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Review

Decoding disease-relevant variants with base and prime editors at scale

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Interpreting variants of uncertain significance remains a central challenge in human genomics. Base and prime editors have launched a new era of precision functional genomics, enabling programmable, double-strand break-free introduction of point mutations and small indels directly within the genome. Here, we review the technological evolution of these editors and their transformative application in high-throughput functional screens. We highlight how base and prime editing platforms systematically annotate clinical variants, reveal mechanisms of drug resistance and immune evasion, and dissect fundamental biological processes at single-nucleotide resolution. Crucially, we address current challenges and future perspectives for precision editing screens. By enabling causal genotype-to-phenotype mapping, precision editing screens are redefining genomic variation interpretation and accelerating its translation into precision diagnostics and therapeutics.

Highlights

Precision genome editors (base editors and prime editors) enable *in situ* mutational scanning at single-nucleotide resolution.

Massively parallel screens allow functional annotation of variants of uncertain significance within their native genomic context.

High-resolution mapping of mutational landscapes elucidates mechanisms underlying disease pathogenesis and therapeutic response.

Base editing and prime editing reveal fundamental biological mechanisms, including DNA/RNA processing, post-translational modifications, epigenetic and *epitranscriptomic* regulation, and the functional impact of noncoding and synonymous mutations.

To overcome current limitations in precision editing screens, future efforts will focus on developing next-generation editors integrated with advanced technologies and capable of interpreting complex physiological contexts.

Base and prime editing: a precision revolution

Precise genome manipulation at single-nucleotide resolution is essential for decoding the functional consequences of genetic variation. The advent of base editors (BEs) [1,2] and prime editors (PEs) [3] has provided powerful solutions to longstanding challenges in precision, scalability, and predictability in genome engineering, ushering in a new era for **functional genomics** (see Glossary). Unlike conventional CRISPR/Cas9 approaches that rely on stochastic DNA repair following double-strand breaks (DSBs) (Box 1), BEs and PEs enable programmable installation of point mutations and small indels (insertions and deletions) directly within the native genomic context, without donor templates or inefficient **homology-directed repair (HDR)** [31].

By circumventing DSB-induced toxicity and unpredictable repair outcomes, BEs and PEs offer high editing efficiency and product purity, making them particularly well suited for high-throughput applications. This technological leap has transformed the scope of mutational scanning, moving beyond simple gene-disruption screens to high-resolution saturation mutagenesis, enabling systematic interrogation of **variants of uncertain significance (VUS)** in physiologically relevant settings. By directly linking engineered variants to phenotypic readouts, these approaches provide a scalable path from genetic variation to causal functional insight, overcoming the limitations of earlier semi-random editing strategies (Box 1).

In this review, we outline the technological evolution of BEs and PEs, focusing on their distinct mechanisms and recent optimizations (Figure 1). We discuss their adaptation to large-scale pooled screening platforms (Figure 2) and highlight applications in decoding disease-relevant mutations across diverse biological systems. Finally, we outline current limitations and future perspectives for leveraging high-throughput precision editing to map the functional landscape of the human genome.

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Box 1. The evolution of functional genomics: from genetic association to high-resolution genome editing

Decoding how individual genetic variants shape cellular function and disease remains a major hurdle in the post-GWAS era. Although GWAS has identified thousands of loci linked to complex traits [4], these explain only a modest fraction of heritability. Much of the remaining genetic risk likely resides in rare or poorly characterized variants [5], a problem further compounded by the rapid accumulation of VUS from large-scale sequencing. Bridging statistical association and biological causality therefore requires scalable, high-resolution approaches to interrogate variant function within native genomic contexts.

Early mutational scanning using reporter-based assays and customizable mutant libraries yielded valuable insights into specific amino acids and regulatory elements [6,7]. However, reliance on episomal constructs fails to recapitulate endogenous chromatin environments and native gene control, often yielding context-dependent results. The advent of CRISPR-based technologies enabled *in situ* interrogation of gene function, marking a turning point in functional genomics [8]. High-throughput CRISPR screens have since been widely applied to dissect the functions of protein-coding genes [9–12], long noncoding RNAs (lncRNAs) [13–16], and noncoding regulatory elements [17–19], driving major advances in fields such as cancer biology and immunology.

Nevertheless, conventional Cas9 introduces DSBs that are repaired by error-prone nonhomologous end joining (NHEJ) or inefficient HDR, restricting precise single-base manipulation [20,21]. To achieve finer resolution, strategies such as the PASTMUS pipeline integrate CRISPR/Cas9-based **tiling mutagenesis** with next-generation sequencing (NGS) to map functional amino acids [22]. There remains a growing need for high-precision editing approaches with broader variant coverage to accurately define the functional impact of SNVs. Moreover, nuclease-dependent editing raises safety concerns, including undesired genomic rearrangements [23,24] and activation of p53-mediated DDR, particularly in DSB-sensitive cells [25,26].

To circumvent these limitations, the first-generation base-resolution editors were introduced. These systems typically couple a catalytically impaired Cas9 (nickase nCas9 or nuclease-dead variant dCas9) to a cytidine deaminase, such as activation-induced deaminase (AID) [27], allowing localized base conversion without creating DSBs. Early platforms, including targeted AID-mediated mutagenesis (TAM) and CRISPR-X, were successfully deployed in high-throughput screens to identify drug-resistance mutations [28–30], as reviewed elsewhere [21].

Although these approaches enabled diverse *in situ* point mutations, their utility for systematic, genome-wide functional discovery remained limited. The semi-random mutagenesis within editing windows produces heterogeneous genotypes, complicating direct genotype–phenotype linkage in pooled assays and necessitating deep, locus-specific sequencing to resolve functional outcomes—an approach difficult to scale. These constraints highlight the need for next-generation precision editors, which offer greater accuracy and scalability for comprehensive functional interrogation of SNVs.

Base editors: efficient, predictable transition editing

Similar to earlier cytidine deaminase-based systems such as Target-AID [27], TAM [28], and CRISPR-X [29], BEs employ catalytically impaired Cas9 variants, typically nCas9, fused to DNA deaminases. Guided by a single-guide RNA (sgRNA), the editor catalyzes the chemical modification of a target base within a defined editing window. Two principal BE classes have been established: cytosine base editors (CBEs), which use cytidine deaminases (e.g., APOBEC1) to convert C•G to T•A [1] (Figure 1A), and adenine base editors (ABEs), which use evolved adenosine deaminases derived from TadA to convert A•T to G•C [2] (Figure 1B).

Both platforms have undergone extensive optimization. Early CBEs (BE1 and BE2) fused APOBEC1 to dCas9, with BE2 incorporating a uracil glycosylase inhibitor (UGI) to prevent base excision repair. Replacement of dCas9 with nCas9 in BE3 introduced a strand nick that biased repair toward the edited strand, markedly improving efficiency [1]. Subsequent iterations further refined performance: BE4 incorporated a second UGI to enhance product purity, while BE4max optimized nuclear localization signals (NLSs) and codon usage to maximize intracellular activity. Furthermore, AncBE4max, generated by ancestral sequence reconstruction, augmented robustness and editing activity, particularly under suboptimal conditions [73] (Figure 1D).

ABEs represented a landmark in protein engineering by repurposing a bacterial tRNA deaminase for DNA editing [2]. While the initial ABE7.10 exhibited high specificity, later variants, such as ABEmax, improved activity via codon and NLS optimization, and ABE8e leveraged an evolved TadA to expand the editing window and markedly increase efficiency [74] (Figure 1D). The

Glossary

Bystander edits: unintended modifications of nontarget nucleotides (typically cytosine or adenine) located within the active window of a base editor. These unwanted edits at neighboring sites can complicate the interpretation of functional screens by creating ambiguity about which specific mutation is responsible for a phenotype.
***cis*-regulatory elements (CREs):** regions of noncoding DNA, such as promoters and enhancers, that regulate the transcription of target genes located on the same chromosome (in *cis*). Variations within CREs can significantly impact gene expression and contribute to disease.
DNA damage response (DDR): a complex network of signaling pathways that cells use to sense, signal, and repair DNA lesions, such as DSBs, to maintain genomic integrity. Defective DDR is a hallmark of cancer and drives mutagenesis. DSB-free precision editing avoids the activation of deleterious DDR pathways often triggered by conventional nuclease-based tools.
Functional genomics: a field of molecular biology that aims to understand the relationship between an organism's genome and its phenotype. It utilizes large-scale, systematic approaches, such as the precision editing screens discussed in this review, to determine the biological function of genes and noncoding elements.
Homology-directed repair (HDR): a DNA repair mechanism that uses a homologous DNA template to accurately repair DSBs. In traditional CRISPR/Cas9 genome editing, this pathway is co-opted to introduce precise sequence changes by providing an exogenous donor DNA template containing the desired edit. However, the HDR pathway is often inefficient in many cell types and competes with other error-prone repair pathways, limiting the scalability of nuclease-based saturation editing.
Protospacer adjacent motif (PAM): a short DNA sequence (typically 2–6 bp) that is adjacent to the DNA target sequence and is required for a Cas protein to bind and edit the DNA.
Post-translational modifications (PTMs): covalent chemical modifications that occur on a protein during or after its synthesis, such as phosphorylation, acetylation, and ubiquitination. PTMs act as critical

most recent ABE9 variant narrowed the editing window and minimized **bystander edits**, achieving superior single-base precision [75].

Prior to their widespread application in variant scanning, BEs enabled next-generation, DSB-free knockout screening strategies. Conventional CRISPR/Cas9 knockout screens can suffer from cytotoxicity, false positives in copy-number-amplified regions, and p53 activation [25,26]. Early DSB-independent approaches, including CRISPR-STOP and iSTOP [76,77], used CBEs to introduce premature termination codons, achieving clean loss-of-function (LOF) alleles. The BARBEKO strategy [78] further expanded targeting to start codons and splice sites, increasing gene coverage. Incorporation of sgRNAs with internal barcodes (iBARs) enabled robust screening at high multiplicity of infection, which reduced cell input requirements while enhancing statistical power [79]. Together, these innovations established BEs as a versatile and robust platform for functional genomics, particularly in DSB-sensitive contexts and limited primary samples.

Prime editors: extending beyond transition mutations

Prime editors, developed subsequently, offer substantially broader editing capabilities, enabling all 12 possible base substitutions as well as small indels. The original PE1 system consists of a Cas9 nickase fused to a Moloney murine leukemia virus reverse transcriptase (M-MLV RT) and is guided by a prime editing guide RNA (pegRNA) that contains a spacer sequence targeting the genomic locus, a primer binding site (PBS), and a reverse transcription template (RTT) specifying the desired edit [3] (Figure 1C).

Iterative optimization yielded progressively improved systems (Figure 1D). PE2 incorporated five mutations in the reverse transcriptase (RT) to enhance editing efficiency, while PE3 added an additional sgRNA to nick the nonedited strand, biasing repair toward the edited strand [3]. To suppress DNA mismatch repair, which constrains PE efficiency and promotes indels, PE4 and PE5 incorporated a dominant-negative MLH1 mutant (MLH1dn). Additional refinements, such as codon optimization, enhanced NLSs, and optimized linker design, produced the highly active PEmax system [80].

Further progress targeted both pegRNA stability and enzyme performance. On the RNA level, engineered pegRNAs (epegRNAs) incorporating structured RNA motifs (e.g., evopreQ1 or mpknot) exhibited improved resistance to degradation and more consistent editing outcomes [81]. In parallel, introducing synonymous mutations within the RTT was shown to boost editing efficiency [82]. On the protein side, directed evolution yielded the PE6 series (PE6a–PE6g) with context-specific advantages [83], and PE7 further stabilized pegRNAs via fusion of the La RNA-binding domain to PEmax [84]. Beyond single-site edits, paired pegRNA strategies now enable large and complex genomic modifications, including precise substitutions, insertions up to 250 bp, and deletions up to 10 kb [85–90]. Collectively, these advances greatly broaden the scope of prime editing for precision genomics and systematic functional variant discovery.

Functional annotation at single-nucleotide resolution

Precision mapping of cancer variants using genome editors

Comprehensive cancer genome sequencing has uncovered vast numbers of mutations with uncertain functional and clinical relevance, highlighting the urgent need for systematic functional characterization. Precision genome editors offer powerful, high-throughput approaches to interrogate thousands of single-nucleotide variants (SNVs) directly within their native context, thereby bridging the gap between genetic association and biological consequence (Figure 2; Tables 1 and 2).

regulatory switches, dynamically altering protein activity, stability, localization, or interaction networks in response to cellular signals.

Variants of uncertain significance (VUS): genetic variants identified through sequencing whose impact on gene function and clinical significance is unknown. Resolving the functional status of VUS is a major bottleneck in clinical genetics and a primary goal for large-scale mutational scanning.

Tiling/saturation mutagenesis: an experimental strategy that systematically generates and tests all possible mutations across a genomic region to build a comprehensive functional map. In CRISPR-based screens, this is typically achieved using densely tiled guide RNAs to identify critical residues at high resolution *in situ*.

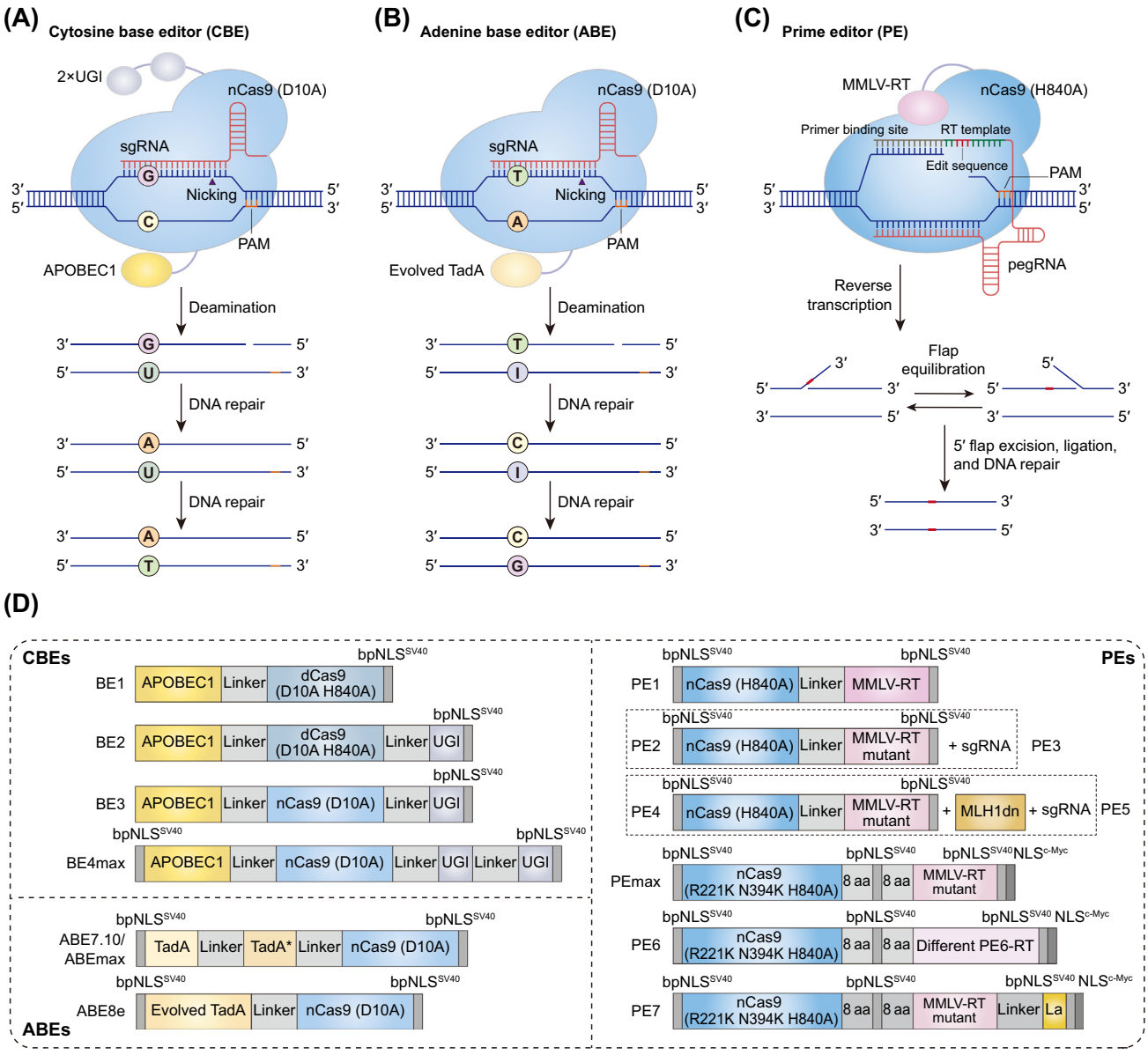

Figure 1. Schematic of representative base editors and prime editors. (A–C) Overall process of cytidine base editing (A), adenosine base editing (B), and prime editing (C) for each gene editor. (D) Representative types of cytidine base editors, adenosine base editors, and prime editors.

Saturation mutagenesis of key cancer-associated genes

The tumor suppressors *BRCA1* and *BRCA2* are central to DNA damage repair and genome stability maintenance [91]. Mutations in these genes are common in human cancers [92], and defining the functional impact of individual point mutations is critical for understanding tumorigenesis and improving clinical interpretation. LOF variants typically reduce cell viability or increase sensitivity to PARP inhibitors and DNA-damaging agents, enabling their identification through negative-selection screens [93]. Early base editing screens using BE3 libraries spanning *BRCA1* exons and splice junctions successfully identified novel LOF variants, including ClinVar-listed VUS [32]. Later studies employing BE3.9max [94] extended tiling mutagenesis to both *BRCA1* and

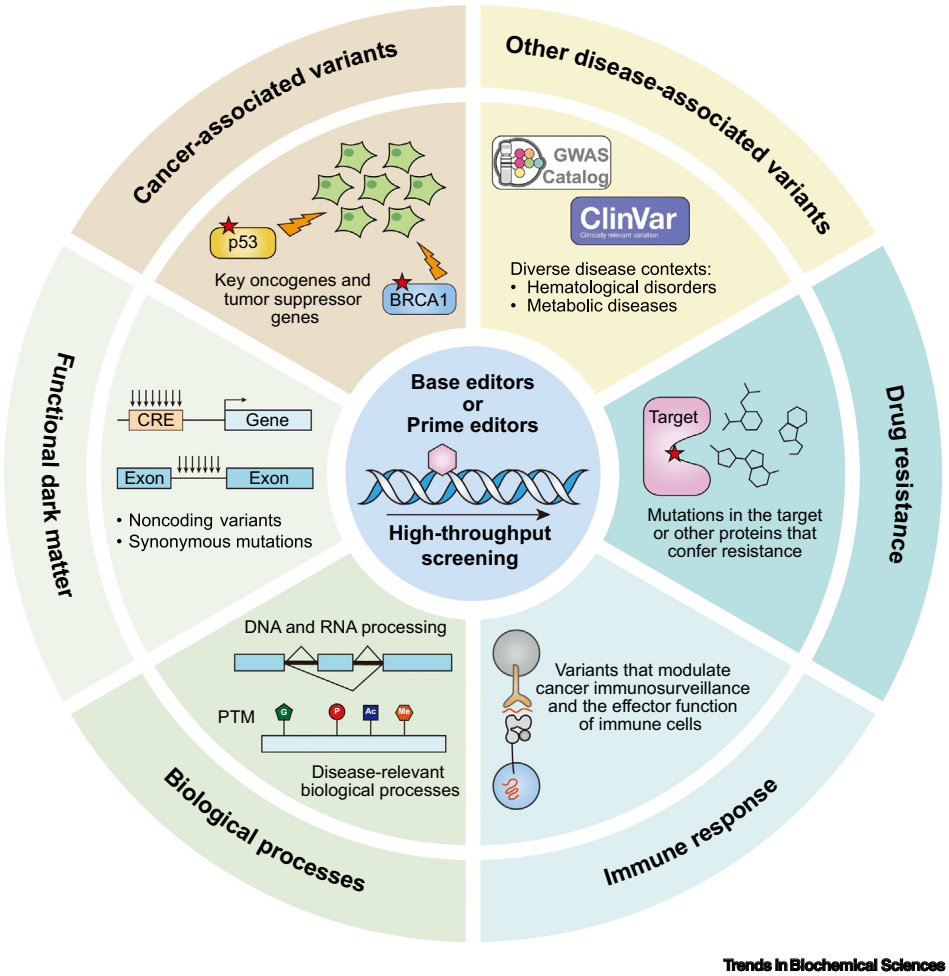

Figure 2. Application of base and prime editor screens in functionally characterizing disease-relevant variants. All representative applications were listed in Table 1 (base editor screens) and Table 2 (prime editor screens).

BRCA2, covering missense, nonsense, synonymous, splice, intronic, and untranslated region (UTR) variants [33]. Further expansion of the editable mutational landscape was achieved by combining CBEs and ABEs with **protospacer adjacent motif (PAM)**-relaxed Cas9 variants [34,35].

TP53, the most frequently mutated tumor suppressor in human cancers [95], has also been extensively interrogated using BEs. A modular BE-sensor platform that links sgRNAs to their corresponding targets in *cis* enabled the empirical assessment of editing efficiency across editor configurations and cell types. Applying this system with a mouse CBE library targeting 62 *Trp53* mutations identified multiple previously uncharacterized variants that drive cancer cell proliferation and tumorigenesis *in vivo* [36]. More recently, researchers developed TISCC-seq to integrate BE-induced mutagenesis with single-cell transcriptomics, evaluating the transcriptional consequences of over 100 recurrent *TP53* mutations across diverse cellular contexts [37].

In parallel, prime editing has emerged as a complementary strategy for functional interrogation of cancer-associated mutations. The first saturation prime editing (SPE) study in *BRCA2*-haploid HEK293T cells identified 78 deleterious mutations with strong concordance to ClinVar

Table 1. Representative studies of base editor-based screens

Target(s)	Editors	Cell line(s)	Assay	Study
Residues associated with cancer development and therapeutic response				
BRCA1	CBE (BE3)	HAP1	Cell survival in the presence of olaparib	Kweon *et al*. [32]
47 genes with known phenotypes, including ten pan-lethal genes and four vemurafenib-resistant genes	CBE (BE3.9max and BE4max)	A375, MELJUSO, OVCAR8, HA1E, and HAP1	Cell fitness (BE3.9max and BE4max for A375 only and BE3.9max for all) and vemurafenib resistance (BE3.9max for A375 only)	Hanna *et al*. [33]
BRCA1 and *BRCA2*	CBE (BE3.9max)	HAP1 and MELJUSO	Cell survival after treatment with talazoparib or cisplatin (MELJUSO only)	
MCL1 and *BCL2L1*	CBE (BE3.9max)	MELJUSO	Cell survival after treatment with BCL2L1 inhibitor (MCL1 screen only) or MCL1 inhibitor (BCL2L1 screen only), or both inhibitors	
PARP1	CBE (BE3.9max)	HAP1	Cell survival after treatment with each of five PARP inhibitors	
52 034 ClinVar mutations (3584 genes)	CBE (BE3.9max)	HT29 and MELJUSO	Cell survival following treatment with cisplatin and hygromycin	
BRCA1 and *BRCA2*	CBE (AncBE4max and AncBE4max-NG) and ABE (ABEmax and ABEmax-NG)	eHAP	Cell fitness	Huang *et al*. [34]
BRCA1	CBE (BE3.9max-NG and BE3.9max-SpG) and ABE (ABE8e, ABE8e-NG, and ABE8e-SpG)	HAP1 and MELJUSO	Cell fitness and cell survival following cisplatin treatment (MELJUSO only)	Sangree *et al*. [35]
BCL2	CBE (BE3.9max-NG) and ABE (ABE8e-NG)	MOLM13	Venetoclax resistance	
MSK-IMPACT mutations (>21 000 tumors, 462 genes)	CBE (FNLS, F2X, and FNLS-NG)	MDA-MB-231, PC9, NIH3T3, PDEC, and KPT1	Cell fitness (using BE sensor libraries)	Sa´nchez-Rivera *et al*. [36]
TP53	CBE (An cBE4max and BE4max-SpG) and ABE (ABEmax, ABEmax (7.10)-SpG)	HCT116 and U2OS	Cell fitness with readout through scRNA-seq (TISCC-seq)	Kim *et al*. [37]
27 DDR genes (sub-library)	CBE (BE3)	MCF7, MCF10A, and HAP1	Cell fitness	Cuella-Martin *et al*. [38]
86 DDR genes (including the 27 genes from the sub-library)	CBE (BE3)	MCF10A	Cell survival following treatment with each of four DNA-damaging agents (cisplatin, olaparib, doxorubicin, and camptothecin)	
All feasible lysine residues	ABE (ABEmax)	RPE1-hTERT	Cell survival following treatment with ionizing radiation (IR) or cisplatin (IC50)	Pan *et al*. [39]
Genome-wide protein-coding genes	AncBE4max (BARBEKO-based gene KO screen)	RPE1-hTERT	Cell survival following cisplatin treatment	
29 060 COSMIC mutations	CBE (AncBE4max) and ABE (ABEmax)	HBEC30KT with *TP53* knockdown	Cell fitness (using a surrogate target system)	Kim *et al*. [40]
Regulators of the EGFR signaling pathway	CBE (AncBE4max)	HBEC30KT with *TP53* knockdown	Cell survival under normal conditions or with simultaneous EGF removal and afatinib treatment	
MAP2K1, KRAS, and *NRAS*	CBE (BE3)	A375	Cell survival following treatment with vemurafenib, with readout through scRNA-seq	Jun *et al*. [41]
KRAS ($KRAS^{G12C}$ and $KRAS^{G12D}$)	CBE (BE3.9max) and ABE (ABE8e)	MIA PaCa-2 ($KRAS^{G12C}$) and AGS ($KRAS^{G12D}$)	Resistance to classical and molecular glue KRAS inhibitors (sotorasib, adagrasib, and	Merz *et al*. [42]

(continued on next page)

Table 1. (continued)

Target(s)	Editors	Cell line(s)	Assay	Study
			RMC-6291 for MIA PaCa-2; MRTX-1133 and RMC-9805 for AGS; and pan-KRAS inhibitors BRD5066, BI-2865, and RMC-6236 for both)	
MEN1	CBE (BE3.9max)	MOLM13 and MV4–11	Cell survival following VTP-50469 (revumenib analog) treatment	Perner *et al*. [43]
STT3A	CBE and ABE (ABE8e-NG)	NALM-6 with *TNFAIP3* knockout and reporter expression	NG-1 resistance	Lampson *et al*. [44]
JAK1	CBE (BE3, BE3.9max-NG, and YE1-modified BE4max-NG) and ABE (ABE8e-NG)	HT-29	Cell survival in the presence of IFN-γ (all) and FACS based on MHC-I and PD-L1 expression with IFN-γ treatment (BE3 only)	Coelho *et al*. [45]
Regulators of the IFN-γ signaling pathway	CBE (BE3)	HT-29 and CRC-9 tumor organoids	Cell survival in the presence of IFN-γ (all) and FACS based on MHC-I and PD-L1 expression with IFN-γ treatment (HT-29 only)	
All feasibly serine, threonine, and tyrosine residues	ABE (ABEmax)	A375	FACS based on MHC-I and PD-L1 expression with or without IFN-γ treatment	Liu *et al*. [46]
385 genes implicated in T cell activity	CBE (evoCDA1-BE4max) and ABE (ABE8e)	Primary human T cells	FACS based on cytokine (TNF and IFN-γ) production and surface marker (PD-1 and CD25) downregulation	Schmidt *et al*. [47]
102 genes involved in major T cell functions	CBE (BE3.9max-NG) and ABE (ABE8e-NG)	Primary human T cells	Cell fitness and FACS based on CD25 expression and cytokine (TNF and IFN-γ) production	Walsh *et al*. [48]
Residues associated with hematological disorders and metabolic diseases				
CD33 (splice site), *HBG1/2* promoters, and *GATA1*	ABE (ABE8e-NG)	Primary HSPCs from two male donors	FACS based on CD33 expression (*CD33*) and HbF expression (*HBG1/2* promoters), cell survival under induction of erythroid differentiation (*GATA1*), with readout through scRNA-seq	Martin-Rufino *et al*. [49]
307 putative CREs	ABE (ABEmax)	HUDEP-2	FACS based on HbF expression	Cheng *et al*. [50]
LDLR	CBE (AID-BE5-SpRY) and ABE (ABE8e-SpRY)	HepG2	FACS based on fluorescent LDL-C uptake (using a surrogate target system and the computational pipeline BEAN)	Ryu *et al*. [51]
Residues associated with fundamental biological processes				
DNA polymerase κ (Polκ)	ABE (ABE8e-SpG)	RPE1-hTERT with *TP53* knockout	Sensitivity to illudin S	Sellés-Baiget *et al*. [52]
Splice sites of >12 000 exons	CBE (APOBEC1, BECON2, and evoCDA1) and ABE (ABE8e)	HAP1	Cell fitness	Xiao *et al*. [53]
153 spliceosomal proteins	CBE (FNLS)	eHAP	Resistance or hypersensitivity to pladienolide B	Beusch *et al*. [54]
All feasibly lysine residues	ABE (ABEmax)	RPE1-hTERT	Cell fitness	Bao *et al*. [55]
All feasibly serine, threonine, and tyrosine residues	ABE (ABEmax)	RPE1-hTERT	Cell fitness	Li *et al*. [56]
PhosphoSitePlus(R) database phosphorylation sites	CBE (split and further optimized A3A-BE3) and ABE (split and further optimized ABE7.10)	HCT116	Cell survival following 5-fluorouracil treatment	Li *et al*. [57]
~11 000 phosphosites	ABE (ABE8e)	Jurkat	Reporter-based screen for altered NFAT transcriptional activity	Kennedy *et al*. [58]

Table 1. (continued)

Target(s)	Editors	Cell line(s)	Assay	Study
DNMT3A	CBE (BE3.9max)	K562	Reporter-based screen for altered DNA methyltransferase activity	Lue *et al.* [59]
6048 m6A sites	ABE (FNLS-ABE7.10)	H1 hESCs	FACS based on CXCR4 expression to assess endodermal differentiation	Cheng *et al.* [60]

Abbreviations: EGF: epidermal growth factor; FACS: fluorescence-activated cell sorting; IFN-γ: interferon-γ; IR: ionizing radiation; LDL-C: low-density lipoprotein cholesterol; PARP: poly ADP-ribose polymerase; Polκ: polymerase κ; TISCC-seq: transcript-informed single-cell CRISPR sequencing; TNF: tumor necrosis factor.

annotations [61]. Subsequent work developed PE sensor libraries to functionally evaluate more than 1000 endogenous *TP53* variants from the MSK-IMPACT database in A549 cells, uncovering functional patterns distinct from those obtained using cDNA-based assays [62]. A parallel *TP53*-targeting PE screen in A549 cells leveraged optimized engineered viruslike particles for efficient PE delivery [63]. PE screens have since been extended to additional cancer genes, including *SMARCB1* and *MLH1*, further illustrating the technology's power for systematic variant annotation [64].

Table 2. Representative studies of prime editor-based screens

Target(s)	Editors	Nicking sgRNA	EpegRNA	Cell line(s)	Assay	Study
NPC1	PE2	Yes	No	HEK293T (*NPC1*-haploid) and RPE1 (*NPC1*-haploid)	FACS-based detection of LysoTracker-stained cell subpopulations with high and low fluorescence intensity	Erwood *et al.* [61]
BRCA2	PE2	Yes	No	HEK293T (*NPC1*-haploid)	Cell fitness	
TP53	PEmax	No	Yes	A549	Cell fitness (using PE sensor libraries)	Gould *et al.* [62]
TP53	PEmax	No	No	A549	Cell fitness	Langley *et al.* [63]
SMARCB1	PEmax	No	Yes	HAP1	Cell fitness (using a surrogate target system)	Herger *et al.* [64]
MLH1	PEmax	No	Yes	HAP1	Cell survival following 6TG treatment (using a surrogate target system)	
MYC enhancer and clinical variants	PE2	Yes	Yes	MCF7	Cell fitness	Ren *et al.* [65]
3644 protein-coding genes	PEmax	No	Yes	HCT116, A549, and KYSE-30	Cell fitness	Niu *et al.* [66]
1231 pan-essential genes	PEmax	No	Yes	K562	Cell fitness	Cirincione *et al.* [67]
ATM	PEmax	No	Yes	HCT116 (*ATM*-haploid)	Cell survival following olaparib treatment	Lee *et al.* [68]
EGFR	PEmax, CBE (BE3.9max), and ABE (ABE8e)	No	Yes	MCF10A	Cell survival following EGF treatment	Belli *et al.* [69]
BRAF, KRAS, *EGFR, RIT1*, *MET, PIK3CA*, *MEK1*, and *AKT*	PEmax	No	Yes	PC-9	Cell survival following treatment with each of three types of TKIs	Chardon *et al.* [70]
EGFR	PEmax	No	Yes	PC-9	Cell survival following treatment with each of two types of TKIs	Kim *et al.* [71]
PPIF promoter	PE2	No	Yes	THP-1	FACS-based detection of distinct cell subpopulations by RNA FISH or fluorescent antibody staining	Martyn *et al.* [72]
IL2RA	PE2	No	Yes	Jurkat		

Abbreviations: EGF: epidermal growth factor; FACS: fluorescence-activated cell sorting; RNA FISH: RNA fluorescence in situ hybridization; TKIs: tyrosine kinase inhibitors.

Systematic interrogation of cancer pathways and clinically relevant phenotypes

Beyond individual genes, precision editing platforms have been applied to explore mutations across entire oncogenic pathways. Mutations in **DNA damage response (DDR)** genes are major drivers of cancer predisposition and genomic instability syndromes [96], with *BRCA1/2* representing well-characterized cases, as noted earlier.

An early BE3-based tiling mutagenesis study spanning 86 human DDR genes profiled cellular fitness under diverse DNA-damaging conditions, identifying both LOF and gain-of-function (GOF) variants. Notable findings included 53BP1 Tudor-domain mutations that disrupt USP28 interaction, ATM kinase variants with opposing effects on genome stability, and clinically relevant CHK2 VUS [38]. A subsequent ABEmax-based screen focused on lysine substitutions under cisplatin stress, identifying novel regulators, such as C17orf53 K494 [39]. Both studies validated functional variants against ClinVar and International Cancer Genome Consortium (ICGC) datasets, accelerating the translation of genomic data into clinically actionable insights.

To further scale variant interpretation, large sgRNA libraries encompassing mutations from major cancer genomics resources have been developed. The BE sensor library [36], drawing on the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) dataset, systematically assessed >200 000 editor–sgRNA pairs to model thousands of cancer-associated mutations. Additional BE screens assessed tens of thousands of variants across cancer cell lines, including 29 060 Catalogue of Somatic Mutations in Cancer (COSMIC) variants [40] and 52 034 ClinVar variants involved in DDR and translational stress pathways [33].

Prime editing further expands the mutational scope accessible to screening. In the first large-scale lentiviral PE study, PE3 was used to interrogate thousands of coding and noncoding variants in MCF-7 cells, including essential *MYC* enhancer nucleotides, breast cancer-associated genome-wide association studies (GWAS) loci, and ClinVar variants [65]. Subsequent studies employing large epegRNA libraries with PEmax substantially broadened genome-wide screening capability [66,67]. One such screen targeted approximately 240 000 sites across 1231 essential genes, identifying nearly 8000 nonsense mutations associated with reduced cellular fitness in K562 cells [67].

Extending precision editing beyond cancer

Precision editing-based screens extend beyond oncology, proving instrumental in dissecting the molecular basis of diverse inherited and acquired diseases. In hematological disorders, large-scale mutagenesis in primary human hematopoietic stem and progenitor cells (HSPCs) has enabled systematic functional mapping of key regulatory genes. For instance, **saturation mutagenesis** of *GATA1*, combined with single-cell transcriptomic profiling, generated a high-resolution genotype–phenotype map linking specific mutations to hematopoietic differentiation outcomes, including validation of a patient-derived VUS that disrupts erythropoiesis and causes hypoplastic anemia [49]. Similarly, ABE-based noncoding screens have pinpointed regulatory elements governing fetal hemoglobin (HbF) expression with single-nucleotide precision, as discussed later [49,50].

In metabolic disorders, BE-based saturation mutagenesis of *LDLR*, coupled with computational normalization for editing efficiency, produced quantitative functional scores for missense variants that correlated strongly with low-density lipoprotein cholesterol (LDL-C) levels of the patients [51]. This framework enables scalable interpretation of *LDLR* VUS while revealing structural

mechanisms underlying pathogenic mutations. The initial PE platform has also been applied to *NPC1*, which is associated with the lysosomal storage disorder Niemann–Pick disease type C, in which functional screening in *NPC1*-haploid cells classified hundreds of missense mutations and provided both pathogenicity predictions and mechanistic insights [61].

Collectively, these studies demonstrate that precision editing-based functional screens can directly link genetic variation to molecular mechanisms, enabling quantitative prediction of clinical outcomes across a broad spectrum of human diseases.

Mapping the genetic determinants of therapeutic response

Beyond identifying pathogenic residues, precision editing-based screens provide a powerful framework for systematically uncovering the genetic determinants of drug response and immune modulation (Figure 2). By modeling disease-relevant mutations at scale within their native genomic context, these approaches elucidate mechanisms of drug–target interactions and therapeutic escape, providing direct guidance for resistance prediction, efficacy optimization, and rational drug development [97].

Mechanisms of drug resistance

A key strength of precision editing screens lies in their capacity to map resistance mutations with residue-level resolution. Tiling mutagenesis across a drug target enables the identification of substitutions that abrogate drug binding while preserving protein function. For example, a *BCL2* tiling screen using PAM-flexible BEs recovered known venetoclax resistance mutations, including F104L and D103E/Y, and uncovered previously unreported variants that also confer resistance [35]. Similarly, systematic mutagenesis of *MEN1* prospectively identified residues at the drug-binding interface (e.g., G331 and T349) that subsequently emerged in patients with clinical resistance to menin inhibitors [43].

Combining base editing with orthogonal technologies yields deeper mechanistic insight. A BE-based resistance screen against the oligosaccharyltransferase inhibitor NGI-1 revealed three mutation clusters in the catalytic subunit STT3A; integration with cryo-electron microscopy precisely delineated the drug-binding pocket and inhibitory mechanism [44]. Likewise, coupling BE screens with single-cell RNA sequencing (scRNA-seq) linked *MAP2K1* and *KRAS* mutations to distinct transcriptional signatures underlying resistance to the BRAF inhibitor vemurafenib [41].

Insights from BE-based resistance mapping also guide the design of next-generation therapeutics. Comparative tiling screens can predict clinical failure modes and identify nonoverlapping resistance mechanisms among distinct therapeutic modalities. For example, tiling mutagenesis of oncogenic *KRAS* revealed that classical inhibitors and induced-proximity molecular glues, despite targeting the same protein pocket, exhibit largely orthogonal resistance profiles, suggesting molecular glues as a potential second-line therapy following resistance to classical KRAS inhibitors [42]. Similarly, systematic screening of *PARP1* against five PARP inhibitors provided a functional map of residues distinguishing broadly resistant residues from inhibitor-specific ones, informing the development of drugs with complementary resistance spectra [33].

Prime editing further extends resistance mapping toward near-saturation coverage. To chart the resistance landscape of EGFR, two independent studies employed PEmax for deep mutational scanning. Kim *et al*. characterized approximately 99% of all possible variants within the tyrosine kinase domain, creating comprehensive resistance maps for afatinib and osimertinib while functionally annotating thousands of VUS [71]. A parallel study combining BE and PE revealed distinct resistance architectures: whereas gefitinib resistance was largely confined to the ATP-binding

pocket, osimertinib resistance also involved residues in the C-terminal regulatory domain, pointing to additional mechanisms involving receptor autoregulation [69].

The versatility of PE also enables multiplexed resistance profiling across multiple oncogenes. The prime-SGE framework simultaneously assayed thousands of SNVs across eight oncogenes for resistance to three EGFR inhibitors [70], identifying both known variants (e.g., EGFR C797S and KRAS G12C) and novel resistance-conferring substitutions (e.g., EGFR Q791 and Y801). Notably, this screen revealed inhibitor-specific resistance patterns: EGFR C797S conferred resistance to covalent inhibitors such as osimertinib and sunvozertinib, but not to noncovalent competitors, demonstrating the approach's precision in mapping mechanism-dependent resistance.

Saturation prime editing has also been applied to large, clinically challenging genes such as ATM, which spans 63 exons and encodes a 3056-amino acid protein densely populated with VUS. Screening over 23 000 SNVs introduced via an epegRNA library under PARP inhibitor (olaparib) selection identified critical residues affecting drug resistance. These data were used to train DeepATM, a deep learning model capable of predicting the functional impact of untested variants, enabling the construction of a genome-wide functional atlas of *ATM* mutations [68]. Together, these studies establish a scalable paradigm for predicting clinical resistance and guiding rational drug design.

Immune evasion and implications for immunotherapy

Precision editing is equally powerful for dissecting the molecular basis of immune regulation and resistance to immunotherapy. Analogous to drug resistance, tumor cells acquire mutations that enable escape from immune surveillance. Residue-level functional screens map this landscape by identifying LOF and GOF mutations that modulate tumor sensitivity to immune pressure.

An early BE-based screen systematically mutagenized components of the interferon-γ (IFN-γ) signaling pathway in colorectal cancer cells, identifying specific LOF and GOF variants in genes, such as *JAK1*, that altered tumor organoid susceptibility to autologous T cell-mediated killing [45]. Expanding on this, a genome-wide ABEmax screen targeting all editable serine, threonine, and tyrosine residues revealed variants controlling the expression of the immune checkpoint ligand PD-L1 and the antigen-presenting molecule HLA-I [46]. This comprehensive analysis uncovered thousands of functional mutations beyond canonical IFN-γ signaling, including clinically relevant alterations in genes, such as *SETD2*, that coregulate PD-L1 and HLA-I, thereby enhancing the efficacy of immune checkpoint blockade in preclinical models.

Beyond profiling tumor-intrinsic mechanisms, precision editing has emerged as a transformative strategy for engineering immune effector cells. Large-scale BE screens in primary human T cells have systematically identified alleles that tune T cell activation, cytokine secretion, and cytotoxicity. Seminal screens from Marson [47] and Izar [48] groups independently uncovered a broad spectrum of GOF mutations in key signaling components, with activating variants in *PIK3CD* consistently enhancing T cell function. Introduction of these variants into melanoma-specific TCR-T cells or CD19 CAR-T cells significantly improved antigen-specific signaling, polyfunctional cytokine production, and tumor cell killing. Notably, this approach proved effective in improving T cell killing of cancer cells that had acquired resistance through loss of antigen presentation, in some contexts outperforming conventional approaches, such as checkpoint gene knockout [48].

Collectively, these findings demonstrate that precision editing-based screens not only map mechanisms of therapeutic resistance and immune evasion but also enable the rational engineering of more potent and durable immunotherapies.

Dissecting fundamental biological mechanisms at single-base resolution

Beyond their roles in disease pathogenesis and therapeutic response, genetic variants can perturb the core molecular processes that sustain cellular function. Base and prime editing screens now offer the resolution needed to interrogate these effects at the level of individual residues, revealing the molecular logic governing DNA and RNA metabolism, **post-translational modification (PTM)**, epigenetic and *epitranscriptomic* regulation, as well as the functional impact of noncoding and synonymous variants (Figure 2; Table 1).

Interrogating DNA and RNA processing machineries

Genome stability depends on the coordinated DNA replication and repair pathways, in which specific residues play indispensable roles in resolving lesions and maintaining fidelity. An ABE-based tiling mutagenesis study of DNA polymerase κ (Polκ) exemplifies this resolution, delineating its dual roles in translesion synthesis. This screen identified residues within the catalytic core, PIP1, and UBZ2 domains required for bypassing minor-groove DNA adducts, while revealing that a distinct noncatalytic function supporting the REV1–Polζ complex during major-groove bypass relies specifically on the REV1-interacting region. This residue-level analysis transcends conventional gene essentiality assays, directly linking molecular determinants to the protein–protein and protein–DNA interactions that underlie DNA damage tolerance and genome maintenance [52].

Similarly, precision editing has transformed the study of RNA processing. By directing BEs to mutate canonical splice sites (5′GT and 3′AG), researchers can systematically induce exon skipping or intron retention. Building on earlier CBE-based knockout strategies [76–78], targeted splice-site editing has validated functional alternative exons identified in large-scale screens. For instance, editing confirmed that the inclusion of alternative exon 8 in *TAF5* is essential for the assembly of the TFIID transcription initiation complex [53].

At a systems level, BEs have enabled comprehensive dissection of the splicing machinery itself. A CBE screen targeting 153 core spliceosomal components under SF3b inhibitor treatment identified resistance mutations not only in direct drug-binding targets but also in the G-patch domain of SUGP1, a factor previously unlinked to SF3b. Mechanistic follow-up demonstrated that SUGP1 activates the spliceosome disassemblase DHX15, uncovering a proofreading pathway that removes stalled early spliceosomes to preserve splicing fidelity [54].

Mapping post-translational modification landscapes

High-resolution functional screening has expanded beyond nucleic acid biology to encompass post-translational regulation, enabling causal interrogation of PTM sites in their native genomic context rather than inferring function from correlational proteomics.

A landmark BE screen focused on lysine residues, which serve as key acceptors for ubiquitination and acetylation. Using ABEmax in combination with the iBAR strategy, roughly 35% of all lysine codons in the human proteome were targeted, identifying more than 1500 residues essential for cellular fitness [55]. These indispensable lysines were strongly enriched for known ubiquitination and acetylation sites, establishing a direct functional link between PTMs and cell viability. Follow-up screens under genotoxic stress further revealed lysine residues critical for the DNA damage response, highlighting the power of BE to map functional PTMs in specific physiological and pathological contexts [39].

Phosphorylation, the most pervasive PTM, has likewise been interrogated at scale. Genome-wide ABEmax screens targeting thousands of serine, threonine, and tyrosine (S/T/Y) residues

identified numerous substitutions that affect cell fitness, many corresponding to known or predicted phosphorylation sites [56]. Related approaches uncovered residues that modulate chemoresistance [57], T cell activation [58], and cancer immunosurveillance [46], revealing both LOF mutations that abolish essential phosphorylation events and GOF variants that constitutively activate signaling pathways.

Dissecting epigenetic and epitranscriptomic regulation

BE-based screens are also reshaping the study of epigenetic and epitranscriptomic control by enabling residue-level functional analysis of chromatin modifiers, DNA methyltransferases, and RNA-modifying enzymes.

Coupling BE with functional reporters, investigators mapped the activity landscape of DNMT3A, the *de novo* DNA methyltransferase, identifying LOF mutations across multiple domains. These included residues at the ATRX-DNMT3-DNMT3L (ADD)–methyltransferase interface that disrupt allosteric activation, as well as unexpected substitutions within the histone reader Pro-Trp-Trp-Pro (PWWP) =domain that alter DNA binding and enzymatic activity [59]. Precision editing has further enabled epigenetic interrogation in disease-relevant contexts. For instance, the aforementioned ABEmax screen for regulators of cancer immunosurveillance uncovered a clinically relevant mutation in the histone methyltransferase SETD2 that compromises enzymatic function and correlates with improved immunotherapy outcomes [46].

Extending into the epitranscriptome, ABE screens have enabled functional annotation of individual N^6-methyladenosine (m6A) sites on RNA. This study revealed that specific methylation events act as regulatory switches, controlling mRNA stability and cell fate decisions during differentiation, moving beyond global m6A remodeling to site-specific functional analysis [60].

Revealing the function of noncoding and synonymous variants

Precision editing is also illuminating genomic regions once considered functionally inert, including noncoding elements and synonymous mutations. High-throughput BE screens now enable systematic, single-nucleotide mapping of ***cis*-regulatory elements (CREs)**. For instance, screens targeting *BRCA1* and *BRCA2* identified 151 LOF variants within noncoding regions, such as 5′ UTRs, highlighting the pathogenic potential of noncoding mutations [34]. Similar strategies have dissected the regulatory architecture of HbF expression, a critical therapeutic target in sickle cell disease. Genome-scale ABEmax screens across predicted CREs in *BCL11A*, *MYB*, and β-globin loci identified numerous regulatory nucleotides that fine-tune HbF levels and modulate disease phenotypes [60]. A subsequent study coupled BE screening with single-cell transcriptomics in primary HSPCs, providing cell-resolved insights into how noncoding variants shape HbF expression and hematopoietic differentiation trajectories [49].

Prime editing has further expanded the functional annotation of noncoding regions. Saturation mutagenesis approaches have characterized hundreds of noncoding LOF variants, including 362 ClinVar-listed mutations in *MLH1* evaluated by 6-thioguanine selection [64], as well as dense profiling of a *MYC* enhancer and multiple GWAS-associated noncoding loci [65]. The PE2-based Variant-EFFECTS framework enabled systematic dissection and reprogramming of regulatory elements; tiling mutagenesis of the *PPIF* promoter and an upstream enhancer mapped transcription factor binding sites and demonstrated that minimal edits can tune gene expression across a broad dynamic range [72].

Concurrently, precision editing is overturning the assumption that synonymous mutations are functionally neutral. BE and PE screens have identified synonymous variants that impair gene function [34,67]. In a large-scale PEmax study using approximately 300 000 epegRNAs to assay approximately 100 000 synonymous variants across diverse cancer cell lines, most variants were benign, but a substantial subset significantly affected cell fitness [66]. Mechanistic analyses revealed disruptions in splicing, mRNA secondary structure, or transcript stability. These insights enabled the development of predictive models, such as DS Finder, to identify potentially pathogenic synonymous mutations.

Together, these findings underscore how precision editing technologies are completing the functional annotation of the genome. By directly linking subtle nucleotide changes in both coding and noncoding regions to cellular phenotypes, BE and PE screens reveal how even minimal genetic variation can reshape gene regulation, cellular function, and disease susceptibility.

Challenges in precision editing screens

Despite remarkable progress, current precision editing technologies retain inherent limitations that pose significant challenges for pooled screening applications (Table 3). With respect to mutational scope and efficiency, commonly used BEs are largely restricted to transition mutations (C•G to T•A or A•T to G•C) and frequently display heterogeneous activity across sgRNAs, as well as bystander edits that complicate data interpretation [31]. PEs, while offering broader editing versatility, have historically been limited by variable efficiency and inconsistent performance across genomic loci and cell types, constraining their application in truly genome-wide screens [31,106].

Specificity represents an additional concern. Like all CRISPR-based modalities, both BEs and PEs can generate off-target effects. Notably, BEs, particularly CBEs, exhibit varying degrees of Cas9-independent off-target activity at both DNA and RNA levels [31,98,99,103,107,108]. Such promiscuous deaminase activity can activate p53-dependent DNA damage responses, leading to cytotoxicity-driven false-positive dropouts or selective enrichment of p53-deficient clones, thereby confounding genotype–phenotype attribution.

Table 3. Comparison of characteristics of representative base editors and PEs

Category		Editing window	Bystander effects	Off-target effects		Refs
				DNA level	RNA level	
CBEs	BE3	C4–C8 (away from the PAM)	High	High	High	[1,98,99]
	A3A-BE3	C4–C8 (away from the PAM)	High	High	High	[100,101]
	BE4max	C4–C8 (away from the PAM)	High	High	High	[73,102]
	YE1-BE4	C5–C6 (away from the PAM)	Reduced	Reduced	Reduced	[101,103]
	transformer BE	C3–C9 (away from the PAM)	Reduced	Low	Low	[104]
	eTd-CBE	C5–C6 (away from the PAM)	Low	Low	Low	[102]
ABEs	ABE7.10	A4–A7 (away from the PAM)	General	Low	High	[2,98,99]
	ABEmax	A4–A7 (away from the PAM)	High	Low	High	[73,98]
	ABE8e	A4–A8 (away from the PAM)	High	High	High	[74]
	ABE9	A5–A6 (away from the PAM)	Low	Low	Low	[75]
PEs	PE2	+1–+60 (relative to the nick)	Low	Low	Unknown	[3,105]
	PEmax	+1–+60 (relative to the nick)	Low	Low	Unknown	[80,105]
	PE7	+1–+60 (relative to the nick)	Low	Low	Unknown	[84]

Addressing these challenges requires a multilayered approach to ensure robust and accurate functional inference. Rigorous library design is a critical first step, incorporating appropriate negative controls (e.g., nontargeting guides, safe-harbor targets such as *AAVS1*, or synonymous mutations) and positive controls (e.g., guides introducing nonsense or splice-site mutations). To streamline this process, computational tools such as BEscreen [109], CRISPR-BEasy [110], and SynDesign [111] have been developed to systematically design diverse classes of guide RNAs. In parallel, advanced readout strategies are essential to account for variable editing efficiencies. 'Sensor' or 'surrogate target' systems, which pair sgRNA or pegRNA sequences in *cis* with their corresponding targets, enable simultaneous sequencing of editing outcomes and guide abundance. This design supports computational normalization frameworks that empirically quantify and correct for editing efficiency [36,40,51,62,64,67]. Finally, rigorous orthogonal validation, such as CRISPR-mediated HDR or complementary assays, is essential to confirm screening hits and exclude artifacts arising from bystander or off-target edits.

Beyond improvements in library design and analytical frameworks, overcoming current bottlenecks depends on continued innovation in editor engineering. Protein engineering has significantly refined the core editing architectures. The integration of PAM-relaxed Cas9 variants, such as NG-Cas9 [112] and SpRY [113], together with evolved effector enzymes, including optimized deaminases and RTs, has greatly expanded the targetable sequence space, with many of these variants now deployed in high-throughput screens (Tables 1 and 2). At the same time, engineering efforts increasingly focus on balancing efficiency and fidelity, yielding editors such as transformer BEs [104], eTd-CBEs [102], and ProPE [114] that combine high activity with improved specificity.

The editable mutational repertoire is also rapidly expanding. Emerging BE systems now enable more complex edits, including transversions through C-to-G [102,115,116], A-to-C [117], and A-to-Y editing [118], as well as deaminase-independent platforms [119–122]. These advances are further amplified by improvements in sgRNA and pegRNA design and by strategies that modulate DNA repair pathways to favor desired outcomes [123]. Importantly, artificial intelligence (AI)-driven protein evolution is increasingly being leveraged as a transformative approach to accelerate the rational design of next-generation editors with optimized performance and specificity [124,125].

Concluding remarks

Base and prime editing have fundamentally transformed functional genomics by enabling precise and scalable links between genetic variation and biological function. These platforms now support systematic, *in situ* mutational scanning, converting vast catalogs of genetic variants, particularly VUS, into high-resolution functional maps of protein activity and disease relevance. By elucidating mechanisms of drug resistance, identifying critical residues across essential pathways, and connecting molecular variation to clinical phenotypes, precision editing has greatly expanded our capacity to generate residue-level insights into human biology.

Realizing the full potential of these advances for precision medicine, however, requires addressing several outstanding challenges, from continued optimization of editor performance to decoding complex genetic interactions (see Outstanding questions). Looking forward, integrating precision editing with complementary technologies, such as structural biology, multiomics profiling, and artificial intelligence, will further propel the evolution of functional genomics. Incorporating scRNA-seq into precision editing screens has already provided molecular-resolution insights that surpass traditional bulk readouts, accelerating VUS annotation and deepening our understanding of genotype–phenotype relationships across diverse human diseases [37,41,49,66].

Outstanding questions

Can artificial intelligence (AI)-driven protein engineering and an expanding editor toolkit overcome the inherent trade-offs between editing scope, efficiency, and precision to create a unified framework for deploying the optimal editor for any therapeutic or research target?

As precision editing screens continue to scale, how can we leverage the resulting rich datasets through the systematic integration of advanced techniques, such as structural biology, multiomics profiling, and artificial intelligence, to construct functional maps that predict and elucidate the phenotypic consequences of genetic variants?

What is the most effective translational pipeline for converting large-scale functional data into clinically actionable variants of uncertain significance classifications, and how can the proactive mapping of resistance landscapes guide the development of more durable therapies?

Beyond simple survival assays (e.g., cell fitness and therapeutic resistance), what innovative readouts and *in vivo* models are required to adapt these screens for characterizing genetic determinants that govern complex biological processes, such as transient signaling, metabolism, or morphological changes?

To what extent can these platforms be repurposed for applications beyond characterizing natural variation, such as driving directed protein evolution to facilitate the rapid discovery of enzymes and therapeutic proteins with optimized functions?

How can we create a closed-loop and self-improving platform where AI not only predicts variant function but also autonomously designs the optimal high-throughput experiments to test its own hypotheses, creating a virtuous cycle of discovery?

How can precision-editing screens be scaled to systematically map the epistatic networks of disease-relevant alleles, deciphering how the pathogenicity of a specific variant is modulated by the broader genetic background?

A key remaining challenge is extending residue-level functional characterization into more complex physiological contexts. Progress will necessitate innovative phenotypic readouts to capture genetic determinants governing biological processes such as transient signaling, metabolism, and cellular morphology, alongside the translation of these screening strategies from *in vitro* systems to *in vivo* microenvironments. Beyond annotating natural genetic variation, precision editing platforms also hold promise for directed protein evolution, enabling rapid engineering of enzymes and therapeutic proteins with optimized properties. Concurrently, the massive datasets generated by saturation mutagenesis provide fertile ground for machine-learning approaches that increasingly enable accurate prediction of variant pathogenicity [51,66,68]. These predictive models, in turn, guide experimental prioritization, establishing a self-reinforcing feedback loop that accelerates comprehensive functional annotation of the human genome.

Ultimately, large-scale mechanistic annotation of clinical variants holds transformative promise for precision medicine. By systematically charting resistance landscapes, researchers can better anticipate therapeutic failure and inform the design of more durable treatment strategies. By elucidating disease mechanisms from single-nucleotide perturbations to complex regulatory networks, precision editing screens are poised to become indispensable tools for future biomedical discovery, bringing the field closer to predictive and programmable biology.

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Declaration of interests

W.W. is a scientific advisor and founder of EdiGene and Therorna. All other authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies

During the preparation of the manuscript, the authors used Gemini 3 Pro and ChatGPT in order to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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