Agilent
Genomic Workbench 6.5

Product Overview Guide

For Research Use Only. Not for use in diagnostic procedures.

Agilent Technologies

Notices

Manual Part Number

G3800-90021

Revision

Revision A1, October 2015

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

Software Revision

This guide is valid for 6.5 and later revisions of the Agilent Genomic Workbench software, until superseded.

Warranty

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

Technology Licenses

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

Restricted Rights Legend

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

Safety Notices

CAUTION

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

WARNING

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

In This Guide...

This guide 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.
- Simultaneously detect and investigate high resolution copy number and copy neutral variations for CGH+SNP (Comparative Genomic Hybridization with Single Nucleotide Polymorphism) microarrays. 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 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 Agilent Genomic Workbench.

4 System Administration and Troubleshooting

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

Contents

Contents

1 Overview

Agilent Genomic Workbench is a comprehensive design and analysis tool that lets you 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 Agilent Genomic Workbench. It shows you how each component of 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 Agilent Genomic Workbench software. 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.

Agilent Technologies

Features of Agilent Genomic Workbench

The Agilent Genomic Workbench software provides a robust data management and integrated data analysis environment for Agilent genomics applications, including comparative genomic hybridization (CGH), CGH+SNP (Comparative Genomic Hybridization with Single Nucleotide Polymorphism), chromatin immunoprecipitation (ChIP), and methylation (CH3).

Through its eArray$_{XD}$ functionality, the Agilent Genomic Workbench lets you design microarrays for CGH, CGH+SNP, ChIP, CH3, and SureSelect array capture applications, in addition to libraries for SureSelect target enrichment applications. You can also use this suite of applications to do quality control for both array and library-based assays.

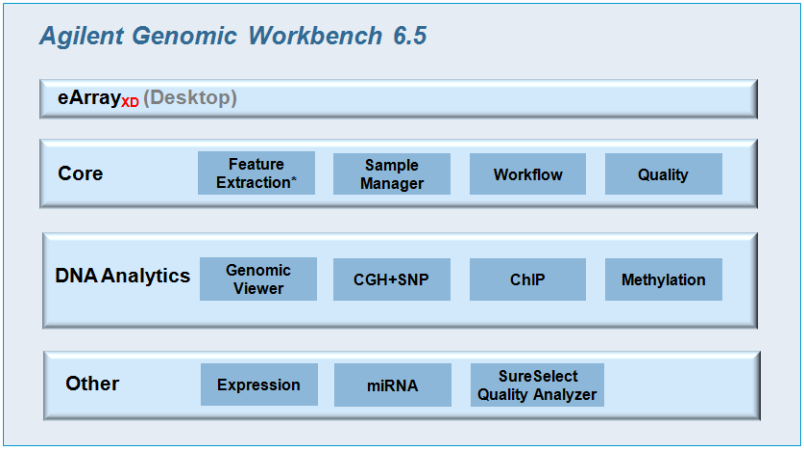

Figure 1 Programs available in Agilent Genomic Workbench

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 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.

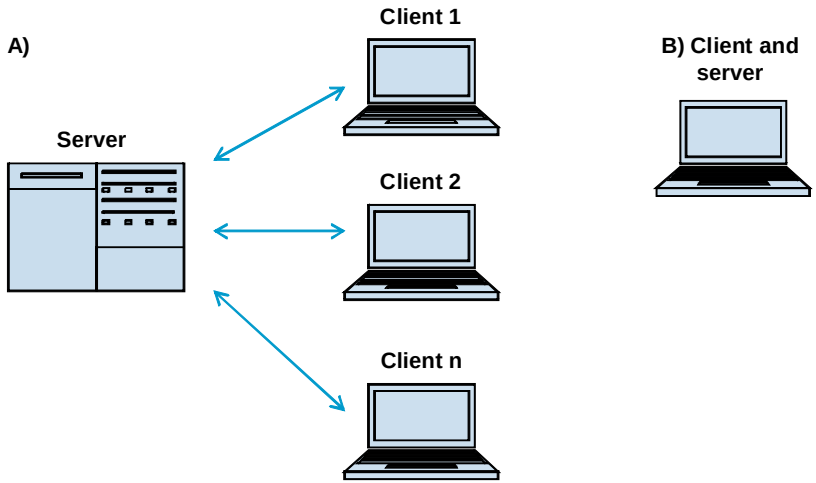

Figure 2 Agilent Genomic Workbench uses a scalable client-server architecture

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 Analytics (CGH, ChIP, and Methylation modules).

The ability to do queries is another advantage of the database. For example, you can query for all female samples 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.

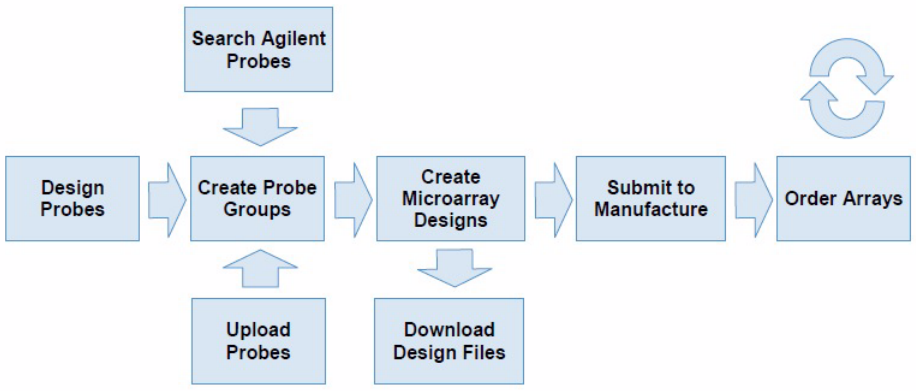

Figure 3 Array creation process

Automated feature extraction and analysis

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).

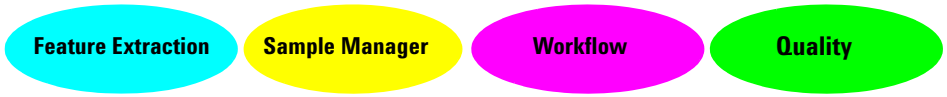

Figure 4 Agilent Genomic Workbench core utilities

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 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.

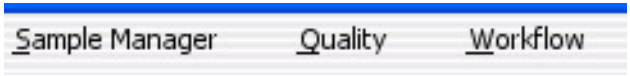

Figure 5 Sample Manager, Quality, and Workflow tabs

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 Agilent Genomic Workbench looks like this:

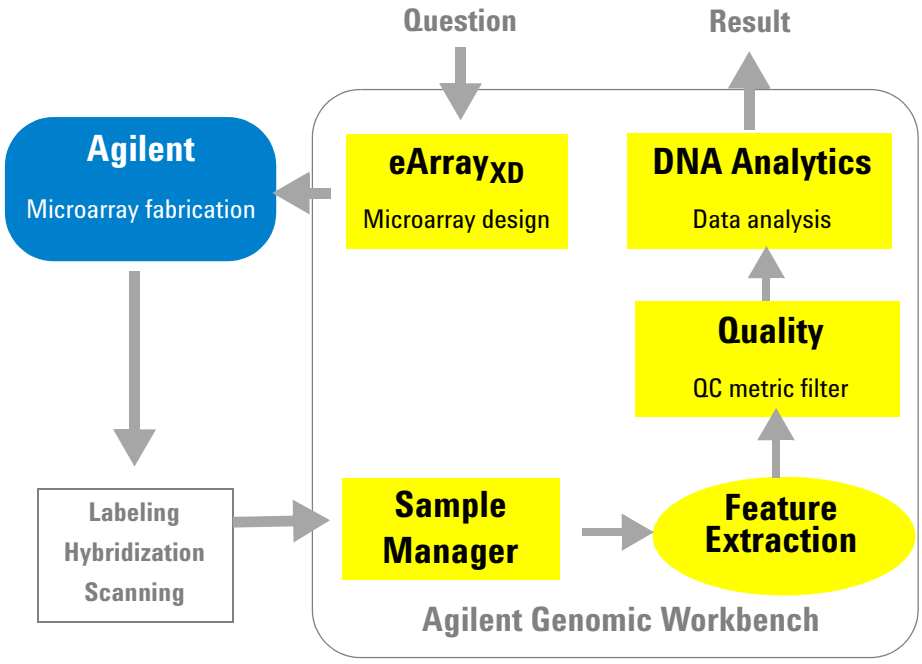

Figure 6 Microarray research pathway with Agilent Genomic Workbench

Interactive data analysis for CGH (including CGH+SNP), 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 module is enhanced with SNP processing algorithms in Preprocessing and Analysis. 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	• Add feature and array filters • Apply GC Content Correction • Turn on Centralization • Add design filters • Combine array designs and replicates • Display QC metrics • Set up and apply metric set filters	• Select/Edit normalization calculation • Select/Edit error model • Combine designs and replicates • Display QC metrics • Set up and apply metric set filters	• Combine array designs
Analysis	• Calculate a moving average on log ratio data • Select an aberration detection algorithm and set up its parameters • Turn on SNP Analysis calculations (SNP Copy Number and LOH)	• Select/Edit/Apply event detection model	• 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

Table 1 DNA Interactive Analysis capabilities (continued)

Tabs	CGH capabilities	ChIP capabilities	Methylation (CH3) capabilities
Discovery	• 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 • Generate a Genotype Reference for SNP	• Not applicable	• Not applicable
Reports	• Aberration report • Penetrance report • Cyto report • SNP Genotype report • SNP Aberration & LOH report • Use Report Manager to manage reports	• Probe report • Gene report • QC report • Use Report Manager to manage reports	• Probe report • Batman report

Capabilities without licenses

You do not need to purchase licenses to get some component capability with 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 36) 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 Agilent Genomic Workbench give you the capabilities shown in Table 2:

Table 2 Capabilities in Agilent Genomic Workbench 6.5

If you want to do this:	Start this program or click this tab: (See Figure 7 on page 22 and Figure 14 on page 36)	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 10.10 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 10.10 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 10.10 or higher license required)	Quality Tools User Guide

Table 2 Capabilities in Agilent Genomic Workbench 6.5 (continued)

If you want to do this:	Start this program or click this tab: (See Figure 7 on page 22 and Figure 14 on page 36)	Read this 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 10.10 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 10.10 or higher license required)	Workflow User 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 10.10 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

Table 2 Capabilities in Agilent Genomic Workbench 6.5 (continued)

If you want to do this:	Start this program or click this tab: (See Figure 7 on page 22 and Figure 14 on page 36)	Read this 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
Interactively analyze CGH data that contains SNP content	DNA Analytics CGH (license required)	CGH Interactive Analysis User 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

This chapter helps you get started with Agilent Genomic Workbench 6.5.

Before you read this chapter, install Agilent Genomic Workbench. For instructions on how to install Agilent Genomic Workbench, or if you need to reinstall the program, see the *Agilent Genomic Workbench 6.5 Installation Guide*. If you do not have the guide, download it from the Agilent Web site.

Agilent Technologies

Starting Your Application and Finding Help

After you install the software and double-click the Agilent Genomic Workbench 6.5 icon, you should see the Open Application tab of the main window. If you see anything else, please see Chapter 4.

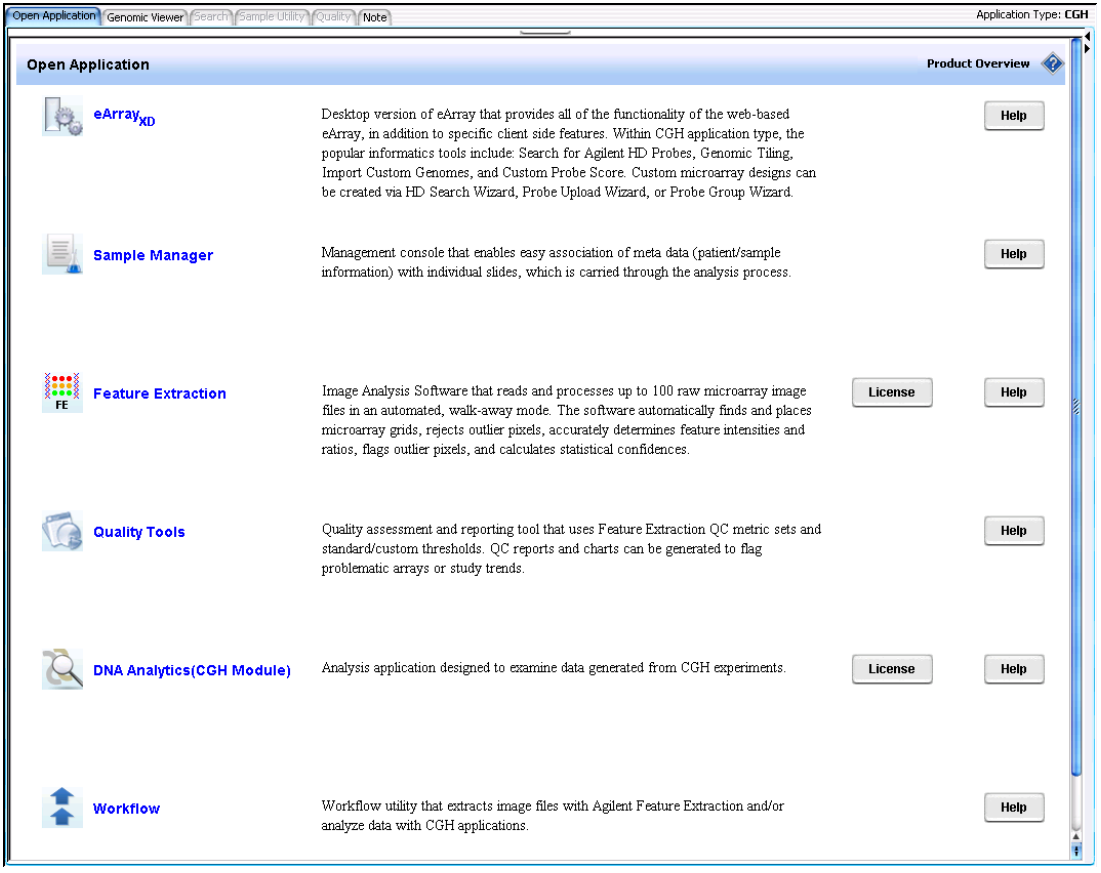

Figure 7 Open Application tab in Agilent Genomic Workbench, shown when CGH is selected under Switch Application

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 desired 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 eArrayXD" on page 48.

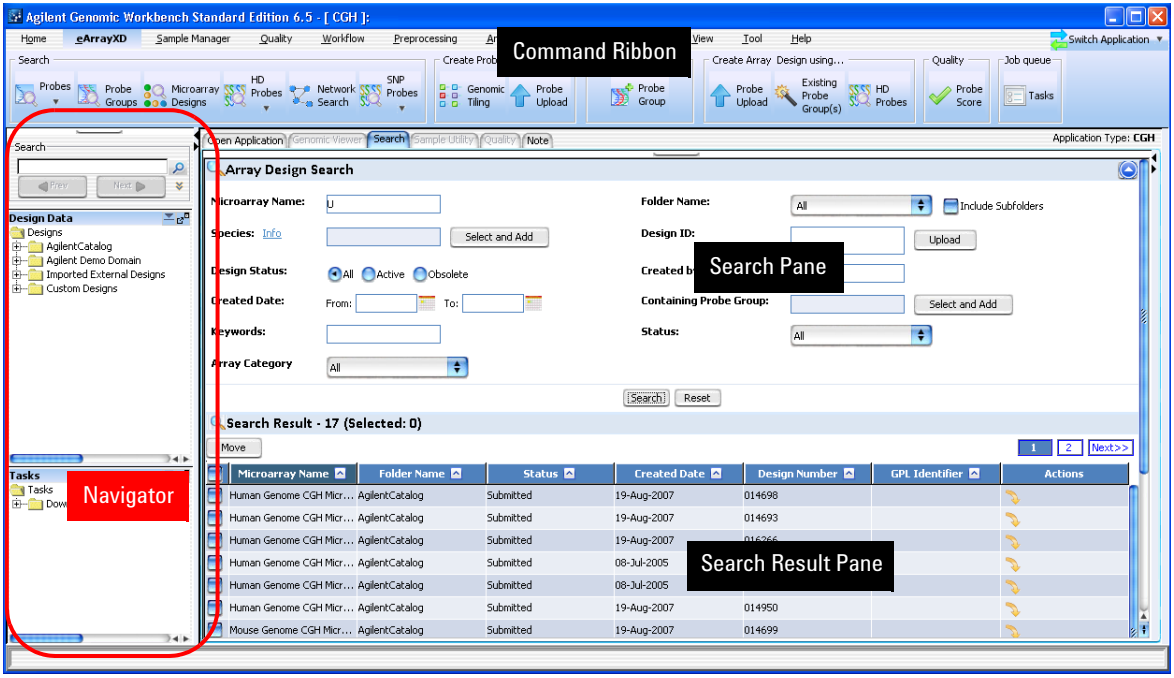

Figure 8 Agilent Genomic Workbench main window – eArray$_{XD}$ tab

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 53.

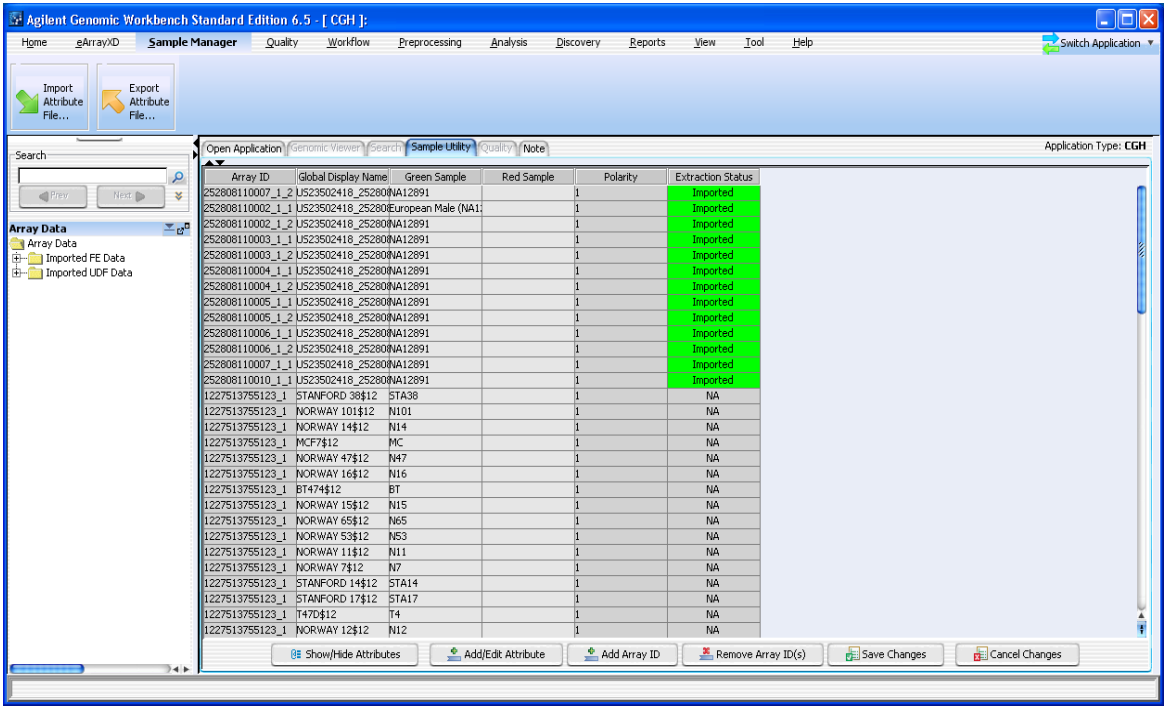

Figure 9 Agilent Genomic Workbench main window – Sample Manager tab

To start and find help for Feature Extraction 10.10

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.

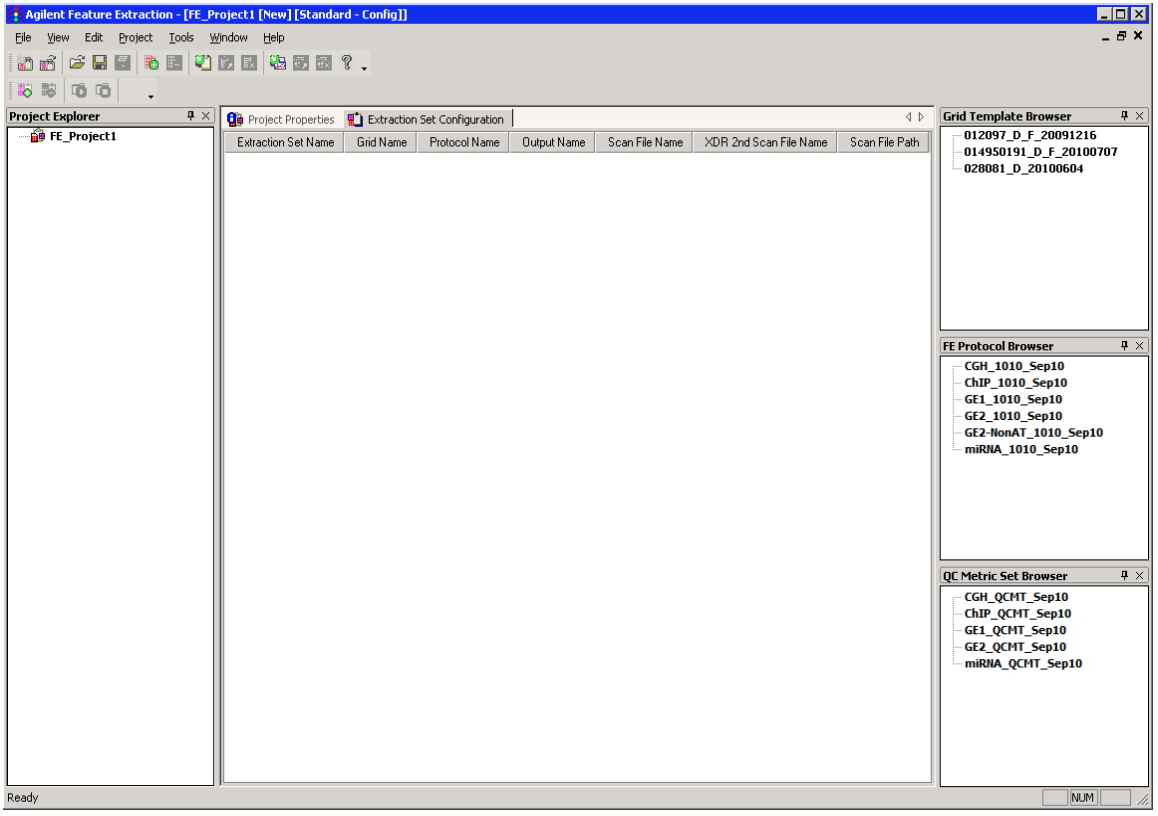

Figure 10 Agilent Genomic Workbench Feature Extraction main window

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.

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**.

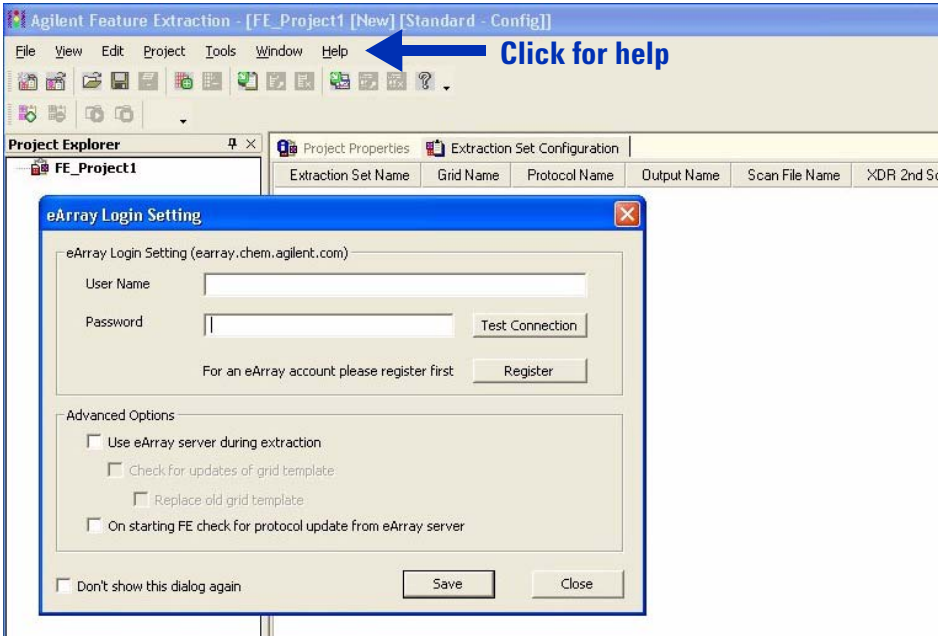

Figure 11 eArray Login Setting dialog box in front of Feature Extraction main window

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.

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. Existing grid templates are left in the database, unless you mark **Replace old grid template**.

   - **Replace old grid template**

     Mark this box to have Feature Extraction 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 55.

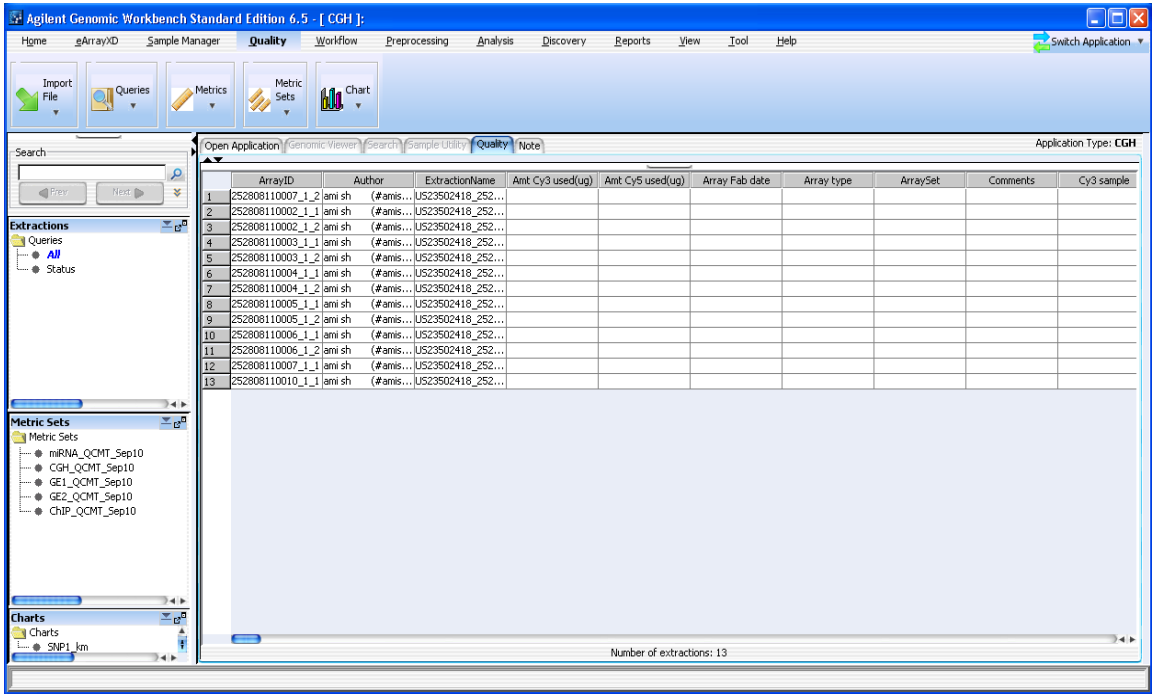

Figure 12 Agilent Genomic Workbench main window – Quality tab, populated with data

See Figure 22 on page 56 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 10.10 (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 37.

b Follow the instructions under "To use Server Location to enter the license(s)" on page 38 or "To use Text License to enter the license(s)" on page 38.

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 58.

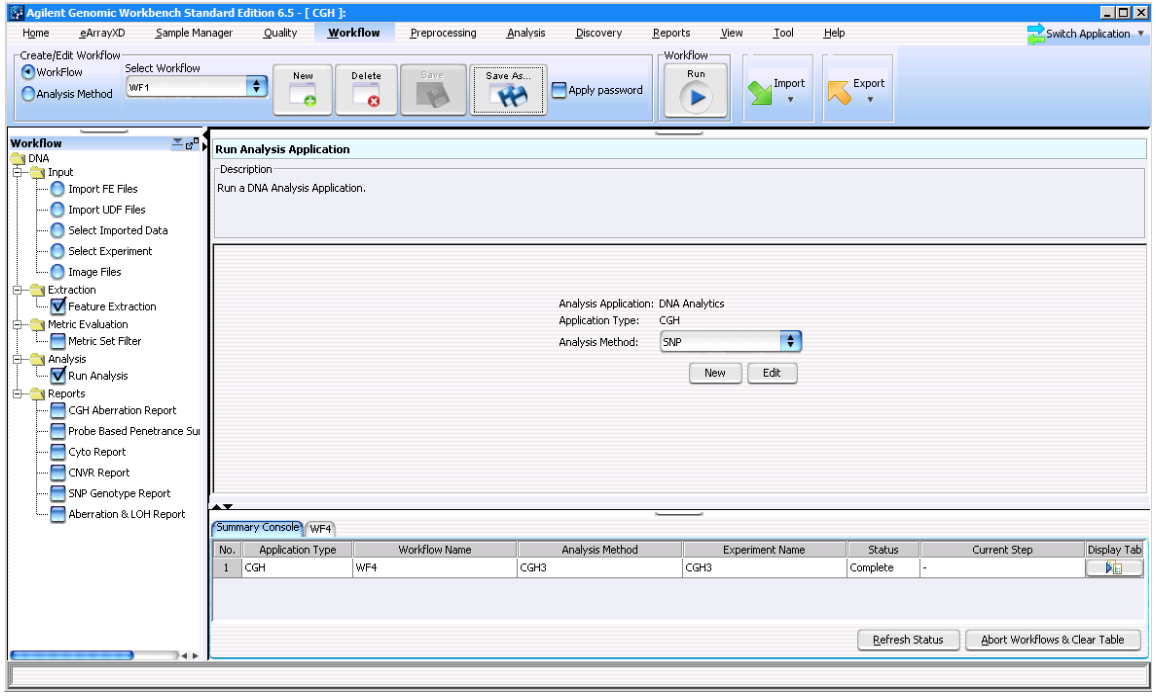

Figure 13 Agilent Genomic Workbench main window – Workflow tab

To start and find help to display CGH (including CGH+SNP), 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 desired 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 60.

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.

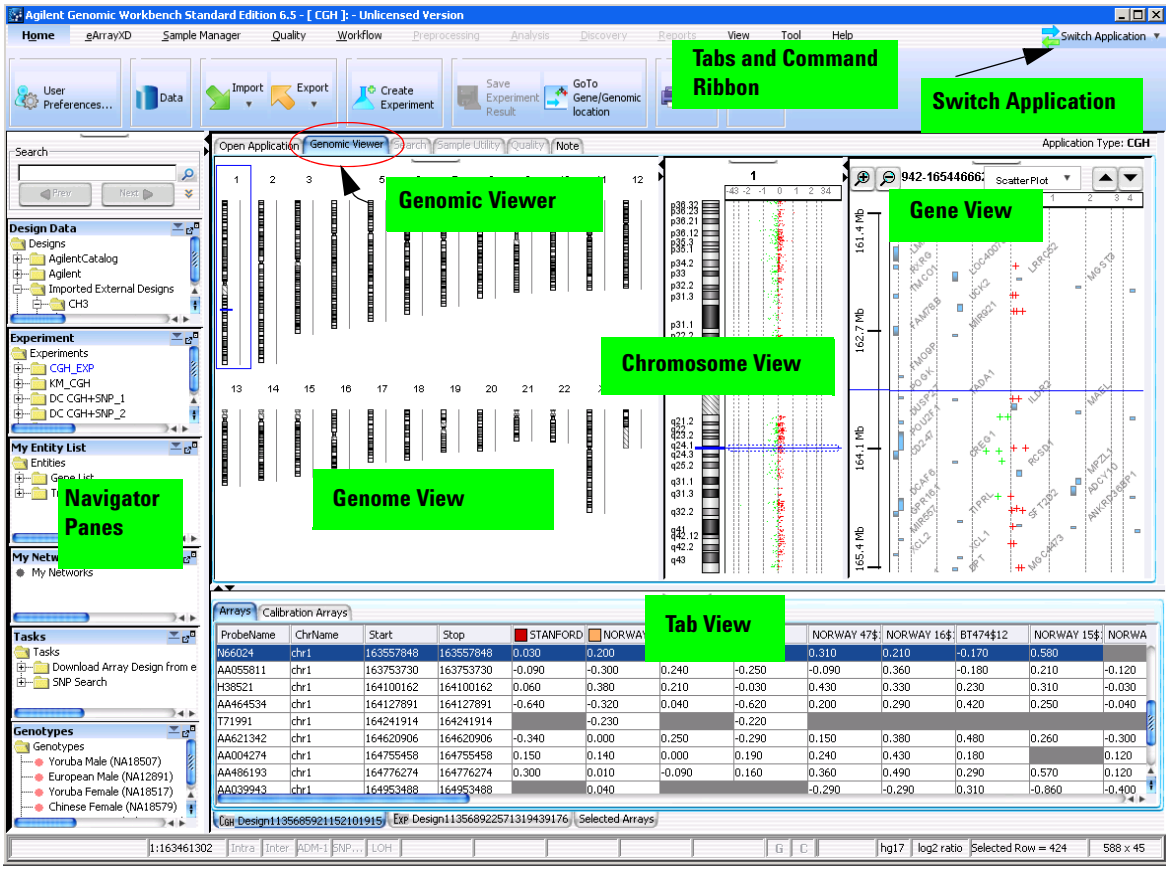

Figure 14 Agilent Genomic Workbench main window – Genomic Viewer tab for unlicensed CGH application type

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

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.

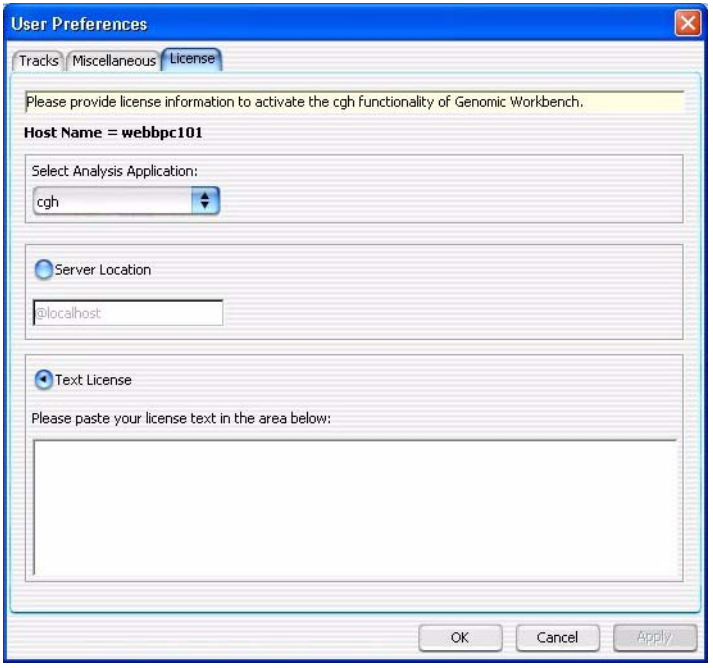

Figure 15 License tab of the User Preferences dialog box

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 37.)

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 37.)

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 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.

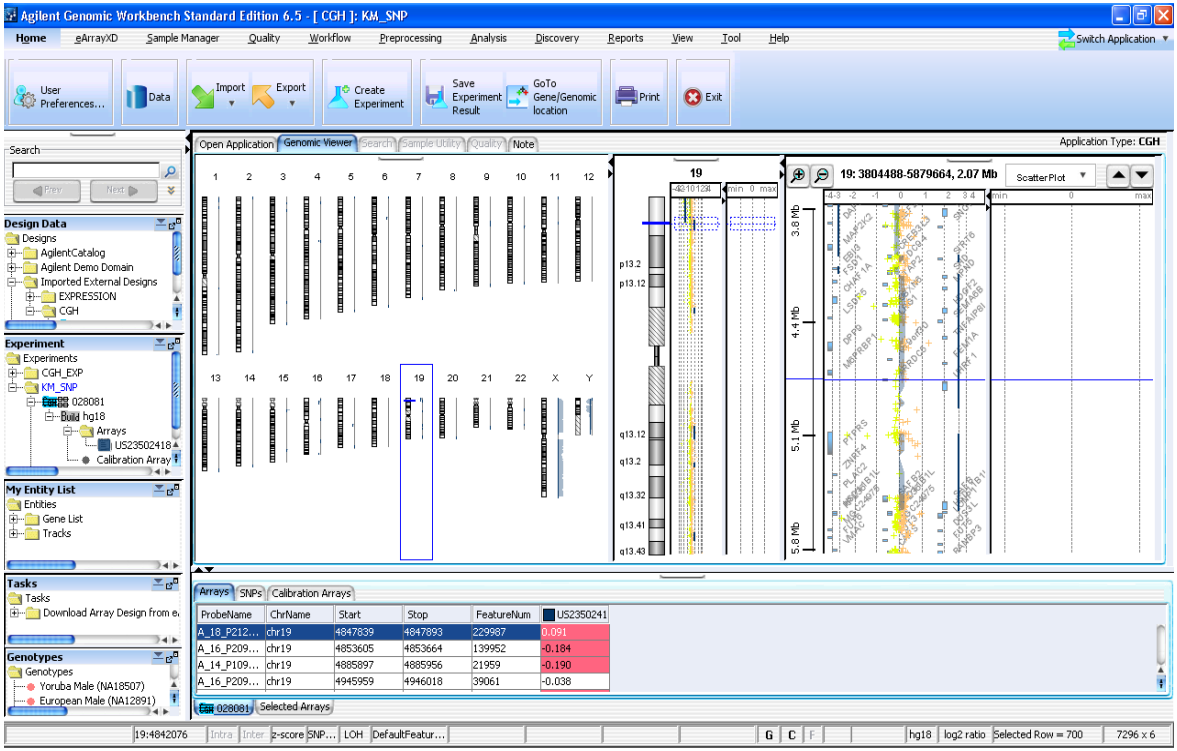

Figure 16 Agilent Genomic Workbench main window – Genomic Viewer tab for CGH application type

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 66.

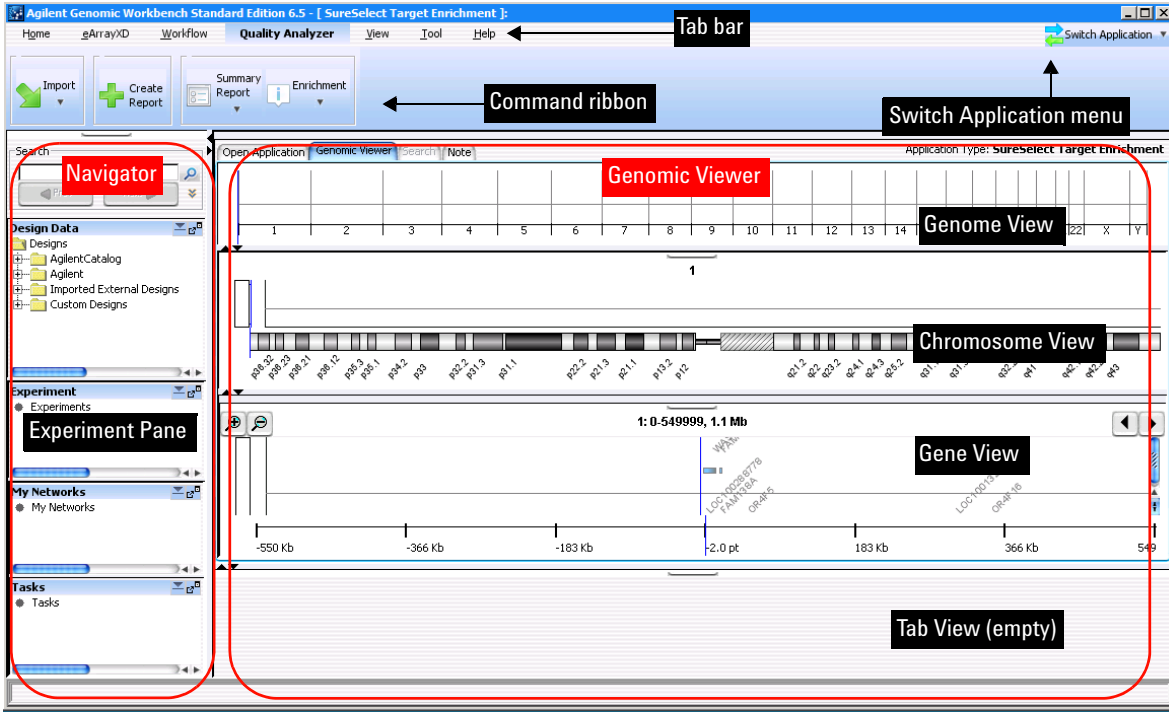

Figure 17 Agilent Genomic Workbench main window – Quality Analyzer tab

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. • 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. **1** Set the desired application type. **2** 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. **3** Click the desired user guide. The selected user guide opens in Adobe Reader.

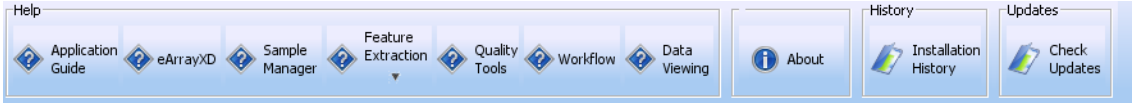

Figure 18 Help ribbon for the CGH and ChIP application types

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	(Available for all application types except Expression and microRNA) For each of these application types, this button opens the indicated user guide: • **CGH** – Opens the *CGH Interactive Analysis User Guide*. 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 *ChIP Interactive Analysis User Guide*. This guide describes how to use the ChIP application of Agilent Genomic Workbench to analyze chromatin immunoprecipitation data and create reports. • **CH3** – Opens the *Methylation (CH3) Analysis User Guide*. 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 *SureSelect Quality Analyzer User Guide*. 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$_{XD}$	(Available for all application types) Opens the *eArray$_{XD}$ User Guide*. This guide describes how to design and submit custom microarray designs and SureSelect Target Enrichment bait libraries.
Sample Manager	(Available for all application types except SureSelect Target Enrichment) Opens the *Sample Manager User Guide*. This guide describes how to use the Sample Manager module of Agilent Genomic Workbench to organize microarrays and edit their attributes.

Button	Description
Feature Extraction	(Available for all application types except SureSelect Target Enrichment) Opens a menu with these options: • **Quick Start** – Opens the *Feature Extraction Quick Start Guide.* 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 *Feature Extraction User Guide.* 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 *Feature Extraction Reference Guide.* 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	(Available for the CGH, ChIP-on-chip, and Methylation application types) Opens the *Quality Tools User Guide.* 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.
Workflow	(Available for the CGH and ChIP-on-chip application types) Opens the *Workflow User Guide.* 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.
Data Viewing	(Available for all application types except Expression and microRNA) Opens the *Data Viewing User Guide.* 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).

Other commands

About Opens a dialog box that displays version and copyright information for your installation of Agilent Genomic Workbench. 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.

3 Detailed Descriptions

This chapter gives you more details about each of the modules of Agilent Genomic Workbench 6.5.

Designing Your Own Microarrays and SureSelect Target Enrichment Kits with eArray$_{XD}$

What is eArray$_{XD}$?

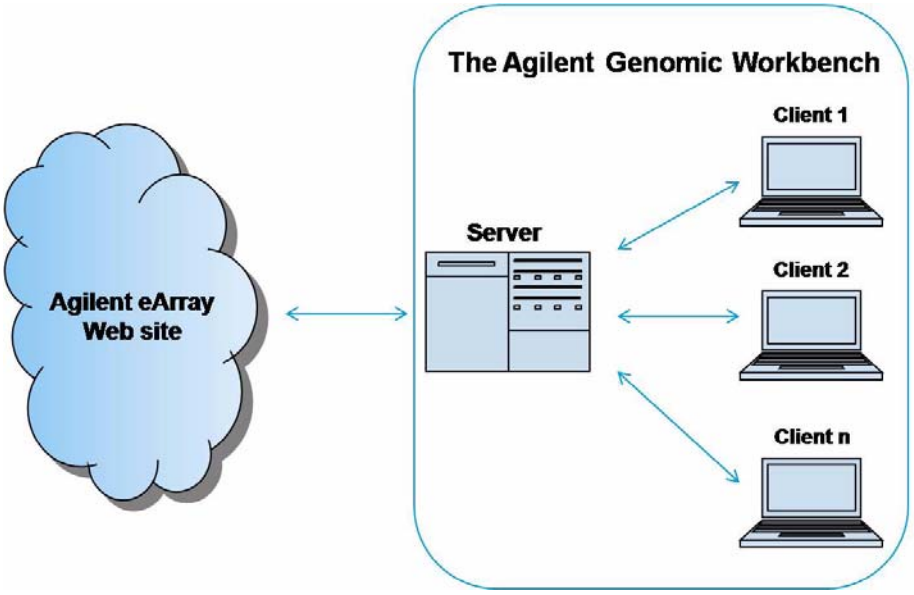

Figure 19 Components of eArray system

eArray$_{XD}$ is a tool that lets you design microarrays (and SSTE kits), and to submit the design files to Agilent for printing. The eArray system, illustrated in Figure 19, 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 Agilent Catalog and 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.

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.

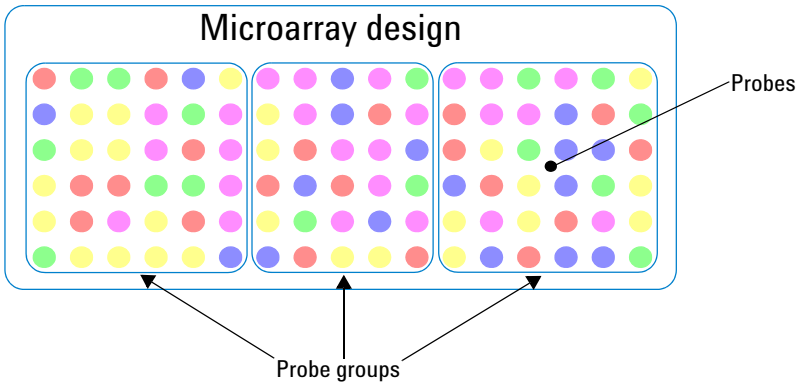

Figure 20 Probes, probe groups, and microarray designs

- **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 required 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 lets you focus your sequencing efforts.

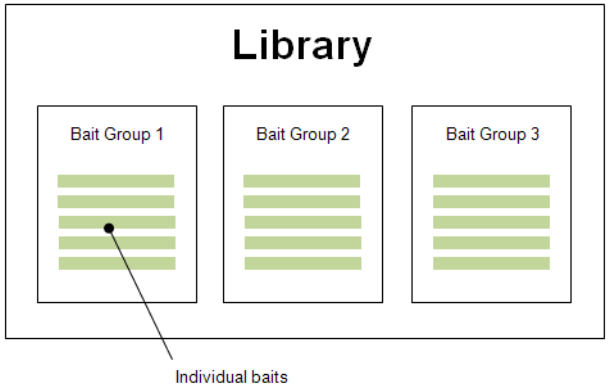

Figure 21 Baits, bait groups, and libraries

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).

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 also use Sample Manager to assign genotype references for CGH+SNP arrays. 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 10.10?

Agilent Feature Extraction 10.10 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 10.10, 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 10.10 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*.

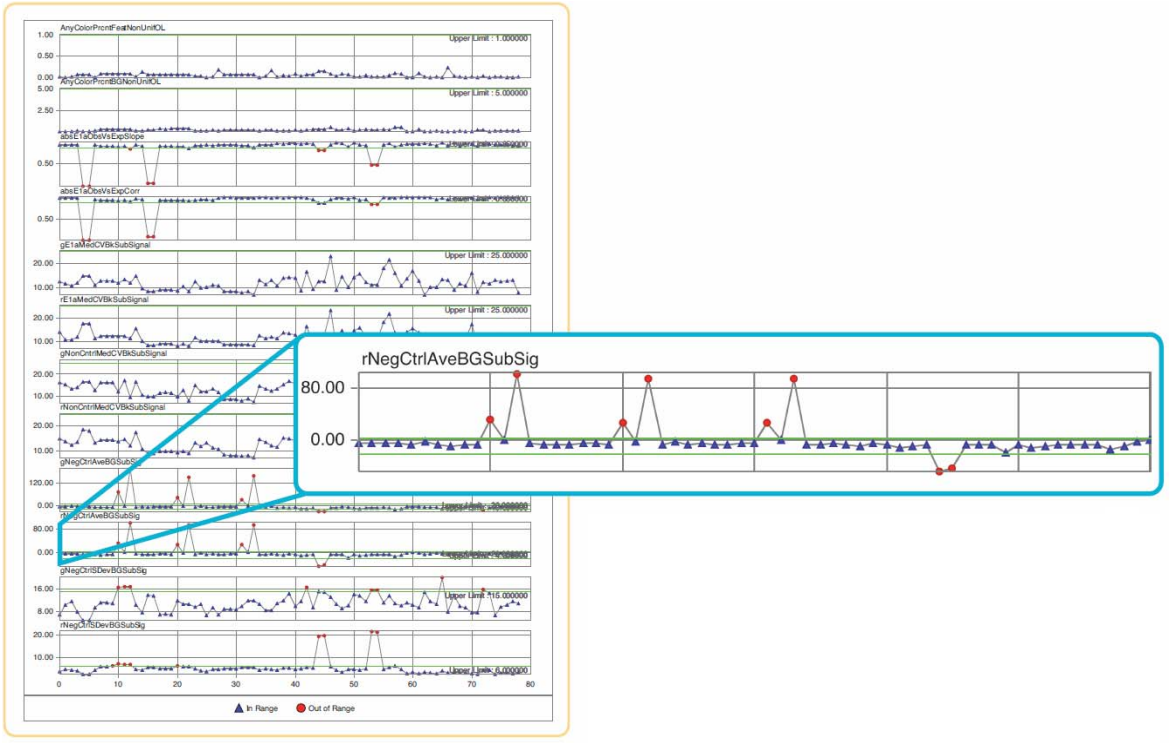

Figure 22 The control chart capability lets you monitor multiple parameters across multiple samples and multiple experiments. It gives you an at-a-glance view of important quality parameters.

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).

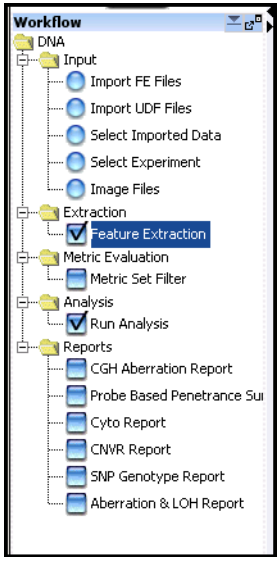

Figure 23 Workflow Navigator for CGH

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
- 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, CGH+SNP, 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, CGH+SNP, 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.

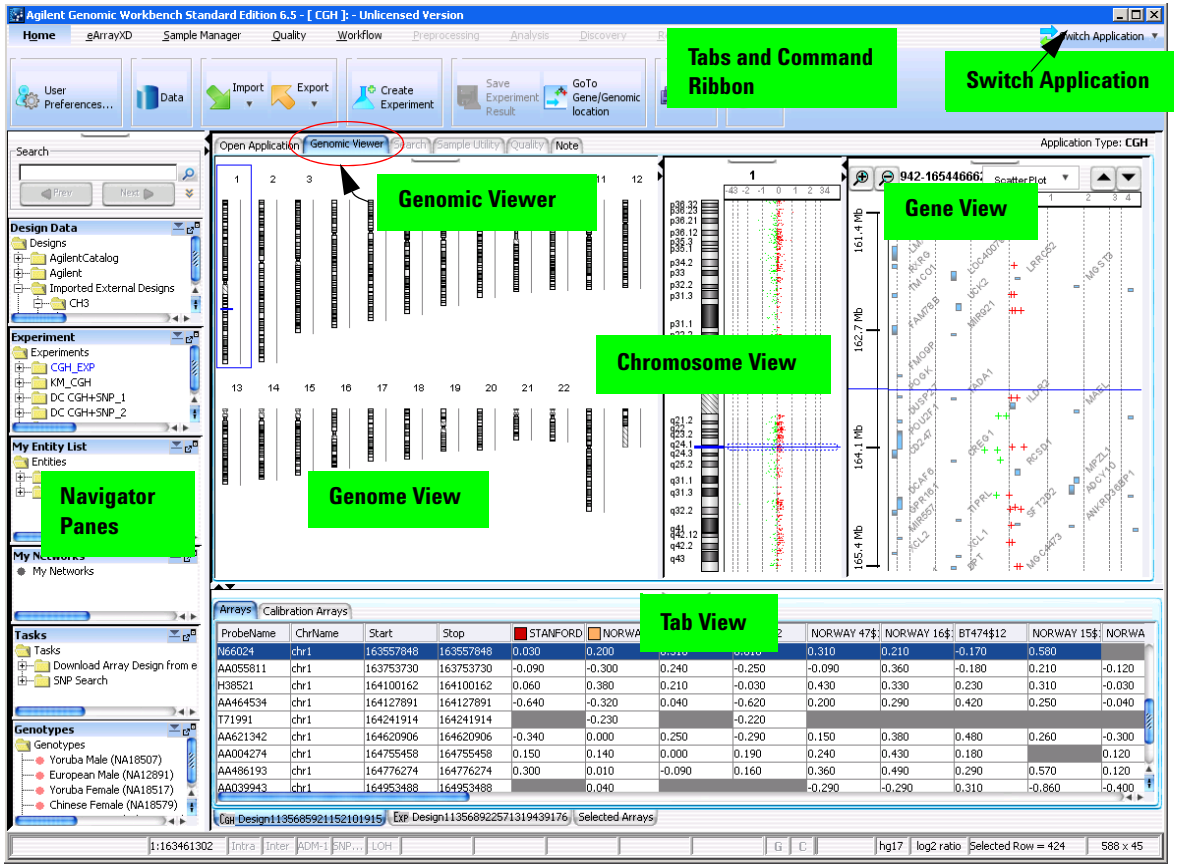

Figure 24 Agilent Genomic Workbench main window with major components – unlicensed CGH version

Analyzing CGH and CGH+SNP Data Interactively

The Agilent Genomic Workbench lets you visually explore, detect, and analyze aberration patterns from multiple Comparative Genomic Hybridization (CGH) or Comparative Genomic Hybridization with Single Nucleotide Polymorphism (CGH+SNP) 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 15. 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
  - Enable GC content correction (required for SNP Copy Number and LOH analysis)
  - Centralize the data, including nonunique probes
  - Display QC metrics on the original data
- Use robust statistical aberration detection algorithms to detect and map aberration regions with high confidence
- Display chromosomal deletions and amplifications at multiple zoom levels simultaneously
- Display allele specific copy numbers (SNP Copy Number) for SNP probes and Lack/Loss of Heterozygosity (LOH) regions
- 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”
  - Generate a genotype reference file for SNP analysis
- Save aberration results in a centralized database
- Make customizable Cytogenetic Reports for individual CGH samples
- Make Genotype and Aberration & LOH reports for CGH+SNP 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 15. 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 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.

4 System Administration and Troubleshooting

This chapter helps you with system administration and troubleshooting for the Agilent Genomic Workbench 6.5.

Agilent Technologies

Changing Login and Database settings

This section describes how to change login and database settings after Agilent Genomic Workbench is installed.

To change the location of the server

To start 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.

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.**

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.

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.

1 From the Home tab, click **User Preferences**.

2 Click the **Miscellaneous** tab.

3 Verify that you see the dialog box shown in Figure 25 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

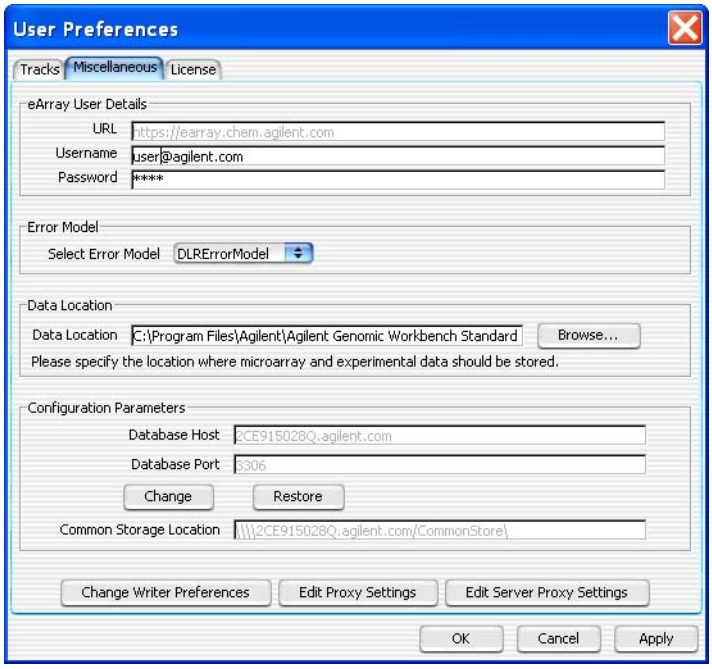

Figure 25 Miscellaneous tab of User Preferences dialog box

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 26.

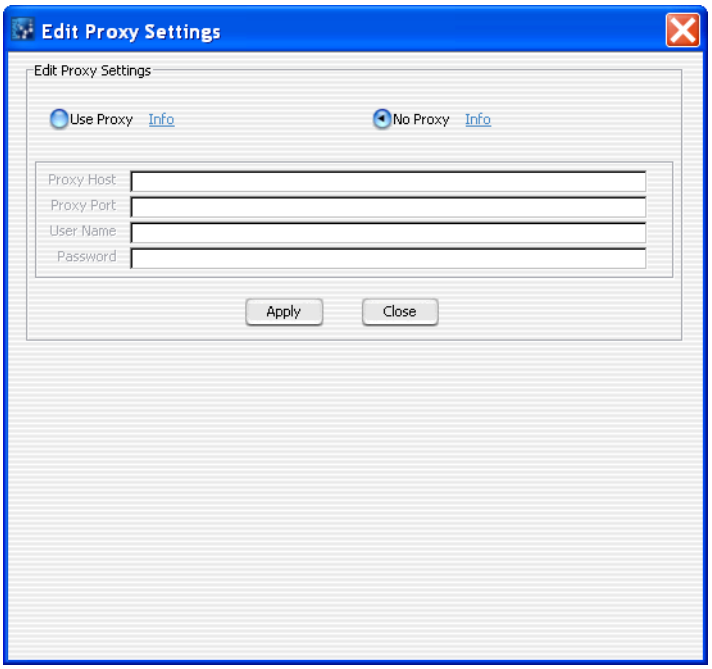

Figure 26 Edit Proxy Settings dialog box – used for the client computer

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 27.

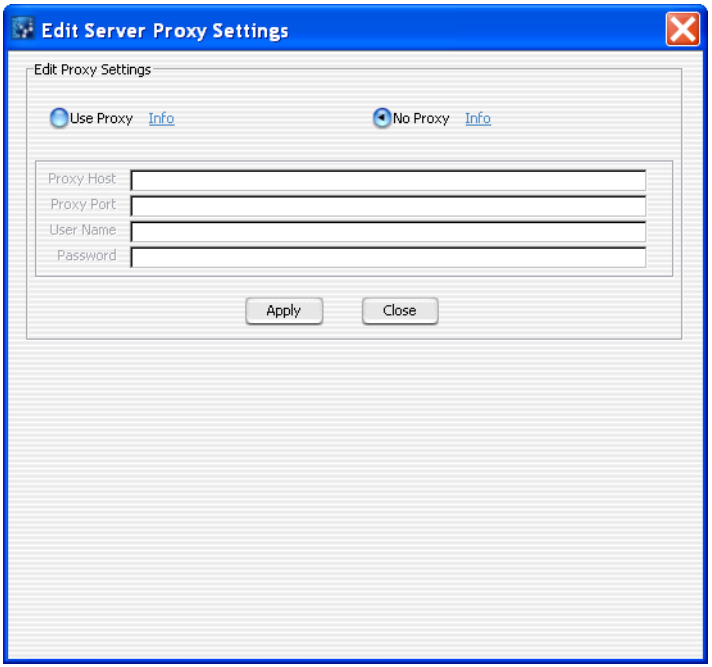

Figure 27 Edit Server Proxy Settings dialog box – used for the client computer

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**.

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.

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.
- **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.

About Agilent Genomic Workbench Services

The Agilent Genomic Workbench server program registers the following services on the Windows computer where it is installed. These services are described in this section.

- RemoteTask Manager
- DataTransfer
- ProbeUpload
- Writer

RemoteTaskManager Service

This service does those tasks that require remote processing. They include loading Catalog and Workgroup data, checking for updates on eArray Web site, transferring updated data, sending out remote search requests (such as HD Search and Probe Design requests) and loading the search results, and submitting a Microarray design on eArray Web site. This service coordinates with DataTransfer service for sending/receiving files. Unless this service retrieves existing user workgroup data and catalog data from the local system, the Writer and ProbeUpload services won't be able to do their work. Client applications won't start in this case either.

Only one instance of RemoteTaskManager service can run at a time for a given Workgroup. If you try to manually start another instance, an error message is displayed that says that another instance is already running.

As it runs, the RemoteTaskManager service creates log files in the folder: **<Installation-folder>/RemoteTaskManager/log/**.

DataTransfer Service

This service sends and receives data in the form of files from the local system to the eArray Web site and from eArray Web site back to the local system. Files transferred from the eArray Web site are then processed by the RemoteTaskManager service for various purposes. This service does not process files that it sends out or receives.

Only one instance of DataTransfer service can run at a time for a given Workgroup. If you try to manually start another instance, an error message is displayed that says that another instance is already running.

As it runs, the DataTransfer service creates log files in the folder: **<Installation-folder>/DataTransfer/log/**.

Writer Service

This service creates the design files for Microarray designs created by users on the local system. This service requires all Catalog and Workgroup data to be present in the local database before it can create the design files. Requests are not processed until catalog and workgroup data is processed and transferred to the local system by the RemoteTaskManager. Writer service processes one request at a time if multiple microarray design output requests are submitted concurrently.

Only one instance of Writer service can run at a time for a given Workgroup. If you try to manually start another instance, an error message is displayed that says that another instance is already running.

As it runs, Writer service creates log files in the folder: **<Installation-folder>/Writer/log/**.

ProbeUpload Service

This service transfers the probes submitted by users to the database. It processes the input file (.txt and .xls files) and loads the probes into the database. These files are kept in Common Storage temporarily once they are uploaded by the user from the user interface. It will not process any request until RemoteTaskManager service processes and transfers Catalog and Workgroup data to the local system, as it first checks to see if the probe is already present in the system.

Only one instance of ProbeUpload service can run at a time for a given Workgroup. If you try to manually start another instance, an error message is displayed that says that another instance is already running.

As it runs, ProbeUpload generates log files in the folder: **<Installation-folder>/ProbeUpload/log/**.

Starting and stopping services

Services installed by Agilent Genomic Workbench server are started automatically when installation is complete and also whenever the computer is rebooted. You can also start and stop the services using the Administration tool, or manually as described later in this guide. For

example, suppose you find out that a service stopped after an exception and you want to start it again. Or, you might want to stop all the services to upgrade your MySQL installation, and need to start them again once the MySQL upgrade is complete.

System Administration

A system administration tool is provided to help you with various troubleshooting and administration tasks. The Administration tool is used to start and stop database services, change common storage location, and to perform a system check.

To start the Administration tool

- On the server computer, click **Start > Programs > Agilent Genomic Workbench Server > Administration**.

NOTE The Administration icon is in the location you selected for the program icons when you installed the server.

The Administration tool opens, and displays the Manage dialog box.

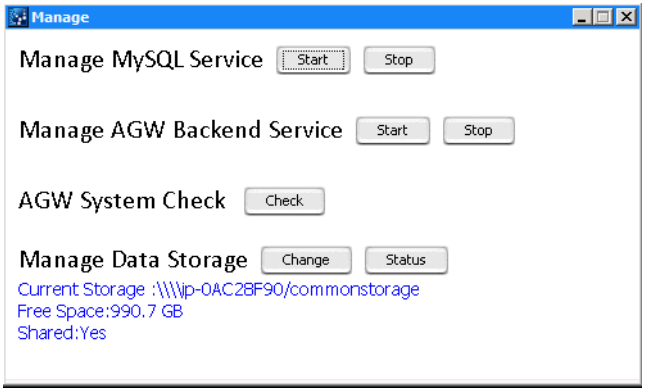

Figure 28 Administration tool Manage dialog box

To start or stop MySQL service

1 Start the Administration tool. See "To start the Administration tool" on page 78.

2 In the Manage dialog box, next to Manage MySQL Service, click **Start** to start the MySQL service, or click **Stop** to stop the MySQL service.

To start or stop Agilent Genomic Workbench services

1 Start the Administration tool. See "To start the Administration tool" on page 78.

2 In the Manage dialog box, next to Manage AGW Backend Service, click **Start** to start the Agilent Genomic Workbench services, or click **Stop** to stop the Agilent Genomic Workbench services.

To perform a system check

A system check examines the basic functions of your system, and generates a log that contains the results. This is helpful when working with Agilent Customer Support and troubleshooting problems.

1 Start the Administration tool. See "To start the Administration tool" on page 78.

2 In the Manage dialog box, next to AGW System Check, click **Check**.

A system check log is generated and displayed.

To change the common storage folder location

1 Start the Administration tool. See "To start the Administration tool" on page 78.

The current common storage location, free space, and share status is displayed at the bottom of the dialog box.

2 In the Manage dialog box, next to Manage Data Storage, click **Change**.

An information box appears with important cautions and instructions.

CAUTION Do not change the location of your common storage location unless you have read this information, and understand it. If you change the location of your common storage, you must copy the contents of the previous location to the new location manually. Failure to do so may cause unexpected behavior in the program.

3 After you read the information, click **OK.**

4 In the Path to common storage directory dialog box, type the path for the new common storage folder, or click **Browse** and select the folder to use.

5 Click **OK** to accept the new location for the common storage folder.

How common storage is used

The common storage is used by Agilent Genomic Workbench to store the following files:

- Design/Annotation files written for microarray designs created using $eArray_{XD}$.
- Design/Annotation files downloaded from eArray Web site upon request.
- Input files when you use $eArray_{XD}$, such as probe files that you uploaded through Probe Upload, target sequence files that you uploaded for gene expression probe design, custom genome files that you imported, etc.
- Error reports created during probe upload process.
- Various temporary files used to update database and communicate with eArray Web site.
- Index files for displaying information on user interface.
- Track files that you can download for various DNA tracks.

To start or stop a service using batch files

Agilent Genomic Workbench server includes one-click batch files to let you start and stop services manually, as needed.

1 Find the batch files in Windows Explorer. They are located in the Agilent Genomic Workbench server installation folder.

2 Double-click the batch file name of interest, depending on whether you want to start or stop a service.

- start_RemoteTaskManager_service.bat
- stop_RemoteTaskManager_service.bat
- Start_MySQL.bat
- stop_mysql.bab

To check memory usage

1 Open the Task Manager window.

2 Click the Processes tab and view the value in the Mem Usage column for the processes of interest.

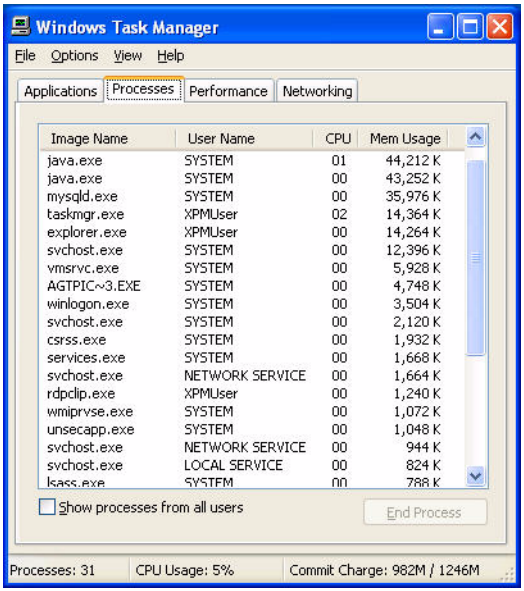

Figure 29 Windows Task Manager - Processes tab

Configuring MySQL

MySQL server configuration is done automatically during the installation of Agilent Genomic Workbench Server software components.

NOTE Under normal operation and circumstances, you won't need to perform the manual configuration steps described in this section. The information is included here in case there is a problem.

Consider backing up your existing MySQL databases if you are using the same MySQL Server installation for other purposes.

Agilent Genomic Workbench software must be able to log in using MySQL's 'root' user (database user) from any host so that it can create its own database, database user and grant appropriate privileges to its new database user.

MySQL automatically creates ‘root’ user account for logging in to MySQL server from the computer where MySQL Server is installed. In addition to the default ‘root’ account, Genomic Workbench also requires a ‘root’ account for logging in from any other host with proper privileges. This is true even if you are running the Agilent Genomic Workbench server software installer on the same computer where the MySQL server is currently installed. Agilent recommends that you install MySQL server and Agilent Genomic Workbench server on the same computer.

1 Check that MySQL is configured to accept ‘root’ user login from other hosts.

   a Log in to the computer where MySQL server is installed.

   b Open a command prompt.

   c Execute the command: `mysql -u root mysql -p`

   If the error message: *' mysql ' is not recognized as an internal or external command, operable program or batch file,* check that the MySQL Server installer's bin directory is included in your PATH environment variable. For help setting PATH variable, see http://dev.mysql.com/doc/refman/5.1/en/server-system-variables.html.

   d When MySQL asks you to enter your password, type the root user password and press **Enter**.

   e After MySQL displays a message that says you are now logged in, execute the following command including the semicolon at the end of the line: `select user, host from user where user = 'root';`

   If the following information is displayed, continue with step 3, as this means that MySQL is configured to accept log in from other hosts.

```
+--------+-----------------+
| user   | host            |
+--------+-----------------+
| root   | %               |
| root   | localhost       |
+--------+-----------------+
```

   If only one record is returned as shown below, then continue with step 2 to configure MySQL to a log in from other hosts.

```
+--------+-----------------+
| user   | host            |
```

```
+--------+-----------------+
| root   | localhost       |
+--------+-----------------+
```

f Execute the command: exit;

2 Configure access to MySQL from other hosts using 'root' user, if you find this is necessary from step 1.

a Log in to the computer where MySQL server is installed.

b Open a command prompt.

c Execute the following command: `mysql -u root mysql -p`

If the error message: *' mysql ' is not recognized as an internal or external command, operable program or batch file*, check that the MySQL Server installer's bin directory is included in your PATH environment variable. For help setting PATH variable, see http://dev.mysql.com/doc/refman/5.1/en/server-system-variables.html.

d When MySQL asks you to enter your password, type the root user password and press **Enter**.

e After MySQL displays a message that says you are now logged in, execute the following command including the semicolon at the end of the line:

```
grant all on *.* to 'root'@'%' identified by
'<your-root-password>';
```

where <your-root-password> is the root password that you entered while logging in to MySQL. Note that the < and > characters are not part of the command, but the single quotation marks (') are.

The system should respond with:

Query OK, 0 rows affected

f Execute the following command: `exit;`

3 Grant appropriate privileges to the 'root' user (configured for other hosts).

a Login to the computer where MySQL server is installed.

b Open a command prompt.

c Execute following command:

```
mysql -u root mysql -p
```

If the error message: *' mysql ' is not recognized as an internal or external command, operable program or batch file*, check that the MySQL Server installer's bin directory is included in your PATH environment variable. For help setting PATH variable, see http://dev.mysql.com/doc/refman/5.1/en/server-system-variables.html.

d When MySQL asks you to enter your password, type the root user password and press **Enter**.

e After MySQL displays a message that says you are now logged in, execute the following commands including the semicolon at the end of the line. Skip the first command if you did it in step 2.

Command	Expected Response
grant all on *.* to 'root'@'%';	Query OK, 0 rows affected
update user set Grant_priv = 'Y' where user='root' and host = '%';	Query OK, 1 row affected (0.00 sec) Rows matched: 1 Changed: 1 Warnings: 0
commit;	Query OK, 0 rows affected
flush privileges;	Query OK, 0 rows affected
exit;	

4 Set MySQL configuration parameters.

a Stop the MySQL service.

b Open the my.ini file located in your MySQL server installation directory.

c Modify/Add the following configuration to the my.ini file:

```
max_allowed_packet=64M

innodb_buffer_pool_size=512M

tmpdir="=<new-location>"
```

where <new-location> is where you want to put temporary files from MySQL. Note that the < and > characters are not part of the command, but the quotation marks (") are.

d Save the file and close it.

e Start the MySQL service.

5 Make sure you have enough space in your MySQL data directory.

Agilent Genomic Workbench uses large scientific data that includes your own customized data and Agilent Catalog data. Because of this, your MySQL server installation must be on a high-end computer with sufficient disk space that is configured to make use of available disk space. If your MySQL data directory is not set to a location with sufficient disk space, then MySQL will not be able to hold data that is created with Agilent Genomic Workbench.

Though MySQL makes use of available disk space, eventually the disk may get full and you will need to add data files to your database or set up MySQL data directory to some other location.

Please visit http://dev.mysql.com/doc/refman/5.1/en/windows-create-option-file.html for information on how to set up a MySQL data directory for your MySQL installation. Or visit http://dev.mysql.com/doc/refman/5.0/en/adding-and-removing.html for information on how to add a new data file to your MySQL database. You may want your Database Administrator to perform these tasks for you.

If you change the location of your MySQL data directory after some data is already stored in the database, you will need to manually copy the contents of the previous data directory to the new data directory before you start the MySQL service again.

NOTE If your MySQL installation runs out of disk space and it cannot hold additional data, you will see exceptions in the application log files. Please refer to page 24 for more information on Agilent Genomic Workbench services.

6 Set up a temporary directory for MySQL.

Agilent Genomic Workbench uses some MySQL features for bulk entry of data into a database. These features require a 'temp' directory for MySQL with sufficient available disk space. This disk space is used

when bulk data is loaded and becomes available again after the data is saved to the database. If a temp directory is not set up, data loading operations for large datasets may fail, which will cause abnormal or unexpected behavior. If MySQL does run out of temporary disk space, the following exception is written to the appropriate application log files:

Caused by: java.sql.SQLException: Incorrect key file for table '<temp-directory-location>\#sql_6c0_0.MYI'; try to repair it

where <temp-directory-location> is location of the temporary directory and the .MYI file name after it may vary.

See http://dev.mysql.com/doc/refman/5.0/en/temporary-files.html to learn how to set up a temp directory location for MySQL server.

The following procedure describes how to set up a one-time temp directory for MySQL.

a Stop the MySQL service.

b Open my.ini file located in your MySQL server installation folder.

c Look for an entry such as tmpdir=<some-location>.

- If you find one, edit the <some-location> to the location you want to configure as the temp directory.
- If you don't find one, add an entry with tmpdir=<new-location>.
- Remember to use forward (/) slashes rather than backslashes for identifying Windows path names. If you do use backslashes, you must double them.

d Save the file and close it.

Start the MySQL service.

www.agilent.com

In this book

This book gives an overview of the capabilities within Agilent Genomic Workbench 6.5. 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.

Revision A1, October 2015

G3800-90021

Agilent Technologies