ENGINEERING OF SYSTEMS, METHODS AND OPTIMIZED GUIDE COMPOSITIONS WITH NEW ARCHITECTURES FOR SEQUENCE MANIPULATION

RELATED APPLICATIONS AND INCORPORATION BY REFERENCE

[0001] This application is a continuation of U.S. patent application Ser. No. 15/172,636, filed on Jun. 3, 2016, which is a continuation-in-part application of International Patent Application No. PCT/US2014/70152, filed on Dec. 12, 2014, which published as PCT Publication No. WO2015/089473 on Jun. 18, 2015. This application claims priority from U.S. Provisional Patent Applications Nos. 61/939,256, filed Feb. 12, 2014, and 61/915,267, filed on Dec. 12, 2013.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under Grant No. MH100706 awarded by the National Institutes of Health. The government has certain rights in the invention.

[0003] The foregoing applications, and all documents cited therein or during their prosecution ("appln cited documents") and all documents cited or referenced in the appln cited documents, and all documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

SEQUENCE LISTING

[0004] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy created on Aug. 10, 2016, is named 47627.99.2066_SL.txt and is 51,285 bytes in size.

FIELD OF THE INVENTION

[0005] The present invention generally relates to systems, methods and compositions used for the control of gene expression involving sequence targeting, such as genome perturbation or gene-editing, that may use vector systems related to Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and components thereof. In particular the present invention comprehends engineered new guide architectures to be used in optimized CRISPR-Cas enzyme systems.

BACKGROUND OF THE INVENTION

[0006] Recent advances in genome sequencing techniques and analysis methods have significantly accelerated the ability to catalog and map genetic factors associated with a diverse range of biological functions and diseases. Precise genome targeting technologies are needed to enable systematic reverse engineering of causal genetic variations by allowing selective perturbation of individual genetic elements, as well as to advance synthetic biology, biotechnological, and medical applications. Although genome-editing techniques such as designer zinc fingers, transcription activator-like effectors (TALEs), or homing meganucleases are available for producing targeted genome perturbations, there remains a need for new genome engineering technologies that are affordable, easy to set up, scalable, and amenable to targeting multiple positions within the eukaryotic genome.

SUMMARY OF THE INVENTION

[0007] There exists a pressing need for alternative and robust systems and techniques for sequence targeting with a wide array of applications. This invention addresses this need and provides related advantages. The CRISPR/Cas or the CRISPR-Cas system (both terms are used interchangeably throughout this application) does not require the generation of customized proteins to target specific sequences but rather a single Cas enzyme can be programmed by a short RNA molecule to recognize a specific DNA target, in other words the Cas enzyme can be recruited to a specific DNA target using said short RNA molecule. Adding the CRISPR-Cas system to the repertoire of genome sequencing techniques and analysis methods may significantly simplify the methodology and accelerate the ability to catalog and map genetic factors associated with a diverse range of biological functions and diseases. To utilize the CRISPR-Cas system effectively for genome editing without deleterious effects, it is critical to understand aspects of engineering and optimization of these genome engineering tools, which are aspects of the claimed invention.

[0008] In a first aspect, the invention provides a non-naturally occurring or engineered CRISPR-Cas system guide RNA or chimeric single guide RNA molecule (sgRNA) capable of effecting the manipulation of a target nucleic acid within a prokaryotic or eukaryotic cell when in complex within the cell with a CRISPR enzyme comprising a CRISPR enzyme, or a non-naturally occurring or engineered composition comprising a CRISPR-Cas system comprising said guide RNA or optionally sgRNA, the guide RNA or optionally sgRNA comprising a guide sequence capable of hybridizing to a target sequence in a genomic locus of interest in a cell wherein architecture of the guide RNA or optionally sgRNA is modified

[0009] In an aspect, the invention provides a non-naturally occurring or engineered composition comprising a CRISPR-Cas system comprising a guide RNA (e.g. sgRNA) comprising a guide sequence capable of hybridizing to a target sequence in a genomic locus of interest in a cell wherein architecture of the guide RNA or optionally sgRNA is modified.

[0010] In some embodiments, the modification of architecture is replacing a poly U tract (22-25 with respect to the position numbering of +85 sgRNA or guide RNA architecture) with poly C.

[0011] In some embodiments, the modification of architecture is replacing a poly A tract (46-49 with respect to the position numbering of +85 sgRNA or guide RNA architecture) with poly G.

[0012] In some embodiments, the modification of architecture is replacing the repeat:anti-repeat duplex A28 and