

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

(19) World Intellectual Property
Organization
International Bureau



(10) International Publication Number
WO 2022/086846 A3

(43) International Publication Date
28 April 2022 (28.04.2022)

(51) International Patent Classification:

C12N 15/11 (2006.01) C12N 9/22 (2006.01)

(88) Date of publication of the international search report:

21 July 2022 (21.07.2022)

(21) International Application Number:

PCT/US2021/055394

(22) International Filing Date:

18 October 2021 (18.10.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/093,459 19 October 2020 (19.10.2020) US
63/127,648 18 December 2020 (18.12.2020) US
63/229,870 05 August 2021 (05.08.2021) US

(71) Applicant: **CARIBOU BIOSCIENCES, INC.** [US/US];
2929 7th Street, Suite 105, Berkeley, CA 94710 (US).

(72) Inventor: **DONOHUE, Paul, Daniel**; 2929 7th Street,
Suite 105, Berkeley, CA 94710 (US).

(74) Agent: **KAY, Olga**; Counsel, I.P., Caribou Biosciences,
Inc., 2929 7th Street, Suite 105, Berkeley, CA 94710 (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, 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))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: DNA-CONTAINING POLYNUCLEOTIDES AND GUIDES FOR CRISPR TYPE V SYSTEMS, AND METHODS OF MAKING AND USING THE SAME

(57) Abstract: The present disclosure provides guides for use in Type V CRISPR systems wherein such guides contain ribonucleotide bases and at least one deoxyribonucleotide base. CRISPR Cas2 guides that contain at least one deoxyribonucleotide base, as well as nucleoprotein complexes of Type V CRISPR-Cas2 proteins and such guides, are also described. Also disclosed are methods for making and using the deoxyribonucleotide-containing polynucleotides and guides, and for making and using the nucleoprotein complexes. Also disclosed are methods for engineering cells using Cas2 chRNA guide/nucleoprotein complexes to produce CAR-expressing cells; and the use of such CAR-expressing cells in adaptive cell therapy.



WO 2022/086846 A3