

FAQs

1: General

1.1 What are the minimum system requirements for BRB-ArrayTools?

BRB-ArrayTools runs under Microsoft Windows. Any version of Windows 7, VISTA, XP, NT or 2000. It also requires Excel 2000 or later. It is recommended that the user have at least 512 MB of RAM; more RAM is better for large numbers of arrays.

1.2 Does BRB-ArrayTools work on a Mac machine?

BRB-ArrayTools has been tested and works well on an Apple Mac book pro machine with Windows XP professional/VISTA installed with Apple's boot camp software.

1.3 Does BRB-ArrayTools work on a 64-bit Windows machine?

Yes.

1.4 Does BRB-ArrayTools work on Linux platform?

No. BRB-ArrayTools works only on windows OS.

1.5 How many arrays can BRB-ArrayTools handle?

We have tested the software on very large data sets (2000 arrays with 54,000 probe sets). Although certain analysis tools will cause an out-of-memory error, using a stringent gene filtering criteria will avoid the problem by reducing the total number of genes in the analysis. We are working on modifications that will utilize 64-bit version of R for large datasets.

1.6 Do I need to be a statistician to use BRB-ArrayTools effectively?

No. The user-interface is menu driven within Excel and most of the complex algorithms are run transparently to the user. Wizards and help screens are available to guide users with importing their data and the selecting and using the appropriate tools for data analysis.

1.7 Does BRB-ArrayTools provide annotations for genes found of importance in analyses?

Extensive biological annotations are provided. The user has the option to annotate their data using Bioconductor packages (for Affymetrix or Illumina data) or download annotations from SOURCE or import their own gene identifiers. Currently SOURCE

annotation can be run on nine species. The output results will show the gene annotations for significant genes found in the analysis.

2:Installation

2.1 Having problems installing BRB-ArrayTools in Windows 7/VISTA and/ or Office 2007/2010.

Please, look at the instructions available on BRB- software download page http://linus.nci.nih.gov/~brb/download_individual_new_v4_3_0.html

2.2 I have installed the program but it requires me to enter a registration email address and password. What should i do to obtain the password or get the registration?

Please, visit the guest book link available on <http://linus.nci.nih.gov/~brb/guestbook.html> to register your email address. A password will be sent via email to the registered address.

2.3 Can I have multiple versions of R installed on my machine?

Yes, as long as the current version installed is the version that BRB-ArrayTools recommends. For current version requirements please refer to the BRB software download page.

2.4 Does BRB ArrayTools work if I have non-English language settings?

You must set the default regional language on your machine to "English". For XP users,

1. Go to the Control Panel -> Regional and Language Options and choose English.
2. From the Start → Programs → Microsoft office → Microsoft office tools -> ->"Microsoft Office language settings" make sure that the "primary editing language" is "English".

You will need to re-boot your machine after the above steps and then proceed to re-install BRB-ArrayTools FULL installer.

Users with the Office 2007/2010, need to have the multi-language pack. Then follow the steps above. If you have purchased a single language pack for Office 2007/2010 then our current release may cause some problems when running some of the analysis tools.

2.5 After registrating, I get "error75:Path/File access error. Need to set full control of the ArrayTools installation folder".

This typically happens to VISTA/ Windows 7 users. If you have not modified the permissions to the “ArrayTools” installation folder, you could see this error. To view instructions on how to change the permissions to this folder please, review the presentation for Vista/ Windows 7 users available on <http://linus.nci.nih.gov/PowerPointSlides/HowtoTakeFullControloftheArrayTools.ppt>

2.6 How can I temporarily remove the ArrayTools add-in from Excel?

From the Excel add-ins options you can de-select BRB-ArrayTools. This will un-load the add-in from Excel but will not un-install the software. You can re-select BRB-ArrayTools from the Excel add-ins when you want to use ArrayTools again.

Using BRB-ArrayTools

3: Data Importing

3.1 Can I import ST arrays using BRB-Arraytools?

BRB-ArrayTools can import Affymetrix Gene 1.0ST arrays, but not the Exon ST arrays. From the “ArrayTools” pull down menu-> select “Import data” and choose the option “Affymetrix Gene 1.0 ST array importer”. For Exon ST arrays, you can use Affymetrix Expression console to obtain the probe-set summary data in .txt format, and then import the .txt file into BRB-ArrayTools through the general format importer with all the spot-filtering and normalization options turned off.

3.2 I have a custom array –how can I import my annotations?

If you have a custom array, as part of the importing step, you can specify that you have your own gene identifier file and would like to use this file to annotate the data. The software will read in this file to create the gene identifier and the corresponding gene annotation worksheets in the project workbook.

3.3 I got a Run time error 75 when importing the data. Please, help.

This sometimes happens to VISTA/Windows 7 users. If you have not modified the permissions to the “ArrayTools” installation folder, you could see this error. To view instructions on how to change the permissions to this folder please, review the

presentation available on
http://linus.nci.nih.gov/~brb/download_individual_v4_2_1_stable.html

4: Analysis

4.1 How can I learn some of the features in the new scatterplot tool?

Please view the video available on
<http://linus.nci.nih.gov/PowerPointSlides/Scatterplot.wmv>

4.2 How do I exclude arrays from an analysis?

Suppose, for example, that you want to find genes differentially expressed between cell lines of lung cancer and cell lines of breast cancer. Set up a column in your experiment descriptor worksheet that has a code (e.g. LC) for the lung cancer cell lines, a different code (e.g. BC) for the breast cancer cell lines, and is blank in the rows for the arrays corresponding to other types of cell lines. The arrays with the blanks in that column will be omitted from the analysis. You will find that you create many new columns of your experiment descriptor worksheet as you proceed with your analysis as you think of new comparisons you'd like to do. You can easily add columns interactively within BRB-ArrayTools. When you re-save your project, the changes are saved.

4.3 Do I need to distinguish between technical replicates (i.e. multiple assays of the same RNA) and biological replicates? How do I do it?

Yes, it is important to distinguish between technical and biological replicates. In the experiment descriptor worksheet, you can add an indicator column that contains the same values for arrays that are replicates. Below is an example where each array has two replicates.

| ArrayID | TechRep |
|------------|---------|
| Array123A1 | 1 |
| Array123A2 | 1 |
| Array123A3 | 2 |
| Array123A4 | 2 |
| Array123A5 | 3 |
| Array123A6 | 3 |

When running various analysis tools, the software provides an option to average these technical replicates. You will need to specify the column that contains the replicate information. In this example the column will be "TechRep"

4.4 I have developed a class predictor. How do I apply it to new samples?

The class prediction tools provide enough details about the predictor for you to classify new samples outside of BRB-ArrayTools if you wish. If you want BRB-ArrayTools to

classify the new samples, and if the new samples were not part of the initial dataset then you need to re-import your data with the test samples. You can then create a column in the experiment descriptor worksheet that includes the key words “training”, “predict” or “exclude” to denote which arrays belong to the training set and which arrays are the new test set.

4.5 What do I need to do in order to run survival analysis?

You would need the survival time and survival status columns in your experiment descriptor worksheet. The status column should contain “1”s and “0”s. If the total time is the time to live, then the status should be 1. Status=0 implies that the survival time is truncated. The survival time is defined by the difference of the time of death and sampling. The program expects these columns to be numeric.

4.6 How are multiple probes/probe sets that correspond to one gene handled for Affmetrix data?

Included, in the Filtering tool is an option to reduce multiple probes(sets) to a single gene. From the “ArrayTools” pull down menu, you can select the “Re-filter, normalize and subset” option. Under the “Gene subset” tab, select the option “Reducing multiple probes/probes sets to one gene”. For single channel data, there are two options either you can choose the most variable probe set or the maximally expressed probe set.

4.7 What are plug-ins?

Plug-ins are analysis tools that are written in R and added to BRB-ArrayTools easily. Users can write their own plug-ins for doing additional analyses on the data in their BRB-ArrayTools projects. The BRB-ArrayTools development team uses plug-ins as a fast way of adding new tools that we want to try out. BRB-ArrayTools, has pre-loaded many plug-ins and users are encouraged to write their own plug-ins as well.

4.8 Is there any guidance available, for me to write my own plug-in?

Yes, in the user’s manual there is a section of “creating your own plug-ins”. Should you need any additional guidance feel free to post a message to the message board or send an email to arraytools@emmes.com

4.9 I get an error message “too many plug-in files open”. What can I do?

You must change the permissions to the “ArrayTools” installation folder. Specifically for Vista/Windows 7 users the folder should have “full control” when you running plug-ins. You can check if you have a full control over the “ArrayTools” installation folder by right clicking the ArrayTools folder in Windows Explorer and then choose Properties. If you are using Windows 7 or Vista, please refer to the power point slides on the

ArrayTools download page http://linus.nci.nih.gov/~brb/download_individual_new.html on how to enable the full control.

4.10 I would like to export the data to use outside of BRB-ArrayTools. How can I do that?

From the “Plug-ins” menu option, you can choose to use the plug-in to export 1-color or 2-color data. You can then directly launch R to further analyze your data or txt files are also created of the log ratio/log intensity data in the specified output. For additional details refer to the ReadMe file.

4.11 What is the difference between the beta version and stable version of BRB-Arraytools?

At most times, BRB-ArrayTools provides users with two supported versions. The stable release is a version that has gone through a series of beta releases. It may not have some of the new tools and enhancements that are included in the next beta release. The beta version has been tested internally but is currently in its “beta” testing cycle. All BRB-ArrayTools users are welcome to be beta testers.

4.12 How do I cite BRB-Arraytools?

The following should suffice: “Analyses were performed using BRB-ArrayTools developed by Dr. Richard Simon and the BRB-ArrayTools Development Team.”

5: Miscellaneous

5.1 When I try to enter my email address to register my license, I get a message something like “No Internet connection or the Internet is too slow. Or a firewall blocked <http://linus.nci.nih.gov>. Try again?”

The cause can be either the computer is connected to the internet through proxy or a firewall of the user’s institution has blocked the connection. BRB-ArrayTools does not support internet connection via proxy.

5.2 When I use BRB-ArrayTools, I see a blue R icon on the taskbar. Can I close it?

From ArrayTools v4.3, the Rserve package is used for communication between Excel and R. The R icon shows Rserve is waiting for accepting commands from Excel. So the icon should not be closed by users. The R icon will be automatically closed if Excel has been closed.

5.3 Does Rserve require any special installation?

Rserve can be installed like a regular R package. BRB-ArrayTools installer automatically installs it and copies Rserve.exe from the package directory (libs\i386 and x64) to the R binary directory (i386 and x64) so it can be launched from a command line (see also 5.1).

5.4 What is the additional system requirement for 3D scatter-plot tools?

In order to run 3D scatter-plot tools, the computer graphic card must support OpenGL. If the computer uses an Intel Graphic Processor, or Nvidia/ATI, you should be fine. Check [here](http://en.wikipedia.org/wiki/Intel_GMA#Specifications) for more details (http://en.wikipedia.org/wiki/Intel_GMA#Specifications).