

Biological Design Doctoral Defense

Designing a Method of Prime Induced Nucleotide Engineering Using a Transient Reporter for Editing Enrichment (PINE-TREE)

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Abstract

The advent of CRISPR/Cas9 revolutionized the field of genetic engineering and gave rise to the development of new gene editing tools including prime editing. Prime editing is a versatile gene editing method that mediates precise insertions and deletions and can perform all 12 types of point mutations. In turn, prime editing represents great promise in the design of new gene therapies and disease models where editing was previously not possible using current gene editing techniques. Despite advancements in genome modification technologies, parallel enrichment strategies of edited cells remain lagging behind in development. To this end, this project aimed to enhance prime editing using transient reporter for editing enrichment (TREE) technology to develop a method for the rapid generation of clonal isogenic cell lines for disease modeling. TREE uses an engineered BFP variant that upon a C-to-T conversion will convert to GFP after target modification. Using flow cytometry, this BFP-to-GFP conversion assay enables the isolation of edited cell populations via a fluorescent reporter of editing. Prime induced nucleotide engineering using a transient reporter for editing enrichment (PINE-TREE), pairs prime editing with TREE technology to efficiently enrich for prime edited cells. This investigation revealed PINE-TREE as an efficient editing and enrichment method compared to a conventional reporter of transfection (RoT) enrichment strategy. Here, PINE-TREE exhibited a significant increase in editing efficiencies of single nucleotide conversions, small insertions, and small deletions in multiple human cell types. Additionally, PINE-TREE demonstrated improved clonal cell editing efficiency in human induced pluripotent stem cells (hiPSCs). Most notably, PINE-TREE efficiently generated clonal isogenic hiPSCs harboring a mutation in the APOE gene for in vitro modeling of Alzheimer's Disease. Collectively, results gathered from this study exhibited PINE-TREE as a valuable new tool in genetic engineering to accelerate the generation of clonal isogenic cell lines for applications in developmental biology, disease modeling, and drug screening.



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Zoom Link: <https://asu.zoom.us/j/4574652505>