CLAIMS

1. A method of generating a cell line having fully penetrant expression of an exogenous gene of interest, comprising:

introducing a non-native nucleic acid molecule comprising a gene of interest and a detectable marker gene into a population of host cells to obtain one or more transformed cell lines comprising the non-native nucleic acid molecule;

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

using flow cytometry to identify at least one transformed cell line demonstrating fully penetrant expression of the detectable marker in culture to identify a fully penetrant expression of the exogenous gene of interest.

2. A method according to claim 1, wherein identifying a transformed cell line demonstrating fully penetrant expression of the detectable marker comprises comparing the flow cytometry trace of the at least one transformed cell line culture with the flow cytometry trace of a control culture that does not express the detectable marker, wherein a flow cytometry trace of a transformed cell line culture than demonstrates a single peak that is shifted to a higher fluorescence level than the flow cytometry trace of the control culture to identify a transformed cell line demonstrating fully penetrant expression of the gene of interest.

3. A method according to claim 1, wherein the non-native nucleic acid molecule further includes a selectable marker gene.

4. A method according to claim 3, wherein the selectable marker gene confers antibiotic resistance.

5. A method according to claim 3, wherein obtaining one or more transformed cell lines comprising the non-native nucleic acid molecule is by selecting transformed colonies on selective media.

6. A method according to claim 1, wherein the gene encoding a gene of interest and the detectable marker gene are operably linked to separate promoters.

7. A method according to claim 1, wherein the gene encoding a gene of interest and the selectable marker gene are operably linked to separate promoters.

8. A method according to any of claims 1-7, wherein a gene of interest encodes a functional RNA or polypeptide.

9. A method according to claim 8, wherein a gene of interest encodes a functional RNA selected from the group consisting of a transactivating RNA (tracrRNA), cripsr RNA (crRNA), single guide RNA, chimeric guide RNA, RNAi construct, shRNA, siRNA, antisense RNA sequence, ribozyme, or microRNA.

10. A method according to claim 8, wherein a gene of interest encodes a polypeptide selected from the group consisting of an enzyme, a ribosomal protein, a structural protein, a component of a secretion system, a transporter, a porin, a membrane receptor, a DNA-binding protein, a recombinase, an endonuclease, a transposase, a transcription factor, or a cell signaling protein.

11. A method according to any of claims 1-10, wherein the detectable marker is a fluorescent protein.

12. A method according to claim 1, wherein the detectable marker gene is flanked by sites recognized by a site-specific recombinase.

13. A method according to claim 2, wherein the selectable marker gene is flanked by sites recognized by a site-specific recombinase.

14. A method according to claim 2, wherein a sequence comprising the selectable marker gene and the detectable marker gene is flanked by sites recognized by a site-specific recombinase.

15. A method according to any of claims 1-14, wherein the non-native nucleic acid molecule comprises two or more genes of interest.

16. A method according to claim 15, comprising three, four, five, six, seven, eight, nine, ten, or more genes of interest.

17. A method according to any of claims 1-16, wherein at least one gene of interest encodes a site-specific recombinase.

18. A method according to claim 17, wherein the site-specific recombinase is operably linked to an inducible promoter.

19. A method according to claim 17 or 18, wherein the site-specific recombinase is cre, flp or Dre.

20. A method according to any of claims 17-19, wherein the detectable marker gene is flanked by sites recognized by a site-specific recombinase.

21. A method according to claim 20, further comprising inducing expression of the site-specific recombinase, whereby the detectable marker gene is excised from the nucleic acid molecule.

22. A method according to any of claims 17-19, wherein the nucleic acid molecule comprises a selectable marker gene, further wherein the selectable marker gene is flanked by sites recognized by a site-specific recombinase.

23. A method according to claim 18, further comprising inducing expression of the site-specific recombinase, whereby the selectable marker gene is recombined out of excised from the nucleic acid molecule.

24. A method according to any of claims 17-19, wherein the nucleic acid molecule comprises a selectable marker gene, wherein the nucleic acid sequence comprising the detectable marker gene and the selectable marker gene are flanked by sites recognized by a site-specific recombinase.

25. A method according to claim 24, further comprising inducing expression of the recombinase, whereby the detectable marker gene and the selectable marker gene are excised from the nucleic acid molecule.

26. The method of claim 1 wherein the host cells are eukaryotic host cells.

27. The method of claim 26 wherein the host cells are plant, mammalian, insect, arachnid, fish, reptile, amphibian, avian, crustacean, or protozoan cells.

28. The method of claim 27 wherein the host cells are fungal cells, optionally of a genus selected from the group consisting of *Aspergillus*, *Mucor*, *Pichia*, *Pullularia*, *Saccharomyces*, *Schizosaccharomyces*, *Trichoderma*, *Rhodotorula*, and *Yarrowia*.

29. The method of claim 27 wherein the host cells are mesomycetozoea, heterokont, or algal cells.

30. The method of claim 29, wherein the host cells are algal cells.

31. The method of claim 30, wherein the algal cells are of a genus selected from the group consisting of *Achnanthes*, *Amphiprora*, *Amphora*, *Ankistrodesmus*, *Asteromonas*, *Boekelovia*, *Bolidomonas*, *Borodinella*, *Botrydium*, *Botryococcus*, *Bracteococcus*, *Chaetoceros*, *Carteria*, *Chlamydomonas*, *Chlorococcum*, *Chlorogonium*, *Chlorella*, *Chroomonas*, *Chrysosphaera*, *Cricosphaera*, *Crypthecodinium*, *Cryptomonas*, *Cyclotella*, *Desmodesmus*, *Dunaliella*, *Elipsoidon*, *Emiliania*, *Eremosphaera*, *Ernodesmius*, *Euglena*, *Eustigmatos*, *Franceia*, *Fragilaria*, *Fragilaropsis*, *Gloeothamnion*, *Haematococcus*, *Hantzschia*, *Heterosigma*, *Hymenomonas*, *Isochrysis*, *Lepocinclis*, *Micractinium*, *Monodus*, *Monoraphidium*, *Nannochloris*, *Nannochloropsis*, *Navicula*, *Neochloris*, *Nephrochloris*, *Nephroselmis*, *Nitzschia*, *Ochromonas*, *Oedogonium*, *Oocystis*, *Ostreococcus*, *Parachlorella*, *Parietochloris*, *Pascheria*, *Pavlova*, *Pelagomonas*, *Phæodactylum*, *Phagus*, *Picochlorum*, *Platymonas*, *Pleurochrysis*, *Pleurococcus*, *Prototheca*, *Pseudochlorella*, *Pseudoneochloris*,

Pseudostaurastrum, *Pyramimonas*, *Pyrobotrys*, *Scenedesmus*, *Schizochlamydella*, *Skeletonema*, *Spyrogyra*, *Stichococcus*, *Tetrachlorella*, *Tetraselmis*, *Thalassiosira*, *Tribonema*, *Vaucheria*, *Viridiella*, *Vischeria*, and *Volvox*.

32. The method of claim 30, wherein the algal cells are Bacillariophyte cells, optionally of a genus selected from the group consisting of *Achnanthes*, *Amphora*, *Amphiprora*, *Chaetoceros*, *Cyclotella*, *Fragilaria*, *Fragilariopsis*, *Navicula*, *Nitzschia*, *Phaeodactylum*, *Skeletonema*, and *Thalassiosira*.

33. The method of claim 31, wherein the algal cells are Eustigmatophyte cells, optionally of a genus selected from the group consisting of *Ellipsoidion*, *Eustigmatos*, *Monodus*, *Nannochloropsis*, *Pseudostaurastrum*, and *Vischeria*.

34. The method of claim 29, wherein the host cells are Labyrinthulomycete cells, optionally of a genus selected from the group consisting of *Labryinthula*, *Labryinthuloides*, *Thraustochytrium*, *Schizochytrium*, *Aplanochytrium*, *Aurantiochytrium*, *Oblongichytrium*, *Japonochytrium*, *Diplophrys*, and *Ulkenia*.

35. A method for generating a high efficiency genome editing cell line, comprising:

introducing a non-native nucleic acid molecule comprising a nucleic acid sequence encoding an RNA-guided endonuclease and a nucleic acid sequence encoding a detectable marker into a population of host cells to obtain one or more RNA-guided endonuclease-transformed cell lines comprising the non-native nucleic acid molecule;

culturing at least one of the one or more RNA-guided endonuclease-transformed cell lines to provide at least one RNA-guided endonuclease-transformed cell line culture;

using flow cytometry to assess the at least one RNA-guided endonuclease-transformed cell line culture for expression of the detectable marker in the cells of the RNA-guided endonuclease-transformed cell line culture; and

identifying a RNA-guided endonuclease-transformed cell line demonstrating fully penetrant expression of the detectable marker in the cell line culture to identify a high efficiency genome editing cell line.

36. A method according to claim 35, wherein identifying a transformed cell line demonstrating fully penetrant expression of the detectable marker comprises comparing the flow cytometry trace of the at least one transformed cell line culture with the flow cytometry trace of a control culture that does not express the detectable marker, wherein a flow cytometry trace of a transformed cell line culture than demonstrates a single peak that is shifted to a higher fluorescence level than the flow cytometry trace of the control culture to

identify a transformed cell line demonstrating fully penetrant expression of the gene of interest.

37. The method of claim 35 wherein the RNA-guided endonuclease is heterologous with respect to the host cells.

38. The method of claim 35, wherein the RNA-guided endonuclease is a Cas protein.

39. The method of claim 38, wherein the RNA-guided endonuclease is a Type II or Type V Cas protein.

40. The method of claim 35, wherein the RNA-guided endonuclease is selected from the group consisting of Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Cbf1, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Cpf1, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, C2c1, C2c2, C2c3, homologs thereof, and modified versions thereof.

41. The method of claim 39, wherein the RNA-guided endonuclease is Cas9, Cpf1, C2c1, C2c2, or C2c3.

42. The method of claim 41, wherein the RNA-guided endonuclease is Cas9.

43. The method of claim 42, wherein the Cas9 protein is a Streptococcus pyogenes, Streptococcus thermophilus, or Neisseria meningitidis Cas9 protein.

44. The method of claim 42 wherein the Cas9 protein comprises at least one mutation with respect to a wild-type Cas9 protein.

45. The method of claim 44, wherein at least one mutation is selected from the group consisting of D10A, H840A, N854A, and N863A.

46. The method of claim 35, wherein the nucleic acid sequence encoding an RNA-guided endonuclease is codon optimized for expression in the host strain.

47. The method of claim 35, wherein the RNA-guided endonuclease comprises at least one nuclear localization sequence (NLS).

48. The method of claim 35, wherein the RNA-guided endonuclease comprises at least epitope tag.

49. The method of claim 48, wherein the epitope tag is a histidine tag, a hemagglutinin (HA) tag, a FLAG tag, or a Myc tag.

50. The method of claim 35, wherein the non-native nucleic acid molecule further comprises a selectable marker.

51. The method of claim 50, wherein the selectable marker confers resistance to an antibiotic or toxin.

52. The method of claim 51, wherein the nucleic acid sequence encoding the detectable marker is codon optimized for expression in the microbial strain.

53. The method of claim 35, wherein the detectable marker is a fluorescent protein.

54. The method of claim 53, wherein the fluorescent protein is green fluorescent protein (GFP), YFP, RFP, CFP, BFP, Cherry, Tomato, Venus, Cerulean, or a variant of any thereof, including but not limited to a monomeric variant.

55. The method of claim 54, wherein the detectable marker and the Cas9 protein are produced as separate proteins.

56. The method of claim 55, wherein the gene encoding the selectable marker and the gene encoding the RNA-guided endonuclease are operably linked to different promoters.

57. The method of claim 35, wherein the host cells are prokaryotic host cells.

58. The method of claim 57, wherein the host cells are of a species belonging to any of the following groups: Archaea, cyanobacteria, green-sulfur bacteria, green non-sulfur bacteria, purple sulfur bacteria, or purple non-sulfur bacteria or any of the following genera: *Arthrobacter*, *Escherichia*, *Bacillus*, *Brevibacteria*, *Clostridium*, *Corynebacteria*, *Desulfovibrio*, *Jeotgalicoccus*, *Kineococcus*, *Lactobacillus*, *Micrococcus*, *Mycobacterium*, *Pantoea*, *Pseudomonas*, *Rhodococcus*, *Rhodopseudomonas*, *Rhodospirillium*, *Rhodomicrobium*, *Stenotrophomonas*, *Vibrio*, *Streptomyces*, or *Zymomonas*,.

59. The method of claim 35, wherein the host cells are eukaryotic host cells.

60. The method of claim 59, wherein the host cells are plant, mammalian, insect, arachnid, fish, reptile, amphibian, avian, crustacean, or protozoan cells.

61. The method of claim 59, wherein the host cells are CHO cells, COS cells, VERO cells, BHK cells, HeLa cells, Cvl cells, MDCK cells, 293 cells, 3T3 cells, or PC12 cells.

62. The method of claim 59, wherein the host cells are fungal cells, optionally of a genus selected from the group consisting of Aspergillus, Mucor, Pichia, Pullularia, Saccharomyces, Schizosaccharomyces, Trichoderma, Rhodotorula, and Yarrowia.

63. The method of claim 59, wherein the host cells are mesomycetozoea, heterokont, or algal cells.

64. The method of claim 63, wherein the host cells are algal cells, optionally of a genus selected from the group consisting of Achnanthes, Amphiprora, Amphora, Ankistrodesmus, Asteromonas, Boekelovia, Bolidomonas, Borodinella, Botrydium, Botryococcus, Bracteococcus, Chaetoceros, Carteria, Chlamydomonas, Chlorococcum, Chlorogonium, Chlorella, Chroomonas, Chrysosphaera, Cricosphaera, Crypthecodinium, Cryptomonas, Cyclotella, Desmodesmus, Dunaliella, Elipsoidon, Emiliania, Eremosphaera,

~~Ernodesmius, Euglena, Eustigmatos, Franceia, Fragilaria, Fragilaropsis, Glocothamnion, Haematococcus, Hantzschia, Heterosigma, Hymenomonas, Isochrysis, Lepocinclis, Micractinium, Monodus, Monoraphidium, Nannochloris, Nannochloropsis, Navicula, Neochloris, Nephrochloris, Nephroselmis, Nitzschia, Ochromonas, Oedogonium, Oocystis, Ostreococcus, Parachlorella, Parietochloris, Pascheria, Pavlova, Pelagomonas, Phæodactylum, Phagus, Picochlorum, Platymonas, Pleurochrysis, Pleurococcus, Prototheca, Pseudochlorella, Pseudoneochloris, Pseudostaurastrum, Pyramimonas, Pyrobotrys, Scenedesmus, Schizochlamydella, Skeletonema, Spyrogyra, Stichococcus, Tetrachlorella, Tetraselmis, Thalassiosira, Tribonema, Vaucheria, Viridiella, Vischeria, and Volvox.~~

~~**65.** The method of claim 63, wherein the host cells are heterokont cells, optionally of a genus selected from the group consisting of Labryinthula, Labryinthuloides, Thraustochytrium, Schizochytrium, Aplanochytrium, Aurantiochytrium, Oblongichytrium, Japonochytrium, Diplophrys, and Ulkenia.~~

~~**66.**~~**1.** A fully penetrant RNA-guided endonuclease-expressing ~~cell line or microbial~~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.

~~**67.**~~**2.** A fully penetrant RNA-guided endonuclease-expressing ~~cell line or microbial~~algal strain according to claim ~~66~~1, wherein the RNA-guided endonuclease is a Cas nuclease.

~~**68.**~~**3.** A fully penetrant RNA-guided endonuclease-expressing ~~cell line or microbial~~algal strain according to claim ~~67~~2, wherein the RNA-guided endonuclease is a Cas9, Cpf1, C2c1, C2c2, or C2c3 nuclease.

~~**69.**~~**4.** A fully penetrant RNA-guided endonuclease-expressing ~~cell line or microbial~~algal strain according to ~~any of~~ claim~~s~~ ~~66-68~~1, wherein the ~~targeted mutation rate of the fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain using a gRNA and donor fragment that~~non-native nucleic acid molecule further comprises a selectable marker ~~is at least 10%~~.

~~**70.** A fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain according to claim 69, wherein the targeted mutation rate of the fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain using a gRNA and donor fragment that comprises a selectable marker is at least 25%.~~

~~**71.**~~**5.** A fully penetrant RNA-guided endonuclease-expressing ~~cell line or microbial~~algal strain according to any one of claims ~~70~~1-4, wherein the strain has a targeted mutation rate ~~of the fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain~~ using a gRNA and donor fragment that comprises a selectable marker ~~is approximately~~of at least 50% ~~or greater~~.

~~**72.** A fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain according to claim 71, wherein the targeted mutation rate of the fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain using a gRNA and donor fragment that comprises a selectable marker is approximately 80% or greater.~~

~~**73.** A fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain according to claim 72, wherein the targeted mutation rate of the fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain using a gRNA and donor fragment that comprises a selectable marker is approximately 90% or greater.~~

~~**74.** A fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain according to any of claims 66-73, wherein the cell line or microbial strain is a microbial strain.~~

~~**75.** A fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain according to claim 74, wherein the microbial strain is a fungus, mesomycetozoea, heterokont, or alga.~~

~~**76.** A fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain according to claim 74, wherein the microbial strain is a fungal strain, optionally of a genus selected from the group consisting of Aspergillus, Mucor, Pichia, Pullularia, Saccharomyces, Schizosaccharomyces, Trichoderma, Rhodotorula, and Yarrowia.~~

~~**77.** A fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain according to claim 74, wherein the microbial strain is an algal strain, optionally of a genus selected from the group consisting of *Achnanthes*, *Amphiprora*, *Amphora*, *Ankistrodesmus*, *Asteromonas*, *Boekelovia*, *Bolidomonas*, *Borodinella*, *Botrydium*, *Botryococcus*, *Bracteococcus*, *Chaetoceros*, *Carteria*, *Chlamydomonas*, *Chlorococcum*, *Chlorogonium*, *Chlorella*, *Chroomonas*, *Chrysosphaera*, *Cricosphaera*, *Crypthecodinium*, *Cryptomonas*, *Cyclotella*, *Desmodesmus*, *Dunaliella*, *Elipsoidon*, *Emiliania*, *Eremosphaera*,~~

~~*Ernodesmius, Euglena, Eustigmatos, Franceia, Fragilaria, Fragilaropsis, Glocothamnion, Haematococcus, Hantzschia, Heterosigma, Hymenomonas, Isochrysis, Lepocinclis, Micractinium, Monodus, Monoraphidium, Nannochloris, Nannochloropsis, Navicula, Neochloris, Nephrochloris, Nephroselmis, Nitzschia, Ochromonas, Oedogonium, Oocystis, Ostreococcus, Parachlorella, Parietochloris, Pascheria, Pavlova, Pelagomonas, Phæodactylum, Phagus, Picochlorum, Platymonas, Pleurochrysis, Pleurococcus, Prototheca, Pseudochlorella, Pseudoneochloris, Pseudostaurastrum, Pyramimonas, Pyrobotrys, Scenedesmus, Schizochlamydella, Skeletonema, Spyrogyra, Stichococcus, Tetrachlorella, Tetraselmis, Thalassiosira, Tribonema, Vaucheria, Viridiella, Vischeria,* and *Volvox.*~~

~~**78.** A fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain according to claim 74, wherein the microbial strain is a heterokont strain, optionally of a genus selected from the group consisting of *Labryinthula, Labryinthuloides, Thraustochytrium, Schizochytrium, Aplanochytrium, Aurantiochytrium, Oblongichytrium, Japonochytrium, Diplophrys*, and *Ulkenia*.~~

~~**79.** A fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain according to any of claims 66-78, wherein the fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain does not include an exogenous selectable marker gene.~~

~~**80.** A fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain according to any of claims 66-79, wherein the fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain does not include an exogenous detectable marker gene.~~

~~81.~~6. A fully penetrant RNA-guided endonuclease-expressing ~~cell line or microbial~~algal strain according to any one of claims ~~79 or 80~~1-5, ~~wherein the RNA-guided endonuclease-expressing cell line or microbial strain~~further comprisinge~~s~~ an exogenous gene encoding a site-specific recombinase.

~~82.~~7. A fully penetrant RNA-guided endonuclease-expressing ~~cell line or microbial~~algal strain according to claim~~s 79 or 80~~6, wherein the site-specific recombinase is cre, frt, or dre.

~~**83.** A fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain according to claims 79 or 80, wherein the site-specific recombinase is cre,.~~

~~**84.** A fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain according to claims 79 or 80, wherein the exogenous gene encoding a site-specific recombinase is codon optimized from expression in the host organism.~~

~~85.~~8. A fully penetrant RNA-guided endonuclease-expressing ~~cell line or microbial~~algal strain according to claim~~s 79 or 80~~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.

~~86.~~11. A method of ~~altering the genome of a cell in vivo~~generating a fully penetrant RNA-guided endonuclease-expressing algal strain of any one of claims 1-10~~, wherein the method~~ comprisinge~~s~~:

~~**87.** introducing at least one guide RNA or at least one construct for expressing at least one guide RNA into a fully penetrant RNA-guided endonuclease-expressing cell line or microbial strain, wherein the guide RNA targets a site in the genome of the cell; and~~

~~screening cells transformed with the guide RNA for alteration of the targeted site in the genome~~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.

~~**88.** A method according to claim 86, wherein the fully penetrant RNA-guided endonuclease-expressing strain is obtained by~~

~~introducing into a cell at least one non-native nucleic acid molecule comprising at least one nucleic acid sequence encoding a RNA-guided endonuclease-expressing protein and at least one nucleic acid sequence encoding a detectable marker to obtain transformants comprising the at least one non-native nucleic acid molecule;~~

~~culturing at least one transformant;~~

~~performing flow cytometry on a culture of at least one transformant to identify a cell line that is fully penetrant for the RNA-guided endonuclease-expressing construct.~~

~~**89.** A method according to claim 86 or 87, wherein the at least one guide RNA is a chimeric guide RNA.~~

~~**90.** A method according to claim 86 or 87, wherein the at least one guide RNA is a crRNA.~~

~~**91.** A method according to claim 86, wherein the fully penetrant RNA-guided endonuclease-expressing strain further comprises a construct encoding a tracrRNA.~~

~~**92.** A method according to claim 86, wherein the method further comprises introducing a tracrRNA into the fully penetrant RNA-guided endonuclease-expressing strain.~~

~~**93.** A method according to claim 91, wherein the tracrRNA comprises at least one chemical modification.~~

~~**94.** A method according to any of claims 86-92, wherein said screening comprises performing PCR to detect a mutation at the targeted site.~~

~~**95.** A method according to any of claims 86-92, wherein said screening comprises employing a phenotypic screen.~~

~~**96.**~~**12.** A method according ~~any of~~to claim~~s~~ ~~86 – 43~~11, wherein the ~~method further comprises transforming a donor DNA into the fully penetrant~~nucleic acid molecule encoding the RNA-guided endonuclease~~-expressing strain for insertion into the targeted site~~ 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.

~~**97.**~~**14.** A method according to any one of claim~~s~~ ~~95~~11-13, wherein the ~~donor DNA~~non-native nucleic acid molecule ~~comprises~~further includes a selectable marker gene.

~~**98.**~~**15.** A method according to claim ~~95~~14, wherein the ~~donor DNA molecule~~selectable marker gene ~~comprises one or more homology regions for recombination into the target~~ is flanked by site~~s~~ recognized by a site-specific recombinase.

~~**99.** A method according to claim 95, wherein the donor DNA molecule does not comprise homology regions for recombination into the target site.~~

~~**100.** A method according to claim 95, wherein screening for alteration of the targeted site in the genome comprises identifying transformants that express the selectable marker of the donor DNA molecule.~~

101. A method according to claim 91 or 92, wherein tracrRNA and the crRNA are allowed to anneal prior to introducing the tracrRNA and the crRNA into the fully penetrant RNA-guided endonuclease-expressing strain.

102.16. A method according to any one of claims 86-10011-15, wherein the RNA-guided endonuclease is a Cas endonuclease.

103.17. AThe method according to any one of claims 10111-16, wherein the RNA-guided endonuclease is a Type II or Type V Cas endonucleasealgal strain is a Eustigmatophyte.

104.18. AThe method according to any one of claims 10211-17, wherein the RNA-guided endonuclease is selected from the group consisting of Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Cbf1, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Cpf1, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, C2c1, C2c2, or C2c3, homologs thereof, and modified versions thereof.

105. A method according to claim 103, wherein the RNA-guided endonuclease is a Cas9 RNA-guided endonuclease.

106. A method according to any of claims 86-104, wherein the cell is a plant, mammalian, insect, arachnid, fish, reptile, amphibian, avian, crustacean, or protozoan cell.

107. The method of claim 105 wherein the cell is a CHO cell, COS cell, VERO cell, BHK cell, HeLa cell, Cvl cell, MDCK cell, 293 cell, 3T3 cell, or PC12 cell.

108. The method of claim 105 wherein the cell is a fungal cell, optionally of a genus selected from the group consisting of Aspergillus, Mucor, Pichia, Pullularia, Saccharomyces, Schizosaccharomyces, Trichoderma, Rhodotorula, and Yarrowia.

109. The method of claim 105 wherein the cell is a mesomycetozoea, heterokont, or algal cell.

110. The method of claim 108, wherein the cell is an algal cell, optionally of a genus selected from the group consisting of *Achnanthes*, *Amphiprora*, *Amphora*, *Ankistrodesmus*, *Asteromonas*, *Boekelovia*, *Bolidomonas*, *Borodinella*, *Botrydium*, *Botryococcus*, *Bracteococcus*, *Chaetoceros*, *Carteria*, *Chlamydomonas*, *Chlorococcum*, *Chlorogonium*, *Chlorella*, *Chroomonas*, *Chrysosphaera*, *Cricosphaera*, *Crypthecodinium*, *Cryptomonas*, *Cyclotella*, *Desmodesmus*, *Dunaliella*, *Elipsoidon*, *Emiliania*, *Eremosphaera*, *Ernodesmius*, *Euglena*, *Eustigmatos*, *Franceia*, *Fragilaria*, *Fragilaropsis*, *Gloeothamnion*, *Haematococcus*, *Hantzschia*, *Heterosigma*, *Hymenomonas*, *Isochrysis*, *Lepocinclis*, *Micractinium*, *Monodus*, *Monoraphidium*, *Nannochloris*, *Nannochloropsis*, *Navicula*, *Neochloris*, *Nephrochloris*, *Nephroselmis*, *Nitzschia*, *Ochromonas*, *Oedogonium*, *Oocystis*, *Ostreococcus*, *Parachlorella*,

~~*Parietochloris*, *Pascheria*, *Pavlova*, *Pelagomonas*, *Phæodactylum*, *Phagus*, *Picochlorum*, *Platymonas*, *Pleurochrysis*, *Pleurococcus*, *Prototheca*, *Pseudochlorella*, *Pseudoneochloris*, *Pseudostaurastrum*, *Pyramimonas*, *Pyrobotrys*, *Scenedesmus*, *Schizochlamydella*, *Skeletonema*, *Spyrogyra*, *Stichococcus*, *Tetrachlorella*, *Tetraselmis*, *Thalassiosira*, *Tribonema*, *Vaucheria*, *Viridiella*, *Vischeria*, and *Volvox*.~~

~~**111.** The method of claim 108, wherein the cell is a heterokont cells, optionally of a genus selected from the group consisting of *Labryinthula*, *Labryinthuloides*, *Thraustochytrium*, *Schizochytrium*, *Aplanochytrium*, *Aurantiochytrium*, *Oblongichytrium*, *Japonochytrium*, *Diplophrys*, and *Ulkenia*.~~