VAMPIRE microarray suite: a web-based platform for the interpretation of gene expression data

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ABSTRACT

Microarrays are invaluable high-throughput tools used to snapshot the gene expression profiles of cells and tissues. Among the most basic and fundamental questions asked of microarray data is whether individual genes are significantly activated or repressed by a particular stimulus. We have previously presented two Bayesian statistical methods for this level of analysis, collectively known as variance-modeled posterior inference with regional exponentials (VAMPIRE). These methods each require a sophisticated modeling step followed by integration of a posterior probability density. We present here a publicly available, web-based platform that allows users to easily load data, associate related samples and identify differentially expressed features using the VAMPIRE statistical framework. In addition, this suite of tools seamlessly integrates a novel gene annotation tool, known as GOby, which identifies statistically overrepresented gene groups. Unlike other tools in this genre, GOby can localize enrichment while respecting the hierarchical structure of annotation systems like Gene Ontology (GO). By identifying statistically significant enrichment of GO terms, Kyoto Encyclopedia of Genes and Genomes pathways, and TRANSFAC transcription factor binding sites, users can gain substantial insight into the physiological significance of sets of differentially expressed genes. The VAMPIRE microarray suite can be accessed at http://genome.ucsd.edu/microarray.

INTRODUCTION

Gene expression microarrays are commonly used to study the transcriptional responses of cells and tissues. Most studies involve comparisons of an experimental treatment with a corresponding control, often with only 2–3 replicates within each treatment group. These experiments are commonly performed on either one-channel or two-channel microarray platforms, which simultaneously measure gene expression in one or two RNA samples, respectively. As biologists devise more sophisticated experimental designs, existing statistical tools for analyzing these data become increasingly unwieldy. For example, in large data sets of >50 arrays, users may wish to begin analysis before all microarrays have been completely processed. As users define more statistical tests to perform on this data, accounting for all of the tests and changes to the underlying data becomes extremely challenging. Furthermore, because of the high-throughput nature of these experiments, and because of their non-uniform error structure, the resulting data can be difficult to interpret. To address these issues, we have devised a tightly integrated, web-based suite of microarray analysis tools based on a robust Bayesian approach known as variance-modeled posterior inference with regional exponentials (VAMPIRE)\textsuperscript{(1)}.

The microarray analysis platform we present here provides an integrated interface for data management, statistical analysis and interpretation of gene expression data. To simplify the analysis of large data sets, this interface allows users to quickly load data and characterize experimental designs within the data set. Users can associate related samples and combine related groups. The variance structure of these groups can then be modeled to identify the coefficients of expression-dependent (A) and expression-independent variance (B). The analysis suite subsequently uses these models, stored in the user’s account, and applies them to identify microarray features that are differentially expressed between treatment groups. Once this analysis is complete, the corresponding gene lists must still be interpreted and related to biological function. We have therefore integrated a novel statistical tool known as GOby, which can identify overrepresentation of previously defined functional categories. This is performed while respecting the hierarchical nature of annotation systems like Gene Ontology (GO)\textsuperscript{(2)}. Together, these data management and analytical tools provide users with a powerful new approach to microarray gene expression analysis.

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IMPLEMENTATION

The web application is implemented as a package of Java 1.5 servlets. In order to separate interface and the underlying content and logic, we applied a commonly used three-tier scheme. At the foundation of the VAMPIRE analysis suite is a structured query language (SQL)-based relational database. The current implementation uses a combination of MySQL and Oracle databases. Java interfaces have been appropriately designed to isolate SQL-specific code, which allows us to quickly re-deploy the system on other database platforms. Each servlet gathers data through these SQL interfaces and constructs its own intermediate extensible markup language (XML) document. XML is subsequently transformed via extensible stylesheet language transformation (XSLT) into HTML. The end-user is ultimately presented with the HTML interface. By implementing VAMPIRE in this fashion, each component can be re-designed or re-implemented without disturbing the remainder of the site.

The VAMPIRE algorithm itself is computationally intensive. On an Intel Xeon 3.0 GHz processor, typical execution time for variance modeling ranges from 5 to 10 min depending on the number of features on the microarray, and whether unpaired or paired analysis is desired. Significance testing and GOby analysis usually complete within 2–3 min. Because of these computational costs, we have devised a Java remote-method invocation (RMI)-based distributed processing solution (Figure 1). Each analysis job constructed by the web-application is placed into a priority queue. An RMI host program provides access to each job as it arrives at the top of the queue to any number of RMI clients. The RMI hosts and clients may be executed on remote machines distant from the web-server. This design provides scalability to the VAMPIRE site, as additional machines may be added to handle the increased computational load. Since all of these transactions are stored in the VAMPIRE database, no data is lost when individual hosts or processing nodes fail. Jobs may be restarted by other RMI nodes or provided through alternative RMI hosts as long as the database itself is accessible.

USER INTERFACE

The web-based interface of the microarray analysis suite can be divided into three major sections: (i) data management, (ii) statistical analysis and (iii) interpretation. Throughout all three divisions is a consistent user-interface with multiple tools for exporting data (Figure 2). To ease integration with third-party tools, results may be downloaded as tab-delimited text files or as XML documents.

Data management

Management of microarray data sets and their corresponding analyses becomes increasingly complex as the numbers of treatment groups grow. Users must manage not only individual samples, but also all subsequent analyses. This is a particular problem for users with large data sets, who wish to begin interpreting gene expression data before all of samples have been completely processed. To accommodate these issues, we have created a data management system that can be quickly used to load, annotate and associate microarray measurements. Summary measures of gene expression, such as those obtained from Affymetrix MAS 5.0 or Agilent processed signal
intensities, can be imported into the server as tab-delimited text files, a file format that is easily accessible to most biologists.

Once data have been loaded into the web application, the user may associate related microarray samples. Characterizing these relationships is crucial, as they help to describe the analyses that will be later performed. For example, in a two-channel tutorial provided on the web site, users create sample groups for (i) replicates of LPS-treated macrophages, (ii) replicates of control-treated macrophages and (iii) paired samples obtained from the same chips. These groups can be further combined to create ‘categories’ of related groups. Once these relationships are recorded, they can be used to compare gene expression across different treatment conditions. Since further changes to each sample group are recorded by the analysis suite, VAMPIRE can subsequently inform users when analyses need to be re-executed.

**Statistical analysis**

Statistical analysis by VAMPIRE requires two distinct steps: (i) modeling of the error structure of sample groups and (ii) significance testing with a priori-defined significance thresholds. This approach to microarray analysis is considerably different from the approaches taken by other analytical methods (3–6). Normalization methods are commonly applied to average out the error structure prior to performing statistical analysis. Statistical tests are then left to address the significance of expression differences found in the remaining data. In contrast, VAMPIRE studies the underlying error structure, without perturbing it, and uses this knowledge to distinguish signal from noise. The cutoff for statistical significance is then defined by the significance threshold and by the magnitude of the variance model coefficients. Because of the additional variance modeling step however, this kind of analysis can be quite challenging without a robust accounting system. In the web application that we present here, users can easily keep track of all variance models and the data sets for which they can be applied. We have initially incorporated two variants of VAMPIRE—classical unpaired analysis (1) and paired analysis (A. Hsiao and S. Subramaniam, manuscript submitted); both of which use variance models to detect significant changes in gene expression.

When users submit a request to model the variance structure of a group of samples, a new ‘processing job’ is immediately
submitted into a processing queue. Individual jobs require an average of 5–10 min to compute on an Intel Xeon 3.06 GHz processor. In the meantime, users may continue to use the remainder of the site, without waiting for each job to complete. An estimate of the date and time of completion is prominently displayed. Similarly, users may request that specific statistical tests be performed. Since these tests rely on variance model results, they will not be executed until their dependent models have been completed. As data can be continually added to the analysis platform, outdated models and tests are automatically flagged by the system to allow users to re-execute analyses with updated data. This particular feature facilitates ‘on-the-fly’ analysis. Users can monitor the results as they collect data, which may help them to decide which analyses require additional replication.

**Interpretation**

Differentially regulated features obtained from any statistical test must be interpreted biologically. We have developed a novel tool, known as GOby, to initiate biological interpretation, independent of whether VAMPIRE itself was used to derive the feature lists. This database-driven application curates annotation data from several sources: National Center for Biotechnology Information (NCBI), GO, Kyoto Encyclopedia of Genes and Genomes (KEGG), TRANSFAC, Biocarta and Superarray. In addition, it can be readily updated with additional user-defined annotation lists.

GOby primarily uses its annotation database to identify overrepresented annotation groups. It does so by comparing a ‘selected’ list to a ‘background’. In our experience, using the comprehensive feature list for each microarray as the background gives quite meaningful results. In a manner similar to other recently published tools (9–11), GOby uses exact probabilities to compute enrichment likelihoods, and displays the enrichment likelihood as a P-value. GOby reports as its P-value, the probability of finding no more than s features annotated with a given term among k ‘selected’ features:

\[
P(\text{annotated} \leq s) = \sum_{i=0}^{s} P(\text{annotated} = i)
\]

\[
P(\text{annotated} = i) = \binom{k}{i} \prod_{j=0}^{i-1} \frac{b-j}{N-j} \prod_{j=0}^{k-i} \frac{(N-b)-j}{(N-i)-j}
\]

\[
= \frac{\binom{b}{i} \frac{N-b}{k-i}}{\binom{N}{k}}
\]

where b is the number of ‘background’ features annotated with the term; s, the number of ‘selected’ features annotated with the term; N, the total number of ‘background’ features; and k, the total number of ‘selected’ features.

Unlike similar tools however, GOby is also able to compute a ‘conditional-enrichment likelihood’, or Q-value, for each term in a hierarchical annotation system. This Q-value is based on the idea that truly meaningful enrichment in a hierarchical system like GO will occur at specific nodes in the annotation tree. The Q-value computes the enrichment likelihood for a particular term conditioned on the enrichment of its parent terms. In other words, instead of using the entire set of array features as the ‘background’, we use only the subset of features that are annotated with one of the parent terms. Unlike the P-value previously described, the Q-value prevents annotation terms from reaching significance simply because they lie in an area of the tree near other terms that are enriched. It can therefore narrow the user’s focus by reporting only the optimal level of functional detail while excluding both more general and more specific terms (unless these terms fall into a second area of functional enrichment independent of the first). Since both methods have their own advantages, both are displayed in the results.

GOby provides two options for viewing the results of its statistical analysis. Results may be downloaded in an XML format, or they may be viewed directly from the VAMPIRE web site (Figure 3). Since each of the result pages is rendered for GOby via XSLT from the XML export file, all necessary information is easily accessible to third-party applications. Highly skilled users may wish to render their own views of GOby reports. Most users, however, will rely on automatically generated web pages. For each annotation system, GOby renders at least two types of web pages. First, a result table displays the annotation terms that are overrepresented given a specified significance cutoff. Significant P-values and Q-values, according to a user-selected significance threshold, are displayed in bold. Users may choose between Bonferroni-corrected thresholds and false-discovery rates to correct for multiple testing (A. Hsiao and S. Subramaniam, manuscript submitted). Second, each entry in the result table can be clicked to view a page that displays differentially expressed features that are annotated with the selected term. Because of careful integration with VAMPIRE statistical tests, fold-changes and P-values for each array feature can be viewed directly from these pages, eliminating the need to manually cross-reference additional lists. Each feature is linked by accession numbers and LocusLink IDs to corresponding web pages at NCBI. When available, each annotation term has also been linked to pages that display term-specific information. For example, clicking on the KEGG link from a KEGG report page will display the relevant KEGG pathway, while highlighting differentially expressed genes. In addition, we have included several Javascript-based functions to show/hide columns and to sort tables by column data. For hierarchical systems such as GO, GOby also renders a third view, the tree view. Here, P-values and Q-values are again visible to allow users to quickly address the importance of each node in annotation tree.

**CONCLUSIONS**

The VAMPIRE microarray analysis suite provides a fundamentally different approach to gene expression analysis. While other tools independently exist for management of microarray data, analysis and interpretation, none are yet equipped with the statistical tools that we have described. The VAMPIRE suite gracefully handles incremental analysis of large data sets, applies some of the most rigorous statistical methods available for microarray analysis, and provides
powerful tools for interpretation of the results. We have also presented a novel tool, GOby, for interpreting of sets of differentially regulated genes. It can compute both ‘global enrichment’ of annotation terms, as well as ‘conditional enrichment’ at specific nodes of a hierarchical annotation system, like GO. This suite therefore represents a substantial advance in bringing the latest analytical algorithms into the hands of biologists.
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