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Enhanced mammalian CRISPR editing with separated retron donor and nickases

Researchers at Stanford and the European Molecular Biology Laboratory (EMBL) have discovered an improved embodiment of bacterial retron-based CRISPR gene editing in mammalian cells. They found that encoding the retron-based donor sequence and guide RNA (gRNA) sequence in separate expression cassettes allows for more efficient editing than the previously demonstrated embodiments of single fusion retron-gRNA transcripts. This "split" system incorporates distinct promoter and RNA processing elements in the respective retron and gRNA cassettes, allowing for greater control and functionality of each component in the retron gene editing system.

In addition, because single-stranded nicks in the host DNA dispel unwanted repair events that commonly occur after double-stranded DNA breaks, the researchers coupled the above split system with the use of a Cas9 nickase rather than a full nuclease. Limiting off-target editing effects in this way is a favorable strategy especially for mammalian editing/therapeutic applications. Strikingly, the researchers found a 2.5-fold increase in editing efficiency with the nickase over the full nuclease, indicating that deleterious impacts of double-strand breaks limit editing efficiency.

This improvement on the novel retron-based gene editing system provides greater editing efficiency and greater stability of the individual editing components, which can be useful for gene therapy, research and industrial applications.

Stage of Development
Proof of concept

Applications

- Genome editing by homology-directed repair in human cell lines for therapeutic applications
- Lineage tracing studies in mammalian systems
- Identifying causal genetic variants in multiplexed screens
- Engineering organisms, pathways, and proteins for industrial, agricultural, and medical applications

Advantages

- Less prone to unwanted indels and off-target edits
- Highly efficient and flexible by separately tuning the retron and guide RNA elements of the editing system

Patents

- Published Application: WO2024044736

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