Practical Bioinformatics

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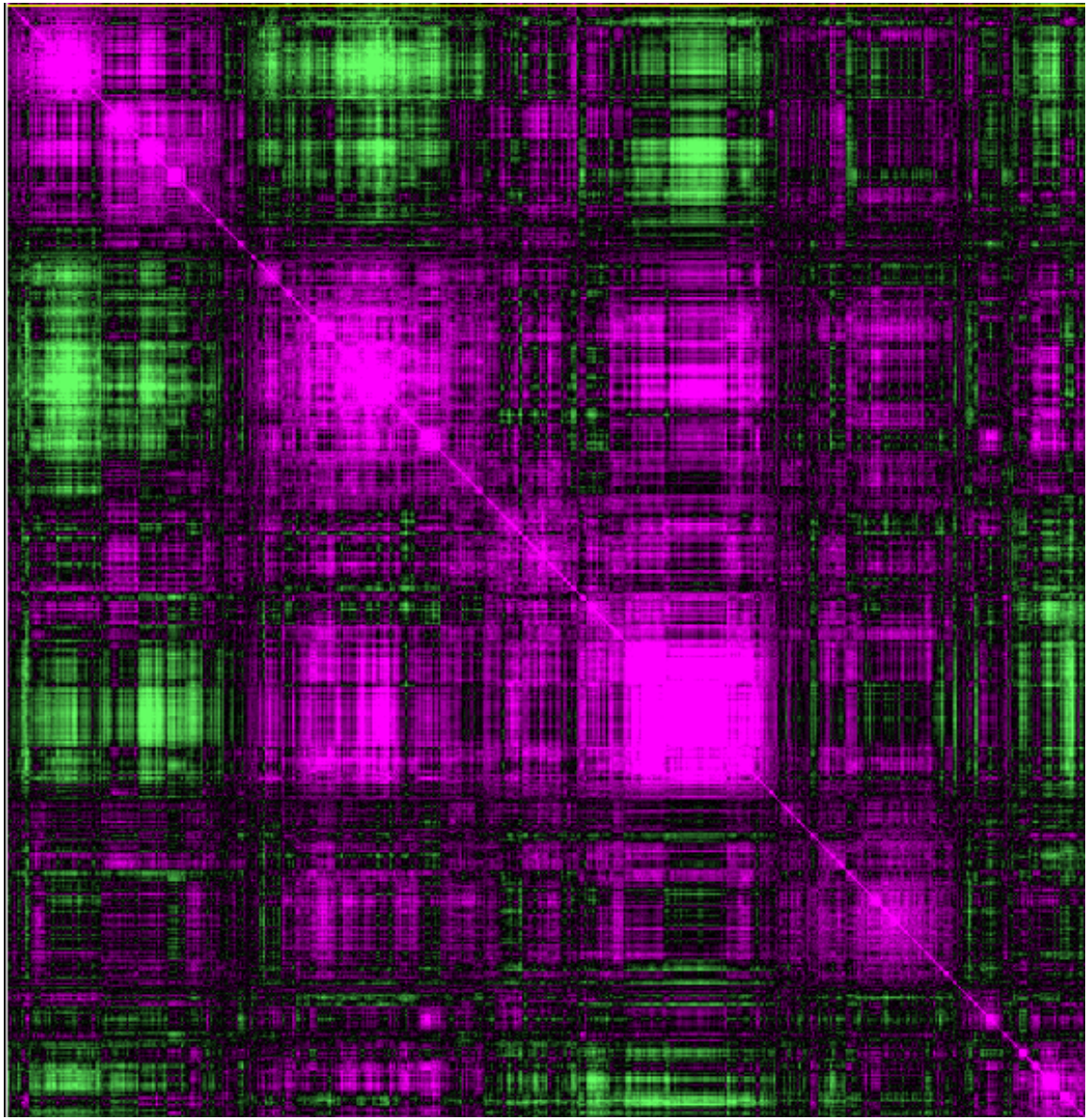
5/15/2015

Gotchas

- Indentation matters

Clustering exercises – Visualizing the distance matrix



Loading and re-loading your functions

```
# Use import the first time you load a module
#   (And keep using import until it loads
#    successfully)
import my_module

my_module.my_function(42)

# Once a module has been loaded, use reload to
#   force python to read your new code
reload(my_module)
```

Setting Canopy's working/import directory

OS X

- Open a terminal
- cd path/to/working/directory
- env PYTHONPATH="$PYTHONPATH:$PWD" canopy

Windows (or OS X)

- Start canopy
- %cd path/to/working/directory
- import sys, os
- sys.path.append(os.getcwd())

Pearson distances

Pearson similarity

$$s(x,y) = \frac{1}{N}\sum_{i}^{N}\left(\frac{x_i - x_{offset}}{\phi_x}\right)\left(\frac{y_i - y_{offset}}{\phi_y}\right)$$

$$\phi_G = \sqrt{\sum_{i}^{N}\frac{(G_i - G_{offset})^2}{N}}$$

Pearson distances

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$$\phi_G = \sqrt{\sum_{i}^{N} (G_i - G_{offset})^2}$$

Pearson distances

Pearson similarity

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

Pearson distances

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 distances

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Pearson distance

$$d_{uncentered}(x,y) = 1 - s(x,y)$$

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Pearson distance

$$d_{uncentered}(x,y) = 1 - s(x,y)$$

Euclidean distance

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

Clustering exercises – Negative controls

Write functions to reproduce the shuffling controls in figure 3 of the Eisen paper (removing correlations among genes and/or arrays).

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```
def shuffleGenes(self, seed = None):
    """Shuffle expression matrix by row."""
    import random
    if(seed != None):
        random.seed(seed)
    indices = range(len(self.genes))
    random.shuffle(indices)
    genes = [self.geneName[i] for i in indices]
    self.geneName = genes
    annotations = [self.geneAnn[i] for i in indices]
    self.geneAnn = genes
    num = [self.num[i] for i in indices]
    self.num = num
```

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```
def shuffleRows(self, seed = None):
    """Permute ratio values within rows."""
    import random
    if(seed != None):
        random.seed(seed)
    for i in self.num:
        random.shuffle(i)
```

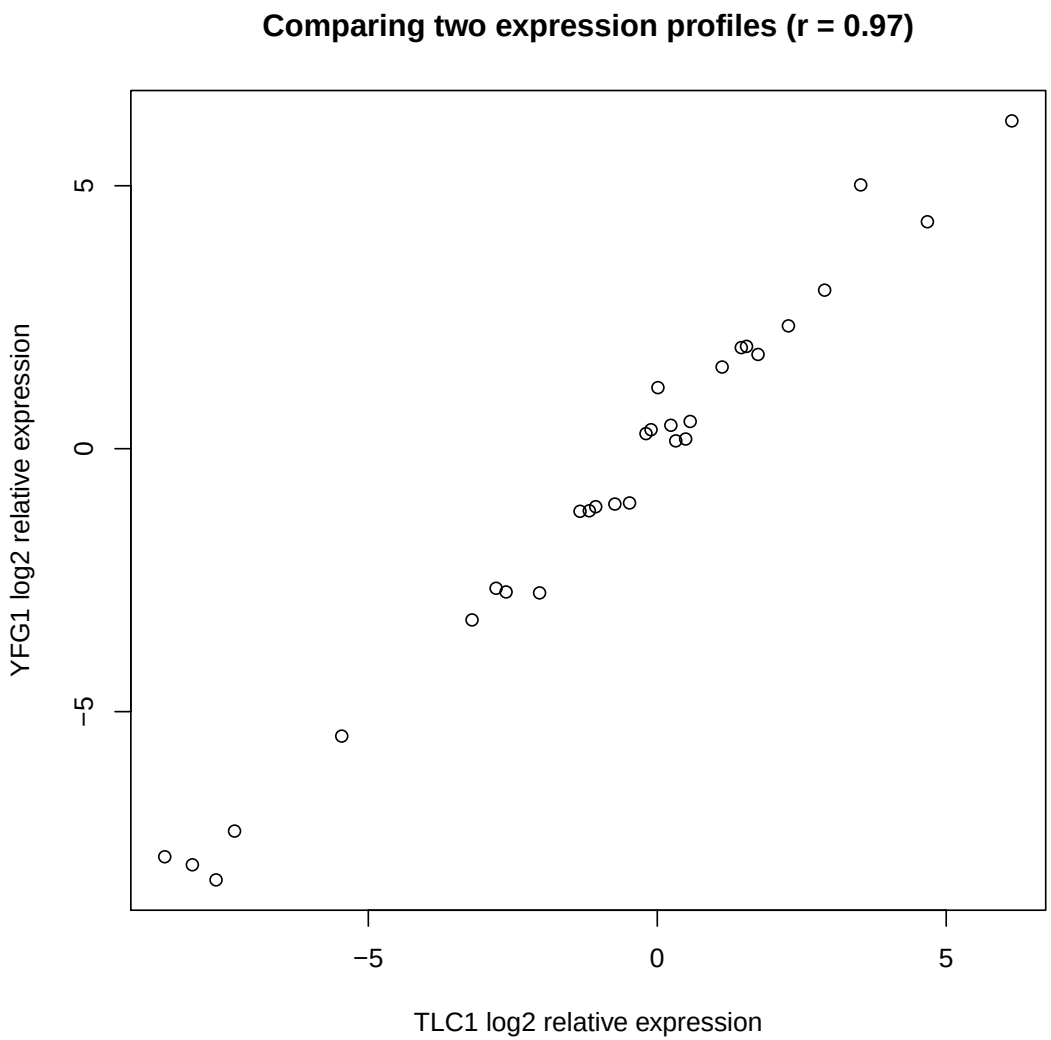
Clustering exercises – Negative controls

Write functions to reproduce the shuffling controls in figure 3 of the Eisen paper (removing correlations among genes and/or arrays).

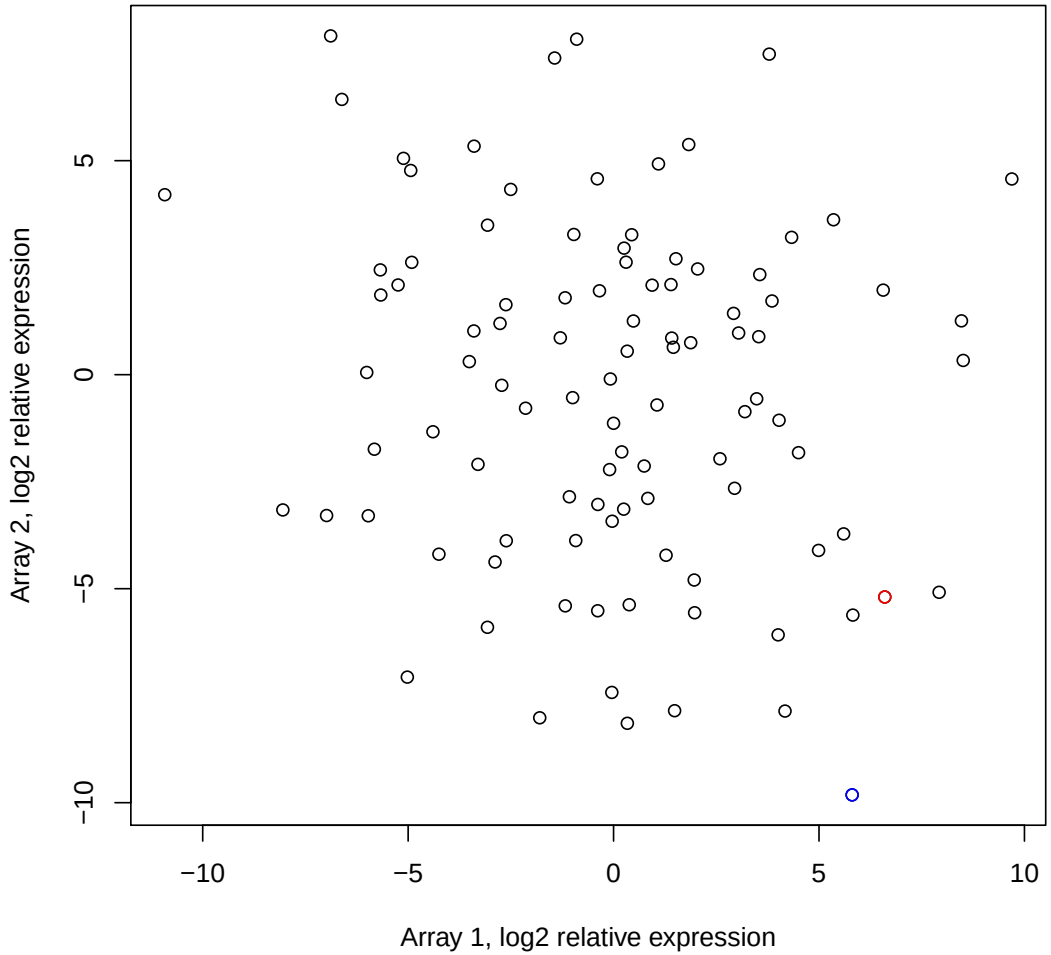
```
def shuffleRows(self, seed = None):
    """Permute ratio values within rows."""
    import random
    if(seed != None):
        random.seed(seed)
    for i in self.num:
        random.shuffle(i)


def shuffleCols(self, seed = None):
    """Permute ratio values within columns."""
    import random
    if(seed != None):
        random.seed(seed)
    # Transpose the expression matrix
    cols = []
    for col in xrange(len(self.num[0])):
        cols.append([row[col] for row in self.num])
    # Shuffle
    for i in cols:
        random.shuffle(i)
    # Transpose back to original orientation
    self.num = []
    for row in xrange(len(cols)):
        self.num.append([col[row] for col in row])
```

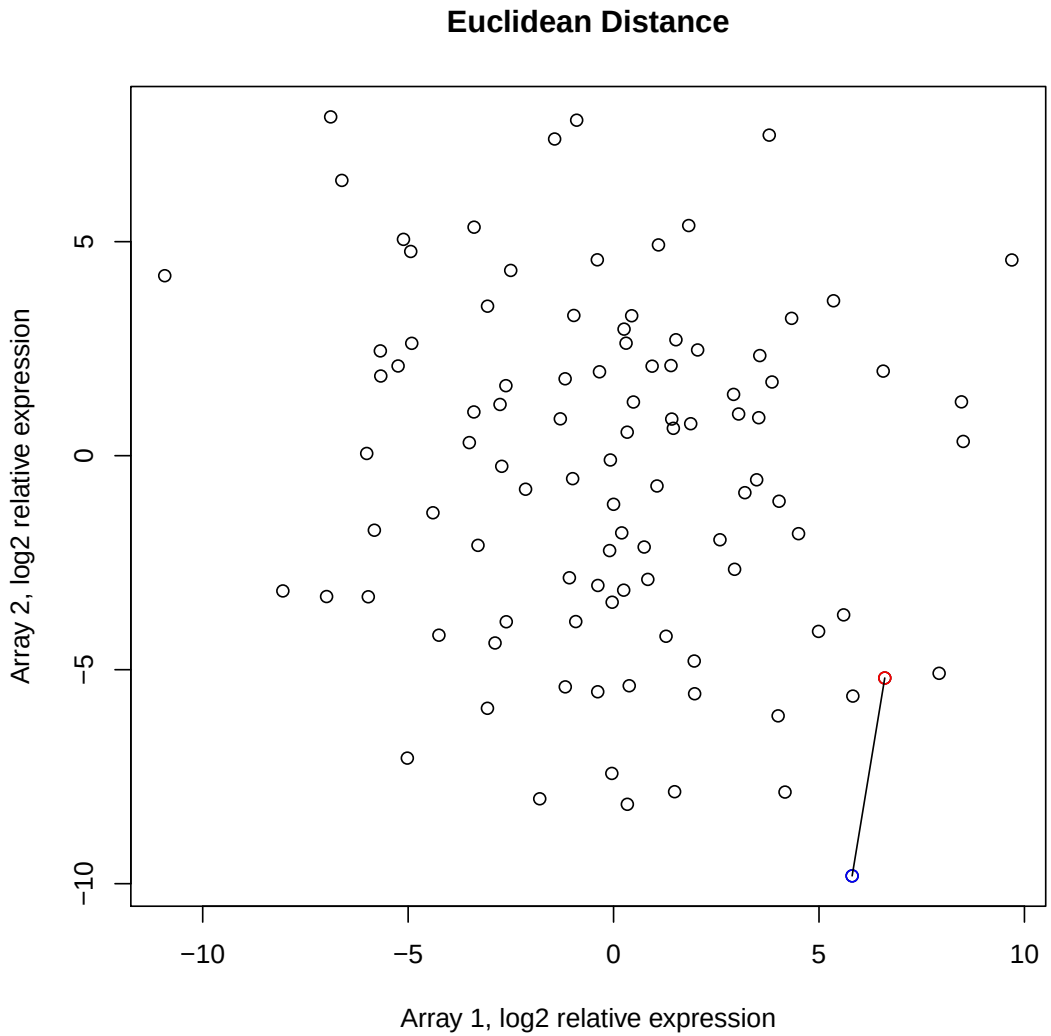
Comparing all measurements for two genes



Comparing all genes for two measurements



Comparing all genes for two measurements



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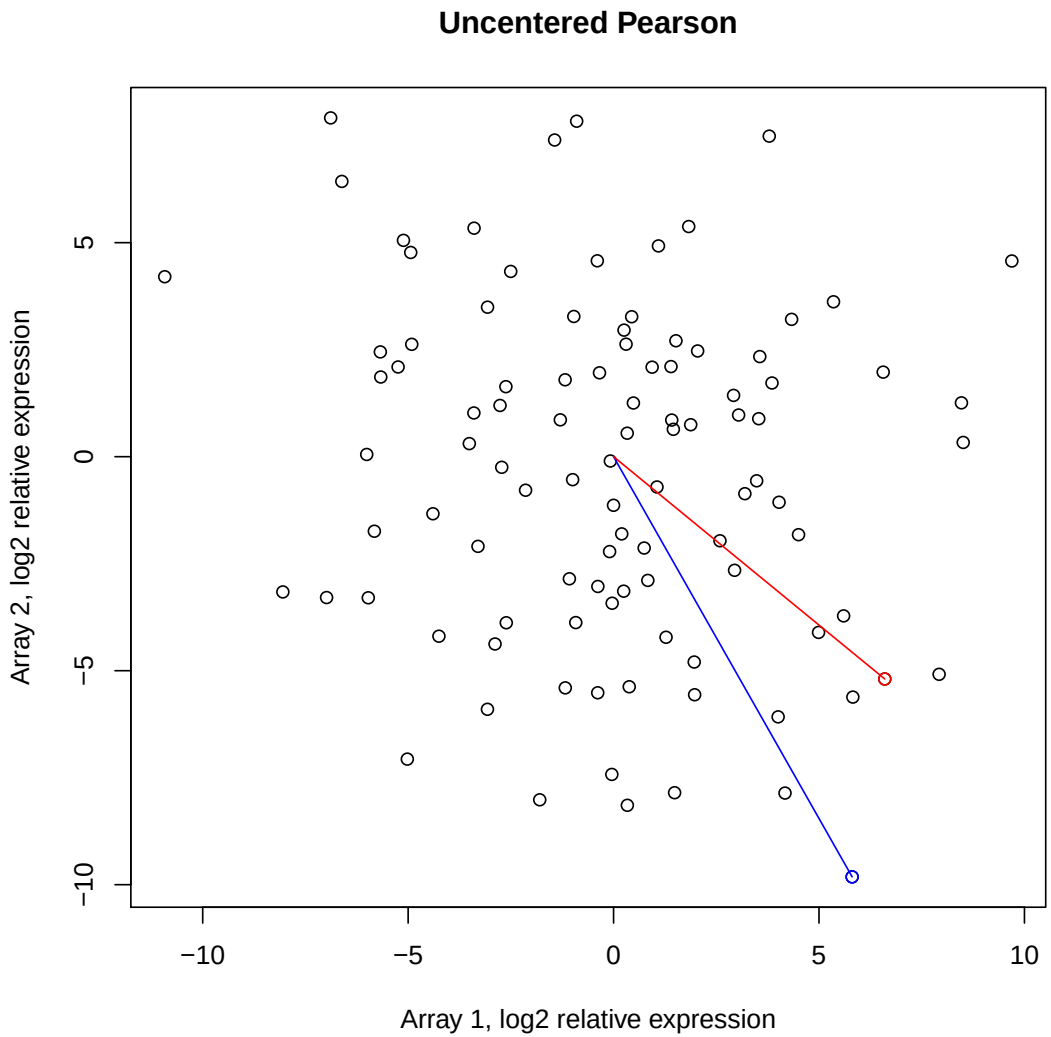
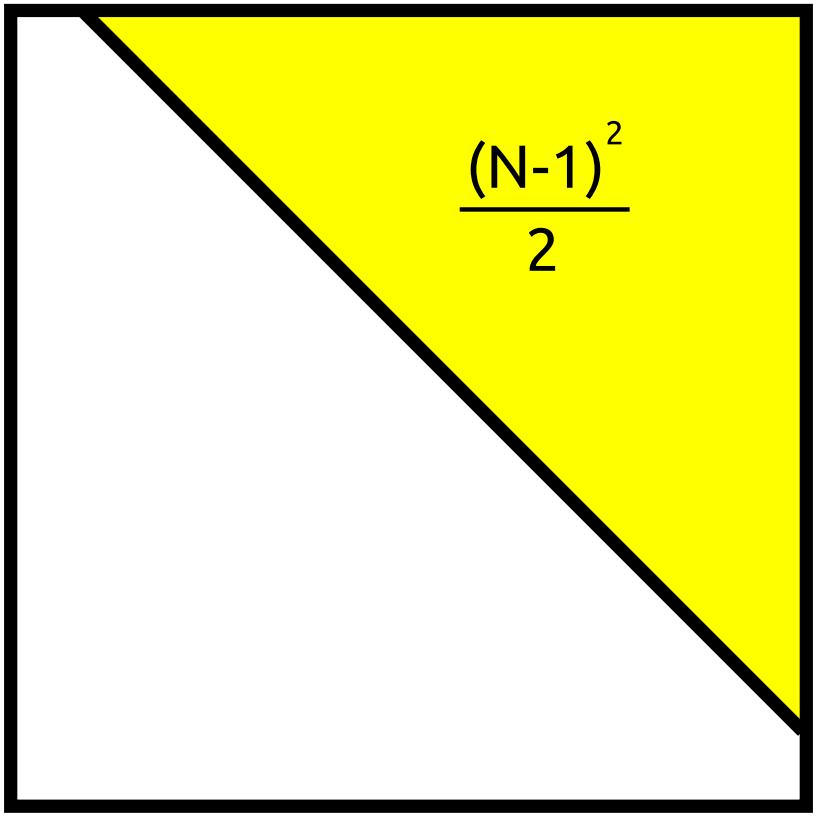
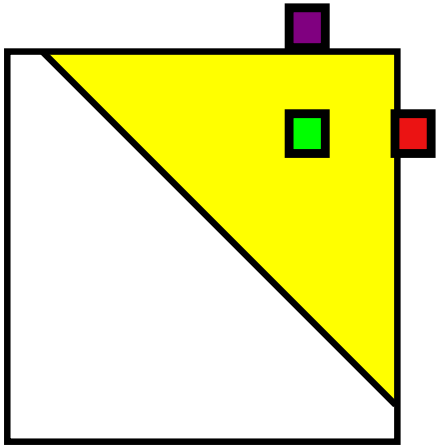
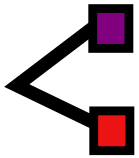


Measure all pairwise distances under distance metric



Hierarchical Clustering





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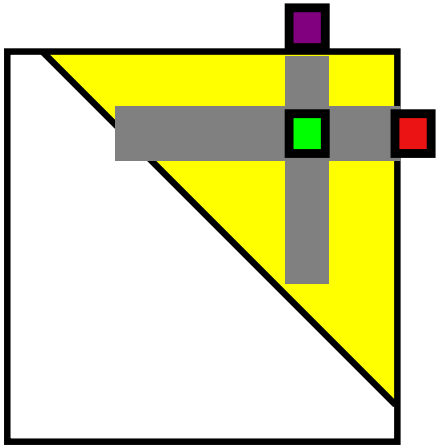

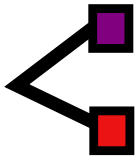

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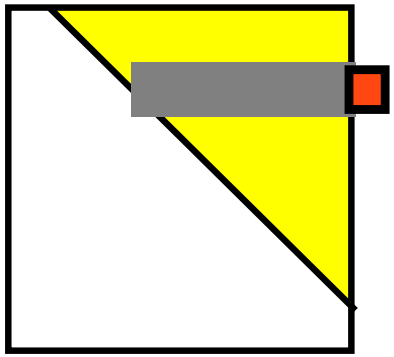

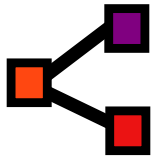

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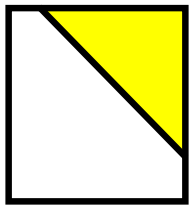

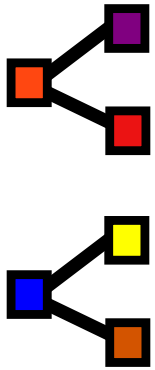

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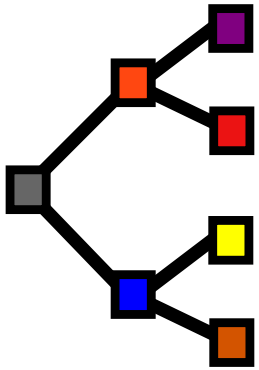


Scripting Cluster

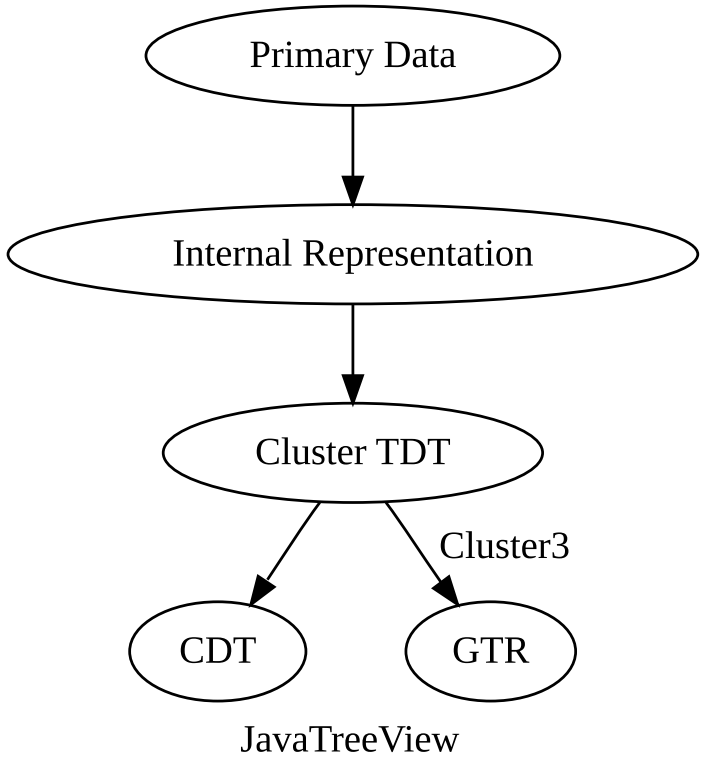
- Running Cluster3 from the command line
  - /Applications/Cluster.app/Contents/MacOS/Cluster
  - /Program Files/Stanford University/Cluster3/Cluster.com
- Command-line programs are like functions
- "man program" is like "help(function)"
- Use the `subprocess` module to run command-line programs from within Python.

USAGE: cluster [options]

-f filename	File loading
-u jobname	Allows you to specify a different name for the output files (default is derived from the input file name)
-g [0..8]	Specifies the distance measure for gene clustering 0: No gene clustering 1: Uncentered correlation 2: Pearson correlation 3: Uncentered correlation, absolute value 4: Pearson correlation, absolute value 5: Spearman's rank correlation 6: Kendall's tau 7: Euclidean distance 8: City-block distance (default: 0)
-m [msca]	Specifies which hierarchical clustering method to use m: Pairwise complete-linkage s: Pairwise single-linkage c: Pairwise centroid-linkage a: Pairwise average-linkage (default: m)

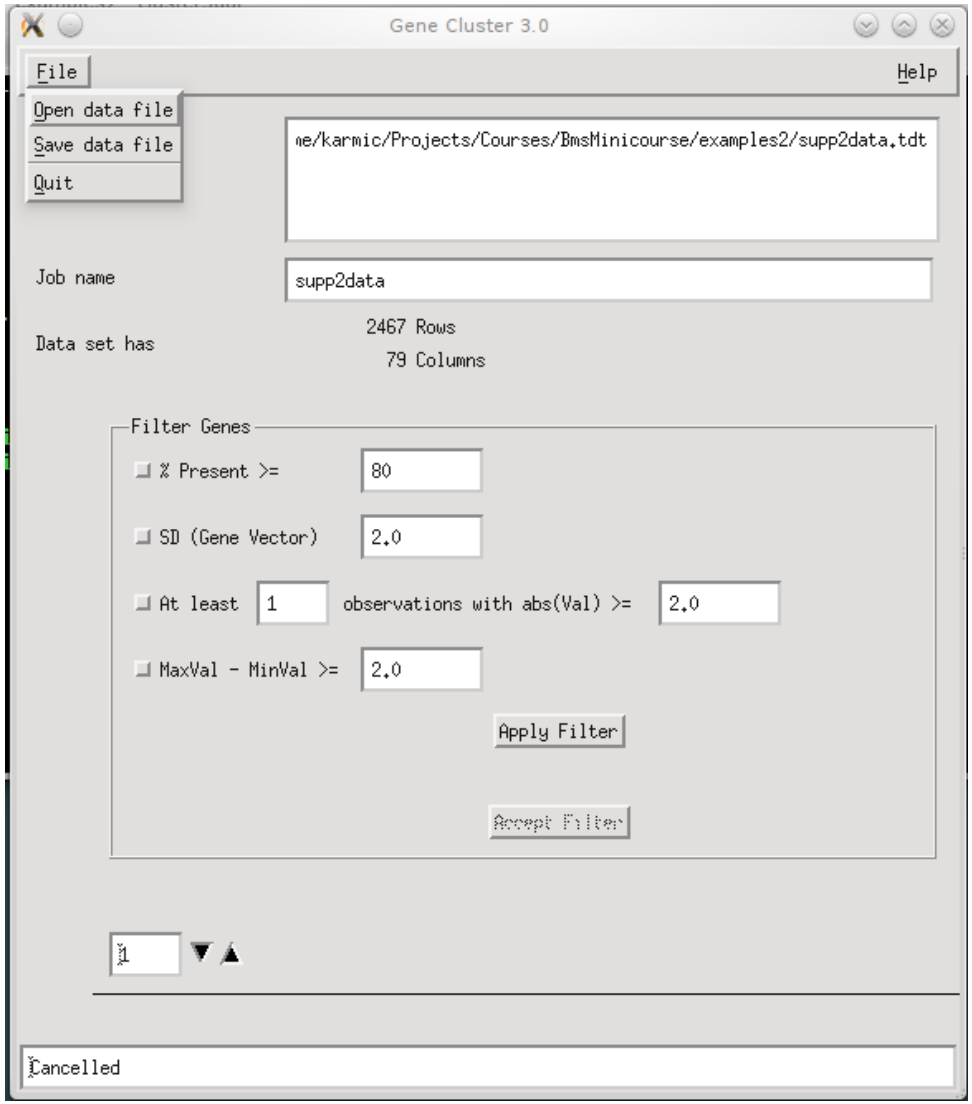
Scripting the Protocol

```
from subprocess import check_call
check_call(
    # Which program to run
    ("cluster",
     # Input file
     "-f","supp2data.tdt",
     # Output prefix
     "-u","supp2data.Uncentered.Complete",
     # Clustering method: complete linkage
     "-m","m",
     # Distance function: uncentered Pearson
     "-g","1"))
```

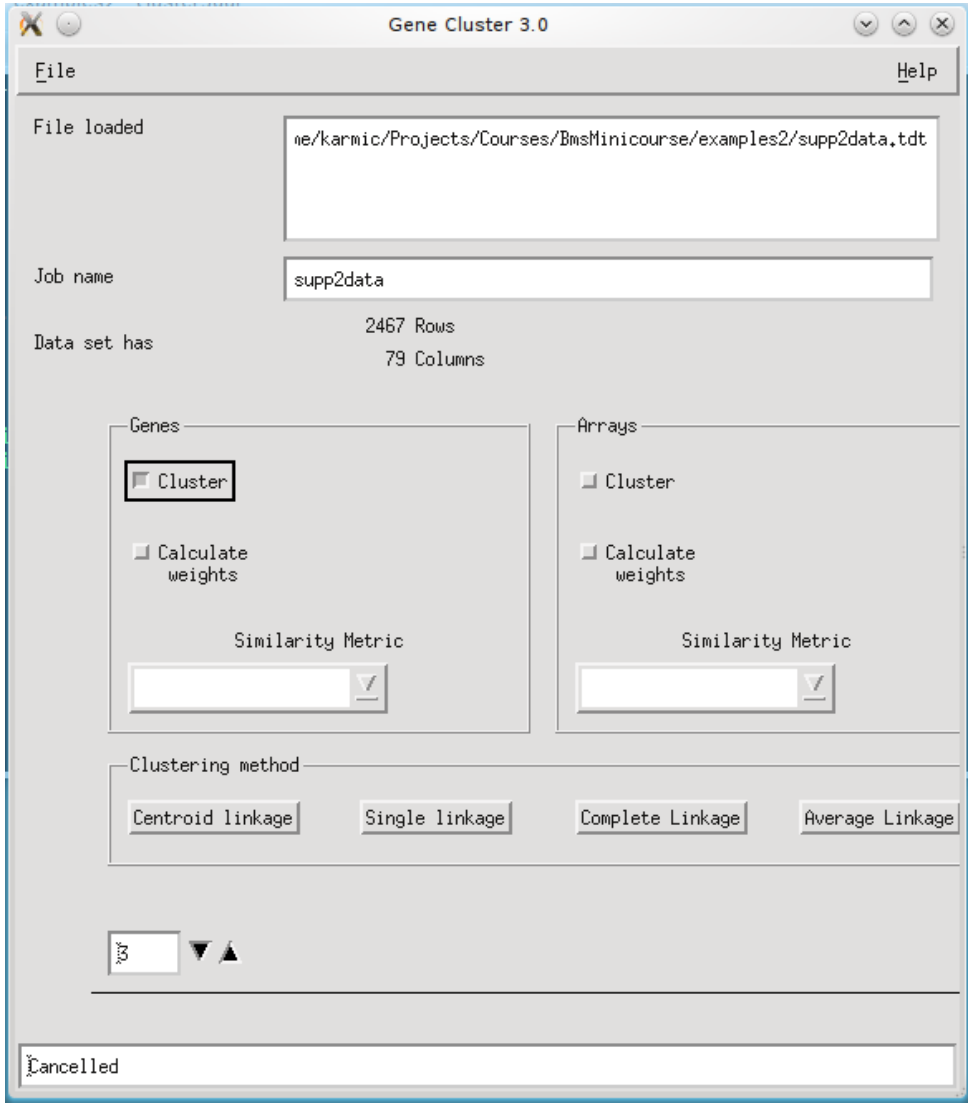


Using the Cluster3 GUI

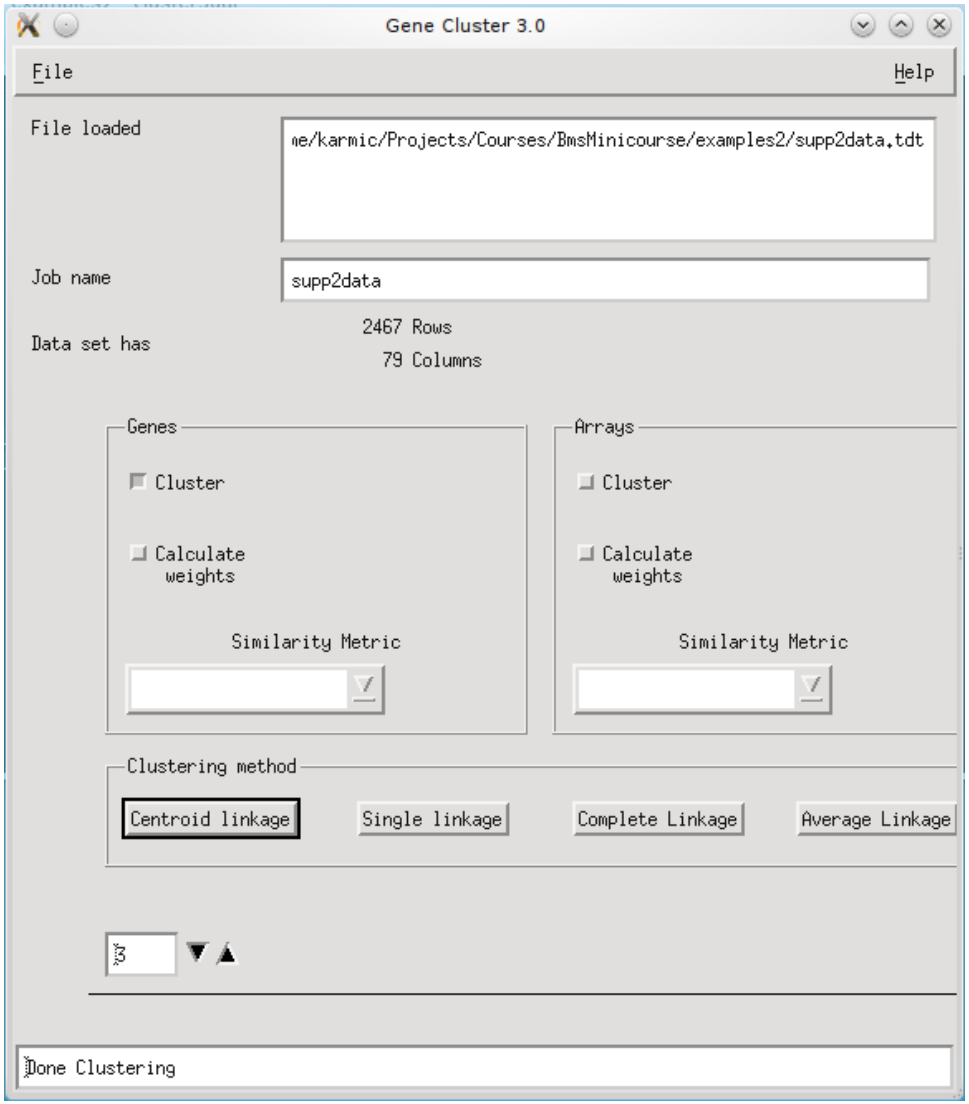
Load your data



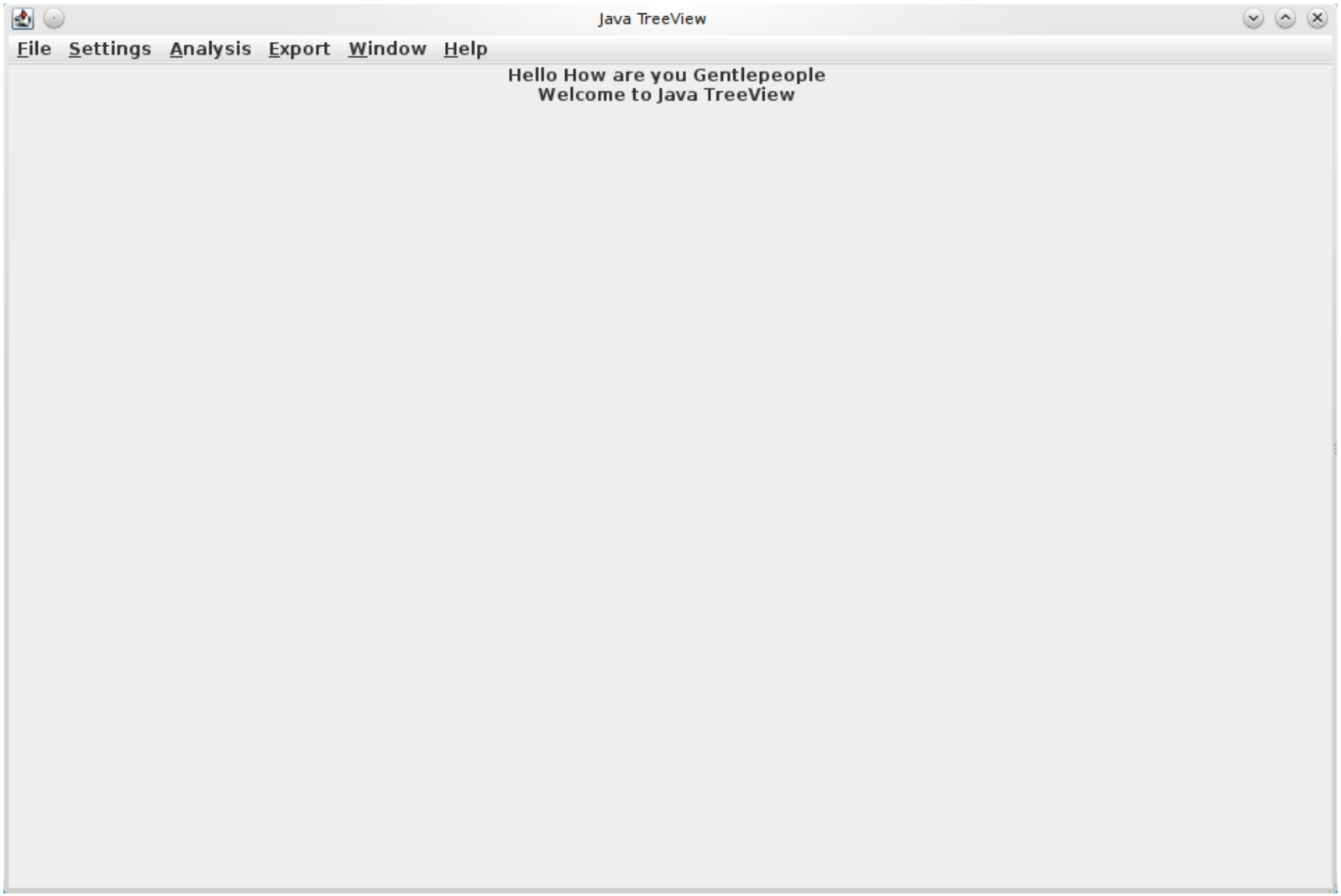
Choose distance function



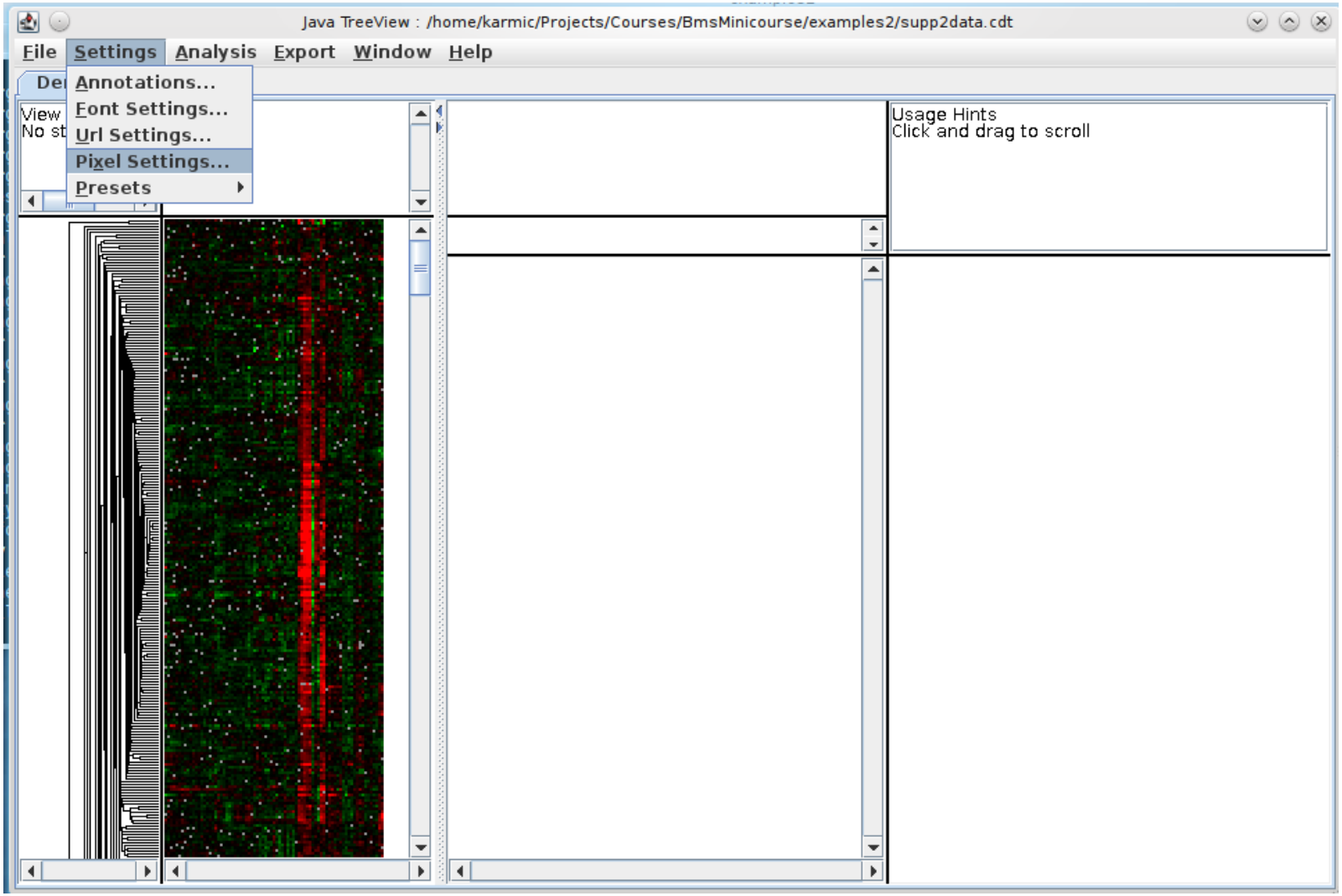
Choose linking method



Using JavaTreeView



Adjust pixel settings for global view



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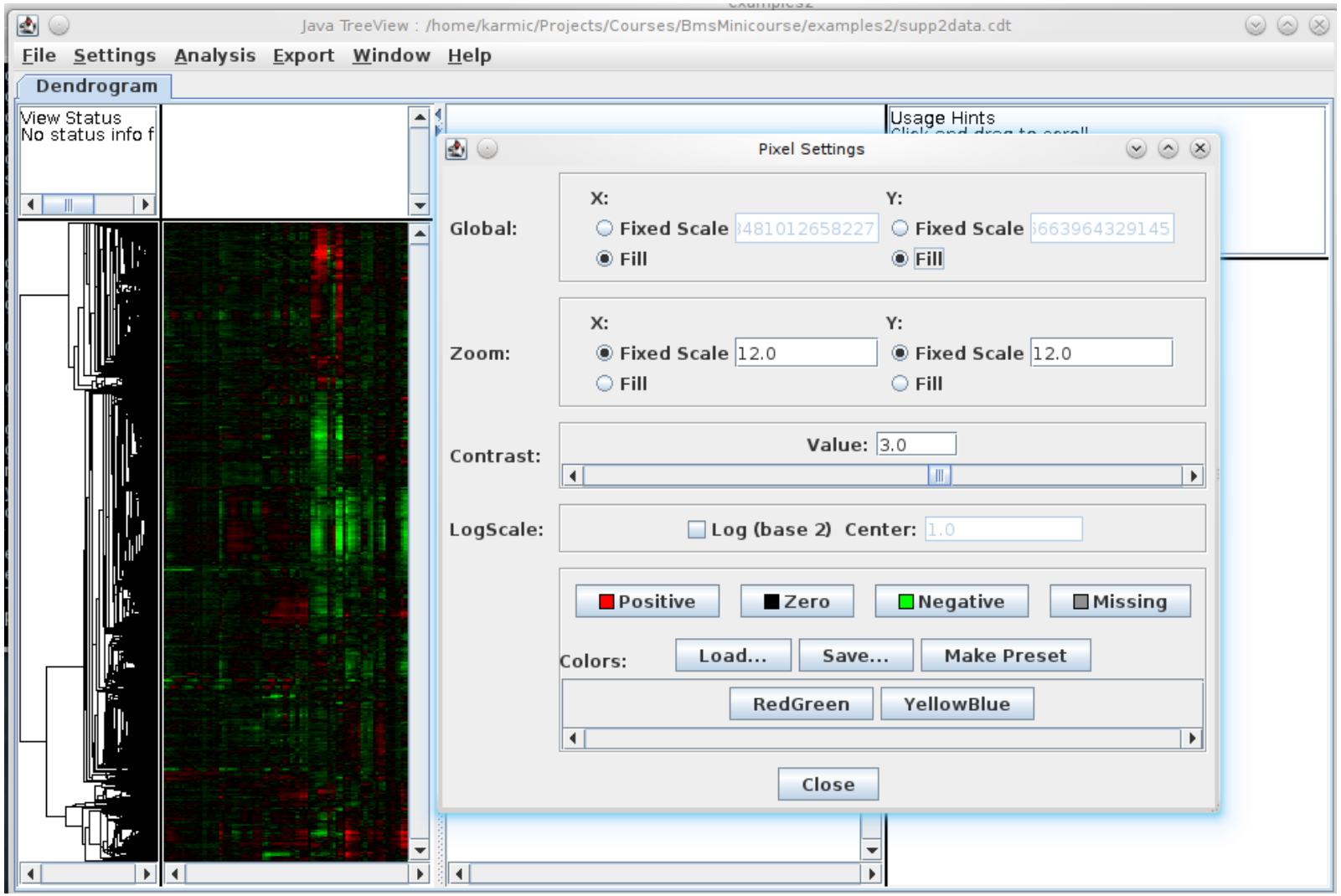
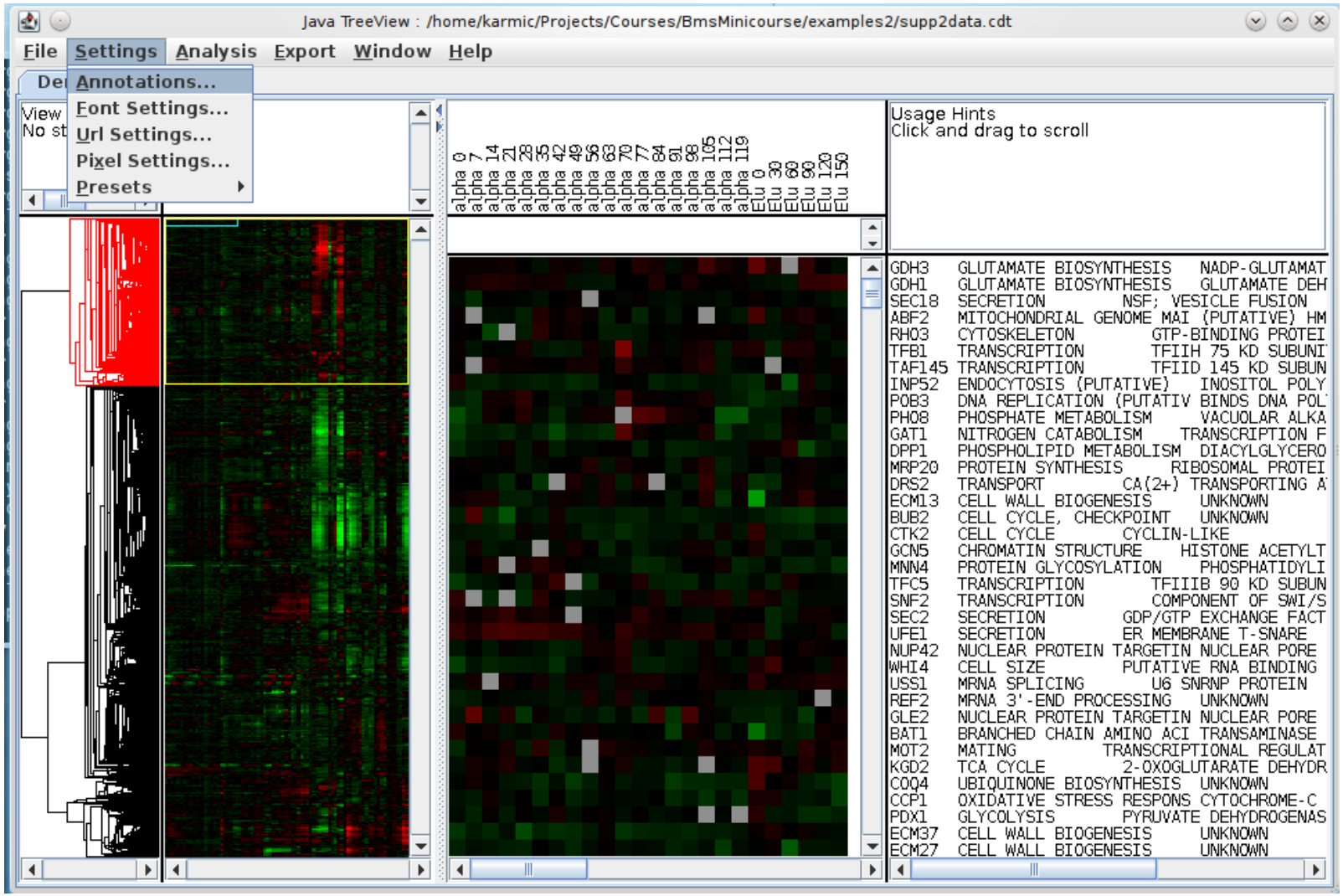


Select annotation columns



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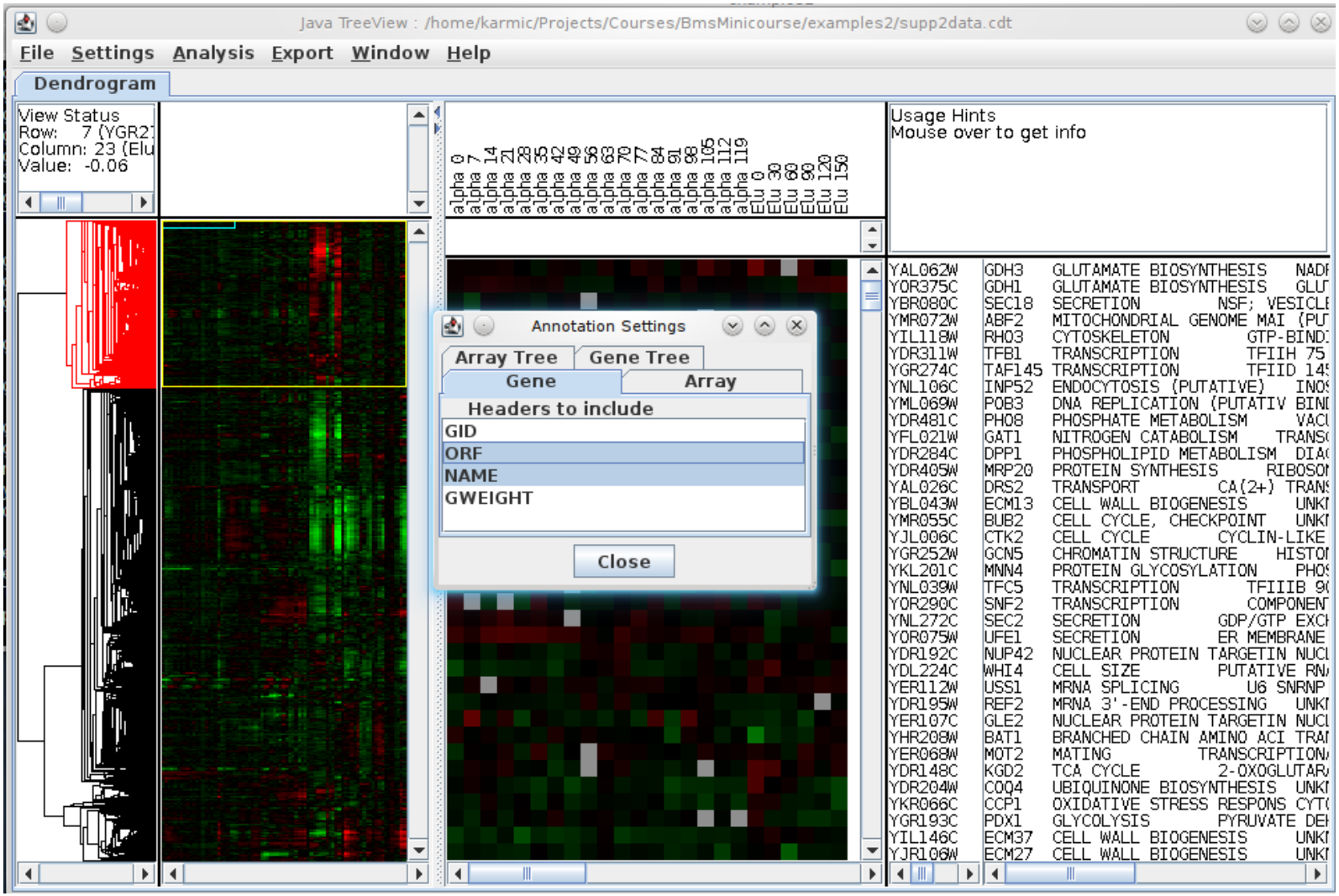
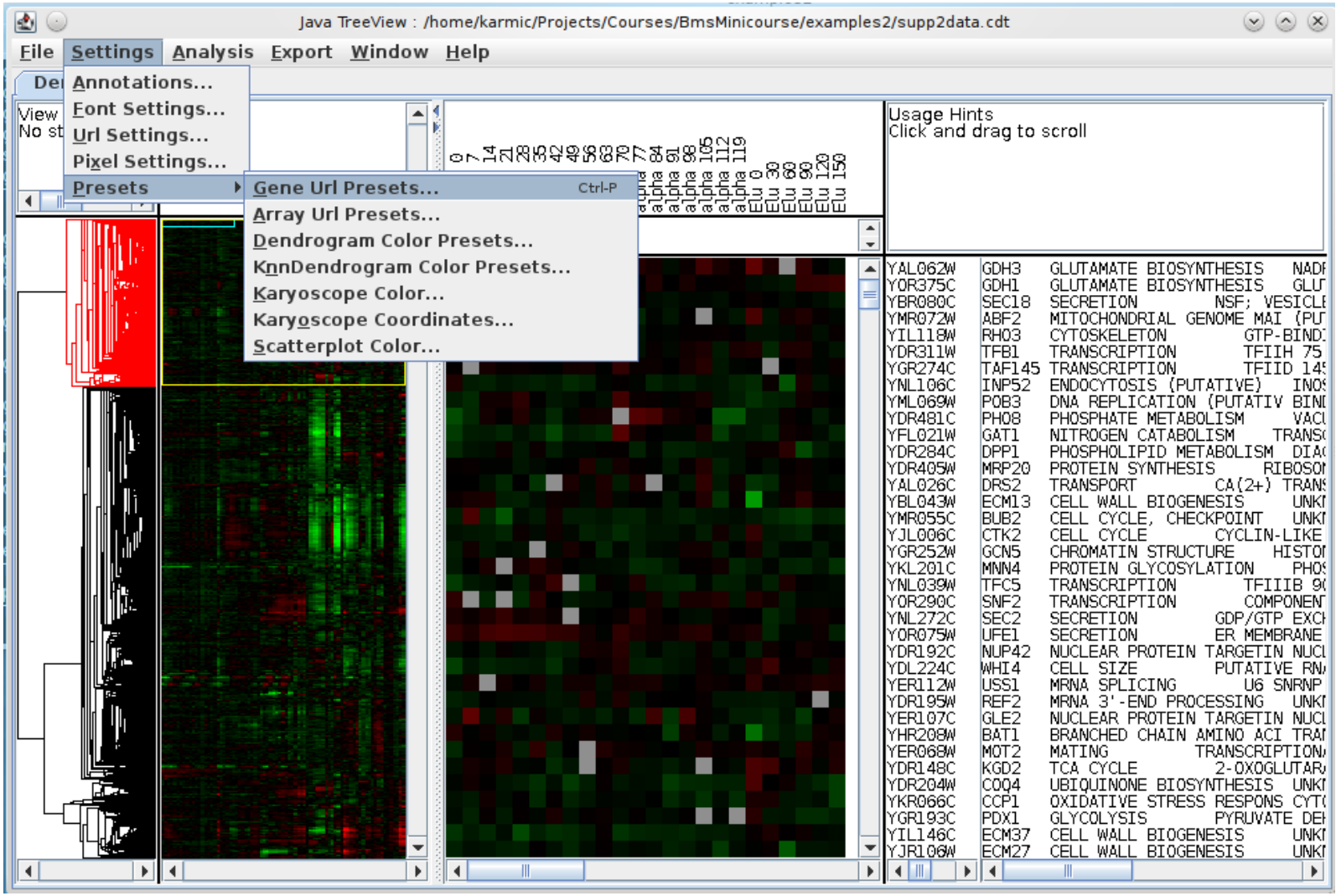


Select URL for gene annotations



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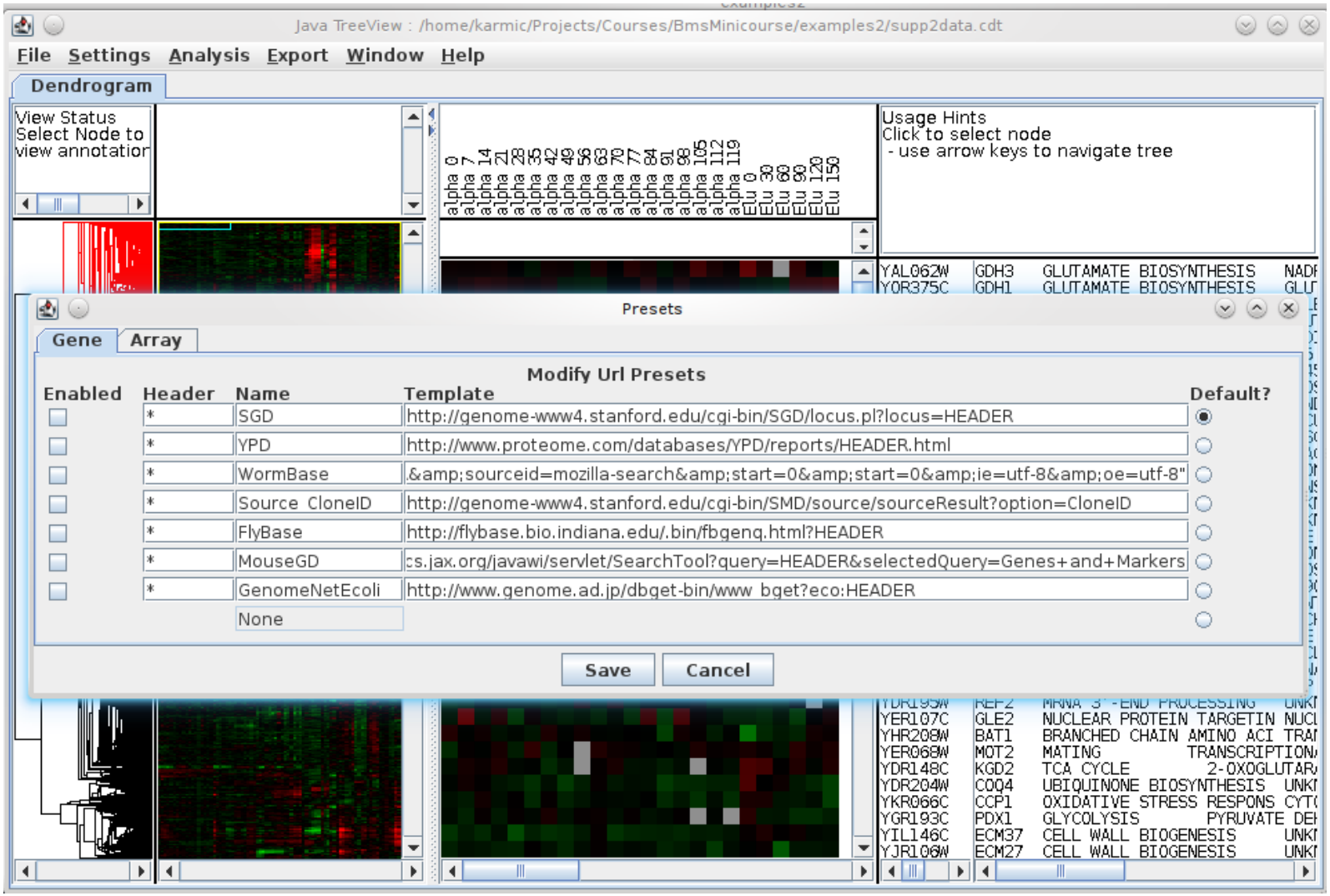
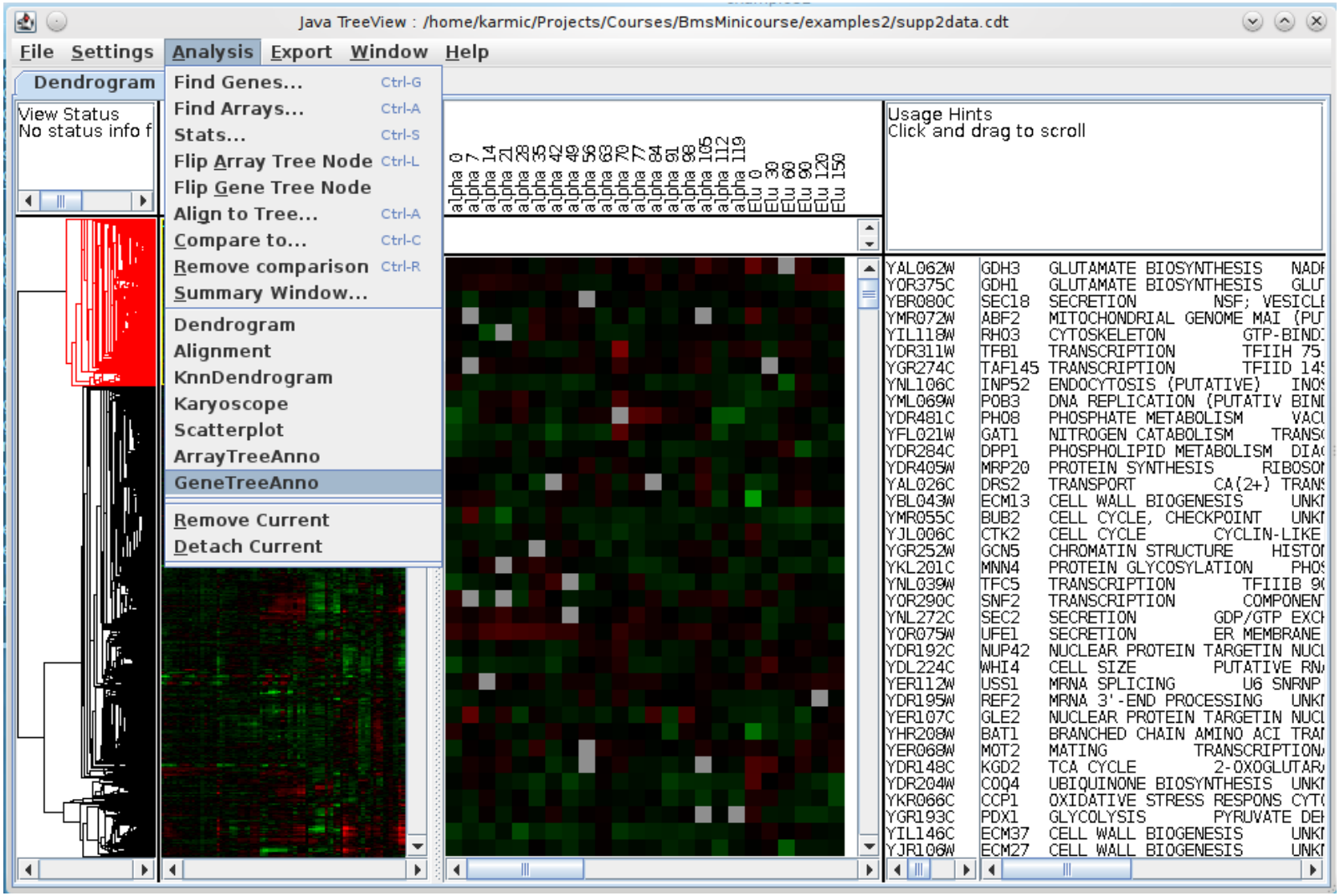


Activate and detach annotation window



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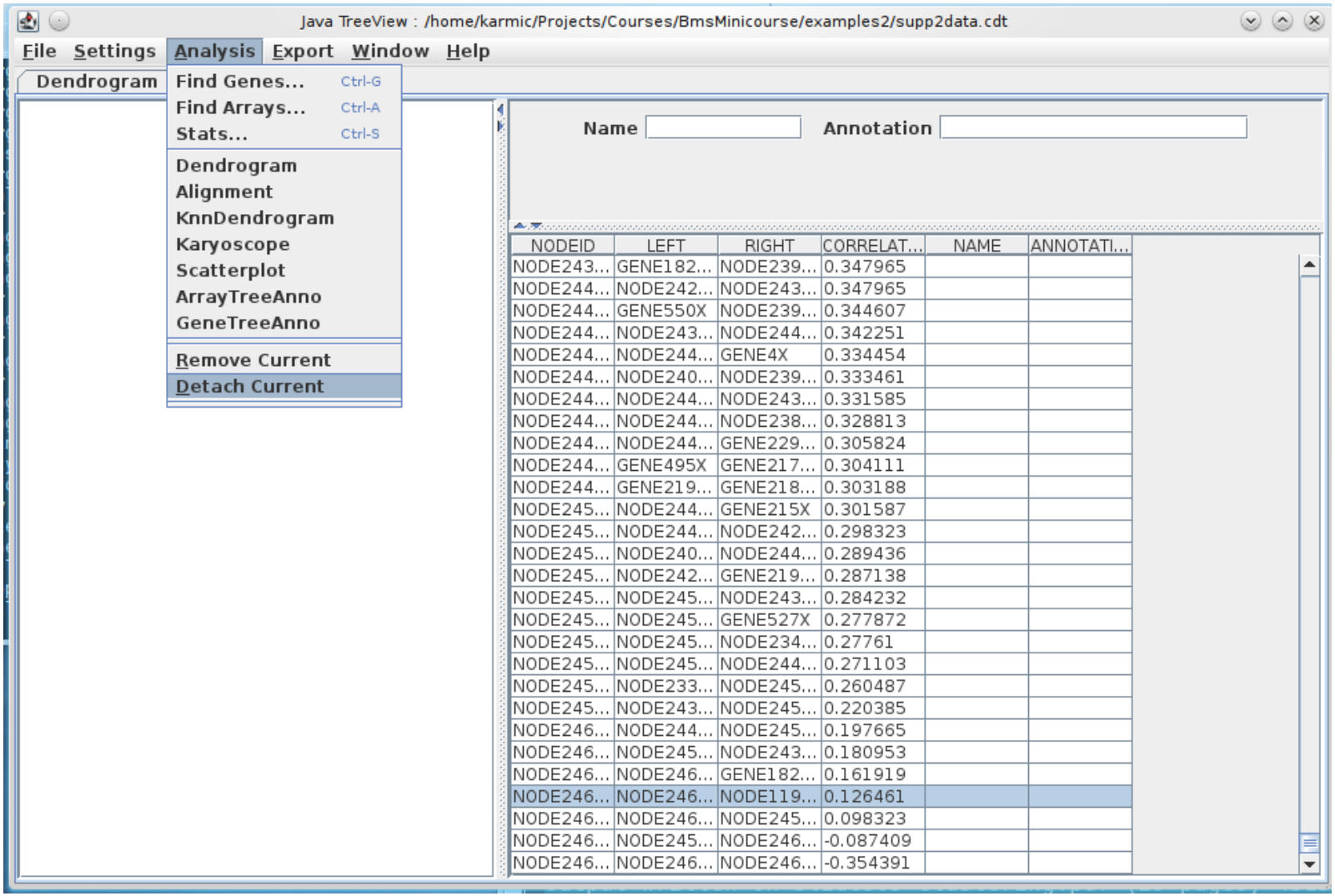

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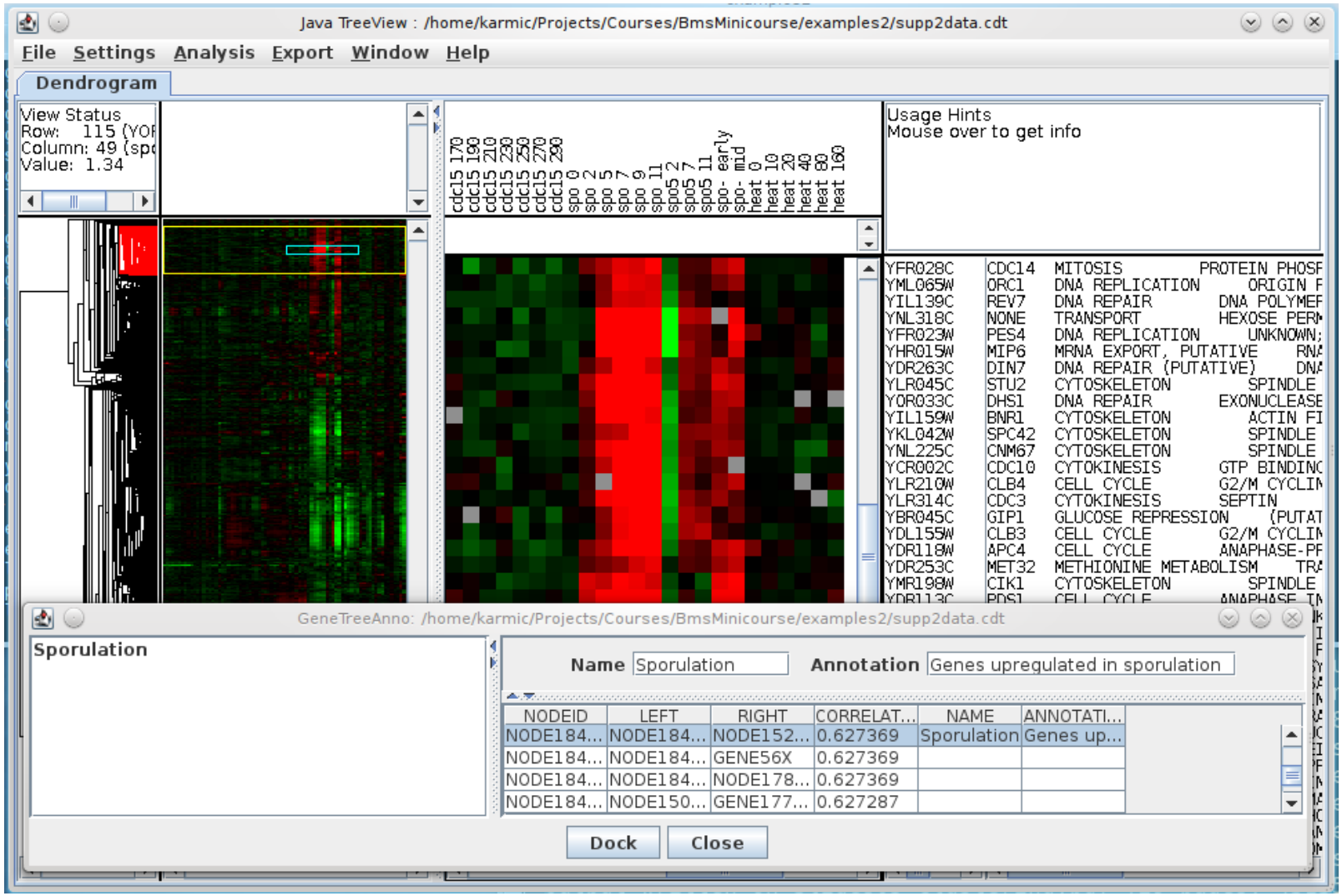

Clustering exercises – Scripting Cluster

Modify the clustering protocol script to run Cluster3 multiple times on the same input, varying distance metric and/or clustering method. Be sure to give the output files distinct names.

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```
metrics = ("None",
           "Uncentered",
           "Pearson",
           "UncenteredAbs",
           "PearsonAbs",
           "Spearman",
           "Kendall",
           "Euclidean",
           "City")
linkage = (("Complete","m"),
           ("Single","s"),
           ("Centroid","c"),
           ("Average","a"))

# Loop over all 32 possible methods
print "Starting hierarchical clustering runs..."
from subprocess import check_call
for metric in xrange(1,len(metrics)):
    print "    ",metrics[metric],"..."
    for (linkname, link) in linkage:
        print "        ",linkname
        check_call(("cluster","-f","shuffled.txt",
                    "-u",".".join(("shuffled",
                                   metrics[metric],
                                   linkname)),
                    "-m",link,"-g",str(metric)))
```

Homework

1. If you haven't done so already, read the PNAS paper
2. Explore the figure 2 data with Cluster3 and JavaTreeView.
   - Can you find/reproduce the clusters described in the paper?
   - Are the annotations consistent with the current annotations in SGD?
   - Are there other patterns that you can find in the data?
   - What follow-up experiments are prompted by this analysis?