

What is DriverMap[™]?

The DriverMap™ Assay measures RNA expression levels of all human protein-coding genes in complex biological samples. A single-tube multiplex RT-PCR amplification of 19,000 mRNAs followed by next-generation sequencing (NGS) provides more complete expression profiles of proteincoding genes than standard RNA-Seq analysis with less sequencing reads and simpler data analysis.

Why DriverMap?

DriverMap overcomes the low sensitivity, specificity and complexity challenges in probe preparation of current RNA-Seq and microarray technologies. It greatly simplifies the protocols for data generation and analysis while providing a significant improvement in specificity, sensitivity and reproducibility of detection of differentially-expressed, protein-coding genes.

Validate existing expression profiling data

DriverMap's targeted primers enable orthogonal confirmation of differentially expressed genes identified by RNA-seq, microarray or other transcription profiling

Advantages of DriverMap

- Functionally validated PCR primers Genome-wide, experimentally validated primer sets that have been selected for highest sensitivity, and specificity of amplification of target genes
- · High throughput Generate NGS probes from up to 96 RNA samples in a single day

Streamlined protocol

Starting with total RNA, simply perform a rapid, single-tube, RT-PCR followed directly by NGS for digital quantitation of each expressed gene

Exceptional flexibility

Customize DriverMap panels to any specific sets of genes of interest

Ideal Applications for DriverMap

- · Biomarker discovery in whole blood Directly uses total RNA isolated from whole blood without PBMC isolation, globin/rRNA depletion
- Profiling of cellular composition in complex tissues More comprehensive results enable analysis of tumor purity, immune, stromal cell composition using cellspecific signatures
- Ultra, sensitive detection of rare cells Reliable, reproducible measurements with ultra-small samples enable expression profiling of CTCs, pathogenic organisms in clinical samples, and more
- · PDX and xenograft mouse models Specific profiling of human genes in the background of mouse transcripts without isolation of human cells

High-content signaling pathway profiling in drug development

Better sensitivity with fewer sequencing reads provides more cost-effective, direct profiling of cell lysates in 96- or 384-plate format for lead optimization and mechanism-of-action studies

DriverMap vs. RNA-Seq

Compared to RNA-Seq, the DriverMap Solution offers:

- Broad dynamic range for increased measurement of differentially expressed abundant transcripts
- · High sensitivity for detection of low-medium abundant transcripts

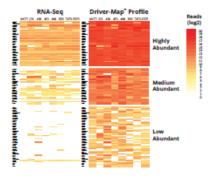


Figure 1:

Comparison of sensitivity of targeted RNA-Seq vs DriverMap assay. NGS read levels for selected high-abundant (10K-100K copies per sample). medium abundant (1K to 10K copies per sample), and low abundant transcripts (100-1K copies per sample) in 50ng of total RNA from seven common cancer cell lines.

DriverMap performance

- · Minimum sample requirement Start with as little as 10 pg total RNA
- Exquisite sensitivity, specificity and reproducibility Quantitate low- and medium- abundance transcripts with high reproducibility (R2 > 0.92) and a specific NGSconfirmed amplicon for each gene.
- · Broad dynamic range 100.000-fold, similar to RT-PCR



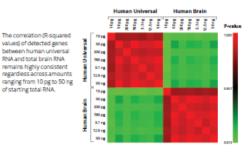
of starting total RNA.

Public Datasets:

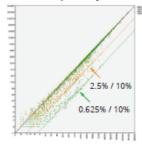
GSE85176 NanoString GX Human Immunology v2 GSE19738 Aglent Whole Genome Microarray G4112A GSE36177 Illumina HumanHT-12 v3 Expression BeadChip GSE83578 Affymetrix Human Genome U219 Array

Figure 2:

Validated sensitivity: The DriverMap Solution demonstrates significantly greater sensitivity for detection of differentially expressed genes compared to data sets generated with other technologies.



10% of spiked T-cvt RNA



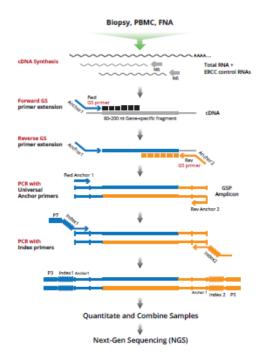
Each dot above represents the number of NGS reads for a gene in duplicate rups of a model system with T-cvt RNA spiked at 0.625%, 2.5%, and 10% into 100ng of MDA231 RNA, Even at the lowest spike-in level, read levels for each of the detected gene remains highly reproducible.

Figure 3:

Exceptional reproducibility in 10pg to 50 ng starting RNA sample range. Allows detection of rare cells in complex tissues at low (0.5%) abundance levels

How the DriverMap Solution Works

Start with total RNA from any source. No mRNA enrichment required. The DriverMap Solution workflow leverages the power of quantitative RT-PCR (upstream) and NGS (downstream read-out). The protocol begins with cDNA synthesis, stranded anchor addition, followed by PCR and NGS. Experimentally validated pool of genespecific primers are used to specifically amplify selected target cDNA regions.



DriverMap Products and Services

Pre-made, ready-to-use DriverMap kits

The Human DriverMap Assay is available as a ready-touse kit to run samples on widely available Illumina NGS instrumentation. DriverMap Kits are available in two configurations:

- 24-multiplex
- · 96-multiplex

Custom designed kits targeting your focused set of genes

Choose your target genes of interest and we deliver a custom primer mix and all reagents needed to profile the set.

Custom DriverMap expression profiling service

Cellecta offers a comprehensive end-to-end service workflow including RNA purification, RNA QC, preparation NGS probes and generation of NGS data analysis package, including data deconvolution, normalization, basic statistics and gene annotation. Extended bioinformatics package includes identification of differentially-expressed genes.

For more information on DriverMap kits, assay customization, or services and ordering, please visit our website and see the DriverMap Service page (https://www.cellecta.com/drivermap), or email your Cellecta customer service representative at orders@cellecta.com.



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