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MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
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(54) Title: CAS9-DNA TARGETING UNIT CHIMERAS

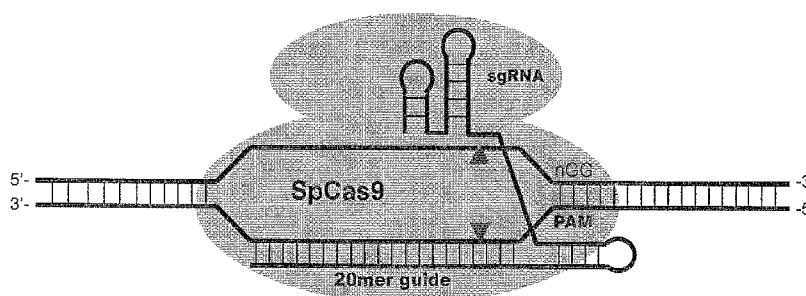


Figure 1

(57) Abstract: The present invention provides a Cas9 platform to facilitate single-site nuclease gene editing precision within a human genome. For example, a Cas9 nuclease/DNA-targeting unit (Cas9-DTU) fusion protein precisely delivers a Cas9/sgRNA complex to a specific target site within the genome for subsequent sgRNA-dependent cleavage of an adjacent target sequence. Alternatively, attenuating Cas9 binding using mutations to the protospacer adjacent motif (PAM) recognition domain makes Cas9 target site recognition dependent on the associated DTU, all while retaining Cas9's sgRNA-mediated DNA cleavage fidelity. Cas9-DTU fusion proteins have improved target site binding precision, greater nuclease activity, and a broader sequence targeting range than standard Cas9 systems. Existing Cas9 or sgRNA variants (e.g., truncated sgRNAs (tru-gRNAs), nickases and FokI fusions) are compatible with these improvements to further reduce off-target cleavage. A robust, broadly applicable strategy is disclosed to impart Cas9 genome-editing systems with the single-genomic-site accuracy needed for safe, effective clinical application.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/67424

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/00, 15/62, 15/63, 9/14 (2016.01)

CPC - C07K 2319/00; C12N 15/87, 15/63, 9/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C12N 15/00, 15/62, 15/63, 9/14 (2016.01)

CPC: C07K 2319/00; C12N 15/87, 15/63, 9/16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

CPC: C07K 2319/00; C12N 15/87, 15/63, 9/16 (text search)

USPC: 435/455, 375, 320.1, 195, 69.7; 536/23.2, 23.4 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Electronic data bases: PatBase; Google Patents; Google Scholar Search terms: Cas9 nuclease, CRISPR, protospacer adjacent motif (PAM) recognition site domain (Arg1333 and Arg1335), off-target cleavage, reduction, fusion protein, Cas9 effector domain (e.g. FokI nuclease, transcriptional activator or repressor), Cas9 fusion to DNA binding prote

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ANDERS et al. Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. NATURE ePub 27 July 2014 Vol 513 No 7519 Pages 569-573. Especially abstract, pg 570 col 2 para 2, pg 570 fig 2E, Extended version fig 3.	1-206
A	US 2014/0295557 A1 (General Hospital Corporation) 2 October 2014 (02.10.2014). Especially para [0020], [0099]	1-206
A	US 2014/0295556 A1 (General Hospital Corporation) 2 October 2014 (02.10.2014). Especially [0006]	1-206
X,P	BOLUKBASI et al. DNA-binding-domain fusions enhance the targeting range and precision of Cas9. Nature Methods ePub 19 October 2015 Vol 12 No 12 Pages 1150-1156. Especially abstract, pg 1150 col 2 para 3, pg 1151 col 2 para 2.	1-206
Y,P	KLEINSTIVER et al. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. NATURE ePub 22 June 2015 Vol 523 No 7561 Pages 481-485. Especially abstract.	1-206



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* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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