Comparison of RNP-mediated editing by Type V Cpf1 variants

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Abstract

The CRISPR-Cpf1 system offers several potential advantages over other nucleases for *ex vivo* genome editing therapies, including a smaller single crRNA that can be readily synthesized, the ability to target T- and C-rich PAMs with the WT and RR variants, respectively, and lastly a 5'-staggered cut which may lead to different repair outcomes (1).

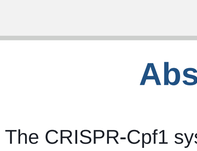
We have optimized several Type V Cpf1 variant ribonucleoproteins which are the preferred delivery mode for *ex vivo* gene-editing therapeutics. Comparing their cellular potency with SpCas9 we show that AsCpf1 and LbCpf1 RNPs show robust editing activity at multiple sites in T-cells.

In addition, we show that delivery of proteins such as Lb2Cpf1 and FnCpf1, that had low activity when expressed as a plasmid (1) show robust editing when delivered as an RNP.

This finding underscores the promise of RNP delivery for Cpf1 nucleases which have desirable properties for genome editing therapeutics.

Cpf1 vs. Cas9

Cpf1

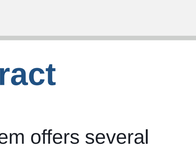


WT TTTV, TTN PAMs along with NYCV and TATV engineered PAMs

Single guide with 20-24 nt protospacer and 19-20 nt direct repeat (~40 nt)

5' staggered DNA cut with 4-5 nt overhangs

Cas9

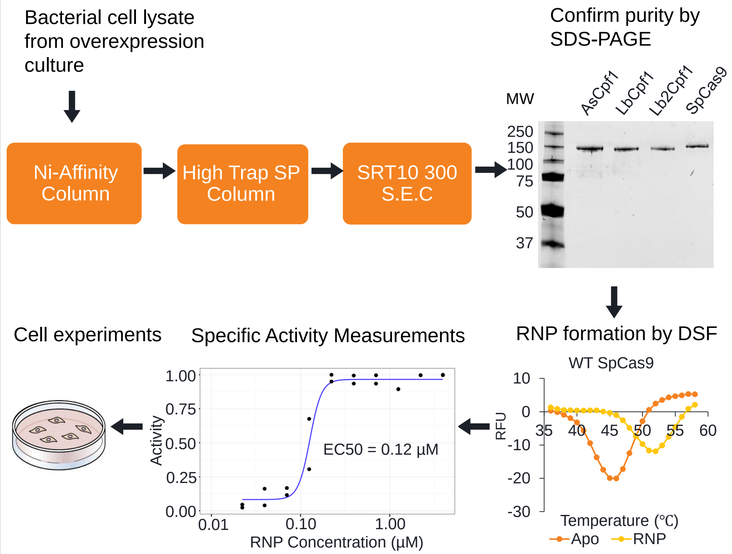


WT NGG PAM along with NGAN, NGCG engineered PAMs

Separate crRNA and tracrRNA that can be linked into a single molecule (~100 nt)

Blunt DNA cut

1. Robust pipeline for production and evaluation of our RNPs



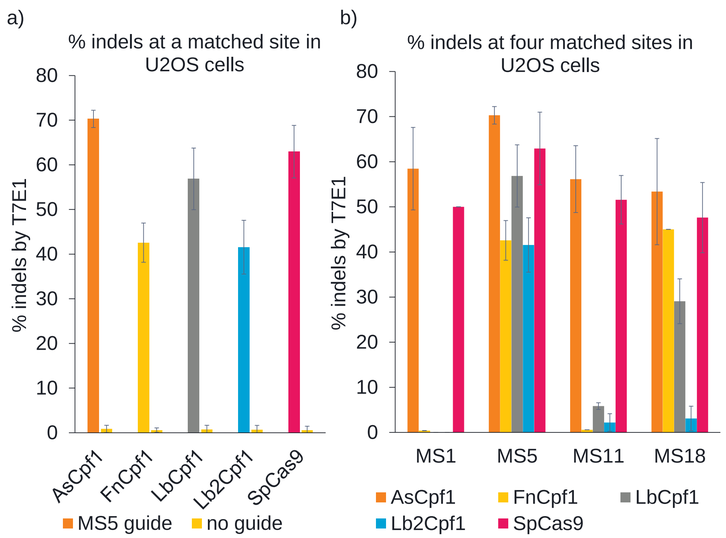
We have developed a robust pipeline for characterizing new enzymes. This begins with a rigorous purification process from which we obtain highly pure material as seen by the SDS-PAGE gel.

We validate formation of RNP using our DSF assay to measure thermal melting of the fully-complexed RNP versus the apo-protein.

We then obtain specific activity measurements for our protein to benchmark it against other known CRISPR enzymes.

Finally, we nucleofect our RNPs into mammalian cells to assess editing efficacy

2. Efficient editing with several Cpf1 orthologs delivered as RNPs



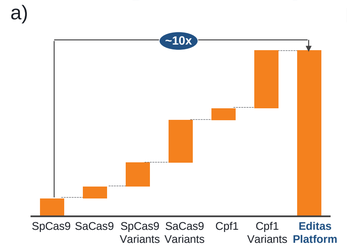
a) FnCpf1 was previously shown to edit poorly while Lb2Cpf1 showed no editing in mammalian cells by DNA transfection (1). We show here that we are able to resurrect the cellular activity of these two Cpf1 orthologs when delivered as RNPs.

b) We show that the AsCpf1 enzyme efficiently edits at multiple published matched target sites (2) with adjacent Cpf1/SpCas9 PAM sites in U2OS cells. At all four tested matched sites AsCpf1 RNP edits comparably or even slightly better than SpCa9 RNPs. LbCpf1, Lb2Cpf1 and FnCpf1 were found to be less efficient than AsCpf1 as RNPs at these matched sites.

References:

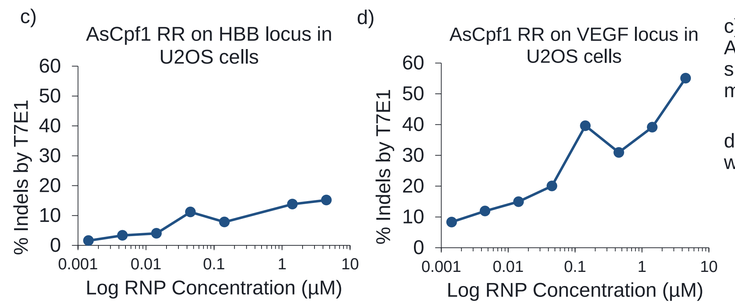
1. Zetsche et al. *Cell* 2015
2. Kleinstiver et al. *Nat Biotech* 2016
3. Gao et al. *Biorxiv* 2016

3. Engineered Cpf1 variants expand our PAM targeting space



b)

Variant	PAM	Expected genome frequency
SpCas9	NGG	1 per 16 bp
SaCas9	NNGRRT	1 per 64 bp
SaCas9 KKH	NNNRRT	1 per 16 bp
AsCpf1	TTTV	1 per 85.3 bp
AsCpf1 RR	**NYCV**	**1 per 10.7 bp**
AsCpf1 RVR	TATV	1 per 85.3 bp



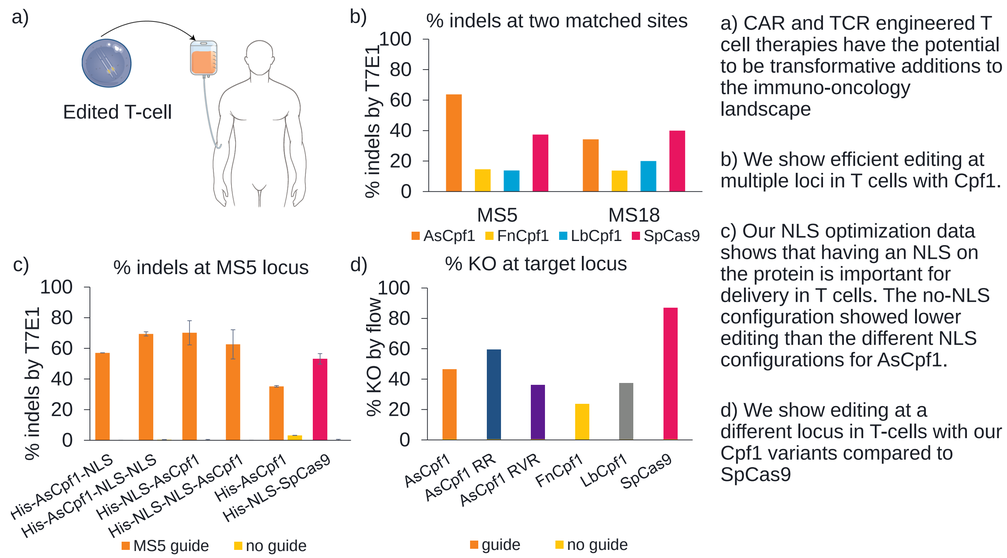
a) An important priority in developing our Cpf1 gene targeting capabilities is to expand the PAM sites that we can target with Cpf1.

b) The AsCpf1 RR and RVR PAM variants from the Zhang group (3) greatly expand the targeting space for Cpf1.

c) Editing of the HBB locus with AsCpf1 RR (no AsCpf1 WT sites near desired Sickle mutation site).

d) Editing of the VEGF locus with AsCpf1 RR.

4. Efficient editing in T cells at multiple loci with Cpf1 RNPs



a) CAR and TCR engineered T cell therapies have the potential to be transformative additions to the immuno-oncology landscape

b) We show efficient editing at multiple loci in T cells with Cpf1.

c) Our NLS optimization data shows that having an NLS on the protein is important for delivery in T cells. The no-NLS configuration showed lower editing than the different NLS configurations for AsCpf1.

d) We show editing at a different locus in T-cells with our Cpf1 variants compared to SpCas9

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