CLAIMS

1. A fully penetrant RNA-guided endonuclease-expressing algal strain comprising,

at least one non-native nucleic acid molecule comprising at least one nucleic acid sequence encoding a heterologous RNA-guided endonuclease;

a nucleic acid sequence encoding a nuclear localization signal linked to the nucleic acid sequence encoding the heterologous RNA-guided endonuclease; and

a nucleic acid sequence encoding a fluorescent protein;

wherein the fully penetrant algal strain exhibits a single fluorescence intensity peak in a flow cytometry histogram, and the single fluorescence intensity peak is greater than an autofluorescence peak of non-transformed cells.

2. A fully penetrant RNA-guided endonuclease-expressing algal strain according to claim 1, wherein the RNA-guided endonuclease is a Cas nuclease.

3. A fully penetrant RNA-guided endonuclease-expressing algal strain according to claim 2, wherein the RNA-guided endonuclease is a Cas9, Cpf1, C2c1, C2c2, or C2c3 nuclease.

4. A fully penetrant RNA-guided endonuclease-expressing algal strain according to claim 1, wherein the non-native nucleic acid molecule further comprises a selectable marker.

5. A fully penetrant RNA-guided endonuclease-expressing algal strain according to any one of claims 1-4, wherein the strain has a targeted mutation rate using a gRNA and donor fragment that comprises a selectable marker of at least 50%.

6. A fully penetrant RNA-guided endonuclease-expressing algal strain according to any one of claims 1-5, further comprising an exogenous gene encoding a site-specific recombinase.

7. A fully penetrant RNA-guided endonuclease-expressing algal strain according to claim 6, wherein the site-specific recombinase is cre, frt, or dre.

8. A fully penetrant RNA-guided endonuclease-expressing algal strain according to claim 6 or 7, wherein the exogenous gene encoding a site-specific recombinase is operably linked to an inducible promoter.

9. A fully penetrant RNA-guided endonuclease-expressing algal strain according to any one of claims 1-8, wherein the targeted mutation rate of the fully penetrant algal strain using a gRNA and donor fragment that comprises a selectable marker is at least 50%.

10. A fully penetrant RNA-guided endonuclease-expressing algal strain according to any one of claims 1-9, wherein the algal strain is a Eustigmatophyte alga.

11. A method of generating a fully penetrant RNA-guided endonuclease-expressing algal strain of any one of claims 1-10 comprising:

introducing a non-native nucleic acid molecule encoding a heterologous RNA-guided endonuclease and a gene encoding a fluorescent protein into a population of algal host cells to obtain one or more transformed cell lines comprising the non-native nucleic acid molecule; wherein the non-native nucleic acid molecule is linked to a nucleic acid sequence encoding a nuclear localization signal;

culturing at least one of the one or more transformed cell lines to provide at least one transformed cell line culture; and

analyzing at least one of the one or more transformed cell lines using flow cytometry to identify at least one transformed cell line exhibiting a single fluorescence intensity peak in a flow cytometry histogram, and the single fluorescence intensity peak is greater than an autofluorescence peak of non-transformed cells.

12. A method according to claim 11, wherein the nucleic acid molecule encoding the RNA-guided endonuclease and the gene encoding the fluorescent protein gene are operably linked to separate promoters.

13. A method according to claim 11 or 12, wherein the gene encoding a fluorescent protein is flanked by sites recognized by a site-specific recombinase.

14. A method according to any one of claims 11-13, wherein the non-native nucleic acid molecule further includes a selectable marker gene.

15. A method according to claim 14, wherein the selectable marker gene is flanked by sites recognized by a site-specific recombinase.

16. A method according to any one of claims 11-15, wherein the RNA-guided endonuclease is a Cas nuclease.

17. The method according to any one of claims 11-16, wherein the algal strain is a Eustigmatophyte.

18. The method according to any one of claims 11-17, wherein the RNA-guided endonuclease is Cas9, Cpf1, C2c1, C2c2, or C2c3.