Therefore, it was an overarching objective to engineer and identify one or more Cas12a nickase variants through a rational design approach and via a directed evolution approach, said nickases allowing for the *in vitro* and particularly also *in vivo* generation of nicks (or pairs of nicks) in chromosomal DNA of a broad range of prokaryotic and also eukaryotic organisms, wherein the Cas12a nickase should have highly specific nickase activity and low off-target activity as well as high flexibility to be used in various genome modification settings, including base editing, prime editing and paired-nickase assays and an overall robustness and stability to provide a broadly applicable genome nicking tool.

In an aspect, there is provided an engineered Cas12a enzyme having nickase activity (nCas12a), or a catalytically active fragment thereof, the engineered Cas12a enzyme comprising at least one mutation in its core lid domain, wherein the mutation in the core lid domain is selected from:

(i) at least three point mutations of three consecutive positions within the core lid domain; or

(ii) a deletion of at least two consecutive positions within the core lid domain; or

(iii) a combination of at least one first point mutation at at least one position within the core lid domain and

(iiia) at least one deletion of at least one position within the core lid domain, and/or

(iiib) at least one, preferably at least two, at least three, or at least four further point mutation(s) at a different position in comparison to the first point mutation within the core lid domain, wherein the position(s) of the further point mutation(s) is/are not in consecutive order with the position(s) of the at least one first point mutation;

(iv) one point mutation at a position within the core lid domain;

wherein the at least one mutation in the core lid domain confers broad spectrum nickase activity, wherein the core lid domain reference sequence comprises a sequence as defined in SEQ ID NO: 13, optionally a complex additionally comprising at least one compatible guide RNA, or a sequence encoding the same, forming a complex with the cognate engineered Cas12a enzyme having nickase activity, or the catalytically active fragment thereof.

In another aspect, there is provided a nucleic acid molecule encoding the Cas12a enzyme or the catalytically active fragment thereof as defined above, optionally, wherein the nucleic acid molecule is codon-optimized, preferably codon-optimized for a fungal cell, including a yeast cell, a prokaryotic cell or an archaeal cell, and/or comprises a nucleic acid molecule encoding at least one guide RNA.

In another aspect, there is provided an expression construct or vector comprising at least one nucleic acid molecule as defined above.

In another aspect, there is provided a cell comprising at least one engineered Cas12a enzyme or a catalytically active fragment thereof as defined above; and/or at least one nucleic acid molecule as defined above; and/or at least one expression construct or vector as defined above.

In another aspect, there is provided a complex, or at least one nucleic acid molecule encoding the components of the complex, the complex comprising at least one engineered Cas12a enzyme having nickase activity or a catalytically active fragment as defined above, and at least one compatible guide RNA, optionally comprising at least one further polypeptide, covalently and/or non-covalently attached to the at least one engineered Cas12a enzyme having nickase activity or the catalytically active fragment thereof within the complex, wherein the at least one further polypeptide is selected from an organellar localization sequence, including a nuclear localization signal (NLS), a mitochondrion localization signal, or a chloroplast localization signal, and/or wherein the at least one further polypeptide is a cell-penetrating polypeptide, preferably, in case the at least one further polypeptide is covalently attached to the at least one engineered Cas12a enzyme having nickase activity or the catalytically active fragment thereof, wherein the at least one further polypeptide is covalently attached to the N-terminus and/or the C-terminus of the at least one engineered Cas12a enzyme having nickase activity.

In another aspect, there is provided a fusion protein or at least one nucleic acid molecule encoding the same, comprising at least one engineered Cas12a enzyme having nickase activity or the catalytically active fragment thereof as defined above, covalently and/or non-covalently attached to at least one further polypeptide domain, the at least one further polypeptide domain having an activity selected from an enzymatic activity, binding activity or targeting activity, and optionally comprising at least one guide RNA compatible with the engineered Cas12a enzyme having nickase activity, wherein the at least one compatible guide RNA covalently and/or non-covalently interacts with the at least one engineered Cas12a enzyme having nickase activity or the catalytically active fragment thereof.

In another aspect, there is provided an adenine or a cytidine base editor, or a base editor complex, or at least one nucleic acid molecule encoding the same, the base editor or base editor complex comprising at least one catalytically active portion of at least one engineered Cas12a enzyme having nickase activity as defined above.

In another aspect, there is provided a prime editor or a prime editor complex, or at least one nucleic acid molecule encoding the same, the prime editor or prime editor complex comprising at least one catalytically active portion of at least one engineered Cas12a enzyme having nickase activity as defined above.

In another aspect, there is provided a kit comprising

(i) an engineered Cas12a enzyme having nickase activity (nCas12a), or a catalytically active fragment thereof as defined above, or an expression construct or vector as defined above, or a complex as defined above, or at least one sequence encoding the same, or a of fusion protein as defined above, or at least one sequence encoding the same, or an adenine or a cytidine base editor, or a base editor complex, or at least one nucleic acid molecule encoding the same as defined above, or prime editor or a prime editor complex, or at least one nucleic acid molecule encoding the same as defined above;

(ii) at least one compatible guide RNA, or a set of compatible guide RNAs, each guide RNA being complementary to target sequences of interest; and

(iii) a set of reagents;

(iv) optionally comprising particles, vesicles, or at least one vector, including a viral vector, for assisting delivery, wherein said particles comprise a lipid, including lipid nanoparticles, a sugar, a metal or a polypeptide, or a combination thereof, or wherein said vesicles comprise exosomes or liposomes.

In another aspect, there is provided a method for modifying the genomic locus of interest of at least one cell or construct at or near at least one target site, the method comprising:

(a) providing at least one cell or construct comprising the genomic locus to be modified;

(b) providing and/or introducing

(i) at least one engineered Cas12a enzyme having nickase activity (nCas12a), or a catalytically active fragment thereof, or at least one nucleic acid molecule encoding the same, as defined above; or

(ii) at least one expression construct or vector as defined above; or

(iii) at least one complex or at least one nucleic acid molecule encoding the same as defined above; or

at least one fusion protein or at least one nucleic acid molecule encoding the same as defined above; or

(iv) at least one adenine or a cytidine base editor, or at least one base editor complex, or at least one nucleic acid molecule encoding the same as defined above; or

(v) at least one prime editor or at least one prime editor complex, or at least one nucleic acid molecule encoding the same as defined above;

to/into the at least one cell or construct;

(c) providing and/or introducing at least one compatible guide RNA or a sequence encoding the same, as defined above;

(d) allowing complex formation of the at least one engineered Cas12a enzyme having nickase activity, or the catalytically active fragment thereof of (a) and the at least compatible guide RNA as defined above and thus allowing the insertion of at least one nick at the genomic locus of interest of the at least one cell or construct at or near at least one target site;

(e) optionally: providing at least one donor repair template, or at least one the nucleic acid molecule encoding the same; and

(f) obtaining at least one edited cell or construct comprising a modification of a genomic locus of interest at or near a target site;

wherein the method excludes processes for modifying the germ line genetic identity of human beings, uses of human embryos for industrial or commercial purposes and

processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and also animals resulting from such processes,

optionally, where the method comprises the following step:

(g) regenerating at least one population of edited cells, tissues, organs, materials or whole organisms from the at least one edited cell or construct.

In another aspect, there is provided an edited cell, tissue, organ, material or whole organism obtained by or obtainable by a method as defined above.

In another aspect, there is provided a use of a compound selected from (i) to (vi):

(i) at least one engineered Cas12a enzyme having nickase activity (nCas12a), or a catalytically active fragment thereof, or at least one nucleic acid molecule encoding the same, as defined above;

(ii) at least one expression construct or vector as defined above; or

(iii) at least one complex or at least one nucleic acid molecule encoding the same as defined above, or a fusion protein or at least one nucleic acid molecule encoding the same as defined above; or

(iv) at least one adenine or a cytidine base editor, or at least one base editor complex, or at least one nucleic acid molecule encoding the same as defined above; or

(v) at least one prime editor or at least one prime editor complex, or at least one nucleic acid molecule encoding the same as defined above; or

(vi) a kit as defined above;

for introducing a nucleotide deletion or insertion or modification in a nucleic acid molecule, preferably in a genome, including uses for metabolic engineering in a cell, including a prokaryotic or eukaryotic cell, preferably in a fungal cell, including a yeast cell, or a prokaryotic cell, including a Gram-positive, Gram-negative or Gram-variable bacterial cell, preferably a Gram-negative bacterial cell, or an archaeal cell.

Definitions

Broad spectrum nickase activity as used herein refers to the capability to efficiently generate specific single-strand DNA breaks (nicks), both *in vitro* and *in vivo*, and with minimal to no residual nuclease activity, preferably wherein residual nuclease activity *in vitro* and/or *in vivo,* preferably *in vitro* and *in vivo,* is less than approximately 20%, more preferably less than approximately 15 %, even more preferably less than approximately 10 %, and most preferably less than approximately 5 % of total enzyme activity, wherein the total enzyme activity is the sum of nickase activity and nuclease activity of a given Cas12a enzyme having nickase activity or catalytically active fragment thereof, wherein the nickase activity and nuclease activity of a given Cas12a enzyme having nickase activity or catalytically active fragment thereof are determined and compared with the same detection system and/or method in a suitable cellular and/or *in vitro* system using suitable and reasonable reaction conditions and further using the same target site(s) under the same conditions within reasonable limits of said cellular and/or *in vitro* system. The skilled person is well aware of various different suitable methods to determine nickase and nuclease activity of a Cas12a enzyme, including methods disclosed herein. The term “nuclease activity” as used herein refers to endonucleolytic activity wherein one nuclease effector is able to generate a double-strand break, whereas for a nickase – to achieve a double-strand break – two individual nicks (by the same, or by at least two different nickases) are needed. Target strand (TS) nickase activity as used herein refers to nickase activity as described above, wherein at least 90 % of the nicking occurs in the target strand. Non-target strand (NTS) nickase activity as used herein refers to nickase activity as described above, wherein at least 90 % of the nicking occurs in the non-target strand.

A target site as used herein refers to both strands of a double-stranded DNA, i.e. a target strand – to which a guide RNA anneals – and a complementary non-target strand, wherein the target site is the stretch of DNA for with a guide RNA has suitable complementarity to the target strand, wherein in embodiments, in which at least two compatible guide RNAs