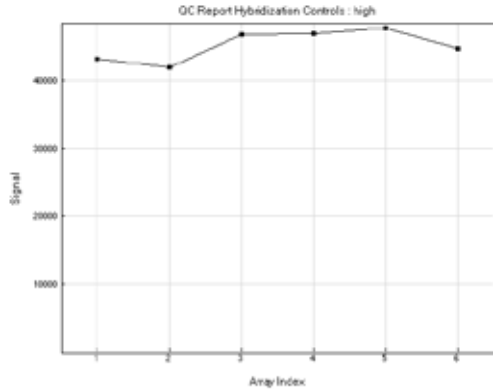


Figure 2: Genomestudio Control Summary Plot



Signal intensity values (y-axis) of hybridization controls are consistently high across all six arrays/samples (x-axis) from a gene expression profiling experiment. This graph does not indicate a data quality problem.

ments from the actual sample of interest. Poor performance of these controls may indicate a problem related to the sample or labeling. Further details of the control plots in GenomeStudio are provided in Table 1, the GenomeStudio Gene Expression Module User Guide, and related Assay Guides.

Normal variations in control plot values can arise due to incidental factors such as system setup, sample origin, and BeadChip type. These factors make it difficult to determine data quality by comparison to a specified expected value for each QC metric. To minimize the influence of these factors, relative—rather than absolute—control values should be used as QC criteria. Relative comparisons of control values can be made by:

1. Identification of outliers by comparison to current and historical data. Outlying samples for any given control metric can be quickly identified in GenomeStudio by using the control summary plot and expanding the plots to view QC values for individual samples (Figure 2). The data can also be exported and examined in other spreadsheet software, enabling further comparisons with historical data.
2. Comparison of samples across two or more control metrics to ensure a consistent ratio between relevant control values. This can be shown by simultaneously displaying and comparing different control plots in GenomeStudio. Alternatively, the control data can be exported and relevant values co-plotted using other software (Figure 3). For example, typical comparisons for the Direct Hyb assay include:
 - Housekeeping & Background. While housekeeping genes are known to fluctuate as a function of tissue type, they should be fairly consistent across arrays when from a similar sample source. Housekeeping genes should produce a higher signal than background.
 - Perfect Match (PM) & Mismatch (MM2). The PM probe signal is expected to be higher than the MM2 probe signal (Figure 3). Deviations in the ratio of PM to MM2 signal for a given sample could indicate a problem with specificity in the experiment.

Table 1: Control Plots in Genomestudio

A: Direct Hyb Control Plots

Control Metric	Expected value
Hybridization Controls*	High > Medium > Low
Low Stringency*	PM > MM2
Biotin and High Stringency*	High
Negative Controls (Background and Noise)	Low
Gene Intensity (Housekeeping and All Genes)	Higher than Background (Housekeeping > All Genes)
Labeling and Background	If used, Labeling > Background; Otherwise, Labeling ≈ Background.

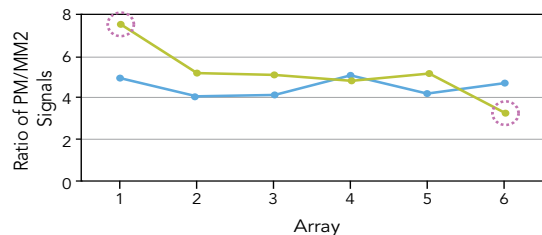
B: WG DASL Control Plots

Control Metric	Expected value
Hybridization Controls*	High > Medium > Low
Contamination	One Code High, Others Low
Stringency† (Low and High)	Low: Red > Green High: Green > Red
Negative Controls (Background and Noise)	Low
Genes (Genes and Variation)	Higher than Background
Gap‡	Higher than Background

*Sample-independent control metrics.

†DASL stringency and gap controls are designed against the glutaminyI-tRNA-synthetase gene (QARS) and are therefore dependent on the expression level of QARS in the sample of interest.

Figure 3: Comparison Across Control Metrics, PM/MM2 Ratio Line Plot



A plot of the ratio of PM/MM2 probe signals across several samples from two different BeadChips (blue and green). In the case of the blue BeadChip, all six samples have similar ratios approximately 4–5 PM/MM2. However, some arrays from the green BeadChip (circled) exhibit deviating ratios, indicating a possible difference in stringency between arrays 1 and 6.

