Improvement and Optimization of Lentiviral Vectors for CRISPR/Cas9 Delivery

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Technology description

Invention Description:
The subject invention is a delivery platform for use in gene editing that includes a relatively short, highly efficient promoter that drives transcription of a nucleic acid sequence that encodes a gene-editing molecule, e.g., either a gRNA or a nuclease. In conjunction with this promoter, the vector includes one or more transcription factor binding elements. More specifically, the transcription factor binding element(s) can be cloned into the vector upstream of a promoter that drives transcription of a gene-editing molecule. The vector can be an all-in-one CRISPR/Cas9 delivery platform and can incorporate one or more of the transcription factor binding elements upstream of a promoter for the gRNA component and/or of a promoter for the nuclease component.
Background
Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (e.g., CRISPR/Cas9) systems have revolutionized the field of genome editing by providing unprecedented control over gene expression in many species, including humans.
Lentiviral vectors are one of the mainstays of current delivery platforms for gene editing systems. While lentiviral vector systems exhibit many advantages such as low immunogenicity, the ability to accommodate large DNA payloads, and efficient transduction of a wide range of dividing and non-dividing cells, low production yields of CRISPR/Cas9 limit its use in many experimental settings including gene screening, disease-modeling and gene correction.
The subject invention introduces transcription factor binding elements to increase upregulation in the vector production efficiency and gene expression. The overall functional titer determined by screening and counting drug-selection-resistant colonies was shown to be upregulated by at least sevenfold. Furthermore, in proof-of-concept, target genes were robustly knocked outin vitroandin vivousing integrating and non-integrating vectors derived from the new system.

Application area

Viral vectors for use in gene editing including to adeno-associated vectors, retroviruses and adenoviruses

Advantages

CRISPR/Cas9 delivered from the novel integrase-deficient lentiviral vector platform showed significant advantages over existing integrase-competent lentiviruses. The main difference is that it delivers its transgene transiently, so in dividing cells the genomes of non-integrating lentiviral vectors disappear. However, since the CRISPR/Cas9 targets DNA, the effect of its activity is permanent. Altogether, this feature is crucial for enhancing the safety of lentiviral vector-delivery. It has been shown that off-target effects of CRISPR/Cas9 delivered by integrase-competent lentiviral vectors can significantly compromise the specificity and safety of the system. Another problem with integrase-proficient lentiviral vectors is that they possess a significantly higher risk of insertional mutagenesis, since they support the integration process.
Introduction of a binding element upstream of a promoter for a gene-editing sequence can dramatically improve transcription efficiency. For instance, the disclosed vectors can exhibit high efficiency in mediating rapid gene knockouts in cells, including both dividing cells as well as non-dividing cells such as brain neurons.

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