



(51) International Patent Classification:

A01K 67/027 (2006.01) C12N 15/85 (2006.01)
C12N 9/22 (2006.01) C12N 15/90 (2006.01)

(21) International Application Number:

PCT/US2015/066681

(22) International Filing Date:

18 December 2015 (18.12.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/094,104 19 December 2014 (19.12.2014) US
62/167,408 28 May 2015 (28.05.2015) US
62/205,524 14 August 2015 (14.08.2015) US

(71) Applicant: **REGENERON PHARMACEUTICALS, INC.** [US/US]; 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US).

(72) Inventors: **VORONINA, Vera**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **MACDONALD, Lynn**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **ZAMBROW-ICZ, Brian**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **MURPHY, Andrew J.**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US).

(74) Agents: **SCHILLING, Stephen H.** et al.; Alston & Bird LLP, Bank of America Plaza, Suite 4000, 101 South Tryon Street, Charlotte, North Carolina 28280-4000 (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, IR, IS, JP, KE, KG, KN, KP, KR, 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).

Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

Published:

— with international search report (Art. 21(3))
— with amended claims (Art. 19(1))
— with sequence listing part of description (Rule 5.2(a))

(54) Title: METHODS AND COMPOSITIONS FOR TARGETED GENETIC MODIFICATION THROUGH SINGLE-STEP MULTIPLE TARGETING

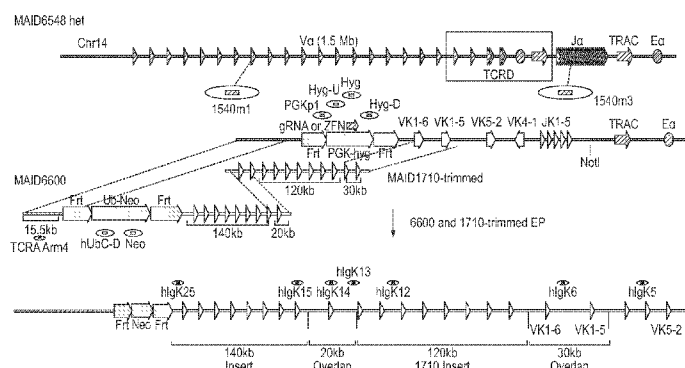


FIG. 1

(57) Abstract: Methods and compositions are provided for making one or more targeted genetic modifications at a target genomic locus within a cell and for producing non-human animals comprising the modified genomic locus. The methods employ two or more large targeting vectors which are capable of recombining with each other and with the target genomic locus in a single genomic targeting step. The methods may also be employed in combination with a nuclease agent. Methods and compositions are also provided for enhancing homologous recombination at a target genomic locus in a cell. The methods employ two or more nucleic acids comprising one or more overlapping sequences. The methods may be employed in combination with a nuclease agent or without a nuclease agent.