Skysong Innovations
Advancing the Arizona State University Knowledge Enterprise

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Base-Edited Isogenic hPSC Line Generation (BIG-TREE)

Human pluripotent stem cells (hPSCs) are a vital tool not only in research and disease modeling but also in developmental biology and regenerative medicine because of their ability to self-renew and differentiate. Current genome editing tactics in hPSCs require the incorporation of deleterious double-stranded DNA breaks followed by inefficient homology-directed repair (HDR). These methods can result in DNA insertion or deletion issues, apoptosis, oncogenic mutations and other negative outcomes.

Researchers at Arizona State University have developed a novel method using CRISPR tools to co-target genomic loci and an episomal reporter to enable single-nucleotide genomic changes in hPSCs without HDR. This method, called base-edited isogenic hPSC line generation using a transient reporter for editing enrichment (BIG-TREE), enables the rapid generation of clonal isogenic hPSC lines. It produces single-nucleotide editing efficiencies greater than 80% across multiple hPSC lines and allows for the efficient generation of loss-of-function hPSC lines and efficient multiplex editing of hPSCs at multiple independent loci.

BIG-TREE advances the implementation of base-editing technologies in hPSCs for use in developmental biology, disease modeling, drug screening, cell-based therapies and more.

Potential Applications

- Isogenic cell line engineering
- Generating knockout cell lines
- Developmental biology
- Disease modeling
- Drug screening
- Regenerative medicine

Benefits and Advantages

- Greater than 80% efficiencies across multiple hPSC lines
- Allows for the precise and efficient base editing of hPSCs
- Efficient generation of loss-of-function hPSC lines
- Efficient editing of multiple loci and across several independent hPSC lines
- Bulk enrichment of base-edited cell populations including hPSCs
- Fast and efficient generation of clonal isogenic hPSC lines with homozygous and heterozygous single base pair edits
- Rapid engineering of isogenic hPSC lines

o Establishes in vitro models to assess pathogenic risk/disease mechanisms

- Allows for biallelic/multiplexed targeting without the need for sequential retargeting
- Compatible with off the shelf chemical transfection reagents

o Doesn't require cloning of complex viral constructs/use of specialized cell transfection systems

- Can be used in conjunction with other base editing variants that have altered PAM specificities and editing windows

For more information about this opportunity, please see

Brookhouseer et al - Stem Cell Reports - 2020

For more information about the inventor(s) and their research, please see

Dr. Brafman's departmental webpage

Dr. Wang’s laboratory webpage