

COMPOSITIONS COMPRISING A VARIANT POLYPEPTIDE AND USES THEREOF

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application 63/158,741 filed on March 09, 2021, U.S. Provisional Application 63/294,224 filed on December 28, 2021, the entire contents of each of which are hereby incorporated by reference.

SEQUENCE LISTING

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 March 7, 2022 is named A2186-7046WO_SL.txt and is 38,750 bytes in size.

BACKGROUND

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) genes, collectively known as CRISPR-Cas or CRISPR/Cas systems, are adaptive immune systems in archaea and bacteria that defend particular species against foreign genetic elements.

SUMMARY OF THE INVENTION

It is against the above background that the present invention provides certain advantages and advancements over the prior art.

Although this invention disclosed herein is not limited to specific advantages or functionalities, the invention provides a variant polypeptide comprising an alteration relative to a parent polypeptide of SEQ ID NO: 3, and wherein the alteration is a substitution of Table 2. In some embodiments, the substitution is a P14R substitution, an E311R substitution, a D32R substitution, an I61R substitution, a G223R substitution, an N109R substitution, and/or a D719R substitution.

In another aspect, the disclosure provides a variant polypeptide comprising an amino acid sequence having at least 95% identity to SEQ ID NO: 3 and comprising: i) a substitution at one or more of positions P14, E311, D32, I61, G223, N109, and D719 relative to SEQ ID NO: 3; ii) one or more of the following substitutions: P14R, E311R, D32R, I61R, G223R, N109R, and D719R; iii) a substitution at one or more of positions K208, D302, D590, E154, D567, L38, D145, C13, T338, P14, D55, K221, K35, and E736 relative to SEQ ID NO: 3; or iv) one or more of the following substitutions: K208G, D302G, D590G, E154G, D567G, L38G, D145G, C13G, T338G, P14G, D55G, K221G, K35G, and E736G.

In another aspect, the disclosure provides a composition comprising the variant polypeptide as described herein, wherein the composition further comprises an RNA guide or a nucleic acid encoding the RNA guide, wherein the RNA guide comprises a direct repeat sequence and a spacer sequence.

5 In another aspect, the disclosure provides a composition comprising a nucleic acid that encodes the variant polypeptide and/or the RNA guide as described herein.

In another aspect, the disclosure provides a cell comprising the variant polypeptide or the composition as described herein.

In another aspect, the disclosure provides a method for editing a gene in a cell, the method comprising contacting the cell with the variant polypeptide or composition as described herein.

10 In certain embodiments, the variant polypeptide comprises a) a P14R substitution, an E311R substitution, and a D32R substitution; b) a P14R substitution, an E311R substitution, and a G223R substitution; c) a P14R substitution, an E311R substitution, a D32R substitution, and an I61R substitution; or d) a D32R substitution, an N109R substitution, an E311R substitution, and a D719R substitution. In some embodiments, the variant polypeptide comprises c) a P14R substitution, an E311R substitution, a
15 D32R substitution, and an I61R substitution.

In some embodiments, the variant polypeptide further comprises a K208G substitution, a D302G substitution, a D590G substitution, an E154G substitution, a D567G substitution, an L38G substitution, a