

# **Generation and tracking of cells with precise edits**

Current methods of genetically-engineered cells require precise integration of genetic material. Genotyping is usually used to detect genetic edits; however, it's difficult to track the exact changes made in each individual cell in a large cellular mixture, especially when the cells receive multiple edits. While whole genome genotyping is available to track each cell in a mixture, the technique is expensive and time-consuming. Thus, there is a need for additional methodologies that enables quicker and cost-efficient tracking of genetic edits within cells of interest.

The Fraser lab at Stanford has invented a genetic engineering platform that enables multiple cellular gene editing while tracking the exact edits being made. The invention consists of two or more CRISPEY modules covalently produced by the vector, where the combination of edits that will be made across all modules are predetermined. Each module applies edits of interest tagged with unique sequences that can be easily genotyped by DNA sequencing or other methods. When needed, the intended combination of precise edits for each can also be inferred by Sanger sequencing, NGS, or other detection methods. Unlike existing methods, the invention can be applied to a mixture of cells as well as single cell or clonal cell lineages, and provides an efficient, high-throughput, and low-cost method for genetic and cellular engineering.

## **Applications**

- Tracking relative abundance of cells targeted by a mixture of edit in parallel, such as high-throughput precision editing genetic screens to:
  - improve industrial microbial growth
  - select strains for improving crop yield
  - track edited cell populations treated by genetic editing medicine
  - track edited cell populations used in-cell therapy

## Advantages

- High-throughput, cost-effective method compared to existing methods
- Does not require integrating of vector into the genome
- Detection of the first edit (label) that installs the unique sequencing indicates that the edit tool is functional, and higher likelihood of the rest of the cells containing the second edit that is intended, important for isolating edited cells of interest.
- Edits made to the same target cell iteratively can be tracked by reading past unique barcodes installed into the genetic material
- The barcode label can also be associated with antibiotic markers or other markers that allow cell purification

## Patents

- Published Application: [WO2023225358](#)

## Innovators

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## Licensing Contact

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