Agilent CytoGenomics 1.0

Running CytoGenomics Analyses

User Guide

Research Use Only. Not for Diagnostic Procedures

Agilent Technologies

Notices

Manual Part Number

G2602-90004

Edition

Revision A0, November 2010

Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051 USA

Trademarks

Microsoft ® is a registered trademark of Microsoft Corporation in the United States and other countries.

Adobe®, Adobe®Acrobat® and Adobe®Reader® are either registered trademarks or trademarks of Adobe Systems Incorporated in the United States and/or other countries.

Software Revision

This guide is valid for the Agilent CytoGenomics 1.0 software and later revisions, until superseded.

Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

U.S. Government Restricted Rights. Software and technical data rights granted to the federal government include only those rights customarily provided to end user customers. Agilent provides this customary commercial license in Software and technical data pursuant to FAR 12.211 (Technical Data) and 12.212 (Computer Software) and, for the Department of Defense, DFARS 252.227-7015 (Technical Data - Commercial Items) and DFARS 227.7202-3 (Rights in Commercial Computer Software or Computer Software Documentation).

Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In This Guide...

This guide describes how to use the Agilent CytoGenomics 1.0 software to run workflows and display reports and aberrations. The functions described in this guide are typically performed by users with an assigned role of "Technician." However, tasks described in this guide are available to users with an assigned role of "Technician," "Scientist," or "Administrator."

1 Getting Started

This chapter gives an overview of the tasks typically performed by users with the role of Technician. This includes running workflows to extract or import and analyze samples, and displaying reports and aberration results.

2 Running and Monitoring Workflows

This chapter describes how to select and run workflows. It also explains how to use the Job Monitor to find and check the status of workflow jobs.

3 Displaying Reports and Aberrations

This chapter describes how to display the reports and aberrations for completed workflows.

Contents

Contents

1 Getting Started

In Agilent CytoGenomics 1.0, the role of the Technician is to run workflows that perform feature extraction, analysis, and reporting for samples. This user guide gives instructions on how to do the tasks commonly performed by Agilent CytoGenomics 1.0 users who have the role of Technician. Users with the role of Scientist or Administrator can also perform these tasks. For information on how to review results, see the *Setup and Data Review User Guide*.

This chapter gives an overview of the tasks typically performed by users with the role of Technician. The chapters that follow contain step-by-step instructions for performing those tasks.

For information on the tasks commonly performed by users with the Administrator role, see the *Installation and Administration Guide*. For information on setting up and signing off results (typical tasks for the Scientist role), see the *Setup and Data Review User Guide*. For information on windows, command ribbons, dialog boxes, and reports you see in the Agilent CytoGenomics 1.0 program, see the *Reference Guide*.

Overview of Tasks for Running Workflows and Displaying Results

The following diagram shows the main tasks performed by users with the role of Technician in Agilent CytoGenomics 1.0. Details on how to perform these tasks are given in the chapters that follow.

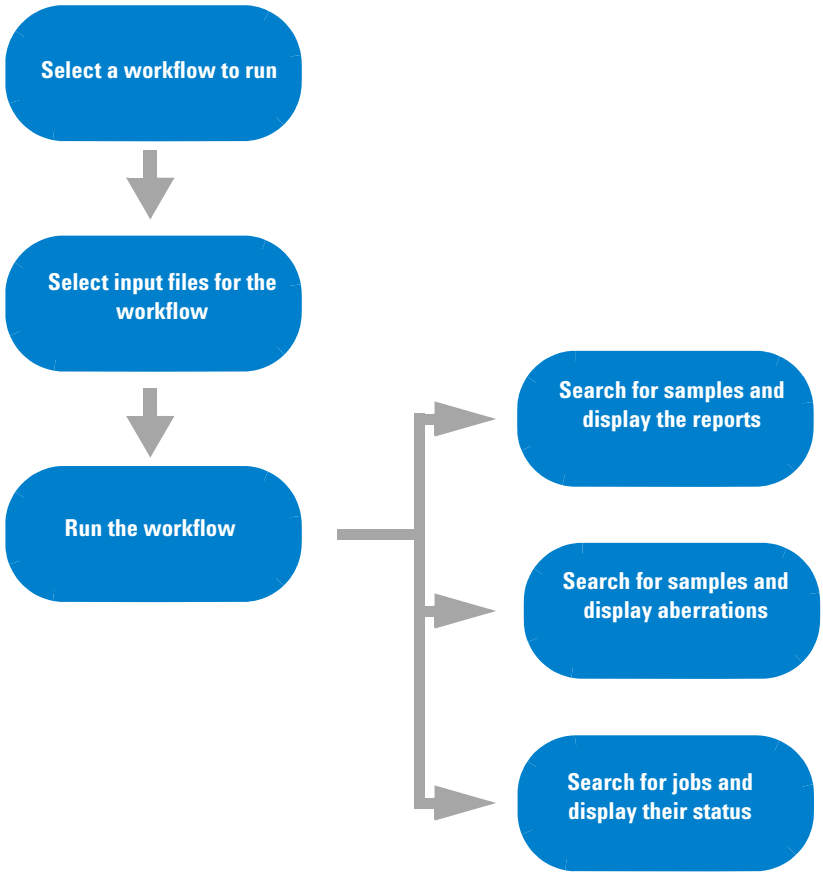

Figure 1 Typical CytoGenomics tasks for running workflows and displaying results

Getting Help

To get help within Agilent CytoGenomics 1.0

Agilent CytoGenomics 1.0 has several help guides. To open a help guide, on the right side of the Agilent CytoGenomics 1.0 tab ribbon, click the **Help** arrow, and then select the help guide you want to display. Help guides are opened in Adobe® Reader® software.

To contact Agilent Technical Support

Technical support is available by phone and/or e-mail. A variety of useful information is also available on the Agilent Technical Support Web site.

Resource	To find technical support contact information
Agilent Technical Support Web site	**1** Go to http://chem.agilent.com. **2** Select a country or area. **3** Under Quick Links, select **Technical Support**. **4** Select from the available links to display support information.
Contact Agilent Technical Support by telephone or e-mail (United States and Canada)	Telephone: (800-227-9770) E-mail: informatics_support@agilent.com
Contact Agilent Technical Support by telephone or e-mail (for your country)	**1** Go to http://chem.agilent.com. **2** Select **Contact Us**. **3** Under Worldwide Sales and Support Phone Assistance, click to select a country, and then click **Go**. Complete e-mail and telephone contact information for your country is displayed.

To learn about Agilent products and services

To view information about the Life Sciences and Chemical Analysis products and services that are available from Agilent, go to www.chem.agilent.com.

1 Getting Started

To learn about Agilent products and services

2 Running and Monitoring Workflows

In Agilent CytoGenomics 1.0, *workflows* are used to automate feature extraction and/or analysis of CGH and CGH+SNP microarrays. Each workflow contains a set of actions and parameters for analyzing the data during the workflow. Workflows are set up by users in your laboratory with the role of Scientist. This chapter describes how to select and run a workflow and how to search for and display the status of workflows.

Running Workflows

This section describes how to select and run workflows. A workflow can extract and/or analyze one or many samples that you select when you start the workflow. Each time a workflow is started, it is assigned a Job Name and Description, which can be used to track and monitor the status of the workflow, as described later in this chapter.

The following diagram shows the steps for running a workflow.

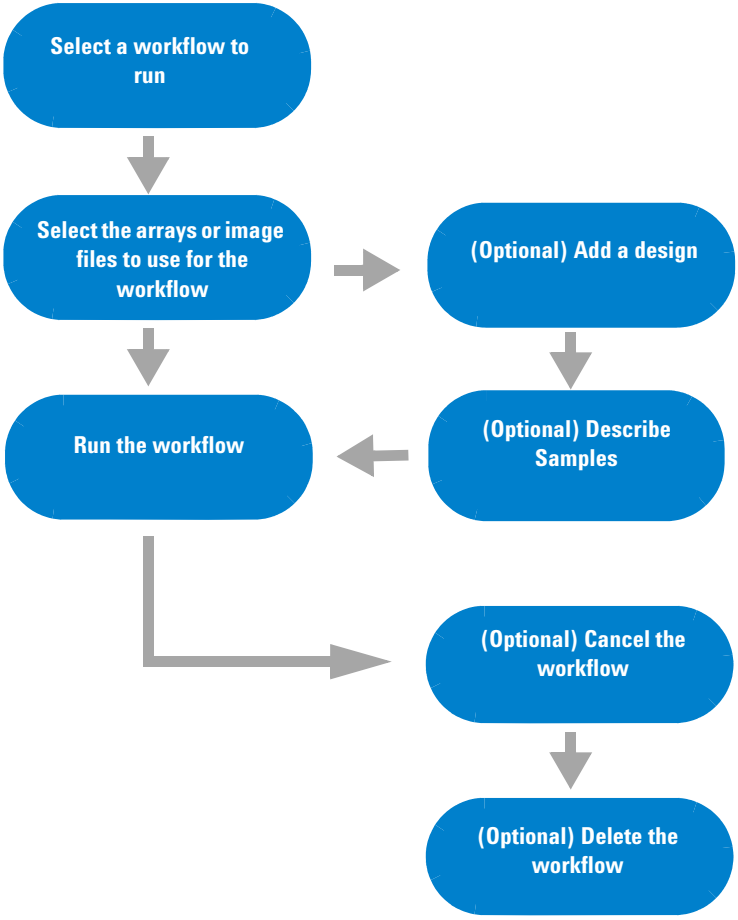

Figure 2 Tasks for running a workflow

Table 1 Tasks for running a workflow

To do this	Follow these instructions	Comments
Select a workflow to run	**1** Under **Analyze**, click **Analyze**  . **2** Under Select Workflow, click the arrow and select the workflow from the list. The program creates a job name and description, based on the workflow name and the date. **3** Under Job Name, type a job name to use for the workflow run. **4** Under Description, type a description for the workflow run.	• You can run published workflows that were created by users with the role of "Scientist" or "Administrator."
Import image files for the workflow	**1** For workflows that require image files as the input, in the Import Image Files pane, click **Add**. The Open dialog box appears. By default, the folder displayed is the default image file folder that was set up by the Scientist. **2** If necessary, browse to the folder where the microarray image (.tif) file is located. **3** Select the image file, and click **Open**. The Add image pack information for FE Extraction dialog box appears. **4** If you selected an image that does not have a matching design in the database, under Number of Packs, select the number of images on the microarray. **5** Click **Add Images**. The images appear in the Import Image Files table. **6** Under Sample ID <Red/Green(ArrayID/Global display Name)>, click the arrow and select the attribute where the Array ID/Global Display Name matches the Array ID and Global Display Name for this sample.	• The input for a workflow can be image files (for feature extraction + analysis workflows), extracted feature extraction files, or files that were already imported. The workflow you select determines what kind of files you must select. • If you select a workflow that is designed to do feature extraction before the analysis, you must select image files for the workflow input. • For information on the Import Image Files pane, see the *Reference Guide*.

Table 1 Tasks for running a workflow (continued)

To do this	Follow these instructions	Comments
Import extracted files for the workflow	**1** For workflows that require extracted FE files as the input, in the Import FE Files pane, click **Add Arrays**. The Open dialog box appears. By default, the folder displayed is the default image file folder that was set up by the Scientist. **2** If necessary, browse to the folder where the feature extracted file (.txt) is located. **3** Select the file to import, and click **Open**. The selected file appears in the Import FE Files table. If the Design Status shows **Not Found**, you must add the design.	• In order to import an FE file in a workflow, the design that matches the file must be present in the database.
Select imported data for the workflow	**1** For workflows that require already-imported data as the input, in the Select Imported Data pane, under Select Design, click to select the design for the imported data. **2** Under Select Genome Build, click to select the build for the design. The available arrays for the selected design and build appear in the Array List. **3** Select the array(s) you wish to analyze with the workflow. Use the > and >> buttons to move one or all of the arrays to the Selected Array List. Use the < and << buttons to remove one or all of the arrays from the Selected Array List.	
(Optional) Add a design to the database	**1** At the bottom of the workflow table, click **Add Designs**. The Open dialog box appears. **2** Search for the location for the design, click to select it, and then click **Open**. The Design Status for the sample changes to **Path Provided.**	• Images or FE files in the workflow for which no matching design exists in the database, will show **Not Found** under Design Status in the workflow table.

Table 1 Tasks for running a workflow (continued)

To do this	Follow these instructions	Comments
(Optional) Describe Samples	**1** At the bottom of the workflow window, click **Describe Samples**. The Describe Samples pane appears. **2** To show or hide attributes for the sample, click **Show/Hide Attributes**. The Show/Hide Attributes dialog box appears where you mark or clear the attributes to display. **3** To select or change an attribute for a sample, select or change it in the column for the selected array. **4** For CGH+SNP arrays, select a genotype reference, if one is not already selected. **5** Click **Save Changes**. **6** To go back to the workflow window, click **<<Back**. **7** To run the workflow, click **Run**.	• The Describe Samples pane shows the samples for the current workflow. You can show or hide attributes for the samples in the workflow, and select or change the attributes. • To go back to the workflow window, use the <<Back button. Otherwise, you will lose the selected samples for the workflow. • For CGH+SNP analysis, each array must have a genotype reference selected for the Green Sample attribute (Red Sample for dye-flipped arrays.)
Run the workflow	• At the bottom of the Analyze workflow window, click **Run**. The job monitor window appears, with the workflow at the bottom of the workflow list.	• You can also start the run from within the Describe Samples window. See (Optional) Describe Samples, above.
(Optional) Cancel the workflow	**1** Under Analyze, click **Job Monitor**.  The workflow job monitor table appears. **2** In the job monitor table, in the row for the workflow you want to cancel, under Actions, click **Cancel**.	• Once a workflow has completed or failed, the Cancel button is no longer available.

Table 1 Tasks for running a workflow (continued)

To do this	Follow these instructions	Comments
(Optional) Delete a workflow	**1** Under Analyze, click **Job Monitor**.  The workflow job monitor table appears. **2** In the job monitor table, in the row for the workflow you want to delete, under Actions, click **Delete**.	• You cannot delete a running workflow. You must first cancel the workflow and then delete it.

Searching for Jobs

When a workflow run is started, it is added to the jobs list in the Analysis Job Monitor window. This window is a convenient way to see the status of all jobs, and to look at the results from completed workflow jobs.

The table below describes how you use the Job Monitor to search for and manage workflow jobs.

Use the Job Monitor to search for and manage jobs

The steps in this section show you how to use the Job Monitor to search for and manage workflow jobs.

Table 2 Using the job monitor

To do this	Follow these instructions	Comments
Search for a job	**1** Under Analyze, click **Job Monitor**.  The workflow job monitor table appears. **2** Under Job Search, next to Search Type, click the arrow and select the parameter to use for the job search. **3** Next to Value, click the arrow, and select the value to match for the job search. OR Type the value to match for the job search. **4** Click **Search**. The jobs that match the selected criterion are displayed in the table.	• Click the **Reset** button to reset the table to display all jobs.
Delete a job	**1** Under Analyze, click **Job Monitor**.  The workflow job monitor table appears. **2** In the row for the job you want to delete, under Actions, click **Delete**.	• You cannot delete a job whose Status is *Running,* or *Waiting***.** To delete a running job, you must Cancel it first.

Table 2 Using the job monitor (continued)

To do this	Follow these instructions	Comments
Display the job summary	**1** Under Analyze, click **Job Monitor**.  The workflow job monitor table appears. **2** In the job row, under Actions, click **View**. The Job Summary is displayed. **3** Optional: Click **Save Log File**. The Save Log file dialog box opens. **4** Browse to a location where you want to save the log file. **5** In File name, type the name for the log file. **6** Click **Save**. **7** To close the Job Summary, click **Close**.	

3 Displaying Reports and Aberrations

This chapter gives instructions on how to display reports and aberration results after workflow analyses are completed.

Searching for Analyzed Samples

In order to display reports or analysis results, you must first search for the samples you want to show. You search for samples using the Annotation Search and/or Saved Queries tabs that appear when you click the Report or View buttons in the command ribbon. The following diagram gives an overview of how to search for analyzed samples.

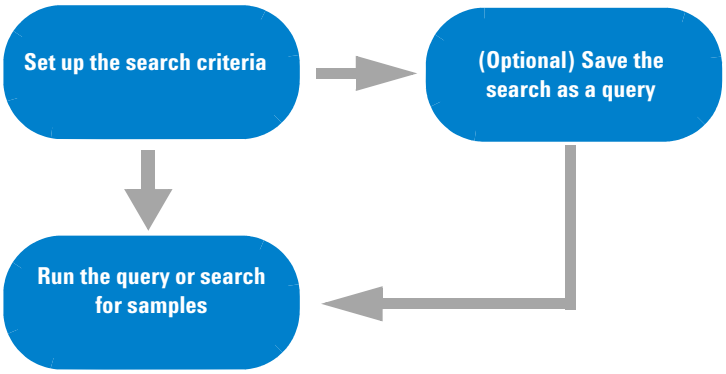

Figure 3 Searching for workflows

The following table gives instructions on how to set up searches and queries for finding analyzed samples.

Table 3 Setting up queries to search for samples

To do this	Follow these instructions	Comments
Set up a simple search	1 In the command ribbon, click **Report**  or **Multi Sample View**  . The Annotation Search tab appears. 2 Next to Search Type, select **Simple Search**. 3 Next to String Search, type a string of text or characters that appears in the Global Sample Name. 4 Click **Run**. The samples that meet the condition are displayed in the table.	• To clear the results of your search, click **Clear Results** at the bottom of the window. • By default, the program displays the last 10 samples with cyto reports or analyzed results.
Set up and run a search query	1 In the command ribbon, click **Report**  or **Multi Sample View**  . The Annotation Search tab appears. 2 Next to Search Type, select **Attribute Search**. 3 Next to Attribute, click the arrow and select one of the available attributes to use for the search. 4 Click the arrow next to Is in range, and select **Is in range** or **Matches**. 5 If you select **Is in range**, under Range, type a start and stop value. The program searches for any value of the attribute that falls within that range. OR If you select **Matches**, under Attribute Value, type the value or text you want to match to the sample attribute. 6 Click **Add**. The criteria is added to the Conditions for the query.	• Logical Operation of **AND** will match samples only if both conditions are true. Logical Operation of **OR** will match samples if any of the conditions are true. • Click **Clear Conditions** to remove all conditions for a query and start again. • To clear the results of your search, click **Clear Results** at the bottom of the window.

Table 3 Setting up queries to search for samples (continued)

To do this	Follow these instructions	Comments
	7 (Optional) Under Logical Operation, click the arrow and select the logic to apply for the next condition. • Perform step 4 through step 6 until all conditions are set up. **8** Click **Execute** to run the query. Samples that match the query appear in the table.	
Save a search query	**1** Set up a search query. See Set up and run a search query, above. **2** Click **Save Query** to save the query conditions. The Input dialog box appears. **3** Type a name for the query. **4** Click **OK**.	
Run a saved search query	**1** In the command ribbon, click **Report**  or **Multi Sample View**  . The Annotation Search tab appears. **2** Click **Saved Queries**. A list of saved queries appears. **3** In the queries list, under Select, mark the query you want to run. **4** Click **Run**. The program runs the query and displays a list of the samples that meet the query conditions.	• If no queries are saved, the Saved Queries tab is not available. • To clear the results of your search, click **Clear Results** at the bottom of the window.

Table 3 Setting up queries to search for samples (continued)

To do this	Follow these instructions	Comments
Change a saved search query	**1** In the command ribbon, click **Report**  or **Multi Sample View**  . The Annotation Search tab appears. **2** Click **Saved Queries**. A list of saved queries appears. **3** In the queries list, under Select, mark the query you want to change. **4** Click **Edit**. The conditions for the query are displayed. **5** Click to select the query condition you want to change. **6** Click **Edit Condition**. The condition parameters are displayed. **7** Change the condition as desired, then click **Add**. **8** (Optional) Click to select a condition you want to remove and then click **Delete**. **9** When you are finished changing the query, click **Update Query**.	

Displaying Cyto Reports

After a workflow is completed, you can search for a sample and display the cyto report that was created for that sample. The diagram below shows the steps you use to search for a sample and display its cyto report.

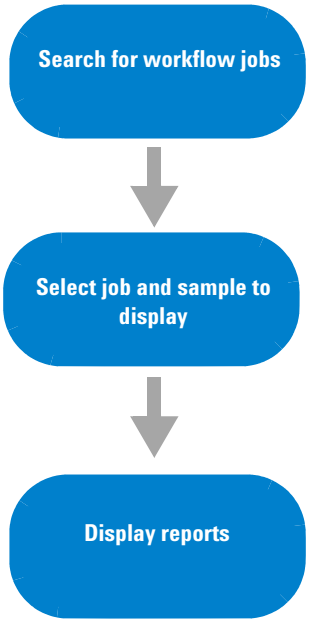

Figure 4 Displaying analysis reports

The table below shows how to display cyto reports for completed workflow samples.

Tasks for displaying cyto reports

The tasks in this section show you how to display cyto reports for samples in a completed workflow.

Table 4 Tasks for displaying reports

To do this	Follow these instructions	Comments
Search for workflow jobs	• In the Annotation Search or Saved Queries tab, search for the workflow(s) of interest. For instructions, see "Searching for Analyzed Samples" on page 18.	• To clear the results of your search, click **Clear Results** at the bottom of the window.
Display cyto report for selected sample	**1** Search for workflow jobs. See Search for workflow jobs, above. **2** In Table View, under Select, mark the workflow sample for which you want to display the cyto report. OR In Genomic View, under Select, mark the box next to the sample depicted in the genomic view of the samples. **3** Click **View Report**. The View Report dialog box appears. **4** Under Select Report, click the arrow, and select the report you want to display (if more than one cyto report exists for the sample.) **5** Click **OK**. The Manage Report dialog box appears with the selected report displayed.	• From within the Manage Report dialog box, you can move through report pages, make changes to editable text fields, export, or print the report. For more information, see the *Reference Guide*.

Displaying Aberrations

After a workflow is completed, you can search for the sample and display the aberrations in the View Aberrations window. In the View Aberrations window, extracted data and analysis results are tabulated and displayed next to depictions of the genome, selected chromosome, and selected genes of the species whose array data you are analyzing. For more information about the View Aberrations window, see the *Reference Guide*.

The flowchart below shows the steps you use to search for analyzed samples and display the results in the View Aberrations window.

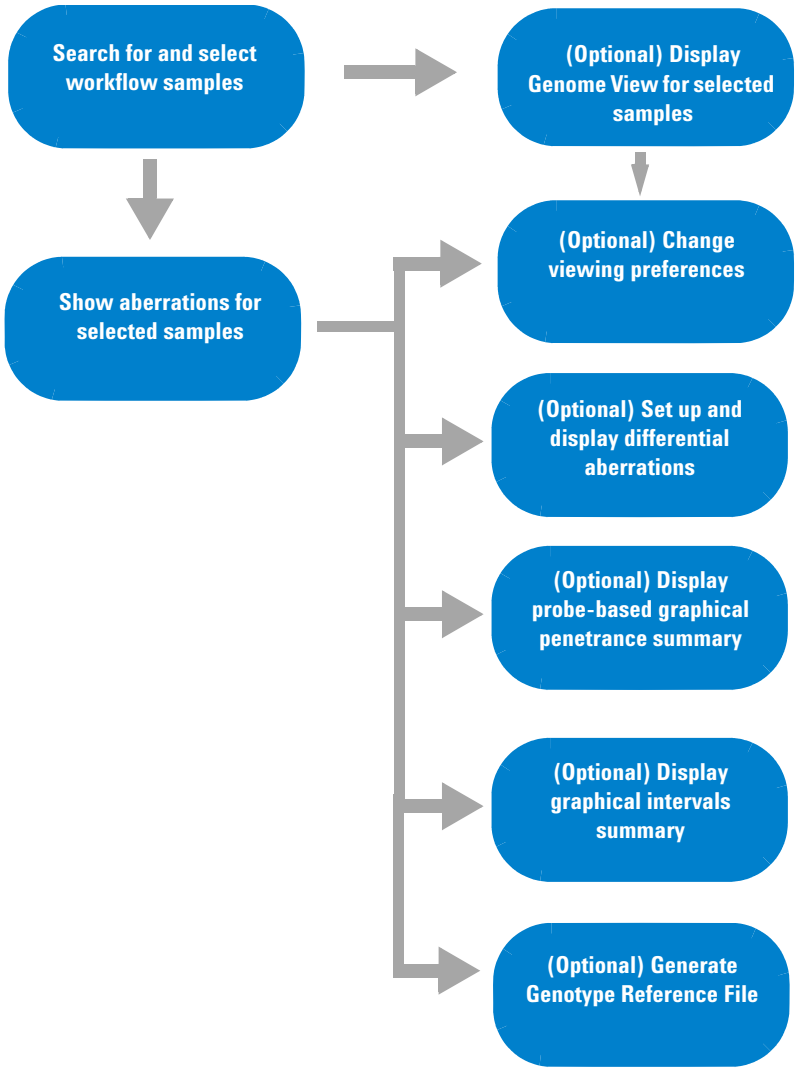

Figure 5 Displaying aberrations for analyzed samples

The following table gives instructions on how to display aberrations within the Genomic View.

Table 5 Tasks for displaying aberration results

To do this	Follow these instructions	Comments
Search for workflow samples	**1** In the command ribbon of the Analysis tab, click **Multi Sample View**  . **2** In the Annotation Search or Saved Queries tab, search for the workflow(s) of interest. For instructions, see "Searching for Analyzed Samples" on page 18. A list of samples that match the search conditions appear in the Table View.	
(Optional) Display Genome View for selected samples	**1** In the View tab, use Annotation Search or Saved Queries to search for workflow samples to display. For instructions, see "Searching for Analyzed Samples" on page 18. **2** Click the **Genomic View** tab. A graphical display of all samples that meet the search conditions appears.	
Show aberrations for selected samples	**1** In the View tab, use Annotation Search or Saved Queries to search for workflow samples to display. See Search for workflow samples, above. **2** In the Table View or Genomic View, under Select, mark the workflow samples for which you want to display aberrations. **3** At the bottom of the Analysis window, click **View Aberrations**. The View Aberrations window appears with the results for the selected samples.	

Table 5 Tasks for displaying aberration results (continued)

To do this	Follow these instructions	Comments
(Optional) Change viewing preferences	**1** In the View tab, show the aberrations for selected samples. See Show aberrations for selected samples, above. **2** In the View Aberrations window, under Setting click **View Settings**  . The View Preferences dialog box appears. **3** In the View Preferences window, mark to select the items you want to display, and select other viewing preferences.	• For information on the items displayed in the View Aberrations window, and the View Preferences options, see the *Reference Guide*.
(Optional) Set up and display differential aberrations	**1** In the View tab, show the aberrations for selected samples. See Show aberrations for selected samples, above. **2** In the View Aberrations window, under Aberration Calls, click **Differential**.  The Differential Aberration Setup dialog box appears. **3** Below the diagram for each array, select one of these options. You must assign at least one array to Set 1 and one array to Set 2. • Set 1 – Assigns the array to the first group of arrays. The program compares the aberrations in this group of arrays to the ones you assign to Set 2. • Set 2 – Assigns the array to the second group of arrays. The program compares the aberrations in this group of arrays to the ones you assign to Set 1.	• You must select at least two samples in order to perform differential aberration. • Differential aberration analysis reveals aberrations that are significantly different in different samples. After you assign one or more arrays to each of two groups, the program compares the groups and evaluates the differences in the detected aberrations between the groups.

Table 5 Tasks for displaying aberration results (continued)

To do this	Follow these instructions	Comments
	• Ignore – The program does not consider the array in the differential aberration analysis. If you have many arrays, it can be easier to use the Define Sets dialog box to assign the arrays to comparison groups. To open this dialog box, click Define Sets. For more information, see the *Reference Guide*. **4** In Select Algorithm, the HyperGeometric algorithm is selected. It is used to evaluate the significance of differences in the detected aberrations between the two comparison groups. **5** Click **Run**. The program evaluates the two sets of arrays, and summarizes the differences in the Graphical Differential Aberration Summary dialog box.	

Table 5 Tasks for displaying aberration results (continued)

To do this	Follow these instructions	Comments
(Optional) Display probe-based graphical penetrance summary	**1** In the View tab, show the aberrations for selected samples. See Show aberrations for selected samples, above. **2** Under Penetrance, click **Probe**  . The Graphical Penetrance Summary dialog box appears. **3** Mark the box(es) for the chromosomes you want to display in the summary, or click **Select All** to mark all chromosomes. To clear all boxes, click **Deselect All**. **4** To copy the summary to the Clipboard, click **Edit** and then click **Copy Summary to Clipboard**. You can then paste the summary information in the Clipboard into a document of your choice.	• Probe penetrance plots display the percentage of selected arrays that have an aberration at each probe position on the array for the selected chromosomes.

Table 5 Tasks for displaying aberration results (continued)

To do this	Follow these instructions	Comments
(Optional) Display graphical intervals summary	**1** In the View tab, show the aberrations for selected samples. See Show aberrations for selected samples, above. **2** Under Penetrance, click **Interval**  . The Graphical Interval Penetrance Summary dialog box appears. **3** To filter the results using an interval filter, **a** Click the arrow next to Filter, and select an interval filter to apply. **b** Click **Apply Filter**. OR To create and apply a new interval filter, **a** Click **Create Filter**. **b** In the Interval Filter dialog box, define the new filter and type a name for it. **c** Click **Apply Filter**.	• Interval penetrance plots display the percentage of selected samples that have an identified aberrant region on the array. You can create and export the plots and tab-delimited tables of the interval penetrance summaries, or copy the summary to the Clipboard and then paste it into another program or document. • For more information on the Graphical Interval Penetrance Summary dialog box, see the *Reference Guide*

Table 5 Tasks for displaying aberration results (continued)

To do this	Follow these instructions	Comments
(Optional) Generate Genotype Reference File	**1** In the View tab, show the aberrations for selected samples. See Show aberrations for selected samples, above. **2** Under SNP, click **Generate Genotype Reference**.  The Generating Genotype Reference File dialog box is displayed. **3** In the Generating Genotype Reference Files dialog box, select the sample(s) to include in the genotype reference file. **4** Under Input Parameters, type a Confidence Threshold. **5** Under Input Parameters, select a Level of confidence. **6** Click **Browse** and select a location to save the new genotype reference file. **7** Click **OK** to generate the new genotype reference file. The file is saved in the specified location, with the name GenotypeReference_<Date>_<Design>_<Build>.txt.	• If you have a reference sample that is not genotyped, this creates a genotype reference for that sample using the Agilent-provided or known genotype references available in the database. The genotype calls generated using the known genotype reference are used, along with a user-supplied confidence threshold and level, to generate a genotype reference file. This "custom" genotype reference file can then be imported to the database, and used to analyze additional CGH+SNP microarrays. • In order to generate a genotype reference file, the Red Sample field for each microarray (Green for dye-flipped,) must contain the unknown reference sample name. A validated genotype reference must be selected in the other sample channel.

www.agilent.com

In this book

This guide describes how to use the Agilent CytoGenomics 1.0 software to run workflows and display reports and aberrations. The functions described in this guide are typically performed by users with an assigned role of "Technician." However, tasks described in this guide are available to users with an assigned role of "Technician," "Scientist," or "Administrator."

Revision A0, November 2010

G2602-90004

Agilent Technologies