Claims

1. An isolated nucleic acid comprising a nucleotide sequence encoding a polypeptide with FVIII activity, wherein the nucleotide sequence comprises at least 95% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 71.

2. The isolated nucleic acid molecule of claim 1, wherein the nucleotide sequence comprises at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 71.

3. The isolated nucleic acid molecule of claim 1, wherein the nucleotide sequence comprises nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 71.

4. The isolated nucleic acid molecule of any one of claims 1 to 3, wherein the nucleotide sequence further comprises a nucleic acid sequence encoding a signal peptide, wherein the nucleic acid sequence encoding the signal peptide has at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to:
   (i) nucleotides 1 to 57 of SEQ ID NO: 1;
   (ii) nucleotides 1 to 57 of SEQ ID NO: 2;
   (iii) nucleotides 1 to 57 of SEQ ID NO: 3;
   (iv) nucleotides 1 to 57 of SEQ ID NO: 4;
   (v) nucleotides 1 to 57 of SEQ ID NO: 5;
   (vi) nucleotides 1 to 57 of SEQ ID NO: 6;
   (vii) nucleotides 1 to 57 of SEQ ID NO: 70;
   (viii) nucleotides 1 to 57 of SEQ ID NO: 71; or
   (ix) nucleotides 1 to 57 of SEQ ID NO: 68.

5. The isolated nucleic acid molecule of any one of claims 1 to 4, wherein the nucleic acid molecule comprises one or more properties selected from the group consisting of:
   (a) the human codon adaptation index the nucleic acid molecule or a portion thereof is increased relative to SEQ ID NO: 16;
   (b) the frequency of optimal codons of the nucleotide sequence or a portion thereof is increased relative to SEQ ID NO:16;
   (c) the nucleotide sequence or a portion thereof contains a higher percentage of G/C nucleotides compared to the percentage of G/C nucleotides in SEQ ID NO: 16;
   (d) the relative synonymous codon usage of the nucleotide sequence or a portion thereof is increased relative to SEQ ID NO: 16;
   (e) the effective number of codons of the nucleotide sequence or a portion thereof is reduced relative SEQ ID NO: 16;

(f) the nucleotide sequence contains fewer MARS/ARS sequences (SEQ ID NOs: 21 and 22) relative to SEQ ID NO: 16;

(g) the nucleotide sequence contains fewer destabilizing elements (SEQ ID NOs: 23 and 24) relative to SEQ ID NO: 16; and

(h) any combination thereof.

6. The isolated nucleic acid molecule of any one of claims 1 to 5 further comprising a heterologous nucleotide sequence encoding a heterologous amino acid sequence.

7. The isolated nucleic acid molecule of claim 6, wherein the heterologous amino acid sequence is an immunoglobulin constant region or a portion thereof, XTEN, transferrin, albumin, or a PAS sequence.

8. The isolated nucleic acid molecule of claim 7, wherein the nucleotide sequence comprises SEQ ID NO: 72.

9. The isolated nucleic acid molecule of claim 6 or 7, wherein the heterologous amino acid sequence is linked to the N-terminus or the C-terminus of the amino acid sequence encoded by the nucleotide sequence or inserted between two amino acids in the amino acid sequence encoded by the nucleotide sequence at one or more insertion sites selected from Table 3.

10. The isolated nucleic acid molecule of any one of claims 1 to 9, wherein the FVIII polypeptide is a full length FVIII or a B domain deleted FVIII.

11. A vector comprising the nucleic acid molecule of any one of claims 1 to 10.

12. The vector of claim 11, wherein the vector is a lentiviral vector.

13. A host cell comprising the nucleic acid molecule of any one of claims 1 to 10 or the vector of claim 11 or 12.

14. A method of producing a polypeptide with FVIII activity, comprising: culturing the host cell of claim 13 under conditions whereby a polypeptide with FVIII activity is produced, and recovering the polypeptide with FVIII activity.

15. Use of a nucleic acid molecule of any one of claims 1 to 10, or the vector of claim 11 or 12, in the manufacture of a medicament for the treatment of a bleeding disorder.