

Engineering an mRNA Prime Editing platform with broad utility in genome editing

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Introduction

The SpCas9 nuclease from *Streptococcus pyogenes* remains one of the most widely employed CRISPR enzymes for genome editing. While insertions, deletions, and homology-dependent repair events can be achieved after cleavage with SpCas9, specificity and toxicity concerns remain with this editing platform. Prime Editing was developed as a next generation solution that can achieve genome editing without the need for a DSB or an exogenous DNA repair template. The Prime Editor consists of reverse transcriptase fused to Cas9 nickase and pegRNA that dually functions as targeting RNA and repair template. Though the prime editing platform requires fewer components, can be delivered entirely in RNA form, and provides intrinsically fewer opportunities for genotoxic effects, it suffers from poor potency. To improve the overall utility of the prime editing platform we (i) engineered the pegRNA to make it more stable without the need for 3' sequence extensions and in vivo expression, (ii) performed end-to-end protein engineering on the entirety of the prime editor open reading frame, and (iii) optimized the mRNA sequence and structure for stability and translatability. The resulting "all RNA" system substantially improves the prime editing platform and allows for the use of chemically synthesized pegRNAs. These improvements are consistent between immortalized cell lines and primary cells, and provide a practical solution to sourcing prime editing reagents beyond research applications.

Engineering of a more potent and stable pegRNA

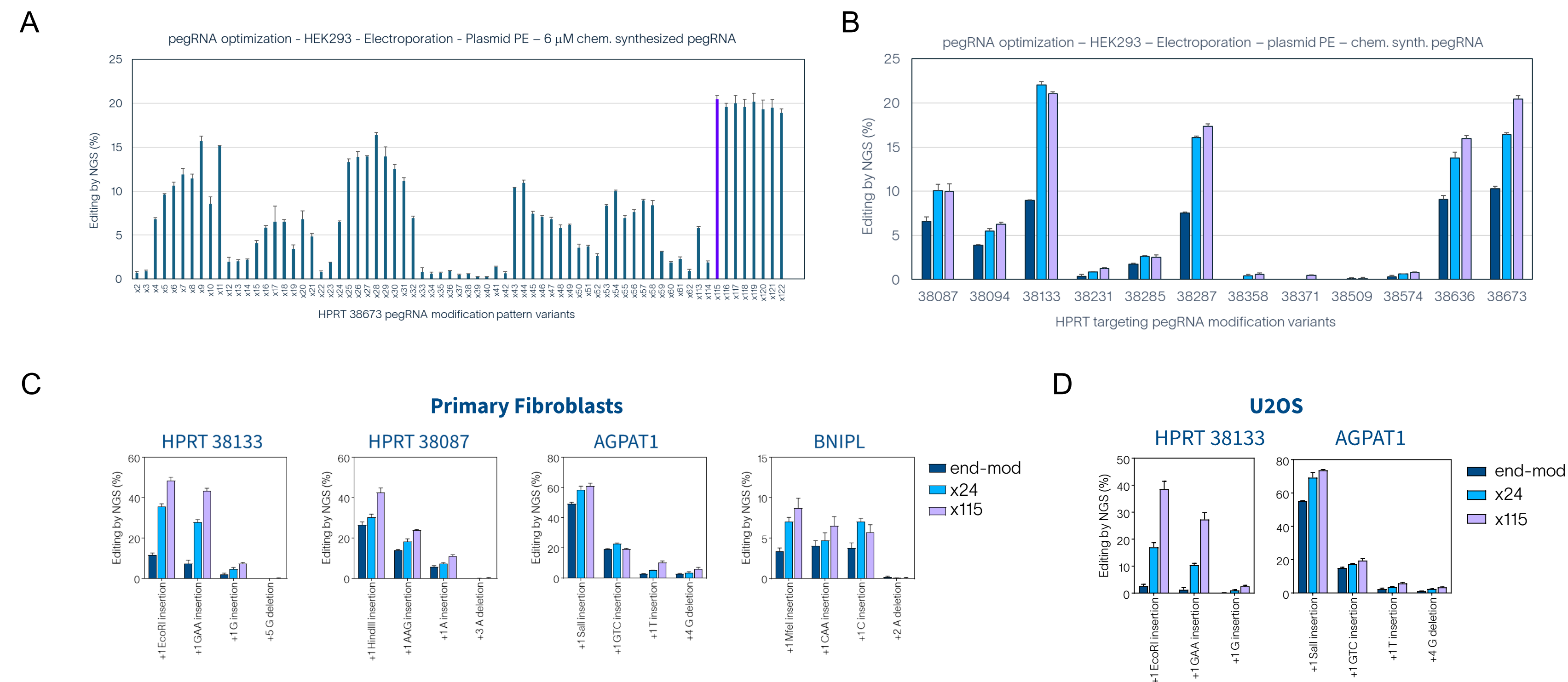


Figure 1. pegRNA chemical modification pattern screening identifies a high-performing pegRNA format that generalizes across targets and cell types. Chemically synthesized pegRNAs encoding restriction-site insertions were used to evaluate how pegRNA chemical modification patterns influence prime editing efficiency. (A) In HEK293 cells, a library of pegRNA modification-pattern variants (x2–x62 and x113–x122) designed to install a +1 EcoRI (GAATTC) insertion at HPRT-38673 was delivered at 6 μ M alongside a prime editor plasmid by electroporation. Genomic DNA was harvested 72 h after delivery and editing frequencies were quantified by NGS; the best-performing pattern (x115) is highlighted. (B) Top patterns were then tested across additional HPRT target sites (as labeled), demonstrating that leading pegRNA chemistries retain improved activity across multiple genomic contexts. (C–D) To assess portability, selected patterns (including end-modified and top-performing patterns as labeled) were applied to additional loci (HPRT 38133, HPRT 38087, AGPAT1, BNIPL) and evaluated in primary fibroblasts and U2OS cells. Multiple edit outcome classes (restriction-site insertions, single-base insertions, and representative deletions shown on x-axes) were quantified by NGS.

High purity gRNAs facilitate efficient Prime Editing

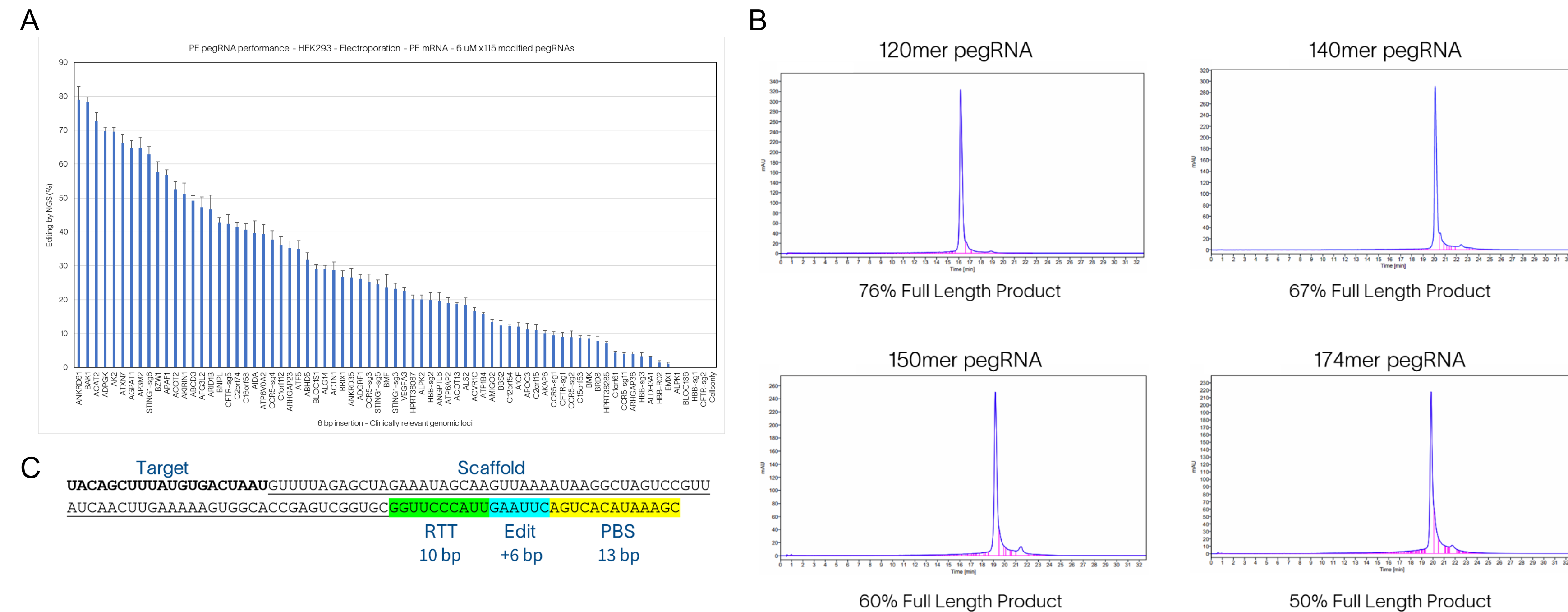


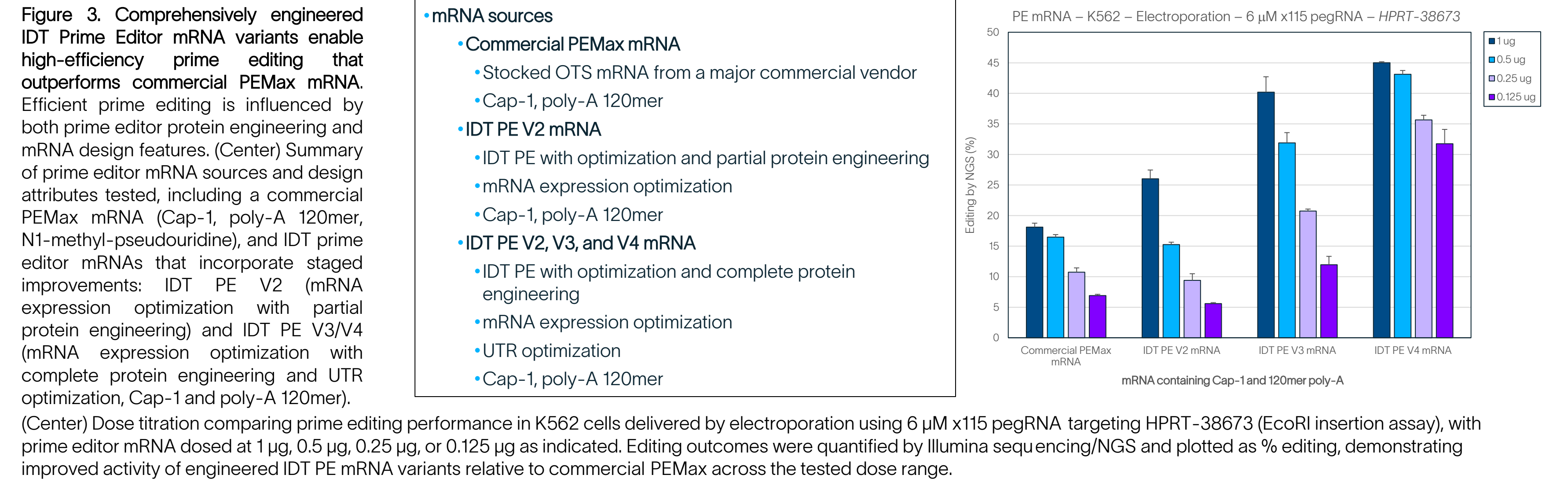
Figure 2. High-purity gRNAs support robust prime editing. Efficient prime editing depends on the quality and integrity of long synthetic guide RNAs. (A) Prime editing efficiencies measured across a broad panel of target sites in HEK293 cells following electroporation delivery (conditions as indicated in the panel title), illustrating the distribution of editing performance when using high-purity guide RNAs. (B) Representative analytical traces for chemically synthesized pegRNAs of increasing length (120-mer, 140-mer, 150-mer, and 174-mer) showing the fraction of full-length product (percentages annotated under each trace), highlighting the progressive reduction in full-length material as pegRNA length increases. (C) Schematic of pegRNA architecture showing the targeting (spacer) region, sgRNA scaffold, and 3' extension encoding the reverse-transcription template (RTT), the intended edit, and the primer-binding site (PBS) (segment boundaries and lengths labeled), illustrating the structural elements that contribute to overall pegRNA length and manufacturing complexity.

CRISPR Custom Guide RNAs

We offer chemically synthesized and modified custom guide RNAs for common as well as specialized research applications. All 5' Custom Guide RNAs are ideal for prime editing (genomics projects, CRISPR-Cas13 applications, and most alternative CRISPR-Cas systems).

- Fast shipping (usually 3–6 business days)
- A range of modification and purity options
- Multi-scale delivery in tubes or plates
- gRNA available up to 275nt in length

Engineered IDT Prime Editor mRNA facilitates high efficiency editing



IDT Prime Editor mRNA achieves a spectrum of efficient small edits

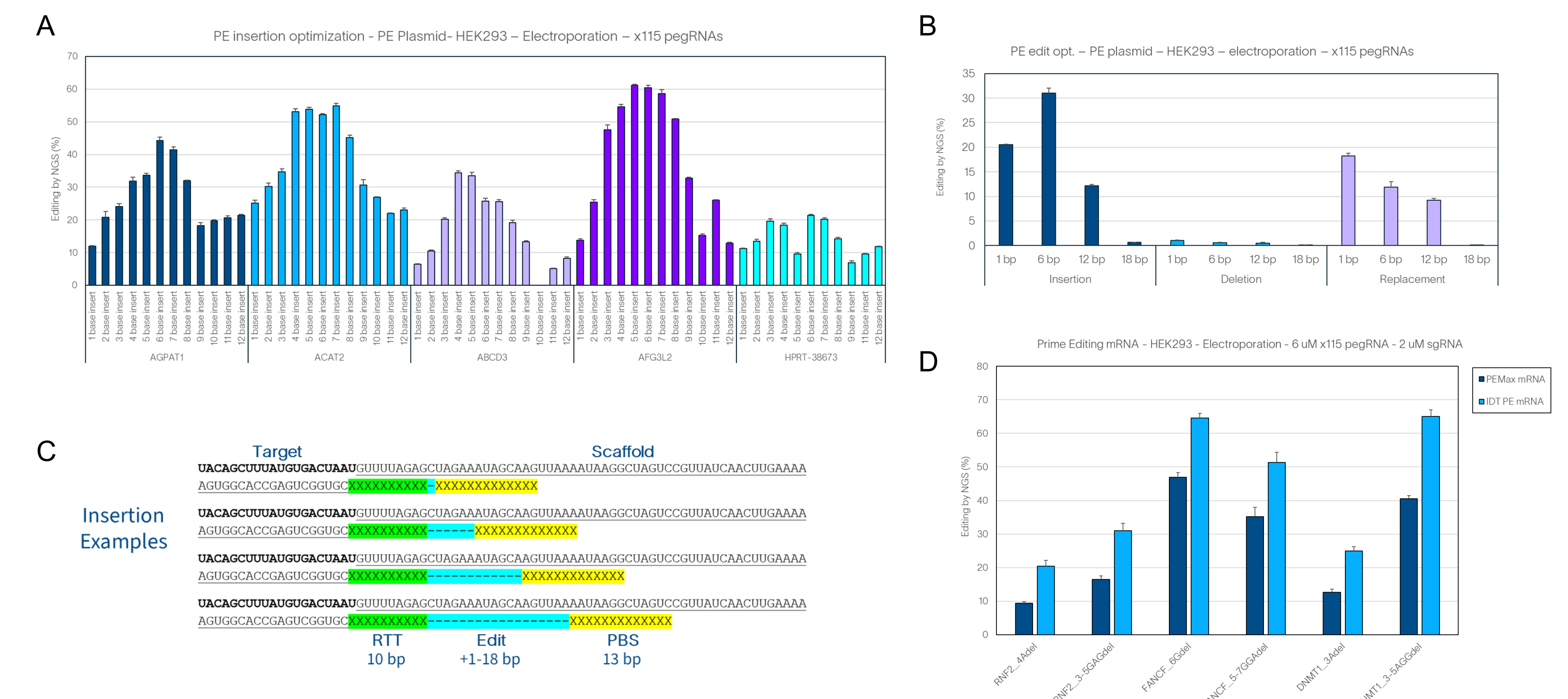
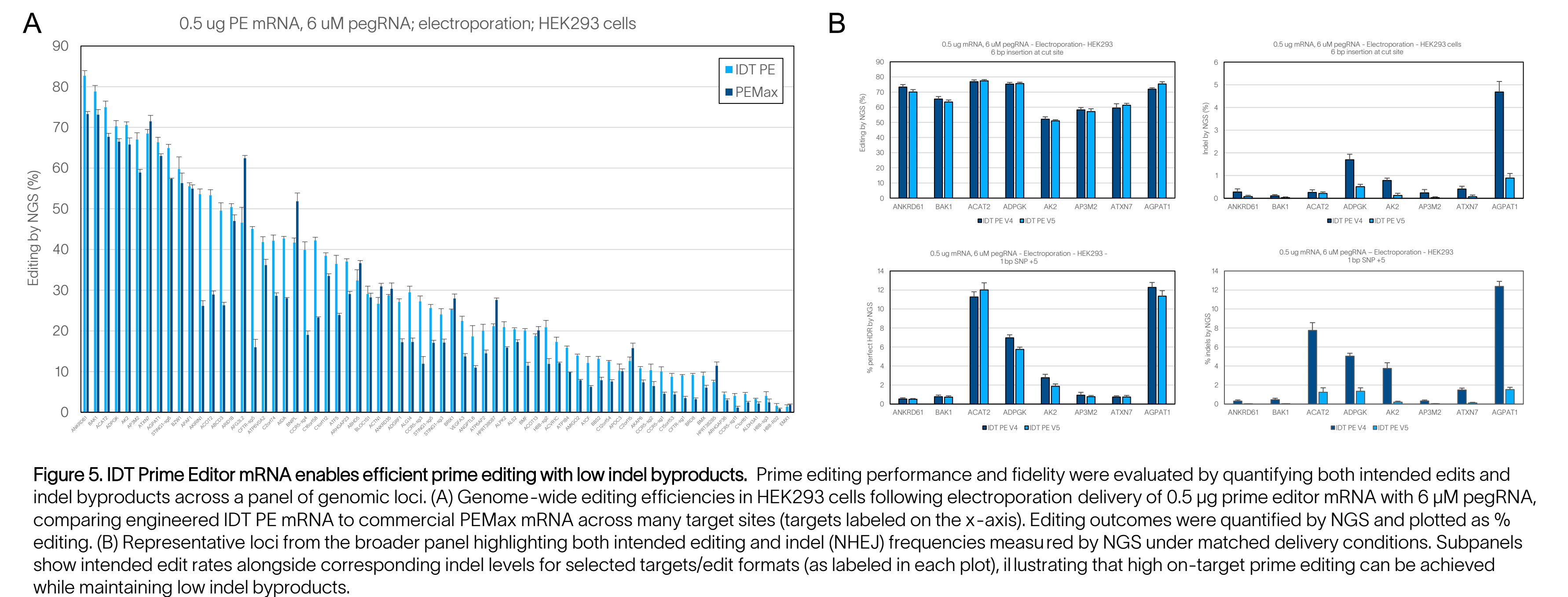


Figure 4. IDT Prime Editor mRNA enables a spectrum of efficient small edits. Prime editing performance was evaluated across multiple small-edit classes and edit sizes using optimized pegRNA designs. (A) Insertion optimization across representative loci (as labeled) in HEK293 cells delivered by electroporation with prime editor plasmid and x115 pegRNAs, testing a range of small insertions (edit sizes as labeled) and quantifying editing by NGS; the dataset highlights that 6-bp insertions are favored on average under these conditions. (B) Aggregate comparison of insertions, deletions, and replacements across increasing edit sizes (e.g., 1, 6, 12, and 18 bp as labeled) in HEK293 cells delivered by electroporation with prime editor plasmid and x115 pegRNAs, illustrating that insertions and replacements are generally more efficient than deletions in this format. (C) pegRNA design schematic used for the insertion series, showing the targeting (spacer) region, sgRNA scaffold, and the 3' extension comprising the reverse-transcription template (RTT; 10 bp), edit region (size range as labeled), and primer-binding site (PBS; 13 bp). (D) Efficient deletion editing with a nicking sgRNA using prime editor mRNA delivered to HEK293 cells by electroporation with 6 μ M x115 pegRNA and 2 μ M sgRNA, comparing performance across multiple deletion outcomes (targets as labeled) by NGS; the plot contrasts commercial and engineered mRNA sources/variants as indicated in the legend.

IDT Prime Editor mRNA facilitates efficient editing with low indels



Conclusions

- A systematically engineered pegRNA format identified a top-performing, chemically stabilized pegRNA design (e.g., x115) that improves prime editing performance and generalizes across additional loci/cell types and multiple edit outcomes (insertions, single-base edits, and deletions).
- High-throughput, iterative engineering of the prime editor architecture (including protein/domain and linker optimization informed by broad screening/selection efforts) was used to identify improved prime editor variants that were advanced into mammalian testing.
- Iterative engineering of the prime editor as an mRNA reagent (protein + mRNA architecture improvements) produced progressive gains across versions (V1→V5), supporting an optimized, higher-performing mRNA prime editor platform.
- The final mRNA design incorporates uridine depletion, UTR optimization, and sequence expression tuning to support an efficient prime editor with minimal toxicity and compatibility with multiple delivery modalities.
- Collectively, combining optimized pegRNA chemistry with engineered prime editor (mutations + linker) and optimized mRNA design yields a robust prime editing platform with broad utility across targets and edit types.