Gene Expression Browser: large-scale and cross-experiment microarray data integration, management, search & visualization

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Abstract

Background: In the last decade, a large amount of microarray gene expression data has been accumulated in public repositories. Integrating and analyzing high-throughput gene expression data have become key activities for exploring gene functions, gene networks and biological pathways. Effectively utilizing these invaluable microarray data remains challenging due to a lack of powerful tools to integrate large-scale gene-expression information across diverse experiments and to search and visualize a large number of gene-expression data points.

Results: Gene Expression Browser is a microarray data integration, management and processing system with web-based search and visualization functions. An innovative method has been developed to define a treatment over a control for every microarray experiment to standardize and make microarray data from different experiments homogeneous. In the browser, data are pre-processed offline and the resulting data points are visualized online with a 2-layer dynamic web display. Users can view all treatments over control that affect the expression of a selected gene via Gene View, and view all genes that change in a selected treatment over control via treatment over control View. Users can also check the changes of expression profiles of a set of either the treatments over control or genes via Slide View. In addition, the relationships between genes and treatments over control are computed according to gene expression ratio and are shown as co-responsive genes and co-regulation treatments over control.

Conclusion: Gene Expression Browser is composed of a set of software tools, including a data extraction tool, a microarray data-management system, a data-annotation tool, a microarray data-processing pipeline, and a data search & visualization tool. The browser is deployed as a free public web service (http://www.ExpressionBrowser.com) that integrates 301 ATH1 gene microarray experiments from public data repositories (viz. the Gene Expression Omnibus repository at the National Center for Biotechnology Information and Nottingham Arabidopsis Stock Center). The set of Gene Expression Browser software tools can be easily applied to the large-scale expression data generated by other platforms and in other species.

Background

A microarray measures the expression of thousands of genes simultaneously. This experimental system has revolutionized biological research by enabling discovery of a large set of genes whose expression levels reflect a given cell type, treatment, disease or development stage. Since the advent of this technology more than a decade ago, a large amount of expression data has been accumulated on more than 100 species [1]. Several initiatives have been undertaken to develop microarray public data repositories and analysis tools for scientists to share and utilize these data [2]. The public data repositories, such as NASC, NCBI GEO [3], EBI ArrayExpress [4,5] and NIG CIBEX [6], have been collecting, annotating, storing and redistributing large amounts of microarray data from diverse experiments. For example, NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/) has collected 366,965 samples from 14,304 experiments. These...
Effective utilization of these datasets has, however, been limited because of a shortage of suitable tools to integrate large-scale and diverse microarray datasets. In most common use case, a scientist performs an experiment-based analysis: he or she downloads microarray data and sample annotations corresponding to a single experiment, inputs the data into a microarray data-analysis tool, such as GeneSpring [2], HDBStat! [7], or Bioconductor packages [2], etc., and carries out single-experiment centered analysis. In another common use case (e.g. for many gene-centric studies), a scientist wants to know how the expression of a given gene changes under various experimental conditions. The latter case is critically important for discovering gene functions, validating biomarkers, and developing new drugs targeted to specific genes. To answer gene-centric questions, we must have a tool that can be used to integrate a large amount of data from different microarray experiments. Developing such a tool presents several challenges.

The first challenge is the heterogeneity of data collected from different microarray experiments. Different microarray experiments from different laboratories are usually designed independently for specific research purposes. Heterogeneity might come from differences in experimental designs, materials sampled, developmental stages, treatment levels (including controls), and so on. The second challenge is to develop an effective software tool to process such a large amount of data at an acceptable speed with currently available hardware resources (i.e., CPU, memory and network). The third challenge is related to the complexity of displaying or visualizing data in a software tool. Most software tools, when applied to large data sets, display items in an extended page or multiple display pages. Therefore, it is impossible for users to get an overall view of the data on a single page. It is also inefficient and inconvenient for users to scroll display pages to find interesting information from thousands of data items. Thus, it is important to design a data display interface that can show both an overall view of a large-scale dataset in its totality and a detailed view of individual data points.

Geneinvestigator [8] and GeneChaser [1] are two web-based gene expression visualization tools that have successfully integrated a large number of microarray datasets and facilitated gene-centric and cross-experiment gene-expression discoveries. Geneinvestigator defines experiment annotation categories as Tissues/ Organs, Developmental Stage, Environmental Factors (Stimulus) and Mutation. The expression data and the analysis results are organized according to these categories. The microarray experiments are discarded if they cannot be classified into one of the predefined categories. GeneChaser, on the other hand, automatically re-annotates and analyzes GDS datasets from NCBI GEO. It segregates all experimental conditions (treatment levels) into groups and then performs group versus group comparisons. However, the display systems of both Geneinvestigator and GeneChaser are limited. These two tools display data with heatmap or bar graphics on a display page with extended dimension or in multiple display pages. Only a limited number of data points can be shown at a time. Users have to scroll down the page to find interesting data points from among hundreds or thousands of total experimental conditions.

The GEB, on the other hand, displays efficiently a large number of data points simultaneously. This has been achieved by developing a set of software tools of data extraction, data management, data annotation, data processing, and gene expression profile search & visualization. This set of software tools can be applied to microarray data in both public and private data repositories. The current public GEB web service (http://www.ExpressionBrowser.com) integrates 301 ATH1 microarray experiments that were originally stored in the data repositories of NCBI and NASC [9]. Arabidopsis, as a model plant, is widely used in various microarray experiments and gene-network modeling [10-12]. The results and knowledge obtained from Arabidopsis studies can be used as a reference for corresponding research on other plants, especially field crops [13,14].

**Implementation**

**Overall design of workflow**

The GEB workflow is shown in Figure 1. Microarray data can be downloaded from public data repositories with the data extraction tool. Alternatively, data owners may upload their data directly into GEB. The data extraction tool harvests raw data files, sample annotations, and experimental designs from data repositories into the GEB data-management system. Data curators use the web-based interfaces of the data-management system to create sample sets by combing all replicated samples in each treatment level into individual groups (i.e. sample sets). Then, the data curators define a T/C by selecting a treatment sample set and a control sample set. In the data-processing pipeline, the microarray data are normalized, and the log2 ratio of treatment-over-control (LOG2R) and its t-test P value are calculated. The normalized intensities of each chip, average intensities of each sample set, LOG2Rs and P values of each T/C are loaded into the GEB database, from which the data can be queried via the web-based search & visualization tool.
Affymetrix probe set annotation
The probe sets on Affymetrix ATH1 chip were annotated via the following procedures: (1) Arabidopsis cDNA sequences and annotations were downloaded from TAIR (http://www.arabidopsis.org/) and ATH1 probe sequences were downloaded from Affymetrix; (2) All probe sequences were BLASTed against all cDNA sequences; (3) A probe set was mapped to a cDNA when nine or more probes in the probe set had a 100% match to a cDNA sequence (each ATH1 probe set contains 11 probes); and (4) The annotation of matched cDNA was used as the annotation of the probe set.

Data extraction and management
The data extraction tool was developed using Java with Jakarta Commons Net Library (http://commons.apache.org/net/). The tool is a web crawler that recursively harvests raw data (such as Affymetrix CEL files), sample annotations, and experiment design descriptions from a repository website and then loads them into GEB database. To download data from different repositories, a corresponding plug-in component was developed for each repository. So far, two data extraction plug-ins have been developed for harvesting data from GEO and NASC.

The data-management system was developed for data curators to view and annotate the microarray data extracted from data repositories or submitted by data owners. Data curators annotate the data via the following steps:

First, a data curator creates a sample set by grouping replicated samples from every treatment level. The user interface for defining a sample set is shown in Figure 2A. A sample set name of “Wildtype_no treatment” is given at Name box and two replicates of “Wildtype_no treatment_Rep1” and “Wildtype_no treatment_Rep2” are assigned to the sample set by moving them from the left panel to the right panel. Other sample sets in the experiment are created via the same procedure as noted above.

Second, a data curator creates a T/C pair by choosing a treatment sample set and the corresponding control sample set from a drop-down menu (Figure 2B). For instance, we selected “ice1_no treatment” as treatment and “Wildtype_no treatment” as control to form a T/C. Then, the curator specifies a name of “ICE1 mutant vs. wild type” at Name box and detailed T/C information is given in Description box at the lower panel of Figure 2B. The control sample set is selected for a given treatment sample set so that only one-factor differs between the treatment and the control. Therefore, the biological effect of the T/C will be clearly distinguished by the differential factor. All possible T/C pairs were created in this way. In the example shown in Figure 2, a total of 10 T/Cs are defined as follows: 3 T/Cs for cold effects in a mutant (viz. “Ice1 mutant with cold treatment for 3 hr vs. Ice1 mutant with no treatment”, “Ice1 mutant with cold treatment for 6 hr vs. Ice1 mutant with no treatment”, and “Ice1 mutant with cold treatment for 24 hr vs. Ice1 mutant with no treatment”); 3 T/Cs for cold effects in wild type (viz. “Wildtype with cold treatment for 3 hr vs. Wildtype with no treatment”, “Wildtype with cold treatment for 6 hr vs. Wildtype with no treatment”, and “Wildtype with cold treatment for 24 hr vs. Wildtype with no treatment”); 3 T/Cs for cold effects under cold treatment (viz. “Ice1 mutant with cold treatment for 3 hr vs. Wildtype with cold treatment for 3 hr”, “Ice1 mutant with cold treatment for 3 hr”, “Ice1 mutant with cold treatment for 6 hr vs.
Wildtype with cold treatment for 6 hr, and "Ice1 mutant with cold treatment for 24 hr vs. Wildtype with cold treatment for 24 hr"; and one T/C for mutation effects without cold treatment (viz. "Ice1 mutant with no treatment vs. Wildtype with no treatment"). All 10 T/Cs are shown at http://expressionbrowser.com/arab/displayExperiment.jsp?id=2202517&tab=1. After all treatment levels in each experiment are transformed into T/Cs, different experiments have same data structure and are comparable to one another and are, thus, easily integrated together. As a result, the heterogeneity caused by the differences in experimental designs is removed. The LOG2R of T/C also removes system errors that affect both treatment and control. Therefore, the ratio data generated based on T/Cs can be more instructive and reliable than intensity data generated from treatment levels.

Data processing and data quality monitoring
The GEB data-processing pipeline is composed of four consecutive programs. The first program is for data normalization using the Robust Multichip Average (RMA) algorithm [15] that was implemented in the Bioconductor Affy package (http://www.bioconductor.org/packages/2.4/bioc/html/affy.html). The second program takes this normalized intensity data as input and computes average intensities, standard deviations, LOG2Rs, and P values of two-sample, two-tailed t-tests. The third program renders JPEG images of MA plots [16,17] with average intensity as the x-axis, LOG2R as the y-axis, and P value as the color. The images are loaded into the GEB application server (Tomcat) for data display when queried by users. The fourth program computes the mean percentage coefficient of variation (%CV) of all microarray features (genes) in a sample set using the following two steps. First, the standard deviation, mean, and %CV of each feature (gene) in a sample set are calculated: that is, %CV = 100 * (Mean intensity/Standard deviation). Second, the mean %CV of all features in the sample set is calculated. The mean %CV of each of individual sample set is computed via the above procedure; the distribution of all mean %CVs is shown in Figure 3. Most sample sets have mean %CV between 0.5 and 4.68. There is a long tail to the right side of the distribution, in which the mean %CV ranges from 4.68 to 16. This result indicates that about 10% of the total sample sets have extremely large mean %CV, and thus probably have poor data quality. Mean %CV of a sample set could be used to monitor quality of the sample set because higher mean %CV implies larger variation among the replicated samples in the sample set. Therefore, any finding or conclusion from a sample set with high mean %CV must be interpreted cautiously. We plan to filter out the sample sets with extremely high mean %CV in the future to guarantee the quality of all the data in GEB.

Some microarray experiments in NASC or GEO were discarded because there were no replicated samples or no suitable controls. As of now, there are a total of 301 experiments, 1450 T/Cs, and 33,074,500 LOG2R data points in the Arabidopsis GEB database. Additional data, when available, can be easily entered into GEB.
Data search and visualization

The Lucene search engine (http://lucene.apache.org/) is used for full-text search. Search index files in GEB are built with the text from gene identifiers, gene symbols, gene annotations, T/C names, T/C descriptions, experiment titles, and experiment descriptions. Genes, T/Cs, and experiments are searchable by matching keywords in the index files.

A 2-layer visualization display is designed to show large-scale data points as both an overall view and a detailed view. This visualization was developed using AJAX technology [18]. The first display layer is a static display (image) generated offline that contains all data points. The second layer is a real-time interactive display built by Web2.0 technology (JavaScript/AJAX). With the 2-layer display, users not only obtain an overall expression profile of the distribution of data points on the static plot, but can also get detailed information on each data point by real-time interactive searching or highlighting. The $P$ value of ratio data is shown by the color of the data. Therefore, data significance level is displayed at the same time as the magnitude of the data is.

Results and Discussion

Full-text search

With full-text searching, users can easily access the information inside GEB. The full text searching method employed by GEB is different from the searching in Genevestigator [8] or GeneChaser [1], in which only gene identifiers or symbols can be used for searching. Users can obtain expression information from Genevestigator or GeneChaser only when they clearly know the gene names or symbols. In contrast, GEB carries out full-text search for any word or letters for a gene symbol, gene annotation, T/C name, T/C description, experiment title and experiment description. Users can freely explore the expression data with any search term they wish.

The full-text search is implemented in three places. The first is the GEB home page (http://www.Expression-Browser.com), where the user can enter keywords and find three types of information: genes, T/Cs and experiments. The second place is in Gene View (Figure 4), where users can search T/Cs and investigate how different T/Cs affect the expression of the selected gene. The third place is in the T/C View (Figure 5), where users can search genes and observe how the expressions of these genes are changed by the selected T/C.

Gene View and co-responsive genes

The GEB backend data model is a matrix with two dimensions, genes and T/Cs. Users visualize the expression profiles as a slice along either of these two dimensions: the Gene View displays data points of all T/Cs for a selected gene, whereas the T/C View displays data points of all genes for a selected T/C.

Figure 4 illustrates the Gene View. Data points from all T/Cs for a gene are displayed in the MA plot [16,17]. Here, M, the y-axis, is the log2 ratio of treatment over...
control (LOG2R) \[\log_2 (\text{treatment intensity}) - \log_2 (\text{control intensity})\] and A, the x-axis, is the average log2 intensity of the treatment and control \[\frac{(\log_2 (\text{treatment intensity}) + \log_2 (\text{control intensity}))}{2}\]. The MA plot provides a quick overview of data points for all T/Cs affecting the selected gene. The data points located in the upper area of the MA plot are ‘up-regulation’ T/Cs, and those located at lower area are ‘down-regulation’ T/Cs. Gene View is a cross-experimental display of the expressions of a gene under all experimental conditions currently available in GEB. With the MA plot, users can get a clear overall view of a gene-expression profile without scrolling down the display page, no matter how many data points might be on the plot.

From a GEB MA plot, users can easily view both the LOG2R changes and also the statistical significance of the LOG2R. Each data point is color coded on the basis of the t-test P value that indicates the significance level of its LOG2R. The data points are coded in blue color when P values are lower than 0.01, in green color when P values are between 0.01 and 0.05, and in yellow color when P values are higher than 0.05. The color-coded data points help users know visually significance levels and reliability of the data. For example, if the data point has both a high-fold change (at the top or bottom of the display) and high P values \(P > 0.05\), yellow color), it suggests that there may be large systematic or experimental errors among replications so that the results should be interpreted cautiously before conclusion are drawn based on such a data point. Therefore, the location and color of the data points on the GEB MA plot give users a clear view of gene expression in both ratio scale and significance level (reliability).

The MA plot is a JPEG image generated by the offline data-processing pipeline. The image is about 60 K in size, with 480 × 480 pixel dimensions, which allows the image to be loaded from host server to users’ browser very quickly so that users can rapidly obtain an overall view of the expression profile of a gene. Most importantly, GEB is equipped with highlighting and search functions that allow users to highlight data points by dragging-and-dropping the mouse and to search data by entering keywords. Figure 4 illustrates how to use the “highlighting window” to locate the up-regulation T/Cs on the MA plot. First, users move the “highlighting window” to cover the data points on the upper panel of the MA plot. The users can resize the window, if needed. The two text boxes to the right of the MA plot are used for listing detailed information about the highlighted data points. Users can click the ‘Select’ button for any T/C on the upper text box and then the selected T/C will be moved to lower text box. At the same time, the selected T/C is also marked on the MA plot with a small rectangle. This two-layer display solution achieves both a quick overview of an expression profile and a detailed view of the selected data points.

*Arabidopsis PR-1* gene, a pathogenesis-related gene [19], was used as an example of Gene View in Figure 4. The up-regulation T/Cs selected in Figure 4 are listed in Table 1. A total of 95 T/Cs were selected when 2-fold and \(P < 0.05\) were used as a double cutoff. Among
Table 1  A list of T/Cs that induces the expression of the *Arabidopsis PR-1* gene

| T/C Name | Treatment type | Fold Change | P-value |
|----------|----------------|-------------|---------|
| Seedling, SA treatment vs. control | Plant defense elicitor | 338.61 | 0.0024 |
| Csn5 (csn5a-2 csn5b) mutant, light vs. wild type, light | Plant defense related mutant | 230.14 | 3.83E-05 |
| gh3.5-1D mutant Pst DC3000(avrRpt2) vs. gh3.5-1D un-inoculated control | Pathogen infection | 220.44 | 2.07E-06 |
| Leaf, eds16 mutant, Golovinomyces orontii infection for 7 d vs. eds16 mutant, 0 d control | Pathogen infection | 160.28 | 2.21E-05 |
| Csn4-1 mutant, light vs. wild type, light | Plant defense related mutant | 130.35 | 6.81E-04 |
| Csn4-1 mutant vs. wild type | Plant defense related mutant | 125.67 | 0.0011 |
| BTH Effect for 24 hr in wrky18 mutant | Plant defense elicitor | 111.62 | 0.0012 |
| Whole plant, mkk1/mkk2 vs. WT | Plant defense elicitor | 108.59 | 5.48E-04 |
| senescence effects in pod | Senescence | 97.64 | 2.67E-05 |
| cpr5scv1 double mutant | Plant defense related mutant | 88.61 | 0.0385 |
| Pst DC3000 infection (12 hr) in WT | Pathogen infection | 83.37 | 0.0181 |
| BTH Effect for 24 hr in WT | Plant defense elicitor | 77.47 | 0.012 |
| Whole plant, WT, 24 h BTH vs. WT control | Plant defense elicitor | 71.71 | 3.44E-07 |
| Csn3-1 mutant, light vs. wild type, light | Plant defense related mutant | 67.19 | 7.58E-05 |
| 120 hr *Erysiphe orontii* infection | Pathogen infection | 64.8 | 0.0053 |
| Whole plant, mkk2, 24 h BTH vs. mkk2 control | Plant defense elicitor | 63.45 | 0.0042 |
| Col-0 WT, Pst DC3000 (avrRpt2) infection vs. un-inoculated control | Pathogen infection | 60.32 | 0.0182 |
| Cold 7 days effects | Others | 58.93 | 0.0061 |
| cpr5 mutant | Plant defense related mutant | 56.63 | 0.0354 |
| Pst DC3000 infection (12 hr) in wrky17 mutant | Pathogen infection | 55.62 | 0.0293 |
| szl-3 mutant drought with treatment vs. Col-0 WT with drought treatment | Pathogen infection | 52.98 | 0.0304 |
| Brm-101 mutant vs. Ler WT | Others | 52.5 | 0.0221 |
| 96 hr *Erysiphe orontii* infection | Pathogen infection | 49.32 | 2.50E-05 |
| Phytophthora infection for 24 hr | Pathogen infection | 47.65 | 3.19E-05 |
| 32 hr PseES4326 infection vs. 9 hr PseES4326 infection | Pathogen infection | 41.11 | 0.0267 |
| szl-3 mutant vs. Col-0 WT | Plant defense related mutant | 38.88 | 0.0031 |
| Pst DC3000 infection (12 hr) in wrky17 mutant | Pathogen infection | 37.07 | 0.0093 |
| 24 hr PseES4326 infection vs. 9 hr PseES4326 infection | Pathogen infection | 33.64 | 0.0297 |
| E2Fa-DPa over-expressing | Others | 32.75 | 0.0099 |
| Cotyledon | Others | 30.2 | 8.69E-05 |
| Chitin receptor mutant, chitooccaose treatment vs. Wild type, chitooccaose treatment | Plant defense elicitor | 29.64 | 7.04E-04 |
| shoot vs root | Others | 29.61 | 8.02E-04 |
| Csn5 (csn5a-2 csn5b) mutant, dark vs. wild type, dark | Plant defense related mutant | 29.26 | 0.007 |
| Chitin receptor mutant vs. Wild type | Plant defense related mutant | 28.92 | 3.09E-05 |
| flower stage 15, sepals | Others | 28.05 | 1.08E-04 |
| Whole plant, mkk1, 24 h BTH vs. mkk1 control | Plant defense elicitor | 26.74 | 0.0174 |
| Leaf, WT, Golovinomyces orontii infection for 7 d vs. 0 d control | Pathogen infection | 26.06 | 2.36E-07 |
| BTH Effect for 8 hr in WT | Plant defense elicitor | 26.06 | 0.0201 |
| BTH Effect for 8 hr in wrky18 mutant | Plant defense elicitor | 25.92 | 3.30E-04 |
| camta3-2 mutant vs. wild type | Plant defense related mutant | 22.87 | 0.0358 |
| odp6-yfp 4 transgene effects | Others | 20.98 | 0.0151 |
| PsmES4326 infection for 32 hr | Pathogen infection | 19.53 | 0.0079 |
| Leaf, WT, Golovinomyces orontii infection for 5 d vs. 0 d control | Pathogen infection | 17.73 | 2.80E-06 |
| S15-118 mutant vs. WT | Others | 17.6 | 0.0368 |
| PsmES4326 infection for 24 hr | Pathogen infection | 16.37 | 0.0072 |
| flower stage 15 | Others | 14.82 | 1.09E-04 |
| BTH treatment in WT vs. WT control | Plant defense elicitor | 14.78 | 3.70E-04 |
| Phenotype                                                                 | Treatment/Condition                                                                 | P-value | FDR  |
|--------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------|------|
| Arabidopsis PR-1 gene expression                                          | Pathogen infection                                                                  | 12.87   | 0.0015 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 12.58   | 0.0232 |
| Arabidopsis PR-1 gene expression                                          | 72 hr *Erysiphe orontii* infection                                                   | 12.55   | 0.0086 |
| Arabidopsis PR-1 gene expression                                          | Senescence                                                                          | 10.82   | 0.0235 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 10.69   | 0.0187 |
| Arabidopsis PR-1 gene expression                                          | Plant defense related mutant                                                        | 10.46   | 0.0293 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 10.19   | 0.0036 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 8.83    | 3.61E-04 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 8.59    | 0.0117 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 8.38    | 2.48E-04 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 7.64    | 0.0015 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 6.92    | 0.0259 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 6.78    | 1.75E-04 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 6.42    | 7.38E-04 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 6.36    | 0.0173 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 6.3     | 5.77E-04 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 6.2     | 3.95E-04 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 6.03    | 0.0034 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 5.88    | 0.0412 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 5.77    | 0.0437 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 5.74    | 0.0402 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 5.52    | 0.0028 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 5.46    | 3.86E-04 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 5.46    | 3.86E-04 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 5.28    | 0.0166 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 5.1     | 8.30E-04 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 4.87    | 0.0001 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 4.64    | 0.0061 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 4.56    | 0.0014 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 4.53    | 1.56E-04 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 4.33    | 3.03E-04 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 4.12    | 3.95E-05 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 4.17    | 0.0247 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 4.08    | 0.0016 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 3.81    | 0.0031 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 3.64    | 3.24E-04 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 3.62    | 0.0028 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 3.22    | 0.0016 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 3.13    | 0.0023 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 3       | 0.0098 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 2.9     | 0.0033 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 2.9     | 0.0061 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 2.76    | 0.0018 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 2.7     | 0.0158 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 2.49    | 0.0364 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 2.45    | 0.0075 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 2.42    | 0.0023 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 2.05    | 0.0063 |
| Arabidopsis PR-1 gene expression                                          | Others                                                                              | 2.03    | 0.0263 |
the 95 T/Cs, 44 T/Cs are pathogen treatments, 13 T/Cs are plant defense elicitor treatments, and 14 T/Cs are plant defense-related mutants. These results clearly suggested that the expression of PR-1 was promoted by infections, plant-defense elicitors, and plant defense-related mutations. In previous studies, PR-1 was defined as a pathogenesis-related gene that was coordinately activated by pathogen infection and functioned as an indicator of the defense reaction [20,21]. The silencing of this gene leads to an increase in extracellular β-(1→3)-glucanase activity at the onset of tobacco defense reactions [22-24]. A decrease in β-(1→3)-glucan deposition in PR-1-silenced lines [22] might cause less deposition of callose that is linked with β-(1→3)-glucan and while the callose deposition is one of the characteristics of defense reactions associated with hypersensitive response of a plant [25]. Morris et al. [26] indicated that chemical induction of maize PR-1 genes increased resistance to downy mildew. The results for PR-1 functions revealed by GEB were impressively consistent with the previous findings. These results strongly suggested that Gene View of GEB would be very useful in gene-function discovery, biomarker validation, and bioprocess identification.

Figure 6 represents a screenshot of “Co-responsive Genes” tab in the PR-1 Gene View (http://www.expressionbrowser.com/arab/displayFeature.jsp?id=1001343&tab=4). The co-responsive relationship of two genes is determined by the following procedure: (1) The up- and down-regulation T/Cs of the two genes are selected using a double cutoff of $P < 0.05$ and of 2-fold; (2) the overlap T/Cs that have the two genes selected are then used to compute the overlap percentage; (3) the Pearson correlation coefficient is calculated using the LOG2R of overlapped T/Cs; and (4) a relationship index is calculated using the overlap percentage multiplied by the square of the correlation coefficient. The relationship between the two co-responsive genes is computed with ratio data from T/C with only a single factor differing between treatment and control. Therefore, the relationship between co-responsive genes solely reflects the effect of a biological treatment because the variations caused by most other factors are removed. On the other hand, if the relationship between co-expressed genes is computed with intensity data where multiple factors vary (such as tissue and cell type of sample, biological treatment, sampling methods, such as time and location, experimental methods, such as sample storage, mRNA extraction, or microarray dying, and systematic errors), then the relationship between co-expression genes reflects the mixed effects from biological treatment and these multiple factors. In the list of PR-1 co-responsive genes (Figure 6), impressively, many well-known plant defense-related genes, such as EXLB3, PR-2, Chitinase, PR-5 and AGP5, were found. Among them, PR-2 and
PR-5 are considered to have a similar function as PR-1 in systemically acquired resistance (SAR) responses [27]. According to a review on the integrated application of online data mining tools by Meier and Gehring [28], PR-1, PR-2 and PR-5 were induced by necrotrophic Botrytis cinerea pathogen. The results shown by GEB are consistent with those from previous studies. The consensus results from multiple experiments in GEB provide reliable clues for gene-expression discoveries.

**T/C View and co-regulation T/Cs**

Figure 5 represents an example of T/C View of “16 hr Pseudomonas infection.” Each data point on the T/C View is the LOG2R of a gene. The MA plot, color codes, two-layer display design, and searching/highlighting functions on the T/C View are exactly the same as those in Gene View described above. The following example shows how to use search function to locate genes in the T/C View. When a string of “PR1 PR2 PR3 PR4 PR5” was used as a search keyword, all genes with any matching word in its annotation are shown in the upper right box (Figure 5). By clicking the ‘Select’ button on each gene, the gene is moved to the lower box. At the same time, the selected gene is marked on an MA plot with a small rectangle. T/C view provides a condition-centric view of microarray data.

Though different T/Cs may stimulate different sets of genes, any two different T/Cs may co-regulate a set of genes such that they have similar gene-expression signatures. The co-regulation relationship between two T/Cs can be constructed from the similarity of gene-expression signatures of the two T/Cs. If we click the “Co-regulation T/Cs” tab in the T/C View of “16 hr Pseudomonas infection” (http://expressionbrowser.com/arab/displayPair.jsp?id=2056966&tab=4), a total of 199 co-regulation T/Cs are listed in a table ordered by their “relation index” to the “16 hr Pseudomonas infection” T/C. The calculation of relation index between the two T/Cs is described in the footnote in Table 2. The T/C of “24 hr Pseudomonas infection” has the closest relationship (with relation index of 0.623816) to “16 hr Pseudomonas infection.” This result is easily understood because they are the same treatment with an 8-hour treatment-time difference. The top 80 (of the 199) co-regulation T/Cs of “16 hr Pseudomonas infection” are listed in Table 2: 29 belong to pathogen-infection, 16 are plant-defense elicitors, and 6 are plant-defense-related mutants. It is interesting to note that 3 T/Cs are negatively correlated with the T/C of “16 hr Pseudomonas infection” (Table 2). Two of the three T/Cs are mutants of Enhanced Disease Susceptibility 16 (EDS16) under infection conditions. EDS genes have special function in basal disease resistance to pathogens as well as R genes [29,30]. Arabidopsis EDS mutants, such as eds1 [31] and eds5 [32], have lower PR gene-expression level and exhibit higher susceptibility to pathogen infection. The reverse relationship of gene-expression signatures between EDS16 under infection and “16 hr Pseudomonas infection” implies that some pathogen-related genes are either not activated or reduced in EDS16 mutants when they are infected by pathogens. Another T/C negatively correlated with the “16 hr Pseudomonas infection” is caused by “high nitrogen effect”. Hoffland et al. [33] reported that high nitrogen application caused higher N concentration in plant tissue, and the effect of tissue N concentration on disease susceptibility was highly pathogen-dependent. They found that disease susceptibility to P. syringae and Oidium lycopersicum was significantly increased with increasing N concentration in tomato tissue [34]. The results obtained from GEB are consistent with the previous independent studies, further suggesting that the results generated by GEB are reliable and the logic/principles implemented in GEB are scientifically sound.

Gene network building has been a hot research topic during the past few years [10,12,34,35]. GEB is not only able to construct gene networks based on the co-responsive relationship described above (Figure 6) but is also able to construct T/C networks based on the co-regulation relationship (Table 2). Another paper will address the details about constructing gene networks and T/C networks in Arabidopsis.

**Slide View**

The slide view of genes or T/Cs is designed to help users discover changes in multiple genes under various T/Cs or vice versa. Users can make a slide show to compare a set of T/Cs with multiple selected genes. For example, the user can search T/C conditions in Gene View (Figure 4) by typing “cold” in the search box and then selecting three T/C conditions with cold treatment of 12 hr, 6 hr, and 3 hr from the upper right box to the lower right box. After selecting the three “cold” conditions, the user can also search another three non-related T/Cs, such as “drought,” “UV-B” and “wounding” with the same procedure. After the six necessary T/C conditions are selected, the user can click the “[slide]” link and then the six MA plots of T/Cs are shown as slides (Figure 7). In Figure 7A, the user highlights a certain number of genes by dragging, dropping and resizing the “highlighting window.” A total of 51 genes with at least 30-fold increase (LOG2R > 4.9) in 12-hr cold condition are selected. To see how the selected genes are changed in other T/Cs, click the “next slide” arrow, and the next slide will appear. The selected genes in the first slide are still highlighted but the positions of the selected genes
| T/C Name                                                                 | Classification          | Gene Number (B)² | Overlapping Gene Number (C)³ | Overlapping Percentage % (OP)⁴ | Correlation Coefficient (CC)⁵ | Relation Index (RI)⁶ |
|-------------------------------------------------------------------------|-------------------------|------------------|-----------------------------|-------------------------------|-----------------------------|---------------------|
| 24 hr *Pseudomonas* infection                                           | Pathogen infection      | 477              | 277                         | 64                            | 0.982917                    | 0.623816            |
| Leaf, WT, *Golovinomyces orontii* infection for 5 d vs. 0 d control    | Pathogen infection      | 716              | 241                         | 43                            | 0.93683                     | 0.385622            |
| BTH Effect for 8 hr in *wrky18* mutant                                 | Plant defense elicitor  | 1245             | 296                         | 36                            | 0.943639                    | 0.3242              |
| BTH Effect for 8 hr in WT                                              | Plant defense elicitor  | 1614             | 308                         | 30                            | 0.951557                    | 0.279581            |
| SA effect at 6 hr (Col-0)                                              | Plant defense elicitor  | 421              | 121                         | 30                            | 0.954483                    | 0.274902            |
| *Pseudomonas syringae pv. tomato* DC3000 hrcC-infiltration for 24 hr   | Pathogen infection      | 1065             | 223                         | 30                            | 0.939357                    | 0.272162            |
| BTH treatment in *mil4* mutant vs. H2O in *mil4* mutant                | Plant defense elicitor  | 652              | 184                         | 35                            | 0.872943                    | 0.271469            |
| BTH Effect for 24 hr in *wrky18* mutant                                | Plant defense elicitor  | 1655             | 307                         | 30                            | 0.935343                    | 0.263835            |
| Leaf, eds16, *Golovinomyces orontii* infection for 5 d vs. WT, infection for 5 d | Plant defense related mutant | 495           | 169                         | 38                            | -0.82538                    | 0.26286             |
| *sid1*-*3* mutant vs. Col-0 WT                                          | Plant defense related mutant | 987         | 221                         | 32                            | 0.8889254                   | 0.255498            |
| *mil4* overexpression line with BTH treatment vs. *mil4* overexpression line control | Plant defense related mutant | 574         | 181                         | 37                            | 0.820014                    | 0.254887            |
| SA effects at 4 hr (MT-0)                                              | Plant defense elicitor  | 702              | 158                         | 29                            | 0.934091                    | 0.254588            |
| BTH Effect for 24 hr in WT                                             | Plant defense elicitor  | 2062             | 341                         | 27                            | 0.94732                     | 0.250527            |
| *pmr5 pmr6* double mutant vs. WT                                        | Plant defense related mutant | 423         | 126                         | 31                            | 0.892795                    | 0.249832            |
| Leaf, WT, *Golovinomyces orontii* infection for 7 d vs. 0 d control    | Pathogen infection      | 2111             | 329                         | 26                            | 0.940966                    | 0.23379             |
| BTH treatment in WT vs. WT control                                     | Plant defense elicitor  | 496              | 141                         | 32                            | 0.847156                    | 0.230768            |
| 120 hr *Erysiphe orontii* infection                                     | Pathogen infection      | 591              | 159                         | 32                            | 0.837777                    | 0.229624            |
| Whole plant, *mkk2*, 24 h BTH vs. *mkk2* control                       | Plant defense elicitor  | 973              | 225                         | 33                            | 0.828929                    | 0.228364            |
| SA effect at 4 hr (Est)                                                | Plant defense elicitor  | 259              | 78                          | 24                            | 0.96622                     | 0.22756             |
| *Pst* ES4326 infection for 9 hr                                         | Pathogen infection      | 340              | 99                          | 27                            | 0.90638                     | 0.225606            |
| Phytophthora infection for 24 hr                                        | Pathogen infection      | 776              | 152                         | 26                            | 0.919965                    | 0.222373            |
| *Pseudomonas syringae pv. phaseolicola* infiltration for 24 hr          | Pathogen infection      | 1667             | 256                         | 25                            | 0.93104                     | 0.216709            |
| *upf3* mutant vs WT                                                    | Others                  | 635              | 123                         | 24                            | 0.938378                    | 0.213205            |
| Whole plant, *WT*, 24 h BTH vs. WT control                             | Plant defense elicitor  | 707              | 165                         | 30                            | 0.834236                    | 0.211088            |
| 10 hr *DC3118 CDS-hrpS* double mutant infection 10 hr                   | Pathogen infection      | 418              | 90                          | 22                            | 0.963317                    | 0.209057            |
| Ozone effects                                                           | Plant defense elicitor  | 1544             | 247                         | 25                            | 0.898834                    | 0.207327            |
| SA effect at 4 hr (Tsu-1)                                              | Plant defense elicitor  | 294              | 83                          | 24                            | 0.911413                    | 0.204284            |
| *cpr5scv1* double mutant                                               | Plant defense related mutant | 742         | 163                         | 29                            | 0.833698                    | 0.20177             |
| Leaf, eds16 mutant, *Golovinomyces orontii* infection for 7 d vs. eds16 mutant, 0 d control | Pathogen infection      | 2644             | 341                         | 22                            | 0.939944                    | 0.199188            |
| Phytophthora infection for 12 hr                                        | Pathogen infection      | 877              | 152                         | 24                            | 0.907767                    | 0.199132            |
Table 2 The co-regulation T/Cs with expression profiles correlated to the T/C of “16 hr Pseudomonas infection” (A)^1 (Continued)

| Shoot under Caesium treatment | Others | 187 | 64 | 22 | 0.938556 | 0.19851 |
|-------------------------------|--------|-----|----|----|-----------|---------|
| E. coli TUV86-2 flc mutant infection 7 hr | Pathogen infection | 859 | 136 | 21 | 0.941937 | 0.194622 |
| Whole plant, mkk1, 24 h BTH vs. mkk1 control | Plant defense elicitor | 1003 | 176 | 25 | 0.867235 | 0.191285 |
| SA effects at 4 hr (Van-0) | Plant defense elicitor | 243 | 66 | 21 | 0.938173 | 0.18619 |
| Pst DC3000 hrpA mutant infection 7 hr | Pathogen infection | 796 | 121 | 20 | 0.947091 | 0.184426 |
| sp2-3 mutant drought with treatment vs. Col-0 WT with drought treatment | Plant defense related mutant | 1713 | 244 | 23 | 0.884965 | 0.182514 |
| Pseudomonas syringae pv phaseolicola infiltration for 6 hr | Pathogen infection | 1090 | 161 | 21 | 0.899435 | 0.177085 |
| upt1 mutant vs WT | Others | 268 | 85 | 26 | 0.82047 | 0.176332 |
| WT (Col-0) Bgh infection vs. WT control | Pathogen infection | 2489 | 296 | 20 | 0.924127 | 0.176158 |
| 6 hr control vs 0 hr control | Others | 1128 | 154 | 20 | 0.924756 | 0.174548 |
| Shoot under potassium starvation | Others | 1293 | 168 | 20 | 0.929743 | 0.173504 |
| Pst DC3118 Coronatine infection 24 hr | Pathogen infection | 483 | 82 | 18 | 0.955476 | 0.173289 |
| ataf1-1 mutant, Bgh infection vs. ataf1-1 mutant control | Pathogen infection | 3165 | 346 | 19 | 0.938368 | 0.171836 |
| Phytophthora infection for 6 hr | Pathogen infection | 1920 | 237 | 20 | 0.912724 | 0.171609 |
| S15-118 mutant vs. WT | Others | 160 | 63 | 23 | 0.854106 | 0.169901 |
| Leaf, eds16 mutant, G. orontii infection for 5 d vs. eds16 mutant, 0 d control | Pathogen infection | 152 | 54 | 20 | 0.905124 | 0.166002 |
| SSS:ERF104, Flg22 treatment vs. SSS:ERF104, control | Plant defense elicitor | 1388 | 160 | 18 | 0.95289 | 0.164251 |
| pmr5 mutant vs. WT | Plant defense related mutant | 93 | 43 | 18 | 0.947634 | 0.16293 |
| E. coli O157:H7 infection 7 hr | Pathogen infection | 582 | 88 | 18 | 0.940332 | 0.161603 |
| SA effects at 4 hr (Kin-0) | Plant defense elicitor | 237 | 59 | 19 | 0.919729 | 0.161515 |
| SS8-2 mutant vs. WT | Others | 175 | 56 | 20 | 0.892642 | 0.160508 |
| Leaf, eds16, G. orontii infection for 7 d vs. WT, infection for 7 d | Plant defense related mutant | 455 | 108 | 25 | -0.78266 | 0.158267 |
| Rosette leaf, flu mutant vs. WT | Others | 1024 | 138 | 19 | 0.89576 | 0.157622 |
| Tnzalopyrimidine herbicide treatment vs. control | Herbicide | 1768 | 191 | 17 | 0.938601 | 0.156599 |
| Col-0 WT, Pst DC3000 (avrRpt2) infection vs. uninoculated control | Pathogen infection | 629 | 110 | 21 | 0.827156 | 0.149031 |
| cpr5snpr1svi1 triple mutant | Plant defense related mutant | 388 | 89 | 23 | 0.799919 | 0.14811 |
| DC3000hrpA vs WT at 14 hr pathogen treatment | Pathogen infection | 891 | 107 | 16 | 0.93812 | 0.148062 |
| 2 hr control vs 0 hr control | Others | 864 | 113 | 18 | 0.898938 | 0.146689 |
| Ogs effects for 1 hr | Plant defense elicitor | 866 | 122 | 19 | 0.86349 | 0.145894 |
| AgNO3 | Others | 807 | 117 | 19 | 0.854076 | 0.143679 |
| Rosette leaf, flu mutant, over-expressing tAPX vs. WT, over-expressing tAPX | Others | 1414 | 149 | 16 | 0.926977 | 0.142656 |
| Elicitor experiment, HrpZ treatment for 2 hr vs. 2 hr control | Plant defense elicitor | 2043 | 211 | 17 | 0.905002 | 0.142587 |
| Imidazolinone herbicide treatment vs. control | Herbicide | 1843 | 176 | 15 | 0.948063 | 0.14226 |
| flg22 effects for 1 hr | Plant defense elicitor | 1714 | 180 | 17 | 0.908522 | 0.141837 |
| Pseudomonas syringae pv phaseolicola infiltration for 2 hr | Pathogen infection | 319 | 65 | 18 | 0.869464 | 0.140394 |
| Pst DC3000 infection (5 hr) in wrky17 mutant | Pathogen infection | 2918 | 271 | 16 | 0.918935 | 0.138735 |

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are changed in different slides. With this slide show, users are able to see the change of these 51 selected genes in different T/Cs. Figures 7B to 7F reveal changes in the selected genes in the T/Cs with treatments of 6-hr cold, 3-hr cold, 12-hr drought, 12-hr UV-B, and 12-hr wounding, respectively. These slides clearly demonstrate that the selected genes had highest fold changes under 12-hour cold treatment (Figure 7A). The fold-changes decreased in 6-hr (Figure 7B) and 3-hr (Figure 7C) cold treatments. The positions of the 51 selected genes in treatments of drought (Figure 7D), UV-B (Figure 7E) and wounding (Figure 7F) showed less similarity to "12 hr cold treatment." The Slide View is a very simple and powerful visualization tool for scientists to compare their candidate genes and see how the genes behave differently in the T/Cs across studies.

**Conclusions**

GEB is composed of a data extraction tool, a microarray data-management system, a data annotation tool, a data-processing pipeline, and a search & visualization tool. The heterogeneity of diverse experimental designs has been greatly mitigated by re-organizing different experimental treatment levels into T/Cs so that cross-experimental data integration is easily achieved. GEB separates data processing from interactive display. It pre-processes data and generates data plot images, and then displays the processed data with a web2.0-based interactive user-interface, according to users’ requests. This design allows heavy computing to be done offline, and thus allows a large number of data points to be queried quickly and displayed interactively in real-time. GEB displays all data points in one view so that users do not need to scroll down display pages to obtain the

| Table 2 The co-regulation T/Cs with expression profiles correlated to the T/C of “16 hr Pseudomonas infection” (A) |  |
|---|---|---|---|---|---|
| high nitrogen effects | Others | 1155 | 131 | 17 | -0.89249 | 0.135869 |
| Pst DC3000 infection (5 hr) in WT | Pathogen infection | 2782 | 264 | 16 | 0.901735 | 0.135735 |
| Whole plant, mkk1/mkk2 vs. WT | Plant defense related mutant | 2559 | 241 | 16 | 0.905859 | 0.134531 |
| Whole plant, mkk2, 24 h BTH treatment vs. WT, 24 h BTH treatment | Plant defense elicitor | 262 | 55 | 17 | 0.886747 | 0.134518 |
| Whole plant, mkk1/mkk2, 24 h BTH vs. WT 24 h BTH treatment | Plant defense elicitor | 1887 | 197 | 17 | 0.879539 | 0.134389 |
| gh3.5-1D mutant Pst DC3000(avnRpt2) vs. gh3.5-1D uninoculated control | Pathogen infection | 2533 | 248 | 17 | 0.888302 | 0.134312 |
| Elicitor experiment, Flg-22 treatment for 4 hr vs. 4 hr control | Plant defense elicitor | 1259 | 134 | 16 | 0.906282 | 0.13422 |
| senescence effects in pod | Senescence | 1722 | 195 | 18 | 0.850641 | 0.134189 |
| snf1 mutant | Plant defense related mutant | 170 | 72 | 26 | 0.713915 | 0.1332 |
| Pst DC3000 infection (5 hr) in wrky11 mutant | Pathogen infection | 3067 | 276 | 16 | 0.909859 | 0.132532 |
| Primisulfuron herbicide treatment vs. control | Herbicide | 2805 | 242 | 15 | 0.931266 | 0.131749 |

1 The number of the genes (B) that are significantly changed (2-fold and P value 0.05 as cutoff)
2 The number of overlapping genes (C) between A and B
3 The Overlap Percentage OP = 2*C/(A + B)
4 The Pearson Correlation Coefficient (CC) of LOG2R of the overlapping genes
5 The Relation Index RI = OP * CC

**Experiment View**

Experiment View shows experiment title, description/ design, lab information, samples, sample sets, biological replicates, and the definitions of T/Cs. This view helps users understand the data in detail. For example, the contents of the Experiment View of “Pathogen Series: Pseudomonas half leaf injection” can be seen at the following link http://expressionbrowser.com/arab/displayExperiment.jsp?id=2020113. There are three tabs in the Experiment View. The first tab, called “Details,” displays experiment title, description, and other detailed information of the experiment. The second tab “T/C” contains information about the T/Cs in the experiment. The third tab “Samples and Data” contains information about all sample sets, samples and raw data files. Users can download raw microarray data files through the “Samples and Data” tab and then input these raw data into other microarray data-analysis software to analyze the data and to validate the results obtained from GEB.
Figure 7 A sample of Slide View for six T/Cs (3 cold treatments, 1 drought treatment, 1 UV-B treatment, and 1 wounding treatment). Users may test this function at http://www.expressionbrowser.com/arab/displayPairSlides.jsp?id=2055336&id=2055335&id=2055334&id=2055599&id=2055979&id=2056077.

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trend or pattern of gene expressions from all data points. The highlighting and searching functions in Gene View, T/C View, and Slide View greatly facilitate dynamically exploring the data points based on users’ interests. As an additional strategy to improve usability, all raw data and calculated data in GEB are accessible via a full-text search engine. GEB also computes relations of co-regulation T/Cs and co-responsive genes. These relations are the foundation for building gene networks and T/C networks.

Availability and requirements
- Project Name: Gene Expression Browser (GEB)
- Public web service: http://www.ExpressionBrowser.com
- Free and no registration.
- Programming Language: Java, R
- Database: MySQL
- Software License: The software license is owned by GeneExp. GeneExp grants free licenses to non-profit organizations and general licenses to commercial organizations.
- License request: support@ExpressionBrowser.com

Abbreviations
A: Average Log2 Intensity; AJAX: Asynchronous JavaScript and XML; ATH1: A light-regulated Arabidopsis thaliana homeobox 1 gene; BTH: light-regulated Arabidopsis thaliana homeobox 1 gene; CC: Correlation Coefficient; CIBEX: Center for Information Biology Gene Expression Database; CPU: Central Processing Unit; EDS: Enhanced Disease Susceptibility; EBI: The European Bioinformatics Institute; GEO: Gene Expression Omnibus; JPEG: Joint Photographic Experts Group; LOG2R: Log2 Ratio of Treatment over Control; M: LOG2R; N: Nitrogen; NASC: Nottingham Arabidopsis Stock Centre; NCBI: The National Center for Biotechnology Information; NIG: The National Institute of Genetics; PR: gene: Pathogenesis-related gene; PR gene: Resistance genes; RI: Relation Index; RMA: Robust Multichip Average; SA: Salicylic Acid; SARC: Systemic Acquired Resistance; TAIR: Texas Association for Institutional Research; T/C: Treatment over Control; TVC: Percentage Coefficient of Variation; OP: Overlapping Percentage; UV-B: Ultraviolet-B Radiation.

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Authors’ contributions
MZ, XF, ST proposed software requirements. MZ, YX, XF, MSK did software specification and design. YZ developed statistical protocols. MZ designed database schema and developed computational algorithms and the software. LL, LY, ST, JT, WY, NY tested the software application and wrote the manual. MZ downloaded and processed raw microarray data from GEO and NASC. LL, LY, ST, JT, WY annotated the data and nominated the T/Cs. MZ, YX, ST, MSK, YA drafted the manuscript. MZ, YX, XF, LL, LY, ST, JT, WY, MSK wrote different parts of the manuscript. MZ, YX, XF, MSK, YA assembled all parts written by different authors together into this manuscript. All authors read and approved this manuscript.

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