Scanning CodeLink™ Bioarrays on Agilent G2565 Microarray Scanner

Key words: microarrays, bioarrays, gene expression, scanners, CodeLink™, Agilent

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.

The Agilent™ miccoarray scanner uses laser-induced fluorescence to read bioarrays labeled with Cy™5 and Cy3 dyes. The scanner uses a circular carousel that can hold up to 48 slides. A slide must be mounted in the holder before inserting it into the carousel.

This application note describes recommended scanning procedures and settings on Agilent G256AA and G2565BA scanners for use with CodeLink™ Bioarrays.

Products Used

CodeLink™ Human Whole Genome Bioarray 300026-6pk

CodeLink™ Expression Analysis v4.2 Software 310030

Other Materials Required

- Agilent G2565BA microarray scanner (Agilent Technologies)
- Software v6.1 (Agilent Technologies)
- Microarray Scanner Calibration slide FMB DS 01 (Full Moon Biosystems)

Protocol

For users who are familiar with the Agilent G2565BA, the following protocol outlines the steps necessary to scan CodeLink™ Bioarrays. For further details on the scanner configuration and operation, please refer to the manufacturer's user manual (1).

1. Preparation and Loading

1.1 Open the cover of the B-type slide holder. Wearing latex gloves, place the bioarray into the holder with the active bioarray surface facing up and toward the cover. Lock the cover by gently pressing it down with one thumb.

Note: Fluorescent spots on the bottom surface increase the background noise and may effect the performance of Dynamic Focus feature, resulting in defective images and failure to complete the scan. Always wear gloves when handling bioarrays, and check the glass surface for non-conformities if this occurs.

1.2 Insert the slide holder into the carousel slot with the tapered end of the holder facing towards the center of the carousel. Close the carousel cover.

1.3 Place the carousel into the scanner.

2. Scanning

2.1 Open the **Modify Default Settings** window and select the following settings:

Bioarray Type	CodeLink UniSet	Whole Genome
Region (scan area)	61 x 21.6 mm	67.2 x 21.6 mm
Dye channel	Red	Red
Red PMT	70%	70%
Scan resolution	10 µm	5 µm

Note: Selecting an incorrect scan area my cause Dynamic Focus to fail due to the laser light scattering from the etched ID and logo.

2.2 Select automatic file name format (Prefix1_Prefix2_scan-number.TIF) as follows:

Prefix1 Instrument Serial Number
Prefix2 Barcode

2.3 Enable 90-degree image rotation by checking **Split and rotate TIFF image**.

2.4 Disable the **GEML** feature by deselecting **Attempt to retrieve from XML files**.

2.5 Click OK to close the **Modify Default Settings** window.

2.6 Open the **Edit Slot Values** window to enter the bioarray ID and the output folder for storing TIFF images for each CodeLink™ Bioarray and then select **Set Value.**

2.7 Select **Scan Slot** to start scanning.

Note: Saved images do not require manipulation for CodeLink™ analysis software if the recommended settings are used.

Results Summary:

- Field uniformity across the entire CodeLink™ array surface was well within 20%.
- Scan-to-scan reproducibility CVs near 1% was observed with multiple scans of the same slide.
- When the recommended settings were applied, each scan took about 16 min, and a high percentage of discovery probes were resolved above threshold in analysis.

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 a single instrument that was set up and calibrated by the vendor. Always contact your vendor for service to guarantee optimal scanner performance before scanning CodeLink™ bioarrays.

- Using a serial dilution calibration slide, dynamic range was measured at 3 logs with CVs better than 20% and over 3.5 logs with CVs better than 30%. This performance was observed for PMT settings above 50%. Good correlation with probe intensities and concordance between differential ratios was observed.

References

1. *Agilent G2565AA and Agilent G2565BA Microarray Scanner System (with SureScan technology) User Manual, Agilent Technologies, G2566-90007 Third Edition (2002).*

Total CV		**Probes within two-fold change**	
CodeLink™ spec	Agilent	CodeLink™ spec	Agilent
20%	9.9%	>95%	95.28%

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 control (PC) and scanned on Agilent G2565BA microarray 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 (CodeLink™ spec).