Base Editing: from animal models to human embryos

Technical Journal Club – Special Series on Laboratory Animal Science

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Overview

I. Introduction

II. Establishment of base editing technology

III. Application of BE in animal models and human embryos

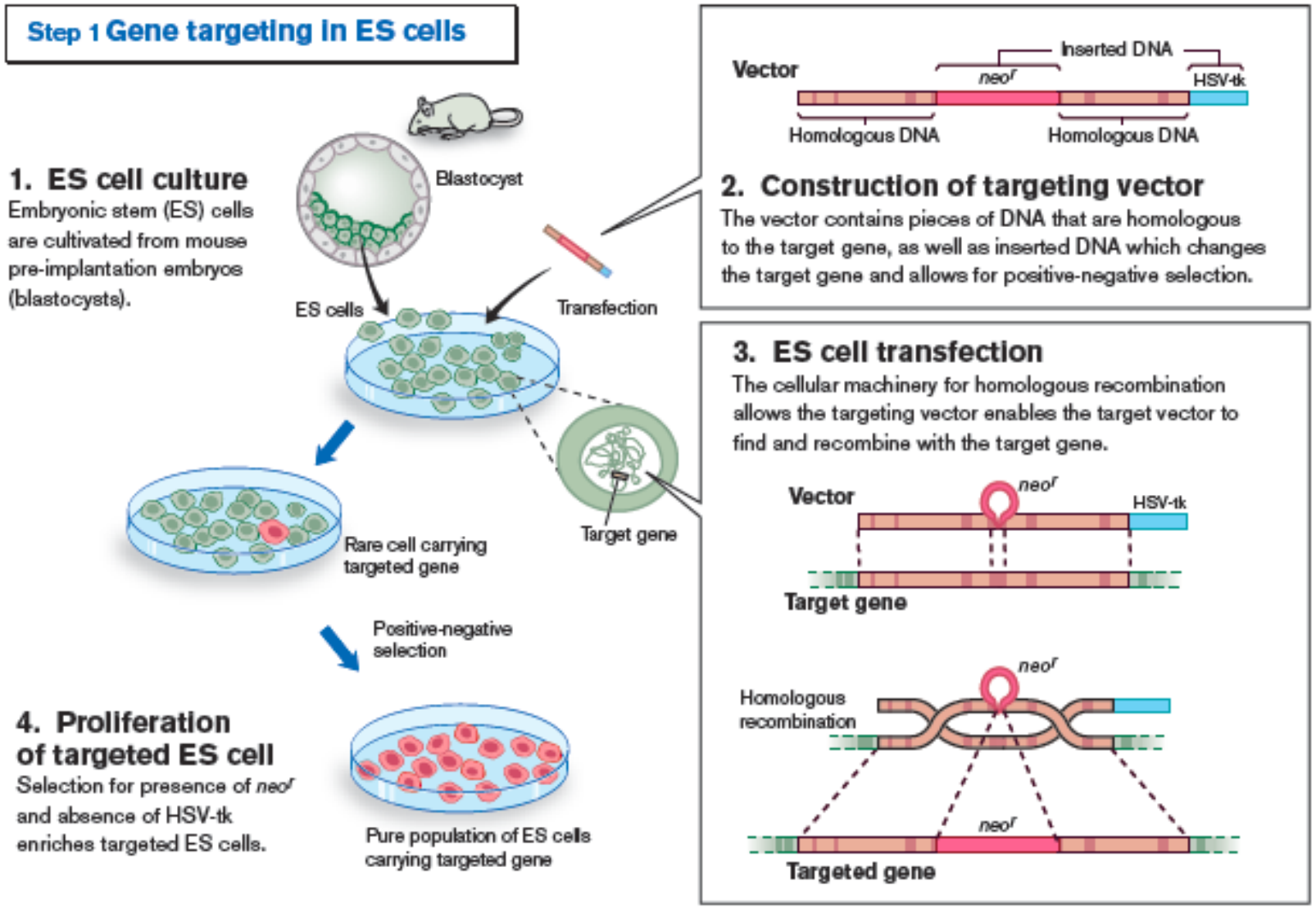
IV. Future directions

Point mutations and human diseases

- The vast majority of human genetic diseases are caused by single-nucleotide substitutions or point mutations rather than small insertions/deletions (indels) or large chromosomal rearrangements in the genome (cystic fibrosis, sickle cell disease, genetic prion diseases etc.).

- Correct these mutated nucleotides to wild-type by base editing holds promises of gene therapy.

General strategy for gene targeting in ES cells

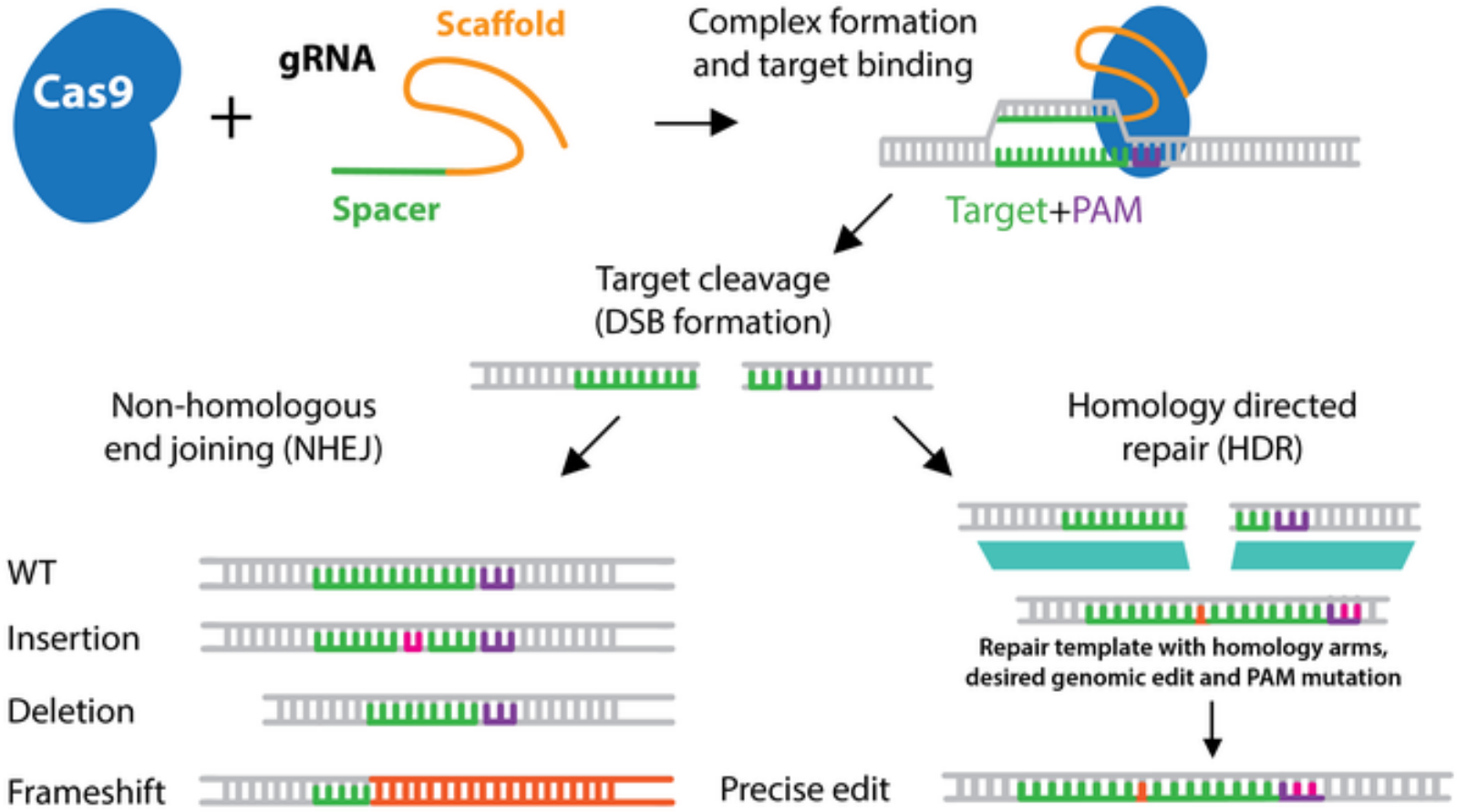


https://www.nobelprize.org/nobel_prizes/medicine/laureates/2007/press.html

Limitations of conventional gene targeting methodology

- Low frequency of gene targeting v.s random integration by conventional method (1 in 10^5-10^6 transfected cells or 0.1% in selected clones);
- One allele is targeted, sometimes not sufficient to completely rescue the diseases;
- Not applicable for gene therapy in vivo.

CRISPR-Cas9 mediated homology directed repair (HDR)



- **HDR is much less efficient than the NHEJ**

https://www.addgene.org/crispr/history/

CRISPR-Cas9 mediated homology directed repair (HDR)

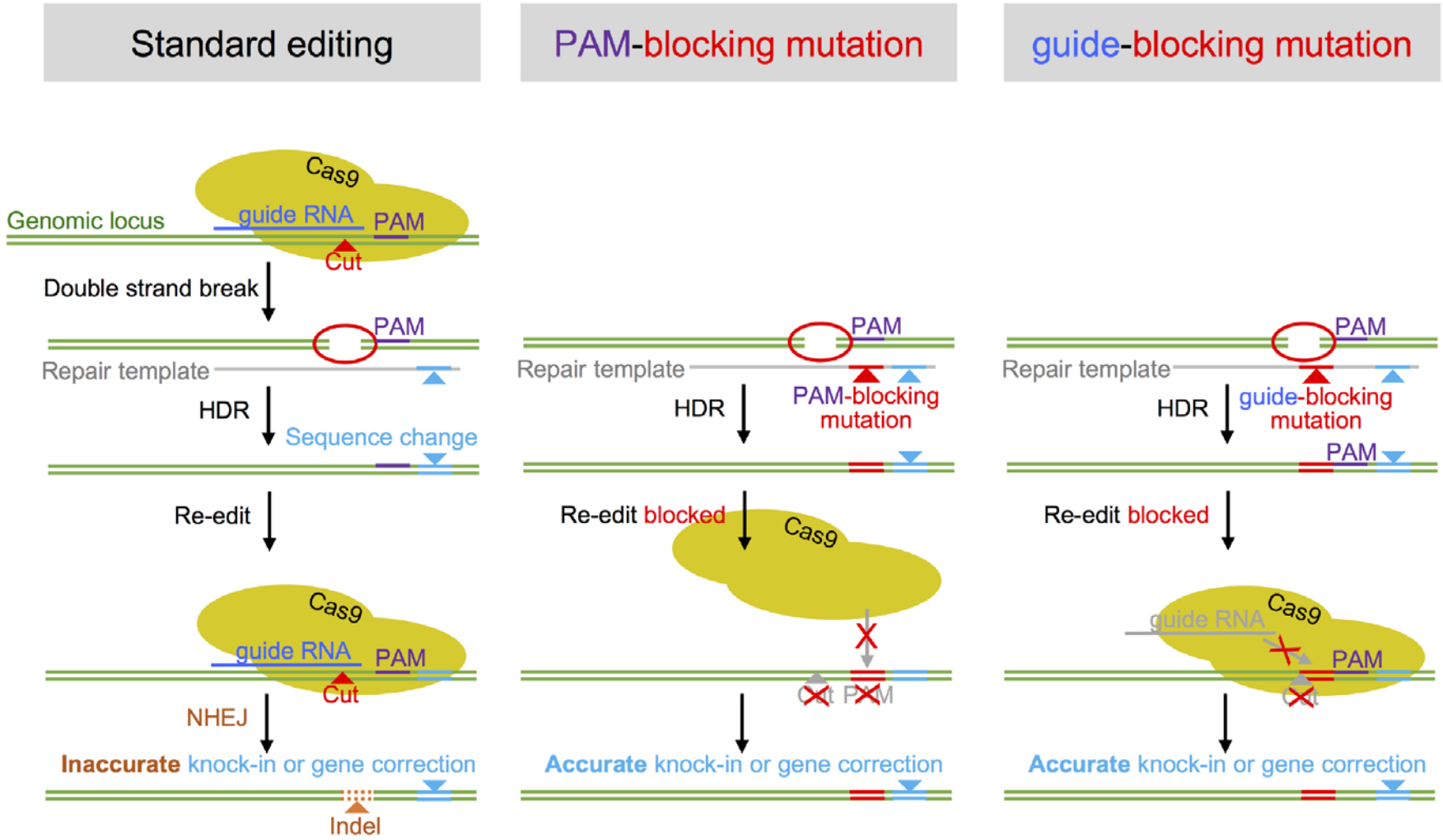


Figure 1: Techniques to improve editing efficiency.

- **Limited improvement: 0.1%-5%**

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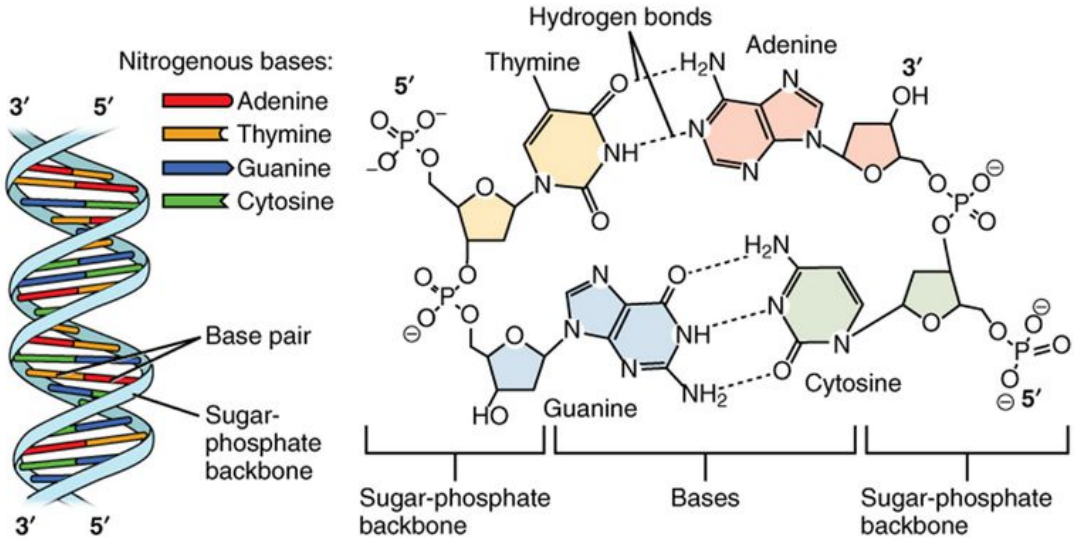
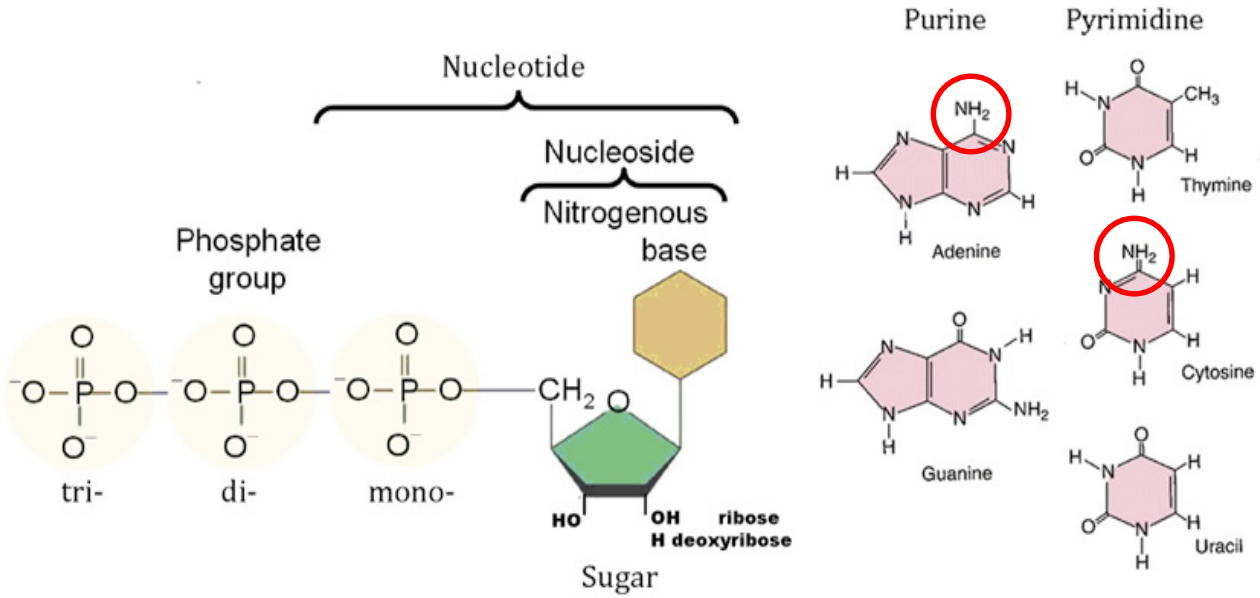
DNA-nucleotides-bases





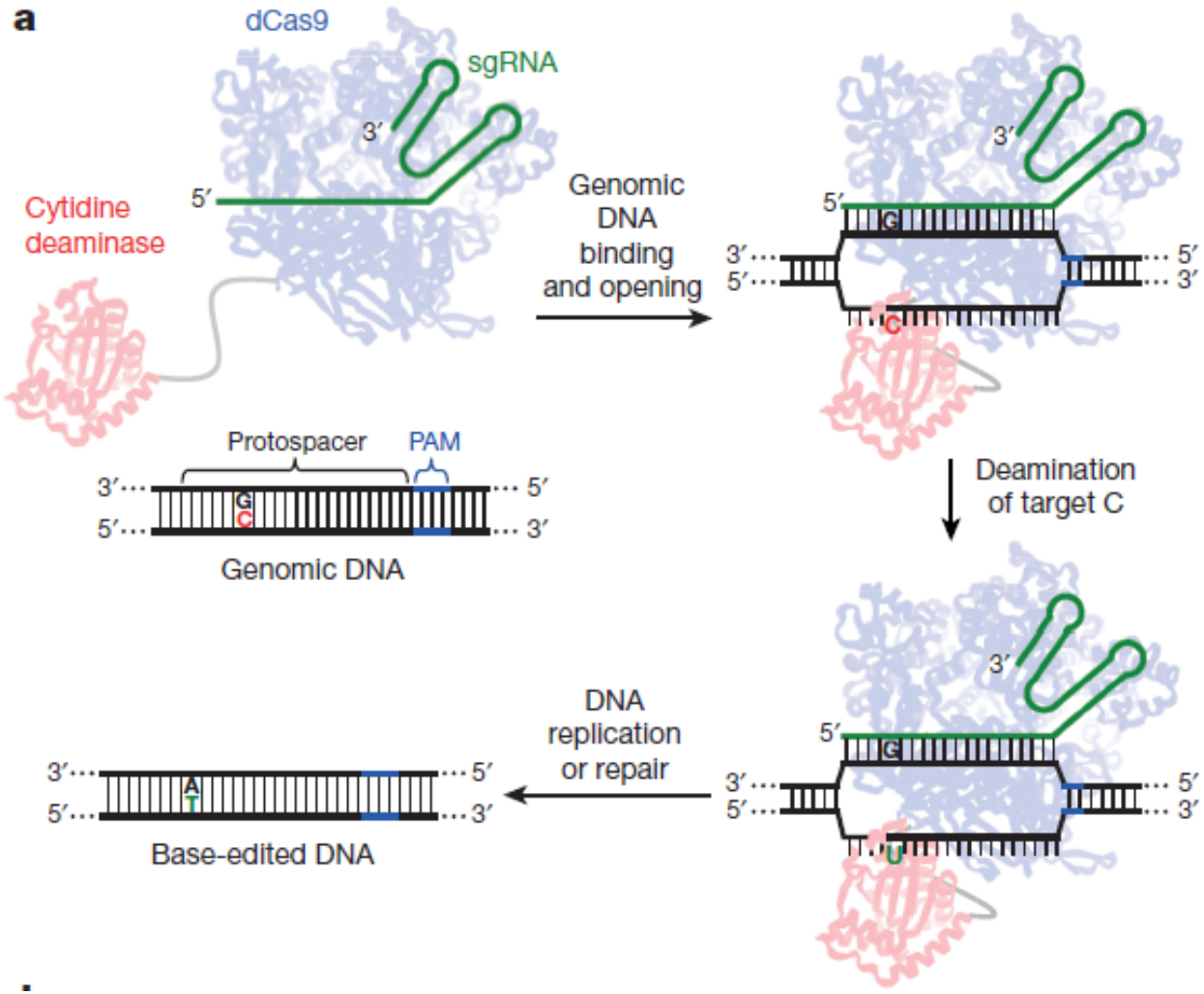
- Use chemistry to modify single nucleotide specifically?

Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage

Alexis C. Komor[1,2], Yongjoo B. Kim[1,2], Michael S. Packer[1,2], John A. Zuris[1,2] & David R. Liu[1,2]

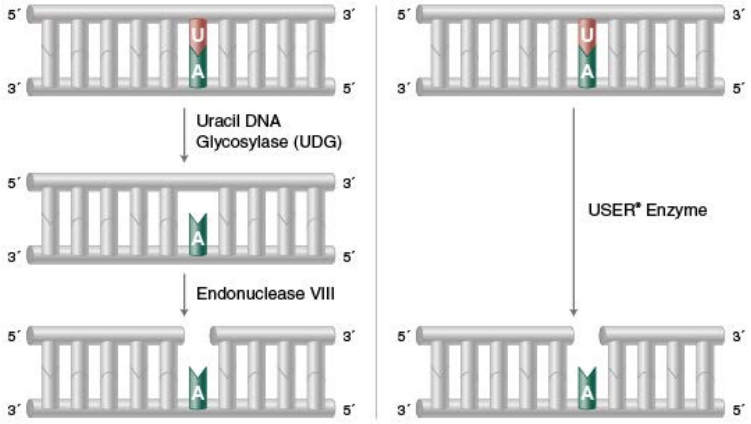
Nature, 2016

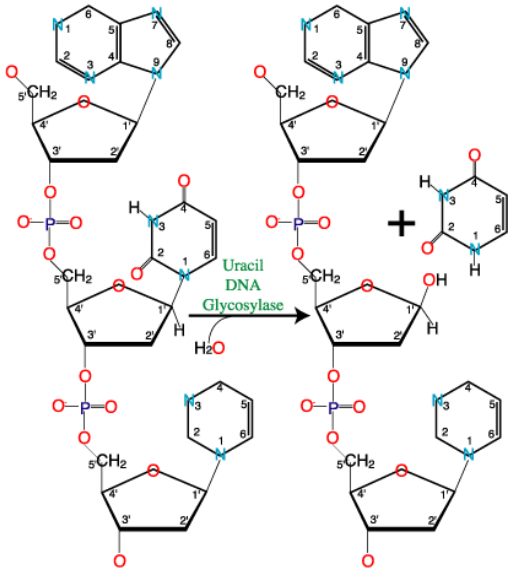
- Most known cytidine deaminases operate on RNA, few accept single strand DNA.



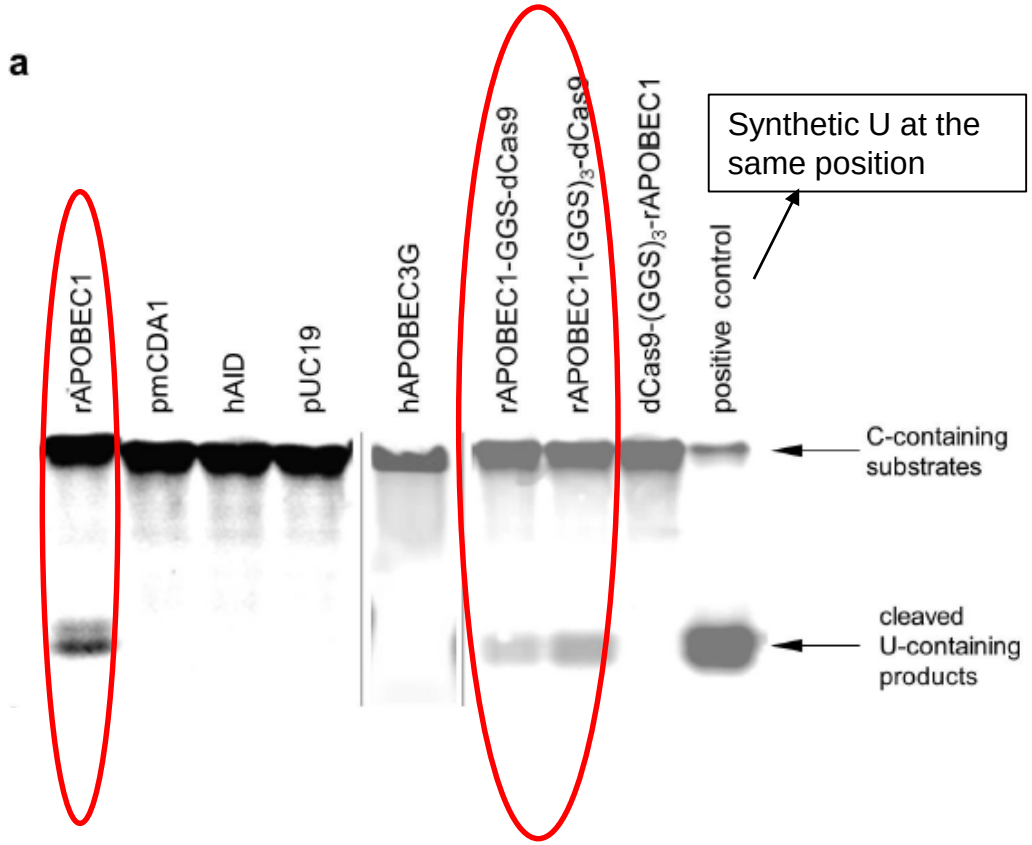
Which cytidine deaminase?

USER: Uracil-Specific Excision Reagent





ssDNA

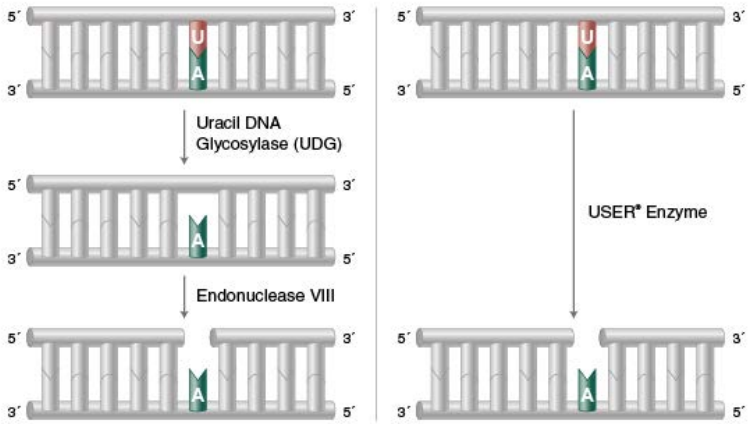


- Rat APOBEC1 (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 1, 229aa, 27kD) showed highest efficiency
- rAPOBEC1 needs to be fused to N- terminus of dCas9

The linker between deaminase-dCas9 and the activity window of the target

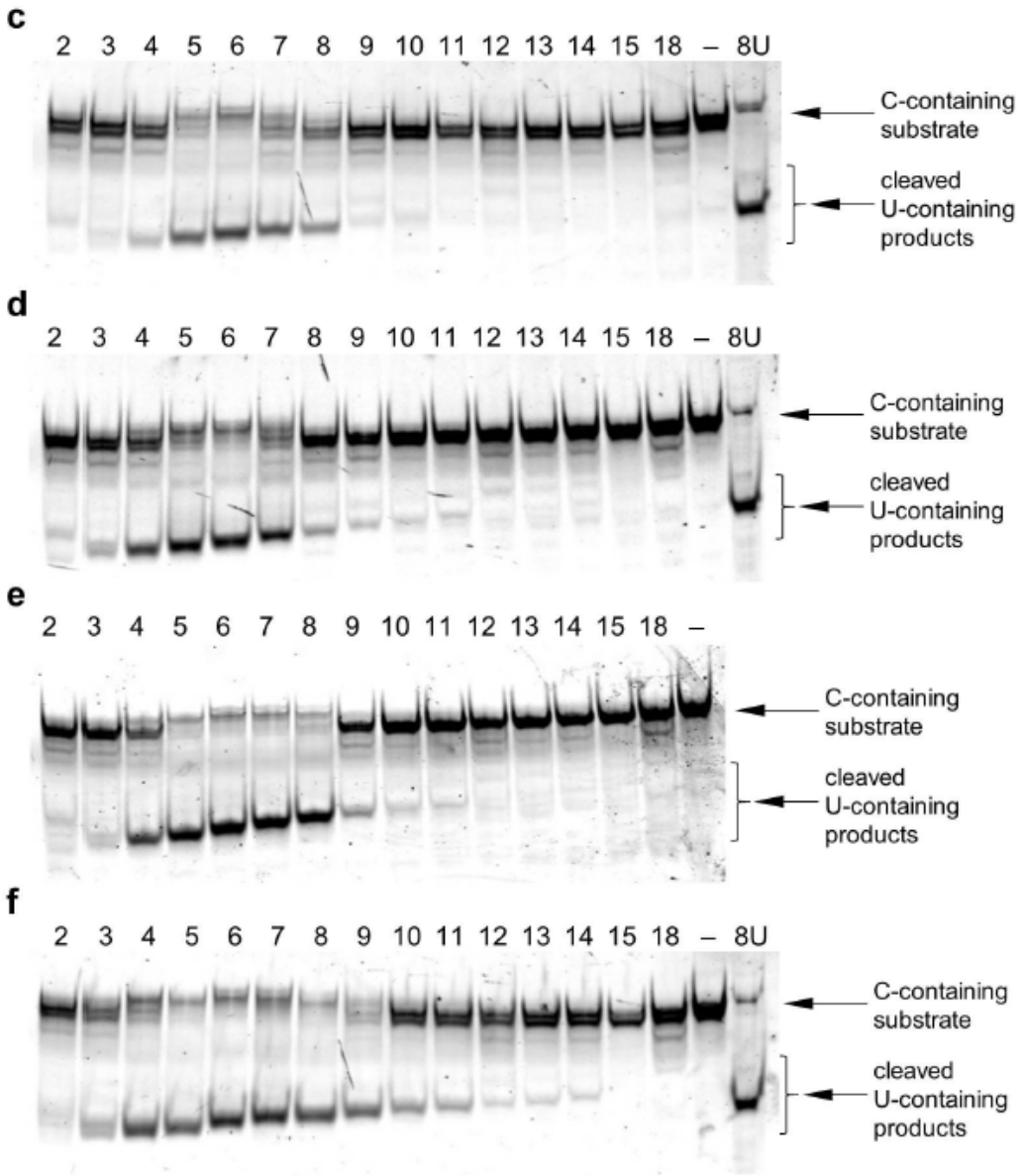
dsDNA

USER: Uracil-Specific Excision Reagent

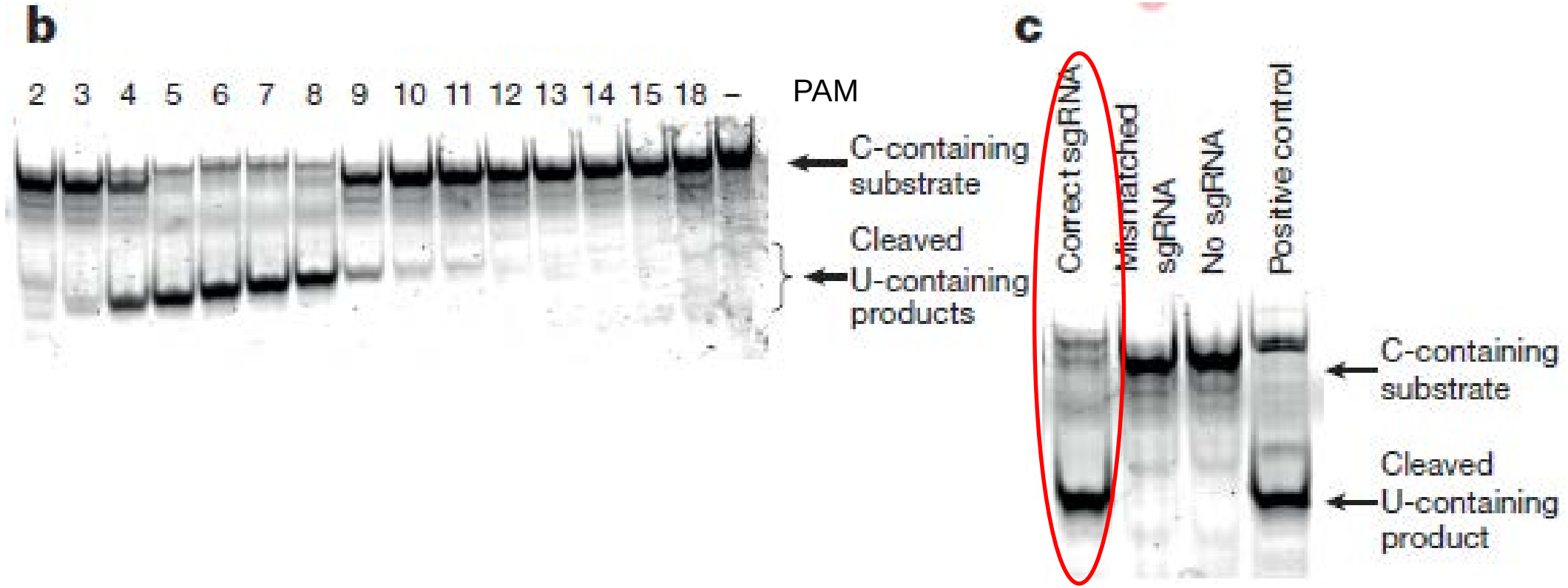


Denature

Gel separation



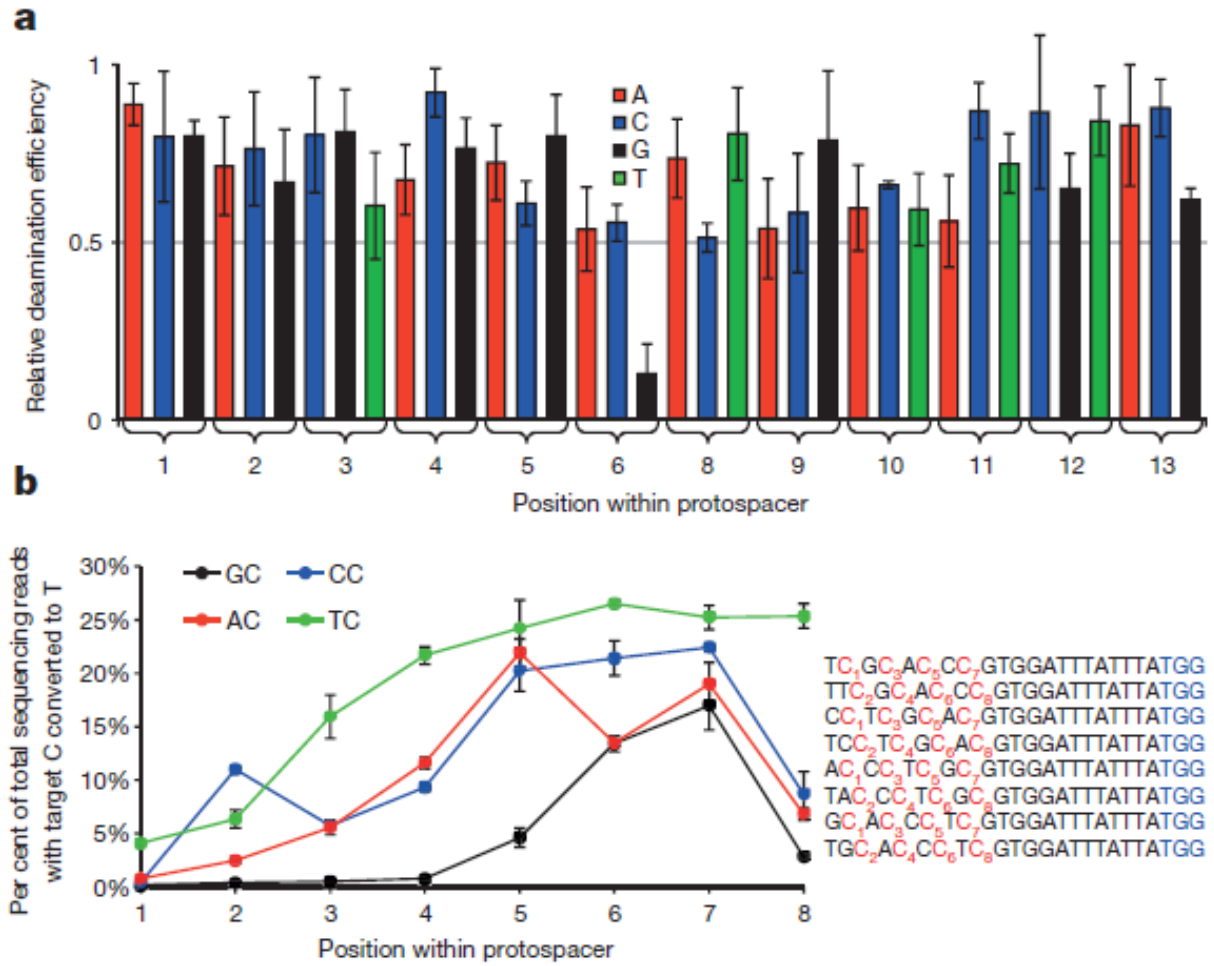
The specificity of BE1: rAPOBEC1-XTEN-dCas9



- BE1 show base editing only when specific sgRNA is presented
- BE1 editing window: position 4-8

Sequence context and target C position

Fixed C_7

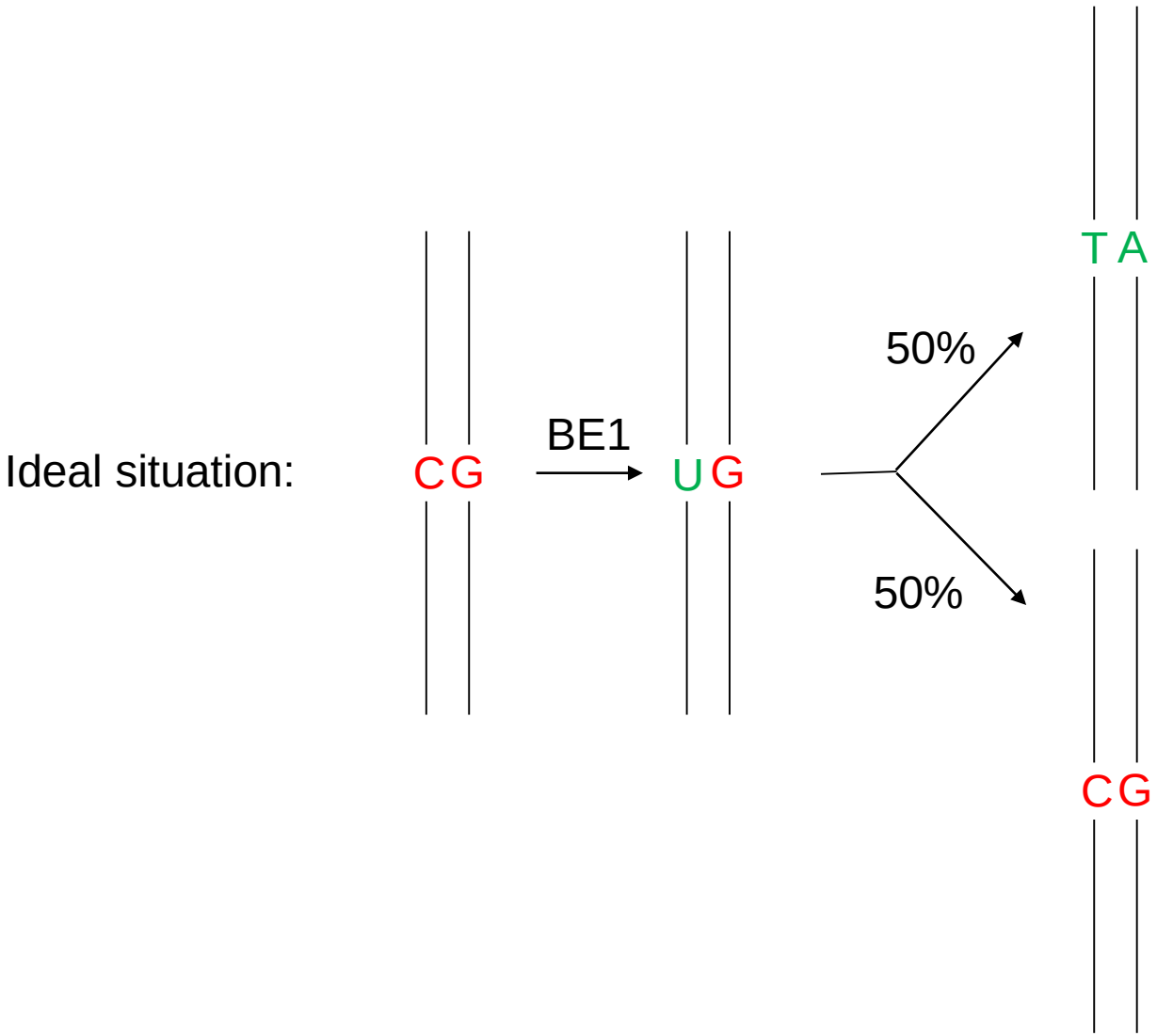


- Independent of sequence context except G_6C_7

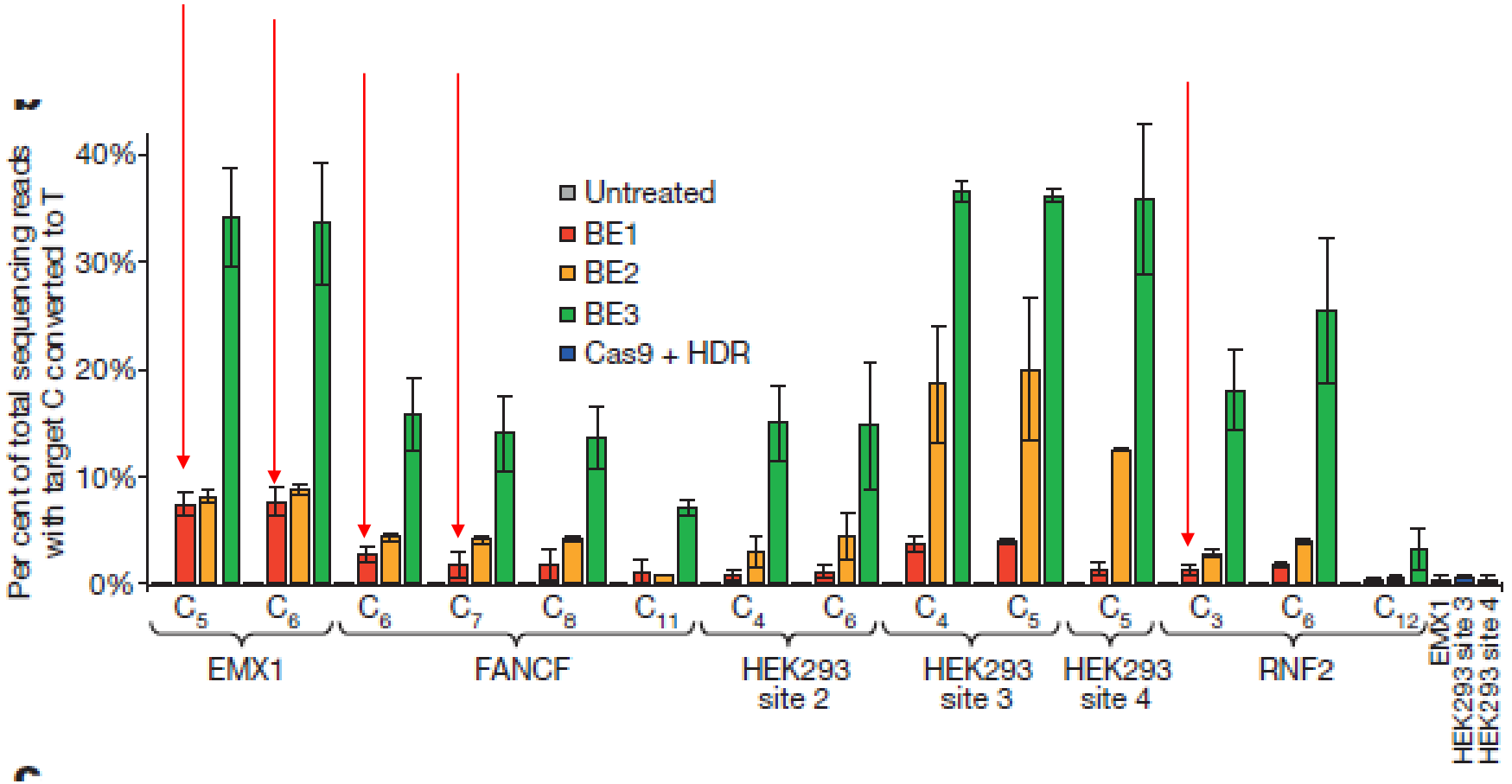
NC

- TC≥CC≥AC > GC
- Max efficiency at C_7

Base editing by BE1 in Cells

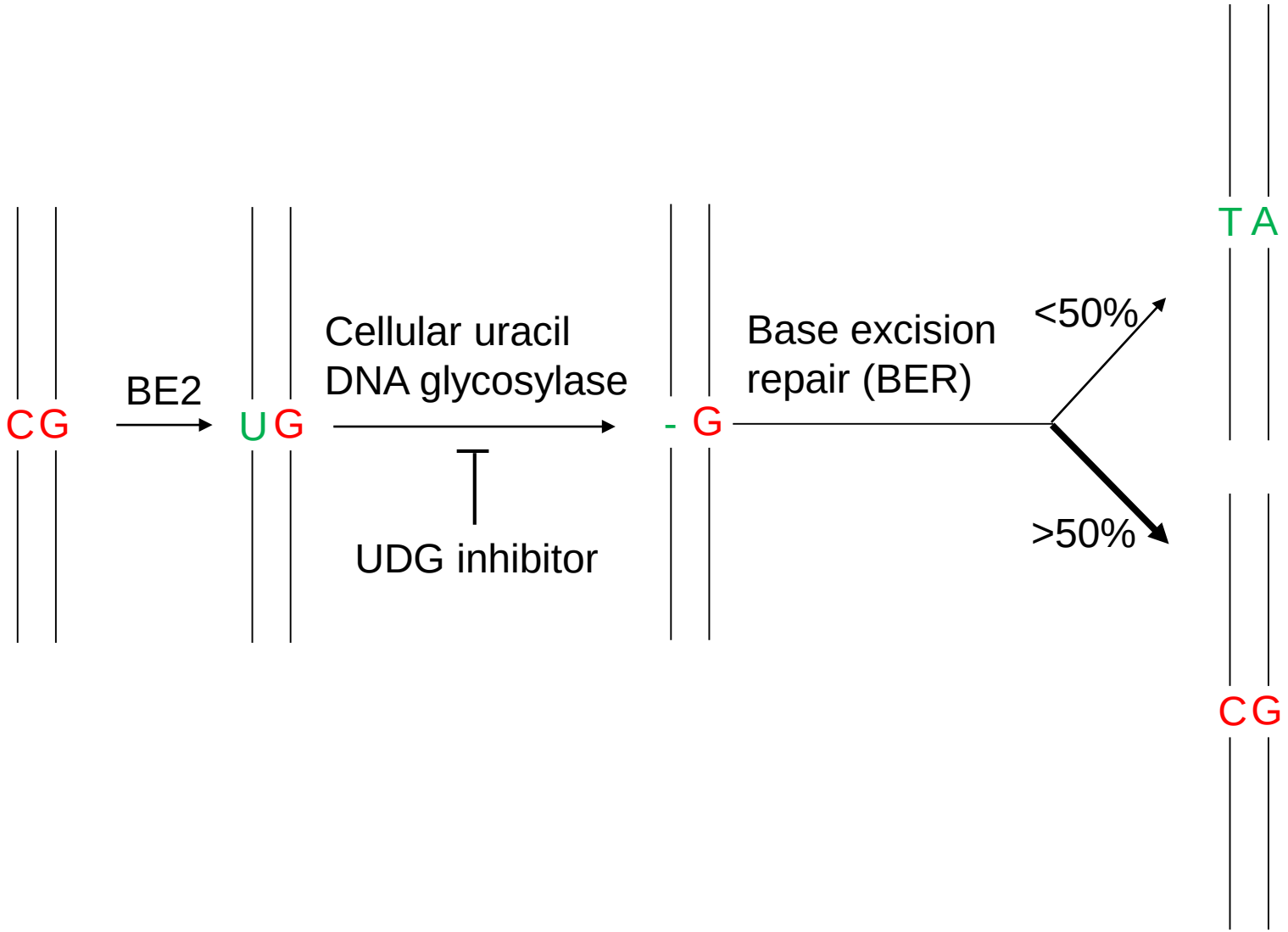


Base editing by BE1 in Cells

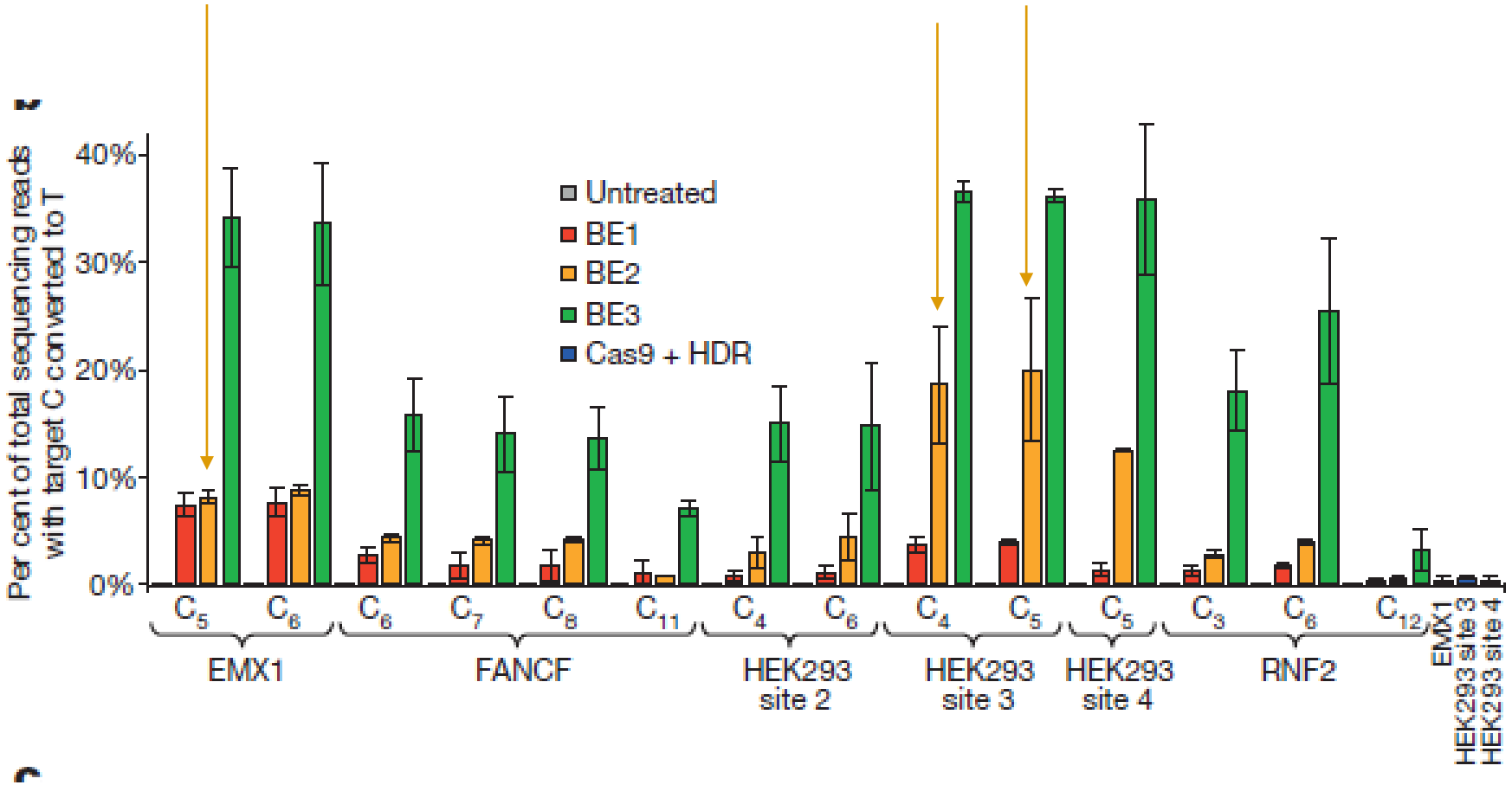


- BE1 editing efficiency: 0.8%-7.7%
- 5- to 36-fold decrease compared to in vitro editing

BE2: rAPOBEC1-XTEN-dCas9-UGI

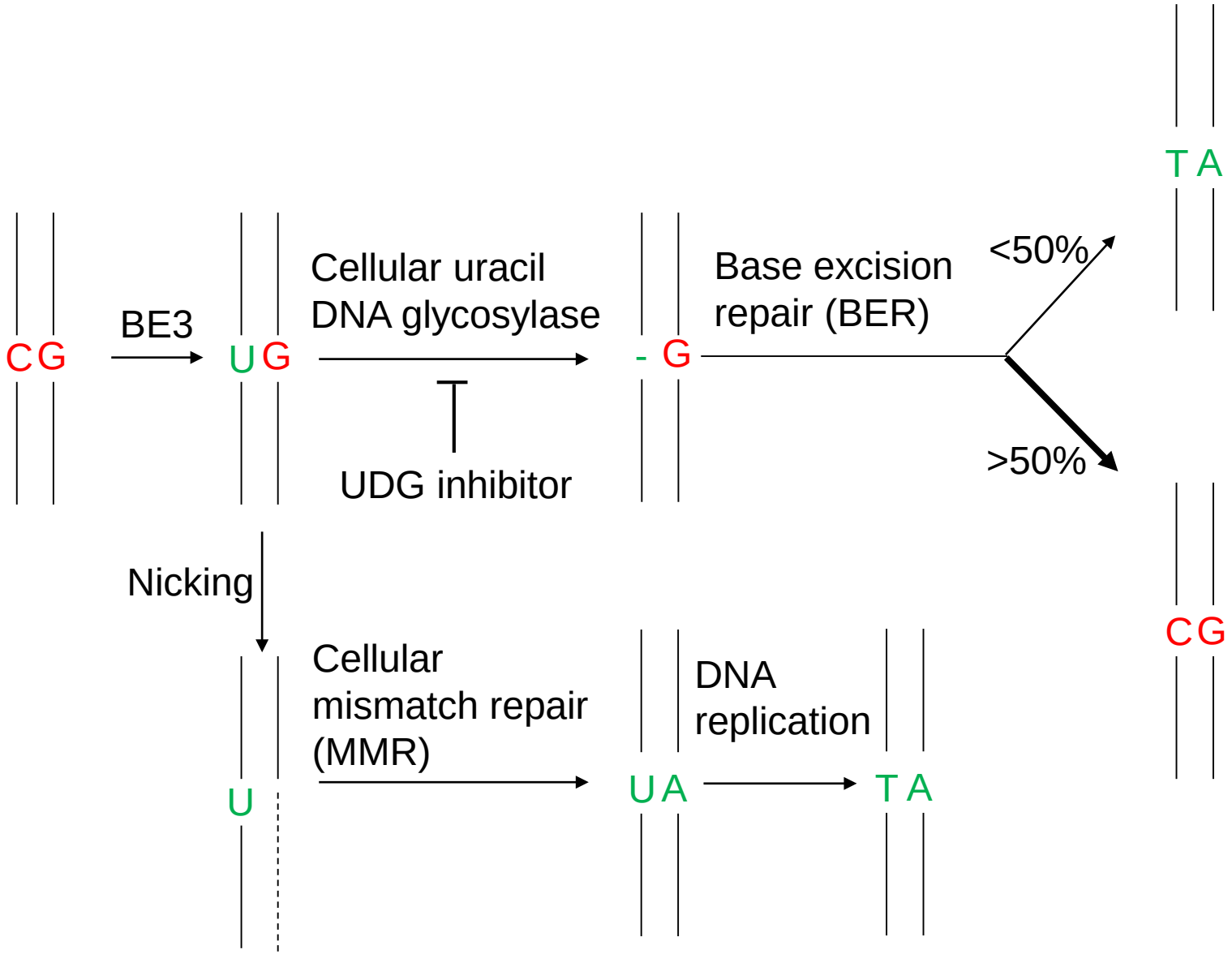


Base editing by BE2 in Cells

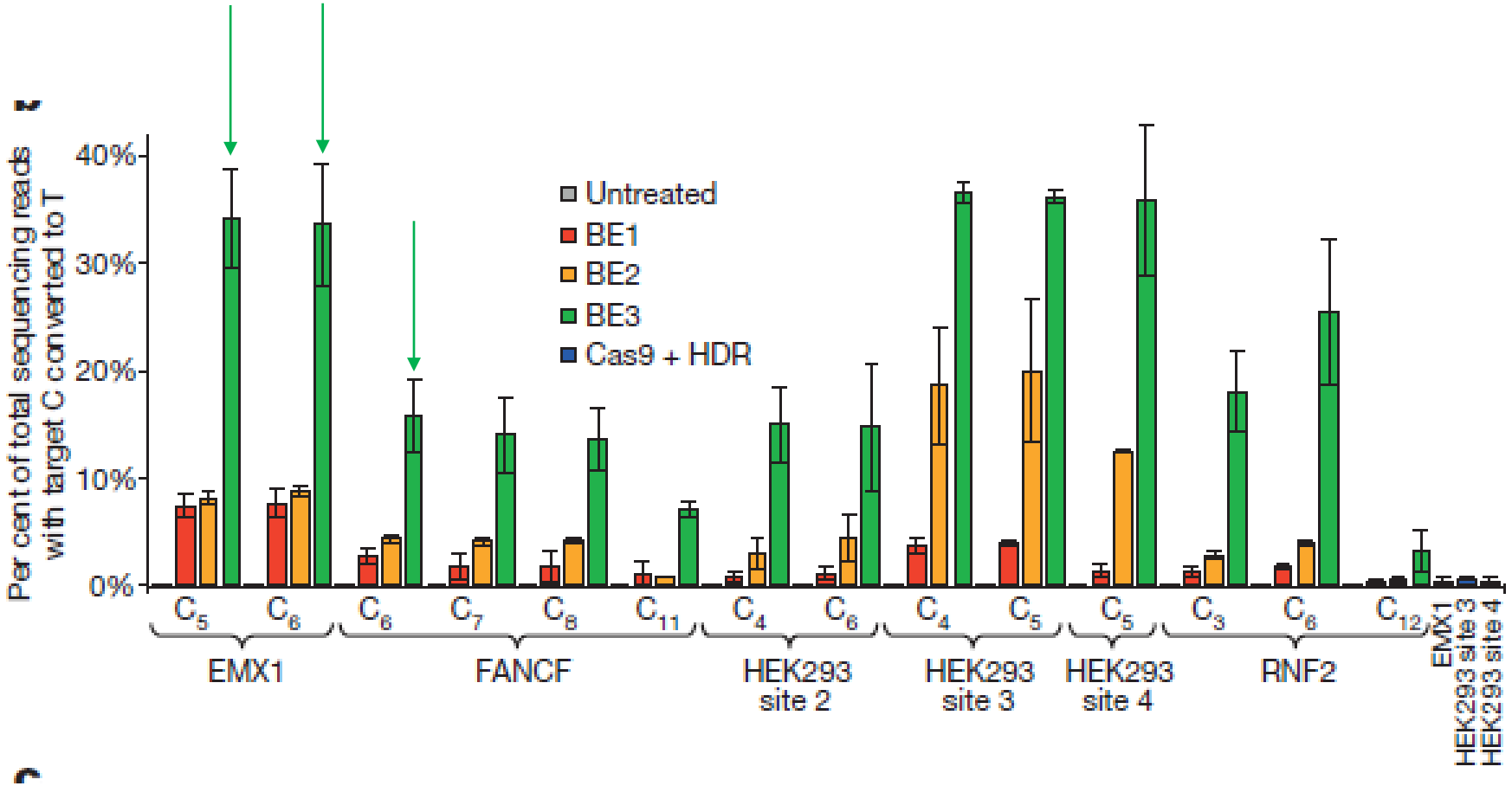


- Editing efficiency up to 20%
- 3-fold decrease compared to BE1

BE3: rAPOBEC1-XTEN-dCas9(D10A)-UGI

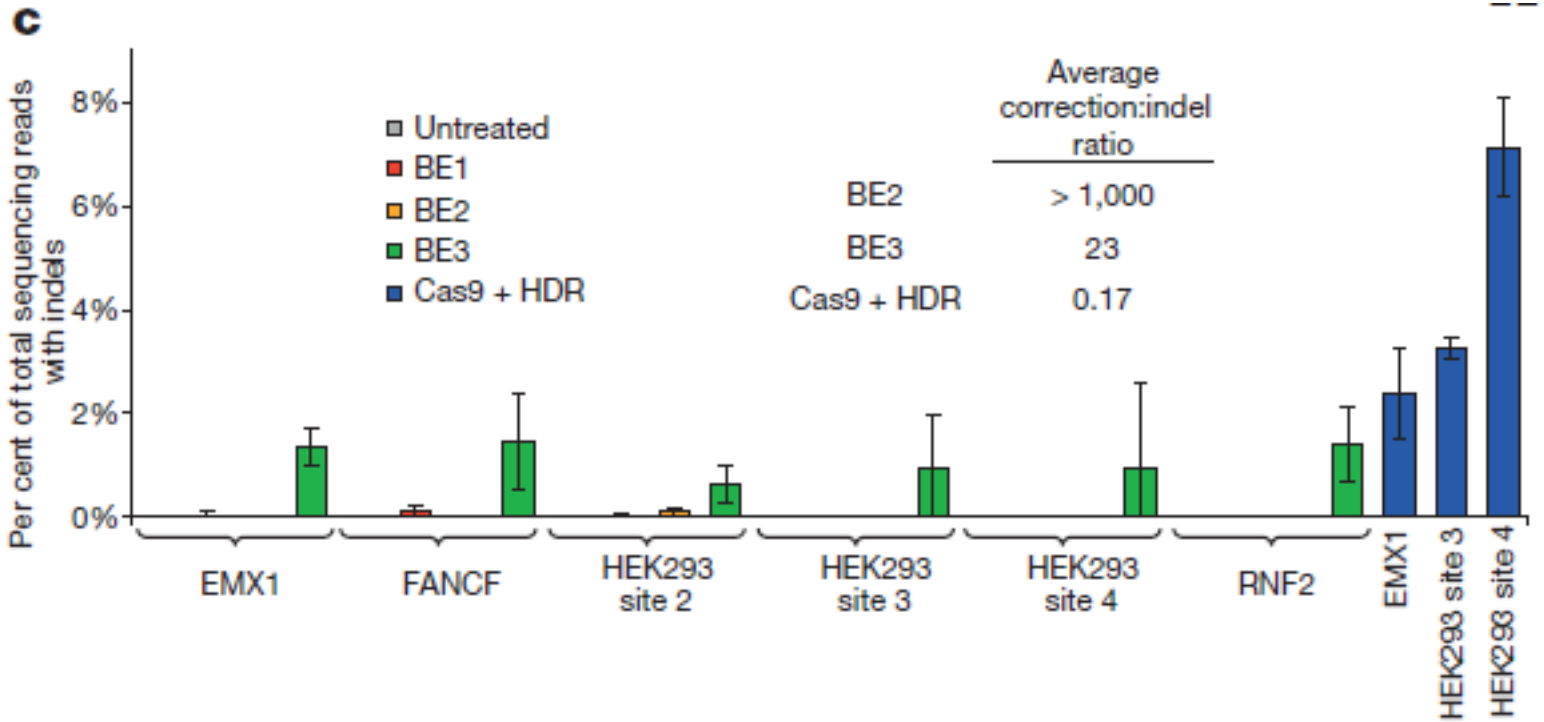


Base editing by BE3 in Cells



- Editing efficiency up to 37%
- 2- to 6-fold decrease compared to BE2

Low indels with BEs



BE3 and Cas9/HDR in editing disease-relevant mutations

a

Untreated		Lys			Arg			Leu			Ala			Val			Tyr			Gln			Indel %
APOE4 C158R	G	A	A	G	C_5	G	C	C	T	G	G	C	A	G	T	G	T	A	C	C	A	G	G	0.0
A	0.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	
C	0.0	0.0	0.0	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0	0.0	
G	100.0	0.0	0.0	100.0	0.0	100.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0	100.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	99.9	100.0	
T	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	

BE3 treated		Lys			Arg → Cys			Leu → Leu			Ala			Val			Tyr			Gln			Indel %
APOE4 C158R	G	A	A	G	C_5	G	C	C	T	G	G	C	A	G	T	G	T	A	C	C	A	G	G	4.6
A	0.1	100.0	100.0	0.0	0.5	0.0	1.3	0.9	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.1	
C	0.0	0.0	0.0	0.0	23.7	0.0	47.4	43.5	0.0	0.0	0.0	99.9	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0	0.0	
G	99.9	0.0	0.0	100.0	0.9	99.9	1.1	0.7	0.0	100.0	100.0	0.0	0.0	100.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	100.0	99.9	
T	0.0	0.0	0.0	0.0	74.9	0.1	50.2	55.0	100.0	0.0	0.0	0.1	0.0	0.0	100.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	

Cas9 + HDR		Lys			Arg → Cys			Leu			Ala			Val			Tyr			Gln			Indel %
APOE4 C158R	G	A	A	G	C_5	G	C	C	T	G	G	C	A	G	T	G	T	A	C	C	A	G	G	26.1
A	0.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	99.4	0.0	0.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	
C	0.0	0.0	0.0	0.0	99.7	0.0	99.9	99.9	0.0	0.0	0.0	100.0	0.5	0.0	0.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0	0.0	
G	100.0	0.0	0.0	100.0	0.0	100.0	0.0	0.0	0.0	100.0	99.9	0.0	0.0	99.6	0.6	99.9	0.2	0.0	0.0	0.0	0.0	100.0	100.0	
T	0.0	0.0	0.0	0.0	0.3	0.0	0.1	0.1	100.0	0.0	0.0	0.0	0.1	0.4	99.3	0.1	99.8	0.0	0.0	0.0	0.0	0.0	0.0	

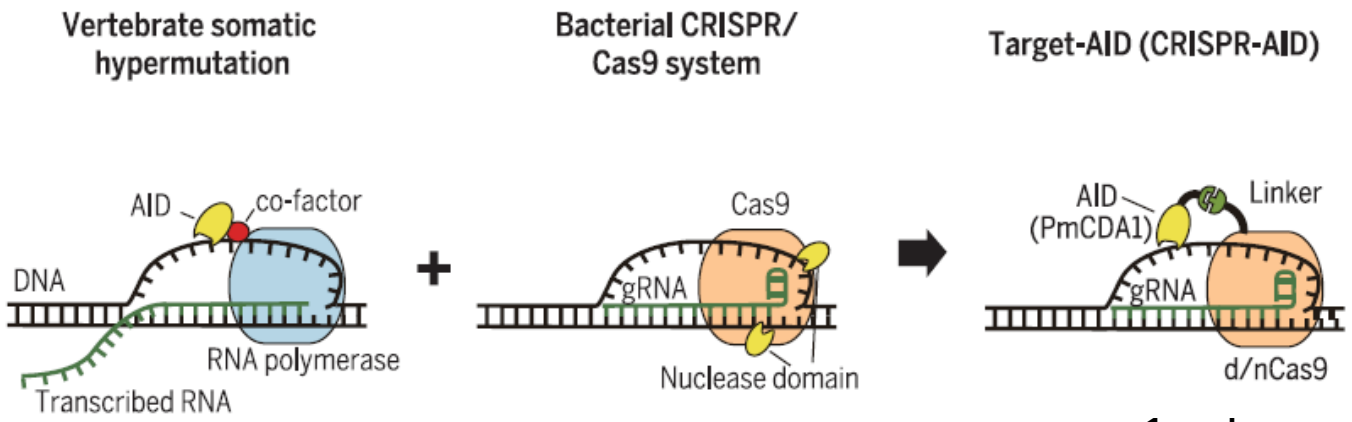
Summary I

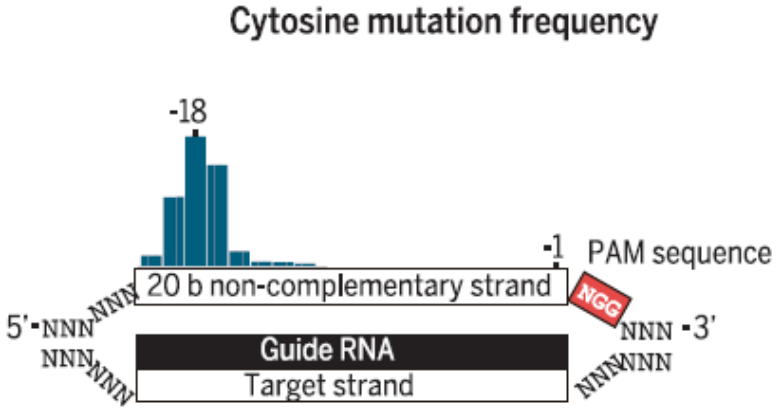
1. Development of base editing tools (BE1, BE2 and BE3) advanced both scope and effectiveness of genome editing;
2. BE2: very little indel (<0.1%); BE3: higher efficiency with ≤1% indel;
3. No DSB, no donor templates and no stochastic DNA repair processes;
4. Only applied to C:G-T:A correction; target C:G need to be in the editing window of PAM sequence NGG;
5. Efficiency…

Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems

Science, 2016

Keiji Nishida, Takayuki Arazoe, Nozomu Yachie, Satomi Banno, Mika Kakimoto, Mayura Tabata, Masao Mochizuki, Aya Miyabe, Michihiro Araki, Kiyotaka Y. Hara, Zenpei Shimatani, Akihiko Kondo*





1. In yeast;
2. AID (PmCDA1, 268aa, 30kD) instead of rAPOBEC1;
3. Deaminase in C- instead of N- terminus of dCas9;
4. Editing window: 3-5bp surrounding -18 (BEs: 5bp surrounding -15 (-12 to -16));
5. UGI and nickase (dCas9(D10A) increased efficiency;
6. C to T editing

Targeted AID-mediated mutagenesis (TAM) enables efficient genomic diversification in mammalian cells

Yunqing Ma[1,4], Jiayuan Zhang[1,4], Weijie Yin[1], Zhenchao Zhang[1], Yan Song[2] & Xing Chang[1,3]

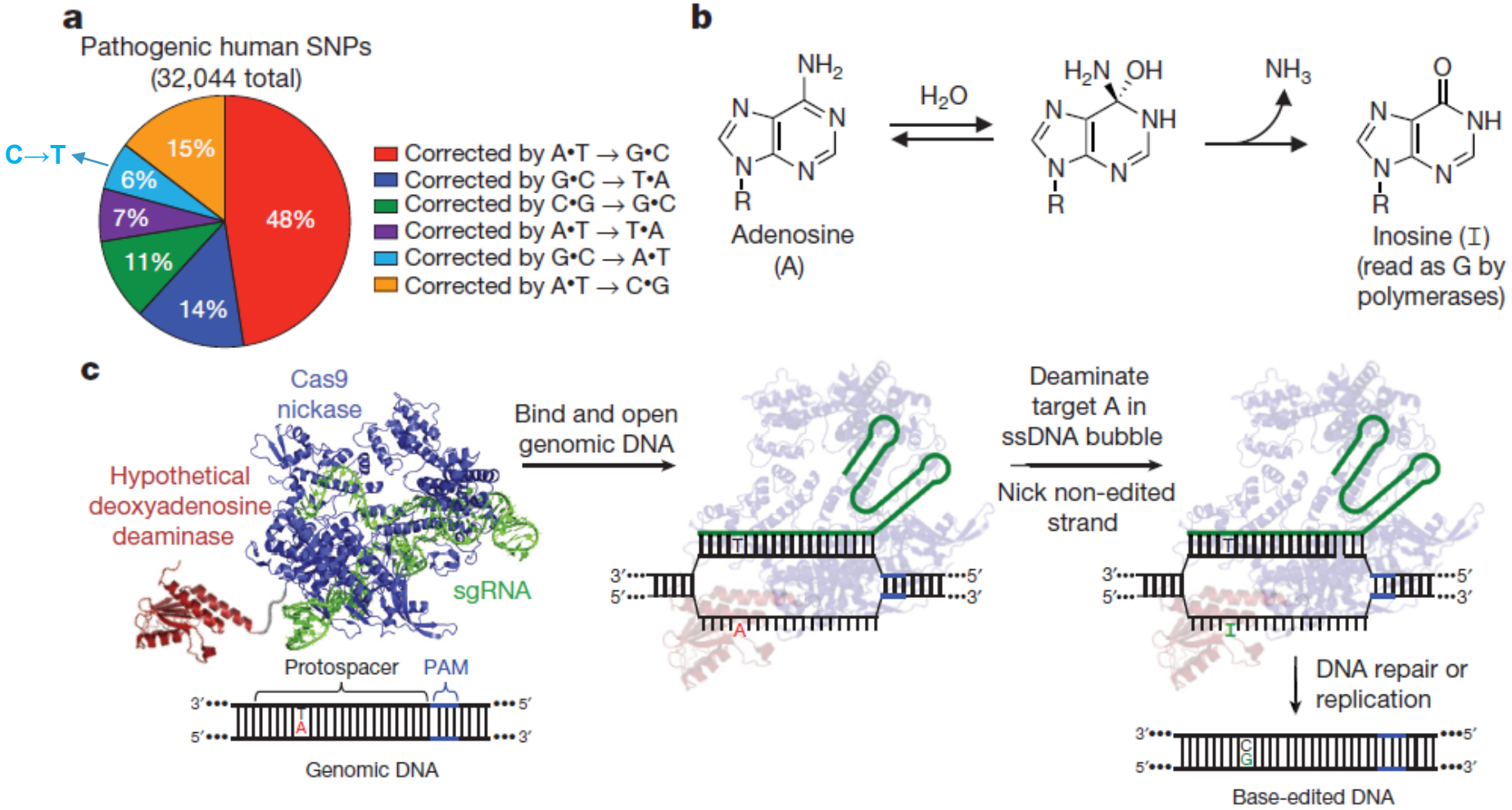
Nature Methods, 2016

1. In mammalian cells HEK293;
2. Mutant human AID P182X (198aa, 24kD), deletion of putative nuclear exporting sequence;
3. dCas9-AID P182X;
4. Editing window: -12 to -16;
5. Co express UGI or Cas9 nickase increases efficiency;
6. C to T editing

Programmable base editing of A·T to G·C in genomic DNA without DNA cleavage

Nature, 2017

Nicole M. Gaudelli[1,2,3], Alexis C. Komor[1,2,3]†, Holly A. Rees[1,2,3], Michael S. Packer[1,2,3]†, Ahmed H. Badran[1,2,3], David I. Bryson[1,2,3]† & David R. Liu[1,2,3]

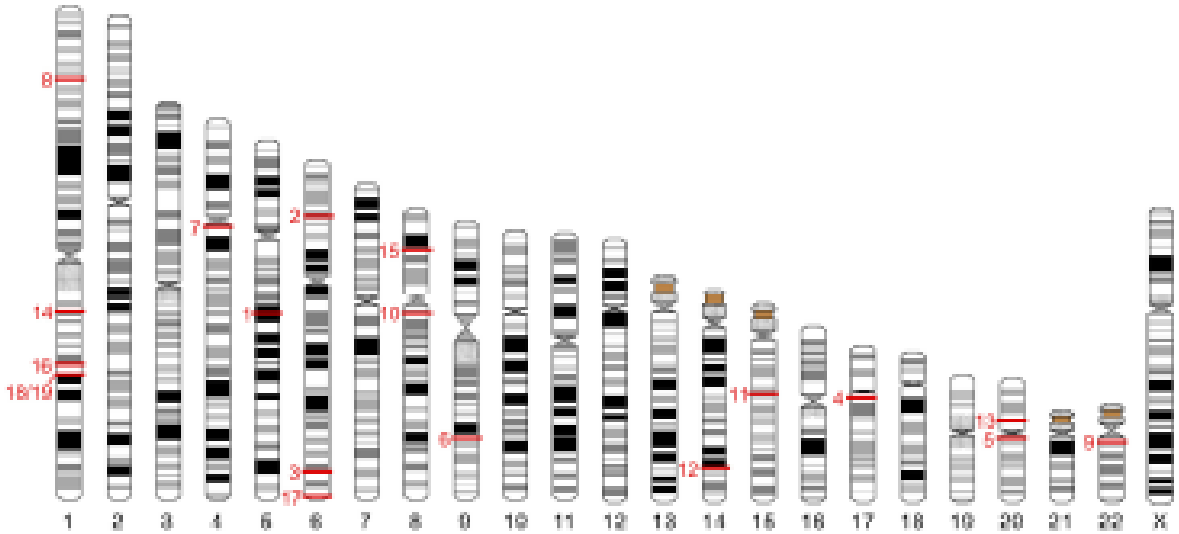


- No enzymes are known to deaminate adenine **in dsDNA**!

Natural adenine deaminase could not edit A to G in HEK293 cells

a

Site	Target motif	Protospacer and PAM sequence
1	CAC	GAACA$_5$CAAAGCATAGACTGCGGG
2	TAT	GAGTA$_5$TGAGGCATAGACTGCAGG
3	AAG	GTCAA$_5$GAAAGCAGAGACTGCCGG
4	CAA	GAGCA$_5$AAGACAATAGACTGTAGG
5	GAG	GATGA$_5$GATAATGATGAGTCAGGG
6	GAC	GGATTGA$_7$CCCAGGCCAGGGCTGG
7	TAC	GAATA$_5$CTAAGCATAGACTCCAGG
8	AAC	GTAAA$_5$CAAAGCATAGACTGAGGG
9	GAC	GAAGA$_5$CCAAGGATAGACTGCTGG
10	CAT	GAACA$_5$TAAAGAATAGAATGATGG
11	CAG	GGACA$_5$GGCAGCATAGACTGTGGG
12	GAA	GTAGA$_5$AAAAGTATAGAGTGCAGG
13	GAT	GAAGA$_5$TAGAGAATAGACTGCTGG
14	TAA	GGCTA$_5$AAGACCATAGACTGTGGG
15	TAG	GTCTA$_5$GAAAGCTTAGACTGCTGG
16	AAT	GGGAA$_5$TAAATCATAGAATCCTGG
17	AAA	GACAA$_5$AGAGGAAGAGAGACGGGG
18	CAC	A$_5$CA$_5$CA$_5$CA$_7$CA$_5$CTTAGAATCTGTGGG
19	CAC	CA$_5$CA$_4$CA$_5$CA$_5$CTTAGAATCTGTGGG



b

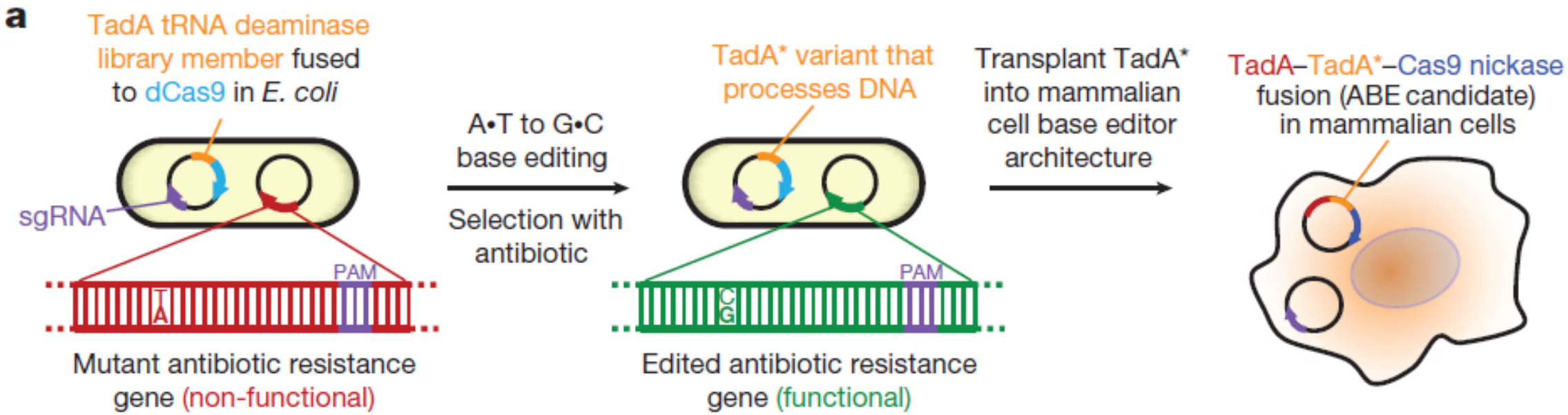
	ecTadA–Cas9 nickase (ABE0.1)	hADAR–Cas9 nickase	mADA–Cas9 nickase	hADAR2–Cas9 nickase	Untreated cells
Site 1	0.21±0.073%	0.14±0.18%	0.080±0.092%	0.080±0.075%	0.17±0.052%
Site 2	0.084±0.035%	0.059±0.017%	0.067±0.012%	0.040±0.025%	0.062±0.068%
Site 3	0.096±0.045%	0.023±0.012%	0.023±0.011%	0.023±0.010%	0.051±0.052%
Site 4	0.034±0.022%	0.029±0.021%	0.028±0.019%	0.029±0.013%	0.026±0.010%
Site 5	0.027±0.015%	0.022±0.008%	0.065±0.057%	0.024±0.016%	0.034±0.015%
Site 6	0.045±0.020%	0.18±0.29%	0.065±0.094%	0.020±0.006%	0.028±0.025%

Evolve an adenine deaminase in bacteria

1. A defective antibiotic resistance genes (chloramphenicol acetyl transferase Cam^R) that carry mutations (A:T to G:C);
2. Reverse the mutations by BE2 (APOBEC1-dCas9-UGI, bacteria lack nick-directed mismatch repair machinery) restore antibiotic resistance (chloramphenicol);
3. BE2 could correct the G:C to A:T, therefore restore antibiotic resistance in bacteria;

4. Introduce another C:G to T:A mutation to Cam^R, confers minimal chloramphenicol resistance;
5. TadA (176aa, 18kD) is a tRNA adenine deaminase that converts A to I in the single-stranded anticodon loop of tRNA in *E.coli*, TadA shares homology with the APOBEC;
6. **Unbiased libraries** of ecTadA-dCas9 fusion containing mutations in the adenine deaminase portion.

Evolve an adenine deaminase in bacteria

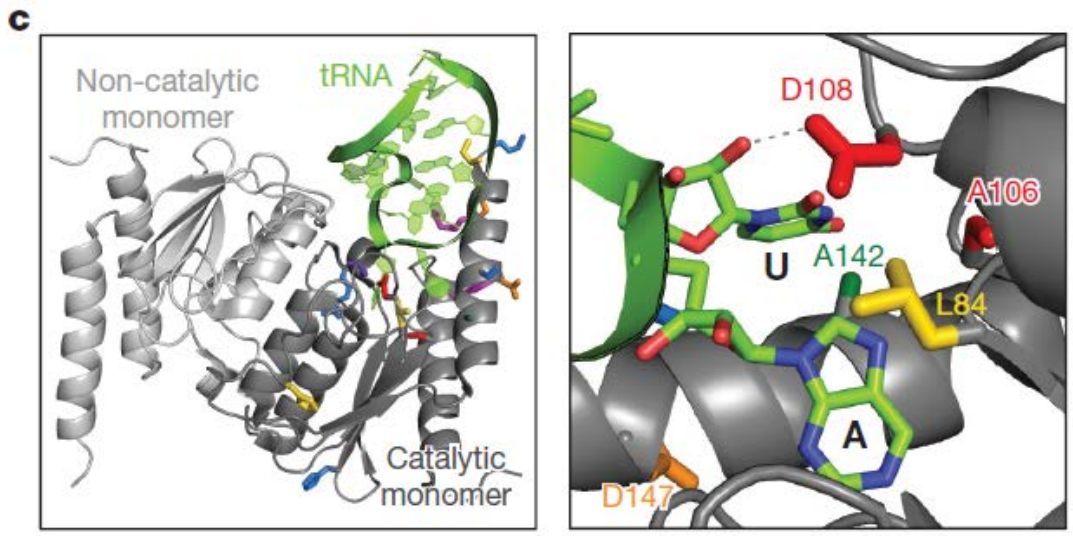


Evolve an adenine deaminase in bacteria

b

	E. coli TadA amino acid															TadA state	Linker 1 length	Linker 2 length
	23	36	48	51	84	106	108	123	142	146	147	152	155	156	157			
ABE0.1	W	H	P	R	L	A	D	H	A	S	D	R	E	I	K	Monomer		16
ABE1.1	W	H	P	R	L	A	N	H	A	S	D	R	E	I	K	Monomer		16
ABE1.2	W	H	P	R	L	V	N	H	A	S	D	R	E	I	K	Monomer		16

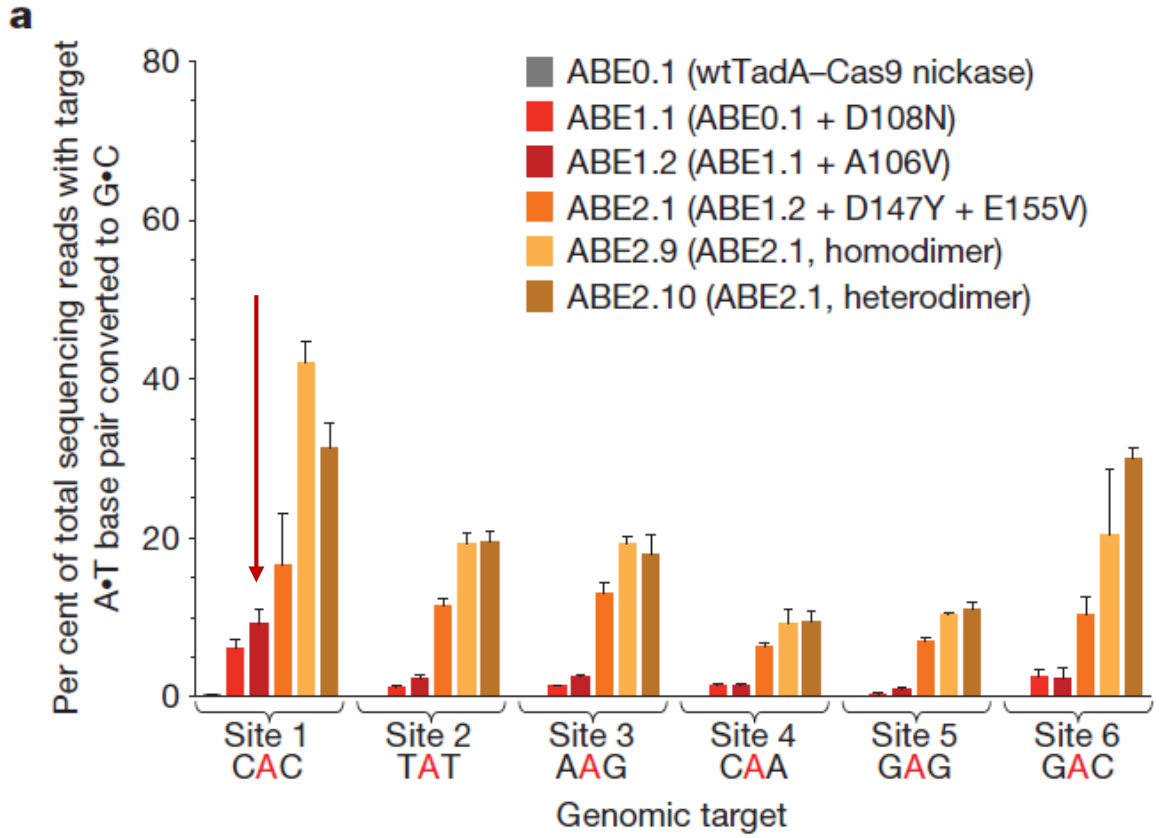
- 1st round of evolution: chloramphenicol resistance
- A106V, D108N enriched



- ❖ D108-OH group in uracil upstream of the adenine.
- ❖ D108N mutation are likely to abrogate the hydrogen bond, decreasing the energetic opportunity cost of binding DNA.
- ❖ Mutations near 108 enable TadA to perform adenine deamination on DNA substrates.

ABE base editing in HEK293 cells

- wtTadA -XTEN-nCas9-NLS: ABE0.1
- TadA* (D108N)-XTEN-nCas9-NLS: ABE1.1
- TadA* (A106V, D108N)-XTEN-nCas9-NLS: ABE1.2



- ABE1.2 resulted in 3.2±0.88% editing efficiency

Evolve an adenine deaminase in bacteria

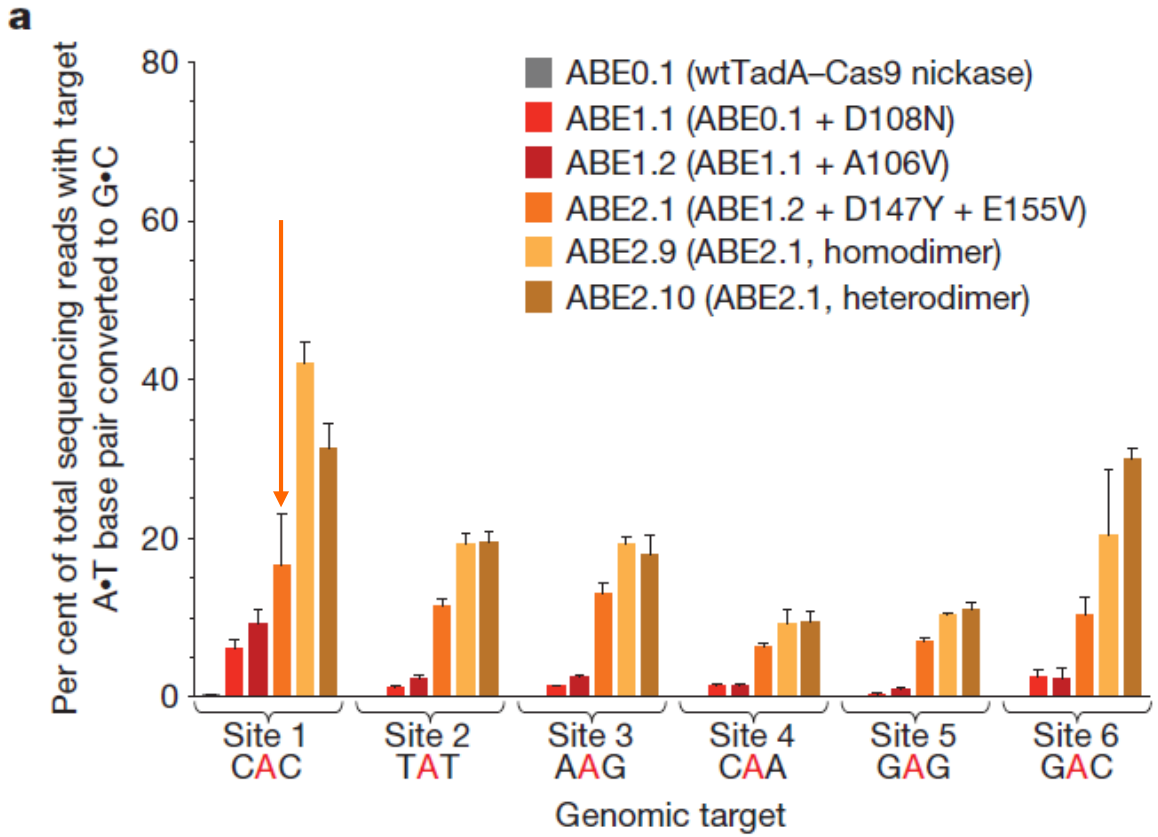
- Based on ABE1.2, another round of evolution using higher concentration of chloramphenicol
- Two mutations enriched: D147Y, E155V

b

	E. coli TadA amino acid																	
	23	36	48	51	84	106	108	123	142	146	147	152	155	156	157	TadA state	Linker 1 length	Linker 2 length
ABE0.1	W	H	P	R	L	A	D	H	A	S	D	R	E	I	K	Monomer		16
ABE1.1	W	H	P	R	L	A	N	H	A	S	D	R	E	I	K	Monomer		16
ABE1.2	W	H	P	R	L	V	N	H	A	S	D	R	E	I	K	Monomer		16
ABE2.1	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Monomer		16
ABE2.9	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Homodimer	32	16
ABE2.10	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Heterodimer	32	16

ABE base editing in HEK293 cells

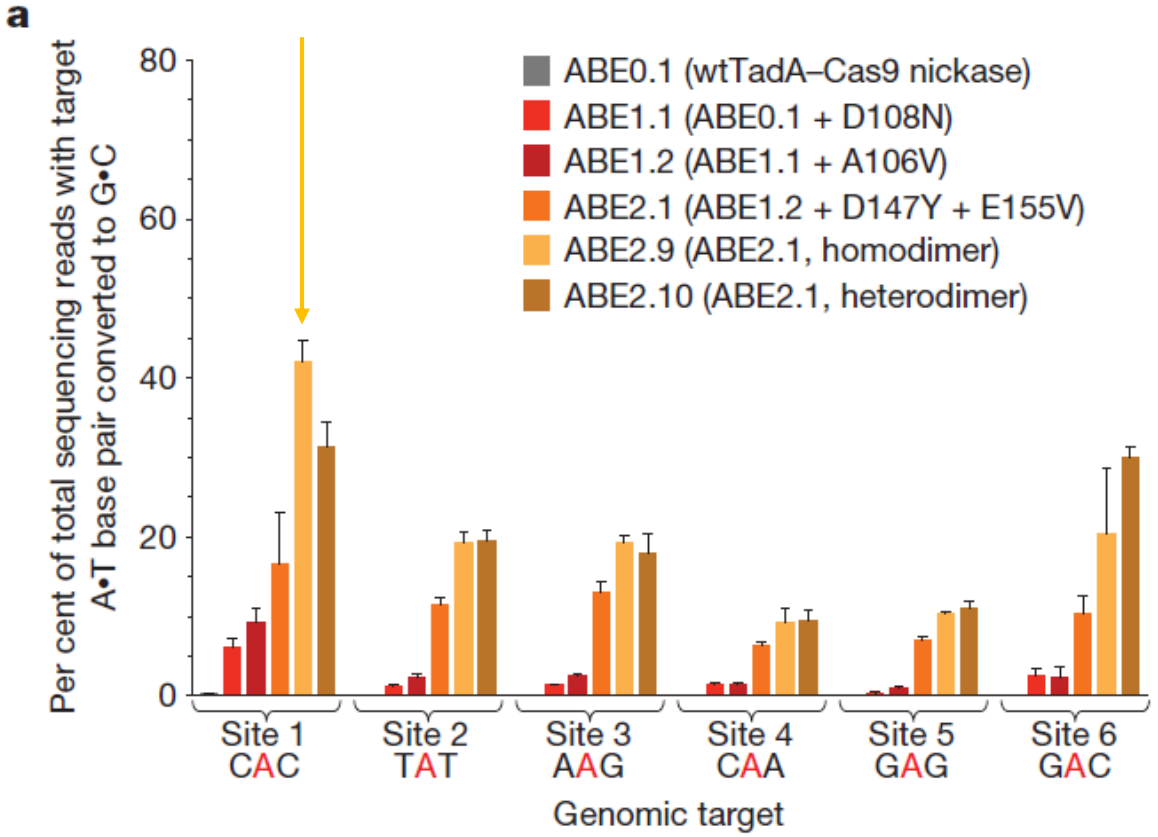
- TadA* (A106V, D108N, D147Y, E155V)-XTEN-nCas9-NLS: ABE2.1



- ABE2.1 increased 2- to 7-fold than ABE1.2, resulting in 11±2.9% editing efficiency

Evolve an adenine deaminase in bacteria

- TadA natively operates as a homodimer, one monomer catalysing deamination, the other monomer acting as docking station for tRNA substrate.
- TadA*-TadA* (A106V, D108N, D147Y, E155V)-XTEN-nCas9-NLS: ABE2.9
- wtTadA-TadA* (A106V, D108N, D147Y, E155V)-XTEN-nCas9-NLS: ABE2.10



- ABE2.9 increased 7.6± 2.6-fold than ABE1.2, resulting in 20±3.8% editing efficiency

Evolve an adenine deaminase in bacteria

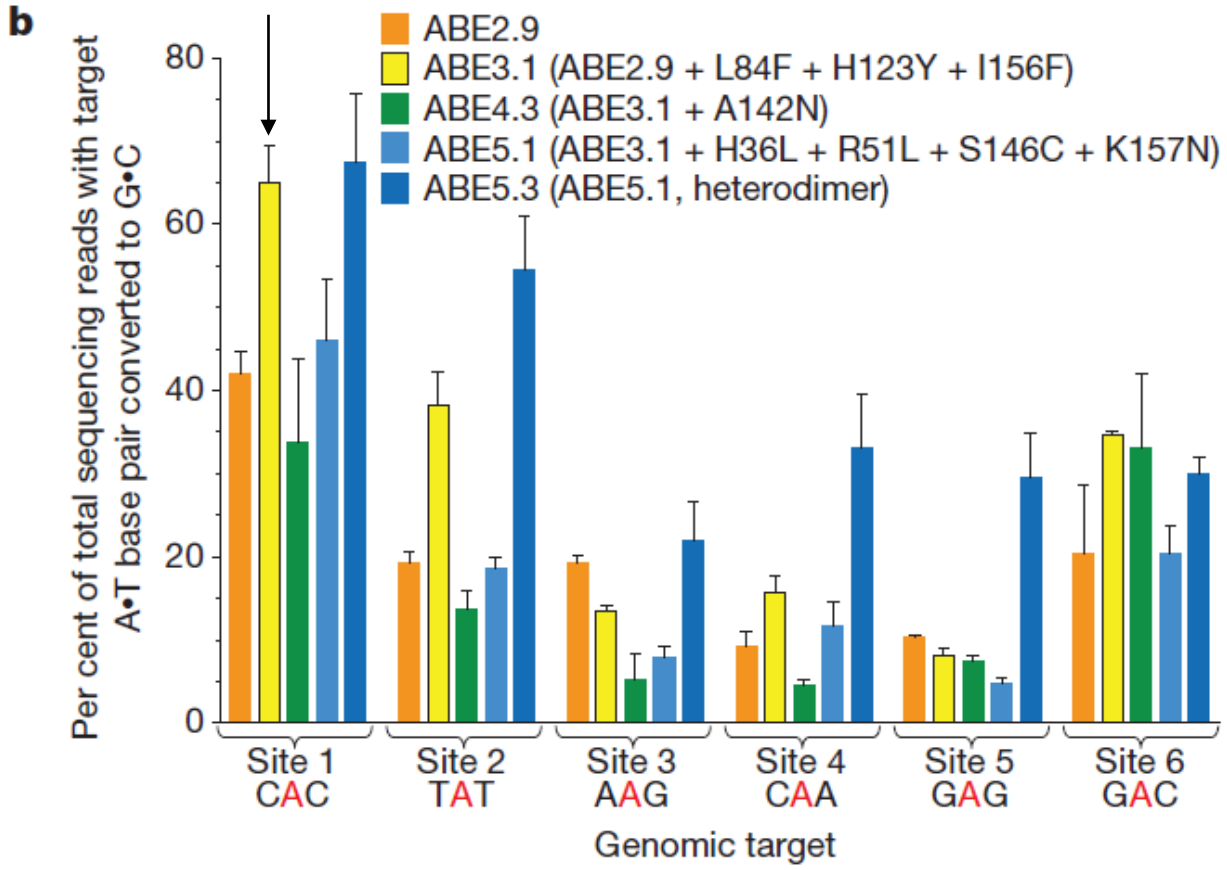
- Based on ABE2.9, 3rd round of evolution using two early stop codons in the kanamycin resistance gene
- Three mutations enriched: L84F, H123Y and I156F

b

	E. coli TadA amino acid																	
	23	36	48	51	84	106	108	123	142	146	147	152	155	156	157	TadA state	Linker 1 length	Linker 2 length
ABE0.1	W	H	P	R	L	A	D	H	A	S	D	R	E	I	K	Monomer		16
ABE1.1	W	H	P	R	L	A	N	H	A	S	D	R	E	I	K	Monomer		16
ABE1.2	W	H	P	R	L	V	N	H	A	S	D	R	E	I	K	Monomer		16
ABE2.1	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Monomer		16
ABE2.9	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Homodimer	32	16
ABE2.10	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Heterodimer	32	16
ABE3.1	W	H	P	R	F	V	N	Y	A	S	Y	R	V	F	K	Homodimer	32	32

ABE base editing in HEK293 cells

- TadA*-TadA* (L84F, A106V, D108N, H123Y, D147Y, E155V, I156F)-XTEN-nCas9-NLS: **ABE3.1**



- **ABE3.1** increased 1.6-fold than ABE2.9, 11-fold then ABE1.2, resulting in 29±2.6% editing efficiency

Evolve an adenine deaminase in bacteria

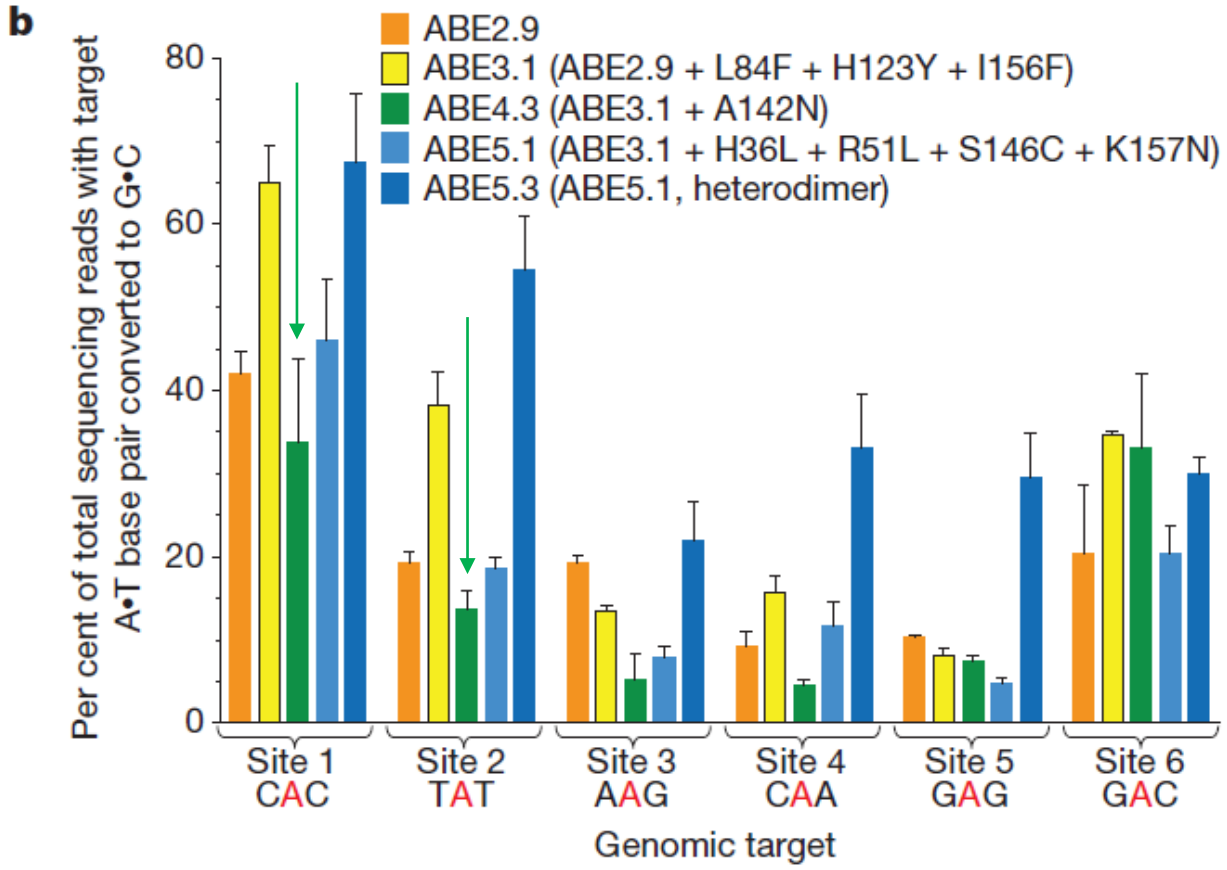
- Based on ABE3.1, 4th round of evolution focusing on the residues that are predicted to interact with nucleotides upstream or down stream of the target adenine (E25, R26m R107, A142 and A143), restore T89I mutation in the spectinomycin resistance gene.
- One mutation enriched: A142N

b

	E. coli TadA amino acid																	
	23	36	48	51	84	106	108	123	142	146	147	152	155	156	157	TadA state	Linker 1 length	Linker 2 length
ABE0.1	W	H	P	R	L	A	D	H	A	S	D	R	E	I	K	Monomer		16
ABE1.1	W	H	P	R	L	A	N	H	A	S	D	R	E	I	K	Monomer		16
ABE1.2	W	H	P	R	L	V	N	H	A	S	D	R	E	I	K	Monomer		16
ABE2.1	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Monomer		16
ABE2.9	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Homodimer	32	16
ABE2.10	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Heterodimer	32	16
ABE3.1	W	H	P	R	F	V	N	Y	A	S	Y	R	V	F	K	Homodimer	32	32
ABE4.3	W	H	P	R	F	V	N	Y	N	S	Y	R	V	F	K	Homodimer	32	32

ABE base editing in HEK293 cells

- TadA*-TadA* (L84F, A106V, D108N, H123Y, A142N, D147Y, E155V, I157F)-XTEN-nCas9-NLS: ABE4.3



- ABE4.3 decreased efficiency compared to ABE3.1, resulting in 16±5.8% editing efficiency.

Evolve an adenine deaminase in bacteria

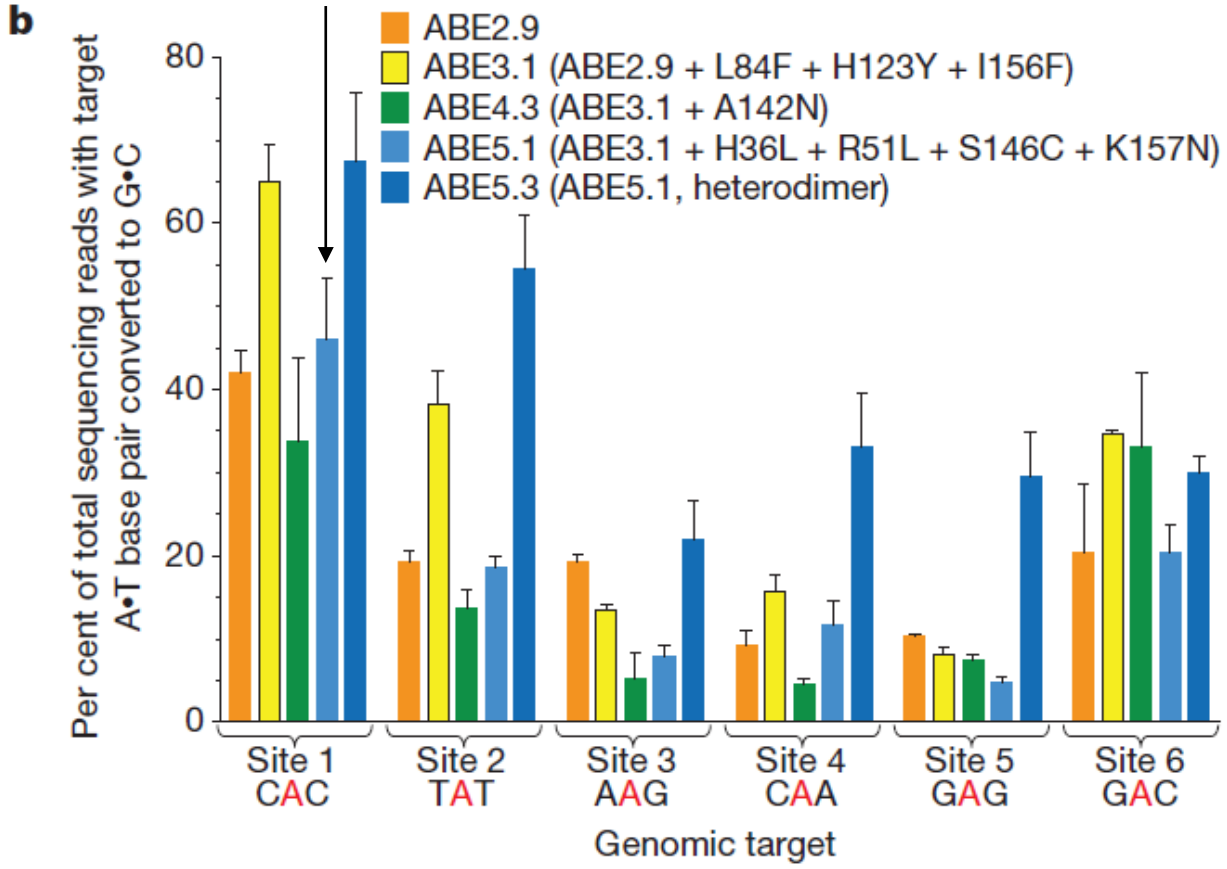
- Based on ABE3.1, 5th round of evolution using higher concentration of chloramphenicol, shorter time for ABE (7 hours instead of 14 hours).
- Four mutations enriched: H36L, R51L, S146C and K157N

b

	E. coli TadA amino acid																	
	23	36	48	51	84	106	108	123	142	146	147	152	155	156	157	TadA state	Linker 1 length	Linker 2 length
ABE0.1	W	H	P	R	L	A	D	H	A	S	D	R	E	I	K	Monomer		16
ABE1.1	W	H	P	R	L	A	N	H	A	S	D	R	E	I	K	Monomer		16
ABE1.2	W	H	P	R	L	V	N	H	A	S	D	R	E	I	K	Monomer		16
ABE2.1	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Monomer		16
ABE2.9	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Homodimer	32	16
ABE2.10	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Heterodimer	32	16
ABE3.1	W	H	P	R	F	V	N	Y	A	S	Y	R	V	F	K	Homodimer	32	32
ABE4.3	W	H	P	R	F	V	N	Y	N	S	Y	R	V	F	K	Homodimer	32	32
ABE5.1	W	L	P	L	F	V	N	Y	A	C	Y	R	V	F	N	Homodimer	32	32
ABE5.3	W	L	P	L	F	V	N	Y	A	C	Y	R	V	F	N	Heterodimer	32	32

ABE base editing in HEK293 cells

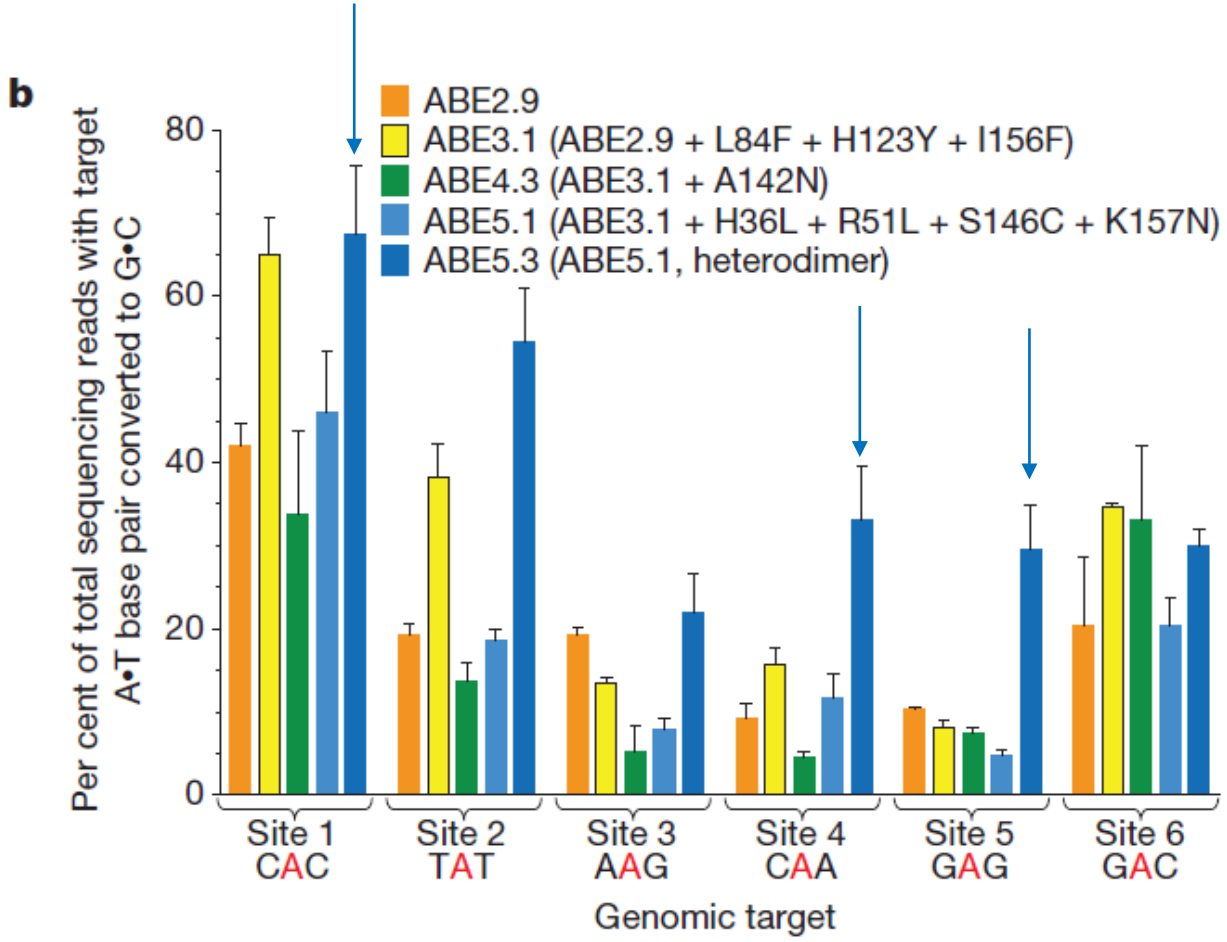
- TadA*-TadA* (H36L, R51L, L84F, A106V, D108N, H123Y, S146C, D147Y, E155V, I156F, K157N)-XTEN-nCas9-NLS: ABE5.1



- ABE5.1 decreased efficiency 1.6-fold compared to ABE3.1.
- Mutations may impair the abilty of non-catalytic N-terminus TadA to play its structural role.

ABE base editing in HEK293 cells

- wtTadA-TadA* (H36L, R51L, L84F, A106V, D108N, H123Y, S146C, D147Y, E155V, I156F, K157N)-XTEN-nCas9-NLS: ABE5.3



- ABE5.3 increased efficiency 2.9± 0.78-fold compared to ABE5.1, resulting in 39±5.9% editing efficiency
- ABE5.3 showed higher editing efficiency in non-YAC target.

Evolve an adenine deaminase in bacteria

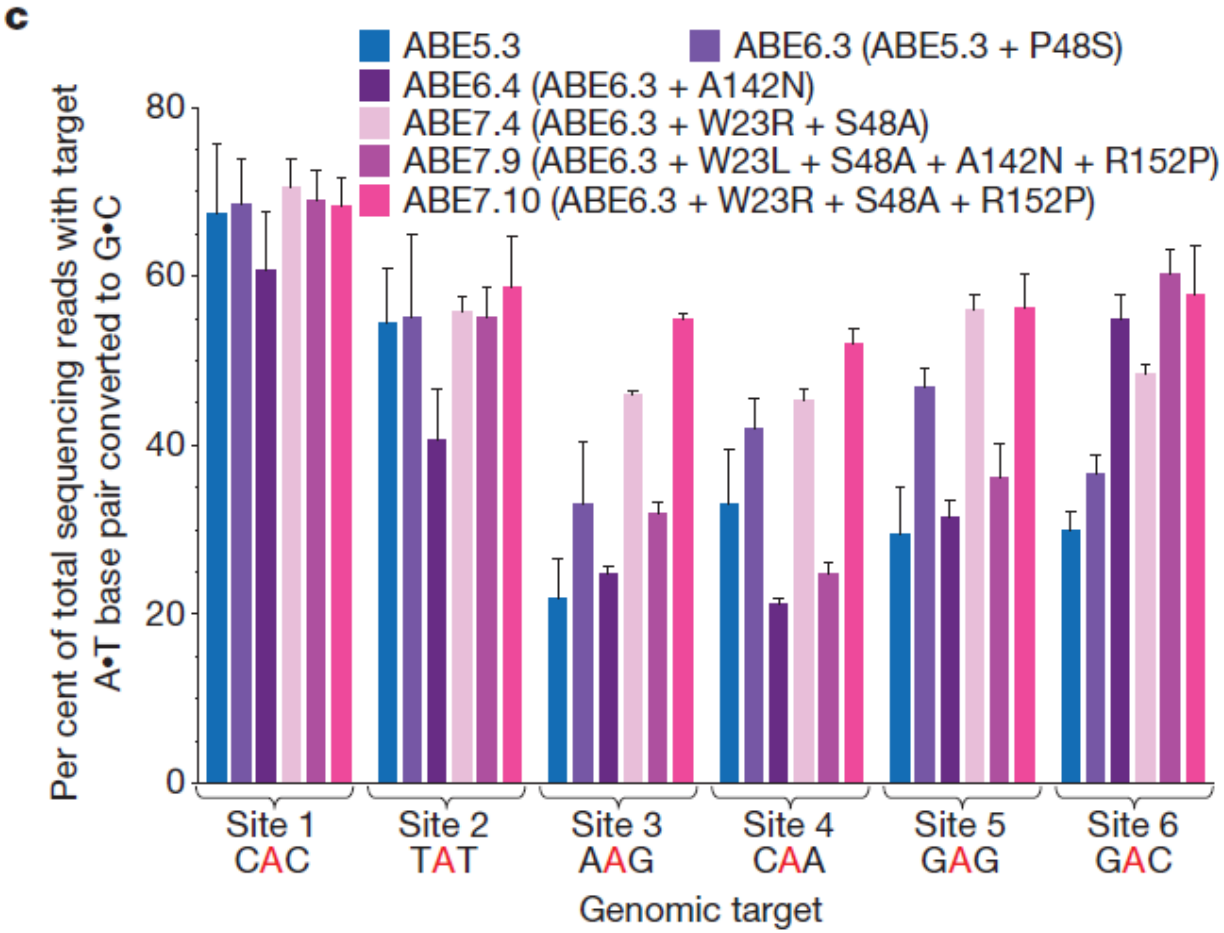
- Based on ABE5.3, 6th round of evolution using T89I mutation in the spectinomycin resistance gene.
- Two mutations enriched: P48S and A142N.

b

	E. coli TadA amino acid																	
	23	36	48	51	84	106	108	123	142	146	147	152	155	156	157	TadA state	Linker 1 length	Linker 2 length
ABE0.1	W	H	P	R	L	A	D	H	A	S	D	R	E	I	K	Monomer		16
ABE1.1	W	H	P	R	L	A	N	H	A	S	D	R	E	I	K	Monomer		16
ABE1.2	W	H	P	R	L	V	N	H	A	S	D	R	E	I	K	Monomer		16
ABE2.1	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Monomer		16
ABE2.9	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Homodimer	32	16
ABE2.10	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Heterodimer	32	16
ABE3.1	W	H	P	R	F	V	N	Y	A	S	Y	R	V	F	K	Homodimer	32	32
ABE4.3	W	H	P	R	F	V	N	Y	N	S	Y	R	V	F	K	Homodimer	32	32
ABE5.1	W	L	P	L	F	V	N	Y	A	C	Y	R	V	F	N	Homodimer	32	32
ABE5.3	W	L	P	L	F	V	N	Y	A	C	Y	R	V	F	N	Heterodimer	32	32
ABE6.3	W	L	S	L	F	V	N	Y	A	C	Y	R	V	F	N	Heterodimer	32	32
ABE6.4	W	L	S	L	F	V	N	Y	N	C	Y	R	V	F	N	Heterodimer	32	32

ABE base editing in HEK293 cells

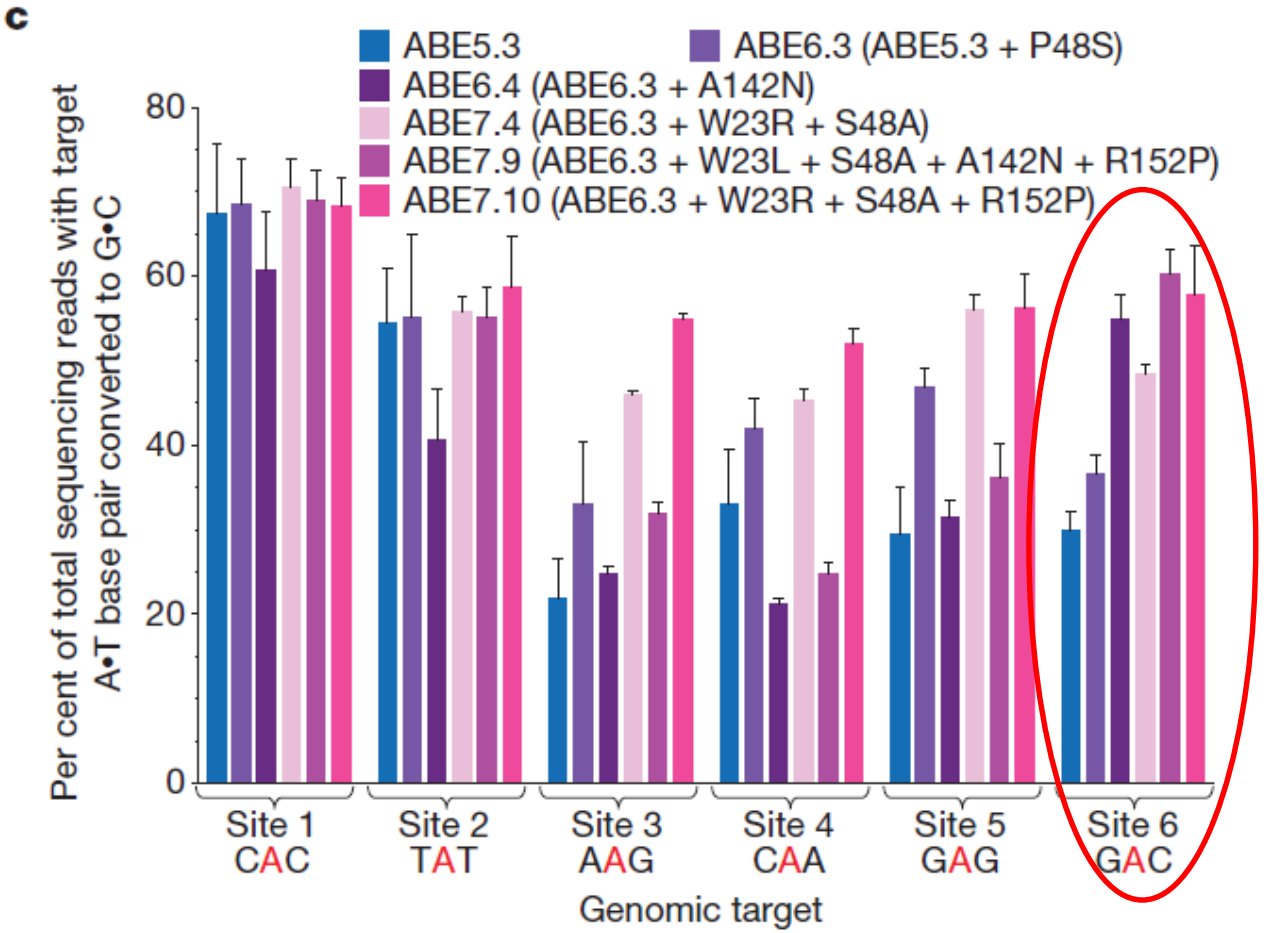
- wtTadA-TadA* (H36L, P48S, R51L, L84F, A106V, D108N, H123Y, S146C, D147Y, E155V, I156F, K157N)-XTEN-nCas9-NLS: ABE6.3



- ABE6.3 increased efficiency 1.3± 0.28-fold compared to ABE5.3, resulting in 47±5.5% editing efficiency

ABE base editing in HEK293 cells

- wtTadA-TadA* (H36L, P48S, R51L, L84F, A106V, D108N, H123Y, A142N, S146C, D147Y, E155V, I156F, K157N)-XTEN-nCas9-NLS: ABE6.4



- ABE6.4 increased efficiency 1.5± 0.13-fold compared to ABE6.3, 1.8± 0.16-fold compared to ABE5.3 at site 6

Evolve an adenine deaminase in bacteria

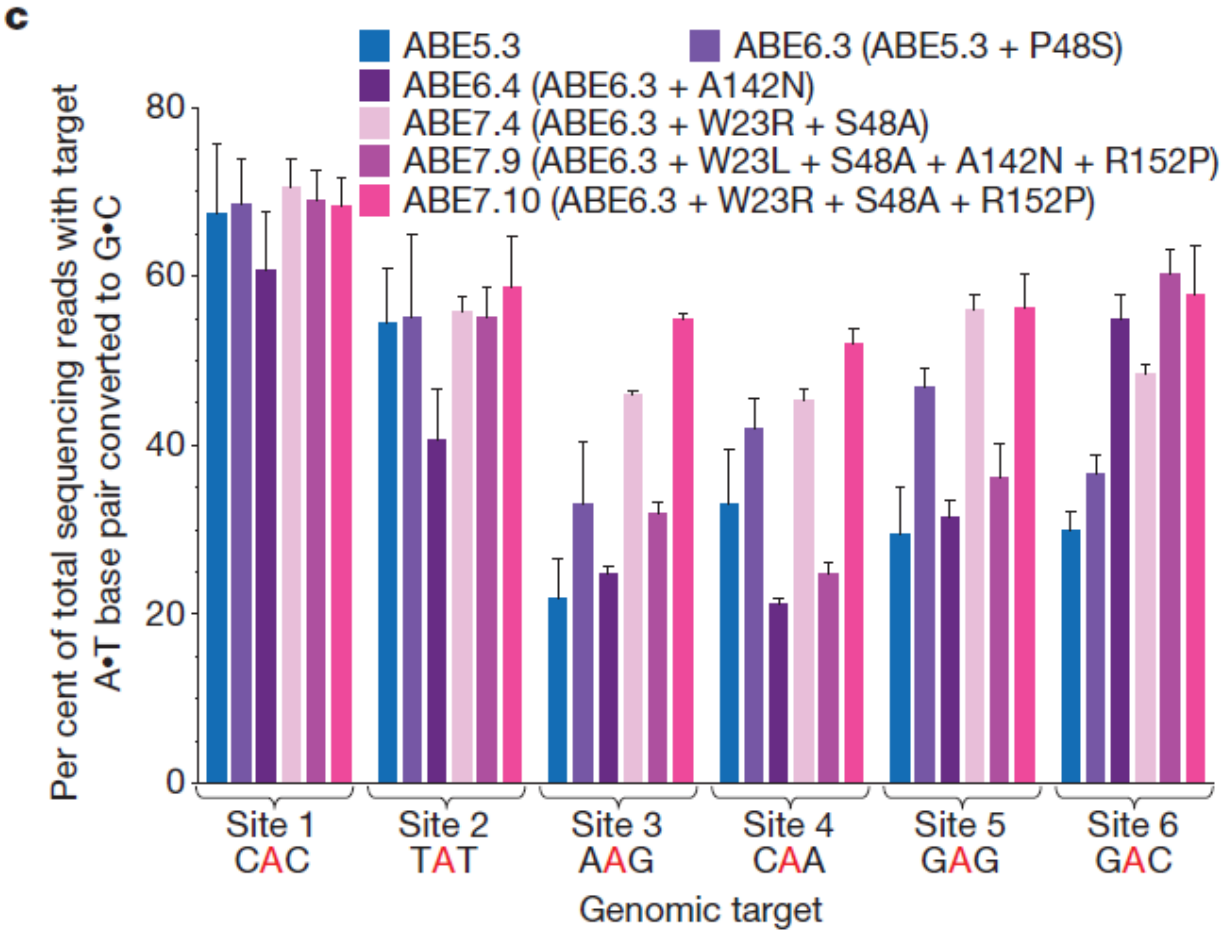
- Based on ABE6, 7th round of evolution using stop codon Q4stop and D208N mutaion in the kanamycin resistance gene
- Three mutations enriched: W23L/R, S48A and R152P

b

	E. coli TadA amino acid																	
	23	36	48	51	84	106	108	123	142	146	147	152	155	156	157	TadA state	Linker 1 length	Linker 2 length
ABE0.1	W	H	P	R	L	A	D	H	A	S	D	R	E	I	K	Monomer		16
ABE1.1	W	H	P	R	L	A	N	H	A	S	D	R	E	I	K	Monomer		16
ABE1.2	W	H	P	R	L	V	N	H	A	S	D	R	E	I	K	Monomer		16
ABE2.1	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Monomer		16
ABE2.9	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Homodimer	32	16
ABE2.10	W	H	P	R	L	V	N	H	A	S	Y	R	V	I	K	Heterodimer	32	16
ABE3.1	W	H	P	R	F	V	N	Y	A	S	Y	R	V	F	K	Homodimer	32	32
ABE4.3	W	H	P	R	F	V	N	Y	N	S	Y	R	V	F	K	Homodimer	32	32
ABE5.1	W	L	P	L	F	V	N	Y	A	C	Y	R	V	F	N	Homodimer	32	32
ABE5.3	W	L	P	L	F	V	N	Y	A	C	Y	R	V	F	N	Heterodimer	32	32
ABE6.3	W	L	S	L	F	V	N	Y	A	C	Y	R	V	F	N	Heterodimer	32	32
ABE6.4	W	L	S	L	F	V	N	Y	N	C	Y	R	V	F	N	Heterodimer	32	32
ABE7.4	R	L	A	L	F	V	N	Y	A	C	Y	R	V	F	N	Heterodimer	32	32
ABE7.8	L	L	A	L	F	V	N	Y	N	C	Y	R	V	F	N	Heterodimer	32	32
ABE7.9	L	L	A	L	F	V	N	Y	N	C	Y	P	V	F	N	Heterodimer	32	32
ABE7.10	R	L	A	L	F	V	N	Y	A	C	Y	P	V	F	N	Heterodimer	32	32

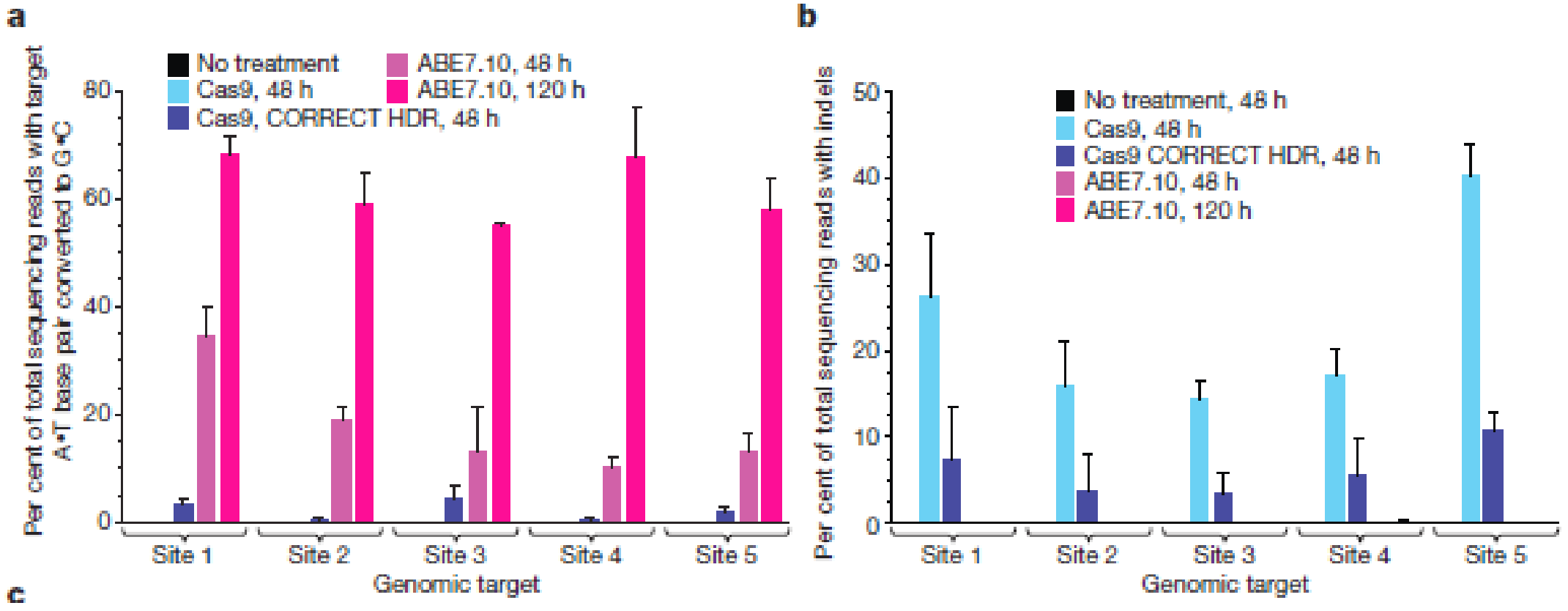
ABE base editing in HEK293 cells

- wtTadA-TadA* (W23R, H36L, S48A, R51L, L84F, A106V, D108N, H123Y, A142N, S146C, D147Y, R152P, E155V, I156F, K157N)-XTEN-nCas9-NLS: ABE7.10

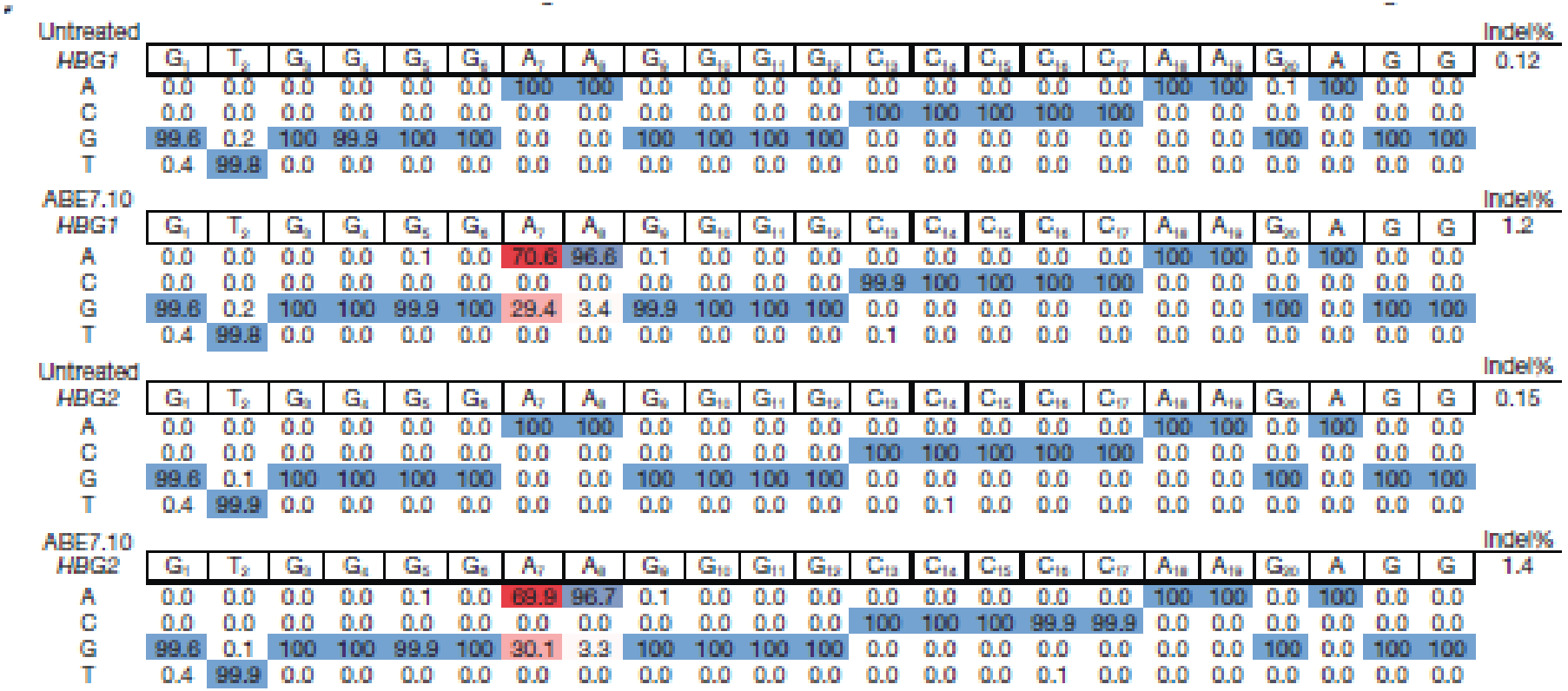


- ABE7.10 increased efficiency 1.3± 0.20-fold compared to ABE6.3, 29± 7.4-fold compared to ABE1.2, resulting in 58±4.0% editing efficiency.
- Comparable to BE3 for C-T editing

Higher efficiency and lower indel by ABE base editing



A-G correction of disease-related mutation by ABE7.10



Untreated *HBG1*	G_1	T_2	G_3	G_4	G_5	G_6	A_7	A_8	G_9	G_{10}	G_{11}	G_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	A_{18}	A_{19}	G_{20}	A	G	G	Indel%
A	0.0	0.0	0.0	0.0	0.0	0.0	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	100	0.1	100	0.0	0.0	
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	
G	99.8	0.2	100	99.9	100	100	0.0	0.0	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	0.0	100	100	
T	0.4	99.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.12

ABE7.10 *HBG1*	G_1	T_2	G_3	G_4	G_5	G_6	A_7	A_8	G_9	G_{10}	G_{11}	G_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	A_{18}	A_{19}	G_{20}	A	G	G	Indel%
A	0.0	0.0	0.0	0.0	0.1	0.0	70.6	96.6	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	100	0.0	100	0.0	0.0	
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	99.9	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	
G	99.8	0.2	100	100	99.9	100	29.4	3.4	99.9	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	0.0	100	100	
T	0.4	99.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2

Untreated *HBG2*	G_1	T_2	G_3	G_4	G_5	G_6	A_7	A_8	G_9	G_{10}	G_{11}	G_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	A_{18}	A_{19}	G_{20}	A	G	G	Indel%
A	0.0	0.0	0.0	0.0	0.0	0.0	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	100	0.0	100	0.0	0.0	
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	
G	99.8	0.1	100	100	100	100	0.0	0.0	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	0.0	100	100	
T	0.4	99.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.15

ABE7.10 *HBG2*	G_1	T_2	G_3	G_4	G_5	G_6	A_7	A_8	G_9	G_{10}	G_{11}	G_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	A_{18}	A_{19}	G_{20}	A	G	G	Indel%
A	0.0	0.0	0.0	0.0	0.1	0.0	69.9	96.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	100	0.0	100	0.0	0.0	
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	100	100	99.9	99.9	0.0	0.0	0.0	0.0	0.0	0.0	
G	99.8	0.1	100	100	99.9	100	30.1	3.3	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	0.0	100	100	
T	0.4	99.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4

Summary II

1. Development of new base editing tools (A:T to G:C);
2. ABE1s and ABE2s with weak efficiency, ABE3s, ABE4s and ABE5s limited efficiency, ABE6s and ABE7s highly active;
3. ABE7.10 for general A to G base editing;
4. Depending on the context sequences, ABE6.3, 7.8 and 7.9 may offer higher efficiency;
5. Greatly expanded the capabilities of base editing for pathogenic SNPs.

Overview

I. Introduction

II. Establishment of base editing technology

III. Application of BE in animal models and human embryos

IV. Future directions

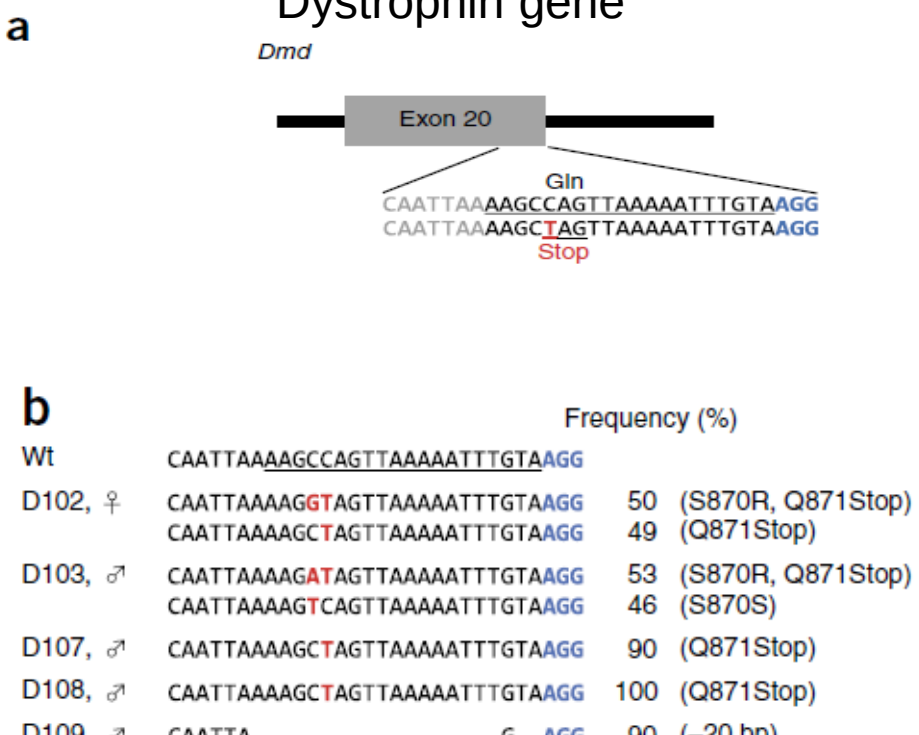
Highly efficient RNA-guided base editing in mouse embryos

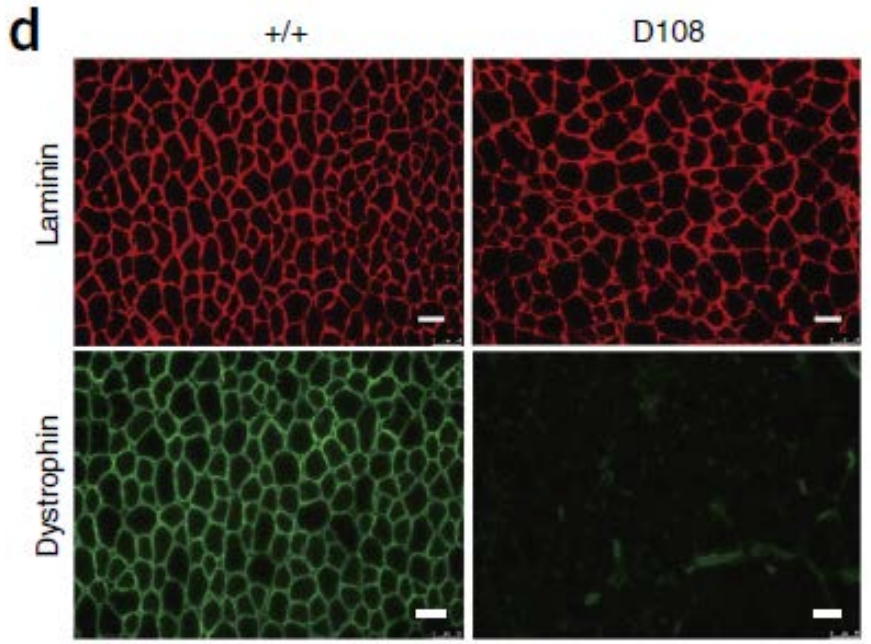
Nature Biotechnology, 2017

Kyoungmi Kim[1,3], Seuk-Min Ryu[1–3], Sang-Tae Kim[1], Gayoung Baek[1], Daesik Kim[2], Kayeong Lim[1,2], Eugene Chung[1,2], Sunghyun Kim[1,2] & Jin-Soo Kim[1,2]

- Microinjection of BE3 and sgRNA or electroporation of BE3 ribonucleoproteins (RNP)
- 11/15 (73%) of blastocysts contain C-T mutation

Dystrophin gene





Phenotype?

Highly efficient RNA-guided base editing in mouse embryos

Kyoungmi Kim[1,3], Seuk-Min Ryu[1–3], Sang-Tae Kim[1], Gayoung Baek[1], Daesik Kim[2], Kayeong Lim[1,2], Eugene Chung[1,2], Sunghyun Kim[1,2] & Jin-Soo Kim[1,2]

b

		Frequency (%)	
Wt	GTGGCACCATCTGGACCTCAGTTCCCCTTC--AAAGGGG		
T110	GTGGCACCATCTGGACCTTAGTTCCCCTTC--AAAGGGG	51	(Q68Stop)
	GTGGCACCATCTGGACCTGAGTTCCCCTTC--AAAGGGG	47	(Q68E)
T111	GTGGCACCATCTGGACCTTAGTTCCCCTTC--AAAGGGG	51	(Q68Stop)
	GTGGCACCATCTGGACCTGAGTTCCCCTTC--AAAGGGG	48	(Q68E)
T112	GTGGCACCATCTGGACCTCA----------GGG	49	(–14 bp)
	GTGGCACCATCTGGACCTAAGTTCCCCTTC--AAAGGGG	46	(Q68K)
	GTGGC----------TTC--AAAGGGG	5	(–22 bp)
T113	GTGGCACCATCTGGACCTTAGTTCCCCTTC--AAAGGGG	100	(Q68Stop)
T114	GTGGCACCATCTGGACCTTAGTTCCCCTTC--AAAGGGG	99	(Q68Stop)
T117	GTGGCACCATCTGGACCTGAGTTCCCCTTC--AAAGGGG	99	(Q68E)
T118	GTGGCACCATCTGGACCTCAGTTCCCCTTCAGAAAGGGG	41	(+2 bp)
	G----------GT----------GG	26	(–31 bp)

- Microinjection of BE3 and sgRNA or electroporation of BE3 ribonucleoproteins (RNP)
- 10/10 (100%) of blastocysts contain C-T mutation

d Albino phenotype in the eyes

Correction of β-thalassemia mutant by base editor in human embryos

Protein & Cell, 2017

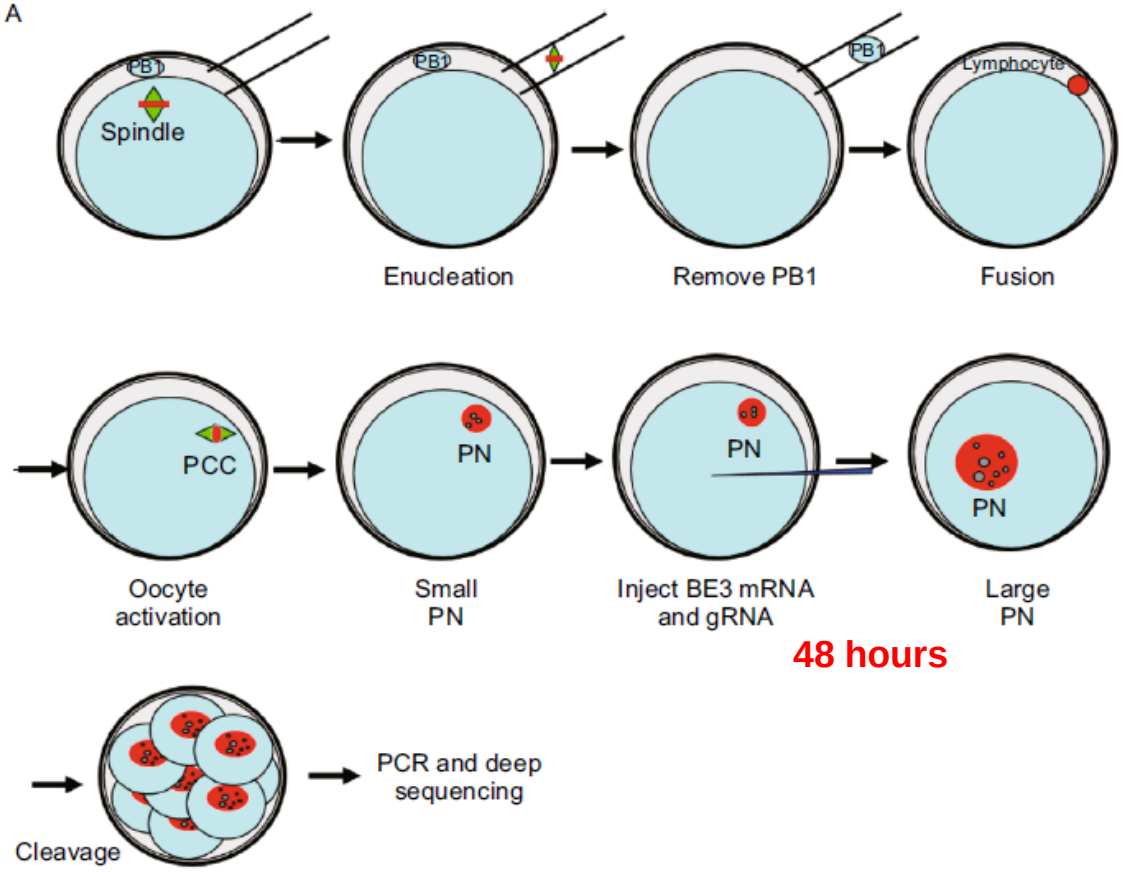
Puping Liang[1,2], Chenhui Ding[2], Hongwei Sun[1], Xiaowei Xie[1], Yanwen Xu[2], Xiya Zhang[1], Ying Sun[1], Yuanyan Xiong[1], Wenbin Ma[1], Yongxiang Liu[2], Yali Wang[2], Jianpei Fang[3], Dan Liu[4], Zhou Songyang[1,2,4]✉, Canquan Zhou[2]✉, Junjiu Huang[1,2]✉

- B-thalassemia, hemoglobin β chain (HBB) A-G mutation at -28 is one of the three most frequent mutations in China and Southeast Asia patients.
- Edit C to T in the antisense strand will correct the G to A in the coding allele.

1) Test BE efficiency in HEK cells expressing an exogenous mutated HBB
2) Do BE in patient-derived primary skin fibroblasts
3) **Do BE in human embryos derived from fusion of lymphocyte to enucleated human oocytes**

BE3 base editing mutant HBB in human embryos

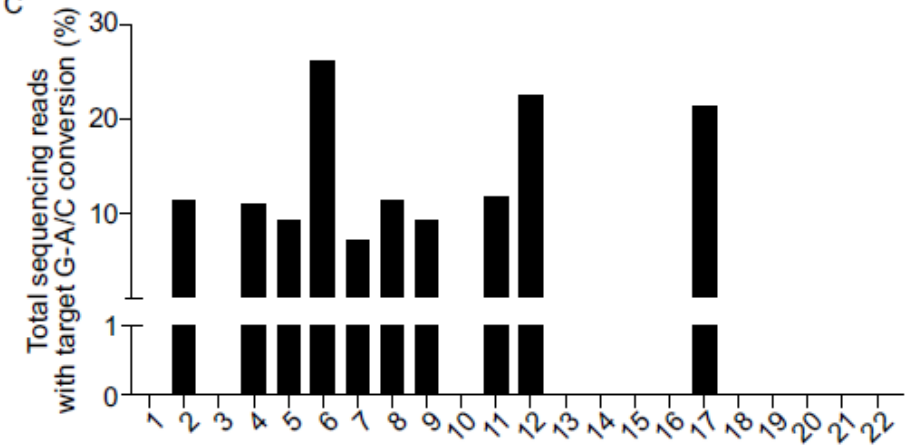
A



B

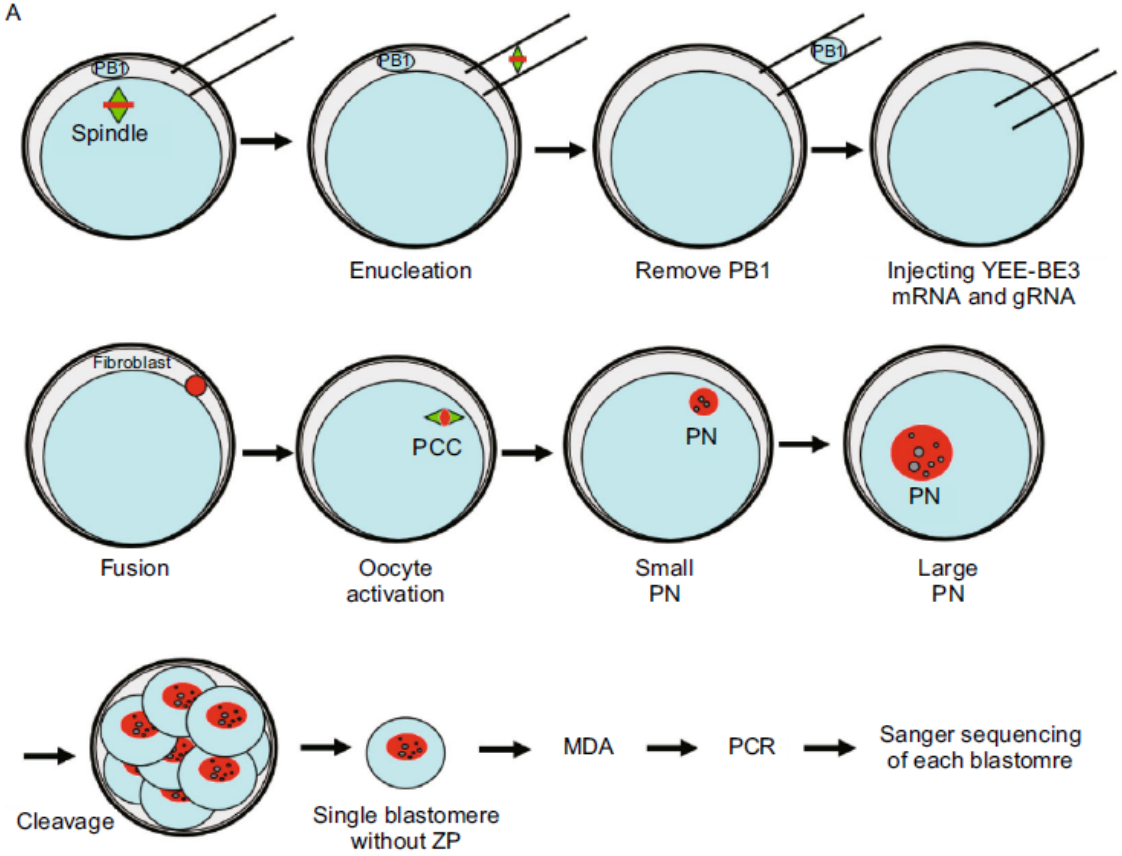
Injected embryo No.	Survived embryo No.	PCR-amplified embryo No.	G>A embryo No. (%)	G>C embryo No. (%)
30	26	22	9 (40.9)	1 (4.5)*

C



BE3 base editing mutant HBB in human embryos

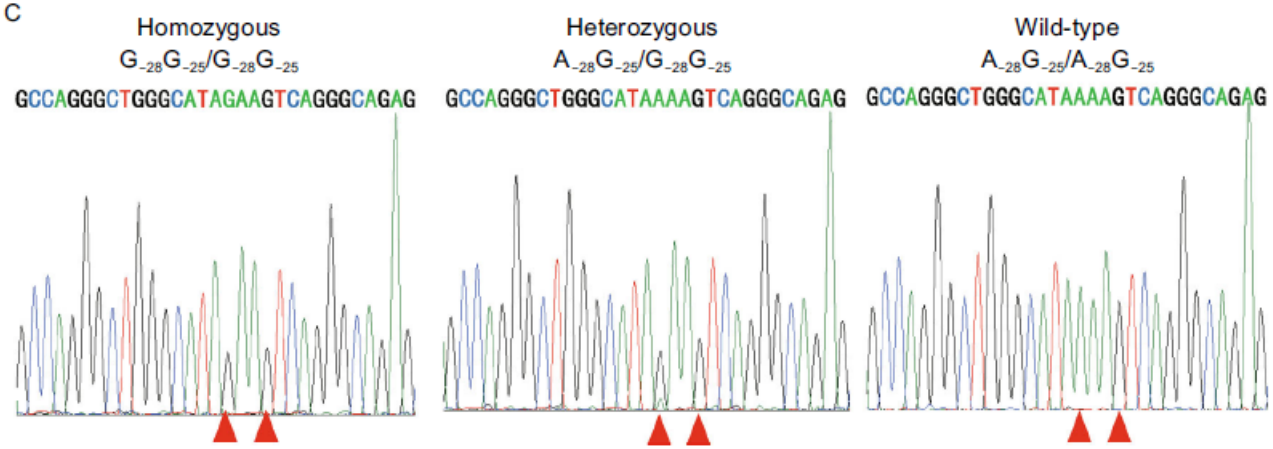
A



B

Survived embryo No. (Injected embryo No.)	Activated embryo No.	Harvested embryo No.	Total blastomere No.	MDA-amplified blastomere No.	PCR-amplified blastomere No.	<Homozygous> $G_{-28}G_{-25}/G_{-28}G_{-25}$ blastomere No. (%)	<Heterozygous> $A_{-28}G_{-25}/G_{-28}G_{-25}$ blastomere No. (%)	<Wild-type> $A_{-28}G_{-25}/A_{-28}G_{-25}$ blastomere No. (%)
28 (35)	24	20#	73	73	48*	37 (77.1)	3 (6.3)	8 (16.7)

C



Application of BE3 in base editing

SCIENTIFIC REPORTS

OPEN

Precise genome-wide base editing by the CRISPR Nickase system in yeast

Received: 16 January 2017
Accepted: 3 April 2017

Atsushi Satomura[1,2], Ryosuke Nishioka[1], Hitoshi Mori[1], Kosuke Sato[1], Kouichi Kuroda[1] & Mitsuyoshi Ueda[1]

ARTICLE

DOI: 10.1038/s41467-017-00175-6 OPEN

Programmable base editing of zebrafish genome using a modified CRISPR-Cas9 system

Yihan Zhang[1,2], Wei Qin[1], Xiaochan Lu[1], Jason Xu[2], Haigen Huang[2], Haipeng Bai[1], Song Li[1] & Shuo Lin[1,2]

Molecular Plant

Volume 10, Issue 3, 6 March 2017, Pages 526-529

Letter to the Editor

Generation of Targeted Point Mutations in Rice by a Modified CRISPR/Cas9 System

Jingying Li [1, 4], Yongwei Sun [1, 4], Jinlu Du [1], Yunde Zhao [2, 3], Lanqin Xia [1]

Molecular Plant

Volume 10, Issue 3, 6 March 2017, Pages 523-525

Letter to the Editor

Precise Editing of a Target Base in the Rice Genome Using a Modified CRISPR/Cas9 System

Yuming Lu [1], Jian-Kang Zhu [1, 2]

Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion

Yuan Zong[1,2,5], Yanpeng Wang[1,2,5], Chao Li[1,2], Rui Zhang[1], Kunling Chen[1], Yidong Ran[3], Jin-Long Qiu[4], Daowen Wang[1] & Caixia Gao[1]

Protein Cell 2017, 8(10):776–779
DOI 10.1007/s13238-017-0458-7

CrossMark Protein & Cell

LETTER

Highly efficient and precise base editing in discarded human tripronuclear embryos

Guanglei Li[1], Yajing Liu[2], Yanting Zeng[1], Jianan Li[2], Lijie Wang[2], Guang Yang[2], Dunjin Chen[1,4], Xiaoyun Shang[3], Jia Chen[2], Xingxu Huang[2]✉, Jianqiao Liu[1]✉

Summary III

1. Base editing BE3 (C to T) is applicable for many species;
2. More applications of ABE will be reported;
3. Efficiency can be improved further;
4. Sites of targets can be broadened by various strategies;
5. Editing window can be narrowed to have higher specificity.

Overview

I. Introduction

II. Establishment of base editing technology

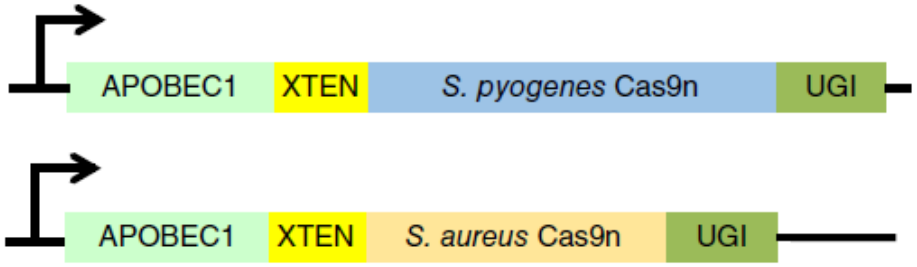
III. Application of BE in animal models and human embryos

IV. Future directions

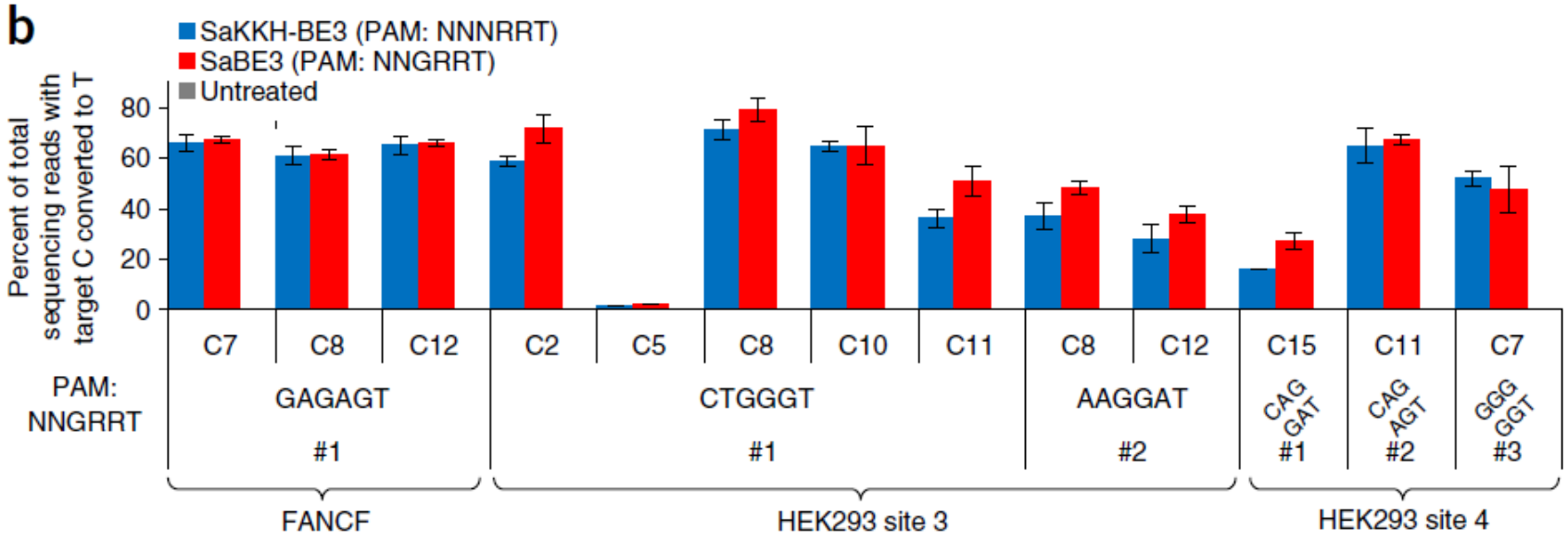
Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions

Nature Biotechnology, 2017

Y Bill Kim[1,2], Alexis C Komor[1,2], Jonathan M Levy[1,2], Michael S Packer[1,2], Kevin T Zhao[1,2] & David R Liu[1–3]



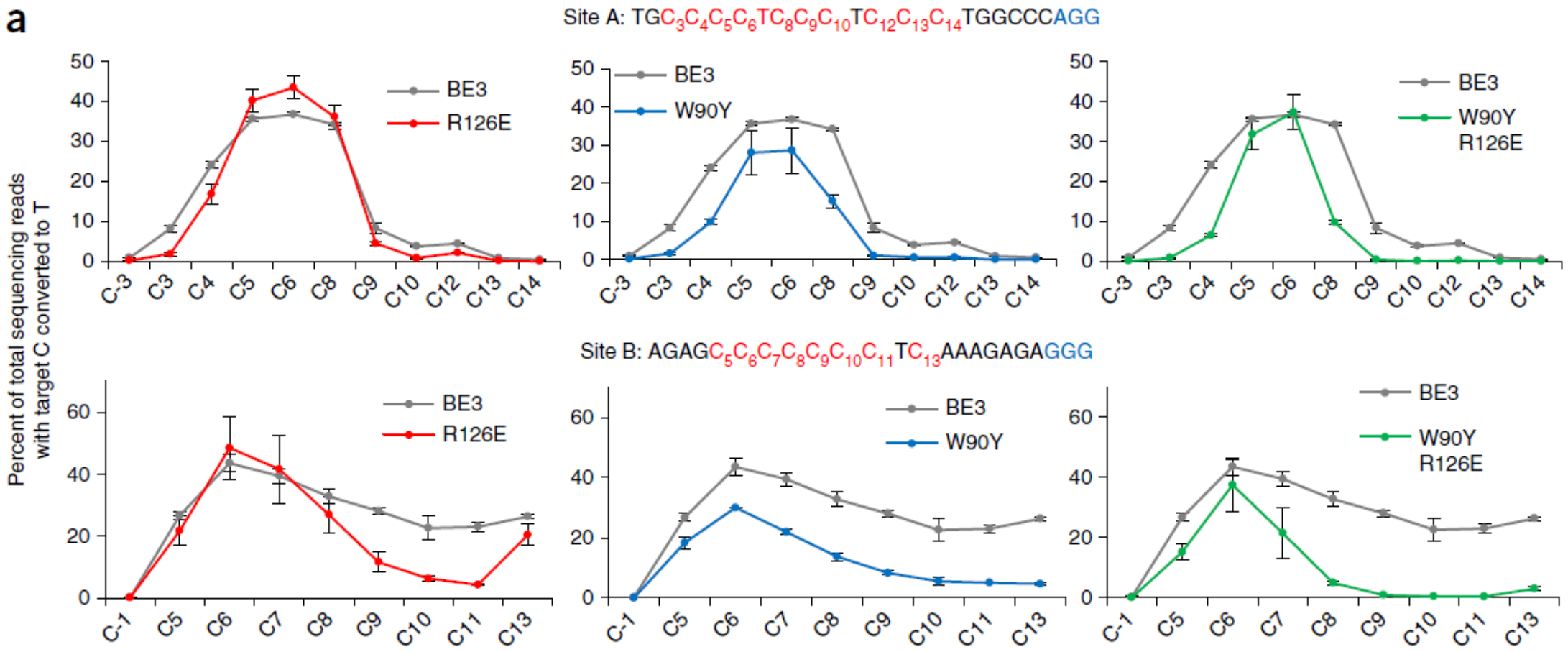
Base editor	PAM
BE3	NGG
VQR-BE3	NGAN
EQR-BE3	NGAG
VRER-BE3	NGCG
SaBE3	NNGRRT
SaKKH-BE3	NNNRRT



Expanded the sites that can be targeted by BE by 2.5-fold.

Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions

Y Bill Kim[1,2], Alexis C Komor[1,2], Jonathan M Levy[1,2], Michael S Packer[1,2], Kevin T Zhao[1,2] & David R Liu[1–3]



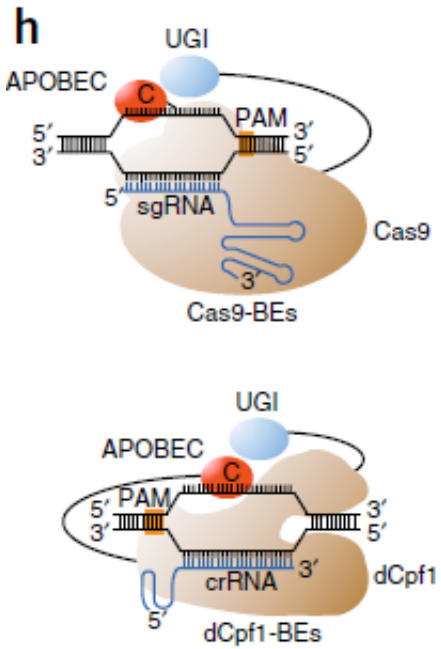
Narrow the editing window from 5 nt to 1-2 nt by engineering deaminase

Base editing with a Cpf1–cytidine deaminase fusion

Xiaosa Li[1–3,6], Ying Wang[4,6], Yajing Liu[1–3,6], Bei Yang[5,6], Xiao Wang[1–3], Jia Wei[4], Zongyang Lu[1–3], Yuxi Zhang[1], Jing Wu[1], Xingxu Huang[1], Li Yang[4] & Jia Chen[1]

Nature Biotechnology, 2018

PAM: TTTV



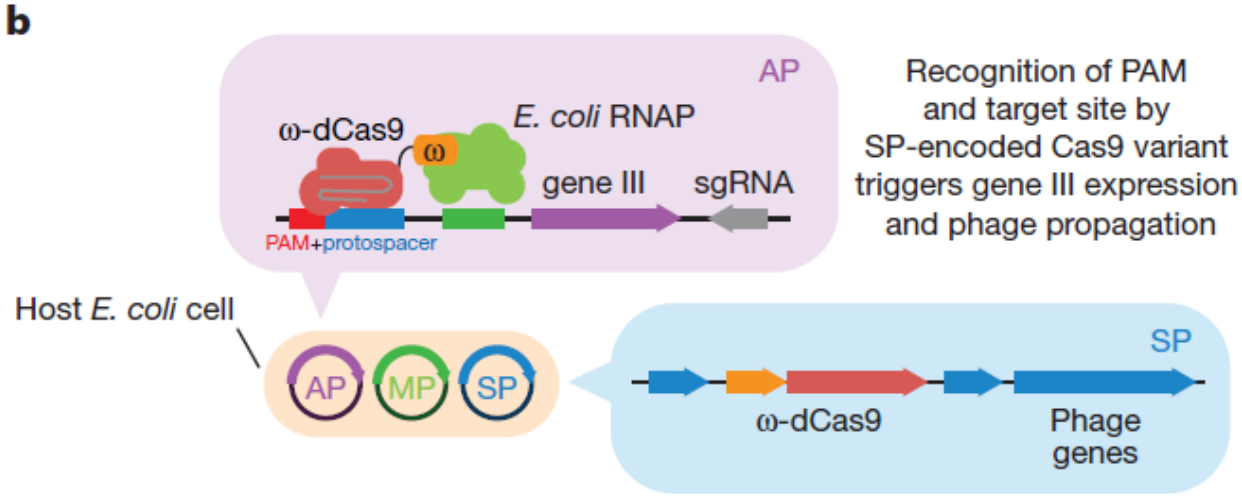
Name	Cas	PAM	APOBEC	N-terminal NLS	Fused UGI	Free UGI	Editing window	Editing efficiency	C-to-T fraction
dCas9-BE2	dCas9	NGG	rA1	–	+	–	4–8	~9–16%	~91–98%
nCas9-BE3	nCas9	NGG	rA1	–	+	–	4–8	~21–46%	~82–99%
dCpf1-BE0	dCpf1	TTTV	rA1	–	+	–	8–13	~10–31%	~89–99%
dCpf1-BE	dCpf1	TTTV	rA1	+	+	–	8–13	~20–44%	~88–99%
dCpf1-BE-YE	dCpf1	TTTV	rA1-YE	+	+	–	10–12	~2–29%	~92–98%
dCpf1-eBE	dCpf1	TTTV	rA1	+	+	+++	8–13	~15–30%	~97–99%
dCpf1-eBE-YE	dCpf1	TTTV	rA1-YE	+	+	+++	10–12	~2–28%	~95–99%

Based on the DYRK1A-, FANCF- and RUNX1-target sites

Evolved Cas9 variants with broad PAM compatibility and high DNA specificity

Johnny H. Hu[1,2,3], Shannon M. Miller[1,2,3], Maarten H. Geurts[1,2,3], Weixin Tang[1,2,3], Liwei Chen[1,2,3], Ning Sun[1,2,3], Christina M. Zeina[1,2,3], Xue Gao[1,2,3], Holly A. Rees[1,2,3], Zhi Lin[1,2,3] & David R. Liu[1,2,3]

Nature, 2018



Phage assisted continuous evolution (PACE)

e

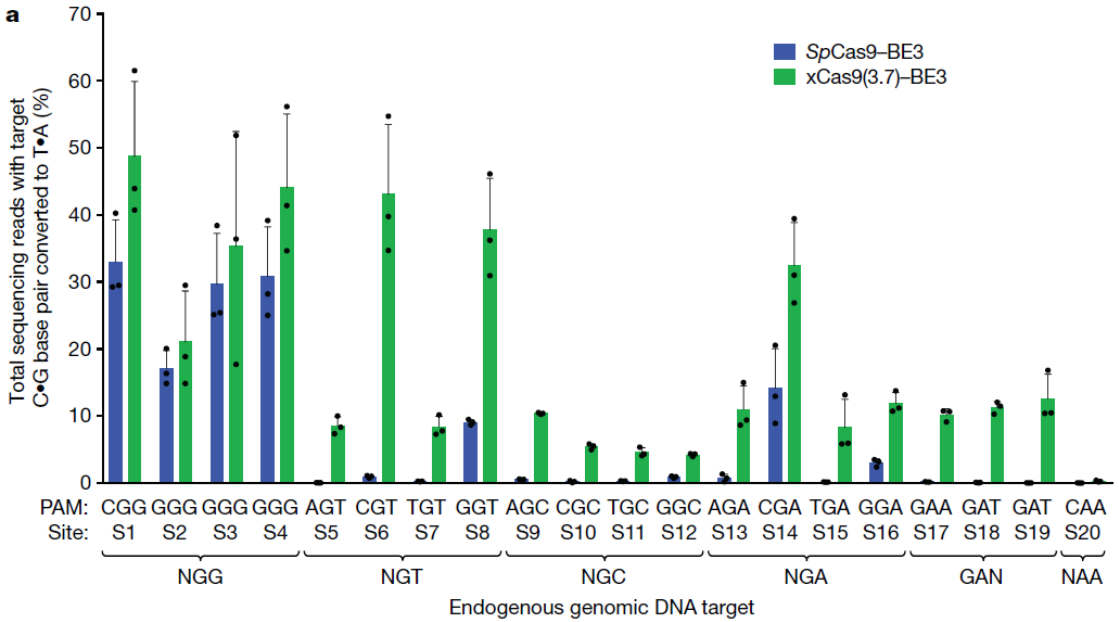
S. pyogenes Cas9 amino acid

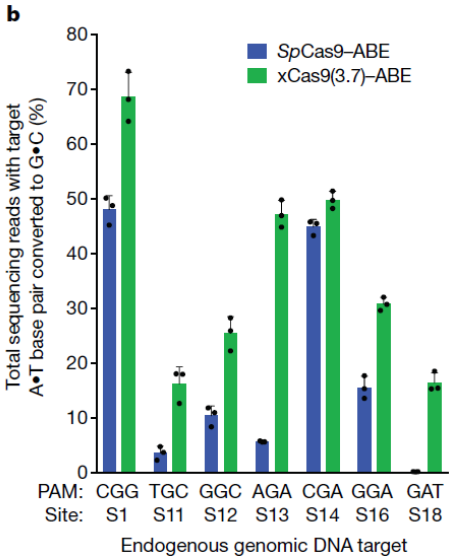
	108	217	262	324	409	480	543	694	1219
*Sp*Cas9	E	S	A	R	S	E	E	M	E
xCas9-1.2	E	S	A	R	S	K	D	M	V
xCas9-1.3	E	S	A	R	S	K	D	M	V
xCas9-2.0	E	S	T	R	I	K	D	M	V
xCas9-2.2	E	S	T	R	I	K	D	I	V
xCas9-3.6	G	A	T	R	I	K	D	I	V
xCas9-3.7	E	S	T	L	I	K	D	I	V

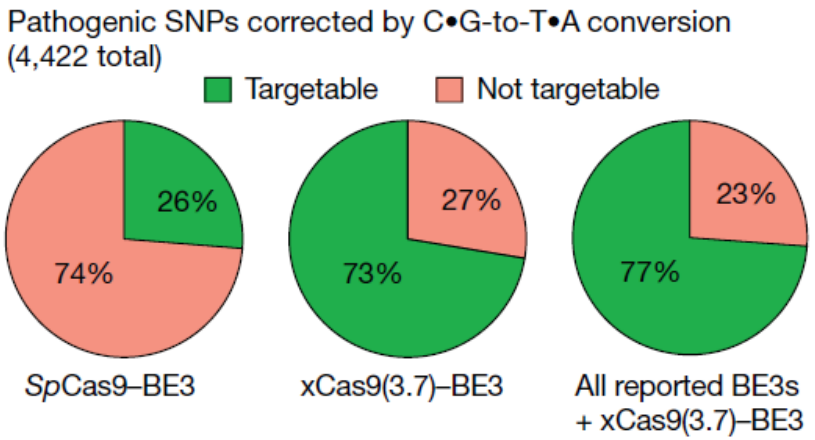
Cas9 variants bind broader PAMs

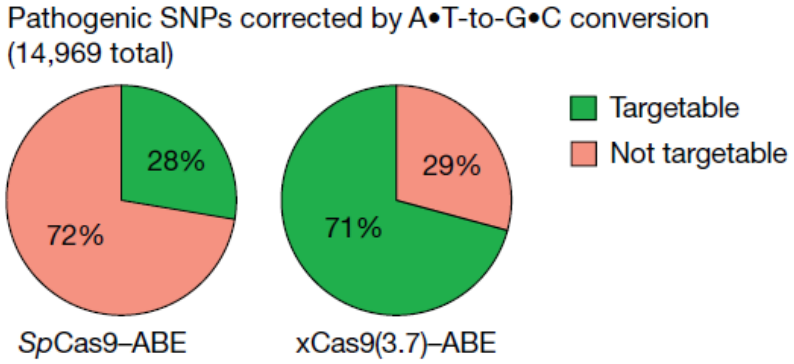
Evolved Cas9 variants with broad PAM compatibility and high DNA specificity

Johnny H. Hu[1,2,3], Shannon M. Miller[1,2,3], Maarten H. Geurts[1,2,3], Weixin Tang[1,2,3], Liwei Chen[1,2,3], Ning Sun[1,2,3], Christina M. Zeina[1,2,3], Xue Gao[1,2,3], Holly A. Rees[1,2,3], Zhi Lin[1,2,3] & David R. Liu[1,2,3]





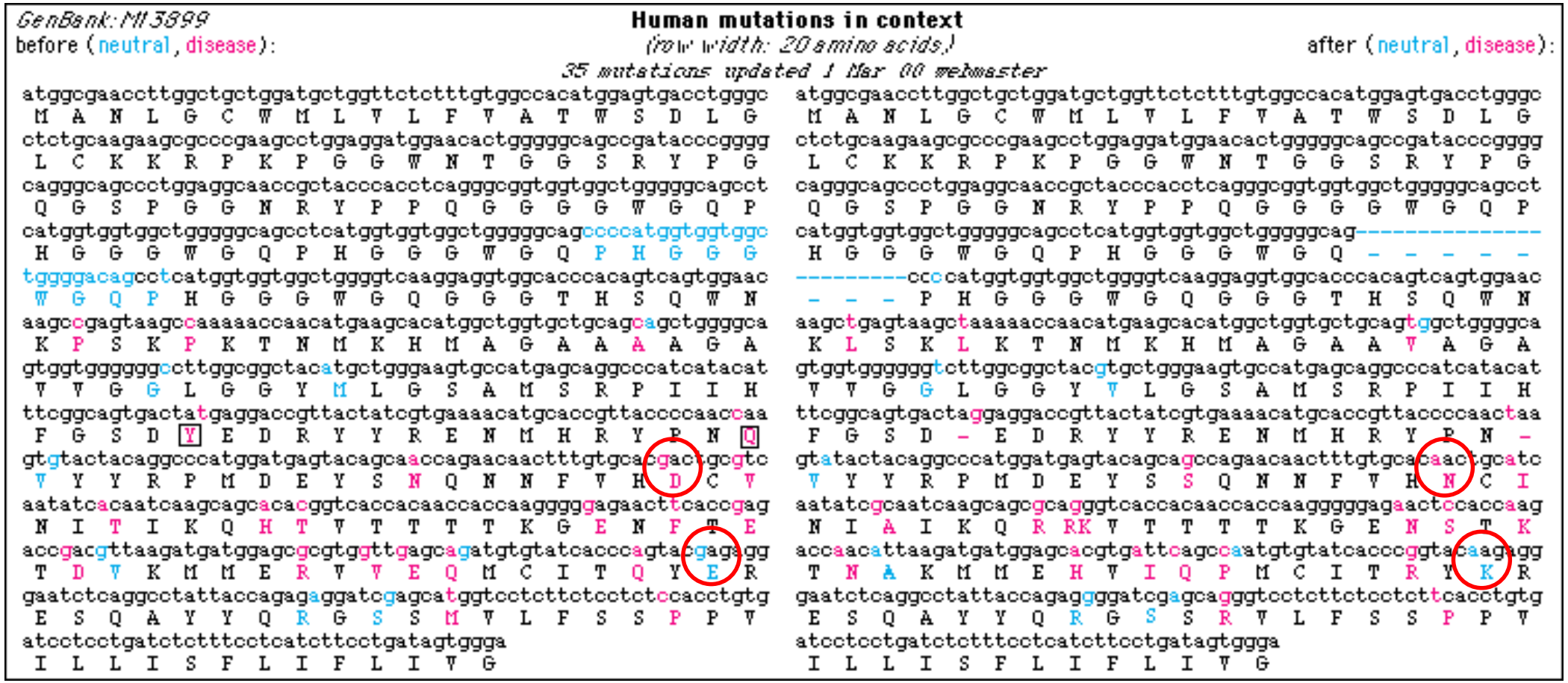




Summary IV

1. Tools for base editing C:G →T:A or A:T →G:C;
2. High efficiency and little unwanted indels;
3. Applicable for in vivo;
4. By protein engineering of Cas9 or other nuclease, more sites can be targeted;
5. Careful validation of the efficiency and specificity in vitro before applied for patients.

Human PRNP mutations



D178N: G→A mutation
E200K: G→A mutation
ABE7.10 could potentially be used for the correction

Thank you!