Statistical

Microarray Data Analysis

Time Course Microarray Experiments

96 陽明大學生物資訊與系統生物學學分班
Course: 系統生物學實驗

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Outlines

- Time Series Microarray Experiments
- Overview of Analyzing Software

- Some Issues
  - P-values
  - Multiple Hypothesis Testing
  - Permutation Test
  - Gene Set Enrichment Analysis

- SAM: Significance Analysis of Microarrays
  - Algorithm
  - Interpretation

Differential Expressed Genes

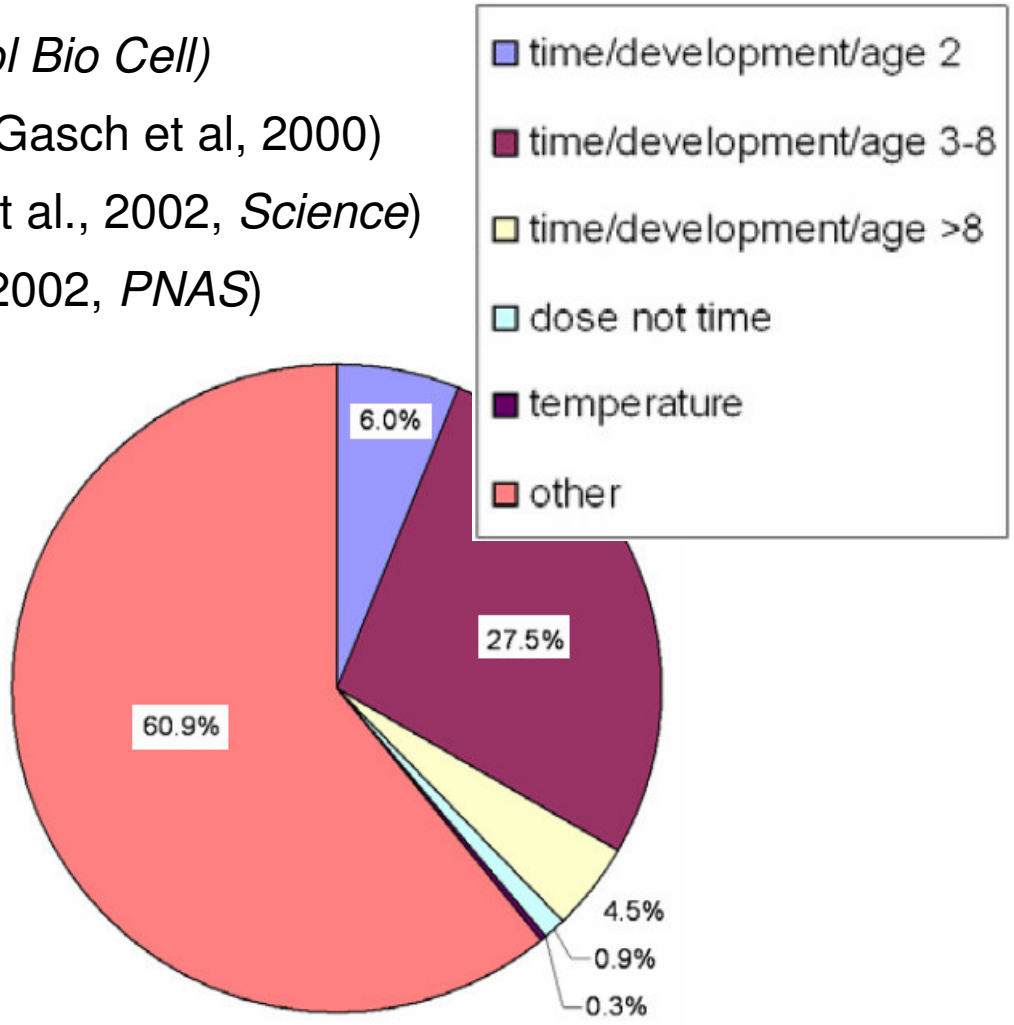
- STEM: Short Time-Series Expression Miner
  - Algorithm
  - Example

Clustering

Time Series Microarray Experiments

Study dynamic biological process

- Cell cycle (Spellman et al., 1998, *Mol Bio Cell)*
- Response to temperature changes (Gasch et al, 2000)
- Developmental studies (Arbeitman et al., 2002, *Science*)
- Immune response (Guillemin et al., 2002, *PNAS*)



Distribution of Microarray Data Sets in the Gene Expression Omnibus

NCBI GEO Gene Expression Omnibus

Distribution of microarray experiments by type. Summary of the 786 microarray datasets for human, mouse, rat, and yeast in the Gene Expression Omnibus as of August 2005.

Source: Ernst and Bar-Joseph. 2006, BMC *Bioinformatics.*

Time Course Experiments

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Gene Search

Experiment: AtGenExpress: Heat stress time course

Experiment Summary | Samples | Slides & Datasets | Array Design | View All

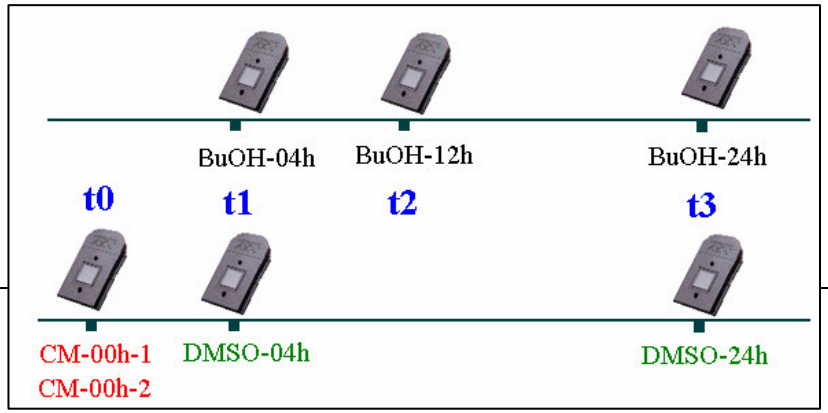
Submission Number	ME00339
TAIR Accession	ExpressionSet:1007967124
Author(s)	Lutz Nover , Pascal von Koskull-Döring
Organization(s)	AtGenExpress
Experimental Variables	root , shoot , time , cultured cell , heat
Variable Type	Plant Material
Experiment Category	abiotic treatment , tissue comparison
Experiment Goals	response to heat
Description	This experiment studies the effect of hea in shoot and root tissue of 16 days old A Columbia-0 ecotype and in cell culture a two samples from each time point, whic were taken in duplicate in a time course (0.25h, 0.5h, 1h, 3h of heat stress and at heat stress). Control samples for the ce non-treated cells (0h; 3h; 6h; 12h; 24h at expression data of the samples were ge the ATH1 chip from Affymetrix. The stud which is funded by the DFG.
Data Counts	Number of Slides: 94 — Number of Replic: 47

NCGR ABRC

General comments or questions: curator@a

Seed or DNA stock questions (donations, availa

abrc@arabidopsis.org

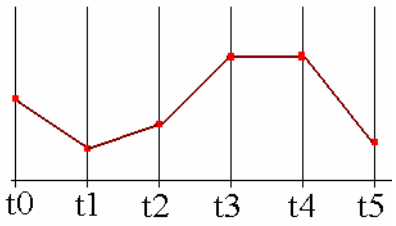


Root (r)
Shoot (s)
Cell Culture (c)

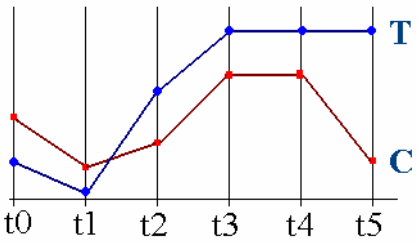
Treatment
3 x 8 x 2 = 48

Control
23 x 2 = 46

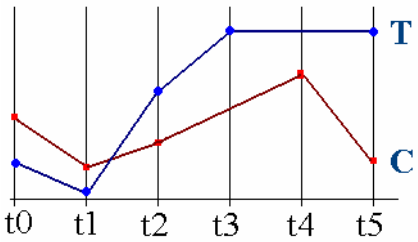
0	.25	.5	1	3	4	6	12	24
c r s	c r s	c r s	c r s	c r s	c r s	c r s	c r s	c r s
c r s				c r s		c r s	c r s	c r s



One Class



Two Class (Paired)



Two Class (Unpaired)

Short Time Series Microarray Experiments

- About 80 % of microarray time series experiments are short:
  - 3-8 time points.
  - Cost of microarray.
  - limited availability of biological material.

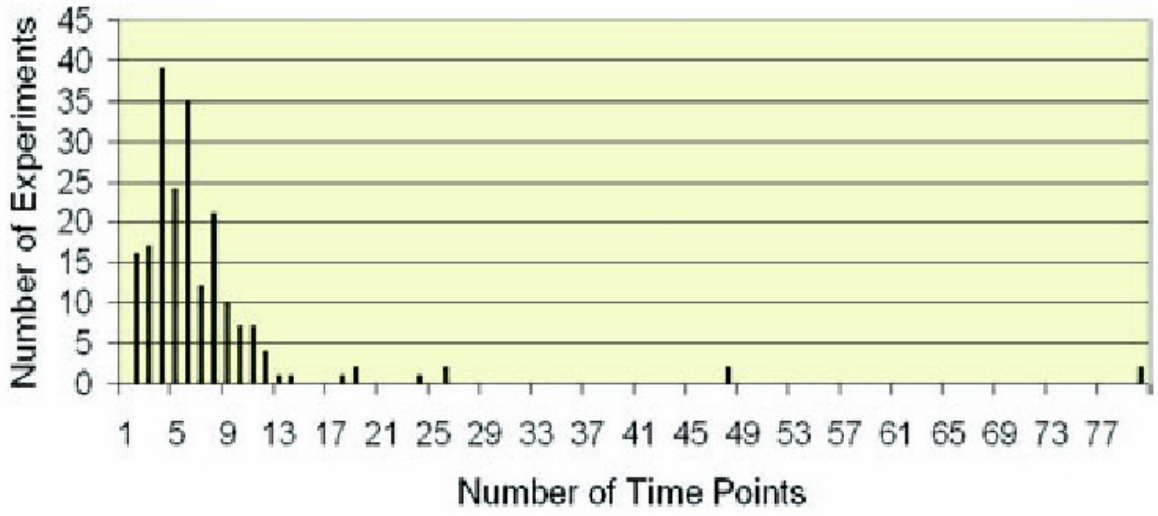


Fig. 1. Distribution of lengths of times series in the SMD as of June 2004.

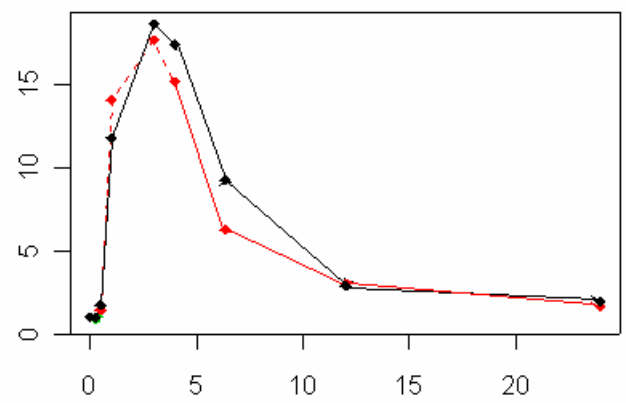
NOTE: SMD (June, 2004): ~170 published papers, ~30% are time series.

Source: Ernst et al., 2005, *Bioinformatics*.

Analyzing Software

Software for *Static* Gene Expression Data

- Do not take advantage of the sequential information in time series data.
- Popular clustering: hierarchical clustering, kmeans clustering, self-organizing maps.
  - ignore the temporal dependency among successive time points.
  - random permute the order of time points, the results would not change.

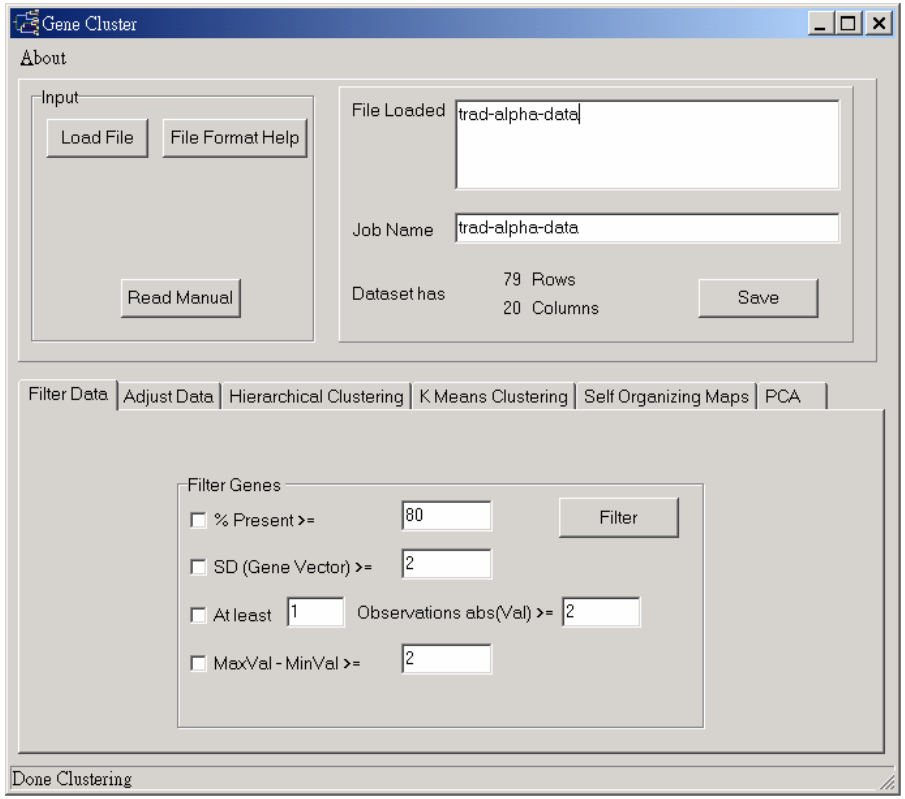


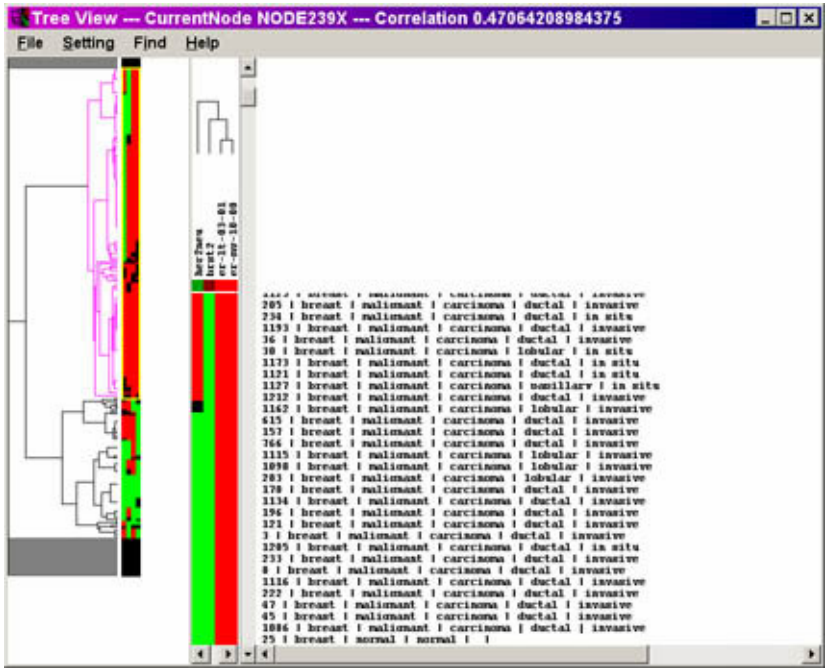
Software for *Time Series* Gene Expression Data

Software for *Static* Gene Expression Data (Clustering and Visualization)

Cluster & TreeView

Eisen et al., 1998, *PNAS*



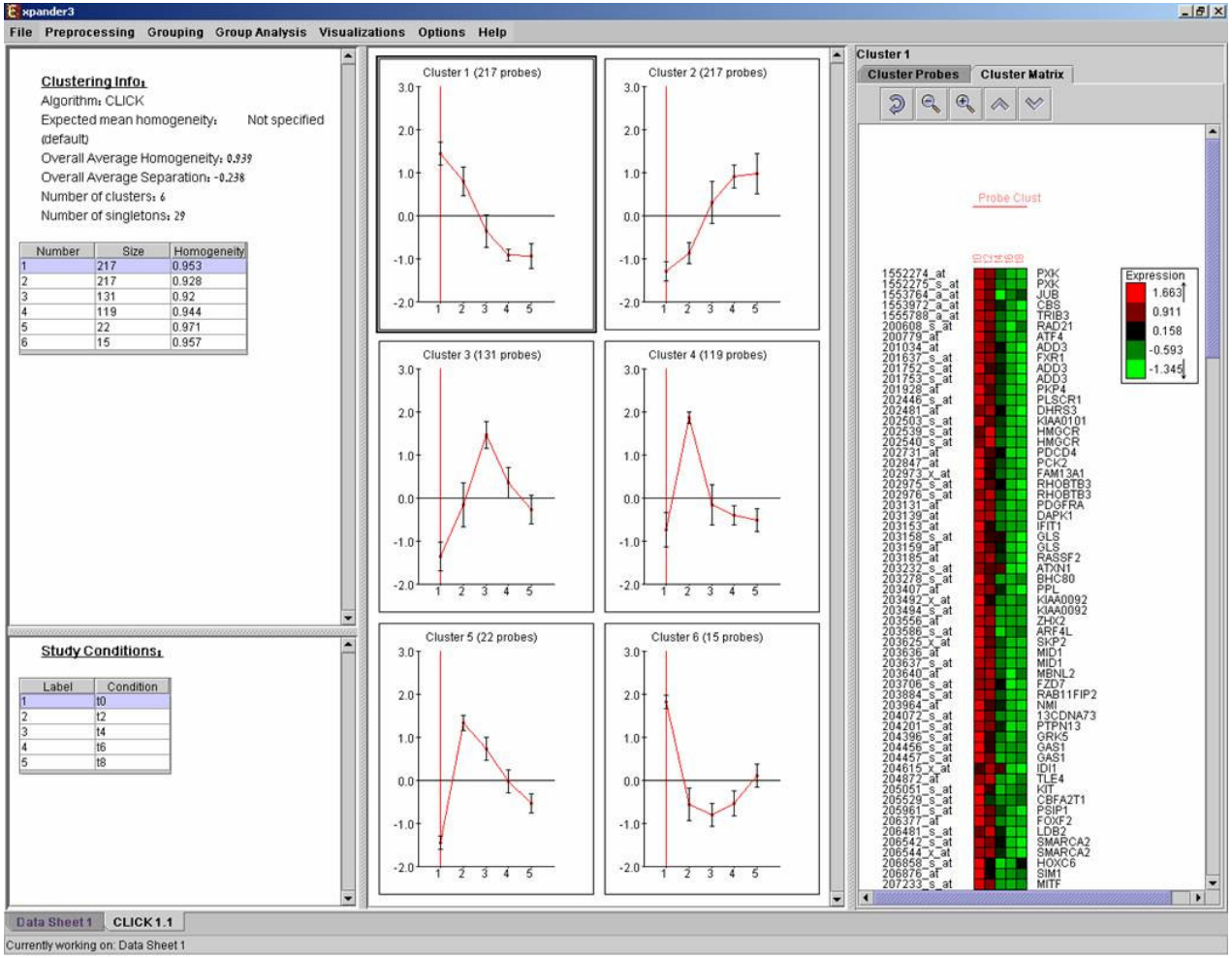


http://rana.lbl.gov/EisenSoftware.htm

Software for *Static* Gene Expression Data (Clustering and Visualization)

EXPANDER

(EXpression Analyzer and DisplayER) Shamir et al., 2005, *BMC Bioinformatics*

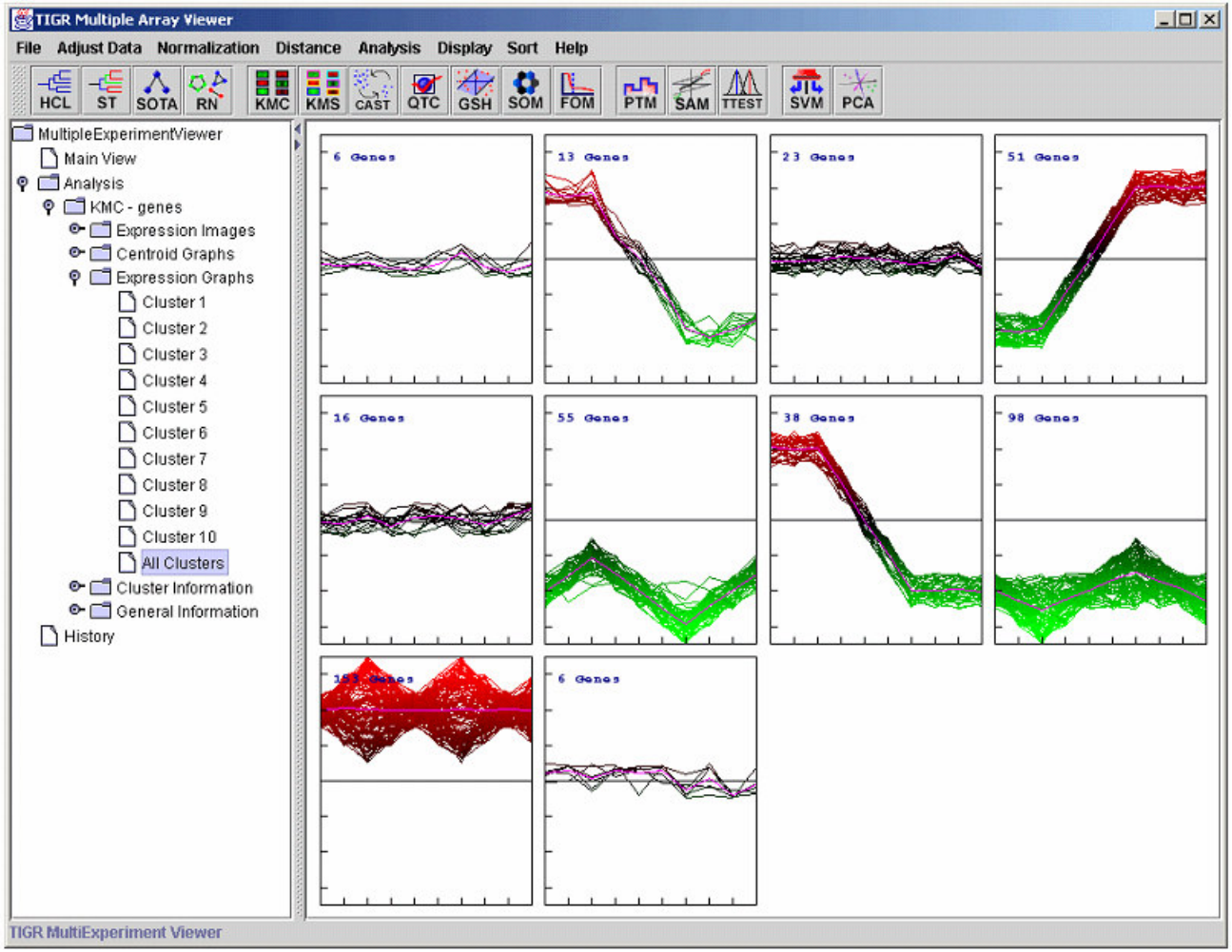


http://www.cs.tau.ac.il/~rshamir/expander/expander.html

Software for *Static* Gene Expression Data
(General Purpose)

TM4: **MeV**

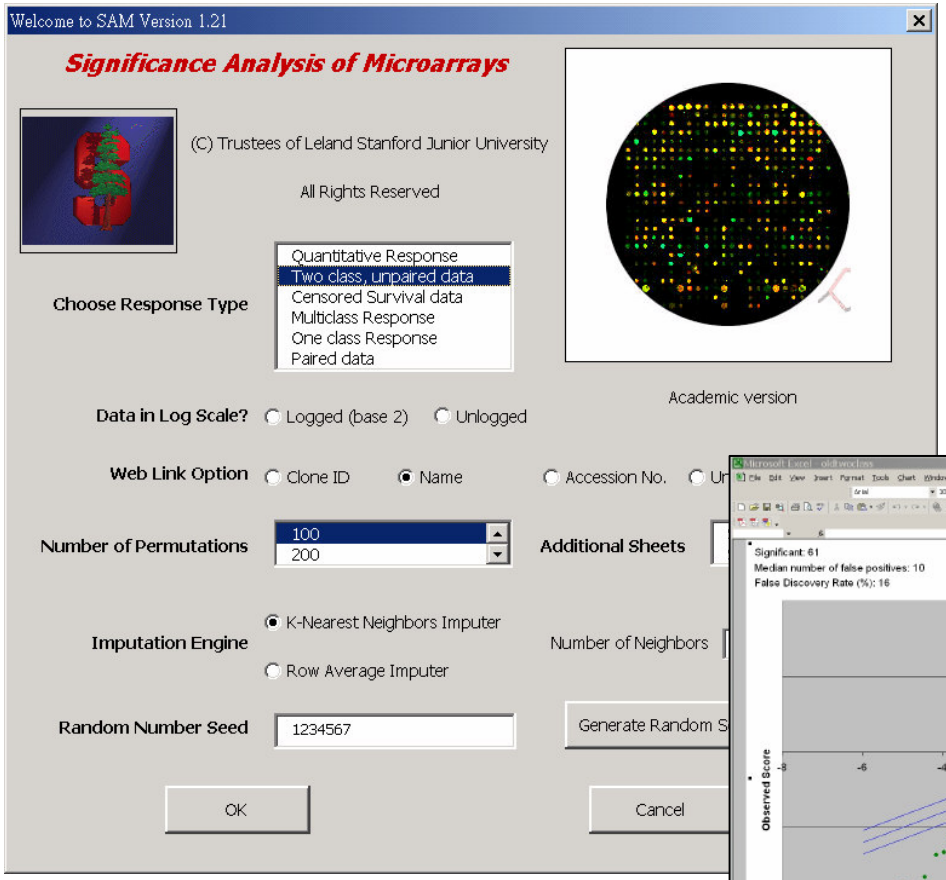
(MultiExperiment Viewer) Saeed et al., 2003, *Biotechniques*



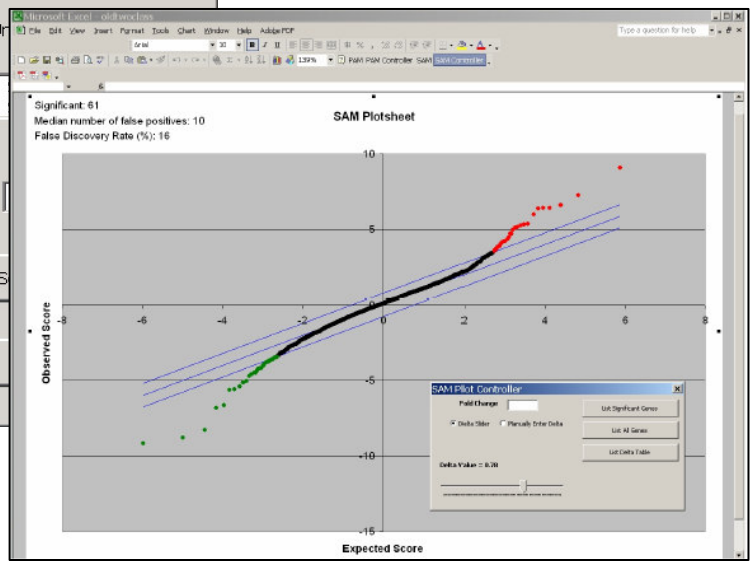
http://www.tm4.org/mev.html

Software for *Static / Time Series* Gene Expression Data (Differential Expressed Genes)

SAM:
Significance Analysis of Microarrays, Detect differentially expressed gene in time series data. (Tusher et al., 2001, *PNAS*)



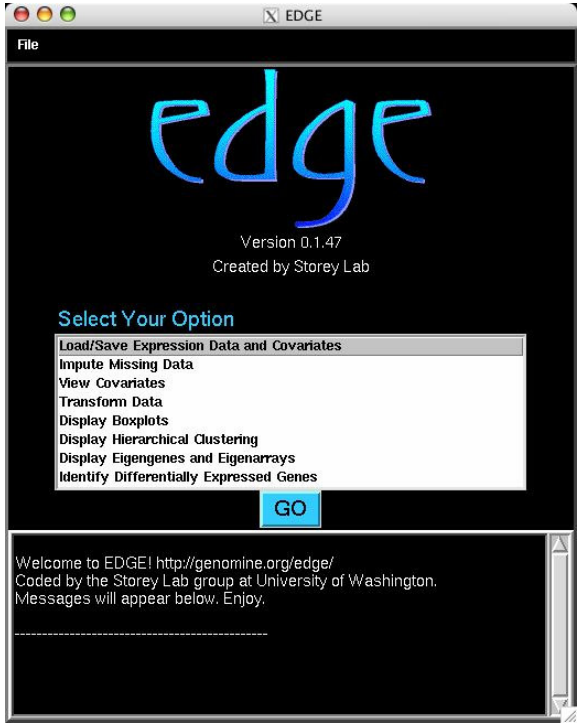
SAM plot

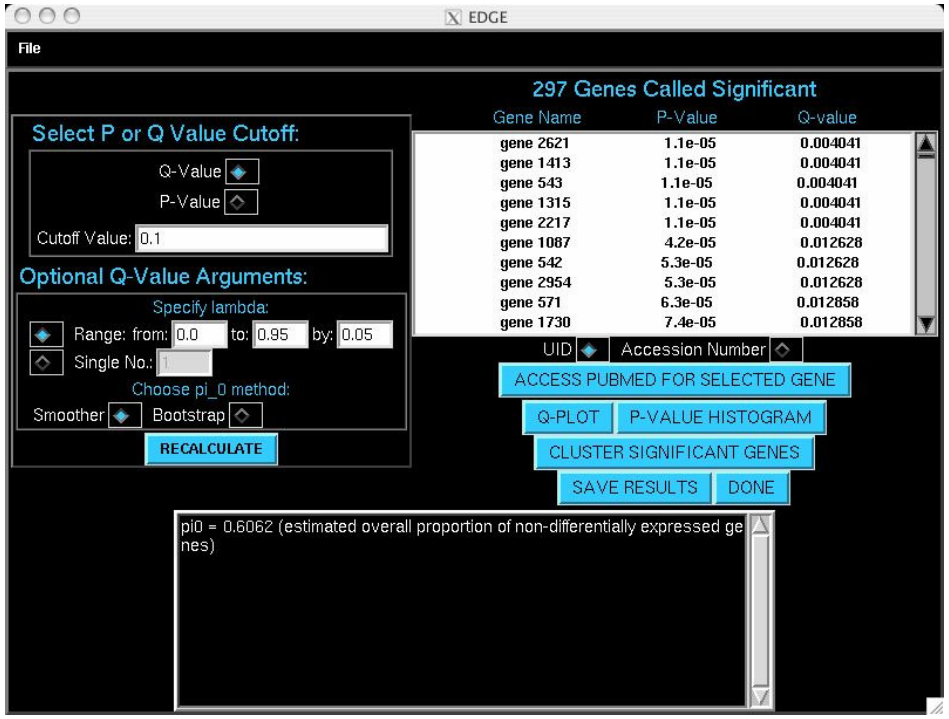


http://www-stat.stanford.edu/~tibs/SAM/

Software for *Time Series* Gene Expression Data (Differential Expressed Genes)

EDGE:
Extraction of Differential Gene Expression
(Leek et al., 2006, *Bioinformatics*)



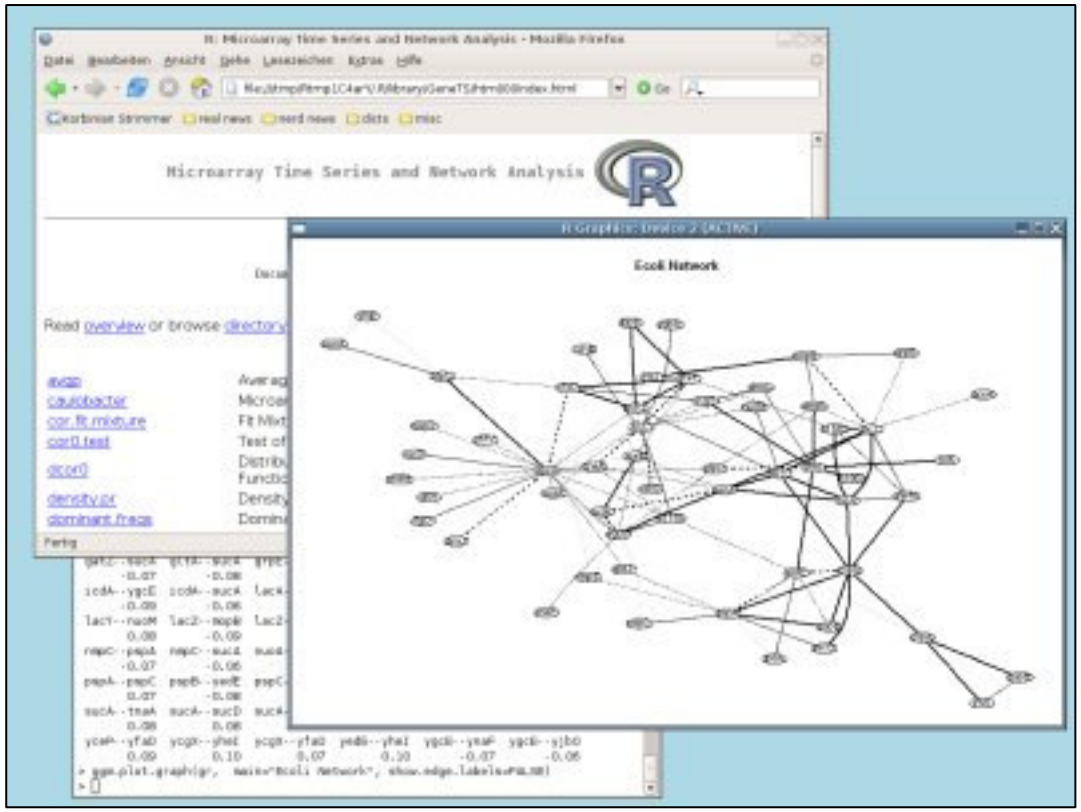


http://www.biostat.washington.edu/software/jstorey/edge/

Timecourse differential expression method: Storey JD, Xiao W, Leek JT, Tompkins RG, and Davis RW. (2005) Significance analysis of time course microarray experiments. Proceedings of the National Academy of Sciences, 102: 12837-12842.

Software for *Time Series* Gene Expression Data (Differential Expressed Genes and Networks)

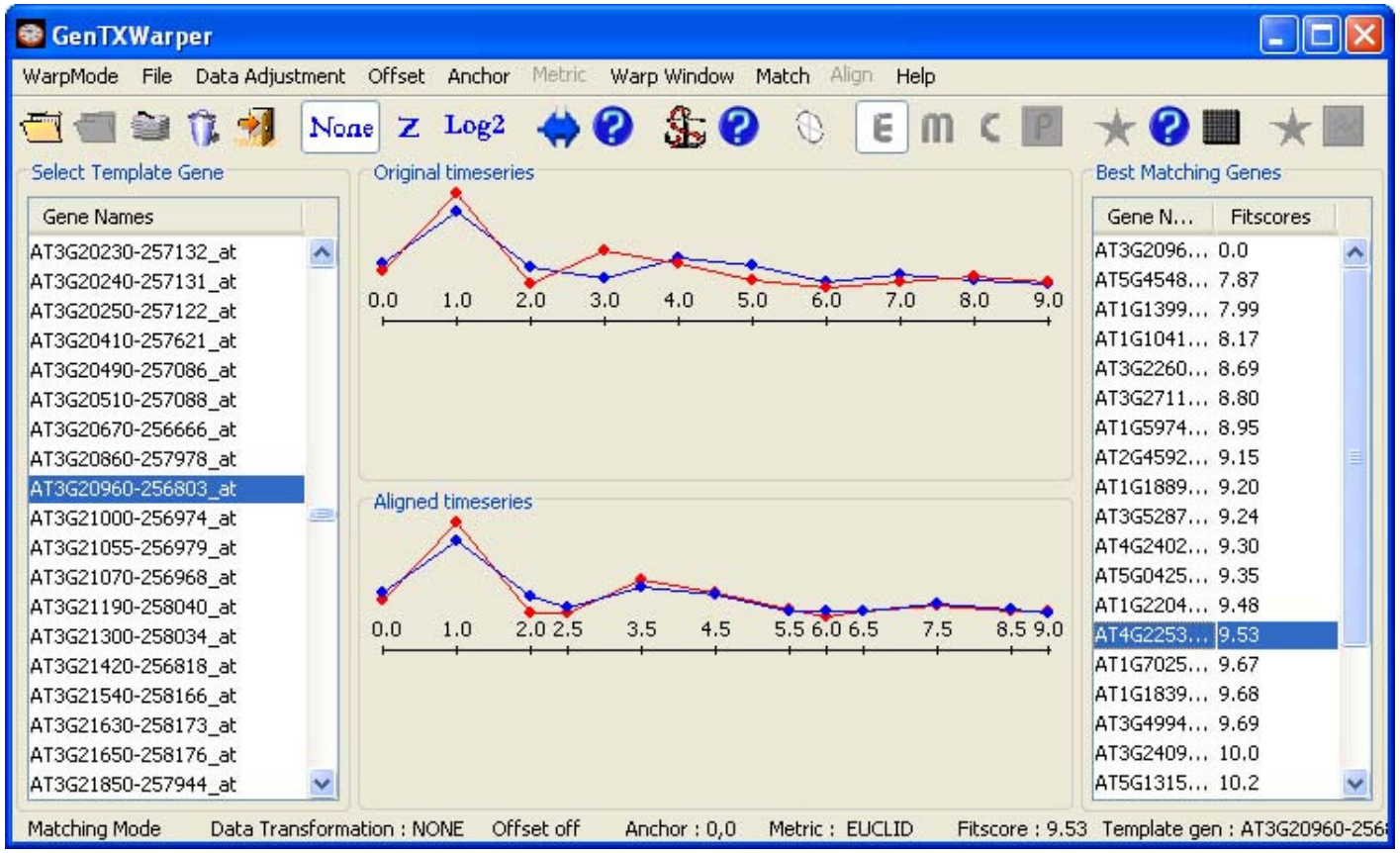
R package, **GeneTS**:
Microarray Time Series and Network Analysis. Detect periodically expressed gene. (Wichert et al., 2004, *Bioinformatics*)



http://www.strimmerlab.org/software/genets/

Software for *Time Series* Gene Expression Data (Visualization)

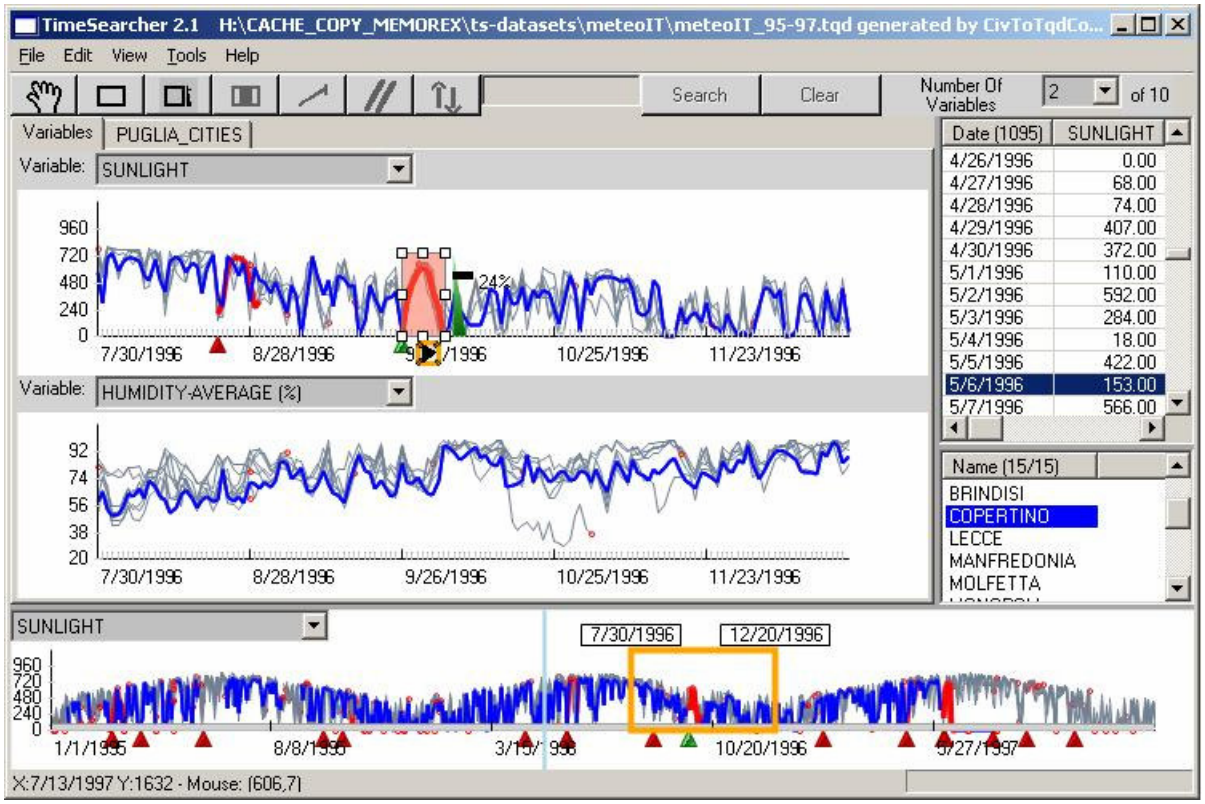
GenT χ Warper:
Mining of gene expression time series with dynamic time warping techniques (Criel and Tsiporkova, 2005, *Bioinformatics*)



http://www.psb.ugent.be/cbd/papers/gentxwarper/

Software for *Time Series* Gene Expression Data (Visualization)

TimeSearcher:
Visual Exploration of Time-Series Data
(Hochheiser et al, 2003)



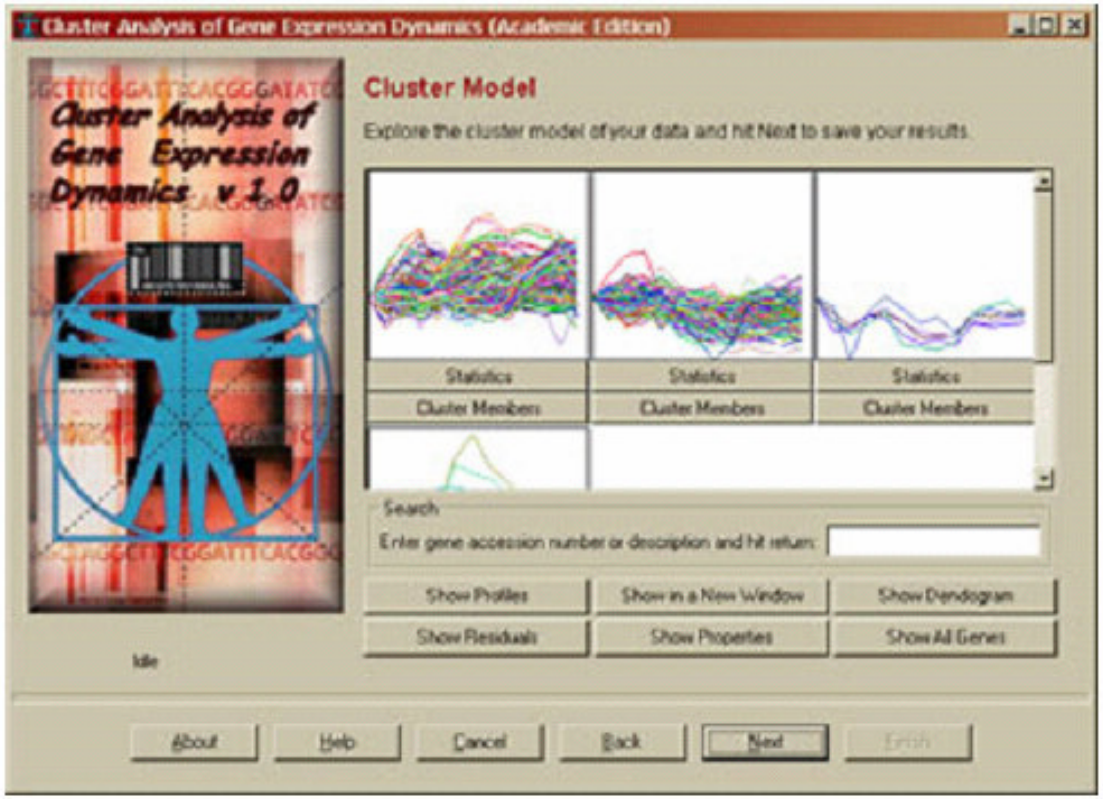
http://www.cs.umd.edu/hcil/timesearcher/

ORIOGEN:
Order Restricted Inference for Ordered Gene ExpressioN clustering for time series.
(Peddada et al., 2005, *Bioinformatics*) http://dir.niehs.nih.gov/dirbb/oriogen1/index.cfm

Software for *Time Series* Gene Expression Data (Visualization and Clustering)

CAGED:

Cluster analysis of gene expression dynamics based on autoregressive equations (Ramoni et al., 2002, *PNAS*)

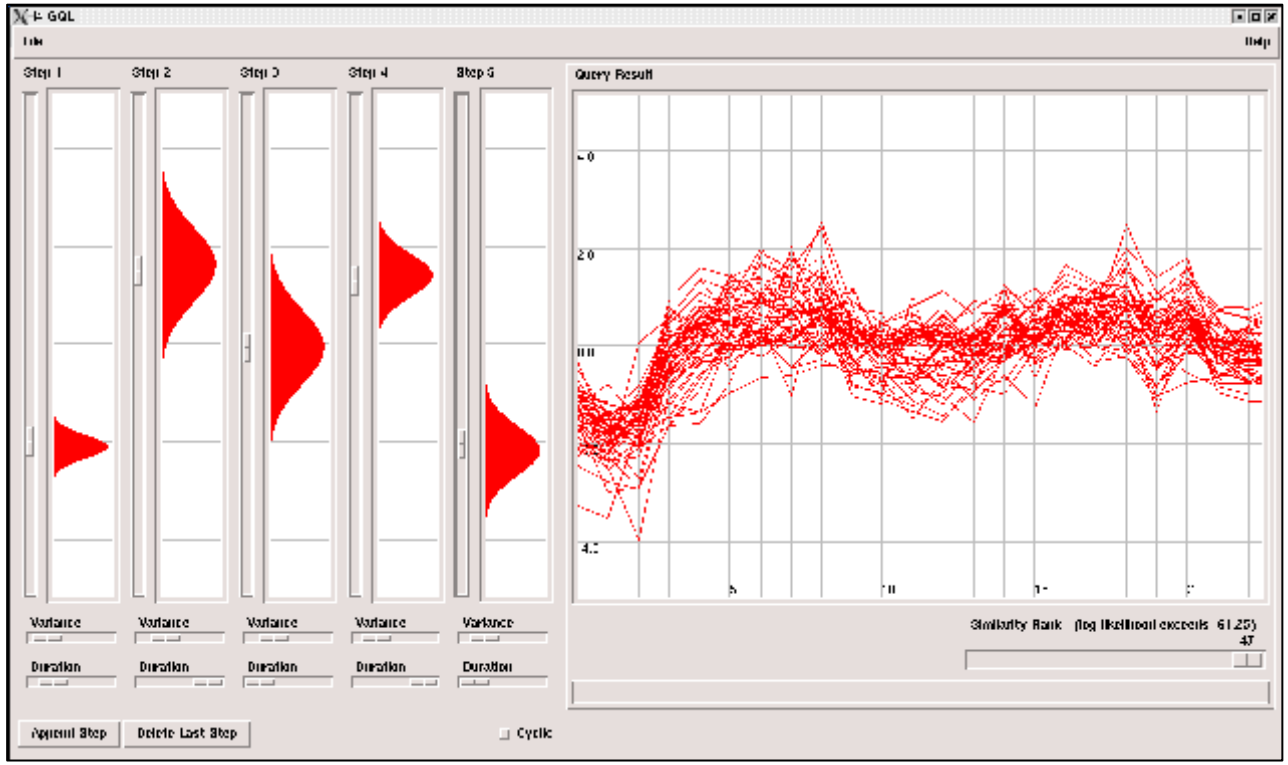


http://genomethods.org/caged/

Software for *Time Series* Gene Expression Data (Visualization and Clustering)

GQL:

The Graphical Query Language: A GHMM-based tool for querying and clustering Gene-Expression time-course data (Costa et al., 2005, *Bioinformatics*)



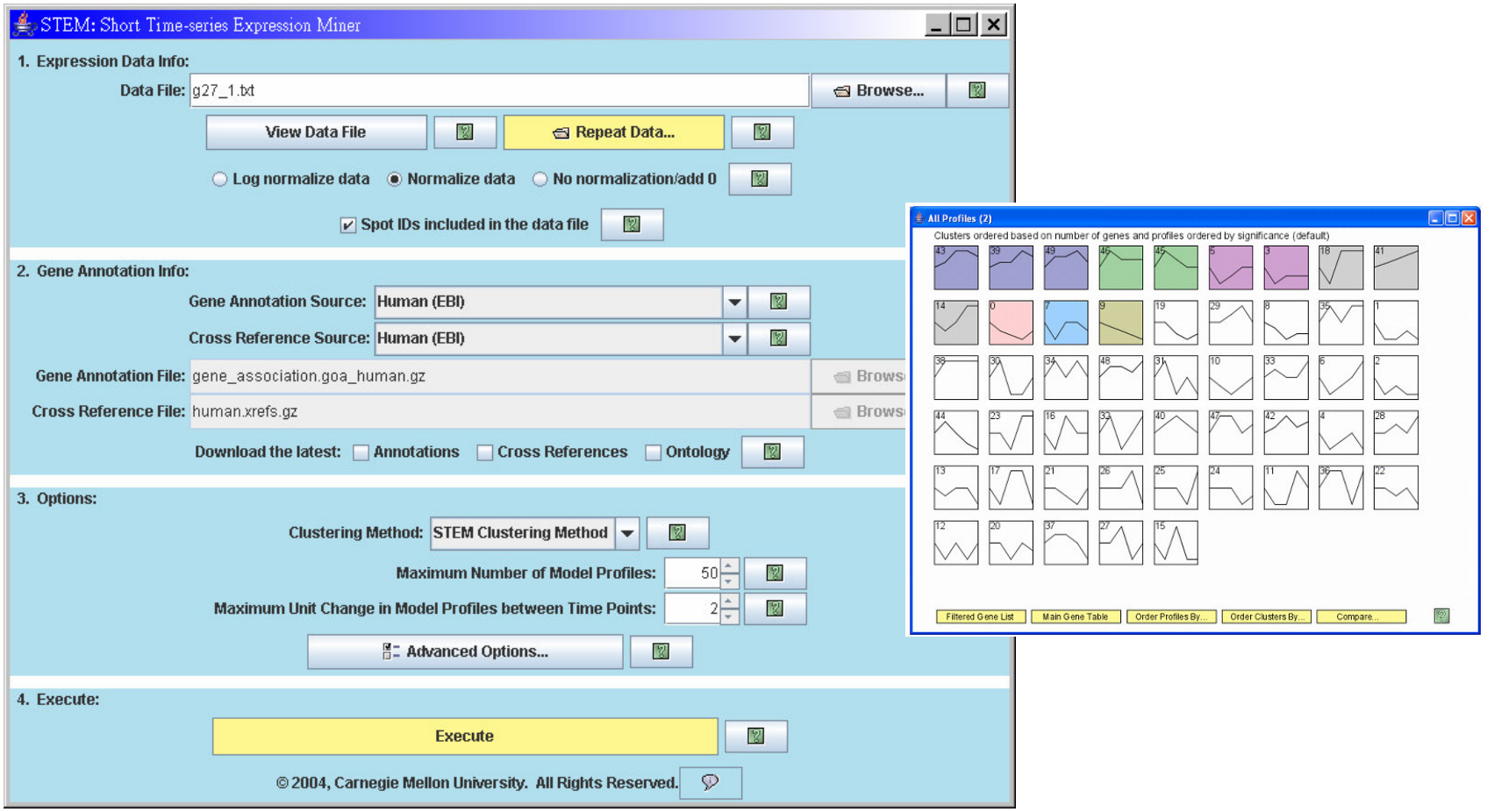
http://www.ghmm.org/gql

Software for *Short Time Series* Gene Expression Data (clustering and visualization)

STEM:

Short Time-series Expression Miner

(Ernst and Bar-Joseph. 2006, *BMC Bioinformatics*.)

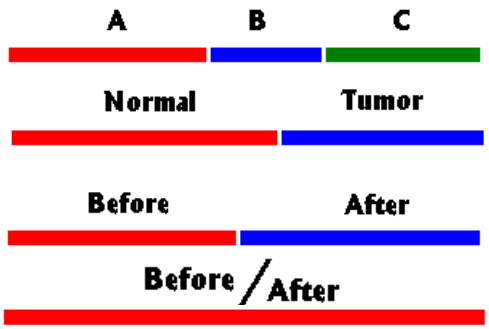


http://www.cs.cmu.edu/~jernst/stem/

Some Issues

- The p-values
- Multiple Testing Corrections
- Permutation Test
- Correlation Coefficient
- Gene Set Enrichment Analysis

Finding Differentially Expressed Genes



→ More than two samples

→ Two-sample (independent)

Cy 5: treatment

Cy 3: control

MA Table	exp01	exp02	exp03	exp04	exp05	exp•••	exp p
gene001	-0.48	-0.42	0.87	0.92	0.67		-0.35
gene002	-0.39	-0.58	1.08	1.21	0.52		-0.58

→ Paired-sample (dependent)

MA Table	exp01	exp02	exp03	exp04	exp05	exp•••	exp p	p-values	
gene001	-0.48	-0.42	0.87	0.92	0.67		-0.35	0.067	
gene002	-0.39	-0.58	1.08	1.21	0.52		-0.58	0.052	
gene003	0.87	0.25	-0.17	0.18	-0.13		-0.13	0.013	*
gene004	1.57	1.03	1.22	0.31	0.16		-1.02	0.016	*
gene005	-1.15	-0.86	1.21	1.62	1.12		-0.44	0.112	
gene006	0.04	-0.12	0.31	0.16	0.17		0.08	0.017	*
gene007	2.95	0.45	-0.40	-0.66	-0.59		-0.76	0.059	
gene008	-1.22	-0.74	1.34	1.50	0.63		-0.55	0.063	
gene009	-0.73	-1.06	-0.79	-0.02	0.16		0.03	0.516	
gene010	-0.58	-0.40	0.13	0.58	-0.09		-0.45	0.009	*
gene011	-0.50	-0.42	0.66	1.05	0.68		0.01	0.068	
gene012	-0.86	-0.29	0.42	0.46	0.30		-0.63	0.030	*
gene013	-0.16	0.29	0.17	-0.28	-0.02		-0.04	0.002	*
gene014	-0.36	-0.03	-0.03	-0.08	-0.23		-0.21	0.423	
gene015	-0.72	-0.85	0.54	1.04	0.84		-0.64	0.084	
gene016	-0.78	-0.52	0.26	0.20	0.48		0.27	0.048	
gene017	0.60	-0.55	0.41	0.45	0.18		-1.02	0.018	*
gene018	-0.20	-0.67	0.13	0.10	0.38		0.05	0.538	
gene019	-2.29	-0.64	0.77	1.60	0.53		-0.38	0.053	
gene020	-1.46	-0.76	1.08	1.50	0.74		-0.70	0.074	
gene021	-0.57	0.42	1.03	1.35	0.64		-0.40	0.764	
gene022	-0.11	0.13	0.41	0.60	0.23		0.19	0.423	
gene•••									
gene n	-1.79	0.94	2.13	1.75	0.23		-0.66	0.723	

Microarray Data Matrix

gene001	-0.48	-0.42	0.87	0.92	0.67		-0.35
gene022	-0.11	0.13	0.41	0.60	0.23		0.19

Significance Level: alpha

H0: no differential expressed.

- The test is significant = Reject **H0**

- *p*: probability of observing your data under the assumption that the null hypothesis is true.
- *p*: probability that you will be in error if you reject the null hypothesis.
- *p*: probability of **false positives** (Reject **H0** | **H0** true).

The *p*-values for detecting DE genes

The p-value is the probability that a gene's expression level are different between the two groups **due to chance**.

False Positive = (Reject **H0** | **H0** true)
= concluding that a gene is differentially expressed when in fact it is not.

Decision Rule

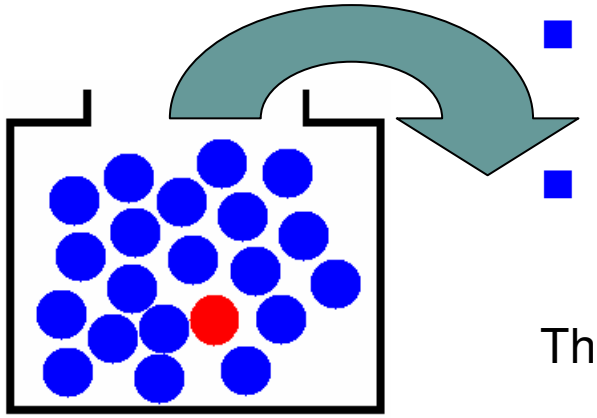
- Reject H_0 if *P* is less than alpha.
- $P < 0.05$ commonly used. (Reject $\mathbf{H_0}$, the test is significant)
- The lower the p-value, the more significant the difference between the groups.

Type I Error (alpha): calling genes as differentially expressed when they are NOT
Type II Error: NOT calling genes as differentially expressed when they ARE

Hypothesis Testing		Truth	
		H0	**H1**
Decision	**Reject H0**	Type I Error (alpha) (false positive)	Right Decision (true positive)
	Don't Reject H0	Right Decision	Type II Error (beta)

Power $= 1 - \beta$.

Multiple Hypothesis Correction



Population

20 marbles:

19:

1:

- What are the odds of randomly sampling the red marble by chance? It is 1 out of 20.
- Sample a single marble (and put it back) 20 times. Have a much higher chance to sample the red marble.

This is exactly what happens when testing several thousand genes at the same time.

Imagine that the red marble is a false positive gene: the chance that false positives are going to be sampled is higher the more genes you apply a statistical test on.

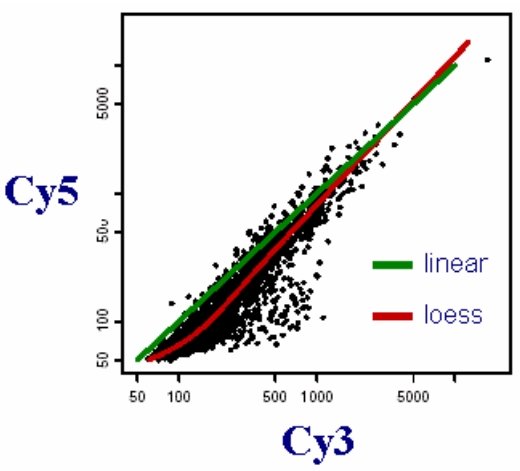
X: false positive gene

$$P(X \geq 1)$$
$$= 1 - P(X = 0)$$
$$= 1 - 0.95^n$$

Multiplicity of Testing

Number of genes tested (N)	False positives incidence	Probability of calling 1 or more false positives by chance ($100(1-0.95^N)$)
1	1/20	5%
2	1/10	10%
20	1	64%
100	5	99.4%

Multiplicity of Testing (for detecting DE genes)



- Label reference sample with Cy3 and Cy5:
  - No genes are DE.
  - Differences are experimental error.
- p-value=0.01: each gene would have a 1% chance of having a p-value of less than 0.01, and thus be significant at the 1% level.

Example: 10000 genes

- Expect to find 100 significant genes at the 1% level.
- Expect to find 10 genes with a p-value less than 0.001.
- Expect to find 1 gene with p-value less than 0.0001

Question:

Truly differentially expressed?, or a false positive results (because we are analyzing a large number of genes?)?

Types of Error Control

- Multiple testing correction adjusts the p-value for each gene to keep the overall error rate (or false positive rate) to less than or equal to the user-specified p-value cutoff or error rate individual.

Multiple Testing

	# Reject H_0	# not Reject H_0	
# true H_{0j}	V	U	m_0
# true H_{1j}	S	T	m_1
	R	m - R	m

V : *false positives = Type I errors*

T : *false negatives = Type II errors*

PCER: Per-comparison error rate

PFER: Per-family error rate

PWER: Family-wise error rate

FDR: False discovery rate

Type One Errors Rates

$$\text{PCER} = \frac{E[V]}{m}$$

$$\text{PFER} = E[V]$$

$$\text{FWER} = p(V \geq 1)$$

$$\text{FDR} = E\left(\frac{V}{R}\middle| R > 0\right)\Pr(R > 0)$$

Power = Reject the false null hypothesis

$$\text{Any-pair Power} = p(S \geq 1)$$

$$\text{Per-pair Power} = \frac{E[S]}{m_1}$$

$$\text{All-pair Power} = p(S = m_1)$$

Multiple Testing Corrections

Test Type	Type of Error control	Genes identified by chance after correction
Bonferroni	Family-wise error rate	If error rate equals 0.05, expects **0.05** genes to be significant by chance
Bonferroni Step-down		
Westfall and Young permutation		
Benjamini and Hochberg	False Discovery Rate	If error rate equals 0.05, **5%** of genes considered statistically significant (that pass the restriction after correction) will be identified by chance (false positives).

Bonferroni Correction

- The p-value of each gene is multiplied by the number of genes in the gene list.
- If the corrected p-value is still below the error rate, the gene will be significant:
  - Corrected p-value= p-value * n <0.05.
  - If testing 1000 genes at a time, the highest accepted individual un-corrected p-value is 0.00005, making the correction very stringent.
- With a Family-wise error rate of 0.05 (i.e., the probability of at least one error in the family), the expected number of false positives will be 0.05.

Bonferroni, Carlo Emilio
(1892-1960)
- Italian mathematician
- Bonferroni correction (1935-36)
- Bonferroni's Inequality

$$P(\bigcup_{i=1}^{n} E_i) \leq \sum_{i=1}^{n} P(E_i)$$

Benjamini and Hochberg FDR

- This correction is the least stringent of all 4 options, and therefore tolerates more false positives.
- There will be also less false negative genes.
- The error rate is a proportion of the number of called genes.
- FDR: Overall proportion of false positives relative to the total number of genes declared significant.

Corrected P-value= p-value * $(n / R_i) < 0.05$

Let n=1000, error rate=0.05

Gene name	p-value (from largest to smallest)	Rank	Correction	Is gene significant after correction?
A	0.1	1000	No correction	0.1 > 0.05 ➔ No
B	0.06	999	1000/999*0.06 = 0.06006	0.06006 > 0.05 ➔ No
C	0.04	998…	1000/998*0.04 = 0.04008	0.04008 < 0.05 ➔ Yes

The Permutation Test

- The **permutation test** is a test where the null hypothesis allows to reduce the inference to a randomization problem.

Randomization test

- Works of R.A. Fisher and E.J.G. Pitman in the 1930s.
- Possible to ascribe a probability distribution to the difference in the outcome possible under null hypothesis.
- The outcome data are analyzed many times
  - once for each acceptable assignment that could have been possible under H**0**
  - and then compared with the observed result,
  - without dependence on additional distributional or model-based assumptions.

Ref: Mansmann, U. (2002), Practical microarray analysis: resampling and the Bootstraap. Heidelberg.

The Permutation Test (conti.)

Coexpression of genes

H_0: Gene 1 and Gene 2 are not correlated.

Test statistic T:

Pearson (or Spearman) correlation coefficient, calculate t_{obs}

Randomization: Under H_0 it is possible to permute the values observed for Gene 2. There are n! possibilities.

p-value: $p = P(T \geq t_{obs} \mid H_0) \approx \dfrac{\#\{ T^* \geq t_{obs} \}}{n!}$

Data

Gene 1	Gene 2
g_1^1	g_1^2
⋮	⋮
g_n^1	g_n^2

↓

$g_{(1)}^1$	$g_{(1)}^2$
⋮	⋮
$g_{(n)}^1$	$g_{(n)}^2$

Random Permutation for group labels

Gene 1	Gene 2	Group	→	Group
1.4482	1.0709	1		2
0.4850	0.9324	1		1
1.1331	1.2379	1		4
		⋮		⋮
0.8015	0.6765	2		1
		⋮		⋮
1.3726	1.2373	3		4
		⋮		⋮
1.1030	1.735	4		2
0.5148	1.0015	4		3

Perform a Permutation Test (general):

1. Analyze the problem, choice of null hypothesis
2. Choice of test statistic **T**
3. Calculate the value of the test statistic for the observed data: **tobs**
4. Apply the randomization principle and look at all possible permutations, this gives the distribution of the test statistic **T** under H0.
5. Calculation of *p-value*:

$$p = P(T \geq t_{obs} \mid H_0) \approx \frac{\#\{t^* \geq t_{obs}\}}{\#\ \text{permutations}}$$

Correlation Coefficient and Distance

Cov	x1	x2	x3	x4		xp
x1	1.00	0.48	0.10	-0.10		-0.28
x2	0.48	1.00	0.41	0.22		-0.23
x3	0.10	0.41	1.00	0.36		-0.05
x4	-0.10	0.22	0.36	1.00		0.10
xp	-0.28	-0.23	-0.05	0.10		1.00

Proximity Matrix

Pearson Correlation Coefficient

$$r_{xy} = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2}\sqrt{\sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

Data Matrix x y →

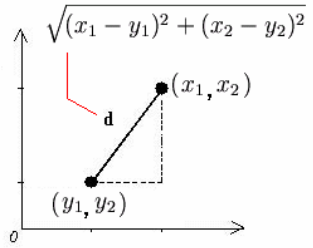
Data	x1	x2	x3	x4	...	xp
subject01	-0.48	-0.42	0.87	0.92		-0.18
subject02	-0.39	-0.58	1.08	1.21		-0.33
subject03	0.87	0.25	-0.17	0.18		-0.44
subject04	1.57	1.03	1.22	0.31		-0.49
subject05	-1.15	-0.86	1.21	1.62		0.16
subject06	0.04	-0.12	0.31	0.16		-0.06
subject07	2.95	0.45	-0.40	-0.66		-0.38
subject08	-1.22	-0.74	1.34	1.50		0.29
subject09	-0.73	-1.06	-0.79	-0.02		0.44
subject10	-0.58	-0.40	0.13	0.58		0.02
subject11	-0.50	-0.42	0.66	1.05		0.06
subject12	-0.86	-0.29	0.42	0.46		0.10
subject13	-0.16	0.29	0.17	-0.28		-0.55
subject14	-0.36	-0.03	-0.03	-0.08		-0.25
subject15	-0.72	-0.85	0.54	1.04		0.24
subject16	-0.78	-0.52	0.26	0.20		0.48
subject17	0.60	-0.55	0.41	0.45		-0.66
⋮						
subject n	-2.29	-0.64	0.77	1.60		0.55
mean	0.07	-0.04	0.44	0.31	...	-0.21

$$x = (x_1, x_2, \cdots, x_n)$$

$$y = (y_1, y_2, \cdots, y_n)$$

Euclidean Distance

$$d_{xy} = \sqrt{\sum_{i=1}^{n}(x_i - y_i)^2}$$



Pearson Correlation coefficient: great success in computational biology, especially in clustering algorithm.

Advantage

it can group together genes with similar expression profiles even if their units of change are different.

Disadvantage

The Correlation Coefficient can take negative values and does not satisfy the triangle inequality and thus not a metric.

Correlation Coefficient and Distance

- Use $d = 1 - r$: (Eisen *et al.* 1998) $d_{rs} = 1 - c_{rs}$
  - still not a metric, does not satisfy the triangle inequality.

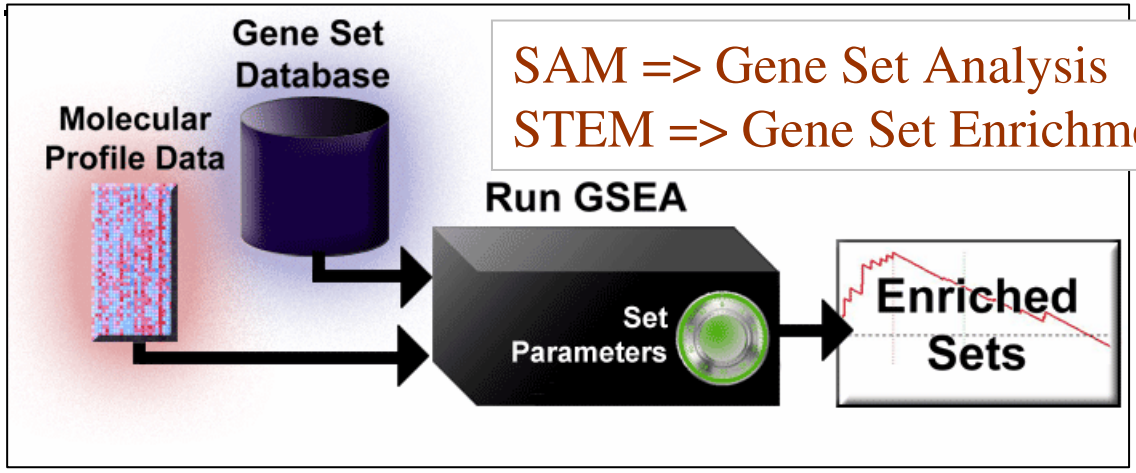
- Generalized version of the triangle inequality:
  - $g_m(x, z) <= 2(g_m(x, y)+g_m(y, z))$ ➔ a transitive measure.
  - When using the correlation coefficient two highly dissimilar profiles can't be very similar to a third profile.

- The standard transformation from a similarity matrix C to a distance matrix D is given by $d_{rs} = (c_{rr} - 2c_{rs} + c_{ss})^{1/2}$.
- Other transformations (Chatfield and Collins 1980, Section 10.2)

Gene Set Enrichment Analysis

- **Single-gene analysis** may miss important effects on metabolic pathways, transcriptional programs and stress response.
- Study same biological system, little overlap statistically significant genes.

- Gene Set Enrichment Analysis (GSEA) is a computational method that determines whether an a priori defined set of genes shows *statistically significant*, concordant differences between two biological states (e.g. phenotypes).



GSEA-p
Molecular Signature Database (MSigDB)

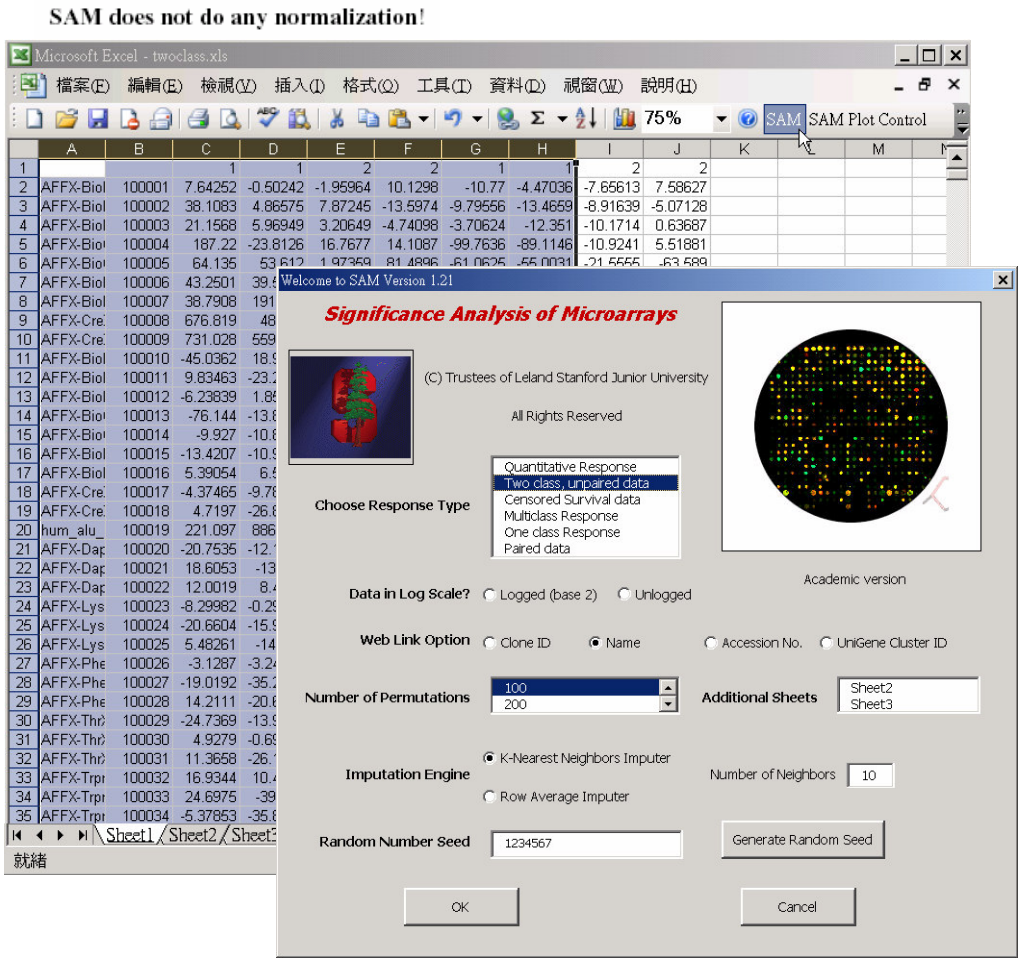
Source: http://www.broad.mit.edu/gsea/

Subramanian, Tamayo, et al. (2005), Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. PNAS 102, 15545-15550

SAM

SAM assigns a score to each gene in a microarray experiment based upon its change in gene expression relative to the standard deviation of repeated measurements.

- ***SAM plot***: the number of observed genes versus the expected number. This visualizes the outlier genes that are most dramatically regulated.
- ***False discovery rate*****:** is the percent of genes that are expected to be identified by chance.
- ***q-value***: the lowest false discovery rate at which a gene is described as significantly regulated.



Tusher VG, Tibshirani R, Chu G.(2001). Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci* 98(9):5116-21.

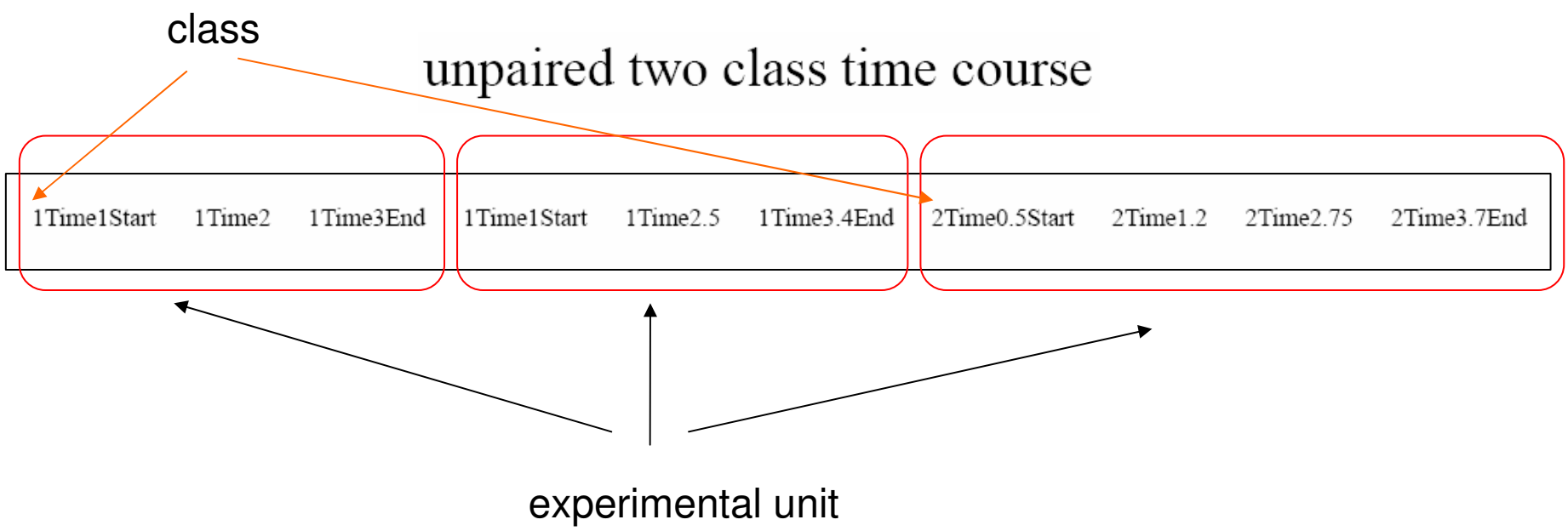
SAM: Significance Analysis of Microarrays
http://www-stat.stanford.edu/~tibs/SAM/

SAM: Response Type

Response type	Coding
Quantitative	Real number eg 27.4 or -45.34
Two class (unpaired)	Integer 1, 2
Multiclass	Integer 1, 2, 3, ...
Paired	Integer -1, 1, -2, 2, etc. eg - means Before treatment, + means after treatment -1 is paired with 1, -2 is paired with 2, etc.
Survival data	(Time, status) pair like (50,1) or (120,0) First number is survival time, second is status (1=died, 0=censored)
One class	Integer, every entry equal to 1
Time course, two class (unpaired)	(1 or 2)Time(t)[Start or End]
Time course, two class (paired)	(-1 or 1 or -2 or 2 etc)Time(t)[Start or End]
Time course, one class	1Time(t)[Start or End]
Pattern discovery	eigengenek, where k is one of 1,2,... number of arrays

SAM Users guide and technical document

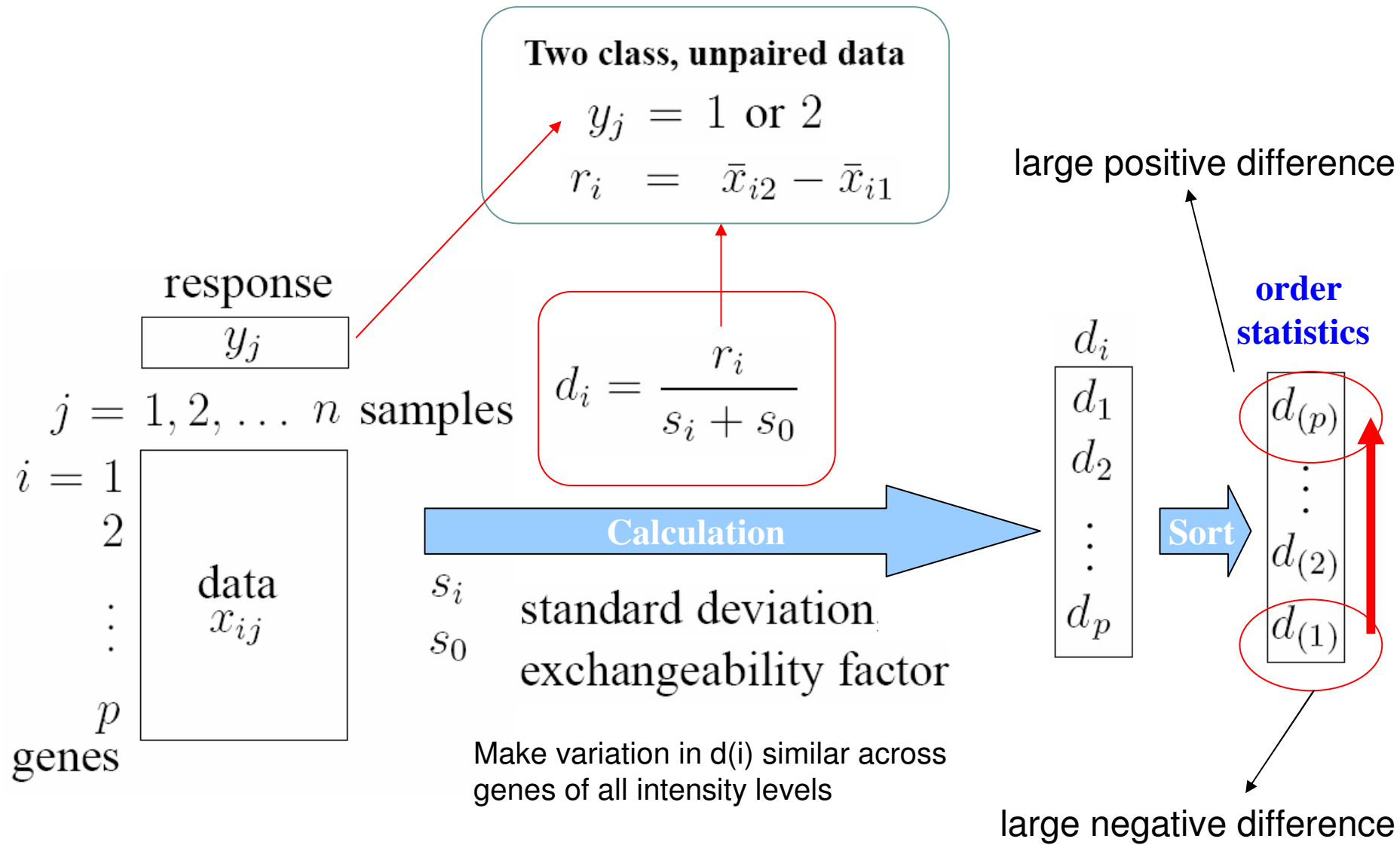
SAM: Time Series



- Paired data time courses: class label is -1, or 1 or -2 or 2.
- One class time courses: class label is a 1.

NOTE: SAM summarizes each time course by a ***slope*** or a ***signed area***, and then treats the summarized data in the same way as it treats two class, one class, or a two-class paired design.

SAM: Significance Analysis of Microarrays



SAM: Expected Test Statistics

response

y_j

$1, 1, \ldots, 2, \ldots, 2$

Permutation

$1, 2, 1, 2, 1, \ldots, 1$

$$r_i^* = \bar{x}_{i2}^* - \bar{x}_{i1}^*$$

$$d_i^* = \frac{r_i^*}{s_i^* + s_0^*}$$

$$\begin{bmatrix} d_{(p)}^{*b} \\ \vdots \\ d_{(2)}^{*b} \\ d_{(1)}^{*b} \end{bmatrix} \quad b = 1, 2, \ldots B$$

$$\bar{d}_{(i)} = (1/B) \sum_b d_{(i)}^{*b}$$

expected order statistics

$$\begin{bmatrix} \bar{d}_{(p)} \\ \vdots \\ \bar{d}_{(2)} \\ \bar{d}_{(1)} \end{bmatrix}$$

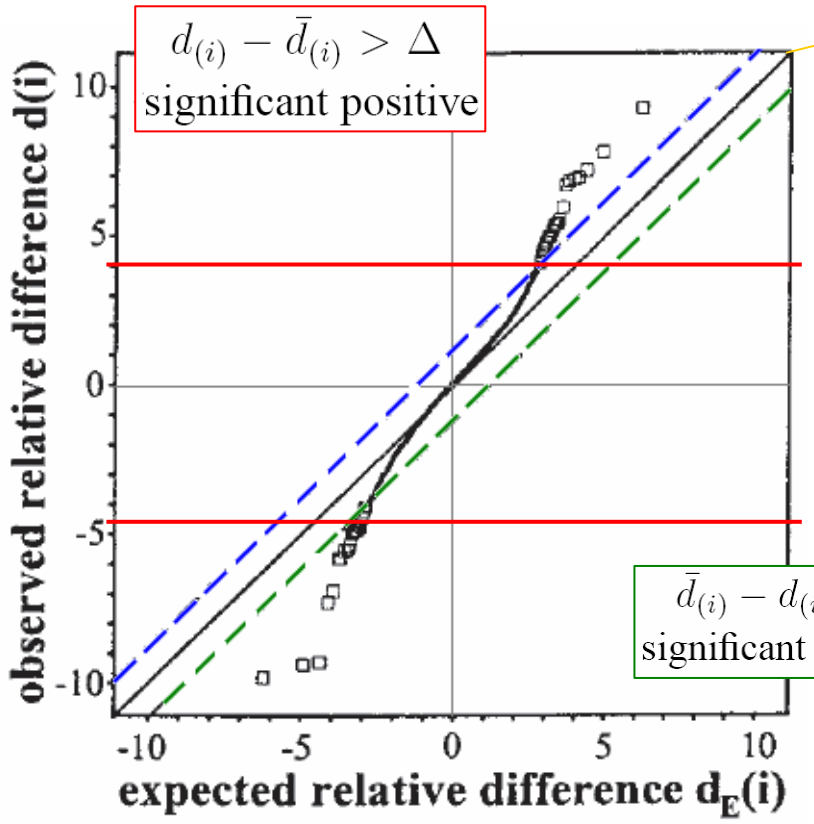
SAM Plot

Points for genes with evidence of induction

$$\begin{bmatrix} d_{(p)} \\ \vdots \\ d_{(2)} \\ d_{(1)} \end{bmatrix}$$

vs

$$\begin{bmatrix} \bar{d}_{(p)} \\ \vdots \\ \bar{d}_{(2)} \\ \bar{d}_{(1)} \end{bmatrix}$$



$d_{(i)} = \bar{d}_{(i)}$

upper cut-point $\text{cut}_{up}(\Delta)$

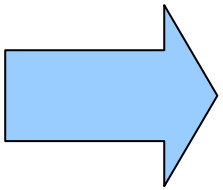
lower cut-point $\text{cut}_{low}(\Delta)$

$\bar{d}_{(i)} - d_{(i)} > \Delta$
significant negative

Points for genes with evidence of repression

Estimating FDR for a Selected Δ

$$\begin{matrix} d^{*b}_{(p)} \\ \vdots \\ d^{*b}_{(2)} \\ d^{*b}_{(1)} \end{matrix} \quad b = 1, 2, \ldots B$$



number of falsely called genes

fall above $\text{cut}_{up}(\Delta)$
or
fall below $\text{cut}_{low}(\Delta)$

False Discovery Rate (FDR) =

$$\frac{\text{[median (or 90th percentile) of the number of falsely called genes]}}{\text{[the number of genes called significant]}}$$

in the original data.

The q-value of a gene is the false discovery rate for the gene list that includes that gene and all genes that are more significant. It is computed by finding the smallest value of $\hat{\Delta}$ for which the gene is called significant, and then is the FDR corresponding to $\hat{\Delta}$.

John D. Storey (2002) A direct approach to false discovery rates, Journal of the Royal Statistical Society: Series B (Statistical Methodology) 64 (3), 479–498.

- The **q-value** gives the scientist a hypothesis testing error measure for each observed statistic with respect to pFDR.
- The **p-value** accomplishes the same goal with respect to the **type I error**, and the **adjusted p-value** with respect to **FWER**.

Interpretation of Results for Time Series Data by SAM

SAM Summarize each time course by a **slope** (least squares slope of expression vs time), or a **signed area**.

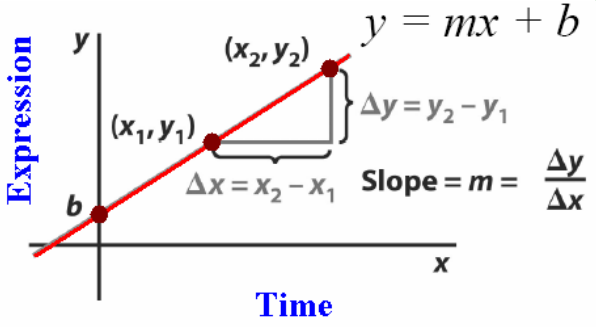
$$r_i = \bar{x}_{i2} - \bar{x}_{i1}$$

$$d_i = \frac{r_i}{s_i + s_0}$$

For two class unpaired data:

Slope: summarizes each time series by a slope.

- Compare slopes across the two groups.
- Useful for finding genes with a consistent increase or decrease over time.



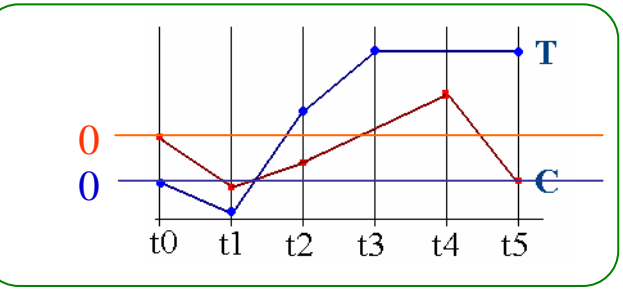
Least-squares slope

$$m = \frac{n\sum(x_i y_i) - \sum x_i \sum y_i}{D}$$

$$D = n\sum(x_i^2) - (\sum x_i)^2$$

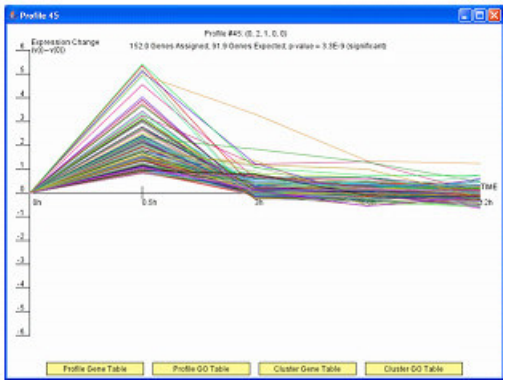
Signed area: the time course profile is shifted so that it is zero at the first time point.

- Counting positive area above the line and negative below the line.
- Compares the areas across the groups.
- Useful for finding genes that rise and then level off or come back down to their baseline.



STEM

Purpose: Identifying Significant Expression Patterns
(Clustering Short Time Series Gene Expression Data)



- Unique challenges
  - Thousands of genes are being profiled simultaneously while the number of time points is few.
  - Many genes will have the same expression pattern just by random chance.
  - Generally require the estimation of many parameters and are less appropriate for short time series data.
  - Do not differentiate between real and random patterns.

http://www.cs.cmu.edu/~jernst/stem/

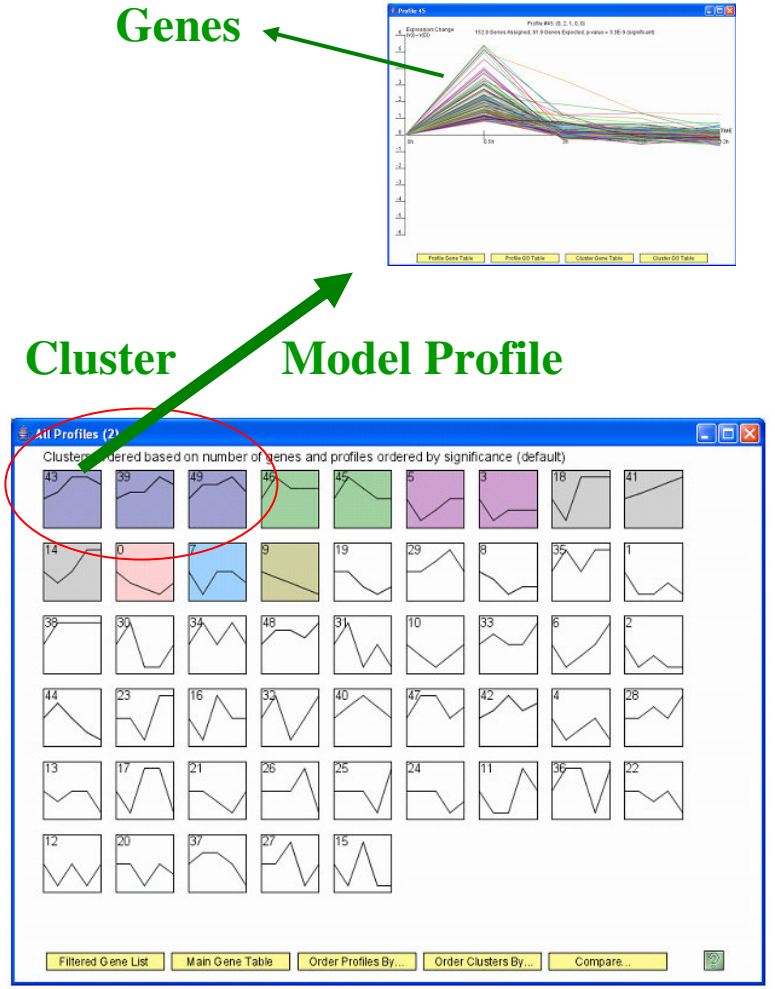
STEM: 4 Steps

1. **Selecting Model Profiles**
   - select a set of distinct and representative temporal expression profiles (Model Profiles), selected independent of the data.

2. **Assigning Genes to Model Profiles**
   - Assign each gene passing the filtering criteria to the model profile that most closely matches the gene's expression profile as determined by the correlation coefficient.

3. **Identifying Significant Model Profiles**
   - Algorithm can determine which profiles have a statistically significant higher number of genes assigned using a permutation test.

4. **Grouping Significant Profiles**
   - Significant model profiles can be grouped based on similarity to form clusters of significant profiles.

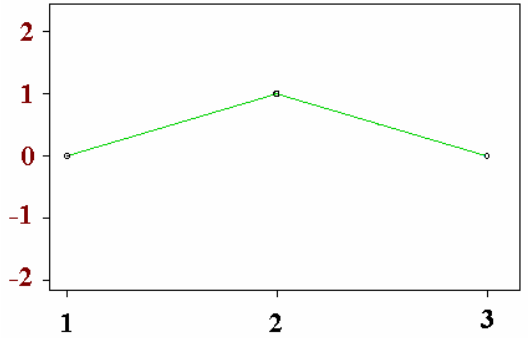


1. Selecting Model Profiles (pre-defined)

- Expression values (*log ratios*), where the ratios are with respect to the expression of the first time point (always be 0).

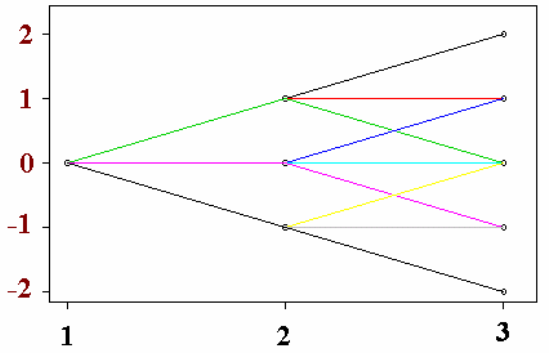
- Select a set of model expression profiles all of which are distinct from one another.

A parameter c: controls the amount of change a gene can exhibit between successive time points.



Example:

$c = 1, n = 3 \Rightarrow Np = 9$



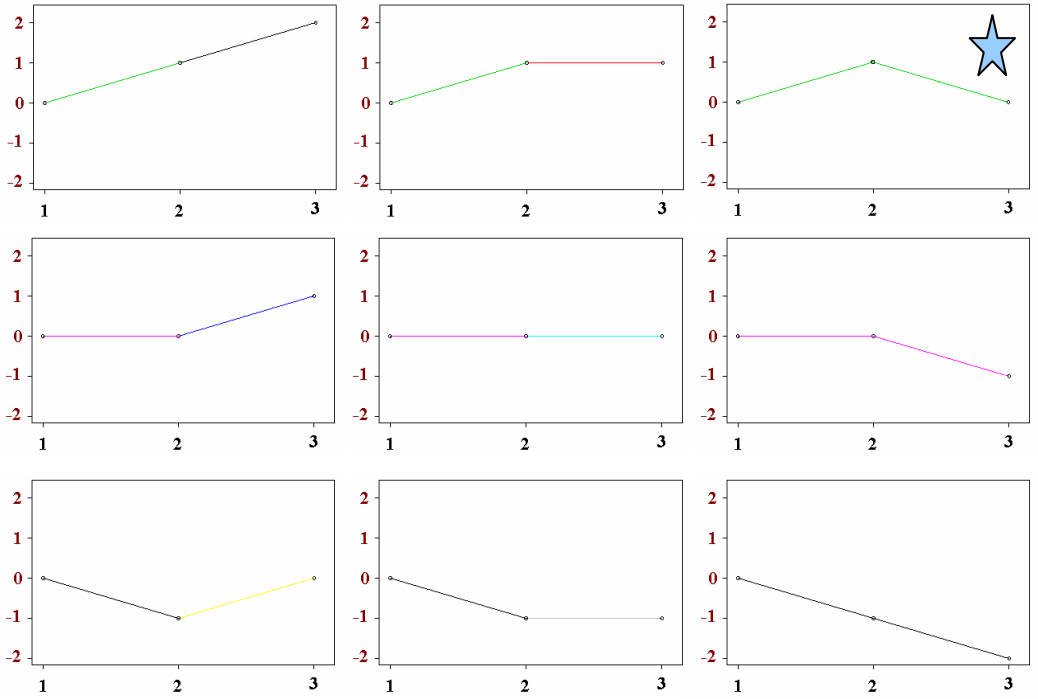
- c = 2: a gene can go up either one or two units, stay the same, or go down one or two units.

- n time points, ➔ $(2c+1)^{n-1}$ distinct profiles.
  - 5 time points and c =1, would result in 81 model profiles.
  - 6 time points and c =2, would result in 3125 model profiles.

- Select m representative profiles (a subset of profiles)
  - Greedy approximation algorithm (see Ernst et al., 2005, *Bioinformatics*).

2. Assigning Genes to Model Profiles

- Given a set m of model profiles and a set of genes G, each gene g in G is assigned to a model expression profiles m_i in m such that $dist(e_g, m_i)$ is the minimum over all m_i in m.
  - e_g is the temporal expression profile for gene g.
  - Ties: assign g to all of these profiles (h), weights 1/h.

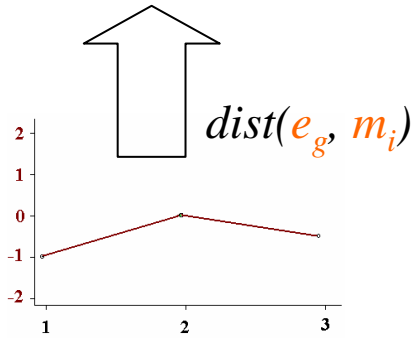


Example:

$c = 1, n = 3 \Rightarrow Np = 9$

- $T(m_i)$: The number of genes assigned to each model profile.

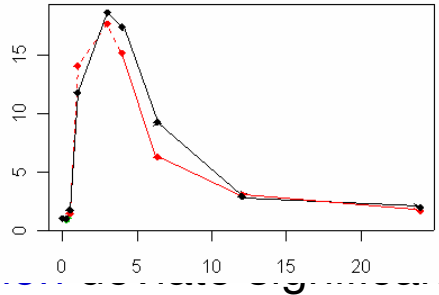
	A	B	C	D	E
1	SPOT	Gene Symbol	0h	0.5h	3h
2	1	ZFX	-1.027	0.158	-0.569
3	2	ZNF133	0.183	-0.068	-0.134
4	3	USP2	-0.67	-0.709	-0.347
5	4	DSCR1L1	-0.923	-0.51	-0.718
6	5	WNT5A	-0.471	-0.264	-0.269
7	6	VHL	-0.327	-0.378	-0.229
8	7	TCF3	-0.021	0.129	-0.209
9	8	TCN2	-0.492	-0.41	-0.306
10	9	TIMP1	-0.111	0.351	0.168
11	10	SERPINA7	-0.468	-0.488	-0.199
12	11	THBD	-1.013	-0.895	-0.743
13	12	EPHA2	0.13	0.313	0.645



3. Identifying Significant Model Profiles

Identify model profiles that are significantly enriched for genes.

- Null hypothesis: the data are *memoryless*.
  - i.e., the probability of observing a value at any time point is independent of past and future values.
  - Under null hypothesis:
    any profile we observe is a results of *random fluctuation* in the measured values for genes assigned to that profile.

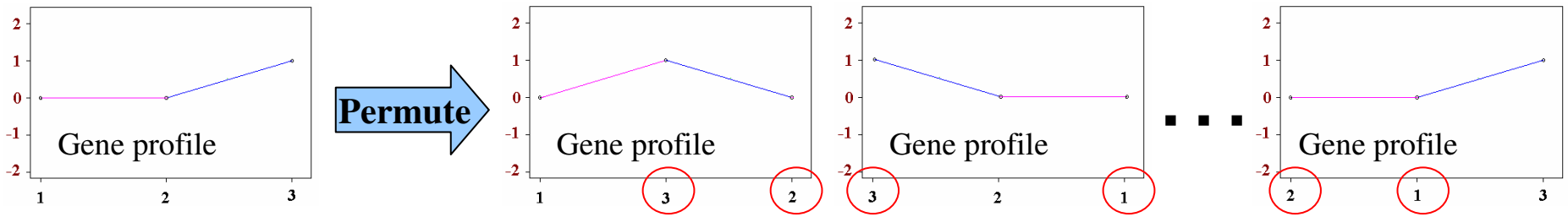


  - Model profiles that represent true biological funct... ...tly from null hypothesis since many more gene than expected by random change are assign to them.

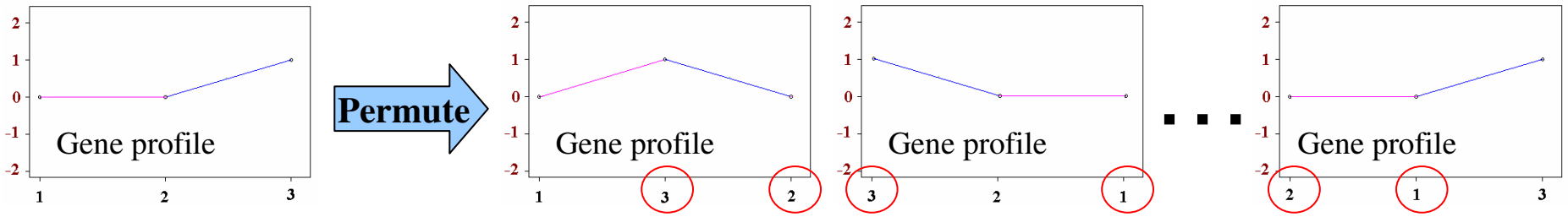
- **Permutation Test**: permutation is used to quantify the expected number of gene that would have been assigned to each profile if the data were generated at random.

3. Identifying Significant Model Profiles (conti.)

- Under the null hypothesis, the order of the observed values is random.
  - as each point is independent of any other point.
  - permutations are expected to result in profiles that are similar to the null distribution.
- Since there are n time points, each gene has $n!$ possible permutations (can be computed for small n).
- For each possible permutation, assign genes to their closet model profile.
  - Let s_{ij} be the number of genes assigned to model profile i in permutation j.
  - Set $Si=\sum_j s_{ij}$, then $E_i=Si/n!$ is the expected number of genes for each profile model if the data were indeed generated according to the null hypothesis.



3. Identifying Significant Model Profiles (conti.)



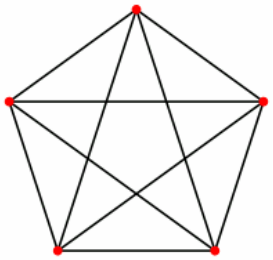
- **Assume**: The number of genes in each profile is distributed as a Binomial with parameters $|G|$ and $E_i/|G|$.
  - Thus the p-value of seening $T(m_i)$ genes assigned to profile m_i is $P(X>= T(m_i))$, where $X \sim Binomial(|G|, E_i/|G|)$.

- **Bonferroni Correction**: consider the number of genes assigned to m_i to statistically be significant if $P(X>= T(m_i)) < \alpha / m$.

4. Grouping Significant Profiles

Graph theoretic problem

- **Graph** (V, E):
  - V : the set of significant model profiles.
  - E : the set of edges.
- Two profiles v_1, v_2 in V are connected with an edge *iff* $dist(v_1,v_2) <$
- **Cliques** in this graph correspond to sets of significant profiles which are all similar to one another.



a clique of size 5

- **To identify large cliques of profiles which are all very similar to each other.**

Greedy algorithm: to partition the graph into cliques and thus to group significant profiles.

- Cluster for a significant profile $C_i = \{p_i\}$,
- Initial $C_i = \{p_i\}$, look for a profile p_j such that p_j is the closet profile to p_i that is not already included in C_i.
  - If $dist(p_j, p_k) <= \delta$ for all profiles p_k in C_i, add p_j to C_i and repeat process,
  - otherwise stop and declare C_i as the cluster for p_i.
- After obtaining clusters for all significant profiles, select the cluster with *largest number of genes* (by counting the number of genes in each of the profiles that are included in this cluster), remove all profiles in that cluster and repeat the above process.
- The algorithm terminates when all profiles have been assigned to clusters.

Example by STEM

- Data: immune response data from Guillemin et al. (2000, *PNAS*)
- Use hmuan cDNA microarray to study the gene expression profile of gastric AGS cells infected with various strains of Helicobacter pylori.
  - H.pylori is one of the most abundant human pathogenic bacteria.
  - **Cy3** (for the reference), **Cy5** (for the experimental sample)
- Analyze data from the response of the wild-type G27 strain.

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Microbiology

Cag pathogenicity island-specific responses of gastric epithelial cells to *Helicobacter pylori* infection

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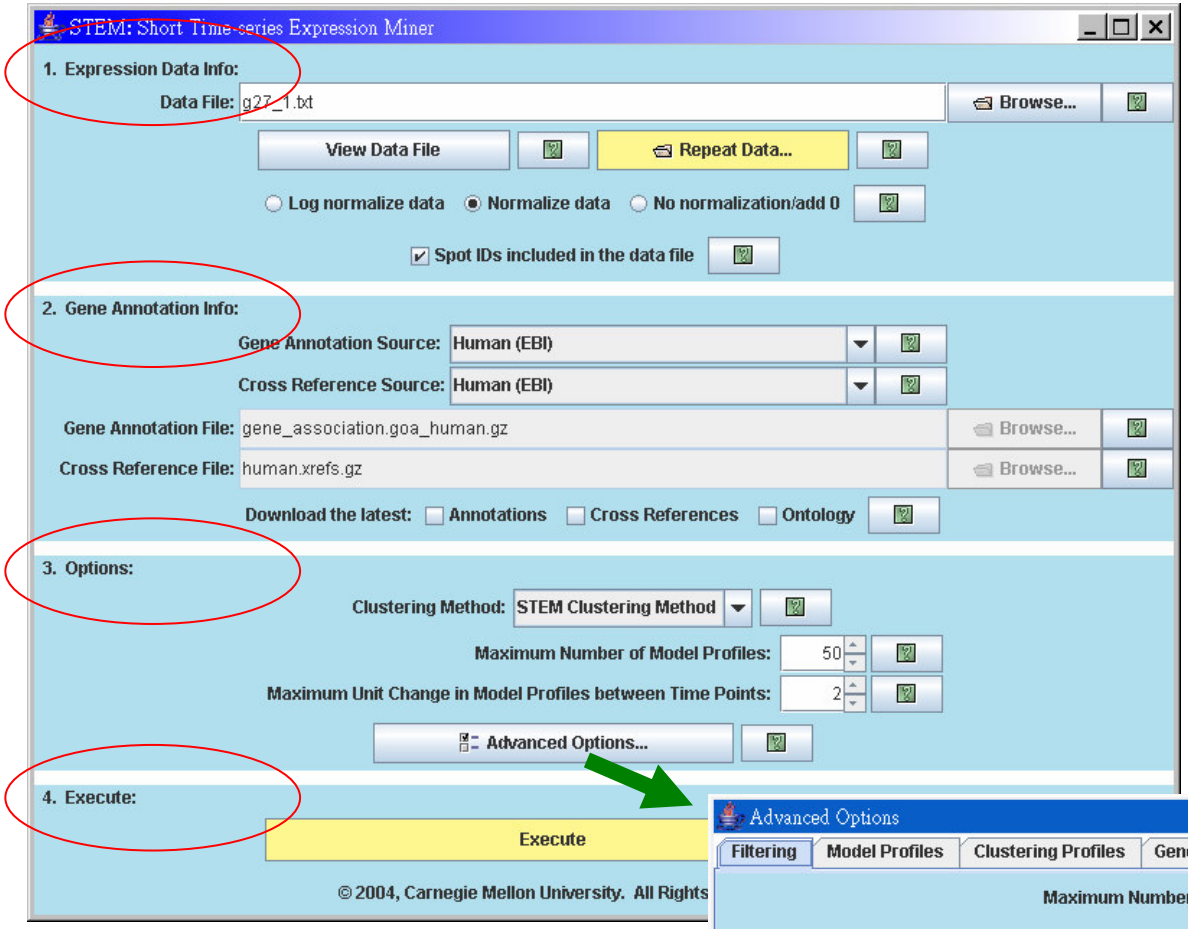
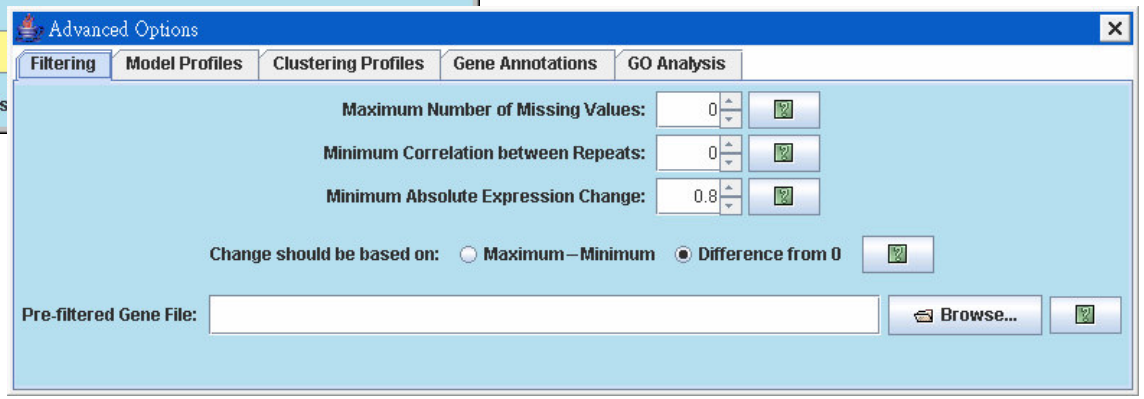
Contributed by Stanley Falkow and approved September 13, 2002

- Two replicates on the same biological sample in which time series data were collected at 5 time points: 0, 0.5, 3, 6, 12 hours.
- Select 2243 genes from 24192 array probes.
- Set m=50 model profiles and c=2.

STEM Interface



	A	B	C	D	E	F	G
1	SPOT	Gene Symbol	0h	0.5h	3h	6h	12h
2	1	ZFX	-0.027	0.158	0.169	0.193	-0.165
3	2	ZNF133	0.183	-0.068	-0.134	-0.252	0.177
4	3	USP2	-0.67	-0.709	-0.347	-0.779	-0.403
5	4	DSCR1L1	-0.923	-0.51	-0.718	-0.512	-0.668
6	5	WNT5A	-0.471	-0.264	-0.269	-0.154	-0.254
7	6	VHL	-0.327	-0.378	-0.229	-0.264	-0.072
8	7	TCF3	-0.021	0.129	-0.209	-0.245	0.036
9	8	TCN2	-0.492	-0.41	-0.306	-0.494	-0.273
10	9	TIMP1	-0.111	0.351	0.168	0.129	-0.293
11	10	SERPINA7	-0.468	-0.488	-0.199	-0.144	-0.185
12	11	THBD	-1.013	-0.895	-0.743	-0.601	-0.543
13	12	EPHA2	0.13	0.313	0.645	-0.155	0.28

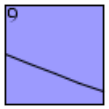


Example: Clustering Results

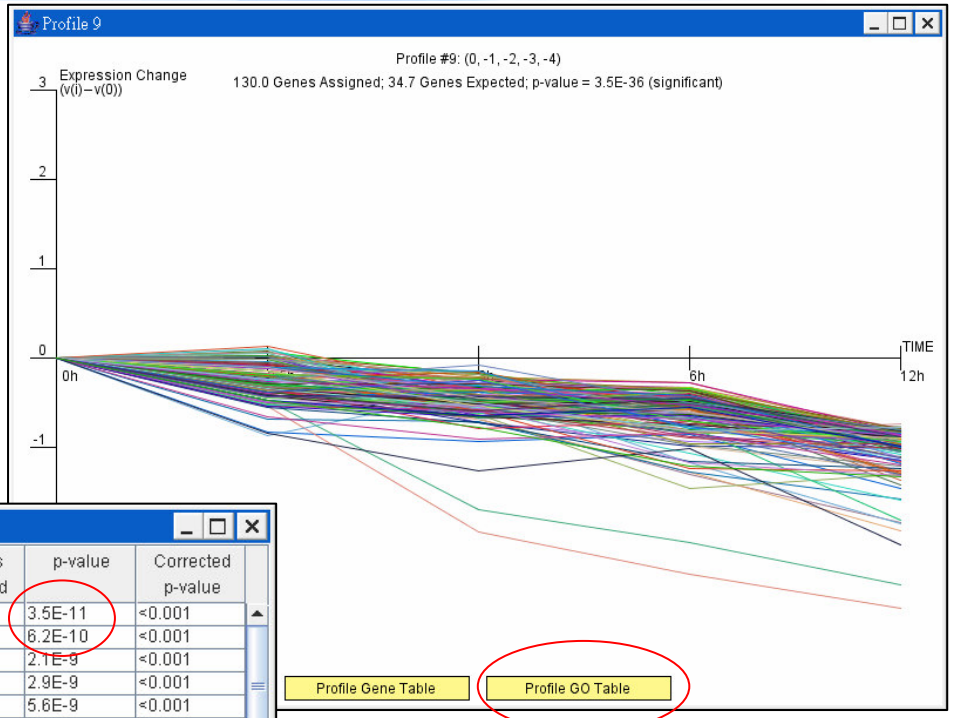
- Colored profiles are significant.
- Profiles with the same shade belong to the same cluster.
- Corr=0.7➔ δ =0.3 in grouping method.
- one: 3 profiles,
  one: 2 profiles,
  five: single profiles.

Four of the 10 significant model profiles were significantly enriched for **GO categories.**

Example: GO Interpretation



- Profile 9 (0, -1, -2, -3, -4): 131 down-regulated genes during the entire experiment duration.
- This profile was significantly enriched for cell-cycle genes (p-value < 10^{-10}).



Profile GO Table

GO Results for Profile 9 based on the actual number of genes assigned to the profile

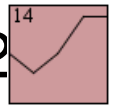
Category ID	Category Name	#Genes Category	#Genes Assigned	#Genes Expected	#Genes Enriched	p-value	Corrected p-value
GO:0007049	cell cycle	432	19.0	2.7	+16.3	3.5E-11	<0.001
GO:0006259	DNA metabolism	344	16.0	2.2	+13.8	6.2E-10	<0.001
GO:0006260	DNA replication	110	10.0	0.7	+9.3	2.1E-9	<0.001
GO:0006139	nucleobase, nucleoside, nucleotide and nuc...	1490	31.0	9.5	+21.5	2.9E-9	<0.001
GO:0000074	regulation of progression through cell cycle	293	14.0	1.9	+12.1	5.6E-9	<0.001
GO:0051726	regulation of cell cycle	294	14.0	1.9	+12.1	5.8E-9	<0.001
GO:0006261	DNA-dependent DNA replication	49	7.0	0.3	+6.7	2.5E-8	<0.001
GO:0005634	nucleus	1667	31.0	10.6	+20.4	3.9E-8	<0.001
GO:0044238	primary metabolism	3112	43.0	19.8	+23.2	2.8E-7	<0.001
GO:0006281	DNA repair	141	9.0	0.9	+8.1	3.0E-7	<0.001
GO:0043283	biopolymer metabolism	1295	25.0	8.2	+16.8	5.3E-7	<0.001
GO:0006974	response to DNA damage stimulus	159	9.0	1.0	+8.0	8.2E-7	<0.001
GO:0044237	cellular metabolism	3175	42.0	20.2	+21.8	1.4E-6	<0.001
GO:0009719	response to endogenous stimulus	170	9.0	1.1	+7.9	1.4E-6	<0.001
GO:0050875	cellular physiological process	4335	51.0	27.6	+23.4	2.1E-6	<0.001
GO:0008152	metabolism	3379	43.0	21.5	+21.5	2.7E-6	<0.001
GO:0043231	intracellular membrane-bound organelle	2476	35.0	15.7	+19.3	3.3E-6	<0.001
GO:0043227	membrane-bound organelle	2477	35.0	15.7	+19.3	3.3E-6	<0.001
GO:0044424	intracellular part	3287	42.0	20.9	+21.1	3.4E-6	<0.001
GO:0005622	intracellular	3450	43.0	21.9	+21.1	4.7E-6	<0.001
GO:0043229	intracellular organelle	2840	37.0	18.1	+18.9	1.1E-5	0.002
GO:0048015	phosphoinositide-mediated signaling	48	5.0	0.3	+4.7	1.3E-5	0.002
GO:0044464	cell part	4598	50.0	29.2	+20.8	2.8E-5	0.010
GO:0051301	cell division	97	6.0	0.6	+5.4	3.6E-5	0.012
GO:0016779	nucleotidyltransferase activity	61	5.0	0.4	+4.6	4.3E-5	0.012

Click for GO Results Based on the Profile's Expected Size | Save Table

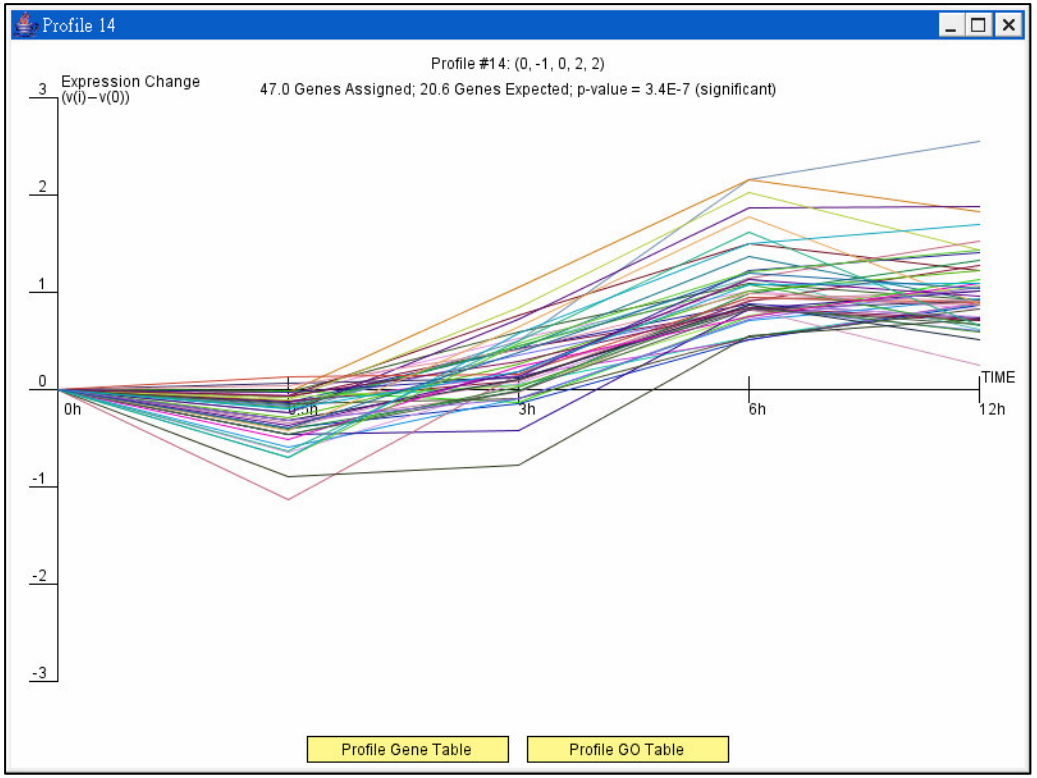
Many of the cycling genes in this profile are known transcription factors, which could contribute to repression of cell-cycle genes, and ultimately, the cell cycle.

Example: GO Interpretation

- Profile 14 (0, -1, 0, 2) contained 49 genes.



- GO analysis indicates that many of these genes were relevant to **cell structure** and annotated as belonging to the categories
  - cytoskeleton ($p=9x\ 10^{-5}$),
  - extracellular matrix ($9x\ 10^{-4}$),
  - membrance ($2x\ 10^{-6}$).



Profile GO Table

Structural elongation of cells is a known phenotypical response to pathogens, and thus the enrichment of such genes in up-regulated expression profiles is consistent with this biological response.

STEM: Other Functionalities

Bidirectional Integration

- Determine for a given model profile what GO terms are significantly enriched.
- Determine for a given GO category what model profiles were most enriched for genes in that category.

Comparing Data Sets

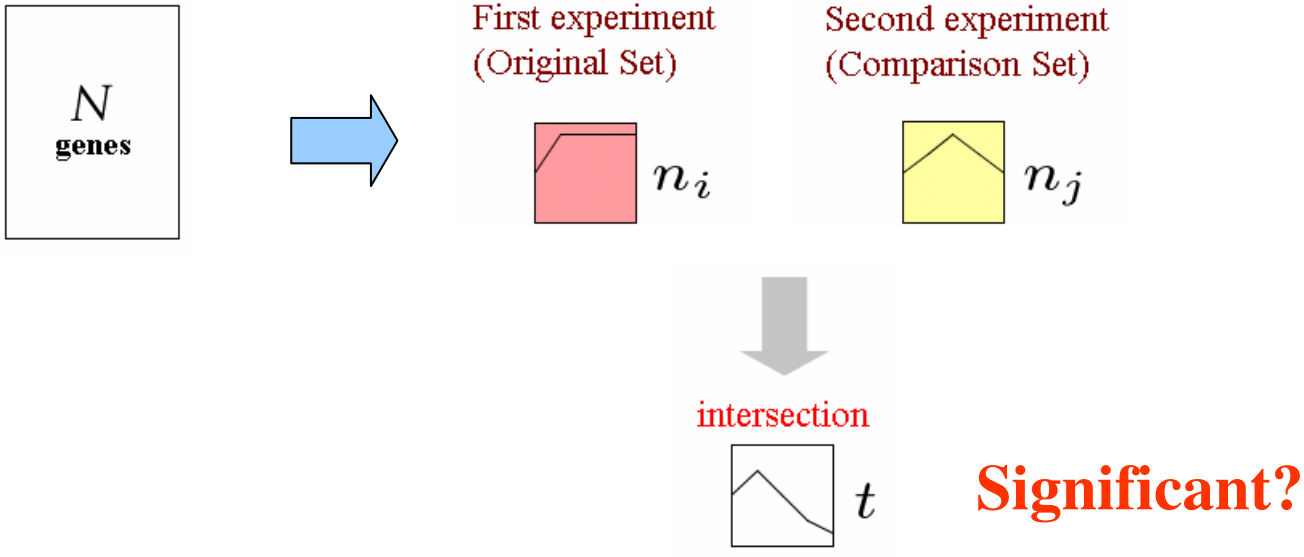
- For a set of genes which had temporal response X in experiment A, which significant responses did they have in experiment B?
- use hypergeometric distribution to compute the significance of overlap between gene sets of model profiles of two experiments.

Example

- Compare the temporal response of gene infected with a wildtype pathogen to those infected woth a knockout mutant version of the pathogen (Guillemin, PNAS, 2002).

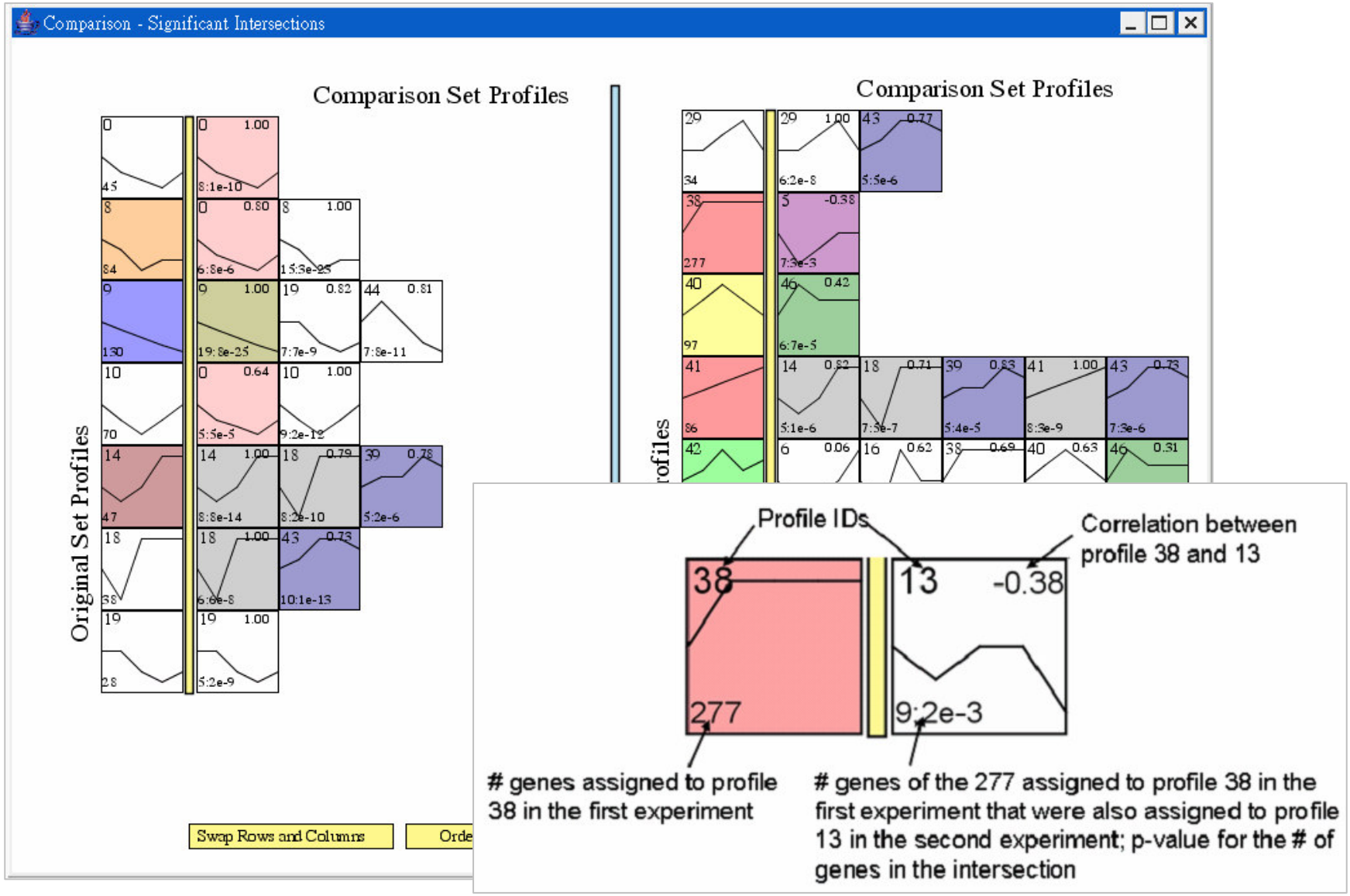
- The response of genes when exposed to a certain chemical substance to their response when not exposed. (Jorgensen et al., *Cell Cycle*, 2004)

STEM: Comparing Data Sets



$$
\begin{aligned}
& \textit{p-value} \\
= \; & \mathrm{P}(\textit{seening } t \textit{ or more genes}) \\
= \; & \sum_{m=t}^{\min(n_i,n_j)} \frac{\binom{n_j}{m}\binom{N-n_j}{n_i-m}}{\binom{N}{n_i}}
\end{aligned}
$$

STEM: Comparing Data Sets (conti.)



Review

- Time Series Microarray Experiments
- Overview of Analyzing Software

- Some Issues
  - P-values
  - Multiple Hypothesis Testing
  - Permutation Test
  - Gene Set Enrichment Analysis

- SAM: Significance Analysis of Microarrays
  - Algorithm
  - Interpretation

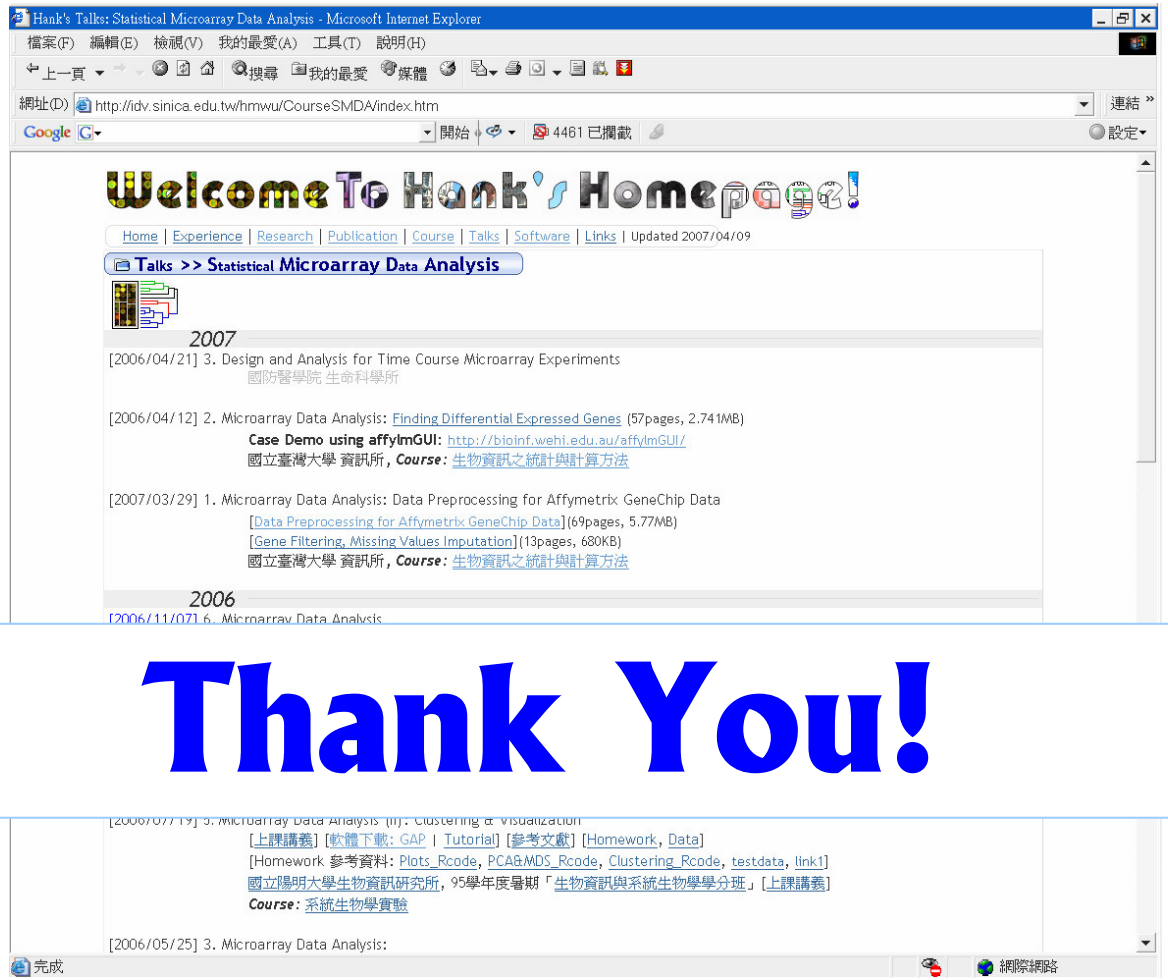
Differential Expressed Genes

- STEM: Short Time-Series Expression Miner
  - Algorithm
  - Example

Clustering

Questions?

Reference: http://idv.sinica.edu.tw/hmwu/SMDA/TimeCourse/index.htm



Thank You!

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