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Inducible AAV Rep Genes

The present invention relates to host cells comprising a nucleic acid encoding Adeno-associated virus (AAV) Rep proteins Rep78 and Rep68, wherein the internal
5 AAV promoter p19 has been inactivated by one or more mutations that maintain the functionality of said Rep78 and Rep68 proteins. The present invention further relates to respective nucleic acids and vectors comprising the same, as well as respective methods for the production of AAV.

10 Recently there has been a rapid increase in the number of gene therapy trials and products based on Adeno-associated virus (AAV)-derived vectors. Advantages of AAV vectors in gene therapy are a good safety profile, the fact that such vectors are not pathogenic, *i.e.*, are not associated with any disease, the stable expression of transgenes, and the possibility of transducing dividing as well as non-dividing cells.

15 The production of recombinant AAV *inter alia* requires the expression of AAV Rep and Cap proteins, usually encoded by the AAV genome, for production of recombinant virus supplied *in trans*. Further, helper genes must be used which can be derived from different helper viruses, the most common being helper virus genes
20 taken from Adenovirus (AV), such as E1A, E1B, E2A, E4orf6, or VA RNA. Furthermore, a transfer vector containing the gene of interest (GOI) is needed.

Current production systems for AAV rely mostly on the following techniques which, however, have several drawbacks. Transient transfection of AAV rep genes, e.g.
25 using a three-plasmid system comprising a transfer vector containing the gene of interest, a plasmid with adenoviral helper functions, and a plasmid supplying the capsid and replicase functions, lacks scalability, robustness, reproducibility, and entails high costs of GMP-grade DNA. Producer cell lines which are mostly based on HeLa cells still need infection with helper virus, thus requiring extensive
30 purification and costly proof of the absence of helper viruses. Inducible expression of AAV rep genes by way of insertion of a stop cassette into the rep locus downstream of the p19 promoter requires the insertion of an artificial intron that contains a stop signal (e.g. SV40 Poly(A)) flanked by two loxP sites. In addition,

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cells need the Cre recombinase that recognizes the loxP sites and excises the stop cassette. This has either to be supplied inducibly or by a modified adenoviral vector, requiring the costly proof of the absence of helper virus. Further, Cre-mediated recombination often shows only a low efficiency.

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Accordingly, current AAV production systems are limited with respect to scalability, robustness, reproducibility, ease of use and cost efficiency. Thus, a scalable system for the stable production of AAV vectors that does not require transient transfection or helper virus is highly desirable.

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Accordingly, the technical problem underlying the present invention is to provide respective host cells, nucleic acids, vectors and methods constituting such a system.

The solution to the above technical problem is achieved by the embodiments
15 characterized in the claims.

In particular, in a first aspect, the present invention relates to a host cell comprising a nucleic acid encoding Adeno-associated virus (AAV) Rep proteins Rep78 and Rep68, wherein the internal AAV promoter p19 has been inactivated by one or more
20 mutations that maintain the functionality of said Rep78 and Rep68 proteins.

In this context, stable producer cell lines for the production of AAV are difficult to generate, as Rep proteins are toxic for cells. The expression of Rep proteins is regulated by E1A that is also necessary for AAV production. In cell lines such as
25 CAP cells, HEK293 cells or Per.C6 cells, E1A is already constitutively expressed. In total, four Rep proteins exist: two long ones (Rep78, Rep68) which are expressed from the p5 promoter, and two short ones (Rep52, Rep40) which are expressed from the internal p19 promoter that is located within the coding region of the long Rep proteins (see Figure 1 for a schematic overview).

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The p5 promoter can be replaced by an inducible promoter but not the internal p19 promoter which is part of the Rep78 and Rep68 coding region. Thus, the generation of packaging/producer cell lines based on cells constitutively expressing E1A is

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impossible, since this would result in a constitutive expression of toxic levels of Rep52 and Rep40. Other cell lines not constitutively expressing EA1 need inducible E1A or E1A supply, e.g. by infection with Adenovirus, entailing the drawbacks indicated above.

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This problem is advantageously solved by the present invention which is based on the inactivation of the internal AAV p19 promoter by mutations which prevent constitutive Rep52 and Rep40 expression while at the same time maintaining the functionality of said Rep78 and Rep68 proteins.

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The terms "Adeno-associated virus" and "AAV" as used herein are not limited to particular AAV serotypes. In this context, it should be noted that AAV Rep proteins are highly conserved among the different AAV serotypes. In particular embodiments, the above terms refer to Adeno-associated virus serotype 2 (AAV2).

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The term "maintain the functionality of said Rep78 and Rep68 proteins" as used herein refers to the fact that the mutations according to the present invention do not reduce the functional activity of said proteins, or reduce said activity at most by 30%, preferably at most 25%, at most 20%, at most 15%, at most 12.5%, at most 10%, at

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most 7.5%, at most 5%, at most 4%, at most 3%, at most 2%, or at most 1%.

In preferred embodiments, the one or more mutations according to the present invention are within at least one of the regulatory sites of the p19 promoter, preferably the SP1 -50 region (nucleotides 817 to 829), the TATA -20 region (nucleotides 843 to 849), or the TATA -35 region (nucleotides 830 to 835). Specifically, said one or more mutations can be within the SP1 -50 region, within the TATA -20 region, within the TATA -35 region, within both the SP1 -50 region and the TATA -20 region, within both the SP1 -50 regions and the TATA -35 region, within both the TATA -20 region and the TATA -35 region, or within all three regions, *i.e.*, in the SP1 -50 region, the TATA -20 region, and the TATA -35 region. In particular, it has been shown in the present invention that even mutation of a single nucleotide within one of said regions advantageously leads to significant reduction of Rep52 and Rep40 expression.

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In this context, all nucleotide positions as indicated herein refer to the AAV2 complete genome sequence available under GenBank accession number AF043303. The same applies to all amino acid positions. Further, Table 1 below shows an excerpt of the one-letter nucleotide nomenclature according to IUPAC.

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Table 1: One-letter nucleotide nomenclature according to IUPAC.

Letter	Nucleotides
W	A; T
S	C; G
M	A; C
K	G; T
R	A; G
Y	C; T
B	C; G; T
D	A; G; T
H	A; C; T
V	A; C; G

10 In preferred embodiments, the one or more mutations according to the present invention which inactivate the internal p19 promoter are silent mutations, *i.e.*, mutations that do not alter the encoded amino acid.

15 Preferably, said one or more mutations comprise at least one mutation, selected from the group consisting of mutations 731C>D, 732A>C, 734A>B, 737T>C, 746A>G, 749C>D, 752G>H, 758G>A, 761G>H, 764G>H, 818G>A, 824G>H, 830T>V, 833T>C, 845T>C, 846T>C, 848A>B or 848A>G, 849A>T, 850G>C, and 851C>D.

20 In cases where said one or more mutations are within the SP1 -50 region of the p19 promoter, said mutations comprise at least one mutation, selected from the group consisting of mutations 818G>A and 824G>H. In cases wherein said one or more

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mutations are within the TATA -20 region of the p19 promoter, said mutations comprise at least one mutation, selected from the group consisting of mutations 845T>C, 846T>C, 848A>B or 848A>G, and 849A>T. In cases wherein said one or more mutations are within the TATA -35 region of the p19 promoter, said mutations
5 comprise at least one mutation, selected from the group consisting of mutations 830T>V, and 833T>C.

In preferred embodiments, said one or more mutations comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or all 20 of the above mutations. Accordingly,
10 the host cells of the present invention can comprise a nucleic acid comprising any one, any two, and three, any four, any five, any six, any seven, any eight, any nine, any ten, any eleven, any twelve, any 13, any 14, any 15, any 16, any 17, any 18, any 19, or all 20 of the mutations 731C>D, 732A>C, 734A>B, 737T>C, 746A>G, 749C>D, 752G>H, 758G>A, 761G>H, 764G>H, 818G>A, 824G>H, 830T>V,
15 833T>C, 845T>C, 846T>C, 848A>B or 848A>G, 849A>T, 850G>C, and 851C>D.

The nucleic acid used in the present invention, encoding Adeno-associated virus (AAV) Rep proteins Rep78 and Rep68, wherein the internal AAV promoter p19 has been inactivated by silent mutations, is preferably a nucleic acid comprising at least
20 one nucleotide sequence, selected from the group consisting of the nucleotide sequences according to SEQ ID NO: 1 to 8, 11 to 14, and 34 to 42. In particularly preferred embodiments, said nucleic acid comprises the nucleotide sequence according to SEQ ID NO: 1, 6, 8, 13, 14, 34 to 39, 41, and 42.

25 In this context, the term "internal promoter" as used herein indicates the fact that the AAV p19 promoter is located within the coding sequence of Rep78 and Rep68, and forms a part of said coding sequence. Further, the term "inactivated" with respect to the AAV p19 promoter indicates the fact that by way of introducing silent mutations into the AAV p19 promoter region, said promoter has abolished or at least strongly
30 reduced (e.g. reduced by at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99%) promoter activity.

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The nucleotide sequences according to SEQ ID NOs: 1 to 8, 11 to 14, and 34 to 42 represent the AAV p19 promoter region (nucleotides 651 to 1053 of the AAV2 genome), wherein the mutation patterns indicated in Table 2 below are present.

- 5 In preferred embodiments, the nucleic acid used in the present invention, encoding Adeno-associated virus (AAV) Rep proteins Rep78 and Rep68, wherein the internal AAV promoter p19 has been inactivated by silent mutations, is preferably a nucleic acid comprising a nucleotide sequence, selected from the group consisting of the nucleotide sequences according to SEQ ID NOs: 15 to 22, 25 to 28, and 43 to 51.
- 10 In particularly preferred embodiments, said nucleic acid comprises the nucleotide sequence according to SEQ ID NO: 15, 20, 22, 27, 28, 43 to 48, 50, and 51.

The nucleotide sequences according to SEQ ID NOs: 15 to 22, 25 to 28, and 43 to 51 represent the AAV coding region for the Rep proteins Rep78, Rep68, Rep52 and

15 Rep40 (nucleotides 321 to 2252 of the AAV2 genome), wherein the p19 promoter region (nucleotides 651 to 1053 of the AAV2 genome) has been replaced by the mutated p19 promoter region containing the mutations indicated in Table 2 below.

Table 2: Preferred p19 promoter region mutation patterns.

SEQ ID NO:	Mutation pattern	Mutations
1/15	mut19	731C>D; 732A>C; 734A>B; 737T>C; 746A>G; 749C>D; 752G>H; 758G>A; 761G>H; 764G>H; 818G>A; 824G>H; 830T>V; 833T>C; 845T>C; 848A>G; 849A>T; 850G>C; 851C>D
2/16	mut5	845T>C; 848A>G; 849A>T; 850G>C; 851C>D
3/17	mut1	848A>G
4/18	mut2	849A>T; 850G>C
5/19	mut1-2	845T>C
6/20	mut14	731C>D; 732A>C; 734A>B; 737T>C; 746A>G; 749C>D; 752G>H; 758G>A; 761G>H; 764G>H; 818G>A; 824G>H; 830T>V; 833T>C

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7/21	mut10	731C>D; 732A>C; 734A>B; 737T>C, 746A>G; 749C>D; 752G>H; 758G>A; 761G>H; 764G>H
8/22	mut10-2	818G>A; 824G>H, 830T>V; 833T>C, 845T>C; 846T>C; 848A>B; 849A>T; 850G>C; 851C>D
11/25	mut2-3	846T>C; 848A>B
12/26	mut1-3	846T>C
13/27	mut5-2	845T>C, 846T>C; 848A>B, 849A>T, 850G>C
14/28	mut20	731C>D; 732A>C; 734A>B; 737T>C, 746A>G; 749C>D; 752G>H, 758G>A; 761G>H; 764G>H, 818G>A; 824G>H, 830T>V; 833T>C, 845T>C, 846T>C; 848A>B, 849A>T, 850G>C, 851C>D
34/43	L (SP1 -50)	818G>A; 824G>H
35/44	M (TATA -20)	830T>V; 833T>C
36/45	N (SP1 -50 1)	818G>A
37/46	O (SP1 -50 2)	824G>H
38/47	P (TATA -20 1)	830T>V
39/48	Q (TATA -20 2)	833T>C
40/49	R (SP1 -50 & TATA -35)	818G>A; 824G>H; 830T>V; 833T>C
41/50	S (SP1 -50 & TATA -20)	818G>A; 824G>H; 845T>C, 846T>C; 848A>B, 849A>T, 850G>C
42/51	T (TATA -20 & TATA -35)	830T>V; 833T>C; 845T>C, 846T>C; 848A>B, 849A>T, 850G>C

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In other preferred embodiments, the one or more mutations according to the present invention which inactivate the internal p19 promoter are mutations that result in one or more conservative amino acid exchanges, *i.e.*, amino acid exchanges that change an amino acid to a different amino acid with similar biochemical properties (e.g. regarding charge, hydrophobicity or size). Preferably, said one or more amino acid exchanges are amino acid exchanges occurring within the class of aliphatic amino acids (Gly, Ala, Val, Leu, Ile), within the class of hydroxyl- or sulphur/selenium-containing amino acids (Ser, Cys, Sec, Thr, Met), within the class of basic amino acids (His, Lys, Arg), or within the class of acidic amino acids (Asp, Glu, Asn, Gln).

In particular embodiments, said one or more amino acid exchanges comprise the amino acid exchanges Leu176>Ala and/or Ala168>Gly.

In this respect, the nucleic acid used in the present invention, encoding Adeno-associated virus (AAV) Rep proteins Rep78 and Rep68, wherein the internal AAV promoter p19 has been inactivated by mutations resulting in conservative amino acid exchanges, is preferably a nucleic acid comprising at least one nucleotide sequence, selected from the group consisting of the nucleotide sequences according to SEQ ID NOs: 9 and 10.

In this context, the term "inactivated" with respect to the AAV p19 promoter indicates the fact that by way of introducing mutations resulting in conservative amino acid exchanges into the AAV p19 promoter region, said promoter has abolished or at least strongly reduced (e.g. reduced by at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99%) promoter activity.

The nucleotide sequences according to SEQ ID NOs: 9 and 10 represent the AAV p19 promoter region (nucleotides 651 to 1053 of the AAV2 genome), wherein the mutation patterns indicated in Table 3 below are present.

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In preferred embodiments, the nucleic acid used in the present invention, encoding Adeno-associated virus (AAV) Rep proteins Rep78 and Rep68, wherein the internal AAV promoter p19 has been inactivated by mutations resulting in conservative amino acid exchanges, is a nucleic acid comprising at least one nucleotide sequence, selected from the group consisting of the nucleotide sequences according to SEQ ID NOs: 23 and 24.

The nucleotide sequences according to SEQ ID NOs: 23 and 24 represent the AAV coding region for the Rep proteins Rep78, Rep68, Rep52 and Rep40 (nucleotides 321 to 2252 of the AAV2 genome), wherein the p19 promoter region (nucleotides 651 to 1053 of the AAV2 genome) has been replaced by the mutated p19 promoter region containing the mutations indicated in Table 3 below.

Table 3: Preferred p19 promoter region mutation patterns.

SEQ ID NO:	Mutation pattern	Mutations	Resulting amino acid exchange
9/23	mut3	846T>G, 847T>C, 848A>B	Leu176>Ala
10/24	mut2-2	823C>G, 824G>H	Ala168>Gly

The nucleic acid comprised in the host cell of the present invention can further comprise at least one element, selected from the group consisting of inducible promoters, poly(A) regions, selection markers, IRES sequences and enhancing elements. Suitable inducible promoters are not particularly limited and are known in the art, e.g. Tet-inducible promoters such as the third generation TRE3G-promoter. Suitable poly(A) regions are not particularly limited and are known in the art, e.g. the SV40 poly(A) region. Suitable selection markers are not particularly limited and are known in the art, e.g. antibiotic resistance cassettes such as blasticidin or ampicillin resistance cassettes.

The present invention also relates to host cells comprising a nucleic acid having at least 70% sequence identity to a nucleic acid as defined above, provided that the specific mutations defined above are present. In this context, the term "provided that

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the specific mutations defined above are present" refers to the following situations (i) to (xxiii):

- (i) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 1 or 15, the mutations 731C>D; 732A>C; 734A>B; 737T>C; 746A>G; 749C>D; 752G>H; 758G>A; 761G>H; 764G>H; 818G>A; 824G>H; 830T>V; 833T>C; 845T>C; 848A>G; 849A>T; 850G>C; and 851C>D are present;
- (ii) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 2 or 16, the mutations 845T>C; 848A>G; 849A>T; 850G>C; and 851C>D are present;
- (iii) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 3 or 17, the mutation 848A>G is present;
- (iv) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 4 or 18, the mutations 849A>T; and 850G>C are present;
- (v) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 5 or 19, the mutation 845T>C is present;
- (vi) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 6 or 20, the mutations 731C>D; 732A>C; 734A>B; 737T>C; 746A>G; 749C>D; 752G>H; 758G>A; 761G>H; 764G>H; 818G>A; 824G>H; 830T>V; and 833T>C are present;
- (vii) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 7 or 21, the mutations 731C>D; 732A>C; 734A>B; 737T>C; 746A>G; 749C>D; 752G>H; 758G>A; 761G>H; and 764G>H are present;
- (viii) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 8 or 22, the mutations 818G>A; 824G>H; 830T>V; 833T>C; 845T>C; 846T>C; 848A>B; 849A>T; 850G>C; and 851C>D are present;
- (ix) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 9 or 23, the mutations 846T>G, 847T>C, and 848A>B are present;
- (x) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 10 or 24, the mutations 823C>G, and 824G>H are present;
- (xi) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 11 or 25, the mutations 846T>C; and 848A>B are present;

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- (xii) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 12 or 26, the mutation 846T>C is present;
- (xiii) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 13 or 27, the mutations 845T>C, 846T>C; 848A>B, 849A>T, and 850G>C are present;
- (xiv) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 14 or 28, the mutations 731C>D; 732A>C; 734A>B; 737T>C, 746A>G; 749C>D; 752G>H, 758G>A; 761G>H; 764G>H, 818G>A; 824G>H, 830T>V; 833T>C, 845T>C, 846T>C; 848A>B, 849A>T, 850G>C, and 851C>D are present;
- (xv) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 34 or 43, the mutations 818G>A and 824G>H are present;
- (xvi) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 35 or 44, the mutations 830T>V and 833T>C are present;
- (xvii) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 36 or 45, the mutation 818G>A is present;
- (xviii) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 37 or 46, the mutation 824G>H is present;
- (xix) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 38 or 47, the mutation 830T>V is present;
- (xx) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 39 or 48, the mutation 833T>C is present;
- (xxi) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 40 or 49, the mutations 818G>A; 824G>H; 830T>V; and 833T>C are present;
- (xxii) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 41 or 50, the mutations 818G>A; 824G>H; 845T>C, 846T>C; 848A>B, 849A>T, and 850G>C are present; and
- (xxiii) in nucleotide sequences derived from the nucleotide sequences according to SEQ ID NOs: 42 or 51, the mutations 830T>V, 833T>C, 845T>C, 846T>C; 848A>B, 849A>T, and 850G>C are present.

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Preferably, said nucleic acids have at least 70%, 72%, 74%, 76%, 78%, 80%, 82%, 84%, 86%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.2%, 98.4%, 98.6%, 98.8%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to a nucleic acid as defined above. In particular embodiments, such nucleic acids have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotide deletions, insertions or replacements (exchanges).

Respective nucleic acids having a defined sequence identity to a nucleic acid as defined above or having specific nucleotide deletions, insertions or replacements with respect to a nucleic acid as defined above exclude any nucleic acids having any kind of frameshift mutation, as well as any nucleic acids encoding non-functional Rep78 and Rep68 proteins, *i.e.*, said nucleic acids still encode functional Rep78 and Rep68 proteins.

In preferred embodiments, the nucleic acid comprised in the host cells of the present invention is stably integrated into the host cell genome. In other preferred embodiments, the nucleic acid comprised in the host cells of the present invention is comprised in a vector, *i.e.*, is part of a vector. Said vector is preferably a vector selected from the group consisting of plasmid vectors, viral vectors, cosmid vectors, and artificial chromosome vectors. Preferably said vector is an expression vector.

According to the present invention, the internal AAV p19 promoter is inactivated by introduction of silent mutations or mutations resulting in conservative amino acid exchanges. In this manner, a nucleic acid encoding Rep78 and Rep68 can be placed under the control of an inducible promoter, wherein no constitutive expression of Rep52 and Rep40 occurs. However, since production of AAV requires all four Rep proteins, an additional nucleic acid encoding Rep52 and Rep40 can be placed under the control of the same promoter, an identical promoter or a different promoter. Thus, in specific embodiments, the host cell of the present invention further comprises a nucleic acid encoding AAV Rep proteins Rep52 and Rep40 under the control of an inducible promoter. This nucleic acid can be part of the vector as defined above, or of the nucleic acid encoding Rep78 and Rep68.

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Host cells suitable for the present invention are not particularly limited and are known in the art. Preferably, said host cells display constitutive E1A expression. In preferred embodiments, said host cells are CAP cells, HEK293 cells or Per.C6 cells, *i.e.*, are derived from said cell lines.

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Methods for generating the host cells of the present invention, *i.e.*, methods for the introduction of the nucleic acids of the present invention into suitable host cells, are not particularly limited and are known in the art. The same applies to methods for the generation of the nucleic acids and vectors of the present invention.

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In a second aspect, the present invention relates to the nucleic acids and vectors as defined above.

In a third aspect, the present invention relates to a method for the production of Adeno-associated virus (AAV), comprising the step of recombinantly expressing AAV Rep proteins Rep78 and Rep68 in a host cell according to the present invention.

Respective methods for generating the necessary nucleic acids, vectors, and/or host cells, as well as respective expression methods, are not particularly limited and are known in the art.

Preferably, the method of the present invention further comprises the step of recombinantly expressing AAV Rep proteins Rep52 and Rep40 in said host cells.

As used herein, the term "comprising"/"comprises" expressly includes the terms "consisting essentially of"/"consists essentially of" and "consisting of"/"consists of", *i.e.*, all of said terms are interchangeable with each other.

According to the present invention, host cells are provided that can express AAV Rep proteins Rep78 and Rep68 without constitutive expression of Rep52 and Rep40. This is achieved by providing nucleic acids wherein the internal AAV p19 promoter region is inactivated by introducing specific mutations.

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Promoters are activated by binding of specific transcription factors and basal transcription complex; these factors recognize specific binding sites within the promoter region that have previously been described for the p19 promoter. Mutating these binding sites abolishes activation of the promoter. Since the integrity of the long Rep proteins has to be maintained, mutations are chosen that do alter the nucleotide sequence but within the genetic code encode the same amino acid and, therefore, result in formation of the same protein (silent mutations), or mutations are chosen that alter the nucleotide sequence resulting in conservative amino acid exchanges.

After introducing the above mutations, it is possible to separate the expression units for long and short Rep proteins: The expression cassette for the long Rep proteins contains the mutated p19 promoter with said mutations. The isolated expression unit for the short Rep proteins starts downstream of the p19 promoter with the start codon. Expression of both expression units can then be placed under regulation of an inducible promoter (as e.g. Tet inducible promoters). Based on this, a stable packaging/producer cell line can be generated.

The host cells, nucleic acids, vectors, and methods of the present invention represent a system for the production of AAV that is advantageously characterized by superior reproducibility, ensuring consistent quality, scalability, and cost efficiency, which does not need the use of helper virus.

The figures show:

Figure 1:

Schematic overview of the rep locus.

Figure 2:

Schematic overview of expression construct for inducible Rep proteins.

Figure 3:

Overview of analyzed mutation patterns with SEQ ID NOs. of the corresponding AAV2 Rep proteins coding region (cf. also Table 2). The indication "SEQ-ID" in Figure 3 refers to the respective SEQ ID NOs.

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Figure 4:

Expression of different Rep proteins in CAP cells upon transfection with different constructs containing silent mutations within the regulatory sequences of the p19 promoter (Table 2) and the wildtype construct (wt). Protein levels were detected in cell lysates of transiently transfected CAP-T cells 72 h post transfection using anti-replicase antibody (Progen). As control, cell lysate of non-transfected cells was included (BI). The indication of "ID" numbers in Figure 4 refers to the respective SEQ ID NOs.

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15 Figure 5:

Quantification of anti-Rep western blots. Rep protein bands were quantified by densitometric analysis using ImageJ. Ratio of long to short Rep protein bands was calculated and wt was set to 1. All other values were normalized to wt. The indication of "ID" numbers in Figure 5 refers to the respective SEQ ID NOs.

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Figure 6:

Inducible expression of the Rep proteins in a stable CAP derived cell line upon induction with 1 µg/mL doxycycline. Rep proteins were detected by immunoblot with anti-Replicase antibody (Progen). As control, cell lysate of non-induced cells was loaded (-Dox). At ~ 80 kDa, an unspecific background band is detected.

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Figure 7:

Induction of Rep proteins by addition of 1 µg/mL doxycycline in single cell clones derived from the stable cell line. Cells were transiently transfected with necessary components for rAAV5 production and induced with 1 µg/mL doxycycline 5 h after transfection. 72 h post transfection, cell lysates were prepared and expression of Rep proteins was detected by immunoblot with anti-Replicase antibody (Progen). The clones 5B6 and 2E3 do express very low levels of the long Rep proteins.

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Figure 8:

Viral titers of rAAV5 production by inducible Rep expressing clones. Viral genomes/mL were measured by qPCR with a primer/probe combination detecting the CMV-promoter using linearized transfer plasmid as standard. The single cell clones 5B6 and 2E3 do not show clear expression of long and short Rep proteins and therefore, do also only produce very low titers of rAAV5.

Figure 9:

(A & B): Expression of different Rep proteins in CAP cells upon transfection with different constructs containing mutations within the regulatory sequences of the p19 promoter (Table 3) resulting in conservative amino acid exchanges and the wildtype construct (wt). Protein levels were detected in cell lysates of transiently transfected CAP-T cells 72 h post transfection using anti-replicase antibody (Progen). As control, cell lysate of non-transfected cells was included (BI).

(C): Viral production upon transfection of CAP cells with the following construct combination: pStbl-Rep-p19mut-ID 47, 48 or 37 or wt, pStbl-TRE3G-Rep50/42, pCMV-Tet3G, pHelper, pStbl-CMV-Cap5, pAAV-GFP. Viral genomes/mL were measured by qPCR with a primer/probe combination detecting the SV40 PolyA using linearized transfer plasmid as standard.

The indication of "ID" numbers in Figure 9 refers to the respective SEQ ID NOs.

The present invention will be further illustrated in the following examples without being limited thereto.

Examples

Experimental procedures:

Cloning of expression constructs.

Synthesis of the rep locus of AAV2 with *HpaI* restriction sites at each end and regulatory sites within the p19 promoter. The genomic sequence of AAV2 was

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derived from GenBank: AF043303 (nucleotides 162 to 2332). The sequence of the synthetic locus is shown in SEQ ID NO: 29.

5 Different constructs for the p19 promoter region (nucleotides 651 to 1053 of the AAV2 genome) were designed containing different numbers of silent mutations within the regulatory sequences (Table 2) and produced synthetically.

Respective expression constructs were produced by standard cloning techniques and verified by sequencing. Components of the final expression constructs pStbl-
10 bsd-Rep, pStbl-bsd-Rep-p19mut-SEQ ID NO: 1-14, 34-42 were the p5 promoter, the Rep locus containing either mutated or wt p19 promoter, a SV40 poly(A), a blasticidin selection cassette under the control of human Ubc promoter, an enhancing element for stable transcription of integrated ORFs, a pUC ori for propagation in *E. coli*, and an ampicillin resistance cassette for selection in *E. coli*.

15

A construct placing the Rep proteins under the control of a Tet-inducible promoter of the third generation (TRE3G-promoter) (Figure 2) was produced by standard cloning techniques and verified by sequencing. The final sequence of this construct pStbl-bsd-TRE3G-Rep52/40-IRES-Rep78/68, starting from the TRE3G promoter
20 until the SV40 poly(A) is shown in SEQ ID NO: 30. Components of pStbl-bsd-TRE3G-Rep52/40-IRES-Rep78/68 are the TRE3G promoter, the Rep locus starting from start codon of short Rep proteins, an IRES sequence of ECMV, the Rep locus containing mutated p19 promoter starting from start codon of long Rep proteins, a SV40 poly(A), a blasticidin selection cassette under the control of human Ubc
25 promoter, and an enhancing element for stable transcription of integrated ORFs.

Cell culture.

30 CAP cells were routinely cultivated in chemically defined, serum-free PEM medium (Thermo Fisher Scientific) supplemented with 4 mM L-alanyl-L-glutamine (Biochrom, Germany) in shake flasks (125 mL; Corning) on a shaking incubator at 185 rpm (5 cm orbit), 5 % CO₂ and 37 °C.

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During routine cultivation, cells were diluted with fresh medium to a viable cell density of 1×10^6 cells/ml every 72 to 96 h. Viable cell density and viability were determined by trypan blue exclusion using a CEDEX XS cell counter (Innovatis, Roche Applied Science). Stable cell line expressing the Tet-on-3G-activator was cultivated in presence of 25 µg/mL G418; upon nucleofection with the pStbl-bsd-TRE3G-Rep50/42-IRES-Rep78/68 5 µg/mL blasticidin were added.

Transient transfection and Western blot to test for Rep protein expression.

Transient transfection was performed using PEI_{max} (PolySciences) in FreeStyle 293 medium (Thermo Fisher Scientific). 5 h post transfection, cells were fed with complete PEM medium (Thermo Fisher Scientific). An overview of generated transient transfection pools is found in Table 4, below.

Western Blot analysis was performed with cell lysates from 1×10^5 transfected cells utilizing mouse-anti-Replicase antibody (Progen, Germany) and horseradish peroxidase labeled anti-mouse antibody (Cell Signaling). Proteins were detected using the Pierce ECL WB Substrate Kit via chemiluminescence detector (INTAS).

Nucleofection and generation of stable pools.

Stable pools were generated using Lonza's Nucleofector according to the manufacturer's instructions. A stable CAP cell line expressing the Tet-on-3G transactivator of the third generation was used for nucleofection with the inducible Rep-expression construct. For each nucleofection reaction, 1×10^7 cells were harvested by centrifugation ($150 \times g$, 5 min). The cells were resuspended in 100 µL complete nucleofector solution V (Lonza) and mixed with 5 µg of the linearized expression vector. The DNA/cell suspension was transferred into a cuvette and the nucleofection was performed using the X001 program. The transfected cells were transferred into 12.5 mL growth medium and cultured as described before at 37 °C, 5 % CO₂ at 185 rpm.

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For generation of stable pools, cells were pelleted by centrifugation and resuspended in selection medium (see Table 4) 72 to 96 h post-transfection following cultivation in a shaking incubator as described before.

- 5 Table 4: Generated CAP-T transient expression and CAP stable pools with corresponding expression vectors and media.

Cell line	Plasmid	Selection medium
CAP-T	pStbl-bsd-Rep wt	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 15	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 16	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 17	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 18	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 19	transient
CAP-T	pStbl-bsd-Rep p19mut ID 20	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 21	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 22	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 25	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 26	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 27	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 28	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 43	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 44	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 45	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 46	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 47	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 48	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 49	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 50	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 51	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 23	transient
CAP-T	pStbl-bsd-Rep p19mut-ID 24	transient
CAP-Tet-on-3G	pStbl-bsd-TRE3G-Rep50/42-IRES-Rep78/68	PEM + 4 mM Gln + 5 µg/mL blasticidin + 25 µg/mL G418

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The indication of "ID" numbers in the above Table 4 refers to the respective SEQ ID NOs.

5 *Induction and transient transfection to test for AAV production.*

To test for AAV production by the stable single cell clones with inducible Rep expression, transient transfections were performed as described before. The following three constructs were used to provide the additional components for
10 production of rAAV5 in a ratio of 1:1:0.5 (pAAV-GFP : pHelper : pStbl-CMV-Cap5). 5 h post transfection, a final concentration of 1 µg/mL doxycycline (Clontech) was added to induce expression of the Rep proteins.

72 h post transfection, cell suspension was harvested. Cells were lysed by addition
15 of 0.5 % Triton-X and incubation for 30 min at 37 °C with 1300 rpm. After centrifugation, supernatants were diluted 10-fold with buffer (50 mM Tris/HCl, pH 8.0; 2 mM MgCl₂) and incubated with 125 U/mL benzonase (Merck Millipore) for 2 h at 37 °C. Addition of 2 mM EDTA and incubation at 70 °C for 10 min was used to inactivate the benzonase. Viral DNA was purified via the Pure Link Viral RNA/DNA
20 mini kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

qPCR to determine viral titer.

25 The following primer/dual-labelled probe combination (ordered at MWG, Eurofins; Table 5) directed against the CMV promoter or the SV40 poly A were used to measure the viral titer:

Table 5: Primer/probe combination used for measuring the viral titer

Primer/Probe	Sequence
CMV Primer for (SEQ ID NO: 31)	5'-AAATGGCCCGCCTGGCATTATG-3'

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CMV Primer rev (SEQ ID NO: 32)	5'-AAACCGCTATCCACGCCCATTG-3'
CMV Probe (SEQ ID NO: 33)	ROX-5'- ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATC- 3'-BHQ2
SV40 PolyA fw (SEQ ID NO: 52)	5'-AGCAATAGCATCACAAATTTACAA-3'
SV40 PolyA rev (SEQ ID NO: 53)	5'-CCAGACATGATAAGATACATTGATGAGTT-3'
SV40 PolyA Probe (SEQ ID NO: 54)	FAM-5'-AGC ATT TTT TTC ACT GCA TTC TAG TTG TGG TTT GTC-3'-BHQ1

As standard, linearized transgene plasmid with a defined copy number was used. The qPCR reaction contained the following components: 2 × Brilliant Multiplex qPCR Master Mix (Agilent), nuclease-free H₂O (Thermo Fisher Scientific), primer/probe mix and sample/standard. qPCR was run on an Agilent Mx3005P according to the manufacturer's instructions.

10 Example 1:

Effects of silent mutations on expression of REP proteins.

Introduction:

15 CAP cells are human amniocyte-derived suspension cells, which have been immortalized by stable transfection with a construct encoding E1A/E1B.

AAV Rep proteins are encoded by the AAV genome. There are in total four Rep proteins: Rep78 and Rep68 (expressed from p5 promoter); as well as Rep52 and Rep40 (expressed from p19 promoter located within coding region of long Rep proteins). These proteins mediate the replication and packaging of the AAV genome. However, said proteins are toxic for cells when stably expressed.

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For the inactivation of the internal p19 promoter by introduction of silent mutations, regulatory elements of the p19 promoter were identified and different silent mutations not affecting the final protein sequence were inserted into the nucleotide sequence of these regulatory elements.

5

Results:

Expression of long and short Rep proteins was successfully separated by introducing silent mutations in the regulatory sequences of the p19 promoter. Most versions with mutations showed significantly reduced expression of the short Rep proteins. The reduction in expression of the short Rep proteins was already visible upon introducing one single mutation becoming more pronounced when introducing up to 20 mutations (Figure 4, Figure 5). Separating the 5' and 3' motifs showed that especially the 3' motifs next to the promoter (SP1 -50; TATA -35, TATA -20) are important for the activation of the internal p19 promoter. Mutating only the TATA -20 motif led to a significant but not full reduction of expression of the short rep proteins suggesting that also SP1 -50 and TATA -35 are important for the activation of expression, supported further by the fact that mutating everything except the TATA -20 also resulted in a clear decrease of expression of the short rep proteins. Looking closer at the single motifs, especially the SP1 -50 motif and more exactly the mutation 824G>H resulted in a very clear reduction of the expression of the short rep proteins. In addition, mutation of TATA -20 clearly reduced expression of short rep proteins. For TATA -35, only the combination of the different mutations within the motif resulted in a very clear decrease with the mutation 848A>G having the biggest effect on expression.

To generate a stable cell line inducibly expressing Rep proteins, the genes for the long Rep proteins carrying the silent mutations and the genes for the short Rep proteins were separated and each put under control of an inducible promoter. For the further experiments, the p19mut construct with the 19 mutations was chosen.

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Example 2:

Generation of stable CAP clones inducibly expressing Rep proteins of AAV2.

Introduction:

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For the generation of an inducible expression cassette for AAV2 Rep proteins the p19mut variant with 19 silent mutations was selected.

10 Tet-inducible promoters of the third generation (TRE3G-promoter) were used to regulate expression of the Rep proteins by doxycycline addition (overview of construct see Figure 2). CAP cells expressing the Tet-on-3G transactivator were previously generated by nucleofection and selection with 25 µg/mL G418. The pStbl-bsd-TRE3G-Rep52/40-IRES-Rep78/68 construct was nucleofected in this stable pool and selected with 5 µg/mL blasticidin. Single cell cloning using limiting dilutions
15 was performed and clones were screened by western blot for Rep proteins.

Results:

20 A stable CAP cell pool carrying the Tet-on-3G transactivator and the pStbl-bsd-TRE3G-Rep52/40-IRES-Rep78/68 was analyzed by Western blot for expression of the different rep protein before and after induction with doxycyclin (Figure 6). Without Doxycyclin induction, no signals specific for the Rep proteins could be detected, whereas both long and short Rep proteins were expressed upon Doxycyclin induction.

25

From the stable pool, single cell-derived clones were generated by limiting dilution. The single cell-derived clones were analyzed for expression of the Rep proteins after Doxycyclin induction. Out of 5 clones, 3 clones expressed both the long and the short Rep proteins in the expected ratio, whereas 2 of the clones displayed
30 reduced levels of the long Rep proteins (Figure 7).

The data shows that using rep constructs with silent mutations in the p19 promoter, clonal cell populations with inducible expression of both long and short Rep proteins

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can be generated. Such clones can serve as a basis for the generation of packaging/producer cell lines.

5 Example 3:

Production of AAV in stable CAP clones inducibly expressing Rep proteins of AAV2.

Introduction:

- 10 To proof that the inducible Rep cell lines are capable of producing AAV vectors, the missing components for AAV production were transiently introduced into cells of the 5 different clones:

- Capsid proteins from AAV5, cloned under control of CMV promoter in pStbl vector,
15 the additional helper genes E2A, E4orf6, VA RNA, as well as the transfer vector with gene of interest (GOI): pAAV-GFP.

Results:

- 20 Using single cell clones of the stable Rep inducible expressing cell line, AAV production could be shown upon transfecting the cells with the lacking necessary components and doxycycline induction (Figure 8). The two clones with lower levels of long Rep proteins also showed lower AAV titers as expected. This proves that this approach results in production of viral vectors, and that the generation of a high
25 producing AAV packaging cell line is now possible.

Example 4:

AAV production using Rep proteins with conservative amino acid exchange

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For the inactivation of the internal p19 promoter by introduction of mutations in the promoter regions resulting in conservative amino acid exchanges, regulatory elements of the p19 promoter were identified. Three distinct mutations, 846T>G,

- 25 -

847T>C, 848A>B, were inserted into the nucleotide sequence of the TATA -20 region resulting in a conservative Leu176>Ala exchange (ID23). Two distinct mutations, 823C>G, 824G>H, were inserted into the nucleotide sequence of the SP1 -50 region resulting in a conservative Ala168>Gly exchange (ID24). The different constructs were transiently introduced into CAP-T cells and the expression level of the long and short Rep proteins was analyzed and compared to the wt and mut-20 (ID28) (Fig. 9 A, B).

To proof the functionality of the long rep proteins with conservative amino acid exchanges in the TATA -20 or the SP1 -50 region, AAV was produced by introducing the following construct combination transiently into the CAP-T cells: a construct coding for the long Rep proteins, either pStbl-Rep-p19mut-ID23, or -ID24, or -mut20, or -wt, together with the a construct encoding for the short Rep proteins (pStbl-TRE3G-Rep50/42), pCMV-Tet3G, and the additional helper genes E2A, E4orf6, VA RNA, as well as the transfer vector with gene of interest (GOI), pAAV-GFP (Fig. 9 C).

Results:

Western blot analysis revealed that the expression of long and short Rep proteins was successfully separated by introducing distinct mutations in the regulatory sequences of the p19 promoter resulting in conservative aa exchanges either in the SP1 -50 or in the TATA -20 region.

As before the mutation in the SP1 -50 region had a more pronounced effect that the introduced mutations in the TATA -20 box, confirming the important role of SP1 -50 (Fig. 9 A, B).

Importantly the functionality of the long Rep proteins harboring the Leu176>Ala (ID23) or Ala168>Gly (ID24) exchange could be proven by the production of AAV particle via transient production. The titer of the AAV particle produced with the Rep proteins harboring the conservative amino acid exchange are in the same range as

- 26 -

the wt or the mut-20 control, with some reduction of the AAV titer in the ID24 sample (Fig. 9C).

- 5 The present invention relates to the following nucleotide sequences.

SEQ ID NO: 1

AAV2 mutated p19 promoter region mut19

- 10 **bold**: regulatory sites within the p19 promoter
underlined: mutations in the p19 promoter region

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
 GGTTTCGCGGTCACAAAGAC**CD****CB****AA****CG****GC****CC****GG****GG****DG****GH****AA****CA****AG****TH****GT****TH**GATGAGTG
 15 CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCA**AT****TGGG****CH****TGG****AC****VA****AC**
AT**GGA****AC****AG****TACT****TGT****CD****GC****CT****GTT****TGA****AT****CT****CAC****GG****AG****CG****TAA****AC****GG****TT****TGG****CG****CAG****C**
 ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
 TCGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
 GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

20

SEQ ID NO: 2

AAV2 mutated p19 promoter region mut5

- bold**: regulatory sites within the p19 promoter
 25 underlined: mutations in the p19 promoter region

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
 GGTTTCGCGGTCACAAAGAC**CC****AG****AA****AT****TGG****CG****CC****GG****AG****GC****GG****GA****CA****AG****GT****TGG****AT****GAG****TG**
 CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCC**AG****TGGG****CG****TGG****ACT****TA****AT**
 30 **AT****GGA****AC****AG****TACT****TGT****CD****GC****CT****GTT****TGA****AT****CT****CAC****GG****AG****CG****TAA****AC****GG****TT****TGG****CG****CAG****C**
 ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
 TCGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
 GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

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SEQ ID NO: 3AAV2 mutated p19 promoter region mut1**bold:** regulatory sites within the p19 promoter5 underlined: mutations in the p19 promoter region

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAGTGGGCGTGGACTAAT
10 ATGGAACAGTATTTGAGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

15 SEQ ID NO: 4AAV2 mutated p19 promoter region mut2**bold:** regulatory sites within the p19 promoterunderlined: mutations in the p19 promoter region

20

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAGTGGGCGTGGACTAAT
ATGGAACAGTATTTATCCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
25 ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

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SEQ ID NO: 5AAV2 mutated p19 promoter region mut1-2**bold:** regulatory sites within the p19 promoter5 underlined: mutations in the p19 promoter region

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGAC**CCAGAAATGG**CGCCGG**GAGCGGGA**ACAAGGTGGTGGATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCC**AGTGGGCGTGGACTAAT**
10 **ATGGAACAGTACTTAAG**CGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

15 SEQ ID NO: 6AAV2 mutated p19 promoter region mut14**bold:** regulatory sites within the p19 promoterunderlined: mutations in the p19 promoter region

20

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGAC**DCGBAACGG**CGCCGG**GGGDGGHA**ACAA**AGTHGTH**GATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCC**AAATGGGCHTGGACVAAC**
ATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
25 ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

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SEQ ID NO: 7AAV2 mutated p19 promoter region mut10**bold:** regulatory sites within the p19 promoter5 underlined: mutations in the p19 promoter region

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACDCGBAACGGCGCCGGGGDGGHAACAAAGTHGTHGATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAGTGGGCGTGACTAAT
10 ATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

15 SEQ ID NO: 8AAV2 mutated p19 promoter region mut10-2**bold:** regulatory sites within the p19 promoterunderlined: mutations in the p19 promoter region

20

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGAC**CAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG**
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCC**AATGGGCHTGGACVAAC**
ATGGAACAGTACCTBTCDGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
25 ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

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SEQ ID NO: 9AAV2 mutated p19 promoter region mut3**bold:** regulatory sites within the p19 promoter5 underlined: mutations in the p19 promoter region

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAACT
 GGTTTCGCGGTCACAAAGAC**CAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG**
 CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCC**AGTGGGCGTG**GACT**TAAT**
 10 **ATGGAACAGTATGCB**AGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
 ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
 TCGCCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCTGGGTGGCTCGTG
 GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

15 SEQ ID NO: 10AAV2 mutated p19 promoter region mut2-2**bold:** regulatory sites within the p19 promoterunderlined: mutations in the p19 promoter region

20

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAACT
 GGTTTCGCGGTCACAAAGAC**CAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG**
 CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCC**AGTGGGGHT**GACT**TAAT**
ATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
 25 ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
 TCGCCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCTGGGTGGCTCGTG
 GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

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SEQ ID NO: 11AAV2 mutated p19 promoter region mut2-3**bold:** regulatory sites within the p19 promoter5 underlined: mutations in the p19 promoter region

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAACT
GGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAGTGGGCGTGGACTAAT
10 ATGGAACAGTATCTBAGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

15 SEQ ID NO: 12AAV2 mutated p19 promoter region mut1-3**bold:** regulatory sites within the p19 promoterunderlined: mutations in the p19 promoter region

20

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAACT
GGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAGTGGGCGTGGACTAAT
ATGGAACAGTATCTAAGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
25 ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

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SEQ ID NO: 13

AAV2 mutated p19 promoter region mut5-2**bold:** regulatory sites within the p19 promoter5 underlined: mutations in the p19 promoter region

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAGTGGGCGTGACTAAT
10 ATGGAACAGTACCTBTCCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

15 SEQ ID NO: 14AAV2 mutated p19 promoter region mut20**bold:** regulatory sites within the p19 promoterunderlined: mutations in the p19 promoter region

20

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACDCGBAACGGCGCCGGGGDGGHAACAAAGTHGTHGATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAATGGGCHTGGACVAAC
ATGGAACAGTACCTBTCDGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
25 ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

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SEQ ID NO: 15

AAV2 Rep proteins coding region with mutated p19 promoter region mut19**bold:** regulatory sites within the p19 promoter5 underlined: mutations in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
10 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAACCAACGGGGTGAAATC
CATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAACTGATTCAGAGAATTTACCGC
GGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTCACAAAGACDCGBAACGGCGCCGGG
GDGGHAACAAAGTHGTHGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
15 TGAGCTCCAATGGGCHTGGACVAACATGGAACAGTACTTGTCDGCCTGTTTGAATCTCACG
GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACTTCAGCCAGGTA
CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
20 CTGCCTTGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACTAAAC
GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA
AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
25 AACGACTGTGTGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTGCG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
30 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
GTACCAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA

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TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
 AGTGCTTTCCCGTGTGAGAATCTCAACCCGTTTCTGTGTCGTCAAAAAGGCGTATCAGAACT
 GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
 5 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 16

AAV2 Rep proteins coding region with mutated p19 promoter region mut5

10 **bold**: regulatory sites within the p19 promoter
underlined: mutations in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAAGTCCCCAGCGACCTTGACGAGCATCTGCCCC
 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
 15 TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
 TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAACACCGGGGTGAAATC
 CATGGTTTTTGGGACGTTTCCTGAGTCAGATTGCGGAAAACTGATTTCAGAGAATTTACCGC
 GGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAG
 20 GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
 TGAGCTCCAGTGGGCGTGACTAATATGGAACAGTACTTGTCDGCCTGTTTGAATCTCACG
 GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTGCGAGACGCAGGAGCAGAACA
 AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
 CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
 25 GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACTCGCGGTCCCAAATCAAGG
 CTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
 GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAATAAAC
 GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTTCGGCA
 AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
 30 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAACTGGACCAATGAGAACTTTCCCTTC
 AACGACTGTGTGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTGC
 TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
 CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG

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ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
 TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
 GGTGGAGCCAAGAAAAGACCCGCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
 5 GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
 GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
 TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
 AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTCTGTCAAAAAGGCGTATCAGAACT
 GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
 10 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 17AAV2 Rep proteins coding region with mutated p19 promoter region mut1

15

bold: regulatory sites within the p19 promoterunderlined: mutation in the p19 promoter region

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25

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ATGCCGGGGTTTTACGAGATTGTGATTAAAGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
 TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCCCGAGGCCCTTTTCTTTGTGCAAT
 TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGAAACCACCGGGGTGAAATC
 CATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAACTGATTCAGAGAATTTACCGC
 25 GGGATCGAGCCGACTTTGCCAACTGGTTTCGCGGTCAAAAGACCAGAAATGGCGCCGGAG
GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
 TGAGCTCC**AGTGGGCGTGGA**CT**AA**TATGGAACAGT**ATTTG**AGCGCCTGTTTGAATCTCACG
 GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTGCGAGACGCAGGAGCAGAAC
 AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
 30 CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
 GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACTCGCGGTCCCAATCAAGG
 CTGCCTTGGAACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
 GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACATAAC

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GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCCGGCA
 AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCTTC
 AACGACTGTGTGACAAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCG
 5 TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGACAGAAATGCAAGTC
 CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
 ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
 TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
 10 GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
 GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
 GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
 TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
 AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTGCTCAAAAAGGCGTATCAGAACT
 15 GTGCTACATTATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 1820 AAV2 Rep proteins coding region with mutated p19 promoter region mut2**bold:** regulatory sites within the p19 promoterunderlined: mutation in the p19 promoter region

25 ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTCT
 TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGCCGAGAAGCTGCAGCGC
 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
 TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAACCACCGGGGTGAAATC
 30 CATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAACTGATTCAGAGAATTTACCGC
 GGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTCAAAAGAC**CAGAAATGGCGCCGGAG**
GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
 TGAGCTCC**AGTGGGCGTGACTAATATGGAACAGTATTTAT**CCGCCTGTTTGAATCTCAGC

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GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
5 CTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACTAAAC
GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTTCGGCA
AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
10 AACGACTGTGTCGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCTG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
15 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
20 AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTGTCGTCAAAAAGGCGTATCAGAACT
GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

25 SEQ ID NO: 19AAV2 Rep proteins coding region with mutated p19 promoter region mut1-2**bold:** regulatory sites within the p19 promoterunderlined: mutation in the p19 promoter region

30

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCC
GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC

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GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCCGGAGGCCCTTTTCTTTGTGCAAT
TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAACCACCGGGGTGAAATC
CATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAACTGATTCAGAGAATTTACCGC
GGGATCGAGCCGACTTTGCCAACTGGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAG
5 GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
TGAGCTCCAGTGGGCGTGGACTAATATGGAACAGTACTTAAGCGCCTGTTTGAATCTCACG
GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
10 GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
CTGCCTTGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACTAAAC
GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA
AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
15 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCTTC
AACGACTGTGTCGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
20 TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
GCGAGTCAGTTGCGCAGCCATCGACGTGAGACGCGGAAGCTTCGATCAACTACGCAGACAG
GTACCAAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
25 TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTGTCGTCAAAAAGGCGTATCAGAACT
GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

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SEQ ID NO: 20

AAV2 Rep proteins coding region with mutated p19 promoter region mut14**bold:** regulatory sites within the p19 promoter5 underlined: mutation in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
10 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAACCAACGGGGTGAAATC
CATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAACTGATTCAGAGAATTTACCGC
GGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTCACAAAGACDCGBAACGGCGCCGGGG
GDGGHAACAAAGTHGHATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
15 TGAGCTCCAATGGGCHTGGACVAACATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACG
GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTGCGAGACGCAGGAGCAGAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACTTCAGCCAGGTA
CATGGAGCTGGTTCGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
20 CTGCCTTGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGAACATAAAC
GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA
AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGAAGACCAACATCGCGGAGGC
CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
25 AACGACTGTGTGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTGCG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
30 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
GTACCAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA

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TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
 AGTGCTTTCCCGTGTGAGAATCTCAACCCGTTTCTGTCTGTCAAAAAGGCGTATCAGAAACT
 GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
 5 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 21AAV2 Rep proteins coding region with mutated p19 promoter region mut10

10 **bold**: regulatory sites within the p19 promoter
underlined: mutation in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCC
 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
 15 TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCCGGAGGCCCTTTTCTTTGTGCAAT
 TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGAAACCACCGGGGTGAAATC
 CATGGTTTTGGGACGTTTCCTGAGTCAGATTTCGCGAAAACTGATTCAGAGAATTTACCGC
 GGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTCACAAAGACDCGBAACGGCGCCGGG
 20 GDGGHAACAAAGTHGTHGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
 TGAGCTCCAGTGGGCGTGACTAATATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACG
 GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTGCGAGACGCAGGAGCAGAACA
 AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACTTCAGCCAGGTA
 CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
 25 GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACTCGCGGTCCCAATCAAGG
 CTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
 GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACATAAC
 GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTTCGGCA
 AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
 30 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
 AACGACTGTGTGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTGC
 TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
 CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG

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ATTGACGGGAACTCAACGACCTTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
5 GCGAGTCAGTTGCGCAGCCATCGACGTGACACGCGGAAGCTTCGATCAACTACGCAGACAG
GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTCTGTCAAAAGGCGTATCAGAAACT
GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
10 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 22AAV2 Rep proteins coding region with mutated p19 promoter region mut10-2

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bold: regulatory sites within the p19 promoterunderlined: mutation in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
20 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAACCACCGGGGTGAAATC
CATGGTTTTGGGACGTTTCCTGAGTCAGATTTCGCGAAAACTGATTTCAGAGAATTTACCGC
25 GGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAG
GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
TGAGCTCCAATGGGCHTGGACVAACATGGAACAGTACCTBTCDGCCTGTTTGAATCTCACG
GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTGCGAGACGCAGGAGCAGAAAC
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
30 CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
GAGGACCAGGCCTCATACTCTCCTTCAATGCGGCCTCCAACTCGCGGTCCCAATCAAGG
CTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACATAAC

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GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTTCGGCA
 AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
 AACGACTGTGTGACACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCTG
 5 TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
 CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
 ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
 TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
 10 GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
 GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
 GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
 TGCAGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
 AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTCTGTCAAAAAGGCGTATCAGAACT
 15 GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 2320 AAV2 Rep proteins coding region with mutated p19 promoter region mut3**bold:** regulatory sites within the p19 promoterunderlined: mutation in the p19 promoter region

25 ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
 TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCCGGAGGCCCTTTTCTTTGTGCAAT
 TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAACCACCGGGGTGAAATC
 30 CATGGTTTTGGGACGTTTCTGAGTCAGATTCGCGAAAACTGATTCAGAGAATTTACCGC
 GGGATCGAGCCGACTTTGCCAACTGGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAG
 GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
 TGAGCTCCAGTGGGCGTGGACTAATATGGAACAGTATGCBBAGCGCCTGTTTGAATCTCACG

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GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
5 CTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACTAAAC
GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTTCGGCA
AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
10 AACGACTGTGTCGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
15 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
GCGAGTCAGTTGCGCAGCCATCGACGTGACGCGGAAGCTTCGATCAACTACGCAGACAG
GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
20 AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTGTCGTCAAAAGGCGTATCAGAACT
GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

25 SEQ ID NO: 24AAV2 Rep proteins coding region with mutated p19 promoter region mut2-2**bold:** regulatory sites within the p19 promoterunderlined: mutation in the p19 promoter region

30

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCC
GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTCT
TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC

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GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCCGGAGGCCCTTTTCTTTGTGCAAT
TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAACCACCGGGGTGAAATC
CATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAACTGATTCAGAGAATTTACCGC
GGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTACAAAGACCAGAAATGGCGCCGGAG
5 GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
TGAGCTCCAGTGGGGHTGGACTAATATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACG
GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
10 GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
CTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACTAAAC
GGGTACGATCCCCAATATGCGGCCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA
AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
15 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
AACGACTGTGTGCGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCTG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
20 TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTACCAAGCAGGAAGTCAAAGA
CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
25 TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTGTCGTCAAAAAGGCGTATCAGAACT
GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

30

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SEQ ID NO: 25

AAV2 Rep proteins coding region with mutated p19 promoter region mut2-3**bold**: regulatory sites within the p19 promoter5 underlined: mutation in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCC
GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATT
TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
10 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGAAACCACCGGGGTGAAATC
CATGGTTTTGGGACGTTTCCTGAGTCAGATTTCGCGAAAACTGATTCAGAGAATTTACCGC
GGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAG
GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
15 TGAGCTCCAGTGGGCGTGGAATAATATGGAACAGTATCTBAGCGCCTGTTTGAATCTCACG
GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTGCGCAGACGCAGGAGCAGAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACTTCAGCCAGGTA
CATGGAGCTGGTGGGTGGCTCGTGGAACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
20 CTGCCTTGGAACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGAACATAAAC
GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA
AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
25 AACGACTGTGTCGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
30 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
GTACCAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA

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TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
 AGTGCTTTCCCGTGTGAGAATCTCAACCCGTTTCTGTGTCGTCAAAAAGGCGTATCAGAAACT
 GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
 5 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 26

AAV2 Rep proteins coding region with mutated p19 promoter region mut1-3

- 10 **bold**: regulatory sites within the p19 promoter
underlined: mutation in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
 15 TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
 TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGAAACCACCGGGGTGAAATC
 CATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAACTGATTCAGAGAATTTACCGC
 GGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAG
 20 GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
 TGAGCTCCAGTGGGCGTGGACTAATATGGAACAGTATCTAAGCGCCTGTTTGAATCTCACG
 GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACAA
 AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
 CATGGAGCTGGTGGGTGGCTCGTGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
 25 GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
 CTGCCTTGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
 GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACATAAC
 GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA
 AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
 30 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
 AACGACTGTGTCGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCTG
 TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
 CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG

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ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
5 GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
AGTGCTTTCCCGTGTGAGAATCTCAACCCGTTTCTGTGCTCAAAAAGGCGTATCAGAACT
GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
10 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 27AAV2 Rep proteins coding region with mutated p19 promoter region mut5-2

15

bold: regulatory sites within the p19 promoterunderlined: mutation in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCC
20 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAACACCAGGGGTGAAATC
CATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAACTGATTTCAGAGAATTTACCGC
25 GGGATCGAGCCGACTTTGCCAACTGGTTCGCGGTCACAAAGAC**CAGAAATGGCGCCGGAG**
GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
TGAGCTCC**AGTGGGCGTGGA****CTAATATGGAACAGTACCTBT**CCGCCTGTTTGAATCTCACG
GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACAA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
30 CATGGAGCTGGTCGGGTGGCTCGTGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
CTGCCTTGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAATAAAC

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GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA
 AGAGGAACACCATCTGGCTGTTTGGGCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAACTGGACCAATGAGAACTTTCCCTTC
 AACGACTGTGTCGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCG
 5 TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
 CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
 ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
 TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
 10 GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
 GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
 GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
 TGCAGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
 AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTGCTCAAAAAGGCGTATCAGAACT
 15 GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 28

20 AAV2 Rep proteins coding region with mutated p19 promoter region mut20

bold: regulatory sites within the p19 promoter

underlined: mutation in the p19 promoter region

25 ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTCTG
 TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
 TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGAAACCACCGGGGTGAAATC
 30 CATGGTTTTGGGACGTTTCCCTGAGTCAGATTGCGGAAAACTGATTGAGAGAATTTACCGC
 GGGATCGAGCCGACTTTGCCAACTGGTTGCGGGTCACAAAGACDCGBAACGGCGCCGGGG
GDGGHAACAAAGTHGTHGATGAGTGCTACATCCCCAATTAATTGCTCCCCAAAACCCAGCC
 TGAGCTCCAATGGGCHTGGACVAACATGGAACAGTACCTBTCDGCCTGTTTGAATCTCACG

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GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTGCGAGACGCAGGAGCAGAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
5 CTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACTAAAC
GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTTCGGCA
AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
10 AACGACTGTGTCGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTGCG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
15 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
GCGAGTCAGTTGCGCAGCCATCGACGTGACGCGGAAGCTTCGATCAACTACGCAGACAG
GTACCAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
20 AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTGCTCAAAAAGGCGTATCAGAACT
GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

25 SEQ ID NO: 29Synthetic AAV2 rep locus**bold:** regulatory sites within the p19 promoterunderlined: HpaI restriction sites

30

GTAACTGACGTGAATTACGTCATAGGGTTAGGGAGGTCCTGTATTAGAGGTCACGTGAGT
GTTTTGCGACATTTTGGCAGACCATGTGGTCACGCTGGGTATTTAAGCCCGAGTGAGCACG
CAGGGTCTCCATTTTGAAGCGGGAGGTTTGAACGCGCAGCCGCCATGCCGGGGTTTTACGA

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GATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCGCATTTCTGACAGCTTT
GTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTCTGACATGGATCTGAATC
TGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGCGACTTTCTGACGGAATG
GCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAATTTGAGAAGGGAGAGAGC
5 TACTTCCACATGCACGTGCTCGTGGAAACCACCGGGGTGAAATCCATGGTTTTTGGGACGTT
TCCTGAGTCAGATTTCGCGAAAACTGATTGAGAGAATTTACCGCGGGATCGAGCCGACTTT
GCCAACTGGTTCGCGGTCAAAAGAC**CCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTG**
GATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCC**AGTGGGCGT**
GGACTAATATGGAACAG**TATTTAAG**CGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGT
10 GGCGCAGCATCTGACGCACGTGTGCGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCC
AATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCCGGT
GGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGGAGGACCAGGCCTCATA
CATCTCCTTCAATGCGGCCTCCAACTCGCGGTCCCAAATCAAGGCTGCCTTGGACAATGCG
GGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGTGGGCCAGCAGCCCGTGG
15 AGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAATAAACGGGTACGATCCCCAATA
TGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAGTTTCGGCAAGAGGAACACCATCTGG
CTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGCCATAGCCACACTGTGC
CCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTCAACGACTGTGTGACAA
GATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCGTGGAGTCGGCCAAAGCC
20 ATCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTCCTCGGCCCAGATAGACC
CGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTGATTGACGGGAAC**TC**AAC
GACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAATTTGAACTCACCCGCCGT
CTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGACTTTTTCCGGTGGGCAA
AGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAGGGTGGAGCCAAGAAAAG
25 ACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGCGCGAGTCAGTTGCGCAG
CCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAGGTACCAAAACAAATGTT
CTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAATGCGAGAGAATGAATCA
GAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAGAGTGCTTTCCCGTGTC
GAATCTCAACCCGTTTCTGTGCTCAAAAAGGCGTATCAGAACTGTGCTACATTCATCATA
30 TCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTCAATGTGGATTTGGATGA
CTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCCGATGGTTATCTTCCAGA
TTGGCTCGAGGACACTCTCTCTGAAGGAATAAGACAGTGGTGGAAGCTCAAACCTGGCCCA
CCACCACCAAAGCCCGCAGAGCGGCATAAGGACGACAGCAGGGGTTAAC

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SEQ ID NO: 30Inducible expression construct for AAV2 Rep proteins

5 GTTTACTCCCTATCAGTGATAGAGAACGTATGAAGAGTTTACTCCCTATCAGTGATAGAGA
ACGTATGCAGACTTTACTCCCTATCAGTGATAGAGAACGTATAAGGAGTTTACTCCCTATC
AGTGATAGAGAACGTATGACCAGTTTACTCCCTATCAGTGATAGAGAACGTATCTACAGTT
TACTCCCTATCAGTGATAGAGAACGTATATCCAGTTTACTCCCTATCAGTGATAGAGAACG
TATAAGCTTTAGGCGTGACGGTGGGCGCCTATAAAAGCAGAGCTCGTTTAGTGAACCGTC
AGATCGCCTGGAGCAATTCCACAACACTTTTGTCTTATACCAACTTTCCGTACCACTTCCT
10 ACCCTCGTAAAGTCGACACCGGGGCCAGATCTATCGATCGGCCGGATAACGCCACCATGG
AGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGGAGGA
CCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGGCTGCC
TTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGTGGGCC
AGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACATAACGGGTA
15 CGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCAAGAGG
AACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGCCATAG
CCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTCAACGA
CTGTGTGCGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCGTGGAG
TCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTCCTCGG
20 CCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTGATTGA
CGGGAACTCAACGACCTTCTGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAATTTGAA
CTCACC CGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGACTTTT
TCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAGGGTGG
AGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGCGCGAG
25 TCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAGGTACC
AAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAATGCGA
GAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAGAGTGC
TTTCCCGTGTCAGAATCTCAACCCGTTTCTGTGTCGTCAAAAGGCGTATCAGAACTGTGCT
ACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTCAATGT
30 GGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCCGATGG
TTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGAGATAACTGAGGGATAGAATTCGCC
CCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTAT
ATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTG

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TCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTT
GAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCG
ACCCTTTGCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCAC
GTGTATAAGATACACCTGCAAAGGCGGCACAACCCAGTGCCACGTTGTGAGTTGGATAGT
5 TGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAG
AAGGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTT
AGTCGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAA
AACACGATGATAATAGTTATCGCCGCCATGCCGGGGTTTTACGAGATTGTGATTAAGGTCC
CCAGCGACCTTGACGAGCATCTGCCCGGCATTTCTGACAGCTTTGTGAACTGGGTGGCCGA
10 GAAGGAATGGGAGTTGCCGCCAGATTCTGACATGGATCTGAATCTGATTGAGCAGGCACCC
CTGACCGTGGCCGAGAAGCTGCAGCGCGACTTTCTGACGGAATGGCGCCGTGTGAGTAAGG
CCCCGGAGGCCCTTTTCTTTGTGCAATTTGAGAAGGGAGAGAGCTACTTCCACATGCACGT
GCTCGTGGAACACCAGGGGTGAAATCCATGGTTTTTGGGACGTTTCCTGAGTCAGATTCGC
GAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAACTGGTTCGCGG
15 TCACAAAGACACGGAACGGCGCCGGGGGAGGCAACAAAGTCGTCGATGAGTGCTACATCCC
CAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAATGGGCTTGGACCAACATGGAACAG
TACTTGTGCGCCTGTTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGCATCTGACGC
ACGTGTGCGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGATGCGCCGGT
GATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTTCGGGTGGCTCGTGGACAAGGGG
20 ATTACCTCGGAGAAGCAGTGGATCCAGGAGGACCAGGCCTCATACATCTCCTTCAATGCGG
CCTCCAACCTCGCGGTCCCAAATCAAGGCTGCCTTGGACAATGCGGGAAAGATTATGAGCCT
GACTAAAACCGCCCCCGACTACCTGGTGGGCCAGCAGCCCGTGGAGGACATTTCCAGCAAT
CGGATTTATAAAATTTTGGAACTAAACGGGTACGATCCCCAATATGCGGCTTCCGTCTTTC
TGGGATGGGCCACGAAAAAGTTCGGCAAGAGGAACACCATCTGGCTGTTTGGGCCTGCAAC
25 TACCGGGAAGACCAACATCGCGGAGGCCATAGCCACACTGTGCCCTTCTACGGGTGCGTA
AACTGGACCAATGAGAACTTTCCCTTCAACGACTGTGTCGACAAGATGGTGATCTGGTGGG
AGGAGGGGAAGATGACCGCCAAGGTCGTGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAA
GGTGCGCGTGGACCAGAAATGCAAGTCCTCGGCCCAGATAGACCCGACTCCCGTGATCGTC
ACCTCCAACACCAACATGTGCGCCGTGATTGACGGGAACTCAACGACCTTCGAACACCAGC
30 AGCCGTTGCAAGACCGGATGTTCAAATTTGAACTCACCCGCCGTCTGGATCATGACTTTGG
GAAGGTCACCAAGCAGGAAGTCAAAGACTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAG
GTGGAGCATGAATTCTACGTCAAAAAGGGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACG
CAGATATAAGTGAGCCCAAACGGGTGCGCGAGTCAGTTGCGCAGCCATCGACGTCAGACGC

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GGAAGCTTCGATCAACTACGCAGACAGGTACCAAAACAAATGTTCTCGTCACGTGGGCATG
AATCTGATGCTGTTTCCCTGCAGACAATGCGAGAGAATGAATCAGAATTCAAATATCTGCT
TCACTCACGGACAGAAAGACTGTTTAGAGTGCTTTCCCGTGTTCAGAATCTCAACCCGTTTC
TGTCGTCAAAAAGGCGTATCAGAAACTGTGCTACATTCATCATATCATGGGAAAGGTGCCA
5 GACGCTTGCACTGCCTGCGATCTGGTCAATGTGGATTTGGATGACTGCATCTTTGAACAAT
AAATGATTTAAATCAGGTATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACACTC
TCTCTGAGTTATCATTTAAATGGCGCGCCACGTGGGTACCGCGGCCGCGGGGATCCAGAC
ATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAATGCT
TTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACA
10 AGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTT
TTTTTCGGATCCTCTAGAGTCGACCTGCAGGCA

SEQ ID NO: 31CMV forward primer

15

AAATGGCCCGCCTGGCATTATG

SEQ ID NO: 32CMV reverse primer

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AAACCGCTATCCACGCCCATTG

SEQ ID NO: 33CMV probe

25

ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATC

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SEQ ID NO: 34AAV2 mutated p19 promoter region L (SP1 -50)**bold:** regulatory sites within the p19 promoter5 underlined: mutations in the p19 promoter region

CAGATTTCGCGAAAACTGATTTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAATGGGCHTGGACTAAT
10 ATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

15 SEQ ID NO: 35AAV2 mutated p19 promoter region M (TATA -20)**bold:** regulatory sites within the p19 promoterunderlined: mutations in the p19 promoter region

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CAGATTTCGCGAAAACTGATTTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAGTGGGCGTGGACVAAC
ATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
25 ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

30

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SEQ ID NO: 36AAV2 mutated p19 promoter region N (SP1 -50 1)**bold:** regulatory sites within the p19 promoter5 underlined: mutations in the p19 promoter region

CAGATTTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACC**CAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG**
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCC**AATGGGCGTG**GACTAAT
10 **ATGGAACAGTATTTAAG**CGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
ATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

15 SEQ ID NO: 37AAV2 mutated p19 promoter region O (SP1 -50 2)**bold:** regulatory sites within the p19 promoterunderlined: mutations in the p19 promoter region

20

CAGATTTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACC**CAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG**
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCC**AGTGGGCHTGGACTAAT**
ATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
25 **ATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA**
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

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SEQ ID NO: 38AAV2 mutated p19 promoter region P (TATA -20 1)**bold:** regulatory sites within the p19 promoter5 underlined: mutations in the p19 promoter region

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCC**AGTGGGCGTGGAC**VAAT
10 **ATGGAACAGTATTTAAG**CGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
ATCTGACGCACGTGTGCGAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

15 SEQ ID NO: 39AAV2 mutated p19 promoter region Q (TATA -20 2)**bold:** regulatory sites within the p19 promoterunderlined: mutations in the p19 promoter region

20

CAGATTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCC**AGTGGGCGTGGACTAAC**
ATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
25 ATCTGACGCACGTGTGCGAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

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SEQ ID NO: 40AAV2 mutated p19 promoter region R (SP1 -50 & TATA -35)**bold:** regulatory sites within the p19 promoter5 underlined: mutations in the p19 promoter region

CAGATTTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACC**CAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG**
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCA**ATGGGCHTGGACVAAC**
10 **ATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC**
ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

15 SEQ ID NO: 41AAV2 mutated p19 promoter region S (SP1 -50 & TATA -20)**bold:** regulatory sites within the p19 promoterunderlined: mutations in the p19 promoter region

20

CAGATTTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACC**CAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG**
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCA**ATGGGCHTGGACTAAT**
ATGGAACAGTACCTBTCDGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
25 **ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA**
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

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SEQ ID NO: 42AAV2 mutated p19 promoter region T (TATA -20 & TATA -35)**bold:** regulatory sites within the p19 promoter5 underlined: mutations in the p19 promoter region

CAGATTTCGCGAAAACTGATTTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACT
GGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTG
CTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAGTGGGCGTGGACVAAC
10 ATGGAACAGTACCTBTCDGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGC
ATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATCCCAATTCTGA
TGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTTCGGGTGGCTCGTG
GACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG

15 SEQ ID NO: 43AAV2 Rep proteins coding region with mutated p19 promoter region L (SP-1)**bold:** regulatory sites within the p19 promoterunderlined: mutations in the p19 promoter region

20

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
25 TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGAAACCACCGGGGTGAAATC
CATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAACTGATTTCAGAGAATTTACCGC
GGGATCGAGCCGACTTTGCCAAACTGGTTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAG
GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
TGAGCTCCAATGGGCHTGGACTAATATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACG
30 GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCTGCAGACGCAGGAGCAGAAC
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
CATGGAGCTGGTTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACTCGCGGTCCCAAATCAAGG

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CTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
 GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACTAAAC
 GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA
 AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
 5 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
 AACGACTGTGTGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCTG
 TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
 CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
 ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
 10 TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
 GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
 GCGAGTCAGTTGCGCAGCCATCGACGTGACGCGGAAGCTTCGATCAACTACGCAGACAG
 GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
 15 TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
 AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTGTCGTCAAAAGGCGTATCAGAACT
 GTGCTACATTTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

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SEQ ID NO: 44AAV2 Rep proteins coding region with mutated p19 promoter region M (TATA -20)**bold:** regulatory sites within the p19 promoter25 underlined: mutations in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTTC
 TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
 30 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
 TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGAAACCACCGGGGTGAAATC
 CATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAACTGATTCAGAGAATTTACCGC
 GGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTACAAAGAC**CAGAAATGGCGCCGGAG**

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GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
TGAGCTCCAGTGGGCGTGGACVAACATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACG
GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
5 CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
CTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAATAAAC
GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA
10 AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGAAGACCAACATCGCGGAGGC
CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
AACGACTGTGTCGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
15 ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
CTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
GCGAGTCAGTTGCGCAGCCATCGACGTGACGCGGAAGCTTCGATCAACTACGCAGACAG
20 GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTGTCGTCAAAAAGGCGTATCAGAACT
GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
25 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

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SEQ ID NO: 45

AAV2 Rep proteins coding region with mutated p19 promoter region N (SP1 -50 1)**bold:** regulatory sites within the p19 promoter5 underlined: mutations in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
10 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAACCACCGGGGTGAAATC
CATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAACTGATTCAGAGAATTTACCGC
GGGATCGAGCCGACTTTGCCAACTGGTTCGCGGTCACAAAGAC**CCAGAAATGGCGCCGGAG**
GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
15 TGAGCTCC**AATGGGCGTGGACTAATAT**GGAAACAGT**ATTTAAG**CGCCTGTTTGAATCTCACG
GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACTTCAGCCAGGTA
CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
GAGGACCAGGCCTCATACTCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
20 CTGCCTTGACAAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACATAAC
GGGTACGATCCCCAATATGCGGCTTCCGTCTTCTGGGATGGGCCACGAAAAAGTTCGGCA
AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
25 AACGACTGTGTGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
30 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA

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TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTCTGTCAAAAAGGCGTATCAGAAACT
GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
5 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 46

AAV2 Rep proteins coding region with mutated p19 promoter region O (SP1 -50 2)

- 10 **bold**: regulatory sites within the p19 promoter
underlined: mutations in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
15 TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAACCAACCGGGGTGAAATC
CATGGTTTTGGGACGTTTCCTGAGTCAGATTGCGGAAAACTGATTCAGAGAATTTACCGC
GGGATCGAGCCGACTTTGCCAACTGGTTCGCGGTCAAAAGACCAGAAATGGCGCCGGAG
20 GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
TGAGCTCCAGTGGGCHTGGACTAATATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACG
GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTGCGAGACGCAGGAGCAGAAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
CATGGAGCTGGTGGGTGGCTCGTGGAACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
25 GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
CTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACTAAAC
GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA
AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
30 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
AACGACTGTGTCGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCTG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG

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ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
5 GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
AGTGCTTTCCCGTGTGAGAATCTCAACCCGTTTCTGTGCTCAAAAAGGCGTATCAGAAACT
GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
10 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 47AAV2 Rep proteins coding region with mutated p19 promoter region P (TATA -20 1)

15

bold: regulatory sites within the p19 promoterunderlined: mutations in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
20 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAACACCAGGGGTGAAATC
CATGGTTTTGGGACGTTTCTTGAGTCAGATTCGCGAAAACTGATTTCAGAGAATTTACCGC
25 GGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTCACAAAGACCAGAAATGGCGCCGGAG
GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
TGAGCTCCAGTGGGCGTGGACVAATATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACG
GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTGCGAGACGCAGGAGCAGAACAA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
30 CATGGAGCTGGTGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACTCGCGGTCCCAAATCAAGG
CTGCCTTGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACATAAC

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GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA
 AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
 AACGACTGTGTGACAAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCTG
 5 TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
 CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
 ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
 TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
 10 GGTGGAGCCAAGAAAAGACCCGCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
 GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
 GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
 TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
 AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTGCTCAAAAAGGCGTATCAGAACT
 15 GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 48

20 AAV2 Rep proteins coding region with mutated p19 promoter region Q
(TATA -20 2)

bold: regulatory sites within the p19 promoter

underlined: mutations in the p19 promoter region

25

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCC
 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
 TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
 30 TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGAAACCACCGGGGTGAAATC
 CATGGTTTTGGGACGTTTCTGAGTCAGATTCGCGAAAACTGATTCAGAGAATTTACCGC
 GGGATCGAGCCGACTTTGCCAACTGGTTCGCGGTCAAAAGAC**CCAGAAATGGCGCCGGAG**
GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC

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TGAGCTCCAGTGGGCGTGGACTAACATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACG
GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTGCGCAGACGCAGGAGCAGAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACTTCAGCCAGGTA
CATGGAGCTGGTCTGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
5 GAGGACCAGGCCTCATA CATCTCCTTCAATGCGGCCTCCA ACTCGCGGTCCCAAATCAAGG
CTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGA ACTAAAC
GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCTGGCA
AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
10 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
AACGACTGTGTCGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCCAGATAGACCCGACTCCCGTGATCGTCACTCCAACACCAACATGTGCGCCGTG
ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
15 TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
GTACCAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
20 TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTGTCGTCAAAAAGGCGTATCAGAACT
GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

25

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SEQ ID NO: 49AAV2 Rep proteins coding region with mutated p19 promoter region R (SP1 -50 & TATA -35)

- 5 **bold**: regulatory sites within the p19 promoter
 underlined: mutations in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
10 TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGAAACCACCGGGGTGAAATC
CATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAACTGATTCAGAGAATTTACCGC
GGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTCAAAAGACCAGAAATGGCGCCGGAG
15 GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
TGAGCTCCAATGGGCHTGGACVAACATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACG
GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTGCGAGACGCAGGAGCAGAAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACTTCAGCCAGGTA
CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
20 GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACTCGCGGTCCCAAATCAAGG
CTGCCTTGGAACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAATAAAC
GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA
AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
25 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
AACGACTGTGTGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTGCG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
30 TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG

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GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
 TGCAGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
 AGTGCTTTCCCGTGTGAGAATCTCAACCCGTTTCTGTGCTCAAAAAGGCGTATCAGAAACT
 GTGCTACATTTCATCATATCATGGGAAAGGTGCCAGACGCTTGCCTGCGATCTGGTC
 5 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 50

AAV2 Rep proteins coding region with mutated p19 promoter region S (SP1 -50 &
 10 TATA -20)

bold: regulatory sites within the p19 promoter

underlined: mutations in the p19 promoter region

15 ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTC
 TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
 TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGAAACCACCGGGGTGAAATC
 20 CATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAACTGATTCAGAGAATTTACCGC
 GGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTCAAAAGACC**CAGAAATGGCGCCGGAG**
GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
 TGAGCTCCA**ATGGGCHTGGACTAATATGGAACAGTACCTBT**CDGCCTGTTTGAATCTCACG
 GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTGCGAGACGCAGGAGCAGAAACA
 25 AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
 CATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG
 GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACTCGCGGTCCCAATCAAGG
 CTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGT
 GGGCCAGCAGCCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACATAAAC
 30 GGGTACGATCCCCAATATGCGGCTTCCGTCTTCTGGGATGGGCCACGAAAAAGTTTCGGCA
 AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
 CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
 AACGACTGTGTGACAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTGCG

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TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
 CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
 ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
 TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTACCAAGCAGGAAGTCAAAGA
 5 CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
 GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
 GCGAGTCAGTTGCGCAGCCATCGACGTGACGCGGAAGCTTCGATCAACTACGCAGACAG
 GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
 TGCAGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
 10 AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTCTGTCAAAAAGGCGTATCAGAACT
 GTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
 AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

15 SEQ ID NO: 51

AAV2 Rep proteins coding region with mutated p19 promoter region T (TATA -20 & TATA -35)

bold: regulatory sites within the p19 promoter

20 underlined: mutations in the p19 promoter region

ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCCG
 GCATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTCTG
 TGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGC
 25 GACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGCCCTTTTCTTTGTGCAAT
 TTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGAAACCACCGGGGTGAAATC
 CATGGTTTTGGGACGTTTCCTGAGTCAGATTGCGGAAAACTGATTCAGAGAATTTACCGC
 GGGATCGAGCCGACTTTGCCAAACTGGTTTCGCGGTCACAAAGAC**CAGAAATGGCGCCGGAG**
GCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCC
 30 TGAGCTCC**AGTGGGCGTG**GAC**VAACAT**GGAAACAGT**ACCTBT**CDGCCTGTTTGAATCTCACG
 GAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACA
 AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGGTA
 CATGGAGCTGGTGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAG

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GAGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGG
CTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCGACTACCTGGT
GGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAATAAAC
GGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA
5 AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCAACATCGCGGAGGC
CATAGCCCACACTGTGCCCTTCTACGGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTC
AACGACTGTGTGACAAAGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTGCG
TGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTC
CTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTG
10 ATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAAT
TTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGA
CTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGC
GCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAG
15 GTACCAAAACAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAA
TGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAG
AGTGCTTTCCCGTGTCAGAATCTCAACCCGTTTCTGTGTCGTCAAAAAGGCGTATCAGAACT
GTGCTACATTTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATCTGGTC
AATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCC
20 GATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA

SEQ ID NO: 52SV40 polyA forward primer

25 AGCAATAGCATCACAAATTTCAAA

SEQ ID NO: 53SV40 polyA reverse primer

30 CCAGACATGATAAGATACATTGATGAGTT

- 70 -

SEQ ID NO: 54

SV40 polyA probe

AGCATTTTTTTCACCTGCATTCTAGTTGTGGTTTGTC

Claims

1. A host cell comprising a nucleic acid encoding Adeno-associated virus (AAV) Rep proteins Rep78 and Rep68, wherein the internal AAV promoter p19 has been inactivated by one or more mutations that maintain the functionality of said Rep78 and Rep68 proteins,
wherein said one or more mutations are within at least one of the p19 promoter SP1 -50 region, TATA -20 region, and TATA -35 region, and wherein
 - i) at least one mutation is within the p19 promoter SP1-50 region and/or
 - ii) at least one mutation each is within both the p19 promoter SP1-50 region and the TATA -20 region, within both the p19 promoter SP1-50 region and the TATA -35 region, and/or within both the p19 promoter TATA -20 region and the TATA -35 region,wherein said nucleic acid comprises at least one nucleotide sequence, selected from the group consisting of the nucleotide sequences according to SEQ ID NOs: 1, 6, 8, 10, 14, 15, 20, 22, 24, 28, 34, 36, 37, 40 to 43, 45, 46, and 49 to 51.
2. A host cell comprising a nucleic acid having at least 90% sequence identity to a nucleic acid as defined in claim 1, provided that the specific mutations defined in said claim are present and the internal AAV promoter p19 has been inactivated by one or more of the specific mutations that maintain the functionality of Rep78 and Rep68 proteins.
3. The host cell according to any one of claims 1 to 2, wherein said nucleic acid is stably integrated into the host cell genome or is comprised in a vector.
4. The host cell according to any one of claims 1 to 3, further comprising a nucleic acid encoding AAV Rep proteins Rep52 and Rep40 under the control of an inducible promoter.

5. The host cell according to any one of claims 1 to 4, selected from the group consisting of CAP cells, HEK293 cells, and Per.C6 cells.
6. A nucleic acid as defined in any one of claims 1 to 2 or a vector as defined in claim 3.
7. A method for the production of Adeno-associated virus (AAV), comprising the step of recombinantly expressing AAV Rep proteins Rep78 and Rep68 in a host cell according to any one of claims 1 to 5.