



- (51) **International Patent Classification:**
C12N 15/10 (2006.01) C12N 15/113 (2010.01)
- (21) **International Application Number:**
PCT/US2020/063084
- (22) **International Filing Date:**
03 December 2020 (03.12.2020)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
62/943,158 03 December 2019 (03.12.2019) US
63/031,262 28 May 2020 (28.05.2020) US
- (71) **Applicant: BEAM THERAPEUTICS INC.** [US/US]; 26
Landsdowne Street, 2nd Floor, Cambridge, MA 02139
(US).
- (72) **Inventors: CAFFERTY, Brian;** c/o Beam Therapeutics
Inc., 26 Landsdowne Street, 2nd Floor, Cambridge, MA
02139 (US). **LARSEN, Aaron;** c/o Beam Therapeutics
Inc., 26 Landsdowne Street, 2nd Floor, Cambridge, MA
02139 (US).
- (74) **Agent: MENDEZ, Julio, J. et al.;** Proskauer Rose LLP,
One International Place, Boston, MA 02110 (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, IT, 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, 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, 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,

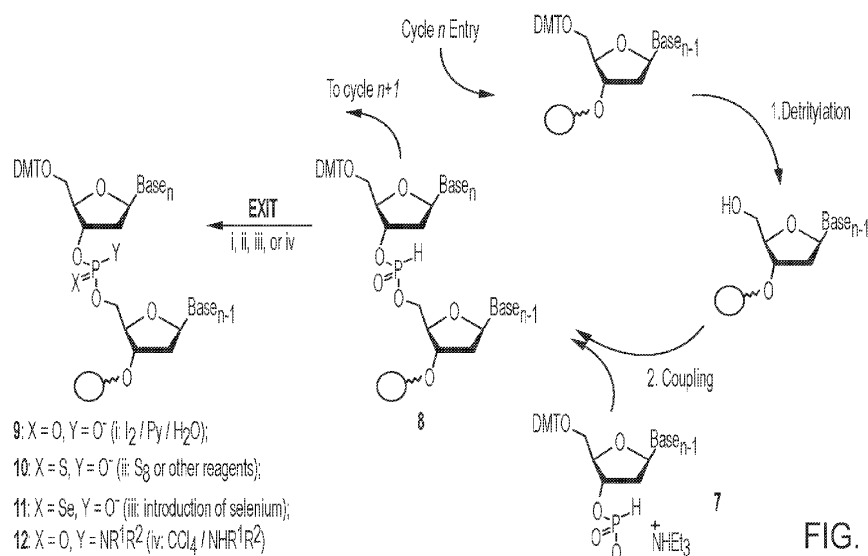
(54) **Title:** SYNTHETIC GUIDE RNA, COMPOSITIONS, METHODS, AND USES THEREOF

FIG. 1

(57) **Abstract:** The present invention provides, among other things, a method of producing a synthetic RNA using a self-templating approach. For example, in some embodiments a synthetic gRNA is produced comprising: contacting a first RNA with a second RNA, wherein the first RNA and the second RNA comprise at least five RNA nucleotides that are complementary, and wherein the contacting forms a stem structure or a stem loop structure, and ligating the first RNA and the second RNA with a ligating enzyme (i) within the stem structure, or (ii) at an end of the stem structure, thereby forming a loop at the end of the stem structure.

[Continued on next page]

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).

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))*