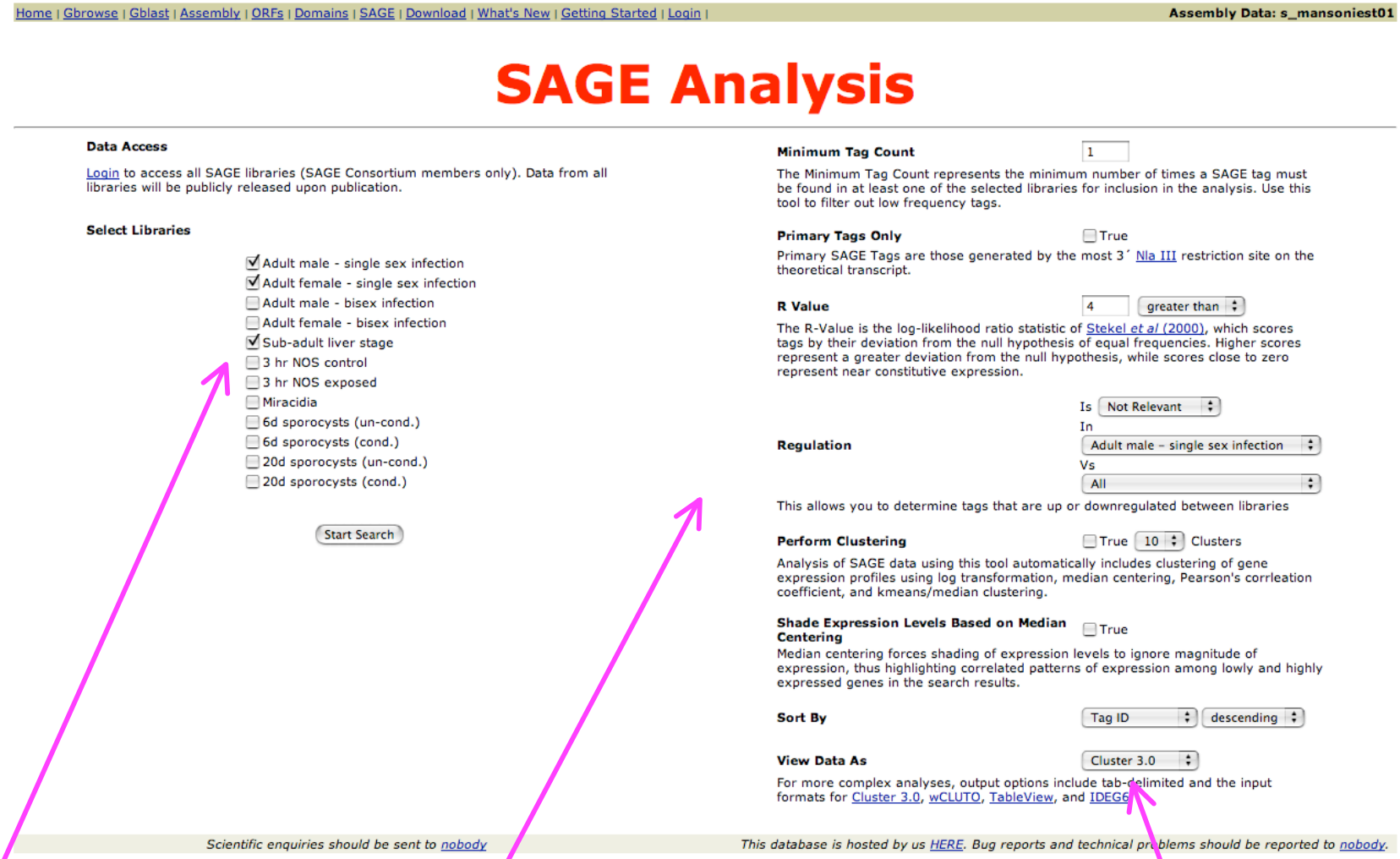
Cluster Analysis of SAGE Data

What you need…

- SAGE Data in Cluster 3.0 format
- Obtain the CLUSTER and TREEVIEW programs
  - http://bonsai.ims.u-tokyo.ac.jp/~mdehoon/software/cluster/software.htm#ctv
- Read the Cluster 3.0 manual to familiarize yourself with cluster analysis

Step 1 - Get the SAGE data from GMOD



Select libraries for analysis

Select data filters

Save data in Cluster 3.0 format

Step 1 - Get the SAGE data from GMOD

After you press submit, you should get a file called “sageresults.txt”. This is the SAGE data in Cluster 3.0 format. You can rename the file if you like.

sageresults.txt

```
tagid   NAME    AM_SS   AF_SS   SA_LIVER
34563   tagtype:UK tagID:34563R-Value:6.2855 BLASTN of SAGETAG : gi|15778807|gb|AC080089.5||Homo sapiens BAC clone RP11-785J10 from 4, complete sequence|0.036  1       1       19
26573   tagtype:UK tagID:26573R-Value:5.5081 BLASTN of SAGETAG : gi|25809521|emb|AL845428.5||Zebrafish DNA sequence from clone CH211-133D24 in linkage group 20, complete sequence|0.14 1       3       20
25464   tagtype:AA tagID:25464R-Value:4.0495 Orf:864 gi|22094807|gb|AAM91993.1||egg secreted protein ESP15 [Schistosoma mansoni]|2e-39  1       13      1
25004   tagtype:PS tagID:25004R-Value:4.4212 Orf:5034 gi|19745168|ref|NP_604448.1||lamin B receptor [Rattus norvegicus] gi|7513997|pir||JC5567 lamin B receptor - rat gi|2204062|dbj|BAA20471.1| Rat NBP60
[Rattus norvegicus]|1e-32       1       4       18
23099   tagtype:UK tagID:23099R-Value:5.0395    1       1       16
22531   tagtype:PS tagID:22531R-Value:4.0679 Orf:8124 gi|56757568|gb|AAW26946.1||unknown [Schistosoma japonicum]|3e-50  1       4       17
21385   tagtype:UK tagID:21385R-Value:5.2544 BLASTN of SAGETAG : gi|15420527|gb|AF358445.1||Schistosoma mansoni glutaminyl-tRNA synthetase mRNA, complete cds|0.009     1       9       23
21114   tagtype:UK tagID:21114R-Value:8.8321 BLASTN of SAGETAG : gi|33632062|emb|BX569689.1||Synechococcus sp. WH8102 complete genome; segment 1/7|8.7  1       1       25
20795   tagtype:AS tagID:20795R-Value:4.8593 Orf:642 gi|66866675|gb|AAY57921.1||22.6 kDa tegument antigen [Schistosoma mansoni] gi|135578|sp|P14202|TEGU_SCHMA Tegument antigen (I(H)A) (Antigen SmA22.6)
(A12) gi|161087|gb|AAA29922.1| SM22.6 antigen (A12) gi|160933|gb|AAA29856.1| antigen|1e-108     2       6       23
20113   tagtype:AS tagID:20113R-Value:5.1422 Orf:1959 gi|160955|gb|AAC14467.1||Cu/Zn-superoxide dismutase [Schistosoma mansoni]|5e-86   2       3       21
17699   tagtype:UK tagID:17699R-Value:4.4279 BLASTN of SAGETAG : gi|68639430|emb|CR339059.15||Zebrafish DNA sequence from clone CH211-121I18 in linkage group 3, complete sequence|0.14 2       1       16
16710   tagtype:UK tagID:16710R-Value:4.4709 BLASTN of SAGETAG : gi|46240545|emb|CR387786.1||Gallus gallus finished cDNA, clone ChEST533d6|8.7  1       14      1
16373   tagtype:AS tagID:16373R-Value:9.0481 Orf:3234 gi|66526630|ref|XP_392104.2||PREDICTED: similar to CG31075-PA [Apis mellifera]|1e-166     1       29      4
15835   tagtype:UK tagID:15835R-Value:5.3058 BLASTN of SAGETAG : gi|18698807|gb|AC090696.6||Homo sapiens chromosome 15, clone RP11-595N10, complete sequence|8.7       1       22      8
15604   tagtype:UK tagID:15604R-Value:4.2835    1       15      2
15598   tagtype:AS tagID:15598R-Value:6.3453 Orf:7727 gi|56753475|gb|AAW24941.1||unknown [Schistosoma japonicum]|1e-25  1       20      2
15463   tagtype:PS tagID:15463R-Value:8.5004 Orf:4630 gi|67463829|pdb|1TD1|C|Chain C, Crystal Structure Of The Purine Nucleoside Phosphorylase From Schistosoma Mansoni In Complex With Acetate gi|67463828|
pdb|1TD1|B Chain B, Crystal Structure Of The Purine Nucleoside Phosphorylase From Schistosoma Mansoni In Complex With Acetate gi|1e-162 1       33      12
15405   tagtype:UK tagID:15405R-Value:7.0170 BLASTN of SAGETAG : gi|26103723|dbj|AK086729.1||Mus musculus 15 days embryo head cDNA, RIKEN full-length enriched library, clone:D930048E06 product:unknown EST,
full insert sequence|0.14       1       24      4
15402   tagtype:UK tagID:15402R-Value:5.7568 BLASTN of SAGETAG : gi|55467283|emb|BX510316.11||Zebrafish DNA sequence from clone CH211-194J3 in linkage group 12, complete sequence|0.14 1       17      1
15359   tagtype:UK tagID:15359R-Value:5.7568 BLASTN of SAGETAG : gi|20197018|gb|AC003096.3||Arabidopsis thaliana chromosome 2 clone T29F13 map ve016, complete sequence|0.14    1       17      1
13441   tagtype:PS tagID:13441R-Value:5.8825 Orf:1095 gi|60692116|gb|AAX30610.1||unknown [Schistosoma japonicum]|8e-40  3       20      1
13148   tagtype:UK tagID:13148R-Value:5.1224 BLASTN of SAGETAG : gi|9581783|emb|AL117374.39|HSDJ47A22|Human DNA sequence from clone RP1-47A22 on chromosome 20q12 Contains STSs and GSSs, complete sequence|
0.14    2       17      1
12165   tagtype:PS tagID:12165R-Value:5.0856 Orf:3629 gi|7494503|pir||T30855|multidrug resistance protein 2 - fluke (Schistosoma mansoni) gi|425476|gb|AAA66477.1| SMDR2|7e-61  3       18      1
11265   tagtype:UK tagID:11265R-Value:5.5802    4       3       25
11156   tagtype:PS tagID:11156R-Value:4.0269 Orf:1997 gi|30794206|ref|NP_084385.1||splicing factor 3b, subunit 2 [Mus musculus] gi|29144992|gb|AAH49118.1| Splicing factor 3b, subunit 2 [Mus musculus]|
1e-126  1       18      8
11086   tagtype:UK tagID:11086R-Value:4.3637 BLASTN of SAGETAG : gi|26984795|emb|AL845171.5||Mouse DNA sequence from clone RP23-38N8 on chromosome 4, complete sequence|8.7     3       18      2
10809   tagtype:UK tagID:10809R-Value:38.2557 BLASTN of SAGETAG : gi|3063366|dbj|AB003713.1||Protula magnifica mRNA for elongation factor-1alpha, partial cds|0.14      1       1       91
10802   tagtype:UK tagID:10802R-Value:11.4264 BLASTN of SAGETAG : gi|1791220|gb|U82283.1|SMU82283|Schistosoma malayensis 18S ribosomal RNA gene, partial sequence, ITS1, complete sequence and 5.8S ribosomal
RNA gene, partial sequence|0.036        1       1       31
10787   tagtype:UK tagID:10787R-Value:4.2234 BLASTN of SAGETAG : gi|30349104|gb|AC124499.3||Mus musculus BAC clone RP24-68G23 from chromosome 12, complete sequence|0.14        1       1       14
10596   tagtype:PS tagID:10596R-Value:10.6517 Orf:702 gi|56753077|gb|AAW24748.1||unknown [Schistosoma japonicum]|4e-70  1       41      17
10542   tagtype:AS tagID:10542R-Value:11.5700 Orf:3275 gi|15986447|gb|AAL11633.1||putative histamine-releasing factor [Schistosoma mansoni] gi|20140691|sp|Q95WA2|TCTP_SCHMA Translationally controlled tumor
protein homolog (TCTP) (Histamine-releasing factor)|2e-92       2       32      1
10467   tagtype:UK tagID:10467R-Value:26.0714 BLASTN of SAGETAG : gi|161027|gb|J04017.1|SCMHSP86|S.mansoni heat shock protein 86 mRNA, complete cds|0.14        1       1       64
10260   tagtype:UK tagID:10260R-Value:4.3064 BLASTN of SAGETAG : gi|25168716|emb|AL928696.6||Mouse DNA sequence from clone RP23-387G11 on chromosome 2, complete sequence|8.7   3       16      1
7449    tagtype:UK tagID:7449R-Value:4.0537 BLASTN of SAGETAG : gi|13157532|emb|AL159154.16||Human DNA sequence from clone RP11-428G23 on chromosome 13 Contains part of a novel gene (KIAA0916), complete
sequence|0.009  2       16      2
7020    tagtype:UK tagID:7020R-Value:4.9445 BLASTN of SAGETAG : gi|11095132|gb|AC084686.1|CBRMM39H11|Caenorhabditis briggsae cosmid MM39H11, complete sequence|0.14     3       21      3
6957    tagtype:UK tagID:6957R-Value:7.4940 BLASTN of SAGETAG : gi|42569710|ref|NM_129333.3||Arabidopsis thaliana aldo/keto reductase family protein (At2g37770) mRNA, complete cds|0.036       12      32
        62
6907    tagtype:AS tagID:6907R-Value:9.6772 Orf:4528 gi|60600029|gb|AAX26558.1||unknown [Schistosoma japonicum]|1e-44   13      1       38
6767    tagtype:UK tagID:6767R-Value:53.3903 BLASTN of SAGETAG : gi|22094806|gb|AF527012.1||Schistosoma mansoni egg secreted protein ESP15 (ESP15) mRNA, partial cds|2.2       3       125     1
6728    tagtype:PS tagID:6728R-Value:14.7359 Orf:1683 gi|605647|gb|AAA57567.1||fructose 1,6 bisphosphate aldolase [Schistosoma mansoni] gi|1703248|sp|P53442|ALF_SCHMA Fructose-bisphosphate aldolase gi|
2598926|gb|AAB84014.1| fructose bisphosphate aldolase [Schistosoma mansoni]|0   3       58      15
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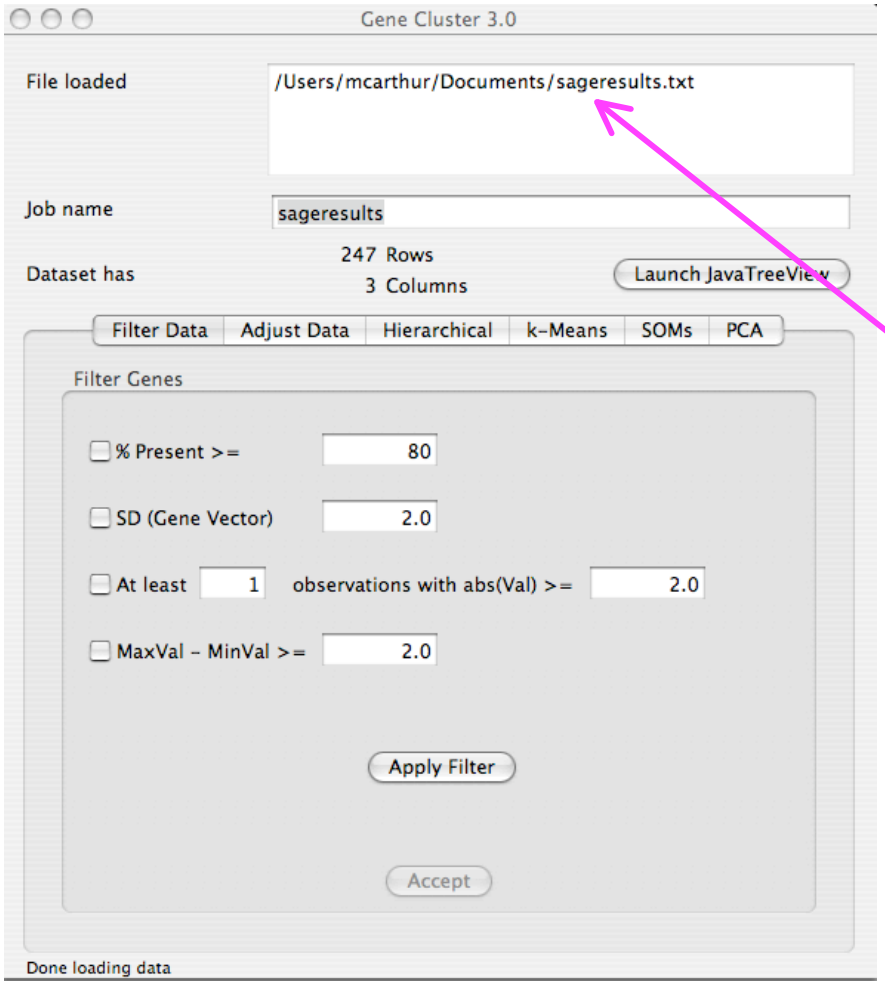
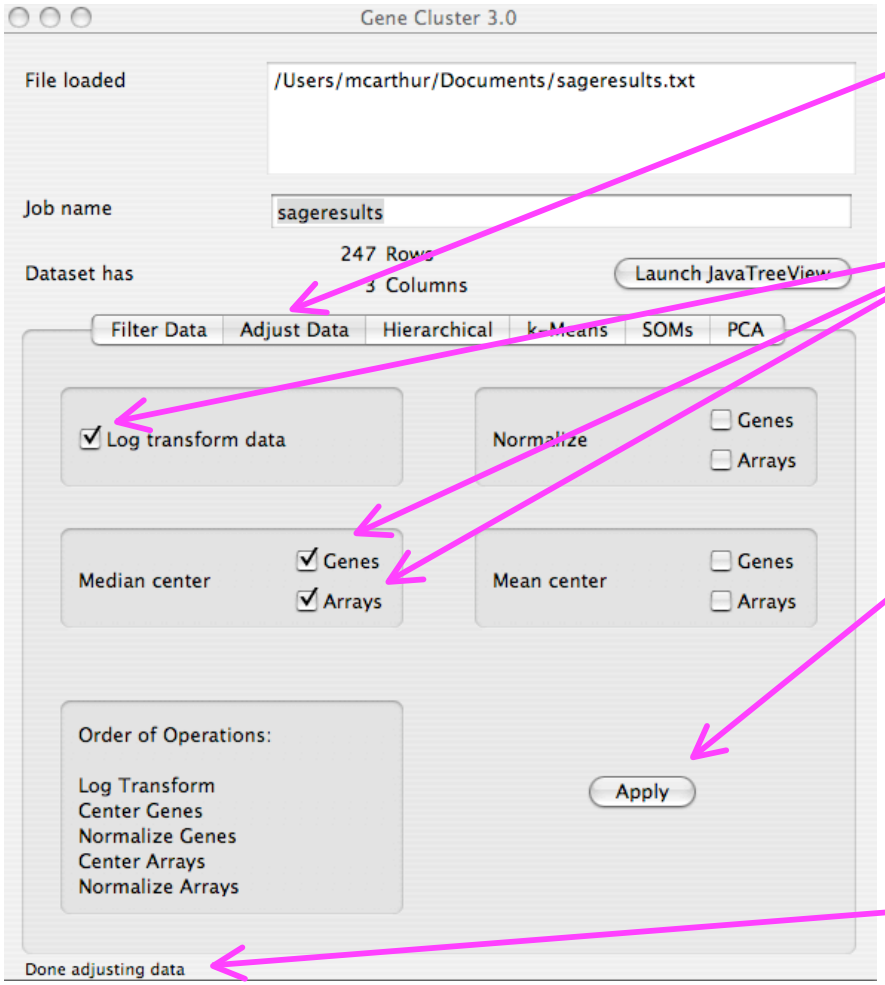
Step 2 - CLUSTER Analysis



- Start the CLUSTER software
- From the *File* menu, open the "sageresults.txt" file
- If you have succeeded, the file should now be listed

Step 2 - CLUSTER Analysis



Change to the *Adjust Data* section

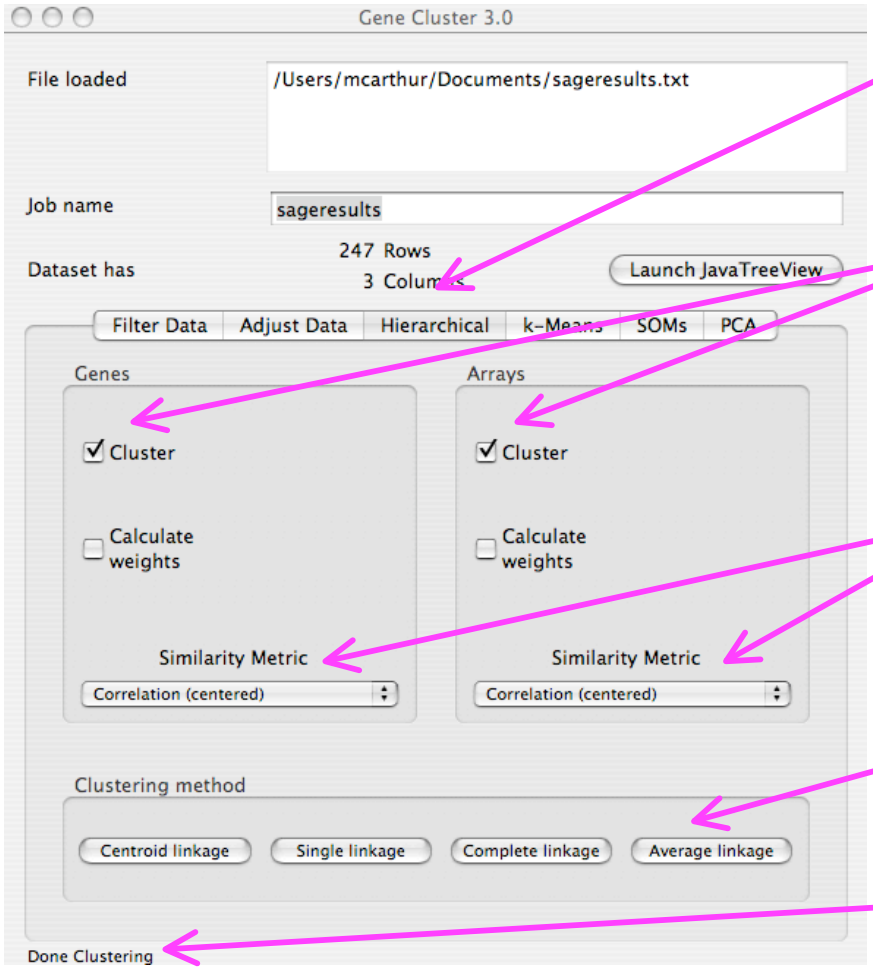
Select *Log transform data* and *Median center* of *Genes* and *Arrays*

Press *Apply*

If you have succeeded, you should see this message

A log transformation is used to reduce the power relationship that exists with SAGE data (i.e. the standard deviation of tag frequencies is not independent of the mean of tag frequencies). Note that this is a different reason for log transforming than for microarray data (see manual). Median centering adjusts tag frequencies to a range of -1 to +1, with the median being zero. This reduces the influence of tag magnitude - lowly expressed genes are treated the same way as highly expressed genes such that only that pattern of change counts, not the magnitude. Normalization is not selected here as the distance metric we use does not require it (see manual).

Step 2 - CLUSTER Analysis



Change to the *Hierarchical* section

Select *Cluster* for both *Genes* (i.e. tags) and *Arrays* (i.e. SAGE libraries)

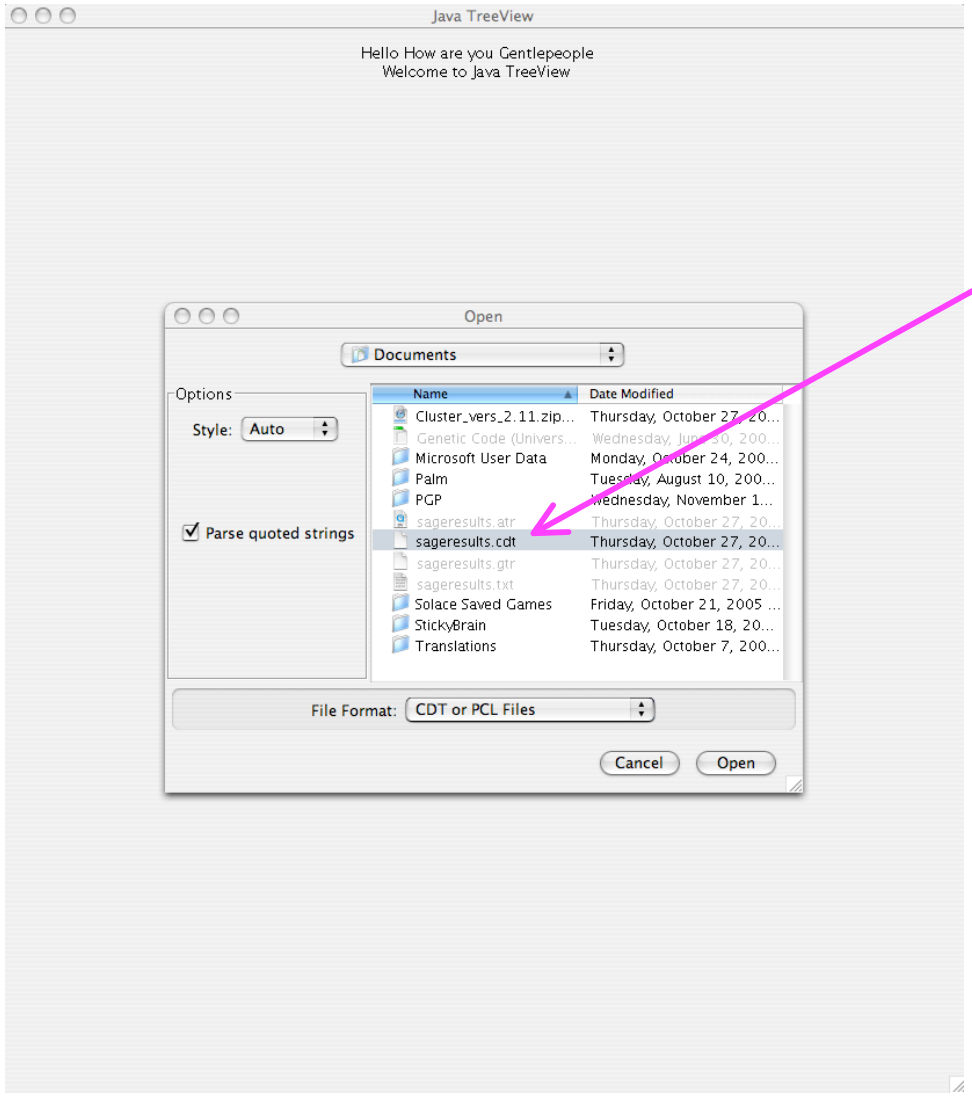
Select *Correlation (centered)* for both *Genes* and *Arrays*

Press *Average Linkage*

If you have succeeded, you should see this message

We are clustering both SAGE tags (Genes) to find tags with correlated expression profiles and SAGE libraries (Arrays) to discern correlated global gene expression among libraries. Centered correlation (i.e. Pearson's correlation) is a reasonable metric for SAGE data (Poisson would be better!). Note that this metric results in an analysis that is insensitive to magnitude of expression. This means our cluster analysis is focused upon correlated patterns of expression, whether genes are lowly or highly expressed. This can be particularly important for highlighting tags from less abundant transcripts (which otherwise do not have striking R-values). Other metrics in CLUSTER may be more robust to outliers, but are sensitive to magnitude. See the manual for an explanation of different clustering methods - we like to focus on average differences between tags, but each method has its strengths and weaknesses.

Step 3 - View the Results



- Start the TREEVIEW software
- From the *File* menu, open the "sageresults.cdt" file

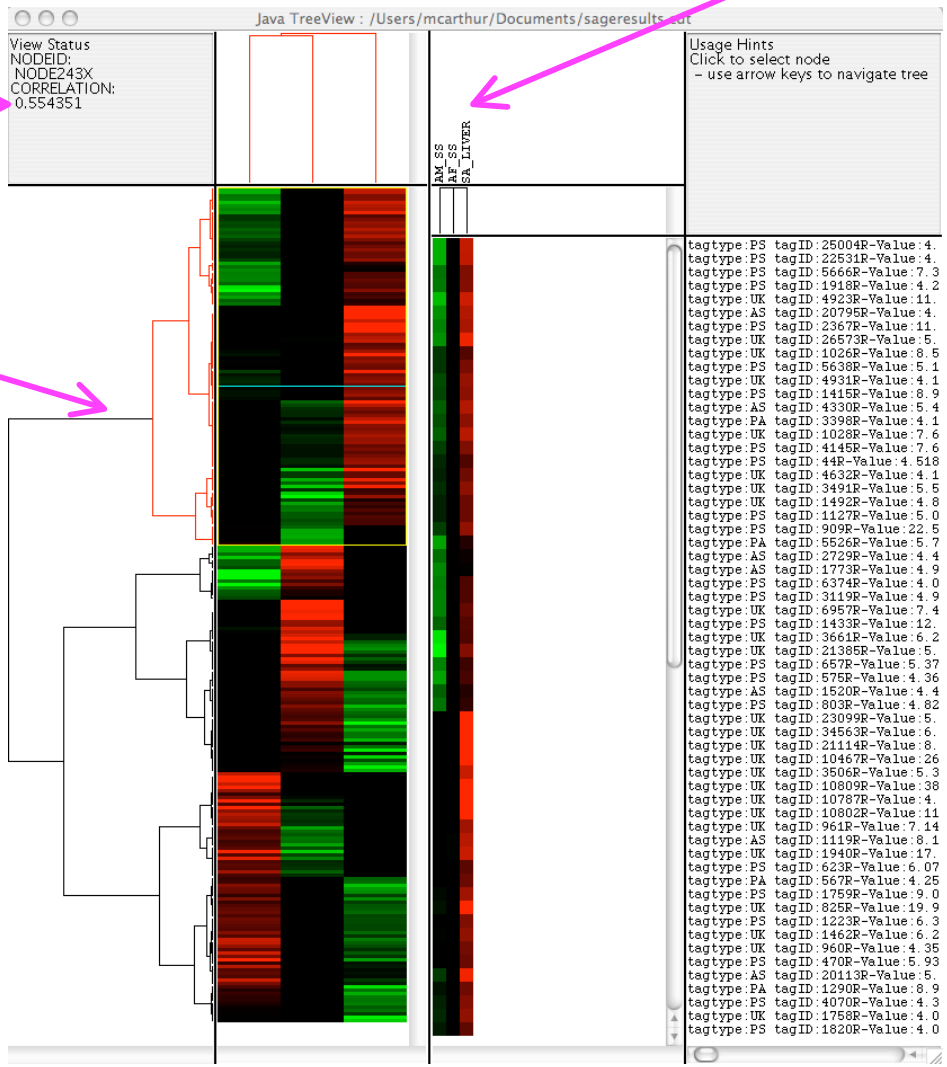
Step 3 - View the Results

SAGE libraries, with global correlation results

This cluster has 55.4% correlated expression

Select a cluster to obtain details

Tags and their annotations



Black = median, Red = upregulated, Green = downregulated