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(54) **PLASMID ENCODING AN INSULIN PEPTIDE AND FC FUSION PROTEIN**

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(57) **ABSTRACT**

Some embodiments of the present disclosure relate to one or more compositions that upregulate the production of one or more sequences of mRNA. The sequences of mRNA may encode for translation of a target biomolecule, thereby causing an increase in bioavailability of the target biomolecule within a subject that is administered the one or more compositions. In some embodiments of the present disclosure, the target biomolecule is a fusion protein with an Fc fragment, such as a toll-like receptor 3-Fc (TLR3-Fc). In some embodiments of the present disclosure, the target biomolecule is toll-like receptor 9-Fc (TLR9-Fc). In some embodiments of the present disclosure, the target biomolecule is deoxyribonuclease I-Fc (DNAse I-Fc). In some embodiments of the present disclosure, the target biomolecule is neural growth factor-Fc (NGF-Fc). In some embodiments of the present disclosure, the target biomolecule is insulin-Fc.

Specification includes a Sequence Listing.

PLASMID ENCODING AN INSULIN PEPTIDE AND FC FUSION PROTEIN

[0001] This application contains a Sequence Listing electronically submitted via Patent Center to the United States Patent and Trademark Office as an XML Document file entitled "A8149440US—Sequence Listing.xml" created on 2024 Feb. 8 and having a size of 68,245 bytes. The information contained in the Sequence Listing is incorporated by reference herein.

TECHNICAL FIELD

[0002] The present disclosure generally relates to compositions for regulating the production of fusion proteins. In particular, the present disclosure relates to compositions for regulating gene expression and, consequently, the production of fusion proteins.

BACKGROUND

[0003] Bioactive molecules, including toll-like receptors, enzymes, and hormones, are necessary for the homeostatic control of biological systems.

[0004] When bioactive molecules are over-expressed, under-expressed or mis-expressed, homeostasis is lost, and disease is often the result.

[0005] As such, it may be desirable to establish therapies, treatments and/or interventions that address when homeostasis and the regulation of bioactive molecules are lost in order to prevent or treat the resulting disease.

SUMMARY

[0006] Some embodiments of the present disclosure relate to one or more compositions that upregulate the production of one or more sequences of mRNA. The sequences of mRNA may encode for translation of a target biomolecule, thereby causing an increase in bioavailability of the target biomolecule within a subject that is administered the one or more compositions. In some embodiments of the present disclosure, the target biomolecule is a fusion protein with an Fc fragment, such as a toll-like receptor 3-Fc (TLR3-Fc). In some embodiments of the present disclosure, the target biomolecule is toll-like receptor 9-Fc (TLR9-Fc). In some embodiments of the present disclosure, the target biomolecule is deoxyribonuclease I-Fc (DNAse I-Fc). In some embodiments of the present disclosure, the target biomolecule is neural growth factor-Fc (NGF-Fc). In some embodiments of the present disclosure, the target biomolecule is insulin-Fc.

[0007] In some embodiments of the present disclosure the compositions comprise a plasmid of deoxyribonucleic acid (DNA) that includes one or more insert sequences of nucleic acids that encode for the production of mRNA and a backbone sequence of nucleic acids that facilitates introduction of the one or more insert sequences into one or more of a subject's cells where it is expressed and/or replicated. Expression of the one or more insert sequences by one or more cells of the subject results in an increased production of the mRNA and, consequently, increased translation of the target biomolecule by one or more of the subject's cells.

[0008] Some embodiments of the present disclosure relate to a recombinant plasmid (RP). In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 2. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding an mRNA sequence that encodes for the fusion protein TLR3-Fc.

[0009] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 3. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding an mRNA sequence that encodes for the fusion protein TLR9-Fc.

[0010] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 4. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding an mRNA sequence that encodes for the fusion protein DNAse I-Fc.

[0011] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 5. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding an mRNA sequence that encodes for the fusion protein NGF-Fc.

[0012] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 6. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding an mRNA sequence that encodes for the fusion protein insulin-Fc.

[0013] Some embodiments of the present disclosure relate to a method of making a composition/target cell complex. The method comprising a step of administering a RP comprising SEQ ID NO. 1 and one of SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5 or SEQ ID NO. 6 to a target cell for forming the composition/target cell complex, wherein the composition/target cell complex causes the target cell to increase production of one or more sequences of mRNA that increases production of a target biomolecule.

[0014] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of mRNA that encodes for a target biomolecule, for example TLR3-Fc. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of mRNA, which are complete or partial sequences and/or combinations thereof of TLR3-Fc, which can be administered to a subject to increase the subject's production of one or more sequences of the mRNA.

[0015] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of mRNA that encodes for a target biomolecule, for example TLR9-Fc. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of mRNA, which are complete or partial sequences and/or combinations thereof of TLR9-Fc, which can be administered to a subject to increase the subject's production of one or more sequences of the mRNA.

[0016] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of mRNA that encodes for a target biomolecule, for example DNAse I-Fc. A first approach

utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of mRNA, which are complete or partial sequences and/or combinations thereof of DNAse I-Fc, which can be administered to a subject to increase the subject's production of one or more sequences of the mRNA.

[0017] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of mRNA that encodes for a target biomolecule, for example NGF-Fc. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of mRNA, which are complete or partial sequences and/or combinations thereof of NGF-Fc, which can be administered to a subject to increase the subject's production of one or more sequences of the mRNA.

[0018] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of mRNA that encodes for a target biomolecule, for example insulin-Fc. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of mRNA, which are complete or partial sequences and/or combinations thereof of insulin-Fc, which can be administered to a subject to increase the subject's production of one or more sequences of the mRNA.

DETAILED DESCRIPTION

[0019] Unless defined otherwise, all technical and scientific terms used therein have the meanings that would be commonly understood by one of skill in the art in the context of the present description. Although any methods and materials similar or equivalent to those described therein can also be used in the practice or testing of the present disclosure, the preferred compositions, methods and materials are now described. All publications mentioned therein are incorporated therein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0020] As used therein, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. For example, reference to "a composition" includes one or more compositions and reference to "a subject" or "the subject" includes one or more subjects.

[0021] As used therein, the terms "about" or "approximately" refer to within about 25%, preferably within about 20%, preferably within about 15%, preferably within about 10%, preferably within about 5% of a given value or range. It is understood that such a variation is always included in any given value provided therein, whether or not it is specifically referred to.

[0022] As used therein, the term "ameliorate" refers to improve and/or to make better and/or to make more satisfactory.

[0023] As used therein, the term "cell" refers to a single cell as well as a plurality of cells or a population of the same cell type or different cell types. Administering a composition to a cell includes in vivo, in vitro and ex vivo administrations and/or combinations thereof.

[0024] As used therein, the term "complex" refers to an association, either direct or indirect, between one or more particles of a composition and one or more target cells. This association results in a change in the metabolism of the target cell. As used therein, the phrase "change in metabolism" refers to an increase or a decrease in the one or more target cells' production of one or more proteins, and/or any post-translational modifications of one or more proteins.

[0025] As used therein, the term "composition" refers to a substance that, when administered to a subject, causes one or more chemical reactions and/or one or more physical reactions and/or one or more physiological reactions and/or one or more immunological reactions in the subject. In some embodiments of the present disclosure, the composition is a plasmid vector.

[0026] As used therein, the term "endogenous" refers to the production and/or modification of a molecule that originates within a subject.

[0027] As used therein, the term "exogenous" refers to a molecule that is within a subject but that did not originate within the subject. As used therein, the terms "production", "producing" and "produce" refer to the synthesis and/or replication of DNA, the transcription of one or more sequences of RNA, the translation of one or more amino acid sequences, the post-translational modifications of an amino acid sequence, and/or the production of one or more regulatory molecules that can influence the production and/or functionality of an effector molecule or an effector cell. For clarity, "production" is also used therein to refer to the functionality of a regulatory molecule, unless the context reasonably indicates otherwise.

[0028] As used therein, the term "subject" refers to any therapeutic target that receives the composition. The subject can be a vertebrate, for example, a mammal including a human. The term "subject" does not denote a particular age or sex. The term "subject" also refers to one or more cells of an organism, an in vitro culture of one or more tissue types, an in vitro culture of one or more cell types, ex vivo preparations, and/or a sample of biological materials such as tissue, and/or biological fluids.

[0029] As used therein, the term "target biomolecule" refers to a protein-Fc fusion molecule that is found within a subject. A biomolecule may be endogenous or exogenous to a subject.

[0030] As used therein, the term "target cell" refers to one or more cells and/or cell types that are affected, either directly or indirectly, by a biomolecule.

[0031] As used therein, the term "therapeutically effective amount" refers to the amount of the composition used that is of sufficient quantity to ameliorate, treat and/or inhibit one or more of a disease, disorder or a symptom thereof. The "therapeutically effective amount" will vary depending on the composition used, the route of administration of the composition and the severity of the disease, disorder or symptom thereof. The subject's age, weight and genetic make-up may also influence the amount of the composition that will be a therapeutically effective amount.

[0032] As used therein, the terms "treat", "treatment" and "treating" refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing an occurrence of a disease, disorder or symptom thereof and/or the effect may be therapeutic in providing a partial or complete amelioration or inhibition of a disease, disorder, or symptom thereof. Additionally, the term "treatment" refers to any treatment of a disease, disorder, or symptom thereof in a subject and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not

yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) ameliorating the disease.

[0033] As used therein, the terms "unit dosage form" and "unit dose" refer to a physically discrete unit that is suitable as a unitary dose for patients. Each unit contains a predetermined quantity of the composition and optionally, one or more suitable pharmaceutically acceptable carriers, one or more excipients, one or more additional active ingredients, or combinations thereof. The amount of composition within each unit is a therapeutically effective amount.

[0034] Where a range of values is provided therein, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also, encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

[0035] In some embodiments of the present disclosure, a composition is a recombinant plasmid (RP) for introducing genetic material, such as one or more nucleotide sequences, into a target cell for reproduction or transcription of an insert that comprises one or more nucleotide sequences that are carried within the RP. In some embodiments of the present disclosure, the RP is delivered without a carrier, by a viral vector, by a protein coat, or by a lipid vesicle. In some embodiments of the present disclosure, the vector is an adeno-associated virus vector.

[0036] In some embodiments of the present disclosure, the insert comprises one or more nucleotide sequences that encode for production of at least one sequence of mRNA that increases the production of target biomolecules, such as a fusion protein with an Fc fragment. An Fc fragment is the distal portion of the heavy chain of an antibody.

[0037] In some embodiments of the present disclosure, the target biomolecule is TLR3-Fc.

[0038] In some embodiments of the present disclosure, the target biomolecule is TLR9-Fc.

[0039] In some embodiments of the present disclosure, the target biomolecule is DNAse I-Fc.

[0040] In some embodiments of the present disclosure, the target biomolecule is NGF-Fc.

[0041] In some embodiments of the present disclosure, the target biomolecule is insulin-Fc.

[0042] Some embodiments of the present disclosure relate to a composition that can be administered to a subject with a condition that results, directly or indirectly, from the dysregulated production of a biomolecule. When a therapeutically effective amount of the composition is administered to the subject, the subject may change production and/or functionality of one or more biomolecules.

[0043] In some embodiments of the present disclosure, the subject may respond to receiving the therapeutic amount of the composition by changing production and/or functionality of one or more intermediary molecules by changing production of one or more DNA sequences, one or more RNA sequences, and/or one or more proteins that regulate the levels and/or functionality of the one or more interme-

diary molecules. The one or more intermediary molecules regulate the subject's levels and/or functionality of the one or more biomolecules.

[0044] In some embodiments of the present disclosure, administering a therapeutic amount of the composition to a subject upregulates the production, functionality or both one or more sequences of mRNA that each encode for one or more biomolecules.

[0045] In some embodiments of the present disclosure, the composition is an RP that may be used for gene therapy. The gene therapy is useful for increasing the subject's endogenous production of one or more sequences of mRNA that encode for a target biomolecule. For example, the RP can contain one or more nucleotide sequences that cause increased production of one or more nucleotide sequences that cause an increased production of one or more mRNA sequences that encode for one biomolecule, such as TLR3-Fc, TLR9-Fc, DNAse I-Fc, NGF-Fc or insulin-Fc.

[0046] In some embodiments of the present disclosure, the delivery vehicle of the RP used for gene therapy may be a vector that comprises a virus that can be enveloped, or not (unenveloped), replication effective or not (replication ineffective), or combinations thereof. In some embodiments of the present disclosure, the vector is a virus that is not enveloped and not replication effective. In some embodiments of the present disclosure, the vector is a virus of the Parvoviridae family. In some embodiments of the present disclosure, the vector is a virus of the genus *Dependoparvovirus*. In some embodiments of the present disclosure, the vector is an adeno-associated virus (AAV). In some embodiments of the present disclosure, the vector is a recombinant AAV. In some embodiments of the present disclosure, the vector is a recombinant AAV6.2FF.

[0047] In some embodiments of the present disclosure, the delivery vehicle of the RP used for gene therapy may be a protein coat.

[0048] In some embodiments of the present disclosure, the delivery vehicle of the RP used for gene therapy may be a lipid vesicle.

[0049] The embodiments of the present disclosure also relate to administering a therapeutically effective amount of the composition. In some embodiments of the present disclosure, the therapeutically effective amount of the composition that is administered to a patient is between about 10 and about 1×10^{16} TCID$_{50}$/kg (50% tissue culture infective dose per kilogram of the patient's body mass). In some embodiments of the present disclosure, the therapeutically effective amount of the composition that is administered to the patient is about 1×10^{13} TCID$_{50}$/kg. In some embodiments of the present disclosure, the therapeutically effective amount of the composition that is administered to a patient is measured in TPC/kg (total particle count of the composition per kilogram of the patient's body mass). In some embodiments of the present disclosure, the therapeutically effective amount of the composition is between about 10 and about 1×10^{16} TCP/kg.

[0050] Some embodiments of the present disclosure relate to an adeno-associated virus (AAV) genome consisting of a RP that when operable inside a target cell will cause the target cell to produce a mRNA sequence that upregulates production of a biomolecule, with examples being TLR3-Fc, TLR9-Fc, DNAse I-Fc, NGF-Fc, or insulin-Fc. The RP is comprised of AAV2 inverted terminal repeats (ITRs), a

composite CASI promoter, and a human growth hormone (HGH) signal peptide followed by a mRNA expression cassette encoding for TLR3-Fc, TLR9-Fc, DNAse I-Fc, NGF-Fc, or insulin-Fc, followed by a Woodchuck Hepatitis Virus post-transcriptional regulatory element (WPRE) and a Simian virus 40 (SV40) polyadenylation (polyA) signal.

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SEQ ID NO. 1 (backbone sequence No. 1):
5'  TTCTAGAATAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTT

AACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCT

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CCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCT

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5

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GATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCT

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GAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTTGTAGTTAATGATTA

ACCCGCCATGCTACTTATCTACGTAGCCATGCTCTAGGACATTGATTATTGACTAGTGGA

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TGATCCTTCCGCCCGGACGCTCAGGACAGCGGCCCGCTGCTCATAAGACTCGGCCTTAGA

ACCCCAGTATCAGCAGAAGGACATTTTAGGACGGGACTTGGGTGACTCTAGGGCACTGGT

TTTCTTTCCAGAGAGCGGAACAGGCGAGGAAAAGTAGTCCCTTCTCGGCGATTCTGCGGA

GGGATCTCCGTGGGCGGTGAACGCCGATGATGCCTCTACTAACCATGTTCATGTTTTCT

TTTTTTTTCTACAGGTCCTGGGTGACGAACAGGGTACCGCCACC 3'

SEQ ID NO. 2 (mRNA expression cassette No. 2-TLR3-Fc):
5' ATGAGGGGCATGAAGCTGCTGGGGGCGCTGCTGGCACTGGCGGCCCTACTGCAGGGG

GCCGTGTCCCTGAAGATCGCAGCCTTCAACATCCAGACATTTGGGGAGACCAAGATGTCC

AATGCCACCCTCGTCAGCTACATTGTGCAGATCCTGAGCCGCTATGACATCGCCCTGGTC

CAGGAGGTCAGAGACAGCCACCTGACTGCCGTGGGGAAGCTGCTGGACAACCTCAATCAG

GATGCACCAGACACCTATCACTACGTGGTCAGTGAGCCACTGGGACGGAACAGCTATAAG

GAGCGCTACCTGTTCGTGTACAGGCCTGACCAGGTGTCTGCGGTGGACAGCTACTACTAC

GATGATGGCTGCGAGCCCTGCGGGAACGACACCTTCAACCGAGAGCCAGCCATTGTCAGG

TTCTTCTCCCGGTTCACAGAGGTCAGGGAGTTTGCCATTGTTCCCCTGCATGCGGCCCCG

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GCCTATGGCCTGAGTGACCAACTGGCCCAAGCCATCAGTGACCACTATCCAGTGGAGGTG

ATGCTGAAGGGCGGATCAGGCGGATCACCCAAATCTTGTGACAAAACTCACACATGCCCA

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CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCG

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TAG 3'

SEQ ID NO. 3 (mRNA expression cassette No. 3-TLR9-Fc):
5' GCCAGACCCTGCCGTGCATTTATTTTTGGGGCGGCCTGCTGCCGTTTGGCATGCTGT

GCGCGAGCAGCACCACCAAATGCACCGTGAGCCATGAAGTGGCGGATTGCAGCCATCTGA

AACTGACCCAGGTGCCGGATGATCTGCCGACCAACATTACCGTGCTGAACCTGACCCATA

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AAATAG 3'

SEQ ID NO. 4 (miRNA expression cassette No. 4-DNAse I-Fc):
5' ATGAGGGGCATGAAGCTGCTGGGGGCGCTGCTGGCACTGGCGGCCCTACTGCAGGGG

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CTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATAG 3'

SEQ ID NO. 5 (mRNA expression cassette No. 5-NGF-Fc):
5' ATGAGGGGCATGAAGCTGCTGGGGGCGCTGCTGGCACTGGCGGCCCTACTGCAGGGG

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GCGGAACCGCATAGCGAAAGCAACGTGCCGGCGGGCCATACCATTCCGCAGGCGCATTGG

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AAATAG 3'

SEQ ID NO. 6 (mRNA expression cassette No. 6-insulin-Fc):
5' ATGAGGGGCATGAAGCTGCTGGGGGCGCTGCTGGCACTGGCGGCCCTACTGCAGGGG

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GCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATAG 3'

SEQ ID NO: 7 = SEQ ID NO: 1 + SEQ ID NO: 2
5' TTCTAGAATAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTT

AACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCT

ATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTT

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[0051] As will be appreciated by those skilled in the art, because the recombinant plasmid is a circular vector, the one or more sequences of the mRNA expression cassettes may be connected at the 3' end of SEQ ID NO. 1, as shown in SEQ ID NO. 7-11 or at the 5' end of SEQ ID NO. 1.

[0052] As will be appreciated by those skilled in the art, a perfect match of nucleotides with each of the miRNA expression cassette sequences is not necessary in order to have the desired result of increased bioavailability of the target biomolecule as a result of the target cell producing the miRNA sequence that will bind to and degrade the mRNA of the target biomolecule. In some embodiments of the present disclosure, about 80% to about 100% nucleotide sequence matching with each of the mRNA expression cassettes causes the desired result. In some embodiments of the present disclosure, about 85% to about 100% nucleotide sequence matching with each of the mRNA expression cassettes causes the desired result. In some embodiments of the present disclosure, about 90% to about 100% nucleotide sequence matching with each of the mRNA expression cassettes causes the desired result. In some embodiments of the present disclosure, about 95% to about 100% nucleotide sequence matching with each of the mRNA expression cassettes causes the desired result.

Example 1—Expression Cassette

[0053] Expression cassettes for expressing mRNA were synthesized. The synthesized miRNA expression cassettes were cloned into the pAVA-00200 plasmid backbone containing the CASI promoter, multiple cloning site (MCS), Woodchuck Hepatitis Virus post-transcriptional regulatory element (WPRE), and Simian virus 40 (SV40) polyadenylation (polyA) sequence, all flanked by the AAV2 inverted terminal repeats (ITR). pAVA-00200 was cut with the restriction enzymes KpnI and XbaI in the MCS and separated on a 1% agarose gel. The band of interest was excised and purified using a gel extraction kit. Each mRNA expression cassette was amplified by polymerase chain reaction (PCR) using Taq polymerase and the PCR products were gel purified and the bands on interest were also excised and purified using a gel extraction kit. These PCR products contained the mRNA expression cassettes in addition to 15 base pair 5' and 3' overhangs that aligned with the ends of the linearized pAVA-00200 backbone. Using in-fusion cloning, the amplified mRNA expression cassettes are integrated with the pAVA-00200 backbone via homologous recombination. The resulting RP contained the following: 5' ITR, CASI promoter, miRNA expression cassette, WPRE, SV40 polyA and ITR 3'.

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1. An isolated plasmid comprising messenger ribonucleic acid (mRNA) encoding a fusion protein comprising an insulin peptide and an Fc domain, wherein the isolated plasmid comprises a nucleotide sequence of SEQ ID NO: 6.

2. The isolated plasmid of claim 1, wherein the isolated plasmid is inserted within one or more suitable pharmaceutically acceptable carriers.

3. (canceled)

4. (canceled)

5. (canceled)

6. An isolated plasmid comprising messenger ribonucleic acid (mRNA) encoding a fusion protein comprising an insulin peptide and an Fc domain, wherein the isolated plasmid comprises a nucleotide sequence of SEQ ID NO: 11.

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