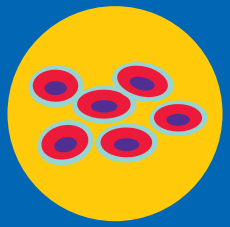
THE RESEARCHER'S GUIDE TO CRISPR SCREENING

STEP 1



Determine Cell Line

Identify which cell lines will work for your requirements.

1. Ensure the cell line is a good model in terms of relevance, biological process & genotype
2. Do you need a primary, transformed or stem cell platform?
3. Determine if the cell line can be adapted to your workflow
4. Consider the doubling time and ploidy of the cell line

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STEP 2

Design/Choose a gRNA Library and Screening Strategy

CRISPR libraries typically contain thousands of plasmids and multiple gRNAs per target gene. First you need to select an appropriate library.

1. Are you interested in the whole genome or a more focused pathway?
2. Lentivirus or ribonucleoprotein?
3. Pooled or arrayed?
   - Pooled: maximize the number of gRNA per gene target
   - Arrayed: optimize the gRNA design
4. Controls: use non-targeting guides and consider controls for enrichment and depletion depending on your screening approach
5. Use optimal designs for gRNA and—if designing your own libraries—spread them to avoid clusters in inaccessible genomic regions

STEP 3

Determine Optimal Conditions

Low transduction efficiency can result in insufficient representation of the modified cell population.

1. Perform a kill curve to determine the concentration of selection antibiotic needed to kill the untransfected or untransduced cells
2. Determine the functional titer in your intended cell line using
   - A colony forming unit assay based on antibiotic resistance, or
   - A vector containing a fluorescence marker like GFP
3. Use a control vector to optimize the multiplicity of infection (MOI). Use the lowest MOI that offers one gRNA per cell

STEP 4

Evaluate Your Cas9 Source

Establish a Cas9-expressing cell line or provide in an "all-in-one" vector.

1. Cas9 expressing cell lines: perform clonal isolation or use the mixed population of Cas9 expressing cells for screening.
2. All-in-one vectors: deliver both the Cas9 effector and gRNA by introducing one construct
3. Considerations for the optimal Cas9 source:
   - Ensures constant expression levels in a uniform genetic background
   - Eliminates concerns about co-transduction of gRNAs
   - Supports high-throughput sgRNA applications

STEP 5

Perform Your Screen

Pooled and arrayed screens have similar workflows with some differences:

STEP	POOLED	ARRAYED
Library Preparation	1000s gRNAs per tube	1 gRNA per well
Library Delivery	Lentivirus required	Multiple feasible formats
Screen Duration	Efficient whole genome screening	Time to screen increases with the number of clones
Screen Capability	*in vivo* screening possible	*in vivo* screening not possible
Analysis	Deep sequencing/ deconvolution required to analyze data/identify hits	NGS is not required to understand results
Readout	Limited options (e.g. cell death or proliferation) but can be coupled with single cell analysis	Multiple options e.g. fluorescence, luminescence, high content, live cell imaging

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