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| UNM SOM EXPERIMENTAL BIOTECHNOLOGY LABORATORY: MICROARRAYS Standard Operating Procedure | | |
| Title: Scanning Slides with GenePix Pro 3 | | |
| SOP#:7.1 | Revision level: 2 | Effective Date: 5/07/03 |
| Author: V. Bain | Primary Reviewers: B. Griffith | |

1. PURPOSE

Post-hybridization with fluorescent targets, we scan the slides with lasers that excite the Cy3 and Cy5 dyes. The scanned images are collected using the GenePix Pro 3 scanning, imaging and quantitation software and the GenePix 4000A scanner. After the images are acquired and stored on the server as multimage tiff files, we use the software to overlay the GenePix Array List (GAL file) to allow quantitation of the fluorescent intensities of the features and association of the features with the gene identifiers and descriptions. Balancing the pmt (photomultiplier tube) settings for each of the lasers over many features, and preferably the whole slide, is critical to balance out differences in the specific fluorescent activities of the two targets. For more information on analytical tools and detail directions please refer to the *GenePix Pro Microarray acquisition and Analysis Software User's Guide*.

2. SCOPE

This procedure is used by the Experimental Biotechnology Laboratory under the direction of Drs. Rick Lyons and Tom Williams. This protocol is adapted from prior protocols written by Axon Instruments for the GenePix 4000A Scanner and GenePix Pro 3 software.

3. MATERIALS

1. Axon 4000A Scanner
2. Black Slide Boxes to hold 25 microscope slides (VWR Cat# 48444-004 ; Becton Dickinson 423843)
3. TZ Labels, 3/4 inch (Brother, TZ-241)

4. SLIDE LABELING

1. The following information should be included on the post hybridization slide label
 1. Cy3 cDNA identification number (eg. 3-0007-24) and number of pmoles of dye hybridized (eg 200pmoles)
 2. Cy5 cDNA identification number (eg. 5-0013-40) and number of pmoles of dye hybridized (eg 200pmoles)
 3. Hybridization date
 4. Experiment title
 5. Date that cDNA was synthesized
 6. Place a post hybridization brother TZ label onto the DNA side of each slide. Place this label in alignment with the slide print date label.

5. PROCEDURE

1. Turn on scanner
 - a. Turn power box to scanner and the scanner on 15 to 30 minutes before using to allow the laser to warm up.
 - b. May have to restart computer so that it recognized the device.
 - c. Open GenePix Pro 3.0 on desktop
2. Insert slide
 - a. When scanner door is ready to be opened the light on the top will be yellow and the lower left of GenePix software window will say "ready"
 - b. Slide scanner door open
 - c. Open clip holder
 - d. Place slide in **feature side down, original print date label side up, and label toward front of scanner.**

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- e. Clip the holder easily around slide. Do not force clip down or push directly down on clip.

3. Set scan settings



- a. Open the hardware settings
- b. Set the PMTs for both 635 (Cy5) and 532 (Cy3) to 600. This is a good PMT setting to start with because you will usually see the blocks that were printed even if they hybridization didn't work well. The PMTs can be adjusted later. We often use PMT's of 480 to 540.

4. Preview scan



- a. Perform a preview scan to determine the location of the spots and the initial intensities. The whole surface of the slide will be scanned rapidly.

5. Set Scan area



- a. Set the area to be scanned by drawing a box around the arrays using the set scan area box. This reduces the time of upcoming full scanning by not scanning unnecessary parts of the slides. Exclude the gasket from the scan area.



- b. Zoom in to assure that all columns and rows are within the scan area.

6. Adjust scan settings as needed

- a. Make initial adjustment to the PMTs by viewing the histograms. The goal for a cDNA hybridization is to maximize the Cy3 and Cy5 signals, with few or no saturated features (>65,000) and with the Cy3/Cy5 histogram plots overlapping giving a ratio near 1.
- b. When doing a cDNA/cDNA hyb looking at gene expression the ratio of red and green in the histogram should be at 1.0. If adjustments of the PMT of one or both colors needs to be done do so now. Note that all of the signals visible in the "image" tab are included in the red/green histograms. So if you are zoomed into one block, the histogram only reflects those features visible in the image on the monitor.

7. High resolution scan

- a. Once the minor adjustments have been made it is time for a full scan. Go to the "hardware settings" box and use "1 line to average" for the first full scan.



- b. Click the full scan button. Features will be at a higher resolution.
- c. As the image is scanning go to the histogram tab located at the top of the screen. The histogram allows for dynamic observations of the relative intensities of both channels as you scan. The histogram setting should be:
 - i. Image: Ratio (635/532)

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- ii. X-axis: Full-scale, 0 min and 66,000 max
- iii. Y-axis: Log axis on, Full-scale, min = 10^{-7} or 10^{-8} and max=1
- iv. Image balance-intensity ratio: near 1

The histogram will only reflect the pixels you are viewing in the image tab so if you are zoomed into a single block, red/green balance is just for that block. Generally, you want to perform the final balancing of the histogram when the full array is in view, but with the gasket edge effects excluded from view through the usage of the “set scan area”. Dirt particles or the gasket edge will alter or skew the readings for appropriate PMT settings so try zooming on a portion of the slide without dirt particles.

8. Observe histogram and adjust PMTs if needed
 - a. The entire intensity range should be captured in the full scan. Saturated pixels will be white, are not desirable and will skew data or be filtered out. Spots with intensities close to background will result in poor data. Try to have a majority of the spots in the middle of the intensity range (1000 to 50,000).
 - b. Balance the PMTs via the histogram to obtain a ratio close to 1.00 and scan slide next with “2 lines to average” setting in the Tools box.

9. Perform real Data scan



- a. Scan the slide again with “2 lines to average” setting.

10. Save your image



- a. Save the image in the print run folder on the H drive. (example: h/home/bgriffit/crfddata/axonscans/4th full print M1 82902)
- b. Save as a multi-image TIFF file.
- c. Name the file using an appropriate naming convention including the date prefix.
- d. Do not save the preview scans or “1 line to average” scans. Full scans occupy approx 40Mb of disk space so only save a single scan per array, in general.

11. Eject slide



- a. Eject the slide.
- b. Wait for light to turn yellow and the monitor text to read “ready” before opening door.
- c. *To conserve the scanner’s lasers, we scan ALL slides in the set before printing images or performing the overlays and analysis.* After all scans are completed, we turn off the scanner and proceed with printing images, doing overlays, and doing analysis.


12. Printing images

- a. If you would like a hard copy of the image click on the report tab located at the top of the screen. Click “print image”.
- b. If you would like to edit the text that appears above the picture click the edit button. Add or remove any text that you would like.
- c. Click the preview image button.



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- d. The image preview will appear then click "Print". The image is set up to print down the hall room 301 to the Phaser Textronix color printer.


13. Data analysis within GenePix

- a.  Select the appropriate GAL file for the slide that was scanned. The GAL file is the GenePix array list that is generated by the OmniGrid software after the slides were printed. The GAL file incorporates the number of arrays/slide, subarrays/array, features/subarray, and locations of the features on the slide to create an overlay which will allow quantitation and association of the features with their gene descriptions. (example: h/home/bgriffit/crf data/full mouse library/gal files/m1 082902 print 22x24)
- b. Line the overlay up with the blocks and features. If you do not have the appropriate number of blocks then you may have the wrong GAL file. Recheck the print date and load the correct GAL file.




- c.  Using the zoom in and zoom out functions it is easier to see how closely aligned the overlay is with the features. All the blocks can be moved together or the blocks can be moved independently from one another.
- d.  Draw a box in block mode (not by zooming) around all the blocks to link them together. Click off the blocks, then click on one particular block to move it. Use the > and < keys to move between blocks rapidly to view whether each block is properly overlaid.




- e.  Select the "align features in all blocks" to have to software locate all the features and draw the correct circle around each feature. If there is a problem with the feature, the feature will be marked differently or "flagged". Double-check how the software aligned the features because it isn't always correct. You can move a spot to the correct feature if for example it drew the circle around a dust particle rather than the feature. By clicking on the feature when the cursor is in



feature mode  you can move, enlarge or shrink the circle size. "control ↑" increases the size. "control ↓" decreases the size. "↑" alone moves the circle up. "↓" alone moves the circle down. "←" alone moves the circle leftward. "→" moves the circle rightward.



- f.  Click analysis to have the software quantitate the data. When the analysis is finished it will tab to the results tab located at the top. The raw data is presented. You can choose the columns of data that you would like to work with and save the results. Click the "save as" button and your results will be saved as a gpr file (GenePix results). The gpr files are tab-delimited text but can be opened in Excel. It is best to save the gpr files as the same name as the image file. Save the GPR files on the H drive or server, in the folder associated with the slide print run. (example: h/home/bgriffit/crf data/axonscans/4thfull print m1 082902/gpr files)

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- g. Your data set is then ready to be normalized, filtered, queried, and clustered in Acuity 3 or GeneSprings.