

Practical Bioinformatics

Mark Voorhies

4/28/2017

Pearson similarity

$$s(x, y) = \frac{\sum_i^N (x_i - x_{offset})(y_i - y_{offset})}{\sqrt{\sum_i^N (x_i - x_{offset})^2} \sqrt{\sum_i^N (y_i - y_{offset})^2}}$$

Pearson similarity

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Pearson distance

$$d(x, y) = 1 - s(x, y)$$

Pearson similarity

$$s(x, y) = \frac{\sum_i^N (x_i - x_{offset})(y_i - y_{offset})}{\sqrt{\sum_i^N (x_i - x_{offset})^2} \sqrt{\sum_i^N (y_i - y_{offset})^2}}$$

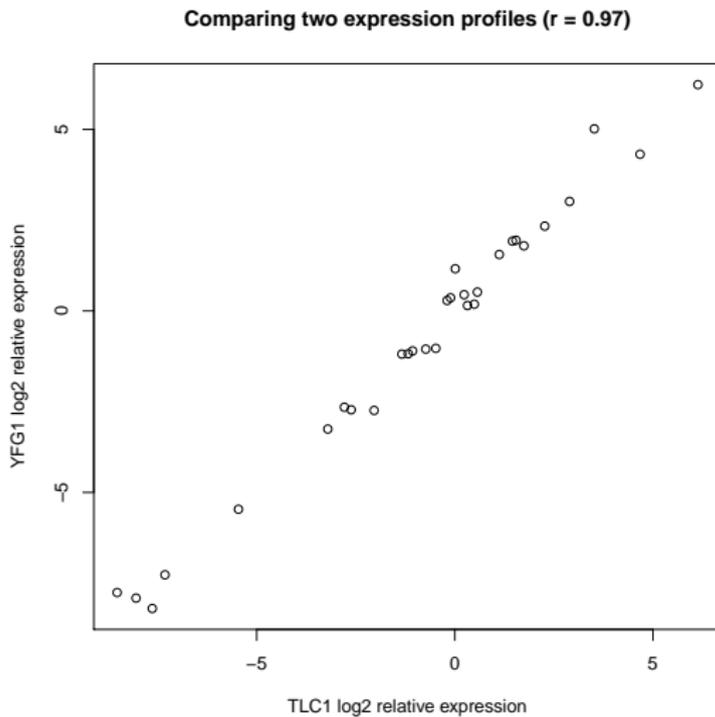
Pearson distance

$$d(x, y) = 1 - s(x, y)$$

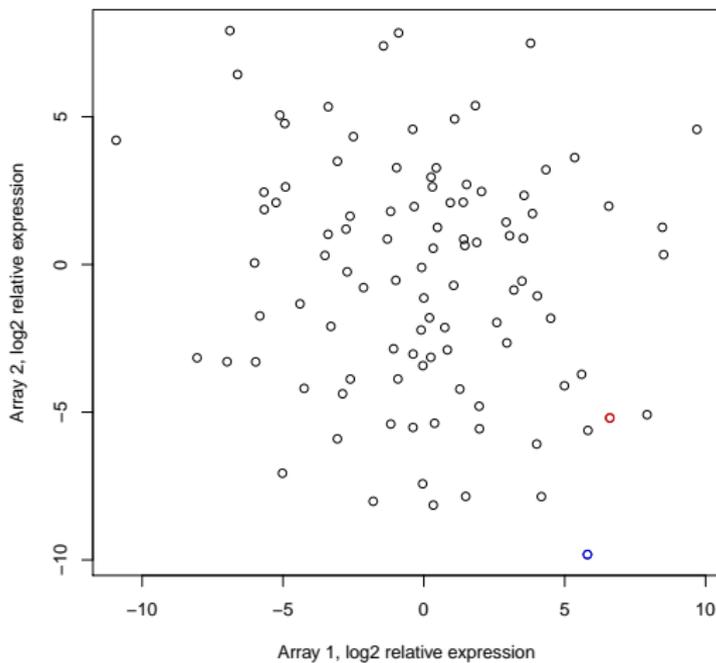
Euclidean distance

$$\frac{\sum_i^N (x_i - y_i)^2}{N}$$

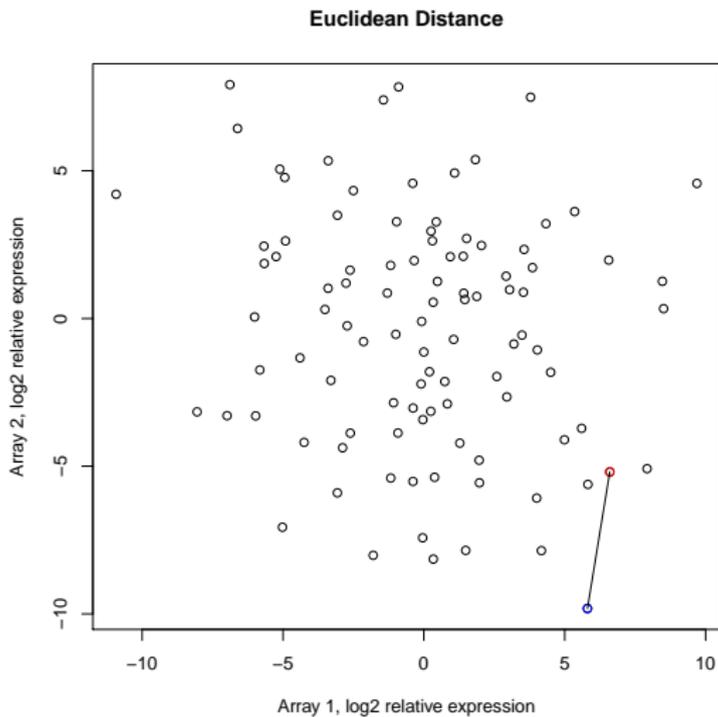
Comparing all measurements for two genes



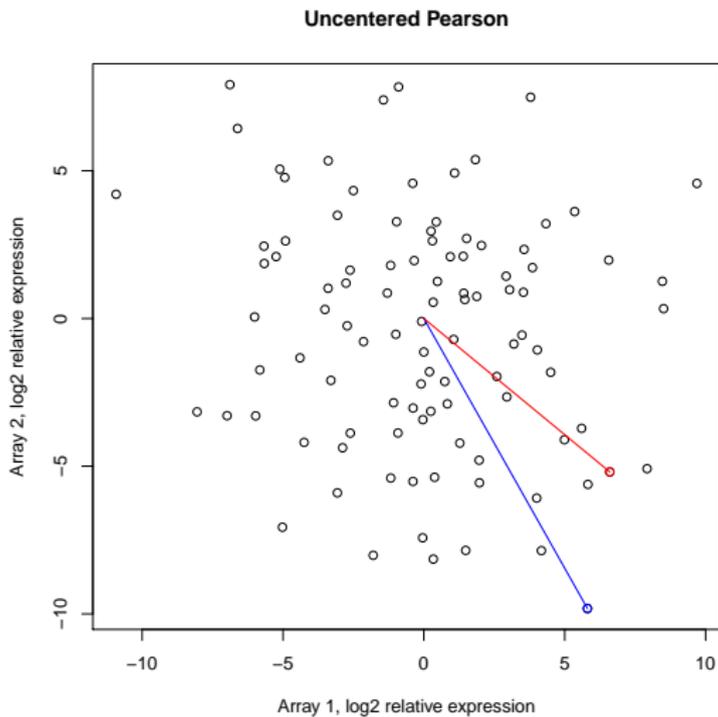
Comparing all genes for two measurements



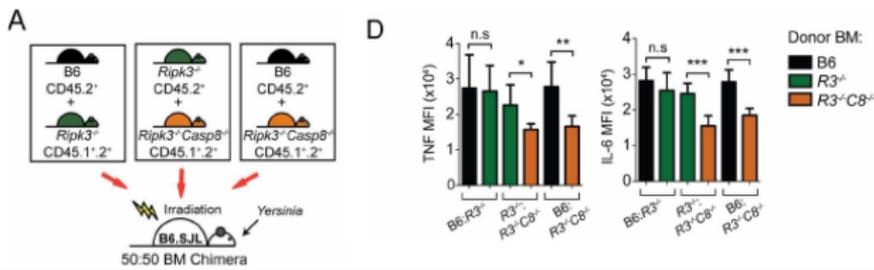
Comparing all genes for two measurements



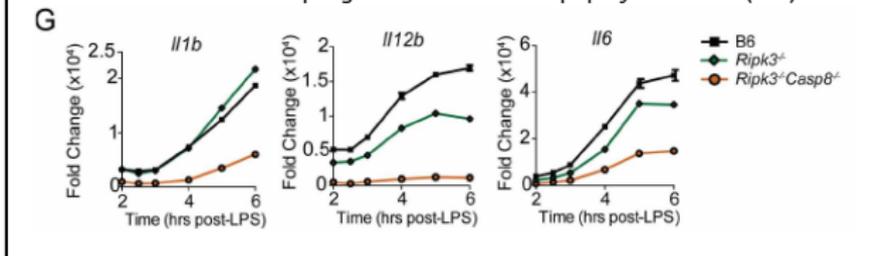
Comparing all genes for two measurements



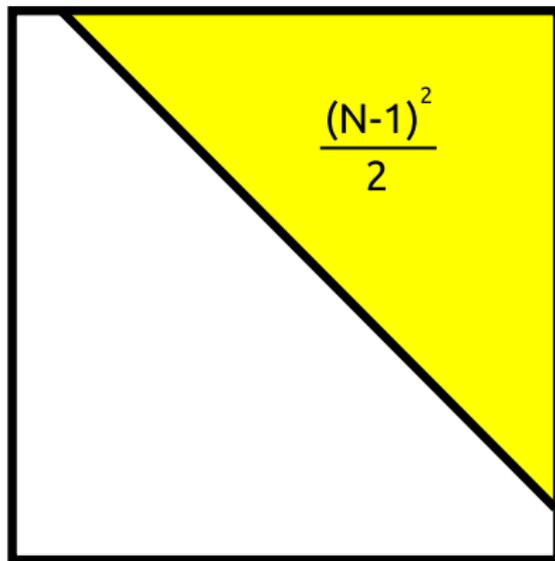
1) *in vivo* Lower inflammatory response for *Ripk3*^{-/-}/*Casp8*^{-/-} transplanted macrophages in mice infected with *Yersinia pestis*



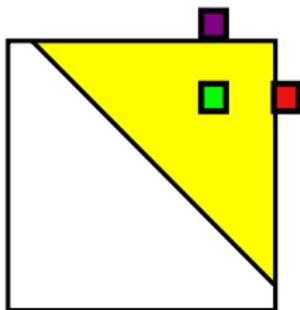
2) *ex vivo* Lower inflammatory response *at the mRNA level* in *Ripk3*^{-/-}/*Casp8*^{-/-} macrophages stimulated with lipopolysaccharide (LPS)



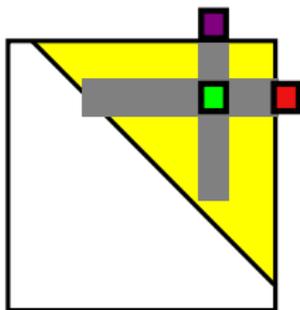
Measure all pairwise distances under distance metric



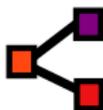
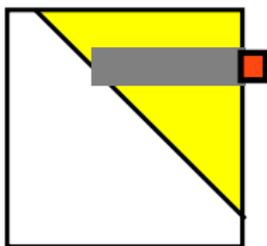
Hierarchical Clustering



Hierarchical Clustering



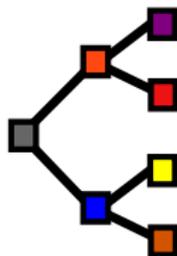
Hierarchical Clustering



Hierarchical Clustering



Hierarchical Clustering

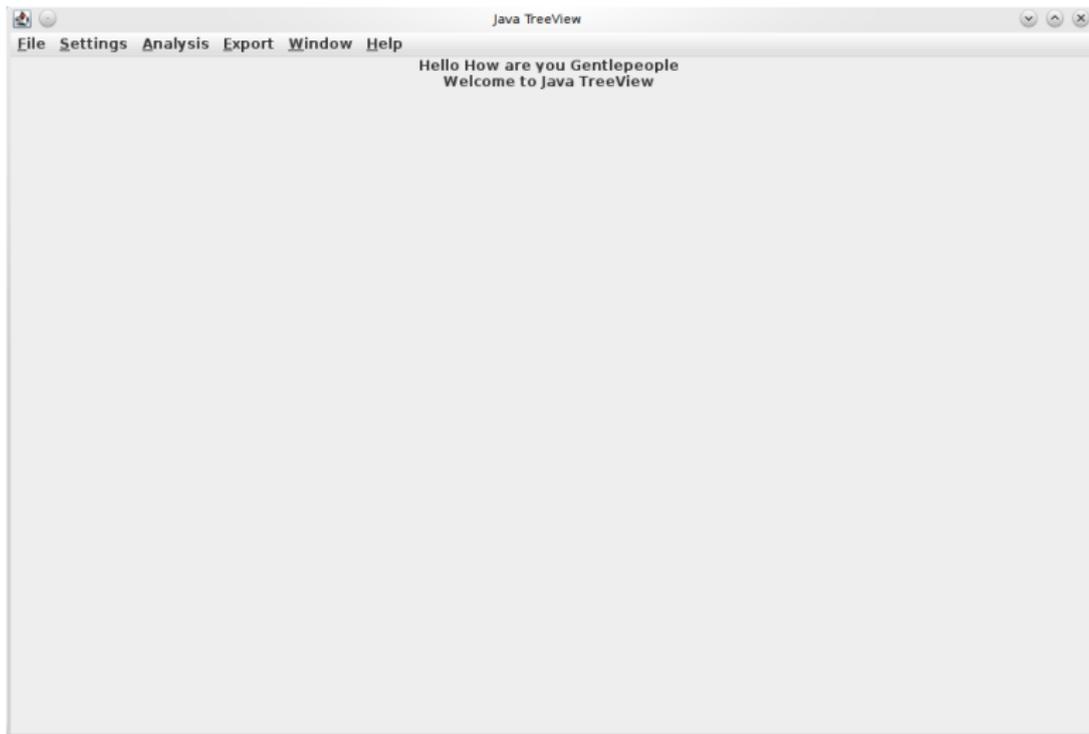


It's hard work at times, but you have to be realistic. If you have a large database with many variables and your goal is to get a good understanding of the interrelationships, then, unless you get lucky, this complex structure is bound to require some hard work to understand.

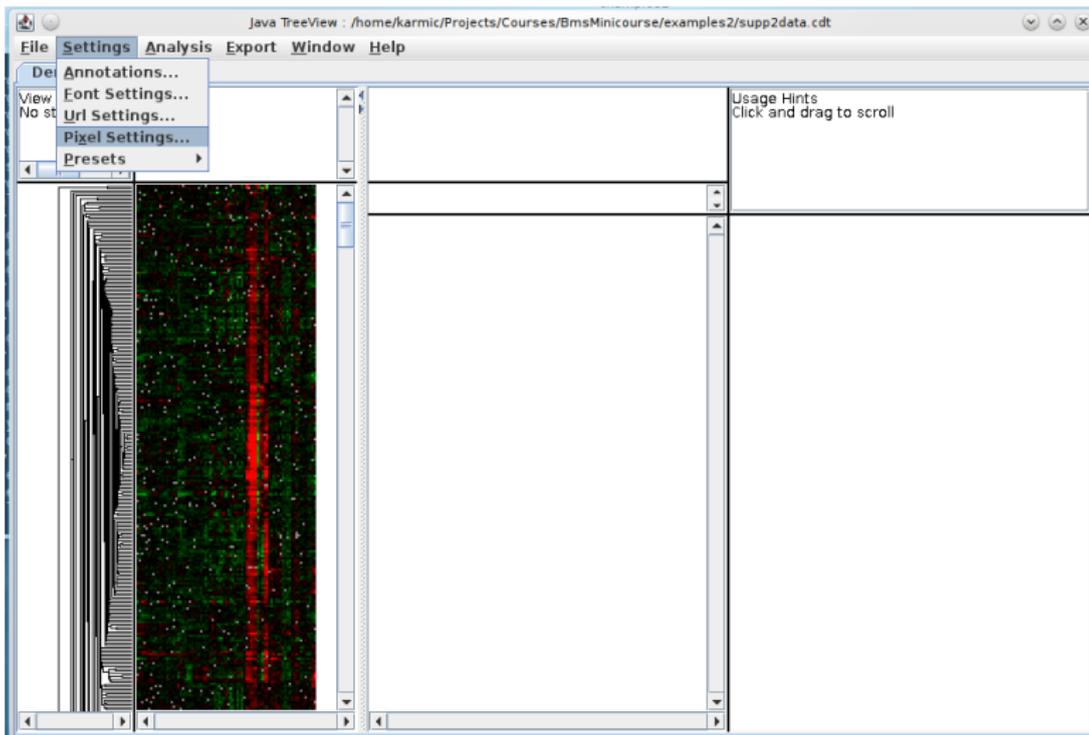
Bill Cleveland and Rick Becker

<http://stat.bell-labs.com/project/trellis/interview.html>

Using JavaTreeView



Adjust pixel settings for global view



Adjust pixel settings for global view

The screenshot shows the Java TreeView application window. The main view displays a heatmap with a dendrogram on the left. A 'Pixel Settings' dialog box is open in the foreground, allowing for adjustments to the heatmap's appearance. The dialog includes the following controls:

- Global:** Radio buttons for 'Fixed Scale' (with input fields for X: 481012658227 and Y: 663964329145) and 'Fill' (selected).
- Zoom:** Radio buttons for 'Fixed Scale' (with input fields for X: 12.0 and Y: 12.0) and 'Fill'.
- Contrast:** A slider with a 'Value' of 3.0.
- LogScale:** A checkbox for 'Log (base 2)' and a 'Center' input field set to 1.0.
- Colors:** Four color selection buttons: 'Positive' (red), 'Zero' (black), 'Negative' (green), and 'Missing' (grey). Below these are 'Load...', 'Save...', and 'Make Preset' buttons, and a dropdown menu currently showing 'RedGreen' and 'YellowBlue' options.
- A 'Close' button at the bottom of the dialog.

Select annotation columns

The screenshot shows the Java TreeView application interface. The title bar reads "Java TreeView : /home/karmic/Projects/Courses/BmsMinicourse/examples2/supp2.data.txt". The menu bar includes "File", "Settings", "Analysis", "Export", "Window", and "Help". The "Settings" menu is open, showing options like "Annotations...", "Font Settings...", "Url Settings...", "Pixel Settings...", and "Presets".

The main window is divided into three panes:

- Left Pane:** A dendrogram showing hierarchical clustering of samples. A red vertical bar highlights a specific cluster of samples.
- Middle Pane:** A heatmap visualization of gene expression data. The color scale ranges from black (low expression) to red (high expression). A yellow box highlights a specific region of the heatmap.
- Right Pane:** A list of gene annotations. The top of this pane contains the text "Usage Hints" and "Click and drag to scroll". Below this, a list of gene names and their associated biological processes is displayed. The list includes genes such as GDH3, GDH1, SEC18, ABF2, RH03, TFEB1, TAF145, INF52, POB3, PH08, GAT1, DPP1, MRP20, DRS2, ECM13, BUB2, CTK2, GGN5, MNN4, TFC5, SNF2, SEC2, UFE1, NUP42, WHI4, USS1, REF2, GLE2, BAT1, MOT2, KG02, COD4, CPT1, PDX1, ECM37, and ECM27. Each gene name is followed by one or more biological processes, such as "GLUTAMATE BIOSYNTHESIS", "MITOCHONDRIAL GENOME MAI (PUTATIVE) HM", "CYTOSKELETON", "TRANSCRIPTION", "ENDOCYTOSIS (PUTATIVE)", "DNA REPLICATION (PUTATIV BINDS DNA POL", "PHOSPHATE METABOLISM", "NITROGEN CATABOLISM", "PROTEIN SYNTHESIS", "TRANSPORT", "CELL WALL BIOGENESIS", "CELL CYCLE, CHECKPOINT", "CELL CYCLE", "CHROMATIN STRUCTURE", "PROTEIN GLYCOSYLATION", "TRANSCRIPTION", "TRANSCRIPTION", "SECRETION", "SECRETION", "NUCLEAR PROTEIN TARGETIN NUCLEAR PORE", "CELL SIZE", "MRNA SPLICING", "MRNA 3'-END PROCESSING", "NUCLEAR PROTEIN TARGETIN NUCLEAR PORE", "BRANCHED CHAIN AMINO ACI TRANSAMINASE", "MATING", "TCA CYCLE", "UBIQUINONE BIOSYNTHESIS", "OXIDATIVE STRESS RESPONSI CYTOCHROME-C", "GLYCOLYSIS", "PYRUVATE DEHYDROGENAS", "CELL WALL BIOGENESIS", and "CELL WALL BIOGENESIS".

Select annotation columns

The screenshot shows the Java TreeView application interface. The main window displays a dendrogram on the left and a heatmap in the center. A dialog box titled "Annotation Settings" is open, showing a list of columns to include in the annotation. The columns listed are: **GID**, **ORF**, **NAME**, and **GWEIGHT**. The dialog also has tabs for "Array Tree" and "Gene Tree", and a "Close" button.

The annotation table on the right side of the window lists gene IDs and their corresponding annotations. The table is as follows:

Gene ID	Annotation 1	Annotation 2	Annotation 3
YAL062W	GDH3	GLUTAMATE BIOSYNTHESIS	NADP
YOR375C	GDH1	GLUTAMATE BIOSYNTHESIS	GLU
YBR080C	SEC18	SECRETION	NSF; VESICLE
YMR072W	ABF2	MITOCHONDRIAL GENOME MAI (PU	
YIL119W	RH03	CYTOSKELETON	GTP-BIND
YDR311W	TFB1	TRANSCRIPTION	TFIIH 75
YGR274C	TAF145	TRANSCRIPTION	TFIID 145
YNL106C	INP52	ENDOCYTOSIS (PUTATIVE)	INR
YML069W	POB3	DNA REPLICATION (PUTATIVE)	BIN
YDR481C	PH08	PHOSPHATE METABOLISM	VACI
YFL021W	GAT1	NITROGEN CATABOLISM	TRANS
YDR284C	DPPI	PHOSPHOLIPID METABOLISM	DIA
YDR405W	MFP20	PROTEIN SYNTHESIS	RIBOSOM
YAL028C	DRS2	TRANSPORT	CA(2+)
YBL043W	ECM13	CELL WALL BIOGENESIS	UNK
YMR055C	BUB2	CELL CYCLE, CHECKPOINT	UNK
YJL006C	CTK2	CELL CYCLE	CYCLIN-LIKE
YGR252W	GCN5	CHROMATIN STRUCTURE	HISTO
YKL201C	MNN4	PROTEIN GLYCOSYLATION	PHO
YNL035W	TFP5	TRANSCRIPTION	TFIIIB 9K
YOF280C	SMF2	TRANSCRIPTION	COMPONENT
YNL272C	SEC2	SECRETION	GDP/GTP EXC
YOR075W	LEP1	SECRETION	ER MEMBRANE
YDR192C	NUP42	NUCLEAR PROTEIN TARGETIN	NUC
YDL224C	WHI4	CELL SIZE	PUTATIVE RN
YER112W	USS1	MRNA SPLICING	UG SMRNP
YDR195W	REF2	MRNA 3'-END PROCESSING	UNK
YER107C	GLE2	NUCLEAR PROTEIN TARGETIN	NUC
YHF208W	BAT1	BRANCHED CHAIN AMINO ACI	TRAI
YER068W	MOT2	MATING	TRANSCRIPTION
YDR149C	KGD2	TCA CYCLE	2-OXOGLUTAR
YDR204W	COO4	UBIQUINONE BIOSYNTHESIS	UNK
YKR068C	OCP1	OXIDATIVE STRESS RESPON	CYTI
YGR193C	FOX1	GLYCOLYSIS	PYRUVATE DE
YIL146C	ECM37	CELL WALL BIOGENESIS	UNK
YJL106W	ECM27	CELL WALL BIOGENESIS	UNK

Select URL for gene annotations

The screenshot shows the Java TreeView application window. The title bar reads "Java TreeView : /home/karmic/Projects/Courses/BmsMinicourse/examples2/supp2data.cdt". The menu bar includes "File", "Settings", "Analysis", "Export", "Window", and "Help". The "Settings" menu is open, showing options like "Annotations...", "Font Settings...", "Url Settings...", "Pixel Settings...", and "Presets". The "Presets" submenu is also open, listing "Gene Url Presets..." (highlighted), "Array Url Presets...", "Dendrogram Color Presets...", "KnnDendrogram Color Presets...", "Karyoscope Color...", "Karyoscope Coordinates...", and "Scatterplot Color...".

The main window displays a dendrogram on the left and a heatmap on the right. The heatmap has a color scale from 0 (black) to 100 (red). A red vertical bar highlights a specific column in the heatmap. The "Usage Hints" panel on the right side of the window contains the following text: "Click and drag to scroll".

Gene ID	Gene Name	Gene Description	Gene Annotation
YAL062W	GDH3	GLUTAMATE BIOSYNTHESIS	NADI
YOR375C	GDH1	GLUTAMATE BIOSYNTHESIS	GLJ
YBR080C	SEC18	SECRETION	NSF; VESICLI
YMR072W	ABF2	MITOCHONDRIAL GENOME MAI	{PU
YTL118W	RHO3	CYTOSKELETON	GTP-BIND;
YOR311W	TFB1	TRANSCRIPTION	TFIIH 75
YGR274C	TAF145	TRANSCRIPTION	TFIID 14;
YNL106C	INP52	ENDOCYTOSIS (PUTATIVE)	INO1
YML069W	POB3	DNA REPLICATION (PUTATIV	BINI
YDR481C	PHO8	PHOSPHATE METABOLISM	VACI
YFL021W	GAT1	NITROGEN CATABOLISM	TRANS
YDR284C	DPP1	PHOSPHOLIPID METABOLISM	DIAP
YOR405W	MFP20	PROTEIN SYNTHESIS	RIBOSOM
YAL028C	DPS2	TRANSPORT	CA (2+) TRAN
YBL043W	ECM13	CELL WALL BIOGENESIS	UNKI
YMR055C	BUB2	CELL CYCLE, CHECKPOINT	UNKI
YJL006C	CTK2	CELL CYCLE	CYCLIN-LIKE
YGR252W	GCN5	CHROMATIN STRUCTURE	HISTO
YKL201C	MNM4	PROTEIN GLYCOSYLATION	PHO
YNL039W	TF15	TRANSCRIPTION	TFIIIB 9;
YOR290C	SNF2	TRANSCRIPTION	COMPONENT
YNL272C	SEC2	SECRETION	GDP/GTP EXCI
YOR075W	LEF1	SECRETION	ER MEMBRANE
YDR192C	NUP42	NUCLEAR PROTEIN TARGETIN	NUCI
YDL224C	WHI4	CELL SIZE	PUTATIVE RN
YER112W	USS1	MRNA SPLICING	U6 SNRNP
YOR109W	REF2	MRNA 3' END PROCESSING	UNKI
YER107C	GLE2	NUCLEAR PROTEIN TARGETIN	NUCI
YHR208W	BAT1	BRANCHED CHAIN AMINO ACI	TRAI
YER069W	MOT2	MATING	TRANSCRIPTION;
YDR149C	KG02	TCA CYCLE	2-OXOGLUTAR
YDR204W	COO4	UBIQUINONE BIOSYNTHESIS	UNKI
YKR069C	CP1	OXIDATIVE STRESS RESPON	CYTI
YGR193C	POX1	GLYCOLYSIS	PYRUVATE DE
YTL146C	ECM37	CELL WALL BIOGENESIS	UNKI
YJL109W	ECM27	CELL WALL BIOGENESIS	UNKI

http://www.ensembl.org/Mus_musculus/Gene/Summary?g=HEADER

Select URL for gene annotations

The screenshot shows the Java TreeView application interface. At the top, the title bar reads "java TreeView - /home/karmic/Projects/Courses/BmsMinicourse/examples2/supp2data.cdt". The menu bar includes "File", "Settings", "Analysis", "Export", "Window", and "Help".

The main window is divided into several sections:

- Dendrogram:** Located at the top left, it shows a hierarchical tree structure of nodes.
- View Status:** A text box on the left of the dendrogram that says "Select Node to view annotator".
- Usage Hints:** A text box on the right of the dendrogram that says "Click to select node - use arrow keys to navigate tree".
- Heatmaps:** Two heatmaps are visible below the dendrogram, showing gene expression data with red and green colors.
- Presets:** A dialog box titled "Modify Url Presets" is open in the foreground. It has a table with columns for "Enabled", "Header", "Name", "Template", and "Default?".

Enabled	Header	Name	Template	Default?
<input type="checkbox"/>	*	SGD	http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=HEADER	<input checked="" type="radio"/>
<input type="checkbox"/>	*	YPD	http://www.proteome.com/databases/YPD/reports/HEADER.html	<input type="radio"/>
<input type="checkbox"/>	*	WormBase	http://www.wormbase.org/cgi-bin/locate.pl?locus=HEADER&start=0&start=0&ie=utf-8&oe=utf-8	<input type="radio"/>
<input type="checkbox"/>	*	Source CloneID	http://genome-www4.stanford.edu/cgi-bin/SMD/source/sourceResult?option=CloneID	<input type="radio"/>
<input type="checkbox"/>	*	FlyBase	http://flybase.bio.indiana.edu/bin/fbqgenq.html?HEADER	<input type="radio"/>
<input type="checkbox"/>	*	MouseGD	cs.jax.org/avaw/servlet/SearchTool?query=HEADER&selectedQuery=Genes+and+Markers	<input type="radio"/>
<input type="checkbox"/>	*	GenomeNetEcoli	http://www.genome.ad.jp/dbget-bin/www_bqet?eco:HEADER	<input type="radio"/>
<input type="checkbox"/>		None		<input type="radio"/>

Below the dialog box, the main window continues with more heatmaps and a list of gene annotations. The annotations include:

- YER107C
- YHR208W
- YER066W
- YDR148C
- YDR204W
- YKR866C
- YGR190C
- YTL146C
- YJR106W
- PEP2
- GLE2
- BAT1
- MOT2
- KGD2
- COQ4
- COF1
- PDX1
- ECM37
- ECM27
- PNR3
- NUCLEAR PROTEIN TARGETIN
- BRANCHED CHAIN AMINO ACI
- MATING
- TCA CYCLE
- UBIQUINONE BIOSYNTHESIS
- OXIDATIVE STRESS RESPON
- GLYCOLYSIS
- CELL WALL BIOGENESIS
- CELL WALL BIOGENESIS
- TRAI
- TRANSCRIPTION
- 2-OXOGLUTAR
- UNKI

Activate and detach annotation window

The screenshot shows the Java TreeView application window titled "java TreeView : /home/karmac/Projects/Courses/BmsMinicourse/examples2/supp2data.cdt". The interface includes a menu bar (File, Settings, Analysis, Export, Window, Help) and a toolbar. On the left, a vertical toolbar contains various analysis tools, with "GeneTreeAnno" selected. The main workspace is divided into three panes: a dendrogram on the left, a heatmap in the center, and a gene annotation list on the right. The heatmap shows a grid of colored cells (green, red, black) representing data points. The gene list on the right includes gene IDs (e.g., YAL063W, YOR375C) and their corresponding biological functions (e.g., GLUTAMATE BIOSYNTHESIS, SECRETION). A "Usage Hints" box in the top right corner states "Click and drag to scroll".

Gene ID	Gene Name	Function
YAL063W	GDH3	GLUTAMATE BIOSYNTHESIS
YOR375C	GDH1	GLUTAMATE BIOSYNTHESIS
YBR080C	SEC18	SECRETION
YMR072W	ABF2	MITOCHONDRIAL GENOME MAI (PU
YDR311W	RH03	CYTOSKELETON
YOR274C	TFB1	TRANSCRIPTION
YML106C	INP52	ENDOCYTOSIS (PUTATIVE)
YML069W	POB3	DNA REPLICATION (PUTATIV BINI
YDR481C	PH08	PHOSPHATE METABOLISM
YFL021W	GAT1	NITROGEN CATABOLISM
YDR284C	DPP1	PHOSPHOLIPID METABOLISM
YDR495W	MRF20	PROTEIN SYNTHESIS
YAL029C	DPS2	TRANSPORT
YBL043W	ECM13	CELL WALL BIOGENESIS
YMR055C	BUB2	CELL CYCLE, CHECKPOINT
YJL006C	CTK2	CELL CYCLE
YGR252W	GCN5	CHROMATIN STRUCTURE
YKL201C	MNN4	PROTEIN GLYCOSYLATION
YML039W	TFY5	TRANSCRIPTION
YOR290C	SNF2	TRANSCRIPTION
YML272C	SEC2	SECRETION
YOR075W	UFEL	SECRETION
YDR192C	NUP42	NUCLEAR PROTEIN TARGETIN NUCL
YDL224C	WHI4	CELL SIZE
YER112W	USJ1	MRNA SPLICING
YOR185W	REF2	MRNA 3'-END PROCESSING
YER107C	GLE2	NUCLEAR PROTEIN TARGETIN NUCL
YHR206W	BAT1	BRANCHED CHAIN AMINO ACI TRAI
YER066W	MOT2	MATING
YDR148C	KGD2	TCA CYCLE
YDR204W	COQ4	UBIQUINONE BIOSYNTHESIS
YKR066C	COP1	OXIDATIVE STRESS RESPONSA CYT
YGR183C	POX1	GLYCOLYSIS
YJL146C	ECM37	CELL WALL BIOGENESIS
YJR106W	ECM27	CELL WALL BIOGENESIS

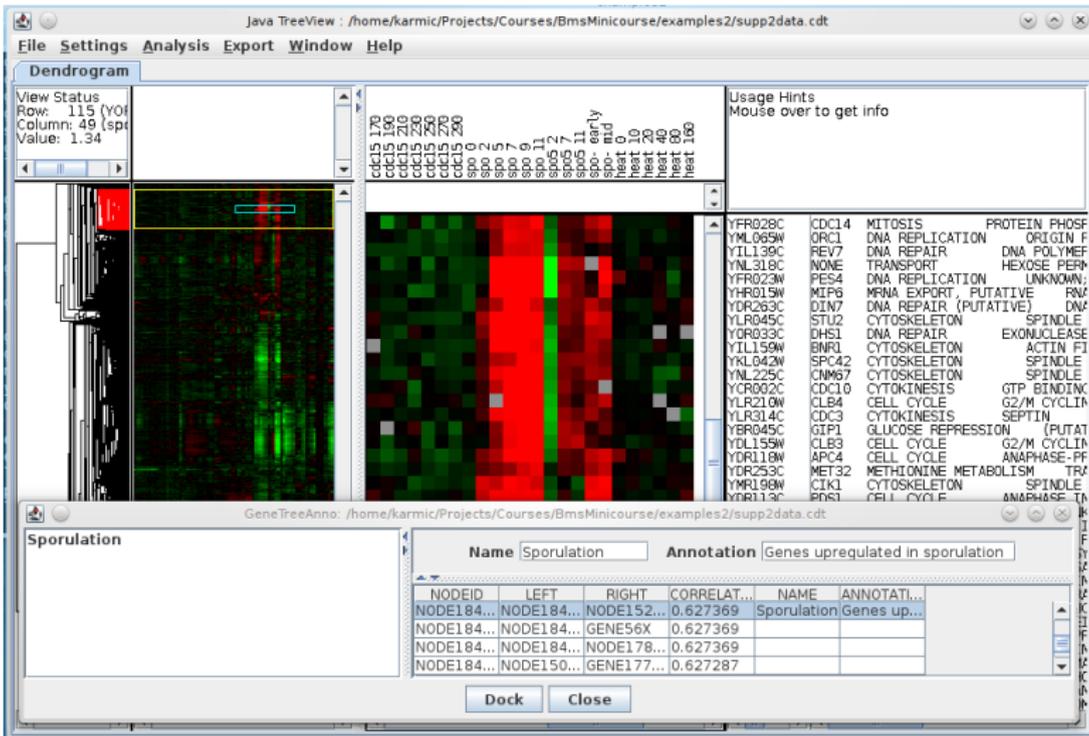
Activate and detach annotation window

The screenshot shows the Java TreeView application window. The title bar reads "Java TreeView : /home/karmic/Projects/Courses/BmsMinicourse/examples2/supp2data.cdt". The menu bar includes "File", "Settings", "Analysis", "Export", "Window", and "Help". The "Analysis" menu is open, showing options: "Find Genes..." (Ctrl-G), "Find Arrays..." (Ctrl-A), "Stats..." (Ctrl-S), "Dendrogram", "Alignment", "KnnDendrogram", "Karyoscope", "Scatterplot", "ArrayTreeAnno", "GeneTreeAnno", "Remove Current", and "Detach Current".

Below the menu, there are input fields for "Name" and "Annotation". The main area contains a table with the following columns: NODEID, LEFT, RIGHT, CORRELAT..., NAME, and ANNOTATI... The table lists various nodes and their associated gene IDs and correlation values.

NODEID	LEFT	RIGHT	CORRELAT...	NAME	ANNOTATI...
NODE243...	GENE182...	NODE239...	0.347965		
NODE244...	NODE242...	NODE243...	0.347965		
NODE244...	GENE550X	NODE239...	0.344607		
NODE244...	NODE243...	NODE244...	0.342251		
NODE244...	NODE244...	GENE4X	0.334454		
NODE244...	NODE240...	NODE239...	0.333461		
NODE244...	NODE244...	NODE243...	0.331585		
NODE244...	NODE244...	NODE238...	0.328813		
NODE244...	NODE244...	GENE229...	0.305824		
NODE244...	GENE495X	GENE217...	0.304111		
NODE244...	GENE219...	GENE218...	0.303188		
NODE245...	NODE244...	GENE215X	0.301587		
NODE245...	NODE244...	NODE242...	0.298323		
NODE245...	NODE240...	NODE244...	0.289436		
NODE245...	NODE242...	GENE219...	0.287138		
NODE245...	NODE245...	NODE243...	0.284232		
NODE245...	NODE245...	GENE527X	0.277872		
NODE245...	NODE245...	NODE234...	0.27761		
NODE245...	NODE245...	NODE244...	0.271103		
NODE245...	NODE233...	NODE245...	0.260487		
NODE245...	NODE243...	NODE245...	0.220385		
NODE246...	NODE244...	NODE245...	0.197665		
NODE246...	NODE245...	NODE243...	0.180953		
NODE246...	NODE246...	GENE182...	0.161919		
NODE246...	NODE246...	NODE119...	0.126461		
NODE246...	NODE246...	NODE245...	0.098323		
NODE246...	NODE245...	NODE246...	-0.087409		
NODE246...	NODE246...	NODE246...	-0.354391		

Activate and detach annotation window



Java TreeView : /home/karmic/Projects/Courses/BmsMinicourse/examples2/supp2data.cdt

File Settings Analysis Export Window Help

Dendrogram

View Status
Row: 115 (YOL001)
Column: 49 (sp05)
Value: 1.34

Usage Hints
Mouse over to get info

cdcl5_170
cdcl5_170
cdcl5_210
cdcl5_210
cdcl5_250
cdcl5_250
cdcl5_270
cdcl5_290
spo_9
spo_9
spo_7
spo_7
spo_11
spo_11
spo5_2
spo5_2
spo5_11
spo5_11
spo_early
spo_mid
heat_0
heat_10
heat_20
heat_30
heat_60
heat_100

YFR028C CDC14 MITOSIS PROTEIN PHOS
YML065W ORC1 DNA REPLICATION ORIGIN F
YIL139C REV7 DNA REPAIR DNA POLYMEF
YNL318C NONE TRANSPORT HEXOSE PERM
YFR023W PES4 DNA REPLICATION UNKNOWN:
YHR015W MIP6 mRNA EXPORT, PUTATIVE RNA
YDR263C DLW7 DNA REPAIR (PUTATIVE) DNA
YLR045C STU2 CYTOSKELETON SPINDLE
YOR033C DHS1 DNA REPAIR EXONUCLEASE
YIL159W BNR1 CYTOSKELETON ACTIN FI
YKL042W SPC42 CYTOSKELETON SPINDLE
YML225C CNM67 CYTOSKELETON SPINDLE
YOR092C CDC10 CYTOKINESIS GTP BINDING
YLR210W CLB4 CELL CYCLE G2/M CYCLIN
YLR314C CDC3 CYTOKINESIS SEPTIN
YBR045C GIP1 GLUCOSE REPRESSION (PUTA
YDL159W CLB3 CELL CYCLE G2/M CYCLIN
YDR118W APC4 CELL CYCLE ANAPHASE-PF
YDR253C MET32 METHIONINE METABOLISM TRP
YML190W CLK1 CYTOSKELETON SPINDLE
YDR113C PDS1 CELL CYCLE ANAPHASE-TA

GeneTreeAnno: /home/karmic/Projects/Courses/BmsMinicourse/examples2/supp2data.cdt

Sporulation

Name Sporulation Annotation Genes upregulated in sporulation

NODEID	LEFT	RIGHT	CORRELAT...	NAME	ANNOTATI...
NODE184...	NODE184...	NODE152...	0.627369	Sporulation	Genes up...
NODE184...	NODE184...	GENE56X	0.627369		
NODE184...	NODE184...	NODE178...	0.627369		
NODE184...	NODE150...	GENE177...	0.627287		

Dock Close

- 1 For a small expression profiling matrix (1000 genes):
 - Cluster the genes
 - Calculate the correlation matrix
 - Write a CDT file of the clustered gene matrix with the correlation matrix appended
 - Visualize the CDT+GTR files in JavaTreeView – how well did the clustering work?
- 2 Repeat the previous exercise, exploring difference clustering methods and/or distance methods
- 3 Read the supplemental RnaSeq methods for PLoS Pathogens 12:e1005910 (Text S2, exported from RStudio). To what extent is this a reproducible method? Is there additional data that would make it more reproducible?