GenomeStudio™ Gene Expression Module v1.0 User Guide

An Integrated Platform for Data Visualization and Analysis

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Revision History

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Introduction

The GenomeStudio Gene Expression Module is a tool for analyzing gene expression data from scanned microarray images generated by the Illumina BeadArray™ Reader, or scanned intensity data generated by the Illumina BeadXpress® Reader. You can use the resulting GenomeStudio output files with most standard gene expression analysis programs.

The GenomeStudio Gene Expression Module allows you to examine data generated from the following assays:

- Direct Hyb Assay
- DASL® Assay
- VeraCode® DASL Assay
- Whole Genome DASL Assay
- miRNA Assay

In addition, it enables two types of data analysis:

- Gene Analysis—quantifying gene expression signal levels
- Differential Analysis—determining whether gene expression levels have changed between two experimental groups

You can perform analyses on individual samples or on groups of samples treated as replicates.

The Gene Expression Module reports experiment performance based on built-in controls that accompany each experiment. In addition, this module includes the following tools, which provide a quick, visual means for exploratory analysis:

- Line plots
- Scatter plots
- Histograms
- Dendrograms
- Box plots
- Heat maps
- Samples table
- Image viewer
- Illumina Genome Viewer (IGV)
- Illumina Chromosome Browser (ICB)

Audience and Purpose

This guide is written for researchers who want to use the GenomeStudio Gene Expression Module to analyze data generated by performing Illumina's DirectHyb, miRNA, DASL, Whole-Genome DASL, or VeraCode DASL assays.

This guide includes procedures and user interface information specific to the GenomeStudio Gene Expression Module. For information about the GenomeStudio Framework, the common user interface and functionality available in all GenomeStudio Modules, refer to the *GenomeStudio Framework User Guide*.

Installing the Gene Expression Module

To install the GenomeStudio Gene Expression Module:

1. Put the GenomeStudio CD into your CD drive.

   If the Illumina GenomeStudio Installation screen appears (Figure 2), continue to Step 3.

   If the CD does not load automatically, double-click the *GenomeStudio<version>.exe* icon in the **GenomeStudio** folder on the CD.

   The GenomeStudio application suite unzips (Figure 1).

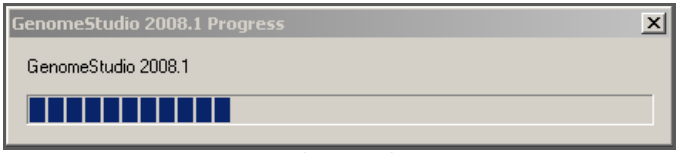


Figure 1 GenomeStudio Application Suite Unzipping

The Illumina GenomeStudio Installation dialog box appears (Figure 2).

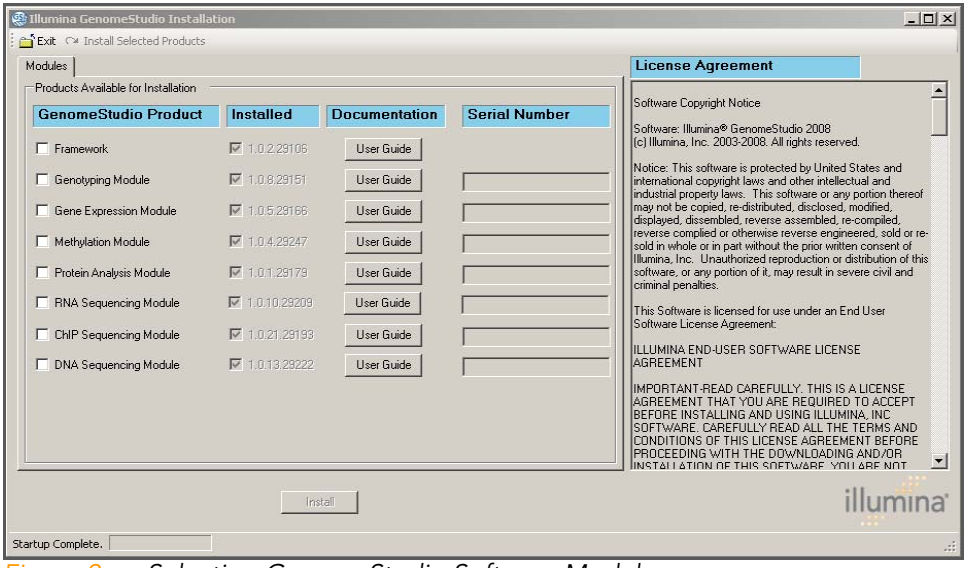


Figure 2 Selecting GenomeStudio Software Modules

2. Read the software license agreement in the right-hand side of the Illumina GenomeStudio Installation dialog box.
3. In the GenomeStudio Product area, select **Gene Expression Module**.

NOTE

The GenomeStudio Framework works in conjunction with GenomeStudio software modules. Select the Framework and one or more GenomeStudio modules to install, and have your serial number(s) available.

4. In the Serial Number area, enter your serial number for the Gene Expression Module.

NOTE

Serial numbers are in the format ####-####-####-#### and can be found on an insert included with your GenomeStudio CD.

5. [Optional] Enter the serial numbers for additional GenomeStudio modules if you have licenses for additional GenomeStudio modules and want to install them now.

6. Click **Install**.

   The Software License Agreement dialog box appears (Figure 3).

Figure 3 License Agreement

7. Click **Yes** to accept the software license agreement.

   The GenomeStudio Framework and Gene Expression Module are installed on your computer, along with any additional GenomeStudio modules you selected (Figure 4).

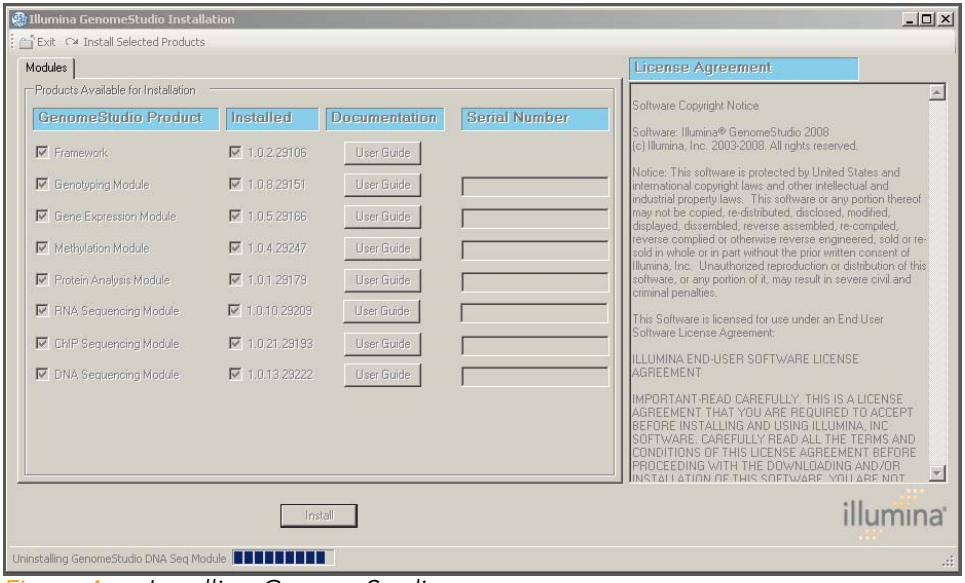


Figure 4 Installing GenomeStudio

The Installation Progress dialog box notifies you that installation is complete (Figure 5).

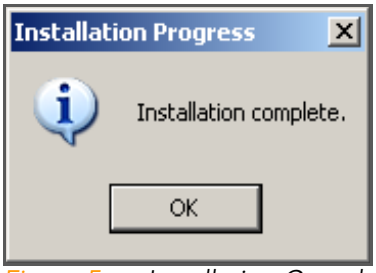


Figure 5 Installation Complete

8. Click **OK**.
9. In the Illumina GenomeStudio Installation dialog box (Figure 4), click **Exit**.

   You can now start a new GenomeStudio project using any GenomeStudio module you have installed.

   See Chapter 2, *Creating a New Project*, for information about starting a new Gene Expression project.

Gene Expression Module Workflow

The basic workflow for gene expression analysis using Illumina's GenomeStudio Gene Expression Module is summarized in Figure 6.

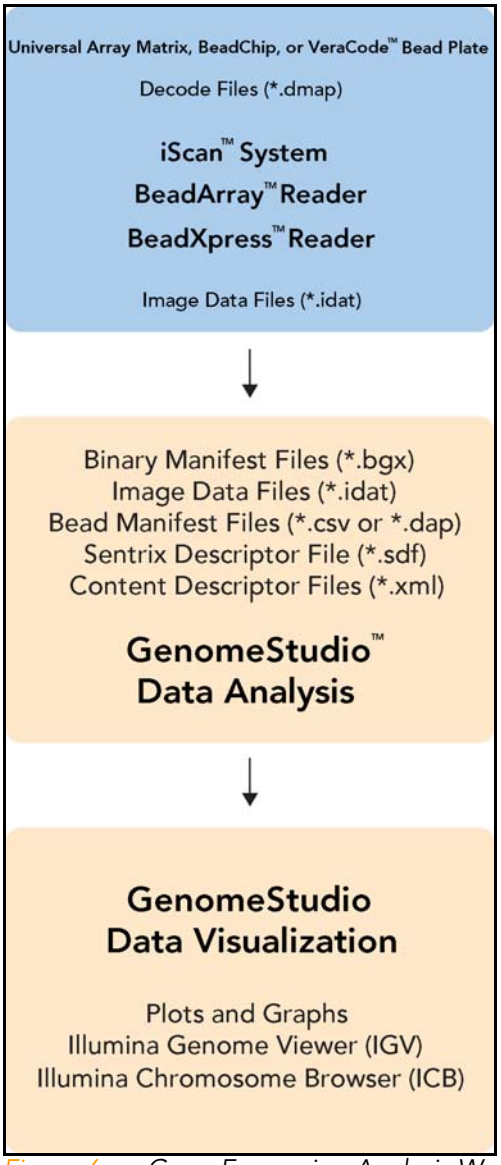


Figure 6 Gene Expression Analysis Workflow

Chapter 2
Creating a New Project

Topics

Introduction

Using intensity (*.idat) files produced by the BeadArray Reader or BeadXpress Reader, the Gene Expression Module's gene analysis tools produce data tables containing:

- Probe and gene lists
- Associated signal intensities (normalized or raw)
- Information about system controls

In addition, the Gene Expression Module's differential analysis tools can produce data tables displaying the probability that a gene's signal has changed between two samples or groups of samples.

Using GenomeStudio's data visualization tools, you can create sophisticated plotting analyses, including:

- Line plots
- Scatter plots
- Bar plots
- Box plots
- Heat maps
- Histograms
- Cluster analysis dendrograms

To run a gene expression analysis, you must first create a GenomeStudio project. In a project, you define one or more groupsets, one or more groups (sample sets that can be compared against each other for the purpose of identifying differences in gene expression), and one or more analyses. For more information about groupsets and groups, see *Defining Groupsets and Groups* later in this chapter.

In the simplest experiment, each group may have only one sample. However, if your experiment includes replicate samples, you can assign these to the same group.

In a project and within a group, GenomeStudio averages the values for each gene across the samples, and algorithms automatically take advantage of beadtype replicates to provide accurate estimates of relative mRNA abundance. This translates into a highly sensitive determination of detection and differential expression.

The following section, *Creating a Project*, provides step-by-step instructions for the following tasks:

- Defining a project
- Creating groupsets and groups
- Defining analysis type and parameters
- Applying normalization and differential expression algorithms
- Viewing and analyzing your data

The GenomeStudio Project Wizard guides you through creating a project, while the GenomeStudio main page provides a starting-point from which you can carry out the same functions independently.

Creating a Project

Follow the instructions in this section to create a GenomeStudio project using data from Illumina's Direct Hyb, DASL, VeraCode DASL, Whole Genome DASL, or miRNA assays with the GenomeStudio Project Wizard.

Starting the Gene Expression Module

1. In GenomeStudio, open the Gene Expression Module by selecting **File** | **New Project** | **Gene Expression**.

   The GenomeStudio Project Wizard—Welcome dialog box appears (Figure 7).

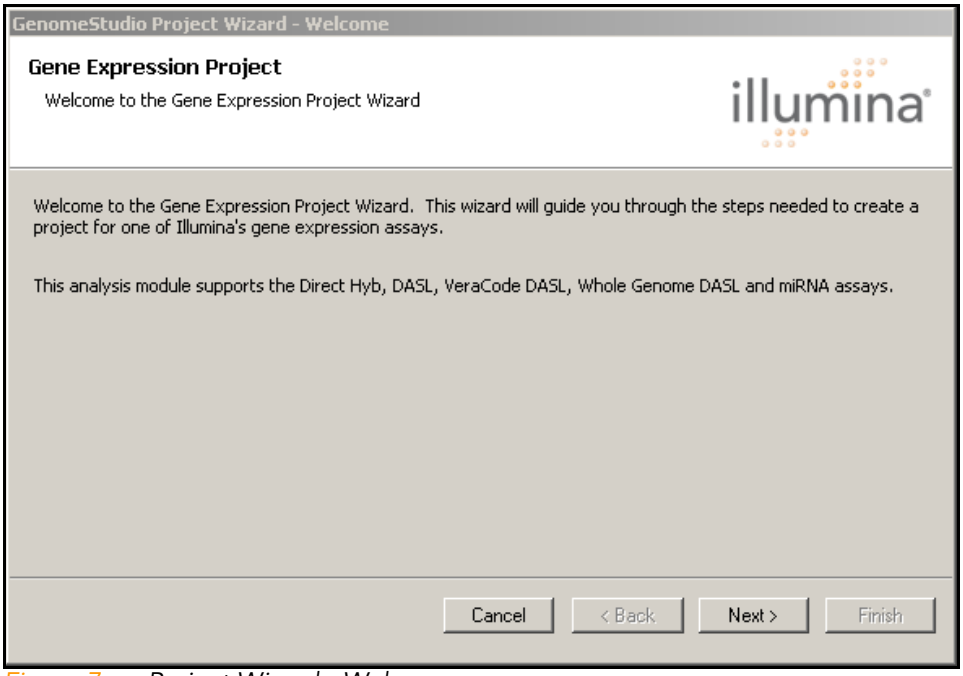


Figure 7 Project Wizard - Welcome

2. Click **Next** to advance to the GenomeStudio Project Wizard—Gene Expression Assay Type dialog box.

Selecting an Assay Type

In the GenomeStudio Project Wizard—Gene Expression Assay Type dialog box (Figure 8), perform the following steps to select an assay type:

1. Specify an assay type by selecting **Direct Hyb**, **DASL**, **VeraCode DASL**, **Whole Genome DASL**, or **miRNA** (Figure 8).

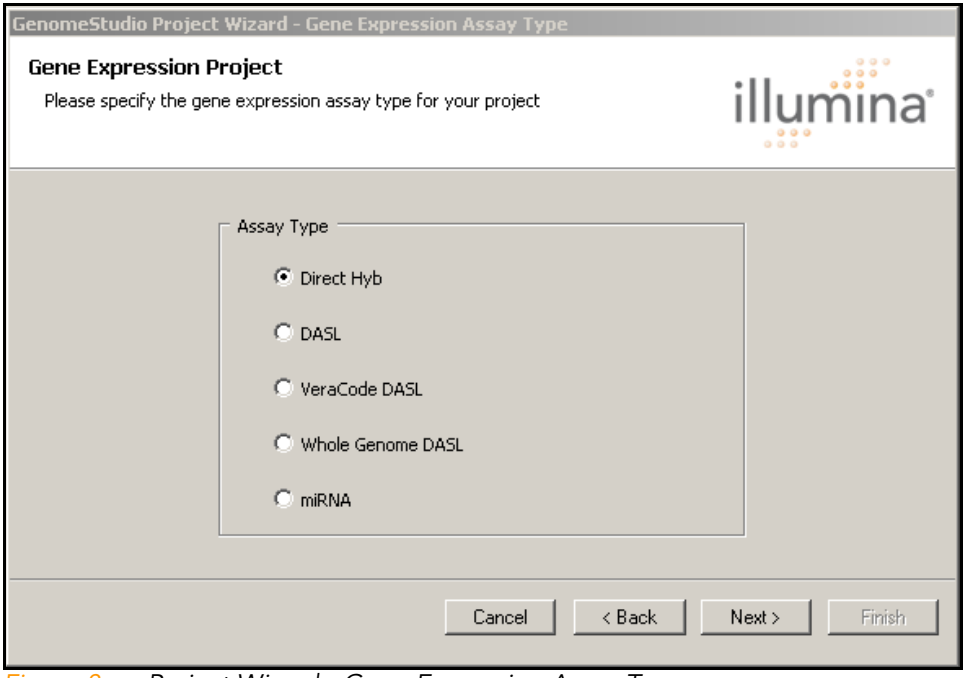


Figure 8 Project Wizard - Gene Expression Assay Type

2. Click **Next** to advance to the GenomeStudio Project Wizard—Project Location dialog box.

Choosing a Project Location

In the GenomeStudio Project Wizard—Project Location dialog box (Figure 9), perform the following steps to choose a project location:

1. In the **Projects Repository** field, browse to the location where you want to save your project.
2. In the **Project Name** field, enter a name for your project.

   The full path for your project appears beneath the name you enter.

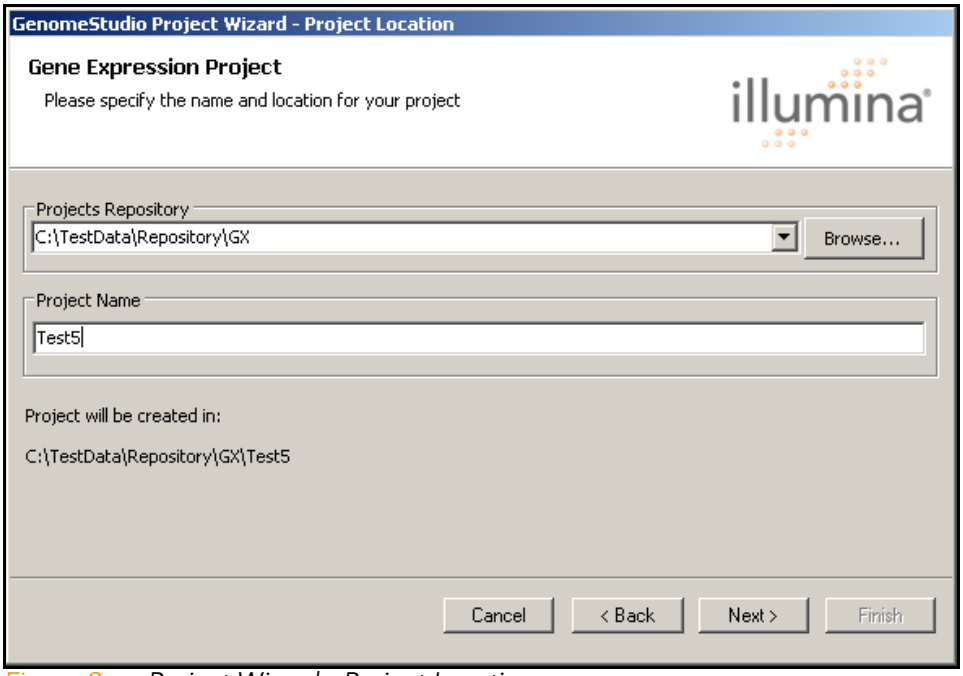


Figure 9 Project Wizard - Project Location

3. Click **Next** to advance to the GenomeStudio Project Wizard—Project Data Selection dialog box.

Selecting Project Data

In the GenomeStudio Project Wizard—Project Data Selection dialog box (Figure 10), perform the following steps to select the project data:

1. In the **Repository** dropdown list, browse to the project repository folder where your data output folders are stored.

   Data output folders are named according to product barcodes, and contain intensity data (*.idat) files.

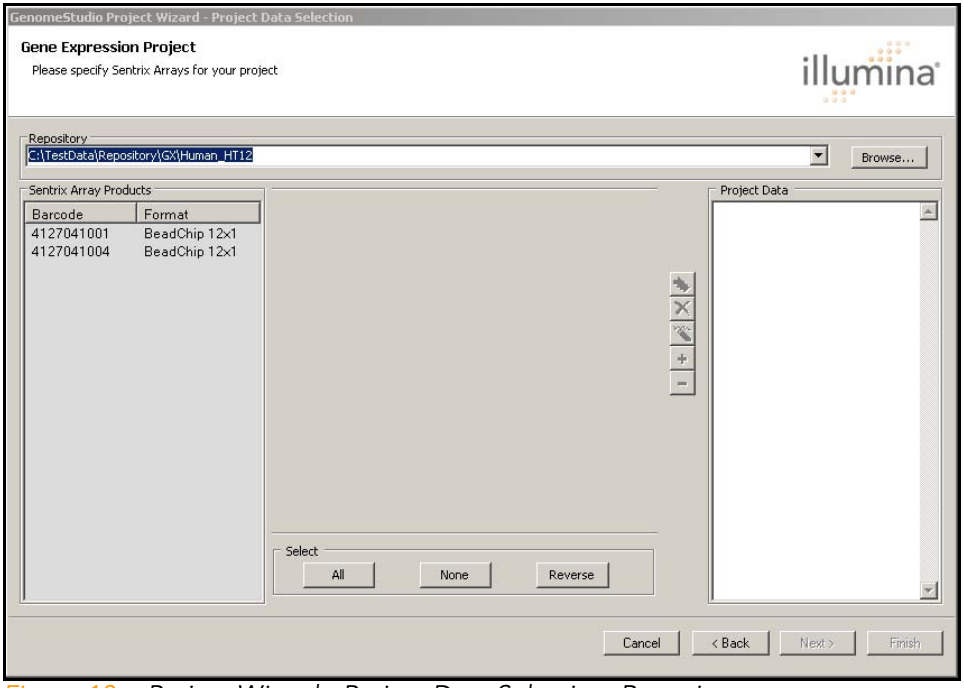


Figure 10 Project Wizard - Project Data Selection, Repository

2. In the Sentrix Array Products pane, select the product(s) you want to include in your project.

Select products from a single species only (e.g., human, mouse, or rat).

Following are some guidelines about which product types can be combined in a single gene expression product.

- Human v2 products ***can*** be combined with Human v3 and future human products in a single gene expression project, but Human v1 products ***cannot*** be combined with Human v2 and future human products.
- Mouse v1.1 products ***can*** be combined with future mouse products in a single gene expression project, but Mouse v1.0 products ***cannot*** be combined with Mouse v1.1 and future mouse products.
- HumanWG-6 products can be combined with HumanRef-8 products.

Example 1

HumanWG-6 v2 products
can be combined with
HumanWG-6 v3 products

Example 2

HumanWG-6 v2 products
can be combined with
HumanRef-8 v2 products

Example 3

HumanWG-6 v1 products
cannot be combined with
HumanWG-6 v2 products

Example 4

HumanWG-6 v2 products
cannot be combined with
RatRef-12 v2 products

Example 5

MouseRef-8 v1.0 products
cannot be combined with
Mouse Ref-8 v1.1 products

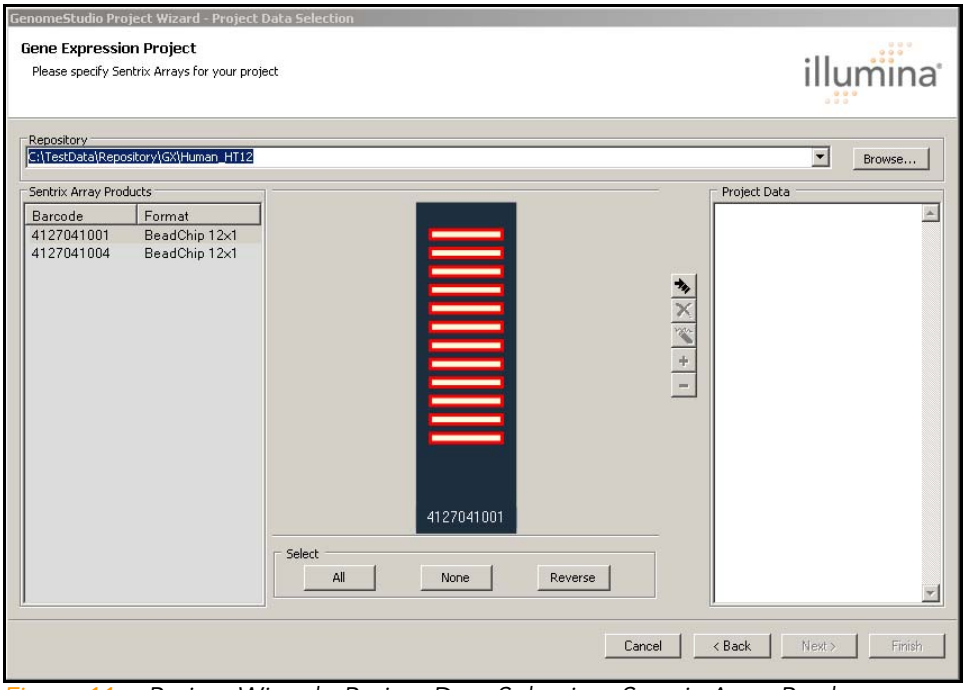


Figure 11 Project Wizard - Project Data Selection, Sentrix Array Products

All of the samples for each product are selected by default.

NOTE

The product image is different for each product.

3. To change the selected samples, use the **All**, **None**, and **Reverse** buttons in the Select area.
   - To select a single sample, click the sample (on the image of the product).
   - To select multiple samples, press and hold **Ctrl** and click each sample you want to select.
   - To select all samples, click **All**.
   - To clear your selection, click **None**.
   - To select the reverse of the samples currently selected (e.g., samples 1, 2, and 3 are currently selected, but you want to select samples 4, 5, and 6), click **Reverse**.

4. Click  to add the selected samples to your project.

   The selected samples appear under the name of the product in the Project Data pane.

5. [Optional] Click  (to the left of the group symbol) to display the list of samples chosen for the current project.

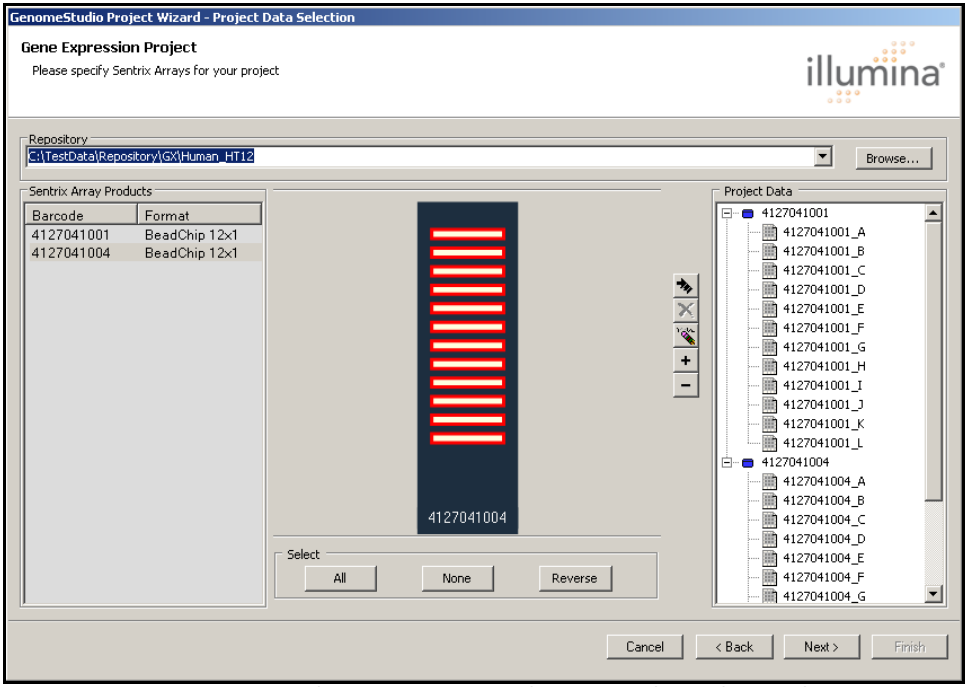


Figure 12 Project Wizard - Project Data Selection, Selected Samples

6. Click **Next** to advance to the GenomeStudio Project Wizard—Groupset Definition dialog box (Figure 15).

   GenomeStudio displays a status dialog box (Figure 13) while it copies your data to the designated location on your computer.

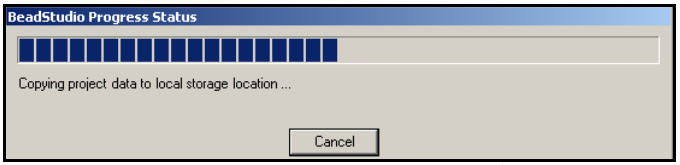


Figure 13 Copying Project Data to Local Storage Location

[Multi-Product Only] If you are combining data from multiple products, GenomeStudio prompts you to select a content descriptor file, also called a binary manifest file or *.bgx file (Figure 14).

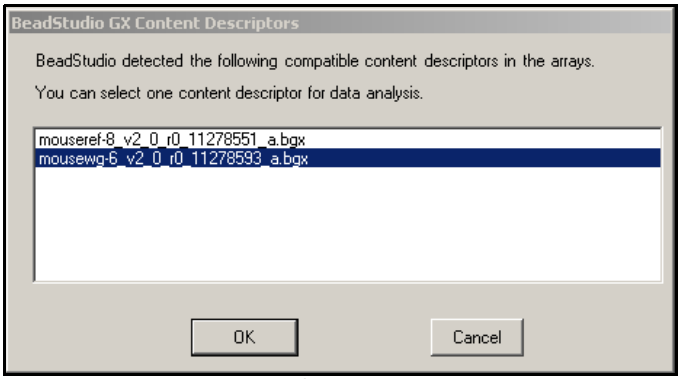


Figure 14 GenomeStudio GX Content Descriptors

Binary manifest files adhere to Illumina naming conventions. For example, for the HumanWG-6 v2 BeadChip, one possible binary manifest file name is: HumanWG-6_V2_0_R1_11223189_B.bgx, where:

- HumanWG-6 is the **product name**
- v2 is the **major version number** of the product
- 0 is the **minor version number** of the product
- R1 is the **annotation revision** of the product
- 11223189_B is the **product identifier**

Early versions of Illumina Gene Expression products, including Human v1, Human v2, Mouse v1, Mouse v1.1, and RatRef-12 v1, require that you use a text-based content descriptor file (*.xml file) or manifest file (*.csv file) instead of a *.bgx file.

Newer products use binary manifest files, which are more compact. *.bgx files contain additional annotation information, such as chromosomal position, which allows you to view gene expression data in the Illumina Genome Viewer (IGV) and the Illumina Chromosome Browser (ICB).

7. Select a content descriptor file (*.bgx file).
8. Click **OK**.

   The GenomeStudio Project Wizard—Groupset Definition dialog box appears (Figure 15). Continue to the next section to define groupsets and groups

Defining Groupsets and Groups

GenomeStudio projects are structured in a hierarchical manner:

- A **project** includes one or more groupsets.
- A **groupset** is a collection of one or more groups that you choose to analyze simultaneously.
- A **group** is a set of arrays that share a functional relationship (e.g., replicates, zero time points, reference group). Within a groupset, an array can be included in more than one group, or it can be analyzed individually.

There are two types of analysis: **gene analysis** and **differential expression analysis**. You perform an analysis on a single groupset at a time.

Perform the following steps to define a groupset for your project.

1. Assign a name to your groupset by doing one of the following:
   - Click **New** and enter a name for your new groupset.
   - Click **Existing** and choose the groupset you want from the dropdown list.

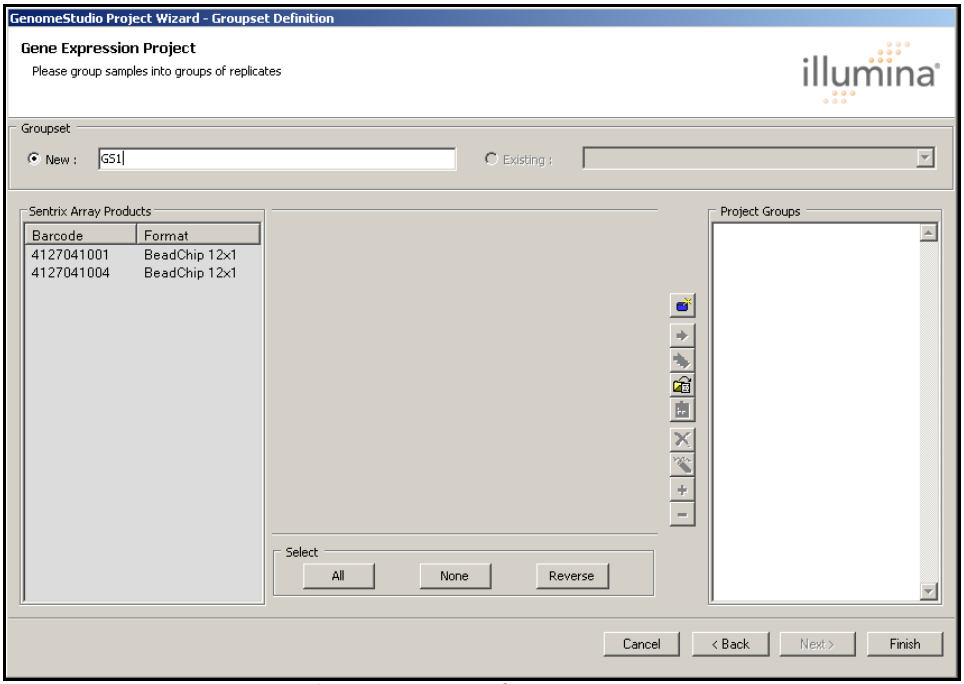


Figure 15 Project Wizard - Groupset Definition, Assigning a Groupset Name

2. In the Sentrix Array Products pane, select the Sentrix Array Product that contains the samples you want to assign to a groupset.

3. Click  to create the first group (Group 1).

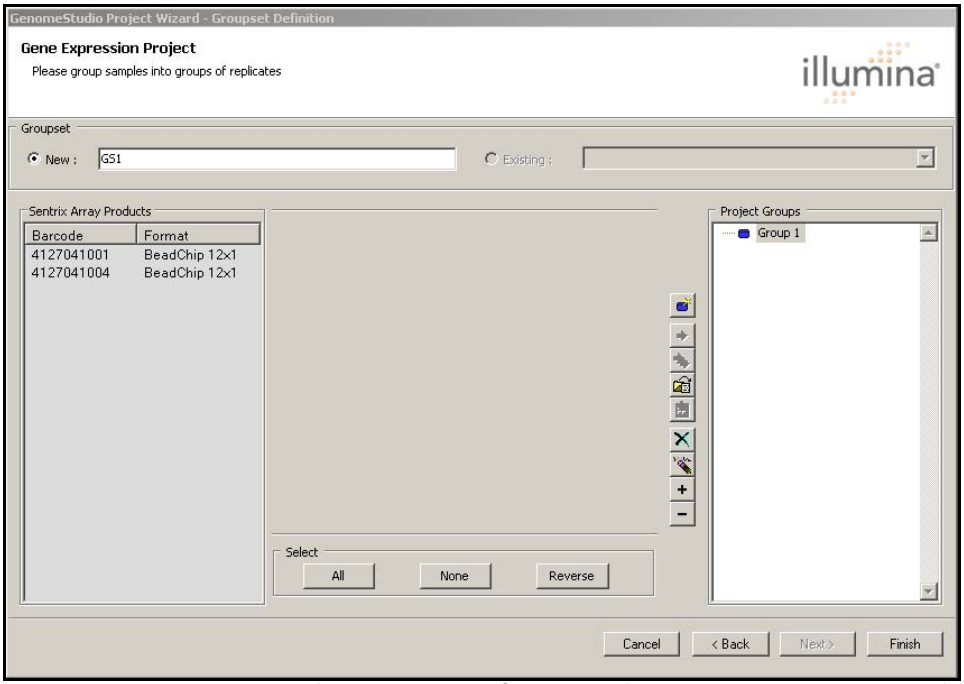


Figure 16 Project Wizard - Groupset Definition, Selecting a Sentrix Array Product

4. Use the **All**, **None**, and **Reverse** buttons to select the specific samples you want to assign to a group.

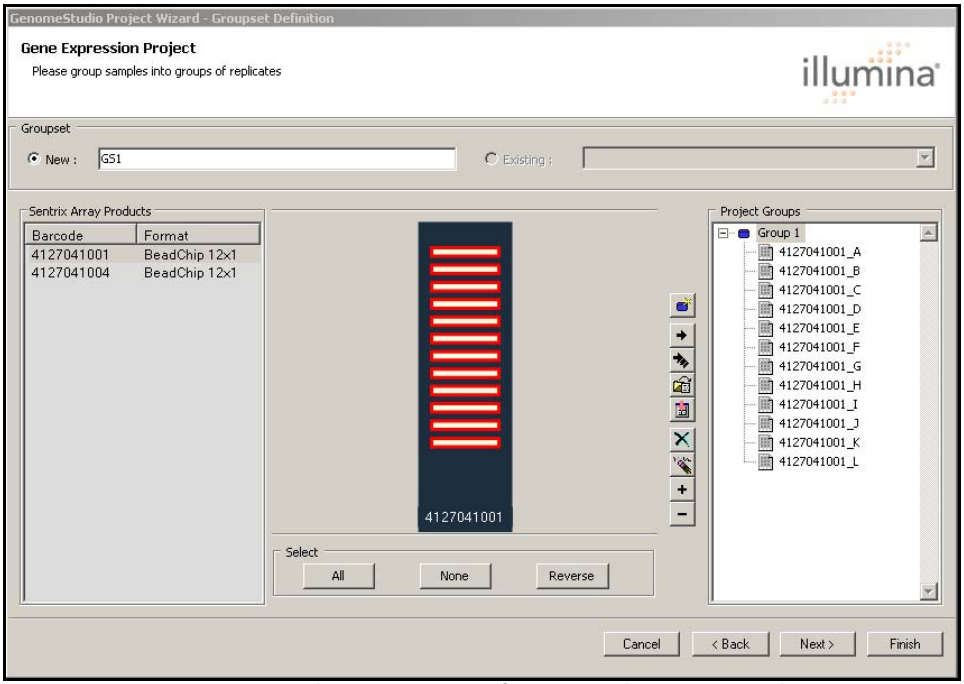


Figure 17 Project Wizard - Groupset Definition, Selecting Samples

5. Use the buttons to the left of the Project Groups area (Figure 18) to define project groups in a groupset:

To...	Click...
Create a new group	
Add selected samples to a group	
Create a group for each selected sample	
Load data from a sample sheet	

To...	Click...
Apply a group layout file For more information about group layout files, see *Applying a Group Layout File* on page 36.	
Remove selected groups and samples from the groupset	
Remove all groups and samples from the project	
Expand all groups	
Collapse all groups	

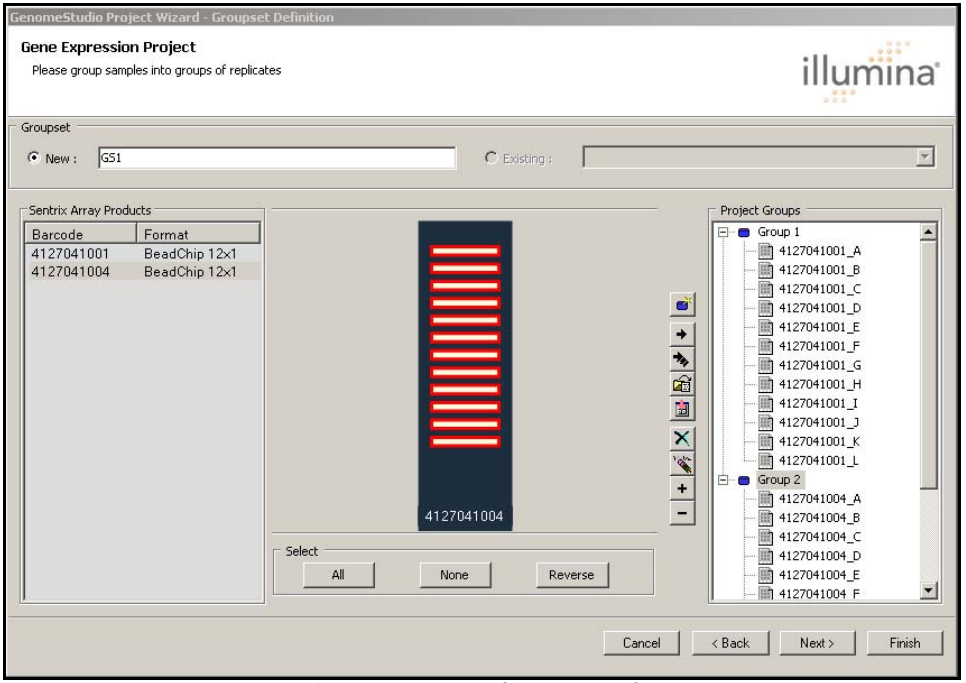


Figure 18 Project Wizard - Groupset Definition, Defining Project Groups

6. [Optional] Click **Finish** to finish building the groupset for your project.
7. Click **Next** to advance to the Project analysis type and parameters dialog box (Figure 19).

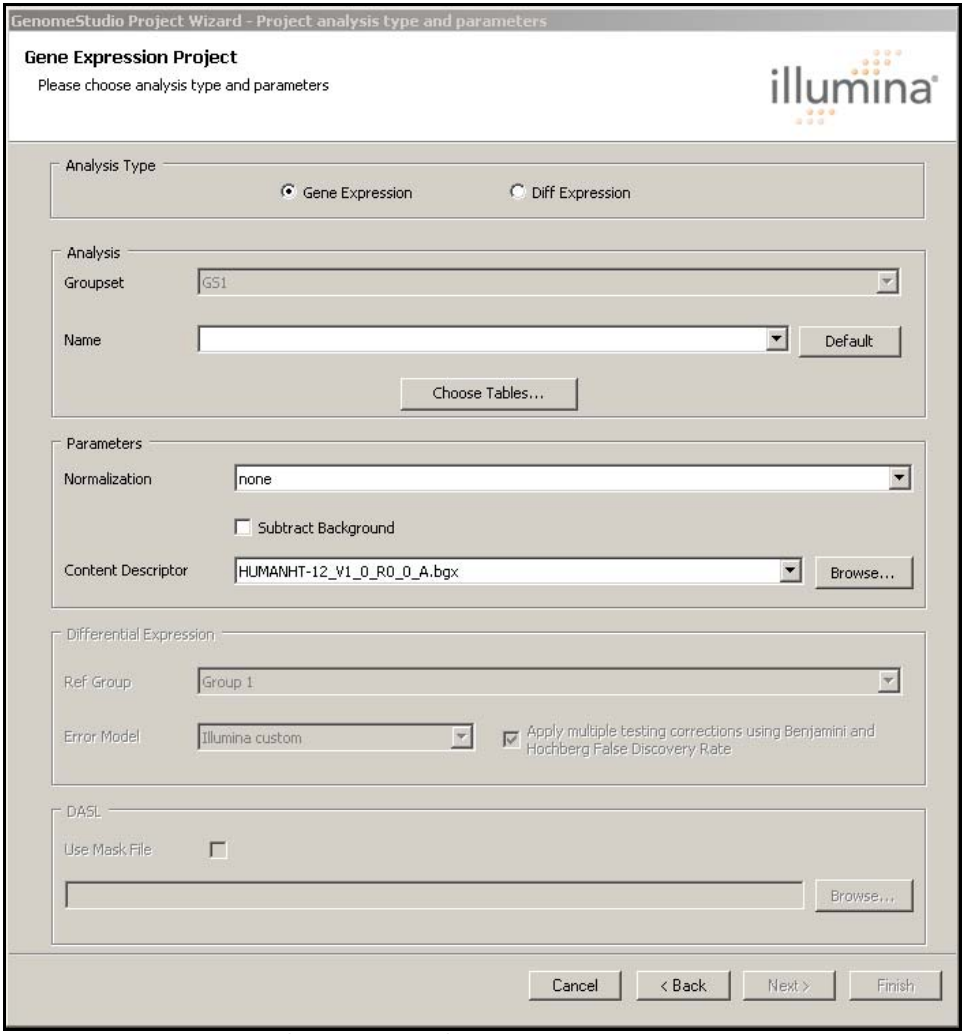


Figure 19 Project Analysis Type and Parameters

Defining the Analysis Type and Parameters

Perform the following steps to define the analysis type and parameters for your project:

1. In the Analysis Type area, select **Gene Expression** or **Diff Expression**.
2. In the Name area, enter a name for this analysis.
3. Do one of the following:
   a. If you want to display the default data tables in this project, continue to step 6.
   b. If you want to customize the data tables you display in this project, click **Choose Tables**.

   The Analysis Tables dialog box appears (Figure 20).

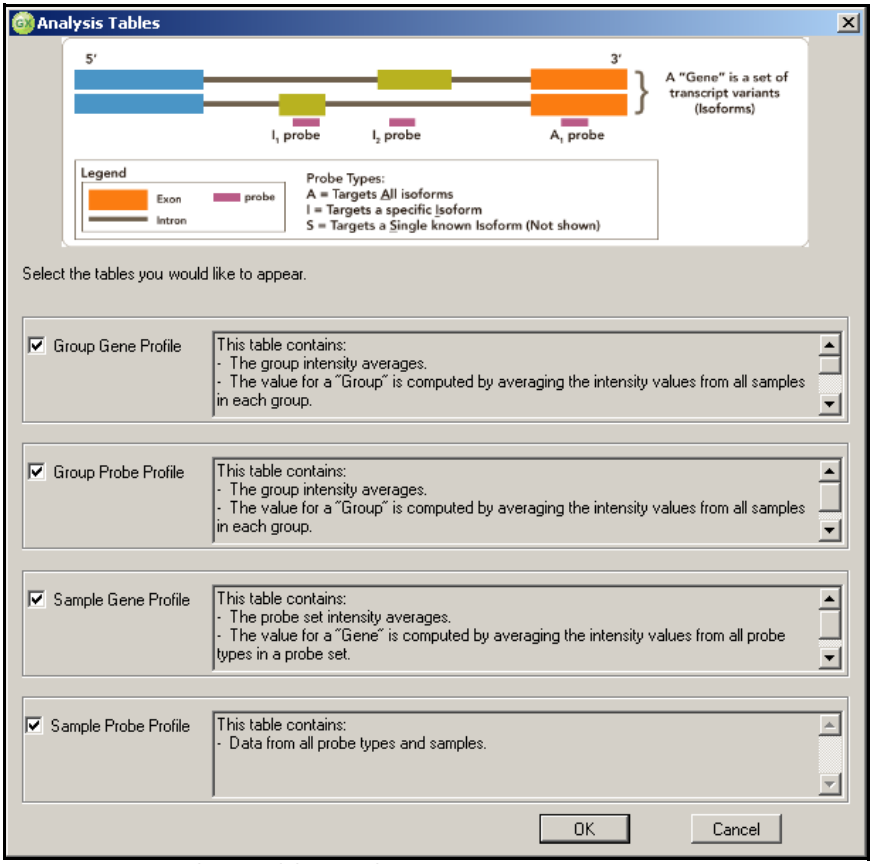


Figure 20 Analysis Tables Dialog Box

4. Select the tables you want to display in this project.
5. Click **OK**.

   The Analysis Tables dialog box closes. The tables you selected will appear in your project.
6. On the Project Analysis Type and Parameters dialog box (Figure 19), in the Parameters area, select the normalization method you want to use:
   - **None**
   - **Average**
   - **Quantile**
   - **Rank invariant**
   - **Cubic spline**

   For information about normalization methods, see *Normalization Methods & Algorithms* on page 98.

If you have run a DASL or miRNA assay, you must decide whether to enable sample plate scaling for this analysis. Sample plate scaling allows you to address lot-to-lot variation. This feature works with common samples across multiple BeadChips, SAMs, or VeraCode Bead Plates.

Sample plate scaling scales the intensities of all probes to create equal average intensities of all common samples for all plates for each probe. This is done on a per-probe basis.

Sample plate scaling does not take detection level into account. There is no noise correction.

7. [DASL or miRNA only] If you would like to enable sample plate scaling for this analysis, select the **With Sample Plate Scaling** checkbox.
8. If you are performing a differential expression analysis, select a **Ref Group** and an **Error Model** in the Differential Expression area.
9. [Optional] If you want to compute the false discovery rate, select **Apply multiple testing corrections using Benjamini and Hochberg False Discovery Rate**.

The name of this option has changed from Compute False Discovery Rate to **Apply multiple testing corrections using Benjamini and Hochberg False Discovery Rate**, but the functionality is the same as in previous versions of GenomeStudio

If you select Apply multiple testing corrections..., the p-values (in the p-value column) are adjusted accordingly. If you do not select Apply multiple testing corrections..., p-values are not adjusted.

The Benjamini and Hochberg correction tolerates more false positive genes than the Bonferroni correction, the Bonferroni Step-down (Holm) correction, and the Westfall and Young Permutation. Applying the Benjamini and Hochberg correction also results in fewer false negative genes.[1]

1. Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: a Practical and Powerful Approach to Multiple Testing. J R Statist Soc B(57): 289-300.

The Benjamini and Hochberg correction works as follows:

- The p-values of each gene are ranked from the smallest to the largest.

  The largest p-value is left as it is.
- The second largest p-value is multiplied by the total number of genes in the gene list divided by its rank.

  If the result is less than 0.05, it is significant. If the corrected p-value = p-value*(n/n-1) < 0.05, the gene is significant.
- The third p-value is multiplied as in the previous step.

  If the corrected p-value = p-value*(n/n-2) < 0.05, the gene is significant.
- These steps are repeated for all p-values.

10. Select a binary manifest (*.bgx) file or a content descriptor (*.xml or *.csv) file.

11. Click **Finish**.

If you selected With Sample Plate Scaling in step 7, the Select Common Sample File for Sample Plate Scaling dialog box appears (Figure 21).

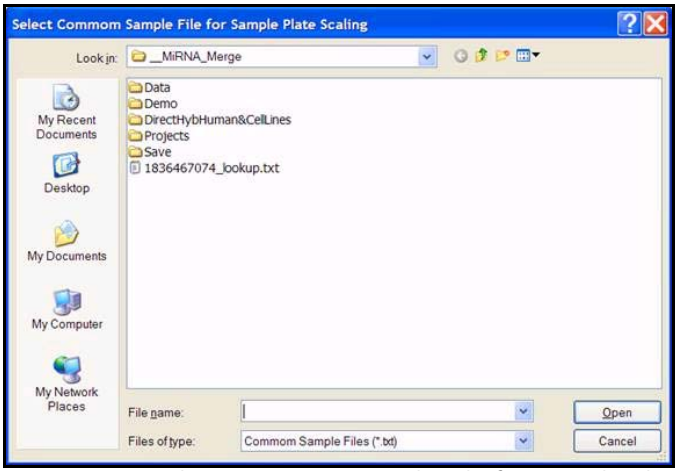


Figure 21 Select Common Sample File for Sample Plate Scaling Dialog Box

A common sample file is a text file (*.txt) that defines common samples across BeadChips, SAMs, or VeraCode Bead Plates. The common sample file should specify all common samples you are using in this analysis.

Sample names in the common sample file should be specified in the format **12345678_R001_C001**, where **12345678** is the plate number, **R001** is the row number, and **C001** is the column number (Figure 22).

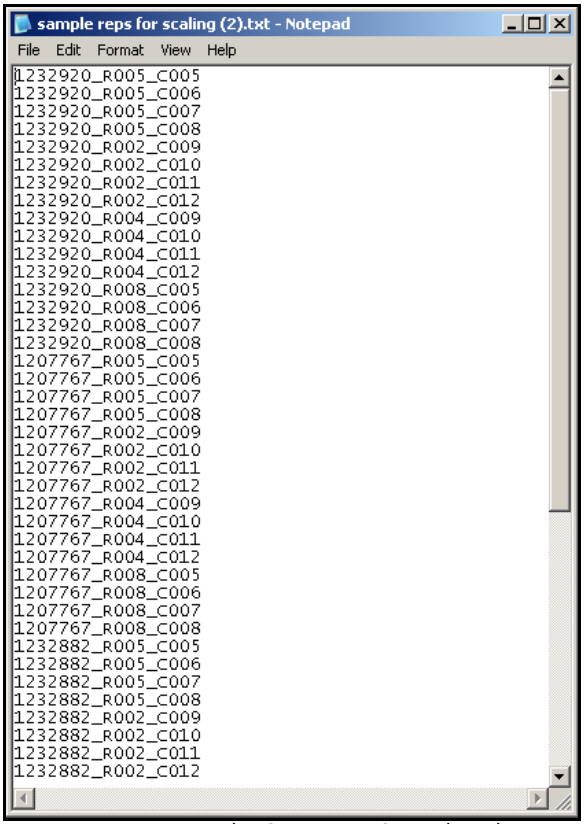


Figure 22 Example Common Sample File

GenomeStudio requires that each BeadChip, SAM, or VeraCode Bead Plate in an analysis has at least one common sample among them. If there is more than one common sample, the normalization value is averaged.

12. Select a common sample file and click **Open**.

GenomeStudio verifies the content of the selected text file.

NOTE

If the BeadChips, SAMs, or VeraCode Bead Plates referenced in the file do not contain common samples, GenomeStudio displays the warning shown in Figure 23 and runs the analysis without sample scaling.

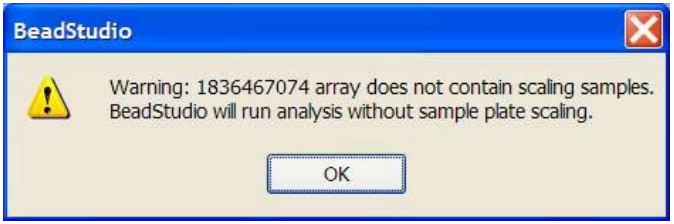


Figure 23 Sample Plate Scaling Warning

13. [Optional, DASL Only] Select the **Use Mask File** checkbox to choose the mask file you want to use. For more information about mask files, see *Creating a Mask File* on page 35.

NOTE

It is possible that not all assay probes are functional, due to design or synthesis. You can use a mask file to filter out these non-functional probes from your data analysis. For more information about mask files, see *Creating a Mask File* on page 35.

14. Click **Finish**.

    GenomeStudio begins to run your analysis. A progress bar indicates the completion level.

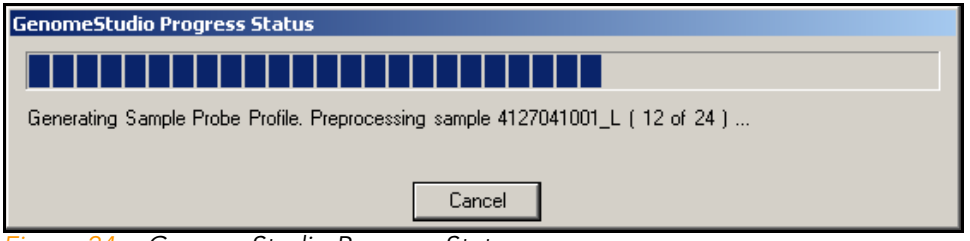


Figure 24 GenomeStudio Progress Status

NOTE

The time it takes for your analysis to be processed depends on the number of samples, groups, and groupsets, and on the type of analysis you wish to perform.

If GenomeStudio detects missing bead types in your data, the Impute or Exclude dialog box appears (Figure 25).

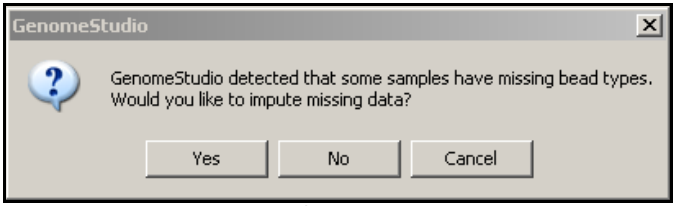


Figure 25 Missing Bead Types

NOTE

GenomeStudio considers data to be "missing" if fewer than three beads exist for a given bead type. HumanHT-12 BeadChips may have missing data, because by design they include fewer beads per bead type.

If GenomeStudio does not identify missing data, your project is created and displays in the GenomeStudio main window. Continue to Chapter 3, *Viewing Your Data*.

If GenomeStudio identifies missing data, you are given two options: you can impute the missing data, or you can exclude the missing data from the project.

Imputing Missing Data

When a bead type is missing from a sample, other samples with valid intensity values for this bead type are used to calculate an imputed value for the missing bead type.

EXAMPLE: There are 10 samples in a gene expression project. In two of the samples, bead type A is missing. In the other eight samples, bead type A has a valid intensity value (is not missing). The Euclidean distance of the intensities of bead type A to all other bead types is calculated across the eight valid samples.

The goal is to find the bead types closest to bead type A from the intensities of all valid samples. From these, the values of the 15 closest bead types are used to calculate the imputed value of bead type A for the missing sample. The imputed value is calculated as the weighted average of the 15 closest probe intensities.

Bead standard error (BEAD_STDERR) is also imputed for the missing bead type. This is calculated as the average of the bead standard error values of this bead type for all valid samples.

The average number of beads (Avg_NBEADS) value is modified in the data tables (e.g., Sample Probe Profile Table) to account for the missing bead type. The number of beads is set to 1 for the missing bead type in the data tables, but the actual number of beads is shown in the Excluded and Imputed Probes Table.

The Excluded and Imputed Probes table appears in a gene expression project only if the project contains imputed or excluded data. If there is no imputed or excluded data in a project, this table is not generated and does not appear.

Excluding Missing Data

You might decide to exclude missing data if you want to use only raw data in your project. However, most of the time you will probably want to impute missing data. Excluded data is removed from GenomeStudio's main tables, and moved to the Excluded and Imputed Probes table.

15. [Missing Data Only] Do one of the following:

- If you would like to impute missing data, click **Yes**.
- If you prefer to exclude missing data, click **No**.

Your GenomeStudio Gene Expression project appears (Figure 26).

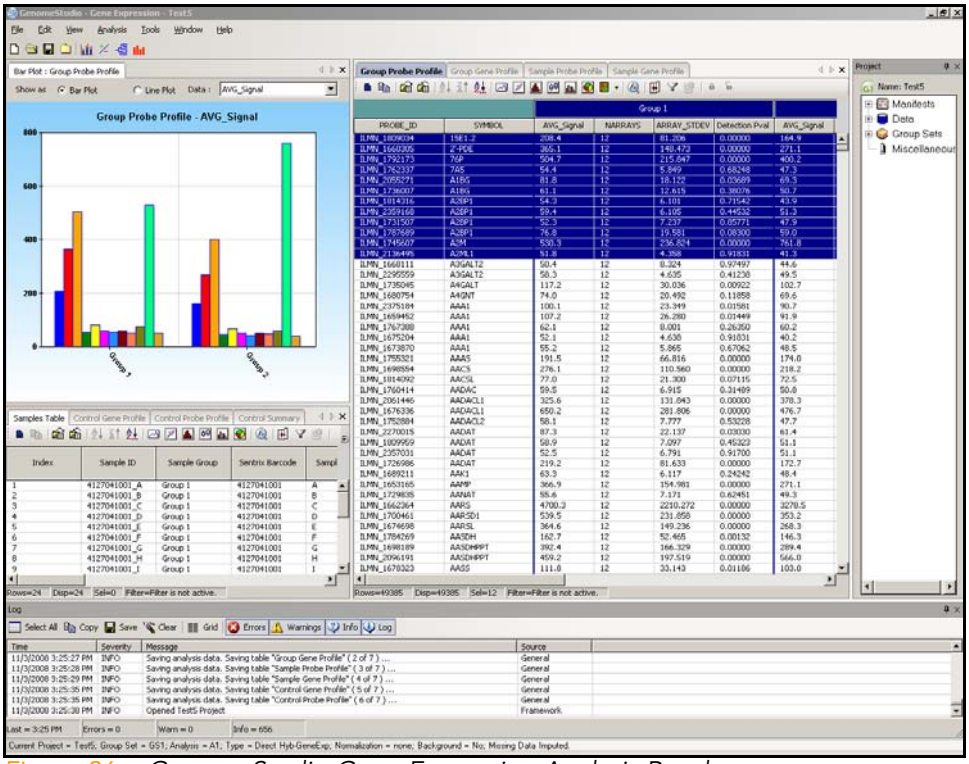


Figure 26 GenomeStudio Gene Expression Analysis Results

All missing data, whether imputed or excluded, appears in the Excluded and Imputed Probes table (Figure 27).

Bar Plot : Group Probe Profile | Excluded and Imputed Probes

TargetID	ProbeID	Excluded/Imputed	AVG_Signal	Avg_NBE
BAMBI	430050	imputed	5341.9	10
CASP8	290592	imputed	97.4	5
CSNK2A1	430025	imputed	798.5	7
CTXN1	360114	imputed	1707.1	7
ERCC-00103-01	4260279	imputed	108.1	12
HS.359754	1940717	imputed	95.0	17
KCNN3	5570068	imputed	61.2	21
NDUFS2	430717	imputed	128.8	14
RHOBTB1	360343	imputed	93.8	7
SPAG8	430451	imputed	84.5	7
SPATA9	3120639	imputed	66.0	31
UBXD5	360528	imputed	93.1	4

Figure 27 Excluded and Imputed Probes Table

For more information about the Excluded and Imputed Probes table and other elements of the Gene Expression Module graphical user interface, see Chapter 7, *User Interface Reference*.

Creating a Mask File

If genomic DNA was used in a DASL Assay to verify probe performance, you can select probes that should be excluded from further analysis. Because all probes are designed to be intraexonic, all probes should be detectable when genomic DNA is used as a sample. Therefore, the Detection p-value reported in the Group Probe Profile Table or the Sample Probe Profile Table can be used as an objective measure of probe performance on genomic DNA.

Illumina recommends excluding probes that have a detection p-value of greater than 0.01 on genomic DNA. However, you may define your own exclusion criteria.

To exclude a probe:

1. Export the ProbeID, Detection Pval, and TargetID columns from one of the Probe Profile Tables for the genomic DNA samples to a text file.
2. Edit the text file so that all p-values above your detection cutoff are set to 0, and all p-values below your detection cutoff are set to 1.

   For example, if you want to use a p-value cutoff of 0.01 for detection, set all values above 0.01 to 0 and all values below 0.01 to 1.
3. Change the Detection Pval column header to "0/1" and save the file as a *.csv file in the same repository where the content descriptor file is stored. The file need not conform to a naming convention.

Any *.csv file present in the same repository as content descriptor files appears in the **Experiment Parameters** pulldown menu. To avoid confusion, Illumina recommends using separate repositories for content descriptor *.csv files and SAM/BeadChip/VeraCode Bead Plate data.

Applying a Group Layout File

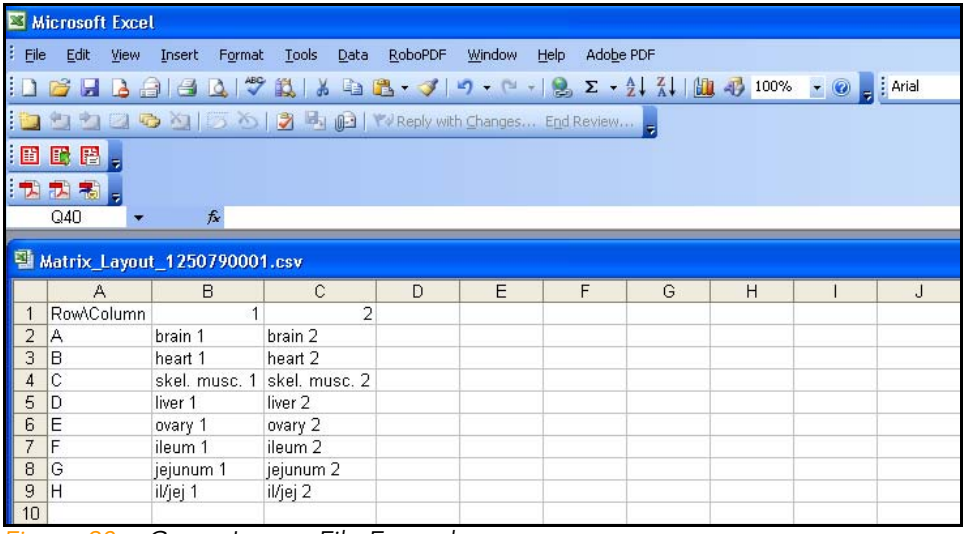
GenomeStudio provides an optional alternative method for creating large numbers of groups in complex experiments that reduces project set-up time. The Apply Group Layout File option allows you to apply a group layout file you previously created in Excel (or a similar application) to a single SAM, BeadChip, or VeraCode Bead Plate.

NOTE

Group layout files must be saved in *.csv format.

GenomeStudio creates groups according to the specifications in the group layout file, and adds selected samples to those groups.

Perform the following steps to create a group layout file (Figure 28).



	A	B	C	D	E	F	G	H	I	J
1	RowAColumn	1	2							
2	A	brain 1	brain 2							
3	B	heart 1	heart 2							
4	C	skel. musc. 1	skel. musc. 2							
5	D	liver 1	liver 2							
6	E	ovary 1	ovary 2							
7	F	ileum 1	ileum 2							
8	G	jejunum 1	jejunum 2							
9	H	il/jej 1	il/jej 2							
10										

Figure 28 Group Layout File Example

1. In the Groups area, click **Apply Group Layout**.
   The Open dialog box appears (Figure 29).

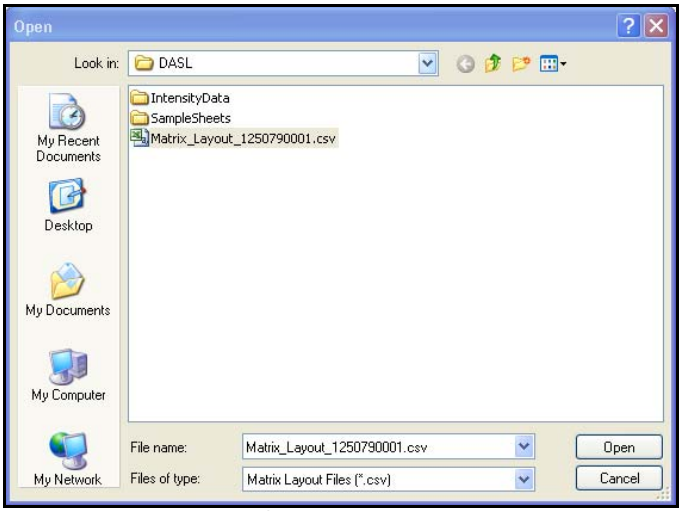


Figure 29 Open Dialog Box

2. Navigate to your group layout file and click **Open**.

   Samples on the selected SAM, BeadChip, or VeraCode Bead Plate are mapped into groups according to the specifics of the group layout file you applied.

   The groups are displayed in the Groups area.

3. [Optional] To display the samples in a group, click the plus sign to the left of that group.

NOTE

Each SAM, BeadChip, or VeraCode Bead Plate layout must be saved in a separate *.csv file.

Chapter 3
Viewing Your Data

Topics

Introduction

This chapter describes the data visualization functions of the GenomeStudio Gene Expression Module, which are used to create and display:

- Scatter plots
- Bar plots
- Line plots
- Box plots
- Heat maps
- Cluster analysis dendrograms
- Control summary reports
- Histograms
- Images

Use these tools to explore the data you create using the Gene Analysis or Differential Expression Analysis tools (described in Chapter 4, *Normalization and Differential Analysis*).

The Gene Expression Module also includes the Illumina Genome Viewer (IGV), the Illumina Chromosome Browser (ICB), and the Illumina Sequence Viewer (ISV). For more information about these tools, see the GenomeStudio Framework User Guide, Part # 11204578.

Scatter Plots

Once gene analysis and/or differential analysis have been completed, you can create scatter plots.

To create a scatter plot:

1. Click  **Scatter Plot**.

   The Plot Columns dialog box appears (Figure 30).

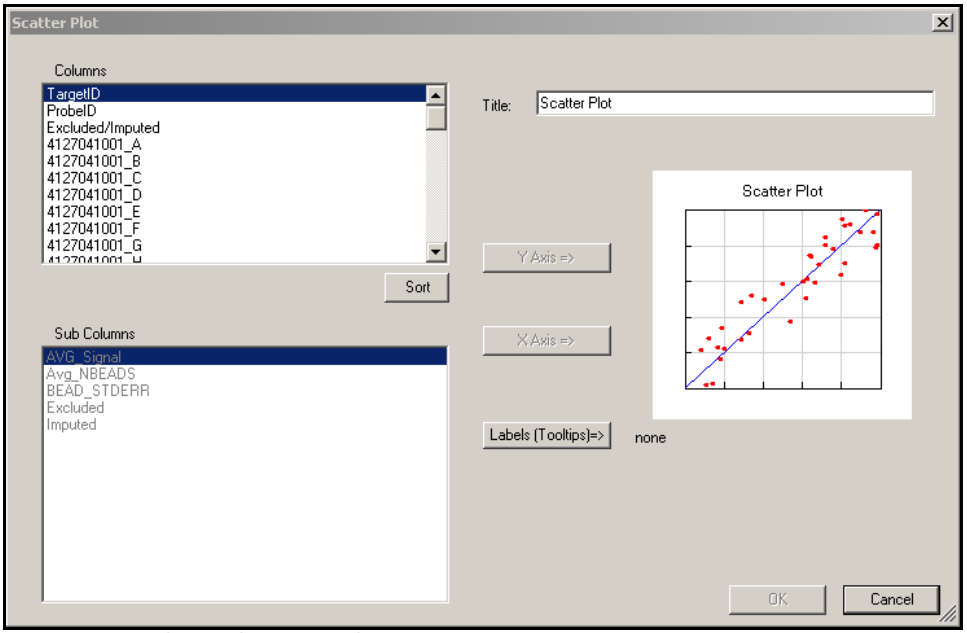


Figure 30 Plot Columns Dialog Box

2. In the Plot Columns dialog box, select options from the Columns and Sub Columns areas for:
   - **Y-axis**
   - **X-axis**
   - **Labels**

   You can choose any subcolumn that contains numerical data for the axes.

   The label you chose appears when you position your cursor over a point in the scatter plot.
3. Click **OK** to create and display the scatter plot (Figure 31).

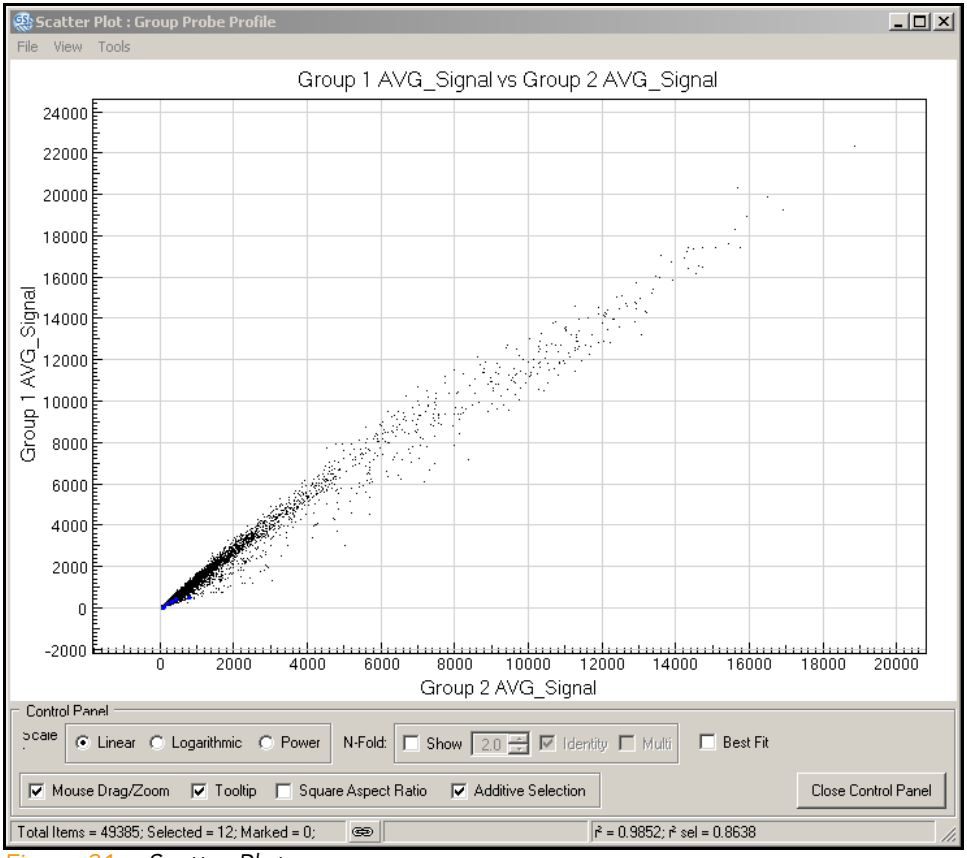


Figure 31 Scatter Plot

The Control Panel appears (lower portion of Figure 31).

Control Panel

Table 1 describes scatter plot Control Panel functions.

Table 1 Scatter Plot Control Panel Functions & Descriptions

Function	Item	Description
Scale	Linear	When enabled, X and Y axes are on a linear scale.
	Logarithmic	When enabled, X and Y axes are on a logarithmic scale.
	Power	When enabled, X and Y axes are on an nth root scale, where n is an odd number from 3 to 9. This allows visual separation of negative values from positive values.
N-Fold	Show	When checked, shows n-fold lines and allows you to select the fold value.
	N-fold setting selector	When **Show** is checked, allows you to select the fold change.
	Identity	When checked, GenomeStudio displays the identity line in bold red color. If a gene is on this line, its X and Y intensities are equal.
	Multi	When checked, GenomeStudio displays additional incremental fold-change regions.

Table 1 Scatter Plot Control Panel Functions & Descriptions (continued)

Function	Item	Description
Options	Best Fit	When checked, presents the Scatter Plot in the optimal fit for the genes of interest. The linear equation is displayed in Control Panel next to R^2 values.
	MouseDrag /Zoom	When checked, allows you to drag and zoom in or out using the mouse wheel. If your mouse does not have a wheel: • Press and hold the **Shift** key while clicking the left mouse button. • Drag to create a rectangle around an area to zoom in on. • Release **Shift** and the mouse button to zoom. • To return to normal view, select Scatter Plot Tools I **Auto Scale Axes**.
	Tooltip	When checked, the scatter plot displays the label you chose.
	Square Aspect Ratio	When checked, the X axis scale is equal to Y axis scale.
	Additive Selection	When checked, any new gene selection will be added to the scatter plot, along with previous selections. When not checked, any new selection replaces the previous selection(s).
	Close Control Panel	When clicked, closes the Control Panel.
Status Bar	Total Items =	The number of total items visible in the Scatter Plot.
	Selected =	The number of selected genes in the Scatter Plot.
	Marked =	The number of marked items visible in the Scatter Plot.
	Linking	 When clicked, toggles synchronization of marking and selection of the data shown in the scatter plot with data in the table.
	Position	Displays current X/Y position of gene (mouse pointer) on the Scatter Plot.

Table 1 Scatter Plot Control Panel Functions & Descriptions (continued)

Function	Item	Description
	R^2	Square of the correlation coefficient. Note: If the scatter plot is in linear scale, the R^2 value is calculated in linear space; if the scatter plot is in logarithmic scale, R^2 is calculated in log space.

4. To use additional plot tools, open the **Tools** menu.

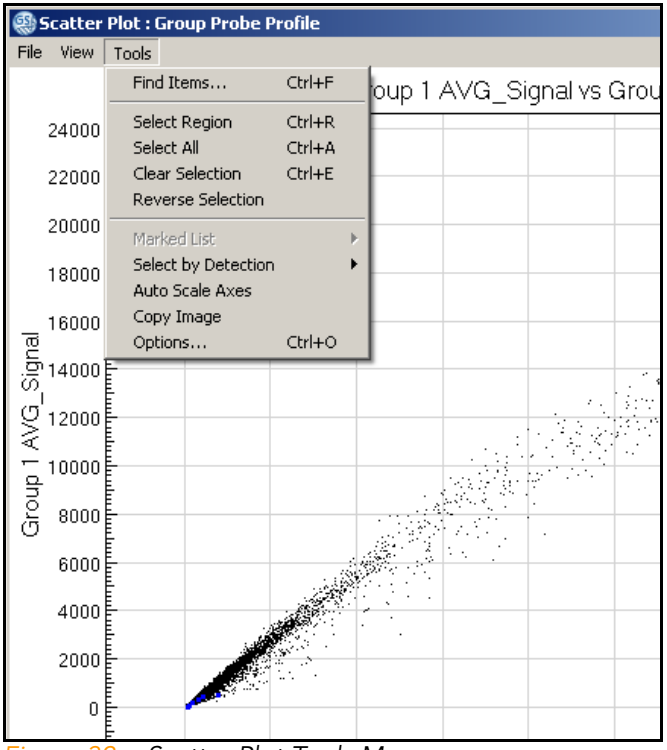


Figure 32 Scatter Plot Tools Menu

Tools Menu

Table 2 describes various scatter plot tools.

Table 2 Scatter Plot Tools Menu Item Descriptions

Tool Name	Description
Find Items	Opens the Find Items dialog box, from which you can enter a list of items separated by commas, or load a search item list from a text file.
Select Region	Converts the cursor to a crosshair tool, which you can use to draw a boundary around any region in the scatter plot. All genes within the boundary are selected.
Select All	Selects all genes in the scatter plot. Genes are displayed in the currently-selected color.
Clear Selection	Clears any previous selections.
Reverse Selection	Reverses the current selection (selects genes that are unselected and clears genes that are selected).
Marked List	Includes operations you can perform on genes you mark in the scatter plot: ▸ **View in Web Browser**—Displays a list of the marked genes in a web browser. ▸ **Save in Text File**—Allows you to save genes in a file in a location you specify. ▸ **Show Item Labels**—Shows item labels, if you applied a label when you created the scatter plot.
Select by Detection	Allows you to select points in a scatter plot based on a detection p-value cut-off. ▸ Both Samples—Uses the same cut-off for both samples. ▸ Sample X—Uses the cut-off only for the X-axis sample. ▸ Sample Y—Uses the cut-off only for the Y-axis sample.
Select by Diff Score	Allows you to select points in a scatter plot based on a Diff Score cut-off for the sample chosen as the Y-axis.
Auto Scale Axes	Automatically scales the X and Y axes of the scatter plot.
Copy Image	Copies the current image to the clipboard.

Table 2 Scatter Plot Tools Menu Item Descriptions

Tool Name	Description
Options	Opens the Scatter Plot dialog box, in which you can set the following parameters: ▸ **Axes**—Displays the minimum and maximum X and Y axis values. When Square Aspect Ratio is not checked, you can set new X and Y axis values. ▸ **Labels**—Allows you to choose font properties for the scatter plot title and axes. ▸ **Data Points**—Allows you to select a point size and style for the Scatter Plot data points. ▸ **Scale**—Allows you to select a power (3, 5, 7, or 9) for the Power setting. ▸ **Colors**—Allows you to set colors for: • Axes • Background • Grid • Data Points • Selection

Context Menu

The context menu contains options that can be applied to the selected project.

To view the scatter plot context menu:

- Right-click anywhere in the scatter plot.

  The scatter plot context menu appears (Figure 33).

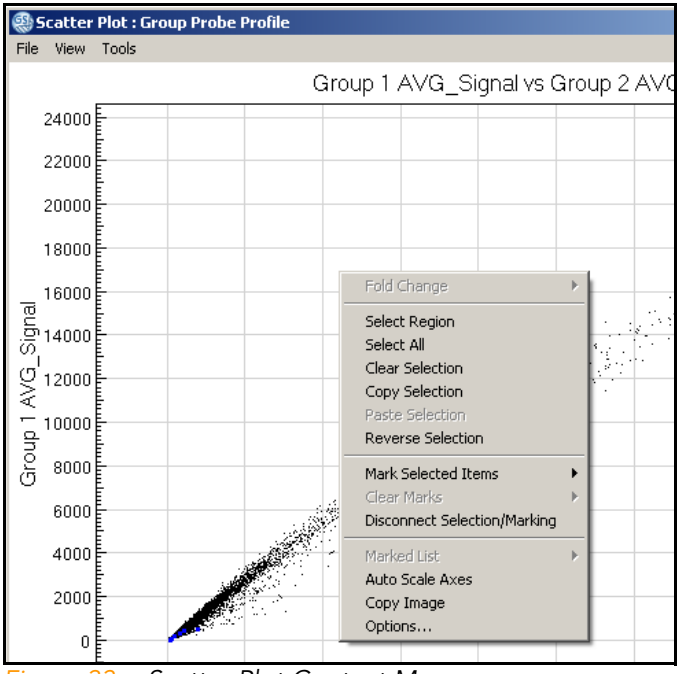


Figure 33 Scatter Plot Context Menu

Table 3 lists context menu items and their functions.

Table 3 Scatter Plot Context Menu Item Descriptions

Item	Description
Fold Change	If fold change lines are present, displays the fold change limits for the current cursor location. Allows you to select or deselect all genes inside the fold change.
Select Region	Allows you to select a region that contains samples of interest.
Select All	Allows you to select all samples.

Table 3 Scatter Plot Context Menu Item Descriptions (continued)

Item	Description
Clear Selection	Clears your selection.
Copy Selection	Copies your selection to the clipboard.
Paste Selection	Pastes the contents of the clipboard to the current location.
Reverse Selection	Allows you to select the samples that were previously unselected.
Mark Selected Items	Allows you to mark items of interest.
Clear Marks	Clears your marks.
Disconnect Selections/Marking	Disconnects synchronization between the graph and the table.
Marked List	Includes operations you can perform on genes you mark in the scatter plot: ▸ **View in Web Browser**—Displays a list of the marked genes in a web browser. ▸ **Save in Text File**—Allows you to save genes in a file in a location you specify. ▸ **Show Item Labels**—Shows item labels, if you applied a label when you created the scatter plot.
Auto Scale Axes	When selected, automatically scales the Scatter Plot X and Y axes.
Copy Image	When selected, places the Scatter Plot image on the clipboard.

Table 3 Scatter Plot Context Menu Item Descriptions (continued)

Item	Description
Options	Opens the Scatter Plot dialog box, in which you can set the following parameters: ▸ **Axes**—Displays the minimum and maximum X and Y axis values. When Square Aspect Ratio is not checked, you can set new X and Y axis values. ▸ **Labels**—Allows you to choose font properties for the scatter plot title and axes. ▸ **Data Points**—Allows you to select a point size and style for the Scatter Plot data points. ▸ **Scale**—Allows you to select a power (3, 5, 7, or 9) for the Power setting. ▸ **Colors**—Allows you to set colors for: • Axes • Background • Grid • Data Points • Selection

Finding Items

The GenomeStudio Gene Expression Module provides a path to gene property information, including gene ID, intensities, and gene ontology information.

To find items in a scatter plot:

1. From the menu bar, select **Tools** | **Find Items** (Figure 34).

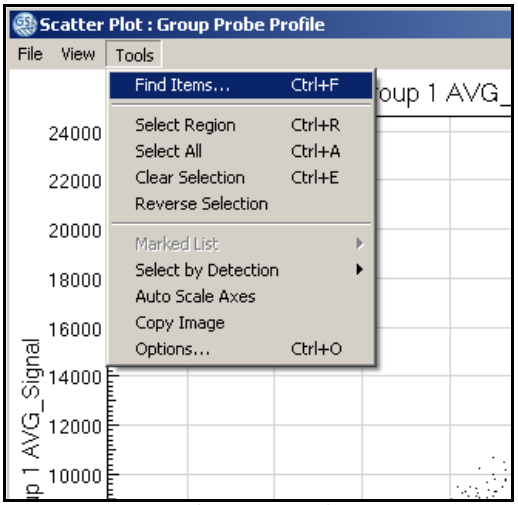


Figure 34 Find Items Tool

2. In the Find Items dialog box (Figure 35), select specific items based on the following fields in the manifest, which includes GenBank database information:
   - **Accession**
   - **Array Address ID**
   - **Chromosome**
   - **Definition**
   - **GI**
   - **Ontology Component**
   - **Ontology Function**
   - **Ontology Process**
   - **Probe Chr Orientation**
   - **Probe Coordinates**
   - **Probe ID**
   - **Probe Sequence**

- **Probe Start**
- **Probe Type**
- **Search Key**
- **Source**
- **Source Reference ID**
- **Species**
- **Symbol**
- **Synonyms**
- **Transcript**

In the Search in pane, select the manifest column you want to search.

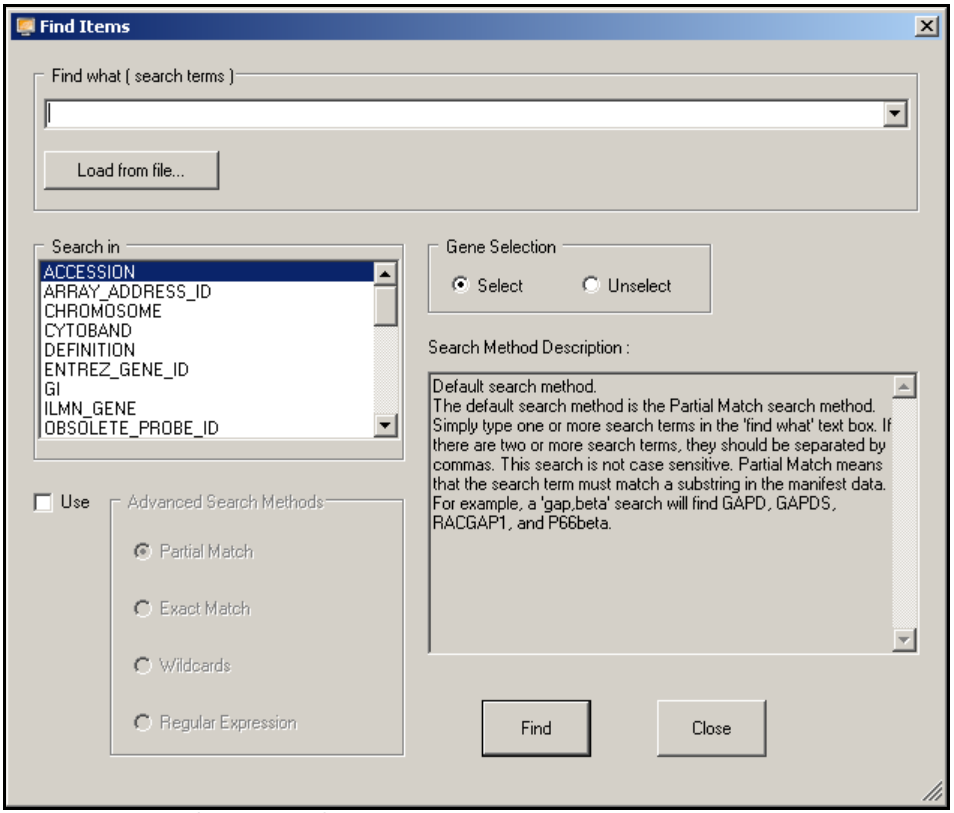


Figure 35 Find Items Dialog Box

3. In the **Find what (search terms)** field, enter the search text.

By default, searches are partial. For example, if you search the word 'VEGF' in the **Symbol** field, the search will return not only VEGF, but also VEGFB and VEGFC.

Multiple search terms can be used, separated by commas.

Search terms can also be loaded from a text file. The file should have each term on a separate line.

4. Do one of the following:
   - Click **Select** to select found genes.
   - Click **Unselect** to clear found genes that were previously selected.
5. Click **Find** to return to the scatter plot with the identified genes highlighted.

   For more advanced search options, click **Use**, to the left of the Advanced Search Methods pane.

   Advanced search methods are described in the Search Method Description area (Figure 35).

   The scatter plot displays the selected gene (Figure 36).
6. [Optional] Use the mouse wheel to zoom in for a magnified view.

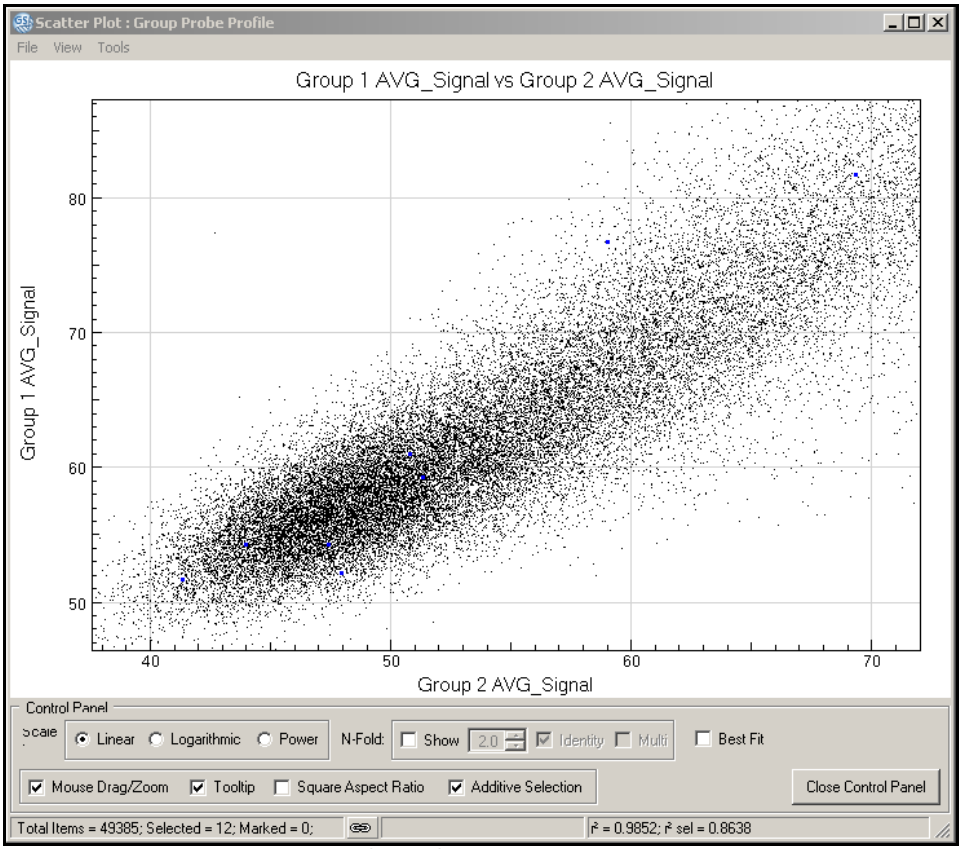


Figure 36 Zoom in to See Selected Genes

7. To display the Gene Properties dialog box,
   c. Right-click the selected gene (Figure 36).
   d. Click **Gene Symbol** in the context menu.

   The Gene Properties dialog box appears (Figure 37).

The following paragraphs illustrate the functions of the Gene Properties dialog box.

Data Tab

Figure 37 illustrates the Data tab of the Gene Properties dialog box.

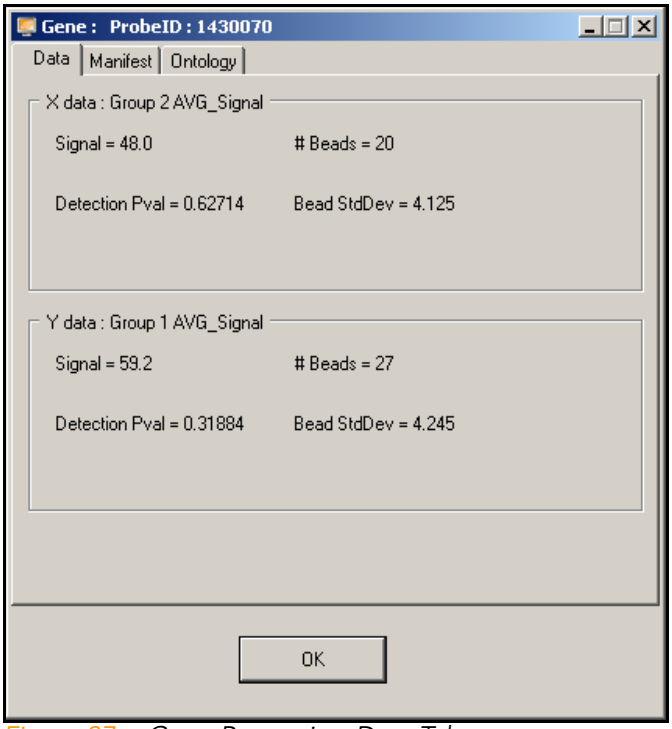


Figure 37 Gene Properties, Data Tab

Manifest Tab

Figure 38, Figure 39, and Figure 40 illustrate functions of the Manifest tab of the Gene Properties dialog box.

1. Click the **Accession** link (Figure 38).

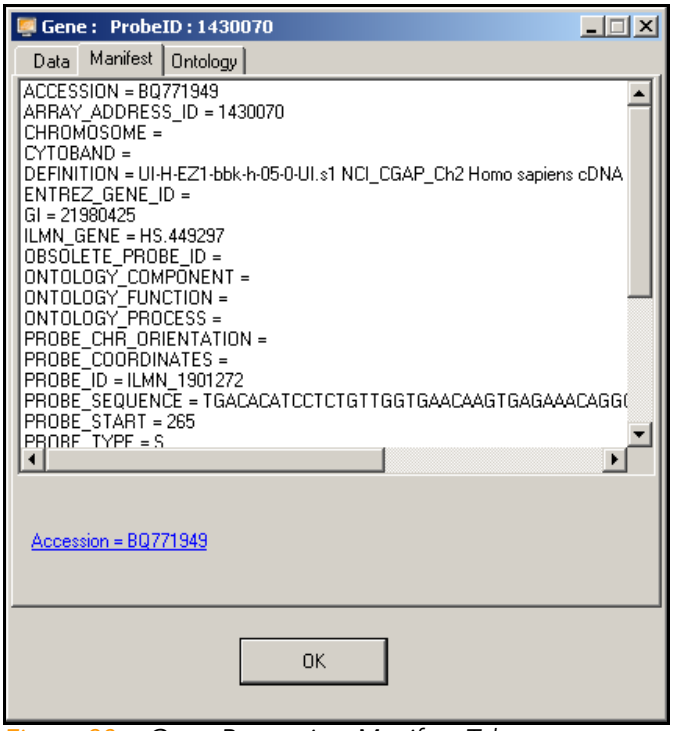


Figure 38 Gene Properties, Manifest Tab

GenomeStudio jumps to the National Center for Biotechnology Information (NCBI) website (Figure 39) where you can view the record for the selected gene (Figure 40).

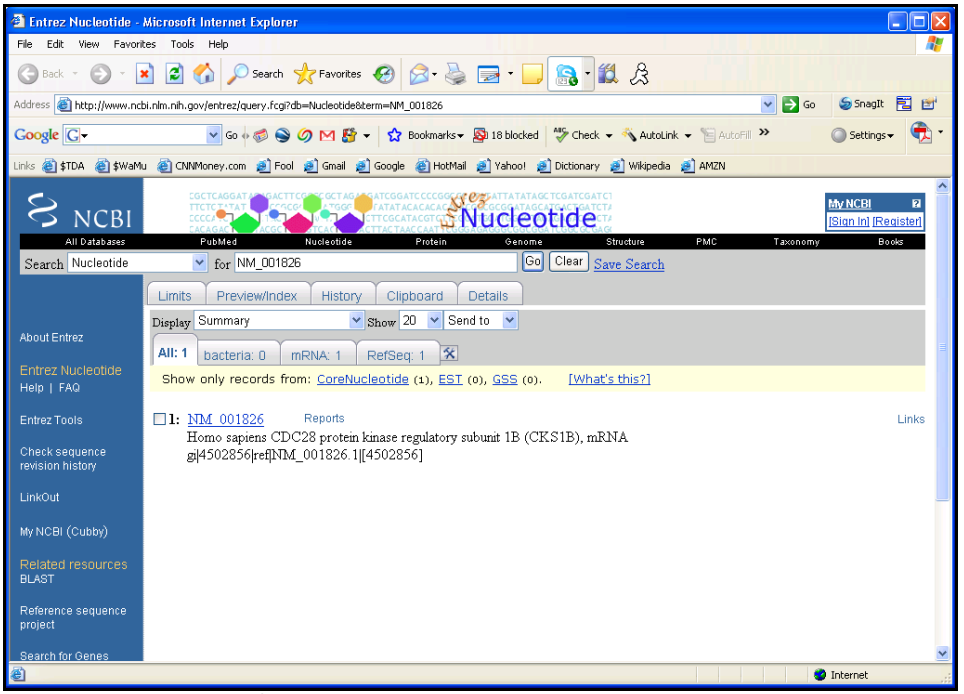


Figure 39 NCBI Website

2. Click the gene name to see the GenBank record (Figure 40).

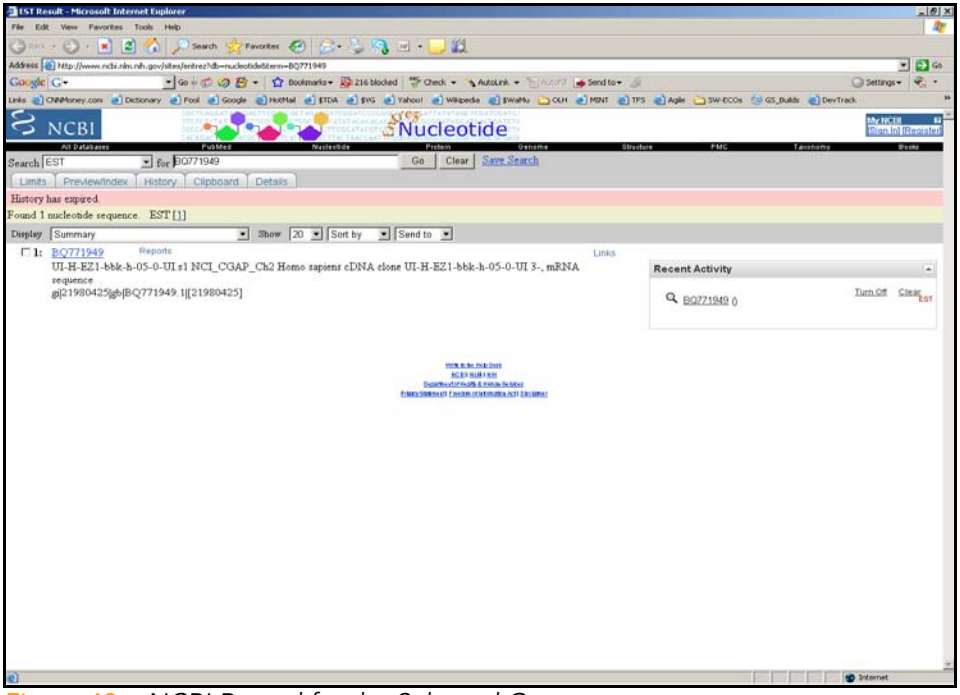


Figure 40 NCBI Record for the Selected Gene

Ontology Tab

Figure 41 illustrates the Ontology tab of the Gene Properties dialog box. This tab provides a quick reference to NCBI gene ontology information.

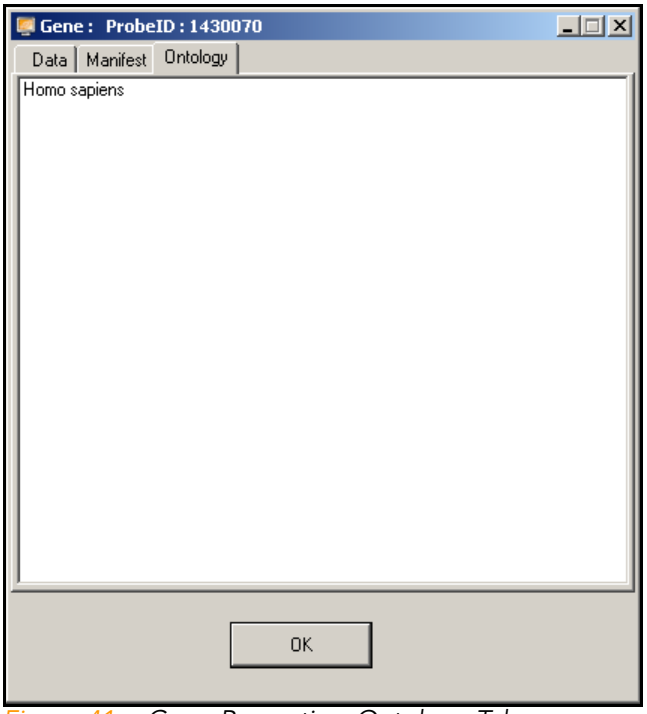


Figure 41 Gene Properties, Ontology Tab

Viewing Marked Data

If you mark your data, you can use the scatter plot to view it in various ways.

For detailed information about marking data, refer to the *GenomeStudio Framework User Guide*, Part # 11204578.

Once you have marked your data, you can select one of the following options from the Marked List option in the scatter plot context menu (Figure 42):

- **View in Web Browser**—displays your marked data in a web browser.
- **Save in Text File**—saves your marked data in a *.txt file.
- **Show Item Labels**—displays item labels in the scatter plot.

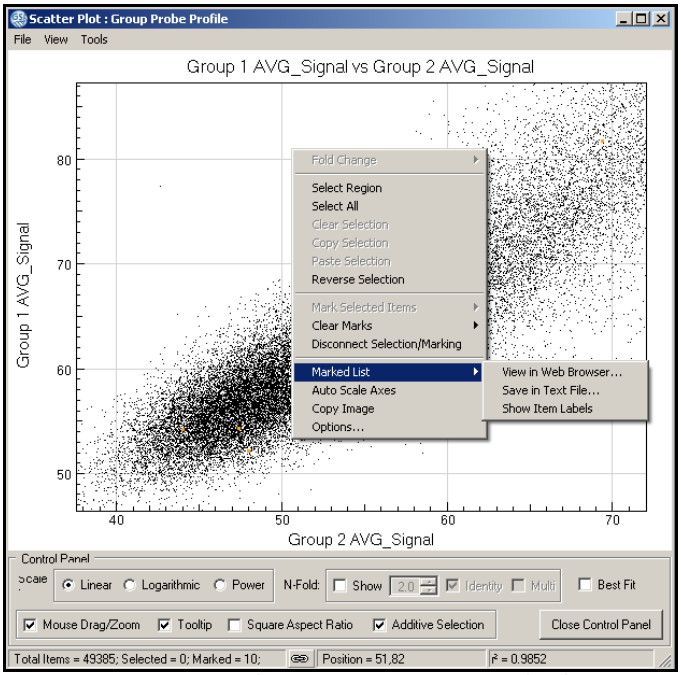


Figure 42 Scatter Plot Context Menu, Marked List Options

Viewing Marked Data in a Web Browser

To view marked data in a web browser, do the following:

1. In the scatter plot context menu, select **Marked List** | **View in Web Browser**.

   The GenomeStudio Scatter Plot Output Data dialog box appears (Figure 43).

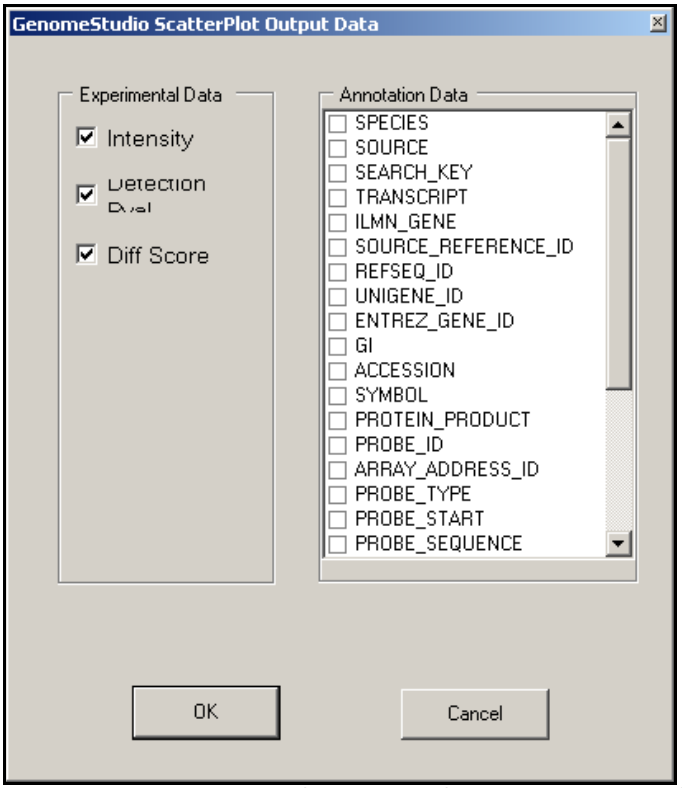
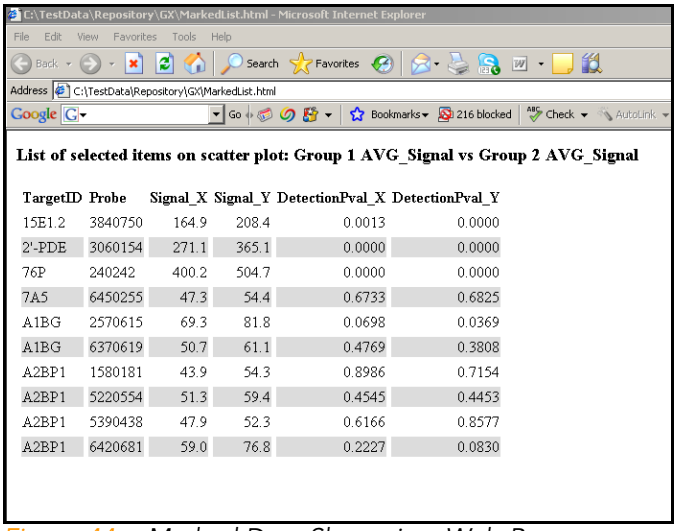


Figure 43 GenomeStudio Scatter Plot Output Data Dialog Box

2. In the Experimental Data and Annotation Data areas, double-click selections for the data you want to include in the web browser.
3. Click **OK**.
4. The data is displayed in your default web browser (Figure 44).



TargetID	Probe	Signal_X	Signal_Y	DetectionPval_X	DetectionPval_Y
15E1.2	3840750	164.9	208.4	0.0013	0.0000
2'-PDE	3060154	271.1	365.1	0.0000	0.0000
76P	240242	400.2	504.7	0.0000	0.0000
7A5	6450255	47.3	54.4	0.6733	0.6825
A1BG	2570615	69.3	81.8	0.0698	0.0369
A1BG	6370619	50.7	61.1	0.4769	0.3808
A2BP1	1580181	43.9	54.3	0.8986	0.7154
A2BP1	5220554	51.3	59.4	0.4545	0.4453
A2BP1	5390438	47.9	52.3	0.6166	0.8577
A2BP1	6420681	59.0	76.8	0.2227	0.0830

Figure 44 Marked Data Shown in a Web Browser

Saving Marked Data in a Text File

To save marked data in a text file, do the following:

1. In the scatter plot context menu, select **Marked List** | **Save in Text File**.

   The Save Marked Genes List As screen appears (Figure 45).

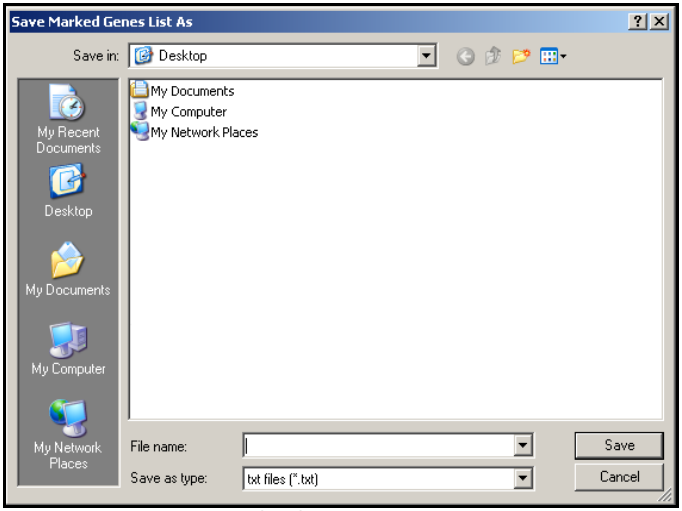


Figure 45 Save Marked Genes List As

2. Browse to the location where you want to save your file.

3. Enter a name for your marked genes list in the **File name** field.
4. Click **Save**.

   The GenomeStudio Scatter Plot Output Data dialog box appears (Figure 46).

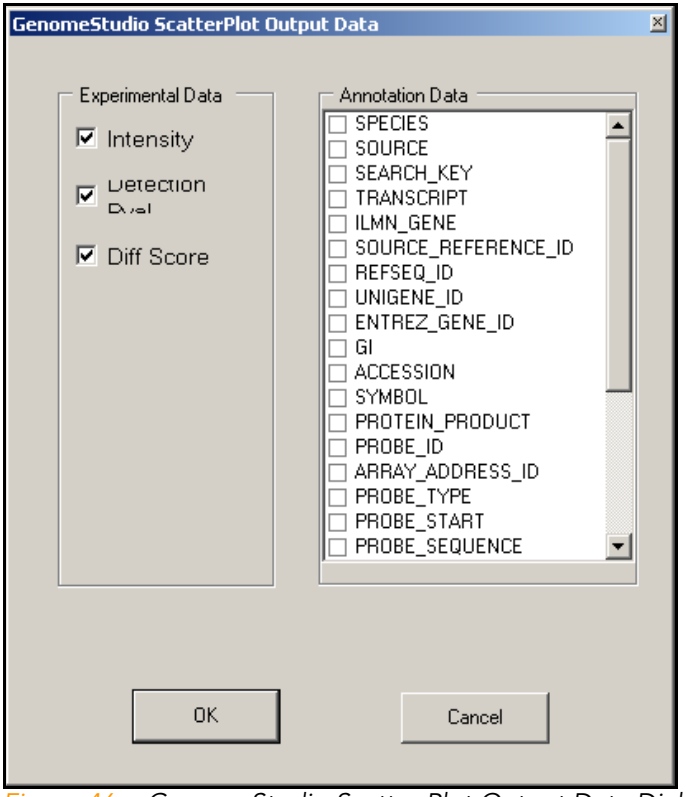


Figure 46 GenomeStudio Scatter Plot Output Data Dialog Box

5. In the Experimental Data and Annotation Data areas, double-click selections for the data you want to include in the web browser.
6. Click **OK**.
7. The data is saved in a text file in the location you specified (Figure 47).

SMG1.txt - Notepad

File Edit Format View Help

TargetID	Probe	Signal_X	Signal_Y	DetectionPval_X	DetectionPval_Y	SPECIES	SOURCE
SEARCH_KEY	TRANSCRIPT	ILMN_GENE					
15E1.2	3840750	164.9	208.4	0.0013	0.0000	Homo sapiens	RefSeq	ILMN_16367	ILMN_16367
15E1.2									
2'-PDE	3060154	271.1	365.1	0.0000	0.0000	Homo sapiens	RefSeq	ILMN_16583	ILMN_16583
2'-PDE									
76P	240242	400.2	504.7	0.0000	0.0000	Homo sapiens	RefSeq	ILMN_19158	ILMN_19158
76P									
7A5	6450255	47.3	54.4	0.6733	0.6825	Homo sapiens	RefSeq	ILMN_5579	ILMN_5579
7A5									
A1BG	2570615	69.3	81.8	0.0698	0.0369	Homo sapiens	RefSeq	ILMN_175569	ILMN_175569
A1BG									
A1BG	6370619	50.7	61.1	0.4769	0.3808	Homo sapiens	RefSeq	ILMN_18893	ILMN_18893
A1BG									
A2BP1	1580181	43.9	54.3	0.8986	0.7154	Homo sapiens	RefSeq	ILMN_5821	ILMN_5821
A2BP1									
A2BP1	5220554	51.3	59.4	0.4545	0.4453	Homo sapiens	RefSeq	ILMN_9081	ILMN_9081
A2BP1									
A2BP1	5390438	47.9	52.3	0.6166	0.8577	Homo sapiens	RefSeq	ILMN_9675	ILMN_9675
A2BP1									
A2BP1	6420681	59.0	76.8	0.2227	0.0830	Homo sapiens	RefSeq	ILMN_5821	ILMN_5821
A2BP1									

Figure 47 Saving Marked Data in a Text File

Showing Item Labels in a Scatter Plot

You can show item labels in a scatter plot if you assigned a label when you created the scatter plot (Figure 48).

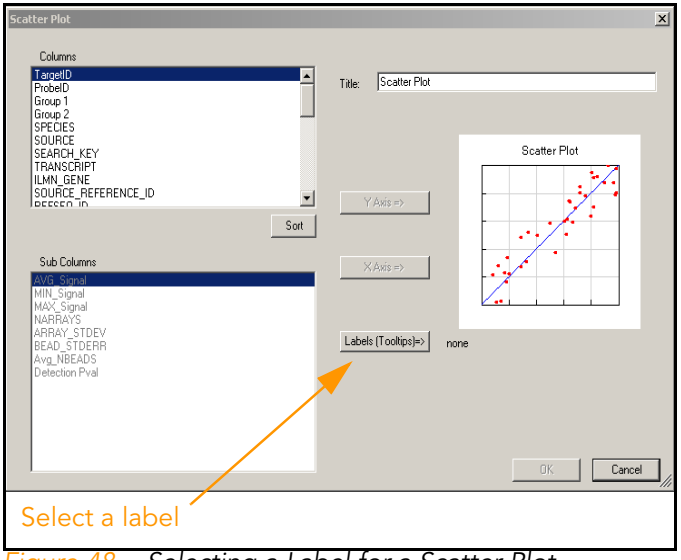


Figure 48 Selecting a Label for a Scatter Plot

For more information about creating a scatter plot, see the Scatter Plots section of the *GenomeStudio 2008.1 Framework User Guide*, Part # 11318815.

To show item labels in the scatter plot, do the following:

1. In the scatter plot context menu, select **Marked List** | **Show Item Labels**.

   The item labels are displayed in the scatter plot (Figure 49).

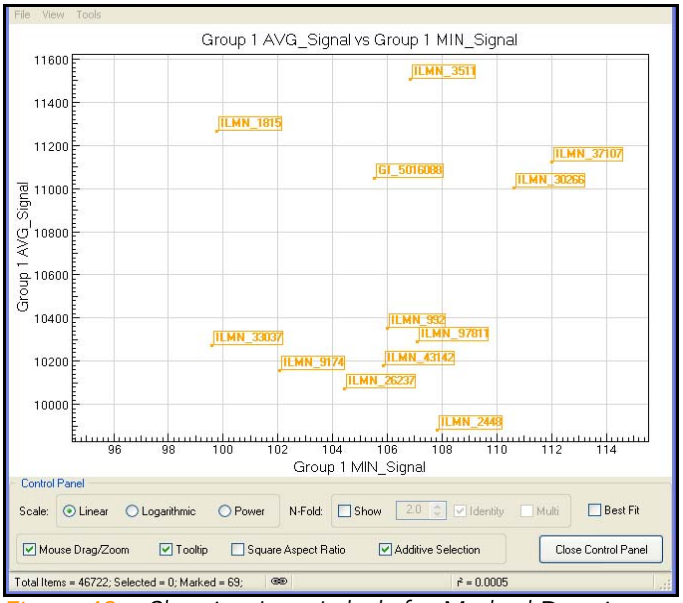


Figure 49 Showing Item Labels for Marked Data in a Scatter Plot

If the labels are too close together to read, you can change their appearance in the scatter plot.

- Using the mouse wheel, scroll up or down to change the resolution of the scatter plot.

Other Scatter Plot Functions

- Click and drag to move the scatter plot.
- Shift-click to zoom into a particular region of the scatter plot.
- Control-click, hold, and move the mouse to select a specific gene or group of genes.

Bar Plots

Once gene analysis or differential analysis is complete, you can create bar plots using GenomeStudio data tables.

To create a bar plot:

1. Click **Show Bar Plot**.

   The bar plot appears.

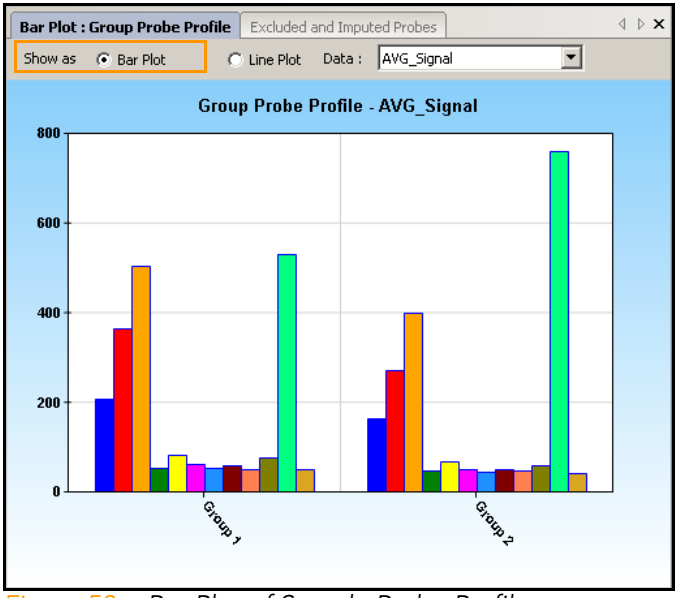


Figure 50 Bar Plot of Sample Probe Profile

NOTE

If you want to view the same data in a line plot, select **Line Plot**.

2. In the **Data** dropdown list (Figure 50), select the type of data to plot.

NOTE

The graph only displays data that are shown in the table. To change which columns are displayed in the table, use the **Column Chooser** tool described in the GenomeStudio Framework User Guide, Part # 11204578.

3. Right-click and select **Properties** from the context menu.
   The Plot Settings dialog box appears (Figure 51).

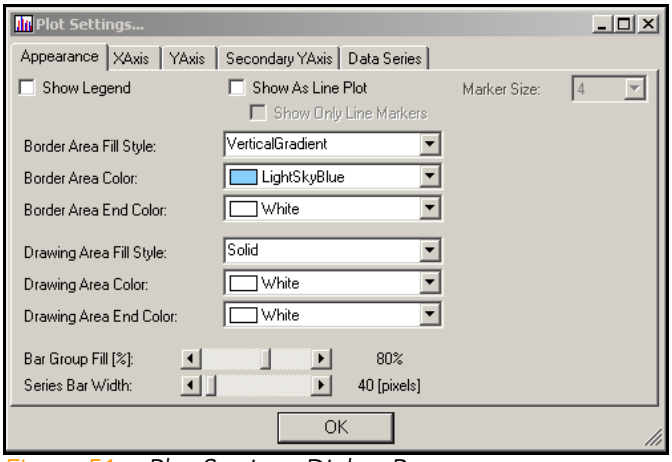


Figure 51 Plot Settings Dialog Box

4. Select attributes for the following aspects of the bar plot:
   - **Appearance**
   - **X-axis**
   - **Y-axis**
   - **Secondary Y-axis**
   - **Data series**
5. Click **OK**.
   The bar plot appears with the attributes you chose (Figure 52).

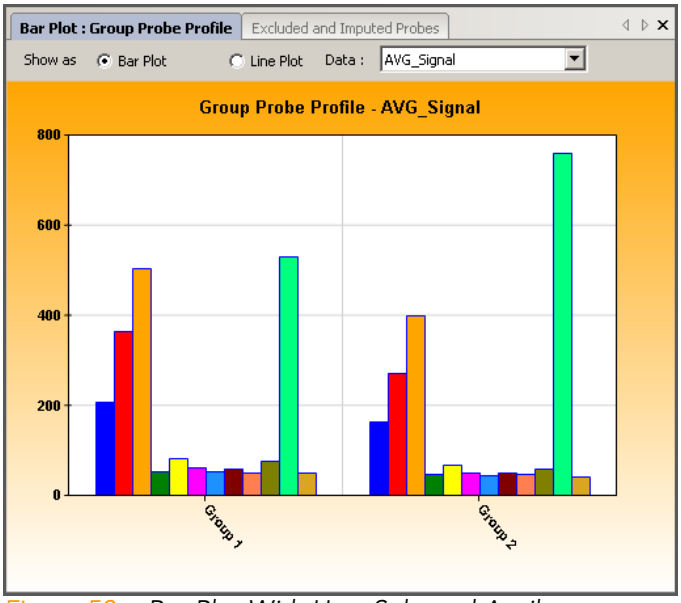


Figure 52 Bar Plot With User-Selected Attributes

Bar Plot Context Menu

Right-click anywhere in the bar plot to view the context menu (Figure 52). The context menu contains features that can be applied to the selected project.

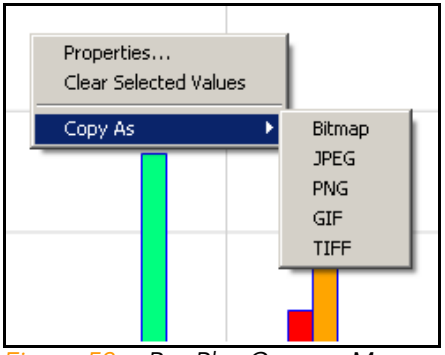


Figure 53 Bar Plot Context Menu

Table 4 lists context menu items and their functions.

Table 4 Bar Plot Context Menu Item Descriptions

Item	Description
Properties	Displays the **Plot Settings** dialog box, which allows you to change the characteristics of the bar plot.
Clear Selected Values	Clears the selected values.
Copy As	Copies the bar plot image to the clipboard in any of the following file formats: BMP, JPEG, PNG, GIF, or TIFF.

Heat Maps

Once gene analysis or differential analysis has been completed, you can create heat maps using GenomeStudio output files.

To create a heat map:

1. Click  **Column Chooser**.

   The Column Chooser dialog box appears.

2. Select rows and columns from the data table that you want to display in a heat map.

3. Click **OK**.

   The Column Chooser dialog box closes.

4. In the table toolbar, click  **Heat Map**.

   The Plot Sample Subcolumns in a Heat Map dialog box appears.

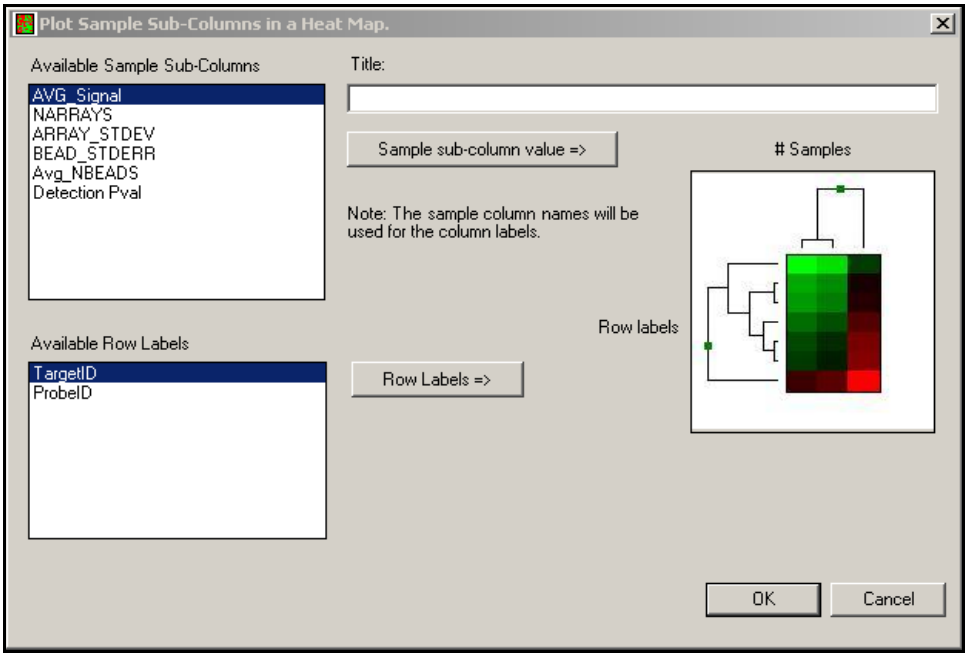


Figure 54 Creating a Heat Map

5. [Optional] Enter a title in the **Title** field.
6. Select an attribute in the Available Sample Subcolumns area.

NOTE

Available sample subcolumns must contain plottable (numerical) data.

7. Select an attribute in the Available Row Labels area.
8. Click **OK** to create and display the heat map (Figure 55).

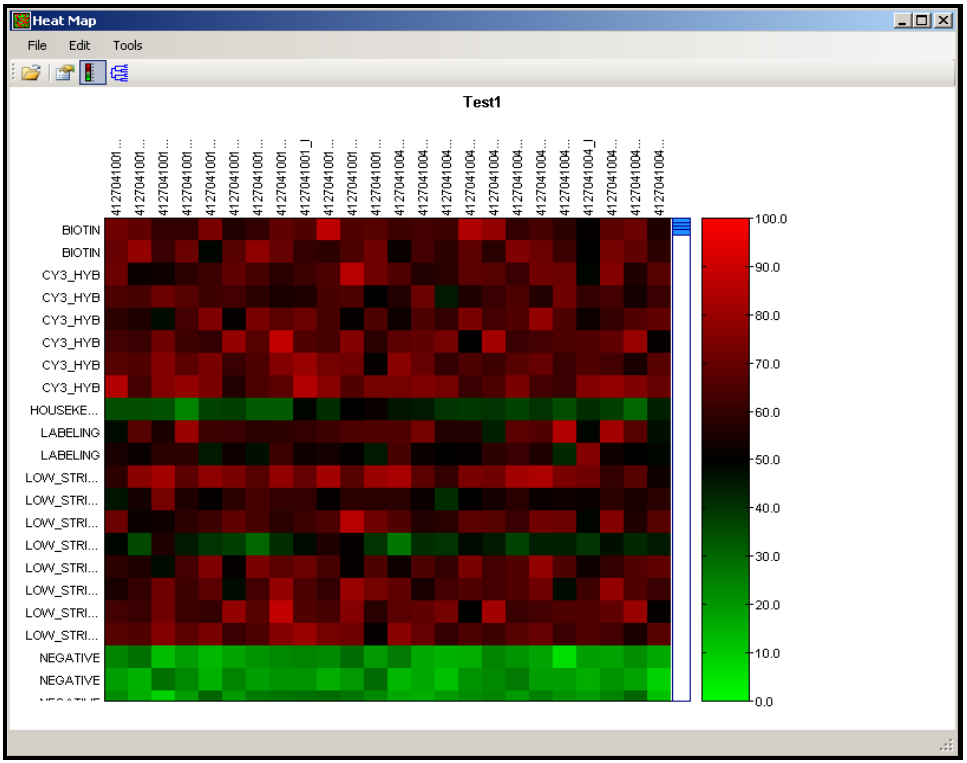


Figure 55 Heat Map

Heat Map Tools Menu

9. To use additional heat map tools, click **Tools** on the menu bar.
10. Select **Cluster** or **Generate Presentation Image** (Figure 56).

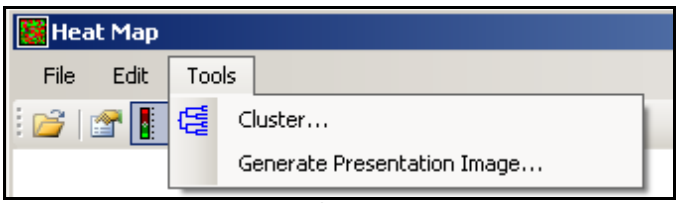


Figure 56 Heat Map Tools Menu

Table 5 describes the available heat map tools.

Table 5 Heat Map Tools Menu Item Descriptions

Tool Name	Description
Cluster	Displays the **Cluster Options** window, from which you can select whether to cluster rows or columns, as well as the hierarchical clustering method to use (COR, ACOR, Manhattan, or Euclidian). See *Cluster Analysis Dendrograms* on page 73 for more information about clustering methods.
Generate Presentation Image	Displays the **Presentation Image Setup** window, from which you can set options for and generate a presentation image.

Heat Map Context Menu

Right-click anywhere in the heat map to view the context menu (Figure 57). The context menu contains options that can be applied to the selected heat map.

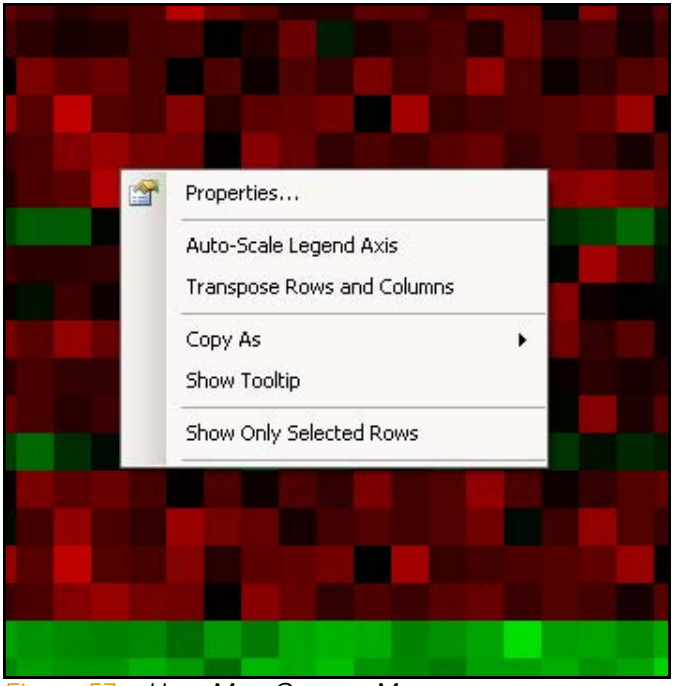


Figure 57 Heat Map Context Menu

Table 6 lists describes map context menu items.

Table 6 Heat Map Context Menu Item Descriptions

Item	Description
Properties	Displays the **Heat Map Properties** dialog box, from which you can alter the visual properties of the title, legend, rows, columns, and scroll bars of the heat map.
Auto-Scale Legend Axis	Automatically scales the axis to a level appropriate for the data being displayed.
Transpose Rows and Columns	Switches the positions of rows and columns.
Copy As	Copies the heat map to the clipboard as any of the following image types: BMP, JPEG, TIFF, PNG, or GIF.
Show Tooltip	Displays a tooltip when the cursor is positioned over the heat map.
Show Only Selected Rows	Displays only selected rows in the heat map.

For more information about working with heat maps, see the *Heat Maps* section of the *GenomeStudio Framework 2008.1 User Guide*, Part # 11318815.

Cluster Analysis Dendrograms

Clustering is an analysis method used to group sets of objects into subsets or clusters. Objects assigned to the same cluster are more closely related to one another than to objects assigned to different clusters. In the context of gene expression, clustering can be used to answer two basic questions:

- Which genes show similar patterns of gene expression across a series of samples?

Knowing this is useful for identifying genes in common pathways, or genes that coordinately respond to the same stimuli.

- Which samples are most similar based on the expression levels of genes within them?

  Knowing this is useful for identifying conditions that generate a common metabolic response. For example, in a toxicology study, if an unknown compound induces a pattern of expression similar to that induced by a panel of genotoxins, it is likely that the unknown is a genotoxin.

Mathematicians have devised dozens of clustering methods using different metrics to establish relationships between sets of values. In GenomeStudio, clustering occurs using the nesting with average linkage method. GenomeStudio offers four clustering metrics for calculating dissimilarities:

- **Correlation (COR)**—Computes the Pearson correlation using a 1—r distance measure.
- **Absolute Correlation (ACOR)**—Computes the Pearson correlation using a 1—|r| distance measure.
- **Manhattan**—Computes the distance between two points if a grid-like path is followed.
- **Euclidian**—Computes the shortest distance between two points.

Illumina recommends using multiple clustering methods to validate results. Groupings with a true biological basis will usually show up regardless of the algorithm used.

Similarities and Distances

There are several ways to compute the similarity of two series of numbers. The most commonly used similarity metric is the Pearson correlation. The Pearson correlation coefficient between any two series of numbers $X = \{x_1, x_2, \ldots x_N\}$ and $Y = \{y_1, y_2, \ldots y_N\}$ is defined as:

$$\mathbf{r} = \frac{1}{N}\sum i = 1, N\left(\frac{x_i - \bar{x}}{\sigma_x}\right)\left(\frac{y_i - \bar{y}}{\sigma_y}\right)$$

Distance is then defined as $1 - r$ for Correlation and $1 - |r|$ for Absolute Correlation. GenomeStudio also uses Manhattan $(\Sigma|X_1 - Y_1|)$ and squared Euclidean $(\Sigma(X_1 - Y_1)^2)$ distances.

GenomeStudio presents the clustering information in the form of a dendrogram, a tree-like structure with branches that correspond to genes or samples, depending on how the analysis is run. The distance on the X axis establishes the similarity relationships among the genes or samples. For example, if the dendrogram plots the similarity of samples based on gene expression, samples C and D are very similar to each other, less similar to B, and even less similar to A (Figure 58).

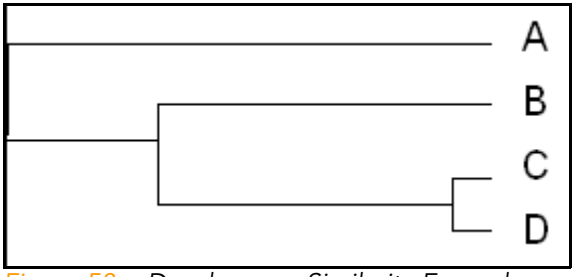


Figure 58 Dendrogram Similarity Example

After clustering, nodes are reordered starting near the top to ensure that node "ar" is closer to "B" than node "al", and node "bl" is closer to "A" than node "br" (Figure 59).

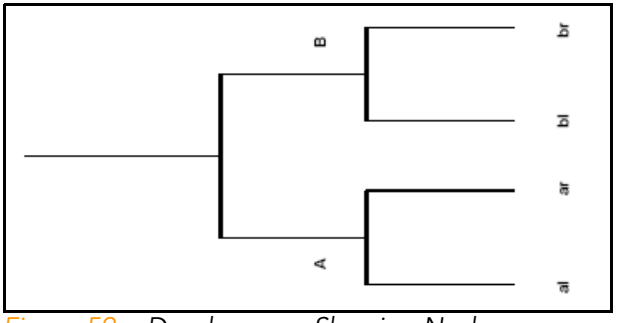


Figure 59 Dendrogram, Showing Nodes

Analyze Clusters

To analyze clusters:

1. Click  **Run Cluster Analysis** to open the cluster analysis tool.
2. In the Cluster Analysis dialog box (Figure 60), perform the following steps:
   a. Groups pane—Highlight the group(s) whose clusters you wish to analyze. Select the **Sort** checkbox to sort the items in the Groups listbox alphabetically in ascending order.
   b. Cluster pane—Click **Genes** or **Samples**.

      If you select Genes, the dendrogram displays a cluster of genes.

      If you select Samples, the dendrogram displays a cluster of samples.

      
      NOTE

      Clustering samples is much faster than clustering genes. Clustering thousands of genes can take hours.

   c. Metric pane—Select the metric you would like GenomeStudio to use to calculate clusters.

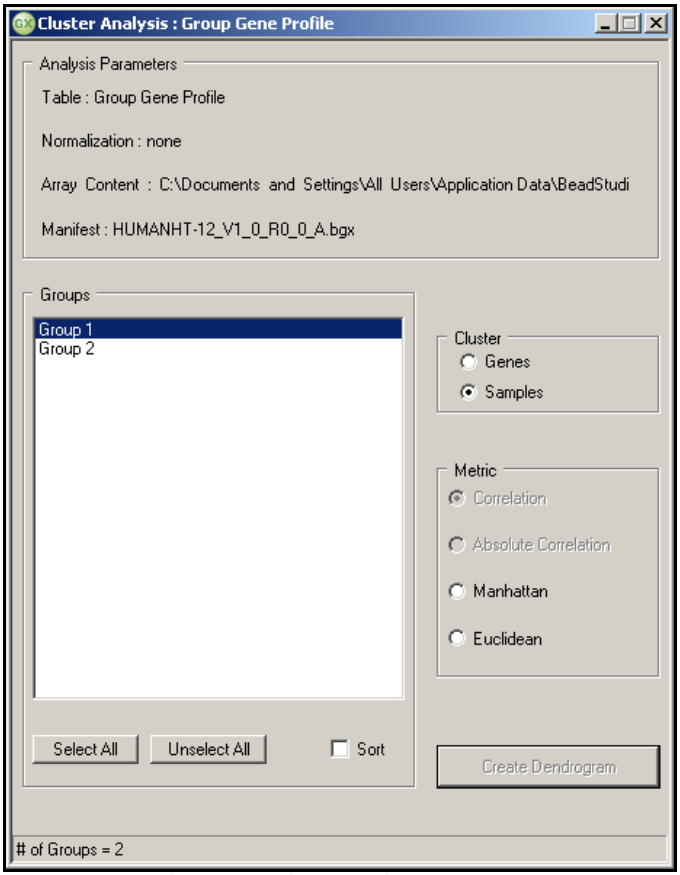


Figure 60 Cluster Analysis Dialog Box

d. Click **Create Dendrogram** to view the graph (Figure 62). A status bar displays your progress (Figure 61).

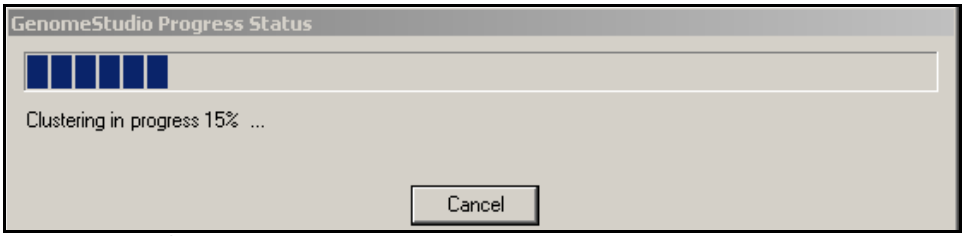


Figure 61 Clustering Progress Status

NOTE

The scale at the bottom of the dendrogram shows dissimilarity between nodes. See *Similarities and Distances* on page 74.

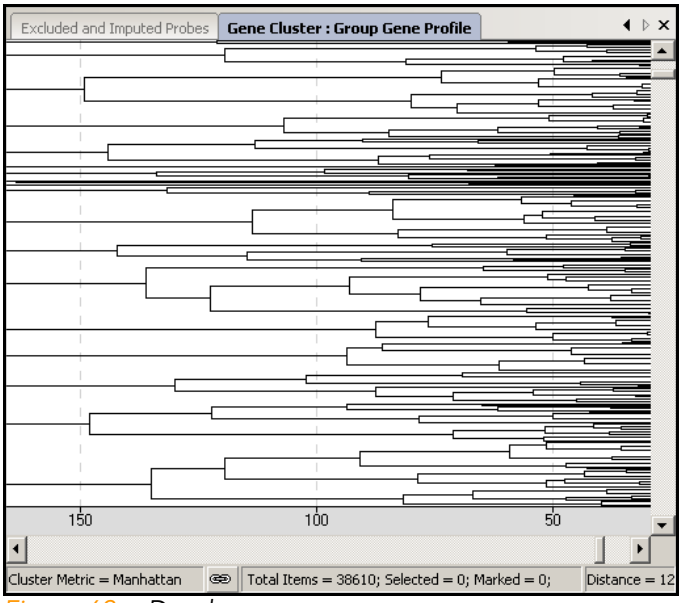


Figure 62 Dendrogram

3. Right-click in the dendrogram to view the context menu (Figure 63).

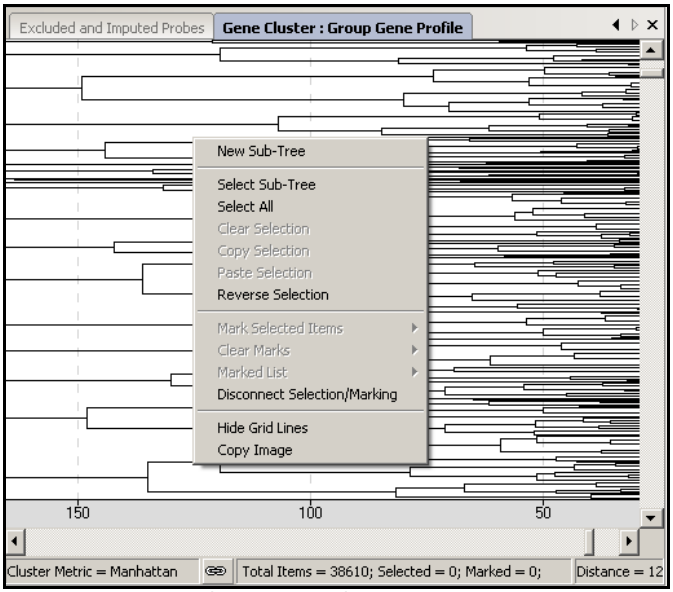


Figure 63 Dendrogram with Context Menu

Dendrogram Context Menu Selections

Table 7 describes dendrogram context menu selections.

Table 7 Dendrogram Context Menu Selections

Item	Description
New Sub-Tree	Displays the selected sub-tree in a new window. This feature is disabled when the cursor is outside of any tree.
Select Sub-Tree	Highlights the sub-tree in blue. This feature is disabled when the cursor is outside of any tree.
Select All	Selects all sub-trees.
Clear Selection	Clears any selection.
Copy Selection	Copies the current selection(s) to the clipboard.
Paste Selection	Pastes the current clipboard contents to a location you choose.
Reverse Selection	Reverses the last selection made.
Mark Selected Items	Marks the currently-selected items.
Clear Marks	Clears all marks.
Marked List	Includes operations you can perform on genes you mark in the scatter plot: • **View in Web Browser**—Displays a list of the marked genes in a web browser. • **Save in Text File**—Allows you to save genes in a file in a location you specify. • **Show Item Symbols**—Shows item symbols.
Disconnect Selection/Marking	Disconnects synchronization between the graph and the table.
Hide Grid Lines	Hides background grid lines.
Copy Image	Copies the current image to the clipboard.

View the Sub-Tree List Directly in the Dendrogram

- To view the sub-tree list directly in the dendrogram, zoom in by using the mouse wheel. The sub-tree list appears to the right of the dendrogram (Figure 64).
- To resize the dendrogram, press **Ctrl** and the right or left arrow keys on your keyboard. The scale adjusts appropriately.
- To return the dendrogram to its default size, click the mouse button.

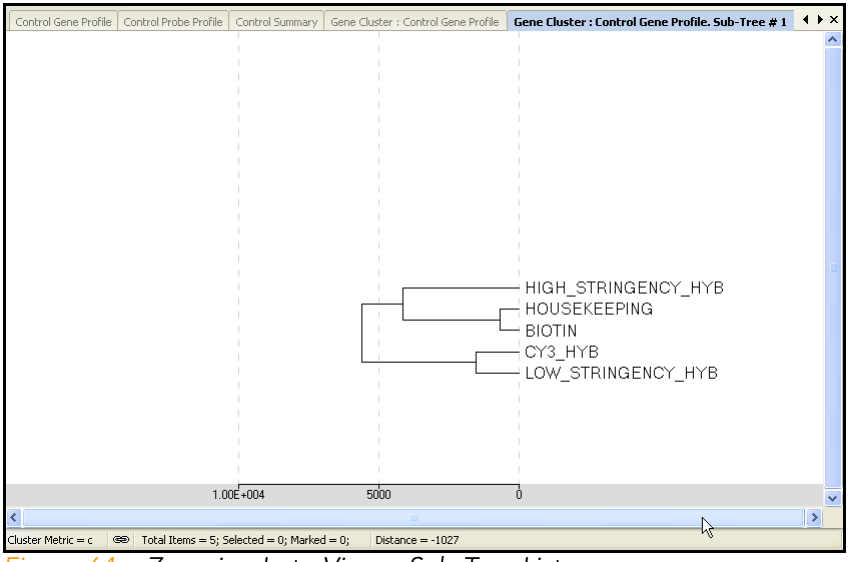


Figure 64 Zooming In to View a Sub-Tree List

Copy/Paste Clusters

You can copy and paste gene clusters from a scatter plot to a dendrogram and vice versa. Refer to Figure 65 through Figure 67.

From Scatter Plot to Dendrogram

To select genes within clusters that you want to copy from a scatter plot and paste into a dendrogram:

1. Select **Tools** | **Select Region**.
2. Using the crosshair tool, draw around the genes you wish to copy.

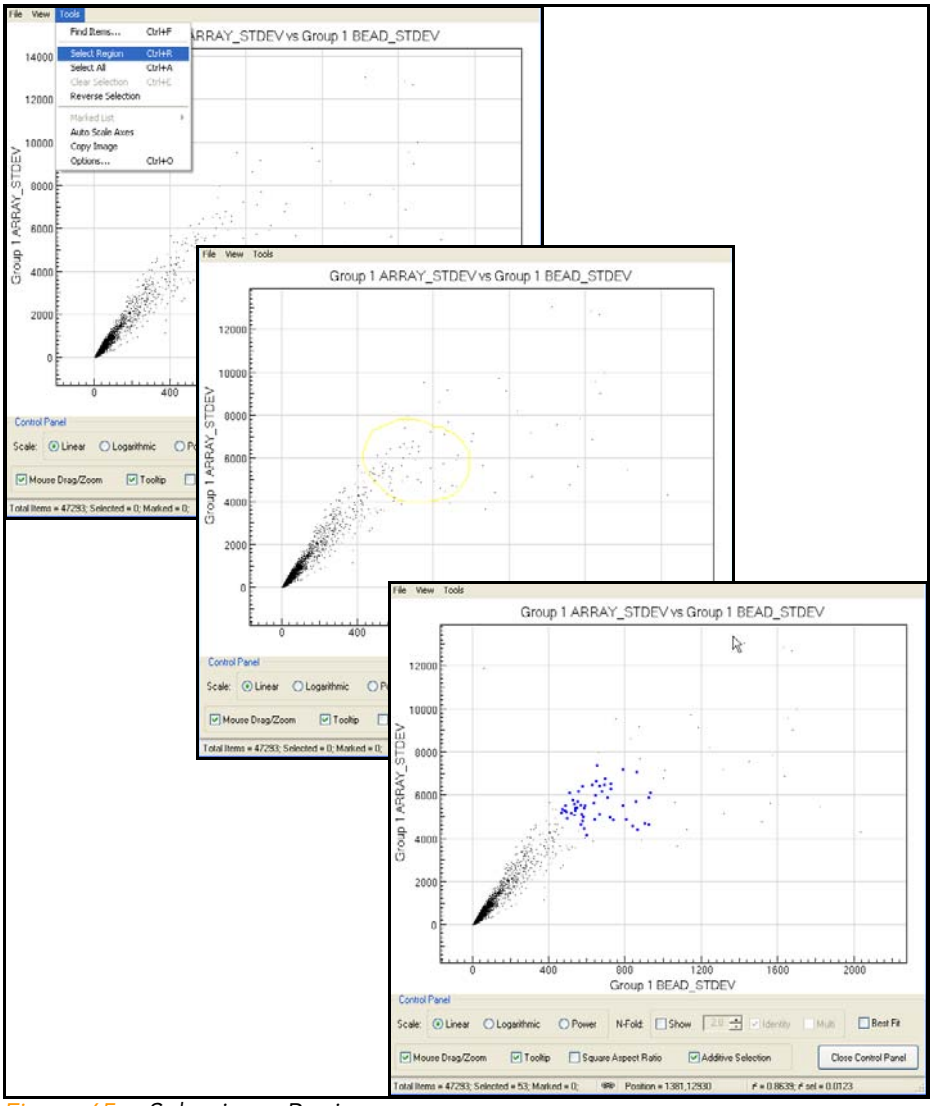


Figure 65 Selecting a Region

NOTE

The selected genes will change color: to blue by default, or to the color you set in Scatter Plot Options.

3. To copy the selection to the clipboard, select **Tools** | **Copy Selection**.
4. To paste the selection into the dendrogram, select **Tools** | **Paste Selection**.

From Dendrogram to Scatter Plot

To select clusters for copying from the dendrogram:

1. Position the cursor over the sub-tree you want to copy.
2. Right-click and click **Select Sub-Tree** from the context menu.

Click ***inside*** the sub-tree you want to select. The sub-tree you select appears in blue.

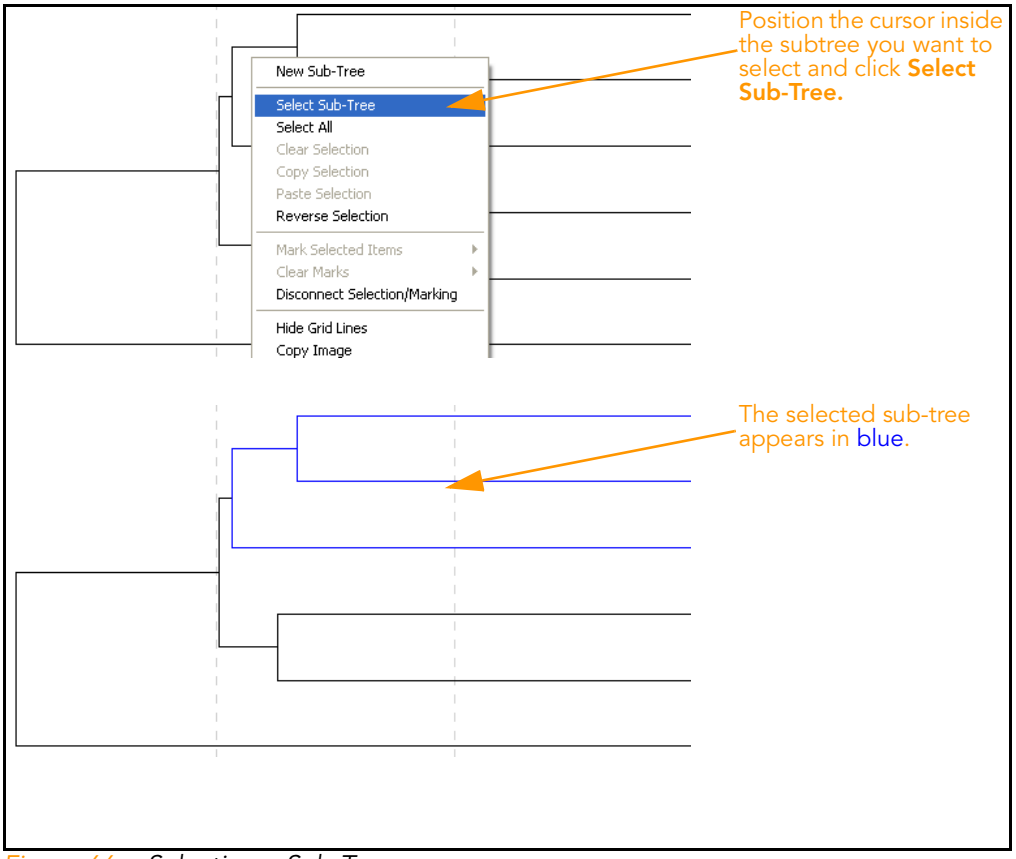


Figure 66 Selecting a Sub-Tree

3. Right-click and select **Copy Selection** from the context menu.

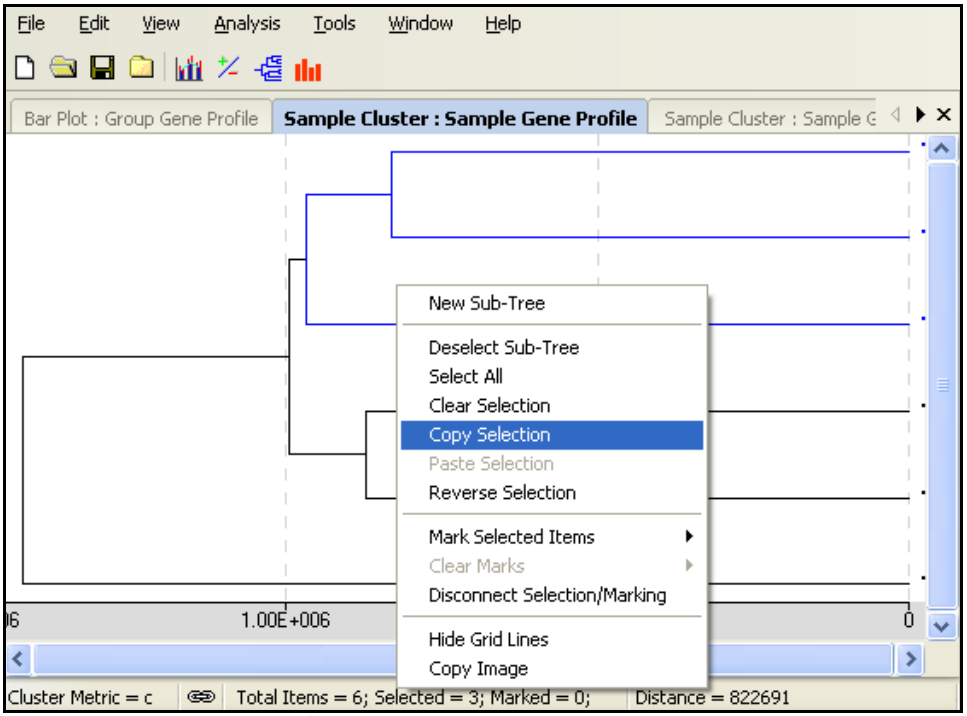


Figure 67 Copying a Sub-Tree

4. To paste your selection into a scatter plot, select **Tools** | **Paste Selection**.

   The genes you selected in a subtree are pasted from the dendrogram into the scatter plot.

Control Summary Reports

The following sections describe Control Summary Reports for the Direct Hyb assay and the DASL assay.

For the DirectHyb Assay

GenomeStudio displays a graphic Control Summary Report for selected samples based on the performance of the built-in controls (Figure 68).

For more detailed information about the controls, see the *System Controls* appendix in the appropriate Illumina product guide.

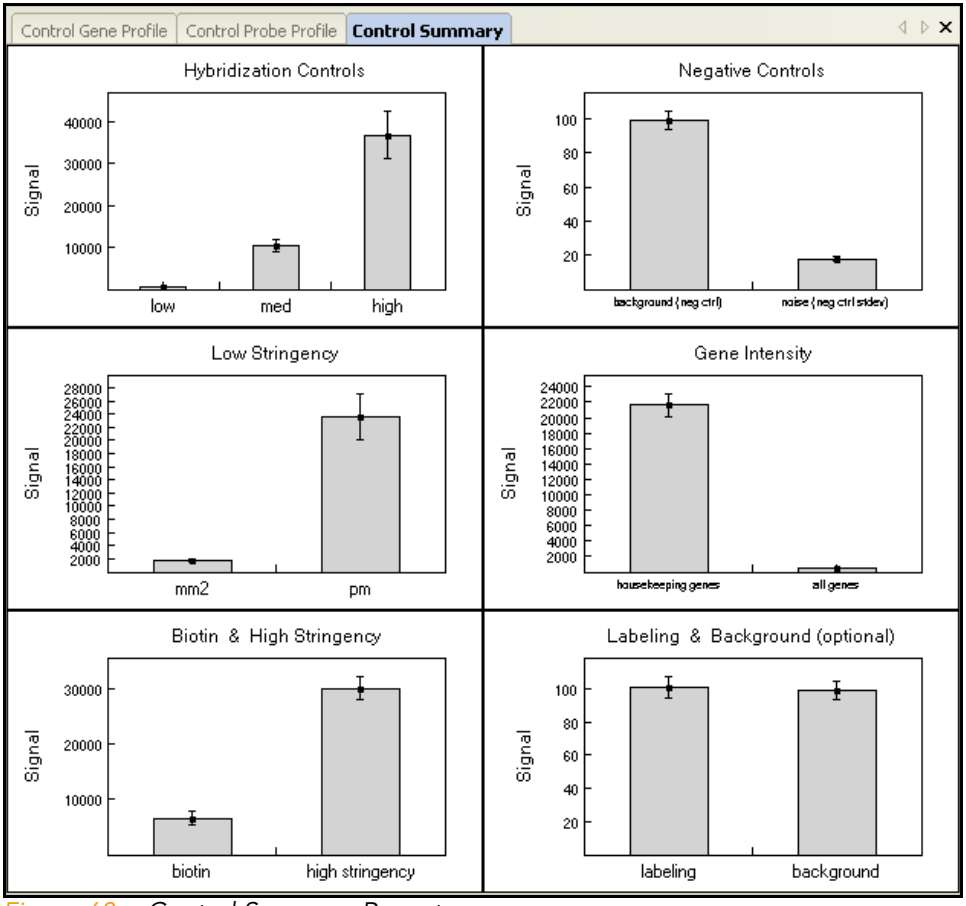


Figure 68 Control Summary Report

NOTE

Negative controls undergo outlier removal. Intensity of negative controls three standard deviations from the mean are identified as outliers. These are removed before the average intensity is computed.

5. To view secondary graph(s), click on a data point at the top of a bar in any of the graphs shown in Figure 68. Each point in the secondary graph represents a sample.

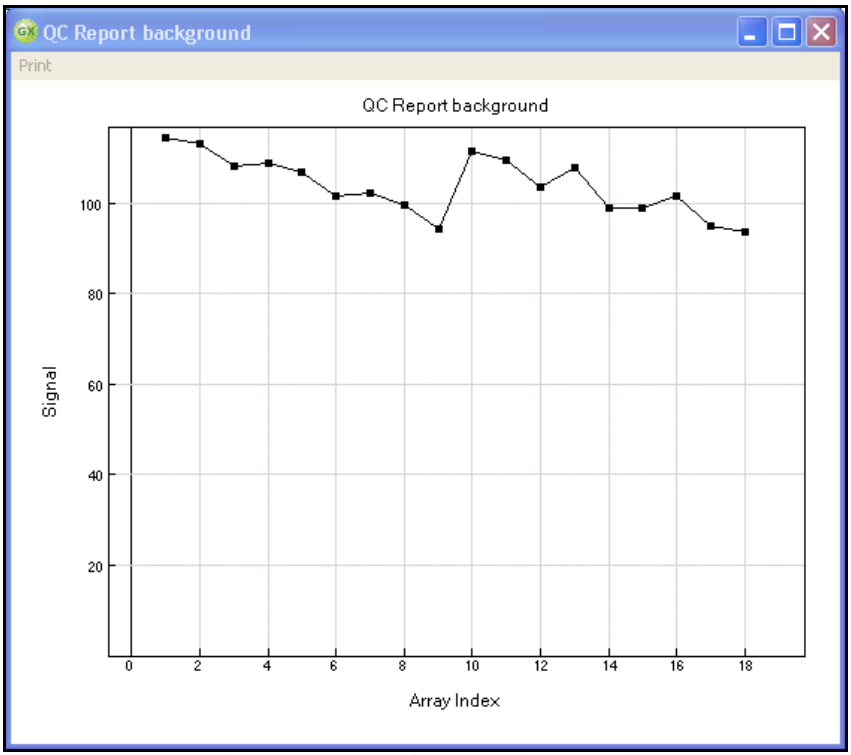


Figure 69 Housekeeping Controls Secondary Graph

6. To copy, change the page setup, or see a print preview, right click in any graph to use the context menu.
7. To print the graph, click **Print** in the menu bar (Figure 70).

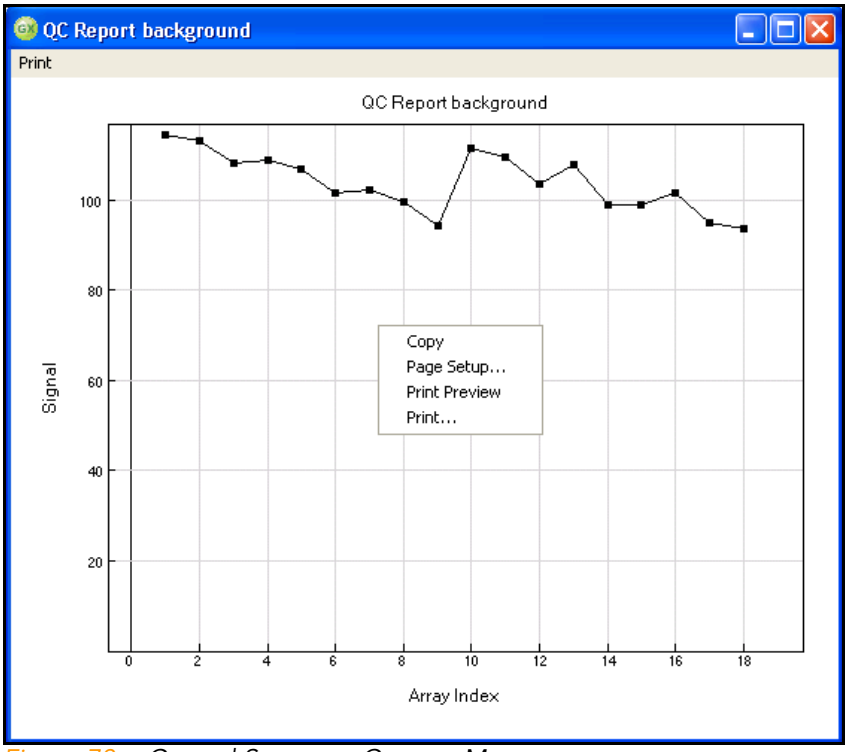


Figure 70 Control Summary Context Menu

For the DASL Assay

GenomeStudio can display a graphic Control Summary for the selected samples based on the performance of the built-in controls (Figure 71).

For more detailed information on the controls, see the *System Controls* appendix in the appropriate Illumina product guide.

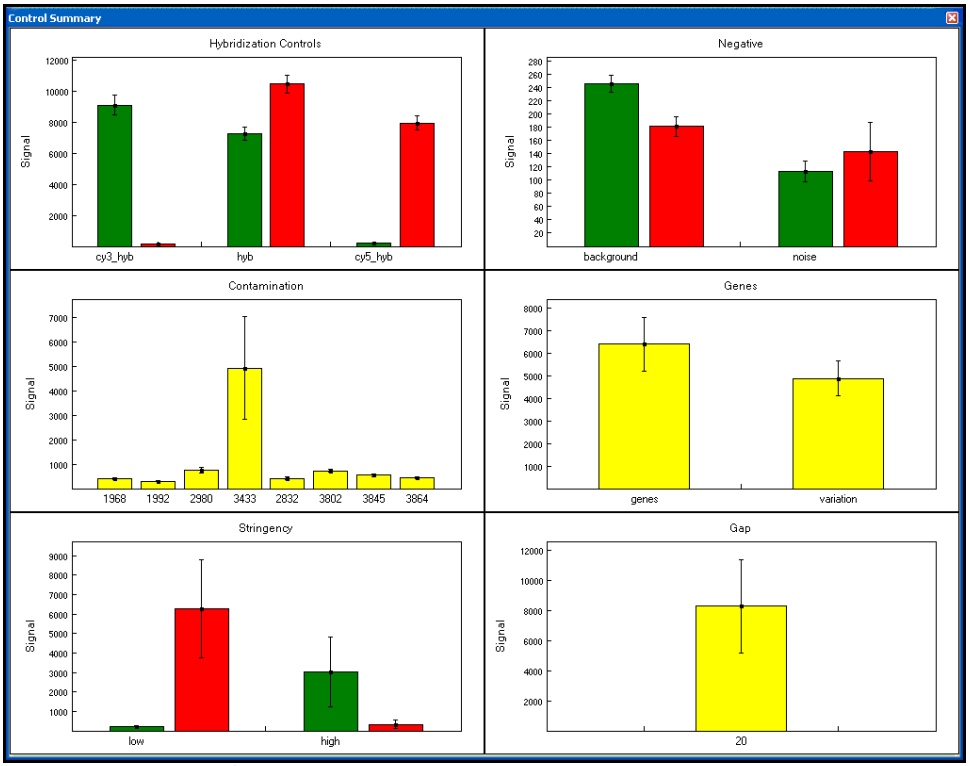


Figure 71 Control Summary Report

8. To view secondary graph(s), click on a data point in any of the graphs shown in Figure 71. Each point in the secondary graph represents a sample.

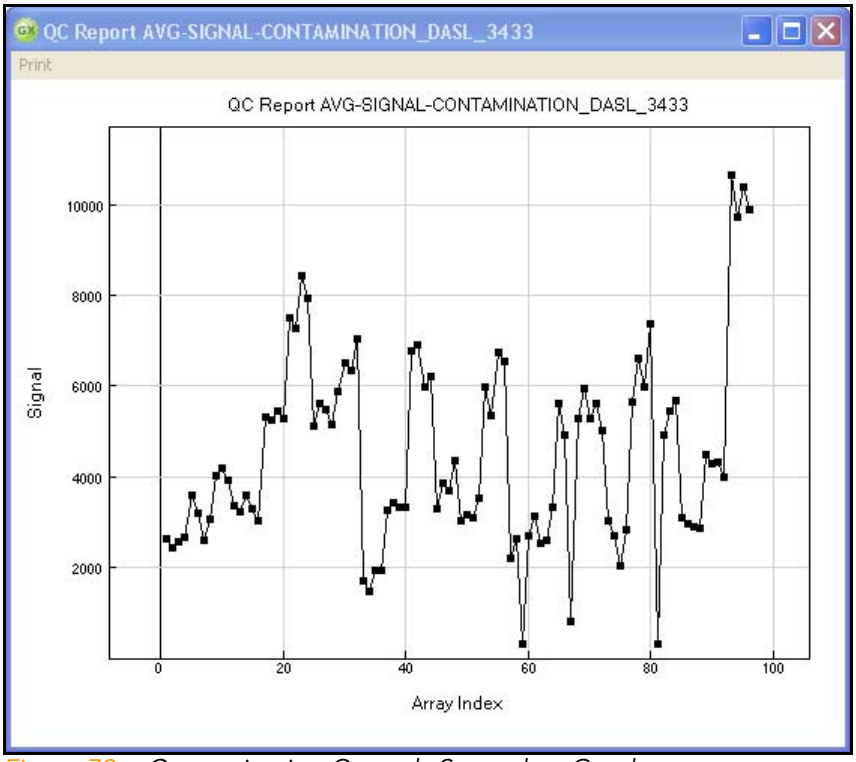


Figure 72 Contamination Controls Secondary Graph

9. To copy, change the page setup, or see a print preview, right click in any graph to use the context menu (Figure 73).
10. To print the graph, click **Print** in the menu bar.

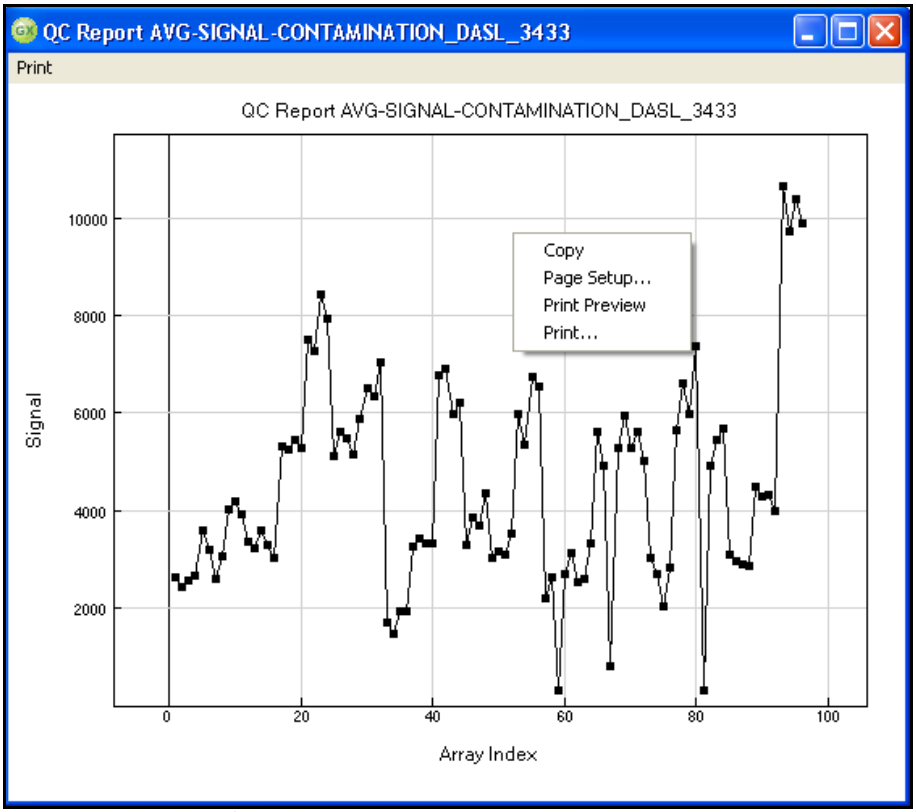


Figure 73 Control Summary Context Menu

Image Viewer

You can visually inspect any sample using the GenomeStudio Image Viewer. The Image Viewer allows you to see images for the purpose of determining whether or not you want to include a particular sample or samples in your experiment.

Using the Image Viewer, you can do the following things:

- See registration information for individual samples
- See registration for GenomeStudio-processed images
- Adjust the contrast of an image
- Zoom in or out
- See pixel intensities

Selecting an Image to View

To select an image to view, do the following:

1. Go to **Analysis** | **View Image**.
   The GenomeStudio View Image dialog box appears (Figure 74).

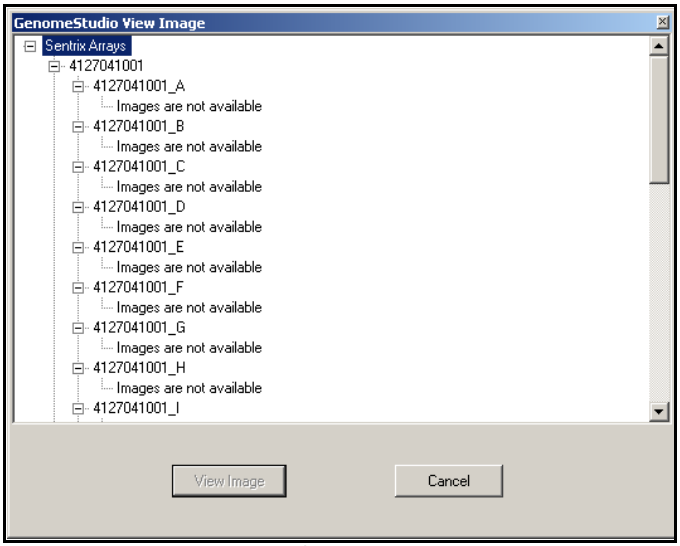


Figure 74 GenomeStudio View Image

2. Click to select an image from the list of available images.
3. Click **View Image**.
   The Image Viewer window appears (Figure 75).

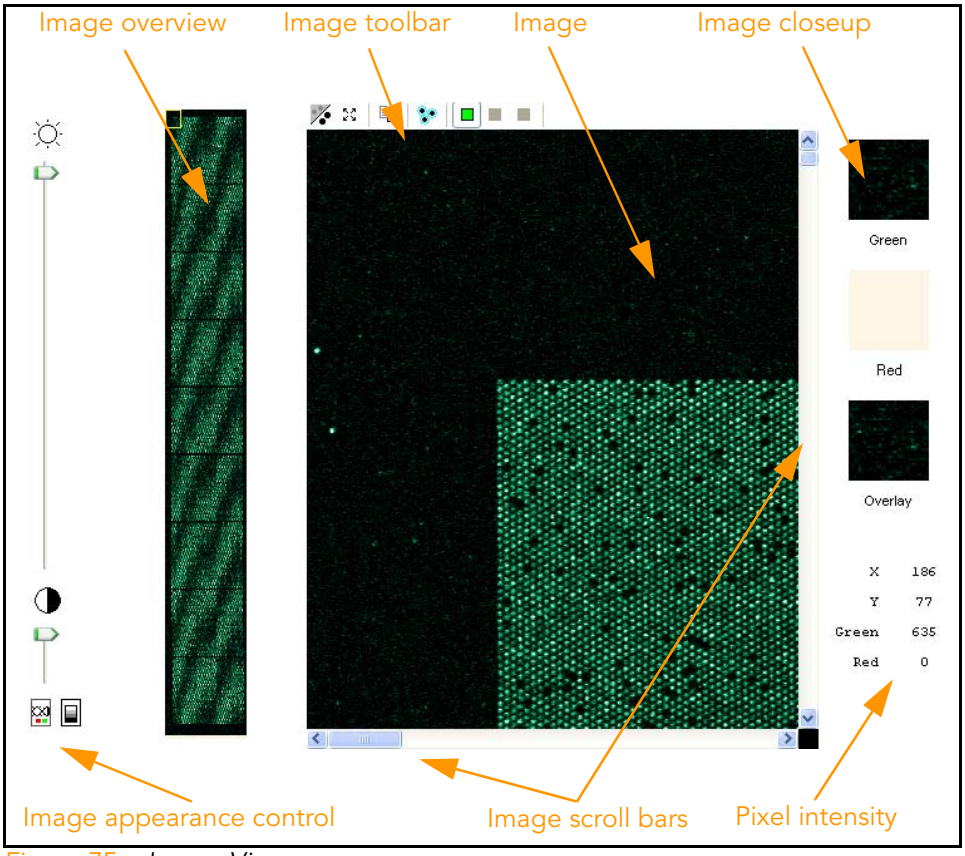


Figure 75 Image Viewer

Table 8 describes the features of the image viewer.

Table 8 Image Viewer Features

Feature	Description	
Image overview pane	Displays a high-level view of the sample image. Use the mouse to move the yellow box, which determines the field of view.	
Image toolbar		Click **Auto Contrast** to reset the image contrast to the default value.

Table 8 Image Viewer Features (continued)

Feature	Description	
Image toolbar (cont.)		Click **Zoom Out** to return to the default image view.
		Click **Copy to Clipboard** to save an image to the clipboard for pasting into another application.
		Click **Overlay Cores** to verify successful registration during data extraction on the BeadArray Reader. See Overlay Cores on page x-x for details.
		Click **Show Green Channel** to see the green channel only.
		Click **Show Red Channel** to see the red channel only. (This option is disabled for monochrome Direct-Hyb images.)
		Click **Overlay Channels** to see both green and red channels. (This option is disabled for monochrome Direct-Hyb images).
Image pane	Allows detailed inspection of the sample image. Use the mouse wheel to control the zoom level. If your mouse lacks a wheel, zoom in to a region by pressing the **Shift** key and the left mouse button at the same time, then dragging to select the zoom area, then releasing. To zoom out, click **Zoom Out** on the Image toolbar.	
Image closeup pane	Displays a closeup view of your image in the red and green color channels, and in a merged channel (overlay) view. The view region is determined by the location of your cursor on the image. Note: the red color channel is disabled for monochrome direct hyb images.	
Image appearance control	Use these controls to change image brightness, contrast, and color balance. Note: these controls affect only the appearance of the image on the screen. They do not change the underlying image file.	
Image scroll bars	Allow you to change the viewing region in the image pane.	
Pixel intensity	Reports the X and Y coordinates of your mouse pointer on the image pane, along with the pixel intensity at that location.	

Displaying Overlay Cores

Click **Overlay Cores** to display the image pane as shown in Figure 76. This feature allows you to verify that registration succeeded during data extraction on the BeadArray Reader.

NOTE

The overlay cores feature is enabled only when viewing a single channel (green or red). This feature is not enabled when viewing both channels simultaneously.

Zoom in on a corner of the image to see blue circles overlaying the scanned image sample spots. Successful registration is indicated when the boundary of the blue-circle grid coincides with the sample pixel boundary.

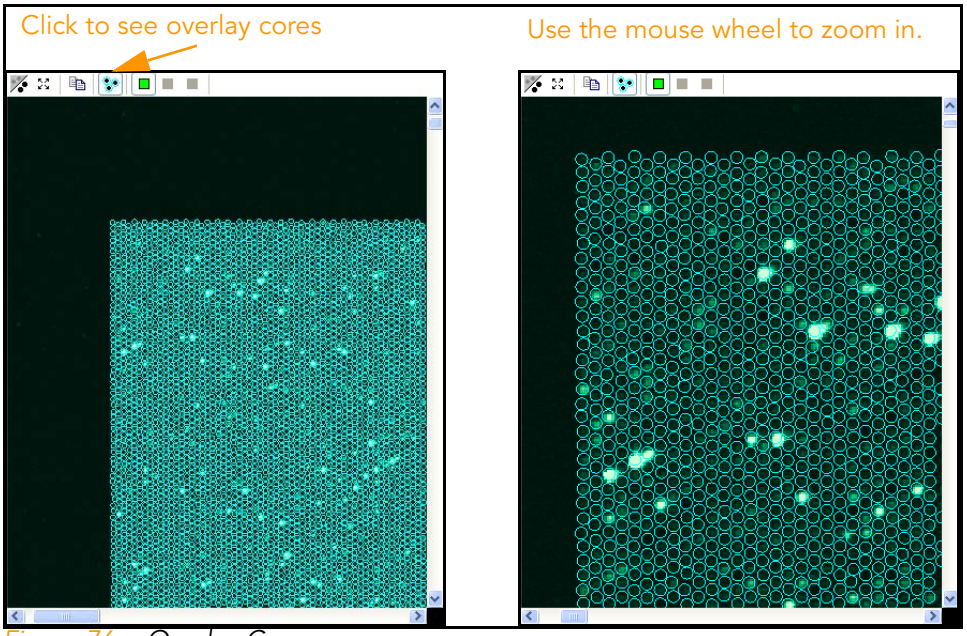


Figure 76 Overlay Cores

NOTE

In rare cases, registration may fail. If this occurs, contact Illumina Customer Solutions.

Changing Image Appearance

You can change the appearance of your images in two ways:

- Brightness/Contract mode
- Intensity Threshold mode

Use the selection buttons at the bottom of the pane to select the mode you want to change.

The components of the image appearance pane are described in Figure 77.

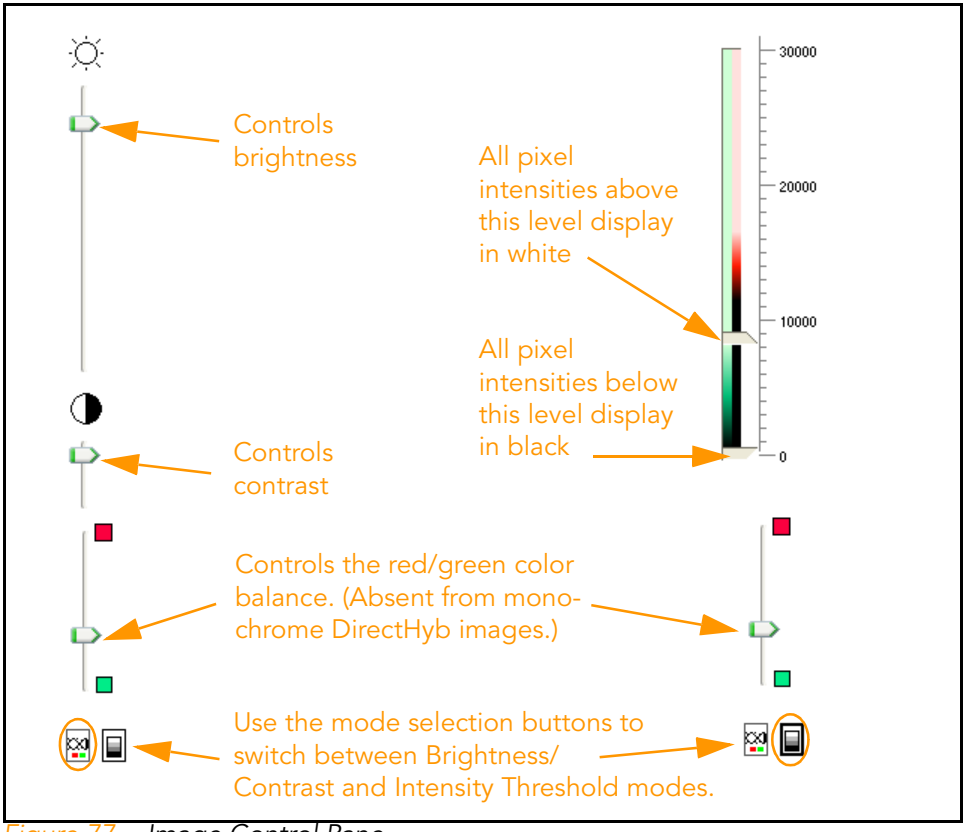


Figure 77 Image Control Pane

Chapter 4

Normalization and Differential Analysis

Topics

Introduction

This chapter describes the statistical algorithms used in GenomeStudio gene expression analysis.

Normalization Methods & Algorithms

Normalization algorithms adjust sample signals in order to minimize the effects of variation arising from non-biological factors. The GenomeStudio Gene Expression Module offers several routines that are described in the following sections.

For all algorithms, normalization is computed with respect to a mathematically calculated "virtual" sample that represents averaged probe intensities across a group of samples.

In the case of cubic spline or rank invariant normalization, the virtual sample is computed differently for SAMs than it is for BeadChips:

- For SAMs, the virtual sample is computed based on the content of the reference group. If there is no reference group, the first group displayed in the Project Group pane is used for group analysis.
- For BeadChips, the virtual sample is computed based on the average of all samples in the experiment.

For quantile normalization, all samples are used to calculate the virtual array. Group information is not used.

The following sections contain detailed description of these normalization algorithms:

- Average
- Sample scaling
- Quantile
- Cubic spline
- Rank invariant

Sample Scaling

Sample scaling normalization is applied when technical replicates are present in the sample set. This is a scaling normalization that is performed on a per-probe basis.

Let i range from 1 to the number of probes, let j range from 1 to the number of replicates, let m range from 1 to the number of samples, and let I equal the intensity for any given probe.

For the case where only one replicate is present between two SAMs or BeadChips, a scaling factor (sf_i) is computed as follows:

$$sf_i = \frac{I_{i1}}{I_{i2}}$$

where j = 1, 2.

For multiple replicates, the per-probe intensities are averaged before computing the scaling factor.

Next, each probe for each sample is normalized as follows:

$$I_{im}^{norm} = sf_i(I_{im})$$

See Figure 22 in Chapter 2.

When you use a sample sheet, the Sample_Name in the Common Samples File must match the Sample_Name in the sample sheet.
If no sample sheet is used, the common sample names must be in the form Sentrix Barcode Number_Array Position.
e.g., 12345678_R000_C000.

Average

Sample intensities are scaled by a factor equal to the ratio of average intensity of virtual sample to the average intensity of the given sample. Background is subtracted prior to the scaling.

Average normalization adjusts for differences in overall intensity between arrays and chips. Sample intensities are scaled by a factor so that the average signal of all samples becomes equal to the global average of all sample signals. Background subtraction is done prior to scaling, so half of the unexpressed targets are expected to have negative signals.

Quantile

Quantile normalization is a method used to make the distribution, median, and mean of probe intensities the same for every sample. The normalization distribution is chosen by averaging each quantile across samples. Like cubic spline, this method assumes that all samples have similar distributions of transcript abundance.

The quantile normalization algorithm works as follows:

1. Given n samples with p probes, form X from the dimensions p x n where each sample is a column and each probe is a row.

Negative control probes are normalized together with analytical probes.

2. Sort each column of X to get X_s.
3. Take the means across rows of X_s.
4. Assign this mean to each element in a row to get X_{sm}.
5. Get $X_{normalized}$ by rearranging each column of X_{sm} to have the same ordering as the original X.
6. $X_{normalized}$ now contains the normalized intensity for all samples (columns) and all probes (rows).

Quantile is not recommended if you have titration samples in your project. In this case, Illumina recommends average normalization.

Cubic Spline

Cubic spline normalization is similar to the method proposed by Workman et al.[1] The normalization uses quantiles of sample intensities to fit smoothing B-splines.

Let $q_i = \frac{i - 0.5}{N}, i = 1, 2 \ldots N$

be a vector of N quantiles $\left(N = \max\left(15, \frac{N_{probes}}{100}\right)\right)$

Here, N_{probes} is the number of probes represented on a sample.

For each sample, the vector of quantile intensities is computed. Similarly, quantiles for the "virtual" averaged sample after background subtraction are computed. Cubic B-spline is computed and used for interpolation. For points with intensities ranked outside the interval, linear extrapolation rather than cubic spline is used to avoid nonlinear effects outside the region of interpolation.

1. Workman C, Jensen LJ, Jarmer H, Berka R, Gautier L, Nielser HB, Saxild HH, Nielsen C, Brunak S, Knudsen S. A new non-linear normalization method for reducing variability in DNA microarray experiments. Genome Biol. 2002 Aug 30; 3(9):research0048. PMID: 12225587 [PubMed - indexed for MEDLINE]

Cubic spline normalization is capable of minimizing effects that cause nonlinear transformation of data, such as saturation. This is similar to the method proposed by Workman et. al. Just as in the rank invariant method, cubic spline normalization is computed with respect to the virtual reference sample. The assumption of this method is that all samples have similar distributions of transcript abundance. The transformation is computed using smoothing B-splines. The number of quantiles equals 1% of the number of probes, but cannot be lower than 15. For points outside of the interpolation interval, linear extrapolation is used. This method is applied after the background normalization method described above.

For the DASL assay, red and green channels are normalized independently.

Rank Invariant

Rank invariant normalization uses a set of probes that is rank invariant between a given sample and a virtual sample.

The rank invariant set is found as follows: we start by considering probes with intensities ranked between LowRank = 50th percentile and HighRank = 90th percentile. If the probe's relative rank changes $\frac{r_x - r_v}{r_v} \leq 0.05$ the probe is considered to be rank invariant. If less than 2% of all probes in the region are identified as rank invariant, LowRank is gradually decreased until it reaches the 25th percentile.

Rank invariant normalization operates under the assumption that probes with similar ranking between samples have similar expression levels. This method minimizes the effects of additive and multiplicative factors. The subset of probes with low relative rank change is defined as follows.

1. The virtual reference sample is created by averaging the content of the reference group (in differential analysis) or the first group of the group set (in gene analysis).
2. All probes ranked between LowRank = 50th and HighRank = 90th percentiles are considered. If the change of rank relative to the virtual reference is less than 0.05, the probe is considered to be "rank invariant."
3. If less than 2% of all probes are picked as rank invariant, LowRank is gradually decreased until it reaches the 25th percentile.
4. Normalization coefficients are computed using iteratively re-weighted least squares. This method is applied after the background normalization method described above.

Differential Expression Algorithms

All algorithms compare a group of samples (referred to as the condition group) to a reference group. The comparison is done using the following error models:

- Illumina Custom
- Mann-Whitney
- T-Test

Illumina Custom

This model assumes that target signal intensity (*I*) is normally distributed among replicates corresponding to some biological condition. The variation has three components: sequence specific biological variation (σ_{bio}), nonspecific biological variation (σ_{neg}), and technical error (σ_{tech}).

$$I = N(\mu, \sigma)$$

$$\sigma = \sqrt{\sigma^2_{tech} + \sigma^2_{neg} + \sigma^2_{bio}}$$

$$\sigma_{tech} = a + b < I >$$

Variation of nonspecific signal σ_{neg} is estimated from the signal of negative control sequences (using median absolute deviation). For σ_{tech}, we estimate two sets of parameters a_{ref}, b_{ref} and a_{cond}, b_{cond} for reference and condition groups respectively.

We estimate σ_{tech} using iterative robust least squares fit, which reduces the influence of highly variable genes. This implicitly assumes that the majority of genes do not have high biological variation among replicates. When this assumption does not hold we overestimate technical error by some averaged biological variation.

When groups contain biological replicates, we produce p-values using the following approach:

$$S_{ref} = (\max(s_{ref}, a_{ref} + b_{ref} I_{ref}))$$

$$S_{cond} = (\max(s_{cond}, a_{cond} + b_{cond} I_{cond}))$$

$$p = z\left(\frac{|I_{cond} - I_{ref}|}{\sqrt{\frac{S_{ref}^2 + S_{neg(ref)}^2}{N_{ref}} + \frac{S_{cond}^2 + S_{neg(cond)}^2}{N_{cond}}}}\right)$$

where S_{ref} and S_{cond} are standard deviations of probe signals.

N_{ref} and N_{cond} denote the number of samples in the reference and condition groups, respectively.

We consider that standard deviations exceeding σ_{tech} reflects biological variation. However, we assume that estimates smaller than σ_{tech} are caused by random errors. Therefore, we use the larger of two estimates. Usage of σ_{neg} provides regularization for low abundance targets. Z is two-sided tail probability of standard normal distribution.

When reference and conditions groups contain one sample each, we can neither estimate sequence specific biological variation nor sample processing variation. Instead, we can only assess σ using bead type variation. Therefore, we penalize for that by a factor of 2.5 applied to parameter b:

$$p = z\left(\frac{|I_{cond} - I_{ref}|}{\sqrt{(a_{ref} + 2.5 b_{ref} I_{ref})^2 + \sigma_{neg(ref)}^2 + (a_{cond} + 2.5 b_{cond} I_{cond})^2 + \sigma_{neg(cond)}^2}}\right)$$

or by a factor of 15 applied to parameter b for VeraCode DASL:

$$p = z\left(\frac{|I_{cond} - I_{ref}|}{\sqrt{(a_{ref} + 15 b_{ref} I_{ref})^2 + \sigma_{neg(ref)}^2 + (a_{cond} + 15 b_{cond} I_{cond})^2 + \sigma_{neg(cond)}^2}}\right)$$

These factors were determined empirically by examining real sample data.

A Diff Score for a probe is computed as:

$$\mathrm{DiffScore} = (10\,\mathrm{sgn}(I_{cond} - I_{ref})\log_{10}(p))$$

For the gene, Diff Scores of corresponding probes are averaged. In addition, concordance between probes is reported.

For the DASL assay, the red and green channel signals are added together before computing diff scores.

Mann-Whitney

This implementation produces exact p-value if:

$$\min(N_{ref}, N_{cond}) < 3$$

or

$$\max(N_{ref}, N_{cond}) < 22$$

Otherwise, normal approximation with continuity correction is used. Differential scores are computed as described for the Illumina Custom model (page 103).

T-Test

When either the reference group or a condition group contains at least two samples, variance is estimated across replicate samples. Otherwise, variance is estimated from bead-to-bead variation. We use t-test with the assumption of equal variance. Differential scores are computed the same way as described for the Illumina Custom model (page 103).

Detection P-Value

Detection p-value is a statistical calculation that provides the probability that the signal from a given probe is greater than the average signal from the negative controls.

Whole Genome BeadChips

Detection p-value is calculated with the equation:

$$DPV = 1 - \frac{R}{N}$$

where R is the rank of the Z score of the analytical probes, and N is the number of negative controls.

The Z score is calculated with the equation:

$$Z_{ig} = \frac{I - \mu_i^{neg}}{\sigma_i^{neg}}$$

where μ_i^{neg} and σ_i^{neg} are the mean and the standard deviation of signals of the negative controls on the i^{th} sample and the g^{th} gene.

When samples are combined together to form a group, the Z score is averaged:

$$\bar{Z}_{ig} = \frac{1}{m}\sum_i Z_{ig}$$

The value for R is returned by a function that compares the Z score for the probe intensity to the Z score of the negative controls.

If the Z score for the probe intensity is smaller than the lowest negative control Z score, the function returns a 0 and the p-value is 1.

If the Z score for the probe intensity falls within the range of the Z scores of the negative controls, R is the rank of the Z score of the probe, and the p-value is in the range of 0 to 1.

If the Z score for the probe intensity is greater than the largest negative control Z score, the function returns a 1 and the p-value is 0.

DASL, miRNA, VeraCode DASL, and Focused Arrays

Because DASL, miRNA, VeraCode DASL, and Focused arrays contain relatively few negative controls, GenomeStudio uses a normal distribution to model their signals. For DASL products, the Cy3 and Cy5 channels are added for the computation of selected p-values.

The detection p-value is given by:

$$p = F\left(\frac{I_{probe} - \mu_{neg}}{\sigma_{neg}}\right)$$

where F is 1 - the normal cumulative probability distribution function.

For gene-level p-values, the calculation is as follows:

$$p = F\left(\sqrt{N}\frac{|I_{gene} - \mu_{neg}|}{\sigma_{neg}}\right)$$

where N is the number of probes for gene g.

For groups containing multiple samples, the average Z score is computed as described in *Whole Genome BeadChips* on page 106. The detection p-value is then computed using the following formula:

$$p = F\left(\frac{|Z_g|}{\sigma_{Z_{neg}}}\right)$$

Where $\sigma_{Z_{neg}}$ is the standard deviation of the average Z scores of the negative controls.

Chapter 5
Analyzing miRNA Data

Topics

Introduction

When viewing and analyzing miRNA data with the GenomeStudio Gene Expression Module, you can use the same plots, normalizations, statistical analyses, and genome viewer options available for Direct Hyb and DASL data.

NOTE

Illumina recommends quantile normalization for miRNA data. Rank invariant normalization is not appropriate for miRNA data.

The GenomeStudio Gene Expression Module also offers an additional miRNA-related feature: the ability to import analyses from Direct Hyb projects to be viewed against miRNA data. This is accomplished by using the Import Analysis wizard and, if sample names are specified, a lookup table that associates sample names between projects.

NOTE

A lookup table is required only if sample names are not the same in the GenomeStudio products you want to associate.

Importing an Analysis for Comparison

To import a Direct Hyb or DASL analysis to compare to miRNA data, do the following:

1. In the GenomeStudio Gene Expression Module, select **Analysis** | **Import Gene Expression Analysis** (Figure 78).

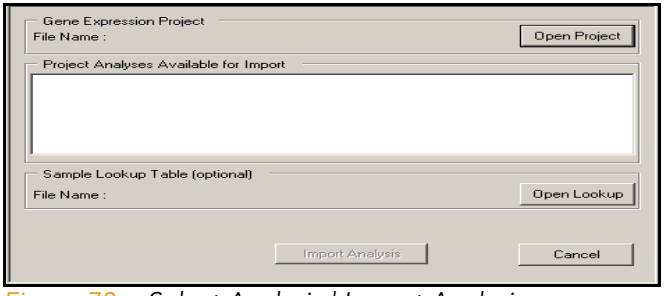


Figure 78 Select Analysis | Import Analysis

The Import Gene Expression Analysis wizard appears (Figure 79).

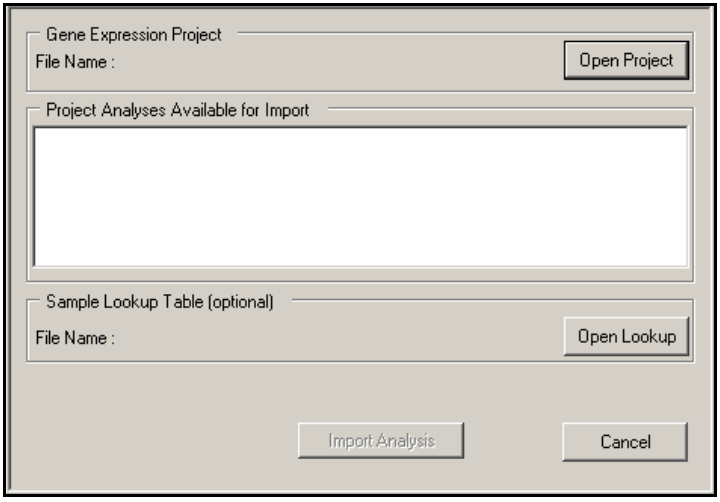


Figure 79 Import Analysis Wizard

Next, you will use the Import Gene Expression Analysis wizard to locate and load a previously-saved GenomeStudio project of interest and the associated lookup table (if needed).

2. To import a previously saved GenomeStudio project, click **Open Project**.
3. Browse to the folder where the project of interest is saved.
4. Click **GenomeStudio**.

   The project you selected is loaded into GenomeStudio.

If the sample names in the projects you want to compare ***are not*** the same, continue to *Loading a Lookup Table* on page 112.

If the sample names in the projects you want to compare ***are*** the same, continue to *Generating a Dendrogram* on page 113.

Loading a Lookup Table

A lookup table allows you to associate group names and BeadChip or SAM (Sentrix Array Matrix) sample names from one project with those from another project if the sample names in these projects are not the same.

In Figure 80, miRNA sample names (SAM bundle IDs) are shown in the column on the left, and Direct Hyb or DASL sample names (Array barcodes or SAM bundle IDs) are shown in the column on the right.

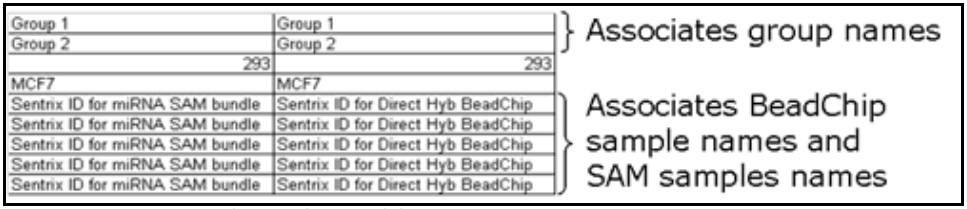


Figure 80 Example Lookup Table

To load a lookup table, do the following:

1. Browse to the location where the lookup table is saved.
2. Select the lookup table of interest.
3. Click **OK**.

   The lookup table you selected is associated with your project.

Generating a Dendrogram

The GenomeStudio Gene Expression module allows you to generate a dendrogram that allows you to identify positive and negative correlations between miRNA and gene expression levels.

To create a gene-based dendrogram using the clustering tool:

1. In the GenomeStudio toolbar, click **Run Cluster Analysis**.

   The Group Gene Profile dialog box appears (Figure 81).

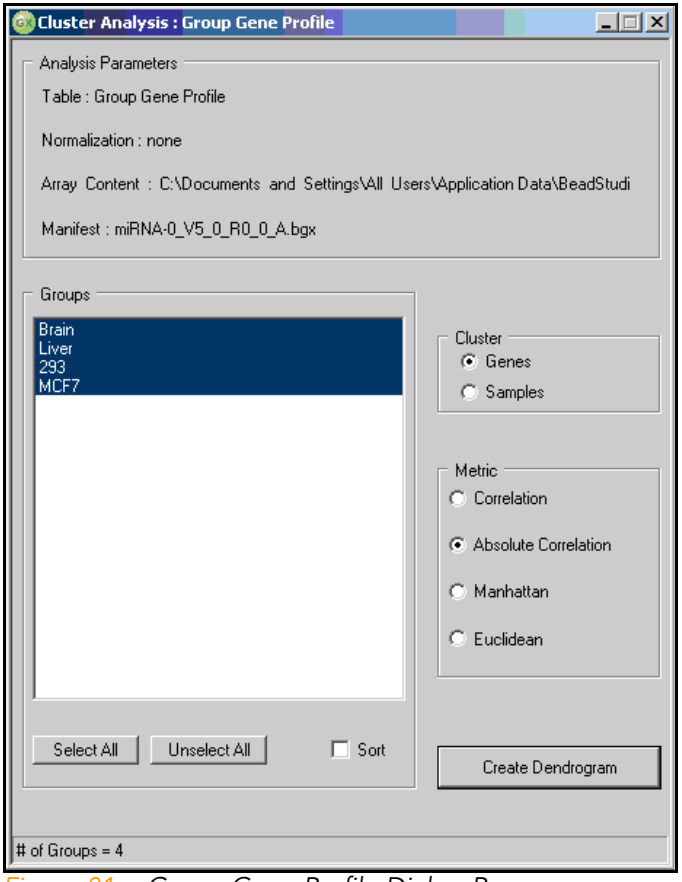


Figure 81 Group Gene Profile Dialog Box

2. In the Groups area, select all samples.

3. In the Cluster area, select **Genes**.
4. In the Metric area, select **Absolute Correlation**.
5. Click **Create Dendrogram**.
   The dendrogram displays (Figure 82).

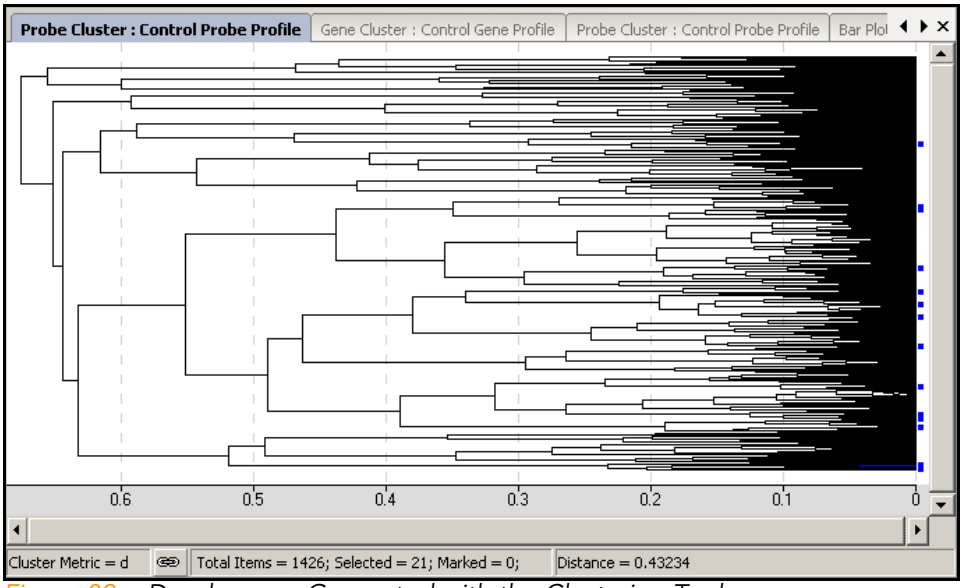


Figure 82 Dendrogram Generated with the Clustering Tool

Identifying Correlated miRNA and mRNA Expression Values

Once you have generated a dendrogram, you can identify positively- and negatively-correlated miRNA and mRNA expression values.

To identify correlated expression values:

1. In the data table, select a miRNA entry.
2. Place the cursor over the dendrogram, and use the mouse wheel to zoom in on the cluster that contains the miRNA entry of interest.
3. Highlight the members of the cluster of interest by double-clicking the cluster node.

NOTE

Because the dendrogram and the table are related, the genes you select in the dendrogram also appear highlighted in the data table.

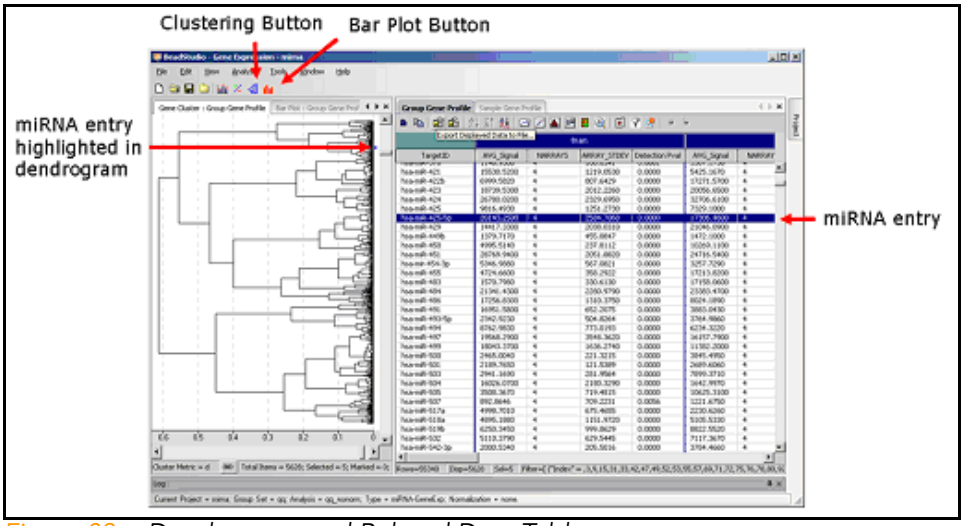


Figure 83 Dendrogram and Related Data Table

4. In the icon toolbar, select **Bar Plot**.

5. Select **Line Plot**.

   The display changes from dendrogram to line plot.

   The cluster members appear as shown in Figure 84, which shows two examples of negative correlation identified using the process described above.

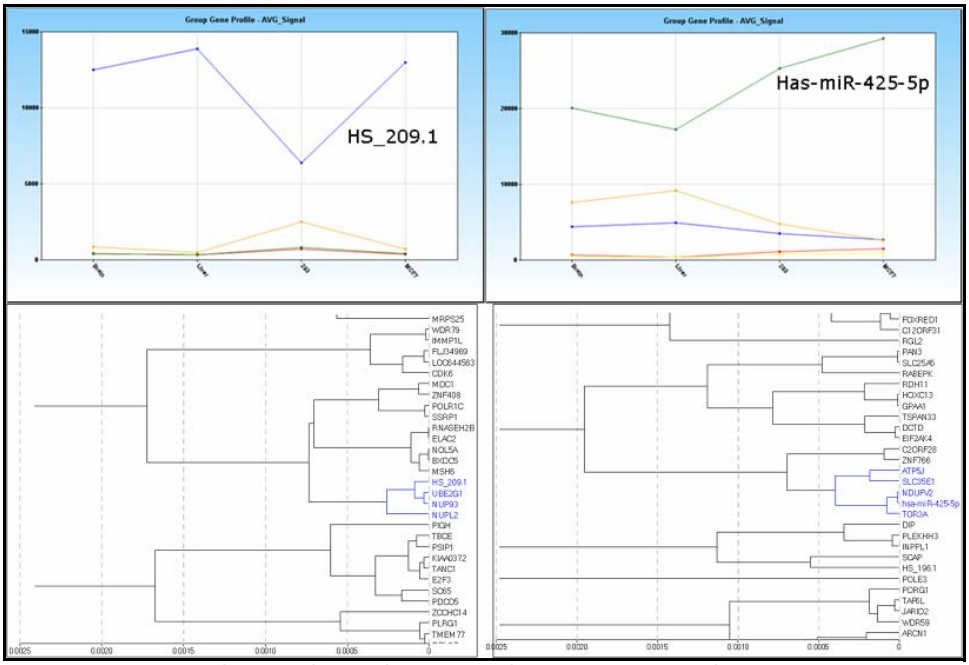


Figure 84 Line Plots and Dendrograms Showing Anticorrelation Between miRNA and mRNA Expression Levels

Viewing miRNA Controls

miRNA Assay controls allow you to perform basic quality control of data generated by performing the miRNA Assay. The controls are displayed in the Control Summary tab (Figure 85).

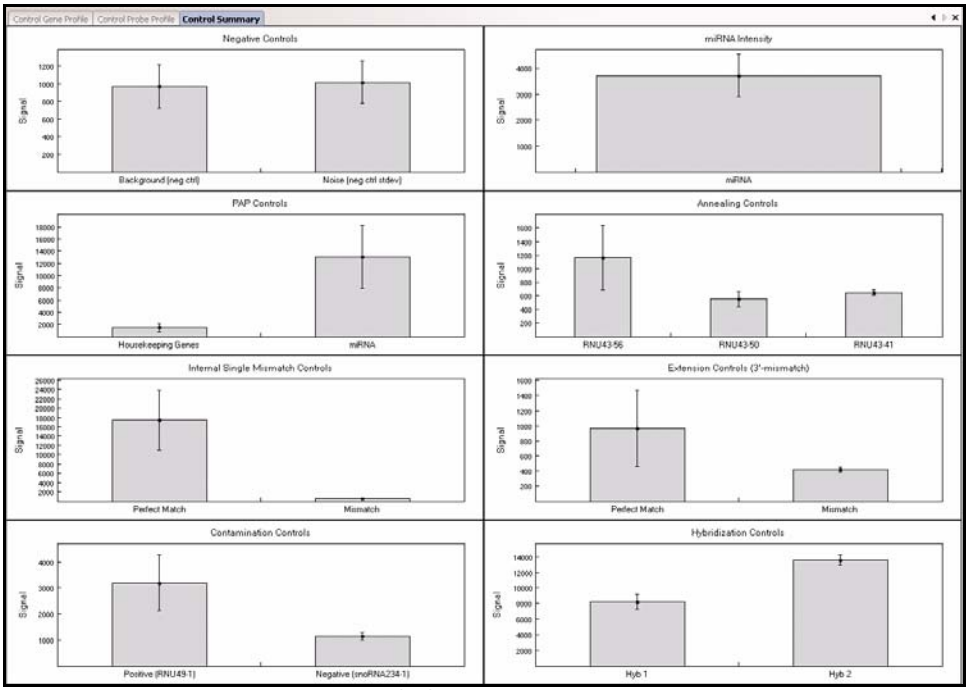


Figure 85 miRNA Assay Control Plots

NOTE

Figure 85 is an example plot. It does not reflect typical values for control element intensity.

Table 9 includes a list of miRNA control types and their functions.

Table 9 Control Features for the miRNA Assay

Control Feature	Purpose
Negative	Determination of background signal
miRNA Intensity	Average intensity of miRNA sample
PAP	Control for polyadenylation step
Annealing	Control for MSO annealing
Internal Single Mismatch	Stringency control
Extension	Positive and negative control for extension reaction
Contamination	Control for PCR contamination
Hybridization	Positive control for hybridization

For more detailed descriptions of miRNA assay controls, refer to the miRNA Assay Protocol Guide, Illumina Part # 11251981.

Chapter 6

Generating a Final Report

Topics

Introduction

GenomeStudio v3 includes the ability to generate a final report. You can save a final report to view later, or to use with downstream, third-party software applications.

This chapter describes how to generate, save, and view a GenomeStudio Gene Expression Module final report.

Generating a Final Report

A final report is the final output of the GenomeStudio Gene Expression Module.

To generate a final report, do the following:

1. Select **Analysis** | **Reports** (Figure 86).

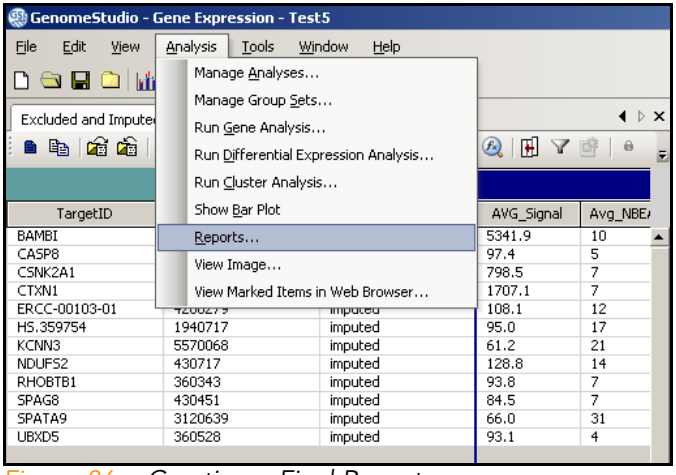


Figure 86 Creating a Final Report

The GenomeStudio Gene Expression Reports dialog appears (Figure 87).

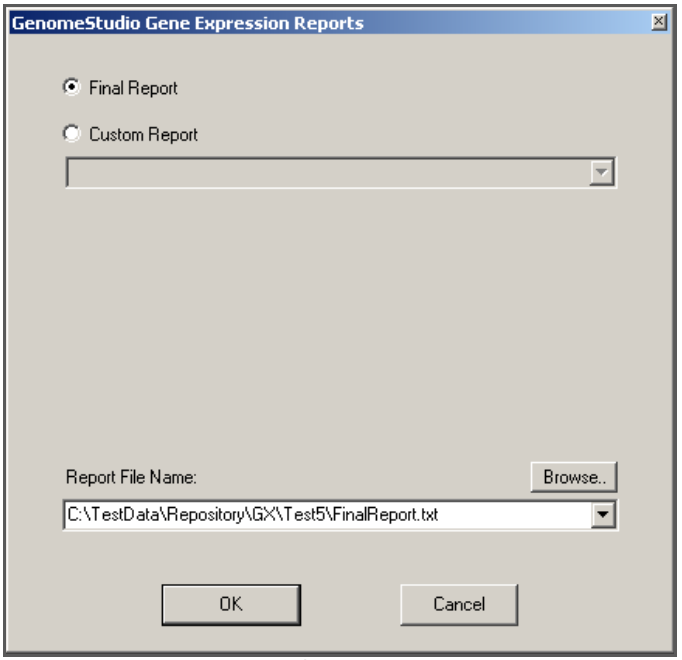


Figure 87 GenomeStudio Gene Expression Reports

2. Select the type of report you would like to generate:
   - Final Report
   - Custom Report
3. Click **OK**.

   The GenomeStudio Gene Expression Final Report dialog box appears (Figure 88).

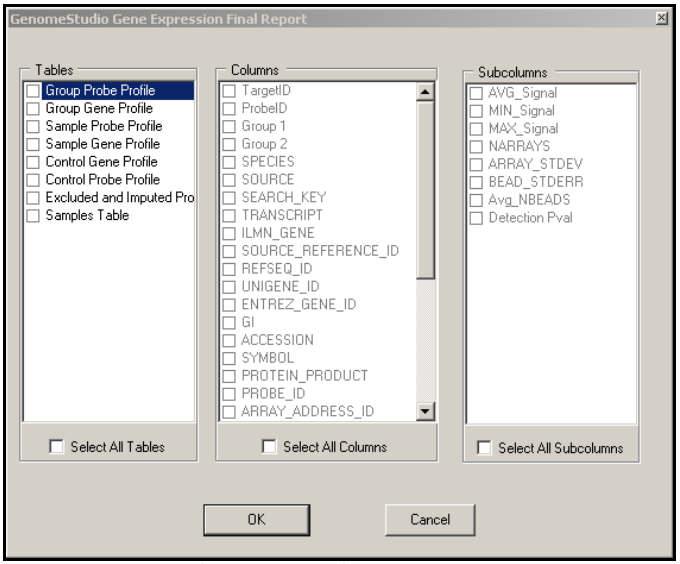


Figure 88 Final Report Dialog Box

4. Select **Table**, **Columns**, and **Subcolumns** options to include in the final report (Figure 89):

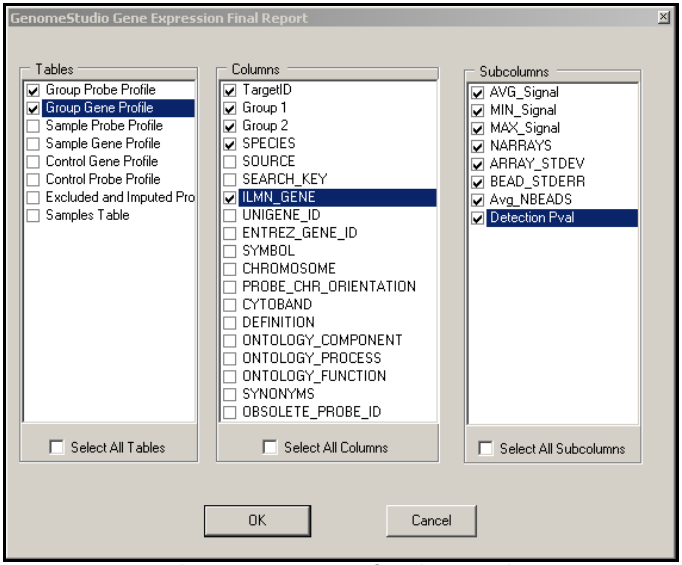


Figure 89 Selecting Options for the Final Report

5. Click **OK**.

6. Click **Browse** to browse to the location where you would like to save the final report.

   The Final Report File window appears (Figure 90).

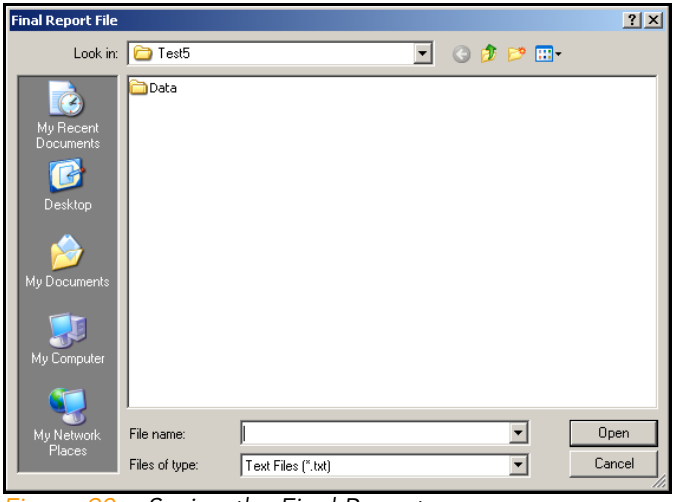


Figure 90 Saving the Final Report

7. In the **Filename** field, enter a name for the final report (Figure 91).

Figure 91 Naming the Final Report

8. Click **Open** on the Final Report File dialog box.

   The Final Report File dialog box closes.

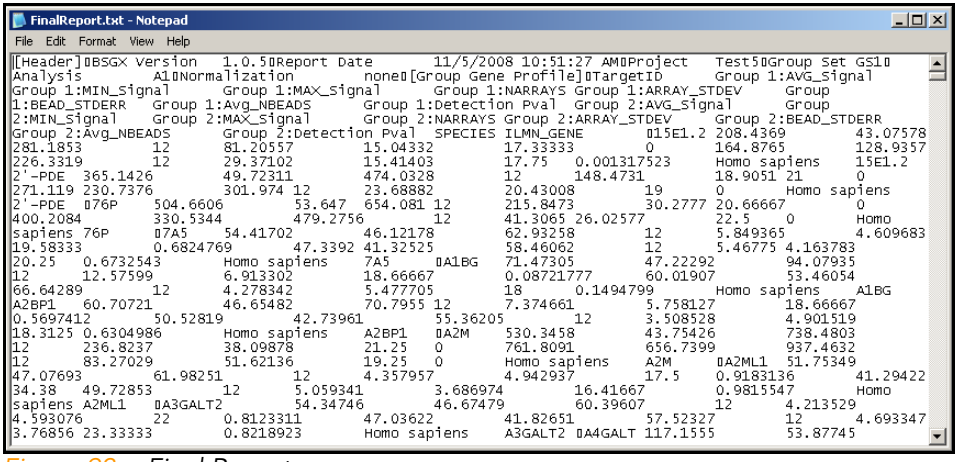
9. Click **OK** on the GenomeStudio Gene Expression Final Report dialog box.

   The GenomeStudio Gene Expression Final Report dialog box closes.

   The final report is saved as a tab-delimited *.txt file in the location you specified.

Viewing a Final Report

- Open the final report in Excel or a similar spreadsheet program or text editor (Figure 92).


FinalReport.txt - Notepad

File Edit Format View Help

[Header]▯BSGX Version 1.0.5▯Report Date 11/5/2008 10:51:27 AM▯Project Test5▯Group Set GS1▯
Analysis A1▯Normalization none▯[Group Gene Profile]▯TargetID Group 1:AVG_Signal
Group 1:MIN_Signal Group 1:MAX_Signal Group 1:NARRAYS Group 1:ARRAY_STDEV Group
1:BEAD_STDERR Group 1:Avg_NBEADS Group 1:Detection Pval Group 2:AVG_Signal Group
2:MIN_Signal Group 2:MAX_Signal Group 2:NARRAYS Group 2:ARRAY_STDEV Group 2:BEAD_STDERR
Group 2:Avg_NBEADS Group 2:Detection Pval SPECIES ILMN_GENE ▯15E1.2 208.4369 43.07578
281.1853 12 81.20557 15.04332 17.33333 0 164.8765 128.9357
226.3319 12 29.37102 15.41403 17.75 0.001317523 Homo sapiens 15E1.2
2'-PDE 365.1426 49.72311 474.0328 12 148.4731 18.9051 21 0
271.119 230.7376 301.974 12 23.68882 20.43008 19 0 Homo sapiens
2'-PDE ▯76P 504.6606 53.647 654.081 12 215.8473 30.2777 20.66667 0
400.2084 330.5344 479.2756 12 41.3065 26.02577 22.5 0 Homo
sapiens 76P ▯7A5 54.41702 46.12178 62.93258 12 5.849365 4.609683
19.58333 0.6824769 47.3392 41.32525 58.46062 12 5.46775 4.163783
20.25 0.6732543 Homo sapiens 7A5 ▯A1BG 71.47305 47.22292 94.07935
12 12.57599 6.913302 18.66667 0.08721777 60.01907 53.46054
66.64289 12 4.278342 5.477705 18 0.1494799 Homo sapiens A1BG
A2BP1 60.70721 46.65482 70.7955 12 7.374661 5.758127 18.66667
0.5697412 50.52819 42.73961 55.36205 12 3.508528 4.901519
18.3125 0.6304986 Homo sapiens A2BP1 ▯A2M 530.3458 43.75426 738.4803
12 236.8237 38.09878 21.25 0 761.8091 656.7399 937.4632
12 83.27029 51.62136 19.25 0 Homo sapiens A2M ▯A2ML1 51.75349
47.07693 61.98251 12 4.357957 4.942937 17.5 0.9183136 41.29422
34.38 49.72853 12 5.059341 3.686974 16.41667 0.9815547 Homo
sapiens A2ML1 ▯A3GALT2 54.34746 46.67479 60.39607 12 4.213529
4.593076 22 0.8123311 47.03622 41.82651 57.52327 12 4.693347
3.76856 23.33333 0.8218923 Homo sapiens A3GALT2 ▯A4GALT 117.1555 53.87745

Figure 92 Final Report

Chapter 7

User Interface Reference

Topics

Introduction

This chapter explains how to use the detachable docking windows, main window menus, and context menus in the GenomeStudio Gene Expression Module.

Detachable Docking Windows

Detachable docking windows let you customize GenomeStudio's user interface to suit your analysis needs.

Figure 93 shows the default view of the GenomeStudio Gene Expression Module. Detachable docking windows are outlined in orange.

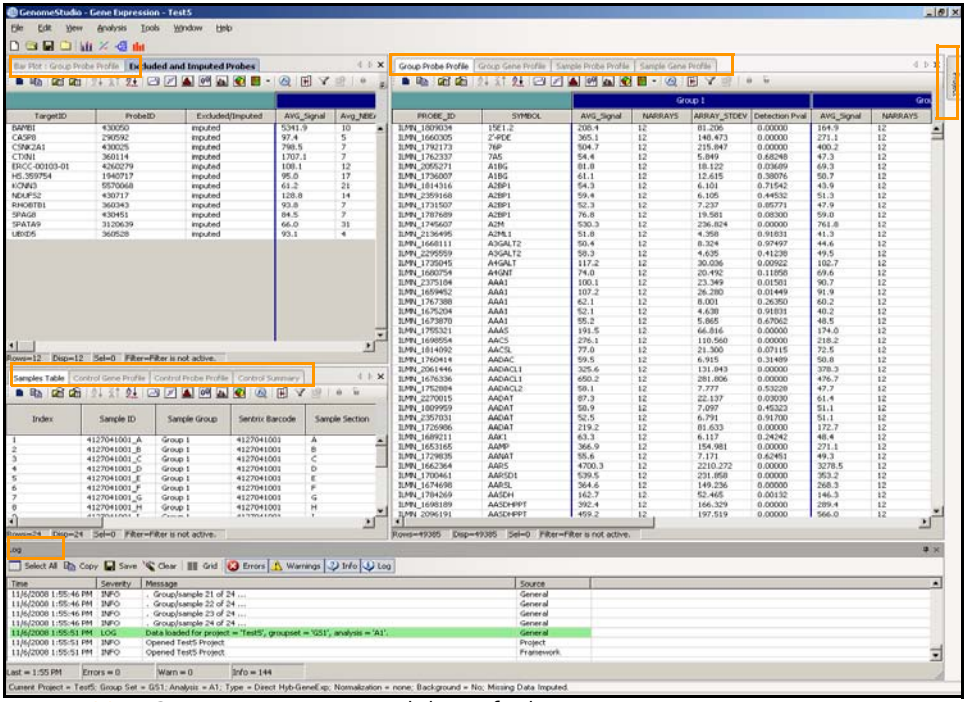


Figure 93 Gene Expression Module Default View

The following sections describe each of the Gene Expression Module's detachable docking windows.

Line Plot, Group Gene Profile

Figure 94 shows an example of a Group Gene Profile line plot.

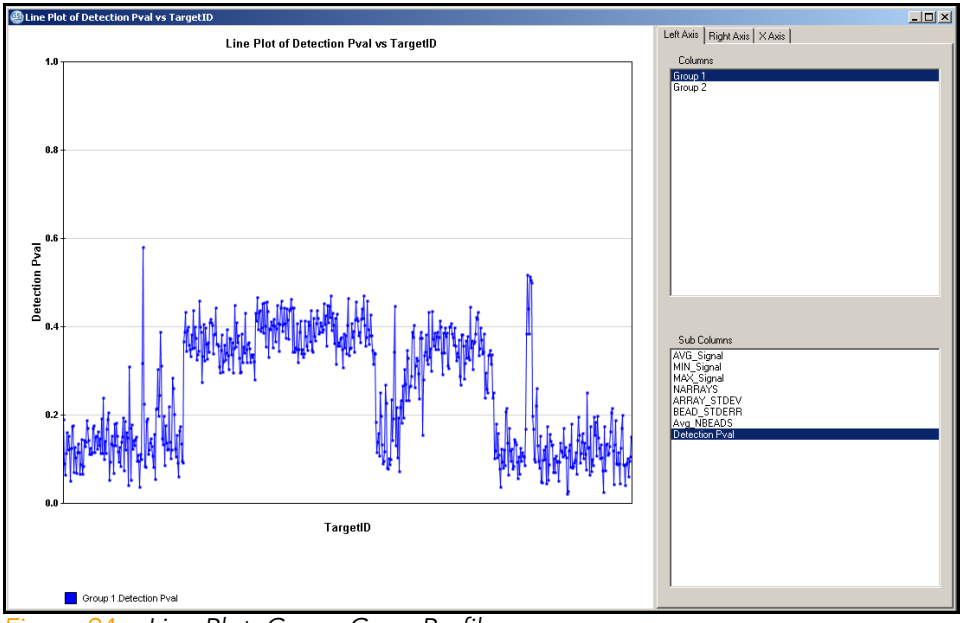


Figure 94 Line Plot, Group Gene Profile

To customize the view of the line plot, right click on the plot and make selections in the Plot Settings dialog box.

Bar Plot, Group Gene Profile

Figure 95 shows an example of a Group Gene Profile bar plot.

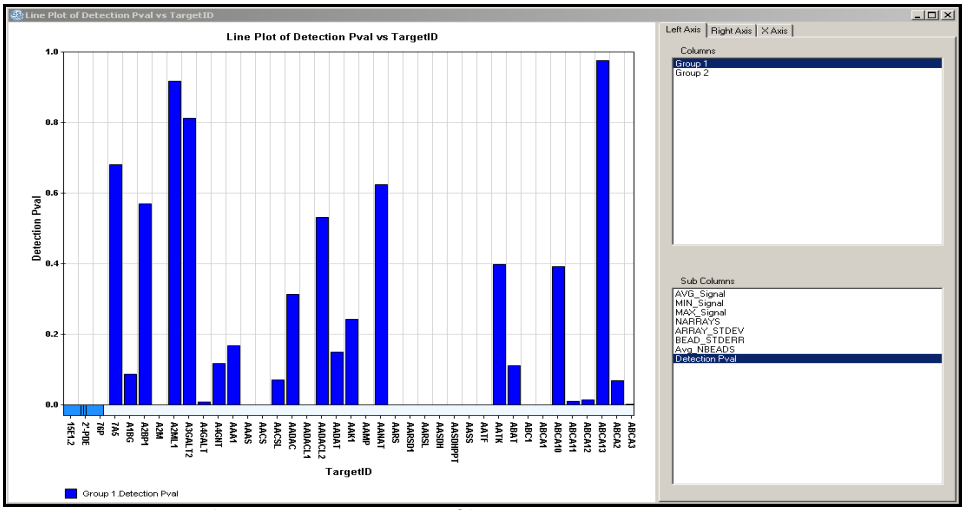
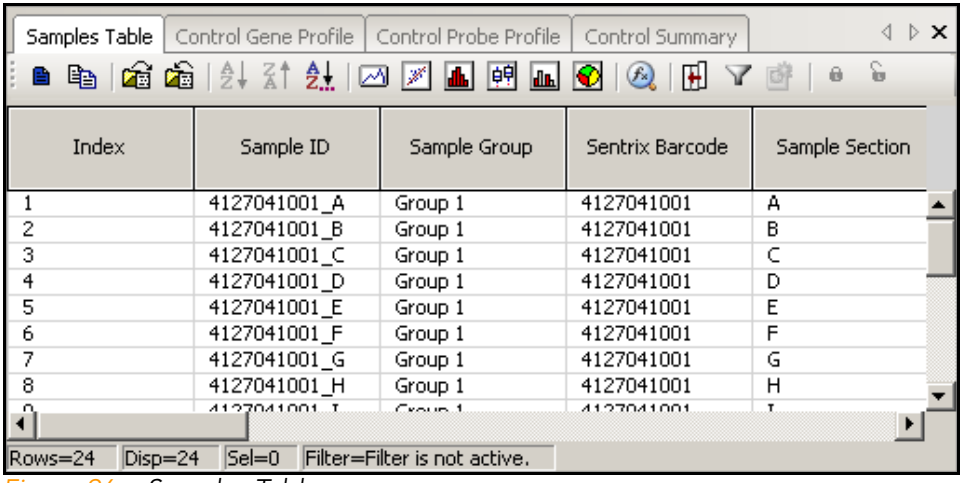


Figure 95 Bar Plot, Group Gene Profile

Samples Table Figure 96 shows an example Samples Table.



Index	Sample ID	Sample Group	Sentrix Barcode	Sample Section
1	4127041001_A	Group 1	4127041001	A
2	4127041001_B	Group 1	4127041001	B
3	4127041001_C	Group 1	4127041001	C
4	4127041001_D	Group 1	4127041001	D
5	4127041001_E	Group 1	4127041001	E
6	4127041001_F	Group 1	4127041001	F
7	4127041001_G	Group 1	4127041001	G
8	4127041001_H	Group 1	4127041001	H

Figure 96 Samples Table

The annotation columns of the Samples Table are described in Table 10.

Table 10 Samples Table Columns

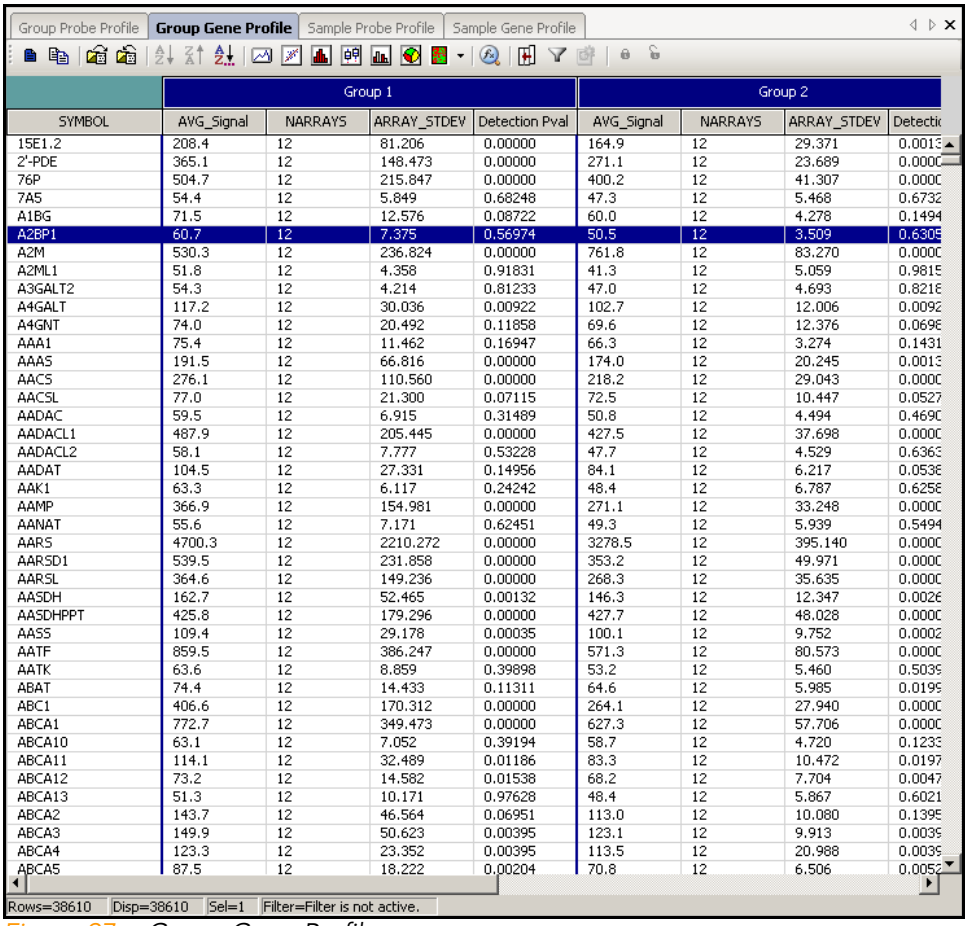
Column	Description	Type	Visible by Default?
Index	The row index of the sample	int	Y
Sample ID	The sample identifier	string	Y
Sample Group	The group the sample belongs to, as defined in a sample sheet or in the **Groupset Definition** dialog box	string	Y
Sentrix Barcode	The barcode of the Universal Array Product	int	Y
Sample Section	The section on the Universal Array Product	string	Y
Detected Genes (0.01)	The number of genes with a detection p-value of 0.01 or less.	int	Y
Detected Genes (0.05)	The number of genes with a detection p-value of 0.05 or less	int	Y
Signal Average	The average signal for the sample across all probes	int	Y
Signal P05	The fifth percentile average intensity across all probes	int	Y
Signal P25	The twenty-fifth percentile average intensity across all probes	int	Y
Signal P50	The fiftieth percentile average intensity across all probes	int	Y
Signal P75	The seventy-fifth percentile average intensity across all probes	int	Y
Signal P95	The ninety-fifth percentile average intensity across all probes	int	Y
Sample_Well	The well within the sample plate	string	Y
Sample_Plate	The sample plate	string	Y

Table 10 Samples Table Columns (continued)

Column	Description	Type	Visible by Default?
Pool_ID	The bead pool ID	string	Y
BIOTIN	The average biotin control intensity across all probes	float	Y
CY3_HYB	The average CY3_HYB control intensity across all probes	float	Y
HIGH_ STRINGENCY_HYB	The average HIGH_STRINGENCY_ HYB control intensity across all probes	float	Y
HOUSEKEEPING	The average HOUSEKEEPING control intensity across all probes	float	Y
LABELING	The average LABELING control intensity across all probes	float	Y
LOW_ STRINGENCY_HYB	The average LOW_STRINGENCY_HYB control intensity across all probes	float	Y
NEGATIVE (background)	The average NEGATIVE (background) control intensity across all probes	float	Y
NOISE	The average noise control intensity across all probes	float	Y

Group Gene Profile

Figure 97 shows an example Group Gene Profile.



Group Probe Profile | **Group Gene Profile** | Sample Probe Profile | Sample Gene Profile

	Group 1				Group 2			
SYMBOL	AVG_Signal	NARRAYS	ARRAY_STDEV	Detection Pval	AVG_Signal	NARRAYS	ARRAY_STDEV	Detecti
15E1.2	208.4	12	81.206	0.00000	164.9	12	29.371	0.0013
2'-PDE	365.1	12	148.473	0.00000	271.1	12	23.689	0.0000
76P	504.7	12	215.847	0.00000	400.2	12	41.307	0.0000
7A5	54.4	12	5.849	0.68248	47.3	12	5.468	0.6732
A1BG	71.5	12	12.576	0.08722	60.0	12	4.278	0.1494
A2BP1	60.7	12	7.375	0.56974	50.5	12	3.509	0.6305
A2M	530.3	12	236.824	0.00000	761.8	12	83.270	0.0000
A2ML1	51.8	12	4.358	0.91831	41.3	12	5.059	0.9815
A3GALT2	54.3	12	4.214	0.81233	47.0	12	4.693	0.8218
A4GALT	117.2	12	30.036	0.00922	102.7	12	12.006	0.0092
A4GNT	74.0	12	20.492	0.11858	69.6	12	12.376	0.0698
AAA1	75.4	12	11.462	0.16947	66.3	12	3.274	0.1431
AAAS	191.5	12	66.816	0.00000	174.0	12	20.245	0.0013
AACS	276.1	12	110.560	0.00000	218.2	12	29.043	0.0000
AACSL	77.0	12	21.300	0.07115	72.5	12	10.447	0.0527
AADAC	59.5	12	6.915	0.31489	50.8	12	4.494	0.4690
AADACL1	487.9	12	205.445	0.00000	427.5	12	37.698	0.0000
AADACL2	58.1	12	7.777	0.53228	47.7	12	4.529	0.6363
AADAT	104.5	12	27.331	0.14956	84.1	12	6.217	0.0538
AAK1	63.3	12	6.117	0.24242	48.4	12	6.787	0.6258
AAMP	366.9	12	154.981	0.00000	271.1	12	33.248	0.0000
AANAT	55.6	12	7.171	0.62451	49.3	12	5.939	0.5494
AARS	4700.3	12	2210.272	0.00000	3278.5	12	395.140	0.0000
AARSD1	539.5	12	231.858	0.00000	353.2	12	49.971	0.0000
AARSL	364.6	12	149.236	0.00000	268.3	12	35.635	0.0000
AASDH	162.7	12	52.465	0.00132	146.3	12	12.347	0.0026
AASDHPPT	425.8	12	179.296	0.00000	427.7	12	48.028	0.0000
AASS	109.4	12	29.178	0.00035	100.1	12	9.752	0.0002
AATF	859.5	12	386.247	0.00000	571.3	12	80.573	0.0000
AATK	63.6	12	8.859	0.39898	53.2	12	5.460	0.5039
ABAT	74.4	12	14.433	0.11311	64.6	12	5.985	0.0199
ABC1	406.6	12	170.312	0.00000	264.1	12	27.940	0.0000
ABCA1	772.7	12	349.473	0.00000	627.3	12	57.706	0.0000
ABCA10	63.1	12	7.052	0.39194	58.7	12	4.720	0.1233
ABCA11	114.1	12	32.489	0.01186	83.3	12	10.472	0.0197
ABCA12	73.2	12	14.582	0.01538	68.2	12	7.704	0.0047
ABCA13	51.3	12	10.171	0.97628	48.4	12	5.867	0.6021
ABCA2	143.7	12	46.564	0.06951	113.0	12	10.080	0.1395
ABCA3	149.9	12	50.623	0.00395	123.1	12	9.913	0.0039
ABCA4	123.3	12	23.352	0.00395	113.5	12	20.988	0.0039
ABCA5	87.5	12	18.222	0.00204	70.8	12	6.506	0.0052

Rows=38610 | Disp=38610 | Sel=1 | Filter=Filter is not active.

Figure 97 Group Gene Profile

The annotation columns of the Group Gene Profile are described in Table 11.

Table 11 Group Gene Profile Columns

Column	Description	Type	Visible by Default?
TargetID	Probe name. Also used as a key column for data import.	string	Y
SPECIES	The species of the BeadChip product	string	N
SOURCE	The database from which the annotation data was acquired	string	N
SEARCH_KEY	Gene identifier provided by the customer (for the DASL assay). Generally equivalent to SYMBOL (for Direct Hyb).	string	Y
TRANSCRIPT	RefSeq entry specifying an isoform (GI number).	string	N
SOURCE_ REFERENCE_ID	Database accession number	string	N
GI	RefSeq entry identifier (GI number).	string	N
ACCESSION	RefSeq entry (NM or XM number).	string	N
SYMBOL	Gene name as reported in RefSeq.	string	Y
PROBE_ID	Illumina identifier for probe sequence.	int	N
ARRAY_ ADDRESS_ID	Internal ID used by Illumina software	int	N

Table 11 Group Gene Profile Columns (continued)

Column	Description	Type	Visible by Default?
PROBE_TYPE	A, I, or S • For transcripts with a single isoform, we design "-S" probes (S=single) For transcripts with multiple isoforms, we design two types of probes: • "-I" (I=isoform-specific) are probes designed to query only one of multiple isoforms • "-A" (A=all) are probes designed to query all known isoforms of that transcript	string	N
PROBE_START	Coordinate in database entry where probe sequence begins	int	N
PROBE_ SEQUENCE	Sequence used as a probe on the array.	string	N
CHROMOSOME	The chromosome on which the probe is located	string	Y
PROBE_CHR_ ORIENTATION	The DNA strand on which the probe is located (positive or negative)	string	N
PROBE_ COORDINATES	The start and end positions of the probe on the chromosome	string	N
DEFINITION	Single-line description of gene in RefSeq	string	Y
ONTOLOGY_ COMPONENT	The gene ontology (GO) component classification(s) for this probe	string	N
ONTOLOGY_ PROCESS	The gene ontology (GO) process classification(s) for this probe	string	N
ONTOLOGY_ FUNCTION	The gene ontology (GO) function classification(s) for this probe	string	N
SYNONYMS	Other names (aliases) for the same gene.	string	Y

The per-group columns of the Group Gene Profile are described in Table 16.

Table 12 Group Gene Profile Per-Group Columns

Column	Description	Type	Visible by Default?
AVG_Signal	Average intensity of the bead type/target in the group.	float	Y
NARRAYS	Number of samples in the group.	int	Y
ARRAY_STDEV	Standard deviation associated with sample-to-sample variability within the group. Undefined when the group contains a single sample.	float	Y
Detection Pval	P-value computed from the background model characterizing the chance that the target sequence signal was distinguishable from the negative controls.	float	Y
MIN_Signal	Minimum intensity of the bead type/target in the group.	float	Y
MAX_Signal	Maximum intensity of the bead type/target in the group.	float	Y
BEAD_STDERR	Average standard error associated with bead-to-bead variability for the samples in the group.	float	Y
Avg_NBEADS	Average number of beads per bead type representing probes for the gene.	int	Y
In the case of groups containing only one sample, MIN, AVG, and MAX signals are equal.			

Group Probe Profile

Figure 98 shows an example Group Probe Profile.

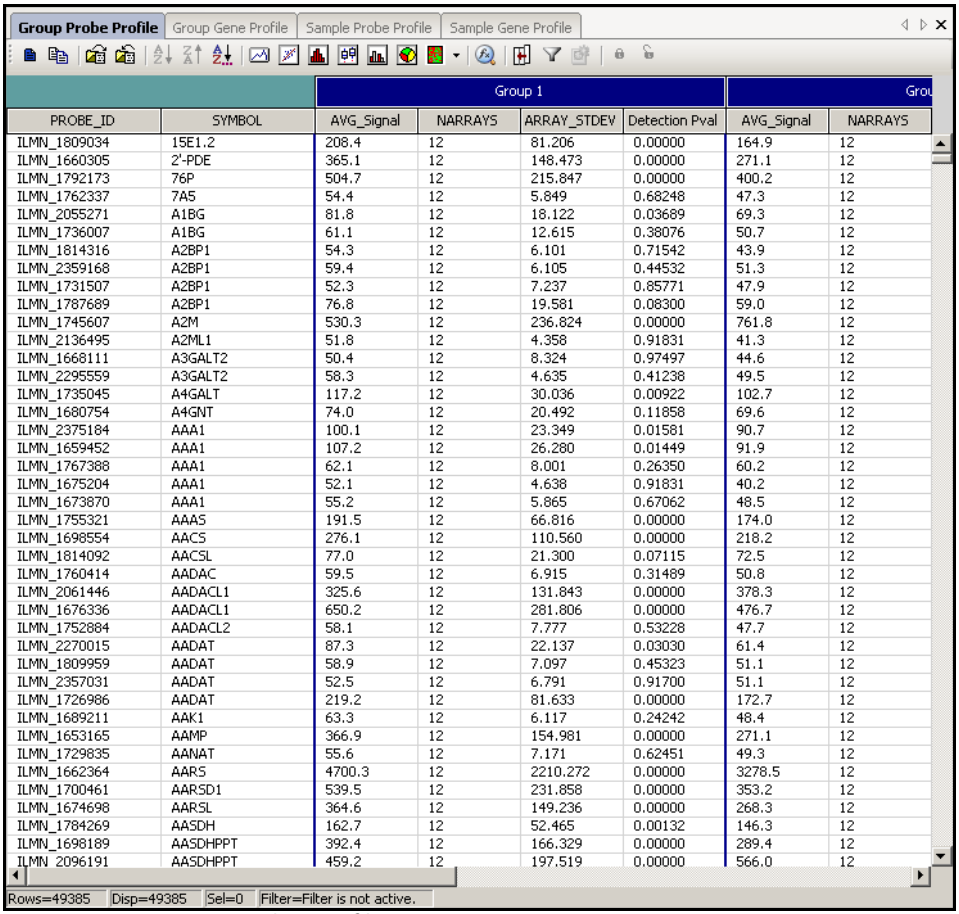


Figure 98 Group Probe Profile

The annotation columns of the Group Probe Profile are described in Table 13.

Table 13 Group Probe Profile Columns

Column	Description	Type	Visible by Default?
Target_ID	Probe name. Also used as a key column for data import.	string	Y
Probe_ID	Bead type.	int	Y
SPECIES	The species of the BeadChip product	string	N
SOURCE	The database from which the annotation data was acquired	string	N
SEARCH_KEY	Gene identifier provided by the customer (for the DASL assay). Generally equivalent to SYMBOL (for Direct Hyb).	string	Y
TRANSCRIPT	RefSeq entry specifying an isoform (GI number).	string	N
SOURCE_ REFERENCE_ID	Database accession number	string	N
GI	RefSeq entry identifier (GI number).	string	N
ACCESSION	RefSeq entry (NM or XM number).	string	N
SYMBOL	Gene name as reported in RefSeq.	string	Y
PROBE_ID	Illumina identifier for probe sequence.	int	N
ARRAY_ ADDRESS_ID	Internal ID used by Illumina software	int	N

Table 13 Group Probe Profile Columns (continued)

Column	Description	Type	Visible by Default?
PROBE_TYPE	A, I, or S • For transcripts with a single isoform, we design "-S" probes (S=single) For transcripts with multiple isoforms, we design two types of probes: • "-I" (I=isoform-specific) are probes designed to query only one of multiple isoforms • "-A" (A=all) are probes designed to query all known isoforms of that transcript	string	N
PROBE_START	Coordinate in database entry where probe sequence begins.	int	N
PROBE_ SEQUENCE	Sequence used as a probe on the array.	string	N
CHROMOSOME	The chromosome on which the probe is located	string	Y
PROBE_CHR_ ORIENTATION	The DNA strand on which the probe is located (positive or negative)	string	N
PROBE_ COORDINATES	The start and end positions of the probe on the chromosome	string	N
DEFINITION	Single-line description of gene in RefSeq.	string	Y
ONTOLOGY_ COMPONENT	The gene ontology (GO) component classification(s) for this probe	string	N
ONTOLOGY_ PROCESS	The gene ontology (GO) process classification(s) for this probe	string	N
ONTOLOGY_ FUNCTION	The gene ontology (GO) function classification(s) for this probe	string	N
SYNONYMS	Other names for the same gene (aliases).	string	Y

The per-group columns of the Group Probe Profile are described in Table 14.

Table 14 Group Probe Profile Per-Group Columns

Column	Description	Type	Visible by Default?
AVG_Signal	Average intensity of the bead type/target in the group.	float	Y
NARRAYS	Number of samples in the group.	int	Y
ARRAY_ STDEV	Standard deviation associated with sample-to-sample variability within the group (undefined when the group contains a single sample).	float	Y
Detection Pval	P-value computed from the background model characterizing the chance that the target sequence signal was distinguishable from negative controls.	float	Y
MIN_Signal	Minimum intensity of bead type/target in the group.	float	N
MAX_Signal	Maximum intensity of bead type/target in the group.	float	N
BEAD_ STDERR	Average standard error associated with bead-to-bead variability for the samples in the group.	float	N
Avg_ NBEADS	Average number of beads per bead type representing probes for the gene.	int	N
In the case of groups containing only one sample, MIN, AVG, and MAX signals are equal.			

Sample Gene Profile

Figure 99 shows an example of a Sample Gene Profile.

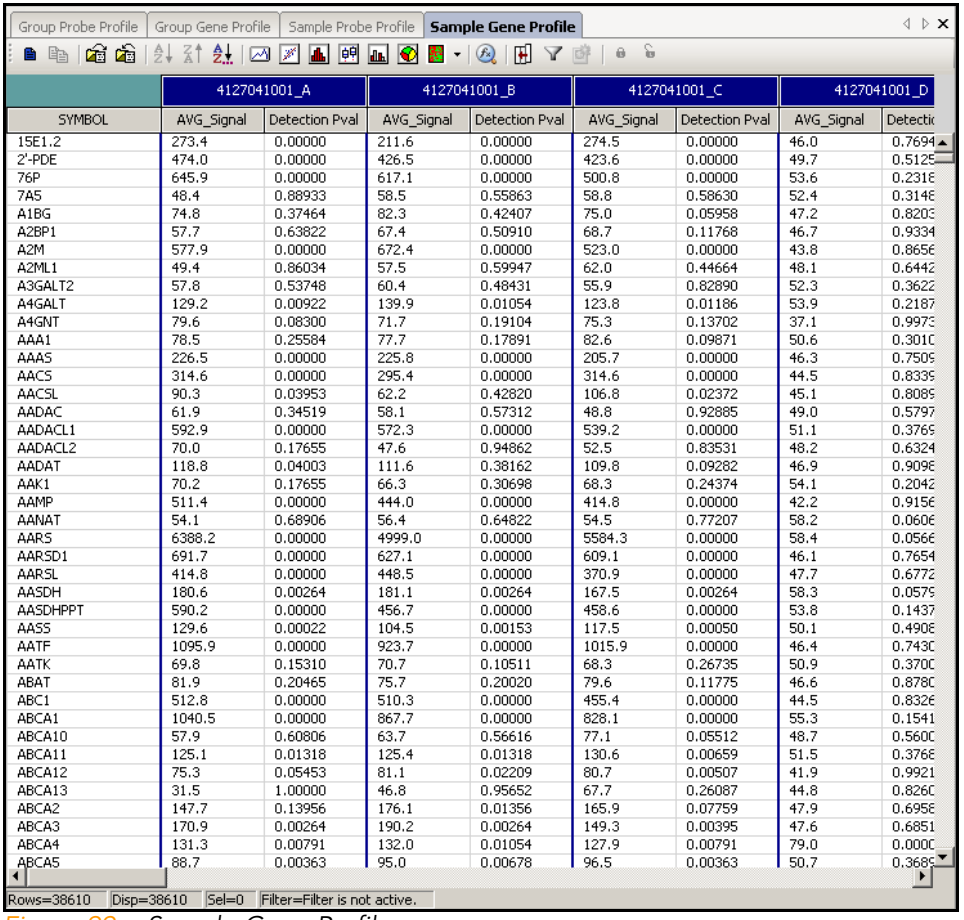


Figure 99 Sample Gene Profile

The annotation columns of the Sample Gene Profile are described in Table 15.

Table 15 Sample Gene Profile Columns

Column	Description	Type	Visible by Default?
TargetID	Probe name. Also used as a key column for data import.	string	Y
SPECIES	The species of the BeadChip product	string	N
SOURCE	The database from which the annotation data was acquired	string	N
SEARCH_KEY	Gene identifier provided by the customer (for the DASL assay). Generally equivalent to SYMBOL (for Direct Hyb).	string	Y
TRANSCRIPT	RefSeq entry specifying an isoform (GI number).	string	N
SOURCE_ REFERENCE_ID	Database accession number	string	N
GI	RefSeq entry identifier (GI number).	string	N
ACCESSION	RefSeq entry (NM or XM number).	string	N
SYMBOL	Gene name as reported in RefSeq.	string	Y
PROBE_ID	Illumina identifier for probe sequence.	int	N
ARRAY_ ADDRESS_ID	Internal ID used by Illumina software	int	N

Table 15 Sample Gene Profile Columns (continued)

Column	Description	Type	Visible by Default?
PROBE_TYPE	A, I, or S • For transcripts with a single isoform, we design "-S" probes (S=single) For transcripts with multiple isoforms, we design two types of probes: • "-I" (I=isoform-specific) are probes designed to query only one of multiple isoforms • "-A" (A=all) are probes designed to query all known isoforms of that transcript	string	N
PROBE_START	Coordinate in database entry where probe sequence begins.	int	Y
PROBE_ SEQUENCE	Sequence used as a probe on the array.	string	N
CHROMOSOME	The chromosome on which the probe is located	string	Y
PROBE_CHR_ ORIENTATION	The DNA strand on which the probe is located (positive or negative)	string	N
PROBE_ COORDINATES	The start and end positions of the probe on the chromosome	string	N
DEFINITION	Single-line description of gene in RefSeq.	string	Y
ONTOLOGY_ COMPONENT	The gene ontology (GO) component classification(s) for this probe	string	N
ONTOLOGY_ PROCESS	The gene ontology (GO) process classification(s) for this probe	string	N
ONTOLOGY_ FUNCTION	The gene ontology (GO) function classification(s) for this probe	string	N
SYNONYMS	Other names for the same gene (aliases).	string	Y

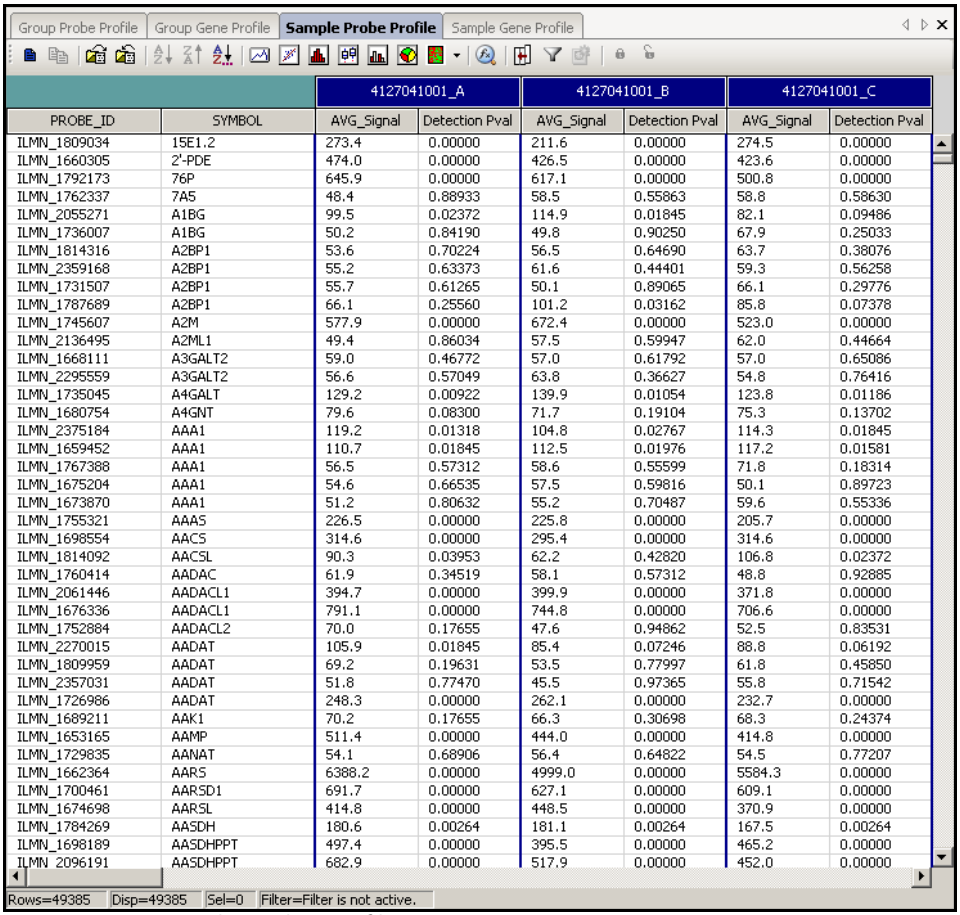
The per-sample columns of the Sample Gene Profile are described in Table 16.

Table 16 Sample Gene Profile Per-Sample Columns

Column	Description	Type	Visible by Default?
AVG_Signal	Average intensity of the bead type/target in the group.	float	Y
Detection Pval	P-value computed from the background model characterizing the chance that the target sequence signal was distinguishable from the negative controls.	float	Y
NARRAYS	Number of samples in the group.	int	N
ARRAY_ STDEV	Standard deviation associated with sample-to-sample variability within the group. Undefined when the group contains a single sample.	string	N
BEAD_ STDERR	Average standard error associated with bead-to-bead variability for the samples in the group.	float	N
Avg_ NBEADS	Average number of beads per bead type representing probes for the gene.	int	N

Sample Probe Profile

Figure 100 shows an example of a Sample Probe Profile.



Group Probe Profile | Group Gene Profile | **Sample Probe Profile** | Sample Gene Profile

		4127041001_A		4127041001_B		4127041001_C	
PROBE_ID	SYMBOL	AVG_Signal	Detection Pval	AVG_Signal	Detection Pval	AVG_Signal	Detection Pval
ILMN_1809034	15E1.2	273.4	0.00000	211.6	0.00000	274.5	0.00000
ILMN_1660305	2'-PDE	474.0	0.00000	426.5	0.00000	423.6	0.00000
ILMN_1792173	76P	645.9	0.00000	617.1	0.00000	500.8	0.00000
ILMN_1762337	7A5	48.4	0.88933	58.5	0.55863	58.8	0.58630
ILMN_2055271	A1BG	99.5	0.02372	114.9	0.01845	82.1	0.09486
ILMN_1736007	A1BG	50.2	0.84190	49.8	0.90250	67.9	0.25033
ILMN_1814316	A2BP1	53.6	0.70224	56.5	0.64690	63.7	0.38076
ILMN_2359168	A2BP1	55.2	0.63373	61.6	0.44401	59.3	0.56258
ILMN_1731507	A2BP1	55.7	0.61265	50.1	0.89065	66.1	0.29776
ILMN_1787689	A2BP1	66.1	0.25560	101.2	0.03162	85.8	0.07378
ILMN_1745607	A2M	577.9	0.00000	672.4	0.00000	523.0	0.00000
ILMN_2136495	A2ML1	49.4	0.86034	57.5	0.59947	62.0	0.44664
ILMN_1668111	A3GALT2	59.0	0.46772	57.0	0.61792	57.0	0.65086
ILMN_2295559	A3GALT2	56.6	0.57049	63.8	0.36627	54.8	0.76416
ILMN_1735045	A4GALT	129.2	0.00922	139.9	0.01054	123.8	0.01186
ILMN_1680754	A4GNT	79.6	0.08300	71.7	0.19104	75.3	0.13702
ILMN_2375184	AAA1	119.2	0.01318	104.8	0.02767	114.3	0.01845
ILMN_1659452	AAA1	110.7	0.01845	112.5	0.01976	117.2	0.01581
ILMN_1767388	AAA1	56.5	0.57312	58.6	0.55599	71.8	0.18314
ILMN_1675204	AAA1	54.6	0.66535	57.5	0.59816	50.1	0.89723
ILMN_1673870	AAA1	51.2	0.80632	55.2	0.70487	59.6	0.55336
ILMN_1755321	AAAS	226.5	0.00000	225.8	0.00000	205.7	0.00000
ILMN_1698554	AACS	314.6	0.00000	295.4	0.00000	314.6	0.00000
ILMN_1814092	AACSL	90.3	0.03953	62.2	0.42820	106.8	0.02372
ILMN_1760414	AADAC	61.9	0.34519	58.1	0.57312	48.8	0.92885
ILMN_2061446	AADACL1	394.7	0.00000	399.9	0.00000	371.8	0.00000
ILMN_1676336	AADACL1	791.1	0.00000	744.8	0.00000	706.6	0.00000
ILMN_1752884	AADACL2	70.0	0.17655	47.6	0.94862	52.5	0.83531
ILMN_2270015	AADAT	105.9	0.01845	85.4	0.07246	88.8	0.06192
ILMN_1809959	AADAT	69.2	0.19631	53.5	0.77997	61.8	0.45850
ILMN_2357031	AADAT	51.8	0.77470	45.5	0.97365	55.8	0.71542
ILMN_1726986	AADAT	248.3	0.00000	262.1	0.00000	232.7	0.00000
ILMN_1689211	AAK1	70.2	0.17655	66.3	0.30698	68.3	0.24374
ILMN_1653165	AAMP	511.4	0.00000	444.0	0.00000	414.8	0.00000
ILMN_1729835	AANAT	54.1	0.68906	56.4	0.64822	54.5	0.77207
ILMN_1662364	AARS	6388.2	0.00000	4999.0	0.00000	5584.3	0.00000
ILMN_1700461	AARSD1	691.7	0.00000	627.1	0.00000	609.1	0.00000
ILMN_1674698	AARSL	414.8	0.00000	448.5	0.00000	370.9	0.00000
ILMN_1784269	AASDH	180.6	0.00264	181.1	0.00264	167.5	0.00264
ILMN_1698189	AASDHPPT	497.4	0.00000	395.5	0.00000	465.2	0.00000
ILMN_2096191	AASDHPPT	682.9	0.00000	517.9	0.00000	452.0	0.00000

Rows=49385 | Disp=49385 | Sel=0 | Filter=Filter is not active.

Figure 100 Sample Probe Profile

The annotation columns of the Sample Probe Profile are listed and described in Table 17.

Table 17 Sample Probe Profile Columns

Column	Description	Type	Visible by Default?
TargetID	Identifies the probe name. Also used as a key column for data import.	string	Y
ProbeID	Identifies the bead type.	int	Y
SPECIES	The species of the BeadChip product	string	N
SOURCE	The database from which the annotation data was acquired	string	N
SEARCH_KEY	Gene identifier provided by the customer (for the DASL assay). Generally equivalent to SYMBOL (for Direct Hyb).	string	Y
TRANSCRIPT	RefSeq entry specifying an isoform (GI number).	string	N
SOURCE_ REFERENCE_ID	Database accession number	string	N
GI	RefSeq entry identifier (GI number).	string	N
ACCESSION	RefSeq entry (NM or XM number).	string	N
SYMBOL	Gene name as reported in RefSeq.	string	Y
PROBE_ID	Illumina identifier for probe sequence.	int	N
ARRAY_ ADDRESS_ID	Internal ID used by Illumina software	int	N

Table 17 Sample Probe Profile Columns (continued)

Column	Description	Type	Visible by Default?
PROBE_TYPE	A, I, or S • For transcripts with a single isoform, we design "-S" probes (S=single) For transcripts with multiple isoforms, we design two types of probes: • "-I" (I=isoform-specific) are probes designed to query only one of multiple isoforms • "-A" (A=all) are probes designed to query all known isoforms of that transcript	string	N
PROBE_START	Coordinate in database entry where probe sequence begins.	int	N
PROBE_ SEQUENCE	Sequence used as a probe on the array.	string	N
CHROMOSOME	The chromosome on which the probe is located	string	Y
PROBE_CHR_ ORIENTATION	The DNA strand on which the probe is located (positive or negative)	string	N
PROBE_ COORDINATES	The start and end positions of the probe on the chromosome	string	N
DEFINITION	Single-line description of gene in RefSeq.	string	Y
ONTOLOGY_ COMPONENT	The gene ontology (GO) component classification(s) for this probe	string	N
ONTOLOGY_ PROCESS	The gene ontology (GO) process classification(s) for this probe	string	N
ONTOLOGY_ FUNCTION	The gene ontology (GO) function classification(s) for this probe	string	N
SYNONYMS	Other names for the same gene (aliases).	string	Y

The per-sample columns of the Sample Probe Profile are listed and described in Table 18.

Table 18 Sample Probe Profile Per-Sample Columns

Column	Description	Type	Visible by Default?
AVG_Signal	Average intensity of the bead type/target in the group.	float	Y
Detection Pval	P-value computed from the background model characterizing the chance that the target sequence signal was distinguishable from the negative controls.	float	Y
NARRAYS	Number of samples in the group.	int	N
ARRAY_ STDEV	Standard deviation associated with sample-to-sample variability within the group. Undefined when the group contains a single sample.	string	N
BEAD_ STDERR	Average standard error associated with bead-to-bead variability for the samples in the group.	float	N
Avg_NBEADS	Average number of beads per bead type representing probes for the gene.	int	N

Groups (columns in Group tabs) are named according to the names given when defining groupsets, or from the sample sheet.
Samples (columns in Sample tabs) are named according to the Universal Array product.

Control Gene Profile

Figure 101 shows an example Control Gene Profile.

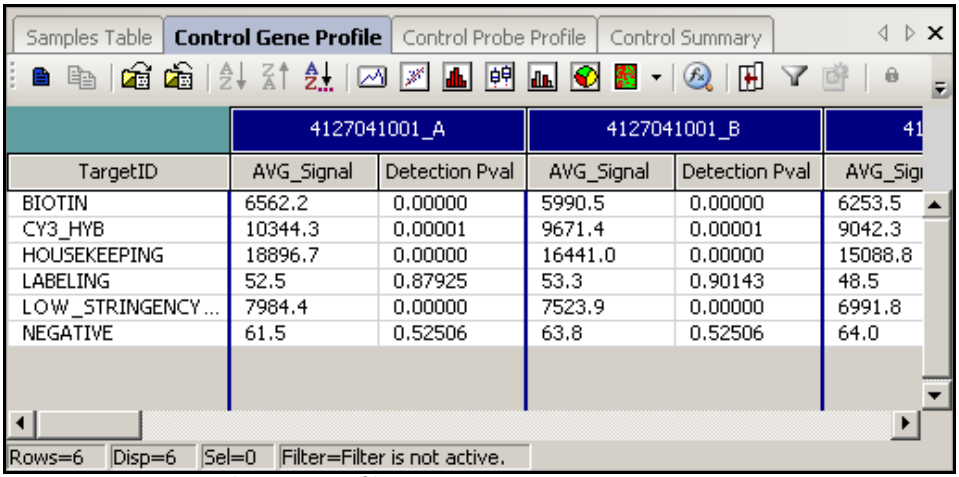


Figure 101 Control Gene Profile

The annotation columns of the Control Gene Profile window are described in Table 19.

Table 19 Control Gene Profile Columns

Column	Description	Type	Visible by Default?
TargetID	Probe name. Also used as a key column for data import.	string	Y

The per-sample columns of the Control Gene Profile are described in Table 16.

Table 20 Control Gene Profile Per-Sample Columns

Column	Description	Type	Visible by Default?
AVG_Signal	Average intensity of the bead type/target in the group.	float	Y
Detection Pval	P-value computed from the background model characterizing the chance that the target sequence signal was distinguishable from the negative controls.	float	Y
NARRAYS	Number of samples in the group.	int	N
ARRAY_ STDEV	Standard deviation associated with sample-to-sample variability within the group. Undefined when the group contains a single sample.	string	N
BEAD_ STDERR	Average standard error associated with bead-to-bead variability for the samples in the group.	float	N
Avg_NBEADS	Average number of beads per bead type representing probes for the gene.	int	N

Control Probe Profile

Figure 102 shows an example Control Probe Profile.

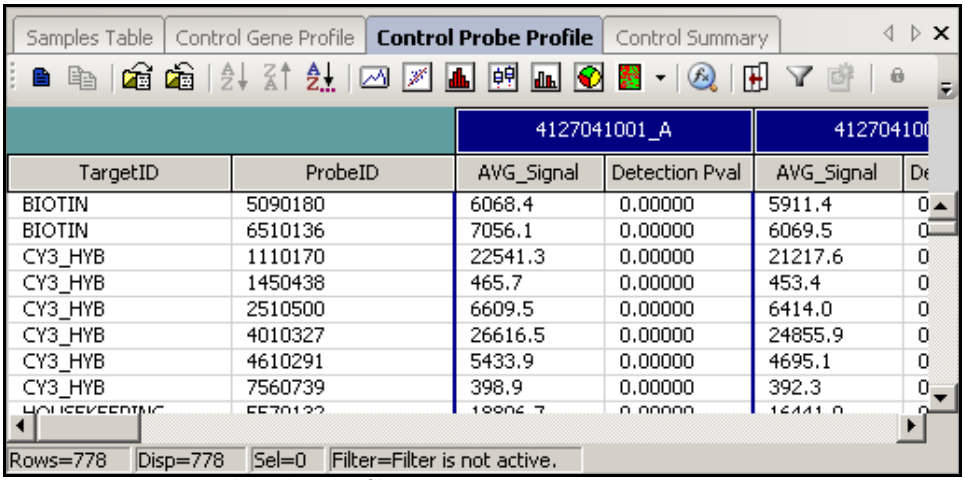


Figure 102 Control Probe Profile

The annotation columns of the Control Probe Profile are described in Table 21.

Table 21 Control Probe Profile Columns

Column	Description	Type	Visible by Default?
TargetID	Probe name. Also used as a key column for data import.	string	Y
ProbeID	Bead type.	int	Y

The per-sample columns of the Control Probe Profile are listed and described in Table 16.

Table 22 Control Probe Profile Per-Sample Columns

Column	Description	Type	Visible by Default?
AVG_Signal	Average intensity of the bead type/target in the group.	float	Y
Detection Pval	P-value computed from the background model characterizing the chance that the target sequence signal was distinguishable from the negative controls.	float	Y
NARRAYS	Number of samples in the group.	int	N
ARRAY_ STDEV	Standard deviation associated with sample-to-sample variability within the group. Undefined when the group contains a single sample.	string	N
BEAD_ STDERR	Average standard error associated with bead-to-bead variability for the samples in the group.	float	N
Avg_NBEADS	Average number of beads per bead type representing probes for the gene.	int	N

Excluded and Imputed Probes Table

The Excluded and Imputed Probes table appears in a gene expression project only if the project contains missing data. If there is no missing data in a project, the Excluded and Imputed Probes table is not generated and does not appear in the project.

Figure 103 shows an example Excluded and Imputed Probes table.

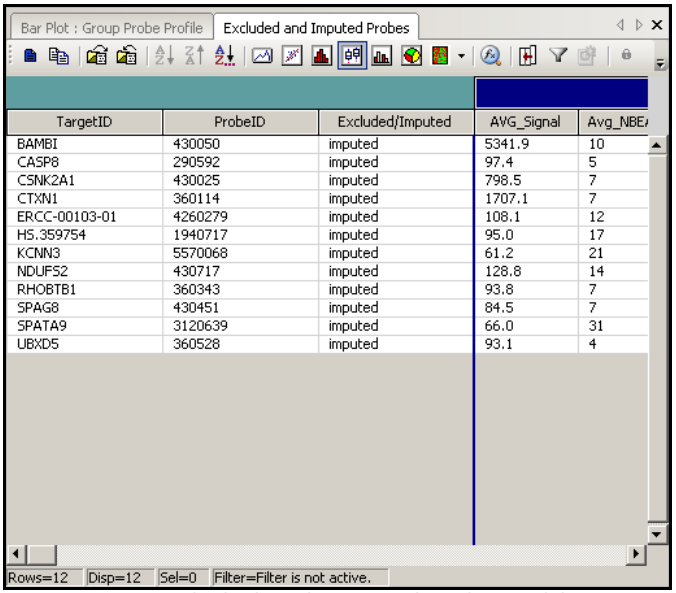


Figure 103 Excluded and Imputed Probes Table

The annotation columns of the Excluded and Imputed Probes table are described in Table 23.

Table 23 Excluded and Imputed Probes Table Columns

Column	Description	Type	Visible by Default?
TargetID	Probe name. Also used as a key column for data import.	string	Y
ProbeID	Bead type.	string	Y
Excluded/ Imputed	Indication whether the data has been excluded from the project or the value has been imputed	string	Y

The per-sample columns of the Excluded and Imputed Probes table are listed and described in Table 24.

Table 24 Excluded and Imputed Probes Table Per-Sample Columns

Column	Description	Type	Visible by Default?
AVG_Signal	Average intensity of the bead type/target in the group.	float	Y
AVG_NBEADS	Average number of beads per bead type representing probes for the gene.	int	Y
BEAD_STDERR	Average standard error associated with bead-to-bead variability for the samples in the group.	float	Y
Excluded	1 indicates that this data has been excluded from the project; 0 otherwise.	int	Y
Imputed	1 indicates that the AVG_Signal value is imputed; 0 otherwise.	int	Y

Control Summary

The Gene Expression Module displays a graphic Control Summary for the selected arrays based on the performance of the built-in controls.

Figure 104 shows an example Control Summary for a Direct Hyb project.

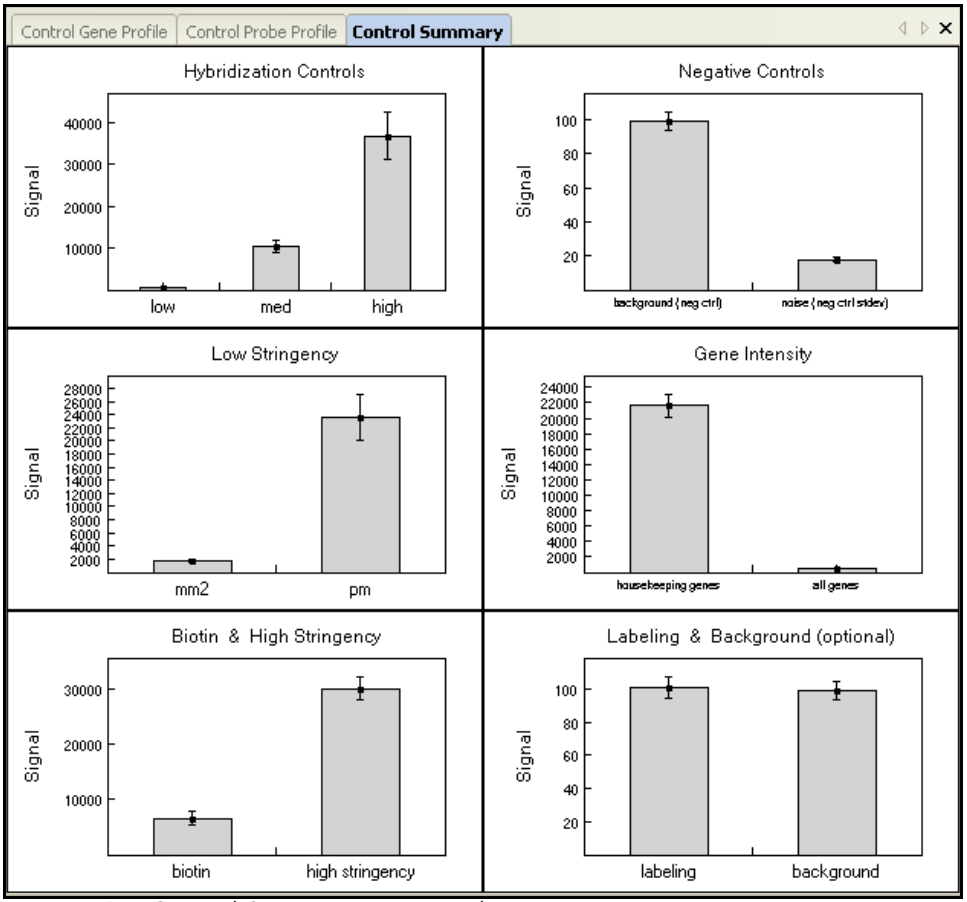


Figure 104 Control Summary, Direct Hyb

Figure 105 shows an example Control Summary for a DASL or VeraCode DASL project.

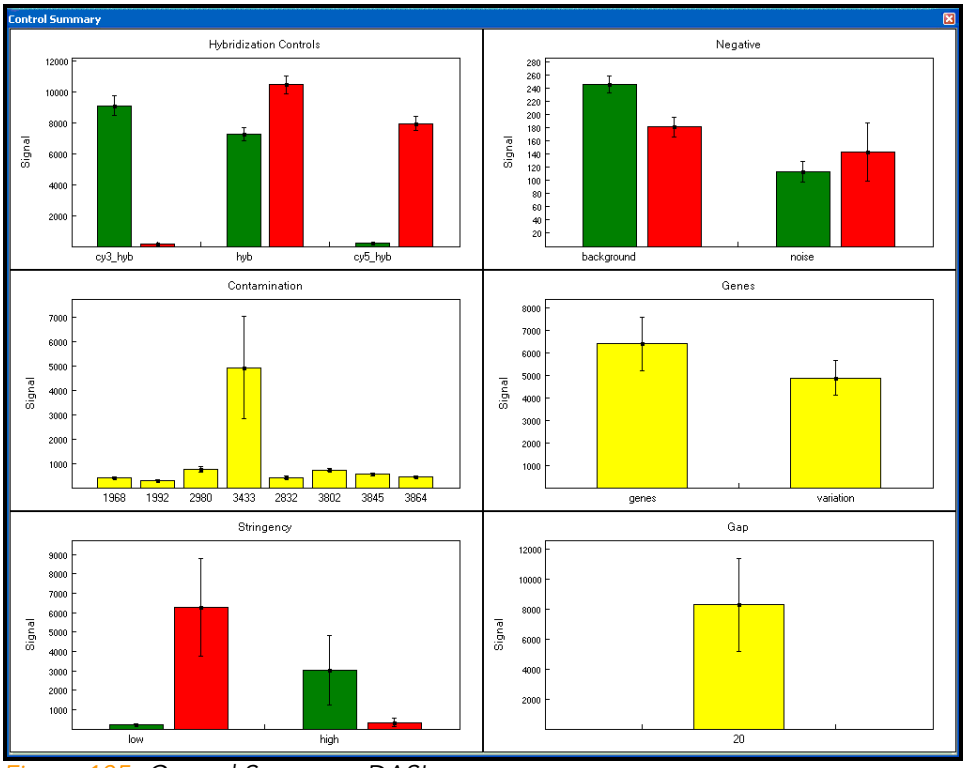


Figure 105 Control Summary, DASL

Figure 106 shows an example Control Summary for a Whole Genome DASL project.

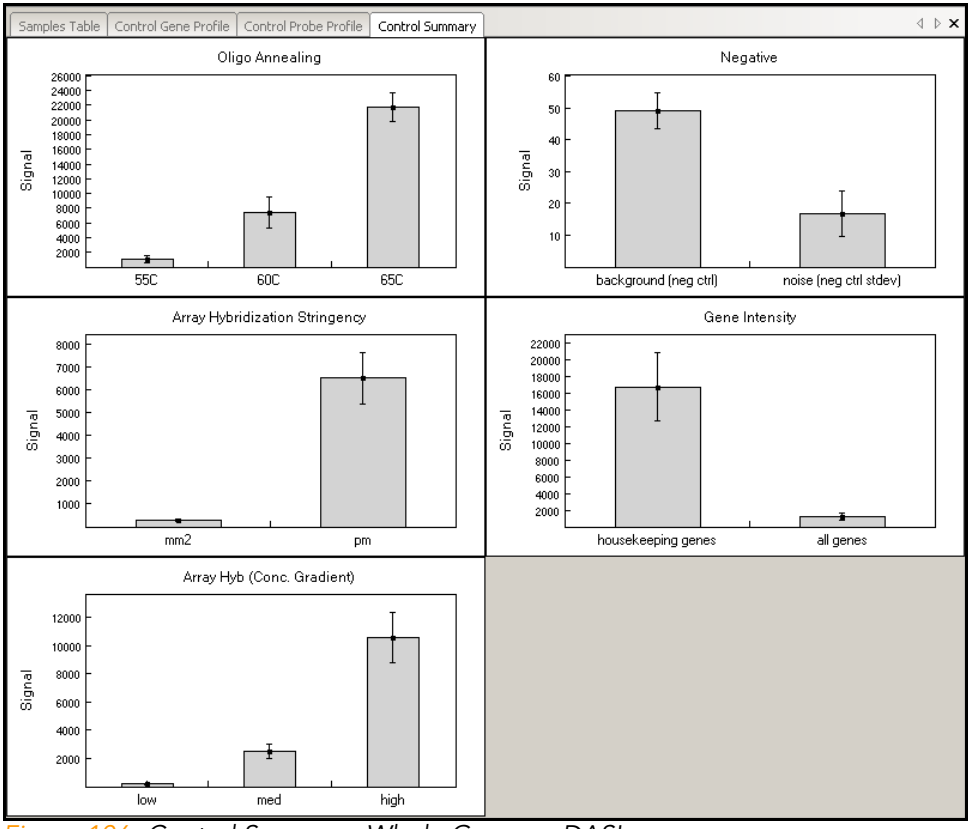


Figure 106 Control Summary, Whole Genome DASL

Figure 107 shows an example Control Summary for a miRNA project.

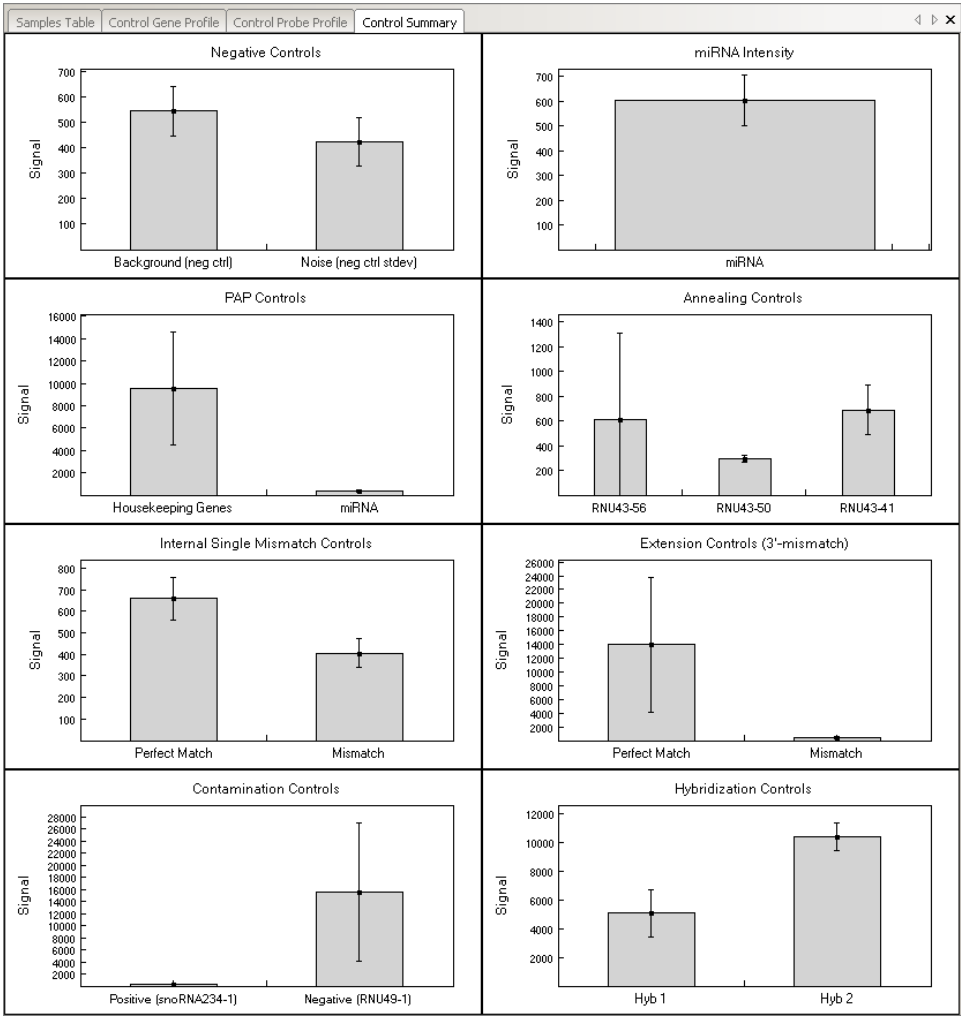


Figure 107 Control Summary, miRNA

For detailed information about the controls, see the *System Controls* appendix in the appropriate Illumina product guide.

Project Window

The Project window (Figure 108) identifies the manifest(s) loaded for your project and has a data section that identifies all of the barcodes used in your project. You can expand a barcode and view the samples loaded on that Universal Array Product.

Figure 108 Project Window

Log Window

The Log window (Figure 109) is a simple console that provides feedback on GenomeStudio processes. The Log window displays any errors in red.

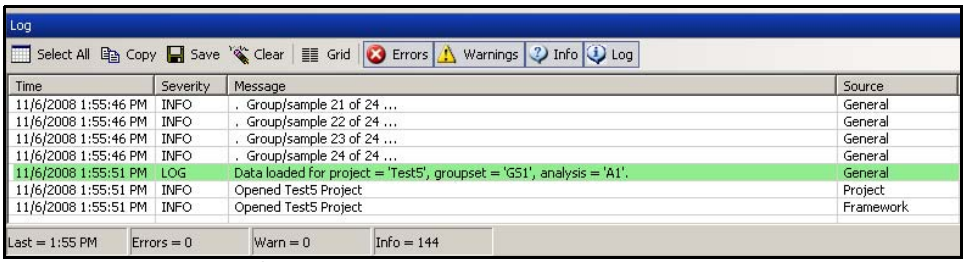


Figure 109 Log Window

Table 25 Log Window Selections & Functions

Selection	Function	Toolbar Button (if used)
Select All	Selects all log entries.	
Copy	Copies log entries to the clipboard.	
Save	Saves all log entries.	
Clear	Clears all log entries.	
Grid	Toggles the grid off and on.	
Errors	Toggles errors off and on.	
Warnings	Toggles warnings off and on.	
Info	Toggles info off and on.	
Log	Toggles the log off and on.	
Time	Displays the time the log entry was generated.	
Severity	Displays the severity of the log entry.	
Message	Displays the text description of the log entry.	
Source	Displays the source of the log entry.	

Main Window Menus

Table 26 lists the selections available from the GenomeStudio Gene Expression Module main window menus and corresponding toolbar buttons.

Table 26 Main Menu Selections & Functions

Selection	Function	Toolbar Button (if used)
	File Menu	
New Project	Opens a new project	
Open Project	Opens a previously saved project.	
Save Project	Saves all current information in this project, so you can return to it later.	
Save Project Copy As	Allows you to specify a file name and location where you would like to save a copy of the current project.	
Close Project	Closes the current project and returns you to the start window of the Gene Expression module.	
Export to GeneSpring GX Format	**Group Gene Profile**—Exports the Group Gene Profile to a *.txt file in a location you specify. **Group Probe Profile**—Exports the Group Probe Profile to a *.txt file in a location you specify. **Sample Gene Profile**—Exports the Sample Gene Profile to a *.txt file in a location you specify. **Sample Probe Profile**—Exports the Sample Probe Profile to a *.txt file in a location you specify.	
Manage Project Data	Opens the GenomeStudio Project Wizard - Project Data Selection dialog box, from which you can specify the Universal Array Products to include in your project.	
Page Setup	Opens the Windows Page Setup dialog box, which you can use to set up the page properties and configure the printer properties.	

Table 26 Main Menu Selections & Functions (continued)

Selection	Function	Toolbar Button (if used)
Print Preview	Opens the Print Preview window, which you can use to see how the selected graph will print.	
Print	Displays the print dialog box. Use this dialog box to select options for printing the currently displayed graph.	
Recent Project	Allows you to select a project you have recently worked on.	
Exit	Closes GenomeStudio.	
Edit Menu		
Cut	Cuts the current selection.	
Copy	Copies the current selection to the clipboard.	
Paste	Pastes the current selection from the clipboard.	
Delete	Deletes the current selection.	
Select All	Selects all rows in the current table.	
View Menu		
Save Default View	Allows you to save the default view of the open project.	
Restore Default View	Restores the default view of the open project.	
Save Custom View	Allows you to save a custom view to use again later.	
Load Custom View	Allows you to load a previously-saved custom view.	
Log	Shows or hides the **Log** window.	
Project	Shows or hides the **Project** window.	

Table 26 Main Menu Selections & Functions (continued)

Selection	Function	Toolbar Button (if used)
	Analysis Menu	
Manage Analyses	Displays the **GenomeStudio Manage Analyses** dialog box.	
Manage Group Sets	Displays the **GenomeStudio Project Wizard—Groupset Definition** dialog box.	
Run Gene Analysis	Performs gene analysis for the current experiment.	
Run Differential Expression Analysis	Performs differential expression analysis for the current experiment.	
Run Cluster Analysis	Creates a dendrogram for the current experiment.	
Show Bar Plot	Creates a bar plot for the current experiment.	
Show Genome Viewer	Launches the Illumina Genome Viewer (IGV).	
Create Final Report	Displays the **GenomeStudio Gene Expression Final Report** dialog box, from which you can create a Final Report.	
View Image	Displays the **GenomeStudio View Image dialog box, from** which you can select an image to view.	
View Marked Items in Web Browser	Displays the **GenomeStudio Gene Expression Web Browser** dialog box, from which you can select columns and subcolumns to display in a web browser.	

Table 26 Main Menu Selections & Functions (continued)

Selection	Function	Toolbar Button (if used)
	Tools Menu	
Options	**Project**—Opens the Project Properties window, in which you can make changes to project settings. **GenomeStudio**—Opens the GenomeStudio Options window, in which you can select GenomeStudio options including the maximum number of project files and display attributes such as font name, size, and style. **Module**—Opens the module Properties window, in which you can select file-based storage or memory-based storage.	
	Windows Menu	
This menu is populated with the windows that are currently available. Check marks indicate the windows that are currently displayed.		
	Help Menu	
About GenomeStudio	Opens the **About** box for the GenomeStudio application, which contains: • Version information for the GenomeStudio Framework as well as any GenomeStudio modules you have installed • GenomeStudio copyright information • Software copyright notice	

Context Menus

The following tables list GenomeStudio Gene Expression Module context menu elements and descriptions.

Table 27 includes Bar Plot: Gene Profile window context menu elements and descriptions.

Table 27 Bar Plot: Group Gene Profile Window Context Menu

Element	Description
Properties	Displays the Plot Settings dialog box, from which you can alter the visual properties of the bar plot.
Clear Selected Values	Clears selected values from the bar plot.
Copy As	Copies the bar plot to the clipboard as one of the following file types: BMP, JPEG, PNG, GIF, or TIFF.

Table 28 includes context menu elements and descriptions for other tabs.

Table 28 Other Tabbed Window Context Menu

Element	Description
Show Only Selected Rows	Shows only selected rows.
View Image	Displays Image Viewer for selected samples.
Configure Marks	Allows you to configure the properties of your marks.
Mark Selected Rows | <Add New>	Creates a new mark and marks selected rows.
Select Marked Rows	Selects marked rows.
Clear Marks <All>	Clears all marks.

Table 29 includes Project window context menu elements and descriptions.

Table 29 Project Window Context Menu

Element	Description
Project Window	**Export Project**—Exports a project.
	Expand All—Expands all project repositories in the **Project** window.
	Collapse All—Collapses all project repositories in the **Project** window.
	Style—Selects a style for your project. Available project styles include: Standard, Plain, Explorer, Navigator, Group, Office Light, and Office Dark.

Table 30 includes Log window context menu elements and descriptions.

Table 30 Log Window Context Menu

Element	Description
Log	Toggles the **Log** window (visible/hidden).
Project	Toggles the **Project** window (visible/hidden).
Show All	Displays both the **Project** and **Log** windows.
Hide All	Hides both the **Project** and **Log** windows.

Appendix A

Sample Sheet Format

Topics

Introduction

The sample sheet is a comma delimited text file (*.csv). It is divided into sections, indicated by lines with the section name enclosed by square brackets. The Data section is the only required section. You can also include a Header section, or other user-defined sections.

Data Section

The first row of the Data section must indicate the column names of the data to follow. The columns can be in any order, and additional user-defined columns can be included in the file.

Table 31 Data Section, Optional and Required Columns

Column	Description	Optional (O) or Required (R)
Sample_Name	For example, S12345. Name of the sample (used only for display in the table). GenomeStudio assigns a default sample name, concatenating the sample plate and sample well names.	O
Sample_Well	For example, A01. The well within the sample plate for this sample. Used only for display in the table.	O
Sample_Plate	For example, XXXXXXXXX-RNA. The barcode of the sample plate for this sample. Used only for display in the table.	O
Sample_Group	For example, Group_1 User-specified name of the sample group. Note: If Sample_Group is missing, GenomeStudio creates one group with the name "Default Group."	R
Pool_ID	Not used for Direct Hyb.	
Sentrix_ID	For example, 1167988 SAM or BeadChip ID.	R

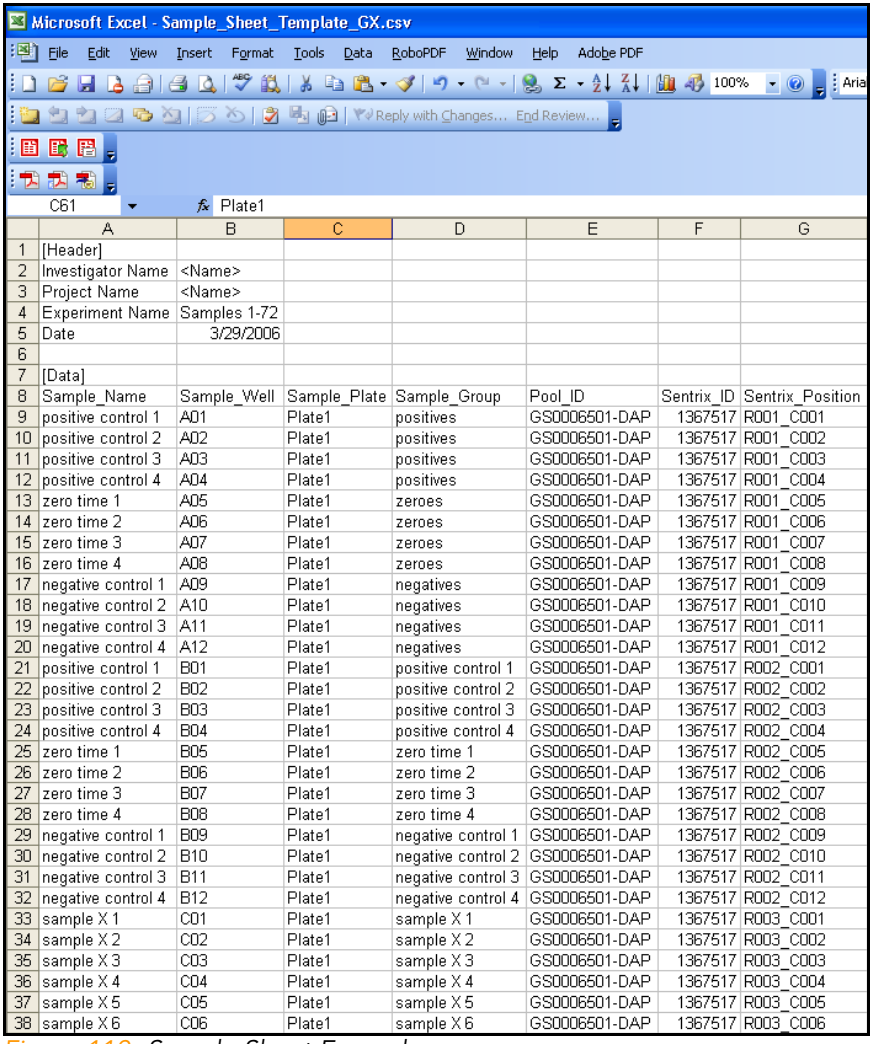
Table 31 Data Section, Optional and Required Columns (continued)

Column	Description	Optional (O) or Required (R)
SentrixPosition	For example, R001_C001 for a SAM, A1 for a BeadChip. For SAMs, the SAM sample to which the sample is hybridized. For BeadChips, the section to which the sample is hybridized.	R
NOTES:	Figure 110 is an example of a sample sheet. Your sample sheet header may contain whatever information you choose. Your sample sheet may contain any number of columns you choose. Your sample sheet must be in a comma-delimited (*.csv) file format.	

Sample Sheet Template

A template for a sample sheet is provided on the GenomeStudio CD.

Sample Sheet Example



Microsoft Excel - Sample_Sheet_Template_GX.csv

File Edit View Insert Format Tools Data RoboPDF Window Help Adobe PDF

C61 Plate1

	A	B	C	D	E	F	G
1	[Header]						
2	Investigator Name	<Name>					
3	Project Name	<Name>					
4	Experiment Name	Samples 1-72					
5	Date	3/29/2006					
6							
7	[Data]						
8	Sample_Name	Sample_Well	Sample_Plate	Sample_Group	Pool_ID	Sentrix_ID	Sentrix_Position
9	positive control 1	A01	Plate1	positives	GS0006501-DAP	1367517	R001_C001
10	positive control 2	A02	Plate1	positives	GS0006501-DAP	1367517	R001_C002
11	positive control 3	A03	Plate1	positives	GS0006501-DAP	1367517	R001_C003
12	positive control 4	A04	Plate1	positives	GS0006501-DAP	1367517	R001_C004
13	zero time 1	A05	Plate1	zeroes	GS0006501-DAP	1367517	R001_C005
14	zero time 2	A06	Plate1	zeroes	GS0006501-DAP	1367517	R001_C006
15	zero time 3	A07	Plate1	zeroes	GS0006501-DAP	1367517	R001_C007
16	zero time 4	A08	Plate1	zeroes	GS0006501-DAP	1367517	R001_C008
17	negative control 1	A09	Plate1	negatives	GS0006501-DAP	1367517	R001_C009
18	negative control 2	A10	Plate1	negatives	GS0006501-DAP	1367517	R001_C010
19	negative control 3	A11	Plate1	negatives	GS0006501-DAP	1367517	R001_C011
20	negative control 4	A12	Plate1	negatives	GS0006501-DAP	1367517	R001_C012
21	positive control 1	B01	Plate1	positive control 1	GS0006501-DAP	1367517	R002_C001
22	positive control 2	B02	Plate1	positive control 2	GS0006501-DAP	1367517	R002_C002
23	positive control 3	B03	Plate1	positive control 3	GS0006501-DAP	1367517	R002_C003
24	positive control 4	B04	Plate1	positive control 4	GS0006501-DAP	1367517	R002_C004
25	zero time 1	B05	Plate1	zero time 1	GS0006501-DAP	1367517	R002_C005
26	zero time 2	B06	Plate1	zero time 2	GS0006501-DAP	1367517	R002_C006
27	zero time 3	B07	Plate1	zero time 3	GS0006501-DAP	1367517	R002_C007
28	zero time 4	B08	Plate1	zero time 4	GS0006501-DAP	1367517	R002_C008
29	negative control 1	B09	Plate1	negative control 1	GS0006501-DAP	1367517	R002_C009
30	negative control 2	B10	Plate1	negative control 2	GS0006501-DAP	1367517	R002_C010
31	negative control 3	B11	Plate1	negative control 3	GS0006501-DAP	1367517	R002_C011
32	negative control 4	B12	Plate1	negative control 4	GS0006501-DAP	1367517	R002_C012
33	sample X 1	C01	Plate1	sample X 1	GS0006501-DAP	1367517	R003_C001
34	sample X 2	C02	Plate1	sample X 2	GS0006501-DAP	1367517	R003_C002
35	sample X 3	C03	Plate1	sample X 3	GS0006501-DAP	1367517	R003_C003
36	sample X 4	C04	Plate1	sample X 4	GS0006501-DAP	1367517	R003_C004
37	sample X 5	C05	Plate1	sample X 5	GS0006501-DAP	1367517	R003_C005
38	sample X 6	C06	Plate1	sample X 6	GS0006501-DAP	1367517	R003_C006

Figure 110 Sample Sheet Example

Appendix B

Troubleshooting

Introduction

Use this troubleshooting guide to assist you with any questions you may have about the GenomeStudio Gene Expression Module.

Frequently Asked Questions

Table 32 lists frequently asked questions and associated responses.

Table 32 Frequently Asked Questions

#	Question	Response
1.	What is the difference between a group and a groupset?	A **group** is a collection of arrays combined according to experimental criteria (e.g., arrays hybridized to similar or replicated samples). A **groupset** is a collection of groups.
2.	What is the minimum statistically-significant detectable fold change of Gene Expression BeadChips?	1.35 fold for single replicates Much lower for multiple replicates
3.	Over what range of intensities can I detect the minimum significant detectable fold change?	The intensity range over which a fold change of ~1.35 are significantly distinguishable is >3 logs.
4.	How can I calculate a p-value from the diff score?	p = 1/(10^(diff score/10*(sgn(μcond - μref)))

Table 32 Frequently Asked Questions (continued)

#	Question	Response
5.	What do the designators "A," "S," and "I" mean in the manifest files?	• For transcripts with a single isoform, we design "-S" probes (S=single) For transcripts with multiple isoforms, we design two types of probes: • "-I" (I=isoform-specific) are probes designed to query only one of multiple isoforms • "-A" (A=all) are probes designed to query all known isoforms of that transcript
6.	How do I determine which genes are accurately detected?	Filter the genes using the detection p-value. Setting detection at 0.01 means that you have a 1% false positive rate. 0.05 is a commonly-used cut-off.
7.	What do the column headers "GeneSymbol," "GID," and "Accession" reference on the gene list for Illumina's standard BeadChips, and where do the numbers come from?	Descriptions for all of the column headers can be found in the document "Bead Manifest Field Descriptors" located on the documentation CD included in the startup kit.
8.	Is it possible to get the data for each feature on the BeadChip?	Yes, this is known as bead-level data. Contact your Field Application Scientist (FAS) or Tech Support for assistance.
9.	What is a Diff Score and how can I use it to get the p-value I want?	The Diff Score is a transformation of the p-value that provides directionality to the p-value based on the difference between the average signal in the reference group vs. the comparison group. The formula is: $Diffscore = 10*sgn(\mu_{ref}-\mu_{cond})*\log_{10}(p)$ For a p-value of 0.05, Diff Score = ± 13 For a p-value of 0.01, Diff Score = ±20 For a p-value of 0.001, Diff Score = ± 30