**UW Medicine - Pathology**

400-11-01-03

Array Scanning and Processing Procedure

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| Adopted Date: 10/12/09  Review Date: 07/2010  Revision Date: 07/2010 |

PURPOSE

To scan two-color NimbleGen arrays with Agilent Microarray Scanner and prepare data to load onto Genoglyphix for case review

PROCEDURE

# Equipment

1. Agilent 2um scanner
2. Computer with softwares below
3. Color Printer
4. SciGene Ozone Filter box
5. Microarray slide holder
6. Microarray slide

# Software

1. Agilent Scan Control
2. NimbleScan
3. NGconverter
4. GGXconverter
5. DNA Analytics
6. Excel
7. [www.genoglyphix.com](http://www.genoglyphix.com)
   1. **Procedure**

**Part I: Loading and Scanning Slides**

***Note:*** *Keep arrays in a dark desiccator until you are ready to scan them. When handling slides, wear powder-free gloves and use care to touch only the slide’s edges*

1. Loading Slides
2. Turn on the Agilent Scanner and the SciGene Ozone Filter for about 10 minutes before start scanning
3. Load slide into slide holder with proper orientation (barcode should be on the back side. DO NOT touch the surface of the slide)
4. Load slide holder into the slot of the carousal. (use gloves when open and close scanner to minimize fingerprint and dusts)
5. Record slide’s position
6. Close the cover and close door of the Ozone box
7. Scanning Slides
8. Open Agilent Scan Control (It takes about 5 minutes for the scanner to be ready)
9. Select the starting and ending slot to scan
10. Profile should be set as <Default>
    1. Slide ID: <Auto detect>, Channels: R+G, Scan Region: Full Slide (71 x 21.6 mm), Resolution: 3um, TIFF: 16 bit, R PMT: 100%, G PMT: 100%, XDR: <NO XDR>, Output Path: D:\ScanData
11. Click on “Scan Slot 1-xx” (lower right corner) to start scanning
12. After the scanning is completed, a summary of the scanning process will pop up. Make sure there is no critical error (red text) before move on to next step.
13. Close Agilent Scan Control
14. Sources of Error for Part I: Loading and scanning slides
15. Not using proper handing technique when handing slides
16. Loading slide in the wrong orientation
17. Carousel is not properly in place
18. Scanner has not warm up
19. Scanning at the incorrect resolution

# Part 2: NimbleScan to Burst, Align, and Extract Features

***Note:*** *NimbleScan is picky. Make sure to close the software and open it again after each step.*

1. Rename images
2. Open “D:\Scan Data” folder and search for scanned tiff files
3. Original file name should be: slideID\_date\_S01\_Green/Red.tif
4. Rename it as: slideID\_532.tif for Green Signal and slideID\_635.tif for Red
   1. If scanner did not pick up slideID (barcode), enter it manually
5. Crop image
6. Open NimbleScan software
7. Click on file -> open
8. Open the folder where the tiff images are stored
9. Select one color channel at a time
10. Select the appropriate design file (.ndf) (design file is located on the desktop inside the SignatureSelect2p03plex folder. Or can be downloaded on [www.genoglyphix.com](http://www.genoglyphix.com))
11. Click open
12. Crop the image (select the region of interest, then click on edit-> crop)
13. Save image
14. Repeat 1-8 for the rest of the image files
15. Close NimbleScan
16. Burst Multiplex Images…
17. Open NimbleScan software
18. Click on file -> Burst Multiplex Images…
19. Click on “add images” from pop up screen and select all images to be burst and Add to batch
20. Layout file = “SignatureSelect2p03plex.ncd” in the same folder as “.ndf”
21. Select the output folder and click on Burst to start
22. Close NimbleScan
23. Align images
24. Open NimbleScan software
25. Click on file->open
26. Choose bursted image (one channel at a time). (note: bursted image end with A01, 2, 3)
27. Choose the design (.ndf) file. (Same file as in part B)
28. Click open
29. Click on View -> auto contrast/brightness to enhance image quality
30. Check and record the image quality for physical abrasions, integrity of the array, and global intensity of the array features.
31. Click on Analysis -> auto align to align the grid to the image
32. Check and record alignment values. (note: lower left corner, this value should be <0.2.)
33. Check the green dots using navigation panel on the top left corner to check for correct alignment.
34. Save aligned image. (aligned image will have an “Aligned” description in the open image pop-up)
35. Align another image by clicking on File->New Images (instead of File->open)
36. Repeats step 3 to 11 for the rest of the unaligned images
37. Close NimbleScan
38. Feature Extraction
39. Open NimbleScan
40. Go to Analysis -> CGH -> segMNT…
41. Add all the aligned files from “part D”
42. **Make sure the data channels ratios is the correct one (usually 532/635)**
43. Deselect the following from “Choose the desired algorithm settings…”
    1. Averaging windows: 1x & 10x
    2. Generate GFF
    3. PDF Formats: Single panel, rainbow & Multi-panel
44. Choose the destination folder… (note: if the folder is changed, the software will not let you click Run. Repeat step 2-5 and the Run option should be active.)
45. Click on Run to generate…segMNT.txt
46. Close NimbleScan
47. Sample Tracking
48. Open NimbleScan
49. Go to Analysis -> Sample Tracking…
50. Add files - Add only the Green channel (532 file)
51. Check the destination file
52. Run. This will generate a stc.xls file. (**double check to make sure everything matches up**)
53. Close NimbleScan
54. Sources of Error for Part 2: NimbleScan to Burst, Align, and Extract Features
    1. Make sure to close and reopen NimbleScan after each process
    2. Bursted file is not aligned when running feature extraction
    3. Data channel might have switched
    4. Use the incorrect \*.ndf and \*.ncd file
    5. Cropping off part of the image
    6. Not saving image after each step
    7. Selection of the appropriate algorithm settings

# Part 3: NG & GGX file converter

***Note: Both software will overwrite existing file without any warning.***

1. NG file converter – converts \*.txt file to \*.udf file
2. Open “ngconverter.exe” file from the SGL\_files folder on the desktop
3. Choose the segMNT.txt file created from Feature Extraction
4. Click on open. (this will create \*.udf file and save in the same folder)
5. Repeat step 2-3 for the others files
6. Close ngcnoverter
7. GGXconverter.exe – converts \*.udf file to \*.ggx file
8. Open “ggxconverter.exe” from the same folder as part A
9. Choose \*.udf file that was created from part A
10. Click on open. (this will create \*.ggx file and save in the same folder)
11. Repeat step 2-3 for the others files
12. Close ggxconverter.exe
13. Sources of Error for Part 3: NG & GGX file converter
14. Not selecting the right file format for the right program
15. Will overwrite existing file without any warning

# Part 4: DNA Analytics for flagging aberrations

1. Import file
2. Open DNA Analytics from the desktop
3. Click on File -> Import -> Array files -> \*.udf files
4. Select the \*.udf file created from Part 3-A (make sure file name end in \*.udf before click open.)
5. “Select data type for experiments” pop-up: select log2 ratio from Data Type. Design type: cgh
6. Click on continue
7. “Universal Data Importer – Map column headers” pop-up:
   1. Mapping Info: select ggx to match headers
   2. Leave everything else as default
8. Click import (note: if you find an error here, Repeat steps 3-6 and make sure you import \*.udf file)
9. “UDF” pop-up: Select NO (on importing more UDF file for the same mapping)
10. “UDF Import Summary” pop-up: Lines skipped should be zero…Click ok to continue
11. Creating Interval Report
12. Open Experiment folders and select the imported file. (Folder on the left hand side under DNA.)
13. Right click on the file added and choose “Select Experiment” (***this step is important, make sure the right file is selected before moving on*)**
14. Click “Yes” on confirmation pop-up
    1. Click “No” on pop-up about saving the previous experiment
15. Double check Settings recommended:
    1. Aberration detection method
       1. Show: checked
       2. Algorithm: ADM-1
       3. Threshold: 10.0
    2. Go to Tools -> User preference -> miscellaneous
       1. Fuzzy zero: checked turn on
       2. Centralization: checked turn on. Threshold 10.0, Bin size 10
16. Go to Reports -> Text Aberration Summary:
    1. Report Type: Interval Based
    2. Output format: Complete Genome
    3. Select file location: example of file name: “SlideID\_Interval Report.xls”
    4. Click on save
17. Repeat Part A and B for the other \*.udf files
18. Close *without* saving
19. Sources of Error for Part 4: DNA Analytics for flagging aberration
20. Saving the wrong interval report for the wrong experiment
21. Importing the wrong file
22. Make the wrong selection for each pop-up screen
23. Overwrite interval report from previous experiment

# Part 5: Loading files into Genoglyphix

1. Log into www.genoglyphix.com
2. Click on Add Subject and enter subject information
3. Click on Add GGX file and select \*.ggx file from Part 3-B and the \*.xls file from Part 4-B-5.
4. Enter the unique slideID in the slideID box.
5. Click on submit data (this will upload the 2 files into the genoglyphix server)
6. Click on Process GGX files to create dataset once the upload is complete
   1. Repeat step 4-6 to add new dataset
7. Check and record the standard Deviation. (Cut off value should be <0.2)
8. Mark and initial Data reviewed on Genoglyphix.
9. Inform technologists that Data is ready for case review

REFERENCES

1. NimbleGen Arrays User’s Guild: CGH Analysis v5.1
2. Agilent G2565CA Microarray Scanner System User Guide
3. Agilent DNA Analytics 4.0 CGH Module User Guide
4. [www.genoglyphix.com](http://www.genoglyphix.com)

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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Procedure / Policy Title: Array Scanning and Processing Procedure

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