

[0013] In additional embodiments, targeted cleavage of cellular chromatin in a region of interest is achieved by expressing two fusion proteins in a cell, each fusion protein comprising a zinc finger binding domain and a cleavage half-domain. One or both of the zinc finger binding domains of the fusion proteins can be engineered to bind to a target sequence in the vicinity of the intended cleavage site. If expression of the fusion proteins is by polynucleotide delivery, each of the two fusion proteins can be encoded by a separate polynucleotide, or a single polynucleotide can encode both fusion proteins.

[0014] Accordingly, a method for cleaving cellular chromatin in a region of interest can comprise (a) selecting a first sequence in the region of interest; (b) engineering a first zinc finger binding domain to bind to the first sequence; (c) expressing a first fusion protein in the cell, the first fusion protein comprising the first zinc finger binding domain and a first cleavage half-domain; and (d) expressing a second fusion protein in the cell, the second fusion protein comprising a second zinc finger binding domain and a second cleavage half-domain, wherein the first fusion protein binds to the first sequence, and the second fusion protein binds to a second sequence located between 2 and 50 nucleotides from the first sequence, thereby positioning the cleavage half-domains such that the cellular chromatin is cleaved in the region of interest.

[0015] In certain embodiments, binding of the first and second fusion proteins positions the cleavage half-domains such that a functional cleavage domain is reconstituted.

[0016] In certain embodiments, the second zinc finger binding domain is engineered to bind to the second sequence. In further embodiments, the first and second cleavage half-domains are derived from the same endonuclease, which can be, for example, a restriction endonuclease (e.g., a Type IIS restriction endonuclease such as Fok I) or a homing endonuclease.

[0017] In other embodiments, any of the methods described herein may comprise (a) selecting first and second sequences in a region of interest, wherein the first and second sequences are between 2 and 50 nucleotides apart; (b) engineering a first zinc finger binding domain to bind to the first sequence; (c) engineering a second zinc finger binding domain to bind to the second sequence; (d) expressing a first fusion protein in the cell, the first fusion protein comprising the first engineered zinc finger binding domain and a first cleavage half-domain; (e) expressing a second fusion protein in the cell, the second fusion protein comprising the second engineered zinc finger binding domain and a second cleavage half-domain; wherein the first fusion protein binds to the first sequence and the second fusion protein binds to the second sequence, thereby positioning the first and second cleavage half-domains such that the cellular chromatin is cleaved in the region of interest.

[0018] In certain embodiments, the first and second cleavage half-domains are derived from the same endonuclease, for example, a Type IIS restriction endonuclease, for example, Fok I. In additional embodiments, cellular chromatin is cleaved at one or more sites between the first and second sequences to which the fusion proteins bind.

[0019] In further embodiments, a method for cleavage of cellular chromatin in a region of interest comprises (a) selecting the region of interest; (b) engineering a first zinc finger binding domain to bind to a first sequence in the region of interest; (c) providing a second zinc finger binding domain

which binds to a second sequence in the region of interest, wherein the second sequence is located between 2 and 50 nucleotides from the first sequence; (d) expressing a first fusion protein in the cell, the first fusion protein comprising the first zinc finger binding domain and a first cleavage half-domain; and (e) expressing a second fusion protein in the cell, the second fusion protein comprising the second zinc finger binding domain and a second cleavage half domain; wherein the first fusion protein binds to the first sequence, and the second fusion protein binds to the second sequence, thereby positioning the cleavage half-domains such that the cellular chromatin is cleaved in the region of interest.

[0020] In any of the methods described herein, the first and second cleavage half-domains may be derived from the same endonuclease or from different endonucleases. In additional embodiments, the second zinc finger binding domain is engineered to bind to the second sequence.

[0021] If one or more polynucleotides encoding the fusion proteins are introduced into the cell, an exemplary method for targeted cleavage of cellular chromatin in a region of interest comprises (a) selecting the region of interest; (b) engineering a first zinc finger binding domain to bind to a first sequence in the region of interest; (c) providing a second zinc finger binding domain which binds to a second sequence in the region of interest, wherein the second sequence is located between 2 and 50 nucleotides from the first sequence; and (d) contacting a cell with (i) a first polynucleotide encoding a first fusion protein, the fusion protein comprising the first zinc finger binding domain and a first cleavage half-domain, and (ii) a second polynucleotide encoding a second fusion protein, the fusion protein comprising the second zinc finger binding domain and a second cleavage half domain; wherein the first and second fusion proteins are expressed, the first fusion protein binds to the first sequence and the second fusion protein binds to the second sequence, thereby positioning the cleavage half-domains such that the cellular chromatin is cleaved in the region of interest. In a variation of this method, a cell is contacted with a single polynucleotide which encodes both fusion proteins.

[0022] For any of the aforementioned methods, the cellular chromatin can be in a chromosome, episome or organellar genome. In addition, in any of the methods described herein, at least one zinc finger binding domain is engineered, for example by design or selection methods.

[0023] Similarly, for any of the aforementioned methods, the cleavage half domain can be derived from, for example, a homing endonuclease or a restriction endonuclease, for example, a Type IIS restriction endonuclease. An exemplary Type IIS restriction endonuclease is Fok I.

[0024] For any of the methods of targeted cleavage, targeted mutagenesis and/or targeted recombination disclosed herein utilizing fusion proteins comprising a cleavage half-domain, the near edges of the binding sites of the fusion proteins can be separated by 5 or 6 base pairs. In these embodiments, the binding domain and the cleavage domain of the fusion proteins can be separated by a linker of 4 amino acid residues.

[0025] In certain embodiments, it is possible to obtain increased cleavage specificity by utilizing fusion proteins in which one or both cleavage half-domains contains an alteration in the amino acid sequence of the dimerization interface.

[0026] Targeted mutagenesis of a region of interest in cellular chromatin can occur when a targeted cleavage event, as