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Removing the Barrier between Cellular-Based and Biochemical Methods

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Commentary

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ABOUT THE STUDY

The latest version of the USFDA guidance document for Human Gene Therapy Products Incorporating Human Genome Editing recommends the use of multiple orthogonal methods, such as *in silico*, biochemical, and cellular-based assays, that include an unbiased genome-wide analysis to identify potential off-target sites [1]. According to the most recent report on the use of three orthogonal methods by Intellia, the predictions made by each method were not as orthogonal as expected; the biochemical method identified seven validated off-target loci, which included all the validated off-target loci predicted by the cellular-based method (missing four validated off-target loci with a false negative ratio of 57%) and the *in silico* method (missing four validated off-target loci with a false negative ratio of 57%)[2]. It appears that the biochemical method that does not miss any candidate off-target loci is sufficient. However, the true positive rate of the biochemical method was 1.5%, as only seven loci were validated out of 475 candidate off-target loci. As a result, there is still an unmet need for the development of a genome-wide off-target prediction method with a low false negative rate and a high true positive rate.

For example, NIST initiated a genome editing consortium with more than 40 members from big pharma and biotech, which develop standardized off-target prediction tools [3]. The high false negative ratio of the cellular-based method compared to the biochemical method originates from DNA repair mechanism of the living cells, as the Double-Strand Break (DSB) made by CRISPR-Cas9 is quickly repaired in the living cells before the probe DNA sequence has any chance to be incorporated. In the biochemical assays, DNA repair mechanism no longer exists which results in a low false negative ratio. However, other cellular components that chromatins are also absent in the biochemical method; that too many false-positive loci are predicted, resulting in a low true positive rate.

Therefore, a method with a low false-negative ratio and a high true positive rate could be developed if one could selectively switch off the DNA repair mechanism while retaining other cellular components.

The latest paper by Kwon, et al. successfully addressed the issue by tearing down the boundary between the cellular-based and the biochemical methods [4]. In the paper, a new protocol named 'Extru-seq' was developed by which the cells are mixed with Cas9 RNP and passed through pores smaller than the size of the cells. Since the cell membrane and nucleus are disrupted and the content of the cytosol is exchanged with the surrounding buffer, all the chemical reactions including the DNA repair mechanism would cease instantly, which is also proved experimentally by the authors. On the other hand, other cellular components that chromatin would still exist since no detergents were added during the process which could loosen the interaction between chromatin and DNA. Therefore, cells that are treated by an Extru-seq process would exist in superposition of live cell and biochemical states, similar to Schrodinger's cat analogy. Indeed, the prediction made by Extru-seq showed high p-values only with the cell-based method GUIDE-seq, when normalized rank sum test was performed. Finally, Extru-seq showed a 2-fold higher true-positive rate compared to the biochemical method Digenome-seq, a 13-fold lower false-positive rate compared to GUIDE-seq, and the highest area under receiver operating characteristic curve among all the cell-based, biochemical, in silico methods compared. All in all, these results suggest that the Extru-seq protocol, which selectively removes chemical processes like a DNA repair mechanism while leaving other cellular components intact, showed improved predictive ability compared to other prediction methods.

Muromonab-CD3 was the first monoclonal antibody drug approved by the FDA in 1986 for preventing transplant rejection [5]. Since then, the FDA has approved more than 100 monoclonal antibody products for various diseases5. This year, the first CRISPR-based therapeutics, CTX001, will likely reach the market [6]. Since it is much easier to screen CRISPR-based drugs with high efficacy compared to antibody-based drugs, it is anticipated that more than 100 CRISPR-based drugs will likely be approved by the FDA within next 40 years. Extru-seq may not be the final protocol and improved versions will likely be developed. However, the selective removal of the DNA repair mechanism, whilst leaving other cellular components intact, would surely be a key strategy to improve the performance of the genome-wide off-target prediction methods that will survive the years of challenges to come.

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