Agilent MassCode PCR Research Solution

Quick Start Guide

Revision A, August 2011

Use this guide to help you get started setting up and running samples with the Agilent MassCode PCR Research Solution.

What Is the Agilent MassCode PCR Research Solution?

Its purpose

The Agilent MassCode PCR Research Solution provides a rapid and sensitive means of analyzing samples for 20-40 targets simultaneously using multiplex PCR (Polymerase Chain Reaction) and LC/MS (Liquid Chromatograph/Mass Spectrometry) technologies. The application analyzes for viral and bacterial targets or any other set of nucleic acid targets for which primers can be designed.

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Agilent Technologies

Its process (Figure 1)

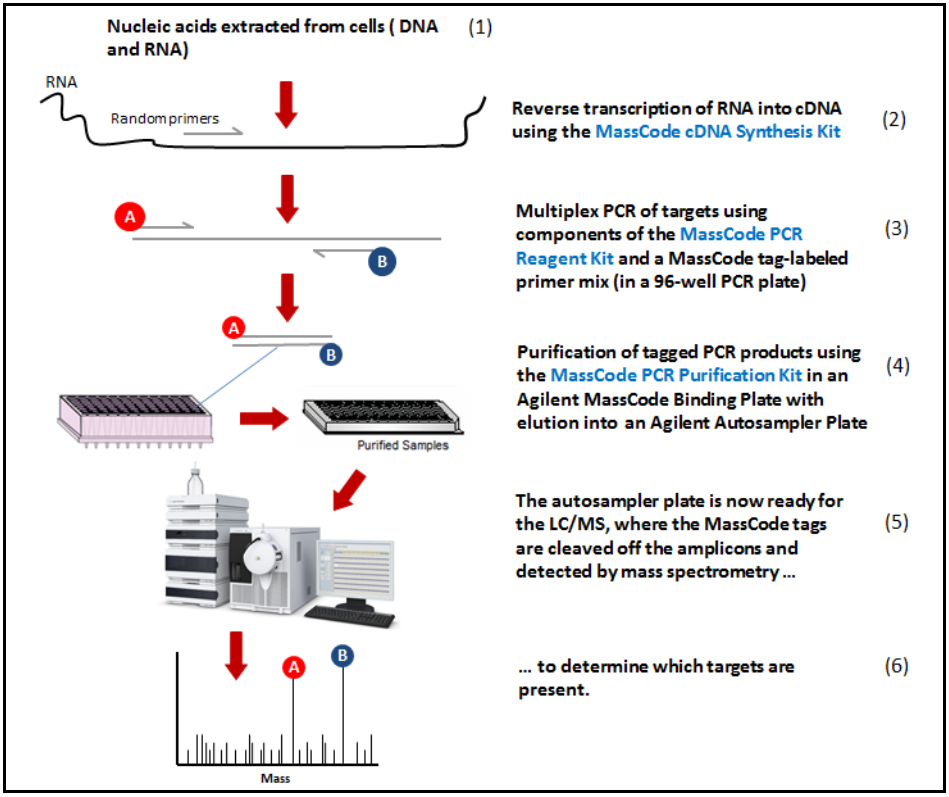


Figure 1 Sample Path for the Agilent MassCode PCR Research Solution

After the DNA/RNA is extracted from the sample (1) and the RNA converted into cDNA (2), targets are amplified by PCR using primers specific for various target sequences (3). Each primer has a tag, which provides a digital code specific to a target sequence, resulting in two tags per amplicon.

The PCR products are then purified to remove excess primers, primer dimers and other reaction side products, buffer components and enzyme (4). A 96-well plate containing the purified PCR products is placed in the autosampler, where each sample is injected in a specified order and passed through a Liquid Chromatograph (LC)/Mass Spectrometer (MS) system for cleavage of tags by UV irradiation, detection on the mass spectrometer (MS) (5), and analysis with the MassCode PCR software (6).

Its components

The Agilent MassCode PCR Research Solution comprises these hardware, software and reagent components (number is the sample path step in Figure 1):

- Agilent kits for MassCode PCR
  - Sample Preparation Kit (1)
  - cDNA Synthesis Kit (2)
  - PCR Reagent Kit (3 and 4)
  - Agilent panel or custom primer panels for target detection (3) (Respiratory Pathogen Panel is available.)
- Agilent hardware and software components for MassCode PCR
  - SureCycler (2 and 3)
  - HPLC (High Pressure Liquid Chromatograph) with MassCode UV module for cleaving tags (5)
  - LC/MS (Liquid Chromatograph/Mass Spectrometer) with APCI source (5)
  - MassCode PCR software for data acquisition and analysis (6)

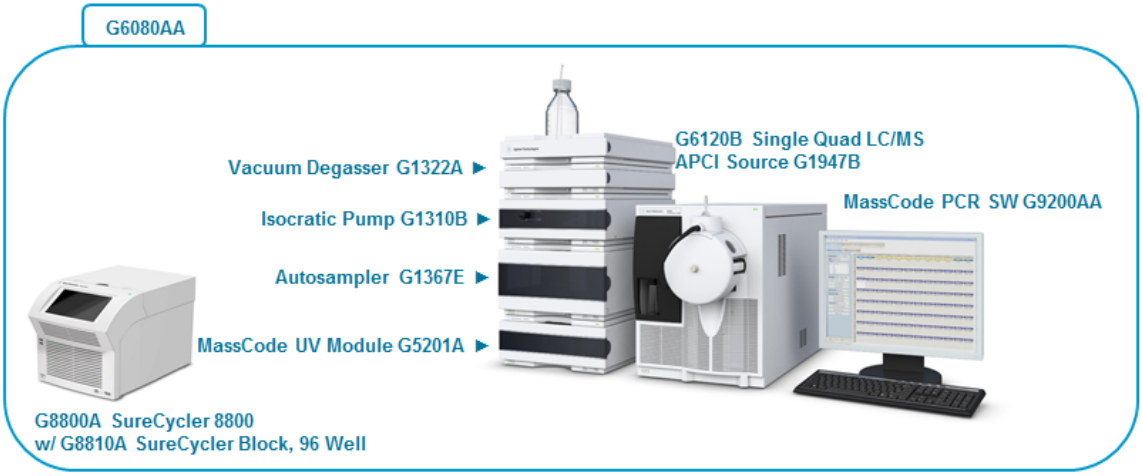


Figure 2 Hardware/Software Components for the Agilent MassCode PCR Research Solution

NOTE This guide assumes that the LC/MS hardware and MassCode PCR software are installed. If you are using an already existing Agilent 6100 series LC/MS and intend to install the MassCode PCR software yourself, see the *Installation Guide*.

How does MassCode PCR Technology work?

The PCR step uses two tagged (forward and reverse) primers specific for each target DNA or cDNA. The two tags are small organic molecules whose molecular weights are distinct from each other and from the tags used for labeling the other target sequences. See Figure 3.

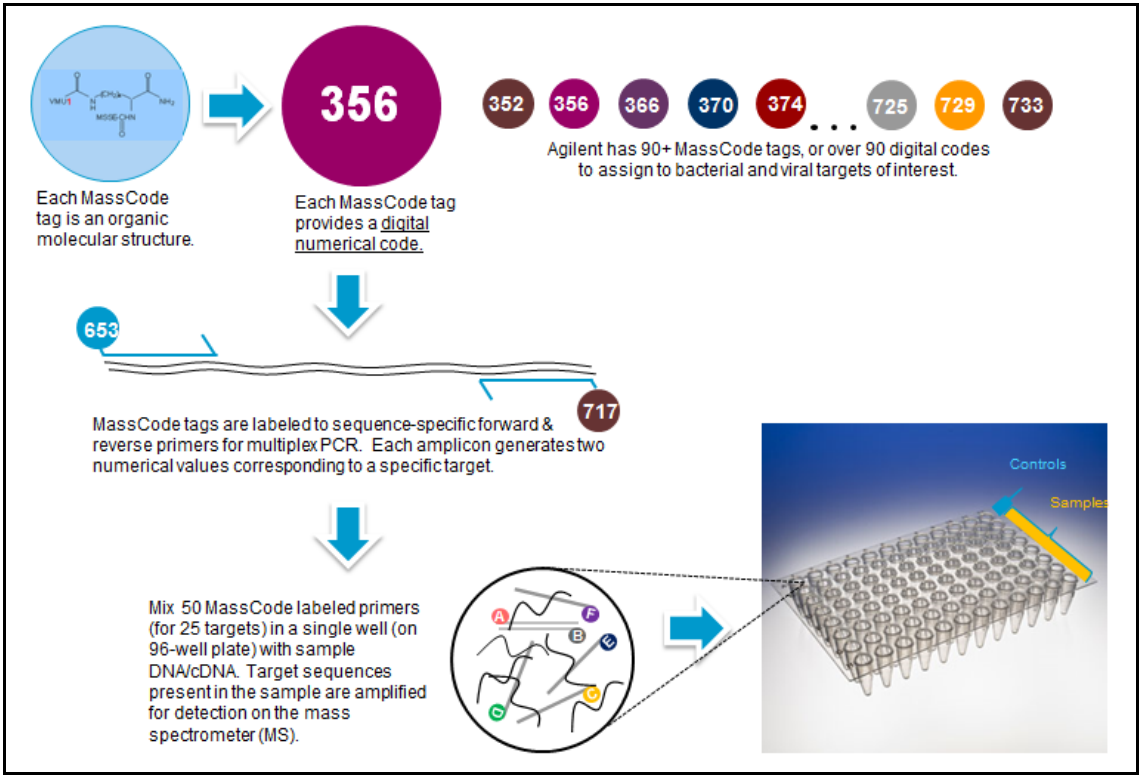


Figure 3 Production of tagged target sequences for cleavage and detection

When a sample containing one or more targets (Agilent's Respiratory Pathogen Panel contains primers for 24 targets and an internal control) passes through the MassCode UV module of the liquid chromatograph (HPLC), it is exposed to UV light and the attached MassCode tags are cleaved, producing small compounds of different molecular weights, which are subsequently detected by mass spectrometry.

Both tags for each target must be observable above a certain cutoff value in order for the target to be called “positive” in the sample. See Figure 4.

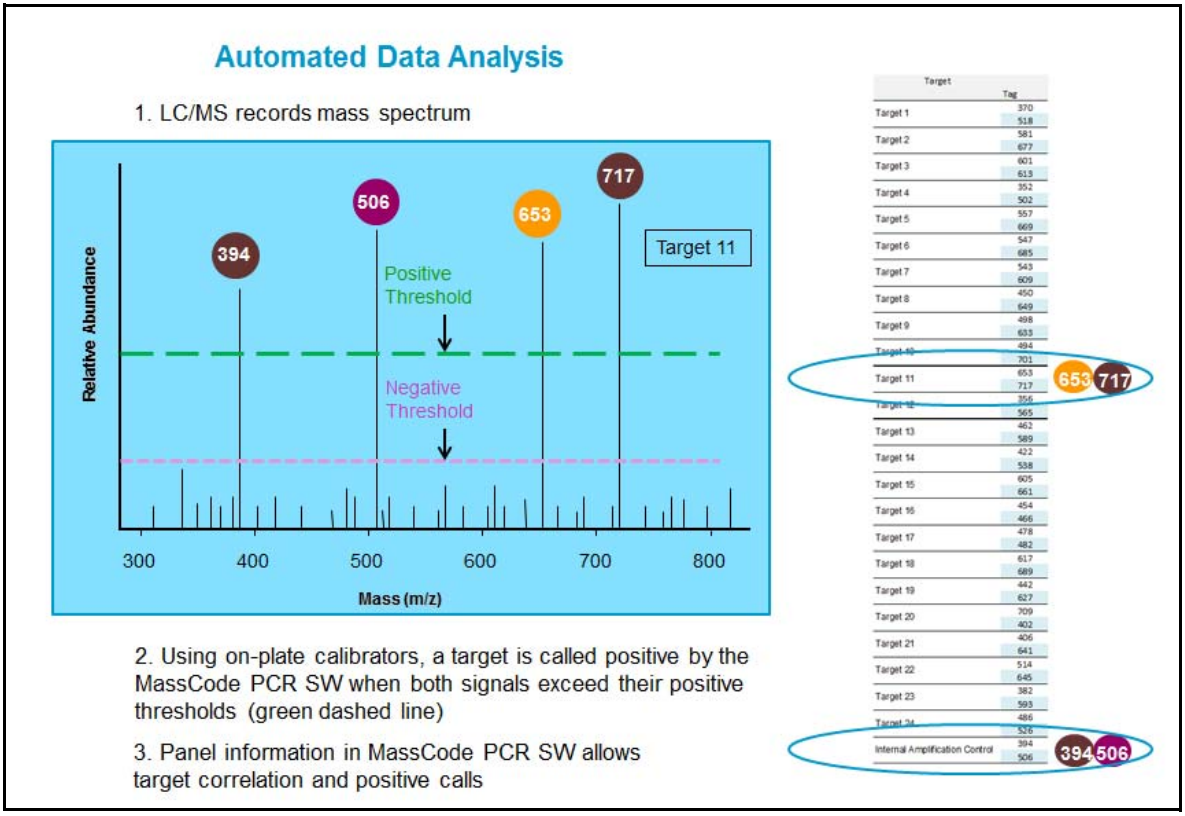


Figure 4 Analysis of cleaved target tags

Finding Help

Before using the help to get started, the LC/MS hardware and software must be installed. Have Agilent Support install the system unless you have received only the MassCode PCR software for an existing Agilent 6100 series LC/MS. In that case refer to the *Installation Guide,* which covers installation of the software and modification of your LC/MS system to include the Agilent MassCode UV Module.

NOTE Do not remove/rename any installation files while using the software. The program may not operate correctly afterwards. If one or more installation files have accidentally been removed or renamed, they can be reinstalled by closing and then reopening the program.

Find the Getting Started Help

Quick Start Guide – Gives an overview of the entire solution and each major workflow step you must perform to get meaningful results

Getting Started section of the Online Help – Provides you instructions for working with the Getting Started screen of the software: creating target panels, starting new experiments and opening existing experiments

Familiarization Guide – Takes you through plate and run setup in the software, then guides you through an analysis with an example data file that comes with the system

Find more detailed Help

To find more detailed information for the entire workflow, see:

Well-Plate Preparation Protocols – instructions for preparing the well plates, from sample extraction, RT-PCR, and purification to well-plate placement in the autosampler

Respiratory Sample Preparation Protocol

cDNA Synthesis, PCR and PCR Purification Protocol

Online Help – help for all facets of the software, from instructions for getting started to explanations of algorithms

- For **ToolTips,** pass the cursor over each button, field and menu item.
- Press **F1** to see descriptions of each field, button, chart and table for each main screen and dialog box. (These are the same descriptions found in the User Interface Help for main screens and dialog boxes.)

- Click **Help** in a dialog box.
- After you start the program, in the menu bar click **Help > Contents** to view the entire Help system.

  The Online Help is divided into these categories:

  Finding Help - Presents instructions to find help for the whole solution

  Getting Started - Shows an overview of the program, concepts for successful operation and instructions to start using the program

  How-To Help - Gives instructions on how to use the software

  User Interface Help - Provides explanations for every field, button, chart and table for each main screen, dialog box and menu

  Reference Help - Shows the equations and thinking behind the System Suitability calculations and results interpretation

  Troubleshooting Help - Helps you troubleshoot the solution; explains the actions you need to take for each System Suitability failure, unexpected result, and error message
- Agilent also provides manuals with each of the instrument modules you use for this application.

Find help maintaining and troubleshooting the LC/MS

Refer to LC/MS Prep and Maintenance Guide, or

Contact Agilent Tech Support

E-mail: masscode.support@agilent.com or click *Help* > *Email Feedback*.

Phone: For Agilent's worldwide sales and support center telephone numbers, go to www.agilent.com/chem/contactus. From the US and Canada, call 800-227-9770 (select options 3-4-3).

Solution Workflow

The Agilent MassCode PCR Research Solution *workflow* consists of steps needed to prepare the LC/MS and well plate, and take the prepared well plate through cleavage, detection and analysis (Table 1).

Table 1 Solution workflow for preparing and running MassCode PCR samples

	To do this workflow step:	Use Agilent Product(s):	And follow the instructions in:
1	Prepare the LC/MS for a run. • Prepare solvents, LC/MS and System Suitability (SS) calibrator for the run.	G6120B Single Quad LC/MS with APCI Source MassCode PCR software PCR Reagent Kit (SS calibrator)	LC/MS Prep and Maintenance Guide
2	Prepare the well plate for a run.	Respiratory Sample Preparation Kit; cDNA Synthesis Kit; PCR Reagent Kit	Respiratory Sample Preparation Protocol and cDNA Synthesis, PCR and PCR Purification Protocol
3	Define a plate setup and enter the run parameters.	MassCode PCR SW (Online Program)	Familiarization Guide Online Help (How To and User Interface Help)
4	Run and monitor the well plate.	G6120B Single Quad LC/MS with APCI Source MassCode PCR SW (Online Program)	Online Help (How To and User Interface Help)
5	Review results.	MassCode PCR SW (Offline or Online Program)	Familiarization Guide Online Help (How To, User Interface and Reference Help)

Step 1: Prepare the LC/MS for a Run

Preparing the appropriate solvents and calibrators and equilibrating the LC/MS are the first steps of the Agilent MassCode PCR Research Solution workflow.

What's Required

- Completely installed LC/MS hardware and MassCode PCR software (Figure 2). See the *Installation Guide*.
- Instructions in the *LC/MS Prep and Maintenance Guide*

How to prepare the LC/MS for a run

1) Prepare the LC solvent

Before each run, check the solvent volume levels to make sure you have enough for the run.

Make sure that the LC solvent bottle contains 500 ml of the solvent before each run. An entire 96-well plate requires about 360 ml of solvent, but 500 ml prevents air bubble formation.

2) Equilibrate the LC/MS

Make sure the system contains no air bubbles and the pressure fluctuates within about 1 bar.

3) Prepare System Suitability vials

Before running samples, you must run the MS grade water that was used to elute the purified PCR samples to make sure it is free of background ions (Vial 1) and a calibrator to ensure the MS measurements are accurate (Vial 2).

The System Suitability methods using these samples check mass alignment and reagent contamination, and prepare the system for data analysis.

4) Place System Suitability vials in the autosampler

See the *Reference Help* (Online Help) for information on the calculations for the System Suitability measurements.

Step 2: Prepare the Well Plate for a Run

Preparing a well plate comprises the first four steps of the sample path.

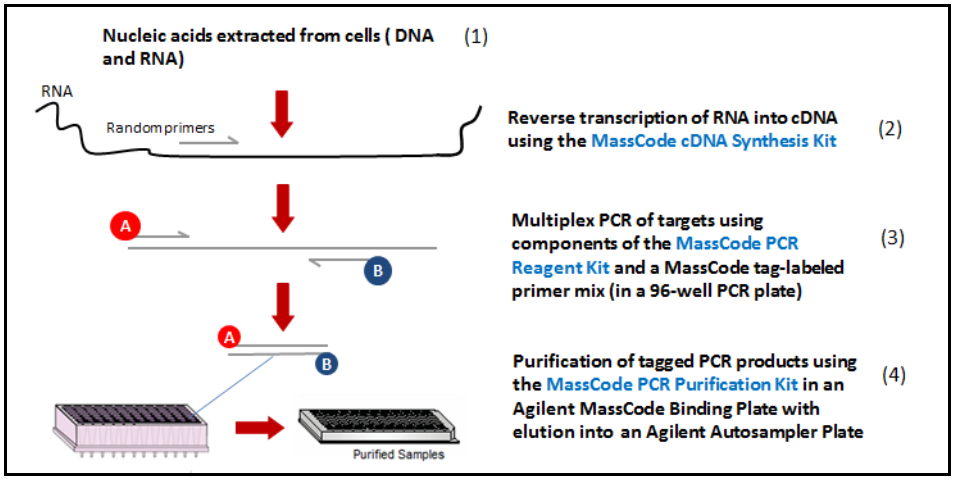


Figure 5 Preparing a well plate

What's required

Table 2 Kits and Instructions for Well-Plate Preparation

Sample Path Step	Kit	Protocol
1	Sample Preparation Kit (Respiratory Sample Preparation Kit is available.)	Sample Preparation Protocol (Respiratory Sample Preparation Protocol is available.)
2	cDNA Synthesis Kit	cDNA Synthesis, PCR and PCR Purification Protocol
3	PCR Reagent Kit and Target Panel Kit (Respiratory Pathogen Panel is available.)	Same protocol as above
4	PCR Purification Kit (inside PCR Reagent Kit)	Same protocol as above

How to design a well-plate setup

Your testing for unknown nucleic acid targets takes place in a 96-well plate. Although you do not need to place samples in the 96-well plate until the PCR step of the well-plate preparation, it helps to design the setup of your plate before beginning to prepare it.

The easiest way to design a plate setup is to use the Plate Setup screen in the software after you start a new experiment. See "How to define a plate setup and enter run parameters" on page 17.

Agilent recommends you set up your plate as depicted in the figure below and run the plate by row.

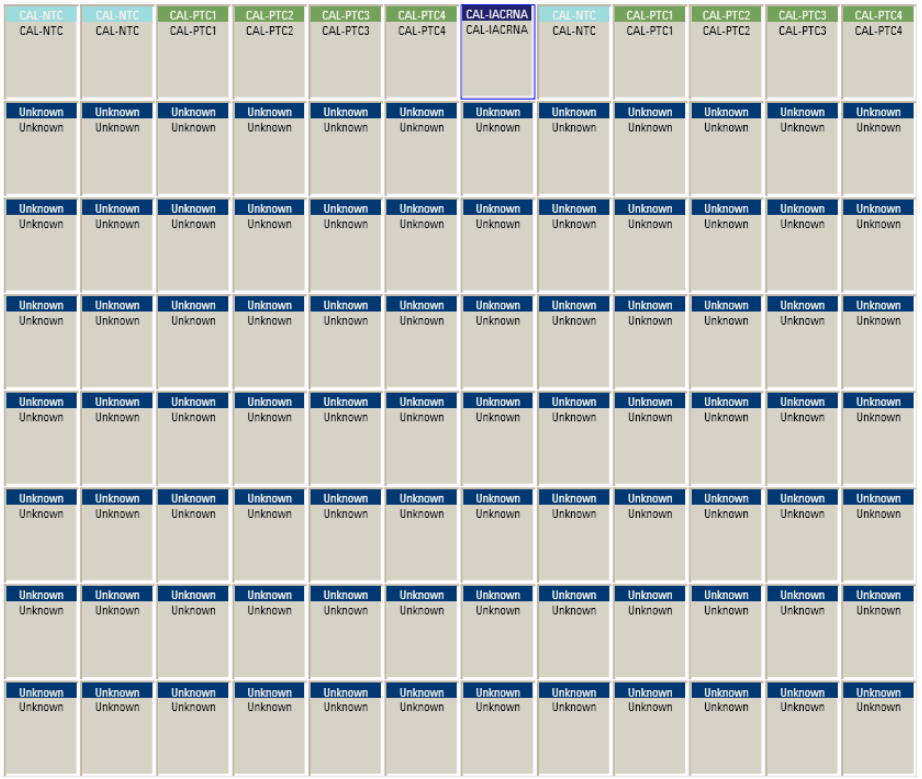


Figure 6 Well-Plate Setup with 12 Calibrators and 84 Unknowns

Calibrators

Agilent MassCode PCR calibrators (three types) are used for calculating the background against which the signal abundance of the unknown is compared. Without a reliable calculation of the background, target detection is unreliable. To learn how targets are detected, see the **Online Help > Reference Help > Target Detection Algorithms**.

For this reason Agilent recommends that Row A on the plate be populated with a mix of 12 calibrators.

Table 3 Recommended Set of Calibrators for Reliable Target Detection

Calibrator Type	Description and Number Needed
CAL-NTC	No Template Calibrator – should contain no unexpected target ions • Place three of these on the plate.
CAL-PTC's	Positive Template Calibrators – each contain several targets from the target panel used • For the Agilent Respiratory Pathogen Panel four different targets are included in each of four different CAL-PTC's. See the Respiratory Pathogen Panel kit for a list of the PTC targets. • Place duplicate sets of CAL-PTC 1-4 on the plate.
CAL-IACRNA	Internal Amplification Calibrator – contains the internal control used for the unknowns; confirms proper preparation of the plate, and contributes to the background calculation • For the Agilent Respiratory Pathogen Panel an MS2 phage is used because most of the targets are RNA targets. If you are using another target panel with primarily DNA targets, use an appropriate DNA control. • Place only one on the plate.

CAUTION Using fewer than 12 calibrators or any other combination of Agilent or non-Agilent calibrators may result in unreliable results.

As you prepare the plate, you introduce these calibrators at different steps of the preparation:

CAL-IACRNA – Set up one reaction at the extraction step.

CAL-NTC – Set up three reactions at the cDNA synthesis step.

CAL-PTC's – Set up two reactions for each PTC at the PCR step.

How to prepare a well plate

You can find the details for the steps below in the Agilent MassCode PCR Research Solution protocols.

1) Extract nucleic acids

After extracting the nucleic acids with Proteinase K, you immobilize, wash and elute them.

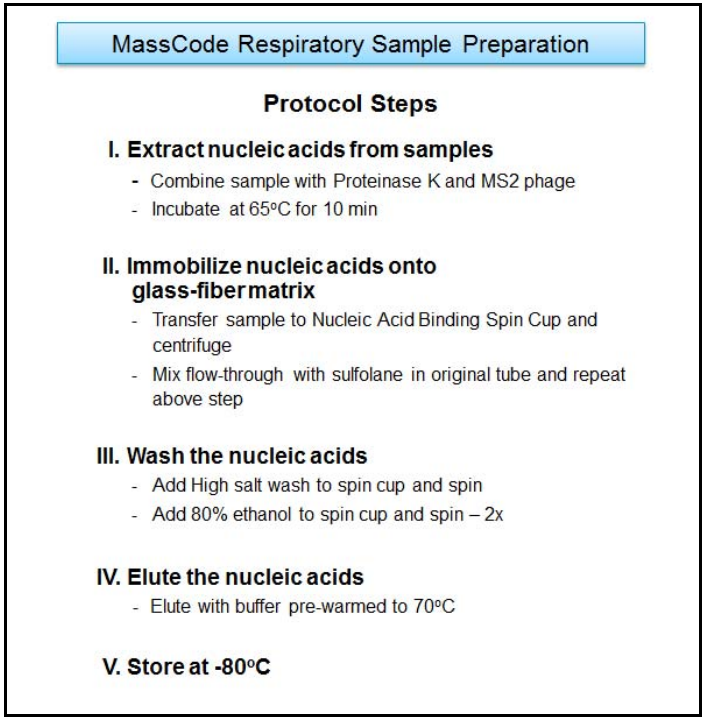


Figure 7 MassCode Respiratory Sample Preparation

2) Synthesize cDNA

Agilent optimized reagents and incubation times to produce the highest signal to noise (S/N).

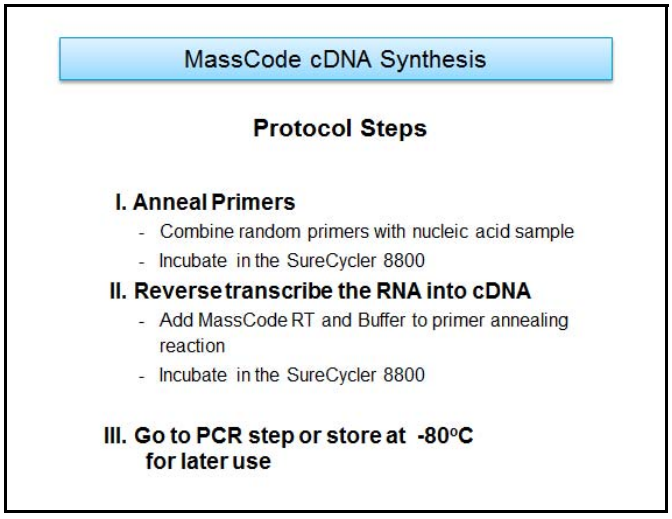


Figure 8 Synthesizing cDNA from RNA

3) Amplify targets with PCR

Agilent optimized PCR reagents and cycling times to produce the highest S/N for as many targets as possible.

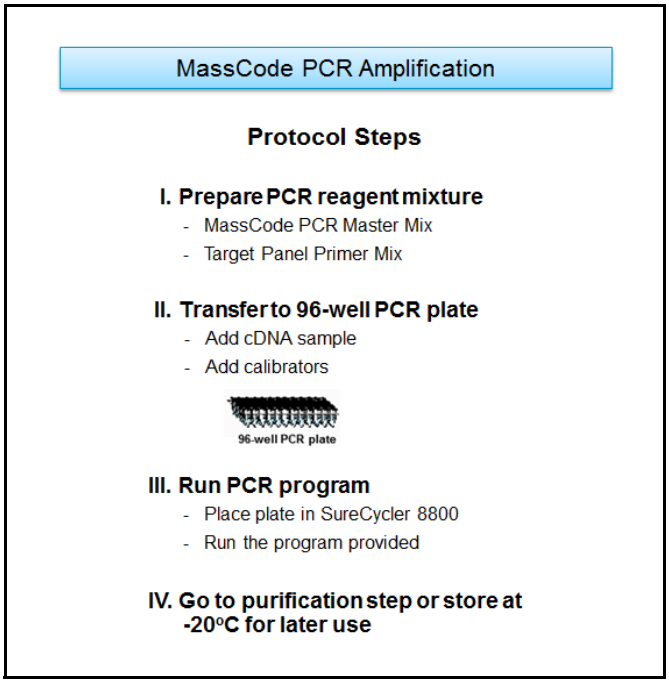


Figure 9 PCR Amplification

Current Target Panels (tagged primers) At present Agilent offers a Respiratory Pathogen Panel kit that contains 24 pathogen primers, an Internal Amplification Calibrator (CAL-IACRNA) and Positive Template Calibrators (PTCs), each containing a set of four standard target amplicons that are used for calibrating the cutoff values needed to identify the corresponding targets as present or absent in the samples.

See the list of respiratory targets and PTCs that accompany the Agilent Respiratory Pathogen Panel kit.

You can also create custom panels and order the corresponding primers from Agilent.

4) Purify PCR products

The next step in the sample path is to separate extra tagged primers and primer dimers from the PCR amplicons.

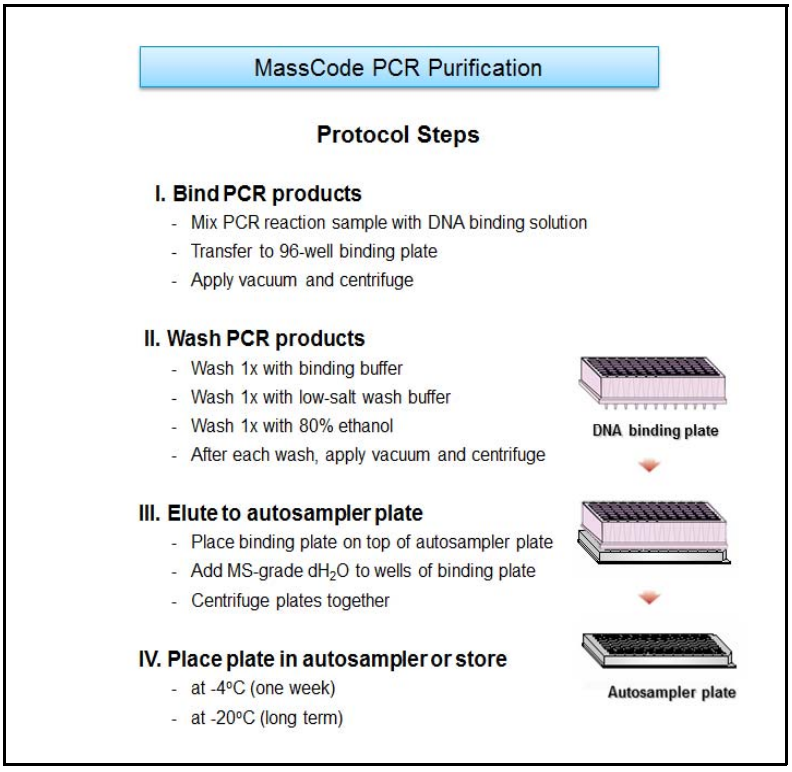


Figure 10 PCR Purification

NOTE You can find the instructions for placing the well plate in the autosampler in the *cDNA Synthesis, PCR and PCR Purification Protocol*.

You are now ready to set up the plate and run parameters with the MassCode PCR program.

Step 3: Define a Plate Setup and Enter Run Parameters

What's required

- The online program of the MassCode PCR software

  The online program opens both the Agilent ChemStation program and the MassCode PCR program. Agilent recommends that only Advanced users access the ChemStation program, which runs in the background supporting communication between MassCode PCR and the LC/MS. See **Online Help > "How To" Help > Running Samples > Working with ChemStation (Advanced)**.
- For instructions on getting started, defining a plate setup, changing the run parameters and saving the experiment settings, see the *Online Help*.

What is a MassCode PCR experiment?

A MassCode PCR experiment is a set of files that can contain plate-setup, run and analysis settings or the settings and results. The contents of an experiment depend on whether it is a new one or an existing one.

A new experiment, or a "pre-run" experiment, contains plate set-up and run settings to be used for acquiring data, as well as Experiment Notes, all of which are saved to the experiment settings file (.MT5).

An existing experiment, or a "post-run" experiment, is essentially the data file (.D) containing the acquired data plus the experiment settings file.

How to define a plate setup and enter run parameters

1) Launch the online MassCode PCR program

- Double-click the **MassCode PCR Online** icon. 

  The Getting Started screen for the Basic mode appears. You can switch to Advanced mode.

Basic mode For users who intend to work with only Agilent-supplied target panels and LC/MS methods and have no or little experience with LC/MS technology

Advanced mode For users who may customize their own target panels and/or have used LC/MS technology and the Agilent ChemStation before

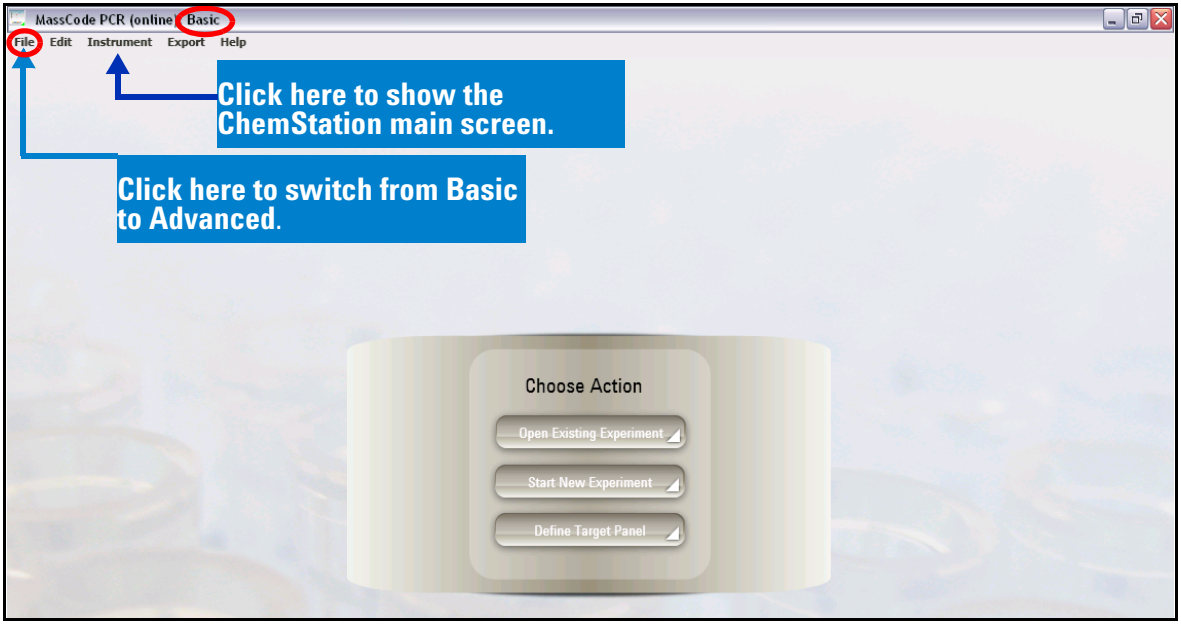


Figure 11 Getting Started screen in Basic mode

For those who intend to operate in Advanced mode and use the ChemStation: See Figure 11.

NOTE

For most of setup and operation you will not need to access the ChemStation main window. In fact, you can look at both LC and MS diagnostics, turn on ChemStation and manually put the instrument in standby after a run from the MassCode PCR menus. MassCode PCR also uses ChemStation methods.

On the other hand, you must do some troubleshooting tasks, such as MS tuning, and some instrument control tasks, such as setting solvent levels in the ChemStation program.

The MassCode PCR program also overrides some ChemStation method settings. See the *Online Help > "How To" Help > Running Samples > Prepare your own acquisition methods, if necessary (Advanced)*.

2) Start a new experiment

When you start a new experiment, the plate setup screen appears with the Experiment Workflow Toolbar at the top.

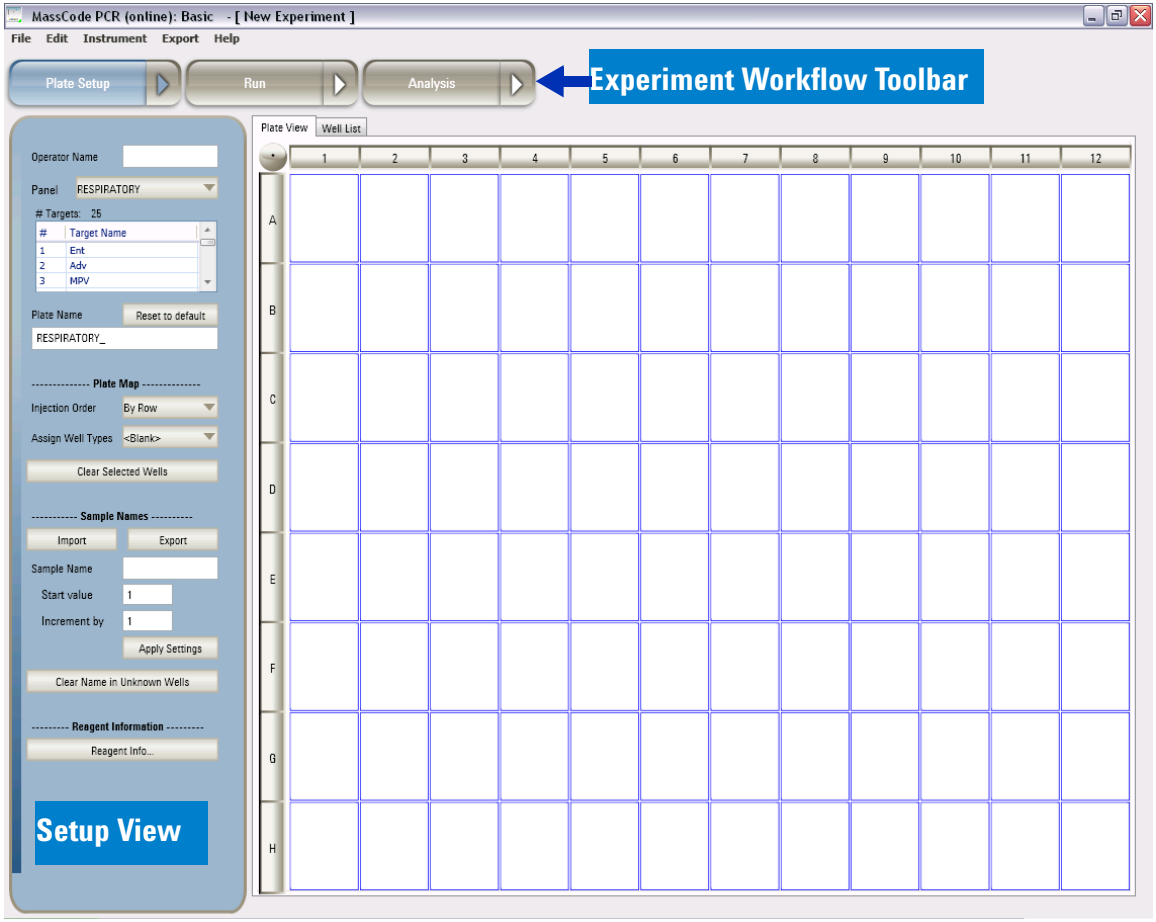


Figure 12 Plate Setup Screen (identical for Basic and Advanced modes)

3) Define a plate setup

In the center of the Plate View in the Plate Setup screen is a representation of a 96-well plate.

Set up a plate so that it matches the layout of the plate containing your purified PCR reactions, including calibrators and samples.

> After the run, you can change the layout to match the layout of the actual plate if they are not the same.

The setup of the plate provides key information to the program on how the data should be acquired, and analyzed after the run is complete.

Setting up the plate includes entering the operator name, choosing the target panel, specifying well types, assigning sample names and designating a run order for the samples. You can set up a plate in either online or offline mode.

You can also save the plate setup for re-use as a template for future experiments.

4) Set up the run

You can set up the run only in the MassCode PCR Online version.

If you are using an Agilent target panel, most of the parameters are set for you. For example, The MassCode_Master.M method is the method optimized for best results with the Agilent Respiratory Pathogen Panel. Basic users must use the MassCode_Master.M method.

The settings you can change depend on the mode you are in—Basic or Advanced.

If you are in Basic mode you can change these settings:

- Data File Name and Data File Directory
- Amplitude for the Total Ion Chromatogram (TIC) and pressure of the well data plots

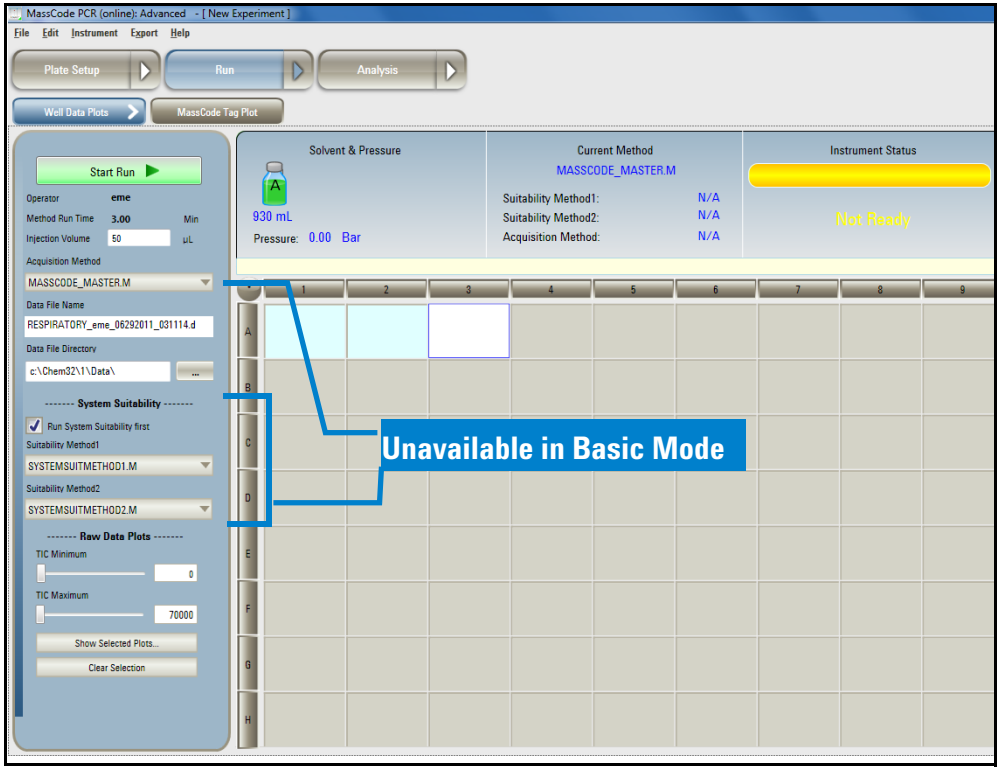


Figure 13 Run Screen

If you are in Advanced mode you can change these settings:

- Acquisition Method
- Data File Name and Data File Directory
- System Suitability Methods
- Amplitude for the TIC and pressure of the well data plots

NOTE If you are in Advanced mode, you can also change the peak start and end points, or the peak window for the analysis. See **Online Help > How To > Setting Up Runs > Enter peak start and end times (Advanced)**.

5) Save the experiment settings

You can save new experiment settings or save modified experiment settings to the same file or a new file.

This file can be saved to the Storage folder or to the .D folder for which the settings are used.

You can reuse these settings by opening the experiment settings file (.MT5) file when you start a new experiment.

You are now ready to start and monitor the run.

CAUTION If you choose to exit the program at this time, close the MassCode PCR program first, then close the ChemStation program. Do NOT close the ChemStation program before the MassCode PCR program. If you do, close the MassCode PCR program immediately.

Step 4: Start and Monitor the Run

After preparing the LC/MS and well plate for a run, defining the plate setup and entering run parameters, you are now ready to run the plate.

What's required

- The online version of the MassCode PCR software
- Instructions for starting and monitoring a run -- *Online Help*

How to start and monitor the run

1) Make sure that the pressure is stable.

You can read the pressure in the Instrument Status bar (Figure 14). It should not fluctuate outside a 1 Bar window (+/- 1 Bar).

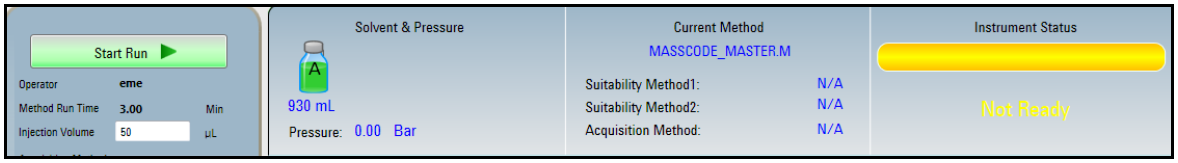


Figure 14 Start Run button and System Status View in Run screen before run

2) Start the run

The Instrument Status bar does not have to be green in order to start the run. After you click on Start Run, the HPLC pump turns on and System Suitability Method 1 runs once the APCI source has reached operating conditions (Figure 15).

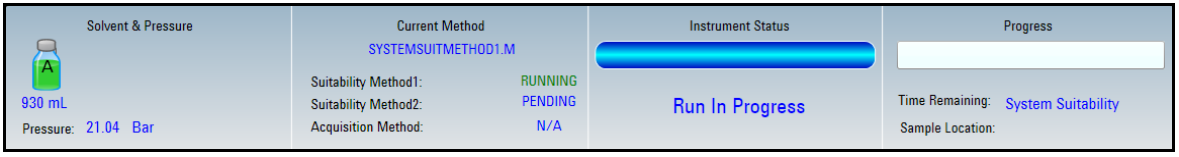


Figure 15 System Suitability Method 1 running

3) Monitor the run

The two System Suitability vials run first, then the calibrators and unknown samples in their order on the plate.

Monitor System Suitability runs If a system suitability method fails, the run stops and a message appears giving the reason for its failure. You can run the plate later.

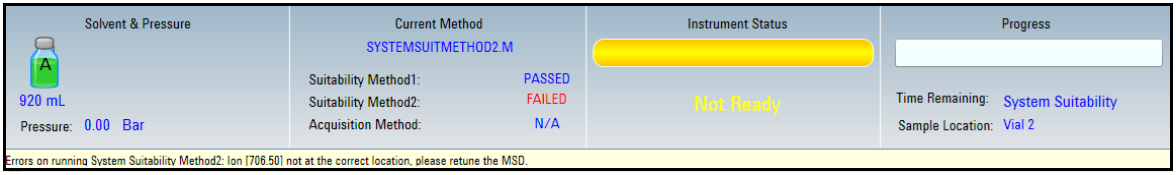


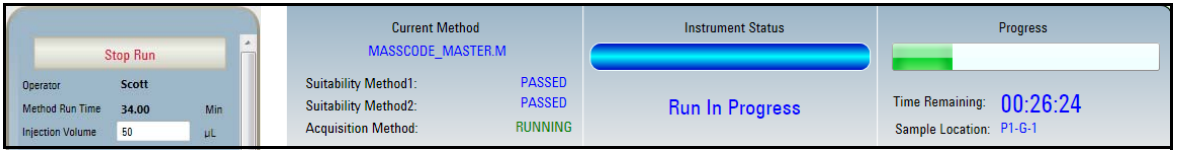
Figure 16 Failure of System Suitability Method 2 with reason for failure

NOTE

If there is a System Suitability or LC/MS failure during a run and you must wait to run the LC/MS again, use these general rules for storing the well plate until the next run:

- If the wait is a day or less, keeping the well plate at room temperature is fine.
- If the wait is overnight or several days, store the well plate at 4°C.
- For long-term storage, it is best to prepare the plate again and store it at -20°C after the PCR reaction and before purification.

Monitor acquisition runs The Stop button does not appear and the Progress bar does not begin until the Acquisition Method starts acquiring data.



As you monitor the acquisition run, you can look at one of two plots:

- Well Data Plots
- MassCode Tag Plot

Well Data Plots

You can monitor the data plots for individual wells either within the wells or as an expanded version within which you can view the TIC (Total Ion Chromatogram) and pressure of each sample.

To learn how to do this, see **Online Help > "How To" Help > Running Samples > Starting and Monitoring the Run > Monitor Raw Data Plots for Plate Wells**.

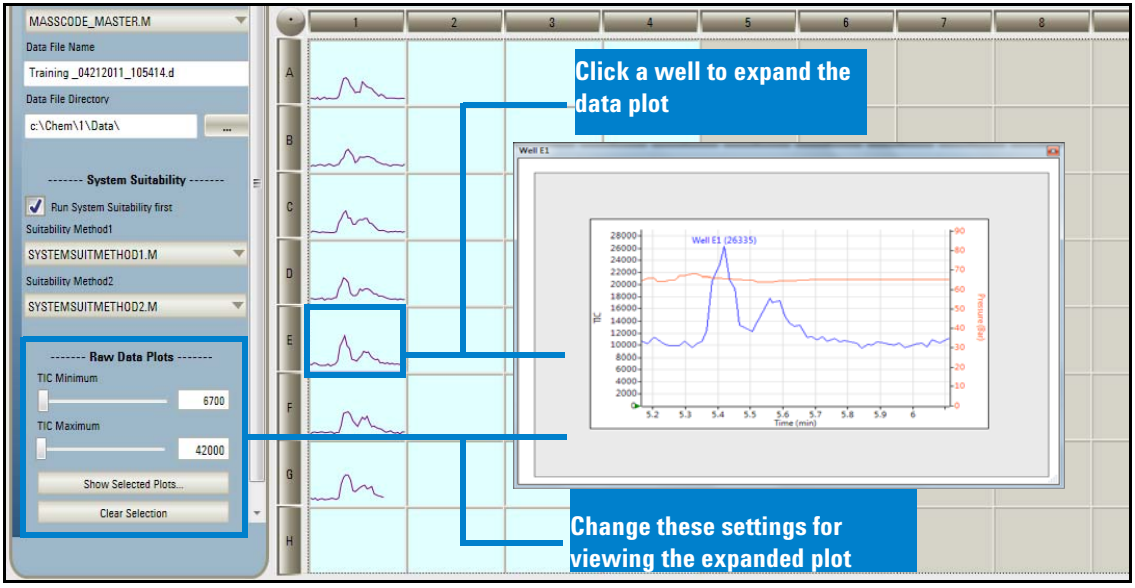


Figure 17 Well data plots and an individual well expanded plot

MassCode Tag Plot

You can see the abundance and masses of the MassCode tags as they are being detected by the MS.

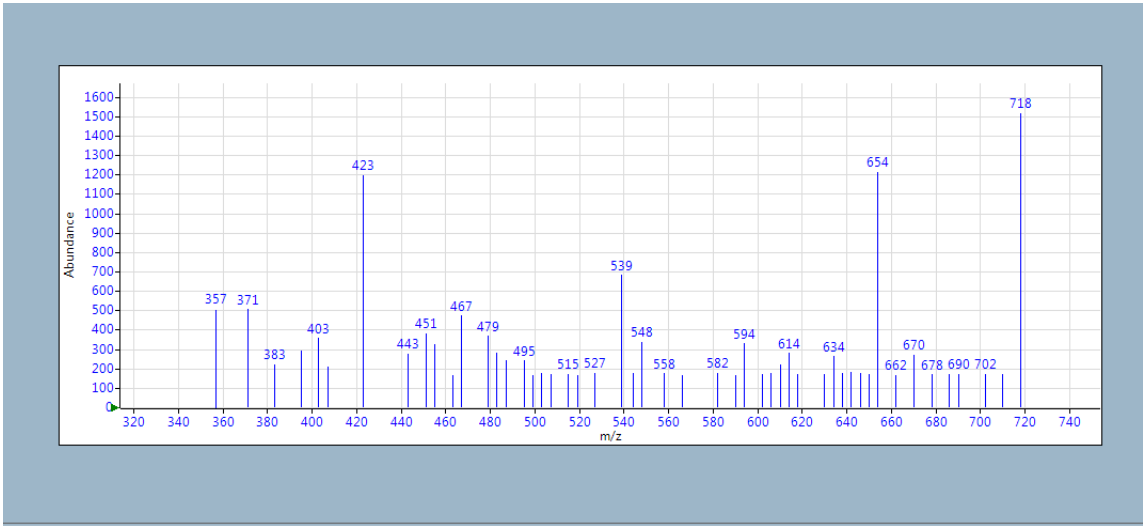


Figure 18 Plot showing the abundance and mass of MassCode tags as they appear in real time

When the run is complete you are ready to review results.

CAUTION If you choose to exit the program at this time, close the MassCode PCR program first, then close the ChemStation program. Do NOT close the ChemStation program before the MassCode PCR program. If you do, close the MassCode PCR program immediately.

Step 5: Review Results

In the Analysis step, you can view the results in multiple formats, modify a few analysis settings, generate reports and export the results to other applications. You can do these tasks in either online or offline mode.

The views that are available in the Analysis screen depend on which mode you are working in—Basic or Advanced.

What's required

- The offline or online version of the MassCode PCR software
- Instructions for viewing results — **Online Help > "How To" Help > Reviewing Analysis Results**
- Tables for interpreting results to determine if a plate or a well needs to be repeated — **Online Help > Reference Help > Results Interpretation Tables** (Print these out; you may refer to them frequently.)

How to review results

The Batch Report appears automatically at the end of a run for Basic mode, and the Plate Results appear in Advanced mode at the end of a run.

If you want to see the results later after you have closed the program, open an existing experiment in the Getting Started screen. The Batch Report opens if you are in Basic mode, and the Plate Results open if you are in Advanced mode.

You can switch between Analysis Views by clicking on one of the buttons shown in Figure 19.

Figure 19 Analysis results buttons (Advanced Mode)

See the descriptions of the Analysis Views on the next few pages:

- Report – Batch Report is the default for Basic mode; you can also choose an Individual Report.
- Plate Results – Default for Advanced mode
- Batch Results
- MS Analysis (Advanced mode only)

You may need to change the well-type assignment or sample names for a few wells in order to reflect the actual contents of a well. You use the Plate Setup screen to do this, just as you would when you set up a new plate.

You can save your changes to the original data file or save them to a new file name.

CAUTION If you choose to exit the program at this time, close the MassCode PCR program first, then close the ChemStation program. Do NOT close the ChemStation program before the MassCode PCR program. If you do, close the MassCode PCR program immediately.

Batch Report

This report contains a listing of the results for all samples on the plate by sample identifier. This is the report that automatically appears after a run is complete for the Basic mode.

The default colors for highlighting results are yellow (positive), pink (indeterminate), and red (no call, invalid results or a negative internal control). If a target is not highlighted in the Batch Report, it is negative. You can change colors in the Preferences dialog box (click Edit > Preferences).

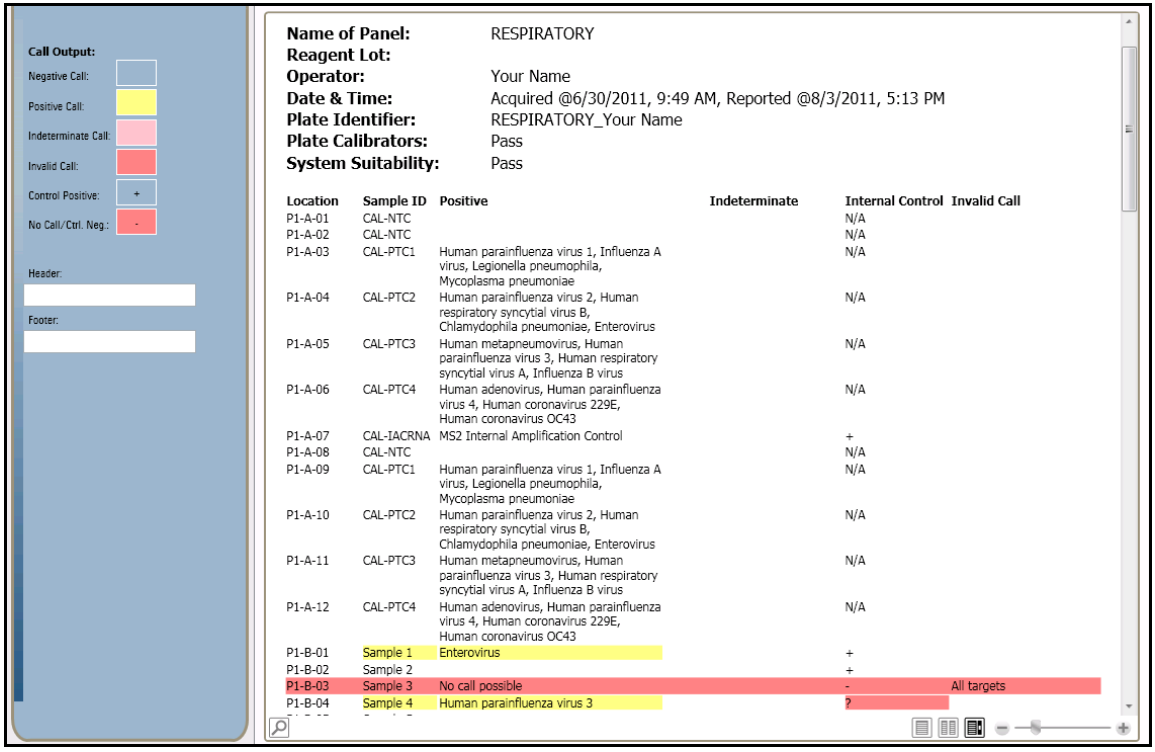


Figure 20 Batch report with legend

Plate Results

This is the screen that automatically appears after a run is complete for the Advanced mode.

The colors and V and F for the calibrator wells indicate whether a calibrator has passed (V) or failed (F).

The colors and +, -, or ? for the unknown wells indicate whether a sample is positive (+), negative (-) or indeterminate (?) for the target.

You can also see the results of the system suitability runs. Each reason for a system suitability failure is described to the left of the plate results.

Figure 21 Plate Results

Individual report

You can access the individual report by clicking the Report button in the Analysis screen and selecting Individual Report.

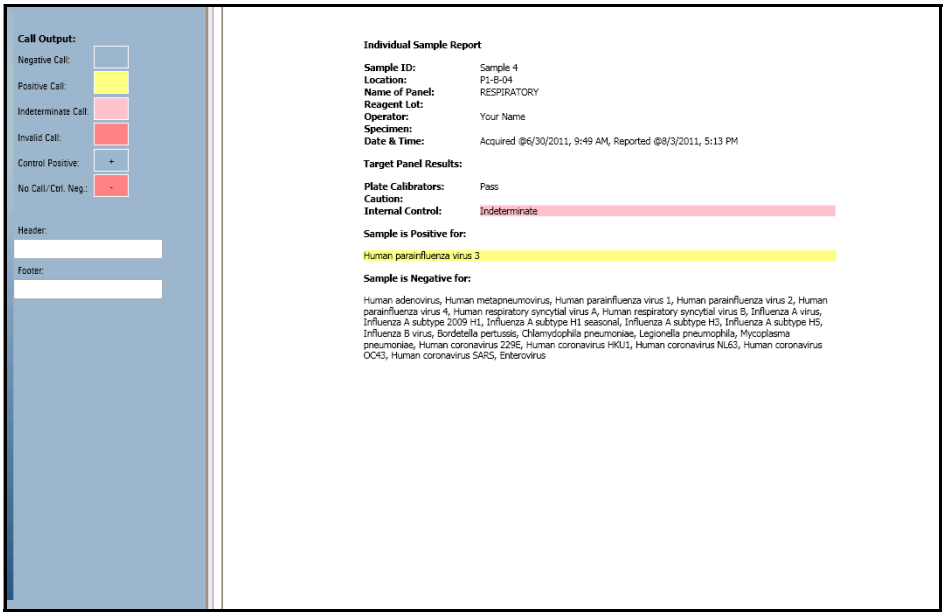


Figure 22 Individual Report with legend

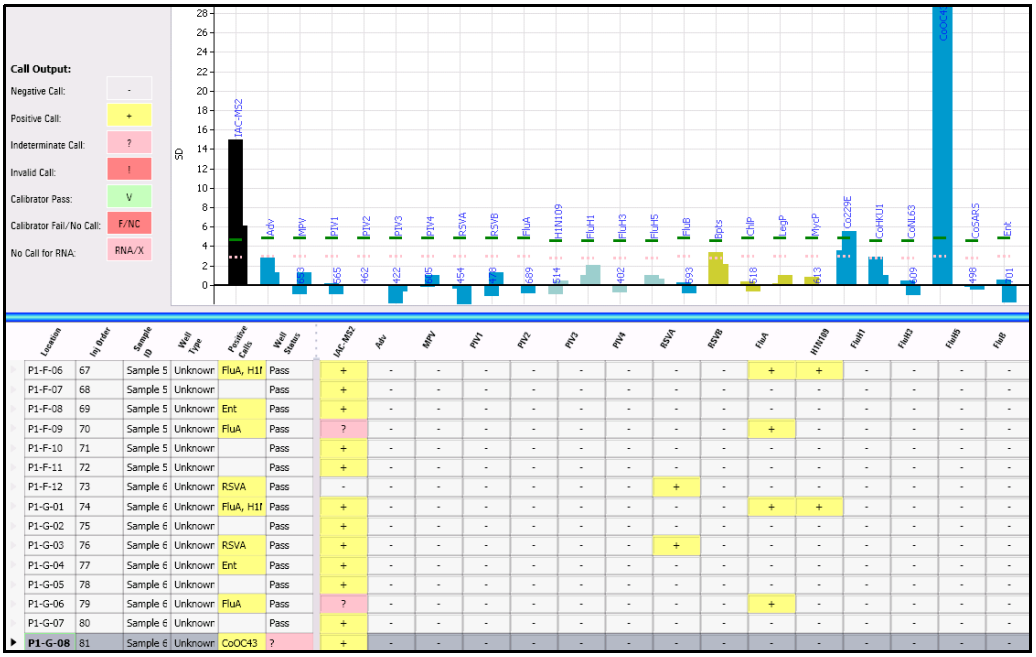
Batch Results

You can graphically view the results of all targets in one sample (click on a row), of one target in all samples (click on a column) or of one target in one sample (click on a cell). View these results to understand why a result is called positive (+), indeterminate (?) or negative (-) or why a well has failed. See Results Interpretation Tables in the Reference Help for more information.

Positive The SD of both ions for the target must be above the green cutoff value. Default color is yellow. (See “Target Detection Algorithms” in the Reference Help for an explanation of how the SD is calculated.)

Indeterminate The SD of one ion is above the green cutoff value, and the second ion abundance is between the pink and green cutoff values, or both ions are between the pink and green cutoff values. Default color is pink.

Negative The SD of at least one ion is below the pink cutoff value, or the ion is nonexistent. Default color is the background color (gray).



Location	Inj Order	Sample ID	Well Type	Positive Calls	Well Status	IAC-MS2	Adv	MPV	PIV1	PIV2	PIV3	PIV4	RSVA	RSVB	FluA	H1N1B	FluH1	FluH3	FluB	FluB
P1-F-06	67	Sample 5	Unknowr	FluA, H1I	Pass	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-
P1-F-07	68	Sample 5	Unknowr		Pass	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P1-F-08	69	Sample 5	Unknowr	Ent	Pass	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P1-F-09	70	Sample 5	Unknowr	FluA	Pass	?	-	-	-	-	-	-	-	-	+	-	-	-	-	-
P1-F-10	71	Sample 5	Unknowr		Pass	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P1-F-11	72	Sample 5	Unknowr		Pass	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P1-F-12	73	Sample 6	Unknowr	RSVA	Pass	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
P1-G-01	74	Sample 6	Unknowr	FluA, H1I	Pass	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-
P1-G-02	75	Sample 6	Unknowr		Pass	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P1-G-03	76	Sample 6	Unknowr	RSVA	Pass	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-
P1-G-04	77	Sample 6	Unknowr	Ent	Pass	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P1-G-05	78	Sample 6	Unknowr		Pass	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P1-G-06	79	Sample 6	Unknowr	FluA	Pass	?	-	-	-	-	-	-	-	-	+	-	-	-	-	-
P1-G-07	80	Sample 6	Unknowr		Pass	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P1-G-08	81	Sample 6	Unknowr	CoOC43	?	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

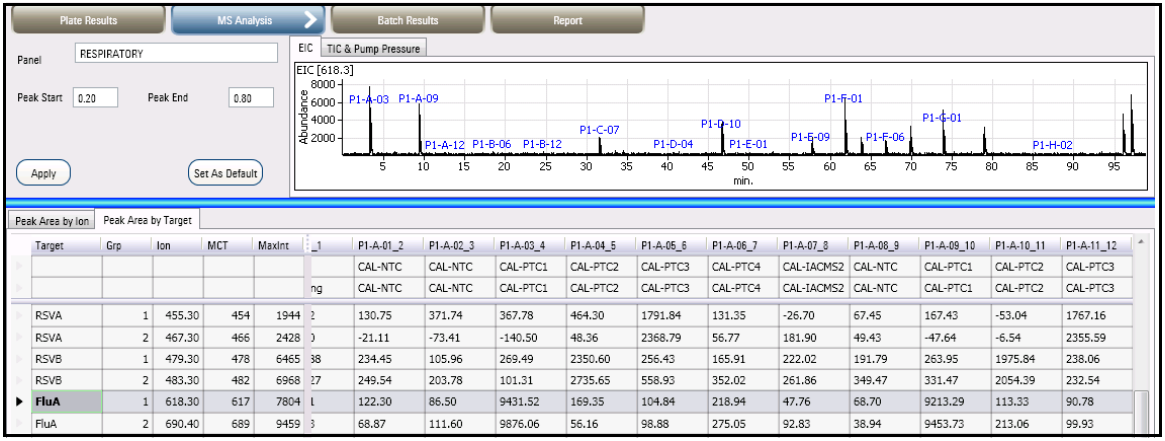
Figure 23 Batch Results

Note in Figure 23 the call for Co229E is indeterminate in the sample G-08 because the SD of one ion is above the green line (positive threshold) and the other is between the pink and green cutoff values.

To zoom in to view a target's results more closely: Right-click the mouse, draw a rectangle around the target(s) and release the mouse.

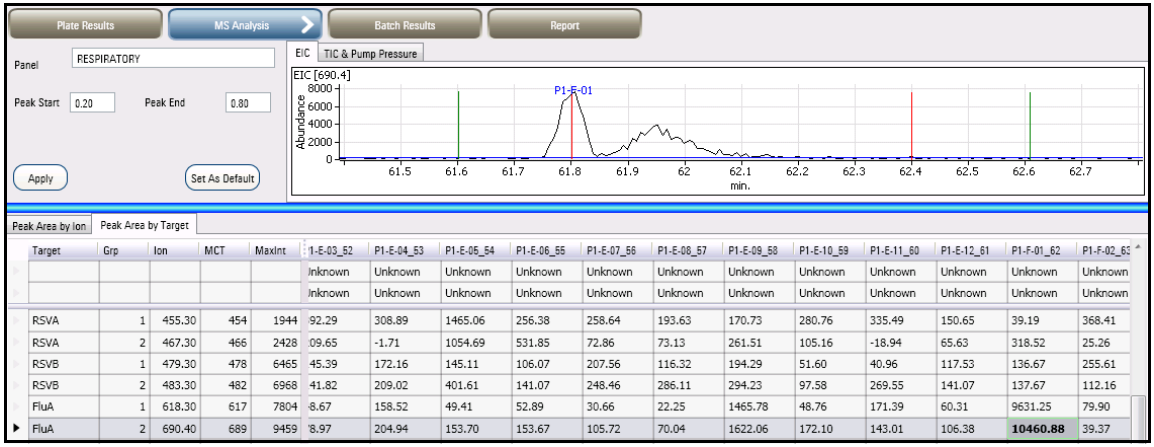
MS Results

These results appear only in the Advanced mode. The plot on this screen shows you the Extracted Ion Chromatogram (EIC) for either a single target or ion in all samples or a single target or ion in one sample. You can also change parameters for modifying peak areas (displayed in the table), as well as view the TIC (Total Ion Chromatogram) and pressure profiles in all samples or in one sample.



Target	Grp	Ion	MCT	MaxInt	_1	P1-A-01_2	P1-A-02_3	P1-A-03_4	P1-A-04_5	P1-A-05_6	P1-A-06_7	P1-A-07_8	P1-A-08_9	P1-A-09_10	P1-A-10_11	P1-A-11_12
						CAL-NTC	CAL-NTC	CAL-PTC1	CAL-PTC2	CAL-PTC3	CAL-PTC4	CAL-IACMS2	CAL-NTC	CAL-PTC1	CAL-PTC2	CAL-PTC3
					ng	CAL-NTC	CAL-NTC	CAL-PTC1	CAL-PTC2	CAL-PTC3	CAL-PTC4	CAL-IACMS2	CAL-NTC	CAL-PTC1	CAL-PTC2	CAL-PTC3
RSVA	1	455.30	454	1944	2	130.75	371.74	367.78	464.30	1791.84	131.35	-26.70	67.45	167.43	-53.04	1767.16
RSVA	2	467.30	466	2428	0	-21.11	-73.41	-140.50	48.36	2368.79	56.77	181.90	49.43	-47.64	-6.54	2355.59
RSVB	1	479.30	478	6465	38	234.45	105.96	269.49	2350.60	256.43	165.91	222.02	191.79	263.95	1975.84	238.06
RSVB	2	483.30	482	6968	27	249.54	203.78	101.31	2735.65	558.93	352.02	261.86	349.47	331.47	2054.39	232.54
FluA	1	618.30	617	7804	1	122.30	86.50	9431.52	169.35	104.84	218.94	47.76	68.70	9213.29	113.33	90.78
FluA	2	690.40	689	9459	3	68.87	111.60	9876.06	56.16	98.88	275.05	92.83	38.94	9453.73	213.06	99.93

Figure 24 EIC (Extracted ion chromatogram) for one target in all samples



Target	Grp	Ion	MCT	MaxInt	1-E-03_52	P1-E-04_53	P1-E-05_54	P1-E-06_55	P1-E-07_56	P1-E-08_57	P1-E-09_58	P1-E-10_59	P1-E-11_60	P1-E-12_61	P1-F-01_62	P1-F-02_63
					Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
					Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
RSVA	1	455.30	454	1944	92.29	308.89	1465.06	256.38	258.64	193.63	170.73	280.76	335.49	150.65	39.19	368.41
RSVA	2	467.30	466	2428	09.65	-1.71	1054.69	531.85	72.86	73.13	261.51	105.16	-18.94	65.63	318.52	25.26
RSVB	1	479.30	478	6465	45.39	172.16	145.11	106.07	207.56	116.32	194.29	51.60	40.96	117.53	136.67	255.61
RSVB	2	483.30	482	6968	41.82	209.02	401.61	141.07	248.46	286.11	294.23	97.58	269.55	141.07	137.67	112.16
FluA	1	618.30	617	7804	8.67	158.52	49.41	52.89	30.66	22.25	1465.78	48.76	171.39	60.31	9631.25	79.90
FluA	2	690.40	689	9459	8.97	204.94	153.70	153.67	105.72	70.04	1622.06	172.10	143.01	106.38	**10460.88**	39.37

Figure 25 EIC for one target in one sample

Safety Warnings

LC/MS Safety Warnings

See the individual manuals for the LC modules and for the LC/MS for safety warnings about handling the LC/MS.

Well-Plate Preparation Safety Warnings

See the *Respiratory Sample Preparation Protocol and the cDNA Synthesis, PCR and PCR Purification Protocol* for safety warnings about handling the biological and chemical materials used for preparing the well plates for analysis.

WARNING **When preparing biological samples using the Agilent MassCode PCR Research Solution, follow safety guidelines for handling biological and chemical materials and wear protective eyewear and gloves.**

WARNING **Always take proper precautions for handling and disposing of solvents and other chemicals. Consult the material data safety sheets supplied by Agilent and the solvent and chemical vendors.**

NOTE For Material Safety Data Sheets (MSDSs) and Certificates of Analysis, visit www.agilent.com/chem/msds or www.genomics.agilent.com.

www.agilent.com

In this Book

The *Quick Start Guide* presents guidelines for using the Agilent MassCode PCR Research Solution.

Printed in USA
Revision A, August 2011

G9200-90000

Agilent Technologies