CLAIMS

What is claimed is:

1. A protein complex comprising:

(a) a first fusion protein comprising a DNA-binding domain and at least one catalytically inactive *FokI* cleavage domain or cleavage half-domain, wherein the cleavage domain or cleavage half-domain comprises a mutation at one or more of amino acid residues 450, 467 and 469; and

(b) a second fusion protein comprising a zinc finger domain and a *FokI* catalytically active cleavage half domain,

wherein the catalytically inactive *FokI* cleavage half domain forms a heterodimer with the catalytically active *FokI* cleavage half-domain of the second fusion protein and further wherein the protein complex induces a single-stranded break in a target double-stranded sequence.

2. A composition comprising a protein complex according to claim 1 or one or more polynucleotides encoding the first and second fusion proteins of the protein complex of claim 1.

3. An isolated cell or cell line comprising a protein complex according to claim 1.

4. An *in vitro* method of generating a single-stranded break in a target double-stranded sequence in a cell, the method comprising:

providing a protein complex according to claim 1, wherein the heterodimer generates a single-stranded break in the target double-stranded sequence.

5. The method of claim 4, wherein the target sequence is in cellular chromatin.

6. An *in vitro* method of inserting an exogenous sequence into a region of interest in a cell, the method comprising

generating a single-stranded break in the region of interest according to the method of claim 4; and

providing an exogenous sequence that is inserted into the region of interest following generation of a single-stranded break.

7. The method of claim 6, wherein the exogenous sequence replaces a wild-type genomic sequence.

8. An *in vitro* method of inactivating a target sequence in a region of interest of a cell, the method comprising

generating a single-stranded break in the region of interest according to the method of claim 4, and

providing an exogenous sequence that replaces the wild-type sequence in the region of interest and wherein the exogenous sequence partially or fully inactivates the target sequence.

What is claimed is:

1. A protein complex comprising:

(a) a first fusion protein comprising a DNA-binding domain and at least one catalytically inactive *FokI* cleavage domain or cleavage half-domain, wherein the cleavage domain or cleavage half-domain comprises a mutation at one or more of amino acid residues 450, 467 and 469; and

(b) a second fusion protein comprising a zinc finger domain and a *FokI* catalytically active cleavage half domain,

wherein the catalytically inactive *FokI* cleavage half domain forms a heterodimer with the catalytically active *FokI* cleavage half-domain of the second fusion protein and further wherein the protein complex induces a single-stranded break in a target double-stranded sequence.

2. A composition comprising a protein complex according to claim 1 or one or more polynucleotides encoding the first and/or second fusion proteins of the protein complex of claim 1.

3. An isolated cell or cell line comprising a protein complex according to claim 1.

4. An *in vitro* method of generating a single-stranded break in a target double-stranded sequence in a cell, the method comprising:

providing a protein complex according to claim 1, wherein the heterodimer generates a single-stranded break in the target double-stranded sequence.

5. The method of claim 3, wherein the target sequence is in cellular chromatin.

6. An *in vitro* method of inserting an exogenous sequence into a region of interest in a cell, the method comprising

generating a single-stranded break in the region of interest according to the method of claim 4; and

providing an exogenous sequence that is inserted into the region of interest following generation of a single-stranded break.

7. The method of claim 6, wherein the exogenous sequence replaces a wild-type genomic sequence.

8. An *in vitro* method of inactivating a target sequence in a region of interest of a cell, the method comprising

generating a single-stranded break in the region of interest according to the method of claim 4, and

providing an exogenous sequence that replaces the wild-type sequence in the region of interest and wherein the exogenous sequence partially or fully inactivates the target sequence.