(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 August 2025 (28.08.2025)

WIPO | PCT

(10) International Publication Number
WO 2025/179170 A3

(51) International Patent Classification:
C12P 19/34 (2006.01) *C12N 15/10* (2006.01)
C12Q 1/6855 (2018.01) *C12N 9/00* (2006.01)
C12Q 1/686 (2018.01) *C40B 40/06* (2006.01)
C12Q 1/6869 (2018.01)

(21) International Application Number:
PCT/US2025/016835

(22) International Filing Date:
21 February 2025 (21.02.2025)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/556,538 22 February 2024 (22.02.2024) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UY, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:
02 October 2025 (02.10.2025)

(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.

WO 2025/179170 A3