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CRISPR/Cas9-Based Imaging: Challenges and Future Prospects

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ABSTRACT

In 1987, CRISPR (clustered regularly interspaced short palindromic repeats), was discovered in the *Escherichia coli* genome. It is a component of the adaptive immune system in prokaryotic, archaeal, and bacterial organisms. since it was discovered in bacteria, understanding CRISPR structure and function has made it a powerful gene editing tool that used in gene therapy and diagnostics purpose. Imaging is another application of CRISPR that is also used by researchers for diagnostic purposes. This short communication aims to discuss the CRISPR imaging field and the challenges facing it.

Keywords: *CRISPR, Imaging, d Cas9, gRNA*

Short Communication

Clustered regularly interspaced short palindromic repeats (CRISPR) that are part of an adaptive immune system in prokaryotic organisms, archaea, and bacteria, firstly discovered in the *Escherichia coli* genome in 1987 (Wang *et al*., 2022). According to the CRISPR system, after the first attack of the phage on the bacteria, the foreign gene fragments of the phage insert into the bacterial genome and memorize the genetic information of the foreign species (LaFleur *et al*., 2019). These unique sequences are interspersed within the host array of short repetitive DNA sequences, creating Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). The CRISPR region transcribes a small piece of RNA or guide RNA (gRNA) that contains the genetic information of the foreign DNA (Phage DNA) and translates it into a CRISPR-associated protein (Cas) with endonuclease activity. After a second attack on the phage, the Cas and gRNA are complexed and cleave the same phage DNA using the genetic information stored in the genome (LaFleur *et al*., 2019). Since the discovery of the CRISPR system in bacteria, understanding of CRISPR structure and function has made it a powerful gene editing tool used in gene therapy, and diagnostics fields (Liu *et al*., 2021). Promising results are shown for gene therapy of genetic diseases such as sickle cell anemia, thalassemia, Duchene muscular dystrophy, leukemia, and even types of solid tumors using CRISPR technology (Rheney, 2023; Choi and Koo, 2021; Vuelta *et al*., 2021; Tang *et al*., 2020). Although some of

these studies are in the research phase, others, such as gene therapy for sickle cell anemia, have entered the clinical trial phase (Anliker *et al*., 2022).

Moreover, the CRISPR system has been highly successful and effective in detecting infectious and non- infectious diseases so far that it could be used as a replacement for currently available laboratory diagnostic tests (Kim *et al*., 2021). Today, CRISPR has attracted the attention of researchers as a tool for imaging DNA and genomic loci of interest (Lucansky *et al*., 2023).

For imaging with CRISPR technology, used a modified Cas9 protein without cleavage activity named dCas9. The dCas9 has been tagged with an enhanced green fluorescent protein, EGFP, and a particular gRNA is designed for a target sequence (Selvaraj *et al*., 2021). The modified Cas9 and gRNA were introduced into cells. If the desired genomic locations are present in the sample or cell, a fluorescent signal is emitted when the guide RNA/dCas9 complex binds to the target sequence, and the operator or researcher can detect EGFP inside the cells (Selvaraj *et al*., 2021) (Fig. 1). This innovative work makes it possible to see cells at a high resolution using CRISPR imaging, providing scientists with new ways of understanding cell dynamics (Wu *et al*., 2019). Research efforts about CRISPR imaging are divided into two main groups due to its relatively new technology: chromatin remodels and telomere health (Wu *et al*., 2019; Thuma *et al*., 2023). Although many advances have been made in the field of CRISPR imaging, several challenges remain. To improve this technology, many efforts have been made to find solutions to the described challenges (Wu *et al*., 2019). The first challenge is to correctly identify the signal emitted from the sample, which can cause false positive results because background intensity and non-specific signals can affect the results (Thuma *et al*., 2023). To reduce and overcome this problem, the transfection method should be optimized. The use of CRISPR imaging is mainly used to image repetitive sequences of the genome. This is despite the fact that a small percentage of the genome consists of repetitive sequences. This problem limits the application of CRISPR imaging (Mao *et al*., 2019). To enhance the use of CRISPR imaging to target non-repetitive sequences, one solution is to use multiple sgRNAs that target sites flanking the target sequence (Lyu *et al*., 2022). Unwanted binding of gRNA to non-specific regions (off-targets) is one of the important challenges that the CRISPR system faces in both editing and detection as well as imaging (Chaudhary *et al*., 2021). Despite careful consideration during gRNA design, the off-target occurrence is inevitable. Overcoming this important challenge can increase the application and use of the CRISPR system in various fields of treatment, diagnosis, and imaging.

Conclusion

In addition to diagnosis and gene therapy, the CRISPR system can also be used successfully in the field of imaging.

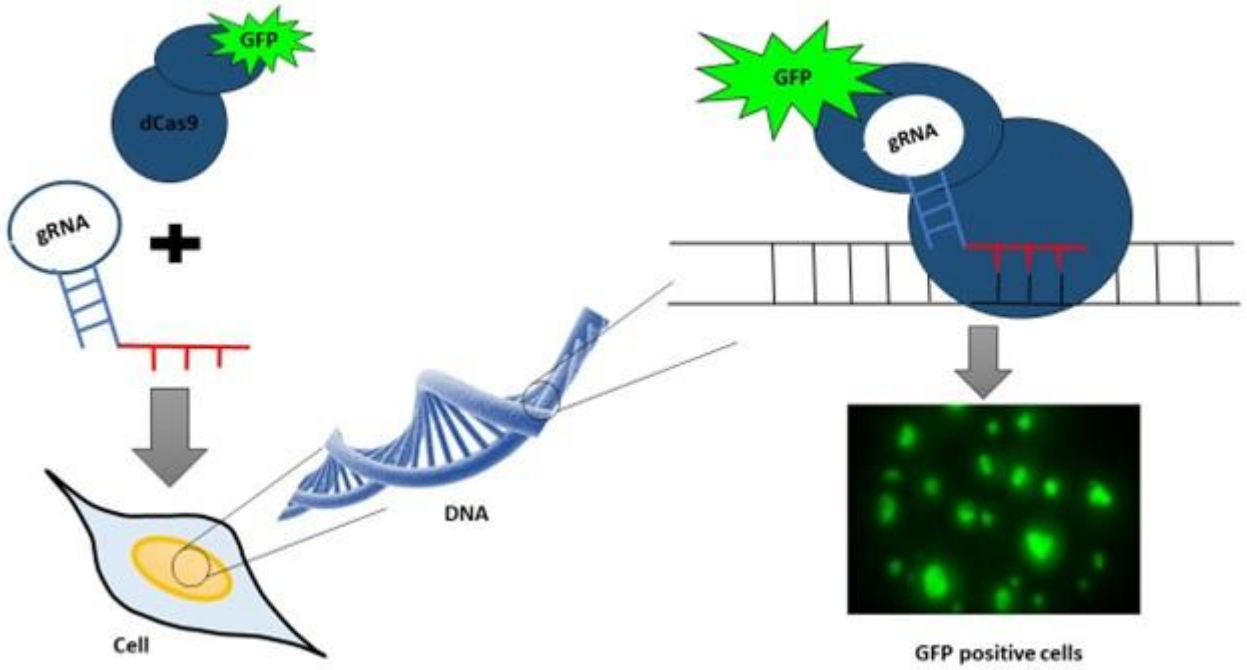

Figure 1: Schematic figure of CRISPR imaging technology. dCas9 that tagged to an enhanced green fluorescent protein (EGFP) guided to the target sequence of the genome by gRNA. The modified Cas9 and gRNA were introduced into cells and the researcher was able to detect EGFP inside the cells.

Conflict of Interest: Mahintaj Dara declares that she has no conflict of interest.

Ethics Approval: Not applicable.

Consent to Participate: Not applicable.

Consent for Publication: Not applicable.

Availability of Data and Material: The datasets collected during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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References

Anliker B, Childs L, Rau J, Renner M, Schüle S, Schuessler-Lenz M, *et al*. Regulatory considerations for clinical trial applications with CRISPR-based medicinal products. *The CRISPR Journal* 2022; 5: 364-376.

Chaudhary N, Im JK, Nho SH, Kim H. Visualizing Live Chromatin Dynamics through CRISPR-Based Imaging Techniques. *Mol Cells* 2021; 44: 627-636.

Choi E, Koo T. CRISPR technologies for the treatment of Duchenne muscular dystrophy. *Molecular Therapy* 2021; 29: 3179-3191.

Kim S, Ji S, Koh HR. CRISPR as a Diagnostic Tool. *Biomolecules* 2021; 11: 1162.

LaFleur MW, Nguyen TH, Coxe MA, Yates KB, Trombley JD, Weiss SA, Brown FD, Gillis JE, Coxe DJ, Doench JG, Haining WN. A CRISPR-Cas9 delivery system for *in vivo* screening of genes in the immune system. *Nat commun* 2019; 10: 1668.

Liu G, Lin Q, Jin S, Gao C. The CRISPR-Cas toolbox and gene editing technologies. *Molecular cell* 2021.

Lucansky V, Holubekova V, Kolkova Z, Halasova E, Samec M, Golubnitschaja O. Multi-faceted CRISPR/Cas technological innovation aspects in the framework of 3P medicine. *EPMA J* 2023; 22: 1-7.

Lyu XY, Deng Y, Huang XY, Li ZZ, Fang GQ, Yang D, Wang FL, Kang W, Shen EZ, Song CQ. CRISPR FISHer enables high-sensitivity imaging of nonrepetitive DNA in living cells through phase separation-mediated signal amplification. *Cell Res* 2022; 32: 969-981.

Mao S, Ying Y, Wu X, Krueger CJ, Chen AK. CRISPR/dual-FRET molecular beacon for sensitive live-cell imaging of non-repetitive genomic loci. *Nucleic Acids Res* 2019; 47: e131-e31.

Rheney J. Using Crisper in the Treatment of Sickle Cell Disease. *J Stud Res* 2023; 12.

Selvaraj D, Dawar R, Sivakumar PK, Devi A. Clustered regularly interspaced short palindromic repeats, a glimpse–impacts in molecular biology, trends and highlights. *Horm Mol Biol Clin Investig* 2021; 28: 105-112.

Tang N, Cheng C, Zhang X, Qiao M, Li N, Mu W, Wei XF, Han W, Wang H. TGF-β inhibition via CRISPR promotes the long-term efficacy of CAR T cells against solid tumors. *JCI Insight* 2020; 5: e133977.

Thuma J, Chung YC, Tu LC. Advances and challenges in CRISPR-based real-time imaging of dynamic genome organization. *Front in Mol Biosci* 2023; 10: 1173545.

Vuelta E, Garcia-Tunon I, Hernandez-Carabias P, Mendez L, Sanchez-Martin M. Future approaches for treating chronic myeloid leukemia: CRISPR therapy. *Biology* 2021; 10: 118.

Wang SW, Gao C, Zheng YM, Yi L, Lu JC, Huang XY, Cai JB, Zhang PF, Cui YH, Ke AW. Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. *Mol cancer* 2022; 21: 1-27.

Wu X, Mao S, Ying Y, Krueger CJ, Chen AK. Progress and challenges for live-cell imaging of genomic loci using CRISPR-based platforms. *Int J Genomics Proteomics* 2019; 17: 119-128.