RNA-GUIDED FUSION PROTEINS FOR TARGETED DIVERSIFICATION OF CYTOPLASMIC DNA

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PATENT STATUS

Country	Type	Number	Dated	Case
Patent Cooperation Treaty	Published Application	WO 2024/011173.	01/11/2024	2022-106

Additional Patent Pending

BRIEF DESCRIPTION

The inventors have developed a method of mutagenizing user-defined regions of cytoplasmic DNA using a single guide RNA (sgRNA) or combinations of sgRNAs and a highly engineered fusion polypeptide comprising:

a nuclear export sequence (NES)-containing, engineered nuclear localization sequence (NLS)-lacking, enzymatically active, RNA-guided endonuclease that introduces a single-stranded break in cytoplasmic DNA, and
an error-prone DNA polymerase.

This novel technology encompasses and provides evidence for the use of RNA-guided nucleases with relaxed PAM requirements, which are particularly useful for AT-rich targets such as the vaccinia virus genome. The inventors show that the truncation of up to several base pairs from the PAM-distal template binding region of the sgRNAs significantly increases the functional activity and specificity of the targeted mutagenesis complex. Moreover, the invention describes specific methods for the use of this technology to edit cytoplasmically replicating viruses with large DNA genomes, using poxviruses as a model system. The novel editing platform and methods selectively and continuously accelerate diversification of user-defined sites in the vaccinia genome during infection, while retaining most library members, due to significantly lowering deleterious off-target mutations.

BACKGROUND

Nucleocytoplasmic large DNA viruses (NCLDVs) are a group of viruses that harbor large (150 kbp - 1.2 mbp) double-stranded DNA genomes and replicate in the cytoplasm of eukaryotic cells. An example of an NCLDV that has historically been among the most prominent tools in human health is vaccinia, a poxvirus. Hundreds of millions of humans have been intentionally inoculated with vaccinia as part of a global effort to eliminate smallpox, which was declared eradicated in 1980.

Vaccinia and some other poxviruses remain highly scientifically relevant in the post-eradication world. They are useful as vaccines against deadly poxvirus outbreaks that could potentially arise from natural spillover, bioterrorism, or biowarfare, as well as due to their therapeutic promise as oncolytic agents to selectively deliver anti-cancer transgenes and recruit adaptive immunity while leaving healthy cells unharmed. Directed evolution is a powerful engineering technique for evolving new phenotypes that are beneficial for biotechnological applications but for which there may have never been a selective pressure to evolve in nature.

Both natural and directed evolution depend upon generation of genetic diversity, followed by a selective pressure. While natural evolution generates genetic diversity randomly and throughout the entirety of the genome, directed evolution ideally focuses mutations within specific genomic windows connected to the phenotype that one wishes to engineer. However, there is a need in the art for compositions and methods for mutagenizing a target DNA in the cytoplasm of mammalian cells. NCLDVs, which either partially or entirely express their own replicative and translational machinery independent of the nucleus, are difficult, and in many cases impossible, to produce from plasmid DNA in cells. Thus, NCLDVs are not amenable to standard in vitro molecular diversification strategies.

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OTHER INFORMATION

KEYWORDS

vaccine, vaccination

CATEGORIZED AS

» **Agriculture & Animal Science**
» Animal Science
» **Biotechnology**
» Health
» **Engineering**
» Engineering
» **Medical**
» Vaccines
» **Research Tools**
» Other
» **Security and Defense**
» Other
» **Veterinary**
» Vaccines

RELATED CASES

2022-106-0

SUGGESTED USES

This technology could be used to perform directed evolution screens on large DNA viruses that replicate in the cytoplasm such that they can be used as gene delivery vectors with improved qualities for cancer, gene therapy, and vaccination in humans. Additionally, other related viruses could be used for similar purposes relevant to agriculturally relevant animal health.

ADVANTAGES

RELATED MATERIALS

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