Factor IX deficiencies (e.g., hemophilia B). The above deficiencies and other problems associated with the treatment of Factor IX deficiencies (e.g., hemophilia B) are reduced or eliminated by the disclosed codon-altered nucleic acids encoding Factor IX proteins.

[0014] In one example, nucleic acid compositions (e.g., codon-altered polynucleotides) encoding Factor IX and Factor IX variants are described. The nucleic acid compositions include polynucleotides with high sequence identity to the CS02, CS03, CS04, CS05, and CS06 sequences encoding Factor IX, as described herein. The nucleic acid compositions described herein provide increased Factor IX expression relative to wild-type Factor IX coding sequences. The nucleic acid compositions also allow for increased production of AAV-based gene therapy virions. In some examples, the nucleic acid compositions described herein have decreased GC content and or include fewer CpG dinucleotides, as compared to wild-type sequences encoding Factor IX.

[0015] In some examples, a nucleic acid composition includes a polynucleotide encoding Factor IX that has a nucleotide sequence with at least 95% sequence identity (e.g., at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to a disclosed sequence selected from CS02-FL-NA (SEQ ID NO:5), CS02-MP-NA (SEQ ID NO:13), CS03-FL-NA (SEQ ID NO:6), CS03-MP-NA (SEQ ID NO:14), CS04-FL-NA (SEQ ID NO:7), CS04-MP-NA (SEQ ID NO:15), CS05-FL-NA (SEQ ID NO:8), CS05-MP-NA (SEQ ID NO:16), CS06-FL-NA (SEQ ID NO:9), and CS06-MP-NA (SEQ ID NO:17).

[0015a] In one aspect, the present disclosure refers to a nucleic acid composition, comprising a Factor IX polynucleotide encoding a Factor IX protein, said Factor IX polynucleotide comprising a nucleic acid sequence that is at least 99% identical to the nucleic acid sequence of CS06-MP-NA (SEQ ID NO:17), wherein the Factor IX polynucleotide encodes for leucine at nucleotide positions 1012-1014 relative to SEQ ID NO: 17, and wherein the Factor IX polynucleotide has no more than 3 CpG dinucleotides.

[0015b] In another example, the present disclosure refers to a nucleic acid composition, comprising a Factor IX polynucleotide encoding a Factor IX protein, said Factor IX polynucleotide comprising a nucleic acid sequence that is least 95% identical to the nucleic acid sequence of CS02-MP-NA (SEQ ID NO:13).

[0015c] In yet another example, the present disclosure refers to a nucleic acid composition, comprising a Factor IX polynucleotide encoding a Factor IX protein, said Factor IX polynucleotide comprising a nucleic acid sequence that is least 98% identical to the nucleic acid sequence of CS03-MP-NA (SEQ ID NO:14).

[0015d] In yet another example, the present disclosure refers to a nucleic acid composition, comprising a Factor IX polynucleotide encoding a Factor IX protein, said Factor IX polynucleotide comprising a nucleic acid sequence that is least 99% identical to the nucleic acid sequence of CS04-MP-NA (SEQ ID NO:15).

[0015e] In a further example, the present disclosure refers to a nucleic acid composition, comprising a Factor IX polynucleotide encoding a Factor IX protein, said Factor IX polynucleotide comprising a nucleic acid sequence that is least 98% identical to the nucleic acid sequence of CS05-MP-NA (SEQ ID NO:16).

[0016] In some examples, a nucleic acid composition includes a polynucleotide encoding Factor IX that has a nucleotide sequence with at least 95% sequence identity (e.g., at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to a disclosed sequence encoding a Factor IX light chain (e.g., CS02-LC-NA (SEQ ID NO:42), CS03-LC-NA (SEQ ID NO:44), CS04-LC-NA (SEQ ID NO:46), CS05-LC-NA (SEQ ID NO:48), or CS06-LC-NA (SEQ ID NO:50)) and a disclosed sequence encoding a Factor IX heavy chain (e.g., CS02-HC-NA (SEQ ID NO:41), CS03-HC-NA (SEQ ID NO:43), CS04-HC-NA (SEQ ID NO:45), CS05-HC-NA (SEQ ID NO:47), or CS06-HC-NA (SEQ ID NO:49)).

[0017] In some examples, a nucleic acid composition includes a polynucleotide that encodes a Factor IX polypeptide having a light chain, a heavy chain, and a polypeptide linker joining the C-terminus of the light chain to the N-terminus of the heavy chain (e.g., an activation

WHAT IS CLAIMED IS:

1. A nucleic acid composition, comprising a Factor IX polynucleotide encoding a Factor IX protein, said Factor IX polynucleotide comprising a nucleic acid sequence that is at least 99% identical to the nucleic acid sequence of CS06-MP-NA (SEQ ID NO:17) , wherein the Factor IX polynucleotide encodes for leucine at nucleotide positions 1012-1014 relative to SEQ ID NO: 17, and wherein the Factor IX polynucleotide has no more than 3 CpG dinucleotides.

2. The nucleic acid composition of claim 1, wherein the Factor IX polynucleotide comprises the nucleic acid sequence of CS06-MP-NA (SEQ ID NO:17).

3. The nucleic acid composition according to claim 1 or 2, wherein the Factor IX protein encoded by the Factor IX polynucleotide has from 1 to 10 amino acid substitutions as compared to FIXp-MP-AA (SEQ ID NO:12).

4. The nucleic acid composition according to claim 1or 2, wherein the Factor IX protein encoded by the Factor IX polynucleotide has the amino acid sequence of FIXp-MP-AA (SEQ ID NO:12).

5. The nucleic acid composition according to any one of claims 1-4, wherein the Factor IX polynucleotide further comprises a pre-pro-leader polynucleotide encoding a pre-pro-leader peptide, said pre-pro-leader peptide comprising the amino acid sequence of FIX-PPP-AA (SEQ ID NO:36).

6. The nucleic acid composition of claim 5, wherein the pre-pro-leader polynucleotide has the nucleic acid sequence of CS06-PPP-NA (SEQ ID NO:23).

7. The nucleic acid composition according to any one of claims 1-6, wherein the Factor IX polynucleotide has a nucleic acid sequence that is at least 99% identical to the nucleic acid sequence of CS06-FL-NA (SEQ ID NO:9).

8. The nucleic acid composition according to any one of claims 1-6, wherein the Factor IX polynucleotide has the nucleic acid sequence of CS06-FL-NA (SEQ ID NO:9).

9. The nucleic acid composition according to any one of claims 1-8, further comprising a liver-specific promoter element operably linked to the Factor IX polynucleotide.

10. The nucleic acid composition of claim 9, wherein the liver-specific promoter element comprises one copy of a promoter polynucleotide, said promoter polynucleotide comprising a nucleic acid sequence that is at least 95% identical to CRM8 (SEQ ID NO:39).

11. The nucleic acid composition of claim 9, wherein the liver-specific promoter element comprises three copies of a promoter polynucleotide, said promoter polynucleotide comprising a nucleic acid sequence that is at least 95% identical to CRM8 (SEQ ID NO:39).

12. The nucleic acid composition of claim 10 or 11, wherein said promoter polynucleotide comprises the nucleic acid sequence of CRM8 (SEQ ID NO:39).

13. The nucleic acid composition according to any one of claims 1-8, further comprising an intron operatively linked to the Factor IX polynucleotide.

14. The nucleic acid composition of claim 13, wherein the intron comprises an MVM intron polynucleotide comprising a nucleic acid sequence that is at least 95% identical to MVMI (SEQ ID NO:53).

15. The nucleic acid composition of claim 14, wherein said MVM intron polynucleotide comprises the nucleic acid sequence of MVMI (SEQ ID NO:53).

16. The nucleic acid composition according to any one of claims 13-15, wherein said intron is positioned between a promoter element and the translation initiation site of the nucleotide sequence encoding a Factor IX polypeptide.

17. The nucleic acid composition of claim 1, comprising the nucleic acid sequence of CS06-CRM8.3-ssV (SEQ ID NO:40).

18. The nucleic acid composition according to any one of claims 1-17, wherein said composition is integrated into a mammalian gene therapy vector.

19. The nucleic acid composition of claim 18, wherein the mammalian gene therapy vector is an adeno-associated virus (AAV) vector.

20. The nucleic acid composition of claim 19, wherein said adeno-associated virus vector is a serotype 8 adeno-associated virus (AAV-8) vector.

21. The nucleic acid composition according to any one of claims 18-20, wherein the mammalian gene therapy vector comprises a single-stranded polynucleotide encoding the Factor IX protein.

22. A nucleic acid composition according to any one of claims 1-21 for use as a medicament for treating hemophilia B.

23. Use of a nucleic acid composition according to any one of claims 1-21 for the manufacture of a medicament for the treatment of hemophilia B.

24. An *in vitro* method for producing an adeno-associated virus (AAV) particle comprising introducing a nucleic acid composition according to any one of claims 1-21 into a mammalian host cell, wherein the nucleic acid composition is competent for replication in the mammalian host cell.