Optimized Retron Editors for Enhanced Genome Engineering in Mammalian Cells

Efficient and scalable method for complex genome editing in mammalian cells that are compatible with lentiviral delivery.

Technology

The investigators have developed an optimized retron system for efficient genome engineering in mammalian cells. Retrons are retroelements that have been adapted into genome editing tools, but remain largely unutilized due to inherit low efficiency in mammalian cells. The team has re-engineered the non-coding RNA (ncRNA) components of the retron system, optimizing steady-state RNA levels using RNA pseudoknot structures, and improving Cas9 activity and genome editing rates via ribonuclease RNA processing strategies. The resulting retron system improves steady state RNA levels as measured by northern blot, which increased editing rates 27-fold when compared to the unoptimized retron editor ncRNA in a flow cytometry-based mutation assay. This system offers significantly enhanced genome editing efficiency and is compatible with viral vector delivery, a critical requirement for scalable forward genetic screens.

Background

Genome engineering is a powerful tool for interrogating the relationship between genotype and phenotype, and holds promise for therapeutic applications. While CRISPR-Cas9 technology has revolutionized genome editing, certain limitations persist, particularly in the context of lentiviral delivery in mammalian cells. Retron systems have recently been adapted for genome editing due to their ability to produce *in situ* single-stranded DNA repair templates. However, their application has been restricted due to relatively low editing efficiency. This innovation addresses this limitation by optimizing the retron system for enhanced genome editing efficiency in mammalian cells.

Development Status

Researchers have demonstrated the delivery of their retron system by either viral vector or plasmid transfection in two distinct cell lines to have improved stability and gene editing efficiency. NYU now seeks a partner to commercialize this technology as a research reagent.

Applications

- Functional genomic screens in mammalian cells
- Therapeutic genome engineering
- Synthetic biology applications

Advantages

Technology ID

POI01-07

Category

Life Sciences/Platform Technology
Life Sciences/Materials/Vectors
Life Sciences/Genetic Engineering
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- **Improved editing efficiency:** The optimized retron system enhances genome editing efficiency in mammalian cells.
- **Compatibility with lentiviral delivery:** The system is compatible with low-copy lentiviral delivery, facilitating scalable forward genetic screens.
- **Broad editing potential:** The system permits efficient introduction of a wide spectrum of mutation types.
- **Enhanced stability:** The re-engineered ncRNA components offer improved RNA stability, contributing to increased editing efficiency.
- **Predictable activity:** The foundational retron biology is well characterized and abides to strict specificity as prescribed by the msDNA template.

Intellectual Property

NYU has filed a provisional patent application covering composition and method of use.

References

1. Matthew A. Cattle, et al. , https://www.biorxiv.org/content/10.1101/2024.08.05.606586v2