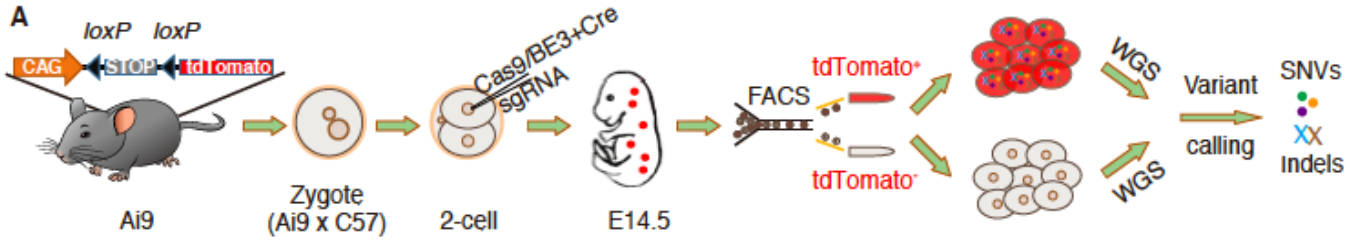
Docket #: S19-079

GOTI- method to identify off target mutations caused by gene editors

Researchers at Stanford and their colleagues have developed a method to detect off-target mutations caused by gene editing tools. Genome editing, by CRISPR-Cas9, cytosine base editor 3 (BE3) or adenine base editor 7.10 (ABE7.10), holds great promise for treating diseases caused by pathogenic mutations. However, these methods can have off-target, undesired effects. Thus, a comprehensive analysis of off-target effects is needed. Methods have been developed to detect genome-wide gene editing of off-target sites, but these approaches are limited as they cannot detect single-nucleotide variants (SNVs) in vivo. To help overcome this limitation, the inventors have developed the GOTI (genome-wide off-target analysis by two-cell embryo injection) method. GOTI can be used to evaluate the off-target effects and identify SNVs caused by a variety of gene editors. Further, use of the GOTI method enabled the inventors to develop and provide an improved version of the BE3 editor which performs clean on-target edits without off-target effects. This technology can be used to evaluate and reduce the off-target effects of gene editing tools thereby increasing their potential for use in correcting pathogenic mutations.



Schematic of GOTI method. It can detect off-target mutations by editing one blastomere of two-cell mouse embryos using CRISPR-Cas9, BE3 or ABE7.10-mediated gene editing.

Stage of research
Using GOTI, the inventors found that BE3 caused SNVs at off-target sites with frequencies more than 20-fold higher than the spontaneous mutation rate.

Applications

- Gene editing research tool- method to detect off-target mutations

Advantages

- Can be used to examine the off-target effects of a variety of gene editing tools
- Potential to improve the fidelity of base editing enzymes
- GOTI examines cell populations derived from one gene edited blastomere
- Maintains signal to allow random off-target effects to be detected

Publications

- Erwie Zuo, Yidi Sun, Wu Wei, Tanglong Yuan, Wenqin Ying, Hao Sun, Liyun Yuan, Lars M. Steinmetz, Yixue Li, Hui Yang, Cytosine base editor generates substantial off-target single nucleotide variants in mouse embryos," Science, 19 Apr 2019: Vol. 364, Issue 6437, pp. 289-292 (published online Feb 28, 2019).

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