ENGINEERED CELLS FOR RECOMBINANT VIRUS PRODUCTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application Nos. 63/414,890, filed on October 10, 2022, and 63/478,742, filed on January 6, 2023, the disclosures of each of which is incorporated by reference herein in its entirety for all purposes.

BACKGROUND

[0002] Viral vectors are commonly used to deliver therapeutic genes to humans. Production of viral vectors is a complex process. For example, several different issues can arise with design and manufacturing, including but not limited to scalability, quality, and potency of the recombinant viruses. Production of clinical grade gene therapies remains a major hurdle in advancing cures for a number of otherwise unpreventable, incurable and/or untreatable diseases, disorders, and conditions. Improved processes are needed to achieve reproducible, consistent, scalable manufacturing solutions and provide commercially viable viral products that can be safely and reliably delivered to patients.

SUMMARY OF THE DISCLOSURE

[0003] The present disclosure provides, among other things, technologies for production of virus-producing cells. As is known to those of skill in the art, even as gene therapy technologies have rapidly improved, there are still challenges present related to engineering and manufacturing cells that reliably, consistently, and accurately achieve viral production at qualities and quantities suitable for, *e.g.*, administration to subjects in need of one or more gene therapies. Technologies provided herein address certain unmet needs in production of virus-producing cells and overcome certain challenges to improve engineering and manufacturing of virus-producing cells. These engineered cells and methods of making and using them solve production challenges and quality (*e.g.*, safety) concerns that are critical to advancing gene therapies.

[0002] The present disclosure also provides a virus-producing cell, the virus-producing cell having an engineered genome comprising a first nucleic acid comprising a first enhancer sequence and a Rep gene or functional fragment thereof; a second nucleic acid comprising a second enhancer sequence, a first Kozak sequence, and a Cap gene or functional

fragment thereof, wherein the first Kozak sequence is an engineered Kozak sequence; and a third nucleic acid comprising a gene-of-interest (GOI).

[0002a] In an aspect, the present disclosure provides a virus-producing cell, the virus-producing cell having an engineered genome comprising: a) a first nucleic acid comprising a first enhancer sequence and a Rep gene or functional fragment thereof; b) a second nucleic acid comprising a second enhancer sequence, a first Kozak sequence, and a Cap gene or functional fragment thereof, wherein the first Kozak sequence is an engineered Kozak sequence; and c) a third nucleic acid comprising a gene-of-interest (GOI), wherein the engineered Kozak sequence of the second nucleic acid comprises a sequence with at least 80% identity to that of any of SEQ ID NOs: 11-191 or any functional fragment or functional derivative thereof.

[0003] The present disclosure also provides method for generating an rAAV vector, comprising: a first nucleic acid comprising a first enhancer sequence and a Rep gene or functional fragment thereof; a second nucleic acid comprising a second enhancer sequence, a first Kozak sequence, and a Cap gene or functional fragment thereof, wherein the first Kozak sequence is an engineered Kozak sequence; and a third nucleic acid comprising a gene-of-interest (GOI).

[0003a] In another aspect, the present disclosure provides a method for generating an rAAV vector, comprising: a) a first nucleic acid comprising a first enhancer sequence and a Rep gene or functional fragment thereof; b) a second nucleic acid comprising a second enhancer sequence, a first Kozak sequence, and a Cap gene or functional fragment thereof, wherein the first Kozak sequence is an engineered Kozak sequence; and c) a third nucleic acid comprising a gene-of-interest (GOI), wherein the engineered Kozak sequence of the second nucleic acid comprises a sequence with at least 80% identity to that of any of SEQ ID NOs: 11-191 or any functional fragment or functional derivative thereof.

[0004] In some examples, the first enhancer sequence has at least 80% identity to the nucleic acid sequence of any of SEQ ID NOs: 1-10 or any functional fragment or functional derivative thereof.

[0005] In some examples, the second enhancer sequence has at least 80% identity to the nucleic acid sequence of any of SEQ ID NOs: 1-10 or any functional fragment or functional derivative thereof.

[0006] In some examples, the first and the second enhancer sequences are the same.

[0007] In some examples, the first nucleic acid further comprises a second Kozak sequence, wherein the second Kozak sequence is an engineered Kozak sequence.

[0008] In some examples, the third nucleic acid comprises a third Kozak sequence, optionally wherein the third Kozak sequence is an engineered Kozak sequence.

[0009] In some examples, any of the first, second, and/or third Kozak sequences facilitates translation in an insect cell and/or a mammalian cell.

[0010] In some examples, the first and/or second Kozak sequence preferentially facilitate translation in an insect cell.

[0011] In some examples, the third Kozak sequence preferentially facilitates translation in a mammalian cell.

[0012] In some examples, the cell is clonal.

[0013] In some examples, the engineered Kozak sequence of the second nucleic acid comprises a sequence with at least 80% identity to that of any of SEQ ID NOs: 11-191 or any functional fragment or functional derivative thereof.

[0014] In some examples, the Kozak sequence of the first nucleic acid, which is optionally engineered, and the engineered Kozak sequence of the second nucleic acid comprise the same nucleic acid sequence.

[0015] In some examples, the cell is rhabdovirus-free.

[0016] In some examples, the GOI is flanked by a first by a first inverted terminal repeat (ITR) sequence and a second ITR sequence.

[0017] In some examples, each of the first ITR sequence and the second ITR sequences has a different nucleic acid sequence.

[0018] In some examples, the first ITR and the second ITR are derived from a viral genome and wherein the first ITR is flanked on its 5′ end and the second ITR flanked on its 3′ end by a total of about 500 nucleotides or less of viral genome.

[0019] In some examples, the Cap gene comprises a sequence having at least 80% sequence identity to the nucleic acid sequence of any of SEQ ID NOs: 192-203 or any functional fragment or functional derivative thereof.

[0020] In some examples, the Cap gene encodes an amino acid sequence having at least about 80% sequence identity to the amino acid sequence of any of SEQ ID NOs: 204-209 or any functional fragment or functional derivative thereof.

[0021] In some examples, the first nucleic acid further comprises a promoter, and, optionally, further comprises one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or any combination thereof; the second nucleic acid further comprises a promoter, and, optionally, further comprises one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or any combination thereof; and/or the third nucleic acid further comprises a promoter, and, optionally, further comprises one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or any combination thereof.

[0022] In some examples, the promoter is selected from: (i) a constitutive promoter; (ii) an inducible promoter, (iii) a mini promoter; and (iv) a functional derivative of any of (i), (ii), or (iii). In some examples, the promoter is selected from: CMV, CBA, EF1a, CAG, PGK, TRE, U6, UAS, T7, Sp6, lac, araBad, trp, Ptac, p5, p10, p19, p40, Synapsin, CaMKII, GRK1, polH, EM7, OpIE1, and any functional fragment and/or functional derivative thereof. In some examples, the Rep gene is derived from an adeno-associated virus (AAV). In some examples, the AAV is selected from: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV6TM, AAV7, AAV7TM, AAV8, AAV8TM, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-

HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional fragment and/or functional derivative thereof.

[0023] In some examples, the Cap gene is derived from an AAV. In some examples, the AAV is selected from: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV6TM, AAV7, AAV7TM, AAV8, AAV8TM, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional derivative thereof.

[0024] In some examples, the first nucleic acid further comprises a first antibiotic resistance gene; the second nucleic acid further comprises a second antibiotic resistance gene; and/or the third nucleic acid further comprises a third antibiotic resistance gene. In some examples, each of the first and the second nucleic acids comprise an antibiotic resistance gene or functional fragment or derivative thereof. In some examples, each of the first and the second antibiotic resistance genes comprise the same antibiotic resistance gene. In some examples, the third nucleic acid does not comprise an antibiotic resistance gene. In some examples, each of the first, second, and/or third antibiotic resistance genes is selected from: a gene encoding an aminoglycoside, a beta-lactam, a macrolide, a tetracycline, or any functional fragment and/or functional derivative thereof. In some examples, each of the first, second, and/or third antibiotic resistance genes is selected from: a gene encoding kanamycin, spectinomycin, streptomycin, ampicillin, carbenicillin, blasticidin, bleomycin, erythromycin, polymyxin B, puromycin, tetracycline, chloramphenicol, neomycin, zeocin, or any functional fragment and/or functional derivative thereof.

[0025] In some examples, the first nucleic acid further comprises a first origin of replication; the second nucleic acid further comprises a second origin of replication; and/or the third nucleic acid further comprises a third origin of replication. In some examples, the first, second, and/or third origin of replication is selected from: pMB1, pBR322, ColE1, R6K, p15A, pSC101, ColE2, F1, pUC, and any functional fragment and/or functional derivative thereof.

[0026] In some examples, the first nucleic acid comprises a nucleic acid sequence having at least 80% sequence identity to that of SEQ ID NO: 210 or to any functional fragment or

functional derivative thereof. In some examples, the second nucleic acid comprises a nucleic acid sequence having at least 80% sequence identity to that of SEQ ID NOs: 211 – 216 or to any functional fragment or functional derivative thereof.

[0027] In some examples, the cell is a eukaryotic cell, a mammalian cell, an immortalized cell, an insect cell, a yeast cell, a plant cell, a fungal cell, or a prokaryotic cell. In some examples, the cell is an A549 cell, a HEK-293 cell, a HEK-293T cell, a BHK cell, a CHO cell, a HeLa cell, an MRC5 cell, an Sf9 cell, an Sf2 cell, an Sf21 cell, a High Five™ cell, a Cos-1 cell, a Cos-7 cell, a Vero cell, a BSC 1 cell, a BSC 40 cell, a BMT 10 cell, a WI38 cell, a Saos cell, a C2C12 cell, an L cell, an HT1080 cell, a HepG2 cell, a Huh7 cell, a K562 cell, a primary cell, or any derivative thereof. In some examples, the cell is an Sf9 cell.

[0028] In some examples, the first nucleic acid, the second nucleic acid, and the third nucleic acid remain stably integrated in the genome after at least 5 passages. In some examples, the cell is able to grow to at least about 1 x 10^7 cells/mL after at least about 24 hours.

[0029] In some examples, the cell is infected with a virus. In some examples, the virus is selected from: an alphavirus, a parvovirus, an adenovirus, an AAV, a baculovirus, a Dengue virus, a lentivirus, a herpesvirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, and a retrovirus. In some examples, the virus is a wild-type virus. In some examples, the virus does not comprise a nucleic acid necessary for AAV packaging.

[0030] In some examples, the virus has been engineered to: remove one or more endogenous genes or functions; and prevent one or more endogenous genes for production a functional gene product. In some examples, the infection induces expression of one or more of the first nucleic acid, the second nucleic acid, the third nucleic acid, or any combinations thereof. In some examples, the infection induces the cell to produce a recombinant virus. In some examples, the recombinant virus is selected from an alphavirus, a parvovirus, an adenovirus, an AAV, a baculovirus, a Dengue virus, a lentivirus, a herpesvirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, or a retrovirus. In some examples, the AAV is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV6TM, AAV7, AAV7TM, AAV8, AAV8TM, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-

HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional derivative thereof.

[0031] In some examples, the recombinant virus comprises a recombinant AAV (rAAV) vector. In some examples, the rAAV vector comprises less than 5%, 4%, 3%, 2%, or 1% contaminants from one or more non-AAV components and/or a GOI. In some examples, the contaminants are from an alphavirus, a parvovirus, a baculovirus, a Dengue virus, a lentivirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, a herpesvirus, or a retrovirus. In some examples, the contaminants are from a baculovirus. In some examples, the contaminants are not from a rhabdovirus.

[0032] In some examples, a plurality of cells infected in accordance with the present disclosure results in a plurality that produces $1X10^9$ vg/L to $1x10^{15}$ vg/L. In some examples, a plurality of cells infected in accordance with the present disclosure results in a plurality that produces $1x10^{15}$ vg/L or greater.

[0033] In another aspect, the present disclosure provides a recombinant virus produced by methods provided herein. In another aspect, the present disclosure provides a composition comprising a plurality of viral particles produced by infecting a cell as provided herein with a virus. In some examples, the cell is an insect cell. In some examples, the insect cell is an Sf9 cell. In some examples, the virus is a baculovirus. In some examples, the plurality of viral particles comprises $1X10^9$ vg/L to $1x10^{15}$ vg/L. In some examples, the plurality of viral particles comprises $1x10^{15}$ vg/L or greater.

[0034] In some examples, the present disclosure provides a method of infecting cells in a subject in need thereof, the method comprising administering to the subject a composition as provided in accordance with the present disclosure.

[0035] In some examples, the present disclosure provides a method of treating a subject having a disease, disorder, or condition associated with a dysfunctional gene of interest (GOI), the method comprising administering a composition as provided herein to produce a functional gene product of the GOI and treat the disease.

[0035a] In another aspect, the present disclosure provides use of the composition as provided herein in the manufacture of a medicament for treating a subject having a disease, disorder, or condition associated with a dysfunctional gene of interest (GOI).

[0035b] In another aspect, the present disclosure provides the composition as provided herein for use in therapy.

[0036] In another aspect, the present disclosure provides a system of generating a recombinant virus comprising: a virus-producing cell as provided herein; and a virus for infecting the virus-producing cell, which when infected with the virus induces the production of the recombinant virus.

[0037] In some examples, the cell is infected with a virus. In some examples, the virus is selected from: an alphavirus, a parvovirus, an adenovirus, an AAV, a baculovirus, a

Dengue virus, a lentivirus, a herpesvirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, and a retrovirus. In some examples, the virus is a wild-type virus. In some examples, the virus does not comprise a nucleic acid necessary for AAV packaging. In some examples, the virus has been engineered to remove one or more endogenous genes or functions; and prevent one or more endogenous genes for production a functional gene product.

[0038] In some examples, the infection induces expression of one or more of the first nucleic acid, the second nucleic acid, the third nucleic acid, or any combinations thereof. In some examples, the infection induces the cell to produce a recombinant virus. In some examples, the recombinant virus is selected from an alphavirus, a parvovirus, an adenovirus, an AAV, a baculovirus, a Dengue virus, a lentivirus, a herpesvirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, or a retrovirus. In some examples, the AAV is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV6TM, AAV7, AAV7TM, AAV8, AAV8TM, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional derivative thereof.

[0039] In some examples, the recombinant virus comprises a recombinant AAV (rAAV) vector. In some examples, the rAAV vector comprises less than 5%, 4%, 3%, 2%, or 1% contaminants from one or more non-AAV components and/or a GOI. In some examples, the contaminants are from an alphavirus, a parvovirus, a baculovirus, a Dengue virus, a lentivirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, a herpesvirus, or a retrovirus. In some examples, the contaminants are from a baculovirus. In some examples, the contaminants are not from a rhabdovirus.

[0040] In some examples, the system produces 1×10^9 vg/L to 1×10^{15} vg/L of recombinant virus. In some examples, the system produces $1x10^{15}$ vg/L or greater of recombinant virus.

[0041] In some examples, the present disclosure provides virus-producing cells, the virus-producing cell having an engineered genome comprising: (a) a first nucleic acid comprising a first enhancer sequence and a Rep gene or functional fragment thereof; (b) a second nucleic

acid comprising a second enhancer sequence and a Cap gene or functional fragment thereof; and (c) a third nucleic acid comprising a gene-of-interest (GOI). In some examples, the first enhancer sequence has at least 80% (*e.g.*, 85%, 90%, 95%, 99%, or 100%) identity to the nucleic acid sequence of any of SEQ ID NOs: 1-10. In some examples, the second enhancer sequence has at least 80% (*e.g.*, 85%, 90%, 95%, 99%, or 100%) identity to the nucleic acid sequence of SEQ ID NOs: 1-10. In some examples, the first and the second enhancer sequences are the same. In some examples, the first nucleic acid further comprises a first engineered Kozak sequence. In some examples, the first engineered Kozak sequence has at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the nucleic acid sequence of any one of SEQ ID NOs: 11- 191.

[0042] In some examples, the second nucleic acid further comprises a second engineered Kozak sequence. In some examples, the second engineered Kozak sequence has at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the nucleic acid sequence of any one of SEQ ID NOs: 11- 191. In some examples, the first and the second engineered Kozak sequences are the same.

[0043] In some examples, the GOI is flanked by a first inverted terminal repeat (ITR) sequence and a second ITR sequence. In some examples, the first ITR sequence and the second ITR sequences are the same. In some examples, the Cap gene comprises a sequence having at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 192-203. In some examples, the Cap gene encodes an amino acid sequence having at least about 80% (*e.g.*, 85%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 204-209.

[0044] In some examples, the first nucleic acid further comprises a promoter (and, optionally further comprises one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or any combinations thereof). In some examples, the promoter is selected from a constitutive promoter, an inducible promoter, a mini promoter, or any functional fragment and/or functional derivative thereof. In some examples, the promoter is selected from: CMV, CBA, EF1a, CAG, PGK, TRE, U6, UAS, T7, Sp6, lac, araBad, trp, Ptac, p5, p10, p19, p40, Synapsin, CaMKII, GRK1, polH, EM7, OpIE1, and any functional fragment and/or functional derivative thereof.

[0045] In some examples, the second nucleic acid further comprises a promoter (and, optionally further comprises one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or combinations thereof). In some examples, the promoter is

selected from a constitutive promoter, an inducible promoter, a mini promoter, or a functional fragment and/or functional derivative thereof. In some examples, the promoter is selected from: CMV, CBA, EF1a, CAG, PGK, TRE, U6, UAS, T7, Sp6, lac, araBad, trp, Ptac, p5, p10, p19, p40, Synapsin, CaMKII, GRK1, polH, EM7, OpIE1, and any functional fragment and/or functional derivative thereof. In some examples, the third nucleic acid further comprises a promoter (and, optionally further comprises one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or any combinations thereof). In some examples, the promoter is selected from a constitutive promoter, an inducible promoter, a mini promoter, or any functional fragment and/or functional derivative thereof. In some examples, the promoter is selected from: CMV, CBA, EF1a, CAG, PGK, TRE, U6, UAS, T7, Sp6, lac, araBad, trp, Ptac, p5, p10, p19, p40, Synapsin, CaMKII, GRK1, polH, EM7, OpIE1, and any functional fragment and/or functional derivative thereof. In some examples, the Rep gene is derived from a parvovirus. In some examples, the parvovirus is a adeno-associated virus (AAV). In some examples, the AAV is selected from: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional derivative thereof. In some examples, the Cap gene is derived from a parvovirus. In some examples, the parvovirus is an AAV. In some examples, the AAV is selected from: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional derivative thereof.

[0046] In some examples, the first nucleic acid further comprises an antibiotic resistance gene. In some examples, the antibiotic resistance gene encodes an aminoglycoside, a

beta-lactam, a macrolide, a tetracycline, or any derivative thereof. In some examples, the antibiotic resistance gene encodes kanamycin, spectinomycin, streptomycin, ampicillin, carbenicillin, blasticidin, bleomycin, erythromycin, puromycin, polymyxin B, tetracycline, chloramphenicol, neomycin, zeocin, or any functional fragment and/or functional derivative thereof.

[0047] In some examples, the second nucleic acid further comprises an antibiotic resistance gene. In some examples, the antibiotic resistance gene encodes an aminoglycoside, a beta-lactam, a macrolide, a tetracycline, or any functional fragment and/or functional derivative thereof. In some examples, the antibiotic resistance gene encodes kanamycin, spectinomycin, streptomycin, ampicillin, carbenicillin, blasticidin, bleomycin, erythromycin, polymyxin B, tetracycline, chloramphenicol, neomycin, zeocin, or any functional fragment and/or functional derivative thereof.

[0048] In some examples, the third nucleic acid further comprises an antibiotic resistance gene. In some examples, the antibiotic resistance gene encodes an aminoglycoside, a beta-lactam, a macrolide, a tetracycline, or any functional fragment and/or functional derivative thereof. In some examples, the antibiotic resistance gene encodes kanamycin, spectinomycin, streptomycin, ampicillin, carbenicillin, blasticidin, bleomycin, erythromycin, polymyxin B, tetracycline, chloramphenicol, neomycin, zeocin, or any functional fragment and/or functional derivative thereof.

[0049] In some examples, the first nucleic acid further comprises an origin of replication. In some examples, the origin of replication is selected from: pMB1, pBR322, ColE1, R6K, p15A, pSC101, ColE2, F1, pUC, and any functional fragment and/or functional derivative thereof. In some examples, the first nucleic acid comprises a sequence having at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the nucleic acid sequence of SEQ ID NO: 210.

[0050] In some examples, the second nucleic acid further comprises an origin of replication. In some examples, the origin of replication is selected from: pMB1, pBR322, ColE1, R6K, p15A, pSC101, ColE2, F1, pUC, and any functional fragment and/or functional derivative thereof. In some examples, the second nucleic acid comprises a sequence having at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 211-216.

[0051] In some examples, the third nucleic acid further comprises an origin of replication. In some examples, the origin of replication is selected from: pMB1, pBR322,

ColE1, R6K, p15A, pSC101, ColE2, F1, pUC, and any functional fragment and/or functional derivative thereof.

[0052] In some examples, the cell is a eukaryotic cell, a mammalian cell, an immortalized cell, an insect cell, a yeast cell, a plant cell, a fungal cell, or a prokaryotic cell. In some examples, the cell is an A549 cell, an HEK-293 cell, an HEK-293T cell, a BHK cell, a CHO cell, a HeLa cell, an MRC5 cell, an Sf9 cell, an Sf2 cell, an Sf21 cell, a High Five™ cell, a Cos-1 cell, a Cos-7 cell, a Vero cell, a BSC 1 cell, a BSC 40 cell, a BMT 10 cell, a WI38 cell, a Saos cell, a C2C12 cell, an L cell, an HT1080 cell, a HepG2 cell, a Huh7 cell, a K562 cell, a primary cell, or any derivative thereof.

[0053] In some examples, the cell is an Sf9 cell.

[0054] In some examples, the first nucleic acid, the second nucleic acid, and the third nucleic acid remain stably integrated in the genome after at least 5 (*e.g.*, 10, 20, 40) passages. In some examples, the cell is able to grow to at least about 1 x 10^7 cells/mL after at least about 24 hours. In some examples, the cell is infected with a virus. In some examples, the virus is selected from: an alphavirus, a parvovirus, an adenovirus, an AAV, a baculovirus, a Dengue virus, a lentivirus, a herpesvirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, and a retrovirus. In some examples, the virus is a wild-type virus. In some examples, the infection induces expression of one or more of the first nucleic acid, the second nucleic acid, the third nucleic acid, or any combinations thereof. In some examples, the infection induces the cell to produce a recombinant virus. In some examples, the recombinant virus is selected from an alphavirus, a parvovirus, an adenovirus, an AAV, a baculovirus, a Dengue virus, a lentivirus, a herpesvirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, or a retrovirus. In some examples, the AAV is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional derivative thereof. In some examples, the recombinant virus comprises a recombinant adeno-associated viral (rAAV) vector. In some examples, the rAAV vector comprises less than 5%, 4%, 3%, 2%, or 1% contaminants from non-AAV components and a GOI. In some examples, the

contaminants are from an alphavirus, a parvovirus, a baculovirus, a Dengue virus, a lentivirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, a herpesvirus, or a retrovirus. In some examples, the contaminants are from a baculovirus.

[0055] Provided herein are methods for generating an rAAV vector, comprising: (a) providing a virus-producing cell having an engineered genome, wherein the cell comprises: (i) a first nucleic acid comprising a first enhancer sequence and a Rep gene or functional fragment thereof; (ii) a second nucleic acid comprising a second enhancer sequence and a Cap gene or functional fragment thereof; and iii. a third nucleic acid comprising a GOI; (b) contacting the cell of step (a) with a virus; and (c) after step (b), culturing the cells to produce a recombinant virus comprising the rAAV vector. In some examples, the first enhancer sequence has at least 80% (*e.g.*, 85%, 90%, 95%, 99%, or 100%) identity to the nucleic acid sequence of any of SEQ ID NOs: 1-10. In some examples, the second enhancer sequence has at least 80% (*e.g.*, 85%, 90%, 95%, 99%, or 100%) identity to the nucleic acid sequence of any of SEQ ID NOs: 1-10. In some examples, the first and the second enhancer sequences are the same. In some examples, the first nucleic acid further comprises a first engineered Kozak sequence. In some examples, the first engineered Kozak sequence has at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the nucleic acid sequence of any one of SEQ ID NOs: 11-191. In some examples, the second nucleic acid further comprises a second engineered Kozak sequence. In some examples, the second engineered Kozak sequence has at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the nucleic acid sequence of any one of SEQ ID NOs: 11-191. In some examples, the first and the second engineered Kozak sequences are the same. In some examples, the GOI is flanked by a first ITR sequence and a second ITR sequence. In some examples, the first ITR sequence and the second ITR sequences are the same. In some examples, the Cap gene comprises a sequence having at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 192203. In some examples, the Cap gene encodes an amino acid sequence having at least about 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 204-209. In some examples, the first nucleic acid further comprises a promoter (and, optionally further comprises one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or any combinations thereof). In some examples, the promoter is selected from a constitutive promoter, an inducible promoter, a mini promoter, or any functional fragment and/or functional derivative thereof. In some examples, the promoter is selected from:

CMV, CBA, EF1a, CAG, PGK, TRE, U6, UAS, T7, Sp6, lac, araBad, trp, Ptac, p5, p10, p19, p40, Synapsin, CaMKII, GRK1, polH, EM7, OpIE1, and any functional fragment and/or functional derivative thereof. In some examples, the second nucleic acid further comprises a promoter (and, optionally further comprises one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or any combinations thereof). In some examples, the promoter is selected from a constitutive promoter, an inducible promoter, a mini promoter, or any functional fragment and/or functional derivative thereof. In some examples, the promoter is selected from: CMV, CBA, EF1a, CAG, PGK, TRE, U6, UAS, T7, Sp6, lac, araBad, trp, Ptac, p5, p10, p19, p40, Synapsin, CaMKII, GRK1, polH, EM7, OpIE1, and any functional fragment and/or functional derivative thereof. In some examples, the third nucleic acid further comprises a promoter (and, optionally further comprises one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or any combinations thereof). In some examples, the promoter is selected from a constitutive promoter, an inducible promoter, a mini promoter, or any functional fragment and/or functional derivative thereof. In some examples, the promoter is selected from: CMV, CBA, EF1a, CAG, PGK, TRE, U6, UAS, T7, Sp6, lac, araBad, trp, Ptac, p5, p10, p19, p40, Synapsin, CaMKII, GRK1, polH, EM7, OpIE1, and any functional fragment and/or functional derivative thereof. In some examples, the Rep gene is derived from a parvovirus. In some examples, the parvovirus is an AAV (*e.g.,* a wild-type AAV; *e.g.,* an rAAV. In some examples, the AAV is selected from: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional derivative thereof. In some examples, the Cap gene is derived from a parvovirus. In some examples, the parvovirus is an AAV. In some examples, the AAV is selected from: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-

HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional derivative thereof. In some examples, the first nucleic acid further comprises an antibiotic resistance gene. In some examples, the antibiotic resistance gene encodes an aminoglycoside, a beta-lactam, a macrolide, a tetracycline, or any functional fragment and/or functional derivative thereof. In some examples, the antibiotic resistance gene encodes kanamycin, spectinomycin, streptomycin, ampicillin, carbenicillin, blasticidin, bleomycin, erythromycin, polymyxin B, tetracycline, chloramphenicol, neomycin, zeocin, or any functional fragment and/or functional derivative thereof. In some examples, the second nucleic acid further comprises an antibiotic resistance gene. In some examples, the antibiotic resistance gene encodes an aminoglycoside, a beta-lactam, a macrolide, a tetracycline, or any functional fragment and/or functional derivative thereof. In some examples, the antibiotic resistance gene encodes kanamycin, spectinomycin, streptomycin, ampicillin, carbenicillin, blasticidin, bleomycin, erythromycin, polymyxin B, tetracycline, chloramphenicol, neomycin, zeocin, or any functional fragment and/or functional derivative thereof. In some examples, the third nucleic acid further comprises an antibiotic resistance gene. In some examples, the antibiotic resistance gene encodes an aminoglycoside, a beta-lactam, a macrolide, a tetracycline, or any functional fragment and/or functional derivative thereof. In some examples, the antibiotic resistance gene encodes kanamycin, spectinomycin, streptomycin, ampicillin, carbenicillin, blasticidin, bleomycin, erythromycin, polymyxin B, tetracycline, chloramphenicol, neomycin, zeocin, or any functional fragment and/or functional derivative thereof. In some examples, the first nucleic acid further comprises an origin of replication. In some examples, the origin of replication is selected from: pMB1, pBR322, ColE1, R6K, p15A, pSC101, ColE2, F1, pUC, and any functional fragment and/or functional derivative thereof. In some examples, the second nucleic acid further comprises an origin of replication. In some examples, the origin of replication is selected from: pMB1, pBR322, ColE1, R6K, p15A, pSC101, ColE2, F1, pUC, and any functional fragment and/or functional derivative thereof. In some examples, the third nucleic acid further comprises an origin of replication. In some examples, the origin of replication is selected from: pMB1, pBR322, ColE1, R6K, p15A, pSC101, ColE2, F1, pUC, and any functional fragment and/or functional derivative thereof. In some examples, the first nucleic acid comprises a sequence having at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the nucleic acid sequence of SEQ ID NO: 210. In

some examples, the second nucleic acid comprises a sequence having at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 211-216. In some examples, the cell is a eukaryotic cell, a mammalian cell, an immortalized cell, an insect cell, a yeast cell, a plant cell, a fungal cell, or a prokaryotic cell. In some examples, the cell is an A549 cell, an HEK-293 cell, an HEK-293T cell, a BHK cell, a CHO cell, a HeLa cell, an MRC5 cell, an Sf9 cell, an Sf2 cell, an Sf21 cell, a High Five™ cell, a Cos-1 cell, a Cos-7 cell, a Vero cell, a BSC 1 cell, a BSC 40 cell, a BMT 10 cell, a WI38 cell, a Saos cell, a C2C12 cell, an L cell, an HT1080 cell, a HepG2 cell, a Huh7 cell, a K562 cell, a primary cell, or a derivative thereof. In some examples, the cell is an Sf9 cell. In some examples, the first nucleic acid, the second nucleic acid, and the third nucleic acid remain stably integrated in the genome after at least 5 (*e.g.*, 10, 20, 40) passages. In some examples, the cell is able to grow to at least about 1×10^7 cells/mL after at least about 24 hours. In some examples, the cell is infected with a virus. In some examples, the virus is selected from: an alphavirus, a parvovirus, an adenovirus, an AAV, a baculovirus, a Dengue virus, a lentivirus, a herpesvirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, and a retrovirus. In some examples, the virus is a wild-type virus. In some examples, the infection induces expression of one or more of the first nucleic acid, the second nucleic acid, the third nucleic acid, or any combinations thereof. In some examples, the infection induces the cell to produce a recombinant virus. In some examples, the recombinant virus is selected from an alphavirus, a parvovirus, an adenovirus, an AAV, a baculovirus, a Dengue virus, a lentivirus, a herpesvirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, or a retrovirus. In some examples, the AAV is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional derivative thereof. In some examples, the recombinant virus comprises a rAAV vector. In some examples, the rAAV vector comprises less than 5%, 4%, 3%, 2%, or 1% contaminants from non-AAV components and a GOI. In some examples, the contaminants are from an alphavirus, a parvovirus, a baculovirus, a Dengue virus, a

lentivirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, a herpesvirus, or a retrovirus. In some examples, the contaminants are from a baculovirus.

[0056] In some examples, the present disclosure provides recombinant viruses produced by methods of the present disclosure.

[0057] In some examples, the present disclosure provides systems of generating a recombinant virus comprising: (a) a virus-producing cell, the virus-producing cell having an engineered genome comprising: (i) a first nucleic acid comprising a first enhancer sequence and a Rep gene or functional fragment thereof; (ii) a second nucleic acid comprising a second enhancer sequence and a Cap gene or functional fragment thereof; and (iii) a third nucleic acid comprising a GOI; and (b) a virus for infecting the virus-producing cell, which when infected with the virus induces the production of the recombinant virus.

[0058] In some examples, the first enhancer sequence has at least 80% (*e.g.*, 85%, 90%, 95%, 99%, or 100%) identity to the nucleic acid sequence of any of SEQ ID NOs: 1-10. In some examples, the second enhancer sequence has at least 80% (*e.g.*, 85%, 90%, 95%, 99%, or 100%) identity to the nucleic acid sequence of SEQ ID NOs: 1-10. In some examples, the first and the second enhancer sequences are the same.

[0059] In some examples, the first nucleic acid further comprises a first engineered Kozak sequence. In some examples, the first engineered Kozak sequence has at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the nucleic acid sequence of any one of SEQ ID NOs: 2- 42. In some examples, the second nucleic acid further comprises a second engineered Kozak sequence. In some examples, the second engineered Kozak sequence has at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the nucleic acid sequence of any one of SEQ ID NOs: 2- 42. In some examples, the first and the second engineered Kozak sequences are the same.

[0060] In some examples, the GOI is flanked by a first ITR sequence and a second ITR sequence. In some examples, the first ITR sequence and the second ITR sequences are the same. In some examples, the Cap gene comprises a sequence having at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 192-203. In some examples, the Cap gene encodes an amino acid sequence having at least about 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 204-209.

[0061] In some examples, the first nucleic acid further comprises a promoter (and, optionally further comprises one or more of an intron, a microRNA, a linker, a splicing

element, a polyA signal, or any combinations thereof). In some examples, the promoter is selected from a constitutive promoter, an inducible promoter, a mini promoter, or any functional fragment and/or functional derivative thereof. In some examples, the promoter is selected from: CMV, CBA, EF1a, CAG, PGK, TRE, U6, UAS, T7, Sp6, lac, araBad, trp, Ptac, p5, p10, p19, p40, Synapsin, CaMKII, GRK1, polH, EM7, OpIE1, and any functional fragment and/or functional derivative thereof. In some examples, the second nucleic acid further comprises a promoter (and, optionally further comprises one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or combinations thereof). In some examples, the promoter is selected from a constitutive promoter, an inducible promoter, a mini promoter, or any functional fragment and/or functional derivative thereof. In some examples, the promoter is selected from: CMV, CBA, EF1a, CAG, PGK, TRE, U6, UAS, T7, Sp6, lac, araBad, trp, Ptac, p5, p10, p19, p40, Synapsin, CaMKII, GRK1, polH, EM7, OpIE1, and any functional fragment and/or functional derivative thereof. In some examples, the third nucleic acid further comprises a promoter (and, optionally further comprises one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or any combinations thereof). In some examples, the promoter is selected from a constitutive promoter, an inducible promoter, a mini promoter, or any functional fragment and/or functional derivative thereof. In some examples, the promoter is selected from: CMV, CBA, EF1a, CAG, PGK, TRE, U6, UAS, T7, Sp6, lac, araBad, trp, Ptac, p5, p10, p19, p40, Synapsin, CaMKII, GRK1, polH, EM7, OpIE1, and any functional fragment and/or functional derivative thereof. In some examples, the Rep gene is derived from a parvovirus. In some examples, the parvovirus is an AAV. In some examples, the AAV is selected from: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional derivative thereof. In some examples, the Cap gene is derived from a parvovirus. In some examples, the parvovirus is an AAV. In some examples, the AAV is selected from: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-

rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional derivative thereof. In some examples, the first nucleic acid further comprises an antibiotic resistance gene. In some examples, the antibiotic resistance gene encodes an aminoglycoside, a beta-lactam, a macrolide, a tetracycline, or any functional fragment and/or functional derivative thereof. In some examples, the antibiotic resistance gene encodes kanamycin, spectinomycin, streptomycin, ampicillin, carbenicillin, blasticidin, bleomycin, erythromycin, polymyxin B, tetracycline, chloramphenicol, neomycin, zeocin, or any functional fragment and/or functional derivative thereof. In some examples, the second nucleic acid further comprises an antibiotic resistance gene. In some examples, the antibiotic resistance gene encodes an aminoglycoside, a beta-lactam, a macrolide, a tetracycline, or any derivative thereof. In some examples, the antibiotic resistance gene encodes kanamycin, spectinomycin, streptomycin, ampicillin, carbenicillin, bleomycin, erythromycin, polymyxin B, tetracycline, chloramphenicol, neomycin, zeocin, or any functional fragment and/or functional derivative thereof. In some examples, the third nucleic acid further comprises an antibiotic resistance gene. In some examples, the antibiotic resistance gene encodes an aminoglycoside, a beta-lactam, a macrolide, a tetracycline, or any functional fragment and/or functional derivative thereof. In some examples, the antibiotic resistance gene encodes kanamycin, spectinomycin, streptomycin, ampicillin, carbenicillin, blasticidin, bleomycin, erythromycin, puromycin, polymyxin B, tetracycline, chloramphenicol, neomycin, zeocin, or any functional fragment and/or functional derivative thereof.

[0062] In some examples, the first nucleic acid further comprises an origin of replication. In some examples, the origin of replication is selected from: pMB1, pBR322, ColE1, R6K, p15A, pSC101, ColE2, F1, pUC, and any functional fragment and/or functional derivative thereof. In some examples, the second nucleic acid further comprises an origin of replication. In some examples, the origin of replication is selected from: pMB1, pBR322, ColE1, R6K, p15A, pSC101, ColE2, F1, pUC, and any functional fragment and/or functional derivative thereof. In some examples, the third nucleic acid further comprises an origin of replication. In some examples, the origin of replication is selected from: pMB1, pBR322, ColE1, R6K, p15A, pSC101, ColE2, F1, pUC, and any functional fragment

and/or functional derivative thereof. In some examples, the first nucleic acid comprises a sequence having at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the nucleic acid sequence of SEQ ID NO: 210. In some examples, the second nucleic acid comprises a sequence having at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 211-216. In some examples, the cell is a eukaryotic cell, a mammalian cell, an immortalized cell, an insect cell, a yeast cell, a plant cell, a fungal cell, or a prokaryotic cell. In some examples, the cell is a A549 cell, a HEK-293 cell, a HEK-293T cell, a BHK cell, a CHO cell, a HeLa cell, an MRC5 cell, an Sf9, an Sf2 cell, an Sf21 cell, a High Five™ cell, a Cos-1 cell, a Cos-7 cell, a Vero cell, a BSC 1 cell, a BSC 40 cell, a BMT 10 cell, a WI38 cell, a Saos cell, a C2C12 cell, an L cell, an HT1080 cell, a HepG2 cell, a Huh7 cell, a K562 cell, a primary cell, or any derivative thereof. In some examples, the cell is an Sf9 cell.

[0063] In some examples, the first nucleic acid, the second nucleic acid, and the third nucleic acid remain stably integrated in the genome after at least 5 (*e.g.*, 10, 20, 40) passages.

[0064] In some examples, the cell is able to grow to at least about 1 x 10^7 cells/mL after at least about 24 hours. In some examples, the cell is infected with a virus. In some examples, the virus is selected from: an alphavirus, a parvovirus, an adenovirus, an AAV, a baculovirus, a Dengue virus, a lentivirus, a herpesvirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, and a retrovirus. In some examples, the virus is a wild-type virus. In some examples, the infection induces expression of one or more of the first nucleic acid, the second nucleic acid, the third nucleic acid, or combinations thereof. In some examples, the infection induces the cell to produce a recombinant virus. In some examples, the recombinant virus is selected from an alphavirus, a parvovirus, an adenovirus, an AAV, a baculovirus, a Dengue virus, a lentivirus, a herpesvirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, or a retrovirus. In some examples, the AAV is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-

NP22, AAV-NP66, AAV-HSC16, and any functional derivative thereof. In some examples, the recombinant virus comprises a rAAV vector.

[0065] In some examples, the rAAV vector comprises less than 5%, 4%, 3%, 2%, or 1% contaminants from non-AAV components and a GOI. In some examples, the contaminants are from an alphavirus, a parvovirus, a baculovirus, a Dengue virus, a lentivirus, a poxvirus, an anellovirus, a bocavirus, a vaccinia virus, a herpesvirus, or a retrovirus. In some examples, the contaminants are from a baculovirus.

BRIEF DESCRIPTION OF THE DRAWINGS

[0066] The disclosure is more completely understood with reference to the following drawings.

[0067] **FIG. 1A** shows exemplary nucleic acid construct designs for exemplary Rep and Cap proteins.

[0068] **FIG. 1B** shows a Western blot of capsid proteins in adeno-associated virus (AAV) serotype 1 (AAV1) and AAV5. **FIG. 1C** shows affinity purified capsid proteins from AAV7, AAV8, and AAV9.

[0069] **FIGS. 2A-2E** are schematic illustrations showing an exemplary method for producing recombinant virus beginning from seeding and selecting a clonal cell (**FIG. 2A** and **FIG. 2B**, respectively) grown into a monolayer and infected with an exemplary non-recombinant virus to produce exemplary recombinant viral particles comprising a gene-of-interest (GOI) (**FIG. 2C**) and ending by discarding the media comprising excess components (**FIG. 2D**) and harvesting the recombinant virus from the remaining cell monolayer, including a final separation of virus from cell debris (**FIG. 2E**). Abbreviations: BEV, baculovirus; GOI, gene-of-interest; PCR, polymerase chain reaction; WT, wild-type.

[0070] **FIG. 3** shows results of a clone screen to identify top producing clones as determined by yield (vg/mL).

[0071] **FIG. 4** is a graph showing quantified differences in scale times shown as number of passages (x-axis) and cells/mL (y-axis) in traditional and optimized methods.

[0072] **FIGS. 5A-5D** show measurements of amount of host-cell-protein and activating virus present over the course of production and pre- and post-purification, demonstrating that both host cell proteins and activating virus impurities were reduced over the course of purification. **FIG. 5A** and **FIG. 5B** are gels showing that impurities were reduced over the course of purification. **FIG. 5C** is a bar graph showing host cell protein (HCP) in ng/mL detected in fractions from various purification steps. **FIG. 5D** is a bar graph showing DNA

CLAIMS

1. A virus-producing cell, the virus-producing cell having an engineered genome comprising:
    a) a first nucleic acid comprising a first enhancer sequence and a Rep gene or functional fragment thereof;
    b) a second nucleic acid comprising a second enhancer sequence, a first Kozak sequence, and a Cap gene or functional fragment thereof, wherein the first Kozak sequence is an engineered Kozak sequence; and
    c) a third nucleic acid comprising a gene-of-interest (GOI),
    wherein the engineered Kozak sequence of the second nucleic acid comprises a sequence with at least 80% identity to that of any of SEQ ID NOs: 11-191 or any functional fragment or functional derivative thereof.

2. A method for generating an rAAV vector, comprising:
    a) a first nucleic acid comprising a first enhancer sequence and a Rep gene or functional fragment thereof;
    b) a second nucleic acid comprising a second enhancer sequence, a first Kozak sequence, and a Cap gene or functional fragment thereof, wherein the first Kozak sequence is an engineered Kozak sequence; and
    c) a third nucleic acid comprising a gene-of-interest (GOI),
    wherein the engineered Kozak sequence of the second nucleic acid comprises a sequence with at least 80% identity to that of any of SEQ ID NOs: 11-191 or any functional fragment or functional derivative thereof.

3. The cell of claim 1 or the method of claim 2, wherein:
    (a) the first enhancer sequence has a nucleotide sequence having at least 80% sequence identity to any of SEQ ID NOs: 1-10 or any functional fragment or functional derivative thereof; and/or
    (b) the second enhancer sequence has a nucleotide sequence having at least 80% identity to any of SEQ ID NOs: 1-10 or any functional fragment or functional derivative thereof.

4. The cell of claim 1 or 3 or the method of claim 2 or 3, wherein the first and the second enhancer sequences are the same nucleotide sequence.

5. The cell of any one of claims 1, 3 and 4 or the method of any one of claims 2-4, wherein the first nucleic acid further comprises a second Kozak sequence, wherein the second Kozak sequence is an engineered Kozak sequence.

6. The cell of any one of claims 1 and 3-5 or the method of any one of claims 2-5, wherein the third nucleic acid comprises a third Kozak sequence, optionally wherein the third Kozak sequence is an engineered Kozak sequence.

7. The cell of any one of claims 1 and 3-6 or the method of any one of claims 2-6, wherein any of the first, second, and/or third Kozak sequences facilitates translation in an insect cell and/or a mammalian cell.

8. The cell or method of claim 7, wherein the first and/or second Kozak sequence preferentially facilitate translation in an insect cell.

9. The cell or method of claim 7 or 8, wherein the third Kozak sequence preferentially facilitates translation in a mammalian cell.

10. The cell of any one of claims 1 and 3-9 or the method of any one of claims 2-9, wherein the first Kozak sequence and the second Kozak sequence are the same nucleotide sequence.

11. The cell of any one of claims 1 and 3-10, wherein the cell is rhabdovirus-free.

12. The cell of any one of claims 1 and 3-11 or the method of any one of claims 2-10, wherein the GOI is flanked by a first inverted terminal repeat (ITR) sequence and a second ITR sequence, optionally wherein each of the first ITR sequence and the second ITR sequence has a different nucleic acid sequence.

13. The cell or method of claim 12, wherein the first ITR sequence and the second ITR sequence are derived from a viral genome and wherein the first ITR sequence is flanked on its 5′ end and the second ITR sequence flanked on its 3′ end by a total of about 500 nucleotides or less of viral genome.

14. The cell of any one of claims 1 and 3-13 or the method of any one of claims 2-10 and 12-13, wherein the Cap gene comprises a nucleotide sequence having at least 80% sequence identity to any of SEQ ID NOs: 192-203 or any functional fragment or functional derivative thereof.

15. The cell of any one of claims 1 and 3-14 or the method of any one of claims 2-10 and 12-14, wherein the Cap gene encodes an amino acid sequence having at least about 80% sequence identity to any of SEQ ID NOs: 204-209 or any functional fragment or functional derivative thereof.

16. The cell of any one of claims 1 and 3-15 or the method of any one of claims 2-10 and 12-15, wherein:

a) the first nucleic acid further comprises a promoter, and one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or any combination thereof;

b) the second nucleic acid further comprises a promoter, and one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or any combination thereof; and/or

c) the third nucleic acid further comprises a promoter, and one or more of an intron, a microRNA, a linker, a splicing element, a polyA signal, or any combination thereof.

17. The cell or method of claim 16, wherein the promoter is selected from the group consisting of: (i) a constitutive promoter; (ii) an inducible promoter, (iii) a mini promoter; and (iv) a functional derivative of any of (i), (ii), or (iii).

18. The cell or method of claim 16 or 17, wherein the promoter is selected from the group consisting of: CMV, CBA, EF1a, CAG, PGK, TRE, U6, UAS, T7, Sp6, lac, araBad, trp, Ptac, p5, p10, p19, p40, Synapsin, CaMKII, GRK1, polH, EM7, OpIE1, and any functional fragment and/or functional derivative thereof.

19. The cell of any one of claims 1 and 3-18 or the method of any one of claims 2-10 and 12-18, wherein the Rep gene is derived from an adeno-associated virus (AAV), optionally wherein the AAV is selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV6TM, AAV7, AAV7TM, AAV8, AAV8TM, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional fragment and/or functional derivative thereof.

20. The cell of any one of claims 1 and 3-19 or the method of any one of claims 2-10 and 12-19, wherein the Cap gene is derived from an AAV, optionally wherein the AAV is selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV6TM, AAV7, AAV7TM, AAV8, AAV8TM, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, AAV16, AAV-rh8, AAV-rh10, AAV-rh20, AAV-rh39, AAV-rh74, AAV-rhM4-1, AAV-hu37, AAV-Anc80, AAV-Anc80L65, AAV-7m8, AAV-PHP.B, AAV-PHP.EB, AAV-2.5, AAV2tYF, AAV-3B, AAV-LK03, AAV-HSC1, AAV-HSC2, AAV-HSC3, AAV-HSC4, AAV-HSC5, AAV-HSC6, AAV-HSC7, AAV-HSC8, AAV-HSC9, AAV-HSC10, AAV-HSC11, AAV-HSC12, AAV-HSC13, AAV-HSC14, AAV-

HSC15, AAV-TT, AAV-DJ, AAV-DJ/8, AAV-Myo, AAV-NP40, AAV-NP59, AAV-NP22, AAV-NP66, AAV-HSC16, and any functional derivative thereof.

21. The cell of any one of claims 1 and 3-20 or the method of any one of claims 2-10 and 12-20, wherein:

a) the first nucleic acid further comprises a first origin of replication;

b) the second nucleic acid further comprises a second origin of replication; and/or

c) the third nucleic acid further comprises a third origin of replication,

optionally wherein the first, second, and/or third origin of replication is selected from: pMB1, pBR322, ColE1, R6K, p15A, pSC101, ColE2, F1, pUC, and any functional fragment and/or functional derivative thereof.

22. The cell of any one of claims 1 and 3-21 or the method of any one of claims 2-10 and 12-21, wherein the first nucleic acid comprises a nucleotide sequence having at least 80% sequence identity to that of SEQ ID NO: 210 or to any functional fragment or functional derivative thereof.

23. The cell of any one of claims 1 and 3-22 or the method of any one of claims 2-10 and 12-22, wherein the second nucleic acid comprises a nucleotide sequence having at least 80% sequence identity to that of SEQ ID NOs: 211 – 216 or to any functional fragment or functional derivative thereof.

24. The cell of any one of claims 1 and 3-23, wherein the cell is a eukaryotic cell, a mammalian cell, an immortalized cell, an insect cell, a yeast cell, a plant cell, a fungal cell, or a prokaryotic cell, optionally wherein the cell is an A549 cell, a HEK-293 cell, a HEK-293T cell, a BHK cell, a CHO cell, a HeLa cell, an MRC5 cell, an Sf9 cell, an Sf2 cell, an Sf21 cell, a High Five™ cell, a Cos-1 cell, a Cos-7 cell, a Vero cell, a BSC 1 cell, a BSC 40 cell, a BMT 10 cell, a WI38 cell, a Saos cell, a C2C12 cell, an L cell, an HT1080 cell, a HepG2 cell, a Huh7 cell, a K562 cell, a primary cell, or any derivative thereof.

25. The cell of any one of claims 1 and 3-24, wherein the cell is an Sf9 cell.

26. A recombinant virus produced by the method of any of claims 2-10 or 12-23.

27. A composition comprising a plurality of viral particles produced by infecting the cell of any one of claims 1 and 3-25 with a virus.

28. Use of the composition according to claim 27 in the manufacture of a medicament for treating a subject having a disease, disorder, or condition associated with a dysfunctional gene of interest (GOI).

29. The composition of claim 27 for use in therapy.

30. A system of generating a recombinant virus comprising:

a) a virus-producing cell of any one of claims 1 and 3-25; and

b) a virus for infecting the virus-producing cell, which when infected with the virus induces the production of the recombinant virus.