Consistent Assay Performance Across Universal Arrays and Scanners

There are multiple Universal Array and scanner options for running Illumina DASL® and GoldenGate® Assay-based products. All substrates and platforms have been tested thoroughly and generate high-quality results equivalent to previously benchmarked standards.

FLEXIBLE TECHNOLOGY

Since Illumina's inception in 1998, the company has leveraged the inherent flexibility of BeadArray™ technology to build a broad portfolio of highly multiplexed genotyping and gene expression profiling products for genetic analysis. Now, for several assay types (genotyping, DNA methylation, gene expression, and miRNA expression), researchers can choose from a variety of array substrates and scanner platforms. No matter what experimental designs or sample sizes are at hand, there is an optimal platform and workflow to support it.

It is obviously essential that all product options generate consistent data and perform equally well to be truly useful in the research setting. Illumina scientists rigorously test all products during development to ensure that researchers can be confident of the data quality. This technical note describes some of these experiments and shows examples of data and conclusions from tests of Universal BeadChips and the Universal Array Matrix using the BeadArray Reader and iScan™ System.

Assays

Four assays utilize the Universal BeadArray substrates:

- GoldenGate Genotyping Assay
- GoldenGate Assay for Methylation
- DASL Expression Profiling Assay
- MicroRNA Expression Profiling Assay

Universal Arrays

There are currently two types of universal BeadArray substrates to choose from. Both make use of the high density self-assembly of oligonucleotide-coated beads, but their unique form factors enable different sample throughput and workflow options. The Universal-12 and Universal-32 BeadChips are 12- and 32-sample slide-sized substrates, while the Universal Array Matrix supports processing 96 samples at a time (*Figure 1*). The Universal-32 BeadChip is only available for processing GoldenGate Genotyping Assays. A core feature common to all universal BeadArray substrates is a high level of feature redundancy, which helps drive the high data quality and consistency generated by Illumina products.

FIGURE 1: UNIVERSAL BEADCHIPS AND ARRAY MATRIX

The Universal-12 and Universal-32 BeadChips (left) and 96-sample Universal Array Matrix (right) offer flexible workflow options with Illumina assays.

Scanners

The results from any BeadArray-based assay type can be read on either of Illumina's scanner platforms when the appropriate substrate is used. Universal-12 and -32 BeadChips can be read by the iScan System or BeadArray Reader (the older Universal-16 BeadChip is only compatible with BeadArray Reader). The Universal Array Matrix can only be scanned by the BeadArray Reader. Both scanner systems generate reproducible high-quality data in combination with Illumina assays due to their high sensitivity, dynamic range, and signal-to-noise ratios. The principal difference between the two platforms is the significantly higher throughput rate using the iScan System. The newer iScan System incorporates cutting-edge optics and detection systems to dramatically speed up scan times and provide higher resolution imaging.

CONCORDANT RESULTS

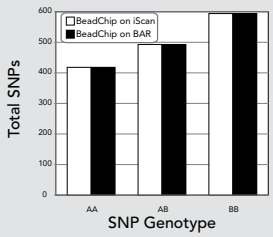
Of primary interest during testing of new substrates and platforms is the confirmation that new products perform equivalently to previous products that have been repeatedly tested for years both internally and in labs around the world. Although it is not recommended to combine sample data across platforms into one project, these experiments are useful for technology comparison purposes. Generally, any workflow changes to a highly precise assay have the potential for shifting its performance. However, Illumina scientists and engineers have been successful in ensuring equivalent performance *(Figure 2)* on the Universal Array Matrix and Universal BeadChips (r^2 = 0.944–0.981). Likewise, performance is equivalent when a Universal BeadChip is imaged with either scanner (r^2 = 0.984–0.994), as measured by the correlations between the two measurements. Genotyping results are discrete (AA, AB, or BB), so a slightly different analysis of concordance between calls is required, which shows consistency between platforms of greater than 99.7%.

It is also important for an assay system to generate consistently reproducible measurements on a given platform. Reproducibility is crucial to having the power to precisely identify relatively minor genetic variations contributing to disease. Here again, replicates of all four assays run on the Universal BeadChip are highly repro-

FIGURE 2: ASSAY CONCORDANCE BETWEEN PLATFORMS

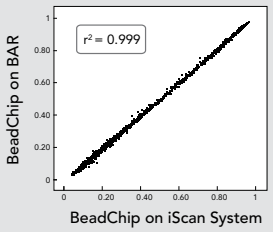
A: GOLDENGATE GENOTYPING (n = 16)

	U-12 ON ISCAN	U-32 ON BAR	U-32 ON ISCAN	UAM ON BAR
U-12 ON ISCAN	100.00%	99.76%	99.80%	99.85%
U-32 ON BAR		100.00%	99.88%	99.76%
U-32 ON ISCAN			100.00%	99.80%
UAM ON BAR				100.00%



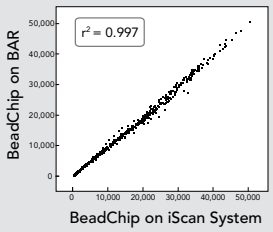
B: GOLDENGATE ASSAY FOR METHYLATION (n = 17)

	U-12 ON ISCAN	U-12 ON BAR	UAM ON BAR
U-12 ON ISCAN	0.989	0.984	0.982
U-12 ON BAR		0.987	0.981
UAM ON BAR			0.991



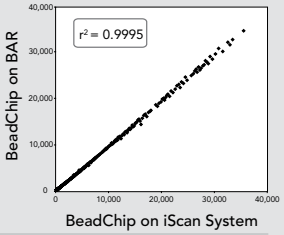
C: DASL GENE EXPRESSION PROFILING (n = 6)

	U-12 ON ISCAN	U-12 ON BAR	UAM ON BAR
U-12 ON ISCAN	0.992	0.992	0.944
U-12 ON BAR		0.992	0.944
UAM ON BAR			0.979



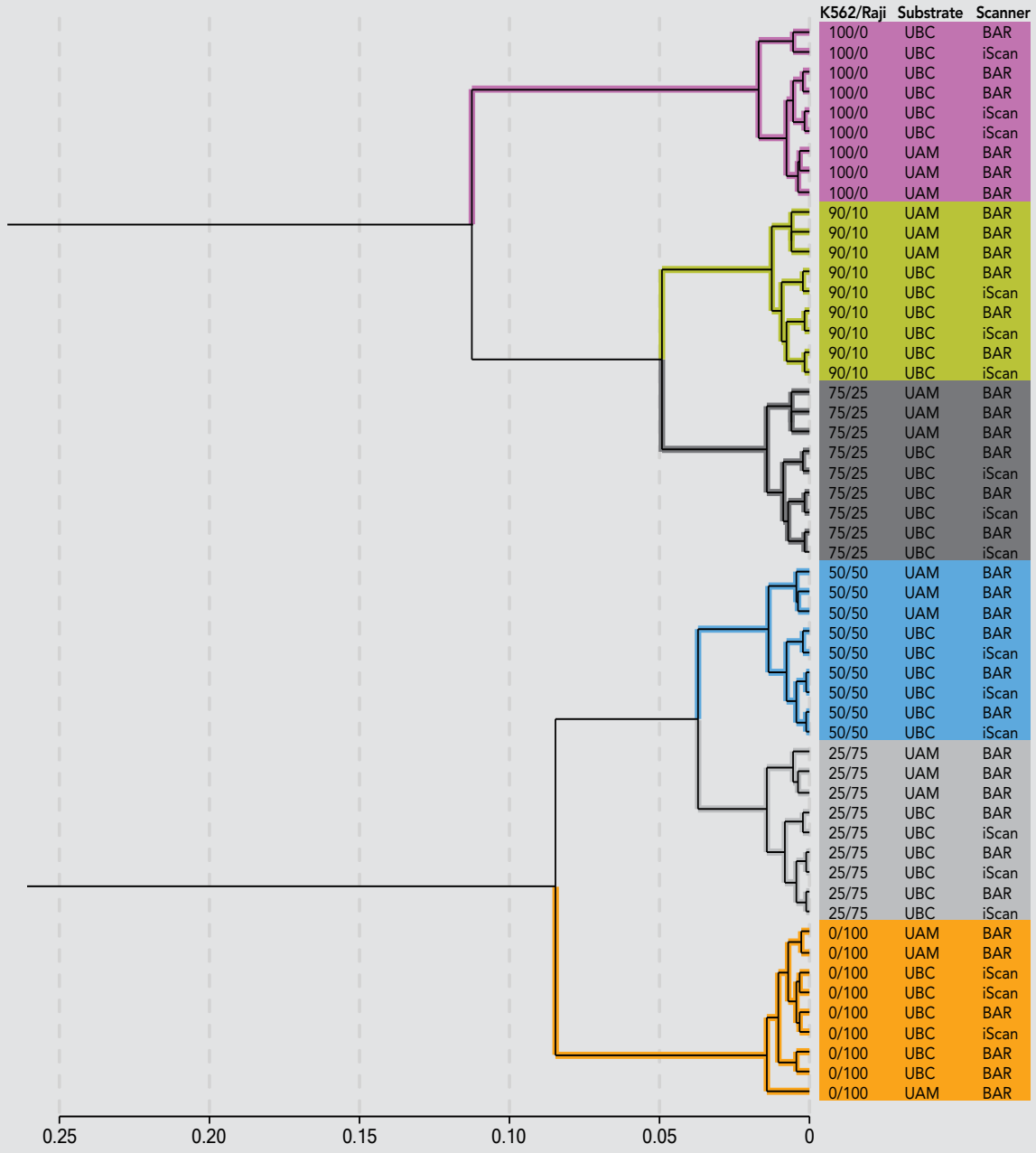
D: MICRORNA EXPRESSION PROFILING (n = 36)

	BC ON ISCAN	BC ON BAR	UAM ON BAR
BC ON ISCAN	0.994	0.994	0.974
BC ON BAR		0.994	0.973
UAM ON BAR			0.989



For all four assay types making use of the Universal Array substrates, inter-platform concordance and technical replicate concordances are shown in the matrices at left. Each value is the mean r^2 correlation (or genotype concordance) of multiple replicates. Example plots used to derive these data are shown on the right for each assay type.
U-12 = Universal-12 BeadChip, U-32 = Universal-32 BeadChip, UAM = Universal Array Matrix, BAR = BeadArray Reader

FIGURE 3: METHYLATION SIGNALS CLUSTER ACCORDING TO BIOLOGICAL REPLICATES



Replicate samples of Raji and K562 cell line extracts mixed at varying proportions were run with the GoldenGate Assay for Methylation using the Universal Array Matrix (UAM) or Universal-12 BeadChip (UBC) and scanned using the BeadArray Reader (BAR) or iScan System. Percentages of Raji and K562 extract content in the sample are listed in the first column of text, and the substrate and scanner are listed in the rightmost two columns of text. The dendrogram resulting from this analysis is depicted on the left, showing that replicates cluster closely and biological differences are easily discernible. Lines are color coded according to sample mixture composition.

TABLE 1: IDENTICAL GOLDENGATE GENOTYPING SPECIFICATIONS FOR ALL UNIVERSAL ARRAYS

	UNIVERSAL BEADCHIPS	UNIVERSAL ARRAY MATRIX
Call Rate	> 99%	> 99%
Reproducibility	> 99.9%	> 99.9%
Mendelian Inconsistencies	< 0.1%	< 0.1%

ducible, when using either the BeadArray Reader or iScan System (r^2 = 0.974–0.992 and 100.00% genotype concordance, *Figure 2*).

HIGH-QUALITY DATA

In addition to assay reproducibility and concordance between platforms, individual assays have more specialized performance metrics that predict the quality of data generated in an experimental setting. Illumina scientists ensure that all products perform to the highest levels in these tests as well.

GoldenGate Genotyping Assay

The GoldenGate Assay deployed on BeadChips continues to exceed the standard specifications for average call rate (> 99%), reproducibility (> 99.9%), and Mendelian inconsistencies (< 0.1%), regardless of whether the iScan System or BeadArray Reader is used and for any Universal Array *(Table 1)*. High call rates ensure that important SNPs are included in analyses. High reproducibility and low Mendelian inconsistency indicate high genotype call accuracy.

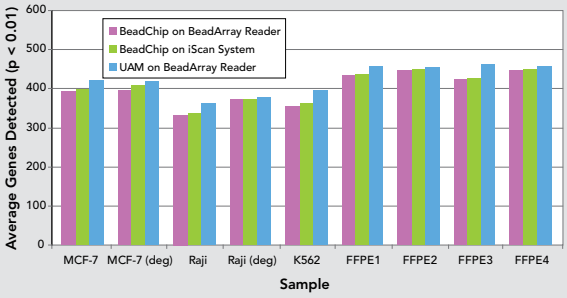
GoldenGate Assay for Methylation

When profiling the methylation status of various biological samples, it is crucial that any differences seen are the result of biological differences, not experimental artifacts. As confirmation of the robustness of the GoldenGate Assay for Methylation to differences in platform, scientists mixed two cell lines in varying proportions. The signals clustered according to their biological composition with very little systematic difference seen between different substrates or scanners used *(Figure 3)*.

DASL Expression Profiling Assay

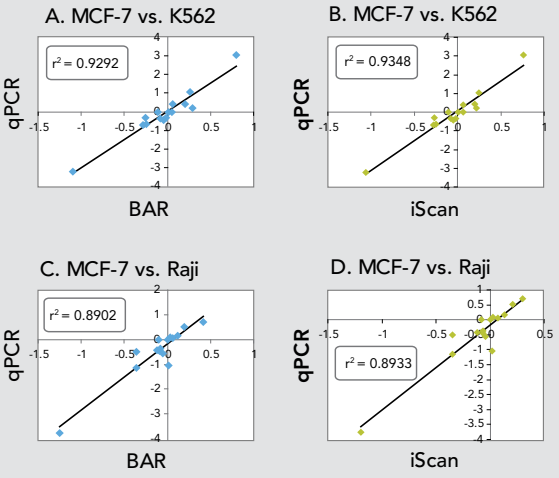
Ensuring that a high number of genes are detected by an expression profiling assay is important for confirming that the assay is performing as expected. Performance depends on a high signal-to-noise ratio and is efficient generation of data from biological samples. In addition, when FFPE samples are used, the assay must be robust

FIGURE 4: EQUIVALENT NUMBER OF GENES DETECTED USING DIFFERENT PLATFORMS AND SCANNERS WITH DASL ASSAY



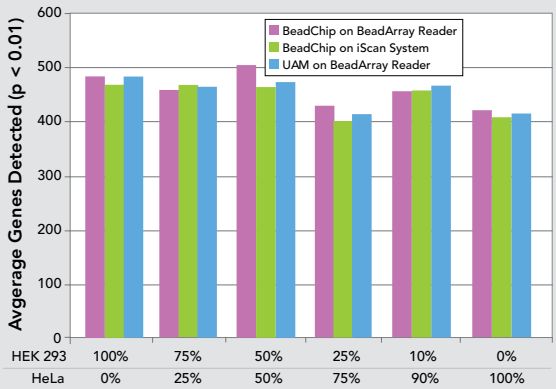
Several cell line extracts, artificially degraded cell line extracts (deg), and FFPE tissue samples were run with the DASL Expression Assay using the Universal Array Matrix (UAM) or Universal-12 BeadChip and scanned using the BeadArray Reader or iScan System. For each sample type, the average number of genes detected (n = 2–6 per sample, p < 0.01) is shown for each of three conditions. There is very little difference between results from different conditions.

FIGURE 5: HIGH CONCORDANCE OF FOLD-DIFFERENCE DETECTION WITH QPCR



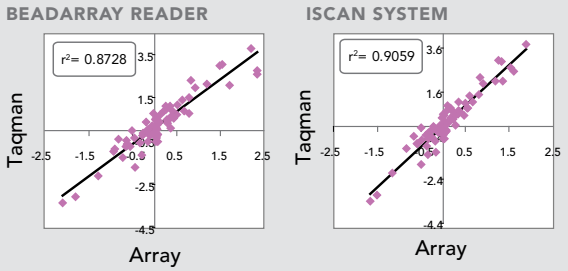
Fold difference of expression was determined for a panel of 15 genes between extracts of MCF-7 and K562 (A and B) or Raji cell lines (C and D) with both the DASL Assay and qPCR. Plots show concordance between the two assays when either the BeadArray Reader (A and C) or iScan System (B and D) were used to scan the arrays that had been run using the DASL Assay.

FIGURE 6: CONSISTENT NUMBER OF MIRNAS DETECTED USING DIFFERENT PLATFORMS AND SCANNERS



Total RNA from HEK293 and HeLa cell lines were mixed in various proportions. These samples were run on the microRNA Profiling Assay using the Universal Array Matrix (UAM) or Universal-12 BeadChip and scanned using the BeadArray Reader or iScan System. The graph shows the average number of genes detected at a threshold of $p < 0.01$. There is very little difference between results from different substrates or scanners.

FIGURE 7: QPCR CONCORDANCE



The two plots show the concordance between data from Illumina microRNA Profiling Assay and qPCR for 12 miRNA genes from four human samples (six combinations). Concordance is equivalently high when the assay is scanned on the BeadArray Reader (left) or iScan System (right).

to effectively detect gene expression signals from the degraded sample. The DASL Assay shows similar numbers of genes detected from cell lines or FFPE samples when either array substrate is used, or when BeadChips are run on either scanner platform *(Figure 4)*.

To further validate the accuracy of the DASL Expression Assay with an independent methodology, Illumina scientists confirmed that the DASL Assay results for fold differences between two cell lines are highly concordant with qPCR results on a panel of genes. The high concordance with qPCR holds for either array substrate or for Universal BeadChips on either scanner platform *(Figure 5)*.

MicroRNA Expression Profiling Assay

In similar fashion to the analysis for the DASL Assay above, Illumina scientists verified that the microRNA Profiling Assay detects similar numbers of genes in replicate samples (*Figure 6*). Comparison with qPCR serves as an orthogonal confirmation of the accuracy of the microRNA Profiling Assay, which is consistent on either scanner (*Figure 7*). Furthermore, three more important assay parameters are the same when using either Universal Array or either scanner: fold-difference detection limit (1.2–1.3-fold), system limit of detection (10^5 molecules in the background of 200 ng total RNA), and dynamic range (approximately 4 logs).

SIMILAR WORKFLOWS

Not only are the results similar, the assay workflow procedures and durations are nearly equivalent when using any Universal Array substrate (*Table 2*). In terms of hands-on time, processing 96 samples with BeadChips is

TABLE 2: GOLDENGATE WORKFLOW DURATIONS ARE NEARLY IDENTICAL WHEN USING BEADCHIP OR ARRAY MATRIX

UNIVERSAL-32 BEADCHIP*		STEP	UNIVERSAL ARRAY MATRIX	
TOTAL DURATION	HANDS-ON TIME		TOTAL DURATION	HANDS-ON TIME
1:45	1:00	MAKE ACTIVATED DNA	1:45	1:00
3:15–16:15	0:50	EXTEND AND LIGATE	3:15–16:15	0:50
3:30	0:46	UNIVERSAL PCR	3:30	0:46
16:40	1:40	HYBRIDIZE	15:15	1:45
1:30	0:15	IMAGE†	2:00	0:30
26:25–39:25	**4:31**	TOTAL	25:45–38:45	**4:51**

* based on running three BeadChips at a time using the iScan System
† includes scanning and other processing steps

TABLE 3: SCAN SETTINGS FOR UNIVERSAL ARRAYS

SCANNER PLATFORM	ARRAY SUBSTRATE	REQUIRED SCAN SETTING			
		GOLDENGATE GENOTYPING	GOLDENGATE METHYLATION	DASL	MICRORNA
iScan System	Universal-12 BeadChip	Universal BC (GGGT, Methylation, DASL)			miRNA
	Universal-32 BeadChip	Universal X (GGGT)	N/A		
BeadArray Reader	Universal-12 BeadChip	Universal BC (GGGT, Methylation)		Universal BC (DASL)	miRNA
	Universal-32 BeadChip	Universal X (GGGT)	N/A		
	Universal Array Matrix	GoldenGate Genotyping		DASL Gene Expression	Direct Hyb Gene Expression

15–20 minutes faster than with a Universal Array Matrix. In sum, processing the GoldenGate Genotyping Assay on BeadChips and imaging with the iScan System takes a negligible hour or less longer total during the three day process.

Scan Setting Differences

A result of the high consistency between Illumina assays is that the iScan System only requires fewer scan settings for all four assays. The one-color microRNA Profiling Assay uses the "miRNA" scan setting, whereas the other two-color assays use the "Universal BC (GGGT, Methylation, DASL)" scan setting. The Universal-32 BeadChip uses a separate "Universal X (GGGT)" scan setting on either scanner. The scan settings required with the Universal-12 BeadChip on the BeadArray Reader are different *(Table 3)*. The BeadArray Reader uses the separate "Universal BC (DASL)" scan setting for DASL Assays and the combined "Universal BC (GGGT, Methylation)" for GoldenGate Genotyping or Methylation Assays.

SUMMARY

The data above are a brief review of some of the testing Illumina has undertaken to ensure equivalent and high-quality performance of all genetic assays, independent of the array substrate or scanner used. With this information in hand, researchers can confidently use the products that best fit their study design and workflow.

ADDITIONAL INFORMATION

Visit www.illumina.com or contact us at the address below to learn more about Illumina genetic analysis assays and platforms.

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