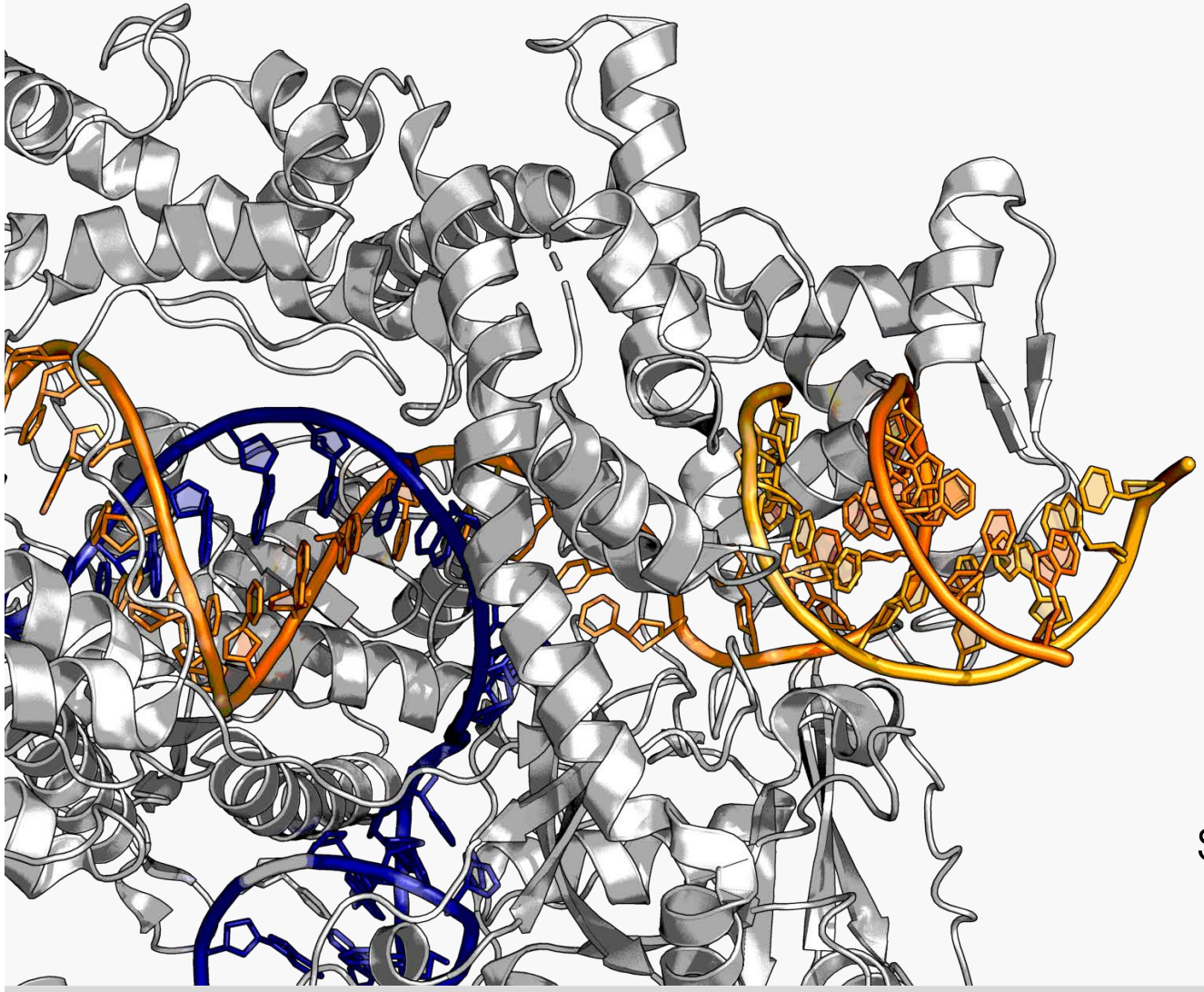
Enhancing Genome Editing with Engineered CRISPR Enzymes



KLEINSTIVER LAB

CENTER FOR GENOMIC MEDICINE

MASSACHUSETTS GENERAL HOSPITAL

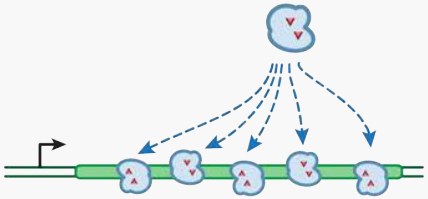
HARVARD MEDICAL SCHOOL

Sunday April 28th, 2019

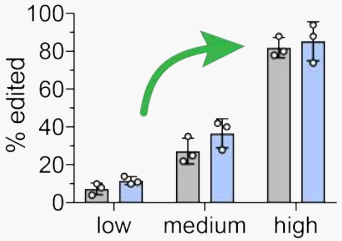
Outline: Expanding the genome editing toolbox

Introduction: Genome editing and targeting range

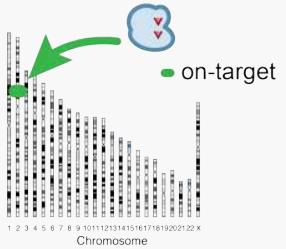
Part 1: Engineering improved CRISPR-**Cas12a** enzymes



targeting range

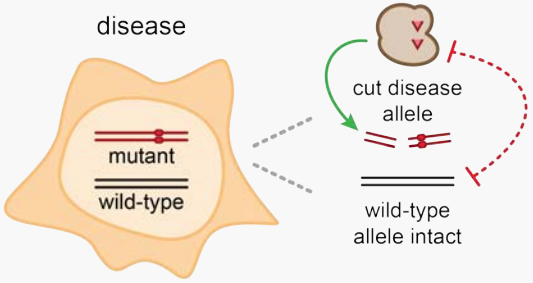


activity



specificity

Part 2: Challenges & applications



- assay development
- protein engineering
- molecular medicines

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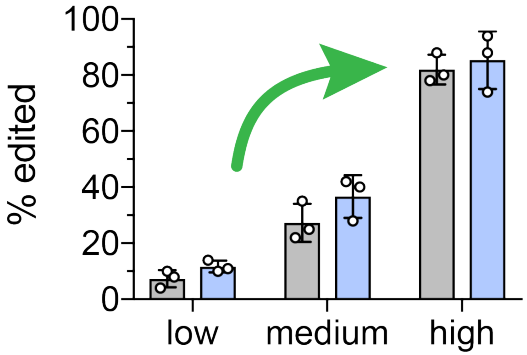
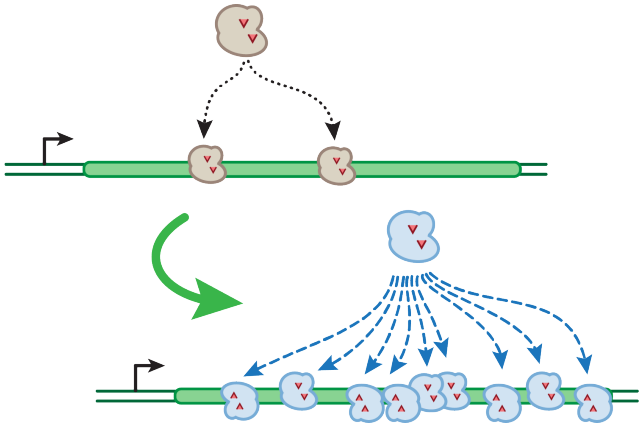
Ideal genome editing properties

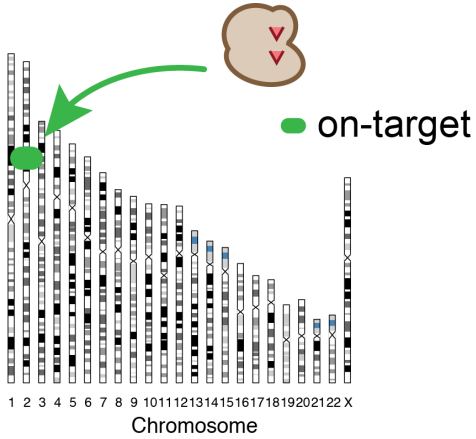
High activity

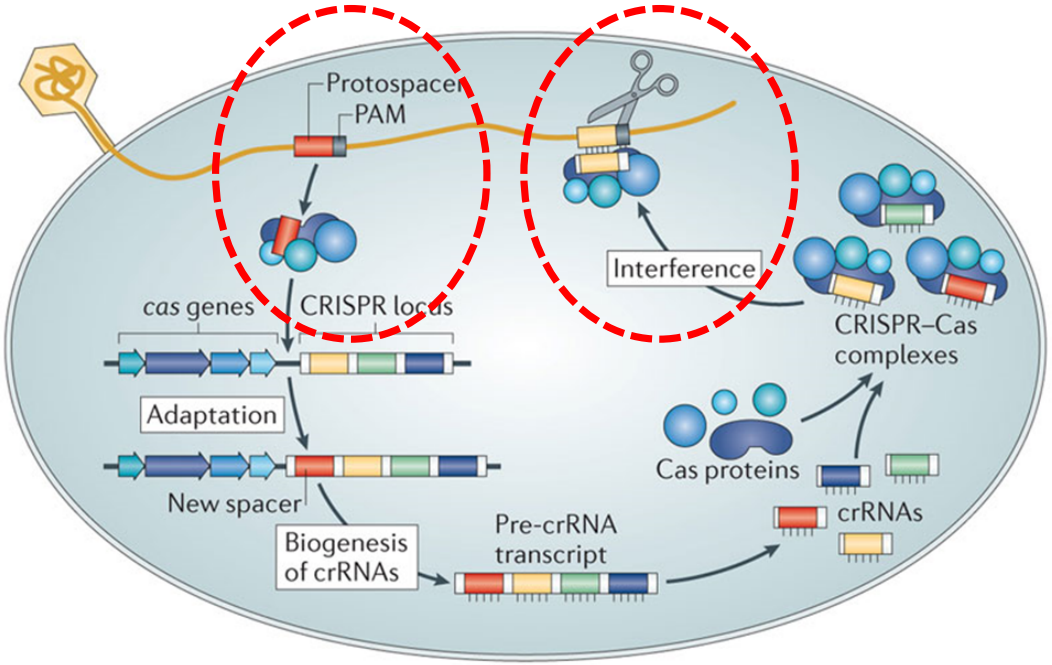


Broadly targetable

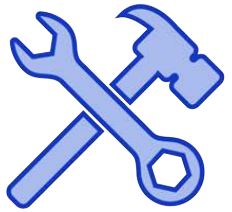


Safe / specific



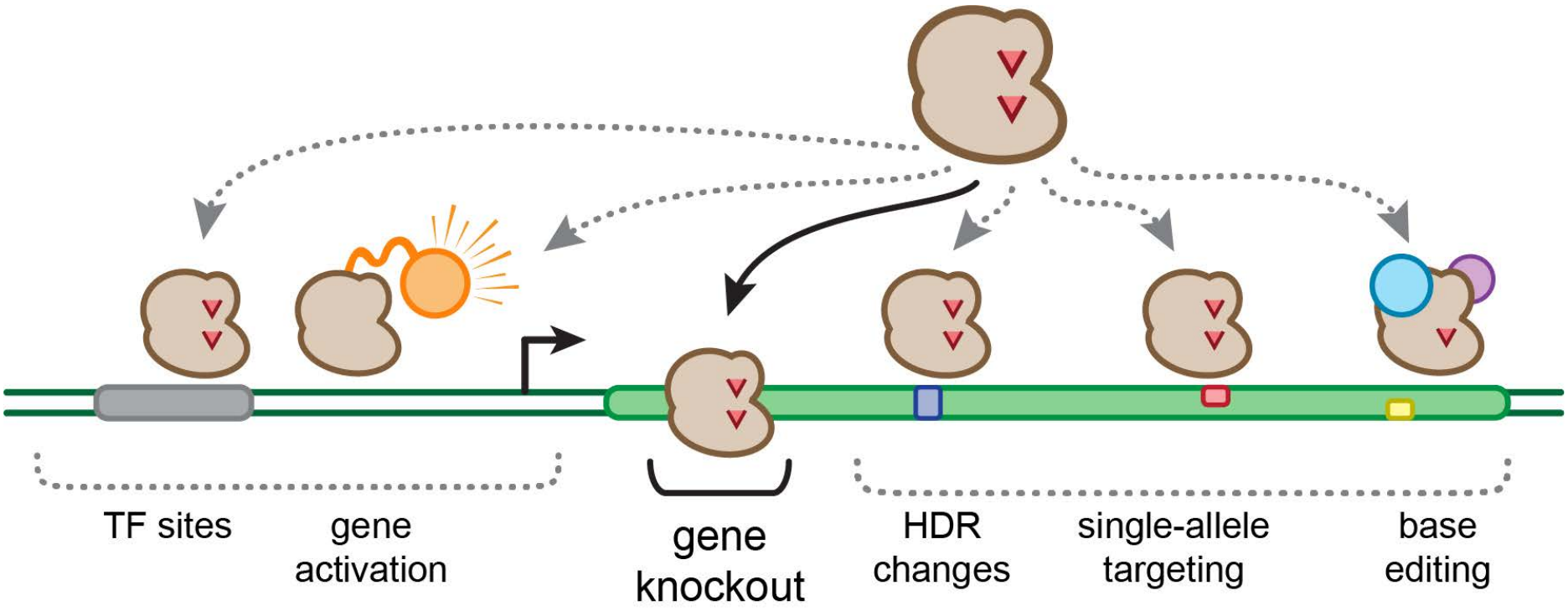


Protein engineering to impart desirable properties



Samson et al. 2013 *Nat. Rev. Micro*

PAM preference can limit editing applications



SpCas9 PAM = NGG = $\frac{1}{8}$ *bp*

SaCas9 PAM = NNGRRT = $\frac{1}{32}$ *bp*

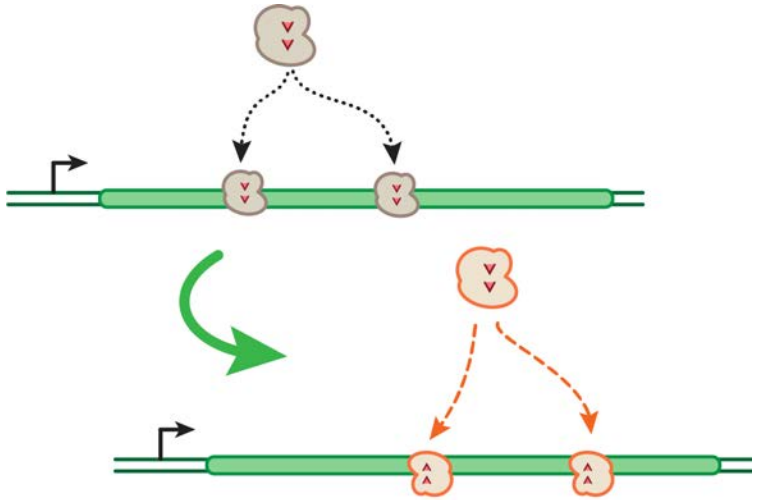
Cas12a PAM = TTTV = $\frac{1}{43}$ *bp*

Ways to expand targeting via protein engineering

wild-type SpCas9 = NGG

SpCas9-VRQR = NGA
Kleinstiver et al., *Nature*, 2016

SpCas9-VRER = NGCG
Kleinstiver et al., *Nature*, 2015

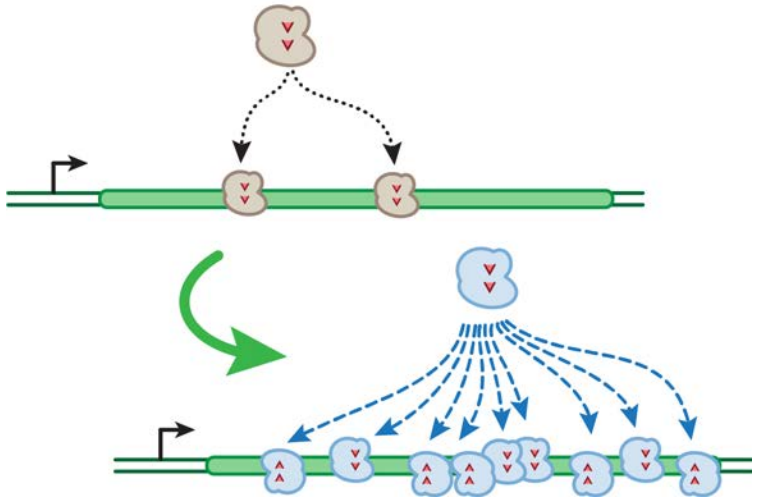


[1]
alter PAM

SELECTIVE

xCas9 = NGN
Hu et al., *Nature*, 2018

SpCas9-NG = NGN
Nishimasu et al., *Science*, 2018



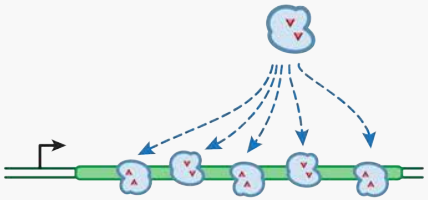
[2]
relax PAM

EXPANDED

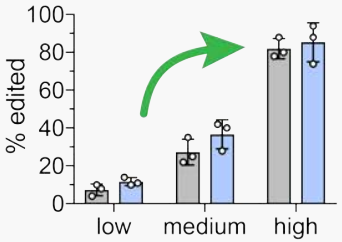
Outline: Expanding the genome editing toolbox

Introduction: Genome editing technologies

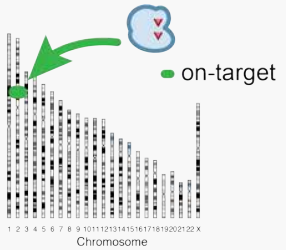
Part 1: Engineering improved CRISPR-**Cas12a** enzymes



targeting range

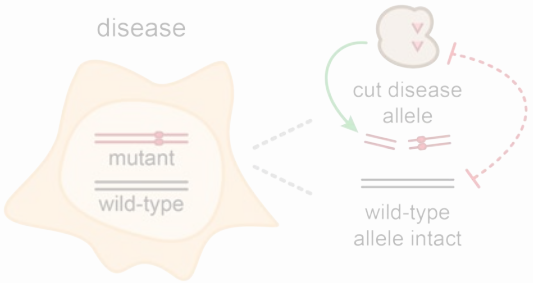


activity



specificity

Part 2: Challenges & applications



- assay development
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- molecular medicines

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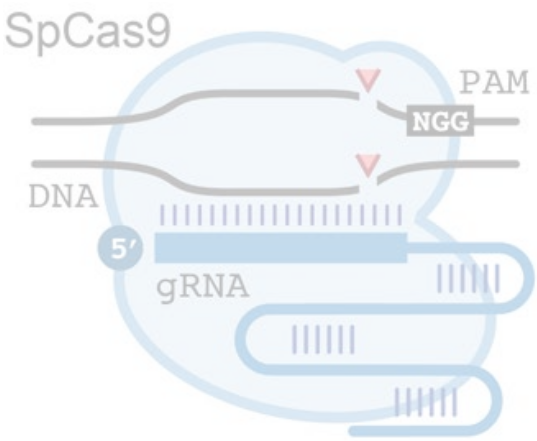
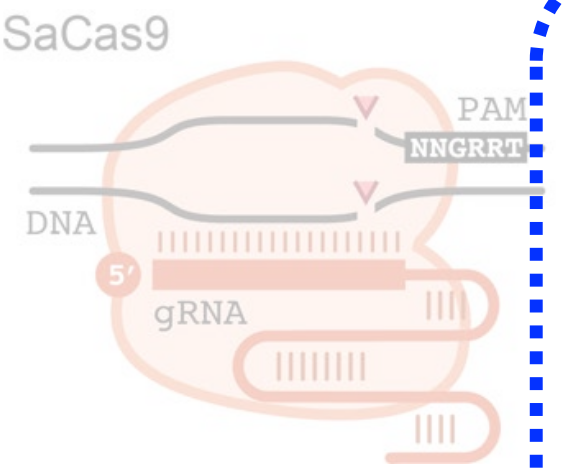
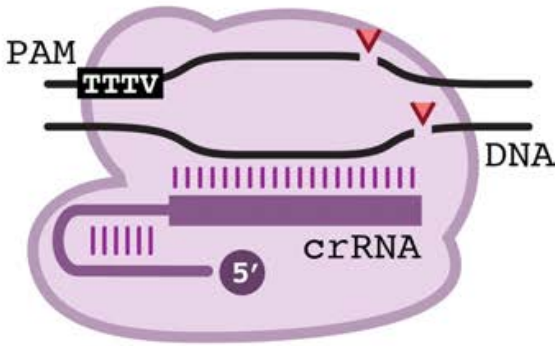
PAM requirement prohibits wide use of Cas12a







CRISPR type	type II-A	type II-A	type V-A
Size (# AA)	1368	1053	~1300
PAM	NGG (3’)	NNGRRT (3’)	TTTV (5’)
DNA break	blunt, PAM prox.	blunt, PAM prox.	5’ overhang, PAM distal
guide RNA	~100nt (sgRNA)	~100nt (sgRNA)	~40nt, single RNA
multiplex	challenging	challenging	simple

Improving CRISPR-Cas12a targeting range

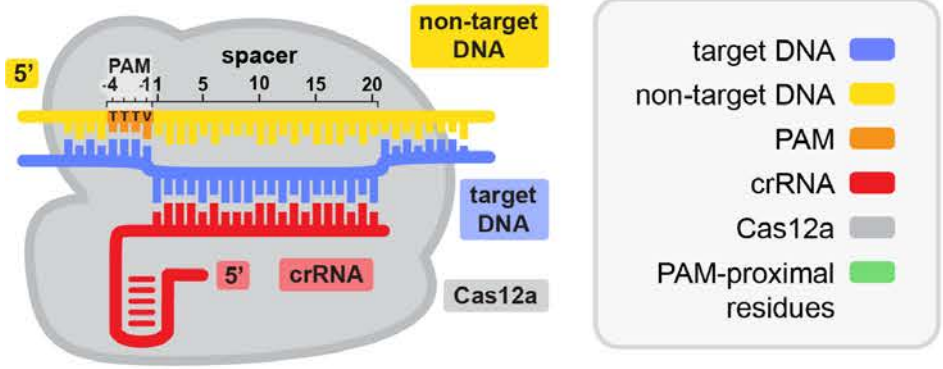
Methods to improve properties:

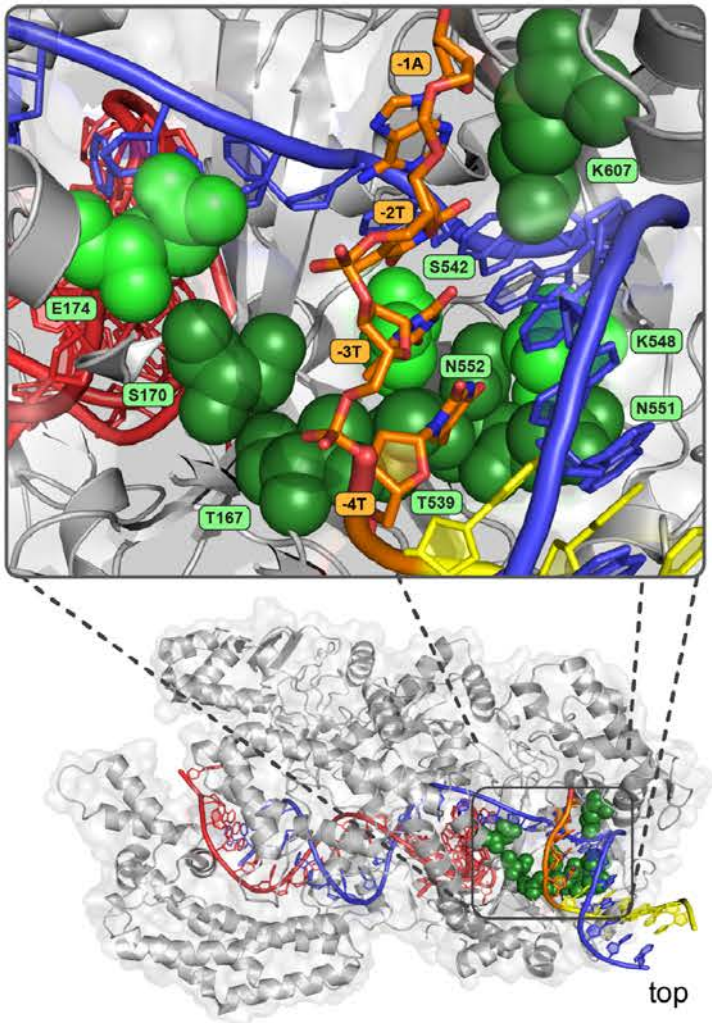
a. Orthologs

b. Engineering

i. Directed evolution

ii. Structure-guided



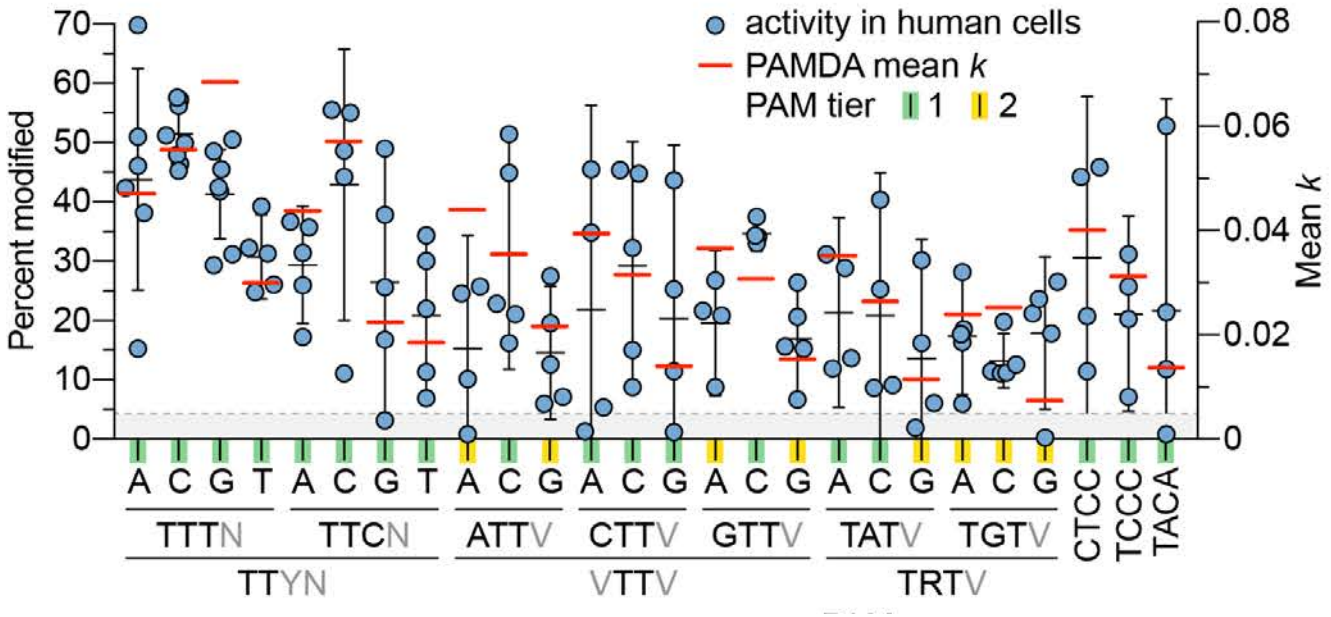


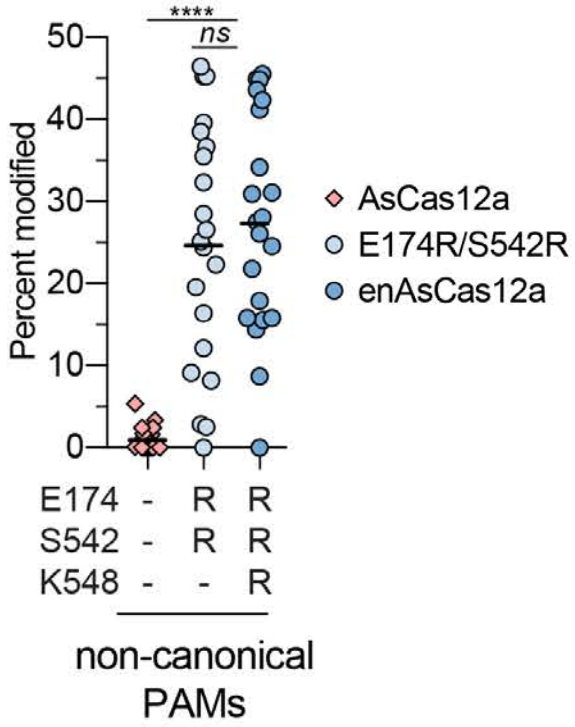
enAsCas12 → expanded PAM preference

Alex Russell

Kleinstiver et al., *Nature Biotechnol.*, 2019

Expanded targeting in human cells with enAsCas12a





AsCas12a = **TTTV**

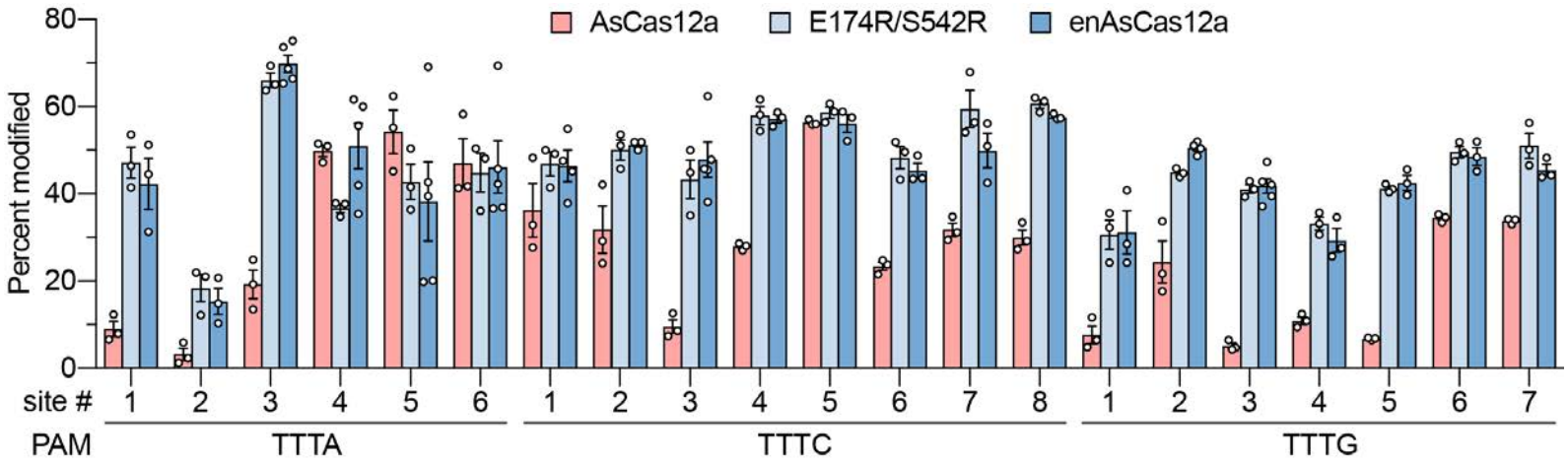
enAsCas12a = **TTTV**, TTTT, TTCN, VTTV, TRTV, and more

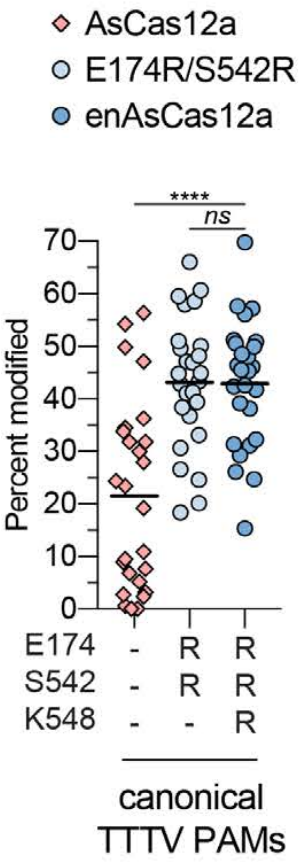
~7-fold expanded targeting range

[2] relax PAM preference

Kleinstiver et al., *Nature Biotechnol.*, 2019

Improved nuclease activity with enAsCas12a





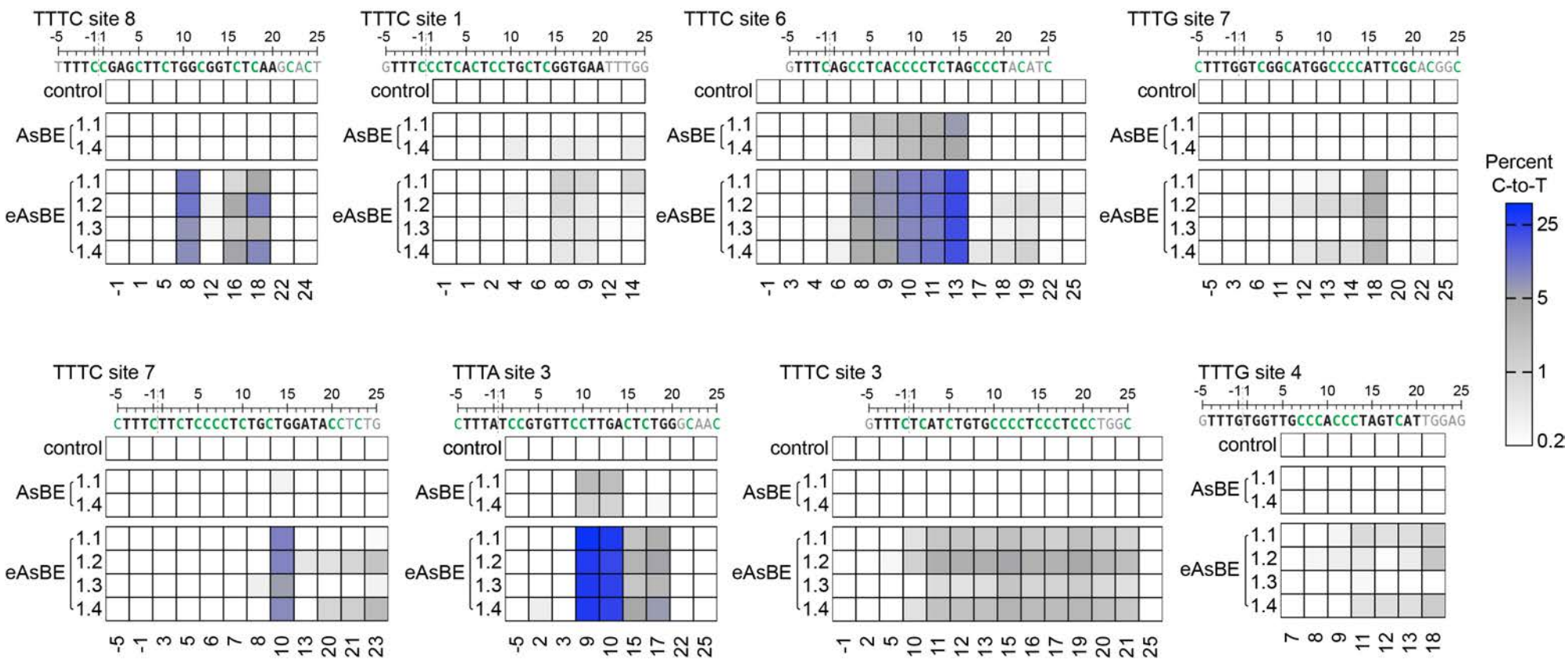
~7-fold expanded targeting range

~2-3 fold improved on-target activity

enAsCas12a

Kleinstiver et al., *Nature Biotechnol.*, 2019

Improved base editing with enAsCas12a



enAsCas12a

~5-10 fold improved base editor activity

Kleinstiver et al., *Nature Biotechnol.*, 2019

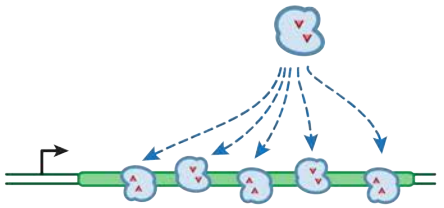
Summary 1: Improved activities of enAsCas12a

enAsCas12a

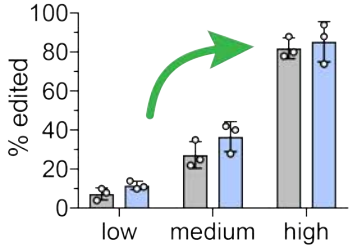
Engineered CRISPR-Cas12a variants with increased activities and improved targeting ranges for gene, epigenetic and base editing

Benjamin P. Kleinstiver[1,2,3,4,10*], Alexander A. Sousa[1,2,3,12], Russell T. Walton[1,2,3,10,12], Y. Esther Tak[1,2,3,4], Jonathan Y. Hsu[1,2,3,5], Kendell Clement[1,2,4,6], Moira M. Welch[1,2,3], Joy E. Horng[1,2,3], Jose Malagon-Lopez[1,2,3,4,7,11], Irene Scarfò[2,8,9], Marcela V. Maus[2,8,9], Luca Pinello[1,2,4,6], ...rtin J. Aryee[1,2,4,6,7] and J. Keith Joung[1,2,3,4*]

Kleinstiver et al., *Nature Biotechnology*, 2019

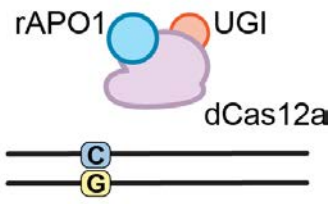


targeting range

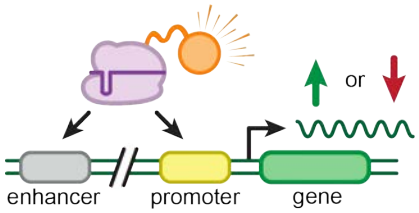


activity

- ~7-fold **expanded** targeting range
- ~2-3 fold improved on-target activity
- enhanced activities at lower temperatures
- can improve other variants (~2-fold, enRVR & enRR)
- improved base editing & gene activation
- efficient editing in primary human T cells



base editing

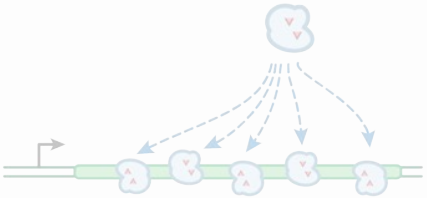


epigenome editing

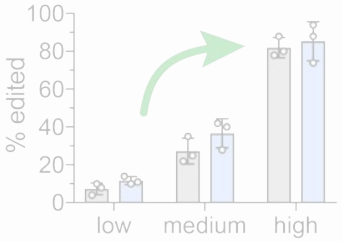
Outline: Expanding the genome editing toolbox

Introduction: Genome editing technologies

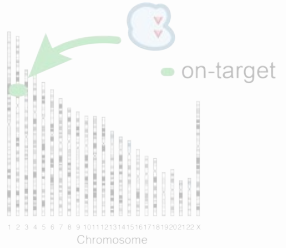
Part 1: Engineering improved CRISPR-**Cas12a** enzymes



targeting range

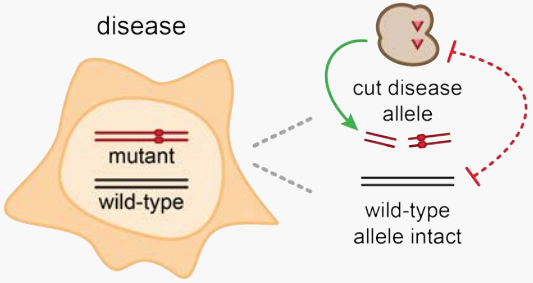


activity



specificity

Part 2: Challenges & applications



- assay development
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- molecular medicines

Russell Katie Joey

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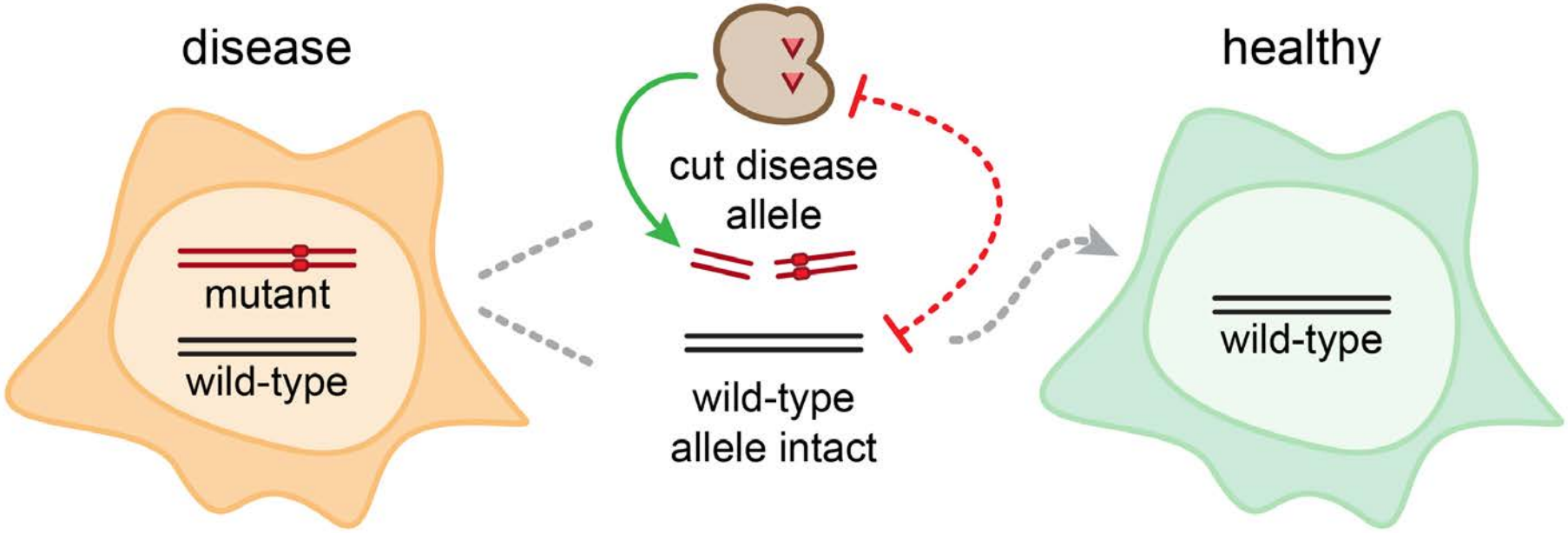
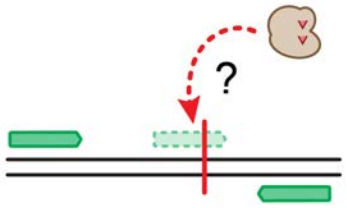
Challenge: Single allele editing



Important properties for allele-specific editing

targeting range



Position the SNP in PAM

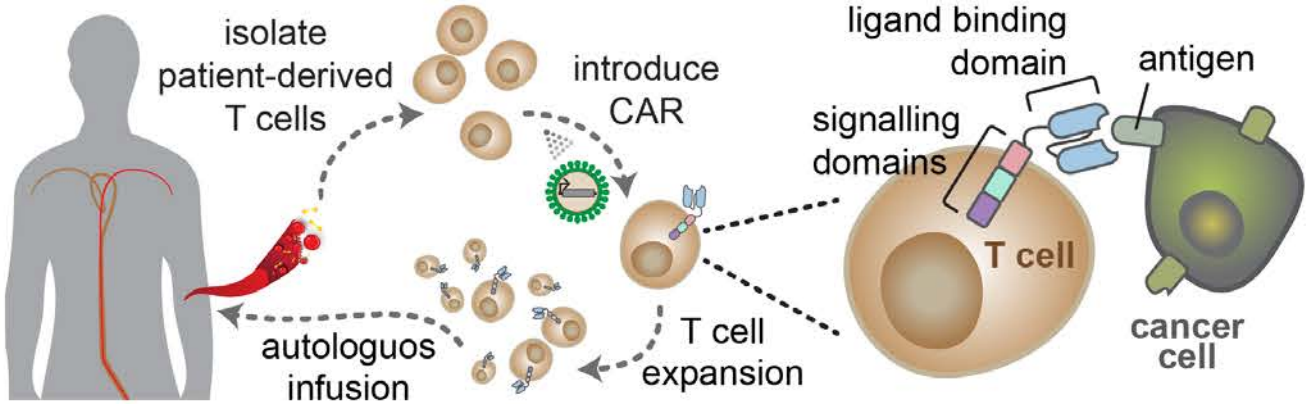
NGG

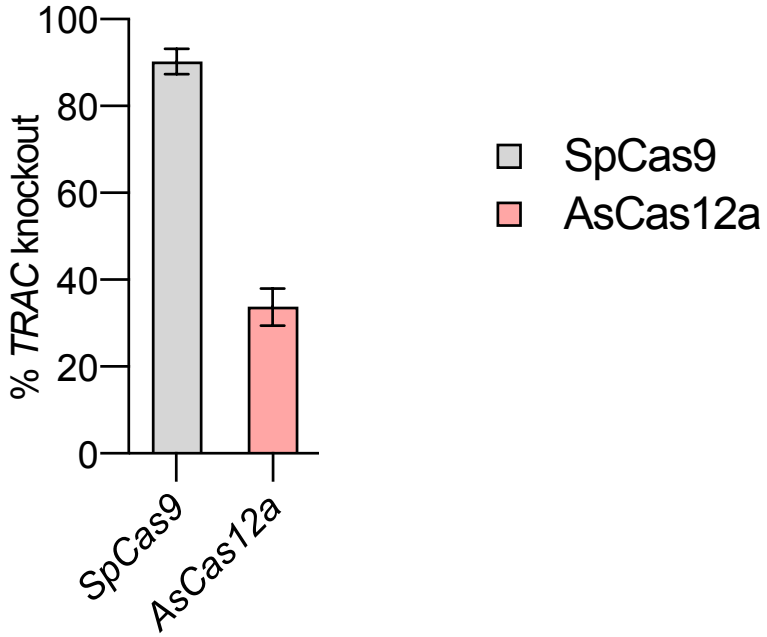
Mut-GATCCAATCGGAATCGGATTTCG

WT-GATCCAATCGGAATCGTATTTCG

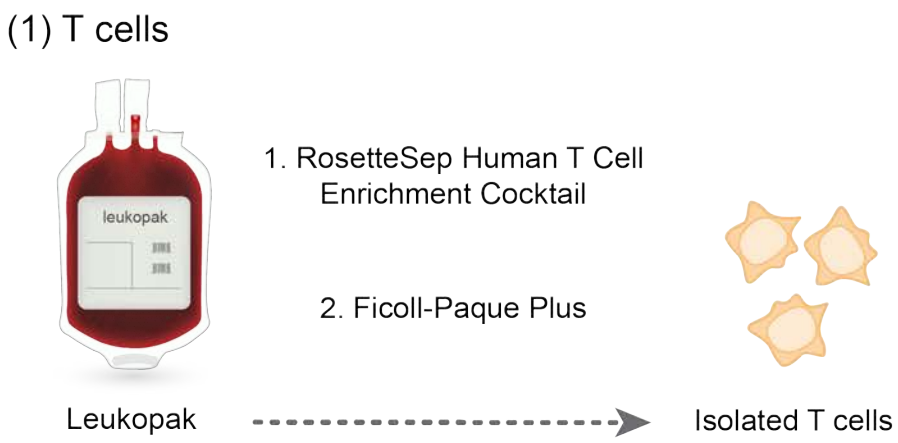
Need library of PAM selective variants

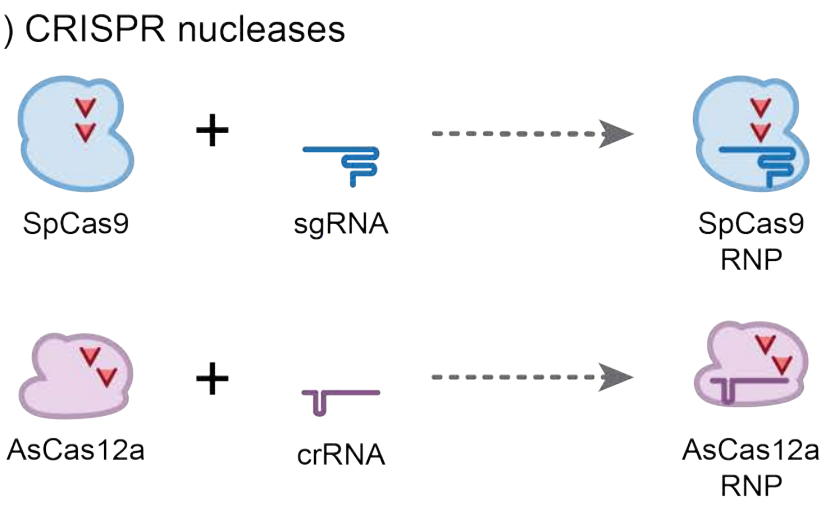
Challenge: Assessing improved tools in primary cells

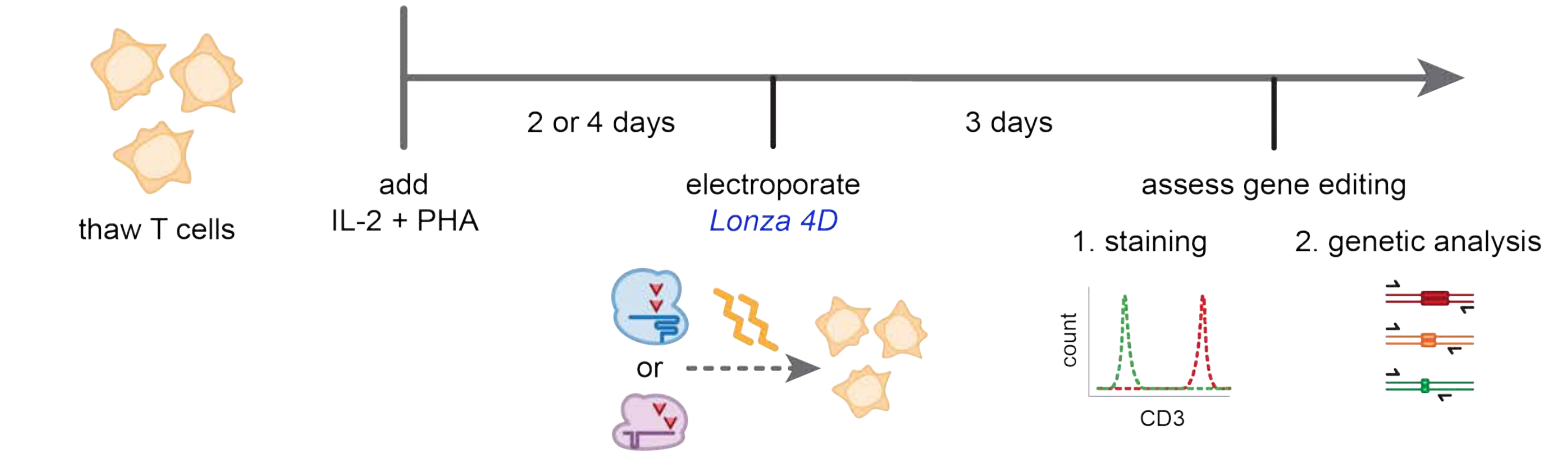




Building a primary human T cell editing workflow

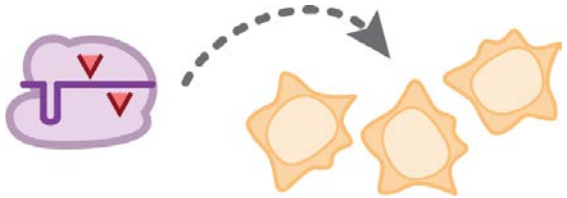


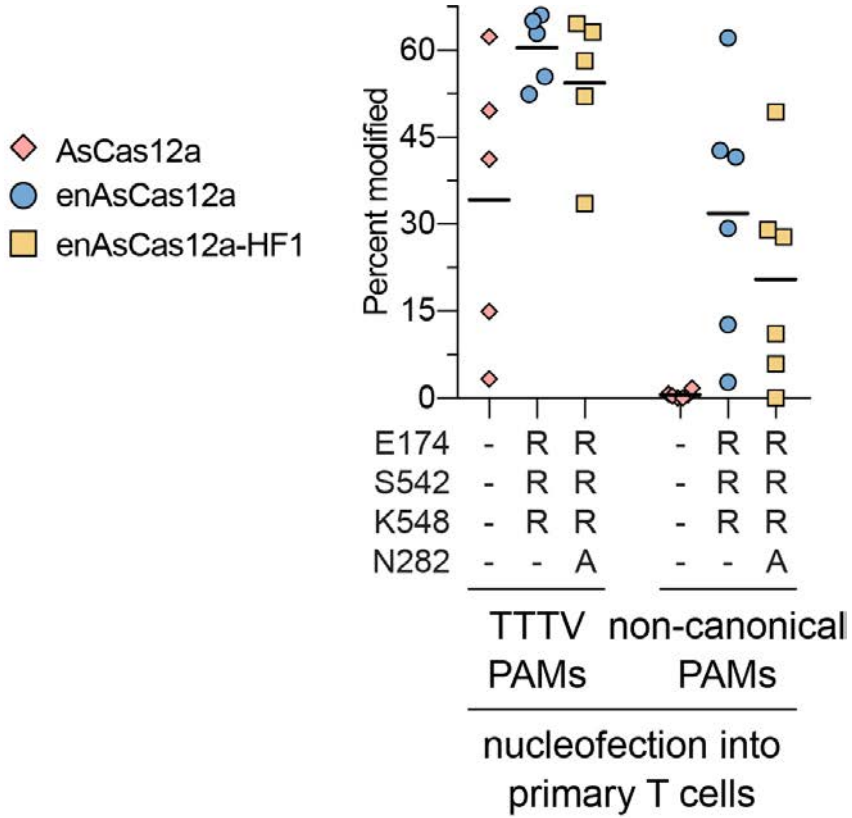




Kleinstiver et al., *Nature Biotechnol.*, 2019

RNP delivery of enAsCas12a in human cells

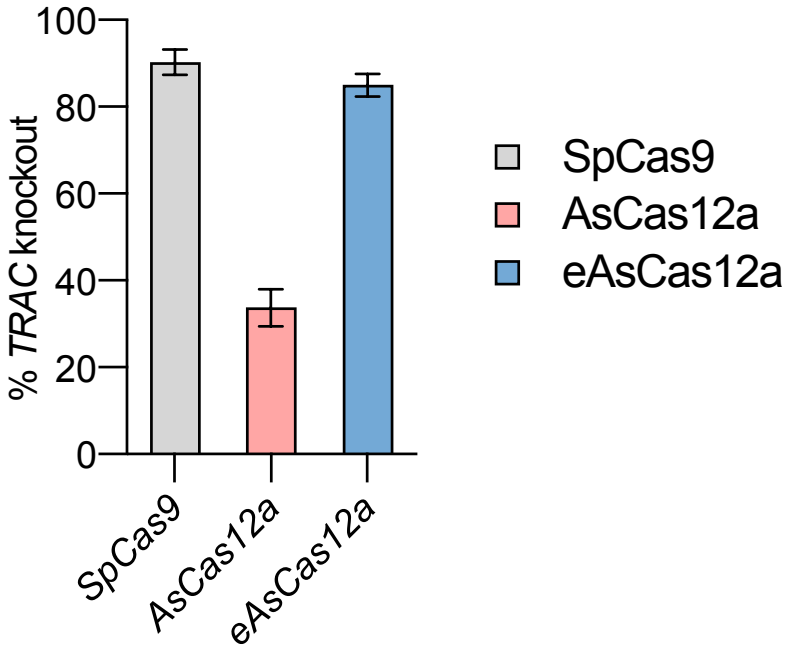


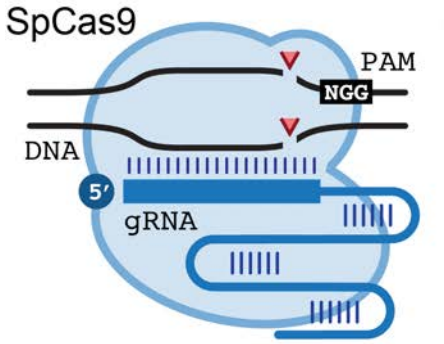


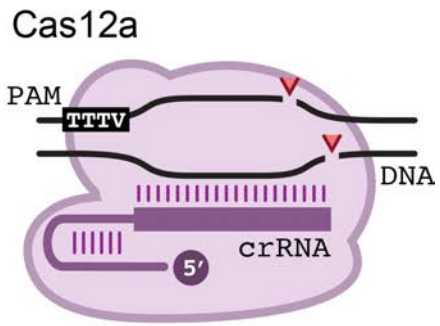
Cas12a

Kleinstiver et al., *Nature Biotechnol.*, 2019

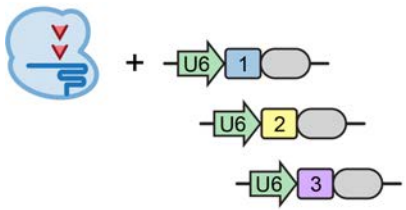
RNP delivery of CRISPR nucleases in primary T cells

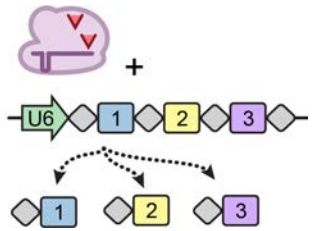






CRISPR type	type II-A	type V-A
Size (# AA)	1368	~1300
PAM	NGG (3')	TTTV (5')
DNA break	blunt, PAM prox.	5' overhang, PAM distal
guide RNA	~100nt (sgRNA)	~40nt, single RNA
multiplex	challenging	simple

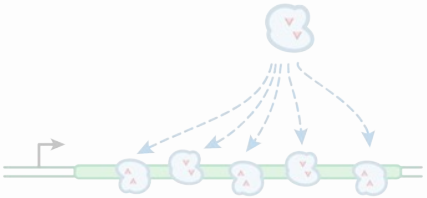




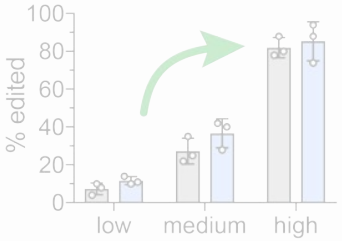
Summary 2: Addressing challenges of genome editing

Introduction: Genome editing technologies

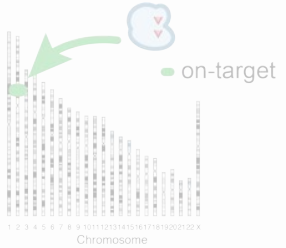
Part 1: Engineering improved CRISPR-**Cas12a** enzymes



targeting range

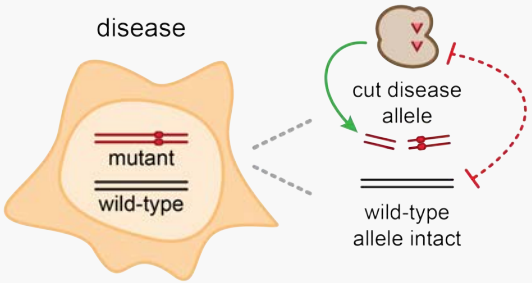


activity



specificity

Part 2: Challenges & applications



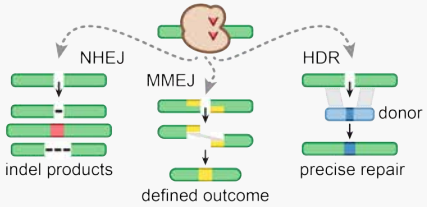
- assay development
- **PAM selective** Cas9 variants
- Activities in 1º T cells

Russell Katie Joey

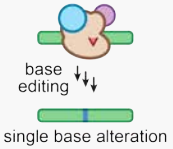
Kleinstiver Lab

Summary + Research in the Kleinstiver lab

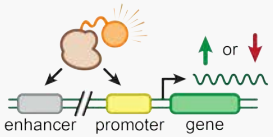
Genome editing technologies



genome editing

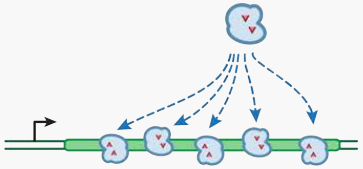


base editing

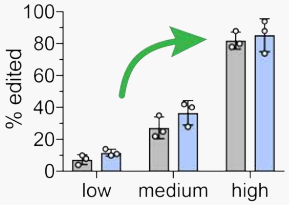


epigenome editing

Protein engineering to enhance CRISPR enzymes



targeting range



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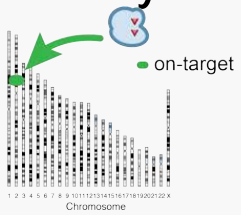
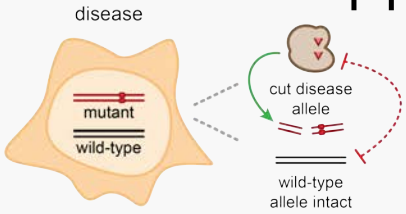
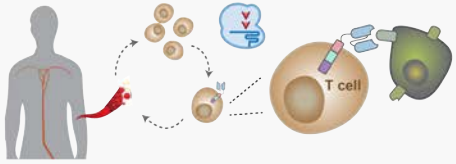


specificity

Molecular medicines + applications



allele specificity



disease applications

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Katie Christie


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Recruiting postdocs, students, & technicians