FACTOR IX ENCODING NUCLEOTIDES

Field of the Invention

The present invention relates to polynucleotides comprising a nucleotide sequence encoding Factor IX, viral particles comprising the polynucleotides and treatments utilising the polynucleotides.

Background to the Invention

Haemophilia B, an X-linked life threatening bleeding disorder affects 1:30,000 males. Current treatment involves frequent intravenous injections (2-3 times per week) of Factor IX (FIX) protein. This treatment is highly effective at arresting bleeding but it is not curative and is extremely expensive (£150,000/patient/year), thus making it unaffordable by the majority of haemophilia B patients in the world. Gene therapy for haemophilia B offers the potential for a cure through persistent, endogenous production of Factor IX following the transfer of a functioning copy of the Factor IX gene to an affected patient.

The present application relates to a gene therapy approach for treating haemophilia B, involving administering a vector comprising a polynucleotide encoding Factor IX. Such a gene therapy approach would avoid the need for frequent intravenous injections of Factor IX. However, it is difficult to provide an effective gene therapy vector, *i.e.* one that allows for a high level of Factor IX expression and of the expression of Factor IX which is highly active.

Summary ~~of the Invention~~

The present application demonstrates that various modifications to a polynucleotide comprising a Factor IX nucleotide sequence can help to improve the expression level and the activity of the expressed Factor IX polypeptide. For example, the present application demonstrates that the following can improve the efficacy of a polynucleotide comprising a Factor IX nucleotide sequence for treatment of haemophilia B:

- using a codon optimised sequence;
- maintaining a portion of the Factor IX polypeptide that is not codon optimised;
- including an intron or a fragment of an intron;
- providing sequences flanking the intron or fragment of an intron that are not codon optimised;
- using a gain of function mutation;
- using a specific promoter; and/or
- maintaining an AAV genome, comprising the nucleotide, in single stranded form.

These modifications provide a Factor IX sequence which is expressed highly, and which encodes a highly active Factor IX polypeptide or fragment thereof. As demonstrated in the Examples, the polynucleotide of the ~~invention~~disclosure expresses, and provides overall Factor IX activity, at higher levels than other Factor IX encoding polynucleotides, for example those disclosed in WO16/075473.

In one aspect, the present disclosure provides a polynucleotide comprising a Factor IX nucleotide sequence corresponding to a reference wild-type Factor IX sequence of SEQ ID NO: 9, wherein: (i) the Factor IX nucleotide sequence comprises a coding sequence that encodes a Factor IX protein or fragment thereof; (ii) a portion of the coding sequence is codon optimized compared to the reference wild-type Factor IX sequence of SEQ ID NO: 9; (iii) the portion of the coding sequence that is codon optimized is at least 1100 nucleotides in length; (iv) in the portion that is codon optimized at least 73% of the codons are selected from the group consisting of: TTC encoding phenylalanine, CTG encoding leucine, ATC encoding isoleucine, GTG or GTC encoding valine, AGC encoding serine, CCC encoding proline, ACC encoding threonine, GCC encoding alanine, TAC encoding tyrosine, CAC encoding histidine, CAG encoding glutamine, AAC encoding asparagine, AAA or AAG encoding lysine, GAC encoding aspartate, TGC encoding cysteine, AGG encoding arginine, GGC encoding glycine, and GAG encoding glutamate; (v) the portion of the coding sequence that is codon optimized is CpG free; (vi) the polynucleotide further comprises a transcription regulatory element comprising: (a) an A1 AT promoter or a fragment of an A1 AT promoter between 150 and 300 nucleotides in length; and/or (b) an HCR enhancer or a fragment of an HCR enhancer; between 100 and 250 nucleotides in

length and (vii) the Factor IX nucleotide sequence comprises a codon that encodes a leucine at a position corresponding to position 384 of the reference wild type Factor IX sequence of SEQ ID NO: 9.

Accordingly, in a first ~~aspect~~example of the ~~invention~~disclosure, there is provided a polynucleotide comprising a Factor IX nucleotide sequence, wherein the Factor IX nucleotide sequence comprises a coding sequence that encodes a Factor IX protein or fragment thereof and wherein a portion of the coding sequence is not wild type.

In a second ~~aspect~~example of the ~~invention~~disclosure, there is provided a polynucleotide comprising a Factor IX nucleotide sequence, wherein the Factor IX nucleotide sequence comprises a coding sequence that encodes a Factor IX protein or a fragment thereof and the coding sequence comprises: (i) a sequence that is at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO.1; and (ii) a sequence that is at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO. 15.

In a third ~~aspect~~example of the ~~invention~~disclosure, there is provided a polynucleotide comprising a Factor IX nucleotide sequence, wherein the Factor IX nucleotide sequence encodes a Factor IX protein or fragment thereof and has at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identity to SEQ ID NO. 5.

In a fourth ~~aspect~~example of the ~~invention~~disclosure, there is provided a viral particle comprising a recombinant genome comprising the polynucleotide of the ~~invention~~disclosure.

In a fifth ~~aspect~~example of the ~~invention~~disclosure, there is provided a composition comprising the polynucleotide or viral particle of the ~~invention~~disclosure and a pharmaceutically acceptable excipient.

In a sixth ~~aspect~~example of the ~~invention~~disclosure, there is provided a method of treatment comprising administering an effective amount of the polynucleotide or viral particle of the ~~invention~~disclosure to a patient.

In a seventh ~~aspect~~example of the ~~invention~~disclosure, there is provided a use of the polynucleotide, viral particle or composition of the ~~invention~~disclosure in the manufacture of a medicament for use in a method of treatment.

In an eighth ~~aspect~~example, the polynucleotide, viral particle or composition of the ~~invention~~disclosure is for use in expressing a polypeptide encoded by the Factor IX nucleotide sequence at a level at least 2, or at least 3 times greater than a polypeptide encoded by a nucleotide sequence comprising a Factor IX nucleotide sequence of SEQ ID NO. 12 and a transcription regulatory element of SEQ ID NO. 7, thereby treating a disease.

In a ninth ~~aspect~~example, the polynucleotide, viral particle or composition of the ~~invention~~disclosure is for use in treating a disease by expressing a polypeptide encoded by the Factor IX nucleotide sequence at a level at least 2, or at least 3 times greater than a polypeptide encoded by a nucleotide sequence comprising a Factor IX nucleotide sequence of SEQ ID NO. 12 and a transcription regulatory element of SEQ ID NO. 7.

In a tenth ~~aspect~~example, there is provided a method of expressing a polypeptide encoded by a Factor IX nucleotide sequence at a level at least 2, or at least 3 times greater than a polypeptide encoded by a nucleotide sequence comprising a Factor IX nucleotide sequence of SEQ ID NO. 12 and a transcription regulatory element of SEQ ID NO. 7 comprising

administering the polynucleotide, viral particle or composition of the ~~invention~~disclosure to a patient.

In an eleventh ~~aspect~~example, there is provided a method of treating a disease by expressing a polypeptide encoded by the Factor IX nucleotide sequence at a level at least 2, or at least 3 times greater than a polypeptide encoded by a nucleotide sequence comprising a Factor IX nucleotide sequence of SEQ ID NO. 12 and a transcription regulatory element of SEQ ID NO. 7 comprising administering the polynucleotide, viral particle or composition of the ~~invention~~disclosure to a patient.

In a twelfth ~~aspect~~example, there is provided a use of the polynucleotide, viral particle or composition of the ~~invention~~disclosure in expressing a polypeptide encoded by the Factor IX nucleotide sequence at a level at least 2, or at least 3 times greater than a polypeptide encoded by a nucleotide sequence comprising a Factor IX nucleotide sequence of SEQ ID NO. 12 and a transcription regulatory element of SEQ ID NO. 7, thereby treating a disease.

In a thirteenth ~~aspect~~example, there is provided a use of the polynucleotide, viral particle or composition of the ~~invention~~disclosure in treating a disease by expressing a polypeptide encoded by the Factor IX nucleotide sequence at a level at least 2, or at least 3 times greater than a polypeptide encoded by a nucleotide sequence comprising a Factor IX nucleotide sequence of SEQ ID NO. 12 and a transcription regulatory element of SEQ ID NO. 7.

In a fourteenth ~~aspect~~example, the polynucleotide, viral particle or composition of the ~~invention~~disclosure is for use in expressing a polypeptide encoded by the Factor IX nucleotide sequence at a level at least 2, or at least 3 times greater than a polypeptide encoded by a nucleotide sequence comprising a Factor IX nucleotide sequence of SEQ ID NO. 18 and a transcription regulatory element of SEQ ID NO. 6, thereby treating a disease.

In a fifteenth ~~aspect~~example, the polynucleotide, viral particle or composition of the ~~invention~~disclosure is for use in treating a disease by expressing a polypeptide encoded by the Factor IX nucleotide sequence at a level at least 2, or at least 3 times greater than a polypeptide encoded by a nucleotide sequence comprising a Factor IX nucleotide sequence of SEQ ID NO. 18 and a transcription regulatory element of SEQ ID NO. 6.

In a sixteenth ~~aspect~~example, there is provided a method of expressing a polypeptide encoded by a Factor IX nucleotide sequence at a level at least 2, or at least 3 times greater than a polypeptide encoded by a nucleotide sequence comprising a Factor IX nucleotide sequence of SEQ ID NO. 18 and a transcription regulatory element of SEQ ID NO. 6 comprising administering the polynucleotide, viral particle or composition of the ~~invention~~disclosure to a patient.

In a seventeenth ~~aspect~~example, there is provided a method of treating a disease by expressing a polypeptide encoded by the Factor IX nucleotide sequence at a level at least 2, or at least 3 times greater than a polypeptide encoded by a nucleotide sequence comprising a Factor IX nucleotide sequence of SEQ ID NO. 18 and a transcription regulatory element of SEQ ID NO. 6 comprising administering the polynucleotide, viral particle or composition of the ~~invention~~disclosure to a patient.

In an eighteenth ~~aspect~~example, there is provided a use of the polynucleotide, viral particle or composition of the ~~invention~~disclosure in expressing a polypeptide encoded by the Factor IX nucleotide sequence at a level at least 2, or at least 3 times greater than a polypeptide encoded by a nucleotide sequence comprising a Factor IX nucleotide sequence of SEQ ID NO. 18 and a transcription regulatory element of SEQ ID NO. 6, thereby treating a disease.

In a nineteenth ~~aspect~~example, there is provided a use of the polynucleotide, viral particle or composition of the ~~invention~~disclosure in treating a disease by expressing a polypeptide encoded by the Factor IX nucleotide sequence at a level at least 2, or at least 3 times greater than a polypeptide encoded by a nucleotide sequence comprising a Factor IX nucleotide sequence of SEQ ID NO. 18 and a transcription regulatory element of SEQ ID NO. 6.

In a twentieth ~~aspect~~example, the polynucleotide, viral particle or composition of the ~~invention~~disclosure is for use in treating a disease by expressing Factor IX protein or a fragment thereof having a Factor IX activity greater than the activity of Factor IX expressed from a viral particle comprising a Factor IX nucleotide sequence of SEQ ID NO:

12 and a transcription regulatory element of SEQ ID NO. 7 and/or a viral particle comprising a Factor IX nucleotide sequence of SEQ ID NO. 18 and a transcription regulatory element of SEQ ID NO. 6.

In a twenty-first ~~aspect~~example, there is provided a method of treating a disease by expressing Factor IX protein or a fragment thereof having a Factor IX activity greater than the activity of Factor IX expressed from a viral particle comprising a Factor IX nucleotide sequence of SEQ ID NO: 12 and a transcription regulatory element of SEQ ID NO. 7 and/or a viral particle comprising a Factor IX nucleotide sequence of SEQ ID NO. 18 and a transcription regulatory element of SEQ ID NO. 6, comprising administering an effective amount of the polynucleotide, viral particle or composition of the ~~invention~~disclosure to a patient.

In a twenty-second ~~aspect~~example, there is provided a use of the polynucleotide, viral particle or composition of the ~~invention~~disclosure in treating a disease by expressing Factor IX protein or a fragment thereof having a Factor IX activity greater than the activity of Factor IX expressed from a viral particle comprising a Factor IX nucleotide sequence of SEQ ID NO: 12 and a transcription regulatory element of SEQ ID NO. 7 and/or a viral particle comprising a Factor IX nucleotide sequence of SEQ ID NO. 18 and a transcription regulatory element of SEQ ID NO. 6.

In a twenty-third ~~aspect~~example, the polynucleotide, viral particle or composition of the ~~invention~~disclosure is for use in achieving a stable Factor IX activity level of at least 20% in a patient having haemophilia B.

In a twenty-fourth ~~aspect~~example, there is provided a method of achieving a stable Factor IX activity level of at least 20% in a patient having haemophilia B, comprising administering an effective amount of the polynucleotide, viral particle or composition of the ~~invention~~disclosure to a patient.

In a twenty-fifth ~~aspect~~example there is provided a use of the polynucleotide, viral particle or composition of the ~~invention~~disclosure in achieving a stable Factor IX activity level of at least 20% in a patient having haemophilia B.

In a twenty-sixth ~~aspect~~example, there is provided the polynucleotide, viral particle or composition of the ~~invention~~disclosure for use in treating haemophilia B comprising administering the polynucleotide, viral particle or composition to a patient at a dose effective to achieve a stable Factor IX activity level of at least 20% in a patient.

In a twenty-seventh ~~aspect~~example, there is provided a method of treating haemophilia B comprising administering the polynucleotide, viral particle or composition of the ~~invention~~disclosure to a patient at a dose effective to achieve a stable Factor IX activity level of at least 20% in a patient.

In a twenty-eighth ~~aspect~~example, there is provided a use of the polynucleotide, viral particle or composition of the ~~invention~~disclosure in treating haemophilia B comprising administering the polynucleotide, viral particle or composition to a patient at a dose effective to achieve a stable Factor IX activity level of at least 20% in a patient.

Description of the Figures

Figure 1 – Schematic of FIX transgene cassettes ssLP1.FIXco, ssHLP2.TI-codop-FIX-GoF (HTFG) and ssHLP2.TI-ACNP-FIX-GoF (HTAG). ITR = Inverted Terminal Repeat; HLP2 and LP1 are transcription regulatory elements of SEQ ID NOs: 6 and 7 respectively; E = Exon; T1 = Truncated Intron 1A; WT = Wild Type; CO = Codon Optimised; ACNP = a codon optimised sequence of the invention.

Figure 2 – Results from HUH7 transduction with AAV2/Mut C vectors – Experiment 1; (A) shows the level of FIX antigen in supernatant; (B) shows the level of FIX antigen after normalisation using the number of vector genomes present in cell lysate; Error bars represent mean ± SD of n=2. 1e3 = 1 x 10^3; 5e3 = 5 x 10^3; 1e4 = 1 x 10^4; MOI = multiplicity of infection.

Figure 3 – Results from HUH7 transduction with AAV2/Mut C vectors – Experiment 2, showing the level of FIX antigen in supernatant. Error bars represent mean ± SD of n=3. 1e3 = 1 x 10^3; 5e3 = 5 x 10^3; 1e4 = 1 x 10^4, MOI = multiplicity of infection.

CLAIMS

1. A polynucleotide comprising a Factor IX nucleotide sequence corresponding to a reference wild-type Factor IX sequence of SEQ ID NO: 9, wherein:

(i) the Factor IX nucleotide sequence comprises a coding sequence that encodes a Factor IX protein or fragment thereof;

(ii) a portion of the coding sequence is codon ~~optimised~~optimized compared to the reference wild-type Factor IX sequence of SEQ ID NO: 9;

(iii) the portion of the coding sequence that is codon ~~optimised~~ optimized is at least 1100 nucleotides in length;

(iv) in the portion that is codon ~~optimised~~ optimized at least ~~60~~73% of the codons are selected from the group consisting of:

TTC encoding phenylalanine,

CTG encoding leucine,

ATC encoding isoleucine,

GTG~~,~~ or GTC encoding valine,

AGC encoding serine,

CCC encoding proline,

ACC encoding threonine,

GCC encoding alanine,

TAC encoding tyrosine,

CAC encoding histidine,

CAG encoding glutamine,

AAC encoding asparagine,

AAA~~,~~ or AAG encoding lysine,

GAC encoding aspartate,

TGC encoding cysteine,

AGG encoding arginine,

GGC encoding glycine, and

GAG encoding glutamate;

(v) the portion of the coding sequence that is codon ~~optimised~~ optimized is CpG free ~~comprises a reduced number of CpGs compared to a corresponding portion of SEQ ID NO. 9~~;

(vi) the polynucleotide further comprises a transcription regulatory element comprising:

(a) an A1 AT promoter or a fragment of an A1 AT promoter between 150 and 300 nucleotides in length; and/or

(b) an HCR enhancer or a fragment of an HCR enhancer; between 100 and 250 nucleotides in length and

(vii) the Factor IX nucleotide sequence comprises a codon that encodes a leucine at a position corresponding to position 384 of the reference wild type Factor IX sequence of SEQ ID NO: 9.

2. The polynucleotide of claim 1, wherein the polynucleotide comprises a promoter selected from the group consisting of:

(i) a promoter comprising at least 80% identity to SEQ ID NO. 14;

(ii) a promoter comprising at least 98% identity to SEQ ID NO. 14;

(iii) a promoter comprising the sequence of SEQ ID NO. 14; and

(iv) a liver-specific promoter.

~~2. The polynucleotide of the preceding claim, wherein the portion of the coding sequence that is codon optimised is codon optimised for expression in the human liver.~~

3. The polynucleotide of ~~any one of the preceding~~ claim~~s~~ 1, wherein:

~~(i) a polypeptide encoded by the Factor IX nucleotide sequence is expressed in human liver cells at higher levels compared to a reference wild type Factor IX nucleotide sequence; and/or~~

~~(ii)~~ the portion of the coding sequence that is codon ~~optimised~~ optimized ~~is at least 800, at least 900, at least 1100, less than 1500, less than 1300, less than 1200, between 800 and 1500, between 900 and 1300, between 1100 and 1200, or around 1191 nucleotides in length; and/or~~

~~(iii) the portion of the coding sequence that is codon optimised~~ comprises at least a portion ~~1, 2, 3, 4, 5 or all~~ of:

~~a)~~ exon 3, ~~or a portion of at least 10, at least 15, at least 20, less than 25, between 10 and 25, between 15 and 25, or between 20 and 25 nucleotides of exon 3;~~

~~b)~~ at least a portion of exon 4, ~~or a portion of at least 80, at least 90, at least 100, less than 114, between 80 and 114, between 90 and 114, or between 100 and 114 nucleotides of exon 4;~~

~~c)~~ at least a portion of exon 5, ~~or a portion of at least 90, at least 100, at least 110, less than 129, between 90 and 129, between 100 and 129, or between 110 and 129 nucleotides of exon 5~~;

~~d)~~ at least a portion of exon 6, ~~or a portion of at least 150, at least 180, at least 200, less than 203, between 150 and 203, between 180 and 203, or between 200 and 203 nucleotides of exon 6;~~

~~e)~~ at least a portion of exon 7, ~~or a portion of at least 70, at least 80, at least 90, at least 100, less than 115, between 70 and 115, between 80 and 115, between 90 and 115, or between 100 and 115 nucleotides of exon 7; and/or~~

~~f)~~ and at least a portion of exon 8, ~~or a portion of at least 400, at least 450, at least 500, less than 548, between 400 and 548, between 450 and 548, or between 500 and 548 nucleotides of exon 8; and/or~~

~~(iv) the portion of the coding sequence that is codon optimised comprises a portion of at least 20 nucleotides of exon 3, a portion of at least 100 nucleotides of exon 4, a portion of at least 110 nucleotides of exon 5, a portion of at least 180 nucleotides of exon 6, a portion of at least 100 nucleotides of exon 7, and a portion of at least 500 nucleotides of exon 8; and/or~~

~~(v) the portion of the coding sequence that is codon optimised comprises exon 3, exon 4, exon 5, exon 6, exon 7, and exon 8; and/or~~

~~(vi) the portion of the coding sequence that is codon optimised comprises a portion of exon 2, and the portion of exon 2 is less than 160, less than 150, less than 100, less than 75, less than 60, at least 20, at least 30, at least 40, at least 50, between 20 and 160, between 30 and 150, between 30 and 100, between 40 and 75, or around 56 nucleotides in length; and/or~~

~~(vii) the portion of the coding sequence that is codon optimised comprises a portion of exon 2 that is between 30 and 100 nucleotides in length.~~

~~4. The polynucleotide of any one of the preceding claims, wherein:~~

~~(i) the portion of the coding sequence that is codon optimised comprises less than less than 40, less than 20, less than 18, less than 10, less than 5, or less than 1 CpG; and/or~~

~~(ii) the portion of the coding sequence that is codon optimised is CpG free.~~

~~5~~4. The polynucleotide of ~~any one of the preceding~~ claim~~s~~ 1, wherein, in the portion of the coding sequence that is codon ~~optimised~~optimized, the codon optimization is selected from the group consisting of:

(i) a) at least 1, at least 2, at least 4, or at least 5 codons that encode phenylalanine is/are replaced with TTC compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 60%, at least 65%, or at least 70% of the codons that encode phenylalanine are TTC;

c) at least 60%, at least 65%, or at least 70% of the codons that encode phenylalanine are TTC and the remainder are TTT; ~~and/~~or

d) the codons that encode phenylalanine are TTC, except where the following codon starts with a G; ~~and/or~~

(ii) a) at least 5, at least 10, at least 15, or at least 16 codons that encode leucine is/are replaced with CTG compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 90%, or at least 94% of the codons that encode leucine are CTG; ~~and/~~or

c) at least 90%, or at least 94% of the codons that encode leucine are CTG and the remainder are CTC; ~~and/or~~

(iii) a) at least 5, at least 10, at least 11, or at least 12 codons that encode isoleucine is/are replaced with ATC compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 1 of codon ATC is/are replaced with ATT compared to a reference wild type Factor IX sequence, where the following codon starts with a G;

c) at least 60%, at least 70%, or at least 75% of the codons that encode isoleucine are ATC;

d) at least 60%, at least 70%, or at least 75% of the codons that encode isoleucine are ATC and the remainder are ATT; ~~and/~~or

e) the codons that encode isoleucine are ATC, except where the following codon starts with a G; ~~and/or~~

(iv) a) at least 10, at least 15, at least 20, or at least 25 codons that encode valine is/are replaced with GTG compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 1 codon that encodes valine is/are replaced with GTC compared to a reference wild type Factor IX sequence of SEQ ID NO: 9;

c) at least 80%, at least 90%, or at least 95% of the codons that encode valine are GTG; ~~and/~~or

d) at least 80%, at least 90%, or at least 95% of the codons that encode valine are GTG and the remainder are GTC; ~~and/or~~

(v) a) at least 5, at least 10, at least 12, or at least 13 codons that encode serine is/are replaced with AGC compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 1, at least 2, or at least 4 codons that encode serine is/are replaced with TCT compared to a reference wild type Factor IX sequence of SEQ ID NO: 9, where the following codon starts with a G;

c) at least 60%, at least 65%, or at least 70% of the codons that encode serine are AGC; ~~and/~~or

d) at least 60%, at least 65%, or at least 70% of the codons that encode serine are AGC and the remainder are TCT or TCC; ~~and/or~~

(vi) a) at least 1, at least 2, or at least 5 codons that encode proline is/are replaced with CCC compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 1 codon~~s~~ that encode proline is/are replaced with CCT compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9, where the following codon starts with a G;

c) at least 50%, at least 55%, or at least 60% of the codons that encode proline are CCC;

d) at least 50%, at least 55%, or at least 60% of the codons that encode proline are CCC and the remainder are CCA or CCT; ~~and/~~or

e) the codons that encode proline are CCC, except where the following codon starts with a G; ~~and/or~~

(vii) a) at least 6, at least 7, at least 8, or at least 10 codons that encode threonine is/are replaced with ACC compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 1, or at least 2, codons that encode threonine is/are replaced with ACT compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9, where the following codon starts with a G;

c) at least 45%, at least 50%, or at least 55% of the codons that encode threonine are ACC;

d) at least 45%, at least 50%, or at least 55% of the codons that encode threonine are ACC and the remainder are ACT; ~~and/~~or

~~e)~~ the codons that encode threonine are ACC, except where the following codon starts with a G; ~~and/or~~

(viii) a) at least 1, at least 2, or at least 3~~, or at least 4~~ codons that encode alanine is/are replaced with GCC compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 1, at least 2, ~~or~~ at least 3, or at least 4 codons that encode alanine is/are replaced with GCT compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9, where the following codon starts with a G;

c) at least 35%, at least 40%, or at least 43% of the codons that encode alanine are GCC;

d) at least 35%, at least 40%, or at least ~~45~~43% of the codons that encode alanine are GCC and the remainder are GCT; ~~and/~~or

~~e)~~ the codons that encode alanine are GCC, except where the following codon starts with a G; ~~and/or~~

(ix) a) at least 1, or at least 2 codons that encode tyrosine is/are replaced with TAC compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 1 of codon TAC is/are replaced with TAT compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9, where the following codon starts with a G;

c) at least 40%, at least 45%, or at least 48% of the codons that encode tyrosine are TAC;

d) at least 40%, at least 45%, or at least 48% of the codons that encode tyrosine are TAC and the remainder are TAT; ~~and/~~or

e) the codons that encode tyrosine are TAC, except where the following codon starts with a G; ~~and/or~~

(x) a) at least 1 codon~~s~~ that encode histidine is/are replaced with CAC compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 50%, at least 60%, or at least 65% of ~~the~~ codons that encode histidine are CAC;

c) at least 50%, at least 60%, or at least 65% of the codons that encode histidine are CAC and the remainder are CAT; ~~and/~~or

d) the codons that encode histidine are CAC, except where the following codon starts with a G; ~~and/or~~

(xi) a) at least 1, at least 2, at least 4, or at least 5 codons that encode glutamine is/are replaced with CAG compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 1 of codon CAG is/are replaced with CAA compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

c) at least 80%, at least 85%, or at least 90% of the codons that encode glutamine are CAG; ~~and/~~or

d) at least 80%, at least 85%, or at least 90% of the codons that encode glutamine are CAG and the remainder are CAA; ~~and/or~~

(xii) a) at least 1, at least 2, at least 4, or at least 5 codons that encode asparagine is/are replaced with AAC compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 60%, at least 65%, or at least 70% of the codons that encode asparagine are AAC;

c) at least 60%, at least 65%, or at least 70% of the codons that encode asparagine are AAC and the remainder are AAT; ~~and/~~or

~~e~~d) the codons that encode asparagine are AAC, except where the following codon starts with a G; ~~and/or~~

(xiii) a) at least 5, at least 7, at least 8, or at least 9 codons that encode lysine is/are replaced with AAG compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 1 of codon AAG is/are replaced with AAA compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

c) at least 80%, at least 90%, or at least 95% of the codons that encode lysine are AAG; ~~and/~~or

d) at least 80%, at least 90%, or at least 95% of the codons that encode lysine are AAG and the remainder are AAA; ~~and/or~~

(xiv) a) at least 1, at least 2, at least 3, or at least 4 codons that encode aspartate is/are replaced with GAC compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 1 of codon GAC is/are replaced with GAT compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9, where the following codon starts with a G;

c) at least 45%, at least 50%, or at least 60% of the codons that encode aspartate are GAC;

d) at least 45%, at least 50%, or at least 60% of the codons that encode aspartate are GAC and the remainder are GAT; ~~and/~~or

e) the codons that encode aspartate are GAC, except where the following codon starts with a G; ~~and/or~~

(xv) a) at least 15, at least 20, at least 25, or at least 26 codons that encode glutamate is/are replaced with GAG compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 80%, at least 90%, or at least 95% of the codons that encode glutamate are GAG; ~~and/~~or

c) at least 80%, at least 90%, or at least 95% of the codons that encode glutamate are GAG and the remainder are GAA; ~~and/or~~

(xvi) a) at least 5, at least 6, at least 7, or at least 8 codons that encode cysteine is/are replaced with TGC compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 1 of codon TGC is/are replaced with TGT compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9, where the following codon starts with a G;

c) at least 40%, at least 50%, or at least 55% of the codons that encode cysteine are TGC;

d) at least 40%, at least 50%, or at least 55% of the codons that encode cysteine are TGC and the remainder are TGT; ~~and/~~or

e) the codons that encode cysteine are TGC, except where the following codon starts with a G; ~~and/or~~

(xvii) wherein, the codons that encode tryptophan are TGG; ~~and/or~~

(xviii) a) at least 5, at least 8, at least 10, or at least 11 codons that encode arginine is/are replaced with AGG compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 1 codon that encodes arginine is/are replaced with AGA compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

c) at least 60%, at least 70%, or at least 75% of the codons that encode arginine are AGG; ~~and/~~or

d) at least 60%, at least 70%, or at least 75% of the codons that encode arginine are AGG and the remainder are AGA; and~~/or~~

(xix) a) at least 5, at least 10, at least 12, or at least 13 codons that encode glycine is/are replaced with GGC compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 5, at least 6, at least 7, or at least 8 codons that encode glycine is/are replaced with GGG compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9, where the following codon starts with a G;

c) at least 50%, at least 55%, or at least 60% of the codons that encode glycine are GGC;

d) at least 50%, at least 55%, or at least 60% of the codons that encode glycine are GGC and the remainder are GGG; ~~and/~~or

e) the codons that encode glycine are GGC, except where the following codon starts with a G.

~~65~~. The polynucleotide of ~~any one of the preceding~~ claim~~s~~ 1 wherein the portion of the coding sequence that is codon ~~optimised~~ optimized comprises codons encoding phenylalanine, leucine, isoleucine, valine, serine, proline, threonine, alanine, tyrosine, histidine, glutamine, asparagine, lysine, aspartate, glutamate, cysteine, tryptophan, arginine, and glycine, and in the portion of the coding sequence that is codon ~~optimised~~ optimized~~portion~~:

a) at least 5 codons that encode phenylalanine is/are replaced with TTC compared to ~~a~~ the reference wild type Factor IX sequence of SEQ ID NO: 9;

b) at least 16 codons that encode leucine is/are replaced with CTG compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

c) at least 12 codons that encode isoleucine is/are replaced with ATC compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

d) at least 25 codons that encode valine is/are replaced with GTG compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

e) at least 13 codons that encode serine is/are replaced with AGC compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

f) at least 5 codons that encode proline is/are replaced with CCC compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

g) at least 10 codons that encode threonine is/are replaced with ACC compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

h) at least 4 codons that encode alanine is/are replaced with GCC compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

i) at least 2 codons that encode tyrosine is/are replaced with TAC compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

j) at least 1 codons that encode histidine is/are replaced with CAC compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

k) at least 5 codons that encode glutamine is/are replaced with CAG compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

l) at least 5 codons that encode asparagine is/are replaced with AAC compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

m) at least 9 codons that encode lysine is/are replaced with AAG compared to the a reference wild type Factor IX sequence of SEQ ID NO: 9;

n) at least 4 codons that encode aspartate is/are replaced with GAC compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

o) at least 26 codons that encode glutamate is/are replaced with GAG compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

p) at least 8 codons that encode cysteine is/are replaced with TGC compared to thea reference wild type Factor IX sequence of SEQ ID NO: 9;

q) the codons that encode tryptophan are TGG;

r) at least 11 codons that encode arginine is/are replaced with AGG compared to the ~~a~~ reference wild type Factor IX sequence of SEQ ID NO: 9; and

s) at least 13 codons that encode glycine is/are replaced with GGC compared to the~~a~~ reference wild type Factor IX sequence of SEQ ID NO: 9.

~~7~~6. The polynucleotide of ~~any one of the preceding~~ claim~~s~~ 1, wherein the portion of the coding sequence that is codon ~~optimised~~ optimized comprises codons encoding phenylalanine, leucine, isoleucine, valine, serine, proline, threonine, alanine, tyrosine, histidine, glutamine, asparagine, lysine, aspartate, glutamate, cysteine, tryptophan, arginine, and glycine, and in the portion of the coding sequence that is codon optimised ~~portion~~:

a) at least 70% of ~~the~~ codons that encode phenylalanine are TTC;

b) at least 94% of ~~the~~ codons that encode leucine are CTG;

c) at least 75% of ~~the~~ codons that encode isoleucine are ATC;

d) at least 95% of ~~the~~ codons that encode valine are GTG;

e) at least 70% of ~~the~~ codons that encode serine are AGC;

f) at least 60% of ~~the~~ codons that encode proline are CCC;

g) at least 55% of ~~the~~ codons that encode threonine are ACC;

h) at least 43% of ~~the~~ codons that encode alanine are GCC;

i) at least 48% of ~~the~~ codons that encode tyrosine are TAC;

j) at least 65% of ~~the~~ codons that encode histidine are CAC;

k) at least 90% of ~~the~~ codons that encode glutamine are CAG;

l) at least 70% of ~~the~~ codons that encode asparagine are AAC;

m) at least 95% of ~~the~~ codons that encode lysine are AAG;

n) at least 60% of ~~the~~ codons that encode aspartate are GAC;

o) at least 95% of ~~the~~ codons that encode glutamate are GAG;

p) at least 55% of ~~the~~ codons that encode cysteine are TGC;

q) ~~the~~ codons that encode tryptophan are TGG;

r) at least 75% of ~~the~~ codons that encode arginine are AGG; and

s) at least 60% of ~~the~~ codons that encode glycine are GGC.

~~87~~. The polynucleotide of ~~any one of the preceding~~ claim~~s~~ 1, wherein the portion of the coding sequence that is codon ~~optimised~~ optimized comprises codons encoding phenylalanine, leucine, isoleucine, valine, serine, proline, threonine, alanine, tyrosine, histidine, glutamine, asparagine, lysine, aspartate, glutamate, cysteine, tryptophan, arginine, and glycine, and in the portion of the coding sequence that is codon ~~optimised~~ optimized~~portion~~:

a) at least 70% of the codons that encode phenylalanine are TTC and the remainder are TTT;

b) at least 94% of ~~the~~ codons that encode leucine are CTG and the remainder are CTC;

c) at least 75% of ~~the~~ codons that encode isoleucine are ATC and the remainder are ATT;

d) at least 95% of ~~the~~ codons that encode valine are GTG;

e) at least 70% of ~~the~~ codons that encode serine are AGC;

f) at least 60% of ~~the~~ codons that encode proline are CCC and the remainder are CCA or CCT;

g) at least 55% of ~~the~~ codons that encode threonine are ACC and the remainder are ACT;

h) at least 43% of ~~the~~ codons that encode alanine are GCC and the remainder are GCT;

i) at least 48% of ~~the~~ codons that encode tyrosine are TAC and the remainder are TAT;

j) at least 65% of ~~the~~ codons that encode histidine are CAC and the remainder are CAT;

k) at least 90% of ~~the~~ codons that encode glutamine are CAG and the remainder are CAA;

l) at least 70% of ~~the~~ codons that encode asparagine are AAC and the remainder are AAT;

m) at least 95% of ~~the~~ codons that encode lysine are AAG and the remainder are AAA;

n) at least 60% of ~~the~~ codons that encode aspartate are GAC and the remainder are GAT;

o) at least 95% of ~~the~~ codons that encode glutamate are GAG and the remainder are GAA;

p) at least 55% of ~~the~~ codons that encode cysteine are TGC and the remainder are TGT;

q) ~~the~~ codons that encode tryptophan are TGG;

r) at least 75% of ~~the~~ codons that encode arginine are AGG and the remainder are AGA; and

s) at least 60% of ~~the~~ codons that encode glycine are GGC and the remainder are GGG.

~~9. The polynucleotide of any one of claims 4-8, wherein the reference wild type Factor IX sequence is SEQ ID NO. 9 or SEQ ID NO. 19.~~

~~10. The polynucleotide of any one of the preceding claims, wherein the transcription regulatory element comprises a liver-specific promoter.~~

~~11. The polynucleotide of any one of the preceding claims, wherein the fragment of an A1AT promoter is:~~

~~(i) at least 100, at least 120, at least 150, at least 180, less than 255, between 100 and 255, between 150 and 225, between 150 and 300, or between 180 and 255 nucleotides in length; and/or~~

~~(ii) between 150 and 300 nucleotides in length.~~

~~12. The polynucleotide of any one of the preceding claims, wherein the fragment of an HCR enhancer is:~~

~~(i) a fragment of at least 80, at least 90, at least 100, less than 192, between 80 and 192, between 90 and 192, between 100 and 250, or between 117 and 192 nucleotides in length; and/or~~

~~(ii) between 100 and 250 nucleotides in length.~~

~~13. The polynucleotide of any one of the preceding claims wherein:~~

~~(i) the transcription regulatory element is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO. 6; and/or~~

~~(ii) the transcription regulatory element has a sequence of SEQ ID NO. 6.~~

~~14~~8. The polynucleotide of ~~any one of the preceding~~ claim~~s~~ 1, wherein the polynucleotide comprises an enhancer selected from the group consisting of:

(i) ~~the polynucleotide comprises~~ an enhancer comprising ~~that is~~ at least 80%~~, at least 85%, at least 90%, at least 95% at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical~~ identity to SEQ ID NO. 13; ~~and/or~~

(ii) ~~the polynucleotide comprises~~ an enhancer comprising ~~that is~~ at least 98%~~, at least 99%, at least 99.5%, at least 99.8%, or 100% identical~~ identity to SEQ ID NO. 13; and~~/or~~

(iii) the polynucleotide comprises an enhancer comprising the sequence of SEQ ID NO. 13~~; and/or~~

~~(iv) the polynucleotide comprises a promoter that is at least 80%, at least 85%, at least 90%, at least 95% at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO. 14; and/or~~

~~(v) the polynucleotide comprises a promoter that is at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO. 14; and/or~~

~~(vi) the polynucleotide comprises a promoter of SEQ ID NO. 14~~.

9. The polynucleotide of claim 2, wherein a polypeptide encoded by the Factor IX nucleotide sequence is expressed in human liver cells at higher levels compared to the reference wild type Factor IX sequence of SEQ ID NO: 9.

~~15~~10. The polynucleotide of ~~any one of the preceding~~ claim~~s~~ 1, wherein the codon that encodes an amino acid at ~~a~~ the position corresponding to position 384 of the reference wild type Factor IX sequence of SEQ ID NO: 9 is ~~CTX~~CTC~~, wherein X is any nucleotide, optionally wherein X is C or G~~.

11. The polynucleotide of claim 1, wherein the codon that encodes an amino acid at the position corresponding to position 384 of the reference wild type Factor IX sequence of SEQ ID NO: 9 is CTG.

~~16. A polynucleotide comprising a Factor IX nucleotide sequence, wherein the Factor IX nucleotide sequence comprises a coding sequence that encodes a Factor IX protein or fragment thereof and wherein a portion of the coding sequence is not wild type.~~

~~17~~12. An AAV viral particle comprising a recombinant genome comprising the polynucleotide of ~~any one of the preceding~~ claim~~s~~ 1.

~~18~~13. The AAV viral particle of claim ~~17~~12,

~~(i) which is an AAV, adenoviral, or lentiviral viral particle; and/or~~

~~(ii) which is an AAV viral particle; and/or~~

~~(iii)~~ wherein the recombinant genome ~~further~~ comprises~~:~~

~~a) AAV2 ITRs;~~

~~b) a poly A sequence;~~

~~c) an origin of replication; and/or~~

~~d)~~ two resolvable ITRs~~; and/or~~

~~(iv) wherein the recombinant genome is single-stranded~~.

~~19~~14. The AAV viral particle of claim ~~17~~ 12~~or claim 18~~, wherein ~~on~~ after transduction into a population of Huh7 cells, the AAV viral particle expresses the Factor IX protein or a fragment thereof, wherein the Factor IX protein or the fragment thereof has an activity ~~having a Factor IX activity~~ greater than ~~the~~ an activity of a Factor IX protein or a fragment thereof expressed from a comparable AAV viral particle comprising a Factor IX nucleotide sequence of SEQ ID NO: 12 and a transcription regulatory element of SEQ ID NO. 7 transduced into a comparable population of Huh7 cells.

~~20. The polynucleotide or viral particle of any one of the preceding claims, wherein the Factor IX fragment is at least 200, at least 250, at least 300, between 200 and 415, between 250 and 415, or between 300 and 415 amino acids in length.~~

~~21. A composition comprising the polynucleotide or viral particle of any one of the preceding claims and a pharmaceutically acceptable excipient.~~

~~22. The polynucleotide, viral particle or composition of any one of the preceding claims for use in a method of treatment.~~

~~23. The polynucleotide, viral particle or composition for use of claim 22, wherein the method of treatment comprises administering an effective amount of the polynucleotide, viral particle or composition of any one of claims 1-21 to a patient.~~

~~24. The polynucleotide, viral particle, or composition, for use of claim 22 or claim 23, wherein:~~

~~(i) the method of treatment is a method of treating haemophilia; and/or~~

~~(ii) the method of treatment is a method of treating haemophilia B; and/or~~

~~(iii) the patient has antibodies or inhibitors to Factor IX.~~

~~25. The polynucleotide, viral particle or composition of any one of claims 1-21 for use in achieving a stable Factor IX activity level of at least 20% in a patient having haemophilia B, optionally wherein achieving a stable Factor IX activity level comprises administering the polynucleotide, viral particle or composition of any one of claims 1-21 to a patient.~~

~~26. The polynucleotide, viral particle or composition of any one of claims 1-21 for use in treating haemophilia B comprising administering the polynucleotide, viral particle or composition to a patient at a dose effective to achieve a stable Factor IX activity level of at least 20% in a patient.~~

~~27. The polynucleotide, viral particle, or composition for use of claim 25 or 26, wherein:~~

~~(a) the Factor IX activity level is stable after at least 10 weeks, at least 15 weeks, at least 20 weeks, at least 30 weeks, at least 40 weeks, or at least 50 weeks from administration of the polynucleotide, viral particle or composition;~~

~~(b) the patient is a patient who is refractory to treatment with Factor IX having an activity lower than the activity of Factor IX expressed from a viral particle~~

~~comprising (i) a Factor IX nucleotide sequence of SEQ ID NO. 4 or 5 and (ii) a transcription regulatory element of SEQ ID NO. 6;~~

~~(c) the patient is a patient who is refractory to treatment with a Factor IX polypeptide that is expressed at a level lower than that encoded by a nucleotide sequence comprising (i) a Factor IX nucleotide sequence of SEQ ID NO. 4 or 5 and (ii) a transcription regulatory sequence of SEQ ID NO. 6;~~

~~(d) the polynucleotide, viral particle or composition is administered at a dose effective to achieve a stable Factor IX activity level of at least 20% in a patient having haemophilia B;~~

~~(e) the polynucleotide, viral particle or composition is administered at a dose of at least 4.5×10^{11} vg/kg, or at a dose of less than 5×10^{11} vg/kg, or at a dose of between 4.5×10^{11} and 1×10^{12} vg/kg, or at a dose of between 4.5×10^{11} vg/kg and 4.9×10^{11} vg/kg, optionally wherein the dose is determined by quantifying the number of vector genomes using qPCR, optionally wherein the qPCR uses primers which bind to a promoter region, optionally wherein the promoter region is the promoter region of a transgene cassette;~~

~~(f) the polynucleotide or viral particle that is administered is produced from a mammalian cell and/or possesses characteristics which result from use of mammalian viral vector production cells and distinguish from vectors produced in insect viral vector production cells (e.g. baculovirus system);~~

~~(g) the stable Factor IX activity level is a stable Factor IX activity level of at least 25% or of at least 30%, optionally wherein the stable Factor IX activity level is a stable Factor IX activity level of at least 35%, optionally wherein the stable Factor IX activity level is of at least or around 40%;~~

~~(h) the Factor IX activity level is at least 20% at a time point at least 50 weeks or at around 52 weeks after administration of the polynucleotide, viral particle or composition, optionally wherein the Factor IX activity level maintains at at least 20% for a continuous period of at least 40 weeks immediately preceding the time point;~~

~~(i) the Factor IX activity level is at least 25% at a time point at least 50 weeks or at around 52 weeks after administration of the polynucleotide, viral particle or composition, optionally wherein the Factor IX activity level maintains at at least~~

~~25% for a continuous period of at least 40 weeks immediately preceding the time point;~~

~~(j) the Factor IX activity is at least or around 40% at a time point at least 50 weeks or at around 52 weeks after administration of the polynucleotide, viral particle or composition, optionally wherein the Factor IX activity level maintains at at least 40% for a continuous period of at least 40 weeks immediately preceding the time point;~~

~~(k) the Factor IX activity is measured using a clotting assay to determine the Factor IX activity in a blood sample from the patient, optionally wherein the clotting assay uses a SynthASil™ kit;~~

~~(l) the Factor IX activity is measured by taking a blood sample from the patient and using a clotting assay to determine the Factor IX activity in the blood sample, optionally wherein the clotting assay uses a SynthASil™ kit;~~

~~(m) the clotting assay is a one-stage clotting assay, optionally wherein the one-stage clotting assay uses a SynthASil™ kit; and/or~~

~~(n) the use comprises co-administration of a steroid, optionally wherein the steroid is prednisolone.~~