operably connected to a second polynucleotide encoding the single guide RNA. This construct allows for the tRNA to direct RNA Polymerase III dependent production of a fusion RNA including the tRNA linked to the single guide RNA with a tRNase Z cleavage site. Action of the endogenous cellular tRNase Z enzyme cleaves the tRNA from the single guide RNA and allows for efficient production of the single guide RNA using a very small promoter element. Kits including these constructs are also included.

In another aspect, there is provided a construct comprising a first promoter operably connected to a first polynucleotide encoding a first single guide RNA and a second promoter operably connected to a second polynucleotide encoding a Cas9 polypeptide, the first single guide RNA comprising a first portion complementary to a strand of a target sequence of a DNA virus and a second portion capable of interacting with the Cas9 polypeptide.

In another aspect, there is provided a recombinant vector comprising the construct of any one of the preceding claims.

In another aspect, there is provided a pharmaceutical composition comprising the recombinant vector of as disclosed herein and a pharmaceutical carrier.

In another aspect, there is provided the use of the recombinant vector of as disclosed herein or the construct of as disclosed herein in an amount effective to allow delivery of the recombinant vector to the cell and production of the single guide RNA and the Cas9 polypeptide in the manufacture of a medicament for inhibiting viral replication or target sequence expression in a cell infected with a DNA virus, wherein the medicament is to be contacted with the cell.

In another aspect, there is provided the use of an effective amount of the recombinant vector of as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a viral infection in a subject, wherein the medicament is to be administered to the subject to treat the viral infection.

In another aspect, there is provided an *in vitro* method of inhibiting viral replication or target sequence expression in a cell infected with a DNA virus comprising contacting the cell with the recombinant vector as disclosed herein or the construct as disclosed herein in an amount effective to allow delivery of the recombinant vector to the cell and production of the single guide RNA and the Cas9 polypeptide.

In another aspect, there is provided a recombinant Sau Cas9 polypeptide of SEQ ID NO: 57.

In another aspect, there is provided a recombinant Sau Cas9 construct comprising a promoter operably connected to a polynucleotide encoding a recombinant Sau Cas9 polypeptide, the polynucleotide comprising SEQ ID NO: 55.

In another aspect, there is provided a recombinant construct for expression of a single guide RNA comprising a first polynucleotide encoding a mammalian or viral tRNA operably connected to a second polynucleotide encoding at least a second portion of a single guide RNA capable of interacting with a Cas9 polypeptide.

In another aspect, there is provided a kit comprising the recombinant Sau Cas9 construct as disclosed herein.

In another aspect, there is provided a kit comprising the recombinant construct as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a set of figures showing an Spy Cas9 HPV-18-specific sgRNA screen in which two sgRNAs were designed to target DNA sequences encoding the amino terminus of the HPV-18 E6 or E7 proteins, and screened to identify the most effective candidate. Fig. 1A is a schematic depicting the fusion protein-based reporter assay, which includes an amino-terminal HIV-1 Rev fragment that acts as an epitope tag, an in-frame HPV-18 derived target sequence, and a carboxy-terminal eGFP open reading frame (ORF). In other assays a firefly luciferase (FLuc) ORF was used in place of GFP. Fig. 1B is a graph showing eGFP expression data from 293T cells co-transfected with plasmids expressing the FLAG-tagged Spy Cas9 protein and sgRNAs specific for the HPV-18 E6 gene, or a control construct, and their cognate indicator plasmids. Transfected cells were processed for flow cytometry at 72 hours. The number of GFP-positive cells and the mean fluorescence intensity (MFI) of these cells is indicated. Average of three independent experiments with SD indicated. Fig. 1C is similar to panel B but using two HPV-18 E7-specific sgRNAs. Fig. 1D depicts a Western blot using an HIV-1 Rev specific antiserum to detect expression of the Rev-GFP indicator fusion protein, thus demonstrating sgRNA efficacy and specificity.

Figure 2 is a photograph of SURVEYOR assay showing HPV-18 E6 and E7-specific Spy Cas9 sgRNAs induce mutagenesis at the predicted cleavage site in the HPV-18 genome. E6 and E7 sgRNA and Spy Cas9 expression constructs were transfected and the SURVEYOR assay performed. The predicted size of the SURVEYOR cleavage product is indicated by an arrow. DNA markers (left lane) are indicated by base pairs.

Figure 3 is a set of photographs showing that HPV-18 E6- and E7-specific RNA guided DNA endonucleases (RGNs) induce tumor suppressor gene expression in HeLa cells. HeLa cells were transfected with vectors expressing Spy Cas9 and HPV-18-specific sgRNAs, as indicated,

29. The construct of any one of the preceding claims, further comprising a first inverted terminal repeat and a second inverted terminal repeat, wherein the inverted terminal repeats flank the construct for packaging in an adeno-associated virus (AAV) vector.
30. The construct of any one of the preceding claims, wherein the construct is an expression vector.
31. The construct of any one of the preceding claims, further comprising a third promoter operably connected to a third polynucleotide encoding a second single guide RNA comprising a first portion complementary to a strand of a second target sequence of a DNA virus, wherein the third promoter is an RNA polymerase III-dependent promoter.
32. The construct of claim 31, wherein the first target sequence and the second target sequence are located within 30 base pairs of each other on opposite strands of the DNA virus.

33. A recombinant vector comprising the construct of any one of the preceding claims.
34. The recombinant vector of claim 33, wherein the vector is a viral vector.
35. The recombinant vector of claim 34, wherein the viral vector is selected from the group consisting of a retrovirus, a lentivirus, an adenovirus or an adeno-associated virus.
36. The recombinant vector of claim 35, wherein the vector is an adeno-associated virus (AAV).
37. A pharmaceutical composition comprising the recombinant vector of any one of claims 33-36 and a pharmaceutical carrier.
38. ~~A method of inhibiting viral replication or target sequence expression in a cell infected with a DNA virus comprising contacting the cell with~~Use of the recombinant vector of any one of claims 33-36 or the construct of any one of claims 1-32 in an amount effective to allow delivery of the recombinant vector to the cell and production of the single guide RNA and the Cas9 polypeptide in the manufacture of a medicament for inhibiting viral replication or target sequence expression in a cell infected with a DNA virus, wherein the medicament is to be contacted with the cell.
39. The ~~method~~ use of claim 38, wherein the single guide RNA and the Cas9 polypeptide cleave the target sequence.
40. The ~~method~~ use of claim 38 or 39, wherein two or more single guide RNAs are present in the recombinant vector or construct and more than one target sequence is cleaved.

41. The ~~method~~ use of any one of claims 38-40, wherein the expression of a gene product encoded by the target sequence is decreased after contact with the recombinant vector or construct and production of the single guide RNA and the Cas9 polypeptide.
42. The ~~method~~ use of any one of claims 38-41, wherein the target sequence is altered after contact with the recombinant vector or construct.
43. The ~~method~~ use of claim 42, wherein the single guide RNA and the Cas9 polypeptide cause a deletion or insertion in the target sequence.
44. The ~~method~~ use of any one of claims 38-43, wherein the DNA virus is unable to replicate in the cell after contact with the recombinant vector or construct.
45. The ~~method~~ use of any one of claims 38-44, wherein the DNA virus is eliminated from the cell after contact with the recombinant vector or construct.

~~46. The method of any one of claims 38-45, wherein the contacting occurs *in vitro*.~~

~~47.~~46. The ~~method~~ use of any one of claims 38-4~~5~~6, wherein the contacting occurs *in vivo* by administering the recombinant vector to a subject in an amount effective to contact the cell.

47. An *in vitro* method of inhibiting viral replication or target sequence expression in a cell infected with a DNA virus comprising contacting the cell with the recombinant vector of any one of claims 33-36 or the construct of any one of claims 1-32 in an amount effective to allow delivery of the recombinant vector to the cell and production of the single guide RNA and the Cas9 polypeptide.
48. ~~A method of treating a viral infection in a subject comprising administering~~Use of an effective amount of the recombinant vector of any one of claims 33-36 or the pharmaceutical composition of claim 37 in the manufacture of a medicament for treating a viral infection in a subject, wherein the medicament is to be administered to the subject to treat the viral infection.
49. The ~~method~~ use of claim 48, wherein administration reduces the genome load or number of viral episomes of the DNA virus in the subject.
50. The ~~method~~ use of claim 48, wherein administration reduces replication of the DNA virus in the subject.
51. The ~~method~~ use of claim 48, wherein administration reduces expression of a gene product encoded by the target sequence in the subject.
52. A recombinant Sau Cas9 polypeptide of SEQ ID NO: 57.
53. A recombinant Sau Cas9 construct comprising a promoter operably connected to a polynucleotide encoding a recombinant Sau Cas9 polypeptide, the polynucleotide comprising SEQ ID NO: 55.
54. The recombinant Sau Cas9 construct of claim 53, further comprising a poly (A) addition site and a nuclear localization signal.
55. The recombinant Sau Cas9 construct of claim 53 or 54, further comprising an intron.