

Agilent Genomic Workbench 6.0

Product Overview Guide



Agilent Technologies

Notices

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This guide is valid for 6.0 and later revisions of the Agilent Genomic Workbench software, until superseded.

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

In This Guide...

This guide gives high-level descriptions of the programs within Agilent Genomic Workbench, a suite of tools that allows you to:

- Design microarrays and SureSelect Target Enrichment Kits
- Manage information about samples
- Use Feature Extraction to generate data from microarray scan images
- Use Quality tools to select the most reliable data for analysis
- Interactively investigate aberration patterns, protein-DNA binding events, and DNA methylation
- Run a workflow to automate feature extraction, the assessment of data quality, and data analysis

1 Overview

This chapter gives a high-level overview of the major features of the Standard Edition of Agilent Genomic Workbench.

2 Getting Started

This chapter describes how to start the programs in Agilent Genomic Workbench and find Help, and how to enter your license information.

3 Detailed Descriptions

This chapter gives more detailed descriptions of the major features of the Standard Edition of Agilent Genomic Workbench.

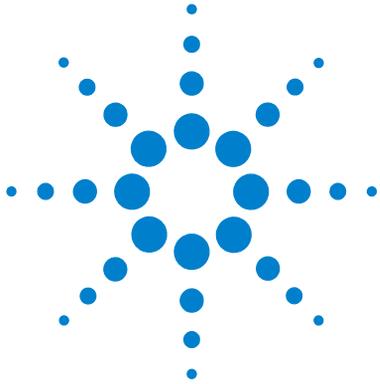
4 System Administration and Troubleshooting

This chapter describes how to perform system administration and fix problems you may encounter.

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1 Overview

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The Agilent Genomic Workbench is a comprehensive design and analysis tool that enables you to manage the data creation and analysis process, for both microarrays and SureSelect Libraries, from a single user interface.

This chapter helps you learn about the organization and capabilities of the Agilent Genomic Workbench. It shows you how each component of the Agilent Genomic Workbench helps you do your work – from design of microarray and target enrichment libraries through analysis and reporting.

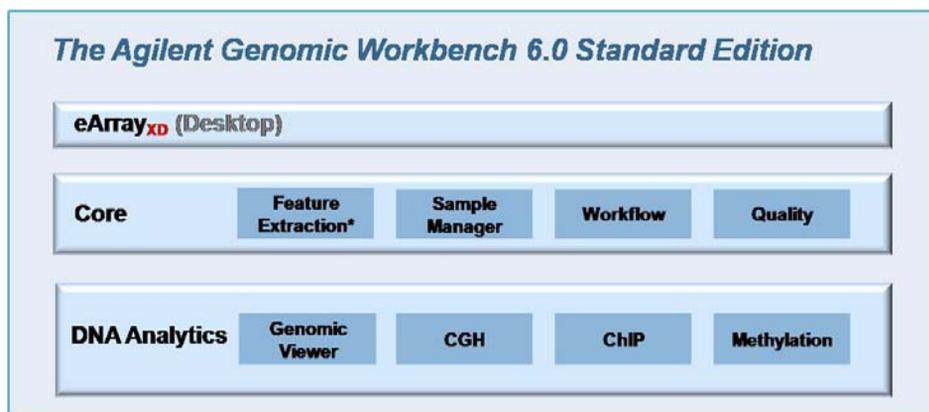
This chapter gives you a high-level overview of the Standard Edition of Agilent Genomic Workbench. Chapter 3 gives you more details about each application, while Chapter 2 tells you how to start and find Help for each module. Chapter 4 gives information about system administration and troubleshooting.



Benefits of the Agilent Genomic Workbench

The Agilent Genomic Workbench (Standard Edition) software provides a robust data management and integrated data analysis environment for Agilent genomics applications, including comparative genomic hybridization (CGH), chromatin immunoprecipitation (ChIP), and methylation (CH3).

It also lets you design microarrays for CGH, ChIP, methylation (CH3), gene expression and microRNA studies. And if you use Agilent SureSelect Target Enrichment, you can use Agilent Genomic Workbench to design bait libraries that retrieve specific DNA fragments for sequencing. You can also use the Agilent Genomic Workbench to do quality control (QC) analysis of the pull-down of target fragments.



*Can also be used as a stand-alone application

Figure 1 Programs available in Agilent Genomic Workbench Standard Edition

The software helps you:

- Design microarrays and SureSelect Target Enrichment Kits
- Manage sample information through the analysis process
- Start a program to extract features from microarray *.tif files
- Perform quality control
- Analyze data interactively
- Run a workflow to automate feature extraction, the assessment of data quality, and data analysis

Agilent Genomic Workbench Standard Edition provides a scalable architecture that enables a multiuser workgroup to maximize the value of its research, and to automate the process from content design and creation, through image processing and data analysis. Centralized data storage allows you to share data and reports within the workgroup.

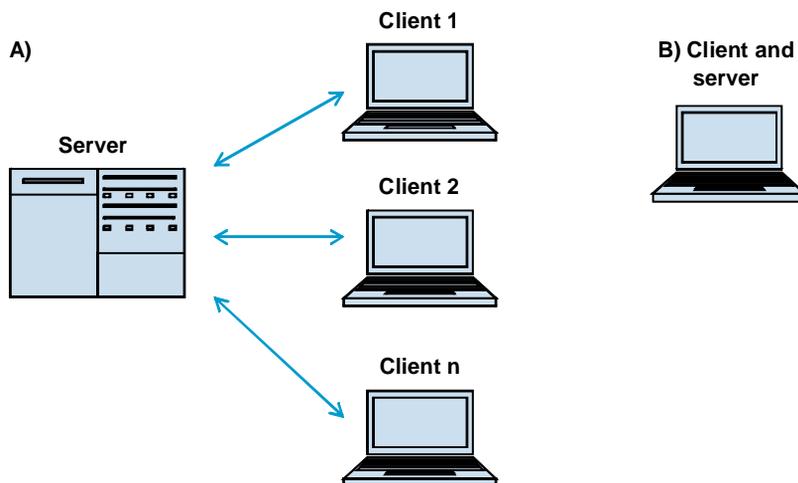


Figure 2 Agilent Genomic Workbench uses a scalable client-server architecture

The software has a database that enables data sharing and a more efficient way to work. For example, if you download a design file, it is available both in Feature Extraction and in DNA Interactive Analysis (CGH, CHIP, and Methylation).

The ability to do queries is another advantage of the database. For example, you can query for all female patients with *de novo* deletions on Chromosome 15. Or you could return all records that have aberrations in a defined genomic region.

Custom microarray design with eArray_{XD}

The eArray_{XD} program lets you create and manage custom microarray content for CGH, ChIP, methylation, gene expression, and microRNA applications. It also lets you design oligonucleotide bait libraries for target enrichment experiments.

With eArray_{XD}, you can submit microarray designs to Agilent for custom printing. You can also search and download any Agilent Catalog Microarray content from the Agilent eArray Web site through your Agilent Genomic Workbench server.

You can use the designs created in eArray_{XD}, whose files become part of Agilent Genomic Workbench, to set up the matching image files for automated feature extraction and analysis.

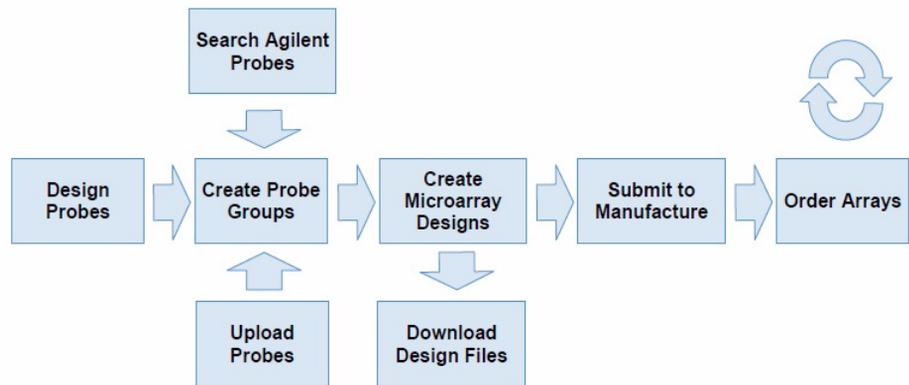


Figure 3 Array creation process

Automated feature extraction and analysis

The Agilent Genomic Workbench Workflow is uniquely designed to meet the needs of users who are extracting and analyzing microarray data from a large number of samples.

With an Agilent Feature Extraction license and a DNA Analytics program license (CGH or ChIP), you can use Sample Manager to associate a list of image files with Array IDs and sample attributes, and then set up and run a workflow that extracts selected images. The Feature Extraction results are then automatically analyzed with a DNA Analytics analysis method.

Behind the scenes are four Agilent Genomic Workbench core utilities that work together to create the final analytical results (Figure 4).

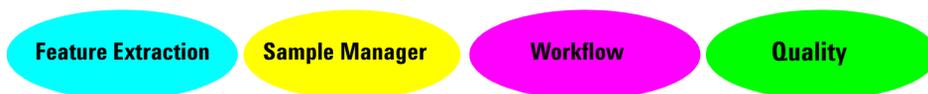


Figure 4 Agilent Genomic Workbench core utilities

Workflow is used with Sample Manager and Feature Extraction to keep sample IDs and their results together during extraction; then Workflow calls a specified analysis method to run a selected algorithm on the extracted results. You can save the workflow and the analysis method for later use.

With the Standard Edition of Agilent Genomic Workbench, you can also:

- Use the CGH and ChIP interactive interfaces to create and configure metric set filters from existing metric sets, or to create array-level filters or design filters
- Apply the filters to arrays in the workflow

The Agilent Genomic Workbench user interface has Sample Manager and Workflow tabs. You can set up samples and run the workflow with the commands under these tabs. You can use the Quality tab to determine the quality of microarray extractions.

1 Overview

Automated feature extraction and analysis

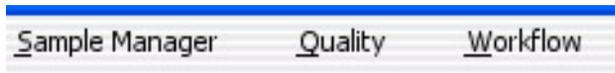


Figure 5 Sample Manager, Quality, and Workflow tabs

Feature Extraction runs in the background and uses a grid template that matches the array ID in the Sample Manager and a default Feature Extraction protocol associated with the grid template. If you want to check images before a run, or run a sample interactively with a different Feature Extraction protocol, you use the Feature Extraction user interface, which is separate from the Agilent Genomic Workbench user interface. Refer to the *Agilent Feature Extraction User Guide* for more information on grid templates.

The entire Agilent microarray research pathway with the Standard Edition of Agilent Genomic Workbench looks like this:

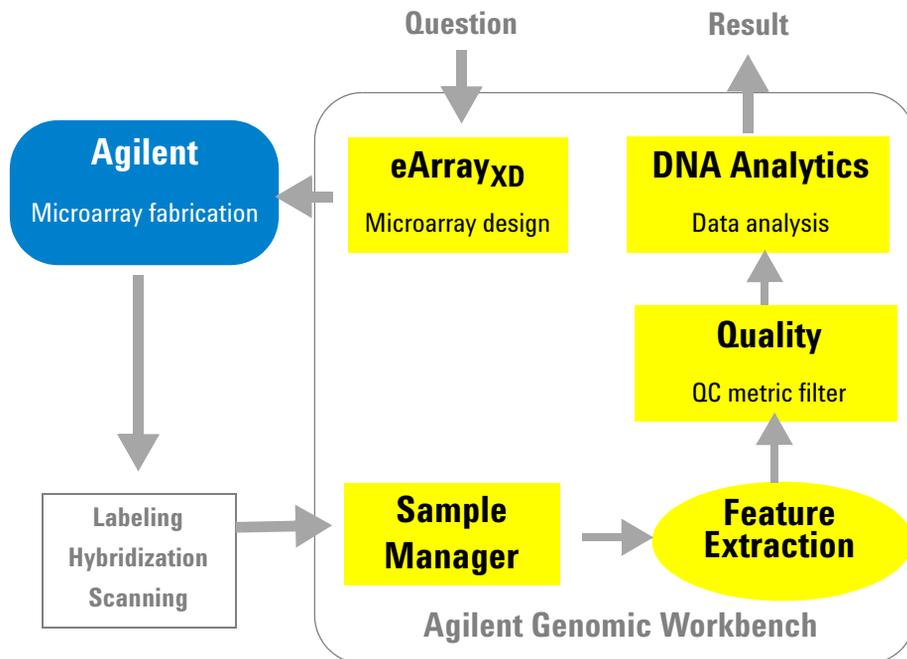


Figure 6 Microarray research pathway with Agilent Genomic Workbench – Standard Edition

Interactive data analysis for CGH, ChIP, or Methylation (CH3)

With the DNA Analytics licenses – CGH, ChIP and/or Methylation (CH3) – you can set up preprocessing, analysis, and reporting parameters interactively. The CGH program also has many postprocessing capabilities, called Discovery.

Table 1 DNA Interactive Analysis capabilities

Tabs	CGH capabilities	ChIP capabilities	Methylation (CH3) capabilities
Preprocessing	<ul style="list-style-type: none"> • Add feature and array filters • Turn on Centralization • Add design filters • Combine array designs and replicates • Display QC metrics • Set up and apply metric set filters 	<ul style="list-style-type: none"> • Select/Edit normalization calculation • Select/Edit error model • Combine designs and replicates • Display QC metrics • Set up and apply metric set filters 	<ul style="list-style-type: none"> • Combine array designs
Analysis	<ul style="list-style-type: none"> • Calculate a moving average on log ratio data • Select an aberration detection algorithm and set up its parameters 	<ul style="list-style-type: none"> • Select/Edit/Apply event detection model 	<ul style="list-style-type: none"> • Calculate a moving average on log ratio data • Apply probe methylation • Calculate a moving average on Z-scores generated by probe methylation algorithm • Apply Batman algorithm

1 Overview

Capabilities without licenses

Table 1 DNA Interactive Analysis capabilities (continued)

Tabs	CGH capabilities	ChIP capabilities	Methylation (CH3) capabilities
Discovery	<ul style="list-style-type: none">• Add aberration filters• Compare arrays with common aberrations• Make graphical penetrance diagrams• Set up to view CNVRs (copy number variant regions)• Compare CGH data with expression data• Compare arrays with different aberrations• Do a cluster analysis• Do a heatmap analysis	<ul style="list-style-type: none">• Not applicable	<ul style="list-style-type: none">• Not applicable
Reports	<ul style="list-style-type: none">• Aberration report• Penetrance report• Cyto Report• Use Report Manager to manage reports	<ul style="list-style-type: none">• Probe report• Gene report• QC report• Use Report Manager to manage reports	<ul style="list-style-type: none">• Probe report• Batman report

Capabilities without licenses

You do not need to purchase licenses to get some component capability with the Standard Edition of Agilent Genomic Workbench. Without an Agilent Feature Extraction or DNA Analytics program license you can still:

- Use the Home commands and Navigator (see [Figure 14](#) on page 34) to import, manage and display extracted log ratio data and other content in Genomic Viewer
- Use eArray_{XD} to design and manage your own microarrays and target enrichment libraries at your desktop, and keep track of all of their components
- Use SureSelect Quality Analyzer to assess the quality of results from the Agilent SureSelect Target Enrichment system

Summary of capabilities

The modules in the Standard Edition of Agilent Genomic Workbench give you the capabilities shown in [Table 2](#):

Table 2 Capabilities in Agilent Genomic Workbench 6.0 Standard Edition

If you want to do this:	Start this program or click this tab: (See Figure 7 on page 20 and Figure 14 on page 34)	Read this guide:
Design a microarray or SureSelect Target Enrichment library	eArray _{XD}	eArray _{XD} User Guide
Manage samples – Associate array IDs and attributes (sample information) with image files and/or imported extracted data	Sample Manager (Agilent Feature Extraction 10.9 or higher license required. Note: Feature Extraction has no tab in the Agilent Genomic Workbench user interface.)	Sample Manager User Guide
Set up and run Feature Extraction interactively – Get raw intensity values from scanned microarray images; calculate background, signal biases and errors; calculate dye bias; and calculate intensity ratios	Feature Extraction (This is a separate program that you can start from Agilent Genomic Workbench; Feature Extraction 10.9 or higher license required.)	Feature Extraction User Guide
Define quality metrics for microarrays and set their thresholds; define a set of metrics as custom metric sets	Start Quality Tools or click Quality tab (Agilent Feature Extraction 10.9 or higher license required)	Quality Tools User Guide
Produce QC Charts – Create queries and evaluate the results against a metric set, to produce a QC Chart that highlights feature extraction data points that fall outside of quality thresholds	Start Quality Tools or click Quality tab (Agilent Feature Extraction 10.9 or higher license required)	Quality Tools User Guide
Run Feature Extraction Workflow – Set up and run a workflow where scanned image files are automatically extracted, with or without a metric set filter	Workflow (Agilent Feature Extraction 10.9 or higher license required)	Workflow User Guide

1 Overview

Summary of capabilities

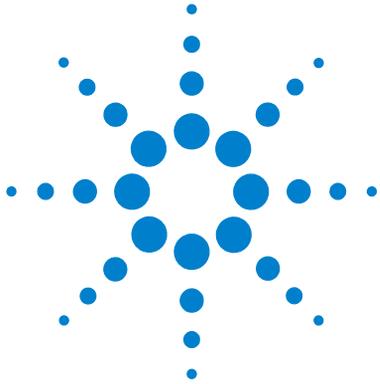
Table 2 Capabilities in Agilent Genomic Workbench 6.0 Standard Edition (continued)

If you want to do this:	Start this program or click this tab: (See Figure 7 on page 20 and Figure 14 on page 34)	Read this guide:
Run Analysis Workflow – Set up and run a workflow for automated, unattended CGH or ChIP analyses, with or without a metric set filter for the extracted data	Workflow (CGH or ChIP license required)	Workflow User Guide
Run Feature Extraction and Analysis Workflow – Set up and run a workflow where image files are automatically extracted and the results are automatically analyzed, with or without a metric set filter for the extracted data	Workflow (Agilent Feature Extraction 10.9 or higher license required and either a CGH or ChIP license required)	Workflow User Guide
Display Feature Extraction data – Import Feature Extraction data and view it next to chromosomes and genes	Genomic Viewer	Data Viewing User Guide (if you do not have a CGH, ChIP, or Methylation license), OR CGH Interactive Analysis User Guide ChIP Interactive Analysis User Guide Methylation (CH3) Analysis User Guide
Create and apply design filters in the CGH Interactive interface	DNA Analytics (CGH license required)	CGH Interactive Analysis User Guide
Create and apply metric set filters in the CGH Interactive and ChIP Interactive interfaces	DNA Analytics (CGH or ChIP license required)	CGH Interactive Analysis User Guide ChIP Interactive Analysis User Guide Quality Tools User Guide
Analyze extracted data interactively for CGH, ChIP or Methylation (CH3) application types	DNA Analytics (CGH, ChIP or Methylation license required)	CGH Interactive Analysis User Guide ChIP Interactive Analysis User Guide Methylation (CH3) Analysis User Guide

Table 2 Capabilities in Agilent Genomic Workbench 6.0 Standard Edition (continued)

If you want to do this:	Start this program or click this tab: (See Figure 7 on page 20 and Figure 14 on page 34)	Read this guide:
Manage and view reports	DNA Analytics (CGH or ChIP license required)	CGH Interactive Analysis User Guide ChIP Interactive Analysis User Guide
Assess quality of pull-downs of targeted genomic fragments when you use the Agilent SureSelect Target Enrichment System	SureSelect Quality Analyzer	SureSelect Quality Analyzer User Guide

1 Overview
Summary of capabilities



2 Getting Started

Starting Your Application and Finding Help 20
Getting Help Within the Applications 40

This chapter helps you get started with the Standard Edition of Agilent Genomic Workbench 6.0.

Before you read this chapter, install the Agilent Genomic Workbench Standard Edition. If you need to reinstall the program, see the *Standard Edition Installation Guide*. If you do not have the guide, download it from the [Agilent Web site](#).



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Starting Your Application and Finding Help

After you install the software and double-click the Agilent Genomic Workbench 6.0 icon, you should see the Open Application tab of the main window. If you see anything else, please see [Chapter 4](#).

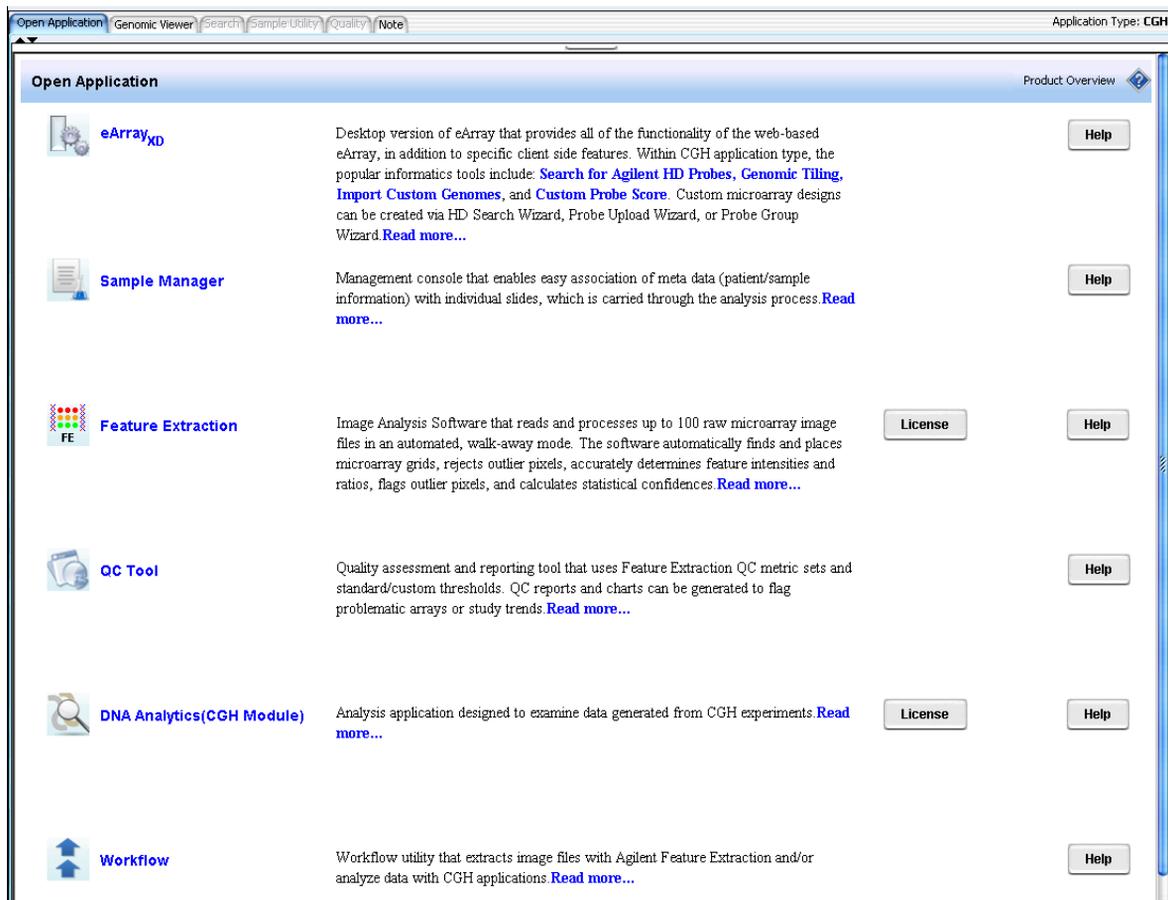


Figure 7 Open Application tab in Agilent Genomic Workbench Standard Edition, shown when CGH is selected under Switch Application

This section describes how to start each application and find help, and how to enter license(s) for individual modules.

To start and find help for eArray_{XD}

This application requires no license.

NOTE

To use eArray_{XD}, you must be a registered user on the Agilent eArray Web site. Go to <https://earray.chem.agilent.com> and click **Request for Registration**.

1 (Optional) To display the *eArray_{XD} User Guide*, click **Help** next to the description of eArray_{XD} in the Open Application tab.

2 In the Open Application tab, click the icon by **eArray_{XD}**.

OR

Click the **eArray_{XD}** tab.

The eArray_{XD} window appears. See [Figure 8](#). For more information about the window components and how to use them, see the “Getting Started” chapter in the *eArray_{XD} User Guide*.

eArray_{XD} is available for all of the application types in Agilent Genomic Workbench – CGH, ChIP, Methylation (CH3), Gene Expression, microRNA, and SureSelect Target Enrichment. If you are already using one of the DNA Analytics programs, you can just click the eArray_{XD} tab to use eArray_{XD} for that application type.

3 (Optional) If you want to use eArray for another type of data, click **Switch Application** in the upper right corner of the application window, and click the appropriate application type.

NOTE

In the Switch Application menu, you actually select the “application type.” Your selection then makes available all of the various programs for the application type. For example, when you select **CGH** as the application type, you gain access to the CGH functionality of eArray_{XD}, and to the Sample Manager program, the Quality tools for CGH, and the CGH Interactive Analysis program.

4 (Optional) To display the *eArray_{XD} User Guide*, click the **Help** tab, then click **eArray_{XD}**.

See also “[Designing Your Own Microarrays and SureSelect Target Enrichment Kits with eArray_{XD}](#)” on page 46.

2 Getting Started

To start and find help for eArrayXD

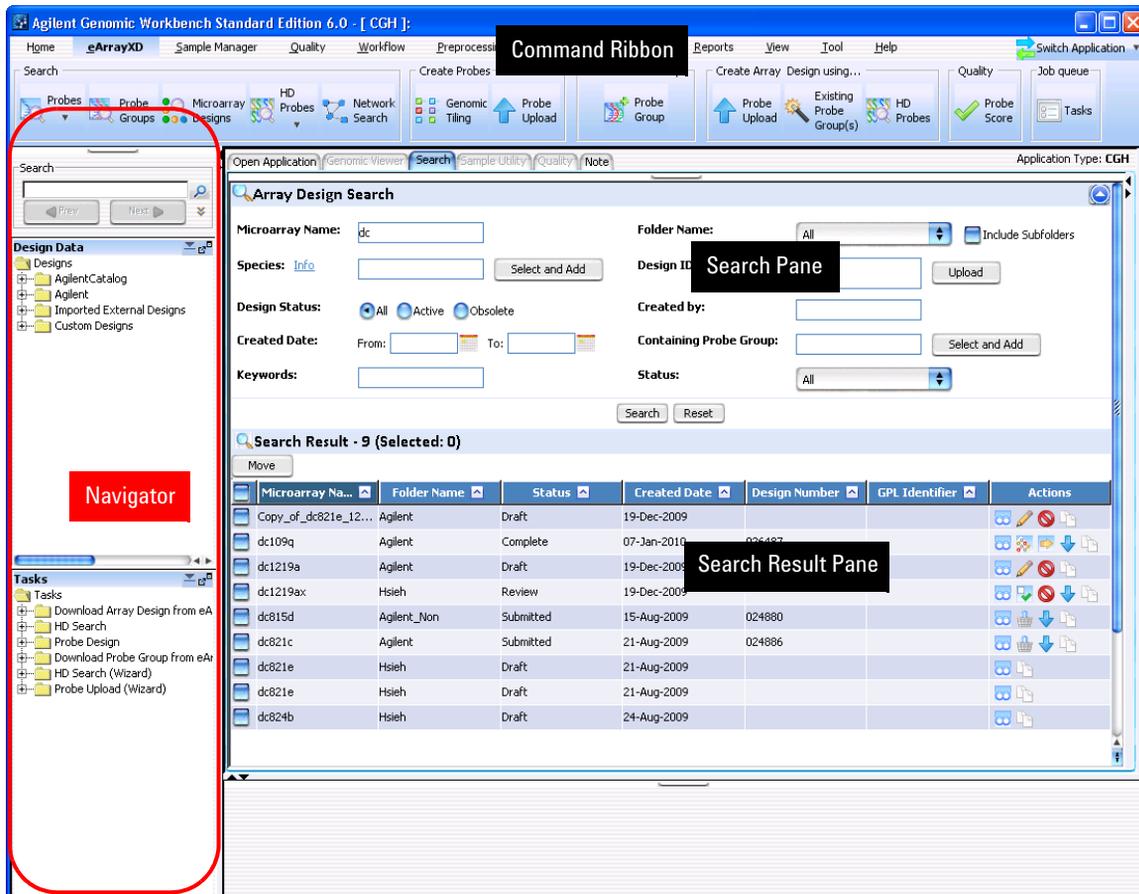


Figure 8 Agilent Genomic Workbench main window – eArray_{XD} tab

To start and find help for Sample Manager

This application requires a Feature Extraction license.

- 1 (Optional) To display the *Sample Manager User Guide*, in the Open Application tab, click **Help** next to the description of Sample Manager.
- 2 In the Open Application tab, click the icon by **Sample Manager**.

OR

Click the **Sample Manager** tab.

The Sample Manager window appears. Two folders with demo data appear in the Navigator. See [Figure 9](#). For more information about the window components and how to use them, see the “Getting Started” chapter in the *Sample Manager User Guide*.

You do not have to change applications to work with Sample Manager for a different program – CGH, ChIP, or Methylation (CH3).

- 3 (Optional) To display the *Sample Manager User Guide*, click the **Help** tab, then click **Sample Manager**.

See also [“Organizing and Assigning Array Attributes with Sample Manager”](#) on page 51.

2 Getting Started

To start and find help for Feature Extraction 10.9

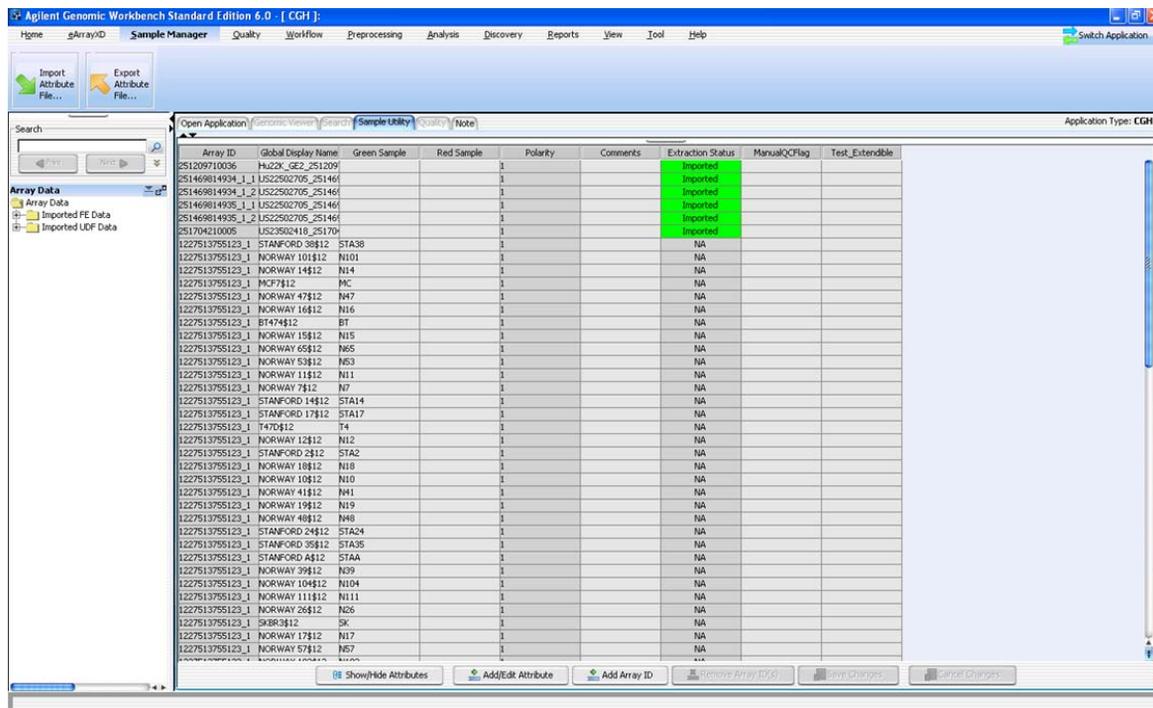


Figure 9 Agilent Genomic Workbench main window – Sample Manager tab

To start and find help for Feature Extraction 10.9

This application requires a Feature Extraction license.

1 (Optional) To view the *Feature Extraction User Guide*, click **Help** next to the description of **Feature Extraction** in the Open Application tab.

2 In the Open Application tab, click the icon by **Feature Extraction**.

The Feature Extraction program appears. See Figure 10. For more information about the window components and how to use them, see the “Getting Started” chapter in the *Feature Extraction User Guide*.

NOTE

To start Feature Extraction, you can also click **Start > All Programs > Agilent Genomic Workbench Standard Edition <version> > AGW Feature Extraction <version>**. Or you can click the Feature Extraction desktop icon. When you start the program for the first time, if you have not yet entered valid license information, the program asks you for the license.

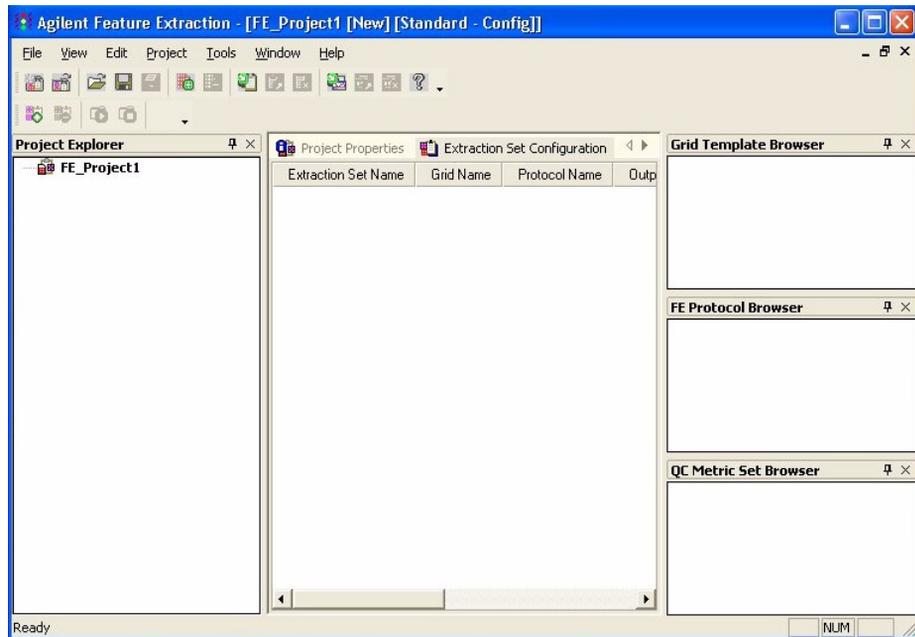


Figure 10 Agilent Genomic Workbench Feature Extraction main window

To redeem your Feature Extraction license

When you purchase Agilent Feature Extraction software, you receive a Software Entitlement Certificate (SEC). To redeem your purchased license, go to the Agilent Software License Redemption Web site and use the information on the certificate.

Without a valid license, you can still open an image, import templates and protocols, set up preferences, and change protocols. You cannot open a project or open Grid Mode to set up grids manually.

2 Getting Started

To start and find help for Feature Extraction 10.9

If you try to open a project in Feature Extraction without a valid license, an error message appears.

To redeem your Feature Extraction license:

- 1 Start the Feature Extraction program and select **Help > Agilent License**. Have the following items available:
 - **Software Entitlement Certificate (SEC)** - This PDF contains the Order Number and Certificate Number that you need to provide to redeem your purchased license.
 - **Host ID** - You can find this under the menu **Help > About**. You need to provide the host ID to redeem your purchased license.
- 2 Follow the instructions on the Agilent Software License Redemption Web site to redeem your purchased license.

After you redeem the license online, you will receive an e-mail with the license attached.
- 3 Save the unzipped license file to the folder on the drive where you installed the software. By default, Feature Extraction is installed in the Agilent Genomic Workbench installation folder:
Program Files\Agilent\Agilent Genomic Workbench Standard Edition <version>\FeatureExtraction
- 4 Follow one of the procedures below to enter the license from Feature Extraction or from Agilent Genomic Workbench.

To set up the license from Feature Extraction

- 1 If the Feature Extraction program is already running, open a project. Otherwise, double-click the Feature Extraction icon to open a project.
- 2 Click **Retry**.
- 3 Find the directory that contains the license file.
- 4 Select the license file (*.lic), and click **Open**.

To set up the license from Agilent Genomic Workbench

- 1 Locate your license file and double-click it to open the file in Notepad.
- 2 Highlight the license text, and copy it to the Clipboard.
- 3 In the Open Application tab in Agilent Genomic Workbench, next to Feature Extraction, click **License**.

- 4 Click **License** and then paste the license into the blank license field.
(To paste the text, press **Ctrl + V** on your keyboard.)

To set up an eArray login within Feature Extraction

This section describes how to set up an eArray login, so you can automatically update grid templates (design files), protocols, and QC metric sets. When you first open Feature Extraction, the **eArray Login Setting** dialog box appears in front of the Feature Extraction main window. If you do not see the eArray Login Setting dialog box, you can open it under **Tools > eArray Settings**.

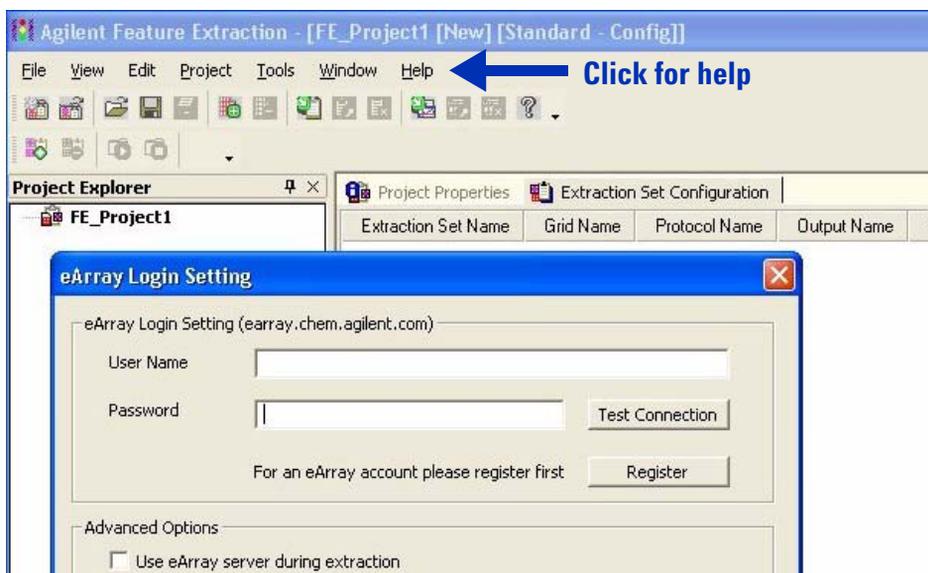


Figure 11 eArray Login Setting dialog box in front of Feature Extraction main window

Feature Extraction can connect to the Agilent eArray Web site to automatically download, install, and use the required grid templates (design files), Feature Extraction protocols, and QC metric sets during extraction.

2 Getting Started

To start and find help for Feature Extraction 10.9

To take advantage of this feature, Agilent highly recommends that you set up an eArray login before you run the extraction project. You need an internet connection for Feature Extraction to log into the eArray Web site.

1 If you already have an existing eArray account, type the **User Name** and **Password** in the eArray Login Setting window. Hint: The user name is the e-mail address you gave when you created an eArray account.

If you do not have an eArray account, you must first click the **Register** button to register for one. This will take you to the Agilent eArray registration Web site, where you can request a free eArray account. Follow the instructions on the eArray Web site to complete the registration.

2 Click **Test Connection**. The message “Login Successful!!!” is displayed if the connection to the eArray Web site is successful. If you get the message “Login denied”, make sure that the user name and password you typed are correct.

3 In the Advanced Options, mark the appropriate boxes:

- **Use eArray server during extraction**

Mark this box to let Feature Extraction log into the eArray Web site to download, install, and use grid templates (design files), Feature Extraction protocols, and QC metric sets during extraction.

- **Check for updates of grid template**

Mark this box to let Feature Extraction download updates to grid templates that are already in the Grid Template Browser.

- **Replace old grid template**

Mark this box to let Feature Extraction silently replace old grid templates in the Grid Template Browser with the latest ones from the eArray server. See the *Feature Extraction User Guide* for more information.

- **On starting FE check for protocol update from eArray server**

Mark this box to let Feature Extraction download updates to existing default protocols and QC metric sets in the Feature Extraction Protocol Browser.

4 To save the eArray login settings, click **Save**. A message appears: “Setting updated successfully”. Click **OK** to close the message, and click **Close** to exit the eArray Login Setting dialog box.

The Feature Extraction main window appears.

To start and find help to assess the quality of Feature Extraction data

This application requires a Feature Extraction license.

1 (Optional) To display the *Quality Tools User Guide*, in the Open Application tab, click **Help** next to the description of the Quality Tools.

2 In the Open Application tab, click the icon by **Quality Tools**.

OR

Click the **Quality** tab.

The Quality window appears. See [Figure 12](#). For more information about the window components and how to use them, see the “Getting Started” chapter in the *Quality Tools User Guide*.

3 (Optional) To display the *Quality Tools User Guide*, click the **Help** tab, then click **Quality**.

See also [“Using the Quality Tools to Monitor Array Quality”](#) on page 53.

2 Getting Started

To start and find help to assess the quality of Feature Extraction data

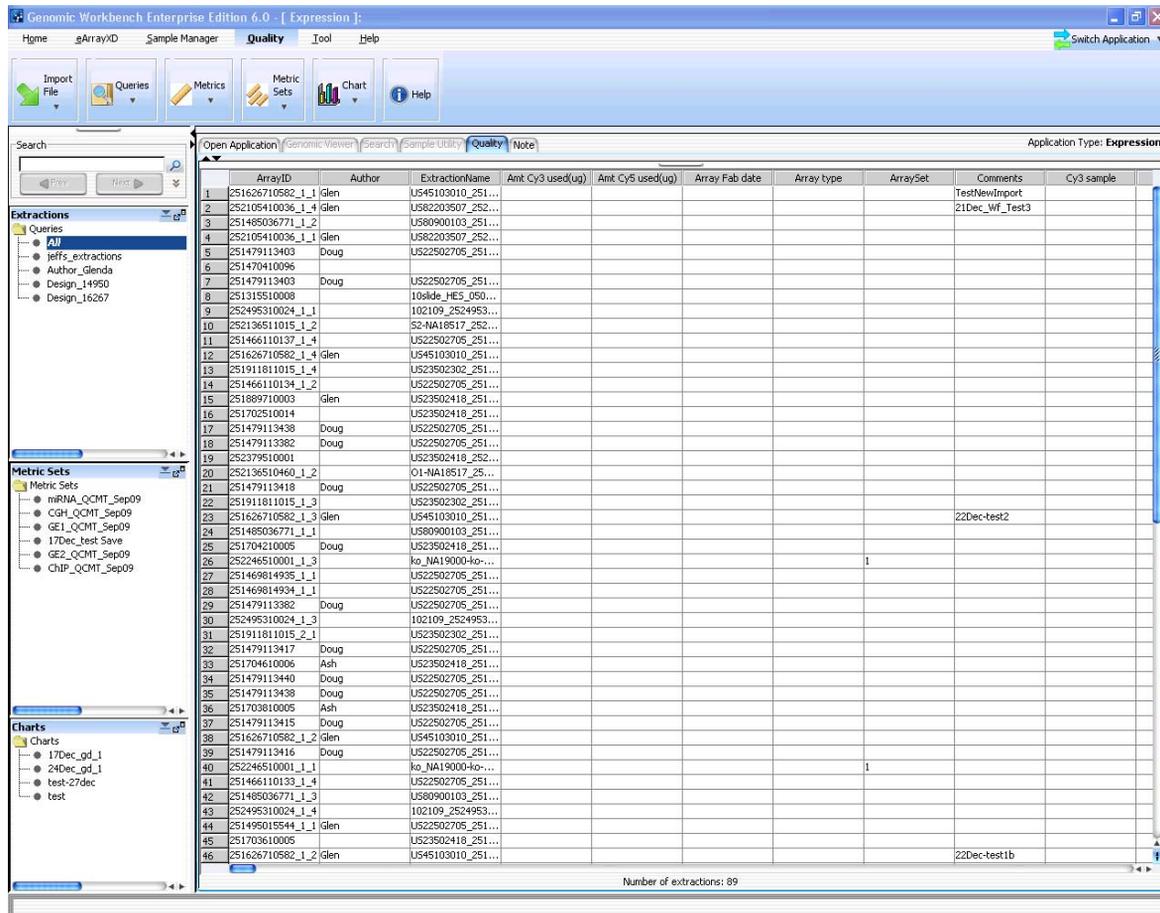


Figure 12 Agilent Genomic Workbench main window – Quality tab, populated with data

See Figure 22 on page 54 for an example of a QC Chart you can produce with the Quality tools application.

To start and find help to run workflows

This application requires one or more licenses. You must have an Agilent Feature Extraction 10.9 (or higher) license to run an *extraction* workflow. You can run an *extraction* workflow for many types of arrays, including CGH, ChIP, and Methylation (CH3).

You must have a CGH and/or ChIP license to run an *analysis* workflow. You can run an *analysis* workflow only for CGH and ChIP arrays. For details on how to activate a CGH or ChIP license, see [step 3](#) below.

1 (Optional) To display the *Workflow User Guide* in the Open Application tab, click **Help** next to the description of Workflow.

2 In the Open Application tab, click the icon by **Workflow**.

OR

Click the **Workflow** tab.

The Workflow window appears and the content of the Navigator changes. You initially see the Workflow Navigator. See [Figure 13](#). For more information about the window components and how to use them, see the “Getting Started” chapter in the *Workflow User Guide*.

You must change applications to set up a workflow for a different DNA Analytics program (CGH or ChIP).

3 Either before or after you set up a workflow, enter your CGH or ChIP license if you have not already done so. Follow these steps:

a Click the **Home** tab, then click **User Preferences**, then click the **License** tab. See [Figure 15](#) on page 35.

b Follow the instructions under “[To use Server Location to enter the license\(s\)](#)” on page 36 or “[To use Text License to enter the license\(s\)](#)” on page 36.

4 (Optional) To display the *Workflow User Guide*, click the **Help** tab, then click **Workflow**.

See also “[Setting Up and Running Workflows for Extraction and/or Analysis](#)” on page 56.

2 Getting Started

To start and find help to run workflows

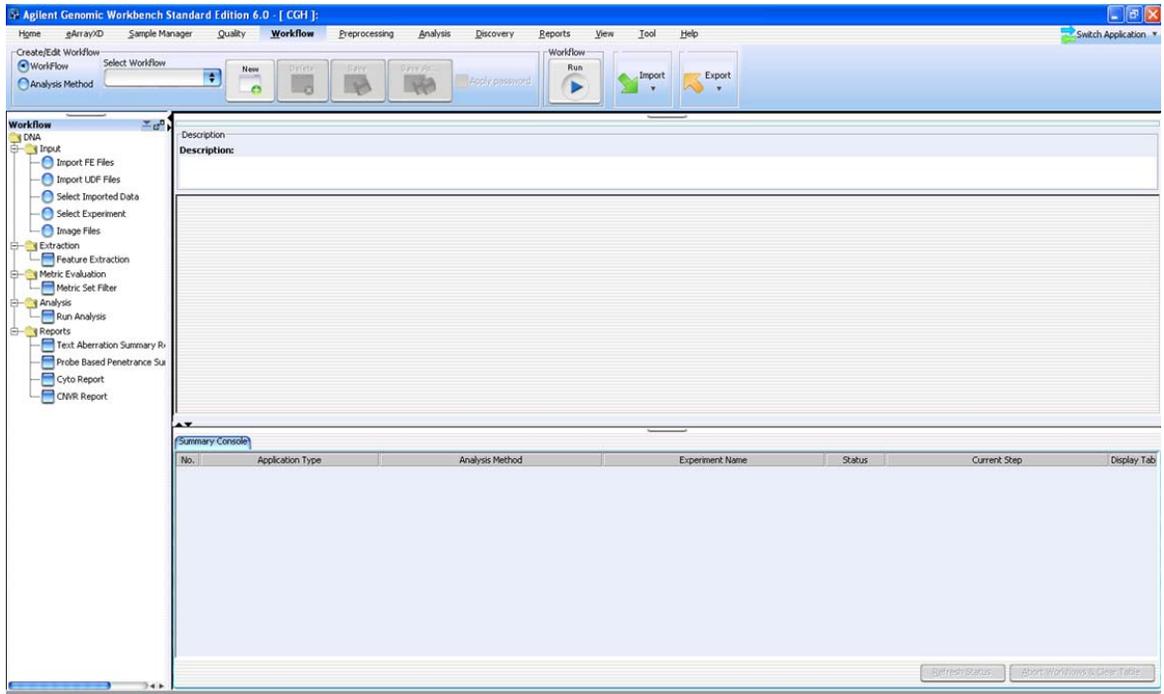


Figure 13 Agilent Genomic Workbench main window – Workflow tab

To start and find help to display CGH, ChIP, or Methylation (CH3) data

This application requires no license.

- 1 Click the **Genomic Viewer** tab at the top of the Agilent Genomic Workbench main window to display the Genomic Viewer pane. See [Figure 14](#). For more information about the window components and how to use them, see the “Getting Started” chapter in the *Data Viewing User Guide*.

You can now display the data for the application you selected when you started the program.

- 2 If you want to display another type of data, click **Switch Application** in the upper right corner of the application window, and click the appropriate application type.

You must change applications to view other data types because some of the options are different for each data type.

- 3 To display the *Data Viewing User Guide*, click the **Help** tab, then click **Data View**.

See also “[Displaying Data/Results in Genomic Viewer](#)” on page 58.

NOTE

In the Switch Application menu, you actually select the “application type.” Your selection then makes available all of the various programs for the application type. For example, when you select **CGH** as the application type, you gain access to the CGH functionality of eArray_{XD}, and to the Sample Manager program, the Quality tools for CGH, and the CGH Interactive Analysis program.

2 Getting Started

To start and find help to display CGH, ChIP, or Methylation (CH3) data

The screenshot displays the Agilent Genomic Workbench Standard Edition 6.0 interface. The main window is titled "Agilent Genomic Workbench Standard Edition 6.0 - [CGH] - Unlicensed Version". The interface is divided into several sections:

- Top Ribbon:** Contains tabs for Home, gArrayXD, Sample Manager, Quality, Workflow, Preprocessing, Analysis, Discovery, Reports, View, Tool, and Help. Below the tabs are buttons for User Preferences, Import, Export, Create Experiment, Save Experiment Result, Go To Gene/Genomic location, Print, and Exit.
- Search and Application Management:** A search bar and "Open Application" button are visible. The "Genomic Viewer" tab is selected and highlighted with a red circle.
- Left Navigator Panes:** Includes "Design Data" (Designs, Agilent Catalog, Agilent, Imported External Designs, Custom Designs), "Experiment" (Experiments, CGH_EXP, Design 11256659211521016), "My Entry List" (Entities, Gene List, Tracks), and "Tasks".
- Main View Area:** Displays four views: "Genomic Viewer" (a grid of data points), "Gene View" (a detailed view of a gene with associated probes), "Chromosome View" (a chromosome ideogram), and "Genome View" (a genome-wide overview).
- Bottom Array Table:** A table titled "Arrays" with columns: ProbeName, ChrName, Start, Stop, MCF7#12, and BT47#12. The table lists various probes and their coordinates on chromosome 1.
- Bottom Status Bar:** Shows the current design "Design1135665921152101915", selected arrays, and other parameters like "hg17", "log2 ratio", "Selected Row = 166", and "588 x 6".

Green callout boxes with arrows point to specific features: "Tabs and Command Ribbon", "Switch Application", "Genomic Viewer", "Gene View", "Chromosome View", "Genome View", "Navigator Panes", and "Tab View".

ProbeName	ChrName	Start	Stop	MCF7#12	BT47#12
AA121360	chr1	46366378	46366378	-0.150	-0.150
AA489847	chr1	46541549	46541549	-0.320	-0.340
T70327	chr1	46613602	46613602	-0.150	0.060
AA431884	chr1	46735830	46735830	-0.620	0.060
T71965	chr1	46812730	46812730	-0.920	-0.220
W68541	chr1	46853146	46853146	-0.920	-0.270
AA291484	chr1	46996538	46996538	-0.370	-0.130
W84867	chr1	47107599	47107599	-0.320	-0.270
R56123	chr1	47228075	47228075	-0.320	-0.250
A1668602	chr1	47294350	47294350	-0.420	-0.140
T51236	chr1	47396381	47396381	-0.510	-0.040
R97066	chr1	47406058	47406058	-0.470	-0.380
W78843	chr1	48425225	48425225	-0.620	-0.400
W70062	chr1	50965691	50965691	-0.250	-0.190
N72115	chr1	51151831	51151831	-1.180	-0.140
W94868	chr1	51450718	51450718	-0.090	0.000
AA490223	chr1	51531955	51531955	0.210	-0.400
T67223	chr1	51555906	51555906	-0.170	-0.510

Figure 14 Agilent Genomic Workbench main window – Genomic Viewer tab for unlicensed CGH application type

To start and find help to analyze CGH, ChIP or Methylation (CH3) data interactively

To start and find help to analyze CGH, ChIP or Methylation (CH3) data interactively

This application requires a DNA Analytics license (CGH, ChIP, or Methylation).

- 1 (Optional) To view the *CGH* or *ChIP Interactive Analysis User Guide* or the *Methylation (CH3) User Guide*, click **Help** next to the description of DNA Analytics in the Open Application tab.

The appropriate User Guide is displayed.

- 2 Click **License** next to the description of DNA Analytics in the Open Application tab.

The License tab of the User Preferences dialog box appears.

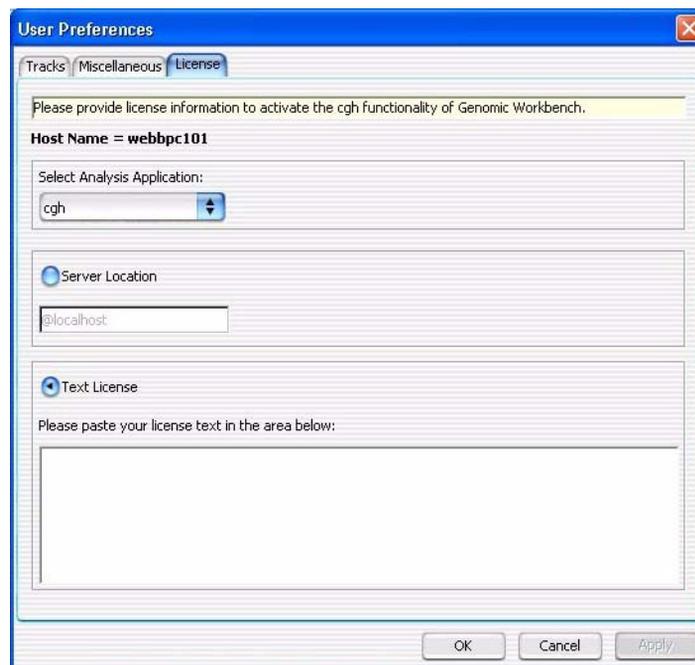


Figure 15 License tab of the User Preferences dialog box

2 Getting Started

To start and find help to analyze CGH, ChIP or Methylation (CH3) data interactively

You can enter your license information in one of two ways:

- Let Agilent Genomic Workbench automatically read the license(s) – uses Server Location shown in [Figure 15](#)
- Copy the text for the license(s) into the box – uses Text License shown in [Figure 15](#)

To use Server Location to enter the license(s)

- 1 Under Select Analysis Application, select the application for the license you wish to enter. (See [Figure 15](#) on page 35.)
- 2 Unzip all your DNA Analytics text license file(s) into a folder on your server. Make sure this is a folder to which the program has access. Copy this path to the Clipboard.
- 3 In the License tab of the User Preferences dialog box, click **Server Location** and paste the path where the license file(s) are located.
- 4 Click **Apply**. Agilent Genomic Workbench automatically reads the license for the selected application.
- 5 If you have no other license, click **OK**.
If you have another license, repeat [step 1](#) and [step 4](#).

To use Text License to enter the license(s)

- 1 Under Select Analysis Application, select the application for the license you wish to enter. (See [Figure 15](#) on page 35.)
- 2 Find the folder that contains the DNA Analytics program license.
- 3 Double-click the license name.
- 4 Copy the text in the Notepad window, and paste it into the License text box, then click **Apply**.
- 5 If you have no other license, click **OK**.
If you have another license, repeat [step 1](#) through [step 5](#).

To start and find help to analyze CGH, ChIP or Methylation (CH3) data interactively

To open the program

- 1 In the Open Application tab, click the icon by **DNA Analytics**.

The application program – CGH, ChIP, or Methylation (CH3) – appears with the Preprocessing tab displayed. See [Figure 16](#). For more information about the window components and how to use them, see the “Getting Started” chapter in a DNA Analytics *User Guide* (CGH or ChIP Interactive Analysis or Methylation (CH3) Analysis).

- 2 (Optional) To change application type, click **Switch Application** in the upper right corner of the application window, and click the program for which you entered another license.
- 3 (Optional) To view a DNA Analytics *User Guide* (CGH or ChIP Interactive Analysis or Methylation (CH3) Analysis), click the **Help** tab, then click **Help** in the ribbon.

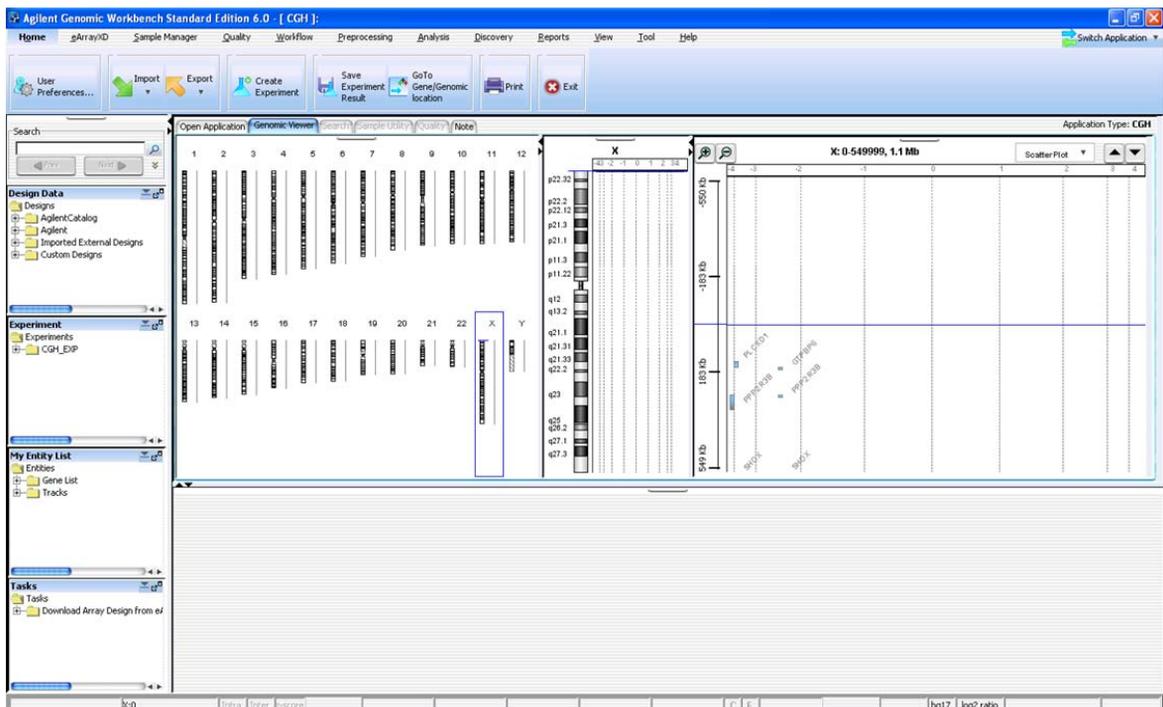


Figure 16 Agilent Genomic Workbench main window – Genomic Viewer tab for CGH application type

To start and find help to assess the effectiveness of the SureSelect Target Enrichment System

This application requires no license.

- 1 Click **Switch Application** in the upper right corner of the application window, and click **SureSelect Target Enrichment**.
- 2 (Optional) To display the *SureSelect Quality Analyzer User Guide*, in the Open Application tab, click **Help** next to the description of the SureSelect Quality Analyzer.
- 3 In the Open Application tab, click the icon by **SureSelect Quality Analyzer**.

OR

Click the **Quality Analyzer** tab.

The Quality Analyzer window appears. See [Figure 17](#). For more information about the window components and how to use them, see the “Getting Started” chapter in the *SureSelect Quality Analyzer User Guide*.

See also [“Using the SureSelect Quality Analyzer”](#) on page 63.

To start and find help to assess the effectiveness of the SureSelect Target Enrichment System

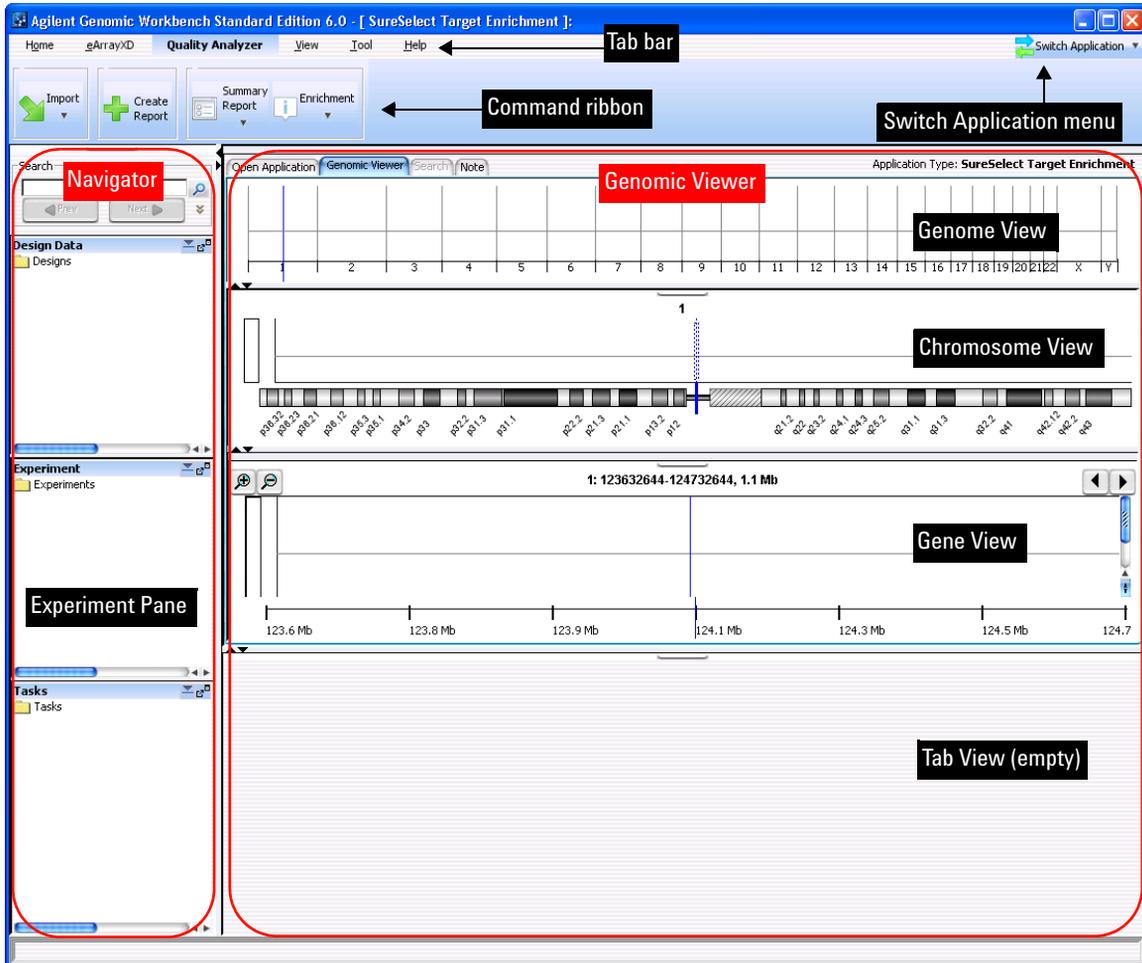


Figure 17 Agilent Genomic Workbench main window – Quality Analyzer tab

Getting Help Within the Applications

To get help within Agilent Genomic Workbench

The program has several built-in help resources:

Help resource	Description/instructions
Info links	Info links within the program display contextual help. <ul style="list-style-type: none">Next to a parameter or criterion, click Info, if it appears. A message appears with a description of the item and/or instructions that relate to it.
User Guides	The Help tab in the program lets you view any of the available user guides that apply to the currently selected application type. <ol style="list-style-type: none">Set the desired application type.In the Agilent Genomic Workbench tab bar, click Help. The names of the available user guides appear in the command ribbon. See Figure 18 for an example.Click the desired user guide. The selected user guide opens in Adobe Reader.



Figure 18 Help ribbon for the CGH and ChIP application types

Help tab

The Help tab contains commands that open the user guides that are available for Agilent Genomic Workbench. It also contains additional commands that let you display version and license information for the program, view information about the software updates that have been installed, and check the Agilent Web site for software updates that are available.

Help buttons

These buttons let you view the user guides that are available for Agilent Genomic Workbench. The guides that are available vary by application type. Each opens in Adobe Reader. These buttons can appear:

Button	Description
Application Guide	<p>(Available for all application types except Expression and microRNA) For each of these application types, this button opens the indicated user guide:</p> <ul style="list-style-type: none"> • CGH – Opens the <i>CGH Interactive Analysis User Guide</i>. This guide describes how to use the CGH application of Agilent Genomic Workbench to analyze comparative genomic hybridization data and create reports. • ChIP-on-chip – Opens the <i>ChIP Interactive Analysis User Guide</i>. This guide describes how to use the ChIP application of Agilent Genomic Workbench to analyze chromatin immunoprecipitation data and create reports. • CH3 – Opens the <i>Methylation (CH3) Analysis User Guide</i>. This guide describes how the use the Methylation (CH3) application of Agilent Genomic Workbench to apply algorithms that help identify methylated regions. • SureSelect Target Enrichment – Opens the <i>SureSelect Quality Analyzer User Guide</i>. This guide describes how to use the SureSelect Quality Analyzer application of Agilent Genomic Workbench to assess the effectiveness of fragment pull-down for target enrichment experiments
eArray _{XD}	<p>(Available for all application types) Opens the <i>eArray_{XD} User Guide</i>. This guide describes how to design and submit custom microarray designs and SureSelect Target Enrichment bait libraries.</p>
Sample Manager	<p>(Available for all application types except SureSelect Target Enrichment) Opens the <i>Sample Manager User Guide</i>. This guide describes how to use the Sample Manager module of Agilent Genomic Workbench to organize microarrays and edit their attributes.</p>

Button	Description
Feature Extraction	<p>(Available for all application types except SureSelect Target Enrichment) Opens a menu with these options:</p> <ul style="list-style-type: none"> • Quick Start – Opens the <i>Feature Extraction Quick Start Guide</i>. This guide gives an overview of how to use the Feature Extraction software to extract and generate QC reports for Agilent microarrays. • User Guide – Opens the <i>Feature Extraction User Guide</i>. This guide shows you how to set up and run Feature Extraction to automatically extract a batch of image files. It also describes how to extract image files in real time. • Reference Guide – Opens the <i>Feature Extraction Reference Guide</i>. This guide contains tables that list default parameter values and results for Feature Extraction analyses, and explanations of how Feature Extraction uses its algorithms to calculate results.
Quality Tools	<p>(Available for the CGH, ChIP-on-chip, and Methylation application types) Opens the <i>Quality Tools User Guide</i>. This guide describes how to query, filter, and evaluate microarray extractions within Agilent Genomic Workbench. It also describes how to visualize current and historical batch microarray extraction processes.</p>
Workflow	<p>(Available for the CGH and ChIP-on-chip application types) Opens the <i>Workflow User Guide</i>. This guide describes how to use the workflow module of Agilent Genomic Workbench to extract image files with Agilent Feature Extraction software and/or analyze data using the CGH and ChIP analysis applications.</p>
Data Viewing	<p>(Available for all application types except Expression and microRNA) Opens the <i>Data Viewing User Guide</i>. This guide describes how to import, organize, manage, export, and display data and other content (experiments, gene lists, tracks) within Agilent Genomic Workbench. It is targeted for users who have no DNA Analytics application license(s).</p>

Other commands

About Opens a dialog box that displays version and copyright information for your installation of Agilent Genomic Workbench Standard Edition. You can also use this dialog box to display the License Agreement for the Agilent Genomic Workbench software.

Installation History	Opens a dialog box that lets you view information about the server and client updates that you have installed.
Check Updates	Checks the Agilent Web site for software updates that are available for Agilent Genomic Workbench.

To get help with the eArray Web site

The eArray Web site contains a comprehensive online Help system that describes how to use the Web site and all of its available tools. You do not need to log in to the site, or to be a registered user on the site to view the online Help.

1 In Internet Explorer 7, go to <https://earray.chem.agilent.com>

The login page of the eArray Web site appears.

2 At the top of the page, click **Help**.

The online Help system for the eArray Web site opens in a new window.

In addition, Info links appear throughout the site that give additional details and instructions about selected parameters, criteria, and commands. Click [Info](#) where it appears.

2 Getting Started

To get help with the eArray Web site



3

Detailed Descriptions

Designing Your Own Microarrays and SureSelect Target Enrichment Kits with eArrayXD [46](#)

Organizing and Assigning Array Attributes with Sample Manager [51](#)

Using Feature Extraction Interactively [52](#)

Using the Quality Tools to Monitor Array Quality [53](#)

Setting Up and Running Workflows for Extraction and/or Analysis [56](#)

Displaying Data/Results in Genomic Viewer [58](#)

Analyzing CGH Data Interactively [60](#)

Analyzing ChIP Data Interactively [61](#)

Analyzing Methylation (CH3) Data [62](#)

Using the SureSelect Quality Analyzer [63](#)

This chapter gives you more details about each of the modules within the Standard Edition of Agilent Genomic Workbench 6.0.



Designing Your Own Microarrays and SureSelect Target Enrichment Kits with eArray_{XD}

What is eArray_{XD}?

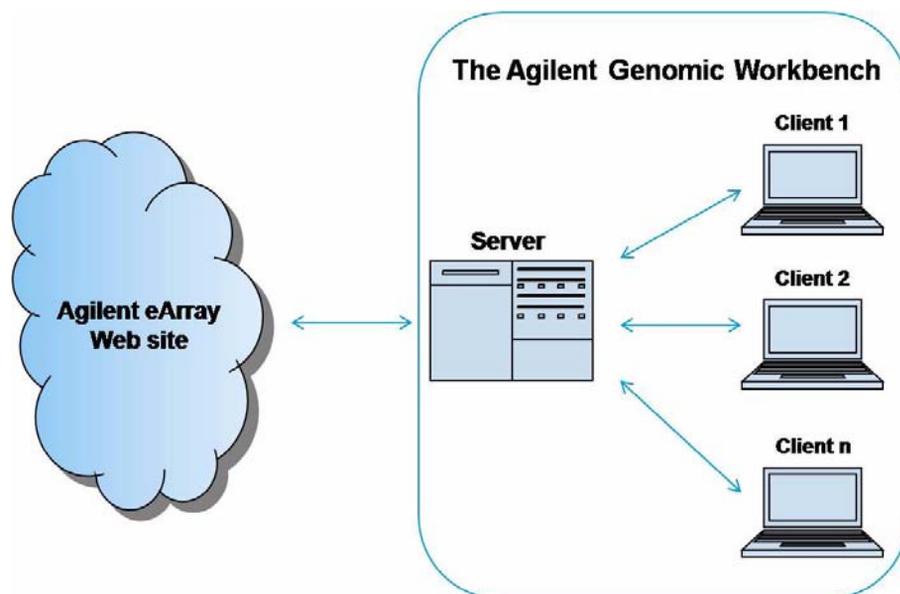


Figure 19 Components of eArray system

eArray_{XD} is a tool that lets you design and print microarrays. The eArray system, illustrated in [Figure 19](#), is a client-server system that contains several main components:

- **Agilent eArray Web site** – An Agilent site that contains several databases, including the Agilent Catalog Probe Database, the Agilent HD Probe Database, and a database of content that your workgroup may have previously created on the site. The site also contains several useful utilities that can create new custom content, and lets you submit your microarray designs to Agilent Manufacturing for subsequent quotation, ordering, and fabrication.

To use eArray_{XD}, your workgroup must be registered on the eArray Web site, and you must have a user account with a valid login name and password.

- **Agilent Genomic Workbench server** – A single, central repository for the microarray-related and SureSelect Target Enrichment library-related content of your workgroup. This system is a set of programs that you can set up on your computer, or on a networked computer at a different location. It stores workgroup content, and it communicates with the eArray Web site to download content as needed. In addition, it submits probe design and other types of jobs to the eArray Web site and monitors their progress.
- **eArray_{XD} on Agilent Genomic Workbench client(s)** – A program that is installed with Agilent Genomic Workbench and that lets you create and manage custom microarray content for CGH, ChIP, methylation, gene expression, and microRNA applications. It also lets you work with oligonucleotide bait libraries for target enrichment experiments.

eArray_{XD} is a client program that communicates with your Agilent Genomic Workbench server to upload or retrieve data as needed. In general, it stores all content on your Agilent Genomic Workbench server, but you also can transfer specific types of files to your local computer.

In addition, it lets you submit microarray designs and retrieve data from the Agilent eArray Web site through your Agilent Genomic Workbench server. If your workgroup has multiple users, they can all run eArray_{XD} on their own computers and connect to the Agilent Genomic Workbench server through a standard network connection.

Once you are connected to your Agilent Genomic Workbench server and the eArray Web site, you can do all of your design work on your own computer in eArray_{XD}.

NOTE

The Agilent eArray Web site provides additional capabilities and support for additional application types, including SureSelect Capture Array.

3 Detailed Descriptions

Probes, probe groups, and microarray designs and sets

Probes, probe groups, and microarray designs and sets

When you use eArray_{XD} to create custom microarray designs, you work with content on several levels of organization. Figure 20 illustrates these levels.

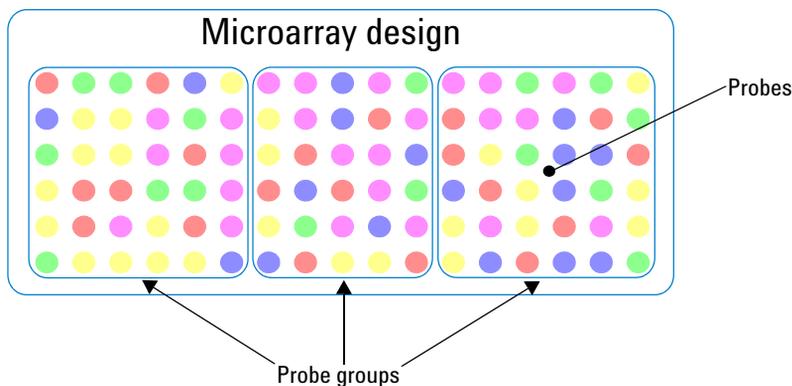


Figure 20 Probes, probe groups, and microarray designs

- **Probes** are single-stranded oligonucleotide molecules of defined sequence that are the fundamental units of microarrays. eArray_{XD} contains their nucleotide sequences, as well as annotation and accession information. You can use the extensive set of tools available in the program to search for, create, display, manage, and analyze probes.
- **Probe groups** are collections of probes that share one or more criteria, and are used to organize probes. Probe groups are organizational units that you use as the building blocks of microarray designs.
- **Microarray designs** contain one or more probe groups. A given design is a set of files that contains all of the information necessary to manufacture a specific microarray slide, as well as relevant information for downstream analysis.

SureSelect Target Enrichment Kits

Target enrichment is a method that isolates specific fragments of genomic DNA for sequencing. You use a **library** of complementary oligonucleotide **baits** to harvest fragments of interest (target DNA). The target DNA hybridizes well with the baits, but other DNA does not, which forms the basis of a powerful selection method that enables you to focus your sequencing efforts.

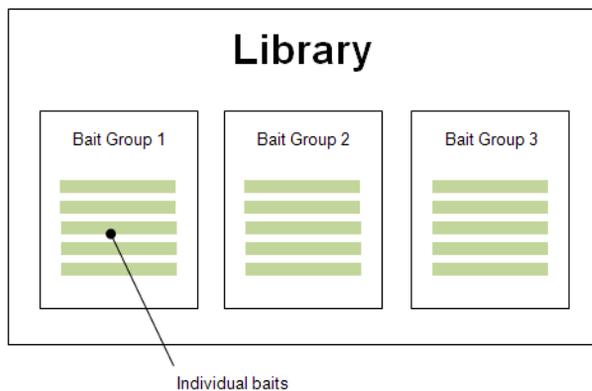


Figure 21 Baits, bait groups, and libraries

When you order a SureSelect Target Enrichment library from Agilent, the final product that you receive is a kit that contains a set of biotinylated RNA oligonucleotides. However, as part of the library manufacturing process, Agilent first creates DNA oligonucleotides, and then later transcribes them into RNA. Thus, when you create a library in eArray_{XD}, you specify bait sequences in terms of DNA bases (A, C, G, T).

With eArray_{XD}, you can access existing baits and bait libraries or create custom libraries of baits. A bait library is a collection of oligonucleotides in a tube, not a microarray, but you can use the eArray microarray creation infrastructure to easily design and obtain the libraries that you need for your research.

Custom genomes are supported only in eArray_{XD} (not on the eArray Web site).

3 Detailed Descriptions

Capability available only on eArray Web site

Capability available only on eArray Web site

eArray_{XD} contains a subset of the functionality available on the eArray Web site. To learn the full functionality on the Web site, please see “Comparison of eArray_{XD} and the eArray Web site” in the *eArray_{XD} User Guide*.

Organizing and Assigning Array Attributes with Sample Manager

When you scan a hybridized microarray slide, the Agilent Microarray Scanner creates a *.tif image file that includes the Array ID from the slide. This Array ID, contained in the header of the *.tif file, uniquely identifies the slide. A microarray slide can contain one array, or for multi-pack arrays, multiple replicate arrays on the same slide. The scanned data from each microarray represents a particular sample, and each array on a slide has a unique Array ID; thus each sample has a specific Array ID.

In Sample Manager, you can define and edit the sample attributes for each Array ID, such as the biological sample used, the amount of label used, or the hybridization date. You can export attributes to an Attribute File. Later, you can import the file into Agilent Genomic Workbench to use the saved attributes.

An Attribute File contains identification information (Array ID) and attributes for one or more microarray samples. An Attribute File for a two-color experiment must at a minimum include Array ID, Global Display Name, Red Sample, Green Sample, and Polarity values. An Attribute File for a one-color experiment must include the Array ID and Green Sample. Other information may be included as well. You can create Attribute Files with a spreadsheet program, and save them as a tab-delimited text file or *.xls file.

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

The attributes that you assign for each Array ID stay with the Array ID for the rest of the analysis in Agilent Genomic Workbench. This sample information can appear in reports. By default, the Global Display Name is the same as the Array ID. However, you can change the Global Display Name and your new name will appear throughout the rest of the user interface for that array (for example, in experiments, reports, etc.).

Using Feature Extraction Interactively

What is Feature Extraction 10.9?

Agilent Feature Extraction 10.9 software automatically reads and processes up to 100 raw microarray image files. It finds and adjusts microarray grids, rejects outlier pixels, accurately calculates feature intensities and ratios, flags outlier features, and calculates statistical confidences. QC reports are application-specific and contain results for multiple analyses. Feature Extraction is a key component of Agilent's comprehensive informatics platform that integrates complementary technologies and multidisciplinary approaches.

Feature Extraction 10.9, the Quality tools, eArray_{XD}, and the DNA Analytics programs (CGH, ChIP, and Methylation) use the same database, which gives you an efficient way to share data between programs. For example, if you load a GEML design file in Feature Extraction, it is automatically available in the other Agilent Genomic Workbench modules, and vice versa.

Agilent Feature Extraction software extracts microarray image data from:

- Agilent microarrays scanned on an Agilent scanner
- Non-Agilent microarrays scanned on an Agilent scanner

Feature Extraction handles these image data types differently.

Interaction of Feature Extraction 10.9 and Workflow

You set parameters in Feature Extraction, then run a Workflow that uses these settings. You can view and change some of the settings in Workflow. For details, see the *Workflow User Guide*.

Using the Quality Tools to Monitor Array Quality

What are the Quality tools?

Quality tools are integrated into Agilent Genomic Workbench and can be used to determine the quality of extractions. When you process a batch of microarrays with the Feature Extraction program, Feature Extraction generates a batch summary and a quality report for each microarray. The Quality tools compile a single graphical report – a QC Chart – that is a summary of the individual quality reports. You can quickly review one document to find the quality of each microarray and select which data to submit to further processing.

For example, the chart in [Figure 22](#) confirms the presence of processing artifacts and replicate microarray outliers. The chart shows that several microarrays have more than one metric out of normal range (represented by red circles). Values in range are also displayed (blue triangles). The inset window zooms in on the “rNegCtrlAveGBSubSig” metric, which is the average of the red-channel negative-control background-subtracted signals. For more information about Feature Extraction statistics used for metrics, see the *Feature Extraction User Guide*.

3 Detailed Descriptions

What are the Quality tools?

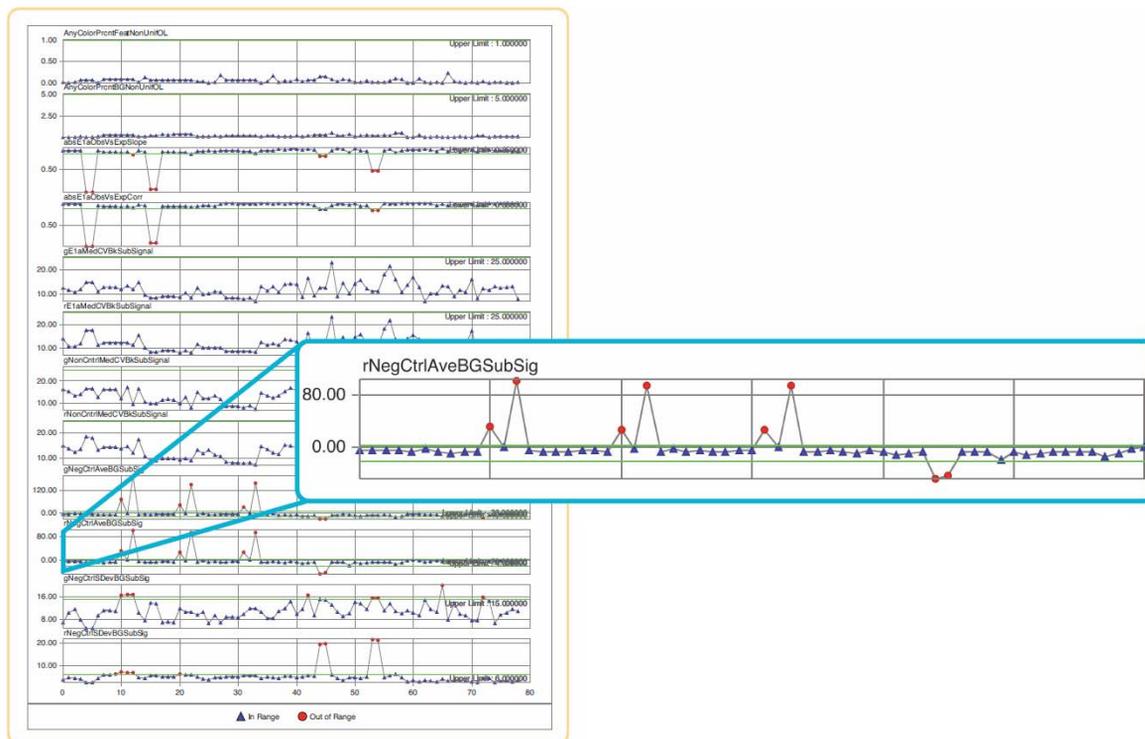


Figure 22 The control chart capability lets you monitor multiple parameters across multiple samples and multiple experiments. It gives you an at-a-glance view of important quality parameters.

The Quality tools let you use custom protocols developed with earlier versions of the software.

With the Quality tools, you can:

- Monitor hundreds of extractions in the database
- Import global statistics from Feature Extraction text files
- Create new metric sets and thresholds for your own workflow
- Customize Agilent-supplied metric sets and thresholds
- Use filters to search the database for specific data
- Graphically display the metrics as applied to imported microarray data sets

QC metric sets with thresholds help you identify key metrics and set normal ranges of metrics for Agilent microarrays. QC metric sets let you:

- Monitor each microarray as it is processed in Feature Extraction
- Monitor historical processing performance of microarrays that were extracted by Feature Extraction

How the Quality tools work

To monitor microarray processing performance, the Quality tools collect, combine, and analyze summary statistics from Feature Extraction output files and optional annotation files. The Quality tools keep the data in a relational database, so you can make queries and save the results. This lets you retrieve the data by various criteria, such as experiment, batch, or date of processing.

Using the data, you can create metrics that monitor aspects of the microarray processing workflow. You can make metric sets that combine metrics that monitor different aspects of microarray processing. With metric sets, you can graphically display the results from historical data and set thresholds for the metrics that are appropriate for your experimental conditions and processing environment.

To monitor processing performance, you can use the metric sets and thresholds in the Quality tools on a regular basis. You can also import them to Feature Extraction software, to monitor each array and batch as it is processed.

The Quality tools can be used in a production environment where:

- Microarray processing protocols are standardized and you need to know the effect of specific variables on performance
- Monitoring run-to-run consistency is an important goal to identify extractions that fall out of the established normal range, and to find trends in the data.

Setting Up and Running Workflows for Extraction and/or Analysis

After you organize your microarrays and define their attributes, you can set up and run a workflow that runs Feature Extraction to automatically create log ratio data from the scanned images of your arrays. You can use this capability for many types of arrays, including CGH, ChIP, and Methylation (CH3). If you have a CGH or ChIP license, you can also set up the workflow to analyze the CGH or ChIP log ratio data right after the extraction.

Agilent Genomic Workbench lets you set up and run a workflow with no need for intervention (Figure 23).

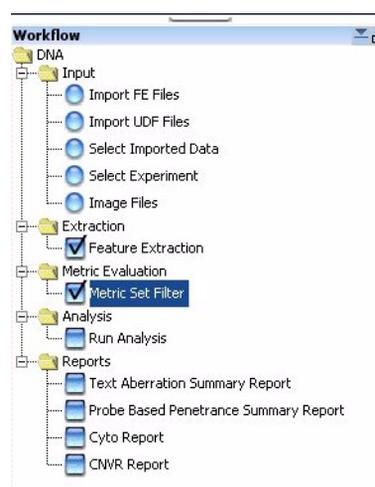


Figure 23 Workflow Navigator for CGH

You can:

- Select the source of data you want to process
- Extract the data (from image files) or analyze it (from four different sources of extracted data) with a DNA Analytics analysis method, or do both

Setting Up and Running Workflows for Extraction and/or Analysis

- Apply a metric set filter to the extracted data to include or exclude arrays based on threshold values of selected metrics (CGH workflow only)
- Do one of the following:
 - Run the configured workflow to extract image files with Agilent Feature Extraction and create a QC report that contains statistics for the extraction
 - Run the configured workflow to analyze CGH or ChIP (not Methylation, Expression, or microRNA) Feature Extraction data with DNA Analytics and create DNA Analytics reports
 - Run the configured workflow to extract image files and then analyze the extracted results to create both sets of reports.

With Workflow analysis, you configure an analysis method ahead of time and then run it in a workflow. This method is useful for unattended operation and consistent analyses of multiple data sets. To review workflow results, you use the Genomic Viewer. For more details and quick-start instructions to set up and run a workflow, see the *Workflow User Guide*.

Displaying Data/Results in Genomic Viewer

What is Genomic Viewer?

Genomic Viewer is the graphics and tabular display section of the Agilent Genomic Workbench main window. You can use this data *viewing* capability in Agilent Genomic Workbench without a license. You can view data for many types of arrays, including CGH, ChIP, and Methylation (CH3). You can use the data *analysis* capability in Agilent Genomic Workbench only if you have a license for one or more of the DNA Analytics programs (CGH, ChIP, or Methylation).

Figure 24 shows the main window of Agilent Genomic Workbench, and identifies the names of its components. In the Genomic Viewer, extracted data and analysis results can be tabulated and displayed next to depictions of the genome, selected chromosome, and selected genes of the species whose array data you are analyzing.

Genomic Viewer contains four main views:

- **Genome View** – A graphical representation of the entire genome for the selected species. Use this view to select the chromosome to show in the other views.
- **Chromosome View** – A graphical representation of the selected chromosome, displayed with cytobands and a plot area. Click or drag the mouse to select a region to display in the Gene View.
- **Gene View** – A more detailed view of the chromosomal region selected in the Chromosome View.
- **Tab View** – A view that displays design annotation and log ratio data related to the chromosome you select in Chromosome View

To learn how to display log ratio data and content (experiments, gene lists and tracks), see the *Data Viewing User Guide*.

To learn how to display log ratio data, content, and results, see the *User Guide* for which you have a DNA Analytics program license.

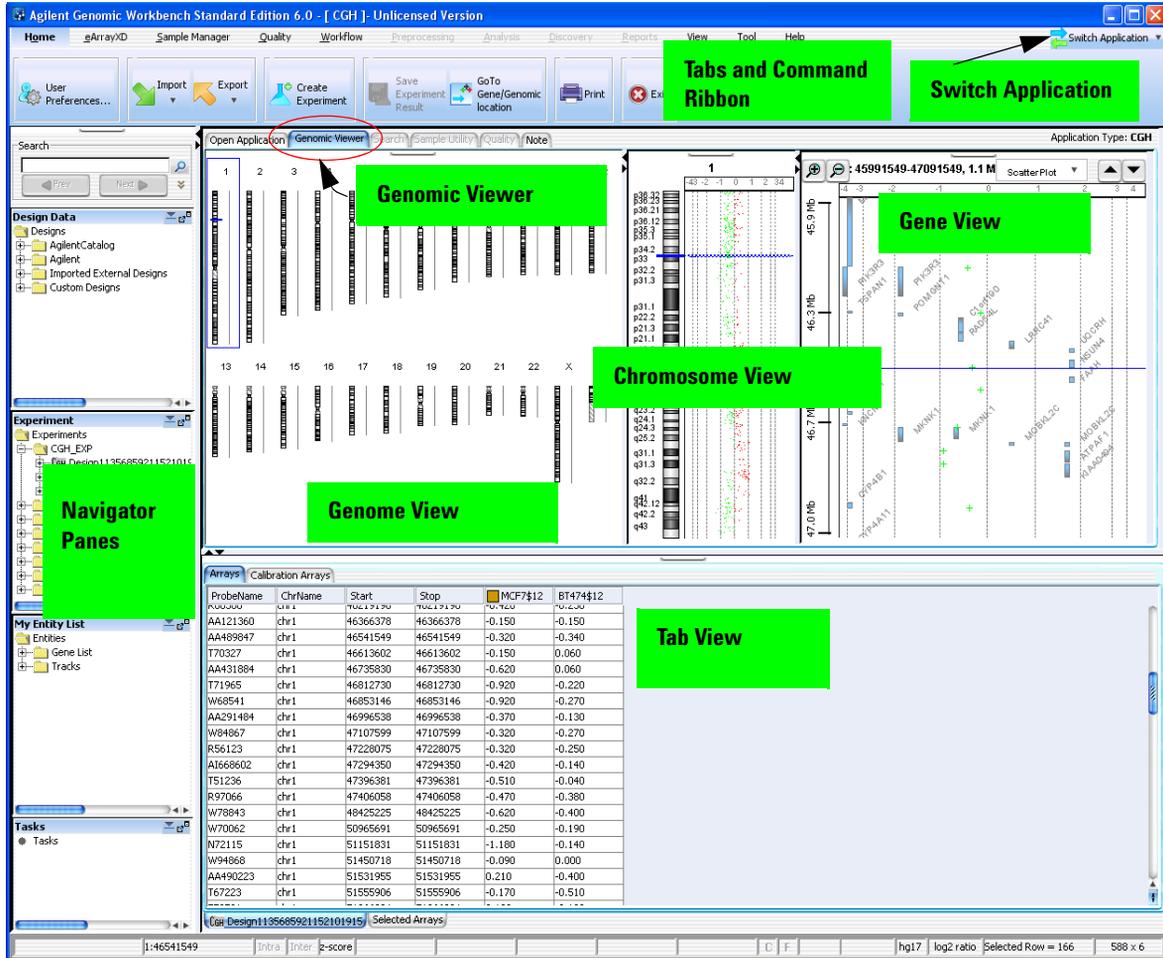


Figure 24 Agilent Genomic Workbench Standard Edition main window with major components – unlicensed CGH version

Analyzing CGH Data Interactively

The Agilent Genomic Workbench lets you visually explore, detect, and analyze aberration patterns from multiple Comparative Genomic Hybridization (CGH) microarray profiles, either interactively or by setting up an analysis method to use with a workflow. With CGH Interactive Analysis, you set up an experiment and apply analysis algorithms to your data one experiment at a time. You also set parameters for some report templates for use in Workflow analysis.

You do most of your operations with the Preprocessing, Analysis, Discovery and Reports tabs. See [Table 1](#) on page 13. Also see *CGH Interactive Analysis User Guide*.

With the interactive portion of the Agilent Genomic Workbench, you can:

- Import Agilent Feature Extraction data, Axon data, or UDF files and use the Genomic Viewer to visualize this data along the chromosome
- Preprocess the data:
 - Apply feature, array, metric set, and design filters
 - Combine designs and/or inter- or intra-array replicates
 - Centralize the data, including nonunique probes
 - Display QC metrics on the original data
- Use five different robust statistical aberration detection algorithms to detect and map aberration regions with high confidence
- Display chromosomal deletions and amplifications at multiple zoom levels simultaneously
- Use Discovery options to display the analysis results in many different ways
 - Apply aberration filters on the results
 - Use the CGH program for CNV (Copy Number Variation) applications
 - Find common aberrations between several CGH samples, as well as do a differential aberration analysis
 - Do a correlation analysis of gene expression and CGH data, do a cluster analysis, and display data with a “heatmap”
- Save aberration results in a centralized database
- Make customizable Cytogenetic Reports for individual CGH samples

Analyzing ChIP Data Interactively

The ChIP program is a complete environment that lets you analyze chromatin immunoprecipitation (ChIP) microarray data. ChIP microarray analysis can identify the genomic loci that contain proteins that bind to DNA, including individual transcription factors, chromatin modifiers, and components of the general transcription machinery.

With ChIP interactive analysis, you set up an experiment and apply analysis algorithms to your data one experiment at a time. You also set parameters for some report templates for use in Workflow analysis.

You do most of your operations with the Preprocessing, Analysis, and Reports tabs. See [Table 1](#) on page 13. Also see *ChIP Interactive Analysis User Guide*.

With the interactive portion of the ChIP program, you can:

- Import data from the Agilent Feature Extraction and Axon programs
- Use an intuitive graphical interface to display data and annotations in the context of an organism's genome, at several simultaneous levels of detail
- Normalize your data with several statistical methods
- Use error modeling and event detection algorithms to identify probes, genes, and genomic loci that have significant binding
- Display significant binding events graphically within the program, and export report files that you can analyze further with other programs

Analyzing Methylation (CH3) Data

The Methylation (CH3) program is a framework used to identify methylation events in your samples. CH3 microarray analysis can help identify the CpG islands where methylation has occurred. The software helps you:

- Identify molecular events associated with DNA methylation
- Find and validate gene regulation and regulatory networks by creating high-resolution, genome-wide methylation profiles
- Show modes of action and potential therapeutic activities of compounds and target genes by understanding the relation of DNA methylation to transcriptional control

With the Methylation (CH3) program, you can:

- Import data from the Agilent Feature Extraction and Axon programs, and import UDF files
- Use an intuitive graphical interface to display data and annotations in the context of an organism's genome, at several simultaneous levels of detail
- Use a *Z*-score or Batman algorithm to calculate the probability that probes are methylated or unmethylated
- Compare the moving average of your log ratio and *Z*-score data within the boundaries of CpG Island tracks

You cannot run methylation event detection within a Workflow.

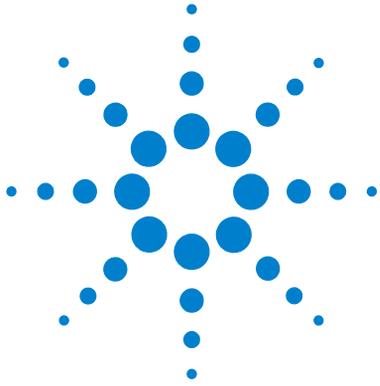
Using the SureSelect Quality Analyzer

The SureSelect Quality Analyzer is a program that lets you assess the effectiveness of the pull-down of targeted genomic fragments when you use the Agilent SureSelect Target Enrichment system. This system uses libraries of biotinylated RNA oligonucleotide “baits” to harvest genomic DNA fragments of interest for sequencing. This forms the basis of a powerful selection method that lets you focus your sequencing efforts.

The selective nature of the process makes it ideal for targeted resequencing using next-generation sequencing technology. After you sequence the harvested fragments, you can use the SureSelect Quality Analyzer to calculate statistical metrics and perform read depth analysis. You can also perform enrichment analysis, and view the results in the UCSC Genome Browser next to the annotation tracks of your choice.

3 Detailed Descriptions

Using the SureSelect Quality Analyzer



4 System Administration and Troubleshooting

If you see the eArray login and/or other dialog boxes when you start the software [66](#)

If you need to change the location of the server [67](#)

If you need to change proxy settings [68](#)

If you need to change eArray login settings within an application [71](#)

This chapter helps you with system administration and troubleshooting for the Standard Edition of Agilent Genomic Workbench 6.0.



4 System Administration and Troubleshooting

If you see the eArray login and/or other dialog boxes when you start the software

If you see the eArray login and/or other dialog boxes when you start the software

- 1 If this is the first time you have opened Agilent Genomic Workbench after installation, you will see the eArray login dialog box. Ask your system administrator for the eArray login. Then type it into the boxes and click **OK**.



Figure 25 Dialog box for eArray login

- 2 Select an application type and click **OK**.



Figure 26 Select Application Type dialog box

- 3 If you see a message about a missing license, click **OK**.
- 4 If you see a message that proxy settings are not yet configured, click **Yes**, then see “[If you need to change proxy settings](#)” on page 68.
- 5 Confirm that the Open Application window appears. See [Figure 7](#) on page 20.
- 6 Continue with the instructions that follow [Figure 7](#) on page 20. They describe how to start programs and enter license information.

If you need to change the location of the server

To start the Agilent Genomic Workbench, you must have already specified the location of a valid server. However, within the program, you can view the current settings, or enter settings for a different server.

If you have installed Agilent Genomic Workbench server on multiple computers, the client can access each installation. You can change the server location to access a different installation.

CAUTION

Agilent Genomic Workbench client relies on the server for essential functions. For the program to work properly, you must specify configuration parameters for a location that contains a valid Agilent Genomic Workbench server program.

- 1 In the **Home** tab, click **User Preferences**.
The User Preferences dialog box appears.
- 2 Click the **Miscellaneous** tab.
The database host name and port for your server appear under Configuration Parameters.
- 3 To change the host name or port, click **Change**.
A dialog box asks if you are sure that you want to change the database configuration parameters.
- 4 Click **Yes**.
The Database Configuration Parameters become available.
- 5 Type the desired database host name and port, then click **OK**.

If you need to change proxy settings

A proxy server is an intermediate computer between your computer and the Internet. Most large organizations have proxy servers to increase security, filter access to Web sites, and improve performance by storing Web pages that someone within the company downloaded previously.

You do not normally need to change the proxy settings in Agilent Genomic Workbench. Do not change these settings unless you have a problem with the current settings, such as the following:

- The server cannot communicate with the Agilent eArray Web site.
- The client cannot use the Cytoscape network feature.

Even if you experience these problems, you may not need to change the settings. Ask your network administrator if a change has occurred within your company network (for example, a company-wide change in proxy settings). If so, ask your network administrator for any new settings you may need.

CAUTION

If you set the wrong proxy settings for your client, your Agilent Genomic Workbench software will not function on your client. If you set the wrong proxy settings for your server, your Agilent Genomic Workbench software will not function for your entire workgroup. Ask your network administrator to help with this task.

To change the proxy settings:

- 1 From the Home tab, click **User Preferences**.
- 2 Click the **Miscellaneous** tab.
- 3 Verify that you see the dialog box shown in [Figure 27](#) and note the two buttons to change proxy settings. Because the server and client are installed on different computers, it is possible that the proxy settings are different.
 - **Edit Proxy Settings** – for the client
 - **Edit Server Proxy Settings** – for the server

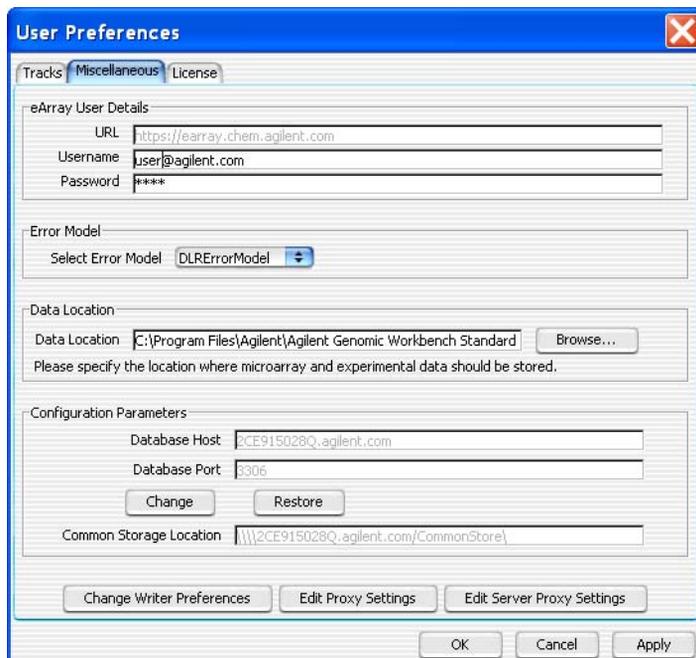


Figure 27 Miscellaneous tab of User Preferences dialog box

4 System Administration and Troubleshooting

If you need to change proxy settings

- 4 Change the proxy settings for your client.
 - a In the User Preferences dialog box, click **Edit Proxy Settings**.
 - b Confirm that you see the dialog box shown in [Figure 28](#).



Figure 28 Edit Proxy Settings dialog box – used for the client computer

- c If you need to change from the default (and most commonly used) setting of No Proxy, click **Use Proxy**.
 - d Type the correct information in the boxes. Ask your network administrator to help.
 - e Click **Close**.
 - 5 Change the proxy settings for your server.
 - a In the User Preferences dialog box, click **Edit Server Proxy Settings**.
 - b Confirm that you see the dialog box shown in [Figure 29](#).

If you need to change eArray login settings within an application

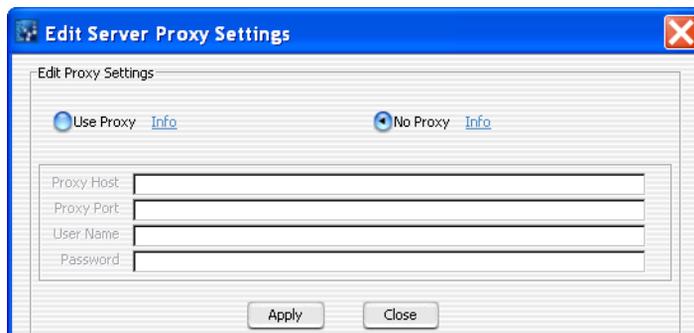


Figure 29 Edit Server Proxy Settings dialog box – used for the client computer

- c If you need to change from the default (and most commonly used) setting of No Proxy, click **Use Proxy**.
- d Type the correct information in the boxes. Ask your network administrator to help.
- e Click **Close**.

For more information, see a description of this dialog box in the Reference section of one of the following *User Guides*: Data Viewing, CGH, ChIP, Methylation (CH3), or eArray_{XD}.

If you need to change eArray login settings within an application

The Agilent Genomic Workbench client software on your computer is linked to a specific account on the eArray Web site. This account information is initially entered during the installation process.

Your login account affects the content you see within eArray_{XD} and your ability to edit or delete. If you need to link the client program to a different account, you can do so from within the client program.

- 1 In the **Home** tab, click **User Preferences**.

The User Preferences dialog box appears.

- 2 In the dialog box, click the **Miscellaneous** tab.

- 3 In **eArray User Details**, type the following information:

- **Username** – The desired existing login name on the eArray Web site.

4 System Administration and Troubleshooting

If you need to change eArray login settings within an application

- **Password** – The current password for the account on the eArray Web site. For security, the actual characters of your password do not appear.

4 Click **OK**.

You must close the program and restart it for your new login information to take effect.

NOTE

- The procedure above links the Agilent Genomic Workbench client program to a different user account that already exists on the eArray Web site. To create a new user account, or to change the login information of an existing account, see the *eArray_{XD} User Guide*.
 - If you log in to the eArray Web site and change the password for your account, this information is automatically updated in eArray_{XD} within approximately one day. You do not need to change this information in the User Preferences. After this update occurs, the program asks you to type your new password the next time you open the program.
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In this book

This book gives an overview of the capabilities within the Standard Edition of Agilent Genomic Workbench 6.0. It also describes how to start each of the component programs and find Help, and how to enter your license information. In addition, it helps you with system administration and troubleshooting.

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