Newly Discovered and Engineered CRISPR-Associated Nucleases as a Robust, Efficient, Allele- and Target-Specific Editing Tool

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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 gene editing is a promising novel technology that holds great potential for treating genetic diseases. However, several limitations in current CRISPR-Cas systems restrict harnessing the full potential of genome-wide editing. First, commonly used nucleases share a narrow repertoire of PAM recognition motifs, which confines editing to only limited genomic targets. Second, editing efficiency may be low and occasionally inadequate to achieve the desired therapeutic requirement. Third, the off-target effect remains a major concern for most CRISPR-Cas systems. Fourth, achieving allele-specific targeting, required for treating diseases caused by dominant negative mutations, remains a major challenge.

EmendoBio developed the OMNI nuclease platform, a panel of nucleases designed to overcome key challenges in the CRISPR field. By applying a discovery pipeline to identify active nucleases in mammalian cells, we established a portfolio of novel nucleases (OMNI-nucleases) with high activity and specificity. These nucleases recognize a diverse range of PAM sequences, enabling coverage of approximately 80% of the human genome. Thus, our platform enables gene-editing of genomic regions that are inaccessible by commonly used nucleases.

By applying a variety of protein engineering techniques, we optimized the OMNI nucleases resulting in improved variants performing high editing efficiency with no detectable off-targets, allele-specific and optimized-stable nucleases. Directed evolution was used to generate OMNI nucleases with increased specificity and activity. Machine-learning based rational design, which was guided by EmendoBio's team, led to OMNI variants with increased activity and reduced off targets, which broke the trade-off between activity and specificity. Additionally, EmendoBio has an optimized and innovative zero-shot AI based engineering systems that generated highly stable OMNI nucleases with improved expression yield, thermal stability, and solubility. These highly stable OMNI variants enable producibility, ultimately leading to increased editing efficiency.

Our optimized OMNI nuclease platform supplies a diverse set of solutions for discovering and optimizing nucleases, that help to overcome the limitations of CRISPR based gene editing field and expanding the existing CRISPR toolbox for a wide range of diseases.

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

Genome accessibility

The OMNI™ nuclease panel overcomes PAM constraints

Short nucleases

OMNI™	PAM	Length (aa)	MaxEdit
OMNI-A2	NGGNNNNN	1062	0.86
OMNI-A7	NNGNRMNN	1097	0.87
OMNI-A8	NYRRVNNN	1109	0.88
OMNI-A13	NNNNCMAN	1091	0.91
OMNI-A31	NNGRVNNN	1054	0.86
OMNI-A34	NNNNCVKA	1078	0.91
OMNI-A54	NNRNRYNN	1091	0.85

Long nucleases

OMNI™	PAM	Length (aa)	MaxEdit
OMNI-A1	NGGNNNNN	1370	0.98
OMNI-A4	NNRACTNN	1348	0.93
OMNI-A21	NRGGNCRN	1215	0.89

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**.

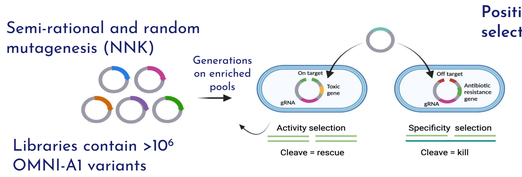
Optimized solution

Emendo's unique Engineering Platform enables efficient navigation of the sequence landscape to increase activity and specificity

Super-specific and highly active OMNI™ nucleases

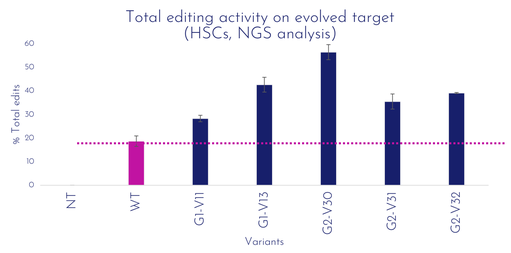
- No off-target effects
- No translocations
- Allele specific editing

Engineering Approach 1: Directed evolution



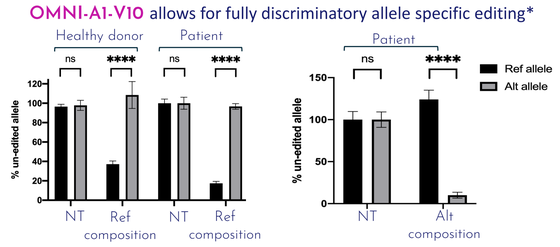
Extremely high positive selection pressure to select over-the-WT activity → highly active Variants

Activity



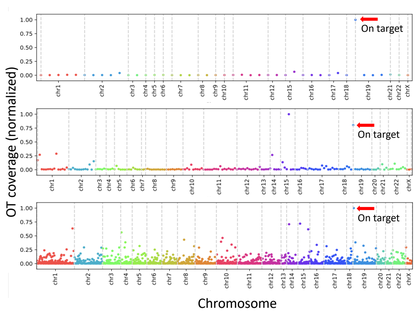
Allele discrimination

OMNI-A1-V10 allows for fully discriminatory allele specific editing*



High fidelity

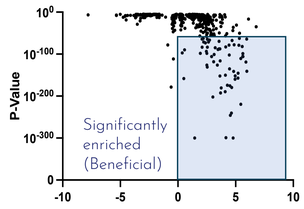
OMNI-A1-V10 has significantly less off-target effects than OMNI-A1 or spCas9



*Sabo *et al.* Mutant allele knockout with novel CRISPR nuclease promotes myelopoiesis in ELANE neutropenia. *Mol Ther Methods Clin Dev*, 2022.

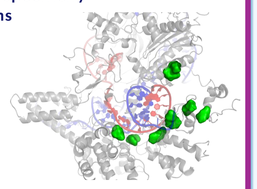
Approach 2: Rational design with machine-learning

Positive Negative selections enable the identification of key substitutions

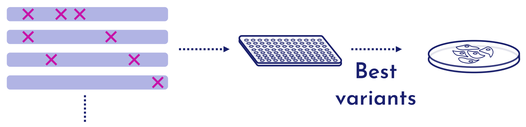


PUL analysis as described in Song et al., *Cell Systems* 2021

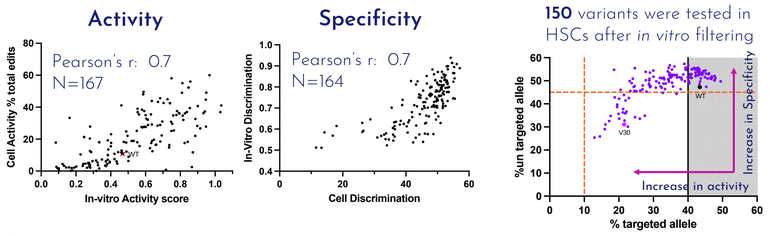
OMNI-A1 CryoEM structure with top specificity mutations



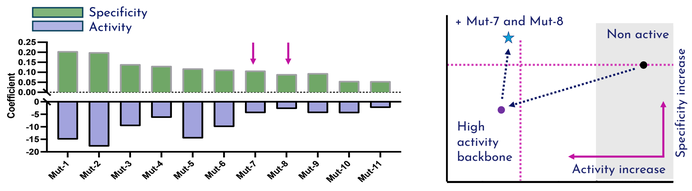
Rational and semi-rational libraries of variants with combinations of enriched mutations



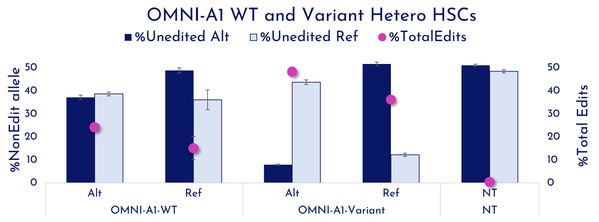
High throughput and quantitative biochemical assays enable quick variant screening and correlate between sequence and function



Ridge regression model identifies key beneficial mutations



Optimized OMNI break the activity – specificity trade-off



Approach 3: Zero-shot Protein design

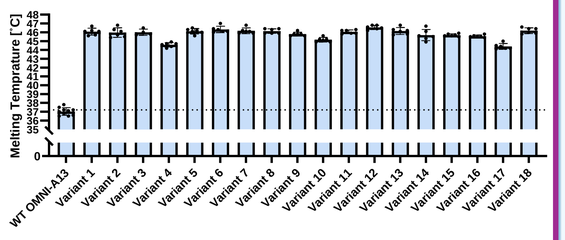
Multiple AI tools were integrated to create an in-house novel protein design approach to overcome the nuclease engineering challenges.

Improve OMNI-A13 producibility:

- Higer yield
- Higher Tm
- More soluble
- Active

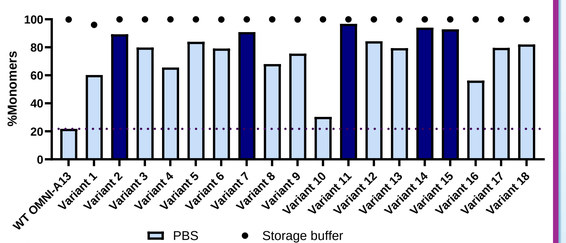
Thermo-stability

All the designed variants obtained Tm increase of 9-11°C compared to WT



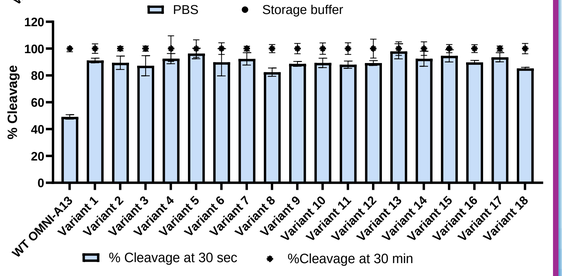
Solubility

% 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 five the leading variants that exhibited improved producibility parameters in mammalian cells confirmed that their activity remained intact after the protein engineering process.