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**Development of clustered regularly interspaced short palindromic repeats/CRISPR-associated technology for potential clinical applications**

Huang YY *et al*. Application of CRISPR technology

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**Abstract**

The clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated (Cas) proteins constitute the innate adaptive immune system in several bacteria and archaea. This immune system helps them in resisting the invasion of phages and foreign DNA by providing sequence-specific acquired immunity. Owing to the numerous advantages such as ease of use, low cost, high efficiency, good accuracy, and a diverse range of applications, the CRISPR-Cas system has become the most widely used genome editing technology. Hence, the advent of the CRISPR/Cas technology highlights a tremendous potential in clinical diagnosis and could become a powerful asset for modern medicine. This study reviews the recently reported application platforms for screening, diagnosis, and treatment of different diseases based on CRISPR/Cas systems. The limitations, current challenges, and future prospectus are summarized; this article would be a valuable reference for future genome-editing practices.

**Key Words:** CRISPR-Cas; Gene editing; Molecular diagnostics; Gene targeting

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**Core Tip:** This review mainly discusses and explores the potential clinical applications of the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) technology. The detection technologies for nucleic acids and small molecules of different pathogens based on the CRISPR/Cas system are summarized. The advantages and disadvantages of the CRISPR/Cas technology from the aspects of gene editing, disease treatment, multi-drug resistance, and treatment are enumerated, asserting that CRISPR/Cas system has unlimited potential in clinical applications with certain challenges.

**INTRODUCTION**

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system, an adaptive immune system present in numerous bacteria and archaea, is a nucleic acid-targeted defense mechanism composed of numerous short and conserved repeat regions and spacers that protect themselves from exogenous mobile genetic elements such as plasmids and phages. A similar arrangement in the chromosomes of Gram-negative bacteria (*Escherichia coli, E. coli*) was also reported by Ishino *et al* in 1987 and was again confirmed in 2010[1]. The type II CRISPR/Cas system from *Streptococcus pyogenes* could specifically recognize and cleave target DNA guided by gRNA[2]; thus, laying the foundation for the development and utilization of the CRISPR/Cas system. Over the past few years, several CRISPR/Cas systems belonging to Cas proteins with different characteristics have been developed, which in turn have produced many CRISPR/Cas system-related toolboxes, offering functional robustness, efficiency, and ease of implementation in multiple organisms[3]. Various gene-editing tools based on CRISPR/Cas9 were introduced in 2013[4], followed by successful implementation in the modern medical field. Within a few years, CRISPR/Cas technology gradually made crucial breakthroughs and is now widely employed in gene-editing, treatment of genetic diseases, clinical diagnosis of common pathogenic molecules, and alleviating antimicrobial resistance. This review compiles the recent advancements in CRISPR technology and summarizes the achievements of CRISPR/Cas technology in clinical applications to provide opportunities for programmable genomic editing for translational medicine.

**CLASSIFICATION OF CRISPR/CAS SYSTEM**

In CRISPR/Cas systems, researchers have discovered various Cas proteins with different characteristics. There are two classes of CRISPR/Cas systems based on the composition of their effector subunits, Classes 1 and 2 (Table 1). Class 1 system contains numerous RNA-effector complexes, and Class 2 system comprises a solitary protein like Cas 9 that conducts all effector complex activities[4]. The Class 1 CRISPR–Cas system includes type I, III, and putative IV subtypes. The type I subtype contains the signature gene Cas3 encoding a single-stranded DNA (ssDNA), which acts as a candidate for guiding cascade in type I CRISPR module like Cas8 for DNA invasion, but this theory has not been thoroughly investigated. The type III CRISPR-Cas system containing gene Cas10 includes nonspecific degradation of both ssDNA and RNA molecules and requires the target DNA transcription for immunity[5,6]. The Cas10 subunit cleaves the transcribed ssDNA and activates the Csm6 nonspecific-RNase activity, while as the Csm3 cleaves the RNA target, the RuvC domain introduces staggered double-stranded DNA (dsDNA) breaks[7-9]. Since the Cas10 subunit HD domain cleaves ssDNA from the transcription bubble[10-12], due to activation of the Palm domain, ATP is converted into four or six-member cyclic oligoadenylate (CoA) rings. These six-member CoA rings then act as a secondary messenger and activate Csm6 by binding to its CARF (CRISPR-Associated Rossman Fold) domain, causing activation of the Higher Eukaryotes and Prokaryotes Nucleotide-binding (HEPN) domain and unleashing nonspecific RNA cleavage[13-15]. Putative type IV CRISPR/Cas systems include a large subunit, Csf1, Cas5, and Cas7 (a solitary unit), and usually lack the known Cas proteins involved in adaptation and target cleavage[16].

Class 2 effectors are composed of a single Cas unit that associates with the CRISPR RNAs (crRNA) for gene targeting in various biotechnological applications. This class includes CRISPR types II, V, and VI. Type II uses the mature crRNA directed CRISPR-associated protein Cas9 base-paired to trans-activating crRNA (tracrRNA) while introducing double-stranded breaks (DSB) in target DNA (Figure 1). Although Cas9 also harbors RuvC and HNH domains, at sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand. In contrast, the Cas9 RuvC-like domain cleaves the noncomplementary strand, thereby stressing the fact that target recognition by Cas9 requires both a seed sequence in the crRNA as well as a GG dinucleotide-containing protospacer adjacent motif (PAM) sequence adjacent to the crRNA-binding region in the DNA target[2,16-20]. The majority of type V CRISPR modules can recognize dsDNA targets. For instance, a single crRNA processed by Cas12 RNase domain guides Cas12 with a T-rich PAM sequence to cleave dsDNA targets, generating sticky ends[21] (Figure 1). Cas12a cleaves both the target and non-target strands of a targeted dsDNA by a single active site in the RuvC catalytic pocket[22]. Besides Cas12, Cas14 is the smallest RNA-guided nuclease discovered to date with 400-700 amino acids and does not require a target sequence such as PAM in the ssDNA substrate[23]. Lastly, Cas13 (also named C2c2) belongs to type VI recognizing RNA targets. A study discovered that Cas13 has two HEPN domains commonly associated with ribonucleases (RNases)-one for cutting its RNA target and the other for processing the crRNA[24-26] (Figure 1). It is also suggested that both type V and VI CRISPR modules work collaterally, *i.e.*, Cas12 and Cas14 can cleave ssDNA nonspecifically. In contrast, Cas13 can initiate nonspecific RNA cutting[16], which has not been observed when Cas12, Cas14, and Cas13 have been applied in either human or plant cell lines[27,28].

**CLINICAL APPLICATION OF CRISPR/CAS TECHNOLOGY**

***Application of CRISPR/Cas technology in clinical disease diagnosis***

**Application of CRISPR/Cas technology in pathogen detection:** Zhang *et al*[24] developed a detection platform called Specific High-sensitivity Enzymatic Reporter Unlocking (SHERLOCK) that combined isothermal amplification and Cas13a technology for the detection of either single DNA or RNA molecules. As activated Cas13 cleaves quenchable fluorescent RNA, it produces a quantifiable signal indicating the presence of the target nucleic acid (Figure 1). SHERLOCK was demonstrated to detect closely related Zika virus and dengue virus due to its property of rapidly detecting nucleic acids with high sensitivity. Subsequently, SHERLOCK was updated to SHERLOCKv2, based on simple four-channel multiplexing with orthogonal nucleic acid sequences of LwaCas13a, PsmCas13b, CcaCas13b, and AsCas12a[29], breaking through the quantitative and fluorescence limitations of SHERLOCK. SHERLOCKv2 uses fewer primers during the pre-amplification process to achieve better quantification without affecting sensitivity. Furthermore, the introduction of the test strip aids in determining whether the target DNA or RNA is present in the sample by visualization. SHERLOCKv2, due to its efficiency, specificity, ease of use, and portability, can detect ssRNA of dengue or Zika virus, as well as mutations in liquid biopsy samples from patients by the lateral flow assay system. Chen *et al*[30] also reported that Cas12a could be used as a potential nucleic acid detection platform and developed DNA endonuclease-targeted CRISPR trans reporter (DETECTR), whose working principle was similar to that of SHERLOCK as this platform used isothermal amplification and Cas12a ssDNase activation for detection (Figure 1). Their system could specifically identify two human papillomavirus strains [Human papillomavirus (HPV16) and HPV18] from human SiHa and HeLa cells with higher accuracy.

However, as the above-mentioned nucleic acid detection processes require repeated uncapping and extraction, Joung *et al*[31] introduced the “one-pot” detection technology, while updating the COVID-19 detection technology, in which RNA does not need to be purified from patient samples. Moreover, the reaction steps required to detect COVID-19 are done in a tube during the test. It was then named SHERLOCK Testing in One Pot (STOP). While STOP employs loop-mediated isothermal amplification (LAMP) as a method to amplify RNA and utilizes AapCas12b, an enzyme that can remain active at 60 °C (the temperature required for the LAMP reaction), the steps of RNA extraction were simplified enough that could easily detect viral RNA only by adding viral lytic releasers to throat wipes or saliva samples containing new crown viruses without purifying and isolating RNA.

Although STOP simplifies the complexity of the detection process and reduces the contamination caused by reagent transfer, it also requires heating, amplification, and other steps when compared with SHERLOCKv2. To overcome these hindrances, a new system, CRISPR-Cas9-assisted DNA detection (CADD), was developed. This system employed the Beads-HCR method, in which a pair of dCas9- single-guide RNA (sgRNA), after being attached to the target DNA, was captured by the bead surface, followed by the addition of two hybridization chain reaction (HCR) hairpins (hairpin 1 and hairpin 2). Since the hairpin was labeled with fluorescein, HCR could produce a fluorescent signal on the bead’s surface. As the hairpin was connected continuously, the fluorescence signal became brighter in the positive direction, thereby making it easier to detect the fluorescence signals to the greatest extent without amplifying the target gene[32].

Due to the advantages of simple design, high efficiency, convenience, and a wider scope of application, CRISPR-Cas systems have become the most frequent genome editing technology in molecular biology while simultaneously promoting the development of basic scientific research, molecular innovations, and advanced clinical approaches. Freije *et al*[33] combined the Cas13 antiviral activity with its diagnostic ability and established a powerful and rapidly programmable diagnostic and antiviral system, named Cas13-assisted restriction of viral expression and readout. This system could detect RNA-based viruses such as influenza A virus in human cells within two hours on the test strip itself. Fozouni *et al*[34] also developed a COVID-19 detection technology connected to a smartphone that took only 15-30 min from sampling to reporting results on a mobile phone. This technique omitted the reverse transcription and pre-amplification steps and used CRISPR to directly detect viral RNA. It was a new diagnostic test that, apart from producing positive or negative results, could also measure the viral load in a given sample. Since Csm6 can sense the presence of RNA small loops and cleaves various RNA molecules, Liu *et al*[35] utilized Cas13, Csm6, and their activators in combination to create a tandem nuclease method for detecting COVID-19. This method avoided the original amplification of RNA and the possibility of sample cross-contamination caused by amplification (Table 2).

**Application of CRISPR/Cas technology in tumor pathogenesis monitoring:** Tumor monitoring is still an important parameter of global chronic disease monitoring. In recent years, due to complicated operations, long detection time, and low specificity, genetic sequencing for tumor marker detection has lost its popularity. To overcome this recurrent issue, Chow *et al*[36] developed *in vivo* CRISPR screening that identified both oncogenes and tumor suppressors that are the regulators of tumor immunotherapy in the tissue microenvironment[37]. This method could be suitable for personalized cancer modeling and tumor-driven analysis in the future to provide guidance for precision medicine. In recent years, as microRNAs have been reported to be associated with tumorigenesis, diagnosis, and prognosis, the CRISPR/Cas technology promises great potential for an early diagnosis of miRNA-related disease[38,39]. Qiu *et al*[40] developed the RCA-CRISPR-split-HRP (RCH) detection system based on dCas9, which facilitated CRISPR/Cas9 technology in miRNA detection for the first time. It offers great advantages such as a low detection cost and significant genetic effects. To demonstrate the potential application value of RCH, it was applied to detect circulating let-7a in serum samples from patients with non-small cell lung cancer (NSCLC) and healthy volunteers. As it was reported that since the expression of circulating let-7a was significantly down-regulated in patients with NSCLC, it could become a useful biomarker for NSCLC[41]. It was also discovered that the detection results were highly consistent with RT-PCR results and the literature reports, suggesting that this method could be used for the screening and diagnosis of tumors in the near future. Simultaneously, Lee *et al*[42] developed CRISPR-mediated Ultrasensitive Detection of Target DNA)-PCR (CUT) using Cas9/sgRNA for specific cleavage of wild-type DNA in blood samples from colorectal cancer patients, which in turn enriched the circulating tumor DNA content in plasma to improve the specificity and sensitivity of early tumor diagnosis. While the SHERLOCK and SHERLOCKv2 molecular detection platforms can detect the BRAF V600E mutation in simulated circulating DNA samples and the EGFR L858R mutation in liquid biopsy samples from adenocarcinoma patients[26,29,43], the DETECTR method can also be used to rapidly detect tumor mutations in the reproductive system[30]. For example, human papillomavirus, closely related to the occurrence of cervical cancer, is detected in human anal wipe DNA extracts[41]. The development of this technology is expected to bring a significant breakthrough in the early screening of cervical cancer (Table 3).

***Application of CRISPR/Cas technology in gene-editing disease therapy***

In clinical medicine, gene-editing has changed from a niche research technique to an extensive and highly precise tool for disease treatment[44], which has shown surprising results in the treatment of a variety of clinical diseases, such as genetic disorders[45] and cancer immunotherapy[46].

**Application of the CRISPR/Cas9 System in gene editing disease therapy**: An *in vitro* study by Egelie *et al*[47] observed that Cas9 protein could target and edit genes in bacterial cells, human stem cells, zebrafish, and human cell lines. In the targeting phase, Cas9 is guided to the DNA target site beside the PAM (spacer sequence adjacent motif) site (3’ GGN) by gRNA. The Cas9 nuclease domains like RuvC and HNH cleave single strands of DNA to form DNA DSBs. Although non-homologous end joining is subsequently activated in the host for repair, resulting in frameshift mutations, the body can also perform precise repair by homologous recombination repair in the presence of homologous sequences[48]. Lately, the CRISPR/Cas9-mediated gene therapy has become a quick and effective gene-editing tool as it corrects single-gene mutations, thus saving the disease phenotypes by achieving prompt treatment[49]. Leber congenital amaurosis (LCA) is an autosomal recessive retinopathy with both early and severe onset, causing severe visual loss due to premature transcription termination due to the presence of point mutations in the CEP290 gene at the intron branch point, followed by complete loss of pyramidal cell function in both eyes at birth or within one year of birth in infants[50,51]. Many previous studies on LCA disclosed that the adeno-associated virus type 5 (AAV5) vector delivers Cas9 and CEP290-specific gRNAs to the retina by targeting the point mutation region to invert or delete it as a whole, thereby restoring the normal expression of CEP290 gene[52-54]. Although still in the early clinical trial stage, this recent approach is currently being used to treat type 10 congenital mongolism patients[55]. It has also been suggested that using mRNAs encoding nucleases, like CRISPR/Cas9 and gRNA, and their DNA editing property in target cells has the potential to be effective in Cystic fibrosis (CF) patients with the impact of potential mutations[56]. Many past literary insights reported that an adeno-associated virus vector might be used to express the Cas9 of *Campylobacter jejuni* with adenine deaminase activity as well as the corresponding sgRNA to achieve accurate correction of oncogenic mutations in the telomerase gene promoter region of glioma cells[57].

Additionally, numerous prior studies have also demonstrated that CRISPR/Cas9 technology also has application prospects in the treatment of hematological diseases: For example, when congenital glucose-6-phosphatase-dehydrogenase (G6PD) deficiency patients ingest fava beans, acute hemolytic anemia with several manifestations occur along with sickle cell anemia and β-thalassemia, a condition caused by mutations in the β-globin gene (HBB). Wu *et al*[45] effectively corrected G6PD and HBB point mutations by giving composite injections into single-cell human embryos in Cas9-sgRNA and homologous donors[58,59], thereby demonstrating that CRISPR/Cas9 might also become a valuable therapeutic tool in human genetic diseases.

**Application of the CRISPR/Cas12 System in gene editing therapy:** In 2015, Zetsche *et al*[21] characterized the Cas12a protein and identified two candidate enzymes from Eosinophilaceae and Lactobacillaceae, and demonstrated that Cas12a protein was able to conduct effective genome editing activity in human cells. Unlike Cas9, the crRNA used by cas12a has only 42 nt[21], which offers many advantages in design and facilitates delivery and simplification of the multiplex gene-editing process[60]. According to Verwaal, LbCas12a and FnCas12a show editing efficiency comparable to Cas9 in yeast cells and are expected to be good alternatives to Cas9 in the future[61]. However, it is not widely used in practice, so there are very few instances of genome editing for disease treatment. Recently, some researchers engineered wild-type AsCas12a and designed a nuclease called enAsCas12a[62], which can show better gene editing activity at the TTT PAM site, greatly improving the editing efficiency of base C to base T. DeWeirdt *et al*[63] also applied enAsCas12a for genetic screening in human cells, and in the future, the CRISPR/Cas12 system might also play a larger role in disease treatment.

**Application of the CRISPR/Cas13 System in gene editing therapy:** On the contrary, the Cas13 protein family also seems to be promising in RNA knockout and editing, as reported by Cox *et al*[64]. In their study, catalytically inactive Cas13 (dcas13) was combined with ADAR2 to target transcripts to mammalian cells for editing of RNA bases A to I and correcting certain mutations in genetic diseases[27,64]. Recently, Li *et al*[65] also developed a brand-new CRISPR/Cas gene-editing technology using dCas13, called RESCUE, in which ADAR2 enzyme targeted both base C in RNA and unwanted base C in tRNA and precisely modified them to base U, thereby achieving the purpose of changing the protein without modifying the DNA by altering the mRNA injunctions.

N6-methyladenosine (m6A) is a common post-transcriptional RNA methylation modification in eukaryote mRNA responsible for mRNA modifications, which sometimes in an abnormal state can trigger a series of diseases[66]. This was also confirmed by Wilson *et al*[67] using an m6A RNA editing tool constructed by phase fusion of dCas13 and gRNA, and precisely edited m6A in the nucleus and cytoplasm. It could correct the methylase abnormality, bringing the latest breakthroughs in RNA editing. Thus, CRISPR/Cas technology can provide broader research ideas and application prospects for the treatment of many diseases by precisely editing genes and rectifying the diversity of gene variants that cause diseases.

In recent years, CRISPR/Cas technology has emerged as a potentially powerful tool in cancer research and treatment. It offers genetic screening of oncogenic mutations and can alter expressions of tumor suppressor genes[68]. It can be applied in CAR-T cell immunotherapy and immune checkpoint blocking therapy[46,69,70], thereby exploring and validating novel therapeutic targets in several preclinical studies involving tumors.

***Application of CRISPR/Cas technology in multi-drug resistance analysis and treatment***

CRISPR/Cas technology can be utilized to assess the surmounting multi-drug resistance (MDR) that has emerged as a serious public health threat due to inappropriate clinical antibiotic usage[71]. MDR usually occurs due to horizontal gene transfer of antibiotic resistance genes mediated by plasmids into other pathogenic bacterial forms[72]. Guk *et al*[73] discovered a significant negative correlation between CRISPR/Cas loci in bacteria and detected the acquired antibiotic resistance by sequencing the *Enterococcus faecalis* genome. It was revealed that strains without CRISPR/Cas loci were more likely to acquire external resistance genes than strains with CRISPR/Cas loci[74].

Nearly 26000 Enterobacteriaceae infections per year in China are caused by extended-spectrum β-lactamases (ESBLs) producing *E. coli*[72]. Bader *et al*[75] developed a technique called Re-Sensitization to Antibiotics from Resistance (ReSAFR).In this technique, the CRISPR/Cas9 system facilitates the intracellular delivery of antimicrobials, followed by sgRNA-guided Cas9 specific cleavage of resistance-mediating genes present on the same plasmid as the target genes so that antibiotic-resistant cells become re-sensitive to antibiotics. As ReSAFR improves the practical value of the CRISPR/Cas9 system, it might become an effective approach to curb the formation of multidrug-resistant bacteria[72,76]. Since in recent years, methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major nosocomial pathogen worldwide, Guk *et al*[73] developed a simple, rapid, and highly sensitive method to detect MRSA, *i.e.*, DNA-FISH to rapidly and reliably detect MRSA and provide effective treatment. In this technique, the dCas9/sgRNA complex is used as the targeting material and a nucleic acid stain SYBR Green I as the fluorescent probe to capture the MRSA DNA by specifically recognizing the mecA gene sequence with sgRNA. It offers a detection sensitivity of 10 CFU/mL, which is sufficient for effective detection of MRSA as the mecA gene, mainly present in resistant bacteria, is the prime underlying cause of MRSA resistance to β-lactam antibiotics. Therefore, MRSA can be promptly identified based on the mecA gene for effective antimicrobial therapy[77]. Kiga *et al*[78] also reported a positive outcome while developing the CRISPR-Cas13a-based antimicrobials capable of sequence-specific killing of MRSA, which is expected to be put into practical use as a therapeutic agent.Many of the emerging technologies mentioned above have tremendous potential to combat some of our most critical clinical predicaments in world public health problems.

***Challenges to the application of CRISPR/Cas technology***

CRISPR/Cas technology is indeed a convenient, easy-to-use biomedical tool having a wide range of potential applications, but at the same time, there are certain unsolved problems in its application that should be accurately recognized.

***Off-target effect***

CRISPR has proven to be a highly versatile gene-editing tool with great potential in a wide range of problems such as gene therapy, drug discovery, and gene modification in plant technology. However, the accuracy and reliability of CRISPR technology might be severely hampered by off-target effects due to unintended cleavage at untargeted genomic loci that do not match sgRNA, thus resulting in severe genomic aberrations[79].

When CRISPR technology is used for nucleic acid detection, the presence of an off-target effect can cause false positive or false negative results, affecting the accuracy of clinical diagnosis. The off-target effect is the main limiting factor affecting the application of CRISPR technology in clinical practice. In 2019, Grünewald *et al*[80] reported that the Cas9-based DNA editor experienced a severe off-target phenomenon while editing single bases and mutating a large number of unrelated DNA and RNA. A correct interpretation of genomic data, along with the strategies for the detection of off-target mutations and minimizing off-target cleavage efficiency, are still some of the urgent problems that need to be addressed at present. For example, technologies such as GUIDE-seq[81], Digenome-seq[77], and CIRCLE-seq[82] have been widely used to detect off-target effects. In recent years, after an in-depth study of the factors affecting off-target effects, it has been found that the current strategies to solve off-target effects mainly include: (1) Predicting off-target sites: Using CRISPOR, CHOPCHOP, and other tools, gRNAs can be designed online to analyze potential off-target sites, so that researchers can select gRNAs with low off-target effects as much as possible. The recently introduced DISCOVER-Seq technology[83] can identify the exact site of CRISPR cutting genome with simple processes and accurate results. In addition, the structure of sgRNAs and the activity of Cas protein are closely related to the off-target effects[84]; (2) optimizing the design strategy of sgRNAs: Doench *et al*[85] found a significant reduction in off-target effects after establishing new sgRNA design rules by optimizing the composition of sgRNAs; and (3) altering the structure of the Cas enzyme: Slaymaker *et al*[86] improved the binding rate to the target sequences using mutants of the Cas9 protein.

***Safety and ethical issues***

Recently, a study reported that a large number of unnecessary repetitive fragments appear when gene insertion is performed in mice using the CRISPR/Cas technology, which cannot be detected using the standard PCR analysis[87]. On the other hand, it was also proposed that in human embryonic cells, frequent DNA breakage can lead to the loss of the entire chromosome or sometimes a large part of the chromosome, which can pose significant challenges for mutation corrections[88]. Another therapeutic intervention is the usage of AAV vectors that are often used with CRISPR/Cas9 for targeted gene editing. They are sometimes unable to load larger genes and might adversely affect the functioning of AAV vectors carrying exogenous DNA fragments inserted into human chromosomes due to insertional mutagenesis. Henceforth, all combined efforts should be directed towards implementing the strong gene-editing abilities of CRISPR/Cas technology for the treatment of related diseases, while preventing any untoward genome-editing behaviors and similar negative events, as well as avoiding irreversible and unethical mutational consequences.

***Quantitative and high-throughput detection and sample pretreatment***

As CRISPR/Cas technology accurately conducts a quantitative analysis of target nucleic acids, it is deemed important for providing rapid and ultrasensitive data relevant for prompt disease management and treatment. However, HOLMES and DETECTR, two detected CRISPR-Cas effectors, might not be capable of precise target quantification for Cas12-based detection platforms[30,89]. In addition to that, multi-channel detection is also very crucial for distinguishing different pathogens as well as single-base polymorphisms for an accurate diagnosis, but only SHERLOCKv2 contains multi-channel detection ability at present[29], highlighting its innate potential as a rapid and quantitative detection platform.

With the more and more extensive application of CRISPR/Cas technology in clinical diagnosis, the sample sources, as well as the influencing factors within the samples, will be gradually expanded and diversified[90]. Therefore, it is very important to select a simple, efficient and economical sample pretreatment strategy for opening up novel avenues to tackle genetic diseases in a precise manner.

Despite significant technical improvements, the above-mentioned challenges should be further addressed for optimizing genomic stability in future studies.

**CONCLUSION**

As stated by Barrangou *et al*[91], “The potential for CRISPR/Cas technology applications is enormous, affecting almost all aspects of life and providing inspiration for future technological breakthroughs”. CRISPR/Cas technology may revolutionize diagnostic and therapeutic research in clinical diseases and become a versatile tool in practice in the field of clinical medicine as a pathogen detection platform due to its high efficiency, portability, and low-cost factor. Moreover, the developed diagnostic tools based on the CRISPR/Cas system are highly suitable for large-scale screening tasks in the frontline, community hospitals, and resource-limited environments[90], thereby initiating rapid and accurate detection and further promoting the development of point-of-care testing. Simultaneously, emerging CRISPR/Cas technology is being used with renewed efforts for discovering new therapeutic targets and detecting biomarkers to provide more accurate and scientific avenues for the early diagnosis and clinical treatment of oncological diseases[38,69,70]. Since the application of CRISPR/Cas technology in bacterial resistance detection has the advantages of simplicity, rapidity, and sensitivity, the recently developed platform can be more optimized by applying more detection methods for discovering various pathogens, which will be essential to prevent the spread of drug-resistant bacterial infections in hospitals[73,78].

Owing to the fact that as CRISPR/Cas technology has an undeniable tremendous potential in clinical applications and scientific research technology, many long-term clinical studies are required to elucidate biological mechanisms behind disease development and progression. This will make CRISPR/Cas technology an emerging discipline that provides better health care and improves human health more efficiently. It is also suggested that CRISPR will certainly provide more exciting results in the future, bringing us unlimited possibilities, thus providing novel molecular therapies and promoting the development of the life sciences.

**REFERENCES**

1 **Garneau JE**, Dupuis MÈ, Villion M, Romero DA, Barrangou R, Boyaval P, Fremaux C, Horvath P, Magadán AH, Moineau S. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature* 2010; **468**: 67-71 [PMID: 21048762 DOI: 10.1038/nature09523]

2 **Bharathkumar N**, Sunil A, Meera P, Aksah S, Kannan M, Saravanan KM, Anand T. CRISPR/Cas-Based Modifications for Therapeutic Applications: A Review. *Mol Biotechnol* 2022; **64**: 355-372 [PMID: 34741732 DOI: 10.1007/s12033-021-00422-8]

3 **Hidalgo-Cantabrana C**, Goh YJ, Barrangou R. Characterization and Repurposing of Type I and Type II CRISPR-Cas Systems in Bacteria. *J Mol Biol* 2019; **431**: 21-33 [PMID: 30261168 DOI: 10.1016/j.jmb.2018.09.013]

4 **Liu X**, Hussain M, Dai J, Li Y, Zhang L, Yang J, Ali Z, He N, Tang Y. Programmable Biosensors Based on RNA-Guided CRISPR/Cas Endonuclease. *Biol Proced Online* 2022; **24**: 2 [PMID: 35067222 DOI: 10.1186/s12575-021-00163-7]

5 **Varble A**, Marraffini LA. Three New Cs for CRISPR: Collateral, Communicate, Cooperate. *Trends Genet* 2019; **35**: 446-456 [PMID: 31036344 DOI: 10.1016/j.tig.2019.03.009]

6 **Deng L**, Garrett RA, Shah SA, Peng X, She Q. A novel interference mechanism by a type IIIB CRISPR-Cmr module in Sulfolobus. *Mol Microbiol* 2013; **87**: 1088-1099 [PMID: 23320564 DOI: 10.1111/mmi.12152]

7 **Javaid N**, Choi S. CRISPR/Cas System and Factors Affecting Its Precision and Efficiency. *Front Cell Dev Biol* 2021; **9**: 761709 [PMID: 34901007 DOI: 10.3389/fcell.2021.761709]

8 **Staals RH**, Zhu Y, Taylor DW, Kornfeld JE, Sharma K, Barendregt A, Koehorst JJ, Vlot M, Neupane N, Varossieau K, Sakamoto K, Suzuki T, Dohmae N, Yokoyama S, Schaap PJ, Urlaub H, Heck AJ, Nogales E, Doudna JA, Shinkai A, van der Oost J. RNA targeting by the type III-A CRISPR-Cas Csm complex of Thermus thermophilus. *Mol Cell* 2014; **56**: 518-530 [PMID: 25457165 DOI: 10.1016/j.molcel.2014.10.005]

9 **Tamulaitis G**, Kazlauskiene M, Manakova E, Venclovas Č, Nwokeoji AO, Dickman MJ, Horvath P, Siksnys V. Programmable RNA shredding by the type III-A CRISPR-Cas system of Streptococcus thermophilus. *Mol Cell* 2014; **56**: 506-517 [PMID: 25458845 DOI: 10.1016/j.molcel.2014.09.027]

10 **Elmore JR**, Sheppard NF, Ramia N, Deighan T, Li H, Terns RM, Terns MP. Bipartite recognition of target RNAs activates DNA cleavage by the Type III-B CRISPR-Cas system. *Genes Dev* 2016; **30**: 447-459 [PMID: 26848045 DOI: 10.1101/gad.272153.115]

11 **Estrella MA**, Kuo FT, Bailey S. RNA-activated DNA cleavage by the Type III-B CRISPR-Cas effector complex. *Genes Dev* 2016; **30**: 460-470 [PMID: 26848046 DOI: 10.1101/gad.273722.115]

12 **Kolesnik MV**, Fedorova I, Karneyeva KA, Artamonova DN, Severinov KV. Type III CRISPR-Cas Systems: Deciphering the Most Complex Prokaryotic Immune System. *Biochemistry (Mosc)* 2021; **86**: 1301-1314 [PMID: 34903162 DOI: 10.1134/S0006297921100114]

13 **Han W**, Stella S, Zhang Y, Guo T, Sulek K, Peng-Lundgren L, Montoya G, She Q. A Type III-B Cmr effector complex catalyzes the synthesis of cyclic oligoadenylate second messengers by cooperative substrate binding. *Nucleic Acids Res* 2018; **46**: 10319-10330 [PMID: 30239876 DOI: 10.1093/nar/gky844]

14 **Huang F**, Zhu B. The Cyclic Oligoadenylate Signaling Pathway of Type III CRISPR-Cas Systems. *Front Microbiol* 2020; **11**: 602789 [PMID: 33552016 DOI: 10.3389/fmicb.2020.602789]

15 **Rouillon C**, Athukoralage JS, Graham S, Grüschow S, White MF. Control of cyclic oligoadenylate synthesis in a type III CRISPR system. *Elife* 2018; **7** [PMID: 29963983 DOI: 10.7554/eLife.36734]

16 **Pinilla-Redondo R**, Mayo-Muñoz D, Russel J, Garrett RA, Randau L, Sørensen SJ, Shah SA. Type IV CRISPR-Cas systems are highly diverse and involved in competition between plasmids. *Nucleic Acids Res* 2020; **48**: 2000-2012 [PMID: 31879772 DOI: 10.1093/nar/gkz1197]

17 **Smits JPH**, Meesters LD, Maste BGW, Zhou H, Zeeuwen PLJM, van den Bogaard EH. CRISPR-Cas9‒Based Genomic Engineering in Keratinocytes: From Technology to Application. *JID Innov* 2022; **2**: 100082 [PMID: 35146483 DOI: 10.1016/j.xjidi.2021.100082]

18 **Dort EN**, Tanguay P, Hamelin RC. CRISPR/Cas9 Gene Editing: An Unexplored Frontier for Forest Pathology. *Front Plant Sci* 2020; **11**: 1126 [PMID: 32793272 DOI: 10.3389/fpls.2020.01126]

19 **Burmistrz M**, Pyrć K. CRISPR-Cas Systems in Prokaryotes. *Pol J Microbiol* 2015; **64**: 193-202 [PMID: 26638527 DOI: 10.5604/01.3001.0009.2114]

20 **Sapranauskas R**, Gasiunas G, Fremaux C, Barrangou R, Horvath P, Siksnys V. The Streptococcus thermophilus CRISPR/Cas system provides immunity in Escherichia coli. *Nucleic Acids Res* 2011; **39**: 9275-9282 [PMID: 21813460 DOI: 10.1093/nar/gkr606]

21 **Zetsche B**, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, van der Oost J, Regev A, Koonin EV, Zhang F. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell* 2015; **163**: 759-771 [PMID: 26422227 DOI: 10.1016/j.cell.2015.09.038]

22 **Dronina J**, Samukaite-Bubniene U, Ramanavicius A. Towards application of CRISPR-Cas12a in the design of modern viral DNA detection tools (Review). *J Nanobiotechnology* 2022; **20**: 41 [PMID: 35062978 DOI: 10.1186/s12951-022-01246-7]

23 **Aquino-Jarquin G**. CRISPR-Cas14 is now part of the artillery for gene editing and molecular diagnostic. *Nanomedicine* 2019; **18**: 428-431 [PMID: 30935995 DOI: 10.1016/j.nano.2019.03.006]

24 **Zhang D**, Li Z, Yan B, Li JF. A novel RNA-guided RNA-targeting CRISPR tool. *Sci China Life Sci* 2016; **59**: 854-856 [PMID: 27436421 DOI: 10.1007/s11427-016-0192-5]

25 **Aquino-Jarquin G**. Recent progress on rapid SARS-CoV-2/COVID-19 detection by CRISPR-Cas13-based platforms. *Drug Discov Today* 2021; **26**: 2025-2035 [PMID: 34147688 DOI: 10.1016/j.drudis.2021.06.005]

26 **Caliendo AM**, Hodinka RL. A CRISPR Way to Diagnose Infectious Diseases. *N Engl J Med* 2017; **377**: 1685-1687 [PMID: 29069564 DOI: 10.1056/NEJMcibr1704902]

27 **Abudayyeh OO**, Gootenberg JS, Essletzbichler P, Han S, Joung J, Belanto JJ, Verdine V, Cox DBT, Kellner MJ, Regev A, Lander ES, Voytas DF, Ting AY, Zhang F. RNA targeting with CRISPR-Cas13. *Nature* 2017; **550**: 280-284 [PMID: 28976959 DOI: 10.1038/nature24049]

28 **Li SY**, Cheng QX, Liu JK, Nie XQ, Zhao GP, Wang J. CRISPR-Cas12a has both cis- and trans-cleavage activities on single-stranded DNA. *Cell Res* 2018; **28**: 491-493 [PMID: 29531313 DOI: 10.1038/s41422-018-0022-x]

29 **Priya Swetha PD**, Sonia J, Sapna K, Prasad KS. Towards CRISPR powered electrochemical sensing for smart diagnostics. *Curr Opin Electrochem* 2021; **30**: 100829 [PMID: 34909513 DOI: 10.1016/j.coelec.2021.100829]

30 **Chen JS**, Ma E, Harrington LB, Da Costa M, Tian X, Palefsky JM, Doudna JA. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science* 2018; **360**: 436-439 [PMID: 29449511 DOI: 10.1126/science.aar6245]

31 **Joung J**, Ladha A, Saito M, Segel M, Bruneau R, Huang MW, Kim NG, Yu X, Li J, Walker BD, Greninger AL, Jerome KR, Gootenberg JS, Abudayyeh OO, Zhang F. Point-of-care testing for COVID-19 using SHERLOCK diagnostics. *medRxiv* 2020 [PMID: 32511521 DOI: 10.1101/2020.05.04.20091231]

32 **Xu X**, Luo T, Gao J, Lin N, Li W, Xia X, Wang J. CRISPR-Assisted DNA Detection: A Novel dCas9-Based DNA Detection Technique. *CRISPR J* 2020; **3**: 487-502 [PMID: 33346711 DOI: 10.1089/crispr.2020.0041]

33 **Freije CA**, Myhrvold C, Boehm CK, Lin AE, Welch NL, Carter A, Metsky HC, Luo CY, Abudayyeh OO, Gootenberg JS, Yozwiak NL, Zhang F, Sabeti PC. Programmable Inhibition and Detection of RNA Viruses Using Cas13. *Mol Cell* 2019; **76**: 826-837.e11 [PMID: 31607545 DOI: 10.1016/j.molcel.2019.09.013]

34 **Fozouni P**, Son S, Díaz de León Derby M, Knott GJ, Gray CN, D'Ambrosio MV, Zhao C, Switz NA, Kumar GR, Stephens SI, Boehm D, Tsou CL, Shu J, Bhuiya A, Armstrong M, Harris AR, Chen PY, Osterloh JM, Meyer-Franke A, Joehnk B, Walcott K, Sil A, Langelier C, Pollard KS, Crawford ED, Puschnik AS, Phelps M, Kistler A, DeRisi JL, Doudna JA, Fletcher DA, Ott M. Amplification-free detection of SARS-CoV-2 with CRISPR-Cas13a and mobile phone microscopy. *Cell* 2021; **184**: 323-333.e9 [PMID: 33306959 DOI: 10.1016/j.cell.2020.12.001]

35 **Liu TY**, Knott GJ, Smock DCJ, Desmarais JJ, Son S, Bhuiya A, Jakhanwal S, Prywes N, Agrawal S, Díaz de León Derby M, Switz NA, Armstrong M, Harris AR, Charles EJ, Thornton BW, Fozouni P, Shu J, Stephens SI, Kumar GR, Zhao C, Mok A, Iavarone AT, Escajeda AM, McIntosh R, Kim S, Dugan EJ; IGI Testing Consortium, Pollard KS, Tan MX, Ott M, Fletcher DA, Lareau LF, Hsu PD, Savage DF, Doudna JA. Accelerated RNA detection using tandem CRISPR nucleases. *Nat Chem Biol* 2021; **17**: 982-988 [PMID: 34354262 DOI: 10.1038/s41589-021-00842-2]

36 **Chow RD**, Chen S. Cancer CRISPR Screens In Vivo. *Trends Cancer* 2018; **4**: 349-358 [PMID: 29709259 DOI: 10.1016/j.trecan.2018.03.002]

37 **Song CQ**, Li Y, Mou H, Moore J, Park A, Pomyen Y, Hough S, Kennedy Z, Fischer A, Yin H, Anderson DG, Conte D Jr, Zender L, Wang XW, Thorgeirsson S, Weng Z, Xue W. Genome-Wide CRISPR Screen Identifies Regulators of Mitogen-Activated Protein Kinase as Suppressors of Liver Tumors in Mice. *Gastroenterology* 2017; **152**: 1161-1173.e1 [PMID: 27956228 DOI: 10.1053/j.gastro.2016.12.002]

38 **Shan Y**, Zhou X, Huang R, Xing D. High-Fidelity and Rapid Quantification of miRNA Combining crRNA Programmability and CRISPR/Cas13a trans-Cleavage Activity. *Anal Chem* 2019; **91**: 5278-5285 [PMID: 30873832 DOI: 10.1021/acs.analchem.9b00073]

39 **Durán-Vinet B**, Araya-Castro K, Calderón J, Vergara L, Weber H, Retamales J, Araya-Castro P, Leal-Rojas P. CRISPR/Cas13-Based Platforms for a Potential Next-Generation Diagnosis of Colorectal Cancer through Exosomes Micro-RNA Detection: A Review. *Cancers (Basel)* 2021; **13** [PMID: 34572866 DOI: 10.3390/cancers13184640]

40 **Qiu XY**, Zhu LY, Zhu CS, Ma JX, Hou T, Wu XM, Xie SS, Min L, Tan DA, Zhang DY, Zhu L. Highly Effective and Low-Cost MicroRNA Detection with CRISPR-Cas9. *ACS Synth Biol* 2018; **7**: 807-813 [PMID: 29486117 DOI: 10.1021/acssynbio.7b00446]

41 **Cook PJ**, Ventura A. Cancer diagnosis and immunotherapy in the age of CRISPR. *Genes Chromosomes Cancer* 2019; **58**: 233-243 [PMID: 30382614 DOI: 10.1002/gcc.22702]

42 **Lee SH**, Yu J, Hwang GH, Kim S, Kim HS, Ye S, Kim K, Park J, Park DY, Cho YK, Kim JS, Bae S. CUT-PCR: CRISPR-mediated, ultrasensitive detection of target DNA using PCR. *Oncogene* 2017; **36**: 6823-6829 [PMID: 28846115 DOI: 10.1038/onc.2017.281]

43 **Granados-Riveron JT**, Aquino-Jarquin G. CRISPR-Cas13 Precision Transcriptome Engineering in Cancer. *Cancer Res* 2018; **78**: 4107-4113 [PMID: 30021724 DOI: 10.1158/0008-5472.CAN-18-0785]

44 **Bak RO**, Gomez-Ospina N, Porteus MH. Gene Editing on Center Stage. *Trends Genet* 2018; **34**: 600-611 [PMID: 29908711 DOI: 10.1016/j.tig.2018.05.004]

45 **Wu Y**, Zeng J, Roscoe BP, Liu P, Yao Q, Lazzarotto CR, Clement K, Cole MA, Luk K, Baricordi C, Shen AH, Ren C, Esrick EB, Manis JP, Dorfman DM, Williams DA, Biffi A, Brugnara C, Biasco L, Brendel C, Pinello L, Tsai SQ, Wolfe SA, Bauer DE. Highly efficient therapeutic gene editing of human hematopoietic stem cells. *Nat Med* 2019; **25**: 776-783 [PMID: 30911135 DOI: 10.1038/s41591-019-0401-y]

46 **Qasim W**, Zhan H, Samarasinghe S, Adams S, Amrolia P, Stafford S, Butler K, Rivat C, Wright G, Somana K, Ghorashian S, Pinner D, Ahsan G, Gilmour K, Lucchini G, Inglott S, Mifsud W, Chiesa R, Peggs KS, Chan L, Farzeneh F, Thrasher AJ, Vora A, Pule M, Veys P. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. *Sci Transl Med* 2017; **9** [PMID: 28123068 DOI: 10.1126/scitranslmed.aaj2013]

47 **Egelie KJ**, Graff GD, Strand SP, Johansen B. The emerging patent landscape of CRISPR-Cas gene editing technology. *Nat Biotechnol* 2016; **34**: 1025-1031 [PMID: 27727218 DOI: 10.1038/nbt.3692]

48 **Nishiga M**, Liu C, Qi LS, Wu JC. The use of new CRISPR tools in cardiovascular research and medicine. *Nat Rev Cardiol* 2022 [PMID: 35145236 DOI: 10.1038/s41569-021-00669-3]

49 **Khadempar S**, Familghadakchi S, Motlagh RA, Farahani N, Dashtiahangar M, Rezaei H, Gheibi Hayat SM. CRISPR-Cas9 in genome editing: Its function and medical applications. *J Cell Physiol* 2019; **234**: 5751-5761 [PMID: 30362544 DOI: 10.1002/jcp.27476]

50 **Cideciyan AV**, Jacobson SG. Leber Congenital Amaurosis (LCA): Potential for Improvement of Vision. *Invest Ophthalmol Vis Sci* 2019; **60**: 1680-1695 [PMID: 31009524 DOI: 10.1167/iovs.19-26672]

51 **Maeder ML**, Stefanidakis M, Wilson CJ, Baral R, Barrera LA, Bounoutas GS, Bumcrot D, Chao H, Ciulla DM, DaSilva JA, Dass A, Dhanapal V, Fennell TJ, Friedland AE, Giannoukos G, Gloskowski SW, Glucksmann A, Gotta GM, Jayaram H, Haskett SJ, Hopkins B, Horng JE, Joshi S, Marco E, Mepani R, Reyon D, Ta T, Tabbaa DG, Samuelsson SJ, Shen S, Skor MN, Stetkiewicz P, Wang T, Yudkoff C, Myer VE, Albright CF, Jiang H. Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. *Nat Med* 2019; **25**: 229-233 [PMID: 30664785 DOI: 10.1038/s41591-018-0327-9]

52 **Yu W**, Wu Z. Use of AAV Vectors for CRISPR-Mediated In Vivo Genome Editing in the Retina. *Methods Mol Biol* 2019; **1950**: 123-139 [PMID: 30783971 DOI: 10.1007/978-1-4939-9139-6\_7]

53 **Ruan GX**, Barry E, Yu D, Lukason M, Cheng SH, Scaria A. CRISPR/Cas9-Mediated Genome Editing as a Therapeutic Approach for Leber Congenital Amaurosis 10. *Mol Ther* 2017; **25**: 331-341 [PMID: 28109959 DOI: 10.1016/j.ymthe.2016.12.006]

54 **Wang D**, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov* 2019; **18**: 358-378 [PMID: 30710128 DOI: 10.1038/s41573-019-0012-9]

55 **Ledford H**. CRISPR treatment inserted directly into the body for first time. *Nature* 2020; **579**: 185 [PMID: 32157225 DOI: 10.1038/d41586-020-00655-8]

56 **Da Silva Sanchez A**, Paunovska K, Cristian A, Dahlman JE. Treating Cystic Fibrosis with mRNA and CRISPR. *Hum Gene Ther* 2020; **31**: 940-955 [PMID: 32799680 DOI: 10.1089/hum.2020.137]

57 **Li X**, Qian X, Wang B, Xia Y, Zheng Y, Du L, Xu D, Xing D, DePinho RA, Lu Z. Programmable base editing of mutated TERT promoter inhibits brain tumour growth. *Nat Cell Biol* 2020; **22**: 282-288 [PMID: 32066906 DOI: 10.1038/s41556-020-0471-6]

58 **Tang L**, Zeng Y, Du H, Gong M, Peng J, Zhang B, Lei M, Zhao F, Wang W, Li X, Liu J. CRISPR/Cas9-mediated gene editing in human zygotes using Cas9 protein. *Mol Genet Genomics* 2017; **292**: 525-533 [PMID: 28251317 DOI: 10.1007/s00438-017-1299-z]

59 **Park SH**, Lee CM, Dever DP, Davis TH, Camarena J, Srifa W, Zhang Y, Paikari A, Chang AK, Porteus MH, Sheehan VA, Bao G. Highly efficient editing of the β-globin gene in patient-derived hematopoietic stem and progenitor cells to treat sickle cell disease. *Nucleic Acids Res* 2019; **47**: 7955-7972 [PMID: 31147717 DOI: 10.1093/nar/gkz475]

60 **Jiang DJ**, Xu CL, Tsang SH. Revolution in Gene Medicine Therapy and Genome Surgery. *Genes (Basel)* 2018; **9** [PMID: 30486314 DOI: 10.20944/preprints201810.0010.v1]

61 **Verwaal R**, Buiting-Wiessenhaan N, Dalhuijsen S, Roubos JA. CRISPR/Cpf1 enables fast and simple genome editing of Saccharomyces cerevisiae. *Yeast* 2018; **35**: 201-211 [PMID: 28886218 DOI: 10.1002/yea.3278]

62 **Kleinstiver BP**, Sousa AA, Walton RT, Tak YE, Hsu JY, Clement K, Welch MM, Horng JE, Malagon-Lopez J, Scarfò I, Maus MV, Pinello L, Aryee MJ, Joung JK. Engineered CRISPR-Cas12a variants with increased activities and improved targeting ranges for gene, epigenetic and base editing. *Nat Biotechnol* 2019; **37**: 276-282 [PMID: 30742127 DOI: 10.1038/s41587-018-0011-0]

63 **DeWeirdt PC**, Sanson KR, Sangree AK, Hegde M, Hanna RE, Feeley MN, Griffith AL, Teng T, Borys SM, Strand C, Joung JK, Kleinstiver BP, Pan X, Huang A, Doench JG. Optimization of AsCas12a for combinatorial genetic screens in human cells. *Nat Biotechnol* 2021; **39**: 94-104 [PMID: 32661438 DOI: 10.1038/s41587-020-0600-6]

64 **Cox DBT**, Gootenberg JS, Abudayyeh OO, Franklin B, Kellner MJ, Joung J, Zhang F. RNA editing with CRISPR-Cas13. *Science* 2017; **358**: 1019-1027 [PMID: 29070703 DOI: 10.1126/science.aaq0180]

65 **Li G**, Wang Y, Li X, Wang Y, Huang X, Gao J, Hu X. Developing PspCas13b-based enhanced RESCUE system, eRESCUE, with efficient RNA base editing. *Cell Commun Signal* 2021; **19**: 84 [PMID: 34380502 DOI: 10.1186/s12964-021-00716-z]

66 **Tang F**, Chen L, Gao H, Xiao D, Li X. m6A: An Emerging Role in Programmed Cell Death. *Front Cell Dev Biol* 2022; **10**: 817112 [PMID: 35141221 DOI: 10.3389/fcell.2022.817112]

67 **Wilson C**, Chen PJ, Miao Z, Liu DR. Programmable m6A modification of cellular RNAs with a Cas13-directed methyltransferase. *Nat Biotechnol* 2020; **38**: 1431-1440 [PMID: 32601430 DOI: 10.1038/s41587-020-0572-6]

68 **Moses C**, Garcia-Bloj B, Harvey AR, Blancafort P. Hallmarks of cancer: The CRISPR generation. *Eur J Cancer* 2018; **93**: 10-18 [PMID: 29433054 DOI: 10.1016/j.ejca.2018.01.002]

69 **Yin H**, Xue W, Anderson DG. CRISPR-Cas: a tool for cancer research and therapeutics. *Nat Rev Clin Oncol* 2019; **16**: 281-295 [PMID: 30664678 DOI: 10.1038/s41571-019-0166-8]

70 **Wang G**, Chow RD, Bai Z, Zhu L, Errami Y, Dai X, Dong MB, Ye L, Zhang X, Renauer PA, Park JJ, Shen L, Ye H, Fuchs CS, Chen S. Multiplexed activation of endogenous genes by CRISPRa elicits potent antitumor immunity. *Nat Immunol* 2019; **20**: 1494-1505 [PMID: 31611701 DOI: 10.1038/s41590-019-0500-4]

71 **Gupta D**, Bhattacharjee O, Mandal D, Sen MK, Dey D, Dasgupta A, Kazi TA, Gupta R, Sinharoy S, Acharya K, Chattopadhyay D, Ravichandiran V, Roy S, Ghosh D. CRISPR-Cas9 system: A new-fangled dawn in gene editing. *Life Sci* 2019; **232**: 116636 [PMID: 31295471 DOI: 10.1016/j.lfs.2019.116636]

72 **Kim JS**, Cho DH, Park M, Chung WJ, Shin D, Ko KS, Kweon DH. CRISPR/Cas9-Mediated Re-Sensitization of Antibiotic-Resistant Escherichia coli Harboring Extended-Spectrum β-Lactamases. *J Microbiol Biotechnol* 2016; **26**: 394-401 [PMID: 26502735 DOI: 10.4014/jmb.1508.08080]

73 **Guk K**, Keem JO, Hwang SG, Kim H, Kang T, Lim EK, Jung J. A facile, rapid and sensitive detection of MRSA using a CRISPR-mediated DNA FISH method, antibody-like dCas9/sgRNA complex. *Biosens Bioelectron* 2017; **95**: 67-71 [PMID: 28412663 DOI: 10.1016/j.bios.2017.04.016]

74 **Wang G**, Song G, Xu Y. Association of CRISPR/Cas System with the Drug Resistance in *Klebsiella pneumoniae*. *Infect Drug Resist* 2020; **13**: 1929-1935 [PMID: 32606841 DOI: 10.2147/IDR.S253380]

75 **Bader MS**, Loeb M, Leto D, Brooks AA. Treatment of urinary tract infections in the era of antimicrobial resistance and new antimicrobial agents. *Postgrad Med* 2020; **132**: 234-250 [PMID: 31608743 DOI: 10.1080/00325481.2019.1680052]

76 **Shabbir MAB**, Shabbir MZ, Wu Q, Mahmood S, Sajid A, Maan MK, Ahmed S, Naveed U, Hao H, Yuan Z. CRISPR-cas system: biological function in microbes and its use to treat antimicrobial resistant pathogens. *Ann Clin Microbiol Antimicrob* 2019; **18**: 21 [PMID: 31277669 DOI: 10.1186/s12941-019-0317-x]

77 **Kim D**, Bae S, Park J, Kim E, Kim S, Yu HR, Hwang J, Kim JI, Kim JS. Digenome-seq: genome-wide profiling of CRISPR-Cas9 off-target effects in human cells. *Nat Methods* 2015; **12**: 237-243, 1 p following 243 [PMID: 25664545 DOI: 10.1038/nmeth.3284]

78 **Kiga K**, Tan XE, Ibarra-Chávez R, Watanabe S, Aiba Y, Sato'o Y, Li FY, Sasahara T, Cui B, Kawauchi M, Boonsiri T, Thitiananpakorn K, Taki Y, Azam AH, Suzuki M, Penadés JR, Cui L. Development of CRISPR-Cas13a-based antimicrobials capable of sequence-specific killing of target bacteria. *Nat Commun* 2020; **11**: 2934 [PMID: 32523110 DOI: 10.1038/s41467-020-16731-6]

79 **Wang DC**, Wang X. Off-target genome editing: A new discipline of gene science and a new class of medicine. *Cell Biol Toxicol* 2019; **35**: 179-183 [PMID: 31030279 DOI: 10.1007/s10565-019-09475-7]

80 **Grünewald J**, Zhou R, Garcia SP, Iyer S, Lareau CA, Aryee MJ, Joung JK. Transcriptome-wide off-target RNA editing induced by CRISPR-guided DNA base editors. *Nature* 2019; **569**: 433-437 [PMID: 30995674 DOI: 10.1038/s41586-019-1161-z]

81 **Höijer I**, Emmanouilidou A, Östlund R, van Schendel R, Bozorgpana S, Tijsterman M, Feuk L, Gyllensten U, den Hoed M, Ameur A. CRISPR-Cas9 induces large structural variants at on-target and off-target sites in vivo that segregate across generations. *Nat Commun* 2022; **13**: 627 [PMID: 35110541 DOI: 10.1038/s41467-022-28244-5]

82 **Tsai SQ**, Nguyen NT, Malagon-Lopez J, Topkar VV, Aryee MJ, Joung JK. CIRCLE-seq: a highly sensitive in vitro screen for genome-wide CRISPR-Cas9 nuclease off-targets. *Nat Methods* 2017; **14**: 607-614 [PMID: 28459458 DOI: 10.1038/nmeth.4278]

83 **Fu R**, He W, Dou J, Villarreal OD, Bedford E, Wang H, Hou C, Zhang L, Wang Y, Ma D, Chen Y, Gao X, Depken M, Xu H. Systematic decomposition of sequence determinants governing CRISPR/Cas9 specificity. *Nat Commun* 2022; **13**: 474 [PMID: 35078987 DOI: 10.1038/s41467-022-28028-x]

84 **Pattanayak V**, Lin S, Guilinger JP, Ma E, Doudna JA, Liu DR. High-throughput profiling of off-target DNA cleavage reveals RNA-programmed Cas9 nuclease specificity. *Nat Biotechnol* 2013; **31**: 839-843 [PMID: 23934178 DOI: 10.1038/nbt.2673]

85 **Doench JG**, Fusi N, Sullender M, Hegde M, Vaimberg EW, Donovan KF, Smith I, Tothova Z, Wilen C, Orchard R, Virgin HW, Listgarten J, Root DE. Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nat Biotechnol* 2016; **34**: 184-191 [PMID: 26780180 DOI: 10.1038/nbt.3437]

86 **Slaymaker IM**, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F. Rationally engineered Cas9 nucleases with improved specificity. *Science* 2016; **351**: 84-88 [PMID: 26628643 DOI: 10.1126/science.aad5227]

87 **Skryabin BV**, Kummerfeld DM, Gubar L, Seeger B, Kaiser H, Stegemann A, Roth J, Meuth SG, Pavenstädt H, Sherwood J, Pap T, Wedlich-Söldner R, Sunderkötter C, Schwartz YB, Brosius J, Rozhdestvensky TS. Pervasive head-to-tail insertions of DNA templates mask desired CRISPR-Cas9-mediated genome editing events. *Sci Adv* 2020; **6**: eaax2941 [PMID: 32095517 DOI: 10.1126/sciadv.aax2941]

88 **Zuccaro MV**, Xu J, Mitchell C, Marin D, Zimmerman R, Rana B, Weinstein E, King RT, Palmerola KL, Smith ME, Tsang SH, Goland R, Jasin M, Lobo R, Treff N, Egli D. Allele-Specific Chromosome Removal after Cas9 Cleavage in Human Embryos. *Cell* 2020; **183**: 1650-1664.e15 [PMID: 33125898 DOI: 10.1016/j.cell.2020.10.025]

89 **Li SY**, Cheng QX, Wang JM, Li XY, Zhang ZL, Gao S, Cao RB, Zhao GP, Wang J. CRISPR-Cas12a-assisted nucleic acid detection. *Cell Discov* 2018; **4**: 20 [PMID: 29707234 DOI: 10.1038/s41421-018-0028-z]

90 **Li Y**, Li S, Wang J, Liu G. CRISPR/Cas Systems towards Next-Generation Biosensing. *Trends Biotechnol* 2019; **37**: 730-743 [PMID: 30654914 DOI: 10.1016/j.tibtech.2018.12.005]

91 **Barrangou R**, Doudna JA. Applications of CRISPR technologies in research and beyond. *Nat Biotechnol* 2016; **34**: 933-941 [PMID: 27606440 DOI: 10.1038/nbt.3659]

**Footnotes**

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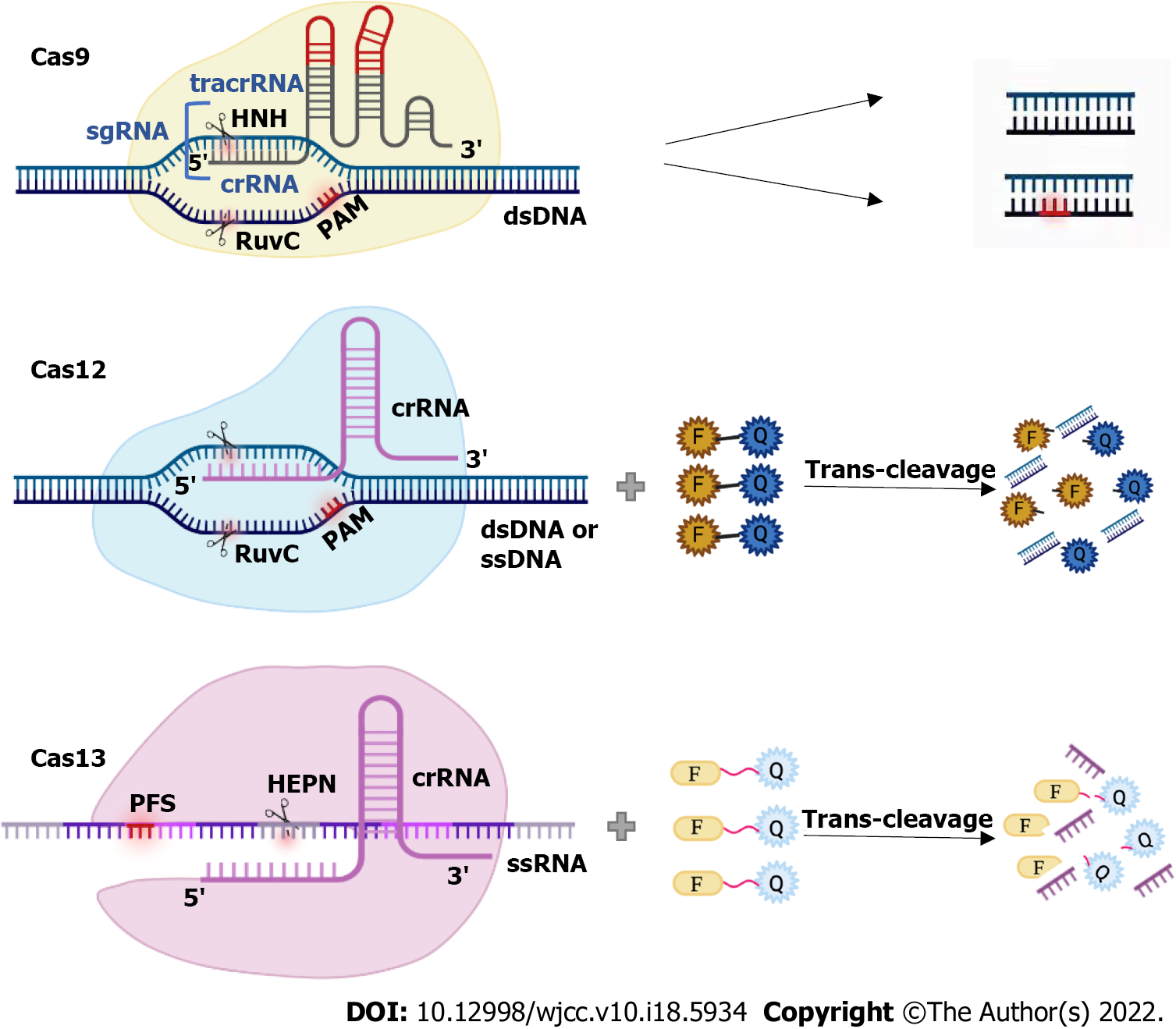
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**Figure Legends**



**Figure 1 Collateral activity of Cas9, Cas12 and Cas13.** PAM: Protospacer adjacent motif; dsDNA: Double-stranded DNA; ssDNA: Single-stranded DNA; crRNA: Clustered regularly interspaced short palindromic repeats RNAs; HEPN: Higher Eukaryotes and Prokaryotes Nucleotide-binding.

**Table 1 Features of clustered regularly interspaced short palindromic repeats/CRISPR-associated system**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **CRISPR type** | | **Specific cleavage** | **Collateral cleavage** | **Secondary messenger** | **CRISPR inhibitors** | **Ref.** |
| Class 1 | I (Cas8) | DNA | Not identified to date | Not identified to date | Yes | [4] |
| III (Cas10) | RNA | RNA, ssDNA | cOA | Not identified to date | [6-8] |
| IV (Csf1) | Not studied yet | Not studied yet | Not studied yet | Not studied yet | [16] |
| Class 2 | II (Cas9) | DNA | Not identified to date | Not identified to date | Yes | [4,5,16-20] |
| V (Cas12, Cas14) | DNA | ssDNA | Not identified to date | Yes | [21-23] |
| VI (Cas13) | RNA | RNA | Not identified to date | Not identified to date | [24-26] |

CRISPR: Clustered regularly interspaced short palindromic repeats; ssDNA: Single-stranded DNA; cOA: Cyclic oligoadenylate.

**Table 2 Clustered regularly interspaced short palindromic repeats-based pathogen nucleic acid detection system**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Nucleic acid detection system** | **Cas** | **Target DNA** | **Amplification method** | **Test method** | **Time** | **Sensitivity** | **Specificity** | **Ref.** |
| SHERLOCK | Cas13a | Zika virus *etc*. | RPA | Fluorescence | 2-5 h | 2 × 10-8 M | High | [26] |
| SHERLOCKv2 | Cas13a, Cas12a, Csm6 | Zika virus, Gordon fever virus, yellow fever virus, *etc*. | RPA | Fluorescence  Test strip | 0.5-4.0 h | 8 × 10-21 M | High | [29] |
| DETECTR | Cas12a | HPV | RPA | Fluorescence | 1 h | 10-18 M | High | [30] |
| STOP | Cas12b | 2019-nCoV | LAMP | Fluorescence | 1 h | 100 copies | High | [31] |
| CADD | Cas9 | HPV | - | Fluorescence | 30 min | 10-15 M | High | [32] |
| CARVER | Cas13a | Influenza A | RPA | Test strip | 2 h | - | High | [33] |
| Smartphone testing | Cas13a | 2019-nCoV | - | Fluorescence | 15-30 min | 10-4 M | High | [34] |
| Tandem nuclease | Cas13a + Csm6 | 2019-nCoV | - | Fluorescence | 20 min | 3.1 × 10-5 M | High | [35] |

Cas: CRISPR-associated protein; RPA: Recombinase Polymerase Amplification; SHERLOCK: Specific high-sensitivity enzymatic reporter unlocking; STOP: SHERLOCK Testing in One Pot; CADD: CRISPR-Cas9-assisted DNA detection; CARVER: LAMP: Loop-mediated isothermal amplification; 2019-nCoV: 2019 novel coronavirus; HPV: Human papillomavirus.

**Table 3 Clustered regularly interspaced short palindromic repeats-based tumor diagnostic system**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **System** | **Cas** | **Tumor type** | **Sensitivity** | **Specificity** | **Ref.** |
| RCH | Cas9 | NSCLC | 10-15 M | High | [40] |
| CUT | Cas9 | Colorectal cancer | < 0.01% | High | [41] |
| SHERLOCK | Cas13a | NSCLC | 0.1% | High | [26] |
| SHERLOCKv2 | Cas13a, Cas12a, Csm6 | NSCLC | 8 × 10-21 M | High | [29] |
| DETECTR | Cas12a | HPV | 10-18 M | High | [30] |

RCH: RCA-CRISPR-split-HRP; NSCLC: Non-small cell lung cancer; HPV: Human papillomavirus; CUT: CRISPR-mediated Ultrasensitive Detection of Target DNA-PCR; SHERLOCK: Specific high-sensitivity enzymatic reporter unlocking; DETECTR: DNA endonuclease-targeted CRISPR trans reporter.



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