

Variant Flow-FISH Technology to Modulate Gene Expression Using Genome Editing

Stanford researchers have developed a new technology, Variant-FlowFISH, to enable high-throughput, highly sensitive measurements of how variants, introduced via CRISPR, affect gene expression.

It is challenging to therapeutically modulate gene expression only in specific cell types to treat disease. One solution would be to reprogram the gene regulatory sequences in the genome, which control gene expression in cell-type specific ways. Stanford researchers have developed a new technology, called Variant Flow-FISH, that efficiently edits the human genome as desired and is also able to measure the effects of the variants on gene expression. The technology involves three steps: i) pools of edits are introduced into the human genome using CRISPR prime editing, ii) cells are sorted via FACS into 6 bins based on gene expression levels using RNA FlowFISH, iii) DNA sequencing is performed to determine the frequency of edits across the 6 bins and determine the effect of the variants on gene expression.

Stanford inventors have successfully applied the technology to identify specific synthetic sequences that can be introduced into a non-coding regulatory sequence at the *PPIF* locus, to change expression of the *PPIF* gene in monocytes, a gene linked to inflammatory bowel disease. Variant Flow-FISH was used to introduce hundreds of noncoding variants into cells in a single experiment and measure each of the variants' effects on the expression of the *PPIF* gene.

a Variant-FlowFISH

Introduce variants at an enhancer

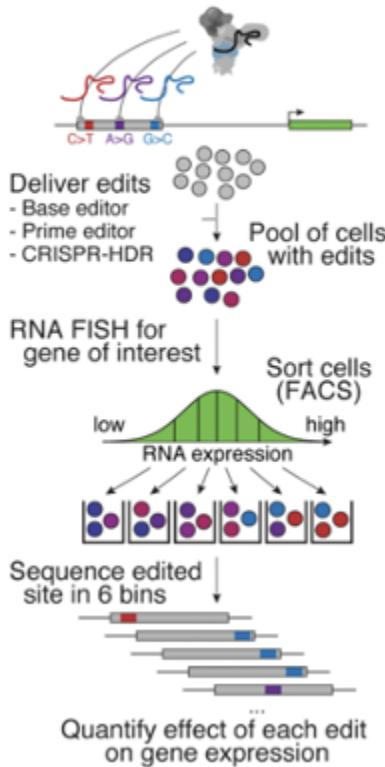


Figure Description: Schematic of Variant-FlowFISH. Edits are introduced into a pool of cells (e.g. via prime-editing), which are then stained for an RNA of interest and sorted into expression bins. The edited sites are sequenced, and the distribution of variant frequencies across the 6 bins, allows us to infer the effect of each variant. (Image credit: inventors)

Stage of Development

Proof of concept - in vitro data

Applications

- Characterizing disease variants in their endogenous genomic context
 - Identify gene sequence changes to tune gene expression
- Treatment of inflammatory bowel disease
 - Decrease PPIF gene expression in monocytes
- Therapeutics for Coronary Artery Disease

Advantages

- Avoids deriving single-cell clonal populations
- Quantitative
- Detects small effects on gene expression (5-10%)
- Fast: ~3-4 weeks for editing and reading out effects on the gene expression
- Overcomes inherent inefficiencies/inaccuracies of CRISPR: inefficient editing or multiple indels
 - Variant Flow-FISH measures the effect of each allele through pooled sequencing and analysis of allele frequencies in a population of cells

Patents

- Published Application: [WO2024064761](#)

Innovators

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