Pooled Lentiviral RNAi & CRISPR Libraries

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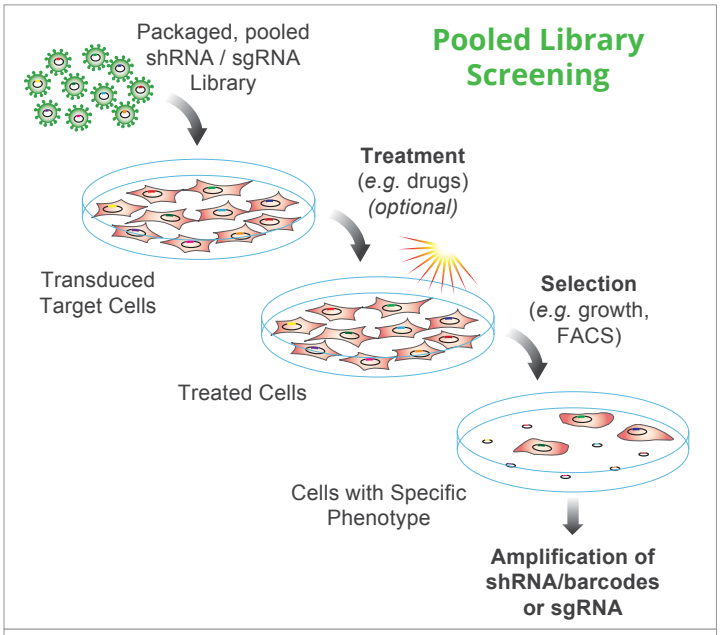
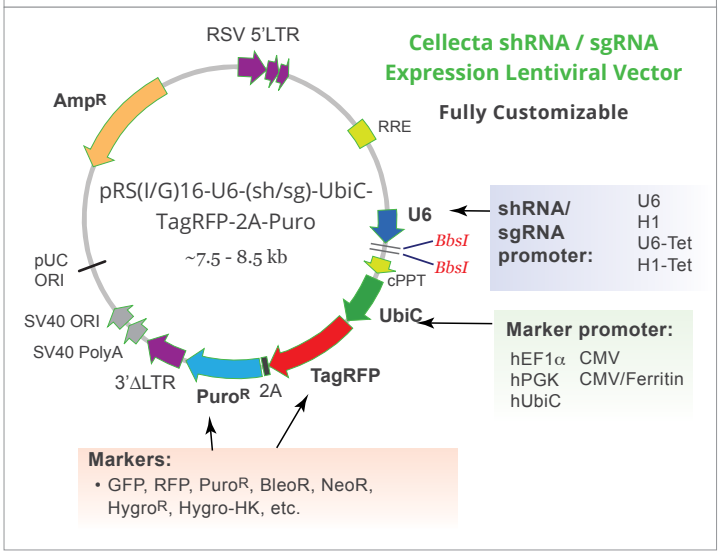
Pooled lentiviral-based libraries allow you to assay the effects of many thousands of effector constructs simultaneously in one experiment. Producing a reliable and effective screening tool with such complexity requires considerable expertise. Cellecta has extensively optimized library construction protocols and has overcome a number of technical challenges to produce quality effector libraries. Over the last several years, we have built and tested over 400 shRNA, CRISPR, and other effector libraries.

High-Quality Pooled RNAi & CRISPR Screening Libraries

- **Flexible** array-based oligonucleotide synthesis enables rapid creation of complex libraries expressing any set of shRNAs or sgRNA to any targets
- **Optimized** shRNA / sgRNA expression vectors with various markers, selections and both inducible and constitutive promoters
- Lentiviral-based system ensures **efficient** delivery of high complexity, pooled libraries into a wide range of cell types
- Internal non-targeting negative controls and positive dropout controls ensure **robust screening analysis**





Exceptional Oligonucleotides

- Synthesized using microarray-based oligonucleotide synthesis platform
- **Minimal mutation rate** (typically ~0.2%)
- Each oligo sequence in pool is fully defined without randomizations
- Solid support synthesis **minimizes bias** by providing similar yields of each individual oligonucleotide

Range of Well-Designed Vectors

- Lentiviral shRNA and sgRNA cloning vectors provide efficient delivery of complex libraries into a wide range of cell types
- Constitutive or tet-regulated promoters for expression of shRNA or sgRNA
- Single or dual selection marker (GFP, RFP, Puro^R, Bleo^R, Neo^R, Hygro^R, etc.) expressed from a single CMV, hEF1a, hUbiC, hPGK, or other promoter.

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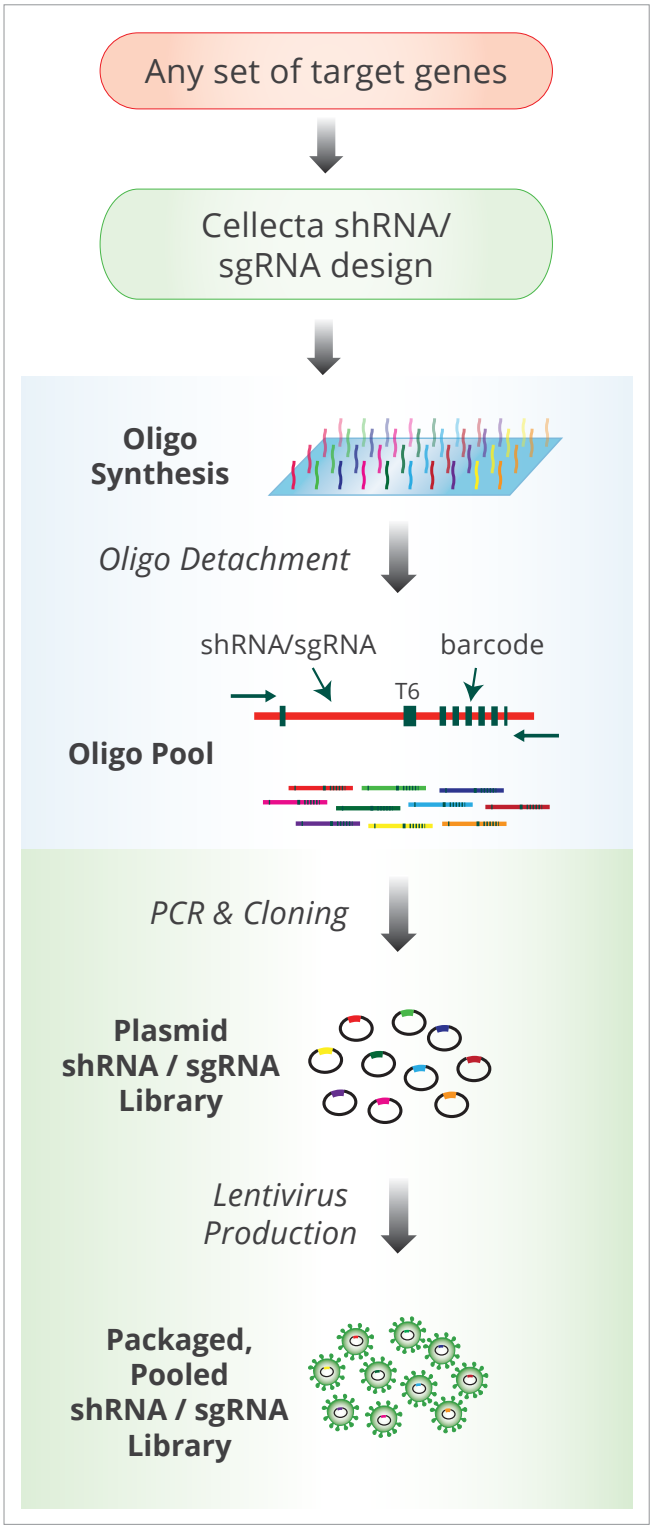
Custom shRNA & CRISPR Libraries

You Give us Your List of Targets, and We Do the Rest!

- Target list may be gene identifiers or sequences, or shRNA / sgRNA sequences (other formats also acceptable)
- You choose the type of vector you would like us to use
- Cellecta provides the oligo design and synthesis and library construction
- All libraries are checked for shRNA / sgRNA representation by NGS
- A portion of individual clones are fully sequenced to assess mutation levels
- Library can be provided as ready-to-use viral particles or in plasmid form
- Turnaround time is approximately 3 months

Screen 100 to 10,000 Targets Simultaneously

- Library sizes from a few thousand to over 50,000 shRNAs or sgRNAs
- As many shRNA or sgRNA per target as desired--even "tiling" of targets
- Optional packaging of the libraries as ready-to-transduce pseudoviral particles



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Human Genome-Wide shRNA & CRISPR Libraries

NEW!

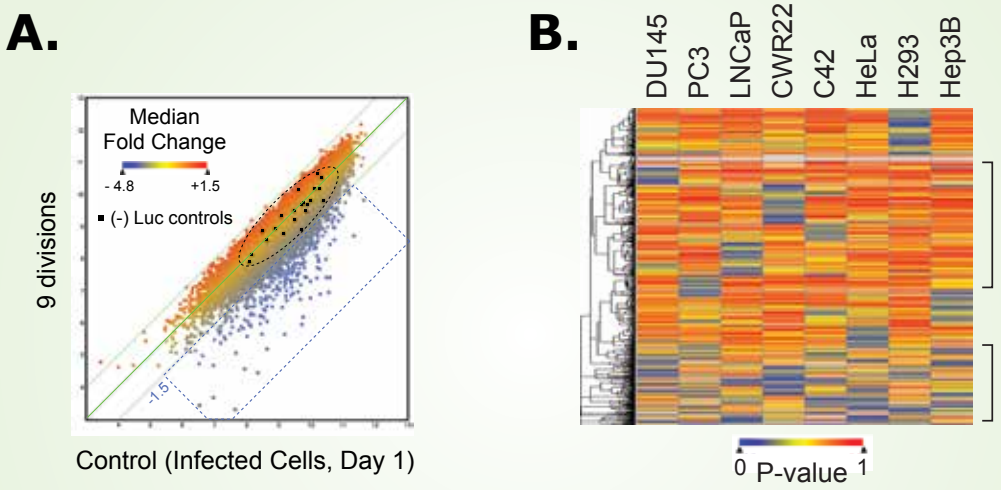
For Genome-Wide Knock-down or Knock-out Coverage in Human

- 3 Modules, together targeting nearly all protein coding genes
- Each gene is targeted by 8 shRNA or sgRNA for a total of 55,000 shRNA or sgRNA per module
- Modules can be combined for a single genome-wide screen

Library Modules

- Module 1 targets signal transduction genes
  - Members of major signal transduction pathways
  - Top-ranking genes from the Cancer Genome Atlas
  - Approved drug targets
  - Many disease-associated genes
- Module 2 targets other disease-associated genes and known drug targets
- Module 3 targets cell surface markers, extracellular matrix genes, and DNA binding genes

Dropout viability RNAi screen to identify disease-specific essential genes



Panel A plots the number of reads (out of 20 million) of each shRNA specific barcode present in DU145 cells compared with the corresponding number of barcodes.

Panel B is a "heat map" that depicts significance of depletion levels for each shRNA in each of 8 cell lines screened. Red or orange indicates no significant change in the shRNAs targeting the gene over time, whereas blue indicates genes with the most significantly depleted shRNAs.

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Control Constructs for RNAi & CRISPR Libraries

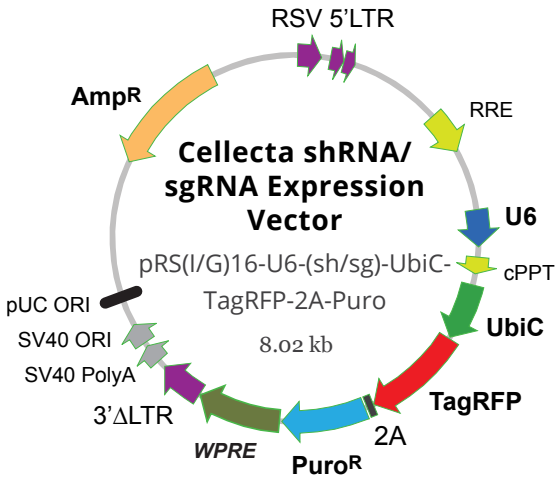
Cellecta provides predesigned control constructs for optimization of protocols and screening procedures. Constructs are available in plasmid form or as ready-to-transduce pseudoviral particles.

shRNA Controls

- Luciferase
- Non-targeting
- P53
- PSMA1
- PSMA6
- RPL30
- EIF3A
- KRAS

CRISPR sgRNA Controls

- CopGFP (variant)
- Non-targeting (empty)
- PCNA
- POLR2L



Next Generation Sequencing and Analysis Services

For laboratories needing support with sequencing and analysis, Cellecta can provide NGS and Barcode Enumeration of samples. You provide Cellecta with frozen cell pellets or DNA after screening, and Cellecta does the rest:

- Extracts genomic DNA
- Amplifies barcodes
- Performs NGS on the Illumina NextSeq or HiSeq
- Enumerates barcodes from raw sequencing data
- Additional bioinformatics and pathway analysis available