

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
29 June 2023 (29.06.2023)



(10) International Publication Number
WO 2023/122303 A3

(51) International Patent Classification:

C12N 15/11 (2006.01) C12N 15/64 (2006.01)
C07H 21/04 (2006.01)

(21) International Application Number:

PCT/US2022/053868

(22) International Filing Date:

22 December 2022 (22.12.2022)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/293,337 23 December 2021 (23.12.2021) 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, 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, 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).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

(54) Title: SCALABLE AND HIGH-PURITY CELL-FREE SYNTHESIS OF CLOSED-ENDED DNA VECTORS

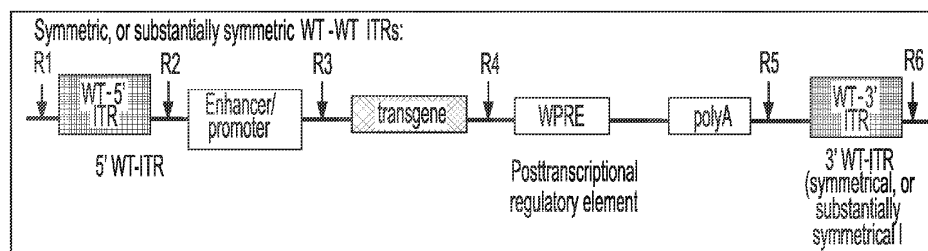


FIG. 1A

(57) Abstract: This disclosure provides methods for scalable and high-purity cell-free synthesis of DNA vectors, particularly closed-ended DNA vectors (e.g., ceDNA vectors) having linear and continuous structure for delivery and expression of a transgene. The cell-free synthesis includes digesting a double-stranded DNA construct with at least one restriction endonuclease that is capable of cleaving the construct at cleavage sites, which are distinct from the recognition sites, to release an insert having unique overhangs that regulate the high specificity of the subsequent ligation reaction. The insert is then ligated with inverted terminal repeat (ITR) oligonucleotides to form the closed-ended DNA vector. Corresponding DNA vectors prepared by these methods and related products as well other base and intermediate vectors and constructs associated with the methods are also provided in this disclosure.

[Continued on next page]

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:

16 November 2023 (16.11.2023)