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(54) **Title:** PRIME EDITING GUIDE RNAS, COMPOSITIONS THEREOF, AND METHODS OF USING THE SAME

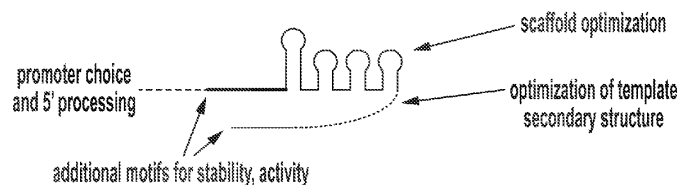


FIG. 55B

(57) **Abstract:** The disclosure provides modified pegRNAs comprising one or more appended nucleotide structural motifs which increase the editing efficiency during prime editing, increase half-life in vivo, and increase lifespan in a cell. Modifications include, but are not limited to, an aptamer (e.g., prequeosim-1 riboswitch aptamer or "evopreQi-1") or a variant thereof, a pseudoknot (the MMLV viral genome pseudoknot or "Mpknot-1") or a variant thereof, a tRNA (e.g., the modified tRNA used by MMLV as a primer for reverse transcription) or a variant thereof, or a G-quadruplex or a variant thereof. The disclosure further provides prime editor complexes comprising the modified pegRNAs and having improved characteristics and/or performance, including stability, improved cellular lifespan, and improved editing efficiency. The disclosure also provides methods of editing a genome using the prime editor complexes with modified pegRNAs, and to nucleotide sequences and expression vectors encoding said prime editors and modified pegRNAs, and to cells, kits, and pharmaceutical compositions comprising the improved prime editor complexes.

[Continued on next page]

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