Notices

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Software Revision
This guide is valid for 3.5 and later revisions of the Agilent QC Chart Tool software, until superseded.

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In This Guide...

This guide describes how use the Agilent QC Chart Tool software to query, filter, and evaluate microarray extractions. It also describes how to visualize current and historical batch microarray extraction processes.

1 Getting Started

This chapter gives an overview of how to use the software to evaluate microarray extraction quality. It gives instructions for how to do common tasks.

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.

3 QC Chart Tool Reference

This chapter describes the parts of the Agilent QC Chart Tool main window that you use to query and evaluate microarray extractions. It also describes any dialog boxes that appear during quality evaluation of the extractions.
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Getting Started

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

Agilent QC Chart Tool is installed when you install the Agilent Feature Extraction software. It analyzes summary statistics from Feature Extraction output files in order to monitor microarray processing performance.

The data is kept in the shared Feature Extraction database, that you can query. Queries enable you to search this data. You can save the queries to allow the display of specific subsets of the data. For instance, queries can select the data found in specific designs, batches, or dates of processing. Using the data, you can create metrics that monitor aspects of the microarray processing workflow.

Additionally, you can create metric sets that combine metrics, and you can set thresholds for metrics within a metric set. Agilent includes default metric sets for each microarray type in the database. These metric sets were optimized for the workflow using the Agilent microarray scanner, Feature Extraction default protocols, and Agilent laboratory protocols. You can change and save a default metric set using a new name, to create one or more custom metric sets; for example, to optimize the thresholds for your custom protocols.

With QC Chart Tool, you can select a query to define which extractions to view and select a metric set to define which metrics to view. You 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.

The QC Chart Tool software is designed for use in a production environment where:

- Microarray processing protocols are standardized and you want to examine the effect of variables on performance 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, go to the website: www.genomics.agilent.com/article.jsp?pageId=2040.

Example use cases

Feature Extraction generates output to assist in quality assessment. A table of array-wide, or global statistics (the “Stats” table in FE), is 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 are sometimes cumbersome without a tool for quality assessment of each extraction. QC Chart Tool captures key global statistics to use as metrics and create graphs for easy visual assessment of metrics. Additionally, you can use the metrics that are used for analysis for specific monitoring requirements.

This section provides several common use cases for the software. Although these examples are from an earlier version of the 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 QC Chart Tool software 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 were 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 23.
By default, the chart generated by QC Chart Tool shows extractions in the order they were performed. Because QC Chart Tool has customized sorting, color-by, and shape-by attributes, it is a powerful tool for visualizing and highlighting trends in patterns.

**Figure 1** 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*.
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 QC Chart Tool:

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 18.

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 25.

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 User Guide.
Use Case 3  Analysis of the effect of changing the FE parameter protocols

Analysis of Extraction statistics used for metrics with the QC Chart Tool software 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. It was then processed using the standard metric set in QC Chart Tool.

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. It is 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 User Guide.

**Figure 3**  Comparison of the effect of FE parameter protocols on extraction quality
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Building Queries, Metric Sets, and Charts to Evaluate Extractions

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This chapter provides a list of how-to instructions for the tasks available in the QC Chart Tool software. Most functionality in the QC Chart Tool is accessible from either the command ribbon or from the object under consideration in the Navigator.

For a description of each part of this software, including all of the dialog boxes that can appear when you click buttons and other elements, see Chapter 3, “QC Chart Tool Reference”.
Importing Data

This chapter describes how to import data into QC Chart Tool. After running an extraction in Feature Extraction, you can import quality information from the extraction manually using the Import function in QC Chart Tool 3.5.

To import FE statistics and parameter information

1. On the command ribbon, click **Import** and then click **FE Stats and Parameters**. The Import FE Files dialog box appears.
2. Navigate to the Feature Extraction output files.
3. Click **Open**.
   The Agilent Feature Extraction Importer dialog box appears with the list of selected microarrays.
4. For microarrays that are dye flipped, under Dye Flip, click the arrow and select **Flipped**. Otherwise, leave the selection **Normal**.
5. Click **OK**.
   The FE quality information is added to the available extractions that you can query and evaluate.

**NOTE**
If you import an extraction whose ArrayID (barcode) and ExtractionName are the same as an extraction in the database, the program prompts you to rename the duplicate extraction, or ignore it and import the remaining extractions.

If you import an extraction that has the same Array ID (barcode) as another extraction in the database, you are notified there are duplicate extractions for that barcode. The extraction is imported as a new row. This can occur if you perform a second feature extraction on the same image file, or if you washed and reused a microarray.

**See Also**
Refer to “Chapter 3” of the *Feature Extraction Reference Guide* for a description of the statistics and parameters that are imported.
To import a query result

1. On the command ribbon, click **Import** and then click **Query Result**. The Import Query Result dialog box appears.

2. Navigate to a previously exported query result file and click **Open**. The program imports the extractions from the query result file. Each extraction in the file appears as a row in the extraction table.

**NOTE**
If there is an extraction in the table that matches an extraction in the query result file, the extraction row in the extraction table is updated. The existing attribute values in the extraction table for that row are overwritten with the values from the file. The values of any attributes that are not present in the query result file will not be updated in the extraction table.

To remove an extraction

1. In the extractions table, select the extraction to remove.

   OR

   To select more than one extraction to remove,

   • Hold down the Ctrl key and then click noncontiguous extractions to remove.

   • Hold down the Ctrl key and then click the first row and then the last row for contiguous extractions to remove.

2. Right click and select **Delete**. A Confirmation dialog box appears.

3. Click **Yes**.
Building and Running Queries

QC Chart Tool has a Query builder where you can create a query for a subset of the extractions from the Agilent QC Chart Tool 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 54 for information about using processing attributes. See “Example use cases” 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, on the command ribbon, 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 text box on the right, select the value with which to compare the value of the Column Name parameter.
5. Click Add.
6. In the Query Name area, type a name for the query.
7. Click Save.

You can now use the query to develop thresholds for metrics or to define a Quality chart. You can run the query, and export and save the results. You can also rename and delete queries.
To create a composite query

1. Create a basic query as described in “To create a query” on page 18.
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 4 on page 20.
6. Click **Save**.

These components and attributes are described in “Query Builder dialog box” on page 66.

Figure 4 shows an example of a query that finds all 1-color gene expression extractions.
To edit a query

1. In the Navigator, under **Queries** in the Extractions pane, select the query to edit.
2. On the command ribbon, 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. Click **Clear** to remove all query conditions.
4. Click **Save** to save the query with the existing name. OR
   Click **Save As** to type a new name for the query and save it.

---

**Figure 4** Query Builder showing a composite query.
To run a query

1 In the Navigator, under Queries in the Extractions pane, select the query to run.
2 On the command ribbon, 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, under Queries in the Extractions pane, select the query to delete.
2 On the command ribbon, 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.

NOTE
If a query is used in a chart, you cannot delete the query.

To rename a query

1 In the Navigator, under Queries in the Extractions pane, select the query to rename.
2 On the command ribbon, 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, type the new name for the query.
4 Click OK.
To import a query result

1. On the command ribbon, click **Import** 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 result

1. In the Navigator, in the Extractions pane, under Queries, select the query to export.
2. On the command ribbon, 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, type 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 are associated in a metric set.

To create a new metric

1. On the command ribbon, click Metrics and then click New. The Create a new Metric dialog box opens.
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 Metric Name text box, type a name to save the new metric.
5. Click Save.

The new metric is saved to the database.

See Also

These components and attributes are described in “Create a new Metric dialog box” on page 55.

Example

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

To create this metric:
1. Click New from the Metric area of the command ribbon 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. Save the metric as Abs_eQCSlope.
To delete a metric

1. On the command ribbon, click **Metrics** and then click **Delete**.

The Delete Metrics dialog box appears. Metrics that can be removed from the database appear in the table. Default metrics cannot be removed from the database.

2. Select one or more metrics you want to delete.

3. Click **Delete**.

If the delete button is gray, the metric is associated with one or more metric sets. You must 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. You can save and export metric sets for future use.

To create a metric set

1. On the command ribbon, 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 metrics from the Existing Metrics checklist that you want to associate with the selected metric set.
4. Set the appropriate thresholds for the Metric. See “To set metric thresholds in metric sets” on page 27.
5. Optional: assign an Extraction Query to the metric set. This option is available in Standard, Robust, and Percentage Threshold Calculation modes. An Extraction Query filters the appropriate extractions from the database. Therefore, just the data from the queried extractions are used in the calculation of the statistical summary values used to set the thresholds.
6. In the Metric Set Name area, type the name for the Metric Set.
7. Click Save.

You can now display, export, and save the Metric Set. You can also delete and rename Metric Sets.

Tip You can also create a 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 26.

See Also

“To view a metric set” on page 26
“To set metric thresholds in metric sets” on page 27
“To export metric sets” on page 30
“To import metric sets” on page 31
To edit a metric set

1. Select the Metric Set of interest in the Metric Sets pane of the Navigator.
2. Click Metric Sets on the command ribbon and click Edit.
3. When the Metric Set Configuration dialog box appears, select the Add Metrics to Metric Set tab.
4. Select or clear the metrics from the Existing Metrics checklist to add or remove from the selected metric set.
5. Set the appropriate thresholds for the Metric. See “To set metric thresholds in metric sets” on page 27.
6. Optional: assign an Extraction Query to the metric set. This option is available in Standard, Robust, and Percentage Threshold Calculation modes. An Extraction Query filters the appropriate extractions from the database. Therefore, just the data from the queried extractions are 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. Typing a new name creates 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. On the command ribbon, click Metric Sets and then click View. Alternatively, double-click the metric set in the Navigator.
3. The metric set is displayed in the window.
To set metric thresholds in metric sets

You can associate a threshold with a given metric 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 25 or “To edit a metric set” on page 26.

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

3. Select the metric from the Existing Metrics checklist.

4. In the Threshold Type area, select the type of threshold to associate 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. An Extraction Query filters the appropriate extractions from the database. Therefore, just the data from the queried extractions are 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, type or change the name for the metric set in the Metric Set Name area.

9. In the Edit Thresholds dialog box, click **Save Threshold** to save the threshold values for the selected metric.

10. In the Metric Set Name area at the bottom of the dialog box, click **Save**. The chosen metric is saved in the metric set, along with its limits.
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 60.

**Threshold example**

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

1. Select the metric 'gNegCtrlISDevBGSubSig' from the Existing Metrics checklist to associate to 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 number is the SD multiplier value).
5. Click **Save Threshold**.
6. In the Metric Set Name area, enter the name for the metric set.
7. Click **Save**.

The statistics of the gNegCtrlISDevBGSubSig 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 + SD* 3, which is shown to be the value 5.6552 in **Figure 5**.
If you select the **Robust** type of calculation, then the median and IQR (interquartile 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 (for example, 99%), lower limit (for example, 1%), or range (for example, 99%, 1%).
To export metric sets

You can manually export a metric set as an XML file and reimport it in QC Chart Tool.

- On the command ribbon, 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**.

The following is an example of an XML Metric Set file:

```xml
<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 were exported, or that were downloaded to your computer from the Agilent website at www.genomics.agilent.com/article.jsp?pageId=2040.

1 From the command ribbon, click Metric Sets and then select Import. The Import Metric Set dialog box appears.

1 Browse to a location, select the metric set file, and then click Open.

2 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, and thresholds.

To delete a metric set

1 In the Metric Sets panel of the Navigator, select the metric set to remove.

2 On the command ribbon, select Metric Sets and then click Delete. Alternatively, right-click the metric set in the Navigator and select Delete Metric set.

NOTE You cannot delete a metric set that is used in a chart, and you cannot delete any of the default metric sets.

To remove a metric from a metric set

1 Open the Metric Set Configuration dialog box. See “To create a metric set” on page 25.

2 Select the Add Metrics to Metric Set tab.

3 Clear the box next to the metrics in the Existing Metrics list you want removed from the selected metric set.
To rename a metric set

1 In the Metric Sets pane of the Navigator, right-click a metric set and then select **Rename**.

2 In the Enter new name dialog box, type the new name for the metric set.

3 Click **Ok**.

**OR**

1 Open the Metric Set Configuration dialog box. See “To create a metric set” on page 25.

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**.

**NOTE**

You cannot rename a default metric set.

You cannot change any of the default metric sets. To change a default metric set, save it using a new name.
Producing and Displaying Charts

You can chart a metric set for a chosen query. The chart is defined in the Chart Configuration dialog box. See “Chart Configuration dialog box” on page 54 for more information.

To create a chart

1. On the command ribbon, click Chart and then select New. The Chart Configuration dialog box appears.
2. Select the metric set use for the chart from the Metric Set drop-down list.
3. Optional: select the Extraction Query from the drop-down list to apply to the metric set.
   If the metric set already includes an extraction query as part of the threshold operations, then this extraction query can further 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: Select the Color and shape by in/out of the threshold range check box. This selection sets 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.
To view the chart

1 In the Navigator, in the Charts panel, select the chart to view.
2 In the command ribbon, click Chart and then click View. Alternatively, double-click the chart in the Navigator.
3 The selected chart is displayed.
   To return the view to another metric set, chart, or query result, click the appropriate entity in the Navigator.

See Also “Disassociate Metric” on page 48

To edit a chart

1 In the Navigator, in the Charts panel, select the chart to edit.
2 In the command ribbon, click Chart and then click Edit. 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 chart” on page 33.
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 command ribbon, 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 command ribbon, 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.
2  Building Queries, Metric Sets, and Charts to Evaluate Extractions

To rename a chart
This chapter provides a description of each part of this software, including all of the dialog boxes that appear when you click buttons and other elements.
The QC Chart Tool software is divided into the following functional areas.

- "Command ribbon" on page 39
- "Navigator" on page 43
- "Data window" on page 47
Command ribbon

The command ribbon has the following functions:

**Import**

The QC Chart Tool software uses microarray extractions imported into the Agilent QC Chart Tool database to create meaningful metrics and thresholds.

The following submenu options are available for Import:

**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 Reference Guide* for a description of the statistics and parameters that are imported.

**Query Results** Imports results of a query previously exported using the QC Chart Tool software.
Queries

The Agilent QC Chart Tool database captures quality columns from microarray extractions. The software lets you query using any combination of those quality columns.

The following submenu options are available for Queries:

- **New** Launches the Query Builder dialog box to create a query.
- **Run** Executes the selected query on the database and displays the extractions from that query in the window.
- **Edit** Opens the Query Builder dialog box to allow the query to be changed.
- **Delete** Permanently removes the selected query from Agilent QC Chart Tool software.
- **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 are defined as any combination of existing metrics. They are created and deleted as necessary.

The following submenu options are available for Metrics:
New
Opens the Create a new Metric dialog box to allow definition of a new metric.

Delete
Permanently removes the selected metric from Agilent QC Chart Tool.

Metric Sets

Metric Sets are a set of metrics with optional thresholds. You can create, delete, import, or export metric sets as necessary. You cannot delete the default Agilent metric sets.

The following submenu options are available for Metric Sets:

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

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

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 QC Chart Tool.
3 QC Chart Tool Reference

Command ribbon

**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.

**Charts**

Chart functions graphically present the software 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 Charts:

![Charts submenu](image)

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

**View** Opens the selected chart.

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

**Delete** Permanently removes the selected chart from Agilent QC Chart Tool.

**Rename** Opens the Enter New Name dialog box to change the name of the selected chart.
The Navigator consists of the Search pane, the Extractions pane, the Metric Sets pane, and the Charts panes. These panes are described in this section.

**Search pane**

The Search pane lets you find all occurrences of an entire name or specific search string in the Navigator. It also contains several buttons that you can use to move, hide, show, or resize the Navigator.

![Navigator Search pane](image)

<table>
<thead>
<tr>
<th><strong>Resize buttons</strong></th>
<th>Click to hide, show, or expand the Navigator.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Search term box</strong></td>
<td>The place where you type your desired search term. Search terms are not case-sensitive, but they must reflect the entire name of an array or other content item that you want to find. You can use asterisks (*) as wildcards to represent groups of unspecified characters. For example, a search term <em>25887</em> searches for any content that contains the string “25887”.</td>
</tr>
<tr>
<td><strong>Pane list</strong></td>
<td>Lets you limit a search to a specific pane. Select the name of the desired pane from the list. To select all panes, select <strong>All Panels</strong>. By default, the software searches all panes.</td>
</tr>
</tbody>
</table>

**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:

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add New Query</td>
<td>Opens the Query Builder dialog box, where you define and save a new Quality query. See “Query Builder dialog box” on page 66.</td>
</tr>
<tr>
<td>Import Query</td>
<td>Opens the Import Query Result dialog box, where you browse to and select a query file to import.</td>
</tr>
<tr>
<td>Run Query</td>
<td>Runs the selected query on the database and displays the extractions from that query in the data window.</td>
</tr>
<tr>
<td>Delete Query</td>
<td>Permanently removes the selected query.</td>
</tr>
<tr>
<td>Rename Query</td>
<td>Opens the Enter New Name dialog box to change the name of the selected query.</td>
</tr>
<tr>
<td>Edit Query</td>
<td>Opens the Query Builder dialog box, identical to clicking Query in the command ribbon and then selecting Edit.</td>
</tr>
<tr>
<td>Export Query</td>
<td>Opens the Export Query dialog box, identical to clicking Query in the command ribbon and then selecting Export.</td>
</tr>
</tbody>
</table>

**Metric Sets Pane**

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

Right-click to see the following options in the Metric Sets pane:
<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add New Metric set</td>
<td>Opens the Metric Set Configuration dialog box, where you define and save a new metric set. See “Metric Set Configuration dialog box: Add Metrics to Metric Set tab” on page 60.</td>
</tr>
<tr>
<td>Import Metric set</td>
<td>Opens the Import Metric Set dialog box, where you browse to and select a metric set file to import.</td>
</tr>
<tr>
<td>Delete Metrics</td>
<td>Opens the Delete Metrics dialog box, where you select metrics you want to remove from the database. See “Delete Metrics” on page 58.</td>
</tr>
<tr>
<td>Edit Metric set</td>
<td>Opens the Metric Set Configuration dialog box, identical to when you click Metric Sets in the command ribbon and then select Edit. See “Metric Set Configuration dialog box: Add Metrics to Metric Set tab” on page 60.</td>
</tr>
<tr>
<td>Duplicate Metric set</td>
<td>Opens the Duplicate Metric Set dialog box, which lets you enter a name for the duplicate metric set.</td>
</tr>
<tr>
<td>Delete Metric set</td>
<td>Permanently removes the selected metric set from the software.</td>
</tr>
<tr>
<td>Refresh Metrics</td>
<td>Updates the list of displayed metrics.</td>
</tr>
<tr>
<td>Rename Metric set</td>
<td>Opens the Enter New Name dialog box to change the name of the selected metric set.</td>
</tr>
<tr>
<td>Export Metric set</td>
<td>Opens the Export Metric Set dialog box, identical to when you click Metric Sets in the command ribbon and then select Export.</td>
</tr>
</tbody>
</table>
### Charts Pane

This pane displays a list of charts that were created in or imported into the software.

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

![Figure 16 Metric Sets Navigator options](image)

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add New Chart</td>
<td>Appears when you right-click Charts folder. Opens the Chart Configuration dialog box where you set up and name a new chart. See “Chart Configuration dialog box” on page 54.</td>
</tr>
<tr>
<td>Edit Chart</td>
<td>Opens the Chart Configuration dialog box, identical to clicking Charts in the command ribbon and then selecting Edit. See “Chart Configuration dialog box” on page 54.</td>
</tr>
<tr>
<td>Delete Chart</td>
<td>Permanently removes the selected chart.</td>
</tr>
<tr>
<td>Rename Chart</td>
<td>Opens the Enter new name dialog box to change the name of the selected chart.</td>
</tr>
</tbody>
</table>
**Data window**

The data window 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 in this section.

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

**Data window - Extractions Table**

The extractions loaded into the Agilent QC Chart Tool database are displayed in the form of a grid-view. See Figure 17:

![Figure 17](image)

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

**Data window - Metric Sets**

The Metric Sets created in or imported into the software are displayed in the form of a grid-view. See the following figure:
Data window

### Disassociate Metric

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

### Data window - 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 view supports the operations shown in Figure 19:
Figure 19  Data Window - Charts

**Plot**  Draws the chart according to the data selected in the 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 you can paste in MS Word and MS Paint, or in any other appropriate software.
The plots in this dialog box represent the selected columns in the QC Metrics Table. Each plot shows the value of a given metric for all arrays.

These plots have several additional features:

- The background colors in each plot correspond to quality guidelines developed by Agilent. These guidelines are based on normal ranges observed for analyses of well-established cell lines using standard Agilent protocols. See the descriptions of each of these metrics in the Feature Extraction Reference Guide.

- A “Box & Whisker” plot appears to the right of the main plot for each metric. See the following description of “Box & Whisker’ Plot”.

- You can right-click any bar to open a shortcut menu for the corresponding array.

**“Box & Whisker” Plots**

A small plot appears to the right of each of the main plots. It represents the overall distribution of values for the metric. Two examples appear in Figure 20. The symbols have the following meanings:

- The lower and upper edges of the box represent the 25th and 75th percentiles, respectively.

- The black horizontal line in the box is the median.

- The black dot is the mean.

- The “whiskers” represent the range of values that are not outliers. An outlier is a point that is out of the 25th to 75th percentile range by more than 150%.

- Open circles represent outliers, and an open triangle represents outliers that plot beyond the available space on the graph.
Figure 20  Two “Box & Whisker” plots
Dialog Boxes

The following dialog boxes appear when using QC Chart Tool. 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.

Agilent Feature Extraction Importer

![Agilent Feature Extraction Importer dialog box]

**Figure 21**  Agilent Feature Extraction Importer dialog box

**Purpose:** Lets you edit the name of the FE data file you intend to import. Also, lets you select to flip the red/green ratio for the data.

**To open:** In the command ribbon, click **Import**, and then click **FE Stats and Parameters**. In the Import FE Files dialog box, select the desired FE data files, then click **Open**.
Extraction Name  Shows the name of each microarray to import. You can change the names of the files to names that you are more likely to recognize or remember.

Dye Flip  For each array:

Select **Normal** if:
- The test samples were labeled with cyanine-5 (red).
- The control samples were labeled with cyanine-3 (green).
- The imported ratio (test/control) must be reported directly.

Select **Flipped** if:
- The test samples were labeled with cyanine-3 (green).
- The control samples were labeled with cyanine-5 (red).
- The imported ratio (control/test) must be reported with the ratio inverted (test/control).

The software does not combine dye-flip pairs.

**OK** Imports the files in the foreground. You cannot use your computer for other purposes while the import occurs.

**Cancel** Cancels the entire import process without importing anything.
Chart Configuration dialog box

**Purpose:** To configure the appearance of a Quality chart.

**To open:** On the Quality ribbon, click Chart and then select New.

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

- **Metric Set**
  Select the metric set to use with the chart.

- **Extraction Query**
  Select the extraction query to evaluate for a chart.

- **Sort by Columns**
  Select how you want to order the extractions in the chart. You can select three conditions, 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 selected, 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 \textit{light-gray} line. If this check box is selected, the ShapeBy and ColorBy columns are disabled.

**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** Saves the chart using the name you entered.

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

**Create a new Metric dialog box**

![Create a new Metric dialog box](image)

**Figure 23** Create a new Metric dialog box
**Purpose:** Lets you create a metric in the command ribbon to track selected statistical values for a set of extractions.

**To open:** On the command ribbon, click Metrics, and then select New.

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

- **Choose Metric Column**
  - A list of metrics that are used to create a calculation as a new metric.

- **Add**
  - 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.

- **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.
<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abs</td>
<td>Returns the absolute value of a metric or a collection of metrics grouped by matching parenthesis.</td>
</tr>
<tr>
<td>Numerical Constant</td>
<td>Lets you enter a value to add to the metric calculation formula.</td>
</tr>
<tr>
<td>Add Constant</td>
<td>Accepts the value entered in the Numerical Constant field and adds it to the metric calculation formula.</td>
</tr>
</tbody>
</table>

**Metric Calculations**

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

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear</td>
<td>Removes all metrics, mathematical operators, and constants from the formula in the Metric Calculations area.</td>
</tr>
<tr>
<td>Save Metric</td>
<td>Lets you type a name to be associated with the metric.</td>
</tr>
<tr>
<td>Save</td>
<td>Saves the metric using the name you entered.</td>
</tr>
</tbody>
</table>
Delete Metrics

Figure 24  Delete Metrics dialog box

**Purpose:** Used to select unnecessary metrics from the database. When you delete a metric, it is permanently removed from the database.

**To open:** In the command ribbon, in the Metric Sets Navigator, right-click the Metric Sets folder and select **Delete Metrics.**

- **Delete** Deletes the selected metrics from the database.
- **Select All** Selects all metrics.
- **Deselect All** Removes the selection from all metrics.
- **Close** Closes the dialog box.
Frequency Distribution

Purpose: The plots in this dialog box represent the selected columns in a Quality chart. Each plot shows the number of arrays within each value range for a metric.

To open: Click Show Frequency Distribution in the quality charts window.

Edit Opens a menu with a Copy command that copies the plots in the dialog box to the Clipboard. You can then paste the image into a document.

Close Closes the dialog box.
Metric Set Configuration dialog box: Add Metrics to Metric Set tab

Purpose: Used to define a metric set that consists of existing metrics and user-defined thresholds.

To open: On the command ribbon, click Metric Sets, and then click New.

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 metrics that you can use in the metric set.

**Threshold Type**
The following fields are threshold types, which are used to select which thresholds to apply and display.
**Upper Limit** Sets a limit where extraction values 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 further separate the extractions.

**Upper Warning Limit** Sets a limit where extraction values 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 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 further separate the extractions.

**Lower Limit** 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 further separate the extractions.

**Threshold Calculation**

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

**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 62.

**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 62.

**Percentage** Takes a percentage range of the data to calculate the limit. For detailed information, see “Percentage Threshold Calculations” on page 63.
Optional: Lets you assign an Extraction Query to the metric set for Standard, Robust, and Percentage Threshold Calculation modes. This query filters the appropriate extractions from the database, so that just the data from the queried extractions are 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 add to the mean to create the Upper Limit. For example, to apply an upper limit of two 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 add to the mean to create the Upper Warning Limit. The Upper Warning Limit is defined as the Median + constant*IQR.

**Lower Warning Limit**

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

**Lower Limit**

Lets you define a multiplier for the number of standard deviations to subtract from 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 add 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 Median + constant*IQR.

**Upper Warning Limit**

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

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

Lower Limit

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

**Percentage Threshold Calculations**

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

Upper Limit

Lets you define percentage from the uppermost 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 uppermost 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 percentage from the bottommost values of the data to calculate the Lower Warning Limit. For example, to set a 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 percentage from the bottommost values of the data to calculate the Lower Limit. For example, to set a 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

Saves the threshold settings for the selected metrics. These metrics with their associated thresholds become available in the “Metric Set Configuration dialog box: Selected/Existing Metrics tab” on page 64.

Metric Set Name

Lets you enter a name for the new metric set.

Save

Saves the metric set.

Cancel

Closes the Metric Set Configuration dialog box without saving any changes.
3 QC Chart Tool Reference

Metric Set Configuration dialog box: Selected/Existing Metrics tab

**Purpose:** Used to display existing/selected metrics in a metric set.

**To open:** In the Metric Set Configuration dialog box, click the Existing Metrics or Selected Metrics tab.

The Selected/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 the software 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

```

Figure 27 Metric Set Configuration dialog box - Selected/Existing Metrics tab.
Metric Set Configuration dialog box: Selected/Existing Metrics tab

- 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 28 The Query Builder dialog box

**Purpose:** Used to define a subset of extractions for a representative data set, for use in metric and threshold development, and in producing Charts.

**To open:** In the command ribbon, click **Queries** and then click **New**.

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

- **Column Name** Displays a list of the metrics that you can use to create an expression with which to query the database.

- **Operator** Displays a list of the relational operators that you can use with the selected metric.

- **Enter Value** A text area where a value is typed that is compared to the metric for each extraction. If the chosen relation between the metric and value is valid, the query produces extractions that pass that condition (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 previously. Each logical operation 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.

()  Lets you create a subset for 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**  Saves the query using the name specified in the Query Name field.

**Cancel**  Cancels all query operations and closes the Query Builder dialog box.
In This Book

This book contains information to set up and use the QC Chart Tool software with Agilent Feature Extraction.