



(51) International Patent Classification:

C07K 16/00 (2006.01) C12N 9/90 (2006.01)
C07K 16/24 (2006.01) C12N 15/70 (2006.01)

(21) International Application Number:

PCT/US2019/059661

(22) International Filing Date:

04 November 2019 (04.11.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/755,915 05 November 2018 (05.11.2018) US

(71) Applicant (for all designated States except AL, AT, BE, BG, CH, CN, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IN, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR): **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, California 94080 (US).

(71) Applicant (for AL, AT, BE, BG, CH, CN, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IN, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR only): **F. HOFFMANN-LA ROCHE AG** [CH/CH]; Grenzacherstrasse 124, 4070 Basel (CH).

(72) Inventors: **VEERAVALLI, Karthik**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US). **MCKENNA, Rebekah**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US).

(74) Agent: **LEKUTIS, Christine**; Morrison & Foerster LLP, 425 Market Street, San Francisco, California 94105 (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,

(54) Title: METHODS OF PRODUCING TWO CHAIN PROTEINS IN PROKARYOTIC HOST CELLS

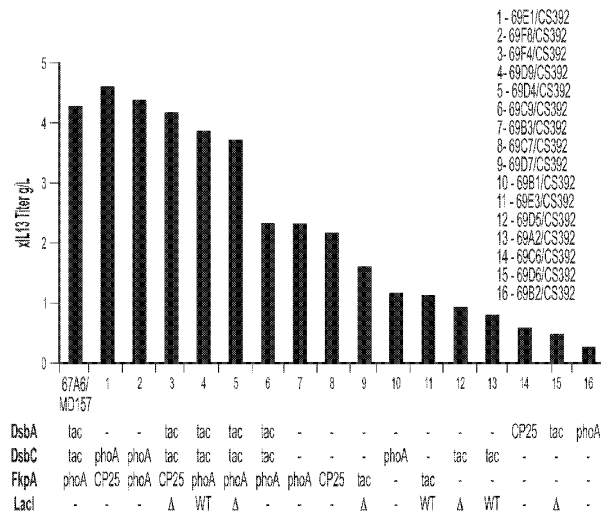


FIG. 5

(57) Abstract: Provided herein are methods and host cells for producing a polypeptide containing two chains, such as an antibody, half-antibody, antibody fragment, or one-armed antibody. The methods and host cells allow for two-chain polypeptide production using expression of polynucleotides encoding the polypeptide chains from extra-chromosomal polynucleotide(s), and expression of one or more chaperone protein(s) (e.g., peptidyl-prolyl isomerases and/or protein disulfide oxidoreductases) from the host cell chromosome using non-native combination(s) of promoters and translational units encoding a chaperone protein.

[Continued on next page]

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:

- *of inventorship (Rule 4.17(iv))*

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