Defining and characterizing the components of a CRISPR-Cas9 genomic medicine

Hari Jayaram

CRISPR International Conference 2017

June 10, 2017

Presentation Overview

Platform for CRISPR medicines

RNP based Lead Discovery platform

Fully synthetic modular dgRNA

Ex-vivo T-cell and HSC editing with Cpf1

Pipeline strategy to enable successful medicines

Medical Need

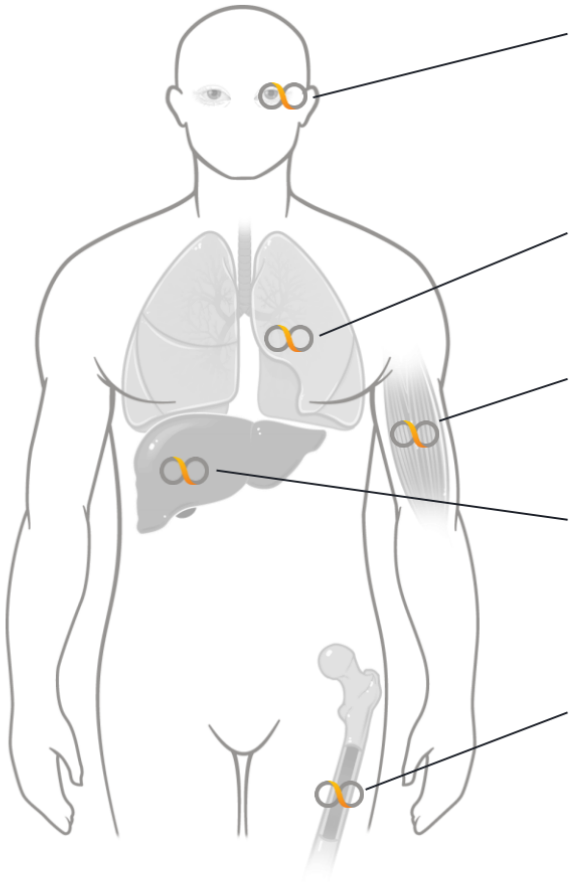
- Severe diseases where current treatments, if any, are poor
- Potential for durable therapies to provide unique benefit

Biology & Clinical

- Clear biological hypothesis for genomic intervention
- Favorable clinical and regulatory path

Technical

- Validated delivery approaches
- Mutation feasibly corrected



Eye

- Leber Congenital Amaurosis 10
- Ocular HSV
- Additional ocular indications

Lung

- Cystic Fibrosis

Muscle

- Duchenne Muscular Dystrophy

Liver

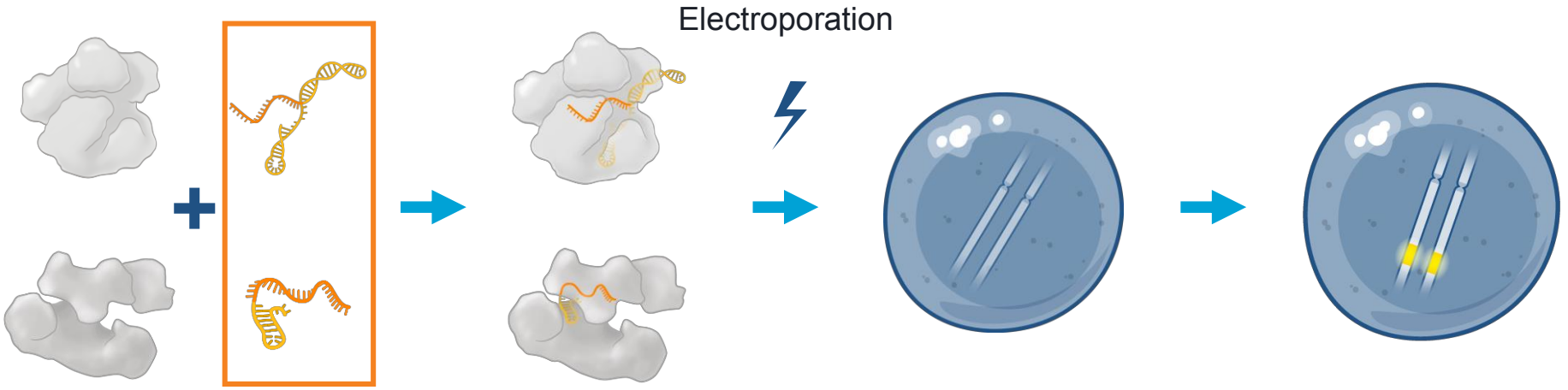
- Alpha-1 Antitrypsin Deficiency
- Infectious diseases of liver

Bone Marrow & Blood

- Hemoglobinopathies
- Engineered T cells for cancer
- Additional bone marrow and blood indications

Scalable, Consistent Engineered Cell Therapies

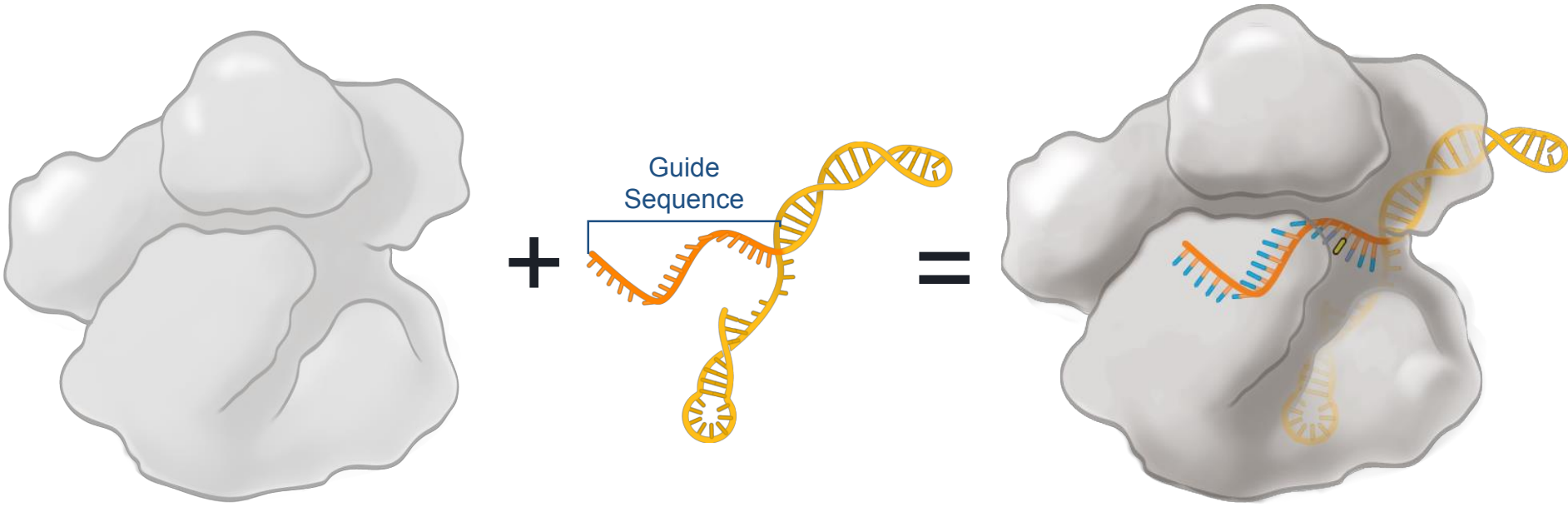
Platform for CRISPR Medicines



Engineer multiple components to T-cell & HSC sensitivity (maintain cell viability, potency)

Defining every component : key to a successful CRISPR medicine

Aspects of a Ribonucleoprotein medicine



- Efficacy: well optimized cellular and well characterized in vitro efficacy
- Stability: track RNP activity through gene-editing workflow and therapeutic window
- Fidelity: Define every component and characterize trace elements
- Pharmacokinetics

Presentation Overview

Platform for CRISPR medicines

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Fully synthetic modular gRNA

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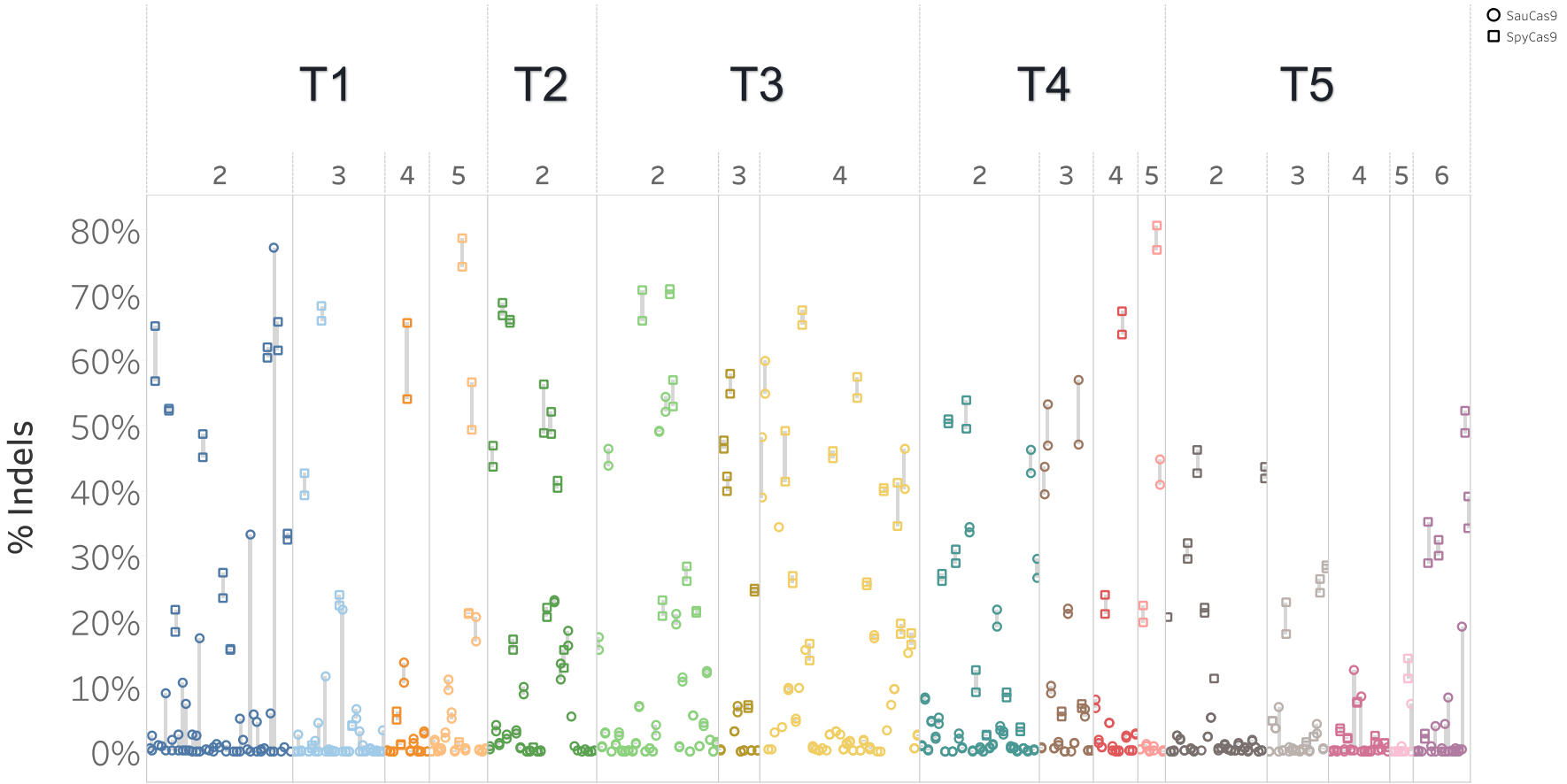
RNP lead discovery

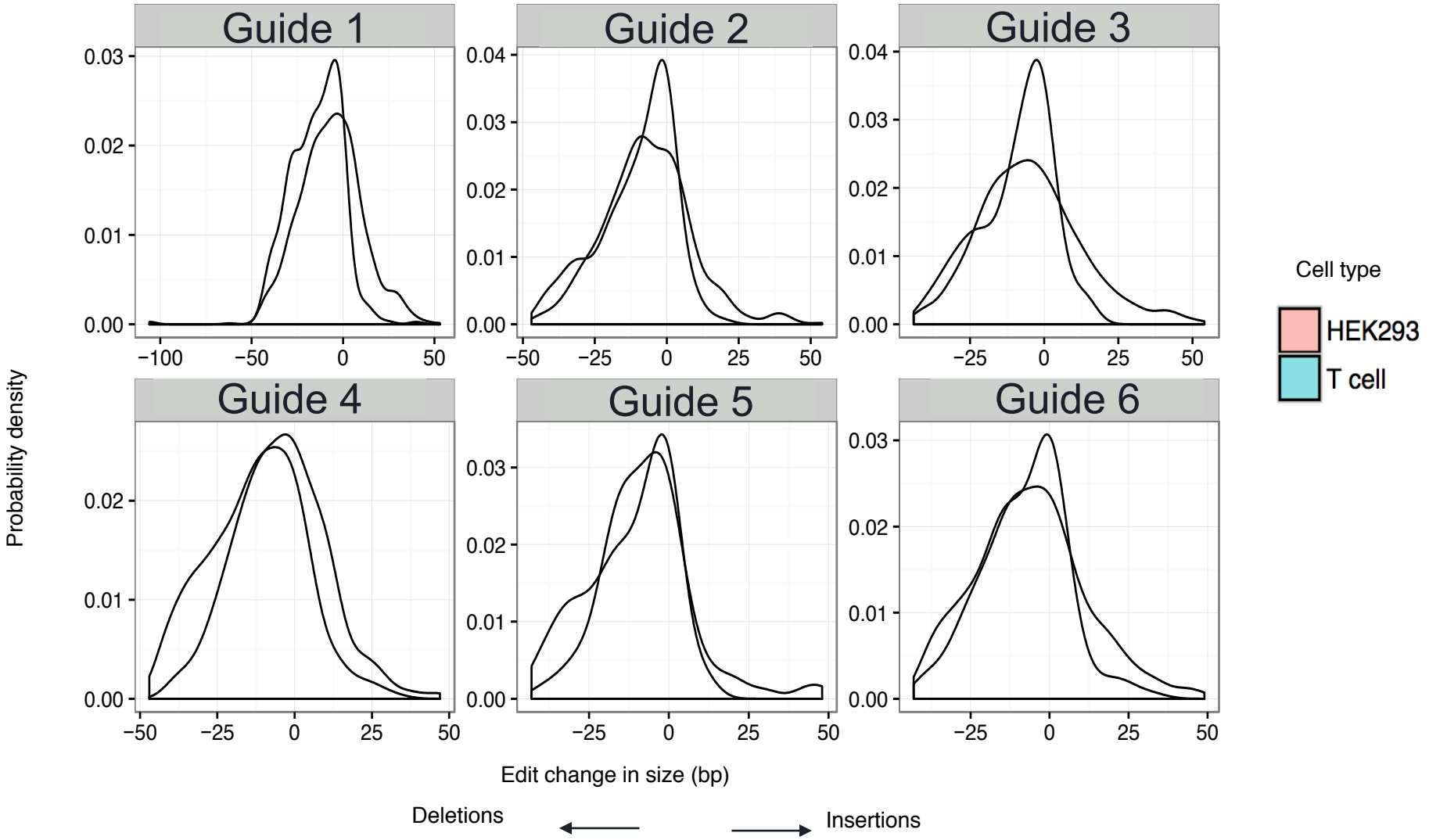
- Wealth of standardized data across cell types, nucleases, gRNA formats



Many factors influence gRNA efficacy: gRNA sequence, Nuclease type, cell type

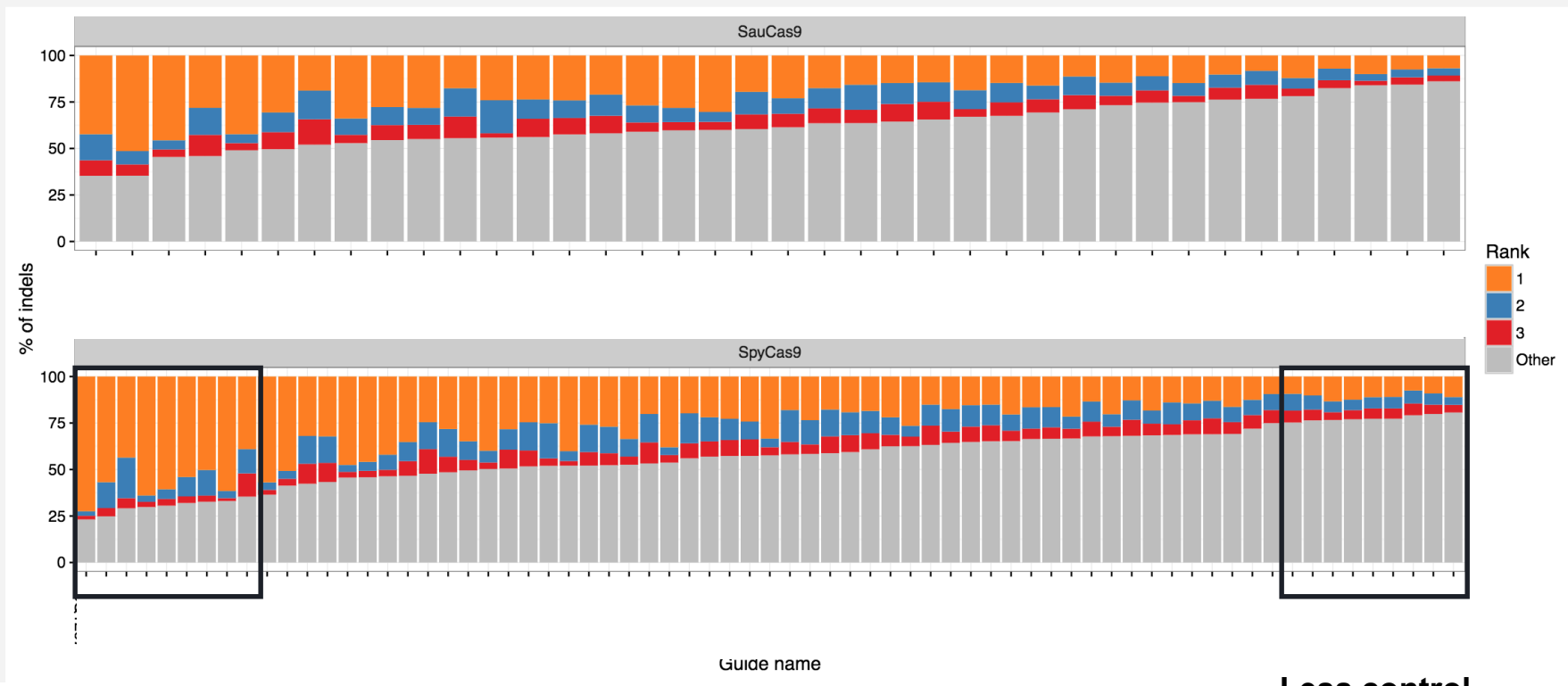
Cell type dependence

T-cells skew to deletions as compared to insertions in HEK-293T cells



Guide dependence of edit

Normalized indel distributions display range of levels of control



More control

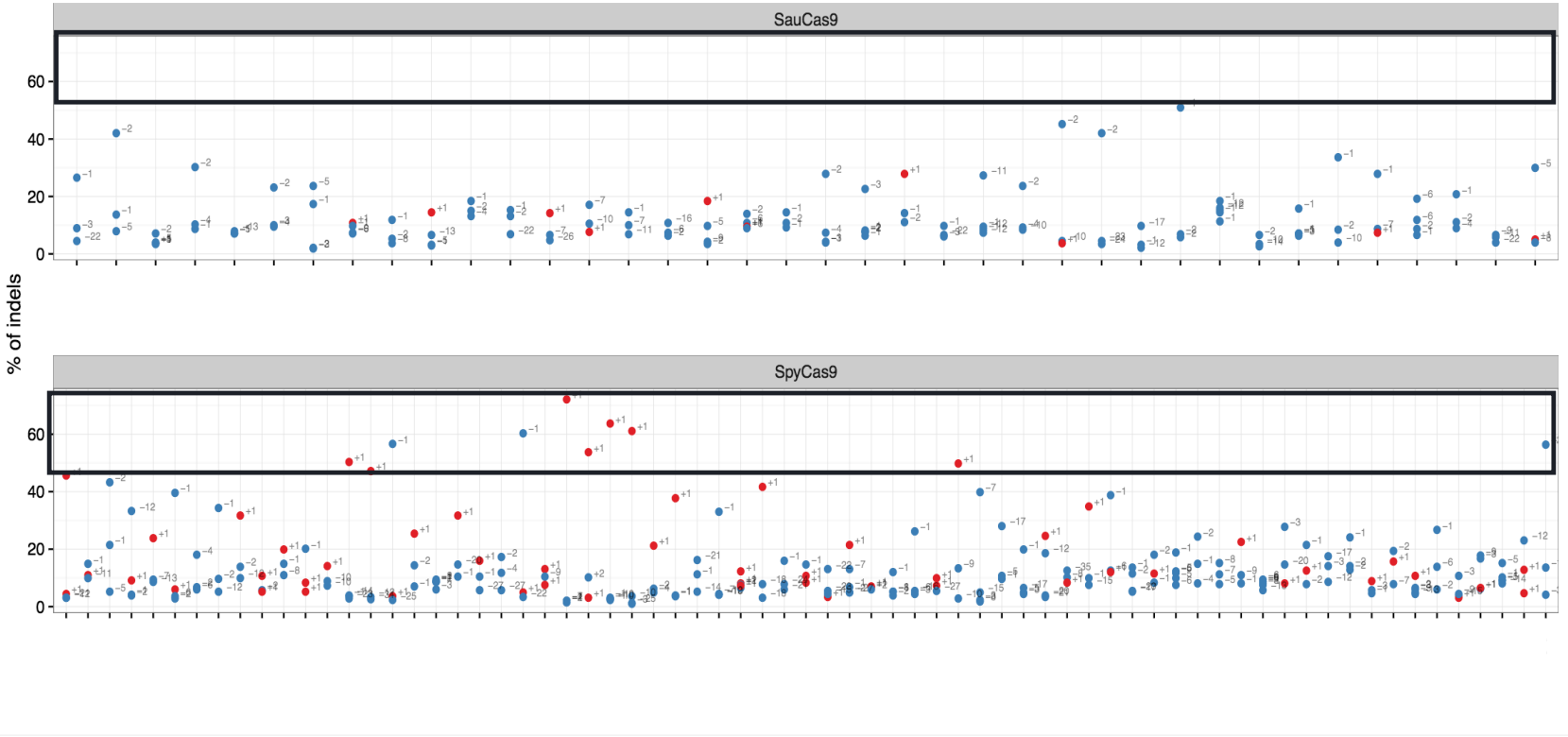
Less control

Normalized edit profiles

Indel pattern dependence on nuclease choice

- Spy-Cas9 shows the +1 insertion more often than Spy-Cas9

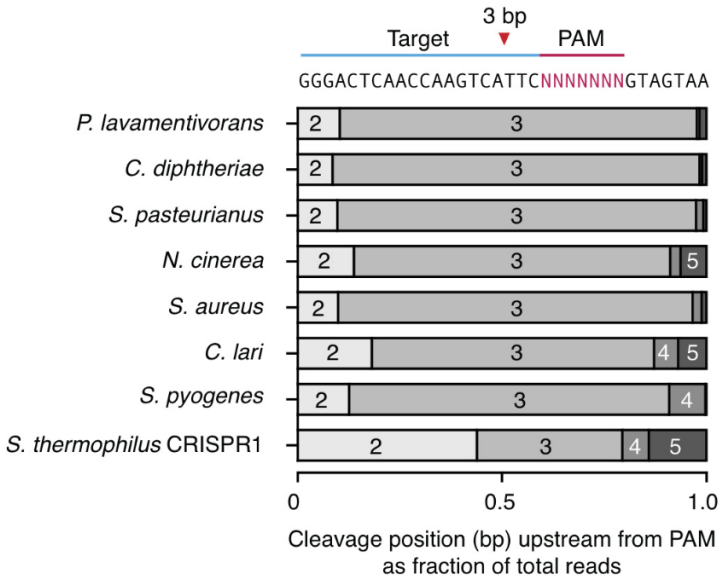
%age of indels with a specific edit



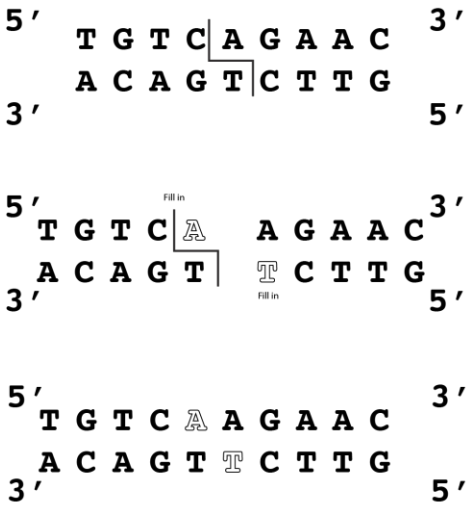
+1 insertions

+1 overhangs a result of nuclease properties

Pyogenes cuts with a 1bp stagger more often than Sau Cas9



Extended Data Figure 2 | Cas9 orthologue cleavage pattern *in vitro*. Stacked bar graph indicates the fraction of targets cleaved at 2, 3, 4, or 5 bp upstream of PAM for each Cas9 orthologue; most Cas9 enzymes cleave stereotypically at 3 bp upstream of PAM (red triangle).



+1 insertions come from base 5' of cut site

In vivo genome editing using Staphylococcus aureus Cas9.
Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, Zetsche B, Shalem O, Wu X, Makarova KS, Koonin EV, Sharp PA, Zhang F.
Nature. 2015 Apr 9;520(7546):186-91. doi: 10.1038/nature14299.

Presentation Overview

- Platform for CRISPR medicines
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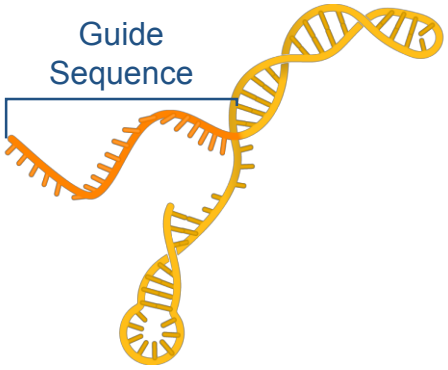
- **Fully synthetic modular gRNA**
- Ex-vivo T-cell and HSC editing with Cpf1

gRNA component at the core of the RNP medicine

Maturation of the synthetic gRNA field allow for multiple formats

- Chemical synthesis of gRNA allows for greater flexibility, fidelity , scale and purity
- Synthetic chemistry goes from 3’ to 5’ , 100-mers were challenging till recently
- Safety and Specificity of a medicine rely on a precise characterization and control of every component



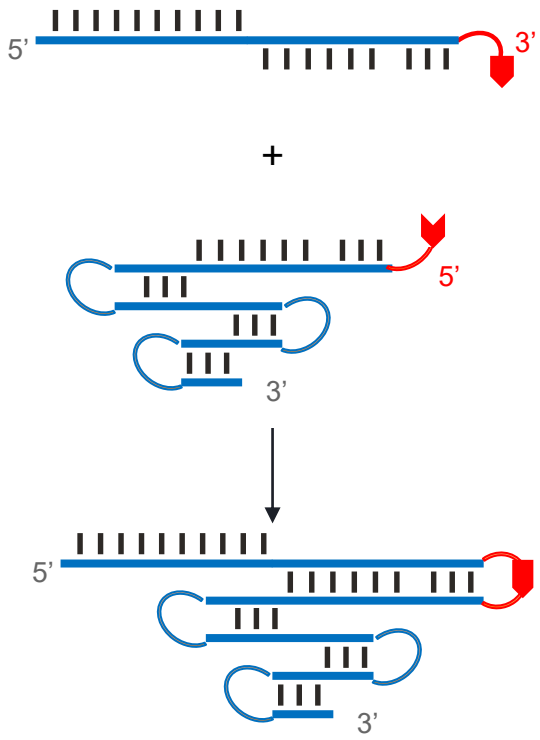
100-mer sgRNA

Generating Synthetic Covalently-Coupled Dual gRNA

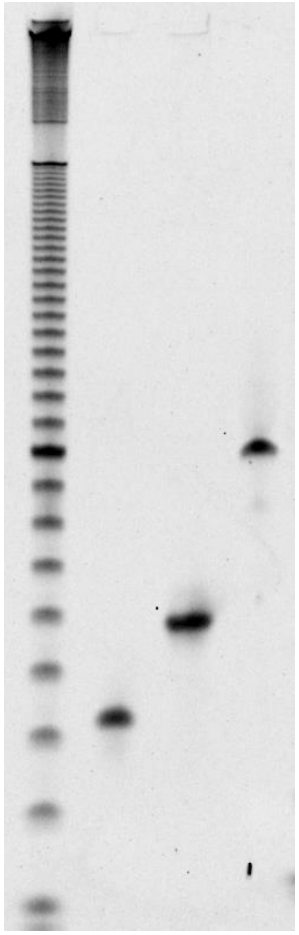
A completely non-enzymatic process for guide production

Why make a synthetic guide?

- Targeted chemistries anywhere in the molecule
- Unhindered ends and modifications
- Scale up and purity are more compatible with CMC requirements

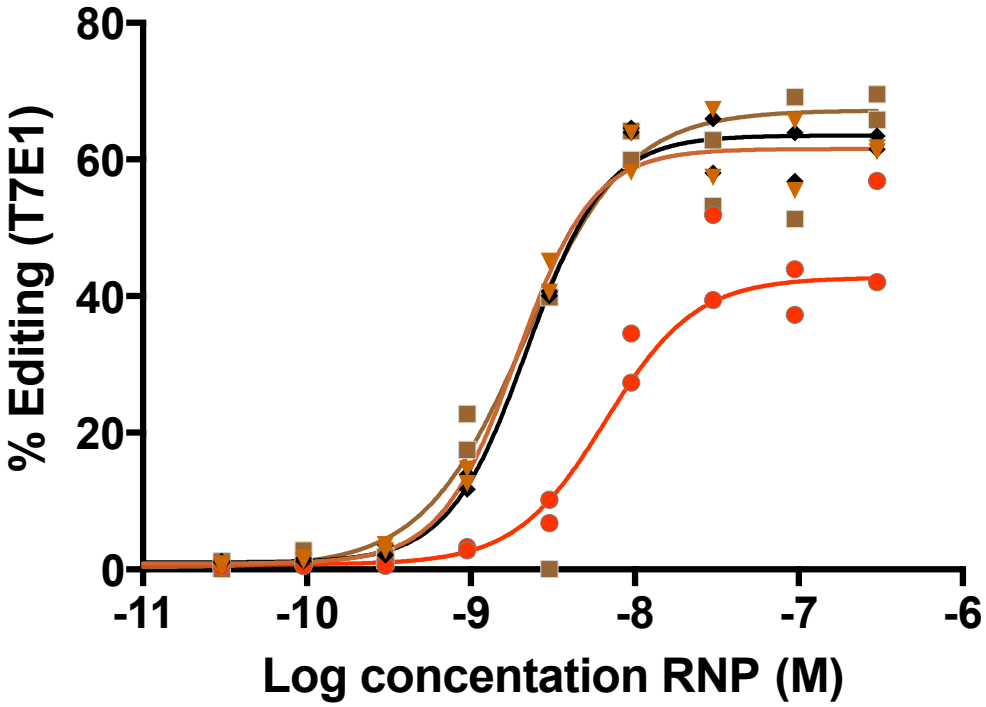


covalently-coupled dual gRNA (dgRNA)



Cellular Editing Activity

In vitro transcribed and synthetic covalently-coupled dgRNA are equivalent in cells

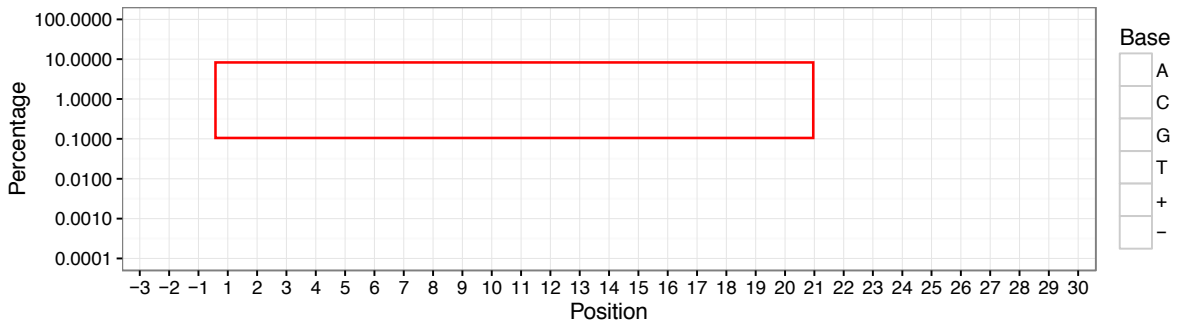


- IVT purified by vendor
- IVT purified by collaborator
- covalently-coupled dgRNA
- 2-part synthetic

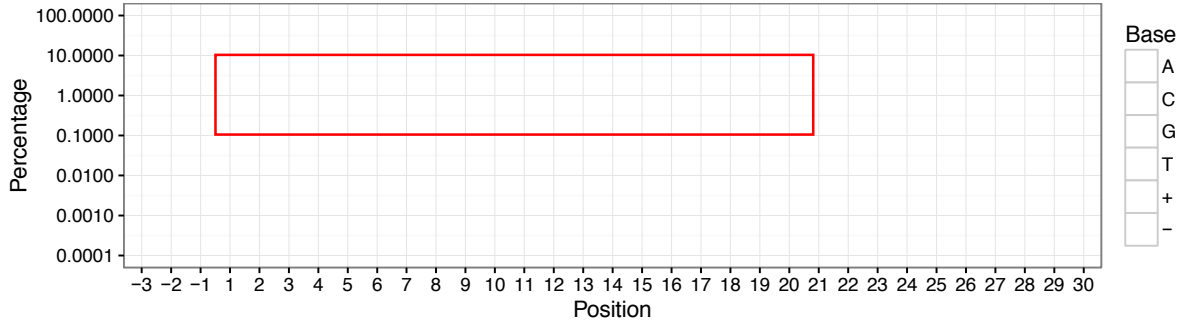
gRNA purity and sequence fidelity

Covalently-coupled dgRNA result in greater sequence fidelity in target region

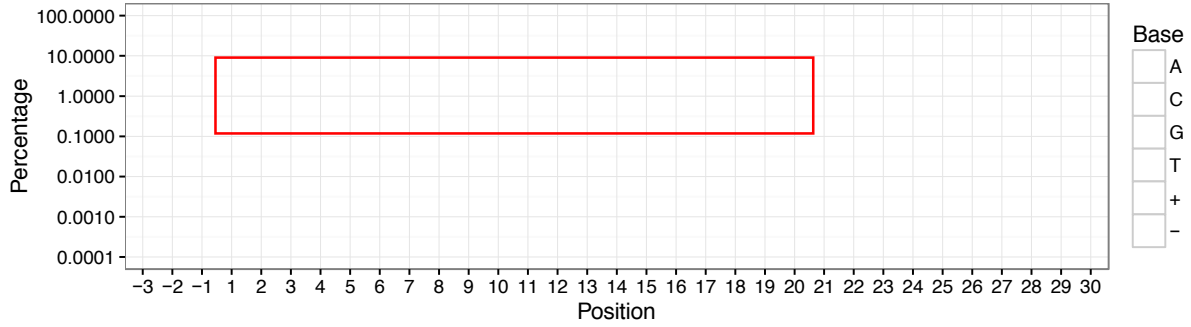
A



B



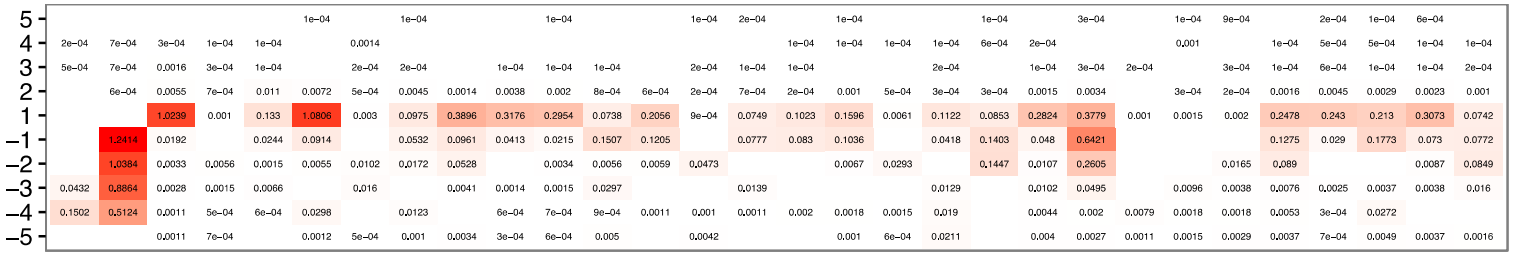
Covalently-Coupled dgRNA



gRNA Purity and Sequence Fidelity

Covalently-coupled dgRNA result in fewer truncated molecules

A



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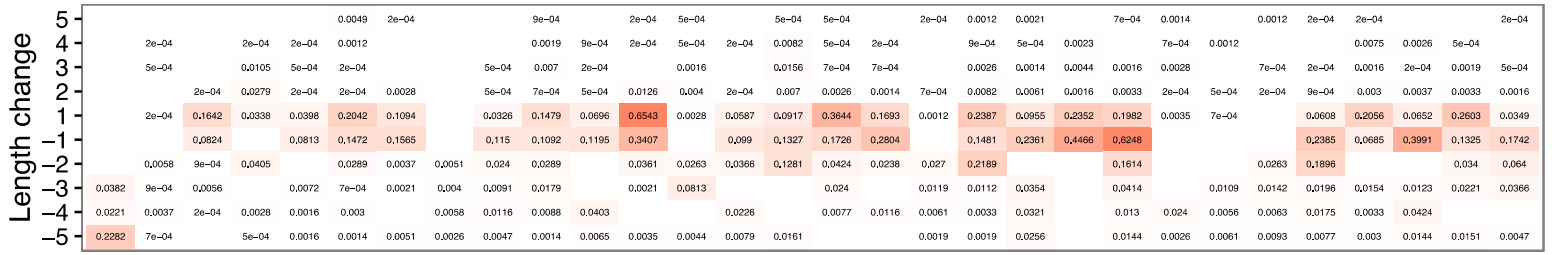
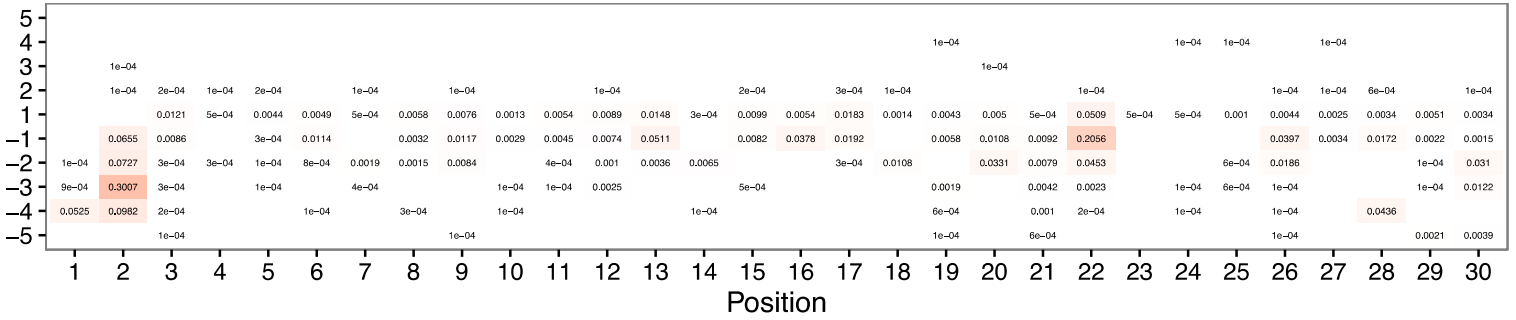


Covalently-Coupled dgRNA



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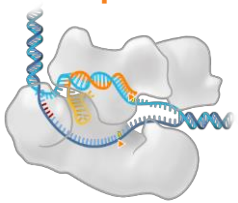
- **Ex-vivo T-cell and HSC editing with Cpf1**

Type VI nuclease Cpf1 with many benefits

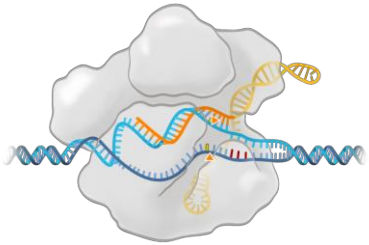
- Smaller gRNA just 44 nucleotides
- The business end of gRNA is at 3’
- dsDNA Cutting profile different from Spy and Sau Cas9
- More targets with enzyme and evolved variants

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

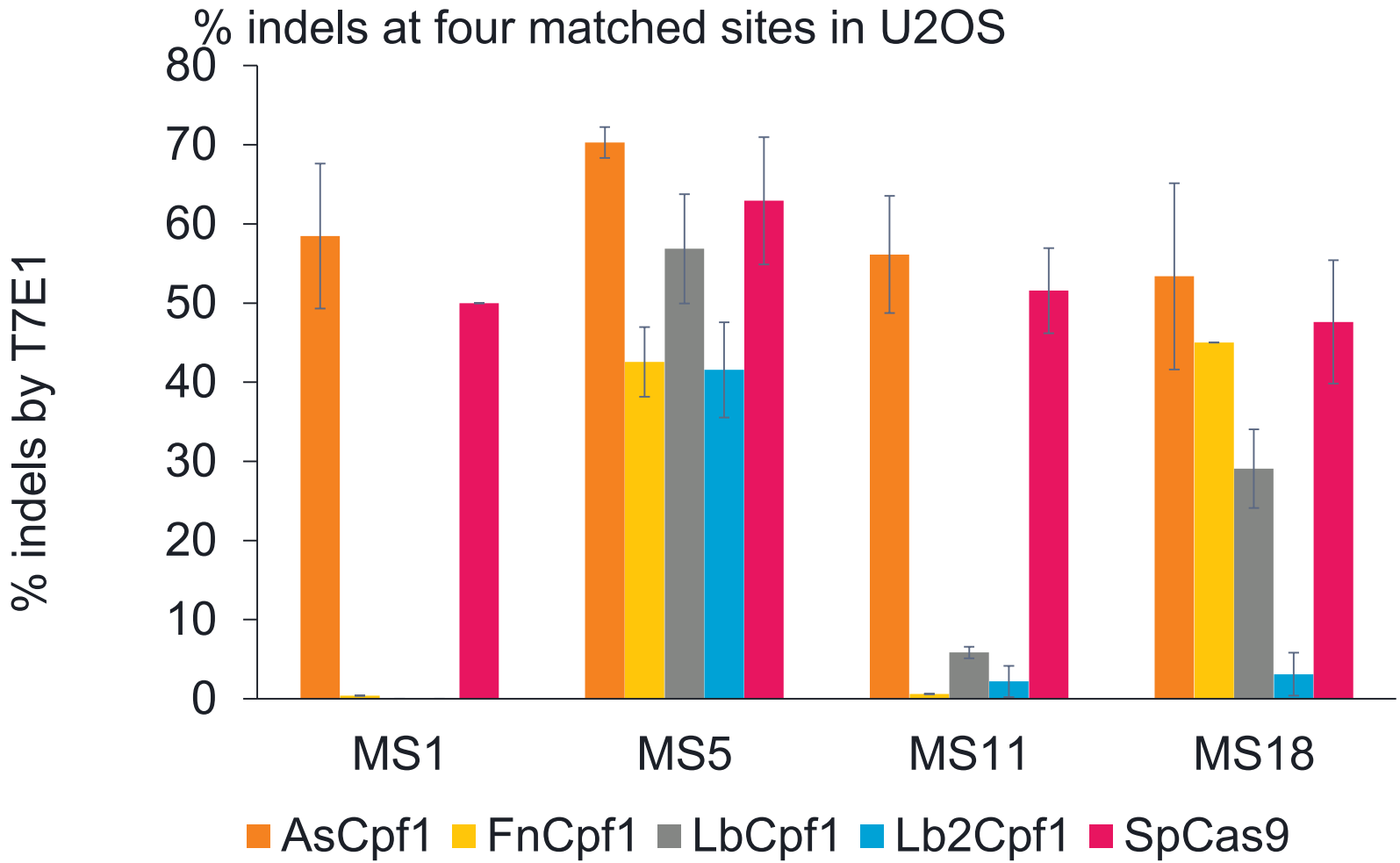
Cas9



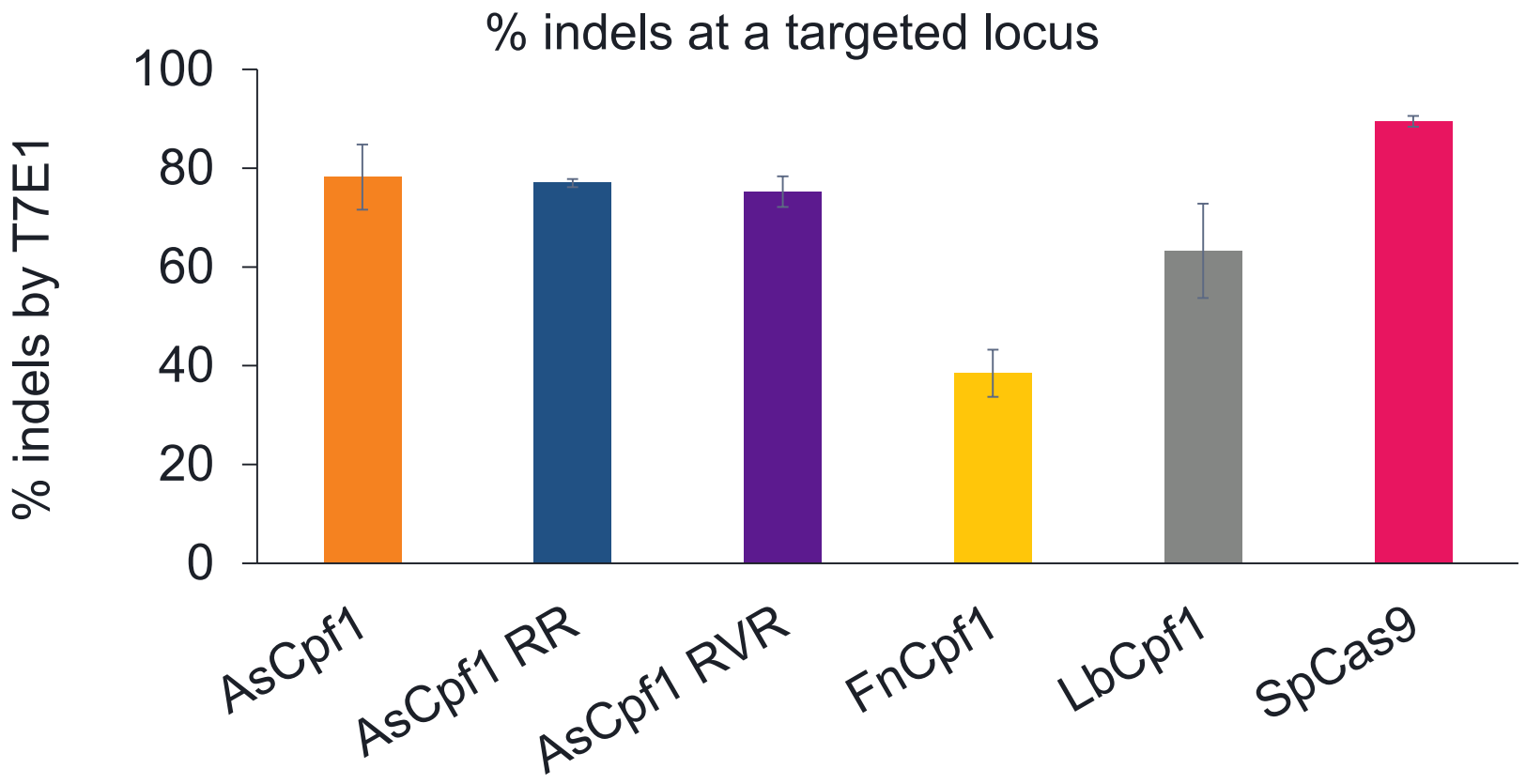
- WT NGG PAM plus NGAN, NGCG engineered PAM variants
- Separate crRNA and tracrRNA that can be linked into one molecule (~100 nt)
- Blunt DNA cut

Screening of multiple Cpf-1 orthologs and variants

AsCpf1 emerging as the “go to” Cpf1 with Robust activity

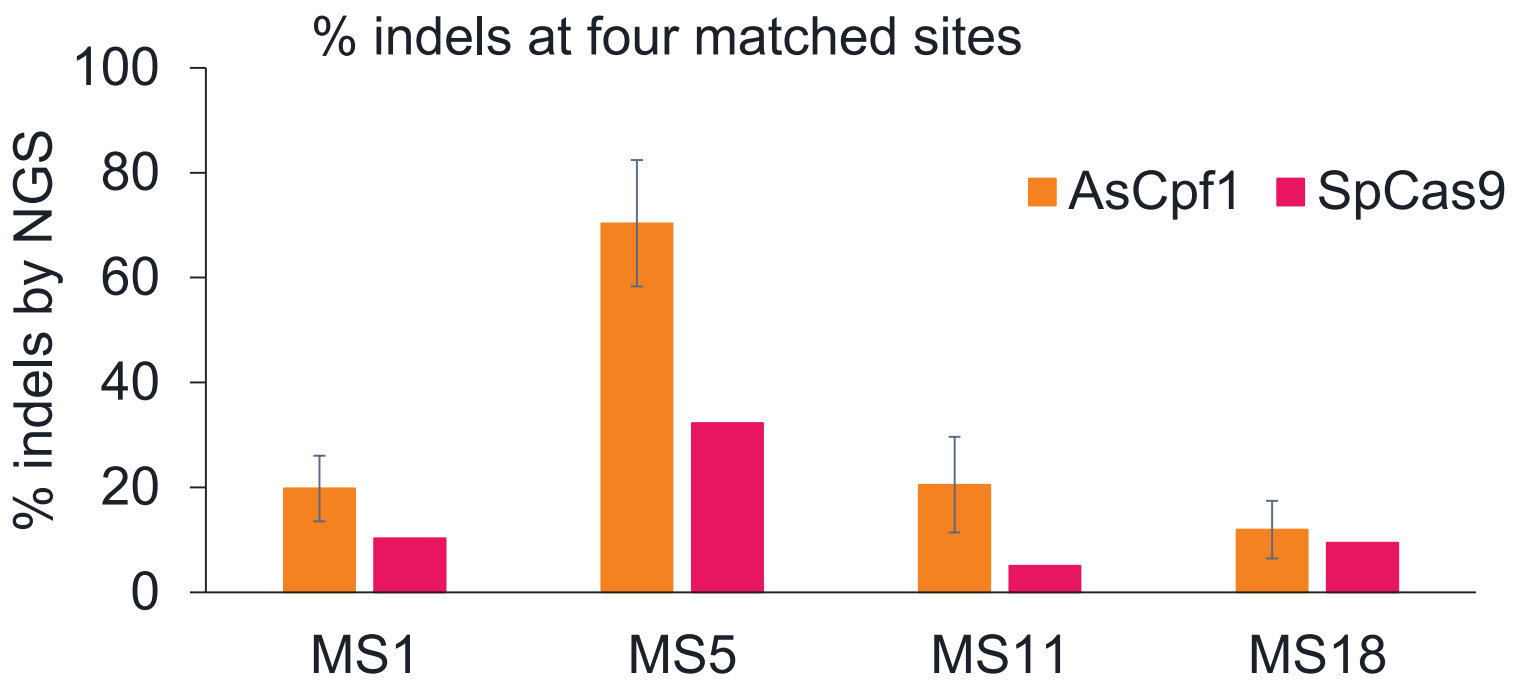


Cpf1 editing at T-cell targets



Ex-vivo editing of Cpf1 at sample locus

Sample editing by AsCPf1 in HSCs



Summary and Conclusions

- A flexible platform allows for lead discovery screening and optimization at scale
- Wealth of standardized data allows for insights into nuclease function and its interplay with biology to engineer a therapeutic outcome
- Modular gRNA allow for controlling the core of the RNP medicine
- Cpf1 editing points to greater flexibility in developing CRISPR medicines

Poster : 7, 33 and 68