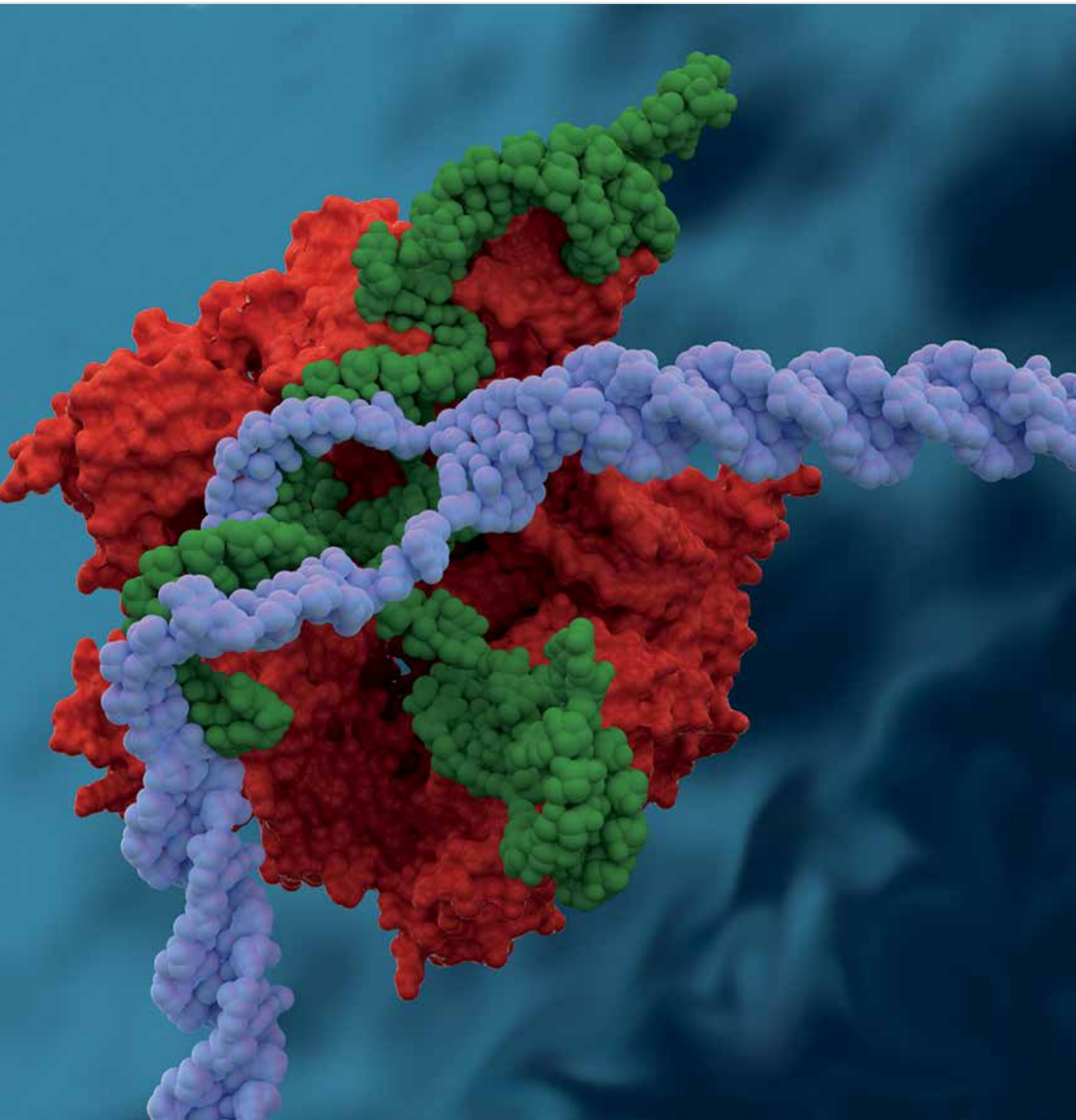
Gene Editing Research Review

An Overview of Recent Gene Editing Research Publications Featuring Illumina® Technology



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This document highlights recent publications that demonstrate the use of Illumina technologies in single-cell research. To learn more about the platforms and assays cited, visit www.illumina.com.

INTRODUCTION

Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas technology is a revolutionary gene editing method in which a programmable RNA guides a nuclease to find a specific target location in the genome. This simple approach replaces the laborious and expensive protein-based DNA editing techniques, such as the use of zinc finger proteins (ZNFs) or transcription activator–like effector nucleases (TALENs). From the time of the initial publications describing its application for genome editing in both prokaryotic[1, 2] and eukaryotic cells,[3, 4] the use of CRISPR-Cas technology has spread exponentially to laboratories worldwide.[5] Potential applications are in the fields of basic research, therapeutics, agriculture, and environmental research. In 2016, 2 years after gene editing was first introduced in human trials,[6] the National Institutes of Health (NIH) approved the first application of CRISPR-Cas technology in a human trial.[7, 8] This decision holds promise for future treatment of rare genetic diseases, human immunodeficiency virus (HIV) infection, and hematologic malignancies through cell replacement.

The CRISPR-Cas system originates from the prokaryotic adaptive immune system that targets and cuts invading genetic elements from phages or plasmids.[9-15]

The efficacy and safety of any DNA editing tool are highly dependent on specificity. Some studies have found that the use of CRISPR-Cas may lead to undesirable off-target effects.[16-28] For this reason, several groups are developing methods to detect[29-32] and reduce[33-45] off-target effects.

The CRISPR Locus and the Mechanism for CRISPR-Cas Technology

Bacteria and archaea use CRISPR-Cas systems for adaptive immunity. The CRISPR locus consists of short palindromic repeats separated by short nonrepetitive sequences called “spacers”. Spacers originate from invading sources of DNA, such as phages, that are copied and incorporated into the CRISPR locus when an infection occurs. Near this locus, a second locus includes a set of genes that encode CRISPR-associated endonucleases (Cas), which introduce cuts in the genome. When a repeat infection occurs from the same invading DNA, an RNA molecule (CRISPR RNA, or crRNA) will form a complex with Cas and a transactivating crRNA (tracrRNA) to guide the nuclease to the exogenous sequence. The Cas-RNA complex will recognize the DNA target that is complementary to the crRNA and adjacent to a specific 3-nucleotide locus (the protospacer-adjacent motif, or PAM). The complex then cuts and deactivates the invading DNA (Figure 1).

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3. Cong L, Ran FA, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. *Science*. 2013;339:819-823.
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5. Dow LE. Modeling Disease In Vivo With CRISPR/Cas9. *Trends Mol Med*. 2015;21:609-621.
6. Tebas P, Stein D, Tang WW, et al. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. *N Engl J Med*. 2014;370:901-910.
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8. Cyranoski D. CRISPR gene-editing tested in a person for the first time. *Nature News*. 2016;
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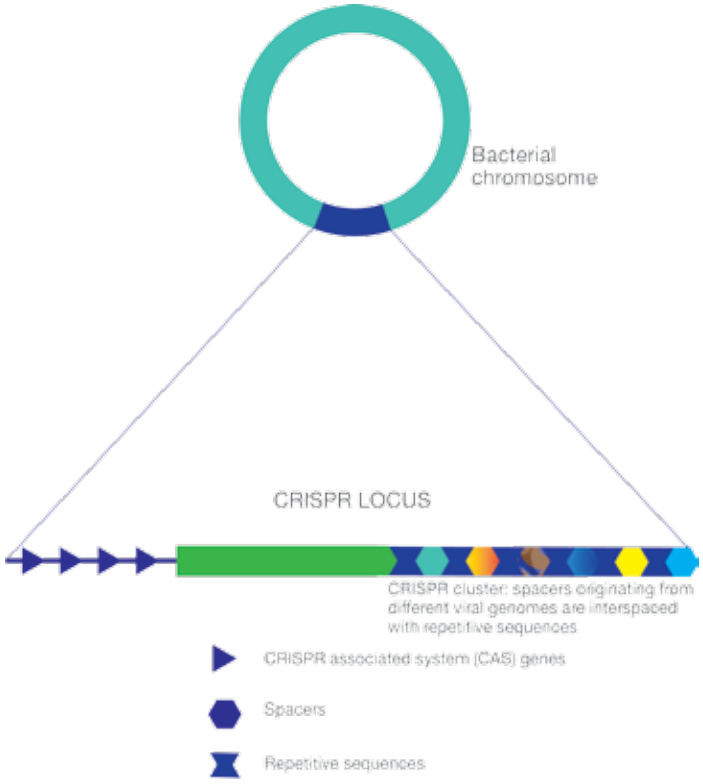

Figure 1. The CRISPR locus consists of short palindromic repeats separated by short nonrepetitive sequences, called "spacers". Spacers originate from invading sources of DNA that are copied and incorporated into the CRISPR locus when an infection occurs. Near this locus, another includes a set of genes that encode Cas endonucleases, enzymes that introduce cuts in the genome.

Although different nucleases are associated with CRISPR activity in different bacteria, most of the topics in this review refer to *Streptococcus pyogenes*, which relies on the endonuclease Cas9. For this reason, the review refers to CRISPR-Cas9 technology.

The CRISPR-Cas9 system requires 2 RNA molecules: crRNA, transcribed from the DNA spacers, and tracrRNA, whose interaction with crRNA is a structural requirement for the recruitment of Cas9 (Figure 2). In a landmark study,[46] these 2 RNAs were hybridized to create a single-guide RNA (sgRNA). This simplified Cas9-sgRNA system demonstrated gene editing properties.

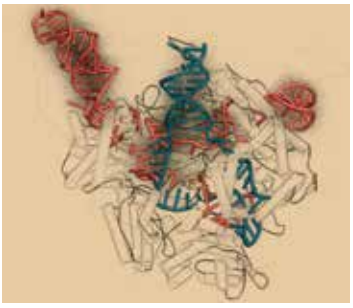

Figure 2. Cartoon representation of the molecular structure of Cas9 (transparent) and sgRNA (red) interacting with DNA (blue).

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28. Zhang H, Zhang J, Wei P, et al. The CRISPR/Cas9 system produces specific and homozygous targeted gene editing in rice in one generation. *Plant Biotechnol J*. 2014;12:797-807.
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In mammalian cells[47, 48] Cas9 introduces nuclease-induced double-stranded breaks (DSBs) that can be repaired by 1 of 2 possible competing repair mechanisms (Figure 3). The first is nonhomologous end-joining (NHEJ), which is more frequent but error-prone, and it results in insertion and deletion mutations (indels). The second pathway is homology-directed repair (HDR), which can be used for precise gene corrections or insertions in the presence of a donor DNA template.

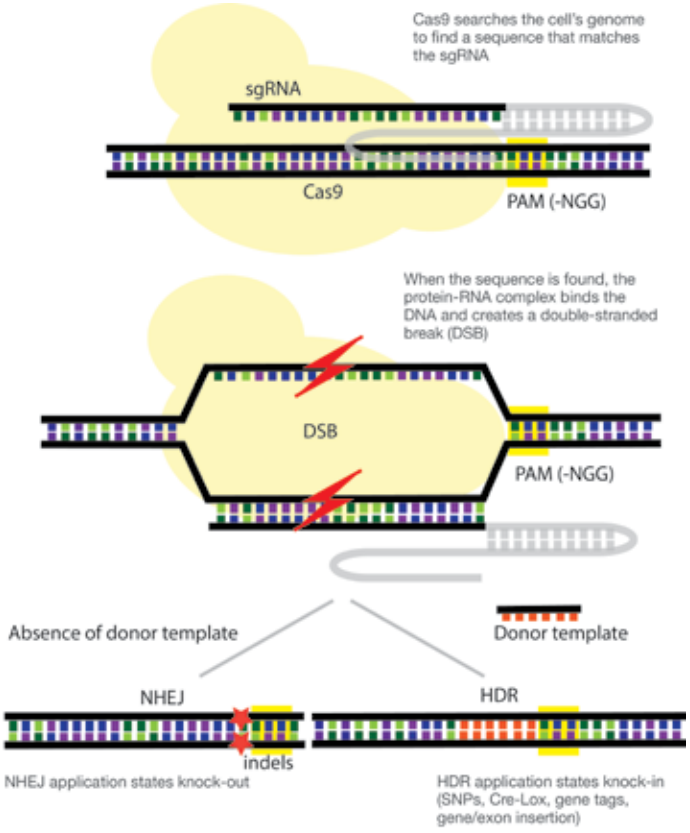

Figure 3. A schematic representation of the CRISPR-Cas9 mechanism. Cas9 searches the cell's genome for a sequence that matches the 20 bp sgRNA. Once found, Cas9 introduces a DSB in the matching sequence. At this point, 2 pathways will compete to repair the introduced breaks: 1) NHEJ ligates the break ends with no need for a homologous template in an error-prone mechanism, resulting in small insertions and deletions. 2) The HDR mechanism requires a homologous sequence that guides the repair and will result in the insertion of the donor template into the sequence.

Reviews

Dow L. E. Modeling Disease In Vivo With CRISPR/Cas9. *Trends Mol Med*. 2015;21: 609-621

Lander E. S. The Heroes of CRISPR. *Cell*. 2016;164: 18-28

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47. Cong L, Ran FA, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. *Science*. 2013;339:819-823.
48. Mali P, Yang L, Esvelt KM, et al. RNA-guided human genome engineering via Cas9. *Science*. 2013;339:823-826.

APPLICATIONS OF CRISPR-CAS9 TECHNOLOGY

Gene editing using CRISPR-Cas9 technology has provided genomics researchers with a powerful new tool, as evidenced by the exponential increase in the number of publications mentioning "CRISPR" in association with "gene editing" in the last few years (Figure 4). While a significant number of studies address technical aspects of the methodology itself, the versatility of the system has already enabled its application to a multiplicity of biological systems (Table 1).

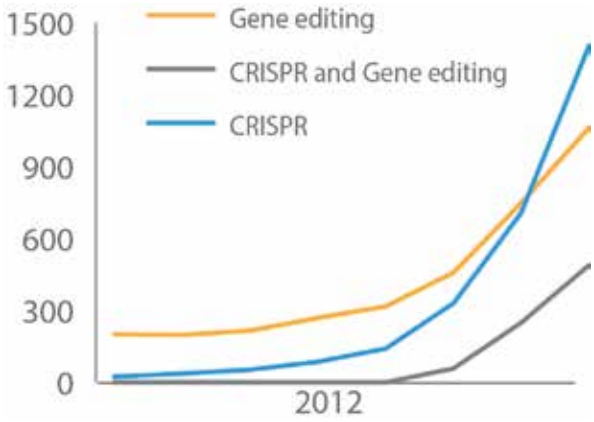

Figure 4. The increased occurrence of CRISPR-Cas9 and related keywords in scientific literature abstracts, obtained by PubMed searches.

The ability of the CRISPR-Cas9 system to insert or delete DNA sequences, simply and accurately, within living cells has been a long-held dream of researchers. This system allows for the deletion and insertion of genetic elements (eg, promoters and regulatory sequences) and syntenic regions to test the impact of these elements on a phenotype or disease.

The success and rapid adoption of CRISPR-Cas9 technology are, in part, due to the availability of next-generation sequencing (NGS). NGS can be used to detect target regions in the genome, validate accurate modification of the target, and detect any off-target effects. The expression and function of the gene can then be followed by next-generation RNA sequencing (RNA-Seq) and chromatin immunoprecipitation sequencing (ChIP-Seq). These approaches can also be used to guide the optimization of the CRISPR-Cas9 system and to discover alternative gene-editing systems.

Efficiency, versatility, and ease of use will make CRISPR-Cas9 and similar gene-editing systems indispensable for genomic research. It is likely that the technology will be integrated into every molecular biology laboratory, similar to the polymerase chain reaction (PCR).

Table 1. Examples of Recent Applications of the CRISPR System for Gene Editing

Field	Application	References
Research	Method development	49, 50, 51, 52, 53, 54, 55, 56, 57
	High-throughput genomic screening	58, 59, 60, 61, 62, 63, 64
	Editing of non-model species for research or medical interest	65
Medicine	Phase 1 trials	66, 67
Agriculture	Editing of crops and animals	68, 69
Environment	Gene drive and disease prevention	70, 71, 72

Research

CRISPR-Cas9 genome editing allows rapid, precise genomic manipulations to screen for multiple mutations *in vitro* or *in vivo*. The use of genetically modified cells and animals has been a key tool in molecular biology since the creation of the first knockout mouse model in the 1980s.[73] Now, the application of CRISPR-Cas9 technology allows researchers to mutate, silence, induce, or replace genetic elements simply and with precision, to investigate gene function and the biology of disease.

Genome-wide association studies (GWAS) have identified multiple loci associated with complex traits, most of which have low penetrance.[74, 75, 76] Thanks to its precision and practicality, the CRISPR-Cas9 system can be used to screen the effect of these polymorphisms on phenotypic expression. Furthermore, it may allow researchers to edit multiple loci in parallel to model complex phenotypes.[77]

49. Tsai SQ, Zheng Z, Nguyen NT, et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nat Biotechnol*. 2015;33:187-197.
50. Frock RL, Hu J, Meyers RM, Ho YJ, Kii E and Alt FW. Genome-wide detection of DNA double-stranded breaks induced by engineered nucleases. *Nat Biotechnol*. 2015;33:179-186.
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64. Parnas O, Jovanovic M, Eisenhaure TM, et al. A Genome-wide CRISPR Screen in Primary Immune Cells to Dissect Regulatory Networks. *Cell*. 2015;162:675-686.
65. Yang L, Guell M, Niu D, et al. Genome-wide inactivation of porcine endogenous retroviruses (PERVs). *Science*. 2015;350:1101-1104.
66. Kaisler J. First proposed human test of CRISPR passes initial safety review. *Science*. 2016;

The CRISPR-Cas9 system has also opened the door to generate new animal models, including species that were previously dismissed due to difficulties in manipulation (eg, the absence of meiosis and plasmids in *Candida albicans* is challenging for traditional editing methods).[78] It has also made possible other previously unachievable studies, such as those of humanized organs in pigs, that were dismissed due to the possibility of rejection by the human immune system and infection by retroviruses embedded in the pig genome.[79, 80]

Chen S., Sanjana N. E., Zheng K., Shalem O., Lee K., et al. Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis. *Cell*. 2015;160: 1246-1260.
In this study, the authors used CRISPR-Cas9 technology to perform a genome-wide loss-of-function screen in tumor growth and metastasis. Using a genome-scale library with 67,405 sgRNAs, they mutagenized a nonmetastatic mouse cancer cell line. Once mutagenized, the mutant cell pool rapidly generated metastases if transplanted in immunocompromised mice. Enriched sgRNAs in lung metastasis and late-stage primary tumors targeted a small set of genes, suggesting that tumor growth and metastasis is regulated by a subset of specific loss-of-function mutations. By using individual sgRNAs or small pools of sgRNAs targeting the top-scoring genes identified by the primary screen, the researchers significantly accelerated the development of metastases. This study demonstrates the utility of systematic Cas9-based screenings to study cancer evolution *in vivo*.

Illumina Technology: MiSeq® Sequencer, HiSeq® 2000 Sequencer, and HiSeq® 2500 Sequencer

Korkmaz G., Lopes R., Ugalde A. P., Nevedomskaya E., Han R., et al. Functional genetic screens for enhancer elements in the human genome using CRISPR-Cas9. *Nat Biotechnol*. 2016;34: 192-198.
To characterize noncoding regulatory elements, the authors applied CRISPR-Cas9 gene editing to cell lines to edit transcription factor binding sites in enhancer regions of p53 and ERα. They identified several functional enhancer elements and characterized the role of two of them in regulating gene expression. They also demonstrated that that CRISPR-Cas9 tiling screens can map functional domains within enhancer elements. Their work demonstrates the utility of *in vitro* CRISPR-Cas9 editing to study the noncoding regions of the genome.

Illumina Technology: TruSeq® Small RNA Kit, TruSeq RNA Library Prep Kit, HiSeq 2500 Sequencer

Maresch R., Mueller S., Veltkamp C., Ollinger R., Friedrich M., et al. Multiplexed pancreatic genome engineering and cancer induction by transfection-based CRISPR/Cas9 delivery in mice. *Nat Commun*. 2016;7: 10770.
Pancreatic ductal adenocarcinoma (PDAC) is among the most deadly forms of cancer, with very limited therapeutic opportunities. In the past, genetic association studies have created catalogues of genes that are involved in PDAC development, but much still remains to be understood. In this study, the authors developed and applied an electroporation-based vector delivery approach to deliver multiple CRISPR-Cas9 vectors to the pancreas of adult mice to simultaneously edit multiple genes. They were able to induce pancreatic tumors and to demonstrate the utility of their approach to key applications such as combinatorial gene-network analysis, *in vivo* synthetic lethality screening, and chromosome engineering. They also demonstrated the feasibility of negative-selection screenings, which provides an opportunity to address questions of biological relevance. Furthermore, they modeled chromosomal deletions and targeted editing of inter-chromosomal translocations to analyze complex structural variation, which plays a significant role in pancreatic cancer.

Illumina Technology: MiSeq Sequencer

Parnas O., Jovanovic M., Eisenhaure T. M., Herbst R. H., Dixit A., et al. A Genome-wide CRISPR Screen in Primary Immune Cells to Dissect Regulatory Networks. *Cell*. 2015;162: 675-686.
In this study, the authors introduced genome-wide pooled CRISPR-Cas9 libraries into dendritic cells (DCs) to detect the genes that control the induction of tumor necrosis factor (Tnf) by bacterial lipopolysaccharide (LPS). This is an important process in host response, which is mediated by the Tlr4 pathway. Through RNA sequencing, they identified many of the regulators of Tlr4 signaling, as well as many previously unknown candidates. Based on protein and mRNA in DCs that are deficient in the known or candidate genes, they divided the genes in three functional modules with distinct effects on the canonical responses to LPS, highlighting functions for the PAF complex and the oligosaccharyltransferase (OST) complex.

Illumina Technology: Nextera® XT DNA Sample Preparation Kit, HiSeq 2500 System

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79. Reardon S. Gene-editing record smashed in pigs. *Nature*. 2015;
80. Yang L, Guell M, Niu D, et al. Genome-wide inactivation of porcine endogenous retroviruses (PERVs). *Science*. 2015;350:1101-1104.

Swiech L., Heidenreich M., Banerjee A., Habib N., Li Y., et al. In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9. *Nat Biotechnol*. 2015;33: 102-106.
The authors used adeno-associated viral (AAV) vectors to deliver SpCas9 and guide RNAs to target either a single gene (*Mecp2*) or multiple (*Dnmt1, Dnmt3a, and Dnmt3b*) genes in adult mice brains *in vivo*. They then characterized the effects of these genomic edits in post-mitotic neurons with behavioral, electrophysiological, biochemical, and genetic approaches. Their study demonstrates the usefulness of AAV-mediated SpCas9 genome editing to enable reverse functional studies in mice brain *in vivo*.

Illumina Technology: Nextera XT DNA Sample Preparation Kit, MiSeq Sequencer

Weber J., Ollinger R., Friedrich M., Ehmer U., Barenboim M., et al. CRISPR/Cas9 somatic multiplex-mutagenesis for high-throughput functional cancer genomics in mice. *Proc Natl Acad Sci U S A*. 2015;112: 13982-13987.
In this work, the authors used hepatic delivery of sgRNAs and CRISPR-Cas9 to induce targeted somatic multiplex mutagenesis. They were able to induce hepatocellular carcinomas (HCC) and intrahepatic cholangiocarcinoma (ICC). They observed Darwinian selection of target genes, which suppressed tumorigenesis in the respective cellular or tissue context. With this study, they demonstrated the utility of multiplexed CRISPR-Cas9 for recessive genetic screening or high-throughput cancer validation in mice.

Illumina Technology: MiSeq Sequencer

Yang L., Guell M., Niu D., George H., Lesha E., et al. Genome-wide inactivation of porcine endogenous retroviruses (PERVs). *Science*. 2015;350: 1101-1104.
Porcine organs are promising options for human transplantation. However, their use in the past has been subject to concerns about the transmission of porcine endogenous retroviruses (PERVs) to humans. After estimating a total number of 62 PERVs, the authors in this study eradicated all PERVs on a porcine kidney epithelial cell line (PK15). They used CRISPR-Cas9 to disrupt all copies of the PERV pol gene and demonstrated a >1000-fold reduction in PERV transmission to human cells.

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Applications in the Medical Field

The application of CRISPR-Cas9 gene editing may hold promise in the treatment of disease. As in other species, gene editing could be applied to human cells at both the germline and somatic cellular level. However, germline editing is subject to safety and ethical considerations. It has been put on hold by request of the scientific community itself.[81, 82, 83]

A recently approved phase 1 trial in China has been the first to inject engineered immune cells in individuals with metastatic non–small cell lung cancer that are nonresponsive to chemotherapy or radiotherapy.[84] This study will extract T cells from 10 participants' blood and use the CRISPR-Cas9 system to knock out the gene encoding the programed death protein 1 (PD-1), a checkpoint in the immune response. Edited cells will be then cultured, multiplied, and reintroduced into the individual's bloodstream.[85] In the United States, the NIH Recombinant DNA Advisory Committee (RAC) approved a similar proposal. This study will focus on individuals with myeloma, sarcoma, and melanoma who are terminally ill and nonresponsive to standard therapies. In addition to PD-1 knockout, the researchers will also insert a gene encoding a receptor for NY-ESO-1, a protein often (and only) expressed in cancer cells. The team will also knock out 2 gene segments that encode different parts of the main T-cell receptor in order to enhance the specificity of NY-ESO-1.[86, 87]

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Because of their novelty, both these trials are focused on safety (phase 1) and will be limited to a small number of individuals. Critically, both the procedures will screen the engineered cells for off-target edits before reinjecting them into the subjects.

The first demonstration of somatic gene editing in an adult mammal was carried out in murine hepatocytes. In this study, CRISPR-Cas9 was used to correct mutations associated with hereditary tyrosinemia[88] representing a proof-of-principle of CRISPR-Cas9 somatic editing in medicine. However, this study also highlighted the necessity of improvements in delivery methods to increase the rate of gene correction and to deliver transgenes in other tissues. Because of these reasons, as well as considerations of the efficacy and specificity of editing, scientists recommend caution as the technique is applied in the medical space.[89]

Kataoka K., Shiraishi Y., Takeda Y., Sakata S., Matsumoto M., et al. Aberrant PD-L1 expression through 3'-UTR disruption in multiple cancers. *Nature*. 2016;534: 402-406.
PD-1/PD-L1-mediated immune escape is a crucial mechanism in cancer development. Through whole-genome sequencing of 49 cases of adult T-cell lymphoma (ATL), the authors demonstrated that immune escape is partly mediated through structural variations that disrupt the 3' region of the gene encoding PD-L1. RNA-Seq revealed that these variations are associated with an elevation of aberrant PD-L1 transcripts that are stabilized by the truncation. By using CRISPR-Cas9 technology, they showed that the disruption of the 3' untranslated region (3'-UTR) of the PD-L1 gene in mice leads to immune evasion of tumor cells. These results demonstrate a new regulatory mechanism for PD-L1 expression and suggest that the 3'-UTR could serve as a genetic marker to detect those cancers that evade antitumor immunity through PD-L1 overexpression.

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Yin H., Song C. Q., Dorkin J. R., Zhu L. J., Li Y., et al. Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. *Nat Biotechnol*. 2016;34: 328-333.
In this study, the authors combined lipid nanoparticle-mediated delivery of Cas9 mRNA with adeno-associated viruses (AAV) encoding a sgRNA and a repair template, with the aim of inducing repair of the gene in a mouse model of human hereditary tyrosinemia. They showed that the treatment generated fumarylacetoacetate hydrolase (Fah)-positive hepatocytes by correcting the causative Fah-splicing mutation. They used genome-wide, unbiased identification of DSBs enabled by sequencing (GUIDE-Seq) in cultured liver cells and targeted deep sequencing to monitor for off-target effects, and they found that the *in vivo* off-target rate was low for the proposed method.

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Agriculture and Environmental Science

CRISPR-Cas9 also holds great potential for agriculture, food science, and environmental science. In the past, gene editing in crops and animals has been applied only to large commodity species, such as maize and soybeans. This limitation is due to the technical and regulatory hurdles that are necessary to obtain approval for a genetically modified crop. The simplicity and low costs of CRISPR-Cas9 technology have already enabled scientists to apply it to different species, such as hexaploid bread wheat,[90] minipigs,[91] and mushrooms. Importantly, some engineered species will not be covered by the current regulatory process. For example, the recently engineered mushroom *Agaricus bisporus* does not contain exogenous genes and, therefore, does not require regulation by the US Department of Agriculture.[92]

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CRISPR-Cas9 technology also has potential applications in environmental science, particularly in gene-drive technology—a process through which an edited gene can propagate through a population, potentially wiping out disease. Some studies have suggested combining gene drives with CRISPR to eliminate disease-carrying mosquitoes or ticks.[93-96] However, as in the case of human germline editing, this approach has been put on hold in order to evaluate the potential risks of irreversibility or unexpected effects and to elaborate on proper regulation.[97]

Kistler K. E., Vosshall L. B. and Matthews B. J. Genome engineering with CRISPR-Cas9 in the mosquito Aedes aegypti. *Cell Rep*. 2015;11: 51-60.

In this study, the authors used the CRISPR-Cas9 system to edit the genome of the mosquito *Aedes aegypti*. Through RNA-DNA pairing, the system resulted in efficient editing and yielded high survival rates. The authors used deep sequencing to verify the presence of the edits, and they were able to integrate single- and double-stranded donors into the genome. The edits resulted in stable germline transmission of mutant alleles from one generation to the next.

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Guo R., Wan Y., Xu D., Cui L., Deng M., et al. Generation and evaluation of Myostatin knock-out rabbits and goats using CRISPR/Cas9 system. *Sci Rep*. 2016;6: 29855.

To investigate whether the gene encoding myostatin (Mstn) can be disrupted in livestock to improve meat quality, the authors applied CRISPR-Cas9 technology to generate Mstn-knockout rabbits and goats. In the 24 Mstn-knockout rabbits obtained, the authors observed morphological changes and severe health problems, such as stillbirth and early-stage death. The 1 out of 4 goats obtained outperformed the controls in early-stage growth. The authors recommend considering the potential health effects on animals before applying gene editing for modifying animal reproduction.

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Reviews

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WORKFLOW AND SPECIFICITY

The CRISPR-Cas9 genome-editing workflow is relatively simple. Scientists are able to achieve modifications in 1–2 weeks and derive modified clonal cell lines in 2–3 weeks.[98] This section will outline the steps in a genome-editing experiment, the tools available to check its efficiency and specificity, and the use of sequencing.

Overview of the Procedure

For a detailed explanation of the CRISPR-Cas9 gene-editing protocol, refer to the appropriate scientific literature.[99] Briefly, the procedure is divided into the following steps (Figure 5):

1. *Target selection for sgRNA* and design of reagents
2. *Construction of reagents*
3. *Delivery of sgRNA and Cas enzyme* to the required cells
4. *Design of repair template*: This is an optional step to leverage the HDR pathway over the NHEJ for more precise editing. It is required when a specific edit needs to be inserted in a defined location.
5. *Clonal isolation and expansion of modified cell lines*
6. *Functional testing and validation*
7. *Clonal expansion of desired cells*

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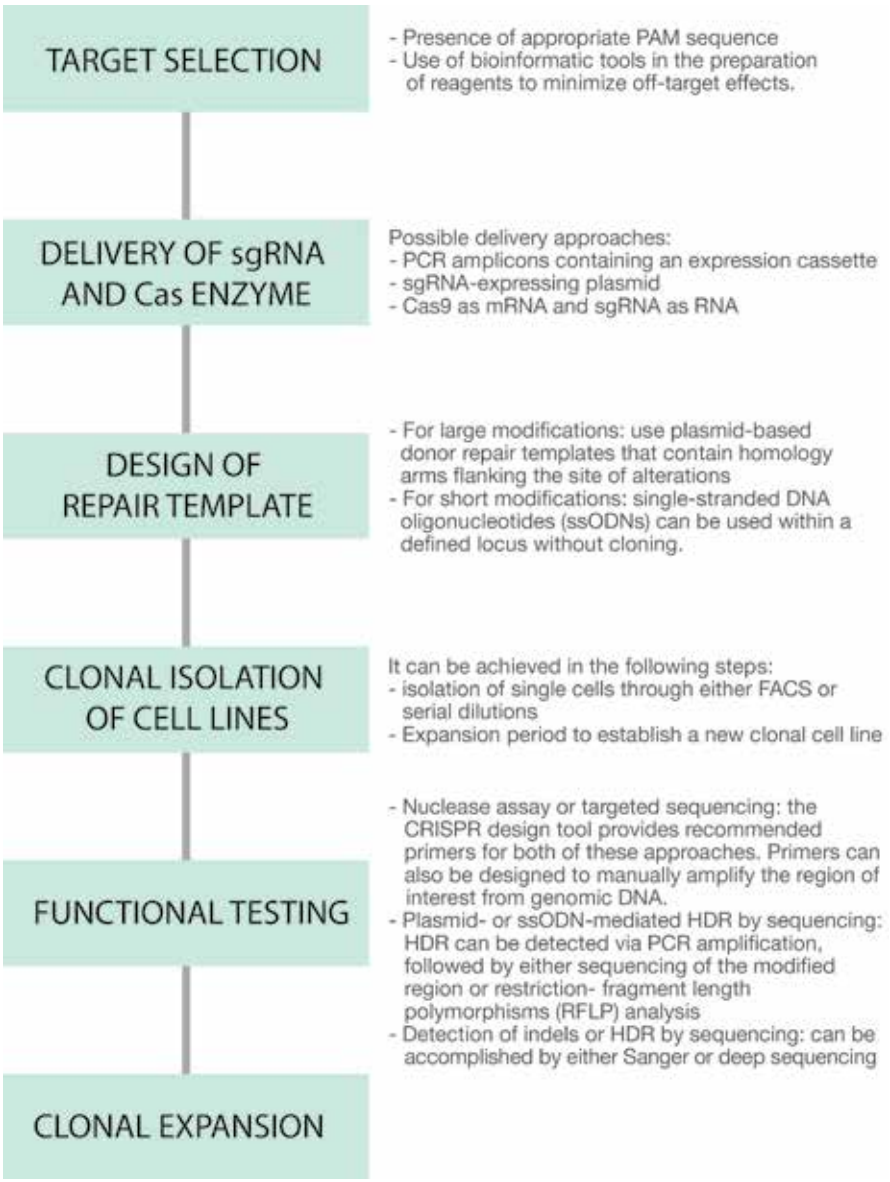

Figure 5. Workflow for genome editing using the CRISPR-Cas9 system.

On-Target Effects

A genome-editing experiment will result in a mixed population of cells. Some of these cells will not carry the desired mutation, some will carry a homozygous mutation, and some will carry heterozygous mutations of varying allele frequencies. Further, as the less precise NHEJ pathway is preferred endogenously over HDR, most edited cells will contain small indels. For this reason, the initial steps after an editing experiment are to detect those cells in which the desired mutation has occurred, isolate them, and expand the colony when the edit allows expansion. The methods to evaluate edits at the nucleotide level vary by intended edit and cell line but can generally be summarized as shown in Table 2.[100]

Mismatch cleavage assays use mismatch-sensitive nucleases to detect *single mismatches and indels*. Briefly, an initial PCR amplifies the region of interest in mutated and reference wild type cells. Following this initial amplification, the denaturation and reannealing of the DNA of interest leads to heteroduplexes containing a mismatch at the level of the mutation. These mismatches are then recognized by the mismatch-sensitive nucleases upon digestion. The SURVEYOR™ assay[101] is an example of this approach. Although this method is simple and cost-effective, it is not sensitive to frequencies below 5%, is low throughput, and does not provide sequence data.[102]

PCR amplification followed by restriction fragment length polymorphisms (RFLP) analysis is useful to analyze HDR insertions, while PCR assays with primers flanking the deleted region can quickly detect deletions. These methods are all cost-effective and have simple workflows; however, they are low throughput and do not provide sequence data.[103] Sanger sequencing provides accurate sequence data and can be used for any type of edit; however, it can be expensive, has a laborious workflow, and is not high throughput.[104]

NGS can assess all of the described edits in both a quantitative and qualitative fashion.[105] This approach is particularly useful when the number of samples is high, and when both on-target and off-target effects need to be validated.[106]

Table 2. Available methods to check editing efficiency

Assay	Type of Identified Variation	Results
Cleavage assay	Single mismatches and indels	Qualitative, semi-quantitative
PCR and targeted sequencing	HDR insertions	Qualitative
PCR and RFLP	HDR insertions	Quantitative
PCR	Deletions	Qualitative
NGS	All edits, genome-wide off-target effects	Quantitative + qualitative

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Specificity: What It Means and Why It Matters

The *on-target efficacy* is a measure of how well a sgRNA modifies the intended gene targets, while *off-target effects* are the unintended perturbation of genetic elements and global cellular responses.[107]

In general, the *specificity* of a gene-editing method is defined as its ability to avoid modifications where they are not required. It is inversely proportional to the number of off-target effects.

Off-target effects are dependent on the following factors:

- Position—The 3' end of the guide sequence is less tolerant of mismatches than the 5' end.
- Quantity—Depending on the cell and the edit, there is a maximum number of off-target effects beyond which the cell cannot survive.
- Guide Sequence—Some guides are less tolerant of mismatches than others.
- Off-Target Cleavage—It is sensitive to the ratios of reagents.
- Nuclease—Some nucleases are more specific than others.

Refer to the original publications for more details on each point.[108, 109]

The routine use of CRISPR-Cas9 genome editing should include strategies to successfully detect and reduce off-target effects.[110]

Best Practices for Evaluating Guide Specificity

Several approaches can be used to predict and evaluate guide RNA specificity and off-target effects (Table 3).[111, 112]

The most broadly adopted approach to detecting off-target effects is to use computational methods that predict where these sites could occur, in combination with a mismatch cleavage assay or targeted sequencing.[113, 114, 115] Bioinformatic methods may use either sequence similarity analysis or scoring systems based on other parameters to predict potential off-target cleavage sites *in silico*.[116, 117, 118] Publicly available programs are listed in Table 3. Refer to the original publications for details about each method.

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113. Fu Y, Foden JA, Khayter C, et al. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat Biotechnol*. 2013;31:822-826.
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Table 3. Publicly Available Software to Predict Off-Target Effects

Assay	Website	References
Benchling	https://benchling.com/	119, 120
Cas-OFFinder	http://www.rgenome.net/cas-offinder/	121
MIT CRISPR Design Tool	http://crispr.mit.edu/	122
Deskgen	https://www.deskgen.com/landing/	123
DNA 2.0 CRISPR gRNA Design Tool	https://www.dna20.com/products/crispr#4	124, 125
E-CRISP	http://www.e-crisp.org/E-CRISP/	126
EuPaGDT	http://grna.ctegd.uga.edu/	127
GenScript gRNA Design Tool	http://www.genscript.com/gRNA-design-tool.html	128
ZiFiT	http://zifit.partners.org/ZiFiT/	129, 130
Broad GPP Portal	http://portals.broadinstitute.org/gpp/public/	131
CROP-IT	http://cheetah.bioch.virginia.edu/AdliLab/CROP-IT/homepage.html	132

Targeted methods also include the *in vitro* interrogation of libraries of partially randomized target sites—based on the circularization of partially degenerate oligonucleotides—followed by rolling circle amplification, *in vitro* cleavage by SpCas9, ligation of adapters, and high throughput sequencing.[133] This approach has the advantage of checking for many diverse sets of sequence libraries that are similar to the target site. The disadvantage is that many of the randomized target sites may not occur in the genome of interest. The use of machine-learning algorithms partially addresses this limitation.[134]

Targeted approaches are biased by necessary *a priori* assumptions. Genome-wide approaches are necessary to discover off-target cleavage sites that might escape prediction algorithms.[135] These approaches can generally be divided into cell-based[136-143] and *in vitro*,[144, 145] methods (Table 4).[146, 147]

127. Doench JG, Fusi N, Sullender M, et al. Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nat Biotechnol*. 2016;34:184-191.
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Table 4. Unbiased Methods to Detect Off-Target Effects [148, 149]

Method	Type	Description	Advantage	Limitation	References
Integrase-defective lentiviral vector (IDLV) capture	Cell-based (live cells)	Integration of IDLVs with a selectable marker into DSB sites, recovery of integration sites through LAM-PCR, high-throughput sequencing	Can detect DSBs in living cells	Relatively insensitive	150, 151, 152
Genome-wide unbiased identification of DNBs enabled by sequencing (GUIDE-Seq)	Cell-based (live cells)	Integration of an end-protected dsODN into DSBs sites in living cells, tag-specific amplification, high-throughput sequencing	Simple, efficient, and precise, straightforward protocol, and availability of open-source software for data analysis	The efficient delivery of dsODN may be potentially harmful to cells, and it has not been tested *in vivo*	153, 154
High-throughput genome-wide translocation sequencing (HGTST)	Cell-based (live cells)	Generation of a prey and bait DSB through the expression of 2 nucleases, use of a biotinylated primer against the bait DSB junction, LAM-PCR to recover translocations between prey and bait, streptavidin-based enrichment, high-throughput sequencing	Requires the delivery of only the editing complex and can potentially be used *in vivo*	Nuclease-induced translocations are rare events and tend to occur between sites on the same chromosome	155, 156
Breaks labeling, enrichment on streptavidin and next-generation sequencing (BLESS)	Cell-based (fixed cells)	Isolation and fixation of treated cells, isolation and permeabilization of intact nuclei, *in situ* ligation of adapters to transient nuclease-induced DSBs, enrichment, high-throughput sequencing	Has been used in tissues where Cas9 had been delivered in vivo, independent from endogenous DNA repair machinery	Technically challenging, only allows identification of DSBs present at a specific moment, cannot detect DSBs before permeabilization	157
Digested genome sequencing (Digenome-Seq)	*In vitro*	Isolation of genomic DNA from *in vitro* treated cells, ligation of sequencing adapters, whole-genome sequencing	No limitations related to cell-based factors	Sequencing-inefficient, high background noise	158, 159

Frock R. L., Hu J., Meyers R. M., Ho Y. J., Kii E., et al. Genome-wide detection of DNA double-stranded breaks induced by engineered nucleases. *Nat Biotechnol*. 2015;33: 179-186.

In this study, the authors leverage a previously described emulsion PCR method to perform high-throughput, genome-wide, translocation sequencing (HTGTS) to detect DNA DSBs generated by nucleases across the human genome. This method is based on the identification of translocations between nuclease-induced and off-target DSBs. The application of HTGTS revealed that off-target hotspot numbers for given nucleases ranged from a few or none to dozens or more, and it extended the number of known off-target sites for certain previously characterized nucleases more than tenfold. The authors were also the first to detect translocations between nuclease targets on homologous chromosomes.

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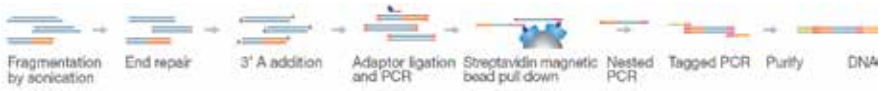

Figure 6. Schematic overview of HTGTS.

Tsai S. Q., Zheng Z., Nguyen N. T., Liebers M., Topkar V. V., et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nat Biotechnol*. 2015;33: 187-197.

The authors describe GUIDE-Seq, a method for the genome-wide identification of DNA DSBs introduced by CRISPR RNA-guided nucleases (RGNs). In the first stage, the method uses an end-joining process to tag RGN-induced DSBs in human cells by inserting a dsODN. The second stage uses unbiased amplification and NGS to map the dsODN integration sites. The application of GUIDE-Seq to 13 RGNs in human cell lines revealed wide variability in RGN off-target activities, most of which had not been identified with previously existing methods. GUIDE-Seq also identified RGN-independent genomic breakpoint hotspots. Finally, the authors also observed that truncated guide RNA led to a reduction in the number of RGN-induced off-target DSBs.

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Tsai S. Q., Topkar V. V., Joung J. K. and Aryee M. J. Open-source guideseq software for analysis of GUIDE-seq data. *Nat Biotechnol*. 2016;34: 483.

The authors developed guideseq, a Python package that enables the analysis of data from GUIDE-Seq experiments. Briefly, this program initially demultiplexes a pooled multisample run into sample-specific read files to prepare them for alignment. It then identifies off-target effects through alignment of reads, identification of sites, testing of false positives, and reporting. The guideseq package is open-source and can be downloaded at http://github.com/aryeelab/guideseq.

Illumina Technology: MiSeq Sequencer

Figure 7. Schematic overview of GUIDE-Seq.

Kim D., Bae S., Park J., Kim E., Kim S., et al. Digenome-seq: genome-wide profiling of CRISPR-Cas9 off-target effects in human cells. *Nat Methods*. 2015;12: 237-243, 231 p following 243.

The authors developed digested genome sequencing (Digenome-Seq), an *in vitro* method that enables the profiling of genome-wide Cas9 off-target effects in human cells. This method consists of digesting genomic DNA with purified Cas9-gRNA ribonucleoprotein (RNP) complexes *in vitro*, followed by deep sequencing. Cas-9 induced cleavage is then distinguished from random DNA breaks by detecting sites with relative enrichment of reads that possess the same start or end mapping positions. The authors validated the method on CRISPR-Cas9–engineered HAP1 cells, detecting off-target sites at which insertions or deletions were induced at frequencies less than 0.1%, barely within the detection limits of targeted deep sequencing. They then demonstrated that Cas9 off-target effects can be avoided by using modified sgRNAs.

Illumina Technology: HiSeq X Ten System

Kim D., Kim S., Kim S., Park J. and Kim J. S. Genome-wide target specificities of CRISPR-Cas9 nucleases revealed by multiplex Digenome-seq. *Genome Res*. 2016;26: 406-415.

The authors developed multiplex Digenome-Seq, to analyze the specificity of up to 11 CRISPR-Cas9 nucleases simultaneously, and a new DNA cleavage scoring system. They digested cell-free human genomic DNA with multiple sgRNAs and Cas9, performed whole-genome sequencing, and used the new system to detect cleavage patterns and on- and off-target sites. They observed that, while sgRNAs that were transcribed from an oligonucleotide duplex cleaved false-positive, bulge-type off-target sites, this result was not true for those transcribed from a plasmid template. They also observed that multiplex Digenome-Seq could detect several bona fide off-target effects that were missed by other genome-wide methods. Finally, after analyzing 964 cleavage sites, they provide guidelines on how to choose target sites in order to minimize off-target effects.

Illumina Technology: TruSeq DNA Sample Prep Kit, HiSeq X Ten System

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154. Tsai SQ, Topkar VV, Joung JK and Aryee MJ. Open-source guideseq software for analysis of GUIDE-seq data. *Nat Biotechnol*. 2016;34:483.
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Figure 8. Schematic overview of Digenome-Seq.

Osborn M. J., Webber B. R., Knipping F., Lonetree C. L., Tennis N., et al. Evaluation of TCR Gene Editing Achieved by TALENs, CRISPR/Cas9, and megaTAL Nucleases. *Mol Ther*. 2016;24: 570-581.

The authors developed a method that leverages the propensity of DNA double strand breaks (DSBs) to incorporate double stranded integrase-defective lentiviral vectors (IDLVs) by a nonhomologous end joining mechanism. They then test this method, they then target two regions on HEK293T cell lines with CRISPR-Cas9 and TALEN assays. They found frequent off-target effects with a one-base bulge or up to 13 mismatches between the sgRNA and its genomic target.

Illumina Technology: MiSeq Sequencer

Wang X., Wang Y., Wu X., Wang J., Wang Y., et al. Unbiased detection of off-target cleavage by CRISPR-Cas9 and TALENs using integrase-defective lentiviral vectors. *Nat Biotechnol*. 2015;33: 175-178.
In this study, the authors used three different TCR-α-targeted nucleases to disrupt T-cell receptor in primary human T cells and test for off-target effects using the integrase-defective lentiviral vectors (IDLV) approach. They found that megaTAL and CRISPR-Cas9 reagents had the highest disruption efficiency combined with lower levels of toxicity and off-target effects. They then propose a manufacturing process to produce safe cellular substrates for next-generation immunotherapies.

Illumina Technology: MiSeq Sequencer

Method Development

CRISPR-Cas9 technology has a variety of applications as a gene editing tool. CRISPR has utility in both cell lines and animal models at the somatic or germline level. However, given the issue of off-target effects, efforts are needed to develop methods to increase specificity, as well as to look for potential alternative editing technologies.[160, 161]

In general, the studies that aim to reduce CRISPR's off-target effects focus on 2 approaches:

- *Increasing the specificity of the nuclease cleavage*, either engineering Cas9 or replacing it with another enzyme[162-165]
- *Reducing the duration of the nuclease expression*, limiting its time to accumulate off-target mutations[166, 167]

As knowledge about the mechanistic function of the process increases, it is likely that new strategies to improve specificity will become available.

Abudayyeh O. O., Gootenberg J. S., Konermann S., Joung J., Slaymaker I. M., et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science. 2016;353: aaf5573.
The authors characterized the class 2 type IV CRISPR-Cas effector C2c2 from *Leptotrichia shahii*, which provides protection against RNA phages *in vivo*. Through a biochemical analysis performed *in vitro*, they demonstrated that C2c2 can be programmed to cleave single-stranded RNA targets with the use of a single CRISPR RNA guide. The cleavage was mediated by catalytic residues in the 2 conserved higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domains. Mutations that targeted these domains generated catalytically inactive RNA-binding proteins.

Illumina Technology: MiSeq Sequencer

Davis K. M., Pattanayak V., Thompson D. B., Zuris J. A. and Liu D. R. Small molecule-triggered Cas9 protein with improved genome-editing specificity. *Nat Chem Biol*. 2015;11: 316-318.
The authors developed Cas9 enzymes that can be activated by small molecules, by insertion of an evolved 4-hydroxytamoxifen (4-HT)-responsive intein at a specific position in Cas9. They then tested their method in human cells and used deep sequencing to monitor its performance. They demonstrated that the conditionally active Cas9 had an editing activity with a specificity up to 25-fold higher than wild-type Cas9.

Illumina Technology: MiSeq Sequencer

Kim D., Kim J., Hur J. K., Been K. W., Yoon S. H., et al. Genome-wide analysis reveals specificities of Cpf1 endonucleases in human cells. *Nat Biotechnol*. 2016;34: 863-868.
To test whether CRISPR-Cpf1 could replace CRISPR-Cas9, improving the precision of gene editing, the authors used a mismatched crRNA. They found that Cpf1 could tolerate single or double mismatches in the 3'-protospacer-adjacent motif (PAM) distal region, but not in the 5'-PAM proximal region. They then used Digenome-Seq to perform a genome-wide analysis of cleavage sites for 8 Cpf1 nucleases. Of these, *Lachnospiraceae bacterium* Cpf1 (LbCpf1) and *Acidaminococcus sp* Cpf1 (AsCpf1) cut 6 and 12 sites in the human genome, while Cas9 nucleases generally cut over 90 sites. Most of the identified Cpf1 cleavage sites did not produce mutations in cells.

Illumina Technology: TruSeq DNA Sample Prep Kit, HiSeq X Ten System

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Kleinstiver B. P., Tsai S. Q., Prew M. S., Nguyen N. T., Welch M. M., et al. Genome-wide specificities of CRISPR-Cas Cpf1 nucleases in human cells. Nat Biotechnol. 2016;34: 869-874.
The authors found that AsCpf1 and LbCpf1 have on-target editing accuracies in human cells that are comparable to that of *Streptococcus pyogenes* Cas9. Through GUIDE-Seq and targeted deep sequencing analysis performed on both nucleases, they found no off-target effects for most of the crRNAs used. Their results suggest that AsCpf1 and LpCpf1 are highly specific in human cells.

Illumina Technology: MiSeq Sequencer

Komor A. C., Kim Y. B., Packer M. S., Zuris J. A. and Liu D. R. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*. 2016;533: 420-424.
CRISPR-Cas9 genome editing relies on the generation of targeted DSBs and 1 of 2 repair mechanisms: either NHEJ or HDR, in the presence of a homologous template. These mechanisms are a limitation when it is necessary to correct a point mutation, as in many genetic diseases. In this study, the authors developed an approach that enables the direct conversion of a target DNA base to another in a programmable manner, without requiring DSBs or a donor template. To do so, they fused CRISPR-Cas9 and a cytidine deaminase enzyme that can mediate the direct conversion of cytidine to uridine, thereby enabling C -> T (or G -> A) substitutions.

Illumina Technology: MiSeq Sequencer

Nishida K., Arazoe T., Yachie N., Banno S., Kakimoto M., et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. *Science*. 2016; .
In this study, the authors engineered the nuclease-deficient type II CRISPR-Cas9 and the activation-induced cytidine deaminase (AID) ortholog PmCDA1 to create a synthetic complex (Target-AID). They used it to demonstrate the targeted single-nucleotide substitution of DNA. Using deep sequencing, they showed the high specificity of Target-AID in performing targeted mutagenesis. Target-AID induced specific point mutations mainly at cytidines within the target range of 5 bases. The authors further improved efficiency by using uracil DNA glycosylase inhibitor (UGI) to suppress indel formation.

Illumina Technology: MiSeq Sequencer

Slaymaker I. M., Gao L., Zetsche B., Scott D. A., Yan W. X., et al. Rationally engineered Cas9 nucleases with improved specificity. *Science*. 2016;351: 84-88.
The authors used structure-guided engineering to increase the specificity of *Streptococcus pyogenes* Cas9 by inserting individual alanine substitutions at 31 positively charged residues within the non–target-strand groove of the enzyme. They then used both targeted and whole-genome deep sequencing (breaks labeling, enrichment on streptavidin and NGS, or BLESS) to assess on-target activity and specificity. They demonstrated that the "enhanced specific" eSpCas9 variants they created were able to edit genomes in human cells while maintaining a robust on-target activity.

Illumina Technology: TruSeq Nano LT Kit

Zetsche B., Volz S. E. and Zhang F. A split-Cas9 architecture for inducible genome editing and transcription modulation. *Nat Biotechnol*. 2015;33: 139-142.
Strategies using catalytically inactive Cas9 are useful to direct the protein to the correct target and regulate transcription. In this study, the authors created a split-Cas9 system in which the 2 fragments can be chemically induced to dimerize and are activated in presence of rapamycin. They then tested the system in HEK293 cells and observed that it reduces off-target effects when the split enzyme is expressed from an integrated low-copy lentiviral vector.

Illumina Technology: MiSeq Sequencer

Reviews

Kim J. S. Genome editing comes of age. *Nat Protoc*. 2016;11: 1573-1578

Tsai S. Q. and Joung J. K. Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases. *Nat Rev Genet*. 2016;17: 300-312

Tycko J., Myer V. E. and Hsu P. D. Methods for Optimizing CRISPR-Cas9 Genome Editing Specificity. *Mol Cell*. 2016;63: 355-370

Shalem O., Sanjana N. E. and Zhang F. High-throughput functional genomics using CRISPR-Cas9. *Nat Rev Genet*. 2015;16: 299-311

Integration of CRISPR-Cas9 Technology in a Research Workflow

CRISPR-Cas9 genome editing now allows scientists to perform precise genomic manipulations quickly and conveniently, in order to screen for multiple mutations *in vitro* or *in vivo*. Researchers can then use a vast repertoire of sequencing methods to determine the impact of the edited sequence on the structure and function of the genes. For example, ChIP-Seq[168] can determine the impact of mutations on DNA-protein binding. When the protein is a transcription factor, researchers can study the impact on expression (RNA-Seq)[169, 170, 171] RNA structure (chemical inference of RNA structures by sequencing, CIRS-Seq),[172] protein binding (RNA immunoprecipitation sequencing, RIP-Seq; crosslinking immunoprecipitation sequencing, CLIP-Seq)[173, 174] and modification or splicing (RNA-mediated oligonucleotide annealing, selection, and ligation with NGS, RASL-Seq).[175] Alternatively, protein coding regions can be systematically edited to determine the structure and function of the encoded protein. For example, if the protein is a methylation factor, the downstream impact of changes in methylation can be measured directly through whole-genome bisulfite sequencing (BS-Seq);[176] additionally, scientists can study its impact on protein binding (ChIP-Seq) or on the conformation of the chromatin (Hi-C).[177] This ability to make rapid and precise changes to the genome, followed by careful measurements of the implications on the gene and ultimately cellular functions, will profoundly change how genomes are studied.

Flavahan W. A., Drier Y., Liau B. B., Gillespie S. M., Venteicher A. S., et al. Insulator dysfunction and oncogene activation in IDH mutant gliomas. *Nature*. 2016;529: 110-114.

In this study, the authors used ChIP-Seq and BS-Seq to demonstrate that human mutant IDH gliomas exhibit hypermethylation at binding sites for cohesion and CCCTC-binding factor (CTCF), compromising its binding ability. They then used high-throughput chromatin capture maps, to detect structural and regulatory domains, and RNA-Seq, to demonstrate that genes in the same domains correlate across samples while genes separated by a boundary show less correlation. They demonstrated that loss of CTCF at a domain boundary permits a constitutive enhancer to interact aberrantly with the receptor tyrosine kinase gene *PDGFRA*, a prominent glioma oncogene. Through chromosome-conformation capture, the authors demonstrated that loss of CTCF at a domain boundary permits a constitutive enhancer to interact aberrantly with *PDGFRA*. Using CRISPR-Cas9 technology, they also showed that disruption of the CTCF motif in IDH wild-type gliomaspheres upregulates *PDGFRA* and increases proliferation.

Illumina Technology: NextSeq 500 Sequencer

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Wu J., Huang B., Chen H., Yin Q., Liu Y., et al. The landscape of accessible chromatin in mammalian preimplantation embryos. *Nature*. 2016;534: 652-657.
In this study, the authors reconstructed the spatiotemporal chromatin configurations that accompany mouse development. They combined the assay for transposase-accessible chromatin using sequencing (ATAC-Seq) with CRISPR-Cas9–assisted mitochondrial DNA depletion. They also used switching mechanism at 5' end of RNA template (Smart-Seq) to validate each phase of RNA-Seq, and ChIP-Seq to investigate histone modifications. Despite extensive parental asymmetry in DNA methylomes, the authors observed that chromatin accessibility between the parental genomes is globally comparable after major zygotic genome activation (ZGA). Accessible chromatin in early embryos is widely shaped by transposable elements and overlaps extensively with putative cis-regulatory sequences. Accessible chromatin was also found near the transcription end sites of active genes. They constructed the regulatory network of early development by integrating the maps of cis-regulatory elements and single-cell transcriptomes. The authors observed that the activities of cis-regulatory elements and their associated open chromatin diminished before major ZGA.

Illumina Technology: HiSeq 1500 Sequencer, HiSeq 2500 Sequencer

Dixit A., Parnas O., Li B., Chen J., Fulco C. P., et al. Perturb-Seq: Dissecting Molecular Circuits with Scalable Single-Cell RNA Profiling of Pooled Genetic Screens. *Cell*. 2016;167: 1853-1866 e1817.
The authors developed Perturb-seq, a method that combines single-cell RNA-Seq (scRNA-Seq) and CRISPR-based perturbations to perform genetic screens to infer gene function on complex phenotypes in pools of mammalian cells. Briefly, this method uses pools of lentiviral constructs encoding sgRNAs to infect cells. These lentiviral vectors also report on the identity of the expressed sgRNA by an expressed guide barcode (GBC). Infected cells are grown, differentiated, and/or stimulated. ScRNA-Seq is then used to tag each cell's RNA with a unique cell barcode (CBC) that associates the cell's transcriptional profile with the delivered genetic perturbation (or perturbations) encoded by the GBC. The authors then applied Perturb-Seq to 200,000 immune cells or cell lines, with a focus on transcription factors regulating the response of dendritic cells to lipopolysaccharide. They were able to detect individual gene targets, gene signatures, and cell states affected by single genetic perturbations and their epistatic effects, demonstrating the power of Perturb-Seq to increase the scope of pooled genomic assays.

Illumina Technology: MiSeq Sequencer, NextSeq Sequencer

Adamson B., Norman T. M., Jost M., Cho M. Y., Nunez J. K., et al. A Multiplexed Single-Cell CRISPR Screening Platform Enables Systematic Dissection of the Unfolded Protein Response. *Cell*. 2016;167: 1867-1882 e1821.
Perturb-Seq is a method that allows the parallel phenotypic screening from single cells by delivering and detecting up to three CRISPR perturbations. The authors applied Perturb-Seq to perform a genome-wide screening to dissect the mammalian unfolded protein response (UPR) using single and combinatorial CRISPR perturbation. They performed two genome-scale CRISPR interference screens detecting genes whose repression perturbs ER homeostasis. Perturb-Seq on ~100 hits enabled high-precision functional clustering of genes, providing insights into how the three sensors of ER homeostasis react to different stress types. This study highlights the ability of Perturb-Seq to dissect complex phenotypes.

Illumina Technology: MiSeq Sequencer, NextSeq Sequencer

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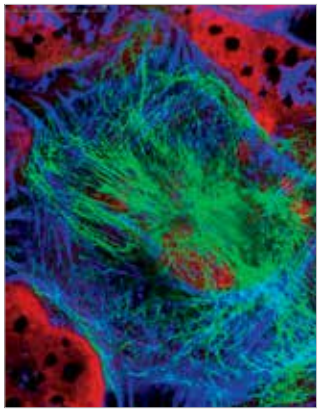
Genomic Solutions for Cell Biology and Complex Disease Research

Complex diseases are the result of multiple genetic and environmental factors. They are distinguished from Mendelian traits (or simple traits) as they do not follow a specific model of inheritance and are usually more frequent in the population. Although some of these diseases are highly heritable, currently known genetic variants can explain only some of the estimated heritability. This review gives a general overview on how genomic technologies and NGS can help in the study of complex diseases.



Single-cell Research

Much of the initial impetus for single-cell tissue sequencing has come from cancer, but single-cell approaches are currently used to improve our understanding of other complex biological systems including the central nervous system, immune system, and mammalian development. Single-cell sequencing is also an effective approach to characterize organisms that are difficult to culture in vitro. This review highlights recent publications demonstrating how Illumina technology is being used in single-cell sequencing applications and techniques.

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