

CodeLink ADME Rat 16-Assay Bioarray:

Multi-assay bioarray processing for higher throughput and robust experimental results from focused bioarrays

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Introduction

CodeLink™ Expression Bioarrays are part of a system solution for high quality, reproducible gene expression profiling. CodeLink ADME Rat 16-Assay Bioarray features a new multi-assay chamber that allows up to 16 samples to be hybridized, washed, and detected in parallel on a single slide (Fig 1).

- 16 identical arrays spotted on each bioarray slide and separated by individual hybridization chambers.
- Increased throughput in a robust assay that can be automated. With three bioarrays used together on a microtiter tray, 48 arrays can be processed as a single unit, making this format ideal for automation. Especially for high-volume microarray users that require flexibility for custom assays, this latest CodeLink Bioarray format offers increased throughput with robust assay performance.
- Open system format. The 16-assay bioarrays can be detected by scanning using commonly available microarray scanners. Images generated can be easily analyzed with CodeLink Expression Analysis v4.0 software and the data further visualized and mined with commercially available microarray data analysis applications (Fig 2).

Dynamic Range (Fig 5) - Rat Liver (LVR) cRNA samples were generated including 6 cRNA spiked bacterial transcripts at multiple concentrations (38 fM-38 pM). Eight bioarrays tested the dynamic range of the new platform where there are three replicates for each bacterial transcript for each array. Assays were performed according to standard 16-assay protocol using Cy™5-Streptavidin and were analyzed using CodeLink Expression Analysis v4.0 software.

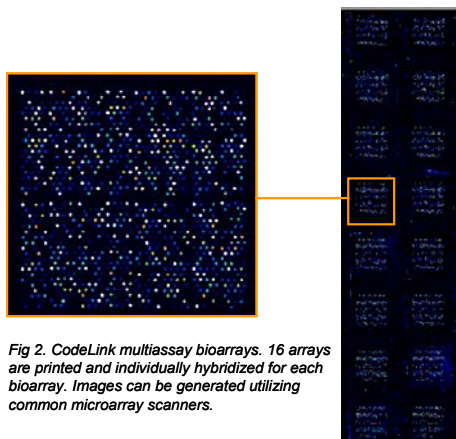


Fig 2. CodeLink multiassay bioarrays. 16 arrays are printed and individually hybridized for each bioarray. Images can be generated utilizing common microarray scanners.



Fig 1. CodeLink multiassay bioarray chamber allows for processing of up to 16 arrays and samples in parallel on a single slide.

Materials and methods

CV Reproducibility (Fig 3) - Mouse Heart (HRT) cRNA sample was generated and tested across 32 internally designed bioarrays (2 slides) to determine CV variations from array-to-array.

Array Plot Reproducibility (Fig 4) - Rat Brain (BRN) and Rat Liver (LVR) cRNA were generated and tested across 16-assay (n=24 array) and 1-Assay Flex Chamber (N=36 array) Bioarrays. 16-Assay ADME bioarray content was used with two different chambers types. Assays were performed according to each platform's standard protocol using Cy™5-Streptavidin and were analyzed using CodeLink Expression Analysis v4.0 software.

Optimized Assays for High Array to Array Reproducibility

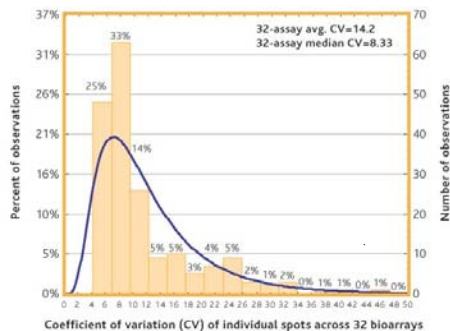


Fig 3. As shown here, Median CVs are considerably below the acceptable level with the majority of CodeLink spots within 4-12% Median CV across 32 arrays.

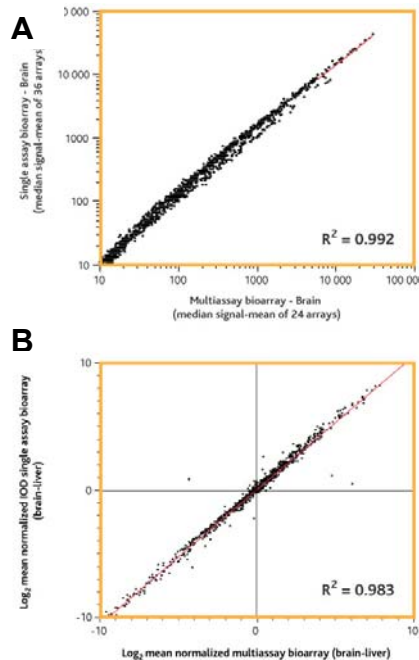


Figure 4. High correlation between expression data generated with a CodeLink single assay bioarray and CodeLink multiassay bioarray. CodeLink multiassay bioarrays exhibit high signal and expression ratio correlation with the CodeLink single assay bioarrays. Panel A: Signal reproducibility between two array formats. Panel B: Differential ratio reproducibility between the two bioarray formats.

Substrate Chemistry for high sensitivity

CodeLink Bioarray is a 3-D polyacrylamide aqueous gel matrix, which immobilizes amine-terminated oligonucleotide probes, allowing for greater target access to probes and better sensitivity—a key benefit over 2-D matrix. For the 16-assay bioarrays, the new CodeLink high density substrate with improved 3-D aqueous gel matrix enables efficient interaction between probe and target. This is the same substrate used in CodeLink's whole genome bioarrays. With this new substrate, sensitivity of detection is as low as 1:1 024 000 mass ratio with a dynamic range linearity of response observed to be over three logs of concentration (38 fM pM – 38 pM) by spiking experiments at the cRNA level for enhanced detection of low abundance transcripts (Fig 5).

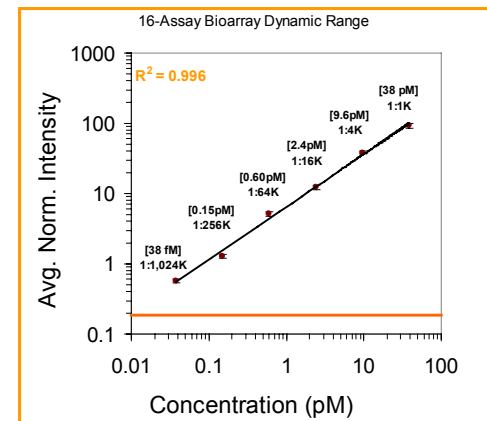


Figure 5. CodeLink ADME Rat 16-Assay show sensitivity of detection down to 1:1 024 000 mass ratio as determined by cRNA spiking experiments. Linearity of response is observed over three logs of concentration (38 fM – 38 pM). Error bars are +/- 1 stdev, N=24 data points (3 target replicates across 8 ADME arrays). The orange line represents the lower limit of detection, which is the upper 99th percentile of the negative controls.

CONCLUSION:

CodeLink 16-Assay Bioarrays enable sensitive, accurate and precise expression profiling for each of the 16 ADME Rat Bioarrays across a single slide.

Demonstrated performance:

	Percent of Observations	Typical performance Benefit
Sensitivity	mass ratio < 1:1 000 000 with bacterial spiking into cRNA and total RNA level	Detection of more low abundance genes (< 0.3 copies per cell)
Dynamic range	Linear signal response of three orders of concentration magnitude (38 fM – 38 pM)	More usable/accurate data across a broad range of gene expression levels
Signal reproducibility	Typically < 15% total median CV among batches, all probes included; ~8% median CV among production batches, only probes above noise included	Reproducible differential, expression results in every experiment (Fig 3)

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