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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 June 2014 (12.06.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/089290 A1

(51) International Patent Classification:
A61K 45/00 (2006.01)

(21) International Application Number:
PCT/US2013/073307

(22) International Filing Date:
5 December 2013 (05.12.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/734,256	6 December 2012 (06.12.2012)	US
61/758,624	30 January 2013 (30.01.2013)	US
61/761,046	5 February 2013 (05.02.2013)	US
61/794,422	15 March 2013 (15.03.2013)	US

(71) Applicant: **SIGMA-ALDRICH CO. LLC** [US/US]; 3050 Spruce Street, St. Louis, Missouri 63103 (US).

(72) Inventors; and

(71) Applicants : **CHEN, Fuqiang** [US/US]; 3050 Spruce Street, St. Louis, Missouri 63103 (US). **DAVIS, Gregory D.** [US/US]; 3050 Spruce Street, St. Louis, Missouri 63103 (US). **KANG, Qiaohua** [US/US]; 3050 Spurce Street, St. Louis, Missouri 63103 (US). **KNIGHT, Scott W.** [US/US]; 3050 Spruce Street, St. Louis, Missouri 63103 (US).

(74) Agents: **BISSEN, Shirley T.** et al.; Polsinelli PC, 100 South Fourth Street, Suite 1000, St. Louis, Missouri 63102-1825 (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, 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, 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, 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,

[Continued on next page]

(54) Title: CRISPR-BASED GENOME MODIFICATION AND REGULATION

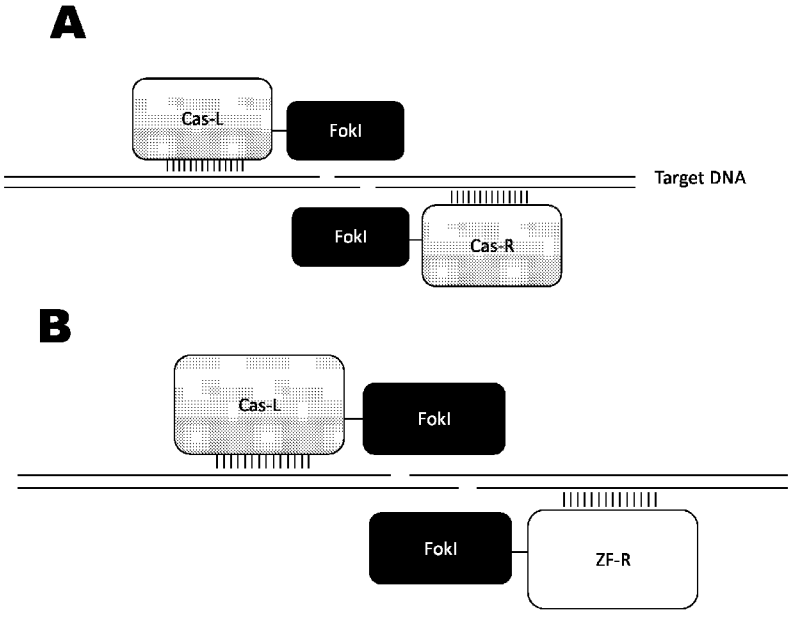


FIG. 1

(57) Abstract: The present invention provides RNA-guided endonucleases, which are engineered for expression in eukaryotic cells or embryos, and methods of using the RNA-guided endonuclease for targeted genome modification in in eukaryotic cells or embryos. Also provided are fusion proteins, wherein each fusion protein comprises a CRISPR/Cas-like protein or fragment thereof and an effector domain. The effector domain can be a cleavage domain, an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. Also provided are methods for using the fusion proteins to modify a chromosomal sequence or regulate expression of a chromosomal sequence.

WO 2014/089290 A1

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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

Published:

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