse to the data file for the sample experiment: C:\MassCode PCR\Storage\Sample.D. **c** With the Sample.D folder selected, click **OK** in the dialog box. The experiment opens to the Plate Results view. **d** Check the title bar to verify that you are in the Advanced mode.	  
3 Save a copy of the experiment.	**a** Click **File > Save Experiment As**. The **Save Experiment As** dialog box opens. **b** Next to the **Parent Folder** field, click **Browse**. The **Browse For Folder** dialog box opens. **c** Browse to the folder C:\MassCode PCR\Storage. Select the folder and click **OK**. **d** In the **New Experiment Name** field, type "Sample_" followed by your name. End the experiment name with ".D". **e** Click **Save Experiment**. The dialog box closes.	  After you have saved the experiment, the title bar indicates that you are working in the copy of the experiment that you created.

Task 2. Print the Results Interpretation Tables – Advanced

Task 2 walks you through opening and printing the help system topic that contains the results interpretation tables for advanced users.

Task 2. Print the Results Interpretation Tables – Advanced

Steps	Detailed Instructions	Screen Images
1 Open the MassCode PCR help system.	• Click **Help > Contents**. The MassCode PCR Help window opens to the topic *Navigate the Software Help System*.	
2 Locate the help topic *Results Interpretation Table (Advanced)* in the *Reference Help* chapter.	**a** Double-click the *Reference Help* book. **b** Double-click the sub-book *Results Interpretation Tables*. **c** Click the topic *Results Interpretation Table (Advanced)*.	
3 Print out the topic. Keep this printout at hand. We will refer to it as we complete the exercises for analyzing the results of the sample experiment.	**a** Click the Print icon at the top of the Help window. **b** In the **Print Topics** dialog box, select the option **Print the selected topic** and click **OK**. **c** Close the Help window.	

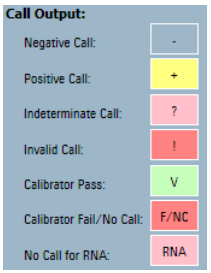
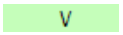
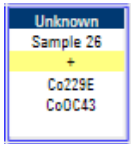
Exercise 5. Analyze the experiment results in the Plate Results view

The tasks in this exercise show you how to view the results of the experiment using the Plate Results view of the program and edit the color code scheme that is used to depict calls in the individual wells. This exercise also demonstrates how to change certain features of the plate setup in a post-run experiment.

Task 1. Review the results in the Plate Results view

Task 1 shows you how the data in the Plate Results view are displayed. The task also shows you how to copy the plate results image to another application.

Task 1. Review the results in the Plate Results view

Steps	Detailed Instructions	Screen Images
1 If you are not already in the Plate Results view, navigate to that screen.	• In the workflow toolbar, click **Analysis** then **Plate Results**.	
2 Review the color code key on the left side of the screen.	• Unknown wells can be called as positive (+), negative (–), indeterminate (?), no call for all targets (NC), or no call for RNA targets only (RNA). • Calibrator wells can be called as passed (V) or failed (F).	
3 Examine the top row of wells containing the calibrator reactions.	All wells in this row are marked with a green "V" which means all the calibrator reactions passed and are valid.	
4 Examine the Unknown wells in rows B through H.	The wells called as positive are color coded yellow and the positive targets are listed in the well. In samples with a co-infection (e,g. Sample 26) both positive targets are listed.	

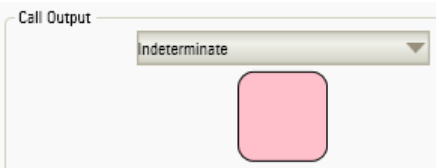
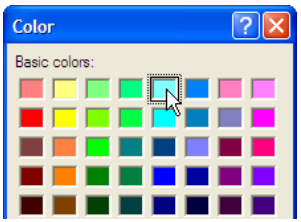
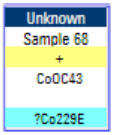
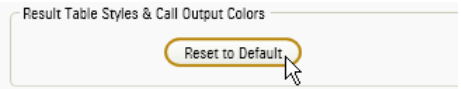
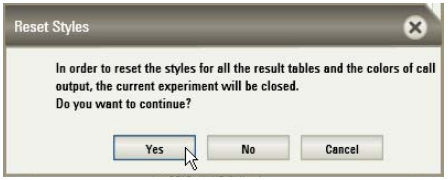
Task 1. Review the results in the Plate Results view

Steps	Detailed Instructions	Screen Images
5 Copy the plate results image into another program.	**a** Click **Export > Export Chart to Clipboard**. The image is on the clipboard. **b** Open Microsoft Word or Powerpoint. **c** Click **Edit > Paste**. The image of the plate results is pasted into the file. **d** Close the application without saving changes.	

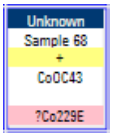
Task 2. Change the color code and reset back to default

Task 2 shows you how to change the color code scheme for a call and then reset the color code back to the default setting.

Task 2. Change the color code and reset back to default

Steps	Detailed Instructions	Screen Images
1 Change the color code for indeterminate calls from pink to aqua.	**a** Click **Edit > Preferences** to open the **Preferences** dialog box. **b** Click the **ColorCode/Table Styles** tab. **c** Under **Call Output**, select **Indeterminate**. **a** Click **Edit Color**. The **Color** dialog box opens. **b** Click on the aqua square and click **OK** to close the **Color** dialog box. **c** Click **OK** to close the **Preferences** dialog box.	 
2 View your changes on the Plate Results view.	The well for sample 68, which has an indeterminate call for the Co229E target, is now aqua.	
3 Reset the indeterminate color code back to the default setting. In order to reset the color codes, the program needs to close the experiment.	**a** Click **Edit > Preferences** to open the **Preferences** dialog box. **b** Click the **ColorCode/Table Styles** tab. **c** Click **Reset to Default**. The **Reset Styles** dialog box opens notifying you that the experiment needs to be closed to continue. **d** Click **Yes** in this dialog box. **e** If prompted to save changes to the open experiment, click **Yes**. The experiment closes and the Getting Started screen opens. **f** Click **OK** on the **Preferences** dialog box to close the dialog box.	 

Task 2. Change the color code and reset back to default

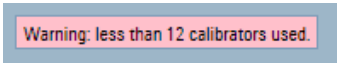
Steps	Detailed Instructions	Screen Images
4 Re-open your copy of the sample experiment and verify that the color code for indeterminate calls has returned to the default pink color	**a** Click **Open Existing Experiment**. The **Browse for Folder** dialog box opens. **b** Browse to your copy of the sample experiment (*Sample_your name.D*) that you saved earlier. **c** Click **OK**. The experiment opens to the Plate Results view. **d** Examine the color code in Sample 68. The indeterminate call has the default pink color code.	

Task 3. Change plate setup if well is not labeled or mislabeled

Task 3 shows you how to change the well type and sample name of a well on the Plate Setup screen and view the effects of these changes in the Plate Results screen. Specifically, the task walks you through the process of swapping the plate setup information for two wells on the plate and then returning the wells to their original setup.

You may need to make changes to the plate setup of a post-run experiment if you ever run an experiment with incomplete plate setup information or with the wrong plate setup information assigned to one or more wells.

Task 3. Change the plate setup if well is not labeled or mislabeled

Steps	Detailed Instructions	Screen Images
1 Change the 1st CAL-NTC well to an Unknown well.	**a** In the workflow toolbar, click **Plate Setup** to navigate to the Plate Setup view. **b** Click well A-01 to select it. **c** In the **Assign Well Types** drop-down list, select **Unknown**.	
2 View the results with the new plate setup in the Plate Results view.	• In the workflow toolbar, click **Analysis** to return to Plate Results. • Note the warning on the left side of the screen.	 When the plate setup includes fewer than 12 calibrator wells, confidence in the calls is not optimal and a warning is given on the Plate Setup and Report screens.
3 Change the Sample 3 well to a CAL-NTC well and assign well A-01 as Sample 3.	**a** Navigate back to the Plate Setup view. **b** Select well B-03. **c** In the **Assign Well Types** drop-down list, select **CAL-NTC**. **d** In the **Sample Name** field, delete the name *Sample 3*. **e** Select well A-01. **f** In the **Sample Name** field, type the name *Sample 3*.	

Task 3. Change the plate setup if well is not labeled or mislabeled

Steps	Detailed Instructions	Screen Images
4 View the results with the new plate setup in the Plate Results view.	• In the workflow toolbar, click **Analysis** to return to Plate Results. Note that the warning no longer appears because the plate setup now has 12 calibrators.	
5 Return the plate setup to its original configuration.	**a** Navigate back to the Plate Setup view. **b** Select well A-01 and set the well type assignment and sample name back to their original settings: • In the **Assign Well Types** drop-down list, select **CAL-NTC**. • In the **Sample Name** field, delete the name *Sample 3*. **c** Select well B-03 and set the well type assignment and sample name back to their original settings: • In the **Assign Well Types** drop-down list, select **Unknown**. • In the **Sample Name** field, type the name *Sample 3*.	

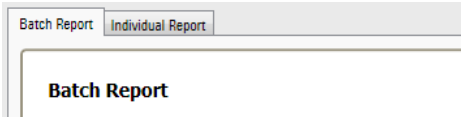
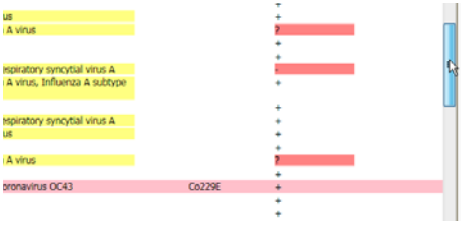
Exercise 6. Analyze the experiment results in the Report view

The tasks in this exercise show you how to view the results of the experiment using the Report view of the program. Two tabs are available for the Report view: *Batch Report* and *Individual Report*.

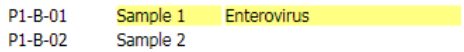
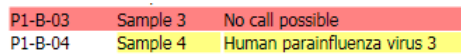
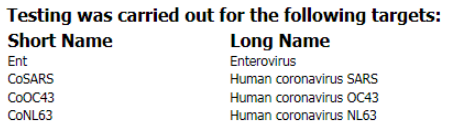
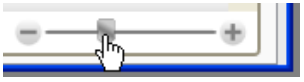
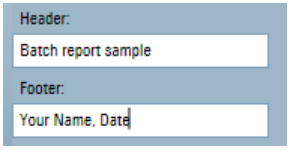
Task 1. Review the experiment results in the batch report and export to Excel

Task 1 demonstrates the features of the batch report. The task also shows you how to export the report to a text file.

Task 2. Review the experiment results in the batch report and export to Excel

Steps	Detailed Instructions	Screen Images
1 Navigate to the Report view.	• In the workflow toolbar, click **Analysis**. • Below the workflow toolbar, click **Report**. The screen opens to the batch report.	
2 Review the summary information at the top of the batch report.	You are viewing the batch report when the **Batch Report** tab is selected on the Report screen.	
3 Review the contents of the batch report.	• Use the scroll bar on the right side of the report to scroll through the report. Each row summarizes the results for a single well.	 The color coding of the rows indicates the call in that well. The color code key is on the left side of the screen under **Call Output**.

Task 2. Review the experiment results in the batch report and export to Excel

Steps	Detailed Instructions	Screen Images
4 Compare the batch report results for wells B-01 and B-02. In both wells, the internal control target was detected (the **Internal Control** column has a +).	• Well B-01 is called as positive because the Enterovirus target was detected. • Well B-02 was called as negative because no targets, other than the internal control target (IAC), were detected.	
5 Compare the results in wells B-03 and B-04. In both wells, the internal control target was not positively detected (the **Internal Control** column has a –).	• Well B-03 is called as NC (no call possible) because no targets (including the IAC) were successfully amplified. • Well B-04 is positive because, although the IAC target is indeterminate, another RNA target (human para-influenza virus 3) was positive. Refer to your printout of the results interpretation table for more information.	
6 Review the information at the bottom of the batch report.	• Scroll to the bottom of the report. The long and short names of the targets in the target panel are listed.	
7 Zoom in and out on the report.	**a** In the lower right corner of the report, click and drag on the zoom scroll bar and note the change in magnification. **b** Select a magnification level that you prefer for ease of viewing.	
8 Add a header and footer. The header and footer appear on the printed report.	**a** In the **Header** field on the left side of the screen, type "Batch report sample". **b** In the **Footer** field, type your name and the date.	

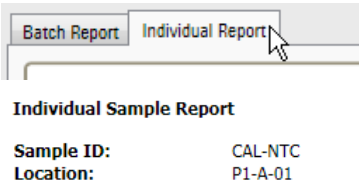
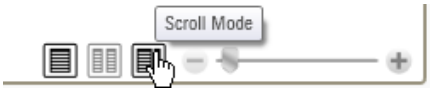
Task 2. Review the experiment results in the batch report and export to Excel

Steps	Detailed Instructions	Screen Images
9 Export the batch report to a text file. This file can be opened in Microsoft Excel as a tab-delimited text file.	**a** Click **Export > Export Report to File**. The **Save As** dialog box opens prompting you to choose a location and file name for the text file. The default location is the experiment data folder. **b** In the **File name** field, type "Batch report sample data". **c** Click **Save**. You can view the contents of this file in Excel if desired.	

Task 2. Review the experiment results in the individual report

Task 2 demonstrates the features of the individual report and describes options for viewing the report and finding information.

Task 2. Review the experiment results in the individual report

Steps	Detailed Instructions	Screen Images
1 Navigate to the individual report and review page 1.	• Click the **Individual Report** tab at the top of the report window. Page 1 of the individual report is for well A-01, a CAL-NTC well. In Advanced mode, the individual report includes the calibrator wells.	
2 Review the individual report for well G-08.	• You can click through page by page using the forward arrow at the bottom center o f the report, or use the Find tool in the bottom left corner. The sample in this well is positive for one target (human coronavirus OC43) and indeterminate for another (human coronavirus 229E).	
3 Switch the view to Scroll Mode.	• Click the Scroll Mode button near the bottom right corner of the report. You can now use the scroll bar on the right side of the report to scroll through the list of wells.	

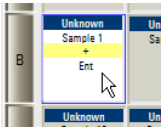
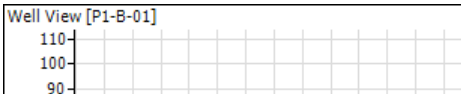
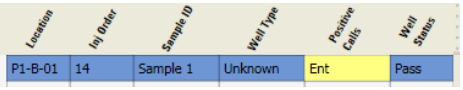
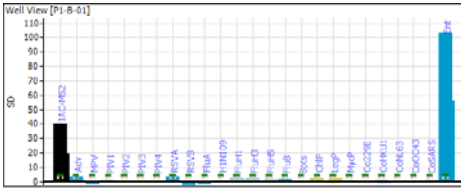
Exercise 7. Analyze the experiment results in the Batch Results view

The tasks in this exercise show you how to view the results of the experiment using the Batch Results view of the program. The Batch Results can be displayed for a single well (called the well view) or a single target (called the target view).

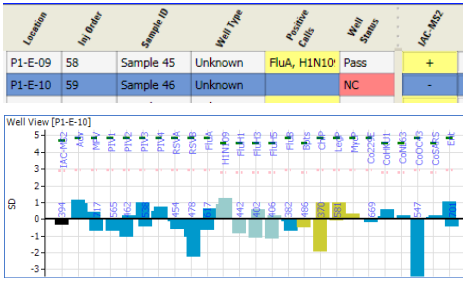
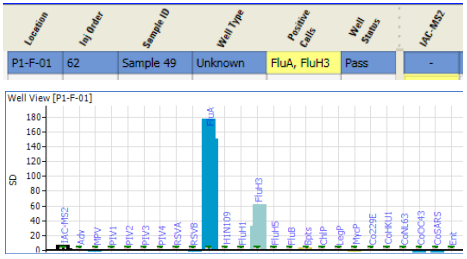
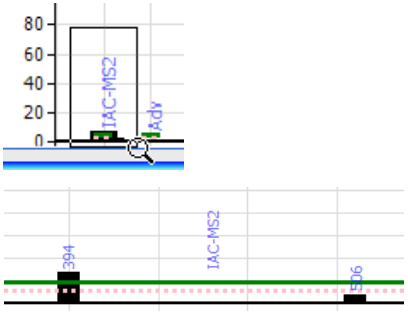
Task 1. Review the results in the Batch Results Well View

Task 1 shows you how to examine the Batch Results for individual wells.

Task 1. Review the results in the Batch Results Well View

Steps	Detailed Instructions	Screen Images
1 Display the Batch Results well view for well B-01. The well view displays the results for all targets within a single well.	**a** While still on the Plate Results screen, click directly on well B-01 (Sample 1). **b** In the workflow toolbar, click **Batch Results** to navigate to the Batch Results view. The Batch Results Well View for well B-01 is displayed in the graph on the top half of the screen. Note that the selected well is indicated in the upper left corner of the graph.	  
2 Review the Batch Results table and graph for well B-01. The table appears on the bottom half of the Batch Results view. The graph appears on the top half.	The Batch Results table indicates that well B-01 is positive for the *Ent* target and that the IAC-MS2 target was detected. The graph indicates that IAC-MS2 and Ent are the only targets in which the SD over Avg of both ions for each target passes the green cutoff value. (The green line is the cutoff for positive calls; see your printout of the Results Interpretation Table for details.)	 

Task 1. Review the results in the Batch Results Well View

Steps	Detailed Instructions	Screen Images
3 Display the Batch Results for well E-10.	• In the Batch Results table, click the row corresponding to well E-10. You may need to scroll down to find this row.	
4 Review the Batch Results table and graph for well E-10.	In the table, the IAC-MS2 target is listed as negative (and no other targets are detected), resulting in a well status of NC (no call possible for any target.) In the graph, none of the ions pass the pink cutoff value. (The pink line is the cutoff for indeterminate calls; see your printout of the Results Interpretation Table for details).	
5 Display the Batch Results for well F-01.	• In the Batch Results table, click the row corresponding to well F-01.	
6 Review the Batch Results table and graph for well F-01.	In the table, the IAC-MS2 target is listed as negative but the well status is *Pass* (indicating that target calls are valid). In the graph, note that both ions for the FluA target and both ions for the FluH3 target exceed the green line, resulting in a positive call for those targets.	
7 Zoom in on the IAC-MS2 target on the graph.	**a** Right click on the graph near the IAC-MS2 target and drag across the area you want to zoom in on. **b** Note that one ion (394) crosses the green cutoff line but the other ion (506) is below the pink line, resulting in a negative call for the IAC-MS2 target in this well. **c** Double-click anywhere on the graph to zoom out again.	

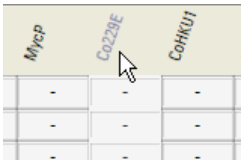
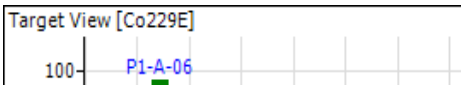
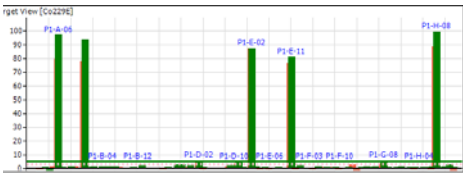
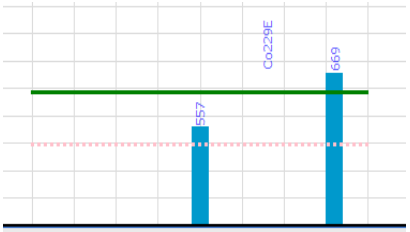
Task 1. Review the results in the Batch Results Well View

Steps	Detailed Instructions	Screen Images
8 Compare the results in wells E-10 and F-01. Refer to your printout of the Results Interpretation Table.	Although the IAC-MS2 target was not detected in either well, only well E-10 received a NC call. The difference is that well E-10 had no positive targets (potentially indicating that the cDNA synthesis or PCR failed) while well F-01 was positive for FluA and FluH3 (indicating that the cDNA synthesis and PCR were successful even though IAC-MS2 was not detected).	

Task 2. Review the results in the Batch Results Target View

Task 2 shows you how to examine the Batch Results for an individual target.

Task 2. Review the results in the Batch Results Target View

Steps	Detailed Instructions	Screen Images
1 Display the Batch Results in target view for the Co229E target.	**a** Locate the column for the Co229E target in the Batch Results table. You may have to scroll over to see this column. **b** Click on the Co229E target name in the column heading. The Batch Results for target Co229E are displayed, as indicated in the upper left corner of the graph.	 
2 Review the Batch Results graph for target Co229E.	In the graph, the results for target Co229E are displayed for each well. You can quickly identify the wells in which this target was detected (specifically, wells A-06, A-12, E-02, E-11 and H-08).	
3 Display the Batch Results for the Co229E target in the indeterminate well (well G-08). To be called indeterminate, a target must have both ions between the pink and green cutoff lines or one ion between the pink and green lines and one ion above the green line.	**a** In the Batch Results table, scroll down to locate the row for well G-08. The Co229E column has a pink "?" in this row. **b** Click directly on the "?" in the Co229E column of the table. The graph displays the Co229E results specifically for the selected well.	  Note that both ions are between the pink and green cutoff lines, resulting in the indeterminate call.

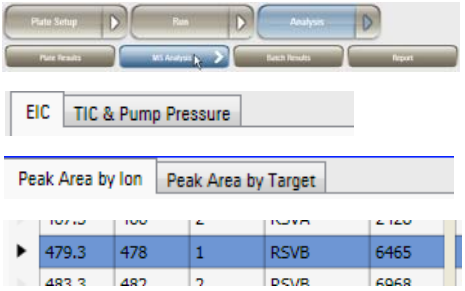
Exercise 8. Analyze the experiment results in the MS Analysis view

The tasks in this exercise show you how to view the results of the experiment using the MS Analysis view of the program. The MS Analysis graph can display the extracted ion chromatogram (EIC) or the total ion chromatogram (TIC) and pump pressure. The graph displays the results for a single ion at a time (in all wells or in one well). The MS Analysis table displays peak areas and can be organized by ion (sorted in order of mass) or by target name (with the two ions for each target listed next to each other).

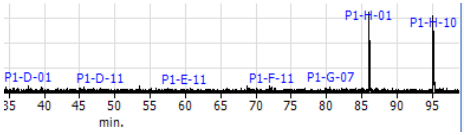
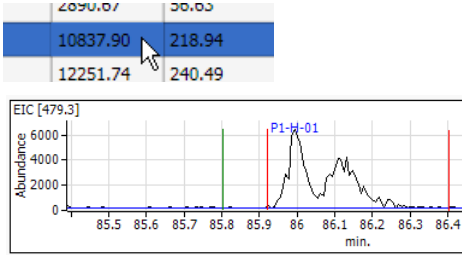
Task 1. Review the EIC and peak areas for an ion

Task 1 shows you how to review the extracted ion chromatogram (EIC) and peak area for a specific ion in all wells and in a single well.

Task 1. Review the EIC and peak areas for an ion

Steps	Detailed Instructions	Screen Images
1 Navigate to the MS Analysis view and display the EIC for the ion with a mass of 479.3.	**a** In the workflow toolbar, click **MS Analysis** to navigate to the MS Analysis view. **b** Check that the **EIC** tab is selected in the MS Analysis graph (top half of the screen) and that the **Peak Area by Ion** tab is selected in the table (bottom half of the screen). **c** In the left most column, click on the ion 479.3 to select it. The EIC for this ion is displayed in the graph.	
2 Review the contents of the table for ion 479.3.	The table identifies which target this ion is associated with (RSVB) and displays the peak area for this ion in each well.	

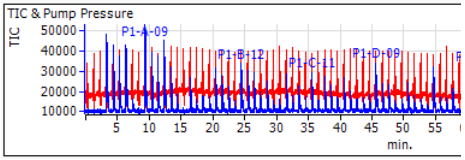
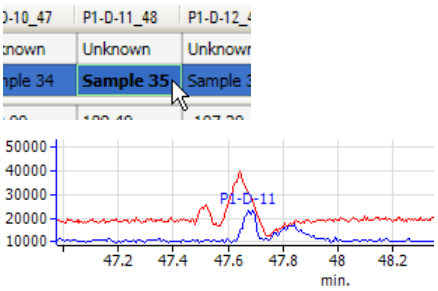
Task 1. Review the EIC and peak areas for an ion

Steps	Detailed Instructions	Screen Images
3 Review the results of the EIC graph for ion 479.3.	You can quickly identify which wells have the highest peak area for this ion (specifically, wells H-01 and H-10).	
4 View the EIC for ion 479.3 in well H-01 alone.	• In the table, click on the peak area for ion 479.3 in the column for well H-01. You may need to scroll over to find the column for well H-01. The EIC graph zooms in on the peak for ion 479.3 specifically in well H-01.	

Task 2. Review the TIC and pump pressure

Task 2 shows you how to display the total ion chromatogram (TIC) and pump pressure for the entire plate and for a single well.

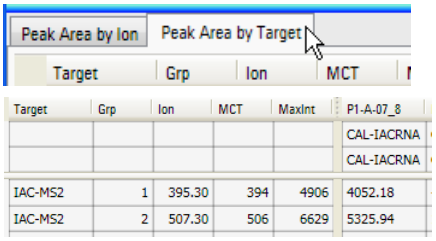
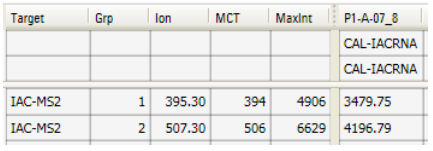
Task 2. Review the TIC and pump pressure

Steps	Detailed Instructions	Screen Images
1 Display the TIC and pump pressure for all wells.	**a** On the graph, click the **TIC & Pump Pressure** tab. **b** To ensure that the graph includes all wells, double-click anywhere on the graph (double-clicking causes the graph to zoom out to include all wells).	
2 Display the TIC and pump pressure for the well containing Sample 35 (well D-11).	**a** In the table, locate the column for Sample 35 (use the horizontal scroll bar at the bottom of the graph). **b** Click on the cell displaying the sample name or click any of the peak area values in that same column. The TIC and pump pressure graph zooms in on the selected well.	

Task 3. Change the peak window for calculating peak areas

Task 3 shows you how to change the peak start and peak end values and demonstrates how these values alter the calculated peak areas. The default peak start and peak end values for the software are 0.20 and 0.80, respectively, but the sample experiment uses the values 0.12 and 0.60. The optimal values are dependent on the tubing length on your particular LC/MS instrument. This task will show you how to change the peak start and end values in case you need to do so.

Task 3. Change the peak window for calculating peak areas

Steps	Detailed Instructions	Screen Images
1 Note the peak areas of the IAC-MS2 target in the CAL-IACRNA well.	**a** Click the tab **Peak Area by Target**. The 2 ions for the IAC-MS2 target are the top 2 rows of the table. **b** Locate the column for the CAL-IACRNA well. The peak areas are 4052.18 for the Grp 1 ion and 5325.94 for the Grp 2 ion.	
2 Change peak start and peak end values. • **Peak Start:** 0.20 • **Peak End:** 0.80	**a** In the **Peak Start** field, type 0.2. **b** In the **Peak End** field, type 0.8. **c** Click **Apply**.	 The fields are highlighted yellow until you click **Apply**.
3 Note the new peak areas of the IAC-MS2 target in the CAL-IACRNA well.	The new peak areas are 3479.75 and 4196.79.	
4 Change peak start and peak end values back to their original settings. • **Peak Start:** 0.12 • **Peak End:** 0.60	**a** In the **Peak Start** field, type 0.12. **b** In the **Peak End** field, type 0.6. **c** Click **Apply**.	
5 Save changes to the experiment.	• Click **File > Save Sample_your name.D**, or press **Ctrl+S**.	

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In this Book

This guide takes you through a series of exercises to familiarize you with the operation and data analysis capabilities of the MassCode PCR software.

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