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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 October 2013 (03.10.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/148249 A1

(51) International Patent Classification:
C12P 21/00 (2006.01)

(21) International Application Number:
PCT/US2013/031383

(22) International Filing Date:
14 March 2013 (14.03.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/616,297 27 March 2012 (27.03.2012) 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, CH-4070 Basel (CH).

(72) Inventors: **LAIRD, Michael W.**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US). **ST. JOHN, Richard**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US). **GUNSON, Jane V.**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US). **KALEAS, Kim**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US). **NADARAJAH, Deepa**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US). **ADAMS, Rachel**; 142 Stephen Drive, Guelph, Ontario N1H 7R2 (CA). **SNEDECOR, Bradley R.**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US).

(74) Agents: **WONG, Connie** et al.; Genentech, Inc., 1 DNA Way, Mail Stop 49, South San Francisco, California 94080 (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, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, 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,

[Continued on next page]

(54) Title: IMPROVED HARVEST OPERATIONS FOR RECOMBINANT PROTEINS

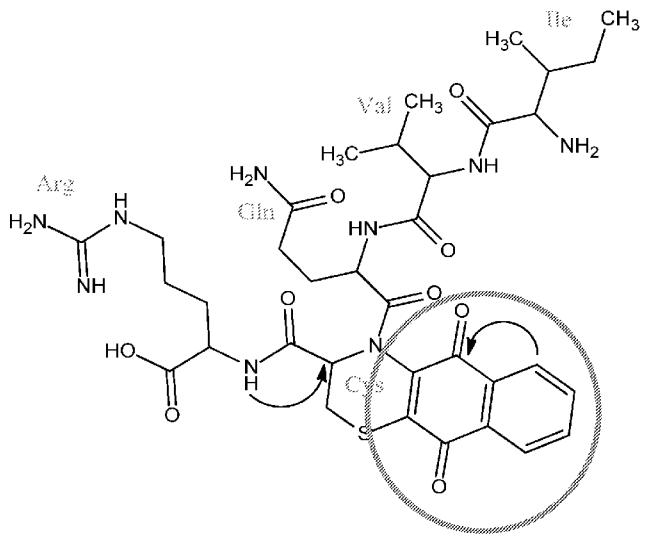


FIG. 9

(57) Abstract: The present invention contemplates methods of producing a recombinant protein comprising fermenting a prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, harvesting said recombinant protein under conditions where dO_2 levels are greater than 0%, purifying said recombinant protein to a filtered bulk, wherein said filtered bulk does not contain detectable DHNA-recombinant protein adduct, as measured by an IEC assay at 310 nm. Furthermore, method of producing a recombinant protein comprising fermenting a menE gene-deleted prokaryotic host cell wherein said prokaryotic host cell has been transformed with a nucleic acid encoding said recombinant protein, harvesting said recombinant protein, purifying said recombinant protein to a filtered bulk, wherein said filtered bulk does not contain detectable DHNA-recombinant protein adduct, as measured by an IEC assay at 310 nm, wherein the recombinant protein yield is increased by about 20% or greater is contemplated.

WO 2013/148249 A1

RW, 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, 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, ML, MR, NE, SN, TD, TG).

Published:

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