Exploring the roles of DNA repair processes across diverse Prime Editing strategies with knock-knock’

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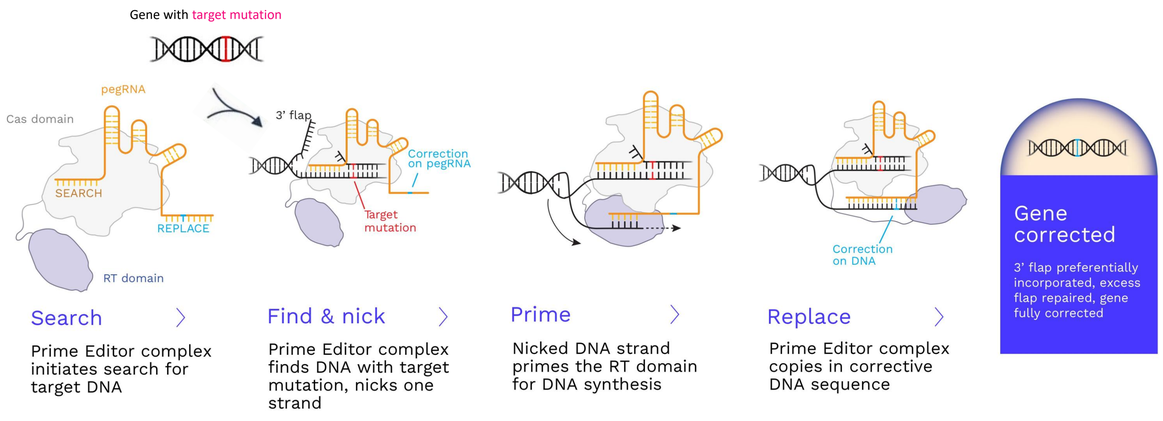
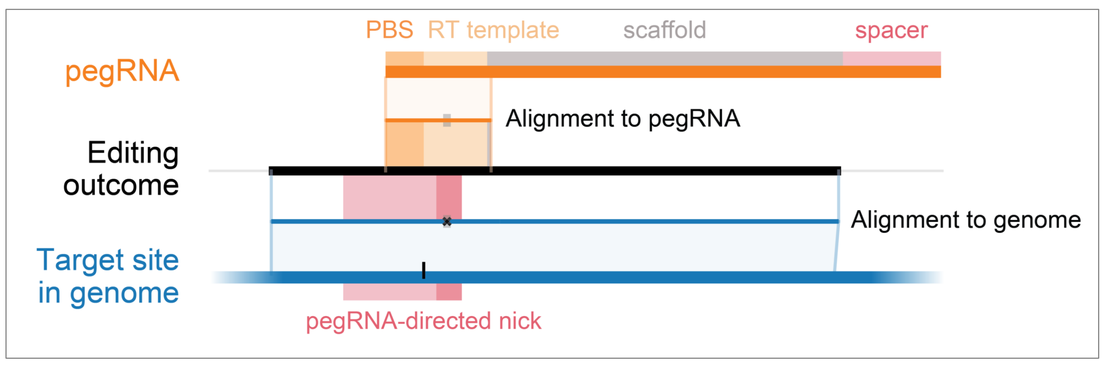
Background

Prime Editing allows for the precise installation of a wide range of programmed changes to genomic sequence [1].



To quantify the full spectrum of editing outcomes produced by diverse Prime Editing strategies, we developed **knock-knock’**, an improved computational pipeline for analysis of high-throughput sequencing of Prime-Edited loci [2, 3].

knock-knock’ decomposes reads into local alignments to genomic and pegRNA sequences, then analyzes the architecture of these alignments to identify different categories of Prime Editing outcomes.



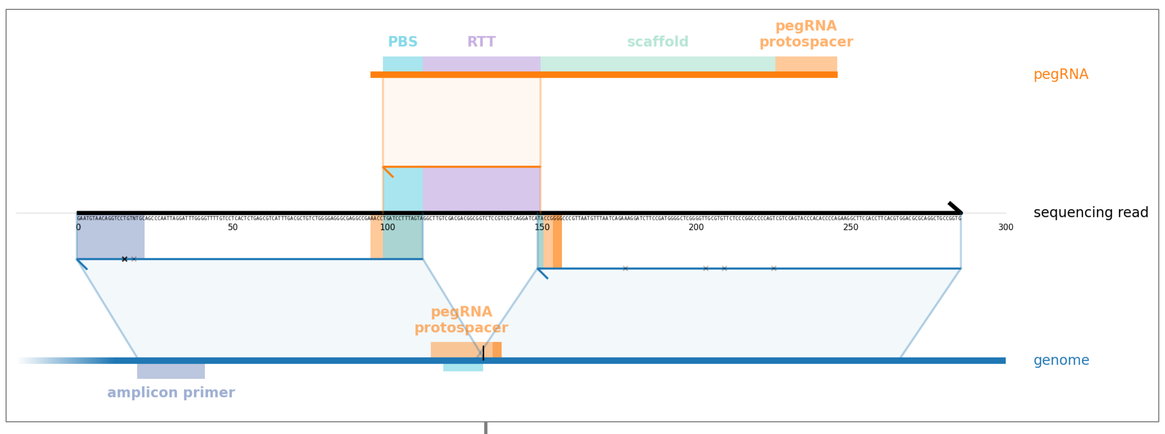
Comparison of high-resolution editing outcome frequencies across many experiments allows for identification of DNA repair processes that produce different outcomes and of pegRNA sequence features that promote efficient, precise editing.

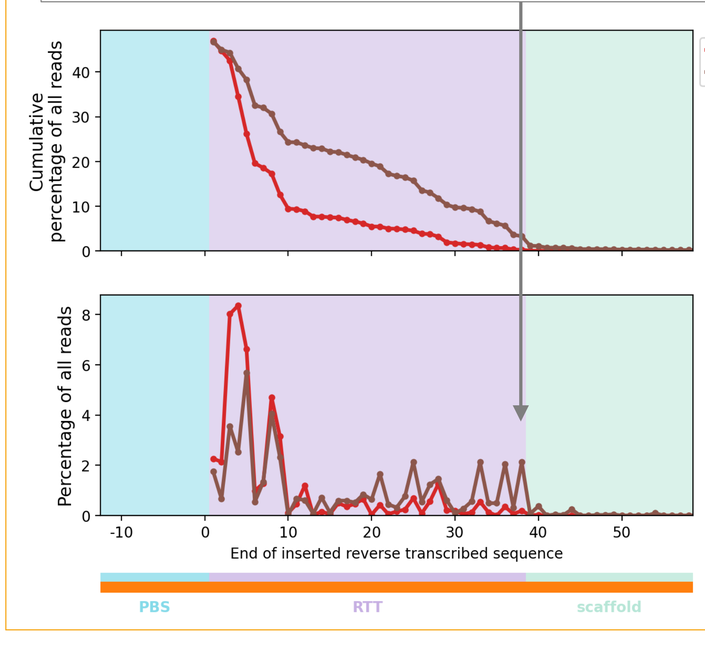
Application of knock-knock’ to PE-Nuclease Experiments for Screening Purposes

Prime Editing with a fully nuclease active Cas9 (PE-Nuclease) is a screening tool that can be used to dissect activity of the different steps required for Prime Editing.

PE-Nuclease produces editing outcomes in which variable amounts of reverse transcribed sequence are inserted at the targeted double strand break.

Example PE-nuclease editing outcome:

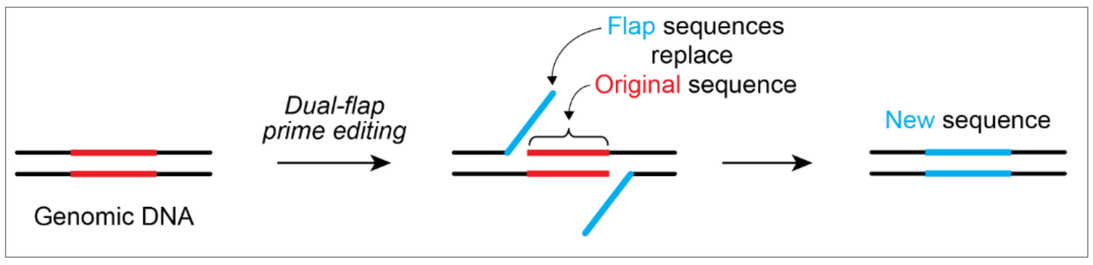




Comparison of the distributions of insertion lengths across different PE-Nuclease experiments allows identification of conditions that maximize reverse transcription activity and processivity.

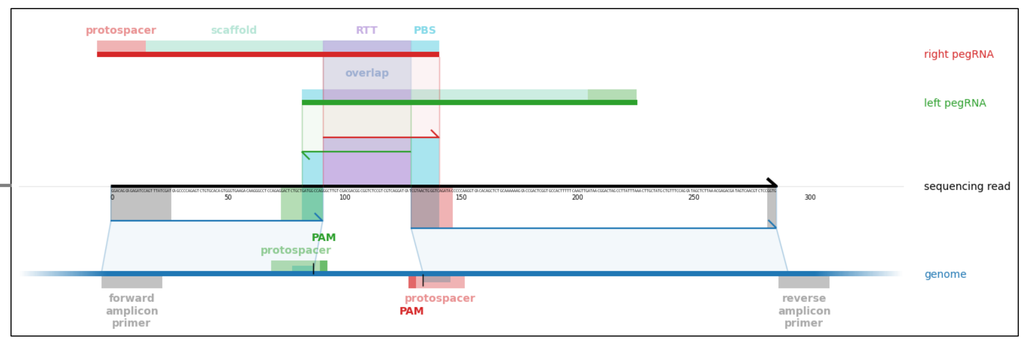
Application of knock-knock’ to Dual Flap Prime Editing

Dual Flap Prime Editing uses two pegRNAs that target nearby genomic locations and program complementary flap sequences to allow longer range insertions, deletions, or sequence replacements [4].

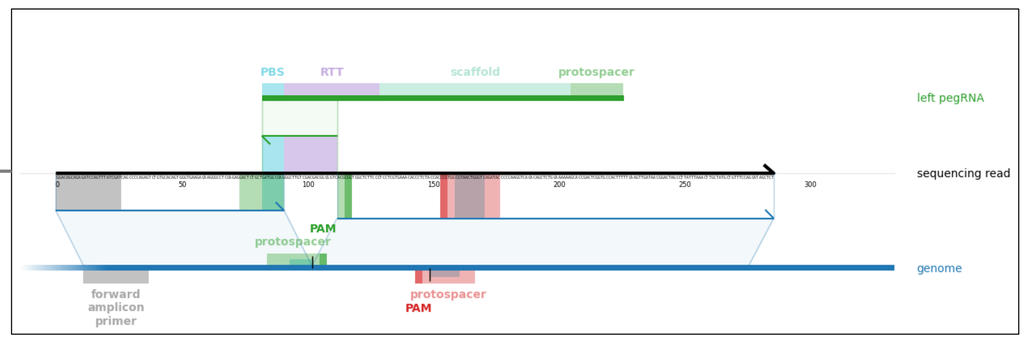


In addition to the intended edit programmed by a pair of Dual Flap pegRNAs, knock-knock’ identifies unintended editing outcomes categories containing different configurations of reverse transcribed sequence from one or both pegRNAs.

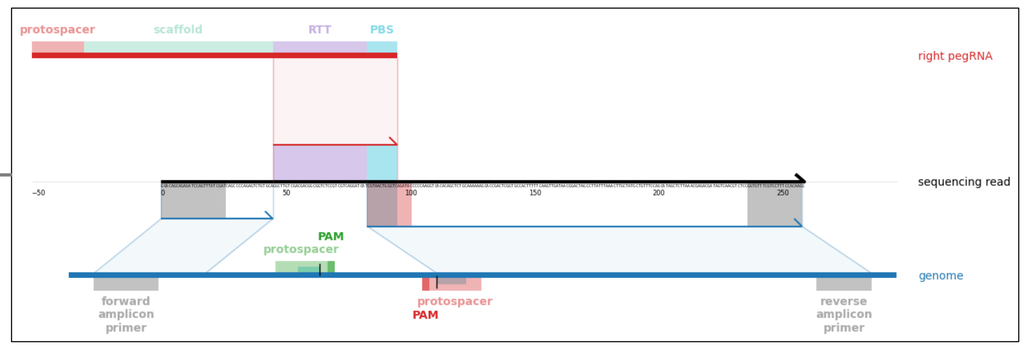
Intended edit:



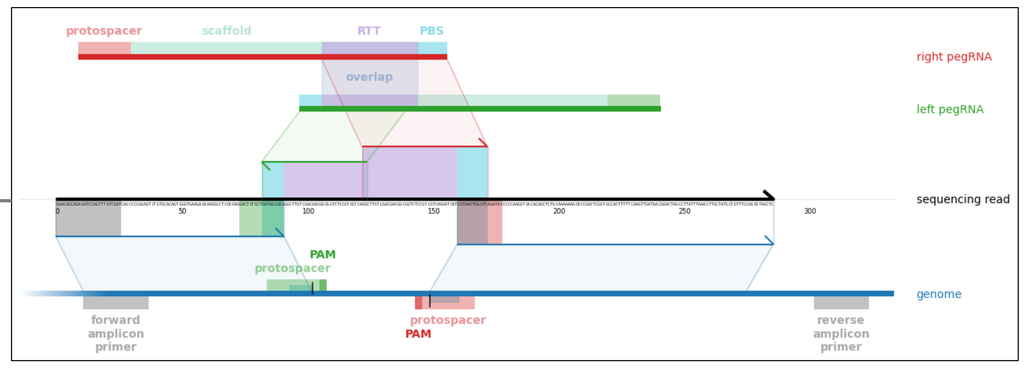
Example unintended rejoining of just left flap:

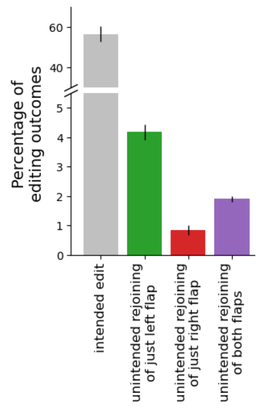


Example unintended rejoining of just right flap:



Example unintended rejoining of both flaps:





Quantification of different categories of unintended outcomes across experimental conditions allows for systematic, hypothesis-driven optimization of pegRNA designs that minimize formation of these outcome categories to maximize edit purity.

References

1. Anzalone, …, Liu, *Nature* (2019)
2. Canaj, Hussmann, …, Leonetti, *bioRxiv* (2019)
3. Chen, Hussmann, …, Liu, Adamson, *Cell* (2021)
4. Anzalone, Gao, Podracky, …, Liu, *Nature Biotechnology* (2021)