Comparison of RNP-mediated editing by Type V Cpf1 variants across multiple cell types

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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 multiple Cpf1 variants show robust editing activity at multiple sites in cells.

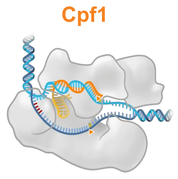
In addition, we show that Cpf1 orthologs such as Lb2Cpf1 and FnCpf1 can lead to robust editing in cells when delivered as RNPs.

Lastly, we demonstrate efficient editing with several Cpf1 variants delivered as RNPs in T cells and AsCpf1 delivered as an RNP in adult hematopoietic stem cells (HSCs).

These findings underscore the promise of RNP delivery for Cpf1 nucleases which have desirable properties for *ex vivo* genome editing therapeutics.

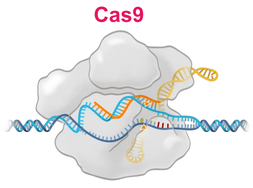
Cpf1 vs. Cas9

Cpf1



- WT TTTV, TTN PAMs plus TYCV/CCCC, TATV engineered PAM variants
- 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 plus NGAN, NGCG engineered PAM variants
- Separate crRNA and tracrRNA that can be linked into one molecule (~100 nt)
- Blunt DNA cut

References:

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

Robust pipeline for production and evaluation of our RNPs

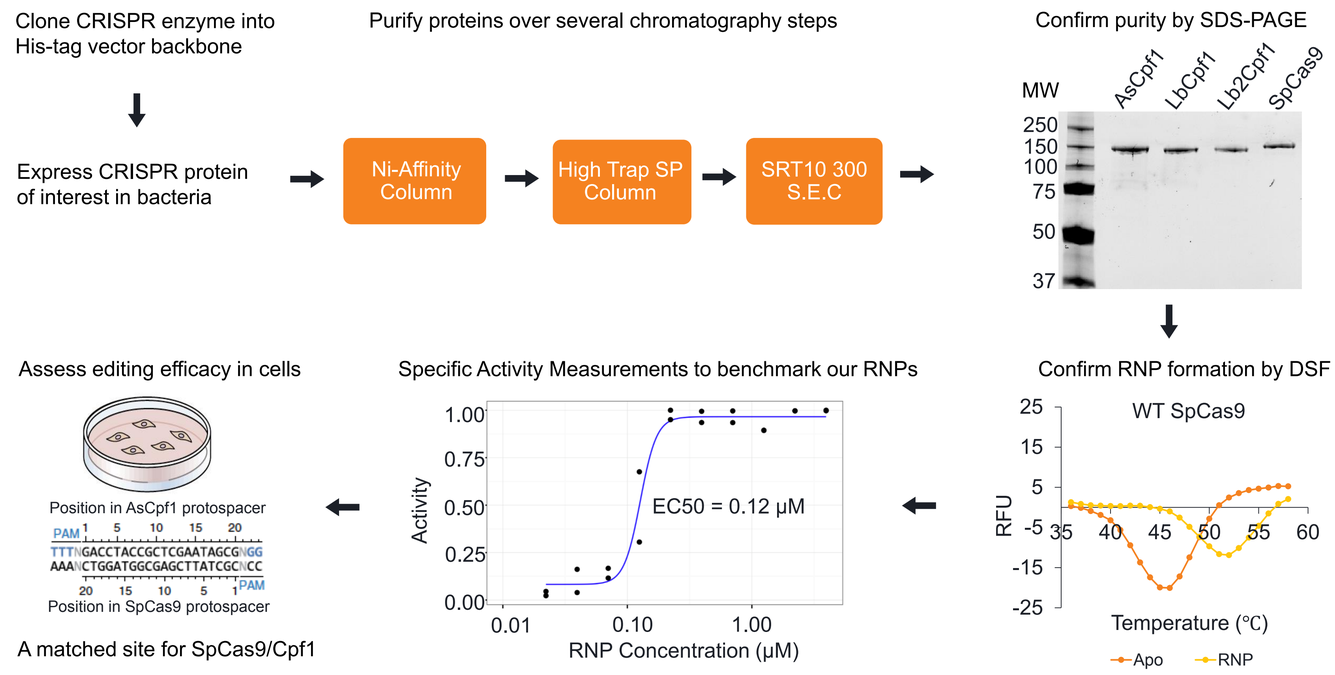


Figure 1. We have developed a robust pipeline for characterizing new enzymes. This begins with a rigorous purification process from which we obtain highly pure material as seen by the SDS-PAGE gel.

Bacterial cell lysates containing our His-tagged protein are incubated with Ni-NTA resin and then further purified over both a cation exchange column and a size-exclusion column to yield material with high purity that is free of nucleic acid contaminants.

We validate formation of RNP using our DSF assay to measure thermal melting of the fully-complexed RNP versus the apo-protein. A uniform thermal shift to the right indicates that our enzymes is fully complexed with guide.

We obtain specific activity measurements for our protein to benchmark a given enzyme against other known CRISPR enzymes.

Finally, we deliver our RNPs into cells to assess editing efficacy in a cellular context. Matched genomic sites for SpCas9/Cpf1 (2) allow us to make the fairest comparisons between these two enzymes.

Efficient editing with Cpf1 orthologs delivered as RNPs

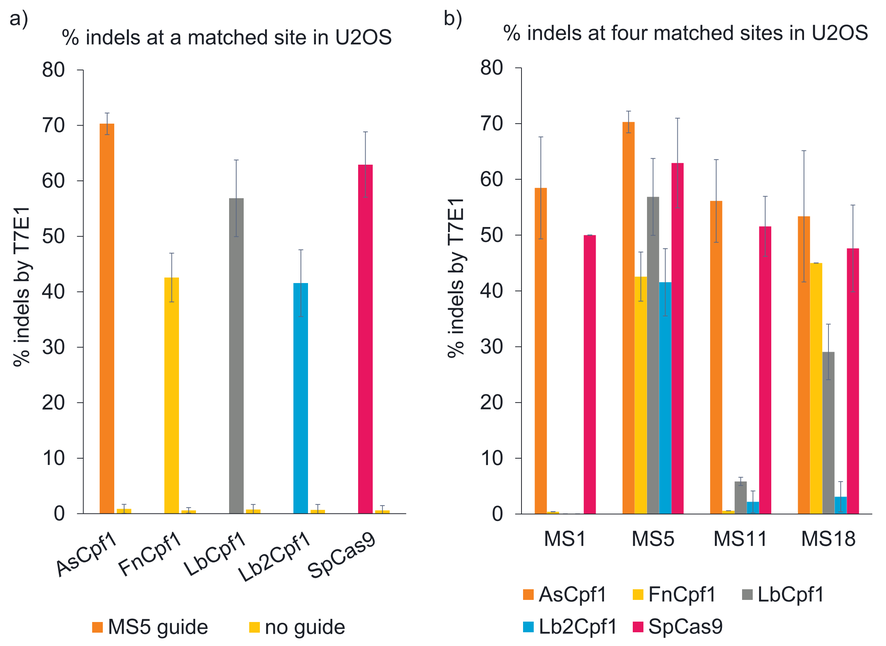
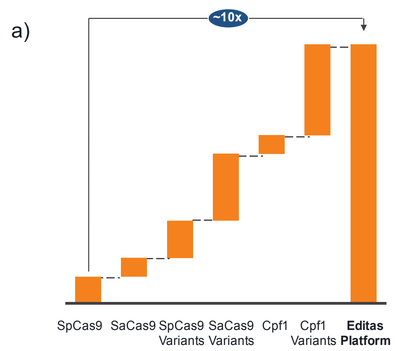


Figure 2. a) We show that we are able to achieve efficient cellular editing with four different Cpf1 orthologs targeting a matched site when delivered as RNPs to U2OS cells. b) We then show that several of these Cpf1 orthologs can efficiently edit multiple published matched target sites (2) with adjacent Cpf1/SpCas9 PAMs. We show that AsCpf1 can edit at greater than or equal efficacy compared to SpCas9 at four matched sites using the same RNP dose.

Engineered Cpf1 variants expand PAM targeting space



b)

Variant	PAM	Expected genome frequency (nt)
SpCas9	NGG	1 per 16
SaCas9	NNGRRT	1 per 64
SaCas9 KKH	NNNRRT	1 per 16
AsCpf1 WT	TTTV	1 per 85
AsCpf1 RR	**TYCV/CCCC**	**1 per 42**
AsCpf1 RVR	**TATV**	**1 per 85**
FnCpf1	TTN	1 per 16

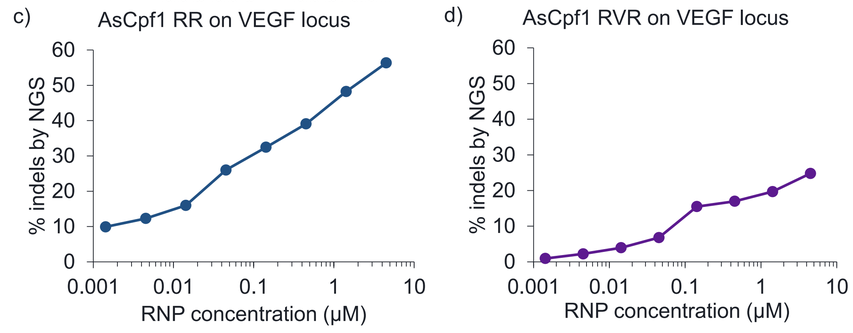


Figure 3. a) An important priority in developing our Cpf1 gene targeting capabilities is to expand the PAM sites that we can target with Cpf1. b) The AsCpf1 RR and RVR PAM variants from the Zhang group (3) greatly expand the targeting space for Cpf1. c) Editing of the VEGF locus with AsCpf1 RR PAM variant in dose response. d) Editing of the VEGF locus with AsCpf1 RVR PAM variant in dose response.

Efficient editing in T cells at multiple loci with Cpf1 RNPs

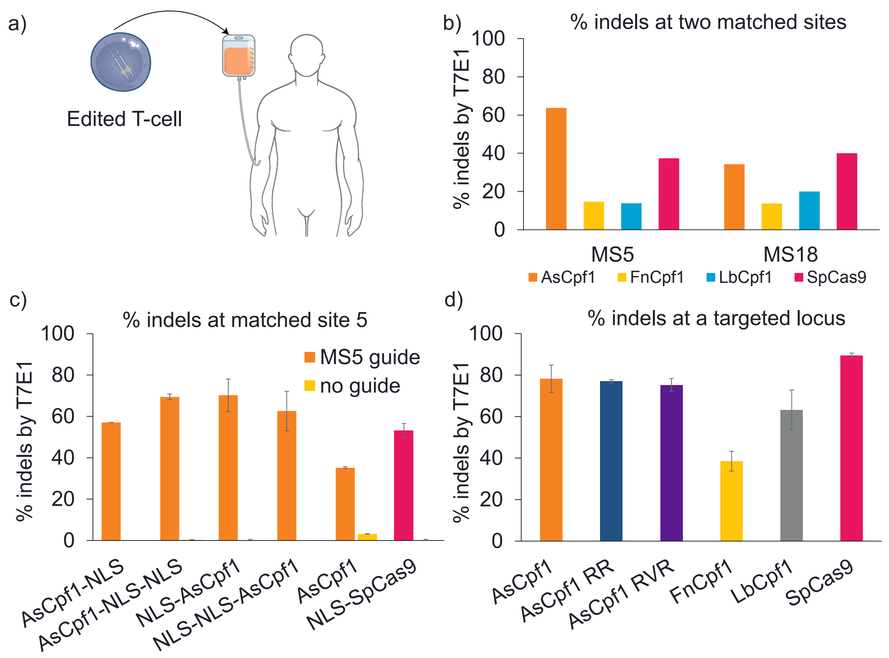


Figure 4. a) CAR and TCR engineered T cell therapies have the potential to be transformative additions to the immuno-oncology landscape. b) We show efficient editing at multiple loci in T cells with multiple Cpf1 variants. c) Our NLS optimization data shows that having an NLS on the protein is important for delivery in T cells. All constructs begin with an N-terminal His-tag. d) We show editing at a different locus in T-cells with our Cpf1 variants along with SpCas9.

Efficient editing in HSCs at multiple loci with Cpf1 RNPs

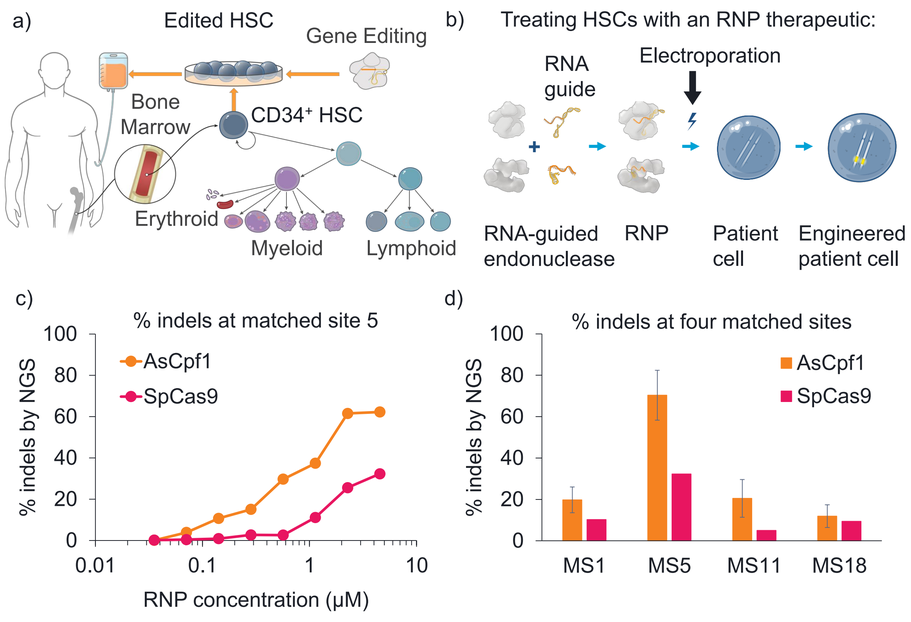


Figure 5. a) Edited HSCs have the potential to provide a durable therapy for patients with β-hemoglobinopathies. b) Steps involved in generating an HSC RNP therapeutic. c) Editing in adult HSCs at a matched site with AsCpf1 and SpCas9 enzymes delivered as RNPs with indels detected by next-generation sequencing (NGS). d) Editing at four matched sites with AsCpf1 and SpCas9 at an equal maximal dose of RNP in adult HSCs.

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