

## SCI-121 – Medical Science For AI Engineering CRISPR Application

**Name: Thanawin Pattanaphol**

**ID: 01324096**

In the paper “Search-and-replace genome editing without double-strand breaks or donor DNA”, authors Andrew V. Anzalone, Peyton B. Randolph, Jessie R. Davis, Alexander A. Sousa, Luke W. Koblan, Jonathan M. Levy, Peter J. Chen, Christopher Wilson, Gregory A. Newby, Aditya Raguram, and David R. Liu (et al.) introduced a new genome-editing tool.

The paper discusses a central problem in genome editing that many disease-causing genetic variants; including point mutations, small insertions, or deletions are difficult to correct precisely and efficiently with existing methods. Conventional CRISPR Cas9 editing typically relies on making double-strand breaks (DSBs) and providing an external donor DNA template for repair, however, this often leads to unintended insertions or deletions (indels), chromosomal rearrangements, or low efficiency in many clinically relevant cell types.

To overcome these limitations, the authors developed “Prime editing”, in which, a “search-and-replace” strategy that can precisely write new genetic information into a targeted DNA site without generating DSBs or requiring a donor DNA template. In practice, they constructed a fusion protein of a catalytically impaired Cas9 (a nickase that cuts only one strand of DNA) and an engineered reverse transcriptase (RT). Instead of a standard guide RNA, they used a specially designed prime editing guide RNA (pegRNA), which both directs the Cas9 nickase to the target site and encodes the desired edited sequence. When the nickase creates a single-strand break, the reverse transcriptase copies the new genetic sequence from the pegRNA onto the DNA at that position; the cell then repairs and incorporates this change.

Using this system, the researchers managed to carry out over 175 different edits in human cells including all 12 possible base-to-base conversions, small insertions, and deletions demonstrating remarkable versatility. They also corrected the mutation in the human  $\beta$ -globin gene (HBB) that causes the most common form of sickle-cell disease, and removed a pathogenic four-base duplication in the HEXA gene (implicated in a form of neurodegenerative disorder) along with introducing a protective variant in the PRNP gene, and demonstrated the insertion of precise tags at endogenous loci.

Prime editing proved to offer significant advantages over both traditional DSB-based editing (homology-directed repair) and earlier base-editing methods. Due to it avoiding DSBs, it reduces the risk of unintended indels or large genomic rearrangements along with how there is no external donor DNA is required meaning delivery into cells is simplified. Its capacity to introduce many kinds of edits including substitutions, insertions, deletions makes it far more flexible than base editors, which are limited to certain base conversions.

In my opinion, the development of prime editing is a major leap forward for genome engineering essentially transforming CRISPR from a blunt “cut-and-repair” tool into a precise “search-and-replace” editor. This improved precision and flexibility has a huge probability for correcting disease-causing mutations in a safe and efficient way, especially those involving subtle base changes or small indels that were previously hard to correct. As delivery methods improve and off-target effects are further characterized, prime editing could become a foundation for future gene therapies that treat a wide range of genetic disorders.

**SCI-121 – Medical Science For AI Engineering**  
**CRISPR Application**

**Name: Thanawin Pattanaphol**

**ID: 01324096**

## **References**

Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., Chen, P. J., Wilson, C., Newby, G. A., Raguram, A., & Liu, D. R. (2019). *Search-and-replace genome editing without double-strand breaks or donor DNA*. *Nature*.