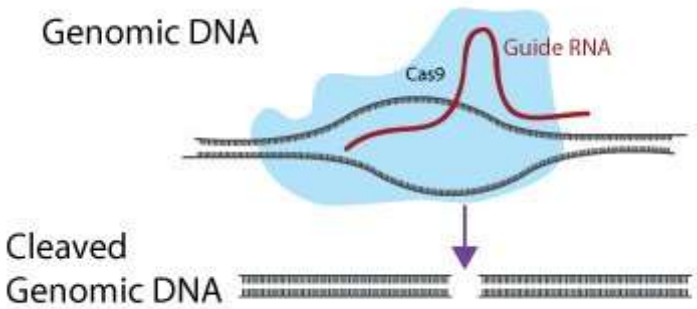
1. CRISPR Pooled Lentiviral sgRNA Libraries

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes denoted as CRISPR/Cas9 system is a targeted gene-editing tool adapted from *Streptococcus pyogenes* that enables the permanent knockout of target genes. Single Guide RNAs (sgRNA or gRNA) direct the Cas9 nuclease to a specific genomic region, upon which the Cas9 cleaves the target gene and permanently knocks it out (see figure below).



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Variations of the CRISPR system have also been developed using engineered versions of the deactivated Cas9 (dCas9) protein to either interfere (i.e., repress) or activate gene expression without modifying genomic DNA. These gene-regulating variations of CRISPR use libraries with the same basic sgRNA design but target gene promoter regions rather than the coding sequence. The promoter targeting sgRNA, then, recruits a modified dCas9 protein which either activates or represses gene expression—depending on the dCas9 modification. Selective activation of targeted genes occurs when CRISPRa sgRNA expression constructs are used in cells expressing dCas9-VPH (or other dCas9-activator variations compatible with spCas9 sgRNA designs) and selective expression inhibition of targeted genes occurs when CRISPRi sgRNA constructs are used in cells expressing dCas9-KRAB (or other deactivated Cas9 repressors compatible with spCas9 sgRNA designs). Pooled screens can be run with both the CRISPR-activation (CRISPRa) and CRISPR-interference (CRISPRi) systems very similarly to standard knockout CRISPR screens using appropriately designed libraries.

Cellecta has employed a Two-Vector CRISPR/Cas9 lentiviral system for nearly all pooled CRISPR sgRNA libraries. With the Two-Vector CRISPR system, Cas9 is transduced into target cells, which are then selected for a high level of Cas9 expression. Generally, the higher the expression of Cas9, the more efficient is the knockout of the target gene. After a population of high-expressing Cas9 cells are obtained, they are then transduced with the sgRNA library such that most cells receive only one copy of a single sgRNA, thus knocking out only one target gene per cell. Upon selecting the transduced cells for a specific phenotype (e.g., cell death, cell proliferation, etc.), Next-Generation Sequencing (NGS) of the sgRNA sequences present in the genomic DNA of the remaining cells quantifies the fractional representation of each sgRNA sequence remaining in the population.

The protocols included provide the instructions on how to package the plasmid form of a pooled sgRNA library into viral particles, perform a CRISPR-based screen, and prepare experimental samples for NGS and deconvolution of raw sequencing data sets.

References and Product Citations for all Cellecta products can be found on the Cellecta website: https://cellecta.com/pages/citations-publications.

Please read the entire user manual before proceeding with your experiment. Also, please note that, when working with pseudoviral particles, you should follow the recommended guidelines for working with Biosafety Level 2 (BSL-2) materials.

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Last modified: 8 March 2023

2. Materials Required for sgRNA Screening