Prime editing in zebrafish: a precise path to gene modification?

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CRISPR/Cas9 has become a staple in genetic research, but its reliance on stochastic integration of insertions and deletions often compromises precision. The field has long sought a method for programmed, exact genomic alterations, particularly for modeling human disease in animal systems.

A recent study published in eLife explored optimised prime editing (PE) in zebrafish, demonstrating its capacity for precise DNA insertion and substitution without exogenous donor DNA. This work offers a potential solution to the long-standing challenge of precise genome manipulation in animal models, a critical step for understanding gene function and developing new therapies.

KEY TAKEAWAYS

- The Pivot: Prime editing offers a more precise alternative to traditional CRISPR/Cas9 for targeted DNA insertions and substitutions in zebrafish.
- The Data: Nuclease-based PEn achieved up to 27.3% precise insertion, while nickase-based PE2 mediated a higher ratio of precise prime edits to total edits.
- The Action: Clinicians and researchers should consider prime editing for applications requiring highly precise genomic modifications in animal models, particularly when avoiding random indels is paramount.

Traditional CRISPR/Cas9 genome editing, while revolutionary, presents inherent limitations for precise genetic engineering. The system often generates random insertions and deletions (indels) at the target site, making it challenging to introduce specific, desired DNA modifications. This imprecision complicates the creation of accurate disease models and the study of gene function, particularly when subtle genetic changes are critical to phenotype. The need for a more controlled and predictable method for genomic manipulation has been a significant hurdle in the advancement of gene therapy and functional genomics.1

Y. Ono and colleagues at the University of Cambridge addressed this challenge by investigating prime editing, an advanced CRISPR/Cas9 technology, in zebrafish.1 Prime editors (PEs) consist of a Cas9 nickase or nuclease fused to a reverse transcriptase, guided by a prime editing guide RNA (pegRNA) that specifies the target DNA sequence and encodes the desired edit. This system allows for direct copying of new genetic information into the target site, theoretically bypassing the need for double-strand breaks and exogenous donor DNA, which are common sources of off-target effects and random indels in conventional CRISPR. The investigators aimed to optimise PE for precise DNA insertion and substitution at various loci within the zebrafish genome, a model organism widely used for developmental biology and disease modeling due to its genetic tractability and rapid external development.1

The Mechanics of Precision Editing

The study specifically evaluated two types of prime editors: nickase-based PE2 and nuclease-based PEn. PE2 employs a Cas9 nickase, which creates a single-strand break, while PEn uses a Cas9 nuclease, which generates a double-strand break. The choice between a nickase and a nuclease can influence editing efficiency and the ratio of precise edits to total edits. The researchers designed pegRNAs to target specific loci in the zebrafish genome, including regions within reporter transgenes and endogenous genes. They delivered the PE components, including mRNA encoding the Cas9-reverse transcriptase fusion and the pegRNA, via microinjection into one-cell stage zebrafish embryos. This direct delivery method ensures broad distribution of the editing machinery during early development, maximising the chance of germline transmission.1

Ono and colleagues first tested the ability of PE2 to introduce short DNA substitutions. They targeted a specific site in a reporter transgene, aiming to change a few base pairs. The nickase-based PE2 mediated a higher ratio of precise prime edits to the total edits compared to traditional CRISPR/Cas9 methods. This indicates that while the overall editing efficiency might vary, the edits that did occur were more likely to be the intended, precise modifications rather than random indels. This is a critical distinction for applications requiring exact genetic alterations, such as correcting point mutations associated with genetic diseases.1

But, the overall efficiency of PE2 for short DNA modifications was not always optimal for all target sites. This variability in efficiency is a known challenge in genome editing and often depends on factors such as chromatin accessibility, pegRNA design, and the specific sequence context of the target locus. The researchers systematically varied pegRNA designs, including the length of the primer binding site (PBS) and the reverse transcriptase template (RTT), to identify optimal parameters for different types of edits. They found that longer RTTs generally improved the efficiency of insertions, while PBS length needed careful optimisation to balance specificity and activity.1

Comparing Nickase and Nuclease Approaches

The investigators then explored the nuclease-based PEn system, which uses a Cas9 nuclease to create a double-strand break, theoretically enhancing the accessibility of the target site for reverse transcription. PEn proved more efficient for short DNA modifications, achieving up to 27.3% precise insertion at certain loci. This efficiency is a significant improvement over many previous prime editing attempts in animal models and approaches the levels seen with conventional CRISPR/Cas9 for indel generation, but with the added benefit of precision. The higher efficiency of PEn likely stems from the more disruptive nature of the double-strand break, which may facilitate the annealing of the pegRNA and subsequent reverse transcription.1

To further validate their approach, the team inserted a nuclear localisation signal (NLS) into a reporter transgene. This modification involved incorporating a longer DNA fragment, a more challenging task for genome editing technologies. The successful insertion of the NLS sequence demonstrated the capability of PE-mediated prime editing to incorporate fragments longer than simple base substitutions, expanding its utility for more complex genetic engineering tasks. The NLS insertion was confirmed by sequencing and functional assays, showing that the modified reporter protein correctly localised to the nucleus. This experiment highlights the potential of prime editing for creating functional knock-ins, where specific protein domains or regulatory elements are added to existing genes.1

A critical aspect of any genome editing technology intended for heritable modifications is germline transmission. Ono and colleagues confirmed that these gene modifications were transmitted to the next generation. They bred the edited

zebrafish and genotyped their offspring, demonstrating that the precise prime edits were stably integrated into the germline and passed on to subsequent generations. This finding is crucial for establishing stable transgenic lines for research purposes and for potential therapeutic applications where heritable correction of genetic defects is desired. The ability to generate stable lines without the mosaicism often associated with early embryonic editing is a major advantage.1

“PE-mediated prime editing can efficiently manipulate genome information in zebrafish without using exogenous donor DNA.”Y. Ono, M. Peterka, M. LoveThe study also addressed the issue of off-target editing, a perennial concern with all CRISPR-based technologies. While prime editing is generally considered to have a lower off-target rate than traditional CRISPR/Cas9 due to its reliance on both a nickase and a reverse transcriptase, the researchers performed computational predictions and targeted sequencing of potential off-target sites. They found minimal evidence of off-target activity at the sites examined, suggesting a relatively high specificity for the optimised pegRNAs and PE systems used. This low off-target rate is essential for clinical translation and for ensuring the integrity of genetic models.1

Still, the efficiency varied significantly depending on the target locus and the specific pegRNA design. Some sites showed high editing rates, while others proved more refractory to modification. This variability underscores the need for continued optimisation of pegRNA design rules and delivery methods to achieve consistent, high-efficiency editing across diverse genomic contexts. The current study focused on relatively short insertions and substitutions; the efficiency of prime editing for larger insertions or more complex genomic rearrangements remains an area for further investigation. The Oxford Handbook of Genetics provides a concise overview of these evolving technologies.

The study's reliance on microinjection into one-cell stage embryos, while effective for zebrafish, may not be directly transferable to all animal models or human therapeutic applications. Other delivery methods, such as viral vectors or electroporation, might be necessary for different systems, each with its own set of challenges regarding efficiency, specificity, and immunogenicity. The long-term stability and potential immunogenicity of the Cas9-reverse transcriptase fusion protein in a living organism also warrant further investigation, particularly for therapeutic applications where sustained expression might be required.1

The work by Ono and colleagues provides a clear demonstration that PE-mediated prime editing can efficiently manipulate genome information in zebrafish without using exogenous donor DNA. This eliminates a major hurdle associated with traditional homology-directed repair (HDR) based methods, which often require the introduction of a DNA template that can be difficult to deliver and integrate efficiently. The ability to perform precise insertions and substitutions with high fidelity and germline transmission opens new avenues for creating sophisticated genetic models of human disease, enabling researchers to study the precise effects of specific mutations on gene function and organismal phenotype.1

Where it falls short

The study was limited to zebrafish, and while a powerful model, the direct translation of these efficiencies and specificities to mammalian systems, including humans, is not guaranteed. Mammalian genomes are more complex, and factors like chromatin structure and DNA repair pathways can significantly influence editing outcomes. The efficiency rates, while impressive for precise insertions, still mean that a substantial proportion of cells may not undergo the desired edit, which could be a concern for therapeutic applications requiring widespread correction. Further research is needed to refine prime editing protocols for higher efficiency and broader applicability across different species and cell types.1

CLINICAL IMPLICATIONS

The ability to precisely insert and substitute DNA sequences without relying on exogenous donor DNA represents a significant step forward for genetic engineering. For researchers developing disease models, this means more accurate representations of human genetic conditions, free from the confounding effects of random indels. This precision is paramount when studying subtle genetic variants that drive complex disease phenotypes.

The higher ratio of precise edits with nickase-based PE2, even if overall efficiency is lower, offers a compelling trade-off for applications where fidelity is more critical than sheer volume of edits. Conversely, the efficiency of nuclease-based PEn for short insertions could accelerate the creation of knock-in models. Clinicians will eventually benefit from the improved understanding of disease mechanisms that these refined models enable, potentially leading to more targeted therapeutic strategies.

But, the variability in editing efficiency across different genomic loci and the current reliance on microinjection in early embryos remain practical hurdles. Translating these techniques to clinical settings will require robust and efficient delivery methods applicable to somatic cells, along with further optimisation to ensure consistent, high-level editing in diverse tissues. The long-term safety profile, including potential off-target effects and immunogenicity in humans, also requires extensive investigation before any therapeutic application can be considered.

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REFERENCES

1. 1. Ono Y, Peterka M, Love M. Optimised genome editing for precise DNA insertion and substitution using prime editors in zebrafish. eLife. 2026;107475. doi:10.7554/eLife.107475

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