MicroArray Explorer - A Java-based Tool For Data Mining Microarrays

http://www.lecb.ncifcrf.gov/MAExplorer

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Data Mining - Finding Putative Relevant Patterns



Quantified array spot data for multiple samples

Organize by sample, gene expr, gene sets

Change views, normalization, data filters

Visualize and query: plots, cluster, reports

Explore external genomic databases

What is the MicroArray Explorer?

- MAExplorer is a flexible Java stand-alone (off-line) or applet (Web-based) microarray data-mining tool
- Handles multiple cDNA arrays with replicate spots
- Handles intensity or ratio (Cy3/Cy5) quantified array data
- Analyzes data for 2-conditions and expression profiles
- Data-filters gene sets by statistics, clustering, gene set membership
- Direct data manipulation in graphics, spreadsheets
- Accesses genomic Web servers from plots and reports
- Currently oriented for cDNA, <u>extending to protein arrays</u>

MAExplorer was Developed for the Mammary Genome Anatomy Program

- MGAP models, histology, expression http://mammary.nih.gov/
- Mouse models of
 - 1. normal mammary development C57B6
 - 2. Knock-outs: Stat5a (-,-) & (+,-), CEBP null, TGF α etc.
 - 3. Tumor models: WAP taq, p53 mutant, etc.
 - 4. Transgenics: WAP Int3, BRCA1, etc.
- Initially, MAExplorer was a <u>Java applet</u> for analyzing MGAP arrays http://www.lecb.ncifcrf.gov/mae
- MAExplorer identified genes in preferentially expressed during lactation, *Nucleic Acids Res.* (2000) 28:4452

Mammary Geneome Anatomy Program MAExplorer http://www.lecb.ncifcrf.gov/mae



MAExplorer Home Page http://www.lecb.ncifcrf.gov/MAExplorer



MAExplorer GUI-based User Interface



Data Sources

- <u>Schema</u>: tab-delimited experiment data files:
 - 1. GIPO (array print file)
 - 2. List of samples in DB
 - 3. Configuration
 - 4. Separate spot quantification data files
- Use with any experiment data that uses this schema
- Files are read from:
 - 1. Local disk for stand-alone application or
 - 2. Web server for <u>Web-browser applet</u> optionally cached locally
- Used with data from NCI/CIT-ATC mAdb Web server
- The <u>Cvt2Mae</u> tool is used to convert generic data to this schema

Lists of Hybridized Probe (HP) Samples

- <u>Multiple-sample database</u> may consist of many conditions with replicate samples
- Organize samples as lists of conditions:
 - 1. X and Y pairs of individual samples
 - 2. Sets of X and Y replicates (X-set, Y-set)
 - 3. Ordered expression profile list (E-list)
- Manipulate <u>named lists</u> of sample conditions
- <u>Save and restore named sample lists</u> during analysis and between sessions

Operations on 2- & N-conditions of Samples⁽¹⁾



Operations on <u>order lists of samples</u>: e.g. clustering, Expression Profile plots

⁽¹⁾ "HP" is hybridization probe i.e. the sample hybridized to the chip

Choosing HP-X, HP-Y Sets and HP-E Lists

😹 MGAP DB - MicroArray Explo		
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Gene Data Filter is Intersection of Tests



MAExplorer User Interface - Filter Menu



Plots

Plots allow visualization and direct manipulation of gene data

- 1. <u>Pseudo array image</u> intensity, ratio (X/Y)
- 2. Zoomable scatter plots X vs Y, Cy3 vs Cy5, duplicate spots
- 3. <u>Histograms</u> ratio and intensity
- 4. Expression profiles individual genes and overlay plots
- 5. <u>Silhouette plots</u> similarity clusters, K-means clustering
- 6. <u>Hierarchical clustering</u> clustergram, dendrogram

Scatter Plots of Two Conditions

X-Y scatter plot of two 13-day pregnancy samples: C57B6 vs Stat5a (-,-) [MGAP]



Zoomed X-Y Scatter Plot

- Zoomed-in on Raf-related oncogene (green circle) using scrollbars
- Genes not passing Filter may be grayed out in the plot



Genes Filtered by Intensity-Histogram Bin



Expression Profile Plots of N-conditions



Cluster Genes With Similar Expression



K-means Clustering of Expression Data

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List of Expression Profiles Plots of Clusters

• Scrollable list of EP plots showing genes from clusters #1, #2, #3



Mean Expression Profiles of K-means Clusters



Hierarchical Clustering of Expression Data



Reports

- Data reported as:
 - 1. Web-accessible dynamic spreadsheets or
 - 2. <u>tab-delimited text</u> exportable to Excel
- <u>Gene set</u> reports linked to UniGene, GenBank, mAdb, LocusLink, etc.
- <u>Array sample</u> reports linked to histology and model Web pages
- <u>Pop-up Web browser</u> on specific data from dynamic reports or plots

Gene Set Operations Help Manage Data and Search Results

- <u>All gene sets are named</u> with a directory of existing sets
- <u>Set operations</u> (AND, OR, DIFFERENCE) may be used to create new derived sets
- Special sets:
 - <u>Filtered genes set</u> holds genes passing the data filter
 <u>Edited Gene List</u> holds results of clustering or editing
 <u>Normalization set</u> may be used as normalization method
 <u>User data filter set</u> may be used as a data filter
- Sets are <u>saved</u> when the session is saved, <u>restored</u> when MAExplorer restarted

Find Gene or Gene Subset by Name Guesser



Gene Set Operations - e.g. 'AND' of Two Sets

MGAP DB - MicroArray Explorer - V0.90.03-Beta - C	57B6 💶 🗙 👹 User Gene Sets	
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*C57B6-virgin-3	ALL NAMED GENES	
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Scrollable Dynamic Gene Reports

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Scrollable Dynamic Gene Reports

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Correlation Report of all Samples

• Sample vs Sample correlation coefficient report for current set of Filtered genes

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Tab-Delimited Reports Exportable to Excel

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HP-Y: Stat5a (-,-) pregnancy day 13	
Genes with highest HP-X/HP-Y ratios	
There are 405 genes passing the Filter.	_
HP-X: C57B6 pregnancy day 13	
HP-Y: Stat5a (-,-) pregnancy day 13	
Norm.: median intensity 🛛 😹 GENE REPORT - Filtered genes with 50 Highest ratios HP-X[C57B6 preg	
HP-XY 'set' ratio	tat5a 🔺
3.25 Grid-Coord Ratio HP-X/HP-Y Clone-ID Gene-Name Plate-G,R,C mA	
2.5 C [1-06,21] 1.8030 1032272 Mids missionersching-zind miger protein 1 (Mi21) mitrix,	8264
1.75 [1-A3,17] 1.8456 1248170 Mouse mRNA for SDF2, complete cds plate[4,A,5] 124	18170
[1-H4,15] 1.8449 1248272 ADRENODOXIN PRECURSOR plate[6,H,3] 1248272 124	8272
1.0 [1-D5,3] 1.8256 1248351 Abl-interactor 1 plate[7,D,3] 1248351 1248351 Al4	8337:
0.571 (1-F7,7) 1.8118 1382525 Acetyl coenzyme A dehydrogenase, medium chain plate[11,F,7]	
0.4 1-5 0 [1-C2,19] 1.7997 1247627 Mus musculus mRNA for osteomodulin, complete cds pla	rte[2,(
0.307 [1-A3,0] 1.7677 1247777 Mus musculus metalloprotease/disintegrin/cysteine rich protein pr	ecurs
50.25 [1-86,7] 1.7302 1381034 TKOPOMITOSING, CTTOSKEEPACTIFE plate[9,8,7]	
[1-A5,23] 1.7377 1248527 Mus musculus ubiquitin-conjugating enzyme HR6A mRNA, comp	lete c
X Active Probe [1-C3,10] 1.7316 1247708 Ephrin A1 plate[3,C,10] 1247708 1247708 AA959770	
*C57 B8-p13-total [1-D3,5] 1.7249 1247564 Erythrocyte protein band 7.2 plate[3,D,5] 1247564 124	7564
*C6788.c13.1 (1-C6,2) 1.7190 1381920 Mus musculus mRNA for NEFA protein, complete cds pla	/te[9,(
2 C6798 p13 2 poly 1-C [1-D7,16] 1.7081 1382671 Mouse MA-3 (apoptosis-related gene) mRNA, complete cds pla	ite[12
C37 66 p 13.2 poly [1-H3,12] 1.7073 1248169 Histocompatibility 2, T region locus 22 plate[3,H,12] 124	8169
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[1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-04.6] [1-	molet
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C67B6-virgin-3h	
SaveAs (Close

Plug-in Extensions to MAExplorer



- <u>Plug-ins</u> will allow investigators to extend capabilities of core MAExplorer program to other analysis methods
- Open Java API, Java examples, donated plug-ins & links to Plugins will be published on our Web site
- <u>Typical plug-ins</u>: normalization, Filters, PCA, clustering, Web-server functional analysis of cluster results, etc.
- <u>Plug-ins</u> will have three types of implementations:
 - 1. Using 100% Java code
 - 2. Accessing local programs written in any language
 - 3. Web-CGI or client-server to specialized genomic DBs

Data Format Conversion for MAExplorer

- <u>Cvt2Mae</u> reads a variety of array data for both oneof-a kind academic and commercial arrays (eg. Affymetrix, Incyte, etc).
- Users can create an <u>Array Layout</u> description that may be used in subsequent conversions
- Will add generic "MAML" (now "MGED") standard compatibility (MicroArray XML) for data portability
- Cvt2Mae is undergoing Beta-testing

Select a Chip Array-Layout

📸 Cvt2Mae: convert array data to MAExplorer files - Ve	ersion: 08-21-2001 (Beta)	a to ABA Fundarar format	_ 🗆 🗵
Enter data for steps 1, 2, and 3. Then press	run to convert your dat	a to MAExplorer format.	
1. Select Chipset:	select a chip	layout 🗾	
	select a chip . iser-defineds</td <td>layout</td> <td></td>	layout	
1.1 (opt.) Select Quant. software used: [NONE] pic	k array dincyte - Mouse		-
	Incyte - Human Affumetrix - Ma	1160	
2. Select Input Data Files:	E Affymetrix - Hu	man older	
L			
2.1 Samples to use '< <file>> sample name'.</file>	Remove sample	Rename sample	
Vendor			
Layout nam	ne		
Species			
Spotsimici	roarray		
3. Select Project Output Folder:	tout Folder		
	·····		
Project output folder:			
MAExplorer startup File:			
Edit Layout	Assign GIPO fields	Assign Quant fields	Abort
4. E ON and Run 74111 Save Layout	Expert assign-mode		Reset
Status:			

Converted Affymetrix files for Analysis

👹 Cvt2Mae: convert array data to MAExpl	orer files - Version: 08-21-2001 (Beta		_ 🗆 ×
Enter data for steps 1, 2, and 3). Then press 'Run' to convert your de	ata to MAExplorer format.	
1. Select Chipset:	Affymetrix - H	luman 🔽	
	•		
1.1 (opt.) Select Quant. software used:	[NONE] pick array data ()		-
2. Select Input Data Files:	Browse file nam	e Browse folder	
U937-Affymetrx-2subciones.txt			
l			
2.1 Samples to use '< <file>> sample name</file>	Remove samp	le Rename sample	
< <c:\temp\affydata\u937-affymetrx-2sub< td=""><td>clones.txt>> [DSD-1-Tel10A-1-U95A</td><td></td><td>-</td></c:\temp\affydata\u937-affymetrx-2sub<>	clones.txt>> [DSD-1-Tel10A-1-U95A		-
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< <c:\temo\affvdata\u937-affvmetrx-2sub< td=""><td>clones.txt>> IDSD-1-Tel17B-1-U95A</td><td></td><td>•</td></c:\temo\affvdata\u937-affvmetrx-2sub<>	clones.txt>> IDSD-1-Tel17B-1-U95A		•
	Vendor	Affymetrix	
	Layout name	Affymetrix - Human	
	Species	Human	
	Spots/microarray	12630	
2 Salact Project Output Falder	Croote New preject folder		
5. Selectri vject valpat Folder.	Create New project Torder		
Project output folder:	C:\Temp\junk\		
MAExplorer startup File:	C:\Temp\junk\MAE\Start.mae		
EditLa	yout Assign GIPO fields	Assign Quant fields	Done
4. Edit and Run Run Save La	expert assign-mode		Reset
Status:	===> Finished writing out data files	Press 'Done' to exit	
	To start MAExplorer, go to project t	older & click on Start.mae.	

Affymetrix Data from Cvt2Mae Conversion

😹 C:\Temp\AffyData\ DB - MicroArray Explor	er - V0.90.05-Beta - 10A&B vs 17A&B	
File HybProbe Edit Analysis View Help		
Enter gene name or Normalization Se-over int	0 HP-X: DSD-1-Tel10A-1-U95A	
Filter 🕨 🗸 Filter by (GeneClass membership	
Plot Filter by	User Filter Gene Set' membership	
Boody elick on a go Report Filter by	Edited Gene List' membership	
Filter by	good genes list' membershin	
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Filter by r	atio histogram bin	
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Filter by	oositive intensity data 🛛 🔸	Filter by spots with positive inte
Norm.: median intensity Filter by (Good Spot data 🔹 🕨	Check spots for positive values
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Active GeneClass		
Active GeneClass	00000000000000000000000000000000000000	00000000000000000000000000000000000000

Converted Incyte files Ready to Analyze

👹 Cvt2Mae: convert array data to MAExp	orer files - Version: 08-21-2001 (Beta)		_ 🗆 ×
Enter data for steps 1, 2, and	3. Then press 'Run' to convert your da	ta to MAExplorer format.	
1. Select Chipset	Affymetrix - H	ıman 🔽	
1.1 (opt.) Select Quant. software used:	[NONE] pick array data ()		~
2. Select Input Data Files:	Browse file nam	Browse folder	
U937-Affymetrx-2subciones.txt			
l			
2.1 Samples to use '< <file>> sample name</file>	Remove sample	Rename sample	
< <c:\temp\affydata\u937-affymetrx-2sut< th=""><th>clones.txt>> [DSD-1-Tel10A-1-U95A]</th><th></th><th><u> </u></th></c:\temp\affydata\u937-affymetrx-2sut<>	clones.txt>> [DSD-1-Tel10A-1-U95A]		<u> </u>
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	Vendor	Affymetrix	
	Layout name	Affymetrix - Human	
	Species	Human	
	Spots/microarray	12630	
3. Select Project Output Folder:	Create New project folder		7
Project outout folder	C:\Temo\unk\		
MAExplorer startup File	C:\Temp\unk\MAE\Start.mae		
Editle	Accian GIPO fielde	Acciun Quant fielde	Done
4. Edit and Run Run Save L		Noorgh Quant Incido	Pacat
Savel			Acset
Status	===> Finished writing out data files	Press 'Done' to exit	
510143.	To start MAExplorer, go to project for	older & click on Start.mae.	



- MAExplorer is a flexible microarray data-mining tool running on the user's computer
- Uses direct-manipulation, data filtering, built-in graphics, statistics, clustering, gene and sample set operations
- Manages multiple samples, replicates, sets, expression profile lists where the session state may be saved on the disk for later use
- The Cvt2Mae data conversion tools allows use with common chips
- Plug-ins will allow extension with new analytic methods by users
- MAExplorer identified genes in preferentially expressed during lactation, *Nucleic Acids Res.* (2000) 28:4452
- Freely available for download with documentation on Web site