Docket #: S22-339

Recruitment of donor DNA from in vivo assembled plasmids for genome editing screens

Researchers at Stanford previously described a method under Stanford Docket S17-020 for introducing a large number of gene edits in parallel, termed Multiplexed Accurate Genome Editing with Short, Trackable, Integrated Cellular barcodes (MAGESTIC). MAGESTIC enables thousands of edits to be introduced into a cell population whereby each cell receives a single distinct edit along with a barcode for tracking cell identity and abundance in pooled assays. Now, researchers at Stanford disclose a new enhanced system, titled MAGESTIC 3.0. This new invention is characterized by three orthogonal enhancements to the homology-directed repair (HDR) mechanism for high efficiency gene editing: 1) donor DNA recruitment with the FHA domain of the yeast Forkhead family transcription factor Fkh1p; 2) single-stranded donor DNA synthesis with the bacterial retron system; and 3) in vivo assembly of linearized donor plasmids. Each enhancement functions at different stages in the editing process. Combined into a single system, MAGESTIC 3.0 improves editing efficiency to the highest overall levels of any CRISPR gene editing system described to date.

Stage of Development
Proof-of-concept, plus validation in large-scale workflows

Applications

- Highly parallelized multi-site genome-wide editing for basic research and industry
- High-throughput functional genomics studies on variants in genes, pathways, and entire genomes

- Engineering industrial yeast strains with desirable traits for improved production of high volume or high value chemicals and biologics

Advantages

- Unparalleled rates of editing efficiency and accuracy by integrating three orthogonal mechanisms for donor DNA enhancement
- Improved editing survival rates following transformation
- Each transformant receives a unique barcode, yielding many internal replicates for each variant

Publications

- Roy et al., "Dissecting quantitative traits with saturation genome editing," *in preparation*.

Patents

- Published Application: WO2024044767

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