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The following instructions are specific to GenePixPro 3.0 software and gene expression hybridizations. This protocol is intended to help new users get started, for further explanations, search the GenePix Help tab using the GenePix term found indicated as *"ITALICS"* below.

1. Turn on scanner, start GenePix Pro software.

2. Slide scanner door open. Insert chip hyb side down with label facing towards you into scanner. Clip the chip holder easily around the slide. DO NOT PUSH DIRECTLY DOWN ON THE CLIP.

3. Set PMT's to 600 in both 635nm(Cy 5) and 532nm(Cy3) channels. These settings are controlled in the *"HARDWARE SETTINGS"* window.

4. Perform low resolution *"PREVIEW SCAN"* to determine location of spots and initial hyb intensities.

5. Once you have determined scan location, draw a *"SCAN AREA"* marquis around the entire array.

6. Perform a quick visual inspection of hyb and make initial adjustments to *PMT's*.

7. For gene-expression hybs, you would like the ratio over the entire scan area to be 1.0. You will want to raise or lower the red and green PMTs to achieve this color balance. This will be performed more stringently later.

8. Before you perform your data scan, change the *"LINES TO AVERAGE"* in the *"HARDWARE SETTINGS"* to 2. The scanner will now scan each pixel twice and average the data counts collected which reduces any background noise that may be present.

9. Perform a high– resolution "*DATA-SCAN*". As the image is scanning, go to the *"HISTOGRAM"* tab located at the top of the screen. The histogram allows you to dynamically observe the relative intensities of both channels as you scan. Important components of the histogram tab are:

-histogram settings should be:
Image: Both
X-axis: Fullscale
Y-axis: Log Axis On
Fullscale

-the histogram is basically showing you the percentage of Normalized Counts that are at a given Intensity or quanta.
-the histogram only shows you the pixels you are viewing in the image tab, i.e., if you are zoomed in on the image it will only show you the pixels you are zoomed on.
-remember that EVERY pixel is represented in the histogram, so artifacts and dirt will skew the readings. If you have a lot of dirt or artifacts, try and zoom in on a clean portion of the array to determine accurate PMT settings.

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10. Observe the histograms and make adjustments to PMT's. In general, you would like to see pixels represented across the entire Intensity range. However, saturated pixels (with counts greater than approx. 67,000) will be thrown out and spots with pixel counts close to background will result in poor data.

11. Once the PMT levels have been set so that the Intensity Ratio is near 1.00 perform a "*DATA SCAN*" over the "*SCAN AREA*" and save the results.

12. To save your image, go to the *"OPEN/SAVE"* button and select *"SAVE IMAGES"*.

13. We like to save as type= Multi-image TIFF Files and using a naming convention which includes a date prefix. In general, it is not necessary to save the Preview or Export images, and instead only save the Wavelength 635nm and Wavelength 532nm.

14. Once the image is successfully scanned and saved you are ready to assign spot identities and calculate results. GenePixPro offers easy to use software that will deconvolute the identities of your PCR products from the 96 well text file into 384 well format, saving this information in the form of a *GAL* file. It will then further transform the 384 well files into a grid (the *GPS, GenePixSettingsFile*) that assigns each spot on the chip its identity based on your printing parameters.

Print parameters include:

- **the number of tips used to print**
- **the spacing between spots**
- **number of plates printed**

15. To create the "*GAL(GenepixArrayList"*) refer to the HELP section associated with the *"ARRAY LIST GENERATOR"* button found in the *IMAGE* tab.

16. Once you have created a *"GAL"* and *"GPS"* file you are ready to obtain your *RESULTS.* The results provided by GenePixPro are very extensive. For all further analysis information, refer to software documentation.