

Extending MicroArray Explorer with R Language Scripts

Peter F. Lemkin⁽¹⁾, Gregory Thornwall⁽²⁾, W. Gregory Alvord⁽³⁾, Mariusz Lubomirski⁽³⁾, Shyam Sundaram⁽⁴⁾

¹LECB/NCI-Frederick, lemkin@ncifcrf.gov;

²SAIC-Frederick; ³CSS/DMS-Frederick; ⁴SRA/BIMAS/CIT-NIH

<http://maexplorer.sourceforge.net/>

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Abstract

The Microarray Explorer (MAExplorer) is a versatile open-source Java-based data mining bioinformatic tool for analyzing quantitative DNA expression profiles across multiple microarray platforms and DNA labeling systems
<http://maexplorer.sourceforge.net/>

MAExplorer analyses may now be extended by writing R scripts in the powerful R data, statistics, and graphics language (www.r-project.org).

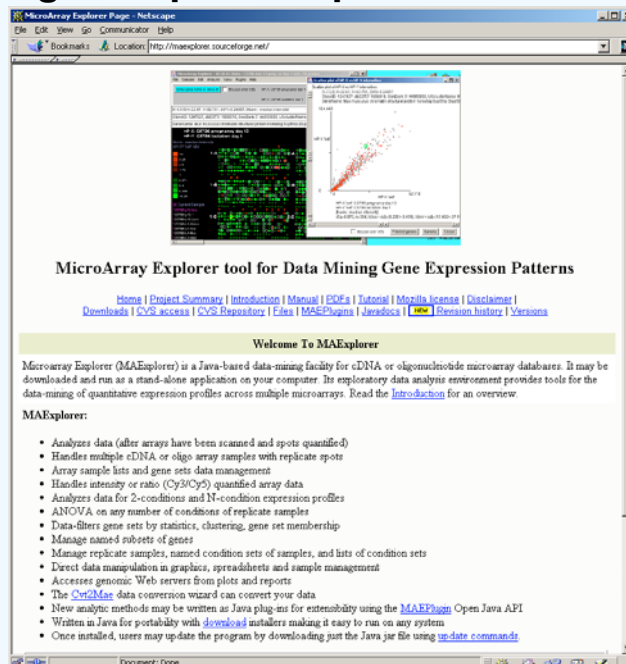
The RtestPlugin is a Java plugin for use with MAExplorer to extend its statistical data analysis capabilities using the R program. Scripts in the R language may invoke statistical and other methods in R.

Abstract (continued)

Users may create and edit R scripts and associated R LayOut (RLO) descriptions of the interface between MAExplorer and R. The RLO describes data exported from MAExplorer into R (R input files), output files from R after the R script has been evaluated, and actions to import R results back into MAExplorer.

Once a RLO and its associated R script has been created and tested, it may be saved as a special purpose “permanent” plugin so it automatically appears as a plugin extension to MAExplorer.

Home Page: <http://maexplorer.sourceforge.net/>



The screenshot shows a Netscape browser window displaying the MAExplorer website. The browser's address bar shows the URL <http://maexplorer.sourceforge.net/>. The website content includes a navigation menu with links for Home, Project Summary, Introduction, Manual, FAQs, Tutorial, Mozilla license, Disclaimer, Downloads, CVS access, CVS Repository, Files, MAExplorer, JavaDocs, Revision history, and Versions. A green banner reads "Welcome To MAExplorer". Below this, a paragraph describes MAExplorer as a Java-based data-mining facility for cDNA or oligonucleotide microarray databases. A section titled "MAExplorer:" lists various features and capabilities, such as analyzing data, handling multiple samples, and managing gene sets.

MicroArray Explorer tool for Data Mining Gene Expression Patterns

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Welcome To MAExplorer

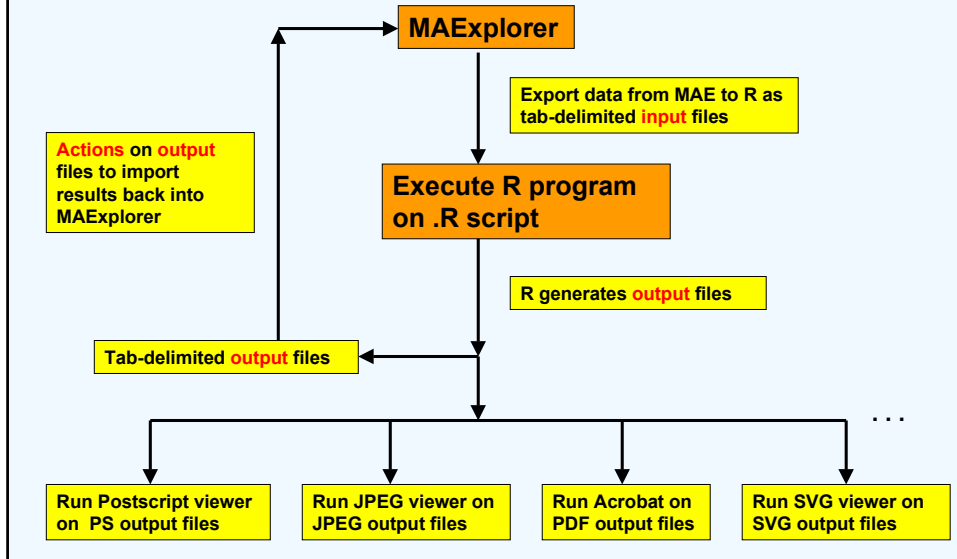
Microarray Explorer (MAExplorer) is a Java-based data-mining facility for cDNA or oligonucleotide microarray databases. It may be downloaded and run as a stand-alone application on your computer. Its exploratory data analysis environment provides tools for the data-mining of quantitative expression profiles across multiple microarrays. Read the [Introduction](#) for an overview.

MAExplorer:

- Analyzes data (after arrays have been scanned and spots quantified)
- Handles multiple cDNA or oligo array samples with replicate spots
- Array sample lists and gene sets data management
- Handles intensity or ratio (Cy3/Cy5) quantified array data
- Analyzes data for 2-condition and N-condition expression profiles
- ANOVA on any number of conditions of replicate samples
- Data-filters gene sets by statistics, clustering, gene set membership
- Manage named sub-sets of genes
- Manage replicate samples, named condition sets of samples, and lists of condition sets
- Direct data manipulation in graphics, spreadsheets and sample management
- Accesses genomic Web servers from plots and reports
- The [CytosMin](#) data conversion wizard can convert your data
- New analytic methods may be written as Java plug-ins for extensibility using the [MAExplorer](#) Open Java API
- Written in Java for portability with [download](#) installers making it easy to run on any system
- Once installed, users may up-date the program by downloading just the Java jar file using [un-date commands](#).

Evaluating R Scripts on MAExplorer Data

The “R Layout” (RLO) specifies the R script, menu name, input/output files, actions, etc.



Functionality of RtestPlugin

- Create/edit R scripts and associated R LayOut (RLO) descriptions of the interface between MAExplorer and R
- Evaluate R scripts using data exported from MAExplorer and generate R output files (text, PDF, image, etc.)
- Display PDF or image files generated by R after the script has been evaluated
- Import data from R output back into MAExplorer after the script has been evaluated
- Create user-defined RLOs using data templates to generate R code
- Use to learn the R interface to MAExplorer with editable demonstration examples for various data MAExplorer data types

R Methods: User Defined Analyses

- Use Templates to generate data-dependent R code
- Allows totally general analyses
- May import R script code files to serve as a basis for a new RLO which may then be edited
- Invoke an editing wizard when create a "New RLO" or use "Edit RLO" on a previously saved RLO
- Permanently saved with "Save RLO" command
- When "saved", the RLO menu name will automatically be added to MAExplorer menu

R Methods: Demonstrations

- Data-specific demos allow selecting 'canned' R analysis methods specific for various MAExplorer data
- Simple methods are intended for demonstration purposes to illustrate how to access MAExplorer from R scripts
- User may modify the generated code by specifying which R analyses (e.g., histogram, plots, t-test, summaries, etc.) should be added to the R script
- Editing is invoked using the "Edit RLO" command.
- RLO's may be saved for invoking from MAExplorer (Plugins | RLO Methods) menu

R LayOut (RLO) Schema

- **R script name** (e.g., "SomeAnalysis.R")
- Analysis method **menu name** (e.g., "Perform some analysis on the data")
- **Menu stub name** to place this RLO method in the MAExplorer menu tree
- Optional list of exported MAExplorer data in **R input files** (file names, data-types)
- Optional list of **R output files** written by R during script execution (file names, data-types)
- Optional list of **actions** to be taken on R output files used to import the R results back into MAExplorer (specified by: output-file-name, MAE-object-name, MAE-object-action)
- **Processing options** for MAExplorer data to be exported to R (e.g., raw vs. normalized data, filtered vs. unfiltered, etc., horizontal vs. vertical data stacking, etc.)
- Optional **R command line options** (e.g., increase memory, etc).
- (Demo mode only) **generate demonstration R script methods** to let you configure different types of R analyses in your script.

R Data Types Exported from MAExplorer as Input data to R

- Single HP-sample expression data
- HP-sample (Cy3,Cy5) or (F1,F2) channels expression data
- HP-X and HP-Y samples expression data
- HP-X 'set' and HP-Y 'set' samples expression data

- HP-X 'set' samples expression data
- HP-Y 'set' samples expression data
- HP-E 'list' expression profile samples expression data
- Current Condition list of samples expression data
- Multiple Conditions List (OCL) of samples expression data

- Gene annotation data (e.g., gene ids, gene names)
- MAExplorer state thresholds (e.g, p-value & other sliders)

R Output Data Types

These are files that may be specified to be created by R

- Stdout is the normal text output of R
- Tab-delimited table (to import data to MAExplorer from R)
- XML table (to importing data to programs from R)
- Report (free form)
- PDF file
- JPEG image file
- PNG image file
- Postscript file
- SVG file

MAExplorer Actions on R Output Data Types

Create named data structures as part of the MAExplorer state by importing R output data back into MAExplorer

- Gene set
- Gene set with p-Value
- Gene set with cluster data (#, cluster distance)
- Filter gene set
- Normalization gene set
- Condition List of (replicate or similar) samples
- Multiple Conditions List (OCL) of condition lists
- Named normalization map [Future]
- 2D plot overlay map [Future]
- Normalization polynomial [Future]

Resources Required

- Must install MAExplorer and the R program
- Both are open source and free
<http://maexplorer.sourceforge.net/>
<http://www.r-project.org/>
- MAExplorer and R run on Windows, Linux, Solaris, MacOS-X
- To view PDF generated plots, you could use Adobe Acrobat
- No other software is required

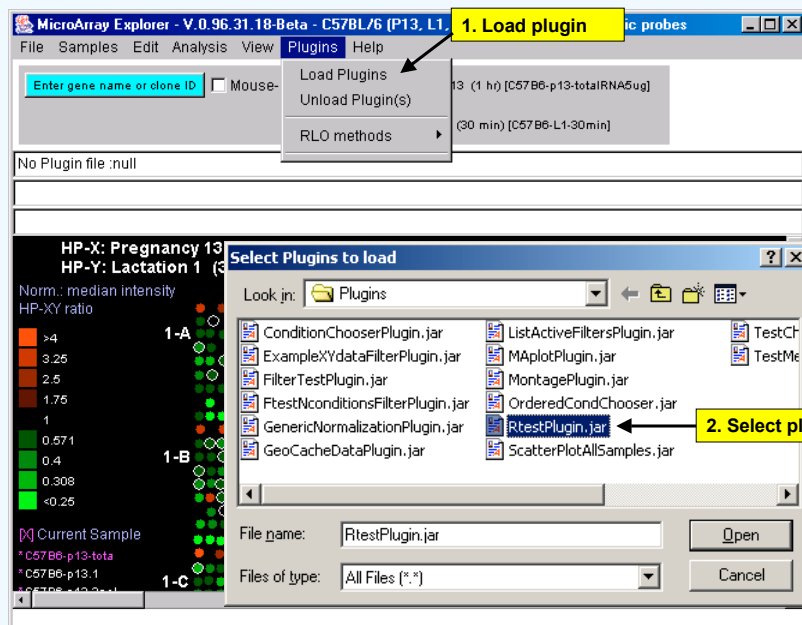
Disk directories used

- *<Current MAExplorer project directory>*
 - /Temp** - holds all files that communicate with the R program from MAExplorer
 - /Report** - holds all files that are generated by your R script as specified by the RLO for that script
- *<MAExplorer installation directory>*
 - /RLO** - holds all permanent **.rlo** RLO specification files in your RLO database. Read by MAExplorer when it starts if the **.rlo** files exist
 - /R** - holds all permanent **.R** script files for the RLO database
 - /MAERlibr** - is R style project library to support MAExplorer-R interface

Running RtestPlugin

- First load the RtestPlugin
 1. In MAExplorer, select the (Plugins menu | Load plugin) command.
 2. Specify "RtestPlugin.jar". This installs the plugin in the MAExplorer Plugins menu with the new menu entry "R test".
 3. To run RtestPlugin, select (Plugins menu | R test)
- Select demo or user-created RLO to edit or evaluate
- If you want, edit or create new RLOs and save them using the "Save RLO" button in the permanent RLO database
- Press "Evaluate" to invoke R on the selected RLO script
- Or, after saving RLOs, they may be run created directly from MAExplorer (Plugins | RLO methods) submenu

Loading RtestPlugin in MAExplorer



Running RtestPlugin

The screenshot shows the MicroArray Explorer interface. The 'Plugins' menu is open, showing 'RLO methods' and 'R test'. A yellow box labeled '3. Run plugin' points to the 'R test' option. Another yellow box labeled 'Current list of RLO methods' points to the 'RLO methods' sub-menu. Below the menu, a heatmap visualization is displayed with the following text:

HP-X: Pregnancy 13 (1 hr) [C57B6-p13-totalRNA5ug]
 HP-Y: Pregnancy 13 (15 min) [Stat5a.--.p13-15min]

Norm.: median intensity
 HP-XY ratio

Legend for HP-XY ratio:

- >4
- 3.25
- 2.5
- 1.75
- 1
- 0.571
- 0.4
- 0.308
- <0.25

Current Sample: *C57B6-p13-tota

Select a Data Analysis and Its RLO for Analyzing Current MAExplorer Data*

The screenshot shows the Rtest application window. The 'Database: MGAP DB' is selected. A list of demo data analyses and RLOs is shown, with a red oval highlighting the list. A yellow box labeled 'Select MAExplorer data to export' points to the list. A yellow box at the bottom contains the text:

* If you add a "New RLO" analysis method, it will appear at the end of this pull-down menu

Buttons at the bottom include: Evaluate, Show generated plot(s), Clear report, Show R script, SaveAs, Close, DEBUG, New RLO, Edit RLO, Show RLO, Delete RLO, Help Msg.

Help Message - RtestPlugin

MicroArray Explorer - V.0.96.31.18-Beta - C57BL/6 (P13, L1, L10), stat5a (-), 15 public probes

Database: MGAP DB

Demo 0 - random (no MAE) data Show Demo RLOs Save ALL Demo RLOs

Instructions:

A. To generate a report from an R analysis (see www.r-project.org):

1. select an R LayOut (RLO),
2. press 'Evaluate' button to perform the analysis if the analysis generated plot(s),
3. press 'Show generated plot(s)' button to popup windows with the plots,
4. To view the current RLO R script, press 'Show R script' button.
5. press 'Help' button to re-print this message,

B. To Edit an existing RLO

1. select the RLO to edit,
2. press 'Edit RLO' to popup the RLO editor,
3. enable/disable generation checkboxes to modify the generated R script that is update as you make the changes.
4. press 'Close' to exit the RLO editor.

C. To Create a new RLO database entry

1. press 'New RLO' to popup the RLO editor,
2. either type in an R script in the edit window, or
 - 2.1 press 'Import R script' button read a R script file into the window.
 - 2.2 go through the other wizards and fill out the relevant responses.

Press 'Next' to advance to the next wizard and 'Prev' to backup to the previous one.

Evaluate Show generated plot(s) Clear report Show R script SaveAs Close

DEBUG New RLO Edit RLO Show RLO Delete RLO Help Msg

An R Script Contains Three Parts

1. **Prologue R code** reads the exported MAExplorer data as R input tab-delimited data files using R `read.table()` or `scan()` methods to fixed tables.
2. **Computation R code** performs the analysis of the data read in prologue.
3. **Epilogue R code** to write data to output files using plot files, or text tab-delimited table files using R `write.table()` methods.

Simple Example of Three Part R Script

```
####--- Start of Prologue ---####
# RLO Number 13, Rscript [ DemoMaeRscript13.R ]
print("Method:Demo 13 - MAExplorer State-Scroller thresholds")
maeStateThr <- read.table("R-inputStateThresholds.txt",
                          header=TRUE, sep="\t")
####--- End of Prologue - Don't edit above this line ---####

print("MAExplorer state-scroller threshold values")
maeStateThr

####--- Start of Epilogue - Don't edit below this line ---####
####--- End of Epilogue ---####
```

Note: there is no epilogue R code in this example

Viewing R Script of Currently Selected RLO

The screenshot displays the Rtest software interface. At the top, the title bar reads "Rtest - [alpha] \$Revision: 1.74 \$ [Date: 2003/06/12 21:02:56 \$]". Below the title bar, the "Database: MGAP DB" is shown. A dropdown menu is set to "Demo 6 - HP-X and HP-Y 'sets' samples expression", with a yellow callout box labeled "Select Current demo" pointing to it. To the right of the dropdown are checkboxes for "Show Demo RLOs" (checked) and a button "Save ALL Demo RLOs". The main area contains the R script for "Demo 6", which includes a prologue, main processing code, and an epilogue. A yellow callout box labeled "Current R script" points to the main code block. At the bottom, there is a toolbar with buttons: "Evaluate", "Show generated plot(s)", "Clear report", "Show R script", "SaveAs", "Close", "DBG" (checked), "New RLO", "Edit RLO", "Show RLO" (circled in red), "Delete RLO", and "Help Msg".

Viewing the Currently Selected RLO

Database: MGAP DB

Demo 6 - HP-X and HP-Y 'sets' samples expression Show Demo RLOs Save ALL Demo RLOs

R LayOut #6
 Menu name: 'Demo 6 - HP-X and HP-Y 'sets' samples expression'
 Submenu to place Menu entry in for MAExplorer: 'Plugins:RLOmethods'
 ,R script name: 'DemoMaeRscript06.R'
 RLO creation date: 'Thu May 08 16:00:35 EDT 2003'
 # of input data files: 1
 Input data file[1]: 'R-inputHP-XYsets.txt'
 Input data type[1]: 'HP-X and HP-Y 'sets' of samples'
 Process MAExplorer data when export it to R (R input files)
 Using data filtered genes
 Using normalized spot expression data
 Don't display plots generated by R after R script execution
 Use mean(F1,F2) or (Cy3/Cy) ratio
 Don't add separate expression background data channels
 Use "--vanilla" option for R to start up R faster
 Generate demonstration R script
 R 'summary()'
 R 'plot()'
 # of output data files: 2
 Output data file[1]: 'R-Stdout.txt'
 Output data type[1]: 'Stdout'
 Output data file[2]: 'R-OutputFile6.pdf'
 Output data type[2]: 'Pdf'
 # of R2Mae action:
 scriptSpecificOptions: ''

Buttons: Evaluate, Show generated plot(s), Clear report, Show R script, SaveAs, Close, DEBUG, **Edit RLO**, Show RLO, Delete RLO, Help Msg

Callouts:
 - Yellow box: Summary of current R LayOut (RLO) (points to R script content)
 - Blue box: See next page for editing RLO (points to Edit RLO button)

1. Edit Wizard for Current RLO: R Script File Name [.R file]

Edit existing R script LayOut (RLO)

RLO [6] R script [DemoMaeRscript06.R]

Menu Name: 'Demo 6 - HP-X and HP-Y 'sets' samples expression'

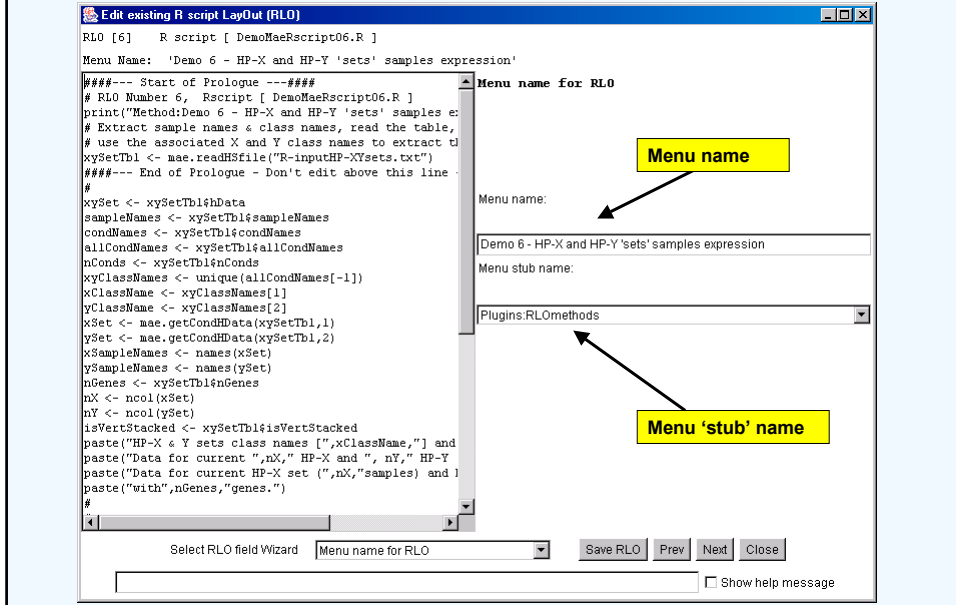
Start of Prologue #####
 # RLO Number 6, Rscript [DemoMaeRscript06.R]
 print("Method:Demo 6 - HP-X and HP-Y 'sets' samples ex
 # Extract sample names & class names, read the table,
 # use the associated X and Y class names to extract t
 xySetTbl <- mae.readRFile("R-inputHP-XYsets.txt")
 ##### End of Prologue - Don't edit above this line

R script file name [.R file]
 R script file: DemoMaeRscript06.R

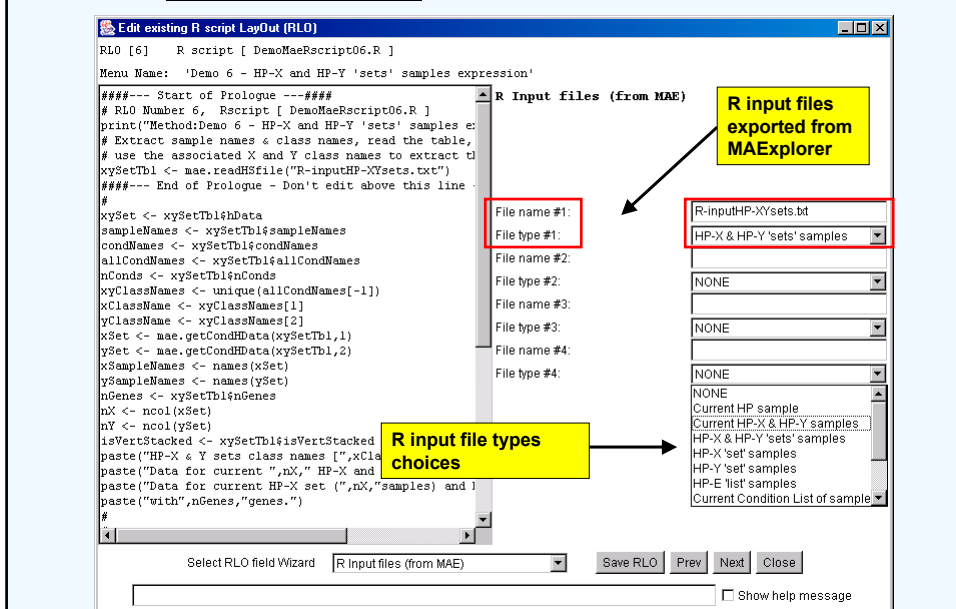
Buttons: Save RLO, Prev, Next, Close

Callouts:
 - Yellow box: R script for the current RLO (points to R script code)
 - Yellow box: R script name (points to R script file name field)
 - Yellow box: User selects a wizard to edit one of the RLO fields or use 'Prev' or 'Next' buttons (points to wizard options)

2. Edit Wizard for Current RLO: Menu name - for RtestPlugin or MAExplorer



3. Edit Wizard for Current RLO: R Input Files - from MAExplorer



4. Edit Wizard for Current RLO: R Output Files

RLO [6] R script [DemoMaeRscript06.R]

Menu Name: 'Demo 6 - HP-X and HP-Y 'sets' samples expression'

```
##### Start of Prologue #####
# RLO Number 6, Rscript [ DemoMaeRscript06.R ]
print("Method:Demo 6 - HP-X and HP-Y 'sets' samples e:
# Extract sample names & class names, read the table,
# use the associated X and Y class names to extract t
xySetTbl1 <- mae.readHSfile("R-inputHP-XYsets.txt")
##### End of Prologue - Don't edit above this line #####
#
xySet <- xySetTbl1$data
sampleNames <- xySetTbl1$sampleNames
condNames <- xySetTbl1$condNames
allCondNames <- xySetTbl1$allCondNames
nConds <- xySetTbl1$nConds
xyClassNames <- unique(allCondNames[-1])
xClassName <- xyClassNames[1]
yClassName <- xyClassNames[2]
xSet <- mae.getCondHData(xySetTbl1,1)
ySet <- mae.getCondHData(xySetTbl1,2)
xSampleNames <- names(xSet)
ySampleNames <- names(ySet)
nGenes <- xySetTbl1$nGenes
nX <- ncol(xSet)
nY <- ncol(ySet)
isVertStacked <- xySetTbl1$isVertStacked
paste("HP-X & Y sets class names [",xClassName,") and
paste("Data for current ",nX," HP-X and ",nY," HP-Y
paste("Data for current HP-X set
paste("with",nGenes,"genes.")
#
```

R Output files

File name #1: R-Stdout.txt
File type #1: Stdout

File name #2: R-OutputFile6.pdf
File type #2: PDF image

File name #3: Stdout
File type #3: Stdout

File name #4: Stdout
File type #4: Stdout

File type #4 options: NONE, Tab-delimited table, XML, Report (free form), JPEG image, PDF image, PNG image

Select RLO field Wizard: R Output files

Buttons: Save RLO, Prev, Next, Close

Checkbox: Show help message

5. Edit Wizard for Current RLO: MAExplorer Actions Applied to R Output Files

RLO [14] R script [DemoMaeRscript14.R]

Menu Name: 'Demo 14 - HP-X and HP-Y 'sets' - t-test 0.05, save Gene Set'

```
##### Start of Prologue #####
# RLO Number 14, Rscript [ DemoMaeRscript14.R ]
print("Method:Demo 14 - HP-X and HP-Y 'sets' - t-test
# Extract sample names & class names, read the table,
# use the associated X and Y class names to extract t
xySetTbl1 <- mae.readHSfile("R-inputHP-XYsets.txt")
##### End of Prologue - Don't edit above this line #####
#
xySet <- xySetTbl1$data
sampleNames <- xySetTbl1$sampleNames
condNames <- xySetTbl1$condNames
allCondNames <- xySetTbl1$allCondNames
nConds <- xySetTbl1$nConds
xyClassNames <- unique(allCondNames[-1])
xClassName <- xyClassNames[1]
yClassName <- xyClassNames[2]
xSet <- mae.getCondHData(xySetTbl1,1)
ySet <- mae.getCondHData(xySetTbl1,2)
xSampleNames <- names(xSet)
ySampleNames <- names(ySet)
nGenes <- xySetTbl1$nGenes
nX <- ncol(xSet)
nY <- ncol(ySet)
isVertStacked <- xySetTbl1$isVertStacked
paste("HP-X & Y sets class names [",xClassName,") and
paste("Data for current ",nX," HP-X and ",nY," HP-Y
paste("Data for current HP-X set ("",nX,"samples) and
paste("with",nGenes,"genes.")
#
```

MAE Actions taken on R output

File name #1: R-GeneSet.txt
Object name #1: Rtest GeneSet
Object Action #1: Gene set

Object Action #1 options: Gene set, Gene set with p-Values, Gene set with cluster #s & distar, Data filter Gene set, Normalization Gene set, Condition list, Ordered Condition List, Normalization map

Select RLO field Wizard: MAE Actions taken on R output

Buttons: Save RLO, Prev, Next, Close

Checkbox: Show help message

6. Edit Wizard for Current RLO: MAExplorer Export/Import Data Processing

The screenshot shows the 'Edit existing R script LayOut (RLO)' window. The title bar reads 'Edit existing R script LayOut (RLO)'. The main content area is split into two panes. The left pane contains R code for a script named 'DemoMaeRscript06.R'. The right pane is titled 'Export/import data processing options' and contains several checkboxes: 'Gene-filter, else no gene-filter', 'Raw-data, else normalized-data', 'use vertically stacking for multiple samples [Future]', 'use (F1,F2) or (Cy3/Cy5) channels else mean or ratio', 'add separate expr. bkgd data channels data [Future]', 'Post display plots generated by the R execution', and 'Use '-vanilla' option for R to start up R faster' (which is checked). A yellow callout box with an arrow points to the right pane, containing the text 'MAExplorer export/import data processing options'. At the bottom, there is a 'Select RLO field Wizard' dropdown menu set to 'Export/import data processing options', and buttons for 'Save RLO', 'Prev', 'Next', and 'Close'. A text field at the bottom left contains 'Saved existing RLO #6' and a 'Show help message' checkbox is on the bottom right.

7. Edit Wizard for Current RLO: Optional R Startup Switches

The screenshot shows the 'Edit existing R script LayOut (RLO)' window. The title bar reads 'Edit existing R script LayOut (RLO)'. The main content area is split into two panes. The left pane contains R code for a script named 'DemoMaeRscript06.R'. The right pane is titled 'Optional R startup switches' and contains a text area labeled 'Switches:'. A yellow callout box with an arrow points to this text area, containing the text 'Optional command line for R startup'. At the bottom, there is a 'Select RLO field Wizard' dropdown menu set to 'Optional R startup switches', and buttons for 'Save RLO', 'Prev', 'Next', and 'Close'. A text field at the bottom left contains 'Saved existing RLO #6' and a 'Show help message' checkbox is on the bottom right.

8. Edit Wizard for Current RLO: Generate Demo R Script Methods**

Generate demo R scripts options

- add summary()
- add ttest()
- add cor()
- add hist()
- add density()
- add boxplot()
- add plot()

** Only for demo scripts - not for user defined scripts

Example: Edit Current Demo RLO Generate Demo Methods - Add t-test

Generate demo R scripts options

- add summary()
- add ttest()
- add cor()
- add hist()
- add density()
- add boxplot()
- add plot()

Added t-test

```

#Exporting the pValueThreshold from MAExplorer
pValueThreshold <- 0.05
#
paste("Using ", nGenes, " MAExplorer genes")
paste("Summary of X set samples", xClassName)
summary(xSet)
paste("Summary of Y set samples", yClassName)
summary(ySet)
#
print("Doing individual spot t-tests for X and Y sets")
# compute t-test p-value over all genes
xyPvalues <- mae.maxPvalue(xSet, ySet)
# compute thresholded P-value list (fail and meet p-value)
genesActionList <- (xyPvalues < pValueThreshold)
paste("The thresholded p-values < ", pValueThreshold,
summary(genesActionList)
# Output the set of genes passing the t-test
print("Doing t-test for entire X and Y sets")
xyTtest <- t.test(xSet, ySet, conf.level=pValueThreshold)
summary(xyTtest)

```


Example: Edit Current Demo RLO Generate Demo Methods - Also Add histogram

Then added histogram and density plot

Evaluate R Script for Current RLO

Press "Evaluate"

```
[1] "Method: Demo 6 - HP-X and HP-Y 'sets' samples expression"
Read 8 items
Read 8 items
[1] "HP-X & Y sets class names [ C57B6.p13 ] and [ C57B6.L1 ]"
[1] "Data for current 3 HP-X and 4 HP-Y 'sets' samples)"
[1] "Data for current HP-X set ( 3 samples) and HP-Y set ( 4 samples)"
[1] "with 393 genes."
[1] "Using 393 MAExplorer genes"
[1] "Summary of X set samples C57B6.p13"
C57B6.p13.totalRNAsug C57B6.p13.1 C57B6.p13.2poly.A
Min. : 0.1635 Min. : 0.4508 Min. : 0.3369
1st Qu.: 0.7606 1st Qu.: 0.9049 1st Qu.: 0.8968
Median : 2.2720 Median : 1.5930 Median : 1.8883
Mean : 15.1545 Mean : 1.6472 Mean : 1.8956
3rd Qu.: 18.6924 3rd Qu.: 2.3583 3rd Qu.: 2.8921
Max. : 135.5656 Max. : 2.7843 Max. : 3.5821
[1] "Summary of Y set samples C57B6.L1"
C57B6.L1.30min C57B6.L1.3hrs C57B6.L1.4hrs C57B6.L1.total
Min. : 0.4288 Min. : 0.1384 Min. : 0.4779 Min. : 0.1384
1st Qu.: 0.8634 1st Qu.: 0.7459 1st Qu.: 0.8827 1st Qu.: 0.7459
Median : 2.0662 Median : 2.6245 Median : 2.0020 Median : 2.6245
Mean : 14.1429 Mean : 21.1851 Mean : 13.2889 Mean : 21.1851
3rd Qu.: 19.4648 3rd Qu.: 27.4533 3rd Qu.: 17.3382 3rd Qu.: 27.4533
Max. : 102.8328 Max. : 167.8087 Max. : 99.9495 Max. : 167.8087
[1] "Doing individual spot t-tests for X and Y sets"
[1] "The thresholded p-values < 0.05 is:"
Mode FALSE TRUE
logical 258 135
[1] "Doing t-test for entire X and Y sets"
Length Class Mode
```

Creating a New User-defined RLO

Rtest - (alpha) \$Revision: 1.62 \$ (\$Date: 2003/05/20 23:10:25 \$)

Database: MGAP DB

Demo 14 - HP-X and HP-Y 'sets' - t-test 0.05, save Gene Set Show Demo RLOs Save ALL Demo RLOs

B. To Edit an existing RLO

1. select the RLO to edit,
2. press 'Edit RLO' to popup the RLO editor,
3. enable/disable generation checkboxes to modify the generated R script that is update as you make the changes.
4. press 'Close' to exit the RLO editor.

C. To Create a new RLO database entry

1. press 'New RLO' to popup the RLO editor,
2. either type in an R script in the edit window, or
 - 2.1 press 'Import R script' button read a R script file into the window.
 - 2.2 go through the other wizards and fill out the relevant responses. Press 'Next' to advance to the next wizard and 'Prev' to backup to the previous one.
3. select one of the RLO wizards and follow the instructions,
4. press 'Save RLO' button save the RLO in the permanent RLO database at any time,
5. press 'Close' button save the RLO and exit the RLO editor,
- 5.1. press 'Cancel' button exit the editor and NOT save the RLO.

Buttons: Evaluate Show generated plot(s) Clear report Show R script SaveAs Close

DEBUG New RLO **Edit RLO** Show RLO Delete RLO Help Msg

Create new RLO using editor - see next slide

View Help Message for RLO Wizard Editor

Define new R script LayOut (RLO) #13

RLO [13] R script [--not defined--]

Menu Name: '--not defined--'

How to create a new R LayOut (RLO) entry

For each entry in the 'select wizard' below, fill in the relevant fields. You can review the fields in the demo RLOs as examples. Press 'Next' to advance to the next RLO field wizard, and 'Prev' to backup to the previous one. You can also go to a particular wizard by selecting the RLO field in the pull-down menu at the bottom of the window.

When you are done reading this message, unset 'Show help' check box in the lower right corner of this window. This lets you edit the .R script in this window. You may switch to this message at any time by enabling the 'Show help message'.

You may then type the .R script in this window. Or alternatively, you may also import an .R script file into this text window as a basis and edit it further. When you save the RLO, it will save the R script in the file you specify. When you are done, press 'Close' to save the RLO in the RLO database. You may save the RLO any time by pressing the 'Save RLO' button.

Before you save a RLO, you must define both the 'R script name' and 'Menu name' to save a new RLO. Although you may edit the .R script text for a demonstration script, it is overridden and regenerated when 'Evaluate' it in the main window.

R script file name [.R file]

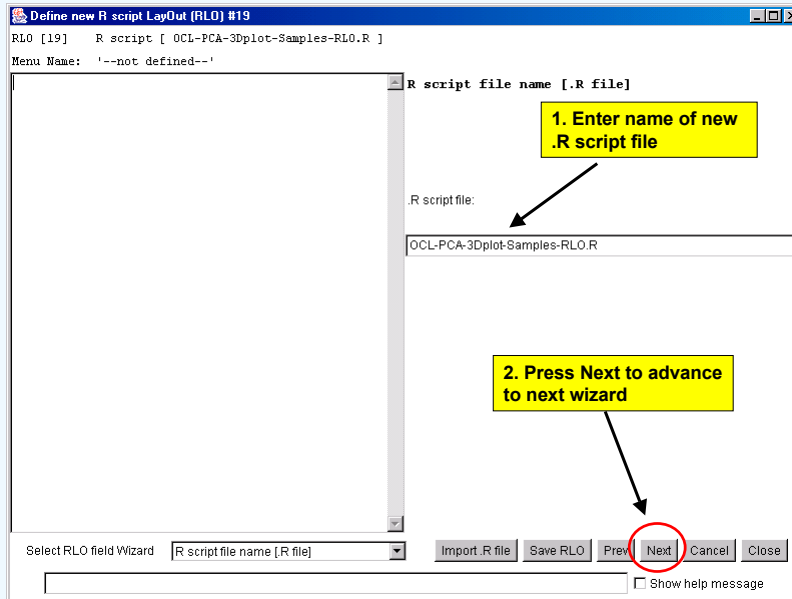
.R script file:

Buttons: Import R file Save RLO Prev **Next** Cancel Close

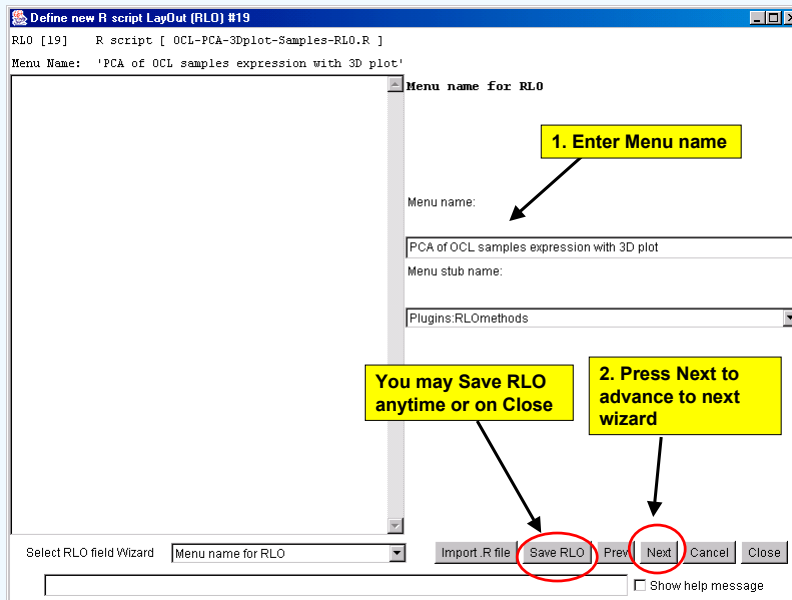
Show help message

Toggle help checkbox

1. Define New R script name Using RLO Wizard



2. Define New RLO Menu name Using Wizard



3. Define New RLO R Input files and R template Using Wizard

The screenshot shows the 'Define new R script LayOut (RLO) #19' wizard. The left pane displays R script code with annotations: 'Start of Prologue' and 'End of Epilogue' are circled in red, and 'Enter your analysis R script here ...' is highlighted in yellow. The right pane is titled 'R Input files (from MAE)' and contains the following fields:

File name #1:	R-inputCurOCL.txt
File type #1:	Current OCL of Conditions lists
File name #2:	
File type #2:	NONE
File name #3:	
File type #3:	NONE
File name #4:	
File type #4:	NONE

Below these fields are two sections: '1. Select template type' (set to 'Current OCL of Conditions lists') and '2. Add template code' (with an 'Add template' button). At the bottom, the 'Next' button is circled in red. Yellow callout boxes with arrows point to: '1. Define input file(s)' (pointing to the first file type), '2. Define R template' (pointing to the template type dropdown), '3. Define R template' (pointing to the 'Add template' button), and '4. Press Next' (pointing to the 'Next' button).

4. Define New RLO R Output files Using Wizard

The screenshot shows the 'Define new R script LayOut (RLO) #19' wizard. The left pane displays R script code. The right pane is titled 'R Output files' and contains the following fields:

File name #1:	R-Stdout.txt
File type #1:	Stdout
File name #2:	simplePCAGenes.pdf
File type #2:	PDF image
File name #3:	simplePCAarrays.pdf
File type #3:	PDF image
File name #4:	points3Darray.pdf
File type #4:	PDF image
File name #5:	
File type #5:	Stdout
File name #6:	
File type #6:	Stdout

At the bottom, the 'Next' button is circled in red. A yellow callout box with an arrow points to '1. Define output files' (pointing to the first file type) and another points to '2. Press Next' (pointing to the 'Next' button).

5. Define New RLO Actions Using Wizard - If Any

Define new R script LayOut (RLO) #19

RLO [19] R script [OCL-PCA-3Dplot-Samples-RL0.R]

Menu Name: 'PCA of OCL samples expression with 3D plot'

```
##### Start of Prologue #####
# RLO Number 19, Rscript [ OCL-PCA-RL0.R ]
print("Method:PCA of current OCL samples expression")
print("Data for current Ordered Condition List")
oclTbl <- mae.readHSfile("R-inputCurOCL.txt")
##### End of Prologue - Don't edit above this line
# ... Enter your analysis R script here ...
##### Start of Epilogue - Don't edit below this line
##### End of Epilogue #####
```

MAE Actions taken on R output

File name #1:

Object name #1:

Object Action #1:

File name #2:

Object name #2:

Object Action #2:

File name #3:

Object name #3:

Object Action #3:

Select RLO field Wizard MAE Actions taken on R output

Import R file Save RLO Prev **Next** Cancel Close

Show help message

Press Next

6. Define New RLO Export/Import Data Processing Options Using Wizard

Define new R script LayOut (RLO) #19

RLO [19] R script [OCL-PCA-3Dplot-Samples-RL0.R]

Menu Name: 'PCA of OCL samples expression with 3D plot'

```
##### Start of Prologue #####
# RLO Number 19, Rscript [ OCL-PCA-RL0.R ]
print("Method:PCA of current OCL samples expression")
print("Data for current Ordered Condition List")
oclTbl <- mae.readHSfile("R-inputCurOCL.txt")
##### End of Prologue - Don't edit above this line
# ... Enter your analysis R script here ...
##### Start of Epilogue - Don't edit below this line
##### End of Epilogue #####
```

Export/import data processing options

Gene-filter, else no gene-filter

Raw-data, else normalized-data

use vertically stacking for multiple samples [Future]

use (F1,F2) or (Cy3/Cy5) channels else mean or ratio

add separate expr. bkgnd data channels data [Future]

Post display plots generated by the R execution

Use '--vanilla' option for R to start up R faster

Select RLO field Wizard Export/import data processing options

Import R file Save RLO Prev **Next** Cancel Close

Show help message

1. Set processing options

2. Press Next

Import R Code From file(s) to New RLO

Define new R script LayOut (RLO) #19

```

RLO [19] R script [ OCL-PCA-3Dplot-Samples-RL0.R ]
Menu Name: 'PCA of OCL samples expression with 3D plot'

###--- Start of Prologue ---###
# RLO Number 19, Rscript [ OCL-PCA-RL0.R ]
print("Method:PCA of current OCL samples expression")
print("Data for current Ordered Condition List")
oclTbl <- mae.readH5file("R-inputCurOCL.txt")
###--- End of Prologue - Don't edit above this line -
# ... Enter your analysis R script here ...

## Analyze the current MAExplorer array data using PCA.
# PDF and out files.
# Authors: P. Leakin, S. Sundaram
# $Date: 6-14-2003$ $Revision: 1.1$

# The input file contains the format as genes(rows) x
oclData <- oclTbl$hData
condNames <- oclTbl$condNames
allCondNames <- oclTbl$allCondNames
sampleNames <- oclTbl$sampleNames
nConds <- oclTbl$nConds
nGenes <- oclTbl$nGenes
isVertStacked <- oclTbl$isVertStacked
paste("The Ordered Condition List has ",nGenes," genes")
paste("The Ordered Condition List has ",nConds," cond")
paste("The Ordered Condition List has ",length(sampleNames))
# -- [1] Compute the PCA both for Arrays and for Gene.
print("Computing the PCA of the current MAExplorer data")
maePCAarrays <- mae.simplePCAanalysis(data=oclData,pl
mode="arrays",
uniqGroups=allC
# -- [1.1] Write out the data into files
    
```

Please enter .R script to read

Look in: R

- Test.R
- simpleRscriptForXY.R
- simpleRscriptForPCA-3DplotSamples.R
- OCL-PCA-RL0.R

Type: R File
Size: 2.58 KB

File name: simpleRscriptForPCA-3DplotSamples.R [Open]

Files of type: All Files (*.*) [Cancel]

Buttons: Import R file, Save RLO, Prev, Next, Cancel, Close

1. Imported R script

2. Press Next

3. Press Close

Executing the New RLO from RtestPlugin

Rtest - (alpha) \$Revision: 1.77 \$ (\$Date: 2003/06/14 20:35:07 \$)

Database: MGAP DB

PCA of OCL samples expression with 3D plot [Show Demo RLOs] [Save ALL Demo RLOs]

Number of Eigen Vectors= 6

Length	Class	Mode
3	prcomp	list
15	-none-	numeric
90	-none-	numeric
1	-none-	numeric
1179	-none-	numeric
3	-none-	list

Importance of components:

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Standard deviation	3.723	0.822	0.5249	0.3025	0.17989	0.15676	0.1092	0.09083
Proportion of Variance	0.924	0.045	0.0184	0.0061	0.00216	0.00164	0.0008	0.00055
Cumulative Proportion	0.924	0.969	0.9876	0.9937	0.99585	0.99748	0.9983	0.99883
	PC9	PC10	PC11	PC12	PC13	PC14	PC15	
Standard deviation	0.06854	0.05886	0.05740	0.05057	0.04736	0.03586	2.22e-17	
Proportion of Variance	0.00031	0.00023	0.00022	0.00017	0.00015	0.00009	0.00e+00	
Cumulative Proportion	0.99914	0.99937	0.99959	0.99976	0.99991	1.00000	1.00e+00	

Buttons: Evaluate, Show generated plot(s), Clear report, Show R script, SaveAs, Close

Buttons: [checked] DEBUG, New RLO, Edit RLO, Show RLO, Delete RLO, Help Msg

1. Press Evaluate to run the R script

2. Results appear in window

Executing the New RLO From MAExplorer RLO Methods menu

1. Select command

The screenshot shows the MicroArray Explorer interface. The 'RLO methods' menu is open, with 'PCA of OCL samples expression with 3D plot' selected and circled in red. A yellow callout box points to this menu item. Below the main window, a 'PCA of OCL samples expression with 3D plot' popup window is displayed, containing technical details about the analysis. A second yellow callout box points to this popup window. To the right, an Adobe Acrobat window shows a 3D scatter plot of the results.

MicroArray Explorer - V.0.96.32.06-Beta - C57BL/6 (P13, L1, L10), sta5a (-), 15 public probes

File Samples Edit Analysis View Plugins Help

Enter gene name or clone ID Mouse- Load Plugins Unload Plugin(s)

Ready - click on a gene to query database

RLO methods

- PCA of OCL samples expression with 3D plot
- PCA of current OCL samples expression
- Example of test R analysis of X and Y data

HP-X: Pregnancy 13 (1 hr) [C57B6-p13-totalRNA5ug]
HP-Y: Lactation 1 (30 min) [C57B6-L1-30min]

Norm HP-X
HP-Y

PCA of OCL samples expression with 3D plot

- [1] "Method - PCA of OCL samples expression with 3D plot"
- [1] "Data for current Ordered Condition List (OCL)"
- [1] "The OCL consists of multiple conditions of samples:"
- Read 16 items
- Read 16 items
- [1] "The Ordered Condition List has 393 genes"
- [1] "The Ordered Condition List has 4 conditions"
- [1] "The Ordered Condition List has 16 samples"
- [1] "Computing the PCA of the current MAExplorer data by 'arrays'"
- Loading required package: mva
- Number of Eigen Vectors= 6
- Length Class Mode
- poa 3 prcomp list
- eigenVals 15 -none-numeric
- eigenVecs 2399 -none-numeric
- nEigenVecs 1 -none-numeric
- points3D 45 -none-numeric
- loadings 3 -none-list
- Importance of components:
- PC1 PC2 PC3 PC4 PC5 PC6 PC7 PC8
- Standard deviation 13.716 9.638 8.865 3.4286 2.7622 2.4786 1.71162 1.38125

SaveAs Close

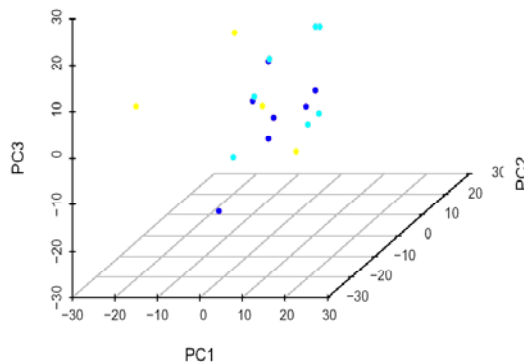
Adobe Acrobat - [points3Darray.pdf]

File Edit Document Tools View Window Help

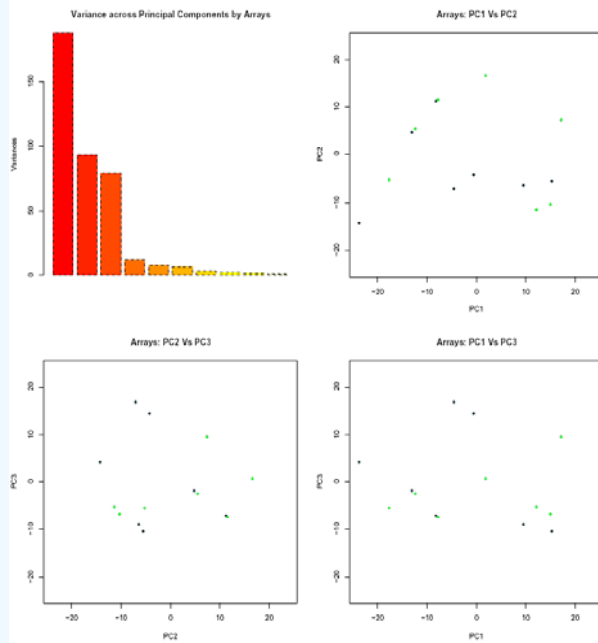
3D plot of PC1 vs PC2 vs PC3 of Arrays

Plot (1 of 5) Generated for New RLO: PCA of Multiple Conditions w/Replicate Samples

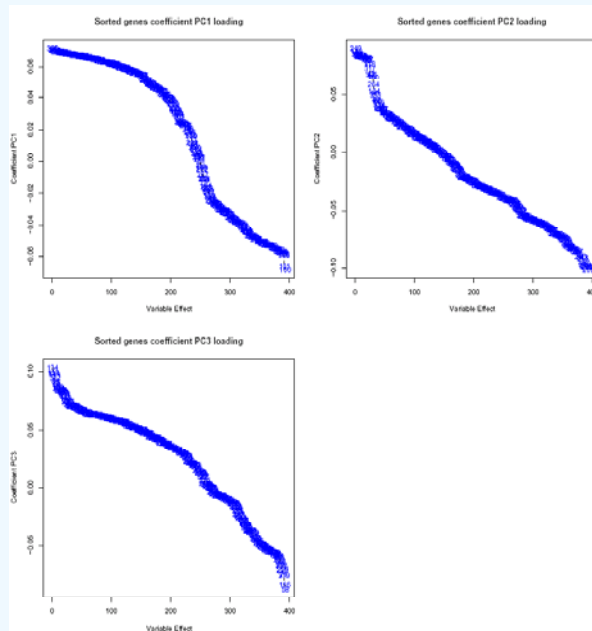
3D plot of PC1 vs PC2 vs PC3 of Arrays



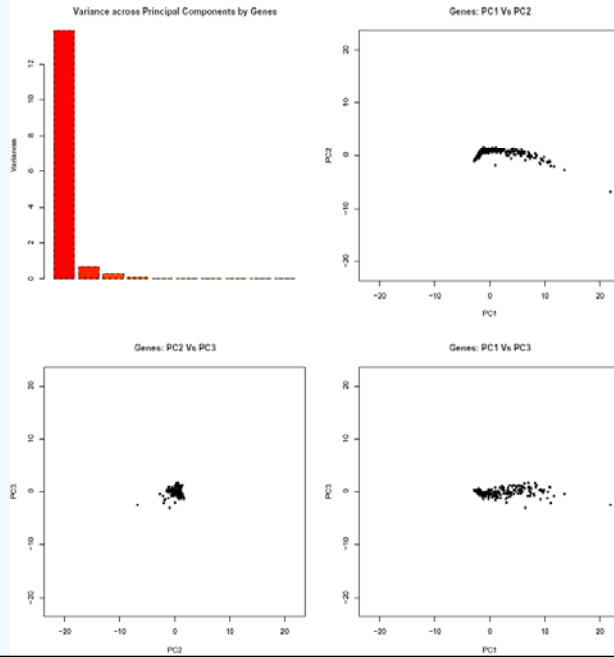
Plot (2 of 5) Variance across PCA by Arrays



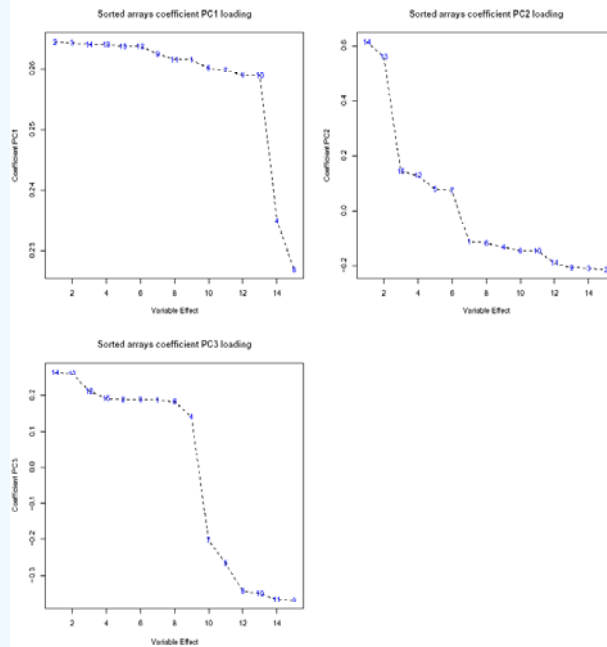
Plot (3 of 5) PCA loadings, by Arrays



Plot (4 of 5) Variance across PCA by Genes



Plot (5 of 5) PCA loadings, by Genes



MAExplorer Java API - MJAReval Class

- MJAReval Application Programming Interface (API) class supports the R evaluation and RLO database functionality
- Define and maintain a permanent R LayOut (RLO) database and evaluate RLOs directly from MAExplorer “RLO methods” menu
- Create new R scripts using RLO wizard templates & imported R code
- Evaluate R scripts using MAExplorer data in the context of their RLO
- Post-process R output to
 - a) show R text output (stdout)
 - b) display PDF, JPEG, SVG, etc. files
 - c) apply actions to import R output back into MAExplorer database

Summary

- MAExplorer is a flexible fully Open Source Java-based microarray data-mining tool available at <http://maexplorer.sourceforge.net/>
- RtestPlugin is a plugin for MAExplorer to create R programs to analyze MAExplorer data
- RtestPlugin lets user's edit and create R scripts and associated R LayOut (RLO) methods using RLO templates and imported R code
- RtestPlugin has demonstration RLO examples
- RLO methods may be added to the permanent MAExplorer menus
- RLO methods may then be used to analyze any MAExplorer data
- Results from an R evaluation may be imported back into the MAExplorer database