

TECHNOLOGY SPOTLIGHT

DASL® Assay vs. Direct Hybridization

Understanding the Differences Between Illumina's Two Gene Expression Assays

INTRODUCTION

Illumina offers two assays for studying gene expression: 1) Direct Hybridization (using IVT as originally described by Eberwine and colleagues¹), and 2) cDNA-mediated Annealing, Selection, extension, and Ligation (DASL). While both assays provide sensitive and reproducible expression data, they involve significantly different approaches and are best deployed in distinct situations and with different types of samples. This document explains the basic differences between the two assays and provides guidance for assay selection.

ASSAY OVERVIEWS

The Direct Hybridization assay is based on standard protocols originally described by Eberwine and colleagues¹. In brief, cDNA is produced by reverse transcription of mRNA by priming off the polyA tail. Amplification follows with an in vitro transcription step generating biotin-labeled cRNA. This amplified pool is then assembled onto the array platform. Visualization of the array is performed by staining with Streptavidin-Cy3 following hybridization to the array. Illumina's Focused Array product line uses a direct hybridization method and profiles up to 1400 genes using one probe per gene.

The DASL Assay, on the other hand, generates cDNA using both poly(T) and random priming with biotinylated nine-mers. Amplicon cDNAs are probed in solution, rather than with 50-mer oligos covalently attached to beads in the array, as with the Direct Hybridization assay. The DASL Assay incorporates universal primers for a subsequent PCR amplification step, and a short universal address sequence (IllumiCode) for decoding the array. Gene-specific probe groups, comprised of the universal primers and an address sequence, are synthesized and pooled to form the DASL Assay Oligo Pool (DAP). A total of up to 1536 genes can be queried using a single probe group per gene. For optimal performance, three probe groups per gene are recommended, corresponding to 512 genes.

THE DIFFERENCES

1) Targeted vs. global RNA amplification:

- Direct Hybridization involves a *linear* amplification of the *entire transcriptome* of a particular sample of interest.

- The DASL Assay involves the *exponential* amplification of only the *specific genes* or transcripts being targeted by the DAP.
- Impact:
 - While no expression array can offer absolute quantitation, Direct Hybridization comes closer to this ideal simply because it involves a linear amplification of all transcripts. Genes that do not exhibit differential expression, even between dramatically different tissue samples, will have roughly the same intensity on both arrays. The DASL Assay amplifies a subset of transcripts, as opposed to all transcripts. The cDNA templates all compete for a finite number of primer molecules in the PCR mix. As a result, the expression signal observed for any particular gene is always *relative* to the other genes present in the pool.

- This aspect of the DASL Assay has specific advantages and disadvantages. By selecting appropriate gene subsets, the DASL Assay can be tuned to high levels of detection sensitivity. This renders the DASL Assay particularly well-suited for "needle in a haystack" applications, such as the detection of transcripts found in rare cell types within heterogeneous tissue. Even with its exponential amplification, DASL is highly precise and can be used to detect small changes in expression. However, adding probe groups to monitor other transcripts in a pool will result in slight changes to the relative expression values of genes. Sensitivity and fold-change measures are only valid within a given pool. For this reason, the DAP should be carefully selected because it can not be augmented once it is synthesized.

2) Random vs. poly(T) priming:

- Direct Hybridization assay: primes off the polyA tail of mRNA using a poly(T) primer.
- DASL Assay: uses both poly(T) and random nine-mers for priming
- Impact:
 - Partially degraded RNA, such as that obtained from formalin-fixed, paraffin-embedded (FFPE) samples, suffers from breaks occurring throughout the RNA transcript, making it very difficult or impossible to generate high-quality, full-length cDNA by priming

COMPARISON BETWEEN ASSAYS FOR FOCUSED SETS: DIRECT HYBRIDIZATION VS. DASL

Feature	Direct Hybridization	DASL
Input to assay	Intact total RNA	Intact or partially degraded total RNA
Array formats offered	Sentrix® Array Matrix (96 samples) Sentrix BeadChip (16 samples)	Sentrix Array Matrix (96 samples) Sentrix BeadChip (16 samples)
Array content	Gene-specific probe sequences	Universal probe sequences
Custom or catalog	Both	Both
Amount of starting material (ng)	50-100	100 (intact), 200 (degraded)
# genes profiled	up to 1400 for human, mouse and rat; up to 700 for all other species	up to 1536*
# probes (or probe groups) per gene	1 probe for human, mouse and rat; 2 for all other species	1 probe group*
Probes	50-mer oligos, attached to beads in the arrays	Two ~25-mer probes targeting a ~50-base site on a transcript
Analytical hybridization	Hybridize directly to gene-specific probes on the array	Hybridize to oligo probe sets in solution
Software	BeadStudio	BeadStudio (with DASL upgrade)
Automation	Not available	Currently available

*For optimum performance, 3 probe groups per gene are recommended, which profile up to 512 genes.

from the polyA tail (3' end). The DASL Assay is much more effective in profiling partially degraded RNA due to the use of random priming.

3) Hybridization and assay readout:

- Direct Hybridization assay involves hybridization of cRNA directly to 50-mer probes attached to beads on the array.
- The DASL Assay takes place in solution. The resultant cDNAs are hybridized to the array via IllumiCodes.
- Impact:
The DASL Assay can take advantage of Illumina's universal arrays, which are routinely manufactured, readily available and always in stock. When one wishes to change study of specific gene expression, only the DAP is changed, not the array.

WHICH ASSAY SHOULD BE USED?

Use Direct Hybridization if any of the following apply:

- Intact RNA (e.g., obtained from fresh or frozen tissue samples or cell lines) is being studied and current sample preparation employs the Eberwine protocol.
- Plans are in place to perform whole-genome discovery studies with the Illumina platform, which uses the same assay, to follow-up on a targeted set of genes.
- Data will be compared to results from other platforms that use the Eberwine protocol.

Use the DASL Assay if any of the following apply:

- When working with partially degraded RNA samples, such as FFPE samples.
- When interested in detecting very subtle sample to sample differences such as in disease classification and progression.
- When already using Illumina's system for genotyping and comfortable using the GoldenGate® workflow.

REFERENCE

(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.

ADDITIONAL INFORMATION

To learn more about the DASL and Direct Hybridization Assays or about any of Illumina's products and services, contact us.

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