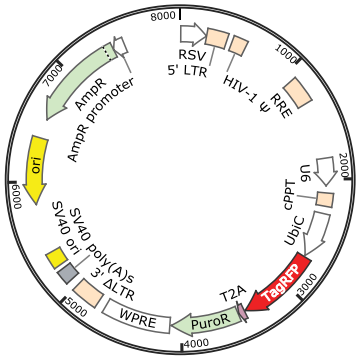
Two Vector CRISPR/Cas9 System

CELLECTA

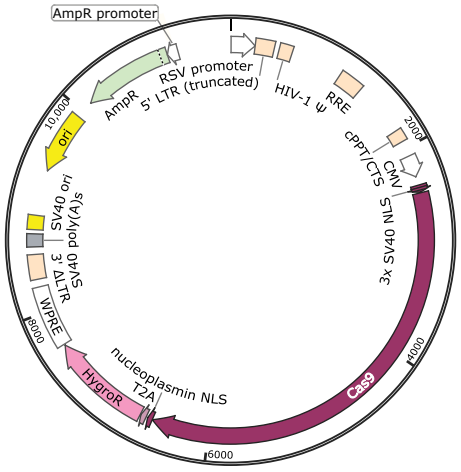
The CRISPR/Cas9 system can be used for knocking out gene expression by using a combination of sgRNA and the Cas9 nuclease. The Two Vector CRISPR/Cas9 system allows for faster knockout of the target gene in cells by first selecting cells expressing high levels of Cas9. The Two Vector CRISPR/Cas9 system is also great for creating custom sgRNA libraries for screening assays.



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

Expression of Cas9 and sgRNA from Separate Vectors to Optimize Knockouts

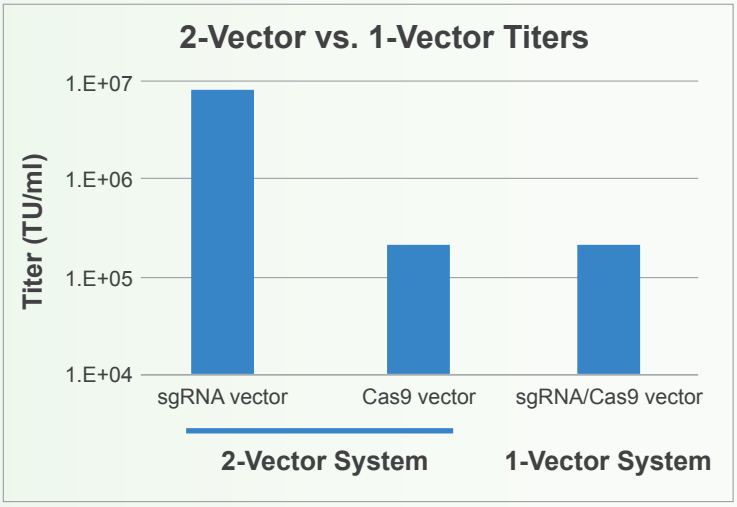
- Obtain higher titers for Cas9 for difficult-to-transduce cells
- Reduce noise in screening experiments by standardizing expression of Cas9
- Cas9 and sgRNA vectors contain different antibiotic resistance genes for easy selection
- Tet-inducible and constitutive sgRNA expression available



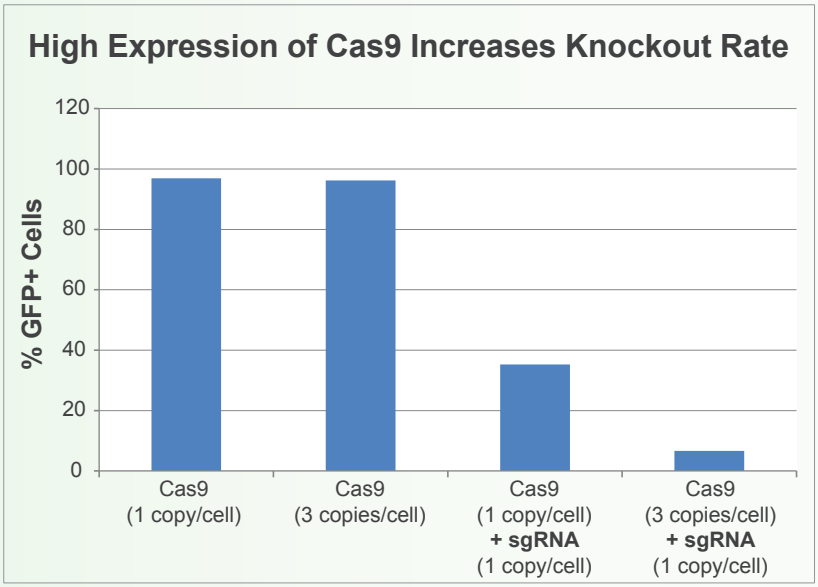
pR-CMV-Cas9-2A-Hygro
11.6 kb

Knock Out Targets with Lentiviral sgRNA Constructs

- You provide the RefSeq number or gene ID
- We design 3-5 sgRNA constructs that target the transcript



When transducing many thousands of constructs into large populations of cells, as is required for loss of function screens with pooled sgRNA libraries, high titers are a necessity. The 2-Vector CRISPR system provides higher titers than the 1-vector system and allows selection of cells expressing a high level of Cas9 before introducing the sgRNA, which leads to more efficient knockouts with less variability for screens.



Cells expressing GFP were transduced with Cas9 at high MOI producing a population with approximately 3 Cas9 per cell on average, or a low MOI generating a population of approximately 1 per cell on average. After selecting the Cas9 transductants with hygromycin, each population of cells was then transduced with the same sgRNA to GFP and grown for 9 more days in media containing puromycin. Cells with a higher number of integrated copies of Cas9 have 5-fold fewer GFP-positive cells.

Related CRISPR Products & Services

Human CRISPR Genome-Wide sgRNA Library

- Built using solid support oligonucleotide synthesis method
- Provided as either plasmid or packaged pooled sgRNA library

Custom CRISPR / sgRNA Libraries

- You decide which genes you want to knock out
- Cellecta designs 3-5 sgRNAs per gene (or to your specifications)

Create Knockout Cell Lines with CRISPR

- Design and cloning of sgRNA constructs
- Viral packaging for sgRNA constructs and transduction into desired cell type
- Confirmation by PCR that both alleles are out-of-frame
- Functional assays such as proliferation, viability screens, or pathway activation assays available