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Efficient CRISPR-based gene editing for stem cells

A CRISPR-based gene editing protocol for induced pluripotent stem cells (iPSCs) with improved editing efficiency and cell survival.

IP Status: US Patent Pending / Application No.: 17/811,048

Applications

- CRISPR-based gene editing
- iPSC disease modeling
- Research tool

Key Benefits & Differentiators

- **Improved efficiency:** This protocol enhances the homology-directed repair (HDR) rate by 8-fold, leading to a significant reduction in the number of clones needed for screening.
- **Faster editing timeline:** By reducing the time required for gene editing, the protocol enables completion in as little as 8 weeks from start to finish.
- **Enhanced recombination rates:** A series of optimization steps improves nucleoporation efficiency and cell survival, resulting in higher recombination rates.

Technology Overview

CRISPR-based gene editing has gained popularity because it offers significant advantages in creating specialized cell lines for disease modeling. However, its use in CRISPR gene editing of induced pluripotent stem cells (iPSC) is inefficient resulting in high cell death, low recombination rates, and long recovery times for surviving cells. iPSCs, with their versatility in modeling various biological processes, underscore the urgent need for more efficient CRISPR protocols tailored specifically to this cell type.

Researchers at the University of Minnesota have developed a CRISPR-based editing protocol specifically designed for human iPSCs, resulting in an eight-fold increase in the homology-directed repair (HDR) rate. This advancement significantly reduces the number of clones needed for screening and shortens the gene editing process to just 8 weeks, three times faster than current commercially available solutions. The protocol achieves higher recombination rates through optimization steps that improve nucleoporation and cell survival, involving the inhibition of p53 and optimization of commercially available gene editing reagents. Notably, this protocol does not require specialized equipment, making it easily adoptable by industry and academic labs seeking to create disease-relevant cell lines with knockout or knock-in mutations to test novel therapies or study disease processes.

Phase of Development

TRL: 3-5
Proof of concept demonstrated in human iPSCs cells. A comparison with existing protocols showed a 5-fold increase in efficiency for knockins of single nucleotide changes.

Technology ID

2021-185

Category

Life Sciences/Biologics
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Researchers

- **Shauna Yuan, MD** Associate Professor, Department of Neurology

References

1. Avinash Singh, G. Dalton Smedley, Jamee-Grace Rose, Kristina Fredriksen, Ying Zhang, Ling Li & Shauna H. Yuan(30 April 2024) , https://doi.org/10.1038/s41598-024-60766-4, Scientific Reports