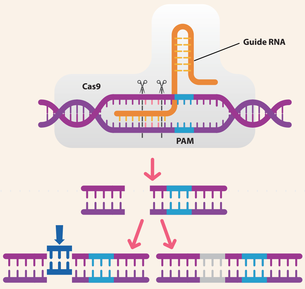
Beyond Sequencing

CRISPR Gene Editing Assessment and Quality Control Using Next-Generation Sequencing

Derived from a prokaryotic adaptive immune system for cleavage of invasive nucleic acids, the CRISPR-Cas genome editing system, one of the most important breakthroughs in modern biotechnology, enhances the efficiency of DNA modifications in living cells (base and prime editing). CRISPR-Cas9 allows precise gene targeting and mutations in virtually any cell type and organism relying on cellular DNA repair pathways.

One ongoing concern is that the CRISPR-Cas9 system induces mutations in the genome other than the target site. Meanwhile, off-target mutations can potentially disrupt the function or regulation of genes unpredictably. Therefore, efficient and rapid strategies for genome editing validation are essential to facilitate the application of CRISPR-Cas9 for disease model conductions, gene and cell therapies, agriculture, bioenergy, and the development of more accurate CRISPR editing tools.

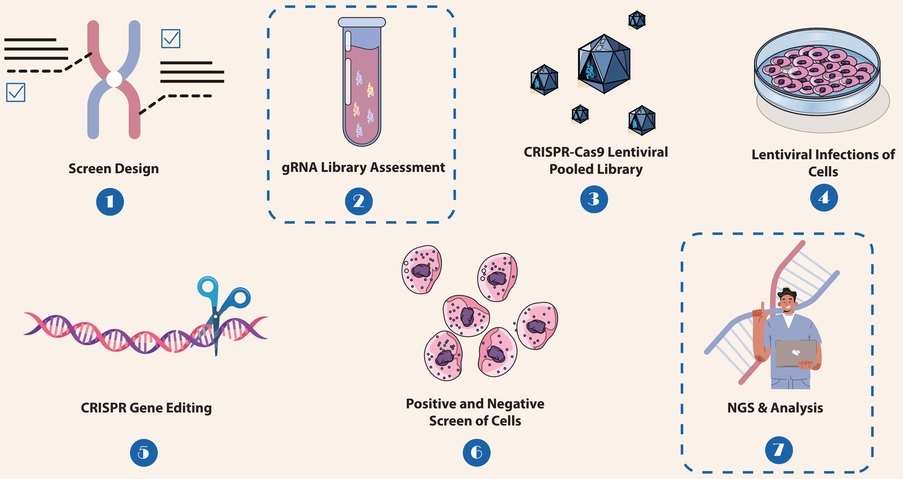


Sequencing-Based CRISPR Gene Editing Assays

Sequencing-based genome editing validation can detect whether alleles of a gene have been edited correctly, identify insertion and deletion sites at the whole-genome level, as well as screen and identify monoclonal cell lines. In addition, it is an excellent way to study the off-target effects of CRISPR gene editing.

NGS is a massively parallel sequencing strategy that allows for higher throughputs. It is widely used for CRISPR library screening, genome editing target validation, and off-target analysis because it is highly sensitive to identifying low-frequency mutations in cell populations.

CRISPR Genome Editing Workflow



Screen Design. gRNA libraries can be designed to target the whole genome, coding genes, non-coding genes, and other regions of interest as needed.

gRNA Library Assessment. The distribution abundance and quality of the libraries are examined using NGS, including sequencing quality statistics, base content distribution statistics, gRNA localization and coverage statistics, *etc.*

NGS & Analysis. Covering gRNA and gene count analyses, sample correlation analysis, inter-sample difference quantification analysis, gene annotation, and COSMIC data analysis.

CRISPR Library Screen Sequencing

CRISPR-Cas9 gRNA library screening is based on PCR amplification and deep sequencing analysis to uncover genes relevant to the screening phenotype through functional screening and enrichment of genome-wide mutant libraries associated with a particular class of function. CRISPR library screen sequencing not only allows for a more comprehensive assessment of the sgRNA library and quantification of sgRNA abundance but also analyzes gene function and regulatory networks in a high-throughput manner. It is widely used in projects such as mechanism research and biopharmaceutical development.

CRISPR Validation Sequencing

Although the CRISPR system is effective for genome editing, only a small percentage of cells are edited successfully due to its efficiency. Hence, it is important to validate the genome after CRISPR-Cas9 editing experiments. By using amplicon-based sequencing or other analysis methods, creating a population of clonal cells containing mutations is unnecessary. Meanwhile, large samples can be used to identify rare mutations in subpopulations of cells.

CRISPR Off-Target Detection

Regardless of which CRISPR gene editing system is used, the observed phenotypes are likely to be caused by off-target-ings. In addition to introducing insertions and deletions to base pairs, another potential side-effect of CRISPR-Cas9 editing is related to structural variants (SVs). Theoretically, off-target mutations may have serious consequences and disrupt the function or regulation of non-target regions.

Typically, off-target analysis is one of the most important steps for the CRISPR experiment. In addition to strategies such as target site selection and optimization of sgRNA design and Cas9, two other major off-target detection strategies are applied: biased and unbiased detection. To further explore, biased techniques sequence only certain regions of the genome that are predicted to contain off-target events, while unbiased techniques search the entire genome without regard to computer predictions.

Approach	Principle	Features
PCR	Design PCR primers to amplify predicted off-target sites, and detect off-target using sequencing or enzymatic digestion.	Easy to operate and low cost Bias prediction
Whole genome sequencing	Detects off-target mutations using high-throughput sequencing, which requires an appropriate reference genome to filter background mutations and perform data comparison analysis, thus obtaining potential modification sites containing PAM motifs and further gene amplification to verify mutation status.	High-throughput genome-wide analysis SNPs, Indels and chromosome level changes can be detected
Digenome-Seq	in vitro Cas9-digested whole genome sequencing is performed to detect off-target mutations.	Whole Genome Sequencing Direct detection of cleavage sites Ability to detect low-frequency off-target mutations