Claims

1. An in vitro method of modulating expression of two or more target nucleic acids in a cell comprising

introducing into the cell a first foreign nucleic acid encoding two or more RNAs complementary to the two or more target nucleic acids,

introducing into the cell a second foreign nucleic acid encoding two or more orthogonal RNA guided nuclease null DNA binding proteins that respectively bind to the two or more target nucleic acids and are guided by the two or more RNAs,

introducing into the cell a third foreign nucleic acid encoding two or more transcriptional regulator proteins or domains,

wherein the RNAs, the orthogonal RNA guided nuclease-null DNA binding proteins, and the transcriptional regulator proteins or domains are expressed,

wherein two or more co-localization complexes form between an RNA, an orthogonal RNA guided nuclease null DNA binding protein, a transcriptional regulator protein or domain and a target nucleic acid and wherein the transcriptional regulator protein or domain regulates expression of the target nucleic acid.

2. The method of claim 1 wherein:

(1) the second foreign nucleic acid and the third foreign nucleic acid are expressed to produce a transcriptional regulator protein or domain fused to the two or more orthogonal RNA guided nuclease null DNA binding proteins; or

(2) the first foreign nucleic acid encoding two or more RNAs further encodes a target of an RNA-binding domain and the third foreign nucleic acid encoding the transcriptional regulator proteins or domains further encodes an RNA-binding domain fused to the transcriptional regulator proteins or domains.

3. The method of claim 1 wherein:

(1) the cell is a eukaryotic cell;

(2) the cell is a yeast cell, a plant cell or an animal cell;

(3) the RNAs are between about 10 to about 500 nucleotides; or

(4) the RNAs are between about 20 to about 100 nucleotides.

4. The method of claim 1 wherein:

(1) at least one of the transcriptional regulator proteins or domains is a transcriptional activator;

(2) at least one of the transcriptional regulator proteins or domains upregulates expression of at least one of the target nucleic acids;

(3) at least one of the transcriptional regulator proteins or domains upregulates expression of a target nucleic acid associated with a disease or detrimental condition;

(4) at least one of the transcriptional regulator proteins or domains is a transcriptional repressor;

(5) at least one of the transcriptional regulator proteins or domains downregulates expression of at least one of the target nucleic acids; or

(6) at least one of the transcriptional regulator proteins or domains downregulates expression of a target nucleic acid associated with a disease or detrimental condition.

5. The method of claim 1 wherein at least one of the target nucleic acids is associated with a disease or detrimental condition.

6. The method of claim 1 wherein:

(1) the two or more RNAs are a guide RNAs; or

(2) the two or more RNAs are a tracrRNA-crRNA fusions.

7. The method of claim 1 wherein the target nucleic acids are genomic DNA, mitochondrial DNA, viral DNA, or exogenous DNA.

8. The method of claim 1 wherein:

(1) a first target nucleic acid is activated and a second target nucleic acid is repressed;

(2) a first plurality of target nucleic acids are repressed and a second plurality of target nucleic acids are repressed; or

(3) a first one or more target nucleic acids are activated and a second one or more target nucleic acids are repressed.

9. An in vitro method of altering one or more target nucleic acids in a cell simultaneous with regulating expression of one or more target nucleic acids in a cell comprising

introducing into the cell a first foreign nucleic acid encoding two or more RNAs with at least one RNA being complementary to the one or more target nucleic acids to be altered and at least one RNA being complementary to the one or more target nucleic acids to be regulated,

introducing into the cell a second foreign nucleic acid encoding 1) one orthogonal RNA-guided protein nickase having one inactive nuclease domain and being guided by the one or more RNAs complementary to the one or more target nucleic acids to be altered, and 2) one or more orthogonal RNA guided nuclease null DNA binding proteins that bind to and are guided by the one or more RNAs complementary to the target nucleic acids to be regulated,

introducing into the cell a third foreign nucleic acid encoding one or more transcriptional regulator proteins or domains,

wherein the RNAs, the orthogonal RNA guided protein nickase, the orthogonal RNA guided nuclease-null DNA binding proteins, and the transcriptional regulator proteins or domains are expressed,

wherein at least one co-localization complex forms between an RNA, an orthogonal RNA guided protein nickase, and a target nucleic acid to be altered and wherein the protein nickase nicks the target nucleic acid to be altered,

wherein at least one co-localization complex forms between an RNA, an orthogonal RNA guided nuclease null DNA binding protein, a transcriptional regulator protein or domain and a target nucleic acid to be regulated and wherein the transcriptional regulator protein or domain regulates expression of the target nucleic acid to be regulated.

10. An in vitro method of altering one or more target nucleic acids in a cell simultaneous with regulating expression of one or more target nucleic acids in a cell comprising

introducing into the cell a first foreign nucleic acid encoding two or more RNAs with at least one RNA being complementary to the one or more target nucleic acids to be altered and at least one RNA being complementary to the one or more target nucleic acids to be regulated,

introducing into the cell a second foreign nucleic acid encoding 1) one orthogonal RNA-guided protein nuclease and being guided by the one or more RNAs complementary to the one or more target nucleic acids to be altered, and 2) one or more orthogonal RNA guided nuclease null DNA binding proteins that bind to and are guided by the one or more RNAs complementary to the target nucleic acids to be regulated,

introducing into the cell a third foreign nucleic acid encoding one or more transcriptional regulator proteins or domains,

wherein the RNAs, the orthogonal RNA guided protein nuclease, the orthogonal RNA guided nuclease-null DNA binding proteins, and the transcriptional regulator proteins or domains are expressed,

wherein at least one co-localization complex forms between an RNA, an orthogonal RNA guided protein nuclease, and a target nucleic acid to be altered and wherein the nuclease cuts the target nucleic acid to be altered,

wherein at least one co-localization complex forms between an RNA, an orthogonal RNA guided nuclease null DNA binding protein, a transcriptional regulator protein or domain and a target nucleic acid to be regulated and wherein the transcriptional regulator protein or domain regulates expression of the target nucleic acid to be regulated.

11. A cell comprising

a first foreign nucleic acid encoding two or more RNAs complementary to two or more respective target nucleic acids,

a second foreign nucleic acid encoding two or more RNA guided nuclease null DNA binding proteins, and

a third foreign nucleic acid encoding two or more transcriptional regulator proteins or domains wherein each RNA, orthogonal RNA guided nuclease null DNA binding protein and transcriptional regulator protein or domain is a member of a co-localization complex for a respective target nucleic acid.

12. The cell of claim 11 wherein:

(1) the second foreign nucleic acid and the third foreign nucleic acid are expressed to produce a transcriptional regulator protein or domain fused to the two or more orthogonal RNA guided nuclease null DNA binding proteins; or

(2) the first foreign nucleic acid encoding two or more RNAs further encodes a target of an RNA-binding domain and the third foreign nucleic acid encoding the transcriptional regulator proteins or domains further encodes an RNA-binding domain fused to the transcriptional regulator proteins or domains.

13. The cell of claim 11 wherein:

(1) the cell is a eukaryotic cell;

(2) the cell is a yeast cell, a plant cell or an animal cell;

(3) the RNAs include between about 10 to about 500 nucleotides;

(4) the RNAs include between about 20 to about 100 nucleotides;

(5) a target nucleic acid is associated with a disease or detrimental condition;

(6) the two or more RNAs are guide RNAs;

(7) the two or more RNAs are a tracrRNA-crRNA fusions; or

(8) the target nucleic acids are genomic DNA, mitochondrial DNA, viral DNA, or exogenous DNA.

14. The cell of claim 11 wherein:

(1) at least one of the transcriptional regulator proteins or domains is a transcriptional activator;

(2) at least one of the transcriptional regulator proteins or domains upregulates expression of the respective target nucleic acid; or

(3) at least one of the transcriptional regulator proteins or domains upregulates expression of a target nucleic acid associated with a disease or detrimental condition.

15. The method of claim 1 wherein:

(1) the RNA guided nuclease null DNA binding proteins are DNA binding proteins of a Type II CRISPR system;

(2) the RNA guided nuclease null DNA binding proteins are orthogonal nuclease-null Cas9 proteins.