

Agilent Genomic Workbench 7.0 Workflow

User Guide

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Notices

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Manual Part Number

G3800-90050

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 7.0 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 7.0. 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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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 10.7 (or higher) license and a CGH or ChIP license to run a *feature extraction* workflow.

NOTE

To run a Feature Extraction workflow on CGH+SNP microarrays, you must have Feature Extraction 10.10 or higher.

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](#).

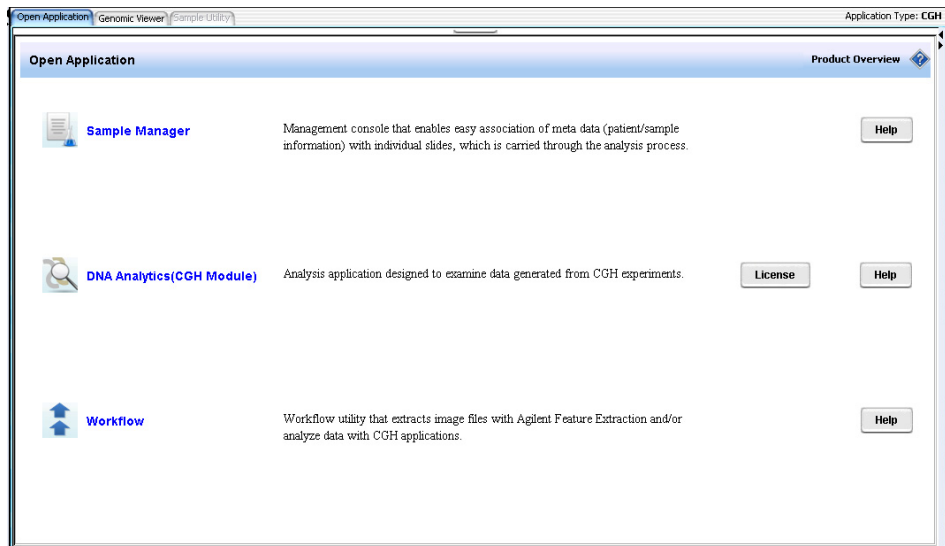


Figure 1 Open Application pane

OR

At the top of the Agilent Genomic Workbench window, click the **Workflow** tab. See [Figure 2](#).

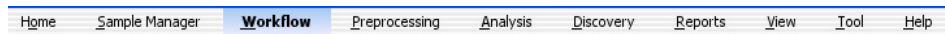


Figure 2 Workflow tab

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

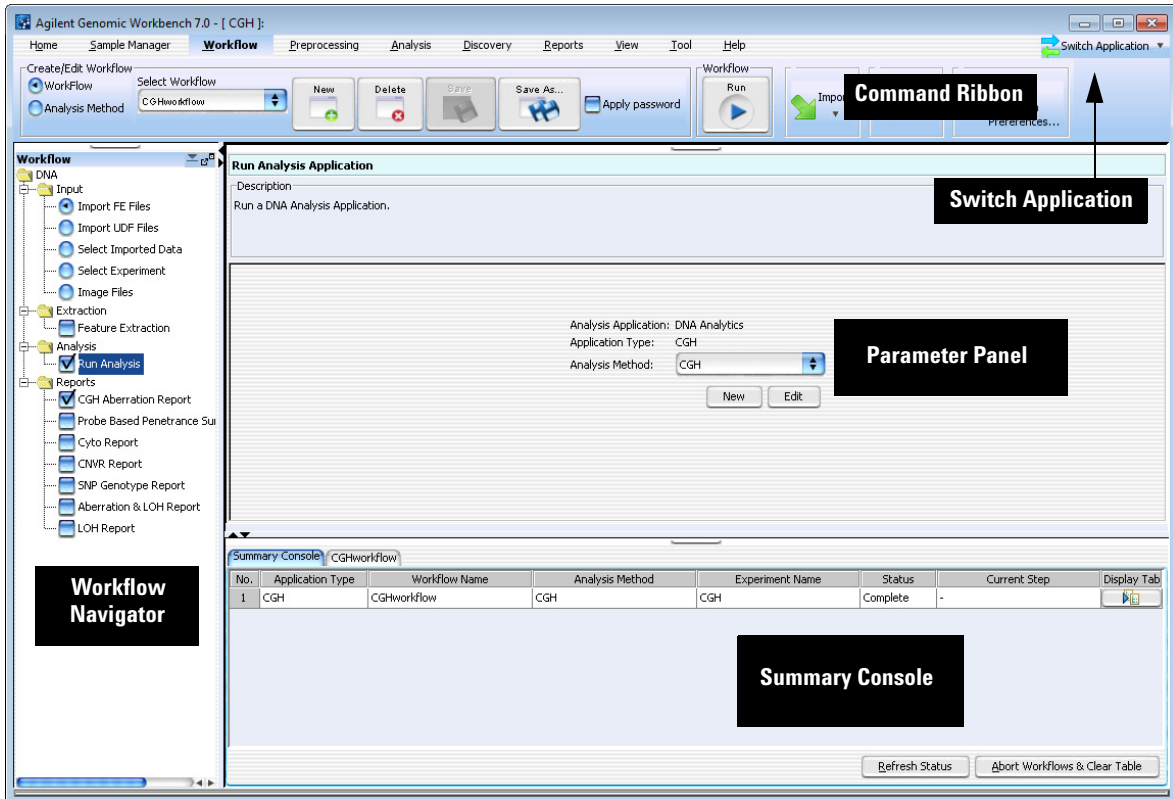


Figure 3 Workflow Main Window

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 96.

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

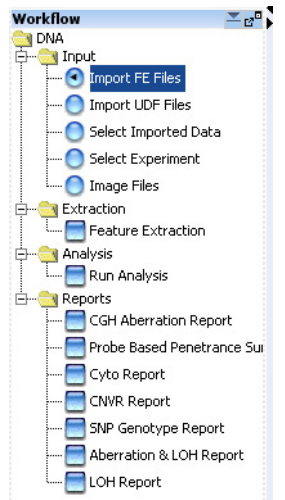


Figure 4 Workflow Navigator

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 19.

On the next page is a typical CGH workflow analysis.

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

1 Getting Started

To change settings for CGH workflow analysis

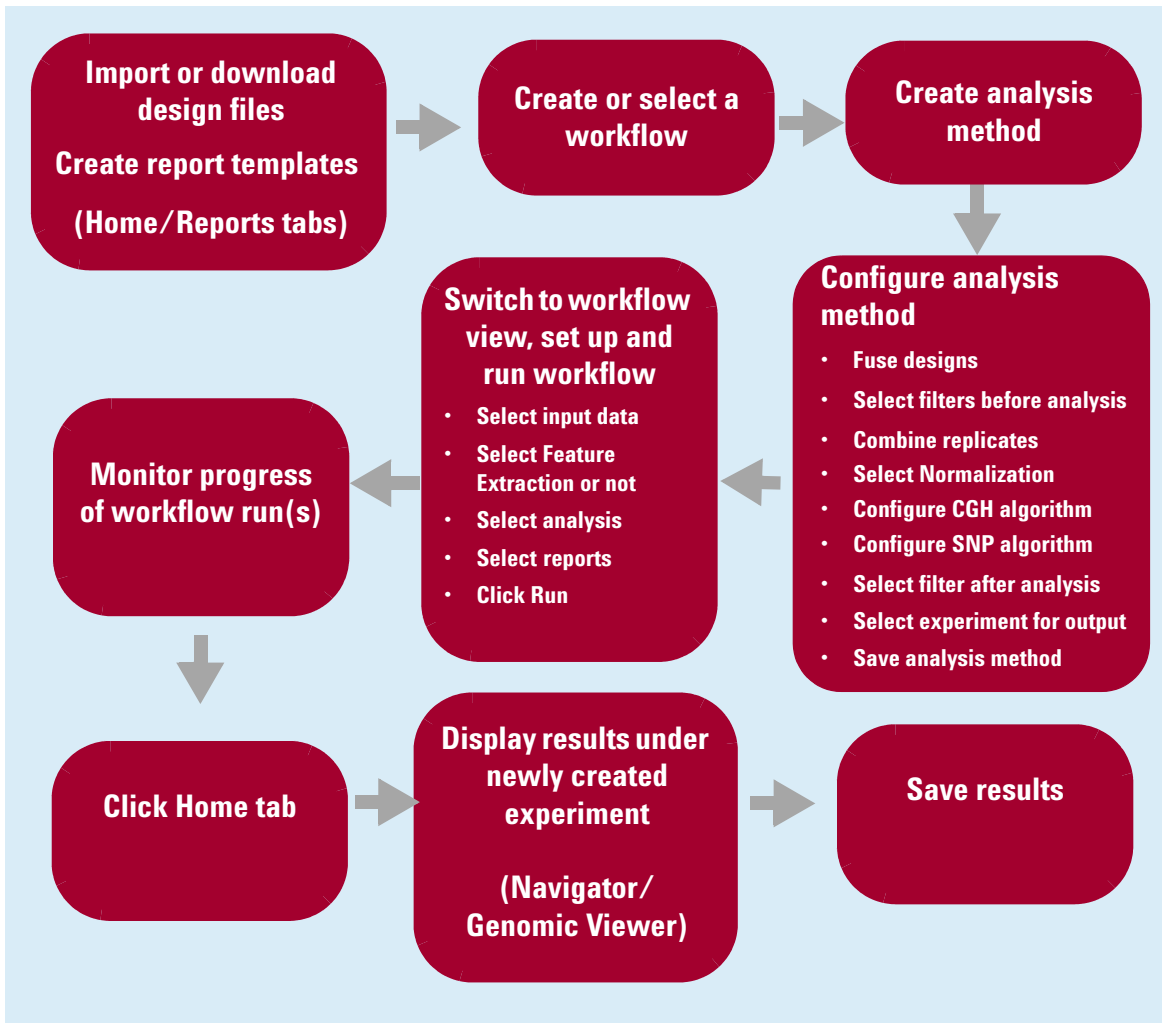
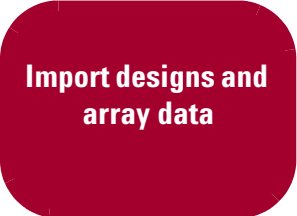


Figure 5 Typical CGH Workflow analysis pathway

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
 <p>Import designs and array data</p>	<p>In order to perform Feature Extraction in a workflow,</p> <ul style="list-style-type: none"> The design(s) for the data you want to extract must be in the Feature Extraction database <ul style="list-style-type: none"> To automatically add designs during workflow, your eArray settings for Feature Extraction must contain a valid Username and Password, and you must select Use eArray server during extraction and Check for updates of grid template in the Advanced Options of the eArray Login Settings dialog box OR Manually add the designs to the Feature Extraction program The design(s) for the data you want to extract must be in the Agilent Genomic Workbench database <ul style="list-style-type: none"> Manually import the design(s) from the Home tab of Agilent Genomic Workbench OR Make sure the design file is in the workflow output path for Feature Extraction. <p>In order to perform an analysis workflow (no Feature Extraction), the design(s) for the extractions you want to analyze must be in the Agilent Genomic Workbench database.</p> <ul style="list-style-type: none"> Manually import the design(s) from the Home tab of Agilent Genomic Workbench OR Select the location (path) of the design file(s) when you select the files for the workflow run. The workflow will automatically import them during the workflow. 	<ul style="list-style-type: none"> For information on how to add designs (grid templates) to the Feature Extraction database, see the <i>Feature Extraction User Guide</i>. For information on how to add data files and designs to Agilent Genomic Workbench, see the <i>CGH Interactive Analysis User Guide</i>. To set the output folder for Feature Extraction see “To set the output path for Feature Extraction results” on page 44.

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)


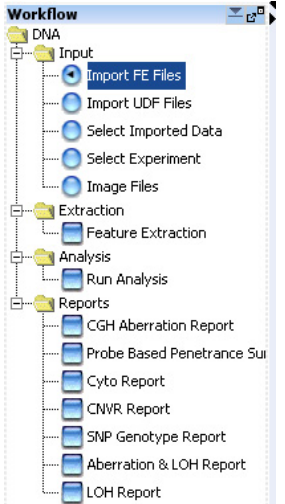

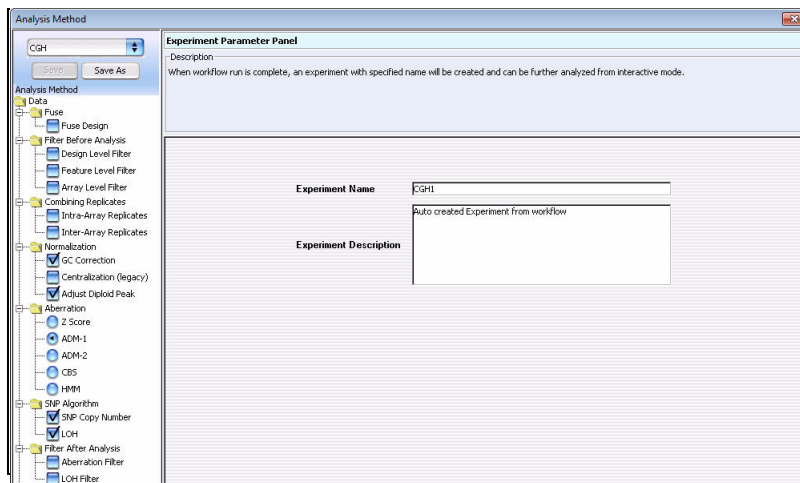
To do this	Follow these instructions	Comments
	<ol style="list-style-type: none">1 Click the Workflow tab. You see the Workflow Navigator.2 On the command ribbon, under Select Workflow, select an existing workflow to run. OR On the command ribbon, click New. Type a name for the workflow, and then click OK.	<ul style="list-style-type: none">• Using a workflow, you can run Feature Extraction with image files before the CGH analysis, if you have an Agilent Feature Extraction 10.7 (or higher) license. 

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"> 1 On the Workflow command ribbon, under Create/Edit Analysis Method, click Analysis Method. 2 Click New to create a new analysis method. A dialog box appears. 3 Type a Name for the analysis method, then click Ok. 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. 4 Type a name to change the name of the Experiment. 	<ul style="list-style-type: none"> • The name you type for the analysis method appears in the selection list under Create/Edit Analysis Method. • You can create more than one analysis method for use in multiple workflows.



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

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

To do this	Follow these instructions	Comments
 <p>Set up to combine replicates</p>	<ol style="list-style-type: none"> <li data-bbox="468 340 929 591"> <p>In the Analysis Method Navigator, under Combining Replicates, select any of these options:</p> <ul style="list-style-type: none"> <li data-bbox="496 427 929 479">• Intra-Array Replicates – Combines replicate probes within arrays. <li data-bbox="496 482 929 591">• Inter-Array Replicates – Combines replicate probes from multiple arrays, that are marked as replicate arrays using one of the available attributes. <li data-bbox="468 604 929 687"> <p>If you select the Inter-Array option, in the Parameters tab, select an array attribute next to Group By.</p> <p data-bbox="496 722 929 829">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.</p>	<ul style="list-style-type: none"> <li data-bbox="953 340 1272 626">• 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 data-bbox="953 630 1272 861">• 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.
 <p>Normalize the data</p>	<ul style="list-style-type: none"> <li data-bbox="468 887 929 999">• To correct for artifacts by performing a regression fit to GC content in a specified region flanking the probes, select GC Correction. <li data-bbox="468 1003 929 1086">• To centralize the data so that zero represents the diploid state, select the Adjust Diploid Peak check box. 	<ul style="list-style-type: none"> <li data-bbox="953 887 1272 965">• GC Correction is recommended for SNP Copy Number and LOH analyses. <li data-bbox="953 968 1272 1086">• The Adjust Diploid Peak centralization option is recommended for SNP Copy Number and LOH analyses. <li data-bbox="953 1090 1272 1260">• If you prefer to centralize the data using the algorithm from previous versions of Agilent Genomic Workbench, mark Centralization (legacy) under Normalization.

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)


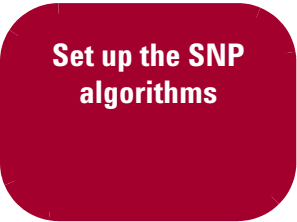

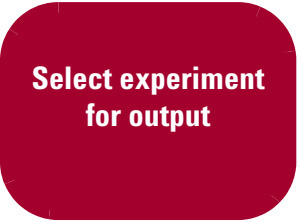

To do this	Follow these instructions	Comments
	<ol style="list-style-type: none">1 Select one of the aberration algorithms.2 Type or change parameters. <p>To remove long, low aberrations from the ADM1 or ADM2 results, select the Fuzzy Zero check box.</p> <p>If you use HMM (Hidden Markov Model), you must also select the State Parameters.</p>	<ul style="list-style-type: none">• ADM1 and 2 can use Fuzzy Zero to make the results more reliable by taking into account the global error across the chromosomes.• See the “Statistical Algorithms” chapter of the <i>CGH Interactive Analysis User Guide</i> for information on the aberration algorithm calculations and suggested thresholds.
	<ol style="list-style-type: none">1 Select one or both of the SNP calculations for analysis of CGH+SNP data.2 Type or change the parameters.<ul style="list-style-type: none">• SNP Copy Number – detects allele-specific copy numbers for SNP probes.• LOH – Detects regions that show loss of heterozygosity.	<ul style="list-style-type: none">• In order to select SNP Copy Number, you must also select an aberration algorithm (other than z-score or HMM).• In order to select LOH, you must first select SNP Copy Number.
	<ol style="list-style-type: none">1 Under Filter After Analysis, select the Aberration Filter check box.2 Select the DefaultAberrationFilter or another filter from the list, or create a new one.3 Under Filter After Analysis, select the LOH Filter check box.4 Select a filter from the list, or create a new one.	<ul style="list-style-type: none">• Suggested aberration filter for CNV analysis: 2 probes, 0.25 log ratio• To filter out all “nested” aberrations, mark Maximum Nesting Level in the Aberration Filter Parameter Panel and set the value to 0.• To create a new filter, see the <i>CGH Interactive Analysis User Guide</i>.

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

To do this	Follow these instructions	Comments
 <p data-bbox="182 430 421 499">Select experiment for output</p>	<ol style="list-style-type: none"> <li data-bbox="465 331 928 392">1 To change the name of the workflow experiment, under Output, click Experiment. <li data-bbox="465 392 928 453">2 Type the name you want to use for the experiment. <li data-bbox="465 453 928 513">3 Type a description for the experiment. 	<ul style="list-style-type: none"> <li data-bbox="943 331 1285 513">• 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.
 <p data-bbox="182 682 421 751">Save the analysis method</p>	<ol style="list-style-type: none"> <li data-bbox="465 609 928 730">1 Click Save. To save the analysis method with a new name, click Save As and then type the name of the new analysis method. 	

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)


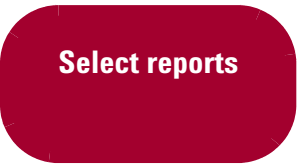
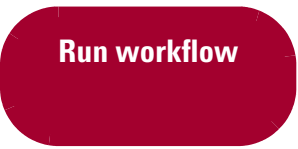

To do this	Follow these instructions	Comments
 <p>Set up a workflow and select input files</p>	<ol style="list-style-type: none">1 To display the Workflow Navigator to set up and run a workflow, on the Workflow command ribbon, click Workflow.2 In the Workflow Navigator, under Input, click one of these option buttons:<ul style="list-style-type: none">• Import FE Files – Select these files to analyze Feature Extraction log ratio data.• Import UDF Files – Select these text-delimited files to analyze non-Agilent data.• Select Imported Data – Select to analyze data that appears in the Navigator for Interactive Mode.• Select Experiment – Select an existing experiment to analyze data in the experiment.• Image Files – Select to run Feature Extraction.	<ul style="list-style-type: none">• The program creates a new experiment when you run a workflow.• If you select Image Files, the Feature Extraction check box must be selected.• Image files are the only input allowed for Feature Extraction.<ul style="list-style-type: none">• If you select image files, you must have a Feature Extraction license.• 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.• You may want to use the imported data from the design file 014698 that comes with the program to run a workflow.

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

To do this	Follow these instructions	Comments
	<p>3 Select any of the following Reports:</p> <ul style="list-style-type: none"> • CGH Aberration Report – Gives overall deletion and amplification tabular results, along with p-values (log 10). Select Probe Based or Interval Based report type. • Probe-based Penetrance Summary Report– Gives percent penetrance for each probe across all the selected arrays for amplification or deletion. • Cyto Report – Gives deletion and amplification tabular and graphical results with all the parameter settings in pdf format. Select a report template. • CNVR Report – 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. • SNP Genotype Report – Reports genotype and p-values for SNP probes in the microarray. • Aberration & LOH Report – Reports SNP data on a per-interval basis for aberration and LOH regions. • LOH Report – Reports SNP data on a per-interval basis for LOH regions. 	<ul style="list-style-type: none"> • See the <i>CGH Interactive Analysis User Guide</i> for more detailed instructions, and for the column formats of each report. • You must type a name and location for all the reports. • 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>.
	<p>1 Click the Run button. </p> <p>The Provide Workflow Identifier dialog box opens.</p> <p>2 Type a name for the workflow.</p> <p>3 Click OK to start the workflow.</p>	<ul style="list-style-type: none"> • 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.

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)


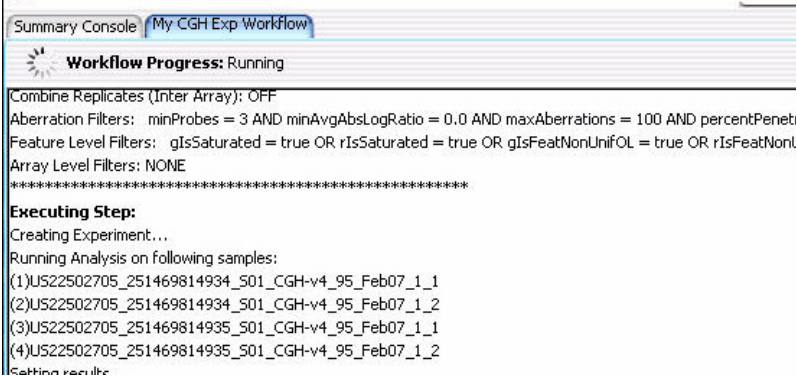

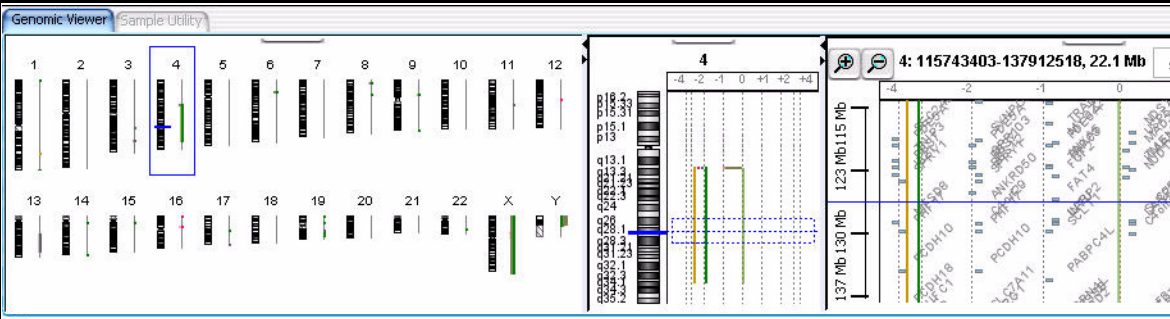
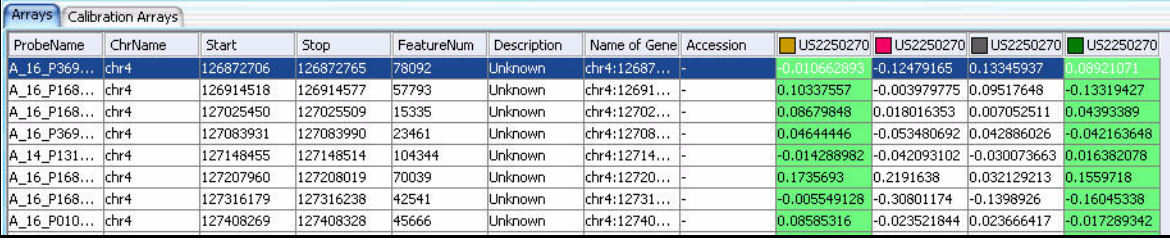
To do this	Follow these instructions	Comments
	 <pre>Summary Console My CGH Exp Workflow Workflow Progress: Running Combine Replicates (Inter Array): OFF Aberration Filters: minProbes = 3 AND minAvgAbsLogRatio = 0.0 AND maxAberrations = 100 AND percentPenet Feature Level Filters: gIsSaturated = true OR rIsSaturated = true OR gIsFeatNonUnifOL = true OR rIsFeatNon Array Level Filters: NONE ***** Executing Step: Creating Experiment... Running Analysis on following samples: (1)US22502705_251469814934_S01_CGH-v4_95_Feb07_1_1 (2)US22502705_251469814934_S01_CGH-v4_95_Feb07_1_2 (3)US22502705_251469814935_S01_CGH-v4_95_Feb07_1_1 (4)US22502705_251469814935_S01_CGH-v4_95_Feb07_1_2 Setting results</pre>	<ul style="list-style-type: none">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.
	<ol style="list-style-type: none">Click the Home tab.In the Navigator, double-click the newly created workflow experiment, and click Yes when asked if you want to select this experiment.Expand the Results folder under the experiment. Note that the WF result label is also in blue, to indicate this set of results is active.Select a chromosome in Genome View that appears to have a significant number of aberrations. (Chr 4 was selected in figure below).Move the blue cursor in Chromosome View to a region of interest for display in Gene View.In the Home command ribbon, click Save Experiment Result, and click Yes, then OK.	<ul style="list-style-type: none">The new workflow experiment appears in the Experiment pane. The experiment is marked with a W while the workflow is running.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.

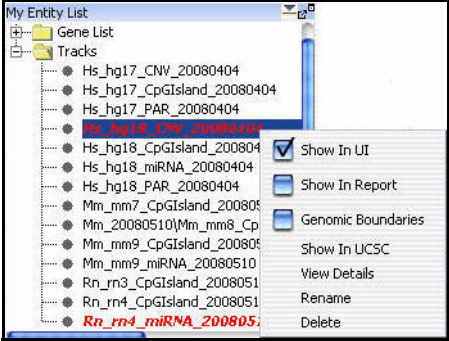
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To do this	Follow these instructions	Comments																																																																																																												
																																																																																																														
	 <table border="1"> <thead> <tr> <th>ProbeName</th> <th>ChrName</th> <th>Start</th> <th>Stop</th> <th>FeatureNum</th> <th>Description</th> <th>Name of Gene</th> <th>Accession</th> <th>US2250270</th> <th>US2250270</th> <th>US2250270</th> <th>US2250270</th> </tr> </thead> <tbody> <tr> <td>A_16_P369...</td> <td>chr4</td> <td>126872706</td> <td>126872765</td> <td>78092</td> <td>Unknown</td> <td>chr4:12687...</td> <td>-</td> <td>0.010662893</td> <td>-0.12479165</td> <td>0.13345937</td> <td>0.08921071</td> </tr> <tr> <td>A_16_P168...</td> <td>chr4</td> <td>126914518</td> <td>126914577</td> <td>57793</td> <td>Unknown</td> <td>chr4:12691...</td> <td>-</td> <td>0.10337557</td> <td>-0.003979775</td> <td>0.09517648</td> <td>-0.13319427</td> </tr> <tr> <td>A_16_P168...</td> <td>chr4</td> <td>127025450</td> <td>127025509</td> <td>15335</td> <td>Unknown</td> <td>chr4:12702...</td> <td>-</td> <td>0.08679848</td> <td>0.018016353</td> <td>0.007052511</td> <td>0.04393389</td> </tr> <tr> <td>A_16_P369...</td> <td>chr4</td> <td>127083931</td> <td>127083990</td> <td>23461</td> <td>Unknown</td> <td>chr4:12708...</td> <td>-</td> <td>0.04644446</td> <td>-0.053480692</td> <td>0.042886026</td> <td>-0.042163648</td> </tr> <tr> <td>A_14_P131...</td> <td>chr4</td> <td>127148455</td> <td>127148514</td> <td>104344</td> <td>Unknown</td> <td>chr4:12714...</td> <td>-</td> <td>-0.014288982</td> <td>-0.042093102</td> <td>-0.030073663</td> <td>0.016382078</td> </tr> <tr> <td>A_16_P168...</td> <td>chr4</td> <td>127207960</td> <td>127208019</td> <td>70039</td> <td>Unknown</td> <td>chr4:12720...</td> <td>-</td> <td>0.1735693</td> <td>0.2191638</td> <td>0.032129213</td> <td>0.1559718</td> </tr> <tr> <td>A_16_P168...</td> <td>chr4</td> <td>127316179</td> <td>127316238</td> <td>42541</td> <td>Unknown</td> <td>chr4:12731...</td> <td>-</td> <td>-0.005549128</td> <td>-0.30801174</td> <td>-0.1398926</td> <td>-0.16045338</td> </tr> <tr> <td>A_16_P010...</td> <td>chr4</td> <td>127408269</td> <td>127408328</td> <td>45666</td> <td>Unknown</td> <td>chr4:12740...</td> <td>-</td> <td>0.08585316</td> <td>-0.023521844</td> <td>0.023666417</td> <td>-0.017289342</td> </tr> </tbody> </table>	ProbeName	ChrName	Start	Stop	FeatureNum	Description	Name of Gene	Accession	US2250270	US2250270	US2250270	US2250270	A_16_P369...	chr4	126872706	126872765	78092	Unknown	chr4:12687...	-	0.010662893	-0.12479165	0.13345937	0.08921071	A_16_P168...	chr4	126914518	126914577	57793	Unknown	chr4:12691...	-	0.10337557	-0.003979775	0.09517648	-0.13319427	A_16_P168...	chr4	127025450	127025509	15335	Unknown	chr4:12702...	-	0.08679848	0.018016353	0.007052511	0.04393389	A_16_P369...	chr4	127083931	127083990	23461	Unknown	chr4:12708...	-	0.04644446	-0.053480692	0.042886026	-0.042163648	A_14_P131...	chr4	127148455	127148514	104344	Unknown	chr4:12714...	-	-0.014288982	-0.042093102	-0.030073663	0.016382078	A_16_P168...	chr4	127207960	127208019	70039	Unknown	chr4:12720...	-	0.1735693	0.2191638	0.032129213	0.1559718	A_16_P168...	chr4	127316179	127316238	42541	Unknown	chr4:12731...	-	-0.005549128	-0.30801174	-0.1398926	-0.16045338	A_16_P010...	chr4	127408269	127408328	45666	Unknown	chr4:12740...	-	0.08585316	-0.023521844	0.023666417	-0.017289342	
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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
<ul style="list-style-type: none">See the <i>CGH Interactive Analysis User Guide</i> for instructions on how to:<ul style="list-style-type: none">Create and use gene listsCreate and use tracksCustomize the appearance of the displaySee <i>"To set up the Run Analysis"</i> on page 46 for instructions on how to change the analysis method.See the <i>CGH Interactive Analysis User Guide</i> for instructions on how to use postprocessing Discovery statistics on the results.	<p>At this point you can add tracks with other gene information to the Gene View.</p> <ol style="list-style-type: none">In the My Entity List pane, double-click the Tracks folder.Right-click one of the tracks in the list.Select the Show in UI check box.  <ol style="list-style-type: none">To clear the tracks, right-click the Gene View, and click Preferences.Click Tracks, and clear the Show in UI check boxes, and click OK.	<ul style="list-style-type: none">With these tracks, you can see if the aberrations correlate with copy number variant regions. You can import other tracks as well.Select the Show in Report check boxes to add track information in the report.Select Genomic Boundaries to limit the analysis within the boundaries defined in the tracks.

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 19. 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 83.

1 Getting Started

To change settings for ChIP Workflow Analysis

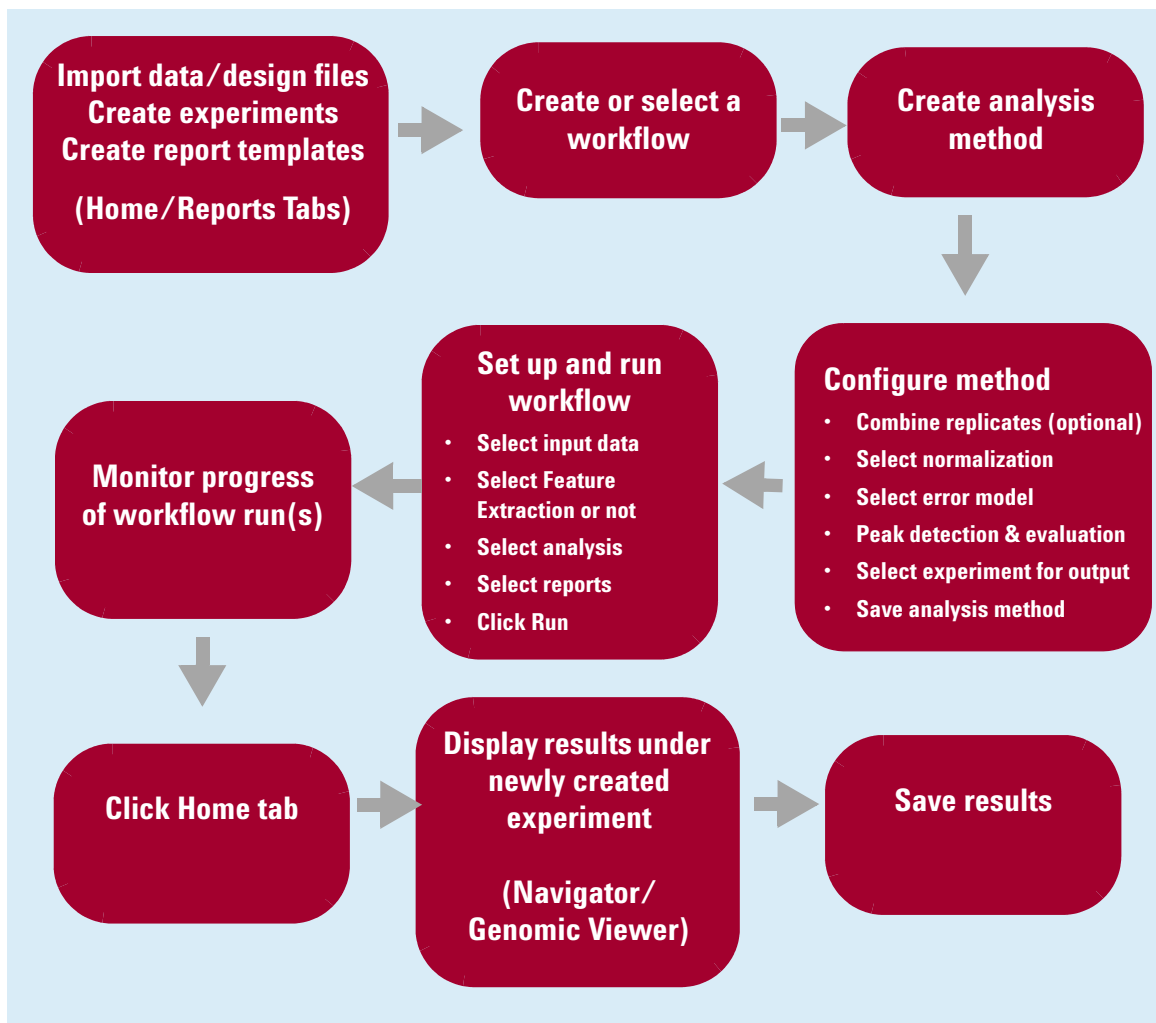


Figure 6 Typical ChIP Workflow analysis pathway

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">1 In any tab of Agilent Genomic Workbench, click the Help tab.2 On the Help Ribbon, click Workflow. The Workflow User Guide opens.
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">1 Set the desired application type from the Switch Application menu.2 In the Agilent Genomic Workbench tab bar, click Help. The names of the available user guides appear in the command ribbon.3 Click the desired help guide. The selected guide opens.

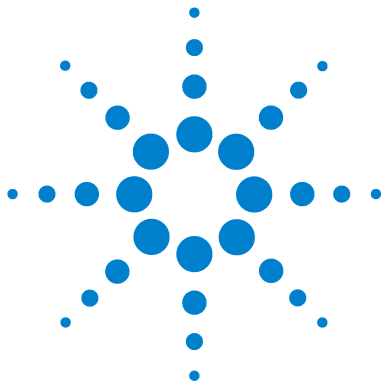
To contact Agilent Technical Support

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

To learn about Agilent products and services

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



2 Setting Up and Running Workflows

Creating and Managing Workflows	36
Setting up a Workflow for Feature Extraction	38
Setting up an Analysis Workflow	45
Running Workflows	59

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 96, and for the command ribbons of the Workflow tab, see [“Workflow Command Ribbons”](#) on page 98.

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 182.
- 3 In Enter Workflow Name, type a name.
If you intend to restrict access to this workflow, select **Apply Password**.
- 4 Click **OK**.
If you selected the Apply Password check box, the Set Password dialog box appears. See [“Set Password”](#) on page 196.
 - 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 45.
- 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 45.

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, select **Apply Password**.
The Set Password dialog box appears. See “[Set Password](#)” on page 196.
- 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 45.

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 Data folder for workflow or interactive analysis and data display
- The Array IDs in Sample Manager are updated

For more information on using Sample Manager, see the *Sample Manager User Guide*.

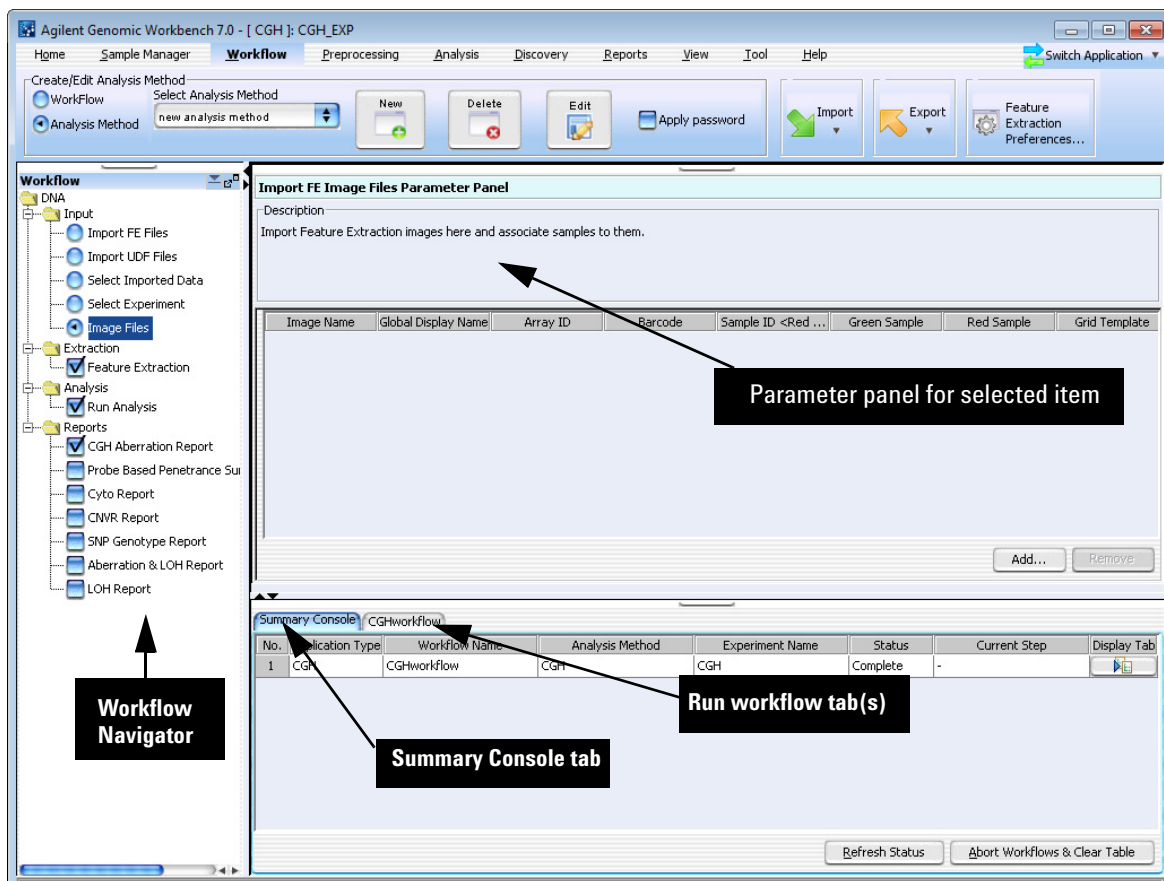


Figure 7 Workflow Main Window for Feature Extraction

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.

2 Setting Up and Running Workflows

To use eArray to update design/template files

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 selected **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 select **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*.

To set the location of the Feature Extraction software

In order for **Workflow** to automate Feature Extraction, you need to tell it where your Feature Extraction software is installed.

NOTE

Feature Extraction software must be version 10.7 (or higher) to work with Agilent Genomic Workbench 7.0. If you intend to perform feature extraction with CGH+SNP microarrays, you need Feature Extraction 10.10 (or higher).

1 From the **Genomic Workbench** menu bar, click **Workflow**.

On the command ribbon, click **Feature Extraction Preferences**.

The Feature Extraction Preferences dialog box opens. See “[Feature Extraction Preferences](#)” on page 186.

2 Type the location where your Feature Extraction software is installed. To browse for the location, click **Browse** and search for the correct folder. The default location for the Feature Extraction software is C:\Program Files\Agilent\MicroArray\Feature Extraction. Click on the folder, and then click **Open**. The path will appear in the **FE Location** box.

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 Setting Up and Running Workflows

To import an image file

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 148.

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 180.

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 148.

- 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.

2 Setting Up and Running Workflows

To set the output path for Feature Extraction results

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 check mark 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 167.
- 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 10.7 (or higher) installed on your computer. To run Feature Extraction for CGH+SNP microarrays, you must have 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 check mark 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 187.

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 96.

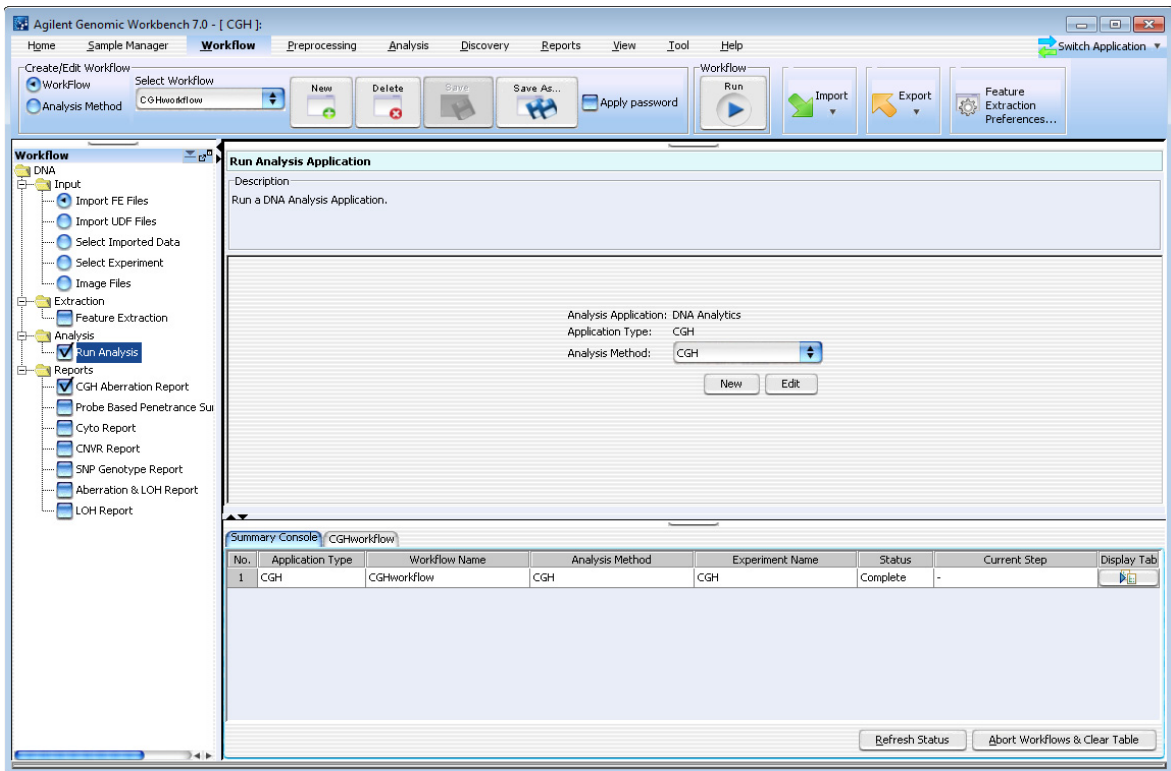


Figure 8 Workflow Window showing Run Analysis

2 Setting Up and Running Workflows

To set up the Run Analysis

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 68. 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 164 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 47.

- 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 70 for more information.
- 4 (optional) To create a new analysis method, click **New**. See [“To create a new analysis method”](#) on page 69 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 “Import FE Image Files Parameter Panel” on page 148. This is the only input option allowed for running a Feature Extraction in a workflow.

2 Setting Up and Running Workflows

To select workflow input

Table 3 Workflow Input for CGH Analysis

Option	Description
Import FE Files	Extracted microarray data that you have not yet imported into Agilent Genomic Workbench. For instructions on how to set the parameters for this option, see “To select FE data files for the workflow to import” on page 49.
Import UDF Files	Tab delimited Universal Data Files created by non-Agilent programs. For instructions on how to set the parameters for this option, see “To select UDF data files for the workflow to import” on page 50.
Select Imported Data	CGH microarray data that you have previously imported into Agilent Genomic Workbench. For instructions on how to set the parameters for this option, see “To use previously imported data as the workflow input” on page 51.
Select Experiment	CGH microarray data from an existing Agilent Genomic Workbench experiment. For instructions on how to set the parameters for this option, see “To use an experiment’s arrays as the workflow input” on page 52.

Table 4 Workflow Input for ChIP Analysis

Option	Description
Import Data Files	Extracted microarray data that you have not yet imported into Agilent Genomic Workbench. For instructions on how to set the parameters for this option, see “To select data files for the workflow to import” on page 52.

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 “To use previously imported data as the workflow input” on page 51.
Select Experiment	ChIP microarray data from an existing Agilent Genomic Workbench experiment. For instructions on how to set the parameters for this option, see “To use an experiment’s arrays as the workflow input” on page 52.

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 146.

- 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, select **Overwrite arrays with duplicate names**.

2 Setting Up and Running Workflows

To select UDF data files for the workflow to import

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 150.

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.

- 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 166.
- 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 166.

2 Setting Up and Running Workflows

To use an experiment's arrays as the workflow input

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 165.

- 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 146.

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

An Open dialog box appears.

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

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, select **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 reports (CGH)

When you run a workflow, the program can produce six 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, select 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.

2 Setting Up and Running Workflows

To select reports (CGH)

Table 5 CGH module reports

Report	Description/Instructions
CGH Aberration Report	<p>To set parameters for the report</p> <ol style="list-style-type: none"> 1 In the Workflow Navigator, in Reports, select CGH Aberration Report. Three settings appear in the parameter panel. See “Variance Stabilization” on page 170. 2 Under Report Type, select to generate a Probe Based report, an Interval Based report, or both a Probe & Interval Based report. 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. 4 Under Select File Location, select Report Flat Intervals to have aberration intervals reported without any nested structure. 5 Under Select File Location, select Generate report per array 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. 6 Under Select File Location, click Browse. The Select report folder dialog box appears. 7 Select a location for the report, and if necessary, change the File name. 8 Click Open. The location of the CGH Aberration Report appears in the parameter panel, in Select File Location. 9 Under Select File Location, select Overwrite if file exists to overwrite a previous report saved as the same filename and location.
Probe Based Penetrance Summary Report	<p>To set parameters for the report</p> <ol style="list-style-type: none"> 1 In the Workflow Navigator, in Reports, select Probe Based Penetrance Summary Report. Two settings appear in the parameter panel. See “Probe Based Penetrance Summary Report Parameter Panel” on page 161. 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. 3 Under Select File Location, click Browse. The Select report folder dialog box appears. 4 Select a location for the report, and if necessary, change the File name. 5 Click Open. The location of the Text Penetrance Summary Report appears in the parameter panel, in Report Location. 6 Under Select File Location, select Overwrite if file exists to overwrite a previous report saved as the same filename and location.

Table 5 CGH module reports (continued)

Report	Description/Instructions
Cyto Report	<p>To set parameters for the report</p> <ol style="list-style-type: none"> 1 In the Workflow Navigator, in Reports, select Cyto Report. Three settings appear in the parameter panel. See “Cyto Report Parameter Panel” on page 134. 2 In Select Report, select an existing Cyto Report. 3 Under Select File Location, click Browse. The Select report folder dialog box appears. 4 Select a location for the report, and if necessary, change the File name. 5 Click Open. The location of the Cyto Report appears in the parameter panel, in Report Location. 6 Under Select File Location, select Overwrite if file exists to overwrite a previous report saved as the same filename and location.
CNVR Report	<p>To set parameters for the report</p> <ol style="list-style-type: none"> 1 In the Workflow Navigator, in Reports, select CNVR Report. Three settings appear in the parameter panel. See “CNVR Report Parameter Panel” on page 133. 2 In CNVR Node Name, type a node name under which to access the CNVR report in interactive mode. 3 Under Select File Location, click Browse. The Select report folder dialog box appears. 4 Select a location for the report, and if necessary, change the File name. 5 Click Open. The location of the CNVR Report appears in the parameter panel, in Report Location. 6 Under Select File Location, select Overwrite if file exists to overwrite a previous report saved as the same filename and location.

2 Setting Up and Running Workflows

To select reports (CGH)

Table 5 CGH module reports (continued)

Report	Description/Instructions
SNP Genotype Report	<p>To set parameters for the report</p> <ol style="list-style-type: none">1 In the Workflow Navigator, in Reports, select SNP Genotype Report. Three settings appear in the parameter panel. See “SNP Genotype Report Parameter Panel” on page 169.2 In Output Format, select to format the report on a Full Genome or Per-Chromosome basis.3 Under Select File Location, click Browse. The Select report folder dialog box appears.4 Select a location for the report, and if necessary, change the File name.5 Click Open. The location of the SNP Genotype Report appears in the parameter panel, in Report Location.6 Under Select File Location, select Overwrite if file exists to overwrite a previous report saved as the same filename and location.
Aberration & LOH Report	<p>To set parameters for the report</p> <ol style="list-style-type: none">1 In the Workflow Navigator, in Reports, select Aberration & LOH Report. Three settings appear in the parameter panel. See “Aberration & LOH Report Parameter Panel” on page 121.2 Under Select File Location, click Browse. The Select report folder dialog box appears.3 Select a location for the report, and if necessary, change the File name.4 Click Open. The location of the Aberration & LOH Report appears in the parameter panel, in Report Location.5 Under Select File Location, select Overwrite if file exists to overwrite a previous report saved as the same filename and location.
LOH Report	<p>To set parameters for the report</p> <ol style="list-style-type: none">1 In the Workflow Navigator, in Reports, select LOH Report. Three settings appear in the parameter panel. See “LOH Report Parameter Panel” on page 158.2 Under Select File Location, click Browse. The Select report folder dialog box appears.3 Select a location for the report, and if necessary, change the File name.4 Click Open. The location of the LOH Report appears in the parameter panel, in Report Location.5 Under Select File Location, select Overwrite if file exists to overwrite a previous report saved as the same filename and location.

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, select 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>To set parameters for the Probe Report</p> <ol style="list-style-type: none"> 1 In the Analysis Method Navigator, in Reports select the box next to Probe Report. 2 In the Probe Report Settings parameter panel, click Browse. A Select report folder dialog box appears. 3 Select a location for the report, and if desired, change the File name. 4 Click Open. The location of the Probe Report appears, in Report Location.

2 Setting Up and Running Workflows

To select and configure reports (ChIP)

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>To set parameters for the Gene Report</p> <ol style="list-style-type: none">1 In the Analysis Method Navigator, select the box next to Gene Report. Three settings appear under Gene Report Settings.2 Select one of these check boxes:<ul style="list-style-type: none">• 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.• Show probe information – The resulting Gene Report contains additional information about the probes in the array.3 Under Gene Report Settings, click Browse. A Select report folder dialog box appears.4 Select a location for the report, and if desired, change the File name.5 Click Open. The location of the Gene Report appears, in Report Location.
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>To set parameters for the QC Report</p> <ol style="list-style-type: none">1 In the Analysis Method Navigator, select the box next to ChIP QC Report. One setting appears under QC Report Settings.2 In the QC Report Settings parameter panel, click Browse. A Select report folder dialog box appears.3 Select a location for the report, and if desired, change the File name.4 Click Open. The location of the QC Report appears, in Report Location.

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 192.

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 176.

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 178.

2 Setting Up and Running Workflows

To run a series of workflows

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 59.
- 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 61.

To run a series with the same workflow

- 1 Run the first workflow.
Follow the instructions in [“To run a workflow”](#) on page 59.
- 2 Click **Run** again.

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 192.

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 61.

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 176.

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 176.
- 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 178.

- 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.

2 Setting Up and Running Workflows

To abort a single workflow

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.

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 47.
- 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 52
 - [“To select FE data files for the workflow to import”](#) on page 49
 - [“To select UDF data files for the workflow to import”](#) on page 50
 - [“To use previously imported data as the workflow input”](#) on page 51
 - [“To use an experiment’s arrays as the workflow input”](#) on page 52
- 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 139.
- 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 63.

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).

2 Setting Up and Running Workflows

To export a workflow

- 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 185 for more information.
The Export dialog box appears. See “[Export](#)” on page 183.
- 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 190.

- 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 188.

- 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**.

2 Setting Up and Running Workflows

To import a workflow



3 Setting up Workflow Analysis Methods

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- Setting up CGH Analysis Methods 73
- Setting up CHIP Analysis Methods 83

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.

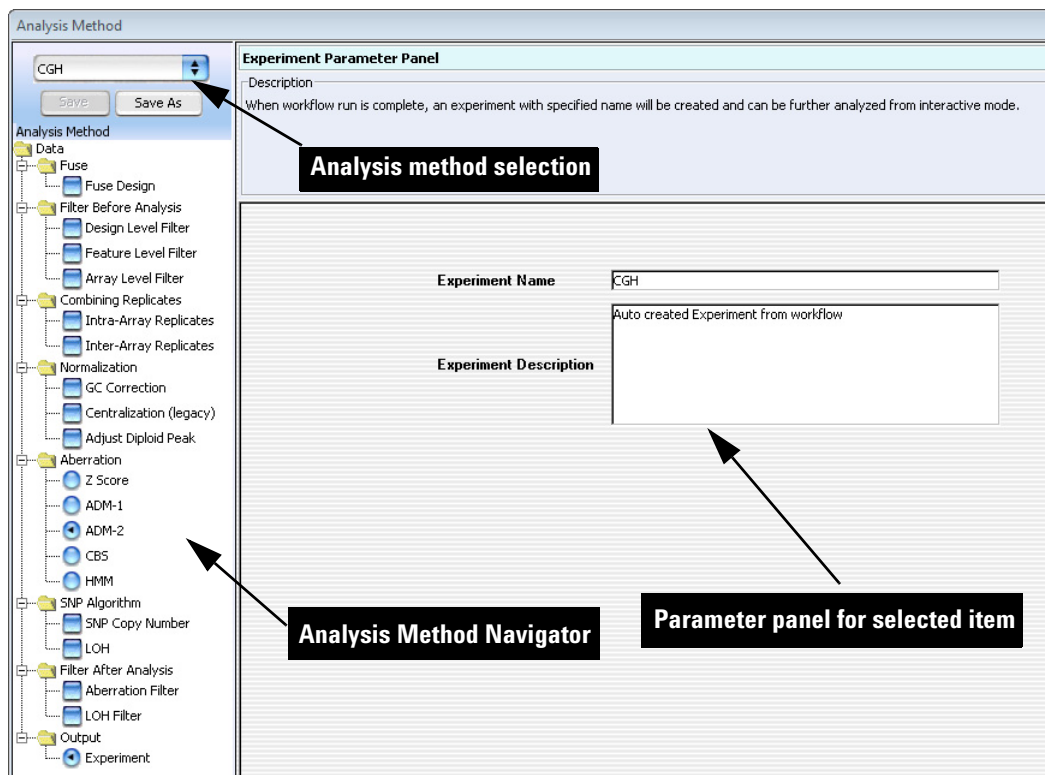


Figure 9 Analysis Method window for CGH

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 69).

- 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 181.

- 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 164 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 69.

3 Setting up Workflow Analysis Methods

To edit an existing analysis method

- 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 73 or “[Setting up ChIP Analysis Methods](#)” on page 83.

- 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 193.

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 193.

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 184 for more information.
The Export dialog box appears. See [“Export”](#) on page 183.
- 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 189.
- 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 188.
- 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 142.

- 2 Click **Select Normalization** and select None or Centralization (legacy).
- 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 83.

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, select **Design Level Filter**.

The Design Level Filter Parameter Panel appears. See “[Design Level Filter Parameter Panel](#)” on page 135.

- 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, select **Feature Level Filter**.

The Feature Level Filter Parameter Panel appears. See “[Feature Level Filter Parameter Panel](#)” on page 140.

- 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 75.

To configure the analysis method to apply an array level filter

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

The Array Filter Parameter Panel appears. See “[Array Level Filter Parameter Panel](#)” on page 127.

- 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 76.

To create or modify a feature level filter

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

The Feature Level Filter Parameter Panel appears. See “[Feature Level Filter Parameter Panel](#)” on page 140.

- 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.

3 Setting up Workflow Analysis Methods

To create or modify an array level filter

To create or modify an array level filter

- 1 In the Analysis Method Navigator, in Filter Before Analysis, select **Array Level Filter**.
The Array Level Filter Parameter Panel appears. See “[Array Level Filter Parameter Panel](#)” on page 127.
- 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, select **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, select **Inter-Array Replicates**.

The Inter-Array Replicates Parameter Panel appears. See “[Inter-Array Replicates Parameter Panel](#)” on page 154.

- 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 recommended for calculation of SNP Copy Number and LOH.

- 1 In the Analysis Method Navigator, in Normalization, select **GC Correction**.

The GC Correction parameter panel appears. See “[GC Correction Parameter Panel](#)” on page 143.

- 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.

- In the Analysis Method Navigator, in Normalization, select **Adjust Diploid Peak**.

3 Setting up Workflow Analysis Methods

To select aberration algorithms (CGH)

The Diploid Peak Centralization Parameter Panel appears. No further input is required. See “Diploid Peak Centralization Parameter Panel” on page 137.

The Diploid Peak Centralization algorithm is new to Agilent Genomic Workbench 7.0. To use the centralization algorithm offered in earlier versions of the program, select **Centralization (legacy)** in the Analysis Method Navigator.

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 diploid peak centralization algorithm finds a constant value to add to or subtract from all log ratio measurements, to recenter the log ratio values so that the zero-point reflects the diploid 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, select 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, select **Z score**.
The Z score Parameter Panel appears. See “[Z Score Parameter Panel](#)” on page 174.
- 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, select 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 125 and “[ADM-2 Parameter Panel](#)” on page 126.
- 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 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, select **CBS**.
The CBS algorithm is selected for the analysis method. See the *CGH Interactive Analysis User Guide* for more information.

3 Setting up Workflow Analysis Methods

To configure the analysis method to apply the HMM algorithm

To configure the analysis method to apply the HMM algorithm

- 1 In the Workflow Navigator, in Aberration, select **HMM**.
The HMM Parameter Panel appears. See “[HMM Parameter Panel](#)” on page 145.
- 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 or to LOH regions. The aberration 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, select **Aberration Filter** or **LOH Filter**.
The Parameter Panel for the selected filter appears. See “[Aberration Filter Parameter Panel](#)” on page 122 and “[LOH Filter Parameter Panel](#)” on page 156.
- 2 In Select Filter, choose one of the default filters 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 an aberration algorithm (except z-score or HMM). GC Correction and Diploid Peak Centralization are recommended.

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

The SNP Copy Number Parameter Panel appears. See “[SNP Copy Number Parameter Panel](#)” on page 168.

- 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 **SNP Copy Number** for SNP Algorithm. GC Correction and Diploid Peak Centralization are recommended.

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

The LOH Parameter Panel appears. See “[LOH Parameter Panel](#)” on page 155.

- 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*.

3 Setting up Workflow Analysis Methods

To define an output experiment

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 165. 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, select **Intra-Array Replicates**.

To configure the analysis method to combine interarray replicates

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

The Inter-Array Replicates Parameter Panel appears in the parameter panel. See “[Inter-Array Replicates Parameter Panel](#)” on page 154.

- 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, select 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">• No additional parameters are necessary.• 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.
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">• In the Analysis Method Navigator, in Normalization, select the check box next to Blank Subtraction. The Median method is used to estimate central tendency of blank probes. No other selection is possible.

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"> • In the Analysis Method Navigator, in Normalization, select Inter-array Median.
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"> 1 In the Analysis Method Navigator, in Normalization, select Dye-bias (intra-array) Median. 2 In the parameter panel, in Normalize by, select the way that the program compensates for dye bias. <ul style="list-style-type: none"> • 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. • 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. <p>Note: 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>Note: 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">1 In the Analysis Method Navigator, in Normalization, select Intra-Array Lowess (Intensity dependent) Normalization.2 In the parameter panel, select the data to be used to compute the regression curve. <p>Note: 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>Note: 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">1 In the Analysis Method Navigator, in Normalization, select Variance Stabilization.2 In the parameter panel, select how to fit the regression curve. <p>Note: 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, select 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 171.
 - 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">• Standard deviation of background pixels• Additive error as computed by Agilent Feature Extractor• Observed spread of negative controls
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> <ol style="list-style-type: none">1 Select Custom defined f-value.2 In the box, type the desired f-value.

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).

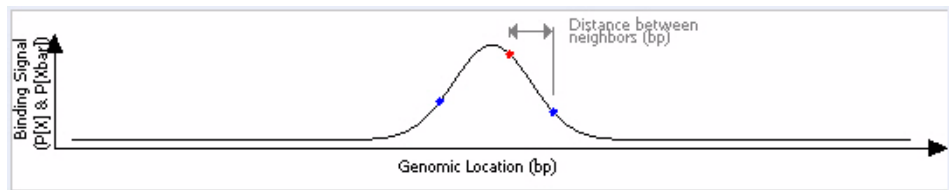


Figure 10 Central probe flanked by two neighbor probes.

The program accepts the probe as “bound” if the p -value of the composite error-corrected ratio (“X”) of all three probes (“ X_{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*.

3 Setting up Workflow Analysis Methods

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

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.	<p>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> <ul style="list-style-type: none">• To change the value, delete the old value and type a new one in the box.
$P(X_{\text{bar}}) <$	<ul style="list-style-type: none">• This parameter refers to the p-value of the average error-corrected ratio ("X") of the central probe and its left and right neighbors. (The "bar" indicates the average.)• The default value is 0.001.• To make detection more stringent, decrease the value.
Central probe has $P(X) <$	<ul style="list-style-type: none">• The central probe is the red probe in Figure 10.• The default value is 0.001.• To make detection more stringent, decrease the value.
At least one neighboring probe has $P(X) <$	<ul style="list-style-type: none">• Neighboring probes are probes to either side of the central probe. The blue probes in Figure 10 are the neighbors of the central (red) probe.• The default value is 0.1.• To make detection more stringent, decrease the value.
At least n of the neighbors has $P(X) <$	<ul style="list-style-type: none">• The default value for n is 1.• The default cut-off value for $P(X)$ is 0.005.• To make detection more stringent, decrease the value.

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"> 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. To change the value, delete the old value and type a new one in the box.
A probe is considered bound if: $P(X_{\text{bar}}) <$	<ul style="list-style-type: none"> This parameter refers to the p-value of the average error-corrected ratio (“X”) of the central probe and its left and right neighbors. (The “bar” indicates the average.) The default value is 0.001. To make detection more stringent, decrease the value.
Central probe has $P(X) <$	<ul style="list-style-type: none"> The central probe is the red probe in Figure 10. The default value is 0.001. To make detection more stringent, decrease the value.
At least one neighboring probe has $P(X) <$	<ul style="list-style-type: none"> Neighboring probes are probes to either side of the central probe. The blue probes in Figure 10 are the neighbors of the central (red) probe. The default value is 0.1. To make detection more stringent, decrease the value.
At least n of the neighbors has $P(X) <$	<ul style="list-style-type: none"> The default value for n is 2. The default cut-off value for $P(X)$ is 0.005. To make detection more stringent, decrease the value.

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
Thresholds	
<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.)
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.

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

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.
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. 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 p -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.

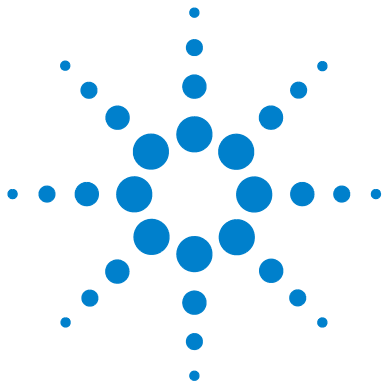
- 1 In the Analysis Method Navigator, in Output, click **Experiment**.

Two parameters appear in the parameter panel. See “[Select Experiment Parameter Panel](#)” on page 165. 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

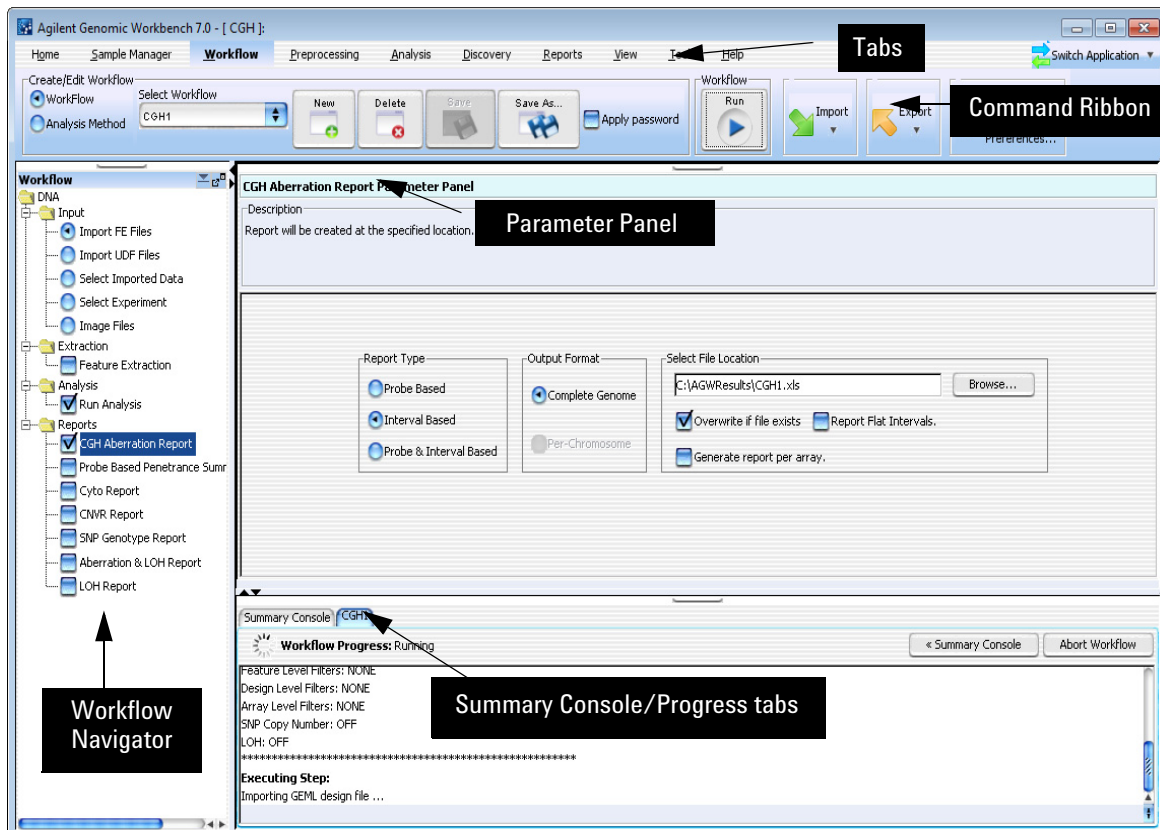


Figure 11 Workflow Main Window

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"> • Summary Console tab that displays the application type, workflow name, experiment name, status, and current step for each workflow completed or in process. • Workflow Progress tab for each named workflow, that displays the status of the workflow progress, including any errors that might have occurred.

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 OK .
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 Yes .
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 "To export a workflow" on page 64.

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 OK . 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).
Feature Extraction Preferences	Lets you set the path for the installation folder of your Feature Extraction Software.

Command ribbon for Analysis Method

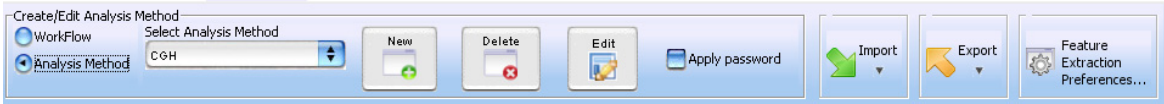


Figure 13 Command ribbon for Analysis Method

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 OK . 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 Yes .
Edit	Opens the Analysis Method window. Select the analysis method to edit and change the parameters. Click Save to save the changes in the selected analysis method. Click Save As 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).
Feature Extraction Preferences	Lets you set the path for the installation folder of your Feature Extraction Software.

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](#).

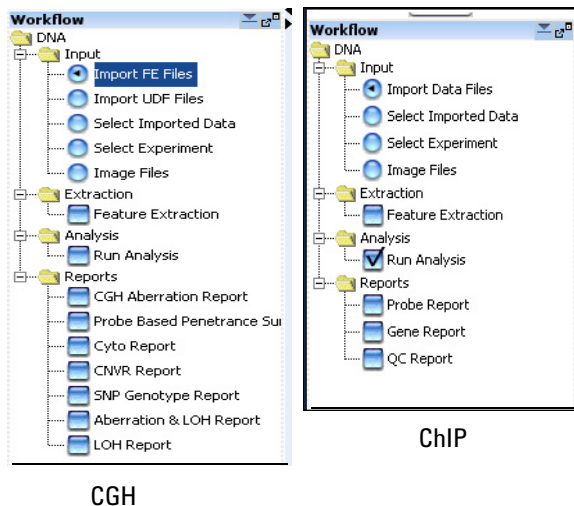


Figure 14 Workflow Navigator for CGH and ChIP

To select any workflow option, click the option button or select 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 47. 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 “Import Data Files Parameter Panel” on page 146. 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 “Import UDF Files Parameter Panel” on page 150.</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 “Select Imported Data Parameter Panel” on page 166.</p>
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 “Experiment Parameter Panel” on page 139.</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 Experiment 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 “Import FE Image Files Parameter Panel” on page 148.</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 “Import Data Files Parameter Panel” on page 146. 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 “Select Imported Data Parameter Panel” on page 166.</p>
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 “Select Experiment Parameter Panel” on page 165.</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 Experiment 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 “Import FE Image Files Parameter Panel” on page 148.</p>

Feature Extraction

You can use the Workflow application to automatically perform Feature Extraction on a series of microarray images. In order to run a Feature Extraction workflow, you must have both a Feature Extraction license and either a CGH or ChIP license. You run Feature Extraction workflows from either the CGH or the ChIP Workflow Navigator. 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.

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®.

Select 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 select 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 “Variance Stabilization” on page 170.</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 select 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. “Probe Based Penetrance Summary Report Parameter Panel” on page 161.</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 select 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 “Cyto Report Parameter Panel” on page 134.</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 select 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 “CNVR Report Parameter Panel” on page 133.</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 select this box, the SNP Genotype Report Parameter Panel appears, where you select the format and a location for the report. See “SNP Genotype Report Parameter Panel” on page 169.</p>
Aberration & LOH Report	<p>The Aberration & LOH Report contains aberration and log ratio information for significant intervals.</p> <p>When you select this box, the Aberration & LOH Report Parameter Panel appears, where you select the location for the report. See “Aberration & LOH Report Parameter Panel” on page 121.</p>
LOH Report	<p>The LOH Report contains aberration and log ratio information for significant LOH intervals.</p> <p>When you select this box, the LOH Report Parameter Panel appears, where you select the location for the report. See “LOH Report Parameter Panel” on page 158.</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 "Probe Report Settings" on page 162.</p>
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 "Gene Report Settings" on page 144.</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 "QC Report Settings" on page 163.</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.

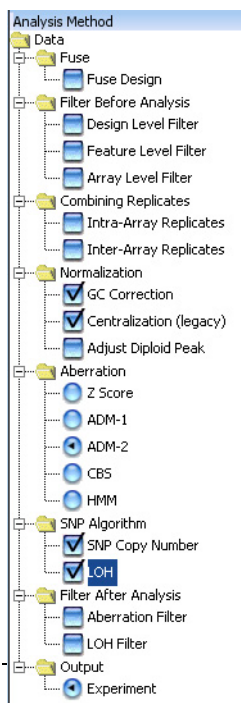


Figure 15 CGH Analysis Method Navigator with selections for SNP analysis

To select an analysis method option, click the option button or select 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 73.

To fuse designs, select **Fuse Design**. The Fuse Design Parameter Panel appears. See [“Fuse Design Parameter Panel”](#) on page 142.

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.

Select 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 “Design Level Filter Parameter Panel” on page 135.
Feature Level Filter	Opens the Feature Level Filter Parameter Panel. See “Feature Level Filter Parameter Panel” on page 140. 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 “Array Level Filter Parameter Panel” on page 127. 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.

Select any of these options:

Option	Description
Intra-Array Replicates	Combines replicate probes within each array. If you select 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 select 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 “Inter-Array Replicates Parameter Panel” on page 154.

Normalization

Select 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 recommended for SNP Copy Number and LOH analyses.

Option	Description
Centralization (legacy)	The Centralization (legacy) option applies the centralization algorithm offered in earlier versions of Agilent Genomic Workbench. The algorithm recenters log ratio values to ensure that the zero-point reflects the most-common-ploidy state. For a description of the legacy centralization algorithm, see the <i>CGH Interactive Analysis User Guide</i> .
Adjust Diploid Peak	Diploid Peak Adjustment is a centralization algorithm new to Agilent Genomic Workbench 7.0. It sets the log ratios of the probes that are in copy number 2 regions to be as centered around zero as possible. It finds a constant value to subtract from or add to all values, and ensures that the zero-point reflects the diploid state. Adjust Diploid Peak is recommended for SNP Copy Number and LOH analyses. For a description of diploid peak centralization, see the <i>CGH Interactive Analysis User Guide</i> .

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 “ Z Score Parameter Panel ” on page 174. 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 “ ADM-1 Parameter Panel ” on page 125. For a discussion of the ADM-1 algorithm, see the <i>CGH Interactive Analysis User Guide</i> .

4 Workflow Reference

Analysis Method Navigator for CGH

Option	Description
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 “ADM-2 Parameter Panel” on page 126. For a discussion of the ADM-2 algorithm, see the <i>CGH Interactive Analysis User Guide</i> .
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 “HMM Parameter Panel” on page 145. 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. Select one or both of the following options:

Option	Description
SNP Copy Number	<p>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 “SNP Copy Number Parameter Panel” on page 168. For more information on the SNP Copy Number algorithm, see the <i>CGH Interactive Analysis User Guide</i>.</p> <p>Note: In order to select SNP Copy Number, you must first select an Aberration (except z-score or HMM).</p>
LOH	<p>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 “LOH Parameter Panel” on page 155. For more information on the LOH algorithm, see the <i>CGH Interactive Analysis User Guide</i>.</p> <p>Note: In order to select LOH, you must first select SNP Copy Number.</p>

Filter After Analysis

Aberration filters and LOH 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, or to apply a nesting filter, select **Aberration Filter**. To apply a filter specifically for LOH regions, select **LOH Filter**. The parameter panel for the selected filter appears, where you can select filter parameters. See [“Aberration Filter Parameter Panel”](#) on page 122 and [“LOH Filter Parameter Panel”](#) on page 156. You create and edit aberration and LOH filters in the analysis. See [“To select to filter the results after analysis \(CGH\)”](#) on page 80.

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 139.

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.

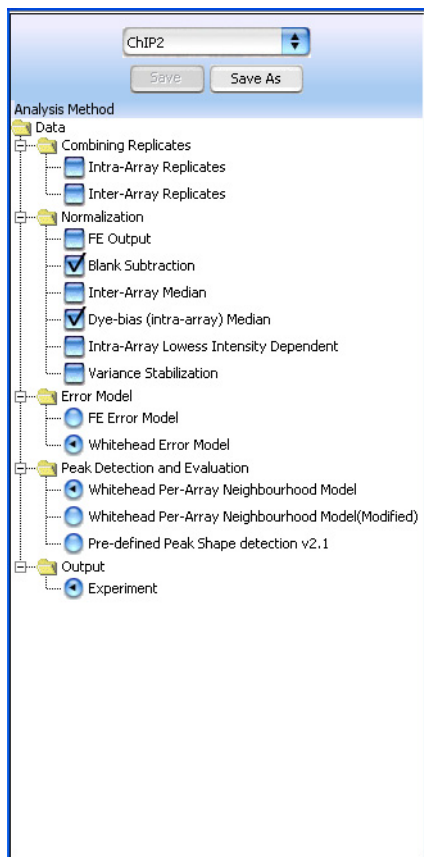


Figure 16 The Analysis Method Navigator for ChIP

To select any analysis method option, click the option button or select 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.

Select any of these options:

Option	Description
Intra-Array Replicates	Combines replicate probes within each array. If you select 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 select 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 “Inter-Array Replicates Parameter Panel” on page 154.

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>Select FE Output 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 select this type of normalization, central tendencies are calculated using the median of the negative control probes as a baseline. See “Blank Subtraction Normalization” on page 129.</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 select 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 “Dye-bias (Intra-Array) Median Normalization” on page 138.</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 “Inter-Array Median Normalization” on page 153.</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 “Intra-Array Lowess (Intensity Dependent) Normalization” 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 “Variance Stabilization” on page 170.

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).

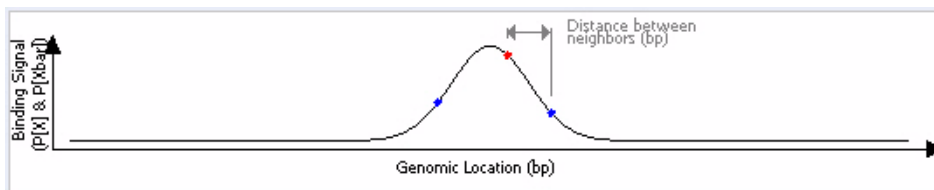


Figure 17 Central probe flanked by two neighbor probes.

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.

4 Workflow Reference

Analysis Method Navigator for ChIP

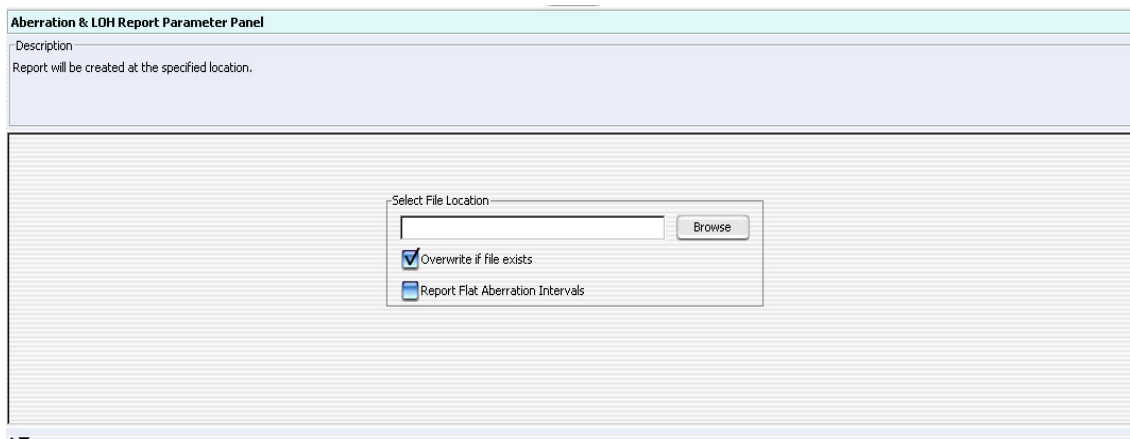
The program always creates an output experiment when it successfully runs a workflow. To customize the name or description of the experiment, click **Experiment** in the Analysis Method Navigator. The Experiment Parameter Panel appears. See “[Experiment Parameter Panel](#)” on page 139.

Parameter Panels

As you select options in the Workflow Navigator and 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 either the Workflow Navigator or Analysis Method Navigator, organized alphabetically by name.

Aberration & LOH Report Parameter Panel



The screenshot shows a software interface window titled "Aberration & LOH Report Parameter Panel". At the top, there is a description: "Report will be created at the specified location." Below this is a large, empty text area. In the center of this area, there is a smaller dialog box titled "Select File Location". This dialog box contains a text input field, a "Browse" button, a checked checkbox labeled "Overwrite if file exists", and an unchecked checkbox labeled "Report Flat Aberration Intervals".

Figure 18 Aberration & LOH Report Parameter Panel

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

To open: In the Workflow Navigator for CGH, under Reports, select **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**.

4 Workflow Reference

Aberration Filter Parameter Panel

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

Report Flat Aberration Intervals Select this option to have aberrations reported without any nested structure.

Aberration Filter Parameter Panel

The screenshot shows the 'Aberration Filter Parameter Panel' within a software interface. On the left is a tree view of the 'Analysis Method' with 'Aberration Filter' selected. The main panel has a description: 'You can select an existing filter from the drop-down list below, or create a new filter.' Below this is an 'Edit Aberration Filter' dialog box. The dialog shows a name 'DefaultAberrationFilter_v1' and a table of filter conditions:

	AMP	DEL	
<input checked="" type="checkbox"/> Minimum Number of Probes in Region	3	3	Reset
<input type="checkbox"/> Minimum Size (Kb) of Region	0.0	0.0	Delete
<input type="checkbox"/> Minimum Absolute Average Log Ratio of Region	0.0	0.0	Rename

Below the table are options for 'Maximum Nesting Level' and 'Use Nesting in Legacy Mode'. A note at the bottom states: '* Each condition must be met before an amplification or deletion is reported.'

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 select **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.
Update	Saves any changes you make to the filter criteria.
Reset	Restores the values of the filter criteria 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 currently 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 .
Minimum Number of Probes in Region	Mark the check box to exclude putative amplification regions (AMP) and/or deletion regions (DEL) that contain fewer probes than the numbers you type in the AMP and DEL columns.
Minimum Size of Region	Mark the check box to exclude putative amplification regions (AMP) and/or deletion regions (DEL) that are smaller than the numbers of Kb you type in the AMP and DEL columns.
Minimum Absolute Average Log Ratio of Region	Mark the check box to exclude putative amplification regions (AMP) and/or deletion regions (DEL) if the average \log_2 ratio within the region is less than the value you type in the AMP and DEL columns.

4 Workflow Reference

Aberration Filter Parameter Panel

Maximum Nesting Level Mark the check box to apply a nesting-level filter, and type a whole number from 0 to 2147483647 into the adjacent field. Nested aberrations are aberrations within other aberrations. If the maximum nesting level is set to zero, the program reports only the parent aberration, without any child (nested) aberrations. If the maximum nesting level is set to 1, the programs reports the parent aberrations and the first level of child aberrations.

Use Nesting in Legacy Mode Mark the check box to apply a nesting filter using the algorithm used in earlier versions of Agilent Genomic Workbench. You must first mark the **Maximum Nesting Level** check box before you can mark the **Use Nesting in Legacy Mode** check box.

When nesting is used in legacy mode, the program first applies the nesting filter, then applies the aberration conditions. In some cases, this can cause the program to omit child aberrations that would have passed the aberration conditions had they not been excluded by the nesting filter.

In contrast, when legacy mode is not selected, the program applies the nesting filter after the aberration conditions. In this case, if a parent aberration is omitted, but the child aberrations pass the aberration conditions, then the child aberrations are promoted a level in the nesting order and the nesting filter determines whether or not to keep them.

*** Each condition must be met before an amplification or deletion is reported** This note is present at all times in the Aberration Filter Parameter Panel. It explains that in order for a putative amplification or deletion to be reported in the results, it must meet all of the specifications designated in the aberration filter parameter panel. The filter excludes any putative aberrations that do not meet all of the specifications.

ADM-1 Parameter Panel

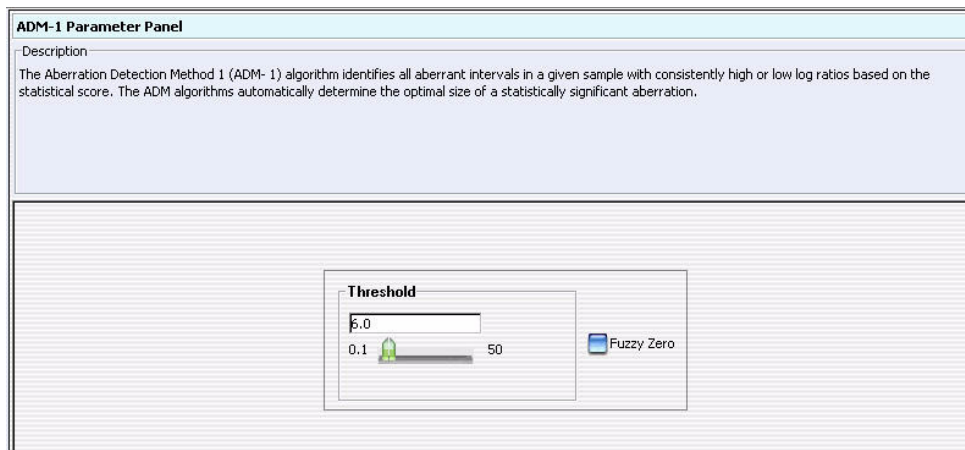


Figure 20 ADM-1 Parameter Panel

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.

Fuzzy Zero Select **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

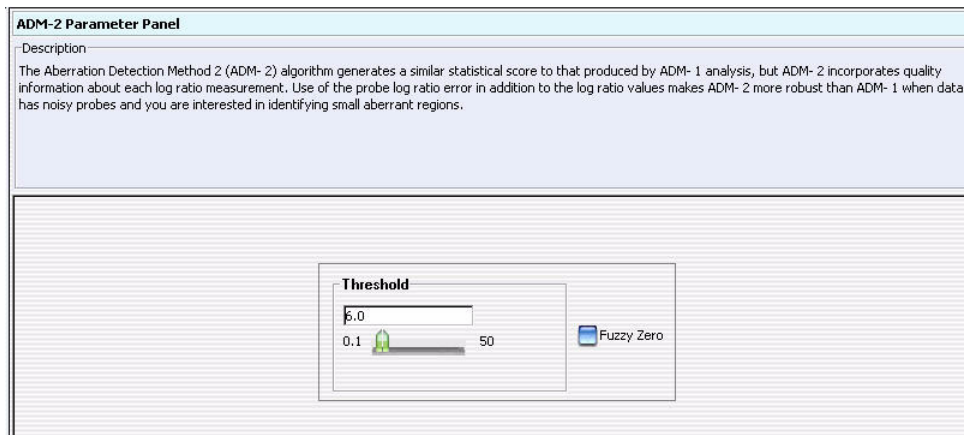


Figure 21 ADM-2 Parameter Panel

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.

Fuzzy Zero Select **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

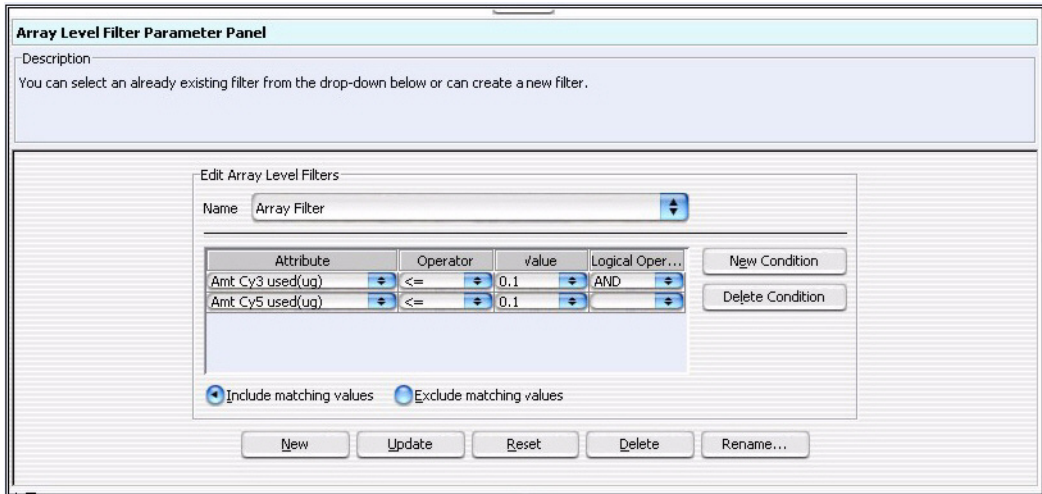


Figure 22 Array Level Filter Parameter Panel

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 select **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 array attribute evaluated by the filter

4 Workflow Reference

Array Level Filter Parameter Panel

- **Operator** – How the filter uses the entry in Value to evaluate arrays. For example, the \geq operator configures the filter to include or exclude arrays 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 an attribute. For example, if you select the **AmtCy3used(ug)** attribute, the = operator, and a value of **5**, the filter includes or excludes an array if its AmtCy3used(ug) attribute is equal to 5.
- **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.

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

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

Include/Exclude matching values Select one of these options:

- **Include matching values** – If an attribute passes the filter conditions, the program includes the array in the analysis.
- **Exclude matching values** – If an attribute passes the filter conditions, the program excludes the array 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 without closing the dialog box.

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**.

Blank Subtraction Normalization

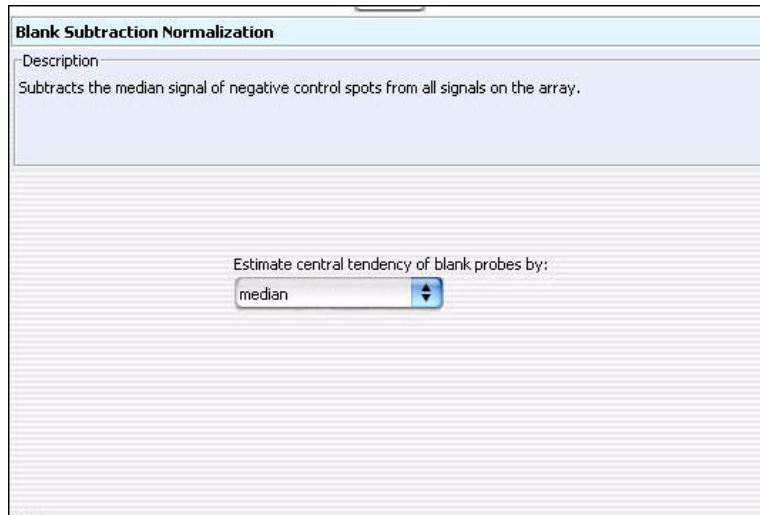


Figure 23 Blank Subtraction Normalization

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 84](#).

To open: This parameter panel appears when you click (or select 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 (legacy) Parameter Panel

Centralization (legacy) Parameter Panel

Description

Linear normalization routine for 2 color CGH data. By adding or subtracting a constant from the log ratios of all the probes, it makes the most common p data the new zero value. The two parameters specified below can be left to the default values.

Centralization Threshold

Centralization Bin Size

Figure 24 Centralization (legacy) Parameter Panel

Purpose: This parameter panel lets you set up centralization parameters. Centralization (legacy) 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 select **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.

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 select **CGH Aberration Report** in Reports in the CGH Workflow Navigator.

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

4 Workflow Reference

CGH Aberration Summary Report Parameter Panel

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 select 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 Select this to have aberration intervals reported without any nested structure.

Generate report per array Select 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

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 select **CNVR Report** in Reports in the CGH Workflow Navigator.

CNVR Node Name Type a name. If you select **CNVR Report** 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.

Select File Location The location where the workflow saves the generated CNVR report file. Click **Browse**. The Select report folder dialog box opens, where you can type a name and select a location for the CNVR report file.

Overwrite if file exists If you select 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

Cyto Report Parameter Panel

Description
You have to create a template from the interactive mode, which will be available from the drop-down below. Only formatting settings will be used from the selected Cyto Report template. Analysis settings will be used from the selected Analysis Method.

Select Report
Select Report

Select File Location
Browse...
 Overwrite if file exists

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 select **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 select 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

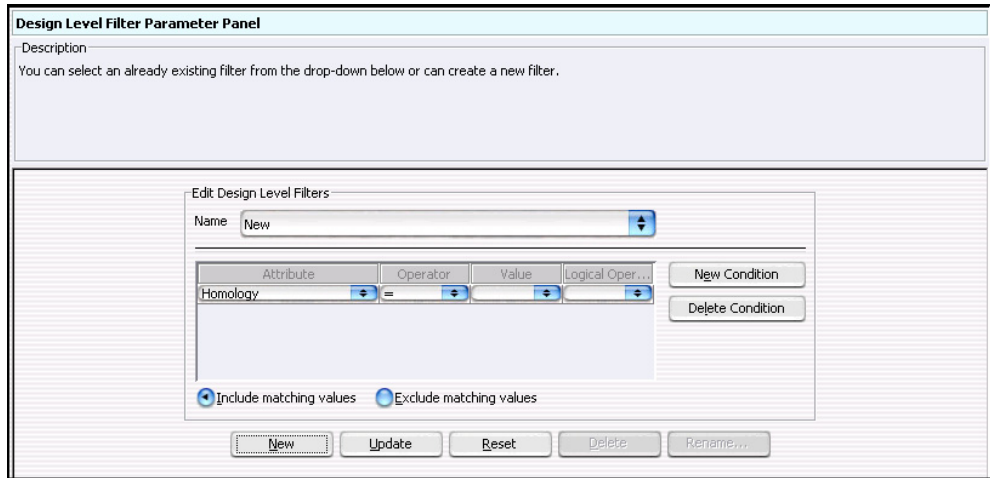


Figure 28 Design Level Filter Parameter Panel

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

To open: In the Analysis Method Navigator, under Filter Before Analysis, select 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 condition (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 design attribute evaluated by the filter.
- **Operator** – How the filter uses the entry in Value to evaluate data. For example, the \geq operator configures the filter to include or exclude probes where the selected attribute is greater than or equal to the entry in Value.

4 Workflow Reference

Design Level Filter Parameter Panel

- **Value** – The value the filter uses as the basis to evaluate an attribute. 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 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 a probe 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 a probe passes the filter condition, the program *includes* it in the analysis.
- **Exclude matching values** – If a probe passes the filter condition, 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 without closing the dialog box.

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**.

Diploid Peak Centralization Parameter Panel



Figure 29 Diploid Peak Centralization Parameter Panel

Purpose: This parameter panel has no input parameters. To include diploid peak centralization in your analysis method, all you need to do is mark the Adjust Diploid Peak check box. No further settings are required. For a description of the diploid peak centralization algorithm, see the *CGH Interactive Analysis User Guide*.

To open: The Diploid Peak Centralization Parameter Panel appears when you select **Adjust Diploid Peak** in Normalization in the CGH Analysis Method Navigator.

NOTE

Diploid Peak Centralization is recommended for SNP Copy Number and LOH analyses.

Dye-bias (Intra-Array) Median Normalization

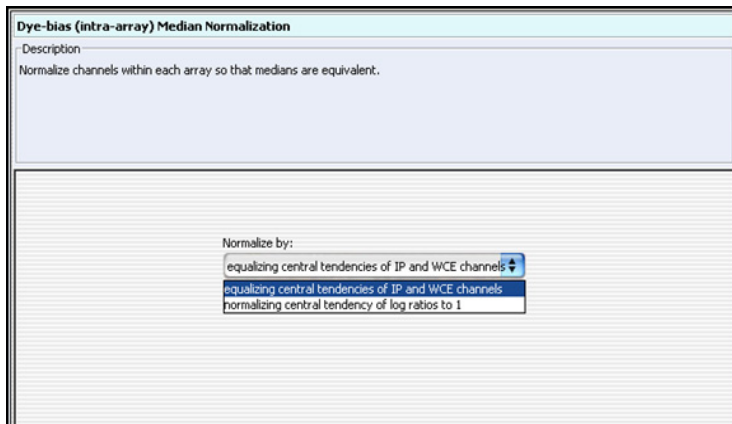


Figure 30 Dye-bias (intra-array) Median Normalization

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 84.

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

Experiment Description

Figure 31 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 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

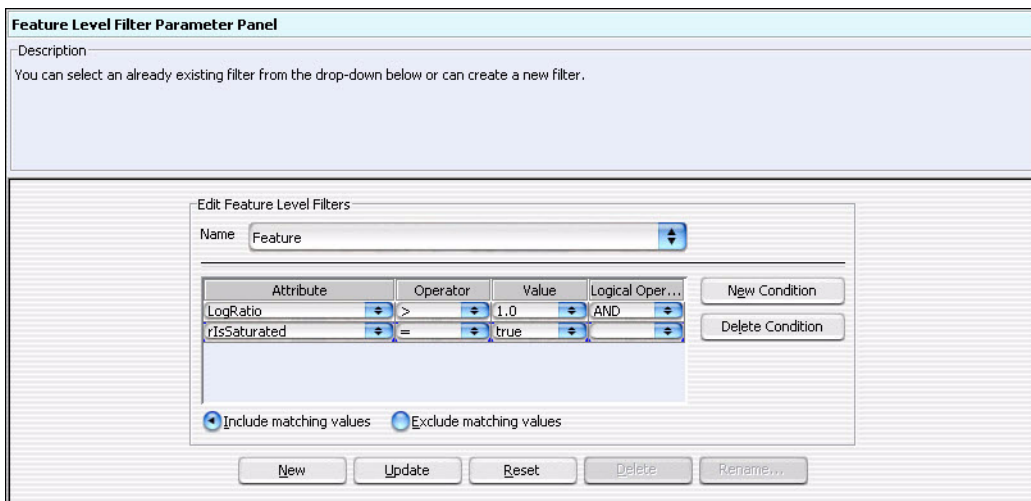


Figure 32 Feature Level Filter Parameter Panel

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 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 a feature 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 row, then click **Delete Condition**.

Include/Exclude matching values Select one of these options:

- Include matching values – If a feature passes the filter condition, the program includes it in the analysis.
- Exclude matching values – If a feature passes the filter condition, 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 criteria without closing the dialog box.

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 currently 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**.

Fuse Design Parameter Panel

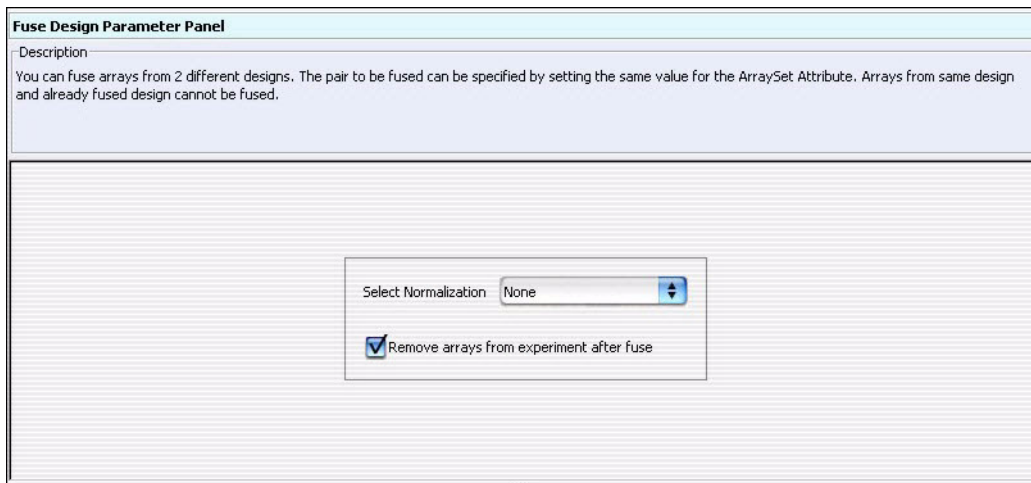


Figure 33 Fuse Design Parameter Panel

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 select **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 (legacy) 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 select 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

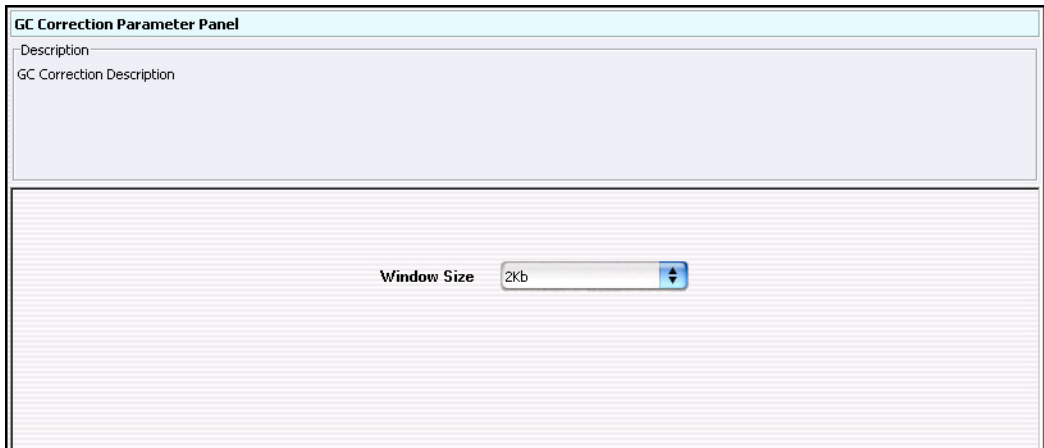


Figure 34 GC Correction parameter panel

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

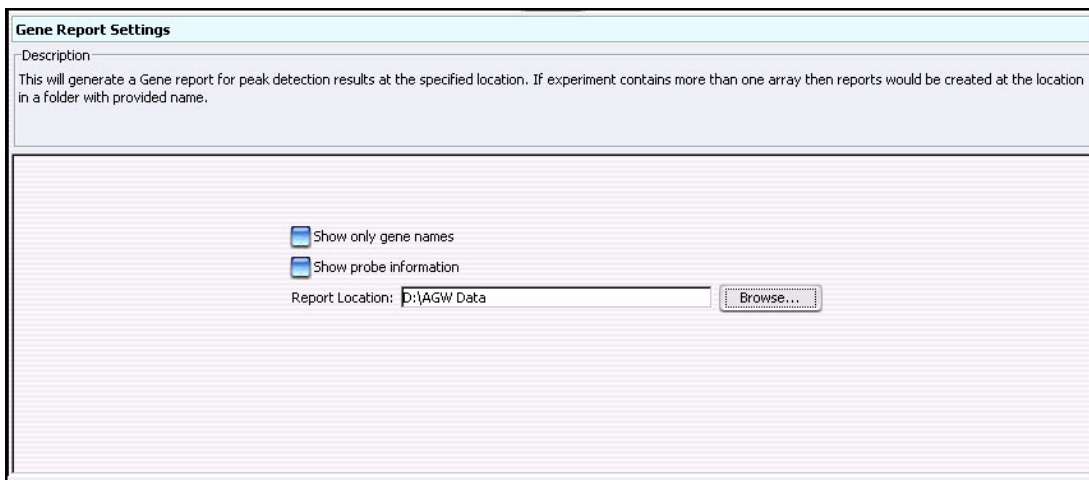


Figure 35 Gene Report Settings

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 select 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 select 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 194.

HMM Parameter Panel

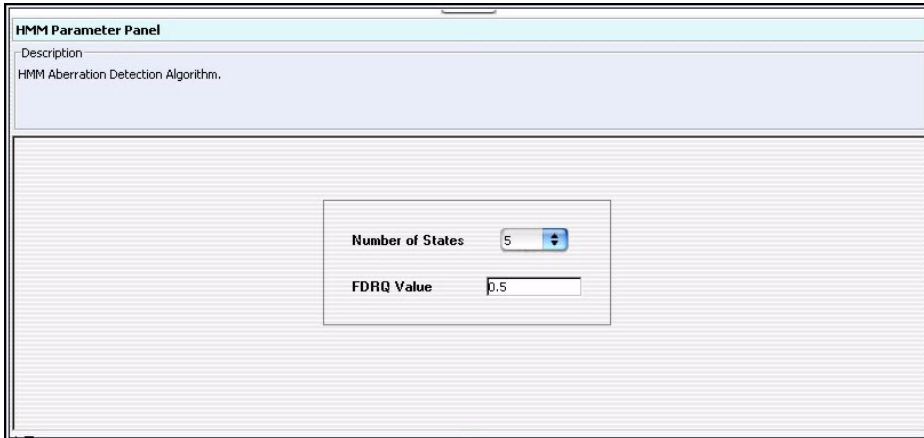


Figure 36 HMM Parameter Panel

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

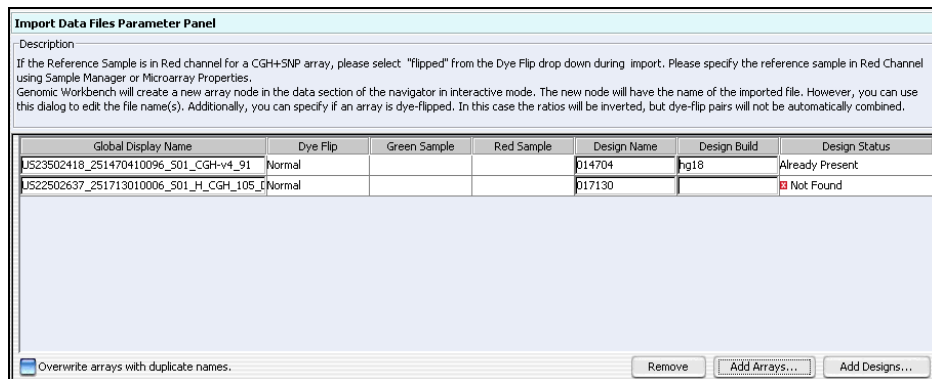


Figure 37 Import Data Files Parameter Panel

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"> • 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.
Flipped	<ul style="list-style-type: none"> • 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)

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 select 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.

4 Workflow Reference

Import FE Image Files Parameter Panel

Add Designs This option is enabled after you add an array to the list that does not have an associated design 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. After you select the design, **Path Provided** appears under Design Status for the array.

Import FE Image Files Parameter Panel

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>

Figure 38 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 41 and [“To remove an image from the list”](#) on page 43.

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.

Array ID (Read-only) The unique identifier for the microarray.

- Barcode** (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.
- Sample ID**
<Red/Green
(ArrayID)> If the Red/Green attributes for the array are assigned in Sample Manager, they appear here. Otherwise, a ? appears in the field.
- Grid Template** 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.
- Protocol** By default, the Feature Extraction program automatically determines the Protocol for the microarray. Or, you can select a Protocol from the list.
- Add** Click this button to open the Open dialog box, where you can select the image file(s) to be added. See “Open” on page 191.
- Remove** 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 **Ctrl** key and then click the microarrays to remove. To select a contiguous series of microarrays, hold down the **Shift** 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 39 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
- **log₂ ratio** – binary log ratio
- **log₁₀ ratio** – common log ratio
- **In 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**.

4 Workflow Reference

Intra-Array Lowess (Intensity Dependent) Normalization

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.

Intra-Array Lowess (Intensity Dependent) Normalization

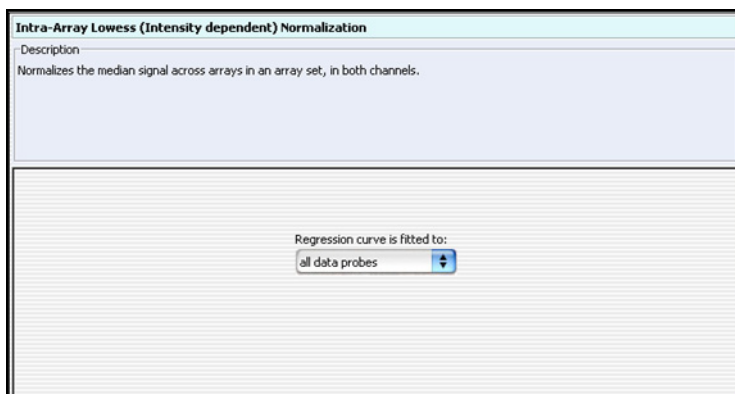


Figure 40 Intra-Array Lowess (Intensity dependent) Normalization

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”.

Inter-Array Median Normalization

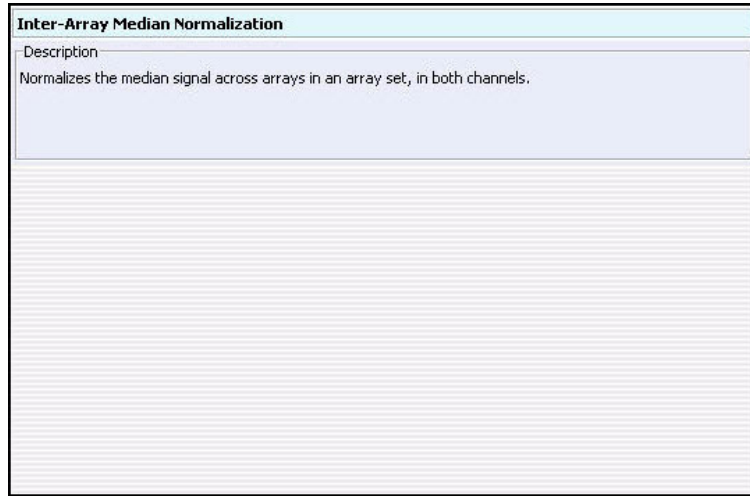


Figure 41 Inter-Array Median Normalization

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 84.

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.

Inter-Array Replicates Parameter Panel

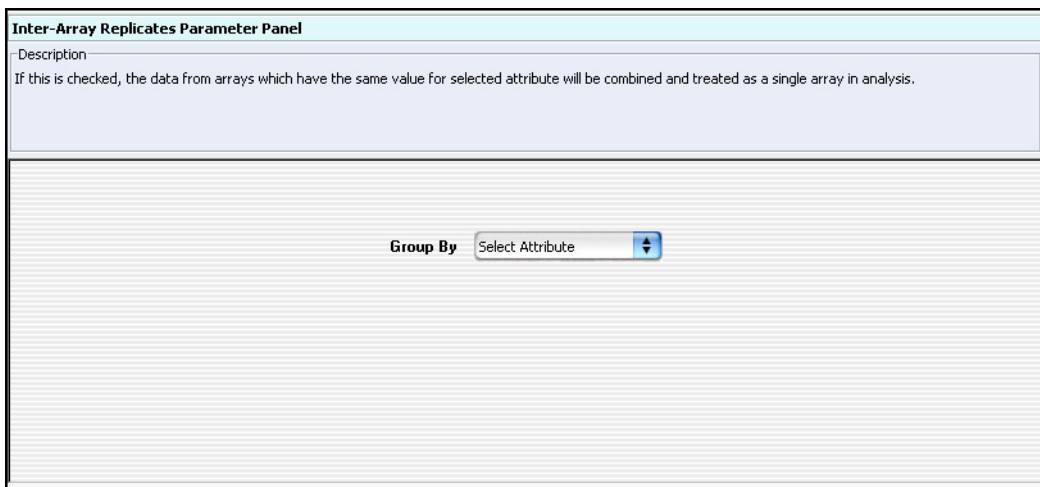


Figure 42 Inter-Array Replicates Parameter Panel

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 select **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*.

LOH Parameter Panel

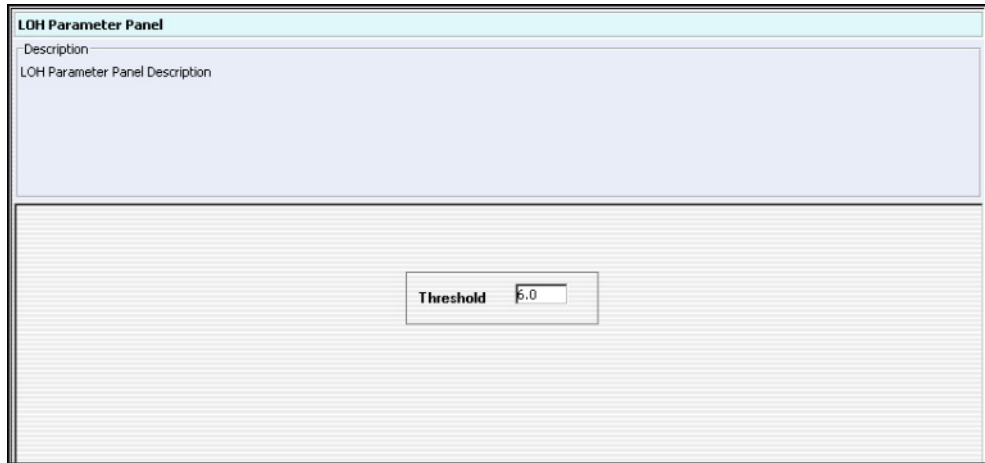


Figure 43 LOH Parameter Panel

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, select **LOH**.

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

LOH Filter Parameter Panel

LOH Filter Parameter Panel

Description
You can select an already existing filter from the drop-down below or can create a new filter.

Edit LOH Filter

Name: LOH Filter1 [New]

Minimum Number of Probes in Region: 10 [Update]

Minimum Size (Mb) of Region: 2 [Reset]

Maximum Fraction of Heterozygous Probes in...: 0.25 [Delete]

[Rename]

Figure 44 LOH Filter Parameter Panel

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

To open: The LOH Filter Parameter Panel appears when you select **LOH 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 LOH 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.

- Update** Saves any changes you make to the filter criteria.
- Reset** Restores the values of the filter criteria 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 currently 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**.
- Minimum Number of Probes in Region** Mark the check box to exclude putative LOH regions that contain fewer probes than the number you type in the adjacent field.
- Minimum Size (Mb) of Region** Mark the check box to exclude putative LOH regions that are smaller than the number of Mb you type in the adjacent field.
- Maximum Fraction of Heterozygous Probes in Region** Mark the check box to exclude putative LOH regions that have a higher fraction of heterozygous probes than the maximum that you set. In the adjacent field, type a fraction (between 0 and 1) to set the maximum fraction. Any putative LOH regions in which the fraction of heterozygous probes exceeds the maximum are excluded by the filter.

LOH Report Parameter Panel

The screenshot shows a software interface titled "LOH Report Parameter Panel". It features a light blue header bar with the title. Below the header is a "Description" section with a light blue background, containing the text "Report will be created at the specified location." The main content area is white and contains a "Select File Location" section. This section includes a text input field, a "Browse" button, and a checked checkbox labeled "Overwrite if file exists".

Figure 45 LOH Report Parameter Panel

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

To open: In the Workflow Navigator for CGH, under Reports, select **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 select this option, the workflow deletes an existing file if it has the same name and location as a generated report.

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 46 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.)

4 Workflow Reference

Predefined peak shape detection v2.1

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

Probe Based Penetrance Summary Report Parameter Panel

Description
Report will be created at the specified location.

Output Format
 Complete Genome Per-Chromosome

Select File Location

 Overwrite if file exists

Figure 47 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 select **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.

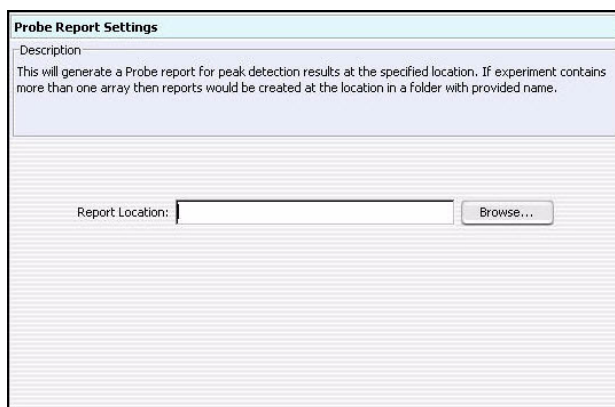
4 Workflow Reference

Probe Report Settings

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 select this option, the workflow deletes an existing file if it has the same name and location as a generated report.

Probe Report Settings



Probe Report Settings

Description:
This will generate a Probe 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.

Report Location:

Figure 48 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 194.

QC Report Settings

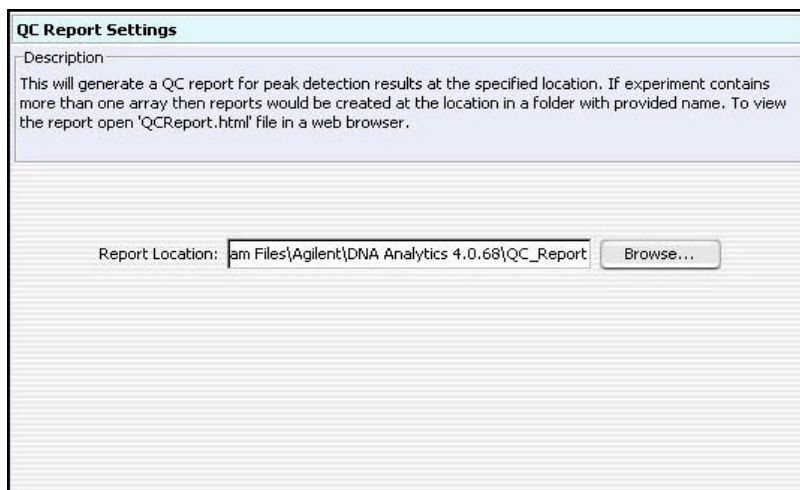


Figure 49 QC Report Settings

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.

4 Workflow Reference

Run Analysis Application Panel

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 194.

Run Analysis Application Panel

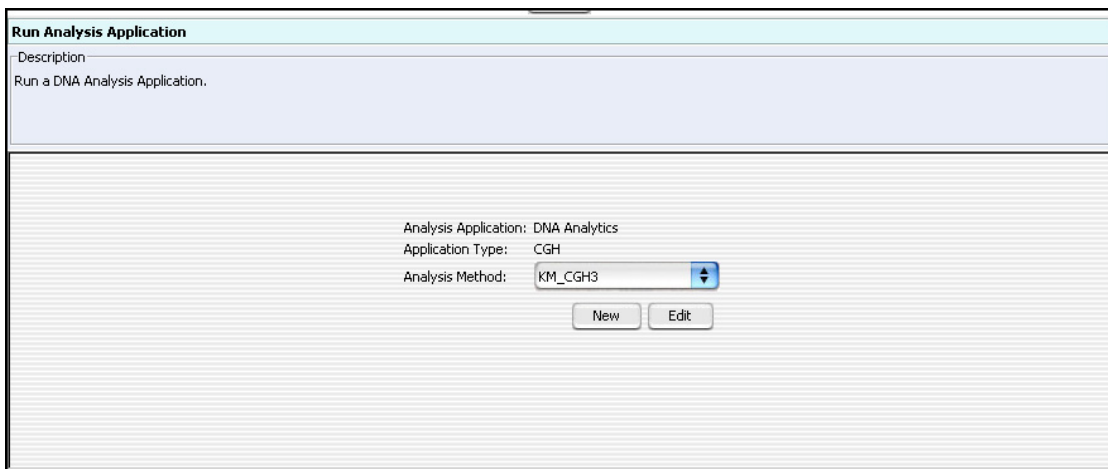



Figure 50 Workflow – Run Analysis Application panel

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

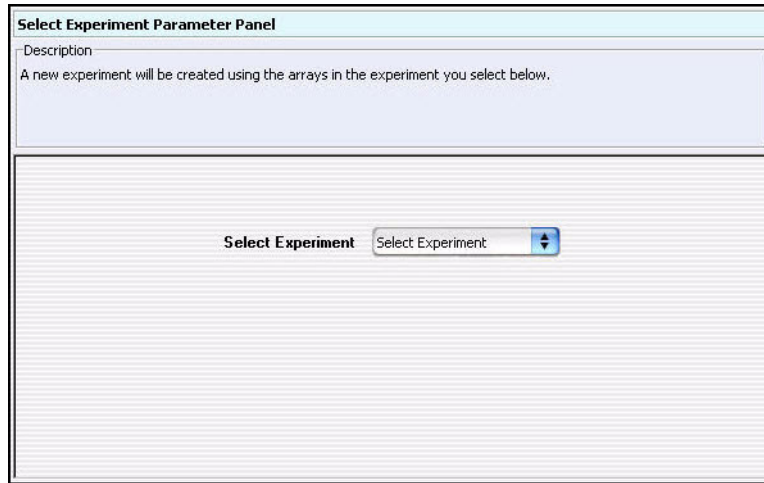


Figure 51 Select Experiment Parameter Panel

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

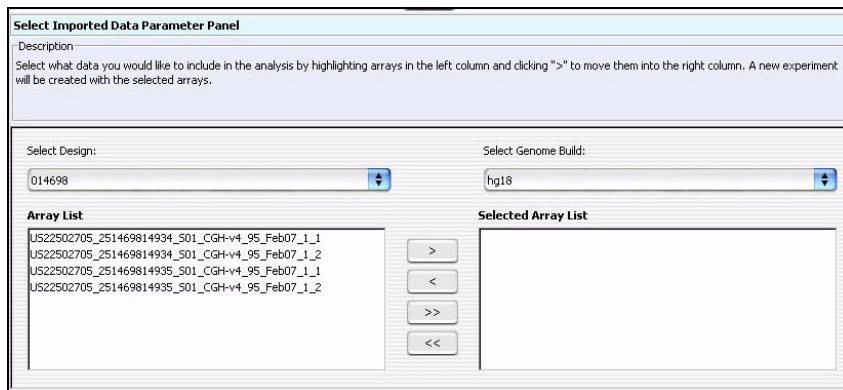


Figure 52 Select Imported Data Parameter Panel

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.

-  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

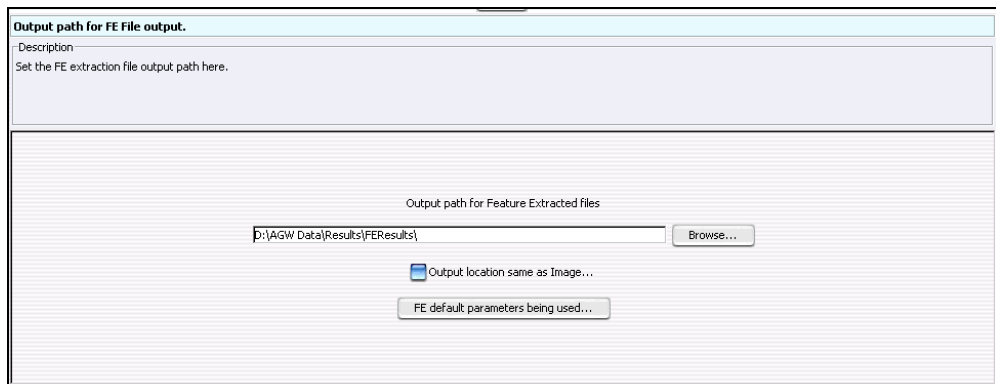


Figure 53 Workflow – Output path for FE File output

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 191.

4 Workflow Reference

SNP Copy Number Parameter Panel

- Browse** Click this button to browse for the folder where you want to save the FE files.
- Output location same as Image** Select this to set the output path for extracted files to the location of the image files.
- FE default parameters being used** Click this to display the Feature Extraction Properties. These are the parameters that are used when you run Feature Extraction using the Workflow. See “[Feature Extraction Properties](#)” on page 187.

SNP Copy Number Parameter Panel

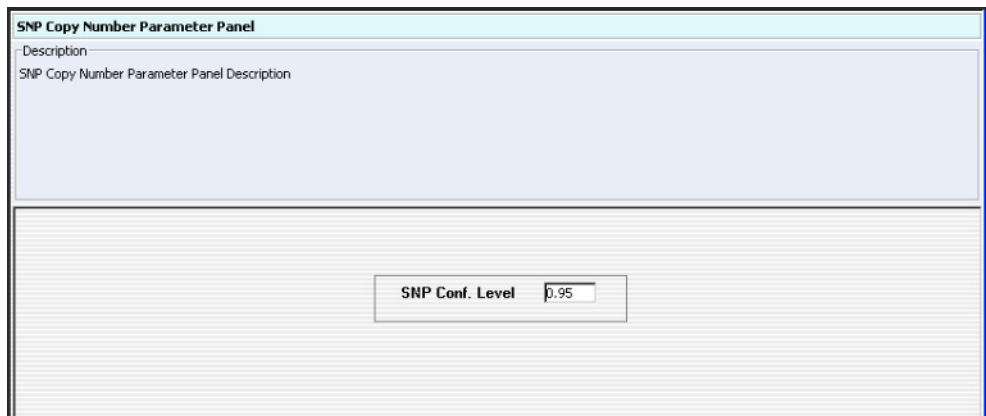


Figure 54 SNP Copy Number Parameter Panel

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, select **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

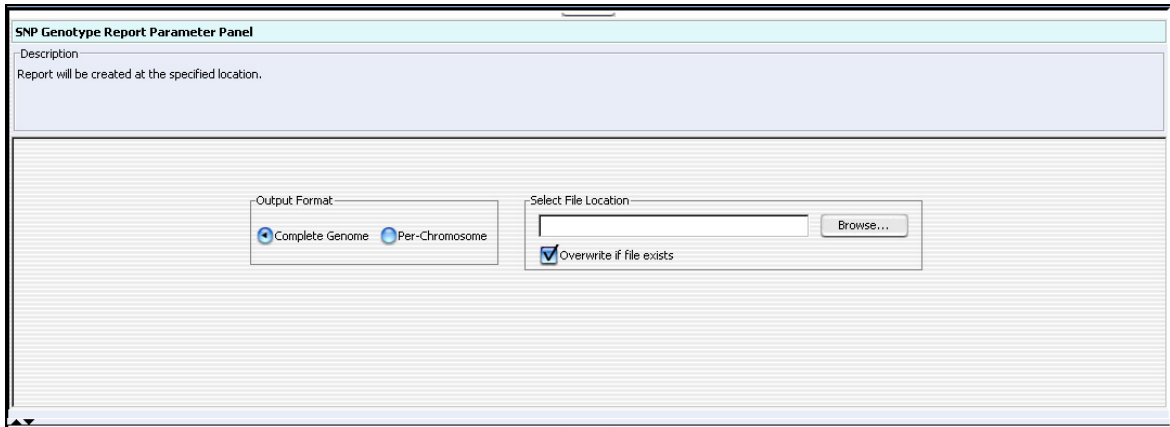


Figure 55 SNP Genotype Report Parameter Panel

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, select **SNP Genotype Report**

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 select this option, the workflow deletes an existing file if it has the same name and location as a generated report.

Variance Stabilization

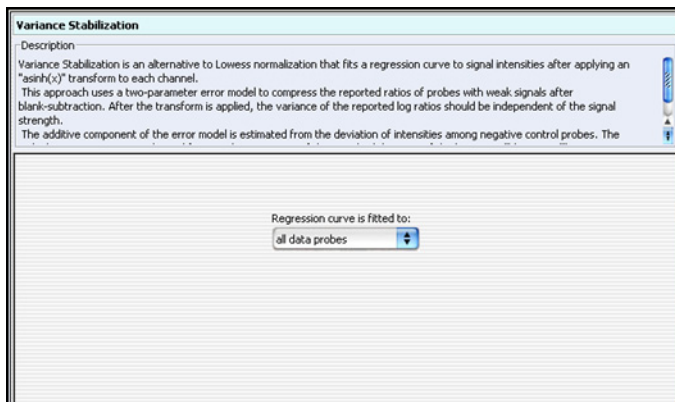


Figure 56 Variance Stabilization

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

Figure 57 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 87.

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

Select 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

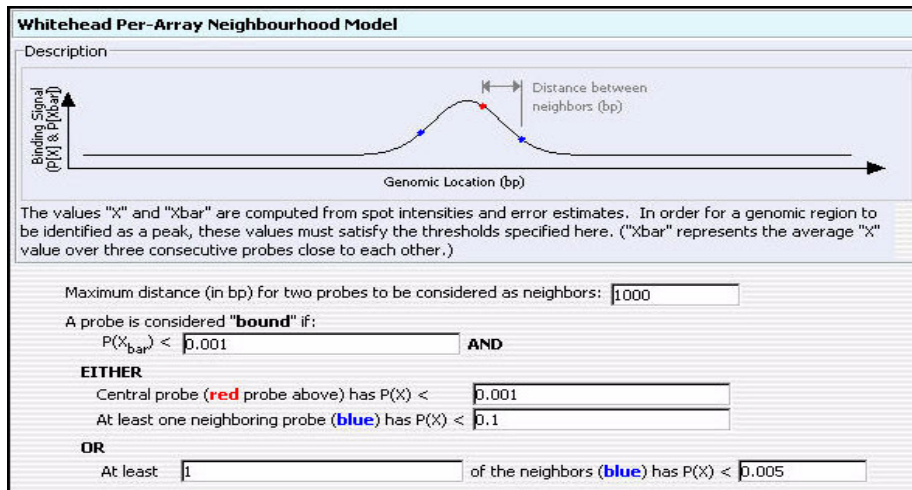


Figure 58 Whitehead Per-Array Neighbourhood Model

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 89.

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 58](#). 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"> • This parameter refers to the average p-value for the central probe and its neighbors. • The default cut-off value is 0.001. • Decreasing the cut-off value makes the selection more stringent.
Central probe has $P(X) <$	<ul style="list-style-type: none"> • The central probe is the red probe in Figure 58. • The default cut-off value is 0.001. • Decreasing the cut-off value makes the selection more stringent.
At least one neighboring probe has $P(X) <$	<ul style="list-style-type: none"> • Neighboring probes are probes to either side of the central probe. The blue probes in Figure 58 are the neighbors of the central (red) probe. • The default cut-off value is 0.1. • Decreasing the cut-off value makes the selection more stringent.
At least n of the neighbors has $P(X) <$	<ul style="list-style-type: none"> • The default value for n is 1. • The default cut-off value for $P(X)$ is 0.005. • Decreasing the cut-off value makes the selection more stringent.

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 89.

Z Score Parameter Panel

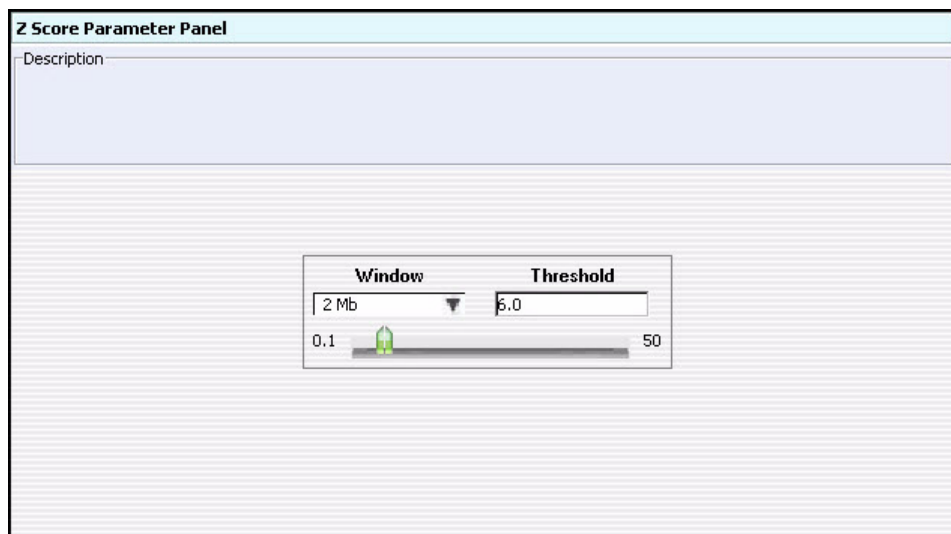


Figure 59 Z Score Parameter Panel

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.

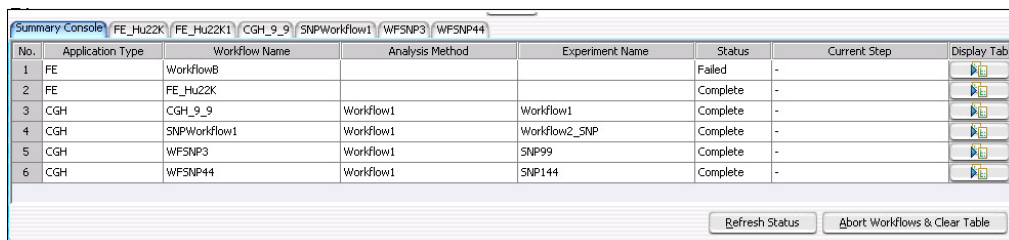
Alternatively, you can specify the window size in terms of data points (pt). This sets the window size to the specified number of contiguous data points. Type the desired number of data points, for example 30 pt, or select a value from the list.

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



No.	Application Type	Workflow Name	Analysis Method	Experiment Name	Status	Current Step	Display Tab
1	FE	WorkflowB			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	-	

Refresh Status Abort Workflows & Clear Table

Figure 60 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.

Workflow Name	(Read-only) The name of the workflow described in the row.
Analysis Method	(Read-only) The name of the analysis method used in the workflow.
Experiment Name	(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.
Status	(Read-only) Indicates whether the workflow is Running or Complete .
Current Step	(Read-only) For a workflow that is running, shows the progress through the steps of the workflow.
Display Tab	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.
Refresh Status	Updates the status of all workflows. The program also updates the status of the workflows in the workflow list in real time.
Abort Workflows & Clear Table	Opens a Confirm dialog box that asks if you are sure you want to abort all workflows. If you click Yes , 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

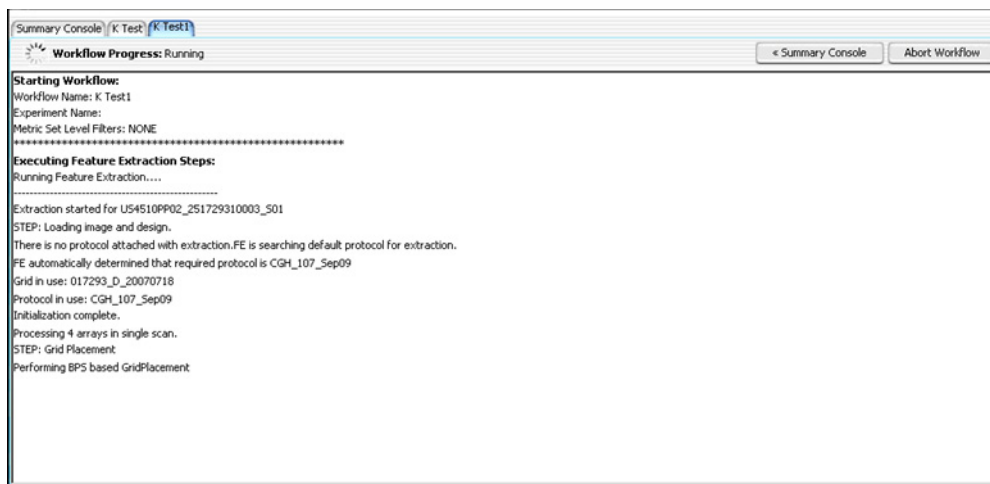


Figure 61 Workflow progress tab.

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 176.
- 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.



(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

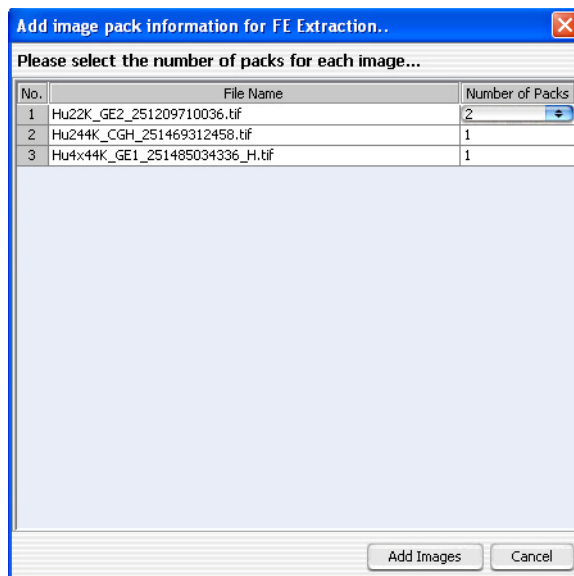


Figure 62 Add image pack information for FE Extraction dialog box

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 63 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

Select this box if you want to protect the analysis method with a password. When this box is selected, the Set Workflow Password dialog box will appear when you click **OK**. See “Set Password” on page 196.

OK

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

Cancel

Click to cancel the operation.

Create Workflow

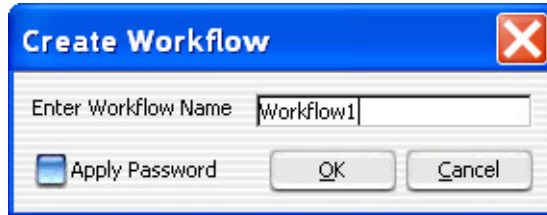


Figure 64 Create Workflow dialog box

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

Select the box to protect the workflow with a password. If this check box is selected, when you click **OK**, the Set Password dialog box appears, where you type a password for the workflow. See “[Set Password](#)” on page 196.

Export

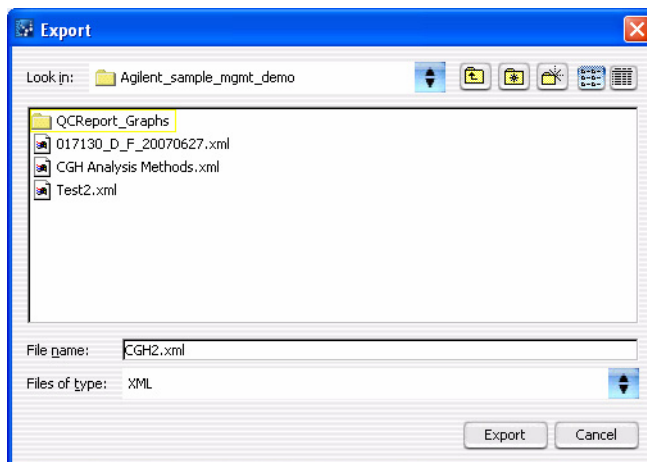


Figure 65 Export dialog box

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)

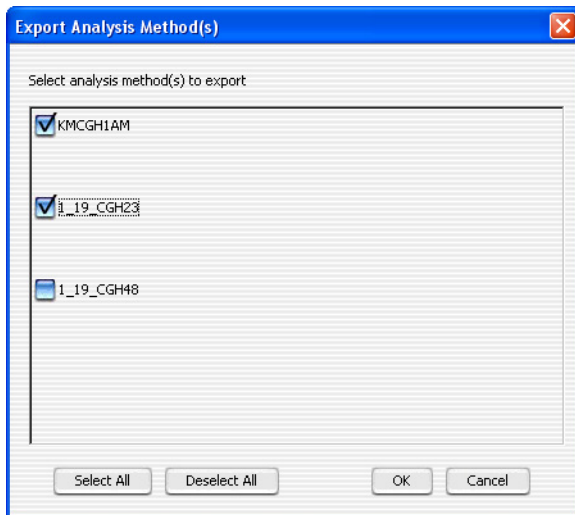


Figure 66 Export Analysis Method(s) dialog box

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)

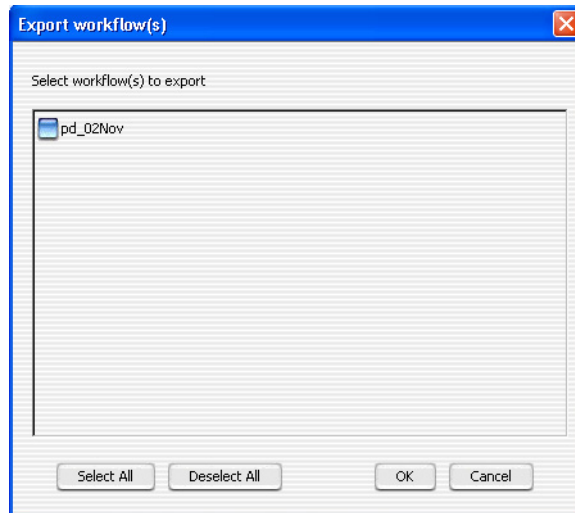


Figure 67 Export Workflow(s) dialog box

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 Preferences

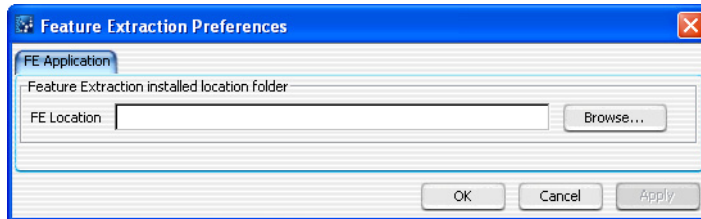


Figure 68 Feature Extraction Preferences dialog box

Purpose: Used to designate the location where your Feature Extraction software is installed.

To open: On the Workflow Command Ribbon, click **Feature Extraction Preferences**.

FE location Enter the path where the Feature Extraction software is installed.

Browse Click to browse for the folder where the Feature Extraction software is installed.

OK Click to accept the displayed location and close the dialog box.

Cancel Click to cancel the operation.

Apply Click to apply the displayed location but keep the dialog box open.

Feature Extraction Properties

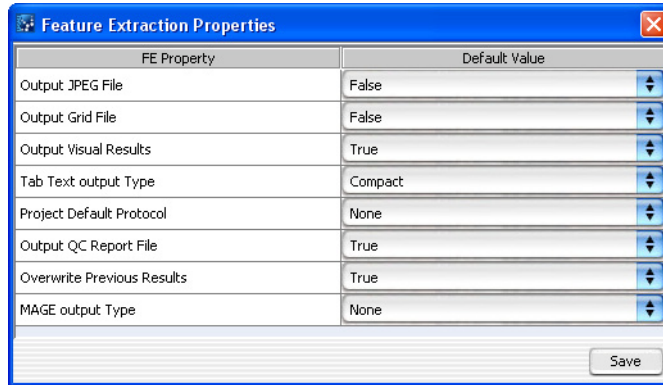


Figure 69 Feature Extraction Properties dialog box

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

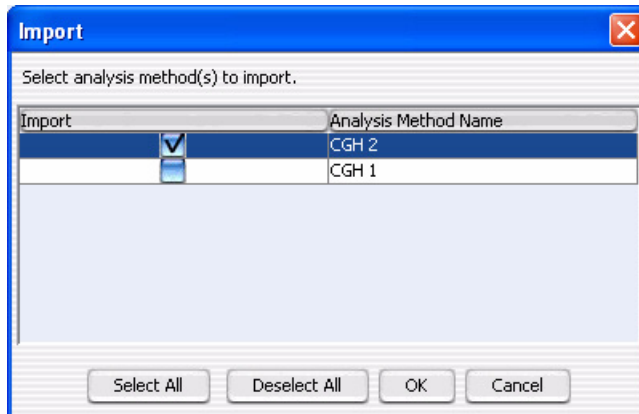


Figure 70 Import dialog box

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)

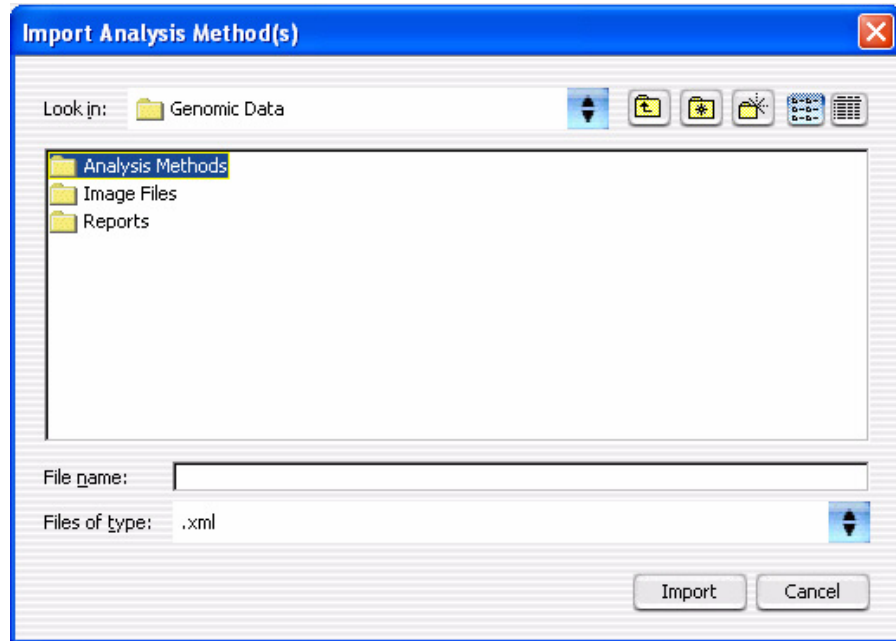


Figure 71 Import Analysis Method(s) dialog box

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.

4 Workflow Reference

Import Workflow(s)

Cancel Click to cancel the operation.

Import Workflow(s)

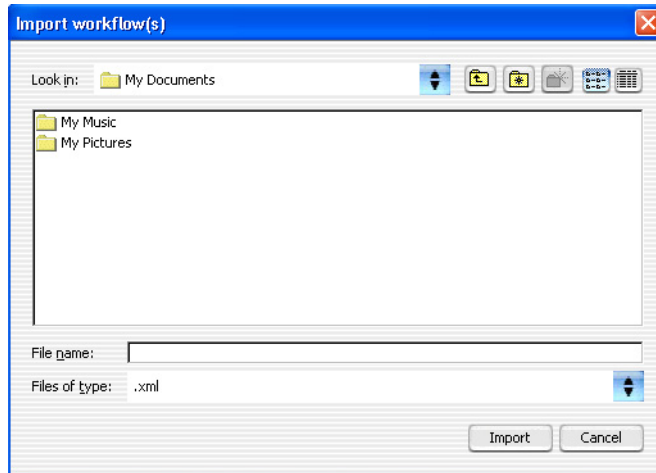


Figure 72 Import Workflow(s) dialog box

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

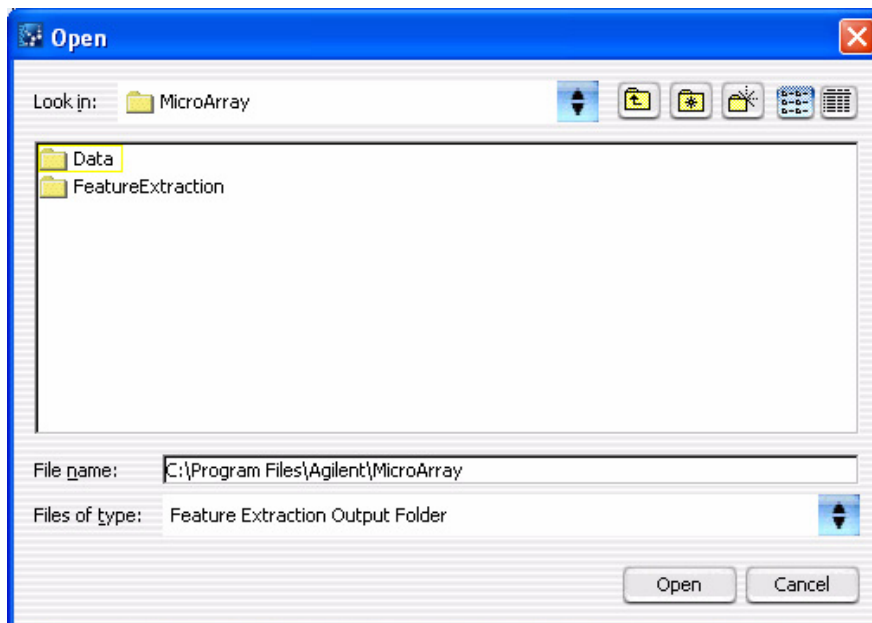


Figure 73 Open dialog box

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 148.

4 Workflow Reference

Provide Workflow Identifier

Cancel Click this to cancel the operation.

Provide Workflow Identifier

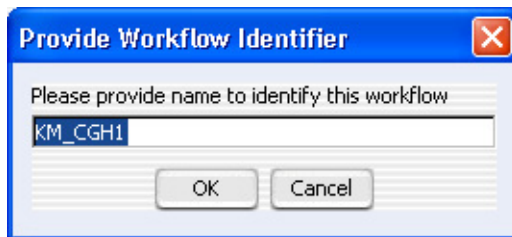


Figure 74 Provide Workflow Identifier dialog box

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 75 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 selected, the Set Analysis Method Password dialog box opens. See “[Set Password](#)” on page 196.

Cancel

Click to cancel the operation.

Select Report Folder

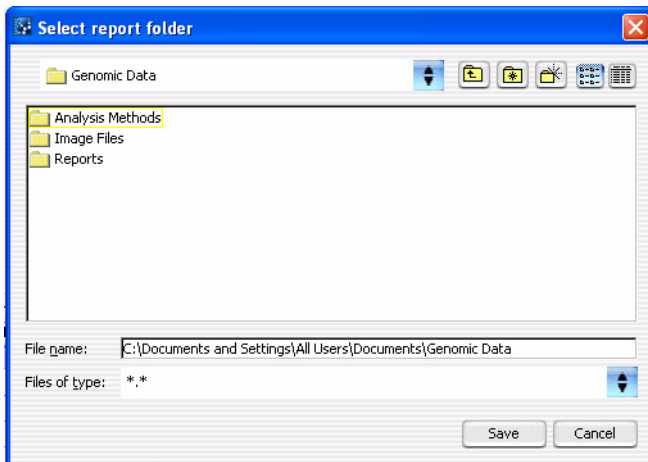


Figure 76 Select report folder dialog box

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

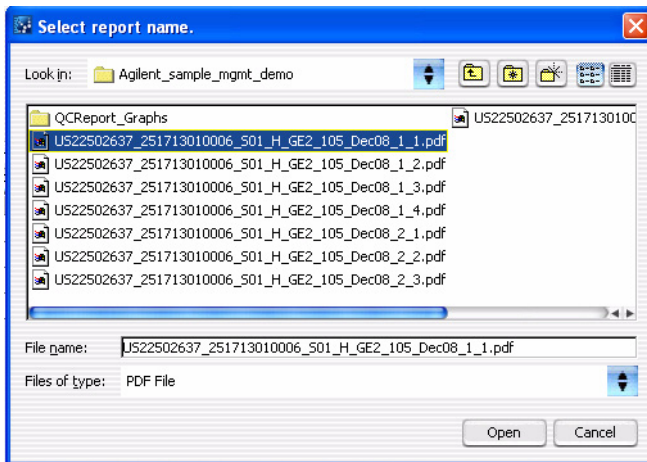


Figure 77 Select report name dialog box

Purpose: Used to select the folder location and name to store a CGH report.

To open: From any of the Cyto, CNVR, SNP Genotype, or Aberration & LOH 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

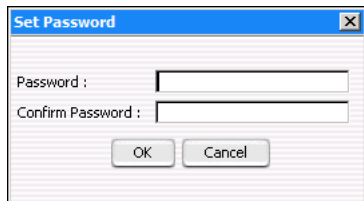


Figure 78 Set Password dialog box

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 7.0 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



G3800-90050



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