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Precise Identification of Clustered Regularly Interspaced Short Palindromic Repeats-Induced Mutations via Hidden Markov Model-Based Sequence Alignment

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Abstract : CRISPR genome editing technology has transformed molecular biology by accurately targeting and altering an organism's DNA. Despite the state-of-art precision of CRISPR genome editing, the imprecise mutation outcome and off-target effects present considerable risk, potentially leading to unintended genetic changes. Targeted deep sequencing, combined with bioinformatics sequence alignment, can detect such unwanted mutations. Nevertheless, the classical method, Needleman-Wunsch (NW) algorithm may produce false alignment outcomes, resulting in inaccurate mutation identification. The key to precisely identifying CRISPR-induced mutations lies in determining optimal parameters for the sequence alignment algorithm. Hidden Markov models (HMM) are ideally suited for this task, offering flexibility across CRISPR systems by leveraging forward-backward algorithms for parameter estimation. In this study, we introduce CRISPR-HMM, a statistical software to precisely call CRISPR-induced mutations. We demonstrate that the software significantly improves precision in identifying CRISPR-induced mutations compared to NW-based alignment, thereby enhancing the overall understanding of the CRISPR gene-editing process.

Keywords : CRISPR, HMM, sequence alignment, gene editing
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