

Scanning CodeLink™ Bioarrays on GenePix 4000B Array Scanner



Key words: microarrays, bioarrays, gene expression, scanners, CodeLink™, GenePix 4000B microarray scanner

CodeLink™ Expression Bioarray System is a high-performance gene expression system that includes high-quality bioarrays (pre-arrayed oligonucleotide slides), reagents and optimized protocols, parallel processing kits and instrumentation, analysis software, and full product support.

GenePix™ 4000B Array Scanner from Axon Instruments, Inc., is an integrated scanner and software system for simultaneous single-slide, dual laser scanning in a small bench top platform. The scanner has been optimized for dual laser scanning at 532- and 635-nm wavelengths for the detection of two of the most commonly used fluorophores, Cy™3 and Cy5.

This application note describes a recommended scanning procedure and settings on GenePix 4000B Array Scanner for use with CodeLink™ Bioarrays.

Products Used

CodeLink™ Human Whole Genome Bioarray 300026-6pk

CodeLink™ Expression Analysis v4.0 Software 310030

Other Materials Required

- GenePix 4000B Array Scanner (Axon Instruments, Inc.)
- GenePix Pro 4.0 software (Axon Instruments, Inc.)
- GP-7, GP-8 PMT calibration slides (Axon Instruments, Inc.)
- Microarray Scanner Calibration slide FMB DS 01 (Full Moon Biosystems)

Protocol

For further details on the scanner configuration and operation, please refer to the manufacturer's user manual (1).

1. Preparation and Loading

1.1 Turn the scanner on using the switch on the left panel of the scanner.

Note: Wait at least 15 min for the lasers to stabilize.

1.2 Slide the cover to the left to expose the slide holder.

1.3 Lift the latch of the slide holder and lift the upper clip.

1.4 Wearing latex gloves, load the bioarray into the tray with the label side down and closest to the front of the scanner.

1.5 Pull the clip on the left of the bioarray out and let the bioarray fall into place. Release the clip to put pressure against the bioarray.

1.6 Grab bioarray by the edges and move the bioarray toward you.

1.7 Lower upper clip and press down on the latch until it clicks.

1.8 Slide the cover to the right to cover the slide holder.

2. Scanning

2.1 Open the GenePix software and select the following settings:

Bioarray Type	CodeLink UniSet	Whole Genome	Multiassay	SNP
Setting file name	Expression_10um.gps	Expression_5um.gps	Expression 16UP 5um.gps	SNP.gps
Wavelength	635 nm	635 nm	635 nm	635 nm
PMT gain	600 v	600 v	600 v	450 v
Laser power	100%	100%	100%	100%
Pixel size	10 µm	5 µm	5 µm	10 µm
Focus position	0 µm	0 µm	0 µm	0 µm
Lines to average	1	1	1	1

2.2 In the Reports tab, open the scanning output script by clicking **Scan CodeLink Slide**.

- 2.3 Enter the bioarray serial number and press **Next**. The Experiment and Scan Information interface is displayed.
- 2.4 Type in the project name, experiment name, and sample name. The user name is automatically captured. If a message box asks whether to allow an ActiveX interpretation to proceed, click **Yes**.
- 2.5 Refer to the table in Step 2.1 to select the correct setting (.gps) file. If a settings file was previously selected, the name and path are displayed under Current Settings File. To select a new file, click **Browse** under Select New Settings File. The values for project name, experiment name, user name, and setting file that were entered for a previous bioarray are retained but may be changed.
- 2.6 In the Load and Scan Slide screen, the standard TIF file name for the current bioarray is displayed but may be changed.
- 2.7 Click **Browse** and select the image path or the location where the image files will be stored.
- 2.8 Click **Scan Slide**. The Image tab will display, and the instrument will perform the scan.
- 2.9 When complete, the view will then return to the Reports tab.
- 2.10 Click **Save Image** to save the scanned image.
- 2.11 Slide the cover to the left and remove the bioarray. Handle bioarray as outlined in step 1.
- 2.12 To scan the next bioarray, click **New slide** and enter the serial number for the next bioarray. The setting information previously entered will be displayed.

Total CV		Probes within two-fold change	
CodeLink™ spec	GenePix 4000B	CodeLink spec	GenePix 4000B
<20%	10.48%	>95%	98.27%

Table 1. Typical CodeLink Expression Bioarray performance. CodeLink Human Whole Genome Bioarrays were hybridized with human brain cRNA with 1:300K spike level for Positive controls (PC) and scanned on GenePix 4000B array scanner. Observed CV values And minimum fold change results are averages obtained from a batch of three bioarrays. All observed values are compared with the corresponding CodeLink™ specification.

Expected Performance

Provided the scanner is property calibrated and the recommended scan settings are employed, CodeLink™ Expression Bioarrays are expected to perform according to specification. The results generated from the set of experiments performed in this application note (Table 1) were achieved with multiple instruments. Always contact your vendor for service to guarantee optimal scanner performance before scanning CodeLink™ bioarrays.

Results Summary:

- Field uniformity across the entire CodeLink™ array surface was well within 20%. An elevated nonuniformity value may be evidence of stage wear, and the vendor should be contacted for repair.
- Scan-to-scan reproducibility CV is less than 1%.
- Dynamic range was measured at 4 logs and over 3 logs for linear dynamic range.

- Scanner to scanner variability did not exceed 3-4% when monthly PMT calibrations were performed with GP-7 and GP-8 PMT calibration slides.

References

1. GenePix 4000B User's Guide, Axon Instruments, Inc., 2500-136 Rev E, (2001).

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