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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 August 2019 (29.08.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/165050 A1

(51) International Patent Classification:
C12N 15/86 (2006.01) *C12N 15/87* (2006.01)
C12N 15/864 (2006.01)

(21) International Application Number:
PCT/US2019/018927

(22) International Filing Date:
21 February 2019 (21.02.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

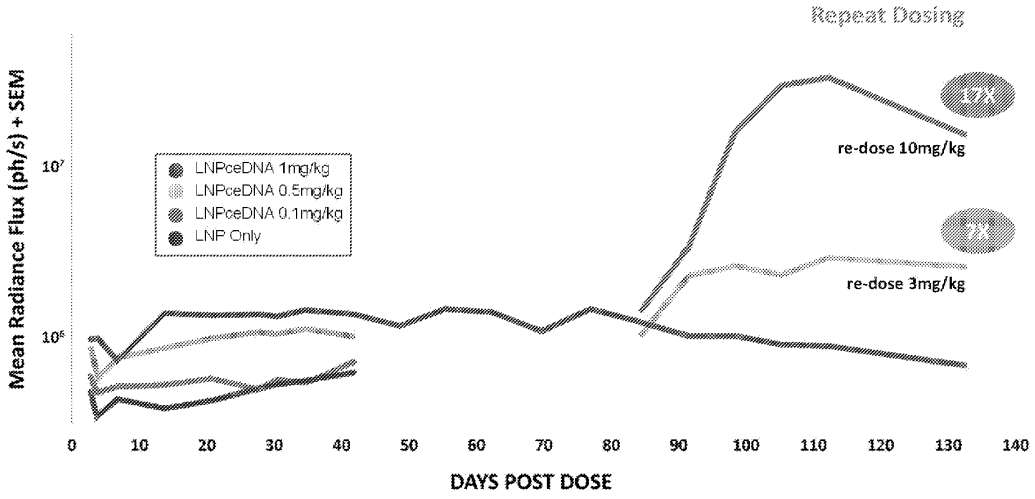
62/633,757	22 February 2018 (22.02.2018)	US
62/633,882	22 February 2018 (22.02.2018)	US
62/633,795	22 February 2018 (22.02.2018)	US
62/746,762	17 October 2018 (17.10.2018)	US

(71) Applicant: **GENERATION BIO CO.** [US/US]; 215 First Street, Suite 150, Cambridge, Massachusetts 02142 (US).

(72) Inventors: **KERR, Douglas A.**; c/o Generation Bio Co., 215 First Street, Suite 150, Cambridge, Massachusetts 02142 (US). **STANTON, Matthew G.**; c/o Generation Bio Co., 215 First Street, Suite 150, Cambridge, Massachusetts 02142 (US). **CHIOCCO, Matt**; c/o Generation Bio Co., 215 First Street, Suite 150, Cambridge, Massachusetts 02142 (US). **ANGELINO, Mark D.**; c/o Generation Bio Co., 215 First Street, Suite 150, Cambridge, Massachusetts 02142 (US). **KOTIN, Robert M.**; c/o Generation Bio Co., 215 First Street, Suite 150, Cambridge, Massachusetts 02142 (US). **SAMAYOA, Phillip**; c/o Generation Bio Co., 215 First Street, Suite 150, Cambridge, Massachusetts 02142 (US).

(54) Title: CONTROLLED EXPRESSION OF TRANSGENES USING CLOSE-ENDED DNA (CEDNA) VECTORS

FIG. 6



(57) **Abstract:** Provided herein are methods and constructs comprising close-ended DNA (ceDNA vectors) for maintaining or sustaining a level of transgene expression at a predetermined level or range for a predefined time, or increasing the level of transgene expression in a cell or a subject, where the transgene expression level can be modulated (e.g., increased) with one or more subsequent administrations (e.g., a re-dose or a booster administration) after an initial priming administration. Provided are methods for personalizing gene therapy throughout an individuals' lifespan to express a transgene at a level that meets an individual's needs, by modulating expression levels of a transgene expressed by ceDNA vector incrementally, or in a step-by-step manner, with one or more administrations after an initial priming administration (e.g., at time 0), thereby enabling titration of the level of expression of the transgene to a desired predetermined expression level or to a desired expression level range.

[Continued on next page]

WO 2019/165050 A1

(74) **Agent: BENN, Susanna C.** et al.; Nixon Peabody LLP, Exchange Place, 53 State Street, Boston, Massachusetts 02109 (US).

(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, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, 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, 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))*

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*