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(54) Title: METHODS AND COMPOSITIONS FOR SIMULTANEOUS EDITING OF BOTH STRANDS OF A TARGET DOUBLE-STRANDED NUCLEOTIDE SEQUENCE

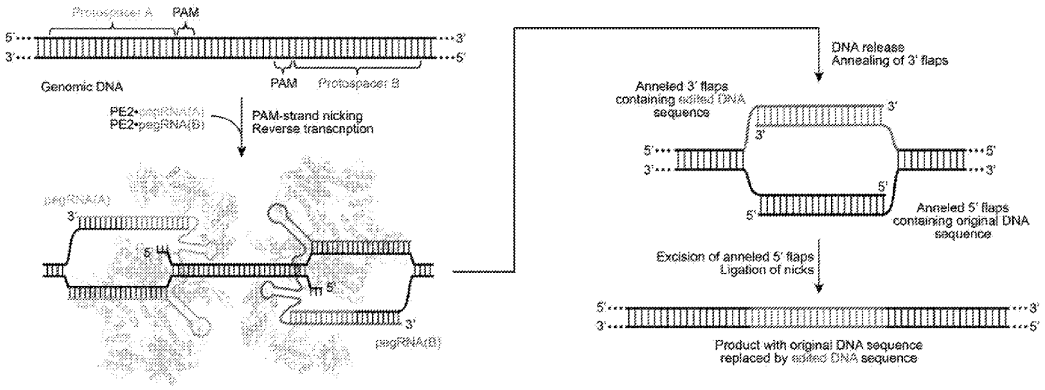

FIG. 90

(57) Abstract: The present disclosure provides systems, compositions, and methods for simultaneously editing both strands of a double-stranded DNA sequence at a target site to be edited. In some aspects, the systems comprise a first and second prime editor complex, wherein each of the first and second prime editor complexes comprises (1) a prime editor comprising (i) a nucleic acid programmable DNA binding protein (napDNAbp), and (ii) a polypeptide having an RNA-dependent DNA polymerase activity; and (2) a pegRNA comprising a spacer sequence, gRNA core, a DNA synthesis template, and a primer binding site, wherein the DNA synthesis template encodes a desired DNA sequence or a complement thereof, wherein the desired DNA sequence and the complement thereof form a duplex comprising an edited portion which integrates into the target site to be edited. In some aspects, the systems comprise a first, second, third, and fourth prime editor complex, each comprising a prime editor and a PEgRNA. Also provided herein are methods

[Continued on next page]

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for simultaneously editing both strands of a double- stranded DNA sequence at a target site to be edited. Further provided herein are pharmaceutical compositions, polynucleotides, vectors, cells, and kits.