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(54) Title: SINGLE-STRANDED END PRESERVING ADAPTORS

(57) Abstract: Provided herein are compositions, kits, systems, and methods employing single-stranded end-preserving adaptors. Such single-stranded adaptors are attached to DNA duplex molecules while preserving original 5' or 3' single-strand protruding ends (e.g., present in cell-free DNA) by attaching such adapters to 3' ends the DNA duplex molecules using a single strand ligase that has step 3 ligase activity, but not step 2 adenylyl transfer activity, and attaching such adapters to the 5' ends of the DNA duplex molecules using a ligase enzyme (e.g., a circligase), thereby forming loop-like structures on one or each end of the DNA duplex molecules. In further embodiments, the loop-like structures are cleaved (e.g., by an endonuclease) as the single-stranded adapters have a cleavable portion, thereby generating a two-part adapter on one or both ends of the DNA duplex molecules that preserves the initial 5' or 3' single-strand protruding ends, along with any methylation present.

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