Enhancing Base Editing by DNA Gap Induction

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Base editors hold great promise in the treatment of genetic diseases as nearly half of known pathogenic genetic variants are point mutations. However, despite recent progress, the base editors are limited by the low editing efficiency and a narrow editing window. In this study, we present a novel gap-driven base editing system in which paired Cas9 D10A-sgRNAs are designed to induce a gap, not just a single nick, on the DNA target strand, thereby enhancing editing efficiency. Using a dye-labeled probe, we validated the ability of two Cas9 D10A-sgRNAs to generate a gap on the target strand in vitro. In mouse embryonic stem (ES) cells, gap-directed adenine base editors (Gap-ABEs) and gap- directed cytosine base editors (Gap-CBEs) exhibited around 2-fold increase in editing efficiency compared to the best efficiency of single base editors. The gap introduced by two Cas9 D10A could further expand the editing range, potentially offering a broader target region for modifying previously unreachable bases by deaminases. This novel strategy in base editing opens new avenues for further improvement of current base editors and advances base editing technology.

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