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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 June 2013 (27.06.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/092743 A2

(51) International Patent Classification:
C07K 16/00 (2006.01) *C12N 15/67* (2006.01)

(21) International Application Number:
PCT/EP2012/076203

(22) International Filing Date:
19 December 2012 (19.12.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
11195361.8 22 December 2011 (22.12.2011) EP

(71) Applicant *(for all designated States except DE, JP, US)*: **F. HOFFMANN-LA ROCHE AG** [CH/CH]; Grenzacher Strasse 124, CH-4070 Basel (CH).

(72) Inventors; and
(71) Applicants *(for US only)*: **HUELSMANN, Peter Michael** [DE/DE]; Im Sonnental 20, 82392 Habach (DE). **KNOETGEN, Hendrik** [DE/DE]; Agathe-Fleissner-Weg 16, 82377 Penzberg (DE).

(74) Agents: **SKOLAUT, Alexander** et al.; Roche Diagnostics GmbH, Patent Department (LPP.....6164), P.O. Box 11 52, 82372 Penzberg (DE).

(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, 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).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

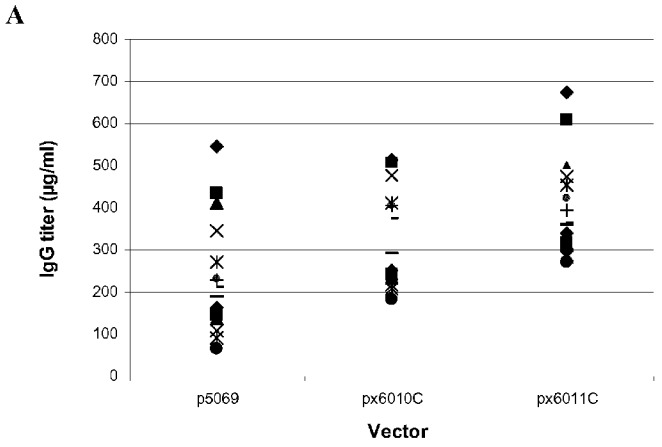
Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

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

(54) Title: EXPRESSION VECTOR ELEMENT COMBINATIONS, NOVEL PRODUCTION CELL GENERATION METHODS AND THEIR USE FOR THE RECOMBINANT PRODUCTION OF POLYPEPTIDES

Figure 10



(57) Abstract: Herein is reported that for transient transfections the use of the human elongation factor 1 alpha promoter (with Intron A) provides for an enhanced productivity (in LC-HC-SM organization), the use of the bovine growth hormone polyA signal sequence provides for an enhanced productivity compared to use of the SV40 polyA signal sequence, the addition of the HGT to the bGH PolyA signal sequence results in an increased productivity in vectors containing the hCMV promoter and the vector organization LC(3` -5 ')-HC-SM results in improved expression. For stable pools it is reported that pools generated with vectors containing the hEF1α promoter show an enhanced productivity in batch analysis, clones generated with vectors containing the hEF1α promoter show a reduced number of low producing clones, and clones generated with vectors containing the hEF1α promoter show a higher stability of IgG expression. For single clones it is reported that the vector organization with downstream position of selection marker (LC-HC-SM) has a positive effect on productivity of single clones and that clones generated with vectors containing the bGH polyA signal sequence and the hGT have higher productivities.

WO 2013/092743 A2