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Small guide rna crispr definition chemistry examples biology





(1) Virus invades bacterial cell



Small guide rna.

The spacer region of the guide undergoes Watson and Crick base pairing with the complimentary stand to the DNA protospacer. doi: 10.1038/nmeth.3433 PubMed Abstract | CrossRef Full Text | Google Scholar Shmakov, S., Smargon, A., Scott, D., Cox, D., Pyzocha, N., Yan, W., et al. 17, 1097-1107. doi: 10.1126/sciadv.aaz0051 PubMed Abstract CrossRef Full Text | Google Scholar Liu, Y., Zou, R. doi: 10.1038/nature16526 PubMed Abstract | CrossRef Full Text | Google Scholar Kocak, D. Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. The aforementioned work by Yin et al. Taken together, the mode of intracellular delivery of gRNA-Cas9 complexes, whether gRNA is delivered with Cas9 mRNA or protein, and the number and positions of chemical modifications are all key factors that must be considered when planning CRISPR-Cas9 gene editing experiments. demonstrated that the addition of 2'-O-Me and PS groups on the 2'-OH and phosphate backbone within synthesized gRNAs completely abolished any immune response (Schubert et al., 2018). This significantly hinders the ability to study the full spectrum of DSB formation and subsequent DNA repair dynamics. ACS Cent. 22, 169-174. L., Strezoska, Z., He, K., Vermeulen, A., and Smith, A. A later study by Kocak et al. Adding modifications only on the 3' and 5' ends of gRNAs would protect the gRNA from exonucleolytic but not endonucleolytic activity inside the cells, which also may impair the editing efficiency by reducing gRNA stability. The donor DNA is fused to the 5' end of the guide region. A codon consists of three continuous nucleotide bases that code for an amino acid or signal the end of translation. A different approach relied on simultaneously expressing engineered gRNAs containing MS2/PCP aptamers, MS2/PCP binding proteins fused to fluorescent proteins, and dCas9. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. W., and Liu, D. Physiol. Exp. Several studies have shown that gRNA modifications in Type V CRISPRs (Cas12a), including 3' terminal chemical modifications (Li et al., 2017; McMahon et al., 2018) and crRNA elongation (Bin Moon et al., 2018), stabilize the complex and enhance editing efficiency. G., Cho, H. H., Camarena, J., Srifa, W., et al. E., Curry, B., Lucas, A. F., and Rahdar, M. Y., Lee, G., Lim, D. S., Li, L., Wu, X., Choi, U., Allen, C., Koontz, S., et al. Nonetheless, caution should be exercised when introducing RNA modifications since further analysis found that recognize the target DNA closest to the PAM sequence, also known as the PAM-proximal portion, inhibits proper DNA:RNA hybridization and can significantly hinder efficiency (Rahdar et al., 2017; Yin et al., G. A., Fraietta, J. D., Goeppert, A. Acad. Basila et al. V., Bielas, J. P., Bak, R. H., Bratovic, M., Charpentier, E., et al. However, since primary cells are known to mount an innate immune response to the foreign DNA (Sun et al., 2013), as well as to the in vitro transcribed gRNAs (as discussed below), chemical synthesis represents a cost-effective, expeditious alternative that produces highly purified gRNA at scalable quantities. Incorporation of bridged nucleic acids into CRISPR RNAs improves Cas9 endonuclease specificity. J., Clark, J., Punjya, N., Sebastiano, V., et al. 45:e98. The crRNA sequence can be divided into a guide region and a repeat region, while the tracrRNA sequence consists of an anti-repeat region and three stem-loop (numbered 1-3) structures. conducted a genome editing screen in Cas9 expressing HEK293T cells, which revealed that in living cells, the tail region, or the PAM-distal portion of the guide sequence was more amenable to DNA replacement than the seed region. 9:3313. Therefore, when translating these discoveries to the clinic, the relevant modifications must be validated in primary cells and animal models. C., and MacLachlan, I. 15, 169-182. 11, 198-200. Ribosomal RNA is responsible for creating the peptide bonds between the amino acids in the polypeptide chain. A., and Gagnon, K. Methods 14, 891-896. N., Castro, M., and Cooper, T. These sequences are transcribed together into CRISPR RNAs (crRNAs), which are subsequently utilized to recognize and destroy the invading complementary DNA or RNA molecules by Cas nucleases (Horvath and Barrangou, 2010; Terns and Terns, 2011; Morange, 2015). Though much more work remains to be done to optimize modified gRNAs for future routine human genome-editing-based therapies, there is no denying that the future of modified gRNAs and CRISPR-based therapeutics remains exceptionally bright. This method of modifying the gRNA to facilitate light-induced Cas9 activation allows for synchronous DNA cleavage across a population of cells. doi: 10.1002/cam4.2257 PubMed Abstract | CrossRef Full Text | Google Scholar Ge, Z., Zheng, L., Zhao, Y., Jiang, J., Zhang, E. Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells. Structure and specificity of the RNA-guided endonuclease stimulates homologous recombination in mammalian cells. patient with HIV and acute lymphocytic leukemia. 24, 1216-1224. doi: 10.1089/oli.2009.0180 CrossRef Full Text | Google Scholar Romero, Z., Lomova, A., Said, S., Miggelbrink, A., Kuo, C. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. In addition, BNANC nucleotides have been shown to be less toxic than LNA nucleotides when delivered to cultured cells (Manning et al., 2017). R., Patel, M. Class 2 is distinguished by a multi-domain effector Cas nuclease and uses trans-activating CRISPR RNA (tracrRNA), in addition to crRNA, for target recognition and cleavage (Makarova et al., 2020). Biosci. They demonstrated that using a phosphorothioate (PS) (Figure 2) modified backbone in tandem with 2'-O-Me modifications on the terminal five nucleolytic cleavage. With three types in each class and more than a dozen subtypes, the CRISPR-Cas system represents a fruitful field for developing bioengineering tools. E., Joung, J., Abudayyeh, O. 2162, 37-48. Science 339, 819-823. Notably, chemically-modified gRNAs are not restricted to the genome-editing via DSBs but can be exploited for a variety of applications involving catalytically-inactive Cas9 nucleases, Cas9 nickases, base editors and prime editors. doi: 10.1126/scitranslmed.aaf9336 PubMed Abstract | CrossRef Full Text Dow, L. Production of gRNAs Like other types of RNA, gRNAs consist of ribonucleotides covalently bound together by phosphodiester bonds. doi: 10.7554/eLife.25312 PubMed Abstract | CrossRef Full Text | Google Scholar Lennox, K. Therefore, Adeno Associated Virus (AAV) vectors have become a method of choice to introduce donor templates (Gaj et al., 2017). Cell 155, 1479-1491. S., Dawlaty, M. G., Lee, N. RNA interference in mammalian cells by chemically-modified RNA. The popular method for CRISPR-mediated gene editing in cultured cells involves transfection with plasmid DNA that expresses both gRNA and Cas9 protein under constitutive promoters (Ran et al., 2013). (2015). The partial replacement of RNA nucleotides with DNA nucleotides in the crRNA has emerged as a potential approach to enhance CRISPR-Cas9 complex specificity by reducing off-target activity (Rueda et al., 2017; Kartje et al., 2018). revealed that although PS, 2'-F, and 2'-O-Me modifications are tolerated in all of the non-Cas9 interacting nucleotides to improve gRNA stability, the extent of off-target editing between unmodified and modified and modified and modified sgRNA was comparable in both cultured cell lines and mice liver cells (Yin et al., 2017). doi: 10.1038/nbt.2808 PubMed Abstract | CrossRef Full Text | Google Scholar Gaj, T., Staahl, B. Following the CRISPR-induced DSB, the endogenous cellular DNA repair mechanism, called non-homologous end joining (NHEJ), can repair the break, often resulting in small insertions, thereby inactivating the target gene (Yang et al., 2020). Therefore, for clinical purposes, the CRISPR-Cas9 system must possess a limited intracellular lifespan to allow for quick and efficient gene editing while minimizing off-target effects. doi: 10.1371/journal.pbio.2005840 PubMed Abstract | CrossRef Full Text | Google Scholar Wu, Y., Zeng, J., Roscoe, B. Together, these advancements in the engineering of synthetically modified gRNA have enabled tremendous improvements in CRISPR-mediated genome editing's stability, specificity, and safety. Engl. E., Roy, S., et al. Contrary to the in vitro activity assays, in cultured cells, any hydrogen-bond-disrupting modifications on the Cas9-interacting nucleotides reduced editing activity (O'Reilly et al., 2019). (2009). On the other hand, G-rich hairpins or G4 structures at the 5' end completely abolished Cas9-mediated cleavage (Nahar et al., 2018). Additionally, we thank the Israel Science Foundation (ISF) (Grant No. 19-701-IPG) for their funding contributions. CRISPR-Cas for fungal genome editing: a new tool for the management of plant diseases. U.S.A. 112, 3002-3007. Rapidly inducible Cas9 and DSB-ddPCR to probe editing kinetics. Although single stranded, RNA is not always linear. R. Benjamin Cummings, 2011. doi: 10.1126/science.aax7852 PubMed Abstract | CrossRef Full Text | Google Scholar Wang, H., Yang, H., Shivalila, C. 5:101. Extensive work has been done to produce inducible Cas9 systems to control nuclease activity by modifying the Cas9 protein to be activated only when induced chemically (Dow et al., 2015; Rose et al., 2017; Polstein and Gersbach, 2017; Polstein and 2017; Polstein and 2017; Polstein | CrossRef Full Text | Google Scholar Schubert, M. Rep. By taking advantage of this endogenous repair pathway, efficient gene editing and gene knock-in are possible. Science 351, 84-88. Photocaging with light-sensitive 6'-nitropiperonyloxymethyl (NPOM) thymidine modifications on the distal portion of the guide region prevents the gRNA from binding completely to its DNA target. Funding We gratefully acknowledge the funding support from the European Research Council (ERC) under the Horizon 2020 Research and Innovation Program (Grant Agreement No. 755758). During translation, the anticodon region of tRNA recognizes a specific area on messenger RNA (mRNA) called a codon. Truncated gRNAs (tru-gRNAs), as short as seventeen nucleotides, have been shown to destabilize the cleavage complex formation and reduce the time spent in the zipped conformation, allowing for more specific editing (Fu et al., 2014). E., et al. conducted an extensive, high-throughput analysis of Cas9 cleavage specificity both in vitro and in cultured cells, combined with mechanistic studies to identify the precise stage during the Cas9-cleavage reaction that was affected by the BNANC and LNA substitutions (Cromwell et al., 2018). doi: 10.1021/ja512664v PubMed Abstract | CrossRef Full Text | Google Scholar Hendel, A., Bak, R. Lastly, this research was supported by the Ministry of Science, Technology & Space (Grant No. 3-14679). This can be achieved by chemically modifying the gRNA ends to reduce degradation by exonucleases, thus improving the guide's stability (Hendel et al., 2015a). The study focused solely on modifying crRNA while being mindful of the impact on the RNA's helix conformation. GFP and BFP were shown solely as examples since CRISPRainbow covers the full spectrum of combinations. doi: 10.1038/s41467-019-11962-8 PubMed Abstract | CrossRef Full Text | Google Scholar Goodwin, M., Lee, E., Lakshmanan, U., Shipp, S., Froessl, L., Barzaghi, F., et al. Both ribosomal subunits travel along the mRNA molecule translating the codons on mRNA into a polypeptide chain as they go. Although there is a wide consensus regarding the profile of chemical modifications that improve the intracellular and intra-serum stability of guide RNAs, the proper design of the chemical gRNA modifications to improve the specificity of CRISPR-mediated genome editing is still to be determined. S., Charpentier, E., and Koonin, E. This highlighted the necessity for proper hydrogen bonding for Cas9-gRNA complexes in cultured cells. 9:60. (2015a)], as well as on the internal residues in the crRNA and tracrRNA regions, resulted in more efficient in vivo genome editing compared to the unmodified sgRNA or sgRNA with only terminal modifications (Finn et al., 2018). S., He, S., Nihongaki, Y., Li, X., Razavi, S., et al. 9:2641. P., Liu, P., Yao, Q., Lazzarotto, C. 26, 2431-2442. A light-inducible CRISPR-Cas9 system for control of endogenous gene activation. However, a relatively simple and cost-effective method that allows optically-induced genome editing was recently demonstrated by adding photocaged light-sensitive 6'-nitropiperonyloxymethyl (NPOM) thymidine modifications on the distal portion of the gRNA (Liu et al., 2020; Moroz-Omori et al., 2020). M., and Joung, J. Likewise, a pioneering study by Hendel et al. Nature 529, 490-495. This creates a potential major pitfall for CRISPR-based therapies due to the well-understood correlation between increased DSBs to increased cellular toxicity and elevated immune response (Obe et al., 1992; Lips and Kaina, 2001; Nakad and Schumacher, 2016; Bednarski and Sleckman, 2019). B., Kim, D. Therapeutic potential of CRISPR/Cas9 gene editing in engineered T-cell therapy. P., Pattanayak, V., Prew, M. 8, 4254-4264. Modifying gRNA to Increase HDR Efficiency CRISPR-mediated DSBs can be repaired via the HDR pathway to allow for precise editing of DNA sequences, to correct genetic mutations, or to introduce novel genetic fragments. Table 2. Hence, measuring the extent of indels on the site of interest, following CRISPR-mediated editing, is considered a gold standard for assessing the CRISPR activity in cultured cells and in vivo. R., van Heteren, J., et al. Since it was first reported in 2013 that the CRISPR system could be repurposed into a reliable and straightforward genome editing. Global transcriptional response to CRISPR/Cas9-AAV6-based genome editing in CD34(+) hematopoietic stem and progenitor cells. doi: 10.1038/s41467-020-17626-2 PubMed Abstract | CrossRef Full Text | Google Scholar Ran, F. hypothesized that maximal 2'-modified ribose rings and modified backbone phosphate groups inside the crRNA and tracrRNA should generate the required gRNA formulation for clinical studies; albeit, all of the work in the study was conducted on HEK293 cells, without in vivo validation. Cell Biosci. R., Debacker, A. doi: 10.1016/j.ymthe.2018.06.002 PubMed Abstract | CrossRef Full Text | Google Scholar Cromwell, C. R., Sung, K., Park, J., Krysler, A. They also concluded that the guide region of crRNA, and especially the seed region, favor modifications that closely resemble the native RNA nucleotides, such as 2'-F, while more bulky modifications on ribose ring and phosphodiester bonds, however, since it is well-known that RNA bases undergo a wide spectrum of modifications, such as 5-methylcytidine, or pseudouridine (Harcourt et al., 2017; Pan, 2018), which can ameliorate cellular immune responses (Hu et al., 2020), the potential to incorporate these could be a plausible future direction for engineering gRNAs. Certain modifications, such as the aforementioned MS and MSP modifications on the gRNA termini, are already being used worldwide as the guintessential standard for highly efficient genome editing. N., St. Martin, A., Harris, R. 10:4045. A., Carney, J. Disorders that can be treated by this method include β-globin-associated diseases such as sickle-cell anemia and β-thalassemia (Dever et al., 2016; DeWitt et al., 2016; Park et al., 2019; Romero et al., 2019; Nu et al., 2019), as well as Severe Combined Immunodeficiency (SCID) (Pavel-Dinu et al., 2020), Wiskott-Aldrich Syndrome (IPEX) (Goodwin et al., 2019), as well as Severe Combined Immunodeficiency (SCID) (Pavel-Dinu et al., 2020), Wiskott-Aldrich Syndrome (IPEX) (Goodwin et al., 2019), as well as Severe Combined Immunodeficiency (SCID) (Pavel-Dinu et al., 2020), Wiskott-Aldrich Syndrome (IPEX) (Goodwin et al., 2019), as well as Severe Combined Immunodeficiency (SCID) (Pavel-Dinu et al., 2019), as well as Severe Combined Immunodeficiency (SCID) (Pavel-Dinu et al., 2019), as well as Severe Combined Immunodeficiency (SCID) (Pavel-Dinu et al., 2020), Wiskott-Aldrich Syndrome (IPEX) (Goodwin et al., 2020), Wiskott-Aldrich Syndrome (IPEX) (SCID) (Pavel-Dinu et al., 2020), Wiskott-Aldrich Syndrome (IPEX) (SCID) (SC Mucopolysaccharidosis Type 1 (Gomez-Ospina et al., 2019). Editing the sickle cell disease mutation in human hematopoietic stem cells: comparison of endonucleases and homologous donor templates. Additionally, in Cas12a, modifications in the seed region or on the 5' handle were not well-tolerated (Safari et al., 2019). 8:360ra134. In this procedure, hematopoietic stem and progenitor cells (HSPCs) or T lymphocytes are isolated from the patient's blood, undergo the desired gene correction ex vivo, and are then transfused back to the patient's bloodstream. R-loop formation is depicted with Watson and Crick base pairing of the RNA:DNA heteroduplex. doi: 10.1126/science.1231143 PubMed Abstract | CrossRef Full Text | Google Scholar Cromer, M. doi: 10.1038/s41467-018-06129-w PubMed Abstract | CrossRef Full Text | Google Scholar Braasch, D. Throughout evolution, bacteria and archaea acquired the ability to store copies of portions of invading foreign genetic material such as plasmids, phage genomes, or RNA, as segments between clustered repetitive sequences in the genome. Microbiol. A., Machemer, T., Ragone, F. M., et al. J., Barrera, L. 10:135. M., Gao, L., Zetsche, B., Scott, D. Acknowledgments We thank Dr. Adi Tovin-Recht for her useful support. Natl. Cytokine Biol. During transcription, certain proteins called ranscription factors unwind the DNA strand and allow the enzyme RNA polymerase to transcribe only a single strand of DNA. O., Reinisch, A., Camarena, J., Washington, G., Nicolas, C. On the contrary, any modifications on the 2' carbon in the ribose ring were not tolerated in the PAM-proximal (seed) region, presumably since the seed region is critical for target DNA recognition by CasS (Jiang et al., 2015). P., and Butler, K. doi: 10.1038/s41579-019-0299-x PubMed Abstract | CrossRef Full Text | Google Scholar Mali, P., Yang, L., Esvelt, K. A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. Hence, our goal in this review article was to elucidate the entire repertoire of gRNA chemical modifications in order to allow the researchers in the field to make educated decisions while choosing the appropriate gRNA formulation that would fit the particular study design. 8:1610. A large ribosomal subunit then joins the newly formed complex. This underscored the importance of avoiding modifications on the endonucleaseinteracting 2'-OH groups, maintaining the sgRNA-Cas9 hydrogen bonding, and modifying the other nucleotides to increase editing efficiency (Yin et al., 2017). 38, 824-844. doi: 10.1038/nature24268 PubMed Abstract | CrossRef Full Text | Google Scholar Cho, S. In some cases, these bacterial nucleases have demonstrated significant off-target activity. leading to unintended DNA breaks at ectopic sites in the genome with only partial complementarity to the gRNA sequence (Li et al., 2016). E. S., Brouns, S. (2015b). This method provided efficient and reliable live-cell multicolor labeling of multiple chromosomal loci at the same time in live cells (Shao et al., 2016). A., Harrington, L. Plant Sci. Another possible side effect of gRNA modification can be increased cytotoxicity, leading to cellular death, a major problem many researchers are actively seeking to solve (Basila et al., 2017). This was the first time it was shown that sgRNA chemical modifications enhance intracellular stability, thereby increasing genome editing efficacy when Cas9 and sgRNAs are co-delivered into human primary cells (Hendel et al., 2015a). J. Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. Nat. M., Dang, H. Furthermore, adding two extra guanine residues on the 5' end of the spacer sequence (ggXX20 gRNA) had a variable effect on gene-editing performance in cultured cells, enhancing the guide specificity at specific genomic sites by significantly reducing off-target activity while maintaining the on-target efficiency. M., Liu, H., Wu, J., Chong, A., Mackley, V., Fellmann, C., et al. Adv. K., Gao, Y., Liu, Y., Oi, L. Modifications included: 2'F. 2'F-ANA, 2'.5'-RNA, contributed to the conceptualization of the review and wrote the paper. 1:0066. The spacer region (also known as guide region) is typically 20 nucleotides long but it has been shown that it can be shortened or lengthened (to include hairpin structures) at the 5' end. doi: 10.1126/scitranslmed.aah3480 PubMed Abstract | CrossRef Full Text | Google Scholar Dever, D. Y., Ko, J. 54, 2377-2380. (C,D)-gRNA modifications that utilize CRISPR-dCas9 specificity for high-resolution cellular imaging: (C) sgRNA molecule with fluorophore-bound aptamers binding to either the tetraloop or stem-loop 2 (for the same reason as mentioned above). Assembly of CRISPR ribonucleoproteins with biotinylated oligonucleotides via an RNA aptamer for precise gene editing, A., Kohlenberg, L., Goedland, M., Molugu, K., Lou, M., et al. This method can potentially be adapted for therapeutic purposes in other hematopoietic cells such as T and B lymphocytes, and Natural Killer (NK) cells. One-step gene editing, A., Kohlenberg, L., Goedland, M., Molugu, K., Lou, M., et al. This method can potentially be adapted for therapeutic purposes in other hematopoietic cells such as T and B lymphocytes. CRISPR/Cas-mediated genome engineering. Transfer RNA along with ribosomes read the mRNA codons and produce a polypeptide chain. M., Cheng, A. Due to the incomplete gRNA base pairing with the DNA site, the Cas9 remains catalytically inactive. W., Bennett, C. doi: 10.1021/acs.biochem.8b00107 PubMed Abstract | CrossRef Full Text | Google Scholar Kelley, M. 1:54. doi: 10.1093/nar/gkv137 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Full Text | Google Scholar O'Reilly, D., Kartje, Z. Am. Chem. doi: 10.1016/j.celrep.2014.02.040 PubMed Abstract | CrossRef Fu with completely modified crRNA exhibited satisfactory editing activity (Mir et al., 2018). doi: 10.1016/j.cell.2013.04.025 PubMed Abstract | CrossRef Full Text | Google Scholar Wang, S., Su, J. (B) sgRNA molecule with streptavidin-binding aptamers that attach to either the tetraloop or stem-loop 2 (the two loops protruding from the Cas9 molecule). 9:1448. The CRISPRainbow method further expanded the number of loci that can be viewed simultaneously by exploiting aptamer-carrying gRNA species (Ma et al., 2016) (Figure 3C). doi: 10.1073/pnas.91.13.6064 PubMed Abstract | CrossRef Full Text | Google Scholar Rueda, F. HDR uses a homologous DNA template, either endogenous (sister chromatid or homologous chromosomes) or exogenously introduced (donor template) sequences for genetic manipulation, and is, therefore, significantly less error-prone (Rouet et al., 1994; Porteus, 2016). O., Mantri, S., Quadros, R. S., and Anderson, D. Cell. CRISPR-engineered T cells in patients with refractory cancer. C., Stephany, J. C., Lindsay, H., Berk, C., Bargsten, K., Li, Y., et al. 44:e86. K., et al. The enhanced efficacy of the subsequent HDR showed that the conjugated gRNA and donor DNA without the need for viral transduction (Lee et al., 2017b) (Figure 3A). 44, 10003-10014. They were able to obtain complete gRNA modification by combining the PS, 2'-F, and 2'-O-Me modifications which resulted in decreased Cas9 activity and as well as in precise HDR efficiency (Carlson-Stevermer et al., 2017) (Figure 3B). Additionally, Cas12a has been shown to be more specific than Cas9 at certain genomic sites (Kim et al., 2016) and may be more useful in particular settings. S., Wolf, Y. Figure 1. Science 339, 823-826. Interestingly, the hairpin structures had a strong negative effect on the in vitro nuclease activity due to the slower kinetics of the cleavage reaction. 208, 44-53. B., and Sinha, R. Reece, Jane B., and Neil A. P., Rettig, G. Adding RNA aptamers on either the tetraloop or stem-loop 2, which both protrude from the Cas9 protein, leaving it into an active nuclease (Josephs et al., 2015; Sternberg et al., 2015). (2010 Cas9 which in turn activates the nuclease domain, and DNA cleavage which is induced almost instantaneously. Modifying gRNA to Produce Inducible and Controlled Editing Although tremendous progress in the quest to adapt the bacterial defense system to human cells has been made, much remains to be learned about the cellular response mechanisms and repair pathways in response to Cas-induced DSBs. Delivering CRISPR as an RNP complex is the most effective gene-editing method, but even then, cleavage is neither immediate nor synchronous across the treated cell population. Previous work has shown that there are ~20 positions of nucleotides in both crRNA and tracrRNA that interact with the Cas9 protein via the 2'-OH group, and thus do not tolerate any 2'-OH modifications. Biol. W. doi: 10.1073/pnas.1520883112 PubMed Abstract | CrossRef Full Text | Google Scholar Rai, R., Romito, M., Rivers, E., Turchiano, G., Blattner, G., Vetharoy, W., et al. 381, 1240-1247. Wienert et al., Kim et al., and Schubert et al. (1992). doi: 10.1038/s41587-020-0561-9 PubMed Abstract | CrossRef Full Text | Google Scholar Basila, M., Kelley, M. The synthetic sgRNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficiency compared to the two-part RNA system has been shown to achieve equivalent or higher efficience equivalent or higher efficience equivalent or higher efficience equivale specificity by altering the thermodynamic and kinetic properties of the gRNA-DNA heteroduplex formation, such as melting temperature (Ryan et al., 2018). A., and Marszalek, P. CRISPR-Cas9 gene repair of hematopoietic stem cells from patients with X-linked chronic granulomatous disease. 34, 863-868. Very fast CRISPR on demand. Gene editing and CRISPR in the clinic: current and future perspectives. 233, 74-83. J., Fine, E. S., Tsai, S. doi: 10.1038/s41467-018-05641-3 PubMed Abstract | CrossRef Full Text | Google Scholar Park, S. 24, 132-141. systematically evaluated several combinations of MS end modifications in both the two-part system and sgRNA as well as two types of intracellular delivery mechanisms for the editing complexes: electroporation and cationic lipid transfection (Basila et al., 2017). C., Naseri, A., Huisman, M., Zhang, S., Grunwald, D., et al. F., Hassler, M. Moreover, with few exceptions, the increase in the on-target activity was accompanied by only a minor effect on off-target activity, thus achieving favorable ontarget:off-target ratios. The shorter interaction time in this conformation resulted in slower cleavage kinetics on the on-target sites but resulted in lowered Cas9-induced off-target DNA cleavage by several orders of magnitude (Cromwell et al., 2018), which on an overall scale was beneficial for the specificity of the genome editing. Multiplexed labeling of genomic loci with dCas9 and engineered sgRNAs using CRISPRainbow. Research has also shown that the order in which the gRNA some level of protection from degradation when delivered as an RNP complex. CRISPR-Cas9-based photoactivatable transcription system. Conversely, Rueda et al. Modifications to make a phosphodiester bond between the 2' and 5' carbons (2',5'-RNA) of adjacent RNAs as well as a butane 4-carbon chain link between adjacent RNAs as well as a butane performed an extensive study of gRNA modifications in in vivo gene editing in mouse livers using lipid nanoparticles (Yin et al., 2017); however, they used the crystal structure of the CRISPR-Cas9 RNP complex to guide the optimization of combinations of sgRNA modifications. Synthetic CRISPR RNA-Cas9-guided genome editing in human cells. P Nahar et al. Removal of the 5'-ppp groups by in vitro phosphatase treatment yielded 5'-hydroxyl gRNAs that could, in complex with Cas9 or Cas12a, achieve a high degree of mutagenesis in cell lines and primary human cells. Dev. A. doi: 10.1016/j.chembiol.2014.12.011 PubMed Abstract | CrossRef Full Text | Google Scholar Nishimasu, H., Ran, F. Upon encountering a PAMP motif on an RNA molecule, these proteins trigger a signaling cascade, eventually resulting in the upregulation of type 1 interferon-stimulated genes (Kell and Gale, 2015). Elife 7:e33761. Indeed, significant DNA cleavage was generated within 30 seconds of light activation. doi: 10.1038/s41467-018-05073-z PubMed Abstract | CrossRef Full Text | Google Scholar Moon, S. M., Lunstad, B. P., Krishnakumar, R., Timlin, J. Figure 3. Inducible in vivo genome editing with three hairpin loops. Immunol. A., Corn, J. A., Malek-Adamian, E., Habibian, M., Schofield, A., et al. Additionally, Cas12a nuclease cleavage produces cohesive double-strand breaks (DSBs) (compared to the predominantly blunt-end DSB created by Cas9) and relies on different PAM recognize and attack tumor cells (Gao et al., 2019; Stadtmauer et al., 2020). doi: 10.1126/science.1232458 PubMed Abstract | CrossRef Full Text | Google Scholar Taemaitree, L., Shivalingam, A., El-Sagheer, A. However, since the majority of genetic diseases and tumors occur in tissues that cannot be conveniently isolated and edited ex vivo, other therapeutic options must be explored. doi: 10.1038/nature14136 PubMed Abstract CrossRef Full Text | Google Scholar Lee, K., Conboy, M., Park, H. doi: 10.1124/jpet.112.193789 PubMed Abstract | CrossRef Full Text | Google Scholar Carlson-Stevermer, J., Abdeen, A. Rev. In fact, it has been found that modifying the RNA secondary structure by engineering a hairpin onto the 5' end of the sgRNA spacer sequence (hp-sgRNAs) significantly increases gene editing specificity in cells when complexed with various CRISPR effector nucleases (Kocak et al., 2019). The anticodon recognizes a specific area on mRNA called a codon. 32, 279-284. M., Kang, J. L., Rohilla, K. On the contrary, Cas9 endonuclease capability was severely impaired when crRNAs underwent substitutions inside the seed region. I., Iranzo, J., Shmakov, S. Biochemistry 42, 7967-7975. Y., Sung, K., Jeong, E., Lee, S. Phosphonoacetate alterations. (A) Naturally occurring crRNA [~42 nt (striped nucleotides)] containing the DNA-binding spacer sequence and the trans-activating tracrRNA [80 nt (Rahdar et al., 2015) (checkered nucleotides)] annealed together through Watson and Crick base-pairing by the repeat (brown) and anti-repeat (gray) regions. A., Jensen, S., Liu, Y., Kaur, K., White, M. Nature 517, 583-588. DNA double-strand breaks induced by sparsely ionizing radiation and endonucleases as critical lesions for cell death, chromosomal aberrations, mutations and oncogenic transformation. The guide can be adapted to the target site in the genome (providing the genomic sequence). (2015a). has already been conducted in an HIV-positive patient with Acute Lymphoblastic Leukemia (Xu et al., 2019). Pharmacol. D., Josephs, E. doi: 10.1038/s41467-019-10080-9 CrossRef Full Text | Google Scholar Polstein, L. Evolutionary classification of CRISPR-Cas systems: a burst of class 2 and derived variants. By avoiding those 2'-OH sites, a sqRNA was designed with a pattern of PS, 2'-F, and 2'-O-Me modifications on the remaining non-Cas9-interacting nucleotides that maximized the editing efficiency both in HEK293 cells and in live animals. Ther. utilized a broader variety of chemical modifications and linkers to test the compatibility and structure-activity relationships of engineered gRNAs with Cas9-mediated editing activity to try and lay out the foundation for a rational design of modified gRNAs (O'Reilly et al., 2019), 9:3651, All authors approved the submitted version, S., and Kim, Y. PubMed Abstract | CrossRef Full Text | Google Scholar Fu, Y., Sander, I. In addition, adding modifications known to increase RNA affinity to DNA, such as 2'-fluoro (2'-F) and S-constrained ethyl (cEt) (Figure 2), on the crRNA inside of the PAM-distal and tracrRNA-binding regions, respectively, further increased editing activity. K., Been, K. Plasmid donors are problematic in clinical applications due to the risk of insertional mutagenesis and of triggering an immune response to foreign DNA. A., Schnitzbauer, J., Zhang, W., Li, G. Covalent linkage of the DNA repair template to the CRISPR-Cas9 nuclease enhances homology-directed repair. They aimed to preserve sufficient duplex stability and relatively low dissociation rate on the off-target sites with only partial gRNA complementarity. The guide region forms the gRNA:DNA heteroduplex through Watson and Crick base pairing (Jinek et al., 2012; Nishimasu et al., 2014) (Figure 1A). Table 1. Through more extensive testing and development of different gRNA modifications aimed to increase efficiency, specificity, and safety, as well as new applications of the CRISPR-Cas technology will be accelerated for the benefit of human society. T., Zheng, Z., et al. A., et al. Mutagenesis 7, 3-12. MicroRNAs, a type of regulatory RNA, have also been linked to the development of some types of cancer. doi: 10.3389/fimmu.2017.00331 CrossRef Full Text | Google Scholar Mir, A., Alterman, J. A genome-wide analysis of Cas9 binding specificity using ChIP-seq and targeted sequence capture. U.S.A. 112, E7110-E7117. 2115, 23-55. The nucleic acid chemistry of gRNA enables expanding the array of nucleotide formulations from a native 4-letter RNA code to a wide range of phosphodiester, sugar ring, and nitrogen base modifications. each examined various cell lines as well as different clinically

relevant primary cells such as HSPCs, human peripheral blood monocytic cells (PBMCs), and CD4+ T cells. examined the impact of sgRNA modifications on genome editing efficiency in mouse and rat liver in vivo (Finn et al., 2018). Based on prior work in the field of RNA therapeutics, Mir et al. 17, 1865–1867. CRISPR/Cas9 genome editing technology in filamentous fungi: progress and perspective. The second type of gRNA that can complex with Cas is a synthetic sgRNA (~100 nt) where the bridged portion between the crRNA and the tracrRNA is covalently linked by an artificial tetraloop (Jinek et al., 2012) (Figure 1B). Recently, Taemaitree and colleagues presented a simplified method for producing sgRNAs via synthesis of the variable guide sequence (20 nt) and subsequently ligating the product to the remaining constant region (79 nt) by a triazole linkage (Taemaitree et al., 2019). doi: 10.1093/nar/gkx1199 PubMed Abstract | CrossRef Full Text | Google Scholar Safari, F., Zare, K., Negahdaripour, M., Barekati-Mowahed M., and Ghasemi, Y. D., Smith, A. D., Singh, M., et al. Recently, a thoroughly optimized protocol for using end-modified sgRNA in human primary HSPCs was evaluated, demonstrating high editing efficiency and specificity through the delivery of the CRISPR system as an RNP complex (Shapiro et al., 2020, 2021). S., Domm, J., Eustace, B. Mapping the sugar dependency for rational generation of a DNA-RNA hybrid-guided Cas9 endonuclease. Nucleic Acids 2010:592980. Y., Wu, Q., Walsh, S., et al. A., Bhandarkar, V., Adkar, S. The most popular tool developed based on the CRISPR-Cas system is CRISPR-Cas9 (Jiang and Doudna, 2017), derived from Streptococcus pyogenes. A., Alkhnbashi, O. To address this, a study by Rahdar et al. The current solution to these issues is to use formulations of gRNAs together with Cas9 mRNA or protein instead of plasmid DNA. Structure-guided chemical modification of guide RNA enables potent non-viral in vivo genome editing. doi: 10.1007/s11274-018-2518-4 PubMed Abstract | CrossRef Full Text | Google Scholar Yang, H., Ren, S., Yu, S., Pan, H., Li, T., Ge, S., et al. The many years of progress in enhancing small RNA-based technologies, such as antisense RNA and RNA interference (RNAi) (Levin, 2019), includes improving RNA stability by incorporating chemical modifications onto the small RNAs (Braasch et al., 2003; Chiu and Rana, 2003; Behlke, 2008; Bennett and Swayze, 2010; Deleavey and Damha, 2012; Lennox and Behlke, 2020). K., Doudna, J. CRISPR-Cas9-based photoactivatable transcription systems to induce neuronal differentiation. However, in this study, the tracrRNA remained unmodified, and the potential of using these chemical modifications in vivo was not explored. Optical control of CRISPR/Cas9 gene editing. P., Welch, M. Another approach that has been shown to increase HDR efficiency without the need to conjugate the gRNA to the donor DNA utilizes RNA aptamers. doi: 10.1056/NEJMoa2031054. While mutations or mismatches within the PAM sequence ostensibly abrogate Cas9 endonuclease activity (O'Geen et al., 2015a; Jiang and Doudna, 2017), mismatches within the guide region may be permitted (O'Geen et al., 2015b) resulting in the undesired cleavage of off-target DNA sequences. Y., et al. doi: 10.1038/s41467-017-01732-9 PubMed Abstract | CrossRef Full Text | Google Scholar Ryan, D. This is actuated while triggering a reduced immune response similar to the synthesized gRNA species which are manufactured lacking 5'-ppp groups (Kim et al., 2018; Wienert et a was not tolerated (Yin et al., 2018). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. P., Muley, A., Kastenhuber, E. O., Bista, M., Newton, M. Q., Suresh, S., Rhym, L. Nucleic Acids Res. S., and Gordon, W. doi: 10.1159/000486168 PubMed Abstract | CrossRef Full Text | Google Scholar Zhou, X. D., Weber, K. 7:13350. doi: 10.1016/j.celrep.2018.02.014 PubMed Abstract | CrossRef Full Text | Google Scholar Frangoul, H., Altshuler, D., Cappellini, M. 18, 67-83. doi: 10.1038/nature20134 PubMed Abstract | CrossRef Full Text | Google Scholar Frangoul, H., Altshuler, D., Cappellini, M. 18, 67-83. doi: 10.1038/nature20134 PubMed Abstract | CrossRef Full Text | Google Scholar DeWitt, M. K. (Anzalone et al., 2020). They do so by binding to a specific location on mRNA, preventing the molecule from being translated. L., Lancaster, E., et al. CRISPR-Cas systems, which can be engineered and modified with relative ease, provide a tremendous array of groundbreaking and versatile tools for programmable genome editing. B. demonstrated that introducing G-quadruplex (G4) structure at the 3' end of the sgRNA resulted in increased in vitro serum stability and higher editing efficiency in the zebrafish embryos, compared to the unmodified sgRNA (Nahar et al., 2018). 37, 657-666. doi: 10.1016/j.tibtech.2019.01.009 PubMed Abstract | CrossRef Full Text | Google Scholar Moroz-Omori, E. Science 337, 816-821. Cancer Med. V., Satyapertiwi, D., Ramel, M. 43, 3389-3404. demonstrated that in vitro cleavage of DNA duplexes by Cas9 could be facilitated by chimeric DNA-RNA crRNAs. Contrary to expectations, they showed that DNA substitutions inside the crRNA 3' end, but not within the guide sequence, resulted in the Cas9-mediated cleavage being less tolerant of mismatches in the target sequence (Kartje et al., 2018). RNA nitrogenous bases include adenine (A), guanine (G), cytosine (C) and uracil (U). doi: 10.1056/NEJMoa1817426 PubMed Abstract | CrossRef Full Text | Google Scholar Xue, T., Liu, K., Chen, D., Yuan, X., Fang, J., Yan, H., et al. B., Boix, C., Charpentier, M., De Cian, A., Cochennec, J., Duvernois-Berthet, E., et al. K., Vaidyanathan, S., Ryan, D. Cell 153, 910-918. doi: 10.1016/j.cell.2013.12.001 PubMed Abstract | CrossRef Full Text | Google Scholar Chen, J. A., Chou, E., Machado, H. Double-stranded RNA (or dsRNA), as is seen here, can be used to block the expression of specific genes. M., Hacisuleyman, E., Younger, S. This could potentially lead to treatments for a number of diseases including cervical cancer (Zhen and Li, 2017), an inherited form of blindness Leber congenital amaurosis type 10 (LCA10) (Maeder et al., 2019), among others. doi: 10.1093/nar/gky1214 PubMed Abstract | CrossRef Full Text | Google Scholar Park, H. Lastly, we would like to express our appreciation to Dr. Mark Behlke and Dr. Kim Lennox for reading the manuscript and providing insightful suggestions. P., Davis, T. The amino acid sequences are joined together to form a protein. J., Ageely, E. doi: 10.1093/nar/gkx154 PubMed Abstract | CrossRef Full Text | Google Scholar Gao, Q., Dong, X., Xu, Q., Zhu, L., Wang, F., Hou, Y., et al. 10:1634. W., Kim, S., Kim, Y., Kweon, J., Kim, H. Albeit, the application of the CRISPR-Cas9 system for clinical purposes still faces significant obstacles. Hence, synthesized and chemically-modified gRNAs represent an optimal and clinically appropriate option for CRISPR-mediated gene editing in primary cells. Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair To that end, clinically relevant CRISPR-Cas9 systems must be developed that would avoid triggering the innate immune response and increase specificity in primary cells. Cromwell et al. H., Zhang, F., and Zhuang, X. doi: 10.1016/j.jbiotec.2015.04.024 PubMed Abstract | CrossRef Full Text | Google Scholar Lim, Y., Bak, S. PLoS ONE 12:e0188593. (2013). doi: 10.1021/acscentsci.9b01093 PubMed Abstract | CrossRef Full Text | Google Scholar Munoz, I. L., Wang, T., Berman, J. Quantifying genome-editing outcomes at endogenous loci with SMRT sequencing. More extensive ribose modifications such as 2'F-4'-Ca-OMe and 2',4'-di-Ca-OMe an 792-803. doi: 10.1093/nar/gkw930 PubMed Abstract | CrossRef Full Text | Google Scholar Robbins, M., Judge, A., and MacLachlan, I. STRUCTURAL BIOLOGY. D., Trevino, A. doi: 10.1016/j.ymthe.2005.11.002 PubMed Abstract | CrossRef Full Text | Google Scholar Kaczmarek, J. Recently, in order to reduce the costs of producing a large amount of gRNAs, IVT by T7/SP6 phage RNA polymerases has become a popular method. K., Liu, Z., et al. 3, 135-149. Gene correction for SCID-X1 in long-term hematopoietic stem cells. Photoswitchable gRNAs for spatiotemporally controlled CRISPR-cas-based genomic regulation. Type II CRISPR formulations. (D) CRISPR LiveFISH method utilizes crRNAs fused to a fluorophore at the 5' end to actuate live intracellular staining without the need for cellular fixation. 33, 390–394. doi: 10.1038/s41551-017-0137-2 PubMed Abstract | CrossRef Full Text | Google Scholar Lee, K., Mackley, V. The CRISPR system can be utilized to knock-out genes by creating a DSB at the site of interest in the genome. Various applications of engineered gRNAs. (A,B)-gRNA modifications to improve HDR: (A) crRNA-donor DNA conjugate. Rapid and highly efficient mammalian cell engineering via Cas9 protein transfection. doi: 10.1093/nar/gkz475 PubMed Abstract | CrossRef Full Text | Google Scholar Pavel-Dinu, M., Wiebking, V., Dejene, B. Front. (2015a). RNA stands for ribonucleic acid and like DNA, RNA nucleotides contain three components: A Nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of three main elements: a nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of three main elements: a nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of three main elements: a nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of three main elements: a nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of three main elements: a nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of three main elements: a
nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of three main elements: a nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of three main elements: a nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of three main elements: a nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of three main elements: a nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of three main elements: a nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of three main elements: a nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of three main elements: a nitrogenous BaseA Five-Carbon Sugar A Phosphate Group RNA is a single-stranded nucleic acid that is composed of the single-stranded nucleic acid that is composed of the single-stranded nucleic acid that is composed o (rRNA) are the three major types of RNA.mRNA is involved in the transcription of DNA while tRNA has an important role in the translation component of protein synthesis. As the name implies, ribosomal RNA (rRNA) is found on ribosomes. A less common type of RNA known as small regulatory RNAs possess the ability to regulate the expression of genes. 34:154. Sci. F., and Swayze, E. doi: 10.1038/s41467-017-01875-9 PubMed Abstract | CrossRef Full Text | Google Scholar Chen, B., Gilbert, L. Annu. Methods 12, 664-670. U.S.A. 91, 6064-6068. B., et al. CRISPR-Cas9/Cas12a biotechnology and application in bacteria. doi: 10.1146/annurev.pharmtox.010909.105654 PubMed Abstract | CrossRef Full Text | Google Scholar Bin Moon, S., Lee, J. S., et al. doi: 10.3390/ijms21186461 PubMed Abstract | CrossRef Full Text | Google Scholar Yao, R., Liu, D., Jia, X., Zheng, Y., Liu, W., and Xiao, Y. It has the ability to fold into complex three dimensional shapes and form hairpin loops. When a termination codon is reached on the mRNA molecule, the translation process ends. (B) Synthetic sgRNA formulation where the crRNA and tracrRNA are covalently fused by a tetraloop. doi: 10.1101/gr.162339.113 PubMed Abstract | CrossRef Full Text | Google Scholar Song, R., Zhai, Q., Sun, L., Huang, E., Zhang, Y., Zhu, Y., et al. Q., Suresh, S., Kwan, S. Mol. Commun. In nature, gRNA is found as a two-part molecule consisting of crRNA (~67-89 nt) (Jinek et al., 2012). In addition, the researchers achieved higher specificity using the engineered hairpin structures than with the tru-gRNA analog when tested side by side. CRISPR Cpf1 proteins: structure, function and implications for genome editing. 8:1711. In addition to engineering the Cas9 protein (Aird et al., 2018; Savic et al., 2018; Ling et al., 2018; Savic et al., 2018; S gRNA itself have great potential to enhance HDR efficiency in a non-viral manner to increase the relevance of the CRISPR-Cas9 gene-editing tool for many biotechnological applications. To that end, a combination of the truncated or hairpin-modified sgRNAs in tandem with the previously discussed terminal chemical modifications could prevent hairpin removal by intrinsic intracellular nuclease activity, thus maximizing the editing capabilities of engineered sgRNAs. It is important to note that the hairpin structures' design must meet stringent constraints for thermodynamic stability since below a specific free energy cut-off, the nuclease activity is severely impaired. Improving the efficiency of precise genome editing with site-specific Cas9-oligonucleotide conjugates. S., Dagdas, Y. The cationic lipid delivery technique previously suggested that liposomes protect gRNA molecules from RNase degradation in the cytosol or culture medium (Anderson et al., 2015; Liang et al., 2015). A., Magis, W., Bray, N. Y., Campo-Fernandez, B., et al. doi: 10.4061/2010/592980 PubMed Abstract | CrossRef Full Text | Google Scholar Renaud, J. Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors, transposases and and translation of the genetic code to produce proteins. (E) Light-activated CRISPR to allow for control over synchronous editing across a cell population. Simple modifications at the 2'OH include 2'-O-Me, 2'-F, and 2'F-ANA. When this occurs, the nitrogenous bases bind to one another. Biomed. Crystal structure of Cas9 in complex with guide RNA and target DNA. doi: 10.1021/acschembio.7b00416 PubMed Abstract | CrossRef Full Text | Google Scholar McMahon, M. To address these issues simultaneously, conjugated gRNA-donor DNAs, which ensures the proximity of the DNA donor to the cut site, have been engineered and have indeed showed improved HDR efficiency. allow greater simplicity, albeit limited to two colors unless applying additional dCas9 species fused to fluorescent proteins. Modifying gRNA to Utilize CRISPR-Cas9 as a Robust Method for Nuclear Imaging Another application that modified gRNAs seek to improve upon is the existing imaging tools of chromosomal dynamics and genomic mapping, which are essential for comprehending a plethora of basic cellular nuclear processes. The polypeptide chain is released from the tRNA molecule and the ribosome splits back into large and small subunits. was proof that chemically modified gRNAs work efficiently in concert with Cas9 mRNA or protein in primary cells, which do not tolerate the introduction of plasmid DNA. LNAs are conformationally restricted RNA nucleotides in which the 2' oxygen on the ribose forms a covalent bond with the 4' carbon (You et al., 2006). B., Ryan, D. doi: 10.1126/science.1225829 PubMed Abstract | CrossRef Full Text | Google Scholar Josephs, E. Therefore, quantifying (Hendel et al., 2014, 2015b) and improving the accuracy, precision, and specificity of these nucleases (Tsai and Joung, 2016) is of major significance. With additional clinical trials.gov, #NCT03655678, and # NCT03745287, (Frangoul et al., 2020)] we expect synthetically modified gRNA-based therapeutics to take a major leap in the years to come. S., Cedrone, E., Neun, B., Behlke, M. doi: 10.1038/s41587-019-0095-1 PubMed Abstract | CrossRef Full Text | Google Scholar Konermann, S., Brigham, M. (2019). They also observed only a small increase in editing efficiency when gRNAs were delivered together with Cas9 mRNA into HeLa or U2OS cell lines, while the number and placement of modifications on gRNA termini showed a significant effect on cellular toxicity (Basila et al., 2017). 6:eaaz0051. At the end of transcription, mRNA is transported to the cytoplasm for the completion of protein synthesis. E., Gordon, E., et al. An additional study by O'Reilly et al. W., Yoon, S. At the same time, an initiator tRNA molecule recognizes and binds to a specific codon sequence on the same mRNA molecule. RNA Secondary Structures and Modified Spacer Length There are at least five stages in the gRNA-mediated Cas9 cleavage reaction, most of which involve conformational changes both within the Cas9 protein and in the RNA-DNA helix (Lim et al., 2016). Transl. Partial DNA gRNA It is well-documented that RNA residues in the crRNA and tracrRNA can be partially substituted for DNA residues without significantly impairing Cas9 activity both in in vitro cleavage assays and cultured cells (Rueda et al., 2017; Kartje et al., 2018; Vin et al., 2018; O'Reilly et al., 2019). doi: 10.4172/2576-3881.1000121 PubMed Abstract | CrossRef Full Text | Google Scholar Shao, S., Zhang, W., Hu, H., Xue, B., Qin, J., Sun, C., et al. V., Sarrocco, S., Malfatti, L., Baroncelli, R., and Vannacci, G. 47, 7955-7972. Albeit, with CRISPR-mediated genome editing being a rapidly developing field, no standardized protocol for gRNA modifications has been generated yet for clinical studies, and every gRNA should be examined on an individual basis. M., Aach, J., Guell, M., DiCarlo, J. Med. BNA(NC) gapmers revert splicing and reduce RNA foci with low toxicity in myotonic dystrophy cells. 28, 367-373. P., Cleveland, D. Genome-wide analysis reveals specificities of Cpf1 endonucleases in human cells. in cultured cells after 2'-OH modification. Genome Med. doi: 10.1038/nmeth.4368 PubMed Abstract | CrossRef Full Text | Google Scholar Rouet, P., Smih, F., and Jasin, M. J., Dewitt, M. BNANCs are molecules with a six-membered bridged structure where the 2' oxygen and the 4' carbon are linked by a methyl-bound nitrogen. R., et al. References Aird, E. This is done by the enzyme RNA polymerase (purple). However, AAV vectors can also elicit immune responses, especially when used in primary cells or in human subjects, posing a critical caveat for gene therapy (High and Roncarolo, 2019). When RNA polymerase transcribes the DNA into a mRNA molecule, adenine pairs with guanine (A-U and C-G). Ribosomal subunits are synthesized in the nucleolus. B., Camarena, J., et al. A., Kocak, D. T. H., and Kim, J. G. Science 367:eaba7365. M., McNeill, M. Through a deeper understanding of the structure of the gRNAs. Removal of 5' Triphosphate and Introduction of 2'-O-Me Uridine or Guanosine Residues In human cells, foreign RNAs are recognized in the cytosol by pathogen-associated molecular pattern (PAMP) binding receptors, Retinoic acid-inducible gene 1 (RIG-1), also known as DExD/H-Box Helicase 58 (DDX58), and melanoma differentiationassociated gene 5 (MDA5). Minimal 2'-O-methyl phosphorothioate linkage modification pattern of synthetic guide RNAs for increased stability and efficient CRISPR-Cas9 gene editing avoiding cellular toxicity. As mentioned above, a significant issue with gRNAs is their marked tendency to be degraded by exonucleases. E., Bennett, C. and Wang et al. They showed that replacing the ten RNA nucleotides in the PAM-distal region with DNA residues maintained on-target genome-editing activity (Yin et al., 2018). A ribosome consists of
ribosomal proteins and rRNA. Messenger RNA (mRNA) plays an important role in the transcription of DNA. New formulations of Cas9-gRNA complexes with various RNA modifications are continually being developed to achieve the proper balance between benefits and side effects. Yin et al. Improving CRISPR-Cas specificity with chemical modifications in single-guide RNAs. Nucleic Acids Res. 6:26857. (2006). They designed lipid nanoparticles containing Cas9 mRNA and discovered that 2'-O-Me and PS chemical modifications on both termini of sgRNA [similar to the MS used by Hendel et al. Targeted gene correction of human hematopoietic stem cells for the treatment of wiskott - aldrich syndrome. [Epub ahead of print]. Increasing the Safety of CRISPR-mediated Gene Editing by Curbing Cellular Toxicity and Immune Responses CRISPR-Cas systems are bacterial mechanisms that researchers have worked determinedly to adapt to mammalian cells. This illustration shows the process of transcription of deoxyribonucleic acid (RNA, green). I., Dohmae, N., et al. R., Jovel, J., Kim, S. 22, 2227–2235. These modifications provide a complementary copy of ribonucleic acid (RNA, green). I., Dohmae, N., et al. R., Jovel, J., Kim, S. 22, 2227–2235. These modifications provide a complementary copy of ribonucleic acid (RNA, green). the CRISPR-Cas9 system the versatility to not only be used for genome editing but also for a deeper understanding of nuclear dynamics and mechanisms of action, including transcription, DNA replication, and DNA repair. undergoes seed nucleation to form an A-form-like helical RNA:DNA hybrid duplex. D., Wright, J., Agarwala, V., Scott, D. Taking advantage of the strong natural interaction between streptavidin and biotin, it was shown that the addition of a streptavidin-binding RNA aptamer on the loop domains of the gRNA along with biotinylated single-stranded oligodeoxynucleotides (ssODNs) formed a highly effective tertiary complex (streptavidin-gRNA, biotin-ssODN, and Cas9). Extensive research has been done on other nucleic acids therapies, such as siRNAs, mRNAs, and antisense oligodeoxynucleotides (ODNs) (Robbins et al., 2012; Kaczmarek et al., 2017; Meng and Lu, 2017) which can trigger immune responses; however, less is known about the immune recognition of gRNAs and the CRISPR system. Increasing Cas9-mediated homology-directed repair template. Engineered xCas9 and SpCas9-NG variants broaden PAM recognition sites to generate mutations in Arabidopsis plants. 11:4034. L., Stefanidakis, M., Wilson, C. One such direction that is pursued using CRISPR-based genome editing is in vivo delivery of the editing complexes to the target tissues, with a focus on more accessible tissues such as the eye, liver, muscle, and cervix (Hirakawa et al., 2020). O., Clark, J. 13, 443-448. doi: 10.1038/nbt.4005 PubMed Abstract | CrossRef Full Text | Google Scholar Zhen, S., and Li, X. Methods Mol. To that end, the first clinical trial, using C-C chemokine receptor type 5 (CCR5) knockout CD34+ HSPCs edited by gRNAs with the chemical modifications described in Hendel et al. (2003). 14, 311-316. Human genome-edited hematopoietic stem cells phenotypically correct Mucopolysaccharidosis type I. Chemical modifications in RNA interference and CRISPR/Cas genome editing reagents. The three primary types of RNA molecules are messenger RNA, transfer RNA and ribosomal RNA. Only once the RNA and DNA complete R-loop formation, also known as the zipped conformation, and structural rearrangement of the nuclease domains commence, can the endonuclease cut the DNA creating a DSB (Jiang et al., 2015; Jiang and Doudna, 2017). Increasing CRISPR efficiency and measuring its specificity in hspcs using a clinically relevant system. Multicolor CRISPR labeling of chromosomal loci in human cells. A., Yan, W. Med 25, 776-783. A much less pronounced effect was observed with G-rich hairpin at the 3' end. doi: 10.1038/s41467-019-09600-4 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. doi: 10.1038/s42003-018-0054-2 PubMed Abstract | CrossRef Full Text | Google Scholar Vakulskas, C. Google Scholar Ling, X., Xie, B., Gao, X., Chang, L., Zheng, W., Chen, H., et al. Methods Clin. Structural roles of guide RNAs in the nuclease activity of Cas9 endonuclease. Naturally, the cell can use the sister chromatid or the homologous chromosome as a template for HDR; however, researchers have shown the ability to use an exogenous donor template to introduce genes into the CRISPR cut site (Porteus, 2016). They first examined the on- and off-target editing by testing gRNA modifications 2'-O-Me, 2'-O-methyl-3'-phosphonoacetate (MP), MS, and MSP (Figure 2) in in vitro cleavage assays and then continued to assess the editing by NHEJ in cultured K562 cells, primary CD34+ HSPCs, and induced pluripotent stem cells. After crRNA and tracrRNA anneal together to form a guide RNA (gRNA), they assemble a ribonucleoprotein (RNP) complex with a Cas9 molecule to direct site-specific DNA cleavage. Advances in the delivery of RNA therapeutics: from concept to clinical reality. doi: 10.1038/nbt.3526 PubMed Abstract | CrossRef Full Text | Google Scholar Maeder, M. World J. F., and Cleveland, D. gRNA modifications to improve CRISPR-Cas9 specificity in cultured mammalian cells. J., et al. doi: 10.1093/nar/gku241 CrossRef Full Text | Google Scholar Cong, L., Ran, F. Chemical modifications on the ribose rings and phosphate backbone of gRNAs. Ribose modifications are typically placed at the 2'OH as it is readily available for manipulation. doi: 10.1038/nprot.2013.143 CrossRef Full Text | Google Scholar Rastogi, R. 27, 1389-1406. Ryan et al. The lower thermodynamic stability of the DNA-DNA duplex compared to the RNA-DNA duplex renders the partially DNA-substituted guide sequence of crRNA less tolerable to mismatches when interacting with genomic DNA. (1994). J., Hudgens, E., Echeverria, D., et al. X., and Zhang, F. G., et al. 33, 985-989. A., Zhang, S., and Pederson, T. Cas9 belongs to the Class 2 type II system and is a multi-domain endonuclease that requires both crRNA and tracRNA to introduce a double-strand break (DSB) at the target genomic site. Science 368, 1265–1269. These modified RNA-DNA hybrid molecules were engineered by conjugating an azide terminated DNA molecules are produced in the nucleus of our cells and can also be found in the cytoplasm. Combinations of the ribose and phosphodiester modifications have given way to formulations such as 2'-O-methyl-3'-phosphorothioate (MS), or 2'-O-methyl-3'-phosphonoacetate (MP) RNAs. Locked and unlocked nucleic acid (LNA), bridged nucleic acids (BNA), S-constrained ethyl (cEt), and unlocked nucleic acid (UNA) are examples of sterically hindered nucleotide modifications. D., Reyon, D., Cascio, V. Science 365, 1301-1305. observed an increase in specificity in in vitro cleavage by replacing RNA residues and betathalassemia. CRISPR RNAs trigger innate immune responses in human cells. 12, 2503-2509. Two independent studies systematically assessed the effect of modifying internal gRNA residues on Cas9 cleavage specificity. Extension of the crRNA enhances Cpf1 gene editing in vitro and in vivo. RNA molecules are polymers of nucleotides joined to one another by covalent bonds between the phosphate of one nucleotide and the sugar of another. G., Mostrel, N., Bak, R. Accordingly, Fu et al. 137, 5642-5645. All cell types eventually exhibited a similar immune response to 5'-ppp gRNAs. Interestingly, the intracellular delivery method was deterministic in the immune response with nucleofection in HEK293 cells triggering a weaker and short-lasting type 1 interferon response, compared to lipofection (Wienert et al., 2018). Oncogenic human papillomavirus: application of CRISPR/Cas9 therapeutic strategies for cervical cancer. Lastly, they noted that it is possible to shorten the crRNA down to 29 nucleotides and still maintain its efficiency (Rahdar et al., 2015). In vitro-transcribed guide RNAs trigger an innate immune response via the RIG-I pathway. Its job is to translate the message within the nucleotide sequences of mRNA into specific amino acid sequences. Although equally as much work has been done to modify the Cas9
protein to improve on its characteristics, herein, we discussed within the nucleotide sequences. ications that have been used specifically on the gRNA to adapt this bacterial element to a more effective, accurate, and versatile genome-editing tool while concurrently attempting to improve safety in order to achieve therapeutic relevance. Development of a gene-editing approach to restore vision loss in Leber amaurosis type 10. (2017b). 26, 1228-1240. 37, 870-881. RNA-guided human genome engineering via Cas9. doi: 10.1186/s13073-017-0450-0 PubMed Abstract | CrossRef Full Text | Google Scholar Kartje, Z. Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. Chemical modification of CRISPR gRNAs eliminate type I interferon responses in human peripheral blood mononuclear cells. 103, 6919-6932. Nature 550, 407-410. Plant Biotechnol. E., Taussig, D., Steinfeld, I., Phadnis, S. CRISPR-based gene editing enables FOXP3 gene repair in IPEX patient cells. 43, 8924-8941. R., Livshits, G., et al. demonstrated that one MS modification at the 5' end of the tracrRNA were enough to improve editing efficiency when electroporated with Cas9 mRNA into K562 cells (Basila et al., 2017). This finding supported an earlier study that showed that the introduction of as few as two 2'-O-Me uridine or guanosine residues into either strand of a siRNA duplex eliminated any immune response (Judge et al., 2006). Conflict of Interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. doi: 10.1038/s41392-020-0207-x PubMed Abstract | CrossRef Full Text | Google Scholar Jiang, F., Zhou, K., Ma, L., Gressel, S., and Doudna, J. The CRISPR system components are modified from the prokaryotic adaptive immune system. Stem-loop 2 and tetraloop do not interact with Cas9 as they protrude from the nuclease (Konermann et al., 2015). Similar work was performed by Mir et al. D., Konermann, S., Shehata, S. However, it should be emphasized that manipulating the cleavage complex stability via truncated gRNAs is obtained at the expense of on-target activity (Pavel-Dinu et al., 2019) such that the balance between efficiency and specificity of genome editing should be carefully weighed. The tRNA molecule forms base pairs with its complementary codon sequence on the mRNA molecule. gRNAs contain 4 loop structures: tetraloop (green), Stem-loop 2 (orange), and Stem-loop 3 (magenta). Even more effective than LNAs, BNANCs can provide additional conformational flexibility for nucleic acid binding and greater nuclease resistance. Long-term dual-color tracking of genomic loci by modified sgRNAs of the CRISPR/Cas9 system. Increasing CRISPR Specificity by Limiting Off-target Editing It is important to remember that CRISPR/Cas9 system. specific gene-editing tool to edit mammalian genomes. doi: 10.1016/j.ymthe.2019.05.014 PubMed Abstract | CrossRef Full Text | Google Scholar Rose, J. Due to CRISPR's wide-ranging applications, as well as its relative simplicity and highly flexible nature, it has been catapulted to the forefront of research in a remarkably vast number of organisms, from bacteria to humans (Wang et al., 2013; Guo and Li, 2015; Sid and Schusser, 2018; Xue et al., 2018; Yao et al., 2019; Song et al., 2019; Munoz et al., 2019; Song et al., 2019; Song et al., 2019; Song et al., 2019; Munoz et al., 2019; Song et al., 2019; Son architecture for light-inducible gene editing and transcription. LNAs display improved base stacking and thermal stability compared to unmodified RNA, resulting in highly efficient binding to complementary nucleic acids and improved mismatch discrimination (You et al., 2006). 3:121. T., and Rinn, J. Cell Rep. There are a few conventional ways to produce gRNAs (Moon et al., 2019), including chemical synthesis using oligonucleotide synthesizers, in vitro transcription (IVT), and intracellular production via gRNA-expressing DNA vectors which hijack the host cell's transcription (IVT), and intracellular production via gRNA-expressing DNA vectors which hijack the host cell's transcription machinery. direction in which CRISPR-based gene editing is currently being exploited is ex vivo gene therapy using cells of hematopoietic origin. T., Dewitt, M. doi: 10.1093/nar/gkv892 PubMed Abstract | CrossRef Full Text | Google Scholar Judge, A. Diversity and evolution of class 2 CRISPR-Cas systems. doi: 10.1073/pnas.1420024112 PubMed Abstract | CrossRef Full Text | Google Scholar Ma, H., Tu, L. Biochemistry 57, 3027-3031. Elife 6:e25312. Multiplexable, locus-specific targeting of long RNAs with CRISPR-display. N. T., Kennedy, A. Gunilla Elam / Science Photo Library / Getty Images Plus RNA molecules are single-stranded nucleic acids composed of nucleotides. demonstrated that manipulating the spacer length reduced off-target editing (Fu et al., 2014). However, the orthogonal approach attempts to elevate CRISPR-Cas9 genome editing precision via chemical modifications on the gRNA, as discussed below (Table 2). doi: 10.1016/j.synbio.2018.09.004 PubMed Abstract | CrossRef Full Text | Google Scholar Yin, H., Song, C. doi: 10.1038/nchembio.1753 PubMed Abstract | CrossRef Full Text | Google Scholar Rahdar, M., McMahon, M. Both BNAs mentioned above improve specificity by inducing a more dynamic RNA-DNA duplex, thereby reducing the time the nuclease spends in the zipped conformation where cleavage is activated. 'A' symbolizes the nitrogen base of the RNA. CRISPR/Cas9 beta-globin gene targeting in human haematopoietic stem cells. S., Rao, A. C., Kowalski, P. Improved genome editing efficiency and flexibility using modified localization method to deploy large cargo, including protein-binding cassettes, to specific DNA loci (Shechner et al., 2015). Unregulated constitutive expression of integrated CRISPR-Cas9 can also destabilize the genome through persistent DSB generation. doi: 10.1093/nar/gkw066 PubMed Abstract | CrossRef Full Text | Google Scholar Shapiro, J., Iancu, O., Jacobi, A. doi: 10.1101/gr.231936.117 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossRef Full Text | Google Scholar Kleinstiver, B. doi: 10.1038/nrmicro.2016.184 PubMed Abstract | CrossR Stabilization of the gRNA In order to use CRISPR-Cas9 genome editing in a therapeutic setting, the first problem that needs to be addressed is gRNA stability. focused on modifying the crRNA and Cas9 separately from plasmid DNA in HEK293T cells (Rahdar et al., 2015). doi: 10.1038/nbt.3290 PubMed Abstract | CrossReting, the first problem that needs to be addressed is gRNA stability. Full Text | Google Scholar Hendel, A., Kildebeck, E. (2014). Modifications can be made both on the ribose ring as well as on the phosphodiester bond to reduce nuclease susceptibility. Its unique shape contains an amino acid attachment site Unique O-methoxyethyl ribose-DNA chimeric oligonucleotide induces an atypical melanoma differentiation-associated gene 5-dependent induction of type I interferon response. The spacer region can be divided into two regions: the PAM-proximal (seed) region and the PAM-distal region. Target Ther. Furthermore, Schubert et al. These linkages are called phosphodiester linkages. Although single-stranded, RNA is not always linear. Mammalian cells recognize the CRISPR complex as foreign and mount an immune response as a result (Cromer et al., 2018; Kim et al., 2018; Kim et al., 2018; Kim et al., 2018; Kim et al., 2018; Moon et al., 2018; Kim et al., 2018; Moon et al., 2018; Kim et al., 2018; Kim et al., 2018; Moon et al., 2018; Kim 10.1021/bi0343774 PubMed Abstract | CrossRef Full Text | Google Scholar Burel, S. Previous attempts relied on the fusion of nuclease-deficient dead Cas9 (dCas9) with fluorescent proteins (Chen et al., 2013; Ma et al., 2013; Ma et al., 2013; Ma et al., 2013; Ma et al., 2014; Ma et al., 2015), which would be directed to the target loci by expressed sgRNAs. Furthermore, in order to improve the
assay sensitivity by increasing sgRNA expression, Chen et al. E., Fisher, J., O'Rourke, K. Conclusion The FDA, EMA, and other oversight drug approval bodies implement rigorous and demanding tests before approving a given drug or therapy. First and foremost, safety is a critical parameter. L., Kato, H., Cauntay, P., Greenlee, S., et al. Engineering CRISPR-Cpf1 crRNAs and mRNAs to maximize genome editing efficiency. M., Sousa, A. Therefore, to improve HDR efficiency and eliminate virus-induced immune responses, non-viral donor DNA delivery is crucial. H., et al. M., Haupt, A., Schiel, J. a Cas9-guide RNA complex with the Cas protein, gRNAs can come in one of two basic formulations: a two-part molecule or a single-guide molecule (sgRNA). Targeted gene knock-in by homology-directed gene knock-in by homologyproximity of the donor DNA to the break site. 8:331. Synthetically modified Cpf1-CRISPR RNAs mediate efficient genome editing in mammalian cells. These improvements have also expanded the applications of CRISPR-Cas9, such as techniques for enhanced HDR and improved genome imaging tools. Int. J., Barkau, C. T., Rodrigues, G. doi: 10.1126/science.aab1452 PubMed Abstract | CrossRef Full Text | Google Scholar Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. Highly efficient editing of the beta-globin gene in patient-derived hematopoietic stem and progenitor cells to treat sickle cell disease. doi: 10.1016/j.celrep.2016.02.018 PubMed Abstract | CrossRef Full Text | Google Scholar Richter, F., Fonfara, I., Bouazza, B., Schumacher, C. Similar to Cas12a, it targets complementary RNA sequences instead of DNA (Chylinski et al., 2014; Shmakov et al., 2017; Tang and Fu, 2018). doi: 10.1038/nbt.3609 CrossRef Full Text | Google Scholar Kim, S., Koo, T., Jee, H. R., Urbinati, F., et al. J., Lovendahl, K. Partial DNA-guided Cas9 enables genome editing with reduced off-target activity. Engineering of temperature- and light-switchable Cas9 variants. doi: 10.1186/s13578-019-0298-7 PubMed Abstract | CrossRef Full Text | Google Scholar Savic, N., Ringnalda, F. Cell 156, 935-949. Oligonucleotides 19, 89-102. However, the major contribution of Hendel et al. Q., Nguyen, N. Ribosomes contain a binding site for tRNA located in the large ribosomal subunit. One of the main challenges facing researchers since the beginning of the CRISPR era is how to optimize the CRISPR system for translation to clinical therapies (Zhang, 2020). A., and Behlke, M. doi: 10.1016/j.cell.2014.02.001 PubMed Abstract | CrossRef Full Text | Google Scholar Obe, G., Johannes, C., and Schulte-Frohlinde, D. A., and Dobrovolskaia, M. P., Swayze, E. Genome Res. Hendel et al. Following exposure to light, the NPOM modifications are released and complete binding and subsequent editing tools, such as zinc-finger nucleases (ZFNs) and Transcription-Activator Like-Effector-Nucleases (Porteus and Carroll, 2005; Carroll, 2011, 2014). Transfer RNA (tRNA) is an RNA molecule that assists in protein synthesis. gRNA modifications to improve CRISPR-Cas9 efficiency in cultured mammalian cells. A., Rao, A., Chong, A. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Transcription is the process in protein synthesis that involves copying the genetic information contained within DNA into an RNA message. modified the sgRNA sequence, as well as extending a Cas9-binding hairpin structure (Chen et al., 2013). Hence, DNA-RNA hybrid crRNAs seem to present a plausible and cost-effective formulation for efficient and more accurate in vitro gene editing; however, it has yet to be validated in primary cells and animal models. DNA contains the four nucleotide bases adenine (A), guanine (G), cytosine (C) and thymine (T) which are paired together (A-T and C-G). Science 348, 1477-1481 47, 546-558. Highly efficient therapeutic gene editing of human hematopoietic stem cells. 6:eaaz0571. We thank the other members of the Hendel Lab for critically reading the manuscript and providing practical advice. doi: 10.1016/j.omtm.2020.04.027 PubMed Abstract | CrossRef Full Text Shapiro, J., Tovin, A., Iancu, O., Allen, D., and Hendel, A. X., Zou, X., Chung, H. Multiplex genome engineering using CRISPR/Cas systems. However, the extended sgRNAs showed a tendency to undergo intracellular digestion back to the original size. highlighted the complete abolishment of genome-editing capability when all 2'-OH sites were modified. The guide RNA (sgRNA) has ~20 nucleotides of sequence complementary to a target site, followed by a Protospacer Adjacent Motif (PAM) sequence (NGG) which is critical for binding to Cas9. From: Methods in Enzymology, 2014 Up until the discovery of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system, genome editing was limited in its capabilities. M., Cohen, A. M. Collingwood, M. 44, 2455-2466. K., Brown, K., Asokan, A., and Deiters, A. doi: 10.1126/science.aba7365 PubMed Abstract | CrossRef Full Text | Google Scholar Anzalone, A. Due to the short length of the gRNA chemical synthesis allows for the swift and uncomplicated formational changes as well as the addition of different moieties. Chemical modification of guide RNAs for improved CRISPR activity in CD34+ human hematopoietic stem and progenitor cells. the CRISPR-Cas9 system to improve its editing capabilities as well as its ability to be tolerated in human cells. Hairpin loops are commonly observed in RNA (tRNA). A., Prakash, T. The complex then scans the DNA for a complementary sequence to the 20 nucleotides on its 5' end, termed the guide region (spacer region), with an adjacent upstream protospacer adjacent motif (PAM) sequence (5'-NGG-3' in S. 35, 1179-1187. Genome engineering using the CRISPR-Cas9 system. doi: 10.1038/s41591-018-0137-0 PubMed Abstract | CrossRef Full Text | Google Scholar Wang, H., Nakamura, M., Abbott, T. (2012). Chem. V., Koblan, L. Chemical modifications comprising of 2'-O-methyl (M or 2'-O-methyl 3' phosphorothioate (MS), or 2'-O-methyl-3'-thioPACE (MSP) (Figure 2) were incorporated at three terminal nucleotides at both the 5' and 3' ends of individual sgRNAs. These modifications, specifically MS and MSP, substantially increased stability, resulting in a high level of indels at the on-target site compared to the indel frequencies obtained with the unmodified sgRNA. doi: 10.1042/BSR20200127 PubMed Abstract | CrossRef Full Text | Google Scholar Hu, B., Zhong, L., Weng, Y., Peng, L., Huang, Y., Zhao, Y., et al. Some RNAs, known as small regulatory RNAs, have the ability to regulate gene expression. The attached amino acid on the tRNA molecule is therefore placed in its proper position in the growing protein chain. doi: 10.1007/s00253-019-10007-w PubMed Abstract | CrossRef Full Text | Google Scholar Stadtmauer, E. In addition, the CRISPR LiveFISH method, with fluorophore-labeled gRNAs, presented a robust and novel approach using both dCas9 and dCas13 to enable real-time imaging of both DNA and RNA to track nuclear dynamics during genome editing and transcription in a wide range of live cells, including human primary cells (Wang et al., 2019) (Figure 3D). 10:1610. 21:6461. Appl. R., Zhao, D., Luo, K., Yu, C., et al. I., Kim, D. Additionally, it has been shown that adding two types of bridged nucleic acids (BNAs), N-methyl substituted BNAs (2',4'-BNANC[N-Me]) and, to a lesser extent, locked nucleic acids (LNAs) (Figure 2), within the central portion of the guide regions (Cromwell et al., 2018). S., Meckler, J. Improving CRISPR genome editing by engineering guide RNAs. Trends Biotechnol. D., Chen, Y. The ability to chemically modify gRNAs opened the door for the development of more efficient and safer gene-editing methods that can be appropriate for clinical applications in primary cells. 40:BSR20200127. Below we review the types of chemical modifications and their impact on various aspects of CRISPR-Cas9 applications in vitro and in vivo (Table 1). Nature 539, 384-389. Therapeutic siRNA: state of the art. siRNA and innate immunity, off-target effect, or immune adjuvant? Adenine pairs with uracil (A-U) and guanine pairs with cytosine (G-C). Researchers also have used the CRISPR system to knock-in specific genes by taking advantage of the homology-directed repair (HDR) pathway (Yang et al., 2020), where the cell uses a template to repair the DSB. R., and Gersbach, C. Syst. H., and Brown, T. One of the benefits of the two-component system is that the gRNA can be modified independently from the Cas nuclease, making the alteration of CRISPR as a genome-editing tool easy and flexible with almost unlimited target capability and high efficiency (Hsu et al., 2014; Moon et al., 2019). R., Turk, R., Jacobi, A. PLoS Biol. During translation, a small ribosomal subunit attaches to a mRNA molecule. An artificial triazole backbone linkage provides a split-and-click strategy to bioactive chemically modified CRISPR sgRNA. D., Bola, G., Lee, A. A., Davis, M. doi: 10.1038/s41467-018-03927-0 PubMed Abstract | CrossRef Full Text De Ravin, S. Nat. The polypeptide chain undergoes several modifications before becoming a fully functioning protein. pyogenes) (Jiang and Doudna, 2017). A., Cox, D., Lin, S., Barretto, R., Habib, N., et al. Steric hindrance from these NPOM residues prevents binding of those residues. The current nomenclature identifies two classes of the CRISPR-Cas systems, Class 1 and 2 (Makarova et al., 2020). Methods 14, 963-966. Transfer RNA (tRNA) plays an important role in the translation portion of protein synthesis. doi: 10.1371/journal.pone.0188593 PubMed Abstract | CrossRef Full Text | Google Scholar Bennett, C. Indeed, more accurate genome editing has been achieved via Cas9 nuclease modification itself (Kleinstiver et al., 2016; Slaymaker et al., 2016; Chen et al., 2017; Vakulskas et al., 2018). 1, 889-901. Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. A., Cimini, B. doi: 10.1016/j.ymthe.2018.02.031 PubMed Abstract |
CrossRef Full Text | Google Scholar Meng, Z., and Lu, M. ACS Chem. U., Cuomo, M. This new CRISPR-Cas9 formulation is sure to lead to higher resolution, real-time DNA-repair analyses to better elucidate CRISPR-Cas9-induced DSB repair (Liu et al., 2020; Moroz-Omori et al., 2020; Moroz-Omor PubMed Abstract | CrossRef Full Text | Google Scholar However, when electroporated as an RNP complex, these modifications did not significantly increase editing efficiency. (2020). Increasing the specificity of CRISPR systems with engineered RNA secondary structures. D., Fitzgibbon, C. P., Richa, K., umar, A., Tyagi, M. Signal Transduct. (2017). Highly efficient genome editing by CRISPR-Cpf1 using CRISPR RNA with a uridinylate-rich 3'-overhang. Extensive CRISPR RNA modification reveals chemical activity. B., Strezoska, Z., et al. Science 339, 786-791. doi: 10.1038/s41591-018-0327-9 PubMed Abstract | CrossRef Full Text | Google Scholar Makarova, K. Toxicol. Protoc. Figure 2. Together, these CRISPR-Cas formulations confer a convenient technology for researchers to conduct sequence-specific editing of nucleic acids in a wide variety of cell types and experimental set-ups. J., Valente, W. Proc. M. doi: 10.1016/j.jbiotec.2016.06.011 PubMed Abstract | CrossRef Full Text | Google Scholar Kim, D., Kim, J., Hur, J. doi: 10.1126/science.1232033 PubMed Abstract | CrossRef Full Text | Google Scholar Manning, K. C., Hogset, H., Sunyovszki, I. 34, 528-530. Ribosomal RNA (rRNA) is a component of cell organelles called ribosomes. The analysis of the relationship between these extensive modifications, the resulting structure of the RNA and RNP complex, and the subsequent intrinsic complex activity in vitro emphasized the necessity for maintaining an A-form-like helical structure of the crRNA in both the guide and the repeat regions. revealed that at off-target sites where RNA:DNA mispairing exists, and binding affinity is reduced, R-loop formation is hindered while R-loop formation can commence normally at on-target sites (Kocak et al., 2019). MicroRNAs have also been linked to the development of some types of cancers and a particular chromosome mutation called a translocation. 6, 695-703. M., Jiang, F., Kim, H. Ribosomes are typically composed of two subunits: a large subunit and a small subunit. Biochem. It contains an amino acid attachment site on one end and a special section in the middle loop called the anticodon site. Heavily and fully modified RNAs guide efficient SpyCas9-mediated genome editing. A., Hsu, P. Biotechnol. On the other hand, after sufficient time in cultured cells, the reduced cleavage rate proved beneficial for the overall specificity of the modified sgRNA-mediated editing. S., Kwon, J. Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells. It was shown that MP modifications, incorporated at select sites in the ribose phosphate backbone of gRNAs (positions 5 and 11), along with modifications which protect the terminal positions (Hendel et al., 2015a), can reduce off-target cleavage activities while maintaining on-target cleavage editing (Ryan et al., 2018). doi: 10.1007/978-1-0716-0687-2_3 PubMed Abstract | CrossRef Full Text | Google Scholar Nihongaki, Y., Furuhata, Y., Otabe, T., Hasegawa, S., Yoshimoto, K., and Sato, M. However, as mentioned earlier, the CRISPR-Cas systems can evoke unwanted cellular and immune responses. showed that sgRNAs with three different independent chemical modifications at both termini increased editing efficacy by protecting the exposed ends from degradation (Hendel et al. 2015a). M., Dever, D. doi: 10.1111/pbi.13148 PubMed Abstract | CrossRef Full Text | Google Scholar Gomez-Ospina, N., Scharenberg, S. However, the plasmid DNA, as well as any foreign DNA, can trigger an innate intracellular immune response, especially in primary cells (Sun et al. 2013). In addition to Cas9 (Type II), other members of the Class 2 system have also been exploited for targeted editing, including Cas12a (formally Cpf1), that belongs to Type V). doi: 10.1126/science.aay8204 PubMed Abstract | CrossRef Full Text | Google Scholar Ma, H., Naseri, A., Reyes-Gutierrez, P., Wolfe, S. An RNAaptamer-based two-color CRISPR labeling system. doi: 10.1126/sciadv.aaz0571 PubMed Abstract | CrossRef Full Text | Google Scholar Hemphill, J., Borchardt, E. 25, 229-233. Indeed, multiple research groups have reported cytotoxicity due to RNA-sensing, specifically via the RIG-1 pathway, and innate immune responses in human cells triggered by the 5'-triphosphate groups present on CRISPR gRNAs (Kim et al., 2018; Schubert et al., 2018; Wienert et al., 2018). (2017a). demonstrated that for optimal gRNA efficiency, the guide must be modified in a way that protects it from degradation by RNA nucleases. Synth. RNA is highly unstable compared to DNA and is extremely vulnerable to both endo- and exo-nucleases. However, since 5'-triphosphate (5'-ppp), which remains on the 5'-end of IVT RNA, is recognized as a PAMP, introducing IVT gRNA species into human cells can potentially trigger an innate immune response. Methods favoring homology-directed repair choice in response to CRISPR/Cas9 induced-double strand breaks. E., Ha D. S. S., Turk, R., Rettig, G. H., Kim, J. Since, as mentioned earlier, the chemical modifications that affect zipped conformation influence Cas9-gRNA complex off-target activity (Cromwell et al., 2018), it is plausible that manipulating the secondary structure or the length of the gRNA may improve genome editing precision as well. A codon consists of three continuous nucleotide bases that specify a particular amino acid or signal the end of translation. L., and Smith, A. MicroRNAs (miRNAs) are a type of regulatory RNA that can inhibit gene expression by halting translation. L., Srifa, W., Mantri, S., Nicolas, C. 42, 6091–6105. Systematic analysis of CRISPR-Cas9 mismatch tolerance reveals low. levels of off-target activity. L. 13, 494-505. doi: 10.1007/978-1-0716-0290-4 2 PubMed Abstract | CrossRef Full Text | Google Scholar Li, B., Zhao, W., Luo, X., Zhang, X., Li, C., Zeng, C., et al. It has the ability to fold into complex three-dimensional shapes and form hairpin loops. Campbell Biology. S., Kleinstiver, B. In contrast to Cas9, Cas12a utilizes a single molecule gRNA with a 3' oriented spacer region and a 5' pseudoknot (5' handle). C., Shaw, L., Youniss, M. O., Barcena, C., et al. The engineered crRNA and complexed with Cas9. 342, 150-162. doi: 10.1038/nbt.3155 PubMed Abstract | CrossRef Full Text | Google Scholar Finn, J. W., Zhang, F., et al. doi: 10.1038/s41591-019-0401-y PubMed Abstract | CrossRef Full Text | Google Scholar Xu, L., Wang, J., Liu, Y., Xie, L., Su, B., Mou, D., et al. Rationally engineered Cas9 nucleases with improved specificity. V. where the modification pattern relied on the CRISPR-Cas9 complex crystal structure (Mir et al., 2018). gRNA and Donor DNA Conjugates In order to improve the CRISPR-Cas9 system to actuate more efficient HDR two parameters must be improved upon: increasing the transfection efficiency of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the immediate vicinity of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the immediate vicinity of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the immediate vicinity of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the immediate vicinity of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DNA donor to the edited cells (Lee et al., 2017a) and localizing the transfection efficiency of the DN between the Cas9 activity in in vitro activity assays vs. 7, 293-305. 9:36. 16:e2005840. CRISPR-mediated live imaging of genome editing and transcription. Campbell. (2018). Enhanced proofreading governs CRISPR-Cas9 targeting accuracy. doi: 10.3389/fpls.2019.00135 PubMed Abstract | CrossRef Full Text | Google Scholar Nahar, S., Sehgal, P., Azhar, M., Rai, M., Singh, A., Sivasubbu, S., et al. doi: 10.1038/srep26857 PubMed Abstract | CrossRef Full Text Wienert, B., Shin, J., Zelin, E., Pestal, K., and Corn, J. A., and Charpentier, E. B., and Gersbach, C. A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and

progenitor cells. To show the significance of maintaining these gRNA-Cas9 interactions, Yin et al.

Nov 04, 2021 · The U5 and U6 small nuclear RNAs as active site components of the spliceosome. Science. Dec. 24, 1993;262(5142):1989-96. cited by applicant . Sousa, R. et al., T7 RNA polymerase. Prog Nucleic Acid Res Mol Biol. 2003;73:1-41. cited by applicant . Sousa, R., Use of T7 RNA polymerase and its mutants for incorporation of nucleoside analogs into RNA. Concepts of Biology is designed for the introductory biology course for nonmajors taught at most two- and four-year colleges. The scope, sequence, and level of the program are designed to match typical course syllabi in the market. Concepts of Biology includes interesting applications, features a rich art program, and conveys the major themes of biology.

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