

## Performance and Validation of GeneChip® HT Human, Mouse and Rat PM Array Plates

### Abstract

**GeneChip® HT Human, Mouse and Rat PM Array Plates provide whole-genome coverage on a single array plate, enabling a more cost-effective and efficient option for medium- and high-throughput, whole-genome expression studies.**

**This technical note demonstrates the performance of the GeneChip HT PM Array Plate design, including sensitivity, specificity, reproducibility and backwards compatibility.**

### Introduction

Affymetrix GeneChip® HT Human, Mouse and Rat PM Array Plates provide whole-genome coverage on a single array plate, enabling a more cost-effective and efficient option for medium- and high-throughput, whole-genome expression studies.

GeneChip HT PM Array Plates contain the same content as the popular whole-genome cartridge arrays—the GeneChip® Human Genome U133 Plus 2.0 Array, GeneChip® Mouse 430 2.0 Array and GeneChip® Rat Genome 230 2.0 Array.

Two important design changes were introduced with the GeneChip HT PM Array Plates. Due to the popularity of probe set summarization algorithms such as RMA and PLIER, which do not use mismatch (MM) probes, these probes were removed and only the perfect match probes (PM) were retained. On the human array plate, empirical data was used to select the best-performing probes, resulting in a reduction of PM probes within a probe set. Among 42,461 probe sets, the number of PM probes was reduced from 11 to nine, and another six probe sets were reduced from 11 to 10.

This technical note demonstrates the performance of the GeneChip HT PM Array Plate design, including sensitivity, specificity, reproducibility and backwards compatibility.

### Methodology

Internal and external studies at four sites were completed to ensure that GeneChip HT PM Array Plates displayed high levels of sensitivity, specificity and reproducibility. Further studies were performed to show backwards compatibility with previous array designs. GeneChip® HT HG-U133+ PM Array Plates were

compared internally to GeneChip® HG-U133 Plus 2.0 Array cartridges and GeneChip® HT HG-U133A Array Plates. GeneChip® HT MG-430 and GeneChip® HT RG-230 PM Array Plates were compared to previous-generation array plates at an external test site.

All studies used cRNA target prepared with GeneChip® HT One-Cycle Target Labeling and Control Reagents. Twenty-eight pre-labeled spikes were added at four concentrations (0.0, 0.75 pM, 1.5 pM, 3.0 pM) in four pools to a complex background using a Latin square design.

### Sensitivity and specificity

Spike-in experiments were used to address system sensitivity and specificity. This was measured by generating receiver-operator characteristic (ROC) curves and comparing true changes versus the detection of false positives. Area under the curve (AUC) values were calculated to assess sensitivity and specificity in a single metric. In this study, AUC values are on a scale of zero to one, with one representing the highest sensitivity and specificity. The same protocol was used for internal and external validation studies.

### Validation studies

The internal validation studies tested three 24-format array plates from three synthesis lots each of human, mouse and rat arrays. During the external phase, four test sites were chosen to perform independent validation studies using human array plates. Three of the test sites had limited experience processing HT array plate sets and the fourth test site had no experience with this array format. Each external test site hybridized samples to two HT HG-U133+ PM 96-Array Plates. Both internal and external validation studies used the methodology described above.

### Reproducibility

Reproducibility was assessed by examining the relative logarithmic expression (RLE), a measure of the deviation of signal compared to a group median which is commonly used to assess variation in microarray studies. RLE was examined for individual arrays and for whole plates

### Backwards compatibility

Backwards-compatibility studies were performed to compare GeneChip HT PM Array Plates to GeneChip whole-genome cartridge arrays and previous-generation HT array plates.

Human studies were performed internally using reference samples MAQC-A (Stratagene’s Human Universal Reference RNA) and MAQC-B (Ambion’s Human Brain Reference RNA) with four technical replicates. Mouse and rat studies were performed externally, with non-disclosed samples using six treatments with three biological replicates and eight technical replicates.

When comparing GeneChip HT HG-U133+ PM Array Plates to previous-generation GeneChip HT Array Plates, only the more commonly used HT HG-U133A Array Plate was examined, and the correlation was limited to the 22,277 perfect match probe sets on this plate. All 54,675 perfect match probe sets of the HG-U133 Plus 2.0 Array cartridge were used to examine backwards compatibility.

### Performance

Performance was assessed by examining the ability to resolve two-fold changes when comparing 0 to 1.5 pM spike-in concentrations (three copies per cell) and 1.5 pM to 3.0 pM (six copies per cell) spike-in concentrations.

All human, mouse and rat arrays performed well, with AUC values near 1.0 across all spike-in concentrations, indicating that measurements are well resolved between concentration levels (Figure 1A). Average AUC values were 0.99 for human, 0.98 for mouse and 0.99 for rat when comparing positive signal changes at 1.5 pM to 3.0 pM. Similar results were seen when comparing positive signal changes at 0 to 1.5 pM, with average AUC values of 0.99 (human), 0.984 (mouse) and 0.979.

All eight HT HG-U133+ PM Array Plates tested at the four beta sites performed well (Figure 1B). When comparing 1.5 pM to 3.0 pM spike-in concentrations, the four test sites produced average AUC values of 0.999, 0.996, 1.000 and 0.999. When comparing 0 to 1.5 pM spike-in concentrations, all four test sites showed an average AUC value of 1.0.

Overall, HT PM Array Plates demonstrated superb performance with AUC across all arrays at internal and external test sites. Across all human arrays, the average AUC value was 0.998, demonstrating extremely high levels of sensitivity and specificity. Mouse and rat arrays demonstrated similarly high sensitivity and specificity, with average AUC values greater than 0.98 across all concentrations tested.

### Reproducibility

Reproducibility was assessed by examining the relative logarithmic expression (RLE), a measure of the deviation of signal compared to a group average which is commonly used to assess variation in microarray studies. Because RLE is a relative value, there is no set cutoff point for assessing reproducibility. However, low RLE values are characteristic of high reproducibility and unusually high values indicate outliers. This metric is most useful for studies with similar sample types to detect outlier arrays. For a set of different tissues, for example, this metric is less useful.

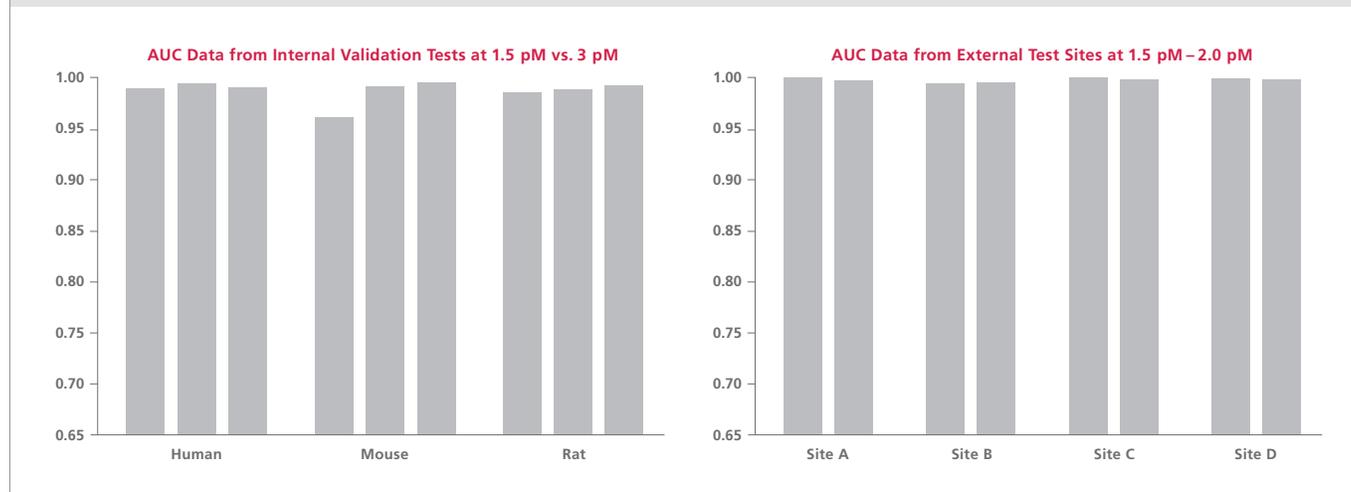
Of 216 arrays tested in the internal validation study, there were only two possible outliers with higher-than-expected mean absolute MA(RLE) values. Thus, more than 99 percent of the arrays displayed low MA(RLE) values and high levels of reproducibility. Figure 2 shows RLE values for 72 individual HT HG-U133+ PM Arrays across three 24-array plates from different synthesis lots.

Across the three plates, there were no outliers and all arrays had similarly low RLE values, indicating high reproducibility. Mouse and rat data (not shown) had similar RLE values. At the plate level, all human, mouse and rat plates tested displayed low MA(RLE) values with no outliers. Thus, no arrays have a noticeably higher MA(RLE) value, thus demonstrating the high reproducibility of array plates.

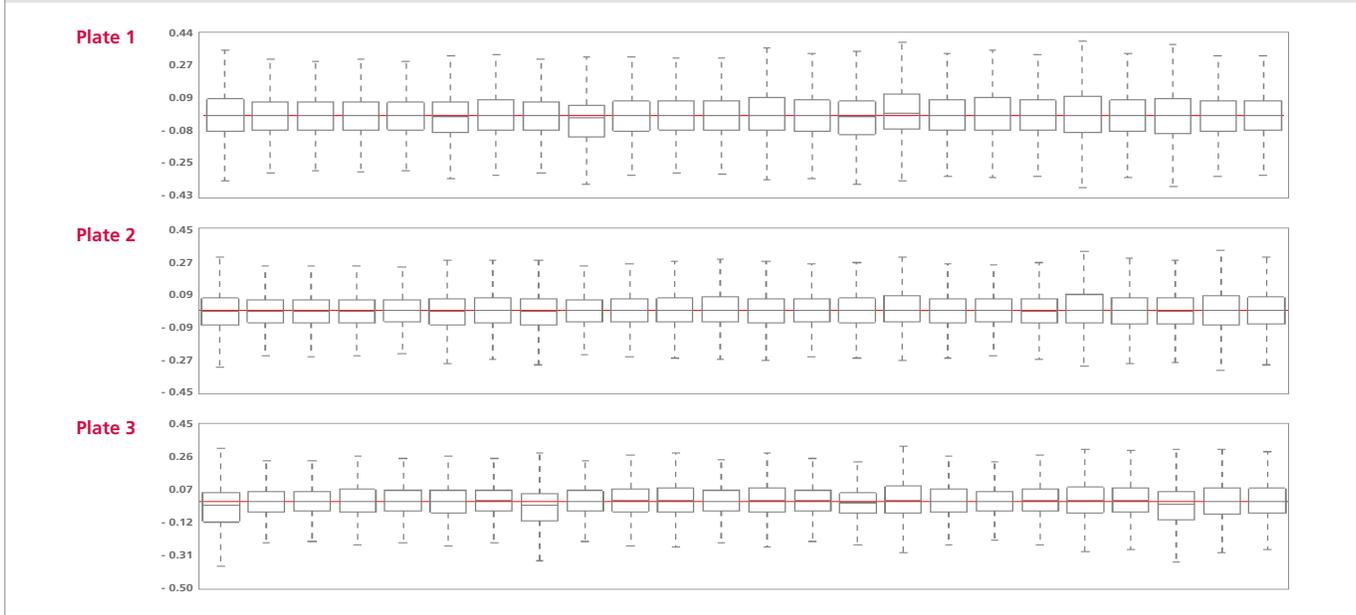
### Backwards compatibility

HT HG-U133+ PM Array Plates were compared to previous-generation HT HG-U133A Array Plates and corresponding

**Figure 1A & 1B:** Area under curve for ROC using RMA signal showing performance of HT PM Array Plates from (A) internal validation tests and (B) data from external test sites.



**Figure 2:** RLE values for all arrays within three HT HG-U133+ PM Array Plates from different synthesis lots. Individual arrays within mouse and rat array plates (not shown) displayed similar patterns.



HG-U133 Plus 2.0 Array cartridges to examine backwards compatibility at the RMA signal level and the fold change level. This was done by examining the fold change between two commonly used test samples, MAQC-A and MAQC-B. HT MG-430 PM and HT RG-230 PM Array Plates were compared to previous-generation array plates using mouse and rat tissue samples.

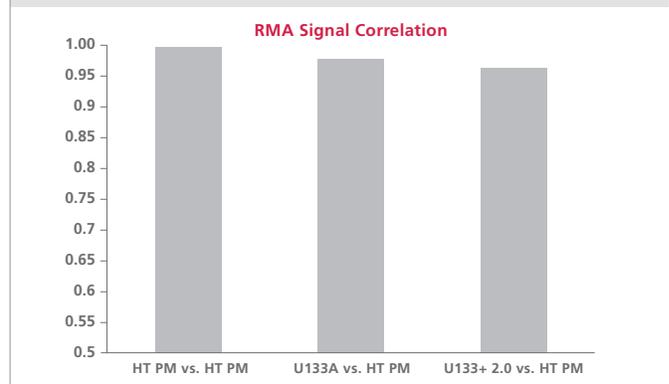
RMA signal correlation (Figure 3A) was greater than:

- 0.99 between individual HT HG-U133+ PM Array Plates
- 0.97 between HT HG-U133+ PM Array Plates and HT U133A Array Plates
- 0.96 between HT HG-U133+ PM Array Plates and HG-U133 Plus 2.0 Array cartridges

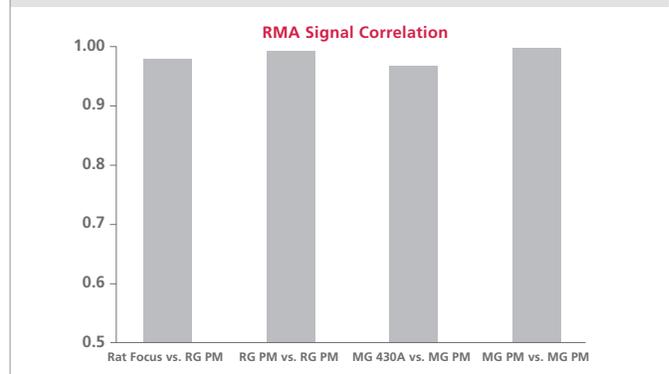
RMA signal correlation was extremely high when comparing HT RG-230 PM Array Plates and HT MG-430 PM Array Plates to previous-generation array plates, with greater than 0.97 and 0.96 correlation for rat and mouse, respectively (Figure 3B). This suggests an extremely high signal-level concordance when comparing HT HG-U133+ PM Array Plates to previous plate and cartridge designs, and when comparing HT MG-430 and HT RG-230 PM Array Plates to previous plate designs.

The correlation in fold change between HT HG-U133A probe sets and the corresponding probe sets on the HT HG-U133+ PM Array Plate was greater than 0.97, demonstrating an extremely high level of correlation (Figure 4A). HG-U133 Plus 2.0 Array cartridges also showed a very high level of concordance, with a fold change correlation greater than 0.97 (Figure 4B). This data illustrates an extremely high level of fold change concordance, indicative of backwards compatibility.

**Figure 3A:** Average Pearson correlation and standard deviation for all possible pair-wise combinations for HT HG-U133+ PM Array Plate versus HT HG-U133+ PM Array Plate, HT HG-U133A Array Plate versus HT HG-U133+ PM Array Plate and HG-U133 Plus 2.0 Array cartridge versus HT HG-U133+ PM Array Plate.



**Figure 3B:** Average Pearson correlation and standard deviation for all possible pair-wise combinations for HT Rat Focus Array Plate versus HT RG-230 PM Array Plate, HT RG-230 PM Array Plate versus HT RG-230 PM Array Plate, HT MG-430A Array Plate versus HT MG-430 PM Array Plate and HT MG-430 PM Array Plate versus HT MG-430 PM Array Plate.



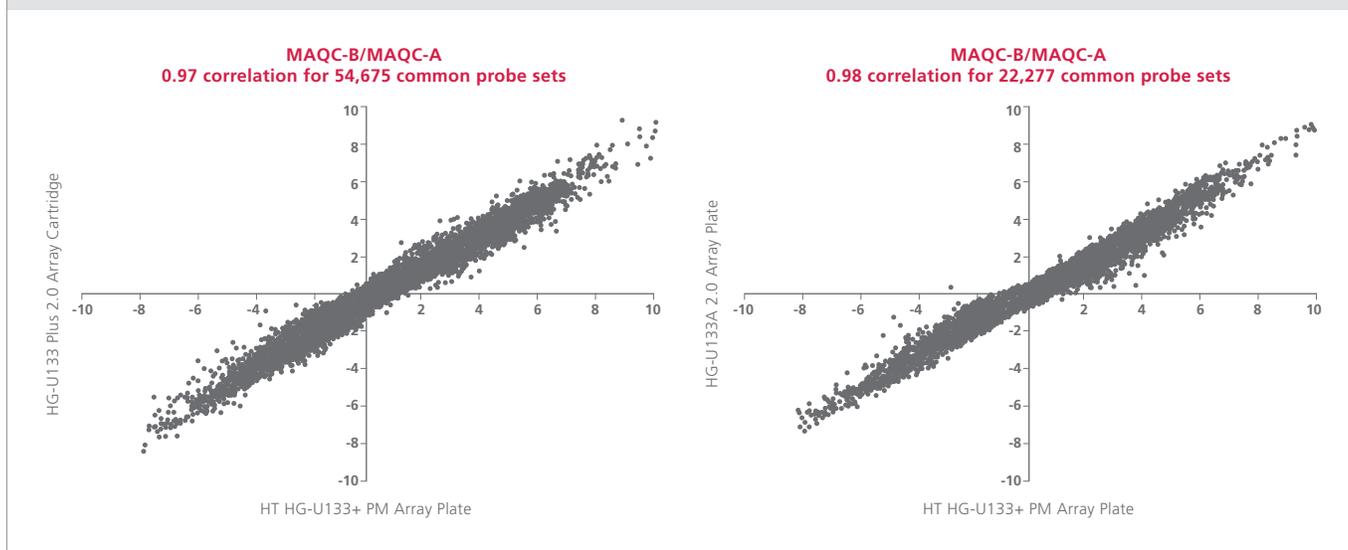


## Conclusion

Testing and evaluation of HT PM Array Plates focused on sensitivity, specificity, reproducibility and backwards compatibility. These arrays demonstrated extremely high performance in internal and external studies and displayed high levels of reproducibility within and between plates. Comparisons with previous-generation array plate sets and cartridge arrays showed high levels of correlation at the signal and fold change level.

Overall, HT HG-U133+, HT MG-430 and HT RG-230 PM Array Plates are powerful tools that enable a medium- and high-throughput option for whole-genome expression studies. With high levels of backwards compatibility, users can leverage the vast amount of data and publications produced from previous designs.

**Figure 4A & 4B:** Fold change correlation for HT HG-U133+ PM Array Plates versus HG-U133 Plus 2.0 Array cartridges (54,675 common probe sets) using MAQC A and B samples. Scatter plots represent median MAQC B - MAQC A signals for each array type. The high correlation coefficients indicate that similar biology is being observed.



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