CLAIMS

WHAT IS CLAIMED IS:

1. A nucleic acid molecule comprising a functional promoter of a herpesvirus, a functional enhancer of a herpesvirus, and one or more internal elements of the CpG island of the aprt (adenine phosphoribosyl transferase) gene and/or a functional variant thereof.

2. The nucleic acid molecule of claim 1, wherein the aprt gene is one of the hamster aprt gene, the mouse aprt gene, the rat aprt gene, the human aprt gene, the bovine aprt gene, the Zebrafish aprt gene, the Yersinia pestis aprt gene, the Xenopus tropicalis aprt gene, the mold aprt gene, the Drosophila melanogaster aprt gene, the Saccharomyces cerevisiae aprt gene, the Schizosaccharomyces pombe aprt gene, the E. coli aprt gene, the Lactobacillus rhamnosus aprt gene and the Salmonella typhimurium aprt gene.

3. The nucleic acid molecule of claims 1 or 2, wherein the hamster is Cricetulus griseus or Cricetulus sp..

4. The nucleic acid molecule of any one of claims 1-3, wherein at least one of the one or more internal elements of the CpG island of the aprt gene comprises one or more binding sites for the transcription factor Sp1.

5. The nucleic acid molecule of claim 4, wherein the aprt gene is the hamster aprt gene and the SP1 binding site of the CpG island of the aprt gene has the sequence: 5'-GCCCCGCCCCGTCCCGCCCC-3'(SEQ ID NO: 1).

6. The nucleic acid molecule of claim 4, wherein the aprt gene is the mouse aprt gene and the SP1 binding site of the CpG island of the aprt gene has the sequence 5'-CCCGCCC-3' (SEQ ID NO: 2) or the sequence 5'-TCCGCCC-3' (SEQ ID NO: 3).

7. The nucleic acid molecule of any one of claims 1 to 5, wherein the aprt gene is the hamster aprt gene and the internal element of the CpG island of the aprt gene has the sequence:

5'-
TCCAGCAAATGCGTTACTTCCTGCCAAAAGCCAGCCTCCCCGCAACCCACT
CTCCCAGAGGCCCCGCCCCGTCCCGCCCCCTCCCGGCCTCTCCTCGTGCTG
GATCGCTCCCTAAGGA-3' (SEQ ID NO: 4).

8. The nucleic acid molecule of any one of claims 1-4 or 6, wherein the aprt gene is the mouse aprt gene and the internal element of the CpG island of the aprt gene has the sequence:

5'-
AGGATGGACATCGCACATCCCCTTTCCACCCATATATCTTTGAGGTAGGGA
TGCTTGTGTTTAGGCAGCTCAAGAAATCTAACCCCTGACTCAGGCCCCACA
CACACCTCGCAGAGGCCCCGCCTCTCAGCCTGTCCCGCCCCTCGTGCTAGA
CCAACCCGCACCCAGAAGCCCCGCCCATCGAGGACGCTCCGCCCTTGTTC
CCCCCGGGATTGACGTG-3' (SEQ ID NO: 5).

9. The nucleic acid molecule of any one of claims 1 to 8, wherein the one or more internal elements of the CpG island of the aprt gene are independently arranged in the nucleic acid molecule in the forward orientation or in the reverse orientation, relative to the sequence of the promoter.

10. The nucleic acid molecule of any one of claims 1 to 9, comprising a plurality of the internal element of the CpG island of the aprt gene and/or of the functional variant thereof.

11. The nucleic acid molecule of any one of claims 1 to 10, further comprising an expressible nucleotide sequence coding for a polypeptide of interest, the expressible nucleotide sequence being operably linked to the promoter of the herpesvirus and the enhancer of the herpesvirus.

12. The nucleic acid molecule of claim 11, wherein the expressible nucleotide sequence is a heterologous nucleotide sequence.

13. The nucleic acid molecule of any one of claims 1 to 12, wherein the expressible nucleotide sequence is arranged downstream from the promoter of the herpesvirus and the enhancer of the herpesvirus.

14. The nucleic acid molecule of any one of claims 1 to 13, wherein the promoter and the enhancer are a promoter and an enhancer of the same herpesvirus.

15. The nucleic acid molecule of any one of claims 1 to 14, wherein the herpesvirus is selected from the group consisting of a cytomegalovirus, a rhadinovirus, a roseolovirus, a simplexvirus, a varicellovirus, a mardivirus, an iltovirus, a betaherpesvirus, a gammaherpesvirus, an Epstein-Barr virus, a varicella zoster virus, murine gammaherpesvirus-68, and Herpes Simian B virus.

16. The nucleic acid molecule of claim 15, wherein the cytomegalovirus is the human cytomegalovirus.

17. The nucleic acid molecule of any one of claims 1-16, wherein the functional promoter and the functional enhancer are comprised in the sequence:

5'-
TTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATT
AGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGG
CCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGA
CGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGG
TGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATA
TGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGG
CATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATC
TACGTATTAGTCATCGCTATTACTCGAGTGATGCGGTTTTGGCAGTACATC
AATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCC
CATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCC
AAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTG
TACGGTGGGAGGTCTATATAAGCAGAGCTC-3' (SEQ ID NO: 6).

18. The nucleic acid molecule of any one of claims 1 to 17, wherein the functional promoter has the sequence:

5'-
TGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCA
C
GGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGC
ACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTG
ACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAG
CTC-3' (SEQ ID NO: 7).

19. The nucleic acid molecule of any one of claims 1 to 18, wherein the functional enhancer has the sequence:

5'-
TGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTA
GTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGC
CCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGAC
GTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGT
GGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATAT
GCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGC
ATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCT
ACGTATTAGTCATCGCTATTA-3' (SEQ ID NO: 8).

20. The nucleic acid molecule of any one of claims 1 to 19, wherein the enhancer of the herpesvirus is arranged upstream of the promoter of the herpesvirus.

21. The nucleic acid molecule of any one of claims 1 to 20, wherein at least one of the one or more internal elements of the CpG island of the aprt gene, or the functional variant thereof, is arranged upstream of the promoter of the herpesvirus or adjacently downstream of the promoter of the herpesvirus.

22. The nucleic acid molecule of claim 21, wherein at least one of the one or more internal elements of the CpG island of the aprt gene, or the functional variant thereof, is arranged (i) between the enhancer of the herpesvirus and the promoter of the herpesvirus or (ii) adjacently upstream of the enhancer of the herpesvirus.

23. The nucleic acid molecule of claims 21 or 22, wherein at least one of the one or more internal elements of the CpG island of the aprt gene, or the functional variant thereof, is arranged adjacent to the enhancer of the herpesvirus or adjacent to the promoter of the herpesvirus.

24. The nucleic acid molecule of any one of claims 1 to 23, wherein the nucleic acid molecule is a DNA molecule.

25. The nucleic acid molecule of any one of claims 1 to 24, wherein the functional promoter of the herpesvirus is the only promoter comprised in the nucleic acid molecule.

26. The nucleic acid molecule of any one of claims 1 to 25, wherein the nucleic acid molecule is comprised in a vector.

27. The nucleic acid molecule of claim 26, wherein the vector is an eukaryotic or a prokaryotic expression vector.

28. The nucleic acid molecule of claims 26 or 27, wherein the vector is a plasmid.

29. The nucleic acid molecule of any one of claims 1 to 28, wherein the nucleic acid molecule is comprised in a suitable host cell.

30. The nucleic acid molecule of claim 29, wherein the host cell is an eukaryotic or a prokaryotic cell.

31. The nucleic acid molecule of any one of claims 1 to 30, wherein the nucleic acid molecule is an isolated nucleic acid molecule.

32. A method of producing a desired polypeptide, the method comprising:

- providing a nucleic acid molecule according to claim 1, wherein the nucleic acid molecule further comprises a nucleotide sequence coding for the desired polypeptide, the nucleotide sequence being operably linked to the promoter of the herpesvirus and the enhancer of the herpesvirus, and
- allowing expression of the desired polypeptide.

33. The method of claim 32, wherein the nucleotide sequence coding for the desired polypeptide is a heterologous nucleotide sequence.

34. The method of claims 32 or 33, wherein allowing expression of the desired polypeptide comprises introducing the nucleic acid molecule according to claim 1 into a suitable host cell.

35. The method of claim 34, wherein the nucleic acid molecule according to claim 1 is comprised in a vector suitable for expression in the host cell.

36. The method of claims 34 or 35, wherein expression of the desired polypeptide is allowed in the host cell over about 30 generations or more.

37. The method of claim 36, wherein the level of expression of the desired polypeptide after about 30 generations or more of the host cell is enhanced when compared to a nucleic acid molecule with a heterologous nucleotide sequence coding for the polypeptide of interest that does not have an internal element of the CpG island of the aprt gene and/or a functional variant thereof.

38. The method of claims 36 or 37, wherein the desired polypeptide is expressed in a plurality of host cells, and wherein the number of host cells still expressing the desired polypeptide after about 30 generations or more of the host cell is enhanced when compared to a plurality of host cells comprising a nucleic acid molecule with a heterologous nucleotide sequence coding for the polypeptide of interest that does not have an internal element of the CpG island of the aprt gene and/or a functional variant thereof.

39. The method of any one of claims 34 to 38, wherein expression of the desired polypeptide is allowed over a period of about four weeks or more.

40. The method of claim 38, wherein the level of expression of the desired polypeptide after the period of four weeks or more is enhanced when compared to a nucleic acid molecule with a heterologous nucleotide sequence coding for the polypeptide of interest that does not have an internal element of the CpG island of the aprt gene and/or a functional variant thereof.

41. The method of claims 39 or 40, wherein the desired polypeptide is expressed in a plurality of host cells, and wherein the number of host cells still expressing the desired polypeptide after the period of four weeks or more is enhanced when compared to a plurality of host cells comprising a nucleic acid molecule with a heterologous nucleotide sequence coding for the polypeptide of interest that does not have an internal element of the CpG island of the aprt gene and/or a functional variant thereof.

42. The method of any one of claims 34 to 41, wherein expression of the desired polypeptide is allowed in the host cell over about 60 generations or more.

43. The method of any one of claims 34 to 42, wherein expression of the desired polypeptide is allowed over a period of eight weeks or more.

44. The method of claim 43, wherein the level of expression of the desired polypeptide after the period of eight weeks or more is enhanced when compared to a nucleic acid molecule that does not have an internal element of the CpG island of the aprt gene and/or a functional variant thereof.

45. The method of claims 43 or 44, wherein the desired polypeptide is expressed in a plurality of host cells, and wherein the number of host cells still expressing the desired polypeptide after the period of eight weeks or more is enhanced when compared to a plurality of host cells comprising a nucleic acid molecule with a heterologous nucleotide sequence coding for the polypeptide of interest that does not have an internal element of the CpG island of the aprt gene and/or a functional variant thereof.

46. A vector comprising the nucleic acid molecule according to any one of claims 1 to 25.

47. The vector of claim 46, wherein the vector is an eukaryotic or a prokaryotic expression vector.

48. The vector of claims 46 or 47, wherein the vector is a plasmid.

49. A host cell comprising the nucleic acid molecule according to any one of claims 1 to 28.

50. Use of the nucleic acid molecule according to any one of claims 1 to 28 for enhancing the expression of a polypeptide of interest, wherein the nucleic acid molecule comprises a nucleotide sequence coding for the polypeptide of interest, the nucleotide sequence being operably linked to the promoter of the herpesvirus and the enhancer of the herpesvirus.

51. The use of claim 50, wherein the polypeptide of interest is expressed in a suitable host cell.

52. The use of claim 51, wherein expression of the polypeptide of interest is allowed in the host cell over about 30 generations or more.

53. The use of claims 51 or 52, wherein expression of the polypeptide of interest is allowed in the host cell over about 60 generations or more.

54. The use of claims 52 or 53, wherein the level of expression of the desired polypeptide after about 30 generations or more of the host cell is enhanced when compared to a nucleic acid molecule with a heterologous nucleotide sequence coding for the polypeptide of interest that does not have an internal element of the CpG island of the aprt gene and/or a functional variant thereof.

55. The use of claims 53 or 54, wherein the level of expression of the desired polypeptide after about 60 generations or more of the host cell is enhanced when compared to a nucleic acid molecule with a heterologous nucleotide sequence coding for the polypeptide of interest that does not have an internal element of the CpG island of the aprt gene and/or a functional variant thereof.

56. The use of claims 52 to 55, wherein the desired polypeptide is expressed in a plurality of host cells, and wherein the number of host cells still expressing the desired polypeptide after about 30 generations or more of the host cell is enhanced when compared to a plurality of host cells comprising a nucleic acid molecule with a heterologous nucleotide sequence coding for the polypeptide of interest that does not have an internal element of the CpG island of the aprt gene and/or a functional variant thereof.

57. The use of claims 53 to 56, wherein the desired polypeptide is expressed in a plurality of host cells, and wherein the number of host cells still expressing the desired polypeptide after about 60 generations or more of the host cell is enhanced when compared to a plurality of host cells comprising a nucleic acid molecule with a heterologous nucleotide sequence coding for the polypeptide of interest that does not have an internal element of the CpG island of the aprt gene and/or a functional variant thereof.

58. The use of any one of claims 50 to 57, wherein the internal element of the CpG island of the aprt gene and/or the functional variant thereof prevents methylation of the functional promoter and/or the functional enhancer, thereby decreasing and/or preventing the reduction of the level of expression of the desired polypeptide.

CLAIMS

WHAT IS CLAIMED IS:

1. A nucleic acid molecule comprising a functional promoter of a cytomegalovirus, a functional enhancer of a cytomegalovirus, and one or more internal elements of the CpG island of the aprt (adenine phosphoribosyl transferase) gene, wherein at least one of the one or more internal elements of the CpG island of the aprt gene comprises one or more binding sites for the transcription factor Sp1, wherein

   (1) the aprt gene is a hamster aprt gene and the SP1 binding site of the CpG island of the aprt gene has the sequence: 5'-GCCCCGCCCCGTCCCGCCCC-3'(SEQ ID NO: 1); or
   (2) the aprt gene is the mouse aprt gene and the SP1 binding site of the CpG island of the aprt gene has the sequence 5'-CCCGCCC-3' (SEQ ID NO: 2) or the sequence 5'-TCCGCCC-3' (SEQ ID NO: 3).

2. The nucleic acid molecule of claim 1, wherein

   (i) when the aprt gene is the hamster aprt gene, the internal element of the CpG island of the aprt gene has the sequence:

   5'-
   TCCAGCAAATGCGTTACTTCCTGCCAAAAGCCAGCCTCCCCGCAACCCAC
   TCTCCCAGAGGCCCCGCCCCGTCCCGCCCCCTCCCGGCCTCTCCTCGTGCT
   GGATCGCTCCCTAAGGA-3' (SEQ ID NO: 4);

   or
   (ii) when the aprt gene is the mouse aprt gene, the internal element of the CpG island of the aprt gene has the sequence:

   5'-
   AGGATGGACATCGCACATCCCCTTTCCACCCATATATCTTTGAGGTAGGG
   ATGCTTGTGTTTAGGCAGCTCAAGAAATCTAACCCCTGACTCAGGCCCCA
   CACACACCTCGCAGAGGCCCCGCCTCTCAGCCTGTCCCGCCCCTCGTGCTA
   GACCAACCCGCACCCAGAAGCCCCGCCCATCGAGGACGCTCCGCCCTTGT
   TCCCCCCGGGATTGACGTG-3' (SEQ ID NO: 5).

3. The nucleic acid molecule of claim 1 or 2, wherein the one or more internal elements of the CpG island of the aprt gene are independently arranged in the nucleic acid molecule in the forward orientation or in the reverse orientation, relative to the sequence of the promoter.

4. The nucleic acid molecule of any one of claims 1 to 3, wherein the nucleic acid molecule comprises a plurality of the internal element of the CpG island of the aprt gene.

5. The nucleic acid molecule of any one of claims 1 to 4, wherein the nucleic acid molecule further comprises an expressible nucleotide sequence coding for a polypeptide of interest, the expressible nucleotide sequence being operably linked to the promoter of the cytomegalovirus and the enhancer of the cytomegalovirus and preferably being a heterologous nucleotide sequence.

6. The nucleic acid molecule of claim 5, wherein the expressible nucleotide sequence is arranged downstream from the promoter of the cytomegalovirus and the enhancer of the cytomegalovirus.

7. The nucleic acid molecule of any one of claims 1 to 5, wherein

(1) the functional promoter and the functional enhancer are comprised in the sequence:

5'-

TTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATT
AGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATG
GCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATG
ACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATG
GGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATC
ATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCC
TGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTAC
ATCTACGTATTAGTCATCGCTATTACTCGAGTGATGCGGTTTTGGCAGTAC
ATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCA
CCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACT
TTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGG

CGTGTACGGTGGGAGGTCTATATAAGCAGAGCTC-3' (SEQ ID NO: 6);

and/or
(2) the functional promoter has the sequence:

5'-

TGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCA
CGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGG
CACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATT
GACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGA
GCTC-3' (SEQ ID NO: 7) ;

and/or
(3) the functional enhancer has the sequence:

5'-

TGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTA

GTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGC

CCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGAC

GTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGT

GGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATA

TGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGG

CATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATC

TACGTATTAGTCATCGCTATTA-3' (SEQ ID NO: 8).

8. The nucleic acid molecule of any one of claims 1 to 7, wherein the enhancer of the cytomegalovirus is arranged upstream of the promoter of the cytomegalovirus.

9. The nucleic acid molecule of any one of claims 1 to 8, wherein at least one of the one or more internal elements of the CpG island of the aprt gene is arranged upstream of the promoter of the cytomegalovirus or adjacently downstream of the promoter of the cytomegalovirus, wherein optionally (1) at least one of the one or more internal elements of the CpG island of the aprt gene is arranged (i) between the enhancer of the cytomegalovirus and the promoter of the cytomegalovirus, or (ii) adjacently upstream of the enhancer of the cytomegalovirus; and/or (2) at least one of the one or more internal elements of the CpG island of the aprt gene is arranged adjacent to the enhancer of the cytomegalovirus or adjacent to the promoter of the cytomegalovirus.

10. The nucleic acid molecule of any one of claims 1 to 9, wherein the nucleic acid molecule is a DNA molecule.

11. A method of producing a desired polypeptide in cell culture, the method comprising:

- providing a nucleic acid molecule according to claim 1, wherein the nucleic acid molecule further comprises a nucleotide sequence coding for the desired polypeptide, the nucleotide sequence being operably linked to the promoter of the cytomegalovirus and the enhancer of the cytomegalovirus, and

- allowing expression of the desired polypeptide.

12. The method of claim 11, wherein (1) the nucleotide sequence coding for the desired polypeptide is a heterologous nucleotide sequence; and/or (2) allowing expression of the desired polypeptide comprises introducing the nucleic acid molecule according to claim 1, optionally comprised in a vector suitable for expression in a host cell, into a suitable host cell.

13. A vector comprising the nucleic acid molecule according to any one of claims 1 to 10, wherein optionally (1) the vector is an eukaryotic or a prokaryotic expression vector; and/or (2) the vector is a plasmid.

14. A host cell comprising the nucleic acid molecule according to any one of claims 1 to 10.

15. Use of the nucleic acid molecule according to any one of claims 1 to 10 for enhancing the expression of a polypeptide of interest in cell culture, wherein the nucleic acid molecule comprises a nucleotide sequence coding for the polypeptide of interest, the nucleotide sequence being operably linked to the promoter of the cytomegalovirus and the enhancer of the cytomegalovirus.