Agilent
Genomic Workbench 6.5

Quality Tools

User Guide

Agilent Technologies

Notices

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In This Guide...

This guide describes how to query, filter, and evaluate microarray extractions within Agilent Genomic Workbench 6.5. It also describes how to visualize current and historical batch microarray extraction processes. The Quality tools application can be used with any of the DNA Analytics applications, or it can be accessed without an application license as part of the general Data Viewing application in Agilent Genomic Workbench 6.5.

1 Getting Started

This chapter gives an overview of how to use Agilent Genomic Workbench 6.5 Quality tools to evaluate microarray extraction quality. It gives instructions for how to do common tasks in Quality tools.

2 Building Queries, Metric Sets, and Charts to Evaluate Extractions

This chapter describes how Queries are applied to Extractions, how Metric Sets are built and used to filter and evaluate Extractions, and how to visualize batch processes using Charts. It gives instructions for how to do common tasks in Quality tools.

3 Quality Tools Reference

This chapter describes the parts of the Agilent Genomic Workbench 6.5 Quality tools main window that you use to query and evaluate microarray extractions. It also describes any dialog boxes that can appear during quality evaluation of the extractions.

Contents

1 Getting Started

This chapter provides an overview of how to use Agilent Genomic Workbench 6.5 Quality tools to query, evaluate, and chart microarray extraction data. For a list of how-to instructions for the tasks available in the Quality tools application, see Chapter 2, “Building Queries, Metric Sets, and Charts to Evaluate Extractions”. For a description of each part of this application, including all of the dialog boxes that can appear when you click buttons and other elements, see Chapter 3, “Quality Tools Reference”.

What is the Quality Tools Application?

Quality tools is an application within Agilent Genomic Workbench 6.5 that analyzes summary statistics from Feature Extraction output files and optional annotation files in order to monitor microarray processing performance.

The data is kept in a relational database that can be queried. The queries can be saved to allow the viewing of user-specified subsets of the data. For instance, queries can select the data that is in specific experiments, batches, or dates of processing. Using the data, metrics can be created that monitor aspects of the microarray processing workflow.

Additionally, metric sets can be created that combine metrics, and you can set thresholds for metrics within a metric set. Agilent includes default metric sets for each array application in the Quality tools database. These metric sets have been optimized for the workflow using the Agilent microarray scanner, Feature Extraction default protocols, and Agilent laboratory protocols. These can be modified and saved with a new name to create custom metric sets. For example, you may need to optimize the thresholds for your custom protocols.

With Quality tools, you can select a query to define which extractions to view and select a metric set to define which metrics to view. You can then graphically plot the results from current or historical microarray data and create thresholds for the metrics that are appropriate for your experimental conditions and processing environment.

Metric sets and thresholds can also be used to create Metric Set filters (see the Preprocessing tab description in the *CGH and ChIP Interactive Analysis User Guides*). The Metric Set filters can then be used in workflow extractions or pre-processing filtering of data.

The Quality tools module is designed for use in a production environment where:

- Microarray processing protocols are standardized and the effect of specific variables on performance is needed such as those related to:
  - Operators
  - Wet-lab protocols
  - FE parameter protocols
- Monitoring run-to-run consistency is an important goal to:

- Identify extractions that fall outside the established normal range
- Identify systematic data trends

For the most recent information and to download QC metric sets, please go to the Web site: **http://www.agilent.com/chem/feqcmetrics**.

Example Use Cases for Quality Tools

Agilent Feature Extraction (FE) generates output to assist in quality assessment. A table of array-wide, or global statistics (the "Stats" table in FE), is extremely useful in data quality determination for each extraction. These global statistics capture information from every independent FE step - for example, the numbers of outliers, the averages of negative control signal statistics, and spike-in regression values.

The number of output fields can be cumbersome without a tool for quality assessment of each extraction. Quality tools captures key global statistics to use as metrics and creates graphs for easy visual assessment of metrics. Additionally, the metrics that are used for analysis can be defined for specific monitoring requirements.

This section provides several common use cases for the Quality tools application. Although these examples are from an earlier version of the Quality tools software, the use cases and results are the same in the current software version.

Use Case 1 Analysis of Feature Extraction output

Feature Extraction analysis is a common way to use the Quality tools application with everyday extraction monitoring. An example of this type of analysis was performed using a collection of microarrays from several experiments.

Some of these microarrays had been previously annotated by the operator as having issues in the labeling, hybridization, and/or washing steps. These microarrays had poor correlation with their replicate microarray sets, and were chosen because they each had at least one metric flagged as having values outside of the normal range.

This analysis of Feature Extraction consisted of:

1 A comparison to all other extractions within the extraction set.

2 A comparison to thresholds associated with the default metric set. For information on creating metric sets, see "Defining Metrics" on page 24.

By default, the chart generated by Quality tools shows extractions in the order they were performed. Because Quality tools has customized sorting, color-by, and shape-by attributes, it can be a powerful tool for visualizing and highlighting trends in patterns, as seen in the results of this analysis.

Figure 1 on page 10 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 10.10 User Guide*.

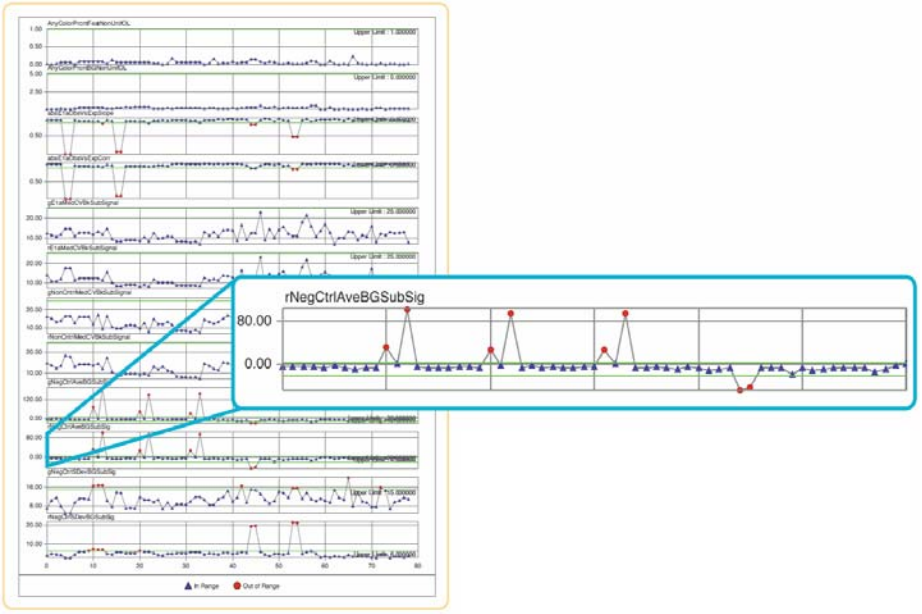

Figure 1 Assessment of extraction statistics used for metrics with Quality tools

Use Case 2 Analysis of the user effect on extraction quality

Often the effects of specific variables on extraction quality are needed in a production setting. One such variable is the effect of the user. A retrospective analysis of different users was generated using the following steps in Quality tools:

1. A query was created to select only those extractions of interest and applied to a chart as the X-axis. For information on creating queries, see "Building and Running Queries" on page 20.
2. A two-color gene expression metric set was chosen and applied to the chart as the Y-axis. For information on creating metric sets, see "Defining Metric Sets and Thresholds" on page 26.
3. The extractions were then color-coded to reflect the three different operators who had processed the arrays in those extractions.

Figure 2 on page 12 shows data from microarrays processed by users A (represented by blue squares), B (red circles), and C (green triangles). Threshold limits appear in upper right-hand corners and as green lines within each plot. 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 Extraction statistics used for metrics, see the *Feature Extraction 10.10 User Guide*.

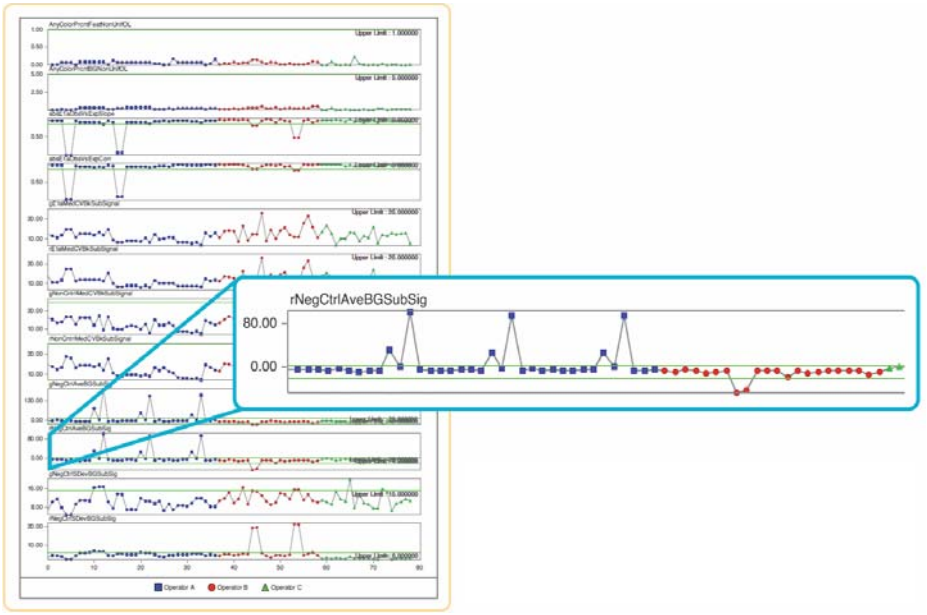

Figure 2 Comparison of the effect of variable users on microarray performance using Quality tools

Use Case 3 Analysis of the effect of changing the FE parameter protocols

Analysis of Extraction statistics used for metrics with the Quality tools application gives an intuitive evaluation of competing protocol methods, such as background processing algorithms. An 18-array set was extracted with either the default FE parameter protocol “Spatial Detrend” background method or an alternative “Minimum Signal” background method and processed using the standard metric set in Quality tools.

Figure 3 on page 13 shows extractions with either the default Spatial Detrend background (represented by blue squares), or the alternate Minimum Signal background data (red circles). For this data set, more favorable metric values were clearly seen with the Spatial Detrend method. This improvement is seen especially with the average negative control background-subtracted signal, which is closer to the expected value of zero, and therefore a more accurate estimate of background.

Additionally, under subtraction of background, as seen with the Minimum Signal method, results in compression of log ratios. The inset window shows a plot of observed versus expected spike-in ratios (the "absE1aObsVsExpSlope" metric), where Spatial Detrend background yields a slope closer to 1.0. For more information about Feature Extraction statistics used for metrics, see the *Feature Extraction 10.10 User Guide*.

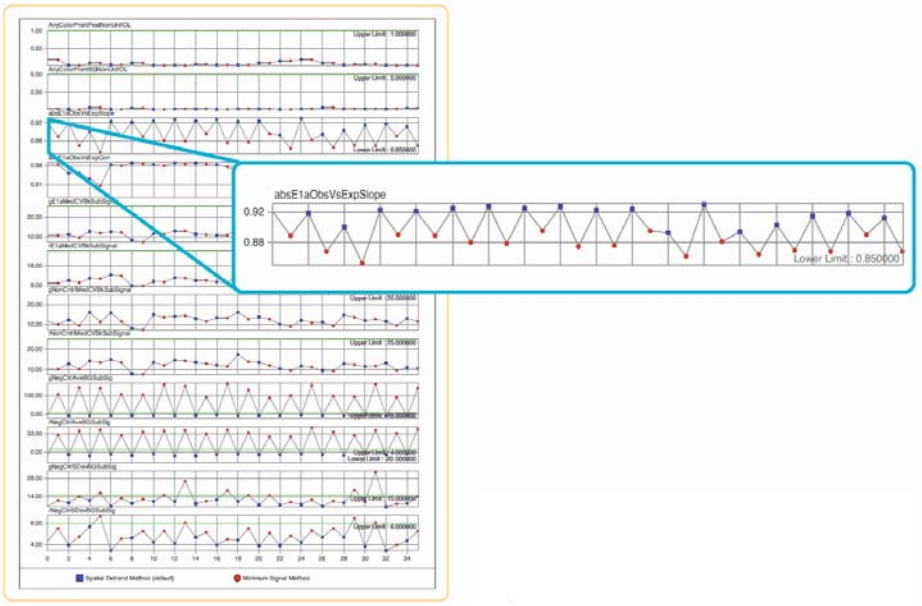

Figure 3 Comparison of the effect of FE parameter protocols on extraction quality

Starting the Quality Tools Application

When you start Agilent Genomic Workbench 6.5 for the first time, the program opens in the **Home** tab, with the **Open Application** tab displayed. From this tab, you can click any of the application areas, or click **Help** to open the User Guide for that application.

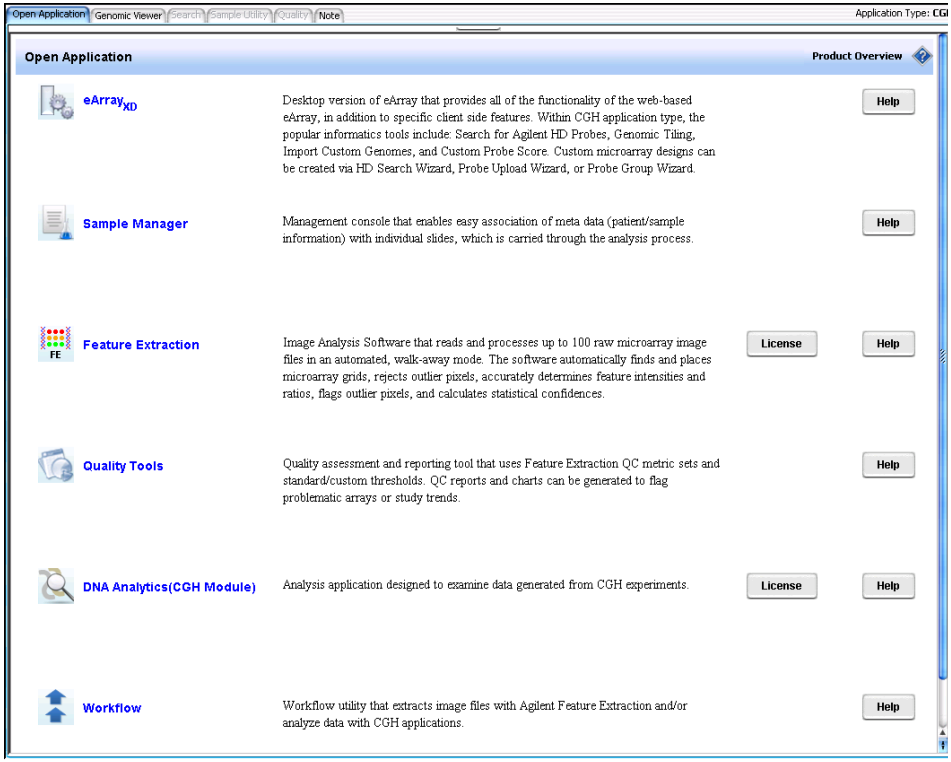

Figure 4 Open Application tab for Agilent Genomic Workbench 6.5

To open Quality tools, do either of the following:

- From the **Open Application** tab, click on the **Quality Tools** icon to launch the Quality tools application.

- Alternatively, launch Quality tools from any application area by clicking the **Quality** tab in the Main Menu ribbon (see "Quality Tab Ribbon" on page 39 for more information).

The Quality tab opens, as shown in the following example:

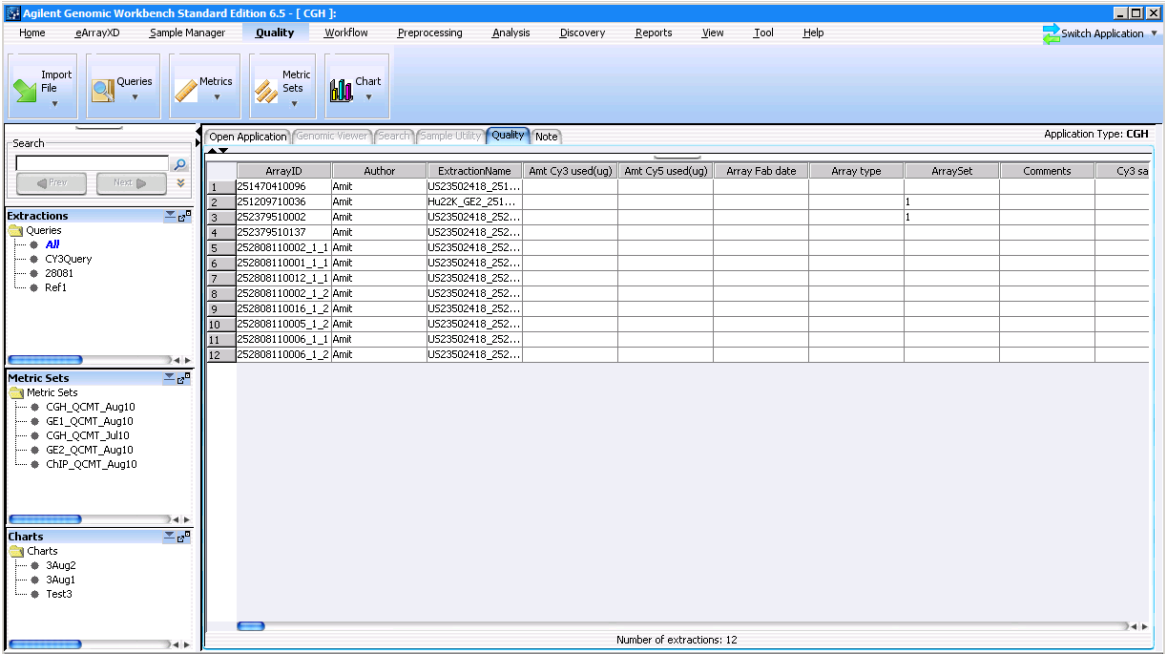

Figure 5 Agilent Genomic Workbench main window – Quality tab, populated with data

For a description of each part of this application, including all of the dialog boxes that can appear when you click buttons and other elements, see Chapter 3, “Quality Tools Reference”.

2 Building Queries, Metric Sets, and Charts to Evaluate Extractions

This chapter provides a list of how-to instructions for the tasks available in the Quality tools application. Most functionality in the Quality tools has been standardized to be accessible from either the Quality tab ribbon interface or from the object under consideration in the Navigator.

For example, to edit a query you can select the query in the Navigator and then select the **Query** button in the Quality tab, which will display a list of options including an edit function for that query.

Alternatively, after selecting the query in the Navigator, you can right-click on that query to display a list of options including an edit function for that query.

For a description of each part of this application, including all of the dialog boxes that can appear when you click buttons and other elements, see Chapter 3, “Quality Tools Reference”.

Importing Data

The Quality tools application can easily access the database of feature extractions (global statistics and FE parameters) that are imported into or extracted within Agilent Genomic Workbench 6.5, making it easy to monitor microarray quality control as well as analysis historical quality control trends.

The Quality tools application also includes a way to import the quality information from Feature Extraction output files that are not in Agilent Genomic Workbench 6.5. The import will contain only the quality information (the Stats and Parameter tables) from the Feature Extraction output so that you can add external extractions to your Quality tools analysis.

The following topics in this chapter describe how to import data into the Quality tools application:

"To import FE statistics and parameter information" on page 19

"To import a query" on page 23

"To import metric sets" on page 32

You can also import FE files (extracted microarray data) into Agilent Genomic Workbench 6.5 from the Workflow, CGH, ChIP, and CH3 applications. When an FE file is imported into the Agilent Genomic Workbench 6.5 database, the appropriate FE statistics and parameters are also imported into the Quality database. See the User Guide for the selected application or the *Agilent Genomic Workbench 6.5 Data Viewing Guide*.

When you set up a Workflow input to import an extracted FE file or input as an image to be extracted, the extraction is imported into the Agilent Genomic Workbench 6.5 database. The appropriate FE statistics and parameters are also imported into the Quality database. See the *Agilent Genomic Workbench 6.5 Workflow User Guide*.

To import FE statistics and parameter information

1 In the Quality tab, click **Import File** and then click **FE Stats and Parameters**. The Import FE Files dialog box appears.

2 Navigate to the Feature Extraction output file(s).

3 Click **Open**. The FE quality information is added to the available extractions to be queried and evaluated.

See Also Refer to "Chapter 3" of the *Feature Extraction 10.10 Reference Guide* for a description of the statistics and parameters that are imported.

Building and Running Queries

Quality Tools has a Query builder that lets you to select a subset of the extractions from the Agilent Genomic Workbench 6.5 database, based on criteria that you select.

A query is used to define a subset of extractions for a representative data set, for use in metric and threshold development, and in producing Charts. One example is a query that contains data from similar biological samples processed under identical conditions. Another example is to query for different types of samples or for different processing methods. With the latter example, you then use the different processing attributes to color-code a chart. See "Chart Configuration Dialog Box" on page 49 for information about using processing attributes. See "Example Use Cases for Quality Tools" on page 9 for examples of color-coding a chart based on the processing attributes.

A subset of extractions is defined in a query by specific FEParameter fields, or by user-added attribute fields.

To create a query

1. To start the query builder, in the Quality tab, click **Queries** and then click **New.**
2. In the Column Name drop-down list, select the parameter to set.
3. In the Operator drop-down list, select the appropriate operator.
4. In the right-most text box, select the value with which to compare the value of the Column Name parameter.
5. Click **Add**.
6. In the Query Name area, enter a name for the query.
7. Click **Save.**

The Query can now be used in developing thresholds for metrics or used to define a Quality chart. The query can be run, and the results can be exported and saved. Queries can also be renamed and deleted.

See Also

"To edit a query" on page 22
"To run a query" on page 22
"To delete a query" on page 23
"To rename a query" on page 23
"To import a query" on page 23
"Query Builder Dialog Box" on page 61

To create a composite query

1. Create a basic query as described in "To create a query" on page 20.
2. Click **AND** or **OR**.

   Use AND to find extractions that meet all criteria. Use OR to find extractions that meet at least one criterion.
3. Create the next basic query.
4. To group composite queries, click a query, then click **"("** or **")"**. Repeat for the query at the other end of the group.
5. In the **Query Name** area, enter a name for the query. See Figure 6 on page 22.
6. Click **Save.** The Quality Tools main user interface is displayed.

These components and attributes are described in "Query Builder Dialog Box" on page 61.

Figure 6 shows an example of a query that will find all 1-color gene expression extractions.

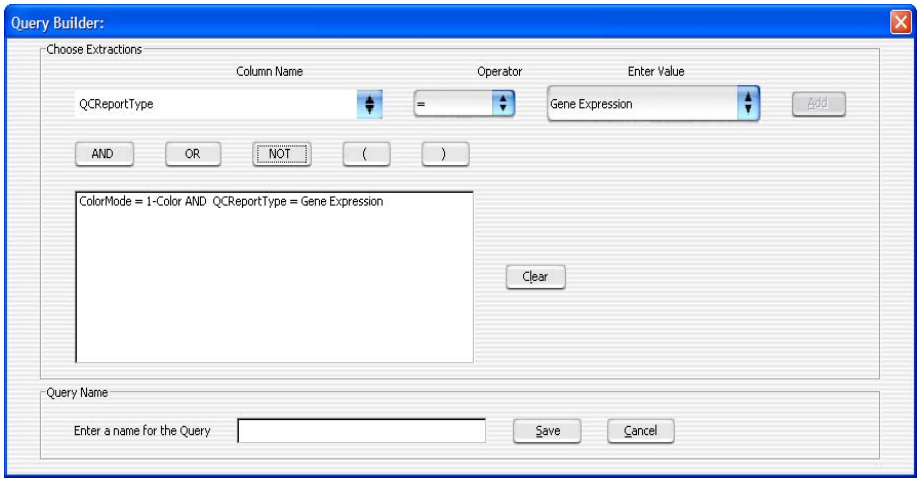

Figure 6 Query Builder showing a composite query.

To edit a query

1. In the Navigator, in the Extractions pane, under Queries, select the query to edit.
2. In the Quality tab, click **Queries** and then click **Edit.** Alternatively, right click on the query of interest and select **Edit Query.**
3. In the Query Builder dialog box, make any necessary changes.
4. Click **Save.**

To run a query

1. In the Navigator, in the Extractions pane, under Queries, select the query to run.
2. In the Quality tab, click **Queries** and then click **Run.** Alternatively, right click on the query of interest and select **Run Query.**

To delete a query

1. In the Navigator, in the Extractions pane, under Queries, select the query to delete.
2. In the Quality tab, click **Queries** and then click **Delete.** Alternatively, right click on the query of interest and select **Delete Query.**
3. In the Delete Query dialog box, click **Yes** to confirm the deletion.

To rename a query

1. In the Navigator, in the Extractions pane, under Queries, select the query to rename.
2. In the Quality tab, click **Queries** and then click **Rename.** Alternatively, right click on the query of interest and select **Rename Query**.
3. In the Enter New Name dialog box, enter the new name for the query.
4. Click **OK**.

To import a query

1. In the Quality tab, click **Import File** and then click **Query Result**. The Import Query Result dialog box appears.
2. Navigate to the previously exported query result file and click **Open**.

To export a query

1. In the Navigator, in the Extractions pane, under Queries, select the query to export.
2. In the Quality tab, click **Queries** and then click **Export.** Alternatively, right click on the query of interest and select **Export Query.**
3. In the Export Query Result dialog box, enter the path and name of the export query result file.
4. Click **OK**.

Defining Metrics

Metrics are defined in order for you to track desired statistical values within a set of extractions. These metrics can be associated in a metric set.

To create a new metric

1. Click **New** from the Metric area of the Quality tab ribbon interface to open the Create a new Metric dialog box.
2. Select a metric from the Choose Metric Column list and then click **Add**.
3. Define a new metric as an expression. Use the operator buttons and type numbers, if needed, in the Numerical Constant text field to create a formula.
4. In the **Save Metric** text box, type a name to save the new metric.
5. Click **Save**.

   The new metric now appears in the Metric list of the Configure Metrics and Thresholds dialog box.

See Also These components and attributes are described in "Create a new Metric Dialog Box" on page 51.

Example

Feature Extraction calculates a slope for the eQC spike-ins (observed versus expected Log Ratio). Depending upon the hybridization, this spike-in mixture may be present as "+1" or "-1" polarity. If it is "-1", then any threshold that is set (e.g. Slope > 0.85), will not pass. Therefore you can make a derivative metric by taking the absolute of the slope.

To do this:

1. Click **New** from the Metric area of the Quality tab ribbon interface to open the Create a new Metric dialog box.
2. Click **Abs**. The term **Abs(** appears in the Metric Calculation box.
3. From the Choose Metric Column list, select the statistic **eQCObsVsExpLRSlope**, click **Add**, then select "**)**" to finish the expression.

4 Validate and save the metric as Abs_eQCSlope.

The new metric now appears in the Metric list of the Create a new Metric dialog box.

To delete a metric

1 In the Quality tab, click **Metrics** and then click **Delete**.

2 Select a metric or hold down the control button and select several metrics previously defined shown in the Delete Metrics dialog box.

3 Click **Delete**.

If the delete button is gray, the metric is associated with one or more metric sets. You need to remove the metric from all metric sets with which it is associated before you can remove the metric globally.

Defining Metric Sets and Thresholds

Metric Sets are combinations of existing metrics applied with optional user-defined thresholds. Metric sets can be saved and exported for future use.

To create a metric set

1. In the Quality tab, click **Metric Sets** and then click **New**.
2. When the Metric Set Configuration dialog box appears, select the **Add Metrics to Metric Set** tab.
3. Select the metric(s) from the Existing Metrics checklist to be associated the selected metric set.
4. Set the appropriate thresholds for the Metric to be added to the Metric Set. See "To set metric thresholds in metric sets" on page 28.
5. Optional: assign an Extraction Query to the metric set.

   This option is available in Standard, Robust, and Percentage Threshold Calculation modes. This query will filter the appropriate extractions from the database, so that just the data from the queried extractions is used in the calculation of the statistical summary values used to set the thresholds.
6. In the Metric Set Name area, enter the name for the Metric Set.
7. Click **Save**.

   The Metric Set can now be viewed, and the results can be exported and saved. Metric Sets can also be renamed and deleted.

Tip You can also create a new metric set by editing an existing metric set, then saving it with a new name as described in "To edit a metric set" on page 27.

See Also
"To view a metric set" on page 27
"To set metric thresholds in metric sets" on page 28
"To export metric sets" on page 31
"To import metric sets" on page 32

"To delete a metric set" on page 32
"To remove a metric from a metric set" on page 33
"To rename a metric set" on page 33

"Metric Set Configuration Dialog Box: Add Metrics to Metric Set Tab" on page 54

To edit a metric set

1 Select the Metric Set of interest in the Metric Sets pane of the Navigator.

2 Click **Metric Sets** in the Quality tab and click **Edit**.

3 When the Metric Set Configuration dialog box appears, select the **Add Metrics to Metric Set** tab.

4 Select or deselect the metric(s) from the Existing Metrics checklist to be associated or with or removed from the selected metric set.

5 Set the appropriate thresholds for the Metric to be added to the Metric Set. See "To set metric thresholds in metric sets" on page 28.

6 Optional: assign an Extraction Query to the metric set.

This option is available in Standard, Robust, and Percentage Threshold Calculation modes. This query will filter the appropriate extractions from the database, so that just the data from the queried extractions is used in the calculation of the statistical summary values used to set the thresholds.

7 Change the name of the Metric Set if necessary in the Metric Set Name area. Entering a new name will create a new custom Metric Set.

8 Click **Save**.

To view a metric set

1 In the Navigator, in the Metric Sets panel, select the metric set to view.

2 In the Quality tab, click **Metric Sets** and then click **View**. Alternatively, double-click the metric set in the Navigator.

3 The metric set is displayed in the Tab View.

To return the Tab View to another metric set, chart, or query result, simply click the appropriate entity in the Navigator.

To set metric thresholds in metric sets

If you want to associate a threshold with a given metric, you can either do this at the same time that the metric is associated with a metric set, or after the association.

1 Open the Metric Set Configuration dialog box. See "To create a metric set" on page 26 or "To edit a metric set" on page 27.

2 Select the **Add Metrics to Metric Set** tab.

3 Select the metric(s) from the Existing Metrics checklist that is currently associated or will be associated with the selected metric set.

4 In the Threshold Type area, select the type of threshold to be associated with the metric:

- Upper Limit
- Upper Warning Limit
- Lower Warning Limit
- Lower Limit

5 In the Threshold Calculation area, select the limit calculation type:

- Manual
- Standard
- Robust
- Percentage

The relevant calculations and their limits are displayed.

6 Optional: assign an Extraction Query to the metric set.

This option is available in Standard, Robust, and Percentage Threshold Calculation modes. This query will filter the appropriate extractions from the database, so that just the data from the queried extractions is used in the calculation of the statistical summary values used to set the thresholds.

7 Edit the limits as appropriate. The text boxes accept floating point constants.

8 If necessary, enter or change the name for the metric set in the Metric Set Name area.

9 In the Edit Thresholds dialog box, click **Save** to add the chosen metric along with its limits to the selected metric-set.

If the metric is already present then it is updated with the new limits.

These components and parameters are described in "Metric Set Configuration Dialog Box: Add Metrics to Metric Set Tab" on page 54.

Threshold Example

To flag extractions with a high standard deviation of background subtracted signals:

1 Select the metric 'gNegCtrlSDevBGSubSig' from the Existing Metrics checklist to be associated the selected metric set.

2 In the Threshold Type area, select **Upper Limit**.

3 In the Threshold Calculation area, select **Standard** as the limit calculation type.

4 In the Calculations area, enter the value of 3 for the Upper Limit: Mean + SD* field (this is the SD multiplier value).

5 In the Metric Set Name area, enter the name for the metric set.

6 Click **Save**.

The statistics of the gNegCtrlSDevBGSubSig values of all extractions are calculated. For this example, calculations include the mean and the standard deviation. An upper limit is then set as mean + 3*SD, which is shown to be the value 61.1024 in Figure 7:

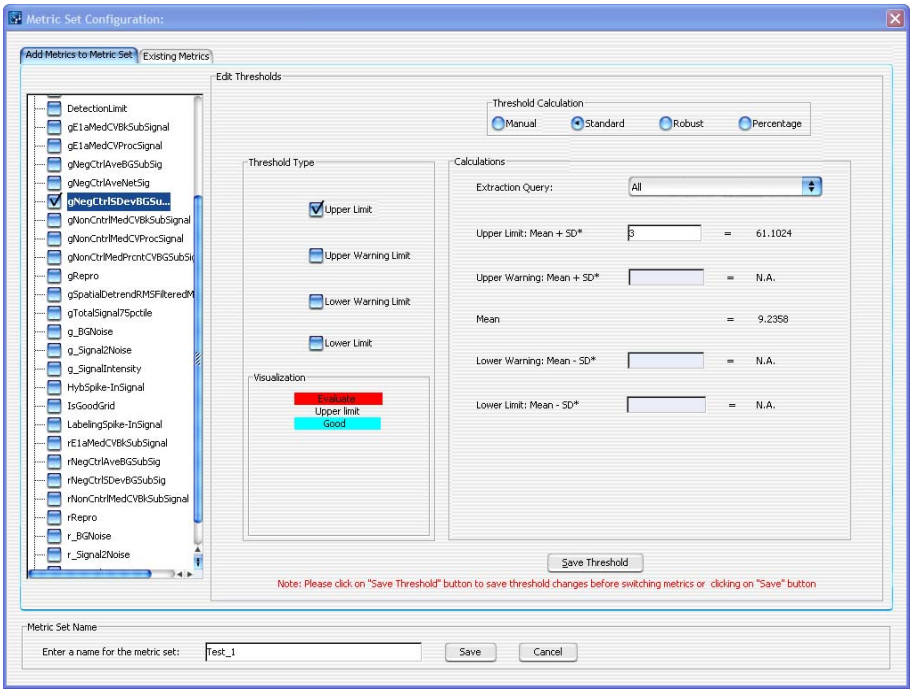

Figure 7 Metric Set Configuration dialog box with settings for the threshold Example

If you select the **Robust** type of calculation, then the median and IQR (inter-quartile range) is calculated. Using the IQR, the robust equivalent of SD is also calculated.

If you select **Percentage** type of calculation, then the percentiles that you choose are calculated for an upper limit (e.g. 99%), lower limit (e.g. 1%), or range (e.g. 99%, 1%).

To export metric sets

A Metric Set can be exported to an XML file and re-imported in Quality Tools or Feature Extraction.

- In the Quality tab, click **Metric Sets** and then click **Export**. The Export Metric Set dialog box appears. Browse to the location where you want to save the file, type a name for the file, and then click **Export**.

An example XML Metric Set file is shown below:

```
<MetricSetFile>
   <MetricSet
      Name = "GE1_QCMT_Oct08"
      isNotRemovable = "false"
      CreatedOn = "13-Mar-2009 15:47">
      <Metric
         Name = "DetectionLimit"
         StatisticType = "0"
         UpperLimitConst = "-10000.00000"
         UpperWarningLimitConst = "-10000.00000"
         LowerWarningLimitConst = "-10000.00000"
         LowerLimitConst = "-10000.00000"
         UpperLimitValue = "2.0"
         UpperWarningLimitValue = "-10000.00000"
         LowerWarningLimitValue = "-10000.00000"
         LowerLimitValue = "0.01"
         Owner = "dgd"
         CreatedOn = "13-Mar-2009 15:47"
         LastUpdated = "13-Mar-2009 15:47">
      </Metric>
   </MetricSet>
</MetricSetFile>
```

To import metric sets

You can import metric sets that have been exported, or that have been downloaded to your computer from the Agilent Web site at http://www.agilent.com/chem/feqcmetrics.

NOTE You can import metric sets from the Agilent Genomic QC Chart Tool program. Export the metric sets as described in the *Agilent QC Chart Tool v1.3 User Guide*. Then select the exported metric set file for import into Agilent Genomic Workbench 6.5 as described in Step 2 below.

1. From the Quality tab, click **Metric Sets** and then select **Import**. The Import Metric Set dialog box appears.
2. Browse to a location, select the metric set file, and then click **Open**.
3. If a warning appears that asks if you want to overwrite Threshold settings for the matching metrics, click **Yes**.

You can also edit metric sets, for example, add or remove thresholds, or edit thresholds.

You can also copy (using “Save As”) or remove Metric Sets. Once you have copied a metric set, you can assign different metrics, thresholds, etc.

To delete a metric set

1. In the Navigator, in the Metric Sets panel, select the metric set to be removed.
2. In the Quality tab, select **Metric Sets** and then click **Delete**. Alternatively, right-click the metric set in the Navigator and select **Delete Metric** set.

To remove a metric from a metric set

1 Open the Metric Set Configuration dialog box. See "To create a metric set" on page 26.

2 Select the **Add Metrics to Metric Set** tab.

3 De-select the metric(s) from the Existing Metrics checklist to be removed from the selected metric set.

4 Click **Save**.

To rename a metric set

1 Open the Metric Set Configuration dialog box. See "To create a metric set" on page 26.

2 Select the **Add Metrics to Metric Set** tab.

3 In the Metric Set Name area, enter a new name for the metric set.

4 Click **Save**.

Producing and Displaying Charts

A chart can be drawn on a metric set for a chosen query. The chart can be defined in the Chart Configuration dialog box. See "Chart Configuration Dialog Box" on page 49 for more information.

To create a new chart

1. In the Quality tab, click **Chart** and then select **New**. The Chart Configuration dialog box appears.
2. Select the metric set to be used for the chart from the Metric Set drop-down list.
3. Optional: select the Extraction Query from the drop-down list to be applied to the metric set.

   If the metric set already includes an extraction query as part of the threshold operations, then this extraction query may additionally reduce the extractions displayed in the chart.
4. Optional: in the Sort by Columns area, select any ordering of the metrics to be displayed in the chart.
5. Optional: Mark the **Color and shape by in/out of the threshold range** check box to set the color and shape of all points by whether the value is in or out of range.

   Otherwise, *clear* the **Color and shape by in/out of the threshold range** check box and set the color and shape for the chart as follows:

   - In the Color By area, select any color for one of the metrics to be displayed in the chart.
   - In the Shape By area, select any shape for one of the metrics to be displayed in the chart.
6. In the Chart Name area, enter a name for the chart.
7. Click **Save**.

   The chart can now be run. Charts can also be edited, renamed and deleted.

See Also

"To view the chart" on page 35
"To edit a chart" on page 35
"To delete a chart" on page 35
"To rename a chart" on page 36
"Chart Configuration Dialog Box" on page 49

To view the chart

1. In the Navigator, in the Charts panel, select the chart to view.
2. In the Quality tab, click **Chart** and then click **View**. Alternatively, double-click the chart in the Navigator.
3. The metric set is displayed in the Tab View.

   To return the Tab View to another metric set, chart, or query result, click the appropriate entity in the Navigator.

See Also

"Disassociate Metric" on page 47

To edit a chart

1. In the Navigator, in the Charts panel, select the chart to edit.
2. In the Quality tab, click **Chart** and then click **View**. Alternatively, right-click the chart in the Navigator and select **Edit Chart**. The Chart Configuration dialog box appears.
3. Configure the dialog box according to your needs. See "To create a new chart" on page 34.
4. If necessary, change the name of the chart in the Chart Name area.
5. Click **Save**.

To delete a chart

1. In the Navigator, in the Charts panel, select the chart to delete.
2. In the Quality tab, click **Chart** and then click **Delete**. Alternatively, right-click the chart in the Navigator and select **Delete Chart**.
3. When the Delete Chart dialog box appears, click **Yes**.

To rename a chart

1. In the Navigator, in the Charts panel, select the chart to rename.
2. In the Quality tab, click **Chart** and then click **Rename**. Alternatively, right-click the chart in the Navigator and select **Rename Chart**. The Enter New Name dialog box appears.
3. Enter the new name for the chart, then click **Ok**.

3 Quality Tools Reference

This chapter provides a description of each part of this application, including all of the dialog boxes that can appear when you click buttons and other elements.

The Quality Application Interface

You can open the Quality tools application in either of the following ways:

- Click the **Quality** tab in the Tab Bar from any of the Agilent Genomic Workbench 6.5 modules, *or*
- In the tab view, click the **Open Application** tab, then click on the **Quality Tools** icon to launch the Quality tools application.

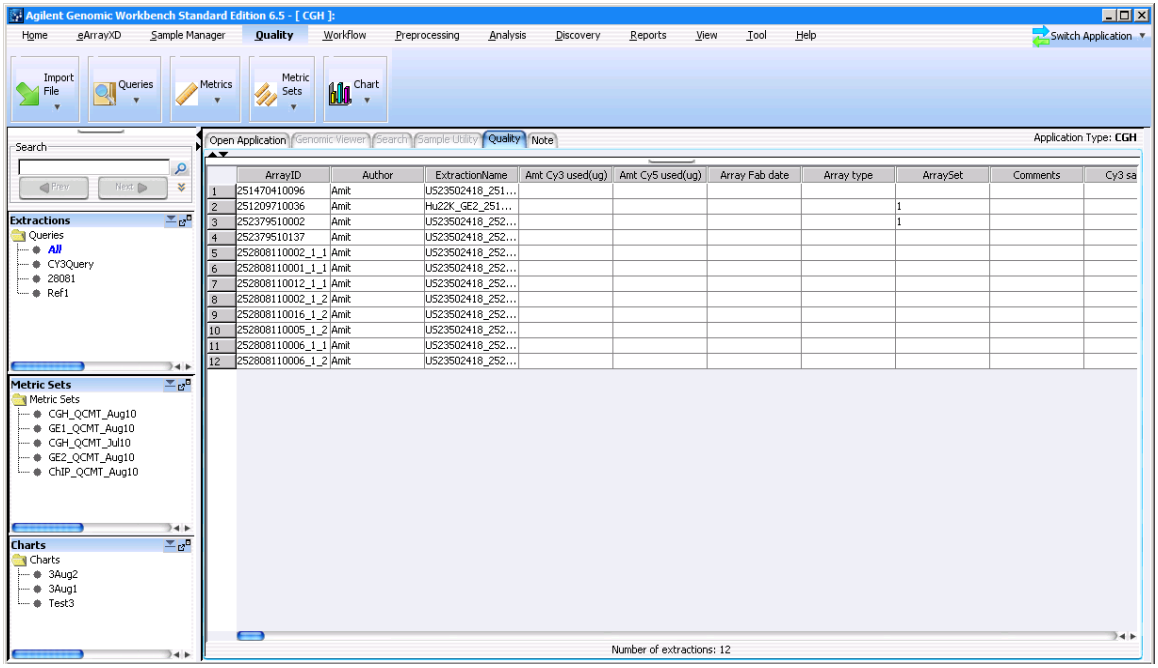

Figure 8 Quality tab

The Quality tools application is divided into the following functional areas.

- "Quality Tab Ribbon" on page 39
- "Navigator" on page 43
- "Tab View" on page 46 (Quality Tab)

Quality Tab Ribbon

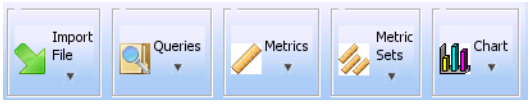

Figure 9 The Main Menu ribbon

The Main Menu ribbon has the following functions:

Import File (from Feature Extraction)

The Quality tools application uses microarray extractions imported into the Agilent Genomic Workbench 6.5 database to create meaningful metrics and thresholds. Extracted data files are imported from the following Agilent Genomic Workbench 6.5 applications: Workflow, CGH, ChIP, and CH3. See the User Guide for the selected application or the *Agilent Genomic Workbench 6.5 Data Viewing Guide* for information about how to import data files.

The following submenu options are available for Import File:

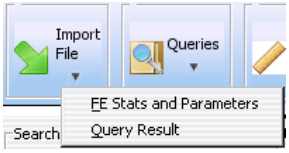

Figure 10 Import File submenu

FE Stats and Parameters Imports file output from Feature Extraction, specifically importing the Stats and Parameter table information. Refer to "Chapter 3" of the *Feature Extraction 10.10 Reference Guide* for a description of the statistics and parameters that are imported.

Query Results Imports a query previously exported using Quality tools.

Queries

The Agilent Genomic Workbench 6.5 database captures quality columns from microarray extractions. The Quality tools application lets you query using any combination of those quality columns.

The following submenu options are available for Queries:

Figure 11 Queries submenu

New Launches the Query Builder dialog box to create a new query.

Run Executes the selected query on the database and displays the extractions from that query in the Tab View.

Edit Opens the Query Builder dialog box to allow the query to be changed.

Delete Permanently removes the selected query from Agilent Genomic Workbench 6.5 Quality tools.

Rename Opens the Enter New Name dialog box to change the name of the selected query.

Export Opens the Export Query dialog box to save the results of a query to an external tab-delimited text file.

Metrics

Metrics can be defined as any combination of existing metrics and can be created and deleted as necessary.

The following submenu options are available for Metrics:

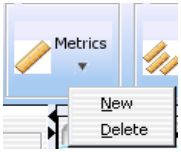

Figure 12 Metrics submenu

New Opens the Create a new Metric dialog box to allow definition of a new metric.

Delete Permanently removes the selected metric from Agilent Genomic Workbench 6.5 Quality tools.

Metric Sets

Metric Sets are a set of metrics with optional thresholds and can be created, deleted (except for the Agilent default Metric Set), imported or exported as necessary.

The following submenu options are available for Metric Sets:

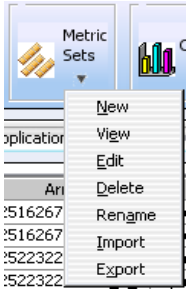

Figure 13 Metric Sets submenu

New Opens the Metric Set Configuration dialog box to allow the creation of a new Metric Set.

View Displays the currently selected metric set in tabular form in the tab view.

Edit Opens the Metric Set Configuration dialog box to allow you to change the selected metric set.

Delete Permanently removes the selected metric set from Agilent Genomic Workbench 6.5 Quality tools.

Rename Opens the Enter New Name dialog box to change the name of the selected metric set.

Import Opens the Import Metric Set dialog box to import a previously exported metric set.

Export Opens the Export Metric Set dialog box to export the selected metric set to a tab-delimited text file.

Chart

Chart functions graphical present the application of the query and metric functions and allow further segmentation of the data by color and shape coding.

The following submenu options are available for Chart:

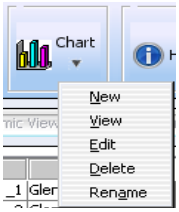

Figure 14 Chart submenu

New Opens the Chart Configuration dialog box to create a new visualization of the extraction metrics, metric sets, and thresholds.

View Opens the selected chart in the tab view.

Edit Opens the Chart Configuration dialog box so that you can change the chart.

Delete Permanently removes the selected chart from Agilent Genomic Workbench 6.5 Quality tools.

Rename Opens the Enter New Name dialog box to change the name of the selected chart.

Navigator

The Navigator consists of the **Extractions** pane, the **Metric Sets** pane, and the **Charts** panes. These panes are described below.

Extractions Pane

This pane displays a list of query views of extractions in the database. The query named "All" is a permanent, unchangeable query and consists of no query parameters; it displays all extractions in the database.

Right-click to see the following options in the Extractions pane:

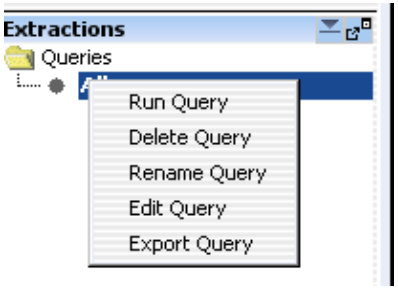

Figure 15 Extractions Navigator Options

Run Query Runs the selected query on the database and displays the extractions from that query in the Tab View.

Delete Query Permanently removes the selected query from Agilent Genomic Workbench 6.5 Quality tools.

Rename Query Opens the Enter New Name dialog box to change the name of the selected query.

Edit Query Opens the Query Builder dialog box, identical to clicking **Query** in the Quality tab and then selecting **Edit**.

Export Query Opens the Export Query dialog box, identical to clicking **Query** in the Quality tab and then selecting **Export**.

Metric Sets Pane

This pane displays a list of metric sets that were created in or imported into Quality tools.

Right-click to see the following options in the Metric Sets pane:

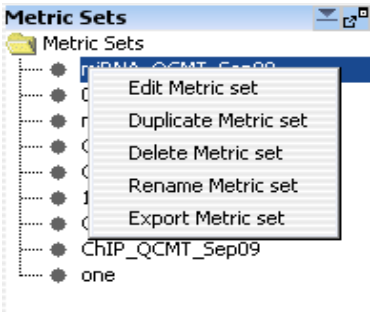

Figure 16 Metric Sets Navigator Options

Edit Metric set Opens the Metric Set Configuration dialog box, identical to clicking **Metric Sets** in the Quality tab and then selecting **Edit**.

Duplicate Metric set Opens the Duplicate Metric Set dialog box, which lets you enter a name for the metric set to be copied.

Delete Metric set Permanently removes the selected metric set from Agilent Genomic Workbench 6.5 Quality tools.

Rename Metric set Opens the Enter New Name dialog box to change the name of the selected metric set.

Export Metric set Opens the Export Metric Set dialog box, identical to clicking **Metric Sets** in the Quality tab and then selecting **Export**.

Charts Pane

This pane displays a list of charts that were created in or imported into Quality tools.

Right-click to see the following options in the Metric Sets pane:

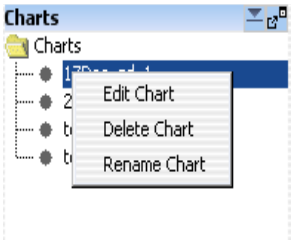

Figure 17 Metric Sets Navigator Options

Edit Chart Opens the Chart Configuration dialog box, identical to clicking **Chart** in the Quality tab and then selecting **Edit**.

Delete Chart Permanently removes the selected chart from Agilent Genomic Workbench 6.5 Quality tools.

Rename Chart Opens the Enter New Name dialog box to change the name of the selected chart.

Tab View

The tab view displays the results of a selection from any pane in the Navigator (Query, Metric set, or Chart) in the Navigator. Examples of each pane are shown below.

Tip When you click on a row heading in the table, the results are sorted by the values in that column.

Tab View - Extractions

The extractions loaded into the Agilent Genomic Workbench 6.5 database are displayed in the form of a grid-view. See the figure below:

	ArrayID	Author	ExtractionName	Amt Cy3 used(ug)	Amt Cy5 used(ug)	Array Fab date	Array type	ArraySet	Comment
1	252136510460_1_2	Ann	O1-NA18517_25...						
2	252136510460_1_2		O1-NA18517_25...					1	
3	252136510317_1_2		O2-NA18517_25...					1	
4	252136511006_1_2		S1-NA18517_252...					1	
5	252136511015_1_2		S2-NA18517_252...					1	
6	252136510356_1_2	Jay	alsc_NA19000-TA...						
7	252136510357_1_1	Jay	alsc_NA19000-TA...						

Figure 18 Tab View - Extractions

The default order of the column is:

- **ArrayID** (the barcode and pack number, if the image is a multi-pack array)
- **Author** (the user who is logged in to Genomic Workbench when the file is imported)
- **Extraction Name** (from FE parameters)
- **Array attributes**
- **FE parameters**
- **FE Stats**

To change the order of the columns in the table, drag the column headings to the desired positions.

Tab View - Metric Sets

The Metric Sets created in or imported into Quality tools are displayed in the form of a grid-view. See the figure below:

	Metric Name	Expression	Upper Limit	Upper Warning Limit	Lower Warning Limit	Lower Limit	Calculation Type	Defined by	Date Create
1	IsGoodGrid	IsGoodGrid	NA	NA	1.0	1.0	Manual		07-Jul-2009 18:
2	AddErrorEstimate...	AddErrorEstimate...	12.0	5.0	NA	NA	Manual		11-Aug-2009 18
3	AnyColorPrcntFe...	AnyColorPrcntFe...	15.0	8.0	NA	NA	Manual		11-Aug-2009 18
4	gNonCtrlMedPrcn...	gNonCtrlMedPrcn...	15.0	10.0	NA	0.0	Manual		11-Aug-2009 18
5	gTotalSignal75pctile	gTotalSignal75pctile	NA	NA	NA	NA	Manual		12-Aug-2009 14
6	LabelingSpike-InS...	0.5 * (gdmr285G...	NA	NA	NA	2.5	Manual		12-Aug-2009 14
7	HybSpike-InSignal	0.5 * (gdmr3Gen...	NA	NA	NA	2.5	Manual		12-Aug-2009 14
8	StringencySpike-I...	gdmr3ProbeRatio	NA	NA	NA	NA	Manual		12-Aug-2009 14

Figure 19 Tab View - Metric Sets

Disassociate Metric A button on the far right that lets you quickly remove a metric from a custom Metric Set.

Tab View - Charts

This pane has both a table and graphical view of the data. In the graphical view, you can zoom in on a chart by using the mouse to drag and release on the area to zoom. Double-click the chart to return to normal view.

The charts Tab View supports the operations shown in the figure below:

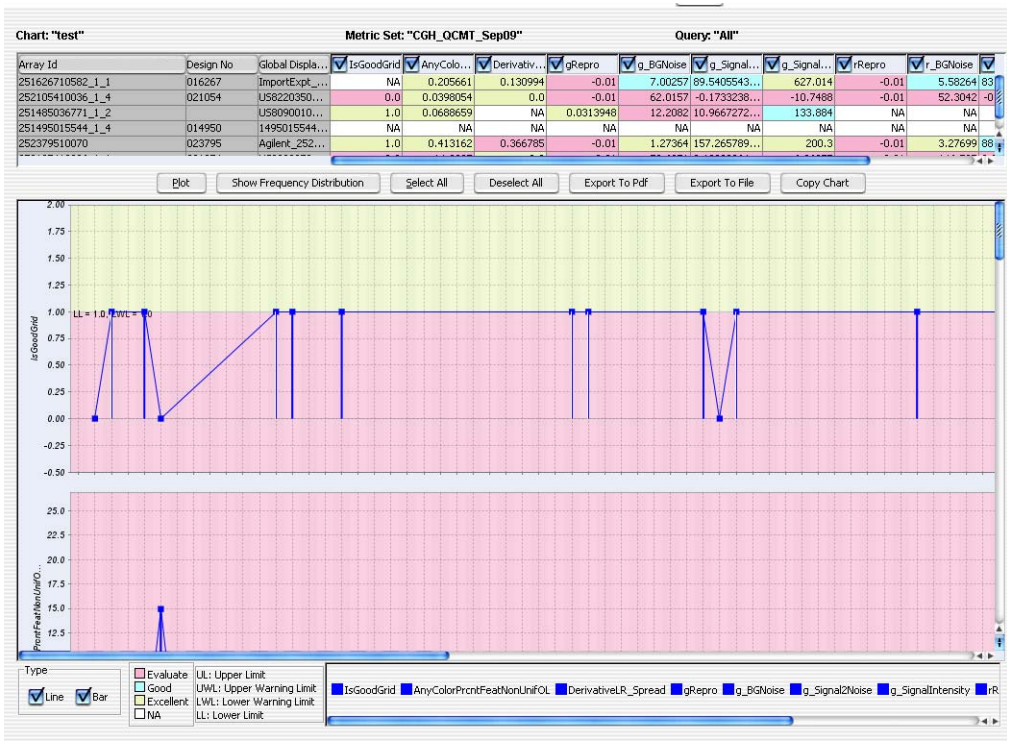

Figure 20 Tab View - Charts

Plot Draws the chart according to the data selected in the table view.

Show Frequency Distribution Displays a binned vertical bar chart (a histogram) of each metric selected in the table view.

Select All/Deselect All Selects all or none of the available metrics to include in the chart.

Export to PDF Saves the chart in PDF format.

Export to File Exports the data from the chart to a tab-delimited text file.

Copy Chart Copies the chart to the Clipboard as a bitmap that can be pasted in MS Word and MS Paint, or in any other appropriate software.

Dialog Boxes

You may encounter the following dialog boxes when using Quality tools. This section describes the components of the dialog boxes and the functions of each component. The dialog boxes are shown in alphabetical order by title.

Chart Configuration Dialog Box

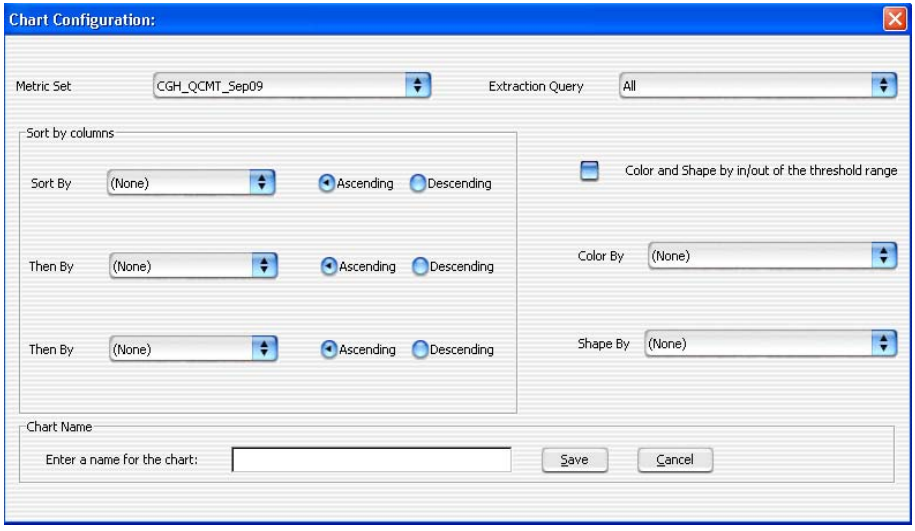

Figure 21 Chart Configuration dialog box

The Chart Configuration dialog box has the following components and functionality:

Metric Set

Select the metric set to be used with the chart.

Extraction Query

Select the extraction query that is to be evaluated to produce a chart.

Sort by Columns

Select how you want to order the extractions in the chart. You can select three criteria, and select whether to sort them in Ascending or Descending order.

Color and shape by in/out of the threshold range

When this check box is marked, the color and shape of all points are set by whether the value is in or out of range. The Color By and Shape By settings are ignored.

Color By

Indicates whether to color-code extraction data points depending on whether they fall inside or outside the threshold level. Points that are outside the limits are color-coded in red and the ones within the limits are color-coded in blue. All the points are connected by a *light-gray* line. The ShapeBy and ColorBy columns are disabled if this check box is marked.

Shape By

Indicates what groups are used to differentiate the data points by shape. For example, selecting Username causes all the extractions that are from a particular user to have the same shape.

Chart Name

Lets you enter a name to be associated with the chart.

Save button Saves the chart using the name you entered.

Cancel button Closes the Chart Configuration dialog box without saving any changes.

Create a new Metric Dialog Box

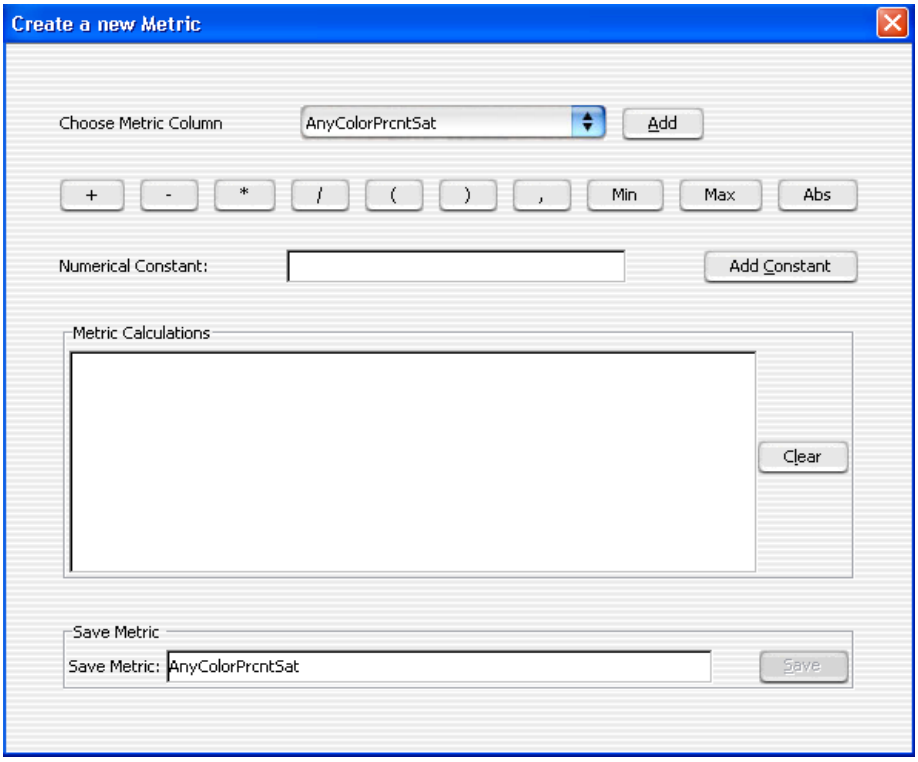

Figure 22 Create a new Metric dialog box

The Create a new Metric dialog box has the following components and functionality:

Choose Metric Column

A list of metrics that can be used to create a calculation as a new metric.

Add button Selects the chosen metric and adds it to the Metric Calculations text area for review.

Operations

The following mathematical operators are available for any metric or collection of metrics that are selected using the Choose Metric Column.

- Precedence of operations is left to right, except when interrupted by parentheses.
- Operations proceed left to right inside any set of parentheses, and inside out in terms of stacked parentheses.

+ Adds any two metrics or collection of metrics grouped by matched parentheses.

- Subtracts any two metrics or collection of metrics grouped by matched parentheses.

***** Multiplies any two metrics or collection of metrics grouped by matched parentheses.

/ Divides any two metrics or collection of metrics grouped by matched parentheses.

( ) Let you subset and prioritize the mathematical operations.

, Lets you list any two metrics or collection of metrics grouped by parenthetical operators for the evaluative operations listed below.

Min Returns the smallest value from a list of metrics (or collection of metrics grouped by matching parenthesis). The list elements are separated by the ',' operator.

Max Returns the largest value from a list of metrics (or collection of metrics grouped by matching parenthesis). The list elements are separated by the ',' operator.

Abs Returns the absolute value of a metric or a collection of metrics grouped by matching parenthesis.

Numerical Constant

Lets you enter a value to be added to the metric calculation formula.

Add Constant button Accepts the value entered in the Numerical Constant field and adds it to the metric calculation formula.

Metric Calculations

The area in which the metric calculation formula is displayed for review.

Clear button Removes all metrics, mathematical operators, and constants from the formula in the Metric Calculations area.

Save Metric

Lets you type a name to be associated with the metric.

Save button

Saves the metric using the name you entered.

Metric Set Configuration Dialog Box: Add Metrics to Metric Set Tab

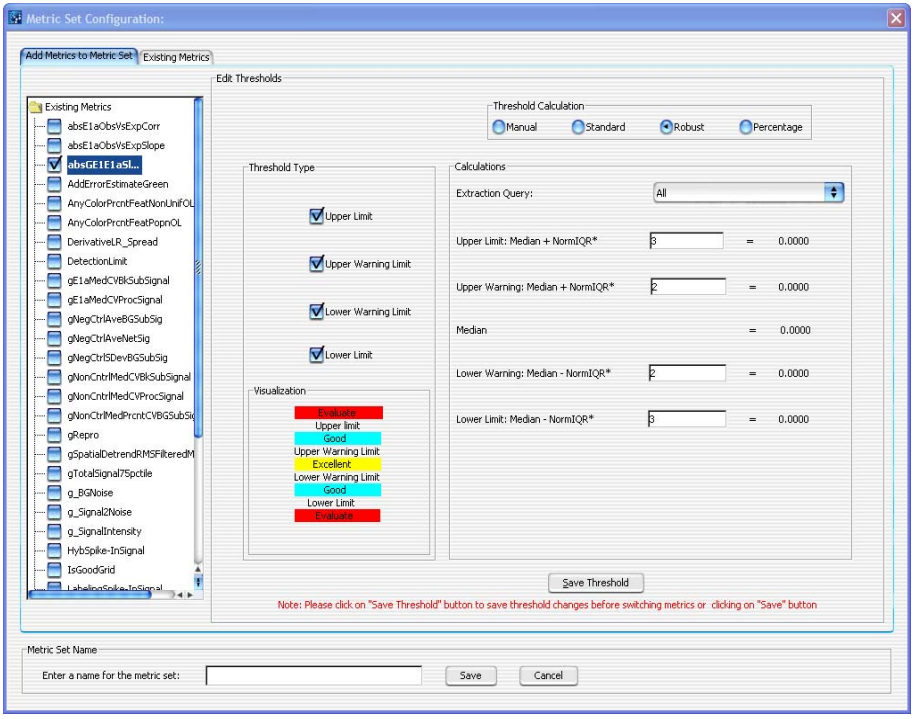

Figure 23 Metric Set Configuration dialog box - Add Metrics to Metric Set tab

The Add Metrics to Metric Set tab of the Metric Set Configuration dialog box has the following components and functionality:

Existing Metrics

Displays a list of the metric(s) that can be used in the metric set.

Threshold Type

The following fields are threshold types, which are used to select which threshold(s) to apply and display.

Upper Limit Sets a limit where extraction values for the appropriate metric that are greater than the limit calculation are displayed in the color red and flagged as “Evaluate”. Extractions with metric values lower than this limit are displayed in the color blue and flagged as “Good”, unless there are additional limits selected that may further separate the extractions.

Upper Warning Limit Sets a limit where extraction values for the appropriate metric that are greater than the limit calculation are displayed in the color blue and flagged as “Good”. Extractions with metric values lower than this limit are displayed in the color yellow and flagged as “Excellent”, unless there are additional limits selected that may further separate the extractions.

Lower Warning Limit Sets a limit where extraction values for the appropriate metric that are less than the limit calculation are displayed in the color blue and flagged as “Good”. Extractions with metric values higher than this limit are displayed in the color yellow and flagged as “Excellent”, unless there are additional limits selected that may further separate the extractions.

LowerLimit Sets a limit where extraction values for the appropriate metric that are less than the limit calculation are displayed in the color red and flagged as “Evaluate”. Extractions with metric values higher than this limit are displayed in the color blue and flagged as “Good”, unless there are additional limits selected that may further separate the extractions.

Threshold Calculation

Manual Applies a constant value for Upper Limit, Upper Warning Limit, Lower Warning Limit, and LowerLimit.

Standard Takes a constant value as the number of standard deviations of the data beyond the mean to calculate the limit. For detailed information, see “Standard Threshold Calculations” on page 56.

Robust Takes a constant value as the number of inter-quartile ranges (IQR) of the data beyond the mean to calculate the limit. For detailed information, see “Robust Threshold Calculations” on page 56.

Percentage Takes a percentage range of the data to calculate the limit. For detailed information, see "Percentage Threshold Calculations" on page 57.

Extraction Query

Optional: Lets you assign an Extraction Query to the metric set for Standard, Robust, and Percentage Threshold Calculation modes. This query will filter the appropriate extractions from the database, so that just the data from the queried extractions is used in the calculation of the statistical summary values used to set the thresholds.

Standard Threshold Calculations

Take a constant value as the number of standard deviations of the data beyond the mean to calculate the limits.

Upper Limit Lets you define a multiplier for the number of standard deviations to be added to the mean to create the Upper Limit. For example, to apply an upper limit of 2 standard deviations, enter the number 2 in the text field. The Upper Limit is defined as the mean + constant*SD.

Upper Warning Limit Lets you define a multiplier for the number of standard deviations to be added to the mean to create the Upper Warning Limit. The Upper Warning Limit is defined as the mean + constant*SD.

Lower Warning Limit Lets you define a multiplier for the number of standard deviations to be added to the mean to create the Lower Warning Limit. The Lower Warning Limit is defined as the mean - constant*SD.

LowerLimit Lets you define a multiplier for the number of standard deviations to be added to the mean to create the Lower Limit. The Lower Limit is defined as the mean - constant*SD.

Robust Threshold Calculations

Take a constant value as the number of inter-quartile ranges (IQR) of the data beyond the mean to calculate the limits.

Upper Limit Lets you define a multiplier for the number of IQRs to be added to the mean to create the Upper Limit. For example, to apply an upper limit of 2 IQR, enter the number 2 in the text field. The Upper Limit is defined as the mean + constant*IQR.

Upper Warning Limit Lets you define a multiplier for the number of IQRs to be added to the mean to create the Upper Warning Limit. The Upper Warning Limit is defined as the mean + constant*IQR.

Lower Warning Limit Lets you define a multiplier for the number of IQRs to be added to the mean to create the Lower Warning Limit. The Lower Warning Limit is defined as the mean - constant*IQR.

LowerLimit Lets you define a multiplier for the number of IQRs to be added to the mean to create the Lower Limit. The Lower Limit is defined as the mean - constant*IQR.

Percentage Threshold Calculations

Take a percentage range of the data to calculate the limits.

Upper Limit Lets you define a multiplier for the percentage from the bottommost values of the data to calculate the Upper Limit. For example, to set an Upper Limit that is equal to the best 1% of the extractions for a particular metric, set the Upper Limit text box to 99%.

Upper Warning Limit Lets you define a multiplier for the percentage from the bottommost values of the data to calculate the Upper Warning Limit. For example, to set an Upper Warning Limit that is equal to the best 25% of the extractions for a particular metric, set the Upper Warning Limit text box to 75%.

Lower Warning Limit Lets you define a multiplier for the percentage from the bottommost values of the data to calculate the Lower Warning Limit. For example, to set an Lower Warning Limit that is equal to the lower 25% of the extractions for a particular metric, set the Lower Warning Limit text box to 25%.

Lower Limit Lets you define a multiplier for the percentage from the bottommost values of the data to calculate the Lower Limit. For example, to set an Lower Limit that is equal to the lower 1% of the extractions for a particular metric, set the Lower Limit text box to 1%.

Save Threshold button

Saves the threshold settings for the selected metric(s). These metric(s) with their associated thresholds become available in the "Metric Set Configuration Dialog Box: Existing Metrics Tab" on page 59.

Metric Set Name

Lets you enter a name for the new metric set.

Save button Saves the metric set.

Cancel button Closes the Metric Set Configuration dialog box without saving any changes.

Metric Set Configuration Dialog Box: Existing Metrics Tab

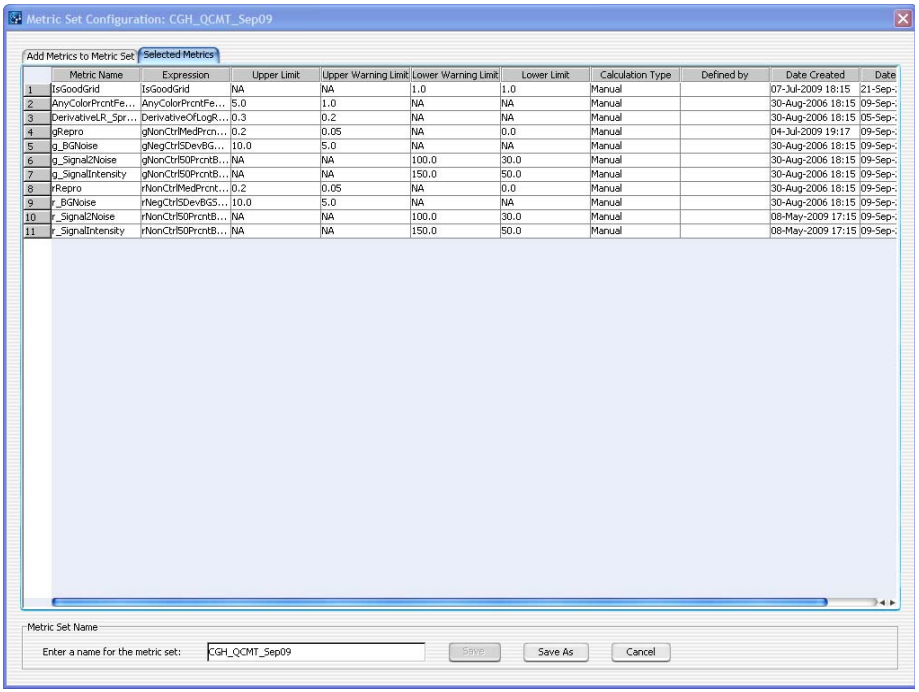

Figure 24 Metric Set Configuration dialog box - Existing Metrics tab.

The Existing Metrics tab of the Metric Set Configuration dialog box has the following components and functionality:

Table

The Metric Sets created in or imported into Quality tools are displayed in the form of a table, or grid-view.

The column-headers appear in this order:

- Metric Name
- Expression
- Upper Limit

- Upper Warning Limit
- Lower Warning Limit
- Lower Limit
- Calculation Type
- Defined By
- Date Created
- Date Modified

Metric Set Name Lets you enter a name to save the metric set.

Save Saves the existing metric set with the previously defined name.

Save As Saves the metric set with a newly defined name.

Cancel Closes the Metric Set Configuration dialog box without saving any changes.

Query Builder Dialog Box

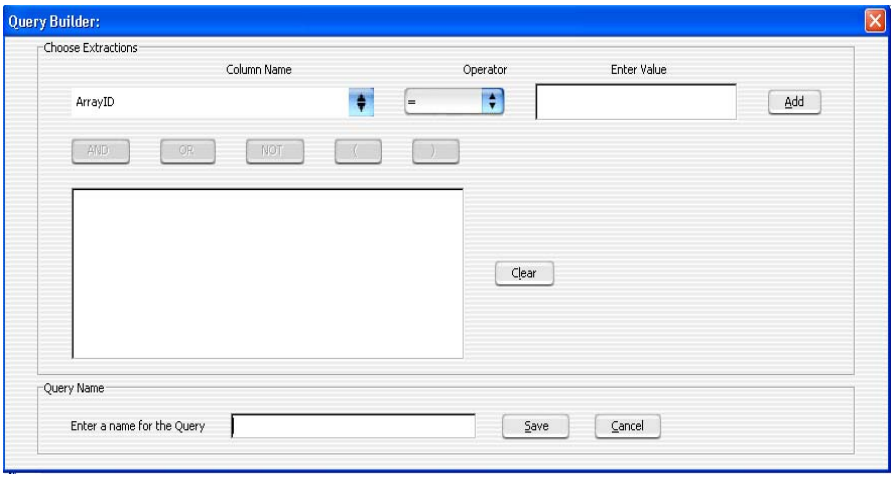

Figure 25 The Query Builder dialog box

The Query Builder dialog box has the following components and functionality:

Column Name

Displays a list of the metrics that can be used to create an expression with which to query the database.

Operator

Displays a list of the relational operators that can be used with the selected metric.

Enter Value

A text area where a value can be compared to the metric for each extraction. If the chosen relation between the metric and value is valid, the query will produce extractions that pass that criteria (for which the relation between the metric and value is true).

Operations

The following buttons are logical operations used to link two or more metric-value relations built using the functions listed above. Each logical operations can link two metric-value relations at a time.

AND Produces a complex query which is true only if *both* metric-value relations are true.

OR Produces a complex query which is true if *either* metric-value relations are true.

NOT Produces a complex query which is true only if both metric-value relations are *not* true.

( ) Let you subset and prioritize the complex query.

Text area

The area in which complex relations using the logical operations are listed for review.

Query Name

A text area for entering a name under which to save the query.

Save button

Saves the query using the name specified in the Query Name field.

Cancel button

Cancels all query operations and closes the Query Builder dialog box.

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In This Book

This book contains information to set up and use the Quality tools application with Agilent Genomic Workbench 6.5.

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