Site-Specific Integration of Large DNA Fragments: Evaluating and Redesigning Genome Editing Systems

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Genome editing technologies have revolutionized our ability to integrate foreign DNA sequences into cells with single nucleotide resolution. A major bottleneck of genome editing today is the tradeoff between the size of the integrated fragment and the ability to control where it is integrated. Currently, there are no techniques to reliably integrate sequences greater than few thousand base pairs in precise locations. PiggyBac transposase (PBase) is an enzyme capable of transposing mobile genetic elements of up to 200 kb. However, PBase integrates randomly into the genome, resulting in a lack of specificity. To address this issue, we introduce a novel technology for genomic insertion, achieving site-specific integration of fragments larger than previously possible. First, we established PBase mediated integration as a safe and viable technique by demonstrating no cytotoxicity upon insertion . Then, we fused PBase to a deactivated Cas9 (CRISPR-based programmable DNA-binding) to specifically direct PBase integration. This fusion was successful at targeting and displacing a red fluorescent protein gene with another genetic cassette. We further optimized this system using structure-guided protein engineering to attenuate PBase and reduce off-target insertions. Our versatile platform enables previously impossible high-value genome modifications for the next generation of genomic surgery. We believe the ability to specifically integrate large DNA fragments will have a transformative impact on multiple fields, with applications such as the bioproduction of pharmaceuticals and fuels, correcting mutated genes, creating vaccine libraries, and storing data on DNA.

Awards Won:
Second Award of $2,000