CLAIMS

1. An engineered host cell comprising:

   a. a first nucleic acid molecule comprising an unnatural nucleotide;

   b. a second nucleic acid molecule comprising:

      (i) a deletion of a gene encoding a transposition-associated protein or transposable element, wherein the transposition-associated protein comprises InsB-4, wherein the transposable element comprises IS*1*, ~~optionally~~ and wherein the deletion is an N-terminal deletion, a C-terminal deletion, a truncation at both termini, an internal deletion, and/or a deletion of the entire gene, wherein when the gene or a portion thereof is deleted, it or the portion thereof is absent; or

      (ii) a gene encoding a mutated transposition-associated protein or transposable element, wherein the transposition-associated protein comprises InsB-4, wherein the transposable element comprises IS*1*, and wherein the mutated transposition-associated protein or transposable element is not expressed, or is expressed at reduced levels; and

   c. a third nucleic acid molecule encoding a nucleoside triphosphate transporter that imports unnatural nucleic acids into the cell, wherein the nucleoside triphosphate transporter comprises a nucleoside triphosphate transporter from *Phaeodactylum tricornutum* and further comprises an N-terminal truncation or a truncation of both termini, wherein the third nucleic acid molecule is incorporated in a genomic sequence of the engineered host cell, or comprises a plasmid encoding the nucleoside triphosphate transporter.

2. The engineered host cell of claim 1, wherein the nucleoside triphosphate transporter exhibits increased stability of expression in the engineered host cell as compared to an expression in an equivalent engineered host cell that does not comprise the second nucleic acid molecule.

~~3. The engineered host cell of claim 1, wherein the nucleoside triphosphate transporter comprises an N-terminal truncation, a C-terminal truncation, or a truncation of both termini.~~

~~4. The engineered host cell of claim 1, wherein the nucleoside triphosphate transporter~~

~~comprises a nucleoside triphosphate transporter from *Phaeodactylum tricornutum.*~~

~~5.~~3. The engineered host cell of ~~claim 1~~any one of claims 1-2, wherein the nucleoside triphosphate transporter comprises *Pt*NTT2.

~~6.~~4. The engineered host cell of claim ~~4 or 5~~3, wherein the nucleoside triphosphate transporter from *Phaeodactylum tricornutum* or *Pt*NTT2 is under the control of a promoter selected from a pSC plasmid or a promoter from a lac operon.

~~7.~~5. The engineered host cell of any one of claims 1-4, further comprising:

a. a Cas9 polypeptide~~ or a variant thereof~~; and

b. a single guide RNA (sgRNA) comprising a crRNA-tracrRNA scaffold, wherein the combination of the Cas9 polypeptide or the variant thereof and the sgRNA modulates replication of the first nucleic acid molecule comprising the unnatural nucleotide.

~~8.~~6. The engineered host cell of ~~claim 1~~any one of claims 1-5, wherein the second nucleic acid molecule comprises a deletion of a gene encoding a transposition-associated protein, wherein the transposition-associated protein comprises InsB-4, or the second nucleic acid comprises a gene encoding a mutated transposition-associated protein, wherein the transposition-associated protein comprises I~~i~~nsB-4~~ and/or insA-4, or the gene encoding a mutated transposition-associated protein comprises a mutated *insB-4* gene and/or a mutated *insA-4* gene~~.

~~9.~~7. The engineered host cell of ~~claim 1~~any one of claims 1-5, wherein the second nucleic acid molecule comprises a deletion of a gene encoding a transposable element, wherein the transposable element comprises IS*1*.

~~10.~~8. The engineered host cell of claim 1, wherein the second nucleic acid molecule comprises a deletion of a gene encoding a transposition-associated protein, wherein the transposition-associated protein comprises InsB-4, and ~~further~~ wherein the deletion comprises an N-terminal deletion, a C-terminal deletion, a truncation at both termini, an internal deletion, and/or a deletion of the entire gene.

~~11.~~9. The engineered host cell of ~~claim 1~~any one of claims 1-8, further comprising a fourth nucleic acid molecule encoding a modified DNA repair response-associated protein, wherein

the DNA repair response comprises recombinational repair, SOS response, nucleotide excision repair, or methyl-directed mismatch repair, or a combination thereof.

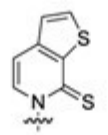
~~12.~~10. The engineered host cell of claim ~~11~~9, wherein the modified DNA repair response-associated protein comprises RecA, RadA, or LexA, or a combination thereof.

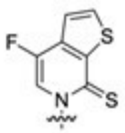
~~13.~~11. The engineered host cell of ~~claim 1~~any one of claims 1-10, wherein the engineered host cell is a prokaryotic cell comprising an *Escherichia coli* cell or an *Escherichia coli* BL21 (DE3) cell.

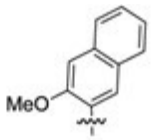
~~14.~~12. The engineered host cell of ~~claim 1~~any one of claims 1-11, wherein the unnatural nucleotide comprises an unnatural base selected from the group consisting of 2-aminoadenin-9-yl, 2-aminoadenine, 2- F-adenine, 2-thiouracil, 2-thio-thymine, 2-thiocytosine, 2-propyl and alkyl derivatives of adenine and guanine, 2-amino-adenine, 2-amino-propyl-adenine, 2-aminopyridine, 2- pyridone, 2'-deoxyuridine, 2-amino-2'-deoxyadenosine 3-deazaguanine, 3-deazaadenine, 4- thio-uracil, 4-thio-thymine, uracil-5-yl, hypoxanthin-9-yl (I), 5-methyl-cytosine, 5- hydroxymethyl cytosine, xanthine, hypoxanthine, 5-bromo, and 5-trifiuoromethyl uracils and cytosines; 5-halouracil, 5-halocytosine, 5-propynyl-uracil, 5-propynyl cytosine, 5-uracil, 5- substituted, 5-halo, 5-substituted pyrimidines, 5-hydroxycytosine, 5-bromocytosine, 5- bromouracil, 5-chlorocytosine, chlorinated cytosine, cyclocytosine, cytosine arabinoside, 5- fluorocytosine, fluoropyrimidine, fluorouracil, 5,6-dihydrocytosine, 5-iodocytosine, hydroxyurea, iodouracil, 5-nitrocytosine, 5- bromouracil, 5-chlorouracil, 5- fluorouracil, and 5-iodouracil, 6-alkyl derivatives of adenine and guanine, 6-azapyrimidines, 6-azo-uracil, 6-azo cytosine, azacytosine, 6-azo-thymine, 6-thio-guanine, 7-methylguanine, 7-methyladenine, 7-deazaguanine, 7-deazaguanosine, 7-deaza-adenine, 7-deaza-8-azaguanine, 8-azaguanine, 8-azaadenine, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, and 8-hydroxyl substituted adenines and guanines; N4-ethylcytosine, N-2 substituted purines, N-6 substituted purines, O-6 substituted purines, those that increase the stability of duplex formation, universal nucleic acids, hydrophobic nucleic acids, promiscuous nucleic acids, size-expanded nucleic acids, fluorinated nucleic acids, tricyclic pyrimidines, phenoxazine cytidine([5,4-b][l,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H- pyrimido[5,4-b][l,4]benzothiazin-2(3H)-one), G-clamps, phenoxazine cytidine (9-(2-aminoethoxy)-H-pyrimido[5,4-b][l,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5- b]indol-2-one), pyridoindole cytidine (H-pyrido [3',2':4,5]pyrrolo [2,3-d]pyrimidin-2-one), 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-

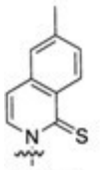
carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methythio-N6-isopentenyladenine, uracil-5oxyacetic acid, wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxacetic acid methylester, uracil-5-oxacetic acid, 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine and those in which the purine or pyrimidine base is replaced with a heterocycle.

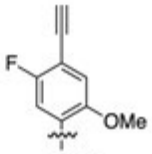
~~15.~~13. The engineered host cell of ~~claim 1~~any one of claims 1-11, wherein the unnatural nucleotide comprises an unnatural base selected from the group consisting of:

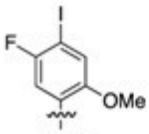


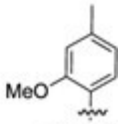
dTPT3 ,


dFTPT3 ,


dNaM ,


d5SICS ,


dFEMO ,


dFIMO and


dMMO2

.

~~16.~~14. The engineered host cell of ~~claim 1~~any one of claims 1-13, wherein the unnatural nucleotide further comprises a modified sugar moiety.

~~17.~~15. The engineered host cell of claim ~~16~~14, wherein the modified sugar moiety is selected from the group consisting of:

(a) a modification at the 2' position comprising:

(i) OH;

(ii) substituted lower alkyl, alkaryl, aralkyl, O-alkaryl, O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2 CH_3, ONO_2, NO_2, N_3, or NH_2F;

(iii) O-alkyl, S-alkyl, or N-alkyl;

(iv) O-alkenyl, S- alkenyl, or N-alkenyl;

(v) O-alkynyl, S-alkynyl, or N-alkynyl; or

(vi) O-alkyl-O-alkyl, 2'-F, 2'-OCH_3, or 2'-$O(CH_2)_2OCH_3$

wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1-C_{10}, alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, -$O[(CH_2)_n O]_mCH_3$, -$O(CH_2)_nOCH_3$, - $O(CH_2)_n NH_2$, -$O(CH_2)_n CH_3$, -$O(CH_2)_n$ -ONH_2, and -$O(CH_2)_nON[(CH_2)_n CH_3)]_2$,

and wherein n and m are from 1 to ~~about~~ 10;

(b) a modification at the 5' position comprising 5'-vinyl, or 5'-methyl (R or S);

(c) a modification at the 4' position comprising 4'-S, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide; and

(d) any combination of (a) to (c).

16. The engineered host cell of any one of claims 1-15, further comprising a derepressed *polB* gene.

17. The engineered host cell of any one of claims 1-15, wherein the engineered host cell constitutively expresses or overexpresses DNA polymerase II.

18. A method of increasing the production of a first nucleic acid molecule comprising an unnatural nucleotide, comprising:

a) incubating an engineered host cell with a plurality of unnatural nucleotides, wherein the engineered host cell comprises a nucleoside triphosphate transporter that imports unnatural nucleic acids into the cell, wherein the nucleoside triphosphate transporter comprises a nucleoside triphosphate transporter from *Phaeodactylum tricornutum* and further comprises an N-terminal truncation or a truncation of both termini, and a second nucleic acid molecule comprising: (i) a deletion of a gene encoding a transposition-associated protein or transposable element, wherein the transposition-associated protein comprises InsB-4, wherein the transposable element comprises IS*1*, and ~~optionally~~ wherein the deletion is an N-terminal

deletion, a C-terminal deletion, a truncation at both termini, an internal deletion, and/or a deletion of the entire gene, wherein when the gene or a portion thereof is deleted, it or the portion thereof is absent; or (ii) a gene encoding a mutated transposition-associated protein or transposable element, wherein the transposition-associated protein comprises InsB-4, wherein the transposable element comprises IS*1*, and wherein the mutated transposition-associated protein or transposable element is not expressed, or is expressed at reduced levels; and

b) incorporating the plurality of unnatural nucleotides into the newly-synthesized first nucleic acid molecule;

wherein the second nucleic acid molecule and the nucleoside triphosphate transporter increases retention of unnatural base pairs comprising the unnatural nucleotides in the first nucleic acid molecule.

19. The method of claim 18, wherein the second nucleic acid molecule comprises a deletion of a gene encoding a transposition-associated protein, wherein the transposition-associated protein comprises InsB-4, or the second nucleic acid comprises a gene encoding a mutated transposition-associated protein, wherein the transposition-associated ~~comprises~~ protein comprises I~~i~~nsB-4 ~~and/or *insA-4*~~.

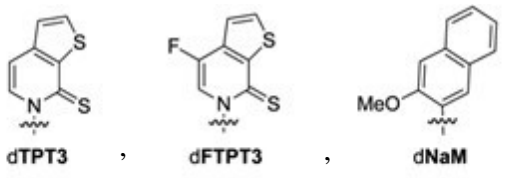
20. The method of claim 18, wherein the second nucleic acid molecule comprises a deletion of a gene encoding a transposable element, wherein the transposable element comprises IS*1*.

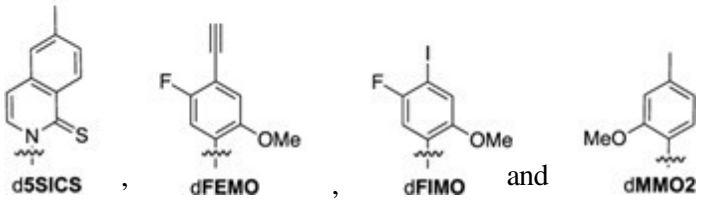
21. The method of ~~claim 18~~any one of claims 18-20, wherein the nucleoside triphosphate transporter comprises a codon optimized nucleoside triphosphate transporter from *Phaeodactylum tricornutum* (*Pt*NTT2~~2~~).

22. The method of ~~claim 18~~any one of claims 18-21, wherein a least one ~~the~~ unnatural nucleotide in the plurality of unnatural nucleotides comprises an unnatural base selected from the group consisting of 2-aminoadenin-9-yl, 2-aminoadenine, 2-F-adenine, 2- thiouracil, 2-thio-thymine, 2-thiocytosine, 2-propyl and alkyl derivatives of adenine and guanine, 2-amino-adenine, 2-amino-propyl-adenine, 2-aminopyridine, 2-pyridone, 2'- deoxyuridine, 2-amino-2'-deoxyadenosine 3-deazaguanine, 3-deazaadenine, 4-thio-uracil, 4-thio-thymine, uracil-5-yl, hypoxanthin-9-yl (I), 5-methyl-cytosine, 5-hydroxymethyl cytosine,

xanthine, hypoxanthine, 5-bromo, and 5-trifiuoromethyl uracils and cytosines; 5-halouracil, 5-halocytosine, 5-propynyl-uracil, 5-propynyl cytosine, 5-uracil, 5-substituted, 5-halo, 5-substituted pyrimidines, 5-hydroxycytosine, 5-bromocytosine, 5-bromouracil, 5-chlorocytosine, chlorinated cytosine, cyclocytosine, cytosine arabinoside, 5-fluorocytosine, fluoropyrimidine, fluorouracil, 5,6-dihydrocytosine, 5-iodocytosine, hydroxyurea, iodouracil, 5-nitrocytosine, 5- bromouracil, 5-chlorouracil, 5- fluorouracil, and 5-iodouracil, 6-alkyl derivatives of adenine and guanine, 6-azapyrimidines, 6-azo-uracil, 6-azo cytosine, azacytosine, 6-azo-thymine, 6-thio-guanine, 7-methylguanine, 7-methyladenine, 7-deazaguanine, 7-deazaguanosine, 7-deaza-adenine, 7-deaza-8-azaguanine, 8-azaguanine, 8-azaadenine, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, and 8-hydroxyl substituted adenines and guanines; N4-ethylcytosine, N-2 substituted purines, N-6 substituted purines, O-6 substituted purines, those that increase the stability of duplex formation, universal nucleic acids, hydrophobic nucleic acids, promiscuous nucleic acids, size-expanded nucleic acids, fluorinated nucleic acids, tricyclic pyrimidines, phenoxazine cytidine([5,4-b][l,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H- pyrimido[5,4-b][l,4]benzothiazin-2(3H)-one), G-clamps, phenoxazine cytidine (9-(2-aminoethoxy)-H-pyrimido[5,4-b][l,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5- b]indol-2-one), pyridoindole cytidine (H-pyrido [3',2':4,5]pyrrolo [2,3-d]pyrimidin-2-one), 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methythio-N6-isopentenyladenine, uracil-5oxyacetic acid, wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxacetic acid methylester, uracil-5-oxacetic acid, 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine and those in which the purine or pyrimidine base is replaced with a heterocycle.

23. The method of ~~claim 18~~any one of claims 18-22, wherein at least one ~~the~~ unnatural nucleotide in the plurality of unnatural nucleotides comprises an unnatural base selected from the group consisting of:





and

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24. The method of ~~claim 18~~any one of claims 18-23, wherein at least one ~~the~~ unnatural nucleotide in the plurality of unnatural nucleotides further comprises a modified sugar moiety.

25. The method of claim 24, wherein the modified sugar moiety is selected from the group consisting of:

(a) a modification at the 2' position comprising:

(i) OH;

(ii) substituted lower alkyl, alkaryl, aralkyl, O-alkaryl, O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2 CH_3, ONO_2, NO_2, N_3, or NH_2F;

(iii) O-alkyl, S-alkyl, or N-alkyl;

(iv) O-alkenyl, S- alkenyl, or N-alkenyl;

(v) O-alkynyl, S-alkynyl, or N-alkynyl; or

(vi) O-alkyl-O-alkyl, 2'-F, 2'-OCH_3, or 2'-$O(CH_2)_2OCH_3$

wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1-C_{10}, alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, -O[(CH2)n O]mCH3, -O(CH2)nOCH3, -O(CH2)n NH2, -O(CH2)n CH3, -O(CH2)n -ONH2, and -O(CH2)nON[(CH2)n CH3)]2, and wherein

n and m are from 1 to ~~about~~ 10;

(b) a modification at the 5' position comprising 5'-vinyl, or 5'-methyl (R or S);

(c) a modification at the 4' position comprising 4'-S, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the

pharmacodynamic properties of an oligonucleotide; and

(d) any combination of (a) to (c).

26. A method of preparing a modified polypeptide comprising an unnatural amino acid that is encoded by a first nucleic acid molecule comprising an unnatural nucleotide, comprising:

a) incubating an engineered host cell with a plurality of unnatural nucleotides, wherein the engineered host cell comprises a nucleoside triphosphate transporter that imports unnatural nucleic acids into the cell and a second nucleic acid molecule comprising: (i) a deletion of a gene encoding a transposition-associated protein or transposable element, wherein the transposition-associated protein comprises InsB-4, wherein the transposable element comprises IS*1*, ~~optionally~~ and wherein the deletion is an N-terminal deletion, a C-terminal deletion, a truncation at both termini, an internal deletion, and/or a deletion of the entire gene, wherein when the gene or a portion thereof is deleted, it or the portion thereof is absent; or (ii) a gene encoding a mutated transposition-associated protein or transposable element, wherein the transposition-associated protein comprises InsB-4, wherein the transposable element comprises IS*1*, and wherein the mutated transposition-associated protein or transposable element is not expressed, or is expressed at reduced levels; and a third nucleic acid molecule encoding a nucleoside triphosphate transporter that imports unnatural nucleic acids into the cell, wherein the nucleoside triphosphate transporter comprises a nucleoside triphosphate transporter from *Phaeodactylum tricornutum* and further comprises an N-terminal truncation or a truncation of both termini; and

b) incorporating the plurality of unnatural nucleotides into the newly-synthesized first nucleic acid molecule;

wherein the second nucleic acid molecule and the nucleoside triphosphate transporter increases retention of unnatural base pairs comprising the unnatural nucleotides in the first nucleic acid molecule which facilitates incorporation of the plurality of unnatural nucleotides into a newly synthesized polypeptide to generate the modified polypeptide.

27. The method of claim 26, wherein the second nucleic acid molecule comprises a deletion of a gene encoding a transposition-associated protein, wherein the transposition-associated protein comprises ~~*insB*~~InsB-4 ~~and/or *insA-4*~~, or the second nucleic acid molecule comprises a gene encoding a mutated transposition-associated protein, wherein the transposition-associated protein comprises ~~a mutated *insB*~~InsB-4 ~~gene and/or a mutated *insA-4* gene~~.

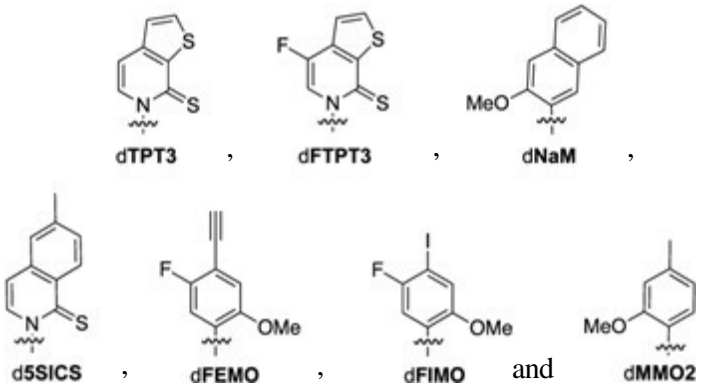
28. The method of claim 26, wherein the second nucleic acid molecule comprises a deletion of a gene encoding a transposable element, wherein the transposable element comprises IS*1*.

29. The method of ~~claim 26~~any one of claims 26-28, wherein the nucleoside triphosphate transporter comprises a codon optimized nucleoside triphosphate transporter from *Phaeodactylum tricornutum* (*Pt*NTT2).

30. The method of ~~claim 26~~any one of claims 26-29, wherein at least one ~~the~~ unnatural nucleotide in the plurality of unnatural nucleotides comprises an unnatural base selected from the group consisting of 2-aminoadenin-9-yl, 2-aminoadenine, 2-F-adenine, 2- thiouracil, 2-thio-thymine, 2-thiocytosine, 2-propyl and alkyl derivatives of adenine and guanine, 2-amino-adenine, 2-amino-propyl-adenine, 2-aminopyridine, 2-pyridone, 2'- deoxyuridine, 2-amino-2'-deoxyadenosine 3-deazaguanine, 3-deazaadenine, 4-thio-uracil, 4- thio-thymine, uracil-5-yl, hypoxanthin-9-yl (I), 5-methyl-cytosine, 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 5-bromo, and 5-trifiuoromethyl uracils and cytosines; 5-halouracil, 5-halocytosine, 5-propynyl-uracil, 5-propynyl cytosine, 5-uracil, 5-substituted, 5-halo, 5-substituted pyrimidines, 5-hydroxycytosine, 5-bromocytosine, 5-bromouracil, 5-chlorocytosine, chlorinated cytosine, cyclocytosine, cytosine arabinoside, 5-fluorocytosine, fluoropyrimidine, fluorouracil, 5,6-dihydrocytosine, 5-iodocytosine, hydroxyurea, iodouracil, 5-nitrocytosine, 5- bromouracil, 5-chlorouracil, 5- fluorouracil, and 5-iodouracil, 6-alkyl derivatives of adenine and guanine, 6-azapyrimidines, 6-azo-uracil, 6-azo cytosine, azacytosine, 6-azo-thymine, 6-thio-guanine, 7-methylguanine, 7-methyladenine, 7-deazaguanine, 7-deazaguanosine, 7-deaza-adenine, 7-deaza-8-azaguanine, 8-azaguanine, 8-azaadenine, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, and 8-hydroxyl substituted adenines and guanines; N4-ethylcytosine, N-2 substituted purines, N-6 substituted purines, O-6 substituted purines, those that increase the stability of duplex formation, universal nucleic acids, hydrophobic nucleic acids, promiscuous nucleic acids, size-expanded nucleic acids, fluorinated

nucleic acids, tricyclic pyrimidines, phenoxazine cytidine([5,4-b][l,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H- pyrimido[5,4-b][l,4]benzothiazin-2(3H)-one), G-clamps, phenoxazine cytidine (9-(2-aminoethoxy)-H-pyrimido[5,4-b][l,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5- b]indol-2-one), pyridoindole cytidine (H-pyrido [3',2':4,5]pyrrolo [2,3-d]pyrimidin-2-one), 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methythio-N6-isopentenyladenine, uracil-5oxyacetic acid, wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxacetic acid methylester, uracil-5-oxacetic acid, 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine and those in which the purine or pyrimidine base is replaced with a heterocycle.

31. The method of ~~claim 26~~any one of claims 26-29, wherein at least one ~~the~~ unnatural nucleotide in the plurality of unnatural nucleotides comprises an unnatural base selected from the group consisting of:



and

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32. The method of ~~claim 26~~any one of claims 26-31, wherein at least one ~~the~~ unnatural nucleotide in the plurality of unnatural nucleotides further comprises a modified sugar moiety selected from the group consisting of:

(a) a modification at the 2' position comprising:

(i) OH;

(ii) substituted lower alkyl, alkaryl, aralkyl, O-alkaryl, O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2 CH_3, ONO_2, NO_2, N_3, or NH_2F;

(iii) O-alkyl, S-alkyl, or N-alkyl

(iv) O-alkenyl, S- alkenyl, or N-alkenyl;

(v) O-alkynyl, S-alkynyl, or N-alkynyl; or

(vi) O-alkyl-O-alkyl, 2'-F, 2'-OCH_3, or 2'-$O(CH_2)_2OCH_3$

wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1-C_{10}, alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, -$O[(CH_2)_n O]_mCH_3$, -$O(CH_2)_nOCH_3$, -$O(CH_2)_n NH_2$, -$O(CH_2)_n CH_3$, -$O(CH_2)_n$ -ONH_2, and -$O(CH_2)_nON[(CH_2)_n CH_3)]_2$, and wherein n and m are from 1 to ~~about~~ 10;

(b) a modification at the 5' position comprising 5'-vinyl, or 5'-methyl (R or S);

(c) a modification at the 4' position comprising 4'-S, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide; and

(d) any combination of (a) to (c).

33. The method of any one of claims 18-32, wherein the engineered host cell further comprises a derepressed *polB* gene.

34. The method of any one of claims 18-32, wherein the engineered host cell constitutively expresses or overexpresses DNA polymerase II.

~~33.~~35. The engineered host cell of any one of claims ~~11~~9-17, wherein the fourth nucleic acid molecule encoding a modified DNA repair response-associated protein comprises an N-terminal deletion, a C-terminal deletion, a truncation at both termini, an internal deletion, and/or a deletion of the entire gene.

~~34.~~36. The engineered host cell of any one of claims ~~11~~9-17, wherein the engineered host cell exhibits improved retention of the nucleic acid molecule comprising the unnatural nucleotide during DNA replication as compared to a nucleic acid molecule comprising the

unnatural nucleotide in an equivalent engineered host cell that does not comprise the fourth nucleic acid molecule encoding the modified DNA repair response-associated protein.

~~35.~~37. The method of ~~claim 18~~any one of claims 18-25, wherein the nucleoside triphosphate transporter exhibits increased stability of expression in the engineered host cell relative to expression in an equivalent engineered host cell that does not comprise the second nucleic acid molecule.