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(54) Title: METHODS AND COMPOSITIONS FOR TREATING A PREMATURE TERMINATION CODON-MEDIATED DISORDER

(57) Abstract: The invention relates generally to expression vectors and pharmaceutical compositions comprising a first, second, and/or third modified tRNA and the use of expression vectors and pharmaceutical compositions to express in a mammalian cell a functional gene product encoded by a gene containing a premature termination codon and/or to treat a disorder mediated by a premature termination codon, e.g., Dravet syndrome.



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**METHODS AND COMPOSITIONS FOR TREATING
A PREMATURE TERMINATION CODON-MEDIATED DISORDER**

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application
5 No. 63/184,514, filed May 5, 2021, which is incorporated herein by reference in its entirety for
all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted
electronically in ASCII format and is hereby incorporated by reference in its entirety. Said
10 ASCII copy, created on April 26, 2022, is named TVD-004WO_SL.txt and is 402,848 bytes in
size.

FIELD OF THE INVENTION

[0003] The invention relates generally to methods and compositions for expressing a gene
product encoded by a gene containing a premature termination codon and/or treating a disorder
15 mediated by a premature termination codon.

BACKGROUND

[0004] Protein synthesis is directed by a genetic code that includes 61 three-base-pair codons
encoding amino acids that are incorporated into the protein being synthesized and 3 three-base-
pair codons (referred to as stop or termination codons) that terminate the synthesis of a protein.
20 When a nucleic acid sequence encoding a protein is mutated to contain a premature termination
codon rather than a codon for the next amino acid, the resulting protein is prematurely
terminated, which is often nonfunctional or less functional than the untruncated or full length
protein. Such mutations, termed nonsense mutations, are often associated with, or are a
causative agent in numerous different genetic diseases.

25 [0005] A number of disorders are associated with, or are caused by, nonsense mutations. These
include epilepsies, for example, Dravet Syndrome, Genetic Epilepsy with Febrile Seizures
(GEFS), Benign Familial Infantile Epilepsy (BFIE), Early Infantile Epileptic Encephalopathy
(EIEE), Lennox-Gastaut Syndrome, Rett Syndrome, PPM-X Syndrome, Ohtahara Syndrome,
Episodic Ataxia, Hemiplegic Migraine, Idiopathic Generalized Epilepsy, FOXG1 Syndrome,
30 Familial Focal Epilepsy with Variable Foci (FFEVF), Childhood-Onset Epileptic
Encephalopathy, SYNGAP1-Related Intellectual Disability, Pyridoxine-Dependent Epilepsy,
Familial Infantile Myoclonic Epilepsy (FIME), Myoclonic Astatic Epilepsy, X-Linked

Intellectual Disability, Partial Epilepsy and Episodic Ataxia, Febrile Seizures, Autosomal Dominant Partial Epilepsy with Auditory Features (ADPEAF), PNPO-Deficiency, Progressive Myoclonus Epilepsy, Action Myoclonus – Renal Failure (AMRF), CDKL5 deficiency disorder, and Benign Familial Infantile Seizures (BFIS).

- 5 [0006] By way of example, Dravet Syndrome is a rare and catastrophic form of intractable epilepsy that begins in infancy. Initially, patients experience prolonged seizures. In their second year, additional types of seizure begin to occur, which typically coincide with a developmental decline, possibly due to repeated cerebral hypoxia. This leads to poor development of language and motor skills. Mutations in SCN1A (encoding the voltage-gated sodium channel α subunit
- 10 Nav1.1), SCN1B (encoding the voltage-gated sodium channel β 1 subunit), SCN2A (encoding Nav1.2), SCN3A (encoding Nav1.3), SCN9A (encoding Nav1.7), GABRG2 (encoding the γ -aminobutyric acid receptor γ 2 subunit), GABRD (encoding the γ -aminobutyric acid receptor Δ subunit) and/or PCDH19 (encoding Protocadherin-19) genes have been linked to Dravet Syndrome.
- 15 [0007] Dravet syndrome may be caused by a nonsense mutation in, for example, the SCN1A gene, resulting in a premature termination codon and a lack of or reduced amount of untruncated or functional protein. The SCN1A gene normally codes for the neuronal voltage-gated sodium channel α subunit, Na(V)1.1. In mouse models, loss-of-function mutations in SCN1A have been observed to result in a decrease in sodium currents and impaired excitability of GABAergic
- 20 interneurons of the hippocampus.
- [0008] Despite the efforts made to date, there is a need in the art for improved compositions and methods for treating disorders mediated by premature termination codons, such as Dravet syndrome.

SUMMARY OF THE INVENTION

- 25 [0009] The invention is based, in part, upon the discovery that is possible to express multiple (*e.g.*, two or three) suppressor tRNAs using a single expression vector. Each suppressor tRNA permits an amino acid to be incorporated into a gene product encoded by a gene in a mammalian cell at a position that would otherwise result in a truncated gene product caused by a premature termination codon (PTC) in the gene. Expression of multiple suppressor tRNAs from a single
- 30 expression vector allows for the single expression vector to treat a disease mediated by multiple, different PTCs in the same subject and/or treat a disease mediated by multiple, different PTCs in multiple, different subjects. The invention is further based, in part, upon the discovery of

optimal combinations of suppressor tRNAs that allow for treatment of the greatest possible patient populations.

[0010] Accordingly, in one aspect, the invention provides an expression vector comprising: (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid.

[0011] In certain embodiments, the first amino acid is selected from arginine, tryptophan, cysteine, serine, glycine, and leucine (*e.g.*, the first amino acid is arginine). In certain embodiments, the second amino acid is selected from glutamine, glutamic acid, tyrosine, tryptophan, lysine, serine, and leucine (*e.g.*, the second amino acid is glutamine). In certain embodiments, the third amino acid is selected from glutamine, glutamic acid, tyrosine, lysine, serine, and leucine. In certain embodiments, the second and third amino acid are the same, for example, the second and third amino acid are selected from glutamine, glutamic acid, tyrosine, lysine, serine, and leucine.

[0012] In certain embodiments: (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is lysine; (ii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamic acid; (iii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is tyrosine; (iv) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is leucine; (v) the first amino acid is arginine, the second amino acid is tryptophan, and the third amino acid is glutamic acid; or (vi) the first amino acid is arginine, the second amino acid is tyrosine, and the third amino acid is glutamic acid. In certain embodiments: (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamine; (ii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamic acid; (iii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is lysine; (iv) the first amino acid is arginine, the second amino acid is tryptophan, and the third amino acid is glutamine; or (v) the first amino acid is arginine, the second amino acid is glutamic acid, and the third amino acid is glutamine. In certain embodiments: (i) the first amino acid is

arginine, the second amino acid is glutamine, and the third amino acid is glutamine; (ii) the first amino acid is tryptophan, the second amino acid is glutamic acid, and the third amino acid is glutamic acid; (iii) the first amino acid is cysteine, the second amino acid is tyrosine, and the third amino acid is tyrosine; (iv) the first amino acid is serine, the second amino acid is lysine, and the third amino acid is lysine; (v) the first amino acid is glycine, the second amino acid is serine, and the third amino acid is serine; or (vi) the first amino acid is leucine, the second amino acid is leucine, and the third amino acid is leucine.

[0013] In certain embodiments, the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**. For example, (i) when the first amino acid is arginine, the first suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-22, and 35, (ii) when the second amino acid is glutamine, the second suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187, and/or (iii) when the third amino acid is glutamine, the third suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45.

[0014] In certain embodiments, the expression vector comprises 1, 2, 3, 4, or more than 4 copy numbers of the nucleotide sequence encoding the first, second, and/or third suppressor tRNA.

[0015] In certain embodiments, the expression vector comprises a nucleotide sequence corresponding to a genomic DNA sequence flanking a wild-type tRNA gene. For example, in certain embodiments, the expression vector comprises a nucleotide sequence set forth in **TABLE 4**. In certain embodiments, the nucleotide sequence set forth in **TABLE 4** is selected from SEQ ID NOs: 869-888. In certain embodiments, the nucleotide sequence set forth in **TABLE 4** is operably linked to the nucleotide sequence encoding the first, second, and/or third suppressor tRNA. In certain embodiments, in the expression vector, the nucleotide sequence set forth in **TABLE 4** is 5' to the nucleotide sequence encoding the first, second, and/or third suppressor tRNA. In certain embodiments, in the expression vector, the nucleotide sequence set forth in **TABLE 4** is immediately 5' to (i.e., adjacent) the nucleotide sequence encoding the first, second, and/or third suppressor tRNA.

[0016] In certain embodiments, the expression vector is a viral vector, *e.g.*, a DNA virus vector, *e.g.*, an adeno-associated virus (AAV) vector.

[0017] In another aspect, the invention provides a pharmaceutical composition comprising any of the foregoing expression vectors and a pharmaceutically acceptable excipient.

[0018] In another aspect, the invention provides a pharmaceutical composition comprising: (a) a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon

(*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid.

[0019] In certain embodiments, the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**. For example, (i) when the first amino acid is arginine, the first suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-22, and 35, (ii) when the second amino acid is glutamine, the second suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187, and/or (iii) when the third amino acid is glutamine, the third suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45.

[0020] In certain embodiments, the first, second, and/or third suppressor tRNA comprises one or more naturally occurring nucleotide modifications, *e.g.*, selected from 5-methyl uridine, 5-carbamoylmethyluridine, 5-carbamoyl-methyl-2-O-methyluridine, 5-methoxy-carbonylmethyluridine, 5-methoxycarbonylmethyl-2-thiouridine, pseudouridine, dihydrouridine, 1-methyladenosine, and inosine. In certain embodiments, the tRNA is not conjugated to, or associated with, another moiety, *e.g.*, a carrier particle, *e.g.*, an aminolipid particle. In certain embodiments, the composition does not comprise a nanoparticle and/or an aminolipid delivery compound.

[0021] In another aspect, the invention provides a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a premature termination codon, the method comprising contacting the cell with an effective amount of any of the foregoing expression vectors or pharmaceutical compositions, thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon.

[0022] In another aspect, the invention provides a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a first, second, and/or third premature termination codon, the method comprising contacting the cell with effective amount of: (a) a first expression vector comprising a nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second expression vector comprising a nucleotide sequence encoding a second suppressor tRNA that comprises an

anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third expression vector comprising a nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid, thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon.

[0023] In another aspect, the invention provides a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a first, second, and/or third premature termination codon, the method comprising contacting the cell with effective amount of: (a) a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid, thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon.

[0024] In certain embodiments of any of the foregoing methods, the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**. For example, (i) when the first amino acid is arginine, the first suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-22, and 35, (ii) when the second amino acid is glutamine, the second suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187, and/or (iii) when the third amino acid is glutamine, the third suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45.

[0025] In certain embodiments of any of the foregoing methods, the gene is a gene set forth in **TABLE 5** or **TABLE 6**. In certain embodiments, the gene is an SCN1A or dystrophin gene.

[0026] In certain embodiments of any of the foregoing methods, the cell is a human cell. In certain embodiments, the cell is a central nervous system cell, *e.g.*, a neuron. In certain embodiments, the tRNA becomes aminoacylated in the cell.

[0027] In another aspect, the invention provides a method of treating a premature termination codon-mediated disorder in a subject (or a population of subjects) in need thereof, wherein the

subject(s) have a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject(s) an effective amount of any of the foregoing expression vectors or any of the foregoing pharmaceutical compositions, thereby to treat the disorder in the subject.

5 **[0028]** In another aspect, the invention provides a method of treating a premature termination codon-mediated disorder in a subject (or a population of subjects) in need thereof wherein the subject(s) have a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject(s) an effective amount of: (a) a first expression vector comprising a nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon
10 that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second expression vector comprising a nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third expression vector comprising a nucleotide sequence
15 encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid; thereby to treat the disorder in the subject(s).

[0029] In another aspect, the invention provides a method of treating a premature termination codon-mediated disorder in a subject (or a population of subjects) in need thereof wherein the
20 subject(s) have a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject(s) an effective amount of: (a) a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is
25 capable of being aminoacylated with a second amino acid; and optionally, (c) a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid; thereby to treat the disorder in the subject(s).

[0030] In certain embodiments of any of the foregoing methods, the first, second, and/or third
30 suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**. For example, (i) when the first amino acid is arginine, the first suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-22, and 35, (ii) when the second amino acid is glutamine, the second suppressor tRNA may comprise a nucleotide sequence

selected from SEQ ID NOs: 178-182, 186, and 187, and/or (iii) when the third amino acid is glutamine, the third suppressor tRNA may comprise a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45.

[0031] In certain embodiments of any of the foregoing methods, the disorder is a disorder set forth in **TABLE 5** or **TABLE 6**. In certain embodiments, the disorder is Dravet Syndrome or Duchenne Muscular Dystrophy.

[0032] In another aspect, the invention provides a method of treating Dravet Syndrome in a subject (or a population of subjects) in need thereof wherein the subject(s) have an SCN1A gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject an effective amount of an expression vector comprising: (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to the first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to the second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to the third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid; thereby to treat Dravet Syndrome in the subject(s). In certain embodiments, (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is lysine; (ii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamic acid; (iii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is tyrosine; (iv) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is leucine; (v) the first amino acid is arginine, the second amino acid is tryptophan, and the third amino acid is glutamic acid; or (vi) the first amino acid is arginine, the second amino acid is tyrosine, and the third amino acid is glutamic acid.

[0033] In another aspect, the invention provides a method of treating Duchenne Muscular Dystrophy in a subject (or a population of subjects) in need thereof wherein the subject(s) have a dystrophin gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject(s) an effective amount of an expression vector comprising: (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to the first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second nucleotide sequence encoding a second

suppressor tRNA that comprises an anticodon that hybridizes to the second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to the third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid; thereby to treat Duchenne Muscular Dystrophy in the subject(s). In certain embodiments: (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamine; (ii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamic acid; (iii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is lysine; (iv) the first amino acid is arginine, the second amino acid is tryptophan, and the third amino acid is glutamine; or (v) the first amino acid is arginine, the second amino acid is glutamic acid, and the third amino acid is glutamine.

[0034] These and other aspects and features of the invention are described in the following detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0035] The invention can be more completely understood with reference to the following drawings.

[0036] **FIGURE 1** is a schematic representation of a transcript (*e.g.*, an SCN1A transcript) containing a premature termination codon (PTC) which leads to a truncated protein product (*e.g.*, a protein product in a subject with Dravet syndrome). Native termination codons are indicated as shaded circles, and premature termination codons are indicated as unshaded circles. Expression of a suppressor tRNA (*e.g.*, an anticodon modified arginine tRNA) charged with its cognate amino acid (A.A.) allows read-through of the PTC and facilitates expression of the full-length protein.

[0037] **FIGURE 2A** is a consensus tRNA secondary structure. The numbering of the residues is based on the tRNA numbering system described in Steinberg *et al.* (1993) NUCLEIC ACIDS RES. 21:3011-15. **FIGURE 2B** is a table showing the modification profile for tRNA sequences from the cytosol of certain eukaryotic organisms. The ratios in the table indicate the frequency of occurrence of listed nucleotide at the numbered position shown in **FIGURE 2A**. The abbreviations for the modified residues are defined in Motorin *et al.* (2005) "Transfer RNA Modification," *ENCYCLOPEDIA OF LIFE SCIENCES*, John Wiley & Sons, Inc.

[0038] **FIGURE 3** is a bar graph showing the global frequencies of nonsense mutations. Data is from ~16,000 entries for pathogenic nonsense mutations in ClinVar.

[0039] **FIGURE 4** is a bar graph showing the frequencies of nonsense mutations in SCN1A. Data is from ClinVar and the Guangzhou SCN1A mutation database.

5 [0040] **FIGURE 5** is a bar graph showing the frequencies of nonsense mutations in Duchenne/Becker muscular dystrophy. Data is from the Leiden database.

[0041] **FIGURE 6** is a schematic representation of an exemplary expression vector encoding three suppressor tRNAs that facilitate read-through of three different premature termination codons (PTC).

10 [0042] **FIGURE 7** depicts an exemplary EGFP reporter with a PTC (TGA) in place of an Arginine codon (CGA) and a suppressor tRNA. Native termination codons are indicated as shaded circles, and premature termination codons are indicated as unshaded circles. In the depicted example, a standard Arginine tRNA (with an anticodon that binds CGA) will result in no read-through of the PTC in EGFP, and a non-functional truncated EGFP protein. An
15 Arg>TGA suppressor tRNA (an Arginine tRNA with a modified anticodon that binds TGA/UGA) allows for read-through of the PTC in EGFP resulting in full-length, functional EGFP protein.

[0043] **FIGURE 8** depicts fluorescent images of EGFP reporter expression in HEK293 cells transiently co-transfected with (i) a plasmid encoding the Tristop suppressor and (ii) a plasmid
20 encoding either an EGFP reporter with a PTC (TGA) in place of an Arginine codon (CGA, “R96*TGA”), an EGFP reporter with a PTC (TAA) in place of an Glutamine codon (CAG, “Q69*TAA”), or an EGFP reporter with a PTC (TAG) in place of an Glutamine codon (CAG, “Q69*TAG”). The readthrough activity of the Tristop suppressor was compared to the activity of separate expression vectors encoding only an Arginine to TGA (R>TGA) suppressor
25 (“R→TGA Suppressor (115)”), only a Glutamine to TAA (Q>TAA) suppressor (“Q→TAA Suppressor (157)”), and only a Glutamine to TAG (Q>TAG) suppressor (“Q→TAG Suppressor (196)”).

[0044] **FIGURE 9** depicts EGFP expression in HEK293 cells co-transfected as described for **FIGURE 8**. EGFP expression was analyzed by flow cytometry and readthrough activity is
30 presented as the percentage of viable cells that express EGFP above background. Controls (without any suppressor tRNA) are depicted on the right, where “R96*TGA” indicates the EGFP reporter with a PTC (TGA) in place of an Arginine codon, “Q69*TAA” indicates the EGFP reporter with a PTC (TAA) in place of an Glutamine codon, “Q69*TAG” indicates the EGFP

reporter with a PTC (TAG) in place of an Glutamine codon, and “EGFP” indicates the wild-type EGFP reporter.

[0045] FIGURE 10 is a bar graph depicting cell viability in cells transfected with the indicated suppressor tRNA. “Mock” indicates mock-transfected cells, and “Control” indicates cells transfected with an expression vector that does not contain a suppressor tRNA.

DETAILED DESCRIPTION

[0046] The invention is based, in part, upon the discovery that is possible to express multiple (*e.g.*, two or three) suppressor tRNAs using a single expression vector. Each suppressor tRNA permits an amino acid to be incorporated into a gene product encoded by a gene in a mammalian cell at a position that would otherwise result in a truncated gene product caused by a premature termination codon (PTC) in the gene. Expression of multiple suppressor tRNAs from a single expression vector allows for the single expression vector to treat a disease mediated by multiple, different PTCs in the same subject and/or treat a disease mediated by multiple, different PTCs in multiple, different subjects. The invention is further based, in part, upon the discovery of optimal combinations of suppressor tRNAs that allow for treatment of the greatest possible patient populations.

[0047] Accordingly, in one aspect, the invention provides an expression vector comprising: (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid.

[0048] In certain embodiments, the first amino acid is selected from arginine, tryptophan, cysteine, serine, glycine, and leucine (*e.g.*, the first amino acid is arginine). In certain embodiments, the second amino acid is selected from glutamine, glutamic acid, tyrosine, tryptophan, lysine, serine, and leucine (*e.g.*, the second amino acid is glutamine). In certain embodiments, the third amino acid is selected from glutamine, glutamic acid, tyrosine, lysine, serine, and leucine. In certain embodiments, the second and third amino acid are the same, for example, the second and third amino acid are selected from glutamine, glutamic acid, tyrosine, lysine, serine, and leucine.

[0049] In certain embodiments: (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is lysine; (ii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamic acid; (iii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is tyrosine; (iv) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is leucine; (v) the first amino acid is arginine, the second amino acid is tryptophan, and the third amino acid is glutamic acid; or (vi) the first amino acid is arginine, the second amino acid is tyrosine, and the third amino acid is glutamic acid. In certain embodiments: (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamine; (ii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamic acid; (iii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is lysine; (iv) the first amino acid is arginine, the second amino acid is tryptophan, and the third amino acid is glutamine; or (v) the first amino acid is arginine, the second amino acid is glutamic acid, and the third amino acid is glutamine. In certain embodiments: (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamine; (ii) the first amino acid is tryptophan, the second amino acid is glutamic acid, and the third amino acid is glutamic acid; (iii) the first amino acid is cysteine, the second amino acid is tyrosine, and the third amino acid is tyrosine; (iv) the first amino acid is serine, the second amino acid is lysine, and the third amino acid is lysine; (v) the first amino acid is glycine, the second amino acid is serine, and the third amino acid is serine; or (vi) the first amino acid is leucine, the second amino acid is leucine, and the third amino acid is leucine.

[0050] In certain embodiments, the expression vector comprises, in order (*e.g.*, in a 5' to 3' orientation): (i) the first nucleotide sequence, the second nucleotide sequence, and the third nucleotide sequence; (ii) the first nucleotide sequence, the third nucleotide sequence, and the second nucleotide sequence; (iii) the second nucleotide sequence, the first nucleotide sequence, and the third nucleotide sequence; (iv) the second nucleotide sequence, the third nucleotide sequence, and the first nucleotide sequence; (v) the third nucleotide sequence, the first nucleotide sequence, and the second nucleotide sequence; or (vi) the third nucleotide sequence, the second nucleotide sequence, and the first nucleotide sequence.

[0051] In another aspect, the invention provides a pharmaceutical composition comprising any of the foregoing expression vectors and a pharmaceutically acceptable excipient.

[0052] In another aspect, the invention provides a pharmaceutical composition comprising: (a) a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon

(*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop
5 codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid.

[0053] In another aspect, the invention provides a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a premature termination codon, the method comprising contacting the cell with an effective amount of any of the foregoing expression vectors or pharmaceutical compositions, thereby permitting an amino acid to be
10 incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon.

[0054] In another aspect, the invention provides a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a first, second, and/or third premature termination codon, the method comprising contacting the cell with effective amount of: (a) a first
15 expression vector comprising a nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second expression vector comprising a nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being
20 aminoacylated with a second amino acid; and optionally, (c) a third expression vector comprising a nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid; thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by
25 the premature termination codon.

[0055] In another aspect, the invention provides a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a first, second, and/or third premature termination codon, the method comprising contacting the cell with effective amount of: (a) a first
30 suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop

codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid; thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon.

[0056] In certain embodiments of any of the foregoing methods, the cell contains less truncated gene product than a cell without the tRNA. For example, in certain embodiments, the cell contains less than about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% of the truncated gene product relative to a cell without the tRNA. In certain embodiments, the cell contains from about 5% to about 80%, about 5% to about 60%, about 5% to about 40%, about 5% to about 20%, about 5% to about 10%, about 10% to about 80%, about 10% to about 60%, about 10% to about 40%, about 10% to about 20%, about 20% to about 80%, about 20% to about 60%, about 20% to about 40%, about 40% to about 80%, about 40% to about 60%, or about 60% to about 80% of the truncated gene product relative to a cell without the tRNA. In certain embodiments, there is no detectable truncated gene product in the cell. Truncated gene product amount or expression may be measured by any method known in the art, for example, Western blot or ELISA.

[0057] In certain embodiments, the cell contains a greater amount of functional gene product than a cell without the tRNA. For example, in certain embodiments, the method increases the amount of functional gene product in a cell, tissue, or subject by about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 110%, about 120%, about 130%, about 140%, about 150%, about 160%, about 170%, about 180%, about 190%, about 200%, about 250%, about 300%, about 350%, about 400%, about 450%, or about 500% relative to a cell, tissue, or subject without the tRNA. In certain embodiments, the method increases the amount of functional gene product in a cell, tissue, or subject, by from about 20% to about 200%, about 20% to about 180%, about 20% to about 160%, about 20% to about 140%, about 20% to about 120%, about 20% to about 100%, about 20% to about 80%, about 20% to about 60%, about 20% to about 40%, about 40% to about 200%, about 40% to about 180%, about 40% to about 160%, about 40% to about 140%, about 40% to about 120%, about 40% to about 100%, about 40% to about 80%, about 40% to about 60%, about 60% to about 200%, about 60% to about 180%, about 60% to about 160%, about 60% to about 140%, about 60% to about 120%, about 60% to about 100%, about 60% to about 80%, about 80% to about 200%, about 80% to about 180%, about 80% to about 160%, about 80% to about 140%, about 80% to about 120%, about 80% to about 100%, about 100% to about 200%, about 100% to about 180%, about 100% to about 160%, about 100% to about 140%, about 100% to about 120%, about 120% to about 200%, about 120% to about 180%, about

120% to about 160%, about 120% to about 140%, about 140% to about 200%, about 140% to about 180%, about 140% to about 160%, about 160% to about 200%, about 160% to about 180%, or about 180% to about 200% relative to a cell, tissue, or subject without the tRNA.

Functional gene product amount or expression may be measured by any method known in the art, for example, Western blot or ELISA.

[0058] In certain embodiments, the tRNA permits an amino acid to be incorporated into the gene product at a position corresponding to a premature termination codon (*i.e.*, the tRNA permits read-through of the premature termination codon), but the tRNA does not permit a substantial amount of amino acid to be incorporated into a gene product at a position corresponding to a native stop codon (*i.e.*, the tRNA does not permit read-through of a native stop codon). For example, in certain embodiments, a disclosed tRNA does not increase read-through of a native stop codon (or all native stop codons) in a cell, tissue, or subject, or increases read-through by less than about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 20%, about 30%, about 40%, or about 50%, relative to a cell, tissue, or subject that has not been contacted with the tRNA. Read-through of a native stop codon may be measured by any method known in the art, for example, ribosome profiling.

[0059] In another aspect, the invention provides a method of treating a premature termination codon-mediated disorder in a subject (or a population of subjects) in need thereof, wherein the subject(s) have a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject an effective amount of any of the foregoing expression vectors or any of the foregoing pharmaceutical compositions, thereby to treat the disorder in the subject(s).

[0060] In another aspect, the invention provides a method of treating a premature termination codon-mediated disorder in a subject (or a population of subjects) in need thereof wherein the subject(s) have a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject(s) an effective amount of: (a) a first expression vector comprising a nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second expression vector comprising a nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third expression vector comprising a nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a third

premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid; thereby to treat the disorder in the subject(s).

[0061] In another aspect, the invention provides a method of treating a premature termination codon-mediated disorder in a subject (or a population of subjects) in need thereof wherein the

5 subject(s) have a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject(s) an effective amount of: (a) a first suppressor tRNA that comprises an anticodon that hybridizes to a first premature stop codon (*e.g.*, TGA), and is capable of being aminoacylated with a first amino acid; (b) a second suppressor tRNA that
10 comprises an anticodon that hybridizes to a second premature stop codon (*e.g.*, TAG), and is capable of being aminoacylated with a second amino acid; and optionally, (c) a third suppressor tRNA that comprises an anticodon that hybridizes to a third premature stop codon (*e.g.*, TAA), and is capable of being aminoacylated with a third amino acid; thereby to treat the disorder in the subject(s).

I. tRNAs and Suppressor tRNAs

15 **[0062]** During protein synthesis, a transfer RNA (tRNA) delivers an amino acid to a ribosome for incorporation into a growing protein (polypeptide) chain. tRNAs typically are about 70 to 100 nucleotides in length, and active tRNAs contain a 3' CCA sequence that may be transcribed into the tRNA during its synthesis or may be added later during post-transcriptional processing. During aminoacylation, the amino acid that is attached to a given tRNA molecule is covalently
20 attached to the 2' or 3' hydroxyl group of the 3'-terminal ribose to form an aminoacyl-tRNA (aa-tRNA). It is understood that an amino acid can spontaneously migrate from the 2'-hydroxyl group to the 3'-hydroxyl group and vice versa, but it is incorporated into a growing protein chain at the ribosome from the 3'-OH position. A loop at the other end of the folded aa-tRNA molecule contains a sequence of three bases known as the anticodon. When this anticodon
25 sequence hybridizes or base-pairs with a complementary three-base codon sequence in a ribosome-bound messenger RNA (mRNA), the aa-tRNA binds to the ribosome and its amino acid is incorporated into the polypeptide chain being synthesized by the ribosome. Because all tRNAs that base-pair with a specific codon are aminoacylated with a single specific amino acid, the translation of the genetic code is effected by tRNAs. Each of the 61 non-termination codons
30 in an mRNA directs the binding of its cognate aa-tRNA and the addition of a single specific amino acid to the growing polypeptide chain being synthesized by the ribosome.

[0063] tRNAs are generally highly conserved and are often functional across species.

Accordingly, a tRNA derived from a bacterial tRNA, a non-mammalian eukaryotic tRNA, or a

mammalian (*e.g.*, human) tRNA may be useful in the practice of the invention. Nucleotide sequences encoding naturally occurring human tRNAs are known and generally available to those of skill in the art through sources such as Genbank. See also Sprinzl *et al.* (2005) NUCLEIC ACIDS RES. 33: D139-40; Buckland *et al.* (1996) GENOMICS 35(1):164-71; Schimmel *et al.* (Eds.) (1979) "Transfer-RNA: Structure, Properties, and Recognition," Cold Spring Harbor Laboratory; Agris (1983) "The Modified Nucleosides of Transfer RNA, II," Alan R. Liss Inc. tRNAs are generally highly conserved and are often functional across species.

[0064] Suppressor tRNAs are modified tRNAs that insert a suitable amino acid at a mutant site, *e.g.*, a PTC, in protein encoding gene. The use of the word in suppressor is based on the fact, that under certain circumstance, the modified tRNA "suppresses" the phenotypic effect of the coding mutation. Suppressor tRNAs typically contain a mutation (modification) in either the anticodon, changing codon specificity, or at some position that alters the aminoacylation identity of the tRNA.

[0065] In certain embodiments, a tRNA (*e.g.*, a suppressor tRNA) contains a modified anticodon region, such that the modified anticodon hybridizes with a different codon than the corresponding naturally occurring anticodon. In certain embodiments, the modified anticodon hybridizes with a termination codon, *e.g.*, a PTC, and as a result, the tRNA incorporates an amino acid into a gene product rather than terminating protein synthesis. In certain embodiments, the modified anticodon hybridizes with a premature termination codon and, and as a result, the tRNA incorporates an amino acid into a gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon.

[0066] In certain embodiments, a tRNA comprises an anticodon that hybridizes to a codon selected from UAG (*i.e.*, an "amber" termination codon), UGA (*i.e.*, an "opal" termination codon), and UAA (*i.e.*, an "ochre" termination codon). In certain embodiments, the anticodon hybridizes to a codon selected from UGA to UAA. In certain embodiments, the anticodon hybridizes to UGA. In certain embodiments, a tRNA comprises an anticodon that hybridizes to a non-standard termination codon, *e.g.*, a 4-nucleotide codon (See, for example, Moore *et al.* (2000) J. MOL. BIOL. 298:195, and Holsaka *et al.* (1999) J. AM. CHEM. SOC. 121:12194).

[0067] In certain embodiments, the tRNA is aminoacylated or is capable of being aminoacylated with any natural amino acid. For example, a tRNA may be capable of being aminoacylated with alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. In certain embodiments the tRNA is capable of being aminoacylated with

serine, leucine, glutamine, or arginine. In certain embodiments the tRNA is capable of being aminoacylated with glutamine or arginine. In certain embodiments the tRNA is capable of being aminoacylated with arginine.

- 5 **[0068]** In certain embodiments, the tRNA (i) comprises an anticodon that hybridizes to a codon as indicated in **TABLE 1**, and (ii) is aminoacylated or is capable of being aminoacylated with an amino acid as indicated in **TABLE 1**.

TABLE 1

codon: UAG amino acid: alanine	codon: UGA amino acid: alanine	codon: UAA amino acid: alanine
codon: UAG amino acid: arginine	codon: UGA amino acid: arginine	codon: UAA amino acid: arginine
codon: UAG amino acid: asparagine	codon: UGA amino acid: asparagine	codon: UAA amino acid: asparagine
codon: UAG amino acid: aspartic acid	codon: UGA amino acid: aspartic acid	codon: UAA amino acid: aspartic acid
codon: UAG amino acid: cysteine	codon: UGA amino acid: cysteine	codon: UAA amino acid: cysteine
codon: UAG amino acid: glutamine	codon: UGA amino acid: glutamine	codon: UAA amino acid: glutamine
codon: UAG amino acid: glutamic acid	codon: UGA amino acid: glutamic acid	codon: UAA amino acid: glutamic acid
codon: UAG amino acid: glycine	codon: UGA amino acid: glycine	codon: UAA amino acid: glycine
codon: UAG amino acid: histidine	codon: UGA amino acid: histidine	codon: UAA amino acid: histidine
codon: UAG amino acid: isoleucine	codon: UGA amino acid: isoleucine	codon: UAA amino acid: isoleucine
codon: UAG amino acid: leucine	codon: UGA amino acid: leucine	codon: UAA amino acid: leucine
codon: UAG amino acid: lysine	codon: UGA amino acid: lysine	codon: UAA amino acid: lysine
codon: UAG amino acid: methionine	codon: UGA amino acid: methionine	codon: UAA amino acid: methionine

codon: UAG amino acid: phenylalanine	codon: UGA amino acid: phenylalanine	codon: UAA amino acid: phenylalanine
codon: UAG amino acid: proline	codon: UGA amino acid: proline	codon: UAA amino acid: proline
codon: UAG amino acid: serine	codon: UGA amino acid: serine	codon: UAA amino acid: serine
codon: UAG amino acid: threonine	codon: UGA amino acid: threonine	codon: UAA amino acid: threonine
codon: UAG amino acid: tryptophan	codon: UGA amino acid: tryptophan	codon: UAA amino acid: tryptophan
codon: UAG amino acid: tyrosine	codon: UGA amino acid: tyrosine	codon: UAA amino acid: tyrosine
codon: UAG amino acid: valine	codon: UGA amino acid: valine	codon: UAA amino acid: valine

[0069] In certain embodiments, the tRNA comprises, consists essentially of, or consists of a nucleotide sequence shown in **TABLE 2**. In certain embodiments, the tRNA comprises, consists essentially of, or consists of a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a nucleotide sequence shown in **TABLE 2**. In certain embodiments, the tRNA comprises, consists essentially of, or consists of a nucleotide sequence selected from SEQ ID NOs: 19-21, 37, 39, 40, 44, 179, 181, 182, and 186, or a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a nucleotide sequence selected from SEQ ID NOs: 19-21, 37, 39, 40, 44, 179, 181, 182, and 186.

It is understood that, throughout the description (*e.g.*, **TABLES 2** and **3**, and the Sequence Listing), in each instance where a tRNA comprises, consists essentially of, or consists of a nucleotide sequence including one or more thymines (T), a tRNA is also contemplated that comprises, consists essentially of, or consists of the same nucleotide sequence including a uracil (U) in place of one or more of the thymines (T), or a uracil (U) in place of all the thymines (T).

Similarly, in each instance where a tRNA comprises, consists essentially of, or consists of a nucleotide sequence including one or more uracils (U), a tRNA is also contemplated that comprises, consists essentially of, or consists of a nucleotide sequence including a thymine (T) in place of the one or more of the uracils (U), or a thymine (T) in place of all the uracils (U).

TABLE 2

SEQ ID NO	Suppressor tRNA Sequence (anticodon shown in lowercase)
2	GGGCCAGTGGCGCAATGGATAACGCGTCTGACTtcaGATCAGAAGATTGTAG GTTTCGACTCCTACCTGGCTCG
5	GGCCGCGTGGCCTAATGGATAAGGCGTCTGATTtcaGATCAGAAGATTGGGG GTTTCGAGTCCCTTCGTGGTTCG
14	GACCACGTGGCCTAACGGATAAGGCGTCTGACTtcaGATCAGAAGATTGAGG GTTTCGAATCCCTTCGTGGTTA
15	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTtcaAGTGACGAGAAAGCGA TTCAAAGGTTGTGGGTTTCGAATCCCACCAGAGTCG
19	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTtcaAGCATGATTGAGAGAT TCAAAGGTTGCGGGTTCGAGTCCCGCCAGAGTCG
20	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTtcaAATTCAAAGGTTGCGG GTTTCGAGTCCCGCCAGAGTCG
21	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTtcaAGACAAATGGAGGCAT TCAAAGGTTGTGGGTTTCGAGTCCCACCAGAGTCG
23	GTCTCTGTGGCGCAATGGACGAGCGCGCTGGACTtcaAATCCAGAGGTTCTG GGTTCGAGTCCCGGCAGAGATG
24	GGCTCTGTGGAGCAATGGATAGCACATTGGACTtcaAGCATGACCGAGAGAT TCAAAGGTTGCGGGTTCGAGTCCCACCAGAGTTG
25	GGCTCTGTGGAGCAATGGATAGCACATTGGACTtcaAATTCAAAGGTTGCGG GTTTCGAGTCCCACCAGAGTTG
37	GGTTCCATGGTGTAATGGTTAGCACTCTGGACTttaAATCCAGCGACCCGAG TTCAAATCTCGGTGGGACCT
39	GGTTCCATGGTGTAATGGTTAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
40	GGTTCCATGGTGTAATGGTGAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
41	GGTTCCATGGTGTAATGGCTAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGATTT
42	GGTTCCATGGTGTAATGGTTAGCACTCTGGACTttaAATCCAGCCATACAAG TTCAAATCTCAGTGGAACCT
43	GGTTCCTTGGTGTAAGATGAGCACTCTGGATTttaAATCCAGCGATCAGAGT TCAAATCTCGGTGGGACCT

SEQ ID NO	Suppressor tRNA Sequence (anticodon shown in lowercase)
44	GGTCCCATGGTGTAATGGTTAGCACTCTGGACTttaAATCCAGCAATCTGAG TTCAAATCTCGGTGGGACCT
46	GGTCTCATGGTGTAATGGTTAGCACACTGGACTttaAGTCCAGCAATCCGAG TTCGAGTCTTGGTGAGACCA
47	GGACCCATGGTGTAATGGTTAGCACTCTGGACTttaAATCCAGCAATCCAAG TTCAAATCTCGGTGGGACCT
48	GTTTCCATGGTGTAATGGTTGGCACTCTGGACTttaAATCCAGCAATCCAAG TTCAAGTCTCTGTGGGACCT
49	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGCTATGGCTTCCTCG CTCTGAGGGTTCTGGTCTCCCTGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA
50	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGCTTAGCTTCCTGT CTGGGGATTCTGGTCTCCGTATGGAGGCGTGGGTTCGAATCCCACCTTCTGAC A
51	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGGTGACAAGCCTTAC CTACGGGTGTTCTGGTCTCCGAATGGAGGCGTGGGTTCGAATCCCACCTTCTG ACA
52	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGCGTTCGCTTCCTCT ACTGAGGGTTCTGGTCTCCGTGTGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA
53	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGCTATGGCTTCCTCG CTCTGAGGGTTCTGGTCTCCCTGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA
54	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGCTTAGCTTCCTGT CTGGGGATTCTGGTCTCCGTATGGAGGCGTGGGTTCGAATCCCACCTTCTGAC A
55	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGGTGACAAGCCTTAC CTACGGGTGTTCTGGTCTCCGAATGGAGGCGTGGGTTCGAATCCCACCTTCTG ACA
56	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGCGTTCGCTTCCTCT ACTGAGGGTTCTGGTCTCCGTGTGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA

SEQ ID NO	Suppressor tRNA Sequence (anticodon shown in lowercase)
57	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGCTATGGCTTCCTCG CTCTGAGGGTTCTGGTCTCCCCTGGAGGCGTGGGTTCGAATCCCACTTCTGA CA
58	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGCTTAGCTTCCCTGT CTGGGGATTCTGGTCTCCGTATGGAGGCGTGGGTTCGAATCCCACTTCTGAC A
59	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGGTGACAAGCCTTAC CTACGGGTGTTCTGGTCTCCGAATGGAGGCGTGGGTTCGAATCCCACTTCTG ACA
60	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGCGTTCGCTTCCTCT ACTGAGGGTTCTGGTCTCCGTGTGGAGGCGTGGGTTCGAATCCCACTTCTGA CA
61	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTctaGAGTTACTAGAATAGT GATCCTTAGGTCGCTGGTTCGAATCCGGCTCGAAGGA
62	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTctaGTCAGTACAATATGGT AATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
63	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTctaAGCTTGTGGCTGTGGA CATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
64	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTctaGCTAACTCCCCGTTAG AAGACATCCTTAGGTCGCTGGTTCGACTCCGGCTCGAAGGA
65	CTTTCGATAGTTTCAGTTGGTAGAGCGGAGGACTctaGAGTATTAACGTTGGT GATCCTTAGGTCGCTGGTTCGAGTCCGGCTCGAAGGA
66	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTttaGAGTTACTAGAATAGT GATCCTTAGGTCGCTGGTTCGAATCCGGCTCGAAGGA
67	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTttaGTCAGTACAATATGGT AATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
68	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTttaAGCTTGTGGCTGTGGA CATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
69	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTttaGCTAACTCCCCGTTAG AAGACATCCTTAGGTCGCTGGTTCGACTCCGGCTCGAAGGA
70	CTTTCGATAGTTTCAGTTGGTAGAGCGGAGGACTttaGAGTATTAACGTTGGT GATCCTTAGGTCGCTGGTTCGAGTCCGGCTCGAAGGA

SEQ ID NO	Suppressor tRNA Sequence (anticodon shown in lowercase)
71	GGGGGTATAGCTCAGTGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
72	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGATGCCCCCT
73	GGGGGTATAGCTCAGGGGTAGAGTATTTGGCTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
74	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGTCCCCCT
75	GGGGGTATAGCTCAGAGGTAGAGCATTTGACTtcaGATCAAGAGATCTCTGG TTCAAATCCAGGTGCCCCCT
76	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTAG TTCAAATCCAGGTGCCCCCT
77	GGTGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGATCCCTGG TTCGAATCCAGGTGCCCCCT
78	GGGGGTATAACTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
79	TGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
80	GGGGGTATAGCTCAGAGGAAGAGCATTTGACTtcaGATCAAGAGGTCCCTGA TTCAAATCCAGGTGCCCCCT
81	GGGGGTAAAGCTCAGGGGTAGAGCATTTGACTtcaGATTAAGAGGTCCCTGG TTCAAATCCAGGTACCCCCCT
83	GGGGTTATAGCTCAGGTGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
84	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCACGAGGTCCCTGG TTCAAATCGAGGTGCCCCCT
85	GGGGGTATAGCTCAGGGGTGGAGCATTTGACTtcaGATCAAGGGGTCCCTGT TTCAAATCCAGGTGCCCCCT
86	GGGGGTATAGCTCAGTGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCCGG TTCAAATCCGGGTGCCCCCT
88	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCGGGTGCCCCCT

SEQ ID NO	Suppressor tRNA Sequence (anticodon shown in lowercase)
89	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCTGG TTCAAATCCAGGTACCCCCT
91	GGGGGCATAGCTCAGGGGTAGAGCATTTGACTtcaGATCAAGAGGTCCCCGG TTCAAATCCGGGTGCTCCCT
92	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTtcaGATTAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
94	TCCCTGGTGGTCTAGTGGTTAGGATTTGGCGCTctaACCGCCGCGGCCTGGG TTCGATTCCCGGTCAGGGAA
95	TCCCTGGTGGTCTAGTGGTTAGGCTTTGGTGCTctaACCTCCATGGCCCAGG TTTGATTCCCTGGTCAGGGAA
97	TCCCTGGTGGTCTAGTGGTTAGGATTTGGCGCTttaACCGCCGCGGCCTGGG TTCGATTCCCGGTCAGGGAA
98	TCCCTGGTGGTCTAGTGGTTAGGCTTTGGTGCTttaACCTCCATGGCCCAGG TTTGATTCCCTGGTCAGGGAA
100	TCCCATATGGTCTAGCGGTTAGGATTCCTGGTTctaACCCAGGCGGCCCCGGG TTCGACTCCCGGTATGGGAA
103	TCCCATATGGTCTAGCGGTTAGGATTCCTGGTTttaACCCAGGCGGCCCCGGG TTCGACTCCCGGTATGGGAA
105	GTTTCCGTAGTGTAGTGGTTAGCGCGTTCGCCTtcaAAAGCGAAAGGTCCCC GGTTCGAAACCGGGCGGAAACA
107	GCATTGGTAGTTCAATGGTAGAATTCTCGCCTtcaACGCGGGTGACCCGGGT TCGATTCCCGGCCAATGCA
108	GCATTGGTGGTTCAATGGTAGAATTCTCGCCTtcaACGCGGGTGACCCGGGT TCGATTCCCGGCCAATGCA
109	GCATTGGTGGTTCAATGGTAGAATTCTCGCCTtcaACTCGGGTGACCCGGGT TCGATTCCCGGCCAATGCA
112	GCATTGGTGGTTCAGTGGTAGAATTCTCGCCTtcaACGCGGGAGGCCCGGGT TTGATTCCCGGCCAATGCA
113	GCATTGGTGGTTCAGTGGTAGAATTCTCGCCTtcaACGCGGGAGGCCCGGGT TCGGTTCCCGGCCAATGCA
115	GGTAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTctaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCACCGCTGCCA

SEQ ID NO	Suppressor tRNA Sequence (anticodon shown in lowercase)
116	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTtctaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCTGCCA
118	GGTAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTtcaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCTGCCA
119	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTtcaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCTGCCA
121	GGTAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTttaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCTGCCA
122	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTttaGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCGCTGCCA
124	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGTTCTGGTCTCCCT GGAGGCGTGGGTTCGAATCCCACCTTCTGACA
126	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGTTCTGGTCTCCGAA TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
127	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTctaGTTCTGGTCTCCGTG TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
128	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGTTCTGGTCTCCCT GGAGGCGTGGGTTCGAATCCCACCTTCTGACA
130	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGTTCTGGTCTCCGAA TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
131	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTtcaGTTCTGGTCTCCGTG TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
132	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGTTCTGGTCTCCCT GGAGGCGTGGGTTCGAATCCCACCTTCTGACA
134	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGTTCTGGTCTCCGAA TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
135	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTttaGTTCTGGTCTCCGTG TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
136	GTCAGGATGGCCGAGCGGTCTAAGGCGCTGCGTTctaGTCGCAGTCTCCCT GGAGGCGTGGGTTCGAATCCCACCTCCTGACA
137	GTCAGGATGGCCGAGCGGTCTAAGGCGCTGCGTTctaGTCGCAGTCTCCCT GGAGGCGTGGGTTCGAATCCCACCTTCTGACA

SEQ ID NO	Suppressor tRNA Sequence (anticodon shown in lowercase)
138	GTCAGGATGGCCGAGTGGTCTAAGGAGCTGTGTTctaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCACTCCTGACA
139	GTCAGGATGGCCGAGCAGTCTAAGGCACTGCGTTctaGTCGCAGTCTCCCCT GGAGGCGTGGATTTCGAATCCCACTCCTGACA
140	GTCAGGATGGCCGAGCGGTCTAAGGCGCTGCGTTtcaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCACTCCTGACA
141	GTCAGGATGGCCGAGCGGTCTAAGGCGCTGCGTTtcaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCACTTCTGACA
142	GTCAGGATGGCCGAGTGGTCTAAGGAGCTGTGTTtcaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCACTCCTGACA
143	GTCAGGATGGCCGAGCAGTCTAAGGCACTGCGTTtcaGTCGCAGTCTCCCCT GGAGGCGTGGATTTCGAATCCCACTCCTGACA
144	GTCAGGATGGCCGAGCGGTCTAAGGCGCTGCGTTttaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCACTCCTGACA
145	GTCAGGATGGCCGAGCGGTCTAAGGCGCTGCGTTttaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCACTTCTGACA
146	GTCAGGATGGCCGAGTGGTCTAAGGAGCTGTGTTttaGTCGCAGTCTCCCCT GGAGGCGTGGGTTCGAATCCCACTCCTGACA
147	GTCAGGATGGCCGAGCAGTCTAAGGCACTGCGTTttaGTCGCAGTCTCCCCT GGAGGCGTGGATTTCGAATCCCACTCCTGACA
148	ACCAGAATGGCCGAGTGGTTAAGGCGTTGGACTctaGATCCAATGGATTTAT ATCCGCGTGGGTTCGAACCCCACTTCTGGTA
149	ACCAGGATGGCCGAGTGGTTAAGGCGTTGGACTctaGATCCAATGGACATAT GTCTGCGTGGGTTCGAACCCCACTCCTGGTA
150	ACTGGGATGGCTGAGTGGTTAAGGCGTTGGACTctaGATCCAATGGGCGGTT GCCTGCGTGGGTTCGAACCCCACTCCCAGTA
151	GATGGGATGGCTGAGAGGTTAAGGCTTTGGACTctaGATCCAATGGGCAGAT GCCTGCGTGGGTTTGAACCCCACTCCCAATA
152	ACCAGAATGGCCGAGTGGTTAAGGCGTTGGACTtcaGATCCAATGGATTTAT ATCCGCGTGGGTTCGAACCCCACTTCTGGTA
153	ACCAGGATGGCCGAGTGGTTAAGGCGTTGGACTtcaGATCCAATGGACATAT GTCTGCGTGGGTTCGAACCCCACTCCTGGTA

SEQ ID NO	Suppressor tRNA Sequence (anticodon shown in lowercase)
154	ACTGGGATGGCTGAGTGGTTAAGGCGTTGGACTtcaGATCCAATGGGCGGTT GCCTGCGTGGGTTTCGAACCCCACTCCCAGTA
155	GATGGGATGGCTGAGAGGTTAAGGCTTTGGACTtcaGATCCAATGGGCAGAT GCCTGCGTGGGTTTGAACCCCACTCCCAATA
156	ACCAGAATGGCCGAGTGGTTAAGGCGTTGGACTttaGATCCAATGGATTTAT ATCCGCGTGGGTTTCGAACCCCACTTCTGGTA
157	ACCAGGATGGCCGAGTGGTTAAGGCGTTGGACTttaGATCCAATGGACATAT GTCTGCGTGGGTTTCGAACCCCACTCCTGGTA
158	ACTGGGATGGCTGAGTGGTTAAGGCGTTGGACTttaGATCCAATGGGCGGTT GCCTGCGTGGGTTTCGAACCCCACTCCCAGTA
159	GATGGGATGGCTGAGAGGTTAAGGCTTTGGACTttaGATCCAATGGGCAGAT GCCTGCGTGGGTTTGAACCCCACTCCCAATA
161	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTctaGCTCCAGTCTCTTCG GAGGCGTGGGTTTCGAATCCCACCACTGCCA
164	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTtcaGCTCCAGTCTCTTCG GAGGCGTGGGTTTCGAATCCCACCACTGCCA
167	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTttaGCTCCAGTCTCTTCG GAGGCGTGGGTTTCGAATCCCACCACTGCCA
169	GCCCAGCTAGCTCAGTTGGTAGAGCGTGGGACTctaAATCCTAGGGTCGTGG GTTTCGAACCCCACTGTTGGGCG
170	GCCCAGCTAGCTCAGTCTGTAGAGCATGAGACTctaAGTCTCAGGGTCATGG GTTGGAGCCCCATGTTGTGCA
171	GCCTAGCTAGTTCAGTCGGTAGAGCATGAGACTctaAATCTCAGGTTTCATGA GTTTGAGCCCCATGTTGGTTTGGCA
172	CCCCGGCTAGCTCAGTCAGTAGAGCTTGAGAATctaAATCTCAGGGTCGTGG GTTGGAGCCCCACGTTGGGCG
179	GGTTCCATGGTGTAATGGTTAGCACTCTGGACTctaAATCCAGCGACCCGAG TTCAAATCTCGGTGGGACCT
181	GGTTCCATGGTGTAATGGTTAGCACTCTGGACTctaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
182	GGTTCCATGGTGTAATGGTTAGCACTCTGGACTctaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT

SEQ ID NO	Suppressor tRNA Sequence (anticodon shown in lowercase)
183	GGTTCCATGGTGTAAATGGCTAGCACTCTGGACTctaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGATTT
184	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCCATACAAG TTCAAATCTCAGTGGAACCT
185	GGTTCCTTGGTGTAAAGATGAGCACTCTGGATTctaAATCCAGCGATCAGAGT TCAAATCTCGGTGGGACCT
186	GGTCCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCAATCTGAG TTCAAATCTCGGTGGGACCT
188	GGTCTCATGGTGTAAATGGTTAGCACACTGGACTctaAGTCCAGCAATCCGAG TTCGAGTCTTGGTGAGACCA
189	GGACCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCAATCCAAG TTCAAATCTCGGTGGGACCT
190	GTTTCCATGGTGTAAATGGTTGGCACTCTGGACTctaAATCCAGCAATCCAAG TTCAAGTCTCTGTGGGACCT
196	GCCCGGCTAGCTCAGTCGGTAGAGCATGGGACTctaAATCCCAGGGTCGTGG GTTTCGAGCCCCACGTTGGGCG
197	GCCCGGCTAGCTCAGTCGGTAGAGCATGAGACTctaAATCTCAGGGTCGTGG GTTTCGAGCCCCACGTTGGGCG
198	GCCCAGCTAGCTCAGTCTGTAGAGCATGAGACTctaAATCTCAGGGTCGTGA GTTTCGAGCCCCACGTTGGGTG
199	GCCCAGATAGCTCAGTGGGTAGAGCATGAGACTctaAATCTCAGGGTCATGG GTTTCATGCCCCATGTTGGGTA
200	GTCCTGCTGGCTCAGTCGGTACAGCATGGGACTctaAATCCCAGGGTCGTGG GTTTCGAGCTCCACGTTGGGTA
201	GCCTGGCTAGCTCAGTCCATAGAGCATGGGACTctaAATCCCAGGGTCATGG GTTTCGAGCCCCATATTAGGCA
202	GCCCAGCTAGCTTAGTTGGTAGAGCATGAGACTctaAATCTCAGAGTCATGG GTTTCAGGCCTCATGTTTGGCA
203	AACCTGGCTAGGTCAGTTGGTAGATCATGAGACTctaAATCTCAGGGTCATG GGTTCAAGCCCCATGTTGGTTT
204	GCCCAGCTAGCTCAGTTGGTAGAGCGTGGGACTttaAATCCTAGGGTCGTGG GTTTCGAACCCCCACGTTGGGCG

SEQ ID NO	Suppressor tRNA Sequence (anticodon shown in lowercase)
205	GCCCAGCTAGCTCAGTCTGTAGAGCATGAGACTttaAGTCTCAGGGTCATGG GTTGGAGCCCCATGTTGTGCA
206	GCCTAGCTAGTTCAGTCGGTAGAGCATGAGACTttaAATCTCAGGTTTCATGA GTTTGAGCCCCATGTTGGTTTGGCA
207	CCCCGGCTAGCTCAGTCAGTAGAGCTTGAGAATttaAATCTCAGGGTCGTGG GTTGGAGCCCCACGTTGGGCG
208	GCCCCGGCTAGCTCAGTCGGTAGAGCATGGGACTttaAATCCCAGGGTCGTGG GTTTCGAGCCCCACGTTGGGCG
209	GCCCCGGCTAGCTCAGTCGGTAGAGCATGAGACTttaAATCTCAGGGTCGTGG GTTTCGAGCCCCACGTTGGGCG
210	GCCCAGCTAGCTCAGTCTGTAGAGCATGAGACTttaAATCTCAGGGTCGTGA GTTTCGAGCCCCACGTTGGGTG
211	GCCCAGATAGCTCAGTGGGTAGAGCATGAGACTttaAATCTCAGGGTCATGG GTTTCATGCCCCATGTTGGGTA
212	GTCCTGCTGGCTCAGTCGGTACAGCATGGGACTttaAATCCCAGGGTCGTGG GTTTCGAGCTCCACGTTGGGTA
213	GCCTGGCTAGCTCAGTCCATAGAGCATGGGACTttaAATCCCAGGGTCATGG GTTTCGAGCCCCATATTAGGCA
214	GCCCAGCTAGCTTAGTTGGTAGAGCATGAGACTttaAATCTCAGAGTCATGG GTTTCAGGCCTCATGTTTGGCA
215	AACCTGGCTAGGTCAGTTGGTAGATCATGAGACTttaAATCTCAGGGTCATG GGTTCAAGCCCCATGTTGGTTT
216	GCCCCGATAGCTCAGTCGGTAGAGCATCAGACTctaAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCGGGCG
218	GCCTGGATAGCTCAATTGGTAGAGCATCAGACTctaAATCTGAGGGTTCAGG GTTCAAGTCCCTGTTTCAGGCG
219	GCCCAGCCAGCTCAGTAGGTAGAGTATGAGACTctaAATCTCAGGGTGGTGG GTTTCGAGCCCCATGTTGGGGG
220	TGTGGTGTAGCTCAGTCGGTAGAGCATCAGACTctaAATCTGAGGGTCCAGG GTTTCAGGTCCCTGTTTCGGGTGCCAAA
221	GCCCCGATAGCTCAGTCGGTAGAGCATCAGACTttaAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCGGGCG

SEQ ID NO	Suppressor tRNA Sequence (anticodon shown in lowercase)
223	GCCTGGATAGCTCAATTGGTAGAGCATCAGACTttaAATCTGAGGGTTCAGG GTTCAAGTCCCTGTTTCAGGCG
224	GCCCAGCCAGCTCAGTAGGTAGAGTATGAGACTttaAATCTCAGGGTGGTGG GTTTCGAGCCCCATGTTGGGGG
225	TGTGGTGTAGCTCAGTCGGTAGAGCATCAGACTttaAATCTGAGGGTCCAGG GTTTCAGGTCCCTGTTTCGGGTGCCAAAA
228	GTAGTCGTGGCCAAGTGAGTAAGGCAATGGACTctaAATCCATTGGGGTCTC CCAGCACAGGTTCAAATCCTGCTGACTATG
231	GTAGTCGTGGCCAAGTGAGTAAGGCAATGGACTtcaAATCCATTGGGGTCTC CCAGCACAGGTTCAAATCCTGCTGACTATG
234	GTAGTCGTGGCCAAGTGAGTAAGGCAATGGACTttaAATCCATTGGGGTCTC CCAGCACAGGTTCAAATCCTGCTGACTATG
237	GCTGTGATGGCCGAGTGGTTAAGGCGTTGGACTctaAATCCAATGGGTTCTT CCCGCGCAGGTTCAAATCCTGCTCACAGCG
240	GCTGTGATGGCCGAGTGGTTAAGGCGTTGGACTtcaAATCCAATGGGTTCTT CCCGCGCAGGTTCAAATCCTGCTCACAGCG
243	GCTGTGATGGCCGAGTGGTTAAGGCGTTGGACTttaAATCCAATGGGTTCTT CCCGCGCAGGTTCAAATCCTGCTCACAGCG
245	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTctaAATCCATTGTGCTCTG CACGCATGGGTTTCGAATCCCATCCTCGTCG
246	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTctaAATCCATTGTGCTTTG CACGCGTGGGTTTCGAATCCCATCCTCGTCG
247	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTctaAATCCATTGTGCTCTG CACGCGTGGGTTTCGAATCCCATCCTCGTCG
248	GATGAGGTGGCCGAGTGGTTAAGGCGATGGACTctaAATCCATTGTGCTCTG CACGCATGGGTTTCGAATCCCATCCTCATCG
250	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTtcaAATCCATTGTGCTCTG CACGCATGGGTTTCGAATCCCATCCTCGTCG
251	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTtcaAATCCATTGTGCTTTG CACGCGTGGGTTTCGAATCCCATCCTCGTCG
252	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTtcaAATCCATTGTGCTCTG CACGCGTGGGTTTCGAATCCCATCCTCGTCG

SEQ ID NO	Suppressor tRNA Sequence (anticodon shown in lowercase)
253	GATGAGGTGGCCGAGTGGTTAAGGCGATGGACTtcaAATCCATTGTGCTCTG CACGCATGGGTTCGAATCCCATCCTCATCG
255	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTttaAATCCATTGTGCTCTG CACGCATGGGTTCGAATCCCATCCTCGTCTG
256	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTttaAATCCATTGTGCTTTG CACGCGTGGGTTCGAATCCCATCCTCGTCTG
257	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTttaAATCCATTGTGCTCTG CACGCGTGGGTTCGAATCCCATCCTCGTCTG
258	GATGAGGTGGCCGAGTGGTTAAGGCGATGGACTttaAATCCATTGTGCTCTG CACGCATGGGTTCGAATCCCATCCTCATCG
259	GCTGAAATAGCTCAGTTGGGAGAGCATTAGACTctaGATCTAAAGGTCCCTG GTTTGATCCCGGGTTTCGGCA
260	GCTGAAATAGCTCAGTTGGGAGAGCATTAGACTtcaGATCTAAAGGTCCCTG GTTTGATCCCGGGTTTCGGCA
261	GCTGAAATAGCTCAGTTGGGAGAGCATTAGACTttaGATCTAAAGGTCCCTG GTTTGATCCCGGGTTTCGGCA
265	GACCTCGTGGCGCAATGGTAGCGCTCTGACTctaGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
270	GACCTCGTGGCACAATGGTAGCACGTCTGACTctaGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
280	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTctaGATCCTTAGGTCGCTG GTTTCGACTCCGGCTCGAAGGA
281	CTTTCGATAGTTTCAGTTGGTAGAGCGGAGGACTctaGATCCTTAGGTCGCTG GTTTCGAGTCCGGCTCGAAGGA
285	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTttaGATCCTTAGGTCGCTG GTTTCGACTCCGGCTCGAAGGA
286	CTTTCGATAGTTTCAGTTGGTAGAGCGGAGGACTttaGATCCTTAGGTCGCTG GTTTCGAGTCCGGCTCGAAGGA

[0070] In certain embodiments, the tRNA comprises, consists essentially of, or consists of a nucleotide sequence shown in **TABLE 3**. In certain embodiments, the tRNA comprises, consists essentially of, or consists of a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a nucleotide sequence shown in **TABLE 3**. In certain embodiments, the tRNA comprises, consists

essentially of, or consists of a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 22, 35, 36, 38, 45, 178, 180, and 187, or a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 22, 35, 36, 38, 45, 178, 180, and 187.

TABLE 3

SEQ ID NO	Suppressor tRNA Sequence
1	GGGCCAGTGGCGCAATGGATAACGCGTCTGACTtcaGATCAGAAGATTCCAG GTTGCGACTCCTGGCTGGCTCG
3	GGGCCAGTGGCGCAATGGATAACGCGTCTGACTtcaGATCAGAAGATTCTAG GTTGCGACTCCTGGCTGGCTCG
4	GGCCGCGTGGCCTAATGGATAAGGCGTCTGATTtcaGATCAGAAGATTGAGG GTTGCGAGTCCCTTCGTGGTTCG
6	GACCCAGTGGCCTAATGGATAAGGCATCAGCCTtcaGAGCTGGGGATTGTGG GTTGCGAGTCCCACCTGCGGTTCG
7	GCCCCAGTGGCCTAATGGATAAGGCACTGGCCTtcaAAGCCAGGGATTGTGG GTTGCGAGTCCCACCTGGGGTA
8	GCCCCAGTGGCCTAATGGATAAGGCACTGGCCTtcaAAGCCAGGGATTGTGG GTTGCGAGTCCCACCTGGGGTG
9	GCCCCGGTGGCCTAATGGATAAGGCATTGGCCTtcaAAGCCAGGGATTGTGG GTTGCGAGTCCCACCCGGGGTA
10	GCCCCAGTGGCCTAATGGATAAGGCATTGGCCTtcaAAGCCAGGGATTGTGG GTTGCGAGTCCCACCTGCGGGTG
11	GGCCGCGTGGCCTAATGGATAAGGCGTCTGACTtcaGATCAGAAGATTGCAG GTTGCGAGTCCCTGCCGCGGTTCG
12	GACCGCGTGGCCTAATGGATAAGGCGTCTGACTtcaGATCAGAAGATTGAGG GTTGCGAGTCCCTTCGTGGTTCG
13	GACCACGTGGCCTAATGGATAAGGCGTCTGACTtcaGATCAGAAGATTGAGG GTTGGAATCCCTTCGTGGTTG
16	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTtcaAATTCAAAGGTTGTGG GTTGGAATCCCACCAGAGTTCG
17	GGCTCCGTGGCGCAATGGATAGCGCATTGGACTtcaAGAGGCTGAAGGCATT CAAAGGTTCCGGGTTTCGAGTCCCGGCGGAGTTCG

SEQ ID NO	Suppressor tRNA Sequence
18	GGCTCCGTGGCGCAATGGATAGCGCATTGGACTtcaAATTCAAAGGTTCCGG GTTTCGAGTCCCGGCGGAGTCG
22	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTtcaAATTCAAAGGTTGTGG GTTTCGAGTCCCACCAGAGTCG
35	GTCTCTGTGGCGCAATGGACGAGCGCGCTGGACTtcaAATCCAGAGGTTCCG GGTTCGAGTCCCGGCAGAGATG
36	GGTCCCATGGTGTAAATGGTTAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
38	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGAACCT
45	GGCCCCATGGTGTAAATGGTTAGCACTCTGGACTttaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
178	GGTCCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
180	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCGATCCGAG TTCAAATCTCGGTGGAACCT
187	GGCCCCATGGTGTAAATGGTTAGCACTCTGGACTctaAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
287	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGGG TTCAAATCCCGTCGGGGTCA
288	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTACGGG TTCAAATCCCGTCGGGGTCA
289	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTCCGGG TTCAAATCCCGGCGGGGTCA
290	CGTCGGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAATTCAAAGGTT GTGGGTTCGAGTCCCAGAGTCG
291	CGTCGCCCCAGTGGCCTAATGGATAAGGCACTGGCCTTCAAAGCCAGGGATT GTGGGTTCGAGTCCCACCTGGGGTG
292	CGTCGGCTCCGTGGCGCAATGGATAGCGCATTGGACTTCAAATTCAAAGGTT CCGGGTTCGAGTCCCGGCGGAGTCG
293	CGTCGCCCCAGTGGCCTAATGGATAAGGCACTGGCCTTCAAAGCCAGGGATT GTGGGTTCGAGTCCCACCTGGGGTG

SEQ ID NO	Suppressor tRNA Sequence
294	CGTCGGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAATTCAAAGGTT GTGGGTTTGAATCCCACCAGAGTCG
295	CGTCGGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAGCTGAGCCTAG TGTGGTCATTCAAAGGTTGTGGGTTTCGAGTCCCACCAGAGTCG
296	CGTCGCCCCGGTGGCCTAATGGATAAGGCATTGGCCTTCAAAGCCAGGGATT GTGGGTTTCGAGTCCCACCCGGGGTA
297	CGTCGGCTCCGTGGCGCAATGGATAGCGCATTGGACTTCAAGAGGCTGAAGG CATTCAAAGGTTCCGGGTTTCGAGTCCCGGCGGAGTCG
298	CGTCGGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAGTGACGAATAG AGCAATTCAAAGGTTGTGGGTTTGAATCCCACCAGAGTCG
299	CGTCGGCCGCGTGGCCTAATGGATAAGGCGTCTGACTTCAGATCAGAAGATT GCAGGTTTCGAGTCCTGCCGCGGTCG
300	CGTCGACCGCGTGGCCTAATGGATAAGGCGTCTGACTTCAGATCAGAAGATT GAGGGTTTCGAGTCCCTTCGTGGTCG
301	CGTCGGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAGATAGTTAGAG AAATTCAAAGGTTGTGGGTTTCGAGTCCCACCAGAGTCG
302	CGTCGGTCCATGGTGTAATGGTGAGCACTCTGGACTCTAAATCCAGCGATC CGAGTTTCGAGTCTCGGTGGAACCT
303	CGTCGGCCCCATGGTGTAATGGTTAGCACTCTGGACTCTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGGACCT
304	CGTCGGTCCCATGGTGTAATGGTTAGCACTCTGGACTCTAAATCCAGCAATC CGAGTTTGAATCTCGGTGGGACCT
305	CGTCGGTCCCATGGTGTAATGGTTAGCACTCTGGACTCTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGGACCT
306	CGTCGGCCCCATGGTGTAATGGTCAGCACTCTGGACTCTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGGACCC
307	CGTCGGTCCATGGTGTAATGGTAAGCACTCTGGACTCTAAATCCAGCGATC CGAGTTTCGAGTCTCGGTGGAACCT
308	CGTCGGTCCATGGTGTAATGGTTAGCACTCTGGACTCTAAATCCGGTAATC CGAGTTCAAATCTCGGTGGAACCT
309	CGTCGGTCCATGGTGTAATGGTTAGCACTCTGGACTCTAAATCCAGCGATC CGAGTTCAAGTCTCGGTGGAACCT

SEQ ID NO	Suppressor tRNA Sequence
310	CGTCGGTTCATGGTGTAATGGTAAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCGAGTCTCGGTGGAACCT
311	CGTCGGCCCCATGGTGTAATGGTTAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGGACCT
312	CGTCGGTTCATGGTGTAATGGTGAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCGAGTCTCGGTGGAACCT
313	CGTCGGTTCATGGTGTAATGGTTAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGAACCT
314	CGTCGGTCCCATGGTGTAATGGTTAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGGACCT
315	CGTCGGTCCCATGGTGTAATGGTTAGCACTCTGGACTTTAAATCCAGCAATC CGAGTTCGAATCTCGGTGGGACCT
316	CGTCGGTTCATGGTGTAATGGTTAGCACTCTGGACTTTAAATCCGGTAATC CGAGTTCAAATCTCGGTGGAACCT
317	CGTCGGCCCCATGGTGTAATGGTCAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCAAATCTCGGTGGGACCC
318	CGTCGGTTCATGGTGTAATGGTTAGCACTCTGGACTTTAAATCCAGCGATC CGAGTTCAAGTCTCGGTGGAACCT
319	CGTCGACCTCGTGGCGCAATGGTAGCGCGTCTGACTCTAGATCAGAAGGTTG CGTGTTCAAGTCACGTCGGGGTCA
320	CGTCGACCTCGTGGCGCAACGGTAGCGCGTCTGACTCTAGATCAGAAGGTTG CGTGTTCAAATCACGTCGGGGTCA
321	CGTCGGCCTCGTGGCGCAACGGTAGCGCGTCTGACTCTAGATCAGAAGGTTG CGTGTTCAAATCACGTCGGGGTCA
322	CGTCGACCTCGTGGCGCAACGGTAGCGCGTCTGACTCTAGATCAGAAGGCTG CGTGTTCAATCACGTCGGGGTCA
323	CGTCGACCTCGTGGCGCAACGGCAGCGCGTCTGACTCTAGATCAGAAGGTTG CGTGTTCAAATCACGTCGGGGTCA
324	CGTCTCCCACATGGTCTAGCGGTTAGGATTCCTGGTTCTAACCCAGGCGGCC CGGGTTCGACTCCCGGTGTGGGAA
325	CGTCTCCCATATGGTCTAGCGGTTAGGATTCCTGGTTCTAACCCAGGTGGCC CGGGTTCGACTCCCGGTATGGGAA

SEQ ID NO	Suppressor tRNA Sequence
326	CGTCTCCCTGGTGGTCTAGTGGCTAGGATTCGGCGCTCTAACCGCCGCGGCC CGGGTTCGATTCCCGGTCAGGGAA
327	CGTCTCCCTGGTGGTCTAGTGGTTAGGATTCGGCGCTCTAACCGCCGCGGCC CGGGTTCGATTCCCGGTCAGGGAA
328	CGTCTCCCTGGTCTAGTGGCTAGGATTCGGCGCTCTAACCGCCGCGGCCCGG GTTTCGATTCCCGGCCAGGGAA
329	CGTCTCCCACATGGTCTAGCGGTTAGGATTCCTGGTTCTAACCCAGGCGGCC CGGGTTCGACTCCCGGTGTGGGAA
330	CGTCTCCCATATGGTCTAGCGGTTAGGATTCCTGGTTCTAACCCAGGTGGCC CGGGTTCGACTCCCGGTATGGGAA
331	CGTCTCCCTGGTGGTCTAGTGGTTAGGATTCGGCGCTCTAACCGCCGCGGCC CGGGTTCGATTCCCGGTCAGGGAA
332	CGTCTCCCTGGTGGTCTAGTGGTTAGGATTCGGCGCTCTAACCGCCGCGGCC CGGGTTCGATTCCCGGTCAGGAAA
333	CGTCTCCCTGGTGGTCTAGTGGCTAGGATTCGGCGCTCTAACCGCCGCGGCC CGGGTTCGATTCCCGGCCAGGGAA
334	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
335	GACCTCGTGGCGCAATGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAGTCACGTCGGGGTCA
336	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
337	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGCTGCGTG TTCGAATCACGTCGGGGTCA
338	GACCTCGTGGCGCAACGGCAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
339	GCGTTGGTGGTATAGTGGTTAGCATAGCTGCCTTCAAAGCAGTTGACCCGGG TTCGATTCCCGGCCAACGCA
340	GCGTTGGTGGTATAGTGGTGAGCATAGCTGCCTTCAAAGCAGTTGACCCGGG TTCGATTCCCGGCCAACGCA
341	GCGTTGGTGGTATAGTGGTAAGCATAGCTGCCTTCAAAGCAGTTGACCCGGG TTCGATTCCCGGCCAACGCA

SEQ ID NO	Suppressor tRNA Sequence
342	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
343	GACCTCGTGGCGCAATGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAGTCACGTCGGGGTCA
344	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
345	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGCTGCGTG TTCGAATCACGTCGGGGTCA
346	GACCTCGTGGCGCAACGGCAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
347	GGCCTCATGGTGCAACAGTAGTGTGTCTGACTTCAGATCAGAAGGTTGTATG TTCAAATCACGTAGGGGTCA
348	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTCTAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
349	GACCTCGTGGCGCAATGGTAGCGCGTCTGACTCTAGATCAGAAGGTTGCGTG TTCAAGTCACGTCGGGGTCA
350	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTCTAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
351	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTCTAGATCAGAAGGCTGCGTG TTCGAATCACGTCGGGGTCA
352	GACCTCGTGGCGCAACGGCAGCGCGTCTGACTCTAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
353	GGCCTCATGGTGCAACAGTAGTGTGTCTGACTCTAGATCAGAAGGTTGTATG TTCAAATCACGTAGGGGTCA
354	GCATTGGTGGTTTCAGTGGTAGAATTCTCGCCTTCAACGCGGGAGACCCGGGT TCAATTCCCGGCCAATGCA
355	GCGCCGCTGGTGTAGTGGTATCATGCAAGATTTCAATTCTTGCGACCCGGGT TCGATTCCCGGGCGGCGCA
356	GCATTGGTGGTTCAATGGTAGAATTCTCGCCTTCAACGCAGGAGACCCAGGT TCGATTCTGGCCAATGCA
357	GCGTTGGTGGTTTGTAGTGGTAGAATTCTCGCCTTCAATGCGGGAGACCCGGGT TCAATTCCCGGGCCACTGCA

SEQ ID NO	Suppressor tRNA Sequence
358	GCCTTGGTGGTGCAGTGGTAGAATTCTCGCCTTCAACGTGGGAGACCCGGGT TCAATTCCCGGCCAATGCA
359	GGTGGTTCAGTGGTAGAATTCTCGCCTTCAACGCGGGAGACCCGGGTTTAAT TCCCGGTCA
360	GTGGTCTAGTGGTTAGGATTGAGCGCTTCAACCGCCGCAGCCCGGGTTTCGAT TCCCGGTCA
361	GCGTCAGTGGTTTTAGTGGTGAATTCTCGCCTTCAATGCACGAGATCCGTGT TCAACTCCTGGTTGGTGCA
362	GCGTCAGTGGTTTTAGTGGTGAATTCTCGCCTTCAATGCACGAGATCCGTG TTCAACTCCTGGTTGGTGCA
363	GCGTTGGCAGTTCAGTGGTAGAATTCTCGCCTTCAACCCGGGAGACCTGGAT TCCATTTCCGGCAAATGCA
364	GCATGGGTGGTTCAGTGGTAGAATTCTCGCCTTCAACGCGGGAGGCCCGGGT TCGATTCCCGGCCCATGCA
365	GCATTGGTGGTTCAGTGGTAGAATTCTCGCCTTCAACGCGGGAGGCCCGGGT TCGATTCCCGGCCAATGCA
366	GCATTGGTGGTTCAGTGGTAGAATTCTCGCCTTCAACGCGGGAGGCCCGGGT TTGATTCCCGGCCAGTGCA
367	GCATAGGTGGTTCAGTGGTAGAATTCTTGCCTTCAACGCAGGAGGCCCAGGT TTGATTCTGGCCCATGCA
368	GCATTGGTGGTTCAGTGGTAGAATTCTCGCCTTCAATGCGGGCGGCCGGGCT TCGATTCTGGCCAATGCA
369	GCATGGGTGATTGAGTGGTAGAATTTTCACCTTCAATGCAGGAGGTCCAGGT TCATTTCTGGCCTATGCA
370	GCGTTGGTGGTATAGTGGTTAGCATAGCTGCCTTCAAAGCAGTTGACCCGGG TTCGATTCCCGGCCAACGCA
371	GCGTTGGTGGTATAGTGGTGAGCATAGCTGCCTTCAAAGCAGTTGACCCGGG TTCGATTCCCGGCCAACGCA
372	GCGTTGGTGGTATAGTGGTAAGCATAGCTGCCTTCAAAGCAGTTGACCCGGG TTCGATTCCCGGCCAACGCA
373	GCGTTGGTGGTATAGTGGTGAGCATAGTTGCCTTCAAAGCAGTTGACCCGGG CTCGATTCCCGGCCAACGCA

SEQ ID NO	Suppressor tRNA Sequence
374	GCGTTGGTGGTATAGTGGTGAGCATAGTTGCCTTCAAAGCAGTTGACCCGGG CTCGATTCCCGGCCAACGCA
375	GGGCCAGTGGCGCAATGGATAACGCGTCTGACTTCAGATCAGAAGATTCCAG GTTCGACTCCTGGCTGGCTCG
376	GGGCCAGTGGCGCAATGGATAACGCGTCTGACTTCAGATCAGAAGATTCTAG GTTCGACTCCTGGCTGGCTCG
377	GGCCGCGTGGCCTAATGGATAAGGCGTCTGATTTAGATCAGAAGATTGAGG GTTCGAGTCCCTTCGTGGTTCG
378	GACCCAGTGGCCTAATGGATAAGGCATCAGCCTTCAGAGCTGGGGATTGTGG GTTCGAGTCCCACCTGGGTTCG
379	GCCCCAGTGGCCTAATGGATAAGGCACTGGCCTTCAAAGCCAGGGATTGTGG GTTCGAGTCCCACCTGGGGTA
380	GCCCCAGTGGCCTAATGGATAAGGCACTGGCCTTCAAAGCCAGGGATTGTGG GTTCGAGTCCCACCTGGGGTG
381	GCCCCGGTGGCCTAATGGATAAGGCATTGGCCTTCAAAGCCAGGGATTGTGG GTTCGAGTCCCACCCGGGGTA
382	GCCCCAGTGGCCTAATGGATAAGGCATTGGCCTTCAAAGCCAGGGATTGTGG GTTCGAGTCCCACCTGGGGTG
383	GCCCCAGTGGCCTGATGGATAAGGTACTGGCCTTCAAAGCCAGGGATTGTGG GTTCGAGTTCCACCTGGGGTA
384	GGCCGCGTGGCCTAATGGATAAGGCGTCTGACTTCAGATCAGAAGATTGCAG GTTCGAGTCCTGCCGCGGTTCG
385	GACCACGTGGCCTAATGGATAAGGCGTCTGACTTCAGATCAGAAGATTGAGG GTTCGAATCCCTCCGTGGTTA
386	GACCGCGTGGCCTAATGGATAAGGCGTCTGACTTCAGATCAGAAGATTGAGG GTTCGAGTCCCTTCGTGGTTCG
387	GACCACGTGGCCTAATGGATAAGGCGTCTGACTTCAGATCAGAAGATTGAGG GTTCGAATCCCTTCGTGGTTA
388	GACCACGTGGCCTAATGGATAAGGCGTCTGACTTCAGATCAGAAGATTGAGG GTTCGAATCCCTTCGTGGTTG
389	GGCCGTGTGGCCTAATGGATAAGGCGTCTGACTTCAGATCAAAGATTGCAG GTTTGAGTTCTGCCACGGTTCG

SEQ ID NO	Suppressor tRNA Sequence
390	GGCTCCGTGGCGCAATGGATAGCGCATTGGACTTCAAGAGGCTGAAGGCATT CAAAGGTTCCGGGTTTCGAGTCCCGGCGGAGTCG
391	GGCTCCGTGGCGCAATGGATAGCGCATTGGACTTCAAATTCAAAGGTTCCGG GTTTCGAGTCCCGGCGGAGTCG
392	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAGTGACGAATAGAGCA ATTCAAAGGTTGTGGGTTTCAATCCCACCAGAGTCG
393	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAATTCAAAGGTTGTGG GTTTCAATCCCACCAGAGTCG
394	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAGCTGAGCCTAGTGTG GTCATTCAAAGGTTGTGGGTTTCGAGTCCCACCAGAGTCG
395	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAATTCAAAGGTTGTGG GTTTCGAGTCCCACCAGAGTCG
396	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAGATAGTTAGAGAAAT TCAAAGGTTGTGGGTTTCGAGTCCCACCAGAGTCG
397	GTCTCTGTGGCGCAATGGACGAGCGCGCTGGACTTCAAATCCAGAGGTTCCG GGTTCGAGTCCCGGCAGAGATG
398	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAGCCTAAATCAAGAGA TTCAAAGGTTGCGGGTTTCGAGTCCCTCCAGAGTCG
399	GGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCAAATTCAAAGGTTGCGG GTTTCGAGTCCCTCCAGAGTCG
400	GGCAGCATAGCAGAGTGTTTCAGGTTACAGGTTCAAGATGTAACTGAGTTC AAATCCCAGTTCTGCCA
401	TGGTGTAATAGGTAGCACAGAGAATTCTAGATTCTCAGGGGTAGGTTCAATT CCTAT
402	TAGGACATGGTGTGATAGGTAGCATGGAGAATTCTAGATTCTCAGGGGTAGG TTCAATTCCTACAGTTCTAG
403	TAGGACGTGGTGTGATAGGTAGCATGGGGAATTCTAGATTCTCAGGGGTGGG TTCAATTCCTATAGTTCTAG
404	TAGGACGTGGTGTAGTAGGTAGCATGGAGAATGCTAAATTCTCAGGGGTAGG TTCAATTCCTATAGTTCTAG
405	TAGGACATGGTGTAAATAGGTAGAATGGAGAATTCTAAATTCTCAGGGGTAGG TTCAATTCCTATAGTTCTAG

SEQ ID NO	Suppressor tRNA Sequence
406	TAGGATGTGGTGTATTAGGTAGCACAGAGAATTCTAGATTCTCAGGGGTAGG TTCGATTCCTATAATTCTAC
407	TAGGACTTGGTGTAAATGGGTAGCACAGAGAATTCTAGATTCTCAGGGGTGGG TTCAATTCCTTTCGTCCTAG
408	TCTAGGATGTGGTGTGATAGGTAGCATGGAGAATTCTAGATTCTCAGGGGTA GGTTCAATTCCTATATTCTAGAA
409	TAGGACGTGGTGTGATAGGTAGCATGGAGAATTCTAGATTCTCAGGGATGGG TTCAATTCCTATAGTCCTAG
410	TAGGACGTGGTGTGATAGGTAGCACGGAGAATTCTAGATTCTCAGGGATGGG TTCAATTCCTGTAGTTCTAG
411	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTCTAAATCCAGCGATCCGAG TTCAAATCTCGGTGGAACCT
412	GGTTCCATGGTGTAAATGGTGACCACTTTGGACTCTAAATACAGTGATCAGAG TTCAAGTCTCACTGGAACCT
413	GGTTCCATGGTGTAAATGGTGAGGGCTTTGGACTCTAACTACAGTGATCAGAG TTCAAGTCTCAGTGGGACCT
414	GGTTCCATGGTGTAAATGGTAAGCACCTGGACTCTAAATCCAGCAACCAGAG TTCCAGTCTCAGCGTGGACCT
415	GGTAGTGTAGTCTACTGGTTAAACGCTTGGGCTCTAACATTAACGTCCTGGG TTCAAATCCCAGCTTTGTCA
416	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTCTAAATCCAGCGATCCGAG TTCAAGTCTCGGTGGAACCT
417	GGTTCCATGGTGTAAATGGTGAGCACTCTGGACTCTAAATCCAGCGATCCGAG TTCGAGTCTCGGTGGAACCT
418	GGTTCCATGGTGTAAATGGTAAGCACTCTGGACTCTAAATCCAGCGATCCGAG TTCGAGTCTCGGTGGAACCT
419	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTCTAAATCCGGTAATCCGAG TTCAAATCTCGGTGGAACCT
420	GGCCCCATGGTGTAAATGGTCAGCACTCTGGACTCTAAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCC
421	GGTTCCATGGTGTAAATGGTAAGCACTCTGGACTCTAAATCCAGCCATCTGAG TTCGAGTCTCTGTGGAACCT

SEQ ID NO	Suppressor tRNA Sequence
422	GGTTCCATGGTGTAAATGGTGAGCACTTTGGACTCTAAATACAGTGATCAGAG TTCAAGTCTCACTGGGACCT
423	GGTTCCATGGGTTAATGGTGAGCACCTGGACTCTAAATCAAGCGATCCGAG TTCAAATCTCGGTGGTACCT
424	GTTTCCATGGTGTAAATGGTGAGCACTCTGGACTCTAAATCCAGAAATACATT CAAAGAATTAAGAACA
425	GGTCCCATGGTGTAAATGGTTAGCACTCTGGACTCTAAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
426	GGTCCCATGGTGTAAATGGTTAGCACTCTGGACTCTAAATCCAGCAATCCGAG TTCGAATCTCGGTGGGACCT
427	GGCCCCATGGTGTAAATGGTTAGCACTCTGGACTCTAAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
428	GGTCCCATGGTGTAAATGGTTAGCACTCTGGGCTCTAAATCCAGCAATCCGAG TTCGAATCTTGGTGGGACCT
429	GGCTGTGTACCTCAGTGGGCAAGGGTATGGACTCTAAAGCCAGACTATTTGG GTTCAAATCCCAGCTTGGCCT
430	GACCATGTGGCCTAAGGGAAAAGACATCTCACTCTAGGTCAGAAGATTGAGG GTTCAAGTCCTTTCATGGTCA
431	GGTACAGTGTTAAAGGGGAGAAAAATTGCTGACTCTAAATACAGTAGACCTA GGTTTGAATCCTGGCTTTACCA
432	TGGTGTAAATAGGTAGCACAGAGAATTTTAGATTCTCAGGGGTAGGTTCAATT CCTAT
433	TAGGACATGGTGTGATAGGTAGCATGGAGAATTTTAGATTCTCAGGGGTAGG TTCAATTCCTACAGTTCTAG
434	TAGGACGTGGTGTGATAGGTAGCATGGGGAATTTTAGATTCTCAGGGGTGGG TTCAATTCCTATAGTTCTAG
435	TAGGACGTGGTGTAGTAGGTAGCATGGAGAATGTTAAATTCTCAGGGGTAGG TTCAATTCCTATAGTTCTAG
436	TAGGACATGGTGTAAATAGGTAGAATGGAGAATTTTAAATTCTCAGGGGTAGG TTCAATTCCTATAGTTCTAG
437	TAGGATGTGGTGTATTAGGTAGCACAGAGAATTTTAGATTCTCAGGGGTAGG TTCGATTCCTATAATTCTAC

SEQ ID NO	Suppressor tRNA Sequence
438	TAGGACTTGGTGTAAATGGGTAGCACAGAGAATTTTAGATTCTCAGGGGTGGG TTCAATTCCTTTCGTCCTAG
439	TCTAGGATGTGGTGTGATAGGTAGCATGGAGAATTTTAGATTCTCAGGGGTA GGTTCAATTCCTATATTCTAGAA
440	TAGGACGTGGTGTGATAGGTAGCATGGAGAATTTTAGATTCTCAGGGATGGG TTCAATTCCTATAGTCCTAG
441	TAGGACGTGGTGTGATAGGTAGCACGGAGAATTTTAGATTCTCAGGGATGGG TTCAATTCCTGTAGTTCTAG
442	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTTTAAATCCAGCGATCCGAG TTCAAATCTCGGTGGAACCT
443	GGTTCCATGGTGTAAATGGTGACCACTTTGGACTTTAAATACAGTGATCAGAG TTCAAGTCTCACTGGAACCT
444	GGTTCCATGGTGTAAATGGTGAGGGCTTTGGACTTTAACTACAGTGATCAGAG TTCAAGTCTCAGTGGGACCT
445	GGTTCCATGGTGTAAATGGTAAGCACCTGGACTTTAAATCCAGCAACCAGAG TTCCAGTCTCAGCGTGGACCT
446	GGTAGTGTAGTCTACTGGTTAAACGCTTGGGCTTTAACATTAACGTCCTGGG TTCAAATCCCAGCTTTGTCA
447	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTTTAAATCCAGCGATCCGAG TTCAAGTCTCGGTGGAACCT
448	GGTTCCATGGTGTAAATGGTGAGCACTCTGGACTTTAAATCCAGCGATCCGAG TTCGAGTCTCGGTGGAACCT
449	GGTTCCATGGTGTAAATGGTAAGCACTCTGGACTTTAAATCCAGCGATCCGAG TTCGAGTCTCGGTGGAACCT
450	GGTTCCATGGTGTAAATGGTTAGCACTCTGGACTTTAAATCCGGTAATCCGAG TTCAAATCTCGGTGGAACCT
451	GGCCCCATGGTGTAAATGGTCAGCACTCTGGACTTTAAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCC
452	GGTTCCATGGTGTAAATGGTAAGCACTCTGGACTTTAAATCCAGCCATCTGAG TTCGAGTCTCTGTGGAACCT
453	GGTTCCATGGTGTAAATGGTGAGCACTTTGGACTTTAAATACAGTGATCAGAG TTCAAGTCTCACTGGGACCT

SEQ ID NO	Suppressor tRNA Sequence
454	GGTTCCATGGGTAAATGGTGAGCACCTGGACTTTAAATCAAGCGATCCGAG TTCAAATCTCGGTGGTACCT
455	GTTTCCATGGTGTAATGGTGAGCACTCTGGACTTTAAATCCAGAAATACATT CAAAGAATTAAGAACA
456	GGTCCCATGGTGTAATGGTTAGCACTCTGGACTTTAAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
457	GGTCCCATGGTGTAATGGTTAGCACTCTGGACTTTAAATCCAGCAATCCGAG TTCGAATCTCGGTGGGACCT
458	GGCCCCATGGTGTAATGGTTAGCACTCTGGACTTTAAATCCAGCGATCCGAG TTCAAATCTCGGTGGGACCT
459	GGTCCCATGGTGTAATGGTTAGCACTCTGGGCTTTAAATCCAGCAATCCGAG TTCGAATCTTGGTGGGACCT
460	GGCTGTGTACCTCAGTGGGCAAGGGTATGGACTTTAAAGCCAGACTATTTGG GTTCAAATCCCAGCTTGGCCT
461	GACCATGTGGCCTAAGGGAAAAGACATCTCACTTTAGGTCAGAAGATTGAGG GTTCAAGTCCTTTCATGGTCA
462	GGTACAGTGTTAAAGGGGAGAAAAATGCTGACTTTAAATACAGTAGACCTA GGTTTGAATCCTGGCTTTACCA
463	TCCCTGGTGGTCTAGTGGTTAGGATTCGGCGCTTTAACCGCCGCGGCCCGGG TTCGATTCCCGGTCAGGGAA
464	TCCCTGGTGGTCTAGTGGTTAGGATTCGGCGCTTTAACCGCCGCGGCCCGGG TTCGATTCCCGGTCAGGAAA
465	CCCCTGGTGGTCTAGTGCTTAGGATTCGGTGCTTTAACCGCTGCTGCCTGCG TTCGATTCCCGGTCAGGGAA
466	TCCTTGATGTCTAGTGGTTAGGATTTGGTGCTTTAACTGCAGCAGCCTGGGT TCATTTCTCAGTCAGGGAA
467	TCCCATATGGTCTAGCGGTTAGGATTCCTGGTTTTAACCAGGTGGCCCCGGG TTCGACTCCCGGTATGGGAA
468	TCCGTGGTGGTCTAGTGGCTAGGATTCGGCGCTTTAACCGCCTGCAGCTCGA GTTTCGATTCTGGTCAGGGAA
469	CCCTGTGGTCTAGTGGCTAAGACTTTGTGCTTTAATTGCTGCATCCTAGGTT CAATTCCCAGTCAGGGA

SEQ ID NO	Suppressor tRNA Sequence
470	TCCCACATGGTCTAGCGGTTAGGATTCCTGGTTTTAACCCAGGCGGCCCCGGG TTCGACTCCCGGTGTGGGAA
471	TCCCTGGTGGTCTAGTGGCTAGGATTCGGCGCTTTAACCGCCGCGGCCCCGGG TTCGATTCCCGGCCAGGGAA
472	TCCCTGGTGGTCTAGTGGCTAGGATTCGGCGCTTTAACCGCCGCGGCCCCGGG TTCGATTCCCGGTCAGGGAA
473	GCGTTGGTGGTGTAGTGGTGAGCACAGCTGCCTTTAAAGCAGTTAACGCGGG TTCGATTCCCGGGTAACGAA
474	TCCTTGGTGGTCTAGTGGCTAGGATTCGGTGCTTTAACCTGTGCGGCCCCGGG TTCAATTCCCGATGAAGGAA
475	TGTCTGGTGGTCAAGTGGCTAGGATTTGGCGCTTTAACTGCCGCGGCCCCGCG TTCGATTCCCGGTCAGGGAA
476	TCCCTGGTGGTCTAGTGGCTAGGATTCGGCGCTTTAACCGCCTGCAGCTCGA GTTTCGATTTCCTGGTCAGGGAA
477	GCAATGGTGGTTCAGTGGTAGAATTCTCGCCTTTAACACAGGAGACCCGGGT TCAATTCCTGACCCATGTA
478	TCCCTGGTGGTCTAGTGGTTAGGATTCGGCGCTCTAACCGCCGCGGCCCCGGG TTCGATTCCCGGTCAGGGAA
479	TCCCTGGTGGTCTAGTGGTTAGGATTCGGCGCTCTAACCGCCGCGGCCCCGGG TTCGATTCCCGGTCAGGAAA
480	CCCCTGGTGGTCTAGTGCTTAGGATTCGGTGCTCTAACCGCTGCTGCCTGCG TTCGATTCCCGGTCAGGGAA
481	TCCTTGATGTCTAGTGGTTAGGATTTGGTGCTCTAACTGCAGCAGCCTGGGT TCATTTCTCAGTCAGGGAA
482	TCCCATATGGTCTAGCGGTTAGGATTCCTGGTTCTAACCCAGGTGGCCCCGGG TTCGACTCCCGGTATGGGAA
483	TCCGTGGTGGTCTAGTGGCTAGGATTCGGCGCTCTAACCGCCTGCAGCTCGA GTTTCGATTTCCTGGTCAGGGAA
484	CCCTGTGGTCTAGTGGCTAAGACTTTGTGCTCTAATTGCTGCATCCTAGGTT CAATTCCCAGTCAGGGA
485	TCCCACATGGTCTAGCGGTTAGGATTCCTGGTTCTAACCCAGGCGGCCCCGGG TTCGACTCCCGGTGTGGGAA

SEQ ID NO	Suppressor tRNA Sequence
486	TCCCTGGTGGTCTAGTGGCTAGGATTCGGCGCTCTAACCGCCGCGGCCCGGG TTCGATTCCCGGCCAGGGAA
487	TCCCTGGTGGTCTAGTGGCTAGGATTCGGCGCTCTAACCGCCGCGGCCCGGG TTCGATTCCCGGTCAGGGAA
488	GCGTTGGTGGTGTAGTGGTGAGCACAGCTGCCTCTAAAGCAGTTAACGCGGG TTCGATTCCCGGGTAACGAA
489	TCCTTGGTGGTCTAGTGGCTAGGATTCGGTGCTCTAACCTGTGCGGCCCGGG TTCAATTCCCGATGAAGGAA
490	TGTCTGGTGGTCAAGTGGCTAGGATTTGGCGCTCTAACTGCCGCGGCCCGCG TTCGATTCCCGGTCAGGGAA
491	TCCCTGGTGGTCTAGTGGCTAGGATTCGGCGCTCTAACCGCCTGCAGCTCGA GTTTCGATTCCCTGGTCAGGGAA
492	GCAATGGTGGTTCAGTGGTAGAATTCTCGCCTCTAACACAGGAGACCCGGGT TCAATTCCTGACCCATGTA
493	CCTTCAATAGTTCAGCTGGTAGAGCAGAGGACTTTAGCTACTTCCTCAGTAG GAGACGTCCTTAGGTTGCTGGTTCGATTCCAGCTTGAAGGA
494	CCTTCAATAGTTCAGCTGGTAGAGCAGAGGACTTTAGGTCCTTAGGTTGCTG GTTTCGATTCCAGCTTGAAGGA
495	GGTAAAATGGCTGAGTAAGCTTTAGACTTTAAATCTAAAGAGAGATTGAGCT CTCTTTTTTACCA
496	GGTAAAATGACTGAGTAAGCATTAGACTTTAAATCTAAAGACAGAGGTCAAG ACCTCTTTTTTACCA
497	GGTAAAATGGCTGAGTAAGCATTAGACTTTAAATCTAAAGACAGAGGTCAAG GCCTCTTTTTTACCA
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499	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTTTAGTTGGCTGTGTCCTTA GACATCCTTAGGTCGCTGGTTCGAATCCGGCTCGAAGGA
500	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTTTAGATCCTTAGGTCGCTG GTTTCGAATCCGGCTCGAAGGA
501	GGGGGTATAGCTCAGGGCTAGAGCTTTTTGACTTTAGAGCAAGAGGTCCCTG GTTCAAATCCAGGTTCTCCCT

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502	TATAGCTCAGTGGTAGAGCATTTAACTTTAGATCAAGAGGTCCCTGGATCAA CTCTGGGTG
503	GTCAGTGTTCACAAACGGTTAAGTGAAGAGGCTTTAAACCCAGACTGGATGG GTTCAATTCCCATCTCTGCCG
504	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTTTAGTGGATAGGGCGTGGC AATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
505	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTTTAGATCCTTAGGTCGCTG GTTTCGATTCCGGCTCGAAGGA
506	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTTTAGGCTCATTAAGCAAGG TATCCTTAGGTCGCTGGTTCGAATCCGGCTCGGAGGA
507	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTTTAGATCCTTAGGTCGCTG GTTTCGAATCCGGCTCGGAGGA
508	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGATTGTATAGACATTT GCGGACATCCTTAGGTCGCTGGTTCGATTCCAGCTCGAAGGA
509	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGATCCTTAGGTCGCTG GTTTCGATTCCAGCTCGAAGGA
510	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGCTACTTCCTCAGCAG GAGACATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
511	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGATCCTTAGGTCGCTG GTTTCGATTCCGGCTCGAAGGA
512	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGGCGCGCGCCCGTGGC CATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
513	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGATCCTTAGGTCGCTG GTTTCGATTCCGGCTCGAAGGA
514	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGCCTGTAGAAACATTT GTGGACATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
515	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGATCCTTAGGTCGCTG GTTTCGATTCCGGCTCGAAGGA
516	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGATTGTACAGACATTT GCGGACATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
517	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGATCCTTAGGTCGCTG GTTTCGATTCCGGCTCGAAGGA

SEQ ID NO	Suppressor tRNA Sequence
518	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGTACTTAATGTGTGGT CATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
519	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGATCCTTAGGTCGCTG GTTTCGATTCCGGCTCGAAGGA
520	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGGGGTTTGAATGTGGT CATCCTTAGGTCGCTGGTTCGAATCCGGCTCGGAGGA
521	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGATCCTTAGGTCGCTG GTTTCAATCCGGCTCGGAGGA
522	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGACTGCGGAAACGTTT GTGGACATCCTTAGGTCGCTGGTTCGAATCCGGCTCGAAGGA
523	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTTTAGATCCTTAGGTCGCTG GTTCAATCCGGCTCGAAGGA
524	CTTTCGATAGCTCAGTTGGTAGAGCGGAGGACTTTAGGTTTCATTAACTAAG GCATCCTTAGGTCGCTGGTTCGAATCCGGCTCGAAGGA
525	CTTTCGATAGCTCAGTTGGTAGAGCGGAGGACTTTAGATCCTTAGGTCGCTG GTTTCAATCCGGCTCGAAGGA
526	TCTTCAATAGCTCAGCTGGTAGAGCGGAGGACTTTAGGTGCACGCCCGTGGC CATTCTTAGGTGCTGGTTTGATTCCGACTTGGAGAG
527	TCTTCAATAGCTCAGCTGGTAGAGCGGAGGACTTTAGATTCTTAGGTGCTGG TTTGATTCCGACTTGGAGAG
528	GGTAAAATGGCTGAGTGAAGCATTGGACTTTAAATCTAAAGACAGGGGTAA GCCTCTTTTTTACCA
529	GGTAAAATGGCTGAGCAAGCATTGGACTTTAAATCTAAAGACAGATGTTGAG CCATCTTTTTTAGCA
530	GGTAAAATGGCTGAGTGAAGCATTGGACTTTAAATCTAAAGACAGGGGCTAA GCCTCTTTTTTACCA
531	GGTAAAATGGCTGAGCAAGCATTAGACTTTAAATCTAAAGACAGAGGTTAAG GCCTCTTTTTTACCA
532	GGTAAAATGGCTGAGTAAGCATTAGACTTTAAATCTAAAGACAGAGGTCAAG GCCTCTTTTTTCCT
533	GGTAAAATGGCTGAGCAAGCATTAGACTTTAAATCTGAAAACAGAGGTCAAA GGTCTCTTTTTTACCA

SEQ ID NO	Suppressor tRNA Sequence
534	GGTAAAATGGCTGAGTAAGCATTAGACTTTAAATCTAAAGACAGAGGTCAAG GCCTCTTTTTTACCA
535	GGTAAAATGACTGAATAAGCCTTAGACTTTAAATCTGAAGACAGAGGTCAAG GCCTCTTTTTTACCA
536	GGTAAAATGGCTGAGTAAGCATTGGACTTTAAATCTAAAGACAGAGGTCAAG ACCTCTTTTTTACCA
537	GGTAAAATGGCTGAGTAAAGCATTAGACTTTAAATCTAAGGACAGAGGCTAA ACCTCTTTTTTACCA
538	CCTTCAATAGTTCAGCTGGTAGAGCAGAGGACTCTAGCTACTTCCTCAGTAG GAGACGTCCTTAGGTTGCTGGTTCGATTCCAGCTTGAAGGA
539	CCTTCAATAGTTCAGCTGGTAGAGCAGAGGACTCTAGGTCCTTAGGTTGCTG GTTTCGATTCCAGCTTGAAGGA
540	GGTAAAATGGCTGAGTAAGCTTTAGACTCTAAATCTAAAGAGAGATTGAGCT CTCTTTTTTACCA
541	GGTAAAATGACTGAGTAAGCATTAGACTCTAAATCTAAAGACAGAGGTCAAG ACCTCTTTTTTACCA
542	GGTAAAATGGCTGAGTAAGCATTAGACTCTAAATCTAAAGACAGAGGTCAAG GCCTCTTTTTTACCA
543	GGTAAAATGGCTGAGTAAGCATTAGACTCTAAATCTAAAGACAGAGGTCAAG GCCTTTTTTACCA
544	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTCTAGTTGGCTGTGTCCTTA GACATCCTTAGGTCGCTGGTTCGAATCCGGCTCGAAGGA
545	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTCTAGATCCTTAGGTCGCTG GTTTCGAATCCGGCTCGAAGGA
546	GGGGGTATAGCTCAGGGCTAGAGCTTTTTGACTCTAGAGCAAGAGGTCCCTG GTTCAAATCCAGGTTCTCCCT
547	TATAGCTCAGTGGTAGAGCATTTAACTCTAGATCAAGAGGTCCCTGGATCAA CTCTGGGTG
548	GTCAGTGTTGCACAACGGTTAAGTGAAGAGGCTCTAAACCCAGACTGGATGG GTTCAATTCCCATCTCTGCCG
549	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTCTAGTGGATAGGGCGTGCC AATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA

SEQ ID NO	Suppressor tRNA Sequence
550	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTCTAGATCCTTAGGTCGCTG GTTTCGATTCCGGCTCGAAGGA
551	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTCTAGGCTCATTAAGCAAGG TATCCTTAGGTCGCTGGTTCGAATCCGGCTCGGAGGA
552	CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTCTAGATCCTTAGGTCGCTG GTTTCGAATCCGGCTCGGAGGA
553	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGATTGTATAGACATTT GCGGACATCCTTAGGTCGCTGGTTCGATTCCAGCTCGAAGGA
554	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGATCCTTAGGTCGCTG GTTTCGATTCCAGCTCGAAGGA
555	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGCTACTTCCTCAGCAG GAGACATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
556	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGATCCTTAGGTCGCTG GTTTCGATTCCGGCTCGAAGGA
557	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGGCGCGCGCCCGTGGC CATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
558	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGATCCTTAGGTCGCTG GTTTCGATTCCGGCTCGAAGGA
559	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGCCTGTAGAAACATTT GTGGACATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
560	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGATCCTTAGGTCGCTG GTTTCGATTCCGGCTCGAAGGA
561	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGATTGTACAGACATTT GCGGACATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
562	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGATCCTTAGGTCGCTG GTTTCGATTCCGGCTCGAAGGA
563	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGTACTTAATGTGTGGT CATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA
564	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGATCCTTAGGTCGCTG GTTTCGATTCCGGCTCGAAGGA
565	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGGGGTTTGAATGTGGT CATCCTTAGGTCGCTGGTTCGAATCCGGCTCGGAGGA

SEQ ID NO	Suppressor tRNA Sequence
566	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGATCCTTAGGTCGCTG GTTCGAATCCGGCTCGGAGGA
567	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGACTGCGGAAACGTTT GTGGACATCCTTAGGTCGCTGGTTCAATTCCGGCTCGAAGGA
568	CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTCTAGATCCTTAGGTCGCTG GTTCAATTCCGGCTCGAAGGA
569	CTTTCGATAGCTCAGTTGGTAGAGCGGAGGACTCTAGGTTTCATTAAACTAAG GCATCCTTAGGTCGCTGGTTTCAATCCGGCTCGAAGGA
570	CTTTCGATAGCTCAGTTGGTAGAGCGGAGGACTCTAGATCCTTAGGTCGCTG GTTCGAATCCGGCTCGAAGGA
571	TCTTCAATAGCTCAGCTGGTAGAGCGGAGGACTCTAGGTGCACGCCCGTGGC CATTCTTAGGTGCTGGTTTGATTCCGACTTGGAGAG
572	TCTTCAATAGCTCAGCTGGTAGAGCGGAGGACTCTAGATTCTTAGGTGCTGG TTTGATTCCGACTTGGAGAG
573	GGTAAAATGGCTGAGTGAAGCATTGGACTCTAAATCTAAAGACAGGGGTAA GCCTCTTTTTTACCA
574	GGTAAAATGGCTGAGCAAGCATTGGACTCTAAATCTAAAGACAGATGTTGAG CCATCTTTTTTAGCA
575	GGTAAAATGGCTGAGTGAAGCATTGGACTCTAAATCTAAAGACAGGGGCTAA GCCTCTTTTTTACCA
576	GGTAAAATGGCTGAGCAAGCATTAGACTCTAAATCTAAAGACAGAGGTAAAG GCCTCTTTTTTACCA
577	GGTAAAATGGCTGAGTAAGCATTAGACTCTAAATCTAAAGACAGAGGTCAAG GCCTCTTTTTTTCCT
578	GGTAAAATGGCTGAGCAAGCATTAGACTCTAAATCTGAAAACAGAGGTCAAA GGTCTCTTTTTTACCA
579	GGTAAAATGGCTGAGTAAGCATTAGACTCTAAATCTAAAGACAGAGGTCAAG GCCTCTTTTTTACCA
580	GGTAAAATGACTGAATAAGCCTTAGACTCTAAATCTGAAGACAGAGGTCAAG GCCTCTTTTTTACCA
581	GGTAAAATGGCTGAGTAAGCATTGGACTCTAAATCTAAAGACAGAGGTCAAG ACCTCTTTTTTACCA

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582	GGTAAAATGGCTGAGTAAAGCATTAGACTCTAAATCTAAGGACAGAGGCTAA ACCTCTTTTTACCA
583	GTTAAGATGGCAGAGCCTGGTAATTGCATTAACTTAAAATTTTATAATCAG AGGTTCAACTCCTCTTCTTAACA
584	GTTAAGATGGCAGAGCCCGCAATTGCATTAGACTTAAACTTTTATAATCAG AGGTTCAACTCCTCTCATTAACA
585	GGTAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTTTAGCTCCAGTCTCTTCG GGGGCGTGGGTTCAAATCCCACCGCTGCCA
586	GGTAGCGTGGCCGAGTGGTCTAAGACGCTGGATTTTAGCTCCAGTCTCTTCG GGGGCGTGGGTTTGAATCCCACCGCTGCCA
587	GGGCCAGTGGCTCAATGGATAATGCGTCTGACTTTAAATCAGAAGATTCCAG CCTTGACTCCTGGCTGGCTCA
588	GGTAGGGTGGCCGAGCGGTCTAAGGCACTGTATTTTAACTCCAGTCTCTTCA GAGGCATGGGTTTGAATCCCACCTGCTGCCA
589	GCCGAGCGGTCTAAGGCTCCGGATTTTAGCGCCGGTGTCTTCGGAGGCATGG GTTCGAATTCCAC
590	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTTAGCTAAGCTTCCTCCG CGGTGGGGATTCTGGTCTCCAATGGAGGCGTGGGTTTGAATCCCACCTTCTGA CA
591	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTTAGTTCTGGTCTCCAAT GGAGGCGTGGGTTTGAATCCCACCTTCTGACA
592	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTTAGCTTGGCTTCCTCGT GTTGAGGATTCTGGTCTCCAATGGAGGCGTGGGTTTGAATCCCACCTTCTGAC A
593	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTTAGTTCTGGTCTCCAAT GGAGGCGTGGGTTTGAATCCCACCTTCTGACA
594	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTTAGCTTACTGCTTCCTG TGTTCCGGTCTTCTGGTCTCCGTATGGAGGCGTGGGTTTGAATCCCACCTTCT GACA
595	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTTAGTTCTGGTCTCCGTA TGGAGGCGTGGGTTTGAATCCCACCTTCTGACA

SEQ ID NO	Suppressor tRNA Sequence
596	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTTAGTTGCTACTTCCCAG GTTTGGGGCTTCTGGTCTCCGCATGGAGGCGTGGGTTCGAATCCCACCTTCTG ACA
597	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTTAGTTCTGGTCTCCGCA TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
598	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTTAGGTAAGCACCTTGCC TGCGGGCTTTCTGGTCTCCGGATGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA
599	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTTAGTTTCTGGTCTCCGG ATGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
600	GCCTCCTTAGTGCAGTAGGTAGCGCATCAGTCTTTAAATCTGAATGGTCCTG AGTTCAAGCCTCAGAGGGGGCA
601	GTCAGGATGGCCGAGCAGTCTTAAGGCGCTGCGTTTTAATCGCACCCCTCCGC TGGAGGCGTGGGTTCGAATCCCACCTTTTGACA
602	GGTTCCATGGTGTAATGGTGAGCACTCTGGACTTTAAATCCAGAAGTAGTGC TGGAACAA
603	GTCAGGGTGGCTGAGCAGTCTGAGGGGCTGCGTTTTAGTCGCAGTCTGCCCT GGAGGCGTGGGTTCGAATCCCACCTCCTGAAA
604	ACCAGGATGGCCGAGTGGTTAAGGCGTTGGACTTTAGATCCAATGGACATAT GTCCGCGTGGGTTCGAACCCCCACTCCTGGTA
605	ACCGGGATGGCCGAGTGGTTAAGGCGTTGGACTTTAGATCCAATGGGCTGGT GCCC GCGTGGGTTCGAACCCCCACTCTCGGTA
606	ACCAGAATGGCCGAGTGGTTAAGGCGTTGGACTTTAGATCCAATGGATTTCAT ATCCGCGTGGGTTCGAACCCCCACTTCTGGTA
607	ACCGGGATGGCTGAGTGGTTAAGGCGTTGGACTTTAGATCCAATGGACAGGT GTCCGCGTGGGTTCGAGCCCCACTCCCGGTA
608	ACTCATTTGGCTGAGTGGTTAAGGCATTGGACTTTAGATCCAATGGAGTAGT GGCTGTGTGGGTTTAAACCCCCACTACTGGTA
609	GAGAAAGTCATCGTAGTTACGAAGTTGGCTTTAACCAGTTTTGGGAGGTTC AATTCCTTCCTTTCTCT
610	ACCAGGATGGCCAAGTAGTTAAAGGCACTGGACTTTAGAGCCAATGGACATA TGTCTGTGTGGGTTTGAACCCCCACTCCTGGTG

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611	GGTAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTTTAGCTCCAGTCTCTTCG GAGGCGTGGGTTCGAATCCCACCGCTGCCA
612	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTTTAGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCACTGCCA
613	GGTAGCGTGGCCGAGTGGTCTAAGGCGCTGGATTTTAGCTCCAGTCATTTTCG ATGGCGTGGGTTCGAATCCCACCGCTGCCA
614	GGTAGTGTGGTTGAATGGTCTAAGGCACTGAATTTTAGCTCCAGTCTCTTTG GGGACGTGGGTTTAAATCCCACCTGCTGCAA
615	GTTAAGATGGCAGAGCCTGGTAATTGCACTAAACTTAAAATTTTATAATCAG AGGTTCAACTCCTCTTCTTAACA
616	GTTAAGATGGCAGAGCCCGGCAATTGCACTAGACTTAAACTTTTATAATCAG AGGTTCAACTCCTCTCATTAACA
617	GGTAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTCTAGCTCCAGTCTCTTCG GGGGCGTGGGTTCAAATCCCACCGCTGCCA
618	GGTAGCGTGGCCGAGTGGTCTAAGACGCTGGATTCTAGCTCCAGTCTCTTCG GGGGCGTGGGTTTGAATCCCACCGCTGCCA
619	GGGCCAGTGGCTCAATGGATAATGCGTCTGACTCTAAATCAGAAGATTCCAG CCTTGACTCCTGGCTGGCTCA
620	GGTAGGGTGGCCGAGCGGTCTAAGGCACTGTATTCTAACTCCAGTCTCTTCA GAGGCATGGGTTTGAATCCCACCTGCTGCCA
621	GCCGAGCGGTCTAAGGCTCCGGATTCTAGCGCCGGTGTCTTCGGAGGCATGG GTTTGAATTCAC
622	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCTAGCTAAGCTTCCTCCG CGGTGGGATTCTGGTCTCCAATGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA
623	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCTAGTTCTGGTCTCCAAT GGAGGCGTGGGTTCGAATCCCACCTTCTGACA
624	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCTAGCTTGGCTTCCTCGT GTTGAGGATTCTGGTCTCCAATGGAGGCGTGGGTTCGAATCCCACCTTCTGAC A
625	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCTAGTTCTGGTCTCCAAT GGAGGCGTGGGTTCGAATCCCACCTTCTGACA

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626	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCTAGCTTACTGCTTCCTG TGTTCTGGGTCTTCTGGTCTCCGTATGGAGGCGTGGGTTCGAATCCCACCTTCT GACA
627	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCTAGTTCTGGTCTCCGTA TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
628	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCTAGTTGCTACTTCCCAG GTTTGGGGCTTCTGGTCTCCGCATGGAGGCGTGGGTTCGAATCCCACCTTCTG ACA
629	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCTAGTTCTGGTCTCCGCA TGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
630	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCTAGGTAAGCACCTTGCC TGCGGGCTTTCTGGTCTCCGGATGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA
631	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTCTAGTTTCTGGTCTCCGG ATGGAGGCGTGGGTTCGAATCCCACCTTCTGACA
632	GCCTCCTTAGTGCAGTAGGTAGCGCATCAGTCTCTAAATCTGAATGGTCCTG AGTTCAAGCCTCAGAGGGGGCA
633	GTCAGGATGGCCGAGCAGTCTTAAGGCGCTGCGTTCTAATCGCACCCCTCCGC TGGAGGCGTGGGTTCGAATCCCACCTTTTGACA
634	GGTTCCATGGTGTAATGGTGAGCACTCTGGACTCTAAATCCAGAAGTAGTGC TGGAACAA
635	GTCAGGGTGGCTGAGCAGTCTGAGGGGCTGCGTTCTAGTCGCAGTCTGCCCT GGAGGCGTGGGTTCGAATCCCACCTCCTGAAA
636	ACCAGGATGGCCGAGTGGTTAAGGCGTTGGACTCTAGATCCAATGGACATAT GTCCGCGTGGGTTCGAACCCCCACTCCTGGTA
637	ACCGGGATGGCCGAGTGGTTAAGGCGTTGGACTCTAGATCCAATGGGCTGGT GCCGCGTGGGTTCGAACCCCCACTCTCGGTA
638	ACCAGAATGGCCGAGTGGTTAAGGCGTTGGACTCTAGATCCAATGGATTTCAT ATCCGCGTGGGTTCGAACCCCCACTTCTGGTA
639	ACCGGGATGGCTGAGTGGTTAAGGCGTTGGACTCTAGATCCAATGGACAGGT GTCCGCGTGGGTTCGAGCCCCACTCCCGGTA
640	ACTCATTTGGCTGAGTGGTTAAGGCATTGGACTCTAGATCCAATGGAGTAGT GGCTGTGTGGGTTTAAACCCCCACTACTGGTA

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641	GAGAAAGTCATCGTAGTTACGAAGTTGGCTCTAACCCAGTTTTGGGAGGTTC AATTCCTTCCTTTCTCT
642	ACCAGGATGGCCAAGTAGTTAAAGGCACTGGACTCTAGAGCCAATGGACATA TGTCTGTGTGGGTTTGAACCCCACTCCTGGTG
643	GGTAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTCTAGCTCCAGTCTCTTCG GAGGCGTGGGTTCGAATCCCACCGCTGCCA
644	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTCTAGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCACTGCCA
645	GGTAGCGTGGCCGAGTGGTCTAAGGCGCTGGATTCTAGCTCCAGTCATTTTCG ATGGCGTGGGTTCGAATCCCACCGCTGCCA
646	GGTAGTGTGGTTGAATGGTCTAAGGCACTGAATTCTAGCTCCAGTCTCTTTG GGGACGTGGGTTTAAATCCCACCTGCTGCAA
647	GTTAAGATGGCAGAGCCTGGTAATTGCATCAAACCTTAAAATTTTATAATCAG AGGTTCAACTCCTCTTCTTAACA
648	GTTAAGATGGCAGAGCCCGCAATTGCATCAGACTTAAACCTTTATAATCAG AGGTTCAACTCCTCTCATTAAACA
649	GGTAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTTTCAGCTCCAGTCTCTTCG GGGGCGTGGGTTCAAATCCCACCGCTGCCA
650	GGTAGCGTGGCCGAGTGGTCTAAGACGCTGGATTTTCAGCTCCAGTCTCTTCG GGGGCGTGGGTTTGAATCCCACCGCTGCCA
651	GGGCCAGTGGCTCAATGGATAATGCGTCTGACTTCAAATCAGAAGATTCCAG CCTTGACTCCTGGCTGGCTCA
652	GGTAGGGTGGCCGAGCGGTCTAAGGCACTGTATTTCAACTCCAGTCTCTTCA GAGGCATGGGTTTGAATCCCACCTGCTGCCA
653	GCCGAGCGGTCTAAGGCTCCGGATTTACGCGCCGGTGTCTTCGGAGGCATGG GTTCGAATTCCAC
654	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTCAGCTAAGCTTCCTCCG CGGTGGGGATTCTGGTCTCCAATGGAGGCGTGGGTTCGAATCCCACCTTCTGA CA
655	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTCAGTTCTGGTCTCCAAT GGAGGCGTGGGTTCGAATCCCACCTTCTGACA

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656	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTCAGCTTGGCTTCCTCGT GTTGAGGATTCTGGTCTCCAATGGAGGCGTGGGTTCGAATCCCACTTCTGAC A
657	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTCAGTTCTGGTCTCCAAT GGAGGCGTGGGTTCGAATCCCACTTCTGACA
658	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTCAGCTTACTGCTTCCTG TGTTCTGGGTCTTCTGGTCTCCGTATGGAGGCGTGGGTTCGAATCCCACTTCT GACA
659	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTCAGTTCTGGTCTCCGTA TGGAGGCGTGGGTTCGAATCCCACTTCTGACA
660	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTCAGTTGCTACTTCCCAG GTTTGGGGCTTCTGGTCTCCGCATGGAGGCGTGGGTTCGAATCCCACTTCTG ACA
661	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTCAGTTCTGGTCTCCGCA TGGAGGCGTGGGTTCGAATCCCACTTCTGACA
662	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTCAGGTAAGCACCTTGCC TGCGGGCTTTCTGGTCTCCGATGGAGGCGTGGGTTCGAATCCCACTTCTGA CA
663	GTCAGGATGGCCGAGTGGTCTAAGGCGCCAGACTTCAGTTTCTGGTCTCCGG ATGGAGGCGTGGGTTCGAATCCCACTTCTGACA
664	GCCTCCTTAGTGAGTAGGTAGCGCATCAGTCTTCAAATCTGAATGGTCCTG AGTTCAAGCCTCAGAGGGGGCA
665	GTCAGGATGGCCGAGCAGTCTTAAGGCGCTGCGTTTCAATCGCACCCCTCCGC TGGAGGCGTGGGTTCGAATCCCACTTTTGACA
666	GGTTCATGGTGTAATGGTGAGCACTCTGGACTTCAAATCCAGAAGTAGTGC TGGAACAA
667	GTCAGGGTGGCTGAGCAGTCTGAGGGGCTGCGTTTCAGTCGCAGTCTGCCCT GGAGGCGTGGGTTCGAATCCCACTCCTGAAA
668	ACCAGGATGGCCGAGTGGTTAAGGCGTTGGACTTCAGATCCAATGGACATAT GTCCGCGTGGGTTCGAACCCCACTCCTGGTA
669	ACCGGGATGGCCGAGTGGTTAAGGCGTTGGACTTCAGATCCAATGGGCTGGT GCCCCGCTGGGTTCGAACCCCACTCTCGGTA

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670	ACCAGAATGGCCGAGTGGTTAAGGCGTTGGACTTCAGATCCAATGGATTTCAT ATCCGCGTGGGTTCGAACCCCACTTCTGGTA
671	ACCGGGATGGCTGAGTGGTTAAGGCGTTGGACTTCAGATCCAATGGACAGGT GTCCGCGTGGGTTCGAGCCCCACTCCCGGTA
672	ACTCATTGCTGAGTGGTTAAGGCATTGGACTTCAGATCCAATGGAGTAGT GGCTGTGTGGGTTTAAACCCCACTACTGGTA
673	GAGAAAGTCATCGTAGTTACGAAGTTGGCTTCAACCCAGTTTTGGGAGGTTT AATTCCTTCCTTTCTCT
674	ACCAGGATGGCCAAGTAGTTAAAGGCACTGGACTTCAGAGCCAATGGACATA TGTCTGTGTGGGTTTGAACCCCACTCCTGGTG
675	GGTAGCGTGGCCGAGCGGTCTAAGGCGCTGGATTTTCAGCTCCAGTCTCTTCG GAGGCGTGGGTTCGAATCCCACCGCTGCCA
676	GGTAGTGTGGCCGAGCGGTCTAAGGCGCTGGATTTTCAGCTCCAGTCTCTTCG GGGGCGTGGGTTCGAATCCCACCACTGCCA
677	GGTAGCGTGGCCGAGTGGTCTAAGGCGCTGGATTTTCAGCTCCAGTCATTTTCG ATGGCGTGGGTTCGAATCCCACCGCTGCCA
678	GGTAGTGTGGTTGAATGGTCTAAGGCACTGAATTTTCAGCTCCAGTCTCTTTG GGGACGTGGGTTTAAATCCCACCTGCTGCAA
679	GAGAAGGTCACAGAGGTTATGGGATTGGCTTTAAACCAGTCTGTGGGGGGTT CGATTCCCTCCTTTTTTCA
680	GAGAAGGTCATAGAGGTTATGGGATTGGCTTTAAACCAGTCTCTGGGGGGTT CGATTCCCTCCTTTTTTCA
681	GAAAAAGTCATAGGGGTTATGAGGCTGGCTTTAAACCAGCCTTAGGAGGTTT AATTCCTTCCTTTTTTTG
682	GGCCGGTTAGCTCAGTTGGTTAGAGCGTGCTGCTTTAAATGCCAGGGTCGAG GTTTCGATCCCCGTACGGGCCT
683	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTTTAAATCCATTGGGGTTTC CCCGCGCAGGTTCGAATCCTGCCGACTACG
684	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTTTAAATCCATTGGGGTTTC CCCACGCAGGTTCGAATCCTGCCGACTACG
685	GTAGTCGTGGCCGAGTGGTTAAGGTGATGGACTTTAAACCCATTGGGGTCTC CCCGCGCAGGTTCGAATCCTGCCGACTACG

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686	GGGTGTATGGCTCAGGGGTAGAGAATTTGACTTTAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
687	AGTTGTAGCTGAGTGGTTAAGGCAACGAGCTTTAAATTCGTTGGTTTCTCTC TGTGCAGGTTTGAATCCTGCTAATTA
688	CAAGAAATTCATAGAGGTTATGGGATTGGCTTTAAACCAGTTTCAGGAGGTT CGATTCCTTCCTTTTTTG
689	GCTGTGATGGCCGAGTGGTTAAGGCGTTGGACTTTAAATCCAATGGGGTCTC CCCGCGCAGGTTCTGAATCCTGCTCACAGCG
690	GCTGTGATGGCCGAGTGGTTAAGGCGTTGGACTTTAAATCCAATGGGGTCTC CCCGCGCAGGTTCAAATCCTGCTCACAGCG
691	GCTGTGATGGCCGAGTGGTTAAGGTGTTGGACTTTAAATCCAATGGGGGTTC CCCGCGCAGGTTCAAATCCTGCTCACAGCG
692	GTCACGGTGGCCGAGTGGTTAAGGCGTTGGACTTTAAATCCAATGGGGTTTC CCCGCACAGGTTCTGAATCCTGTTTCGTGACG
693	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTTTAAATCCATTGTGCTCTG CACGCGTGGGTTCGAATCCACCCCTCGTCG
694	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTTTAAATCCATTGTGCTCTG CACGCGTGGGTTCGAATCCACCTTCGTGCG
695	GGCCGGTTAGCTCAGTTGGTTAGAGCGTGCTTTAACTAATGCCAGGGTCGAG GTTTCGATCCCCGTACGGGCCT
696	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTTTAAATCCATTGTGCTCTG CACACGTGGGTTCGAATCCCATCCTCGTCG
697	GAGGCCTGGCCGAGTGGTTAAGGCGATGGACTTTAAATCCATTGTGCTCTGC ACGCGTGGGTTCGAATCCCATCCTCG
698	GCAGCGATGGCCGAGTGGTTAAGGCGTTGGACTTTAAATCCAATGGGGTCTC CCCGCGCAGGTTCTGAACCCTGCTCGCTGCG
699	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTTTAAATCCATTGGGGTTTC CCCGCGCAGGTTCTGAATCCTGCCGACTACG
700	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTTTAAATCCATTGGGGTCTC CCCGCGCAGGTTCTGAATCCTGCCGACTACG
701	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTTTAAATCCATTGGGGTTTC CCCGCGCAGGTTCTGAATCCTGTCGGCTACG

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702	GAGAAGGTCACAGAGGTTATGGGATTGGCTCTAAACCAGTCTGTGGGGGGTT CGATTCCCTCCTTTTTTCA
703	GAGAAGGTCATAGAGGTTATGGGATTGGCTCTAAACCAGTCTCTGGGGGGTT CGATTCCCTCCTTTTTTCA
704	GAAAAAGTCATAGGGGTTATGAGGCTGGCTCTAAACCAGCCTTAGGAGGTTT AATTCCTTCCTTTTTTG
705	GGCCGGTTAGCTCAGTTGGTTAGAGCGTGCTGCTCTAAATGCCAGGGTCGAG GTTTCGATCCCCGTACGGGCCT
706	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTCTAAATCCATTGGGGTTTC CCCGCGCAGGTTCTGAATCCTGCCGACTACG
707	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTCTAAATCCATTGGGGTTTC CCCACGCAGGTTCTGAATCCTGCCGACTACG
708	GTAGTCGTGGCCGAGTGGTTAAGGTGATGGACTCTAAACCCATTGGGGTCTC CCCGCGCAGGTTCTGAATCCTGCCGACTACG
709	GGGTGTATGGCTCAGGGGTAGAGAATTTGACTCTAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
710	AGTTGTAGCTGAGTGGTTAAGGCAACGAGCTCTAAATTCGTTGGTTTCTCTC TGTGCAGGTTTGAATCCTGCTAATTA
711	CAAGAAATTCATAGAGGTTATGGGATTGGCTCTAAACCAGTTTCAGGAGGTT CGATTCCCTCCTTTTTTG
712	GCTGTGATGGCCGAGTGGTTAAGGCGTTGGACTCTAAATCCAATGGGGTCTC CCCGCGCAGGTTCTGAATCCTGCTCACAGCG
713	GCTGTGATGGCCGAGTGGTTAAGGCGTTGGACTCTAAATCCAATGGGGTCTC CCCGCGCAGGTTCAAATCCTGCTCACAGCG
714	GCTGTGATGGCCGAGTGGTTAAGGTGTTGGACTCTAAATCCAATGGGGGTTC CCCGCGCAGGTTCAAATCCTGCTCACAGCG
715	GTCACGGTGGCCGAGTGGTTAAGGCGTTGGACTCTAAATCCAATGGGGTTTC CCCGCACAGGTTCTGAATCCTGTTTCGTGACG
716	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTCTAAATCCATTGTGCTCTG CACGCGTGGGTTCTGAATCCCACCCTCGTCG
717	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTCTAAATCCATTGTGCTCTG CACGCGTGGGTTCTGAATCCCACCTTCGTGCG

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718	GGCCGGTTAGCTCAGTTGGTTAGAGCGTGCTCTAACTAATGCCAGGGTCGAG GTTTCGATCCCCGTACGGGCCT
719	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTCTAAATCCATTGTGCTCTG CACACGTGGGTTCGAATCCCATCCTCGTCG
720	GAGGCCTGGCCGAGTGGTTAAGGCGATGGACTCTAAATCCATTGTGCTCTGC ACGCGTGGGTTCGAATCCCATCCTCG
721	GCAGCGATGGCCGAGTGGTTAAGGCGTTGGACTCTAAATCCAATGGGGTCTC CCCGCGCAGGTTCGAACCCTGCTCGCTGCG
722	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTCTAAATCCATTGGGGTTTC CCCGCGCAGGTTCGAATCCTGCCGACTACG
723	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTCTAAATCCATTGGGGTCTC CCCGCGCAGGTTCGAATCCTGCCGACTACG
724	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTCTAAATCCATTGGGGTTTC CCCGCGCAGGTTCGAATCCTGTCGGCTACG
725	GAGAAGGTCACAGAGGTTATGGGATTGGCTTCAAACCAGTCTGTGGGGGGTT CGATTCCCTCCTTTTTCA
726	GAGAAGGTCATAGAGGTTATGGGATTGGCTTCAAACCAGTCTCTGGGGGGTT CGATTCCCTCCTTTTTCA
727	GAAAAAGTCATAGGGGTTATGAGGCTGGCTTCAAACCAGCCTTAGGAGGTTC AATTCCTTCCTTTTTTG
728	GGCCGGTTAGCTCAGTTGGTTAGAGCGTGCTGCTTCAAATGCCAGGGTCGAG GTTTCGATCCCCGTACGGGCCT
729	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTTCAAATCCATTGGGGTTTC CCCGCGCAGGTTCGAATCCTGCCGACTACG
730	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTTCAAATCCATTGGGGTTTC CCCACGCAGGTTCGAATCCTGCCGACTACG
731	GTAGTCGTGGCCGAGTGGTTAAGGTGATGGACTTCAAACCCATTGGGGTCTC CCCGCGCAGGTTCGAATCCTGCCGACTACG
732	GGGTGTATGGCTCAGGGGTAGAGAATTTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
733	AGTTGTAGCTGAGTGGTTAAGGCAACGAGCTTCAAATTCGTTGGTTTCTCTC TGTGCAGGTTTGAATCCTGCTAATTA

SEQ ID NO	Suppressor tRNA Sequence
734	CAAGAAATTCATAGAGGTTATGGGATTGGCTTCAAACCAGTTTCAGGAGGTT CGATTCCTTCCTTTTTTGG
735	GCTGTGATGGCCGAGTGGTTAAGGCGTTGGACTTCAAATCCAATGGGGTCTC CCCGCGCAGGTTCTGAATCCTGCTCACAGCG
736	GCTGTGATGGCCGAGTGGTTAAGGCGTTGGACTTCAAATCCAATGGGGTCTC CCCGCGCAGGTTCAAATCCTGCTCACAGCG
737	GCTGTGATGGCCGAGTGGTTAAGGTGTTGGACTTCAAATCCAATGGGGGTTC CCCGCGCAGGTTCAAATCCTGCTCACAGCG
738	GTCACGGTGGCCGAGTGGTTAAGGCGTTGGACTTCAAATCCAATGGGGTTTC CCCGCACAGGTTCTGAATCCTGTTTCGTGACG
739	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTTCAAATCCATTGTGCTCTG CACGCGTGGGTTCTGAATCCCACCCTCGTCG
740	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTTCAAATCCATTGTGCTCTG CACGCGTGGGTTCTGAATCCCACCTTCGTGCG
741	GGCCGGTTAGCTCAGTTGGTTAGAGCGTGCTTCAACTAATGCCAGGGTCGAG GTTTCGATCCCCGTACGGGCCT
742	GACGAGGTGGCCGAGTGGTTAAGGCGATGGACTTCAAATCCATTGTGCTCTG CACACGTGGGTTCTGAATCCCATCCTCGTCG
743	GAGGCCTGGCCGAGTGGTTAAGGCGATGGACTTCAAATCCATTGTGCTCTGC ACGCGTGGGTTCTGAATCCCATCCTCG
744	GCAGCGATGGCCGAGTGGTTAAGGCGTTGGACTTCAAATCCAATGGGGTCTC CCCGCGCAGGTTCTGAACCCTGCTCGCTGCG
745	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTTCAAATCCATTGGGGTTTC CCCGCGCAGGTTCTGAATCCTGCCGACTACG
746	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTTCAAATCCATTGGGGTCTC CCCGCGCAGGTTCTGAATCCTGCCGACTACG
747	GTAGTCGTGGCCGAGTGGTTAAGGCGATGGACTTCAAATCCATTGGGGTTTC CCCGCGCAGGTTCTGAATCCTGTCGGCTACG
748	GCCCAGCTAGCTCAGTCGGTAGAGCATAAGACTTTAAATCTCAGGGTTGTGG ATTCGTGCCCCATGCTGGGTG
749	CTGCAGCTAGCTCAGTCGGTAGAGCATGAGACTTTAAATCTCAGGGTCATGG GTTTCGTGCCCCATGTTGGG

SEQ ID NO	Suppressor tRNA Sequence
750	CCAGCATGTCTCAGTCGGTATAGTGTGAGACTTTAAATCTCAGGGTCGTGGG TTCAAGCCCCACATTGGG
751	GTCTAGCTAGATCAGTTGGTAGAGCATAAGACTTTAAATCTCAGGGTCATGG GTTTGAGCCCTACGTTGGGCG
752	GCCCAGCTAGCTCAGCCGGTAGAGCACAAGACTTTAAATCTCAGGGTCGTGG GTTTGAGCCCTGTGTTGAGCA
753	CCGAATAGCTTAGTTGATGAAGCGTGAGACTTTAAATCTCAGGGTAGTGGGT TCAAGCCCCACATTGGA
754	GCCTGGCTACCTCAGTTGGTAGAGCATGGGACTTTAAATCCCAGAGTCAGTG GGTTCAAGCCTCACATTGAGTG
755	GCCCGGCTAGCTCAGTCGGTAGAGCATGAGACCTTAAATCTCAGGGTCGTGG GTTGAGCCCCACGTTGGGCG
756	GCCCGGCTAGCTCAGTCGGTAGAGCATGGGACTTTAAATCTCAGGGTCGTGG GTTGAGCCCCACGTTGGGCG
757	GCCCGGCTAGCTCAGTCGATAGAGCATGAGACTTTAAATCTCAGGGTCGTGG GTTGAGCCGCACGTTGGGCG
758	GCCCAGCTAGCTCAGTCGGTAGAGCATGAGACTTTAAATCTCAGGGTCATGG GTTTGAGCCCCACGTTTGGTG
759	GCCTGGCTAGCTCAGTCGGCAAAGCATGAGACTTTAAATCTCAGGGTCGTGG GCTCGAGCTCCATGTTGGGCG
760	GCCCGACTACCTCAGTCGGTGGAGCATGGGACTTTACATCCCAGGGTTGTGG GTTGAGCCCCACATTGGGCA
761	CCCCGGCTGGCTCAGTCAGTAGATCATGAGACTTTAAATCTCAGGGTCGTGG GTTACGCCCCACACTGGGCG
762	GCGCTAGTCAGTAGAGCATGAGACTTTAAATCTCAGGGTCGTGGGTTGAGC CCCACATCGGGCG
763	GCCTGGATAGCTCAGTTGGTAGAGCATCAGACTTTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTGAGGCA
764	GCCAGGATAGTTCAGGTGGTAGAGCATCAGACTTTAAACCTGAGGGTTCAGG GTTCAAGTCTCTGTTTGGGCG
765	ACCCAGATAGCTCAGTCAGTAGAGCATCAGACTTTAAATCTGAGGGTCCAAG GTTGATGTCCCTTTTTGGGTG

SEQ ID NO	Suppressor tRNA Sequence
766	ACCTGGGTAGCTTAGTTGGTAGAGCATTGGACTTTAAATTTGAGGGCCCAGG TTTCAAGTCCCTGTTTGGGTG
767	GCCTGGGTAGCTCAGTCGGTAGAGCTATCAGACTTTAAGCCTGAGGATTCAG GGTTCAATCCCTTGCTGGGGCG
768	GATAGCTCAGTTGATAGAGCATCAGACTTTAAATCTGAGGGTCCAGGGTTCA TGTCCCTGTT
769	GTTGGGGTAACTCAGTTGGTAGAGTAGCAGACTTTACATCTGAGGGTCCAGG GTTTAAGTCCATGTCCAGGCA
770	GCCTGGATAGCTCAGTTGGTAGAGCATCAGACTTTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCAGGCG
771	GCCTGGATAGCTCAGTCGGTAGAGCATCAGACTTTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCAGGCG
772	GCCCGGATAGCTCAGTCGGTAGAGCATCAGACTTTAAATCTGAGGGTCCGGG GTTCAAGTCCCTGTTTCGGGCG
773	GCCTGGGTAGCTCAGTCGGTAGAGCATCAGACTTTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTCCAGGCG
774	GCCTGGATAGCTCAGTTGGTAGAACATCAGACTTTAAATCTGACGGTGCAGG GTTCAAGTCCCTGTTTCAGGCG
775	GCCCGGAGAGCTCAGTGGGTAGAGCATCAGACTTTAAATCTGAGGGTCCAGG GTTCAAGTCCTCGTTTCGGGCA
776	ACCTGGGTAGCTCAGTAGGTAGAACATCAGACTTTAAATCTGAGGGTCTAGG GTTCAAGTCCCTGTCCAGGCG
777	GCCTGGATAGCTCCTTCGGTAGAGCATCATCAGACTTTAAATGTGAGGGTCC AGGGTTCAAGTTCCTGTTTGGGCG
778	GCCCAGCTAGCTCAGTCGGTAGAGCATAAGACTCTAAATCTCAGGGTTGTGG ATTCGTGCCCCATGCTGGGTG
779	CTGCAGCTAGCTCAGTCGGTAGAGCATGAGACTCTAAATCTCAGGGTCATGG GTTTCGTGCCCCATGTTGGG
780	CCAGCATGTCTCAGTCGGTATAGTGTGAGACTCTAAATCTCAGGGTCGTGGG TTCAAGCCCCACATTGGG
781	GTCTAGCTAGATCAGTTGGTAGAGCATAAGACTCTAAATCTCAGGGTCATGG GTTTGAGCCCTACGTTGGGCG

SEQ ID NO	Suppressor tRNA Sequence
782	GCCCAGCTAGCTCAGCCGGTAGAGCACAAGACTCTAAATCTCAGGGTCGTGG GTTTGAGCCCTGTGTTGAGCA
783	CCGAATAGCTTAGTTGATGAAGCGTGAGACTCTAAATCTCAGGGTAGTGGGT TCAAGCCCCACATTGGA
784	GCCTGGCTACCTCAGTTGGTAGAGCATGGGACTCTAAATCCCAGAGTCAGTG GGTTCAAGCCTCACATTGAGTG
785	GCCCGGCTAGCTCAGTCGGTAGAGCATGAGACCCTAAATCTCAGGGTCGTGG GTTTCGAGCCCCACGTTGGGCG
786	GCCCGGCTAGCTCAGTCGGTAGAGCATGGGACTCTAAATCTCAGGGTCGTGG GTTTCGAGCCCCACGTTGGGCG
787	GCCCGGCTAGCTCAGTCGATAGAGCATGAGACTCTAAATCTCAGGGTCGTGG GTTTCGAGCCGCACGTTGGGCG
788	GCCCAGCTAGCTCAGTCGGTAGAGCATGAGACTCTAAATCTCAGGGTCATGG GTTTGAGCCCCACGTTTGGTG
789	GCCTGGCTAGCTCAGTCGGCAAAGCATGAGACTCTAAATCTCAGGGTCGTGG GCTCGAGCTCCATGTTGGGCG
790	GCCCGACTACCTCAGTCGGTGGAGCATGGGACTCTACATCCCAGGGTTGTGG GTTTCGAGCCCCACATTGGGCA
791	CCCCGGCTGGCTCAGTCAGTAGATCATGAGACTCTAAATCTCAGGGTCGTGG GTTTCACGCCCCACACTGGGCG
792	GCGCTAGTCAGTAGAGCATGAGACTCTAAATCTCAGGGTCGTGGGTTTCGAGC CCCACATCGGGCG
793	GCCTGGATAGCTCAGTTGGTAGAGCATCAGACTCTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCAGGCA
794	GCCAGGATAGTTCAGGTGGTAGAGCATCAGACTCTAAACCTGAGGGTTCAGG GTTCAAGTCTCTGTTTGGGCG
795	ACCCAGATAGCTCAGTCAGTAGAGCATCAGACTCTAAATCTGAGGGTCCAAG GTTTCATGTCCCTTTTTGGGTG
796	ACCTGGGTAGCTTAGTTGGTAGAGCATTGGACTCTAAATTTGAGGGCCCAGG TTTCAAGTCCCTGTTTGGGTG
797	GCCTGGGTAGCTCAGTCGGTAGAGCTATCAGACTCTAAGCCTGAGGATTTCAG GGTTCAATCCCTTGCTGGGGCG

SEQ ID NO	Suppressor tRNA Sequence
798	GATAGCTCAGTTGATAGAGCATCAGACTCTAAATCTGAGGGTCCAGGGTTCA TGTCCCTGTT
799	GTTGGGGTAACTCAGTTGGTAGAGTAGCAGACTCTACATCTGAGGGTCCAGG GTTTAAGTCCATGTCCAGGCA
800	GCCTGGATAGCTCAGTTGGTAGAGCATCAGACTCTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCAGGCG
801	GCCTGGATAGCTCAGTCGGTAGAGCATCAGACTCTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTTTCAGGCG
802	GCCCGGATAGCTCAGTCGGTAGAGCATCAGACTCTAAATCTGAGGGTCCGGG GTTCAAGTCCCTGTTTCGGGCG
803	GCCTGGGTAGCTCAGTCGGTAGAGCATCAGACTCTAAATCTGAGGGTCCAGG GTTCAAGTCCCTGTCCAGGCG
804	GCCTGGATAGCTCAGTTGGTAGAACATCAGACTCTAAATCTGACGGTGCAGG GTTCAAGTCCCTGTTTCAGGCG
805	GCCCGGAGAGCTCAGTGGGTAGAGCATCAGACTCTAAATCTGAGGGTCCAGG GTTCAAGTCCTCGTTTCGGGCA
806	ACCTGGGTAGCTCAGTAGGTAGAACATCAGACTCTAAATCTGAGGGTCTAGG GTTCAAGTCCCTGTCCAGGCG
807	GCCTGGATAGCTCCTTCGGTAGAGCATCATCAGACTCTAAATGTGAGGGTCC AGGGTTCAAGTTCCTGTTTGGGCG
808	GGCAGAATGGTGCAGCGGTTTCAGCACCCAGGCTCTTCAGCCAGCTGTTGCCT GGGCTCAAATCCCAGCTCTGCCA
809	GGCTGTATAGCTCAGTGGTAGAGCATTTGACTTCAGAATCCTATACTCAGGG GAAGGAGAACTGGGGGTTTCTCAGTGGGTCAAAGGACTTGTAGTGGTAAATC AAAAGCAACTCTATAAGCTATGTAACAAACTTTAAAGTCATATGTAGCTGGG TTCAAATCCTGTTTCTGCCA
810	GGCTGTATAGCTCAGTGGTAGAGCATTTGACTTCAGCTTTAAAGTCATATGT AGCTGGGTTCAAATCCTGTTTCTGCCA
811	GGGGGCATAGCTCAGTGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
812	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCC

SEQ ID NO	Suppressor tRNA Sequence
813	GGGGGTATAGCTTAGCGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCCGG TTCAAATCCGGGTGCCCCCT
814	GGGGGTATAGCTTAGGGGTAGAGCATTTGACTTCAGATCAAAAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
815	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCCAG TTCAAATCTGGGTGCCCCCT
816	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTTCAGATCAAGAAGTCCCCGG TTCAAATCCGGGTGCCCCCT
817	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTTCAGATCAAGAGGTCTCTGG TTCAAATCCAGGTGCCCCCT
818	GGGGGTATAGCTCAGGGGTAGAGCACTTGACTTCAGATCAAGAAGTCCTTGG TTCAAATCCAGGTGCCCCCT
819	GGGGATATAGCTCAGGGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCCGG TTCAAATCCGGGTGCCCCCT
820	GGGGGTATAGTTCAGGGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
821	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTTCAAATCAAGAGGTCCCTGA TTCAAATCCAGGTGCCCCCT
822	GGGCGTATAGCTCAGGGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCCAG TTCAAATCTGGGTGCCCCCT
823	GGGGGTATAGCTCACAGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCCGG TTCAAATCTGGGTGCCCCCT
824	GGGCGTATAGCTCAGGGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCCAG TTCAAATCTGGGTGCCCA
825	GGGGGTATAGCTCACAGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCCGG TTCAAATCCGGTTACTCCCT
826	GGGGGTAGGGCTCAGGGATAGAGCATTTGACTTCAGATCAAGAGGTCCCCGG TTCGAATCTAGGTGCCCCCT
827	GGTATATCTCAGGGGGCAGAGCATTTGACTTCAGATCAAGAGGTCCCCGGTT GAAATCCGGGTGCT
828	GGGGGTATAGCTCAGGGGTAGAGCACTTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT

SEQ ID NO	Suppressor tRNA Sequence
829	GGGGGTATAGCTCAGTGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCGGGTGCCCCCT
830	GGGGGTATAGCTCAGTGGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCCG GTTCAAATCCGGGTGCCCCCT
831	GGGGGTGTAGCTCAGTGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
832	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCCGG TTCAAATCCGGGTGCCCCCT
833	GGGGGTATAGCTCAGGGGTAGAGCATTTGACTTCAGATCAAGAGGTCCCTGG TTCAAATCCAGGTGCCCCCT
834	GACCTCGTGGCGCAATGGTAGCGCTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
835	GACCTCGTGGCACAATGGTAGCACGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
836	GAAGCGGTGGCTCAATGGTAGAGCTTTCGACTTCAATTAAATCTTGAAATT CCACGGAATAAGATTGCAATCGAAGGGTTGCAGGTTCAATTCCTGTCCGTTT CA
837	GAAGCGGTGGCTCAATGGTAGAGCTTTCGACTTCAAATCGAAGGGTTGCAGG TTCAATTCCTGTCCGTTTCA
838	GGCCTCATGGTGCAACAGTAGTGTGTCTGACTTCAGATCAGAAGGTTGTATG TTCAAATCACATAGGGGTCA
839	GACCTCGTGGTGAAATGGTAGCATGTTTGACTTCAAATCAGGAGGTTGTGTG TTCAAGTCACATCAGGGTCA
840	GACCTTGTGGCGCAATGGTAGCATGTTTGACTTCAAATCAGGAGGTTGTGTG TTCAAGTCACATCAGGGTCA
841	GACCTCGTGGCGCAACGGTAGCGCTCTGACTTCAGATCAGAAGGCTGCGTG TTCGAATCACGCCGGGGTCA
842	GACCTTGTGGCTCAATGGTAGCGCATCTGACTTCAGATCAGGAGGTTGCACG TTCAAATCATGCCGGGGTCA
843	GACCTTGTGGCGCAACGGTAGCGCTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
844	GACCTCGTGGCGCAACGGTAGCGCTCTGACTTCAGATCAGAAGGTTGCGTA TTCAAATCACGTCGGGGTCA

SEQ ID NO	Suppressor tRNA Sequence
845	GACCTCGTGGCGCAACGGCAGCGCGTCTGACTTCACATTAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
846	GACCTCATGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACATCGGGGTCA
847	GACCTCGTGGTGCAACGGTAGCGCGTATGATTTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
848	GACCTCGTAGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
849	AGGGGTATAGCTCAATTGGCAGAGCGTCGGTCTTCAAACCGAAGGTTGTAG GTTTCGATTCCCTACTGCCCTGCCA
850	GACCTCATGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
851	GACCTCGTGGCGCAACGGTAGCGCGTCTAACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
852	ACGGGAGTAGCTCAGTTGGTAGAGCACCGGTCTTCAAACCGGGTGTCGGGA GTTTCGAGCCTCTCCTCCCGTG
853	GACCTCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCATG TTCAAATCACGTCGGGGTCA
854	GACTCCGTGGCGCAACGGTAGCGCGTCCGACTTCAGATCGGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
855	GACTCCGTGGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
856	GGCCTCGTGGCGCAACGGTAGCGCGTCTGACTCCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
857	GGCCTCGTGGCGCAACGGTAGCACGTCTGACTCCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
858	CGGCCTCGTGGCGCAACGGTAGCACGTCTGACTTCAGATCAGAAGGTTGCGT GTTCAAATCACGTCGGGGTCA
859	GGCCTCGTCGCGCAACGGTAGCGCGTCTGACTCCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
860	GGCCTCGTCGCGCAACGGTAGCGCGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA

SEQ ID NO	Suppressor tRNA Sequence
861	GGCCTCGTCGCGCAACGGTAGCACGTCTGACTCCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA
862	GGCCTCGTCGCGCAACGGTAGCACGTCTGACTTCAGATCAGAAGGTTGCGTG TTCAAATCACGTCGGGGTCA

[0071] In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 6. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 7. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 8. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 9. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 11. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 16. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 17. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 18. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 19. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 20. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 21. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 22. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 35. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 36. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 37. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 38. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 39. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 40. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 44. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 45. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 178. In certain embodiments, the tRNA comprises, consists essentially

of, or consists of the nucleotide sequence of SEQ ID NO: 179. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 180. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 181. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 182. In certain
5 embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 186. In certain embodiments, the tRNA comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO: 187.

[0072] In certain embodiments, the tRNA may comprise one or more mutations (*e.g.*, nucleotide
10 substitutions, deletions, or insertions) relative to a reference tRNA sequence (*e.g.*, a tRNA disclosed herein). In certain embodiments, the tRNA may comprise, consist, or consist essentially of, a single mutation, or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more than 15 mutations. It is contemplated that the tRNA may comprise, consist, or consist essentially 1-15, 1-10, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-15, 2-10, 2-7, 2-6, 2-5, 2-4, 2-3, 3-15, 3-10,
15 3-7, 3-6, 3-5, or 3-4 mutations.

[0073] Sequence identity may be determined in various ways that are within the skill in the art, *e.g.*, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karin *et al.*,
20 (1990) PROC. NATL. ACAD. SCI. USA 87:2264-2268; Altschul (1993) J. MOL. EVOL. 36, 290-300; Altschul *et al.*, (1997) NUCLEIC ACIDS RES. 25:3389-3402) are tailored for sequence similarity searching. For a discussion of basic issues in searching sequence databases see Altschul *et al.* (1994) NATURE GENETICS 6:119-129. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve
25 maximal alignment over the full length of the sequences being compared. The search parameters for histogram, descriptions, alignments, expect (*i.e.*, the statistical significance threshold for reporting matches against database sequences), cutoff, matrix and filter are at the default settings. The default scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff *et al.*, (1992) PROC. NATL. ACAD. SCI. USA 89:10915-10919).
30 Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every wink.sup.th position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent Blastp parameter settings may be Q=9; R=2; wink=1; and gapw=32. Searches may also be conducted using the NCBI (National Center for Biotechnology Information) BLAST

Advanced Option parameter (*e.g.*: -G, Cost to open gap [Integer]: default = 5 for nucleotides/ 11 for proteins; -E, Cost to extend gap [Integer]: default = 2 for nucleotides/ 1 for proteins; -q, Penalty for nucleotide mismatch [Integer]: default = -3; -r, reward for nucleotide match [Integer]: default = 1; -e, expect value [Real]: default = 10; -W, wordsize [Integer]: default = 11 for nucleotides/ 28 for megablast/ 3 for proteins; -y, Dropoff (X) for blast extensions in bits: default = 20 for blastn/ 7 for others; -X, X dropoff value for gapped alignment (in bits): default = 15 for all programs, not applicable to blastn; and -Z, final X dropoff value for gapped alignment (in bits): 50 for blastn, 25 for others). ClustalW for pairwise protein alignments may also be used (default parameters may include, *e.g.*, Blosom62 matrix and Gap Opening Penalty = 10 and Gap Extension Penalty = 0.1). A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

[0074] It is contemplated that a tRNA may comprise one or more modifications. Exemplary modified tRNAs include: acylated tRNA; alkylated tRNA; a tRNA containing one or more bases other than adenine, cytosine, guanine, or uracil; a tRNA covalently modified by the attachment of a specific ligand or antigenic, fluorescent, affinity, reactive, spectral, or other probe moiety; a tRNA containing one or more ribose moieties that are methylated or otherwise modified; aa-tRNAs that are aminoacylated with an amino acid other than the 20 natural amino acids, including non-natural amino acids that function as a carrier for reagents, specific ligands, or as an antigenic, fluorescent, reactive, affinity, spectral, or other probe; or any combination of these compositions. Exemplary modified tRNA molecules are described in Soll *et al.* (1995) "tRNA: Structure, Biosynthesis, and Function," ASM Press; El Yacoubi *et al.* (2012) ANNU. REV. GENET. 46:69-95; Grosjean *et al.* (1998) "Modification and Editing of RNA." ASM Press; Hendrickson *et al.* (2004) ANNU. REV. BIOCHEM. 73:147-176, 2004; Ibba *et al.* (2000) ANNU. REV. BIOCHEM. 69:617-650; Johnson *et al.* (1995) COLD SPRING HARBOR SYMP. QUANT. BIOL. 60:71-82; Johnson *et al.* (1982) J. MOL. BIOL. 156:113-140; Crowley *et al.* (1994) CELL 78:61-71; Beier *et al.* (2001) NUCLEIC ACIDS RES. 29:4767-4782; Torres *et al.* (2014) TRENDS MOL. MED. 20:306-314; Bjork *et al.* (1987) ANNU. REV. BIOCHEM. 56:263-287; Schaffrath *et al.* (2017) RNA BIOL. 14(9):1209-1222; and Johansson *et al.* (2008) MOL. CELL. BIOL. 28(10):3301-12.

[0075] In certain embodiments, a tRNA comprises a naturally occurring nucleotide modification. Naturally occurring tRNAs contain a wide variety of post-transcriptionally modified nucleotides, which are described, for example, in Machnicka *et al.* (2014) RNA BIOLOGY 11(12): 1619-1629, and include one or more of the residues as shown in **FIGURE 2B**.

In certain embodiments, the tRNA comprises one or more of the residues selected from the group consisting of: 2'-O-methylguanosine or G at position 0; pseudouridine or U at position 1; 2'-O-methyladenosine, A, 2'-O-methyluridine, U, 2'-O-methylcytidine, C, 2'-O-methylguanosine, or G at position 4; N2-methylguanosine or G at position 6; N2-methylguanosine or G at position 7; 1-methyladenosine, A, 1-methylguanosine, G, or a modified G at position 9; N2-methylguanosine or G at position 10; N4-acetylcytidine or C at position 12; pseudouridine, U, 2'-O-methylcytidine, or C at position 13; 1-methyladenosine, A, or a modified A at position 14; dihydrouridine (D) or U at position 16; D or U at position 17; 2'-O-methylguanosine or G at position 18; 3-(3-amino-3-carboxypropyl)uridine, D, or U at position 20; 3-(3-amino-3-carboxypropyl)uridine, D, pseudouridine, U, or a modified U at position 20a; D, pseudouridine, or U at position 20b; pseudouridine or U at position 25; pseudouridine, U, N2,N2-dimethylguanosine, N2-methylguanosine, G, or a modified G at position 26; pseudouridine, U, N2,N2-dimethylguanosine, or G at position 27; pseudouridine or U at position 28; pseudouridine or U at position 30; pseudouridine or U at position 31; 2'-O-methylpseudouridine, 2'-O-methyluridine, pseudouridine, U, 2'-O-methylcytidine, 3-methylcytidine, C, or a modified C at position 32; inosine, A, 2-thiouridine, 2'-O-methyluridine, 5-(carboxyhydroxymethyl)uridine methyl ester, 5-carbamoylmethyluridine, 5-carboxymethylaminomethyl-2'-O-methyluridine, 5-methoxycarbonylmethyl-2-thiouridine, 5-methoxycarbonylmethyluridine, pseudouridine, U, a modified U, 2'-O-methylcytidine, 5-formyl-2'-O-methylcytidine, 5-methylcytidine, C, a modified C, queuosine, mannosyl-queuosine, galactosyl-queuosine, 2'-O-methylguanosine, or G at position 34; pseudouridine or U at position 35; pseudouridine, U, or a modified U at position 36; 1-methylinosine, 2-methylthio-N6-threonylcarbamoyladenosine, N6-isopentenyladenosine, N6-methyl-N6-threonylcarbamoyladenosine, N6-threonylcarbamoyladenosine, A, a modified A, 1-methylguanosine, peroxywybutosine, wybutosine, G, or a modified G at position 37; pseudouridine, U, 5-methylcytidine, C, or a modified C at position 38; 1-methylpseudouridine, 2'-O-methylpseudouridine, 2'-O-methyluridine, pseudouridine, U, 2'-O-methylguanosine, or G at position 39; pseudouridine, U, 5-methylcytidine, or C at position 40; 2'-O-methyluridine, U, or a modified U at position 44; pseudouridine or U at position e11; pseudouridine or U at position e12; pseudouridine or U at position e14; 3-methylcytidine or C at position e2; 7-methylguanosine or G at position 46; D, U, or a modified U at position 47; D, U, 5-methylcytidine, C, or a modified C at position 48; A, a modified A, 5-methylcytidine, C, or a modified C at position 49; pseudouridine, U, 5-methylcytidine, or C at position 50; 5,2'-O-dimethyluridine, 5-methyluridine, pseudouridine, or U at position 54; pseudouridine or U at

position 55; 1-methyladenosine, A, or a modified A at position 58; 2'-O-ribosyladenosine (phosphate), A, 2'-O-ribosylguanosine (phosphate), G, or a modified G at position 64; pseudouridine or U at position 65; pseudouridine, U, N2-methylguanosine, or G at position 67; pseudouridine or U at position 68; and, pseudouridine, U, 5-methylcytidine, or C at position 72.

5 A, C, G, and U, refer to unmodified adenine, cytosine, guanine, and uracil, respectively. The numbering of the residues is based on the tRNA numbering system described in Steinberg *et al.*, (1993) NUCLEIC ACIDS RES. 21:3011-15.

[0076] In certain embodiments, the tRNA comprises one or more nucleotide modifications selected from 5-methyl uridine, 5-carbamoylmethyluridine, 5-carbamoyl-methyl-2-O-
10 methyluridine, 5-methoxy-carbonylmethyluridine, 5-methoxycarbonylmethyl-2-thiouridine, pseudouridine, dihydrouridine, 1-methyladenosine, and inosine.

II. Methods of Making tRNAs

[0077] It is contemplated the tRNA molecules (*e.g.*, suppressor tRNAs) useful in the practice of the invention can be produced by methods known in the art, including extracellular production
15 by synthetic chemical methods, intracellular production by recombinant DNA methods, or purification from natural sources.

[0078] For example, DNA molecules encoding tRNAs can be synthesized chemically or by recombinant DNA methodologies. For example, the sequences of the tRNAs can be synthesized or cloned from libraries by conventional hybridization techniques or polymerase chain reaction
20 (PCR) techniques, using the appropriate synthetic nucleic acid primers. The resulting DNA molecules encoding the tRNAs can be ligated to other appropriate nucleotide sequences, including, for example, expression control sequences to produce conventional gene expression constructs (*i.e.*, expression vectors) encoding the tRNAs. Production of defined gene constructs is within routine skill in the art. Nucleic acids encoding desired tRNAs can be incorporated
25 (ligated) into expression vectors, such as the expression vectors described in the following section, which can be introduced into host cells through conventional transfection or transformation techniques. Exemplary host cells are *E. coli* cells, Chinese hamster ovary (CHO) cells, human embryonic kidney 293 (HEK 293) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), and
30 myeloma cells. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the tRNAs. Specific expression and purification conditions will vary depending upon the expression system employed.

[0079] Alternatively, tRNAs can be chemically synthesized or purified from natural sources by methods known in art. When a tRNA is aminoacylated prior to introduction into the cell or administration to the subject, the tRNA may be aminoacylated with a desired amino acid by any method known in the art, including chemical or enzymatic aminoacylation.

5 **III. Expression Vectors**

[0080] The tRNAs of interest may be expressed in a cell of interest by incorporating a gene encoding a tRNA of interest into an appropriate expression vector. As used herein, "expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression
10 vector comprises sufficient cis- acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (*e.g.*, naked or contained in liposomes), retrotransposons (*e.g.* piggyback, sleeping beauty), and viruses (*e.g.*, lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide of
15 interest.

[0081] In certain embodiments, the expression vector is a viral vector. The term "virus" is used herein to refer to an obligate intracellular parasite having no protein-synthesizing or energy-generating mechanism. Exemplary viral vectors include retroviral vectors (*e.g.*, lentiviral vectors), adenoviral vectors, adeno-associated viral vectors, herpesviruses vectors, epstein-barr
20 virus (EBV) vectors, polyomavirus vectors (*e.g.*, simian vacuolating virus 40 (SV40) vectors), poxvirus vectors, and pseudotype virus vectors.

[0082] The virus may be a RNA virus (having a genome that is composed of RNA) or a DNA virus (having a genome composed of DNA). In certain embodiments, the viral vector is a DNA virus vector. Exemplary DNA viruses include parvoviruses (*e.g.*, adeno-associated viruses),
25 adenoviruses, asfarviruses, herpesviruses (*e.g.*, herpes simplex virus 1 and 2 (HSV-1 and HSV-2), epstein-barr virus (EBV), cytomegalovirus (CMV)), papillomoviruses (*e.g.*, HPV), polyomaviruses (*e.g.*, simian vacuolating virus 40 (SV40)), and poxviruses (*e.g.*, vaccinia virus, cowpox virus, smallpox virus, fowlpox virus, sheeppox virus, myxoma virus). In certain embodiments, the viral vector is a RNA virus vector. Exemplary RNA viruses include
30 bunyaviruses (*e.g.*, hantavirus), coronaviruses, flaviviruses (*e.g.*, yellow fever virus, west Nile virus, dengue virus), hepatitis viruses (*e.g.*, hepatitis A virus, hepatitis C virus, hepatitis E virus), influenza viruses (*e.g.*, influenza virus type A, influenza virus type B, influenza virus type C),

measles virus, mumps virus, noroviruses (*e.g.*, Norwalk virus), poliovirus, respiratory syncytial virus (RSV), retroviruses (*e.g.*, human immunodeficiency virus-1 (HIV-1)) and toroviruses.

[0083] In certain embodiments, the expression vector comprises a regulatory sequence or promoter operably linked to the nucleotide sequence encoding the tRNA. The term "operably linked" refers to a linkage of polynucleotide elements in a functional relationship. A nucleic acid sequence is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a gene if it affects the transcription of the gene. Operably linked nucleotide sequences are typically contiguous. However, as enhancers generally function when separated from the promoter by several kilobases and intronic sequences may be of variable lengths, some polynucleotide elements may be operably linked but not directly flanked and may even function *in trans* from a different allele or chromosome.

[0084] tRNA genes preferably have strong promoters that are active in a variety of cell types. The promoters for eukaryotic tRNA genes typically are present within the structural sequences encoding the tRNA molecule itself. Although there are elements which regulate transcriptional activity within the 5' upstream region, the length of an active transcriptional unit may be considerably less than 500 base pairs.

[0085] Additional exemplary promoters which may be employed include, but are not limited to, the retroviral LTR, the SV40 promoter, the human cytomegalovirus (CMV) promoter, the U6 promoter, or any other promoter (*e.g.*, cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β -actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, TK promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

[0086] In certain embodiments, an expression vector comprises a tRNA coding sequence that encodes a tRNA that comprises, consists essentially of, or consists of a nucleotide sequence shown in **TABLE 2** or **TABLE 3**. In certain embodiments, an expression vector comprises a tRNA coding sequence that encodes a tRNA that comprises, consists essentially of, or consists of a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to a nucleotide sequence shown in **TABLE 2** or **TABLE 3**.

[0087] In certain embodiments, in addition to a tRNA coding sequence, the expression vector comprises a nucleotide sequence corresponding to a genomic DNA sequence flanking a wild-type tRNA gene (*i.e.*, a DNA sequence from the same genome as a wild-type tRNA gene and

which is 5' or 3' to the wild-type tRNA gene in the genome, *e.g.*, immediately 5' or 3' to the wild-type tRNA gene in the genome). In certain embodiments, in addition to a tRNA coding sequence, the expression vector comprises a nucleotide sequence corresponding to an exogenous promoter.

- 5 **[0088]** In certain embodiments, the expression vector comprises a nucleotide sequence shown in **TABLE 4**. In certain embodiments, the expression vector comprises a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a nucleotide sequence shown in **TABLE 4**. In certain embodiments, in the expression vector, the nucleotide sequence set forth in **TABLE 4** is operably linked to the
- 10 nucleotide sequence encoding the tRNA. In certain embodiments, in the expression vector, the nucleotide sequence set forth in **TABLE 4** is 5' or 3' (*e.g.*, immediately 5' or immediately 3') to the nucleotide sequence encoding the tRNA. In certain embodiments, the expression vector comprises a nucleotide sequence selected from SEQ ID NOs: 869-888, or a nucleotide sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or
- 15 99% sequence identity to a sequence selected from SEQ ID NOs: 869-888.

TABLE 4

SEQ ID NO	Location (Relative to tRNA)	Nucleotide Sequence
26	5'	CTACCCAGAGGCAGGCGGGAGACTCCCCGAGCGTCCAATAAGA GCGCCGCCAATGGAGCCGCCGCGGGGGTGCAGAGGGACT TCCGGGTGAGGTCCTCCGCTACTTCCCTCCCCACGGAAAAGATA GACCAGTCTGACGCGAGCCTGAAGGCGGCTACACGCTTTAAGCT AAGTAAAGGCACCTTCTCGCTGGC
27	3'	ACTTGTATGTTGTTTTTATCTGTCAGTTTGTTAATCCCAAGATT CCCTTTGGAAATAAAGCGAAATTGACCGTAGTGTTATGACCAA CTTCTAGTCTAACTTAATTCTTGGAACCAAGGATCTGAGCAA ACAACTGTCAGGGTGACACATTGCTTAAACGGTGACAGCGGTCTG AGAGCCTTGTCCCGGATGGAGAGT
32	3'	ACTTGTATGTTGTTTTTATCTGTCAGTTTGTTAATCCCAAGATT CCCTTTGGAAATAAAGCGAAATTGACCGTAGTGTTATGACCAA CTTCTAGTCTAACTT

SEQ ID NO	Location (Relative to tRNA)	Nucleotide Sequence
33	5'	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATA CAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACA CAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTT CTTGGGTAGTTTGCAGTTTAAATTAATGTTTAAATGGACTA TCATATGCTTACCGTAACTTGAAAGTATTTGATTTCTTGGCTT TATATATCTTGTGGAAAGGACGGGCGGAGGAAGGCACCTTCTCG CTGGC
34	3'	ACTTGTATGTTGTTTTTATCTGTCAGTTTGTAAATCCCAAGATT CC
173	5'	GATCACCGGAAGAGGTGACAGACACCTCGGGGCCCATGAACGTT TGGAATTCGTAAGGACATGAGAATCTCGGTGGTTCCGTGTCTGC CCGCCATCGCGGCCACCGGCCACGGGCCCAAGCCAAGTGTAGCG AAGCTTAGAAAAGGTTGCCCAACGTCATGTGGCTTGAGAAGGCT GCCGGGCGCCTTAAGCCGCCAGCA
174	3'	CACTGAACCTTTTTTTGGCCTTAGAATCCCTGTTTTGGGGCCTG CAGGAAGTAGCAACCAACCCGAGCCTCCGCAGGGAATGCACTGA CCTGTAGAATGGACGTTTCTGCTTCCCTCCCTGTGTCTCAACACG ATTACATTTTCTGGAACAGCCTGGGCTGGGAGGCACTGCGCACGC GCGCCGAGTCGGGCGGAAAAATAA
869	5'	CCAAAACATCTTTTACTGTAGTATCTACTTACCATACTACCCAA GAATGGCACACTGCTCACATCTTCAAAGCTTAAACCAAGAGCA CTACACAGGTGC
870	5'	TGTGTGTCGGGGCCGGTACCCTGCTTCCGGTTCCCGCACGCATT CCCGGATTGCAGTGCGGACCCCTTCTGTAAGCGCGCGATAAAGC GCGGTTTTGGAA
871	5'	TCATGTCATATAAGTAGAACCATAACAATATATATATAAAATCCA GGTTAATAGCCAATCTTACAACATTTCTCATATTTTTTGCAGTT GCTAAGCCATGG
872	5'	ACATTACAATACATATCAACATATCACCATAATTAAATTGCAAG TCTTCGTCAAAGCAAGCCTTAAAGGAGTATCCCAAAAACACAT TTTCCCCAGAAG

SEQ ID NO	Location (Relative to tRNA)	Nucleotide Sequence
873	5'	AGACCTTTAGAGCGTGGTTAAACCCATATGTTGGGATTTATGCT GCTTTTATGGTAGCAATACCCTATATTAAGATTTGAAGTAGACC CGGAAAGTTAGT
874	5'	GTTTCATGAAAGAATAAATAAATGTTTAAAAAAAAAAAAAACTGA GGTAAATTTCTATATTCTTTCATAAAAGCAGTTTAAAGACGAAC GTTTTTCGAGGT
875	5'	GCTGGGTCTCGGTGACACTGACGACGGGAGGCGCGGTTCGGAAGA GCGCGGGGCCGTCGCCTCTGGCTTAACATAGCAGATGCGCTGAG ACTCCAACAGGT
876	5'	CAGTGGCGGCGAAAACTCTCTGCGTTCTGGAGGGAGGGTGCGGG CAGGAGGAGGTAGAGGATGCCTTGTAAGCGGAGCAAAAACAAGG TTCAACGTCTGC
877	5'	CAAATCACTTGCCTCTCGGCGCGAGACCGCGATGCGCGGGGGCG GGAGCGTGATGATGGCATCGCGTAAGGAGAGGGTGTGAGAAGCC GGATCCTGTGGT
878	5'	CCCTGTGTCCGAAGAGGTCTGCGTTGCGACTTACGTGGTAGTGC TTGGAAGGTGCGGAGTAGATGAGAGATAAGTGAATGTGGACAAA CCTGTCACGTAG
879	5'	GAGCGGAGCTCAGAGGGTGCGCGCTCCGCCCTTTCGCGGGCCTG GCATGAGCGCAGTGGTTGTTACACTAAAGTGTCTCCGCCTGTGCG AATATTCTCGTG
880	5'	GTGTCACTGGTTTCAAATCAACCTCAATTTTTTTGGAGACGTGA GTGCTGAGCATTTTTTCTTCAGTGAAGTGACTTGGCAGCCAAAA TCGCCAACGCCC
881	5'	TCCTGGCATGTCCCGCCCCAAGTCCCTTAGCCCCGCTCCCCAACC CTGCCCCATTCCCACTCTAGTACCCGTAAGCTACAAGACGCCGC CGTTCGTCGGGT
882	5'	TGCTCAGTCGTCCTGCCGGGCGGGCCCTGAGGTTGCAAGGGACG GAGGAAGTTTCGTGCGTGCGCCCTTCCTATAGCGCCAGTAGAA CTGACAGTACCT

SEQ ID NO	Location (Relative to tRNA)	Nucleotide Sequence
883	5'	TCCTCGGATTACGCATGCTCAGTGCAATCTTCGGTTGCCTGGAC TAGCGCTCCGGTTTTTCTGTGCTGAACCTCAGGGGACGCCGACA CACGTACACGTC
884	5'	GATAATTTCTGAAAGAAAAGATCAATTCGATGTTACCAAATCT GGGATATCCAGAAAAATTTTCTTCTTCTCCTAGGAGAAAACTA TCAAATGTCAGG
885	5'	TCTCTCACGGCAAACCTGTTGCAGACTGTAGAGACGCTATGCCAA GAATCTTTTACTTAAAAGCAGGAATAGATTCAATAGGCAACTTC ACTGCACATGTA
886	5'	CAACCTCCCCCTTCTCAAGGAGCAGGTGGATTGGTCCCGAGCTAG CTGGTGGGCGGAGGTGACGTTTTTATAAGTTGCTCAAGAGACGG TAACAACCGACG
887	5'	GTGGAACCTTCCACTGAATTACTCTTTTCGCATGTAAGATCACTG AACCGTGATAATCATTGATCCTATTTGTAGAACTGTATGAAACA GTTCCCTAAGGA
888	5'	TCGCTCAACAGGCGGCCAGGGTGCGAGCAGTGAAGCTGCGGCAC GCCGGAGCGTTTAATGGCCATCAAATTGGCCTCTCTAGGAGGTA GCTGCAGCCGGA
895	5'	AAAGGCACCTTCTCGCTGGC
896	3'	ACTTGTATGTTGTTTTT
897	5'	TCTCGCTGGC
900	5'	AGCGCTCCGGTTTTTCTGTGCTGAACCTCAGGGGACGCCGACAC ACGTACACGTC

Adeno-associated virus (AAV) Vectors

[0089] In certain embodiments, an expression vector is an adeno-associated virus (AAV) vector. AAV is a small, nonenveloped icosahedral virus of the genus Dependoparvovirus and family Parvovirus. AAV has a single-stranded linear DNA genome of approximately 4.7 kb. AAV is capable of infecting both dividing and quiescent cells of several tissue types, with different AAV serotypes exhibiting different tissue tropism.

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[0090] AAV includes numerous serologically distinguishable types including serotypes AAV-1 to AAV-12, as well as more than 100 serotypes from nonhuman primates (See, *e.g.*, Srivastava (2008) J. CELL BIOCHEM., 105(1): 17–24, and Gao *et al.* (2004) J. VIROL., 78(12), 6381–6388).

The serotype of the AAV vector used in the present invention can be selected by a skilled person in the art based on the efficiency of delivery, tissue tropism, and immunogenicity. For example, AAV-1, AAV-2, AAV-4, AAV-5, AAV-8, and AAV-9 can be used for delivery to the central nervous system; AAV-1, AAV-8, and AAV-9 can be used for delivery to the heart; AAV-2 can be used for delivery to the kidney; AAV-7, AAV-8, and AAV-9 can be used for delivery to the liver; AAV-4, AAV-5, AAV-6, AAV-9 can be used for delivery to the lung, AAV-8 can be used for delivery to the pancreas, AAV-2, AAV-5, and AAV-8 can be used for delivery to the photoreceptor cells; AAV-1, AAV-2, AAV-4, AAV-5, and AAV-8 can be used for delivery to the retinal pigment epithelium; AAV-1, AAV-6, AAV-7, AAV-8, and AAV-9 can be used for delivery to the skeletal muscle. In certain embodiments, the AAV capsid protein comprises a sequence as disclosed in U.S. Patent No. 7,198,951, such as, but not limited to, AAV-9 (SEQ ID NOs: 1-3 of U.S. Patent No. 7,198,951), AAV-2 (SEQ ID NO: 4 of U.S. Patent No. 7,198,951), AAV-1 (SEQ ID NO: 5 of U.S. Patent No. 7,198,951), AAV-3 (SEQ ID NO: 6 of U.S. Patent No. 7,198,951), and AAV-8 (SEQ ID NO: 7 of U.S. Patent No. 7,198,951). AAV serotypes identified from rhesus monkeys, *e.g.*, rh.8, rh.10, rh.39, rh.43, and rh.74, are also contemplated in the instant invention. Besides the natural AAV serotypes, modified AAV capsids have been developed for improving efficiency of delivery, tissue tropism, and immunogenicity. Exemplary natural and modified AAV capsids are disclosed in U.S. Patent Nos. 7,906,111, 9,493,788, and 7,198,951, and PCT Publication No. WO2017189964A2.

[0091] The wild-type AAV genome contains two 145 nucleotide inverted terminal repeats (ITRs), which contain signal sequences directing AAV replication, genome encapsidation and integration. In addition to the ITRs, three AAV promoters, p5, p19, and p40, drive expression of two open reading frames encoding rep and cap genes. Two rep promoters, coupled with differential splicing of the single AAV intron, result in the production of four rep proteins (Rep 78, Rep 68, Rep 52, and Rep 40) from the rep gene. Rep proteins are responsible for genomic replication. The Cap gene is expressed from the p40 promoter, and encodes three capsid proteins (VP1, VP2, and VP3) which are splice variants of the cap gene. These proteins form the capsid of the AAV particle.

[0092] Because the *cis*-acting signals for replication, encapsidation, and integration are contained within the ITRs, some or all of the 4.3 kb internal genome may be replaced with foreign DNA, for example, an expression cassette for an exogenous gene of interest.

Accordingly, in certain embodiments, the AAV vector comprises a genome comprising an expression cassette for an exogenous gene flanked by a 5' ITR and a 3' ITR. The ITRs may be derived from the same serotype as the capsid or a derivative thereof. Alternatively, the ITRs may be of a different serotype from the capsid, thereby generating a pseudotyped AAV. In certain embodiments, the ITRs are derived from AAV-2. In certain embodiments, the ITRs are derived from AAV-5. At least one of the ITRs may be modified to mutate or delete the terminal resolution site, thereby allowing production of a self-complementary AAV vector.

[0093] The rep and cap proteins can be provided in *trans*, for example, on a plasmid, to produce an AAV vector. A host cell line permissive of AAV replication must express the rep and cap genes, the ITR-flanked expression cassette, and helper functions provided by a helper virus, for example adenoviral genes E1a, E1b55K, E2a, E4orf6, and VA (Weitzman *et al.*, Adeno-associated virus biology. Adeno-Associated Virus: Methods and Protocols, pp. 1–23, 2011). Methods for generating and purifying AAV vectors have been described in detail (See *e.g.*, Mueller *et al.*, (2012) CURRENT PROTOCOLS IN MICROBIOLOGY, 14D.1.1-14D.1.21, Production and Discovery of Novel Recombinant Adeno-Associated Viral Vectors). Numerous cell types are suitable for producing AAV vectors, including HEK293 cells, COS cells, HeLa cells, BHK cells, Vero cells, as well as insect cells (See *e.g.* U.S. Patent Nos. 6,156,303, 5,387,484, 5,741,683, 5,691,176, 5,688,676, and 8,163,543, U.S. Patent Publication No. 20020081721, and PCT Publication Nos. WO00/47757, WO00/24916, and WO96/17947). AAV vectors are typically produced in these cell types by one plasmid containing the ITR-flanked expression cassette, and one or more additional plasmids providing the additional AAV and helper virus genes.

[0094] AAV of any serotype may be used in the present invention. Similarly, it is contemplated that any adenoviral type may be used, and a person of skill in the art will be able to identify AAV and adenoviral types suitable for the production of their desired recombinant AAV vector (rAAV). AAV particles may be purified, for example by affinity chromatography, iodixonal gradient, or CsCl gradient.

[0095] AAV vectors may have single-stranded genomes that are 4.7 kb in size, or are larger or smaller than 4.7 kb, including oversized genomes that are as large as 5.2 kb, or as small as 3.0 kb. Thus, where the exogenous gene of interest to be expressed from the AAV vector is small, the AAV genome may comprise a stuffer sequence. Further, vector genomes may be substantially self-complementary thereby allowing for rapid expression in the cell. In certain embodiments, the genome of a self-complementary AAV vector comprises from 5' to 3': a 5'

ITR; a first nucleic acid sequence comprising a promoter and/or enhancer operably linked to a coding sequence of a gene of interest; a modified ITR that does not have a functional terminal resolution site; a second nucleic acid sequence complementary or substantially complementary to the first nucleic acid sequence; and a 3' ITR. AAV vectors containing genomes of all types are suitable for use in the method of the present invention.

[0096] Non-limiting examples of AAV vectors include pAAV-MCS (Agilent Technologies), pAAVK-EF1 α -MCS (System Bio Catalog # AAV502A-1), pAAVK-EF1 α -MCS1-CMV-MCS2 (System Bio Catalog # AAV503A-1), pAAV-ZsGreen1 (Clontech Catalog #6231), pAAV-MCS2 (Addgene Plasmid #46954), AAV-Stuffer (Addgene Plasmid #106248), pAAVscCBPIGpluc (Addgene Plasmid #35645), AAVS1_Puro_PGK1_3xFLAG_Twin_Strep (Addgene Plasmid #68375), pAAV-RAM-d2TTA::TRE-MCS-WPRE-pA (Addgene Plasmid #63931), pAAV-UbC (Addgene Plasmid #62806), pAAVS1-P-MCS (Addgene Plasmid #80488), pAAV-Gateway (Addgene Plasmid #32671), pAAV-Puro_siKD (Addgene Plasmid #86695), pAAVS1-Nst-MCS (Addgene Plasmid #80487), pAAVS1-Nst-CAG-DEST (Addgene Plasmid #80489), pAAVS1-P-CAG-DEST (Addgene Plasmid #80490), pAAVf-EnhCB-lacZnls (Addgene Plasmid #35642), and pAAVS1-shRNA (Addgene Plasmid #82697). These vectors can be modified to be suitable for therapeutic use. For example, an exogenous gene of interest can be inserted in a multiple cloning site, and a selection marker (*e.g.*, puro or a gene encoding a fluorescent protein) can be deleted or replaced with another (same or different) exogenous gene of interest. Further examples of AAV vectors are disclosed in U.S. Patent Nos. 5,871,982, 6,270,996, 7,238,526, 6,943,019, 6,953,690, 9,150,882, and 8,298,818, U.S. Patent Publication No. 2009/0087413, and PCT Publication Nos. WO2017075335A1, WO2017075338A2, and WO2017201258A1.

[0097] In certain embodiments, the expression vector is an AAV vector capable of targeting the nervous system, *e.g.*, the central nervous system, in a subject, *e.g.*, a human subject. Exemplary AAV vectors that can target the nervous system include the AAV9 variants AAV-PHP.B (See, *e.g.*, Deverman *et al.* (2016) NAT. BIOTECHNOL. 34(2):204–209), AAV-AS (See, *e.g.*, Choudhury *et al.* (2016) MOL. THER. 24:726–35), and AAV-PHP.eB (See, *e.g.*, Chan *et al.* (2017) NAT. NEUROSCI. 20:1172–79). Additional exemplary AAV-based strategies for targeting the nervous system are described in Bedrook *et al.* (2018) ANNU REV NEUROSCI. 41:323–348. In certain embodiments, the AAV vector is an AAV-PHP.eB vector.

Lentivirus Vectors

[0098] In certain embodiments, the viral vector can be a retroviral vector. Examples of retroviral vectors include moloney murine leukemia virus vectors, spleen necrosis virus vectors, and vectors derived from retroviruses such as rous sarcoma virus, harvey sarcoma virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus. Retroviral vectors are useful as agents to mediate retroviral-mediated gene transfer into eukaryotic cells.

[0099] In certain embodiments, the retroviral vector is a lentiviral vector. Exemplary lentiviral vectors include vectors derived from human immunodeficiency virus-1 (HIV-1), human immunodeficiency virus-2 (HIV-2), simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), bovine immunodeficiency virus (BIV), Jembrana Disease Virus (JDV), equine infectious anemia virus (EIAV), and caprine arthritis encephalitis virus (CAEV).

[00100] Retroviral vectors typically are constructed such that the majority of sequences coding for the structural genes of the virus are deleted and replaced by the gene(s) of interest. Often, the structural genes (*i.e.*, gag, pol, and env), are removed from the retroviral backbone using genetic engineering techniques known in the art. Accordingly, a minimum retroviral vector comprises from 5' to 3': a 5' long terminal repeat (LTR), a packaging signal, an optional exogenous promoter and/or enhancer, an exogenous gene of interest, and a 3' LTR. If no exogenous promoter is provided, gene expression is driven by the 5' LTR, which is a weak promoter and requires the presence of Tat to activate expression. The structural genes can be provided in separate vectors for manufacture of the lentivirus, rendering the produced virions replication-defective. Specifically, with respect to lentivirus, the packaging system may comprise a single packaging vector encoding the Gag, Pol, Rev, and Tat genes, and a third, separate vector encoding the envelope protein Env (usually VSV-G due to its wide infectivity). To improve the safety of the packaging system, the packaging vector can be split, expressing Rev from one vector, Gag and Pol from another vector. Tat can also be eliminated from the packaging system by using a retroviral vector comprising a chimeric 5' LTR, wherein the U3 region of the 5' LTR is replaced with a heterologous regulatory element.

[00101] The genes can be incorporated into the proviral backbone in several general ways. The most straightforward constructions are ones in which the structural genes of the retrovirus are replaced by a single gene that is transcribed under the control of the viral regulatory sequences within the LTR. Retroviral vectors have also been constructed which can introduce more than one gene into target cells. Usually, in such vectors one gene is under the regulatory

control of the viral LTR, while the second gene is expressed either off a spliced message or is under the regulation of its own, internal promoter.

[00102] Accordingly, the new gene(s) are flanked by 5' and 3' LTRs, which serve to promote transcription and polyadenylation of the virion RNAs, respectively. The term "long terminal repeat" or "LTR" refers to domains of base pairs located at the ends of retroviral DNAs which, in their natural sequence context, are direct repeats and contain U3, R and U5 regions. LTRs generally provide functions fundamental to the expression of retroviral genes (*e.g.*, promotion, initiation and polyadenylation of gene transcripts) and to viral replication. The LTR contains numerous regulatory signals including transcriptional control elements, polyadenylation signals, and sequences needed for replication and integration of the viral genome. The U3 region contains the enhancer and promoter elements. The U5 region is the sequence between the primer binding site and the R region and contains the polyadenylation sequence. The R (repeat) region is flanked by the U3 and U5 regions. In certain embodiments, the R region comprises a trans-activation response (TAR) genetic element, which interacts with the trans-activator (tat) genetic element to enhance viral replication. This element is not required in embodiments wherein the U3 region of the 5' LTR is replaced by a heterologous promoter.

[00103] In certain embodiments, the retroviral vector comprises a modified 5' LTR and/or 3' LTR. Modifications of the 3' LTR are often made to improve the safety of lentiviral or retroviral systems by rendering viruses replication-defective. In specific embodiments, the retroviral vector is a self-inactivating (SIN) vector. As used herein, a SIN retroviral vector refers to a replication-defective retroviral vector in which the 3' LTR U3 region has been modified (*e.g.*, by deletion or substitution) to prevent viral transcription beyond the first round of viral replication. This is because the 3' LTR U3 region is used as a template for the 5' LTR U3 region during viral replication and, thus, the viral transcript cannot be made without the U3 enhancer-promoter. In a further embodiment, the 3' LTR is modified such that the U5 region is replaced, for example, with an ideal polyadenylation sequence. It should be noted that modifications to the LTRs such as modifications to the 3' LTR, the 5' LTR, or both 3' and 5' LTRs, are also contemplated to be useful in the practice of the invention.

[00104] In certain embodiments, the U3 region of the 5' LTR is replaced with a heterologous promoter to drive transcription of the viral genome during production of viral particles. Examples of heterologous promoters which can be used include, for example, viral simian virus 40 (SV40) (*e.g.*, early or late), cytomegalovirus (CMV) (*e.g.*, immediate early), Moloney murine leukemia virus (MoMLV), Rous sarcoma virus (RSV), and herpes simplex

virus (HSV) (thymidine kinase) promoters. Typical promoters are able to drive high levels of transcription in a Tat-independent manner. This replacement reduces the possibility of recombination to generate replication-competent virus, because there is no complete U3 sequence in the virus production system.

5 **[00105]** Adjacent the 5' LTR are sequences necessary for reverse transcription of the genome (the tRNA primer binding site) and for efficient packaging of viral RNA into particles (the Psi site). As used herein, the term "packaging signal" or "packaging sequence" refers to sequences located within the retroviral genome which are required for encapsidation of retroviral RNA strands during viral particle formation (*see e.g.*, Clever *et al.*, 1995 J. VIROLOGY, 10 69(4):2101-09). The packaging signal may be a minimal packaging signal (also referred to as the psi [Ψ] sequence) needed for encapsidation of the viral genome.

[00106] In certain embodiments, the retroviral vector (*e.g.*, lentiviral vector) further comprises a FLAP. As used herein, the term "FLAP" refers to a nucleic acid whose sequence includes the central polypurine tract and central termination sequences (cPPT and CTS) of a 15 retrovirus, *e.g.*, HIV-1 or HIV-2. Suitable FLAP elements are described in U.S. Patent No. 6,682,907 and in Zennou *et al.* (2000) CELL, 101:173. During reverse transcription, central initiation of the plus-strand DNA at the cPPT and central termination at the CTS lead to the formation of a three-stranded DNA structure: a central DNA flap. While not wishing to be bound by any theory, the DNA flap may act as a cis-active determinant of lentiviral genome 20 nuclear import and/or may increase the titer of the virus. In particular embodiments, the retroviral vector backbones comprise one or more FLAP elements upstream or downstream of the heterologous genes of interest in the vectors. For example, in particular embodiments, a transfer plasmid includes a FLAP element. In one embodiment, a vector of the invention comprises a FLAP element isolated from HIV-1.

25 **[00107]** In certain embodiments, the retroviral vector (*e.g.*, lentiviral vector) further comprises an export element. In one embodiment, retroviral vectors comprise one or more export elements. The term "export element" refers to a cis-acting post-transcriptional regulatory element which regulates the transport of an RNA transcript from the nucleus to the cytoplasm of a cell. Examples of RNA export elements include, but are not limited to, the human 30 immunodeficiency virus (HIV) RRE (*see e.g.*, Cullen *et al.*, (1991) J. VIROL. 65: 1053; and Cullen *et al.*, (1991) CELL 58: 423) and the hepatitis B virus post-transcriptional regulatory element (HPRE). Generally, the RNA export element is placed within the 3' UTR of a gene, and can be inserted as one or multiple copies.

[00108] In certain embodiments, the retroviral vector (*e.g.*, lentiviral vector) further comprises a posttranscriptional regulatory element. A variety of posttranscriptional regulatory elements can increase expression of a heterologous nucleic acid, *e.g.*, woodchuck hepatitis virus posttranscriptional regulatory element (WPRE; *see* Zufferey *et al.*, (1999) J. VIROL., 73:2886); the posttranscriptional regulatory element present in hepatitis B virus (HPRE) (Huang *et al.*, 5 MOL. CELL. BIOL., 5:3864); and the like (Liu *et al.*, (1995), GENES DEV., 9:1766). The posttranscriptional regulatory element is generally positioned at the 3' end the heterologous nucleic acid sequence. This configuration results in synthesis of an mRNA transcript whose 5' portion comprises the heterologous nucleic acid coding sequences and whose 3' portion 10 comprises the posttranscriptional regulatory element sequence. In certain embodiments, vectors of the invention lack or do not comprise a posttranscriptional regulatory element such as a WPRE or HPRE, because in some instances these elements increase the risk of cellular transformation and/or do not substantially or significantly increase the amount of mRNA transcript or increase mRNA stability. Therefore, in certain embodiments, vectors of the 15 invention lack or do not comprise a WPRE or HPRE as an added safety measure.

[00109] Elements directing the efficient termination and polyadenylation of the heterologous nucleic acid transcripts increase heterologous gene expression. Transcription termination signals are generally found downstream of the polyadenylation signal. Accordingly, in certain embodiments, the retroviral vector (*e.g.*, lentiviral vector) further comprises a 20 polyadenylation signal. The term “polyadenylation signal” or “polyadenylation sequence” as used herein denotes a DNA sequence which directs both the termination and polyadenylation of the nascent RNA transcript by RNA polymerase II. Efficient polyadenylation of the recombinant transcript is desirable as transcripts lacking a polyadenylation signal are unstable and are rapidly degraded. Illustrative examples of polyadenylation signals that can be used in a vector of the 25 invention, includes an ideal polyadenylation sequence (*e.g.*, AATAAA, ATTAAA AGTAAA), a bovine growth hormone polyadenylation sequence (BGHpA), a rabbit β -globin polyadenylation sequence (r β gpA), or another suitable heterologous or endogenous polyadenylation sequence known in the art.

[00110] In certain embodiments, a retroviral vector further comprises an insulator 30 element. Insulator elements may contribute to protecting retrovirus-expressed sequences, *e.g.*, therapeutic genes, from integration site effects, which may be mediated by cis-acting elements present in genomic DNA and lead to deregulated expression of transferred sequences (*i.e.*, position effect; *see, e.g.*, Burgess-Beusse *et al.*, (2002) PROC. NATL. ACAD. SCI., USA, 99:16433; and Zhan *et al.*, 2001, HUM. GENET., 109:471). In certain embodiments, the retroviral vector

comprises an insulator element in one or both LTRs or elsewhere in the region of the vector that integrates into the cellular genome. Suitable insulators for use in the invention include, but are not limited to, the chicken β -globin insulator (*see* Chung *et al.*, (1993). CELL 74:505; Chung *et al.*, (1997) PROC. NATL. ACAD. SCI., USA 94:575; and Bell *et al.*, 1999. CELL 98:387).

- 5 Examples of insulator elements include, but are not limited to, an insulator from a β -globin locus, such as chicken HS4.

[00111] Non-limiting examples of lentiviral vectors include pLVX-EF1alpha-AcGFP1-C1 (Clontech Catalog #631984), pLVX-EF1alpha-IRES-mCherry (Clontech Catalog #631987), pLVX-Puro (Clontech Catalog #632159), pLVX-IRES-Puro (Clontech Catalog #632186),
 10 pLenti6/V5-DESTTM (Thermo Fisher), pLenti6.2/V5-DESTTM (Thermo Fisher), pLKO.1 (Plasmid #10878 at Addgene), pLKO.3G (Plasmid #14748 at Addgene), pSico (Plasmid #11578 at Addgene), pLJM1-EGFP (Plasmid #19319 at Addgene), FUGW (Plasmid #14883 at Addgene), pLVTHM (Plasmid #12247 at Addgene), pLVUT-tTR-KRAB (Plasmid #11651 at Addgene), pLL3.7 (Plasmid #11795 at Addgene), pLB (Plasmid #11619 at Addgene), pWPXL
 15 (Plasmid #12257 at Addgene), pWPI (Plasmid #12254 at Addgene), EF.CMV.RFP (Plasmid #17619 at Addgene), pLenti CMV Puro DEST (Plasmid #17452 at Addgene), pLenti-puro (Plasmid #39481 at Addgene), pULTRA (Plasmid #24129 at Addgene), pLX301 (Plasmid #25895 at Addgene), pHIV-EGFP (Plasmid #21373 at Addgene), pLV-mCherry (Plasmid #36084 at Addgene), pLionII (Plasmid #1730 at Addgene), pInducer10-mir-RUP-PheS (Plasmid
 20 #44011 at Addgene). These vectors can be modified to be suitable for therapeutic use. For example, a selection marker (*e.g.*, puro, EGFP, or mCherry) can be deleted or replaced with a second exogenous gene of interest. Further examples of lentiviral vectors are disclosed in U.S. Patent Nos. 7,629,153, 7,198,950, 8,329,462, 6,863,884, 6,682,907, 7,745,179, 7,250,299, 5,994,136, 6,287,814, 6,013,516, 6,797,512, 6,544,771, 5,834,256, 6,958,226, 6,207,455,
 25 6,531,123, and 6,352,694, and PCT Publication No. WO2017/091786.

Adenoviral Vectors

[00112] In certain embodiments, the viral vector can be an adenoviral vector. Adenoviruses are medium-sized (90-100 nm), non-enveloped (naked), icosahedral viruses composed of a nucleocapsid and a double-stranded linear DNA genome. The term "adenovirus"
 30 refers to any virus in the genus Adenoviridae including, but not limited to, human, bovine, ovine, equine, canine, porcine, murine, and simian adenovirus subgenera. Typically, an adenoviral vector is generated by introducing one or more mutations (*e.g.*, a deletion, insertion,

or substitution) into the adenoviral genome of the adenovirus so as to accommodate the insertion of a non-native nucleic acid sequence, for example, for gene transfer, into the adenovirus.

[00113] A human adenovirus can be used as the source of the adenoviral genome for the adenoviral vector. For instance, an adenovirus can be of subgroup A (*e.g.*, serotypes 12, 18, and 31), subgroup B (*e.g.*, serotypes 3, 7, 11, 14, 16, 21, 34, 35, and 50), subgroup C (*e.g.*, serotypes 1, 2, 5, and 6), subgroup D (*e.g.*, serotypes 8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, and 42-48), subgroup E (*e.g.*, serotype 4), subgroup F (*e.g.*, serotypes 40 and 41), an unclassified serogroup (*e.g.*, serotypes 49 and 51), or any other adenoviral serogroup or serotype. Adenoviral serotypes 1 through 51 are available from the American Type Culture Collection (ATCC, Manassas, Virginia). Non-group C adenoviral vectors, methods of producing non-group C adenoviral vectors, and methods of using non-group C adenoviral vectors are disclosed in, for example, U.S. Patent Nos. 5,801,030, 5,837,511, and 5,849,561, and PCT Publication Nos. WO1997/012986 and WO1998/053087.

[00114] Non-human adenovirus (*e.g.*, ape, simian, avian, canine, ovine, or bovine adenoviruses) can be used to generate the adenoviral vector (*i.e.*, as a source of the adenoviral genome for the adenoviral vector). For example, the adenoviral vector can be based on a simian adenovirus, including both new world and old world monkeys (see, *e.g.*, Virus Taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses (2005)). A phylogeny analysis of adenoviruses that infect primates is disclosed in, *e.g.*, Roy *et al.* (2009) PLOS PATHOG. 5(7):e1000503. A gorilla adenovirus can be used as the source of the adenoviral genome for the adenoviral vector. Gorilla adenoviruses and adenoviral vectors are described in, *e.g.*, PCT Publication Nos. WO2013/052799, WO2013/052811, and WO2013/052832. The adenoviral vector can also comprise a combination of subtypes and thereby be a "chimeric" adenoviral vector.

[00115] The adenoviral vector can be replication-competent, conditionally replication-competent, or replication-deficient. A replication-competent adenoviral vector can replicate in typical host cells, *i.e.*, cells typically capable of being infected by an adenovirus. A conditionally-replicating adenoviral vector is an adenoviral vector that has been engineered to replicate under pre-determined conditions. For example, replication-essential gene functions, *e.g.*, gene functions encoded by the adenoviral early regions, can be operably linked to an inducible, repressible, or tissue-specific transcription control sequence, *e.g.*, a promoter. Conditionally-replicating adenoviral vectors are further described in U.S. Patent No. 5,998,205. A replication-deficient adenoviral vector is an adenoviral vector that requires complementation

of one or more gene functions or regions of the adenoviral genome that are required for replication, as a result of, for example, a deficiency in one or more replication-essential gene function or regions, such that the adenoviral vector does not replicate in typical host cells, especially those in a human to be infected by the adenoviral vector.

5 **[00116]** Preferably, the adenoviral vector is replication-deficient, such that the replication-deficient adenoviral vector requires complementation of at least one replication-essential gene function of one or more regions of the adenoviral genome for propagation (*e.g.*, to form adenoviral vector particles). The adenoviral vector can be deficient in one or more replication-essential gene functions of only the early regions (*i.e.*, E1-E4 regions) of the adenoviral genome, only the late regions (*i.e.*, L1-L5 regions) of the adenoviral genome, both the early and late
10 regions of the adenoviral genome, or all adenoviral genes (*i.e.*, a high capacity adenovector (HC-Ad)). See, *e.g.*, Morsy *et al.* (1998) PROC. NATL. ACAD. SCI. USA 95: 965-976, Chen *et al.* (1997) PROC. NATL. ACAD. SCI. USA 94: 1645-1650, and Kochanek *et al.* (1999) HUM. GENE THER. 10(15):2451-9. Examples of replication-deficient adenoviral vectors are disclosed in U.S.
15 Patent Nos. 5,837,511, 5,851,806, 5,994,106, 6,127,175, 6,482,616, and 7,195,896, and PCT Publication Nos. WO1994/028152, WO1995/002697, WO1995/016772, WO1995/034671, WO1996/022378, WO1997/012986, WO1997/021826, and WO2003/022311.

[00117] The replication-deficient adenoviral vector of the invention can be produced in complementing cell lines that provide gene functions not present in the replication-deficient
20 adenoviral vector, but required for viral propagation, at appropriate levels in order to generate high titers of viral vector stock. Such complementing cell lines are known and include, but are not limited to, 293 cells (described in, *e.g.*, Graham *et al.* (1977) J. GEN. VIROL. 36: 59-72), PER.C6 cells (described in, *e.g.*, PCT Publication No. WO1997/000326, and U.S. Patent Nos. 5,994,128 and 6,033,908), and 293-ORF6 cells (described in, *e.g.*, PCT Publication No.
25 WO1995/034671 and Brough *et al.* (1997) J. VIROL. 71: 9206-9213). Other suitable complementing cell lines to produce the replication-deficient adenoviral vector of the invention include complementing cells that have been generated to propagate adenoviral vectors encoding transgenes whose expression inhibits viral growth in host cells (see, *e.g.*, U.S. Patent Publication No. 2008/0233650). Additional suitable complementing cells are described in, for example,
30 U.S. Patent Nos. 6,677,156 and 6,682,929, and PCT Publication No. WO2003/020879. Formulations for adenoviral vector-containing compositions are further described in, for example, U.S. Patent Nos. 6,225,289, and 6,514,943, and PCT Publication No. WO2000/034444.

[00118] Additional exemplary adenoviral vectors, and/or methods for making or propagating adenoviral vectors are described in U.S. Patent Nos. 5,559,099, 5,837,511, 5,846,782, 5,851,806, 5,994,106, 5,994,128, 5,965,541, 5,981,225, 6,040,174, 6,020,191, 6,083,716, 6,113,913, 6,303,362, 7,067,310, and 9,073,980.

- 5 [00119] Commercially available adenoviral vector systems include the ViraPower™ Adenoviral Expression System available from Thermo Fisher Scientific, the AdEasy™ adenoviral vector system available from Agilent Technologies, and the Adeno-X™ Expression System 3 available from Takara Bio USA, Inc.

Viral Vector Production

- 10 [00120] Methods for producing viral vectors are known in the art. Typically, a virus of interest is produced in a suitable host cell line using conventional techniques including culturing a transfected or infected host cell under suitable conditions so as to allow the production of infectious viral particles. Nucleic acids encoding viral genes and/or tRNAs can be incorporated into plasmids and introduced into host cells through conventional transfection or transformation
15 techniques. Exemplary suitable host cells for production of disclosed viruses include human cell lines such as HeLa, Hela-S3, HEK293, 911, A549, HER96, or PER-C6 cells. Specific production and purification conditions will vary depending upon the virus and the production system employed.

- [00121] In certain embodiments, producer cells may be directly administered to a subject,
20 however, in other embodiments, following production, infectious viral particles are recovered from the culture and optionally purified. Typical purification steps may include plaque purification, centrifugation, *e.g.*, cesium chloride gradient centrifugation, clarification, enzymatic treatment, *e.g.*, benzonase or protease treatment, chromatographic steps, *e.g.*, ion exchange chromatography or filtration steps.

25 IV. Pharmaceutical Compositions

- [00122] For therapeutic use, a tRNA and/or expression vector preferably is combined with a pharmaceutically acceptable carrier. The term “pharmaceutically acceptable” as used herein refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings
30 and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00123] The term “pharmaceutically acceptable carrier” as used herein refers to buffers, carriers, and excipients suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable carriers include any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see, *e.g.*, Martin, Remington’s Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, PA [1975]. Pharmaceutically acceptable carriers include buffers, solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art.

[00124] In certain embodiments, a pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. In such embodiments, suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides; and other carbohydrates (such as glucose, mannose or dextrans); proteins (such as serum albumin, gelatin or immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or

pharmaceutical adjuvants (See *Remington's Pharmaceutical Sciences*, 18th ed. (Mack Publishing Company, 1990).

[00125] In certain embodiments, a pharmaceutical composition may contain nanoparticles, *e.g.*, polymeric nanoparticles, liposomes, or micelles (See Anselmo *et al.* (2016) BIOENG. TRANSL.

5 MED. 1: 10-29). In certain embodiments, the composition does not comprise (or is substantially free of, for example, the composition comprises less than 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of) a nanoparticle or an aminolipid delivery compound, *e.g.*, as described in U.S. Patent Publication No. 2017/0354672. In certain embodiments, the tRNA or expression vector introduced into the cell or administered to the subject is not conjugated to or associated with
10 another moiety, *e.g.*, a carrier particle, *e.g.*, an aminolipid particle. As used herein, the term “conjugated,” when used with respect to two or more moieties, means that the moieties are physically associated or connected with one another, either directly or via one or more additional moieties that serves as a linking agent, to form a structure that is sufficiently stable so that the moieties remain physically associated under the conditions in which structure is used, *e.g.*,
15 physiological conditions. Typically the moieties are attached either by one or more covalent bonds or by a mechanism that involves specific binding. Alternately, a sufficient number of weaker interactions can provide sufficient stability for moieties to remain physically associated.

[00126] In certain embodiments, a pharmaceutical composition may contain a sustained- or controlled-delivery formulation. Techniques for formulating sustained- or controlled-delivery
20 means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. Sustained-release preparations may include, *e.g.*, porous polymeric microparticles or semipermeable polymer matrices in the form of shaped articles, *e.g.*, films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides, copolymers of L-glutamic acid and gamma ethyl-L-glutamate, poly (2-
25 hydroxyethyl-inethacrylate), ethylene vinyl acetate, or poly-D(-)-3-hydroxybutyric acid. Sustained release compositions may also include liposomes that can be prepared by any of several methods known in the art.

[00127] Pharmaceutical compositions containing a tRNA and/or expression vector disclosed herein can be presented in a dosage unit form and can be prepared by any suitable method. A
30 pharmaceutical composition should be formulated to be compatible with its intended route of administration. Examples of routes of administration are intravenous (IV), intradermal, inhalation, transdermal, topical, transmucosal, intrathecal and rectal administration. In certain embodiments, a tRNA and/or expression vector is administered intrathecally. In certain

embodiments, a tRNA and/or expression vector is administered by injection. Useful formulations can be prepared by methods known in the pharmaceutical art. For example, see *Remington's Pharmaceutical Sciences*, 18th ed. (Mack Publishing Company, 1990).

Formulation components suitable for parenteral administration include a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose.

[00128] For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). The carrier should be stable under the conditions of manufacture and storage, and should be preserved against microorganisms. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof.

[00129] In general, any method of delivering a nucleic acid molecule can be adapted for use with a tRNA (see *e.g.*, Akhtar *et al.* (1992) TRENDS CELL. BIOL. 2(5):139-144 and PCT Publication No. WO94/02595). The tRNA can be modified or alternatively delivered using a drug delivery system to prevent the rapid degradation of the tRNA by endo- and exo-nucleases *in vivo*. tRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. tRNA molecules can also be conjugated to or otherwise associated with an aptamer. A tRNA can also be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of a tRNA molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of a tRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to the RNA, *e.g.*, tRNA, or induced to form a vesicle or micelle (see *e.g.*, Kim *et al.* (2008) JOURNAL OF CONTROLLED RELEASE 129(2):107-116) that encases the RNA. Methods for making and administering cationic-RNA complexes are well within the abilities of one skilled in the art (see, *e.g.*, Sorensen *et al.* (2003) J. MOL. BIOL 327:761-766; Verma *et al.* (2003) CLIN. CANCER RES. 9:1291-1300; Arnold *et al.* (2007) J. HYPERTENS. 25:197-205). Some non-limiting examples of drug delivery systems useful for systemic delivery of RNAs, *e.g.*, tRNAs include DOTAP (Sorensen *et al.* (2003) *supra*; Verma *et al.* (2003), *supra*), Oligofectamine, solid nucleic acid lipid particles (Zimmermann *et al.* (2006) NATURE

441:111-114), cardiolipin (Chien *et al.* (2005) CANCER GENE THER. 12:321-328; Pal *et al.* (2005) INT J. ONCOL. 26:1087-1091), polyethyleneimine (Bonnet *et al.* (2008) PHARM. RES. 25(12):2972-82; Aigner (2006) J. BIOMED. BIOTECHNOL. 71659), Arg-Gly-Asp (RGD) peptides (Liu (2006) MOL. PHARM. 3:472-487), and polyamidoamines (Tomalia *et al.* (2007) BIOCHEM. SOC. TRANS. 35:61-67; Yoo *et al.* (1999) PHARM. RES. 16:1799-1804). In certain embodiments, a tRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of RNAs and cyclodextrins can be found in U.S. Patent No. 7,427,605.

[00130] Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished by any suitable method, *e.g.*, filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

[00131] The compositions described herein may be administered locally or systemically. Administration will generally be parenteral administration. In a preferred embodiment, the pharmaceutical composition is administered subcutaneously and in an even more preferred embodiment intravenously. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions.

[00132] Generally, a therapeutically effective amount of active component, for example, a tRNA and/or expression vector, is in the range of 0.1 mg/kg to 100 mg/kg, *e.g.*, 1 mg/kg to 100 mg/kg, 1 mg/kg to 10 mg/kg. In certain embodiments, a therapeutically effective amount of a viral expression vector is in the range of 10^2 to 10^{15} plaque forming units (pfus), *e.g.*, 10^2 to 10^{10} , 10^2 to 10^5 , 10^5 to 10^{15} , 10^5 to 10^{10} , or 10^{10} to 10^{15} plaque forming units. The amount administered will depend on variables such as the type and extent of disease or indication to be treated, the overall health of the patient, the *in vivo* potency of the antibody, the pharmaceutical formulation, and the route of administration. The initial dosage can be increased beyond the upper level in order to rapidly achieve the desired blood-level or tissue-level. Alternatively, the initial dosage can be smaller than the optimum, and the daily dosage may be progressively increased during the course of treatment. Human dosage can be optimized, *e.g.*, in a conventional Phase I dose escalation study designed to run from 0.5 mg/kg to 20 mg/kg. Dosing frequency can vary, depending on factors such as route of administration, dosage amount, serum half-life, and the disease being treated. Exemplary dosing frequencies are once per day, once per week and once every two weeks. A preferred route of administration is parenteral, *e.g.*, intravenous infusion. In

certain embodiments, a polypeptide and/or multimeric protein is lyophilized, and then reconstituted in buffered saline, at the time of administration.

[00133] In certain embodiments, the tRNA or expression vector is not conjugated to or associated with another moiety, *e.g.*, a carrier particle, *e.g.*, an aminolipid particle. In certain
 5 embodiments, the tRNA or expression vector is introduced into the cell or administered to subject in a dosage form lacking a nanoparticle. In certain embodiments, the tRNA or expression vector is introduced into the cell or administered to subject in a dosage form lacking an aminolipid delivery compound, *e.g.*, as described in U.S. Patent Publication No. 2017/0354672.

10 **V. Therapeutic Uses**

[00134] The compositions and methods disclosed herein can be used to treat a premature termination codon (PTC)-mediated disorder in a subject. As used herein, the term “PTC-mediated disorder” refers to a disorder that is mediated, enhanced, exacerbated, or otherwise facilitated by or associated with a PTC in a gene.

15 [00135] The invention provides a method of treating a PTC-mediated disorder in a subject in need thereof. The method comprises administering to the subject an effective amount of a tRNA and/or expression vector, *e.g.*, a tRNA and/or expression vector disclosed herein, either alone or in a combination with another therapeutic agent to treat the PTC-mediated disorder in the subject.

20 [00136] In certain embodiments, the premature termination codon-mediated disorder is a disorder listed in **TABLE 5** below, and/or the gene with a premature termination codon is a gene listed in the corresponding row of **TABLE 5** below.

TABLE 5

Gene	Disorder
SCN1A	Dravet Syndrome; Genetic Epilepsy with Febrile Seizures (GEFS)
KCNQ2	Benign Familial Infantile Epilepsy (BFIE); Early Infantile Epileptic Encephalopathy (EIEE)
SCN2A	Benign Familial Infantile Epilepsy (BFIE); Early Infantile Epileptic Encephalopathy (EIEE)

Gene	Disorder
CDKL5	Early Infantile Epileptic Encephalopathy (EIEE); Lennox-Gastaut Syndrome; CDKL5 deficiency disorder
MECP2	Rett Syndrome; PPM-X Syndrome
STXBP1	Early Infantile Epileptic Encephalopathy (EIEE); Ohtahara Syndrome; Dravet Syndrome
SCN8A	Benign Familial Infantile Epilepsy (BFIE); Early Infantile Epileptic Encephalopathy (EIEE)
CACNA1A	Episodic Ataxia; Hemiplegic Migraine
SLC2A1	Idiopathic Generalized Epilepsy
FOXG1	FOXG1 Syndrome
PCDH19	Early Infantile Epileptic Encephalopathy (EIEE)
GRIN2B	Early Infantile Epileptic Encephalopathy (EIEE)
DEPDC5	Familial Focal Epilepsy with Variable Foci (FFEVF)
GRIN2A	Early Infantile Epileptic Encephalopathy (EIEE); Lennox-Gastaut Syndrome
CHD2	Childhood-onset epileptic encephalopathy
SCN9A	Congenital insensitivity to pain, etc
SYNGAP1	SYNGAP1-related intellectual disability
ALDH7A1	Pyridoxine-dependent epilepsy
GRIN1	Early Infantile Epileptic Encephalopathy (EIEE); Lennox-Gastaut Syndrome
TBC1D24	Early Infantile Epileptic Encephalopathy (EIEE); Familial Infantile Myoclonic Epilepsy (FIME)
SLC6A1	Myoclonic Astatic Epilepsy
DNM1	Early Infantile Epileptic Encephalopathy (EIEE)
ARX	Early Infantile Epileptic Encephalopathy (EIEE); X-linked Intellectual Disability
KCNB1	Early Infantile Epileptic Encephalopathy (EIEE)
KCNA1	Partial Epilepsy and Episodic Ataxia
GABRG2	Genetic Epilepsy with Febrile Seizures (GEFS); Early Infantile Epileptic Encephalopathy (EIEE); Febrile seizures
WWOX	Early Infantile Epileptic Encephalopathy (EIEE)

Gene	Disorder
GABRB3	Early Infantile Epileptic Encephalopathy (EIEE); Lennox-Gastaut Syndrome
SZT2	Early Infantile Epileptic Encephalopathy (EIEE)
LGI1	Autosomal Dominant Partial Epilepsy with Auditory Features (ADPEAF)
PNPO	PNPO-Deficiency
SCN1B	Genetic Epilepsy with Febrile Seizures (GEFS); Early Infantile Epileptic Encephalopathy (EIEE)
UBA5	Early Infantile Epileptic Encephalopathy (EIEE)
KCTD7	Progressive Myoclonus Epilepsy
SCARB2	Action Myoclonus – Renal Failure (AMRF); Progressive Myoclonic Epilepsy
SLC13A5	Early Infantile Epileptic Encephalopathy (EIEE)
CSTB	Progressive Myoclonic Epilepsy
EPM2A	Progressive Myoclonic Epilepsy
PRRT2	Benign Familial Infantile Seizures (BFIS)
NHLRC1	Progressive Myoclonic Epilepsy
SLC25A22	Early Infantile Epileptic Encephalopathy (EIEE)
PRRT2	Benign Familial Infantile Seizures (BFIS)
ALG13	Early Infantile Epileptic Encephalopathy (EIEE)

[00137] In certain embodiments, the premature termination codon-mediated disorder is a disorder listed in **TABLE 6** below, and/or the gene with a premature termination codon is a gene listed in the corresponding row of **TABLE 6** below.

TABLE 6

Gene	Disorder
β -globin	β -thalassemia
CHM	Choroideremia
CFTR	Cystic Fibrosis
dystrophin	Duchenne Muscular Dystrophy
α -L-iduronidase	Hurler Syndrome
KIF1A	KIF1A

Gene	Disorder
FBN1	Marfan Syndrome
ARSB	Maroteaux-Lamy Syndrome
SMPD1	Niemann Pick Disease
NAGLU	Sanfilippo Syndrome
DHCR7	Smith-Lemli-Opitz Syndrome
SCN5A	Brugada Syndrome
KCNH2 (hERG)	Long QT Syndrome type 2
KCNQ1	Long QT Syndrome type 1
TTN	Dilated Cardiomyopathy
MYBPC3	Familial Hypertrophic Cardiomyopathy
LMNA	Dilated Cardiomyopathy (sometimes Emery-Dreifuss Muscular Dystrophy)
PKP2	Familial Arrhythmogenic Right Ventricular Dysplasia
PLN	Familial Isolated Dilated Cardiomyopathy
TSC1/2	Tuberous Sclerosis
LDLR	Familial Hypercholesterolemia
SMN1	Spinal Muscular Atrophy

[00138] In certain embodiments, the PTC-mediated disorder is an epilepsy (*e.g.*, Dravet syndrome), wherein the method reduces seizure frequency, seizure severity, and/or cognitive impairment in the subject. For example, in certain embodiments, the method reduces seizure frequency in the subject by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% over the period of, *e.g.*, a day, a week, or a month. In certain embodiments, the method reduces seizure frequency by 50% over the period of, *e.g.*, a day, a week, or a month.

[00139] In certain embodiments, the PTC-mediated disorder is Dravet and/or the gene with a premature termination codon is SCN1A. In certain embodiments, a premature termination codon in the SCN1A gene is caused by a mutation, or a combination of mutations, selected from

c.5745C>G, c.5713G>T, c.5701C>T, c.5677C>T, c.5641C>T, c.5629C>T, c.5623C>T, c.5503A>T, c.5473G>T, c.5437G>T, c.5428C>T, c.5403G>A, c.5402G>A, c.5383G>T, c.5371G>T, c.5049T>G, c.4921G>T, c.4900C>T, c.4873C>T, c.4779del, c.4778G>A, c.4774G>T, c.4761T>G, c.4648G>T, c.4540C>T, c.4516A>T, c.4514C>A, c.4508T>G, c.4488C>G, c.4471G>T, c.4300A>T, c.4269G>A, c.4268G>A, c.4233T>A, c.4222G>T, c.4191G>A, c.4190G>A, c.4186C>T, c.4159A>T, c.4155C>A, c.3964del, c.3952C>T, c.3825G>A, c.3824G>A, c.3819G>A, c.3818G>A, c.3795T>A, c.3789T>G, c.3779G>A, c.3750C>G, c.3724G>T, c.3700C>T, c.3697C>T, c.3657dup, c.3624G>A, c.3604C>T, c.3582G>A, c.3578G>A, c.3574C>T, c.3463C>T, c.3454del, c.3424G>T, c.3422C>A, c.3406G>T, c.3328G>T, c.3273C>A, c.3262G>T, c.3073C>T, c.3060T>A, c.2844T>A, c.2749C>T, c.2695C>T, c.2645T>A, c.2560C>T, c.2551C>T, c.2546C>A, c.2462G>A, c.2298del, c.2228G>A, c.2181G>A, c.2180G>A, c.2101C>T, c.2038A>T, c.1958T>A, c.1837C>T, c.1834C>T, c.1804G>T, c.1795G>T, c.1738C>T, c.1702C>T, c.1660C>T, c.1624C>T, c.1516C>T, c.1378C>T, c.1363C>T, c.1354A>T, c.1348C>T, c.1345G>T, c.1344dup, c.1306G>T, c.1278C>A, c.1278C>G, c.1151G>A, c.1129C>T, c.1118T>A, c.942del, c.751del, c.644T>A, c.327C>G, c.249C>A, c.121A>T, c.4846_4850dup, c.4787_4788del, c.4578_4612dup, c.4211_4212del, c.4125_4130delinsATAATCATACTGATTGCCTAAACTAAT, c.3690_3693del, c.3338_3339del, c.1247_1248insGTAGA, c.825_826insGTATA, and c.278_279dup. In certain embodiments, a premature termination codon in the SCN1A gene is caused by a mutation, or a combination of mutations, selected from c.58G>T, c.575G>A, c.664C>T, c.962C>G, c.1095dupT, c.1129C>T, c.1315C>T, c.1348C>T, c.1366G>T, c.1492A>T, c.1537G>T, c.1624C>T, c.1738C>T, c.1804G>T, c.1837C>T, c.2134C>T, c.2370T>A, c.2495G>A, c.2593C>T, c.2635delC, c.2904C>A, c.3295G>T, c.3311C>A, c.3452C>G, c.3637C>T, c.3656G>A, c.3733C>T, c.3783C>A, c.3829C>T, c.3985C>T, c.4359T>G, c.4547C>A, c.4573C>T, c.4721C>G, c.4954G>T, c.5641G>T, c.5656C>T, and c.5734C>T. In certain embodiments, a premature termination codon in the SCN1A gene is caused by a mutation selected from c.664C>T, c.1129C>T, c.1492A>T, c.1624C>T, c.1738C>T, c.1837C>T, c.2134C>T, c.2593C>T, c.3637C>T, c.3733C>T, c.3985C>T, c.4573C>T, c.5656C>T, and c.5734C>T. In certain embodiments, a premature termination codon in the SCN1A gene is caused by a mutation selected from c.1738C>T and c.3985C>T.

[00140] In certain embodiments, a premature termination codon in the SCN1A gene is caused by a mutation set forth in **TABLE 7**, or a combination of mutations set forth in **TABLE 7**.

TABLE 7

Mutation (coding DNA)	Mutation (Protein)	Suppressor Class
c.664C>T	Arg222Ter	Arg>TGA
c.3637C>T	Arg1213Ter	Arg>TGA
c.3733C>T	Arg1245Ter	Arg>TGA
c.2134C>T	Arg712Ter	Arg>TGA
c.1837C>T	Arg613Ter	Arg>TGA
c.4188C>A	Cys1396Ter	Cys>TGA
c.2877T>A	Cys959Ter	Cys>TGA
c.3183T>A	Cys1061Ter	Cys>TGA
c.3607C>T	Gln1203Ter	Gln>TAA
c.2782C>T	Gln928Ter	Gln>TAA
c.3829C>T	Gln1277Ter	Gln>TAA
c.2893C>T	Gln965Ter	Gln>TAA
c.3106C>T	Gln1036Ter	Gln>TAG
c.3496C>T	Gln1166Ter	Gln>TAG
c.5662C>T	Gln1888Ter	Gln>TAG
c.5461C>T	Gln1821Ter	Gln>TAG
c.3730C>T	Gln1244Ter	Gln>TAG
c.5506G>T	Glu1836Ter	Glu>TAA
c.5470G>T	Glu1824Ter	Glu>TAA
c.3757G>T	Glu1253Ter	Glu>TAA
c.3439G>T	Glu1147Ter	Glu>TAA
c.1345G>T	Glu449Ter	Glu>TAA
c.5404G>T	Glu1802Ter	Glu>TAG
c.1804G>T	Glu602Ter	Glu>TAG
c.5416G>T	Glu1806Ter	Glu>TAG
c.1795G>T	Glu599Ter	Glu>TAG
c.1549G>T	Glu517Ter	Glu>TAG
c.4255G>T	Gly1419Ter	Gly>TGA
c.4954G>T	Gly1652Ter	Gly>TGA
c.4807G>T	Gly1603Ter	Gly>TGA

Mutation (coding DNA)	Mutation (Protein)	Suppressor Class
c.487G>T	Gly163Ter	Gly>TGA
c.1843G>T	Gly615Ter	Gly>TGA
c.539T>A	Leu180Ter	Leu>TAA
c.2678T>A	Leu893Ter	Leu>TAA
c.644T>A	Leu215Ter	Leu>TAG
c.1958T>A	Leu653Ter	Leu>TAG
c.1118T>A	Leu373Ter	Leu>TAG
c.4541T>G	Leu1514Ter	Leu>TGA
c.2627T>G	Leu876Ter	Leu>TGA
c.4549A>T	Lys1517Ter	Lys>TAA
c.5536A>T	Lys1846Ter	Lys>TAA
c.121A>T	Lys41Ter	Lys>TAA
c.4192A>T	Lys1398Ter	Lys>TAA
c.1354A>T	Lys452Ter	Lys>TAA
c.2071A>T	Lys691Ter	Lys>TAG
c.3455C>A	Ser1152Ter	Ser>TAA
c.2579C>A	Ser860Ter	Ser>TAA
c.1883C>A	Ser628Ter	Ser>TAA
c.4547C>A	Ser1516Ter	Ser>TAG
c.2213G>A	Trp738Ter	Trp>TAG
c.3611G>A	Trp1204Ter	Trp>TAG
c.4811G>A	Trp1604Ter	Trp>TAG
c.4223G>A	Trp1408Ter	Trp>TAG
c.5435G>A	Trp1812Ter	Trp>TAG
c.3615G>A	Trp1205Ter	Trp>TGA
c.4224G>A	Trp1408Ter	Trp>TGA
c.4302G>A	Trp1434Ter	Trp>TGA
c.3858G>A	Trp1286Ter	Trp>TGA
c.5436G>A	Trp1812Ter	Trp>TGA
c.3762T>A	Tyr1254Ter	Tyr>TAA
c.3828T>A	Tyr1276Ter	Tyr>TAA

Mutation (coding DNA)	Mutation (Protein)	Suppressor Class
c.4266T>A	Tyr1422Ter	Tyr>TAA
c.3306C>A	Tyr1102Ter	Tyr>TAA
c.249C>A	Tyr83Ter	Tyr>TAA
c.5082T>G	Tyr1694Ter	Tyr>TAG
c.4794T>G	Tyr1598Ter	Tyr>TAG
c.4521C>G	Tyr1507Ter	Tyr>TAG
c.3822T>G	Tyr1274Ter	Tyr>TAG
c.5778C>G	Tyr1926Ter	Tyr>TAG

[00141] Additional exemplary mutations, including exemplary mutations causing a premature termination codon in a gene, *e.g.*, the SCN1A gene, can be found in ClinVar (available on the world wide web at ncbi.nlm.nih.gov/clinvar/), “A catalog of SCN1A variants” Lossin *et al.* (2009) BRAIN DEV. 2009 31(2):114-30, the SCN1A Registry (available on the world wide web at scn1a.net/scn1a-registry/), the SCN1A Mutation Database (available on the world wide web at gzneurosci.com/scn1adatabase), and the Leiden Open Variation Database (LOVD v.3.0; available on the world wide web at databases.lovd.nl/shared/genes/SCN1A). Unless indicated otherwise, any SCN1A mutations described herein are relative to SCN1a isoform 1 (NCBI reference sequence NM_001165963, SEQ ID NO: 863).

[00142] In another aspect, the invention provides a method of treating Dravet syndrome in a subject in need thereof wherein the subject has a SCN1A gene with a mutation set forth in a row **TABLE 7**, the method comprising administering to the subject an effective amount of a suppressor tRNA of the suppressor class indicated in the same row of **TABLE 7** as the mutation, or an expression vector comprising a nucleotide sequence encoding the tRNA. “Suppressor Class” as used in **TABLE 7** (*e.g.*, Arg>TGA) refers to the endogenous tRNA type from which the suppressor tRNA is derived (*e.g.*, an arginine tRNA) and the termination codon recognized by the suppressor tRNA (*e.g.*, TGA). Exemplary Arg>TGA suppressor tRNAs include tRNAs comprising a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 19-22, and 35. Exemplary Gln>TAA suppressor tRNAs include tRNAs comprising a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45. Exemplary Gln>TAG suppressor tRNAs include tRNAs comprising a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187.

[00143] For example, in certain embodiments, the subject has a SCN1A gene with a premature termination codon selected from c.664C>T, c.3637C>T, c.3733C>T, c.2134C>T, and

c.1837C>T, and the method comprises administering to the subject an effective amount of a suppressor tRNA comprising a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-18, 19-22, and 35. In certain embodiments, the subject has a SCN1A gene with a premature termination codon selected from c.3607C>T, c.2782C>T, c.3829C>T, and c.2893C>T, and the method comprises administering to the subject an effective amount of a suppressor tRNA comprising a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45. In certain embodiments, the subject has a SCN1A gene with a premature termination codon selected from c.3106C>T, c.3496C>T, c.5662C>T, c.5461C>T, and c.3730C>T, and the method comprises administering to the subject an effective amount of a suppressor tRNA comprising a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187.

[00144] In certain embodiments, wherein the gene is a SCN1A gene, the SCN1A gene product produced with the tRNA is a functional SCN1A gene product. In certain embodiments, the functional SCN1A gene product has greater activity than the truncated SCN1A gene product, *e.g.*, greater voltage-gated sodium channel activity. In certain embodiments, the method increases voltage-gated sodium channel activity in a cell, tissue, or subject by about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 110%, about 120%, about 130%, about 140%, about 150%, about 160%, about 170%, about 180%, about 190%, about 200%, about 250%, about 300%, about 350%, about 400%, about 450%, about 500%, about 600%, about 700%, about 800%, about 900%, or about 1000% relative to a cell, tissue, or subject without the tRNA. In certain embodiments, the method increases voltage-gated sodium channel activity in a cell, tissue, or subject by from about 20% to about 200%, about 20% to about 180%, about 20% to about 160%, about 20% to about 140%, about 20% to about 120%, about 20% to about 100%, about 20% to about 80%, about 20% to about 60%, about 20% to about 40%, about 40% to about 200%, about 40% to about 180%, about 40% to about 160%, about 40% to about 140%, about 40% to about 120%, about 40% to about 100%, about 40% to about 80%, about 40% to about 60%, about 60% to about 200%, about 60% to about 180%, about 60% to about 160%, about 60% to about 140%, about 60% to about 120%, about 60% to about 100%, about 60% to about 80%, about 80% to about 200%, about 80% to about 180%, about 80% to about 160%, about 80% to about 140%, about 80% to about 120%, about 80% to about 100%, about 100% to about 200%, about 100% to about 180%, about 100% to about 160%, about 100% to about 140%, about 100% to about 120%, about 120% to about 200%, about 120% to about 180%, about 120% to about 160%, about 120% to about 140%, about 140% to about 200%, about 140% to about 180%, about 140% to about 160%, about 160% to about 200%, about 160% to about 180%, or about 180% to

about 200% relative to a cell, tissue, or subject without the tRNA. Voltage-gated sodium channel activity may be measured by any method known in the art, for example, as described in Kalume *et al.* (2007) J. NEUROSCI. 27(41):11065-74, Yu *et al.* (2007) NAT. NEUROSCI. 9(9): 1142-9, and Han *et al.* (2012) NATURE 489(7416): 385–390.

- 5 **[00145]** In certain embodiments, the functional SCN1A gene product is the Na_v1.1 protein. In certain embodiments, the functional SCN1A gene product comprises, consists essentially of, or consists of the amino acid sequence of any one of the following amino acid sequences, or an amino acid sequence having 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of the following
- 10 amino acid sequences (each corresponding to different isoforms of SCN1A):

MEQTVLVPPGPDSFNFFTTRESLAAIERRIAEKAKNPKPKDKDDDENGPKPNSDLEAGKNLPFI
YGDIPPEMVSEPLEDLDPYYINKKTFIVLNKGKAI FRFSATSALYILTPFNPLRKIAIKILVHS
LFSMLIMCTILTNCVFM TMSNPPDWTKNVEYTF TGIYTFESLIKI IARGFCLEDFTFLRDPWNW
LDFTVITFAYVTEFVDLGNVSALRTRFVLRALKTISVIPGLKTIVGALIQSVKKLSDMILTVE
15 CLSVFALIGLQLFMGNLRNKC IQWPPTNASLEEHS IEKNITVNYNGTLINETVFEFDWKS YIQD
SRYHYFLEGFLDALLCGNSSDAGQCPEGYMCVKAGRNP NYGYTSFDTFSWAFSLSLFRLMTQDFW
ENLYQLTLRAAGKTYMIFFVLVIFLGSFYLINLILAVVAMAYEEQNQATLEEAEQKEAEFQQMI
EQLKKQQEAAQQAATATASEHSREPSAAGRLSDSSSEASKLSSKSAKERRNRKRKRQKEQSGG
EEKDEDEFQKSESEDSIRRKGRF RFSIEGNRLTYEKRYSSPHQSLLSIRGSLFSPPRNSRTSLFS
20 FRGRAKDVGSENDFADDEHSTFEDNESRRDSL FVPRRHGERRNSNLSQTSRSSRMLAVFPANGK
MHSTVDCNGVVSLVGGPSVPTSPVGQLLPEVI IDKPADDDNGTTTETEMRKRRSSSFHVSMDFL
EDPSQRQRAMSIASILTNTVEELEE SRQKPCPCWYKFSNIFLIWDCSPYWLKVKHVVNLVMDP
FVDLAITICIVLNTLFMAMEHYPM TDHFNNVLT VGNLVFTGIFTAEMFLKIIAMDPYFFFQEGW
NIFDGFIVTSLSLVELGLANVEGLSVLRSFRLLRVFKLAKSWPTLNMLIKIIGNSVGALGNLTLV
25 LAIIVFIFAVVGMQLFGKSYKDCVCKIASDCQLPRWHMNDFFHSFLIVFRVLCGEWIETMWDCM
EVAGQAMCLTVFMMVMVIGNLVVLNLF LALLLSSFSADNLAATDDD NEMNNLQIAVDRMHKGVA
YVKRKIYEFIQQS FIRKQKILDEIKPLDDLNNKKDSCMSNHTAEIGKDL DYLDKDVNGTTSIGIGT
GSSVEKYIIDESDYMSFINNPSLT VTVPIAVGESDFENLNTEDFSSES DLEESKEKLNESSSSS
EGSTVDIGAPVEEQPVVEPEETLEPEACFTEGCVQRFKCCQINVEEGRGKQWWNLRRTCFRIVE
30 HNW FETFIVFMILLSSGALAFEDIYIDQRKTIKTMLEYADKVFTYI FILEMLLKWVAYGYQTYF
TNAWCWLD FLIVDVSLVSLTANALGYSELGAIKSLRTLRLALRPLRALS RFEGMRVVNALLGAI
PSIMNVLLVCLIFWLIFSIMGVNLFAGKFYHCINTTTGDRFDIEDVNNHTDCLKLIERNETARW
KNVKVNF DNVGFGYLSLLQVATFKGWMDIMYAAVDSRNVELQPKYEESLYMYLYFVIFIIFGSF
FTLNLFIGVIIDNFNQKKKFGGQDI FMTEEQKKYYNAMKKLGSKKPQKPIPRPGNKFQGMVFD

FVTRQVFDISIMILICLNMVTMMVETDDQSEYVTTILSRINLVFIVLFTGECVLKLISLRHYFF
TIGWNI FDFV VVILSIVGMFLAELIEKYFVSPTLFRVIRLARIGRILRLIKGAKGIRTLLFALM
MSLPALFNIGLLLFLVMFIYAI FGMSNFAYVKREVGIDDMFN FETFGNSMICLFQITTSAGWDG
LLAPILNSKPPDCDPNKVNP GSSVKGDCGNPSVGIFFFVSYIIISFLVVVNM YIAVILENFSVA
5 TEESAEPLESEDDFEMFYEVWEKFDPDATQFMFEKLSQFAAALEPPLNLPQPNKLQLIAMDLPM
VSGDRIHCLDILFAFTKRVLGESGEMDALRIQMEERFMASNP SKVSYQPITTTLKRKQEEVSAV
IIQRAYRRHLLKRTVKQASFTYNKNKIKGGANLLIKEDMIIDRINENSITEKTDLT MSTAACPP
SYDRVTKPIVEKHEQEKGDEKAKGK (SEQ ID NO: 863);

MEQTVLVPPGPDSFNFFTRESLAAIERRIAEKAKNP KPDKDDDENGPKPNSDLEAGKNLPFI
10 YGDIPPEMVSEPLEDLDPYYINKKTFIVLNKGKAI FRFSATSALYILTPFNPLRKIAIKILVHS
LFSMLIMCTILTNCVFM TMSNPDPWTKNVEYTF TGIYTFESLIKIIARGFCLEDFTFLRDPWNW
LDFTVITFAYVTEFVDLGNVSALRTFRVLRALKTISVIPGLKTIVGALIQSVKKLS DVMILT VF
CLSVFALIGLQLFMGNLRNKC IQWPPTNASLEEHSIEKNITVNYNGTLINETVFEFDWKS YIQD
SRYHYFLEGFLDALLCGNSSDAGQCPEGYMCVKAGRNP NYGYTSFDTFSWAFLSLFRLMTQDFW
15 ENLYQLTLRAAGKTYMIF FVLVIFLGSFYLINLILAVVAMAYEEQNQATLEEAEQKEAEFQQMI
EQLKKQQEAAQQAATATASEHSREPSAAGRLSDSSSEASKLSSKSAKERRNRKRKQKEQSGG
EEKDEDEFQKSESEDSIRRKGRFSIEGNRLTYEKRYSSPHQSLLSIRGSLFS PRRNSR TSLFS
FRGRAKDVGSENFADDEHSTFEDNESRRDSL FVPRRHGERRNSNLSQTSRSSRMLAVFPANGK
MHSTVDCNGVVSLVGGPSVPTSPVGQLLPEGTTTETEMRKRRSSSFHVSMDFLEDPSQRQRAMS
20 IASILTNTVEELEESRQKCPWCYKFSNIFLIWDCSPYWLKVKHVVNLVVMDFVDLAITICIV
LNTLFMAMEHYPM TDHFNNVLT VGNLVFTGIFTAEMFLKIIAMDPYYYFQEGWNI FDFG FIVTLS
LVELGLANVEGLSVLRSFRLLRVFKLAKSWPTLNMLIKI IGNSVGALGNLT LVLAIIVFI FAVV
GMQLFGKSYKDCVCKIASDCQLPRWHMNDFFHSFLIVFRVLCGEWIETMWD CMEVAGQAMCLTV
FMMVMVIGNLVVLN LFLALLLSSFSADNLAATDDD NEMNNLQIAVDRMHKGVAYVKRKIYEFIQ
25 QSFIRKQKILDEIKPLDDLNNKKDSCMSNHTAEIGKLDLDYLDVNGTTSGIGTGSSVEKYIIDE
SDYMSFINNP SLTVTVPIAVGESDFENLNTEDFSSES DLEESKEKLNESSSSSEGSTVDIGAPV
EEQPVVEPEETLEPEACFTEGCVQRFKCCQINVEEGRGKQWWNLRRTCFRIVEHNWFETFIVFM
ILLSSGALAFEDIYIDQRKTIKTMLEYADKVFTYIIFILEMLLKWVAYGYQTYFTNAWCWLD FLI
VDVSLVSLTANALGYSELGAIKSLRTLRLRPLRALS RFEGMRVVVNALLGAIP SIMNVLLVCL
30 IFWLI FSI MGVNLFAGKFYHCINTTTGDRFDIEDVNNHTDCLKLIERNETARWKNVKVNF DNVG
FGYLSLLQVATFKGWMDIMYAAVDSRNVELQPKYEE SLMYLYFVIFII FGSFFT LNLFIGVII
DNFNQQKKKFGGQDI FMTEEQKKYYNAMKKLGSKKPQKPIPRPGNK FQGMVDFVTRQVFDISI
MILICLNMVTMMVETDDQSEYVTTILSRINLVFIVLFTGECVLKLISLRHYFF TIGWNI FDFV V
VILSIVGMFLAELIEKYFVSPTLFRVIRLARIGRILRLIKGAKGIRTLLFALMMSLPALFNIGL

LLFLVMFIYAI FGMSNFAYVKREVGIDDMFNFETFGNSMICLFQITTSAGWDGLLAPILNSKPP
DCDPNKVNP GSSSVKGDCGNPSVGIFFFVSYIIISFLVVVNM YIAVILENFSVATEESAEP LSED
DFEMFYEVWEKFDPDATQFMEFEKLSQFAAALEPPLNLPQPNKLQLIAMDLPMVSGDRIHCLDI
LFAFTKRVLGESGEMDALRIQMEERFMASNPSKVS YQPI TTTTLKRKQEEVSAV I IQRAYRRHLL
5 KRTVKQASFTYNKNKIKGGANLLIKEDMIIDRINENSITEKTDLT MSTAACPPSYDRVTKPIVE
KHEQEGKDEKAKGK (SEQ ID NO: 864);

MEQTVLVPPGPDSFNFFTRESLAAIERRIAE EKAKNP KPD KDD DENGPKPNSDLEAGKNLPFI
YGDIPPEMVSEPLEDLDPYYINKKTFIVLNKGKAI FRFSATSALYILTPFNPLRKIAIKILVHS
LFSMLIMCTILTNCVFM TMSNPPDWTKNVEYTF TGIYTFESLIK I IARGFCLEDF TFLRDPWNW
10 LDFTVITFAYVTEFVDLGNVSALRTFRVLRALKTISVIPGLKTIVGALIQSVKKLS DVMILT VF
CLSVFALIGLQLFMGNLRNKC IQWPPTNASLEEHSIEKNITVNYNGTLINETVFEFDWKS YIQD
SRYHYFLEGFLDALLCGNSSDAGQCPEGYMCVKAGRNP NYGYTSFDTFSWAFLSL FRLMTQDFW
ENLYQLTLRAAGKTYMIFFVLVIFLGSFYLINLILAVVAMAYEEQNQATLEEAEQKEAEFQQMI
EQ LKKQQEAAQQAATATASEHSREPSAAGRLSDSSSEASKLSSKSAKERRNRKRKQKEQSGG
15 EEKDEDEFQKSESEDSIRRKGFRFSIEGNRLTYEKRYSSPHQSLLSIRGSLFSPRRNSRTSLFS
FRGRAKDVGSEND FADDEHSTFEDNESRRDSL FVPRRHGERRNSNLSQTSRSSRMLAVFPANGK
MHSTVDCNGVVSLGTTTETEMRKRRSSSFHVSMDFLEDPSQRQRAMS IASILTNTVEELEESRQ
KCPPCWYKFSNIFLIWDCSPYWLKVKHVVNLVVMDFVDLAITICIVLNTLFMAMEHYPMTDHF
NNVLTVGNLVFTGIFTAEMFLKIIAMD PYYYFQEGWNI FDFGFI VTL SLVELGLANVEGLSVLRS
20 FRLLRVFKLAKSWPTLNMLIKIIGNSVGALGNLT LVLAIIVFI FAVVGMQLFGKSYKDCVCKIA
SDCQLPRWHMNDFFHSFLIVFRVLCGEW IETMWDCMEVAGQAMCLTVFMMVMVIGNLVVLN LFL
ALLSSFSADNLAATDDD NEMNNLQIAVDRMHKG VAYVKRKIYEFIQQS FIRKQKILDEIKPLD
DLNNKKDSCMSNHTAEIGKDL DYLKDVNGTTSGIGTGSSVEKYIIDESDYMSFINNPSLTVTV P
IAVGESDFENLNTEDFSSES DLEESKEKLNESSSSSEGSTVDIGAPVEEQPVVEPEETLEPEAC
25 FTEGCVQRFKCCQINVEEGRGKQWWNLRRTCFRIVEHNWFETFIVFMILLSSGALAFEDIYIDQ
RKTIKTMLEYADKVFTYIIFILEMLLKWVAYGYQTYFTNAWCWLD FLIVDVSLVSLTANALGYSE
LGAIKSLRTLRLALRPLRALS RFEGMRVVVNALLGAIP SIMNVLLVCLIFWLI FSI MGVNLFAGK
FYHCINTTTGDRFDIEDVNNHTDCLKLIERNETARWKNVKVNF DNVGFGYLSLLQVATFKGWMD
IMYAAVDSRNVELQPKYEESLYMYLYFVIFII FGSFFTLNLFIGV I IDNFNQQKKKFGGQDI FM
30 TEEQKKYYNAMKKLGSKKPQKPIPRPGNK FQGMVDFDVTRQVFDISIMILICLNMVTMMVETDD
QSEYVTTILSRINLVFIVLFTGECVLKLISLRHYYFTIGWNI FDFV VVILSIVGMFLAELIEKY
FVSPTLFRVIRLARIGRILRLIKGAKGIRTLLFALMMSLPALFNIGLLLFLVMFIYAI FGMSNF
AYVKREVGIDDMFNFETFGNSMICLFQITTSAGWDGLLAPILNSKPPDCDPNKVNP GSSSVKGDC
GNPSVGIFFFVSYIIISFLVVVNM YIAVILENFSVATEESAEP LSEDDFEMFYEVWEKFDPDAT

QFMEFEKLSQFAAALEPPLNLPQPNKLQLIAMDLPMVSGDRIHCLDILFAFTKRVLGESGEMDA
LRIQMEERFMASNP SKVSYQPITTTTLKRKQEEVSAV I IQRAYRRHLLKRTVKQASFTYNKNKIK
GGANLLIKEDMIIDRINENS I TEKTDLTMTSTAACPPSYDRVTKPIVEKHEQEGKDEKAKGK
(SEQ ID NO: 865);

5 MEQTVLVPPGPD SFNFFTRESLAAIERRIAE EKAKNP KPD KDD DENGPKPNSDLEAGKNLPFI
YGDIPPEMVSEPLEDLDPYYINKKTFIVLNKGKAI FRFSATSALYILTPFNPLRKIAIKILVHS
LFSMLIMCTILTNCVFMTMSNPDPWTKNVEYTF TGIYTFESLIK I IARGFCLEDF TFLRDPWNW
LDFTVITFAYVTEFVDLGNVSALRTFRVLRALKTISVIPGLKTIVGALIQSVKKLS DVMILT VF
CLSVFALIGLQLFMGNLRNKC IQWPPTNASLEEHS IEKNITVNYNGT LINETVFEFDWKS YIQD
10 SRYHYFLEGFLDALLCGNSSDAGQCPEGYMCVKAGRNP NYGYTSFDTFSWAFLSLFRLMTQDFW
ENLYQLTLRAAGKTYMIFFVLVIFLGSFYLINLILAVVAMAYEEQNQATLEEAEQKEAEFQQMI
EQLKKQQEAAQAATATASEHSREPSAAGRLSDSSSEASKLSKSAKERRNRKRKRKQKEQSGGE
EKDEDEFQKSESEDSIRRKGF RFSIEGNRLTYEKRYSSPHQSLLSIRGSLFSPRRNSRTSLFSF
RGRAKDVGSENFADDEHSTFEDNESRRDSL FVPRRHGERRNSNLSQTSRSSRMLAVFPANGKM
15 HSTVDCNGVSVSLVGGPSVPTSPVGQLLEPGTTTETEMRKRSSSFHVSMDFLEDPSQRQRAMSI
ASILTNTVEELEESRQKCPPCWYKFSNIFLIWDCSPYWLKVHVNLVVMDFVDLAITICIVL
NTLFMAMEHYPMTHFN NVLTVGNLVFTGIFTAEMFLKIIAMD PYYYFQEGWNI FDGFIVTL SL
VELGLANVEGLSVLRSFRLLRVFKLAKSWPTLNMLIKI IGNSVGALGNLTLVLAIIVFIFAVVG
MQLFGKSYKDCVCKIASDCQLPRWHMNDFFHSFLIVFRVLCGEWIETMWD CMEVAGQAMCLTVF
20 MMVMVIGNLVVLNLFLALLLSSFSADNLAATDDDNEMNNLQIAVDRMHKGVAYVKRKIYEFIQQ
SFIRKQKILDEIKPLDDLNNK KDSCMSNHTAEIGKDL DYLDVNGTTS GIGTGSSVEKYI IDES
DYMSFINNP SLTVTVPIAVGESDFENLNTEDFSSES DLEESKEKLNESSSSSSEGSTVDIGAPVE
EQPVVEPEETLEPEACFTEGCVQRFKCCQINVEEGRGKQWWNLRRTCFRIVEHNWFETFIVFMI
LLSSGALAFEDIYIDQRKTIKTMLEYADKVFTYIIFILEMLLKWVAYGYQTYFTNAWCWLD FLIV
25 DVSLVSLTANALGYSELGAIKSLRTLRLRPLRALS RFEGMRVVVNALLGAIPSIMNVLLVCLI
FWLIFSIMGVNLFAGKFYHCINTTTGDRFDIEDVNNHTDCLKLIERNETARWKNVKVNF DNVG F
GYLSLLQVATFKGWMDIMYAAVDSRNVELQPKYEE SLYMYLYFVIFII FGSFFT LNLFIGVIID
NFNQKKKFGGQDIFMTEEQKKYYNAMKKLGSKKPQKPIPRPGNK FQGMVFDFVTRQVFDISIM
ILICLNMVTMMVETDDQSEYVTTILSRINLVFIVLFTGECVLKLISLRHYFTIGWNI FDFVVV
30 ILSIVGMFLAE LIEKYFVSPTLFRVIRLARIGRILRLIKGAKGIRTLLFALMMSLPALFNIGLL
LFLVMFIYAI FGMSNFAYVKREVGIDDMFNFETFGNSMICLFQITTSAGWDGL LAPILNSKPPD
CDPNKVNPGSSVKGDCGNPSVGIFFFVSYIIISFLVVNMYIAVILENFSVATEESAEP LSEDD
FEMFYEVWEKFDPDATQFMEFEKLSQFAAALEPPLNLPQPNKLQLIAMDLPMVSGDRIHCLDIL
FAFTKRVLGESGEMDALRIQMEERFMASNP SKVSYQPITTTTLKRKQEEVSAV I IQRAYRRHLLK

RTVKQASFTYNKNKIKGGANLLIKEDMIIDRINENSITEKTDLTMTAACPPSYDRVTKPIVEK
HEQEGKDEKAKGK (SEQ ID NO: 866);

MEQTVLVPPGPDSFNFFTRESLAAIERRIAEKAKNPKPKDKDDDENGPKNSDLEAGKNLPFI
YGDIPPEMVSEPLEDLDPYYINKKTFIVLNKGKAI FRFSATSALYILTPFNPLRKIAIKILVHS
5 LFSMLIMCTILTNCVFMTMSNPDPWTKNVEYTFGTGIYTFESLIKI IARGFCLEDFTLRDPWNW
LDFTVITFAYVTEFVDLGNVSALRTFRVLRALKTISVIPGLKTIVGALIQSVKKLSVDMILTVF
CLSVFALIGLQLFMGNLRNKC IQWPPTNASLEEHSIEKNITVNYNGTLINETVFEFDWKS YIQD
SRYHYFLEGFLDALLCGNSSDAGQCPEGYMCVKAGRNPNGYTSFDTFSWAFLSLFRLMTQDFW
ENLYQLTLRAAGKTYMIFFVLVIFLGSFYLINLILAVVAMAYEEQNQATLEEAEQKEAEFQQMI
10 EQLKKQQEAAQAATATASEHSREPSAAGRLSDSSSEASKLSSKSAKERRNRKRKQKEQSGGE
EKDEDEFQKSESEDSIRKGFRRFSIEGNRLTYEKRYSSPHQSLLSIRGSLFSPRRNSRTSLFSF
RGRAKDVGSENFADDEHSTFEDNESRRDSL FVPRRHGERNSNLSQTSRSSRMLAVFPANGKM
HSTVDCNGVVSLGTTTETEMRKRRSSSFHVSMDFLEDPSQRQRAMSIASILTNTVEELEESRQK
CPPCWYKFSNIFLIWDCSPYWLKVHVNLVMDPFVDLAITICIVLNTLFMAMEHYPMTDHFN
15 NVLTVGNLVFTGIFTAEMFLKIIAMDPYYYFQEGWNI FDGFI VTL SLVELGLANVEGLSVLRSF
RLLRVFKLAKSWPTLNMLIKI IGNSVGALGNLTLVLAIIVFI FAVVGMQLFGKSYKDCVCKIAS
DCQLPRWHMNDFFHSFLIVFRVLCGEWIETMWDCMEVAGQAMCLTVFMMVMVIGNLVVLNLFLA
LLLSSFSADNLAATDDDNE MNNLQIAVDRMHKGVAYVKRKIYEFIQQS FIRKQKILDEIKPLDD
LNNKKDSCMSNHTAEIGKDL DYLKDVNGTTS GIGTGS SVEKYIIDESDYMSFINNPSLTVTP I
20 AVGESDFENLNTEDFSSES DLEESKEKLNESSSSSEGSTVDIGAPVEEQPVVEPEETLEPEACF
TEGCVQRFKCCQINVEEGRGKQWWNLRRTCFRIVEHNWFETFIVFMILLSSGALAFEDIYIDQR
KTIKTMLEYADKVFTYIFILEMLLKWVAYGYQTYFTNAWCWLD FLIVDVSLSL TANALGYSEL
GAIKSLRTLRLRPLRALS RFEGMRVVVNALLGAIPSIMNVLLVCLIFWLIFSIMGVNLFAGKF
YHCINTTTGDRFDIEDVNNHTDCLKLIERNETARWKNVKVNF DNVGFGYLSLLQVATFKGWMDI
25 MYAAVDSRNVELQPKYEE SLMYLYFVIFII FGSSFTLNLFIGVIIDNFNQQKKKFGGQDIFMT
EEQKKYYNAMKKLGSKPKPKPIPRPGNK FQGMVFD FVTRQVFDISIMILICLNMTMMVETDDQ
SEYVTTILSRINLVFIVLFTGECVLKLISLRHYFTIGWNI FDFV VVILSIVGMFLAE LIEKYF
VSPTLFRVIRLARIGRILRLIKGAKGIRTL LFALMMSLPALFNIGLLLFLVMFIY AIFGMSNFA
YVKREVGIDDMFNFETFGNSMICLFQITTSAGWDG LLAPILNSKPPDCDPNKVNP GSSSVKGDCG
30 NPSVGIFFFVS YIIISFLVVVNM YIAVILENFSVATEESA EPLSEDDFEMFYEVWEKFD PDATQ
FMEFEKLSQFAAALEPPLNLPQPNKLQLIAMDLPMVSGDRIHCLDILFAFTKRVLGESGEMDAL
RIQMEERFMASNPSKVS YQPI TTTLKRKQEEVSAV I IQRAYRRHLLKRTVKQASFTYNKNKIKG
GANLLIKEDMIIDRINENSITEKTDLTMTAACPPSYDRVTKPIVEKHEQEGKDEKAKGK (SEQ
ID NO: 867); or

MFLKIIAMDPYYFQEGWNI FDGFIVTSLVELGLANVEGLSVLRSFRLLRVFKLAKSWPTLNM
 LIKIIIGNSVGALGNLTLLVLAII VFI FAVVGMQLFGKSYKDCVCKIASDCQLPRWHMNDFFHSFL
 IVFRVLCGEWIE TMWDCMEVAGQAMCLTVFMMVMVIGNLVVLNLF LALLLSFSADNLAATDDD
 NEMNNLQIAVDRMHKGVAYVKRKIYE FIIQQSFIRKQKILDEIKPLDDLNNKKDSCMSNHTAEIG
 5 KDL DY LKDVNGTTSGIGTGSSVEKYIIDESDYSFINNPSLTVTVPIAVGESDFENLNTEDFSS
 ESDLEESKEKLNESSSSSEGSTVDIGAPVEEQPVVEPEETLEPEACFTEGCVQRFKCCQINVEE
 GRGKQWWNLRRCTCFRIVEHNWFETFIVFMILLSSGALAFEDIYIDQRKTIKTMLEYADKVFTYI
 FILEMLLKWVAYGYQTYFTNAWCWLD FLIVDVSLVSLTANALGYSELGAIKSLRTLRLRPLRA
 LSRFEGMRVVVNALLGAIP SIMNVLLVCLIFWLI FSIMGVNLFAGKFYHCINTTTGDRFDIEDV
 10 NNHTDCLKLIERNETARWKNVKVNF DNVGFGYLSLLQVATFKGWMDIMYAAVDSRNVELQPKYE
 ESLMYLYFVIFII FGSFFTLNLF IGVIIDNFNQKKKFGGQDI FMTEEQKKYYNAMKKLGSKK
 PQKPIPRPGNKFQGMVDFDVTRQVFDISIMILICLNMVTMMVETDDQSEYVTTILSRINLVFIV
 LFTGECVLKLISLRHYFTIGWNI FDFVVVILSIVGMFLAELIEKYFVSPTLFRVIRLARIGRI
 LRLIKGAKGIRTLLFALMMSLPALFNIGLLLFLVMFIYAI FGMSNFAYVKREVGIDDMFNFETF
 15 GNSMICLFQITTSAGWDGLLAPILNSKPPDCDPNKVNP GSSVKGDCGNPSVGIFFFVSYIIISF
 LVVVNMYIAVILENFSVATEESAEP LSEDDFEMFYEVWEKFDPDATQFMFEKLSQFAAALEPP
 LNLPPQPNKLQLIAMDLPMVSGDRIHCLDILFAFTKRVLGESGEMDALRIQMEERFMASNP SKVS
 YQPITTTLKRKQEEVSAV I IQRAYRRHLLKRTVKQASFTYNKNKIKGGANLLIKEDMIIDRINE
 NSITEKTDLTMTAACPPSYDRVTKPIVEKHEQEGKDEKAKGK (SEQ ID NO: 868).

- 20 **[00146]** The term “effective amount” as used herein refers to the amount of an active agent (*e.g.*, tRNA or expression vector according to the present invention or a secondary active agent in a combination therapy) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route.
- 25 **[00147]** As used herein, “treat”, “treating” and “treatment” mean the treatment of a disease in a subject, *e.g.*, in a human. This includes: (a) inhibiting the disease, *i.e.*, arresting its development; and (b) relieving the disease, *i.e.*, causing regression of the disease state. As used herein, the terms “subject” and “patient” refer to an organism to be treated by the methods and compositions described herein. Such organisms preferably include, but are not limited to,
- 30 mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably includes humans.

[00148] The methods and compositions described herein can be used alone or in combination with other therapeutic agents and/or modalities. The term administered “in combination,” as used herein, is understood to mean that two (or more) different treatments are delivered to the

subject during the course of the subject's affliction with the disorder, such that the effects of the treatments on the patient overlap at a point in time. In certain embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery." In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In certain embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, *e.g.*, an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In certain embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

[00149] In certain embodiments, a method or composition described herein is administered in combination with one or more additional therapeutic agents, *e.g.*, DIACOMIT[®] (stiripentol), EPIODOLEX[®] (cannabidiol), a ketogenic diet, ONFI[®] (clobazam), TOPAMAX[®] (topiramate), fenfluramine, or valproic acid. For example, during the treatment of Dravet Syndrome, a method or composition described herein is administered in combination with one or more additional therapeutic agents, *e.g.*, DIACOMIT[®] (stiripentol), EPIODOLEX[®] (cannabidiol), a ketogenic diet, ONFI[®] (clobazam), TOPAMAX[®] (topiramate), fenfluramine, or valproic acid.

[00150] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

[00151] In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it should be understood that the element or component can be any one of the recited elements or components, or the element or component can be selected from a group consisting of two or more of the recited elements or components.

[00152] Further, it should be understood that elements and/or features of a composition or a method described herein can be combined in a variety of ways without departing from the spirit and scope of the present invention, whether explicit or implicit herein. For example, where reference is made to a particular compound, that compound can be used in various embodiments of compositions of the present invention and/or in methods of the present invention, unless otherwise understood from the context. In other words, within this application, embodiments have been described and depicted in a way that enables a clear and concise application to be written and drawn, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the present teachings and invention(s). For example, it will be appreciated that all features described and depicted herein can be applicable to all aspects of the invention(s) described and depicted herein.

[00153] It should be understood that the expression “at least one of” includes individually each of the recited objects after the expression and the various combinations of two or more of the recited objects unless otherwise understood from the context and use. The expression “and/or” in connection with three or more recited objects should be understood to have the same meaning unless otherwise understood from the context.

[00154] The use of the term “include,” “includes,” “including,” “have,” “has,” “having,” “contain,” “contains,” or “containing,” including grammatical equivalents thereof, should be understood generally as open-ended and non-limiting, for example, not excluding additional unrecited elements or steps, unless otherwise specifically stated or understood from the context.

[00155] Where the use of the term “about” is before a quantitative value, the present invention also includes the specific quantitative value itself, unless specifically stated otherwise. As used herein, the term “about” refers to a $\pm 10\%$ variation from the nominal value unless otherwise indicated or inferred.

[00156] It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present invention remain operable. Moreover, two or more steps or actions may be conducted simultaneously.

[00157] The use of any and all examples, or exemplary language herein, for example, “such as” or “including,” is intended merely to illustrate better the present invention and does not pose a limitation on the scope of the invention unless claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the present invention.

EXAMPLES

[00158] The following Examples are merely illustrative and are not intended to limit the scope or content of the invention in any way.

Example 1

5 [00159] This Example describes an analysis of nonsense mutation frequency in patient populations.

[00160] **FIGURE 3** is a plot depicting the relative share of each nonsense mutation based on global submissions to ClinVar that have been annotated as “pathogenic,” “likely pathogenic,” and “pathogenic / likely pathogenic” (dark columns). A cumulative density plot (light gray
10 region) illustrates the fraction of the total patient population with disorders caused by nonsense mutations who could potentially be treated using combinations of suppressor tRNAs that target each nonsense mutation, starting with the most prevalent and progressing to the least prevalent.

[00161] **FIGURE 4** is plot depicting the relative share of each potential nonsense mutation from SCN1A patient data found on ClinVar and the Guangzhou SCN1A mutation database. All
15 ClinVar nonsense mutations annotated as “pathogenic,” “likely pathogenic,” or “pathogenic / likely pathogenic” are included. All Guangzhou database nonsense mutations tagged as “severe myoclonic epilepsy in infancy” are included.

[00162] **FIGURE 5** is a plot depicting the breakdown of nonsense mutations tagged in human Duchenne muscular dystrophy (DMD) cases from the Leiden LOVD mutation database.

20 [00163] Together, the data provide a rationale for selecting combinations of two or three suppressor tRNAs that can be encoded on a single expression vector in order to maximize coverage of the total patient population with disorders caused by nonsense mutations.

Example 2

[00164] This Example describes the generation of an expression vector encoding three
25 suppressor tRNAs that facilitate read-through of three different premature termination codons (PTC). A schematic representation of such an expression vector is shown in **FIGURE 6**.

[00165] Readthrough activity of suppressor tRNAs was measured using constructs containing EGFP reporters with PTCs (TAG, TAA, or TAG) in place of various amino acid codons that are required for fluorescence. **FIGURE 7** depicts an exemplary EGFP reporter with
30 a PTC (TGA) in place of an Arginine codon (CGA) and an accompanying suppressor tRNA. This approach can be generalized to create EGFP reporter constructs for other classes of

suppressor tRNAs by converting an appropriate amino acid codon within the EGFP open reading frame to a termination codon.

[00166] The activity of a single expression vector (designated the "Tristop" suppressor) encoding an Arginine to TGA (R>TGA) suppressor (including SEQ ID NO: 18), a Glutamine to TAA (Q>TAA) suppressor (including SEQ ID NO: 39), and a Glutamine to TAG (Q>TAG) suppressor (including SEQ ID NO: 178) was assessed in HEK293 cells transiently co-transfected with (i) a plasmid encoding the Tristop suppressor and (ii) a plasmid encoding either an EGFP reporter with a PTC (TGA) in place of an Arginine codon (CGA), an EGFP reporter with a PTC (TAA) in place of an Glutamine codon (CAG), or an EGFP reporter with a PTC (TAG) in place of an Glutamine codon (CAG). The readthrough activity of the Tristop suppressor was compared to the activity of separate expression vectors encoding the three individual suppressors included in the Tristop suppressor: an Arginine to TGA (R>TGA) suppressor only vector, a Glutamine to TAA (Q>TAA) suppressor only vector, and a Glutamine to TAG (Q>TAG) suppressor only vector. Transfections were done using the Lipofectamine 3000 Transfection Reagent according to the manufacturer's protocol. Co-transfections were done using equal amounts of the suppressor tRNA plasmid and the EGFP reporter plasmid. Results are shown in **FIGURE 8** (fluorescent images of EGFP reporter expression) and **FIGURE 9** (in which EGFP expression was analyzed by flow cytometry and readthrough activity is presented as the percentage of viable cells that express EGFP above background). As depicted, in each instance, the Tristop expression construct facilitated readthrough of the PTC.

[00167] The effect of the Tristop suppressor on cell viability was compared to the effect of separate expression vectors comprising only an Arginine to TGA suppressor ("R→TGA"), only a Glutamine to TAA suppressor ("Q→TAA"), and only a Glutamine to TAG suppressor ("Q→TAG"). HEK293 cells were transiently transfected using the Lipofectamine 3000 Transfection Reagent according to the manufacturer's protocol and cell viability was assessed at 24 hours post-transfection using a Pacific Blue Annexin V/SYTOX AADvanced Apoptosis Kit (Thermofisher). This kit detects the externalization of phosphatidylserine in apoptotic cells using annexin V conjugated to violet-fluorescent Pacific Blue dye. Dead cells are detected using SYTOX AADvanced stain. After staining, apoptotic cells show violet fluorescence, dead cells show red fluorescence, and live cells show little or no fluorescence. Staining was performed according to the manufacturer's protocol and cells were assessed by flow cytometry. Results are shown in **FIGURE 10**.

5 [00168] Together, the results demonstrate that Tristop suppressor tRNAs produce readthrough of nonsense mutations that is equivalent to expression vectors that comprise only single suppressor tRNAs. Additionally, the results show that treatment with Tristop suppressor tRNAs is not accompanied by a decrease in cell viability relative to individual suppressor tRNAs or control vectors.

INCORPORATION BY REFERENCE

[00169] The entire disclosure of each of the patent and scientific documents referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

10 [00170] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the
15 claims are intended to be embraced therein.

WHAT IS CLAIMED IS:

1. An expression vector comprising:
 - (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid;
 - (b) a second nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid; and
 - (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid.
2. The expression vector of claim 1, wherein the first amino acid is selected from arginine, tryptophan, cysteine, serine, glycine, and leucine.
3. The expression vector of claim 2, wherein the first amino acid is selected from arginine and tryptophan.
4. The expression vector of claim 3, wherein the first amino acid is arginine.
5. The expression vector of any one of claims 1-4, wherein the second amino acid is selected from glutamine, glutamic acid, tyrosine, tryptophan, lysine, serine, and leucine.
6. The expression vector of claim 5, wherein the second amino acid is selected from glutamine, glutamic acid, tyrosine, and tryptophan.
7. The expression vector of claim 6, wherein the second amino acid is glutamine.
8. The expression vector of any one of claims 1-7, wherein the third amino acid is selected from glutamine, glutamic acid, tyrosine, lysine, serine, and leucine.
9. The expression vector of claim 8, wherein the third amino acid is selected from glutamine, glutamic acid, and tyrosine.
10. The expression vector of any one of claims 1-9, wherein the first amino acid is arginine and the second amino acid is glutamine.
11. The expression vector of any one of claims 1-10, wherein the second and third amino acid are the same.
12. The expression vector of claim 11, wherein the second and third amino acid are selected

from glutamine, glutamic acid, tyrosine, lysine, serine, and leucine.

13. The expression vector of any one of claims 1-10, wherein:
 - (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is lysine;
 - (ii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamic acid;
 - (iii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is tyrosine;
 - (iv) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is leucine;
 - (v) the first amino acid is arginine, the second amino acid is tryptophan, and the third amino acid is glutamic acid; or
 - (vi) the first amino acid is arginine, the second amino acid is tyrosine, and the third amino acid is glutamic acid.
14. The expression vector of any one of claims 1-10, wherein:
 - (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamine;
 - (ii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamic acid;
 - (iii) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is lysine;
 - (iv) the first amino acid is arginine, the second amino acid is tryptophan, and the third amino acid is glutamine; or
 - (v) the first amino acid is arginine, the second amino acid is glutamic acid, and the third amino acid is glutamine.
15. The expression vector of any one of claims 1-12, wherein:
 - (i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamine;
 - (ii) the first amino acid is tryptophan, the second amino acid is glutamic acid, and the third amino acid is glutamic acid;

(iii) the first amino acid is cysteine, the second amino acid is tyrosine, and the third amino acid is tyrosine;

(iv) the first amino acid is serine, the second amino acid is lysine, and the third amino acid is lysine;

(v) the first amino acid is glycine, the second amino acid is serine, and the third amino acid is serine; or

(vi) the first amino acid is leucine, the second amino acid is leucine, and the third amino acid is leucine.

16. The expression vector of any one of claims 1-15, wherein the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**.
17. The expression vector of any one of claims 1-16, wherein the first amino acid is arginine and the first suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-22, and 35.
18. The expression vector of any one of claims 1-17, wherein the second amino acid is glutamine and the second suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187.
19. The expression vector of any one of claims 1-18, wherein the third amino acid is glutamine and the third suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45.
20. The expression vector of any one of claims 1-19, wherein the expression vector comprises 1, 2, 3, 4, or more than 4 copy numbers of the nucleotide sequence encoding the first, second, and/or third suppressor tRNA.
21. The expression vector of any one of claims 1-20, wherein the expression vector further comprises a nucleotide sequence set forth in **TABLE 4**.
22. The expression vector of claim 21, wherein the expression vector comprises a nucleotide sequence selected from SEQ ID NOs: 869-888.
23. The expression vector of any one of claims 1-22, wherein the first nucleotide sequence, the second nucleotide sequence, and the third nucleotide sequence are each operably linked to a nucleotide sequence set forth in **TABLE 4**.
24. The expression vector of any one of claims 1-23, wherein the first nucleotide sequence, the second nucleotide sequence, and the third nucleotide sequence are each operably linked to

a nucleotide sequence selected from SEQ ID NOs: 869-888.

25. The expression vector of any one of claims 1-24, wherein the expression vector is a viral vector.
26. The expression vector of claim 25, wherein the viral vector is a DNA virus vector.
27. The expression vector of claim 25 or 26, wherein the viral vector is an adeno-associated virus (AAV) vector.
28. A pharmaceutical composition comprising the expression vector of any one of claims 1-27 and a pharmaceutically acceptable excipient.
29. A pharmaceutical comprising:
 - (a) a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid;
 - (b) a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid; and
 - (c) a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid.
30. The pharmaceutical composition of claim 29, wherein the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**.
31. The pharmaceutical composition of claim 29 or 30, wherein the first amino acid is arginine and the first suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-22, and 35.
32. The pharmaceutical composition of any one of claims 29-31, wherein the second amino acid is glutamine and the second suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187.
33. The pharmaceutical composition of any one of claims 29-32, wherein the third amino acid is glutamine and the third suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45.
34. The pharmaceutical composition of any one of claims 29-33, wherein the first, second, and/or third suppressor tRNA comprises a naturally occurring nucleotide modification.
35. The pharmaceutical composition of any one of claims 29-34, wherein the first, second,

and/or third suppressor tRNA comprises one or more nucleotide modifications selected from 5-methyl uridine, 5-carbamoylmethyluridine, 5-carbamoyl-methyl-2-O-methyluridine, 5-methoxy-carbonylmethyluridine, 5-methoxycarbonylmethyl-2-thiouridine, pseudouridine, dihydrouridine, 1-methyladenosine, and inosine.

36. The pharmaceutical composition of any one of claims 28-35, wherein the expression vector or tRNA is not conjugated to or associated with another moiety.
37. The pharmaceutical composition of claim 36, wherein the expression vector or tRNA is not conjugated to or associated with a carrier particle.
38. The pharmaceutical composition of claim 37, wherein the carrier particle is an aminolipid particle.
39. The pharmaceutical composition of any one of claims 28-38, wherein the composition does not comprise a nanoparticle.
40. The pharmaceutical composition of any one of claims 28-39, wherein the composition does not comprise an aminolipid delivery compound.
41. A method of expressing in a mammalian cell a functional gene product encoded by a gene containing a premature termination codon, the method comprising contacting the cell with effective amount of the expression vector of any one of claims 1-27, or the pharmaceutical composition of any one of claims 28-40, thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon.
42. A method of expressing in a mammalian cell a functional gene product encoded by a gene containing a first, second, and/or third premature termination codon, the method comprising contacting the cell with effective amount of:
 - (a) a first expression vector comprising a nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid;
 - (b) a second expression vector comprising a nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid; and
 - (c) a third expression vector comprising a nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is

capable of being aminoacylated with a third amino acid;

thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon.

43. A method of expressing in a mammalian cell a functional gene product encoded by a gene containing a first, second, and/or third premature termination codon, the method comprising contacting the cell with effective amount of:

(a) a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid;

(b) a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid;
and

(c) a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid;

thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon.

44. The method of claim 42 or 43, wherein the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**.
45. The method of any one of claims 42-44, wherein the first amino acid is arginine and the first suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-22, and 35.
46. The method of any one of claims 42-45, wherein the second amino acid is glutamine and the second suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187.
47. The method of any one of claims 42-46, wherein the third amino acid is glutamine and the third suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45.
48. The method of any one of claims 41-47, wherein the gene is a gene set forth in **TABLE 5** or **TABLE 6**.
49. The method of claim 48, wherein the gene is a gene set forth in **TABLE 5**.

50. The method of any one of claims 41-49, wherein the gene is SCN1A or dystrophin.
51. The method of any one of claims 41-50, wherein the cell is a human cell.
52. The method of any one of claims 41-51, wherein the tRNA becomes aminoacylated in the cell.
53. A method of treating a premature termination codon-mediated disorder in a subject in need thereof wherein the subject has a gene with a premature termination codon, the method comprising administering to the subject an effective amount of the expression vector of any one of claims 1-27, or the pharmaceutical composition of any one of claims 28-40, thereby to treat the disorder in the subject.
54. A method of treating a premature termination codon-mediated disorder in a subject in need thereof wherein the subject has a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject an effective amount of:
- (a) a first expression vector comprising a nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid;
 - (b) a second expression vector comprising a nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid; and
 - (c) a third expression vector comprising a nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid;
- thereby to treat the disorder in the subject.
55. A method of treating a premature termination codon-mediated disorder in a subject in need thereof wherein the subject has a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject an effective amount of:
- (a) a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid;
 - (b) a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid;

and

(c) a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid; thereby to treat the disorder in the subject.

56. The method of claim 54 or 55, wherein the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in **TABLE 2** or **TABLE 3**.
57. The method of any one of claims 54-56, wherein the first amino acid is arginine and the first suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 6-9, 11, 16-22, and 35.
58. The method of any one of claims 54-57, wherein the second amino acid is glutamine and the second suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 178-182, 186, and 187.
59. The method of any one of claims 54-58, wherein the third amino acid is glutamine and the third suppressor tRNA comprises a nucleotide sequence selected from SEQ ID NOs: 36-40, 44, and 45.
60. The method of any one of claims 53-59, wherein the disorder is a disorder set forth in **TABLE 5** or **TABLE 6**.
61. The method of claim 60, wherein the disorder is a disorder set forth in **TABLE 5**.
62. The method of any one of claims 53-61, wherein the disorder is Dravet Syndrome or Duchenne Muscular Dystrophy.
63. The method of any one of claims 53-62, wherein the subject is a human.

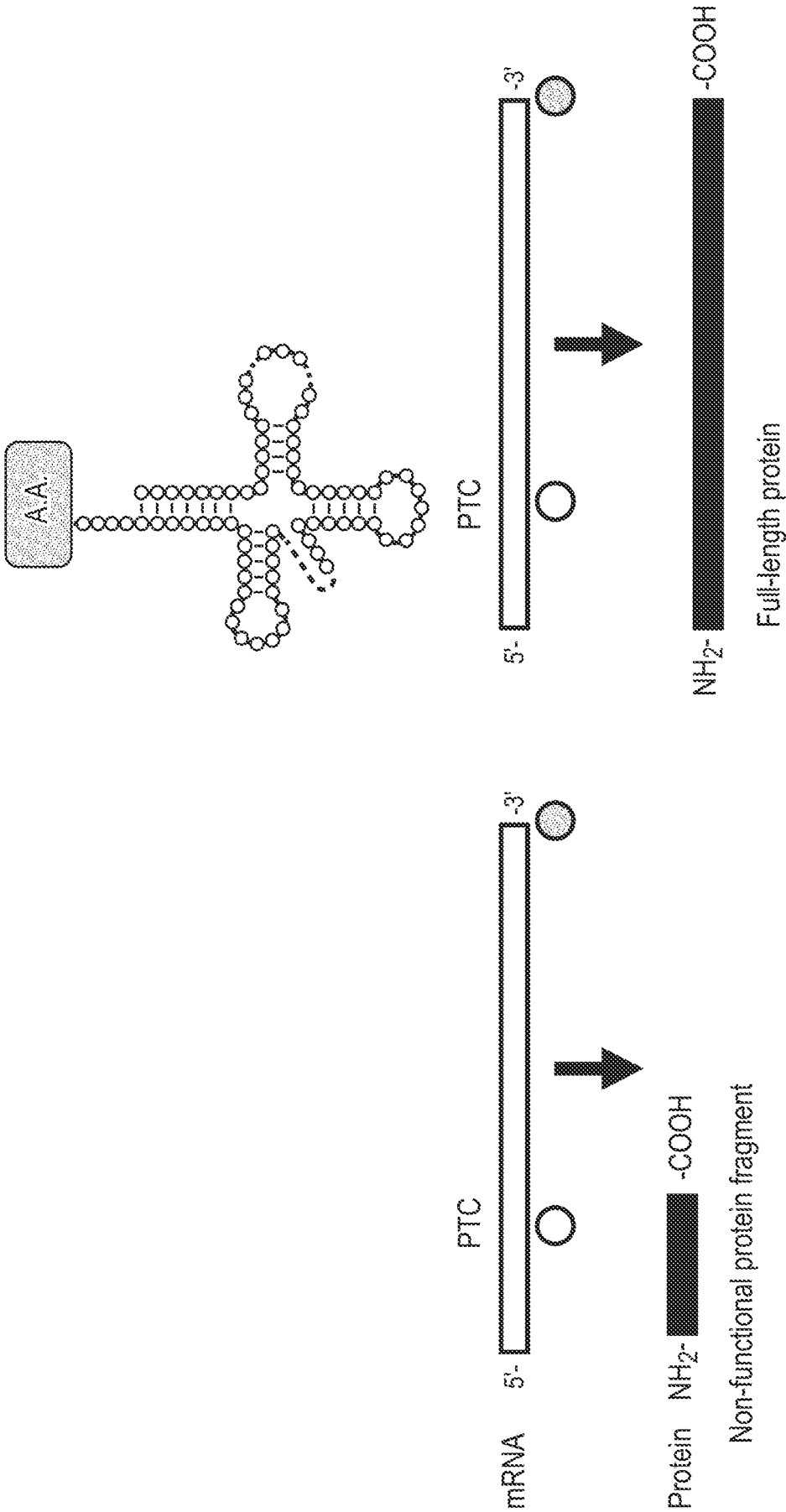


FIG. 1

2/11

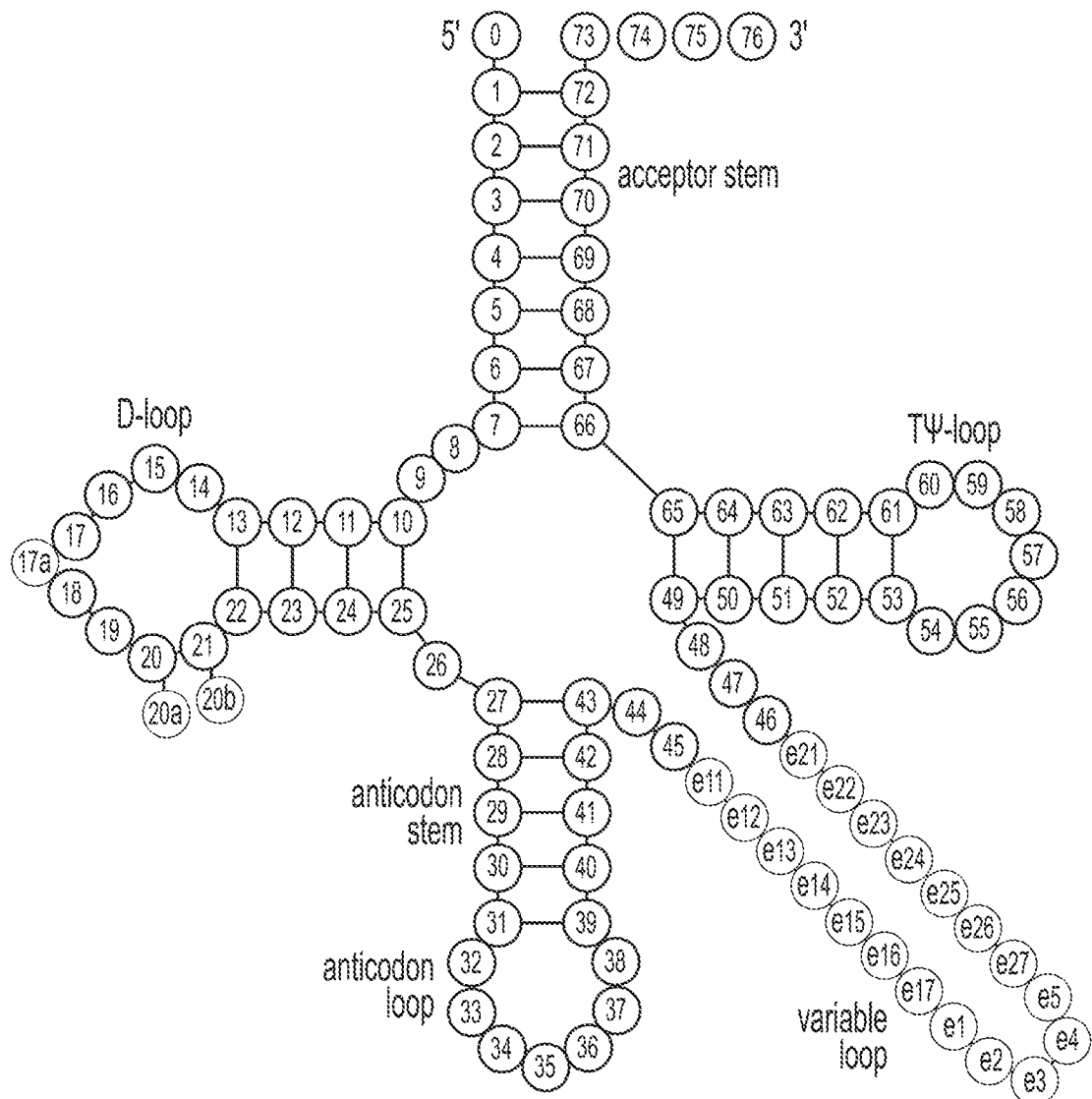


FIG. 2A

3/11

position	residues	ratios	position	residues	ratios
0	Gm/G	1/4	35	ψ/U	7/50
1	ψ/U	3/14	36	ψ/xU/U	2/3/50
4	Am/A	2/28	37	m ¹ I/ms ² t ⁵ A/i ⁶ A/ m ⁶ t ⁶ A/t ⁶ A/xA/A	6/2/22/ 2/48/4/45
4	Um/U	5/45	37	m ¹ G/o ² yW/xG/ yW/G	33/6/2/ 7/1
4	Cm/C	4/51	38	ψ/U	17/1
4	Gm/G	2/41	38	m ⁵ C/xC/C	10/1/26
6	m ² G/G	20/36	39	m ¹ ψ/ψm/ Um/ψ/U	2/6/ 1/79/2
7	m ² G/G	1/69	39	Gm/G	4/32
9	m ¹ A/A	1/69	40	ψ/U	6/1
9	m ¹ G/xG/G	56/1/43	40	m ⁵ C/C	3/139
10	m ² G/G	103/70	44	Um/xU/U	20/2/18
12	ac ⁴ C/C	32/21	e11	ψ/U	2/4
13	ψ/U	47/8	e12	ψ/U	6/11
13	Cm/C	1/83	e14	ψ/U	2/6
14	m ¹ A/xA/A	9/1/165	e2	m ³ C/C	7/7
16	D/U	123/25	46	m ⁷ G/G	86/39
17	D/U	39/0	47	D/xU/U	83/1/16
18	Gm/G	40/138	48	D/U	1/25
20	acp ³ U/D/U	4/118/11	48	m ⁵ C/xC/C	95/1/46
20a	acp ³ U/D/U/ψ/xU/U	6/64/2/2/2	49	xA/A	1/19
20b	D/ψ/U	8/6/2	49	m ⁵ C/xC/C	64/1/13
25	ψ/U	1/28	50	ψ/U	4/45
26	ψ/U	1/24	50	m ⁵ C/C	15/71
26	m ²² G/m ² G/xG/G	90/16/2/8	54	m ⁵ Um/m ⁵ U/ψ/U	16/111/11/17
27	ψ/U	70/10	55	ψ/U	166/12
27	m ²² G/G	3/14	58	m ¹ A/xA/A	151/1/26
28	ψ/U	37/38	64	Ar(p)/A	2/43
30	ψ/U	1/4	64	Gr(p)/xG/G	1/1/87
31	ψ/U	4/2	65	ψ/U	1/32
32	ψm/Um/ψ/U	2/6/24/8	67	ψ/U	2/63
32	Cm/m ³ C/xC/C	40/16/2/80	67	m ² G/G	2/64
34	I/A	32/0	68	ψ/U	1/37
34	s ² U/Um/mchm ⁵ U/ ncm ⁵ U/cnm ⁵ Um/ mcm ⁵ s ² U/mcm ⁵ U/ ψ/xU/U	1/3/1/ 3/1/ 7/4/ 1/11/5	72	ψ/U	1/16
34	Cm/ψ ⁵ Cm/m ⁵ C/xC/C	8/1/1/2/44	72	m ⁵ C/C	5/132
34	QtrRNA/manQtrRNA/ galQtrRNA/Gm/G	5/4/ 3/17/20			

FIG. 2B

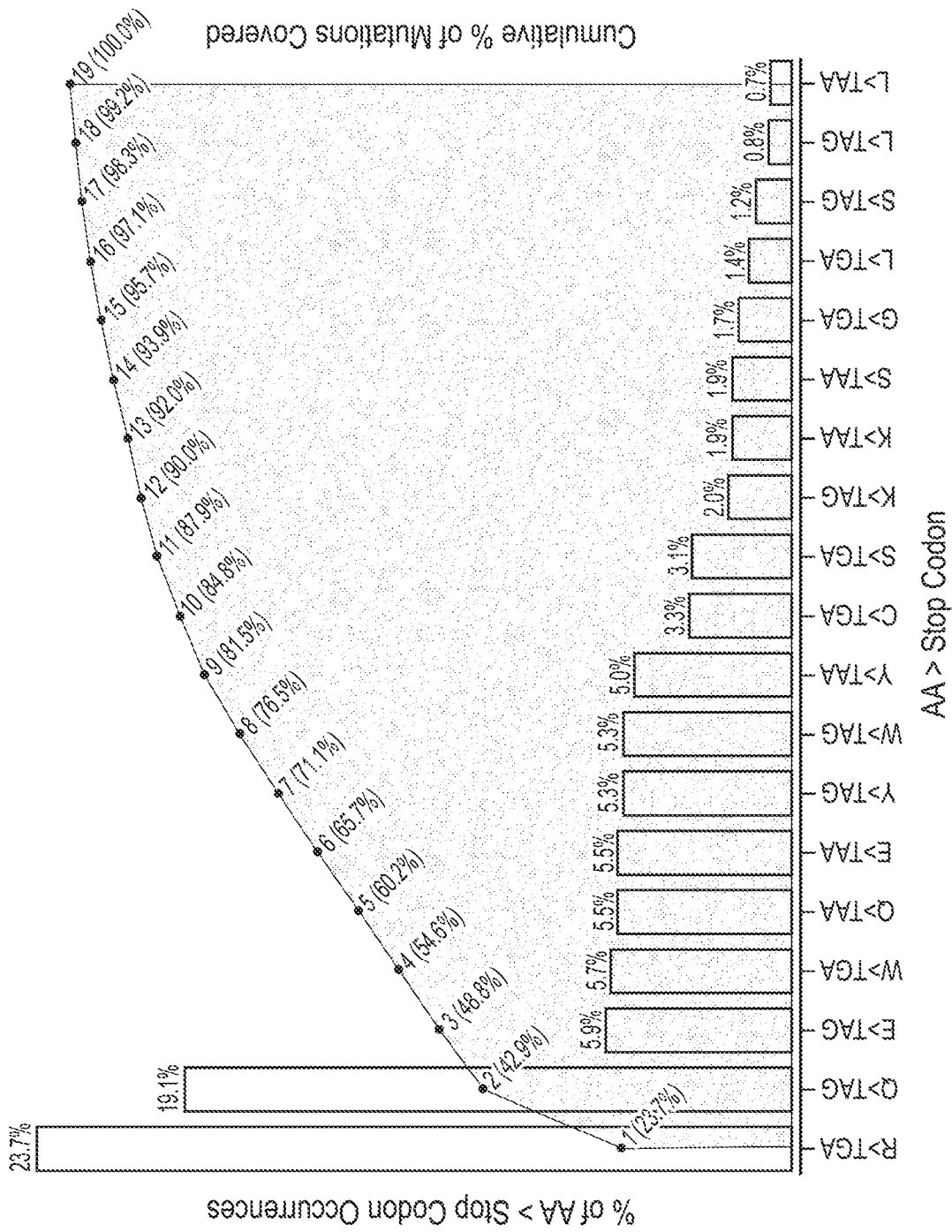


FIG. 3

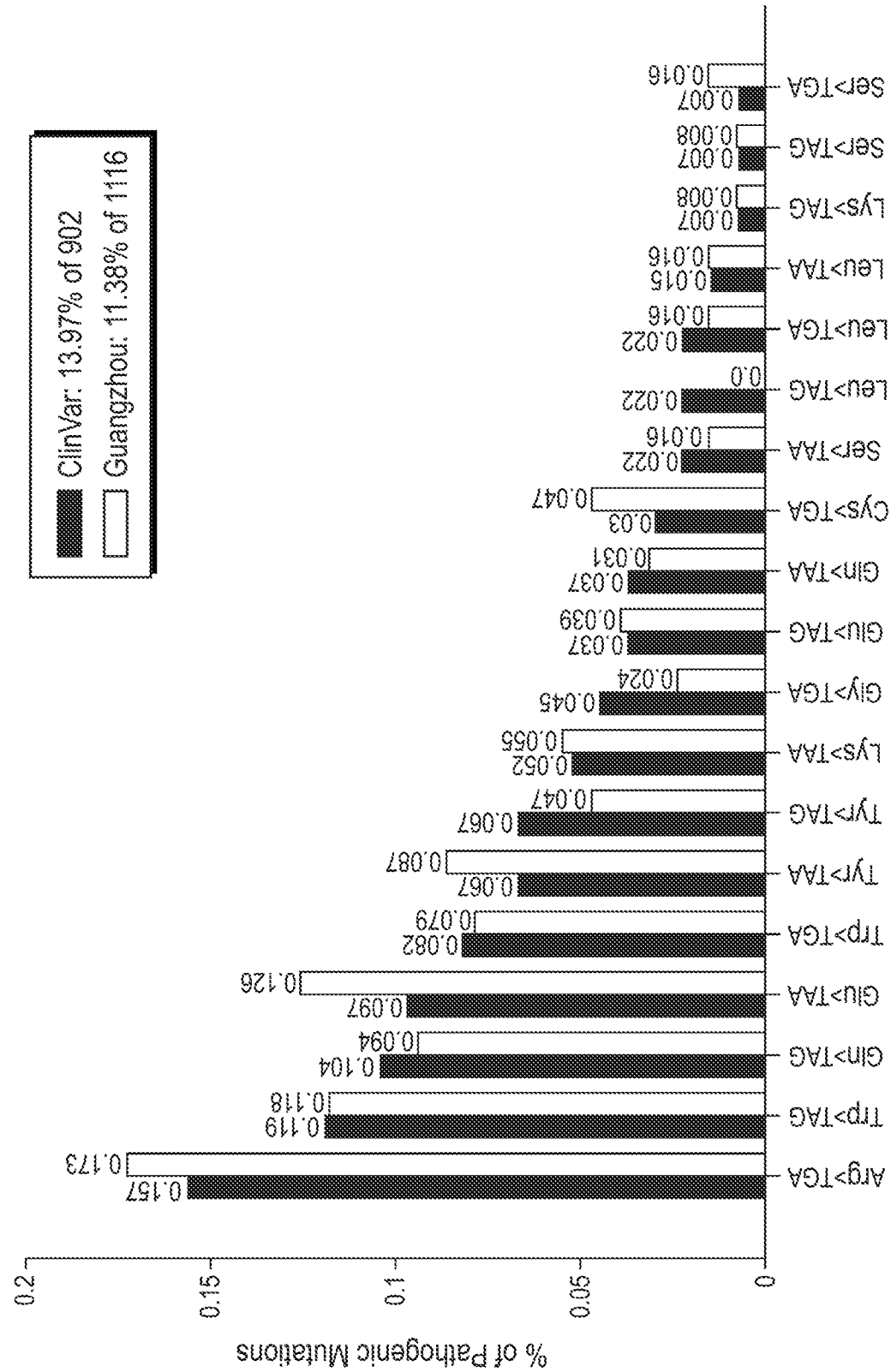


FIG. 4

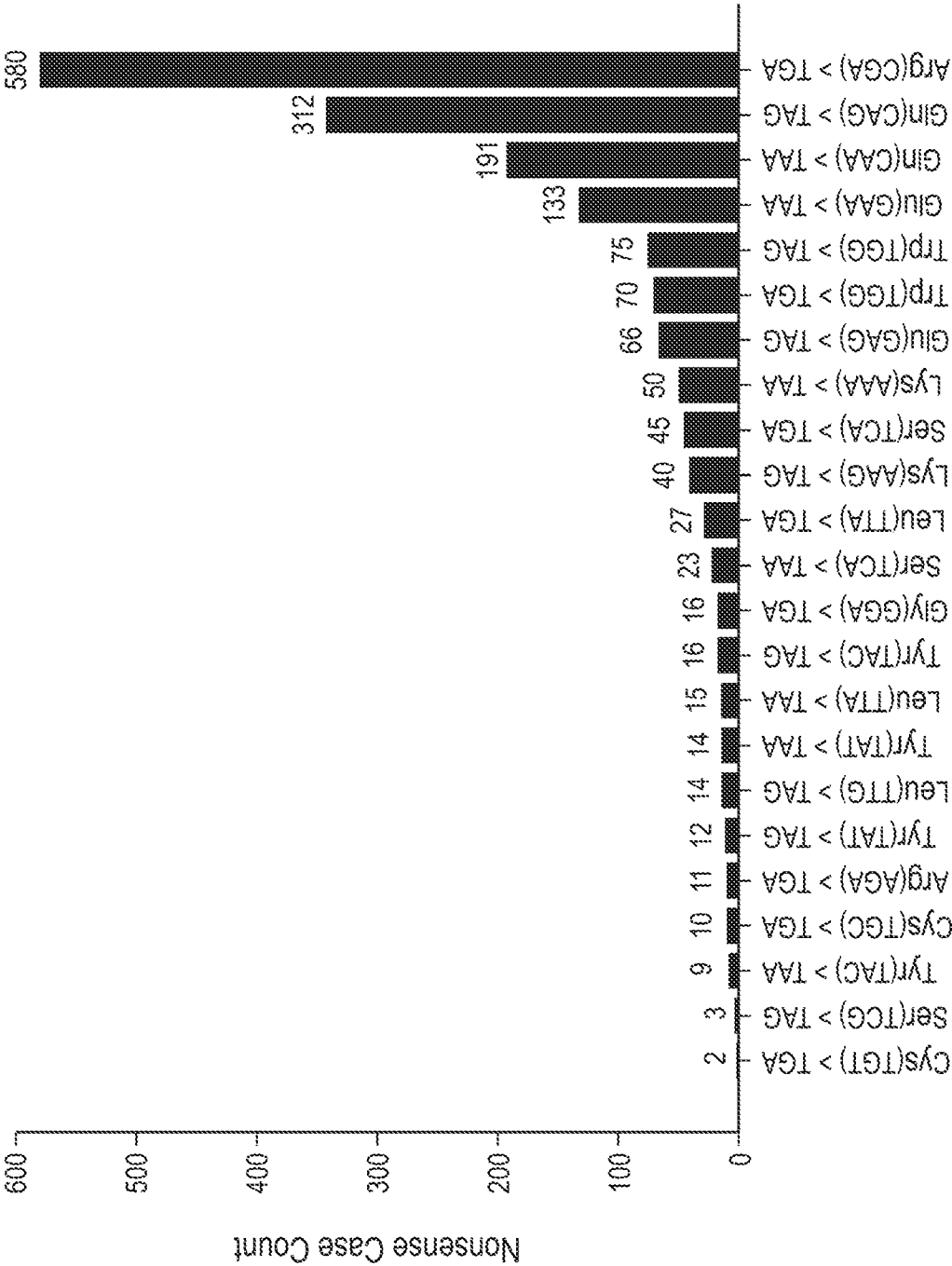
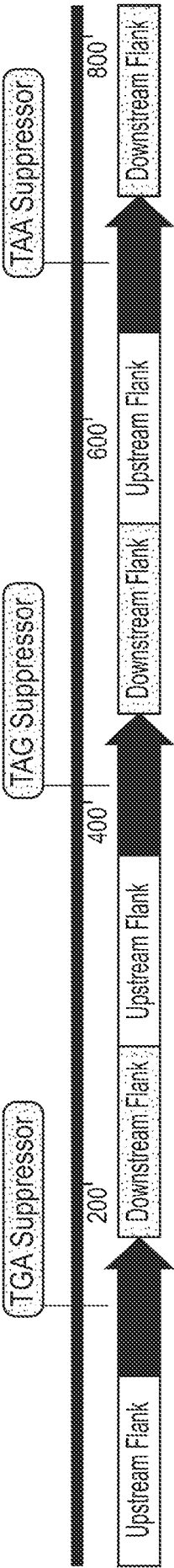


FIG. 5



Example Expression Vector
817 bp

FIG. 6

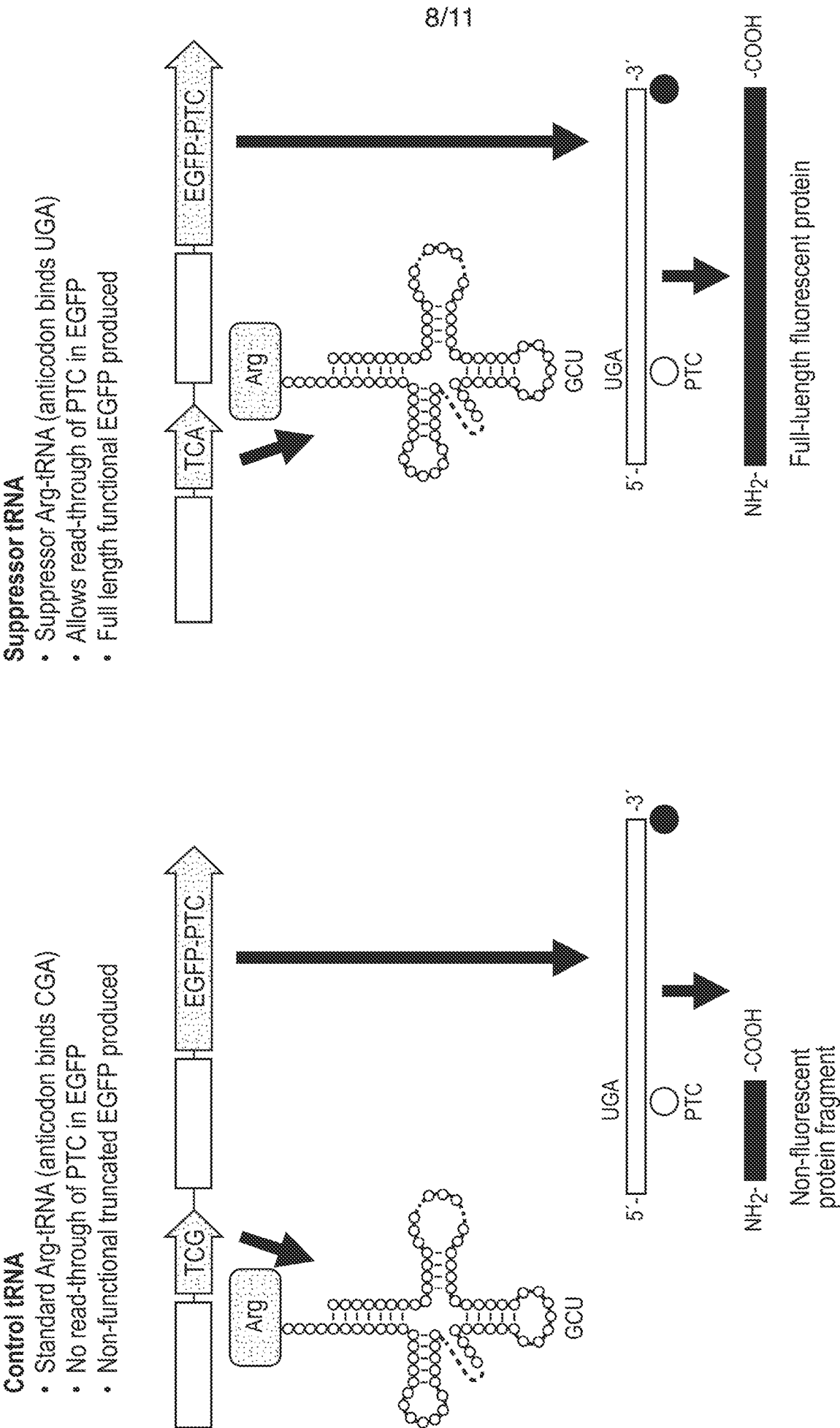


FIG. 7

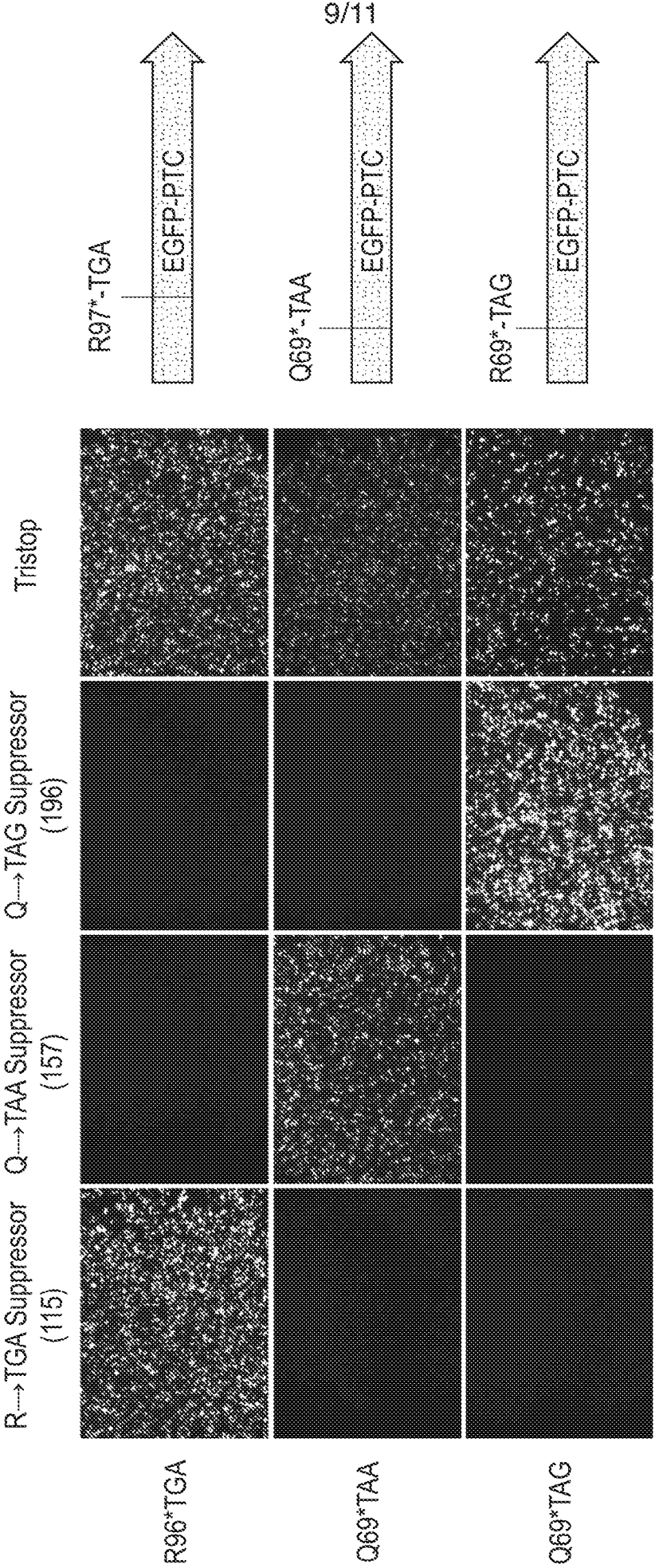


FIG. 8

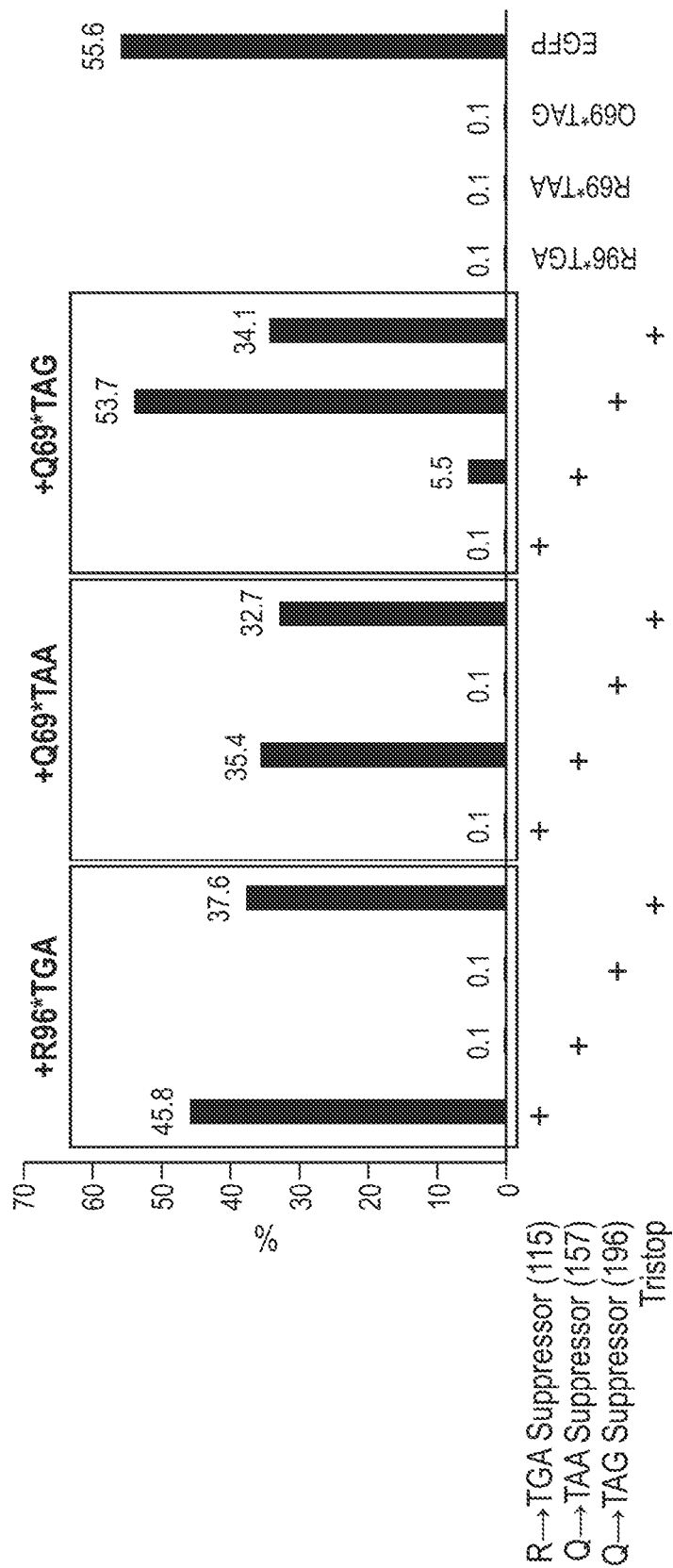


FIG. 9

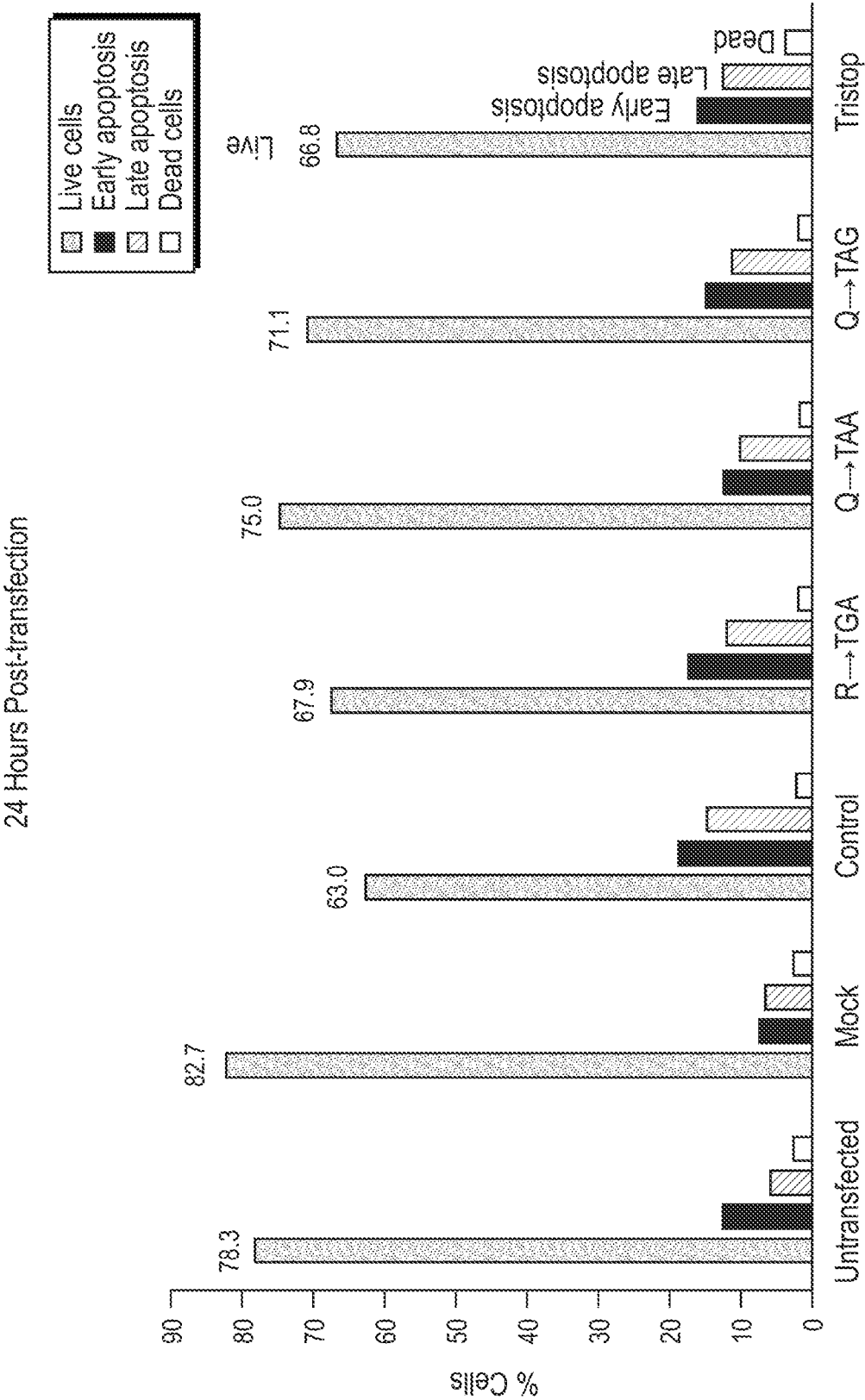


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/027765

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/113; C12N 15/09; C12N 15/67 (2022.01)

CPC - C12N 15/11; C12N 15/113; C12N 15/63; C12N 15/67 (2022.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2020/0291401 A1 (UNIVERSITY OF IOWA RESEARCH FOUNDATION) 17 September 2020 (17.09.2020) entire document	1-7, 29-31, 42-44, 54-56
A	US 2004/0219516 A1 (BENNETT et al) 04 November 2004 (04.11.2004) entire document	1-7, 29-31, 42-44, 54-56
A	US 2020/0101174 A1 (CASE WESTERN RESERVE UNIVERSITY et al) 02 April 2020 (02.04.2020) entire document	1-7, 29-31, 42-44, 54-56
A	US 2016/0222379 A1 (LIFE TECHNOLOGIES CORPORATION) 04 August 2016 (04.08.2016) entire document	1-7, 29-31, 42-44, 54-56
P, X	WO 2021/087401 A1 (TEVARD BIO INC.) 06 May 2021 (06.05.2021) entire document	1-7, 29-31, 42-44, 54-56

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

21 September 2022

Date of mailing of the international search report

OCT 13 2022

Name and mailing address of the ISA/US

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P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Taina Matos

Telephone No. PCT Helpdesk: 571-272-4300

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-7, 29-31, 42-44, and 54-56 are drawn to expression vectors and pharmaceutical compositions comprising a first, second, and/or third modified tRNA, and methods comprising the same.

The first invention of Group I+ is restricted to a first nucleotide sequence, a first amino acid sequence, a second nucleotide sequence, a second amino acid sequence, and a third nucleotide sequence and a third amino acid sequence, where the first nucleotide sequence is selected to be SEQ ID NO:6, the first amino acid is selected to be arginine; the second nucleotide sequence is selected to be SEQ ID NO:178, the second amino acid sequence is selected to be glutamine, the third nucleotide sequence is selected to be SEQ ID NO:36, the third amino acid is selected to be glutamine, and pharmaceuticals, and methods comprising the same. It is believed that claims 1-7, 29-31, 42-44, and 54-56 read on this first named invention and thus these claims will be searched without fee to the extent that they read on SEQ ID NOs: 6, 36, and 178, and arginine, and glutamine.

Applicant is invited to elect additional tRNAs, and aminoacylating amino acids, and their respective, corresponding, SEQ ID NOs, where applicable, to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be a first nucleotide sequence, a first amino acid sequence, a second nucleotide sequence, a second amino acid sequence, and a third nucleotide sequence and a third amino acid sequence, where the first nucleotide sequence is selected to be SEQ ID NO:7, the first amino acid is selected to be arginine; the second nucleotide sequence is selected to be SEQ ID NO:179, the second amino acid sequence is selected to be glutamine, the third nucleotide sequence is selected to be SEQ ID NO:37, the third amino acid is selected to be glutamine, and pharmaceuticals, and methods comprising the same. Additional tRNAs, and aminoacylating amino acids, and their respective, corresponding, SEQ ID NOs, where applicable, treat a disorder mediated by a premature termination codon will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for requiring the selection of alternative treating disorders mediated by premature termination codons where "the first, second, and/or third suppressor tRNA comprises a nucleotide sequence set forth in TABLE 2 or TABLE 3," and where "(i) the first amino acid is arginine, the second amino acid is glutamine, and the third amino acid is glutamine; (ii) the first amino acid is tryptophan, the second amino acid is glutamic acid, and the third amino acid is glutamic acid; (iii) the first amino acid is cysteine, the second amino acid is tyrosine, and the third amino acid is tyrosine; (iv) the first amino acid is serine, the second amino acid is lysine, and the third amino acid is lysine; (v) the first amino acid is glycine, the second amino acid is serine, and the third amino acid is serine; or (vi) the first amino acid is leucine, the second amino acid is leucine, and the third amino acid is leucine."

Additionally, even if Groups I+ were considered to share the technical features of an expression vector comprising: (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid; (b) a second nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid; and (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid; a pharmaceutical comprising: (a) a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid; (b) a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid; and (c) a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid; a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a first, second, and/or third premature termination codon, the method comprising contacting the cell with effective amount of: (a) a first expression vector comprising a nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid; (b) a second expression vector comprising a nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid; and (c) a third expression vector comprising a nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid; thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon; a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a first, second, and/or third premature termination codon, the method comprising contacting the cell with effective amount of: (a) a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid; (b) a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid; and (c) a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid; thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon; a method of treating a premature termination codon-mediated disorder in a subject in need thereof wherein the subject has a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject an effective amount of: (a) a first expression vector comprising a nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid; (b) a second expression vector comprising a nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid; and (c) a third expression vector comprising a nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid; thereby to treat the disorder in the subject; a method of treating a premature termination codon-mediated disorder in a subject in need thereof wherein the subject has a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject an effective amount of

amount of: a) a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid; (b) a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid; and (c) a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid; thereby to treat the disorder in the subject. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2004/0219516 A1 Bennett et al. discloses an expression vector (viral vectors that permit controlled expression, Para. [0045]) comprising: (a) a first nucleotide sequence encoding a first suppressor tRNA that comprises an anticodon that hybridizes to a TGA premature stop codon, and is capable of being aminoacylated with a first amino acid (one or more sequences encoding suppressor tRNAs may be provided on a nucleic acid molecule comprising all or a portion of a viral genome, Para. [0047]; A viral vector may encode one or more suppressor tRNAs that recognize one of the stop codons selected from TAG, TGA, or TAA, Para. [0055]); (b) a second nucleotide sequence encoding a second suppressor tRNA that comprises an anticodon that hybridizes to a TAG premature stop codon, and is capable of being aminoacylated with a second amino acid (a second nucleic acid molecule, Para. [0036]; one or more sequences encoding suppressor tRNAs may be provided on a nucleic acid molecule comprising all or a portion of a viral genome, Para. [0047]; A viral vector may encode one or more suppressor tRNAs that recognize one of the stop codons selected from TAG, TGA, or TAA, Para. [0055]); a pharmaceutical (multiple administrations of viral vectors are contemplated, Para. [0304]; compositions comprising nucleic acids and/or viruses of the invention, as well as methods of making and using such nucleic acids, viruses, and compositions, Para. [0024]) comprising: a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a first, second, and/or third premature termination codon (the invention provides nucleic acid molecules and/or viral vectors that permit controlled expression of fusion polypeptides by suppression of one or more stop codons ... may comprise one or more stop codons (e.g., TAG, TAA, and/or TGA) that may be suppressed, Para. [0045]; genes may be expressed from the same or different promoters that may be the same or different as the promoter used to express the ORF encoding the protein of interest, Para. [0294]), the method comprising contacting the cell with effective amount (contacting the cell with a nucleic acid molecule comprising all or a portion of a viral genome may result in production of a suppressor tRNA and suppression of one or more stop codons present in a sequence of interest, Para. [0052]) thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon ([a] tRNA molecule that results in the incorporation of an amino acid in a polypeptide in a position corresponding to a stop codon in the mRNA being translated, Para. [0193]; The transcribed mRNA molecule comprises at least a first coding sequence corresponding to the sequence of interest and at least one additional sequence containing a second coding region separated from the first coding sequence by a stop codon. Suppression of the stop codon allows expression of both the first and second coding sequences in a single polypeptide molecule. The nucleic acid sequence corresponding to the additional sequence may be contained on the sequence of interest or may be contained in a recombination site or on the nucleic acid molecule. One or more suppressor tRNA molecules may be provided, for example, from any nucleic acid molecule such as a plasmid, a nucleic acid molecule comprising all or a portion of a viral genome and/or a viral vector of the invention, Para. [0045]); a method of expressing in a mammalian cell a functional gene product encoded by a gene containing a first, second, and/or third premature termination codon (the invention provides nucleic acid molecules and/or viral vectors that permit controlled expression of fusion polypeptides by suppression of one or more stop codons ... may comprise one or more stop codons (e.g., TAG, TAA, and/or TGA) that may be suppressed, Para. [0045]; genes may be expressed from the same or different promoters that may be the same or different as the promoter used to express the ORF encoding the protein of interest, Para. [0294]), thereby permitting an amino acid to be incorporated into the gene product at a position that would otherwise result in a truncated gene product caused by the premature termination codon ([a] tRNA molecule that results in the incorporation of an amino acid in a polypeptide in a position corresponding to a stop codon in the mRNA being translated, Para. [0193]; The transcribed mRNA molecule comprises at least a first coding sequence corresponding to the sequence of interest and at least one additional sequence containing a second coding region separated from the first coding sequence by a stop codon. Suppression of the stop codon allows expression of both the first and second coding sequences in a single polypeptide molecule. The nucleic acid sequence corresponding to the additional sequence may be contained on the sequence of interest or may be contained in a recombination site or on the nucleic acid molecule. One or more suppressor tRNA molecules may be provided, for example, from any nucleic acid molecule such as a plasmid, a nucleic acid molecule comprising all or a portion of a viral genome and/or a viral vector of the invention, Para. [0045]); a method of treating a premature termination codon-mediated disorder in a subject in need thereof (vectors, especially those that lead to the production of more than one product, will be particularly useful for the treatment of diseases and/or conditions that result from the expression and/or lack of expression of more than one gene or for the treatment of more than one diseases and/or conditions., Para. [0337]) wherein the subject has a gene with a first, second, and/or third premature termination codon, the method comprising administering to the subject an effective amount and is capable of being aminoacylated with a third amino acid; thereby to treat the disorder in the subject ([a] tRNA molecule that results in the incorporation of an amino acid in a polypeptide in a position corresponding to a stop codon in the mRNA being translated, Para. [0193]; The transcribed mRNA molecule comprises at least a first coding sequence corresponding to the sequence of interest and at least one additional sequence containing a second coding region separated from the first coding sequence by a stop codon. Suppression of the stop codon allows expression of both the first and second coding sequences in a single polypeptide molecule. The nucleic acid sequence corresponding to the additional sequence may be contained on the sequence of interest or may be contained in a recombination site or on the nucleic acid molecule. One or more suppressor tRNA molecules may be provided, for example, from any nucleic acid molecule such as a plasmid, a nucleic acid molecule comprising all or a portion of a viral genome and/or a viral vector of the invention, Para. [0045]).

Further, US 2016/0222379 A1 to Life Technologies Corporation teaches and (c) a third nucleotide sequence encoding a third suppressor tRNA that comprises an anticodon that hybridizes to a TAA premature stop codon, and is capable of being aminoacylated with a third amino acid (transforming host cells which express suppressor tRNAs with the modified vectors; and (f) causing expression of the two coding sequences such that fusion proteins encoded by at least a portion of both of the coding sequences are produced, wherein

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the nucleic acid molecules of (a) and (b) are each flanked by at least one recombination site. Further, the fused nucleic acid molecules or the vector may comprise at least one suppressible stop codon, Para. [0062]; the recombination sites which recombine with each other in compositions and used in methods of the invention comprise ...TAA, Para. [0049]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

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International application No.

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 8-28, 32-41, 45-53, 57-63
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-7, 29-31, 42-44, 54-56

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.