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Unveiling Cas12j Trans-Cleavage Activity for CRISPR Diagnostics: Application to miRNA Detection in Lung Cancer Diagnosis

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Abstract

Cas12j, a hypercompact and efficient Cas protein, has potential for use in CRISPR diagnostics, but has not yet been used because the trans-cleavage activity of Cas12j is veiled. Here, the trans-cleavage behavior of Cas12j1, 2, and 3 variants and evaluate their suitability for nucleic acid detection is unveiled. The target preferences and mismatch specificities of the Cas12j variants are precisely investigated and the optimal Cas12j reaction conditions are determined. As a result, the EXP-J assay for miRNA detection by harnessing the robust trans-cleavage activity of Cas12j on short ssDNA is developed. The EXP-J method demonstrates exceptional detection capabilities for miRNAs, proving that Cas12j can be a pivotal component in molecular diagnostics. Furthermore, the translational potential of the EXP-J assay is validated by detecting oncogenic miRNAs in plasma samples from lung cancer patients. This investigation not only elucidates the trans-cleavage characteristics of Cas12j variants, but also advances the Cas12j-based diagnostic toolkit.

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