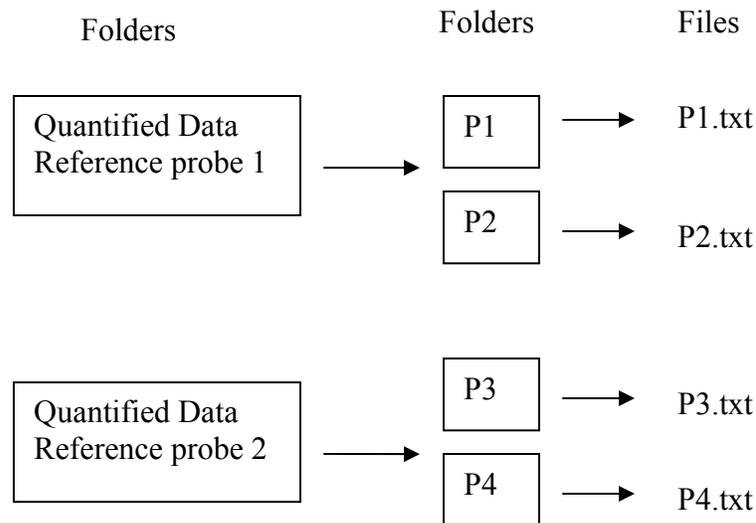
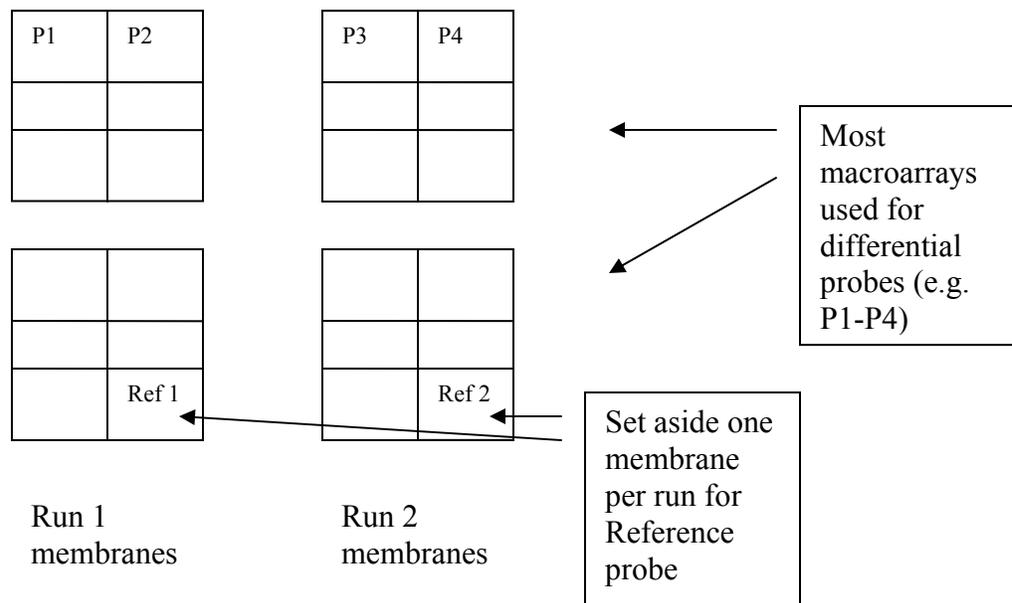


User's Guide to Background Subtraction

To do this step, you will need quantified data from all of the probes that you will be using, as well as from the reference probe. The data for each probe must be saved in a separate folder (ImaGene quantification software will produce two files per image, one picture file and one tab-delimited format text file with the quantified data), and all the folders for all the probes to be subtracted must be included in one folder (see example below). All the files must have data displayed in the same order and have the same number of data points.



You may want to have multiple reference probe hybridizations, one per macroarray gridding run, and analyze each subset of differential probes utilizing membranes from that run separately. To obtain fingerprints with containing information for all probes, you will combine the data from each probe subset at a later point (see Fingerprint Assignment). For example, if I have two gridding runs, I may want to use one macroarray per run for the Reference probe, just to make sure that the spotting of each run was similar:



When I do this, I want to make sure that the membranes from each run are analyzed with the reference probe data from each run (but you don't have to worry about this until the Fingerprint Assignment step, which comes next).

Note that the order of the control clones in the ImaGene output files should match the output of the theoretical fingerprint generating file for those clones (see instructions for Fingerprint Assignment). It may be simpler to change the order of the control clones in the ImaGene output files before starting the subtraction process than changing the order of the clones in the theoretical fingerprint file. To change the order of control clones in an ImaGene output file, use the Data -> Sort function in Excel and sort by the rows/columns as desired. Be sure that all the output columns are highlighted for the rows to be sorted so that you don't introduce errors.

To subtract the background from your data:

- Open the **Background Subtraction** program file (filename will be UPDATED_SUBTRACT) and go to the "readme" page.
- Make sure the number of plates is filled in (should be between 1 and 25)
- Go to the "work" page and clear the information on it. Save the file with a different name than the original name of the file.
- In another window, open My Computer and browse until you find the folder that has your probe data folders in it. Keep this window open.
- Go back to the Excel window and select Tools -> Macro -> Macros -> Main. Click Run.
- A dialog box will pop up asking you to "Find folders of datasource". Go to the file browse window you have open and copy (CTRL + C) the folder path

name (the stuff written in the box on the top of the screen that is right of the word "Address").

- Paste (CTRL + V) the folder path name into the Excel dialog box.
- The results will appear in the "work" page.
- Check that the "flag" column contains data from the reference probe: it may be easier to simply open the reference probe file (tab-delimited text file, remember) and copy and paste the "flag" column data into the appropriate column in the Excel sheet. Any spots that are considered negative or missing for the reference probe should be flagged as "2". (Note: when there are multiple reference probe images, the output of the **Flag Column Tool** should be pasted into this column).
- Using the cut and paste options, sort the probe data into the order that you want them to appear in the final fingerprints. The Reference probe should always be the first column. (Note: probe sorting can also be left until after the fingerprint assignment step, which is performed using the **Main Processing Tool**).
- Save the file again, using the new name you have given it.