AI-Enhanced Optimization of OMNI™ Nucleases Expands the CRISPR Toolbox for Therapeutic Applications

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emendobio

We are open to collaboration on our OMNI™ nuclease panel and editing capabilities
For further information please contact us

ABSTRACT

Clustered regularly interspaced short palindromic repeats (CRISPR)-based genome editing has revolutionized molecular biology and therapeutics development, yet current nucleases face constraints that limit their broader clinical and biotechnological use. Challenges include narrow PAM recognition coverage, suboptimal catalytic activity in mammalian systems, limited protein stability and expression, and off-target activity that compromise safety. Overcoming these limitations requires an efficient, robust and versatile platform capable to optimize nucleases performance across multiple dimensions.

EmendoBio's OMNI portfolio comprises a collection of novel nucleases with high activity and specificity, capable of recognizing a wide range of PAM sequences and enabling gene editing in regions previously inaccessible to conventional systems. While this portfolio addresses many diverse editing needs, an even broader range of nuclease characteristics is still needed to fully meet all potential applications for CRISPR gene editing. To meet this challenge, we developed a proprietary platform that integrates structure-based modeling, rational design, and AI-enhanced protein design. Serving as a zero-shot protein predictor, this new platform guides in silico design of diverse and improved nuclease variants, thereby minimizing the need for extensive experimental screening.

Using EmendoBio's new platform, we generated highly stable non-NGG OMNI nuclease variants with enhanced expression yield, thermal stability, and solubility, improving both producibility and editing efficiency. We also combined this approach with high-throughput PAM profiling, which enabled us to engineer the PAM-interacting domain of another highly active non-NGG nuclease, broadening PAM recognition while maintaining the catalytic efficiency. This new class of non-NGG nucleases offers enhanced PAM flexibility and high activity, expanding possibilities for RT editing beyond the constraints of canonical Cas9 systems and demonstrating the versatility of EmendoBio's new platform for genome editing in both research and therapeutic contexts.

Overall, the optimized OMNI nuclease platform delivers a diverse set of solutions for refining nucleases, addressing limitations in current CRISPR-based gene editing, and expanding the CRISPR toolbox for a wide range of diseases and next-generation technologies.

Application — **Strategy**: Editing site, Specificity, FTO, Delivery → **OMNI™ nuclease panel** (~80% genome coverage) / **Engineering platform** → **Optimized solution**

Genome accessibility

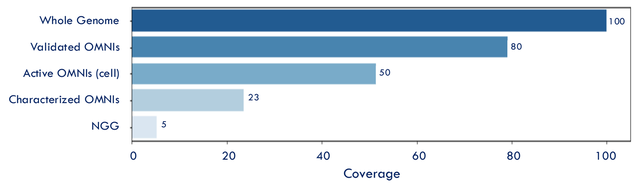
The OMNI™ nuclease panel overcomes PAM constraints

Short nucleases (<1100)

OMNI™	PAM	MaxEdit
OMNI-A2	NGGNNNNN	86%
OMNI-A7	NNGNRMNN	87%
OMNI-A8	NYRRVNNN	88%
OMNI-A13	NNNNCMAN	91%
OMNI-A31	NNGRVNNN	86%
OMNI-A34	NNNNCVKA	91%
OMNI-A54	NNRNRYNN	85%

Long nucleases (>1200)

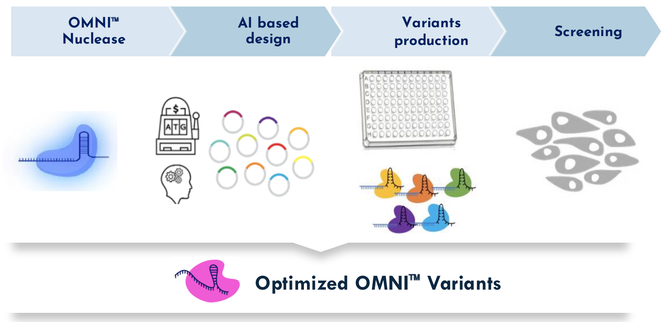
OMNI™	PAM	MaxEdit
OMNI-A1	NGGNNNNN	98%
OMNI-A4	NNRACTNN	93%
OMNI-A21	NRGGNCRN	89%



The diversity of OMNI™ nucleases, and particularly PAM site diversity, significantly enhances genome accessibility, enabling the targeting of genomic sites that are not accessible using NGG nucleases. Cumulatively, the OMNI™ panel covers approximately ~80% of the genome, making **any gene targetable**.

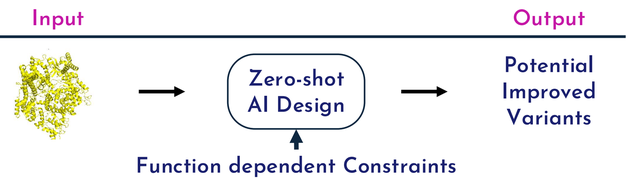
Optimized Solution

Engineered OMNI variants demonstrate improved capabilities compared to non-engineered nucleases



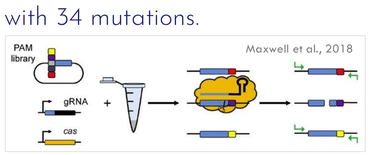
Zero-shot In-House Protein design

Multiple AI tools were integrated to create an in-house novel protein design approach to overcome the nuclease engineering challenges.



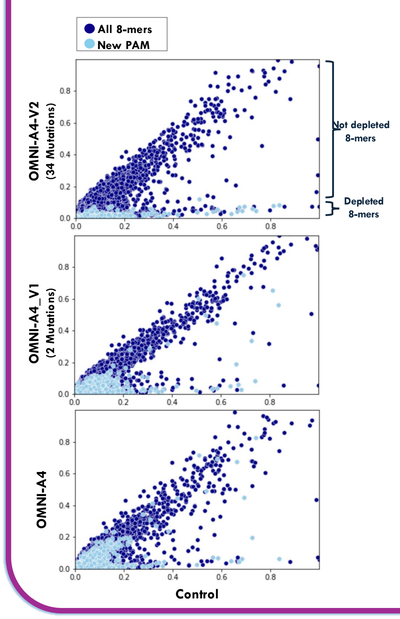
Brader PAM recognition OMNI-A4

Multiple variants were generated to broaden OMNI-A4 PAM recognition: several variants with 1-3 mutations and single variant with 34 mutations.



8-mer	Sample Read Count	Control Read Count
AACGCACT	0	10
AACGCTCT	5	2
AAGGCACT	1	5
AACTTTCT	20	30

PAM Prediction



Variant's PAM

Analysis for each variant separately predicted its PAM.

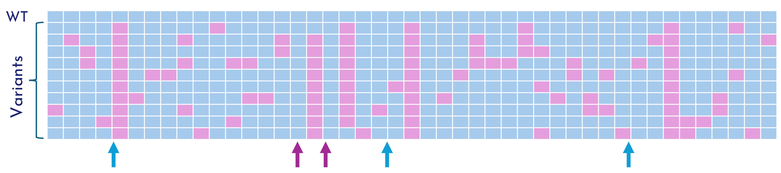
Variant vs. WT

- Analysis of variant's 8-mer coverage compared to WT.
- OMNI-A4-V2 with 34 mutations has a PAM covering ~6% of the 8-mers (Compared to ~0.7% of WT).
- All 1-3 mutations variants (ex: variant 1) showed less depletion on new 8-mers compared to variant 1.

OMNI-A13 Designed Variants Analysis

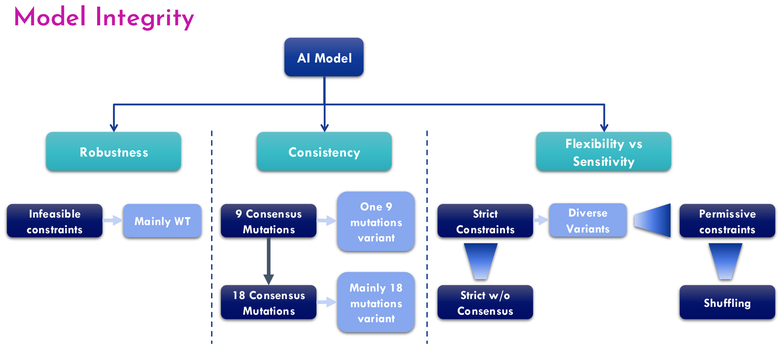
Multiple variants were generated to optimize OMNI-A13 stability. To evaluate the model's integrity and ensure that it does not produce random variants, several analytical assessments were performed.

Variants Alignment

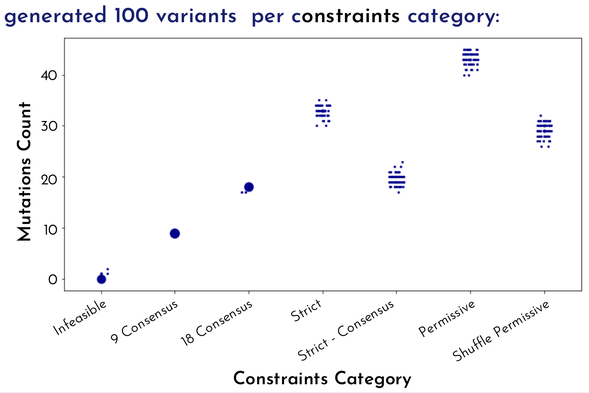


Variants alignment revealed that there are 9 mutations appearing in all variants and 18 mutations appearing in 98% of the variants. In addition, some mutations were rationally selected.

Model Integrity



Model generated 100 variants per constraints category:

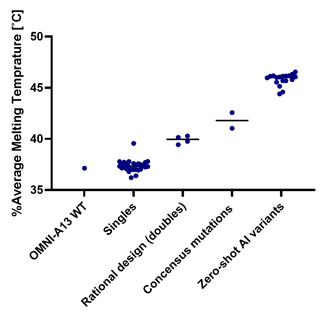


Improve OMNI-A13 Producibility

✓ Higer yield ✓ Higher Tm ✓ More soluble ✓ Active

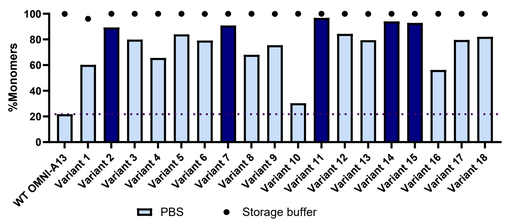
Thermo-stability

All the variants designed by the AI platform obtained Tm increase of 9-11°C compared to WT. Double mutations variants and consensus variants achieved only modest improvements.



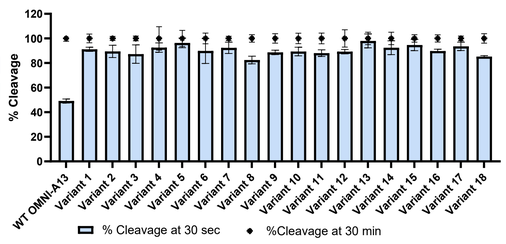
Solubility

% monomer detection showed improvement of 40-75% in monomer performance in variants compare to WT in low salt environment.



In-vitro Kinetics

All the variants showed better kinetic compared to WT.



Activity Validation in mammalian cells

Testing the leading variants in mammalian cells confirmed that their activity remained intact after the protein design process.