Commentary
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CRISPR/Cas13a-based Supersensitive Circulating Tumor DNA Assay for Detecting EGFR Mutations in Plasma

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In the treatment and management of non-small cell lung cancer (NSCLC), accurate detection and monitoring of tumor-associated gene mutations is critical [1]. Traditional detection methods, such as tissue biopsy, are invasive, sampling bias, and cannot be monitored in real time. Liquid biopsies [2], particularly tests based on circulating tumor DNA (ctDNA), are valued for their non-invasive and ability to provide real-time biological information. However, there are still sensitivity and specificity challenges in detecting low-frequency mutations with existing techniques. Therefore, there is an urgent need for the research community to develop new detection techniques to improve the efficiency of early diagnosis, treatment response monitoring, and the study of resistance mechanisms. Recently, CRISPR/Cas13a systems have received a lot of attention for their high sensitivity and specificity in molecular diagnostics [3], and the HiCASE technique presented in the paper brings new ideas to this field.

The HiCASE technique described in the paper is a significant technological advance that significantly improves the detection sensitivity of EGFR mutations in circulating tumor DNA (ctDNA) by combining the CRISPR/Cas13a system, PCR amplification, and restriction enzyme (RE) digestion (Figure1).

- PCR amplification: First, ctDNA extracted from a plasma sample is used as a template for PCR amplification through a specific primer to increase the amount of target DNA. These primers are designed to specifically amplify regions of DNA that contain mutations of interest.
- Restriction enzyme digestion: PCR products are then treated with restriction enzymes. Restriction enzymes are enzymes that recognize a specific DNA sequence and cut DNA at that sequence. In HiCASE, if the target sequence contains a specific restriction enzyme recognition site, the enzyme specifically digests wild-type DNA fragments, leaving those containing mutations undigested. This method helps to reduce background noise and improve the specificity of mutation detection.
- CRISPR/Cas13a system: After digestion by restriction enzymes, undigested DNA (i.e. DNA containing mutations) is used as a target of the CRISPR/Cas13a system. Cas13a is a CRISPR-related protein that is activated and cuts the target DNA when it binds to a specific guide RNA (crRNA) and recognizes the target DNA sequence. In HiCASE, Cas13a binds to a designed crRNA whose sequence is complementary to the target mutation sequence.
- Signal generation: When Cas13a is activated and cuts the target DNA, it also non-specifically cuts the single-stranded RNA reporter molecules added to the reaction. These reporter molecules are labeled with fluorescent labels, and when they are cut, the fluorescence signal changes so that it can be detected by fluorescence detection instruments. The intensity of

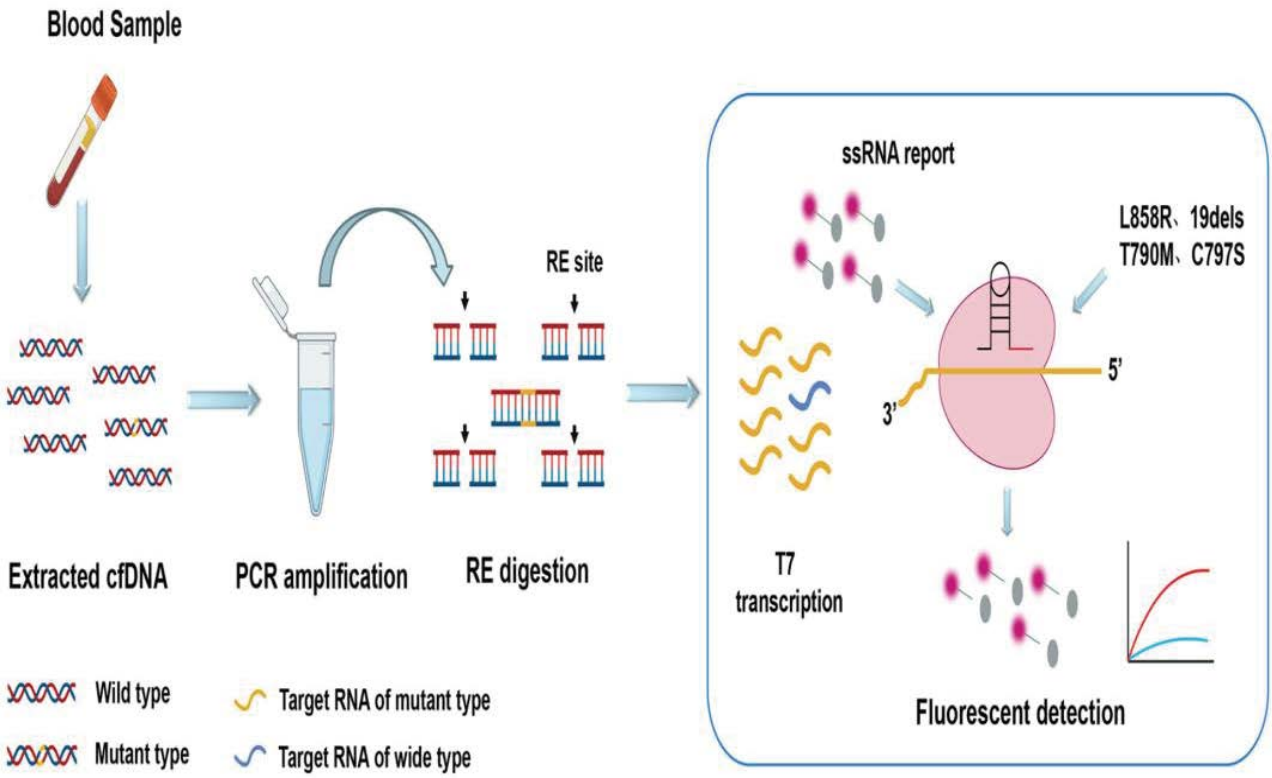

Figure1: Schematic illustration of the HiCASE assay for the detection of cfDNA sample.

the fluorescence signal is proportional to the amount of mutated DNA in the sample, so it can be used to quantify the frequency of mutations.

- Result analysis: By comparing the fluorescence signal of the experimental sample and the control sample, it can be determined whether there is a specific mutation in the sample, and the frequency of mutation can be estimated according to the strength of the signal.

- This method is able to detect mutated allele frequencies (VAF) as low as 0.01%, which is particularly important for early cancer detection and treatment monitoring. In addition, HiCASE technology shows greater sensitivity and specificity compared to traditional digital PCR (ddPCR) and Super-ARMS assays4, which may become a powerful tool in the field of liquid biopsy in the future.
- Increased sensitivity: HiCASE technology can detect mutated allele frequencies as low as 0.01%, which is important for early cancer detection and monitoring of minimal residual disease [5]. For example, HiCASE technology successfully detected low-frequency EGFR T790M/C797S mutations in plasma samples from NSCLC patients, which is of great value for guiding clinical administration and predicting drug response.
- Clinical application potential: The application of HiCASE technology in clinical samples has shown high sensitivity and specificity, which has been difficult to achieve in previous technologies. The advantages of HiCASE in detecting EGFR mutations in plasma samples were demonstrated by comparing HiCASE, ddPCR and Super-ARMS detection methods.
- Simplicity and cost-effectiveness: Although HiCASE technology is relatively complex to operate, it requires a small volume of plasma, which has clear advantages in clinical practice. In addition, HiCASE technology has the potential to reduce overall inspection costs, which is particularly important in resource-limited environments.

The application of HiCASE technology in clinical samples shows great potential. In plasma samples from patients with NSCLC, HiCASE is able to detect EGFR mutations with high sensitivity and specificity, which is essential for personalized medicine and precision therapy. Especially for the detection of EGFR T790M/C797S mutations, HiCASE can distinguish

mutations at different locations, which is of great significance for guiding clinical administration and predicting drug response. In addition, HiCASE technology requires a smaller volume of plasma, which makes it more feasible in clinical practice, especially in resource-limited Settings.

Although HiCASE technology shows great potential, there are still some challenges and room for improvement. First, although HiCASE improves sensitivity, its steps are relatively complex, which may affect its implementation in clinical laboratories. Future research could explore ways to streamline operational processes to improve the utility and accessibility of the technology. Second, while HiCASE performed well in samples of patients with NSCLC, its performance in other types of cancer has not been fully validated. Future studies should evaluate the performance of HiCASE in a wider range of cancer types and patient populations. Finally, the cost-benefit ratio of HiCASE technology is also an important consideration, especially in resource-limited environments. Future studies should evaluate the economics of HiCASE technology and explore strategies to reduce costs.

In summary, HiCASE technology shows significant potential in improving the sensitivity and specificity of ctDNA mutation detection, providing a new tool for the management of NSCLC. However, in order to achieve widespread clinical use, further research is needed to optimize the procedure, validate its performance in different cancer types, and evaluate its cost-benefit ratio. Future studies should also explore the potential of HiCASE technology in other clinical applications, such as early cancer screening and treatment response monitoring. Through these efforts, HiCASE technology is expected to become an important tool in cancer diagnosis and treatment management, providing patients with more accurate medical services.

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