

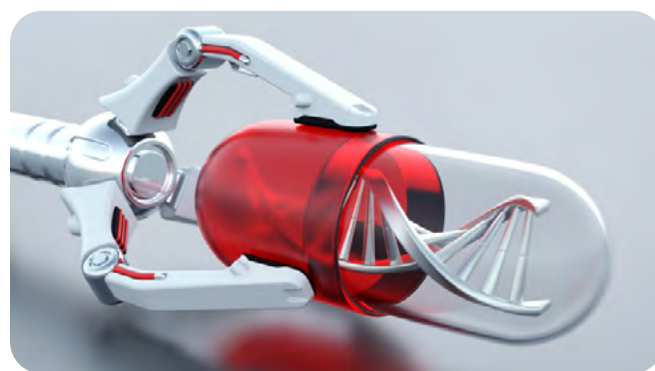
CRISPR-Cas9 screening: a powerful approach to advance drug discovery

Introduction

The capability to perform functional genomic screening has fueled open-ended and hypothesis-generating research to address complex biological questions. CRISPR-Cas9 screening allows parallel genetic perturbation of up to thousands of genes, using a high throughput method for probing gene function and biological pathway interactions. This platform exhibits high reproducibility by allowing efficient homozygous gene knockout and has become the gold standard in functional genomic evaluations.

CRISPR-Cas9 screening requires creating genomic perturbations, measuring a defined phenotypic response, and statistical analysis of the results to identify links between gene disruptions and the measured phenotypes. CRISPR-Cas9 screens with either an unbiased whole genome screen approach or focused on specific gene targets can advance multiple stages of the drug discovery pipeline. They can identify and confirm drug targets and gene interactions, find potential synthetic lethal relationships, determine the biological responses of cells to therapeutics through to patient stratification, therefore providing information as to which patients will be the most receptive or resistant to treatment strategies.

The drug discovery pipeline has much to gain from CRISPR-Cas9 screening, from mechanism of action studies to patient stratification and even further clarification of clinical data results. The choice of which screen format to use, either pooled or arrayed, and the choice of CRISPR-Cas9 technology, depends on the question being asked, the material to be tested, and the functional read-out required.



How does CRISPR-Cas9 screening work?

CRISPR screening is a powerful investigative tool that allows researchers to evaluate the causality relationships of a population of mutant cells to determine the effect of genetic variations on biological pathways at the systemic level. CRISPR screening harnesses the power of Cas9, which, when paired with a single-guide RNA (sgRNA), is targeted to create sgRNA loci-defined double-strand breaks. The error-prone nature of cellular DNA repair ultimately creates mutations that result in the specific loss of gene function. CRISPR screening is not only limited to the generation of knockouts (KO). Utilizing dead Cas9 (dCas9), where both nuclease sites are catalytically inactivated, it is also possible to modulate gene expression. dCas9 retains the ability to bind DNA, allowing modulation by either repression or activation of gene expression using single-guide RNA sequences that recruit dCas9 in or near a specified gene's transcription start site.

This has diversified the CRISPR screening tool kit, where CRISPR screens can be augmented to assess loss-of-function and gain-of-function readouts depending on the question researchers are looking to answer. CRISPR interference (CRISPRi) is a loss-of-function (LoF) screening tool, where dCas9 is targeted to defined loci to block transcriptional access. Fusion of dCas9 to transcriptional repressors enhances silencing further. Performing parallel, dual loss-of-function (CRISPRko and CRISPRi) screening, can prove particularly informative to directly validate hits with orthologous technologies, as well as identify hits unique to each CRISPR screening technology.

CRISPRi can be applied to:

- Study the effect of repressing genes in amplified loci
- Model the effect of druggability more closely
- Simulate hypomorphic mutations and partial LoF
- Study the effect of repressing essential genes
- Target non-protein coding regions (e.g., lncRNA)
- Validation for KO or RNAi with an orthologous tool

In CRISPR activation (CRISPRa), CRISPR-dCas9 specifically targets gene activators to sgRNA-directed loci. CRISPRa systems bring activators to targeted loci to enhance gene expression. In addition to being a stand-alone tool, CRISPRa screening can be used in parallel with CRISPRi LoF assessments. Combinational screening with these approaches can further strengthen screen results by directly validating gene behavior via reverse-orthologous CRISPR-Cas9 screening technologies.

CRISPRa can be applied to:

- Study the effect of activating a gene
- Target non-protein coding regions (e.g., lncRNA)
- Validation of LoF with a reverse-function orthologous tool
- Dual screening application for network analysis.

Pooled versus arrayed screening

Pooled screening is a process where CRISPR components are delivered en masse to a single pool of cells, creating an edited population. This approach is scalable and can easily

accommodate a whole genome scale. However, the readout capability is limited, and the assay typically requires a longer time point, often several weeks in length. Pooled screening is beneficial for answering questions such as how perturbations or treatments affect cell fitness and growth. With a readout of guide abundance, measured by deep sequencing, it is easy to observe which perturbed cells are lost from the population or have become enriched. Pooled screening with a viability readout can be utilized for investigating genetic interactions as well as drug-gene interactions.

Phenotypic pooled screening is also possible by utilizing antibody staining or reporter cells to allow sorting of defined populations from the screen pool and interrogation of guide abundance by deep sequencing. Applications for phenotypic screening include biomarker analysis, linking gene function to cell differentiation, and evaluation of toxicity and apoptosis.

Arrayed screening is more individualized, looking at each sample separately for more discreet details. An example would be looking at a cell that expresses a gene only under certain conditions. Arrayed screening is performed in multi-well plates and has the potential for high throughput and automation. Each well contains individual perturbations, and this arrangement lends itself to having multiple or multiplexed readouts and the potential for more complex models. These assays exhibit an abbreviated timeframe of 48-144 hours compared to the longer assay time points of pooled screening.

| Table 1: Summary of CRISPR screening approaches

Pooled Screening	Arrayed Screening
Pooled edited population	Individual perturbation per well
Up to whole genomic-level screening	From very low to high throughput
Limited readouts (NGS-linked)	Multiple or multiplexed readouts
Proliferation or phenotypic (e.g., FACS)	Complex growth models (e.g., 3D, co-culture)
Longer assay time points	Shorter assay time points (48-144 hours)

Complex model CRISPR screening applications

Complex CRISPR screening models can provide additional scientific context that is more relevant to the clinic. This may include 3D models such as spheroids or organoids, or it could be used to study immunology and co-culture screening paradigms, *ex vivo* or *in vivo* validation, combinatorial discovery, and single-cell RNAseq linking single-cell transcriptome readouts to individual perturbations. Each complex model application requires a customized approach to deliver CRISPR components and perform the screen based on the research questions looking to be answered.

3D models

3D models more accurately recapitulate the complex cellular interactions in tumors and other diseases. 3D screens utilizing spheroids or organoids allow for a more comprehensive assessment and yield data with strong clinical relevance.

Primary T cell models

CRISPR screening can also use primary models, for instance, using primary T cells to reflect full biological capacity, unlike surrogate models.

In Vivo models

CRISPR screening can be performed *in vivo* to study the effect of genomic perturbation on tumor growth. This can be combined with *in vitro* screening to identify phenotypic responses specific to the *in vivo* environment.

Combination models

Combinatorial CRISPR screenings can be tested using a defined combination of guides to create CRISPR perturbations at two simultaneous gene loci. This testing can help discover dependent relationships, validate functional paralogues, and interrogate redundant gene families. However, there is a limitation to the number of genetic perturbations that can be studied in parallel, which can limit the choice of screening format.

Single-cell models

Single-cell RNAseq-linked CRISPR screening is a complex application that can provide ultra-rich transcriptomics data per CRISPR edit. It is also applicable to be performed in a multiplex assay format to enrich the readout gained from each perturbation. Single-cell CRISPR screening combines the strengths of pooled and arrayed CRISPR screening by using pooled edited populations, allowing high throughput, and linking whole transcriptome readouts to individual perturbations. This technology can be used to monitor complex biological phenomena and provide comprehensive datasets, allowing the exploration of complex and multiplexed phenotypic signatures.

Considerations for CRISPR screening

Multiple considerations are needed when planning which CRISPR screening format to use.

1. Model type

The first consideration is the motive for screening and what model type is best suited. Will a 2D cancer cell line provide answers to the question at hand, or is a more complex model required? The cell number availability of the chosen model heavily impacts the choice of screening format, as does whether cells are amendable to lentiviral delivery. Non-lentiviral transduction methods are possible but could affect the screen's scale.

2. Library

The next consideration is what you want to screen for; an unbiased whole genome screen or a focused sub-library where you look at a specific set of proteins or perform a follow-up target validation. The scale of the library utilized may determine which screening approach to take.

3. Technology

Determining what form of CRISPR technology is appropriate to address your research question. For example, whether you should use LoF screening using CRISPR ko or CRISPRi or gain-of-function screening using CRISPRa, or utilizing a combination of CRISPR technologies performed in parallel to strengthen confidence by providing potential cross-validation hits.

4. Treatment

The aim of your research questions will help determine the format of the screen; for example, whether it is a genetic interaction screen to compare different cellular backgrounds or a drug-gene interaction screen to interrogate response to treatment by one or more compounds.

5. Readouts

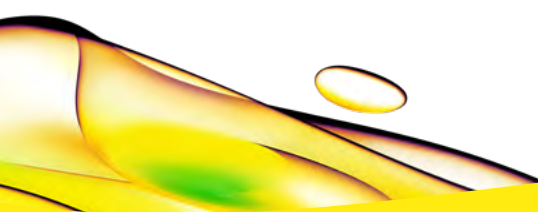
Deciding the required readout type, such as NGS-linked or phenotypic, will help drive which screening approach is appropriate and allow tailored options to answer target questions.

Combining rationale around the model, library, technology, treatment, and readout allows researchers to preemptively plan which format to use, pooled, arrayed, or single-cell screens, and execute highly optimized and efficient CRISPR screening experiments with data outputs integral to studying functional genomics.

Summary

CRISPR screening enables genome-wide interrogation of gene function. A typical CRISPR screening workflow involves sgRNA library design, transduction of cells with CRISPR reagents, assay screen, readout, and hit identification. CRISPR screening, using CRISPRko, CRISPRa, and CRISPRi technologies empowers researchers to interrogate the biological activity of hundreds or even thousands of genes to identify which are critical to specific biological pathways, to find drug targets, to stratify patients, and shed light on many other important biological applications.

Using immortalized cell lines, primary cells, or complex models, CRISPR screening can be performed as pooled, arrayed, or single-cell screens with flexible libraries and formats to prioritize goals, timelines, and budgets. This helps find the most robust and efficient screening method to meet the research requirements.



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