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Assessment and integration of publicly available SAGE, cDNA microarray, and oligonucleotide microarray expression data for global coexpression analyses

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Abstract

Large amounts of gene expression data from several different technologies are becoming available to the scientific community. A common practice is to use these data to calculate global gene coexpression for validation or integration of other "omic" data. To assess the utility of publicly available datasets for this purpose we have analyzed *Homo sapiens* data from 1202 cDNA microarray experiments, 242 SAGE libraries, and 667 Affymetrix oligonucleotide microarray experiments. The three datasets compared demonstrate significant but low levels of global concordance ($r_c < 0.11$). Assessment against Gene Ontology (GO) revealed that all three platforms identify more coexpressed gene pairs with common biological processes than expected by chance. As the Pearson correlation for a gene pair increased it was more likely to be confirmed by GO. The Affymetrix dataset performed best individually with gene pairs of correlation 0.9–1.0 confirmed by GO in 74% of cases. However, in all cases, gene pairs confirmed by multiple platforms were more likely to be confirmed by GO. We show that combining results from different expression platforms increases reliability of coexpression. A comparison with other recently published coexpression studies found similar results in terms of performance against GO but with each method producing distinctly different gene pair lists.

Keywords: Gene expression; Gene expression profiling; Microarray analysis; cDNA microarray; Oligonucleotide microarray; Coexpression; Serial analysis of gene expression; Gene Ontology

Large-scale expression profiling has become an important tool for the identification of gene functions and regulatory elements. The development of three such techniques, cDNA microarrays [1], oligonucleotide microarrays [2], and serial analysis of gene expression (SAGE) [3] has resulted in a plethora of studies attempting to elucidate cellular processes by identifying groups of genes that appear to be coexpressed. Our motivation for this study was to explore the fecundity of large extant expression datasets to identify coexpressed genes and their utility as a resource for biological study. Coexpression data are increasingly used for validation and integration with other "omic" data sources such as sequence conservation [4], yeast two-hybrid interactions [5,6], RNA interference [7], and regulatory element predictions [8], to name only a few. If different platforms or datasets produce widely different measures of coexpression it could have significant impacts on the results of such studies. Furthermore, methods to assess these datasets and identify a coherent, consistent picture of coexpression will be needed.

Abbreviation: SAGE, serial analysis of gene expression; GEO, Gene Expression Omnibus; GO, Gene Ontology; IEA, inferred electronic annotation; MGC, Mammalian Gene Collection; MCE, minimum number of common experiments; r, Pearson correlation; r_c, correlation of Pearson correlations.

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High degrees of consistency within a platform have been reported for cDNA microarrays and Affymetrix oligonucleotide microarrays [9–11]. The reproducibility of SAGE has not been demonstrated as clearly the time and cost required to produce individual SAGE libraries are high. However, a recent study showed a high degree of reproducibility and accuracy for microSAGE (a modification of SAGE) [12] and preliminary analysis of SAGE replicates has demonstrated high levels of correlation, similar to those seen for Affymetrix platforms (A. Delaney, personal communication). Cross-platform comparisons of gene expression values have found "reasonable" correlations for matched samples, especially for more highly expressed transcripts [11,13–19]. Other comparisons have reported "poor" correlations [15,18,20–24]. The correlations reported above were for expression levels or expression changes of individual genes, not coexpression of gene pairs. To our knowledge, only one study has examined the correlation of coexpression results from multiple platforms [25]. The authors compared matched Affymetrix oligonucleotide chips and spotted cDNA microarrays for the NCI-60 cancer cell panel. For each platform, the calculation involved determining the Pearson correlation (r) between expression profiles (across 60 cell lines) for all pair-wise gene combinations. Then, a correlation of correlations (r_c) between the two platforms was determined. When all gene pairs were considered a global concordance of $r_c = 0.25$ was reported. As the correlation cutoff was increased, r_c improved steadily to 0.92 at a correlation cutoff of $r = 0.91$ (but only 28 of 2061 genes remained). Thus, for most gene pairs there is poor correlation of correlations for global coexpression values.

Genome-wide coexpression analyses in *Caenorhabditis elegans* and *Saccharomyces cerevisiae* have been used with some success to identify gene function or genes that are coregulated [26–28]. This "guilt-by-association" approach has received criticism because of high levels of noise and other problems inherent to the methods [29] but still holds great interest for biologists. If matched samples display questionable levels of consistency between expression profiles generated by different platforms the question remains as to how effectively unmatched samples from many different sources will compare. If two genes are coregulated (i.e., controlled by an identical set of transcription factors) they should display similar expression patterns across many conditions and be identified as coexpressed. This is the basic premise of many gene function and regulation studies. If true, large datasets from different expression platforms should identify the same coexpressed gene pairs even if derived from different conditions and tissues. However, it may be that few genes are globally coregulated and thus datasets comprising different samples will identify different sets of coregulated genes. Similarly, noise and biases inherent to the different methods may result in highly discordant measures of coexpression, even for genes with similar function or under similar regulatory control.

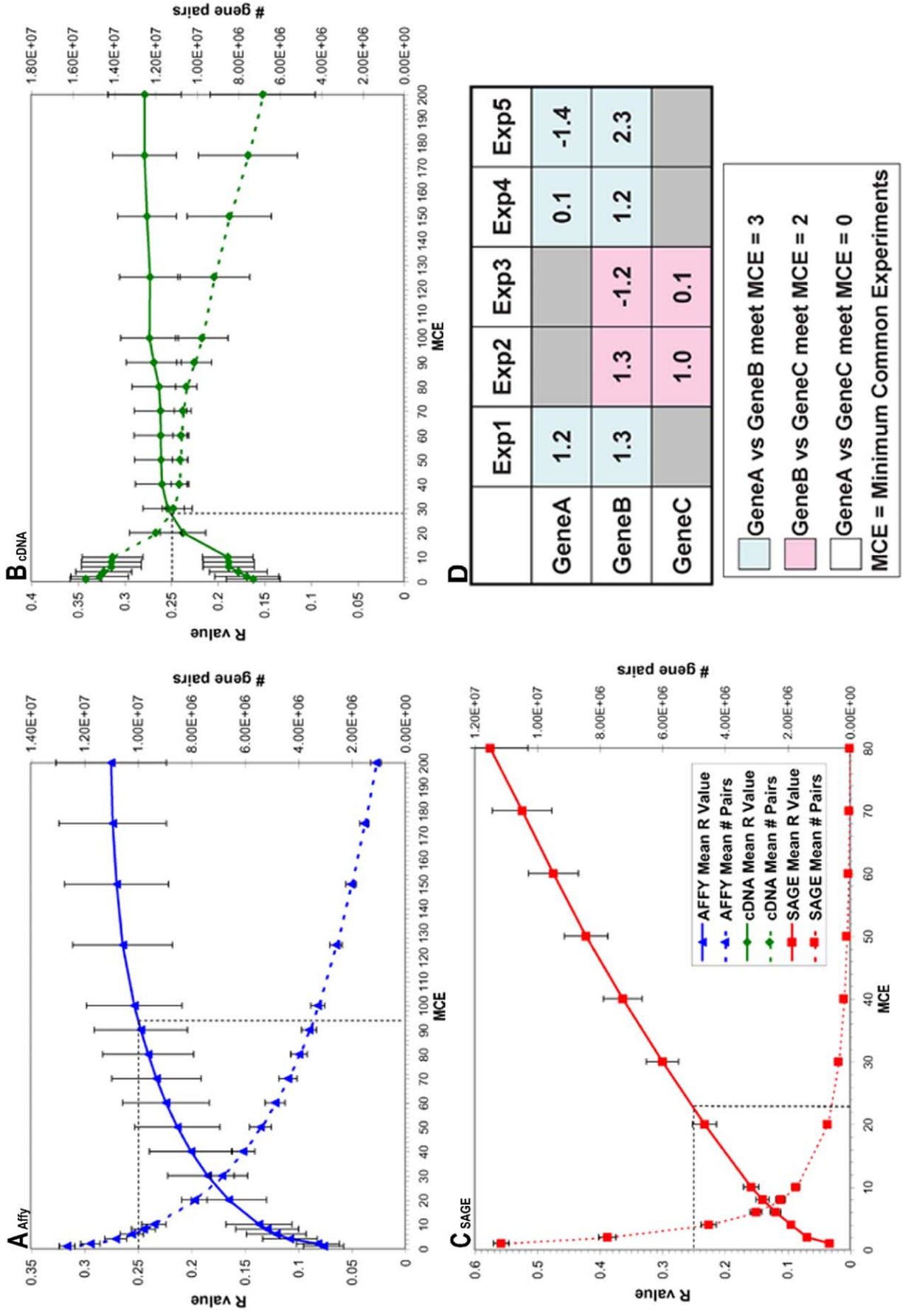
The purpose of this study was to assess the differences between publicly available expression data for global coexpression analyses and investigate the value of combining multiple platforms to decrease noise and improve confidence in coexpression predictions. We have compared large publicly available datasets for SAGE, cDNA microarray (cDNA), and Affymetrix oligonucleotide microarray (Affymetrix) platforms (Supplemental Fig. 1). We calculated all gene-to-gene Pearson correlation coefficients and assessed the platforms for internal consistency, cross-platform concordance, and agreement with the Gene Ontology. The Pearson correlation was chosen as a similarity metric because it is one of the most commonly used, with numerous published examples for Affymetrix [9,30,31], cDNA [5,27,32], and SAGE [33,34]. Because the datasets represent unmatched samples, a direct comparison of platforms is challenging. Our results indicate that the three platforms identify very different measures of coexpression for most gene pairs with a very low correlation of correlations between platforms. However, coexpression predictions become more reproducible with larger datasets and each of the three platforms performs better (identifies more gene pairs with common GO terms) as the Pearson correlation increases. Furthermore, gene pairs confirmed by more than one platform (high two-platform average Pearson) were much more likely to share a GO term than those identified by only a single platform. Other recently published coexpression methods (TMM, ArrayProspector) also performed well against GO at higher scores but identified very different gene pairs. By using the Gene Ontology to choose thresholds of high-confidence pairs for each we identify a set of coexpressed gene pairs that represents the best of each approach.

Results

Internal consistency

Before performing cross-platform comparisons, it is relevant to evaluate each platform individually to determine how consistently different experiments from one technology identify the same levels of gene coexpression. To this end,

Fig. 1. Internal consistency and minimum common experiments analysis using the pseudo-random division method. For each gene pair, the number of common experiments is determined as the number of experiments for which expression values are available for both genes. On the left axis, MCE is plotted against internal consistency. On the right axis, MCE is plotted against number of gene pairs. In general, as more MCE are required, fewer gene pairs meet the criteria but the internal consistency improves as the correlation is based on more expression data. Notice that the (A) Affymetrix and (B) cDNA datasets appear to level off at approximately $r_c = 0.3$ with 100 MCE. However, (C) the SAGE correlation continues to improve up to nearly $r_c = 0.7$ before no genes meet the cutoff and a leveling is not observed. Data represent mean r_c value and gene pair number of 100 pseudo-random divisions at each MCE. Error bars indicate 1 standard deviation.



	Exp1	Exp2	Exp3	Exp4	Exp5
GeneA	1.2			0.1	-1.4
GeneB	1.3	1.3	-1.2	1.2	2.3
GeneC		1.0	0.1		

internal consistency was determined by dividing each of the datasets in half and comparing the gene-to-gene Pearson correlations for each subset (Figs. 1A–1C). We first divided the data in a purely random fashion. To make the internal consistency calculation more comparable to the cross-platform comparisons, we also devised a pseudo-random division, which takes into account the presence of experimental replicates and very similar experimental conditions in the datasets (see Materials and methods).

Internal consistency was found to be dependent on the minimum number of common experiments (MCE) between any two genes on which Pearson correlations are calculated. MCE was defined as the minimum required number of common or shared experiments for which any two genes actually have values available in their respective expression profiles (Fig. 1D).

Increasing the MCE increased the internal consistency but decreased the number of gene pairs considered for both the pseudo-random (Fig. 1) and the random (Supplemental Fig. 2) division methods. With the random division, and an MCE of 100, Affymetrix showed the highest average internal correlation of 0.925, then cDNA microarray with correlation of 0.889, and then SAGE with correlation of 0.776. This MCE cutoff was used by the group that provided the cDNA microarray data [4] (E. Segal, personal communication). As expected, the pseudo-random division, which groups replicates and experimental datasets, reduced internal consistencies with values of 0.253 for Affymetrix, 0.273 for the cDNA microarray, and 0.660 for SAGE with MCE of 100 (Fig. 1). Unfortunately, as the SAGE dataset contains only 242 samples, division into two groups of approximately 120 results in relatively few gene pairs that meet the criteria of 100 MCE (only 1518 pairs on average). Although approximately 60% of these SAGE libraries are derived from cancer samples, we found no evidence of an effect on the coexpression results (Supplemental Fig. 3) and therefore included them in subsequent analysis.

Internal consistency is a measure of the reproducibility or robustness of gene coexpression predictions similar to a cross-validation test. This is based on the assumption that if a gene pair is truly coexpressed based on an expression dataset, it should be predicted as coexpressed by random subsets of the data. The consistency increases with higher MCE but at different rates for the three datasets because of their different natures in terms of number of experiments and experiment composition. Thus, it would be unfair to compare the datasets with MCEs that resulted in different levels of reproducibility. Studies generally choose some cutoff for a minimum number of common experiments, such as 5, 10, or 100 [4,30,35]. In an effort to produce an unbiased comparison of the three platforms, the pseudo-random division was used to determine an appropriate MCE that would generate the same internal consistency ($r_c = 0.25$) for each (Affymetrix MCE = 95; cDNA MCE = 28; SAGE MCE = 23) (Fig. 1). All internal consistency correlations are summarized in Table 1.

Table 1
Summary of r_c values for internal consistency analysis using different sample division methods and MCE cutoffs

Platform	Division	MCE cutoff	Gene pairs	r_c value
Affymetrix	Random	100	4,149,092	0.925
	Pseudo-random	95	3,427,174	0.257
	by GSE series	100	3,260,557	0.253
cDNA microarray	Random	100	10,429,219	0.889
	Pseudo-random	28	11,178,346	0.253
	by author	100	9,747,169	0.273
SAGE	Random	100	2,635	0.776
	Pseudo-random	23	577,820	0.253
	by tissue	100	1,518	0.660

Note that many different divisions are possible for each result (except cancer/normal). Gene pair and r_c values represent mean values from 100 different random or pseudo-random divisions.

Cross-platform correlation analysis

Considering that the levels of consistency between subsets of data from a single platform were relatively low (when replicates and similar experiments were kept together) it is not surprising that datasets from different platforms compared poorly against each other. All comparisons were found to have significant but poor positive correlations compared to randomly permuted data ($p < 0.001$, 1000 permutations). Affymetrix versus cDNA showed the best correlation of 0.102, then Affymetrix versus SAGE with 0.086, and finally cDNA versus SAGE with 0.041 (Supplemental Fig. 4). A Pearson rank analysis also showed significant but poor agreement with only 3–8% better performance than randomly permuted data (Supplemental Fig. 5).

An analysis of correlation at different minimum Pearson cutoffs (r cutoff) for gene pairs was performed as described previously [25] (Supplemental Fig. 6). Lee et al. [25] observed a steady increase in global concordance (r_c = correlation of correlations) up to 0.92 at an r cutoff of 0.91. Our data did not show such an obvious trend. Global concordance stayed close to 0 (or even below) for all three pair-wise platform comparisons up to 0.5–0.6 Pearson cutoff. The Affymetrix/cDNA correlation did show an improvement to $r_c = 0.163$ ($p = 0.003$, $n = 289$ gene pairs) at an r cutoff of 0.65. Similarly the Affymetrix/SAGE comparison improved to $r_c = 0.290$ ($p = 0.028$, $n = 44$ gene pairs) at an r cutoff of 0.7. After these cutoffs, both Affymetrix/cDNA and Affymetrix/SAGE comparisons returned to r_c values close to 0 (or below) and were reduced to insignificant gene pair numbers. The cDNA/SAGE comparison showed no significant increases in r_c with any r cutoff.

Gene ontology analysis

Since the datasets under study demonstrated little agreement, we attempted to determine which dataset was most "biologically relevant." GO biological process

domain knowledge [36] was used to evaluate gene coexpression predictions for each platform. We hypothesized that genes that are coexpressed will be more likely to be involved in the same biological process. The number of gene pairs annotated to the same "most specific" GO (Biological Process) term for each platform was determined (Supplemental Fig. 7). In general, the datasets from all platforms perform better than expected by chance. Affymetrix performed best, followed by cDNA microarray and SAGE, which performed about equally better than randomly permuted data. The analysis was also extended up the GO hierarchy to parent and grandparent terms, and identical trends and relationships were observed (Supplemental Fig. 8).

A second analysis looked at the relationship between the Pearson correlation and the performance against GO. For each platform, the number of gene pairs annotated to the same "most specific term" at different Pearson correlation ranges was determined (Fig. 2). Generally, as Pearson correlation for a gene pair increases it is more likely to be confirmed by GO. With a Pearson value in the range of 0.3–0.4 or better the platforms always performed significantly better than randomly permuted data ($p < 0.001$, 1000 permutations). The improvement over randomly permuted data was very slight for the cDNA and SAGE datasets (2–4%). However, for the Affymetrix data, the trend was striking. Gene pairs identified as coexpressed with a Pearson correlation of 0.9–1.0 were confirmed by GO in 74% of cases. Gene pairs from this list include a large set of highly coexpressed protein biosynthesis genes as well as a few genes involved in translational elongation (a subprocess of protein biosynthesis) and muscle contraction. It should be noted that, in the case of the SAGE and cDNA datasets, only a few gene pairs had Pearson correlations >0.9 (one for cDNA, five for SAGE).

A third analysis examined the effect of averaging platform results and comparing to individual platforms using GO. Requiring coexpression evidence from multiple datasets may represent a method of reducing noise and increase our confidence that coexpressed genes are actually coregulated. The percentage of gene pairs annotated to the same most specific term at different average Pearson correlation ranges was determined as above. The results were again quite striking. With a two-platform combined Pearson of 0.4 or greater the combined platforms all performed significantly better than randomly permuted data ($p < 0.005$, 1000 permutations). Furthermore, for any platform combination, a gene pair with an average Pearson correlation of $r > 0.6$ was much more likely to share a GO term than a gene pair with this level of correlation in only a single platform (Fig. 3). For example, a gene pair with a two-platform average Pearson of 0.7–0.8 was found to share a common GO term 40–50% of the time. Pairs with this same Pearson range in individual datasets shared a common GO term only 5–10% of the time, only a few percent better than expected by chance. Gene pairs confirmed by multiple datasets ($r_{avg} > 0.6$ for any two platforms) covered a wide range of GO categories (52 in total) (Supplemental Fig. 9).

Comparison to other coexpression methods

Finally, an analysis was conducted to assess two other recent coexpression studies that were published while this analysis was in progress. The ArrayProspector method [37], the TMM method [35], and our two-platform combination method (2PC) were each mapped to UniProt IDs and assessed using the same GO analysis as above. In all three cases, we observed significantly more gene pairs with common GO terms at higher scores (Fig. 4). For our method (2PC), the percentage of gene pairs with a common GO term rises sharply at a score of approximately 0.6–0.7. For ArrayProspector this occurs at a score of approximately 0.7–0.8 and for TMM at a score of 5–6. At these cutoffs, each method represents 2500 to 10,000 gene pairs. Each utilizes different genes and identifies different gene pairs as highly coexpressed. Thus, a comparison of the highest scoring 2500 gene pairs for each found only a minimal overlap of less than 10% (Fig. 4D).

Discussion

We have shown that the genes identified as coexpressed are highly dependent on the dataset and expression platform used. In general, we find that the more data a correlation is based on, the more reproducible it is. When division of samples takes similar or replicate experiments into consideration, Affymetrix and cDNA internal consistencies level off at approximately $r_c = 0.25$ with MCE of about 90 and 30–40, respectively. The SAGE dataset continued to improve to nearly $r_c = 0.6$ with MCE of 80. This may reflect the diverse nature of the SAGE dataset for which libraries are rarely constructed from the same or similar tissue. In contrast, it is not uncommon for many Affymetrix or cDNA experiments to measure expression of a very similar series of samples. A recent yeast study found that the ability to identify coregulated genes correctly from coexpression analyses is highly dependent on the number of experiments, with accuracy leveling off at 50 to 100 experiments [38]. Our results agree closely with this observation for human data and suggest that coexpression predictions will be most reproducible if based on 30 to 100 experiments. Furthermore, global coexpression analysis may benefit from a greater representation of tissues and conditions rather than greater numbers.

Given that different experimental subsets of the same platform show poor correlation it is perhaps not surprising that interplatform comparisons show very poor correlations ($r < 0.11$). The fact that none of these datasets agree well raises some serious questions about their use for validation and integration with other data. There are several possible

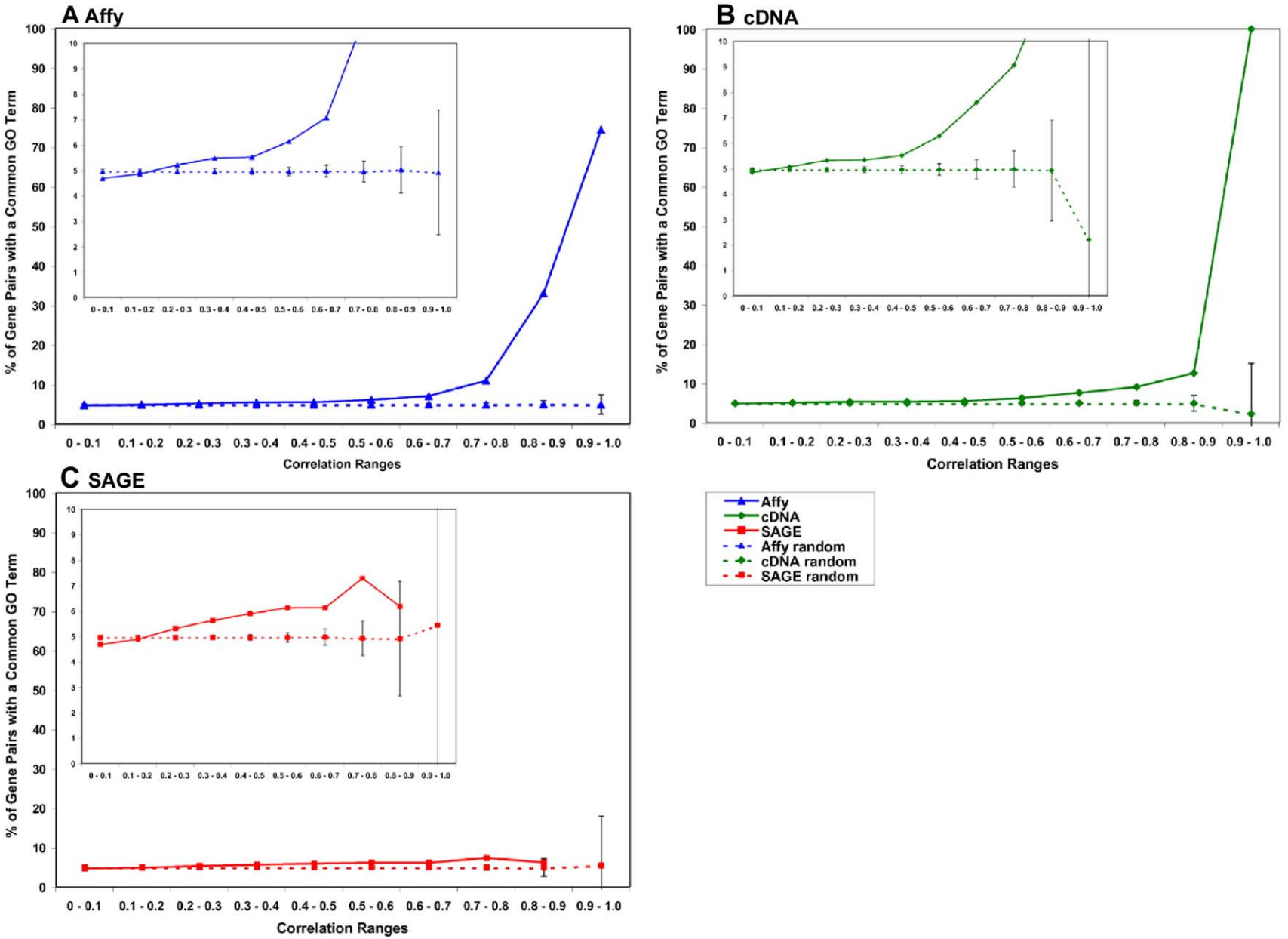


Fig. 2. GO correlation range analysis. At higher Pearson correlations (in particular, $r > 0.8$) gene pairs are more likely to have similar GO biological processes, although very few gene pairs have high correlations in the SAGE and cDNA datasets. 75% of gene pairs with correlation >0.9 calculated from Affymetrix data have the same GO annotation. Interestingly, gene pairs with very low Pearson values are less likely to share a common GO term than randomly permuted data. Random lines represent mean values from 1000 random permutations. Error bars indicate 1 standard deviation.

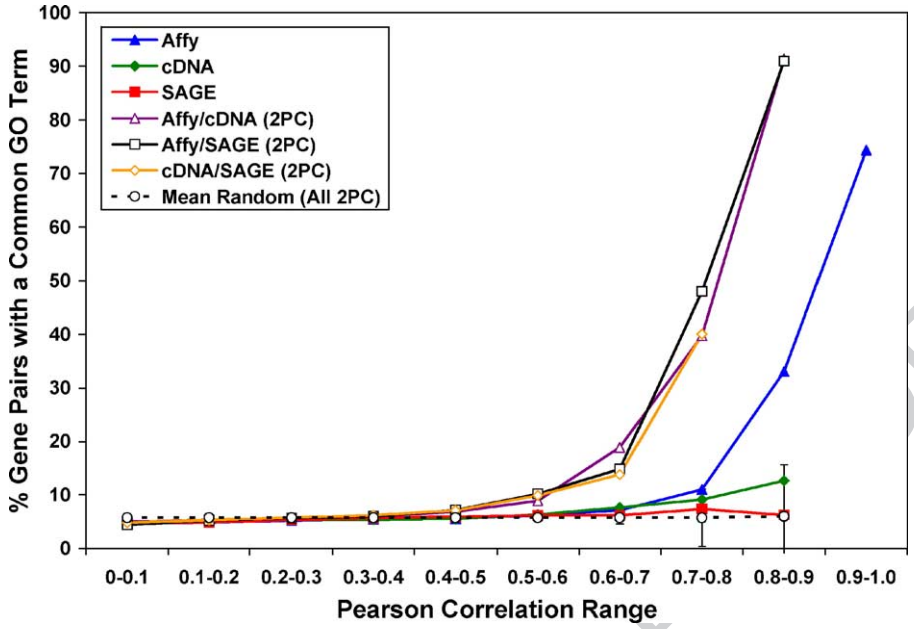


Fig. 3. GO correlation range analysis for multiplatform average. Comparison of two-platform average Pearson to individual platform indicates that gene pairs identified as coexpressed in multiple platforms (higher average Pearson) are much more likely to be confirmed by GO. Random line represents mean values from 1000 random permutations of all two-platform combinations. Error bars indicate 1 standard deviation.

explanations for this observation: (1) The data comprising these datasets are so noisy as to prevent reliable identification of many truly coexpressed genes, (2) the method of identifying coexpressed genes is inadequate, (3) the unmatched and nonoverlapping nature of the samples that make up each dataset results in identification of different subsets of truly coexpressed genes, and (4) genes are under such complex regulatory control that genes coregulated in one cell type or tissue behave in an entirely different manner in other cell types or tissues and are therefore not globally coexpressed. It is likely that each of the explanations outlined above is to some degree responsible for the lack of concordance between coexpression analyses produced from different datasets and different platforms. It is not the purpose of this study to identify which is most important. Rather, we wish to make researchers aware that the choice of dataset or platform for integration or validation of other data could dramatically affect their results, and methods that integrate or combine different platforms may be more appropriate.

The fact that intraplatform comparisons show some correlation and improve with number of data points suggests that some gene pairs identified are truly coexpressed. Furthermore, the GO analysis shows that gene pairs identified as highly coexpressed (higher Pearson correlation) are more likely to share the same biological process and thus actually be related. Similarly, gene pairs with lower Pearson correlations were as or less likely than random chance to share the same biological process. These results suggest that the Pearson correlation is a useful metric and that both high and low Pearson values have the meaning we expect. The GO analysis did not conclusively identify a single "correct" platform or dataset but it did show that the Affymetrix dataset identified more biologically relevant gene pairs than the cDNA or SAGE dataset. However, gene pairs coexpressed in multiple expression platforms were much more likely to be confirmed by GO. Thus, combining platforms appears to act as a filter, producing high-confidence predictions from noisy datasets. This conclusion is based on the assumption that coexpressed genes are more likely to be biologically relevant if they share common biological processes. Assessments using GO are limited by issues such as the incompleteness of the ontology, the potential for circularity (addressed in Materials and methods), experimental bias toward "well-studied" genes, and inconsistencies in structure and depth. Furthermore, it is likely that some coregulated genes will belong to different biological processes while other genes involved in the same process will not be coregulated. As such, an "absolute" performance against GO is difficult or impossible to define. Despite these issues, we believe GO currently represents one of the best resources for a relative assessment of coexpression platforms or methods.

Recent investigations into the utility of combining expression data from different high-throughput platforms have identified highly variable levels of agreement. Based on an analysis of a small set of matched samples using oligonucleotide arrays, SAGE, and EST data, Haverty and colleagues [39] caution against the combination of platforms to confirm expression patterns for specific sets of genes. However, they do suggest that such methods can be used to extract high-confidence subsets of related genes. We agree that for many genes a poor level of agreement between datasets raises questions about their utility.

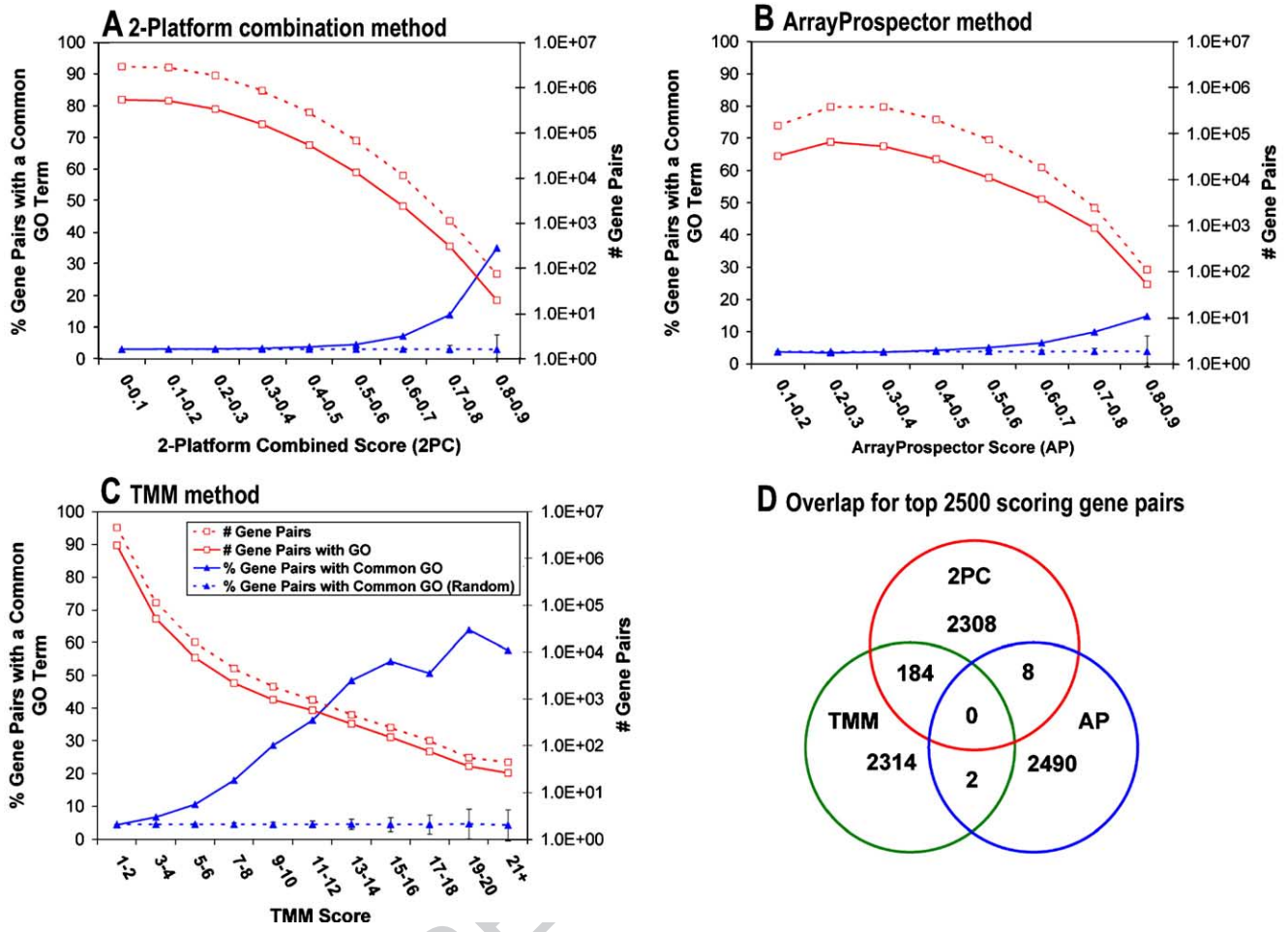


Fig. 4. Comparison of two-platform combination method to other recent coexpression methods. (A–C) For each method, gene pairs with higher scores are more likely to share a common GO term. Lines with open squares represent numbers of gene pairs (right axis). Lines with closed triangles represent percentage of gene pairs with a common GO term. Random lines represent mean values from 1000 random permutations. Error bars indicate 1 standard deviation. (D) Venn diagram indicates overlap between the 2500 top scoring pairs for each method (not required to be in GO). Each method comprises different datasets and has different genes. Therefore a direct comparison of method performance is difficult. Instead, the graphs illustrate that each method is capable of identifying biologically relevant gene-pair relationships and the Venn diagram indicates that they identify very different sets of relationships. Furthermore, the GO analysis provides a means of choosing reasonable score thresholds for each method to generate lists of high-confidence coexpressed genes.

However, our results do show that platform combination methods can be extended to large sets of unmatched publicly available expression data to produce biologically meaningful information.

As we were nearing completion of our analysis, a similar study using multiple microarray datasets (TMM) was published [35]. The authors examined 60 microarray datasets (cDNA and Affymetrix oligonucleotide) for gene pairs identified as coexpressed in multiple datasets. They report that even gene pairs confirmed by only a single dataset have better GO similarity scores than random pairs and GO score increases steadily with the number of confirmed links. Their method differs from ours in that experimental subsets are analyzed separately and a "vote-counting" method was used to identify gene pairs that appear highly coexpressed (above some Pearson cutoff) in multiple sets. Our method combines all experimental subsets into a single dataset for each expression platform and then averages the global Pearson correlations between platforms. Our method is also the first to include SAGE data. A third recently published method (ArrayProspector) used a combination of singular value decomposition and kernel density estimation [37]. This method combines evidence from related arrays and weights the contribution of each array according to how well they correlate with functional annotation.

When attempting to infer function or coregulation from coexpression we should consider that it is likely that genes are biologically related in a number of different ways and therefore different methods will be required to identify each type of relationship. For example, one pair of genes might be "tightly" coexpressed only under very specific conditions, whereas another gene pair might be "loosely" coexpressed across a broad range of conditions depending on the regulatory elements that they share. The three methods discussed above (TMM, ArrayProspector (AP), and 2PC) represent three different approaches to the

problem of identifying high-confidence coexpression for the purpose of inferring function or coregulation. Because the methods use different datasets and scoring methods and comprise different gene sets, a direct comparison of the methods is difficult. Therefore, we chose simply to assess their respective predictions against GO independently. Thus, we do not identify the "best" method but rather show that each method is at least partially effective based on performance against the Gene Ontology. Furthermore, because the highest scoring pairs for each are almost completely nonoverlapping we advocate combining the best results of each into a single set of high-confidence predictions. To this end we have chosen score thresholds for each method based on GO performance (2PC > 0.65; AP > 0.7; TMM > 7) and make available a list of 13,145 high-confidence coexpressed gene pairs (representing 2979 unique genes) (http://www.bcgsc.ca/gc/bomge/coexpression/suppl_materials) for use in regulatory element prediction or other integration studies.

Materials and methods

Data sources

Human gene expression data for three major expression platforms were collected from public sources. We used a recently published dataset of 1202 cDNA microarray experiments [4] representing 13,595 genes, 242 SAGE libraries from the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) representing 15,426 genes, and 667 Affymetrix HG-U133A oligonucleotide microarray experiments (889 were available but 667 had PMA detection calls) representing 8106 genes, also from GEO (Supplemental Fig. 1). cDNA microarray genes provided by Stuart et al. [4] were identified by LocusLink IDs [40]. Therefore this identifier was used for the other two platforms to allow the gene intersection of the three datasets to be determined and used for the subsequent analyses.

Data filtering

cDNA microarray data for 13,595 genes were used as provided by Stuart et al. [4] except for minor formatting changes (see supplementary materials for our data). The 242 SAGE libraries ranged from 1430 to 308,589 total tags in size with an average size of 52,723. SAGE data were first filtered to remove tags with less than one count in at least 10 libraries, reducing the unique tags from 609,224 to 87,521 (and total tags from 12,758,981 to 11,219,373). Next, SAGE tags were mapped to genes by the "lowest" sense-strand tag predicted from RefSeq [40] or MGC [41] sequences and then mapped to LocusLink IDs using the DiscoverySpace software package (Varhol et al., unpublished, http://www.bcgsc.ca/discoveryspace/), reducing the tag set further to 47,263 unique tags. Generally, the lowest tag corresponded to the canonical 3′ -most *Nla*III anchoring enzyme recognition site (position 1) expected for the gene sequence. However, if such a canonical match was not found, higher position (less 3′) mappings were also accepted (see supplementary materials for more details). In the event of discrepancy between RefSeq and MGC, the former was taken as correct because a larger number of tags could be mapped with this resource (9568 vs. 6295) and was thus perceived to be more complete. Only 297 tags with disagreements between RefSeq and MGC are represented in the final gene set (~5%) (see supplementary materials for more details). If a tag mapped to more than one LocusLink or more than one tag mapped to the same LocusLink it was discarded, resulting in a final set of 15,426 unique tags (2,762,500 total tags) confidently mapped to LocusLink IDs. We mapped 22,215 Affymetrix probe IDs to 20,577 LocusLink IDs using the most current Affymetrix annotation file for the HG-U133A chip (www.affymetrix.com, supplementary materials). As with the SAGE tags, probes with ambiguous mapping to LocusLink were discarded, resulting in a final set of 8106 genes from the Affymetrix dataset. Once LocusLink IDs were available for all three platforms, the intersection was determined. This subset of 5881 genes, present in all three platforms, was used for all subsequent analyses. The final 5881 unique SAGE tags represent 1,173,430 total tags sequenced.

Distance calculations

Ratio values for the cDNA microarray data were used as is for the Pearson calculation. Affymetrix probe intensities were converted to natural log values. All ln(intensity) values were normalized by subtracting the median and dividing by the interquartile range for the experiment [42]. Only Affymetrix probe intensities with a "P" call were considered (p value < 0.04). Intensities with "A" or "M" calls were set to null. To compensate for different library sizes SAGE tag counts were normalized to 10,000 tags/library and log-transformed as follows [34]:

$$\text{Tag frequency} = \ln((\text{tag count} \times 10000)/\text{total tags in library})$$

SAGE tag counts of 0 were converted to nulls. In all platforms, genes are represented by a vector of expression values for all the experiments in the dataset. In each case, genes have null values if not represented on that array (cDNA), no tags were observed (SAGE), or intensity was not significantly detected (Affymetrix). Thus, when calculating Pearson correlations between gene pairs, the number of shared data points varied from 0 to the total number of experiments. A minimum number of common experiments was required for each gene pair to provide some confidence in the value calculated (a Pearson correlation based on

observations from only two experiments is meaningless). A range of MCEs was used for the internal consistency analysis (see below) and then one minimum chosen for subsequent analyses.

A Pearson correlation coefficient was calculated for all possible gene pairs for each platform as a measure of expression similarity. These calculations were performed by a modified version of the C clustering library [43] on 64-bit Opteron Linux machines with 8- to 32-GB memory. Please see supplementary materials for modified C source code and explanation of changes.

Correlation of correlations analysis

Correlation of correlations (r_c) for internal consistencies and platform comparisons were performed as previously described [25] using the Pearson correlation function (cor) of the R statistical package (version 1.8.1). This correlation involves millions of data points and thus cannot be graphed easily. Therefore, data were binned and density plots created using the Bioconductor hexbin (version 1.0.3) add-in function for R [44].

Internal consistency analysis

To evaluate the consistency of coexpression observed within each platform, we divided the experiments available and determined coexpression for each subset independently. If a platform consistently finds coexpressed genes regardless of the exact experiments involved, the r_c will be close to 1. To determine whether the observed r_c is significant, we repeat the procedure with randomly permuted gene expression values, expecting an r_c close to 0.

Pseudo-random division method

Division was performed first randomly and then pseudo-randomly. The pseudo-random division was necessary to prevent artificially high internal consistencies resulting from comparing mostly replicates (or very similar experiments) in the two subsets. In many cases (especially for the Affymetrix data) experimental replicates or very similar samples exist in the dataset. The purpose of coexpression analysis is to identify genes that behave similarly across many conditions. The internal consistency analysis is meant to measure how consistently a series of experiments across different conditions would identify the same coexpressed genes. If the two subsets of experiments contain replicates, they are more likely to identify the same coexpressed genes as the expression values of the replicates will be very similar. The cross-platform comparisons do not have this advantage because they consist of different experiments. Thus, to make the internal consistency calculation more comparable to the cross-platform comparisons, we used a pseudo-random division for subsequent analysis. Experiments were randomly divided into two subsets but experiments belonging to the same experimental series (Affymetrix), publication (cDNA), or tissue (SAGE) were required to fall into the same subset.

Minimum common experiments analysis

Differences in the number of common experiments between any two genes result from missing values in the data matrices. In the case of the cDNA microarray data, different arrays were used in different experiments, and not all genes are present on all the arrays. For SAGE, a tag is often observed in one library but will have a 0 tag count in other libraries. For Affymetrix oligonucleotide arrays, an intensity is always reported for every probe but in some cases the Affymetrix statistical software will determine that the probe was not reliably detected and assign an absent (A) or marginal (M) call instead of a present (P) call for that probe. As missing SAGE tags and probes not called P represent genes expressed below the detection threshold of the SAGE and Affymetrix array experiments, we did not include these data in our analysis. Thus, for each dataset, there were gene pairs that were rarely represented in the same experiment and their Pearson correlations were based on very few data points. The effect of number of common experiments on internal consistency was determined by calculating the internal consistency for a series of datasets with different MCE criteria. One hundred different pseudo-random divisions were performed to get an average internal consistency for each MCE. An MCE was chosen for each such that the same internal consistency would result (r = 0.25) (Fig. 1). Thus, all subsequent analyses were based on an MCE of 95 for Affymetrix, 28 for cDNA, and 23 for SAGE. Requiring an MCE removes gene pairs from the datasets. To maintain an unbiased comparison, only the 1,173,330 gene pairs common to all three platform datasets (after application of MCE criteria) were used in the subsequent platform comparisons.

Platform comparisons

As with the internal consistency analysis, a correlation of gene correlations was calculated, but was determined for each of the three pair-wise platform comparisons instead of between subsets of one platform. If the two platforms being compared report the same correlation between each gene pair, we expect the overall correlation between platforms would be near 1. The global concordance (r_c) was determined for increasing gene correlation cutoffs to compare to results obtained in the NCI-60 study [25].

Gene Ontology analysis

The GO is a controlled vocabulary that describes the roles of genes and proteins in all organisms [36]. GO is composed of three independent ontologies: biological

process, molecular function, and cellular component. The GO descriptive terms are represented as nodes connected by directed edges that may have more than one parent node (directed acyclic graph). A gene is annotated to its most specific GO term description and all ancestor GO terms are implied.

The GO MySQL database dump (release 200402 of assocdb) was downloaded from http://www.godatabase.org/dev/database. A GO MySQL database was built and a Perl script was developed to extract three GO information subspaces from the biological process ontology: (1) the most specific GO terms for each gene, (2) the most specific terms along with their associated parent terms, and (3) the most specific terms along with their associated parent and grandparent terms. Two categories of annotations were used for the evaluation of each GO information subspace: (1) gene annotations that did not include those derived from inferred electronic annotations (IEAs) (1007 genes found in common with our dataset) and (2) gene annotations including IEAs (1426 genes found in common with our dataset). Similar results were obtained for both non-IEA and IEA analyses. Only the IEA results are reviewed in the figures and text.

One potential issue with our analysis is that of a circular argument. It is possible that a coexpressed gene pair could be found to share a common GO term that was annotated for both genes by a coexpression analysis. Thus, coexpression data could be confirming coexpression data. To check for this problem we assessed the degree to which our dataset depends on annotations inferred from expression profiles (IEP evidence code). Only 93 of 32,669 biological process annotations use IEP evidence, corresponding to only 73 genes with 1 or more IEP annotations. Of these, only 1 was present in our gene set and this gene also had non-IEP annotations. Therefore the potential for a circular argument is negligible.

Results shown in Supplemental Fig. 7 were extracted from the gene pair correlation data by enumerating the number of gene pairs found at common GO terms across a gene's expression similarity neighborhood for each GO information subspace. Results shown in Fig. 2 were extracted by enumerating the number of gene pairs found at common GO terms for each range of Pearson correlations from 0 to 1 in increments of 0.1. The results summarized in Fig. 3 were enumerated in a similar manner but used average Pearson correlations between two platforms instead of individual Pearson correlations. One thousand random permutations of the data were conducted to determine how often GO confirmation of a gene pair at each neighborhood or Pearson range would occur by chance. Scripts were written in Perl and are available at http://www.bcgsc.ca/gc/bomge/coexpression/suppl_materials.

Comparison to other coexpression methods

Results shown in Fig. 4 were generated using the GO analysis method described above for Figs. 2 and 3. AP data were obtained by request from the author [37]. Only pairs with scores above 0.150 were provided. TMM data were downloaded from the authors' supplemental Web page (see Web references) [35]. Both negative and positive correlations were included and thus a gene pair can appear twice. Only pairs with scores of 1 or greater were provided. The 2PC method represents all two-platform averages (Affymetrix/cDNA, Affymetrix/SAGE, and cDNA/SAGE). Thus, a gene pair can appear as many as three times if all three pair-wise averages fall within the 0–1 range graphed. All datasets were converted from their respective identifiers to UniProt [45] and the percentage of gene pairs found at common GO terms for each range of scores was determined. The top 2500 pairs of each were examined to determine the overlap in results for high-scoring pairs. Thresholds for a high-confidence set of coexpressed gene pairs were chosen for each method at the approximate respective score at which performance was at least three to four times better than random chance (2PC > 0.65; AP > 0.7; TMM > 7).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygeno.2005.06.009.

References

[1] M. Schena, D. Shalon, R.W. Davis, P.O. Brown, Quantitative monitoring of gene expression patterns with a complementary DNA microarray, Science 270 (1995) 467–470.

[2] D.J. Lockhart, et al., Expression monitoring by hybridization to high-density oligonucleotide arrays, Nat. Biotechnol. 14 (1996) 1675–1680.

[3] V.E. Velculescu, L. Zhang, B. Vogelstein, K.W. Kinzler, Serial analysis of gene expression, Science 270 (1995) 484–487.
[4] J.M. Stuart, E. Segal, D. Koller, S.K. Kim, A gene-coexpression network for global discovery of conserved genetic modules, Science 302 (2003) 249–255.
[5] S. Li, et al., A map of the interactome network of the metazoan C. elegans, Science 303 (2004) 540–543.
[6] P. Kemmeren, et al., Protein interaction verification and functional annotation by integrated analysis of genome-scale data, Mol. Cell 9 (2002) 1133–1143.
[7] A.J. Walhout, et al., Integrating interactome, phenome, and transcriptome mapping data for the C. elegans germline, Curr. Biol. 12 (2002) 1952–1958.
[8] E. Segal, et al., Module networks: identifying regulatory modules and their condition-specific regulators from gene expression data, Nat. Genet. 34 (2003) 166–176.
[9] E.J. Yeoh, et al., Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling, Cancer Cell 1 (2002) 133–143.
[10] A. Nimgaonkar, et al., Reproducibility of gene expression across generations of Affymetrix microarrays, BMC Bioinformat. 4 (2003) 27.
[11] P.K. Tan, et al., Evaluation of gene expression measurements from commercial microarray platforms, Nucleic Acids Res. 31 (2003) 5676–5684.
[12] S. Blackshaw, et al., MicroSAGE is highly representative and reproducible but reveals major differences in gene expression among samples obtained from similar tissues, Genome Biol. 4 (2003) R17.
[13] L. Huminiecki, A.T. Lloyd, K.H. Wolfe, Congruence of tissue expression profiles from Gene Expression Atlas, SAGEmap and TissueInfo databases, BMC Genom. 4 (2003) 31.
[14] V. Detours, J.E. Dumont, H. Bersini, C. Maenhaut, Integration and cross-validation of high-throughput gene expression data: comparing heterogeneous data sets, FEBS Lett. 546 (2003) 98–102.
[15] A.K. Jarvinen, et al., Are data from different gene expression microarray platforms comparable? Genomics 83 (2004) 1164–1168.
[16] C.A. Iacobuzio-Donahue, et al., Highly expressed genes in pancreatic ductal adenocarcinomas: a comprehensive characterization and comparison of the transcription profiles obtained from three major technologies, Cancer Res. 63 (2003) 8614–8622.
[17] H.L. Kim, Comparison of oligonucleotide-microarray and serial analysis of gene expression (SAGE) in transcript profiling analysis of megakaryocytes derived from $CD34^{+}$ cells, Exp. Mol. Med. 35 (2003) 460–466.
[18] A.T. Rogojina, W.E. Orr, B.K. Song, E.E. Geisert Jr., Comparing the use of Affymetrix to spotted oligonucleotide microarrays using two retinal pigment epithelium cell lines, Mol. Vision 9 (2003) 482–496.
[19] M. Ishii, et al., Direct comparison of GeneChip and SAGE on the quantitative accuracy in transcript profiling analysis, Genomics 68 (2000) 136–143.
[20] S.J. Evans, et al., Evaluation of Affymetrix Gene Chip sensitivity in rat hippocampal tissue using SAGE analysis: serial analysis of gene expression, Eur. J. Neurosci. 16 (2002) 409–413.
[21] J. Li, M. Pankratz, J.A. Johnson, Differential gene expression patterns revealed by oligonucleotide versus long cDNA arrays, Toxicol. Sci. 69 (2002) 383–390.
[22] W.P. Kuo, T.K. Jenssen, A.J. Butte, L. Ohno-Machado, I.S. Kohane, Analysis of matched mRNA measurements from two different microarray technologies, Bioinformatics 18 (2002) 405–412.
[23] N. Mah, et al., A comparison of oligonucleotide and cDNA-based microarray systems, Physiol. Genom. 16 (2004) 361–370.
[24] J. Lu, A. Lal, B. Merriman, S. Nelson, G. Riggins, A comparison of gene expression profiles produced by SAGE, long SAGE, and oligonucleotide chips, Genomics 84 (2004) 631–636.
[25] J.K. Lee, et al., Comparing cDNA and oligonucleotide array data: concordance of gene expression across platforms for the NCI-60 cancer cells, Genome Biol. 4 (2003) R82.
[26] D.J. Allocco, I.S. Kohane, A.J. Butte, Quantifying the relationship between co-expression, co-regulation and gene function, BMC Bioinformat. 5 (2004) 18.
[27] S.K. Kim, et al., A gene expression map for Caenorhabditis elegans, Science 293 (2001) 2087–2092.
[28] S.A. Jelinsky, P. Estep, G.M. Church, L.D. Samson, Regulatory networks revealed by transcriptional profiling of damaged Saccharomyces cerevisiae cells: Rpn4 links base excision repair with proteasomes, Mol. Cell. Biol. 20 (2000) 8157–8167.
[29] J. Quackenbush, Microarrays—Guilt by association, Science 302 (2003) 240–241.
[30] E.J. Williams, D.J. Bowles, Coexpression of neighboring genes in the genome of Arabidopsis thaliana, Genome Res. 14 (2004) 1060–1067.
[31] B.H. Mecham, et al., Sequence-matched probes produce increased cross-platform consistency and more reproducible biological results in microarray-based gene expression measurements, Nucleic Acids Res. 32 (2004) e74.
[32] D.T. Ross, et al., Systematic variation in gene expression patterns in human cancer cell lines, Nat. Genet. 24 (2000) 227–235.
[33] M. Nacht, et al., Molecular characteristics of non-small cell lung cancer, Proc. Natl. Acad. Sci. USA 98 (2001) 15203–15208.
[34] D.A. Porter, et al., A SAGE (serial analysis of gene expression) view of breast tumor progression, Cancer Res. 61 (2001) 5697–5702.
[35] H.K. Lee, A.K. Hsu, J. Sajdak, J. Qin, P. Pavlidis, Coexpression analysis of human genes across many microarray data sets, Genome Res. 14 (2004) 1085–1094.
[36] M. Ashburner, et al., Gene Ontology: tool for the unification of biology. The Gene Ontology Consortium, Nat. Genet. 25 (2000) 25–29.
[37] L.J. Jensen, J. Lagarde, C. von Mering, P. Bork, ArrayProspector: a Web resource of functional associations inferred from microarray expression data, Nucleic Acids Res. 32 (2004) W445–W448.
[38] K. Yeung, M. Medvedovic, R. Bumgarner, From co-expression to co-regulation: how many microarray experiments do we need? Genome Biol. 5 (2004) R48.
[39] P.M. Haverty, L.L. Hsiao, S.R. Gullans, U. Hansen, Z. Weng, Limited agreement among three global gene expression methods highlights the requirement for non-global validation, Bioinformatics 20 (2004) 3431–3441.
[40] K.D. Pruitt, K.S. Katz, H. Sicotte, D.R. Maglott, Introducing RefSeq and LocusLink: curated human genome resources at the NCBI, Trends Genet. 16 (2000) 44–47.
[41] Mammalian Gene Collection Program Team, et al., Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences, Proc. Natl. Acad. Sci. USA 99 (2002) 16899–16903.
[42] G.S. Davidson, B.N. Wylie, K.W. Boyack, Cluster Stability and the Use of Noise in Interpretation of Clustering, IEEE Comput. Soc, Los Alamitos, CA, 2001, p. 23.
[43] M.J. de Hoon, S. Imoto, J. Nolan, S. Miyano, Open source clustering software, Bioinformatics 20 (2004) 1453–1454.
[44] R. Ihaka, R. Gentleman, A language for data analysis and graphics, J. Comput. Graphical Stat. 5 (1996) 299.
[45] A. Bairoch, et al., The Universal Protein Resource (UniProt), Nucleic Acids Res. 33 (2005) D154–D159.

Web site references

http://www.ncbi.nlm.nih.gov/geo/, The Gene Expression Omnibus.
http://www.r-project.org/, R Statistical Package Home Page.
http://www.bioconductor.org/, Bioconductor Home Page.

http://bonsai.ims.u-tokyo.ac.jp/~mdehoon/software/cluster/software.htm, The C Clustering Library Home Page.
http://www.affymetrix.com/, Affymetrix Home Page.
http://cmgm.stanford.edu/~kimlab/multiplespecies/Supplement/, Stuart et al. Data Home Page.
http://www.cytoscape.org/, Cytoscape Home Page.
http://www.bork.embl.de/ArrayProspector, ArrayProspector.
http://microarray.genomecenter.columbia.edu/tmm/, TMM data.
http://www.bcgsc.ca/discoveryspace/, DiscoverySpace Software References.