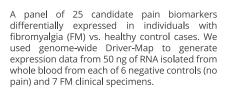# Driver-Map™ Genome-Wide Expression Profiling

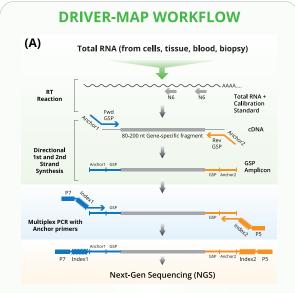## Complete Quantitative Gene Expression Analysis of All Human Genes in a One-Tube Assay

- 100-fold more sensitive than RNA-Seq—detect 20-30% more low-abundance transcripts

- Start with as little as 10 pg total RNA—single-cell level

- Use total RNA from whole blood or tissues—no mRNA enrichment or globin-depletion

- Specific targeted primers—minimal background from mouse when analyzing xenografts

The Driver-Map Assay uses intelligently designed, empirically optimized targeted primers to amplify defined regions of each transcript for all human genes in a single multiplex 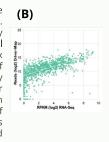RT-PCR reaction. The amplified products of this reaction are then analyzed using Next-Generation Sequencing (NGS) to assess abundance levels. This combination produces an assay that provides the sensitivity of RT-PCR with the dynamic range and quantitation of deep sequencing. Cellecta's novel approach uses total RNA as starting material and provides increased sensitivity for low- abundance genes and a broader linear range for more quantitative differential analysis than RNA-Seq.
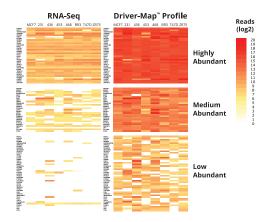
DRIVER-MAP WORKFLOW

The Driver-Map workflow leverages the power of quantitative PCR with NGS. Experimentally-validated primers amplify specific fixed-length regions of all protein-coding genes in a multiplex reaction. The number of reads of each of the resulting amplicons, as determined by NGS, provides a highly quantitative linear measurement of the abundance of each transcript across a range of 5 orders of magnitude (Panel B). Defined amplicons also greatly facilitate alignment and downstream analysis.

A panel of 25 candidate pain biomarkers differentially expressed in individuals with fibromyalgia (FM) vs. healthy control cases. We used genome-wide Driver-Map to generate expression data from 50 ng of RNA isolated from whole blood from each of 6 negative controls (no pain) and 7 FM clinical specimens.

NGS read levels detected RNA-Seq and Driver-Map for selected high-abundant (10K-100K copies per sample), medium-abundant (1,000-10,000 copies per sample), and low-abundant transcripts (100-1,000 copies per sample) in 50ng of total RNA from seven common cancer cell lines.

For more information and to subscribe to updates, email us at info@cellecta.com.
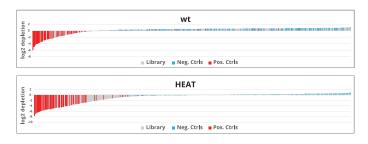
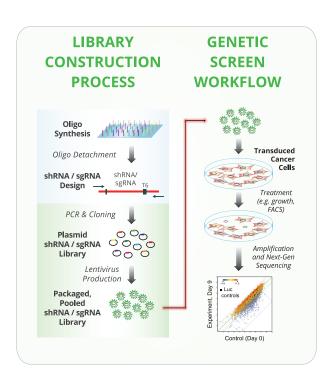# Knockout / Knockdown CRISPR & shRNA Solutions

Genetic Screens with Pooled sgRNA/shRNA Libraries and Custom Knockout/Knockdown Cell Lines
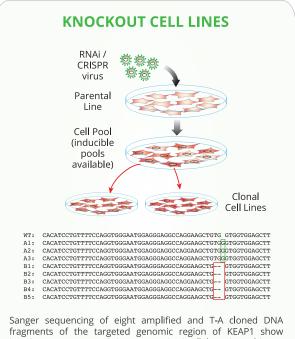
- Complete flexible platform for development and screening of genome-wide and custom CRISPR and RNAi libraries

- Full genetic screening service to identify novel therapeutic targets and genes essential for sensitivity or resistance to compounds or factors

- Customized and effective lentiviral constructs for targeted knockout and knockdown

- Knockout/knockdown cells from virtually any parental line

Cellecta offers effective flexible and scalable services for CRISPR and RNAi screen or target gene disruptions in the cell model of your choice. You give us your gene targets and we will do the rest. Our experience staff will design sgRNA, clone individual constructs or complete libraries, customize constructs for your cells, engineer cell lines, or run full genomic screen and send you the data. Work with us in whatever way allows you to best take advantage of our capabilities.

LIBRARY CONSTRUCTION PROCESS

GENETIC SCREEN WORKFLOW

Waterfall plots of a dropout viability screen testing the standard sgRNA structure against Cellecta's modified HEAT sgRNA structure which contains an A-T substitution to remove a transcription stop signal and a 6-base-pair extension of a stem-loop on the tracr sequence to increase Cas9 binding levels. Dropout levels of sgRNAs targeting essential genes (shown in Red) are ca. 4-fold stronger than the same guides with the standard sgRNA structure. Non-targeting negative-control sgRNA (in Blue) show no significant depletion.

KNOCKOUT CELL LINES


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WT:  CACATCCTGTTTTCCAGGTGGGAATGGAGGGAGGCCAGGAAGCTGTG GTGGTGGAGCTT
A1:  CACATCCTGTTTTCCAGGTGGGAATGGAGGGAGGCCAGGAAGCTGTGGGTGGTGGAGCTT
A2:  CACATCCTGTTTTCCAGGTGGGAATGGAGGGAGGCCAGGAAGCTGTGGGTGGTGGAGCTT
A3:  CACATCCTGTTTTCCAGGTGGGAATGGAGGGAGGCCAGGAAGCTGTGGGTGGTGGAGCTT
B1:  CACATCCTGTTTTCCAGGTGGGAATGGAGGGAGGCCAGGAAGCTG-- GTGGTGGAGCTT
B2:  CACATCCTGTTTTCCAGGTGGGAATGGAGGGAGGCCAGGAAGCTG-- GTGGTGGAGCTT
B3:  CACATCCTGTTTTCCAGGTGGGAATGGAGGGAGGCCAGGAAGCTG-- GTGGTGGAGCTT
B4:  CACATCCTGTTTTCCAGGTGGGAATGGAGGGAGGCCAGGAAGCTG-- GTGGTGGAGCTT
B5:  CACATCCTGTTTTCCAGGTGGGAATGGAGGGAGGCCAGGAAGCTG-- GTGGTGGAGCTT
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Sanger sequencing of eight amplified and T-A cloned DNA fragments of the targeted genomic region of KEAP1 show knockout mutations of the gene in both alleles. The wild-type sequence is shown on the top, followed by 3 sequences from one allele with a "G" insertion and 5 sequences from the second allele with a 2-base deletion.

For more information and to subscribe to updates, email us at info@cellecta.com.