CELLECTA

Single-Cell Screening. Simplified.

CRISPR Libraries for Single-Cell Expression Screening

Custom Pooled Libraries to Target Your Genes of Interest

Single-cell analysis platforms can generate genome-wide expression data from several hundred cells in a single experiment. For Perturb-Seq studies (aka CRISP-Seq, or CROP-seq) expression data need to be obtained from multiple cells for each of the CRISPR guides. Thus, CRISPR Perturb-Seq libraries are typically custom-designed to specifically target just a few hundred genes that are important to the particular study.

Multiple Perturb-Seq CRISPR Library Configurations

Depending on the experimental needs and the specific single-cell RNA (scRNA) profiling workflow, CRISPR libraries configured in different ways may be required for a Perturb-seq experiment. Cellecta can provide CRISPR libraries that accommodate any scRNA system requirements.

The "CROP-Seq" Design (Fig 1): With this configuration (Datlinger, et al., Nature Methods, 2017), the sgRNA expression cassette is expressed on the 3'-UTR of the selection marker for the library vector. Since the sgRNA sequence is expressed in the 3'-UTR, this library design does not require any modification of the sgRNA sequence. However, it does require a separate amplification of the barcodes from the cDNA produced by the scRNA platform.

Incorporating Feature Barcoding (Fig 2): The 10X 3'-Chromium capture beads include unique "feature barcode" capture sequences in addition to the standard poly-dT sequences. As a result, molecules modified with complementary sequences are captured together with the poly-A tailed mRNA. Although this design requires modifying the sgRNA tracr sequence used in the library to include one of the 10X capture sequences, the resulting library can be used with the standard 10X 3'-Chromium protocol and analyzed using Cell Ranger software.

Using a Standard CRISPR sgRNA Library (Fig 3): A typical CRISPR library design made with one of our standard sgRNA vectors can be used with the 10X 5'-Chromium protocol. No special modifications are needed to capture the guide sequences in the cDNA because the 5'-Chromium beads use the 5'-switch oligo to prime first-strand synthesis from the 5'-end of RNA so guide sequences will be present in the cDNA. However, a separate amplification is required to extract the sequence from the guides for sequencing.

Take Advantage of Cellecta's CRISPR Library Expertise

For pooled sgRNA libraries, Cellecta's library construction services includes sgRNA oligo design/synthesis, cloning, and QC by NGS and full insert Sanger sequencing of a couple dozen selected clones. We provide you with the full insert sequences for the guide oligos and the NGS sequencing data. The turn-around time for the service is typically ca. 6-8 weeks. We provide 500ug of the plasmid library, ready for packaging. We can also package the libraries as VSV-G pseudotyped lentiviral particles as an additional service.

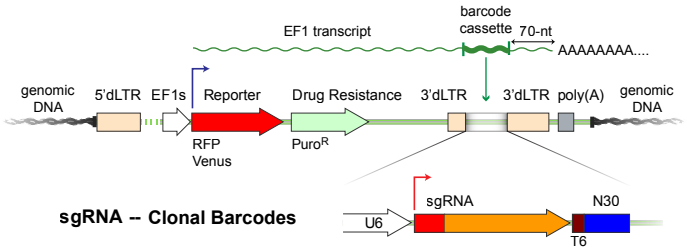


Figure 1

Cellecta's pScribe vectors are designed to incorporate barcodes and/or sgRNA expression cassettes in the 3'-end of the selection marker transcript. As a result, the barcode and/or guide sequence is replicated in the poly-A-primed cDNA that is made during RNA-Seq. When a CRISPR library using this vector design is transduced into cells and then processed using the 10X 3'-Chromium protocol, the guide sequences can be amplified and sequenced in parallel with the cDNA used for the RNA-Seq analysis. The pScribe design is flexible enough to incorporate a clonal barcode adjacent to the sgRNA sequence. This unique index associated with each guide construct uniquely labels each cell transduced with the library.

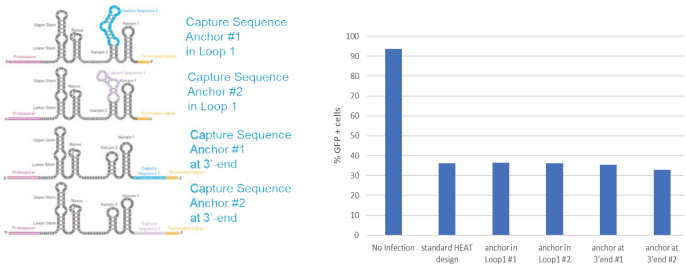
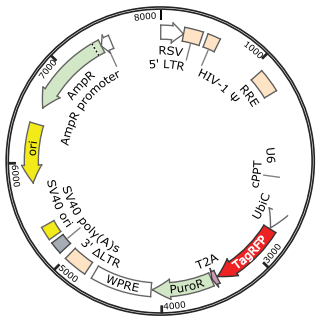


Figure 2

Cellecta made and tested sgRNA with the each of the two different capture sequences inserted at the two recommended locations in the tracr sequence. The capture sequences do not affect the activity of sgRNAs designed with Cellecta's modified HEAT tracr design.



pRSG16-U6-sg-UbiC-TagRFP-2A-Puro

Figure 3

Standard Cellecta CRISPR sgRNA library vector.

For more information on Cellecta's custom sgRNA libraries for single-cell CRISPR screening, visit cellecta.com/services or email info@cellecta.com