

Spot Morphology and Its Effect on Data Accuracy and Reliability

Key words: microarrays, bioarrays, gene expression, CodeLink™, spot morphology

CodeLink™ Expression Bioarray System is a high-performance gene expression analysis system for generating new insights into disease pathogenesis and drug discovery. Most of the elements, or spots, on CodeLink™ Bioarrays have a similar, characteristic donut-shape (Fig. 1). This shape is a direct result of the manufacturing process – a noncontact, piezoelectric deposition of oligonucleotide probe solution onto a 3-D polyacrylamide aqueous gel matrix surface. The dispensed oligonucleotide is initially dispensed over the entire drop area on the surface of the array, but it then becomes concentrated along the outer edge of the spot as it dries.

One likely explanation for this donut-like spot morphology is the common phenomenon of capillary flow and the transport of solutes to the periphery of an evaporating solution on a surface (1). This application note demonstrates that the donut-like morphology observed on CodeLink™ Bioarrays does not impact the accuracy of the data obtained from these spots. In fact, quantitation and comparison of expressed transcript levels using CodeLink™ Expression Bioarrays is extremely accurate and reliable.

Products Used

CodeLink™ UniSet™ Rat I Bioarray	25-6700-10
CodeLink™ Expression Assay Reagent Kit	25-6700-68
Cy™5-Streptavidin	25-0054-31
CodeLink™ Expression Analysis v2.3 Software	24-6700-69

Other Materials Required

- Rat brain total mRNA

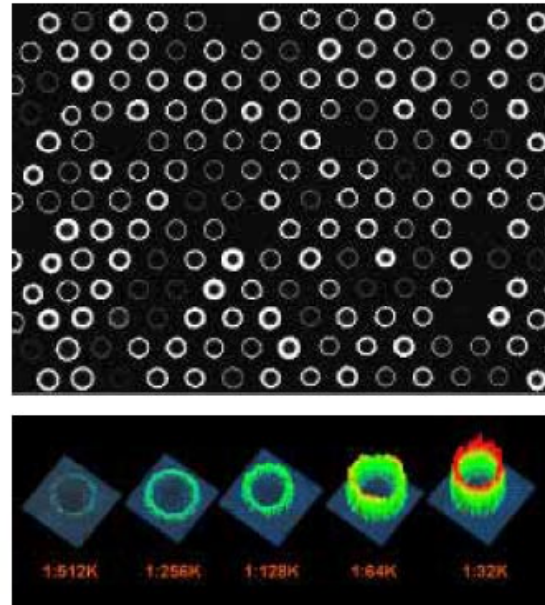


Fig. 1. Representation of spot morphology across target dilution series. Top: A representative region within a typical CodeLink™ Bioarray illustrates the consistent donut-like morphology.

Bottom: This is a three-dimensional pixel representation of a particular spot of five different target levels.

CodeLink™ analysis software is powered by technology from BioDiscovery Inc.

Method

Five synthetic bacterial mRNAs were added into rat brain mRNA at a mass to mass ratio of 1:500 (400 pg of spike in 200 ng of complex target). The size of each spiked poly(A)+ bacterial transcript was approximately 1200 bases. Biotinylated cRNA was prepared using CodeLink™ standard target preparation protocols. A non-spike control sample was also prepared for creating dilutions.

Two-fold serial dilutions were performed by diluting the 1:500 spiked cRNA with no spike cRNA. The lowest spike level created in this fashion represented a 1:512 000 (0.390 pg) bacterial-spike mass ratio to complex mRNA. Following the standard CodeLink™

hybridization protocol with Cy5-Streptavidin staining, three bioarrays were processed for the no-spike target and each of the spike dilution samples. In all, 36 CodeLink™ UniSet Rat I Bioarrays were processed.

Results

The morphology of all spots was examined among hybridizations with various amounts of target. The high concentration of oligonucleotide probe at the periphery of each spot draws increased binding of labeled target and gives the resulting donut-like shape to each hybridized spot. This is most obvious at lower concentrations of target material. As target concentration increases, the center of each spot appears to fill in as target begins to occupy the lower probe concentration center of the spot (Fig. 2). Spot morphology changes as a function of fluorescence, but consistency and accuracy are not compromised. With CodeLink™ Bioarrays, users can consistently detect a two-fold signal differences across at least three logs of target concentration changes. Therefore, the dose dependent response is linear and proportional across the entire range tested. Linear regression fitting of the average intensities across eleven concentrations shows an average R^2 of 0.98 (Fig. 2). Accuracy of calculated changes in transcript abundance based on spot intensity is also not affected (Fig. 3). The average fold change was calculated to be 1.8, or 10% the expected value of 2.0. The values were very constant from point to point even when measuring between two spots with visually different morphologies.

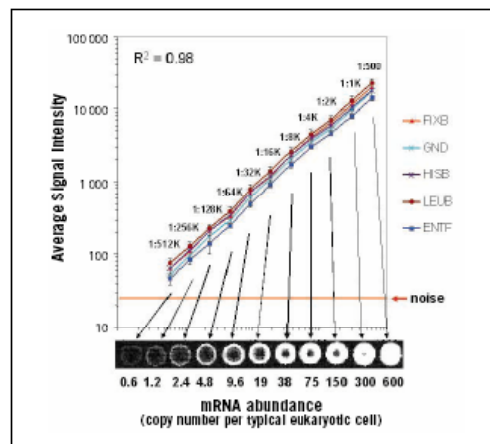


Fig. 2. Differences in spot morphology do not affect bioarray performance.

Each line represents an individual spiked bacterial mRNA transcript. Each data point represents the average signal intensity and standard deviation (error bars) for a given spike mass ratio, obtained from 18 values (three bioarrays with a 6x-probe redundancy per bioarray). The red line represents 95% confidence level of the no spike.

Other reports on array manufacturing have indicated the presence of a donut-like spot morphology, and in one report by Pat Brown, he confirms that this type of spot morphology does not compromise data quality (2). Furthermore, CodeLink™ Expression Bioarray field performance consistently exceeds industry standards (Table 1)(3). Because of the high quality of CodeLink™ Bioarray manufacturing, pre-synthesized and quality control validated probes, and the proprietary 3-D aqueous gel matrix surface, the precise distribution of probe and accessibility of the target is highly consistent. This, coupled with superior spot-finding and signal segmentation analysis software, yields high quality and reproducible gene expression analysis.

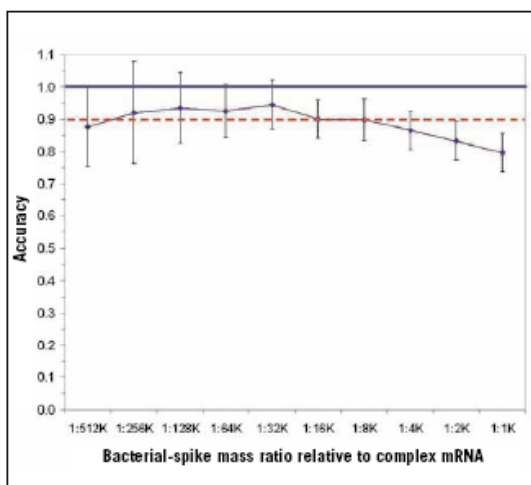


Fig. 3. CodeLink™ Bioarrays are accurate across three logs of target concentration.

This high level of accuracy is retained across all three logs of spike concentration tested. Accuracy is defined as the observed ratio divided by the expected ratio, 2.0 between each spike level. Since each spike level is represented in triplicate, nine combinations of slide-to-slide comparisons are made for all 90 probes, between spike levels. Each data point represents the average and standard deviation (error bars) obtained from 870 individual ratios. The blue line represents expected or target accuracy of 100%. The red-dashed line presents the average accuracy, which is 90% of expected. The x-axis lists the lower of the two spike levels used to make the ratio.

Table 1. Typical field performance of CodeLink™ Expression Bioarray System.

Sensitivity	< 1 transcript per cell
Specificity	90% probe to target sequence identity
Dynamic range	> 3.0 logs
Signal reproducibility (total CV)	< 16% CV
Minimum detectable fold change	95% within 1.5-fold, 98% within two-fold

References

1. Degan, R., *et al.* Capillary flow as the cause of ring stains from dried liquid drops. *Nature* 389, 827-829 (1997).
2. Eisen, M. and Brown P. DNA arrays for analysis of gene expression. *Method Enzymol.* 303, 179-205 (1999).
3. Stafford, P. *et al.* High-quality microarray data using CodeLink Bioarray Platform, *Life Science News* 13, 4-6 (2003).

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