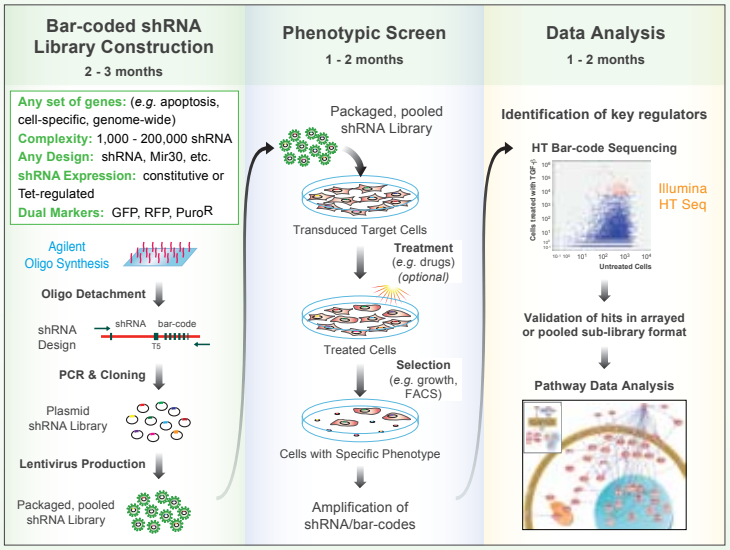
Comprehensive RNAi Screens – Quality Results & Novel Drug Targets

RNAi genetic screens have proven to be an extremely potent and versatile tool to explore the molecular basis of diseases, analyze pathway function, perform gene-disease association studies, and identify drug targets. However, the complexity of high-throughput (HT) genome-wide screening mandates properly constructed libraries and well-designed screening assays to generate good results. As the only provider of libraries specifically built and qualified for pooled RNAi screening, Cellecta's lentiviral shRNA libraries enable true comprehensive genome-wide screening because they…

- Use HT sequencing to quantify each shRNA construct in the library and each shRNA variant in screened cell samples
- Utilize stem-loop structures designed to amplify evenly so that shRNA representation is maximized in the constructed library
- Provide sufficient redundancy of effective shRNA constructs to knock down virtually all gene targets
- Have been designed with a complexity that is practical for effective screening of targeted genes

Cellecta can design, build, and provide you with libraries to your specifications, or perform the entire functional screening assay on a contract basis.

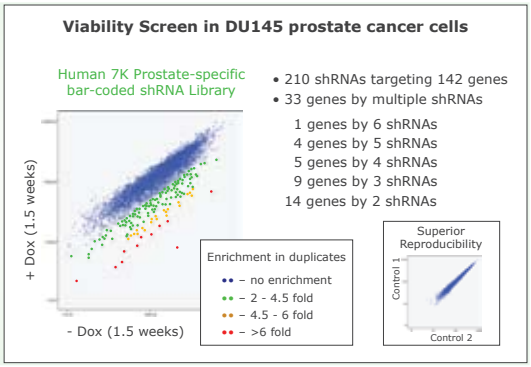
The basis of Cellecta's HT RNAi genetic screening technique is the stable suppression of specific genes on a large-scale allowing for loss-of-function screens in mammalian cell systems. HT genetic screens can be utilized to investigate any aspect of biology that can be recapitulated in a cell culture model. As opposed to expressing and assaying the functional effects of an individual siRNA molecule, development of complex shRNA libraries enables simultaneous screening of thousands of different shRNA molecules on a target population. In general, genetic screens represent an unbiased approach to identify genes that act in specific cellular pathways.



The screening process begins with the introduction of a packaged lentiviral library encoding a highly heterogeneous population of bar-coded shRNA constructs into a population of identical cells under conditions in which each transduced cell will express only a single mRNA-specific siRNA. Cells exhibiting the desired phenotypic changes are isolated, and the shRNA constructs, presumably inducing the phenotypes, are recovered by PCR and identified by HT sequencing of shRNA-specific bar-codes.

The prostate cancer viability screen results summarized on the right demonstrate the exceptional power of Cellecta's HT RNAi screening technology. The screen identified genes and pathways that are critical for viability of prostate cells but are not essential for cells of other tissue types.

DU145 prostate cancer cells were infected with a prostate-specific 7K shRNA / 2K mRNA tet-regulated pooled shRNA library and split into a control pool (no doxycycline treatment) and a pool of cells which was treated with doxycycline to activate tet-dependent expression of shRNAs. Surviving –dox and +dox cells were collected, and genomic DNA purified. Cytotoxic shRNAs responsible for reduced cell viability were identified by HT sequencing of bar-codes.

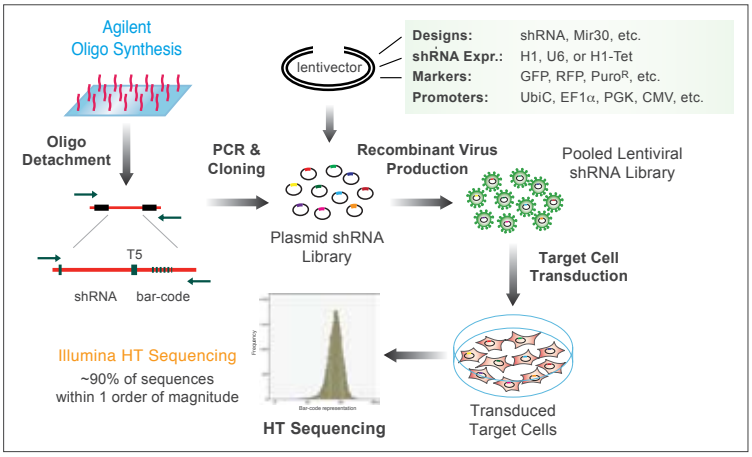


shRNA Libraries Built from Quality Components

Pooled lentiviral-based libraries allow you to assay the effects of many thousands of shRNA-expressing constructs simultaneously in one experiment. Producing a reliable and effective screening tool with such complexity requires considerable expertise. Cellecta has extensively optimized library construction protocols and overcame a number of technical challenges to produce quality shRNA expression libraries. Over the last several years, we have built and tested over 200 shRNA libraries.

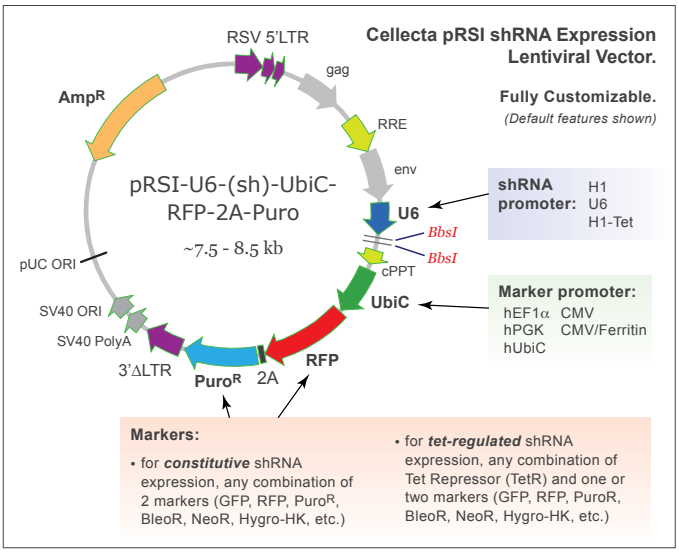
Exceptional Oligonucleotides

For large-scale production of heterogeneous populations of designed oligonucleotides for complex libraries, Cellecta has partnered with Agilent Technologies. Agilent's microarray-based oligonucleotide synthesis platform is unique and unparalleled in terms of providing full-length oligonucleotides over 100 bases in length with minimal mutations (mutation rates approximately 0.2%). Also, of critical importance, the solid support synthesis minimizes bias by providing similar yields of each individual oligonucleotide.



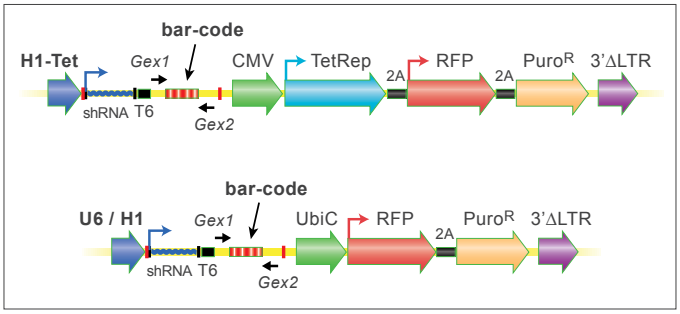
Range of Well-Designed Vectors

To provide efficient delivery of complex shRNA libraries into different cell types for a variety of different screening experiments, we have developed HIV-based lentiviral shRNA cloning vectors with H1, U6, or H1 tet-regulated promoters for expression of shRNA and a choice of a single or dual selection marker (GFP, RFP, PuroR, BleoR, NeoR, Hygro-HK, etc.) expressed from a single CMV, EF1α, PGK, UbiC, or other promoter. Cellecta's HIV-based lentivectors packaged in VSV-g pseudoviral particles have a broad range of tropisms for efficient transduction in a wide variety of cells.



Unambiguous Sequenceable Bar-Codes

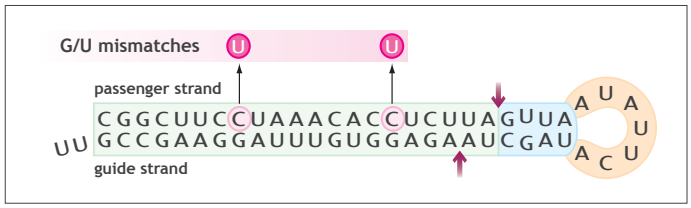
Quality control of the libraries and identification of functional shRNA responsible for specific phenotypes is greatly facilitated by the incorporation of specially designed bar-codes in each shRNA construct. The bar-codes enable unambiguous identification of each shRNA species by HT sequencing since they eliminate the need to amplify problematic stem-loop structures. Also, built-in primer sequences compatible with Illumina HT sequencing greatly simplify sample preparation for analysis.



Unparalleled Experience in Pooled shRNA Library Construction and RNAi Screening

To construct a quality pooled shRNA library, it is critical to both design it for effective screening as well as assess the features of the completed library to ensure they match the required levels for optimal performance. Due to the difficulty in predicting effective sequences, the need to have functional shRNA in all cell types, and the interest in differentiating off-target effects from specific knockdown responses, it is necessary for a library to have multiple shRNA targeting each transcript. To ensure this result after construction, it is essential to select and incorporate effective shRNA in the construction and confirm the actual representation of sequences in the final product.

Effective shRNA



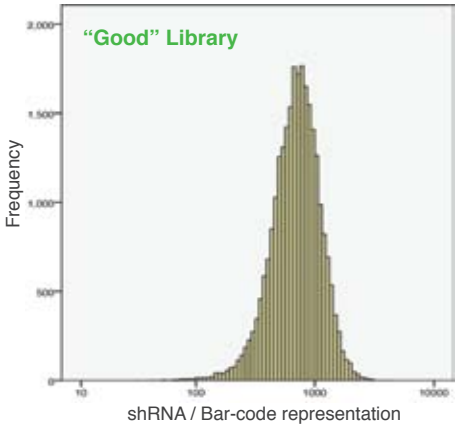
In constructing an shRNA expression library, one key challenge is the selection of highly functional shRNA inserts for the libraries. Cellecta has developed its own in-house shRNA design algorithm that makes use of internal studies primarily focused mostly on optimization of the most efficient structural features for constructing pooled libraries. Specific shRNA structural details greatly affect library amplification and final shRNA representation. Our libraries combine specific structural criteria with published information regarding sequence preferences to optimize knockdown of target genes.

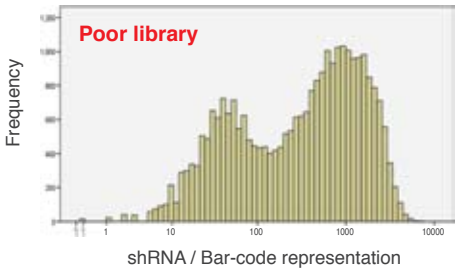
Narrow Representation of shRNA

Cellecta specifically designs and constructs pooled shRNA libraries using proven library construction procedures, not by reamplifying and mixing pre-made individual shRNA constructs. As a result, it is possible to obtain a very narrow representation of virtually all shRNA. The use of our "intelligent" bar-coding technology in combination with HT sequencing enables Cellecta to ensure that at least 99% of shRNA are present and detectable in every library and that at least 90% are within a 10-fold abundance range.

This uniform representation data enables the reliable assessment of which shRNA significantly increase or decrease in abundance during screening, indicating relevant targets. This representational data combined with appropriate experimental screening replicates is essential in differentiating signal vs. noise in a screening assay. It is an essential starting point for interpretable results.

shRNA / Bar-code representation





The histogram on the upper right shows HT sequencing Q.C. data for a 27K library with good representation. Virtually all of the shRNA are present in more than 100 copies and most are around 1,000 copies per 20 million HT sequencing "reads". Also, there is less than 10-fold difference between the most represented and least, so the library has a relatively balanced representation of all shRNA.

The histogram on the lower right shows data from a poor library in which a large portion of the shRNA are present at less than 100 copies and the distribution is very broad. It is only possible to get statistically significant signals for slightly more than half the targets using the library in the lower panel.

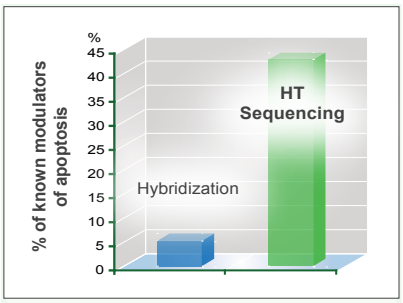
CELLECTA

Comprehensive, Statistically-Reliable Results with Pooled shRNA Libraries

When well-made lentiviral shRNA libraries are used to screen cell populations large enough to ensure that all targets are assayed, pooled format screening provides statistically robust and exceptionally rich data that comprehensively tests thousands of targeted genes in a single experiment.

Quantitative and Digital HT Sequencing

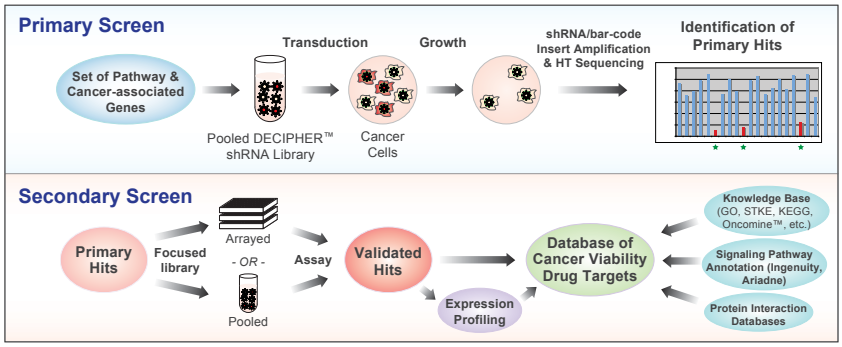
HT sequencing significantly outperforms the hybridization-based approach for identification of individual shRNA species based on the high-quality "digital enumeration data" generated by using bar-codes. Even when using optimized bar-code sequences, array hybridization suffers from a limited dynamic range of approximately 2 orders of magnitude which results in a loss of as many as 30% of the signals that fall outside their effective range. Also, spot-to-spot cross-hybridization on arrays results in a significant false positive rate that does not occur with HT sequencing where virtually every shRNA in the population is detected and counted, from those present in only a few copies to those present in several million. Differences in shRNA species between control and test populations are very easily detected and statistically analyzed, so that hits can be confidently identified.



Digital readouts eliminate the problem with low dynamic range of microarray data by reducing the number of false positives and negatives and increasing the number of positive hits.

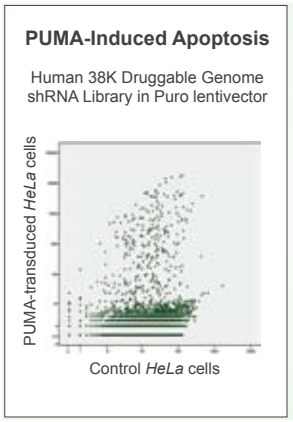
Comprehensive, Data-Rich Results

Cellecta's pooled libraries offer the capability of targeted screens knocking out each transcript with multiple shRNAs that serve to verify a specific functional effect and rule out off-target effects. In addition to unbiased wide-range screens of many thousands of genes to identify ones involved in a particular response, screens with highly-redundant libraries focused on a small set of genes (*e.g.*, 100-500) provide invaluable approaches to thoroughly investigate the function of key pathways, analyze sets of known genes involved in specific responses, or validate targets identified in earlier large-scale screens.



Exceptional Screening Versatility

The flexibility of library construction creates a versatility that enables effective screening of any set of targets against virtually any biological response that can be modeled in cell culture. Cellecta's innovative method enables quick and cost-effective construction of libraries targeting any gene sets. Reporters, drug selection genes, promoters, shRNA designs, and target gene sets can be altered from one screen to the next. Combined with our proprietary vector and shRNA designs, Cellecta Lentiviral shRNA Library Screening offers one of the most powerful and effective functional analysis and drug discovery tools currently available.



HeLa cells expressing recombinant apoptosis-inducing PUMA were transduced with a pooled 38K shRNA library. Surviving cells were harvested and bar-codes sequenced.

About 350 genes are enriched, 150 of which are known modulators of apoptosis related to NF-kB and p53 pathways.