ADAPTATION OF CROFT-SEQ FOR OFF-TARGET DETECTION OF VARIOUS GENOME EDITING TOOLS

Paulius Toliušis[1], Gunda Petraitytė[2], Algirdas Grybauskas[1], Mindaugas Zaremba[1]

[1] Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
[2] Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Lithuania
paulius.toliusis@bti.vu.lt

Programmable genome editing nucleases, such as CRISPR-Cas9, have attracted significant scientific interest due to their immense potential for gene editing and the treatment of human genetic diseases. The successful development of these tools is crucial for both fundamental and clinical applications to ensure that genome editing tools are reliable and safe. Because of their ability to easily target specific DNA sites, these nucleases enable precise and rapid genome editing. Although these nucleases are designed to cut their intended target site (on-target), they often tend to also cut DNA sequences similar to the target site (off-targets). This can lead to harmful gene mutations or chromosomal rearrangements, potentially causing cancerous transformations or even cell death. To address these issues, a variety of methods have been developed to detect off-target sites of Cas9 nucleases both in vitro and in vivo [1]. However, the vast majority of these methods are specifically designed to detect double-strand DNA breaks caused by genome editing nucleases like CRISPR-Cas9, which leave "blunt" DNA ends after cutting. Only a few methods have been developed and adapted for other genome editing tools (non-Cas9), such as Cas12 or base editors, for detecting off-targets in vitro [2, 3]. However, these methods are often expensive, complex and have relatively low sensitivity. Therefore, the ability to detect off-target sequences of various genome editing tools would enable the improvement and selection of more suitable and safer tools for genome editing.

Recently, we have developed a sensitive, simple, fast, and cost-effective CRISPR-Cas9 off-target detection method called CROFT-Seq [4], which, in many aspects, rivals or even outperforms other well-known in vitro off-target detection methods, such as SITE-Seq [5] and CIRCLE-Seq [6]. In this research, we will aim to employ and modify existing CROFT-Seq method by incorporating additional enzymes and experimental stages for detecting off-target sites of various genome editing tools, such as Cas12 and base editors. Additionally, we will modify the bioinformatics algorithm of the CROFT-Seq off-target site analysis to make it suitable for identifying off-target sequences of different genome editing tools. Furthermore, we will perform genome editing of HEK293T cells in vivo and validate wether potential off-target sites identified in vitro are also cleaved during genome editing in living cells. Comparing in vitro off-target sequences with those validated in vivo will allow us to draw significant conclusions regarding the accuracy and sensitivity of the developed methods. With the advancement of the robotics industry, there is a growing demand for adapting off-target detection methods to robotic systems. Therefore, as part of this project, we will also aim to adapt the modified CROFT-Seq methods for use with the Opentrons OT-2 robotic system.

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