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Base Editors: Modular Tools for the Introduction of Point Mutations in Living Cells

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Abstract

Base editors are a new family of programmable genome editing tools that fuse ssDNA (single stranded DNA) modifying enzymes to catalytically inactive CRISPR-associated (Cas) endonucleases to induce highly efficient single base changes. With dozens of base editors now reported, it is apparent that these tools are highly modular; many combinations of ssDNA modifying enzymes and Cas proteins have resulted in a variety of base editors, each with its own unique properties and potential uses. In this perspective, we describe currently available base editors, highlighting their modular nature and describing the various options available for each component. Furthermore, we briefly discuss applications in synthetic biology and genome engineering where base editors have presented unique advantages over alternative techniques.

Introduction

Base editors are new genome editing tools capable of introducing single nucleotide variants (SNVs) with high efficiency in a programmable manner. They are an expansion of clustered regularly interspaced short palindromic repeats (CRISPR)-derived tools, incorporating various elements of this revolutionary technology that have made it so ubiquitous in laboratory applications. CRISPR-associated (Cas) endonucleases utilize a guide RNA molecule (gRNA) to bind to a target genomic DNA sequence and introduce a double-stranded DNA break (DSB)[1]. For this process to occur, the target sequence of interest, (defined as the protospacer), must be both complementary to the sequence of the gRNA and adjacent to a Cas protein-specific recognition sequence motif (the protospacer adjacent motif, or PAM). The Cas9-gRNA complex searches the genome, finds its matching protospacer-PAM sequence, and invades the genomic DNA to form an R-loop, where the gRNA engages in Watson-Crick base pairing with one strand of the protospacer. Once bound, the Cas protein introduces a DSB to initiate the first step of traditional genome editing. DSB-reliant genome editing techniques utilize one of two repair pathways to manipulate the genome following DSB introduction. Non-homologous end joining (NHEJ) will repair the break without requiring a template but results in stochastic nucleotide

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Conflicts of Interest

A.C.K. is a consultant of Pairwise Plants and Beam Therapeutics, companies that are developing and utilizing base editing technologies.

insertions or deletions (indels) at the site of the DSB, while homology directed repair (HDR) will utilize a user-provided homologous DNA template for precise genomic manipulations[2]. Shortcomings of DSB-reliant genome editing include the difficulty in controlling indel sequences and sizes with NHEJ, the low efficiency of HDR in post-mitotic cells, and the inherent competition between these two repair pathways often resulting in intractable mixtures of genome editing products[3,4]. Base editors were developed to provide an alternative, DSB-free, mechanism for making site-specific single nucleotide changes via chemical modification of the DNA nucleobases. This perspective will cover the many iterations of base editors, focusing on their modularity and flexibility, while also highlighting some unique applications these tools are primed for.

Considering the Modular Nature of Base Editors

Original Architectures—Base editors are composed of two key elements: a Cas protein for programmable DNA binding, and a single-stranded DNA (ssDNA) modifying enzyme for targeted nucleobase alteration. The original cytidine base editor (BE1)[5] used the catalytically inactive *Streptococcus pyogenes* Cas9 (dCas9, capable of binding DNA without cleavage) and fused it via its N-terminus to the cytidine deaminase enzyme APOBEC1 from *Rattus norvegicus* for these roles, respectively. To edit, first dCas9 brings the base editor complex to a genomic locus of interest determined by the sequence of its gRNA and the presence of an NGG PAM. This is followed by the dCas9-gRNA invading the double stranded target DNA and forming an R-loop, exposing a small window of single-stranded nucleotides on the opposing displaced strand. These nucleotides, no longer participating in Watson-Crick base pairing, are now suitable substrates for rAPOBEC1, which deaminates any cytidine bases within this ~5-nt window into uracils, creating a U·G mismatch in the genomic DNA (Figure 1). Since uracil, a non-canonical DNA base, has the same base pairing properties as the canonical DNA base thymine, DNA replication and/or repair of the resulting U·G mismatch will result in an overall C·G to T·A base pair edit when the modified uracil-containing strand is used as a template (Figure 1, blue inset). To increase editing efficiency in mammalian cells, where the DNA repair enzyme uracil DNA glycosylase (UNG) efficiently excises uracil from the U·G intermediate, two additional changes were made. First, a UNG inhibitor (UGI) peptide was fused to the C-terminus of the base editor to inhibit this repair pathway (to yield BE2, the second generation base editor[5]). Second, dCas9 was swapped for the nickase Cas9n (Cas9D10A), a variant that induces a nick in the DNA backbone of the G-containing strand of the U·G intermediate to favor replacement of this nicked strand by the cellular DNA replication and/or repair machinery (to yield BE3, the third generation base editor[5], Figure 1 orange inset). Additional studies revealed UNG to be responsible for poor product selectivity (C·G to non-T·A genome editing products) at certain genomic loci[6]. To combat this, an additional copy of UGI and optimized linker lengths were engineered in a follow-up study to yield BE4, the fourth generation original cytosine base editor (CBE).

Cas Protein Modulation—While BE2 and BE3 have been used to make single nucleotide changes in a variety of bacterial[7,8,9] and mammalian cell[10,11,12] applications, their modular natures have also served as scaffolds for further engineering efforts to tune their functionality and expand their substrate scopes. Due to the strict gRNA design criteria for

base editing imposed by the precise PAM-to-target C distance requirements (Figure 2, blue inset), an analysis of the ClinVar dataset[5,13] found that only 27% of pathogenic T·A to C·G SNVs are targetable using base editors relying on NGG PAMs[14]. Fortunately, there are a host of well-characterized Cas endonucleases, both native and engineered, with different PAM requirements. In subsequent follow-up studies, it was demonstrated that the basic base editor architectures of BE2 and BE3 are compatible with replacement of the SpCas9 module with a variety of these homologous and engineered Cas proteins (Figure 2). Specifically, the VQR and VRER mutant variants of SpCas9 allow for base editing at NGA and NGCG PAMs, respectively, and replacement of SpCas9 with the smaller *S. aureus* homolog SaCas9 further expanded PAM availability to include NNGRRT and NNNRRT, albeit with a different editing window (Figure 2, blue inset)[14]. It was additionally demonstrated that the BE2 architecture is compatible with dCas12a, (formerly dCpf1), making T-rich PAM sites amenable to base editing, again with a modified editing window (Figure 2, blue inset)[15]. Base editors derived from xCas9[16] and Cas9-NG[17] have also been reported, yielding the most flexible editors described to date with NG PAM requirements[18]. Additional Cas protein engineering efforts, such as enhanced specificity variants[19], have also been shown to be compatible with various base editor architectures, further demonstrating their modularity.

Cytidine Deaminase Engineering Efforts—Engineering efforts have also been directed at the nucleobase modifying module of base editors. In an early study, the activation-induced cytidine deaminase (AID) enzyme from *Petromyzon marinus*, pmCDA1, was fused to the C-terminus of SpCas9 to create the base editor Target-AID[20]. The combination of an alternate DNA modifying enzyme and overall architecture not only shifted the substrate window, but also altered the sequence specificity of the base editor due to the inherent sequence motif preference of the pmCDA1 enzyme (Figure 2, red inset). While fusion of a UGI domain, as done in BE2 and BE3, helped increase the product selectivity to favor C·G to T·A outcomes, other researchers have taken advantage of UGI-free AID-derived base editors to mutagenize specific genomic loci and create locally hyper-mutagenized, genetically encoded libraries[21]. A variety of homologs of rAPOBEC1 have been repurposed as base editors as well, such as hAID[6], hAPOBEC3G[6], hAPOBEC3A[22,23], hAPOBECB[22], hAPOBEC3B (catalytic domain)[23,24], and hAPOBEC3H[25] yielding base editors with alternate sequence preferences and the ability to efficiently edit methylated cytosines (Figure 2, red inset). Within the APOBEC family of base editors, engineering efforts have been undertaken to both widen and narrow the base editing window, as well as create sequence-specific editors[26]. Linker length and composition have been explored as means to modulate activity window (Figure 2, grey insert)[12, 27]. Additionally, mutations in APOBEC1 known to lower the catalytic activity were found to narrow the editing window[14], creating more precise base editors. Conversely, the fusion complex BE-PLUS[28], designed to recruit multiple antibody-rAPOBEC1 fusions to the Cas9n-gRNA complex, exhibited an expanded editing window (Figure 2, green inset). Engineering efforts have also been made to create sequence-specific deaminases. An hAPOBEC3A editor took advantage of this homolog's native sequence preference to engineer a base editor tailored for deaminating TC motifs[29]. These extensive engineering efforts have produced a litany of mutant cytidine deaminases, each tailored for specific targets and/or applications (Figure 2, red inset).

Additional nucleobase modifications—While engineering the cytidine deaminase produced altered editing windows and sequence preferences, the target nucleobase remained fixed. However, given the modular nature of the base editor architecture, it was proposed that alternative ssDNA modifying enzymes that performed different nucleotide modifying chemistries could be engineered into new base editors capable of introducing additional base transformations. With the repertoire of naturally-occurring ssDNA modifying enzymes exhausted, a new base editor was created by repurposing a tRNA adenosine deaminase enzyme for ssDNA modification[30]. *Escherichia coli* TadA is an enzyme that deaminates the adenosine at the wobble position 34 of $tRNA^{Arg2}$ to inosine[31]. In the context of DNA replication, inosine is interpreted as guanine (Figure 1, blue insert), opening up the possibility of using ecTadA to make an A·T to G·C base editor (ABE). Given its native tRNA substrate, the wild-type ecTadA-derived ABE was not competent for genome editing. However, following seven rounds of directed evolution in which selective pressure was placed on mutated libraries of ecTadA to modify ssDNA, two combinations of mutations were found to result in efficient A·T to G·C base editing, with slightly different editing windows (ABE7.9 and ABE7.10). Analogous to rAPOBEC1 in CBE, the optimized ecTadA* has been appended to several engineered Cas9 variants to create ABEs with varying PAM requirements and editing windows[32,33,34,35] (Figure 2, red inset). Between the development of ABE and CBE, all transition mutations (purine to purine or pyrimidine to pyrimidine) are now possible using base editor technologies, given the proximity to an appropriate PAM (Figure 3).

Enhancing the Specificity of Base Editors—While the use of high-fidelity Cas variants has been shown to greatly reduce gRNA-dependent off-target DNA base editing[19], recent studies reported gRNA-independent off-target C·G to T·A mutations in highly transcribed regions when using BE3 in both mouse embryos[36] and rice[37]. In contrast, gRNA-independent off-target A·T to G·C mutations were not observed following ABE treatment, leading researchers to hypothesize that the engineering used to develop ABE may impart resistance to non-specific ssDNA adenosine deamination. Future work is needed to identify cytidine deaminase mutants that reduce this non-specific activity. In addition to off-target DNA editing, BE3 and ABE have been observed to induce transcriptome-wide gRNA-independent off-target *RNA* editing[38,39,40]. Fortuitously, engineering efforts identified a variety of mutations in both rAPOBEC1 and TadA* that nearly fully abrogated off-target transcriptome-wide editing events without sacrificing on-target DNA editing efficiency (Figure 2, red inset)[40].

Base Editor Applications

Biomedical Applications—Base editors are capable of introducing SNVs efficiently and site-specifically into the genome of mammalian[41,42,43], plant[35,44,45,46], and bacterial cells[7,47]. It is important to note that different codon optimizations of the base editors have been shown to have dramatic effects on editing efficiencies when adapting these tools for use in new cell types/organoids[12,48]. Given their high efficiencies, these tools have been widely applied to develop research models for genetic studies. CBEs and ABEs have been extensively used to both correct pathogenic SNVs and introduce SNVs of clinical interest in model organisms,[42,49,50,51]. Additionally, two techniques called CRISPR-STOP[43] and

iSTOP[52] were recently developed that use CBEs to introduce premature stop codons to generate knockout models. By circumventing the use of indels for gene knockout, the risk of creating an in-frame indel with a functional transcript is avoided. Additionally, base editing induces lower rates of apoptosis than traditional DSB-mediated genome editing[43]. With 99.7% of human genes targetable via iSTOP gRNAs[52], these tools may allow researchers greater control over genome editing outcomes and less cytotoxicity when creating knockout models for further study.

Agricultural Applications—Base editors have been particularly popular in the agricultural field as well, since traditional HDR-mediated genome engineering approaches can be inefficient in plants due to difficulty in delivering donor templates[53,54]. Additionally, many agricultural mutations of interest, such as resistance to herbicides and enhancement of grain production, are SNVs, making base editors well-suited for agricultural engineering. Consequently, several base editor constructs have been codon-optimized to work in rice[35,44,45,55,56], maize[44], tomato[45], wheat[44], cotton[57], potato[58] as well as model organism such as *Arabidopsis thaliana* and *Brassica napus*[59]. Like other CRISPR-edited crops[60], base edited crops are expected to pass USDA GMO-regulation restrictions as they don't deliver viral genetic material. Given the efficiency and ease of use of base editing, BE-modified crops may someday be available in commercial markets.

Synthetic Biology Applications—Beyond using base editors to introduce SNVs for clinical and agricultural purposes, they have also been repurposed for applications in the field of synthetic biology. Due to their high ratio of SNV introduction to indel formation, CBEs have been used to create genetically encoded libraries[21,41]. Specifically, an AID-derived base editor with a wide editing window was programmed to mutagenize the *BCR-ABL* gene, a protein with known SNVs resulting in imatinib-resistance. Under imatinib selection, both known and previously unidentified drug-resistant SNVs were identified[21]. Another unique application of base editors is as molecular recording devices[61]. In one such system, stimulus in the form of light, phage infection, or small molecule treatment resulted in analog intensity of gRNA expression and subsequent accumulation of base editor signal. By stacking base editing targets such that sequential stimuli are required to induce edits, an analog system was created capable of recording the order and intensity of stimuli events. This resulted in a molecular recording device more temporally precise than preceding wild-type Cas9-based recorders.

Current base editor limitations and perspectives moving forward

Base editors, despite their relatively recent inception, have already been widely used for agricultural, biomedical, clinical, and synthetic biology research. Base editing technologies have grown and swiftly expanded in the last 3 years, and there are many exciting opportunities for additional expansions of this technology. Perhaps the most pressing hurdle to overcome is the issue of off-target DNA editing by CBEs, which we envision may be alleviated through additional cytidine deaminase engineering efforts. There is a need for new assays capable of detecting gRNA-independent off-target DNA base editing beyond cost-prohibitive whole genome sequencing to aid in these future engineering efforts to alleviate these issues. For now, researchers must carefully consider whether a base editing application

is tolerable to off-target DNA editing. Additionally, editors capable of facilitating transversion mutations (purine to pyrimidine or vice versa, which account for four of the six possible base conversions) currently do not exist. These presently unavailable tools may be developed from additional nucleobase chemistries or cellular DNA repair manipulation strategies.

Due to the modularity of these tools, it is likely that future Cas protein engineering efforts will be compatible with base editors to create additional variants with expanded targeting scopes (via new PAM recognition) and increased fidelity. The lowered toxicity of base editor intermediates (uracil and inosine) compared to DSBs opens up the possibility of multiplexing base editing and introducing multiple point mutations throughout the genome of a given cell.

Given the substantial engineering efforts that have been placed into its modular components, and the technologies emerging from its application in basic research and as tools for synthetic and chemical biology, base editors hold much promise for the genome engineering community.

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Abbreviations List

A	adenine
ABE	A·T to G·C base editor
AID	activation-induced cytidine deaminase
C	cytosine; Cas, CRISPR-associated
CBE	C·G to T·A base editor
CRISPR	clustered regularly interspaced palindromic repeats
DSB	double stranded break
G	guanine
gRNA	guide RNA
HDR	homology directed repair
indel	insertions and/or deletions
N	any canonical nucleotide
NHEJ	non-homologous end joining
PAM	protospacer adjacent motif

R	purine nucleotide A or G
SNV	single nucleotide variant
ssDNA	single stranded DNA
T	thymine
UGI	UNG inhibitor
UNG	uracil DNA glycosylase

References

1. Jinek M. et al. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science (80-.). 337, 816–822 (2012).
2. Kim H. & Kim JS A guide to genome engineering with programmable nucleases. Nat. Rev. Genet. 15, 321–334 (2014). [PubMed: 24690881]
3. Hsu PD, Lander ES & Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell 157, 1262–1278 (2014). [PubMed: 24906146]
4. Sander JD & Joung JK CRISPR-Cas systems for editing, regulating and targeting genomes. Nat. Biotechnol 32, 347–350 (2014). [PubMed: 24584096]
5. Komor AC, Kim YB, Packer MS, Zuris JA & Liu DR Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 533, 420–424 (2016). [PubMed: 27096365]
6. Komor AC et al. Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity. Sci. Adv 3, eaao4774 (2017).
7. Zheng K. et al. Highly efficient base editing in bacteria using a Cas9-cytidine deaminase fusion. Commun. Biol 1, 1–6 (2018). [PubMed: 29809203]
8. Chen W. et al. CRISPR/Cas9-based genome editing in Pseudomonas aeruginosa and cytidine deaminase-mediated base editing in Pseudomonas species. iScience 6, 222–231 (2018). [PubMed: 30240613]
9. Wang Y. et al. CRISPR-Cas9 and CRISPR-assisted cytidine deaminase enable precise and efficient genome editing in Klebsiella pneumoniae. Appl. Environ. Microbiol 84, e01834–18 (2018). [PubMed: 30217854]
10. Liang P. et al. Correction of β-thalassemia mutant by base editor in human embryos. Protein Cell 8, 811–822 (2017). [PubMed: 28942539]
11. Kim K. et al. Highly efficient RNA-guided base editing in mouse embryos. Nat. Biotechnol 35, 435–437 (2017). [PubMed: 28244995]
12. Zafra MP et al. Optimized base editors enable efficient editing in cells, organoids and mice. Nat. Biotechnol 36, 888–893 (2018). [PubMed: 29969439]
13. Landrum MJ et al. ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res. 44, D862–D868 (2015). [PubMed: 26582918]
14. Kim YB et al. Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. Nat. Biotechnol 35, 371–376 (2017). [PubMed: 28191901]
15. Li X. et al. Base editing with a Cpf1–cytidine deaminase fusion. Nat. Biotechnol 36, 324–327 (2018). [PubMed: 29553573]
16. Hu JH et al. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. Nature 556, 57–63 (2018). [PubMed: 29512652]
17. Endo M. et al. Genome editing in plants by engineered CRISPR-Cas9 recognizing NG PAM. Nat. Plants 5, 14–17 (2019). [PubMed: 30531939]
18. Hua K, Tao X, Han P, Wang R. & Zhu JK Genome Engineering in Rice Using Cas9 Variants that Recognize NG PAM Sequences. Mol. Plant 12, 1003–1014 (2019). [PubMed: 30928636]

19. Rees HA et al. Improving the DNA specificity and applicability of base editing through protein engineering and protein delivery. Nat. Commun 8, 1–10 (2017). [PubMed: 28232747]
20. Nishida K. et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. Science (80-.). 353, (2016).
21. Ma Y. et al. Targeted AID-mediated mutagenesis (TAM) enables efficient genomic diversification in mammalian cells. Nat. Methods 13, 1029–1035 (2016). [PubMed: 27723754]
22. Wang X. et al. Efficient base editing in methylated regions with a human APOBEC3A-Cas9 fusion. Nat. Biotechnol 36, 946–949 (2018). [PubMed: 30125268]
23. St Martin A. et al. A fluorescent reporter for quantification and enrichment of DNA editing by APOBEC-Cas9 or cleavage by Cas9 in living cells. Nucleic Acids Res. 46, e84 (2018).
24. Coelho MA et al. BE-FLARE : a fluorescent reporter of base editing activity reveals editing characteristics of APOBEC3A and APOBEC3B. BMC Biol. 16, 1–11 (2018). [PubMed: 29325545]
25. Martin AS et al. A panel of eGFP reporters for single base editing by APOBEC-Cas9 editosome complexes. Sci. Rep 9, 1–8 (2019). [PubMed: 30626917]
26. Thuronyi BW et al. Continuous evolution of base editors with expanded target compatibility and improved activity. Nat. Biotechnol (2019). doi:10.1038/s41587-019-0193-0
27. Tan J, Zhang F, Karcher D. & Bock R. Engineering of high-precision base editors for site-specific single nucleotide replacement. Nat. Commun 10, 1–10 (2019). [PubMed: 30602773]
28. Jiang W. et al. BE-PLUS: a new base editing tool with broadened editing window and enhanced fidelity. Cell Res. 28, 855–861 (2018). [PubMed: 29875396]
29. Gehrke JM et al. An APOBEC3A-Cas9 base editor with minimized bystander and off-target activities. Nat. Biotechnol 36, 977–982 (2018). [PubMed: 30059493]
30. Gaudelli NM et al. Programmable base editing of A • T to G • C in genomic DNA without DNA cleavage. Nature 551, 464–471 (2017). [PubMed: 29160308]
31. Kim J. et al. Structural and kinetic characterization of Escherichia coli TadA, the wobble-specific tRNA deaminase. Biochemistry 45, 6407–6416 (2006). [PubMed: 16700551]
32. Yang L. et al. Increasing targeting scope of adenosine base editors in mouse and rat embryos through fusion of TadA deaminase with Cas9 variants. Protein Cell 9, 814–819 (2018). [PubMed: 30066232]
33. Huang TP et al. Circularly permuted and PAM-modified Cas9 variants broaden the targeting scope of base editors. Nat. Biotechnol 37, 626–631 (2019). [PubMed: 31110355]
34. Hua K, Tao X. & Zhu J. Expanding the base editing scope in rice by using Cas9 variants. Plant Biotechnol. J 17, 499–504 (2019). [PubMed: 30051586]
35. Hua K, Tao X, Yuan F, Wang D. & Zhu J. Precise A-T to G-C base editing in the rice genome. Mol. Plant 11, 627–630 (2018). [PubMed: 29476916]
36. Zuo E. et al. Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos. Science (80-.). 364, 289–292 (2019).
37. Jin S. et al. Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice. Science (80-.). 364, 292–295 (2019).
38. Grünewald J. et al. Transcriptome-wide off-target RNA editing induced by CRISPR-guided DNA base editors. Nature 569, 433–436 (2019). [PubMed: 30995674]
39. Zhou C. et al. Off-target RNA mutation induced by DNA base editing and its elimination by mutagenesis. Nature 571, 275–278 (2019). [PubMed: 31181567]
40. Rees HA, Wilson C, Doman JL & Liu DR Analysis and minimization of cellular RNA editing by DNA adenine base editors. Sci. Adv 5, eaax5717 (2019).
41. Hess GT et al. Directed evolution using dCas9-targeted somatic hypermutation in mammalian cells. Nat. Methods 13, 1036–1041 (2016). [PubMed: 27798611]
42. Ryu S. et al. Adenine base editing in mouse embryos and an adult mouse model of Duchenne muscular dystrophy. Nat. Biotechnol 36, 536–539 (2018). [PubMed: 29702637]
43. Kuscu C. et al. CRISPR-STOP: gene silencing through nonsense mutations. Nat. Methods 14, 710–712 (2017). [PubMed: 28581493]

44. Zong Y. et al. Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. Nat. Biotechnol 35, 438–440 (2017). [PubMed: 28244994]
45. Shimatani Z. et al. Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. Nat. Biotechnol 35, 441–443 (2017). [PubMed: 28346401]
46. Yan F. et al. Highly efficient A-T to G-C base editing by Cas9n-guided tRNA adenosine deaminase in rice. Mol. Plant 11, 631–634 (2018). [PubMed: 29476918]
47. Li Q. et al. CRISPR–Cas9 D10A nickase-assisted base editing in the solvent producer Clostridium beijerinckii. Biotechnol. Bioeng 116, 1475–1483 (2019). [PubMed: 30739328]
48. Koblan LW et al. Improving cytidine and adenine base editors by expression optimization and ancestral reconstruction. Nat. Biotechnol 36, 843–848 (2018). [PubMed: 29813047]
49. Zhang Y. et al. Programmable base editing of zebrafish genome using a modified CRISPR-Cas9 system. Nat. Commun 8, 1–5 (2017). [PubMed: 28232747]
50. Li Z. et al. Efficient RNA-guided base editing for disease modeling in pigs. Cell Discov. 4, 7–10 (2018). [PubMed: 29449961]
51. Liu Z. et al. Efficient generation of mouse models of human diseases via ABE- and BE-mediated base editing. Nat. Commun 9, 1–8 (2018). [PubMed: 29317637]
52. Billon P. et al. CRISPR-mediated base editing enables efficient disruption of eukaryotic genes through induction of STOP codons. Mol. Cell 67, 1068–1079 (2017). [PubMed: 28890334]
53. Malzahn A, Lowder L. & Qi Y. Plant genome editing with TALEN and CRISPR. Cell Biosci. 7, 1–18 (2017). [PubMed: 28053698]
54. Liu D, Hu R, Palla KJ, Tuskan GA & Yang X. Advances and perspectives on the use of CRISPR/Cas9 systems in plant genomics research. Curr. Opin. Plant Biol 30, 70–77 (2016). [PubMed: 26896588]
55. Li J, Sun Y, Du J, Zhao Y. & Xia L. Generation of targeted point mutations in rice by a modified CRISPR/Cas9 system. Mol. Plant 10, 526–529 (2017). [PubMed: 27940306]
56. Lu Y. & Zhu J. Precise editing of a target base in the rice genome using a modified CRISPR/Cas9 system. Mol. Plant 10, 523–525 (2017). [PubMed: 27932049]
57. Qin L. et al. High-efficient and precise base editing of C • G to T • A in the allotetraploid cotton (Gossypium hirsutum) genome using a modified CRISPR/Cas9 system. Plant Biotechnol. J 1–12 (2019). doi:10.1111/pbi.13168
58. Zong Y. et al. Efficient C-to-T base editing in plants using a fusion of nCas9 and human APOBEC3A. Nat. Biotechnol 36, 950–953 (2018).
59. Kang B. et al. Precision genome engineering through adenine base editing in plants. Nat. Plants 4, 427–431 (2018). [PubMed: 29867128]
60. Waltz E. CRISPR-edited crops free to enter market, skip regulation. Nat. Biotechnol 34, 582 (2016). [PubMed: 27281401]
61. Tang W. & Liu DR Rewritable multi-event analog recording in bacterial and mammalian cells. Science (80-.). 360, eaap8992 (2018).

Summary Points

- Base editors are a new, modular tool for creating single nucleotide variants without introducing double stranded breaks.
- The modularity of base editors allows for control over the precision and specificity of genomic edits, making them flexible tools for basic research, genome engineering and genetically encoded libraries.
- Future engineering efforts on individual modules of these tools are expected to widen the genome editing substrate scope with additional edits made possible.

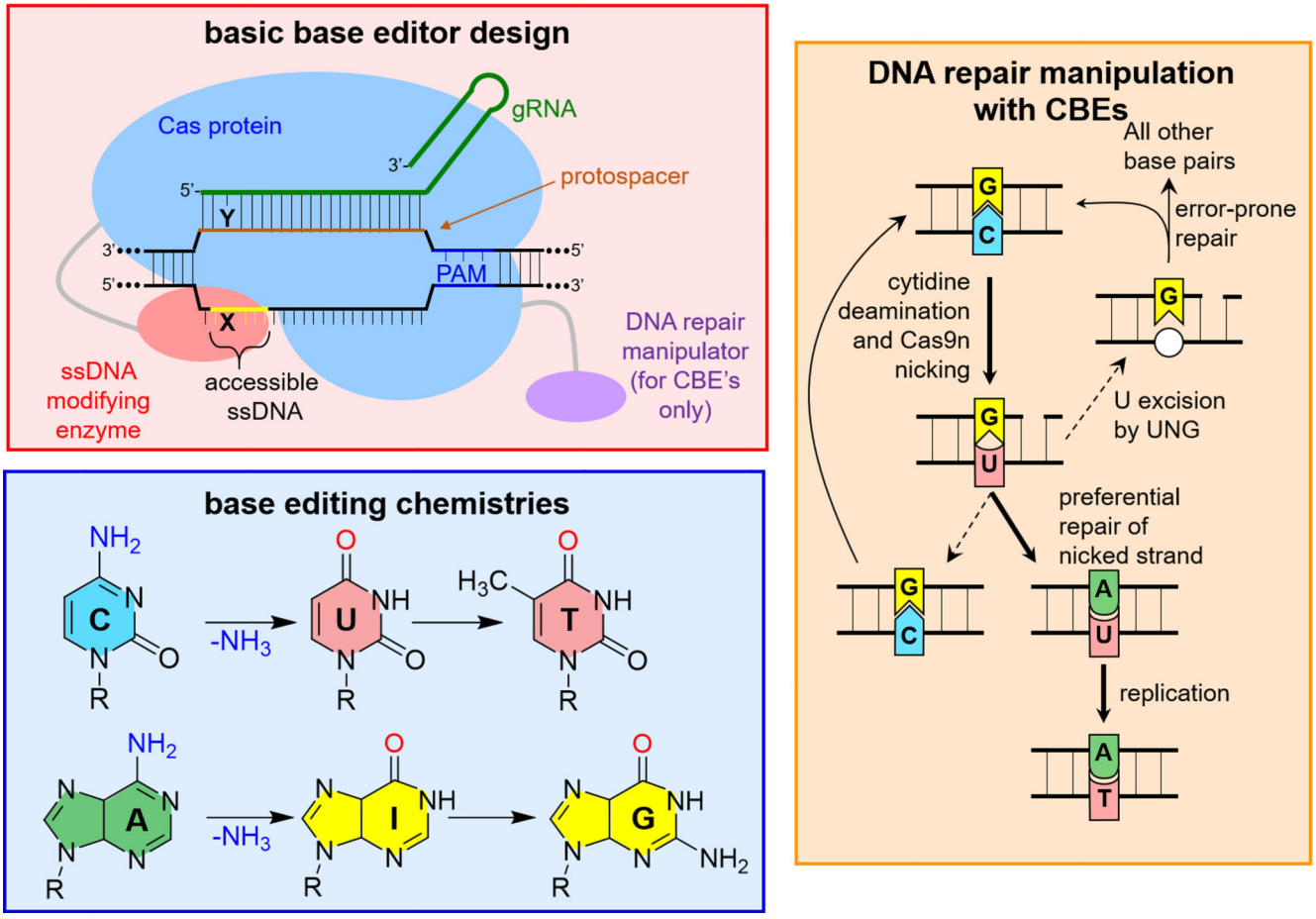
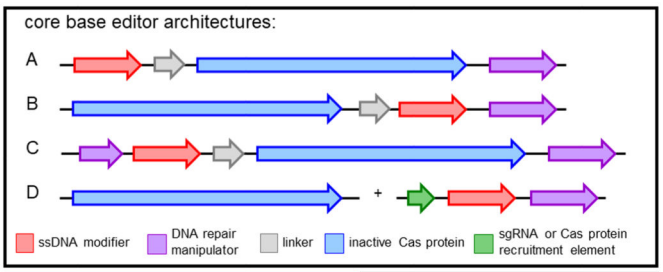


Figure 1. Basic base editor design, editing chemistries, and repair manipulation

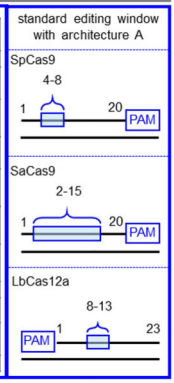
The basic base editor design is illustrated (red inset). Once a Cas-gRNA complex binds and opens up the genomic DNA at a protospacer-PAM sequence, a small stretch of nucleotides within the R-loop (indicated in yellow) becomes accessible to a Cas-tethered ssDNA modifying enzyme. The identity of the target X·Y base pair correlates with the type of base editor; C·G for CBEs and A·T for ABEs. The deamination reactions performed by the ssDNA modifying enzymes and the corresponding non-canonical DNA base intermediates formed by these reactions are also shown (blue insert). CBEs require further DNA repair manipulation to increase their C·G to T·A editing efficiencies (orange inset).



DNA Repair Manipulator	Notes	Architecture[Ref]
UGI	inhibits UNG	A[5], D[27]
2XUGI	enhanced UNG inhibition	A[6]
GAM	binds ends of DSBs, preventing indels	C[6]
none	for random mutagenesis/ usage with ABE	ref. 21

Linker	Sequence length (aa)	Architecture[Ref]
flexible XTEN	16	A[5]
extended XTEN	32	A[6]
SH3	100	B[20]
rigid linker	7	A[26]
2X	33	A[12]

Cas protein (catalytically dead or nickase)	PAM	Architecture[Ref]
SpCas9	NGG	A[5], D[27]
SpCas9 – *VQR* (D1135V+R1335Q+T1337R)	NGA	A[14]
SpCas9 - *VRQR* (D1135V+G1218R+R1335Q+ T1337R)	NGA	A[32]
SpCas9 - *EQR* (D1135E+R1335Q+T1337R)	NGAG	A[14]
SpCas9 – *VRER* (D1135V+G1218R+R1335E+T1337R)	NGCG	A[14]
HF-Cas9 (N497A+R661A+Q695A+Q926A)	NGG	A[19]
xCas9	NG, GAT, GAA	A[16]
Cas9-NG	NG	B[17]
SaCas9	NNGRRT	A[14]
SaCas9-KKH (E782K+N968K+R1015)	NNNRRT	A[14]
LbCas12a	TTTV	A[15]



sgRNA or Cas protein recruitment element	Notes	Architecture[Ref]
MS2 Coat Protein (MCP)	requires MS2 hairpin imbedded in gRNA	D[40]
ScFv	requires GCN4 peptide fused to Cas element	D[27]

ssDNA modifier (CBEs)	Notes	Architecture[Ref]
rAPOBEC1	prefers TC motifs	A[5], D[27]
rAPOBEC1– *YE1* (W90Y+R126E)	less processive variant, prefers editing at positions C5 > C6 > C7 ≈ C4; reduced off-target RNA editing	A[14], A[38]
rAPOBEC1– *EE* (R126E+R132E)	narrows window of activity	A[14]
rAPOBEC1– *YEE* (W90Y+R126E+R132E)	less processive variant, prefers editing at positions C5 > C6 > C7 ≈ C4	A[14]
rAPOBEC1 (R33A+K34A)	no off-target RNA editing (SECURE editor)	A[37]
Anc689 (contains 36 mutations in rAPOBEC1)	increased editing efficiency	A[47]
hAPOBEC3A	deaminates 5-methylcytosine and GpC sites	A[22]
hAPOBEC3A (N57G)	deaminates cytidines with the preference TCR>TCY>VCN	A[28]
hAPOBEC3Bctd (catalytic domain)	prefers TC motifs, narrows catalytic window	A[23]
hAPOBEC3G	little sequence preference, but greatly decreased editing efficiency	A[6]
hAPOBEC3B (full-length)	intermediate editing efficiency	A[25]
hAPOBEC3H-II	intermediate editing efficiency	A[25]
evoAPOBEC1	increased editing efficiency at GC motifs	A[61]
evoFERNY	smaller than APOBEC1, little sequence preference	A[61]
BE4Max	codon optimized for mammalian Cells	A[48]
FNLS	FLAG epitope at N terminus of APOBEC increases BE distribution and efficiency	A[12]
pmCDA1	little sequence preference	B[20], A[6]
hAID	little sequence preference	A[5], B[21], D[40]
AIDΔ (no NLS)	higher activity, used for random mutagenesis	D[40]
AID (P182X)	higher activity, used for random mutagenesis	B[21]

ssDNA modifier (ABEs)	Notes	Architecture[Ref]
ecTadA-ecTadA*7.9 (15 mutations)	edits efficiently at bases 8-10nt from PAM distal end of protospacer with spCas9	A[29]
ecTadA-ecTadA*7.10 (14 mutations)	edits efficiently at bases 4-7nt from PAM distal end of protospacer with spCas9	A[29]
ABEMax	codon optimized for mammalian cells; increased editing efficiency	A[47]
ecTadA(E59A)-ecTadA*7.10(V106W)	greatly reduced off-target RNA editing	A[39]
ecTadA(F148A)-ecTadA*7.10(F148A)	no detectable off-target RNA editing	A[38]

Figure 2. Modularity of Base Editors

The four core architectures used with base editors are shown (black box), with previously used options for each of the components listed in tables. The particular architectures that have been used with each variant are specified, but we encourage readers to mix-and-match components according to their desired application of base editing.

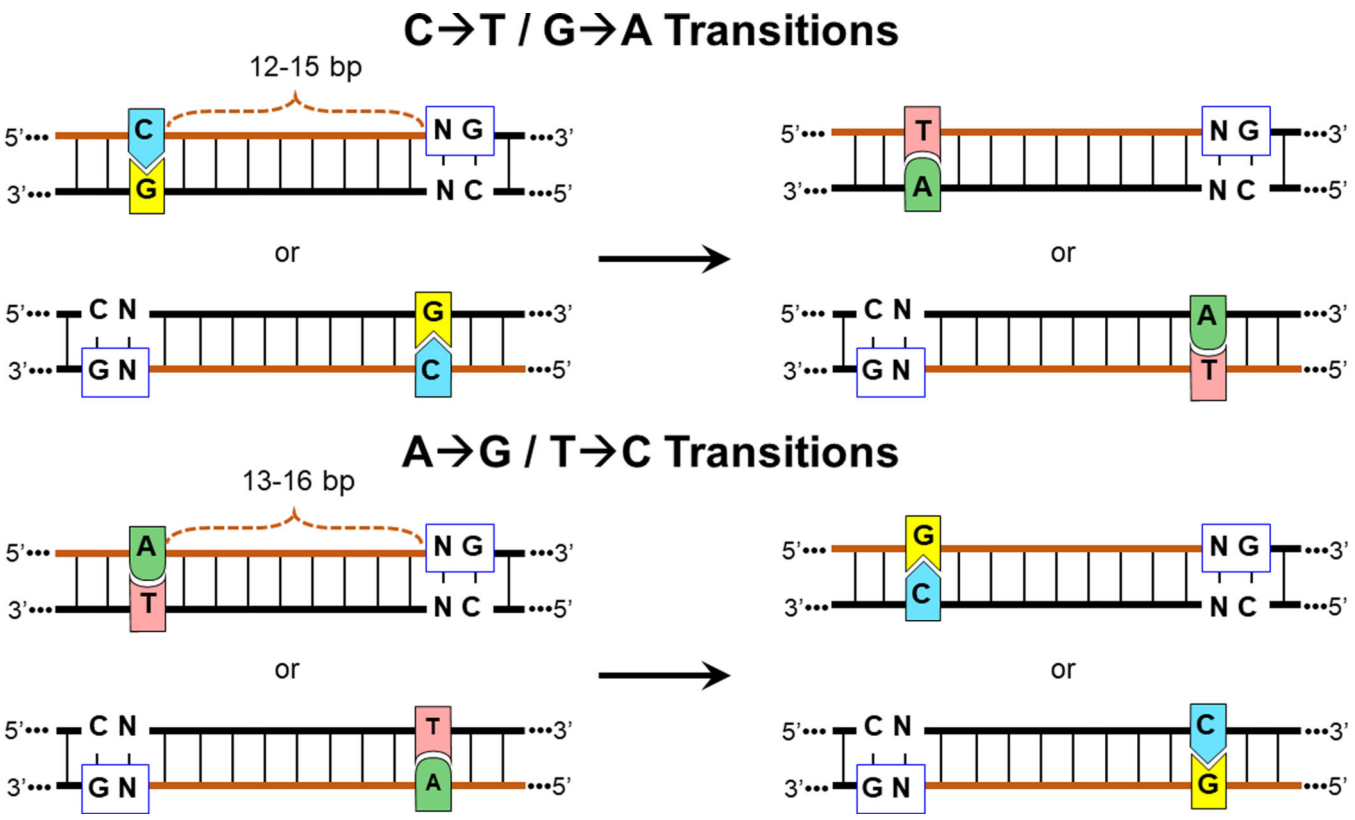


Figure 3. Currently Available Transition Mutations

Base editor chemistries are available to theoretically create all possible transition mutations. While the base editing chemistry is performed on only cytidine or adenine according to Figure 1, due to Watson-Crick base pairing, complement bases will also undergo transition changes. A PAM (indicated with the blue rectangle) must be present within a specific distance from the target base-pair. The strand of the protospacer that will match the gRNA sequence is indicated in orange. Shown here is an example PAM and editing window using an NG-SpCas9 with rAPOBEC1 (top) or ecTadA-ecTadA*7.10 (bottom).