This document describes the experimental design and illustrates some of the data deliverables included in the Human Proficiency Testing program at Expression Analysis.

Expression Analysis offers a Proficiency Testing (PT) service that compares data sets generated in different laboratories and enables individual facilities to monitor their performance over time. These facilities take part in three cycles of PT performed over twelve months. During each cycle, Expression Analysis collects and analyzes expression data for identical RNA samples processed in multiple microarray laboratories. The results reveal the level of sensitivity and repeatability for individual labs as well as the comparability between labs. When the testing is repeated throughout the year, multiple data sets from the same laboratory can be compared to confirm the reproducibility of the results and monitor laboratory performance over time.

Experimental Design

During each PT cycle, a participating lab receives six RNA samples from Expression Analysis. The RNA samples were developed as part of the MicroArray Quality Control (MAQC) project initiated by the FDA (http://www.fda.gov/nctr/science/centers/toxicoinformatics/maqc). They consist of either total RNA from multiple human brain tissues or a pool of total RNA from cell lines representing different human tissues. Each RNA sample also contains external RNA controls, polyadenylated transcripts representing bacterial genes (e.g., Lys, Phe, Thr).

Microarray facilities use their internal protocols for microarray processing. Each lab prepares biotin-labeled cRNA targets from the RNA samples and hybridizes them to the human U133_Plus_2.0 GeneChips (sold separately). Affymetrix files for the six hybridizations are returned to Expression Analysis for “Within-Lab” data analysis. Similar hybridizations are performed in other array facilities using the same starting RNA samples. This design allows Expression Analysis to generate “Between-Lab” data comparisons.

Figure 1: Experimental Design for Proficiency Testing

Expression Analysis tests each of the RNA samples prior to distribution. This hybridization data are included with the results from other laboratories during the analysis. However, the identities of all microarray facilities, including Expression Analysis, are concealed in the final figures. The Data Deliverables do not include one-on-one analyses of each laboratory’s data compared to Expression Analysis.
Each PT experiment includes three replicates of two different RNA samples which include external RNA controls. This design allows us to determine the sensitivity of the assay, to monitor the repeatability of the expression data and to detect differentially expressed genes. Much of the analysis examines the comparability of the results across labs using fold change values and lists of differentially expressed genes.

- Sensitivity
- Accuracy
- Repeatability
- Comparability
- Signal Range
- QC Metrics

Expression Analysis has extensive experience with interlaboratory data comparisons. We have tested a variety of statistical methods and identified the relative value of different quality metrics. This expertise was essential during our analysis of the MAQC data set. In fact, most of the figures in the main MAQC publication (Shi et al., Nat. Biotechnol. 24:1151–1161, 2006) were developed and calculated by Expression Analysis staff. The same metrics are included in the PT reports, enabling direct comparison between your results and the MAQC study.

Expression Analysis prepares a custom report for each lab after every PT cycle. The report includes an evaluation of the individual lab’s performance as well as data on the distribution of the results from all labs. When appropriate, the report also compares data generated in the same laboratory at different times. The report also includes sensitivity and specificity scores, which reveal each laboratory’s ability to detect true positives and avoid true negatives.