



- (51) **International Patent Classification:**
C12N 15/10 (2006.01) *C12N 15/90* (2006.01)
C12N 15/85 (2006.01)
- (21) **International Application Number:**
PCT/US2021/048046
- (22) **International Filing Date:**
27 August 2021 (27.08.2021)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
63/071,764 28 August 2020 (28.08.2020) US
- (71) **Applicants:** **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US). **F. HOFFMANN-LA ROCHE AG** [CH/CH]; 124 Grenzacherstrasse, 4070 Basel (CH). **HOFFMANN-LA ROCHE INC.** [US/US]; 150 Clove Road Overlook at Great Notch, Suite 8, Little Falls, NJ 07424 (US).
- (72) **Inventors:** **SHEN, Amy**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (US). **YUK, Inn**,

Huam; c/o Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (US). **KO, Peggy Wai No**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (US). **MISAGHI, Shahram**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (US). **AUSLAENDER, Simon Thomas**; c/o Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg (DE). **GREENWOOD-GOODWIN, Midori**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (US). **LAIRD, Michael Wilson**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (US). **OSWALD, Benedikt Alois Claudius**; c/o Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg (DE).

(74) **Agent:** **LENDARIS, Steven P.**; Baker Botts LLP, 30 Rockefeller Plaza, New York, NY 10112-4498 (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,

(54) **Title:** CRISPR/CAS9 MULTIPLEX KNOCKOUT OF HOST CELL PROTEINS

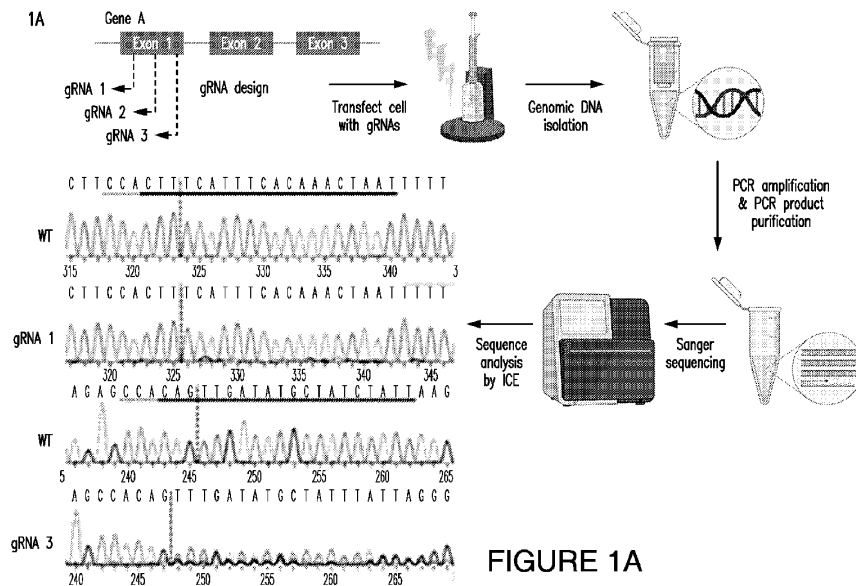


FIGURE 1A

(57) **Abstract:** The present disclosure relates to modified mammalian cells having reduced or eliminated expression of certain cellular proteins, CRISPR/Cas9 multiplex knockout strategies for making such cells, and methods of using such cells, e.g., in the context of cell-based therapy or as host cells in the production of a product of interest.

[Continued on next page]

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))*
- *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:

14 April 2022 (14.04.2022)