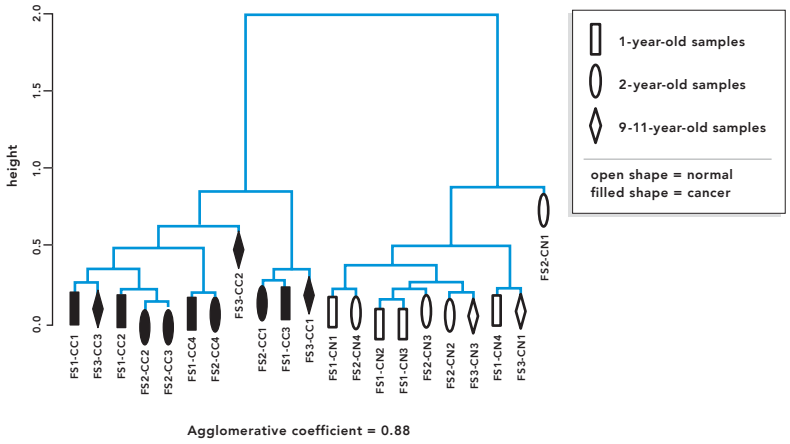
DASL® Assay for RNA Profiling with Paraffin-Embedded Samples

The DASL Assay can streamline discovery processes, allowing researchers to generate expression profiles for target biomarkers from degraded samples and correlate those profiles with known clinical outcomes. The DASL Assay, together with Illumina BeadArray™ technology, provides a high-multiplex, low-cost microarray solution for gene expression analysis.

HIGHLIGHTS OF THE DASL ASSAY

- Reproducible RNA Profiling: profile partially degraded RNA
- Proven Sensitivity: detect low-abundance transcripts
- Ideal for Clinical Sample Types: input of just 100 - 200ng total RNA
- Flexible Formats: process in groups of 16 or 96 samples
- Scalable Solution: automatable platform with low, per-sample pricing

FIGURE 1: CLUSTER ANALYSIS OF PATIENT SAMPLES



When cancer biomarkers are profiled by the DASL assay, FFPE clinical samples cluster consistently by disease type, regardless of sample age (1-11 years old).

INTRODUCTION

Illumina's c**D**NA-mediated **A**nnealing, **S**election, extension and **L**igation—the **DASL** Assay—is part of a powerful gene expression solution designed to generate reproducible RNA profiles from degraded tissue samples such as formalin-fixed, paraffin-embedded (FFPE) tissues (*Figure 1*).

It has been estimated that over 400 million FFPE tissue samples have been archived in North America for cancer alone. Many of these samples represent clinical outcomes — a potential gold mine of information when linked with underlying expression profiles. This is an exciting prospect for the validation and testing of biomarkers associated with cancer or other complex diseases. To date, these samples have been reliably assayed only with expensive, low-multiplex qPCR approaches. Illumina's DASL Assay opens a new avenue for reproducible RNA profiling at high multiplex levels at a low cost per sample (*Figure 2*).

FIGURE 2: ARRAY PLATFORMS

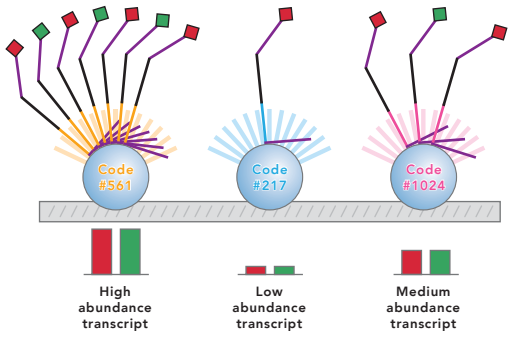
The DASL Assay can be processed on two different array platforms: the Sentrix® Array Matrix or the Sentrix BeadChip to process 96 or 16 samples, respectively.

DASL VS. DIRECT HYBRIDIZATION APPROACHES

Direct hybridization approaches, combined with modifications of the Eberwine protocol[1], have been effective when working with intact RNA. With this method, transcript-specific sequence hybridization occurs on

the array. In addition to intact RNA, the DASL Assay has been designed to also interrogate partially degraded RNA. The assay is performed in solution against cDNA, and only target products are amplified. These amplicons are then hybridized to universal sequences on the arrays. Because the amplicons are of uniform length, the assay maintains an unbiased representation of transcript abundance[2]. The DASL approach delivers specificity, sensitivity and array-to-array reproducibility unachievable with array-based direct hybridization approaches for degraded RNA.

FIGURE 4: HYBRIDIZATION OF TEMPLATES TO ARRAY FOR INTENSITY READOUT.



Hybridization of labeled, universal-address-containing amplicons to complementary universal sequences immobilized to beads on the arrays.

HOW THE DASL ASSAY WORKS

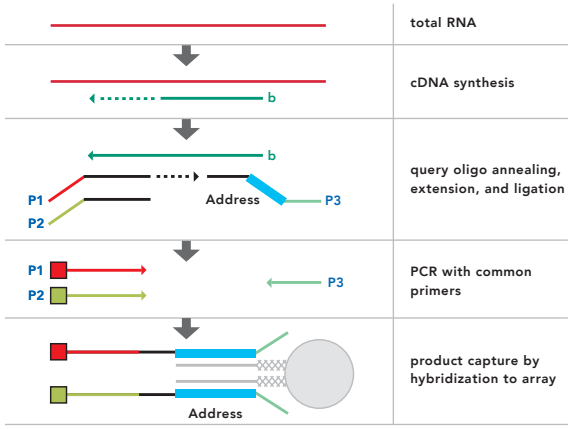
The DASL Assay monitors gene expression with probe groups to query total RNA-generated cDNA target sequences (*Figure 3*). Primer sites, incorporated within each probe group, are used for PCR amplification. Also within the probe group is an address sequence complementary to one of the 1536 sequences on the Sentrix® Array Matrix. Although a single probe group per gene can be used, more probe groups allow detection of even lower fold-changes between samples.

Total RNA is converted to cDNA using both biotinylated random nonamers and biotinylated oligo dT. Probe groups are annealed to the biotinylated cDNA, followed by selection of the duplexes on streptavidin beads to remove unhybridized oligos. Correctly annealed, assay-specific oligos are extended and ligated to a locus-specific oligo. The locus-specific oligo incorporates an address sequence and primer site for the generation of amplifiable products.

Templates are labeled during PCR amplification and subsequently hybridized to the array (*Figure 4*). The array is then scanned to acquire intensity data.

At the core of the DASL Assay is Illumina's GoldenGate® Assay, a robust protocol used broadly for high-throughput genotyping applications. To enable future applications (e.g. allele-specific expression) using the same biochemical approach, the DASL Assay is configured to use both Cy3 and Cy5 fluorescence when amplifying non-polymorphic sequences.

FIGURE 3: RNA PROFILING WITH THE DASL ASSAY



OLIGO DESIGN STRATEGY

In the DASL Assay, query oligos are designed to probe exonic sequences. As a result, genomic DNA may be used as a positive control sample. Since genomic DNA consists of essentially equimolar representation of all sequence targets, the performance of any individual assay can be assessed and verified to decrease false negatives.

Oligo probes for both standard expression panels and custom sets are subjected to a rigorous bioinformatics

TABLE 1: REPRODUCIBILITY METRICS

INTACT RNA

Reproducibility Among Replicates		
	Probe Level	**Gene Level**
Within Array Matrix	0.990	0.994
Across Array Matrices	0.980	0.986
Within BeadChip	0.990	0.995
Across BeadChips	0.985	0.992
Array Matrix vs BeadChip	0.972	0.983

FFPE-DERIVED RNA

Reproducibility Among Replicates		
	Probe Level	**Gene Level**
Within Array Matrix	0.975	0.991

screening procedure. This process assesses each target site for specificity in the transcriptome (RefSeq) and the genome, and also assesses predicted behavior in DASL biochemistry.

Because the probe groups span only about 50 bases, partially degraded RNA can be used in the assay. The availability of additional, unique probe sequences per gene increases the sensitivity of the assay, which allows the quantitation of low-abundance transcripts, even in partially degraded samples.

DASL ASSAY PERFORMANCE

The DASL Human Cancer Panel queries 502 cancer genes with unique probe groups for three different sites per gene[3]. Performance data using Illumina's standard DASL Human Cancer Panel, including reproducibility and differential expression, are discussed in the text that follows.

Reproducibility

The DASL Assay demonstrates excellent reproducibility between intact RNA sample replicates at the individual probe level as well as the gene level. Results from FFPE-derived RNA samples show excellent reproducibility at the probe level; gene-level reproducibility is equivalent to intact RNA *(Table 1)*. Cell-specific RNA expression was evaluated with the DASL Assay. A minimum 1.15-fold difference (with 95% confidence) was observed.

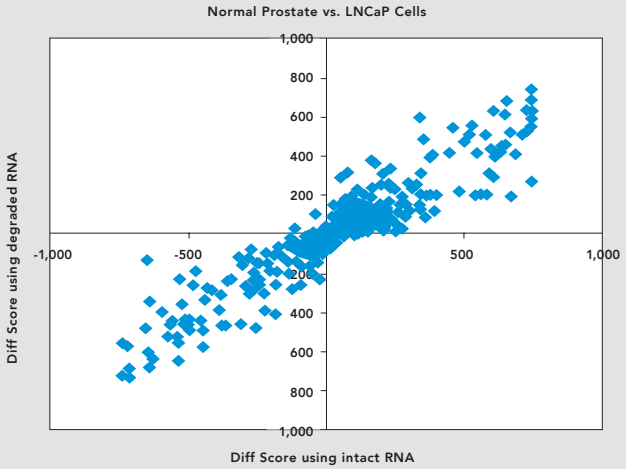
Differential Expression: Intact vs. FFPE RNA

Differential expression results from FFPE-derived RNA was compared to that of intact RNA. Expression profiles from partially degraded RNA in FFPE samples reproduced biological differences found in intact RNA *(Figure 5)*.

Application to Clinical Samples

To demonstrate the capability of the DASL Assay to properly classify FFPE clinical samples, RNA from cancerous breast tissues, cancerous colon tissues and normal tissues were analyzed. The data show dramatic differences in expression when comparing normal to diseased samples as well as excellent reproducibility[3].

FIGURE 5: DIFFERENTIAL RNA EXPRESSION

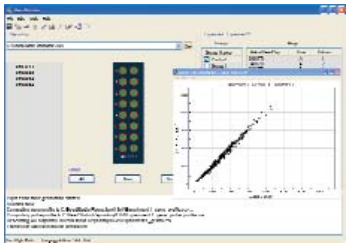


Differential expression of intact RNA compared to degraded RNA samples in normal prostate vs. LNCaP cells.

EASY-TO-USE SOFTWARE FOR DATA ACQUISITION AND ANALYSIS

Illumina's BeadStudio software features a highly intuitive interface for detection and data acquisition of samples hybridized either to the Sentrix Array Matrix or to the Sentrix BeadChip (*Figure 6*). A set of analysis tools provides novel approaches for characterizing, measuring and visualizing gene expression results.

FIGURE 6: SOFTWARE INTERFACE



Illumina's BeadStudio software interface is user-friendly.

GETTING STARTED WITH THE DASL ASSAY

The DASL Assay for gene expression analysis of partially degraded RNA can be used with both the Illumina BeadStation and the Illumina BeadLab. Existing systems are easily upgraded to run the DASL Assay with the DASL Upgrade Kit. This upgrade package includes BeadStudio analysis software, a license to use the assay, installation and training. Pilot evaluations are also available through the Customer Sample Evaluation Program (www.illumina.com/CSE).

ADDITIONAL INFORMATION

To learn more about Illumina's DASL Gene Expression, gene expression products or other products and services, contact us.

ORDERING INFORMATION

PART NO.	PRODUCT	DESCRIPTION
DA-10-100	DASL Human Cancer Panel	Probes for 502 genes collected from ten publicly available databases, three oligo probe sets per gene, for 96 samples
DA-10-480	Custom DASL Oligo Assay Pool- single probe set	Custom panel for expression analysis of partially degraded RNA that probes up to 1536 genes (1 oligo set per gene) per panel (480 samples)
FA-12-104	Sentrix Universal-96 Array Matrix	For processing 96 samples or replicates simultaneously
GT-95-210	Sentrix Universal-16 BeadChip	Six BeadChips, each for processing 16 samples or replicates simultaneously
GT-95-501	Single Use cDNA Synthesis Kit	Kit for preparing cDNA from a 96-well plate of RNA samples for a single DASL reaction per sample
GT-95-503	Multi-Use cDNA Synthesis Kit	Kit for preparing cDNA from six, 96-well plates of RNA samples for six DASL reactions per sample
GT-95-205	GoldenGate Kit with UDG	Prepares reactions for 96 samples including UDG enzyme for contamination control
RELATED PRODUCTS		
UG-10-110	DASL Upgrade Kit for BeadStation 500	Upgrade package for BeadStation 500 that includes DASL software, access to DASL reagents and a license for performing the DASL Assay.
SC-16-200	BeadStation 500	A complete SNP Genotyping and Gene Expression system including: BeadArray Reader, monitor, hybridization oven, assay processing accessories, heat blocks, shaker, analysis software, installation and training.

REFERENCES

(1) Van Gelder, R.N., von Xastrow, M.E., Yool, A., Dement, D.C., Barchas, J.D., Eberwine, J.H. (1990). Amplified RNA synthesized from limited quantities of heterogeneous cDNA. *Proc Natl Acad Sci USA* **87**, 1663-1667.

(2) Fan, J., Yeakley, J.M., Bibikova, M.,Chudin, E.W., Chen, J., Doucet, D., Rigault, P., Zhang, B., Shen, R., McBride, C., Li, H., Fu, X., Oliphant, A., Barker, D.L., Chee, M.S. (2004). A versatile assay for high-throughput gene expression Profiling on Universal Array Martices. *Genome Res* **14**, 878-885.

(3) Illumina Technical Bulletin: *RNA* Profiling with the DASL™ Assay. Pub. No. 470-2005-003.

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