

According to one example, the present disclosure provides a method of providing a linear single stranded nucleic acid to a cell, comprising the use of a delivery vector as herein described.

According to another aspect, the present disclosure provides an *ex vivo* method of providing a linear single stranded nucleic acid to a cell, comprising the use of a nucleic acid delivery vector as herein
5 described.

According to another example, the present disclosure provides a method of providing a linear single stranded donor oligonucleotide or template to a cell for genome editing, comprising the use of a delivery vector as described herein.

According to another aspect, the present disclosure provides an *ex vivo* method of providing a linear
10 single stranded donor template to a cell for genome editing, comprising the use of a nucleic acid delivery vector as described herein, preferably wherein said linear single stranded nucleic acid is a donor template.

According to another aspect, the present disclosure provides an *ex vivo* method as described herein wherein said nuclease is a guided nuclease, optionally Cas9 or a variant thereof.

15 With any of the methods described herein, the delivery vector of the disclosure is introduced into a cell. The delivery vector can be transfected by any suitable means, including chemical, physical or viral.

Detailed Description of the Invention

The construct of the invention is a closed circular polynucleotide comprised of a single strand. The sequence of said construct is designed such that it can be apportioned into at least three sections, although the boundaries of these sections may be adaptable, varying depending on the cleavage site of
20 the nuclease. The construct is preferably a delivery vector, intended for the delivery of a linear single stranded nucleic acid. The first section and third sections include some complementarity, such that under appropriate or physiological conditions, these sections are capable of base-pairing and forming a duplex or stem structure. This duplex is a result of self-complementary sequences within the
25 polynucleotide. The second section intervenes between the first and third sections, and includes the sequence for the linear single stranded nucleic acid for delivery. This second section, since it is positioned between the two complementary sequences, will generally form a "loop" of nucleic acid, which starts and ends at the first and third sections which are duplexed. It will generally be represented as a single strand, since no regions of complementarity with other sequences within the
30 polynucleotide will intentionally be included in order for the delivery vector to work as intended.

Claims

1. A nucleic acid delivery vector comprising a circular single stranded polynucleotide said vector comprising:
 - (a) a duplex formed from a first section and a third section of said polynucleotide, said sections including sequences which are complementary;
 - (b) a loop formed from a second section, said section separating the first and third sections;
 wherein said duplex includes a recognition sequence for a targeted nuclease.
2. The nucleic acid delivery vector of claim 1 wherein said vector delivers a linear single stranded nucleic acid, wherein said single stranded nucleic acid is present within the second section.
3. The nucleic acid delivery vector of claim 2 wherein the linear single stranded nucleic acid may be any one or more of: a nucleic acid enzyme, an aptamer, a donor template, an mRNA, a functional RNA, or an antisense nucleic acid.
4. The nucleic acid delivery vector of claim 2 or 3 wherein the linear single stranded nucleic acid has a free 5' and 3' end once released from the delivery vector.
5. The nucleic acid delivery vector of claim 1 wherein said vector is a closed circular polynucleotide, optionally a closed DNA or closed RNA.
6. The nucleic acid delivery vector of any previous claim wherein said nuclease is a guided nuclease, optionally a nuclease associated with gene editing, preferably Cas9.
7. The nucleic acid delivery vector of any one of claims 1 to 5 wherein the nuclease binds to the recognition sequence without a guide.
8. An *ex vivo* method of providing a linear single stranded nucleic acid to a cell, comprising the use of a nucleic acid delivery vector as claimed in any one of claims 1 to 7.
9. An *ex vivo* method of providing a linear single stranded donor template to a cell for genome editing, comprising the use of a nucleic acid delivery vector as described in any one of claims 1 to 7, preferably wherein said linear single stranded nucleic acid is a donor template.
10. An *ex vivo* method as claimed in claim 9 wherein said nuclease is a guided nuclease, optionally Cas9 or a variant thereof.