

Comparison of L1000 and Affymetrix microarray for *in vitro* concentration-response gene expression profiling

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Abstract

Advances in high-throughput screening technologies and *in vitro* systems have opened doors for cost-efficient evaluation of chemical effects on a diversity of biological endpoints. However, toxicogenomics platforms remain too costly to evaluate large libraries of chemicals in concentration-response. The current study evaluates gene expression profiling using the Genometry L1000 platform, whereby 978 landmark genes are quantified and a computational approach is applied to infer the expression of the ~21000 probes representing the whole genome. Human cell lines (HepG2, HepaRG, Ishikawa, MCF7) were treated with 34 unique chemicals at three concentrations (n=6) for 6 hrs. Purified RNA extracts were halved for gene expression evaluation by Affymetrix microarray and L1000 technologies to be conducted on the same samples. The raw normalized expression values generated by each platform were compared per gene, revealing a linear correlation of R² = 0.63. Comparison of the gene expression profiles revealed differences in the dynamic range of fold-expression achieved per gene as well as differences in the number of differentially expressed genes (as calculated via ANOVA to account for multiple concentrations), though Connectivity Mapping (CMAP; Broad Institute) identified similar profiles. For example, valproic acid (10-1000 μM) treatment in MCF7 cells resulted in the differential expression of 373 and 453 genes in the Affymetrix microarray and L1000 platforms, respectively, with only 22 genes in common. However, the CMAP for both datasets returned a valproic acid signature of histone deacetylase inhibition. In summary, despite differences in raw expression values, fold changes, and genes identified, both platforms succeed in identifying biological signatures of chemical-mediated disruption.

Overview and Study Design

Genometry's L1000 platform presents a novel high-throughput global gene expression profiling methodology for the evaluation chemical-elicited gene expression that could greatly advance to the current toxicogenomics knowledgebase. To evaluate the performance of the L1000 method, RNA samples were split for gene expression profiling by L1000 and Affymetrix microarray, respectively, and compared. The objectives of the comparison were to:

- ▶ Evaluate the dynamic range, in terms of fold change gene expression, from each technology
- ▶ Assess the number and identity of differentially expressed genes from each technology
- ▶ Compare the Connectivity Maps generated based on expression data from each technology

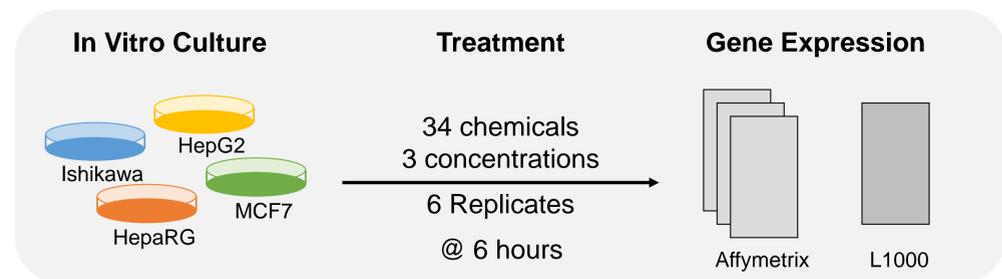


Figure 1. Study design: *In vitro* treatment of human cell lines (HepG2, HepaRG, Ishikawa, MCF7) with 34 unique chemicals at three concentrations (n= 6) was conducted for 6 hrs. Purified total RNA extracts were halved for gene expression evaluation by Affymetrix microarray and L1000 technologies to be conducted on the same samples. Nearly 360 microarrays were run per cell line and processed using RMA normalization, meanwhile L1000 data was processed and normalized by Genometry.

Comparing Raw Normalized and Fold Change Values

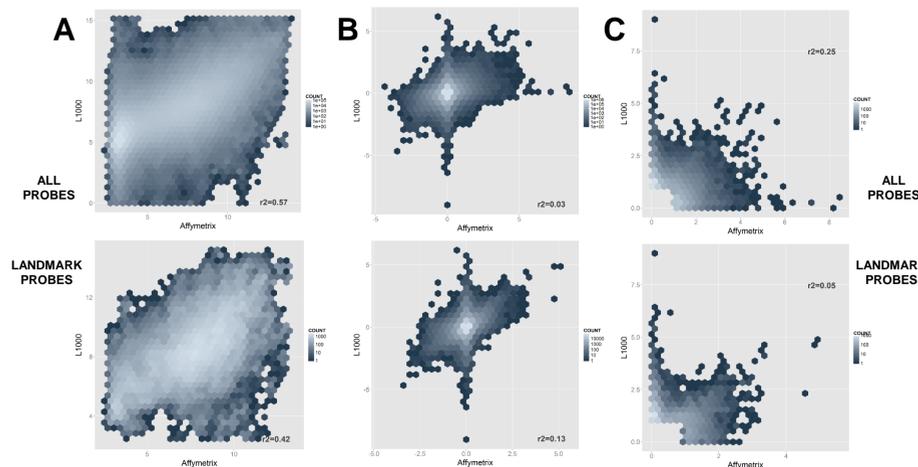


Figure 2. Comparing normalized expression and fold change values between L1000 and Affymetrix microarrays across all cell lines, chemicals and concentrations evaluated: **A.** Comparison, per probe, of raw normalized values for all data generated reveals a correlation (r²) of 0.57. The Landmark (LM) probes alone had an r² = 0.42, whereas the inferred probes alone had an r² = 0.57. Limiting the comparison to LM probes has a limited effect on the overall correlation between L1000 and Affymetrix microarrays and the difference is most likely attributed to the inherent biased selection of the LM probes. **B.** Similarly, the fold change (on a log₂ scale), per probe, was compared revealing low correlation between the two platforms when background expression is removed. **C.** Comparison, per probe, of absolute fold change (on a log₂ scale; filtered >2-fold), showed marginal correlation among the responsive probes, the correlation decreased when limited to the LM probes only.

Differentially Expressed Genes

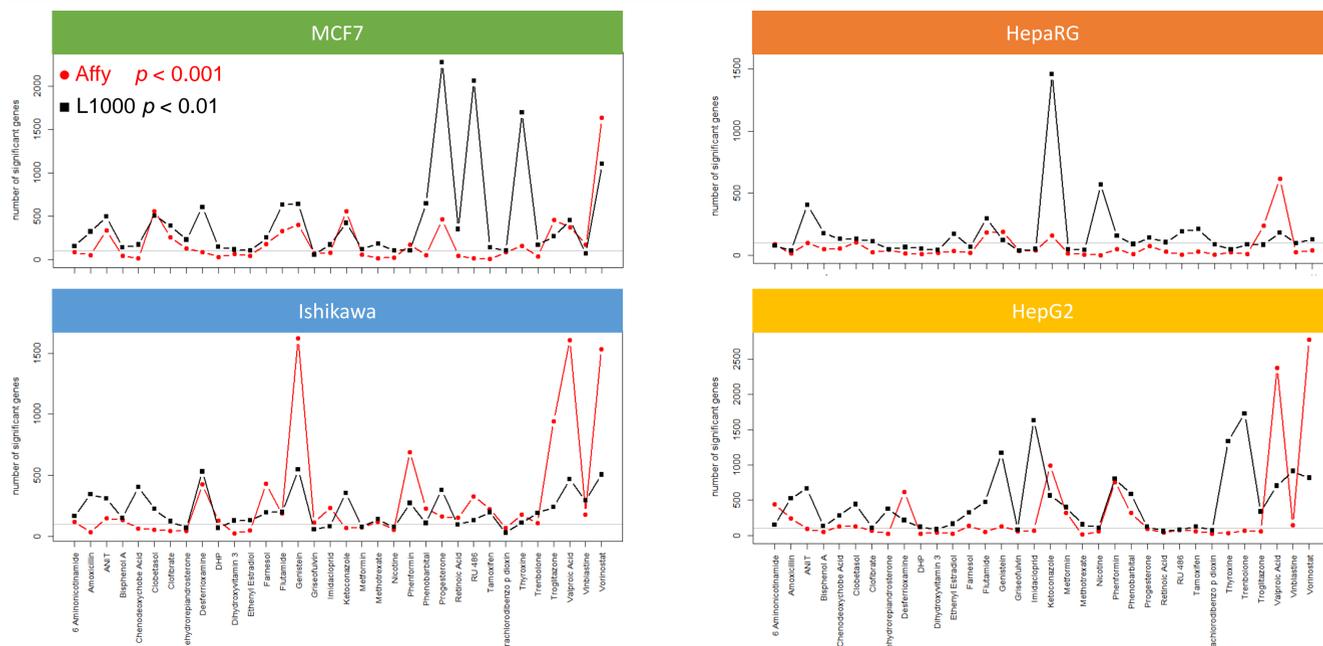


Figure 3. Sums of differentially expressed genes from L1000 and Affymetrix microarrays in four cell lines: The sum of differentially expressed genes in MCF7 (green), Ishikawa (blue), HepaRG (orange), and HepG2 (yellow) cells is shown. Differentially expressed genes were identified using a p-value statistical cutoff calculated by ANOVA, allowing for incorporation of concentration-dependency. Due to the greater variance in the L1000 dataset, the statistical cutoff was set one order of magnitude lower than the cutoff for the Affymetrix microarray. The total number of differentially expressed geneID's (y-axis) were summed per chemical (x-axis) for both Affymetrix microarray (red) and L1000 (black) datasets. These data demonstrate that a subset of chemicals have significantly different number of genes identified with the different technology platforms. Furthermore, it is evident that cellular context plays a role in the gene signature as a chemical can elicit varying numbers of differentially expressed gene sets among cell lines.

Evaluation of Variance

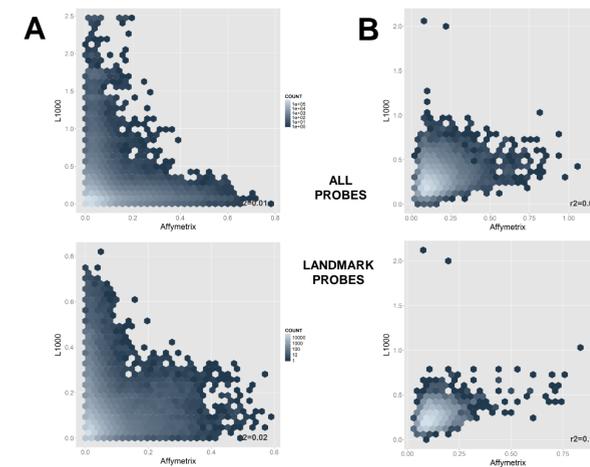


Figure 4. Comparing median absolute deviation (MAD) and coefficient of variance (CV) between L1000 and Affymetrix microarrays across all cell lines, chemicals and concentrations: **A.** The CV, calculated using the raw normalized expression value means and their standard deviations across the 6 technical replicates, for all probes is highly variable in the L1000 dataset with a range of CV values roughly 3-times that of the Affymetrix dataset. **B.** Limited correlation between L1000 and Affymetrix probes based on the median absolute deviation (MAD) across all log₂ fold change values was observed with a marginal increase when compared to the LM probes only (r² from 0.07 to 0.16). The average MAD for the L1000 dataset was roughly double that of the Affymetrix dataset. The increased noise of the L1000 dataset decreases the ability to identify significantly altered genes with confidence.

Connectivity Map Signature

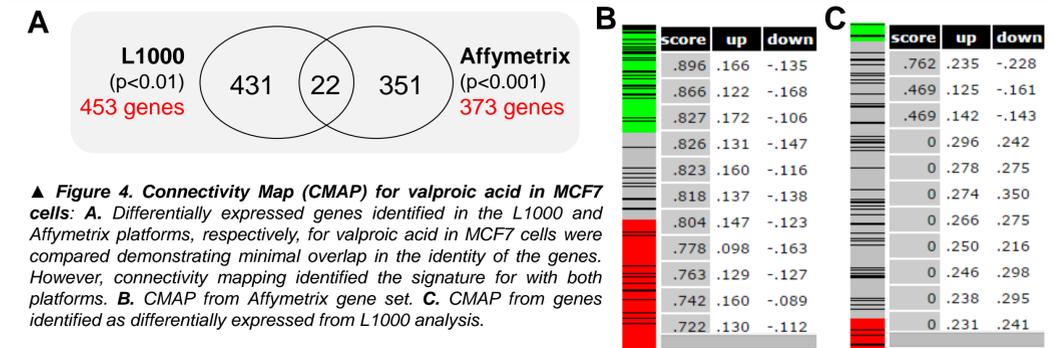


Figure 4. Connectivity Map (CMAP) for valproic acid in MCF7 cells: **A.** Differentially expressed genes identified in the L1000 and Affymetrix platforms, respectively, for valproic acid in MCF7 cells were compared demonstrating minimal overlap in the identity of the genes. However, connectivity mapping identified the signature for with both platforms. **B.** CMAP from Affymetrix gene set. **C.** CMAP from genes identified as differentially expressed from L1000 analysis.

Summary

Gene expression profiles generated by Affymetrix microarray and L1000 were compared:

- ▶ L1000 data had greater variance than the Affymetrix microarray dataset. This resulted in a less stringent statistical cutoff used for identifying differentially expressed genes.
- ▶ Total number of differentially expressed genes varied by platform and cell line, demonstrating a need to carefully select cell lines for toxicogenomic studies and to provide context for differential expression profiles.
- ▶ Connectivity Maps generated with data from each platform both identify a histone deacetylase signature for valproic acid-mediated gene expression in MCF7 cells despite minimal overlap in the identity of genes contributing to the signature.
- ▶ Moderate correlation of raw normalized expression values decreased when fold change values were compared suggesting a limited ability of L1000 to confidently or consistently identify differential expression relative to Affymetrix.
- ▶ Future work will evaluate a more comprehensive L1000 dataset to better evaluate concentration-dependent CMAP profiling for chemicals with known mechanisms of action as well as automate the CMAP analysis process to further our efforts in establishing a workflow for high-throughput toxicogenomics.

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