LYOPHILIZED PHARMACEUTICAL COMPOSITIONS FOR NAKED DNA GENE THERAPY

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/700,655, filed July 19, 2018, which is hereby incorporated by reference in its entirety.

2. SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 16, 2019, is named 40319US_CRF_sequencelisting and is 125,979 bytes in size.

3. BACKGROUND

[0003] There is now substantial clinical evidence that gene therapy involving direct *in vivo* delivery of nucleic acid constructs that have not been packaged in virus or virus-like particles – so-called "naked" DNA or RNA constructs – can be effective in treating various diseases. For example, direct intramuscular injection of a DNA plasmid construct that expresses two isoforms of human HGF protein (*i.e.,* pCK-HGF-X7, also called "VM202") has been demonstrated to be effective in treating neuropathic pain. In a phase II clinical trial, injections of VM202 into the calf muscle of patients with diabetic peripheral neuropathy were shown to significantly reduce pain – two days of treatment, spaced two weeks apart, were sufficient to provide symptomatic relief with improvement in quality of life for 3 months. Kessler *et al.*, *Annals Clin. Transl. Neurology* 2(5):465-478 (2015). The same DNA plasmid construct has also been shown to be effective in treating patients with amyotrophic lateral sclerosis (ALS). In a phase I clinical trial, close to half of the ALS patients remained stable or improved after administration of VM202, with 47%, 50%, and 24% of subjects at months 1, 2, and 3, respectively, experiencing either no decline or an improvement in the ALSFRS-R (Amyotrophic Lateral Sclerosis Functional Rating Scale-revised) score indicating physical functioning of ALS patients, which is better than observed in historical controls. Robert L. Sufit *et al.*, *Amyotrophic Lateral Sclerosis and Frontoemporal Degeneration* 18:269-278 (2017).

[0004] However, despite the recent approval of two antisense oligonucleotide drugs for direct injection – nusinersen, for intrathecal injection, and eteplirsen, for intravenous injection – few naked DNA or RNA constructs have actually been approved for human gene therapy. Thus, despite decades of experience with formulating naked nucleic acids for laboratory use, and more recent experience formulating nucleic acids for *ex vivo* gene therapy approaches, little research has been conducted into formulating DNA as a pharmaceutical product for direct therapeutic delivery. In particular, there have been few investigations into stability of the nucleic acid active ingredient under various storage conditions, or into excipients required to permit uniform reconstitution of the formulation for administration, or into methods for reducing contaminant load, *etc.* These properties are important for better and more reproducible therapeutic efficacy and safety of the DNA-based drugs, as well as for the economic feasibility of scaling up the production and distribution of the drugs.

[0005] There is, therefore, a need for a formulation for a pharmaceutical product of a naked DNA-based drug that provides improved stability, safety and economic feasibility.

4. SUMMARY

[0006] In an aspect, the present invention provides a novel lyophilized pharmaceutical composition that maintains the stability of the DNA construct (*e.g.,* a plasmid) during lyophilization. Specifically, the formulation reduces conformational change of DNA constructs during lyophilization from a more stable supercoiled form to a less stable open circular and linear form. The novel lyophilized formulation further allows uniform reconstitution of the DNA constructs in a pharmaceutically acceptable solution, enabling complete recovery of the active ingredients, minimizing partial loss of potency and allowing administration of the active ingredients in an accurate and consistent manner. Additionally, the novel lyophilized formulation produces a uniform and elegant cake appearance following lyophilization, thus making a visual inspection of the quality of the DNA-based drug feasible.

[0007] In some embodiments, the novel lyophilized formulation comprises plasmid DNA, wherein the pharmaceutical composition is obtained by lyophilizing a liquid composition that comprises, prior to lyophilization: a. DNA of a first plasmid; b. potassium phosphate buffer with pH in the range from 7.0 to 9.0; c. mannitol at a concentration in the range from 0% to 3% (w/v);

d. sucrose at a concentration greater than 0.5% and less than 1.1% (w/v); and e. NaCl at a concentration in the range from 0.1 % to 0.9% (w/v). The first plasmid can be selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0008] In some embodiments, the first plasmid is VM202. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0009] In some embodiments, the first plasmid is pTx-HGF-X7. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0010] In some embodiments, the first plasmid is pTx-IGF-1Ec. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0011] In some embodiments, the first plasmid is pTx-IGF-1Ea. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0012] In some embodiments, the first plasmid is pTx-IGF-1X6. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X10, and pCK-SDF-1α.

[0013] In some embodiments, the first plasmid is pTx-IGF-1X10. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, and pCK-SDF-1α.

[0014] In some embodiments, the first plasmid is pCK-SDF-1α. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, and pTx-IGF-1X10.

[0015] In some embodiments, at least 90% of the plasmid DNA in the liquid composition is supercoiled. In some embodiments, at least 92.5% of the plasmid DNA in the liquid composition is supercoiled. In some embodiments, at least 95% of the plasmid DNA in the liquid composition is supercoiled. In some embodiments, at least 97% of the plasmid DNA in the liquid composition is supercoiled. In some embodiments, at least 98% of the plasmid DNA in the liquid composition is supercoiled.

[0016] In some embodiments, at least 90% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition. In some embodiments, at least 92.5% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition. In some embodiments, at least 95% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition. In some embodiments, at least 97% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition. In some embodiments, at least 98% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition.

[0017] In some embodiments, at least 90% of the plasmid DNA remains supercoiled after storage at 25°C for 3 to 7 days following reconstitution of the lyophilized pharmaceutical composition. In some embodiments, at least 92.5% of the plasmid DNA remains supercoiled after storage at 25°C for 3 to 7 days following reconstitution of the lyophilized pharmaceutical composition. In some embodiments, at least 95% of the plasmid DNA remains supercoiled after storage at 25°C for 3 to 7 days following reconstitution of the lyophilized pharmaceutical composition.

[0018] In some embodiments, at least 80% of the plasmid DNA remains supercoiled 30 minutes after reconstitution, wherein the lyophilized pharmaceutical composition had been stored at 40°C for 10 weeks before reconstitution.

[0019] In some embodiments, the lyophilized pharmaceutical composition comprises supercoiled DNA in an amount of at least 90% of a total amount of supercoiled DNA in the liquid composition. In some embodiments, the lyophilized pharmaceutical composition comprises supercoiled DNA in an amount of at least 92.5% of a total amount of supercoiled DNA in the liquid composition. In some embodiments, the lyophilized pharmaceutical composition comprises supercoiled DNA in an amount of at least 95% of a total amount of supercoiled DNA in the liquid composition.

[0020] In some embodiments, the liquid composition comprises the first plasmid DNA at a concentration in the range from 0.1 to 1 mg/ml. In some embodiments, the liquid composition comprises the first plasmid DNA at a concentration in the range from 0.25 to 0.75 mg/ml. In some embodiments, the liquid composition comprises the first plasmid DNA at a concentration in the range from 0.4 to 0.6 mg/ml. In some embodiments, the liquid composition comprises the first plasmid DNA at a concentration of 0.5 mg/ml.

[0021] In some embodiments, the liquid composition comprises the second plasmid DNA at a concentration in the range from 0.1 to 1 mg/ml. In some embodiments, the liquid composition comprises the second plasmid DNA at a concentration in the range from 0.25 to 0.75 mg/ml. In some embodiments, the liquid composition comprises the second plasmid DNA at a concentration in the range from 0.4 to 0.6 mg/ml. In some embodiments, the liquid composition comprises the second plasmid DNA at a concentration of 0.5 mg/ml.

[0022] In some embodiments, the liquid composition comprises potassium phosphate at a concentration in the range from 5 mM to 15 mM. In some embodiments, the liquid composition comprises potassium phosphate at a concentration in the range from 7.5 mM to 12.5 mM. In some embodiments, the liquid composition comprises potassium phosphate at a concentration in the range from 9 mM to 11 mM. In some embodiments, the liquid composition comprises potassium phosphate at a concentration of 10 mM.

[0023] In some embodiments, the liquid composition comprises potassium phosphate buffer with pH in the range from 7.0 to 8.5. In some embodiments, the liquid composition comprises potassium phosphate buffer with pH in the range from 7.0 to 8.0. In some embodiments, the liquid composition comprises potassium phosphate buffer of pH 8.0.

[0024] In some embodiments, the liquid composition comprises mannitol at a concentration in the range from 1.5% to 3% (w/v). In some embodiments, the liquid composition comprises mannitol at a concentration in the range from 2% to 3% (w/v). In some embodiments, the liquid composition comprises mannitol at a concentration of 2 % (w/v).

[0025] In some embodiments, the liquid composition comprises sucrose at a concentration in the range from 0.75% to 1.1% (w/v). In some embodiments, the liquid composition comprises sucrose at a concentration in the range from 0.9% to 1.0% (w/v). In some embodiments, the liquid composition comprises sucrose at a concentration of 1.0 % (w/v).

[0026] In some embodiments, the liquid composition comprises NaCl at a concentration in the range from 0.1 % to 0.75% (w/v). In some embodiments, the liquid composition comprises NaCl at a concentration in the range from 0.1 % to 0.6 % (w/v). In some embodiments, the liquid composition comprises NaCl at a concentration in the range from 0.4% to 0.5% (w/v). In some embodiments, the liquid composition comprises NaCl at a concentration of 0.45 % (w/v).

[0027] Some embodiments of the present invention relate to a lyophilized pharmaceutical composition comprising plasmid DNA, wherein the pharmaceutical composition is obtained by lyophilizing a liquid composition that comprises, prior to lyophilization: a. DNA of a first plasmid at a concentration of 0.5 mg/ml; b.10mM potassium phosphate buffer of pH 8.0; c. mannitol at a concentration of 2 % (w/v); d. sucrose at a concentration of 1.0 % (w/v); and e. NaCl at a concentration of 0.45 % (w/v), wherein at least 95% of the plasmid DNA is supercoiled, and at least 90% of the plasmid DNA remains supercoiled after storage at 25°C for 3 to 7 days following reconstitution of the lyophilized pharmaceutical composition. The first plasmid can be selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0028] In some embodiments, the first plasmid is VM202. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0029] In some embodiments, the first plasmid is pTx-HGF-X7. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein

the second plasmid is selected from the group consisting of VM202, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0030] In some embodiments, the first plasmid is pTx-IGF-1Ec. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0031] In some embodiments, the first plasmid is pTx-IGF-1Ea. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0032] In some embodiments, the first plasmid is pTx-IGF-1X6. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X10, and pCK-SDF-1α.

[0033] In some embodiments, the first plasmid is pTx-IGF-1X10. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, and pCK-SDF-1α.

[0034] In some embodiments, the first plasmid is pCK-SDF-1α. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, and pTx-IGF-1X10.

[0035] Some aspects of the present invention relate to a reconstituted composition produced by dissolving the lyophilized pharmaceutical composition in water.

[0036] In some embodiments, light absorbance of the reconstituted composition at 450nm is less than 0.002. In some embodiments, the light absorbance of the reconstituted composition at 450nm is 0.001 or less than 0.001. In some embodiments, the light absorbance is measured on the day of reconstitution. In some embodiments, the light absorbance of the reconstituted

composition at 450nm is measured after storage of the lyophilized pharmaceutical composition for 10 weeks.

[0037] Some aspects of the present invention relate to a lyophilized pharmaceutical composition comprising plasmid DNA in a unit dose, wherein the pharmaceutical composition is obtained by lyophilizing a liquid composition that comprises, prior to lyophilization: a.DNA of a first plasmid at a concentration of 0.5 mg/ml; b. 10mM potassium phosphate buffer of pH 8.0; c. mannitol at a concentration of 2 % (w/v); d. sucrose at a concentration of 1.0 % (w/v); and e. NaCl at a concentration of 0.45 % (w/v), wherein the lyophilized pharmaceutical composition is in a vial and the vial contains 2.5 mg of plasmid DNA in total. The first plasmid can be selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0038] In some embodiments, the first plasmid is VM202. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0039] In some embodiments, the first plasmid is pTx-HGF-X7. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0040] In some embodiments, the first plasmid is pTx-IGF-1Ec. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0041] In some embodiments, the first plasmid is pTx-IGF-1Ea. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0042] In some embodiments, the first plasmid is pTx-IGF-1X6. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein

the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X10, and pCK-SDF-1α.

[0043] In some embodiments, the first plasmid is pTx-IGF-1X10. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, and pCK-SDF-1α.

[0044] In some embodiments, the first plasmid is pCK-SDF-1α. In some embodiments, the lyophilized pharmaceutical composition further comprises DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, and pTx-IGF-1X10.

[0045] Some aspects of the present invention relate to a reconstituted composition produced by dissolving the lyophilized pharmaceutical composition.

[0046] Some aspects of the present invention relate to a method of making a lyophilized pharmaceutical composition comprising plasmid DNA, the method comprising: providing a liquid composition comprising: a. DNA of a first plasmid; b. potassium phosphate buffer with pH in the range from 7.0 to 9.0; c. mannitol at a concentration in the range from 0% to 3% (w/v); d. sucrose at a concentration greater than 0.5% and less than 1.1% (w/v); and e. NaCl at a concentration in the range from 0.1 % to 0.9% (w/v); and lyophilizing the liquid composition, thereby generating the lyophilized pharmaceutical composition. The first plasmid can be selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

[0047] In some embodiments, the first plasmid is VM202. In some embodiments, the first plasmid is pTx-HGF-X7. In some embodiments, the first plasmid is pTx-IGF-1X6. In some embodiments, the first plasmid is pTx-IGF-1X10. In some embodiments, the first plasmid is pTx-IGF-1Ec. In some embodiments, the first plasmid is pTx-IGF-1Ea. In some embodiments, the first plasmid is pCK-SDF-1α.

[0048] In some embodiments, the step of lyophilizing comprises: (i) loading the liquid composition; (ii) freezing; (iii) primary drying; and (iv) secondary drying.

[0049] In some embodiments, the step of loading is performed at 5°C.

[0050] In some embodiments, the step of freezing is performed while raising temperatures in the range from -50 °C to -20 °C.

[0051] In some embodiments, the step of primary drying is performed at -20 °C.

[0052] In some embodiments, the step of secondary drying is performed while raising temperatures in the range from -20 °C to 20 °C.

[0053] In some embodiments, at least 90% of the plasmid DNA in the liquid composition is supercoiled. In some embodiments, at least 92.5% of the plasmid DNA in the liquid composition is supercoiled. In some embodiments, at least 95% of the plasmid DNA in the liquid composition is supercoiled. In some embodiments, at least 97% of the plasmid DNA in the liquid composition is supercoiled.

[0054] In some embodiments, at least 90% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition. In some embodiments, at least 92.5% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition. In some embodiments, at least 95% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition. In some embodiments, at least 97% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition. In some embodiments, at least 98% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition.

[0055] In some embodiments, at least 90% of the plasmid DNA remains supercoiled after storage at 25°C for 3 to 7 days following reconstitution of the lyophilized pharmaceutical composition. In some embodiments, at least 92.5% of the plasmid DNA remains supercoiled after storage at 25°C for 3 to 7 days following reconstitution of the lyophilized pharmaceutical composition. In some embodiments, at least 95% of the plasmid DNA remains supercoiled after storage at 25°C for 3 to 7 days following reconstitution of the lyophilized pharmaceutical composition.

[0056] In some embodiments, at least 80% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition, wherein the lyophilized pharmaceutical composition had been stored at 40°C for 10 weeks before reconstitution.

[0057] In some embodiments, the lyophilized pharmaceutical composition comprises supercoiled DNA in an amount of at least 90% of a total amount of supercoiled DNA in the liquid composition. In some embodiments, the lyophilized pharmaceutical composition comprises supercoiled DNA in an amount of at least 92.5% of a total amount of supercoiled DNA in the liquid composition. In some embodiments, the lyophilized pharmaceutical composition comprises supercoiled DNA in an amount of at least 95% of a total amount of supercoiled DNA in the liquid composition.

[0058] In some embodiments, the liquid composition comprises the plasmid at a concentration in the range from 0.1 to 1 mg/ml. In some embodiments, the liquid composition comprises the first plasmid at a concentration in the range from 0.25 to 0.75 mg/ml. In some embodiments, the liquid composition comprises the first plasmid at a concentration in the range from 0.4 to 0.6 mg/ml. In some embodiments, the liquid composition comprises the first plasmid at a concentration of 0.5 mg/ml.

[0059] In some embodiments, the liquid composition comprises potassium phosphate at a concentration in the range from 5 mM to 15 mM. In some embodiments, the liquid composition comprises potassium phosphate at a concentration in the range from 7.5 mM to 12.5 mM. In some embodiments, the liquid composition comprises potassium phosphate at a concentration in the range from 9 mM to 11 mM. In some embodiments, the liquid composition comprises potassium phosphate at a concentration of 10 mM.

[0060] In some embodiments, the liquid composition comprises potassium phosphate buffer with pH in the range from 7.0 to 8.5. In some embodiments, the liquid composition comprises potassium phosphate buffer with pH in the range from 7.0 to 8.0. In some embodiments, the liquid composition comprises potassium phosphate buffer of pH 8.0.

[0061] In some embodiments, the liquid composition comprises mannitol at a concentration in the range from 1.5% to 3% (w/v). In some mebodiments, the liquid composition comprises mannitol at a concentration in the range from 2% to 3% (w/v). In some embodiments, the liquid composition comprises mannitol at a concentration of 2 % (w/v).

[0062] In some embodiments, the liquid composition comprises sucrose at a concentration greater than 0.75 % and less than 1.1 % (w/v). In some embodiments, the liquid composition

comprises sucrose at a concentration greater than 0.9 % and less than 1.1 % (w/v). In some embodiments, the liquid composition comprises sucrose at a concentration of 1.0 % (w/v).

[0063] In some embodiments, the liquid composition comprises NaCl at a concentration in the range from 0.1 % to 0.75% (w/v). In some embodiments, the liquid composition comprises NaCl at a concentration in the range from 0.1 % to 0.6 %. In some embodiments, the liquid composition comprises NaCl at a concentration in the range from 0.4% to 0.5% (w/v). In some embodiments, the liquid composition comprises NaCl at a concentration of 0.45 % (w/v).

[0064] Another aspect of the present invention relates to a method of treating a disease, comprising the step of: administering an effective amount of a reconstituted drug to a patient with the disease, wherein the reconstituted drug is generated by reconstituting the lyophilized pharmaceutical composition of the present invention.

[0065] In some embodiments, the method further comprises the step of dissolving the lyophilized pharmaceutical composition in water, thereby generating the reconstituted drug.

[0066] In some embodiments, light absorbance of the reconstituted drug at 450 nm (A450) is less than 0.003. In some embodiments, the light absorbance is less than 0.002. In some embodiments, the light absorbance is 0.001 or less than 0.001.

[0067] In some embodiments, the disease is selected from the group consisting of neuropathy, ischemic disease, muscle atrophy, vascular disease, and a heart disease. In some embodiments, the disease is selected from ischemic limb disease, diabetic peripheral neuropathy (DPN), amyotrophic lateral sclerosis (ALS), peripheral vascular disease, and coronary artery disease (CAD).

[0068] In some embodiments, the step of administering comprises an intramuscular injection of the reconstituted drug.

5. BRIEF DESCRIPTION OF THE DRAWINGS

[0069] **Figure 1** provides an exemplary result from capillary electrophoresis (CE) of VM202. The result shows two peaks – one for the supercoiled form and the other for the open circle form.

[0070] **Figures 2A-2D** provide subambient DSC analysis results for the liquid state of various formulations from 1st round small scale lyophilization cycle with or without the annealing step.

Specifically, **Figure 2A** provides a result for KP8M2SN, **Figure 2B** provides a result for KP8MS3N, **Figure 2C** provides a result for KP8MT3N, and **Figure 2D** provides a result for Control. The glass transition temperature (Tg'), the eutectic melting temperature (Te), and the devitrification temperature (Td) are marked on the graphs.

[0071] Figure 3 provides a time-lapse change of temperatures (y-axis on the left) and pressures (y-axis on the right) during 1st round lyophilization cycle of KP8M2SN, KP8MS3N, KP8MT3N, and Control.

[0072] Figures 4A-4C provide pictures of vials containing various formulations from the 1st round small scale lyophilization cycle before lyophilization (**Figure 4A**), after lyophilization (**Figure 4B**), and after reconstitution (**Figure 4C**).

[0073] Figure 5 provides a result from capillary electrophoresis (CE) of VM202 formulations from 1st round small scale lyophilization cycle before lyophilization or after reconstitution.

[0074] Figures 6A-6D provide subambient DSC analysis results for the liquid state of various formulations from 2nd round small scale lyophilization cycle with or without the annealing step. Specifically, **Figure 6A** provides a result for 4MSN, **Figure 6B** provides a result for 3MSN, **Figure 6C** provides a result for 2MSN, and **Figure 6D** provides a result for Control.

[0075] Figure 7 provides a time-lapse change of temperatures (y-axis on the left) and pressures (y-axis on the right) during 2nd round lyophilization cycle of the first set of 4MSN, 3MSN, 2MSN, and Control.

[0076] Figures 8A-8C provide pictures of vials containing the first set of 4MSN, 3MSN, 2MSN, and Control from the 2nd round small scale lyophilization cycle before lyophilization (**Figure 8A**), after lyophilization (**Figure 8B**), and after reconstitution (**Figure 8C**).

[0077] Figure 9 provides a result from capillary electrophoresis (CE) of the first set of 4MSN, 3MSN, 2MSN, and Control from 2nd round small scale lyophilization cycle before lyophilization or after reconstitution.

[0078] Figure 10 provides a time-lapse change of temperatures (y-axis on the left) and pressures (y-axis on the right) during the second of 2nd round lyophilization cycle of the second set of 4MSN, 3MSN, 2MSN, and Control.

[0079] Figures 11A-11B provide pictures of vials containing the second set of 4MSN, 3MSN, 2MSN, and Control from the second of 2nd round small scale lyophilization cycle before lyophilization (**Figure 11A**) and after lyophilization (**Figure 11B**). **Figures 11C-11F** show pictures of vials containing the second set of 4MSN (**Figure 11C**), 3MSN (**Figure 11D**), 2MSN (**Figure 11E**), and Control (**Figure 11F**) after reconstitution.

[0080] Figure 12 provides a result from capillary electrophoresis (CE) of the second set of 4MSN, 3MSN, 2MSN, and Control from the second of 2nd round small scale lyophilization cycle before lyophilization or after reconstitution.

[0081] Figure 13 provides a time-lapse change of temperatures (y-axis on the left) and pressures (y-axis on the right) during lyophilization cycle of 2MSN and 2M1SN.

[0082] Figures 14A-14B provide pictures of vials containing 2MSN or 2M1SN before lyophilization (**Figure 14A**) and after lyophilization (**Figure 14B**). **Figures 14C-14D** show pictures of vials containing 2MSN (**Figure 14C**) and 2M1SN (**Figure 14D**) after reconstitution.

[0083] Figure 15 provides a result from capillary electrophoresis (CE) of 2MSN and 2M1SN before lyophilization or after reconstitution.

[0084] Figures 16A-16B provide pictures of vials containing 4MSN, 3MSN, 2MSN, and Control after storage at 25°C for 3 days (**Figure 16A**) or 7 days (**Figure 16B**) following reconstitution at T=0.

[0085] Figures 17A-17B provide results from capillary electrophoresis (CE) of 4MSN, 3MSN, 2MSN and Control following storage at 25°C for 3 days (**Figure 17A**) or 7 days (**Figure 17B**).

[0086] Figure 18A-18C provide pictures of vials containing 4MSN, 3MSN, 2MSN, and Control before or after reconstitution of the lyophilized formulation stored for 10 weeks at 25°C (**Figure 18A**), at 40°C (**Figure 18B**) or at 5°C (**Figure 18C**).

[0087] Figure 19 provides a result from capillary electrophoresis (CE) of 4MSN, 3MSN, 2MSN, and Control after storage at 40°C for 10 weeks.

[0088] Figures 20A-20C provide time-lapse changes of supercoiled DNA percentages measured based on capillary electrophoresis (CE) results for 4MSN, 3MSN, 2MSN and Control, when the formulations were stored at 5°C (**Figure 20A**), 25°C (**Figure 20B**) or 40°C (**Figure 20C**).

[0089] **Figures 21A-21C** provide time-lapse changes of open-circled DNA percentages measured based on capillary electrophoresis (CE) results for 4MSN, 3MSN, 2MSN and Control, when the formulations were stored at 5°C (**Figure 21A**), 25°C (**Figure 21B**) or 40°C (**Figure 21C**).

[0090] **Figures 22A-22B** provide pictures of vials containing 2MSN (**Figure 22A**) or 2M1SN (**Figure 22B**) stored at 25°C for 3 days after reconstitution. **Figures 22C-22D** provide pictures of vials containing 2MSN (**Figure 22C**) or 2M1SN (**Figure 22D**) stored at 25°C for 7 days after reconstitution.

[0091] **Figures 23A-23B** provide results from capillary electrophoresis (CE) of 2MSN and 2M1SN after storage at 25°C for 3 days (**Figure 23A**) or 7 days (**Figure 23B**).

[0092] **Figures 24A-24C** provide pictures of vials containing 2MSN or 2M1SN before or after reconstitution of the lyophilized formulation stored for 10 weeks at 25°C (**Figure 24A**), at 40°C (**Figure 24B**), or at 5°C (**Figure 24C**).

[0093] **Figure 25** provides a result from capillary electrophoresis (CE) of 2MSN and 2M1SN after storage at 40°C for 10 weeks

[0094] **Figures 26A-26C** provide time-lapse changes of supercoiled DNA percentages measured based on capillary electrophoresis (CE) results for 2MSN and 2M1SN, when the formulations were stored at 5°C (**Figure 26A**), 25°C (**Figure 26B**) or 40°C (**Figure 26C**).

[0095] **Figures 27A-27C** provide time-lapse changes of open-circled DNA percentages measured based on capillary electrophoresis (CE) results for 2MSN and 2M1SN, when the formulations were stored at 5°C (**Figure 27A**), 25°C (**Figure 27B**) or 40°C (**Figure 27C**).

[0096] **Figure 28** provide a time-lapse change of temperatures (y-axis on the left) and pressures (y-axis on the right) during lyophilization cycle used in Example 2.

[0097] **Figure 29** provides pictures of vials containing F1 of Example 2 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0098] **Figure 30** provides pictures of vials containing F2 of Example 2 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0099] Figure 31 provides pictures of vials containing F3 of Example 2 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0100] Figure 32 provides pictures of vials containing F4 of Example 2 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0101] Figure 33 provides pictures of vials containing F5 of Example 2 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0102] Figure 34 provides pictures of vials containing F6 of Example 2 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0103] Figure 35 provides a result from capillary electrophoresis (CE) of F1, F2, F3, F4, and F5 of Example 2.

[0104] Figure 36 provides pictures of vials containing F1 of Example 3 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0105] Figure 37 provides pictures of vials containing F2 of Example 3 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0106] Figure 38 provides pictures of vials containing F3 of Example 3 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0107] Figure 39 provides pictures of vials containing F4 of Example 3 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0108] Figure 40 provides pictures of vials containing F5 of Example 3 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0109] Figure 41 provides pictures of vials containing F6 of Example 3 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0110] Figure 42 provides pictures of vials containing F7 of Example 3 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0111] Figure 43 provides pictures of vials containing F8 of Example 3 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0112] Figure 44 provides pictures of vials containing F9 of Example 3 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0113] Figure 45 provides a result from capillary electrophoresis (CE) of F1, F2, F3, F4, F5, F6, F7, F8, and F9 of Example 3 (TABLE 40).

[0114] Figure 46 provides pictures of vials containing F1 of Example 4 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0115] Figure 47 provides pictures of vials containing F2 of Example 4 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0116] Figure 48 provides pictures of vials containing F3 of Example 4 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0117] Figure 49 provides pictures of vials containing F4 of Example 4 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0118] Figure 50 provides pictures of vials containing F5 of Example 4 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0119] Figure 51 provides pictures of vials containing F6 of Example 4 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0120] Figure 52 provides pictures of vials containing F7 of Example 4 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0121] Figure 53 provides pictures of vials containing F8 of Example 4 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0122] Figure 54 provides pictures of vials containing F9 of Example 4 before or after reconstitution of the lyophilized formulation over white background (left) or black background (right).

[0123] Figure 55 provides a result from capillary electrophoresis (CE) of F1, F2, F3, F4, F5, F6, F7, F8, and F9 of Example 4 (TABLE 44).

[0124] The figures depict various embodiments of the present invention for purposes of illustration only. One skilled in the art will readily recognize from the following discussion that alternative embodiments of the structures and methods illustrated herein may be employed without departing from the principles of the invention described herein.

6. DETAILED DESCRIPTION

6.1. Definitions

[0125] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them below.

[0126] The term "**liquid composition**" as used herein refers to a composition in liquid form that comprises plasmid DNA and at least one pharmaceutically acceptable excipient, and that can be lyophilized to generate a lyophilized pharmaceutical composition as described herein.

[0127] The term "**lyophilized composition**" or "**lyophilized pharmaceutical composition**" as used herein refers to any composition or pharmaceutical composition in dry form that is prepared by lyophilization. **"Lyophilizing"** or **"lyophilization"** have the meanings understood by those skilled in the art, referring broadly to any process of freezing followed by dehydration in the frozen state under vacuum. Lyophilized compositions can be reconstituted for injection.

[0128] The term "**reconstituted**" or "**reconstitution**" as used herein refers to the restoration to the original form of a substance previously altered for preservation and storage, such as rehydration, *i.e.*, the restoration to a liquid state of a DNA plasmid formulation that has been previously lyophilized and stored. The lyophilized composition of the present invention may be reconstituted in any aqueous solution which produces a stable solution suitable for pharmaceutical administration. Such aqueous solutions include, but are not limited to, sterile water, Tris-EDTA (TE), phosphate-buffered saline (PBS), Tris buffer and normal saline.

[0129] The term "**isolated**" or "**biologically pure**" as used herein refers to material that is substantially free from components which normally accompany the material as it is found in its native state. Thus, isolated plasmid DNA as used herein is substantially free from components normally associated with the plasmid DNA in its *in situ* environment, such as bacterial proteins, lipids, or cell wall components.

[0130] The term "**VM202**" as used herein refers to a plasmid DNA also called as pCK-HGF-X7, comprising pCK vector (SEQ ID NO: 5) and HGF-X7 (SEQ ID NO: 13) cloned into the pCK vector. VM202 was deposited under the terms of the Budapest Treaty at the Korean Culture Center of Microorganisms (KCCM) under accession number KCCM-10361 on March 12, 2002.

[0131] The term "**isoforms of HGF**" as used herein refers to a polypeptide having an amino acid sequence that is at least 80% identical to the amino acid sequence of a naturally occurring HGF polypeptide in an animal. The term includes polypeptides having an amino acid sequence that is at least 80% identical to any full length wild type HGF polypeptide, and includes polypeptides having an amino acid sequence that is at least 80% identical to a naturally occurring HGF allelic variant, splice variant, or deletion variant. Isoforms of HGF preferred for use in the present invention include two or more isoforms selected from the group consisting of full-length HGF (flHGF) (synonymously, fHGF), deleted variant HGF (dHGF), NKl, NK2, and NK4. According

to a more preferred embodiment of the present invention, the isoforms of HGF used in the methods described herein include flHGF (SEQ ID NO: 1) and dHGF (SEQ ID NO: 2).

[0132] The terms "**human flHGF**", "**flHGF**" and "**fHGF**" are used interchangeably herein to refer to a protein consisting of amino acids 1-728 of the human HGF protein. The sequence of flHGF is provided in SEQ ID NO: 1.

[0133] The terms "**human dHGF**" and "**dHGF**" are used interchangeably herein to refer to a deleted variant of the HGF protein produced by alternative splicing of the human HGF gene. Specifically, "human dHGF" or "dHGF" refers to a human HGF protein with deletion of five amino acids (F, L, P, S, and S) in the first kringle domain of the alpha chain from the full length HGF sequence. Human dHGF is 723 amino acids in length. The amino acid sequence of human dHGF is provided in SEQ ID NO: 2.

[0134] The term "**isoform of IGF-1**," "**human IGF-1 isoform**" or "**IGF-1 isoform**" as used herein refers to a polypeptide having an amino acid sequence that is at least 80% identical to the amino acid sequence of one of naturally occurring pre-pro-IGF-1 polypeptides of humans, or their allelic variant, splice variant, or deletion variant. The naturally occurring pre-pro-IGF-1 polypeptides include Class I, Ec (SEQ ID NO: 25); Class II, Ea (SEQ ID NO: 27); Class I, Eb (SEQ ID NO: 29); and Class I, Ea isoforms (SEQ ID NO: 23).

[0135] The terms "**Isoform #1**," "**Class I, Ec isoform,**" "**Class I, IGF-1 Ec isoform**" or "**Class I, IGF-1 Ec**" are used interchangeably herein to refer to a polypeptide of SEQ ID NO: 25.

[0136] The terms "**Isoform #2**," "**Class II, Ea isoform,**" "**Class II, IGF-1 Ea isoform**" or "**Class II, IGF-1 Ea**" are used interchangeably herein to refer to a polypeptide of SEQ ID NO: 27.

[0137] The terms "**Isoform #3**," "**Class I, Eb isoform,**" "**Class I, IGF-1 Eb isoform**" or "**Class I, IGF-1 Eb**" are used interchangeably herein to refer to a polypeptide of SEQ ID NO: 29.

[0138] The terms "**Isoform #4**," "**Class I, Ea isoform,**" "**Class I, IGF-1 Ea isoform**" or "**Class I, IGF-1 Ea**" are used interchangeably herein to refer to a polypeptide of SEQ ID NO: 23.

[0139] The term "**treatment**" as used herein refers to at least one of (a) suppressing a symptom of a disease; (b) alleviating a symptom of a disease; and (c) eliminating a symptom of a disease.

In some embodiments, the composition of the present invention can treat a symptom associated with neuropathy, ischemic disease, muscle atrophy or a heart disease.

[0140] The term "**therapeutically effective dose**" or "**effective amount**" as used herein refers to a dose or an amount that produces the desired effect for which it is administered. In the context of the present methods, a therapeutically effective amount is an amount effective to treat a symptom of a disease. The exact dose or amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see, e.g.*, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

[0141] The term "**sufficient amount**" as used herein refers to an amount sufficient to produce a desired effect.

[0142] The term "**degenerate sequence**" as used herein refers to a nucleic acid sequence that can be translated to provide an amino acid sequence identical to that translated from the reference nucleic acid sequence.

6.2. Other interpretational conventions

[0143] Ranges recited herein are understood to be shorthand for all of the values within the range, inclusive of the recited endpoints. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50.

[0144] Unless otherwise indicated, reference to a compound that has one or more stereocenters intends each stereoisomer, and all combinations of stereoisomers, thereof.

6.3. Lyophilized pharmaceutical composition

[0145] In a first aspect, lyophilized pharmaceutical compositions are presented. The lyophilized pharmaceutical compositions comprise plasmid DNA, and are obtained by lyophilizing a liquid composition that comprises, prior to lyophilization:

a. plasmid DNA of a first plasmid;
b. potassium phosphate buffer with pH from 7.0 to 9.0;

c. mannitol at a concentration from 0% to 3% (w/v);

d. sucrose at a concentration greater than 0.5% and less than 1.1% (w/v); and

e. NaCl at a concentration from 0.1 % to 0.9% (w/v).

[0146] The first plasmid can be selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

6.3.1. Plasmid DNA

[0147] The liquid composition includes plasmid DNA, which is an active ingredient of the pharmaceutical composition. The plasmid DNA can include a genetic material for gene therapy. Specifically, the plasmid DNA can encode a gene product that can correct the function of a defective gene or transcript, or encode polypeptides, sense or antisense oligonucleotides, or RNAs (coding or non-coding; *e.g.*, siRNAs, shRNAs, micro-RNAs, and their antisense counterparts (*e.g.*, antagoMiR)). A formulation containing any plasmid DNA known in the art to be used for gene therapy falls within the scope of the present invention.

[0148] The liquid composition can comprise the plasmid DNA at a concentration that allows subsequent reconstitution of the lyophilized pharmaceutical composition to provide a concentration effective for the therapeutic application of the reconstituted drug. The concentration of the plasmid DNA can be adjusted depending on various factors, including the amount of a composition to be delivered, diseases to be treated, the age and weight of the subject, the delivery method, and route of administration, *etc.*

[0149] Specifically, the liquid composition can include the plasmid DNA at a concentration from 0.1 to 5mg/ml, from 0.1 to 3mg/ml, from 0.1 to 2mg/ml, from 0.1 to 1mg/ml, from 0.25 to 0.75mg/ml, from 0.4 to 0.6mg/ml, or at a concentration of 0.5mg/ml.

[0150] The plasmid DNA can be a polynucleotide of a length from 3,000 to 15,000 base pairs, from 3,000 to 10,000 base pairs, from 3,000 to 9,000 base pairs, from 3,000 to 8,000 base pairs, from 3,000 to 7,000 base pairs, from 3,000 to 6,000 base pairs, from 3,000 to 5,000 base pairs, from 4,000 to 8,000 base pairs, from 4,000 to 7,500 base pairs, from 4,000 to 6,000 base pairs, from 6,000 to 9,000 base pairs, or from 7,000 to 8,000 base pairs. The plasmid DNA can be a polynucleotide of a length that falls within the scope provided herein.

6.3.1.1. vector

[0151] The plasmid DNA used in the methods of the present invention typically comprises a vector with one or more regulatory sequences (*e.g.*, a promoter or an enhancer) operatively linked to the expressed sequences. The regulatory sequence regulates expression of a protein (e.g., one or more isoforms of HGF or IGF-1).

[0152] It is preferred that the polynucleotide encoding a protein is operatively linked to a promoter in an expression construct. The term "operatively linked" refers to functional linkage between a nucleic acid expression control sequence (such as a promoter, signal sequence, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence affects transcription and/or translation of the nucleic acid corresponding to the second sequence.

[0153] In typical embodiments, the promoter linked to the polynucleotide is operable in, preferably, animal, more preferably, mammalian cells, to control transcription of the polynucleotide, including the promoters derived from the genome of mammalian cells or from mammalian viruses, for example, CMV (cytomegalovirus) promoter, the adenovirus late promoter, the vaccinia virus 7.5K promoter, SV40 promoter, HSV tk promoter, RSV promoter, EFl alpha promoter, metallothionein promoter, beta-actin promoter, human IL- 2 gene promoter, human IFN gene promoter, human IL-4 gene promoter, human lymphotoxin gene promoter and human GM-CSF gene promoter, but not limited thereto. More preferably, the promoter useful in this invention is a promoter derived from the IE (immediately early) gene of human CMV (hCMV) or EFl alpha promoter, most preferably hCMV IE gene-derived promoter/enhancer and 5' -UTR (untranslated region) comprising the overall sequence of exon 1 and exon 2 sequence spanning a sequence immediately before the ATG start codon.

[0154] The expression cassette used in this invention may comprise a polyadenylation sequence, for example, including bovine growth hormone terminator (Gimmi, E. R., et al., Nucleic Acids Res. 17:6983-6998 (1989)), SV40- derived polyadenylation sequence (Schek, N, et al., Mol. Cell Biol. 12:5386-5393 (1992)), HIV-1 polyA (Klasens, B. I. F., et al., Nucleic Acids Res. 26:1870-1876 (1998)), β-globin polyA (Gil, A., et al, Cell 49:399-406 (1987)), HSV TK polyA (Cole, C. N. and T. P. Stacy, Mol. Cell. 5 Biol. 5: 2104-2113 (1985)) or polyoma virus polyA (Batt, D. Band G. G. Carmichael, Mol. Cell. Biol. 15:4783-4790 (1995)), but not limited to.

[0155] In currently preferred embodiments, the vector is pCK, pCP, pVAXl, pTx or pCY. In particularly preferred embodiments, the vector is pCK, details of which can be found in WO 2000/040737 and Lee *et al.*, *Biochem. Biophys. Res. Comm.* 272:230–235 (2000), both of which are incorporated herein by reference in their entireties. *E. coli* transformed with pCK (Top10-pCK) was deposited at the Korean Culture Center of Microorganisms (KCCM) under the terms of the Budapest Treaty on March 21, 2003 (Accession NO: KCCM-10476). *E. coli* transformed with pCK-VEGF165 (*i.e.*, pCK vector with VEGF coding sequence – Top10-pCK/VEGF165') was deposited at the Korean Culture Center of Microorganisms (KCCM) under the terms of the Budapest Treaty on December 27, 1999 (Accession NO: KCCM-10179).

[0156] The pCK vector is constructed such that the expression of a gene, *e.g.*, an HGF gene or an IGF-1 gene, is regulated under enhancer/promoter of the human cytomegalovirus (HCMV), as disclosed in detail in Lee et al., Biochem. Biophys. Res. Commun. 272: 230 (2000); WO 2000/040737, both of which are incorporated by reference in their entirety. pCK vector has been used for clinical trials on human body, and its safety and efficacy were confirmed (Henry et al., Gene Ther. 18:788 (2011)).

[0157] In other preferred embodiments, the vector is pTx (SEQ ID NO: 15), a plasmid vector derived from pCK. pTx was generated by two sequential rounds of mutagenesis of pCK. The first deletion mutagenesis campaign was conducted to remove the unnecessary sequence between Kanamycin resistance gene and ColE1 of pCK. Specifically, deletion mutagenesis PCR was performed using a first primer pair (SEQ ID NOs: 17 and 18). The deletion of 228 base pairs between Kanamycin resistance and ColE1 was confirmed by sequencing the plasmid. The second deletion mutagenesis campaign was then performed using a second primer pair (SEQ ID NOs: 19 and 20), to optimize the size of HCMV intron sequence. HCMV intron sequence (421 base pairs) between IE1 exon 1 and exon 2 was deleted and the deletion was confirmed by sequencing.

6.3.1.2. plasmid DNA encoding a human HGF

[0158] In some embodiments, the plasmid DNA encodes a human HGF or a variant thereof.

[0159] Hepatocyte growth factor (HGF) is a heparin binding glycoprotein also known as scatterfactor or hepatopoietin-A. An endogenous gene encoding human HGF is located at

chromosome 7q21.1 and comprises 18 exons and 17 introns (Seki T., et al., Gene 102:213–219 (1991)). A transcript of about 6 kb is transcribed from the HGF gene, and then, a polypeptide HGF precursor (flHGF) consisting of 728 amino acids is synthesized therefrom. Simultaneously, a polypeptide of dHGF precursor consisting of 723 amino acids is also synthesized by an alternative splicing of the HGF gene. The biologically inactive precursors may be converted into active forms of disulfide-linked heterodimer by protease in serum. In the heterodimers, the alpha chain having a high molecular weight forms four kringle domains and an N-terminal hairpin loop like a preactivated peptide region of plasminogen. The kringle domains of a triple disulfide-bonded loop structure consisting of about 80 amino acids may play an important role in protein-protein interaction. The low molecular weight beta chain forms an inactive serine protease-like domain. dHGF consisting 723 amino acids is a polypeptide with deletion of five amino acids in the 1st kringle domain of the alpha chain, *i.e.*, F. L. P. S and S.

[0160] HGF has various biological functions, *e.g.*, 1) inducing epithelial cells into a tubular structure; 2) stimulating vascularization from endothelial cells *in vitro* and *in vivo*; 3) regeneration of liver and kidney, owing to its anti-apoptosis activity; 4) organogenesis of kidney, ovary and testis; 5) controlling osteogenesis; 6) stimulating the growth and differentiation of erythroid hematopoietic precursor cells; and 7) axon sprouting of neurons (Stella, M. C. and Comoglio, P. M., The International Journal of Biochemistry & Cell Biology 31:1357–1362 (1999)). Based on these various functions, HGF or a gene encoding HGF or a variant thereof, can be developed as a therapeutic agent.

[0161] In fact, plasmids encoding one or more isoforms of human HGF have been developed and used for treating various diseases as described in US Patent Nos. 7,812,146, 8,338,385 and 8,389,492, and US Publication Nos. 20140296142 and 20160250291, which are incorporated by reference in their entirety herein. The plasmids can be used in various embodiments of the present disclosure.

[0162] Specifically, the plasmid can express two or more isoforms of HGF by comprising an expression regulatory sequence for each isoform coding sequence (CDS). In some embodiments, the construct comprises an internal ribosomal entry site (IRES) between two coding sequences, for example, in the order of (1) expression regulatory sequence – (2) coding sequence of first isoform – (3) IRES – (4) coding sequence of second isoform – (5) transcription termination

sequence. IRES allows translation to start at the IRES sequence, thereby allowing expression of two genes of interest from a single construct. In yet further embodiments, a plurality of constructs, each encoding a single isoform of HGF, are used together to induce expression of more than one isoforms of HGF in the subject to whom administered.

[0163] Preferred embodiments of the methods use a construct that simultaneously expresses two or more different types of isoforms of HGF – *i.e.,* flHGF and dHGF – by comprising an alternative splicing site. It was previously demonstrated in U.S. Patent No. 7,812,146, incorporated by reference herein, that a construct encoding two isoforms of HGF (flHGF and dHGF) through alternative splicing has much higher (almost 250-fold higher) expression efficiency than a construct encoding one isoform of HGF (either flHGF or dHGF). In typical embodiments, the construct comprises (i) a first sequence comprising exons 1-4 of a human HGF gene (SEQ ID NO: 3) or a degenerate sequence of the first sequence; (ii) a second sequence comprising intron 4 of the human HGF gene (SEQ ID NO: 6) or a fragment of the second sequence; and (iii) a third sequence comprising exons 5-18 of the human HGF gene (SEQ ID NO: 4) or a degenerate sequence of the third sequence. From the construct, two isoforms of HGF (flHGF and dHGF) can be generated by alternative splicing between exon 4 and exon 5.

[0164] In some embodiments, the construct comprises a full sequence of intron 4. In some embodiments, the construct comprises a fragment of intron 4. In preferred embodiments, the construct comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 7 to SEQ ID NO: 14. The nucleotide sequence of SEQ ID NO:7 corresponds to a 7113bp polynucleotide encoding flHGF and dHGF, and including the full sequence of intron 4. The nucleotide sequences of SEQ ID NOS: 8-14 correspond to polynucleotides encoding flHGF and dHGF and including various fragments of intron 4.

[0165] Various nucleic acid constructs comprising cDNA corresponding exon 1-18 of human HGF and intron 4 of a human HGF gene or its fragment are named "HGF-X" followed by a unique number as described in U.S. Patent No. 7,812,146. The HGF-X tested by Applicant includes, but not limited to, HGF-X1 (SEQ ID NO:7), HGF-X2 (SEQ ID NO:8), HGF-X3 (SEQ ID NO:9), HGF-X4 (SEQ ID NO:10), HGF-X5 (SEQ ID NO: 11), HGF-X6 (SEQ ID NO:12), HGF-X7 (SEQ ID NO:13), and HGF-X8 (SEQ ID NO:14).

[0166] It was previously demonstrated that two isoforms of HGF (*i.e.,* flHGF and dHGF) can be generated by alternative splicing between exon 4 and exon 5 from each of the constructs. In addition, among the various HGF constructs, HGF-X7 showed the highest level of expression of two isoforms of HGF (*i.e.*, flHGF and dHGF) as disclosed in U.S. Pat. No. 7,812,146, incorporated by reference in its entirety herein. Accordingly, a nucleic acid construct comprising HGF-X7 can be used in preferred embodiments of the methods of the present invention.

[0167] In particularly preferred embodiments, the pCK plasmid containing the HGF-X7 expression sequences is used as the nucleic acid construct in the methods of the present invention. Specifically, pCK-HGF-X7 (also called "VM202") can be used. pCK-HGF-X7 is a construct comprising pCK vector (SEQ ID NO: 5) and HGF-X7 (SEQ ID NO: 13) cloned into the pCK vector. pCK-HGF-X7 was deposited under the terms of the Budapest Treaty at the Korean Culture Center of Microorganisms (KCCM) under accession number KCCM-10361 on March 12, 2002.

[0168] The amino acid sequences and nucleotide sequences of HGF isoforms used in the methods described herein may further include amino acid sequences and nucleotide sequences substantially identical to sequences of the wild type human HGF isoforms. The substantial identity includes sequences with at least 80% identity, more preferably at least 90% identity and most preferably at least 95% identity where the amino acid sequence or nucleotide sequence of the wild type human HGF isoform is aligned with a sequence in the maximal manner. Methods of alignment of sequences for comparison are well-known in the art. Specifically, alignment algorithm disclosed in the NCBI Basic Local Alignment Search Tool (BLAST) of the National Center for Biological Information (NBCl, Bethesda, Md.) website and used in connection with the sequence analysis programs blastp, blasm, blastx, tblastn and tblastx can be used to determine the percent identity.

6.3.1.3. plasmid DNA encoding IGF-1

[0169] In some embodiments, the plasmid DNA encodes a human IGF-1 or a variant thereof.

[0170] Insulin-like growth factor 1 (IGF-1) is a hormone similar in molecular structure to insulin which plays an important role in childhood growth, and has anabolic effects in adults. The human IGF-1 gene contains six exons (exons 1, 2, 3, 4, 5, and 6 (6-1 and 6-2)) spanning nearly

90kb of genomic DNA. Exons 1 and 2 are mutually exclusive leader exons, each having multiple promoter sites that are variably used. Further, the IGF-1 gene can be differentially spliced to create multiple transcript variants. Each transcript variant encodes a different pre-pro-IGF-1 protein ("IGF-1 isoform") possessing variable signaling peptide leader sequences. Yet all the transcript isoforms give rise to the same mature 70-amino acid IGF-1 peptide that uses the same receptor after processing.

[0171] The pre-pro-IGF-1 peptides differ in their leader, or signal, sequences and in their carboxy (C) -terminus. Incorporation of exon 1 or exon 2 is mutually exclusive and one of them serves as a leader sequence of the pre-pro-IGF-1 peptide; the different leader exons create different 5'-UTRs. The pre-pro-IGF-1 polypeptides undergo posttranscriptional proteolytic cleavage to remove the leader and the E-peptide carboxy-terminus giving rise to the mature 70-amino acid IGF-1.

[0172] Transcripts containing exon 1 are referred to as Class 1 transcripts (*e.g.,* Class I, Ec; Class I, Eb; and Class I, Ea) whereas those containing exon 2 are referred to as Class 2 transcripts (*e.g.,* Class II, Ea). Nearly all pre-pro peptides include 27 amino acids in the signaling peptide derived from exon 3 with the remaining signal sequences derived from the inclusion of exon 1 or 2. A minority of transcripts utilize a different transcription initiation site within exon 3 generating a shorter signaling peptide of 22 amino acids. Exons 3 and 4 are invariant and encode the B, C, A, and D domains of the mature IGF-1 peptide; exon 4 encodes two thirds of the mature IGF-1 peptide. The human Eb peptide is composed of only exons 4 and 5 whereas Ec contains exons 4, 5, and 6.

[0173] Alternative splicing and mutually exclusive initiation of transcription result in generation of different pre-pro-IGF-1 polypeptides (*i.e.,* IGF-1 isoforms). Specifically, Class I, Ec IGF-1 isoform (SEQ ID NO: 25), comprising at least a fragment of exons 1, 3/4, 5 and 6, is generated from a transcript comprising a sequence of SEQ ID NO: 26. Class II, Ea IGF-1 isoform (SEQ ID NO:27), comprising at least a fragment of exons 2, 3/4 and 6, is generated from a transcript comprising a sequence of SEQ ID NO:28. Class I, Eb IGF-1 isoform (SEQ ID NO:29), comprising at least a fragment of exons 1, 3/4 and 5, is generated from a transcript comprising a sequence of SEQ ID NO:30. Class I, Ea IGF-1 isoform (SEQ ID NO:23), comprising at least a

fragment of exons 1, 3/4 and 6 are generated from a transcript comprising a sequence of SEQ ID NO: 24.

[0174] Although the mature IGF-1 protein derived from the various transcripts does not differ, the various transcript isoforms have been suggested to have different regulatory roles. The variant forms possess different stabilities, binding partners, and activity indicating a pivotal regulatory role for the isoforms. The biological significance of the isoforms remains unclear, although it has been hypothesized that Class I isoforms with exon 1 are autocrine/paracrine forms while Class II isoforms with exon 2 are secreted endocrine forms. This is based on the finding that Class II transcripts include a typical signal peptide motif associated with efficient secretion, whereas Class I transcripts have a longer signal peptide that can possibly interfere with secretion.

[0175] Plasmids encoding one or more isoforms of human IGF-1 have been developed and tested for treating neuropathy as described in US Application Nos. 16/513,560 and/or 16/513,564, which are incorporated by reference in their entirety herein. The plasmids can be used in various embodiments of the present disclosure.

[0176] Specifically, in some embodiments, the plasmid contains a coding sequence of one of the IGF-1 isoforms. For example, the DNA construct can comprise a sequence encoding Class I, Ea (SEQ ID NO: 24); Class I, Eb (SEQ ID NO:30); Class I, Ec (SEQ ID NO:26); or Class II, Ea (SEQ ID NO:28).

[0177] In some embodiments, the DNA construct is a dual expression construct, a DNA construct that can express more than one IGF-1 isoforms, by comprising an expression regulatory sequence for each isoform coding sequence (CDS). In some embodiments, the construct comprises an internal ribosomal entry site (IRES) between two coding sequences, for example, in the order of (1) expression regulatory sequence – (2) coding sequence of first isoform – (3) IRES – (4) coding sequence of second isoform – (5) transcription termination sequence. IRES allows translation to start at the IRES sequence, thereby allowing expression of two protein products from a single transcript. In yet further embodiments, a plurality of constructs, each encoding a single isoform of IGF-1, are used together to induce expression of more than one isoforms of IGF-1 in the subject to whom administered.

[0178] In preferred embodiments, a DNA construct is capable of expressing two or more IGF-1 isoforms simultaneously – *e.g.,* (i) Class I, Ec isoform (Isoform #1) and Class II, Ea isoform (Isoform #2); (ii) Class I, Ec isoform (Isoform #1) and Class I, Eb isoform (Isoform #3); (iii) Class I, Ec isoform (Isoform #1) and Class I, Ea isoform (Isoform #4); (iv) Class II, Ea isoform (Isoform #2) and Class I, Eb isoform (Isoform #3); (v) Class II, Ea isoform (Isoform #2) and Class I, Ea isoform (Isoform #4); (vi) Class I, Eb isoform (Isoform #3) and Class I, Ea isoform (Isoform #4) – by comprising an alternative splicing site.

[0179] For example, the DNA construct can comprise (i) a first sequence comprising exons 1, 3 and 4 of a human IGF-1 gene (SEQ ID NO:31) or a degenerate sequence of the first sequence; (ii) a second sequence comprising intron 4 of the human IGF-1 gene (SEQ ID NO:32) or a fragment of the second sequence; (iii) a third sequence comprising exons 5 and 6-1 of the human IGF-1 gene (SEQ ID NO:33) or a degenerate sequence of the third sequence; (iv) a fourth sequence comprising intron 5 of the human IGF-1 gene (SEQ ID NO:34) or a fragment of the second sequence; and (v) a fifth sequence comprising exon 6-2 of the human IGF-1 gene (SEQ ID NO:35) or a degenerate sequence of the fifth sequence. Introns 4 and 5 can be alternatively spliced, resulting in production of two isoforms of IGF-1 (*e.g.,* Class I, Ec and Class I, Ea).

[0180] In some embodiments, the DNA construct is tested *in vitro* and/or *in vivo* related to its capability to express one or more IGF-1 isoforms. In preferred embodiments, DNA constructs capable of expressing both Class I, Ec and Class I, Ea IGF-1 isoforms are selected.

[0181] Various DNA constructs comprising cDNA corresponding (i) exons 1-6 of the human IGF-1 gene and (ii) introns 4 and 5 of the human IGF-1 gene or various fragments of introns 4 and 5 are named "IGF-1X" followed by a unique number. The IGF-1X constructs tested by Applicant include, but are not limited to, IGF-1X1, IGF-1X2, IGF-1X3, IGF-1X4, IGF-1X5, IGF-1X6, IGF-1X7, IGF-1X8, IGF-1X9 and IGF-1X10. Among the tested constructs, IGF-1X6 and IGF-1X10 were identified to express both Class I, Ec and Class I, Ea IGF-1 isoforms.

[0182] In preferred embodiments, IGF-1X6 (SEQ ID NO:21) or IGF-1X10 (SEQ ID NO:22) is used. IGF-1X6 (SEQ ID NO:21) and IGF-1X10 (SEQ ID NO:22) cloned into a pCK vector are named pCK-IGF-1X6 and pCK-IGF-1X10, respectively. *E.coli* cells transformed with pCK-IGF-1X6 ("DH5α_pCK-IGF1 X6") were deposited under the terms of the Budapest Treaty at the Korea Collection for Type Cultures (KCTC, Korea Research Institute of Bioscience and

Biotechnology (KRIBB) 181, Ipsin-gil, Jeongeup-si, Jeollabuk-do, 56212, Republic of Korea) with accession number KCTC 13539BP on May 30, 2018. *E.coli* cells transformed with pCK-IGF-1X10 ("DH5α_pCK-IGF1 X10") were deposited under the terms of the Budapest Treaty at the Korea Collection for Type Cultures (KCTC, Korea Research Institute of Bioscience and Biotechnology (KRIBB) 181, Ipsin-gil, Jeongeup-si, Jeollabuk-do, 56212, Republic of Korea) with accession number KCTC 13540BP on May 30, 2018.

[0183] In some embodiments, the pTx plasmid contains IGF-1X6 (*i.e.,* pTx-IGF-1X6) or IGF-1 X10 (*i.e.,* pTx-IGF-1X10). For example, pTx-IGF-1X10 (SEQ ID NOs: 16) generated by ligating IGF-1X10 in pTx digested with ClaI enzyme at 5' and Sal1 enzyme at 3' can be used.

[0184] In some embodiments, the pTx plasmid contains IGF-1Ec or IGF-1Ea. For example, pTx-IGF-1Ec is generated by ligating IGF-1Ec (SEQ ID NO: 26) in pTx, and pTx-IGF-1Ea is generated by ligating IGF-1Ea (SEQ ID NO: 24) in pTx. The plasmids express the IGF-1Ec isoform (SEQ ID NO:25) or IGF-1Ea isoform (SEQ ID NO:23), respectively.

[0185] IGF-1 isoforms or plasmids encoding IGF-1 isoforms described herein can include modifications from the wild type human IGF-1 isoforms. The modified sequences can include sequences with at least 80% identity, more preferably at least 90% identity and most preferably at least 95% identity when the modified sequences are aligned with the wild type human IGF-1 isoform sequences in the maximal manner. Methods of alignment of sequences for comparison are well-known in the art. Specifically, alignment algorithm disclosed in the NCBI Basic Local Alignment Search Tool (BLAST) of the National Center for Biological Information (NBCl, Bethesda, Md.) website and used in connection with the sequence analysis programs blastp, blasm, blastx, tblastn and tblastx can be used to determine the percent identity.

6.3.1.4. Plasmid DNA encoding SDF-1α

[0186] The stromal cell-derived factor 1 (SDF-1), also known as C-X-C motif chemokine 12 (CXCL12) is a chemokine protein that in human is encoded by the CXCL12 gene on chromosome 10. It is ubiquitously expressed in many tissues and cell types. Stromal cell-derived factors 1α (SDF-1α) and 1β (SDF-1β) are small cytokines that belong to the chemokine family, members of which activate leukocytes and are often induced by proinflammatory stimuli

such as lipopolysaccharide, TNF, or IL1. SDF-1 is produced in two forms, SDF-1α/CXCL12a and SDF-1β/CXCL12b, by alternate splicing of the same gene.

[0187] Plasmids encoding one or more isoforms of human SDF-1 have been developed and tested for treating peripheral vascular disease as described in US Application No. 15/514,244, incorporated herein by reference in its entirety. Specifically, a polynucleotide encoding the SDF-1α effectively promoted vascular endotherlial cell migration and angiogenesis when administered together with a polynucleotide encoding human HGF.

[0188] Plasmids encoding one or more isoforms of SDF-1 can be used in various embodiments of the present disclosure. In some embodiments, the plasmid encoding one or more SDF-1 disclosed in US Application No. 15/514,244 is used. In some embodiments, the plasmid comprising a coding sequence of SDF-1α is used. In some embodiments, the plasmid comprising a coding sequence of SDF-1β is used. In some embodiments, the plasmid encoding both SDF-1α and SDF-1β is used. In a particular embodiment, the plasmid comprising the sequence of SEQ ID NO: 36 is used.

6.3.2. Buffer

[0189] A liquid composition further comprises a buffer to maintain pH of the pharmaceutical composition. The buffer can include a buffer compound known in the art, such as TAPS, Bicine, Tris, Tricine, TAPSO, HEPES, TES, MPOS, PIPES, Cacodylate, or MES. The buffer can contain citric acid, monopotassium phosphate, boric acid, or diethyl barbituric acid. The buffer can be PBS, HEPES, TRIS or TRIS/EDTA buffer. The buffer can be other phosphate buffer. Phosphate buffers can comprise a mixture of monobasic dihydrogen phosphate and dibasic monohydrogen phosphate.

[0190] Specifically, the buffer can be potassium phosphate buffer. The potassium phosphate buffer can comprise potassium phosphate at a concentration from 5mM to 15mM, from 7.5mM to 12.5mM, from 9mM to 11mM, or at 10mM.

[0191] The buffer contained in the liquid composition can have pH from 7 to 9. In some embodiments, the pH is from 7.0 to 8.5 or 8.0. In some embodiments, the pH is from 7.0 to 8.0.

[0192] In some embodiments, the liquid composition comprises a 7.5-12mM potassium phosphate buffer of pH from 7 to 9. In some embodiments, the liquid composition comprises a

7.5-12mM potassium phosphate buffer of pH from 7.0 to 8.0. In some embodiments, the liquid composition comprises 9-11 mM potassium phosphate buffer of pH from 7.0 to 8.5. In some embodiments, the liquid composition includes 10mM potassium phosphate buffer at pH 8.0.

6.3.3. Carbohydrate

[0193] The stability of the plasmid DNA in the lyophilized pharmaceutical composition can be increased by formulating the plasmid DNA prior to lyophilization with an aqueous solution comprising a stabilizing amount of carbohydrate. The carbohydrate can be mannitol or sucrose.

[0194] The carbohydrate can be added to the liquid composition to have a final concentration of the carbohydrate about 0.05% to about 30%, about 0.1% to about 15%, about 0.2% to about 15%, about 0.2% to about 10%, about 0.5% to about 10%, about 1% to about 5%, about 1% to about 3%, about 0.75% and 1.1%, about 0.9% and 1.1%, about 1.0% or about 1.1%.

[0195] In some embodiments, the liquid composition has a final concentration of at least one carbohydrate, greater than 0.1% and less than 15%, greater than 0.2% and less than 10%, greater than 0.3% and less than 7.5%, greater than 0.5% and less than 5%, greater than 0.5% and less than 3%, greater than 0.5% and less than 2%, greater than 0.5% and less than 1.1%, greater than 0.75% and less than 1.1%, greater than 0.9% and less than 1.1%, or 1.0%.

[0196] Specifically, the liquid composition can have a final concentration of sucrose about 0.05% to about 30%, about 0.1% to about 15%, about 0.2% to about 15%, about 0.2% to about 10%, about 0.5% to about 10%, about 1% to about 5%, about 1% to about 3%, about 0.75% to about 1.1%, about 0.9% to about 1.1%, or about 1.0%.

[0197] In some embodiments, the liquid composition has a final concentration of sucrose, greater than 0.1% and less than 15%, greater than 0.2% and less than 10%, greater than 0.3% and less than 7.5%, greater than 0.5% and less than 5%, greater than 0.5% and less than 3%, greater than 0.5% and less than 2%, greater than 0.5% and less than 1.1%, greater than 0.75% and less than 1.1%, greater than 0.9% and less than 1.1%, or 1.0%.

[0198] In some embodiments, the liquid composition has a final concentration of mannitol less than 5%, less than 4%, less than 3%. In some embodiments, the liquid composition has a final concentration of mannitol from 0% to 10%, from 1% to 9%, from 2% to 7.5%, from 2% to 3%,

from 2% to 5%, from 2% to 4%, from 0% to 3%, from 0% to 2%, from 1% to 2%, from 1.5% to 3%, or 2%.

[0199] In some embodiments, the liquid composition has a final concentration of mannitol greater than 0% and less than 10%, greater than 0% and less than 7.5%, greater than 0% and less than 5%, greater than 0% and less than 4%, greater than 0% and less than 3% or greater than 0% and less than 2.5%, or greater than 1% and less than 2.5%.

[0200] In some embodiments, the liquid composition has a final concentration of sucrose greater than 0.5% and less than 1.1%, and a final concentration of mannitol greater than 0% and less than 3%. In some embodiments, the liquid composition has a final concentration of sucrose from 0.7% to 1.1%, and a final concentration of mannitol from 1.5% to 3% or 2%. In some embodiments, the liquid composition has a final concentration of sucrose from 0.9% to 1.1%, and a final concentration of mannitol from 1.5% to 3% or 2%. In some embodiments, the liquid composition has a final concentration of sucrose at 1.0% and a final concentration of mannitol from 1.5% to 3%, from 2% to 3%, or 2%.

[0201] In some embodiments, other carbohydrate is used in the composition. The carbohydrate can be a mono-, oligo-, or polysaccharide, such as sucrose, glucose, lactose, trehalose, arabinose, pentose, ribose, xylose, galac tose, hexose, idose, mannose, talose, heptose, fructose, glu conic acid, sorbitol, mannitol, methyl a-glucopyranoside, maltose, isoascorbic acid, ascorbic acid, lactone, Sorbose, glucaric acid, erythrose, threose, allose, altrose, gulose, erythrulose, ribulose, xylulose, psicose, tagatose, glucuronic acid, galacturonic acid, mannuronic acid, glucosamine, galactosamine, neuraminic acid, arabinans, fructans, fucans, galactans, galacturonans, glucans, mannans, xylans, levan, fucoidan, carrageenan, galactocarolose, pectins, pectic acids, amylose, pullulan, glycogen, amylopectin, cellulose, dext ran, cyclodextrin, pustulan, chitin, agarose, keratin, chon droitin, dermatan, hyaluronic acid, alginic acid, xantham gum, or starch.

6.3.4. Salt

[0202] The liquid composition further contains a salt. The salt can be NaCl or KCl.

[0203] In some embodiments, the liquid composition comprises a salt at a concentration greater than 0.1% and less than 0.9%, greater than 0.25% and less than 0.75%, greater than 0.4% and less than 0.6%, greater than 0.4% and 0.5% or at a concentration of 0.45%. In some

embodiments, the liquid composition comprises a salt at a concentration from 0.1% to 0.9%, from 0.1% to 0.6%, from 0.25% to 0.75%, from 0.4% to 0.6%, from 0.4% to 0.5% or at a concentration of 0.45%.

[0204] In some embodiments, the liquid composition comprises NaCl at a concentration greater than 0.1% and less than 0.9%, greater than 0.25% and less than 0.75%, greater than 0.4% and less than 0.6%, greater than 0.4% and 0.5% or at a concentration of 0.45%. In some embodiments, the liquid composition comprises NaCl at a concentration from 0.1% to 0.9%, from 0.1% to 0.6%, from 0.25% to 0.75%, from 0.4% to 0.6%, from 0.4% to 0.5% or at a concentration of 0.45%.

[0205] In some embodiments, the liquid composition comprises KCl at a concentration greater than 0.1% and less than 0.9%, greater than 0.25% and less than 0.75%, greater than 0.4% and less than 0.6%, greater than 0.4% and 0.5% or at a concentration of 0.45%. In some embodiments, the liquid composition comprises KCl at a concentration from 0.1% to 0.9%, from 0.25% to 0.75%, from 0.4% to 0.6%, from 0.4% to 0.5% or at a concentration of 0.45%.

[0206] In some embodiments, other salt is used in the composition. The salt can a monovalent cationic halide salt (*e.g.*, sodium chloride, potassium chloride, sodium bromide, lithium chloride, sodium iodide, potassium bromide, lithium bromide, sodium fluoride, potassium fluoride, lithium fluoride, and/or lithium iodide), a divalent or trivalent salt (*e.g.*, calcium chloride, magnesium chloride, calcium sulfate, sodium sulfate, magnesium sulfate, chromium trichloride, chromium sulfate, sodium citrate, Iron (III) chloride, Yttrium (III) chloride, potassium phosphate, potassium sulfate, potassium chloride, sodium acetate, sodium phosphate, potassium phosphate, ferrous chloride, ferric chloride), or a combination thereof.

6.4. Lyophilization conditions

[0207] A lyophilized composition of the present invention is generated by lyophilizing a liquid composition described herein. The liquid composition can be lyophilized under standard lyophilization conditions known in the art or modifications thereof.

[0208] A method for lyophilizing the liquid composition of the invention can comprise (a) loading a container with the liquid composition at a starting temperature of about 5° C to about —50° C; (b) cooling the DNA formulation to subzero temperatures (*e.g.*, -10° C to -50° C); and

(c) substantially drying the DNA formulation. The conditions for lyophilization, *e.g.*, temperature and duration, of the DNA formulation of the invention can be adjusted by a person of ordinary skill in the art taking into consideration factors that affect lyophilization parameters, *e.g.*, the type of lyophilization machine used, the amount of DNA used, and the size of the container used.

[0209] In some embodiments, the steps of (b) cooling and (c) drying are performed while changing temperatures. For example, the step of freezing can be performed while raising temperatures from -50 °C to -40 °C, from -50 °C to -30 °C, from -50 °C to -20 °C, from -50 °C to -10 °C, or from -50 °C to 0 °C. In some embodiments, the step of freezing can be performed while reducing temperatures from -40 °C to -50 °C, from -30 °C to -50 °C, from -20 °C to -50 °C, from -10 °C to -50 °C, from 0 °C to -50 °C, or from 5 °C to -50 °C. In some embodiments, the step of freezing is performed while reducing the temerature and then raising the temperature.

[0210] Some amorphous products (such as mannitol or glycine) form a metastable glass with incomplete crystallization when first frozen. These products can benefit from a thermal treatment process, which is also called annealing. During annealing, the product temperature is cycled (for example: from -40°C to -20°C for a few hours and then back to -40°C; from -50°C to -20°C, holding for two (2) hours, and then introducing the vaccum; or from -50°C to -20°C and then back to -50°C) to obtain more complete crystallization. Annealing has the added advantage of larger crystal growth and corresponding shorter drying times. Water trapped in the amorphous phase can be further removed during the secondary drying.

[0211] In some embodiments, the step of drying is performed in two steps – (i) primary drying and (ii) secondary drying.

[0212] In some embodiments, primary drying is performed while maintaining the temperature, or while raising or reducing temperatures. In some embodiments, primary drying is performed while maintaining the temperature at -50 °C, -40 °C, -30 °C, -20 °C, -10 °C, or 0 °C. In some embodiments, secondary drying is performed while maintaining the temperature, or while raising or reducing temperatures. In some embodiments, the secondary drying can be performed while raising temperatures from -50 °C to 20 °C, from -40 °C to 20 °C, from -30 °C to 20 °C, from -20 °C to 20 °C, from -10 °C to 20 °C, from -50 °C to 10 °C, from -40 °C to 10 °C, from -30 °C to 10 °C, from -20 °C to 10 °C, from -10 °C to 10 °C.

[0213] During lyophilization process, a phase separation can occur. For example, a pure crystalline phase can be separate from saturated amorphus phase. The crystalline phase can include ice or any other crystallizing excipients. Thus, during primary drying, the pure ice phase can be removed, leaving behind other crystalline phases and any saturated amorphous phases. Conditions for primary drying can be adjusted to effectively remove this unbound water while maintaining the cake structure and DNA stability. Primary drying (sublimation) is a slow process conducted at cooler temperatures, safely below the product's critical collapse temperature. Sublimation requires heat energy to drive the phase change process from solid to gas. All three methods of heat transfer - conduction, convection and radiation, must be considered when freeze drying a product

[0214] Each liquid composition has a unique critical temperature. It is necessary to keep the composition at a temperature safely below this critical temperature during primary drying to avoid collapse. The temperature is dependent on the vapor pressure at the ice interface and in turn, this vapor pressure is dependent on both the rate of heat transfer into the composition (which is controlled by adjusting the shelf temperature) and the system vacuum level set point. During primary drying, the system pressure and the shelf temperature are set and controlled in combination to yield the appropriate product temperature. With the temperature and pressure parameters set, primary drying is then continued for a length of time sufficient for all of the ice crystals to be sublimed.

[0215] In addition to the free ice that is sublimed during primary drying, there remains a substantial amount of water molecules that are bound to the product. This is the water that is removed (desorbed) during secondary drying. Since all of the free ice has been removed in primary drying, the product temperature can now be increased considerably without fear of melting or collapse. Secondary drying actually starts during the primary phase, but at elevated temperatures (typically in the 20 to 50°C range), desorption proceeds much more quickly. Secondary drying rates are dependent on the product temperature. System vacuum may be continued at the same level used during primary drying; lower vacuum levels will not improve secondary drying times.

[0216] Secondary drying is continued until the product has acceptable moisture content for long term storage. Depending on the application, moisture content in fully dried products is typically

from 0.5% to 3%. In most cases, the more dry the product, the longer its shelf life will be. However, certain complex biological products may actually become too dry for optimum storage results and the secondary drying process should be controlled accordingly.

[0217] The step of secondary drying can be performed while raising temperatures from -50 °C to 20 °C, from -40 °C to 20 °C, from -30 °C to 20 °C, from -20 °C to 20 °C, from -10 °C to 20 °C, from -50 °C to 10 °C, from -40 °C to 10 °C, from -30 °C to 10 °C, from -20 °C to 10 °C, from -10 °C to 10 °C.

6.5. Lyophilized composition in a unit dose

[0218] Another aspect of the present invention is a lyophilized pharmaceutical composition in a unit dose. In some embodiments, the unit dosage form is a vial, ampule, bottle, or pre-filled syringe. In some embodiments, a unit dose contains about 50 μg to 1 g of plasmid DNA, 100 μg to 1 g of plasmid DNA, 100 μg to 100 mg of plasmid DNA, 1 mg to 100 mg of plasmid DNA, 10 mg to 100 mg of plasmid DNA, or 10 mg to 50 mg of plasmid DNA. A unit dose can comprise about 10 μg, 50 μg, 100 μg, 1 mg, 10 mg, 100 mg or 1g of plasmid DNA. The unit dosage form can contain 0.01 mg, 0.1 mg, 0.2 mg, 0.25 mg, 0.5 mg, 1 mg, 2.5 mg, 5 mg, 8mg, 10 mg, 12.5 mg, 16 mg, 24 mg, 25 mg, 50 mg, 75 mg, 100 mg, 150 mg, or 200 mg of the plasmid DNA.

[0219] In typical embodiments, the unit dosage form is a vial containing 50 mg, 10 mg, 7.5 mg, 5 mg, 1 mg, 100 μg or 50 μg of the lyophilized pharmaceutical composition suitable for administration after reconstitution. The administration includes subcutaneous, intradermal, or intramuscular administration using preloaded syringes, auto-injectors, and auto-inject pens, each containing a predetermined amount of the pharmaceutical composition described hereinabove.

[0220] The unit dose in a container can be determined based on various factors, such as an active ingredient (*e.g.,* plasmid DNA), a disease to be treated, a subject, a route and method of administration. The unit dose can be determined based on *in vitro* or *in vivo* studies, including clinical trials.

[0221] The unit dose in a container can be sealed and stored for an extended period of time at various temperatures (*e.g.*, room temperature to about –180° C., preferably about 2-8°C. to about —80° C., more preferably about -20°C. to about —80°C., and most preferably about -20°C.).

[0222] The lyophilized DNA compositions stored in the container are preferably stable within a range of from about 2-20°C to about —80°C, for a period of at least 1 month, 3 months, 6 months or 1 year without losing significant activity. Storage time may be as long as several months, 1 year, 5 years, 10 years, 15 years, or up to 20 years. Preferably the preparation is stable for a period of at least about 3 years.

6.6. Characterization of the lyophilized composition

[0223] The present invention provides a lyophilized pharmaceutical composition having one or more desired properties as a pharmaceutical product. The properties can include stability and potency of the active ingredient in various storage conditions, cake formation, a uniform reconstitution of the formulation for administration, less contaminations, *etc.* Various properties provided herein can be used to select a preferred lyophilized composition or to determine ideal storage conditions for the lyophilized composition.

[0224] Stability of plasmid DNA in the lyophilized composition can be determined based on methods known in the art. In particular, stability can be determined based on the conformation of plasmid DNA, for example, whether they exist as a more stable supercoiled form or a less stable open circle and linear form. Conformation of plasmid DNA can be determined by capillary electrophoresis of a sample containing plasmid DNA. The supercoiled DNA contents compared to the open circle and linear form can be measured in various conditions. For example, the supercoiled DNA contents can be determined before, during or after lyophilization, or before, during or after reconstitution of the lyophilized composition. The supercoiled DNA contents can be also determined before, during or after a storage at different temperatures to select a stable lyophilized composition as well as to determine an ideal storage condition.

[0225] Another way to determine stability of plasmid DNA is based on DNA concentrations. Various methods of measuring DNA concentrations known in the art can be adopted. For example, DNA concentrations can be measured based on light absorbance at 260 nm. The method can further involve measurement of contaminants to better quantify DNA concentrations in the sample.

[0226] Cake appearance can be another important attribute of a lyophilized product. Uniform and elegant cake appearance is generally preferred. The non-ideal cake appearance can affect

product quality, for example, by making it difficult to determine product quality based on visual inspection or making it difficult to recover the full amount of the active ingredient in a container. In addition, partial or complete meltback of the cake can result in instability and degradation of the active ingredient. Meltback is a form of cake collapse and is caused by the change from the solid to liquid state. That is, there is incomplete sublimation (change from the solid to vapor state) in the vial. These changes can involve a change in the physical form of the drug substance and a pocket of moisture.

[0227] Cake appearance can be determined by visual inspection that may involve taking a photograph. Visual inspections can be performed largely based on historical precedent. A robust qualification program for visual inspection is important before judging product quality. The qualification program can be based on past experience or published information. Visual inspection guidelines for a particular drug product can be developed specific to the drug product, for example, based on the information provided in the inspection guide, "Lyophilization of Parenterals: Guide To Inspections of Lyophilization of Parenterals (7/93)," published by the US Food and Drug Administration.

[0228] Another attribute of a lyophilized product that can affect the quality of a drug product is turbidity of a reconstituted drug obtained by dissolving the lyophilized product. Turbidity of a reconstituted drug can correlate with recovery of active ingredients in the lyophilized composition. In general, a complete dissolution, *i.e.,* a low turbidity of the reconstituted drug, is preferred. Incomplete dissolution can result in waste of active ingredients of the pharmaceutical composition and blockage of a syringe for administering the reconstituted drug.

[0229] Turbidity of a reconstituted drug can be measured by visual inspections or by measuring light absorbance at certain wavelengths, *e.g.,* at 450 nm and 650 nm. Light absorbance can be measured using a device available in the art, for example, a Molecular Devices ThermoMAX microplate.

[0230] Reconstitution time is another factor that can be related with the quality of a drug product. In general, a short reconstitution time is preferred. Increased time for reconstitution at the user stage may result in partial loss of potency if the drug is not completely dissolved, since it is common to use in-line filters during administration to the patient. Time required for reconstitution of the lyophilized product can be determined, for example, by measuring turbidity

of the reconstituted drug at various time points after reconstitution. For example, turbidity can be measured after 1 min, 5 mins, 10 mins, 15 mins, 20 mins, 25 mins, 30 mins, 1 hour, 2 hour, or more.

[0231] Residual moisture after lyophilization can be also important. Various methods known in the art can be adopted to measure residual moisture in the lyophilized composition. For example, a Karl Fisher Coulometer C20 (Mettler Toledo) can be used for moisture content analysis. A pocket of moisture in a lyophilized composition can result in greater instability and increased product degradation. Thus, less moisture content can be preferred.

6.7. Methods of treating a disease using the lyophilized composition

[0232] Methods of treating diseases using the lyophilized composition is within the scope of the present invention.

6.7.1. Reconstitution of the lyophilized composition

[0233] Various methods for reconstitution can be employed, such as the swirling methods, methods using a mechanical orbital shaker or methods of keeping the vial remained stationary.

[0234] The final concentration of reconstituted drug for administration can be adjusted depending on many factors, including the amount of a formulation to be delivered, the age and weight of the subject, the delivery method and route and the immunogenicity of the antigen being delivered.

[0235] The lyophilized composition of the present invention can be reconstituted with an acceptable solution, such as water, TE, PBS, Tris buffer, or normal saline, to the final concentration of about 10 mg/ml, 5 mg/ml, 1 mg/ml, 0.5 mg/ml, 0.3 mg/ml, 0.2 mg/ml, 0.1 mg/ml, or 0.05 mg/ml.

6.7.2. Administration of the reconstituted drug

[0236] The reconstituted drug of the present invention can be administered to a mammalian subject to treat various diseases. The reconstituted lyophilized composition of the invention may be administered by various delivery medthods – *e.g.,* orally or via parenteral routes such as intravenous, intramuscular, intraendocardial, intramyocardial, intrapericardial, intraventricular,

intraar ticular, intradermal, intracerebral, intrarenal, intrahepatic, intrasplenic, intralymphatic, subcutaneous, intraabdominal, intratesticular, intraovarian, intrauterine, sternal, intratra cheal, intraplueral, intrathoracic, intradural, intraspinal, intramedullary, intramural, intrascorionic and arterial injec tion or infusion, or topically through rectal, intranasal, inha lational or intraocular administration. In certain embodiments, the method of delivery is intramuscular, intramyocardial, intravenous, intracerebral, or intrarenal.

[0237] In typical embodiments, the nucleic acid construct is administered by injection of a liquid pharmaceutical composition. In currently preferred embodiments, the polynucleotide construct is administered by intramuscular injection. Typically, the polynucleotide construct is administered by intramuscular injection close to the affected site. In some embodiments, the polynucleotide constructs are administered to the muscles of limbs, heart, or other body parts of the subject.

[0238] In some embodiments, the construct is injected subcutaneously or intradermally. In some embodiments, the polynucleotide construct is administered by intravascular delivery. In certain embodiments, the construct is injected by retrograde intravenous injection.

[0239] It should be understood that the typical daily dose of the reconstituted lyophilized composition of the present invention ought to be determined in light of various relevant factors including the conditions to be treated, the chosen route of administration, the age, sex and body weight of the individual patient, and the severity of the patient's symptom, and can be administrated in a single dose or in divided doses. The polynucleotide construct is administered in a therapeutically effective dose.

[0240] In some embodiments of the methods described herein, the polynucleotide construct is administered at a total dose of 1 μg to 200mg, 1mg to 200mg, 1mg to 100mg, 1mg to 50mg, 1mg to 20mg, 5mg to 10mg, 16 mg, 8 mg, or 4 mg.

[0241] In typical embodiments, the total dose is divided into a plurality of individual injection doses. In some embodiments, the total dose is divided into a plurality of equal injection doses. In some embodiments, the total dose is divided into unequal injection doses.

[0242] In various divided dose embodiments, the total dose is administered to 4, 8, 16, 24, or 32 different injection sites.

[0243] In some embodiments, the injection dose is 0.1 – 5 mg. In certain embodiments, the injection dose is 0.1 mg, 0.15 mg, 0.2 mg, 0.25 mg, 0.3 mg, 0.35 mg, 0.4 mg, 0.45 mg, or 0.5 mg.

[0244] The total dose can be administered during one visit or over two or more visits.

[0245] In typical divided dose embodiments, all of the plurality of injection doses are administered within 1 hour of one another. In some embodiments, all of the plurality of injection doses are administered within 1.5, 2, 2.5 or 3 hours of one another.

[0246] In various embodiments of the methods, a total dose of polynucleotide construct, whether administered as a single unitary dose or divided into plurality of injection doses, is administered only once to the subject.

[0247] In some embodiments, administration of a total dose of polynucleotide construct into a plurality of injection sites over one, two, three or four visits can comprise a single cycle. In particular, administration of 32mg, 16 mg, 8 mg, or 4 mg of polynucleotide construct into a plurality of injection sites over two visits can comprise a single cycle. The two visits can be 3, 5, 7, 14, 21 or 28 days apart.

[0248] In some embodiments, the cycle can be repeated. The cycle can be repeated twice, three times, four times, five times, six times, or more.

[0249] In some embodiments, the cycle can be repeated 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more months after the previous cycle.

[0250] In some embodiments, the total dose administered in the subsequent cycle is same as the total dose administered in the prior cycle. In some embodiments, the total dose administered in the subsequent cycle is different from the total dose administered in the prior cycle.

[0251] In currently preferred embodiments, the nucleic acid construct is administered at a dose of 8 mg per affected limb, equally divided into a plurality of intramuscular injections and plurality of visits, wherein each of the plurality of injections in any single visit is performed at a separate injection site. In certain embodiments, the nucleic acid construct is administered at a dose of 8 mg per affected limb, equally divided into a first dose of 4 mg per limb on day 0 and a second dose of 4 mg per limb on day 14, wherein each of the first and second dose is equally divided into a plurality of injection doses.

[0252] The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease being treated. In typical embodiments, the polynucleotide construct is administered in an amount effective to reduce symptoms of the disease, for example, pain. In some embodiments, the amount is effective to reduce the symptom within 1 week of administration. In some embodiments, the amount is effective to reduce the symptom within 2 weeks, 3 weeks, or 4 weeks of administration.

[0253] The plasmid DNA can be administered alone or in combination with other plasmid DNA, either simultaneously or sequentially dependent upon the condition to be treated.

[0254] In some embodiments, the reconstituted composition comprises a plasmid DNA encoding a human HGF. The reconstituted drug can be administered to treat various diseases, for example, a disease previously demonstrated to be treatable by administration of a plasmid DNA. The plasmid DNA can encode a therapeutic gene, such as human HGF. The disease includes but is not limited to ischemic or liver disease, coronary artery disease ("CAD"), amytrophic lateral sclerosis ("ALS"), peripheral artery disease ("diabetic ulcer"), and diabetic peripheral neuropathy ("DPN") or neuropathy caused by diseases, injuries, infections or vitamin deficiency states. For example, the neuropathy can be caused by diabetes, vitamin deficiencies, autoimmune diseases, genetic or inherited disorders, amyloidosis, uremia, toxins or poisons, trauma or injury, tumors, or can be idiopathic. Methods described in US Pat. Nos. 7,812,146; 7,838,505; 7,745,174; 8,338,385; 8,389,492 and US App. Nos. 12/359,137; 14/355,792; 15/030,999 can be adopted to treat the diseases by administering the lyophilized composition. The references provided herein are incorporated by reference in their entirety herein.

[0255] In some embodiments, the reconstituted composition comprises a plasmid DNA encoding a human IGF-1. The reconstituted drug can be administered to treat various diseases, for example, a disease previously demonstrated to be treatable by administration of a plasmid DNA encoding a human IGF-1 or a human IGF-1 protein. The disease includes but is not limited to neuropathy caused by diseases, injuries, infections or vitamin deficiency states. For example, the neuropathy can be caused by diabetes, vitamin deficiencies, autoimmune diseases, genetic or inherited disorders, amyloidosis, uremia, toxins or poisons, trauma or injury, tumors, or can be idiopathic. In some embodiments, a plasmid encoding a human IGF-1 protein (pTx-IGF-1) is administered with another plasmid encoding human HGF (e.g., VM202) to treat a disease.

Methods described in US App. Nos. 16/513,560 and/or 16/513,564 can be adopted to treat the diseases by administering the lyophilized composition.

[0256] In some embodiments, the reconstituted composition comprises a plasmid DNA encoding human SDF-1α. The plasmid can be administered alone or together with a different plasmid DNA to treat a disease. In some embodiments, a plasmid encoding human SDF-1α (e.g., pCK-SDF-1α) is administered with another plasmid DNA encoding human HGF (e.g., VM202) to treat a disease. The disease includes but is not limited to vascular disease, such as peripheral vescular disease. Methods described in US App. No. 15/514,244 can be adopted to treat a disease by administering a plasmid encoding human SDF-1α.

6.8. Examples

[0257] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations can be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); nt, nucleotide(s); and the like.

[0258] The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art.

6.8.1. Example 1: Lyophilized composition of VM202 (Study 001)

6.8.1.1. Tested pharmaceutical composition comprising VM202

[0259] Various formulations containing VM202 as provided below in TABLE 1 were prepared. The active ingredient, the VM202 plasmid, was obtained from frozen stocks containing either 1.6 mg/mL of VM202 in 0.9% NaCl or 1.3 mg/mL of VM202 in 0.9% NaCl.

TABLE 1					
Code	**Buffer**	**pH**	**Bulking Agent**	**Stabilizer(s)**	**VM202 Concentration (mg/mL)**
KP8M2SN	10 mM Potassium Phosphate	8.0	2 % Mannitol	1.0 % Sucrose, 0.1 % NaCl	0.5
KP8MS3N**	10 mM Potassium Phosphate	8.0	2 % Mannitol	0.5 % Sucrose, 0.45% NaCl	0.5
KP8MT3N	10 mM Potassium Phosphate	8.0	2 % Mannitol	0.5 % Trehalose, 0.45 % NaCl	0.5
4MSN	10 mM Potassium Phosphate	8.0	4% Mannitol	0.5% Sucrose, 0.45% NaCl	0.5
3MSN	10 mM Potassium Phosphate	8.0	3% Mannitol	0.5% Sucrose, 0.45% NaCl	0.5
2MSN**	10 mM Potassium Phosphate	8.0	2% Mannitol	0.5% Sucrose, 0.45% NaCl	0.5
2M1SN	10 mM Potassium Phosphate	8.0	2% Mannitol	1.0% Sucrose, 0.45% NaCl	0.5
Control*	None	-	None	0.9 % NaCl, 1.1 % Sucrose	0.5

* Control is a formulation previously described in U.S. Patent No. 8,389,492, which is incorporated hereinby reference in its entirety.

** KP8MS3N and 2MSN are lyophilized formulations of the same compositions.

[0260] The formulations were prepared using commercially available materials and equipment, for example, Potassium Phosphate monobasic (Spectrum, catalog # PO200), Potassium Phosphate dibasic (EMD, catalog # PX1570-1), Mannitol (J.T.Baker, catalog # 2553-05), Sucrose (J.T.Baker, catalog # 4074-05), Sodium Chloride (Millipore, catalog # 1.06404.5000) and Trehalose (Pfanstiehl, catalog # T-104-04), a dialysis cassette (Thermo Scientific, catalog # 66380 (10,000 MWCO)), glass vials (3 cc or 20 cc glass vials (Schott Type I borosilicate, catalog # 68000316 or 68000321)), and stoppers (13 mm or 20 mm single-vent lyo stopper,

Flurotec®, (West Pharmaceutical, catalog # 19700034 or 19700311)). Excipients such as Dextran (MP Biomedicals, catalog # 101514), Lithium Hydroxide (Sigma Aldrich, catalog # L4533-100G), and Phosphiric acid (J.T.Baker, catalog # 0262-01) were also used.

6.8.1.2. Small scale test of various formulations

6.8.1.2.1. Sample preparation

[0261] To generate various formulations, vials with VM202 at 1.6 mg/mL in 0.9% NaCl were removed from -70°C storage and thawed at ambient temperature.

[0262] **Samples for the 1st round of small scale test:** KP8M2SN (see Table 1) was then prepared by dialyzing out using a 10,000 MWCO Thermo dialysis cassette at ≥ 10,000-fold volume exchange against the target formulation buffer (10 mM potassium phosphate, 2% mannitol, 1.0% sucrose, 0.1% NaCl, pH 8.0) over 24 hours at 2-8°C. Following dialysis, the concentration of KP8M2SN was adjusted to 0.5mg/mL of VM202 with formulation buffer.

[0263] KP8MS3N, KP8MT3N and Control were prepared by diluting the drug substance. Specifically, 1.7 mL of VM202 (*i.e.,* VM202 in 0.9% NaCl) was diluted with 0.85 mL of the dilution buffers (Buffer No. 2 for KP8MS3N, Buffer No. 3 for KP8MT3N, and Buffer No. 8 for Control) provided below in TABLE 2. Following the dilutions, each formulation was adjusted to contain 0.5mg/mL of VM202 for KP8MS3N and KP8MT3N respectively, with a formulation buffer containing 0.45% NaCl.

[0264] **Samples for the 2nd round of small scale tests:** 4MSN, 3MSN, and 2MSN were prepared by diluting 0.84mL of the drug substance (*i.e.,* VM202 in 0.9% NaCl) with 0.42 mL of dilution buffers (Buffer 4 for 4MSN, Buffer 5 for 3MSN, and Buffer 6 for 2MSN) also provided below in TABLE 2. Following dilution, each formulation except Control was adjusted to include 0.5mg/mL of VM202 using each formulation buffer containing 0.45% NaCl.

TABLE 2

Dilution Buffer No.	Buffer	pH	Bulking Agent	Stabilizer(s)
2 (for KP8MS3N)	30 mM Potassium Phosphate	8.0	6 % Mannitol	1.5 % Sucrose
3 (for KP8MT3N)	30 mM Potassium Phosphate	8.0	6 % Mannitol	1.5 % Trehalose

TABLE 2				
Dilution Buffer No.	**Buffer**	**pH**	**Bulking Agent**	**Stabilizer(s)**
4 (for 4MSN)	30 mM Potassium Phosphate	8.0	12% Mannitol	1.5% Sucrose
5 (for 3MSN)	30 mM Potassium Phosphate	8.0	9 % Mannitol	1.5% Sucrose
6 (for 2MSN)	30 mM Potassium Phosphate	8.0	6 % Mannitol	1.5% Sucrose
7 (for 2M1SN)	30 mM Potassium Phosphate	8.0	6 % Mannitol	3.0% Sucrose
8 (for Control)	None	-	None	0.9 % NaCl, 3.3 % Sucrose

[0265] **Sample preparation for accelerated stability test:** A separate set of the formulations was further prepared for accelerated stability test. For the separate set, 4MSN, 3MSN, 2MSN, 2M1SN and Control were prepared by diluting the drug substance (*i.e.,* VM202 in 0.9% NaCl) with dilution buffers provided above in TABLE 2, at a ratio of 2:1 (Buffer 4 for 4MSN, Buffer 5 for 3MSN, Buffer 6 for 2MSN, Buffer 7 for 2M1SN, and Buffer 8 for Control). Following dilutions, all formulations except Control were adjusted to contain VM202 at 0.5 mg/mL with each formulation buffer containing 0.45% NaCl.

[0266] A surrogate formulation was prepared using dextran at 0.5 mg/mL in 0.9% NaCl and 1.1% sucrose.

[0267] **Lyophilization of the samples**: After adjustment of the concentration to 0.5 mg/mL, formulated VM202 was sterile filtered through a 0.2 µm PES filter (small scale and accelerated stability studies) or cellulose acetate (CA) filter (additional accelerated stability study) under aseptic conditions. All samples were filled under aseptic conditions into sterilized 3 cc vials at a fill volume 0.75 mL per vial for small scale lyophilization, or sterilized 20 cc vials at a fill volume 5 mL per vial for accelerated stability studies in a biosafety cabinet. Specifically, the formulations were filled using a syringe filter (PALL Life Science, Acrodisc 13 mm with 0.2 µm PES membrane, catalog # 4602) with a vacuum filtration system (Corning, 1L Vacuum Filter/Storage Bottle System, 0.2 µm CA membrane, catalog # 431-205; Thermo scientific, Rapid Flow Filter Unit, 0.2 µm PES membrane, catalog # 567-0020) under a safety cabinet (NuAire biological safety cabinet, Class II Type A/B3 Model No. NU-425-600)).

[0268] After filling, the vials were partially stoppered with sterile stoppers and loaded into the lyophilizer (VirTis (Model No. 25L Genesis SQ Super XL-70) for freeze-drying using the cycle described in TABLE 3. The surrogate vials were used to completely surround VM202 vials so that the comparable irradiation thermal transfer from neighboring vials are provided during the drying process for small scale lyophilization.

TABLE 3

Step	Temperature	Time (min)	Ramp Rate (° C/min)	Chamber Pressure (mT)
1st round of small scale lyophilization cycle				
Loading	5° C	N/A	N/A	N/A
Freezing	5° C to -50° C	55	1° C	N/A
	-50° C	120	N/A	N/A
	-50° C to -20° C	60	0.5	N/A
	-20° C	120	N/A	N/A
Primary Drying	-20° C	1440	N/A	80
Secondary Drying	-20° C to 20° C	80	0.5	80
	20° C	780	N/A	80
2nd round of small scale lyophilization cycle				
Loading	5° C	N/A	N/A	N/A
Freezing	5° C to -50° C	55	1° C	N/A
	-50° C	120	N/A	N/A
	-50° C to -20° C	60	0.5	N/A
	-20° C	120	N/A	N/A
Primary Drying	-20° C	1500 or 3840 or 3240	N/A	50

TABLE 3				
Step	**Temperature**	**Time (min)**	**Ramp Rate (° C/min)**	**Chamber Pressure (mT)**
Secondary Drying	-20° C to 20° C	80	0.5	50
	20° C	780	N/A	50

[0269] Following lyophilization, the vials were fully stoppered under partial vacuum at 600 mtorr, crimped with aluminum seals, and labeled. Labels included information regarding project, date, incubation temperature/stress condition, and time point. After labeling, samples were placed into their respective stability conditions.

6.8.1.2.2. Assays for testing various formulations

[0270] The lyophilized formulations were exposed to varying stress conditions to assess their relative stability. To thermally stress the samples, all formulations were stored at 5°C, 25°C, or 40°C for up to 10 weeks. For the assessment of stability after reconstitution, samples were reconstituted in a biosafety cabinet using a target volume of 5.0 mL with filtered water for injection (WFI), restoppered, and resealed. Following reconstitution, samples were stored at 25°C for 3 or 7 days. Various conditions tested in this experiment are summarized below in TABLE 4.

TABLE 4		
Stress	**Storage Conditions**	**Time Point(s)**
Temperature	5 °C (Lyophilized)	2 weeks, 4 weeks, 10 weeks
	25 °C (Reconstituted liquid)	0, 3, 7 days
	25 °C (Lyophilized)	2 weeks, 4 weeks, 10 weeks
	40 °C (Lyophilized)	2 weeks, 4 weeks, 10 weeks

[0271] The following assays were performed to analyze stability of various formulations in different conditions.

(1) Visual Inspection: Visual inspection was performed against a dark and white background. Digital photographs were acquired.

(2) UV Spectrophotometry: UV spectrophotometry analysis was performed with an Implen Nanophotometer with a lid factor of 10. The plasmid DNA concentration of the test samples was determined by measuring absorbance at 230, 260, 280 and 350 nm. Concentration analyses were performed using ViroMed's UV/Vis protocol. Calculations were performed using the following equations:

Concentration (µg/mL) = [(D-E)/C] x (B/A)
Recovery = (low µg/mL) / (high µg/mL) x 100%
OD^{260nm} / OD^{280nm} = D / F
OD^{260nm} / OD^{230nm} = D / G

where,

A = weight of sample taken to give the dilution.
B = total weight of sample and buffer to give the dilution.
C = extinction coefficient of a 0.005% solution in a 1cm pathlength cuvette (0.02).
D = optical density for the maximum at 260nm.
E = absorbance measured at 350nm.
F = absorbance measured at 280nm.
G = absorbance measured at 230nm.

Acceptance Criteria:

D shall fall in the range 0.5 to 1.5 OD units.
Recovery shall be $\geq$ 98%
OD_{260nm} / OD_{280nm} = shall lie in the range 1.8 to 2.0
OD_{260nm} / OD_{230nm} = shall be $\geq$ 1.1

(3) Capillary Electrophoresis: A Beckman Coulter, ProteomeLab™ PA 800 CE instrument equipped with a P/ACE™ MDQ UV detector module was used for plasmid DNA structural analysis (purity of supercoiled DNA). 32 Karat software (version 7.0) was used to analyze the results. Prior to capillary electrophoresis analysis, a Beckman Coulter Neutral coated 40 cm capillary with a 50 µm internal diameter (Part No. 477441) and a 8 µm aperture window was conditioned by running HPLC ultrapure grade water at 20 psi for 1 minute, followed by a rinse of 100 mM phosphoric acid (pH 2.6) at 20 psi for 1 minute. The separation occurred by applying a constant voltage of 17 kV for 14 minutes. 40 µL of sample at a concentration of 0.5 mg/mL was added into a polyethylene insert for the analyses. The following parameters were used:

- Pressure/ injection time: 0.5 psi / 11 sec.
- Separation buffer/pressure: 100 mM phosphoric acid (pH 2.60), 85% / 20 psi; 60 sec.

- Wash buffer/pressure: HPLC ultrapure grade water / 20 psi; 60 sec
- Detector wavelength: 254 nm.
- Separation voltage: 17 KV; 0.17 min; 14 min.

An exemplary capillary electrophoresis result of VM202 is provided in Figure 1.

(4) Turbidity: Turbidity was determined by measuring sample absorbance at 450 nm and 650 nm using a Molecular Devices ThermoMAX microplate reader.

(5) Subambient DSC Analysis: Using a Pyris Diamond DSC with an Intercooler II, approximately 10 µL of each formulation was frozen at -60°C. At a ramp rate of 5° C/min, the sample was warmed to 25°C and the heat flow during the warming process was recorded. Also, the program incorporating an annealing step was performed. The annealing step was conducted by warming the sample to -15°C at a ramp rate of 5°C/min.

(6) Karl Fisher Moisture Analysis: A Karl Fisher Coulometer C20 (Mettler Toledo) was used for moisture content analysis. AquaStar Water Standard Oven 1% was used to determine system accuracy. Sample vials were brought to room temperature before caps were removed for analysis. Weigh boats were weighed before and after adding samples to determine the amount of lyophilized powder used for analysis. Approximately 10-160 mg of material was used for analysis.

6.8.1.2.3. Analysis of KP8M2SN, KP8MS3N and KP8MT3N (1st Round of Small Scale Test)

[0272] **Subambient DSC Analysis:** Prior to the lyophilization process, 10 µL samples of formulated VM202 – *i.e.,* KP8M2SN, KP8MS3N, KP8MT3N, and Control (TABLE 1) – at 0.5 mg/mL were analyzed by subambient differential scanning calorimetry (DSC) to characterize candidate formulations. Analyzed signals include collapse temperature (Tg'), devitrification temperature (Td), and the benefit of annealing of various formulations with VM202 in the frozen states. Figures 2A-D illustrate the DSC results of the liquid state for 1st round of small scale lyophilization of KP8M2SN (Figure 2A), KP8MS3N (Figure 2B), KP8MT3N (Figure 2C) and Control (Figure 2D) without annealing (left) or with annealing (right) of VM202. The glass transition (Tg') temperature of KP8M2SN was observed at -44°C. Control displayed the eutectic melting temperature (Te) at ~ -21°C. A devitrification temperature (Td) was observed in all

formulations and disappeared after annealing. Since some of the additives showed devitrification signals which disappeared after annealing, lyophilization cycle 1 (TABLE 3) was designed to incorporate an annealing step during freezing for the 1st round of small scale.

[0273] Ramping of the freezing temperature from -50°C to -20°C and holding for two (2) hours, then subsequently introducing the vacuum allowed for annealing and the induction of the crystallization of mannitol. Primary drying was performed at -20°C shelf temperature (24 hours) with 80 mTorr chamber pressure (TABLE 3). Secondary drying at a shelf temperature of 20°C was designed to remove residual water that was not sublimated during the primary drying step.

[0274] Figure 3 shows the time-lapse graph of temperatures (left y-axis) and pressures (right y-axis) during the lyophilization process for the 1st round of small scale testing. The product temperature dropped below -36°C, due to heat-loss of sublimation when vacuum was applied. The pirani gauge value merged with the capacitance manometer reading at approximately 30 hours during the primary drying stage which confirmed the completion of the primary drying process. Secondary drying was initiated and the samples were allowed to dry for an additional 13 hours. The entire cycle lasted 46 hours, with the potential of a reduction to 43 hours if secondary drying was initiated immediately upon the completion of primary drying.

[0275] Upon completing the 1st round small scale lyophilization cycle, all vials were stoppered with 600 torr vacuum pressure. The lyophilates were removed from the chamber and analyzed. The lyophilized cake of KP8M2SN was elegant in appearance. The other cakes showed signs of collapse, especially Control. Reconstituted samples were clear and comparable to pre-lyophilization samples (Figures 4A-C). Moisture contents of the cakes were 1.37% (KP8M2SN), 2.40% (KP8MS3N), 1.44% (KP8MT3N), and 1.25% (Control), respectively.

[0276] The concentration of VM202 in each sample was measured after reconstitution of the sample from 1st round small scale lyophilization cycle and the results are summarized in TABLE 5. The results showed that each sample contained VM202 at a similar concentration.

TABLE 5		
Code	**Concentration of VM202 (mg/mL)**	
	Pre-Lyophilization	**Reconstituted Sample**
KP8M2SN	0.51	0.49
KP8MS3N	0.52	0.50
KP8MT3N	0.51	0.49
Control	0.52	0.49

[0277] Capillary Electrophoresis: Reconstituted samples were further examined by CE for product purity. Chromatograms and the peak area (%) data following CE are shown in Figure 5 and TABLE 6. Results from CE assays showed a decrease in super coiled peak area in KP8M2SN, KP8MT3N and Control following lyophilization and reconstitution. KP8MS3N had the highest super coiled peak area following lyophilization and reconstitution.

TABLE 6				
Form. Code	**Sample**	**Super Coiled Peak Area (%)**	**Open Circle Peak Area (%)**	**Total Area**
KP8M2SN	Pre-Lyophilization	97.3	2.7	18058
	Reconstituted Sample	95.0	5.0	15523
KP8MS3N	Pre- Lyophilization	97.1	2.9	16328
	Reconstituted Sample	97.7	2.3	14789
KP8MT3N	Pre- Lyophilization	97.5	2.5	15696
	Reconstituted Sample	91.8	8.2	15586
Control	Pre- Lyophilization	97.6	2.4	15104
	Reconstituted Sample	92.4	7.6	13937

[0278] In summary, the results show that KP8M2SN, containing 1.0% Sucrose and 0.1% NaCl produces a better lyophilization cake than KP8MS3N containing 0.5% Sucrose and 0.45% NaCl, or KP8MT3N containing 0.5% Trehalose and 0.45% NaCl. The results further showed that KP8MS3N containing 0.5% Sucrose and 0.45% NaCl had the least amount of degradation as measured by CE. These together suggest that formulations containing Sucrose (KP8M2SN and KP8MS3N) had better properties (*e.g.,* elegant cake formation and stability) than the formulation containing Trehalose (KP8MT3N).

6.8.1.2.4. Analysis of 1st Set of 4MSN, 3MSN and 2MSN (2nd Round of Small Scale Lyophilization Cycle)

[0279] Based on the results from the 1st round of small scale lyophilization cycle, the next lyophilization cycle (2nd Round of small scale test) focused on enhancing the cake appearance by altering the concentrations of mannitol in KP8MS3N while keeping its Sucrose (0.5%) and NaCl (0.45%) concentrations.

[0280] Prior to the lyophilization process, 10 µL samples of formulated VM202 – *i.e.,* 4MSN, 3MSN, 2MSN, and Control (TABLE 1) – at 0.5 mg/mL were analyzed by subambient DSC to characterize candidate formulations. Figures 6A-D illustrate the DSC results of the liquid state from the 2nd round of small scale lyophilization testing. Control (Figure 6D) displayed a eutectic melting temperature (Te) of ~ -21°C. A devitrification temperature (Td) was observed in all formulations (without annealing on the left) and disappeared after annealing (on the right). Due to the observance of devitrification signals, lyophilization cycle 2 (TABLE 3) was designed to incorporate an annealing step during freezing for the 2nd round of small scale testing.

[0281] Ramping of the freezing temperature from -50°C to -20°C and holding for two (2) hours, then subsequently introducing the vacuum allowed for annealing and the induction of the crystallization of mannitol. Primary drying was performed at -20°C shelf temperature (25 hours) with 50 mTorr chamber pressure (TABLE 3). Secondary drying at a shelf temperature of 20°C was designed to remove residual water that was not sublimated during the primary drying step.

[0282] Figure 7 shows the time-lapse graph of temperatures (left y-axis) and pressures (right y-axis) during the lyophilization process for the 2nd round of small scale testing. The product temperature dropped below -37°C, due to heat-loss of sublimation when vacuum was applied. The pirani gauge value merged with the capacitance manometer reading at approximately 23 hours during the primary drying stage which confirmed the completion of the primary drying process. Secondary drying was initiated and the samples were allowed to dry for an additional 13 hours. The entire cycle lasted 47 hours, with the potential of a reduction to 42 hours if secondary drying was initiated immediately upon the completion of primary drying.

[0283] Once the cycle was complete, samples were removed from the lyophilizer and analyzed. By visual inspection, 4MSN displayed elegant cake. 3MSN and 2MSN also showed decent cake

with shrinkage, whereas Control showed signs of cake collapse following lyophilization. The samples were clear and colorless after reconstitution, comparable to pre-lyophilization samples (Figures 8A-C). Moisture contents of the cake were 2.51% (4MSN), 2.15% (3MSN), 2.01% (2MSN), and 1.12% (Control).

[0284] The concentration of VM202 in each sample was measured after reconstitution of the sample from 2nd round small scale lyophilization cycle and the results are summarized in TABLE 7. The results showed that each sample contained VM202 at a similar concentration.

TABLE 7

Form. Code	Concentration (mg/mL)	
	Pre-Lyophilization	Reconstituted Sample
4MSN	0.53	0.51
3MSN	0.53	0.51
2MSN	0.52	0.52
Control	0.53	0.51

[0285] **Capillary Electrophoresis:** The reconstituted samples were further examined by CE for product purity. The chromatograms and the tabular results from CE are detailed in Figure 9 and TABLE 8. 4MSN and 3MSN displayed minimal degradation in the super coiled peak area, whereas 2MSN and Control showed decreases in the super coiled peak are following lyophilization and reconstitution.

TABLE 8

Form. Code	Sample	Super Coiled Peak Area (%)	Open Circle Peak Area (%)	Total Area
4MSN	Pre-Lyo	97.6	2.4	15030
	Reconstituted Sample	97.2	2.8	14482
3MSN	Pre-Lyo	98.2	1.8	14788
	Reconstituted Sample	97.8	2.2	13352
2MSN	Pre-Lyo	97.5	2.5	15143
	Reconstituted Sample	95.6	4.4	14258
Control	Pre-Lyo	98.4	1.6	14761
	Reconstituted Sample	94.1	5.9	12058

[0286] These results suggested that 4MSN (10 mM Potassium Phosphate, 4% Mannitol, 0.5% Sucrose, 0.45% NaCl at pH 8.0) produced the best lyophilized cake and the least amount of degradation by CE in the 2nd round small scale lyophilization cycle.

6.8.1.2.5. Analysis of 2nd Set of 4MSN, 3MSN and 2MSN (2nd Round of Small Scale Lyophilization Cycle)

[0287] Another set of 4MSN, 3MSN, 2MSN and Control was prepared by the 2nd round of small scale lyophilization cycle designed with a primary drying shelf temperature of -20°C at a chamber pressure of 50 mTorr, and a secondary drying shelf temperature of 20°C at a chamber pressure of 50 mTorr (TABLE 3). Figure 10 shows the time-lapse graph of temperatures and pressures obtained during this cycle. The product temperature dropped below -37°C, due to heat-loss of sublimation when vacuum was applied. The pirani gauge values merged with the capacitance manometer reading around 75 hours during primary drying. Secondary drying was initiated and the samples were allowed to dry for an additional 13 hours. The entire cycle lasted 87 hours.

[0288] Once the cycle was complete, samples were removed from the lyophilizer and analyzed. By visual inspection, 4MSN displayed elegant cake. 3MSN and 2MSN also showed decent cake with shrinkage, whereas Control showed signs of cake collapse following lyophilization. Upon reconstitution, some vials in all formulations, except Control, showed haziness, whereas all samples were clear before lyophilization (Figures 11A-F). Moisture contents of the cake were 0.97% (4MSN), 0.95% (3MSN), 1.69% (2MSN), and 1.06% (Control).

[0289] **Turbidity:** Concentration and turbidity results are summarized in TABLE 9. All samples showed similar concentration values following reconstitution. No turbidity was detected for all formulations before lyophilization and after reconstitution.

TABLE 9

Form. Code	Concentration (mg/mL)		Turbidity (A_{650})	
	Pre-Lyophilization	Reconstituted Sample	Pre-Lyophilization	Reconstituted Sample
4MSN	0.51	0.49	-0.001	0.003
3MSN	0.50	0.49	0.000	0.001
2MSN	0.50	0.49	-0.001	0.001
Control	0.52	0.51	0.000	0.000

[0290] **Capillary Electrophoresis:** The reconstituted samples were further examined by CE for product purity. The chromatograms and peak percentages from CE are shown in Figure 12 and TABLE 10. 4MSN, 3MSN, and Control showed a significant decrease in the super coiled peak

area following lyophilization and reconstitution. 2MSN displayed minimal degradation in the super coiled peak area after lyophilization and reconstitution.

TABLE 10

Form. Code	Sample	Average (n=5)		
		Super Coiled Peak Area (%)	Open Circle Peak Area (%)	Total Area
4MSN	Pre-Lyophilization	98.6	1.4	21510.6
	Reconstituted Sample	89.2	10.8	17033.7
3MSN	Pre- Lyophilization	98.4	1.6	17025.0
	Reconstituted Sample	89.5	10.5	16855.6
2MSN	Pre- Lyophilization	98.5	1.5	14671.2
	Reconstituted Sample	96.4	3.6	15813.1
Control	Pre- Lyophilization	98.2	1.8	18755.1
	Reconstituted Sample	92.7	7.3	17806.0

[0291] The results suggested that 2MSN (10 mM Potassium Phosphate, 2% Mannitol, 0.5% Sucrose, 0.45% NaCl at pH 8.0) produced the least amount of degradation by CE in this separate set of experiments for the 2nd round small scale test.

[0292] The samples produced in this lyophilization cycle were used over 10 weeks for the accelerated stability study.

6.8.1.2.6. Analysis of 2MSN and 2M1SN

[0293] Given that KP8MS3N and 2MSN containing 2% Mannitol, 0.5% Sucrose and 0.45% NaCl showed the least amount of degradation as measured by CE in the 1st round and certain 2nd round small scale tests, the KP8MS3N and 2MSN formulations were further optimized by changing the sucrose concentration while keeping the Mannitol (2%) and NaCl (0.45%) concentrations at the same concentrations. Specifically, 2M1SN formulation containing 2% Mannitol, 0.45% NaCl and 1.0% Sucrose was made and analyzed compared to 2MSN.

[0294] 2M1SN and 2MSN were lyophilized using similar lyophilization cycle parameters to those used in the accelerated stability study. Figure 13 displays the temperature and pressure measurements from this cycle. The product temperature dropped below -41°C, due to heat-loss of sublimation when vacuum was applied. The pirani gauge values merged with the capacitance manometer reading around 60 hours during primary drying. Secondary drying was initiated and

the samples were allowed to dry for an additional 13 hours. The entire cycle lasted 76 hours with the potential of a reduction to 70 hours if secondary drying was initiated immediately upon the completion of primary drying.

[0295] Following lyophilization, 2M1SN displayed elegant cake and 2MSN displayed decent cake with minor shrinkage (Figure 14). Upon reconstitution, 2M1SN produced a clear solution, whereas 2MSN displayed haziness. Both formulations were clear before lyophilization. The moisture contents of the cakes were 1.31% (2MSN), and 1.44% (2M1SN).

[0296] **Turbidity:** Formulations were assessed for concentration and turbidity and the results are summarized in TABLE 11. All samples showed similar concentration values following reconstitution. For the additional accelerated stability study, it was determined that turbidity measurements at 450 nm were better able to assess differences in the formulations. No turbidity was detected for 2M1SN before lyophilization and after reconstitution. After reconstitution of 2MSN, a significant increase in turbidity was observed at 450 nm.

TABLE 11

Form. Code	Concentration (mg/mL)		Turbidity (A_{450})	
	Pre-Lyo	Reconstituted Sample	Pre-Lyo	Reconstituted Sample
2MSN	0.51	0.50	0.000	0.013
2M1SN	0.51	0.50	0.000	0.000

[0297] **Capillary Electrophoresis:** The reconstituted samples were further examined by CE for product purity. The chromatograms and tabular results from CE are detailed in Figure 15 and TABLE 12. 2MSN displayed a decrease in the super coiled peak area following lyophilization and reconstitution. 2M1SN displayed minimal degradation in the super coiled peak area after lyophilization and reconstitution

TABLE 12

Form. Code	Sample	Average (n=5)		
		Super Coiled Peak Area (%)	Open Circle Peak Area (%)	Total Area
2MSN	Pre-Lyophilization	98.6	1.4	13755.3
	Reconstituted Sample	91.1	8.9	14135.3
2M1SN	Pre- Lyophilization	98.5	1.5	13928.0
	Reconstituted Sample	96.6	3.4	13998.6

[0298] The samples produced in this lyophilization cycle were used over 10 weeks for the additional accelerated stability study.

6.8.1.3. Accelerated stability study of 4MSN, 3MSN, 2MSN and Control

[0299] Stabilities of lyophilized VM202 formulations, 4MSN, 3MSN, 2MSN, and Control, were evaluated under different storage conditions at refrigerated (5 ± 3°C), ambient (25 ± 3°C), and accelerated (40 ± 3°C) temperature and reconstituted VM202 formulations at ambient temperature for 3 or 7 days.

[0300] The lyophilized samples from the accelerated stability study were reconstituted with 5 mL of filtered water in a biological safety cabinet, re-stoppered, sealed, and incubated at 25°C for 3 and 7 days. Following 3 and 7 days, the incubated samples were removed from the incubator and analyzed by visual, concentration, and CE analysis.

[0301] **Visual inspection:** No changes in visual appearance were observed after storage of the formulations 4MSN, 3MSN, 2MSN and Control at 25°C for 3 days (Figure 16A) and 7 days (Figure 16B) following reconstitution when compared to T=0.

[0302] At each time point, samples were analyzed for absorbance at 260 nm to determine the concentration of VM202. The concentration of each formulation following storage at 25°C for 3 and 7 days is similar to the results obtained at T=0 (TABLE 13). All samples were within 5% of the targeted concentration value. Therefore, temperature stress induced no major changes in VM202 concentration.

TABLE 13

Form. Code	Concentration (mg/mL)		
	T=0	3 days	7 days
4MSN	0.49	0.50	0.50
3MSN	0.49	0.49	0.49
2MSN	0.49	0.50	0.50
Control	0.51	0.52	0.52

[0303] **Capillary Electrophoresis:** CE analysis was employed to monitor product purity during temperature storage. The CE chromatograms and the tabular results of the formulations after

storage are detailed in Figures 17A-B and TABLE 14. All formulations (*i.e.,* 4MSN, 3MSN, 2MSN and Control) displayed decreases in supercoiled peak area following storage at 25°C from 3 to 7 days. 2MSN exhibited the best stability from 3 to 7 days with only a 1.1% decrease in super coiled peak area.

TABLE 14

Form. Code	T=3 Days (n=5)			T=7 Days (n=5)		
	Super Coiled Peak Area (%)	Open Circle Peak Area (%)	Total Area	Super Coiled Peak Area (%)	Open Circle Peak Area (%)	Total Area
4MSN	95.6	4.4	12236.2	91.1	8.9	13628.1
3MSN	96.4	3.6	12019.0	91.5	8.5	13912.7
2MSN	96.7	3.3	14029.2	95.6	4.4	15672.4
Control	96.2	3.8	14029.0	92.6	7.4	17867.4

[0304] The lyophilized VM202 formulations were stored over 10 weeks at 5°C, 25°C, and 40°C (TABLE 4). At each time point, samples were removed from temperature storage and visually assessed. Photographs of the vials were taken and samples were evaluated for cake shape. All lyophilized cakes remained intact and did not show any signs of change upon storage regardless of temperature condition (Control cake remained lifted). The samples were then reconstituted with 5 mL of filtered water and evaluated for clarity and precipitation. Most samples for 4MSN, 3MSN, and 2MSN exhibited slight differences in clarity when compared to T=0. The visual appearance of each formulation following 5°C (Figure 18C), 25°C (Figure 18A), and 40°C (Figure 18B) storage for 10 weeks is shown in Figures 18A-C.

[0305] Concentration, turbidity, moisture content, and capillary electrophoresis analyses were also performed to evaluate the stability of lyophilized VM202 during temperature stresses.

[0306] At each time point, samples were reconstituted and analyzed for absorbance at 260 nm to determine the concentration of VM202 in vials. TABLE 15 shows that the concentration of VM202 in lyophilized samples following 10 weeks of temperature storage is similar to the results obtained at T=0. Therefore, temperature stress induced no major changes in VM202 concentration.

TABLE 15				
Form. Code	Concentration (mg/mL), T=0 or T=10wks			
	T=0	T=10wks		
		5°C	25°C	40°C
4MSN	0.49	0.49	0.49	0.49
3MSN	0.49	0.49	0.49	0.49
2MSN	0.49	0.50	0.49	0.50
Control	0.51	0.52	0.52	0.52

[0307] **Turbidity:** Turbidity of each sample was also evaluated at each time point in the study. The results are detailed in TABLE 16. Turbidity of the reconstituted formulations, 4MSN, 3MSN and 2MSN, did not change significantly following 10 weeks storage, regardless of temperature condition.

TABLE 16				
Form. Code	Turbidity (Abs_{650}), T=0 and T=10wks			
	T=0	5°C	25°C	40°C
4MSN	0.003	0.004	0.004	0.002
3MSN	0.001	0.004	0.004	0.003
2MSN	0.001	0.004	0.002	0.002
Control	0.000	0.001	0.001	0.001

[0308] Moisture content analysis was also performed at each time point. TABLE 17 outlines the results for moisture content following 10 weeks of temperature storage. After 10 weeks of storage at 5°C, 25°C, and 40°C, slight decreases in moisture content were observed in a temperature dependent manner. Higher storage temperatures resulted in lower moisture content. 2MSN had the highest moisture content during the accelerated stability study.

TABLE 17				
Form. Code	Moisture content, T=0 and T=10wks			
	T=0	T=10wks		
		5°C	25°C	40°C
1% Water STD	0.94	0.94		
4MSN	0.97	0.79	0.31	0.30
3MSN	0.95	0.75	0.53	0.35
2MSN	1.69	1.39	0.95	0.67
Control	1.06	1.07	0.79	0.61

[0309] **Capillary Electrophoresis:** Capillary electrophoresis analysis was used to monitor for product purity. The CE chromatograms and peak area values after 10 weeks of incubation at 40°C are shown in Figure 19 and TABLE 18, respectively. All formulations displayed significant decreases in the supercoiled peak area with corresponding increases in the open circle peak area compared to T=0 after reconstitution, following storage for 10 weeks at 40°C. 2MSN displayed the highest supercoiled peak area, although this value was significantly lower at 10 weeks storage at 40°C than at time zero. Higher levels of open circle peak area were observed in the formulations containing 3% and 4% mannitol as well as in the Control formulation.

TABLE 18			
Form. Code	Average (n=5)		
	Super Coiled Peak Area (%)	Open Circle Peak Area (%)	Total Area
4MSN	54.3	45.7	8142.4
3MSN	55.4	44.6	11305.2
2MSN	82.2	17.8	10622.8
Control	70.0	30.0	14767.2

[0310] The CE supercoiled peak purity trends, weighted by standard deviation and including deviation error bars, over 10 weeks at various temperatures are detailed in Figures 20A-C– 5°C (Figure 20A), 25°C (Figure 20B) or 40°C (Figure 20C). Significant differences in purity were observed following incubation at elevated temperature. As illustrated in Figures 20A-C, 2MSN containing 2% mannitol and 0.45% NaCl displayed the highest level of purity following 40°C storage.

[0311] The trends of open circle peak impurities detected by CE after storage at different temperatures are also presented in Figures 21A-C. Slight increases in open circle peak areas were observed at 5°C. However, at ≥ 25°C, all formulations showed considerable degradation. Following 40°C incubation, higher levels of open circle peak impurities were observed for 4MSN, 3MSN and Control. In comparison, 2MSN showed substantially less degradation at all temperatures.

6.8.1.4. Accelerated stability study of 2MSN and 2M1SN

[0312] Stabilities of lyophilized VM202 formulations, 2MSN, and 2M1SN, were also evaluated after incubation at ambient temperature (25°C) for 3 or 7 days. Samples were reconstituted with 5 mL of filtered water in a biological safety cabinet, re-stoppered, sealed, and incubated at 25°C for 3 and 7 days. Following 3 and 7 days, the incubated samples were removed from the incubator and analyzed by visual, concentration, and CE analysis.

[0313] Visual inspection: At both time points, visual inspection was performed (Figures 22A-D). Haziness was observed in 2MSN following 3 and 7 days of storage at 25°C after reconstitution. 2M1SN remained clear at both time points.

[0314] At each time point, samples were analyzed for absorbance at 260 nm to determine the concentration of VM202. VM202 concentrations in both formulations were on target at T=0 and after incubation at 25°C for 3 and 7 days following reconstitution (TABLE 19). Therefore, temperature stress induced no major changes in VM202 concentration.

TABLE 19

Form. Code	Concentration (mg/mL)		
	T=0	3 days	7 days
2MSN	0.50	0.50	0.50
2M1SN	0.50	0.50	0.50

[0315] **Capillary Electrophoresis:** The samples were also analyzed by CE for product purity following temperature storage. The CE chromatograms and the peak area results of the samples after storage are illustrated in Figures 23A-B and TABLE 20. Both formulations exhibited

similar supercoiled peak areas following storage. 2M1SN displayed a higher supercoiled peak area compared to 2MSN.

TABLE 20

Form. Code	T=3 Days (n=5)			T=7 Days (n=5)		
	Super Coiled Peak Area (%)	Open Circle Peak Area (%)	Total Area	Super Coiled Peak Area (%)	Open Circle Peak Area (%)	Total Area
2MSN	92.9	7.1	13364.1	91.4	8.6	18054.2
2M1SN	96.7	3.3	14702.0	97.2	2.8	20771.3

[0316] **Stability during storage:** The lyophilized VM202 formulations for the additional accelerated stability study were stored over a 10 week period at 5°C, 25°C, and 40°C (TABLE 4). At each time point, samples were taken from temperature storage and visually evaluated. Photographs of the vials were taken and samples were assessed for cake shape. Samples were reconstituted with 5 mL of filtered water and evaluated for clarity and precipitation. All lyophilized cakes remained intact and did not show any sign of change upon storage regardless of temperature condition. Haziness was observed in reconstituted 2MSN following storage over 10 weeks. 2M1SN was clear after reconstitution following storage. The visual appearance of each formulation following 5°C, 25°C, and 40°C storage for 10 weeks is shown in Figures 24A-C.

[0317] Concentration, turbidity, moisture content, and capillary electrophoresis analyses were performed to evaluate the stability of lyophilized VM202 during temperature stresses.

[0318] At each time point, samples were reconstituted and analyzed for absorbance at 260 nm to determine the concentration of VM202 in vials. TABLE 21 shows that the concentration of VM202 in lyophilized samples following 10 weeks of temperature storage is similar to the results obtained at T=0. Therefore temperature stress induced no major changes in VM202 concentration.

TABLE 21				
Form. Code	Concentration (mg/mL), T=0 and T=10wks			
	T=0	T=10wks		
		5°C	25°C	40°C
2MSN	0.50	0.50	0.50	0.50
2M1SN	0.50	0.49	0.50	0.50

[0319] Turbidity of each sample was also evaluated at each time point for two (2) different wavelengths. While operators detected visual haziness in samples, meaningful difference in turbidity was not observed when measured at a wavelength of 650 nm. Therefore, it was determined that 450 nm was adequate to assess differences in turbidity between formulations. The results from this evaluation are detailed in TABLE 22. 2MSN exhibited moderate absorbance at 450 nm regardless of the temperature storage condition. 2M1SN exhibited low absorbance at 450 nm at all temperatures.

TABLE 22				
Form. Code	Turbidity (Abs_{450}), T=0 and T=10wks			
	T=0	T=10wks		
		5°C	25°C	40°C
2MSN	0.013	0.011	0.012	0.005
2M1SN	0.000	0.001	0.001	0.001

[0320] Moisture content analysis was also performed at each time point. TABLE 23 outlines the results for moisture content following 10 weeks of temperature storage. After 10 weeks of storage at 5°C, 25°C, and 40°C, similar moisture content was observed in all samples except 2M1SN stored at 5°C, which exhibited slightly elevated moisture content (1.56%).

TABLE 23				
Form. Code	Moisture content, T=0 and T=10wks			
	T=0	T=10wks		
		5°C	25°C	40°C
1% Water STD	0.95	0.94		
2MSN	1.31	1.20	1.18	1.15
2M1SN	1.44	1.56	1.17	1.23

[0321] Capillary electrophoresis analysis was used to monitor product purity. The CE chromatograms and peak area values after 10 weeks of incubation at 40°C are shown in Figure

25 and TABLE 24, respectively. Both formulations displayed significant decreases in the supercoiled peak area with corresponding increases in the open circle peak area compared to T=0 after reconstitution, following storage for 10 weeks at 40°C. 2M1SN displayed the highest supercoiled peak area, although this value was significantly lower at 10 weeks storage at 40°C than at time zero. Higher levels of open circle peak area were observed in 2MSN.

TABLE 24

Form. Code	Average (n=5)		
	Super Coiled Peak Area (%)	Open Circle Peak Area (%)	Total Area
2MSN	79.9	20.1	7410.8
2M1SN	86.0	14.0	9099.4

[0322] The CE supercoiled peak purity trends over 10 weeks at various temperatures are detailed in Figures 26A-C – at 5°C (Figure 26A), at 25°C (Figure 26B) or at 40°C (Figure 26C). Significant differences in purity were observed following elevated temperature incubation. As illustrated in TABLE 24, 2M1SN containing 1% sucrose and 0.45% NaCl displayed the highest level of purity following 40°C storage.

[0323] The trends of open circle peak impurities detected by CE during temperature storage are presented in Figures 27A-C – at 5°C (Figure 27A), at 25°C (Figure 27B) or at 40°C (Figure 27C). These graphs demonstrate increases in open circle peak corresponding to increased temperature storage. Following 40°C incubation, a higher level of open circle peak impurity was observed for 2MSN. 2M1SN exhibited less open circle peak at all temperatures, but followed the same trend as 2MSN.

6.8.1.5. Summary of test results

[0324] Prior to lyophilization, all the tested formulations comprising a plasmid DNA (VM202) remained clear, without discoloration or visible particulates. Additionally, capillary electrophoresis (CE) results consistently showed high purity of all of the compositions.

[0325] The first and second rounds of small scale lyophilization test were executed for VM202 using six (6) different formulations (KP8M2SN, KP8MS3N(=2MSN), KP8MT3N, 4MSN, 3MSN and Control), where the product was filled at 0.75 mL into 3 cc glass vials. The accelerated stability study was executed for VM202 using the same four (4) formulations

(4MSN, 3MSN, 2MSN and Control) of second round of small scale lyophilization filled at 5 mL into 20 cc glass vials, and lyophilization parameters determined from data acquired from the small scale lyophilization studies. Also, two (2) different formulations (2MSN and 2M1SN) were tested in an additional accelerated stability study based on the data from the initial accelerated stability study. The lyophilization cycles were designed from results obtained following subambient DSC analysis. An annealing step was included in the lyophilization cycle, as it was determined that the devitrification temperature was eliminated after annealing.

[0326] Following lyophilization, elegant or decent cakes were observed for all compositions except Control comprising 0.9% NaCl and 1.1% sucrose. The Control is a formulation previously described in U.S. Patent No. 8,389,492, which is incorporated herein by reference in its entirety.

[0327] After reconstitution of the lyophilized compositions, however, all the tested compositions except 2M1SN (10 mM Potassium Phosphate at pH 8.0, 2% Mannitol, 1% Sucrose, 0.45% NaCl) appeared to be hazy. The haziness remained even after storage of the reconstituted compositions at 25 ºC for 3 and 7 days following reconstitution. Only 2M1SN remained clear following reconstitution and following storage at 25 ºC for 3 and 7 days after reconstitution, and there was no change in the concentrations of VM202 in 2M1SN throughout the study.

[0328] Capillary electrophoresis was effective in evaluating VM202 product purity. Following lyophilization and reconstitution, all formulations showed decreases in the supercoiled peak area. A decrease in the supercoiled peak area was also observed in all formulations after 10 weeks of storage at 25°C and 40°C. However, 2M1SN showed the highest supercoiled peak percentage corresponding with the least increase in open circle peak percentage during the 10 week stability study.

[0329] The results suggest that lyophilized VM202 maintains elegant cake appearance and the highest level of purity (supercoiled DNA) after lyophilization and storage when formulated at 0.5 mg/mL concentration with 10 mM Potassium Phosphate at pH 8.0, 2% Mannitol, 1% Sucrose, 0.45% NaCl, at a fill volume of 5 mL in 20 cc vials (2M1SN in TABLE 25). Although the rate of instability increases for VM202 at higher temperatures, less degradation was observed in this 2M1SN formulation than in the other tested formulations.

TABLE 25					
Form. Code	**Buffer**	**pH**	**Bulking Agent**	**Stabilizer(s)**	**VM202 Concentration (mg/mL)**
2M1SN	10 mM Potassium Phosphate	8.0	2% Mannitol	1.0% Sucrose, 0.45% NaCl	0.5

6.8.2. Example 2: Lyophilized composition of VM202 (Study 002)

[0330] Various lyophilized formulations containing VM202 were generated and analyzed to test the quality of VM202 in the drug product formulation following lyophilization as well as the quality of VM202 in formulations with slight variations in pH and/or concentrations of bulking agents and stabilizers.

6.8.2.1. Experimental Design

[0331] **Materials**: The active pharmaceutical ingredient (API) examined in this study was VM202. The material used for this study was comprised of the following: The chemicals and materials used to formulate and analyze VM202 were as follows:

TABLE 26	
Drug Substance (DS):	VM202 in 0.9% (w/v) NaCl, pH 5.9 at 1.6 mg/mL, Lot # 2018#009S
Potassium Phosphate Monobasic	J.T. Baker, Cat# 3248-01, Lot# 0000163254
Potassium Phosphate Dibasic	Sigma Aldrich, Cat# P222-500G, Lot# SLBS0610V
Sucrose	J.T. Baker, Cat# 4074-05, Lot# 0000159304
D-Mannitol	Pfanstiehl, Cat# M-109-6, Lot 35517A
Sodium Chloride	EMD Millipore, Cat## 1.06404.5000, Lot# K49421004743
150 mL Nalgene Rapid-Flow 0.2μm SFCA Filter Unit	Thermo Scientific, Cat# 0974028E, Lot#1233541
150 mL Nalgene Receiver Bottle	Thermo Scientific, Cat# 455-0150
20-cc, 20 mm Borosilicate Vials	Schott, Cat# 68000321
20 mm Stoppers	West Pharmaceutical, Cat#19700033

[0332] **Formulation parameter**: In this study, the following parameters were fixed:

(1) Fill volume: 5 mL
(2) API Concentration: 0.5 mg/mL
(3) Buffer Concentration: 10 mM Potassium Phosphate
(4) Sucrose Concentration: 1%

[0333] The following formulation parameters were examined in these formulations:

(1) pH: 7.0, 8.0, and 9.0
(2) Mannitol Concentration: 1% and 2%
(3) Sodium Chloride Concentration: 0.45%, 0.6%, and 0.9%

[0334] Formulation listed in the below in TABLE 27 were prepared for analysis:

TABLE 27
Formulation Matrix for the Lyophilization and Analysis Study

Form. No.	Form. Code	Buffer (10mM)	pH	Mannitol (%)	Sucrose (%)	NaCl (%)	API (mg/mL)
1	F1	Potassium Phosphate	8.0	2	1	0.45	0.5
2	F2	Potassium Phosphate	7.0	2	1	0.45	0.5
3	F3	Potassium Phosphate	9.0	2	1	0.45	0.5
4	F4	Potassium Phosphate	8.0	1	1	0.45	0.5
5	F5	Potassium Phosphate	8.0	2	1	0.60	0.5
6	F6	Potassium Phosphate	8.0	2	1	0.90	0.5

6.8.2.2. Formulation preparations

6.8.2.2.1. Small scale study

[0335] A small-scale test was performed to determine the buffer pH values required in order to dilute the drug substance (DS) to achieve the target formulation pH values in TABLE 27. To conserve the drug substance, the small-scale study dilutions were performed with a 0.9% NaCl placebo (same formulation as the DS) in lieu of the DS. The 0.9% NaCl placebo was prepared without pH adjustment, to better represent the DS formulation composition. The dilution buffer composition (TABLE 28) was calculated based on dilution of DS from 1.6 mg/mL to 0.5

mg/mL. The small-scale test revealed that after dilution with the formulation buffers, all the final formulations showed pH values on or near the target values in TABLE 27. However, as the pH of formulation F3 (9.0) was beyond the buffering range of phosphate, a downward drift in pH was observed. To best compensate for this, a higher pH diluent buffer was selected (9.5). Small scale testing showed a pH of 9.2 immediately following dilution then a decrease in pH to 9.1 and 8.5 after 24 and 48 hours, respectively.

[0336] To best achieve the target pH values for this study, formulations were prepared within one day of lyophilization and changes in pH following reconstitution were monitored. The dilution buffers shown in TABLE 28 were used for formulation preparation.

TABLE 28
Starting Dilution Buffer Composition

Form. Code	Buffer (14.55 mM)	pH	Mannitol (%)	Sucrose (%)	NaCl (%)
D1	Potassium Phosphate	8.0	2.91	1.46	0.25
D2	Potassium Phosphate	7.0	2.91	1.46	0.25
D3	Potassium Phosphate	9.5	2.91	1.46	0.25
D4	Potassium Phosphate	8.0	1.46	1.46	0.25
D5	Potassium Phosphate	8.0	2.91	1.46	0.46
D6	Potassium Phosphate	8.0	2.91	1.46	0.90

6.8.2.2.2. Lyophilization

[0337] VM202 at 1.6 mg/mL in 0.9% NaCl provided from Cobra was removed from -70°C storage and thawed at 5°C overnight. 35 mL of DS was diluted in 21 mL of dilution buffers (TABLE 28) to a concentration of 1.0 mg/ml. Following dilution, the pH and API concentration were measured. Despite the results of the small scale testing using placebo, in the presence of VM202 the formulations did not produce the target pH values, so the pH of starting dilution buffers were further adjusted during dilution of 1.0 mg/ml DS in 48 mL of dilution buffer (TABLE 29) to a final concentration of 0.5 mg/ml.

TABLE 29

pH and Amount of Dilution Buffers used for Dilution from 1 mg/mL to 0.5 mg/mL

Form. Code	pH	Amount (mL)
D1	8.3	29
	9.3	14
	9.5	5
D2	7.0	10
	7.1	38
D3	9.1	36
	11.0	12
D4	8.3	15
	8.5	20
	8.7	13
D5	8.2	10
	8.7	38
D6	8.8	48

[0338] Following dilution, formulations were sterile filtered using 0.2 µm cellulose acetate filter, filled into 20 cc glass, depyrogenated vials at a 5 mL fill volume (2.5 mg dose) in the BSC. After filling, vials were partially stoppered and loaded into the lyophilizer for freeze-drying. Empty vials were used to completely surround VM202 DS containing vials. Following lyophilization, the vials were fully stoppered inside the lyophilization chamber with 600 Torr partial vacuum, before being removed. Vials were crimped, labeled and placed in -70°C. One (1) vial of each formulation was placed in 5°C. One (1) vial per each formulation was reconstituted with 5 mL of filtered Milli-Q water and analyzed with pre-lyo samples.

6.8.2.2.3. Analysis methods

[0339] Lyophilized formulations were analyzed at different time points as summarized in TABLE 30.

TABLE 30			
Analytical Methods	**Temperature Stress Time Points**		
	Pre-Lyo	**Lyophilized Cake**	**Reconstituted Liquid**
Visual	X	X	X
Concentration (A_{260})	X	-	X
pH	X	-	X
Reconstitution Time	-	X	-
Turbidity (A_{350}, A_{450}, A_{650})	X	-	X
CZE	X	-	X
Residual Moisture Content	-	X	-

[0340] **Visual Inspection:** Visual inspection was performed under a white light source (13W fluorescent tube) against black and white backgrounds. Digital photographs were acquired of all formulations.

[0341] **Concentration Measurement (A260):** Spectrophotometry analysis was performed with a quartz cuvette with a 1 cm path length by a Beckman Coulter DU800. The plasmid DNA concentration of the test samples was determined by measuring absorbance at 230, 260, 280 and 350 nm. Concentration analyses were performed using Helixmith's UV/Vis protocol. Calculations were performed using the following equations:

Concentration (μg/mL) = [(D-E)/C] x (B/A)

Recovery = (low μg/mL) / (high μg/mL) x 100%

OD_{260nm} / OD_{280nm} = D / F

OD_{260nm} / OD_{230nm} = D / G

Where:

A = weight of sample taken for the dilution

B = total weight of sample and buffer used for the dilution

C = extinction coefficient of a 0.005% solution in a 1cm path length cuvette (0.02)

D = optical density for the maximum at 260nm. E = absorbance measured at 350nm

F = absorbance measured at 280nm

G = absorbance measured at 230nm

Acceptance Criteria:

D must fall in the range 0.5 to 1.5 OD units

Recovery must be ≥ 98% OD_{260nm} / OD_{280nm} = must fall in the range 1.8 to 2.0

$OD2_{60nm}$ / OD_{230nm} = must be ≥ 1.1

[0342] **pH:** pH analysis was performed with a SympHony® pH Meter (VWR Scientific, catalog #SB70P), calibrated with three pH standard solutions (pH 4, 7, and 10) with a calibration slope of 95% or higher. Samples were not temperature adjusted, and were allowed to equilibrate to ambient temperature and measured.

[0343] **Turbidity Measurement (A350, A450, A650):** Turbidity was determined by measuring sample absorbance at 350, 450, and 650 nm using a Beckman Coulter DU800. Formulations with an A650 > 0.01 are generally considered to exhibit increased turbidity.

[0344] **Capillary Zone Electrophoresis:** Prior to analysis, a capillary was conditioned by running Milli-Q water, 0.1N NaOH, 0.1N HCl, and Milli-Q water again at 20 psi for 10 minute each (the conditioning lasted a total of 40 minutes). 40 µL of sample at a concentration of 0.5 mg/mL was loaded into a polyethylene insert for analysis.

Instrument: Beckman Coulter PA 800+ CE (S/N 3063309)

Capillary: Neutral coated capillary, 50 µm I.D, 40 cm total length, 30 cm effective length, 8 µm aperture (Beckman Coulter P/N 477441, lot # M812134)

Data Analysis: 32 Karat (version 9.0)

Pressure/injection time: 0.5 psi / 11 sec.

Separation buffer/pressure: 100 mM phosphoric acid (pH 2.60), 85% / 20 psi; 60 sec.

Wash buffer/pressure: Milli-Q water / 20 psi; 60 sec

Detection: UV at 254 nm

Separation voltage: 17 KV; ramp 0.17 min; 14 min.

[0345] **Residual Moisture Content:** A Karl Fisher Coulometer C20 (Mettler Toledo) was used for moisture content analysis. An Apura Water Standard Oven 1% was used to determine system

accuracy. Sample vials were brought to room temperature before caps were removed for analysis. Approximately 100 mg of material was used for each analysis.

6.8.2.3. Summary of test results

[0346] This section summarizes the quality of the lyophilized VM202 in various formulations.

6.8.2.3.1. Lyophilization Cycle Profile

[0347] The previously designed Lyo Cycle was utilized to lyophilize 2.5 mg doses of VM202 in various formulations. The ramping of the freezing temperature from -50°C to -20°C and holding for two (2) hours prior to pulling the vacuum allowed for annealing to occur. The annealing process aids the crystallization of amorphous excipients. Due to unique feature of the lyophilizer used in these experiments, which may abort the program if the vacuum does not reach the set point within a given time frame, a step at the beginning of primary drying where the vacuum is pulled to 100 mTorr initially and adjusted to final vacuum as primary drying starts was utilized. Primary drying was performed at shelf temperature of -20°C (~53.4 hours) with 50 mTorr chamber pressure (TABLE 31). Secondary drying at a shelf temperature of 20°C was designed to remove the residual water that was not sublimated during the primary drying step.

TABLE 31
Lyophilization Cycle Parameters

Step	Temperature (°C)	Time (Min)	Ramp Rate (°C/Min)	Chamber Pressure (mTorr)
Loading	5	N/A	N/A	N/A
Freezing	5 to -50	55	1	N/A
	-50	120	N/A	N/A
	-50 to -20	60	0.5	N/A
	-20	120	N/A	N/A
	-20	60	N/A	100
Primary Drying	-20	3200*	N/A	50
Secondary Drying	-20 to 20	80	0.5	50
	20	780	N/A	50

* Primary drying time was changed from 3240 min to 3200 min due to software limitations. Pirani gauge and the capacitance manometer merged, before advancing to secondary drying.

[0348] Figure 28 shows a chart of the entire lyophilization cycle. The product temperature dropped to around -40°C, due to heat-loss from sublimation when the vacuum was applied. The pirani gauge value merged with the capacitance manometer reading at approximately 41 hours, which confirmed the completion of the primary drying process. Following 62 hours of the entire cycle, secondary drying at 20°C was initiated and the samples were allowed to dry for an additional 13 hours. The entire cycle lasted approximately 75 hours.

[0349] The measured moisture contents of the cakes are shown in TABLE 32.

TABLE 32
Moisture content results

Form. Code	Moisture Content (%)
F1	2.30
F2	1.51
F3	1.78
F4	1.90
F5	2.39
F6	3.02

6.8.2.3.2. Lyophilization study

[0350] **Visual inspection:** Following lyophilization, F1, F2, and F3 displayed decent cakes, with only slight shrinkage. F5 displayed significant cake shrinkage. Total collapse of cakes was observed for F4 (Figure 32) and F6 (Figure 34). F1 (Figure 29), F2 (Figure 30), F3 (Figure 31), F4 (Figure 32), and F5 (Figure 33) reconstituted liquid samples were clear, colorless, and free of visible particulates, comparable to the pre-lyo control. Following reconstitution, F6 was hazy.

[0351] **Concentration (A_{260}):** All samples were at their target concentrations before lyophilization and after reconstitution as provided in TABLE 33.

TABLE 33 Concentration results		
Form. Code	Concentration (mg/mL)	
	Pre-Lyo	Reconstituted Liquid
F1	0.5	0.5
F2	0.5	0.5
F3	0.5	0.5
F4	0.5	0.5
F5	0.5	0.5
F6	0.5	0.5

[0352] **pH and Reconstitution Time:** Due to the buffering limitation of formulation F3, the pH of F3 was not stable and decreased quickly during the fill. By the time the pre-lyo samples were analyzed they had already decreased from pH 9.0 to 8.7. Further decrease of pH value was observed after reconstitution (Δ = -0.4), compared to the pre-lyo control. After reconstitution, F1, F2, F4, F5, F6 formulations showed the same pH values ($\Delta \leq 0.1$), compared to their pre-lyo controls. Reconstituted time analysis indicated that lyophilized cake dissolved between 1 and 1.5 minutes. The pH and reconstitution time results are detailed in TABLE 34.

TABLE 34 pH and Reconstitution Time Results			
Form. Code	pH		Reconstitution Time (min)
	Pre-Lyo	Reconstituted Liquid	Lyo Cake
F1	7.9	8.0	~1.0
F2	7.0	7.1	~1.0
F3	8.7	8.3	~1.5
F4	7.9	8.0	~1.5
F5	7.9	7.9	~1.0
F6	7.9	7.9	~1.5

[0353] **Turbidity (A_{350}, A_{450}, A_{650}):** Slight increases in turbidity were observed for F6 at 350 nm, 450 nm, and 650 nm after reconstitution. All other formulations showed no significant turbidity before lyophilization or after reconstitution as provided below in TABLE 35.

TABLE 35
Turbidity Results

Form. Code	Turbidity					
	Abs_{350}		Abs_{450}		Abs_{650}	
	Pre-Lyo	Reconstituted Liquid	Pre-Lyo	Reconstituted Liquid	Pre-Lyo	Reconstituted Liquid
F1	0.005	0.005	0.002	-0.001	0.000	-0.003
F2	-0.002	0.010	-0.005	0.002	-0.004	-0.001
F3	0.006	0.003	0.001	-0.003	0.000	-0.004
F4	0.000	-0.002	-0.004	-0.005	-0.004	-0.005
F5	-0.002	0.003	-0.005	-0.003	-0.005	-0.004
F6	0.010	0.099	-0.004	0.060	-0.005	0.028

Note: The absorbance of water was subtracted to obtain turbidity values for samples, with $A_{650} \geqslant 0.01$ considered to be turbid.

[0354] **Capillary Zone Electrophoresis (CZE):** The reconstituted samples were further examined by CZE for product purity. F6 was not analyzed due to turbidity. The electropherograms and tabular results from CZE are detailed in Figure 35 and TABLE 36, respectively. After reconstitution, all formulations showed slight increases of open circle peak, with corresponding decreases in supercoil peak (97.5% - 99.0%), compared to pre-lyo (99.1% - 100.0%). F4 and F5 showed the smallest increase of open circle peak percentage, less than or equal to 0.4%, compared to other formulations. Excluding formulations with poor cake appearance, current formulation (F1), showed the highest purity, before (100%) and after lyophilization (98.6%).

TABLE 36
CZE Results

Sample		Supercoil Peak %	Open Circle Peak %	Total Area
F1	Pre-Lyo	100.0	0.0	8082
	Reconstituted Liquid	98.6	1.4	12253
F2	Pre-Lyo	99.8	0.2	9082
	Reconstituted Liquid	98.3	1.7	12848
F3	Pre-Lyo	99.4	0.6	9904
	Reconstituted Liquid	97.5	2.5	12202
F4	Pre-Lyo	99.2	0.8	9535
	Reconstituted Liquid	99.0	1.0	13023
F5	Pre-Lyo	99.1	0.9	9215
	Reconstituted Liquid	98.7	1.3	12542

[0355] **Conclusions:** The objective of the study was to assess the quality of VM202 in the drug product formulation, 0.5 mg/mL VM202 in 10 mM potassium phosphate with 2% mannitol, 1 % sucrose, 0.45% NaCl at pH 8.0, following lyophilization, as well as the quality of VM202 in

formulations with slight variations in pH and/or concentrations of bulking agents and stabilizers from the drug product formulation. The analytical methods implemented in this study included capillary zone electrophoresis (CZE), visual inspection for clarity and cake appearance, turbidity, concentration (A260), pH, and moisture content analyses (Karl Fischer), which have all been shown to be effective stability-indicating assays for the product.

[0356] In this lyophilized formulation study, VM202 was evaluated at 2.5 mg per vial at a fill volume of 5 mL. A small-scale pH testing was performed with 0.9% NaCl placebo to establish proper formulation preparation procedures by dilution method for VM202 on a larger scale.

[0357] Prior to lyophilization, all formulations of VM202 were clear, without discoloration, and free of visible particulates, and concentration was on target. pH of the 10 mM potassium phosphate formulation at pH 9.0 was not stable and decreased quickly during the fill, with further decreases observed after lyophilization and reconstitution. Capillary zone electrophoresis results for all formulations prior to lyophilization showed high purity, with the drug product formulation showing the highest percentage of supercoil peak.

[0358] Following lyophilization, decent cakes were observed for the drug product formulation and the drug product formulations at higher and lower pH. After reconstitution, the formulation with the highest concentration of NaCl exhibited haziness, while the rest of formulations were clear, colorless, and free of visible particulates, comparable to the pre-lyo control. After reconstitution, all formulations showed slight increases of open circle peak, with corresponding decreases in supercoil peak. Excluding formulations with poor cake appearance, the drug product formulation, showed the highest purity by capillary zone electrophoresis after lyophilization.

[0359] Results obtained from this study suggest that lyophilized VM202 maintains decent cake appearance and the highest level of purity (supercoiled DNA) after lyophilization at 0.5 mg/mL with 10 mM potassium phosphate at pH 8.0, 2% mannitol, 1% sucrose, 0.45% NaCl, at a fill volume of 5 mL in 20 cc vials.

6.8.3. Example 3: Lyophilized composition of pTx-IGF-1X10

[0360] Various lyophilized formulations containing pTx-IGF-1X10 were generated and analyzed for the quality of pTx-IGF-1X10 in the formulation following lyophilization. Formulations listed below in TABLE 37 were prepared for analysis.

TABLE 37					
Form. Code	**buffer**	**pH**	**Bulking agent**	**Stabilizers**	**PTX-IGF-1X10 concentration**
F1	10 mM potassium phosphate	8	2% mannitol	1.0% sucrose, 0.45% NaCl	0.5 mg/ml
F2	10 mM potassium phosphate	7	2% mannitol	1.0% sucrose, 0.45% NaCl	0.5 mg/ml
F3	10 mM potassium phosphate	9	2% mannitol	1.0% sucrose, 0.45% NaCl	0.5 mg/ml
F4	10 mM potassium phosphate	8	1% mannitol	1.0% sucrose, 0.45% NaCl	0.5 mg/ml
F5	10 mM potassium phosphate	8	2% mannitol	1.0% sucrose, 0.60% NaCl	0.5 mg/ml
F6	10 mM potassium phosphate	8	2% mannitol	1.0% sucrose, 0.90% NaCl	0.5 mg/ml
F7	10 mM potassium phosphate	8	2% mannitol	1.0% Sucrose, 0.2% NaCl	0.5 mg/ml
F8	10 mM potassium phosphate	8	2% mannitol	0.5% Sucrose, 0.45% NaCl	0.5 mg/ml
F9	10 mM potassium phosphate	8	3% mannitol	1.0% sucrose, 0.45% NaCl	0.5 mg/ml

6.8.3.1. Summary of test results

[0361] **Visual inspection:** Following lyophilization, F1, F2, F7, F8 and F9 displayed decent cakes, with only slight shrinkage. F5 displayed significant cake shrinkage. F3 displayed splashing form. Total collapse of cakes was observed for F4 and F6. F1 (Figure 36), F2 (Figure 37), F3 (Figure 38), F4 (Figure 39), F5 (Figure 40), F6 (Figure 41), F7 (Figure 42), F8 (Figure 43) and F9 (Figure 44) reconstituted liquid samples were clear, colorless, and free of visible particulates, comparable to the pre-lyo control. To determine the turbidity (Clarity) of samples,

samples were compared to Turbidity 4000NTU calibration standard I, II, III, IV. Formulation with "< I Clarity" can be evaluated as not turbid.

[0362] Concentration (A260): All samples were at their target concentrations before lyophilization and after reconstitution as provided below in TABLE 38.

TABLE 38
Concentration results

Form. Code	Concentration (mg/mL)	
	Pre-Lyo	Reconstituted Liquid
F1	0.5	0.5
F2	0.5	0.5
F3	0.5	0.5
F4	0.5	0.5
F5	0.5	0.5
F6	0.5	0.5
F7	0.5	0.5
F8	0.5	0.5
F9	0.5	0.5

[0363] **pH and Reconstitution Time:** The pH value of F3 decreased from pH 9.0 to 7.6 after reconstitution. After reconstitution, F1, F2, F4, F5, F6, F7, F8, F9 formulations showed the same pH values ($\Delta \leq 0.5$), compared to their pre-lyo controls. Reconstituted time analysis indicated that lyophilized cake dissolved between 1min 20sec and 4 min 05sec. The pH and reconstitution time results are detailed in TABLE 39.

TABLE 39
pH and Reconstitution Time Results

Form. Code	pH		Reconstitution Time (min:sec)
	Pre-Lyo	Reconstituted Liquid	Lyo Cake
F1	8.0	7.7	1:50
F2	7.0	6.9	3:40
F3	9.0	7.6	4:05
F4	8.0	7.6	2:00
F5	8.0	7.6	2:20
F6	8.0	7.6	1:20
F7	8.0	7.7	1:40
F8	8.0	7.6	1:50
F9	8.0	7.6	3:20

[0364] **Capillary Electrophoresis (CE):** The reconstituted samples were further examined by CE for product purity. The electropherograms and tabular results from CE are detailed in Figure

45 and TABLE 40, respectively. After reconstitution, all formulations except F3 showed slight increases (0.5-3%) of open circle peak, with corresponding decreases in supercoil peak, compared to pre-lyo. Excluding formulations with poor cake appearance, current formulation (F1), showed the lowest decrease of supercoil peak after lyophilization.

TABLE 40
CE results

Sample		Supercoil Peak %	Open Circle Peak %	Total Area
F1	Pre-Lyo	91.71	8.29	17702.24
	Reconstituted Liquid	91.21	8.79	20779.98
F2	Pre-Lyo	92.67	7.33	15510.04
	Reconstituted Liquid	90.33	9.67	22004.75
F3	Pre-Lyo	95.31	4.69	12198.69
	Reconstituted Liquid	84.62	15.38	22561.09
F4	Pre-Lyo	92.55	7.45	15180.98
	Reconstituted Liquid	91.97	8.03	22858.45
F5	Pre-Lyo	91.85	8.15	18847
	Reconstituted Liquid	90.69	9.31	23597.22
F6	Pre-Lyo	92.19	7.81	19221.12
	Reconstituted Liquid	90.73	9.27	23951.71
F7	Pre-Lyo	91.71	8.29	17919.92
	Reconstituted Liquid	90.51	9.49	22180.31
F8	Pre-Lyo	92.08	7.92	18266.49
	Reconstituted Liquid	90.54	9.46	24314.31
F9	Pre-Lyo	92.54	7.46	15054.62
	Reconstituted Liquid	89.10	10.90	23387.16

6.8.4. Example 4: Lyophilized composition of pCK-SDF-1α

6.8.4.1. Experimental design

[0365] Various lyophilized formulations containing pCK-SDF-1α were generated and analyzed for the quality of pCK-SDF-1α in the formulation following lyophilization. Formulations listed below in TABLE 41 were prepared for analysis.

TABLE 41					
Form. Code	**buffer**	**pH**	**Bulking agent**	**Stabilizers**	**pCK-SDF-1α concentration**
F1	10 mM potassium phosphate	8	2% mannitol	1.0% sucrose, 0.45% NaCl	0.5 mg/ml
F2	10 mM potassium phosphate	7	2% mannitol	1.0% sucrose, 0.45% NaCl	0.5 mg/ml
F3	10 mM potassium phosphate	9	2% mannitol	1.0% sucrose, 0.45% NaCl	0.5 mg/ml
F4	10 mM potassium phosphate	8	1% mannitol	1.0% sucrose, 0.45% NaCl	0.5 mg/ml
F5	10 mM potassium phosphate	8	2% mannitol	1.0% sucrose, 0.60% NaCl	0.5 mg/ml
F6	10 mM potassium phosphate	8	2% mannitol	1.0% sucrose, 0.90% NaCl	0.5 mg/ml
F7	10 mM potassium phosphate	8	2% mannitol	1.0% Sucrose, 0.2% NaCl	0.5 mg/ml
F8	10 mM potassium phosphate	8	2% mannitol	0.5% Sucrose, 0.45% NaCl	0.5 mg/ml
F9	10 mM potassium phosphate	8	3% mannitol	1.0% sucrose, 0.45% NaCl	0.5 mg/ml

6.8.4.2. Summary of test results

[0366] **Visual inspection:** Following lyophilization, F1, F2, F3, F7, F8 and F9 displayed decent cakes, with only slight shrinkage. F5 displayed significant cake shrinkage. Total collapse of cakes was observed for F4 and F6. F1 (Figure 46), F2 (Figure 47), F3 (Figure 48), F4 (Figure 49), F5 (Figure 50), F6 (Figure 51), F7 (Figure 52), F8 (Figure 53) and F9 (Figure 54) reconstituted liquid samples were clear, colorless, and free of visible particulates, comparable to the pre-lyo control. To determine the turbidity (Clarity) of samples, samples were compared to

Turbidity 4000NTU calibration standard I, II, III, IV. Formulation with " < I Clarity" can be evaluated as not turbid.

[0367] **Concentration (A260):** All samples were at their target concentrations before lyophilization and after reconstitution as provided below in TABLE 42.

TABLE 42
Concentration results

Form. Code	Concentration (mg/mL)	
	Pre-Lyo	Reconstituted Liquid
F1	0.5	0.5
F2	0.5	0.5
F3	0.5	0.5
F4	0.5	0.5
F5	0.5	0.5
F6	0.5	0.5
F7	0.5	0.5
F8	0.5	0.5
F9	0.5	0.5

[0368] **pH and Reconstitution Time:** The pH value of F3 decreased from pH 9.0 to 7.9 after reconstitution. After reconstitution, F1, F2, F4, F5, F6, F7, F8, F9 formulations showed the same pH values ($\Delta \leq 0.5$), compared to their pre-lyo controls. Reconstituted time analysis indicated that lyophilized cake dissolved between 1.5min and 4 minutes. The pH and reconstitution time results are detailed below in TABLE 43.

TABLE 43
pH and reconstitution time results

Form. Code	pH		Reconstitution Time (min:sec)
	Pre-Lyo	Reconstituted Liquid	Lyo Cake
F1	8.0	7.7	1:40
F2	7.0	6.9	2:10
F3	9.0	7.9	2:30
F4	8.0	7.8	1:50
F5	8.0	7.7	1:30
F6	8.0	7.6	1:30
F7	8.0	7.7	1:30
F8	8.0	7.7	2:20
F9	8.0	7.7	4:00

[0369] **Capillary Electrophoresis:** The reconstituted samples were further examined by CE for product purity. The electropherograms and tabular results from CE are detailed in Figure 55 and

TABLE 44, respectively. After reconstitution, all formulations showed slight increases (0.5-2%) of open circle peak, with corresponding decreases in supercoil peak, compared to pre-lyo. Excluding formulations with poor cake appearance, current formulation (F1), showed the lowest decrease of supercoil peak after lyophilization.

TABLE 44
CE results

Sample		Supercoil Peak %	Open Circle Peak %	Total Area
F1	Pre-Lyo	92.97	7.03	18689.15
	Reconstituted Liquid	92.35	7.65	21223.38
F2	Pre-Lyo	92.89	7.11	18494.15
	Reconstituted Liquid	92.00	8.00	22748.49
F3	Pre-Lyo	92.88	7.12	17540.79
	Reconstituted Liquid	91.52	8.48	22360.01
F4	Pre-Lyo	93.14	6.86	18019.91
	Reconstituted Liquid	92.80	7.20	21975.46
F5	Pre-Lyo	92.94	7.06	20043.63
	Reconstituted Liquid	92.57	7.43	22977.2
F6	Pre-Lyo	93.15	6.85	19398.54
	Reconstituted Liquid	92.51	7.49	22958.31
F7	Pre-Lyo	92.64	7.36	20243.28
	Reconstituted Liquid	91.86	8.14	21279.46
F8	Pre-Lyo	93.20	6.80	19094.26
	Reconstituted Liquid	91.65	8.35	22253.88
F9	Pre-Lyo	92.82	7.18	18334.58
	Reconstituted Liquid	91.36	8.64	21085.33

7. INCORPORATION BY REFERENCE

[0370] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

8. EQUIVALENTS

[0371] While various specific embodiments have been illustrated and described, the above specification is not restrictive. It will be appreciated that various changes can be made without departing from the spirit and scope of the invention(s). Many variations will become apparent to those skilled in the art upon review of this specification.

SEQUENCE LISTING:

SEQ ID NO:1	Amino acid sequence of flHGF protein	MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIH EFKKSAKTTLIKIDPALKIKTKKVNTADQCANRCTRNKGL PFTCKAFVFDKARKQCLWFPFNSMSSGVKKEFGHEFDLYE NKDYIRNCIIGKGRSYKGTVSITKSGIKCQPWSSMIPHEHSF LPSSYRGKDLQENYCRNPRGEEGGPWCFTSNPEVRYEVC DIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTR WEYCAIKTCADNTMNDTDVPLETTECIQGQGEGYRGTVN TIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNP DGSESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNG KNYMGNLSQTRSGLTCSMWDKNMEDLHRHIFWEPDASK LNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTT PTIVNL DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICG GSLIKESWVLTARQCFPSRDLKDYEAWLGIHDVHGRGDE KCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTID LPNYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMG NEKCSQHHRGKVTLNESEICAGAEKIGSGPCEGDYGGPLV CEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIH KIILTYKVPQS
SEQ ID NO:2	Amino acid sequence of dHGF protein	MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIH EFKKSAKTTLIKIDPALKIKTKKVNTADQCANRCTRNKGL PFTCKAFVFDKARKQCLWFPFNSMSSGVKKEFGHEFDLYE NKDYIRNCIIGKGRSYKGTVSITKSGIKCQPWSSMIPHEHS YRGKDLQENYCRNPRGEEGGPWCFTSNPEVRYEVCDIPQ CSEVECMTCNGESYRGLMDHTESGKICQRWDHQTPHRHK FLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYC AIKTCADNTMNDTDVPLETTECIQGQGEGYRGTVNTIWN GIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGSE SPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYM GNLSQTRSGLTCSMWDKNMEDLHRHIFWEPDASKLNENY CRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVN LDHPVI SCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIK ESWVLTARQCFPSRDLKDYEAWLGIHDVHGRGDEKCKQ VLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLPNYG CTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCS QHHRGKVTLNESEICAGAEKIGSGPCEGDYGGPLVCEQHK MRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKIILTY KVPQS
SEQ ID NO:3	Nucleotide sequence of exons 1-4 of human hgf	ATGTGGGTGACCAAACTCCTGCCAGCCCTGCTGCTGCA GCATGTCCTCCTGCATCTCCTCCTGCTCCCCATCGCCAT CCCCTATGCAGAGGGACAAAGGAAAAGAAGAAATACA ATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAAT

		CAAAATAGATCCAGCACTGAAGATAAAAACCAAAAAA GTGAATACTGCAGACCAATGTGCTAATAGATGTACTAG GAATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTT TGATAAAGCAAGAAAACAATGCCTCTGGTTCCCCTTCA ATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGGCCAT GAATTTGACCTCTATGAAAACAAAGACTACATTAGAAA CTGCATCATTGGTAAAGGACGCAGCTACAAGGGAACAG TATCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGG AGTTCCATGATACCACACGAACACAG
SEQ ID NO:4	Nucleotide sequence of exons 5-18 of human hgf	CCTACAGGAAAACTACTGTCGAAATCCTCGAGGGGAAG AAGGGGGACCCTGGTGTTTCACAAGCAATCCAGAGGTA CGCTACGAAGTCTGTGACATTCCTCAGTGTTCAGAAGTT GAATGCATGACCTGCAATGGGGAGAGTTATCGAGGTCT CATGGATCATACAGAATCAGGCAAGATTTGTCAGCGCT GGGATCATCAGACACCACACCGGCACAAATTCTTGCCT GAAAGATATCCCGACAAGGGCTTTGATGATAATTATTG CCGCAATCCCGATGGCCAGCCGAGGCCATGGTGCTATA CTCTTGACCCTCACACCCGCTGGGAGTACTGTGCAATTA AAACATGCGCTGACAATACTATGAATGACACTGATGTT CCTTTGGAAACAACTGAATGCATCCAAGGTCAAGGAGA AGGCTACAGGGGCACTGTCAATACCATTTGGAATGGAA TTCCATGTCAGCGTTGGGATTCTCAGTATCCTCACGAGC ATGACATGACTCCTGAAAATTTCAAGTGCAAGGACCTA CGAGAAAATTACTGCCGAAATCCAGATGGGTCTGAATC ACCCTGGTGTTTTACCACTGATCCAAACATCCGAGTTGG CTACTGCTCCCAAATTCCAAACTGTGATATGTCACATGG ACAAGATTGTTATCGTGGGAATGGCAAAAATTATATGG GCAACTTATCCCAAACAAGATCTGGACTAACATGTTCA ATGTGGGACAAGAACATGGAAGACTTACATCGTCATAT CTTCTGGGAACCAGATGCAAGTAAGCTGAATGAGAATT ACTGCCGAAATCCAGATGATGATGCTCATGGACCCTGG TGCTACACGGGAAATCCACTCATTCCTTGGGATTATTGC CCTATTTCTCGTTGTGAAGGTGATACCACACCTACAATA GTCAATTTAGACCATCCCGTAATATCTTGTGCCAAAACG AAACAATTGCGAGTTGTAAATGGGATTCCAACACGAAC AAACATAGGATGGATGGTTAGTTTGAGATACAGAAATA AACATATCTGCGGAGGATCATTGATAAAGGAGAGTTGG GTTCTTACTGCACGACAGTGTTTCCCTTCTCGAGACTTG AAAGATTATGAAGCTTGGCTTGGAATTCATGATGTCCA CGGAAGAGGAGATGAGAAATGCAAACAGGTTCTCAAT GTTTCCCAGCTGGTATATGGCCCTGAAGGATCAGATCTG GTTTTAATGAAGCTTGCCAGGCCTGCTGTCCTGGATGAT TTTGTTAGTACGATTGATTTACCTAATTATGGATGCACA ATTCCTGAAAAGACCAGTTGCAGTGTTTATGGCTGGGG CTACACTGGATTGATCAACTATGATGGCCTATTACGAGT GGCACATCTCTATATAATGGGAAATGAGAAATGCAGCC

		AGCATCATCGAGGGAAGGTGACTCTGAATGAGTCTGAA ATATGTGCTGGGGCTGAAAAGATTGGATCAGGACCATG TGAGGGGGATTATGGTGGCCCACTTGTTTGTGAGCAAC ATAAAATGAGAATGGTTCTTGGTGTCATTGTTCCTGGTC GTGGATGTGCCATTCCAAATCGTCCTGGTATTTTTGTCC GAGTAGCATATTATGCAAAATGGATACACAAAATTATT TTAACATATAAGGTACCACAGTCATAG
SEQ ID NO:5	Nucleotide sequence of pCK vector	CGCGTTGACATTGATTATTGACTAGTTATTAATAGTAAT CAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGT TCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCT GACCGCCCAACGACCCCCGCCCATTGACGTCAATAATG ACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCC CCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGC ATTATGCCCAGTACATGACCTTACGGGACTTTCCTACTT GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGG TGATGCGGTTTTGGCAGTACACCAATGGGCGTGGATAG CGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATT GACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACG GGACTTTCCAAAATGTCGTAATAACCCCGCCCCGTTGAC GCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATA TAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGG AGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACA CCGGGACCGATCCAGCCTCCGCGGCCGGGAACGGTGCA TTGGAACGCGGATTCCCCGTGCCAAGAGTGACGTAAGT ACCGCCTATAGACTCTATAGGCACACCCCTTTGGCTCTT ATGCATGCTATACTGTTTTTGGCTTGGGGCCTATACACC CCCGCTTCCTTATGCTATAGGTGATGGTATAGCTTAGCC TATAGGTGTGGGTTATTGACCATTATTGACCACTCCCCT ATTGGTGACGATACTTTCCATTACTAATCCATAACATGG CTCTTTGCCACAACTATCTCTATTGGCTATATGCCAATA CTCTGTCCTTCAGAGACTGACACGGACTCTGTATTTTTA CAGGATGGGGTCCCATTTATTATTTACAAATTCACATAT ACAACAACGCCGTCCCCCGTGCCCGCAGTTTTTATTAAA CATAGCGTGGGATCTCCACGCGAATCTCGGGTACGTGT TCCGGACATGGGCTCTTCTCCGGTAGCGGCGGAGCTTCC ACATCCGAGCCCTGGTCCCATGCCTCCAGCGGCTCATG GTCGCTCGGCAGCTCCTTGCTCCTAACAGTGGAGGCCA GACTTAGGCACAGCACAATGCCCACCACCACCAGTGTG CCGCACAAGGCCGTGGCGGTAGGGTATGTGTCTGAAAA TGAGCTCGGAGATTGGGCTCGCACCGCTGACGCAGATG GAAGACTTAAGGCAGCGGCAGAAGAAGATGCAGGCAG CTGAGTTGTTGTATTCTGATAAGAGTCAGAGGTAACTCC

		CGTTGCGGTGCTGTTAACGGTGGAGGGCAGTGTAGTCT GAGCAGTACTCGTTGCTGCCGCGCGCGCCACCAGACAT AATAGCTGACAGACTAACAGACTGTTCCTTTCCATGGGT CTTTTCTGCAGTCACCGTCCTTGACACGAAGCTTGGTAC CGAGCTCGGATCCACTAGTCCAGTGTGGTGGAATTCTG CAGATATCCAGCACAGTGGCGGCCGCTCGAGTCTAGAG GGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTT CTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGC CTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTT CCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGT AGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGA CAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCAT GCTGGGGAGTCGAAATTCAGAAGAACTCGTCAAGAAGG CGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCGAT ACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGC CAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATG TCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTC GATGAATCCAGAAAAGCGGCCATTTTCCACCATGATAT TCGGCAAGCAGGCATCGCCATGGGTCACGACGAGATCC TCGCCGTCGGGCATGCTCGCCTTGAGCCTGGCGAACAG TTCGGCTGGCGCGAGCCCCTGATGCTCTTCGTCCAGATC ATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTG CTCGCTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGC AGGTAGCCGGATCAAGCGTATGCAGCCGCCGCATTGCA TCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTG AGATGACAGGAGATCCTGCCCCGGCACTTCGCCCAATA GCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGAGC ACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGA TAGCCGCGCTGCCTCGTCTTGCAGTTCATTCAGGGCACC GGACAGGTCGGTCTTGACAAAAAGAACCGGGCGCCCCT GCGCTGACAGCCGGAACACGGCGGCATCAGAGCAGCC GATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTC CACCCAAGCGGCCGGAGAACCTGCGTGCAATCCATCTT GTTCAATCATGCGAAACGATCCTCATCCTGTCTCTTGAT CAGATCTTGATCCCCTGCGCCATCAGATCCTTGGCGGCA AGAAAGCCATCCAGTTTACTTTGCAGGGCTTCCCAACCT TACCAGAGGGCGCCCCAGCTGGCAATTCCGGTTCGCTT GCTGTCCATAAAACCGCCCAGTCTAGCTATCGCCATGTA AGCCCACTGCAAGCTACCTGCTTTCTCTTTGCGCTTGCG TTTTCCCTTGTCCAGATAGCCCAGTAGCTGACATTCATC CGGGGTCAGCACCGTTTCTGCGGACTGGCTTTCTACGTG AAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATG ACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCG TCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGA TCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAA AAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATC

		AAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCA GCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAG CCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACC GCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGC TGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGG ACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCG GGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGA GCGAACGACCTACACCGAACTGAGATACCTACAGCGTG AGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAA GGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACA GGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCT GGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGAC TTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGG AGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACG GTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATG
SEQ ID NO:6	Nucleotide sequence of intron 4 of human hgf	GTAAGAACAGTATGAAGAAAAGAGATGAAGCCTCTGTC TTTTTTACATGTTAACAGTCTCATATTAGTCCTTCAGAA TAATTCTACAATCCTAAAATAACTTAGCCAACTTGCTGA ATTGTATTACGGCAAGGTTTATATGAATTCATGACTGAT ATTTAGCAAATGATTAATTAATATGTTAATAAAATGTAG CCAAAACAATATCTTACCTTAATGCCTCAATTTGTAGAT CTCGGTATTTGTGAAATAATAACGTAAACTTCGTTTAAA AGGATTCTTCTTCCTGTCTTTGAGAAAGTACGGCACTGT GCAGGGGGAGAGGTTGATTGTGAAAAATCAGAGGTAG ATGAGAATCTTACTGAGGGCTGAGGGTTCTTTAACCTTG GTGGATCTCAACATTGGTTGCACATTAAAATCACCTGCT GCAAGCCCTTGACGAATCTTACTTAGAAGATGACAACA CAGAACAATTAAATCAGAATCTCTGGGGAGAATAGGGC ACCAGTATTTTTTGAGCTCCCACCATGATTCCAAAGTGC AGCCAAATTTGAGAACCACTGCTAAAAGCTCAAGCTTC AGATTGACCAGCTTTTCCATCTCACCTATCGCCTAAAGA CCAAATTGGATAAATGTGTTCATTACGACAGATGGGTA CTATTTAAAGATGAGTAAACACAATATACTTAGGCTCG TCAGACTGAGAGTTTTAATCATCACTGAGGAAAAACAT AGATATCTAATACTGACTGGAGTATTAGTCAAGGCTTAT TTCACACACAATTTTATCAGAAACCAAAGTAGTTTAAA ACAGCTCTCCCCTTATTAGTAATGCATTGGAGGGTTTAC TTTACCATGTACCTTGCTGAGCACTGTACCTTGTTAATC TCATTTACTTGTAATGAGAACCACACAGCGGGTAGTTTT ATTGGTTCTATTTTACCTACATGACAAAACTGAAGCATA AAAACACTTAGTAAGTTTTCAGTGTCATGCACAACTAG GAAGTGACATGGCCAGAATATAAGCCCAGTCACCATCA CTCTATAACCTGCGCTTTTAACAACTTCAGGGCATGACA CATTTGGCCGGTCAGTAGAACCCATGCTGTGATTTGTTT TTGCAGTGGTGGTGATGACTGCCTTGTTGAATCCACTTT

		TTATTCTATTCCATTTTGGGGACACAATTCTGCAAGATG ATTCTTCATTAGGAAACAGAGATGAGTTATTGACCAAC ACAGAAAGAAAAAGAGTTTGTTGCTCCACACTGGGATT AAACCTATGATCTTGGCCTAATTAACACTAGCTAGTAA GTGTCCAAGCTGATCATCTCTACAACATTTCAATAACAG AAAACAACAATTTTCAAAATTAGTTACTTACAATTATGT AGAAATGCCTCTAAAACACAGTATTTTCCTTATATTACA AAAACAAAAATTATAATTGGTTTTGTCCTCTTTTGAGAG TTTGCATGGTGTTACTCCCTGCATAGTGAAGAAAACATT TTATTTAAGTAGATGGATCTAAGTTTTTCATGAACAAAG GAATGACATTTGAAATCAATCCTACCCTAGTCCAGGAG AATGCATTAGATTAACCTAGTAGAGGTCTTATTTCACCC TGAGTTTTCTATGATCGTGATTCTCTGCTGGAGGAGTAA TTGTGAAATAGATCTCTCTGGGAACTGGCTTCCTAGTCC AATCAGCTCTTTTACCAATGAACACTTCCTTGTGATATA GATGTTTATGGCCGAGAGGATCCAGTATATTAATAAAA TCCCTTTTTGTATTCAATGAGGGAAACACATAATTTTCA TCAATTAGCAGCTTATTGGAATATCTGCATGATGGTTTA ACACTTTTAAGTGTTGACTAAAGATTAATTTTACAGAAA ATAGAAAAAGAAATATGTTTCTGTCTGGAGGAATGATT TATTGTTGACCCCTAAATTGAAATATTTTACTAGTGGCT TAATGGAAAGATGATGAAAGATGATGAAATTAATGTAG AAGCTTAACTAGAAAATCAGGTGACCTGATATCTACAT CTGTATCCTTCATTGGCCACCCAGCATTCATTAATGAAT CAGATGATGGAATAGATCAAGTTTCCTAGGAACACAGT GAATATTAAAAGAAAACAAAGGGAGCCTAGCACCTAG AAGACCTAGTTTATATTTCAAAGTATATTTGGATGTAAC CCAATTTTAAACATTTCCTCACTTGTCTCTCTTAAAGCCT TGCCAACAGCAAGGACAGAGAACCAAAAATAGTGTAT ATATGAATAAATGCTTATTACAGAATCTGCTGACTGGC ACATGCTTTGTGTGTAATGGGTTCTCATAAACACTTGTT GAATGAACACACATAAGTGAAAGAGCATGGCTAGGCTT CATCCCTTGGTCAAATATGGGGTGCTAAAGAAAAGCAG GGGAAATACATTGGGACACTAACAAAAAAAAACAGTT AATTTAGGTAAAAGATAAAATACACCACAGAATGAAGA AAAGAGATGACCCAGACTGCTCTTTAACCTTCATGTCCT AGAGAGGTTTTTGATATGAATTGCATTCAGAATTGTGG AAAGGAGCCCATCTTTTCTCTTCATTTTGATTTTATTAAC TCCAATGGGGGGAATTTTATTCGTGTTTTGGCCATATCTA CTTTTGATTTCTACATTATTCTCTCTTCCTTTCTACCTGT ATTTGTCCTAATAAATTGTTGACTTATTAATTCACTACTT CCTCACAGCTTTTTTTTGGCTTTACAAATCCACTGGAAA GGTATATGGGTGTATCACTTTGTGTATTTCGGTGTGCAT GTGTAGAGGGGACAAAAATCCTCTCTCAAACTATAAAT ATTGAGTATTTGTGTATTGAACATTTGCTATAACTACTA GGTTTCTTAAATAATCTTAATATATAAAATGATATAGAA

		AAAGGGAAATTATAGTTCGTATTATTCATCTAAGTGAA GAGATTAAAACCCAGGGAGTAAATAAATTGTCTAAGGA CTAAGGTTGTATACTATTTAGGTGATAGATATGGGGCA ACCGTATGGGTTTTATGATTAACAAATAAACTTCTCACC ACTCTACCATATCAACTTTTCCATAAAAGAGAGCTATAG TATTCTTTGCTTAAATAAATTTGATTAGTGCATGACTTC TTGAAAACATATAAAGCAAAAGTCACATTTGATTCTAT CAGAAAAGTGAGTAAGCCATGGCCCAAACAAAAGATG CATTAAAATATTCTGGAATGATGGAGCTAAAAGTAAGA AAAATGACTTTTTAAAAAAGTTTACTGTTAGGAATTGTG AAATTATGCTGAATTTTAGTTGCATTATAATTTTTGTCA GTCATACGGTCTGACAACCTGTCTTATTTCTATTTCCCC ATATGAGGAATGCTAGTTAAGTATGGATATTAACTATT ACTACTTAGATGCATTGAAGTTGCATAATATGGATAAT ACTTCACTGGTTCCCTGAAAATGTTTAGTTAGTAATAAG TCTCTTACACTATTTGTTTTGTCCAATAATTTATATTTTC TGAAGACTTAACTCTAGAATACACTCATGTCAAAATGA AAGAATTTCATTGCAAAATATTGCTTGGTACATGACGC ATACCTGTATTTGTTTTGTGTCACAACATGAAAAATGAT GGTTTATTAGAAGTTTCATTGGGTAGGAAACACATTTGA ATGGTATTTACTAAGATACTAAAATCCTTGGACTTCACT CTAATTTTAGTGCCATTTAGAACTCAAGGTCTCAGTAAA AGTAGAAATAAAGCCTGTTAACAAAACACAAACTGAAT ATTAAAAATGTAACTGGATTTTCAAAGAAATGTTTACTG GTATTACCTGTAGATGTATATTCTTTATTATGATCTTTTG TGTAAAGTCTGGCAGACAAATGCAATATCTAATTGTTG AGTCCAATATCACAAGCAGTACAAAAGTATAAAAAAGA CTTGGCCTTTTCTAATGTGTTAAAATACTTTATGCTGGT AATAACACTAAGAGTAGGGCACTAGAAATTTTAAGTGA AGATAATGTGTTGCAGTTACTGCACTCAATGGCTTACTA TTATAAACCAAAACTGGGATCACTAAGCTCCAGTCAGT CAAAATGATCAAAATTATTGAAGAGAATAAGCAATTCT GTTCTTTATTAGGACACAGTAGATACAGACTACAAAGT GGAGTGTGCTTAATAAGAGGTAGCATTTGTTAAGTGTC AATTACTCTATTATCCCTTGGAGCTTCTCAAAATAACCA TATAAGGTGTAAGATGTTAAAGGTTATGGTTACACTCA GTGCACAGGTAAGCTAATAGGCTGAGAGAAGCTAAATT ACTTACTGGGGTCTCACAGTAAGAAAGTGAGCTGAAGT TTCAGCCCAGATTTAACTGGATTCTGGGCTCTTTATTCA TGTTACTTCATGAATCTGTTTCTCAATTGTGCAGAAAAA AGGGGGCTATTTATAAGAAAAGCAATAAACAAACAAGT AATGATCTCAAATAAGTAATGCAAGAAATAGTGAGATT TCAAAATCAGTGGCAGCGATTTCTCAGTTCTGTCCTAAG TGGCCTTGCTCAATCACCTGCTATCTTTTAGTGGAGCTT TGAAATTATGTTTCAGACAACTTCGATTCAGTTCTAGAA TGTTTGACTCAGCAAATTCACAGGCTCATCTTTCTAACT

		TGATGGTGAATATGGAAATTCAGCTAAATGGATGTTAA TAAAATTCAAACGTTTTAAGGACAGATGGAAATGACAG AATTTTAAGGTAAAATATATGAAGGAATATAAGATAAA GGATTTTTCTACCTTCAGCAAAAACATACCCACTAATTA GTAAAATTAATAGGCGAAAAAAAGTTGCATGCTCTTAT ACTGTAATGATTATCATTTTAAAACTAGCTTTTTGCCTT CGAGCTATCGGGGTAAAGA
SEQ ID NO:7	Nucleotide sequence of HGF-X1	ATGTGGGTGACCAAACTCCTGCCAGCCCTGCTGCTGCA GCATGTCCTCCTGCATCTCCTCCTGCTCCCCATCGCCAT CCCCTATGCAGAGGGACAAAGGAAAAGAAGAAATACA ATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAAT CAAAATAGATCCAGCACTGAAGATAAAAACCAAAAAA GTGAATACTGCAGACCAATGTGCTAATAGATGTACTAG GAATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTT TGATAAAGCAAGAAAACAATGCCTCTGGTTCCCCTTCA ATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGGCCAT GAATTTGACCTCTATGAAAACAAAGACTACATTAGAAA CTGCATCATTGGTAAAGGACGCAGCTACAAGGGAACAG TATCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGG AGTTCCATGATACCACACGAACACAGGTAAGAACAGTA TGAAGAAAAGAGATGAAGCCTCTGTCTTTTTTACATGTT AACAGTCTCATATTAGTCCTTCAGAATAATTCTACAATC CTAAAATAACTTAGCCAACTTGCTGAATTGTATTACGGC AAGGTTTATATGAATTCATGACTGATATTTAGCAAATGA TTAATTAATATGTTAATAAAATGTAGCCAAAACAATAT CTTACCTTAATGCCTCAATTTGTAGATCTCGGTATTTGT GAAATAATAACGTAAACTTCGTTTAAAAGGATTCTTCTT CCTGTCTTTGAGAAAGTACGGCACTGTGCAGGGGGAGA GGTTGATTGTGAAAAATCAGAGGTAGATGAGAATCTTA CTGAGGGCTGAGGGTTCTTTAACCTTGGTGGATCTCAAC ATTGGTTGCACATTAAAATCACCTGCTGCAAGCCCTTGA CGAATCTTACTTAGAAGATGACAACACAGAACAATTAA ATCAGAATCTCTGGGGAGAATAGGGCACCAGTATTTTT TGAGCTCCCACCATGATTCCAAAGTGCAGCCAAATTTG AGAACCACTGCTAAAAGCTCAAGCTTCAGATTGACCAG CTTTTCCATCTCACCTATCGCCTAAAGACCAAATTGGAT AAATGTGTTCATTACGACAGATGGGTACTATTTAAAGA TGAGTAAACACAATATACTTAGGCTCGTCAGACTGAGA GTTTTAATCATCACTGAGGAAAAACATAGATATCTAAT ACTGACTGGAGTATTAGTCAAGGCTTATTTCACACACA ATTTTATCAGAAACCAAAGTAGTTTAAAACAGCTCTCCC CTTATTAGTAATGCATTGGAGGGTTTACTTTACCATGTA CCTTGCTGAGCACTGTACCTTGTTAATCTCATTTACTTGT AATGAGAACCACACAGCGGGTAGTTTTATTGGTTCTATT

		TTACCTACATGACAAAACTGAAGCATAAAAACACTTAG TAAGTTTTCAGTGTCATGCACAACTAGGAAGTGACATG GCCAGAATATAAGCCCAGTCACCATCACTCTATAACCT GCGCTTTTAACAACTTCAGGGCATGACACATTTGGCCG GTCAGTAGAACCCATGCTGTGATTTGTTTTTGCAGTGGT GGTGATGACTGCCTTGTTGAATCCACTTTTTATTCTATTC CATTTTGGGGACACAATTCTGCAAGATGATTCTTCATTA GGAAACAGAGATGAGTTATTGACCAACACAGAAAGAA AAAGAGTTTGTTGCTCCACACTGGGATTAAACCTATGAT CTTGGCCTAATTAACACTAGCTAGTAAGTGTCCAAGCTG ATCATCTCTACAACATTTCAATAACAGAAAACAACAAT TTTCAAAATTAGTTACTTACAATTATGTAGAAATGCCTC TAAAACACAGTATTTTCCTTATATTACAAAAACAAAAA TTATAATTGGTTTTGTCCTCTTTTGAGAGTTTGCATGGTG TTACTCCCTGCATAGTGAAGAAAACATTTTATTTAAGTA GATGGATCTAAGTTTTTCATGAACAAAGGAATGACATT TGAAATCAATCCTACCCTAGTCCAGGAGAATGCATTAG ATTAACCTAGTAGAGGTCTTATTTCACCCTGAGTTTTCT ATGATCGTGATTCTCTGCTGGAGGAGTAATTGTGAAAT AGATCTCTCTGGGAACTGGCTTCCTAGTCCAATCAGCTC TTTTACCAATGAACACTTCCTTGTGATATAGATGTTTAT GGCCGAGAGGATCCAGTATATTAATAAAATCCCTTTTTG TATTCAATGAGGGAAACACATAATTTTCATCAATTAGC AGCTTATTGGAATATCTGCATGATGGTTTAACACTTTTA AGTGTTGACTAAAGATTAATTTTACAGAAAATAGAAAA AGAAATATGTTTCTGTCTGGAGGAATGATTTATTGTTGA CCCCTAAATTGAAATATTTTACTAGTGGCTTAATGGAAA GATGATGAAAGATGATGAAATTAATGTAGAAGCTTAAC TAGAAAATCAGGTGACCTGATATCTACATCTGTATCCTT CATTGGCCACCCAGCATTCATTAATGAATCAGATGATG GAATAGATCAAGTTTCCTAGGAACACAGTGAATATTAA AAGAAAACAAAGGGAGCCTAGCACCTAGAAGACCTAG TTTATATTTCAAAGTATATTTGGATGTAACCCAATTTTA AACATTTCCTCACTTGTCTCTCTTAAAGCCTTGCCAACA GCAAGGACAGAGAACCAAAAATAGTGTATATATGAATA AATGCTTATTACAGAATCTGCTGACTGGCACATGCTTTG TGTGTAATGGGTTCTCATAAACACTTGTTGAATGAACAC ACATAAGTGAAAGAGCATGGCTAGGCTTCATCCCTTGG TCAAATATGGGGTGCTAAAGAAAAGCAGGGGAAATAC ATTGGGACACTAACAAAAAAAAACAGTTAATTTAGGTA AAAGATAAAATACACCACAGAATGAAGAAAAGAGATG ACCCAGACTGCTCTTTAACCTTCATGTCCTAGAGAGGTT TTTGATATGAATTGCATTCAGAATTGTGGAAAGGAGCC CATCTTTTCTCTTCATTTTGATTTTATTAACTCCAATGGG GGAATTTTATTCGTGTTTTGGCCATATCTACTTTTGATTT CTACATTATTCTCTCTTCCTTTCTACCTGTATTTGTCCTA

		ATAAATTGTTGACTTATTAATTCACTACTTCCTCACAGC TTTTTTTTGGCTTTACAAATCCACTGGAAAGGTATATGG GTGTATCACTTTGTGTATTTCGGTGTGCATGTGTAGAGG GGACAAAAATCCTCTCTCAAACTATAAATATTGAGTATT TGTGTATTGAACATTTGCTATAACTACTAGGTTTCTTAA ATAATCTTAATATATAAAATGATATAGAAAAAGGGAAA TTATAGTTCGTATTATTCATCTAAGTGAAGAGATTAAAA CCCAGGGAGTAAATAAATTGTCTAAGGACTAAGGTTGT ATACTATTTAGGTGATAGATATGGGGCAACCGTATGGG TTTTATGATTAACAAATAAACTTCTCACCACTCTACCAT ATCAACTTTTCCATAAAAGAGAGCTATAGTATTCTTTGC TTAAATAAATTTGATTAGTGCATGACTTCTTGAAAACAT ATAAAGCAAAAGTCACATTTGATTCTATCAGAAAAGTG AGTAAGCCATGGCCCAAACAAAAGATGCATTAAAATAT TCTGGAATGATGGAGCTAAAAGTAAGAAAAATGACTTT TTAAAAAAGTTTACTGTTAGGAATTGTGAAATTATGCTG AATTTTAGTTGCATTATAATTTTTGTCAGTCATACGGTC TGACAACCTGTCTTATTTCTATTTCCCCATATGAGGAAT GCTAGTTAAGTATGGATATTAACTATTACTACTTAGATG CATTGAAGTTGCATAATATGGATAATACTTCACTGGTTC CCTGAAAATGTTTAGTTAGTAATAAGTCTCTTACACTAT TTGTTTTGTCCAATAATTTATATTTTCTGAAGACTTAACT CTAGAATACACTCATGTCAAAATGAAAGAATTTCATTG CAAAATATTGCTTGGTACATGACGCATACCTGTATTTGT TTTGTGTCACAACATGAAAAATGATGGTTTATTAGAAGT TTCATTGGGTAGGAAACACATTTGAATGGTATTTACTAA GATACTAAAATCCTTGGACTTCACTCTAATTTTAGTGCC ATTTAGAACTCAAGGTCTCAGTAAAAGTAGAAATAAAG CCTGTTAACAAAACACAAACTGAATATTAAAAATGTAA CTGGATTTTCAAAGAAATGTTTACTGGTATTACCTGTAG ATGTATATTCTTTATTATGATCTTTTGTGTAAAGTCTGGC AGACAAATGCAATATCTAATTGTTGAGTCCAATATCAC AAGCAGTACAAAAGTATAAAAAAGACTTGGCCTTTTCT AATGTGTTAAAATACTTTATGCTGGTAATAACACTAAG AGTAGGGCACTAGAAATTTTAAGTGAAGATAATGTGTT GCAGTTACTGCACTCAATGGCTTACTATTATAAACCAAA ACTGGGATCACTAAGCTCCAGTCAGTCAAAATGATCAA AATTATTGAAGAGAATAAGCAATTCTGTTCTTTATTAGG ACACAGTAGATACAGACTACAAAGTGGAGTGTGCTTAA TAAGAGGTAGCATTTGTTAAGTGTCAATTACTCTATTAT CCCTTGGAGCTTCTCAAAATAACCATATAAGGTGTAAG ATGTTAAAGGTTATGGTTACACTCAGTGCACAGGTAAG CTAATAGGCTGAGAGAAGCTAAATTACTTACTGGGGTC TCACAGTAAGAAAGTGAGCTGAAGTTTCAGCCCAGATT TAACTGGATTCTGGGCTCTTTATTCATGTTACTTCATGA ATCTGTTTCTCAATTGTGCAGAAAAAAGGGGGCTATTTA

		TAAGAAAAGCAATAAACAAACAAGTAATGATCTCAAAT AAGTAATGCAAGAAATAGTGAGATTTCAAAATCAGTGG CAGCGATTTCTCAGTTCTGTCCTAAGTGGCCTTGCTCAA TCACCTGCTATCTTTTAGTGGAGCTTTGAAATTATGTTT CAGACAACTTCGATTCAGTTCTAGAATGTTTGACTCAGC AAATTCACAGGCTCATCTTTCTAACTTGATGGTGAATAT GGAAATTCAGCTAAATGGATGTTAATAAAATTCAAACG TTTTAAGGACAGATGGAAATGACAGAATTTTAAGGTAA AATATATGAAGGAATATAAGATAAAGGATTTTTCTACC TTCAGCAAAAACATACCCACTAATTAGTAAAATTAATA GGCGAAAAAAAGTTGCATGCTCTTATACTGTAATGATT ATCATTTTAAAACTAGCTTTTTGCCTTCGAGCTATCGGG GTAAAGACCTACAGGAAAACTACTGTCGAAATCCTCGA GGGGAAGAAGGGGGACCCTGGTGTTTCACAAGCAATCC AGAGGTACGCTACGAAGTCTGTGACATTCCTCAGTGTTC AGAAGTTGAATGCATGACCTGCAATGGGGAGAGTTATC GAGGTCTCATGGATCATACAGAATCAGGCAAGATTTGT CAGCGCTGGGATCATCAGACACCACACCGGCACAAATT CTTGCCTGAAAGATATCCCGACAAGGGCTTTGATGATA ATTATTGCCGCAATCCCGATGGCCAGCCGAGGCCATGG TGCTATACTCTTGACCCTCACACCCGCTGGGAGTACTGT GCAATTAAAACATGCGCTGACAATACTATGAATGACAC TGATGTTCCTTTGGAAACAACTGAATGCATCCAAGGTC AAGGAGAAGGCTACAGGGGCACTGTCAATACCATTTGG AATGGAATTCCATGTCAGCGTTGGGATTCTCAGTATCCT CACGAGCATGACATGACTCCTGAAAATTTCAAGTGCAA GGACCTACGAGAAAATTACTGCCGAAATCCAGATGGGT CTGAATCACCCTGGTGTTTTACCACTGATCCAAACATCC GAGTTGGCTACTGCTCCCAAATTCCAAACTGTGATATGT CACATGGACAAGATTGTTATCGTGGGAATGGCAAAAAT TATATGGGCAACTTATCCCAAACAAGATCTGGACTAAC ATGTTCAATGTGGGACAAGAACATGGAAGACTTACATC GTCATATCTTCTGGGAACCAGATGCAAGTAAGCTGAAT GAGAATTACTGCCGAAATCCAGATGATGATGCTCATGG ACCCTGGTGCTACACGGGAAATCCACTCATTCCTTGGG ATTATTGCCCTATTTCTCGTTGTGAAGGTGATACCACAC CTACAATAGTCAATTTAGACCATCCCGTAATATCTTGTG CCAAAACGAAACAATTGCGAGTTGTAAATGGGATTCCA ACACGAACAAACATAGGATGGATGGTTAGTTTGAGATA CAGAAATAAACATATCTGCGGAGGATCATTGATAAAGG AGAGTTGGGTTCTTACTGCACGACAGTGTTTCCCTTCTC GAGACTTGAAAGATTATGAAGCTTGGCTTGGAATTCAT GATGTCCACGGAAGAGGAGATGAGAAATGCAAACAGG TTCTCAATGTTTCCCAGCTGGTATATGGCCCTGAAGGAT CAGATCTGGTTTTAATGAAGCTTGCCAGGCCTGCTGTCC TGGATGATTTTGTTAGTACGATTGATTTACCTAATTATG

		GATGCACAATTCCTGAAAAGACCAGTTGCAGTGTTTAT GGCTGGGGCTACACTGGATTGATCAACTATGATGGCCT ATTACGAGTGGCACATCTCTATATAATGGGAAATGAGA AATGCAGCCAGCATCATCGAGGGAAGGTGACTCTGAAT GAGTCTGAAATATGTGCTGGGGCTGAAAAGATTGGATC AGGACCATGTGAGGGGGATTATGGTGGCCCACTTGTTT GTGAGCAACATAAAATGAGAATGGTTCTTGGTGTCATT GTTCCTGGTCGTGGATGTGCCATTCCAAATCGTCCTGGT ATTTTTGTCCGAGTAGCATATTATGCAAAATGGATACAC AAAATTATTTTAACATATAAGGTACCACAGTCATAG
SEQ ID NO:8	Nucleotide sequence of HGF-X2	ATGTGGGTGACCAAACTCCTGCCAGCCCTGCTGCTGCA GCATGTCCTCCTGCATCTCCTCCTGCTCCCCATCGCCAT CCCCTATGCAGAGGGACAAAGGAAAAGAAGAAATACA ATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAAT CAAAATAGATCCAGCACTGAAGATAAAAACCAAAAAA GTGAATACTGCAGACCAATGTGCTAATAGATGTACTAG GAATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTT TGATAAAGCAAGAAAACAATGCCTCTGGTTCCCCTTCA ATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGGCCAT GAATTTGACCTCTATGAAAACAAAGACTACATTAGAAA CTGCATCATTGGTAAAGGACGCAGCTACAAGGGAACAG TATCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGG AGTTCCATGATACCACACGAACACAGGTAAGAACAGTA TGAAGAAAAGAGATGAAGCCTCTGTCTTTTTTACATGTT AACAGTCTCATATTAGTCCTTCAGAATAATTCTACAATC CTAAAATAACTTAGCCAACTTGCTGAATTGTATTACGGC AAGGTTTATATGAATTCATGACTGATATTTAGCAAATGA TTAATTAATATGTTAATAAAATGTAGCCAAAACAATAT CTTACCTTAATGCCTCAATTTGTAGATCTCGGTATTTGT GAAATAATAACGTAAACTTCGTTTAAAAGGATTCTTCTT CCTGTCTTTGAGAAAGTACGGCACTGTGCAGGGGGAGA GGTTGATTGTGAAAAATCAGAGGTAGATGAGAATCTTA CTGAGGGCTGAGGGTTCTTTAACCTTGGTGGATCTCAAC ATTGGTTGCACATTAAAATCACCTGCTGCAAGCCCTTGA CGAATCTTACTTAGAAGATGACAACACAGAACAATTAA ATCAGAATCTCTGGGGAGAATAGGGCACCAGTATTTTT TGAGCTCCCACCATGATTCCAAAGTGCAGCCAAATTTG AGAACCACTGCTAAAAGCTCAAGCTTCAGATTGACCAG CTTTTCCATCTCACCTATCGCCTAAAGACCAAATTGGAT AAATGTGTTCATTACGACAGATGGGTACTATTTAAAGA TGAGTAAACACAATATACTTAGGCTCGTCAGACTGAGA GTTTTAATCATCACTGAGGAAAAACATAGATATCTAAT ACTGACTGGAGTATTAGTCAAGGCTTATTTCACACACA ATTTTATCAGAAACCAAAGTAGTTTAAAACAGCTCTCCC CTTATTAGTAATGCATTGGAGGGTTTACTTTACCATGTA CCTTGCTGAGCACTGTACCTTGTTAATCTCATTTACTTGT

		AATGAGAACCACACAGCGGGTAGTTTTATTGGTTCTATT TTACCTACATGACAAAACTGAAGCATAAAAACACTTAG TAAGTTTTCAGTGTCATGCACAACTAGGAAGTGACATG GCCAGAATATAAGCCCAGTCACCATCACTCTATAACCT GCGCTTTTAACAACTTCAGGGCATGACACATTTGGCCG GTCAGTAGAACCCATGCTGTGATTTGTTTTTGCAGTGGT GGTGATGACTGCCTTGTTGAATCCACTTTTTATTCTATTC CATTTTGGGGACACAATTCTGCAAGATGATTCTTCATTA GGAAACAGAGATGAGTTATTGACCAACACAGAAAGAA AAAGAGTTTGTTGCTCCACACTGGGATTAAACCTATGAT CTTGGCCTAATTAACACTAGCTAGTAAGTGTCCAAGCTG ATCATCTCTACAACATTTCAATAACAGAAAACAACAAT TTTCAAAATTAGTTACTTACAATTATGTAGAAATGCCTC TAAAACACAGTATTTTCCTTATATTACAAAAACAAAAA TTATAATTGGTTTTGTCCTCTTTTGAGAGTTTGCATGGTG TTACTCCCTGCATAGTGAAGAAAACATTTTATTTAAGTA GATGGATCTAAGTTTTTCATGAACAAAGGAATGACATT TGAAATCAATCCTACCCTAGTCCAGGAGAATGCATTAG ATTAACCTAGTAGAGGTCTTATTTCACCCTGAGTTTTCT ATGATCGTGATTCTCTGCTGGAGGAGTAATTGTGAAAT AGATCTCTCTGGGAACTGGCTTCCTAGTCCAATCAGCTC TTTTACCAATGAACACTTCCTTGTGATATAGATGTTTAT GGCCGAGAGGATCTCTTCCTTTCTACCTGTATTTGTCCT AATAAATTGTTGACTTATTAATTCACTACTTCCTCACAG CTTTTTTTTGGCTTTACAAATCCACTGGAAAGGTATATG GGTGTATCACTTTGTGTATTTCGGTGTGCATGTGTAGAG GGGACAAAAATCCTCTCTCAAACTATAAATATTGAGTA TTTGTGTATTGAACATTTGCTATAACTACTAGGTTTCTT AAATAATCTTAATATATAAAATGATATAGAAAAAGGGA AATTATAGTTCGTATTATTCATCTAAGTGAAGAGATTAA AACCCAGGGAGTAAATAAATTGTCTAAGGACTAAGGTT GTATACTATTTAGGTGATAGATATGGGGCAACCGTATG GGTTTTATGATTAACAAATAAACTTCTCACCACTCTACC ATATCAACTTTTCCATAAAAGAGAGCTATAGTATTCTTT GCTTAAATAAATTTGATTAGTGCATGACTTCTTGAAAAC ATATAAAGCAAAAGTCACATTTGATTCTATCAGAAAAG TGAGTAAGCCATGGCCCAAACAAAAGATGCATTAAAAT ATTCTGGAATGATGGAGCTAAAAGTAAGAAAAATGACT TTTTAAAAAAGTTTACTGTTAGGAATTGTGAAATTATGC TGAATTTTAGTTGCATTATAATTTTTGTCAGTCATACGG TCTGACAACCTGTCTTATTTCTATTTCCCCATATGAGGA ATGCTAGTTAAGTATGGATATTAACTATTACTACTTAGA TGCATTGAAGTTGCATAATATGGATAATACTTCACTGGT TCCCTGAAAATGTTTAGTTAGTAATAAGTCTCTTACACT ATTTGTTTTGTCCAATAATTTATATTTTCTGAAGACTTAA CTCTAGAATACACTCATGTCAAAATGAAAGAATTTCATT

		GCAAAATATTGCTTGGTACATGACGCATACCTGTATTTG TTTTGTGTCACAACATGAAAAATGATGGTTTATTAGAAG TTTCATTGGGTAGGAAACACATTTGAATGGTATTTACTA AGATACTAAAATCCTTGGACTTCACTCTAATTTTAGTGC CATTTAGAACTCAAGGTCTCAGTAAAAGTAGAAATAAA GCCTGTTAACAAAACACAAACTGAATATTAAAAATGTA ACTGGATTTTCAAAGAAATGTTTACTGGTATTACCTGTA GATGTATATTCTTTATTATGATCTTTTGTGTAAAGTCTG GCAGACAAATGCAATATCTAATTGTTGAGTCCAATATC ACAAGCAGTACAAAAGTATAAAAAAGACTTGGCCTTTT CTAATGTGTTAAAATACTTTATGCTGGTAATAACACTAA GAGTAGGGCACTAGAAATTTTAAGTGAAGATAATGTGT TGCAGTTACTGCACTCAATGGCTTACTATTATAAACCAA AACTGGGATCACTAAGCTCCAGTCAGTCAAAATGATCA AAATTATTGAAGAGAATAAGCAATTCTGTTCTTTATTAG GACACAGTAGATACAGACTACAAAGTGGAGTGTGCTTA ATAAGAGGTAGCATTTGTTAAGTGTCAATTACTCTATTA TCCCTTGGAGCTTCTCAAAATAACCATATAAGGTGTAA GATGTTAAAGGTTATGGTTACACTCAGTGCACAGGTAA GCTAATAGGCTGAGAGAAGCTAAATTACTTACTGGGGT CTCACAGTAAGAAAGTGAGCTGAAGTTTCAGCCCAGAT TTAACTGGATTCTGGGCTCTTTATTCATGTTACTTCATG AATCTGTTTCTCAATTGTGCAGAAAAAAGGGGGCTATTT ATAAGAAAAGCAATAAACAAACAAGTAATGATCTCAA ATAAGTAATGCAAGAAATAGTGAGATTTCAAAATCAGT GGCAGCGATTTCTCAGTTCTGTCCTAAGTGGCCTTGCTC AATCACCTGCTATCTTTTAGTGGAGCTTTGAAATTATGT TTCAGACAACTTCGATTCAGTTCTAGAATGTTTGACTCA GCAAATTCACAGGCTCATCTTTCTAACTTGATGGTGAAT ATGGAAATTCAGCTAAATGGATGTTAATAAAATTCAAA CGTTTTAAGGACAGATGGAAATGACAGAATTTTAAGGT AAAATATATGAAGGAATATAAGATAAAGGATTTTTCTA CCTTCAGCAAAAACATACCCACTAATTAGTAAAATTAA TAGGCGAAAAAAAGTTGCATGCTCTTATACTGTAATGA TTATCATTTTAAAACTAGCTTTTTGCCTTCGAGCTATCG GGGTAAAGACCTACAGGAAAACTACTGTCGAAATCCTC GAGGGGAAGAAGGGGGACCCTGGTGTTTCACAAGCAAT CCAGAGGTACGCTACGAAGTCTGTGACATTCCTCAGTG TTCAGAAGTTGAATGCATGACCTGCAATGGGGAGAGTT ATCGAGGTCTCATGGATCATACAGAATCAGGCAAGATT TGTCAGCGCTGGGATCATCAGACACCACACCGGCACAA ATTCTTGCCTGAAAGATATCCCGACAAGGGCTTTGATG ATAATTATTGCCGCAATCCCGATGGCCAGCCGAGGCCA TGGTGCTATACTCTTGACCCTCACACCCGCTGGGAGTAC TGTGCAATTAAAACATGCGCTGACAATACTATGAATGA CACTGATGTTCCTTTGGAAACAACTGAATGCATCCAAG

		GTCAAGGAGAAGGCTACAGGGGCACTGTCAATACCATT TGGAATGGAATTCCATGTCAGCGTTGGGATTCTCAGTAT CCTCACGAGCATGACATGACTCCTGAAAATTTCAAGTG CAAGGACCTACGAGAAAATTACTGCCGAAATCCAGATG GGTCTGAATCACCCTGGTGTTTTACCACTGATCCAAACA TCCGAGTTGGCTACTGCTCCCAAATTCCAAACTGTGATA TGTCACATGGACAAGATTGTTATCGTGGGAATGGCAAA AATTATATGGGCAACTTATCCCAAACAAGATCTGGACT AACATGTTCAATGTGGGACAAGAACATGGAAGACTTAC ATCGTCATATCTTCTGGGAACCAGATGCAAGTAAGCTG AATGAGAATTACTGCCGAAATCCAGATGATGATGCTCA TGGACCCTGGTGCTACACGGGAAATCCACTCATTCCTTG GGATTATTGCCCTATTTCTCGTTGTGAAGGTGATACCAC ACCTACAATAGTCAATTTAGACCATCCCGTAATATCTTG TGCCAAAACGAAACAATTGCGAGTTGTAAATGGGATTC CAACACGAACAAACATAGGATGGATGGTTAGTTTGAGA TACAGAAATAAACATATCTGCGGAGGATCATTGATAAA GGAGAGTTGGGTTCTTACTGCACGACAGTGTTTCCCTTC TCGAGACTTGAAAGATTATGAAGCTTGGCTTGGAATTC ATGATGTCCACGGAAGAGGAGATGAGAAATGCAAACA GGTTCTCAATGTTTCCCAGCTGGTATATGGCCCTGAAGG ATCAGATCTGGTTTTAATGAAGCTTGCCAGGCCTGCTGT CCTGGATGATTTTGTTAGTACGATTGATTTACCTAATTA TGGATGCACAATTCCTGAAAAGACCAGTTGCAGTGTTT ATGGCTGGGGCTACACTGGATTGATCAACTATGATGGC CTATTACGAGTGGCACATCTCTATATAATGGGAAATGA GAAATGCAGCCAGCATCATCGAGGGAAGGTGACTCTGA ATGAGTCTGAAATATGTGCTGGGGCTGAAAAGATTGGA TCAGGACCATGTGAGGGGGATTATGGTGGCCCACTTGT TTGTGAGCAACATAAAATGAGAATGGTTCTTGGTGTCA TTGTTCCTGGTCGTGGATGTGCCATTCCAAATCGTCCTG GTATTTTTGTCCGAGTAGCATATTATGCAAAATGGATAC ACAAAATTATTTTAACATATAAGGTACCACAGTCATAG
SEQ ID NO:9	Nucleotide sequence of HGF-X3	ATGTGGGTGACCAAACTCCTGCCAGCCCTGCTGCTGCA GCATGTCCTCCTGCATCTCCTCCTGCTCCCCATCGCCAT CCCCTATGCAGAGGGACAAAGGAAAAGAAGAAATACA ATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAAT CAAAATAGATCCAGCACTGAAGATAAAAACCAAAAAA GTGAATACTGCAGACCAATGTGCTAATAGATGTACTAG GAATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTT TGATAAAGCAAGAAAACAATGCCTCTGGTTCCCCTTCA ATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGGCCAT GAATTTGACCTCTATGAAAACAAAGACTACATTAGAAA CTGCATCATTGGTAAAGGACGCAGCTACAAGGGAACAG TATCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGG AGTTCCATGATACCACACGAACACAGGTAAGAACAGTA

		TGAAGAAAAGAGATGAAGCCTCTGTCTTTTTTACATGTT AACAGTCTCATATTAGTCCTTCAGAATAATTCTACAATC CTAAAATAACTTAGCCAACTTGCTGAATTGTATTACGGC AAGGTTTATATGAATTCATGACTGATATTTAGCAAATGA TTAATTAATATGTTAATAAAATGTAGCCAAAACAATAT CTTACCTTAATGCCTCAATTTGTAGATCTCGGTATTTGT GAAATAATAACGTAAACTTCGTTTAAAAGGATTCTTCTT CCTGTCTTTGAGAAAGTACGGCACTGTGCAGGGGGAGA GGTTGATTGTGAAAAATCAGAGGTAGATGAGAATCTTA CTGAGGGCTGAGGGTTCTTTAACCTTGGTGGATCTCAAC ATTGGTTGCACATTAAAATCACCTGCTGCAAGCCCTTGA CGAATCTTACTTAGAAGATGACAACACAGAACAATTAA ATCAGAATCTCTGGGGAGAATAGGGCACCAGTATTTTT TGAGCTCCCACCATGATTCCAAAGTGCAGCCAAATTTG AGAACCACTGCTAAAAGCTCAAGCTTCAGATTGACCAG CTTTTCCATCTCACCTATCGCCTAAAGACCAAATTGGAT AAATGTGTTCATTACGACAGATGGGTACTATTTAAAGA TGAGTAAACACAATATACTTAGGCTCGTCAGACTGAGA GTTTTAATCATCACTGAGGAAAAACATAGATATCTAAT ACTGACTGGAGTATTAGTCAAGGCTTATTTCACACACA ATTTTATCAGAAACCAAAGTAGTTTAAAACAGCTCTCCC CTTATTAGTAATGCATTGGAGGGTTTACTTTACCATGTA CCTTGCTGAGCACTGTACCTTGTTAATCTCATTTACTTGT AATGAGAACCACACAGCGGGTAGTTTTATTGGTTCTATT TTACCTACATGACAAAACTGAAGCATAAAAACACTTAG TAAGTTTTCAGTGTCATGCACAACTAGGAAGTGACATG GCCAGAATATAAGCCCAGTCACCATCACTCTATAACCT GCGCTTTTAACAACTTCAGGGCATGACACATTTGGCCG GTCAGTAGAACCCATGCTGTGATTTGTTTTTGCAGTGGT GGTGATGACTGCCTTGTTGAATCCACTTTTTATTCTATTC CATTTTGGGGACACAATTCTGCAAGATGATTCTTCATTA GGAAACAGAGATGAGTTATTGACCAACACAGAAAGAA AAAGAGTTTGTTGCTCCACACTGGGATTAAACCTATGAT CTTGGCCTAATTAACACTAGCTAGTAAGTGTCCAAGCTG ATCATCTCTACAACATTTCAATAACAGAAAACAACAAT TTTCAAAATTAGTTACTTACAATTATGTAGAAATGCCTC TAAAACACAGTATTTTCCTTATATTACAAAAACAAAAA TTATAATTGGTTTTGTCCTCTTTTGAGAGTTTGCATGGTG TTACTCCCTGCATAGTGAAGAAAACATTTTATTTAAGTA GATGGATCTAAGTTTTTCATGAACAAAGGAATGACATT TGAAATCAATCCTACCCTAGTCCAGGAGAATGCATTAG ATTAACCTAGTAGAGGTCTTATTTCACCCTGAGTTTTCT ATGATCGTGATTCTCTGCTGGAGGAGTAATTGTGAAAT AGATCTCTCTGGGAACTGGCTTCCTAGTCCAATCAGCTC TTTTACCAATGAACACTTCCTTGTGATATAGATGTTTAT GGCCGAGAGGATCCTGGGTAGGAAACACATTTGAATGG

		TATTTACTAAGATACTAAAATCCTTGGACTTCACTCTAA TTTTAGTGCCATTTAGAACTCAAGGTCTCAGTAAAAGTA GAAATAAAGCCTGTTAACAAAACACAAACTGAATATTA AAAATGTAACTGGATTTTCAAAGAAATGTTTACTGGTAT TACCTGTAGATGTATATTCTTTATTATGATCTTTTGTGTA AAGTCTGGCAGACAAATGCAATATCTAATTGTTGAGTC CAATATCACAAGCAGTACAAAAGTATAAAAAAGACTTG GCCTTTTCTAATGTGTTAAAATACTTTATGCTGGTAATA ACACTAAGAGTAGGGCACTAGAAATTTTAAGTGAAGAT AATGTGTTGCAGTTACTGCACTCAATGGCTTACTATTAT AAACCAAAACTGGGATCACTAAGCTCCAGTCAGTCAAA ATGATCAAAATTATTGAAGAGAATAAGCAATTCTGTTC TTTATTAGGACACAGTAGATACAGACTACAAAGTGGAG TGTGCTTAATAAGAGGTAGCATTTGTTAAGTGTCAATTA CTCTATTATCCCTTGGAGCTTCTCAAAATAACCATATAA GGTGTAAGATGTTAAAGGTTATGGTTACACTCAGTGCA CAGGTAAGCTAATAGGCTGAGAGAAGCTAAATTACTTA CTGGGGTCTCACAGTAAGAAAGTGAGCTGAAGTTTCAG CCCAGATTTAACTGGATTCTGGGCTCTTTATTCATGTTA CTTCATGAATCTGTTTCTCAATTGTGCAGAAAAAAGGG GGCTATTTATAAGAAAAGCAATAAACAAACAAGTAATG ATCTCAAATAAGTAATGCAAGAAATAGTGAGATTTCAA AATCAGTGGCAGCGATTTCTCAGTTCTGTCCTAAGTGGC CTTGCTCAATCACCTGCTATCTTTTAGTGGAGCTTTGAA ATTATGTTTCAGACAACTTCGATTCAGTTCTAGAATGTT TGACTCAGCAAATTCACAGGCTCATCTTTCTAACTTGAT GGTGAATATGGAAATTCAGCTAAATGGATGTTAATAAA ATTCAAACGTTTTAAGGACAGATGGAAATGACAGAATT TTAAGGTAAAATATATGAAGGAATATAAGATAAAGGAT TTTTCTACCTTCAGCAAAAACATACCCACTAATTAGTAA AATTAATAGGCGAAAAAAAGTTGCATGCTCTTATACTG TAATGATTATCATTTTAAAACTAGCTTTTTGCCTTCGAG CTATCGGGGTAAAGACCTACAGGAAAACTACTGTCGAA ATCCTCGAGGGGAAGAAGGGGGACCCTGGTGTTTCACA AGCAATCCAGAGGTACGCTACGAAGTCTGTGACATTCC TCAGTGTTCAGAAGTTGAATGCATGACCTGCAATGGGG AGAGTTATCGAGGTCTCATGGATCATACAGAATCAGGC AAGATTTGTCAGCGCTGGGATCATCAGACACCACACCG GCACAAATTCTTGCCTGAAAGATATCCCGACAAGGGCT TTGATGATAATTATTGCCGCAATCCCGATGGCCAGCCG AGGCCATGGTGCTATACTCTTGACCCTCACACCCGCTGG GAGTACTGTGCAATTAAAACATGCGCTGACAATACTAT GAATGACACTGATGTTCCTTTGGAAACAACTGAATGCA TCCAAGGTCAAGGAGAAGGCTACAGGGGCACTGTCAAT ACCATTTGGAATGGAATTCCATGTCAGCGTTGGGATTCT CAGTATCCTCACGAGCATGACATGACTCCTGAAAATTTC

		AAGTGCAAGGACCTACGAGAAAATTACTGCCGAAATCC AGATGGGTCTGAATCACCCTGGTGTTTTACCACTGATCC AAACATCCGAGTTGGCTACTGCTCCCAAATTCCAAACT GTGATATGTCACATGGACAAGATTGTTATCGTGGGAAT GGCAAAAATTATATGGGCAACTTATCCCAAACAAGATC TGGACTAACATGTTCAATGTGGGACAAGAACATGGAAG ACTTACATCGTCATATCTTCTGGGAACCAGATGCAAGTA AGCTGAATGAGAATTACTGCCGAAATCCAGATGATGAT GCTCATGGACCCTGGTGCTACACGGGAAATCCACTCAT TCCTTGGGATTATTGCCCTATTTCTCGTTGTGAAGGTGA TACCACACCTACAATAGTCAATTTAGACCATCCCGTAAT ATCTTGTGCCAAAACGAAACAATTGCGAGTTGTAAATG GGATTCCAACACGAACAAACATAGGATGGATGGTTAGT TTGAGATACAGAAATAAACATATCTGCGGAGGATCATT GATAAAGGAGAGTTGGGTTCTTACTGCACGACAGTGTT TCCCTTCTCGAGACTTGAAAGATTATGAAGCTTGGCTTG GAATTCATGATGTCCACGGAAGAGGAGATGAGAAATGC AAACAGGTTCTCAATGTTTCCCAGCTGGTATATGGCCCT GAAGGATCAGATCTGGTTTTAATGAAGCTTGCCAGGCC TGCTGTCCTGGATGATTTTGTTAGTACGATTGATTTACC TAATTATGGATGCACAATTCCTGAAAAGACCAGTTGCA GTGTTTATGGCTGGGGCTACACTGGATTGATCAACTATG ATGGCCTATTACGAGTGGCACATCTCTATATAATGGGA AATGAGAAATGCAGCCAGCATCATCGAGGGAAGGTGA CTCTGAATGAGTCTGAAATATGTGCTGGGGCTGAAAAG ATTGGATCAGGACCATGTGAGGGGGATTATGGTGGCCC ACTTGTTTGTGAGCAACATAAAATGAGAATGGTTCTTG GTGTCATTGTTCCTGGTCGTGGATGTGCCATTCCAAATC GTCCTGGTATTTTTGTCCGAGTAGCATATTATGCAAAAT GGATACACAAAATTATTTTAACATATAAGGTACCACAG TCATAG
SEQ ID NO:10	Nucleotide sequence of HGF-X4	ATGTGGGTGACCAAACTCCTGCCAGCCCTGCTGCTGCA GCATGTCCTCCTGCATCTCCTCCTGCTCCCCATCGCCAT CCCCTATGCAGAGGGACAAAGGAAAAGAAGAAATACA ATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAAT CAAAATAGATCCAGCACTGAAGATAAAAACCAAAAAA GTGAATACTGCAGACCAATGTGCTAATAGATGTACTAG GAATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTT TGATAAAGCAAGAAAACAATGCCTCTGGTTCCCCTTCA ATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGGCCAT GAATTTGACCTCTATGAAAACAAAGACTACATTAGAAA CTGCATCATTGGTAAAGGACGCAGCTACAAGGGAACAG TATCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGG AGTTCCATGATACCACACGAACACAGGTAAGAACAGTA TGAAGAAAAGAGATGAAGCCTCTGTCTTTTTTACATGTT AACAGTCTCATATTAGTCCTTCAGAATAATTCTACAATC

		CTAAAATAACTTAGCCAACTTGCTGAATTGTATTACGGC AAGGTTTATATGAATTCATGACTGATATTTAGCAAATGA TTAATTAATATGTTAATAAAATGTAGCCAAAACAATAT CTTACCTTAATGCCTCAATTTGTAGATCTCGGTATTTGT GAAATAATAACGTAAACTTCGTTTAAAAGGATTCTTCTT CCTGTCTTTGAGAAAGTACGGCACTGTGCAGGGGGAGA GGTTGATTGTGAAAAATCAGAGGTAGATGAGAATCTTA CTGAGGGCTGAGGGTTCTTTAACCTTGGTGGATCTCAAC ATTGGTTGCACATTAAAATCACCTGCTGCAAGCCCTTGA CGAATCTTACTTAGAAGATGACAACACAGAACAATTAA ATCAGAATCTCTGGGGAGAATAGGGCACCAGTATTTTT TGAGCTCCCACCATGATTCCAAAGTGCAGCCAAATTTG AGAACCACTGCTAAAAGCTCAAGCTTCAGATTGACCAG CTTTTCCATCTCACCTATCGCCTAAAGACCAAATTGGAT AAATGTGTTCATTACGACAGATGGGTACTATTTAAAGA TGAGTAAACACAATATACTTAGGCTCGTCAGACTGAGA GTTTTAATCATCACTGAGGAAAAACATAGATATCTAAT ACTGACTGGAGTATTAGTCAAGGCTTATTTCACACACA ATTTTATCAGAAACCAAAGTAGTTTAAAACAGCTCTCCC CTTATTAGTAATGCATTGGAGGGTTTACTTTACCATGTA CCTTGCTGAGCACTGTACCTTGTTAATCTCATTTACTTGT AATGAGAACCACACAGCGGGTAGTTTTATTGGTTCTATT TTACCTACATGACAAAACTGAAGCATAAAAACACTTAG TAAGTTTTCAGTGTCATGCACAACTAGGAAGTGACATG GCCAGAATATAAGCCCAGTCACCATCACTCTATAACCT GCGCTTTTAACAACTTCAGGGCATGACACATTTGGCCG GTCAGTAGAACCCATGCTGTGATTTGTTTTTGCAGTGGT GGTGATGACTGCCTTGTTGAATCCACTTTTTATTCTATTC CATTTTGGGGACACAATTCTGCAAGATGATTCTTCATTA GGAAACAGAGATGAGTTATTGACCAACACAGAAAGAA AAAGAGTTTGTTGCTCCACACTGGGATTAAACCTATGAT CTTGGCCTAATTAACACTAGCTAGTAAGTGTCCAAGCTG ATCATCTCTACAACATTTCAATAACAGAAAACAACAAT TTTCAAAATTAGTTACTTACAATTATGTAGAAATGCCTC TAAAACACAGTATTTTCCTTATATTACAAAAACAAAAA TTATAATTGGTTTTGTCCTCTTTTGAGAGTTTGCATGGTG TTACTCCCTGCATAGTGAAGAAAACATTTTATTTAAGTA GATGGATCTAAGTTTTTCATGAACAAAGGAATGACATT TGAAATCAATCCTACCCTAGTCCAGGAGAATGCATTAG ATTAACCTAGTAGAGGTCTTATTTCACCCTGAGTTTTCT ATGATCGTGATTCTCTGCTGGAGGAGTAATTGTGAAAT AGATCTCTCTGGGAACTGGCTTCCTAGTCCAATCAGCTC TTTTACCAATGAACACTTCCTTGTGATATAGATGTTTAT GGCCGAGAGGATCCTTATGTTTCAGACAACTTCGATTCA GTTCTAGAATGTTTGACTCAGCAAATTCACAGGCTCATC TTTCTAACTTGATGGTGAATATGGAAATTCAGCTAAATG

		GATGTTAATAAAATTCAAACGTTTTAAGGACAGATGGA AATGACAGAATTTTAAGGTAAAATATATGAAGGAATAT AAGATAAAGGATTTTTCTACCTTCAGCAAAAACATACC CACTAATTAGTAAAATTAATAGGCGAAAAAAAGTTGCA TGCTCTTATACTGTAATGATTATCATTTTAAAACTAGCT TTTTGCCTTCGAGCTATCGGGGTAAAGACCTACAGGAA AACTACTGTCGAAATCCTCGAGGGGAAGAAGGGGGACC CTGGTGTTTCACAAGCAATCCAGAGGTACGCTACGAAG TCTGTGACATTCCTCAGTGTTCAGAAGTTGAATGCATGA CCTGCAATGGGGAGAGTTATCGAGGTCTCATGGATCAT ACAGAATCAGGCAAGATTTGTCAGCGCTGGGATCATCA GACACCACACCGGCACAAATTCTTGCCTGAAAGATATC CCGACAAGGGCTTTGATGATAATTATTGCCGCAATCCC GATGGCCAGCCGAGGCCATGGTGCTATACTCTTGACCC TCACACCCGCTGGGAGTACTGTGCAATTAAAACATGCG CTGACAATACTATGAATGACACTGATGTTCCTTTGGAAA CAACTGAATGCATCCAAGGTCAAGGAGAAGGCTACAGG GGCACTGTCAATACCATTTGGAATGGAATTCCATGTCA GCGTTGGGATTCTCAGTATCCTCACGAGCATGACATGA CTCCTGAAAATTTCAAGTGCAAGGACCTACGAGAAAAT TACTGCCGAAATCCAGATGGGTCTGAATCACCCTGGTG TTTTACCACTGATCCAAACATCCGAGTTGGCTACTGCTC CCAAATTCCAAACTGTGATATGTCACATGGACAAGATT GTTATCGTGGGAATGGCAAAAATTATATGGGCAACTTA TCCCAAACAAGATCTGGACTAACATGTTCAATGTGGGA CAAGAACATGGAAGACTTACATCGTCATATCTTCTGGG AACCAGATGCAAGTAAGCTGAATGAGAATTACTGCCGA AATCCAGATGATGATGCTCATGGACCCTGGTGCTACAC GGGAAATCCACTCATTCCTTGGGATTATTGCCCTATTTC TCGTTGTGAAGGTGATACCACACCTACAATAGTCAATTT AGACCATCCCGTAATATCTTGTGCCAAAACGAAACAAT TGCGAGTTGTAAATGGGATTCCAACACGAACAAACATA GGATGGATGGTTAGTTTGAGATACAGAAATAAACATAT CTGCGGAGGATCATTGATAAAGGAGAGTTGGGTTCTTA CTGCACGACAGTGTTTCCCTTCTCGAGACTTGAAAGATT ATGAAGCTTGGCTTGGAATTCATGATGTCCACGGAAGA GGAGATGAGAAATGCAAACAGGTTCTCAATGTTTCCCA GCTGGTATATGGCCCTGAAGGATCAGATCTGGTTTTAAT GAAGCTTGCCAGGCCTGCTGTCCTGGATGATTTTGTTAG TACGATTGATTTACCTAATTATGGATGCACAATTCCTGA AAAGACCAGTTGCAGTGTTTATGGCTGGGGCTACACTG GATTGATCAACTATGATGGCCTATTACGAGTGGCACAT CTCTATATAATGGGAAATGAGAAATGCAGCCAGCATCA TCGAGGGAAGGTGACTCTGAATGAGTCTGAAATATGTG CTGGGGCTGAAAAGATTGGATCAGGACCATGTGAGGGG GATTATGGTGGCCCACTTGTTTGTGAGCAACATAAAAT

		GAGAATGGTTCTTGGTGTCATTGTTCCTGGTCGTGGATG TGCCATTCCAAATCGTCCTGGTATTTTTGTCCGAGTAGC ATATTATGCAAAATGGATACACAAAATTATTTTAACAT ATAAGGTACCACAGTCATAG
SEQ ID NO:11	Nucleotide sequence of HGF-X5	ATGTGGGTGACCAAACTCCTGCCAGCCCTGCTGCTGCA GCATGTCCTCCTGCATCTCCTCCTGCTCCCCATCGCCAT CCCCTATGCAGAGGGACAAAGGAAAAGAAGAAATACA ATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAAT CAAAATAGATCCAGCACTGAAGATAAAAACCAAAAAA GTGAATACTGCAGACCAATGTGCTAATAGATGTACTAG GAATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTT TGATAAAGCAAGAAAACAATGCCTCTGGTTCCCCTTCA ATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGGCCAT GAATTTGACCTCTATGAAAACAAAGACTACATTAGAAA CTGCATCATTGGTAAAGGACGCAGCTACAAGGGAACAG TATCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGG AGTTCCATGATACCACACGAACACAGGTAAGAACAGTA TGAAGAAAAGAGATGAAGCCTCTGTCTTTTTTACATGTT AACAGTCTCATATTAGTCCTTCAGAATAATTCTACAATC CTAAAATAACTTAGCCAACTTGCTGAATTGTATTACGGC AAGGTTTATATGAATTCATGACTGATATTTAGCAAATGA TTAATTAATATGTTAATAAAATGTAGCCAAAACAATAT CTTACCTTAATGCCTCAATTTGTAGATCTCGGTATTTGT GGATCCAGTATATTAATAAAATCCCTTTTTGTATTCAAT GAGGGAAACACATAATTTTCATCAATTAGCAGCTTATT GGAATATCTGCATGATGGTTTAACACTTTTAAGTGTTGA CTAAAGATTAATTTTACAGAAAATAGAAAAAGAAATAT GTTTCTGTCTGGAGGAATGATTTATTGTTGACCCCTAAA TTGAAATATTTTACTAGTGGCTTAATGGAAAGATGATG AAAGATGATGAAATTAATGTAGAAGCTTAACTAGAAAA TCAGGTGACCTGATATCTACATCTGTATCCTTCATTGGC CACCCAGCATTCATTAATGAATCAGATGATGGAATAGA TCAAGTTTCCTAGGAACACAGTGAATATTAAAAGAAAA CAAAGGGAGCCTAGCACCTAGAAGACCTAGTTTATATT TCAAAGTATATTTGGATGTAACCCAATTTTAAACATTTC CTCACTTGTCTCTCTTAAAGCCTTGCCAACAGCAAGGAC AGAGAACCAAAAATAGTGTATATATGAATAAATGCTTA TTACAGAATCTGCTGACTGGCACATGCTTTGTGTGTAAT GGGTTCTCATAAACACTTGTTGAATGAACACACATAAG TGAAAGAGCATGGCTAGGCTTCATCCCTTGGTCAAATA TGGGGTGCTAAAGAAAAGCAGGGGAAATACATTGGGA CACTAACAAAAAAAAACAGTTAATTTAGGTAAAAGATA AAATACACCACAGAATGAAGAAAAGAGATGACCCAGA CTGCTCTTTAACCTTCATGTCCTAGAGAGGTTTTTGATA TGAATTGCATTCAGAATTGTGGAAAGGAGCCCATCTTTT CTCTTCATTTTGATTTTATTAACTCCAATGGGGGAATTTT

		ATTCGTGTTTTGGCCATATCTACTTTTGATTTCTACATTA TTCTCTCTTCCTTTCTACCTGTATTTGTCCTAATAAATTG TTGACTTATTAATTCACTACTTCCTCACAGCTTTTTTTTG GCTTTACAAATCCACTGGAAAGGTATATGGGTGTATCA CTTTGTGTATTTCGGTGTGCATGTGTAGAGGGGACAAA AATCCTCTCTCAAACTATAAATATTGAGTATTTGTGTAT TGAACATTTGCTATAACTACTAGGTTTCTTAAATAATCT TAATATATAAAATGATATAGAAAAAGGGAAATTATAGT TCGTATTATTCATCTAAGTGAAGAGATTAAAACCCAGG GAGTAAATAAATTGTCTAAGGACTAAGGTTGTATACTA TTTAGGTGATAGATATGGGGCAACCGTATGGGTTTTATG ATTAACAAATAAACTTCTCACCACTCTACCATATCAACT TTTCCATAAAAGAGAGCTATAGTATTCTTTGCTTAAATA AATTTGATTAGTGCATGACTTCTTGAAAACATATAAAGC AAAAGTCACATTTGATTCTATCAGAAAAGTGAGTAAGC CATGGCCCAAACAAAAGATGCATTAAAATATTCTGGAA TGATGGAGCTAAAAGTAAGAAAAATGACTTTTTAAAAA AGTTTACTGTTAGGAATTGTGAAATTATGCTGAATTTTA GTTGCATTATAATTTTTGTCAGTCATACGGTCTGACAAC CTGTCTTATTTCTATTTCCCCATATGAGGAATGCTAGTT AAGTATGGATATTAACTATTACTACTTAGATGCATTGAA GTTGCATAATATGGATAATACTTCACTGGTTCCCTGAAA ATGTTTAGTTAGTAATAAGTCTCTTACACTATTTGTTTTG TCCAATAATTTATATTTTCTGAAGACTTAACTCTAGAAT ACACTCATGTCAAAATGAAAGAATTTCATTGCAAAATA TTGCTTGGTACATGACGCATACCTGTATTTGTTTTGTGT CACAACATGAAAAATGATGGTTTATTAGAAGTTTCATT GGGTAGGAAACACATTTGAATGGTATTTACTAAGATAC TAAAATCCTTGGACTTCACTCTAATTTTAGTGCCATTTA GAACTCAAGGTCTCAGTAAAAGTAGAAATAAAGCCTGT TAACAAAACACAAACTGAATATTAAAAATGTAACTGGA TTTTCAAAGAAATGTTTACTGGTATTACCTGTAGATGTA TATTCTTTATTATGATCTTTTGTGTAAAGTCTGGCAGAC AAATGCAATATCTAATTGTTGAGTCCAATATCACAAGC AGTACAAAAGTATAAAAAAGACTTGGCCTTTTCTAATG TGTTAAAATACTTTATGCTGGTAATAACACTAAGAGTA GGGCACTAGAAATTTTAAGTGAAGATAATGTGTTGCAG TTACTGCACTCAATGGCTTACTATTATAAACCAAAACTG GGATCACTAAGCTCCAGTCAGTCAAAATGATCAAAATT ATTGAAGAGAATAAGCAATTCTGTTCTTTATTAGGACAC AGTAGATACAGACTACAAAGTGGAGTGTGCTTAATAAG AGGTAGCATTTGTTAAGTGTCAATTACTCTATTATCCCT TGGAGCTTCTCAAAATAACCATATAAGGTGTAAGATGT TAAAGGTTATGGTTACACTCAGTGCACAGGTAAGCTAA TAGGCTGAGAGAAGCTAAATTACTTACTGGGGTCTCAC AGTAAGAAAGTGAGCTGAAGTTTCAGCCCAGATTTAAC

		TGGATTCTGGGCTCTTTATTCATGTTACTTCATGAATCT GTTTCTCAATTGTGCAGAAAAAAGGGGGCTATTTATAA GAAAAGCAATAAACAAACAAGTAATGATCTCAAATAA GTAATGCAAGAAATAGTGAGATTTCAAAATCAGTGGCA GCGATTTCTCAGTTCTGTCCTAAGTGGCCTTGCTCAATC ACCTGCTATCTTTTAGTGGAGCTTTGAAATTATGTTTCA GACAACTTCGATTCAGTTCTAGAATGTTTGACTCAGCAA ATTCACAGGCTCATCTTTCTAACTTGATGGTGAATATGG AAATTCAGCTAAATGGATGTTAATAAAATTCAAACGTT TTAAGGACAGATGGAAATGACAGAATTTTAAGGTAAAA TATATGAAGGAATATAAGATAAAGGATTTTTCTACCTTC AGCAAAAACATACCCACTAATTAGTAAAATTAATAGGC GAAAAAAAGTTGCATGCTCTTATACTGTAATGATTATCA TTTTAAAACTAGCTTTTTGCCTTCGAGCTATCGGGGTAA AGACCTACAGGAAAACTACTGTCGAAATCCTCGAGGGG AAGAAGGGGGACCCTGGTGTTTCACAAGCAATCCAGAG GTACGCTACGAAGTCTGTGACATTCCTCAGTGTTCAGAA GTTGAATGCATGACCTGCAATGGGGAGAGTTATCGAGG TCTCATGGATCATACAGAATCAGGCAAGATTTGTCAGC GCTGGGATCATCAGACACCACACCGGCACAAATTCTTG CCTGAAAGATATCCCGACAAGGGCTTTGATGATAATTA TTGCCGCAATCCCGATGGCCAGCCGAGGCCATGGTGCT ATACTCTTGACCCTCACACCCGCTGGGAGTACTGTGCAA TTAAAACATGCGCTGACAATACTATGAATGACACTGAT GTTCCTTTGGAAACAACTGAATGCATCCAAGGTCAAGG AGAAGGCTACAGGGGCACTGTCAATACCATTTGGAATG GAATTCCATGTCAGCGTTGGGATTCTCAGTATCCTCACG AGCATGACATGACTCCTGAAAATTTCAAGTGCAAGGAC CTACGAGAAAATTACTGCCGAAATCCAGATGGGTCTGA ATCACCCTGGTGTTTTACCACTGATCCAAACATCCGAGT TGGCTACTGCTCCCAAATTCCAAACTGTGATATGTCACA TGGACAAGATTGTTATCGTGGGAATGGCAAAAATTATA TGGGCAACTTATCCCAAACAAGATCTGGACTAACATGT TCAATGTGGGACAAGAACATGGAAGACTTACATCGTCA TATCTTCTGGGAACCAGATGCAAGTAAGCTGAATGAGA ATTACTGCCGAAATCCAGATGATGATGCTCATGGACCC TGGTGCTACACGGGAAATCCACTCATTCCTTGGGATTAT TGCCCTATTTCTCGTTGTGAAGGTGATACCACACCTACA ATAGTCAATTTAGACCATCCCGTAATATCTTGTGCCAAA ACGAAACAATTGCGAGTTGTAAATGGGATTCCAACACG AACAAACATAGGATGGATGGTTAGTTTGAGATACAGAA ATAAACATATCTGCGGAGGATCATTGATAAAGGAGAGT TGGGTTCTTACTGCACGACAGTGTTTCCCTTCTCGAGAC TTGAAAGATTATGAAGCTTGGCTTGGAATTCATGATGTC CACGGAAGAGGAGATGAGAAATGCAAACAGGTTCTCA ATGTTTCCCAGCTGGTATATGGCCCTGAAGGATCAGATC

		TGGTTTTAATGAAGCTTGCCAGGCCTGCTGTCCTGGATG ATTTTGTTAGTACGATTGATTTACCTAATTATGGATGCA CAATTCCTGAAAAGACCAGTTGCAGTGTTTATGGCTGG GGCTACACTGGATTGATCAACTATGATGGCCTATTACG AGTGGCACATCTCTATATAATGGGAAATGAGAAATGCA GCCAGCATCATCGAGGGAAGGTGACTCTGAATGAGTCT GAAATATGTGCTGGGGCTGAAAAGATTGGATCAGGACC ATGTGAGGGGGATTATGGTGGCCCACTTGTTTGTGAGC AACATAAAATGAGAATGGTTCTTGGTGTCATTGTTCCTG GTCGTGGATGTGCCATTCCAAATCGTCCTGGTATTTTTG TCCGAGTAGCATATTATGCAAAATGGATACACAAAATT ATTTTAACATATAAGGTACCACAGTCATAG
SEQ ID NO:12	Nucleotide sequence of HGF-X6	ATGTGGGTGACCAAACTCCTGCCAGCCCTGCTGCTGCA GCATGTCCTCCTGCATCTCCTCCTGCTCCCCATCGCCAT CCCCTATGCAGAGGGACAAAGGAAAAGAAGAAATACA ATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAAT CAAAATAGATCCAGCACTGAAGATAAAAACCAAAAAA GTGAATACTGCAGACCAATGTGCTAATAGATGTACTAG GAATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTT TGATAAAGCAAGAAAACAATGCCTCTGGTTCCCCTTCA ATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGGCCAT GAATTTGACCTCTATGAAAACAAAGACTACATTAGAAA CTGCATCATTGGTAAAGGACGCAGCTACAAGGGAACAG TATCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGG AGTTCCATGATACCACACGAACACAGGTAAGAACAGTA TGAAGAAAAGAGATGAAGCCTCTGTCTTTTTTACATGTT AACAGTCTCATATTAGTCCTTCAGAATAATTCTACAATC CTAAAATAACTTAGCCAACTTGCTGAATTGTATTACGGC AAGGTTTATATGAATTCATGACTGATATTTAGCAAATGA TTAATTAATATGTTAATAAAATGTAGCCAAAACAATAT CTTACCTTAATGCCTCAATTTGTAGATCTCGGTATTTGT GGATCTCTTCCTTTCTACCTGTATTTGTCCTAATAAATTG TTGACTTATTAATTCACTACTTCCTCACAGCTTTTTTTTG GCTTTACAAATCCACTGGAAAGGTATATGGGTGTATCA CTTTGTGTATTTCGGTGTGCATGTGTAGAGGGGACAAA AATCCTCTCTCAAACTATAAATATTGAGTATTTGTGTAT TGAACATTTGCTATAACTACTAGGTTTCTTAAATAATCT TAATATATAAAATGATATAGAAAAAGGGAAATTATAGT TCGTATTATTCATCTAAGTGAAGAGATTAAAACCCAGG GAGTAAATAAATTGTCTAAGGACTAAGGTTGTATACTA TTTAGGTGATAGATATGGGGCAACCGTATGGGTTTTATG ATTAACAAATAAACTTCTCACCACTCTACCATATCAACT TTTCCATAAAAGAGAGCTATAGTATTCTTTGCTTAAATA AATTTGATTAGTGCATGACTTCTTGAAAACATATAAAGC AAAAGTCACATTTGATTCTATCAGAAAAGTGAGTAAGC

		CATGGCCCAAACAAAAGATGCATTAAAATATTCTGGAA TGATGGAGCTAAAAGTAAGAAAAATGACTTTTTAAAAA AGTTTACTGTTAGGAATTGTGAAATTATGCTGAATTTTA GTTGCATTATAATTTTTGTCAGTCATACGGTCTGACAAC CTGTCTTATTTCTATTTCCCCATATGAGGAATGCTAGTT AAGTATGGATATTAACTATTACTACTTAGATGCATTGAA GTTGCATAATATGGATAATACTTCACTGGTTCCCTGAAA ATGTTTAGTTAGTAATAAGTCTCTTACACTATTTGTTTTG TCCAATAATTTATATTTTCTGAAGACTTAACTCTAGAAT ACACTCATGTCAAAATGAAAGAATTTCATTGCAAAATA TTGCTTGGTACATGACGCATACCTGTATTTGTTTTGTGT CACAACATGAAAAATGATGGTTTATTAGAAGTTTCATT GGGTAGGAAACACATTTGAATGGTATTTACTAAGATAC TAAAATCCTTGGACTTCACTCTAATTTTAGTGCCATTTA GAACTCAAGGTCTCAGTAAAAGTAGAAATAAAGCCTGT TAACAAAACACAAACTGAATATTAAAAATGTAACTGGA TTTTCAAAGAAATGTTTACTGGTATTACCTGTAGATGTA TATTCTTTATTATGATCTTTTGTGTAAAGTCTGGCAGAC AAATGCAATATCTAATTGTTGAGTCCAATATCACAAGC AGTACAAAAGTATAAAAAAGACTTGGCCTTTTCTAATG TGTTAAAATACTTTATGCTGGTAATAACACTAAGAGTA GGGCACTAGAAATTTTAAGTGAAGATAATGTGTTGCAG TTACTGCACTCAATGGCTTACTATTATAAACCAAAACTG GGATCACTAAGCTCCAGTCAGTCAAAATGATCAAAATT ATTGAAGAGAATAAGCAATTCTGTTCTTTATTAGGACAC AGTAGATACAGACTACAAAGTGGAGTGTGCTTAATAAG AGGTAGCATTTGTTAAGTGTCAATTACTCTATTATCCCT TGGAGCTTCTCAAAATAACCATATAAGGTGTAAGATGT TAAAGGTTATGGTTACACTCAGTGCACAGGTAAGCTAA TAGGCTGAGAGAAGCTAAATTACTTACTGGGGTCTCAC AGTAAGAAAGTGAGCTGAAGTTTCAGCCCAGATTTAAC TGGATTCTGGGCTCTTTATTCATGTTACTTCATGAATCT GTTTCTCAATTGTGCAGAAAAAAGGGGGCTATTTATAA GAAAAGCAATAAACAAACAAGTAATGATCTCAAATAA GTAATGCAAGAAATAGTGAGATTTCAAAATCAGTGGCA GCGATTTCTCAGTTCTGTCCTAAGTGGCCTTGCTCAATC ACCTGCTATCTTTTAGTGGAGCTTTGAAATTATGTTTCA GACAACTTCGATTCAGTTCTAGAATGTTTGACTCAGCAA ATTCACAGGCTCATCTTTCTAACTTGATGGTGAATATGG AAATTCAGCTAAATGGATGTTAATAAAATTCAAACGTT TTAAGGACAGATGGAAATGACAGAATTTTAAGGTAAAA TATATGAAGGAATATAAGATAAAGGATTTTTCTACCTTC AGCAAAAACATACCCACTAATTAGTAAAATTAATAGGC GAAAAAAAGTTGCATGCTCTTATACTGTAATGATTATCA TTTTAAAACTAGCTTTTTGCCTTCGAGCTATCGGGGTAA AGACCTACAGGAAAACTACTGTCGAAATCCTCGAGGGG

		AAGAAGGGGGACCCTGGTGTTTCACAAGCAATCCAGAG GTACGCTACGAAGTCTGTGACATTCCTCAGTGTTCAGAA GTTGAATGCATGACCTGCAATGGGGAGAGTTATCGAGG TCTCATGGATCATACAGAATCAGGCAAGATTTGTCAGC GCTGGGATCATCAGACACCACACCGGCACAAATTCTTG CCTGAAAGATATCCCGACAAGGGCTTTGATGATAATTA TTGCCGCAATCCCGATGGCCAGCCGAGGCCATGGTGCT ATACTCTTGACCCTCACACCCGCTGGGAGTACTGTGCAA TTAAAACATGCGCTGACAATACTATGAATGACACTGAT GTTCCTTTGGAAACAACTGAATGCATCCAAGGTCAAGG AGAAGGCTACAGGGGCACTGTCAATACCATTTGGAATG GAATTCCATGTCAGCGTTGGGATTCTCAGTATCCTCACG AGCATGACATGACTCCTGAAAATTTCAAGTGCAAGGAC CTACGAGAAAATTACTGCCGAAATCCAGATGGGTCTGA ATCACCCTGGTGTTTTACCACTGATCCAAACATCCGAGT TGGCTACTGCTCCCAAATTCCAAACTGTGATATGTCACA TGGACAAGATTGTTATCGTGGGAATGGCAAAAATTATA TGGGCAACTTATCCCAAACAAGATCTGGACTAACATGT TCAATGTGGGACAAGAACATGGAAGACTTACATCGTCA TATCTTCTGGGAACCAGATGCAAGTAAGCTGAATGAGA ATTACTGCCGAAATCCAGATGATGATGCTCATGGACCC TGGTGCTACACGGGAAATCCACTCATTCCTTGGGATTAT TGCCCTATTTCTCGTTGTGAAGGTGATACCACACCTACA ATAGTCAATTTAGACCATCCCGTAATATCTTGTGCCAAA ACGAAACAATTGCGAGTTGTAAATGGGATTCCAACACG AACAAACATAGGATGGATGGTTAGTTTGAGATACAGAA ATAAACATATCTGCGGAGGATCATTGATAAAGGAGAGT TGGGTTCTTACTGCACGACAGTGTTTCCCTTCTCGAGAC TTGAAAGATTATGAAGCTTGGCTTGGAATTCATGATGTC CACGGAAGAGGAGATGAGAAATGCAAACAGGTTCTCA ATGTTTCCCAGCTGGTATATGGCCCTGAAGGATCAGATC TGGTTTTAATGAAGCTTGCCAGGCCTGCTGTCCTGGATG ATTTTGTTAGTACGATTGATTTACCTAATTATGGATGCA CAATTCCTGAAAAGACCAGTTGCAGTGTTTATGGCTGG GGCTACACTGGATTGATCAACTATGATGGCCTATTACG AGTGGCACATCTCTATATAATGGGAAATGAGAAATGCA GCCAGCATCATCGAGGGAAGGTGACTCTGAATGAGTCT GAAATATGTGCTGGGGCTGAAAAGATTGGATCAGGACC ATGTGAGGGGGATTATGGTGGCCCACTTGTTTGTGAGC AACATAAAATGAGAATGGTTCTTGGTGTCATTGTTCCTG GTCGTGGATGTGCCATTCCAAATCGTCCTGGTATTTTTG TCCGAGTAGCATATTATGCAAAATGGATACACAAAATT ATTTTAACATATAAGGTACCACAGTCATAG
SEQ ID NO:13	Nucleotide sequence of HGF-X7	ATGTGGGTGACCAAACTCCTGCCAGCCCTGCTGCTGCA GCATGTCCTCCTGCATCTCCTCCTGCTCCCCATCGCCAT CCCCTATGCAGAGGGACAAAGGAAAAGAAGAAATACA

		ATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAAT CAAAATAGATCCAGCACTGAAGATAAAAACCAAAAAA GTGAATACTGCAGACCAATGTGCTAATAGATGTACTAG GAATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTT TGATAAAGCAAGAAAACAATGCCTCTGGTTCCCCTTCA ATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGGCCAT GAATTTGACCTCTATGAAAACAAAGACTACATTAGAAA CTGCATCATTGGTAAAGGACGCAGCTACAAGGGAACAG TATCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGG AGTTCCATGATACCACACGAACACAGGTAAGAACAGTA TGAAGAAAAGAGATGAAGCCTCTGTCTTTTTTACATGTT AACAGTCTCATATTAGTCCTTCAGAATAATTCTACAATC CTAAAATAACTTAGCCAACTTGCTGAATTGTATTACGGC AAGGTTTATATGAATTCATGACTGATATTTAGCAAATGA TTAATTAATATGTTAATAAAATGTAGCCAAAACAATAT CTTACCTTAATGCCTCAATTTGTAGATCTCGGTATTTGT GGATCCTGGGTAGGAAACACATTTGAATGGTATTTACT AAGATACTAAAATCCTTGGACTTCACTCTAATTTTAGTG CCATTTAGAACTCAAGGTCTCAGTAAAAGTAGAAATAA AGCCTGTTAACAAAACACAAACTGAATATTAAAAATGT AACTGGATTTTCAAAGAAATGTTTACTGGTATTACCTGT AGATGTATATTCTTTATTATGATCTTTTGTGTAAAGTCT GGCAGACAAATGCAATATCTAATTGTTGAGTCCAATAT CACAAGCAGTACAAAAGTATAAAAAAGACTTGGCCTTT TCTAATGTGTTAAAATACTTTATGCTGGTAATAACACTA AGAGTAGGGCACTAGAAATTTTAAGTGAAGATAATGTG TTGCAGTTACTGCACTCAATGGCTTACTATTATAAACCA AAACTGGGATCACTAAGCTCCAGTCAGTCAAAATGATC AAAATTATTGAAGAGAATAAGCAATTCTGTTCTTTATTA GGACACAGTAGATACAGACTACAAAGTGGAGTGTGCTT AATAAGAGGTAGCATTTGTTAAGTGTCAATTACTCTATT ATCCCTTGGAGCTTCTCAAAATAACCATATAAGGTGTA AGATGTTAAAGGTTATGGTTACACTCAGTGCACAGGTA AGCTAATAGGCTGAGAGAAGCTAAATTACTTACTGGGG TCTCACAGTAAGAAAGTGAGCTGAAGTTTCAGCCCAGA TTTAACTGGATTCTGGGCTCTTTATTCATGTTACTTCATG AATCTGTTTCTCAATTGTGCAGAAAAAAGGGGGCTATTT ATAAGAAAAGCAATAAACAAACAAGTAATGATCTCAA ATAAGTAATGCAAGAAATAGTGAGATTTCAAAATCAGT GGCAGCGATTTCTCAGTTCTGTCCTAAGTGGCCTTGCTC AATCACCTGCTATCTTTTAGTGGAGCTTTGAAATTATGT TTCAGACAACTTCGATTCAGTTCTAGAATGTTTGACTCA GCAAATTCACAGGCTCATCTTTCTAACTTGATGGTGAAT ATGGAAATTCAGCTAAATGGATGTTAATAAAATTCAAA CGTTTTAAGGACAGATGGAAATGACAGAATTTTAAGGT AAAATATATGAAGGAATATAAGATAAAGGATTTTTCTA

		CCTTCAGCAAAAACATACCCACTAATTAGTAAAATTAA TAGGCGAAAAAAAGTTGCATGCTCTTATACTGTAATGA TTATCATTTTAAAACTAGCTTTTTGCCTTCGAGCTATCG GGGTAAAGACCTACAGGAAAACTACTGTCGAAATCCTC GAGGGGAAGAAGGGGGACCCTGGTGTTTCACAAGCAAT CCAGAGGTACGCTACGAAGTCTGTGACATTCCTCAGTG TTCAGAAGTTGAATGCATGACCTGCAATGGGGAGAGTT ATCGAGGTCTCATGGATCATACAGAATCAGGCAAGATT TGTCAGCGCTGGGATCATCAGACACCACACCGGCACAA ATTCTTGCCTGAAAGATATCCCGACAAGGGCTTTGATG ATAATTATTGCCGCAATCCCGATGGCCAGCCGAGGCCA TGGTGCTATACTCTTGACCCTCACACCCGCTGGGAGTAC TGTGCAATTAAAACATGCGCTGACAATACTATGAATGA CACTGATGTTCCTTTGGAAACAACTGAATGCATCCAAG GTCAAGGAGAAGGCTACAGGGGCACTGTCAATACCATT TGGAATGGAATTCCATGTCAGCGTTGGGATTCTCAGTAT CCTCACGAGCATGACATGACTCCTGAAAATTTCAAGTG CAAGGACCTACGAGAAAATTACTGCCGAAATCCAGATG GGTCTGAATCACCCTGGTGTTTTACCACTGATCCAAACA TCCGAGTTGGCTACTGCTCCCAAATTCCAAACTGTGATA TGTCACATGGACAAGATTGTTATCGTGGGAATGGCAAA AATTATATGGGCAACTTATCCCAAACAAGATCTGGACT AACATGTTCAATGTGGGACAAGAACATGGAAGACTTAC ATCGTCATATCTTCTGGGAACCAGATGCAAGTAAGCTG AATGAGAATTACTGCCGAAATCCAGATGATGATGCTCA TGGACCCTGGTGCTACACGGGAAATCCACTCATTCCTTG GGATTATTGCCCTATTTCTCGTTGTGAAGGTGATACCAC ACCTACAATAGTCAATTTAGACCATCCCGTAATATCTTG TGCCAAAACGAAACAATTGCGAGTTGTAAATGGGATTC CAACACGAACAAACATAGGATGGATGGTTAGTTTGAGA TACAGAAATAAACATATCTGCGGAGGATCATTGATAAA GGAGAGTTGGGTTCTTACTGCACGACAGTGTTTCCCTTC TCGAGACTTGAAAGATTATGAAGCTTGGCTTGGAATTC ATGATGTCCACGGAAGAGGAGATGAGAAATGCAAACA GGTTCTCAATGTTTCCCAGCTGGTATATGGCCCTGAAGG ATCAGATCTGGTTTTAATGAAGCTTGCCAGGCCTGCTGT CCTGGATGATTTTGTTAGTACGATTGATTTACCTAATTA TGGATGCACAATTCCTGAAAAGACCAGTTGCAGTGTTT ATGGCTGGGGCTACACTGGATTGATCAACTATGATGGC CTATTACGAGTGGCACATCTCTATATAATGGGAAATGA GAAATGCAGCCAGCATCATCGAGGGAAGGTGACTCTGA ATGAGTCTGAAATATGTGCTGGGGCTGAAAAGATTGGA TCAGGACCATGTGAGGGGGATTATGGTGGCCCACTTGT TTGTGAGCAACATAAAATGAGAATGGTTCTTGGTGTCA TTGTTCCTGGTCGTGGATGTGCCATTCCAAATCGTCCTG

		GTATTTTTGTCCGAGTAGCATATTATGCAAAATGGATAC ACAAAATTATTTTAACATATAAGGTACCACAGTCATAG
SEQ ID NO:14	Nucleotide sequence of HGF-X8	ATGTGGGTGACCAAACTCCTGCCAGCCCTGCTGCTGCA GCATGTCCTCCTGCATCTCCTCCTGCTCCCCATCGCCAT CCCCTATGCAGAGGGACAAAGGAAAAGAAGAAATACA ATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAAT CAAAATAGATCCAGCACTGAAGATAAAAACCAAAAAA GTGAATACTGCAGACCAATGTGCTAATAGATGTACTAG GAATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTT TGATAAAGCAAGAAAACAATGCCTCTGGTTCCCCTTCA ATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGGCCAT GAATTTGACCTCTATGAAAACAAAGACTACATTAGAAA CTGCATCATTGGTAAAGGACGCAGCTACAAGGGAACAG TATCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGG AGTTCCATGATACCACACGAACACAGGTAAGAACAGTA TGAAGAAAAGAGATGAAGCCTCTGTCTTTTTTACATGTT AACAGTCTCATATTAGTCCTTCAGAATAATTCTACAATC CTAAAATAACTTAGCCAACTTGCTGAATTGTATTACGGC AAGGTTTATATGAATTCATGACTGATATTTAGCAAATGA TTAATTAATATGTTAATAAAATGTAGCCAAAACAATAT CTTACCTTAATGCCTCAATTTGTAGATCTCGGTATTTGT GGATCCTTATGTTTCAGACAACTTCGATTCAGTTCTAGA ATGTTTGACTCAGCAAATTCACAGGCTCATCTTTCTAAC TTGATGGTGAATATGGAAATTCAGCTAAATGGATGTTA ATAAAATTCAAACGTTTTAAGGACAGATGGAAATGACA GAATTTTAAGGTAAAATATATGAAGGAATATAAGATAA AGGATTTTTCTACCTTCAGCAAAAACATACCCACTAATT AGTAAAATTAATAGGCGAAAAAAAGTTGCATGCTCTTA TACTGTAATGATTATCATTTTAAAACTAGCTTTTTGCCTT CGAGCTATCGGGGTAAAGACCTACAGGAAAACTACTGT CGAAATCCTCGAGGGGAAGAAGGGGGACCCTGGTGTTT CACAAGCAATCCAGAGGTACGCTACGAAGTCTGTGACA TTCCTCAGTGTTCAGAAGTTGAATGCATGACCTGCAATG GGGAGAGTTATCGAGGTCTCATGGATCATACAGAATCA GGCAAGATTTGTCAGCGCTGGGATCATCAGACACCACA CCGGCACAAATTCTTGCCTGAAAGATATCCCGACAAGG GCTTTGATGATAATTATTGCCGCAATCCCGATGGCCAGC CGAGGCCATGGTGCTATACTCTTGACCCTCACACCCGCT GGGAGTACTGTGCAATTAAAACATGCGCTGACAATACT ATGAATGACACTGATGTTCCTTTGGAAACAACTGAATG CATCCAAGGTCAAGGAGAAGGCTACAGGGGCACTGTCA ATACCATTTGGAATGGAATTCCATGTCAGCGTTGGGATT CTCAGTATCCTCACGAGCATGACATGACTCCTGAAAATT TCAAGTGCAAGGACCTACGAGAAAATTACTGCCGAAAT CCAGATGGGTCTGAATCACCCTGGTGTTTTACCACTGAT CCAAACATCCGAGTTGGCTACTGCTCCCAAATTCCAAA

		CTGTGATATGTCACATGGACAAGATTGTTATCGTGGGA ATGGCAAAAATTATATGGGCAACTTATCCCAAACAAGA TCTGGACTAACATGTTCAATGTGGGACAAGAACATGGA AGACTTACATCGTCATATCTTCTGGGAACCAGATGCAA GTAAGCTGAATGAGAATTACTGCCGAAATCCAGATGAT GATGCTCATGGACCCTGGTGCTACACGGGAAATCCACT CATTCCTTGGGATTATTGCCCTATTTCTCGTTGTGAAGG TGATACCACACCTACAATAGTCAATTTAGACCATCCCGT AATATCTTGTGCCAAAACGAAACAATTGCGAGTTGTAA ATGGGATTCCAACACGAACAAACATAGGATGGATGGTT AGTTTGAGATACAGAAATAAACATATCTGCGGAGGATC ATTGATAAAGGAGAGTTGGGTTCTTACTGCACGACAGT GTTTCCCTTCTCGAGACTTGAAAGATTATGAAGCTTGGC TTGGAATTCATGATGTCCACGGAAGAGGAGATGAGAAA TGCAAACAGGTTCTCAATGTTTCCCAGCTGGTATATGGC CCTGAAGGATCAGATCTGGTTTTAATGAAGCTTGCCAG GCCTGCTGTCCTGGATGATTTTGTTAGTACGATTGATTT ACCTAATTATGGATGCACAATTCCTGAAAAGACCAGTT GCAGTGTTTATGGCTGGGGCTACACTGGATTGATCAACT ATGATGGCCTATTACGAGTGGCACATCTCTATATAATGG GAAATGAGAAATGCAGCCAGCATCATCGAGGGAAGGT GACTCTGAATGAGTCTGAAATATGTGCTGGGGCTGAAA AGATTGGATCAGGACCATGTGAGGGGGATTATGGTGGC CCACTTGTTTGTGAGCAACATAAAATGAGAATGGTTCTT GGTGTCATTGTTCCTGGTCGTGGATGTGCCATTCCAAAT CGTCCTGGTATTTTTGTCCGAGTAGCATATTATGCAAAA TGGATACACAAAATTATTTTAACATATAAGGTACCACA GTCATAG
SEQ ID NO:15	Nucleotide sequence of pTx	CAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGG GAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCT CTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCG GAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTT CCTGGCCTTTTGCTGGCCTTTTGCTCACATGCGCGTTGACATT GATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCAT TAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTA CGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAA TAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT AAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAA GTCCGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCT GGCATTATGCCCAGTACATGACCTTACGGGACTTTCCTACTT GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGA TGCGGTTTTGGCAGTACACCAATGGGCGTGGATAGCGGTTTG ACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAAT GTCGTAATAACCCCGCCCCGTTGACGCAAATGGGCGGTAGGC GTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGA

		ACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACC TCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGGCCGG GAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGA CGTAAGTACCGCCTATAGACTCTATAGGCACACCCCTTTGGC TCTTATGCATGCTATACTGTTTTTGGCTTGGGGCCTATACACC CCCGCTTCCTTATGCTATAGGTGATGGTATAGCTTAGCCTATA GGTGTGGGTTATTGACCATTATTGACCACTCCCCTATTGGTG ACGATACTTTCCATTACTAATCCATAACATGGCTCTAGACTT AAGGCAGCGGCAGAAGAAGATGTAGGCAGCTGAGTTGTTGT ATTCTGATAAGAGTCAGAGGTAACTCCCGTTGCGGTGCTGTT AACGGTGGAGGGCAGTGTAGTCTGAGCAGTACTCGTTGCTGC CGCGCGCGCCACCAGACATAATAGCTGACAGACTAACAGAC TGTTCCTTTCCATGGGTCTTTTCTGCAGTCACCGTCCTTGACA CGAAGCTTATCGATGTCGACCTCGAGTCTAGAGGGCCCGTTT AAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGC CATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGA AGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAAT TGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGG TGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGAC AATAGCAGGCATGCTGGGGAGTCGAAATTCAGAAGAACTCG TCAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGC GGCGATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGC CGCCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGT CCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGA ATCCAGAAAAGCGGCCATTTTCCACCATGATATTCGGCAAGC AGGCATCGCCATGGGTCACGACGAGATCCTCGCCGTCGGGC ATGCTCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGC CCCTGATGCTCTTCGTCCAGATCATCCTGATCGACAAGACCG GCTTCCATCCGAGTACGTGCTCGCTCGATGCGATGTTTCGCTT GGTGGTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGC CGCCGCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGA GCAAGGTGAGATGACAGGAGATCCTGCCCCGGCACTTCGCC CAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGAG CACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATA GCCGCGCTGCCTCGTCTTGCAGTTCATTCAGGGCACCGGACA GGTCGGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGAC AGCCGGAACACGGCGGCATCAGAGCAGCCGATTGTCTGTTGT GCCCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGGA GAACCTGCGTGCAATCCATCTTGTTCAATCATGCGAAACGAT CCTCATCCTGTCTCTTGATCAGATCTTGATCCCCTGCGCCATC AGATCCTTGGCGGCAAGAAAGCCATCCAGTTTACTTTGCAGG GCTTCCCAACCTTACCAGAGGGCGCCCCAGCTGGCAATTCCG GTTCGCTTGCTGCGTCAGACCCCGTAGAAAAGATCAAAGGAT CTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCA AACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGA TCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAG CAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTA GTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATA CCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGG CGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTT ACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGT

		GCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTG AGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCC GAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGT CGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAAC GCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGAC TTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCC TATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGG CCTTTTGCTGGCCTTTTGCTCACATG
SEQ ID NO:16	Nucleotide sequence of pTx-IGF-1X10	CAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGG GAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCT CTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCG GAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTT CCTGGCCTTTTGCTGGCCTTTTGCTCACATGCGCGTTGACATT GATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCAT TAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTA CGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAA TAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT AAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAA GTCCGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCT GGCATTATGCCCAGTACATGACCTTACGGGACTTTCCTACTT GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGA TGCGGTTTTGGCAGTACACCAATGGGCGTGGATAGCGGTTTG ACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAAT GTCGTAATAACCCCGCCCCGTTGACGCAAATGGGCGGTAGGC GTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGA ACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACC TCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGGCCGG GAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGA CGTAAGTACCGCCTATAGACTCTATAGGCACACCCCTTTGGC TCTTATGCATGCTATACTGTTTTTGGCTTGGGGCCTATACACC CCCGCTTCCTTATGCTATAGGTGATGGTATAGCTTAGCCTATA GGTGTGGGTTATTGACCATTATTGACCACTCCCCTATTGGTG ACGATACTTTCCATTACTAATCCATAACATGGCTCTAGACTT AAGGCAGCGGCAGAAGAAGATGTAGGCAGCTGAGTTGTTGT ATTCTGATAAGAGTCAGAGGTAACTCCCGTTGCGGTGCTGTT AACGGTGGAGGGCAGTGTAGTCTGAGCAGTACTCGTTGCTGC CGCGCGCGCCACCAGACATAATAGCTGACAGACTAACAGAC TGTTCCTTTCCATGGGTCTTTTCTGCAGTCACCGTCCTTGACA CGAAGCTTATCGATATGGGAAAAATCAGCAGTCTTCCAACCC AATTATTTAAGTGCTGCTTTTGTGATTTCTTGAAGGTGAAGAT GCACACCATGTCCTCCTCGCATCTCTTCTACCTGGCGCTGTGC CTGCTCACCTTCACCAGCTCTGCCACGGCTGGACCGGAGACG CTCTGCGGGGCTGAGCTGGTGGATGCTCTTCAGTTCGTGTGT GGAGACAGGGGCTTTTATTTCAACAAGCCCACAGGGTATGGC TCCAGCAGTCGGAGGGCGCCTCAGACAGGCATCGTGGATGA GTGCTGCTTCCGGAGCTGTGATCTAAGGAGGCTGGAGATGTA TTGCGCACCCCTCAAGCCTGCCAAGTCAGCTCGCTCTGTCCG TGCCCAGCGCCACACCGACATGCCCAAGACCCAGAAGGTAA

		GCCCACCTGGGTGGGATCCAGCCATCCTCAAGTGGTCTCTCT CTTGTGCATGTGGGTGGGCCAAGCAGAAATCCTGCCCCATAG TCTCCTGGCTTACAAGTCAGAAAAGCTCCTTTGCACCAAAGG GATGGATTACATCCCCATCTCTTTGCTAAACAAACATGGGCT TTGGTGTCAGACAAAAGTGAAGTCCTGGCTTTCTCACACACC AGCTTAGAGAGAAAAGACTTTTAGGTGAATGTGGCAGGAAA GCGTGCTTGCTGGGGCAAAGGCAGATTCATTCTTTCTCTTCCC AGTATCAGCCCCCATCTACCAACAAGAACACGAAGTCTCAG AGAAGGAAAGGAAGTACATTTGAAGAACGCAAGTAGCTTTT TCTCCTTTATTTATAGGAAGTACATTTGAAGAACGCAAGTAG AGGGAGTGCAGGAAACAAGAACTACAGGATGTAGGTCGACC TCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGA CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCC CGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTT TCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGG TGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAA GGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGAGT CGAAATTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGA TGCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGG AAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCA CGGGTAGCCAACGCTATGTCCTGATAGCGGTCCGCCACACCC AGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTTCC ACCATGATATTCGGCAAGCAGGCATCGCCATGGGTCACGAC GAGATCCTCGCCGTCGGGCATGCTCGCCTTGAGCCTGGCGAA CAGTTCGGCTGGCGCGAGCCCCTGATGCTCTTCGTCCAGATC ATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCG CTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGC CGGATCAAGCGTATGCAGCCGCCGCATTGCATCAGCCATGAT GGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGAT CCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCG CTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCC GTCGTGGCCAGCCACGATAGCCGCGCTGCCTCGTCTTGCAGT TCATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGAAC CGGGCGCCCCTGCGCTGACAGCCGGAACACGGCGGCATCAG AGCAGCCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCC TCTCCACCCAAGCGGCCGGAGAACCTGCGTGCAATCCATCTT GTTCAATCATGCGAAACGATCCTCATCCTGTCTCTTGATCAG ATCTTGATCCCCTGCGCCATCAGATCCTTGGCGGCAAGAAAG CCATCCAGTTTACTTTGCAGGGCTTCCCAACCTTACCAGAGG GCGCCCCAGCTGGCAATTCCGGTTCGCTTGCTGCGTCAGACC CCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCT GCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACC AGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTT TCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATA CTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGA ACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTT ACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGG GTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGT CGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAG CGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCT ATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACA

		GGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACG AGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCT GTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGAT GCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAAC GCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTC ACATG
SEQ ID NO:17	F primer of the first primer pair (pTx)	AGCTGGCAATTCCGGTTCGCTTGCTGCGTCAGACCCCGTA
SEQ ID NO:18	R primer of the first primer pair (pTx)	TACGGGGTCTGACGCAGCAAGCGAACCGGAATTGCCAGCT
SEQ ID NO: 19	F primer of the second primer pair (pTx)	CTAATCCATAACATGGCTCTAGACTTAAGGCAGCGGCAGA
SEQ ID NO: 20	R primer of the second primer pair (pTx)	TCTGCCGCTGCCTTAAGTCTAGAGCCATGTTATGGATTAG
SEQ ID NO. 21	IGF-1X6	ATGGGAAAAATCAGCAGTCTTCCAACCCAATTATTTAAGTGC TGCTTTTGTGATTTCTTGAAGGTGAAGATGCACACCATGTCCT CCTCGCATCTCTTCTACCTGGCGCTGTGCCTGCTCACCTTCAC CAGCTCTGCCACGGCTGGACCGGAGACGCTCTGCGGGGCTG AGCTGGTGGATGCTCTTCAGTTCGTGTGTGGAGACAGGGGCT TTTATTTCAACAAGCCCACAGGGTATGGCTCCAGCAGTCGGA GGGCGCCTCAGACAGGCATCGTGGATGAGTGCTGCTTCCGGA GCTGTGATCTAAGGAGGCTGGAGATGTATTGCGCACCCCTCA AGCCTGCCAAGTCAGCTCGCTCTGTCCGTGCCCAGCGCCACA CCGACATGCCCAAGACCCAGAAGGTAAGCCCACCTGGGTGG GATCCAGCCATCCTCAAGTGGTCTCTCTCTTGTGCATGTGGGT GGGCCAAGCAGAAATCCTGCCCCATAGTCTCCTGGCTTACAA GTCAGAAAAGCTCCTTTGCACCAAAGGGATGGATTACATCCC CATCTCTTTGGTCACTCTGCATTGCAAATTTCCCCTCCCACCG CTATGGACGATGTGATGATTGGAAGATGTTACAAAACAGTG GCTAAACAAACATGGGCTTTGGTGTCAGACAAAAGTGAAGT CCTGGCTTTCTCACACACCAGCTTAGAGAGAAAAGACTTTTA GGTGAATGTGGCAGGAAAGCGTGCTTGCTGGGGCAAAGGCA GATTCATTCTTTCTCTTCCCAGTATCAGCCCCCATCTACCAAC AAGAACACGAAGTCTCAGAGAAGGAAAGGAAGTACATTTGA AGAACGCAAGTAGCTTTTTCTCCTTTATTTATAGGAAGTACA TTTGAAGAACGCAAGTAGAGGGAGTGCAGGAAACAAGAACT ACAGGATGTAG
SEQ ID NO. 22	IGF-1X10	ATGGGAAAAATCAGCAGTCTTCCAACCCAATTATTTAAGTGC TGCTTTTGTGATTTCTTGAAGGTGAAGATGCACACCATGTCCT CCTCGCATCTCTTCTACCTGGCGCTGTGCCTGCTCACCTTCAC CAGCTCTGCCACGGCTGGACCGGAGACGCTCTGCGGGGCTG AGCTGGTGGATGCTCTTCAGTTCGTGTGTGGAGACAGGGGCT TTTATTTCAACAAGCCCACAGGGTATGGCTCCAGCAGTCGGA GGGCGCCTCAGACAGGCATCGTGGATGAGTGCTGCTTCCGGA

		GCTGTGATCTAAGGAGGCTGGAGATGTATTGCGCACCCCTCA AGCCTGCCAAGTCAGCTCGCTCTGTCCGTGCCCAGCGCCACA CCGACATGCCCAAGACCCAGAAGGTAAGCCCACCTGGGTGG GATCCAGCCATCCTCAAGTGGTCTCTCTCTTGTGCATGTGGGT GGGCCAAGCAGAAATCCTGCCCCATAGTCTCCTGGCTTACAA GTCAGAAAAGCTCCTTTGCACCAAAGGGATGGATTACATCCC CATCTCTTTGCTAAACAAACATGGGCTTTGGTGTCAGACAAA AGTGAAGTCCTGGCTTTCTCACACACCAGCTTAGAGAGAAAA GACTTTTAGGTGAATGTGGCAGGAAAGCGTGCTTGCTGGGGC AAAGGCAGATTCATTCTTTCTCTTCCCAGTATCAGCCCCCATC TACCAACAAGAACACGAAGTCTCAGAGAAGGAAAGGAAGTA CATTTGAAGAACGCAAGTAGCTTTTTCTCCTTTATTTATAGGA AGTACATTTGAAGAACGCAAGTAGAGGGAGTGCAGGAAACA AGAACTACAGGATGTAG
SEQ ID NO. 23	aa sequence of Class I IGF-1Ea	MGKISSLPTQLFKCCFCDFLKVKMHTMSSSHLFYLALCLLTFTS SATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAP QTGIVDECCFRSCDLRRLEMYCAPLKPAKSARSVRAQRHTDMP KTQKEVHLKNASRGSAGNKNYRM
SEQ ID NO. 24	nucleotide sequence of Class I IGF-1Ea	ATGGGAAAAATCAGCAGTCTTCCAACCCAATTATTTAAGTGC TGCTTTTGTGATTTCTTGAAGGTGAAGATGCACACCATGTCCT CCTCGCATCTCTTCTACCTGGCGCTGTGCCTGCTCACCTTCAC CAGCTCTGCCACGGCTGGACCGGAGACGCTCTGCGGGGCTG AGCTGGTGGATGCTCTTCAGTTCGTGTGTGGAGACAGGGGCT TTTATTTCAACAAGCCCACAGGGTATGGCTCCAGCAGTCGGA GGGCGCCTCAGACAGGCATCGTGGATGAGTGCTGCTTCCGGA GCTGTGATCTAAGGAGGCTGGAGATGTATTGCGCACCCCTCA AGCCTGCCAAGTCAGCTCGCTCTGTCCGTGCCCAGCGCCACA CCGACATGCCCAAGACCCAGAAGGAAGTACATTTGAAGAAC GCAAGTAGAGGGAGTGCAGGAAACAAGAACTACAGGATGTA G
SEQ ID NO. 25	aa sequence of Class I IGF-1Ec	MGKISSLPTQLFKCCFCDFLKVKMHTMSSSHLFYLALCLLTFTS SATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAP QTGIVDECCFRSCDLRRLEMYCAPLKPAKSARSVRAQRHTDMP KTQKYQPPSTNKNTKSQRRKGSTFEERK
SEQ ID NO. 26	nucleotide sequence of Class I IGF-1Ec	ATGGGAAAAATCAGCAGTCTTCCAACCCAATTATTTAAGTGC TGCTTTTGTGATTTCTTGAAGGTGAAGATGCACACCATGTCCT CCTCGCATCTCTTCTACCTGGCGCTGTGCCTGCTCACCTTCAC CAGCTCTGCCACGGCTGGACCGGAGACGCTCTGCGGGGCTG AGCTGGTGGATGCTCTTCAGTTCGTGTGTGGAGACAGGGGCT TTTATTTCAACAAGCCCACAGGGTATGGCTCCAGCAGTCGGA GGGCGCCTCAGACAGGCATCGTGGATGAGTGCTGCTTCCGGA GCTGTGATCTAAGGAGGCTGGAGATGTATTGCGCACCCCTCA AGCCTGCCAAGTCAGCTCGCTCTGTCCGTGCCCAGCGCCACA CCGACATGCCCAAGACCCAGAAGTATCAGCCCCCATCTACCA ACAAGAACACGAAGTCTCAGAGAAGGAAAGGAAGTACATTT GAAGAACGCAAGTAG
SEQ ID NO. 27	aa sequence of Class II IGF-1Ea	MITPTVKMHTMSSSHLFYLALCLLTFTSSATAGPETLCGAELVD ALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRR LEMYCAPLKPAKSARSVRAQRHTDMPKTQKEVHLKNASRGSA GNKNYRM

SEQ ID NO. 28	nucleotide sequence of Class II IGF-1Ea	ATGATTACACCTACAGTGAAGATGCACACCATGTCCTCCTCG CATCTCTTCTACCTGGCGCTGTGCCTGCTCACCTTCACCAGCT CTGCCACGGCTGGACCGGAGACGCTCTGCGGGGCTGAGCTG GTGGATGCTCTTCAGTTCGTGTGTGGAGACAGGGGCTTTTAT TTCAACAAGCCCACAGGGTATGGCTCCAGCAGTCGGAGGGC GCCTCAGACAGGCATCGTGGATGAGTGCTGCTTCCGGAGCTG TGATCTAAGGAGGCTGGAGATGTATTGCGCACCCCTCAAGCC TGCCAAGTCAGCTCGCTCTGTCCGTGCCCAGCGCCACACCGA CATGCCCAAGACCCAGAAGGAAGTACATTTGAAGAACGCAA GTAGAGGGAGTGCAGGAAACAAGAACTACAGGATGTAG
SEQ ID NO. 29	aa sequence of Class I IGF-1Eb	MGKISSLPTQLFKCCFCDFLKVKMHTMSSSHLFYLALCLLTFTS SATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAP QTGIVDECCFRSCDLRRLEMYCAPLKPAKSARSVRAQRHTDMP KTQKYQPPSTNKNTKSQRRKGWPKTHPGGEQKEGTEASLQIRG KKKEQRREIGSRNAECRGKKGK
SEQ ID NO. 30	nucleotide sequence of Class I IGF-1Eb	ATGGGAAAAATCAGCAGTCTTCCAACCCAATTATTTAAGTGC TGCTTTTGTGATTTCTTGAAGGTGAAGATGCACACCATGTCCT CCTCGCATCTCTTCTACCTGGCGCTGTGCCTGCTCACCTTCAC CAGCTCTGCCACGGCTGGACCGGAGACGCTCTGCGGGGCTG AGCTGGTGGATGCTCTTCAGTTCGTGTGTGGAGACAGGGGCT TTTATTTCAACAAGCCCACAGGGTATGGCTCCAGCAGTCGGA GGGCGCCTCAGACAGGCATCGTGGATGAGTGCTGCTTCCGGA GCTGTGATCTAAGGAGGCTGGAGATGTATTGCGCACCCCTCA AGCCTGCCAAGTCAGCTCGCTCTGTCCGTGCCCAGCGCCACA CCGACATGCCCAAGACCCAGAAGTATCAGCCCCCATCTACCA ACAAGAACACGAAGTCTCAGAGAAGGAAAGGTTGGCCAAAG ACACATCCAGGAGGGGAACAGAAGGAGGGGACAGAAGCAA GTCTGCAGATCAGAGGAAAGAAGAAAGAGCAGAGGAGGGA GATTGGAAGTAGAAATGCTGAATGCAGAGGCAAAAAAGGAA AATGA
SEQ ID NO. 31	IGF-1 (exon 1, 3, and 4)	ATGGGAAAAATCAGCAGTCTTCCAACCCAATTATTTAAGTGC TGCTTTTGTGATTTCTTGAAGGTGAAGATGCACACCATGTCCT CCTCGCATCTCTTCTACCTGGCGCTGTGCCTGCTCACCTTCAC CAGCTCTGCCACGGCTGGACCGGAGACGCTCTGCGGGGCTG AGCTGGTGGATGCTCTTCAGTTCGTGTGTGGAGACAGGGGCT TTTATTTCAACAAGCCCACAGGGTATGGCTCCAGCAGTCGGA GGGCGCCTCAGACAGGCATCGTGGATGAGTGCTGCTTCCGGA GCTGTGATCTAAGGAGGCTGGAGATGTATTGCGCACCCCTCA AGCCTGCCAAGTCAGCTCGCTCTGTCCGTGCCCAGCGCCACA CCGACATGCCCAAGACCCAGAAG
SEQ ID NO. 32	IGF-1 (intron 4)	GTAAGCCCACCTGGGTGGGATCCAGCCATCCTCAAGTGGTCT CTCTCTTGTGCATGTGGGTGGGCCAAGCAGAAATCCTGCCCC ATAGTCTCCTGGCTTACAAGTCAGAAAAGCTCCTTTGCACCA AAGGGATGGATTACATCCCCATCTCTTTGGTCACTCTGCATT GCAAATTTCCCCTCCCACCGCTATGGACGATGTGATGATTGG AAGATGTTACAAAACAGTGGCTAAACAAACATGGGCTTTGG TGTCAGACAAAAGTGAAGTCCTGGCTTTCTCACACACCAGCT TAGAGCCCTTGGCAAATAATGTGATGTACCCAAGCCTCAGTT TCATCAGTAACATTGGGATAATAATAATATCTACCACATCAG TTTGTTGTCAAAATTAAGTAGCTCATGCATATACTTTGAGAT

		GCTTTTCACATGCCTGCATAAAGTAATTGTTGGACCATCGTT AATGTCTGCCATAATTGCACTTAATAACAAAGCTTGTAACCT TTCAAGTTCTGAGATTCTACAATCTTCCAAAGAAAATAAAAG GCTAATGGGAACTATTCAAAATTCATATTCAGTAGCAAGCAT AATTAAACATGAAACATTAAAAATAGAAATTTCTGTTTGGCT ATAAGAATGCCTAGACATTTGTAATGATCAAAATCTGCAGGC ATCATTTTCTAAGAGCTAGACTGTAAACAAACCTCAGAGGTA CCAACTATGCCATCAGTAGTACATAAAACATCTGATGCACAT TTAGTCACTTGATCGATTTCTCTTGAATGAGTGAACGAATGA ACAAATGAATATAAGAGATTAAAATTTTAGCCATTAAGTAGA AAGAATAAGAACTAAAGAGAAGGTAAAGGAGGAAAAAGAG AAGGCAAGGAAGTTGAGTAAGGGAAGAAATAGCTCTCGTTT AAGTATTTTGGGGACTCTGTTGAAAAAAGAAATGCCAACATG TGGTTTTAATCTTTGGAGCTAGAACTAATAATATTGTGCAAA AGCACAAGATGAGAGATCAAGAAGTTCACCATGACACCTTC GCTGCTTCCTGGTCTTAAACCTCAGCTGAGGCTGGAAGAGGA CCATGGTGGCTTATTGGAGATGTGACCCCAGGGAGCCCCTCT GAAGGATGGAAGGGGACTGGGCAAGACCCAACACACACAG AACACAGTAGCCACTGGCCAGGCAGGAAGCAAGGATCTCAG AAAAGACTTTTAGGTGAATGTGGCAGGAAAGCGTGCTTGCTG GGGCAAAGGCAGATTCATTCTTTCTCTTCCCAGGTGACCCAG CGCCTCTTGGTTTCTAACTGGGGAGGGGGTAGGTGTCAAGAG ATGAGTCCCAAAGTTCTGGAATGGTGGGTCTTGTGACTGAGG TCTAGACCCCTCTCCAGCATGAGTGCTGTCTCCTGCATCATAT GGAGCCTGGGCATTCTGAGCTCATTCAAAGGGACACCATGG GAACCACTTGTTCTCAATGCAATTATTTTTGTGATGTTTACAG
SEQ ID NO. 33	IGF-1 (exon 5 and 6-1)	TATCAGCCCCCATCTACCAACAAGAACACGAAGTCTCAGAG AAGGAAAGGAAGTACATTTGAAGAACGCAAGTAG
SEQ ID NO. 34	IGF-1 (intron 5)	AGGACAGGAGGATTAAACAGACAGAGGCAAGGATGATGAG AGAGGAGCAGACAGCAAGAATGAAAAGCAGAAAATACAAT AGAGGAAATGAAGAAAAGTAGGCCTGCTGGAGCTAGATGAT GATGTGATGGAAATAGAAGTAACCTTTTAGAGAATCTCGCTA AGAAACATGGAGAAAACGGAAAAGAAAAATGTAATGCCCTA GAAAGCGCAAAGAAAGACAGTGGCAAAAATGAAAAAAAAA AATAAAAATTATAAAAGAGGCAAAAAAAGACACACTATTCT CTGCCTCTAAAACACAATTAAATAAAAGAATTTAAATAAAA ATTAAGGCTTCTATATGCATTTTTAAATTTTGTATGAATCTGT TATGGAAGAATTGCCTATGTCAATATATGTTCAGAGTTAAAT ATTAGCCCCAAATGCTCAGCAAGACTGAATTGTGTCATAGAA GTTCCCAGATTCCCTTTTCCCGCAATGTCATTGGAGGCTGCAT TTCTTAGTCAAGTCCAGGGTTTAGGCCAAAGGGCATCCGGTA TTGCCTAAAACCCTGTGAGGTCTGTGAGGTAACTTTTGAGAA GAGGTCACTGCACTCTTCATCTTTTTTGCACTTTGGAATCAGA TATAAAAGATGTATAAGTTTGCTAGGGCTGCCATAACAAAGT ATCATAGGCTAGGTAGTTTAAACCACAGAAATTGATTTTTTC ATAGTTCTGGGAGTTGAAAGTCCAAAATCAAAGTATCAGCCC TTGCAAGGGCCTTAGAGAAGGCTCTGTCATGGGCTCCTCCCC TCGGCTTGTAGGTGGCCTCCTTCTTCTCCCCCTGTGTCTTCAC TTCATCTTCCCTCCATACATATCTCTGTGTCTAAACATCCTCT GTGTGAAACAACACCAGCCAGGTTGGATTTGGGCCCACCCCA CTGACCTCATTTTAACTTAATTATCTCTGTAAAGACTCTGTCT

		CCAAATACAGTCATATTTTGACGTACTGGGAGTTAGGGCTTC AACACATGAATTTGGACACAATTCAGCCAGTGACAGAAGAC TTCTGATCTCTGATGATAACCACTGCATTTTGATTACAGCTCC TAGAAAACACTCCCCTCCACCACCCCACCACAGATCTATTTT TATATCTGAAACCCTGAGTTTCTGCTCCATGAGAACCCCAGG AACATACTATGTTAGATCTGGAAGAAGCCTCAGAAATCCCCT TATTTTGAAGACTAGGACACTGAGATCCAGAAGTGGGTAAA GATGTGCTTGGGTTCTAAGCTGCTCTTCTTTTGGCCAGGAGA CAACAGCACATAATCAAAGTGGGTCAACTAAGAAAGAATTC CAGAAGGAAAAGAGAGGGCAGAAATGAAGGGAGAGAATGA GAGCAAAAGTGCTGGATTTCCCTGAGGGTGAAGAAAAGTTA AATAGAATCACAGAATTCAGATTTTAGAGATCTTCTCCTTCA GATCCCTTGGTTTAATCAGTAGGATTGGGGTCTTCATAGATA ATAAAGCAAAAACTCTCGCCATCCTCCAAGTTGTGAATTAGA AGAGCTGAGAAAGGGTACAAGACGGAAGTTCTCTACCAAAC AAATGGTGACATTTTGGGGTAAGAATATGACTAACCCAGAA GTGAAGCATTTCATCCAAGTAGTCTATTTTGAAGATGTCATG GTATAAAGGAACCTCCTTTCTGCCTGGTCCTCCATGCCTCTGC CATGCTTTTTACTCCAGGATCACCCTTTCTAGTGGTTCACTGA AAACCCAGGATTACTTAAATATGATGGACATGTTCACGGCTC AATCCAGGAGGAAAAGGTCGAACTGAAAGCATGCCAAAGCC CCACATGGGAGCCAAGCCACTGCTGCTGTGGTTGCAAAGTGG ATCCTGGCTTATCAGAGCAGAGAGAAGCCAGGCTCGTGCCTT AGCCCAAGTGGCCAGTCACCTTATTCAGGAGATACTAAGTTC TCCAGCTAAGACATCCATGCTTTGGGACCAGCTGCAGACAGA AGCCAATTCCTACTACAACCATCACCTTAGAGTAGCATATAG ACACAGATGGCTCTTCAAAGGACCACAGTTCCATGGAATAAC TAAGAATTCATGTCCTGTGGAAAGGTTTGAATAAACTATAAT TATACCCAATCATAAATTTCATTCAAGAAGAACTAAAGCAAA GGCAAAGACAGAGAGAAGAAGGAAGGAAGGAGGGAGGGAG GGAGGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGA AGGAAGGAAGGAAGGAAGGAAAGGGAAGGAAGAACAAAAA GACTTTCTAGTTAAAGAATGCTTAACTAGCAAACTATGTACT ATAAGACAGTTCTTTTCGGAATGAGTTTTATCAACTCTAAAG CAATTATCTTGAATGCCTACATGTGATTACTGAATAATATGA ACCAAGAAAACAGAAAGAATCTATATTATCTTTCCATTTCCT TCTTTCCAGTATCAATACCCAAGCCTCTAGTGATACATGGCA TATAATGTTGGATGGATGGATGGATGGATGGATGGATGGAT GGATGGATGGATGGATGAATGGATGGTTGGATGGACAAATG AGTAACATAGGCTGATGAATAGTGGTAGAAAGACACACCAT AAAAACAAGTGGCACTTCTGAGATGAAATGATTCCTATTCTC CTACACAAGACAGTGAGGCAAGTACAGAGTAAAAAAGGAAA GGCATAGGAGCTATGCTTATACAAGTATTGTATGTTTGGAAT TTCCTTCGCTGGCCAAATTGAAATTGTTCAAGGACCTATTGCT ACAGGTGGCAACTGGCTAAGAATTTCATAGTGAATATTATAC ACCTATTACTCCCCTTAATGTTTCTTTGAAGTAAGCAGAATAT TAATAATCATTTAAAATTCCAGTGTTTCAACTTCAATTGTTTC CTAGGGCAAATTGATAATTGTGTGTAAAACTAATTGGAATAT GTATGGAATAATCATCCTGAAATAAAATTGGTGAAAAGTATT TGTTATTGGGCATCTACAATGTGCAAACCTCTGTACTAGGCA TGAACAAGAGTTATAAGCATTGGAGAGGCTAAAATATAGTC

		CTTAAGGCTGGGCACAGTGGCTCATGCCTGTAATCCTAGCAC TTTGGGAGGCCAAGGCGGGCAGATTGCCTGAGCTCAGGAGT TCAAGACCAGCCTGGGCAACATAGCGAAACCCCATCTCTACT AAAAATACAAAAAAATTACCTGGGCATGGTGGCACGCACCT GTAATCCCAGCTACTCAGGAGGCTGAGGCATGAGAATTCCTT GAACCTGGGAGGCAGAGGTTGCAGCGAGCCGAGATCCTGCC GCTGCATCCCAGCTTGGGTGACAGAGTGAGACTCTGTCTCAA AAAAAAATTAAATAATAAATAAATAGTAAAATACAGTCATT AAGAGTACAAAATGTAGATTCAGACTACCTGGGTTCAAATCT TGGCTCTTACTTGCATTGTGGCTTTGGGCAGATCATGTAACTT ATGTGTGCCTCAGTTTCCTCATCTGTTAAATAGGGGCAACAA CTGAATCTACCTTATTCAGTTGTTGTGAGGGTTTATTGAGATT GTGTGTGTGTATGTGTGTGAGTGTAGTGTGTGCATGTGTGTG TCTGTGCAAGGAGTGGGAGGTGTATATTCAGAGACACATATT ACAGCACTTAAAATGGTATCTAGCACTTAGTAAGCATTATTC AAGTTTTAGTTAACATTATTTTACTTACCTCTGAAAATTGGAG CTATGTGAAAAAGAAGTTGGTCTCCTGAAGTAGAAGCCAGTC TTGTGTCACCAAAAACTTCAAGCCCAAGCTTGCCAACGCTTT TCCATGATGTGGTAGTAGAGTTTCAAGCATGTGGTAGGATAA GAGAACTCAATGACCTAAGAACCATTCCAACCCAGAGAACC CCTGGTTCTATGAATAATTCCAACTTAAATAGGTAGCTTGGC TCTCCCAAGTGAGAGCCATTGCTTCTGTTTCCGGGTCATATA ATGAACTTTCAGAAAACCACCATTTTTCTCAACCAGTTAAAA TTAAGTGTAATACGTGCTTTCATTTCATGGTGCCTGGGGAAA ATTTAATTGTAGTATGAACTCCAGTTATTGGTAGTCTTAAGTA AAATTGCCAAAATAAATAGAAATGCAGGATATTTCTGGGCTC ACACAGCTTCCGGGACACTTTAGTTTCTTGGGCTGCCAATCC AGTGCCTTTCACAAGCATTTGATCTTTTTTCAAACATCTCTTG AAAACAAACAAAACCTCACACAGCTTCTAATGTGTGCACTGT TCGAATGTAAGGGTGGAAAAGGAGGCAAAGAAATGAGCTCC CAAAGAGCAATTCCCCTTCTCTCGCCTCCATCCCTTGACGAC CTCCCTCCCACTAAAGGGAAACATTGTTTTCTTAGGTAATAA ATTCTGCAATTTCTCAAGTCCATTAACATCCACTGGGCAAGA TGAGATCTATTCTTTTTATTTGCCCATAGGAAAAGAATAGTG CTTTTTTGCAATATTCACTAGATAACACAGAGTTGACTTTTAA TCCAAGGGCAACATTGATAGTCTCTAGTTAAAGGGGAAGCCT TCAGGAGCAATGAAAAGATTAATAGTTTTAGATGAAGCAGA ATCCAAATCCCTTTTTATGAGTTTTGAAATATCCAGTTTGTAT GCTCACCTCAATACTTAAAGCCCAGTTACTGATTCCTTTGGCC TAAGCAAGACAGGTCAATTTTTAAAGAGGGAGTAGCTGAGG TTAGCAAAAATTCTCCAGGTCCACAAAACTTCCAGACCTGCA AGGTGAAAATCAGCTTTTCTGTCATCCCTAAAGGCCTAACTG GAATCAGAACTTTTCCCTGATGCCCACATATTTGGAGGTCCT TTTTTAATGGGACTCCTTAATGCCTTTAGTGCCATCCCATTTT CATCCAGTGTCCAAAAGAAATGATTTAAAAATATAAACGTAT GTTTAAATTCCAGAAGAGAGAAATGGAGATTGAGAACAATA GGGAAATGATGAGAGCTATGGGAAAAGAGGTTTATGAGTCC ATGTCTGATTCTTCCAGAGAGCCCCTAAGAAAGTTCTTATCA TACCAGGAACTCAATTATAACTTTCATTGCCTATTGTTAGATG AGTAACAGGAGCTAGAAAACATTTTGGAAATTCCCATCTTTA TTTTTTTAACTAATATGATTATAGTTTTAAGAACCATTGGTCA

		AGAAGCTAACTTTTTAAAAAGTGGAAGTATGATGGTTAGAA ATAAGAATGCTAAAGGTGCATCAAGCTGATTTTAATTCTAAA TGTCCTTGGCAGCAATTTAGAATCTGTAATAAACTACACCAA ACAGTTTTGAGGGGAAGGGGATTAGTTTCTCCCCTTCCTTCG TGTGTGTGTGTGCGCGTGTGTGTGTGTGCACCTTTGTGTTCTA GCATTGTTGCACCCATTACAGAGCTGGGGGGAACTATTTTCC AAAATTATAGGTGAGAACAGTTTCTTGGATTGTCTTTCAGTG AAGGTAAATTCCTCTGTAAAAACTAACCATCATTCAGTAAAA ACTGCAGGATTCCTTTGTCTTCTCAAAAGCCTGTTTCTCATCC TAAATTAAAAATTATTCAGGAAATAGAGAGGACATTATTGG AGGGGTGGAAATAAGTTGGTTTTCTTTTTATTGTATCTTTTGA GGATCCAGGGACTTCTACCATTTCCCATCTAACATACAGAGA AGGATTCTCTAGGTCCCTGTCTATAGACTGCAGTAACTTTCCT ATAGAACCAATTTGCAATTTTAGAAATTTCTAGGTCTAATTA TTGACCCATTACAACCAAAGGTCAATGCATCCAGCCAATCTT CCTTCTATCATCCCCTGCCCTTACTTCTATTAGGGACTGGGAT TACAGGCAAAACCCATCAAATGCCTCTTCTACCACTTTCCCA TTTCTTAACCATTAGCCTCTAACTTCCTCTATTCAGTTTCTCAT ATGCTTTCATGCCCATTGGGTCAGATAAAGGAACATTCATTT ATTTGAGTAGGCATCTGTTATGATCACTCCGGAAAAAAGATG ACAATGGGTTACCTTGTCCTCCTGGGCTTCTCTAACTGACATG GTCAAAATGCCCATATGAAGATAAGATGTTAAGAGCAAGAT TTATGAAAAGCTGAGTATGATGGCAGCTCTTGTCTCATAAAA TAACTCGAAAGTTCCCAGTGAAAGACCAAGAAATTTTACATC AAACCCAAACCGGCCAAATGGTCCAAGCTTCCAAGCTGGGA TCCATGGCTAAAGTTTCTACAAAATTCTGGGTACAATGTATA AACATTCACTTGGGGCTTTCTGTCTAGCCAGCACCAAGAGGT CAAGTAATCAAGGACCAACTAGCCCTGCCATCTGTGAAAATA TGTGCTATTTTCACGGCTTTAGTTCACAATTATGGCAAGACA AAAGTTCCAAATAATTAGGAGCAAGACCATGGCAGGTTGAC GGTTGAGTAAGGTTCTCAATCAGCCGACAATTGTAGAGTTGG GGATGTGCAATGTTTATGTCATGGTGTAAGTATGTGGCATGC TTGACTAGCTTGTGAGGCACTGGAAGACTAGAAGGAATGAA AAATATGAATGAATCAATAAATGCATAGTATAATTACTGTTA TTTTGTCAGTATTGTTTTACCTAGGTCACTATTGAATGCTCTG ATTTGTCTCTTTATAAATAATAATATGTTTTCTTCTTCAAAAG AACACTAGGATGAAGGTAGAGGTGCTTTTGGCACAATGCCA CAATTCTGATTTTTTTAAAACTGTATGCATGCATAAAATGTTC TTGAGCCATTCTCTGCCTTGGAATAGCACTGGCTGGCATTCT GCATGTTTACTTTTATATGCTGAAGGCCCCCATCAACCTCAA ACAGAGGCAAATCAATTTAACTTCTCATAGTGTTATTTTGTTC ATCCTAAAAGTTCAAGAGAGCCTTCCAAACTTCCAAAATTTC TCTCAATTCAGTGAGGAGGAAAATTCAGAACACAGCATTTGA ATGTTCTGCCCAGATTTGTCACACACACAAGGAATGAGTGAA AGAGGGCAACACCCTTTCCTCCTAACCCTGTGAACTCATCAC TATTGCATTGAAATGACACCAAAAGGTAAAAACCCTAGGCCT CACATCTCCCAAGAACACTGCAATAGGAGTTACTGCATACAC CAGTTTAAGTAACTCTAGCATAAATTGTATGTCAGATGAAAC AATGGCATTTTGGAGGCTTAAGAGAAAAAGAATAATCAAAT CCAGTTTTTAGGTACTAATGTGCTGAATCTTTAGCACATAGC AGCAAAATTGCTAGAATCTGGTGTTTCACTTTTTAAAATACC

		ACATTTGAACCTTTCAGCAATTCCAAAATCAACTCCCTCTGC GAAAGATAATAAGCTTAAACATTTTTTAAATTTAAAAATGTA ACACAAACAAACAGCTAAGCAAACAAGCTGCCCATAAAATC AACAGTCTGGGGAGCCCTGATCCTGAAGTATTTTACAACATC CTTCATGACTATTAAAGGCAACATAAACACCTCTTGTCAGCA AGGGAAACTACCCTTGGCATTTTTTTTTCTTTGTTCCCCAGGC TTTTAAACCATTTTGATAGAGATTTTTTTACATCACAGGCAGA AATATTTGAAATAGAGTCAGGTGGTAGTCTTTAAAAGAGTAA GAAAGTTGCTAAGTCAAGATAATCTTGGAATAAAGTCCTCTG ATTCCTGGGGATTCCTAGGGATGCCCCAGTCACTAGAAAACA GAGCTGTAAGTCCACTCTCCCAGCACTCAACGGAGCTCCGGA AACCAAGGAGCTAGCTACTGTTTCCCCACATTCAGCCAGAGA AAGGGCAGCACTCTAGCATGCAAACTGCTTTGACAATAGTAA CAATTAAAAAGTAAATTAAAAAGAATCATAATAGCTGATATT GATTAGGTACTTGCCCTGTGGCAAGAGCTATAGGGAATCACC TCATTTAATCTTCACATGAAGCTTGCAGAGTGAGTACCACAA TTATCACTATTGTATAGACAGGAAAACTCAGGCTGAGTATGG CTAAGTGTCTTGCCAACGTCTTGGGCTAACAAGCGGTCAAGC AGAATCCAAACCCGAGATAGATAGACCACAGTGTGCTAATC AAGCACTGCACTCTCTCCTGCATTTCTTAGTTGATATTTACCA TATACAATCTGTCACTTGTATGAGATGGCAGGGGGTTCTGTG CTATTTGTCCTTGTAGAGAATACCACAGGAAGAAAGTAAGCA GCCATGCAATATTTGCTGTTGACCTGAACTCCATTCCATCATT CCTGCAGGAAATTCGCATCCATTAAATGAGCATTTCCTGGTT TGCCACTTTGCTCAAACACTTTGCTTGGATCTGGAGAGGATA TAGAAGTGAAGGAAATATGCTACCTGCTCTCAAGGAACTTAT GTTTTAGTGGAGAGACAAACATGCAGAATTTACTCTACAGAA CATCAATGCTTGAGCAAATGTAGACCCAGAGAGGGCTCTTAC AGCACACAAGCCAGAACAGACTGATGGTGCTAACAATTAGG TTCAAGGTTTTTCTAAACAGTAGACTCTCCTGCATACAACTAT ACCGCATGCCAGGTAAATGACTGAGGGTTATTACATCCAATT ATAACACCACTGTGATGTAGGTGCTCTTACCCCACACTTTCA TTTTACAGAAGAGGAAATTGAGGACAGCACAATGTAGTGAT TATCAAAGGTCACACGACTACTGTGTGGGAGAGCTAGGATTT AAACCAGATGCATAAGATGAGGTCCTCCAAGAAACAGAAGA TGAGAAGGTGTTAAATGAGCAGGGGTTTTATTAGGGGGAATT AATGTGTGAACAGAAATAGGGGAGGATAGGCAAAGCCATCA GATTGCAAGGCAAGCCTAACCCCAAGGGAAGGAGAGAGAGA GAGTAGATTGGTTGGAAACATTTTTGGTGGGTCTATGGTCTA AGGAAAGTTCAGCAAAGTCATCATGGAGTTTTTGAGCCAAA GTTGGGCAATACAGTTGCCCAACAAATTTCTGTGTTTCTCAG AAATAGGTCTGCCTCAATGTCCCCACCATACTTGGTCACTGG CTCTTGGGAGGGGCCTGCCCTGTTCCAATCCACTAGAGCCAA AGAAGAGCCGTTGTACTGGCAGGGGGTGGGGGAATTCCTAC AACCACATAAAAAGTGGGGTGAGGTTTCCAGAAAAAAACGT GATGCTGGGCTAACCAAAACTGTGTCCAGTAAGTACATATCC CTCACTCTGTTAAAGAAGCAGCCACATAAACAAGGAGTACA CGTTTCTCAAAATGTGCACCTTGTTCTTTGGTTTTGAAGTCAC ATCCCAAAGTGCTGAGTAGATCGCATGACCCTCGCTTTGCCT GGCTGCCAGAGAGGAAAGGCTGATCCAACTCTCCTGGAATTT GAACTTGTGATTCCCTGAAGTAAAGAGATATCAAAGTTGATA

		CTGAGACATCTAAATCATCCTCCACCATTTCACATGTCCCCA GGCCAAGCCAGCAAAATTGCTATAGCACATCCCTTTCAACAG GTAAAGGGCTGATATCTGAGCCCTCTTTCCAATCATCCACTG CTCTTTTCTTCTCATTTTGCCCTTTTTGGGAGCAGGTCAATGC TGAGTTAGTACTTTATGCTGTACAATAAGCTGCTGATATTCC ATGCTGGACAGAATTTTCCCAGTATTTTTTATAGAGTGCCAG GCTTTTCCTAGACTTCATGTCATACAATACTTAACTTGTTTGG AGTGGGTGGAGATGGAAACATAGTCTATTGAAAACATCACT GCTTCCTCCCTGAAGTTTAAAGAGCCTATTTTTATCCTTTTAG ATTCTATCTCTCAGGCAAAATCTCATAAAGATAAGTGGGGAG GAAAAAAAGGGGGTTATAATACCTAGGGAGTTTGCTTTTGCT AATTGAATACTGTGCTCCTAGACTTCTATAAATACCATTACA AATGGGTCCCAGCTTGTGGTAATACTCACCCTCCTCATTGAG TCTTCTGTCCCATGGCACAGCCTTTCCCTCCAAACTAGCATCT ACCCCCATCTGGAAGCATGGGCAGCTCATGATATTATCAACT ATTGCTATTGGAAAGTGATTTGGACTTGAAAGCACTAGATAT TTTTTACCTCTTGGGGAGGCAGTTTAGCAGAGTGGTTAACTG GTGAGCTCCAGAATCAGAAGGAATAGGTCCAAATTCCAACC ACTATTACATCTCCATCATAAGAAATTAGGCAAGTTGTTTAT CCTAAGTTTCAGATTCCTTAAAGATAAAACAGTCAAGACAGT AGTACTTATCCCTGAGAGAAGTATAGGAAACAAGAAAATAT ATGCAATTTACATACATACTACAATCCCCAGCACATGACAAA TGTTCAAGTAATGGGAACTGTTATTATTTTAGCCCTTTGTCTA TCAGTTTGTTCCTCTGTGACCTCAAGCACATTACTAAATGTTA GCGAGCTTCAGCTTGTACGTGGGACTGACAGGAATAACACC GCATCACCTCATGTGGTGATTGTAAGGATTCAGTGATATTAT TTTGTAAACTGTAAAGCCTTTGCAAATGTTAAGCAAGATTAT TATTATTGCCGTTGTTATTAGTCCTCAGTGATCTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTGGAGACAGAGTTTTACT CTGTCGCCAAGGCTGGAATGCAGTGGCACAATCTCAGCTCAC TGCAACCTCCGCCTCCTGGGTTCAAGCAATTTTCCTGCCTCAG CCTCCTGAGTAGCTGAAACTACAGGCACACGCCACCACACCA GGCTAATTTTTTGTATTTTTAGTAGAGACGGGGTTTCACCATG TTGGCCAGGCTGGTCTCCAGCTCCTGACCTCAAGTGATCTGC CCACCTCGGCCTCCCAAAGTGCTGGGATTACAGGTGTGAGCC ACCACACCTGGCACAGTAATCTTAATTGAAAAGTCTGTGGAT AGCTTTCCAAAGGAAAGCTTGGAGCTTGGATAAGAACCAAG AGATAATGGGAGAAGGTGAATGGCCTCTTCAGGGCCTTTTCT AGCACCCTAAATATGCGTGTCTGTCCATAATGGGTAATCATA TATATCACAAATCAAACCCTCCACAAACTTATTTCCTAATGT GTTTGTTAACCTTTCCTTCTAAAGGGTAAACTTCTTTAACCAA CCCCAGTGAGCTGGAGGATCAATGTTTTCTTAATAGTCTTAC CTTCGTTGGTGTCAATAGGAAACAGTATTTACTCACTACTGTT TTCCTTTTAAAAATCTGTCTAGTTGCATACTAGAAACAGTTTC AGCTGGTTTGTTTGTATTGGACAAGCTGCTGAAGTGAAAAGT TTTTGCTTGACTGAATGTGAGACAGTTTCATAACTCTTCAAG AAGTGCACCAAAGGTGGGTGCCAGCTCTGATGACGGCTGCTT CTAACATGCCTCCACTTGCCGCCCATTGTCAAGGGTGGCTGG CGTAATTAAGTTAAGACAATGAGCAAAGCAACAGATGCAAC TGAGACCTAGTCCCTGAGTGCTTTTGTTTTGTCACTGTCATTG TCTGCAACAAAGAAGTCACATGTGACAGCCTGGGAAGAGAG

		CCAAATGCAAACCAGACGATATCCCAGCTGGTTTGAATGGCC TCCACCGTGCACGTGTGTGCATGGGAATCATGCTACTTGGTA CAGCATCTGCTTCACTCAAGTGAGTTTCAGCCCATGGCTTTG CTGTGATGCTGAGACAGACCCAGAAGAAACAGACCAGGGAA TCCCTCCGCTCAGACTTTACACTTTATACCTTGTGCTTTGAGA GAAAAGAAAAAGAATCTCTCTATTGGAGACAAAAAATAGGA TGTATGTGGTTGGTCAATCTAACCTCAATTCTTTTTGCTATAG CCCCCCGCTAATTTAAAGAGTGAAGCATAGATGGTATCTTAA TGTTTTCTTGTAGAAATTTGGGATTAATTTGGCTTGAGAGGA AGAATGGAGATTAAACGCTTTATGAGGCTTTCTTTTAATTTGT TCCCATTTCATTCCTGAATATTTTCTTAGTTTGGGCATTGCAG ATGTTTAAAGAACTTCTTATTTTGAGCTGGTATGCCTCTTAAA CAGAAAAACAAAAGGTAAAATTCAAATTAGTGTGTTTCTCCG CCTGTTAATTAATTTGGTTAGTAGTTAGGCAGAGAGATGGCA TCCTTAATAATATCTATTTTGCGGGTTTGATCAGCTACAGACC ATCAACAGTGTTGATTGAGAATTGAACAAAAACATTTCAAGG AGTTTGGGAACATTAGGGATGCTATTCTGTGGCCCCATGTGT CCTTCTCTCATTTTTCTAGAGAACTCCTATAAGAAAGCAGAA CACGGCCAGGCATGATGGCTCATGCCTGTAATCCCAGCACTT CAGGAGGCTGAGGCAGGCAGATCACCTGAGGTCAGGAGTTC AAGACCAGCCTGGCCAACATGGTGAAACCCTATCTCTATTAA AAATACAAAAAATTAGCTGGGCATGATGGCGCGTGCCTGTA ATCCCAGCTACTTGGGAGGCTGAGGCAGGAGAATCACTTGA ACTGGGAGGCAAAGGTTGCAGTGAGCCTAGATCACACCACT GCACTCCAGCCTGGGTGACAGAGTGAGACTCCAACTCAAAA AAAAGAAAGAAAGAAAGAAAGAAAGCAGAACCCAATGGAA GATTAAGAACACACATTTAGCTTACGCCTGTAATACCAGCAC TTTGGGAGGCCAAGGCGGGTGGATCACAAGGTCAGAAGTTC GAGACCAACCTGGCCAATATGGTGAAACCCCATCTCTACTAA AAAGTACAAAAATTAGCCATGCATGGTGGCAGGCGTCTGTA ATCCCAGCTACTACAGAGGCTGAGGCAGGAGAATCACTTGA ACCCGGGAGGCAGAGGTTGCAGTGAGCTGAGAACGCGCCAC TGCACTCCAGCCTGGGTGACAGAGCGAGACTCCATCTCAAAA AAAAAAAACAACAAAAAAAAAACAAAACACAAGTTTACTGGG AACTTAGCAGTAGATGCTTTGCACCACAACAAATGTATCTTA AGTGGTCTTTTGTGATATTTGAGGGAAAGTGCCAGAATTTAA AACAAATGGCATTTCAAGTTATTCTATACAAATGCCCAGTTT CTTTCTACCATCTTTTTTTCCTTTTTGCAGTGGTCACTGAGCTA TTTTAGTGAATGTTTTTACACAATGATGCCATCTTCCTTCTAC TCAGTCAGTACAAGATGTTGACCATCGACTCATAAAACACTA GCTACCTTTCATGAAGGACTTGGTGATAACTCTCATGTTCCA AGTAGAACCGGAAAACATGTGTAAGAAAACCTGCCGATCCC TATGGGCCTTGGCCAATAGGTATTATTCCCAAGGGGTGGCAG TTTATCTTTTTCCCCAGCCTTCATATTAAAACCTCTCACCTTCT CCAGGTCTCAGGTCTGTGTAATCTCAAATGTGCTTTAGCTCCT CACAATATTGTAACTGTGTGGGTGTTCATTACCTTAGCCAGA AGACAGTTTACAGATTCCAGGTCTCATGGAGAGAACTTTTGT TTTTGGTTATGAACCTCACTGTATACCAATAATTATCCATTAC ATCCTTCTGTAGAGGGCTCTCTGGCTAGAGATAAAACCAAAA AAAGAAGTACCTCAGGTTTATGCATATAAATGCCAGTTCCTC CTTGATTTTATTTCAAAACTCCTGTCTACATACTTTGCAATTT

		AAATACATTCAAGGATAAAGTAATAACTGTAGGAAAAGTAT TATAATATAATGACTTAGTTCTGCACATCACAAGGGGGTCCC TCATACTCATTCATTCATTTCACTCATTTTACAGATATTTATT GAGCACCTGCAATAACCTGCACACTGCTCTAGACACTGGGAC TATAACAGTAAACAGACAGATACATCTCTGGTCTCACAGGGC TTCTATTCTAAGCAAAACTCAATATCCAGGCCGGGTGCAGTG GCTCATGCCTGGAATGCCAGCACTTTGGGAGACCAAGGCCA GGCAGATCACCTGAGCCCACTAGTTGAAGACCAGCCTGGGC AATATAGCAAAACCCCGTCTCTACAAAAAAAAAAAAAAAAA AAAAAAAAAAATTGTCAAGGCATGGTGGCATGCGCCTGTGG TCCCAGCTACTTAGGAGGCTGAGGCAGGAGGATTGTGTAAG CCTGGGAGGCAGAGGTTGCAGTGACCTGAGATGGCACCACC ACACTCCAGCCTGGGCAACAGAGTGAGACCCTGTCCAAAAA AAAAAAACCCTCACTATCCTTAAGATAACATCATTGCTTGTT GATGAGTGAATGTTAACACCAAATTAGGAACCCAGGACTTTT AGTCTTGGCATGGTTACTTTCCAATAAAGATGACAATACTAA GAAGAGAAAAATGATTTAATAATGATAATAGTGGCTAATAC TTATGTAGTGCTTACCATGTGCCAGGTCTATTGTAAGTACTTT TATATATATTAATTATTTAATCTTTGATCCTATAAGGTAGATA TTATTGTTACCCTAGTTTATAGATGAAGAAACGGAAACACAA GAGATTGCCACTCATACAAGTTTACACAGCCAGAAAATAGA AAAGCTACGAGTTGAGCTCAGCCCAGTATGTCTATGATTTTA CAGACTCAAAATTAATTATAAGATTTCCTAATCTTCGATTTCT GAAACTCTGCCTTGCTCTAGAGGAAAACAAGAAAAACAATG AAAAATAAATGTCTCTTTTTTACAAAAATTAAAACAGAACAA ACTGCAATAAAACAACAGAGGATGAATCCAGAATGTGATTG ATTTTTTTTCTTACTAGGAAAGGATCTAGAGGCCAGAAGGCT GGATTTTTCAGGATCTCCTTTCAATCAATGAATCTGTGATAG AAGCAGATGAATCAAATCTCATCTTTGTGTGATTATAAAGCT GTCTGTGGTATTCACGCCACCAGGGGTACATAGAAGATGCCT GAGTGAGGTTTGGCAAAAGTACTAAGGGCCTGTCCACCTATA CATGCCCTTCTCAGGAAAACCAAGGTTCAAGCTCTCTATTAG CTCAACTGGTAAGGCGTAAGACATGGAAGGTTGAGGCCCAA TGTTAGAAATAGATGGATACATAAAACTTCATCAAGTTAATG TCACTTTTTCTCCTTTATTTATAG
SEQ ID NO. 35	IGF-1 (exon 6-2)	GAAGTACATTTGAAGAACGCAAGTAGAGGGAGTGCAGGAAA CAAGAACTACAGGATGTAG
SEQ ID NO: 36	SDF-1α	ATGAACGCCAAGGTCGTGGTCGTGCTGGTCCTCGTGCT GACCGCGCTCTGCCTCAGCGACGGGAAGCCCGTCAGCC TGAGCTACAGATGCCCATGCCGATTCTTCGAAAGCCAT GTTGCCAGAGCCAACGTCAAGCATCTCAAAATTCTCAA CACTCCAAACTGTGCCCTTCAGATTGTAGCCCGGCTGAA GAACAACAACAGACAAGTGTGCATTGACCCGAAGCTAA AGTGGATTCAGGAGTACCTGGAGAAAGCTTTAAACAAG TAA

WHAT IS CLAIMED IS:

1. A lyophilized pharmaceutical composition comprising plasmid DNA, wherein the lyophilized pharmaceutical composition is obtained by lyophilizing a liquid composition that comprises, prior to lyophilization:

a. DNA of a first plasmid, wherein the first plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α;
b. potassium phosphate buffer with pH in the range from 7.0 to 9.0;
c. mannitol at a concentration in the range from 0% to 3% (w/v);
d. sucrose at a concentration greater than 0.5% and less than 1.1% (w/v); and
e. NaCl at a concentration in the range from 0.1 % to 0.9% (w/v).

2. The lyophilized pharmaceutical composition of claim 1, wherein the first plasmid is VM202.

3. The lyophilized pharmaceutical composition of claim 2, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

4. The lyophilized pharmaceutical composition of claim 1, wherein the first plasmid is pTx-HGF-X7.

5. The lyophilized pharmaceutical composition of claim 4, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

6. The lyophilized pharmaceutical composition of claim 1, wherein the first plasmid is pTx-IGF-1Ec.

7. The lyophilized pharmaceutical composition of claim 6, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

8. The lyophilized pharmaceutical composition of claim 1, wherein the first plasmid is pTx-IGF-1Ea.

9. The lyophilized pharmaceutical composition of claim 8, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

10. The lyophilized pharmaceutical composition of claim 1, wherein the first plasmid is pTx-IGF-1X6.

11. The lyophilized pharmaceutical composition of claim 10, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X10, and pCK-SDF-1α.

12. The lyophilized pharmaceutical composition of claim 1, wherein the first plasmid is pTx-IGF-1X10.

13. The lyophilized pharmaceutical composition of claim 12, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, and pCK-SDF-1α.

14. The lyophilized pharmaceutical composition of claim 1, wherein the first plasmid is pCK-SDF-1α.

15. The lyophilized pharmaceutical composition of claim 14, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, and pTx-IGF-1X10.

16. The lyophilized pharmaceutical composition of any one of claims 1-15, wherein at least 90% of the plasmid DNA in the liquid composition is supercoiled.

17. The lyophilized pharmaceutical composition of any one of claims 16, wherein at least 95% of the plasmid DNA in the liquid composition is supercoiled.

18. The lyophilized pharmaceutical composition of claim 17, wherein at least 97% of the plasmid DNA in the liquid composition is supercoiled.

19. The lyophilized pharmaceutical composition of claim 18, wherein at least 98% of the plasmid DNA in the liquid composition is supercoiled.

20. The lyophilized pharmaceutical composition of any of the preceding claims, wherein at least 90% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition.

21. The lyophilized pharmaceutical composition of claim 20, wherein at least 95% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition.

22. The lyophilized pharmaceutical composition of claim 21, wherein at least 97% of the plasmid remains DNA supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition.

23. The lyophilized pharmaceutical composition of claim 22, wherein at least 98% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition.

24. The lyophilized pharmaceutical composition of any of the preceding claims, wherein at least 90% of the plasmid DNA remains supercoiled after storage at 25°C for 3 to 7 days following reconstitution of the lyophilized pharmaceutical composition.

25. The lyophilized pharmaceutical composition of claim 24, wherein at least 95% of the plasmid DNA remains supercoiled after storage at 25°C for 3 to 7 days following reconstitution of the lyophilized pharmaceutical composition.

26. The lyophilized pharmaceutical composition of any of the preceding claims, wherein at least 80% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition, wherein the lyophilized pharmaceutical composition had been stored at 40°C for 10 weeks before reconstitution.

27. The lyophilized pharmaceutical composition of any of the preceding claims, wherein the lyophilized pharmaceutical composition comprises supercoiled DNA in an amount of at least 90% of a total amount of supercoiled DNA in the liquid composition.

28. The lyophilized pharmaceutical composition of claim 27, wherein the lyophilized pharmaceutical composition comprises supercoiled DNA in an amount of at least 95% of a total amount of supercoiled DNA in the liquid composition.

29. The lyophilized pharmaceutical composition of any of the preceding claims, wherein the liquid composition comprises the first plasmid at a concentration in the range from 0.1 to 1 mg/ml.

30. The lyophilized pharmaceutical composition of claim 29, wherein the liquid composition comprises the first plasmid at a concentration in the range from 0.25 to 0.75 mg/ml.

31. The lyophilized pharmaceutical composition of claim 30, wherein the liquid composition comprises the first plasmid at a concentration in the range from 0.4 to 0.6 mg/ml.

32. The lyophilized pharmaceutical composition of any of the preceding claims, wherein the liquid composition comprises the first plasmid at a concentration of 0.5 mg/ml.

33. The lyophilized pharmaceutical composition of any of the preceding claims, wherein the liquid composition comprises potassium phosphate at a concentration in the range from 5 mM to 15 mM.

34. The lyophilized pharmaceutical composition of claim 33, wherein the liquid composition comprises potassium phosphate at a concentration in the range from 7.5 mM to 12.5 mM.

35. The lyophilized pharmaceutical composition of claim 34, wherein the liquid composition comprises potassium phosphate at a concentration in the range from 9 mM to 11 mM.

36. The lyophilized pharmaceutical composition of any of the preceding claims, wherein the liquid composition comprises potassium phosphate at a concentration of 10 mM.

37. The lyophilized pharmaceutical composition of any of the preceding claims, wherein the liquid composition comprises potassium phosphate buffer with pH in the range from 7.0 to 8.0.

38. The lyophilized pharmaceutical composition of claim 37, wherein the liquid composition comprises potassium phosphate buffer of pH 8.0.

39. The lyophilized pharmaceutical composition of any of the preceding claims, wherein the liquid composition comprises mannitol at a concentration in the range from 2% to 3% (w/v).

40. The lyophilized pharmaceutical composition of claim 39, wherein the liquid composition comprises mannitol at a concentration of 2 % (w/v).

41. The lyophilized pharmaceutical composition of any of the preceding claims, wherein the liquid composition comprises sucrose at a concentration in the range from 0.75% to 1.1% (w/v).

42. The lyophilized pharmaceutical composition of claim 41, wherein the liquid composition comprises sucrose at a concentration in the range from 0.9% to 1.0% (w/v).

43. The lyophilized pharmaceutical composition of claim 42, wherein the liquid composition comprises sucrose at a concentration of 1.0 % (w/v).

44. The lyophilized pharmaceutical composition of any of the preceding claims, wherein the liquid composition comprises NaCl at a concentration in the range from 0.1 % to 0.75% (w/v).

45. The lyophilized pharmaceutical composition of claim 44, wherein the liquid composition comprises NaCl at a concentration in the range from 0.1 % to 0.6 % (w/v).

46. The lyophilized pharmaceutical composition of claim 45, wherein the liquid composition comprises NaCl at a concentration in the range from 0.4% to 0.5% (w/v).

47. The lyophilized pharmaceutical composition of claim 46, wherein the liquid composition comprises NaCl at a concentration of 0.45 % (w/v).

48. A lyophilized pharmaceutical composition comprising plasmid DNA, wherein the pharmaceutical composition is obtained by lyophilizing a liquid composition that comprises, prior to lyophilization:

a. DNA of a first plasmid at a concentration of 0.5 mg/ml, wherein the first plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α;
b. 10mM potassium phosphate buffer of pH 8.0;
c. mannitol at a concentration of 2 % (w/v);
d. sucrose at a concentration of 1.0 % (w/v); and
e. NaCl at a concentration of 0.45 % (w/v),

wherein at least 95% of the plasmid DNA in the liquid composition is supercoiled, and at least 90% of the plasmid DNA remains supercoiled after storage at 25°C for 7 days following reconstitution of the lyophilized pharmaceutical composition.

49. The lyophilized pharmaceutical composition of claim 48, wherein the first plasmid is VM202.

50. The lyophilized pharmaceutical composition of claim 49, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

51. The lyophilized pharmaceutical composition of claim 48, wherein the first plasmid is pTx-HGF-X7.

52. The lyophilized pharmaceutical composition of claim 51, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

53. The lyophilized pharmaceutical composition of claim 48, wherein the first plasmid is pTx-IGF-1Ec.

54. The lyophilized pharmaceutical composition of claim 53, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

55. The lyophilized pharmaceutical composition of claim 48, wherein the first plasmid is pTx-IGF-1Ea.

56. The lyophilized pharmaceutical composition of claim 55, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

57. The lyophilized pharmaceutical composition of claim 48, wherein the first plasmid is pTx-IGF-1X6.

58. The lyophilized pharmaceutical composition of claim 57, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X10, and pCK-SDF-1α.

59. The lyophilized pharmaceutical composition of claim 48, wherein the first plasmid is pTx-IGF-1X10.

60. The lyophilized pharmaceutical composition of claim 59, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, and pCK-SDF-1α.

61. The lyophilized pharmaceutical composition of claim 48, wherein the first plasmid is pCK-SDF-1α.

62. The lyophilized pharmaceutical composition of claim 61, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, and pTx-IGF-1X10.

63. A reconstituted composition produced by dissolving the lyophilized pharmaceutical composition of any of the preceding claims in water.

64. The reconstituted composition of claim 63, wherein light absorbance of the reconstituted composition at 450nm is less than 0.002.

65. The reconstituted composition of claim 64, wherein the light absorbance of the reconstituted composition at 450nm is 0.001 or less than 0.001.

66. The reconstituted composition of any of claims 64-65, wherein the light absorbance is measured on the day of reconstitution.

67. The reconstituted composition of claims 64-65, wherein the light absorbance is measured after storage of the lyophilized pharmaceutical composition for 10 weeks.

68. A lyophilized pharmaceutical composition comprising plasmid DNA in a unit dose, wherein the pharmaceutical composition is obtained by lyophilizing a liquid composition that comprises, prior to lyophilization:

a. DNA of a first plasmid at a concentration of 0.5 mg/ml, wherein the first plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α;
b. 10mM potassium phosphate buffer of pH 8.0;
c. mannitol at a concentration of 2 % (w/v);
d. sucrose at a concentration of 1.0 % (w/v); and
e. NaCl at a concentration of 0.45 % (w/v),

wherein the lyophilized pharmaceutical composition is in a vial and the vial contains 2.5 mg of plasmid DNA in total.

69. The lyophilized pharmaceutical composition of claim 68, wherein the first plasmid is VM202.

70. The lyophilized pharmaceutical composition of claim 69, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

71. The lyophilized pharmaceutical composition of claim 68, wherein the first plasmid is pTx-HGF-X7.

72. The lyophilized pharmaceutical composition of claim 71, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

73. The lyophilized pharmaceutical composition of claim 68, wherein the first plasmid is pTx-IGF-1Ec.

74. The lyophilized pharmaceutical composition of claim 73, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

75. The lyophilized pharmaceutical composition of claim 68, wherein the first plasmid is pTx-IGF-1Ea.

76. The lyophilized pharmaceutical composition of claim 75, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α.

77. The lyophilized pharmaceutical composition of claim 68, wherein the first plasmid is pTx-IGF-1X6.

78. The lyophilized pharmaceutical composition of claim 77, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X10, and pCK-SDF-1α.

79. The lyophilized pharmaceutical composition of claim 68, wherein the first plasmid is pTx-IGF-1X10.

80. The lyophilized pharmaceutical composition of claim 79, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, and pCK-SDF-1α.

81. The lyophilized pharmaceutical composition of claim 68, wherein the first plasmid is pCK-SDF-1α.

82. The lyophilized pharmaceutical composition of claim 81, further comprising DNA of a second plasmid, wherein the second plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, and pTx-IGF-1X10.

83. A reconstituted composition produced by dissolving the lyophilized pharmaceutical composition of any one of claims 68-82 in water.

84. A method of making a lyophilized pharmaceutical composition comprising plasmid DNA, the method comprising:

providing a liquid composition comprising:

a. DNA of a first plasmid, wherein the first plasmid is selected from the group consisting of VM202, pTx-HGF-X7, pTx-IGF-1Ec, pTx-IGF-1Ea, pTx-IGF-1X6, pTx-IGF-1X10, and pCK-SDF-1α;
b. potassium phosphate buffer with pH in the range from 7.0 to 9.0;
c. mannitol at a concentration in the range from 0% to 3% (w/v);
d. sucrose at a concentration greater than 0.5% and less than 1.1% (w/v); and
e. NaCl at a concentration in the range from 0.1 % to 0.9% (w/v); and

lyophilizing the liquid composition, thereby generating the lyophilized pharmaceutical composition.

85. The method of claim 84, wherein the first plasmid is VM202.

86. The method of claim 84, wherein the first plasmid is pTx-HGF-X7.

87. The method of claim 84, wherein the first plasmid is pTx-IGF-1Ec or pTx-IGF-1Ea.

88. The method of claim 84, wherein the first plasmid is pTx-IGF-1X6 or pTx-IGF-1X10.

89. The method of claim 84, wherein the first plasmid is pCK-SDF-1α.

90. The method of any one of claims 84-89, wherein the step of lyophilizing comprises:

(i) loading the liquid composition;
(ii) freezing;
(iii) primary drying; and
(iv) secondary drying.

91. The method of claim 90, wherein the step of loading is performed at 5°C.

92. The method of any of claims 84-91, wherein the step of freezing is performed while raising temperatures in the range from -50 °C to -20 °C.

93. The method of claim 92, wherein the step of freezing comprises a step of annealing.

94. The method of any of claims 84-93, wherein the step of primary drying is performed at -20°C.

95. The method of any of claims 84-94, wherein the step of secondary drying is performed while raising temperatures in the range from -20 °C to 20 °C.

96. The method of any of claims 84-95, wherein at least 90% of the plasmid DNA in the liquid composition is supercoiled.

97. The method of claim 96, wherein at least 95% of the plasmid DNA in the liquid composition is supercoiled.

98. The method of claim 96, wherein at least 97% of the plasmid DNA in the liquid composition is supercoiled.

99. The method of claim 98, wherein at least 98% of the plasmid DNA in the liquid composition is supercoiled.

100. The method of any of claims 84-99, wherein at least 90% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition.

101. The method of claim 100, wherein at least 95% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition.

102. The method of claim 100, wherein at least 97% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition.

103. The method of claim 102, wherein at least 98% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition.

104. The method of any of claims 84-103, wherein at least 90% of the plasmid DNA remains supercoiled after storage at 25°C for 3 to 7 days following reconstitution of the lyophilized pharmaceutical composition.

105. The method of claim 104, wherein at least 95% of the plasmid DNA remains supercoiled after storage at 25°C for 3 to 7 days following reconstitution of the lyophilized pharmaceutical composition.

106. The method of claim105, wherein at least 80% of the plasmid DNA remains supercoiled 30 minutes after reconstitution of the lyophilized pharmaceutical composition, wherein the lyophilized pharmaceutical composition had been stored at 40°C for 10 weeks before reconstitution.

107. The method of any of claims 84-106, wherein the lyophilized pharmaceutical composition comprises supercoiled DNA in an amount of at least 90% of a total amount of supercoiled DNA in the liquid composition.

108. The method of claim 107, wherein the lyophilized pharmaceutical composition comprises supercoiled DNA in an amount of at least 95% of a total amount of supercoiled DNA in the liquid composition.

109. The method of any of claims 84-107, wherein the liquid composition comprises the first plasmid at a concentration in the range from 0.1 to 1 mg/ml.

110. The method of claim 109, wherein the liquid composition comprises the first plasmid at a concentration in the range from 0.25 to 0.75 mg/ml.

111. The method of claim 110, wherein the liquid composition comprises the first plasmid at a concentration in the range from 0.4 to 0.6 mg/ml.

112. The method of claim 111, wherein the liquid composition comprises the first plasmid at a concentration of 0.5 mg/ml.

113. The method of any of claims 84-112, wherein the liquid composition comprises potassium phosphate at a concentration in the range from 5 mM to 15 mM.

114. The method of claim 113, wherein the liquid composition comprises potassium phosphate at a concentration in the range from 7.5 mM to 12.5 mM.

115. The method of claim 114, wherein the liquid composition comprises potassium phosphate at a concentration in the range from 9 mM to 11 mM.

116. The method of any of claims 84-113, wherein the liquid composition comprises potassium phosphate at a concentration of 10 mM.

117. The method of any of claims 84-116, wherein the liquid composition comprises potassium phosphate buffer with pH in the range from 7.0 to 8.0.

118. The method of claim 117, wherein the liquid composition comprises potassium phosphate buffer of pH 8.0.

119. The method of any of claims 84-118, wherein the liquid composition comprises mannitol at a concentration in the range from 2% to 3% (w/v).

120. The method of claim 119, wherein the liquid composition comprises mannitol at a concentration of 2 % (w/v).

121. The method of any of claims 84-120, wherein the liquid composition comprises sucrose at a concentration greater than 0.75 % and less than 1.1 % (w/v).

122. The method of claim 121, wherein the liquid composition comprises sucrose at a concentration greater than 0.9 % and less than 1.1 % (w/v).

123. The method of claim 122, wherein the liquid composition comprises sucrose at a concentration of 1.0 % (w/v).

124. The method of any of claims 84-123, wherein the liquid composition comprises NaCl at a concentration in the range from 0.1 % to 0.75% (w/v).

125. The method of claim 124, wherein the liquid composition comprises NaCl at a concentration in the range from 0.1 % to 0.6 % (w/v).

126. The method of claim 125, wherein the liquid composition comprises NaCl at a concentration in the range from 0.4% to 0.5% (w/v).

127. The method of claim 126, wherein the liquid composition comprises NaCl at a concentration of 0.45 % (w/v).

128. A method of treating a disease, comprising the step of:

administering an effective amount of a reconstituted drug to a patient with the disease,

wherein the reconstituted drug is generated by reconstituting the lyophilized pharmaceutical composition of any of claims 1-49 or 68-69.

129. The method of claim 128, further comprising the step of:

dissolving the lyophilized pharmaceutical composition of any of claims 1-62 or 68-82 in water, thereby generating the reconstituted drug.

130. The method of any of claims 128-129, wherein light absorbance of the reconstituted drug at 450 nm (A450) is less than 0.003.

131. The method of claim 130, wherein the light absorbance of the reconstituted drug at 450 nm (A450) is less than 0.002.

132. The method of claim 131, wherein the light absorbance of the reconstituted drug at 450 nm (A450) is 0.001 or less than 0.001.

133. The method of any of claims 128-132, wherein the disease is selected from the group consisting of neuropathy, ischemic disease, muscle atrophy, vascular disease, and a heart disease.

134. The method of claim 133, wherein the disease is selected from ischemic limb disease, diabetic peripheral neuropathy (DPN), amyotrophic lateral sclerosis (ALS), peripheral vascular disease, and coronary artery disease (CAD).

135. The method of any of claims 128-134, wherein the step of administering comprises an intramuscular injection of the reconstituted drug.