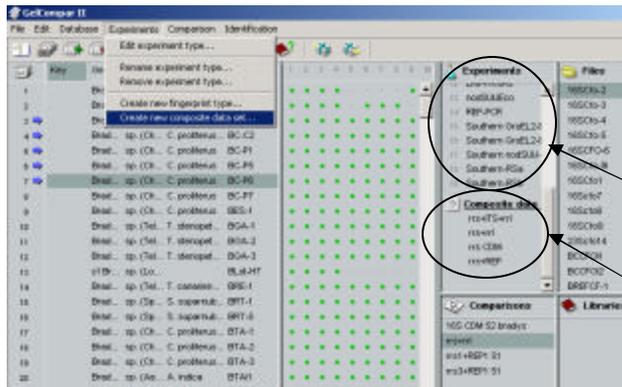


Pablo's step-by-step tutorial on how to perform a Dice/NJ analysis of PCR-RFLP data with GelCompar II using the band-matching algorithm

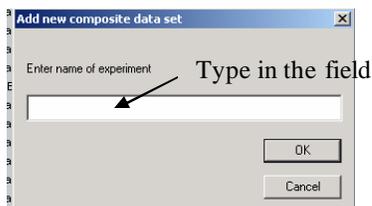
I assume that you have already normalized your data and defined the band position. Here I show only the steps that follow afterwards.



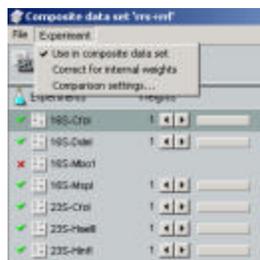
1) You need to create a new **composite data set** (i.e. rrs+rrl)

These are individual experiment types in my database

These are the composite datasets in my database



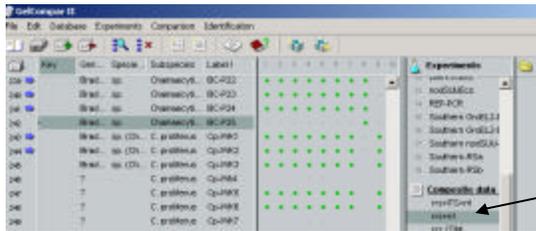
2) You give a name to the new **composite data set** (for example rrs+rrl)



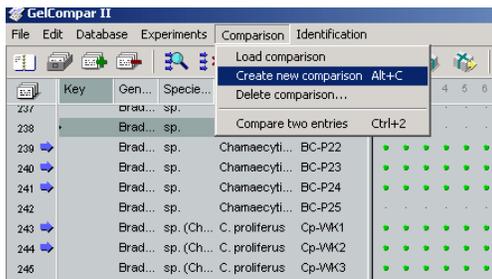
3) You need to define which experiments (i.e. 16S-CfoI+16S-DdeI etc) you want to include in your new composite data set. Give all experiments the same weight, as seen on the picture



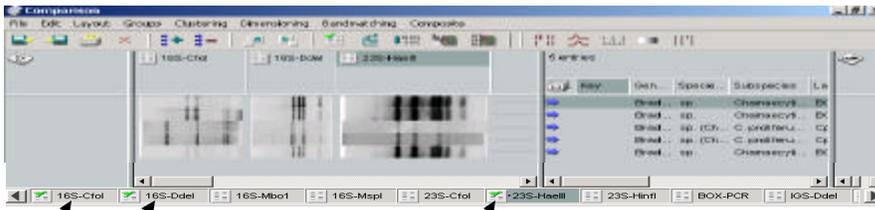
4) You should specify the settings for the composite data set Comparison as indicated in the fig. You may eventually want to Use UPGMA instead of NJ, but this you can specify also later.



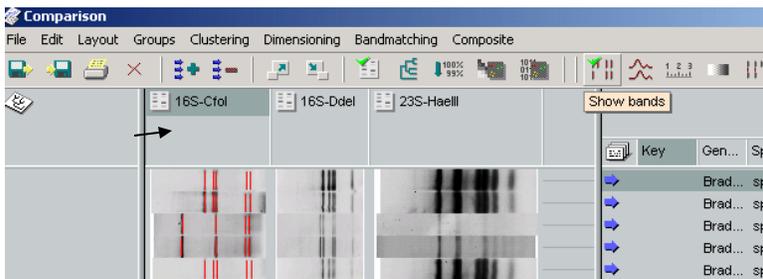
The new composite data set name should appear here now



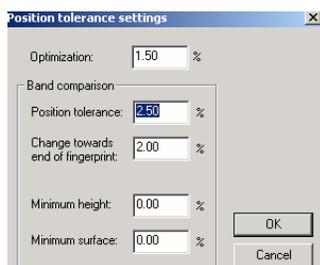
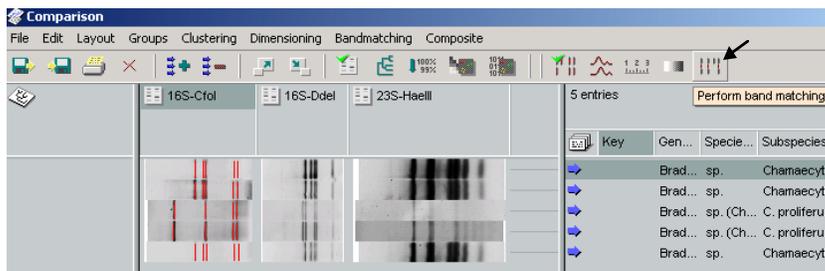
Now you are ready to select the strains you want to include in the new analysis (hold ctrl key and click on the number to the far left, or click with the right mouse button on the strain name and then click "select in list". Once selected (highlighted with the blue arrow, go to comparison and select create new comparison (Alt+C is the shortcut)



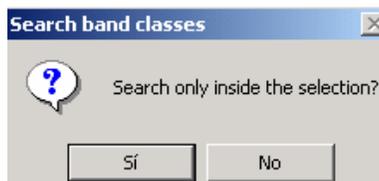
Click on the single experiment type buttons on the bottom line to activate them and display the geltracks



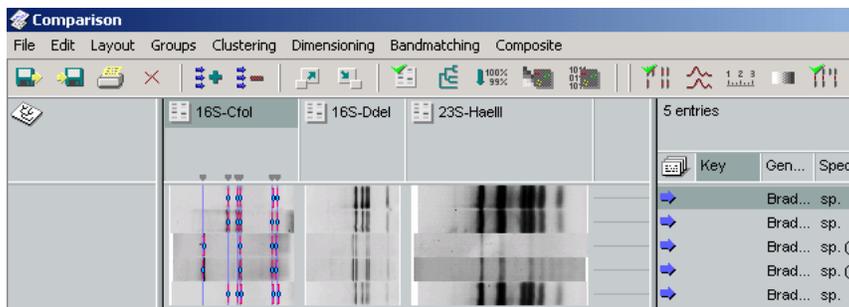
Click on the single experiment fields (arrow) and click on the show bands button on the upper line, as shown. This will display the bands as previously defined.



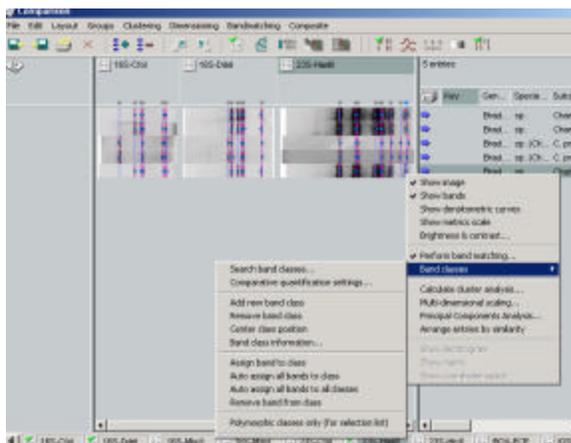
And now the important step of performing the “band matching” I use the settings indicated to the left, which work fine for most RFLP gels



Tell the program yes, to perform the search only within the activated field

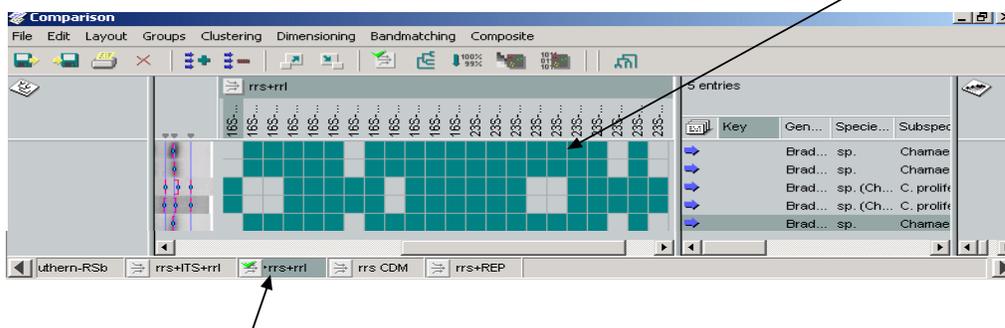


The band matching process should now correctly recognize the different band classes and assign the individual bands to them. In the example (16S-CfoI) there are 6 band classes. Repeat the same for all the gels fields.

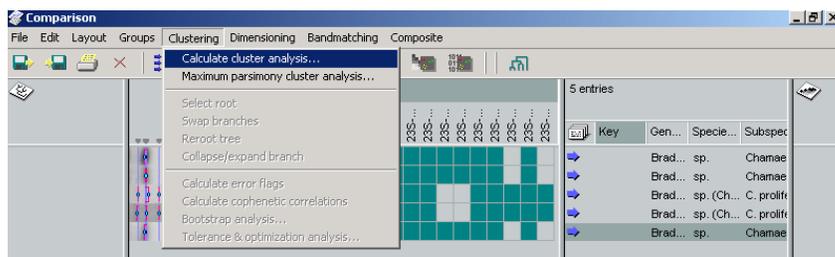


Depending on the gel quality and searching Settings, the program may miss some band classes, Or put too many of them. You should manually Correct eventual mistakes by clicking on the mis- Assigned band with the right mouse button and choose the proper option (delet band class, add new band class etc.). Obviously, a perfect band-class assignation is necessary for a good analysis .

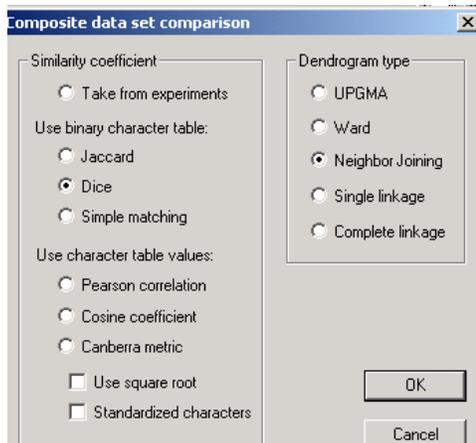
And now you are ready for the cluster analysis . Be shure that you activate the proper experiment type field (see arrow) by simply clicking on it, in our case rrs+rrl. Immediatly the band-matching table will be displayed (gray squares)



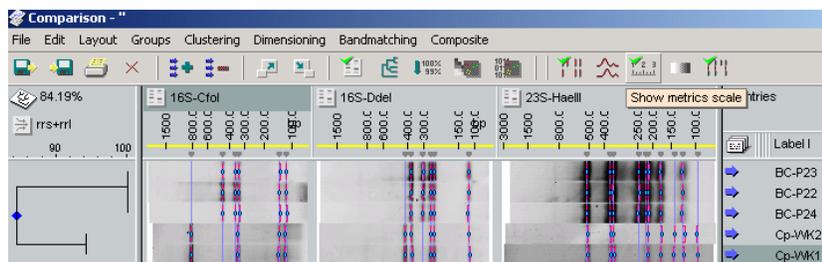
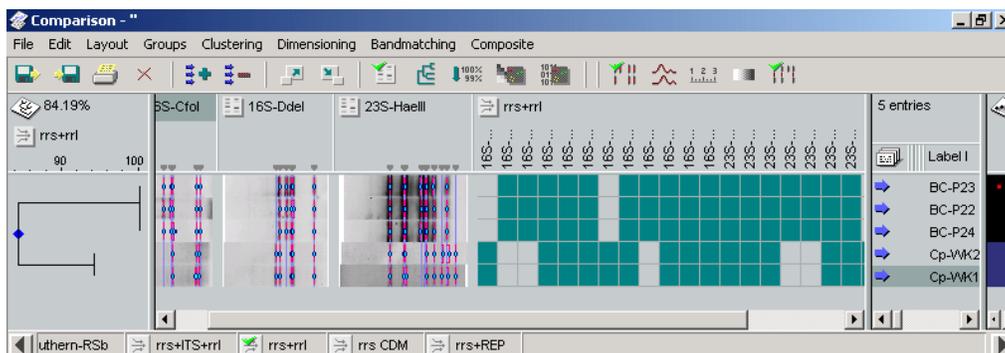
And now you are ready to go to the cluster analysis ; just click on that option



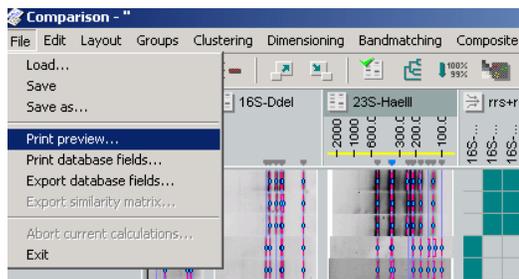
and the following field will appear, which contains the settings previously specified when you created the new composite data set. It is in this window where you can change to UPGMA, if you wish, but leave Dice as the choice for the similarity coefficient (for RFLPs) However, you would use Pearson's correlation coefficient for REP-PCR fingerprints.



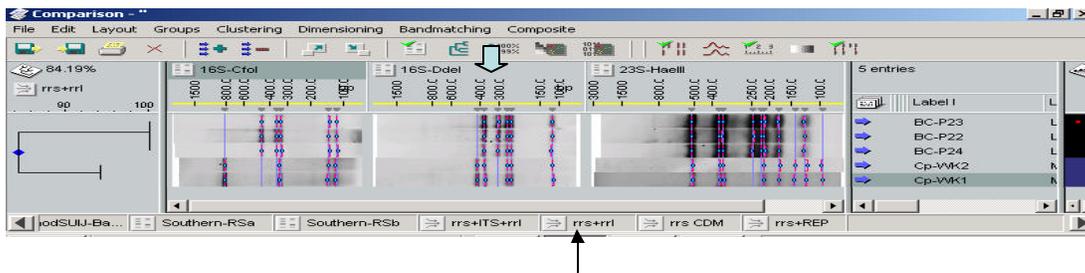
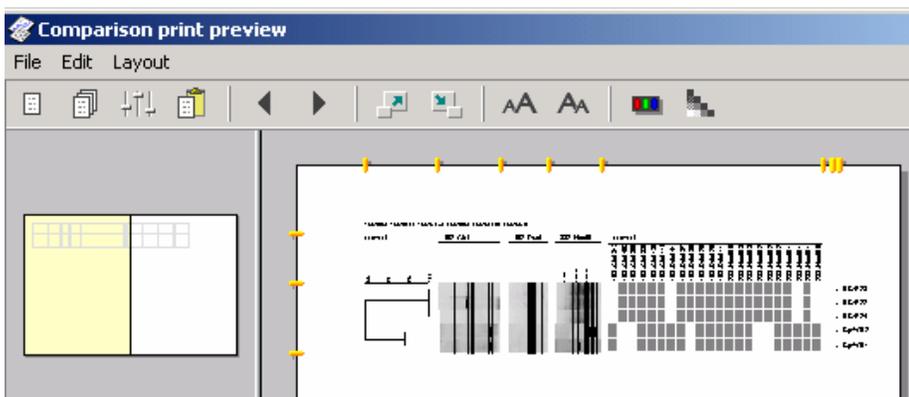
And there you are: On the left panel you see the dendrogram. Notice that only 2 genotypes are found among the 5 strains. That's why you see the straight lines at the end of the tree tips. Notice also that the blue diamond indicates the linkage level of that node (84%). Notice also that below the Linkage level you see the experiment type used for cluster analysis, in this case rrs+rrl.



Optionally, if you have defined the sizes of the DNA MW ladder you used for gel normalization, You can display the metrics scale for each RFLP type.



Now you can print the dendrogram using the print preview option



Deactivating the experiment type fields (for example rrl+rrl) you can get rid of unwanted displays, like for example the band-matching table. Go now again to print preview. If you click with the right Mouse button on the gel fields (filled arrow), you can also specify only to show bands and not the gel Etc...

Good luck