GS01 0163
Analysis of Microarray Data

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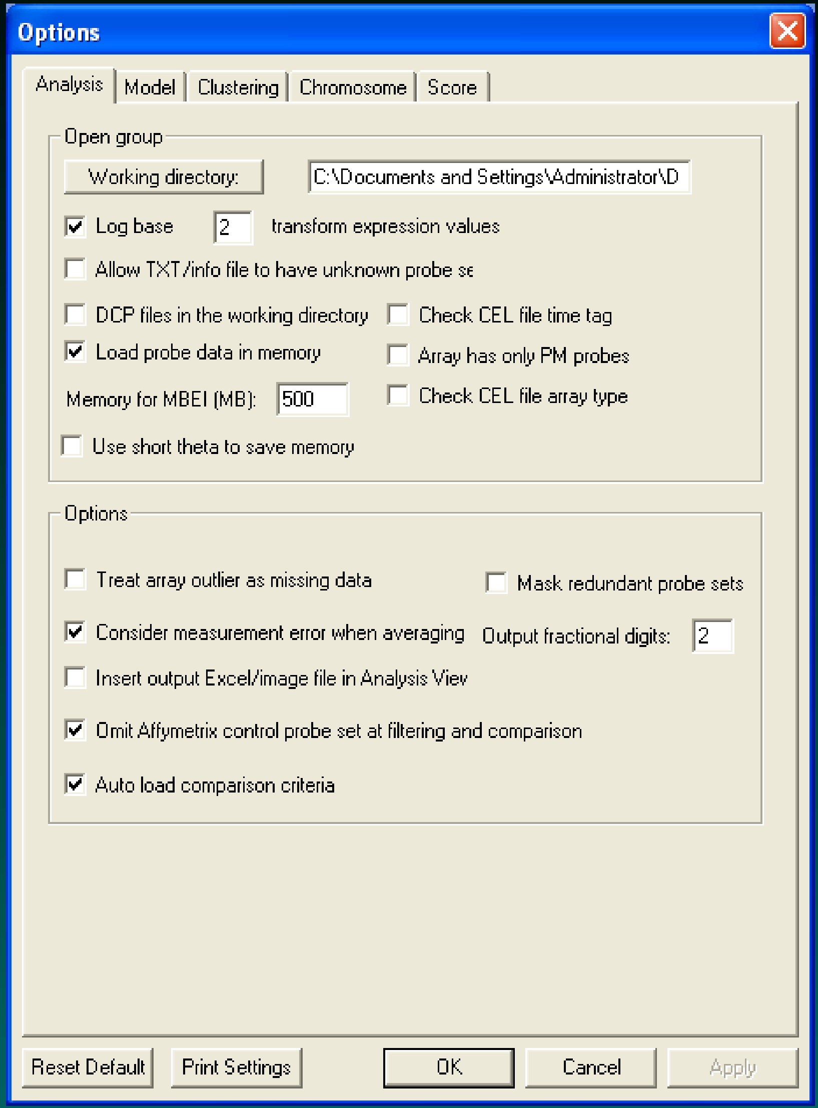
Lecture 15: Hierarchical Clustering

- So, why are we here?
- Clustering in dChip
- Measuring distances
- Hierarchical clustering
- When is a cluster valid?
- Clustering with fewer genes
- Simulating something

Diversion: dChip for TCGA data

- The Affymetrix chip used for the TCGA expression data is the HT_HG-U133A.
- You will need to download the CDF file for this chip from Affymetrix.
- Due to a bug in the current version of dChip, you must unclick the two “Check CEL file” options.



So, why are we here?

We want to learn something about clustering microarray data.

It is a well-known fact that clustering was invented by Michael Eisen, Paul Spellman, Pat Brown, and David Botstein in one of the most widely cited papers of all time:

Cluster analysis and display of genome-wide expression patterns. PNAS 1998; 95:14863-14868.

Digression

1. The ISI lists more than 2900 references to their paper.

2. You can tell they invented clustering, since their paper only has 16 references, 15 of which are to biologists and 1 to a computer scientist (Kohonen, for self-organizing maps). Their erratum, however, does give credit to John Weinstein in 1997 for coloring data matrices after clustering. So maybe Weinstein invented it.

3. They *are* responsible for choosing red-green colormaps, obviously being blithely unconcerned about the fact that this is the most common form of color-blindness.

4. The accuracy of well-known facts should always be questioned.

Clustering in dChip

Let's continue with the ALL-MLL example we have been using for a while. Recall that, when last we visited this data set, we had:

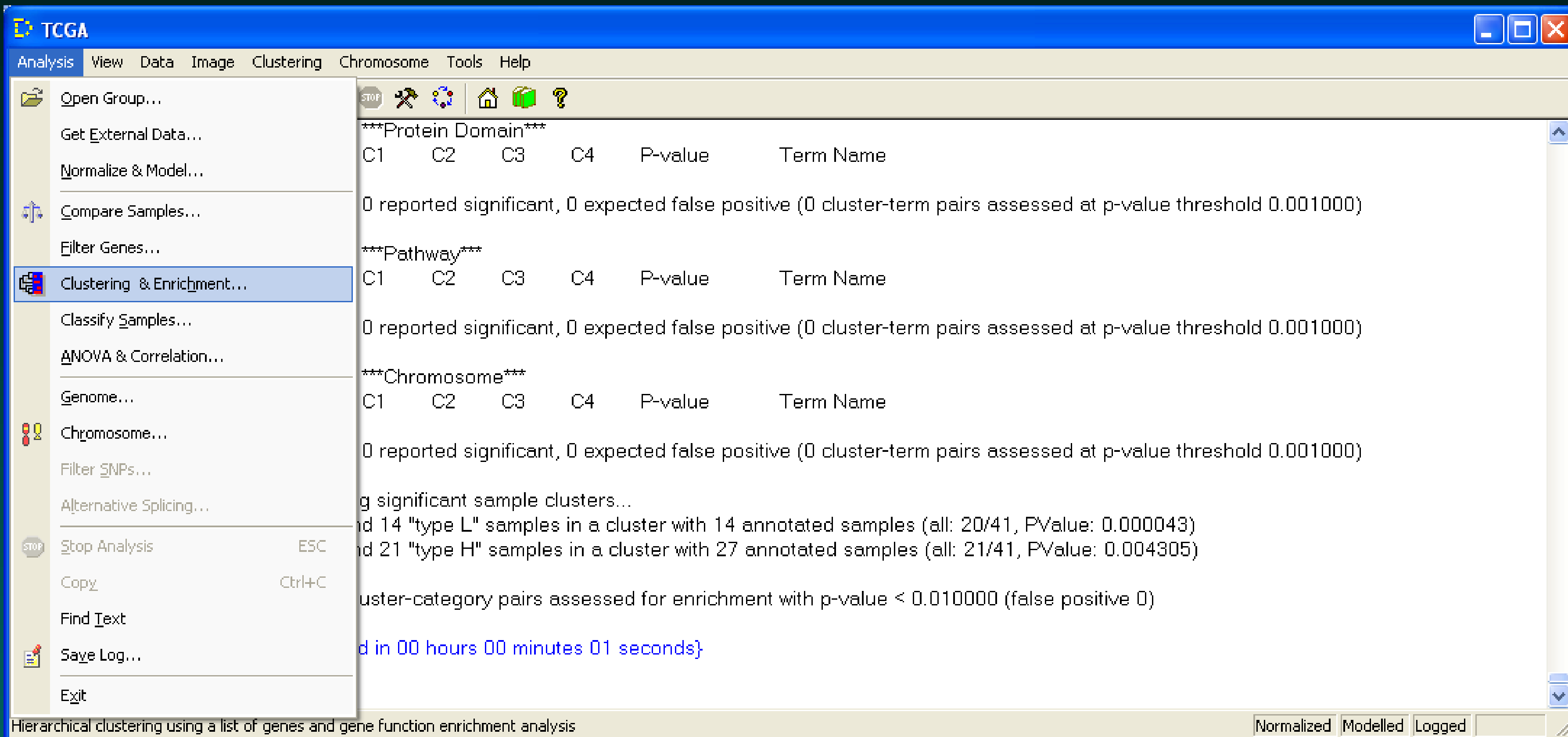
1. Performed a comparison in `dChip` that identified 610 differentially expressed genes (604 with dChip2006)

2. Tried to find out if any functional categories of genes were over-represented on the list of differentially expressed genes.

Now we'd like to take a different approach to grouping the genes and see which ones have similar profiles across the samples.

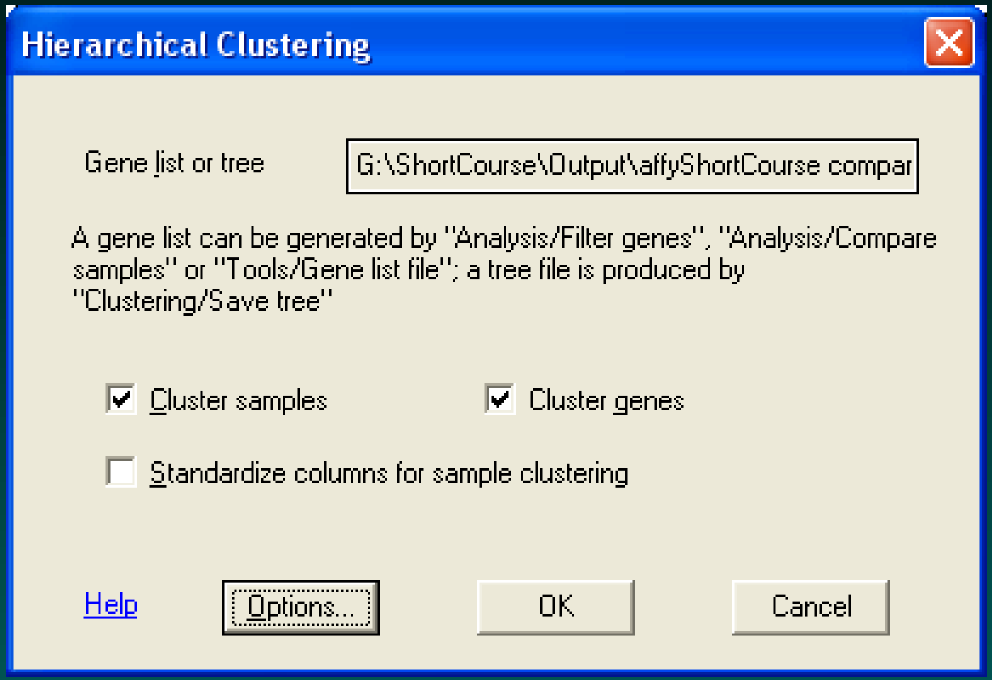
Starting to use hierarchical clustering in dChip

On the main “Analysis” menu, select “Clustering & Enrichment” (was “Hierarchical clustering”).



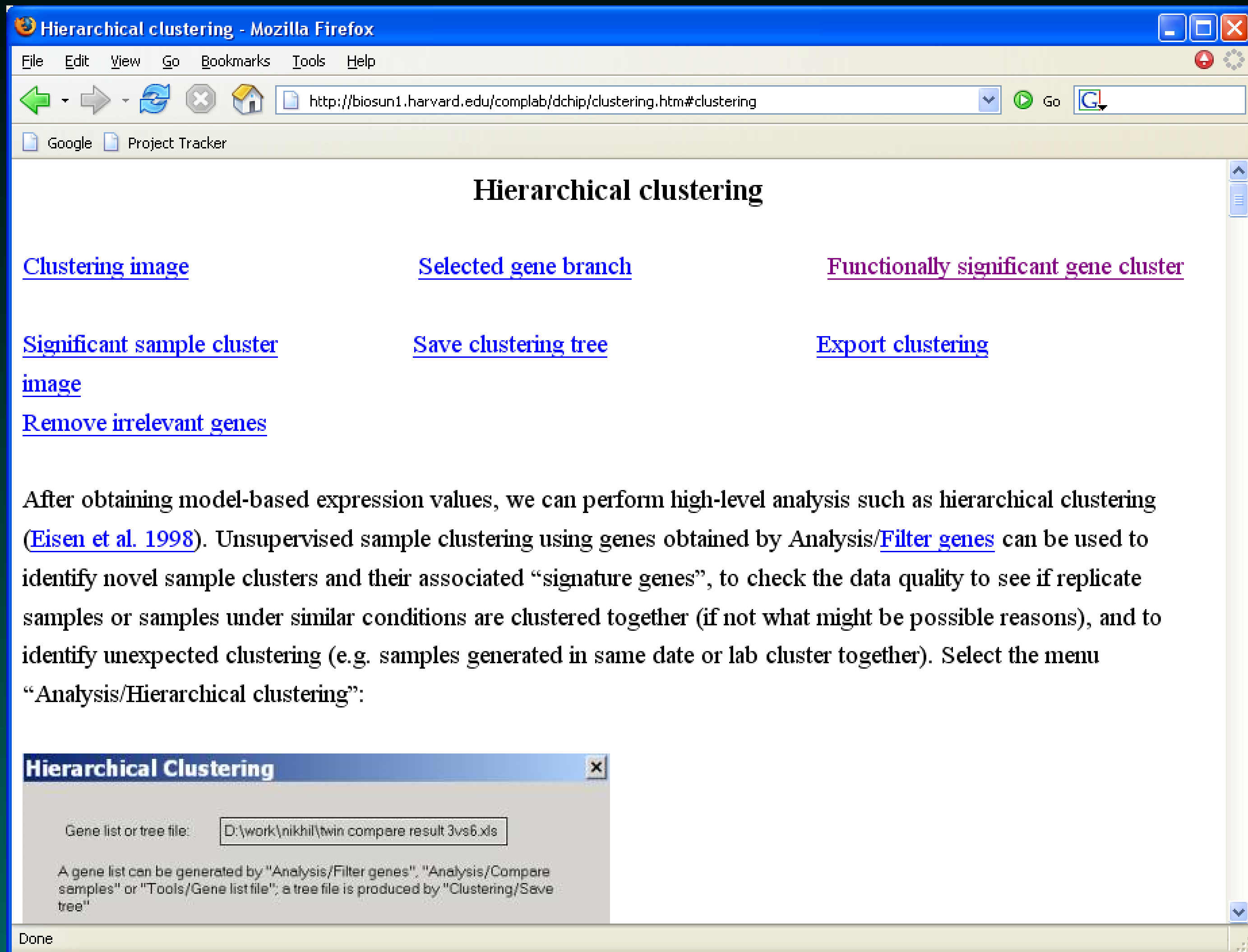
Starting to use hierarchical clustering in dChip

In the resulting dialog box, we can choose to cluster both genes and samples.



Whenever you're not sure about what to do in `dChip`, you can see what "Help" they provide.

dChip help for hierarchical clustering



dChip help for hierarchical clustering

Hierarchical clustering - Mozilla Firefox

File Edit View Go Bookmarks Tools Help

http://biosun1.harvard.edu/complab/dchip/clustering.htm#clustering Go

Google Project Tracker

A "gene list file" is a tab-delimited text file with probe set name in the first column of each line. It can be generated by "Analysis/Filter genes", "Analysis/Compare samples" or "Tools/Gene list file". It may also be a "Tree file" saved by the "Clustering/Save tree" function so that an existing tree structure saved before can be used. dChip will use genes in the file for clustering.

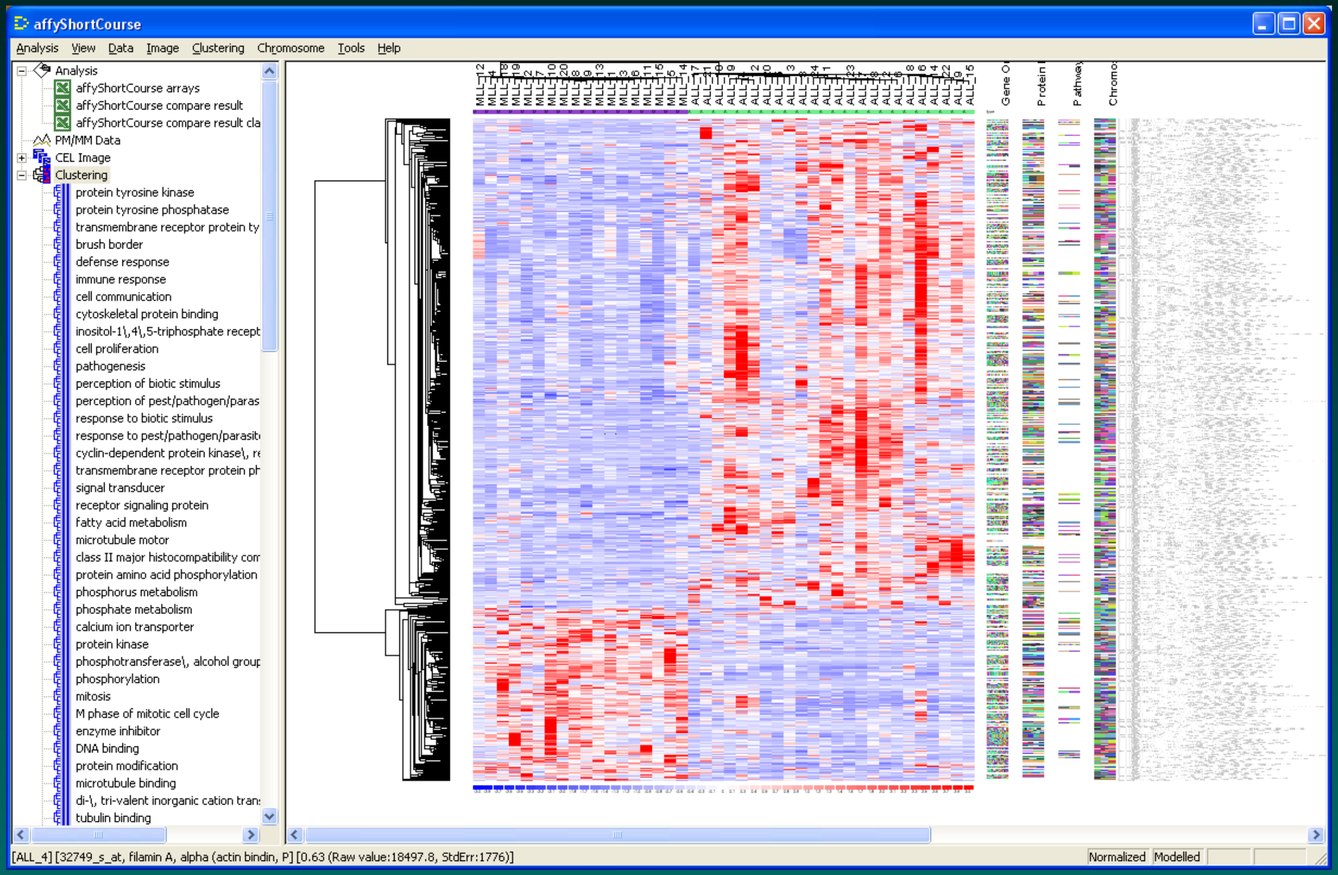
The samples used for clustering are either all the arrays, or the samples in the "Array list file" if it is specified. When a "Filter genes" gene list is used for clustering, it is often desired to use the same "Array list file" used in filtering genes to do gene clustering and sample clustering. This is an unsupervised sample clustering since the genes are selected by large variation across samples and the sample group information is not used. When one specifies a "Compare samples" gene list generated by using only a subset of samples, it is often desired to only specify and order the relevant samples in "Array list file" and view them without sample clustering. In this case the main interest lies in

If the number of genes is large (e.g. 10,000), dChip may report "out of memory" or perform slowly, since storing all the pair-wise distances requires too much memory and may cause virtual-memory swapping. The solution is to uncheck the "Tools/Options/Clustering/Pre-calculate distances" button to calculate the pair-wise distances between genes on the fly.

Done

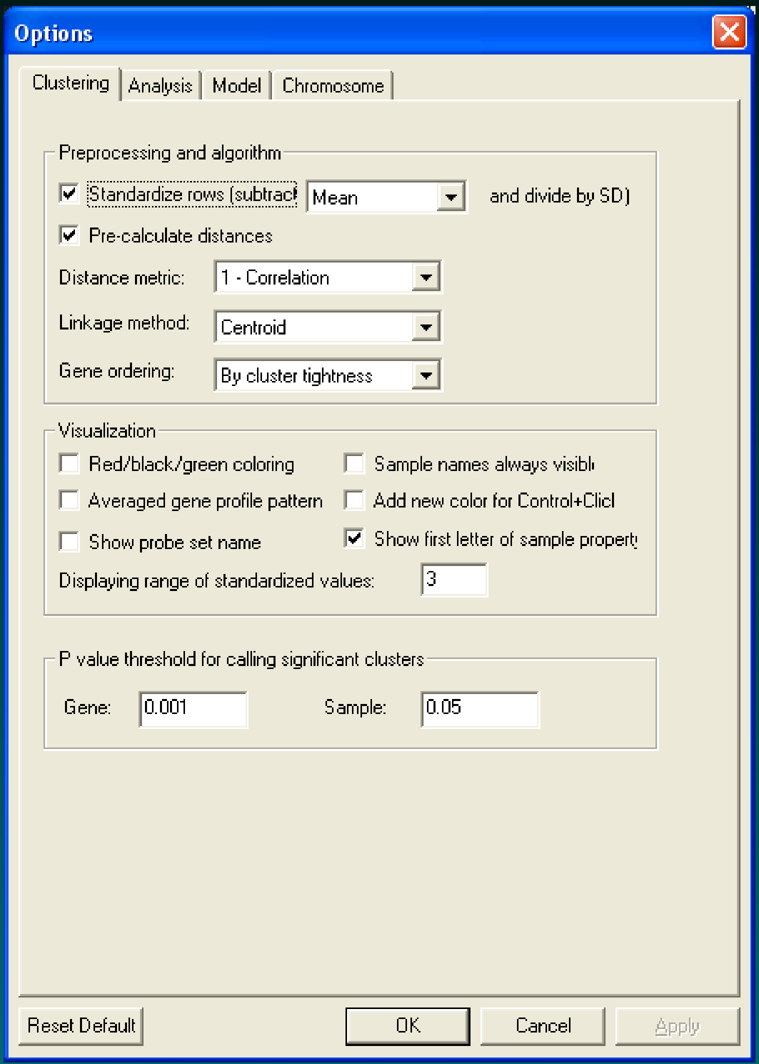
dChip clustering results

Ignoring their advice, we go ahead and cluster samples using the 610 (604) genes selected from our previous sample comparison.



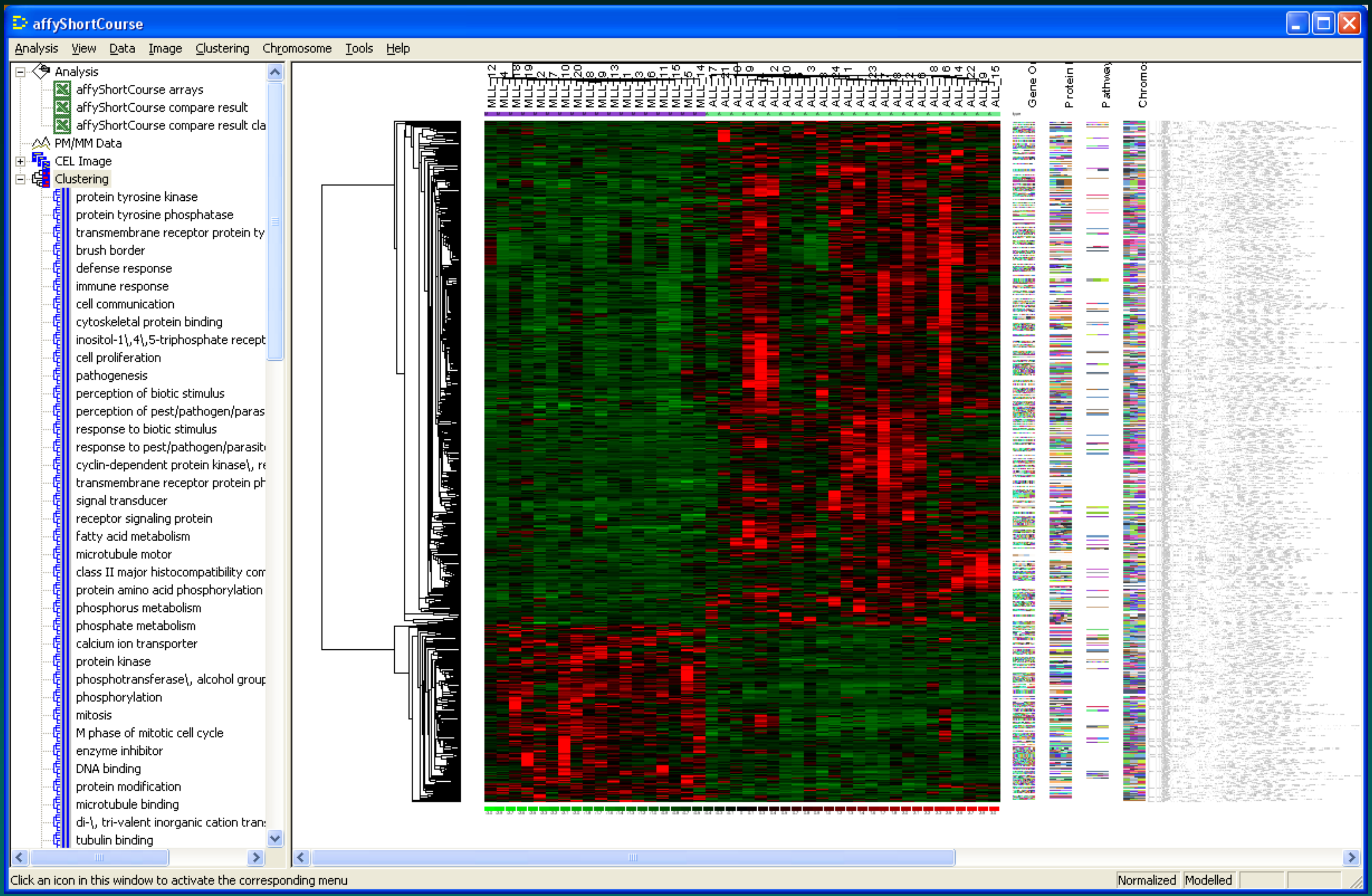
dChip clustering options

Using “Tools” – > “Options”, we get:



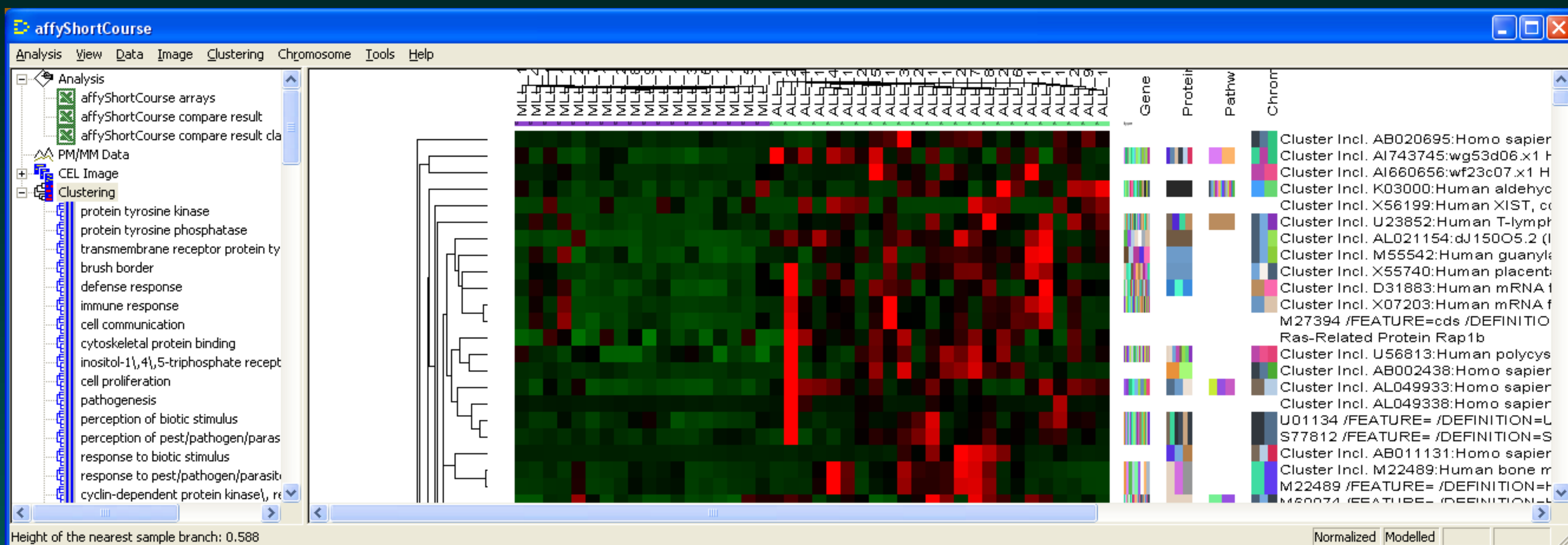
dChip clustering results

Checking the visualization option for red/black/green coloring gives the Eisen colormap.



Exploring the clustering results

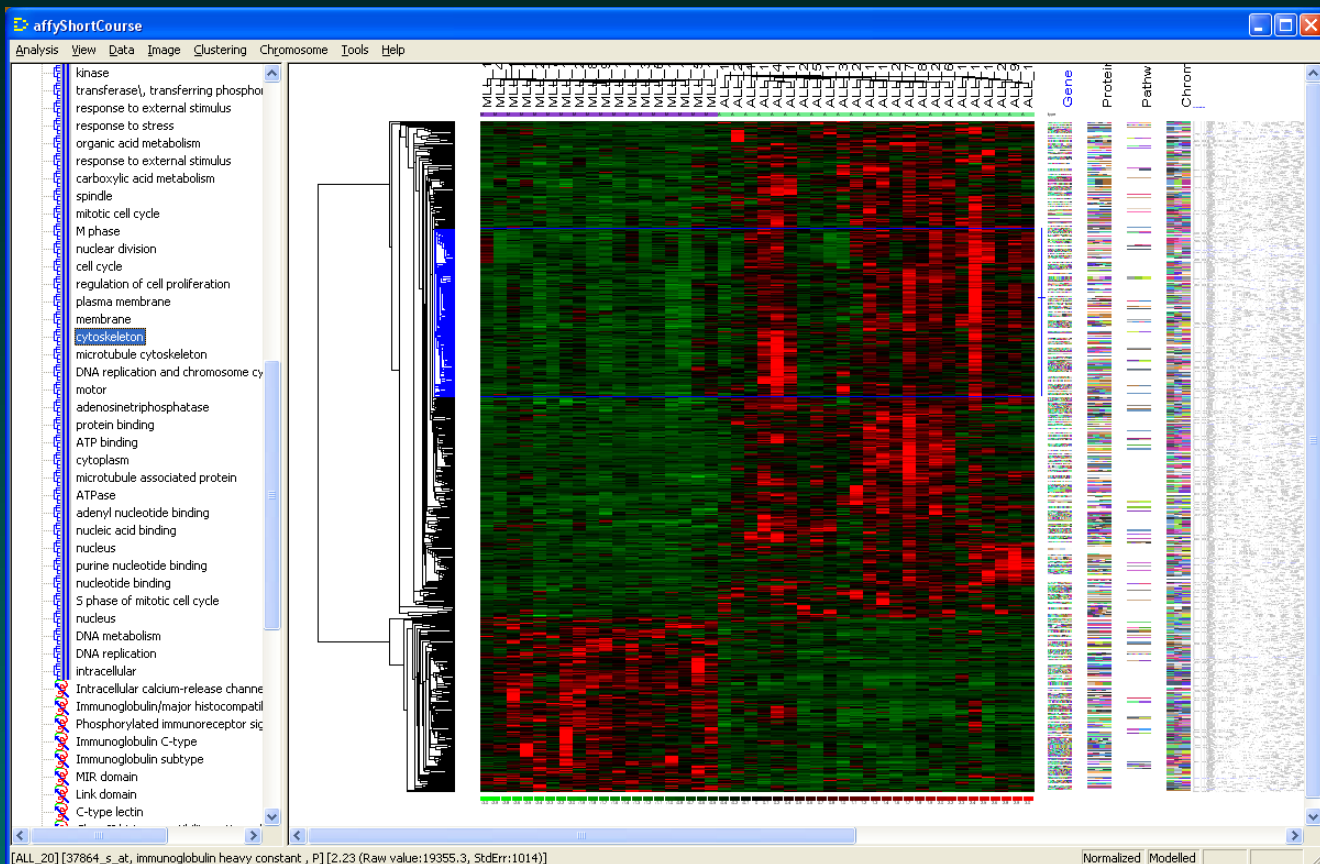
You can use the arrow keys to zoom in or out of the cluster diagram. You may need to zoom in a lot in order to be able to read the gene names.



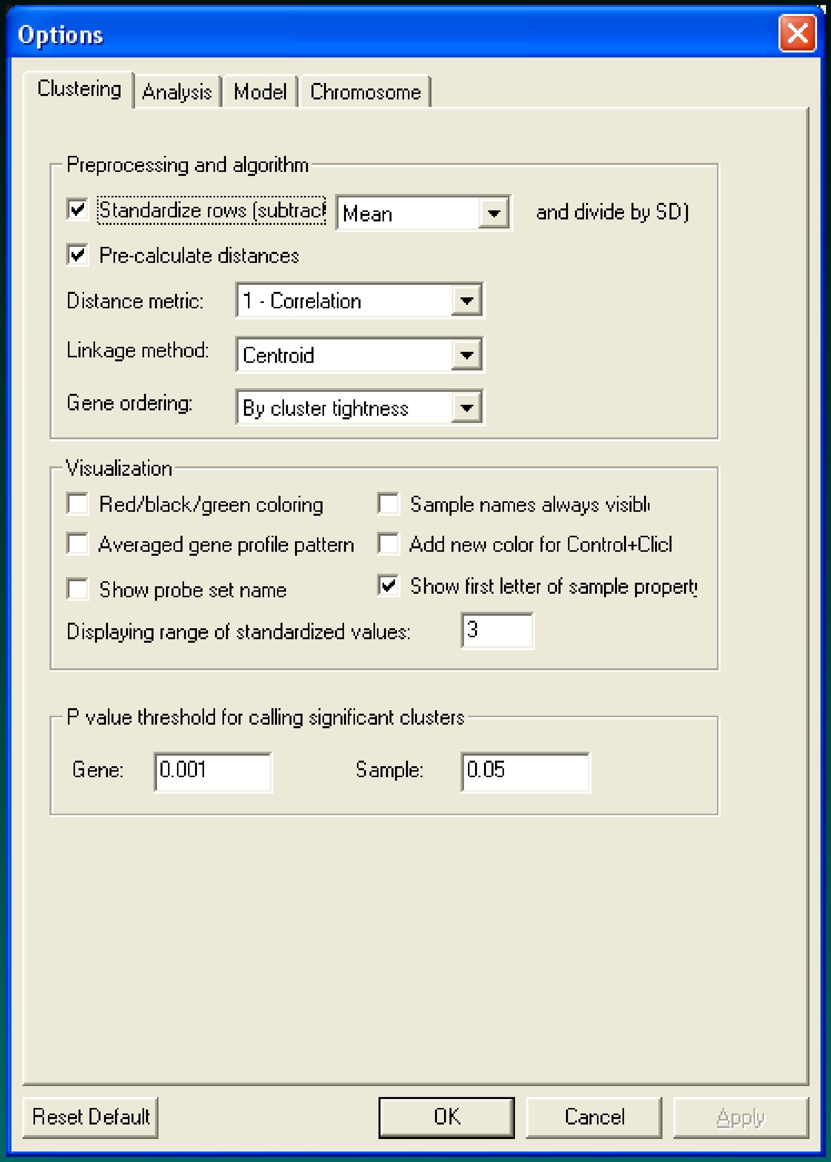
Clicking on a gene name will open a web browser window to the Entrez Gene page for that gene.

Exploring the clustering results

On the left, `dChip` lists “significant” GeneOntology categories, protein domains, and sample types. Click on the names to see where they can be found.



Revisiting the clustering options



Revisiting the clustering options

- Choices for distance metric
  - 1 - correlation
  - 1 - absolute correlation
  - 1 - rank correlation
  - Euclidean

- Choices for linkage
  - centroid
  - average

Which should I choose? What do these mean?

Measuring distances

Ideally, clustering methods tell us that some samples form a more coherent set than the data as a whole, where “more coherent” is generally taken to mean that the samples are closer together.

So, how do we define “closer”?

This requires the specification of a distance or “dissimilarity” matrix. Distances are calculated between each pair of samples. For this purpose, we view each sample as a vector in “gene-space”. The first distance measure most people think of is

Euclidean distance: `sqrt(sum((x - y)^2))`

In the R language,

```
dEuclid <- dist(t(dataMatrix));
```


Alternative definitions of distance

Maximum: `max(abs(x-y))`

Manhattan: `sum(abs(x-y))`

Minkowski: `sum(abs(x-y)^p)^(1/p)`

Canberra: `sum(abs(x-y)/(abs(x)+abs(y)))`

Binary: `sum(xor(x!=0, y!=0))/sum(x!=0 | y!=0)`

Correlation: `(1 - cor(x,y))/2`

Absolute Correlation: `(1 - abs(cor(x,y)))`

Rank Correlation: `(1 - cor(rank(x), rank(y)))/2`

Most clustering methods let you specify the distance measure, or construct any distance matrix you want and work with that matrix.

Hierarchical clustering

Hierarchical clustering produces a dendrogram (a binary tree structure) that displays the distance relationships between clusters.

The most common implementation is agglomerative, which is an unnecessarily big word for bottom-up. The algorithm starts by joining the two samples that are closest together into a cluster. It then keeps repeating this process (joining the two closest clusters into a bigger cluster) until everything has been linked together.

There's only one problem: Distances were defined between individual vectors. How do you measure the distance between clusters of vectors in order to link them?

Linkage rules

Single: Use the minimum distance between cluster members

Complete: Use the maximum distance between cluster members

Average: Use the mean distance between cluster members

Median: Use the median distance between cluster members

Centroid: Use the distance between the “average” member of each cluster

Ward’s: Minimize the increase in variance of the cluster

Simulating nothing

One peculiarity of clustering algorithms is that they always produce clusters. This happens regardless of whether there is actually any meaningful clustering structure present in the data. So, let's simulate some unstructured data and see what happens. We'll write code in R for the simulations.

```
> n.genes <- 1000
> n.samples <- 50
> descr <- sapply(1:50,
                 function(n)sprintf('S%02d', n))
> dataMatrix <- matrix(rnorm(n.genes*n.samples),
+     nrow=n.genes)
> colnames(dataMatrix) <- descr
```

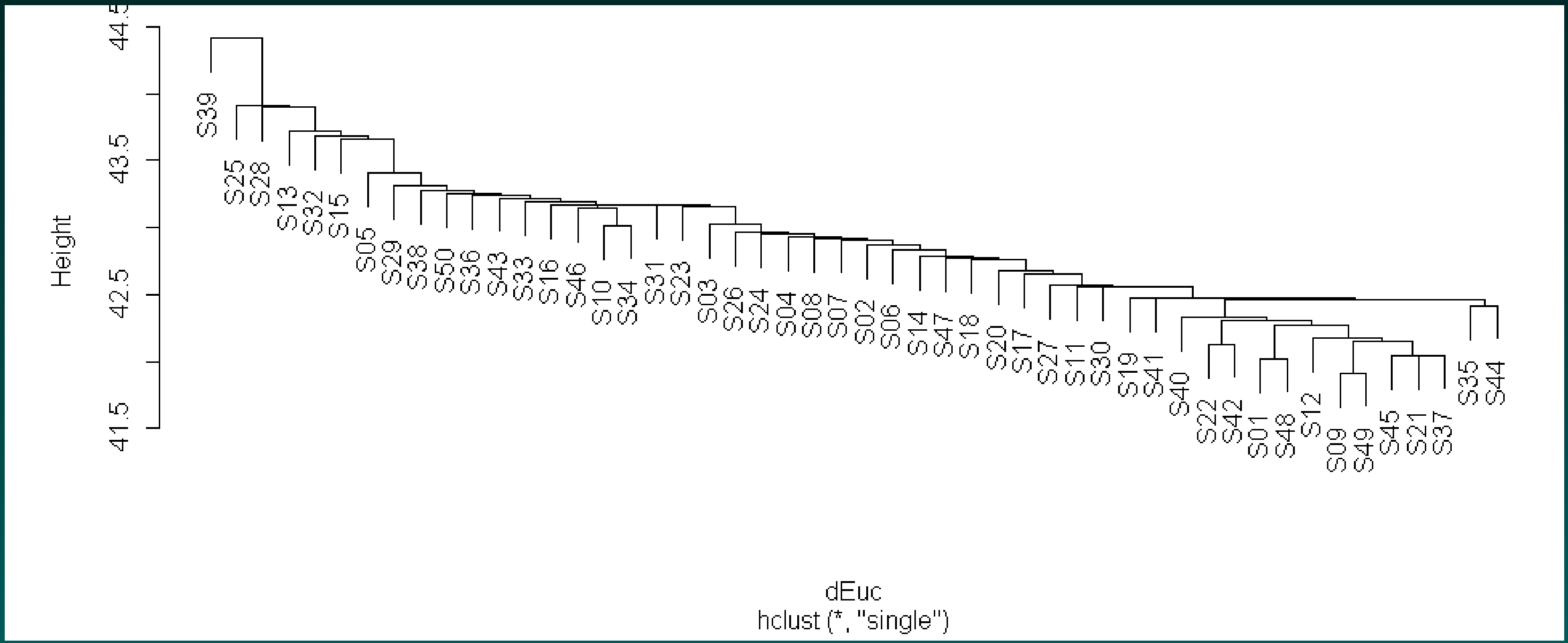

Clustering nothing

```
> dEuc <- dist(t(dataMatrix))
> hAvgEuc <-hclust(dEuc, method='average')
> plclust(hAvgEuc)
```

Euclidean distance, average linkage.

Single linkage often produces "stringlike" clusters

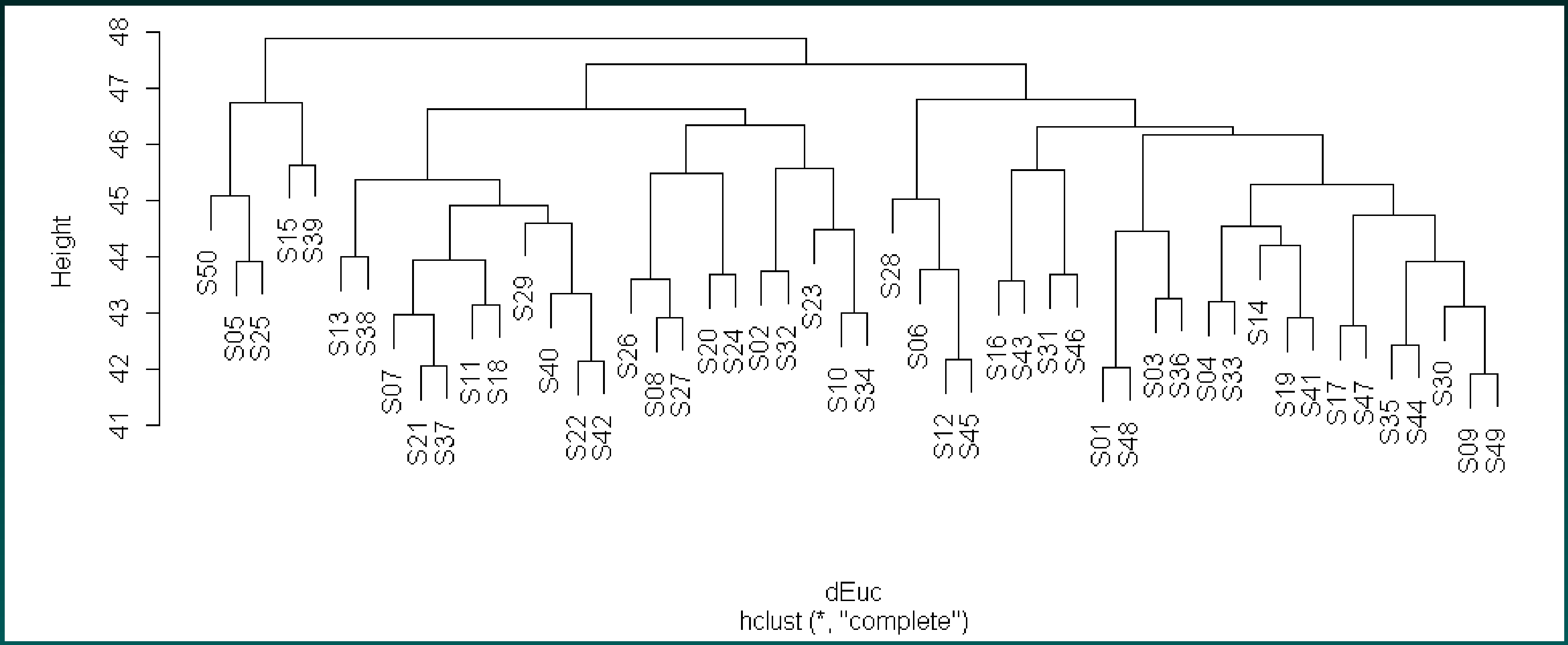
```
> hSinEuc <-hclust(dEuc, method='single')
> plclust(hSinEuc)
```



Euclidean distance, single linkage.

Complete linkage tends to find compact clusters

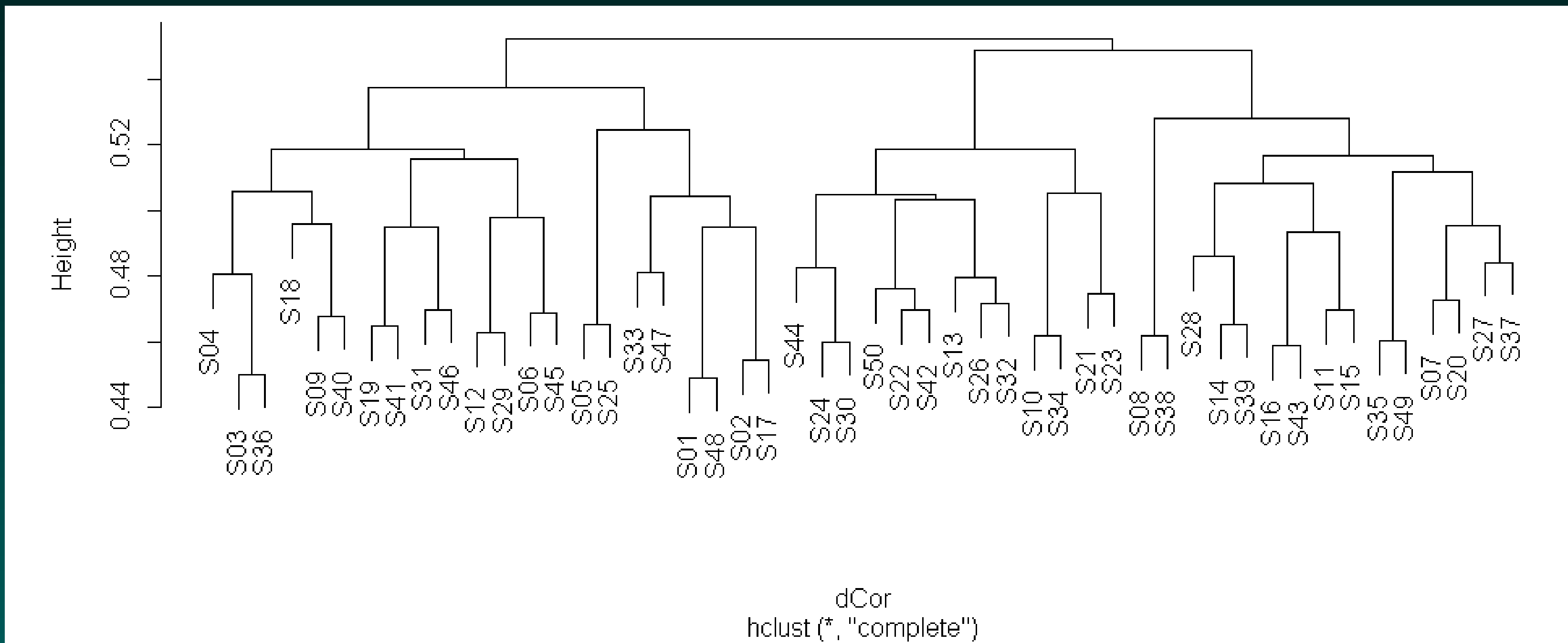
```
> hComEuc <-hclust(dEuc, method='complete')
> plclust(hComEuc)
```



Average linkage tends to produce clusters somewhere in between single and complete linkage.

Clustering with correlation also finds structure

```
> dCor <- as.dist( (1-cor(dataMatrix))/2 )
> hComCor <-hclust(dCor, method=’complete’)
> plclust(hComCor)
```



Correlation distance, complete linkage.

What's Stable?

We can flip branches around without affecting the underlying structure of the data, or changing the meaning of the clustering.

What things are left unchanged by such flips?

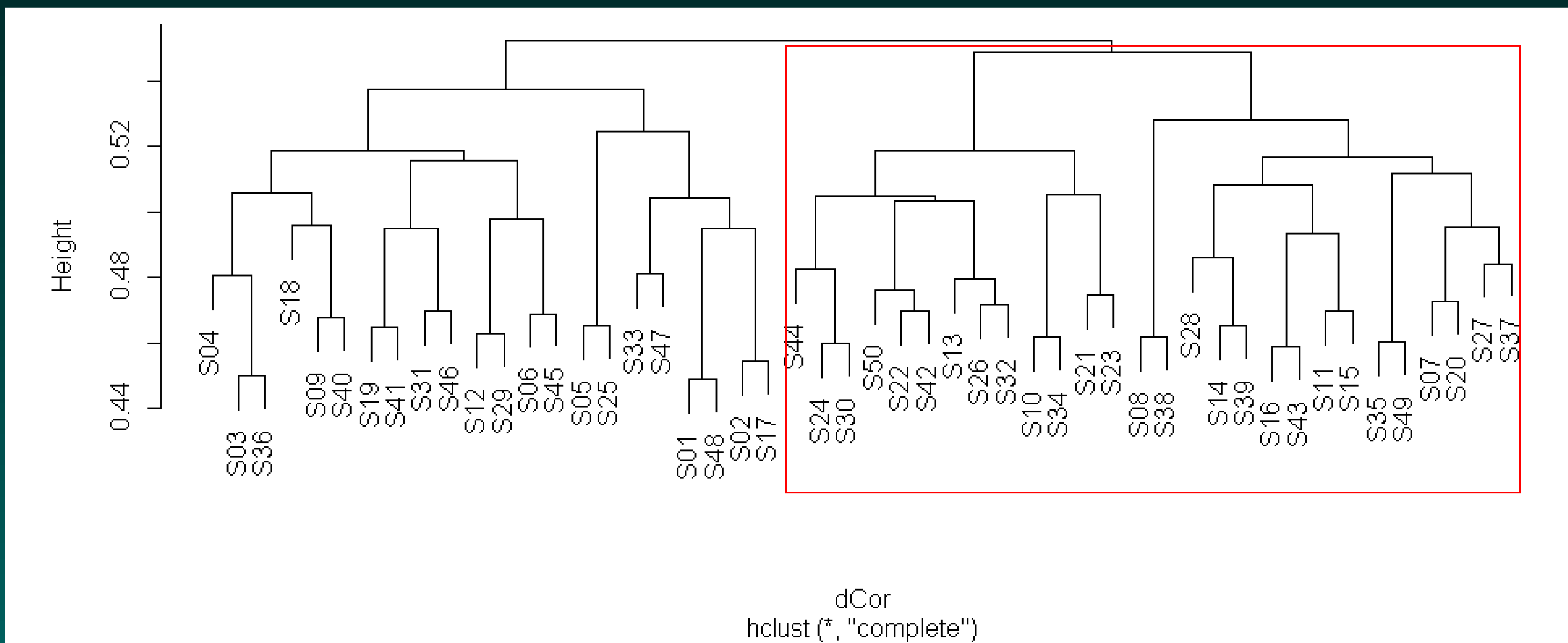
Say we flip a branch at height h_{flip}.

Membership of the sub-branches does not change, but the order can change across the boundary.

How do I define a "cluster"?

What is a cluster?

If we cut the dendrogram at height h, then the sub-branches of each cut branch define clusters. Within a cluster, everything is closer than h to the rest. By varying the cut height, we can produce an arbitrary number of clusters.



What is a cluster?

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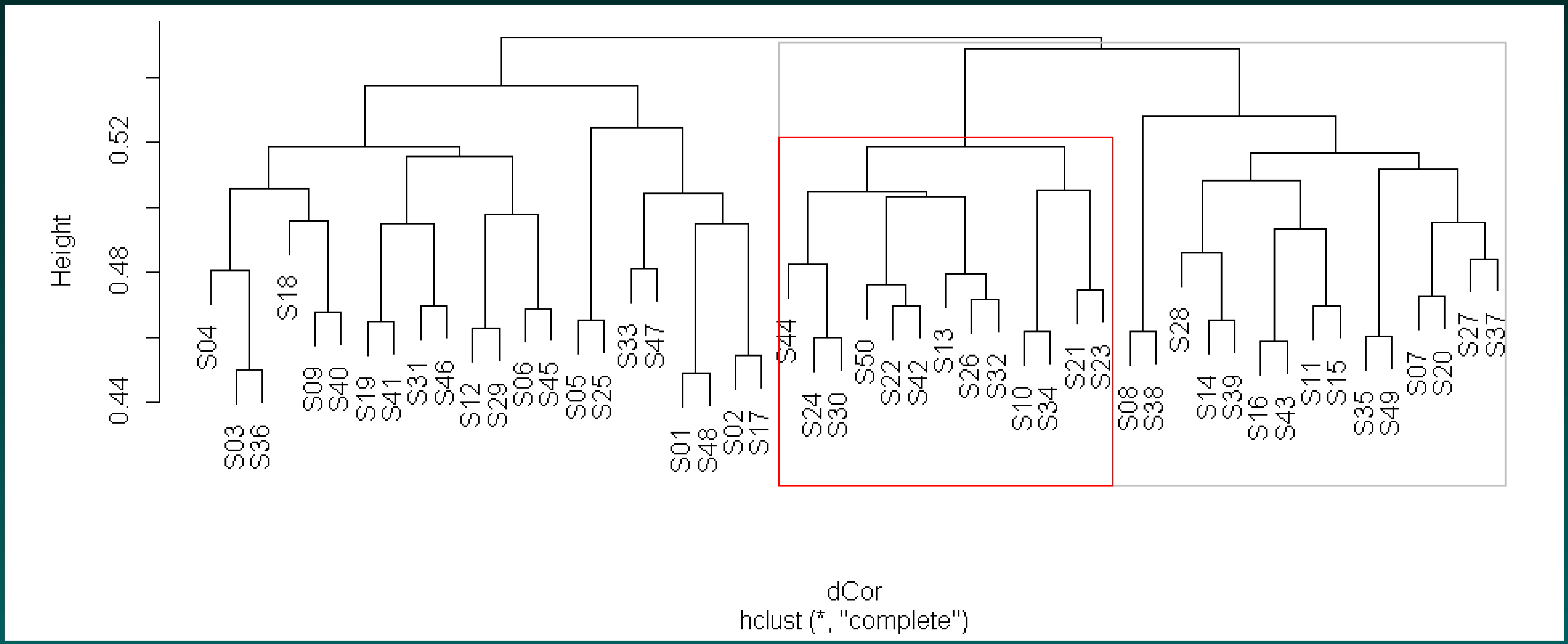


When is a cluster valid?

In other words, where should we cut the tree in order to say that the branches at this point represent something real?

To convince you that this is a real problem, recall that we are using data that was simulated to be completely random. Nevertheless, hierarchical clustering (with complete linkage and either Euclidean distance or correlation) apparently finds structure here.

Bootstrap resampling

Testing cluster validity requires “perturbing” the data.

A cluster consists of pairs of items that are grouped together. If we repeatedly perturb the data, and the pairs still cluster together, this is a good sign that the cluster is “stable”. Samples that cluster in other groups are more questionable.

The simplest way to perturb the data is to “bootstrap” the individual genes, or rows of the data matrix.

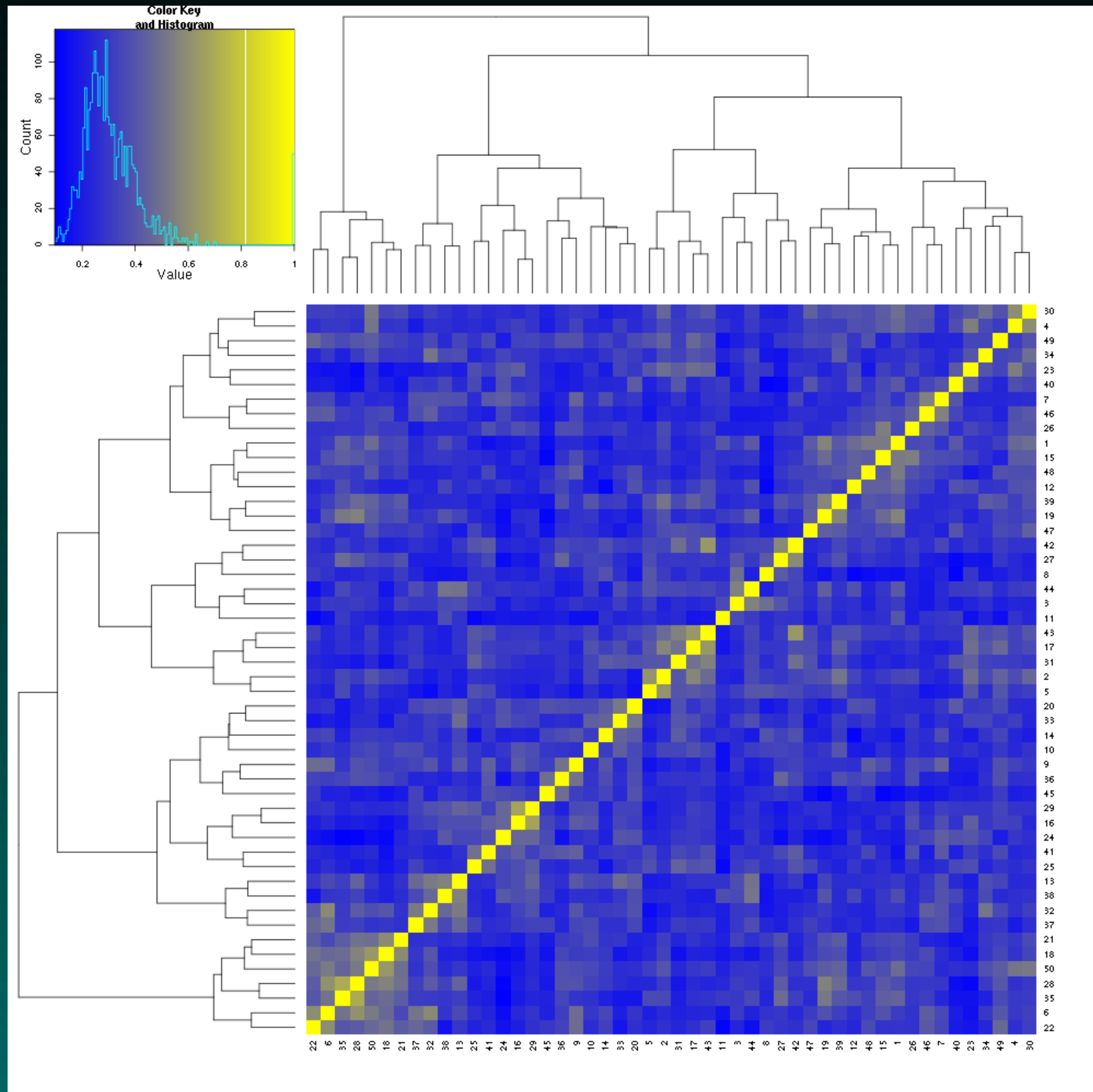
The idea behind the bootstrap is to create a new data matrix (the same size as the original) by randomly selecting rows. Sampling is done with replacement, so some rows will be included multiple times and some rows will be omitted.

Disturbing the universe

We can use the ClassDiscovery package to perform bootstrap resampling for clustering. In order to use $k = 4$ groups, with hierarchical clustering by Pearson correlation distance and average linkage along with an outer loop of $nTimes = 200$ bootstrap samples, we do the following:

```
> require(ClassDiscovery)
> bc <- BootstrapClusterTest(dataMatrix,
+           cutHclust, k=4, method='average',
+           metric='pearson', nTimes=200)
> image(bc)
```


Sometimes it's good to find nothing. . .



Additional Notes

We need to specify the number of clusters to bootstrap, since we record how many times samples are paired. This method extends directly to other clustering techniques.

The image is much more interpretable if the rows and columns of the matching matrix are reordered to match the ordering supplied by the clustering.

Instead of resampling the genes, we can "add noise" to the data from a normal distribution. The scale of noise to use is not obvious with real data.

We can also use "bootstrap subsampling". Instead of reconstructing a sample of the same size as the number of genes on the array, make smaller samples to see how widely the clustering information is spread across the genes.

Clustering with fewer genes

Many times, we cluster using a subset of genes. Maybe we think that other genes are just contributing noise, or maybe we want to focus on genes on a specific chromosome or genes in a specific pathway.

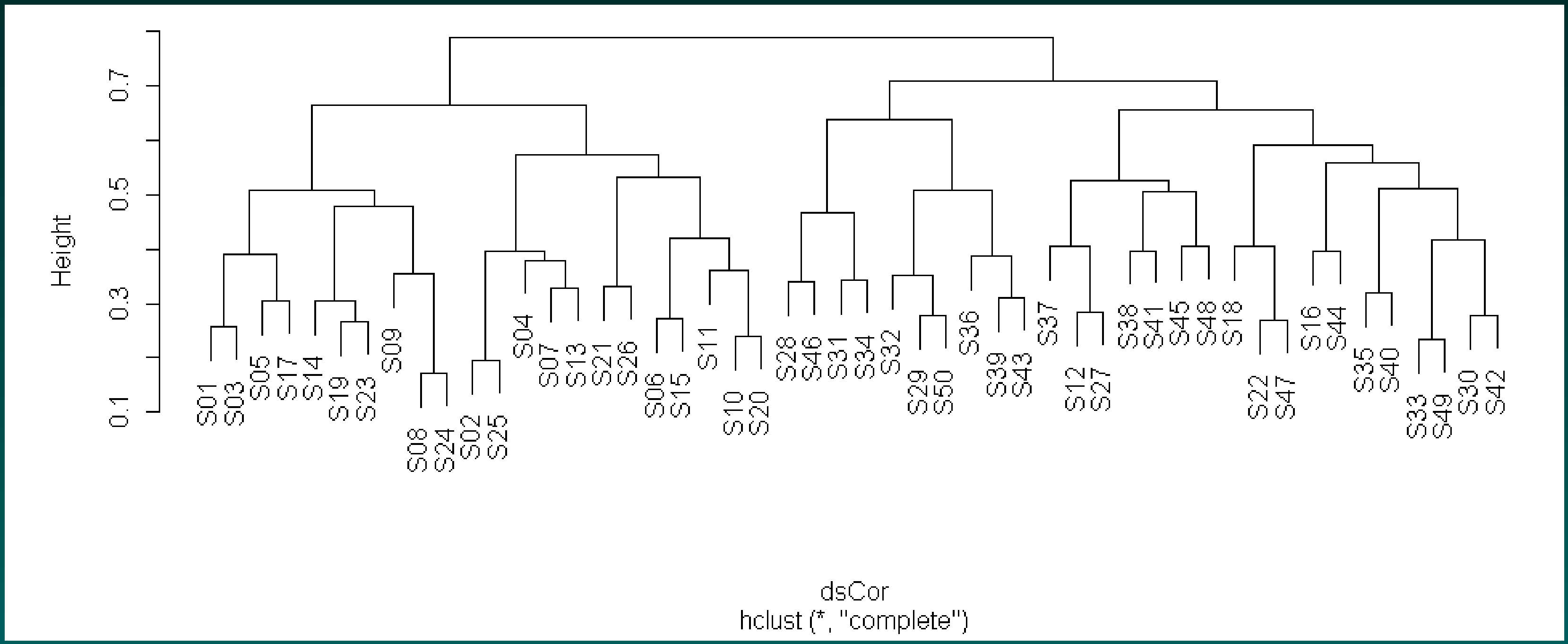
Occasionally, you see papers comparing two known classes of samples that perform the following analysis:

1. Find a list of differentially expressed genes.
2. Cluster data using only the differentially expressed genes.
3. Discover that you can successfully distinguish known classes.

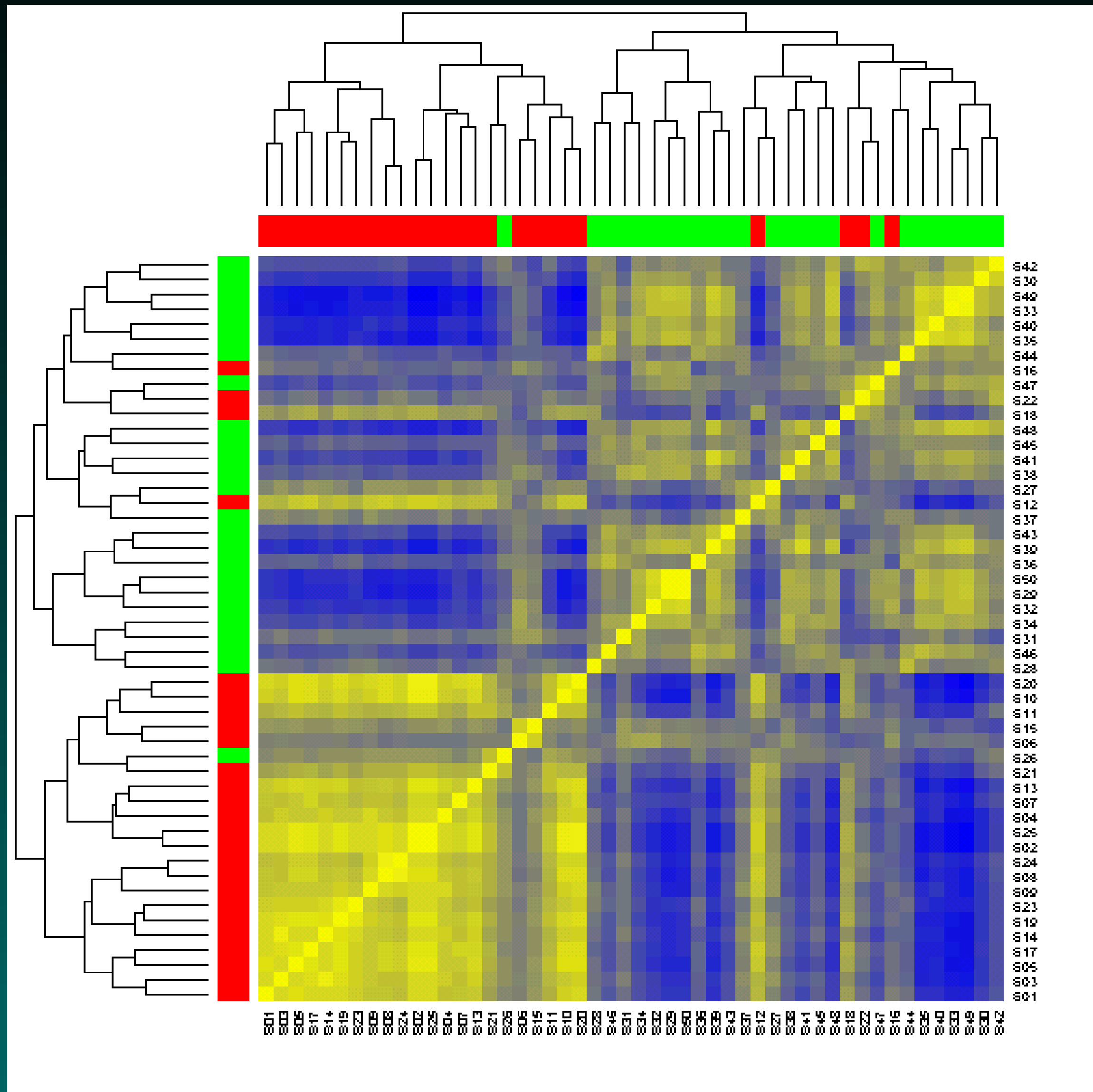
Should this be surprising?

Bending reality to your will

Let's try this on our simulated data. We'll divide the 50 samples into two classes (the first 25 and the last 25). Next, we'll perform t-tests to see how well each gene separates the two classes, and cluster the data using the top 50 genes:



Even the bootstrap doesn't save us. . .



Filtering notes

Filters should not be related to a specific contrast if an overall view is desired.

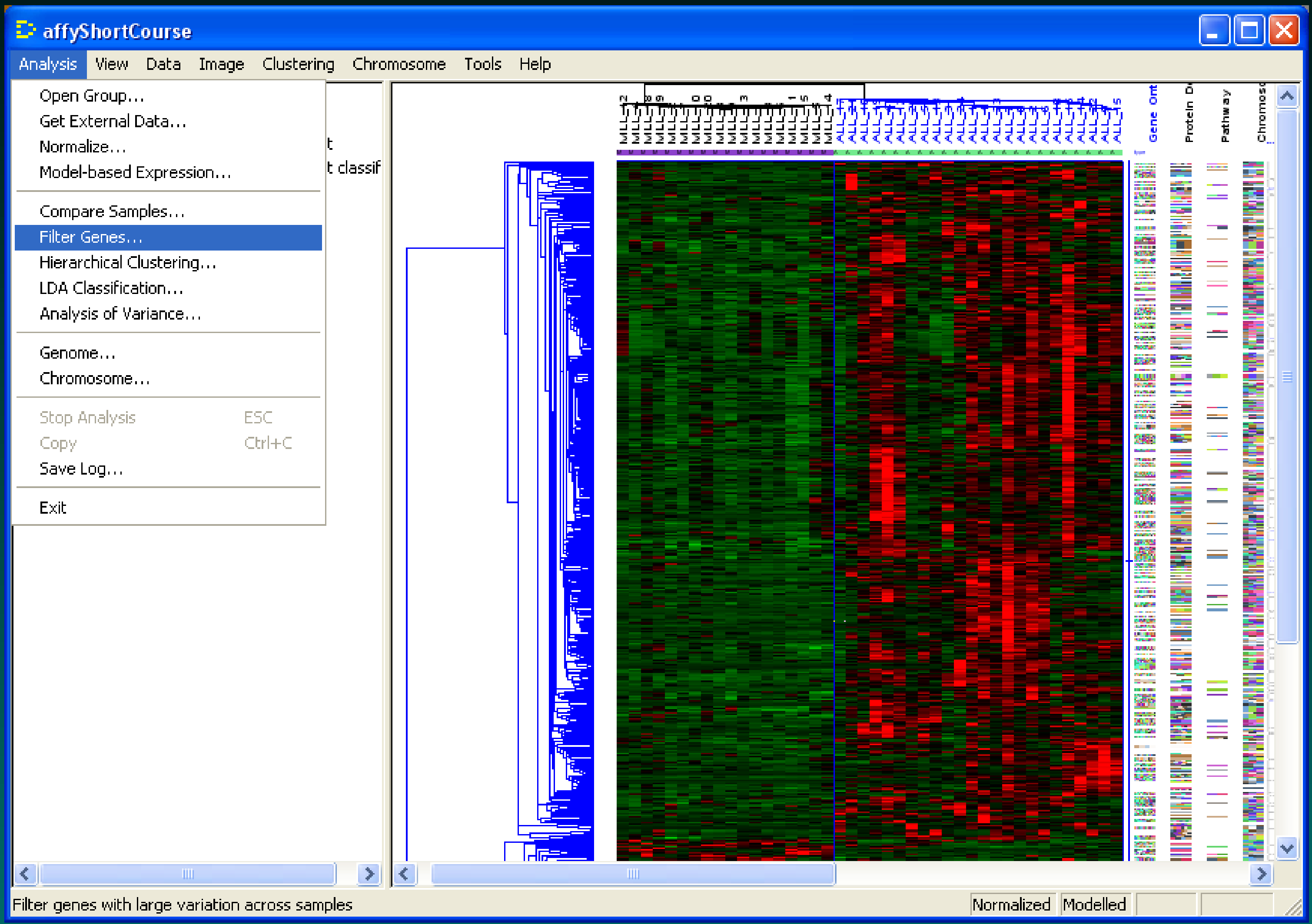
More natural filters exist:

- total variation
- all genes on a given chromosome
- all genes in a given ontology category

Filtering serves a practical purpose – it reduces the number of genes a lot. This is important because we may want to cluster the genes as well as the samples, and clustering thousands of things may make `dChip` (or R) complain. . . .

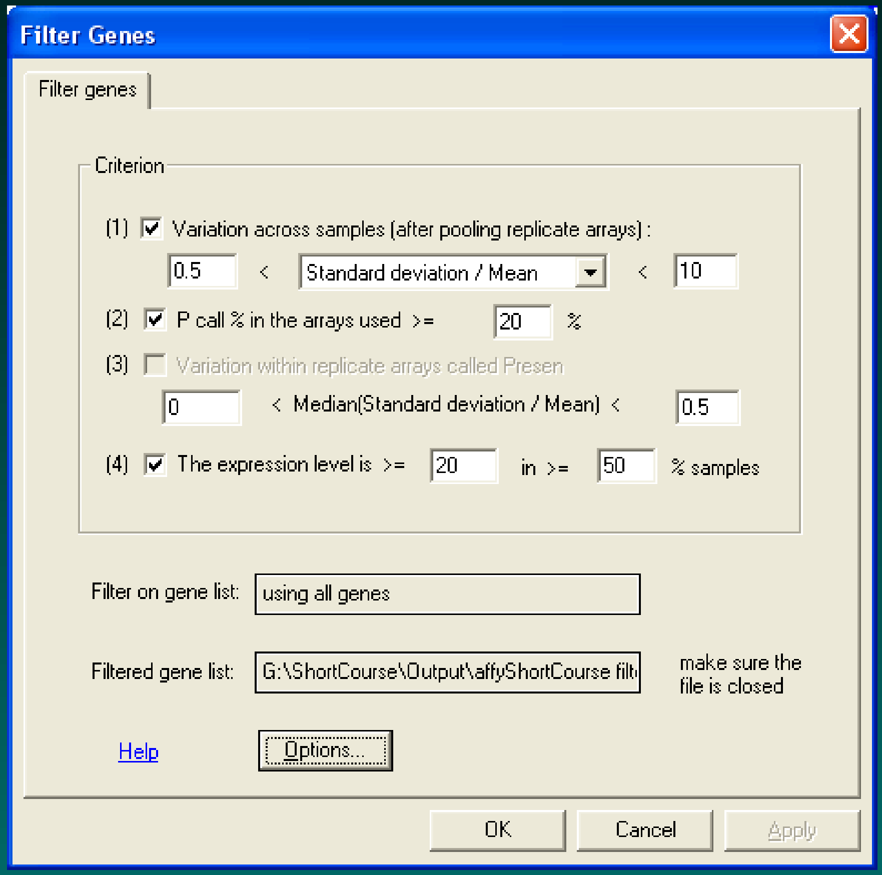
Filtering in dChip

Use “Analysis” – > “Filter genes”.

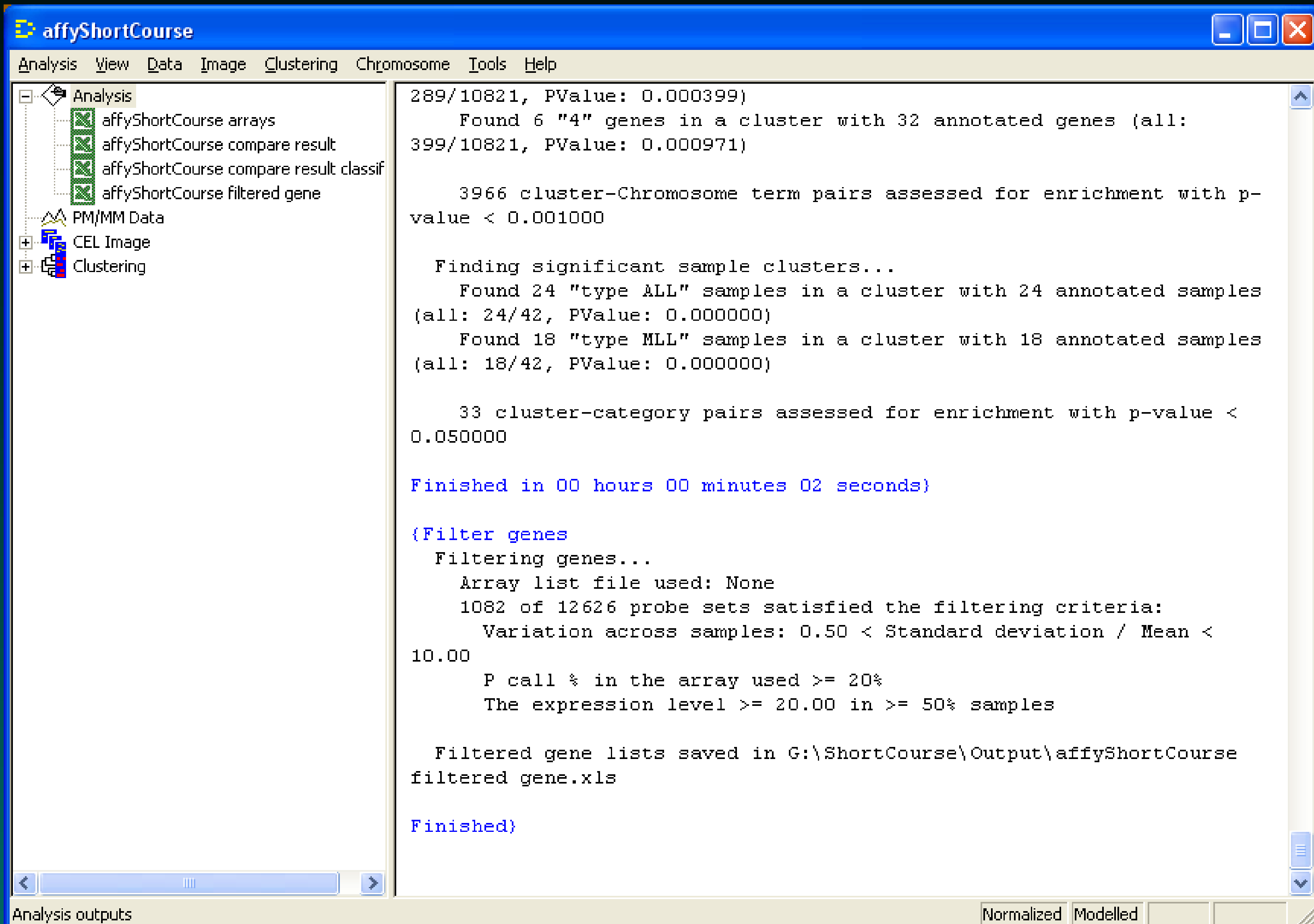


Filtering in dChip

Choose filtering parameters based on variation, expression, or present calls. (I have a bias against variation filters, but those are the default.)

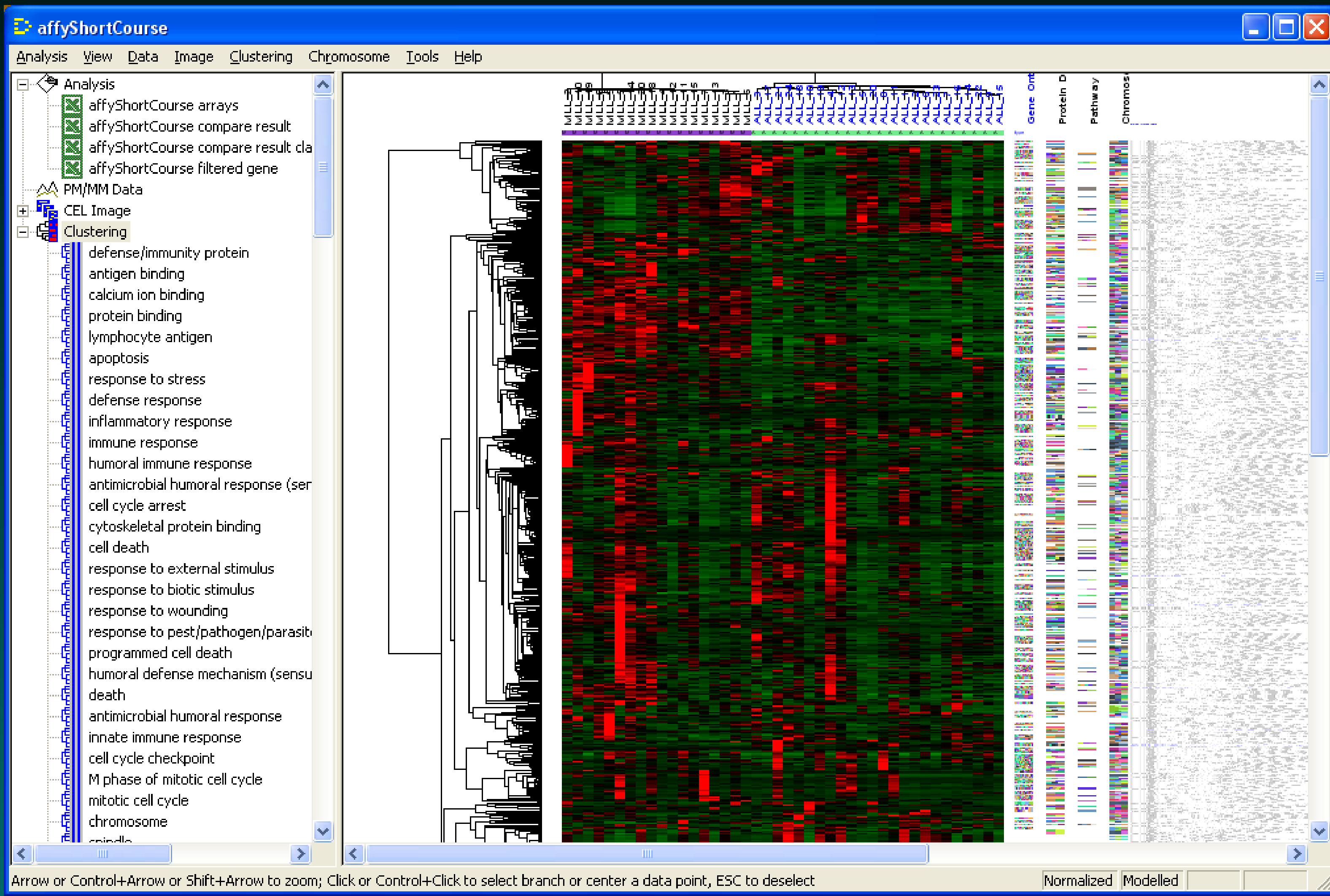


dChip Filter Results



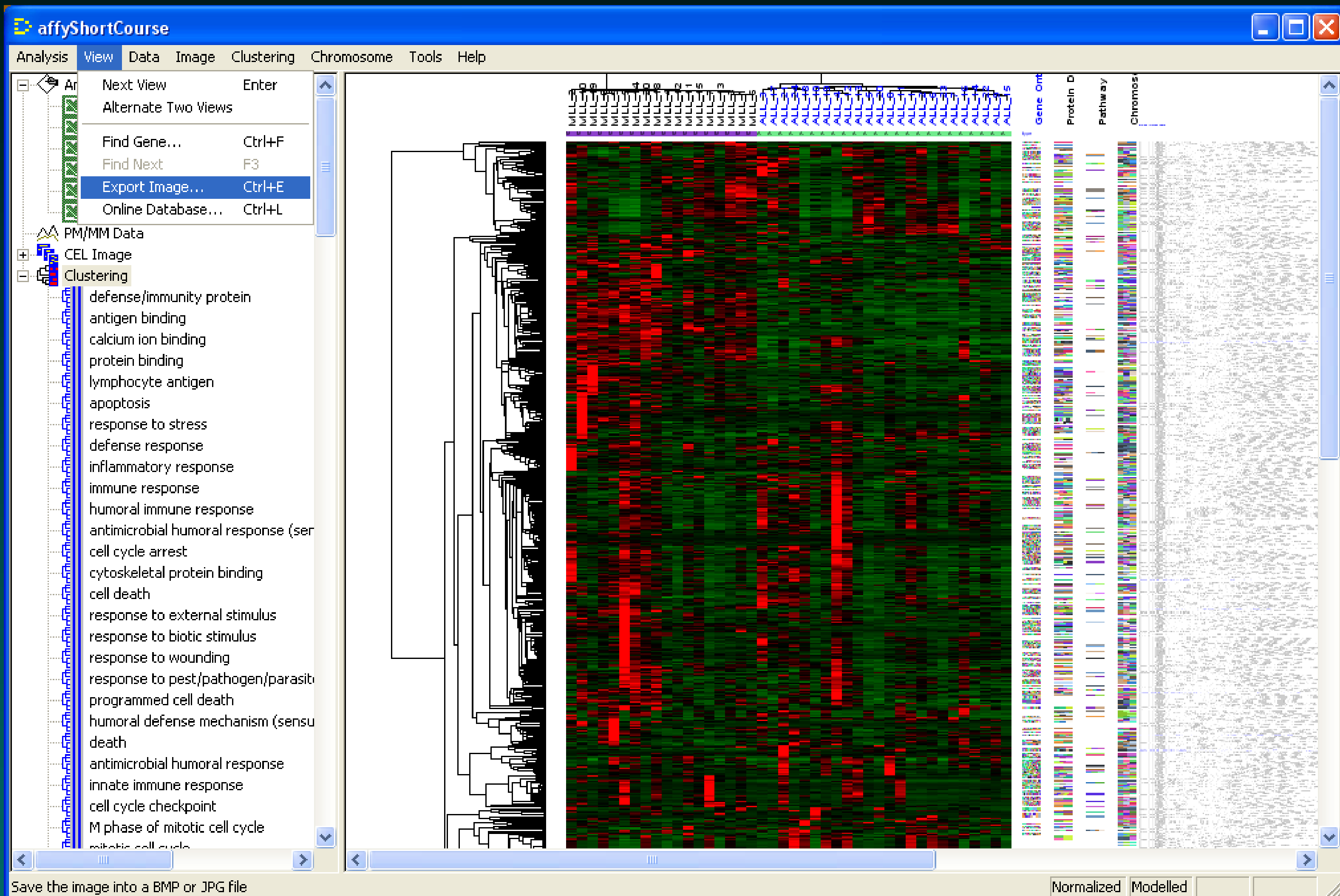
dChip Clusters with Filtered Genes

We can distinguish ALL from MLL in an unsupervised setting.



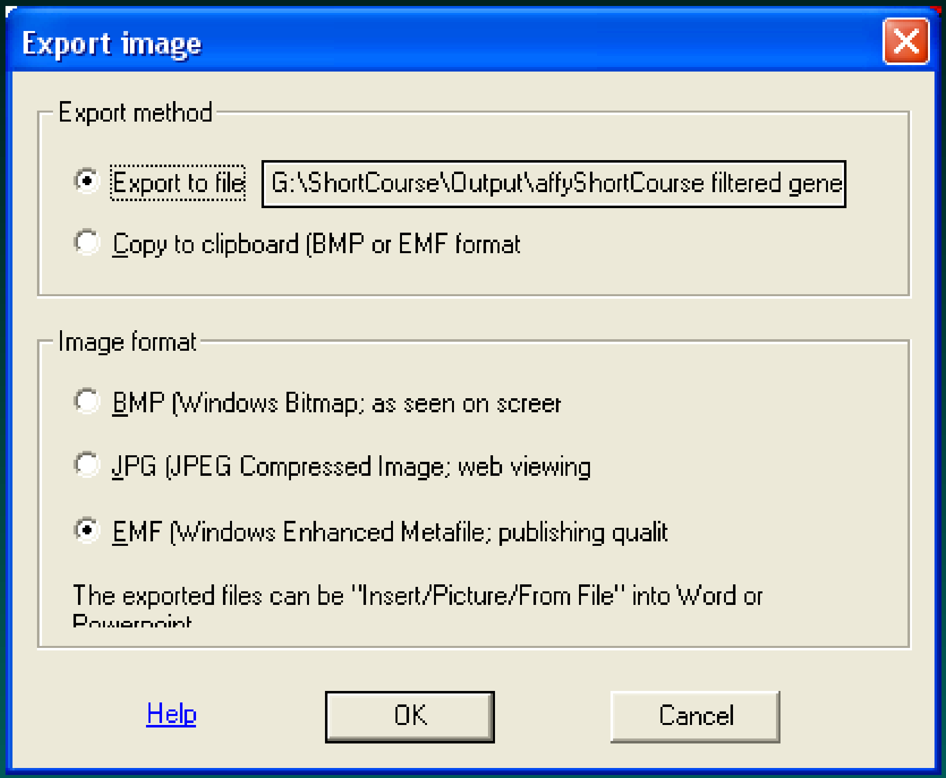
Saving the cluster images

Use “View” – > “Export Image”.



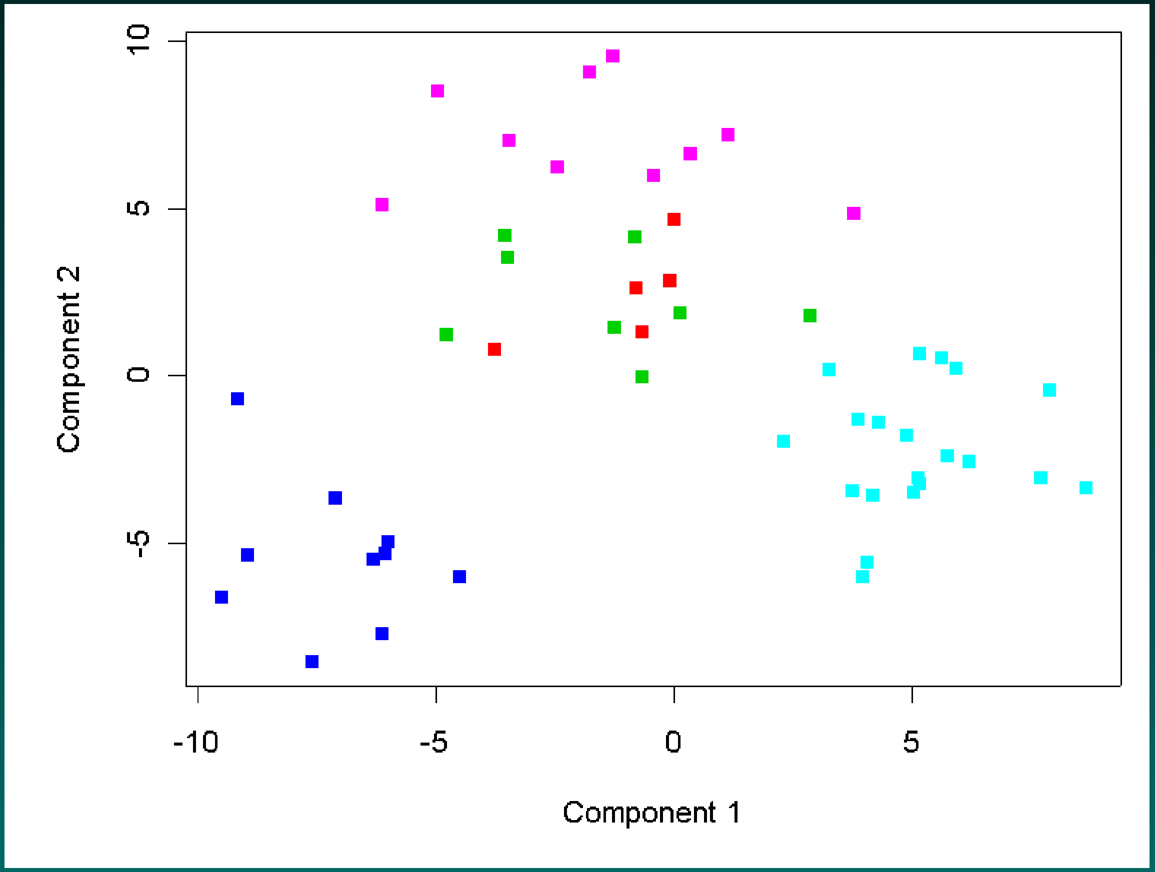
Saving the cluster images

Choose an appropriate format. EMF is probably best if you ever want to zoom in enough to read the gene names.

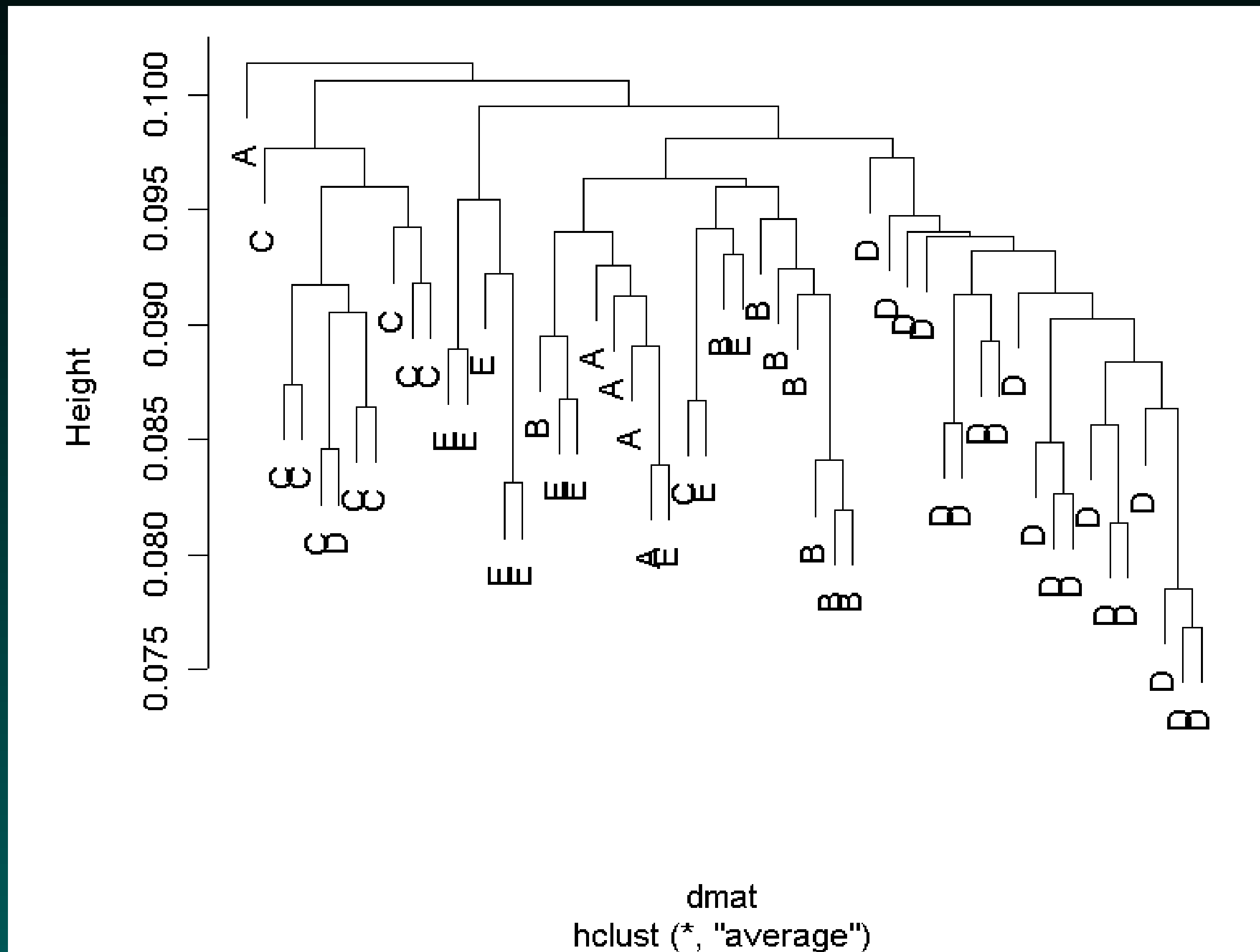


Simulating something

Next, we simulated data with 1000 genes and 5 different sample classes containing different numbers of samples. Here's a two-dimensional picture of the truth:

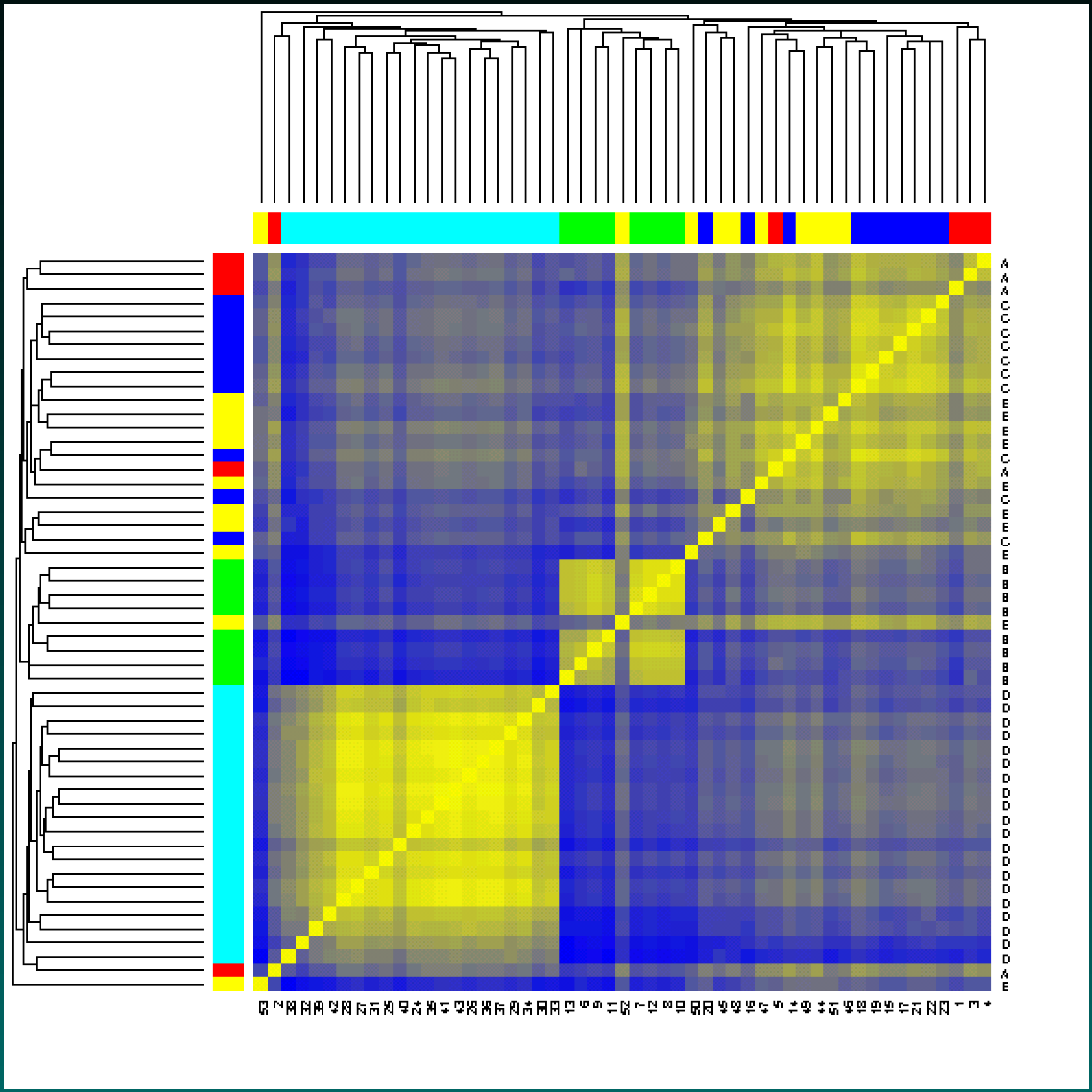


Hierarchical clusters (correlation; average)

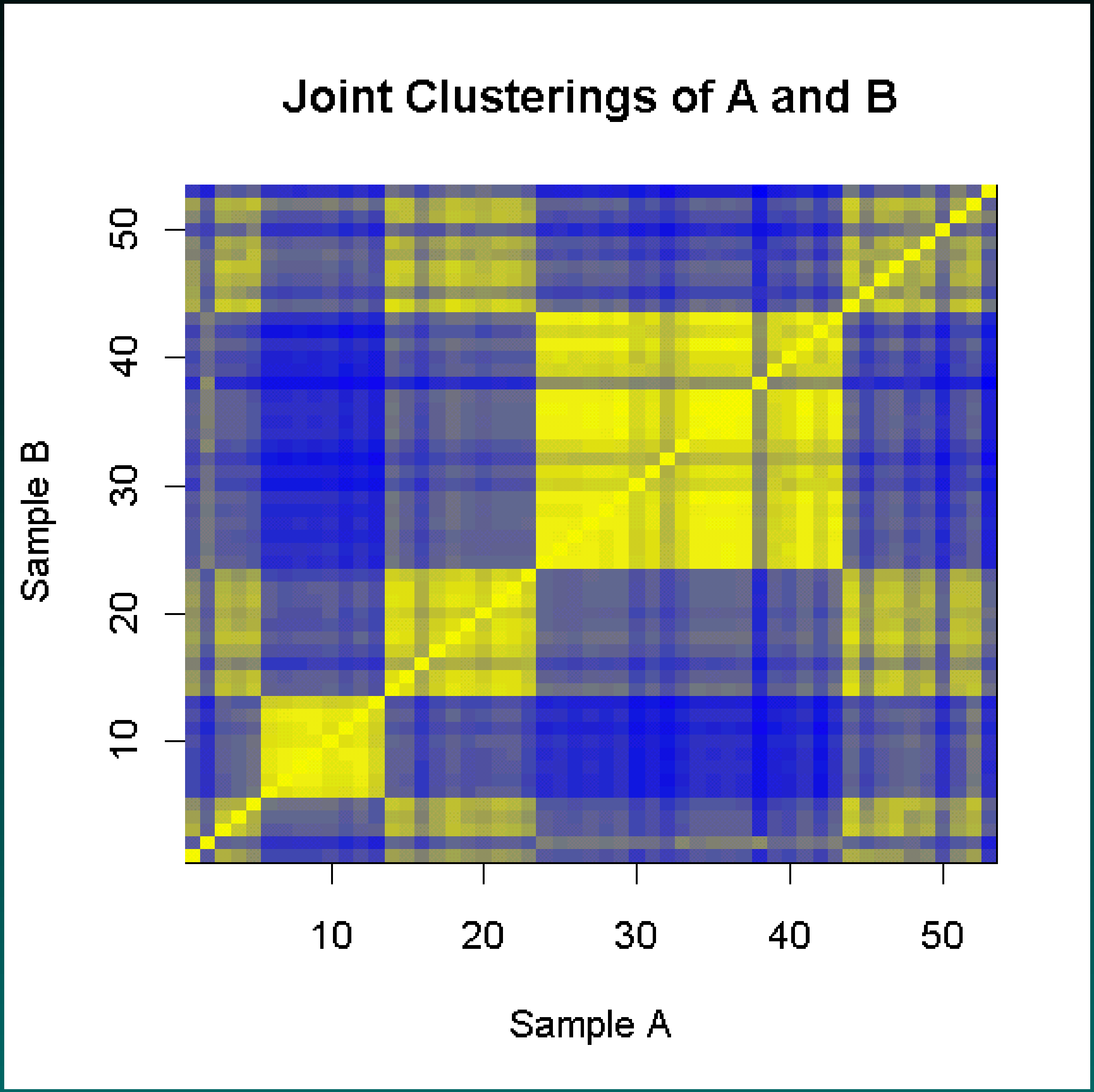


Three of the classes (B, C, D) are mostly correct. The other two classes are less concentrated.

Bootstrap clusters



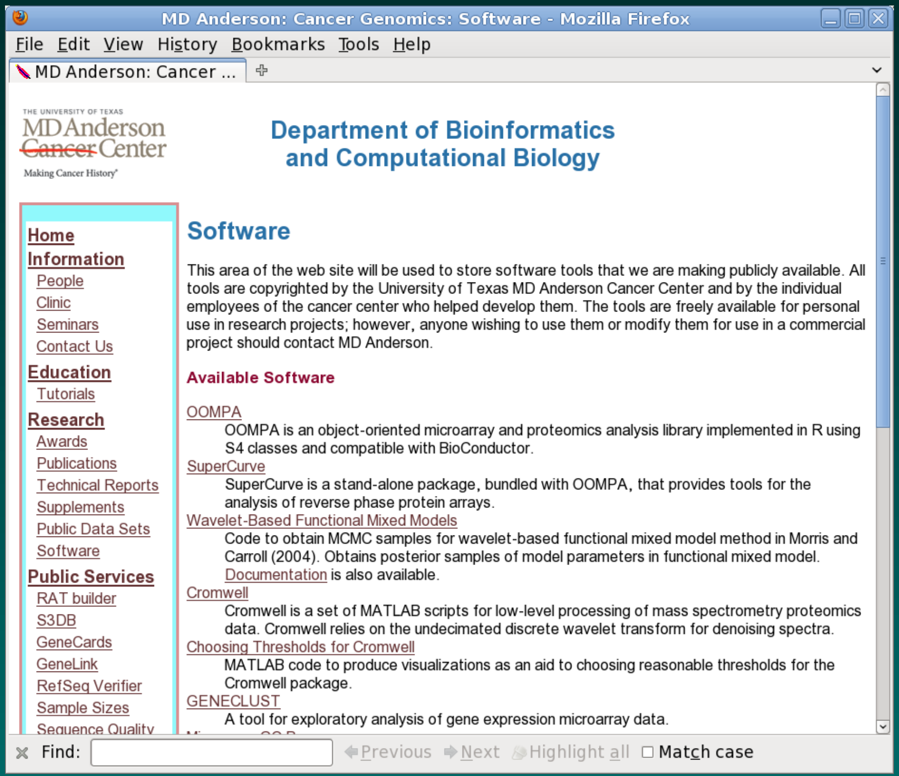
Bootstrap clusters ordered by true groups



R Libraries for Microarray Analysis

We have created R libraries that make it easier for statisticians to perform bootstrap validation of clusters.

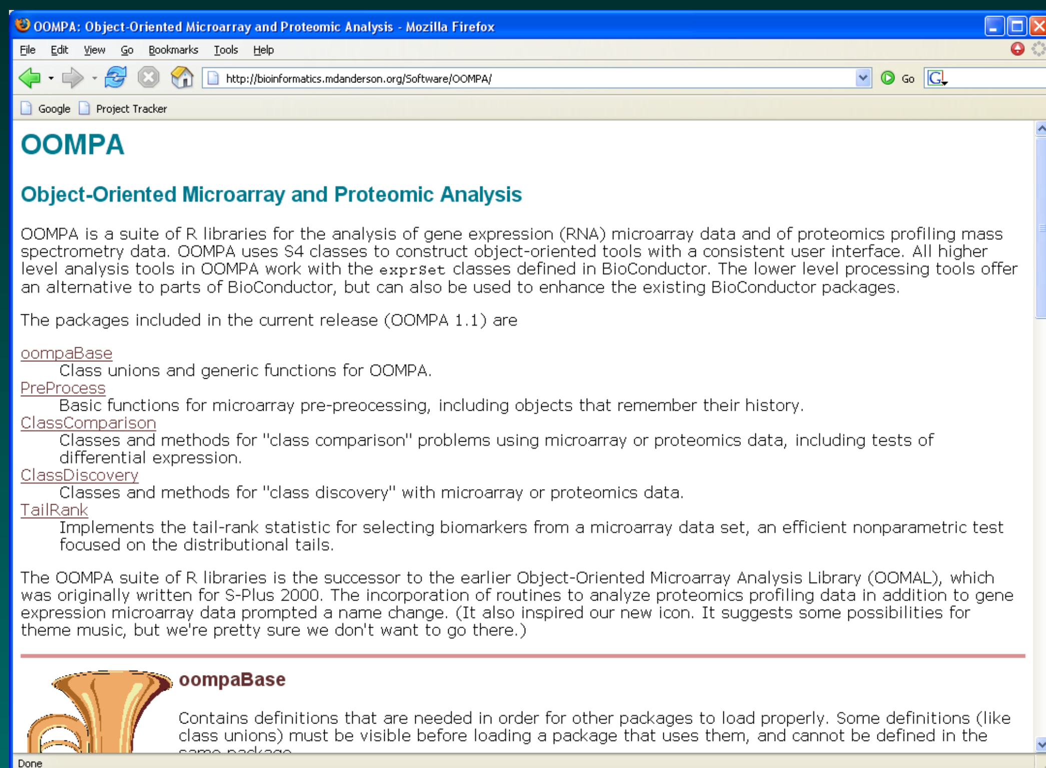
http://bioinformatics.mdanderson.org/software.html



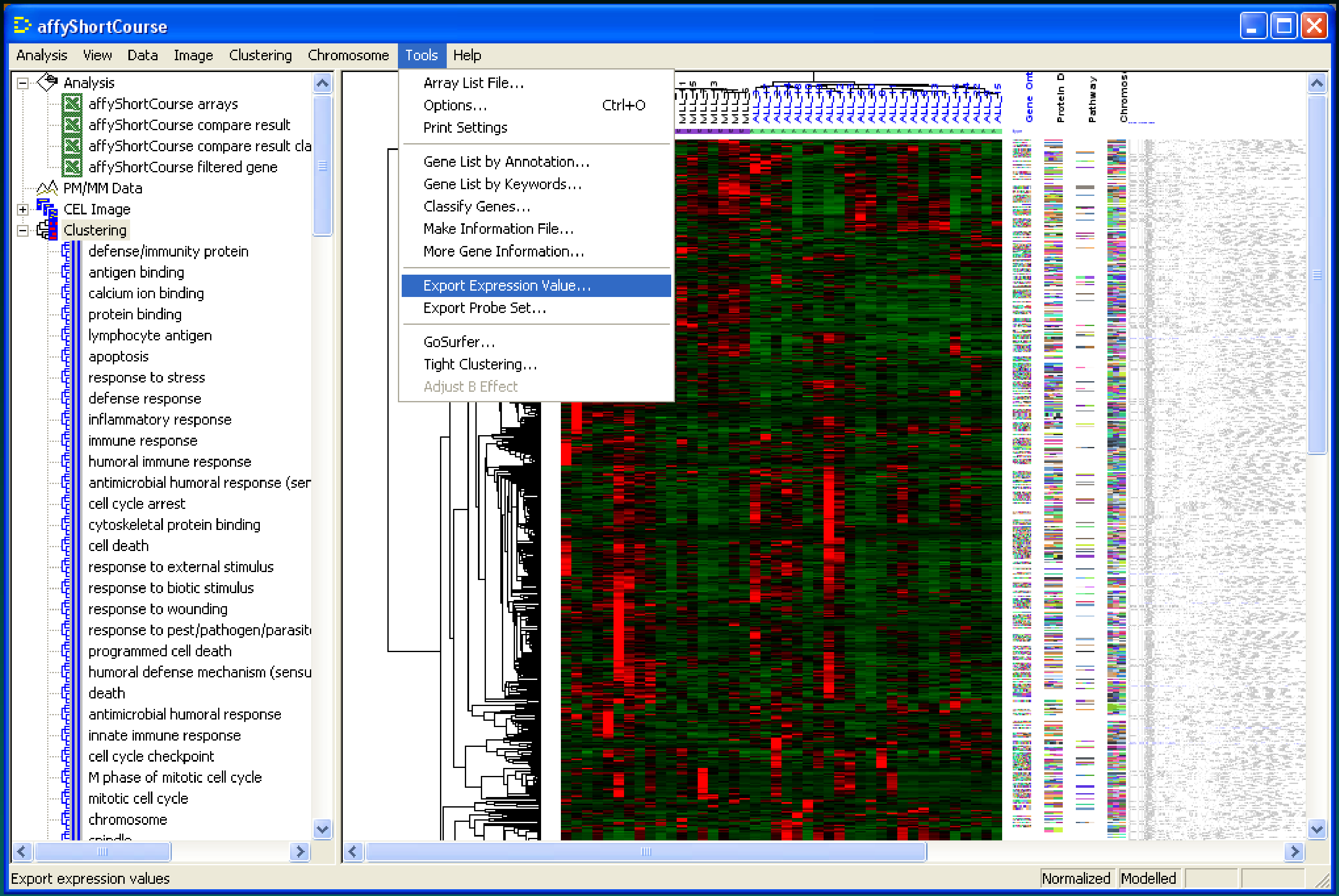
OOMPA

Follow the link for Object-Oriented Microarray and Proteomic Analysis. Then get the libraries.

`http://bioinformatics.mdanderson.org/Software/OOMPA/`

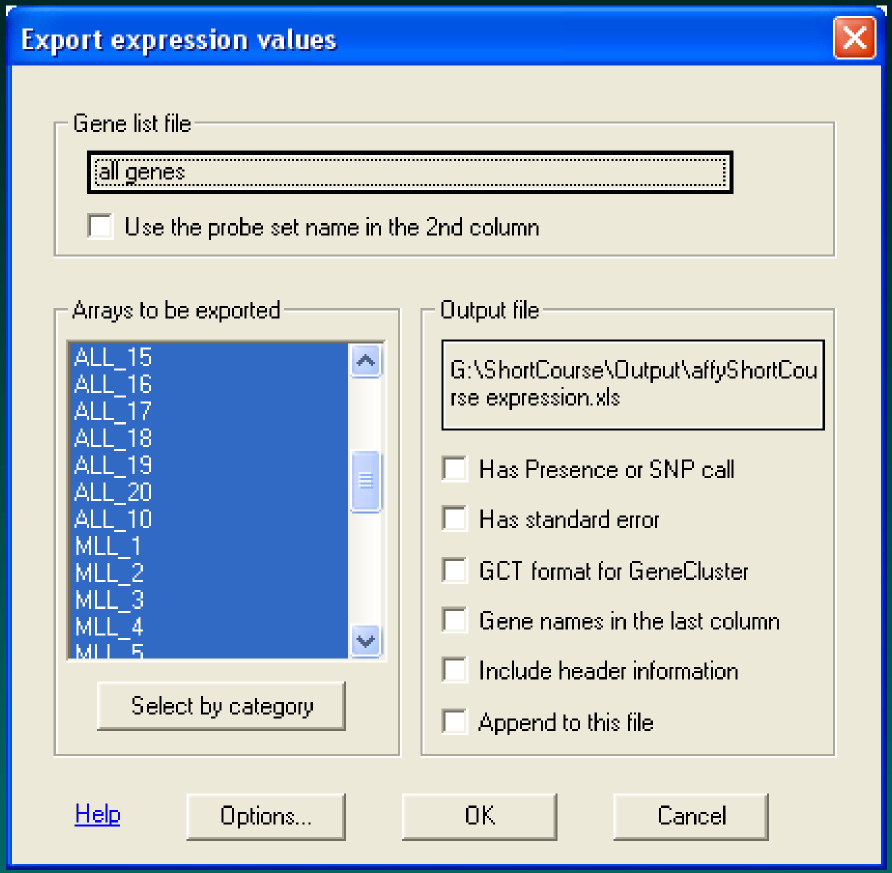


Exporting the data from dChip



Exporting the data from dChip

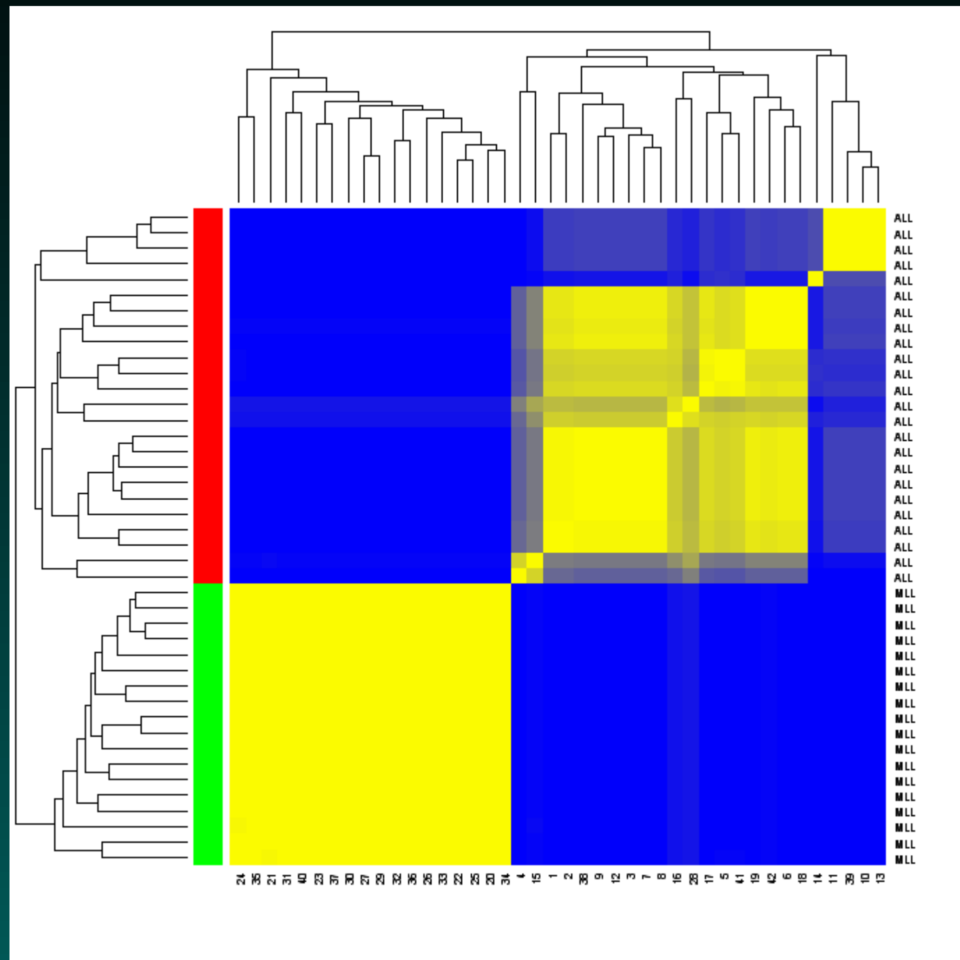
Change the “Gene list file” to “all genes”, or select the file from filtering genes or from comparing samples. Uncheck the boxes.



Using the Exported Data

The exported data lives in a tab-separated values file with an "`.xls`" extension (so that Excel will open it easily). This can be read directly into R using a `read.table` command. If you prepared a sample information file, that can also be read into R using another `read.table` command. The bootstrap clustering routines can then be used on the real data.

Bootstrap validated clusters with filtered genes



Correlation distance, average linkage, 4 clusters, 200 bootstrap samples.

Conclusions

1. Hierarchical clustering always finds clusters.

2. Bootstrap resampling can show that the clusters are fake.

3. Filtering to show a particular grouping can lead to incorrect results.

4. Hierarchical clustering with bootstrap validation may uncover real structure.

