Claims

1. A prime editor complex comprising

(a) at least one prime editing guide RNA (pegRNA) system as defined in any one of claims 6 to 10; and

(b) at least one Cas12a prime editor comprising

(i) at least one Cas12a enzyme, preferably having nickase activity (nCas12a), more preferably having non-target strand (NTS) nickase activity, or an active fragment thereof;

(ii) at least one reverse transcriptase, or an active fragment thereof;

(iii) optionally: at least one linker covalently or non-covalently linking the at least one Cas12a enzyme, or active fragment thereof, and the at least one reverse transcriptase, or an active fragment thereof; and

(iv) optionally: at least one organellar localization signal.

2. The prime editor complex of any of the previous claims, further comprising *in trans* (c) a reverse transcriptase protein, preferably comprising at least one organellar localization signal and preferably comprising an MS2 coat protein (MCP).

3. The prime editor complex of any of the previous claims, wherein the at least one Cas12a enzyme, or active fragment thereof further comprises an RNA binding factor, preferably an N-terminal domain of La or a MCP.

4. The prime editor complex of claim 3, comprising the N-terminal domain of La, optionally comprising the reverse transcriptase protein as defined in claim 2 comprising an MCP, and the pegRNA system as defined in claim 8, and, optionally the nicking crRNA as defined in claim 10, wherein

a) the crRNA comprises in a 5’ to 3’ direction (i) a CRISPR-Cas class 2 type V scaffold sequence, (ii) a spacer sequence; and optionally (vi) a second scaffold sequence, optionally being the same as the first scaffold sequence; and/or wherein

b) the prime editing template RNA (petRNA) comprises in a 5' to 3' direction an MS2 stem loop, optionally a linker sequence, a reverse transcriptase template having a length of 9 to 150 nucleotides; a primer binding site having a length of 5 to 50 nucleotides; optionally a linker sequence; and an MS2 stem loop.

5. The prime editor complex of any of the previous claims, wherein the Cas12a prime editor comprises or consist of an amino acid sequence selected from SEQ ID NO: 65 to 69, 213 or 268 to 277, or a sequence having at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99% sequence identity thereto.

6. A prime editing guide RNA (pegRNA) system comprising the parts

(i) a CRISPR-Cas class 2 type V scaffold sequence;

(ii) a spacer sequence;

(iii) optionally a linker sequence;

(iv) a reverse transcriptase template having a length of 9 to 150 nucleotides;

(v) a primer binding site having a length of 5 to 50 nucleotides; and

(vi) optionally a second scaffold sequence, optionally being the same as the first scaffold sequence;

wherein the primer binding site is complementary to the non-target strand.

7. The pegRNA system of claim 6, comprising

a) a pegRNA comprising parts (i), (ii), optionally (iii), (iv), (v), and optionally (vi); or

b) a crispr RNA (crRNA) and a prime editing template RNA (petRNA), wherein

1) said crRNA comprises parts (i). (ii) and optionally (vi); and

2) said petRNA comprises optionally (iii), (iv) and (v).

8. The pegRNA system of claim 7, comprising a crRNA and a petRNA, wherein the petRNA is linear.

9. The pegRNA system of any one of claims 6 to 8, wherein the pegRNA or the petRNA further comprises

(vii) optionally at least one 3' linker sequence; and

(viii) at least one structured motif, including at least one hairpin, including at least one MS2 stem loop, preferably at least two MS2 stem loops, and/or at least one pseudoknot sequence.

10. The pegRNA system of any one of claims 6 to 9, further comprising a nicking crRNA, said nicking crRNA comprising a CRISPR-Cas class 2 type V scaffold sequence; a spacer sequence, and optionally a second scaffold sequence, wherein the target site of the spacer sequence of said nicking crRNA is in the vicinity of and at the opposite strand of the target site of the spacer sequence of said pegRNA or said petRNA.

11. A Cas12a prime editor comprising

(i) at least one Cas12a enzyme having nickase activity (nCas12a), preferably having non-target strand nickase activity, or an active fragment thereof;

(ii) at least one reverse transcriptase, or an active fragment thereof;

(iii) at least one linker covalently or non-covalently linking the at least one nCas12a, or active fragment thereof, and the at least one reverse transcriptase, or active fragment thereof;

(iv) optionally at least one organellar localization signal;

wherein (i) the at least one nCas12a is fused to (ii) the at least one reverse transcriptase and at least one organellar localization signal is located at the N-terminus of the Cas12a prime editor and at least one organellar localization signal is located at the C-terminus of the Cas12a prime editor; and/or

wherein the at least one nCas12a is an RNase dead nCas12a, optionally comprising a mutation at position H759, including a H759A mutation; and/or

wherein the Cas12a prime editor comprises at least one ssDNA-binding and/or ssDNA-stabilizing protein, domain, or active fragment thereof, preferably Brex27, RPA70-A, RPA70 B, RPA70-C, RPA32-D, BRCA2-OB2, BRCA2-OB3, HNRNPK KH, PUF60RRM, or Rad51DBD and/or

wherein the Cas12a prime editor comprises the reverse transcriptase protein in trans, preferably comprising at least one organellar localization signal and preferably comprising an MS2 coat protein (MCP).

12. The Cas12a prime editor of claim 11, wherein the Cas12a prime editor comprises the reverse transcriptase protein in trans, and further comprises an RNA binding factor, preferably the N-terminal domain of La or of MCP, more preferably wherein the Cas12a prime editor comprises the N-terminal domain of La, optionally comprising the reverse transcriptase protein as defined in claim 2 comprising an MCP, and the pegRNA system as defined in claim 8, and, optionally the nicking crRNA as defined in claim 10, wherein

a) the crRNA comprises in a 5' to 3' direction (i) a CRISPR-Cas class 2 type V scaffold sequence, (ii) a spacer sequence and optionally (vi) a second scaffold sequence, optionally being the same as the first scaffold sequence;

b) a prime editing template RNA the (petRNA) comprising in a 5' to 3' direction an MS2 stem loop, optionally a linker sequence, a reverse transcriptase template having a length of 9 to 150 nucleotides; a primer binding site having a length of 5 to 50 nucleotides; optionally a linker sequence; and an MS2 stem loop.

13. The Cas12a prime editor of claims 11 or 12, wherein (ii) the at least one reverse transcriptase or active fragment thereof, is linked, via (iii) the at least one linker, N-terminally or C-terminally, preferably N-terminally, to (i) the at least one nCas12a, or active fragment thereof.

14. A nucleic acid molecule or more than one nucleic acid molecules encoding

the prime editor complex of any one of claims 1 to 5; and/or

the pegRNA system of any one of claims 6 to 10; and/or

the Cas12a prime editor of any one of claims 11 to 13.

15. An expression vector or construct or more than one expression vectors or constructs comprising the one or more nucleic acid molecules of any one of claims 24 to 30.16. A cell comprising

the prime editor complex of any one of claims 1 to 5; and/or

the pegRNA system of any one of claims 6 to 10; and/or

the Cas12a prime editor of any one of claims 11 to 13; and/or

the nucleic acid molecule or more than one nucleic acid molecules of claim 14; and/or

the expression vector or construct or more than one expression vector or constructs of claim 15.

17. A kit comprising

the prime editor complex of any one of claims 1 to 10; and/or

the pegRNA system of any one of claims 11 to 18; and/or

the Cas12a prime editor of any one of claims 19 to 23; and/or

the nucleic acid molecule or more than one nucleic acid molecules of claim 24; and/or

the expression vector or construct or more than one expression vector or constructs of claim 15; and/or

the cell of any one of claims 26 to 31;

further comprising a set of reagents;

optionally comprising particles, vesicles, or at least one viral vector, or *Agrobacterium* 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.

18. A method for modifying at least one nucleic acid sequence of interest in at least one nucleic acid molecule of at least cell or construct at or near at least one target site, wherein the method does not include treatment of the human or animal body by surgery or therapy,

the method comprising:

(a) providing at least one cell or construct comprising the nucleic acid sequence of interest to be modified;

(b) providing and/or introducing

(b-i) at least one pegRNA system as defined in any one of claims 6 to 10, or at least one nucleic acid molecule or expression vector or construct encoding the same, and

(b-ii) at least one Cas12a prime editor, as defined in any one of claims 11 to 13; or at least one nucleic acid molecule or expression vector or construct encoding the same;

optionally allowing complex formation of (b-i) the at least one pegRNA or crRNA and (b-ii) the at least one Cas12 prime editor before the provision and/or introduction;

or providing and/or introducing:

(b-iii) at least one prime editor complex as defined in any one of claims 1 to 5, or at least one nucleic acid molecule or expression vector or construct encoding the same;

(c) allowing the modification of at least one nucleic acid sequence of interest by (b-i) the at least one pegRNA system and (b-ii) the at least one Cas12 prime editor; or by (b-iii) the at least one prime editor complex;

(d) optionally, obtaining at least one edited cell or construct comprising a modification at at least one nucleic acid sequence of interest at or near a target site;

optionally, where the method comprises the following step:

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

19. A method for producing an edited cell, tissue, and/or organism, the method comprising:

(a) providing at least one cell, tissue, and/or organism comprising at least one nucleic acid sequence of interest in at least one nucleic acid molecule at or near at least one target site;

(b) introducing

(b-i) at least one pegRNA system as defined in any one of claims 6 to 10, or at least one nucleic acid molecule or expression vector or construct encoding the same, and

(b-ii) at least one Cas12a prime editor, as defined in any one of claims 11 to 13; or at least one nucleic acid molecule or expression vector or construct encoding the same;

optionally allowing complex formation of (b-i) the at least one pegRNA or crRNA and (b-ii) the at least one Cas12 prime editor before the provision and/or introduction;

or introducing:

(b-iii) at least one prime editor complex as defined in any one of claims 1 to 5, or at least one nucleic acid molecule or expression vector or construct encoding the same;

(c) allowing the modification of at least one nucleic acid sequence of interest by (b-i) the at least one pegRNA system and (b-ii) the at least one Cas12 prime editor; or by (b-iii) the at least one prime editor complex;

(d) obtaining at least one edited cell or construct comprising a modification at at least one nucleic acid sequence of interest at or near a target site;

optionally, where the method comprises the following step:

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

20. An edited cell, tissue, organ, material or whole organism obtained by or obtainable by a method according to claims 18 or 19.

21. A use of a compound selected from

a prime editor complex as defined in any one of claims 1 to 5; and/or

a pegRNA system as defined in any one of claims 6 to 10; and/or

a Cas12a prime editor as defined in any one of any claims 11 to 13; and/or

a nucleic acid molecule or more than one nucleic acid molecules as defined in claim 14; and/or

an expression vector or construct or more than one expression vector or constructs as defined in claim 15; and/or

a cell as defined in claim 16;

for introducing or modification in a nucleic acid molecule, preferably in a genome, including uses for optimizing or modifying a trait in a plant, including the modification

of a yield-related trait, or a disease-resistance related trait, and/or for metabolic engineering in cell, including a prokaryotic or eukaryotic cell, wherein the use does not include treatment of the human or animal body by surgery or therapy,

optionally wherein the use 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.

22. A compound selected from

a prime editor complex as defined in any one of claims 1 to 5; and/or

a pegRNA system as defined in any one of claims 6 to 10; and/or

a Cas12a prime editor as defined in any one of claims 11 to 13; and/or

a nucleic acid molecule or more than one nucleic acid molecules as defined in claim 14; and/or

an expression vector or construct or more than one expression vector or constructs as defined in claim 15; and/or

a cell as defined in claim 16;

for use in therapy.