III. Biological Variation Analysis (BVA) – Control vs. Experimental

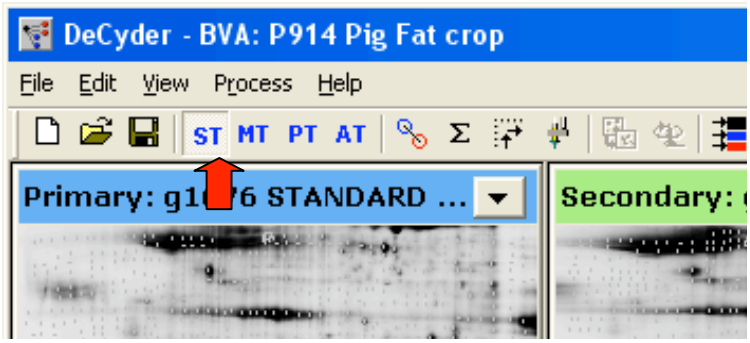
A. Open BVA

B. Click the Create Workspace icon or Click File – Create Workspace

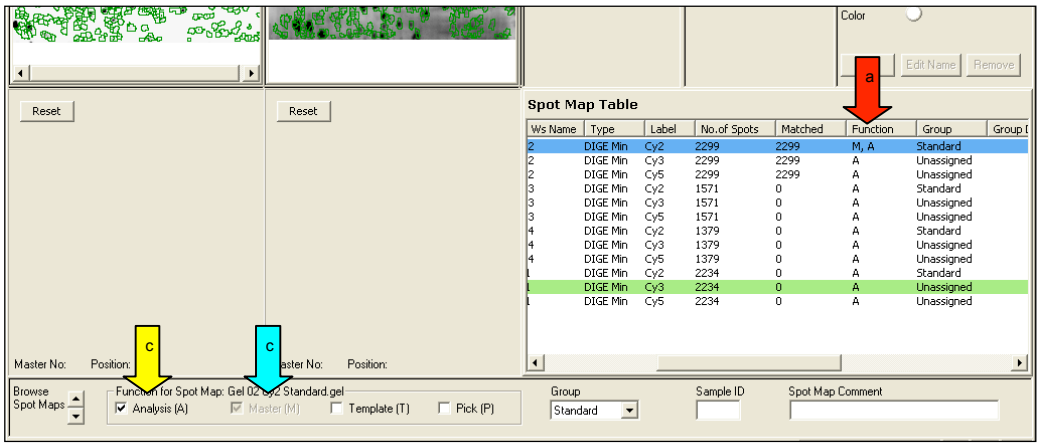
1. Find your project folder in the list and open it
2. Open the DIA folder
3. Select your DIA workspaces for each gel you want
4. Click Add to include it in the right panel
5. Once you have all the DIA workspaces for your project, click Create

C. Function Assignments

1. View the Spot Map Table by clicking on the Spot Map Table icon or click View – Spot Map Table

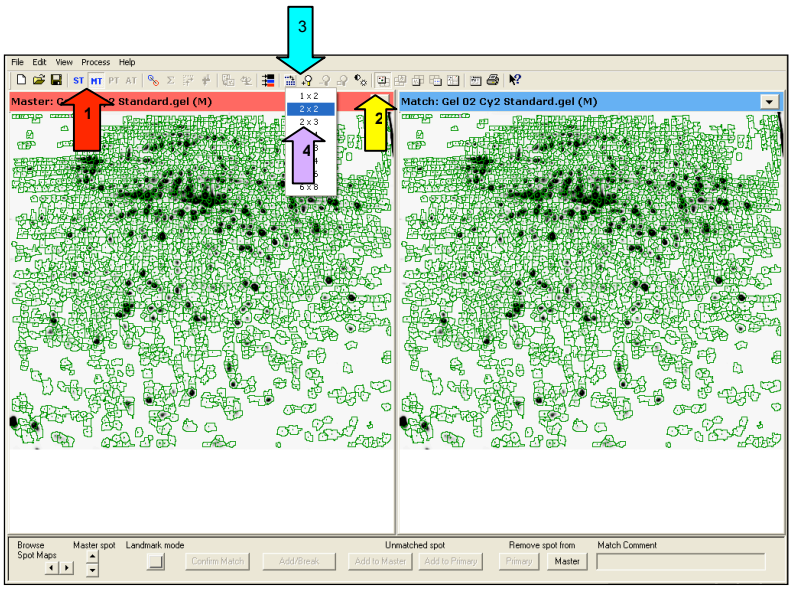


2. The Spot Map Table will list information about all images in the workspace
    a. Look at the Function column
    b. All should have an A for Analysis and one will have an M. This is the Master gel image
    c. Scroll through the images and make sure that they all have a check in the Analysis box and that the Master gel image has a check in the Master box at the bottom of the screen

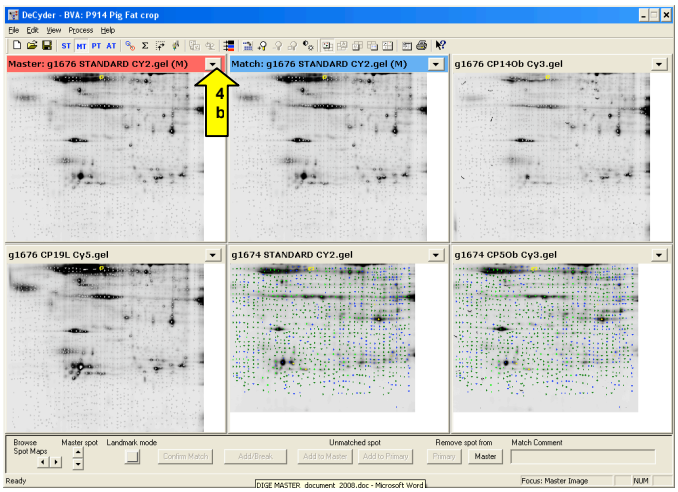


D. Assigning Groups
    1. In the Experimental Design View panel in the upper right of the screen, click Add
    2. Name this group Control and click Confirm
    3. Click Add again and create a group named Experimental
    4. Click on the Unassigned folder and the unassigned images will appear in the center panel
    5. Select the Control images and place them in the Control folder
    6. Select the Experimental images and place them in the Experimental folder

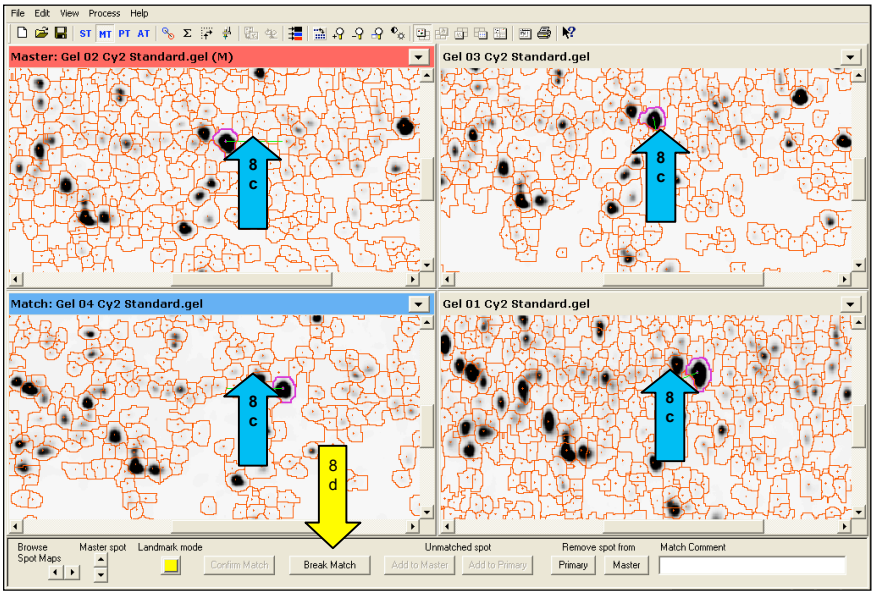
E. Landmarking



1. Click the Match Table icon or click View – Match Table
2. Click the Magnify Image View icon or click View – Image View
3. Click the Multiple Image Views icon or click View – Display Multiple Gel Views
4. Select 2x3
    a. Six gel images will be displayed
    b. Use the drop down arrow on the right side of each images' title bar to select the Cy2 image from each gel



5. Adjust the Contrast and Brightness if necessary
6. Click the Properties icon or click View – Properties
    a. Click the Image View tab
    b. Uncheck the Auto-center selected spots box
7. Zoom in on a region with good spots
8. Click the Landmark Mode button at the bottom of the screen, it should turn yellow
    a. While the Landmark Mode button is activated, it is important to first click on a spot on the Master gel image and then on the corresponding spot in the other gel images. Be careful that you do not have stray clicks because they will lead to erroneous matches.
    b. Select a good spot in the Master Image and then click on the corresponding spot on one of the other images
    c. Note the appearance of the green vectors



    d. Make sure the spots match and then proceed to the next spot
        i. If the spots do not match, select Break Match and try again
    e. Landmark about 10 spots, spread out around the entire gel
    f. Click the Landmark Mode button to deactivate
9. Click the Match icon or click Process – Match – Match All