Claims

1. A method of making multiple alterations to target DNA in a cell expressing an enzyme that forms a co-localization complex with RNA complementary to the target DNA and that cleaves the target DNA in a site specific manner comprising

(a) introducing into the cell a first foreign nucleic acid encoding a plurality of RNAs complementary to different sites of the target DNA and which guide the enzyme to the different sites of the target DNA, wherein the each of the plurality of RNAs and the enzyme are members of a co-localization complex for the target DNA,

introducing into the cell a plurality of donor nucleic acid sequences,

wherein the plurality of RNAs are expressed,

wherein at least one of the plurality of RNAs and the enzyme co-localize to a site of the target DNA, the enzyme cleaves the target DNA and one of the plurality of donor nucleic acids is inserted into the target DNA at the site of cleavage to produce altered DNA in the cell, and

(b) repeating step (a) to introduce the same first foreign nucleic acid and the same plurality of donor nucleic acid sequences to produce multiple donor nucleic acid sequence insertions to the DNA in the cell.

2. The method of claim 1 wherein step (a) is repeated multiple times.

3. The method of claim 1 wherein the enzyme is an RNA-guided DNA binding protein.

4. The method of claim 1 wherein the enzyme is Cas9.

5. The method of claim 1 wherein the cell is a eukaryotic cell, and wherein optionally the cell is a yeast cell, a plant cell or an animal cell.

6. The method of claim 1 wherein each of the plurality of RNAs is between

about 10 to about 500 nucleotides.

7. The method of claim 1 wherein each of the plurality of RNAs is between about 20 to about 100 nucleotides.

8. The method of claim 1 wherein each of the plurality of RNAs is a guide RNA.

9. The method of claim 1 wherein each of the plurality of RNAs is a tracrRNA-crRNA fusion.

10. The method of claim 1 wherein the DNA is genomic DNA, mitochondrial DNA, viral DNA, or exogenous DNA.

11. The method of claim 1 wherein each of the plurality of donor nucleic acid sequences is inserted by recombination.

12. The method of claim 1 wherein each of the plurality of donor nucleic acid sequences is inserted by homologous recombination.

13. The method of claim 1 wherein the plurality of RNAs and the plurality of donor nucleic acid sequences are present on one or more plasmids.

14. The method of claim 1 wherein each of the plurality of donor nucleic acids includes homology sequences or arms flanking the site of cleavage.

15. The method of claim 1 wherein each of the plurality of donor nucleic acids includes a sequence to remove the site of cleavage.