

In another aspect of the RNA guide or the nucleic acid encoding the RNA guide, the spacer sequence comprises about 15 to about 35 nucleotides in length.

In another aspect of the RNA guide or the nucleic acid encoding the RNA guide, the spacer sequence recognizes a target nucleic acid.

In another aspect of the RNA guide or the nucleic acid encoding the RNA guide, the target nucleic acid comprises a target sequence adjacent to a protospacer adjacent motif (PAM) sequence, wherein the PAM sequence comprises a nucleotide sequence set forth as 5'-TTN-3', 5'-NTTN-3', 5'-NTN'-3', 5'-NNTN-3', 5'-VTN-3', or 5'-NVTN-3', wherein N is any nucleotide (e.g., A, G, T, or C) and V is A, G, or C.

The invention yet further provides a composition comprising an RNA guide or a nucleic acid encoding the RNA guide as described herein.

In one aspect of the composition, the composition is a delivery composition comprising a nanoparticle (e.g., a lipid nanoparticle), a liposome, an exosome, a microvesicle, or a gene-gun.

In another aspect of the RNA guide or the nucleic acid encoding the RNA guide described herein, the nucleic acid encoding the RNA guide is operably linked to a promoter.

In another aspect of the RNA guide or the nucleic acid encoding the RNA guide, the nucleic acid encoding the RNA guide is in a vector.

In another aspect of the RNA guide or the nucleic acid encoding the RNA guide, the vector comprises a retroviral vector, a lentiviral vector, a phage vector, an adenoviral vector, an adeno-associated vector, or a herpes simplex vector.

[The invention yet further provides a cell comprising variant Cas12i4 polypeptide, the composition, or the RNA guide or the nucleic acid encoding the RNA guide as described herein.](#)

The invention yet further provides a cell comprising the RNA guide or the nucleic acid encoding the RNA guide described herein.

In one aspect of the cell, the cell is a eukaryotic cell.

In another aspect of the cell, the cell is a mammalian cell or a plant cell.

In another aspect of the cell, the cell is a human cell.

The invention yet further provides a method for editing a gene in a cell, the method comprising contacting the cell with a variant, a composition, an RNA guide, or a nucleic acid molecule as described herein.

The invention yet further provides a nucleic acid molecule encoding a Cas12i4 variant of SEQ ID NO: 4, wherein the sequence of the nucleic acid molecule is 95% identical to the selected from the group consisting of SEQ ID NOs: 222-228.

In one embodiment, the sequence of the nucleic acid molecule comprises a sequence selected from the group consisting of SEQ ID NOs: 222-228