## CLAIMS

1. A method for targeted genomic engineering in an algal cell comprising introducing at least an endonuclease in said algal cell to induce a double-stranded cleavage at a site of interest into the genome of said algal cell.

2. The method of claim 1 comprising:

   a) Providing an endonuclease capable of inducing a double stranded cleavage at a site of interest into the genome of an algal cell ;

   b) Introducing said endonuclease into an algal cell

   c) Isolating algal cell having a modified targeted genomic site of interest.

3. The method according to claims 1 or 2, wherein said endonuclease is introduced into an algal cell by a method selected from the group consisting of: electroporation and bombardment methods.

4. The method according to any one of claims 1 to 3, wherein a targeted knock-out in algae is induced by said endonuclease at the site of interest in said genome.

5. The method according to any one of claims1 to 3, wherein at least one transgene is inserted at the targeted genomic site of interest by further comprising the introduction of a template wherein

said template is flanked by sequences sharing homologies with the region surrounding the genomic DNA cleavage site of interest.

6. The method of claim 5, wherein said template comprises at least one transgene encoding a gene conferring an advantageous trait for biotechnological applications selected from the group consisting of quorum sensing, secretion of hydrocarbons,

fatty acid composition, lipids accumulation, enhanced photosynthesis, pigments production, mercury volatilization, frustule composition or organization, and mitigation genes.

7. The method according to claims 5 or 6 wherein said template comprises a nucleic acid encoding a selectable marker.

8. The method of claim 7 wherein said selectable marker is N-acetyltransferase 1 (Nat1) conferring the resistance to Nourseothricin.

9. The method according to any one of claims 5 to 8, wherein said transgene insertion does not modify expression of genes located in the vicinity of the target sequence.

10. The method according to any one of claims 5 to 9 wherein said template comprises multiple transgenes.

11. The method according to any one of claims 1 to 10 wherein said endonuclease is a meganuclease.

12. The method of claim 11 wherein said meganuclease is selected from the group consisting of a homodimeric, heterodimeric, obligate heterodimers and single chain variant.

13. The method of claims 11 or 12 wherein said endonuclease is an engineered I-CreI.

14. The method according to any one of claims 2 to 10 wherein said endonuclease is an engineered zinc-finger binding domain fused to a restriction enzyme.

15. The method according to anyone of claims 2 to 14 wherein algae are selected from the group consisting of *Amphora, Anabaena, Anikstrodesmis, Botryococcus, Chaetoceros, Chlamydomonas, Chlorella, Chlorococcum, Cyclotella, Cylindrotheca, Dunaliella, Emiliana, Euglena, Hematococcus, Isochrysis, Monochrysis, Monoraphidium, Nannochloris, Nannnochloropsis, Navicula, Nephrochloris, Nephroselmis, Nitzschia, Nodularia, Nostoc, Ochromonas, Oocystis, Oscillartoria, Pavlova, Phaeodactylum, Playtmonas, Pleurochrysis, Porhyra, Pseudoanabaena, Pyramimonas, Stichococcus, Synechococcus, Synechocystis, Tetraselmis, Thalassiosira*, and *Trichodesmium*.

16. A targeted genome engineered algae obtained by the method according to any one of claims 1 to 14.

17. The targeted genome engineered algae according to claim 16, comprising at least one transgene inserted into a targeted genomic site of interest.

18. The targeted genome engineered algae of claim 17, wherein said transgene encodes a gene conferring an advantageous trait for biotechnological applications selected from the group consisting of quorum sensing, secretion of hydrocarbons, fatty acid composition, lipids accumulation, enhanced photosynthesis, pigments production, mercury volatilization, frustule composition or organization, and mitigation genes.

19. Algae comprising a nucleic acid sequence encoding an endonuclease.

20. Use of targeted genome engineered algae according to claims 16 to 19 to produce biofuel.

## CLAIMS

1. A method for targeted genomic engineering in an algal cell comprising introducing at least an endonuclease in said algal cell to induce a double-stranded cleavage at a site of interest into the genome of said algal cell.

2. The method of claim 1, comprising:

   a) Providing an endonuclease capable of inducing a double-stranded cleavage at a site of interest into the genome of an algal cell ;

   b) Introducing said endonuclease into an algal cell

   c) Isolating algal cell having a modified targeted genomic site of interest.

3. The method according to claim 1 or claim 2, wherein said endonuclease is introduced into an algal cell by a method selected from the group consisting of: electroporation and bombardment methods.

4. The method according to any one of claims 1 to 3, wherein a targeted knock-out in algae is induced by said endonuclease at the site of interest in said genome.

5. The method according to any one of claims 1 to 3, wherein at least one transgene is inserted at the targeted genomic site of interest by further comprising the introduction of a template wherein said template is flanked by sequences sharing homologies with the region surrounding the genomic DNA cleavage site of interest.

6. The method of claim 5, wherein said template comprises at least one transgene encoding a gene conferring an advantageous trait for biotechnological applications selected from the group consisting of quorum sensing, secretion of hydrocarbons, fatty acid composition, lipids accumulation, enhanced photosynthesis, pigments production, mercury volatilization, frustule composition or organization, and mitigation genes.

7. The method according to claim 5 or claim 6, wherein said template comprises a nucleic acid encoding a selectable marker.

8. The method of claim 7, wherein said selectable marker is N-acetyltransferase 1 (Nat1) conferring the resistance to Nourseothricin.

9. The method according to any one of claims 5 to 8, wherein said transgene insertion does not modify expression of genes located in the vicinity of the target sequence.

10. The method according to any one of claims 5 to 9, wherein said template comprises multiple transgenes.

11. The method according to any one of claims 1 to 10, wherein said endonuclease is a meganuclease.

12. The method of claim 11, wherein said meganuclease is selected from the group consisting of a homodimeric, heterodimeric, obligate heterodimers and single chain variant.

13. The method of claim 11 or claim 12 wherein said endonuclease is an engineered I-CreI.

14. The method according to any one of claims 2 to 10, wherein said endonuclease is an engineered zinc-finger binding domain fused to a restriction enzyme.

15. The method according to anyone of claims 2 to 14, wherein algae are selected from the group consisting of *Amphora, Anabaena, Anikstrodesmis, Botryococcus, Chaetoceros, Chlamydomonas, Chlorella, Chlorococcum, Cyclotella, Cylindrotheca, Dunaliella, Emiliana, Euglena, Hematococcus, Isochrysis, Monochrysis, Monoraphidium, Nannochloris, Nannnochloropsis, Navicula, Nephrochloris, Nephroselmis, Nitzschia, Nodularia, Nostoc, Oochromonas, Oocystis, Oscillartoria, Pavlova, Phaeodactylum, Playtmonas, Pleurochrysis, Porhyra, Pseudoanabaena, Pyramimonas, Stichococcus, Synechococcus, Synechocystis, Tetraselmis, Thalassiosira*, and *Trichodesmium*.

16. A targeted genome engineered algae obtained by the method according to any one of claims 1 to 14.

17. The targeted genome engineered algae according to claim 16, comprising at least one transgene inserted into a targeted genomic site of interest.

18. The targeted genome engineered algae of claim 17, wherein said transgene encodes a gene conferring an advantageous trait for biotechnological applications selected from the group consisting of quorum sensing, secretion of hydrocarbons, fatty acid composition, lipids accumulation, enhanced photosynthesis, pigments production, mercury volatilization, frustule composition or organization, and mitigation genes.

19. An algal cell into which a nucleic acid encoding an endonuclease has been introduced, wherein said endonuclease induces a double-stranded cleavage at a site of interest into the genome of said algal cell.

20. Use of targeted genome engineered algae according to any one of claims 16 to 19, to produce biofuel.