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Applications of the CRISPR-Cas9 system in cancer biology

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Preface

The prokaryotic type II clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system is rapidly revolutionizing the field of genetic engineering, allowing researchers to alter the genomes of a large variety of organisms with relative ease. Experimental approaches based on this versatile technology have the potential to transform the field of cancer genetics. Here we review current approaches based on CRISPR-Cas9 for functional studies of cancer genes, with emphasis on its applicability for the development of the next-generation models of human cancer.

Cancer is a disease characterized by multiple genetic and epigenetic alterations in oncogenes and tumor suppressor genes[1]. Therefore, experimental approaches to manipulate the genomes of normal and cancer cells are critical for modeling the disease as well as systematically studying the many genes involved in the process. Decades of research and development of genome engineering technologies have made it possible to precisely delete, or otherwise modify, specific DNA sequences in the genomes of cells in culture or of animal models to explore the role of genes implicated in cancer initiation, progression and therapeutic response. Pioneering work by Mario Capecchi, Oliver Smithies, and others on gene targeting in embryonic stem (ES) cells via homologous recombination[2–4] provided the scientific community the means to generate numerous genetically-engineered mouse models (GEMMs) harboring precise mutations in tumor suppressors and oncogenes as well as cell lines with defined loss-of-function or gain-of-function alterations in genes that are relevant to cancer biology. Moreover, this technology has been successfully employed in combination with site-specific recombinases, such as Cre and Flp, to generate conditional alleles of a large number of cancer genes[5]. Although a mainstay of cancer genetics over the past two decades, these gene modification approaches have been limited by the relatively low efficiency of gene targeting by homologous recombination and the time required for ES cell manipulation and subsequent mouse breeding.

One strategy to increase the efficiency of gene targeting is to introduce DNA double-strand breaks (DSBs) at the genomic locus of interest[6–10]. These DSBs are repaired by cellular DNA repair pathways, particularly by the error-prone non-homologous end joining (NHEJ)

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pathway, which frequently leads to insertion or deletion mutations (indels). DSBs are also repaired by the homology-directed repair (HDR) pathway, which can mediate precise DNA modifications in the presence of exogenous donor DNA templates (Figure 1A). Subsequent studies based on these initial observations led to the development of improved site-specific genome engineering methods, of which zinc finger nucleases (ZFNs)[10–12] and transcription activator-like effector nucleases (TALENs)[13–16] have been extensively utilized in a variety of cell types and organisms (reviewed in[17,18]). ZFNs and TALENs greatly facilitated precise genome engineering; however, their widespread adoption has been limited by the cost and complexity of designing these custom-built endonucleases.

The recently described prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR)-Cas system and the successful implementation of the *Streptococcus pyogenes*-derived type II CRISPR-Cas9 system in mammalian cells by the Zhang[19], Church[20], Doudna[21] and Kim[22] groups has rapidly changed the landscape of genome engineering by addressing many of the limitations of earlier methods. This highly versatile system, which is derived from a prokaryotic adaptive immune system, is composed of two biological components: the RNA-guided DNA endonuclease Cas9 and a chimeric single guide RNA (sgRNA). The sgRNA molecule contains both a CRISPR RNA (crRNA) component and a *trans*-activating crRNA (tracrRNA) component, which binds to Cas9 and directs it to a genomic sequence of interest via base pairing to the target sequence[23] (Figure 1B). The only criterion defining the target sequence is that it be adjacent to a protospacer adjacent motif (PAM), consisting of either an NGG or NAG trinucleotide[24] for *S. pyogenes*-derived Cas9 (of note, other Cas9 orthologues recognize different PAM sequences[25,26]). By simply combining the expression of Cas9 with an sgRNA complementary to a target DNA sequence, one can achieve high efficiency cleavage of the target, leading to DSBs, which then get repaired via NHEJ or HDR (Figure 1B). Numerous studies published over just the last few years have demonstrated efficient gene disruption and gene modification in a variety of cells and organisms via CRISPR-Cas9-mediated NHEJ or HDR, respectively (reviewed in[27]).

In this Progress article, we discuss several recent applications of the CRISPR-Cas9 system, with particular emphasis on approaches that promise to transform the field of cancer biology by facilitating the engineering of normal and cancer genomes.

Rapid modeling of genetic events

In the current era of cancer genomics, several large-scale cancer genome sequencing efforts have produced an expanding catalogue of the genetic alterations present in human tumors[28]. Amongst a background of so-called passenger mutations, which are presumed not to directly affect the tumorigenic process, driver mutations directly or indirectly promote the transformation of normal cells to cancer cells through mutational activation of oncogenes and/or inactivation of tumor suppressor genes. Oncogenes are typically activated via gain-of-function mutations whereas tumor suppressor genes are usually inactivated via loss-of-function mutations.

Moderate to large-scale functional genetic studies aimed at dissecting the role of putative oncogenes and tumor suppressor genes in cell culture, xenografts, allografts and, in some cases, transgenic mouse models have traditionally relied on cDNA-based overexpression and RNA interference (RNAi)-mediated knockdown approaches. While these approaches have led to many important discoveries in cancer biology over the last several years, they have a number of important limitations. First, cDNA-based expression systems can lead to supraphysiological levels of gene expression[29], which might cause aberrant and artifactual effects on signaling pathways and cell biological processes. RNAi-based inactivation approaches are limited by the uncertainty of the degree of gene silencing and the stability of the inhibition. This is not problematic for some targets or experimental protocols, but for others complete and permanent inactivation is required to obtain consistent results. RNAi-based approaches can also suffer from substantial off-target effects. The deployment of the CRISPR-Cas9 system for targeted modification of endogenous loci offers a rapid method for overcoming these limitations. In addition to simplifying the study of oncogenes and tumor suppressor genes, the CRISPR-Cas9 system also allows for rapid discrimination between driver and passenger mutations.

Permanent Cas9-mediated modification of single or multiple endogenous loci can be achieved via transient or stable delivery of the CRISPR components. Several groups have reported successful editing of endogenous genes in cells in culture via transient transfection of plasmid DNA encoding Cas9 and sgRNAs[19–22] or Cas9-sgRNA ribonucleoprotein complexes (RNPs)[30,31]. Alternatively, CRISPR components can be stably delivered into cells through the use of retroviruses or lentiviruses[32,33]. To engineer loss-of-function mutations, one relies on NHEJ, which often results in the generation of indels near the Cas9 cleavage site that frequently lead to frameshift mutations. Engineering gain-of-function mutations requires the inclusion of an HDR template in the form of single-stranded or double-stranded DNA carrying the desired mutation (Figure 1B and Box 1). Transient expression of the CRISPR components offers the advantage of a hit-and-run strategy, which should allow for unlimited serial editing of endogenous genes without the need of multiple viral integrations or continuous expression of CRISPR components. Cell lines carrying one or more targeted mutations can then be tested using a battery of cell-based and *in vivo* assays to examine the effects of the mutation(s) on cancer-associated phenotypes. This approach can be used on established cancer cell lines, primary cell lines obtained from mouse or human origins, as well as patient-derived xenografts and organoid cultures, among others (Box 1). Moreover, this technology should allow for systematic analysis of epistatic interactions and comprehensive dissection of oncogenic signaling pathways via sequential or multiplex gene editing. In addition to allowing the functional characterization of true cancer genes, such studies can also help rule out a functional effect of a passenger mutation on cancer initiation and progression. Several review articles[27,34] have recently described in detail most applications of the CRISPR-Cas9 system for genome engineering. We have summarized these applications in Boxes 1–3 and will focus below on the utility of this technology for generating animal models for the study of cancer genes *in vivo*.

Rapid generation of mouse models

Genetically-engineered mouse models (GEMMs)[5] and non-germline GEMMs (nGEMMs)[35] of cancer have played a critical role in uncovering several fundamental aspects of tumor initiation, maintenance and progression. In addition, they have emerged as faithful models with which to test a variety of anti-cancer agents, as well as for uncovering mechanisms of drug resistance[36,37]. However, generating GEMMs is a slow and expensive process, requiring complex ES cell manipulation and/or pronuclear injection, as well as extensive mouse husbandry to obtain animals harboring the alleles of interest[35]. nGEMMs of cancer can simplify this process by bypassing the need for complex genetic crosses through the serial re-targeting of ES cells[35]. Nevertheless, the inability to simultaneously introduce multiple genetic modifications in mice or ES cells remains a considerable barrier.

Jaenisch and colleagues have recently demonstrated that the CRISPR-Cas9 system can be utilized to simultaneously disrupt up to eight alleles in mouse ES cells in a single step[38]. Furthermore, they reported efficient simultaneous disruption of two genes in single-cell mouse embryos and the subsequent one-step generation of double knockout animals[38]. This group also demonstrated efficient simultaneous HDR-mediated genome editing of two endogenous genes[38]. In a subsequent study, they extended their CRISPR-Cas9 methods for rapidly generating mice carrying conditional Cre/loxP-based alleles and reporter alleles, as well as using pairs of sgRNAs to generate mice carrying small deletions[39] (Box 1). These studies have demonstrated the ease with which ES cells or mice harboring multiple gain-of-function and loss-of-function mutations can be generated, an advance that has opened the door for the development of novel GEMMs and nGEMMs of cancer with unprecedented speed and precision. Indeed, we predict that there will be an explosion of novel GEMMs and nGEMMs harboring uniquely complex genetic alterations that will allow for detailed analysis of several stages of tumor evolution with unprecedented speed and efficiency (Figure 2A). For example, CRISPR-mediated engineering will allow for rapid generation of large repositories of ES cell lines harboring multiple combinations of constitutive or conditional mutations in oncogenes and tumor suppressor genes, as well as large chromosomal rearrangements that will capture some of the genetic heterogeneity that is characteristic of human cancer genomes. These ES cell lines can be utilized to generate GEMMs and nGEMMs of cancer harboring multiple distinct mutant genotypes, which will be highly valuable for testing new therapeutic regimens and for personalized oncology efforts.

It is important to note that the majority of mouse cancer models have been based on a rather limited number of mutant genes or alleles, such as the G12D or G12V mutations in the *Kras* oncogene[40,41]. The CRISPR-Cas9 system will allow for systematic generation of models harboring multiple oncogenic alleles, making it possible to investigate allele-specific consequences in tumor progression and therapeutic response. Highly systematic and multiplexable approaches for HDR-mediated editing of specific genomic regions, such as the methods developed by the Shendure laboratory[42], will facilitate rapid analysis of ‘hotspot’ regions with various combinations of mutations and subsequent generation of GEMMs and nGEMMs.

Beyond new model development, the CRISPR-Cas9 system can also be used to refine existing models of cancer. ES cell lines derived from well-studied GEMMs can be readily reengineered to harbor additional constitutive or conditional mutant alleles of oncogenes and tumor suppressor genes[43] (Figure 2A). Thus, candidate cooperating mutations can be easily studied and putative synthetic lethal interactions can be validated. Moreover, this approach will allow for pre-clinical studies consisting of cohorts of mice that better represent the genetic heterogeneity of human cancers (Figure 2A). One can even envision combining comprehensive genomic characterization of tumors from individual patients with the rapid generation of personalized GEMMs, nGEMMs or cell-based xenografts. *In vivo* models carrying the exact complement of driver mutations from a given patient's tumor could then be screened with conventional or experimental anti-cancer agents to identify the most effective therapies.

Somatic genome engineering

As outlined above, the efficiency of genome editing by CRISPR-Cas9 makes the process of germline and ES cell line genetic manipulation more rapid and more powerful. The power of the system is even more evident in the ability to perform somatic genome editing *ex vivo* and *in vivo*.

Ex vivo CRISPR-based somatic genome editing for modeling cancer in vivo

Three recent studies have demonstrated the power of CRISPR-based *ex vivo* somatic genome editing for rapid modeling of cooperating mutations and the generation of mouse models of haematopoietic malignancies[32,44,45]. The Pelletier group demonstrated efficient *ex vivo* editing of the *Trp53* tumor suppressor gene in *Arf*$^{-/-}$Eμ-*Myc* lymphomas that were subsequently transplanted into syngeneic mice to show that *Arf*$^{-/-}$Eμ-*Myc* cells lacking p53 are substantially enriched upon treatment with doxorubicin[32]. Using a similar approach, the Lowe laboratory utilized *ex vivo* CRISPR-mediated disruption of the *Mll3* (also known as *Kmt2c*) tumor suppressor gene in *shNf1;Trp53*$^{-/-}$ primary mouse haematopoietic stem and progenitor cells (HSPCs) to demonstrate that *Mll3* is a haploinsufficient tumor suppressor in acute myeloid leukemia (AML)[44]. The Ebert group employed the CRISPR-Cas9 system to rapidly generate mouse models of AML by lentiviral-mediated *ex vivo* editing of single or multiple genes in primary mouse haematopoietic stem and progenitor cells[45]. These three studies highlight the potential of the CRISPR-Cas9 system for *ex vivo* somatic genome editing of primary cells, which can be further exploited for the rapid generation of mouse models of a variety of human malignancies (Figure 2B).

In vivo CRISPR-based somatic genome editing for modeling cancer

To explore the use of the CRISPR-Cas9 system for directly mutating genes in living animals, our laboratory utilized hydrodynamic gene transfer to simultaneously deliver plasmids encoding Cas9 and sgRNAs targeting the *Pten* and *Trp53* tumor suppressor genes to hepatocytes *in vivo*[46]. Strikingly, delivery of these CRISPR plasmids to the hepatocytes of adult wild-type mice was sufficient to induce liver tumors with identical histopathology to those observed in *Pten*$^{fl/fl}$*;Trp53*$^{fl/fl}$ GEMMs, in which tumors were initiated via delivery of adenoviruses expressing Cre recombinase. These results strongly suggest that CRISPR-

mediated somatic genome editing of cancer genes in adult wild-type mice can efficiently substitute for traditional GEMMs, at least for some cancer types. Moreover, we further demonstrated the feasibility of using the CRISPR-Cas9 system to engineer gain-of-function mutations in the livers of adult wild-type mice via the co-delivery of CRISPR components and a single-stranded DNA template encoding a mutant form of β-catenin, which resulted in the generation of hepatocytes with nuclear β-catenin at a low (0.5%) but detectable frequency[46].

Moving beyond the liver, we also developed an all-in-one lentivirus simultaneously encoding CRISPR components and Cre recombinase. This vector was used to mutate three lung cancer tumor suppressor genes in the developing tumors of the well established *Kras*$^{LSL\text{-}G12D/+}$ and *Kras*$^{LSL\text{-}G12D/+}$;*Trp53*$^{flox/flox}$ GEMMs of lung cancer[40,47,48]. Intratracheal delivery of all-in-one lentiviruses expressing sgRNAs targeting a panel of tumor suppressor genes into *Kras*$^{LSL\text{-}G12D/+}$ or *Kras*$^{LSL\text{-}G12D/+}$;*Trp53*$^{flox/flox}$ mice resulted in lung adenocarcinomas with diverse histopathological and molecular features that depended on the tumor suppressor gene targeted. Moreover, a large fraction of the lung tumors harboured indels in predicted sites within the target genes with no detectable off-target editing, strongly supporting Cas9 on-target activity for somatic genome editing *in vivo*. In a parallel study, the Ventura group demonstrated the feasibility of using the CRISPR-Cas9 system for modeling large oncogenic chromosomal rearrangements (Box 1) in wild-type mice *in vivo* via delivery of an adenovirus encoding Cas9 and two sgRNAs designed to induce an *Eml4-Alk* (echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase) inversion[49,50]. Lung tumors developed with complete penetrance and were exquisitely sensitive to crizotinib, an inhibitor used to treat human lung tumors that harbor this particular oncogenic rearrangement[51]. Moreover, the fact that the *Eml4* and *Alk* loci are separated by ~11 megabases strongly supports the feasibility of the CRISPR-Cas9 system for modeling large genomic rearrangements. A subsequent study utilizing lentiviruses also demonstrated the ability of the CRISPR-Cas9 system to induce chromosomal rearrangements *in vivo*[52]. These studies demonstrated the potential of rapidly generating mouse models of cancer via somatic genome engineering through delivery of all CRISPR components in the form of plasmids or viruses. In addition to these traditional DNA- or viral-based delivery methods, recent advances in engineering of non-viral delivery materials have made it possible to deliver Cas9-sgRNA protein-RNA complexes[53] and sgRNA-nanoparticle complexes[54] *in vivo* utilizing cationic lipid-mediated delivery or 7C1 nanoparticles, respectively. Future advances in materials science and engineering should make it possible to implement additional types of non-viral delivery platforms for the delivery of Cas9, sgRNAs and HDR donor DNA templates for achieving highly efficient genome modification *in vivo* (non-viral materials extensively reviewed in[55]).

To further streamline the generation of CRISPR-based somatic mouse models of cancer, the Zhang and Sharp laboratories reported the generation of mouse models expressing constitutive or Cre-inducible versions of the Cas9 enzyme[54]. By intratracheally delivering a novel adeno-associated virus (AAV) encoding six components: a *Kras*^{G12D} HDR donor DNA template, sgRNAs targeting *Kras*, serine/threonine kinase 11 (*Stk11*; also known as *Lkb1*) and *Trp53*, Cre recombinase and *Renilla* luciferase into mice expressing the Cre-

inducible *Cas9* allele, they were able to induce lung tumors in adult mice by simultaneously disrupting both tumor suppressors and engineering the oncogenic *Kras*^{G12D} mutation. Recently, the Lowe laboratory reported the generation of a highly flexible mouse modeling platform consisting of transgenic mice co-expressing doxycycline-inducible alleles of *Cas9* or the *Cas9*^{D10A} nickase variant[52] and constitutively expressed sgRNA cassettes[56]. Utilizing this conditional platform, they demonstrated effective gene editing *in vivo* with up to 85% target gene modification. Moreover, they demonstrated efficient simultaneous biallelic modification of up to two genes *in vivo* using a pair of sgRNAs and the Cas9 nuclease. This flexible platform allowed them to accommodate up to six sgRNA cassettes that, when combined with the Cas9^{D10A} nickase, led to simultaneous editing of three genes in mouse ES cells with high efficiency.

The development of mouse models expressing the Cas9 nuclease and Cas9^{D10A} nickase represents a major advancement for CRISPR applications in cancer biology, allowing researchers to focus their efforts on delivering single or multiple sgRNAs with or without synthetic HDR donor DNA templates utilizing viral and/or non-viral carriers, bypassing the need to optimize approaches for co-delivery of this large DNA endonuclease. In addition, expression of Cre-inducible or doxycycline-inducible alleles of Cas9 *in vivo* can be rendered tissue-specific via the incorporation of tissue-specific Cre or reverse tetracycline transactivator alleles, respectively. Moreover, the development of constitutive and conditional mouse models for CRISPR-mediated activation[57] or repression[58] of gene expression (Box 2) will serve as powerful complementary approaches for functionally studying both coding and non-coding DNA elements without permanent disruption of the endogenous genomic sequence. Beyond the established *Mus musculus* laboratory organism, the flexibility of CRISPR-Cas9 technologies should allow for rapid generation of novel animal models of cancer utilizing genetically intractable organisms that better recapitulate human tissue architecture and drug metabolism, such as pigs[59] and non-human primates[60].

Future applications to cancer biology

We envision a new era in cancer biology in which CRISPR-based genome engineering will serve as an important conduit between the bench and the bedside (Figure 2C). The successful deployment of sophisticated genetic profiling technologies for comprehensive characterization of a patient's tumor is generating detailed roadmaps to instruct the development of tailored cell-based or whole animal-based experimental systems. These systems will serve as personalized platforms, with which researchers will rapidly and systematically identify genotype-specific vulnerabilities and synthetic lethal interactions via single or multiplex CRISPR-based and small molecule-based approaches. Moreover, such personalized platforms could be studied in parallel to the patients, potentially allowing for the rapid identification of resistance mechanisms and the development of strategies to overcome such shortcomings[61].

Although there are current technical limitations to the use of CRISPR-Cas9 for targeting cancer genes in human patients as a therapeutic strategy, the prospects of this form of gene therapy are nonetheless very exciting. Recent work has demonstrated the potential of this technology to permanently correct genetic mutations *in vivo* in the adult liver of mouse

models of a hereditary genetic disease via HDR, successfully alleviating aspects of the disorder[62]. Therefore, future advancements of this technology for increasing the efficiency of editing and delivery of CRISPR-Cas9 components utilizing both viral and non-viral delivery vehicles will allow for therapeutic genetic correction of single or multiple driver mutations. In addition to permanently correcting cancer-associated mutations, the CRISPR-Cas9 system could be employed for precise *ex vivo* engineering of immune cells for immunotherapeutic applications. For example, the CRISPR-Cas9 system could be utilized for the development of novel chimeric antigen receptor (CAR)-modified T cells[63], in which the CAR is precisely inserted into a safe harbor locus[64].

Ever since the Doudna and Charpentier groups demonstrated the potential of the CRISPR-Cas9 system as a powerful RNA-programmed genome editing platform[23], the field of genome engineering has rapidly undergone a scientific revolution that promises to transform nearly every aspect of basic biological and biomedical research. The application of this technology to several aspects of cancer biology, ranging from basic research to clinical and translational applications, offers numerous exciting opportunities for better understanding and potentially treating this devastating disease.

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Biographies

Francisco J. Sánchez-Rivera received his bachelor’s degree from the University of Puerto Rico at Mayagüez. He is currently a graduate student in the Biology Department at the Massachusetts Institute of Technology. His research in the Jacks laboratory revolves around the application of the CRISPR-Cas9 system to model cancer *in vivo*.

Tyler Jacks received his Ph.D. training in the laboratory of Harold Varmus at the University of California, San Francisco. He was a post-doctoral fellow with Robert Weinberg at the Whitehead Institute at MIT and joined the faculty at MIT in 1992. He is currently the director of the David H. Koch Institute for Integrative Cancer Research at MIT. The Jacks laboratory has pioneered the use of gene targeting technology in the mouse to study cancer-associated genes and to construct mouse models of many human cancer types, including lung cancer, pancreatic cancer, ovarian cancer, astrocytoma, retinoblastoma, peripheral nervous system tumors, soft tissue sarcoma, and invasive colon cancer.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57–70. [PubMed: 10647931]
2. Smithies O, Gregg RG, Boggs SS, Koralewski MA, Kucherlapati RS. Insertion of DNA sequences into the human chromosomal β-globin locus by homologous recombination. Nature. 1985; 317:230–234. [PubMed: 2995814]
3. Thomas KR, Folger KR, Capecchi MR. High frequency targeting of genes to specific sites in the mammalian genome. Cell. 1986; 44:419–428. [PubMed: 3002636]

4. Mansour SL, Thomas KR, Capecchi MR. Disruption of the proto-oncogene int-2 in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. Nature. 1988; 336:348–352. [PubMed: 3194019]
5. Frese KK, Tuveson DA. Maximizing mouse cancer models. Nat Rev Cancer. 2007; 7:645–658. [PubMed: 17687385]
6. Rudin N, Sugarman E, Haber JE. Genetic and physical analysis of double-strand break repair and recombination in Saccharomyces cerevisiae. Genetics. 1989; 122:519–534. [PubMed: 2668114]
7. Plessis A, Perrin A, Haber JE, Dujon B. Site-specific recombination determined by I-SceI, a mitochondrial group I intron-encoded endonuclease expressed in the yeast nucleus. Genetics. 1992; 130:451–460. [PubMed: 1551570]
8. Rouet P, Smih F, Jasin M. Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells. Proc Natl Acad Sci USA. 1994; 91:6064–6068. [PubMed: 8016116]
9. Choulika A, Perrin A, Dujon B, Nicolas JF. Induction of homologous recombination in mammalian chromosomes by using the I-SceI system of Saccharomyces cerevisiae. Mol Cell Biol. 1995; 15:1968–1973. [PubMed: 7891691]
10. Bibikova M, Golic M, Golic KG, Carroll D. Targeted chromosomal cleavage and mutagenesis in Drosophila using zinc-finger nucleases. Genetics. 2002; 161:1169–1175. [PubMed: 12136019]
11. Bibikova M. Enhancing Gene Targeting with Designed Zinc Finger Nucleases. Science. 2003; 300:764–764. [PubMed: 12730594]
12. Urnov FD, et al. Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nat Cell Biol. 2005; 435:646–651.
13. Christian M, et al. Targeting DNA double-strand breaks with TAL effector nucleases. Genetics. 2010; 186:757–761. [PubMed: 20660643]
14. Li T, et al. TAL nucleases (TALNs): hybrid proteins composed of TAL effectors and FokI DNA-cleavage domain. Nucleic Acids Res. 2011; 39:359–372. [PubMed: 20699274]
15. Miller JC, et al. A TALE nuclease architecture for efficient genome editing. Nat Biotechnol. 2011; 29:143–148. [PubMed: 21179091]
16. Hockemeyer D, et al. Genetic engineering of human pluripotent cells using TALE nucleases. Nat Biotechnol. 2011; 29:731–734. [PubMed: 21738127]
17. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. Nature Publishing Group. 2010; 11:636–646.
18. Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol. 2013; 14:49–55. [PubMed: 23169466]
19. Cong L, et al. Multiplex Genome Engineering Using CRISPR/Cas Systems. Science. 2013; 339:819–823. [PubMed: 23287718]
20. Mali P, et al. RNA-Guided Human Genome Engineering via Cas9. Science. 2013; 339:823–826. [PubMed: 23287722]
21. Jinek M, et al. RNA-programmed genome editing in human cells. eLife Sciences. 2013; 2:e00471.
22. Cho SW, Kim S, Kim JM, Kim J-S. Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. Nat Biotechnol. 2013; 31:230–232. [PubMed: 23360966]
23. Jinek M, et al. A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity. Science. 2012; 337:816–821. [PubMed: 22745249]
24. Hsu PD, et al. DNA targeting specificity of RNA-guided Cas9 nucleases. Nat Biotechnol. 2013; 31:827–832. [PubMed: 23873081]
25. Esvelt KM, et al. Orthogonal Cas9 proteins for RNA-guided gene regulation and editing. Nat Methods. 2013; 10:1116–1121. [PubMed: 24076762]
26. Hou Z, et al. Efficient genome engineering in human pluripotent stem cells using Cas9 from Neisseria meningitidis. Proceedings of the National Academy of Sciences. 2013; 110:15644–15649.
27. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014; 346:1258096. [PubMed: 25430774]

28. Vogelstein B, et al. Cancer genome landscapes. Science. 2013; 339:1546–1558. [PubMed: 23539594]
29. Denicola GM, et al. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature. 2011; 475:106–109. [PubMed: 21734707]
30. Kim S, Kim D, Cho SW, Kim J, Kim J-S. Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. genome.cshlp.org.
31. Lin S, Staahl BT, Alla RK, Doudna JA. Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. eLife Sciences. 2014; 4
32. Malina A, et al. Repurposing CRISPR/Cas9 for in situ functional assays. Genes Dev. 2013; 27:2602–2614. [PubMed: 24298059]
33. Shalem O, et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. Science. 2014; 343:84–87. [PubMed: 24336571]
34. Hsu PD, Lander ES, Zhang F. Development and Applications of CRISPR-Cas9 for Genome Engineering. Cell. 2014; 157:1262–1278. [PubMed: 24906146]
35. Heyer J, Kwong LN, Lowe SW, Chin L. Non-germline genetically engineered mouse models for translational cancer research. Nat Rev Cancer. 2010; 10:470–480. [PubMed: 20574449]
36. Engelman JA, et al. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. Nat Med. 2008; 14:1351–1356. [PubMed: 19029981]
37. Chen Z, et al. A murine lung cancer co-clinical trial identifies genetic modifiers of therapeutic response. Nature. 2012; 483:613–617. [PubMed: 22425996]
38. Wang H, et al. One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering. Cell. 2013; 153:910–918. [PubMed: 23643243]
39. Yang H, et al. One-Step Generation of Mice Carrying Reporter and Conditional Alleles by CRISPR/Cas-Mediated Genome Engineering. Cell. 2013; 154:1370–1379. [PubMed: 23992847]
40. Jackson EL, et al. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. Genes Dev. 2001; 15:3243–3248. [PubMed: 11751630]
41. Guerra C, et al. Tumor induction by an endogenous K-ras oncogene is highly dependent on cellular context. Cancer Cell. 2003; 4:111–120. [PubMed: 12957286]
42. Findlay GM, Boyle EA, Hause RJ, Klein JC, Shendure J. Saturation editing of genomic regions by multiplex homology-directed repair. Nature. 2014; 513:120–123. [PubMed: 25141179]
43. Dow LE, Lowe SW. Life in the Fast Lane: Mammalian Disease Models in the Genomics Era. Cell. 2012; 148:1099–1109. [PubMed: 22424222]
44. Chen C, et al. MLL3 is a haploinsufficient 7q tumor suppressor in acute myeloid leukemia. Cancer Cell. 2014; 25:652–665. [PubMed: 24794707]
45. Heckl D, et al. Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing. Nat Biotechnol. 2014; 32:941–946. [PubMed: 24952903]
46. Xue W, et al. CRISPR-mediated direct mutation of cancer genes in the mouse liver. Nature. 2014; 514:380–384. [PubMed: 25119044]
47. Jackson E, et al. The Differential Effects of Mutant p53 Alleles on Advanced Murine Lung Cancer. Cancer Res. 2005; 65:10280–10288. [PubMed: 16288016]
48. Sanchez-Rivera FJ, et al. Rapid modelling of cooperating genetic events in cancer through somatic genome editing. Nature. 2014
49. Maddalo D, et al. In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system. Nature. 2014; 516:423–427. [PubMed: 25337876]
50. Soda M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature. 2007; 448:561–566. [PubMed: 17625570]
51. Shaw AT, et al. Crizotinib versus Chemotherapy in Advanced ALK-Positive Lung Cancer. N Engl J Med. 2013; 368:2385–2394. [PubMed: 23724913]
52. Blasco RB, et al. Simple and Rapid In Vivo Generation of Chromosomal Rearrangements using CRISPR/Cas9 Technology. Cell Rep. 2014; 9:1219–1227. [PubMed: 25456124]
53. Zuris JA, et al. Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo. Nat Biotechnol. 2000 doi:

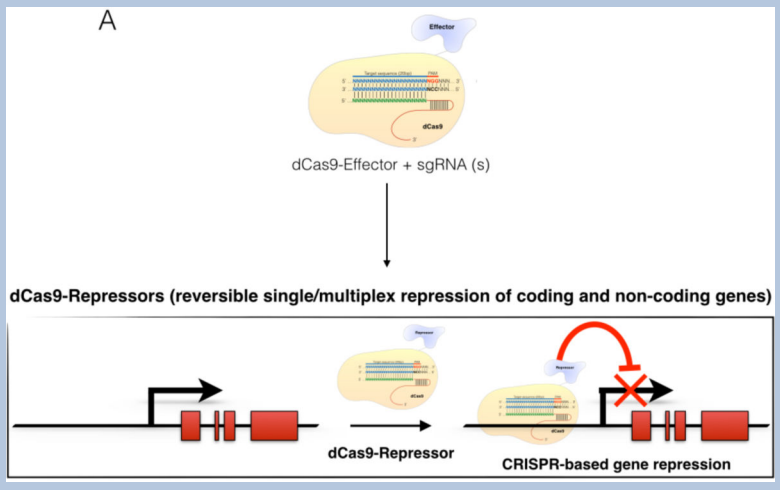
54. Platt RJ, et al. CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Cell. 2014; 159:440–455. [PubMed: 25263330]
55. Yin H, et al. Non-viral vectors for gene-based therapy. Nat Rev Genet. 2014; 15:541–555. [PubMed: 25022906]
56. Dow LE, et al. Inducible in vivo genome editing with CRISPR-Cas9. Nat Biotechnol. 2015
57. Tanenbaum ME, Gilbert LA, Qi LS, Weissman JS, Vale RD. A protein-tagging system for signal amplification in gene expression and fluorescence imaging. Cell. 2014; 159:635–646. [PubMed: 25307933]
58. Gilbert LA, et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. Cell. 2013; 154:442–451. [PubMed: 23849981]
59. Whitworth KM, et al. Use of the CRISPR/Cas9 System to Produce Genetically Engineered Pigs from In Vitro-Derived Oocytes and Embryos. Biology of Reproduction. 2014; 91:78–78. [PubMed: 25100712]
60. Niu Y, et al. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell. 2014; 156:836–843. [PubMed: 24486104]
61. Crystal AS, et al. Patient-derived models of acquired resistance can identify effective drug combinations for cancer. Science. 2014
62. Yin H, et al. Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nat Biotechnol. 2014; 32:551–553. [PubMed: 24681508]
63. Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. Cancer Discovery. 2013; 3:388–398. [PubMed: 23550147]
64. Sadelain M, Papapetrou EP, Bushman FD. Safe harbours for the integration of new DNA in the human genome. Nat Rev Cancer. 2012; 12:51–58. [PubMed: 22129804]
65. Matano M, et al. Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. Nature Medicine. 2015
66. Mansour MR, et al. An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element. Science. 2014; 346:1373–1377. [PubMed: 25394790]
67. Choi PS, Meyerson M. Targeted genomic rearrangements using CRISPR/Cas technology. Nature Communications. 2014; 5
68. Torres R, et al. Engineering human tumour-associated chromosomal translocations with the RNA-guided CRISPR-Cas9 system. Nature Communications. 2014; 5
69. Ghezraoui H, et al. Chromosomal Translocations in Human Cells Are Generated by Canonical Nonhomologous End-Joining. Mol Cell. 2014; 55:829–842. [PubMed: 25201414]
70. Xiao A, et al. Chromosomal deletions and inversions mediated by TALENs and CRISPR/Cas in zebrafish. Nucleic Acids Res. 2013; 41:e141. [PubMed: 23748566]
71. Canver MC, et al. Characterization of Genomic Deletion Efficiency Mediated by Clustered Regularly Interspaced Palindromic Repeats (CRISPR)/Cas9 Nuclease System in Mammalian Cells. Journal of Biological Chemistry. 2014; 289:21312–21324. [PubMed: 24907273]
72. Han J, et al. Efficient in vivo deletion of a large imprinted lncRNA by CRISPR/Cas9. RNA Biol. 2014; 11:829–835. [PubMed: 25137067]
73. Ho T-T, et al. Targeting non-coding RNAs with the CRISPR/Cas9 system in human cell lines. Nucleic Acids Res. 2014
74. Essletzbichler P, et al. Megabase-scale deletion using CRISPR/Cas9 to generate a fully haploid human cell line. Genome Research. 2014; 24:2059–2065. [PubMed: 25373145]
75. Blasco RB, et al. Simple and Rapid In Vivo Generation of Chromosomal Rearrangements using CRISPR/Cas9 Technology. Cell Rep. 2014; 9:1219–1227. [PubMed: 25456124]
76. Bikard D, et al. Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas system. Nucleic Acids Res. 2013; 41:7429–7437. [PubMed: 23761437]
77. Gilbert LA, et al. Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation. Cell. 2014:1–28. [PubMed: 24679520]
78. Mali P, et al. CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. Nat Biotechnol. 2013; 31:833–838. [PubMed: 23907171]

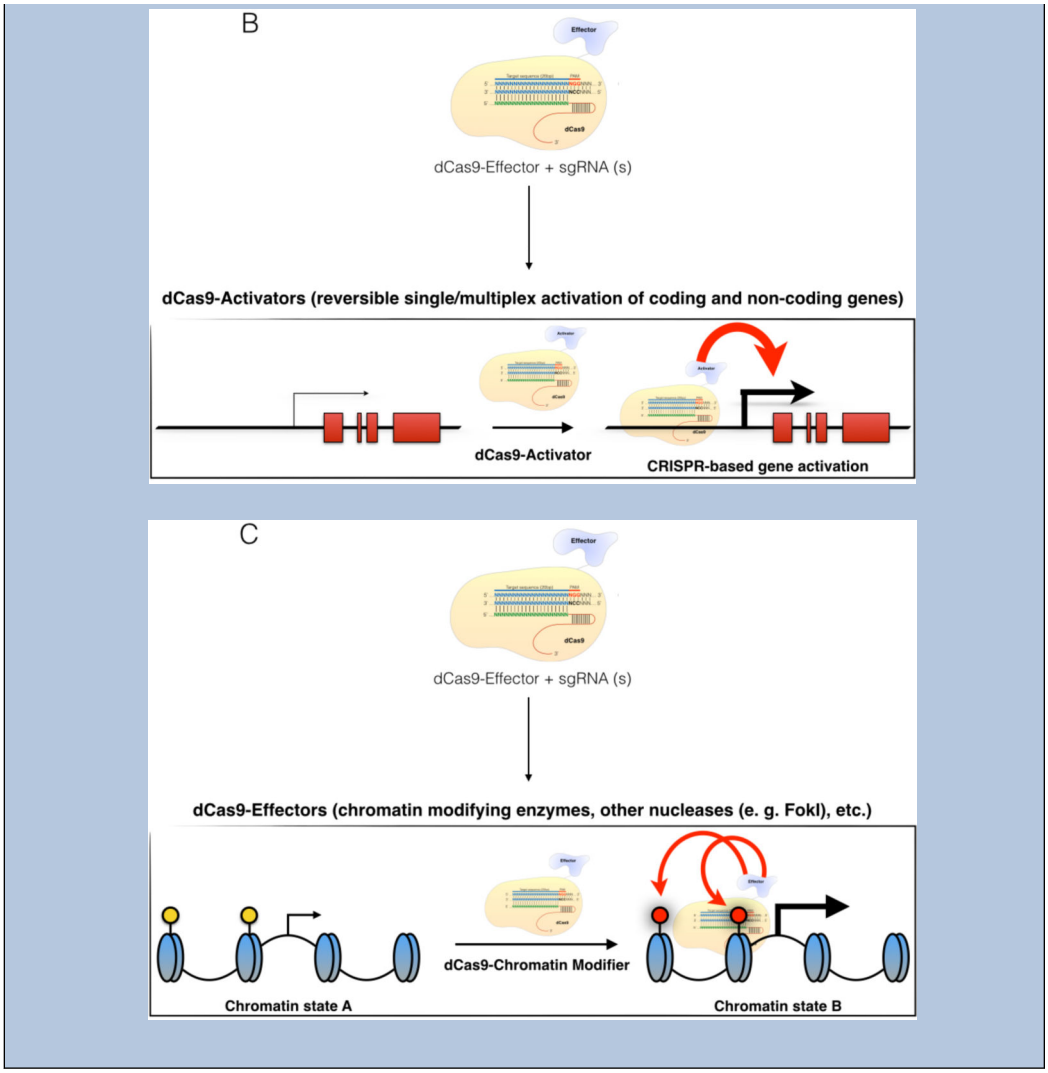
79. Perez-Pinera P, et al. RNA-guided gene activation by CRISPR-Cas9-based transcription factors. Nat Methods. 2013; 10:973–976. [PubMed: 23892895]
80. Cheng AW, et al. Multiplexed activation of endogenous genes by CRISPR-on, an RNA-guided transcriptional activator system. Cell Research. 2013; 23:1163–1171. [PubMed: 23979020]
81. Maeder ML, et al. CRISPR RNA-guided activation of endogenous human genes. Nat Methods. 2013; 10:977–979. [PubMed: 23892898]
82. Konermann S, et al. Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. Nature. 2014; 517:583–588. [PubMed: 25494202]
83. Zalatan JG, et al. Engineering complex synthetic transcriptional programs with CRISPR RNA scaffolds. Cell. 2015; 160:339–350. [PubMed: 25533786]
84. Wang T, Wei JJ, Sabatini DM, Lander ES. Genetic screens in human cells using the CRISPR-Cas9 system. Science. 2014; 343:80–84. [PubMed: 24336569]
85. Koike-Yusa H, Li Y, Tan E-P, Velasco-Herrera MDC, Yusa K. Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library. Nat Biotechnol. 2013; 32:267–273. [PubMed: 24535568]
86. Zhou Y, et al. High-throughput screening of a CRISPR/Cas9 library for functional genomics in human cells. Nature. 2014; 509:487–491. [PubMed: 24717434]
87. Chen S, et al. Genome-wide CRISPR Screen in a Mouse Model of Tumor Growth and Metastasis. Cell. 2015

Box 1

Potential applications of the CRISPR-Cas9 system in cancer biology

The flexibility and modularity of the CRISPR-Cas9 system has led to the development of numerous genome engineering applications, most of which have been carried out successfully in cell culture systems. Many of these can also be adapted for use *in vivo* (see the figure). The power of this technology can be harnessed for rapidly and precisely engineering both loss-of-function (LOF) (part a of the figure) and gain-of-function (GOF) (part b of the figure) mutations in tumor suppressor genes, oncogenes and other modulators of cellular transformation or drug response. For example, Toshiro Sato's group recently demonstrated the utility of the CRISPR-Cas9 system for systematically engineering both LOF and GOF mutations in untransformed human intestinal organoids in order to model human colorectal cancer (CRC)[65]. Remarkably, the serial introduction of five independent mutations frequently associated with human CRC (three LOF mutations and two GOF mutations) did not fully recapitulate the tumorigenic and metastatic characteristics of the human disease, suggesting that additional secondary genetic and/or epigenetic events are required for full malignancy[65]. In addition, the ability to multiplex the CRISPR-Cas9 system offers the opportunity to investigate combinatorial vulnerabilities in cancer cells, as well as systematically test epistatic relationships and synthetic lethal interactions (part a of the figure). This technology also allows for generating endogenous conditional alleles based on site-specific recombinases[39], tagging endogenous alleles[39], and interrogating non-coding DNA elements[66] (part b of the figure). The CRISPR-Cas9 system can also be utilized to trigger two distant DSBs in the same or different chromosomes, leading to inversion, deletion or translocation of the target or translocation of the target sequences, respectively (part c of the figure). This approach has been shown to be efficient in cells[67–74] and *in vivo*[49,75].

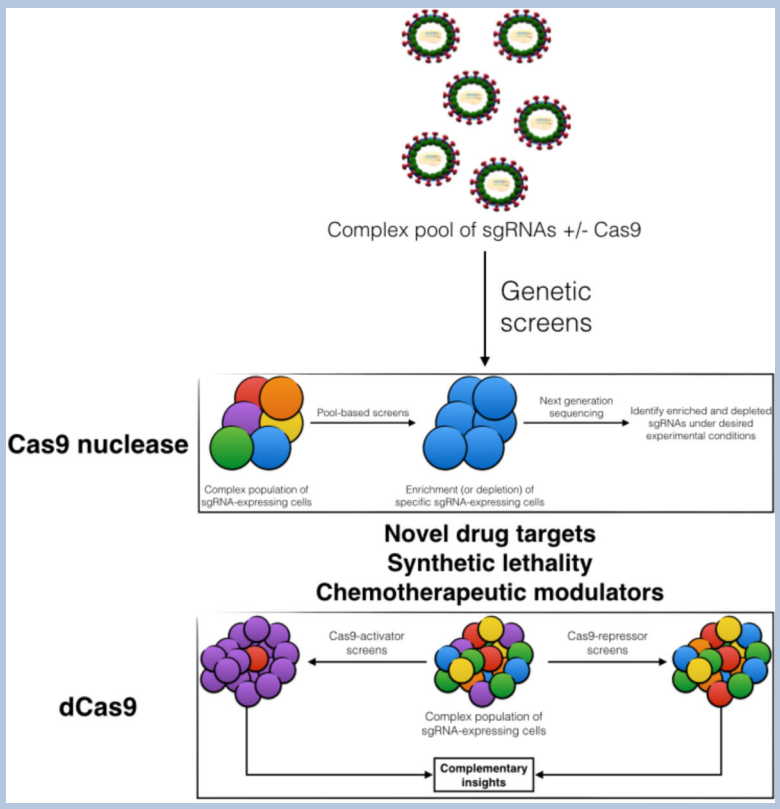




Box 2

Potential applications of dCas9-effector fusions in cancer biology

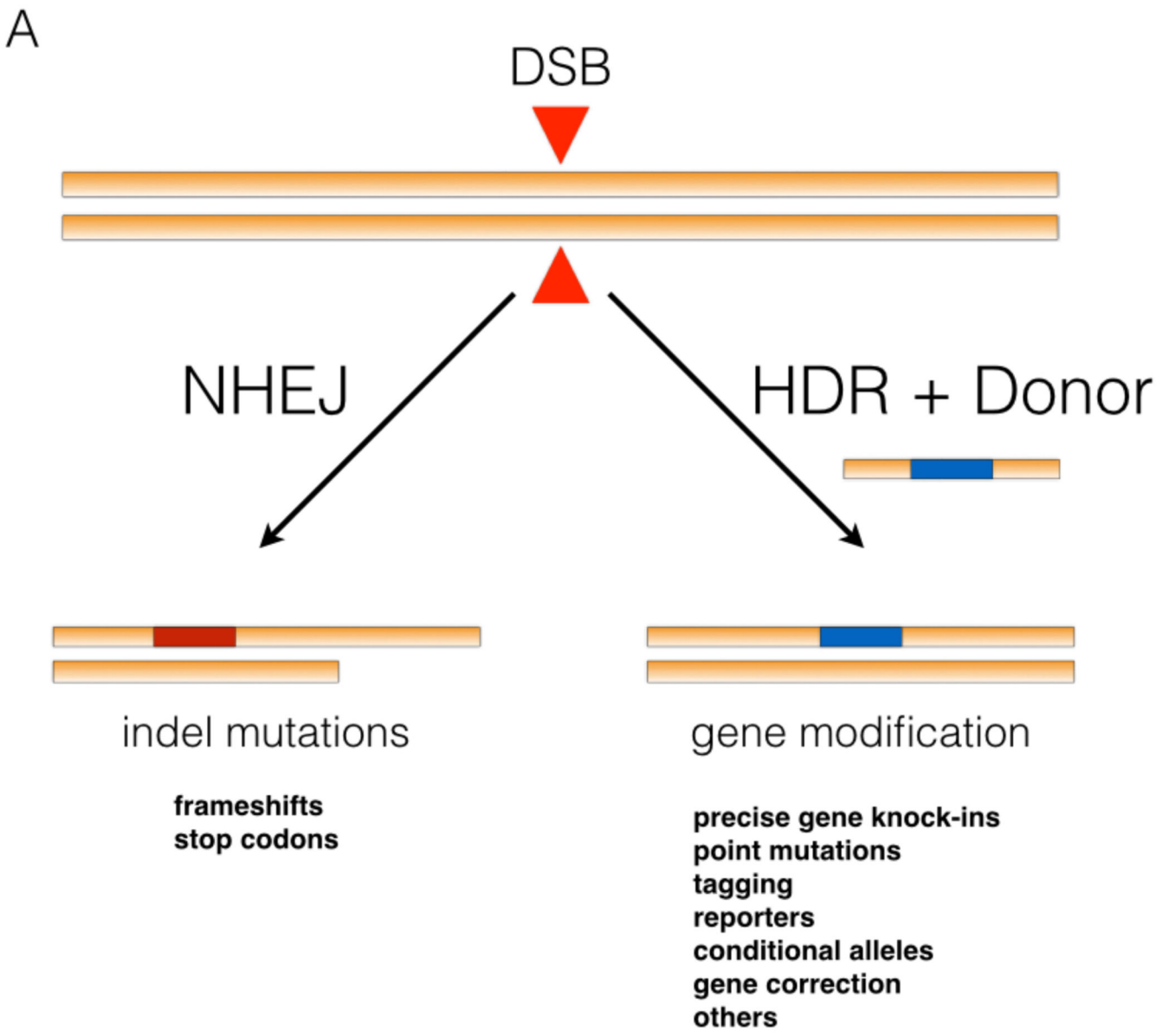
The ability of Cas9 to bind in a specific RNA-dependent fashion can be uncoupled from its nuclease activity by mutating its HNH and RuvC-like catalytic domains. This catalytically inactive form of Cas9, often referred to as dead Cas9 (dCas9), retains its RNA-guided DNA binding activity without any detectable DNA endonuclease activity[23]. A series of studies have demonstrated the power of dCas9-effector fusions (see the figure) for reversible transcriptional repression[58,76,77] or activation[25,57,58,76–82] of endogenous coding and non-coding genes. In addition, the use of scaffold RNAs that encode both targeting and effector-recruitment functions can be utilized for simultaneous multiplex gene repression and activation within a single cell[83].



Box 3

High-throughput genetic screens using CRISPR-Cas9

The flexibility of the CRISPR-Cas9 technology has been recently exploited for carrying out high-throughput CRISPR screens using the Cas9 nuclease[33,84–87] and dCas9-effectors[77,82] for the systematic identification of genes involved in a variety of biological phenotypes (see the figure). The groups of David Sabatini and Eric Lander[84] designed and utilized a library of ~73,000 sgRNAs targeting human genes to screen for genes involved in the DNA-mismatch repair pathway (MMR) in the presence of the nucleotide analogue 6-thioguanine (6-TG) and for genes whose disruption conferred resistance to the topoisomerase IIA (TOP2A) poison etoposide. Strikingly, both CRISPR screens demonstrated a very high signal-to-noise ratio with the top scoring sgRNAs from each screen targeting genes involved in the MMR pathway and *TOP2A* itself, respectively. In a parallel study, the group of Feng Zhang[33] generated and screened a library of ~65,000 sgRNAs targeting human genes and successfully identified essential genes in both cancer cell lines and pluripotent stem cells. Moreover, they utilized this library for performing a positive selection screen in melanoma cell lines to uncover genes whose deletion mediates resistance to the BRAF-V600Einhibitor vemurafenib, successfully identifying several known and novel candidates mediating resistance to this targeted therapy. Additional contemporaneous studies by Koike-Yusa *et al.*[85] and Zhou *et al.*[86] successfully demonstrated the broad applicability of pooled CRISPR-based screening technologies for identifying host factors mediating toxin susceptibility in mouse embryonic stem cells and human cells, respectively. In addition to CRISPR-based screens utilizing the Cas9 nuclease, Jonathan Weissman's group[77] adapted dCas9-based activators and repressors to carry out powerful complementary genome-wide gene activation and repression screens, respectively. A subsequent study by Feng Zhang's laboratory[82] also demonstrated the successful adaptation of dCas9-based activators for genome-wide gene activation screens. Notably, Feng Zhang's group also demonstrated the feasibility of identifying mediators of vemurafenib resistance. These landmark studies have demonstrated the feasibility of carrying out pooled high-throughput screens utilizing CRISPR-Cas9 technologies to uncover genes mediating a variety of biological phenotypes, including uncovering cancer cell vulnerabilities and mechanisms of therapeutic response and resistance. In addition to *in vitro* screens, the Zhang and Sharp laboratories recently demonstrated the utility of the CRISPR-Cas9 system for performing genome-wide *in vivo* screens to uncover genes involved in tumor progression and metastasis[87].



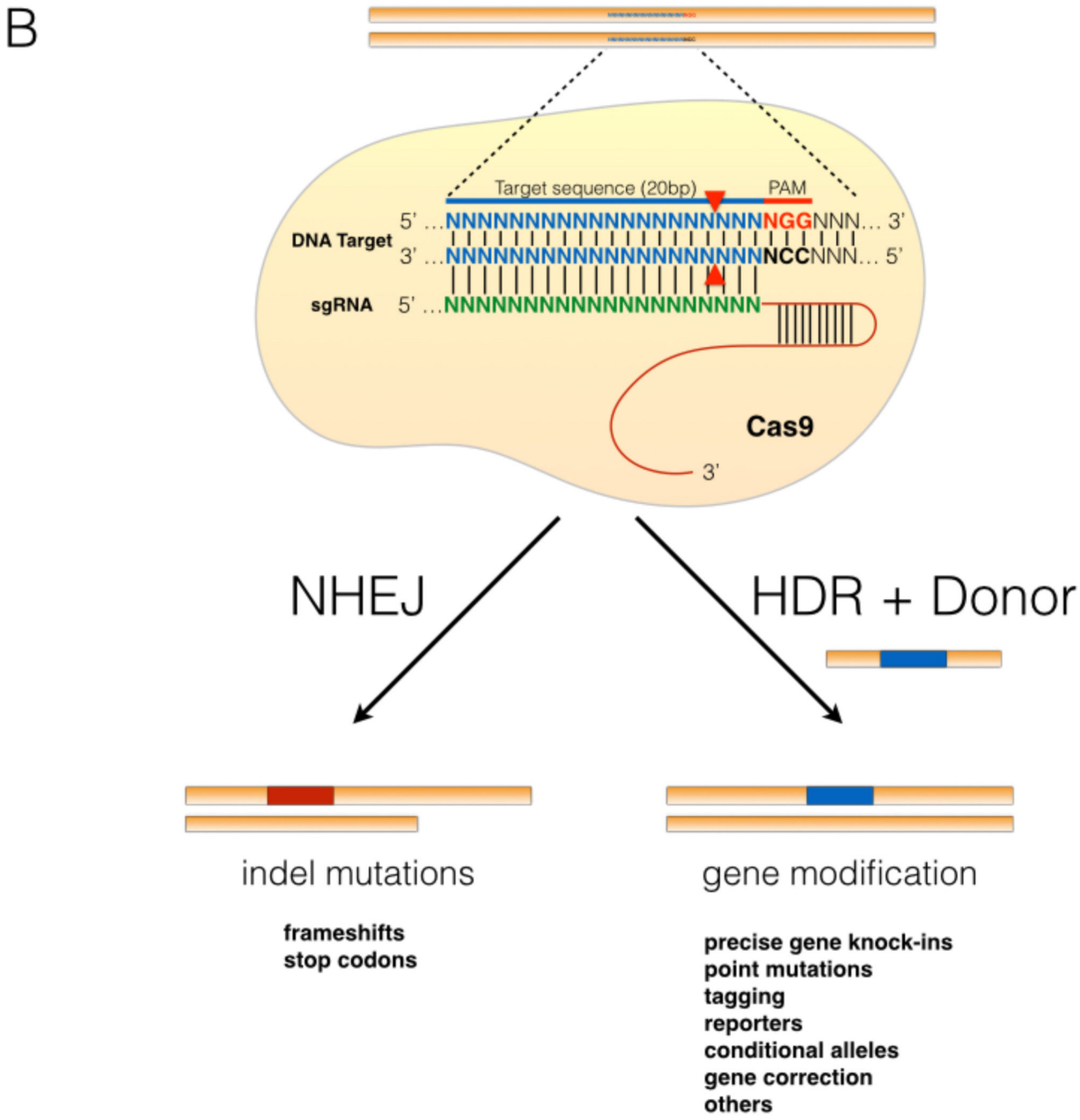
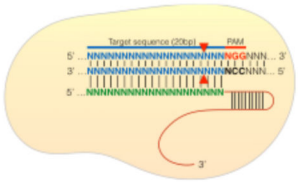


Figure 1. Genome engineering utilizing the CRISPR-Cas9 system

a | DNA double-strand breaks (DSBs) can be repaired by two cellular DNA repair pathways: the non-homologous end joining (NHEJ) pathway or the homology-directed repair (HDR) pathway. Repair via the NHEJ pathway, which is error-prone, frequently leads to insertion or deletion mutations (indels) that can lead to disrupting frameshift mutations and the generation of premature stop codons. Alternatively, in the presence of an exogenous donor DNA template, the DSB can be repaired via the HDR pathway, which can be utilized for engineering precise DNA modifications. **b** | The *S. pyogenes*-derived Cas9 RNA-guided

DNA endonuclease is localized to a specific DNA sequence via a single guide RNA (sgRNA) sequence, which base-pairs with a specific target sequence that is adjacent to a protospacer adjacent motif (PAM) sequence in the form of NGG or NAG. Cas9-mediated induction of a DSB in the DNA target sequence leads to indel mutations via NHEJ or precise gene modification via HDR.

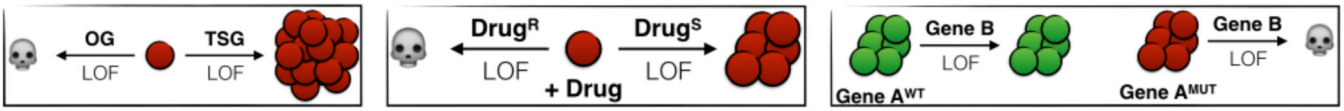
A



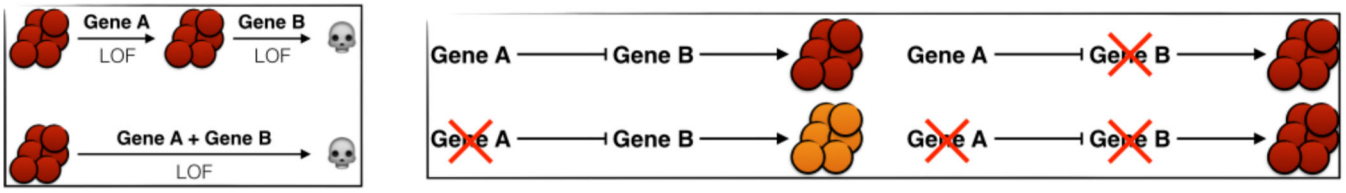
Cas9 + sgRNA

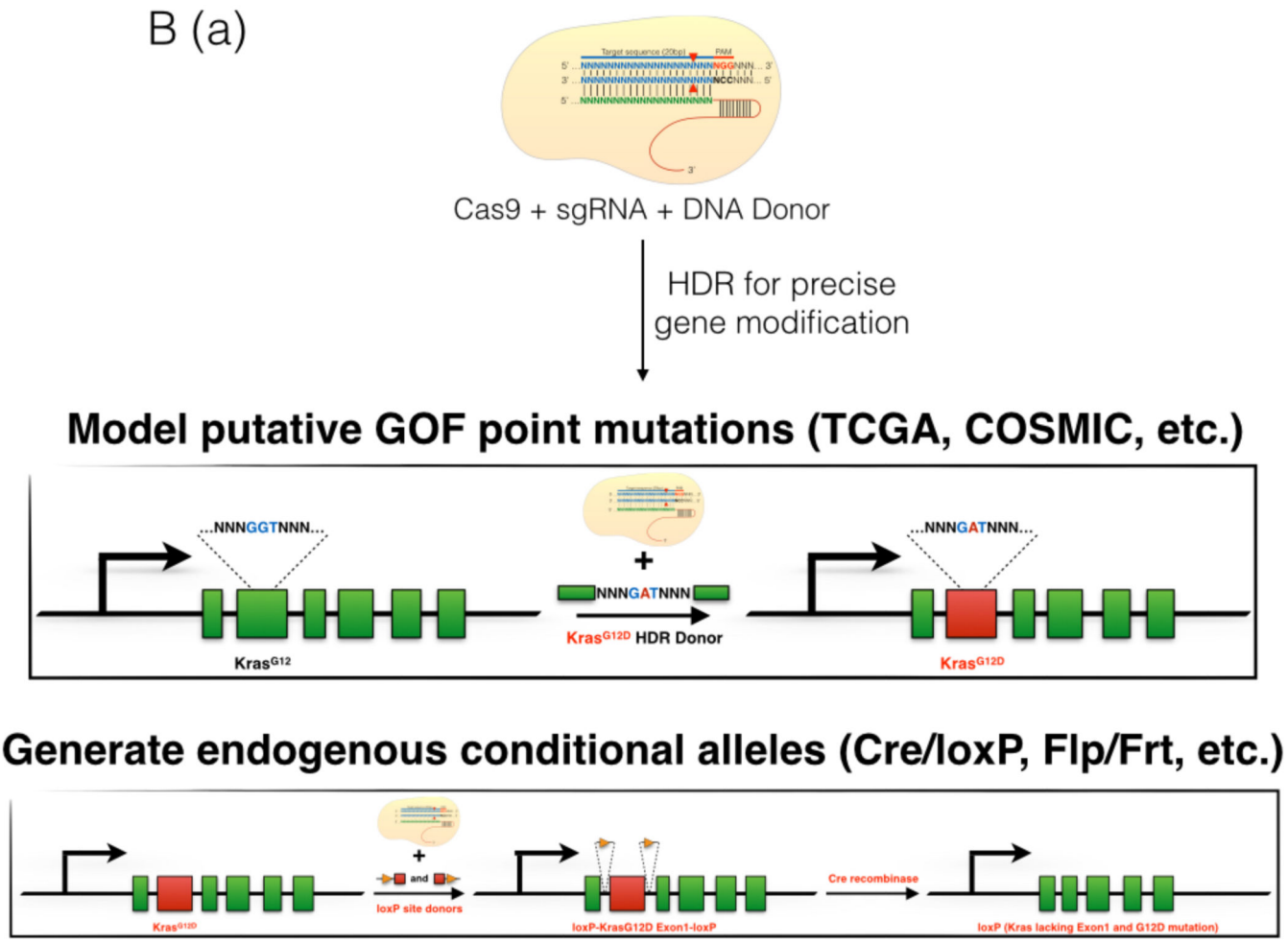
NHEJ for LOF

Single gene KO (individual OGs, TSGs, drug response modulators, synthetic lethality, etc.)

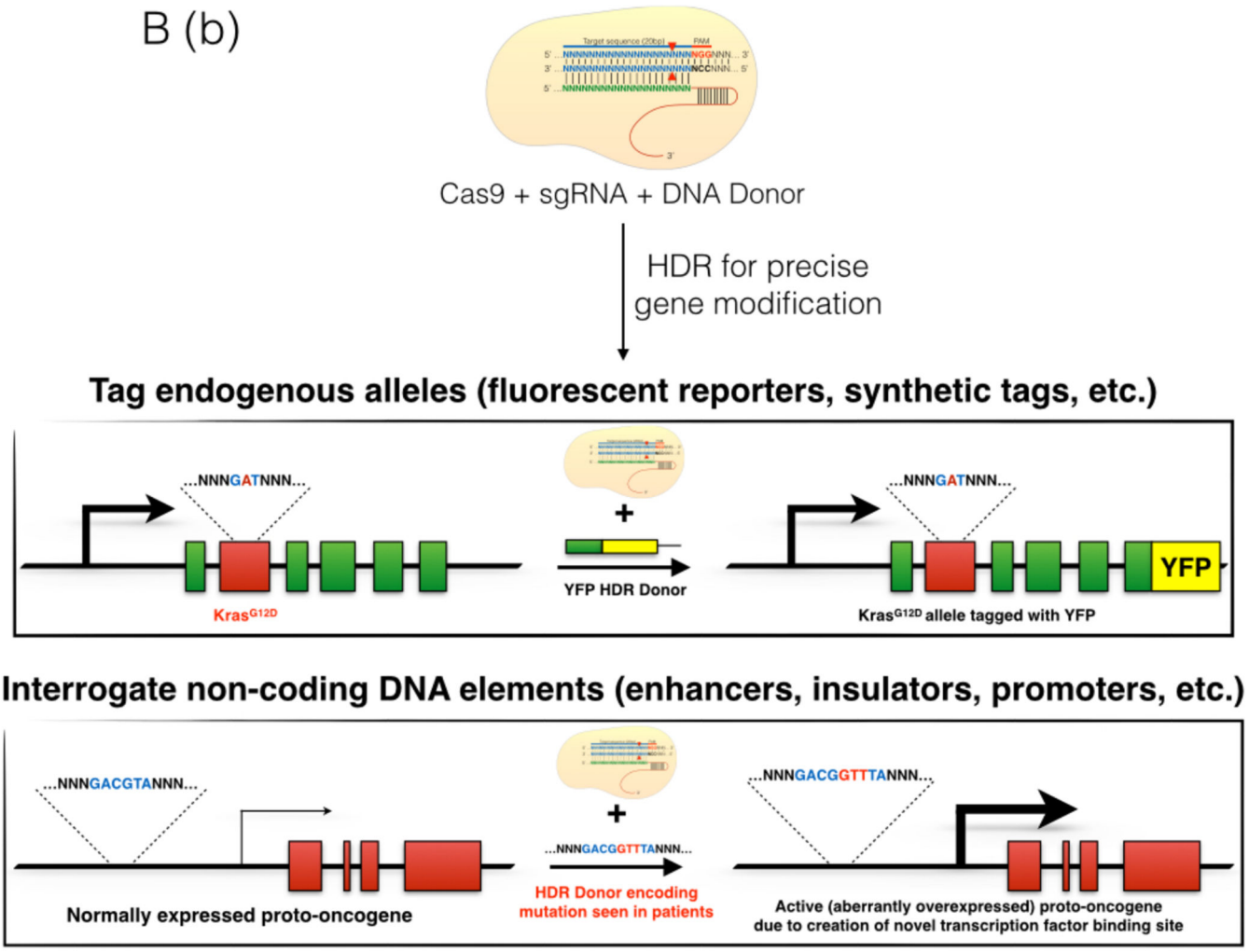


Multiplex gene KO (combinatorial vulnerabilities, epistatic relationships, etc.)





B (b)



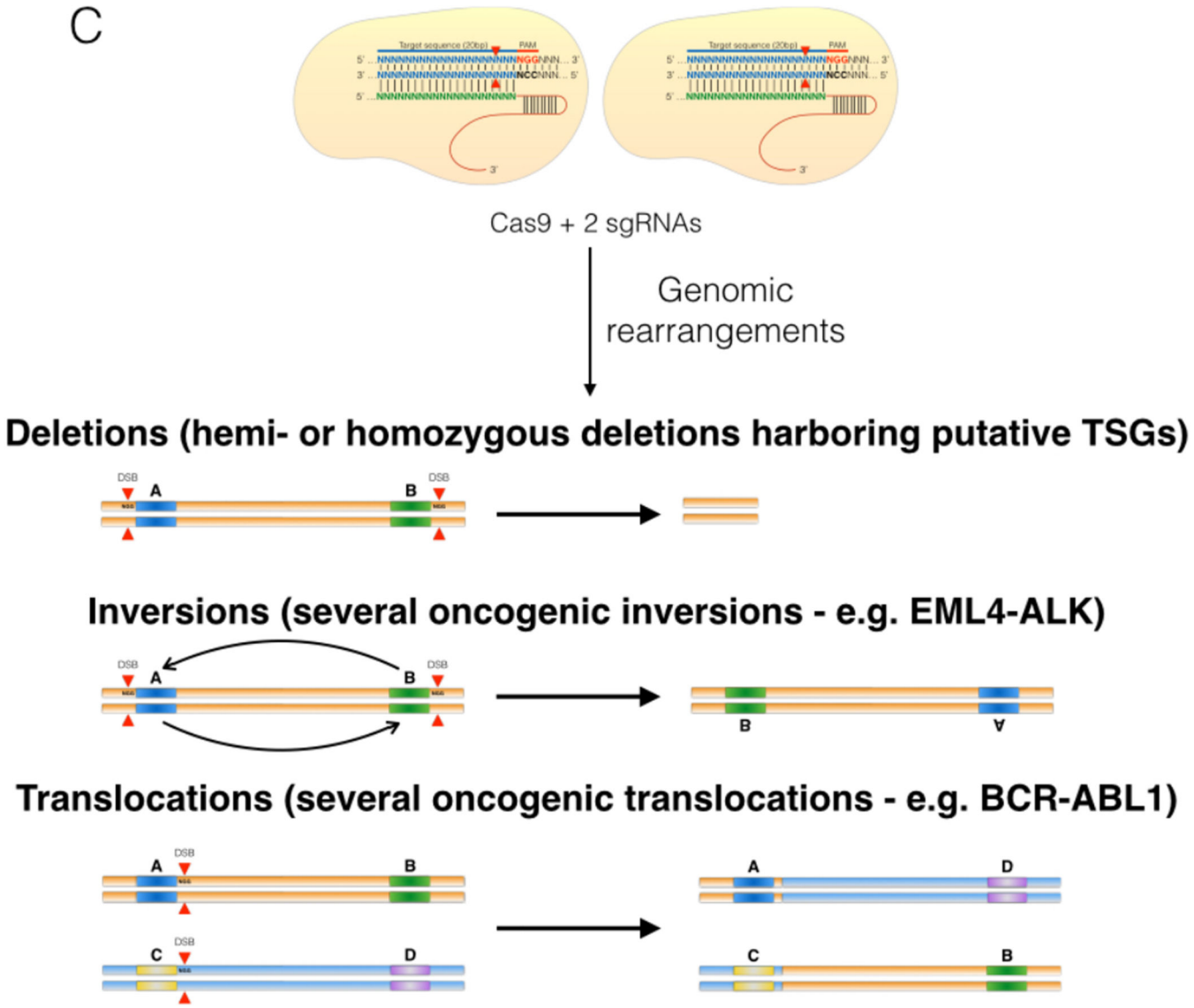
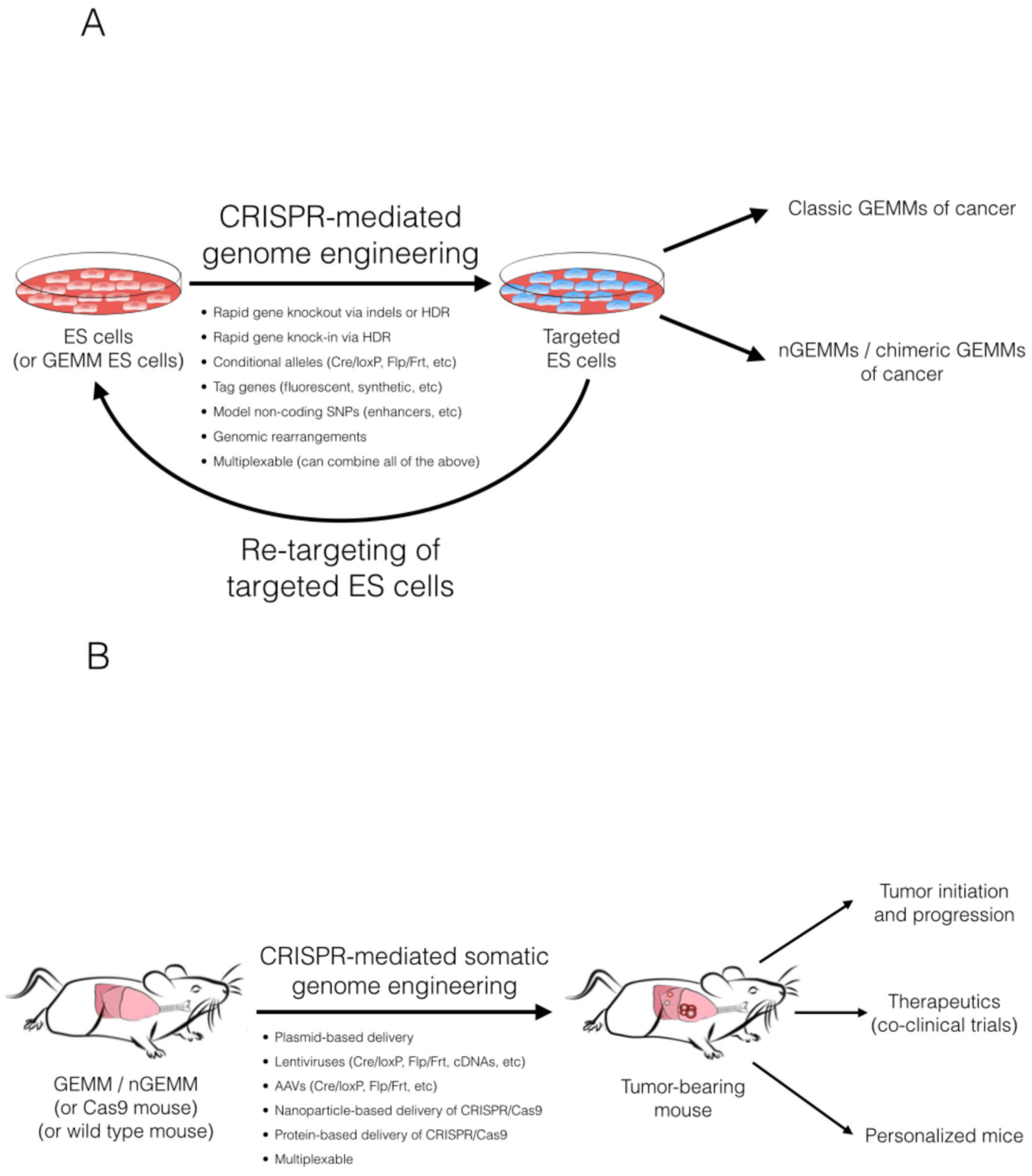


Figure 2. Applications of the CRISPR-Cas9 system in cancer biology

a | CRISPR-mediated genome engineering of embryonic stem (ES) cells or genetically engineered mouse model (GEMM)-derived ES cells can be utilized for rapidly generating novel GEMMs or non-germline GEMMs (nGEMMs) of cancer harbouring multiple genetic alterations, such as constitutive or conditional knockout and knock-in alleles, endogenous synthetic tags or reporters, non-coding single nucleotide polymorphisms (SNPs) and genomic rearrangements, as well as a combination of all of these via re-engineering of ES cells or multiplex CRISPR-mediated genome engineering. **b** | CRISPR-mediated somatic genome engineering *in vivo* can be utilized to rapidly generate cohorts of tumor-bearing mice for studying both basic and translational aspects of cancer biology. For example, the CRISPR-Cas9 system can be deployed *in vivo* for generating cohorts of personalized mice based on the exact complement of mutations observed in individual patients. **c** | The CRISPR-Cas9 system can serve as an important conduit between the bench and the bedside. The combination of sophisticated molecular profiling technologies with CRISPR-based

genome engineering technologies will allow researchers to generate personalized experimental platforms that can be utilized for rapidly and systematically identifying novel genotype-specific vulnerabilities through a battery of cell-based and *in vivo* assays.



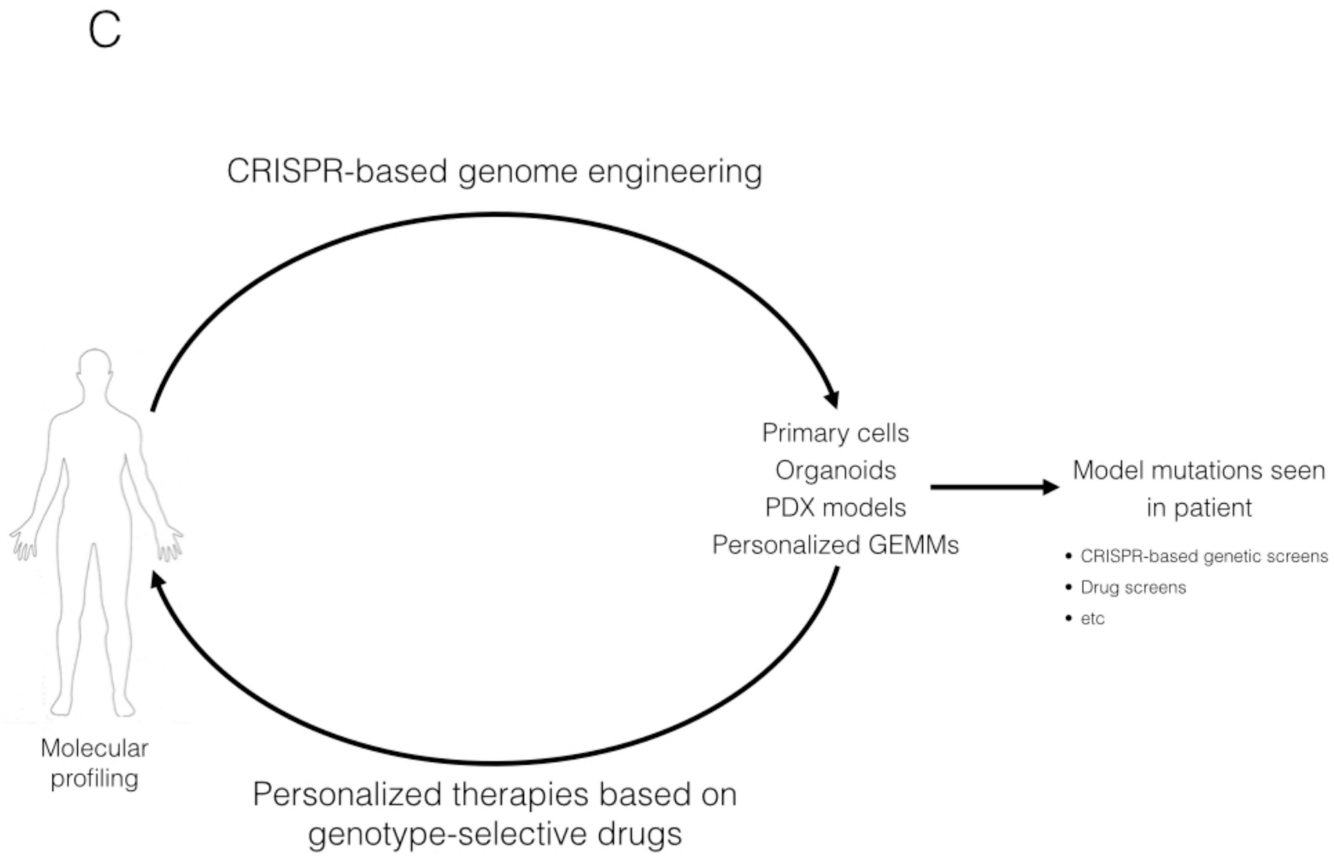


Figure 3.