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Enhanced Diversifying Base Editors for Directed Evolution in S. cerevisiae

Current directed evolution techniques for yeast can be slow and inconvenient

S. cerevisiae is commonly used to screen protein variants to interrogate and improve their structure and performance. While there are many techniques to carry out directed evolution in yeast, there is still a great need to improve their speed and ease of use. This invention presents an optimized and integrated CRISPR diversifying base editor for use in yeast and demonstrate its ability to rapidly improve the affinity of an antibody through yeast display. We enhanced the base editor mutation rate up to 27-fold by characterizing an improved key variant and by optimizing the structure of the CRISPR guide RNAs, attaining a rate of in situ mutations of 1x10-4 mutations/bp/generation, roughly 10-fold higher than the previously reported highest rate of in situ mutations. The diversifying base editor system uses a human enzyme.

An optimized and integrated CRISPR diversifying base editor rapidly improves antibody affinity

This invention is comprised of an optimized and integrated CRISPR diversifying base editor for use in yeast and demonstrate its ability to rapidly improve the affinity of an antibody through yeast display. Its potential uses include enabling studies involving direct evolution via mutagenesis, engineering yeast to mimic human antibody response in immune cells and in the development of novel protein therapeutics and antibodies.

Summary Bullets

- This invention presents an optimized and integrated CRISPR diversifying base editor for use in yeast and demonstrate its ability to rapidly improve the affinity of an antibody through yeast display.
- The innovation has enhanced the base editor mutation rate up has been increased to 27-fold by characterizing an improved key variant and by optimizing the structure of the CRISPR guide RNAs.
- The technology has attained a rate roughly 10-fold higher than state-of-art systems and facilitates ultrarapid antibody diversification.

Solution Advantages

- Enhanced diversification and directed evolution.
- In-situ mutation rate 10X higher than state-of-art systems
- Facilitates ultra-rapid antibody diversification

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IP Status

The patent application has been filed:

Publications

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Images

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