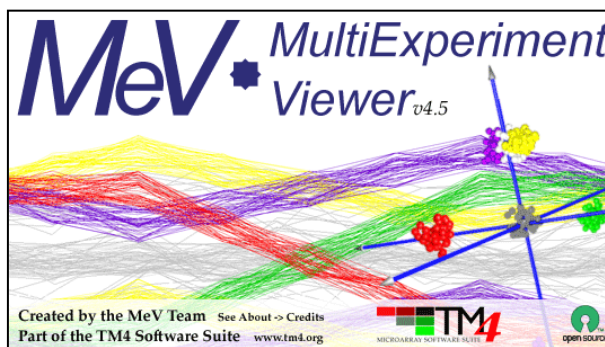


MeV nEASE tutorial

Downloading and Running MeV

MeV for Windows and Linux requires Java v1.6 or higher to run. MeV for Mac OSX requires Java v1.5 or higher. Download the Java Runtime Environment from <http://java.sun.com/> and install it before proceeding with this tutorial.



Download the appropriate version of nEASE-MeV for your operating system from http://www.tm4.org/mev_nease.html. Unzip the downloaded file with Winzip or another unzipping tool. To run MeV on Windows, double-click tmev.bat. To run MeV on Linux/Unix, run tmev.sh. To run MeV on MacOSX, double-click on the MeV application icon.

Loading Data

When you launch MeV, two windows will open. The small narrow window across the top of the screen is called the MeV main menubar. This window is used normally to open new MultipleArrayViewer windows and manage other MeV properties. We will not be using this menubar window for the purposes of this tutorial. The larger window that opens is called a MultipleArrayViewer (MAV). This is where the majority of our work will take place.

Choose *File-> Open Analysis* from the MAV window. In the file chooser that opens, navigate to the folder where you unzipped MeV, and open the folder called Data. Please select the file *ER_status_SAM_1_Miller.anl* and choose *Open*. A saved analysis will be loaded into MeV. This may take some time. These data and the other data files found in the MeV-nEASE package are described more fully in the manuscript. They are based on the data from Miller *et al.* (2005) and Minn *et al.* (2005).

Along the left-hand side of the MAV window is a result tree. As you create results in MeV, new icons will appear in this window. Double-clicking on these icons will reveal the result viewers for these analyses.

Running Nested EASE

Near the top of the MAV is a row of colorful drop-down menus. These menus contain the analysis options available in MeV. After loading an analysis file, click the *Meta-Analysis* drop-down menu, and select *EASE Cluster Analysis*. An initialization dialog will appear.

Step 1: Selecting the EASE file system

Click the button marked “Browse” and navigate to the folder in the unzipped MeV directory named `data\ease\affy_HT_HG-U133A_EASE`. You should see three folders inside, `Data`, `Enhance` and `Lists`. Do not select any of these folders. Simply click *Open*.

Step 2: Population and Cluster Selection Tab

Make sure the option *Select Background Population from File* is selected. Click the button labeled *File Browser* and select the file `ProbesetIDs.txt`.

Step 3: Annotation Parameters Tab

From the drop-down menu labeled *Annotation Key*, select the heading *PROBE_ID*. Click the checkbox labeled *use annotation converter*. Click the button labeled *File Browser*. The file selection dialog that opens should already be set to the correct directory. (If it is not, navigate from the MeV directory to `data\ease\affy_HT_HG-U133A_EASE\Data\Convert`.) Select the file `affy_HT_HG-U133A_ProbesetIDs.txt`.

In the lower half of the tab, under the heading *Gene Annotation / Gene Ontology Linking Files*, click the *Add Files* button. Again, the resulting file browser should already be displaying a list of the available GO term files. (If not, navigate to the `data\ease\affy_HT_HG-U133A_EASE\Data\Class` directory within the

MeV folder.) Hold down the shift key and click on the files, `GO Biological Process.txt`, `GO Molecular Function.txt` and `GO Cellular Component.txt` to select them. When the three files are selected, click *Open*.

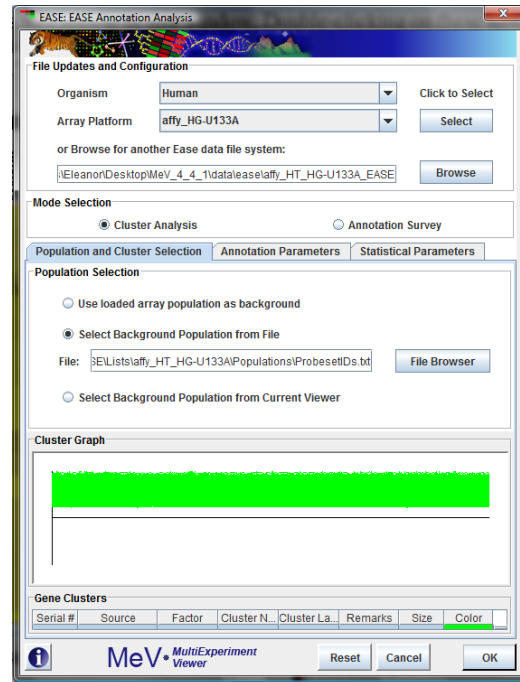


Figure 1: EASE dialog and Population Panel

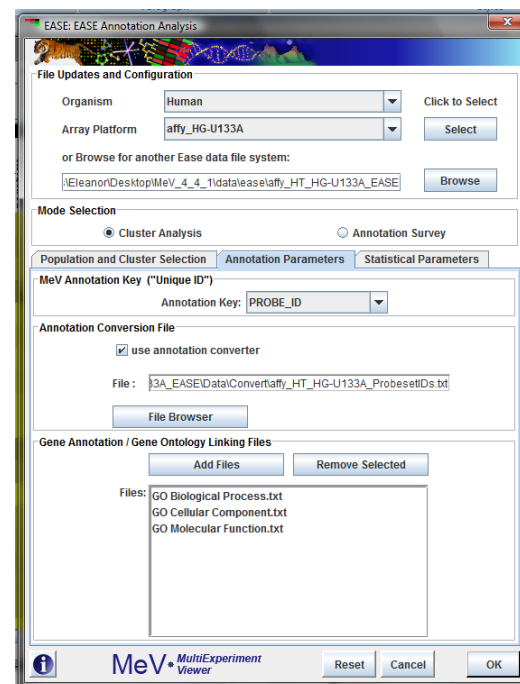


Figure 2: EASE Dialog and Annotation Panel

Step 4: Statistical Parameters Tab

Click to select the check box next to *Run Nested EASE*, near the bottom of the tab. Insure that the box labeled *Benjamini-Hochberg Method* is also checked.

Now click the *Ok* button at the bottom of the initialization dialog to run EASE and Nested EASE. This will take some time.

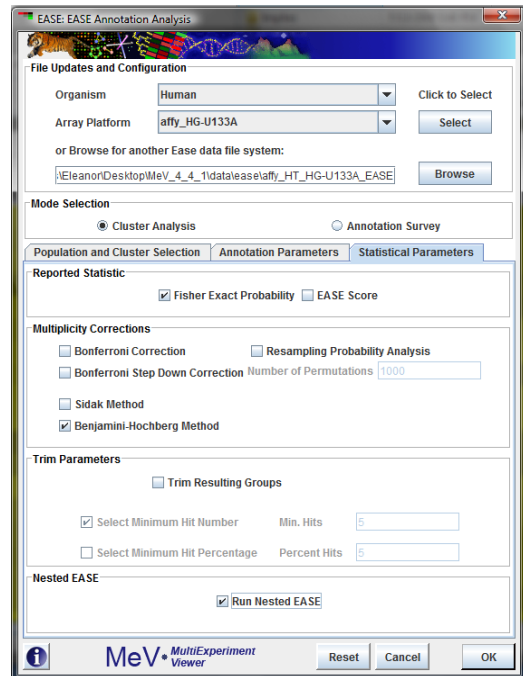


Figure 3: EASE Dialog and Statistical Panel

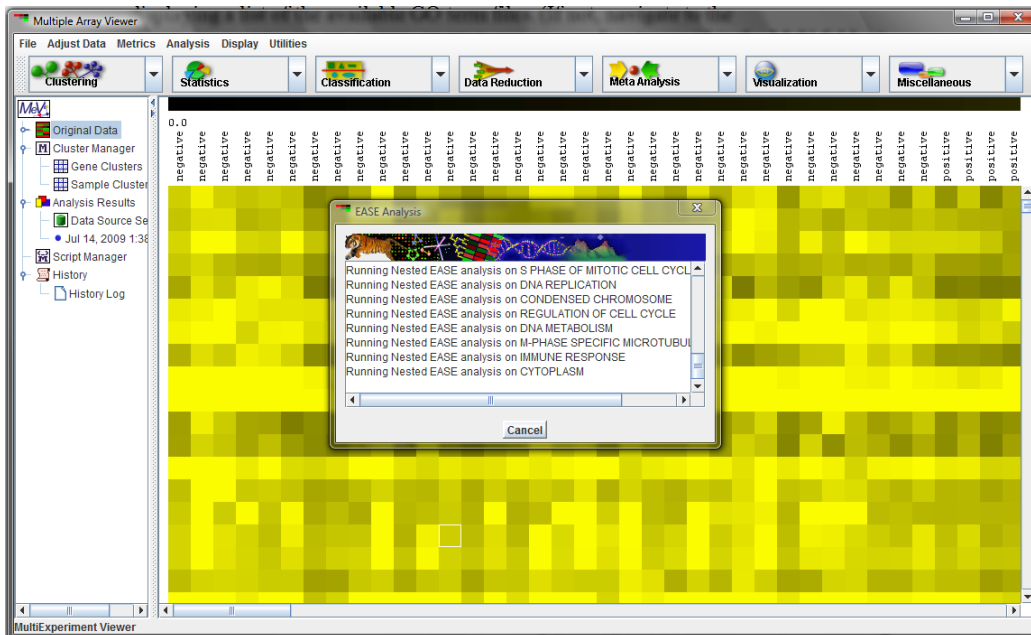


Figure 4: EASE and Nested EASE in Progress. This analysis will take quite a while to run.

Viewing the nEASE results

The standard EASE analysis, as described in Hosack, *et al.* (2003), will run, followed by the Nested EASE analysis. The nEASE results are included as part of the standard EASE results, as a subnode on the result tree, labeled *Nested EASE*. Double-click the result node labeled *EASE Analysis* to see the nested ease results appear.

We recommend that you expand the size of the window containing the Result Tree by clicking and dragging on the dividing bar between the Result Tree and the larger viewing window on the right.

Multiple Array Viewer

File Adjust Data Metrics Analysis Display Utilities

Clustering

Statistics

Classification

Data Reduction

Meta Analysis

Visualization

Miscellaneous

Original Data

Cluster Manager

Gene Clusters

Sample Clusters

Analysis Results

Data Source Selection

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EASE Analysis (1)

Table Viewer

Expression Viewers

General Information

GO Hierarchy – Biological Process

GO Hierarchy – Cellular Component

GO Hierarchy – Molecular Function

Nested EASE: 68 Terms

Nested EASE Summary Table

GO Hierarchy – Biological Process

GO Hierarchy – Cellular Component

GO Hierarchy – Molecular Function

nEASE run: GO Biological Process: CELL CYCLE

Table Viewer

Expression Viewers

General Information

GO Hierarchy – Biological Process

GO Hierarchy – Cellular Component

GO Hierarchy – Molecular Function

nEASE run: GO Biological Process: MITOTIC CELL CYCLE

nEASE run: GO Biological Process: CELL PROLIFERATION

nEASE run: GO Biological Process: M PHASE

nEASE run: GO Biological Process: NUCLEAR DIVISION

nEASE run: GO Biological Process: MITOSIS

nEASE run: GO Biological Process: DNA REPLICATION AND REPAIR

nEASE run: GO Biological Process: M PHASE OF MITOTIC

Index	File	Acc.	Term	List	List	Pop	Pop	Fish	Gen	p-val	nEA	Perc	EAS	EAS	
3	GO BiolGO:0	PHOSPHOINOSITIDE-MED15	341	17	808	1.029E-7	82542	2601	1.654E46	0.032	GO:000CELL				
17	GO BiolGO:0	SECOND-MESSENGER-ME19	341	30	808	1.438E6	33911	8361	19.08621	1.30	GO:000MITOT				
3	GO BiolGO:0	PHOSPHOINOSITIDE-MED15	225	17	486	3.424E7	12961	7360	958641	1.938	GO:000MITOT				
18	GO BiolGO:0	REGULATION OF PROTEIN5	225	5	486	2.076E2	68511	46763	139653	703	GO:000MITOT				
15	GO BiolGO:0	PHOSPHOINOSITIDE-MED15	519	21	1361	1.897E6	99150	99470	820933	294	GO:000MITOT				
13	GO BiolGO:0	DNA DAMAGE RESPONSE7	127	8	255	3.22E-2	3.01560	8010	0.024737	696	GO:000MITOT				
7	GO BiolGO:0	INDUCTION OF APOPTOSIS7	298	9	811	1.458E3	69251	51853	123841	0.033	GO:000MITOT				
11	GO BiolGO:0	MAINTENANCE OF FIDELITY4	298	4	811	1.8E-02	53021	12530	601063	255	GO:000MITOT				
13	GO BiolGO:0	RESPONSE TO TOXIN	4	298	4	811	1.8E-02	53021	45762	202663	255	GO:000MITOT			
14	GO BiolGO:0	MISMATCH REPAIR	4	298	4	811	1.8E-02	53021	28941	311963	255	GO:000MITOT			
17	GO BiolGO:0	ENZYME LINKED RECEPT26	298	51	811	2.261E7	26011	10862	456614	235	GO:000MITOT				
28	GO BiolGO:0	HEART DEVELOPMENT	11	298	19	811	4.736E4	01841	20516	794721	149	GO:000MITOT			
30	GO BiolGO:0	DEPHOSPHORYLATION	3	298	3	811	4.93E-1	89761	12533	432063	255	GO:000MITOT			
32	GO BiolGO:0	NEGATIVE REGULATION O3	298	3	811	4.93E-1	89761	54940	210363	255	GO:000MITOT				
34	GO BiolGO:0	APOPTOTIC NUCLEAR CH3	298	3	811	4.93E-1	89761	9150	968563	255	GO:000MITOT				
36	GO BiolGO:0	SIGNAL COMPLEX FORMA3	298	3	811	4.93E-1	89761	93061	210363	255	GO:000MITOT				
37	GO BiolGO:0	PROTEIN AMINO ACID DER3	298	3	811	4.93E-1	89761	12933	384763	255	GO:000MITOT				
20	GO CellGO:0	MIDBODY	6	1733	9	5649	2.816E3	23850	12930	101735	988	GO:000MITOT			
2	GO MolGO:0	HEPARIN BINDING	20	1684	37	5465	2.667E8	59871	46162	439523	239	GO:000MITOT			
6	GO MolGO:0	CYSTEINE PROTEASE INH7	1684	10	5465	1.234E3	9181	0851	378739	185	GO:000MITOT				
7	GO MolGO:0	CALCIUM-RELEASE CHAN5	1684	6	5465	1.235E3	15110	59530	450152	519	GO:000MITOT				
8	GO MolGO:0	GTP BINDING	50	1684	124	5465	1.455E11	7900	88782	50889	5083	GO:000MITOT			
11	GO MolGO:0	GUANYL NUCLEOTIDE BIN50	1684	128	5465	2.74E-10	55570	85933	86278	2483	GO:000MITOT				
14	GO MolGO:0	DNA (CYTOSINE-5-)METH3	1684	3	5465	2.922E2	07550	40610	225069	185	GO:000MITOT				
16	GO MolGO:0	DNA-METHYLTRANSFERA3	1684	3	5465	2.922E2	07550	70010	512469	185	GO:000MITOT				
20	GO MolGO:0	SULFURIC ESTER HYDRO3	1684	3	5465	2.922E2	07550	92090	524169	185	GO:000MITOT				
24	GO MolGO:0	PHOSPHOFRUCTOKINAS4	1684	5	5465	3.39E-2	45920	51190	470949	185	GO:000MITOT				
25	GO MolGO:0	GDP BINDING	4	1684	5	5465	3.39E-2	45920	2540	183549	185	GO:000MITOT			
28	GO MolGO:0	PROTEIN-TYROSINE-PHO19	1684	43	5465	4.379E5	74960	9934	60231	37	GO:000MITOT				
31	GO MolGO:0	GLYCOSAMINOGLYCAN BI20	1684	46	5465	4.669E5	82540	84653	413612	863	GO:000MITOT				
12	GO BiolGO:0	INDUCTION OF APOPTOSIS6	87	7	191	3.545E2	81151	13232	242340	164	GO:000MITOT				
16	GO BiolGO:0	ACTIN FILAMENT ORGANIZ4	87	4	191	4.143E2	17801	07331	694354	450	GO:000MITOT				
17	GO BiolGO:0	MAINTENANCE OF FIDELITY4	87	4	191	4.143E2	17800	76330	248554	450	GO:000MITOT				
19	GO BiolGO:0	MISMATCH REPAIR	4	87	4	191	4.143E2	17800	92730	959754	450	GO:000MITOT			
10	GO BiolGO:0	SECOND-MESSENGER-ME7	74	7	157	4.418E3	7002	34916	44752	866	GO:000MITOT				
15	GO BiolGO:0	CELL PROJECTION BIOG4	74	4	157	4.724E2	11460	81080	123552	866	GO:000MITOT				
17	GO BiolGO:0	CILIUM BIOGENESIS	4	74	4	157	4.724E2	11460	450552	866	GO:000MITOT				
3	GO CellGO:0	OBSOLETE CELLULAR CO113	1200	250	3827	1.422E34	6062	83844	71813	843	GO:000MITOT				
5	GO CellGO:0	SPINDLE	56	1200	116	3827	8.259E19	6261	58540	749816	918	GO:000MITOT			
10	GO CellGO:0	ENDOSOME	18	1200	28	3827	3.237E9	22022	83564	041132	926	GO:000MITOT			
17	GO CellGO:0	SPINDLE POLE	40	1200	88	3827	3.486E12	4061	16962	078314	098	GO:000MITOT			
26	GO CellGO:0	ENDOSOME MEMBRANE	5	1200	6	3827	1.337E3	11961	79345	71851	977	GO:000MITOT			
27	GO CellGO:0	CYTOSOLIC VESICLE	16	1200	54	3827	1.456E6	27064	734610	65490	256	GO:000MITOT			

MultiExperiment Viewer

Figure 5: Nested EASE Results. The summary table.

Click on the result nodes to explore the result data within. The most useful view is found in a node labeled *Nested EASE Summary Table*, which contains the summarized data for all of the nEASE results. These are the data reported in the manuscript.

To examine the genes which drive a result row in the nEASE Summary Table, left-click to select a row, and right-click to open up a context-sensitive menu. Choose *Open Viewer* -> *Expression Image*. MeV will open a heatmap view of the probe expression values that correspond to the genes that were members of that group.

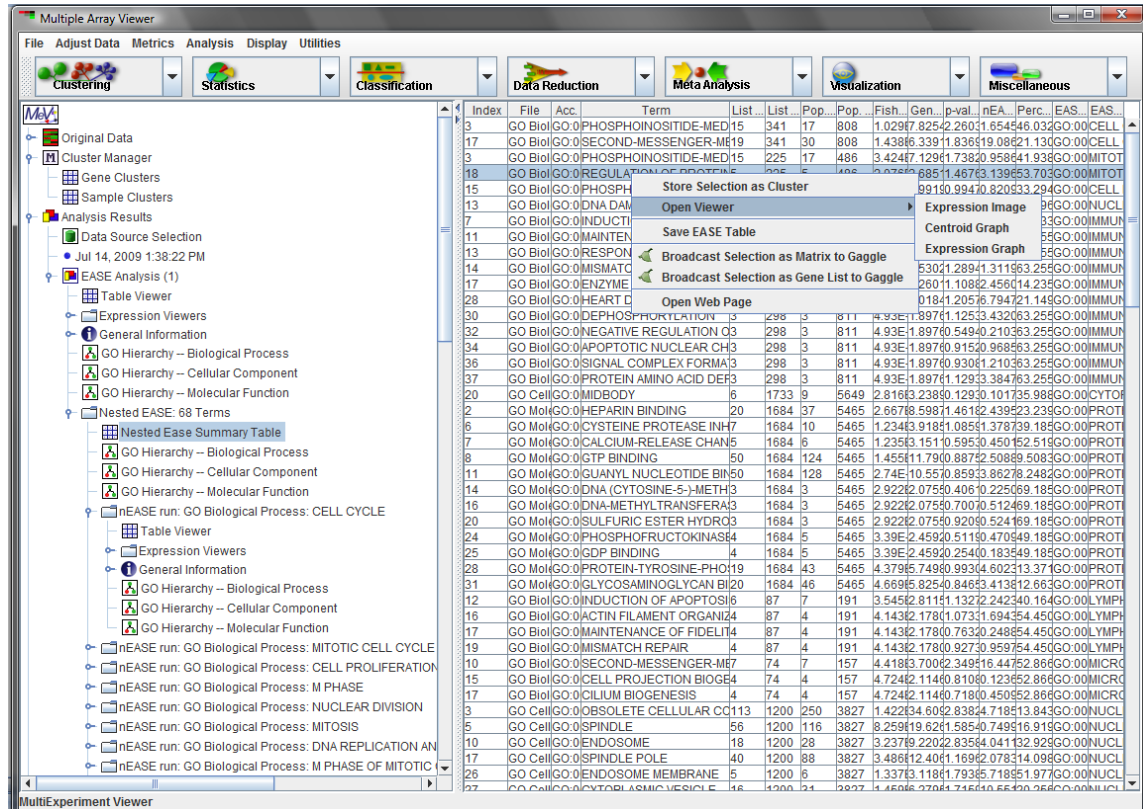


Figure 6: Nested EASE Results. Navigation.

All of the usual heatmap-related options are available in this window, including adjusting the color display, changing the gene annotation displayed, and storing the displayed genes in a new cluster. Note that the left-hand panel, the Result Tree, does not obviously change when this heatmap is opened. However, scrolling the Result Tree down will reveal that a new result node has been selected, corresponding to the Nested EASE result that was selected from the Summary Table.

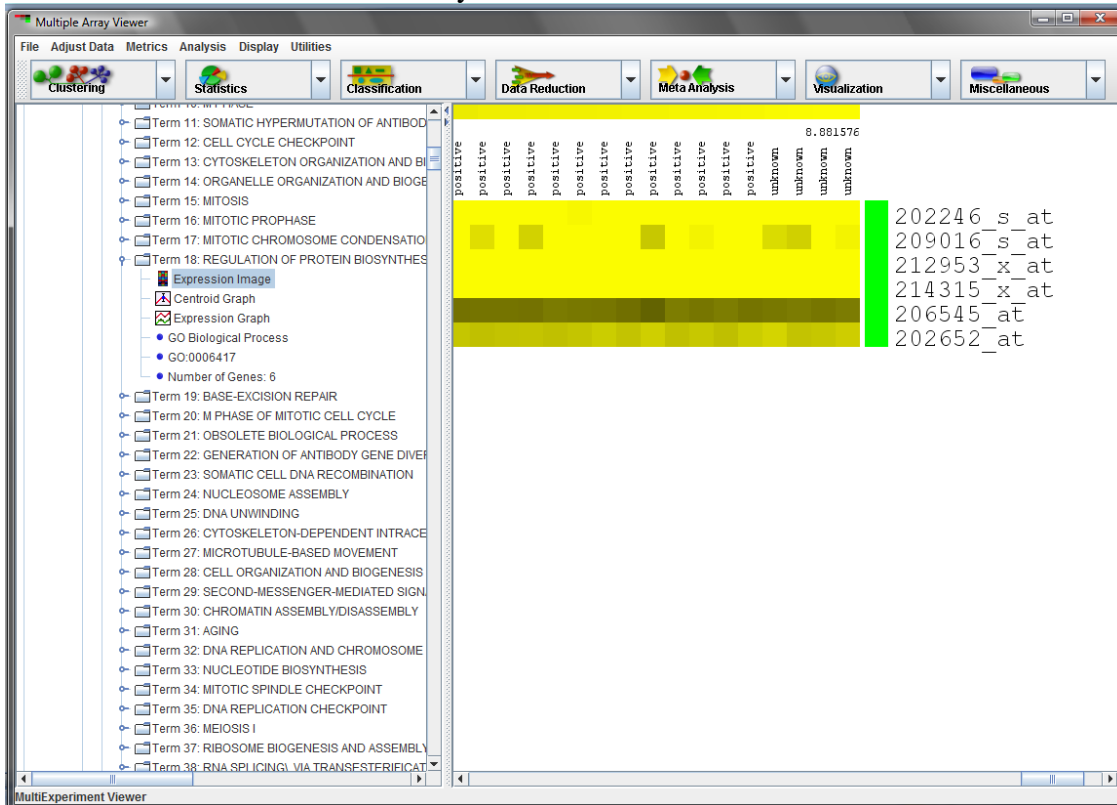


Figure 7: Nested EASE Results. Viewing the individual probe expression values.

References

- D. A. Hosack, G. Dennis, B. T. Sherman, H. C. Lane, R. A. Lempicki, *Genome Biol* **4** (2003).
- A. J. Minn, *et al.* , *Nature* **436**, 518. (2005)
- L. D. Miller, *et al.* , *Proceedings of the National Academy of Sciences* **102**, 13550 (2005).