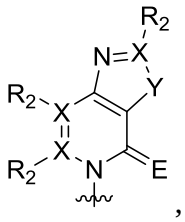
CLAIMS

WHAT IS CLAIMED IS:

1. A nucleobase of the structure:



,

wherein:

each X is independently carbon or nitrogen;

R_2 is present when X is carbon and is independently hydrogen, alkyl, alkenyl, alkynyl, methoxy, methanethiol, methaneseleno, halogen, cyano, or azide group;

Y is sulfur, oxygen, selenium, or secondary amine; and

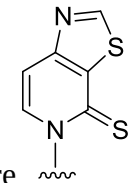
E is oxygen, sulfur, or selenium;

wherein the wavy line indicates a point of bonding to a ribosyl, deoxyribosyl, or dideoxyribosyl moiety or an analog thereof, wherein the ribosyl, deoxyribosyl, or dideoxyribosyl moiety or analog thereof is in free form, is connected to a mono-phosphate, diphosphate, triphosphate, α-thiotriphosphate, β-thiotriphosphate, or γ-thiotriphosphate group, or is included in an RNA or a DNA or in an RNA analog or a DNA analog.

2. The nucleobase of claim 1, wherein X is carbon.

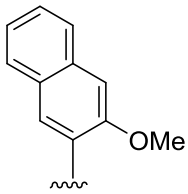
3. The nucleobase of claim 1 or 2, wherein E is sulfur.

4. The nucleobase of any one of claims 1 to 3, wherein Y is sulfur.

5. The nucleobase of claim 1, which has the structure .

6. The nucleobase of any one of claims 1 to 5, which is bound to a complementary base-pairing nucleobase to form an unnatural base pair (UBP).

7. The nucleobase of claim 6, wherein the complementary base-pairing nucleobase is selected from:



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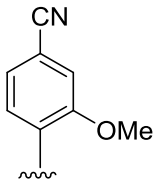

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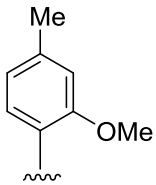

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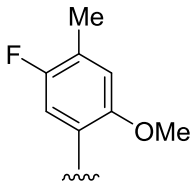

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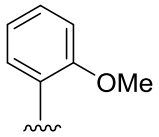

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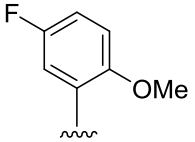

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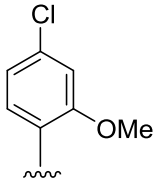

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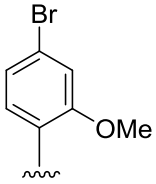

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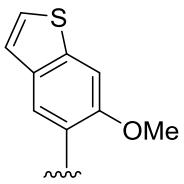

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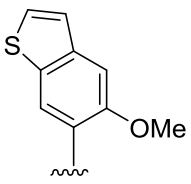

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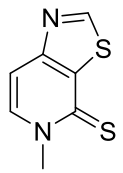
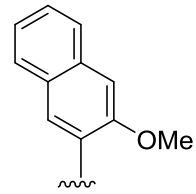
8. A double stranded oligonucleotide duplex wherein a first oligonucleotide strand comprises the nucleobase of any one of claims 1 to 5, and a second complementary oligonucleotide strand comprises a complementary base-pairing nucleobase in a complementary base-pairing site thereof.

9. The double stranded oligonucleotide duplex of claim 8, wherein the first oligonucleotide strand comprises 

; and the second strand comprises a complementary base pairing nucleobase selected from:



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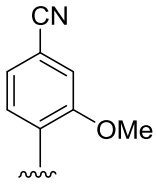

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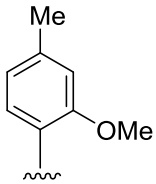

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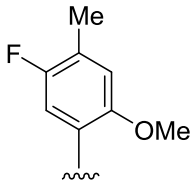

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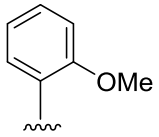

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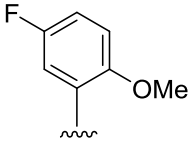

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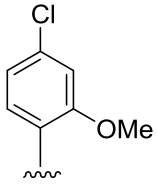

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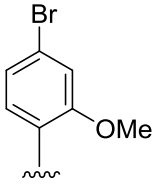

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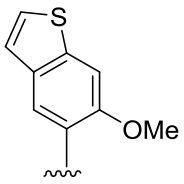

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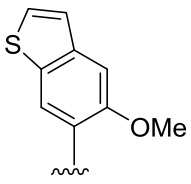
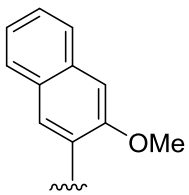


in a complementary base-pairing site thereof.

10. The double stranded oligonucleotide duplex of claim 9, wherein the second strand comprises the complementary base pairing nucleobase 

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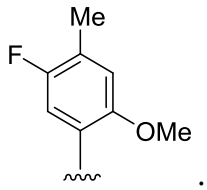
11. The double stranded oligonucleotide duplex of claim 9, wherein the second strand comprises the complementary base pairing nucleobase 

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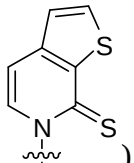
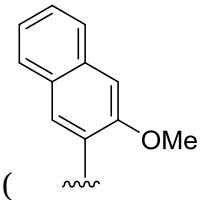
12. A plasmid comprising a gene encoding a transfer RNA (tRNA) and/or a gene encoding a protein of interest, wherein the gene comprises at least one nucleobase of any one of claims 1 to 5 or TPT3 (

) and at least one complementary base-pairing nucleobase of claim 7 or NaM (

), wherein the complementary base-pairing nucleobase is in a complementary base-pairing site.

13. An mRNA encoded by the plasmid of claim 10 encoding the tRNA.

14. An mRNA encoded by the plasmid of claim 10 encoding the protein.

15. A transfer RNA (tRNA) comprising the nucleobase of any one of claims 1 to 5, comprising:

an anticodon, wherein the anticodon comprises the nucleobase, optionally wherein the nucleobase is at the first, second, or third position of the anticodon; and

a recognition element, wherein the recognition element promotes selective charging of the tRNA with an unnatural amino acid by an aminoacyl tRNA synthetase.

16. The tRNA of claim 15, wherein the aminoacyl tRNA synthetase is derived from *Methanosarcina*, or a variant thereof, or *Methanococcus* (*Methanocaldococcus*) or a variant thereof.

17. The tRNA of claim 15, wherein the unnatural amino acid comprises an aromatic moiety.

18. The tRNA of claim 15, wherein the unnatural amino acid is a lysine or phenylalanine derivative.

19. A structure comprising the formula:

N1 - Zx - N2

wherein:

each Z is independently a nucleobase of any one of claims 1 to 7, which is bonded to a ribosyl or deoxyribosyl or analog thereof;

N1 is one or more nucleotides or analogs thereof, or a terminal phosphate group attached at the 5’ end of the ribosyl or deoxyribosyl or analog thereof of Z;

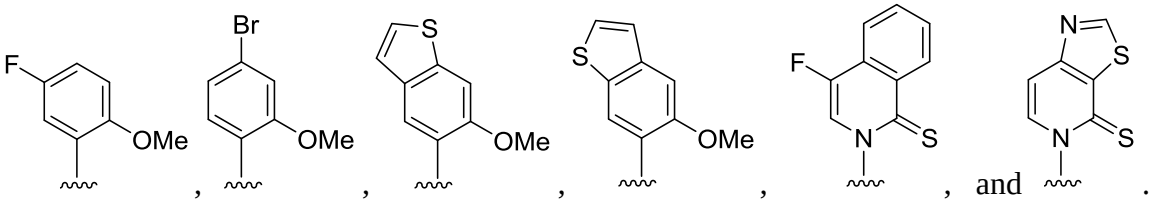
N2 is one or more nucleotides or analogs thereof, or a terminal hydroxyl group attached to the 3’ end of the ribosyl or deoxyribosyl or analog thereof of Z; and

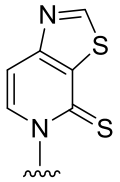
x is an integer from 1 to 20.

20. The structure of claim 19, wherein the structure encodes a gene, optionally wherein Zx is located in a translated region of the gene or wherein Zx is located in an untranslated region of the gene.

21. A polynucleotide library, wherein the library comprises at least 5000 unique polynucleotides, and wherein each polynucleotide comprises at least one nucleobase of any one of claims 1 to 5.

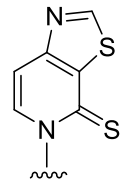
22. A nucleoside triphosphate comprising a nucleobase, wherein the nucleobase is selected from:

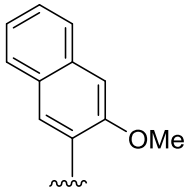




23. The nucleoside triphosphate of claim 22, wherein the nucleobase is .

24. The nucleoside triphosphate of claim 22 or 23, wherein the nucleoside comprises ribose or deoxyribose.

25. A DNA comprising a nucleobase having the structure 

, and a complementary base-pairing nucleobase having the structure 

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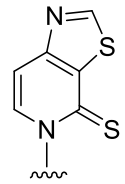
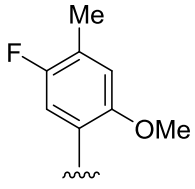
26. A DNA comprising a nucleobase having the structure 

, and a complementary base-pairing nucleobase having the structure 

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