

# **Agilent Genomic Workbench 6.5 Workflow**

## **User Guide**

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## Manual Part Number

G3800-90026

## Edition

Revision A1, October 2015

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## In This Guide...

This guide describes how to use the Workflow utility of Agilent Genomic Workbench to extract image files with Agilent Feature Extraction software and/or analyze data using CGH and ChIP analysis software.

### **1 Getting Started**

This chapter gives an overview of Workflow and how it is used in Agilent Genomic Workbench. It also provides flow charts for setting up and running CGH and ChIP analysis workflows.

### **2 Setting Up and Running Workflows**

This chapter describes how to set up and run Feature Extraction and analysis workflows. It includes instructions for creating new workflows.

### **3 Setting up Workflow Analysis Methods**

This chapter describes how to set up an analysis method for a CGH or ChIP analysis workflow.

### **4 Workflow Reference**

This chapter describes the main window, parameter panels, and the dialog boxes for Workflow.



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# 1

## Getting Started

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In Agilent Genomic Workbench, Workflow is used to automate feature extraction and/or analysis of CGH and ChIP data. This chapter gives an overview of how to use a workflow to automate feature extraction and data analysis.

You must have a Feature Extraction 10.10 (or higher) license and a CGH or ChIP license to run a *feature extraction* workflow.

You must have a CGH and/or ChIP license to run an *analysis* workflow.

For information on how to run a SureSelect Target Enrichment workflow, see the *SureSelect Target Enrichment User Guide*.


For details on how to activate a license, see the *Product Overview Guide* or the *User Guide* for your analysis application.

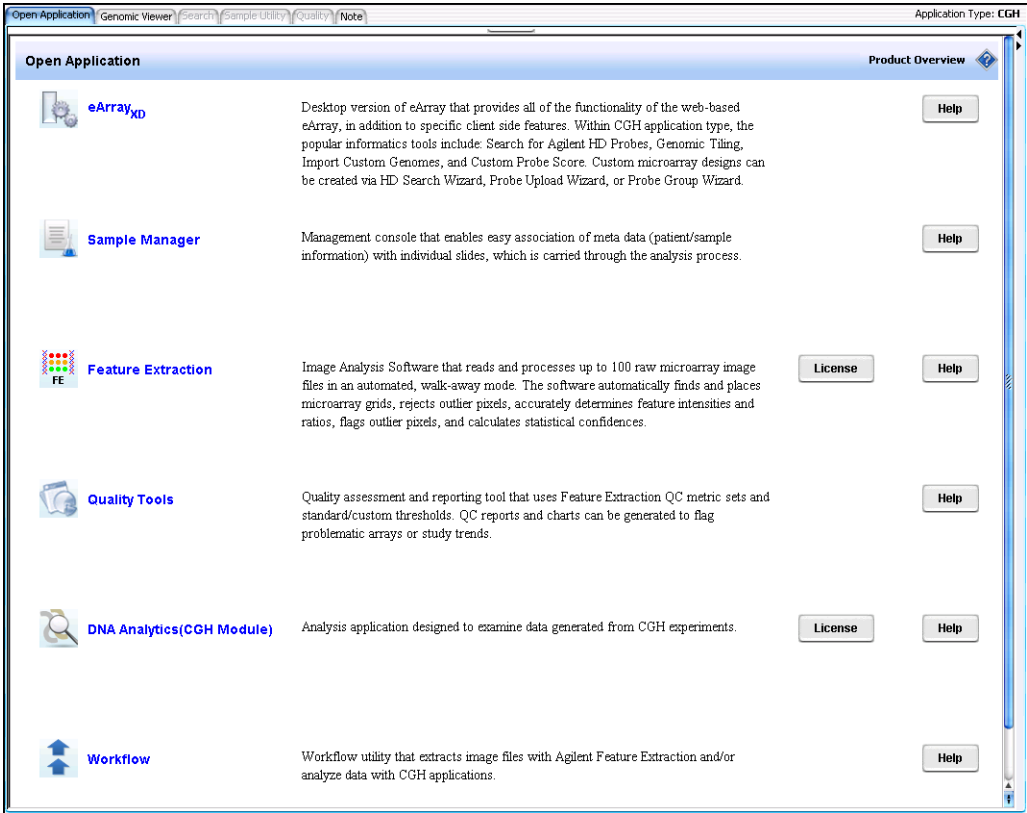


# Starting the Workflow Program

This section describes how to open the Workflow program in Agilent Genomic Workbench, and shows what the program window looks like.

## To start the Workflow program

- 1 To open Workflow, in the Open Application pane click the **Workflow** icon . See [Figure 1](#).



**Figure 1** Open Application pane

OR

At the top of the Agilent Genomic Workbench window, click the **Workflow** tab.

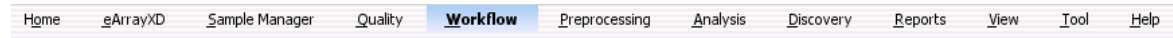


Figure 2 Workflow tab

The Workflow window appears with the Workflow Navigator displayed on the left side of the window.

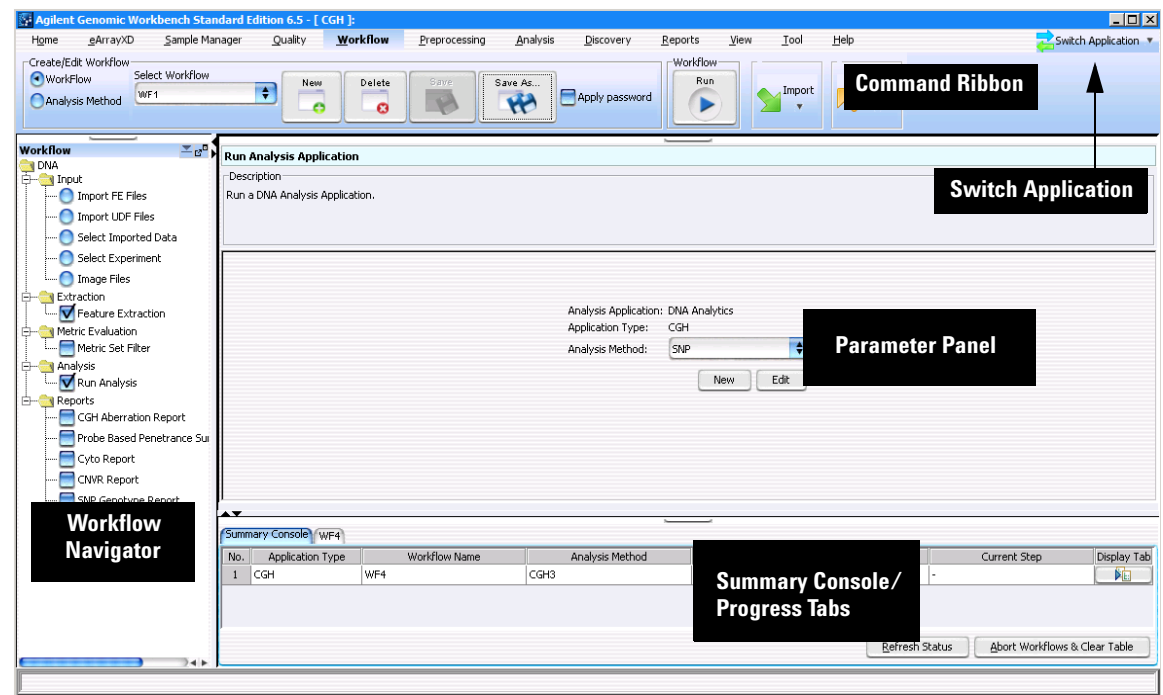


Figure 3 Workflow main window

## 1 Getting Started

### To start the Workflow program

The selected DNA Analytics application appears at the top of the window, in brackets. You must change applications to set up a workflow for a different application (CGH or CHIP.) To change the application type, click **Switch Application** at the top right corner of the Agilent Genomic Workbench tab bar, and click the application type.

For more information on the contents of the main window of Agilent Genomic Workbench, see [“Main Window”](#) on page 94.

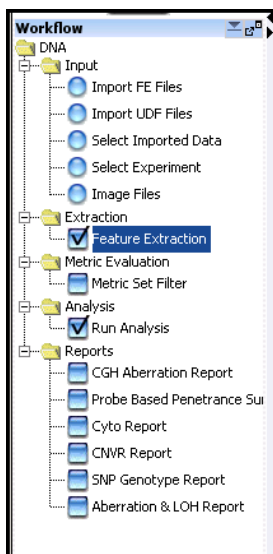


## Setting Up and Running Workflows for Extraction and/or Analysis

Agilent Genomic Workbench Workflow lets you set up and run automatic feature extraction and analysis for multiple samples. You use a workflow to:

- Run the workflow to extract image files with Agilent Feature Extraction software (FE) and produce a QC report that contains sample ID information from the Sample Manager table, or
- Run the workflow to analyze CGH or ChIP (not CH3) data using Agilent Genomic Workbench and create reports, or
- Run the workflow to extract image files and then analyze the extracted results to create both sets of reports

You can also select a metric set filter to pass only extracted data that meets the filter criteria onto the analysis application.



**Figure 4** Agilent Genomic Workbench Workflow Navigator for CGH

#### NOTE

In DNA Analytics 4.0 “workflow” was the name for “analysis method”, used in Agilent Genomic Workbench 5.0 and higher.

## To change settings for CGH workflow analysis

A workflow is helpful if you need to analyze multiple arrays and you know which algorithm settings you intend to use for the analysis.

First, you set all parameters for an analysis in an *analysis method*. When you run the workflow, the program automatically runs the analysis method. On 64-bit computers, you can run up to three workflows simultaneously. On 32-bit computers, workflows are processed sequentially, not simultaneously.

You create experiments for a workflow in one of two ways:

- You create and save a new experiment in interactive mode, and then select the experiment for the workflow.
- Let the workflow create an experiment automatically to hold data from the selected input source. The experiment is saved at the end of the workflow run, and is available in the interactive mode.

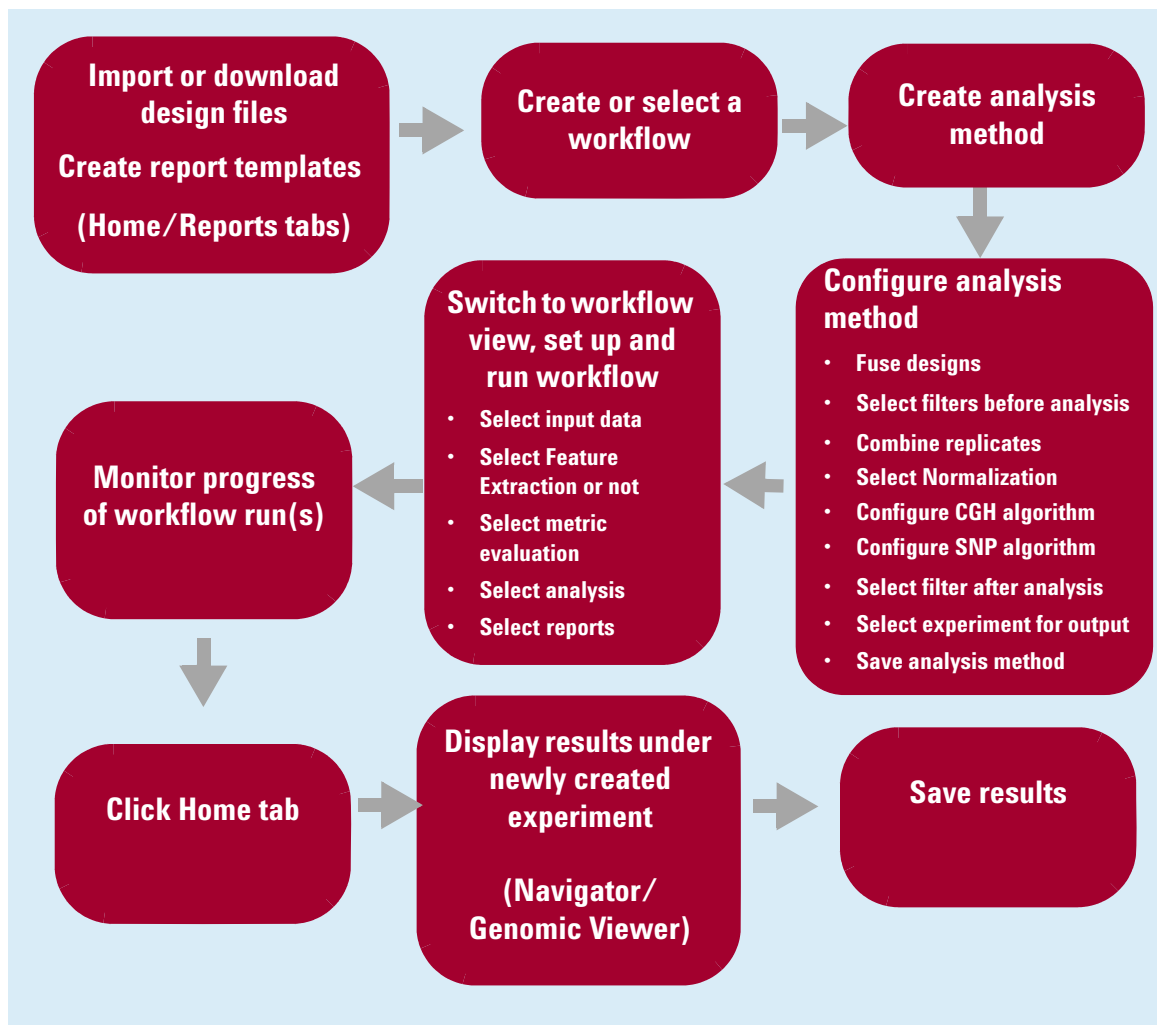
You must create Cyto Report templates interactively before you can use them in an analysis method. After you select the method settings and their options, you set up and run the workflow.

After a workflow run is completed, you return to the Home tab or one of the interactive analysis tabs to select the workflow experiment and display the results in the Genome, Chromosome and Gene Views.

In the procedure described in this section, you configure an analysis method to analyze CGH microarray data. You set up and run the workflow. Then you use the Genomic Viewer to review the result files and data generated from the workflow run. See [“Quick-start instructions for analyzing CGH data in a workflow”](#) on page 20.

On the next page is a typical CGH workflow analysis.

For more information, see [Chapter 3](#), “Setting up Workflow Analysis Methods”.



**Figure 5** Typical CGH Workflow analysis pathway

# Quick-start instructions for analyzing CGH data in a workflow

These instructions apply when you have started the program with the CGH license installed. See the *CGH Interactive Analysis User Guide* for information on how to install the license.

**Table 1** Steps for setting up and running a CGH workflow

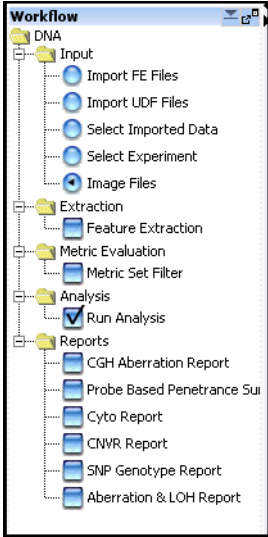
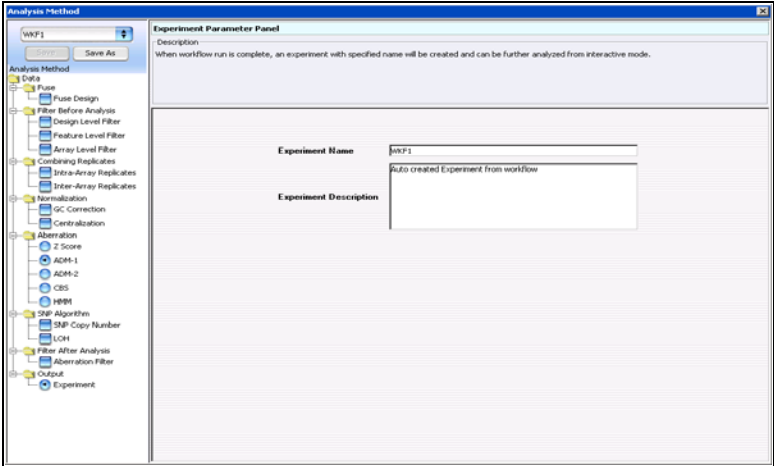
To do this	Follow these instructions	Comments
<div>Create or select a workflow</div>	<div><div>1 Click the Workflow tab. You see the Workflow Navigator.</div><div>2 On the command ribbon, under Select Workflow, select an existing workflow to run.</div><div>OR</div><div>On the command ribbon, click <b>New</b>. Type a name for the workflow, and then click <b>OK</b>.</div></div>	<div><div>• Using a workflow, you can run Feature Extraction with image files before the CGH analysis, if you have an Agilent Feature Extraction 10.10 or higher (or higher) license.</div><div></div></div>

Table 1 Steps for setting up and running a CGH workflow (continued)



To do this	Follow these instructions	Comments
<div>Create/select an analysis method</div>	<div><div>1 On the Workflow command ribbon, under Create/Edit Analysis Method, click <b>Analysis Method</b>.</div><div>2 Click <b>New</b> to create a new analysis method. A dialog box appears.</div><div>3 Type a Name for the analysis method, then click <b>Ok</b>. The Analysis Method window opens, and the Experiment Parameter Panel contains a place to change the name of the experiment that is created. By default, the experiment name is the name of the analysis method.</div><div>4 Type a name to change the name of the Experiment.</div></div>	<div><ul style="list-style-type: none"><li>• The name you type for the analysis method appears in the selection list under Create/Edit Analysis Method.</li><li>• You can create more than one analysis method for use in multiple workflows.</li></ul></div>





# 1 Getting Started

## Quick-start instructions for analyzing CGH data in a workflow

**Table 1** Steps for setting up and running a CGH workflow (continued)

To do this	Follow these instructions	Comments
	<ol style="list-style-type: none"><li>1 In the Analysis Method Navigator, under Fuse, click <b>Fuse Design</b>.</li><li>2 Select whether you want to normalize the data.</li><li>3 Select if you want to remove the arrays from the experiment after the designs are combined.</li></ol> <p>When you set up a workflow and click <b>Select Imported Data</b>, remember to select the designs to be fused.</p>	<ul style="list-style-type: none"><li>• In this step you combine two design files into a larger file when the same sample has been hybridized to multiple designs.</li><li>• Arrays from the same design and already fused designs cannot be fused.</li></ul>
	<ol style="list-style-type: none"><li>1 In the Analysis Method Navigator, under Filter Before Analysis, mark one or more of the check boxes, <b>Design Level Filter</b>, <b>Feature Level Filter</b> or <b>Array Level Filter</b>.</li><li>2 Select <b>DefaultFeatureFilter</b> or another one from the list, or create a new one.</li><li>3 Select a filter from the Array Filter list, or create a new one.</li></ol>	<ul style="list-style-type: none"><li>• When you apply a Design Level probe filter, you include or exclude probes, based on design filter conditions.</li><li>• When you apply an Array filter, microarrays that fail the filter criteria are not included in the evaluation.</li><li>• When you apply a Feature Level filter, features from the array that fail the criteria are not included in the evaluation.</li><li>• To create a new filter, see instructions in the <i>Workflow User Guide</i>.</li></ul>

**Table 1** Steps for setting up and running a CGH workflow (continued)

To do this	Follow these instructions	Comments
 <p><b>Set up to combine replicates</b></p>	<ol style="list-style-type: none"> <li>1 In the Analysis Method Navigator, under Combining Replicates, select any of these options: <ul style="list-style-type: none"> <li>• <b>Intra-Array Replicates</b> – Combines replicate probes within arrays.</li> <li>• <b>Inter-Array Replicates</b> – Combines replicate probes from multiple arrays, that are marked as replicate arrays using one of the available attributes.</li> </ul> </li> <li>2 If you select the Inter-Array option, in the Parameters tab, select an array attribute next to Group By.  For interarray replicates, the program combines replicate arrays into groups by the value for the attribute you select in Group By. Values must be the same.</li> </ol>	<ul style="list-style-type: none"> <li>• If your array(s) contain probes that are replicated in the array, you can combine them to increase the confidence of your analysis. When the program combines replicates, it selects probes with common probe names, and calculates a weighted average of their values to create a single point.</li> <li>• If the probes are from arrays with the same polarity, the algorithm used to combine replicates calculates a weighted average of the probes with the same name. Otherwise, it calculates a straight average.</li> </ul>
 <p><b>Normalize the data</b></p>	<ul style="list-style-type: none"> <li>• To correct for artifacts by performing a regression fit to GC content in a specified region flanking the probes, mark <b>GC Correction</b>.</li> <li>• To normalize the data so that zero represents the most common ploidy, mark the <b>Centralization</b> check box.</li> </ul>	<ul style="list-style-type: none"> <li>• GC Correction is required in order to perform SNP Copy Number and LOH analyses.</li> <li>• All of the aberration algorithms can use the Centralization calculation, used to normalize data.</li> <li>• Centralization is required in order to perform SNP Copy Number and LOH analyses.</li> </ul>

# 1 Getting Started

## Quick-start instructions for analyzing CGH data in a workflow

**Table 1** Steps for setting up and running a CGH workflow (continued)

To do this	Follow these instructions	Comments
<b>Set up the aberration algorithms</b>	<ol style="list-style-type: none"><li>1 Select one of the aberration algorithms.</li><li>2 Type or change parameters.  To remove long, low aberrations from the ADM1 or ADM2 results, mark the <b>Fuzzy Zero</b> check box. To filter out “nested” aberrations, mark the <b>Apply Nesting Filter</b> and type a value for the filter.  If you use HMM (Hidden Markov Model), you must also select the <b>State Parameters</b>.</li></ol>	<ul style="list-style-type: none"><li>• ADM1 and 2 can use Fuzzy Zero to make the results more reliable by taking into account the global error across the chromosomes.</li><li>• If the Nesting Filter value is set to zero, you will filter out all nested aberrations. By default, no filter is applied.</li><li>• See the “Statistical Algorithms” chapter of the <i>CGH Interactive Analysis User Guide</i> for information on the aberration algorithm calculations and suggested thresholds.</li></ul>
<b>Set up the SNP algorithms</b>	<ol style="list-style-type: none"><li>1 Select one or both of the SNP calculations for analysis of CGH+SNP data.</li><li>2 Type or change the parameters.<ul style="list-style-type: none"><li>• <b>SNP Copy Number</b> – detects allele-specific copy numbers for SNP probes.</li><li>• <b>LOH</b> – Detects regions that show loss of heterozygosity.</li></ul></li></ol>	<ul style="list-style-type: none"><li>• In order to select <b>SNP Copy Number</b>, you must also select <b>GC Correction</b>, <b>Centralization</b>, and an aberration algorithm (other than z-score or HMM).</li><li>• In order to select <b>LOH</b>, you must first select <b>SNP Copy Number</b>.</li></ul>
<b>Select filters after the analysis</b>	<ol style="list-style-type: none"><li>1 Under <b>Filter After Analysis</b>, mark the <b>Aberration Filter</b> check box.</li><li>2 Select the <b>DefaultAberrationFilter</b> or another filter from the list, or create a new one.</li></ol>	<ul style="list-style-type: none"><li>• Suggested filter for CNV analysis: 2 probes, 0.25 log ratio</li><li>• To create a new filter, see instructions in the <i>CGH Interactive Analysis User Guide</i>.</li></ul>



Table 1 Steps for setting up and running a CGH workflow (continued)

To do this	Follow these instructions	Comments
<div>Select experiment for output</div>	<div><div>1</div>To change the name of the workflow experiment, under Output, click <b>Experiment</b>.</div> <div><div>2</div>Type the name you want to use for the experiment.</div> <div><div>3</div>Type a description for the experiment.</div>	<div><div>•</div>By default, Workflow creates an experiment and gives it the name of the analysis method, unless you change the experiment name in the analysis method.</div>
<div>Save the analysis method</div>	<div><div>1</div>Click <b>Save</b>. To save the analysis method with a new name, click <b>Save As</b> and then type the name of the new analysis method.</div>	

# 1 Getting Started

## Quick-start instructions for analyzing CGH data in a workflow

**Table 1** Steps for setting up and running a CGH workflow (continued)

To do this	Follow these instructions	Comments
<div>Set up a workflow and select input files</div>	<div><div>1 To display the Workflow Navigator to set up and run a workflow, on the Workflow command ribbon, click <b>Workflow</b>.</div><div>2 In the Workflow Navigator, under <b>Input</b>, click one of these option buttons:<ul style="list-style-type: none"><li><b>Import FE Files</b> – Select these files to analyze Feature Extraction log ratio data.</li><li><b>Import UDF Files</b> – Select these text-delimited files to analyze non-Agilent data.</li><li><b>Select Imported Data</b> – Select to analyze data that appears in the Navigator for Interactive Mode.</li><li><b>Select Experiment</b> – Select an existing experiment to analyze data in the experiment.</li><li><b>Image Files</b> – Select to run Feature Extraction.</li></ul></div></div>	<div><ul style="list-style-type: none"><li>The program creates a new experiment when you run a workflow.</li><li>If you select <b>Image Files</b>, the <b>Feature Extraction</b> check box must be marked.</li><li>Image files are the only input allowed for Feature Extraction.<ul style="list-style-type: none"><li>If you select image files, you must have a Feature Extraction license.</li><li>If you selected Image Files as the input, you can run a CGH analysis in addition to Feature Extraction. If you selected any other input type, you can only run the analysis, not Feature Extraction.</li></ul></li><li>You may want to use the imported data from the design file 014698 that comes with the program to run a workflow.</li></ul></div>
<div>Select metric set filter and analysis method</div>	<div><div>1 Mark the <b>Metric Set Filter</b> check box to select or create a metric set filter.</div><div>2 To run a CGH analysis, mark the <b>Run Analysis</b> check box. Select the analysis method to use for the workflow.</div></div>	


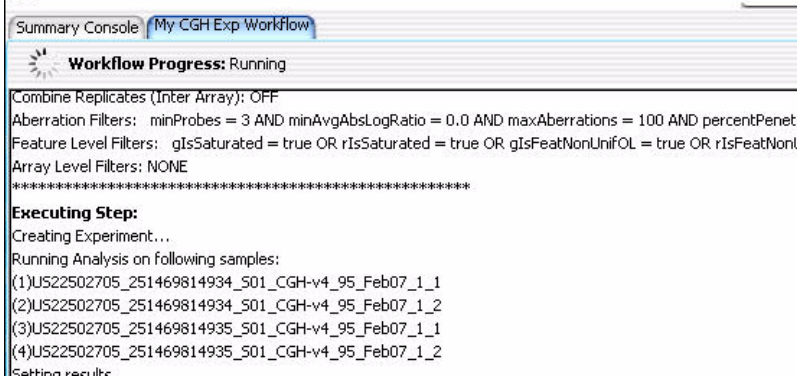

Table 1 Steps for setting up and running a CGH workflow (continued)

To do this	Follow these instructions	Comments
<div>Select reports</div>	<div><div>3</div>Select any of the following Reports:<ul style="list-style-type: none"><li><b>CGH Aberration Report</b> – Gives overall deletion and amplification tabular results, along with <i>p</i>-values (log 10). Select Probe Based or Interval Based report type.</li><li><b>Probe-based Penetrance Summary Report</b>– Gives percent penetrance for each probe across all the selected arrays for amplification or deletion.</li><li><b>Cyto Report</b> – Gives deletion and amplification tabular and graphical results with all the parameter settings in pdf format. Select a report template.</li><li><b>CNVR Report</b> – Reports the CNV regions found during analysis. Type a CNVR Node Name. This node will appear under the experiment in the Experiment pane. You can accept this name for the report, or not.</li><li><b>SNP Genotype Report</b> – Reports genotype and <i>p</i>-values for SNP probes in the microarray.</li><li><b>Aberration &amp; LOH Report</b> – Reports SNP data on a per-interval basis.</li></ul></div>	<ul style="list-style-type: none"><li>See the <i>CGH Interactive Analysis User Guide</i> for more detailed instructions, and for the column formats of each report.</li><li>You must type a name and location for all the reports.</li><li>If no report template exists for the Cyto Report, you must set up the report template under Reports. See the <i>CGH Interactive Analysis User Guide</i>.</li></ul>
<div>Run workflow</div>	<div><div><div>1</div>Click the <b>Run</b> button.<div>The Provide Workflow Identifier dialog box opens.</div></div><div><div>2</div>Type a name for the workflow.</div><div><div>3</div>Click <b>OK</b> to start the workflow.</div></div>	<ul style="list-style-type: none"><li>You can run multiple workflows (3 maximum) simultaneously only on a 64-bit computer. You can start more than one workflow at a time with a 32-bit computer, but they will run one right after the other, not simultaneously.</li></ul>

# 1 Getting Started

## Quick-start instructions for analyzing CGH data in a workflow

**Table 1** Steps for setting up and running a CGH workflow (continued)

To do this	Follow these instructions	Comments
	 <p>The Summary Console tab shows a list of all the runs you have started and their status.</p>	<ul style="list-style-type: none"><li>• The actions are listed in the named workflow tab next to the Summary Console tab during the run. The named workflow tabs show the progress of each workflow run and its completion status.</li></ul>
	<ol style="list-style-type: none"><li>1 Click the <b>Home</b> tab.</li><li>2 In the Navigator, double-click the newly created workflow experiment, and click <b>Yes</b> when asked if you want to select this experiment.</li><li>3 Expand the <b>Results</b> folder under the experiment. Note that the WF result label is also in blue, to indicate this set of results is active.</li><li>4 Select a chromosome in Genome View that appears to have a significant number of aberrations. (Chr 4 was selected in figure below).</li><li>5 Move the blue cursor in Chromosome View to a region of interest for display in Gene View.</li><li>6 In the Home command ribbon, click <b>Save Experiment Result</b>, and click <b>Yes</b>, then <b>OK</b>.</li></ol>	<ul style="list-style-type: none"><li>• The new workflow experiment appears in the Experiment pane. The experiment is marked with a <b>W</b> while the workflow is running.</li><li>• When you select the new experiment, tabular data from the experiment appear in the Tab View, and the aberration results appear in Genome, Chromosome, and Gene views.</li></ul>

**Table 1** Steps for setting up and running a CGH workflow (continued)

To do this

Follow these instructions

Comments

Genomic Viewer

Sample Utility

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13 14 15 16 17 18 19 20 21 22 X Y

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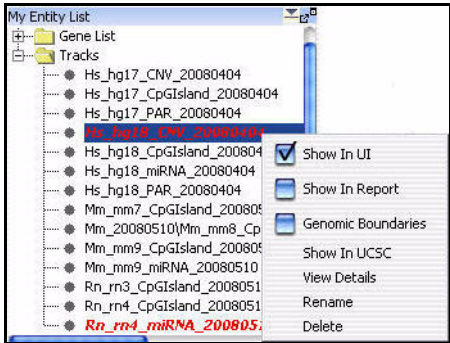
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## Quick-start instructions for analyzing CGH data in a workflow

To do this	Follow these instructions	Comments
<ul style="list-style-type: none"> <li>See the <i>CGH Interactive Analysis User Guide</i> for instructions on how to:               <ul style="list-style-type: none"> <li>Create and use gene lists</li> <li>Create and use tracks</li> <li>Customize the appearance of the display</li> </ul> </li> <li>See “<i>To set up the Run Analysis</i>” on page 45 for instructions on how to change the analysis method.</li> <li>See the <i>CGH Interactive Analysis User Guide</i> for instructions on how to use postprocessing Discovery statistics on the results.</li> </ul>	<p>At this point you can add tracks with other gene information to the Gene View.</p> <ol style="list-style-type: none"> <li>In the My Entity List pane, double-click the <b>Tracks</b> folder.</li> <li>Right-click one of the tracks in the list.</li> <li>Mark the <b>Show in UI</b> check box.</li> </ol>	<ul style="list-style-type: none"> <li>With these tracks, you can see if the aberrations correlate with copy number variant regions or miRNA gene regions. You can import other tracks as well.</li> </ul>
	 <p>The screenshot shows the 'My Entity List' window with a tree view containing 'Gene List' and 'Tracks'. Under 'Tracks', several tracks are listed, including 'Hs_hg18_CNV_20080404' which is highlighted. A right-click context menu is open over this track, showing options: 'Show In UI' (checked), 'Show In Report', 'Genomic Boundaries', 'Show In UCSC', 'View Details', 'Rename', and 'Delete'.</p>	
	<ol style="list-style-type: none"> <li>To clear the tracks, right-click the <b>Gene View</b>, and click <b>Preferences</b>.</li> <li>Click <b>Tracks</b>, and clear the <b>Show in UI</b> check boxes, and click <b>OK</b>.</li> </ol>	<ul style="list-style-type: none"> <li>Mark the <b>Show in Report</b> check boxes to add track information in the report.</li> <li>Mark <b>Genomic Boundaries</b> to limit the analysis within the boundaries defined in the tracks.</li> </ul>

## To change settings for ChIP Workflow Analysis

A ChIP workflow is helpful if you need to analyze multiple arrays and you know which algorithm settings you intend to use for the analysis.

First, you set all parameters for an analysis in an *analysis method*. When you run the workflow, the program automatically runs the analysis method. On 64-bit computers, you can run up to three workflows simultaneously. On 32-bit computers, workflows are processed sequentially, not simultaneously.

With a workflow, you can use already-imported FE data or existing experiments as the source of data for your analysis. Or, you can import data and create experiments automatically as you run the workflow. Workflows also let you analyze different data sets with the same analysis method and multiple data sets with multiple analysis methods.

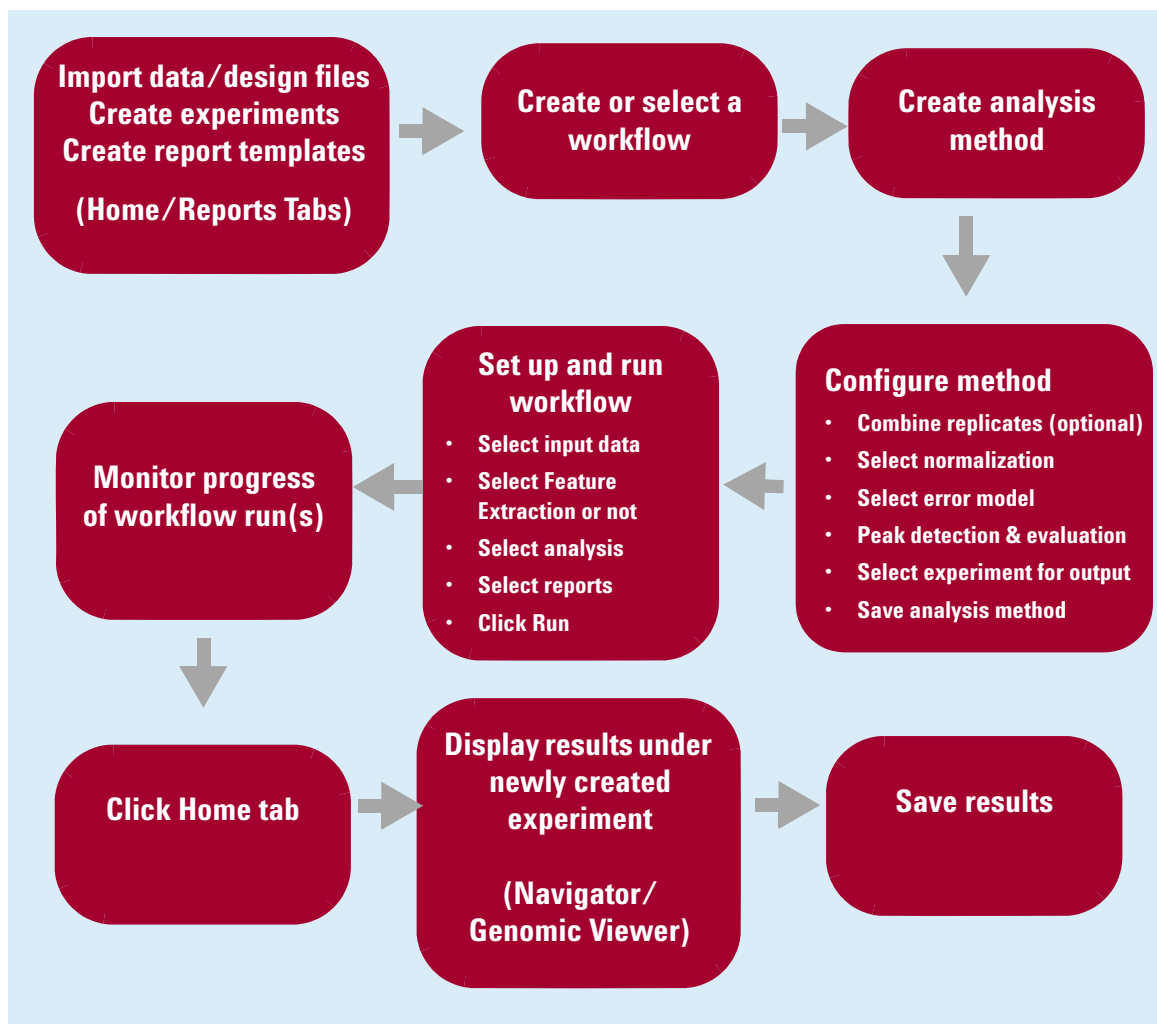
After completion of the workflow run, you use the Navigator to select the workflow experiment to display the results in the Genome, Chromosome and Gene Views.

To learn how to set up an analysis method and workflow and run a workflow, refer to [“Quick-start instructions for analyzing CGH data in a workflow”](#) on page 20. Even though many of the individual settings for ChIP analysis methods are different than those for CGH analysis methods, you set them up the same way and set up and run the workflow the same way. On the next page is a typical ChIP workflow analysis.

To learn more about the individual settings for ChIP analysis methods, see [“Setting up ChIP Analysis Methods”](#) on page 81.

## 1 Getting Started

To change settings for ChIP Workflow Analysis



**Figure 6** Typical ChIP Workflow analysis pathway



# Getting Help

## To get help within Agilent Genomic Workbench

Help guides are opened in Adobe® Reader® software. Agilent Genomic Workbench has several help resources:

Help Resource	Description/Instructions
Workflow User Guide	<p>This user guide, which you are now reading, supplies comprehensive help on all available Data Viewing tasks. You can access it easily from anywhere within the program.</p> <ol style="list-style-type: none"><li>1 In any tab of Agilent Genomic Workbench, click the <b>Help</b> tab.</li><li>2 On the Help Ribbon, click <b>Workflow</b>. The Workflow User Guide opens.</li></ol>
Other User Guides	<p>The Help tab in Agilent Genomic Workbench lets you view any of the available user guides that apply to the currently selected application type.</p> <ol style="list-style-type: none"><li>1 Set the desired application type from the Switch Application menu.</li><li>2 In the Agilent Genomic Workbench tab bar, click <b>Help</b>. The names of the available user guides appear in the command ribbon.</li><li>3 Click the desired help guide. The selected guide opens.</li></ol>

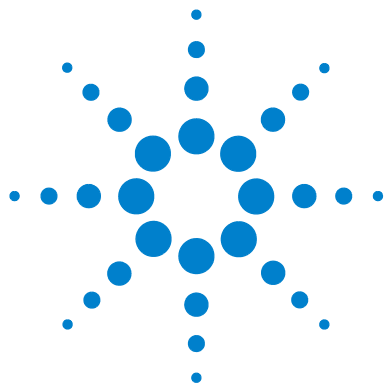
## To contact Agilent Technical Support

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	<ol style="list-style-type: none"><li>1 Go to <a href="http://chem.agilent.com">http://chem.agilent.com</a>.</li><li>2 Select a country or area.</li><li>3 Under Quick Links, select <b>Technical Support</b>.</li><li>4 Select from the available links to display support information.</li></ol>
Contact Agilent Technical Support by telephone or e-mail (United States and Canada)	Telephone: (800-227-9770) E-mail: <a href="mailto:informatics_support@agilent.com">informatics_support@agilent.com</a>
Contact Agilent Technical Support by telephone or e-mail (for your country)	<ol style="list-style-type: none"><li>1 Go to <a href="http://chem.agilent.com">http://chem.agilent.com</a>.</li><li>2 Select <b>Contact Us</b>.</li><li>3 Under Worldwide Sales and Support Phone Assistance, click to select a country, and then click <b>Go</b>. Complete e-mail and telephone contact information for your country is displayed.</li></ol>

## 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](http://www.chem.agilent.com).



## 2 Setting Up and Running Workflows

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This chapter gives instructions on how to set up and run workflows. The first section explains how to set up a workflow using Feature Extraction software for automatic feature extraction of microarray images. The second section describes how to set up a workflow for automatic analysis of data using the Agilent Genomic Workbench CGH or ChIP analysis packages. The third section explains how to run a workflow and review results.



## Creating and Managing Workflows

In this section you learn to create, edit, save and delete workflows. For a detailed description of the window of the Workflow tab, see [“Main Window”](#) on page 94, and for the command ribbons of the Workflow tab, see [“Workflow Command Ribbons”](#) on page 96.

### To create a new workflow

- 1 Start the Workflow program.  
See [“To start the Workflow program”](#) on page 14.
- 2 Click **New**.  
The Create Workflow dialog box appears. See [“Create Workflow”](#) on page 179.
- 3 In Enter Workflow Name, type a name.  
If you intend to restrict access to this workflow, mark **Apply Password**.
- 4 Click **OK**.  
If you marked the Apply Password check box, the Set Password dialog box appears. See [“Set Password”](#) on page 192.
  - Type a password and click **OK**.
- 5 Set up the workflow.  
See [“Setting up a Workflow for Feature Extraction”](#) on page 38 or [“Setting up an Analysis Workflow”](#) on page 44.
- 6 Click **Save**.

### To edit an existing workflow

- 1 Next to the **Select Workflow** list, click the right arrow.
- 2 Select a workflow name from the list.
- 3 Edit the workflow.  
See [“Setting up a Workflow for Feature Extraction”](#) on page 38 or [“Setting up an Analysis Workflow”](#) on page 44.

You can also create a new workflow from a selected one by saving it to another name. See “[To save a workflow to a new name](#)” on page 37.

## To save a workflow

- In the Workflow command ribbon click **Save**.

## To save a workflow to a new name

- 1 Next to the **Select Workflow** list, click the right arrow.
- 2 Select a workflow name from the list.
- 3 In the command ribbon click **Save As**.
- 4 Type the name of the workflow, and click **OK**.

## To delete a workflow

- 1 Next to the **Select Workflow** list, click the right arrow.
- 2 Select a workflow name from the list.
- 3 Click **Delete**.

## To set a password for an existing workflow

- 1 Next to the **Select Workflow** list, click the right arrow.
- 2 Select a workflow name from the list.
- 3 If you intend to restrict access to this workflow, mark **Apply Password**.  
The Set Password dialog box appears. See “[Set Password](#)” on page 192.
- 4 Type a password.
- 5 Type the password again to confirm it, and click **OK**.

## Setting up a Workflow for Feature Extraction

This section provides how-to help for the Feature Extraction tasks available in the Workflow tab of Agilent Genomic Workbench. If you have installed a license for the Agilent Feature Extraction software, you can automatically perform Feature Extraction on image files without exiting Agilent Genomic Workbench, whether or not you have any licenses installed for analysis applications (such as CGH or ChIP). If you have one or more licenses installed for analysis applications, you can also set up workflows to automatically perform feature extraction and analysis.

For more information on setting up Workflows for CGH or ChIP analysis, see [“Setting up an Analysis Workflow”](#) on page 44.

For a detailed description of all of the parameter panel and dialog boxes that appear, see [Chapter 4](#), “Workflow Reference”.

Agilent recommends that you use Sample Manager to set up your samples *before* you run a workflow. After you organize your Array IDs and assign their attributes using Sample Manager, you can use Workflow to automate Feature Extraction (and analyze the data, if you want). In Workflow, you select the image files to extract and then run Feature Extraction on the microarrays. When you run the extraction using Workflow, the following things happen:

- The extraction results are saved on your hard drive
- The extracted arrays are available in the appropriate Design Data folder for workflow or interactive analysis and data display
- The Array IDs in Sample Manager are updated
- Extraction information is available for use in the Quality tools

For more information on using Sample Manager and Quality tools, see the *Sample Manager User Guide* and the *Quality Tools User Guide*.

### To use eArray to update design/template files

To run a Feature Extraction workflow, the design/template file(s) for the files you want to extract must be present in the Feature Extraction Grid Template Browser or in the workflow FE output folder, and also in the Agilent Genomic Workbench database.

NOTE

You can check the status of the design in the Agilent Genomic Workbench database from the Home tab. Use the Search function to find the design in the Design Data pane of the Navigator. If the arrow next to the design is green, or if the arrow is yellow, and the build folder appears under the design, you can run a Feature Extraction workflow for microarrays of this design. See the *Data Viewing User Guide*, the *CGH Interactive Analysis User Guide*, or the *ChIP Interactive Analysis User Guide* for more information.

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When you run a workflow, the program will check the eArray Web site for design/templates and add them to the Feature Extraction database automatically, if

- The eArray Login Settings dialog box in Feature Extraction has a valid Username and Password AND
- You marked **Use eArray server during extraction** and **Check for updates of grid template** in the Advanced Options of the eArray Login Settings dialog box.

NOTE

You cannot do automatic design/template upload from a workflow for CGH+SNP custom designs. You must first import these designs with the Home > Import > Design Files > GEML file command.

---

You must import or download the design/template manually in Feature Extraction *before* you run the workflow if

- The eArray settings in Feature Extraction are blank, or
- If you entered an eArray Username and Password, but did not mark **Use eArray server during extraction** and **Check for updates of grid template** in the Advanced Options of the eArray Login Settings dialog box.
- If you use a design that is not in eArray.

For information on the eArray Login Settings in Feature Extraction, see the *Feature Extraction User Guide*.

## 2 Setting Up and Running Workflows

### To import an image file

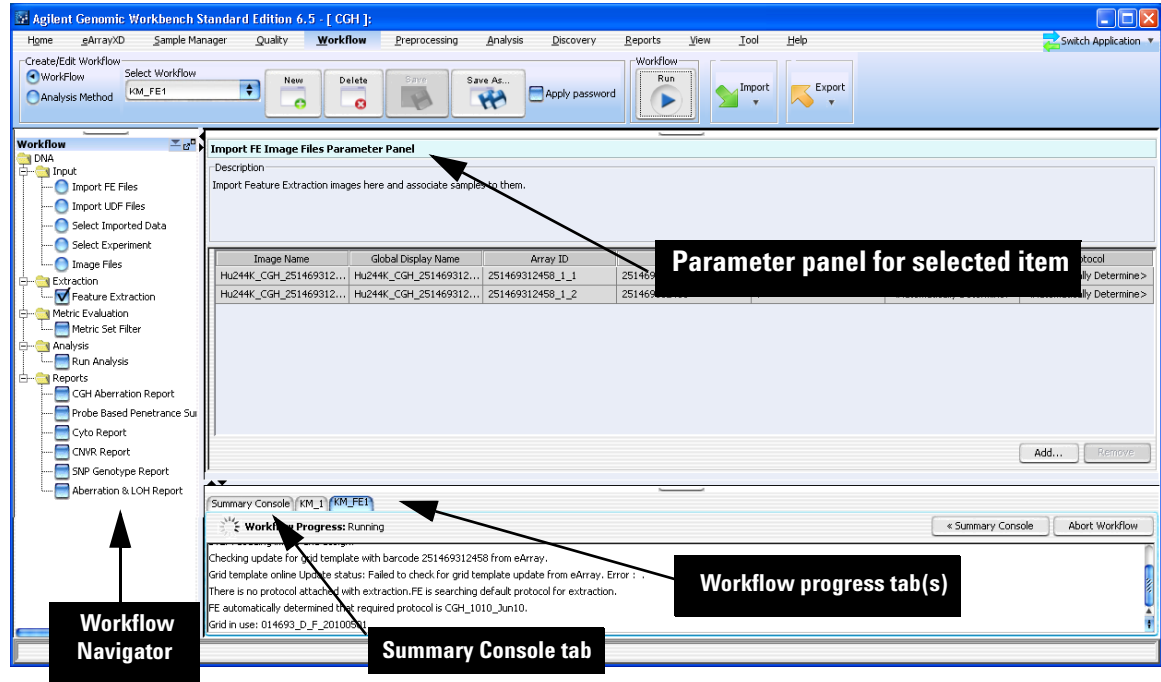


Figure 7 Workflow Main Window for Feature Extraction

### To import an image file

As part of the Workflow to automate extraction, you must import an image file that contains one or more scanned microarrays, along with the Array ID that identifies the microarray. For example, this can be a .tif image file generated by an Agilent scanner.

#### NOTE

The only input allowed for Feature Extraction workflows are image files. If any other input is selected, then Feature Extraction cannot be selected.

- 1 From the Workflow tab, in the command ribbon, select **Workflow**.  
The Workflow Navigator appears in the Navigator Pane.



**2** The DNA folder should be open by default. If not, double-click the **DNA** folder to open the folder. Input, Extraction, and Analysis folders are displayed.

**3** Click the **Input** folder to open it.

**4** Click **Image Files**.

The Import FE Image Files Parameter Panel is displayed. See [“Import FE Image Files Parameter Panel”](#) on page 146.

**5** Click **Add**.

The Open dialog box appears.

**6** Click to highlight the image file you wish to open. Or, click the **Look in** arrow and search for the desired folder. Then click to highlight the image file. To select more than one image file, hold down the **Ctrl** key and click the files you want to open.

**7** Click **Open**.

The Add image pack information for FE Extraction dialog box appears. See [“Add Image Pack Information for FE Extraction”](#) on page 177.

**8** For each image file, select the **Number of packs** for the image file. Select 1 if your image is a 1x1M or 1x244K slide, select 2 if your image is a 2x400K or 2x105K slide, select 4 if your image is a 4x180K or 4x44K slide or select 8 if your image is an 8x60K or 8x15K image.

**9** Click **Add Images**.

The sample images from the file are displayed in the image list.

## To remove an image from the list


- 1 In the Import FE Image Files Parameter Panel, click an image to highlight it. See [“Import FE Image Files Parameter Panel”](#) on page 146.
- 2 Click **Remove**.  
The selected image is removed from the list.

## To correlate sample attributes with workflow microarrays

To correlate sample attributes for image files to use for Workflow input, import an attribute file with the Array ID in Sample Manager or add the Array ID *before* you add the FE image file in Workflow. The successful correlation is indicated by **<red attribute>/<green attribute>(array ID)** displayed in the Sample ID **<Red/Green(Array ID)>** field of the Import FE Image Files Parameter Panel.

If the association of sample attributes has not been successful, a “?” appears in the Sample ID **<Red/Green(Array ID)>** field of the Import FE Image Files Parameter Panel.

To correlate sample attributes when you add image files to the workflow, if the Array ID is already in Sample Manager,

- 1 In the Import FE Image Files Parameter Panel, click the **Sample ID <Red/Green(Array ID)>** field for a microarray.
- 2 Click  and then select the correct Array ID from the list.

See the *Sample Manager User Guide* for more information.

## To set the output path for Feature Extraction results

Once you have opened an image file and the images are displayed in the images list, you must select a location where you want the FE output results to be saved.

- 1 In the Navigator pane of the Workflow tab, click the **Extraction** folder to open it.
- 2 Click the **Feature Extraction** box to select it. A checkmark indicates Feature Extraction is selected.
- 3 In the Set output path for FE File output pane, type the path where you want the program to save results. See [“Set Output Path for Feature Extraction Panel”](#) on page 164.
- 4 Or, click **Browse** and find the location to save results. Click **Save**.

### NOTE

To run a Feature Extraction workflow, you must have a license for Feature Extraction 10.10 or higher installed on your computer.

## To display or change the default FE parameters

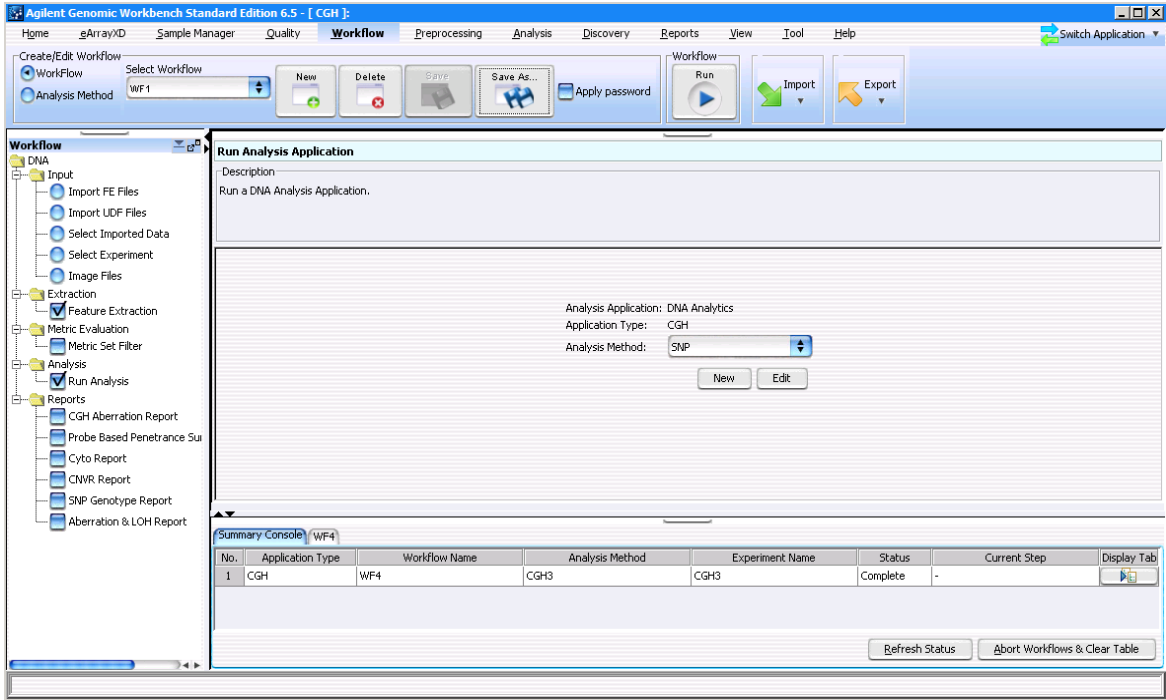
The Feature Extraction parameters that are used for automated feature extraction during a workflow are set using the Feature Extraction program. To display or change the basic default parameters currently in use for a workflow:

- 1 In the Navigator pane of the Workflow tab, click the **Extraction** folder to open it.
- 2 Click the **Feature Extraction** box to select it. A checkmark indicates Feature Extraction is selected.

In the Output path for FE File output parameter panel, click **FE default parameters being used**. See [“Feature Extraction Properties”](#) on page 183.

# Setting up an Analysis Workflow

This section describes how to set up a workflow for automatic analysis using CGH or ChIP. If you have the Agilent Feature Extraction software and license installed, you can use image files as the input and extract the files before you run the ChIP or CGH analysis in the workflow. If you do not have the Agilent Feature Extraction license installed, you can use existing FE files or UDF files, imported data or an existing experiment. For a detailed description of the main window of the Workflow tab, see “Main Window” on page 94.



**Figure 8** Workflow Window showing Run Analysis Application

To use Workflow for automatic analysis of data using CGH or ChIP, you first create and save an analysis method that specifies all the parameters to use for the analysis. For more information on how to create an analysis method, see [“Setting up an Analysis Method”](#) on page 66. You then set up the analysis workflow as described below, and then run the workflow.

## NOTE

To run an analysis workflow, you must have a CGH and/or ChIP application license installed.

## To set up the Run Analysis

To use a workflow to run a CGH or ChIP analysis, you must select an analysis method.

- 1 In the Workflow Run Navigator, in the Analysis folder, click the box to select **Run Analysis**.

The Run Analysis Application panel appears. See [“Run Analysis Application Panel”](#) on page 161 for more information. The Analysis Application is the software application that is used to analyze the data in your workflow. The Application Type is CGH or ChIP, depending on what application is selected. To change this, see [“To change the Application Type”](#) on page 46.

- 2 Click **Analysis Method** and select the analysis method to use for the workflow analysis of the data. If there is no existing analysis method, you must create one. See Step 4 below.
- 3 (optional) To edit the selected analysis method, click **Edit**. See [“To edit an existing analysis method”](#) on page 68 for more information.
- 4 (optional) To create a new analysis method, click **New**. See [“To create a new analysis method”](#) on page 67 for more information.

## To change the Application Type

The application type for a workflow can be CGH or ChIP. The CH3 application does not apply to Workflow. The current application is displayed in the Run Analysis Application pane. To change the application type,

- 1 On the tab menu, click **Switch Application**.  
A pop-up list appears that displays the applications.
- 2 Click to select the application.

**NOTE**

You can run a workflow for Feature Extraction without selecting any analysis application. You can also run a workflow that includes both Feature Extraction and one of the analysis applications.

## To select workflow input

When you set up a workflow, you must select its source of input data.

- In the Workflow Navigator, under Input, click the option button next to the source of microarray data.

See the tables below for a description of the available options.

**Table 2** Workflow Input for Feature Extraction

Option	Description
Image Files	Opens the Import FE Image Files Parameter Panel, where you can add image files to be extracted during a workflow. See <a href="#">“Import FE Image Files Parameter Panel”</a> on page 146. This is the only input option allowed for running a Feature Extraction in a workflow.

**Table 3** Workflow Input for CGH Analysis

Option	Description
Import FE Files	<p>Extracted microarray data that you have not yet imported into Agilent Genomic Workbench.</p> <p>For instructions on how to set the parameters for this option, see <a href="#">“To select FE data files for the workflow to import”</a> on page 48.</p>
Import UDF Files	<p>Tab delimited Universal Data Files created by non-Agilent programs.</p> <p>For instructions on how to set the parameters for this option, see <a href="#">“To select UDF data files for the workflow to import”</a> on page 49.</p>
Select Imported Data	<p>CGH microarray data that you have previously imported into Agilent Genomic Workbench.</p> <p>For instructions on how to set the parameters for this option, see <a href="#">“To use previously imported data as the workflow input”</a> on page 50.</p>
Select Experiment	<p>CGH microarray data from an existing Agilent Genomic Workbench experiment.</p> <p>For instructions on how to set the parameters for this option, see <a href="#">“To use an experiment’s arrays as the workflow input”</a> on page 51.</p>

**Table 4** Workflow Input for ChIP Analysis

Option	Description
Import Data Files	<p>Extracted microarray data that you have not yet imported into Agilent Genomic Workbench.</p> <p>For instructions on how to set the parameters for this option, see <a href="#">“To select data files for the workflow to import”</a> on page 51.</p>

## 2 Setting Up and Running Workflows

### To select FE data files for the workflow to import

Option	Description
Select Imported Data	ChIP microarray data that you have previously imported into Agilent Genomic Workbench.  For instructions on how to set the parameters for this option, see <a href="#">“To use previously imported data as the workflow input”</a> on page 50.
Select Experiment	ChIP microarray data from an existing Agilent Genomic Workbench experiment.  For instructions on how to set the parameters for this option, see <a href="#">“To use an experiment’s arrays as the workflow input”</a> on page 51.

### To select FE data files for the workflow to import

When you do CGH analysis, you can configure a workflow to import Feature Extraction data files and use them as the workflow input. The design files for the extractions must be in Agilent Genomic Workbench database before you run the workflow. For more information on how to import files, see the *CGH Interactive Analysis User Guide*.

#### NOTE

This input option is only available when CGH is selected as the application.

- 1 In the Workflow Navigator, under Input, click the **Import FE Files** option.

The Import Data Files Parameter Panel appears. See [“Import Data Files Parameter Panel”](#) on page 144.

- 2 In the parameter panel, click **Add Arrays**.

An Open dialog box appears.

- 3 Select the file to import, then click **Open**.

The array appears in the parameter panel. You can add as many files as you want; however, all files must use the same genome build.

You can remove existing arrays from the program with the same names as the ones you import. To enable this option, mark **Overwrite arrays with duplicate names**.



To remove an array from the list in the parameter panel, click its name, then click **Remove**.

- 4 In **Dye Flip**, select either **Normal** or **Flipped** for each array.

Select **Normal** if:

- The test samples were labeled with cyanine-5 (red).
- The control samples were labeled with cyanine-3 (green).
- The imported ratio (test/control) should be reported directly.

Select **Flipped** if:

- The test samples were labeled with cyanine-3 (green).
- The control samples were labeled with cyanine-5 (red).
- The imported ratio (control/test) should be reported with the ratio inverted (test/control).

## To select UDF data files for the workflow to import

You can configure a workflow to import custom, tab-delimited UDF data files and to use them as the workflow input.

### NOTE

This input option is only available when CGH is selected as the application.

- 1 In the Workflow Navigator, in Input, click **Import UDF Files**.  
The Import UDF Data Files Parameter Panel appears. See [“Import UDF Files Parameter Panel”](#) on page 148.
- 2 In the parameter panel, click **Add**.  
An Open dialog box appears.
- 3 Select the file(s) to import, then click **Open**.
- 4 Agilent Genomic Workbench attempts to use information in the UDF file to set the data and design type parameters. For more information, see the *CGH Interactive Analysis User Guide*. Make any necessary changes to these parameters.


## 2 Setting Up and Running Workflows

### To use previously imported data as the workflow input

- 5 If necessary, select the proper column correlation from the drop-down lists in the data display view. Alternatively, select a predefined correlation using the **Select Mapping** drop-down box.
- 6 Optionally, click **Save Mapping As** to save the correlated column fields in Agilent Genomic Workbench for future import of UDF data files.

### To use previously imported data as the workflow input

When you configure a workflow, you can use array data that you have previously imported into Agilent Genomic Workbench as the source of data for the workflow. (This applies to both CGH and ChIP analysis types.)

- 1 In the Workflow Navigator, under Input, click **Select Imported Data**.  
The Select Imported Data Parameter Panel appears. See [“Select Imported Data Parameter Panel”](#) on page 163.
- 2 In the parameter panel, in Select Design, select an array design.
- 3 In the parameter panel, in Select Genome Build, select an array design build.  
The arrays associated with the design and genome build appear under Array List.
- 4 In Array List, click the name of an array to include in the workflow. To select additional arrays, hold down the **Ctrl** key and click their names. To select a contiguous block of arrays, click the name of the first one in the block, then hold down the **Shift** key and click the name of the last one.
- 5 Click .

The program moves the selected arrays to the Selected Array List.

You can also use the other buttons in the dialog box to change the array lists. For more information, see [“Select Imported Data Parameter Panel”](#) on page 163.

## To use an experiment's arrays as the workflow input

You can use the arrays selected in an existing CGH or ChIP experiment as the input for a workflow. The program only uses the arrays linked to the experiment, and does not overwrite the original experiment, or use any of its settings.

- 1 In the Workflow Navigator, under Input, click **Select Experiment**.  
The Select Experiment Parameter Panel appears. See [“Select Experiment Parameter Panel”](#) on page 162.
- 2 In **Select Experiment**, select the experiment from the list.

## To select data files for the workflow to import

For ChIP analysis, you can configure a workflow to import data files and use them as the workflow input. Because a single workflow can process many data files, the array design file(s) must be available in the Agilent Genomic Workbench database. Array design files can only be imported from one of the interactive tabs, so it may be necessary to temporarily switch tabs to load a design file for use in an analysis. See the *ChIP Interactive Analysis User Guide* for more information on how to import design files.

### NOTE

This input option is only available when ChIP is selected as the application.

Workflow mode supports these microarray data files:

- Agilent Feature Extraction (\*.txt) array files
- Axon (\*.gpr) array files

- 1 In the Workflow Navigator, under Input, click the **Import Data Files** option.  
The Import Data Files Parameter Panel is displayed. See [“Import Data Files Parameter Panel”](#) on page 144.
- 2 In the parameter panel, click **Add Arrays**.  
An Open dialog box appears.
- 3 Select the file to import, then click **Open**.

## 2 Setting Up and Running Workflows

### To select a metric evaluation

The name of the file appears in Name in the parameter panel. You can add as many files as you want.

You can remove existing data files from the program with the same names as the ones you import. To enable this option, mark **Overwrite arrays with duplicate names**.

To remove a file from the list in the parameter panel, click its name, then click **Remove**.

**4** In **Dye Flip**, select either **Normal** or **Flipped** for each array.

Select **Normal** if:

- The test samples were labeled with cyanine-5 (red).
- The control samples were labeled with cyanine-3 (green).
- The imported ratio (test/control) should be reported directly.

Select **Flipped** if:

- The test samples were labeled with cyanine-3 (green).
- The control samples were labeled with cyanine-5 (red).
- The imported ratio (control/test) should be reported with the ratio inverted (test/control).

#### NOTE

Agilent Feature Extraction \*.txt array files must use GEML (\*.xml) design files. Axon (\*.gpr) array files must use Axon \*.gal design files. This helps Agilent Genomic Workbench to match data and design files correctly.

## To select a metric evaluation

When you run a workflow, you can set up a filter to include or exclude data from the analysis based on the metric set evaluation results calculated during feature extraction.

**1** In the Workflow Navigator, under Metric Evaluation, click **Metric Set Filter**.

The Metric Set Filter Parameter Panel appears.

**2** Select an existing metric set filter, or create a new one. See [“Metric Set Filter Parameter Panel”](#) on page 154 for more information.

## To select reports (CGH)

When you run a workflow, the program can produce four different reports that present the analysis method's experimental results. (See [Table 5](#).) Reports are files that contain output from the CGH module that you can open with other programs.

- In the Workflow Navigator, in Reports, mark the reports you want the analysis method to produce. In the parameter panel, set the parameters for each report.

See [Table 5](#) for a description of the available reports, and instructions on how to set the specific parameter(s) for each.

**Table 5** CGH module reports

Report	Description/Instructions
CGH Aberration Report	<p><b>To set parameters for the report</b></p> <ol style="list-style-type: none"> <li>1 In the Workflow Navigator, in Reports, mark <b>CGH Aberration Report</b>. Three settings appear in the parameter panel. See "<a href="#">Variance Stabilization</a>" on page 167.</li> <li>2 Under Report Type, select to generate a Probe Based report, an Interval Based report, or both a Probe &amp; Interval Based report.</li> <li>3 Under Output Format, select if the report file will contain output from the Complete Genome or if individual files will be generated Per-Chromosome.</li> <li>4 Under Select File Location, mark <b>Report Flat Intervals</b> to have aberration intervals reported without any nested structure.</li> <li>5 Under Select File Location, mark <b>Generate report per array</b> to generate reports as each microarray sample is analyzed. This lets you look at results for samples even though the workflow has not completed the entire analysis.</li> <li>6 Under Select File Location, click <b>Browse</b>. The Select report folder dialog box appears.</li> <li>7 Select a location for the report, and if necessary, change the <b>File name</b>.</li> <li>8 Click <b>Open</b>. The location of the CGH Aberration Report appears in the parameter panel, in Select File Location.</li> <li>9 Under Select File Location, mark <b>Overwrite if file exists</b> to overwrite a previous report saved as the same filename and location.</li> </ol>

## 2 Setting Up and Running Workflows

### To select reports (CGH)

**Table 5** CGH module reports (continued)

Report	Description/Instructions
Probe Based Penetrance Summary Report	<p><b>To set parameters for the report</b></p> <ol style="list-style-type: none"><li>1 In the Workflow Navigator, in Reports, mark <b>Probe Based Penetrance Summary Report</b>. Two settings appear in the parameter panel. See <a href="#">“Probe Based Penetrance Summary Report Parameter Panel”</a> on page 158.</li><li>2 Under Output Format, select if the report file will contain output from the Complete Genome or if individual files will be generated Per-Chromosome.</li><li>3 Under Select File Location, click <b>Browse</b>. The Select report folder dialog box appears.</li><li>4 Select a location for the report, and if necessary, change the File name.</li><li>5 Click <b>Open</b>. The location of the Text Penetrance Summary Report appears in the parameter panel, in Report Location.</li><li>6 Under Select File Location, mark <b>Overwrite if file exists</b> to overwrite a previous report saved as the same filename and location.</li></ol>
Cyto Report	<p><b>To set parameters for the report</b></p> <ol style="list-style-type: none"><li>1 In the Workflow Navigator, in Reports, mark <b>Cyto Report</b>. Three settings appear in the parameter panel. See <a href="#">“Cyto Report Parameter Panel”</a> on page 133.</li><li>2 In Select Report, select an existing Cyto Report.</li><li>3 Under Select File Location, click <b>Browse</b>. The Select report folder dialog box appears.</li><li>4 Select a location for the report, and if necessary, change the File name.</li><li>5 Click <b>Open</b>. The location of the Cyto Report appears in the parameter panel, in Report Location.</li><li>6 Under Select File Location, mark <b>Overwrite if file exists</b> to overwrite a previous report saved as the same filename and location.</li></ol>

**Table 5** CGH module reports (continued)

Report	Description/Instructions
CNVR Report	<p><b>To set parameters for the report</b></p> <ol style="list-style-type: none"> <li>1 In the Workflow Navigator, in Reports, mark <b>CNVR Report</b>. Three settings appear in the parameter panel. See <a href="#">“CNVR Report Parameter Panel”</a> on page 132.</li> <li>2 In CNVR Node Name, type a node name under which to access the CNVR report in interactive mode.</li> <li>3 Under Select File Location, click <b>Browse</b>. The Select report folder dialog box appears.</li> <li>4 Select a location for the report, and if necessary, change the <b>File name</b>.</li> <li>5 Click <b>Open</b>. The location of the CNVR Report appears in the parameter panel, in Report Location.</li> <li>6 Under Select File Location, mark <b>Overwrite if file exists</b> to overwrite a previous report saved as the same filename and location.</li> </ol>
SNP Genotype Report	<p><b>To set parameters for the report</b></p> <ol style="list-style-type: none"> <li>1 In the Workflow Navigator, in Reports, mark <b>SNP Genotype Report</b>. Three settings appear in the parameter panel. See <a href="#">“SNP Genotype Report Parameter Panel”</a> on page 166.</li> <li>2 In Output Format, select to format the report on a <b>Full Genome</b> or <b>Per-Chromosome</b> basis.</li> <li>3 Under Select File Location, click <b>Browse</b>. The Select report folder dialog box appears.</li> <li>4 Select a location for the report, and if necessary, change the <b>File name</b>.</li> <li>5 Click <b>Open</b>. The location of the SNP Genotype Report appears in the parameter panel, in Report Location.</li> <li>6 Under Select File Location, mark <b>Overwrite if file exists</b> to overwrite a previous report saved as the same filename and location.</li> </ol>
Aberration & LOH Report	<p><b>To set parameters for the report</b></p> <ol style="list-style-type: none"> <li>1 In the Workflow Navigator, in Reports, mark <b>Aberration &amp; LOH Report</b>. Three settings appear in the parameter panel. See <a href="#">“Aberration &amp; LOH Report Parameter Panel”</a> on page 120.</li> <li>2 Under Select File Location, click <b>Browse</b>. The Select report folder dialog box appears.</li> <li>3 Select a location for the report, and if necessary, change the <b>File name</b>.</li> <li>4 Click <b>Open</b>. The location of the Aberration &amp; LOH Report appears in the parameter panel, in Report Location.</li> <li>5 Under Select File Location, mark <b>Overwrite if file exists</b> to overwrite a previous report saved as the same filename and location.</li> </ol>

## To select and configure reports (ChIP)

When you run a workflow, the program can produce three different reports that present the analysis method's experimental results. (See [Table 6](#).) Reports are files that contain results from the ChIP module that you can open with other programs.

- In the Analysis Method Navigator, in Reports, mark the reports to produce. In the parameter panel, set the parameters for each report.

See [Table 6](#) for a description of the available reports, and instructions on how to set the parameter(s) for each.

**Table 6** ChIP module reports

Report	Description/Instructions
Probe Report	<p>This report contains information about the probes in the current experimental result in tab-separated value (*.tsv) format. A probe report contains one row for each probe in the array (or array set). The program generates a separate file for each array. See the <i>ChIP Interactive Analysis User Guide</i> for a description of the columns in the report. You can display probe reports and perform further analysis on them with a spreadsheet program.</p> <p><b>To set parameters for the Probe Report</b></p> <ol style="list-style-type: none"><li>1 In the Analysis Method Navigator, in Reports mark the box next to <b>Probe Report</b>.</li><li>2 In the Probe Report Settings parameter panel, click <b>Browse</b>. A Select report folder dialog box appears.</li><li>3 Select a location for the report, and if desired, change the File name.</li><li>4 Click <b>Open</b>. The location of the Probe Report appears, in Report Location.</li></ol>



**Table 6** ChIP module reports (continued)

Report	Description/Instructions
Gene Report	<p>This report contains information about the genes in the current experimental result in tab-separated value (*.tsv) format. It contains one row for each probe in the array (or array set), grouped by the genes to which the probes bind. The program generates a separate file for each array. It also includes loci represented by probes on the array that are not associated with genes. The program creates the Gene Report in several formats. See the <i>ChIP Interactive Analysis User Guide</i> for a description of these formats and the columns in each. You can display gene reports and perform further analysis on them with a spreadsheet program.</p> <p><b>To set parameters for the Gene Report</b></p> <ol style="list-style-type: none"> <li>1 In the Analysis Method Navigator, mark the box next to <b>Gene Report</b>. Three settings appear under Gene Report Settings.</li> <li>2 Mark one of these check boxes: <ul style="list-style-type: none"> <li>• Show only gene names – The resulting gene report contains only accession numbers of genes (or chromosomal locations for probe loci not associated with genes). This check box overrides the next one.</li> <li>• Show probe information – The resulting Gene Report contains additional information about the probes in the array.</li> </ul> </li> <li>3 Under Gene Report Settings, click <b>Browse</b>. A Select report folder dialog box appears.</li> <li>4 Select a location for the report, and if desired, change the File name.</li> <li>5 Click <b>Open</b>. The location of the Gene Report appears, in Report Location.</li> </ol>
ChIP QC Report	<p>This report summarizes the settings of the current analysis, and the overall statistics of each array. In addition to summary tables, it includes plots that summarize the data graphically. The program creates the QC Report in HTML format, and generates a separate folder for each array. For more details about the contents of the report, see the <i>ChIP Interactive Analysis User Guide</i>.</p> <p><b>To set parameters for the QC Report</b></p> <ol style="list-style-type: none"> <li>1 In the Analysis Method Navigator, mark the box next to <b>ChIP QC Report</b>. One setting appears under QC Report Settings.</li> <li>2 In the QC Report Settings parameter panel, click <b>Browse</b>. A Select report folder dialog box appears.</li> <li>3 Select a location for the report, and if desired, change the File name.</li> <li>4 Click <b>Open</b>. The location of the QC Report appears, in Report Location.</li> </ol>

## Running Workflows

When you run a workflow, the selected analysis (Feature Extraction, CGH/ChIP application analysis, or both) is run on the selected input files.

### To run a workflow

- 1 Create a new workflow or select one from the list.  
See [“To create a new workflow”](#) on page 36 or [“To edit an existing workflow”](#) on page 36.
- 2 On the Workflow ribbon, click **Run**.
- 3 At the prompt, type a name into the **Provide Workflow Identifier** dialog box, and then click **OK**.

This name becomes the name of the progress tab in the Summary Console/Progress tabs pane. The default name is the name of the workflow. Typing a new name does not change the name of the experiment or the workflow. See [“Provide Workflow Identifier”](#) on page 188.

An experiment is created in the Experiments pane of the Home tab Navigator. The experiment folder lists the Experiment Name you specified when you set up the analysis method. Otherwise, the experiment has the name of the Analysis Method used for the workflow. Feature Extraction-only workflows do not create experiments. The experiment folder is marked with a “W” while the workflow is running.

The Summary Console tab at the bottom of the window in the Summary Console/Progress tabs pane displays the status of the workflow run(s). See [“Summary Console tab”](#) on page 173.

A workflow progress tab is created in the Summary Console/Progress tabs pane for the named workflow and displays the progress of the run and any errors that occur. A not-yet-completed run has “running” displayed in its progress tab. A completed run has “completed” displayed in its progress tab. See [“Workflow progress tabs”](#) on page 175.

**NOTE**

The Summary Console and workflow progress tabs include workflows for CGH, ChIP, and SureSelect Target Enrichment. For information on SureSelect Target Enrichment workflows, see the *SureSelect Target Enrichment User Guide*.

---

## To run a series of workflows

Once a workflow run has started, you can set up and run additional workflows, which are run in the order in which you started them. Each workflow has its own progress tab.

**NOTE**

On 64-bit computers, you can run up to three workflows simultaneously. On 32-bit computers, workflows are processed sequentially, not simultaneously.

---

### To run a series with different workflows

- 1 Run the first workflow.  
Follow the instructions in [“To run a workflow”](#) on page 58.
  - 2 Create a new workflow or select an existing one from the list.  
See [“To create a new workflow”](#) on page 36 or [“To edit an existing workflow”](#) on page 36.
  - 3 In Output, click **Experiment**.
  - 4 Change the name of the experiment if the named experiment exists in the Experiment pane.
  - 5 Save the workflow.
  - 6 Click **Run**.
  - 7 Repeat steps 2-5 until you are complete.
- See [“To monitor workflow runs”](#) on page 60.

### To run a series with the same workflow

- 1 Run the first workflow.  
Follow the instructions in [“To run a workflow”](#) on page 58.
- 2 Click **Run** again.

## 2 Setting Up and Running Workflows

### To monitor workflow runs

An Input dialog box appears with the name of the experiment in the workflow plus an increment of 1. This will be the name of the experiment created for the second run of the same workflow. You can change the name if you want.

**3 Click OK.**

The Provide Workflow Identifier dialog box appears. See [“Provide Workflow Identifier”](#) on page 188.

**4 Enter the name for the second workflow progress tab, and click OK.**

**5 Repeat steps 2 through 4 until you are complete.**

See [“To monitor workflow runs”](#) on page 60.

The Summary Console tab at the bottom of the window in the Summary Console/Progress tabs pane displays the status of all the in-progress or completed workflow(s). See [“Summary Console tab”](#) on page 173.

## To monitor workflow runs

You can monitor workflow runs in the Summary Console/Progress tabs pane by viewing the Summary Console tab and/or the progress tabs.

- Click **Summary Console** to monitor the status of all the workflows.

See [“Summary Console tab”](#) on page 173.

- Click the button under **Display Tab** in the Summary Console row to go to the progress tab for the selected run, or in the progress tabs, click a named workflow tab.

A progress tab displays the workflow progress and any errors that occur. A not-yet-completed workflow run has “running” displayed in its progress tab. A completed workflow run has “completed” displayed in its progress tab. See [“Workflow progress tabs”](#) on page 175.

- Click **Close Tab** in a progress tab to stop that run and remove the workflow from the list.
- Click **< Summary Console** to return to a summary table of each workflow and experiment.

Click **Abort Workflows & Clear Table** in the Summary Console tab to stop all workflow runs, and remove workflows and workflow experiments from the run list.

## To abort a single workflow

- 1 In the Summary Console/Progress tabs pane, click the workflow progress tab for the workflow to abort.
- 2 Click **Abort Workflow**. You are asked to confirm that you want to abort the workflow. Note that the Abort Workflow button will not appear unless the workflow is currently running.

The workflow is aborted and the workflow progress tab and workflow status in the Summary Console tab are removed.

## To abort all workflows

- 1 In the Summary Console/Progress tabs pane, click the **Summary Console** tab.
- 2 Click **Abort Workflows & Clear Table**.
- 3 You are asked to confirm that you want to abort all workflows. Click **Yes**.
- 4 A warning that workflow information will be lost is displayed for each workflow. Click **Yes** to confirm each.

All workflows are aborted and the Summary Console/Progress tabs pane is cleared of all workflow information.

## To run an existing workflow on a new set of data


You can use an existing workflow to analyze many different sets of data. For each set of data, you change the input and output parameters of the workflow.

## 2 Setting Up and Running Workflows

To display the results of a workflow

### NOTE

Choice of input will depend on whether the CGH or ChIP application is selected.

- 1 On the command ribbon, in Select Analysis Method, select an analysis method.
- 2 In the Workflow Navigator, in the Input folder, select the source of data for the workflow. See [“To select workflow input”](#) on page 46.
- 3 Set the specific parameters for the selected source of data. For more information, see these topics:
  - [“To select data files for the workflow to import”](#) on page 51
  - [“To select FE data files for the workflow to import”](#) on page 48
  - [“To select UDF data files for the workflow to import”](#) on page 49
  - [“To use previously imported data as the workflow input”](#) on page 50
  - [“To use an experiment’s arrays as the workflow input”](#) on page 51
- 4 In the Workflow Navigator, in the Output folder, click **Experiment**.  
In Tab View, the Experiment Parameter Panel appears. By default, the name of the workflow appears in Experiment Name. See [“Experiment Parameter Panel”](#) on page 137.
- 5 In Experiment Name, type a new name for the experiment.
- 6 In the command ribbon, click **Save**.
- 7 In the command ribbon, click **Run** .

The program runs the workflow. Workflow status is displayed in the Summary Console tab during the run. In addition, the program highlights each step of the workflow as it occurs. Both the data and results appear automatically in Genome, Chromosome, and Gene views, and in the Data tab in Tab View. See [“To display the results of a workflow”](#) on page 62.

## To display the results of a workflow

After you configure a workflow and run it, the results are displayed in the interactive tabs of Agilent Genomic Workbench (Preprocessing, Analysis, Discovery, Reports, View, Tool).

- Switch to one of the interactive tabs to review the results that are generated by the completed workflows.

The workflow results appear as a node under the experiment created during the workflow run. This experiment has the letter **W** on the folder during the run to let you know this experiment and its results were generated during a workflow run. The **W** is not displayed after the workflow run is complete.

In addition, you can use other programs to open the reports created by the workflow. Use a spreadsheet program to display and further analyze \*.xls report files (Text Aberration and Text Penetration Summary reports, CNVR report, Probe and Gene reports). Use Adobe Reader to display \*.pdf format report files (Cyto reports). Use an Internet browser to display the QC report.

## To export a workflow

To save workflow in a file on your hard disk, you must export it. To export a workflow, you must have one or more workflows saved in the program.

- 1 On the command ribbon, under Create/Edit Analysis Method click **Workflow**.
- 2 On the command ribbon, click **Export Workflow(s)**.  
A selection menu appears.
- 3 To export workflow, select **Workflow(s)**.
- 4 In the Export Workflow(s) dialog box, click to select the workflow(s) you wish to export. See [“Export Workflow\(s\)”](#) on page 182 for more information.

The Export dialog box appears. See [“Export”](#) on page 180.

- 5 In the Export dialog box, go to the location where you want to save the file, and type the file name.
- 6 Click **Export**.

## To import a workflow

If you have exported one or more workflows and you want to import them into the program,

- 1 On the command ribbon, click **Import Workflow(s)**.

The Import Workflow(s) dialog box appears. See “[Import Workflow\(s\)](#)” on page 186.

- 2 Browse to the location where the file is located, click to select it, and then click **Import**.

The Import dialog box appears. See “[Import](#)” on page 184.

- 3 Click to select the workflow(s) to import.

- 4 Click **OK** to import the workflow(s).

- 5 An import status box will appear. Click **OK**.





### 3

## Setting up Workflow Analysis Methods

- Setting up an Analysis Method [66](#)
- Setting up CGH Analysis Methods [71](#)
- Setting up ChIP Analysis Methods [81](#)

This chapter describes how to create analysis methods to use in the workflow. An *analysis method* is a list of actions and parameters to use for an analysis workflow. (The “analysis method” was called the “workflow” in DNA Analytics 4.0.)

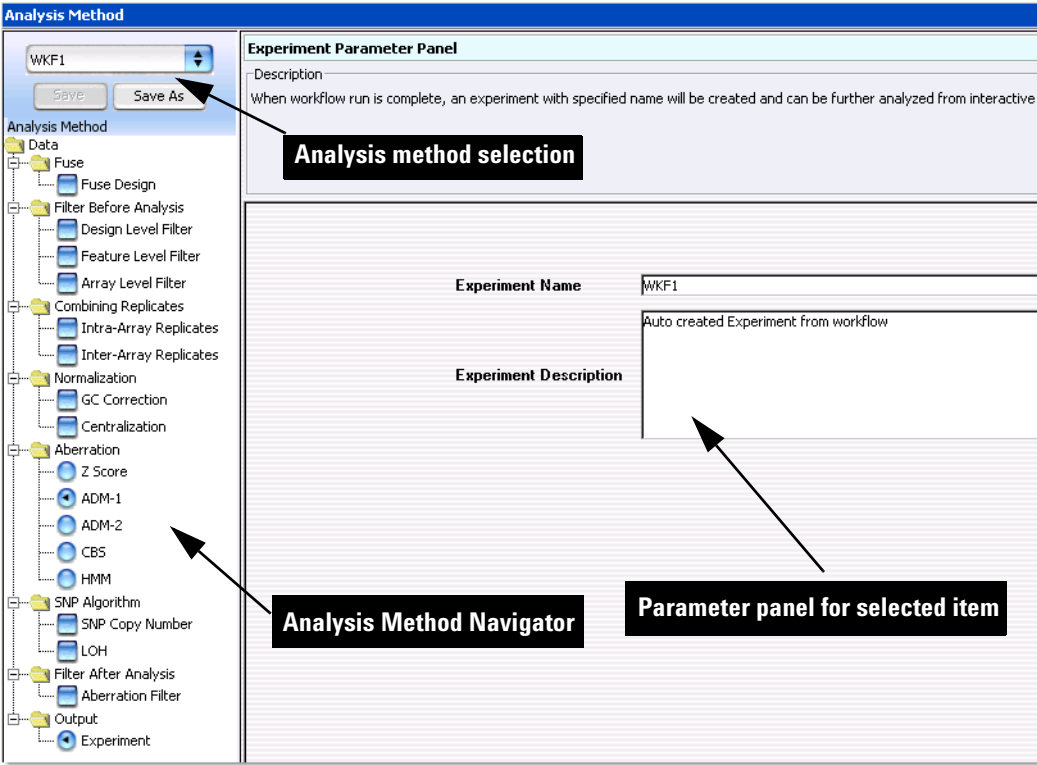
You can save analysis methods, and apply the same method to many different sets of data. Use workflows to analyze data and generate results, and then review the results in Genomic Viewer.

After you set up and run your workflow, you see the results graphically, with chromosomal aberrations or binding events correlated with genes and chromosomal locations. The workflow also produces report files that you can analyze further with a spreadsheet or other analysis program.



# Setting up an Analysis Method

Analysis methods are created, edited and saved from the Analysis Method window. This window appears when you create or edit an analysis method.



**Figure 9** Analysis Method window for CGH

## To create a new analysis method

An analysis method is a group of settings that the program uses to run an analysis in Workflow. Before you run an analysis method, you must create a new one (or select an existing one; see [“To select an existing analysis method”](#) on page 67).

- 1 On the Workflow command ribbon, click **Analysis Method**.

When Analysis Method is selected, the command ribbon displays commands related to the Analysis Method.

- 2 On the Analysis Method command ribbon, click **New**.

The Create Analysis Method dialog box appears. See [“Create Analysis Method”](#) on page 178.

- 3 Type a name for the new analysis method.

By default, the program also gives this name to the experiment it creates when you run the workflow.

- 4 Click **OK**.


The program creates the new analysis method and opens the Analysis Method window. The name of the new analysis method appears in the toolbar under Select Analysis Method. You can now set the parameters of the new analysis method. You can also create a new analysis method from the Run Analysis application pane. See [“Run Analysis Application Panel”](#) on page 161 for more information.

## To select an existing analysis method

The current analysis method appears in the toolbar under Create/Edit Analysis Method. To select a different analysis method, follow these steps:

- 1 On the Workflow command ribbon, click **Analysis Method**.

When Analysis Method is selected, the command ribbon displays commands related to the Analysis Method.

- 2 On the command ribbon, click the arrows next to **Select Analysis Method**, .


A list of existing analysis methods appears. If no analysis methods appear, you must create a new one. See [“To create a new analysis method”](#) on page 67.

- 3 Select the analysis method from the list.

You can now set the parameters for the selected analysis method.

## To edit an existing analysis method

You can edit an existing analysis method, or use an existing analysis method as the basis for a new one.

- 1 On the Workflow command ribbon, click **Analysis Method**.  
When Analysis Method is selected, the command ribbon displays commands related to the Analysis Method.
- 2 On the Workflow command ribbon, under Create/Edit Analysis Method, click .  
A list of analysis methods appears.
- 3 From the list, select the analysis method to change.
- 4 On the Workflow command ribbon, click **Edit**.  
The **Analysis Method** window appears.
- 5 Change the parameters of the analysis method. For specific instructions, see the topics in the sections entitled “[Setting up CGH Analysis Methods](#)” on page 71 or “[Setting up ChIP Analysis Methods](#)” on page 81.
- 6 When you are finished, do one of the following:
  - To save the existing analysis method with the changes, click **Save**.
  - To save the analysis method, with changes, as a new analysis method, click **Save As**. In the dialog box that appears, type a name for the new analysis method, then click **OK**. By default, the program also gives this name to the experiment it creates when you run the workflow. See “[Save As](#)” on page 189.

## To save an analysis method

You must save an analysis method before you can use it in a workflow.

- In the Analysis Method window, do one of the following:
  - To save the analysis method with the same name, click **Save**.

- To save the analysis method with a different name, click **Save As**. In the dialog box that appears, type a name for the new analysis method, then click **OK**. By default, the program also gives this name to the experiment it creates when you run the workflow. See “[Save As](#)” on page 189.

**NOTE**

When you use the Save or Save as command to save an analysis method, it is not saved in an external file on your hard disk. To save an analysis method to a location on your hard disk, use the **Export Workflow(s) > Analysis Method** command.

## To delete an analysis method

When you delete an analysis method, the program removes the name, parameters and settings of the analysis method from the program. The program does not delete any microarray data files.

- 1 On the Workflow command ribbon, click **Analysis Method**.

When Analysis Method is selected, the command ribbon displays commands related to the Analysis Method.

- 2 On the Workflow command ribbon, under Create/Edit Analysis Method, select the analysis method to delete.
- 3 Click **Delete**.  
A Confirm dialog box appears.
- 4 Click **Yes**.

## To export an analysis method

To save an analysis method in a file on your hard disk, you will need to export it. To export an analysis method, you must have one or more analysis methods saved in the program.

- 1 On the command ribbon, under Create/Edit Analysis Method click **Analysis Method**.
- 2 On the command ribbon, click **Export Workflow(s)**.  
A selection menu appears.

### 3 Setting up Workflow Analysis Methods

#### To import an analysis method

- 3 To export an analysis method, select **Analysis Method(s)**.
- 4 In the Export Analysis Method(s) dialog box, click to select the workflow(s) or analysis method(s) you wish to export. See [“Export Analysis Method\(s\)”](#) on page 181 for more information.  
The Export dialog box appears. See [“Export”](#) on page 180.
- 5 In the Export dialog box, go to the location where you want to save the file, and type the file name.
- 6 Click **Export**.

## To import an analysis method

If you have exported one or more analysis methods and you want to import them into the program,

- 1 On the workflow command ribbon, under Create/Edit Analysis Method click **Analysis Method**.
- 2 On the command ribbon, click **Import Workflow(s)**.  
The **Import Analysis Method(s)** dialog box appears. See [“Import Analysis Method\(s\)”](#) on page 185.
- 3 Browse to the location where the file is located, click to select it, and then click **Import**.  
The **Import** dialog box appears. See [“Import”](#) on page 184.
- 4 Click to select the analysis method(s) to import.
- 5 Click **OK** to import the analysis method(s).
- 6 An import status box appears. Click **OK**.

## Setting up CGH Analysis Methods

This section describes how to set up analysis methods to run a workflow for CGH analysis.

### To select to fuse designs (CGH)

If you have two arrays that use different design files, you can combine (fuse) them into one larger virtual array. You do this to increase the coverage of the genome in your design. This can make it easier to work with multiple arrays that are part of an array set. For information about fusing designs, see the *CGH Interactive Analysis User Guide*.

#### NOTE

Agilent Genomic Workbench cannot combine arrays from more than two different design files at a time. Arrays to be fused must have the same value for the ArraySet attribute. This is set interactively.

- 1 In the Analysis Method Navigator, in Fuse, click the **Fuse Design** option button.

The Fuse Design Parameter Panel appears. See [“Fuse Design Parameter Panel”](#) on page 140.

- 2 Click **Select Normalization** and select None or Centralization.

- 3 Select **Remove arrays from experiment after fuse** if you wish to use only fused arrays in the experiment rather than fused and individual arrays.

This deletes the initial unfused arrays from the experiment and reduces the duplication of data within the experiment.

#### NOTE

If the fused arrays have common probes, these probes are treated as replicates in the fused array. You can combine these replicates. See [“To configure the analysis method to combine intra-array replicates”](#) on page 81.

## To filter the data before analysis (CGH)

Some features on an array can lead to erroneous results; for example, saturation in either channel attenuates the true log ratio. With a feature level filter, the workflow can remove these data before analysis. Also, measurable log ratios can be established with a minimum log ratio value. The Default FeatureFilter removes saturated and nonuniform features. See the *CGH Interactive Analysis User Guide* for more information.

Array level filters include or exclude arrays from an experiment based on the values of specific array attributes. It is sometimes useful to filter arrays based on the parameters used in array preparation. For example, if you set a minimum degree of labeling in either channel, this can help produce better results. Agilent Genomic Workbench allows feature and array attribute criteria to be applied during analysis. See the *CGH Interactive Analysis User Guide* for more information.

By default, all probes are present in an experiment (unique and non unique). The design level filter lets you filter out probes based on specified design filters such as homology or probe score. See the *CGH Interactive Analysis User Guide* for more information.

## To configure the analysis method to apply a design level filter

- 1 In the Analysis Method Navigator, in Filter Before Analysis, mark **Design Level Filter**.

The Design Level Filter Parameter Panel appears. See “[Design Level Filter Parameter Panel](#)” on page 134.

- 2 Do one of the following:

- To create a new filter, click **New**. In the dialog box that appears, type a name for your new filter, then click **OK**.
- To edit an existing filter, select its name.

The name of the filter and its conditions appear in the dialog box.

- 3 Change the conditions of the filter.

To restore changed values to their original values, click **Reset**.

- 4 Click **Update** to save the filter.

- 5 Click **Close**.



## To configure the analysis method to apply a feature level filter

- 1 In the Analysis Method Navigator, in Filter Before Analysis, mark **Feature Level Filter**.

The Feature Level Filter Parameter Panel appears. See [“Feature Level Filter Parameter Panel”](#) on page 138.

- 2 In Name, select the Default Feature Filter or any filter previously defined in interactive mode, or click **New** to create a new filter. See [“To create or modify a feature level filter”](#) on page 73.

## To configure the analysis method to apply an array level filter

- 1 In the Analysis Method Navigator, in Filter Before Analysis, mark **Array Filter**.

The Array Filter Parameter Panel appears. See [“Array Level Filter Parameter Panel”](#) on page 126.

- 2 In Name, select any filter previously defined in interactive mode, or click **New** to create a new filter. See [“To create or modify an array level filter”](#) on page 74.

## To create or modify a feature level filter

- 1 In the Analysis Method Navigator, in Filter Before Analysis, mark **Feature Level Filter**.

The Feature Level Filter Parameter Panel appears. See [“Feature Level Filter Parameter Panel”](#) on page 138.

- 2 Do one of the following:

- To create a new filter, click **New**. In the dialog box that appears, type a name for your new filter, then click **OK**.
- To edit an existing filter, select its name. The name of the filter and its conditions appear in the dialog box.

- 3 Change the conditions of the filter. To restore changed values to their original values, click **Reset**.

- 4 Click **Update** to save the filter.

## To create or modify an array level filter

- 1 In the Analysis Method Navigator, in Filter Before Analysis, mark **Array Level Filter**.

The Array Level Filter Parameter Panel appears. See “[Array Level Filter Parameter Panel](#)” on page 126.

- 2 Do one of the following:
  - To create a new filter, click **New**. In the dialog box that appears, type a name for your new filter, then click **OK**.
  - To edit an existing filter, select its name. The name of the filter, and its conditions, appear in the dialog box.
- 3 Change the conditions of the filter, as desired. To restore changed values to their original values, click **Reset**.
- 4 Click **Update** to save the filter.

## To combine replicates (CGH)

You can configure an analysis method to combine intra-array or interarray replicate probes, or both. This increases the statistical power of your analysis. For a discussion of the statistical model Agilent Genomic Workbench uses to combine replicates, see the *CGH Interactive Analysis User Guide*.

Intra-array replicates are features within the same array that contain the same probe. Interarray replicates are features on different arrays that contain the same probe.

## To configure the analysis method to combine intra-array replicates

- In the Analysis Method Navigator, in Combining Replicates, mark **Intra-Array Replicates**.

The Intra-Array Replicates Parameter Panel appears. No parameters are required for this option.

## To configure the analysis method to combine interarray replicates

- 1 In the Analysis Method Navigator, in Combining Replicates, mark **Inter-Array Replicates**.  
The Inter-Array Replicates Parameter Panel appears. See [“Inter-Array Replicates Parameter Panel”](#) on page 150.
- 2 In Group By, select an array attribute. When you run the workflow, the program combines replicates from arrays with matching values for the selected attribute.

## To select to correct for GC content

When you select to correct for GC content, the algorithm corrects for “wave artifacts” by performing a robust regression fit to GC content in a specified region flanking the probes, and then corrects for it. The correction is done for both CGH and SNP probes.

### NOTE

GC Content correction is required in order to calculate SNP Copy Number and LOH.

- 1 In the Analysis Method Navigator, in Normalization, mark **GC Correction**.  
The GC Correction parameter panel appears. See [“GC Correction Parameter Panel”](#) on page 141.
- 2 Click to select the **Window Size** to use for the GC correction. For more information on how this algorithm works, see the *CGH Interactive User Guide*.

## To select to Centralize the data (CGH)

You normalize microarray data to correct it for known factors that cause the reported log ratios to differ from the “true” log ratios.

- 1 In the Analysis Method Navigator, in Normalization, mark **Centralization**.

The Centralization Parameter Panel appears. See [“Centralization Parameter Panel”](#) on page 129.

- 2 In **Centralization Threshold**, type a value as a threshold for probes to be included in the centralization algorithm.
- 3 In Centralization Bin Size, type a value for the number of contiguous probes to be averaged together across the genome.

Many statistical algorithms for aberration detection use log ratio values that are centered around zero if no aberration occurs, that reflects no change between the reference and sample channels. In samples with a high aberration percentage, this can lead to erroneous results because the measured center of the data deviates from a log value of zero.

The centralization algorithm finds a constant value to add to or subtract from all log ratio measurements, to recenter the log ratio values. This causes the zero-point to reflect the most-common-ploidy state. For a discussion of the statistical algorithms the program uses to normalize data, see the *CGH Interactive Analysis User Guide*.

## To select aberration algorithms (CGH)

A variety of detection algorithms are available to provide detection of contiguous aberrant regions. See the *CGH Interactive Analysis User Guide* for more information. To select an aberration algorithm:

- In the Analysis Method Navigator, in Aberration, mark the algorithm to use for the analysis.

## To configure the analysis method to apply the Z-score algorithms

- 1 In the Analysis Method Navigator, in Aberration, mark **Z score**.  
The Z score Parameter Panel appears. See [“Z Score Parameter Panel”](#) on page 171.
- 2 In Window, select the size of the sliding window from the drop-down list. The probe abundance is calculated within the selected size for the genomic region. Note that this is a fixed value in the Z-score algorithm. For more information, see the *CGH Interactive Analysis User Guide*.
- 3 In Threshold, type or select with the green slider the parameter used to calculate whether a region is statistically different from a log ratio value of 0.

## To configure the analysis method to apply the ADM (ADM-1 or ADM-2) algorithms

- 1 In the Analysis Method Navigator, in Aberration, mark either **ADM-1** or **ADM-2**. See the *CGH Interactive Analysis User Guide* for more information.  
The ADM-1 or ADM-2 Parameter Panel appears. See [“ADM-1 Parameter Panel”](#) on page 123 and [“ADM-2 Parameter Panel”](#) on page 124.
- 2 In Threshold, type or select with the green slider the parameter used to calculate whether a region is statistically different from a log ratio value of 0.
- 3 In Nesting Level, select the level of nesting.  
In Nesting Level, the Nesting Filter text box is enabled. Type the maximum number of iterations for the ADM algorithms.
- 4 Click **Fuzzy Zero** to apply the Fuzzy Zero algorithm. See the *CGH Interactive Analysis User Guide* for more information.

## To configure the analysis method to apply the CBS algorithm

- In the Analysis Method Navigator, in Aberration, mark **CBS**.  
The CBS algorithm is selected for the analysis method. See the *CGH Interactive Analysis User Guide* for more information.

## To configure the analysis method to apply the HMM algorithm

- 1 In the Workflow Navigator, in Aberration, mark **HMM**.  
The HMM Parameter Panel appears. See “[HMM Parameter Panel](#)” on page 143.
- 2 Select the number of states and type an FDRQ value to be used.  
THE HMM (Hidden Markov Model) algorithm is selected for the analysis method. See the *CGH Interactive Analysis User Guide* for more information.

## To select to filter the results after analysis (CGH)

Agilent Genomic Workbench can apply a post-analysis filter to aberrant regions. This filter will ignore small, spurious, or low-quality aberrations and is important for commonly aberrant region and CNVR detection. For example, you may want to consider only aberrations that contain three or more probes, or have a minimum log ratio, or have a minimum percent penetrance across samples.

- 1 In the Analysis Method Navigator, in Filter After Analysis, mark **Aberration Filter**.  
The Aberration Filter Parameter Panel appears. See “[Aberration Filter Parameter Panel](#)” on page 121.
- 2 In Select Filter, choose either the DefaultAberrationFilter or any filter predefined in interactive mode. See the *CGH Interactive Analysis User Guide* for details. Or, click **New** to open the Input dialog box to type a name for the new filter.
- 3 Complete the parameter panel for the selected, edited, or new filter.

## To select to calculate SNP Copy Number

This selection causes the program to calculate allele specific copy number for SNP probes.

### NOTE

In order to select this option, you must first select **GC Correction** and **Centralization** methods for Normalization.

---

- 1 In the Analysis Method Navigator, in SNP Algorithm, mark **SNP Copy Number**.

The SNP Copy Number Parameter Panel appears. See “[SNP Copy Number Parameter Panel](#)” on page 165.

- 2 In **SNP Conf Level**, type a value to use for the confidence level in the calculation. For more information, see the *CGH Interactive Analysis User Guide*.

## To select to calculate LOH

Use this selection to identify regions of LOH (loss or lack of heterozygosity) for microarrays with SNP content.

### NOTE

In order to select this option, you must first select **GC Correction** and **Centralization** methods for Normalization, and **SNP Copy Number** for SNP Algorithm.

---

- 1 In the Analysis Method Navigator, in SNP Algorithm, mark **LOH**.

The LOH Parameter Panel appears. See “[LOH Parameter Panel](#)” on page 153.

- 2 In **Threshold**, type a value to use for the confidence level in the calculation. For more information, see the *CGH Interactive Analysis User Guide*.

## To define an output experiment

When you run a workflow, the program creates an Agilent Genomic Workbench experiment that you can display in interactive mode.

- 1 In the Analysis Method Navigator, in Output, click **Experiment**.

Two parameters appear in the parameter panel. See [“Select Experiment Parameter Panel”](#) on page 162. By default the program gives the experiment the same name as the analysis method.

- 2 You can type a new name for the experiment. The name must not already exist in Agilent Genomic Workbench.
- 3 In Description, type an optional description for the experiment.



## Setting up ChIP Analysis Methods

This section describes how to set up analysis methods for ChIP analysis with a workflow.

### To combine replicates (ChIP)

You can configure a workflow to combine intra array or interarray replicate probes, or both. This increases the statistical power of your analysis. For an explanation of the statistical model Agilent Genomic Workbench uses to combine replicates, see the *ChIP Interactive Analysis User Guide*.

Intra-array replicates are features within the same array that contain the same probe. Interarray replicates are features on different arrays that contain the same probe.

When you combine interarray replicates, you select an array attribute. Agilent Genomic Workbench combines replicates from arrays with matching values for the selected attribute.

### To configure the analysis method to combine intra-array replicates

- In the Analysis Method Navigator, in Combining Replicates, mark **Intra-Array Replicates**.

### To configure the analysis method to combine interarray replicates

- 1 In the Analysis Method Navigator, in Combining Replicates, mark **Inter-Array Replicates**.

The Inter-Array Replicates Parameter Panel appears in the parameter panel. See [“Inter-Array Replicates Parameter Panel”](#) on page 150.

- 2 In Group By, select an array attribute. When you run the workflow, the program combines replicates from arrays with values that are the same as the selected attribute.

## To configure normalization methods (ChIP)

Normalization corrects microarray data for known factors that cause the reported signal intensities to be different from the actual signal. For a discussion of the statistical algorithms the program uses to normalize data, see the *ChIP Interactive Analysis User Guide*.

- In the Analysis Method Navigator, in Normalization, mark the kind(s) of normalization you want the program to apply when it runs the workflow. You can select either FE output *or* any combination of the others.

See the table below for a description of the method choices, and instructions on how to set the specific parameter(s) for each.

Normalization method	Description/Instructions
FE Output	<p>By default, the program uses the raw, unprocessed feature intensity data from the output files of your feature extraction program. If you use Agilent Feature Extraction, you can use the processed intensity data from the output file instead.</p> <ul style="list-style-type: none"><li>• No additional parameters are necessary.</li><li>• Array normalization within Agilent Genomic Workbench and from FE output are not compatible. If you select the FE Output option, the other options are unavailable. If you select any of the other normalizations, the FE Output option is unavailable.</li></ul>
Blank subtraction	<p>This kind of normalization corrects for non-specific binding. It first calculates the central tendency of the negative controls on the array for both the immunoprecipitated (IP) and whole cell extract (WCE) channels. It then subtracts these central tendencies from the raw signal intensities of each feature on the array.</p> <ul style="list-style-type: none"><li>• In the Analysis Method Navigator, in Normalization, mark the check box next to <b>Blank Subtraction</b>. The Median method is used to estimate central tendency of blank probes. No other selection is possible.</li></ul>

Normalization method	Description/Instructions
Inter-array Median	<p>This kind of normalization corrects for variations between replicate arrays. Agilent Genomic Workbench calculates and applies it separately for each channel. It first calculates the median signal intensity over the common probes in each replicate array. It then finds the average of these median intensities over all replicates of all arrays. For each array, it computes the ratio of its median signal intensity to the average of the median signal intensities of all arrays. Finally, it normalizes data by multiplying each signal intensity by the applicable ratio.</p> <ul style="list-style-type: none"> <li>In the Analysis Method Navigator, in Normalization, mark <b>Inter-array Median</b>.</li> </ul>
Dye-bias (intra-array) Median	<p>This kind of normalization corrects for dye bias within each array in an experiment, and it normalizes the intensities of the IP channel, to match the median of the WCE channel.</p> <ol style="list-style-type: none"> <li>In the Analysis Method Navigator, in Normalization, mark <b>Dye-bias (intra-array) Median</b>.</li> <li>In the parameter panel, in Normalize by, select the way that the program compensates for dye bias. <ul style="list-style-type: none"> <li>By equalizing central tendencies of IP and WCE channels – This method first calculates the ratio of the median IP signal intensity to the median WCE signal intensity. Then, it multiplies the signal intensities of the data probes by this ratio.</li> <li>By normalizing central tendency of log ratios to 1 – This method multiplies the signal intensities of all data probes on the array by a correction factor. This correction factor adjusts the central tendency of log ratios of the data probes on the array to 1.</li> </ul> </li> </ol> <p><b>Note:</b> If Dye-bias (intra-array) Median normalization is selected, Variance Stabilization normalization and Intra-array Lowess normalization are not available.</p>

**3**    **Setting up Workflow Analysis Methods**  
To configure normalization methods (ChIP)

Normalization method	Description/Instructions
Intra-Array Lowess Intensity Dependent	<p>Intra-array normalization attempts to correct for artifacts caused by nonlinear rates of dye incorporation, as well as inconsistencies in the relative fluorescence intensity between some red and green dyes. The Lowess normalization algorithm normalizes the channels within each array using a nonlinear polynomial fit to the data, and effectively normalizes by probes and by arrays.</p> <p><b>Note:</b> If you are using feature extraction data that have been normalized by the Lowess approach, you do not need to apply the intra-array Lowess normalization here.</p> <ol style="list-style-type: none"><li>1 In the Analysis Method Navigator, in Normalization, mark <b>Intra-Array Lowess (Intensity dependent) Normalization</b>.</li><li>2 In the parameter panel, select the data to be used to compute the regression curve.</li></ol> <p><b>Note:</b> If Intra-array Lowess normalization is selected, Dye-bias (intra-array) Median normalization and Variance Stabilization are not available.</p>
Variance Stabilization	<p>This normalization is useful for data that is either “blank-subtracted” or “spatially detrended” but it may have utility for data processed by other means as well. Variance stabilization is an alternative to Lowess normalization that fits a regression curve to signal intensities after applying an “arcsinh(x)” transform to each channel. This approach uses a two-parameter error model to compress the reported ratios of probes with weak signals after blank-subtraction. After the transform is applied, the variance of the reported log ratios should be independent of the signal strength.</p> <p><b>Note:</b> If you are using feature extraction data that have been normalized by the Lowess approach, you do not need to apply Variance Stabilization here.</p> <ol style="list-style-type: none"><li>1 In the Analysis Method Navigator, in Normalization, mark <b>Variance Stabilization</b>.</li><li>2 In the parameter panel, select how to fit the regression curve.</li></ol> <p><b>Note:</b> If Variance Stabilization normalization is selected, Dye-bias (intra-array) Median normalization and Intra-array Lowess normalization are not available.</p>

## To configure error model for analysis method (ChIP)

The goal of ChIP data analysis is to find the chromosomal locations where protein binding (or other events) occur. The error model calculates the likelihood that probes represent binding events, and assigns  $p$ -values to probes. A  $p$ -value close to 1 indicates that a probe is unlikely to represent a significant binding event. A very small  $p$ -value (for example,  $< 0.001$ ) indicates that the probe is very likely to represent one. Later, the program combines  $p$ -values from groups of probes to make binding calls.

- 1 In the Analysis Method Navigator, in Error Model, click the option button next to the desired error model.

Option	Description
FE error model	Uses the error model from the Agilent Feature Extraction program. Select this model if you have tried both models, and know this one provides a better match to biological truths and/or positive controls you have available for your experiment. If you select this model, the edit button becomes unavailable; you do not need to set any additional parameters.
Whitehead Error Model	The program uses this error model by default. Select this model unless you have tried both models and know the other one provides a better match to biological truths and/or positive controls you have available for your experiment. If you choose this model, the edit button becomes available, and you can set additional advanced parameters. For a description of the statistical algorithm used in this model, see the <i>ChIP Interactive Analysis User Guide</i> .

- 2 If you selected the Whitehead Error Model, you can set several advanced parameters that are optional. You can set them to optimize the statistical calculations of the error model with training data specific to your particular assay. Follow these steps:
  - a In the Analysis Method Navigator, mark the check box next to **Whitehead Error Model**.  
Parameters for the Whitehead Error Model appear in the parameter panel. See [“Whitehead Error Model”](#) on page 168.
  - b Set any of these parameters, described below:

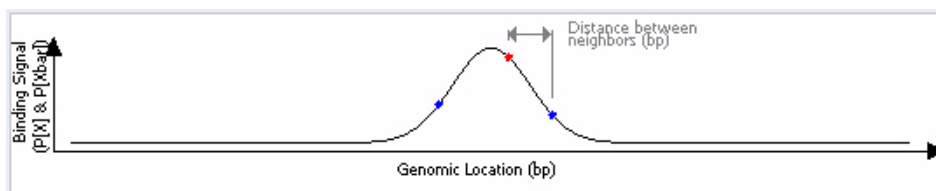
### 3 Setting up Workflow Analysis Methods

#### To configure error model for analysis method (ChIP)

Parameter	Comments/Instructions
Source of additive (intensity-dependent) error of each channel is:	<p>The choices for this parameter change the additive (intensity-dependent) component of the estimate of the error in IP – WCE.</p> <p>Select one of these sources from the list:</p> <ul style="list-style-type: none"><li>• Standard deviation of background pixels</li><li>• Additive error as computed by Agilent Feature Extractor</li><li>• Observed spread of negative controls</li></ul>
Custom defined f-value	<p>The f-value of one replicate of an array is the rate at which the multiplicative error increases with signal intensity. Normally, the ChIP module calculates f-values automatically, but you can define a custom value.</p> <p><b>1</b> Mark <b>Custom defined f-value</b>.</p> <p><b>2</b> In the box, type the desired f-value.</p>

## To configure peak detection and evaluation (ChIP)

The ChIP module uses two versions of the Whitehead Per-Array Neighbourhood Model to make binding calls. These models consider the  $p$ -values of both the probe in question and its neighbors. You can customize the parameters of the models, that include the maximum distance between neighbor probes, and the stringency of the detection process. The models consider probes in groups of three, shown in [Figure 10](#). In this figure, two neighbor probes (blue) are located before and after a central probe (red).



**Figure 10** Central probe flanked by two neighbor probes.

The program accepts the probe as “bound” if the  $p$ -value of the composite error-corrected ratio (“X”) of all three probes (“ $X_{\text{bar}}$ ”) is less than a set cut-off value, and if either of the following is true:

- The  $p$ -values for the central probe and at least one of its neighbors are less than set cut-off values.
- The  $p$ -value of one (or optionally, another number) of the neighbors of the central probe is less than a set cut-off value.

For a detailed description of the statistical calculations involved in event detection, see the *ChIP Interactive Analysis User Guide*.

# To set the parameters for the Whitehead per-array neighbourhood model

- 1 In the Analysis Method Navigator, in Peak Detection and Evaluation, click **Whitehead Per-Array Neighbourhood Model**.  
Parameters for the model appear in the parameter panel.
- 2 Set any of the parameters in the parameter panel. Default values appear for each parameter, but you can change them. Refer to [Table 7](#) for descriptions of each parameter.

**Table 7** Parameters for the Whitehead per-array neighbourhood model

Parameter	Comments
Maximum distance (in bp) for two probes to be considered as neighbors.	<ul style="list-style-type: none"><li>The program only considers probes to be neighbors if their genomic locations are within this threshold distance. The default value for this parameter is 1000 base pairs.</li><li>To change the value, delete the old value and type a new one in the box.</li></ul>
$P(X_{\text{bar}}) <$	<ul style="list-style-type: none"><li>This parameter refers to the <math>p</math>-value of the average error-corrected ratio ("X") of the central probe and its left and right neighbors. (The "bar" indicates the average.)</li><li>The default value is 0.001.</li><li>To make detection more stringent, decrease the value.</li></ul>
Central probe has $P(X) <$	<ul style="list-style-type: none"><li>The central probe is the red probe in <a href="#">Figure 10</a>.</li><li>The default value is 0.001.</li><li>To make detection more stringent, decrease the value.</li></ul>
At least one neighboring probe has $P(X) <$	<ul style="list-style-type: none"><li>Neighboring probes are probes to either side of the central probe. The blue probes in <a href="#">Figure 10</a> are the neighbors of the central (red) probe.</li><li>The default value is 0.1.</li><li>To make detection more stringent, decrease the value.</li></ul>
At least <b>n</b> of the neighbors has $P(X) <$	<ul style="list-style-type: none"><li>The default value for <b>n</b> is 1.</li><li>The default cut-off value for <math>P(X)</math> is 0.005.</li><li>To make detection more stringent, decrease the value.</li></ul>



## To set the parameters of the modified Whitehead per-array neighbourhood model

- 1 In the Analysis Method Navigator, in Peak Detection and Evaluation, click **Whitehead Per-Array Neighbourhood Model (Modified)**.

Parameters for the model appear in the parameter panel.

Set any of the parameters in the parameter panel. Default values appear for each parameter, but you can change them. Refer to [Table 8](#) for descriptions of each parameter.

**Table 8** Parameters for the Whitehead per-array neighbourhood model (modified)

Parameter	Comments
Maximum distance (in bp) for two probes to be considered as neighbors.	<ul style="list-style-type: none"> <li>The program only considers probes to be neighbors if their genomic locations are within this threshold distance. The default value for this parameter is 1000 base pairs.</li> <li>To change the value, delete the old value and type a new one in the box.</li> </ul>
A probe is considered <b>bound</b> if: $P(X_{\text{bar}}) <$	<ul style="list-style-type: none"> <li>This parameter refers to the <math>p</math>-value of the average error-corrected ratio ("<math>X</math>") of the central probe and its left and right neighbors. (The "<math>\text{bar}</math>" indicates the average.)</li> <li>The default value is 0.001.</li> <li>To make detection more stringent, decrease the value.</li> </ul>
Central probe has $P(X) <$	<ul style="list-style-type: none"> <li>The central probe is the red probe in <a href="#">Figure 10</a>.</li> <li>The default value is 0.001.</li> <li>To make detection more stringent, decrease the value.</li> </ul>
At least one neighboring probe has $P(X) <$	<ul style="list-style-type: none"> <li>Neighboring probes are probes to either side of the central probe. The blue probes in <a href="#">Figure 10</a> are the neighbors of the central (red) probe.</li> <li>The default value is 0.1.</li> <li>To make detection more stringent, decrease the value.</li> </ul>
At least <b>n</b> of the neighbors has $P(X) <$	<ul style="list-style-type: none"> <li>The default value for <b>n</b> is 2.</li> <li>The default cut-off value for <math>P(X)</math> is 0.005.</li> <li>To make detection more stringent, decrease the value.</li> </ul>

# To set the parameters for the predefined peak shape detection algorithm v2.1

This peak detection algorithm “slides” a peak shape through the data, and searches for good fits. The peak shape is computed from the estimated mean and standard deviation of DNA lengths of the shear distribution, and the “significance” of a potential fit is judged by comparing it to fits on randomized data, with a nonparametric (rank-significance) test.

For each peak that satisfies the nonparametric test, a “score” is computed by testing the quality of the fit under the assumption of an extreme value distribution of the qualities of the fits to randomized data. The significance derived from this test is converted to a score by computing  $-\log_{10}(\text{significance})$  for the peak fit.

This algorithm assumes that:

- You are trying to detect relatively rare events that occur over small genomic intervals (for example, transcription factor binding.)
- Genomic regions to be analyzed must be covered by stretches of several consecutive probes. (It will not work well with Agilent 44k “Proximal Promoter 2-set” designs.)
- Probes should be spaced fewer than 300 bp apart (depending on your shear distribution).

**Table 9** Parameters for predefined peak shape detection algorithm v2.1

Parameter	Comments
<b>Thresholds</b>	
<i>p</i> -value threshold	Maximum threshold for the nonparametric test for reporting peaks. Value must be greater than 1/number of randomized runs. (Increase the value to find more peaks.)
Score threshold	Minimum threshold for extreme value distribution (EVD)-based score. (Decrease value to find more peaks.)
<b>Peak Shape Parameters</b>	
Estimated mean shear length distribution of sample DNA	Type a mean to be used in the gamma distribution calculation for approximation of the distribution of sheared DNA fragments.

**Table 9** Parameters for predefined peak shape detection algorithm v2.1

Parameter	Comments
Estimated standard deviation of the shear length distribution of sample DNA	Type a standard deviation to be used in the gamma distribution calculation of the distribution of sheared DNA fragments.
<b>Other Algorithmic Parameters</b>	
Precision of peak placement on the chromosome (in base pairs)	This is the window within which the algorithm searches for potential positions for the peak center. When you decrease this window, the time it takes for the algorithm to run increases.
Number of randomizations for determination of peak significance (via nonparametric test) and score	The program computes <i>p</i> -value and peak score through a number of random samplings. Increase the number of samples to increase the accuracy of the prediction; however this also increases the time to do the calculation.
Window size (in bp) for computing local baseline.	Use smaller number for smaller genomes.
Desired spacing of interpolated data points between probe.	Must be less than or equal to probe spacing on the array.
Automatically re-run calculation after learning peak shape?	Selection increases accuracy, but will double the runtime.
Use errors estimated by Error model?	Select to use the estimated error for each probe to weight its contribution to the peak fit measurement.

## To define output experiment (ChIP)

When you run a workflow, the program creates an Agilent Genomic Workbench experiment that you can display in interactive mode.

- 1 In the Analysis Method Navigator, in Output, click **Experiment**.

Two parameters appear in the parameter panel. See “[Select Experiment Parameter Panel](#)” on page 162. By default the program names the experiment with the name of analysis method.

- 2 If desired, type a new name for the experiment. The name must not already exist in Agilent Genomic Workbench.
- 3 In Description, type an optional description to associate with the experiment.

### **3    Setting up Workflow Analysis Methods**

To set the parameters for the predefined peak shape detection algorithm v2.1



## 4 Workflow Reference

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This chapter describes the commands, tabs, views, and parameter panels specific to Agilent Genomic Workbench Workflow.

A *workflow* is a sequence setup, or roadmap, to automatically run an analysis with selected data input and output. An *analysis method* is a method you set up to run in the workflow. The analysis method contains parameters you select to prepare the data for event detection, detect events and generate reports using the CGH or ChIP licensed applications.

If you also have an Agilent Feature Extraction license, you can set up to use image files in the workflow, which can be extracted and then analyzed when the workflow is run, all without intervention.



# Main Window

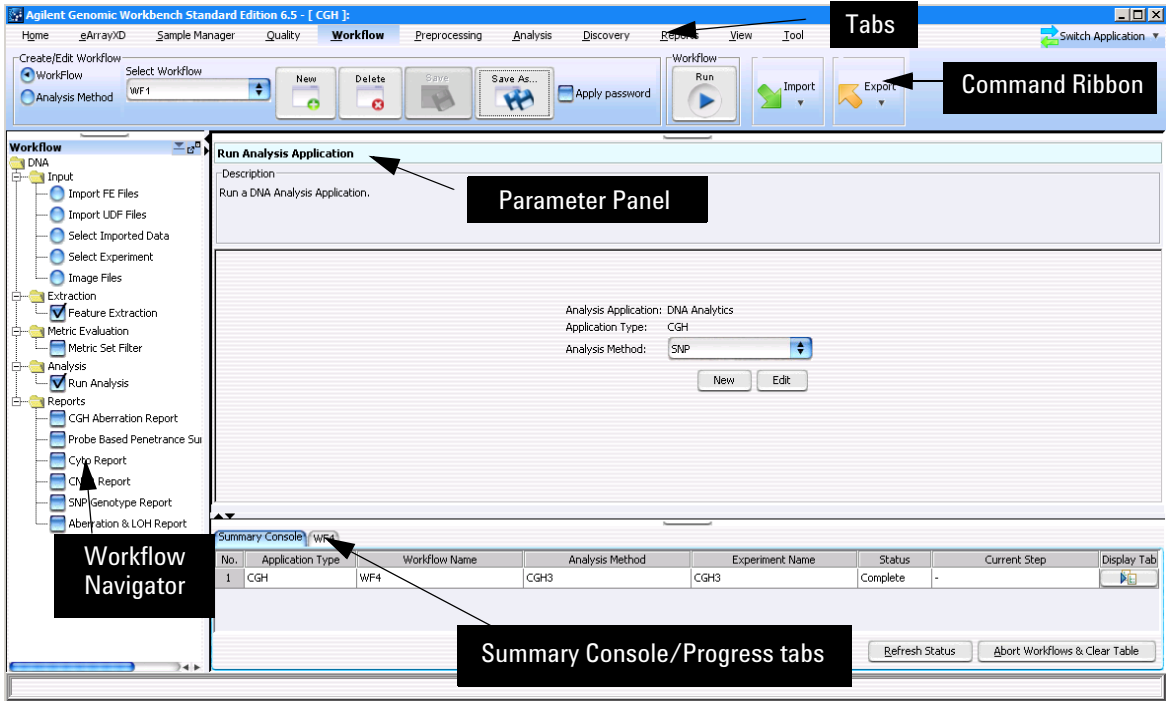


Figure 11 Workflow main window

Figure 11 shows the main window when the Workflow tab is selected. This window contains the elements listed in Table 10 below. More detailed descriptions of each element appear later in this section.

**Table 10** Workflow – Main Window Elements

Element	Purpose
Tabs	Used to change functional areas of Agilent Genomic Workbench. The tab menu also contains the Switch Application command, where you change to another licensed application (CGH/ChIP/CH3).
Command Ribbon	When Workflow is selected, contains the commands used to create, save, delete, and run a workflow, and to set locations for Feature Extraction. When Analysis Method is selected, contains the commands to create, save, edit, and delete an analysis method.
Workflow Navigator	Displays current workflow and options. As you select each option, the relevant parameters appear in the parameter panel.
Parameter Panel	Displays parameters for the selection in the Workflow Navigator.
Summary Console/ Progress View	For Workflow, this view contains the following tabs: <ul style="list-style-type: none"><li>• Summary Console tab that displays the application type, workflow name, experiment name, status, and current step for each workflow completed or in process.</li><li>• Workflow Progress tab for each named workflow, that displays the status of the workflow progress, including any errors that might have occurred.</li></ul>

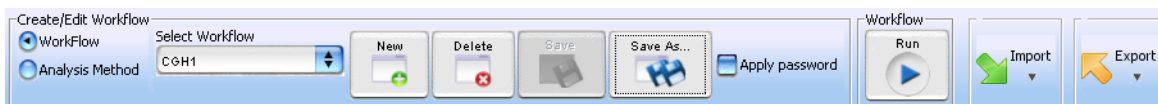
**NOTE**

In Workflow, the program only lets you set up workflow procedural steps, and not explore the data. You must switch to one of the other interactive tabs to review data and perform further analysis.

## Workflow Command Ribbons

The Workflow commands are divided into two groups, located at the top of the window directly below the tabs. The Create/Edit Workflow commands on this ribbon change when you select **Workflow** or **Analysis Method**.

### Command ribbon for Workflow



**Figure 12** Command ribbon for Workflow

The commands in this ribbon are described in the following table.

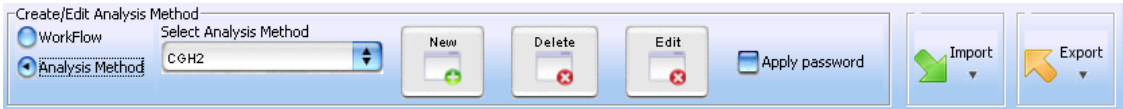
**Table 11** Commands for Workflow command ribbon

Command	Purpose
Workflow/Analysis Method	Select to change the command ribbon for Workflow or Analysis Method.
Select Workflow	Shows the existing workflows in the program. To use an existing workflow, select one from this list.
New	Opens the Create Run Workflow dialog box. To create a new workflow, type a name for the workflow in Enter Workflow Name, then click <b>OK</b> .
Delete	(Available only if a workflow is selected.) Opens a Confirm dialog box that asks if you want to delete the workflow. To delete the current workflow from the program, click <b>Yes</b> .
Save	(Available only if there are unsaved changes in the selected workflow.) Saves the changes in the selected workflow. (Note: This does not save the workflow in a file. To save a workflow in a file, see <a href="#">“To export a workflow”</a> on page 63.



Command	Purpose
Save As	(Available only if workflow is selected.) Opens a Save As dialog box. To save the selected workflow with a new name, type the new name, then click <b>OK</b> . You must type a name that does not match an existing workflow or experiment.
Apply password	Lets you type your password for a password-protected workflow.
Workflow Run	Starts the workflow.
Import	Lets you select to import workflow(s) or analysis method(s).
Export	Lets you select to export workflow(s) or analysis method(s).

## Command ribbon for Analysis Method



**Figure 13** Command ribbon for Analysis Method

The commands in this ribbon are described in the following table.

**Table 12** Commands for Analysis Method command ribbon

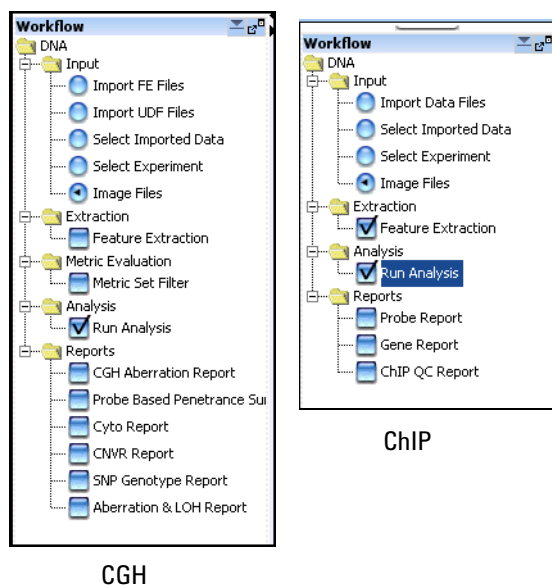
Command	Purpose
Workflow/Analysis Method	Select to change the command ribbon for Workflow or Analysis Method.
Select Analysis Method	Shows the existing analysis methods in the program. To use an existing analysis method, select one from this list.
New	Opens the Create Analysis Method dialog box. To create a new analysis method, type a name for the analysis method in Enter Analysis Method Name, then click <b>OK</b> . The Analysis Method window opens, where you select parameters for the analysis method.
Delete	(Available only if an analysis method is selected.) Opens a Confirm dialog box that asks if you want to delete the analysis method. To delete the selected analysis method, click <b>Yes</b> .
Edit	Opens the Analysis Method window. Select the analysis method to edit and change the parameters. Click <b>Save</b> to save the changes in the selected analysis method. Click <b>Save As</b> to save the changes with a new analysis method name.
Apply password	Lets you type your password for a password-protected analysis method.
Import	Lets you select to import workflow(s) or analysis method(s).
Export	Lets you select to export workflow(s) or analysis method(s).

## Workflow Navigators

The Workflow application has two Navigators: the Run Workflow Navigator and the Analysis Method Navigator. The Run Workflow Navigator is always displayed at the left side of the main Workflow window. The Analysis Method Navigator is displayed in the Analysis Method window when you create or edit an analysis method. The Navigators contain different information, depending on whether you select the CGH or ChIP application.

### Workflow Navigators for CGH and ChIP

When CGH or ChIP is selected as the analysis application, the Workflow Navigator changes to look like [Figure 14](#).



**Figure 14** Workflow Navigator for CGH and ChIP

To select any workflow option, click the option button or mark the check box next to its name. To display the parameters for a workflow option without changing its selection status, click the name of the option.

Input

In Input, you select the source of data for the workflow. See “[To select workflow input](#)” on page 46. Select one of the input options:

**Table 13** Input for CGH

Option	Description
Import FE Files	<p>Configures the workflow to import Agilent Feature Extraction microarray data files. When you select this option, the Import Data Files Parameter Panel appears. Use this pane to select data files for import. See “<a href="#">Import Data Files Parameter Panel</a>” on page 144. To use this option, you must have already imported the representative design files into the program. See the <i>CGH Interactive Analysis User Guide</i> for more information.</p> <p>In Workflow mode, the CGH application supports the import of Agilent and UDF microarray data files only. To use Axon files in a workflow, first import them in Interactive mode. For more information, see the <i>CGH User Guide</i>. Then in Workflow mode, use the Select Imported Data option in the Workflow Navigator to select them.</p>
Import UDF Files	<p>Configures the workflow to import UDF microarray data files. When you select this option, the Import UDF Data Files Parameter Panel appears. Use this pane to select UDF data files for import and to identify their columns. See “<a href="#">Import UDF Files Parameter Panel</a>” on page 148.</p>
Select Imported Data	<p>Configures the workflow to use microarray data that you have previously imported into the CGH application. When you select this option, the Select Imported Data Parameter Panel appears. Use this tab to select previously imported arrays for the workflow. See “<a href="#">Select Imported Data Parameter Panel</a>” on page 163.</p>

**Table 13** Input for CGH

Option	Description
Select Experiment	<p>Configures the workflow to use microarray data from an existing CGH experiment. When you select this option, the Select Experiment Parameter Panel appears in the Parameters tab. Use this tab to select the desired experiment. See <a href="#">“Experiment Parameter Panel”</a> on page 137.</p> <p>When you run the workflow, the program creates a new experiment with the same name as the workflow. The original experiment is unchanged. To customize the name of the new experiment, click <b>Experiment</b> in the Output folder of the Workflow Navigator.</p>
Image Files	<p>If you select this option, you must also select Feature Extraction. This option lets you select the image files to extract during the Feature Extraction workflow. See <a href="#">“Import FE Image Files Parameter Panel”</a> on page 146.</p>

**Table 14** Input for ChIP

Option	Description
Import Data Files	<p>Configures the workflow to import ChIP microarray data files. When you select this option, the Import Data Files Parameter Panel appears in the parameter panel. Use this panel to select data files for import. See <a href="#">“Import Data Files Parameter Panel”</a> on page 144. To use this option, you must have already imported the representative design files into the program.</p> <p>In Workflow mode, the ChIP module supports the import of Agilent microarray data files, only. To use Axon files in a workflow, first import them from the Genomic Viewer. See the <i>ChIP Interactive Analysis User Guide</i> for more information. Then in Workflow, use the Select Imported Data option in the Workflow Navigator to select them.</p>
Select Imported Data	<p>Configures the workflow to use ChIP microarray data that you previously imported into Agilent Genomic Workbench. When you select this option, the Select Imported Data Parameter Panel appears in the parameter panel. Use this tab to select previously imported arrays for the workflow. See <a href="#">“Select Imported Data Parameter Panel”</a> on page 163.</p>

**Table 14** Input for ChIP

Option	Description
Select Experiment	<p>Configures the workflow to use ChIP microarray data from an existing Agilent Genomic Workbench experiment. When you select this option, the Select Experiment Parameter Panel appears in the parameter panel. Use this tab to select the desired experiment. See <a href="#">“Select Experiment Parameter Panel”</a> on page 162.</p> <p>When you run the workflow, the program creates a new experiment with the same name as the workflow. The original experiment is unchanged. To customize the name of the new experiment, click <b>Experiment</b> in the Output folder of the Workflow Navigator.</p>
Image Files	<p>If you select this option, you must also select Feature Extraction. This option lets you select the image files to extract during the Feature Extraction workflow. See <a href="#">“Import FE Image Files Parameter Panel”</a> on page 146.</p>

## Feature Extraction

You can use the Workflow application to automatically perform Feature Extraction on a series of microarray images. You do not need a CGH or ChIP license to perform Feature Extraction using Workflow, however you must have the Feature Extraction license installed. You run Feature Extraction workflows from either the CGH or the ChIP Workflow Navigator, whether or not you have a license for CGH or ChIP. For Feature Extraction, the only workflow selections required are Input > Image Files and Extraction > Feature Extraction. If Image Files is selected as the Input for the workflow, you must also select Feature Extraction, whether or not you choose to do a CGH or ChIP analysis.

## Metric Evaluation

The Metric Set Filter option lets you include or exclude data from the workflow, based on QC metric set filters.

## Reports

The options in the Reports folder let you select the reports that are created by the workflow. The reports contain one or more files that you can open in other programs such as Microsoft® Excel or Adobe® Reader®.

Mark any of these options:

**Table 15** Report selections for CGH

Report	Description
CGH Aberration Report	<p>The CGH Aberration Report describes regions that have detected aberrations. You can report these regions by genomic interval, by probe, or both. The program reports aberrations separately for each array in the workflow, and creates one or more *.xls files that you can work with in Microsoft Excel.</p> <p>When you mark this option, the CGH Aberration Report Parameter Panel appears, where you can configure the report, and type a name and select a location for it. See <a href="#">“Variance Stabilization”</a> on page 167.</p>
Probe Based Penetrance Summary Report	<p>The Probe Based Penetrance Summary Report lists each probe with a significant aberration, and gives the percentage of selected arrays that show a significant deletion or amplification in the region for each probe. The workflow creates one or more *.xls files that you can work with in Microsoft Excel.</p> <p>When you mark this option, the Probe Based Penetrance Summary Report Parameter Panel appears, where you configure the report, and type a name and select a location for it. <a href="#">“Probe Based Penetrance Summary Report Parameter Panel”</a> on page 158.</p>
Cyto Report	<p>Cyto reports summarize analysis settings and detected aberrations by array. The workflow creates a separate PDF file for each array. When you mark this option, the Cyto Report Parameter Panel appears, where you select the desired cyto report template to use, and a location for the report. See <a href="#">“Cyto Report Parameter Panel”</a> on page 133.</p> <p>Note – You create Cyto Report templates in the Reports tab. See the <i>CGH Interactive Analysis User Guide</i>. Also, when you generate a Cyto Report in a workflow, the program uses the data analysis settings from the workflow, not from the selected Cyto Report template. The program uses only the format settings from the template.</p>
CNVR Report	<p>The CNVR report contains a list of the copy number variant regions (CNVRs) detected by the analysis, saved as an *.xls file. The report also contains a list of the parameters of the analysis. You can open this file in Microsoft Excel.</p> <p>When you mark this option, the CNVR Report Parameter Panel appears, where you can select a location for the report. You also provide the name for the CNVR node that appears in the results folder of the output experiment generated by the workflow. See <a href="#">“CNVR Report Parameter Panel”</a> on page 132.</p>



**Table 15** Report selections for CGH

Report	Description
SNP Genotype Report	<p>The SNP Genotype Report contains genotype and <i>p</i>-values for SNP probes. It generates reports in .xls format for the entire genome, or for each chromosome.</p> <p>When you mark this box, the SNP Genotype Report Parameter Panel appears, where you select the format and a location for the report. See <a href="#">“SNP Genotype Report Parameter Panel”</a> on page 166.</p>
Aberration & LOH Report	<p>The Aberration &amp; LOH Report contains aberration and log ratio information for significant intervals.</p> <p>When you mark this box, the Aberration &amp; LOH Report Parameter Panel appears, where you select the location for the report. See <a href="#">“Aberration &amp; LOH Report Parameter Panel”</a> on page 120.</p>

**Table 16** Report selections for ChIP

Option	Description
Probe Report	<p>The Probe Report contains information in tab-separated value (*.tsv) about the probes in the workflow’s arrays. You can use a spreadsheet program to open this file. A Probe Report contains one row for each probe in the array (or array set). See the <i>ChIP Interactive Analysis User Guide</i> for a description of the columns in the report.</p> <p>If you select this option, parameters for the report appear in the parameter panel. Select a location for the report. See <a href="#">“Probe Report Settings”</a> on page 159.</p>

**Table 16** Report selections for ChIP

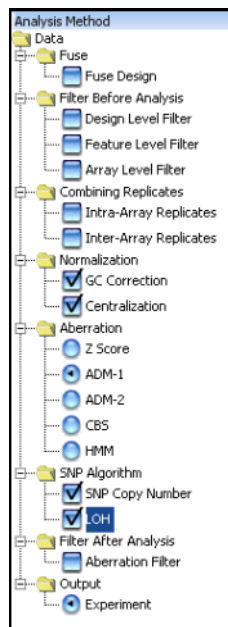
Option	Description
Gene Report	<p>The gene report contains one row for each probe in an array, grouped by the genes to which the probes bind. It is a tab-separated value (*.tsv) file that you open and analyze further with a spreadsheet program. For multiple arrays, the program creates a separate *.tsv file for each array. The program creates gene reports in several formats. See the <i>ChIP Interactive Analysis User Guide</i> for a description of the columns in each.</p> <p>If you select this option, parameters for the report appear in the parameter panel. You can select a location for the report, and customize its content. See <a href="#">“Gene Report Settings”</a> on page 142.</p>
ChIP QC Report	<p>The QC report summarizes the settings of the current analysis, and the overall statistics of each array. In addition to summary tables, it also includes plots that summarize the data graphically.</p> <p>The program creates the QC report in HTML format, and you can display the report with your Internet browser. For more information about the contents of the report, see the <i>ChIP Interactive Analysis User Guide</i>.</p> <p>If you select this option, a parameter for the report appears in the parameter panel. Select a location for the report. See <a href="#">“QC Report Settings”</a> on page 160.</p>

# Analysis Method Navigator

This section describes the options for CGH analysis methods, in the order in which they appear in the Analysis Method Navigator.

## Analysis Method Navigator for CGH

In Workflow mode, you use the Analysis Method Navigator to configure an analysis method. The Analysis Method Navigator is displayed in the Analysis Method window that appears when you create or edit an analysis method. As you select each option, you set the parameters for it as they appear in specific parameter panels. You can select different options and change parameter settings later.



**Figure 15** CGH Analysis Method Navigator with selections for SNP analysis

To select an analysis method option, click the option button or mark the check box next to its name. To display the parameters for an analysis method option without changing its selection status, click the name of the option.

**Fuse Design**

If you have two or more arrays that use different design files, you can combine them into one larger virtual array. This can make it easier to work with multiple array that are part of an array set. See [“To select to fuse designs \(CGH\)”](#) on page 71.

To fuse designs, mark **Fuse Design**. The Fuse Design Parameter Panel appears. See [“Fuse Design Parameter Panel”](#) on page 140.

**Filter Before Analysis**

The options available in the Filter Before Analysis folder let you select filters that remove or include data based on specific criteria. You can also create and edit these filters.

Mark any of these options:

Option	Description
Design Level Filter	Opens the Design Level Filter Parameter Panel. Design level filters let you include or exclude probes, based on criteria set in the filter. For example, a design filter can be used to filter out probes that fail the homology filter or have a low probe score. See <a href="#">“Design Level Filter Parameter Panel”</a> on page 134.
Feature Level Filter	Opens the Feature Level Filter Parameter Panel. See <a href="#">“Feature Level Filter Parameter Panel”</a> on page 138. Feature level filters let you include or exclude data from specific microarray features, based on information from the imported Feature Extraction output files.
Array Level Filter	Opens the Array Level Filter Parameter Panel. See <a href="#">“Array Level Filter Parameter Panel”</a> on page 126. Array level filters let you include or exclude arrays in the current experiment based on their attributes.

## Combining Replicates

For CGH arrays, intra-array replicates are features within the same array that contain the same probe. Interarray replicates are features on different arrays that contain the same probe. For expression arrays, replicate probes are probes correlated with the same gene. When you combine replicates, you define how the program handles replicate probes. The program can combine multiple biological and technical replicates within and among arrays.

Mark any of these options:

Option	Description
Intra-Array Replicates	Combines replicate probes within each array. If you mark this option, the Intra-Array Replicates Parameter Panel appears. However, no input parameters are required.
Inter-Array Replicates	Combines replicate probes within designated groups of arrays. When you mark this option, the Inter-Array Replicates Parameter Panel appears. Use this parameter panel to select the array attribute the program uses to group arrays when it combines interarray replicates. See <a href="#">“Inter-Array Replicates Parameter Panel”</a> on page 150.

## Normalization

Mark any of these options:

Option	Description
GC Correction	Corrects for artifacts by performing a regression fit to GC content in a specified region flanking the probes. GC Correction is required in order to perform SNP Copy Number and LOH analyses.
Centralization	Centralization recenters log ratio values. It finds a constant value to subtract from or add to all values, and ensures that the zero-point reflects the most-common-ploidy state. For a description of the centralization algorithm, see the <i>CGH Interactive Analysis User Guide</i> . All of the aberration algorithms can use the Centralization calculation, used to normalize data. Centralization is required in order to perform SNP Copy Number and LOH analyses.

**Aberration**

The options in the Aberration folder let you select the aberration detection algorithm for the workflow. For a detailed discussion of all aberration detection algorithms, see the *CGH Interactive Analysis User Guide*. Select one of these options:

Option	Description
Z Score	The Z-Score algorithm is a quick method of detecting aberrant regions. It assesses intervals using a sliding window of fixed size, and is especially useful in the exploratory phase of CGH data analysis. When you select this option, the Z Score Parameter Panel appears, where you can set the parameters of the algorithm. See <a href="#">“Z Score Parameter Panel”</a> on page 171. For a discussion of the Z-Score algorithm, see the <i>CGH Interactive Analysis User Guide</i> .
ADM-1	The ADM-1 algorithm searches for intervals in which the average log ratio of the sample and reference channels exceed a threshold that you specify. When you select this option, the ADM-1 Parameter Panel appears, where you can set the parameters of the algorithm. See <a href="#">“ADM-1 Parameter Panel”</a> on page 123. For a discussion of the ADM-1 algorithm, see the <i>CGH Interactive Analysis User Guide</i> .
ADM-2	The ADM-2 algorithm is similar to the ADM-1 algorithm, except that it is more accurate and also takes into account probe quality. It is especially useful for the detection of small aberrant intervals. When you select this option, the ADM-2 Parameter Panel appears, where you can set the parameters of the algorithm. See <a href="#">“ADM-2 Parameter Panel”</a> on page 124. For a discussion of the ADM-2 algorithm, see the <i>CGH Interactive Analysis User Guide</i> .

Option	Description
CBS	The CBS algorithm partitions probes into subsets that share the same copy number, to identify copy number change points. It is useful for putative aberration characterization, copy number estimates, and downstream analysis. You do not set any parameters for this algorithm. For a discussion of the CBS algorithm, see the <i>CGH Interactive Analysis User Guide</i> .
HMM	The Hidden Markov Model (HMM) algorithm identifies all aberrant intervals in a given sample based upon the individual likelihood of such signals in a genomic context. A Hidden Markov Model is a method to partition a large number of observations into a smaller number of (hidden) states. The HMM algorithm differs from other detection algorithms in that it identifies local probabilities in the data. See “ <a href="#">HMM Parameter Panel</a> ” on page 143. For more information on the HMM algorithm, see the <i>CGH Interactive Analysis User Guide</i> .

## SNP Algorithm

These options let you set up the workflow to calculate results for CGH arrays that contain SNP probes. Mark one or both of the following options:

Option	Description
SNP Copy Number	For each SNP site that is represented on the array, SNP Copy Number (ASCN algorithm) calculates an expectation value for the copy number of the uncut SNP allele. See “ <a href="#">SNP Copy Number Parameter Panel</a> ” on page 165. For more information on the SNP Copy Number algorithm, see the <i>CGH Interactive Analysis User Guide</i> . <b>Note:</b> In order to select SNP Copy Number, you must first select GC Correction, Centralization, and an Aberration (except z-score or HMM.)
LOH	LOH algorithm identifies copy-neutral genomic regions with a statistically significant scarcity of heterozygous SNP calls. The algorithm reports the regions where the LOH score exceeds a definable threshold. See “ <a href="#">LOH Parameter Panel</a> ” on page 153. For more information on the LOH algorithm, see the <i>CGH Interactive Analysis User Guide</i> . <b>Note:</b> In order to select LOH, you must first select SNP Copy Number.

### **Filter After Analysis**

Aberration filters exclude certain detected aberrations from the output of the workflow, based on specific criteria. To apply an aberration filter to the results of the workflow, mark Aberration Filter. The Aberration Filter Parameter Panel appears, where you can select a filter. See [“Aberration Filter Parameter Panel”](#) on page 121. You create and edit aberration filters in the analysis. See [“To select to filter the results after analysis \(CGH\)”](#) on page 78.

### **Output**

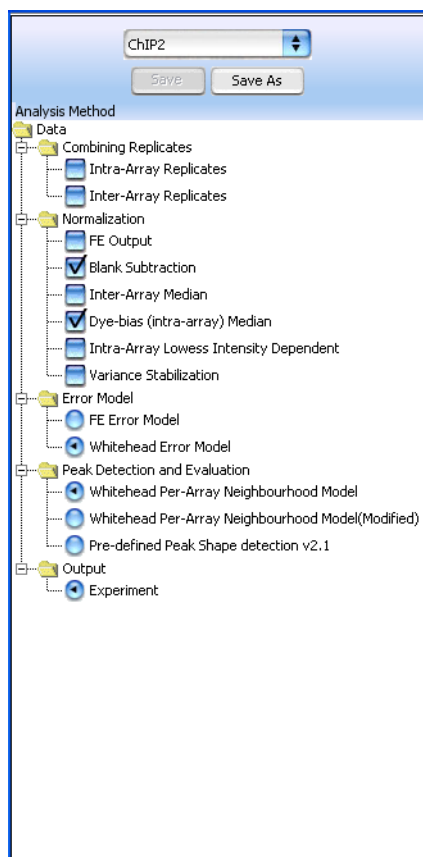
When you run a workflow, the program creates a new experiment that you can display in the interactive tabs. By default, the program uses the name of the workflow as the name of the new experiment.

The program always creates an output experiment when it successfully runs a workflow. To customize the name or description of the experiment, click **Experiment** in the Analysis Method Navigator. The Experiment Parameter Panel appears. See [“Experiment Parameter Panel”](#) on page 137.



## Analysis Method Navigator for ChIP

The Analysis Method Navigator is used to set or edit the ChIP analysis method parameters. As you select each option, you set the parameters for it as they appear in the Parameters Panel. You can select different options and change parameter settings later.



**Figure 16** The Analysis Method Navigator for ChIP

To select any analysis method option, click the option button or mark the check box next to its name. To edit the parameters for an option without changing its selection status, click the name of the option.

This section describes the options for ChIP analysis methods in the order in which they appear in the Analysis Method Navigator.

### Combining Replicates

In the ChIP application, replicate probes are probes that have the same probe name. When you combine replicates, you define how the program handles replicate probes. The program can combine multiple biological and technical replicates within and among arrays. See the *ChIP Interactive Analysis User Guide* for a discussion of the statistical algorithm the program uses to combine replicate probe data.

Mark any of these options:

Option	Description
Intra-Array Replicates	Combines replicate probes within each array. If you mark this option, you do not need to set any additional parameters for it.
Inter-Array Replicates	Combines replicate probes within designated groups of arrays. When you mark this option, the Inter-Array Replicates Parameter Panel appears in the parameter panel. Use this tab to select the array attribute the program uses to group arrays when it combines interarray replicates. See <a href="#">“Inter-Array Replicates Parameter Panel”</a> on page 150.

### Normalization

Normalization corrects data for known factors that cause measured values to deviate from their “true” values.

For ChIP analysis, select any of these options:

Option	Description
FE Output	<p>Mark <b>FE Output</b> to use the processed feature intensity values in the output files of the Agilent Feature Extraction program. The ChIP program uses these values instead of applying its own normalization methods. Normally, the program uses the raw, unprocessed feature intensities.</p> <p>If you use processed FE output instead of applying normalization within the ChIP program, you do not need to set any additional normalization parameters.</p>
Blank Subtraction	<p>This kind of normalization corrects for nonspecific binding. It first calculates the central tendency of the negative controls on the array for both the immunoprecipitated (IP) and whole cell extract (WCE) channels. It then subtracts these central tendencies from the raw signal intensities of each feature on the array. As with all of the normalization methods, if the method causes a probe to have a negative value for intensity, it flags the probe as <i>excluded</i>. See the <i>ChIP Interactive Analysis User Guide</i> for more information.</p> <p>If you mark this type of normalization, central tendencies are calculated using the median of the negative control probes as a baseline. See <a href="#">“Blank Subtraction Normalization”</a> on page 128.</p>
Dye-bias (intra-array) Median	<p>This kind of normalization corrects for dye bias within each array in the workflow, and it normalizes only the intensities of the IP channel. See the <i>ChIP Interactive Analysis User Guide</i> for more information.</p> <p>If you mark this type of normalization, the parameters for it appear in the parameter panel. Use this tab to select the way the program calculates the dye bias, and also how the program calculates central tendencies. See <a href="#">“Dye-bias (Intra-Array) Median Normalization”</a> on page 136.</p>
Inter-Array Median	<p>This kind of normalization corrects for variations from one replicate array to another. The program calculates and applies the normalization separately for each channel. It first calculates the median signal intensity over the common probes in each replicate array. It then finds the average of these median intensities over all replicates of all arrays. For each array, it computes the ratio of its median signal intensity to the average of the median signal intensities of all arrays. Finally, it normalizes data by multiplying each signal intensity by the applicable ratio. See <a href="#">“Inter-Array Median Normalization”</a> on page 151.</p>

Option	Description
Intra-Array Lowess Intensity Dependent	Intra-array normalization attempts to correct for artifacts caused by nonlinear rates of dye incorporation, as well as inconsistencies in the relative fluorescence intensity between some red and green dyes. The Lowess normalization algorithm normalizes the channels within each array using a nonlinear polynomial fit to the data, and effectively normalizes by probes and by arrays. See <a href="#">“Intra-Array Lowess (Intensity Dependent) Normalization”</a> on page 152.
Variance Stabilization	This normalization is useful for data that is either “blank-subtracted” or “spatially detrended” but it may have utility for data processed by other means as well. Variance stabilization is an alternative to Lowess normalization that fits a regression curve to signal intensities after applying an “asinh(x)” transform to each channel. This approach uses a two-parameter error model to compress the reported ratios of probes with weak signals after blank-subtraction. After the transform is applied, the variance of the reported log ratios should be independent of the signal strength. See <a href="#">“Variance Stabilization”</a> on page 167.

**Error Model**

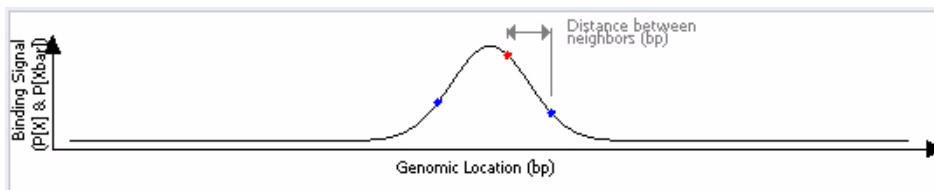
The error model calculates the likelihood that probes represent binding events, and assigns I-values to probes. A *p*-value close to 1 indicates that a probe is unlikely to represent a significant binding event. A very small *p*-value (for example, *p* < 0.001) indicates that the probe is very likely to represent a significant binding event.

Select one of these error models:

Option	Description
FE Error Model	Uses the error model from the Agilent Feature Extraction program. Select this model if you have tried both models, and know this one provides a better match to biological truths and/or positive controls you have available for your experiment. If you select this model, the edit button becomes unavailable; you do not need to set any additional parameters. For more information, see the <i>Agilent Feature Extraction Software Reference Guide</i> .
Whitehead Error Model	The program uses this error model by default. Select this model unless you have tried both models and know the FE Error Model provides a better match to biological truths and/or positive controls you have available for your experiment. If you choose this model, the edit button becomes available, and you can set additional advanced parameters. For a description of the statistical algorithm used in this model, see the <i>ChIP Interactive Analysis User Guide</i> .

## Peak Detection and Evaluation

The ChIP program uses two versions of the Whitehead per-array neighbourhood model to make binding calls. These models consider the  $p$ -values of both the probe in question and its neighbors. You can customize the parameters of the models, including the maximum distance between neighbor probes, and the stringency of the detection process. The models consider probes in groups of three, shown in [Figure 17](#). In this figure, two neighbor probes (blue) flank a central probe (red).



**Figure 17** Central probe flanked by two neighbor probes.

The program accepts the probes as “bound” if the average  $p$ -values for all three probes is less than a set cut-off value, and if either of the following is true:

- The  $p$ -values for the central probe and at least one of its neighbors are less than set cut-off values.
- The  $p$ -value of one (or optionally, another number) of the neighbors of the central probe is less than a set cut-off value.

For a detailed description of the statistical calculations involved in event detection, see the *ChIP Interactive Analysis User Guide*.

You can customize the settings of the model. In the Analysis Method Navigator, click Whitehead per-array neighborhood model. The parameters of the model appear in the parameter panel, where you can edit them.

### **Predefined peak shape detection algorithm**

This peak detection algorithm “slides” a peak shape through the data searching for good fits. The peak shape is computed from the estimated mean and standard deviation of DNA lengths of the shear distribution, and the “significance” of a potential fit is judged by comparing it to fits on randomized data, using a nonparametric (rank-significance) test.

For each peak that satisfies the nonparametric test, a “score” is computed by testing the quality of the fit under the assumption of an extreme value distribution of the qualities of the fits to randomized data. The significance derived from this test is converted to a score by computing  $-\log_{10}(\text{significance})$  for the peak fit.

- If a probe is not inside any of the “peak” objects, then it is “not bound.” Nothing special is drawn for this probe, except a baseline at exactly a ratio of 1 (log ratio of zero).
- If a probe is inside a peak, then it gets the significance value and score value of the associated peak. If it is inside two overlapping peaks, it gets the values for the peak with the better score. Peaks are drawn by computing ratios from the collection of detected peaks.

### **Output**

When you run a workflow, the program creates a new Agilent Genomic Workbench experiment that you can display in the interactive tabs. By default, the program uses the name of the analysis method as the name of the experiment.

The program always creates an output experiment when it successfully runs a workflow. To customize the name or description of the experiment, click **Experiment** in the Analysis Method Navigator. The Experiment Parameter Panel appears. See “[Experiment Parameter Panel](#)” on page 137.

# Parameter Panels

As you select options for an analysis method in the Analysis Method Navigator, the parameters available for each option appear in specific parameter panels. If you do not need to set any parameters for a specific analysis method option, **No input parameters required** appears.

This section describes the parameter panels that appear in Workflow mode, organized alphabetically by name.

## Aberration & LOH Report Parameter Panel

Aberration & LOH Report Parameter Panel

Description

Report will be created at the specified location.

Select File Location

Browse...

☒ Overwrite if file exists

**Figure 18** Aberration & LOH Report Parameter Panel

**Purpose:** To set location for Aberration & LOH Report created by Workflow.

**To open:** In the Analysis Method Navigator for CGH, under Reports, mark **Aberration & LOH Report**

**Select File Location** Displays the location where the workflow saves the files. To select a location for the report, click **Browse**. An Open dialog box appears. Type a name and select a location for the report, then click **Open**.



**Overwrite if file exists** If you mark this option, the workflow deletes an existing file if it has the same name and location as a generated report.

## Aberration Filter Parameter Panel

**Figure 19** Aberration Filter Parameter Panel

**Purpose:** This parameter panel is used to create or edit aberration filters. Aberration filters exclude detected aberrations from the output of the workflow, based on selected criteria.

**To open:** The Aberration Filter Parameter Panel appears when you mark **Aberration Filter** under Filter After Analysis in the Analysis Method Navigator.

**Name** Select the name of the filter you want to use. To create a new aberration filter, click **New**.

**New** Opens an Input dialog box, where you can type a name for the new aberration filter. To accept the name, click **OK**. The program creates the filter, and adds the new name to the Name list.

<b>Update</b>	Saves any changes you make to the filter criteria.
<b>Reset</b>	Restores the values of the filter criteria to what they were before you made any changes to them.
<b>Delete</b>	Opens a Confirm dialog box that asks you if you want to delete the currently selected filter. To delete the filter, click <b>Yes</b> .
<b>Rename</b>	Opens an Input dialog box where you can type a new name for the filter. To accept the name, click <b>OK</b> .
<b>Minimum number of probes in region</b>	Type a whole number. The filter excludes putative aberrant regions that contain fewer probes than the number you type.
<b>Minimum absolute average log ratio for region</b>	Type a value. The filter excludes putative aberrant regions if the average log2 ratio within the region is less than the value you type.
<b>Maximum number of aberrant regions</b>	Type a whole number. For each microarray, the filter includes up to this number of aberrant regions that have the highest statistical significance.
<b>Percent penetrance per feature</b>	Type a value. The filter excludes putative aberrations that have less than the specified minimum percent penetrance across the set of selected arrays.

## ADM-1 Parameter Panel

**ADM-1 Parameter Panel**

**Description**  
ADM1 Aberration detection method.  
By default, Nesting Level is set to maximum integer value(2147483647), which means no nesting level filter will be applied. You can set it to any desired integer value between 1 and 2147483647.

**Threshold**  
6.0  
0.1 50

**Nesting Level**  
☒ Apply Nesting Filter  
2147483647  
Fuzzy Zero

**Figure 20** ADM-1 Parameter Panel

**Purpose:** This panel lets you set the parameters for the ADM-1 aberration detection algorithm. For more information on ADM-1, see the *CGH Interactive Analysis User Guide*.

**To open:** The ADM-1 Parameter Panel appears when you select **ADM-1** under Aberration in the Analysis Method Navigator.

**Threshold** Type a numerical value from 0.1 to 50, or use the slider to set a value. The threshold is the minimum ADM-1 score for the detection algorithm to consider a given genomic interval significant. In general, increase this value to make the detection process more stringent.

**Nesting Level** To apply a nesting-level filter, mark **Apply Nesting Filter**, then type a whole number from 0 to 2147483647 in the box.

The ADM-1 aberration detection algorithm reports nested aberrations; that is, aberrations within other aberrations. If you set the nesting level to 0, the program reports only the parent aberration, without any child (nested) aberrations. If you set it to 1, the programs reports the first level of child

aberrations. By default, the program sets the nesting-level filter to its maximum value, which essentially applies no filter. To make the filter more stringent, decrease the value.

**Fuzzy Zero** Mark **Fuzzy Zero** to apply Fuzzy Zero correction to detected aberrant intervals. This correction applies a “global error model” to the intervals, and can result in fewer errors in aberration calls. For more information on the fuzzy zero algorithm, see the *CGH Interactive Analysis User Guide*.

## ADM-2 Parameter Panel

**ADM-2 Parameter Panel**

Description

ADM2 Aberration detection method.  
By default, Nesting Level is set to maximum integer value(2147483647), which means no nesting level filter will be applied. You can set it to any desired integer value between 1 and 2147483647.

**Threshold**

5.0

0.1 50

**Nesting Level**

☒ Apply Nesting Filter

2147483647

☐ Fuzzy Zero

**Figure 21** ADM-2 Parameter Panel

**Purpose:** This parameter panel lets you set the parameters for the ADM-2 aberration detection algorithm. For more information on ADM-2, see the *CGH Interactive Analysis User Guide*.

**To open:** The ADM-2 Parameter Panel appears when you select **ADM-2** under Aberration in the Analysis Method Navigator.

- Threshold** Type a numerical value from 0.1 to 50, or use the slider to set a value. The threshold is the minimum ADM-2 score for the detection algorithm to consider a given genomic interval significant. In general, increase this value to make the detection process more stringent.
- Nesting Level** To apply a nesting-level filter, mark **Apply Nesting Filter**, then type a whole number from 0 to 2147483647 in the box.
- The ADM-2 aberration detection algorithm reports nested aberrations; that is, aberrations within other aberrations. If you set the nesting level to 0, the program reports only the parent aberration, without any child (nested) aberrations. If you set it to 1, the programs reports the first level of child aberrations. By default, the program sets the nesting-level filter to its maximum value, which applies no filter. To make the filter more stringent, decrease the value.
- Fuzzy Zero** Mark **Fuzzy Zero** to apply Fuzzy Zero correction to detected aberrant intervals. This correction applies a “global error model” to the intervals, and can result in fewer aberration call errors. For more information on the fuzzy zero algorithm, see the *CGH Interactive Analysis User Guide*.

# Array Level Filter Parameter Panel

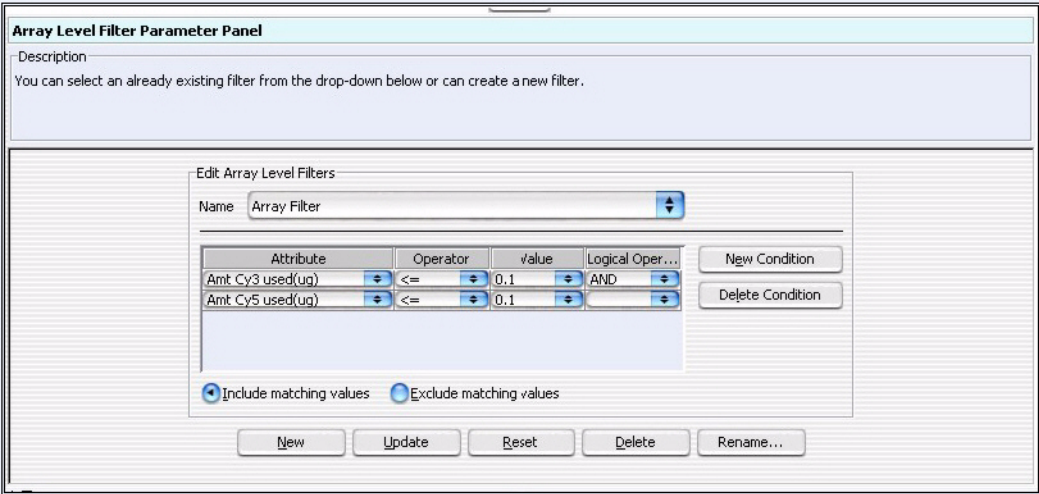


Figure 22 Array Level Filter Parameter Panel

**Purpose:** This parameter panel lets you create or edit array level filters. An array filter excludes arrays from the workflow based on selected criteria.

**To open:** The Array Filter Parameter Panel appears when you mark **Array Level Filter** in Filter Before Analysis in the Analysis Method Navigator.

**Name** Select the name of the array filter you want to edit. To create a new filter, and add its name to the list, click **New**.

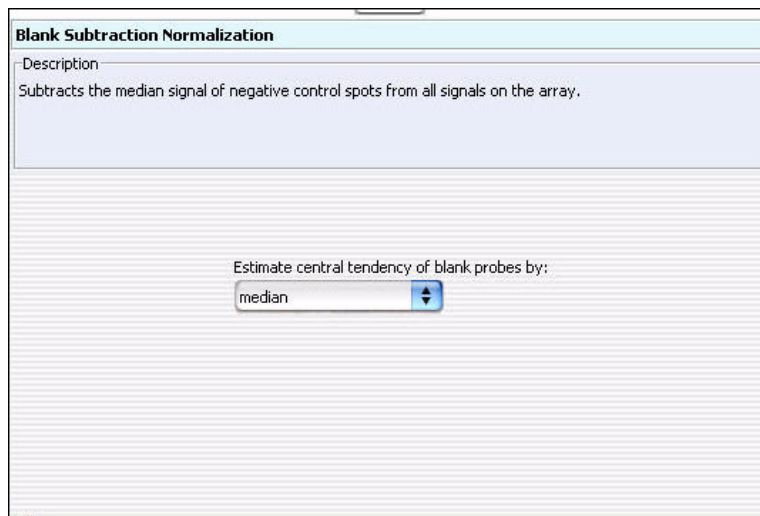
**Filter conditions table** Displays the conditions defined for the selected array level filter. When you create or edit the filter, for each condition (row), select options from the lists. In Value, select an option from the list, if available. Otherwise, type a value, then press **Enter**. To add another row to the table, click **New Condition**. Each condition has these elements:

- Attribute – The feature attribute evaluated by the filter
- Operator – How the filter uses the entry in Value to evaluate arrays. For example, the >= operator configures the filter to include or exclude features where the selected attribute is greater than or equal to the entry in Value.

- Value – The value the filter uses as the basis to evaluate a feature. For example, if you select the **gIsSaturated** attribute, the = operator, and a value of **true**, the filter includes or excludes a feature if its gIsSaturated attribute is true.
- Logical Operator – (Available only if you configure more than one criterion) The relationship between the criterion and the next one in the list. For example, if you select **AND** in Logical Operator for the first criterion, the filter includes or excludes an array if it passes both the first criterion and the next criterion.

<b>New Condition</b>	Adds a new, blank condition (row) to the table.
<b>Delete Condition</b>	Removes a condition from the list. To remove a condition, click anywhere within the condition row, then click <b>Delete Condition</b> .
<b>Include/Exclude matching values</b>	<p>Select one of these options:</p> <ul style="list-style-type: none"> <li>• Include matching values – If a feature passes the filter criteria, the program includes it in the analysis.</li> <li>• Exclude matching values – If a feature passes the filter criteria, the program excludes it from the analysis.</li> </ul>
<b>New</b>	Opens an Input dialog box, where you can type a name for the new filter. To accept the name, click <b>OK</b> . The program creates the filter, and adds the new name to the Name list.
<b>Update</b>	Saves any changes you make to the filter criteria without closing the dialog box.
<b>Reset</b>	Restores the values of the filter criteria to what they were before you made any changes to them.
<b>Delete</b>	Opens a Confirm dialog box that asks you if you want to delete the selected filter. To delete the filter, click <b>Yes</b> .
<b>Rename</b>	Opens an Input dialog box where you can type a new name for the filter. To accept the name, click <b>OK</b> .

## Blank Subtraction Normalization



**Figure 23** Blank Subtraction Normalization

**Purpose:** This parameter panel lets you select parameters for the Blank Subtraction normalization method for ChIP. For more information, see [“To configure normalization methods \(ChIP\)” on page 82](#).

**To open:** This parameter panel appears when you click (or mark the check box beside) **Blank Subtraction** under Normalization in the Analysis Method Navigator.

**Estimate central  
tendency of blank  
probes by**

Defines how the program calculates the central tendency of the negative control probes on an array. The only option for this selection is Median.



## Centralization Parameter Panel

The screenshot shows a software window titled "Centralization Parameter Panel". It features a "Description" label above a large, empty text box. Below this, there are two labeled input fields: "Centralization Threshold" with a value of "5.0" and "Centralization Bin Size" with a value of "10".

**Figure 24** Centralization Parameter Panel

**Purpose:** This parameter panel lets you set up centralization parameters. Centralization recenters log ratio values. It finds a constant value to subtract from or add to all values, and makes sure that the zero-point reflects the most-common-ploidy state. For a description of the centralization algorithm, see the *CGH Interactive Analysis User Guide*.

**To open:** The Centralization Parameter Panel appears when you mark **Centralization** in Normalization in the CGH Analysis Method Navigator.

### Centralization Threshold

This value is the ADM-1 threshold used to call aberrations for the centralization algorithm. Ideally, set this value to the ADM-1 threshold defined in the current analysis. However, because the centralization value is fairly robust over a wide range of threshold values, Agilent does not recommend that you change the default settings for this algorithm.

**Centralization Bin Size** Type a whole number. To improve performance, the algorithm finds the average of groups (or “bins”) of contiguous probes and runs the centralization process on these averages, rather than on individual probes. The centralization bin size is the number of probes that the program groups together for each of these averages. The default value is 10 probes.

**NOTE**

Centralization is required in order to perform SNP Copy Number and LOH analyses.

# CGH Aberration Summary Report Parameter Panel

**Figure 25** CGH Aberration Report Parameter Panel

**Purpose:** This parameter panel lets you configure the CGH Aberration Summary Report for CGH, and select a location for it. This report describes regions that have detected aberrations. You can report these regions by genomic interval, by probe, or both. The program reports aberrations separately for each array in the workflow, and creates one or more \*.xls files that you can work with in Microsoft Excel.

**To open:** The CGH Aberration Report Parameter Panel appears when you mark **CGH Aberration Report** in Reports in the CGH Workflow Navigator.

**Report Type** Configures the organization of reported aberrations. Select one of these options:

Option	Description
Probe Based	Creates a report that contains one line for each probe showing an aberration.
Interval Based	Creates a report that contains one line for each aberrant genomic interval.
Probe & Interval Based	Creates both a probe-based report and an interval-based report.

**Output Format**    Select one of these options:

Option	Description
Complete Genome	Creates a single report file for each requested report type.
Per-Chromosome	(Available only for probe-based reports) Creates a separate report file for each chromosome.

- Select File Location**

Displays the location to which the workflow saves the CGH Aberration Report. To select a location, click **Browse**. An Open dialog box appears. Select a location and type a name for the report, then click **Open**.
- Overwrite if file exists**

If you mark this option, the workflow deletes an existing file if it has the same name and location as a generated CGH Aberration Report.
- Report Flat Intervals**

Mark this to have aberration intervals reported without any nested structure.
- Generate report per array**

Mark this to generate a report after each microarray is analyzed. This lets you look at the results for individual microarrays before the workflow is completed.

# CNVR Report Parameter Panel

CNVR Report Parameter Panel

Description  
Report will be created at the specified location.

CNVR Node Name

Select File Location

Browse...

☒ Overwrite if file exists

Figure 26 CNVR Report Parameter Panel

**Purpose:** You use the CNVR Report Parameter Panel to select a location for the report. You also provide a name for the CNVR node that appears in the results folder of the output experiment generated by the workflow. A CNVR report contains a list of the copy number variant regions (CNVRs) detected by an analysis, saved as an \*.xls file. The report also contains a list of the parameters of the analysis. You can open this file in Microsoft Excel.

**To open:** The CNVR Report Parameter Panel appears when you mark **CNVR Report** in Reports in the CGH Workflow Navigator.

<b>CNVR Node Name</b>	Type a name. If you mark <b>CNVR Report</b> in the Workflow Navigator, the results folder of the output experiment of the workflow will contain a CNVR node with the name that you typed.
<b>Select File Location</b>	The location where the workflow saves the generated CNVR report file. Click <b>Browse</b> . The Select report folder dialog box opens, where you can type a name and select a location for the CNVR report file.
<b>Overwrite if file exists</b>	If you mark this option, the workflow deletes an existing file if it has the same name and location as the generated CNVR report.

## Cyto Report Parameter Panel

**Figure 27** Cyto Report Parameter Panel

**Purpose:** This panel lets you select the Cyto Report template to use, and a location for the report. Cyto Reports summarize analysis settings and detected aberrations by array. The workflow creates a separate PDF file for each array.

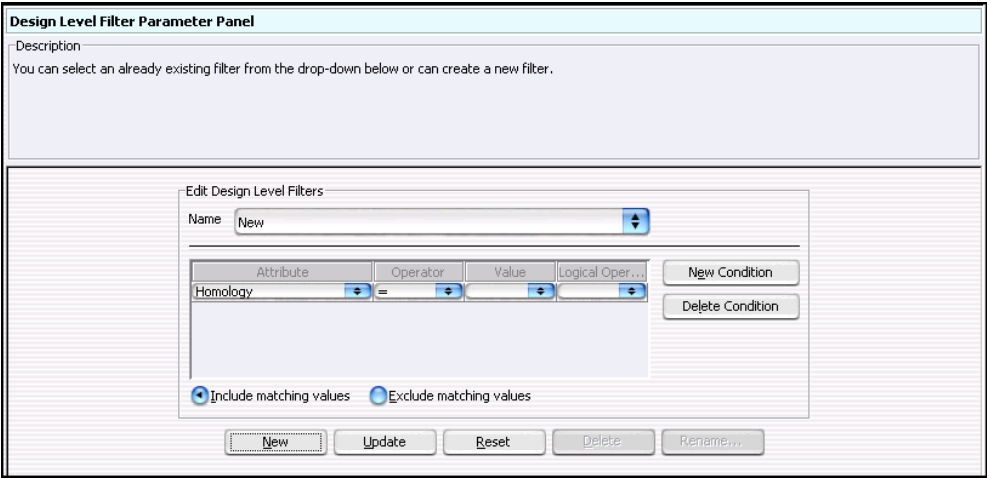
**To open:** The Cyto Report Parameter Panel appears when you mark **Cyto Report** in Reports in the CGH Workflow Navigator.

**Select Report** Select the desired Cyto Report template. You create and edit Cyto Report templates in the Reports tab. See the *CGH Interactive Analysis User Guide* for more information. Also, when you generate a Cyto Report in a workflow, the program uses the data analysis settings from the workflow, not from the selected Cyto Report template. The program uses only the format settings from the template.

**Select File Location** Click **Browse**. The Select report folder dialog box appears, where you can type a name and select a location for the Cyto Report file(s). The workflow creates a new folder that contains all of the files.

**Overwrite if file exists** If you mark this option, the workflow deletes an existing file if it has the same name and location as the generated Cyto Report.

## Design Level Filter Parameter Panel



**Figure 28** Design Level Filter Parameter Panel

**Purpose:** This parameter panel is used to create or edit design filters that let you include or exclude data from analysis based on selected design attributes.

**To open:** In the Analysis Method Navigator, under Filter Before Analysis, mark the box next to **Design Level Filter**.

**Name** Select the name of the design filter you want to edit. To create a new filter, and add its name to the list, click **New**.

**Filter Conditions table** For each criterion (row), select options from the list or type a value, then press **Enter**. To add another row to the table, click **New Condition**.

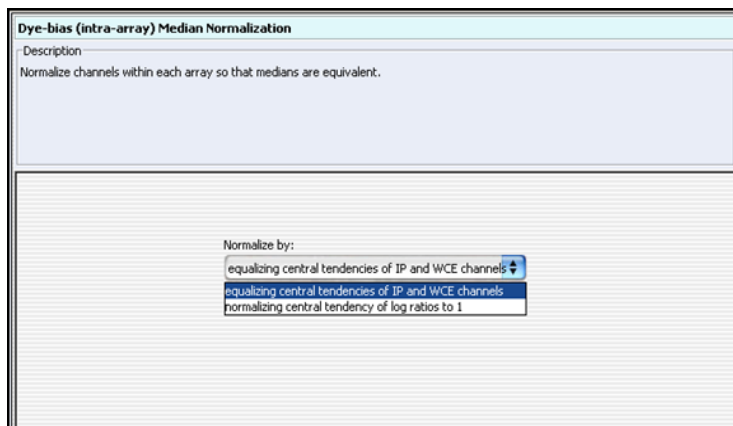
Each criterion has these elements:

- **Attribute** – The design attribute evaluated by the filter.
- **Operator** – How the filter uses the entry in Value to evaluate arrays. For example, the  $\geq$  operator configures the filter to include or exclude features where the selected attribute is greater than or equal to the entry in Value.

- **Value** – The value the filter uses as the basis to evaluate a feature. You either select a value or, for some attributes, type a value. For example, if you select the **Homology** attribute with an operator of =, the filter will include or exclude probes that exhibit homology if the value is set to **1**. (The homology attribute is assigned to probes that have more than one mapping in the genome or probes that have secondary hits that are not perfect matches.)
- **Logical Operator** – (Available only if you configure more than one criterion) The relationship between the criterion and the next one in the list. For example, if you select **AND** in Logical Operator for the first criterion, the filter includes or excludes an array if it passes both the first condition and the next condition.

<b>New Condition</b>	Adds a new, blank condition (row) to the table.
<b>Delete Condition</b>	Removes a condition from the list. To remove a specific condition, click anywhere within the condition, then click <b>Delete Condition</b> .
<b>Include/Exclude matching values</b>	<p>Select one of these options:</p> <ul style="list-style-type: none"> <li>• <b>Include matching values</b> – If a feature passes the filter condition, the program <i>includes</i> it in the analysis.</li> <li>• <b>Exclude matching values</b> – If a feature passes the filter condition, the program <i>excludes</i> it from the analysis.</li> </ul>
<b>New</b>	Opens an Input dialog box, where you can type a name for the new filter. To accept the name, click <b>OK</b> . The program creates the filter, and adds the new name to the Name list.
<b>Update</b>	Saves any changes you make to the filter conditions without closing the dialog box.
<b>Reset</b>	Restores the values of the filter conditions to what they were before you made any changes to them.
<b>Delete</b>	Opens a Confirm dialog box that asks you if you want to delete the selected filter. To delete the filter, click <b>Yes</b> .
<b>Rename</b>	Opens an Input dialog box where you can type a new name for the filter. To accept the name, click <b>OK</b> .

## Dye-bias (Intra-Array) Median Normalization



**Figure 29** Dye-bias (intra-array) Median Normalization

**Purpose:** This parameter panel lets you configure the Dye-Bias (intra-array) Median Normalization method for ChIP.

**To open:** These parameters appear in the parameter panel when you click **Dye-bias (intra-array) Median** under Normalization in the Analysis Method Navigator. See [“To configure normalization methods \(ChIP\)”](#) on page 82.

**Normalize by** Defines how the program computes the dye bias when it applies this kind of normalization. Select one of these options:

- **By equalizing central tendencies of IP and WCE channels** – This method first calculates the ratio of the median IP signal intensity to the median WCE signal intensity. It then multiplies the signal intensities of the data probes by this ratio.
- **By normalizing central tendency of log ratios to 1** – This method multiplies the signal intensities of all data probes on the array by a correction factor. This correction factor adjusts the central tendency of the log ratios of data probes on the array to 1.



# Experiment Parameter Panel

Experiment Parameter Panel

Description

When workflow run is complete, an experiment with specified name will be created and can be further analyzed from interactive mode.

Experiment Name

SNP

Experiment Description

Auto created Experiment from workflow

Figure 30 Experiment Parameter Panel

**Purpose:** This parameter panel lets you customize the name of the output experiment for the workflow, and edit the description that the program saves with the experiment.

**To open:** The Experiment Parameter Panel appears when you click **Experiment** in Output in the Analysis Method Navigator, or after you create a new workflow.

**Experiment Name** Edit the name if desired. By default, the program creates a new experiment when it runs a workflow, and gives the new experiment the same name as the workflow analysis method. After you run the workflow, the program makes the experiment available under this name in the Navigator Experiment pane in the interactive tabs.

**Experiment Description** Type or edit an optional description for the experiment.

# Feature Level Filter Parameter Panel

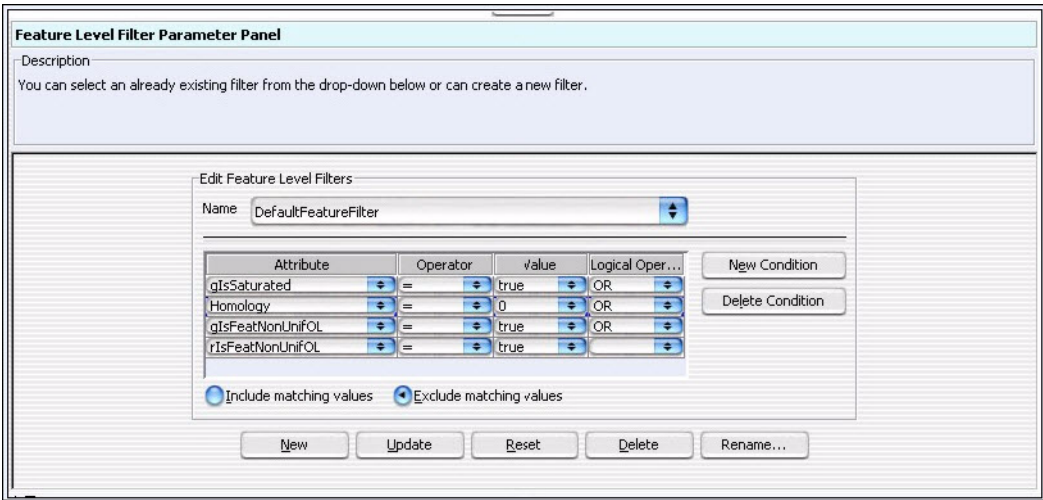


Figure 31 Feature Level Filter Parameter Panel

**Purpose:** This parameter panel lets you select a feature level filter, display a description of its filtering criteria, or create a new filter. Feature level filters include or exclude data from microarray features based on selected criteria.

**To open:** The Feature Filter Parameter Panel appears when you select **Feature Level Filter** under Filter Before Analysis in the Analysis Method Navigator.

**Name** Select the name of the feature filter you want to edit. To create a new filter, and add its name to the list, click **New**.

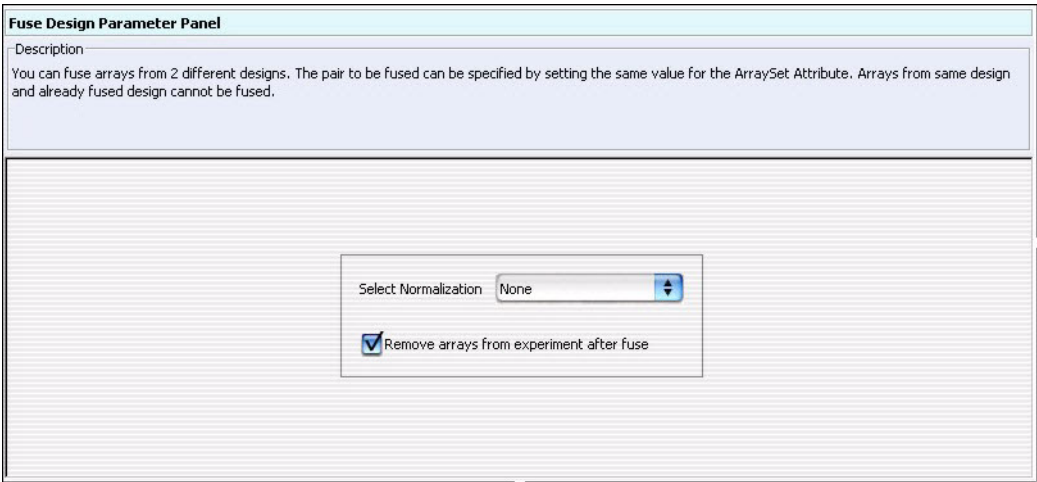
**Filter Conditions table** Below the Name is a list that displays the conditions defined for the selected feature level filter. For each criterion (row), select options from the list or type a value, then press **Enter**. To add another row to the table, click **New Condition**. Each condition has these elements:

- **Attribute** – The feature attribute evaluated by the filter.

- **Operator** – How the filter uses the entry in Value to evaluate arrays. For example, the **>=** operator configures the filter to include or exclude features where the selected attribute is greater than or equal to the entry in Value.
- **Value** – The value the filter uses as the basis to evaluate a feature. For example, if you select the **gIsSaturated** attribute, the **=** operator, and a value of **true**, the filter includes or excludes a feature if its **gIsSaturated** attribute is true.
- **Logical Operator** – (Available only if you configure more than one condition.) The relationship between the condition and the next one in the list. For example, if you select **AND** in Logical Operator for the first condition, the filter includes or excludes an array if it passes both the first condition and the next condition.

<b>New Condition</b>	Adds a new, blank condition (row) to the table.
<b>Delete Condition</b>	Removes a condition from the list. To remove a specific condition, click anywhere within the condition row, then click <b>Delete Condition</b> .
<b>Include/Exclude matching values</b>	<p>Select one of these options:</p> <ul style="list-style-type: none"> <li>• <b>Include matching values</b> – If a feature passes the filter condition, the program includes it in the analysis.</li> <li>• <b>Exclude matching values</b> – If a feature passes the filter condition, the program excludes it from the analysis.</li> </ul>
<b>New</b>	Opens an Input dialog box, where you can type a name for the new filter. To accept the name, click <b>OK</b> . The program creates the filter, and adds the new name to the Name list.
<b>Update</b>	Saves any changes you make to the filter criteria without closing the dialog box.
<b>Reset</b>	Restores the values of the filter conditions to what they were before you made any changes to them.
<b>Delete</b>	Opens a Confirm dialog box that asks you if you want to delete the currently selected filter. To delete the filter, click <b>Yes</b> .
<b>Rename</b>	Opens an Input dialog box where you can type a new name for the filter. To accept the name, click <b>OK</b> .

## Fuse Design Parameter Panel



**Figure 32** Fuse Design Parameter Panel

**Purpose:** This parameter panel lets you select to have the workflow merge arrays from two different array designs into a larger virtual design. This can make it easier to work with arrays that are part of an array set. See the *CGH Interactive Analysis User Guide*, for a description of the requirements that arrays must meet for you to fuse them.

**To open:** The Fuse Design Parameter Panel appears when you mark **Fuse Design** under Fuse in the Analysis Method Navigator.

**NOTE**

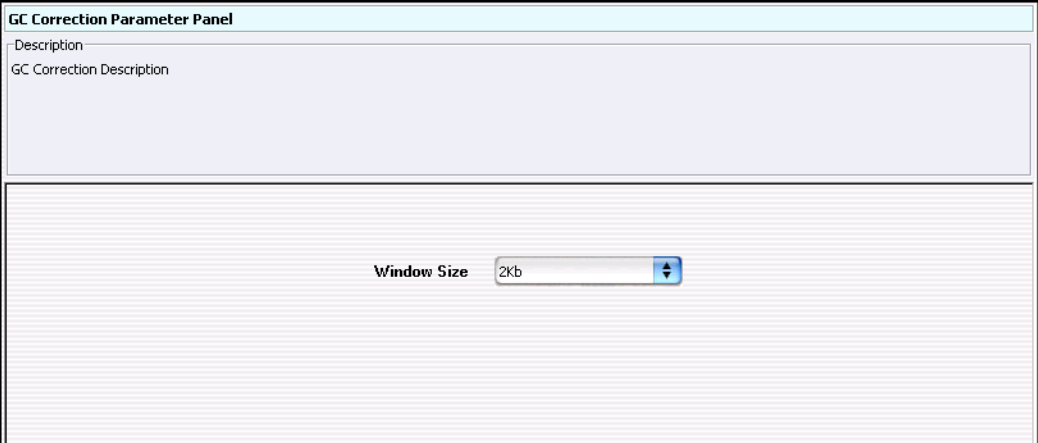
In order to fuse designs, the ArraySet microarray attribute for each array to be fused must have the same value. The ArraySet attribute is entered in the microarray properties from one of the interactive tabs. When you run the workflow, the program fuses all of the arrays that have the same value for the ArraySet attribute.

**Select Normalization**

Select to normalize the data. The workflow always uses the Centralization algorithm to normalize the data in fused arrays. For more information on this algorithm, see the *CGH Interactive Analysis User Guide*.

**Remove arrays from experiment after fuse** If you mark this option, the workflow deletes the original individual arrays after it creates the fused array. This minimizes the duplication of data within the experiment.

## GC Correction Parameter Panel



**Figure 33** GC Correction parameter panel

**Purpose:** To select the window size to use for GC content correction.

**To open:** This panel appears when you click **GC Correction** under Normalization in the CGH Analysis Method Navigator.

**Window Size** Select a window size from the drop-down menu of choices.

# Gene Report Settings

Gene Report Settings

Description

This will generate a Gene report for peak detection results at the specified location. If experiment contains more than one array then reports would be created at the location in a folder with provided name.

Show only gene names

Show probe information

Report Location:

**Figure 34** Gene Report Settings

**Purpose:** This parameter panel lets you configure and select a location for the ChIP gene report.

**To open:** These parameters appear when you click **Gene Report** under Reports in the ChIP Workflow Navigator.

- Show only gene names

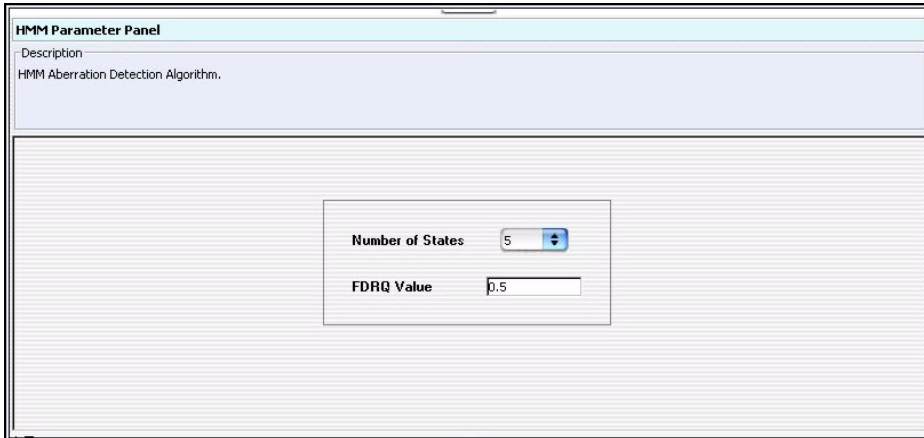
If you mark this check box, the resulting gene report contains only accession numbers of genes (or chromosomal locations for probe loci not associated with genes). A mark in this check box overrides a mark in Show probe information.
- Show probe information

If you mark this check box, the resulting gene report contains additional information about the probes in the array.
- Report Location

Shows the location where the program saves the gene report. Type a new location in the text box, or click **Browse** to select a location.
- Browse

Opens the Select report folder dialog box, where you can select a location for the gene report. See “[Select Report Folder](#)” on page 190.

## HMM Parameter Panel



**Figure 35** HMM Parameter Panel

**Purpose:** This parameter panel lets you set up the parameters required for applying the Hidden Markov Model (HMM) algorithm. See the *CGH Interactive Analysis User Guide* for more information.

**To open:** The HMM Parameter Panel appears when you select **HMM** under Aberration in the Analysis Method Navigator.

**Number of states** Select the number of states. This is the number of distinct aberration states into which the observed data is to be partitioned. For example, if the number of states equals 3, then this would correspond to amplification, deletion and “no change” aberration status. The number of states can be set to either 3, 5, or 7.

**FDRQ value** Type the FDRQ value. This is a False Discovery Rate threshold used in the calculation of the Discrete Haar Wavelet Transform, and is used to keep only high-amplitude coefficients, which mark occurrences of true breakpoints (rather than noise), in the data.

# Import Data Files Parameter Panel

Import Data Files Parameter Panel

Description

Genomic Workbench will create a new array node in the data section of the navigator in interactive mode. The new node will have the name of the imported file. However, you can use this dialog to edit the file name(s). Additionally, you can specify if an array is dye-flipped. In this case the ratios will be inverted, but dye-flip pairs will not be automatically combined.

Global Display Name	Dye Flip	Design Name	Design Build	Design Status
JS23502418_252808110005_501_CGH_109_Feb10_1_1	Normal	028081	hg18	Already Present
JS23502418_252808110005_501_CGH_109_Feb10_1_2	Normal	028081	hg18	Already Present
JS23502418_252808110006_501_CGH_109_Feb10_1_1	Normal	028081	hg18	Already Present
JS23502418_252808110006_501_CGH_109_Feb10_1_2	Normal	028081	hg18	Already Present

☐ Overwrite arrays with duplicate names.

RemoveAdd Arrays...Add Designs...

Figure 36 Import Data Files Parameter Panel

**Purpose:** This panel lets you configure the workflow to import Agilent Feature Extraction (FE) array data files.

**To open:** The Import Data Files Parameter Panel appears when you select **Import FE Files** in Input in the Workflow Navigator.

NOTE

You must import representative design files into the program before you run a workflow that imports FE files.

Global Display Name

Lists the global display names of array data files selected for import. To add files, click **Add**. To edit the global display name of a file, double-click the name, type the name, then press **Enter**.

Dye Flip

Lets you annotate an array as a dye-flip array (an array in which the Cy3 and Cy5 fluorochrome-labeled samples have been reversed). If you select **Flipped**, the program inverts ratios so you can make proper comparisons. Also, the program does not combine dye-flip pairs.

Select one of these options for each array:



Select this option	If:
Normal	<ul style="list-style-type: none"> <li>The test samples were labeled with cyanine 5 (red).</li> <li>The control samples were labeled with cyanine 3 (green).</li> <li>The imported ratio (test/control) should be reported directly.</li> </ul>
Flipped	<ul style="list-style-type: none"> <li>The test samples were labeled with cyanine 3 (green).</li> <li>The control samples were labeled with cyanine 5 (red).</li> <li>The imported ratio (control/test) should be reported with the ratio inverted (test/control)</li> </ul>

**Design Name** The design name for the array is displayed in this field.

**Design Build** The design build for the array is displayed in this field. If this field is blank, you need to import the design file from the Home tab, or add the design before you can run a workflow that imports data files.

**Design Status** This field displays the status of the design file.

- Not Found - The design file is not currently in the program or a path has not been selected.
- Already Present - The design file was imported from the Home tab of Agilent Genomic Workbench.
- Path Provided - The design file was not imported, but a location was selected for the file.

**Overwrite arrays with duplicate names** If you mark this option, the workflow deletes an existing array if it has the same name as one you import.

#### NOTE

For a complete description of what happens when you import FE data files, see the *Sample Manager User Guide*.

**Remove** Removes files from the list of files to be imported. To remove a file from the list, click its name, then click **Remove**.

**Add Arrays.** Opens an Open dialog box, where you can select a data file to import. The file you select appears in the list of arrays to import.

**Add Designs** This option is enabled after you have added arrays to the list, and the design is not already present in the program. When you select this, an Open dialog box appears, where you can select a design file for the arrays in the list. When you select a file, the Design Name appears in the parameter panel along with the Design Build. Click to open a dialog box where you can select the design file from a location on your hard disk.

Import FE Image Files Parameter Panel

Import FE Image Files Parameter Panel						
Description Import Feature Extraction images here and associate samples to them.						
Image Name	Global Display Name	Array ID	Barcode	Sample ID <Red / Green (Arra...	Grid Template	Protocol
US23502418_252152910035...	US23502418_252152910035...	252152910035_1_1	252152910035	?	<Automatically Determine>	<Automatically Determine>
US23502418_252152910035...	US23502418_252152910035...	252152910035_1_2	252152910035	?	<Automatically Determine>	<Automatically Determine>
US23502418_252152910035...	US23502418_252152910035...	252152910035_1_3	252152910035	?	<Automatically Determine>	<Automatically Determine>
US23502418_252152910035...	US23502418_252152910035...	252152910035_1_4	252152910035	?	<Automatically Determine>	<Automatically Determine>
US23502418_252152910037...	US23502418_252152910037...	252152910037_1_1	252152910037	?	<Automatically Determine>	<Automatically Determine>
US23502418_252152910037...	US23502418_252152910037...	252152910037_1_2	252152910037	?	<Automatically Determine>	<Automatically Determine>
US23502418_252152910037...	US23502418_252152910037...	252152910037_1_3	252152910037	?	<Automatically Determine>	<Automatically Determine>
US23502418_252152910037...	US23502418_252152910037...	252152910037_1_4	252152910037	?	<Automatically Determine>	<Automatically Determine>
						Add... Remove

Figure 37 Workflow – Import FE Image Files Parameter Panel

**Purpose:** This panel lets you import or remove FE image files to extract using a workflow. For each image, sample parameters are displayed. Buttons at the bottom of the tab are used to add or remove images from the list. See [“To import an image file”](#) on page 40 and [“To remove an image from the list”](#) on page 42.

**To open:** This parameter panel is shown when you select **Image Files** under Input in the Workflow Navigator.

For additional information on associating samples to images, see the *Sample Manager User Guide*.

**Image Name** (Read-only) The file name of the image.

**Global Display Name** The Global Display Name for the array. To change the Global Display Name, double-click the name and type the new name.

<b>Array ID</b>	(Read-only) The unique identifier for the microarray.
<b>Barcode</b>	(Read-only) This is the barcode identifier for the slide. A microarray slide contains one array, or for multiplex slides, multiple arrays on one slide.
<b>Sample ID &lt;Red/Green (ArrayID)&gt;</b>	If the Red/Green attributes for the array are assigned in Sample Manager, they appear here. Otherwise, a ? appears in the field.
<b>Grid Template</b>	By default, the Feature Extraction program automatically determines the Grid Template for the microarray. Or, you can select a Grid Template from the list, or select a grid file (.grd) from your hard disk.
<b>Protocol</b>	By default, the Feature Extraction program automatically determines the Protocol for the microarray. Or, you can select a Protocol from the list.
<b>Add</b>	Click this button to open the Open dialog box, where you can select the image file(s) to be added. See “ <a href="#">Open</a> ” on page 187.
<b>Remove</b>	Highlight one or more microarrays and then click this button to remove them from the list. To select more than one microarray, hold down the <b>Ctrl</b> key and then click the microarrays to remove. To select a contiguous series of microarrays, hold down the <b>Shift</b> key and click the first microarray and then click the last microarray.

# Import UDF Files Parameter Panel

Import UDF Files Parameter Panel

Description

A tab delimited CGH data can be imported as a new custom-formatted array data file.  
The order of tabular data must follow the column headers in the Universal Data Importer - Map column headers dialog box.

File Name: udf\_sample\_file

Design type: cgh

Select species: H. sapiens

Select Mapping: CUSTOM

Design Id: Custom

Data type: ratio

Select Genome Build: hg18

[Save Mapping As](#)

ProbeName	ChrName	Start	Stop	LogRatio	Sample2	descrip
Select	Select	Select	Select	Select	Select	Select
A101122	1	1000	1060	0.0023	0.0044	ATC66
A102121	1	1100	1160	0.0022	0.1123	ATC66
A103222	1	1200	1260	0.0033	0.1121	ATC67
A104343	1	1300	1360	0.1125	0.1123	ATC66
A105122	1	1400	1460	0.0123	0.0112	ATC67

Add...

Figure 38 Import UDF Files Parameter Panel

**Purpose:** This parameter panel lets you configure the workflow to import one or more Universal Data Files (UDFs) as input for the analysis.

**To open:** The Import UDF Files Parameter Panel appears when you select **Import UDF Files** in Input in the Workflow Navigator.

Most of the parameters help to identify the array and its contents. UDFs are tab-delimited \*.txt files. Use the arrow buttons at the top of the table to map the column to a program parameter. A UDF file must have these columns, in any order:

Column	Description
ProbeName	The name (Probe ID) of the probe
ChrName	The name of the chromosome to which the probe binds
Start	The first base pair on the chromosome to which the probe binds

Column	Description
Stop	The last base pair on the chromosome to which the probe binds
Description	Additional probe annotation, such as gene names
LogRatio	Log ratio data for the probe. The file can contain more than one column of log ratio data.

**Add** Click this button to open an Open dialog box, where you can select a UDF file for the workflow to import.

**Main data table** The first row of the main data table contains the first line of the UDF file. Although the first line of the UDF file must contain column headings, the workflow does not interpret them. Instead, you select the most appropriate label for each column from the lists. The rest of the main data table contains the first few data lines of the file to help you identify the columns properly. You must use all of the labels exactly once, except **LogRatio**, which you can use more than once.

**File Name** (Read-only) The name of the UDF file to be imported.

**Design ID** (Read-only) A unique identifier for the design. If **Custom** appears here, the workflow creates the necessary design ID(s) after it imports the files.

**Design type** Select the application type that best represents the data, either **cgh** or **expression**.

**Data type** Select the mathematical relationship that best exemplifies the data. The following options appear in the list:

- **ratio** – linear ratio
- **log<sub>2</sub> ratio** – binary log ratio
- **log<sub>10</sub> ratio** – common log ratio
- **ln ratio** – natural log ratio, base e

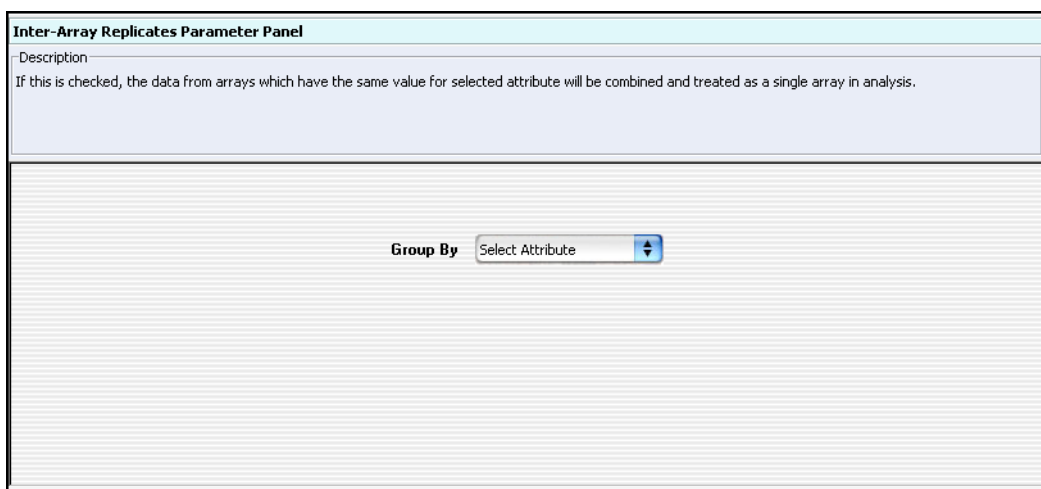
**Select Species** Select the species appropriate to data in the UDF file.

**Select Genome Build** Select the genome build appropriate to the data in the UDF file. The choices available depend on the species you select in **Select Species**.

**Select Mapping** Select a saved column mapping, if desired. To create a new mapping, select **CUSTOM**.

**Save Mapping As** Opens an Input dialog box, where you can type a name for the current column map. This lets you save the current column map for future use. This is especially useful if you want to import many UDF files that have columns arranged in the same manner.

## Inter-Array Replicates Parameter Panel



**Figure 39** Inter-Array Replicates Parameter Panel

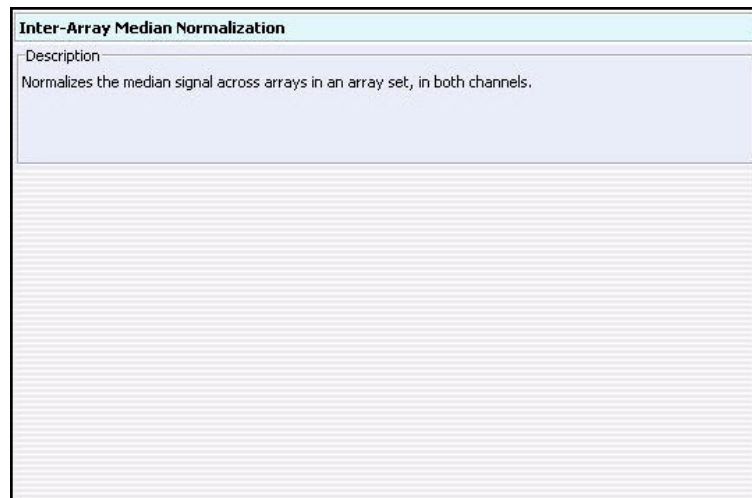
**Purpose:** This parameter panel lets you configure how the workflow combines replicate probes across multiple arrays.

**To open:** The Inter-Array Replicates Parameter Panel appears when you mark **Inter-Array Replicates** under Combining Replicates in the Analysis Method Navigator.

**Group By** In **Select Attribute**, select an array attribute. The program uses the selected array attribute to group arrays when it combines replicate probes. For example, if you have some arrays where the Sample Name attribute is set to **C44** and other arrays with the same array attribute set to **D95**, the program combines the corresponding probes for the C44 arrays to make a virtual array C44. It combines the probes with the same names for the

D95 arrays to make another virtual array. To edit the attributes of an array, see the *CGH Interactive Analysis User Guide* or the *ChIP Interactive Analysis User Guide*.

## Inter-Array Median Normalization



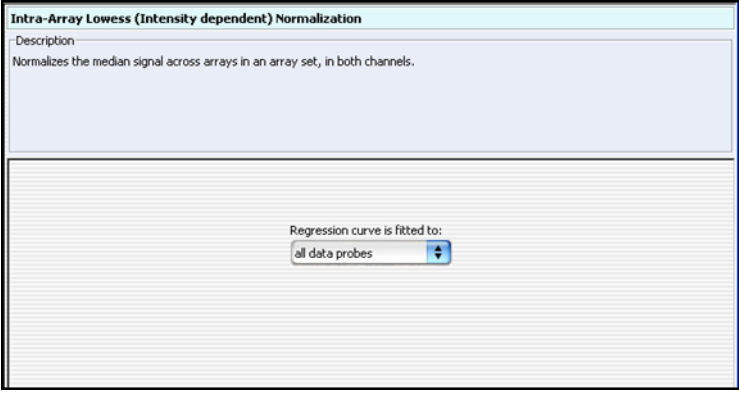
**Figure 40** Inter-Array Median Normalization

**Purpose:** Selects the interarray median normalization method for ChIP. No parameters are required.

**To open:** This parameter panel appears when you click **Inter-Array Median** under Normalization in the Analysis Method Navigator. See [“To configure normalization methods \(ChIP\)”](#) on page 82.

Agilent Genomic Workbench calculates the central tendency of the common probes on replicate arrays using the median of the probe signal intensities. No further parameters are necessary in this panel.

## Intra-Array Lowess (Intensity Dependent) Normalization



The screenshot shows a software interface for "Intra-Array Lowess (Intensity dependent) Normalization". It has a title bar with the same text. Below the title bar is a "Description" section with the text: "Normalizes the median signal across arrays in an array set, in both channels." The main area of the panel contains a label "Regression curve is fitted to:" followed by a dropdown menu. The dropdown menu currently shows "all data probes" and has a small blue arrow icon to its right.

**Figure 41** Intra-Array Lowess (Intensity dependent) Normalization

**Purpose:** This parameter panel is used to configure the intra-array Lowess normalization method for ChIP workflow analysis.

**To open:** This parameter panel is displayed when you select **Intra-Array Lowess Intensity Dependent** under Normalization in the ChIP Analysis Method Navigator.

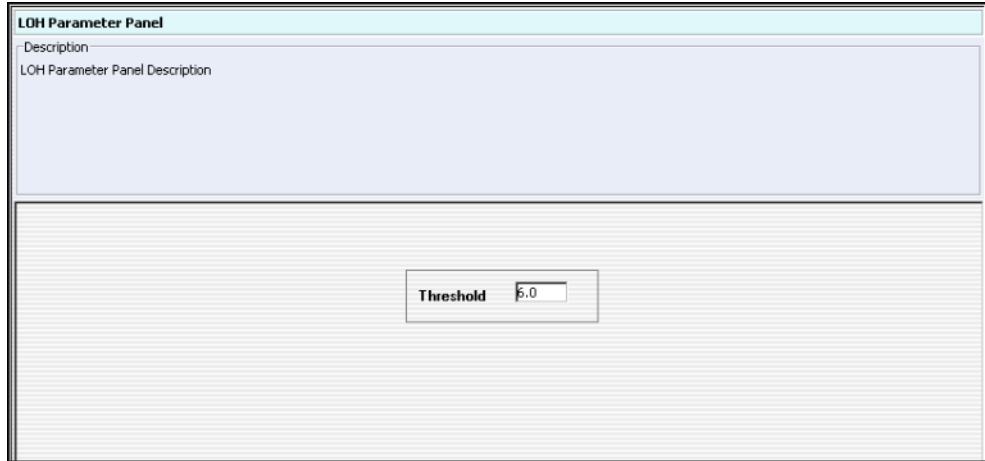
**Regression curve  
is fitted to**

Select the data to use for the regression curve.

- **All data probes** - Includes all of the data probes in the regression curve.
- **All common probes** - Includes probes whose names start with "LACC".
- **Gene desert probes** - Includes data for probes whose names start with "LACC:GD".



## LOH Parameter Panel



**LOH Parameter Panel**

Description  
LOH Parameter Panel Description

Threshold 6.0

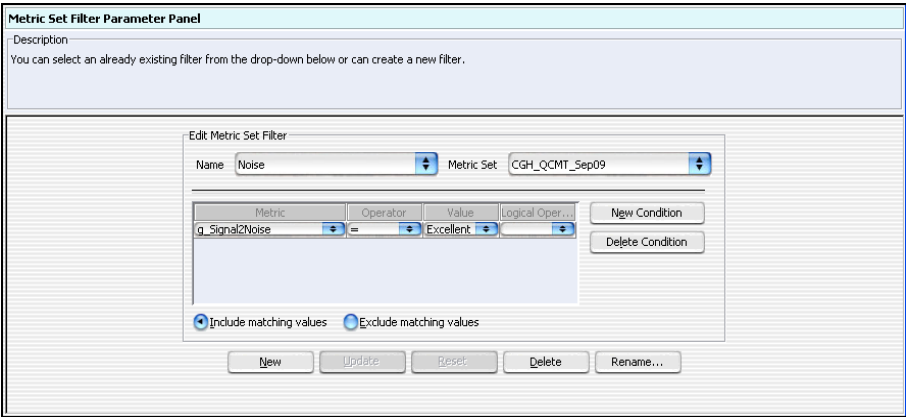
**Figure 42** LOH Parameter Panel

**Purpose:** To set the threshold level for calculation of LOH (Loss of Heterozygosity) regions.

**To open:** In the Analysis Method Navigator for CGH, under SNP Algorithm, mark **LOH**.

**Threshold** Type the threshold to use for the LOH calculation. For more information on this algorithm, see the *CGH Interactive Analysis User Guide*.

# Metric Set Filter Parameter Panel



**Figure 43** Workflow - Metric Set Level Filter Parameter Panel

**Purpose:** In this panel, you create or edit a metric set filter that is used to include or exclude data for the workflow. For each metric selected, you select conditions for how to apply the filter.

**To open:** This panel is displayed when you select **Metric Set Filter** under Metric Evaluation in the Workflow Navigator.

## Edit Metric Set Filter

<b>Name</b>	Select the name of the metric set filter you want to edit. To create a new filter, and add its name to the list, click <b>New</b> .
<b>Metric Set</b>	The default metric set for the selected application is shown. Click the arrow to select a different metric set.
<b>Filter Conditions table</b>	For each criterion (row), select an option from the list, if available. Otherwise, type a value, then press <b>Enter</b> . To add another row to the table, click <b>New Condition</b> .

Each criterion has these elements:

- Metric – The QC metric evaluated by the filter.

- **Operator** – How the filter uses the entry in Value to evaluate arrays. For example, the **Is** operator configures the filter to include or exclude features where the calculated extraction result of the selected attribute is the same as the threshold selected in Value.
- **Value** – The value the filter uses to evaluate an array. For example, if you select the **g\_BGNoise** attribute and the Operator **Is**, when you select the **Evaluate** Value, the array will pass the filter if its extraction result is in the Evaluate threshold range.
- **Logical Operator** – (Available only if you configure more than one criterion) The relationship between the criterion and the next one in the list. For example, if you select **AND** in **Logical Operator** for the first criterion, the filter includes or excludes an array if it passes both the first condition and the next condition.

**New Condition** Adds a new, blank condition (row) to the table.

**Delete Condition** Removes a condition from the list. To remove a specific condition, click anywhere within the condition, then click **Delete Condition**.

**Include/Exclude matching values** Select one of these options:

- **Include matching values** – If an array meets the filter conditions, the program *includes* it in the analysis.
- **Exclude matching values** – If an array meets the filter conditions, the program *excludes* it from the analysis.

**New** Opens an Input dialog box, where you can type a name for the new filter. To accept the name, click **OK**. The program creates the filter, and adds the new name to the Name list.

**Update** Saves any changes you make to the filter conditions.

**Reset** Restores the values of the filter conditions to what they were before you made any changes to them.

**Delete** Opens a Confirm dialog box that asks you if you want to delete the selected filter. To delete the filter, click **Yes**.

**Rename** Opens an Input dialog box where you can type a new name for the filter. To accept the name, click **OK**.

# Predefined peak shape detection v2.1

**Pre-defined Peak Shape detection v2.1**

**Description**

This peak detection algorithm "slides" a peak shape through the data searching for good fits. Please refer to the user guide for a detailed description.

The peak shape is computed from the estimated mean and standard deviation of DNA lengths of the shear distribution, and the "significance" of a potential fit is judged by comparing it to fits on randomized data, using a non-parametric (rank-significance) test.

For each peak that satisfies the non-parametric test, a "score" is computed by testing the quality of the fit under the assumption of an extreme value distribution of the nullities of the fits to randomized data. The significance derived from this test is converted to a

**Thresholds**

**P-value threshold** (maximum) for non-parametric test for reporting peaks:   
Must be greater than  $1/(\text{number of randomization runs})$ . Increase to find more peaks

**Score threshold** (minimum) for EVD-based score. Decrease to find more peaks:

**Peak Shape Parameters**

Estimated **mean** shear length distribution of sample DNA:

Estimated **standard deviation** of the shear length distribution of sample DNA:

**Other Algorithmic Parameters**

**Precision** of peak placement on the chromosome (in base pairs):

**Number of randomizations** for determining peak significance (via non-parametric test) and score:

**Window size** (in bp) for computing local baseline. (Use smaller number for smaller genomes):

**Desired spacing** of interpolated datapoints between probe (must be less than or equal to probe spacing on the array.):

☐ Automatically re-run calculation after learning peak-shape? (Doubles runtime, but increases accuracy.)

☐ Use errors estimated by Error model?  
If selected, the estimated error for each probe is used to weight its contribution to the peak fit measurement

**Figure 44** Pre-defined peak shape detection v2.1

**Purpose:** This parameter panel is used to configure the predefined peak shape detection v2.1 algorithm for peak detection in ChIP analysis.

**To open:** This parameter panel appears when you select **Pre-defined Peak Shape detection v2.1** under Peak Detection and Evaluation in the ChIP Analysis Method Navigator.

## Thresholds

- P-value threshold** Maximum threshold for the nonparametric test for reporting peaks. Value must be greater than  $1/(\text{number of randomized runs})$ . (Increase the value to find more peaks.)
- Score threshold** Minimum threshold for extreme value distribution (EVD) -based score. (Decrease value to find more peaks.)

## Peak Shape Parameters

**Estimated mean  
shear length  
distribution of  
sample DNA**

Type a mean to be used in the gamma distribution calculation for approximation of the distribution of sheared DNA fragments.

**Estimated  
standard  
deviation of the  
shear length  
distribution of  
sample DNA**

Type a standard deviation to be used in the gamma distribution calculation of the distribution of sheared DNA fragments.

## Other Algorithmic Parameters

**Precision of peak  
placement on the  
chromosome (in  
base pairs)**

This is the window within which the algorithm searches for potential positions for the peak center. Decreasing this window increases the time it takes for the algorithm to run.

**Number of  
randomizations  
for determining  
peak significance  
(via  
non-parametric  
test) and score**

The program computes *p*-value and peak score through a number of random samplings. Increasing the number of samples increases the accuracy of the prediction; however, it also increases the time to do the calculation.

**Window size (in  
bp) for computing  
local baseline.**

Use smaller number for smaller genomes.

**Desired spacing  
of interpolated  
datapoints  
between probe.**

Must be less than or equal to probe spacing on the array.

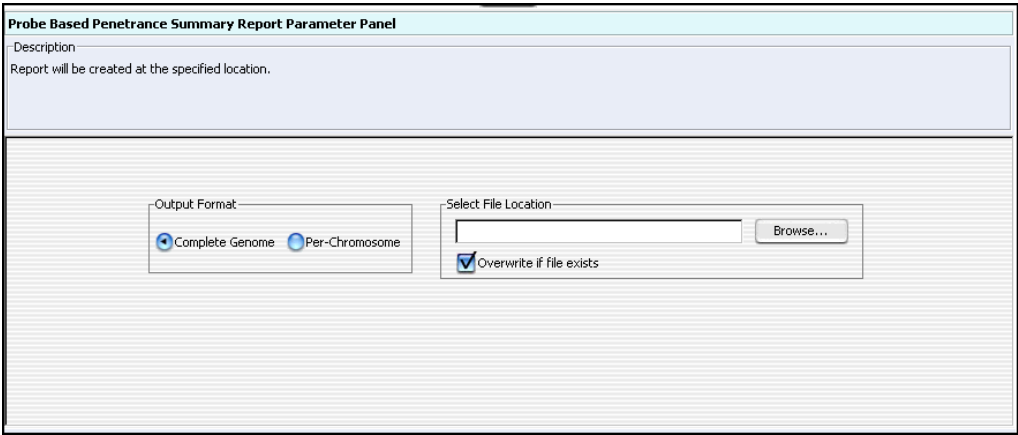
**Automatically  
re-run  
calculation after  
learning peak  
shape?**

Selection increases accuracy, but it doubles the runtime.

**Use errors  
estimated by  
Error model?**

Select to use the estimated error for each probe to weight its contribution to the peak fit measurement.

## Probe Based Penetrance Summary Report Parameter Panel



**Figure 45** Probe Based Penetrance Summary Report Parameter Panel

**Purpose:** This parameter panel lets you configure the Probe Based Penetrance Summary Report, and select a location for it. This report displays each probe that shows a significant aberration, and gives the percentage of selected arrays that show a significant deletion or amplification in the region for each probe. The workflow creates one or more \*.xls files that you can work with in Microsoft Excel.

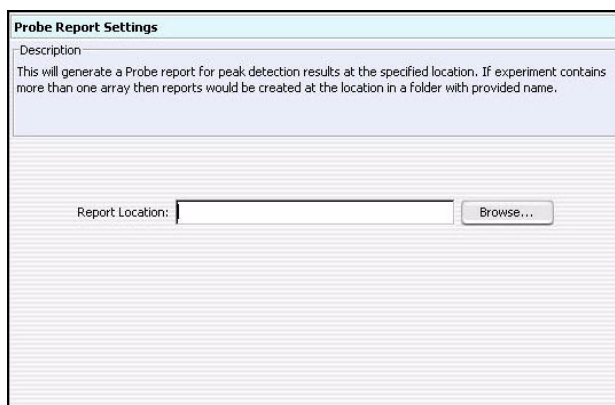
**To open:** The Probe Based Penetrance Summary Report Parameter Panel appears when you mark **Probe Based Penetrance Summary Report** in Reports in the Workflow Navigator for CGH.

**Output Format** Select one of these options:

- **Complete Genome** – Creates a single report file.
- **Per-Chromosome** – Creates a separate report file for each chromosome.

- Select File Location** Displays the location where the workflow saves the files. To select a location for the report, click **Browse**. An Open dialog box appears. Type a name and select a location for the report, then click **Open**.
- Overwrite if file exists** If you mark this option, the workflow deletes an existing file if it has the same name and location as a generated report.

## Probe Report Settings



**Figure 46** Probe Report Settings

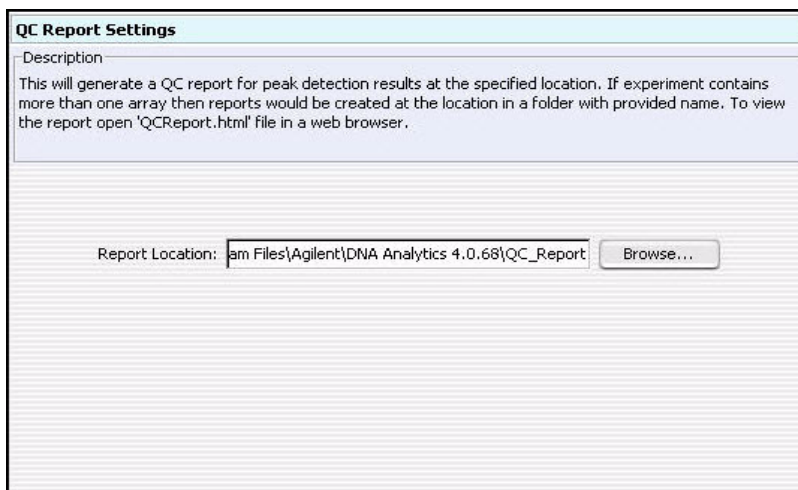
**Purpose:** This parameter panel lets you select the location for Probe Reports generated during ChIP workflow analysis.

**To open:** This parameter panel appears when you click **Probe Report** under Reports in the ChIP Workflow Navigator. For information about the content and format of the Probe Report, see the *ChIP Interactive Analysis User Guide*.

- Report Location** Shows the location of the Probe Report that the program creates during the workflow. You can edit the location. You can also click **Browse** to select a new location for the report. The program saves the Probe Report as a tab-separated value (\*.tsv) file.

**Browse** Opens the Select report folder dialog box, where you can select a location and type a name for the Probe Report file. See “[Select Report Folder](#)” on page 190.

## QC Report Settings



**Figure 47** QC Report Settings

**Purpose:** This parameter panel is used to select a location for QC reports generated in a ChIP workflow analysis.

**To open:** This parameter panel appears when you click **ChIP QC Report** under Reports in the ChIP Workflow Navigator. For information about the content and format of the QC report, see the *ChIP Interactive Analysis User Guide*.

**Report Location** Shows the location of the QC report that the program creates during the workflow. You can edit the location. You can also click **Browse** to select a new location for the report. The program saves the QC report as an HTML file.



**Browse** Opens the Select report folder dialog box, where you can select a location and type a name for the QC report file. See “Select Report Folder” on page 190.

## Run Analysis Application Panel

**Run Analysis Application**

Description  
Run a DNA Analysis Application.


Analysis Application: DNA Analytics  
Application Type: CGH  
Analysis Method: KM\_CGH3

New Edit

**Figure 48** Workflow – Run Analysis Application panel

**Purpose:** This panel is used to display the analysis application and application type, and to select, create, or edit the analysis method to use for the workflow.

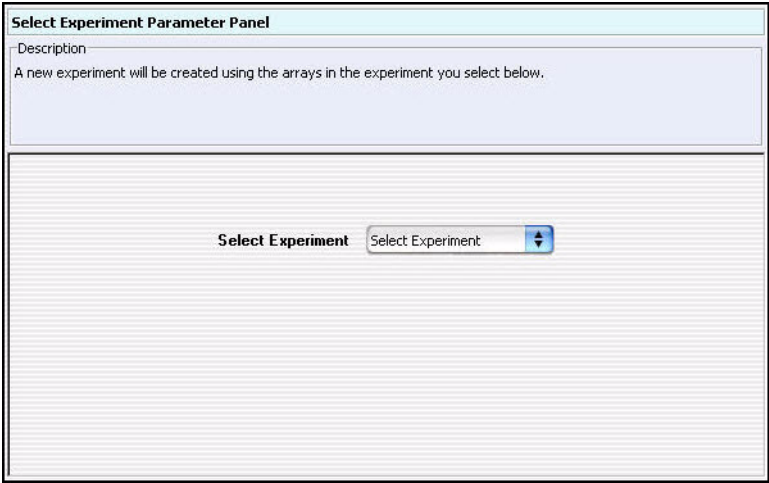
**To open:** This panel is displayed when Run Analysis is selected from Analysis in the Workflow Navigator for either CGH or ChIP analysis.

**Analysis Method** Click the arrows  to select the Analysis Method to run in the workflow.

**New** Opens the Create Analysis Method dialog box, where you type a name for the new analysis method. The Analysis Method window then opens, where you can create and save the new analysis method for the workflow.

**Edit** Opens the Analysis Method window, where you can edit the selected analysis method.

## Select Experiment Parameter Panel



**Figure 49** Select Experiment Parameter Panel

**Purpose:** This parameter panel lets you configure the workflow to use the arrays from a CGH or ChIP experiment as input.

**To open:** The Select Experiment Parameter Panel appears when you select **Select Experiment** under Input in the Workflow Navigator.

**Select  
Experiment**

The names of the available experiments appear in Select Experiment. Select an experiment from the list. The program uses the arrays from the selected experiment as input for the workflow. The program creates a new experiment during the workflow, and does not change the selected experiment.

You create experiments in the Genomic Viewer. See the *Data Viewing Guide* for information on how to use the Genomic Viewer.

## Select Imported Data Parameter Panel

**Select Imported Data Parameter Panel**

Description:  
Select what data you would like to include in the analysis by highlighting arrays in the left column and clicking ">>" to move them into the right column. A new experiment will be created with the selected arrays.

Select Design: 014698 Select Genome Build: hg18

**Array List**

- US22502705\_251469814934\_S01\_CGH-v4\_95\_Feb07\_1\_1
- US22502705\_251469814934\_S01\_CGH-v4\_95\_Feb07\_1\_2
- US22502705\_251469814935\_S01\_CGH-v4\_95\_Feb07\_1\_1
- US22502705\_251469814935\_S01\_CGH-v4\_95\_Feb07\_1\_2

**Selected Array List**

**Figure 50** Select Imported Data Parameter Panel

**Purpose:** This parameter panel lets you select previously imported arrays as input for the workflow.

**To open:** The Select Imported Data Parameter Panel appears when you select **Select Imported Data** under Input in the Workflow Navigator.

**Select Design** Displays the array designs available in Agilent Genomic Workbench. Select the design from the list. The arrays for the design appear in the Array List. Although the program displays the arrays from one design at a time, you can add arrays from more than one design to the Selected Array List.

**Select Genome Build** If the design you select in Select Design has arrays from more than one genome build, select the desired genome build. Although the program displays the arrays from one genome build at a time, you can add arrays from more than one genome build to the Selected Array List.

**Array List** Displays the arrays for the selected design and genome build.

**Selected Array List** Displays the arrays the program uses as input for the workflow.

## 4 Workflow Reference

### Set Output Path for Feature Extraction Panel



Moves selected arrays from the Array List to the Selected Array List. To select an array, click its name. To select additional arrays, hold down the **Ctrl** key and click (command-click on a Mac) their names. To select a block of arrays, click the name of the first array in the block, then hold down the **Shift** key and click the last one.



Moves selected arrays from the Selected Array List to the Array List.

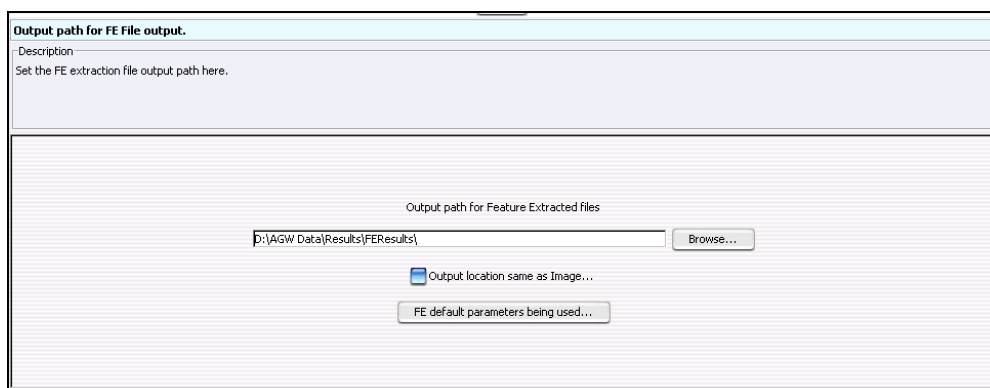


Moves all of the arrays in the Array List to the Selected Array List.



Clears all of the arrays from the Selected Array List, and restores them to their original location(s).

## Set Output Path for Feature Extraction Panel



**Figure 51** Workflow – Output path for FE File output

**Purpose:** This panel is used to select the location for extracted FE files.

**To open:** This panel is displayed when you select **Feature Extraction** under Extraction in the Workflow Navigator.

#### Output path for Feature Extracted files

Type the path to be used for saving FE files. To search for the location, click the **Browse** button. See “[Open](#)” on page 187.

<b>Browse</b>	Click this button to browse for the folder where you want to save the FE files.
<b>Output location same as Image</b>	Mark this to set the output path for extracted files to the location of the image files.
<b>FE default parameters being used</b>	Click this to display the Feature Extraction Properties. These are the parameters that are used when you run Feature Extraction using the Workflow. See <a href="#">“Feature Extraction Properties”</a> on page 183.

## SNP Copy Number Parameter Panel

The image shows a software interface for the 'SNP Copy Number Parameter Panel'. It features a title bar at the top. Below the title bar is a large text area for a description, which currently contains the text 'Description: SNP Copy Number Parameter Panel Description'. At the bottom of the panel, there is a control for the 'SNP Conf. Level' parameter, consisting of a label and a text input field that displays the value '0.95'.

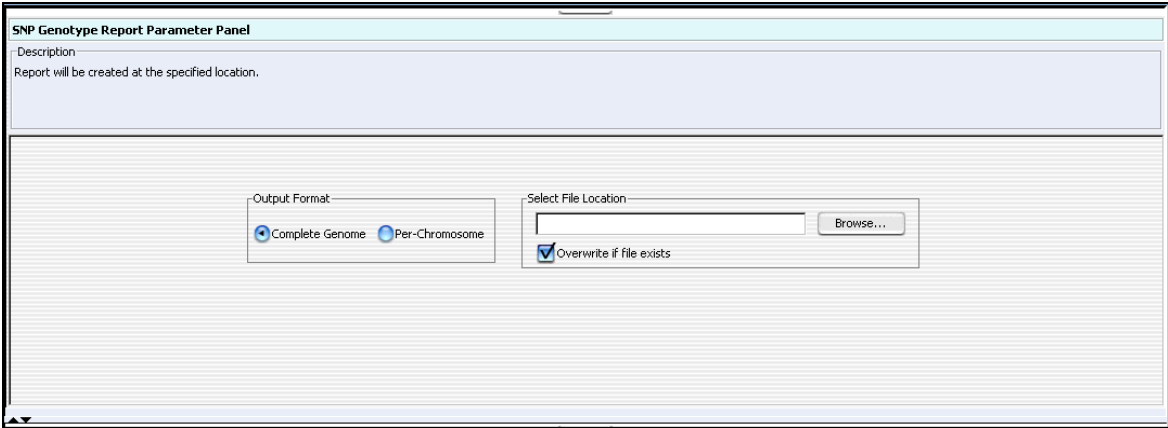
**Figure 52** SNP Copy Number Parameter Panel

**Purpose:** This panel is used to type a confidence level value to use in the SNP Copy Number calculation.

**To open:** In the Analysis Method Navigator for CGH, under SNP Algorithm, mark **SNP Copy Number**.

**SNP Conf. Level** Type a value for the confidence level to use in the SNP copy number calculation.

## SNP Genotype Report Parameter Panel



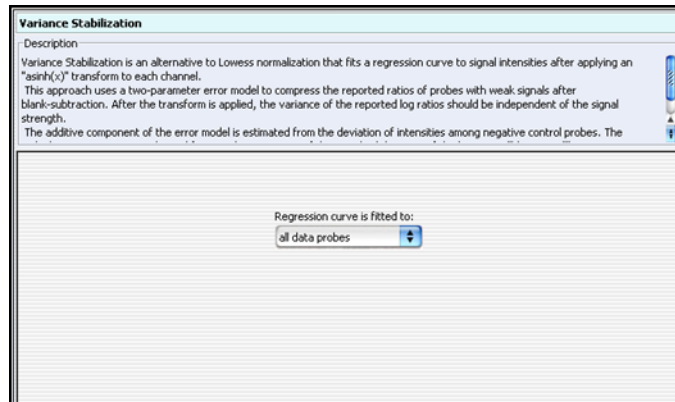
**Figure 53** SNP Genotype Report Parameter Panel

**Purpose:** To set the format and storage location for SNP Genotype Reports generated in the Workflow.

**To open:** In the Analysis Method Navigator for CGH, under Reports, mark **SNP Genotype Report**

- |                                 |   |
|---------------------------------|---|
| <b>Output Format</b>            | Select one of these options: <ul style="list-style-type: none"><li>• <b>Complete Genome</b> – Creates a single report file.</li><li>• <b>Per-Chromosome</b> – Creates a separate report file for each chromosome.</li></ul> |
| <b>Select File Location</b>     | Displays the location where the workflow saves the files. To select a location for the report, click <b>Browse</b> . An Open dialog box appears. Type a name and select a location for the report, then click <b>Open</b> . |
| <b>Overwrite if file exists</b> | If you mark this option, the workflow deletes an existing file if it has the same name and location as a generated report.  |

## Variance Stabilization



**Figure 54** Variance Stabilization

**Purpose:** This parameter panel is used to configure the regression curve for the variance stabilization method of normalization for ChIP workflow analysis.

**To open:** This parameter panel is displayed when you select **Variance Stabilization** under Normalization in the ChIP Analysis Method Navigator.

**Regression curve  
is fitted to**

Select the data to use for the regression curve.

- **All data probes** - Includes all of the data probes in the regression curve.
- **All common probes** - Includes probes whose names start with “LACC”.
- **Gene desert probes** - Includes data for probes whose names start with “LACC:GD”.

# Whitehead Error Model

**Whitehead Error Model**

Description  
If selected, per-probe error estimates will be calculated as per Whitehead Error Model method.

Source of additive(intensity-dependent) error of each channel is:  
Standard deviation of background pixels

The f-value of one replicate of an array is the rate at which the multiplicative error increases with signal intensity. Normally it is computed for you, but you can also provide it here instead.

☒ Custom defined f-value 0.2

**Figure 55** Whitehead Error Model

**Purpose:** This parameter panel lets you customize the advanced parameters of the Whitehead error model. Set parameters to optimize the statistical calculations of the error model using training data specific to your particular assay.

**To open:** This parameter panel appears when you select **Whitehead Error Model** under Error Model in the ChIP Analysis Method Navigator. See [“To configure error model for analysis method \(ChIP\)”](#) on page 85.

**Source of additive (intensity-dependent) error in each channel is:**

The choices for this parameter change the additive (intensity-dependent) component of the estimate of the error in IP – WCE. Select one of these sources:

- Standard deviation of background pixels
- Additive error as computed by Agilent Feature Extractor
- Observed spread of negative controls

**Custom defined f-value**

Mark this check box to define a custom f-value. In the box to the right, type an f-value. The f-value of one replicate of an array is the rate at which the multiplicative error increases with signal intensity. By default, the ChIP program calculates f-values automatically.



## Whitehead Per-Array Neighbourhood Model

Whitehead Per-Array Neighbourhood Model

Description

The values "X" and "Xbar" are computed from spot intensities and error estimates. In order for a genomic region to be identified as a peak, these values must satisfy the thresholds specified here. ("Xbar" represents the average "X" value over three consecutive probes close to each other.)

Maximum distance (in bp) for two probes to be considered as neighbors:

A probe is considered "bound" if:

$P(X_{bar}) < 0.001$  AND

**EITHER**

Central probe (red probe above) has  $P(X) < 0.001$

At least one neighboring probe (blue) has  $P(X) < 0.1$

**OR**

At least  of the neighbors (blue) has  $P(X) < 0.005$

**Figure 56** Whitehead Per-Array Neighbourhood Model

**Purpose:** This parameter panel is used to configure the Whitehead per-array neighbourhood model for peak detection in ChIP workflow analysis.

**To open:** This parameter panel is displayed when you click **Whitehead Per-Array Neighbourhood Model** under Peak Detection and Evaluation in the ChIP Analysis Method Navigator. See [“To configure peak detection and evaluation \(ChIP\)”](#) on page 87.

The ChIP program uses the Whitehead per-array neighbourhood model to make binding calls. This model considers the  $p$ -values of both the probe in question and its neighbors. You can customize the parameters of the model, including the maximum distance between neighbor probes, and the stringency of the detection process. The model considers probes in groups of three, as shown at the bottom of [Figure 56](#). Two neighbor probes (blue) flank a central probe (red).

The program considers a probe “bound” if the average  $p$ -values for all three probes is less than a set cut-off value, and if either of the following is true:

- The  $p$ -values for the central probe and at least one of its neighbors are less than set cut-off values.
- The  $p$ -value of one (or optionally, another number) of the neighbors of the central probe is less than a set cut-off value.

To configure the model, you set the cut-off values of this significance heuristic.

The ChIP program lets you set the following parameters of the Whitehead per-array neighbourhood model:

Parameter	Comments
Maximum distance (in bp) for two probes to be considered as neighbors.	The program only considers probes to be neighbors if their genomic locations are within this threshold distance. The default value for this parameter is 1000 base pairs.
$P(X_{\text{bar}}) <$	<ul style="list-style-type: none"><li>• This parameter refers to the average <math>p</math>-value for the central probe and its neighbors.</li><li>• The default cut-off value is 0.001.</li><li>• Decreasing the cut-off value makes the selection more stringent.</li></ul>
Central probe has $P(X) <$	<ul style="list-style-type: none"><li>• The central probe is the red probe in <a href="#">Figure 56</a>.</li><li>• The default cut-off value is 0.001.</li><li>• Decreasing the cut-off value makes the selection more stringent.</li></ul>
At least one neighboring probe has $P(X) <$	<ul style="list-style-type: none"><li>• Neighboring probes are probes to either side of the central probe. The blue probes in <a href="#">Figure 56</a> are the neighbors of the central (red) probe.</li><li>• The default cut-off value is 0.1.</li><li>• Decreasing the cut-off value makes the selection more stringent.</li></ul>
At least $n$ of the neighbors has $P(X) <$	<ul style="list-style-type: none"><li>• The default value for <math>n</math> is 1.</li><li>• The default cut-off value for <math>P(X)</math> is 0.005.</li><li>• Decreasing the cut-off value makes the selection more stringent.</li></ul>

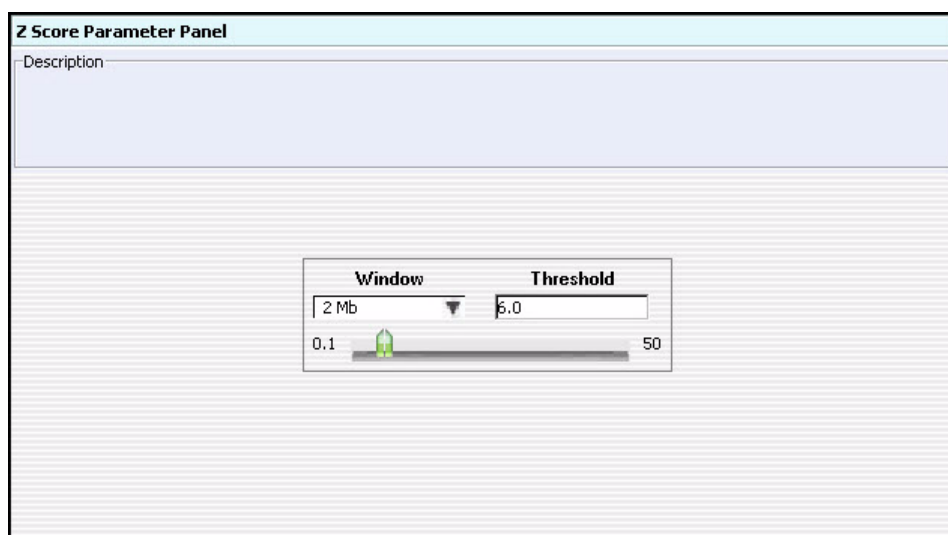
## Whitehead Per-Array Neighbourhood Model (Modified)

This model is exactly like the unmodified model, except that in the modified model, the number of neighbors includes the probe itself, so the default value for **n** is 2 instead of 1.

**Purpose:** This dialog box lets you customize the parameters of the Whitehead per-array neighbourhood model. The ChIP application uses this model to make binding calls based on the *p*-values of each probe and its neighbors.

**To open:** This parameter panel is displayed when you click **Whitehead Per-Array Neighbourhood Model (Modified)** under Peak Detection and Evaluation in the ChIP Analysis Method Navigator. See [“To configure peak detection and evaluation \(ChIP\)”](#) on page 87.

## Z Score Parameter Panel



**Figure 57** Z Score Parameter Panel

**Purpose:** This parameter panel lets you configure the Z-Score aberration detection algorithm for CGH. The Z-Score algorithm is a quick method of detecting aberrant regions. It calculates intervals using a sliding window of fixed size, and is especially useful in the exploratory phase of CGH data analysis. For more information on the Z-Score algorithm, see the *CGH Interactive Analysis User Guide*.

**To open:** The Z Score Parameter Panel appears when you Select **Z Score** under Aberration in the Analysis Method Navigator.

**Window** The size of the sliding window. You can type a window size in Kb or Mb – for example, 2 Mb. You can also select a value from the list.

Alternatively, you can specify the window size in terms of data points (pt). This sets the window size to the specified number of contiguous data points. Type the desired number of data points, for example 30 pt, or select a value from the list.

**Threshold** The minimum Z-Score for the Z-Score algorithm to consider a region aberrant. Type a value from 0.1 to 50, or use the slider to set the value. In general, to increase the stringency of aberration detection, increase the threshold value.

# Summary Console/Progress Tabs

In Workflow mode, the Summary Console and Workflow Progress Tabs provide information on the status of each workflow you run. The Summary Console tab displays the workflows you run, and the status of each. Named workflow progress tabs show information about each single workflow run, including the analysis settings used, and a running log of progress through the workflow.

## Summary Console tab

Summary Console   FE_Hu22K   FE_Hu22K1   CGH_9_9   SNPWorkflow1   WFSNP3   WFSNP44							
No.	Application Type	Workflow Name	Analysis Method	Experiment Name	Status	Current Step	Display Tab
1	FE	Workflow0			Failed	-	
2	FE	FE_Hu22K			Complete	-	
3	CGH	CGH_9_9	Workflow1	Workflow1	Complete	-	
4	CGH	SNPWorkflow1	Workflow1	Workflow2_SNP	Complete	-	
5	CGH	WFSNP3	Workflow1	SNP99	Complete	-	
6	CGH	WFSNP44	Workflow1	SNP144	Complete	-	
							<div>Refresh Status   Abort Workflows &amp; Clear Table</div>

Figure 58 Summary Console tab

The Summary Console tab displays the workflows you run, and gives basic identifying information and the workflow status. It also lets you manage the workflows.

NOTE




The Summary Console and workflow tabs include workflows for CGH, ChIP, and SureSelect Target Enrichment. For information on SureSelect Target Enrichment workflows, see the *SureSelect Target Enrichment User Guide*.

No.

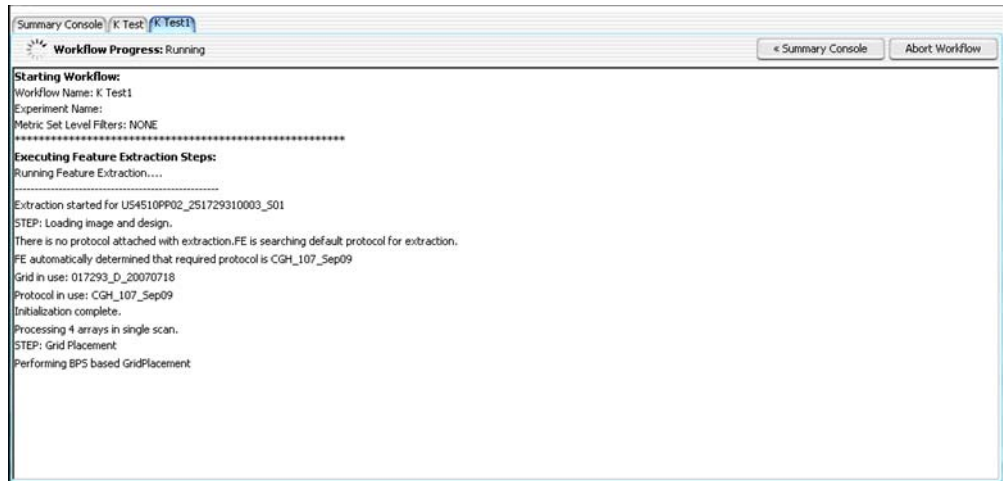
(Read-only) The order in which the workflows were started, from first to most recent.

Application Type

(Read-only) The basic application type (CGH or ChIP, for example) for the workflow.

<b>Workflow Name</b>	(Read-only) The name of the workflow described in the row.
<b>Analysis Method</b>	(Read-only) The name of the analysis method used in the workflow.
<b>Experiment Name</b>	(Read-only) The name of the output experiment that the workflow generates. By default, the program gives the experiment the same name as the Analysis Method. However, you can change the experiment name before you run the workflow. You look at the experiment, including its results, in the interactive tabs.
<b>Status</b>	(Read-only) Indicates whether the workflow is <b>Running</b> or <b>Complete</b> .
<b>Current Step</b>	(Read-only) For a workflow that is running, shows the progress through the steps of the workflow.
<b>Display Tab</b>	In this column, in the row of the desired workflow, click  to open the tab for the selected workflow. This lets you review information about the workflow.
<b>Refresh Status</b>	Updates the status of all workflows. The program also updates the status of the workflows in the workflow list in real time.
<b>Abort Workflows &amp; Clear Table</b>	Opens a Confirm dialog box that asks if you are sure you want to abort all workflows. If you click <b>Yes</b> , the program stops the execution of all workflows, and removes all workflows from the list. It also removes all named workflow tabs.
	(Available if tabs are hidden past the left edge of the pane) Shifts the display of tabs to the left to reveal hidden tabs.
	(Available if tabs are hidden past the right edge of the pane) Shifts the display of tabs to the right to reveal hidden tabs.

## Workflow progress tabs



**Figure 59** Workflow progress tab.

The program creates a separate tab for each workflow that you run. The name displayed is the name you specified as the Workflow Identifier when you started the workflow run. Each workflow progress tab shows the analysis settings, and a log of workflow activity.

- Workflow Progress** Shows the status of the workflow, either **Running** or **Complete**.
- Summary Console** Opens the Summary Console tab, where you see a list of all workflows and check the status of each. See [“Summary Console tab”](#) on page 173.
- Abort Workflow** Appears only when a workflow is running and that workflow’s progress tab is selected. Click to abort the running workflow and remove its tab from the Summary Console/Progress tabs pane.
- Close Tab** Removes the current tab, and removes its row in the Summary Console Tab.
- Text Box** Displays analysis settings, and a running log of workflow activity.

## 4 Workflow Reference

### Workflow progress tabs



(Available if tabs are hidden past the left edge of the pane) Shifts the display of tabs to the left to reveal hidden tabs.

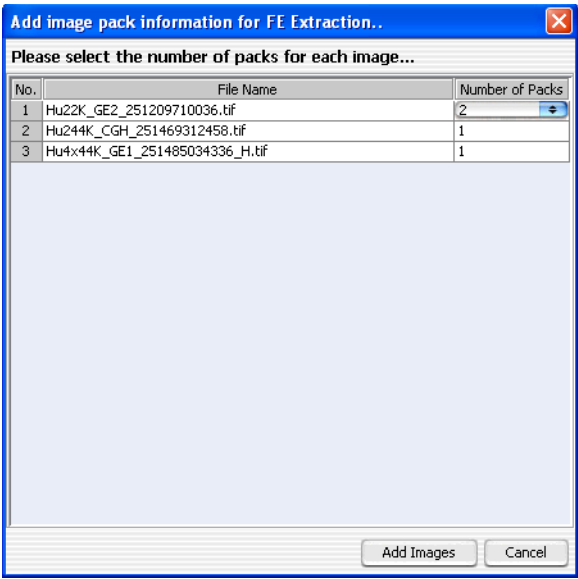


(Available if tabs are hidden past the right edge of the pane.) Shifts the display of tabs to the right to reveal hidden tabs.



# Dialog Boxes

## Add Image Pack Information for FE Extraction



**Figure 60** Add image pack information for FE Extraction dialog box

**Purpose:** To select the number of image packs to open for multi-pack image files to be opened.

**To open:** This dialog box appears when you click **Open** after you select an image file from the Open dialog box.

For each image file, click the **Number of Packs** and select the number of packs included in the image file to be imported. For example, for a 2-pack array, select **2** for Number of Packs. The Number of Packs is set to 1 by default.

- Add Images**
- Click to add the images to the workflow.
- Cancel**
- Closes the dialog box. No images are added.

## Create Analysis Method



**Figure 61** Create Analysis Method dialog box

**Purpose:** Used to give a name, (optionally) apply a password, and create an analysis method.

**To open:** This dialog box appears when you click **New** on the workflow command ribbon.

**Enter Analysis Method Name**

Type the name for the analysis method you are creating.

**Apply Password**

Mark this box if you want to protect the analysis method with a password. When this box is marked, the Set Workflow Password dialog box will appear when you click **OK**. See “[Set Password](#)” on page 192.

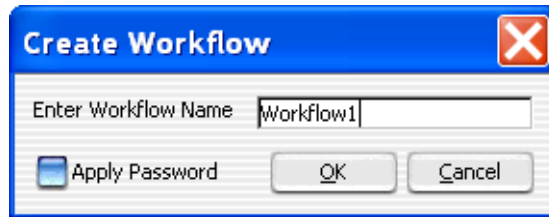
**OK**

Click this button to create the new analysis method with the designated name.

**Cancel**

Click to cancel the operation.

## Create Workflow



**Figure 62** Create Workflow dialog box

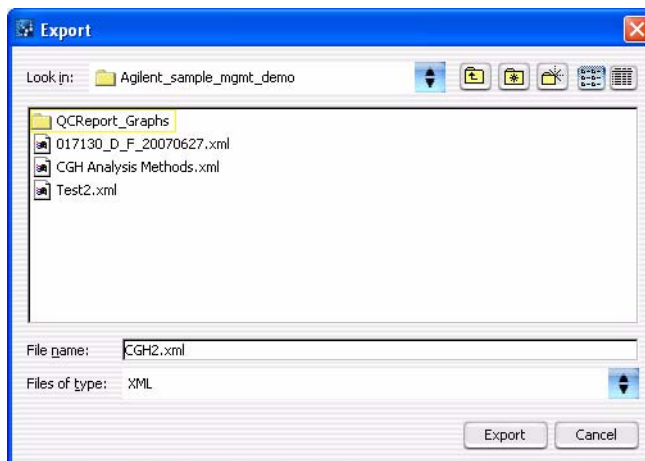
**Purpose:** To name a new workflow, and apply a password to the workflow.

**To open:** From the Workflow command ribbon, click **New**.

**Enter Workflow Name** Type the name of the new workflow here.

**Apply Password** Mark the box to protect the workflow with a password. If this check box is marked, when you click **OK**, the Set Password dialog box appears, where you type a password for the workflow. See “[Set Password](#)” on page 192.

## Export



**Figure 63** Export dialog box

**Purpose:** Used to designate a location and file name to export analysis methods.

**To open:** This dialog box opens when you click **OK** from the Export Analysis Method(s) dialog box.

Use the buttons at the top of the dialog box to change the display.

**Look in** Click the arrow and select the folder where you wish to export the file.

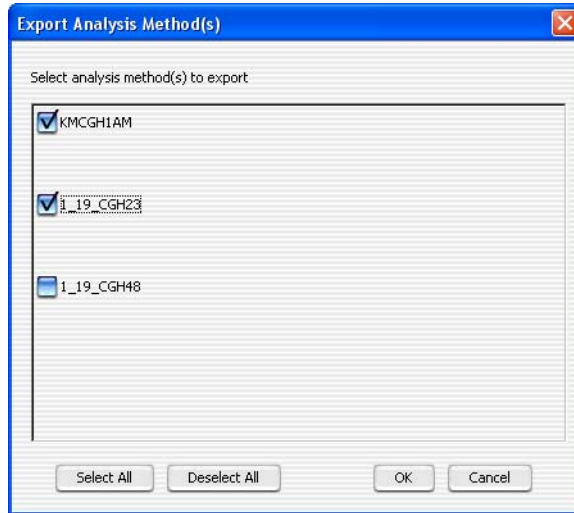
**File name** Type the name you wish to use for the exported file.

**Files of type** Shows the type of files displayed in the window. Click the arrow to change the type of files displayed.

**Export** Click to export the currently-selected analysis methods to the file.

**Cancel** Click to cancel the operation.

## Export Analysis Method(s)



**Figure 64** Export Analysis Method(s) dialog box

**Purpose:** Used to select analysis methods for export.

**To open:** On the command ribbon, click **Export Analysis Method(s)**.

A list of analysis methods currently saved in the program appears. Click the box next to an analysis method to select it for export, or use the buttons as described below.

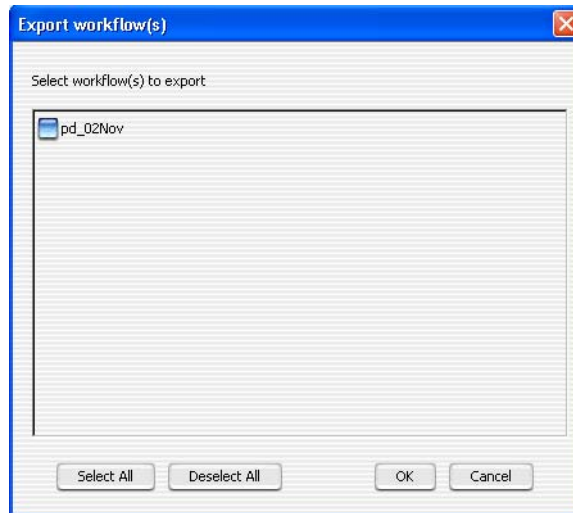
**Select All** Selects all displayed analysis methods.

**Deselect All** Clears the selection from all analysis methods.

**OK** Once one or more analysis method is selected, click **OK** to export those analysis methods to a file. The Export dialog box is opened.

**Cancel** Cancels the export operation and closes the dialog box.

## Export Workflow(s)



**Figure 65** Export Workflow(s) dialog box

**Purpose:** This dialog box is used to select workflows for export.

**To open:** On the command ribbon, click **Export Workflow(s)**.

A list of workflows currently saved in the program appears. Click the box next to a workflow to select it for export, or use the buttons as described below.

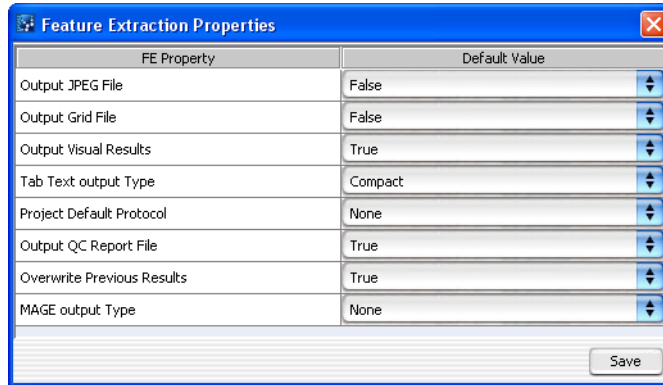
**Select All** Selects all displayed workflows.

**Deselect All** Clears the selection from all workflows.

**OK** Once one or more workflow is selected, click **OK** to export those workflows to a file. The Export dialog box is opened.

**Cancel** Cancels the export operation and closes the dialog box.

## Feature Extraction Properties



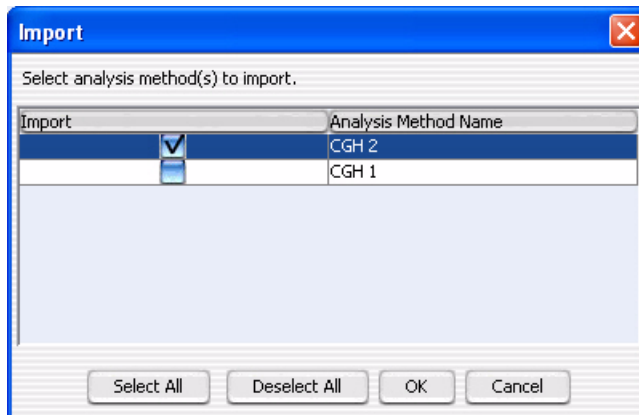
**Figure 66** Feature Extraction Properties dialog box

**Purpose:** To use Workflow to display and change the parameters for Feature Extraction.

**To open:** In the Run Workflow Navigator, make sure image files are already open, click **Feature Extraction**, and then in the Output path for FE output parameter panel, click **FE default parameters being used**.

This dialog box displays the current FE parameters. To change a parameter, click the field next to the parameter and select the new value. Click **Save** to save any changes and close the dialog box.

## Import



**Figure 67** Import dialog box

**Purpose:** Used to select the analysis method(s) or workflow(s) to be imported from a selected workflow or analysis method file.

**To open:** This dialog box appears when you select a file and click **Import** from the Import Workflow(s) or Import Analysis Method(s) dialog box.

A list of workflows or analysis methods in the selected import file is shown. Click to select the workflows or analysis methods to be imported, or use the buttons at the bottom of the dialog box.

**Select All** Selects all displayed workflows or analysis methods.

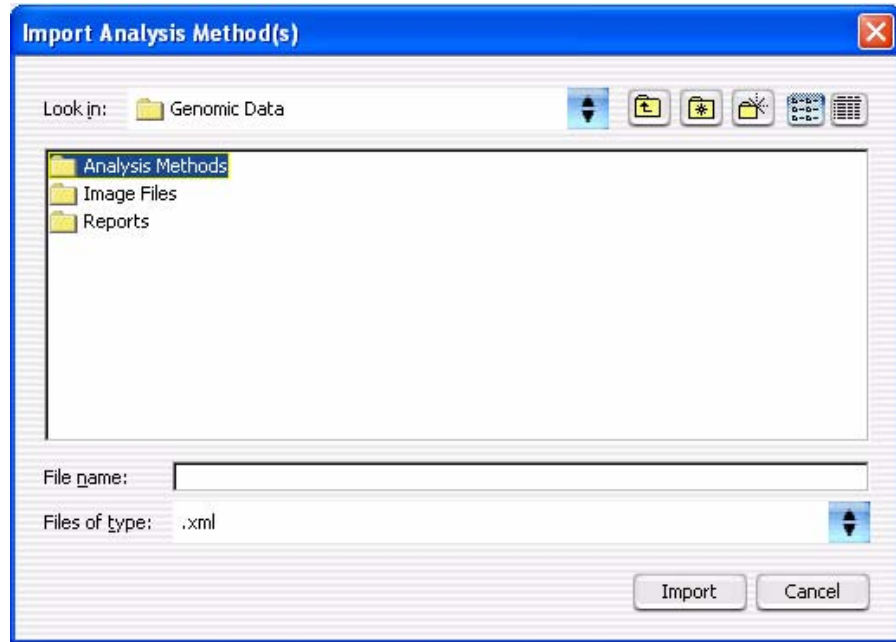
**Deselect All** Clears the selection from all workflows or analysis methods.

**OK** Once one or more workflows or analysis methods is selected, click **OK** to import the workflows or analysis methods. A status box that shows the status of the imported workflows or analysis methods is displayed.

**Cancel** Cancels the import operation and closes the dialog box.



## Import Analysis Method(s)



**Figure 68** Import Analysis Method(s) dialog box

**Purpose:** Used to select an analysis method file to be imported into the program.

**To open:** On the command ribbon, click **Analysis Method**, and then click **Import > Analysis Method(s)**.

**Look in** Click the arrow and select the folder from which you want to import a file.

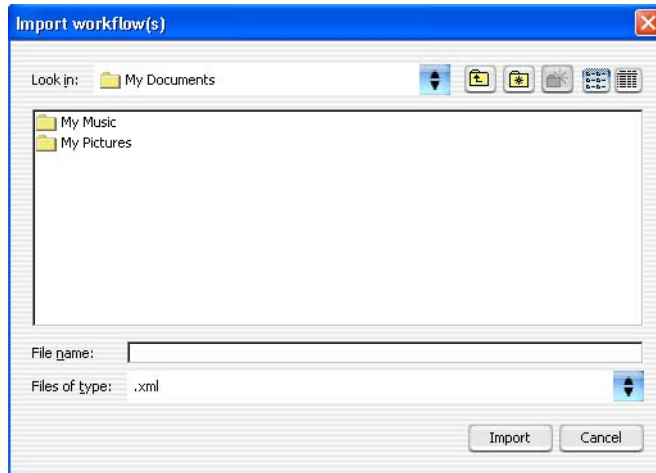
**File name** Type the name of the file you wish to import. Or, click to select the file from the displayed files.

**Files of type** Shows the type of files displayed in the window. Click the arrow to change the type of files displayed.

**Import** Click to import the currently-selected analysis method file into the program.

**Cancel** Click to cancel the operation.

## Import Workflow(s)



**Figure 69** Import Workflow(s) dialog box

**Purpose:** Used to select an workflow file to be imported into the program.

**To open:** Click **Import > Workflow(s)** from the command ribbon.

**Look in** Click the arrow and select the folder from which you want to import a file.

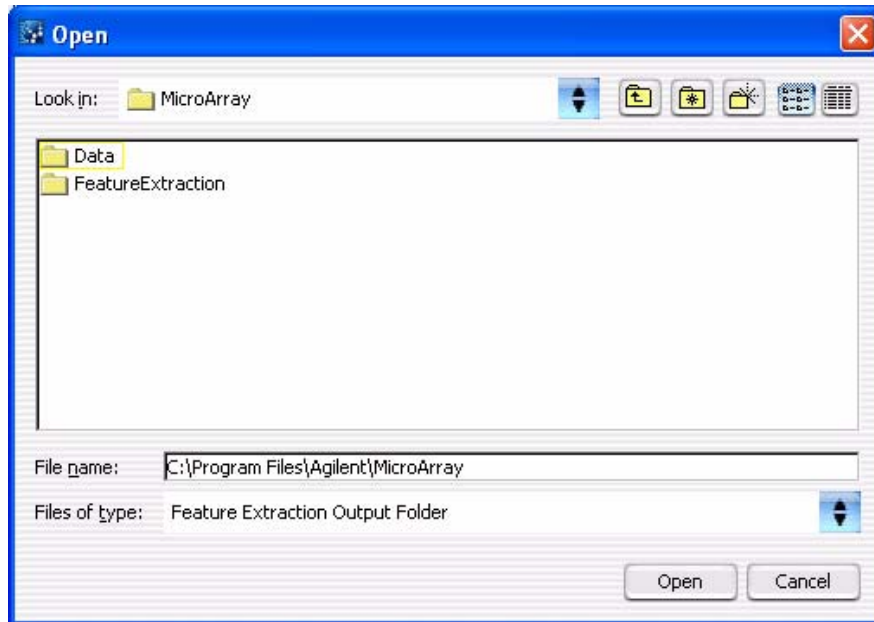
**File name** Type the name of the file you wish to import. Or, click to select the file from the displayed files.

**Files of type** Shows the type of files displayed in the window. Click the arrow to change the type of files displayed.

**Import** Click to import the currently-selected workflow file into the program.

**Cancel** Click to cancel the operation.

## Open



**Figure 70** Open dialog box

**Purpose:** To select the files you want to open, or to identify the location where you want files to be stored.

**To open:** This dialog box appears when you click **Add** at the bottom of the Image Files parameters panel. This also appears when you click **Browse** in a parameter panel or dialog box.

**Look in** Select folder where the files are located. To browse for a location, click the arrow and browse to the desired folder.

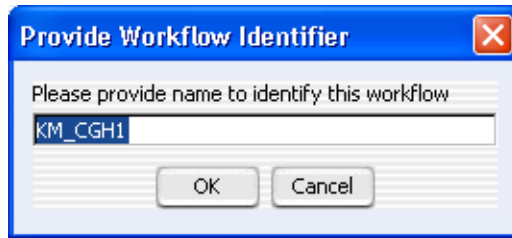
**File name** Type the name for the file you want to open, or click the file to select it. To select multiple files to open, hold down the **Ctrl** key and click the files to open.

**Files of type** Expected file type is displayed. Click to change displayed file types.

**Open** Click open the open the Add Image Pack Information dialog box. See [“Import FE Image Files Parameter Panel”](#) on page 146.

**Cancel** Click this to cancel the operation.

## Provide Workflow Identifier



**Figure 71** Provide Workflow Identifier dialog box

**Purpose:** To designate a name used in the Summary Console to identify a workflow to be run.

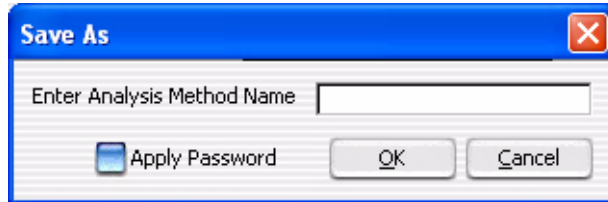
**To open:** This dialog box appears when you click **Run** to run a workflow.

**Provide a name to identify this workflow** Type a name for the workflow progress tab.

**OK** Click to accept the workflow identifier and start the workflow.

**Cancel** Click to cancel the operation without running the workflow.

## Save As



**Figure 72** Save As dialog box

**Purpose:** To type a name, apply a password (optional) and save an analysis method or a workflow.

**To open:** Click **Save As** from the workflow command ribbon.

**Enter Workflow  
Name/ Enter  
Analysis Method  
Name**

Type the name for the analysis method or workflow to save.

**Apply Password**

Click this box to protect the analysis method or workflow with a password.

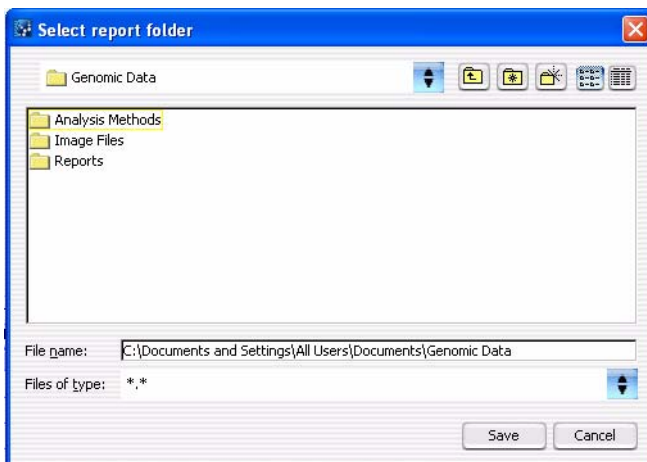
**OK**

Click to accept the password and close the dialog box. If the Apply Password box is marked, the Set Analysis Method Password dialog box opens. See [“Set Password”](#) on page 192.

**Cancel**

Click to cancel the operation.

## Select Report Folder



**Figure 73** Select report folder dialog box

**Purpose:** Used to select the folder location and name to store a CGH or ChIP report.

**To open:** From any of the CGH or ChIP Reports parameter panels, click **Browse**.

Click the arrow and select the folder, or browse to the location where you want to save the report file.

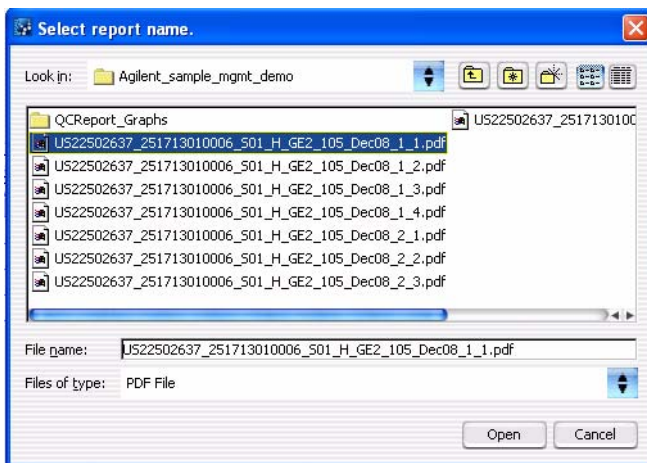
**File name** Type the name of the file to save. Or, click to select the file from the displayed files.

**Files of type** Shows the type of files displayed in the window. Click the arrow to change the type of files displayed.

**Save** Click to save the report file.

**Cancel** Click to cancel the operation.

## Select Report Name



**Figure 74** Select report name dialog box

**Purpose:** Used to select the folder location and name to store a CGH report.

**To open:** From any of the CGH Reports parameter panels, click **Browse**.

Click the arrow and select the folder, or browse to the location where you want to save the report file.

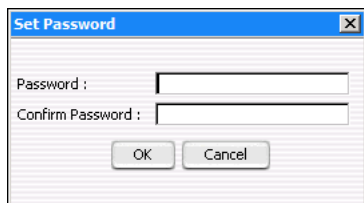
**File name** Type the name of the file you wish to save. Or, click to select the file from the displayed files.

**Files of type** Shows the type of files displayed in the window. Click the arrow to change the type of files displayed.

**Open** Click to save the report file.

**Cancel** Click to cancel the operation.

## Set Password



**Figure 75** Set Password dialog box

**Purpose:** Used to create a password for a newly-created analysis method or workflow, or to type the password when trying to open a password-protected analysis method or workflow.

**To open:** This dialog box appears when you create a password-protected analysis method or workflow, or when you select a password-protected analysis method or workflow.

**Password** Type the password you wish to use.

**Confirm Password** Retype the password to confirm it.

**OK** Click this button to accept the password and close the dialog box.

**Cancel** Click this to cancel the operation.





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## **In this book**

This guide describes how to use the Workflow utility of Agilent Genomic Workbench to extract image files with Agilent Feature Extraction software and/or analyze data using CGH and ChIP analysis software.

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Revision A1, October 2015



G3800-90026



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