

RNA-guided bridge recombinases expand the scope of genome editing

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Over the past decade, technologies based on clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins have transformed programmable genome editing. Yet the editable landscape remains uneven. Current platforms are highly effective for gene knockout and local sequence rewriting, but they remain less capable when the goal is gene-sized DNA insertion or precise restructuring of larger chromosomal intervals. As a result, programmable control of structural genome edits remains a major unmet need.

Canonical nuclease-based systems, represented by Cas9 and Cas12a, rely on DNA double-strand breaks (DSBs) and endogenous repair pathways. Although this strategy is powerful and broadly applicable, it often yields heterogeneous insertions and deletions and can introduce genotoxic risk. Base editors reduce reliance on DSBs and enable efficient nucleotide conversion, but each system is restricted to defined classes of base substitution. Prime editors broaden the range of editable sequences, yet their performance declines as edit size increases, limiting their utility for gene-scale DNA installation. CRISPR-associated transposases (CASTs) and several other targeted integration systems offer alternative routes to site-specific DNA insertion. Still, many remain mechanistically complex and delivery-intensive, and most are designed primarily for cargo integration rather than as unified platforms for programmable excision and inversion. Notable exceptions, such as programmable chromosome engineering (PCE) and Re-pegRNA-assisted PCE (RePCE) chromosome-engineering systems developed by Sun et al., extend beyond insertion to support large deletions and inversions.¹ PCE/RePCE platforms couple engineered recombinase chemistry with pegRNA-enabled scar erasure, thereby enabling scarless genome restructuring across kilobase-to-megabase scales. Zhang et al. reported amplification editing (AE), which enables programmable duplication of endogenous DNA from short sequences to chromosomal scale.² Its high efficiency for megabase-scale copying establishes AE as a powerful tool for programmable copy-number engineering. In parallel, Chen et al. engineered avian R2 retrotransposons into an all-RNA platform for targeted gene integration in mammalian cells.³ The all-RNA platform highlights a distinct all-RNA, insertion-focused editing modality with low persistence and potentially reduced immunogenicity, underscoring its translational potential for targeted gene addition. Together, these platforms are complementary rather than interchangeable. PCE/RePCE and related recombinase-based systems enable programmable chromosomal restructuring but may require recombination-site design and enzyme engineering. AE is well-suited for programmable DNA duplication, while R2-derived retrotransposon systems provide an all-RNA, insertion-focused route for targeted gene addition. This comparison highlights a remaining conceptual gap: a single programmable system that can directly translate RNA-guided target recognition into recombinase-mediated DNA rearrangement. Bridge recombinases fill this gap by coupling RNA-specified target and donor recognition with recombination chemistry, thereby enabling insertion, excision, and inversion at endogenous loci within a unified programmable framework (Figure 1).

RNA-guided bridge recombinases provide a mechanistically distinct solution to this problem. Derived from IS110-family insertion sequences, these enzymes use a bridge RNA to specify both a genomic target site and a donor site, thereby coupling donor mobility with RNA programmability. Earlier work established bridge RNA-guided recombination in vitro and in bacteria, but two recent Science studies extended and engineered this chemistry for human cells.^{4,5} Pelea et al. identified ISCro4 as a mammalian-active bridge recombinase and established the basic framework for programmable insertions, deletions, and inversions in human cells.⁴ In a complementary study, Perry et

al. optimized ISCro4 through bridge-RNA engineering and deep mutational scanning of the recombinase, substantially improving performance and expanding the accessible scale of genome rearrangement.⁵ Together, these studies position bridge recombinases as a new platform for programmable structural genome editing in mammalian systems.

In the study by Pelea et al., ortholog screening identified ISCro4 as the most active bridge recombinase tested in human cells.⁴ Cryo-electron microscopy further revealed molecular features that help explain its superior activity relative to related orthologs. A key conceptual advance was the demonstration that the bridge RNA can be split into independent target-binding loop (TBL) and donor-binding loop (DBL) guides, which substantially increased recombination activity. Using plasmid-based delivery, and in some settings all-RNA delivery, the authors showed that ISCro4 can support deletions and inversions in reporter systems and mediate targeted rearrangements at endogenous loci. These experiments included deletion at the *CNBP* locus, inversion around *CFTR* exon 23, and donor insertion at *AAVS1* and *CCR5*, with targeted insertion of an approximately 3.5-kb donor reaching efficiencies above 6% at selected sites. Notably, the authors did not detect ISCro4-dependent indels at the targeted genomic loci in their sequencing analyses, consistent with a recombination mechanism that is distinct from DSB-and-repair editing.

Perry et al. extended this platform in two important directions: specificity and scale.⁵ The authors first showed that naive insertion designs can be dominated by donor-like off-target integration, reflecting DBL–DBL recombination as a major specificity liability. They then derived practical design principles to suppress this behavior, including the use of genome-orthogonal donor sequences and an orientation in which the DBL recognizes the genome and the TBL recognizes the donor. Combined with enhanced bridge-RNA scaffolds and a deep mutational scan-derived ISCro4 variant, these advances increased targeted insertion efficiency to as high as 20% and genome-wide specificity to as high as 82% at selected loci. Most strikingly, the engineered system supported scarless intrachromosomal inversions approaching 0.93 Mb and excisions exceeding 0.13 Mb, without obvious distance dependence across the tested range. The study also provided disease-relevant proof-of-concept applications, including *BCL11A* enhancer excision and removal of expanded GAA repeats associated with Friedreich's ataxia.

These studies help define a distinct role for bridge recombinases within the genome-editing toolbox. Their strength is not in replacing nuclease-based editors, base editors, or prime editors for local sequence correction. Instead, bridge recombinases seem particularly well suited for structurally defined edits, including excision of pathogenic intervals, inversion of disease-associated segments, compression or removal of repeat expansions, and mutation-agnostic insertion of multi-kilobase DNA cassettes without relying on homology-directed repair. In principle, this could make gene-editing therapies more scalable and more compatible with standardized, industry-level manufacturing. This is precisely the area where current genome-editing modalities still show clear limitations.

Even so, the technology remains early. The effective design space for bridge RNAs, including optimal scaffold architecture, loop orientation, and sequence constraints, is not yet fully mapped. Moreover, recombination efficiency is likely to depend on genomic context, chromatin accessibility, donor configuration, and higher-order properties of the RNA-guided synaptic complex. High-throughput design-build-test strategies, coupled with structural analysis, should help convert the current empirical rules into predictive guide-design principles.

Specificity also remains a major challenge. Because ISCro4 recognizes

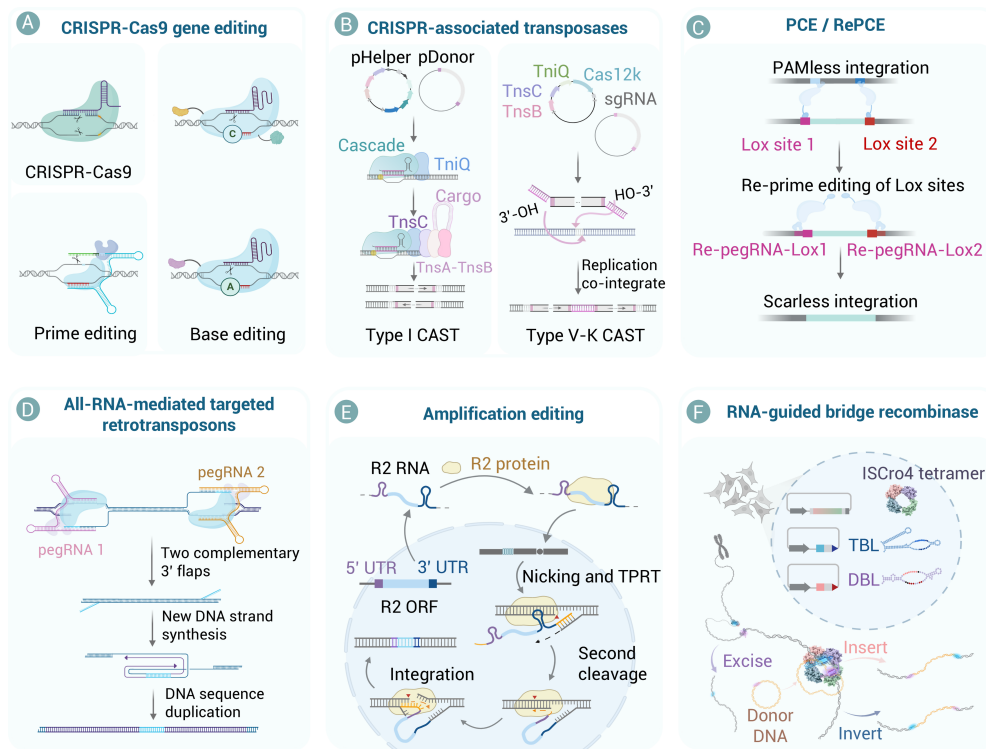


Figure 1. Programmable genome engineering modalities from local sequence editing to chromosome-scale rewriting (A) CRISPR–Cas tools generate indels, base editing and prime editing. (B) CASTs mediate RNA-guided cargo insertion. (C) PCE/RePCE enables large-fragment restructuring and scarless editing. (D) Amplification editing generates endogenous tandem duplications. (E) All-RNA retrotransposons enable RNA-templated gene insertion. (F) Bridge recombinases couple RNA-guided recognition with DNA rearrangement to enable insertion, excision, and inversion.

mammalian genome engineering. They combine RNA-programmable targeting with recombinase-mediated DNA mobility and therefore address a class of structural edits that existing cut-and-repair systems handle only inefficiently or not at all. Continued progress in protein engineering, RNA design, delivery, and specificity profiling should clarify where bridge recombinases can offer decisive advantages over other genome-editing technologies.

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short 14-nt donor and target sites, perfect or near-perfect genomic alternatives can occur in complex genomes. In addition, unintended TBL–DBL and potentially TBL–TBL recombination events remain important concerns. The current studies nevertheless provide a concrete path forward: selecting unique genomic sites, using genome-orthogonal donor sites, exploiting favorable guide orientation, and continuing to engineer both the recombinase protein and bridge-RNA scaffold.

Equally important, the combined literature now shows that bridge recombinases are not limited to kilobase-scale editing. Instead, the field has already progressed from multi-kilobase targeted insertion to megabase-scale chromosomal rearrangement in human cells. At the same time, their therapeutic potential remains at an early proof-of-concept stage. Delivery bottlenecks differ across large-fragment editing platforms. CASTs and PCE/RePCE are constrained mainly by their multi-component and protein-engineering complexity. AE avoids exogenous donor DNA but relies on a large prime-editor architecture. R2-derived systems offer an all-RNA route for targeted insertion, although the RNA template increases with cargo size. By contrast, bridge recombinases have a relatively compact recombinase component and short bridge RNA, but practical translation will still depend on the size of the donor DNA and the coordinated delivery of three components: the recombinase, bridge RNA, and donor DNA. Adeno-associated virus (AAV) vectors may be suitable for compact payloads but are limited when donor DNA and regulatory elements are included. Lentiviral vectors can carry larger payloads but raise concerns related to integration, vector titer, tropism, and vivo delivery. Lipid nanoparticle (LNP)-based delivery may support transient recombinase messenger RNA (mRNA) and bridge RNA delivery, but donor DNA co-delivery and component stoichiometry remain important challenges. Defining these parameters will determine how far RNA-guided recombination can be advanced toward clinically relevant genome restructuring.

Overall, RNA-guided bridge recombinases add a genuinely new modality to