

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

(19) World Intellectual Property
Organization

International Bureau

(43) International Publication Date
14 February 2019 (14.02.2019)



(10) International Publication Number
WO 2019/030373 A1

(51) International Patent Classification:

C12N 15/90 (2006.01) C12N 9/22 (2006.01)

C07K 16/00 (2006.01)

(21) International Application Number:

PCT/EP2018/071733

(22) International Filing Date:

10 August 2018 (10.08.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

17185988.7 11 August 2017 (11.08.2017) EP

(71) Applicant: **BOEHRINGER INGELHEIM INTERNATIONAL GMBH** [DE/DE]; Binger Str. 173, 55216 Ingelheim am Rhein (DE).

(72) Inventors: **MUELLER, Markus**; c/o Boehringer Ingelheim International GmbH, Corporate Patents, Binger Strasse 173, 55216 Ingelheim am Rhein (DE). **SCHAUB, Jochen**; c/o Boehringer Ingelheim International GmbH, Corporate Patents, Binger Strasse 173, 55216 Ingelheim am Rhein (DE). **BERNLOEHR, Christian**; c/o Boehringer Ingelheim International GmbH, Corporate Patents, Binger Strasse 173, 55216 Ingelheim am Rhein (DE). **KOENITZER, Jennifer**; c/o Boehringer Ingelheim International GmbH, Corporate Patents, Binger Strasse 173, 55216 Ingelheim am Rhein (DE).

(74) Agent: **WALLINGER RICKER SCHLOTTER TOST-MANN**; Patent- und Rechtsanwälte, Partnerschaft mbB, Zweibrückenstraße 5-7, 80331 München (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, 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, 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).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))



WO 2019/030373 A1

(54) Title: Integration sites in CHO cells

(57) Abstract: The present invention relates to the identification of a genomic integration site for heterologous polynucleotides in Chinese Hamster Ovary (CHO) cells resulting in high RNA and/or protein production. More specifically it relates to CHO cells comprising at least one heterologous polynucleotide stably integrated into the S100A gene cluster of the CHO genome and to methods for the production of said CHO cells. Further, the invention relates to a method for the production of a protein of interest using said CHO cell and to the use of said CHO cell for producing a protein of interest at high yield. Integration within these specific target regions leads to reliable, stable and high yielding production of an RNA and/or protein of interest, encoded by the heterologous polynucleotide.