

Pomelo II: finding differentially expressed genes

Edward R. Morrissey^{1,2}, Ramón Diaz-Uriarte^{1,3}

¹Structural and Computational Biology Programme, Spanish National Cancer Center (CNIO), Melchor Fernández Almagro 3, Madrid, 28029, Spain

²Current address: Systems Biology DTC, University of Warwick, Coventry House, Gibbet Hill Road, Coventry, CV4 7AL, UK

Abstract

Pomelo II (<http://pomelo2.bioinfo.cnio.es>) is an open-source, web-based, freely-available tool for the analysis of gene (and protein) expression and tissue array data. Pomelo II implements: permutation-based tests for class comparisons (t-test, ANOVA) and regression; survival analysis using Cox model; contingency table analysis with Fisher's exact test; linear models (of which t-test and ANOVA are especial cases) that allow additional covariates for complex experimental designs and use empirical Bayes moderated statistics. Permutation-based and Cox model analysis use parallel computing, which permits taking advantage of multicore CPUs and computing clusters. Access to, and further analysis of, additional biological information and annotations (PubMed references, Gene Ontology terms, KEGG and Reactome pathways) are available either for individual genes (from clickable links in tables and figures) or sets of genes. The source code is available, allowing for extending and reusing the software. A comprehensive test suite is also available, and covers both the user interface and the numerical results. The possibility of including additional covariates, parallelization of computation, open-source availability of the code, and comprehensive testing suite make Pomelo II a unique tool.

³Corresponding author. Email: rdiaz02@gmail.com. Phone: +34-91-224-6900

1 Introduction

There is a continuous demand for web-based applications for the analysis of genomic and proteomic data. For end-users, a key feature of web-based applications is that they make few demands on users' software and hardware, since only a web-browser is needed [1]. Moreover, and of particular relevance when dealing with large data sets, computational capabilities are not limited by the user's hardware (only by the servers'). In this context, web-based applications allow developers to take advantage of the increased availability of multicore processors and clusters built with off-the-shelf components. These are probably the major opportunities for significant performance gains in the near future [2–5]. When deployed in a computing cluster, parallelization (such as provided by MPI [6]), harvests computational resources that are rarely available to individual researchers and can deliver significant decreases in waiting time, while being completely transparent to the end-user. Moreover, web-based applications can offer a user interface and experience very similar to that of desktop applications (e.g., by usage of Javascript [7]). Finally, web-based tools offer the opportunity to quickly bring new methodological developments to many potential users. Interpretation of results [8, 9] can also be easily provided by web-based tools, by linking to additional sources of information (e.g., PubMed references, Gene Ontology terms, etc), which also permits further analysis with this additional information, such as identifying features (e.g., pathways, GO terms, etc) which might be characteristic of the set of differentially expressed genes.

In addition to the above general features of “omics” web-based applications, when searching for differentially expressed genes (and similarly for protein and tissue array analysis) it is of course imperative to incorporate the best statistical practices in the field. Depending on the type of response data, different tests should be applied. The most common type of data (gene expression data for different types of patients) are often analyzed to search for differentially expressed genes using ANOVA, t-tests, and related approaches that compare two or more classes. Tissue-array data, however, are of a categorical or presence/absence nature, and require contingency table methods. Survival data, in contrast, require methods (such as Cox model) that can explicitly deal with right-censored observations. Thus, a tool for the search of differentially expressed genes (proteins) should incorporate the above methods to cover some of the more common needs of wet-lab researchers. In all these cases, and regardless of the type of test, it is by now been well recognized [10, 11] that multiple testing problems should be taken into account. In addition, and since many microarray studies are really observational studies with human patients, it is often necessary to include additional clinical covariates to minimize confounding problems [12, 13]. Finally, in some cases the statistical methodology exists that will allow us to borrow information from all genes in the array when carrying out the test for each gene, using moderated statistics and Empirical Bayes approaches [e.g., 14].

Finally, availability of source code, under an open-source license, is well recognized as an important feature of bioinformatics applications [13, 15]: it allows for fast methodological development based upon previous work by permitting other researchers to extend the methods and provide improvements and bug fixes, it makes it possible to verify claims made by method developers, and ensures that the international research community remains the owner of the tools it needs to carry out its work. The impact of code availability is further enhanced when standard best practices in software development (see review and references in [16]) and the usual open source development mode [17], are followed. Of particular importance, especially with applications that perform complex analysis, is to provide testing suites that allow to verify the results of the analysis performed by the web-based application.

2 Pomelo II: unique features

There are several other web-based applications that can be used to identify differentially expressed genes [18–31]. However, all of these fail one or more of the requirements mentioned in the introduction. Many of them incorporate some of the same procedures as Pomelo II, but few offer as comprehensive a set of analysis as Pomelo does. Most tools are limited to two-class comparisons. Multiclass comparisons are only available from EMAAS [18], EzArray [24], GenePublisher [26], WebArray [27], and GEPAS [32]. Survival analysis and regression are only available in GEPAS [32], but GEPAS is not open source. Contingency tables, however, are not available in other tools except Pomelo II. Several other tools make it explicit that they run in clusters [20, 33], which allows for load balancing and swapping jobs to idle nodes. However, with the exception of EMAAS [18], parallel computing seems not to be used by any other tools.

More importantly in microarray data analysis, a unique characteristic of Pomelo II is that it allows to incorporate additional covariates, such as age or sex, a much needed feature in many microarray studies with human subjects, where these variables can have an effect in gene expression [12, 13]. As part of our

emphasis on this feature, when using additional covariates, the user is alerted to possible aliasing and confounding and to the available degrees of freedom available (see Section 3.2).

3 Functionality, input, output

3.1 Available statistical methods

Pomelo II incorporates a range of validated, well know, statistical methods for identifying differentially expressed genes (or proteins). Fisher’s exact test is available for contingency tables (this test is useful specially with tissue array data). Linear regression, with p-value obtained by permutation test, is of interest when we try to model the values of an interval scaled variable using gene expression data. Cox model is a widely used method for censored data, such as when we want to find the relationship between patient survival and gene expression. Two-class comparisons are available as a permutation-based t-test, as a parametric t-test using moderated statistics with an empirical Bayes approach [14] as implemented in Limma [34], and as a paired t-test (also using Limma). Class comparisons for two or more classes are available as ANOVA, using permutation for significance, or as linear models, using Limma. If using linear models, we can adjust for the possible effects of **additional covariates** (e.g., sex, age, etc). For all the tests implemented, we return unadjusted p-values as well as FDR-adjusted p-values, using the approach of Benjamini and Hochberg (see details and discussion in [10, 11]).

3.2 Input and output

Input are plain text files. For all analysis (except survival analysis), two files are needed: the expression data, and the class labels data. In addition, for linear models, and if additional covariates are used, a file with the additional covariables will be required. For survival analysis, three files are needed: expression data, survival times, and censored indicators. A screenshots of the main input screens showing the methods available is shown in Figure 1 (panel a).

[Figure 1 about here.]

When using linear models, the user can use additional covariables. These are other subject attributes (e.g. subject age, gender, weight, etc), often readily available from the clinical history. This information can allow Pomelo II to check if gene expression differences or similarities may be due to these factors instead of due to belonging to a certain class.

When entering additional covariates for the linear model, the user can choose which of the covariates to use. In addition, we show plots of each of the covariates at the different levels of the class variable (see Figure 1, panel b). This allows the user to check that the program has correctly interpreted the variables showing which are numerical and which are categorical. In addition, it alerts the user of the possible existence of confounding and aliasing [35]. Suppose that in a study comparing expression profiles of breast cancer patients with non-breast cancer control subjects, most breast cancer patients were females and the non-breast cancer subjects were males. This situation would be readily detectable with the plots provided by Pomelo II. In addition, some studies have small numbers of subjects but try to correct for too many covariates; when entering additional covariates, the user is informed about the available degrees of freedom, as well as the degrees of freedom used by any of the covariates included; help is available immediately, explaining the meaning of the table (see Figure 1 c).

The main output from the program is a table with the results of the analysis and a heatmap. The results table contains a header indicating the test you have used, number of permutations and which covariables were used (if any); see Figure 2, a) and b) for two examples, corresponding to a permutation t-test and a Cox model. The table shows an index corresponding to the original ordering in the data file, gene names, p-values (unadjusted), FDR-adjusted p-values, and statistics (and the absolute value of the statistic); in the case of Cox models, an additional column, “Warnings”, might show warnings from the fit (e.g., lack of convergence). At the bottom of the output, there is a figure with a heatmap (see Figure 2, panel c) where you can filter how and which genes to plot, and allows you to choose the color scale. Both tables and heatmap are clickable and will take you to a page with additional information (our IDConverter Light [36]) and will allow you to send selected genes (based on user-specified selection criteria) to PaLS [37] to examine PubMed references, Gene Ontology terms, KEGG pathways or Reactome pathways that

are common to that set of genes.

[Figure 2 about here.]

If you have run an "Anova, linear models (limma)" test, the output will also contain a Class compare section containing a button. By clicking on the button we will be taken to a class compare page (Figure 3, a), where we will be able to compare specific pairs of classes. For each comparison, a table will appear (e.g., Figure 3 b), showing a table with (moderated) t-statistics (and associated p-values and FDR-corrected p-values), similar to the one in Figure 2. The Class compare page is provided because in linear models (ANOVAs) with three or more classes, we might be interested in comparing particular pairs of classes in addition to the overall F test (if our linear model had only two classes originally, this option is not really necessary, since of course the overall F test is equivalent to the t-test for the two class comparison). Note that a particular two-class comparison in a, say, three class analysis is not necessarily identical to conducting just a two-class comparison with a t-test: in linear models, we use all available data to estimate the error term and, moreover, the empirical Bayes method implemented in Limma [14] borrows information from gene expression data across all classes. Thus, in experiments that comprise more than two classes, it is always preferable to carry out specific contrasts after a full, global model, is fitted to all the data, rather than conducting many two-group analyses that discard information from the other groups.

[Figure 3 about here.]

From the Class compare page, we can also obtain differential expression tables which, again, are particularly useful with more than two classes. They are also useful with two classes since the F statistic, which is always of positive sign, gives no indication of whether the mean of the first group is larger or smaller than the mean of the second group. As shown in Figure 3 (panel c), for the user selected group comparisons we obtain Venn diagrams that provide a quick visual information about the number of up- and down-regulated genes in each two-class comparison and their intersection (e.g., the number of genes that are up-regulated in both the contrast between classes 0 and 1 and the contrast between classes 0 and 2 are 656 in the figure). We also obtain a table showing which genes are differentially expressed in each two-class comparison; we use color codes (green and red) and the "<" and ">" signs to allow for fast differentiation between up- and down-regulated genes. The FDR threshold below which genes are considered differentially expressed can be changed by the user, and the Venn diagram and table will be regenerated automatically.

3.3 Documentation, help, tutorials

Online help, including full documentation, pre-run examples, sample files, and loading of sample data sets is available from the main page of Pomelo II. We also provide video tutorials (see http://pomelo2.bioinfo.cnio.es/help/flash_tutorials/video_tutorials.html) of some of the most common or most involved analysis. In most screens, there is help available to options specific of that step, accessible by clicking on the "?" symbol (e.g., see Figure 1 c). The help files are licensed under a Creative Commons license (<http://www.creativecommons.org>), allowing for redistribution and classroom use.

4 Implementation, availability, maturity and testing

Most of the statistical functionality is written in R [38] or in C/C++, with extensive use of parallelization using MPI [6] and R interfaces to MPI (via the R-packages Rmpi [39], by H. Yu, and papply [40] by D. Currie). Parallelization is used in all permutation-based tests and the Cox model computations. Cox model fitting uses the survival package R-package, by T. Therneau [41]. For linear models we use Limma [34], by G. K. Smyth and collaborators. The web interface is written in Python and Javascript. Control of the application, fault-tolerance, and booting and halting the LAM/MPI universes is accomplished by a combination of Python and shell scripts. We create a new LAM/MPI universe for each run of each application, and the actual nodes/CPU's that are used in a LAM/MPI universe are determined at run-time (thus excluding nodes that are down).

Our publicly-accessible installation, available from <http://pomelo2.bioinfo.cnio.es>, runs on a cluster with 31 two dual-core AMD Opteron 2.2 GHz CPUs and 6 GB RAM, running Debian GNU/Linux. Shared storage space uses RAID 50, which provides protection against hard-disk failure, as well as access to results and data from nodes different from the one where computations started. Redundancy and

load-balancing of the web-service is achieved with Linux Virtual Server with heartbeat and mon, which ensures balancing of the master nodes for MPI and of the non-parallelized executions.

All of the code (including repository history) is available under open source licenses (GNU GPL and Affero GPL) from the Launchpad at <http://launchpad.net/pomelo2>.

4.1 Testing, maturity, and number of accesses

Pomelo II includes a comprehensive test suite that uses FunkLoad (<http://funkload.nuxeo.org>). These tests cover the user interface, handling of error conditions and incorrectly formatted files, and the numerical output, and can be run on demand, and wherever new changes are introduced in the software, thus ensuring appropriate quality control and regression testing. The complete code is also available, under the GNU GPL and Affero GPL licenses, from <http://launchpad.net/functional-testing> (go to the Pomelo II directory in the source code). An additional test using Selenium (<http://www.openqa.org/selenium/>) is available (<http://pomelo2.bioinfo.cnio.es/tests.html>); these tests verify that the AJAX component of the application runs correctly under different operating systems and browsers.

Pomelo II is a mature application. The server has been running for more than four years. In the last two years, over 6000 experimental data sets have been analyzed. Usage and testing includes four groups at the developers institution (CNIO), and users worldwide.

Funding

Funding for development provided by Fundacion de Investigacion Medica Mutua Madrileña and Project TIC2003-09331-C02-02 of the Spanish Ministry of Education and Science (MEC). Publication costs covered by Red Tematica de Investigacion Cooperativa COMBIOMED.

Acknowledgments

We want to thank many testers at CNIO and elsewhere for feedback on the applications and bug reports. Bioinformatics.org and Launchpad provide hosting for the repositories. Our applications would not have been possible without the excellent and free R language and its many freely available packages. A. Valencia for covering publication costs.

References

- [1] Graham, P. Hackers and Painters chapter The other road ahead O'Reilly (2004).
- [2] Sutter, H. (March, 2005) The free lunch is over: A fundamental turn toward concurrency in software. *Dr. Dobbs's Journal*, **30**, 202–210.
- [3] Kontoghiorghes, E. J. (2006) Handbook of Parallel Computing and Statistics, Chapman & Hall, CRC, Boca Raton, FL.
- [4] Dongarra, J., Gannon, D., Fox, G., and Kenned, K. (February, 2007) The impact of multicore on computational science software. *CTWatch Quarterly*, **3**(1), 3–10.
- [5] Turek, D. (February, 2007) High performance computing and the implications of multi-core architectures. *CTWatch Quarterly*, **3**(1), 31–33.
- [6] Pacheco, P. (1997) Parallel programming with MPI, Morgan Kufman, San Francisco.
- [7] Geraci, F., Pellegrini, M., and Renda, M. E. (July, 2008) Amic@: All microarray clusterings @ once.. *Nucleic acids research*, **36**(Web Server issue).
- [8] Hyatt, G., Melamed, R., Park, R., Seguritan, R., Laplace, C., Poirot, L., Zucchelli, S., Obst, R., Matos, M., Venanzi, E., Goldrath, A., Nguyen, L., Luckey, J., Yamagata, T., Herman, A., Jacobs, J., Mathis, D., and Benoist, C. (2006) Gene expression microarrays: glimpses of the immunological genome. *Nature Immunology*, **7**, 686–691.
- [9] Rhodes, D. R. and Chinnaiyan, A. M. (2005) Integrative analysis of the cancer transcriptome. *Nat Genet*, **37 Suppl**, S31–7.

- [10] Ge, Y., Dudoit, S., and Speed, T. (2003) Resampling-based multiple testing for microarray data analysis (with discussion). *TEST*, **12**, 1–77.
- [11] Reiner, A., Yekutieli, D., and Benjamini, Y. (2003) Identifying differentially expressed genes using false discovery rate controlling procedures. *Bioinformatics*, **19**, 368–375.
- [12] Potter, J. D. (2003) Epidemiology, cancer genetics and microarrays: making correct inferences, using appropriate designs. *Trends Genet*, **19**, 690–695.
- [13] Díaz-Uriarte, R. (2005) Supervised methods with genomic data: a review and cautionary view. In Azuaje, F. and Dopazo, J., (eds.), *Data analysis and visualization in genomics and proteomics*, chapter 12, pp. 193–214 Wiley New York.
- [14] Smyth, G. K. (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Statistical Applications in Genetics and Molecular Biology*, **3**, Article 3.
- [15] Dudoit, S., Gentleman, R. C., and Quackenbush, J. (2003) Open source software for the analysis of microarray data. *Biotechniques*, **Suppl**, 45–51.
- [16] Baxter, S. M., Day, S. W., Fetrow, J. S., and Reisinger, S. J. (2006) Scientific software development is not an oxymoron. *PLoS Computational Biology*, **2**, e87+.
- [17] Fogel, K. F. (2005) Producing open source software, O’Reilly, Sebastopol, CA.
- [18] Barton, G., Abbott, J., Chiba, N., Huang, D. W., Huang, Y., Krznaric, M., Smith, J. M., Saleem, A., Sherman, B., Tiwari, B., Tomlinson, C., Aitman, T., Darlington, J., Game, L., Sternberg, M., and Butcher, S. (2008) Emaas: An extensible grid-based rich internet application for microarray data analysis and management. *BMC Bioinformatics*, **9**(1).
- [19] Weniger, M., Engelmann, J. C., and Schultz, J. (June, 2007) Genome expression pathway analysis tool - analysis and visualization of microarray gene expression data under genomic, proteomic and metabolic context. *BMC Bioinformatics*, **8**, 179+.
- [20] Argraves, G., Jani, S., Barth, J., and Argraves, S. W. (2005) Arrayquest: a web resource for the analysis of dna microarray data. *BMC Bioinformatics*, **6**(1).
- [21] Rehrauer, H., Zoller, S., and Schlapbach, R. (July, 2007) Magma: analysis of two-channel microarrays made easy.. *Nucleic Acids Res*, **35**(Web Server issue).
- [22] Kapushesky, M., Kemmeren, P., Culhane, A. C., Durinck, S., Ihmels, J., Körner, C., Kull, M., Torrente, A., Sarkans, U., Vilo, J., and Brazma, A. (July, 2004) Expression profiler: next generation—an online platform for analysis of microarray data.. *Nucleic Acids Res*, **32**(Web Server issue).
- [23] Psarros, M., Heber, S., Sick, M., Thoppae, G., Harshman, K., and Sick, B. (July, 2005) Race: Remote analysis computation for gene expression data.. *Nucleic Acids Res*, **33**(Web Server issue).
- [24] Zhu, Y., Zhu, Y., and Xu, W. (2008) Ezarray: a web-based highly automated affymetrix expression array data management and analysis system. *BMC Bioinformatics*, **9**(1).
- [25] Rainer, J., Sanchez-Cabo, F., Stocker, G., Sturn, A., and Trajanoski, Z. (2006) Carmaweb: comprehensive r- and bioconductor-based web service for microarray data analysis. *Nucleic Acids Res*, **34**(Web Server issue), W498–503.
- [26] Knudsen, S., Workman, C., Sicheritz-Ponten, T., and Friis, C. (July, 2003) Genepublisher: automated analysis of dna microarray data. *Nucl. Acids Res.*, **31**(13), 3471–3476.
- [27] Xia, X., McClelland, M., and Wang, Y. (2005) Webarray: an online platform for microarray data analysis.. *BMC Bioinformatics*, **6**.
- [28] Luscombe, N. M., Royce, T. E., Bertone, P., Echols, N., Horak, C. E., Chang, J. T., Snyder, M., and Gerstein, M. (July, 2003) Expressyourself: a modular platform for processing and visualizing microarray data. *Nucl. Acids Res.*, **31**(13), 3477–3482.

- [29] Romualdi, C., Vitulo, N., Favero, M. D., and Lanfranchi, G. (2005) MIDAW: a web tool for statistical analysis of microarray data. *Nucleic Acids Research*, **33**, W644–649.
- [30] Patel, S. and Lyons-Weiler, J. (2004) cageda: a web application for the integrated analysis of global gene expression patterns in cancer.. *Appl Bioinformatics*, **3**(1), 49–62.
- [31] Tarraga, J., Medina, I., Carbonell, J., Huerta-Cepas, J., Minguéz, P., Alloza, E., Al-Shahrour, F., Vegas-Azcarate, S., Goetz, S., Escobar, P., Garcia-Garcia, F., Conesa, A., Montaner, D., and Dopazo, J. (May, 2008) Gepas, a web-based tool for microarray data analysis and interpretation. *Nucl. Acids Res.*, pp. gkn303+.
- [32] Montaner, D., Tarraga, J., Huerta-Cepas, J., Burguet, J., Vaquerizas, J. M., Conde, L., Minguéz, P., Vera, J., Mukherjee, S., Valls, J., Pujana, M. A., Alloza, E., Herrero, J., Al-Shahrour, F., and Dopazo, J. (July, 2006) Next station in microarray data analysis: Gepas.. *Nucleic Acids Res*, **34**(Web Server issue), W486–491.
- [33] Hokamp, K., Roche, F. M., Acab, M., Rousseau, M.-E., Kuo, B., Goode, D., Aeschliman, D., Bryan, J., Babiuk, L. A., Hancock, R. E., and Brinkman, F. S. (July, 2004) Arraypipe: a flexible processing pipeline for microarray data. *Nucl. Acids Res.*, **32**(suppl_2), W457–459.
- [34] Smyth, G. K. (2005) Limma: linear models for microarray data. In Gentleman, R., Carey, V., Dudoit, S., and R. Irizarry, W. H., (eds.), *Bioinformatics and Computational Biology Solutions using R and Bioconductor*, pp. 397–420 Springer New York.
- [35] Woodward, M. (2005) *Epidemiology. Study design and data analysis*, Chapman & Hall, CRC, London.
- [36] Alibés, A., Yankilevich, P., Cañada, A., and Diaz-Uriarte, R. (2007) Idconverter and idclight: conversion and annotation of gene and protein ids. *BMC Bioinformatics*, **8**, 9.
- [37] Alibes, A., Canada, A., and Diaz-Uriarte, R. (May, 2008) Pals: filtering common literature, biological terms and pathway information. *Nucl. Acids Res.*, pp. gkn251+.
- [38] R Development Core Team R: A language and environment for statistical computing R Foundation for Statistical Computing Vienna, Austria (2004) ISBN 3-900051-00-3.
- [39] Yu, H. Rmpi: Interface (wrapper) to mpi (message-passing interface). Technical report Department of Statistics, University of Western Ontario URL:<http://www.stats.uwo.ca/faculty/yu/Rmpi> (2004).
- [40] Currie, D. papply: Parallel apply function using MPI.
- [41] Therneau, T. survival: Survival analysis, including penalised likelihood. (Ported to R by Thomas Lumley) (2008).

Figure legends

Figure 1: Three input screens from Pomelo II. a) Initial input, showing available statistical methods. b) Additional covariates check page, with figures showing distributions at different levels of the class variable. c) Additional covariates check page, showing degrees of freedom available, and help.

Figure 2: Output. Output table from a permutation t-test (a) and a Cox model (b), and a heatmap with dendrogram, showing available options for heatmap redrawing (c).

Figure 3: Output from linear model. a) Class comparison page. b) Output table from one of the two-class comparisons. c) Details of Class comparison, showing Venn diagram and table of up- and down-regulated genes for each two-class comparison.

b)

POMELO II Covariables - Iceweasel

File Edit View History Bookmarks Tools Help

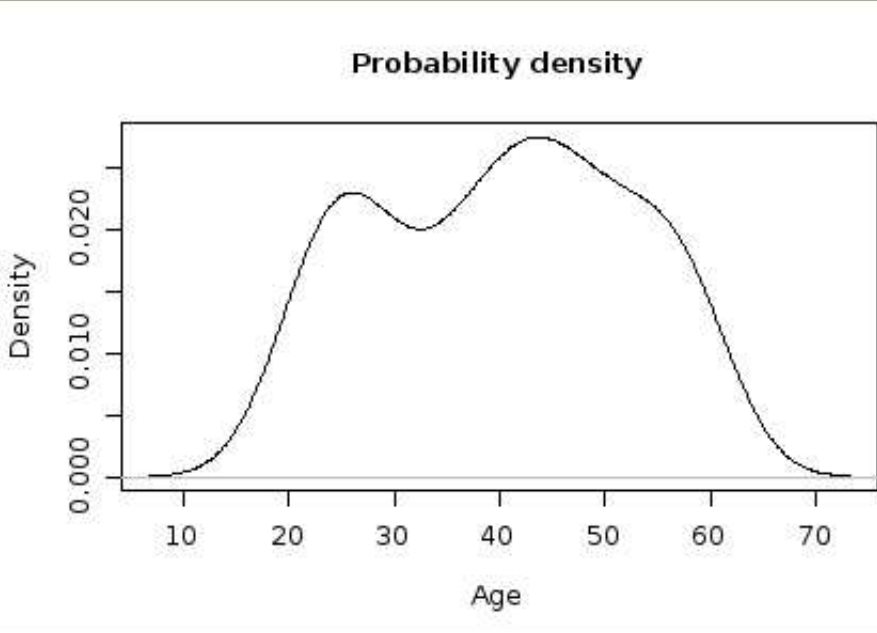
http://pomelo2.bioinfo.cnio.es/cgi-bin/check_covariables.cgi

Check and choose covariables

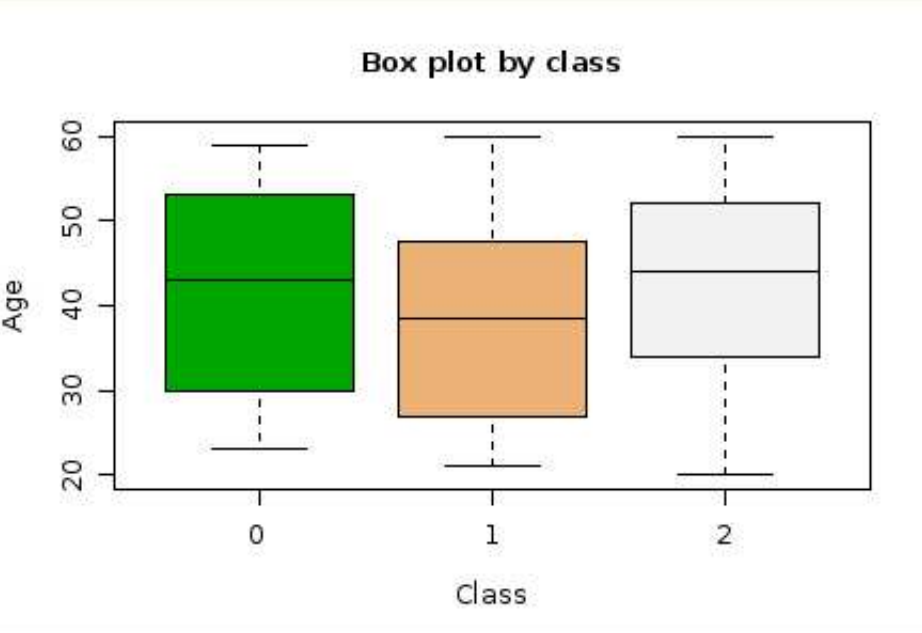
? Check covariables are read properly and choose which to use:

Age (NUMERIC COVARIABLE)

Minimum Value: 20.00
 Maximum Value: 60.00
 Mean Value: 40.25
 25% below(1st Quartile): 29.00
 50% below(median): 41.00
 75% below(3rd Quartile): 50.00



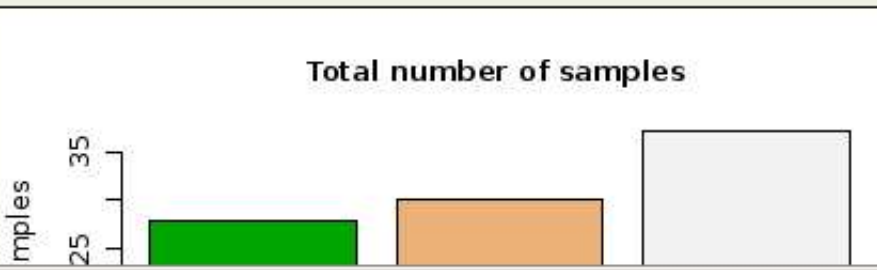
Probability density



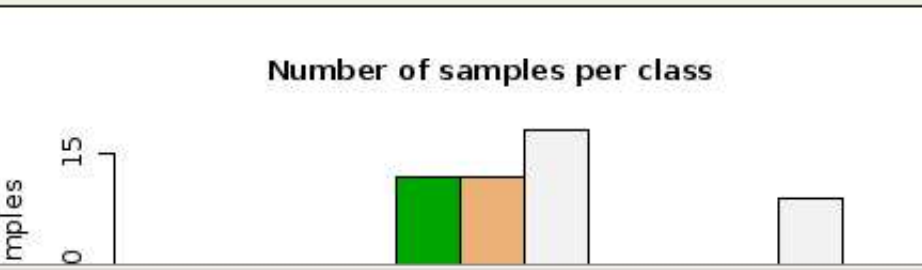
Box plot by class

Loc (NON-NUMERIC COVARIABLE)

A 28
 B 30
 C 37



Total number of samples



Number of samples per class

a)

POMELO II (THE RETURN OF THE POMELO)

UPLOAD DATA

Expression data file Browse...
 Class labels or dependent variable Browse...
 Censored indicator Browse...

TEST OPTIONS

Select test: **t-test (Permutations)** Number of permutations: 200000
 t-test (Permutations)
 Anova (Permutations)
 Regression (Permutations)
 Fisher Ixj
 Cox model
 t-test (limma)
 Paired t-test (limma)
 Anova, linear models (limma)

ID CONVERTER INFORMATION

Type

http://pomelo2.bioinfo.cnio.es - Pomelo II: df table - Iceweasel

Help on: df table

What are df?
 df stands for degrees of freedom. Depending on the number of subjects there are a certain number of degrees of freedom available for the analysis. Each covariable you choose will use up a certain amount of degrees of freedom (numeric variables use one and non-numeric use as many as the number of different values - 1; eg. gender would use 1, and a five class variable would use 4 dfs).

What is the df table for?
 The df table will display the covariables you have selected, the number of degrees of freedom used and the maximum allowed. You cannot select a number of covariables such that the used dfs will be larger than the dfs you have available. And you also do not want to run an analysis with too few dfs.

Close help window Open full help

? **df table:**

Covariable name	Degrees of freedom used
Age	1
Loc	2
Total used	3
Maximum allowed	92

Send selected covariables

c)

a)

http://pomelo2.bioinfo.cnio.es - Pomelo II: modify heatmap - ICGEbase

2 Select values to redraw heatmap

all with unadj.p < 0.05
 all with FDR < 0.15
 all with abs(obs_stat) > 0.0
 all with obs_stat > 1.0000000
 all with obs_stat < -1.0000000
 Max number of genes = 50

Colour type: Green/Red
 Red/Green
 Blue/Yellow

Picture size: Draw new heatmap

Color Key
 Value
 -1 1 3

G321 (<0.001)
 G381 (<0.001)
 G198 (<0.001)
 G1754 (<0.001)
 G1009 (<0.001)
 G2670 (<0.001)
 G413 (<0.001)
 G378 (<0.001)
 G1778 (<0.001)
 G124 (<0.001)
 G808 (<0.001)
 G1911 (<0.001)
 G2500 (<0.001)
 G2683 (<0.001)
 G2714 (<0.001)
 G7749 (<0.001)
 G337 (<0.001)
 G448 (<0.001)
 G565 (<0.001)
 G1987 (<0.001)
 G701 (<0.001)
 G801 (<0.001)
 G1920 (<0.001)

b)

POMELO II Results - ICGEbase

http://pomelo2.bioinfo.cnio.es/Examples/LeukemiaGolub/results.html

POMELO

Table

Output table:
 Chosen test type: t-test
 Permutations used: 199980
 Covariables used: Selected test does not allow additional covariables
 Class labels: [Click here](#)

	Gene Name	Row number	unadj.p	FDR_indep	Obs_stat	abs(Obs_stat)
1	G2124	2124	5e-06	0.0003246	-10.577748	10.577748
2	G829	829	5e-06	0.0003246	-9.775847	9.775847
3	G896	896	5e-06	0.0003246	-8.032939	8.032939
4	G766					
5	G2600					
6	G2939					
7	G1995					
8	G2386					
9	G717					
10	G2489					
11	G394					
12	G1037					
13	G703					
14	G2851					
15	G1834					
16	G1448					
17	G2198					

Table

Output table:
 Chosen test type: Cox model
 Covariables used: Selected test does not allow additional covariables
 Survival time: [Click here](#)

	Gene Name	Row number	unadj.p	FDR_indep	Obs_stat	abs(Obs_stat)
1	Mm.347441	1647	7.4e-06	0.0297334	0.019989	0.019989
2	Mm.29020	292	8.3e-06	0.0297334	0.001584	0.001584
3	Mm.87277	5324	1.97e-05	0.0361792	0.017308	0.017308
4	Mm.3914	4155	2.31e-05	0.0361792	0.002989	0.002989
5	Mm.2036	1322	3.89e-05	0.0361792	0.019754	0.019754
6	Mm.286285	5124	3.8e-05	0.0361792	0.012416	0.012416
7	Mm.32883	4186	4.14e-05	0.0361792	0.002185	0.002185
8	Mm.267410	1625	4.38e-05	0.0361792	0.002947	0.002947
9	Mm.265060	2190	4.63e-05	0.0361792	0.00916	0.00916
10	Mm.177539	3811	5.46e-05	0.0361792	0.001188	0.001188

Find:

a)

Compare classes

Choose classes you wish to compare:

Class **0** vs Class **1**

Calculate comparison | Clear all comparisons

Results Tables:

0-1
0-2
1-2

**POMELO II
(THE RETURN OF THE
POMELO)**

Table

Chosen test type: Class contrasts (Annova Limma)
Chosen contrast: Class0-Class1
Covariables used: Age, Loc

Gene Name	Row number	unadj-p	FDR_indep	Obs_stat	abs(Obs_stat)
1	G804	804	< 0.0000001	-8.112948	8.112948
2	G1413	1413	< 0.0000001	-7.96567	7.96567
3	G2987	2987	< 0.0000001	8.454752	8.454752
4	G3661	3661	< 0.0000001	-8.266648	8.266648
5	G3006	3006	< 0.0000001	7.58206	7.58206
6	G3144	3144	< 0.0000001	7.748828	7.748828
7	G3678	3678	< 0.0000001	-7.727708	7.727708
8	G4511	4511	< 0.0000001	-7.759027	7.759027
9	G44	44	< 0.0000001	-7.417047	7.417047
10	G14	14	< 0.0000001	7.319826	7.319826
11	G1841	1841	< 0.0000001	-7.182047	7.182047
12	G1597	1597	< 0.0000001	7.101902	7.101902
13	G3316	3316	< 0.0000001	7.134453	7.134453

b)

Differential expression

Differential expression criteria:
Genes are considered differentially expressed, for the diagram and table below, when: **FDR**

FDR < Change criteria

Venn diagram:

Choose contrast 1: None 0-1 0-2 1-2
 Choose contrast 2: None 0-1 0-2 1-2
 Choose contrast 3: None 0-1 0-2 1-2

Up-regulated

Down-regulated

Both

Differential expression table:

Gene name	0 vs 1	0 vs 2	1 vs 2
G4	0 > 1	-	-
G5	0 > 1	0 > 2	-
G7	0 > 1	0 > 2	-
G8	0 > 1	0 > 2	-
G10	0 < 1	0 < 2	-
G12	0 < 1	-	-
G13	0 < 1	0 < 2	-