

We claim:

1. A recombinant adeno-associated virus (rAAV) comprising:
 - (a) an AAV capsid comprising an AAV capsid protein; and
 - (b) an rAAV genome comprising a transcriptional regulatory element operably linked to an iduronate-2-sulfatase (IDS) intron-inserted coding sequence comprising an intron.
2. The rAAV of claim 1, wherein the IDS intron-inserted coding sequence encodes a human IDS protein.
3. The rAAV of claim 1 or 2, wherein the IDS intron-inserted coding sequence encodes the amino acid sequence set forth in SEQ ID NO: 23.
4. The rAAV of any one of the preceding claims, wherein the intron is a heterologous intron.
5. The rAAV of any one of the preceding claims, wherein the intron has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 33.
6. The rAAV of any one of the preceding claims, wherein the intron is positioned between nucleotides in the IDS intron-inserted coding sequence that correspond to positions 708 and 709 of the IDS coding sequence set forth in SEQ ID NO: 24.
7. The rAAV of any one of the preceding claims, wherein the IDS intron-inserted coding sequence comprises a nucleotide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 25, 59, or 60.
8. The rAAV of any one of claims 1 to 5, wherein the intron is positioned between nucleotides in the IDS intron-inserted coding sequence that correspond to positions 580 and 581 of the IDS coding sequence set forth in SEQ ID NO: 26.

9. The rAAV of claim 8, wherein the IDS intron-inserted coding sequence comprises a nucleotide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 27.
10. The rAAV of any one of claims 1 to 5, wherein the IDS intron-inserted coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 25, 27, 59, or 60.
11. The rAAV of any one of the preceding claims, wherein the transcriptional regulatory element comprises one or more of the elements selected from the group consisting of a cytomegalovirus (CMV) enhancer element, cytomegalovirus (CMV) promoter, chicken- β -actin (CBA) promoter, a small chicken- β -actin (SmCBA) promoter, a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) promoter, a beta-glucuronidase (GUSB) promoter, a modified human EF-1 α promoter, a CALM1 promoter, a synthetic promoter, and any combination thereof.
12. The rAAV of any one of the preceding claims, wherein the transcriptional regulatory element comprises a nucleotide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a nucleotide sequence set forth in SEQ ID NO: 29, 30, 36, 39, 40, 41, 42, 44, 46, 47, 48, or 55.
13. The rAAV of any one of the preceding claims, wherein the transcriptional regulatory element comprises the nucleotide sequence set forth in SEQ ID NO: 29.
14. The rAAV of any one of the preceding claims, wherein the rAAV genome further comprises a polyadenylation sequence 3' to the IDS intron-inserted coding sequence.
15. The rAAV of claim 14, wherein the polyadenylation sequence is an exogenous polyadenylation sequence.
16. The rAAV of claim 15, wherein the exogenous polyadenylation sequence is an SV40 polyadenylation sequence.

17. The rAAV of claim 16, wherein the SV40 polyadenylation sequence comprises the nucleotide sequence set forth in SEQ ID NO: 34, 35, or 45.
18. The rAAV of any one of the preceding claims wherein the rAAV genome comprises a nucleotide sequence set forth in SEQ ID NO: 37, 43, 52, 54, 61, 63, 65, 69, 75, or 77.
19. The rAAV of any one of the preceding claims, wherein the rAAV genome further comprises a 5' inverted terminal repeat (5' ITR) nucleotide sequence, and a 3' inverted terminal repeat (3' ITR) nucleotide sequence.
20. The rAAV of claim 19, wherein the 5' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 18, 20, or 49, and/or the 3' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 14, 19, 21, or 51.
21. The rAAV of claim 19, wherein:
- (a) the 5' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 18, and the 3' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 14;
 - (b) the 5' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 18, and the 3' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 19;
 - (c) the 5' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 18, and the 3' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 51;
 - (d) the 5' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ

ID NO: 49, and the 3' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 14;

(e) the 5' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 49, and the 3' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 19;

(f) the 5' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 49, and the 3' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 51; or

(g) the 5' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 20, and the 3' ITR nucleotide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 21.

22. The rAAV of claim 19, wherein the 5' ITR nucleotide sequence and the 3' ITR nucleotide, respectively, comprise the sequences of SEQ ID NOs: 18 and 14; 18 and 19; 18 and 51; 49 and 14; 49 and 19; 49 and 51; or 20 and 21.

23. The rAAV of any one of the preceding claims, wherein the rAAV genome comprises a nucleotide sequence set forth in SEQ ID NO: 28, 38, 50, 53, 56, 57, 58, 62, 64, 66, 70, 71, 72, 73, or 74.

24. The rAAV of claim 23, wherein the rAAV genome comprises the nucleotide sequences set forth in SEQ ID NOs: 72 and 74; 72 and 28; 73 and 74; or 73 and 28.

25. The rAAV of any one of claims 1-18, wherein the rAAV genome is self-complementary.

26. The rAAV of claim 25, wherein the rAAV genome comprises a nucleotide sequence set forth in SEQ ID NO: 38, 50, 62, 64, 66, 70, 76, or 78.

27. The rAAV of any one of claims 1-18, wherein the rAAV genome is single-stranded.

28. The rAAV of claim 27, wherein the rAAV genome comprises a nucleotide sequence set forth in SEQ ID NO: 53 or 58.

29. The rAAV of any one of the preceding claims, wherein the AAV capsid protein comprises an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of amino acids 203-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

30. The rAAV of claim 29, wherein: the amino acid in the capsid protein corresponding to amino acid 206 of SEQ ID NO: 16 is C; the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H; the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q; the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A; the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N; the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S; the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 590 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G or Y; the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M; the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K; the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C; or, the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G.

31. The rAAV of claim 30, wherein:

(a) the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G, and the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G;

(b) the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H, the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M;

(c) the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R;

(d) the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A, and the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; or

(e) the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C.

32. The rAAV of claim 29, wherein the capsid protein comprises the amino acid sequence of amino acids 203-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

33. The rAAV of any one of the preceding claims, wherein the AAV capsid protein comprises an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of amino acids 138-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

34. The rAAV of claim 33, wherein: the amino acid in the capsid protein corresponding to amino acid 151 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 160 of SEQ ID NO: 16 is D; the amino acid in the capsid protein corresponding to amino acid 206 of SEQ ID NO: 16 is C; the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H; the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q; the amino acid in the capsid protein corresponding to

amino acid 346 of SEQ ID NO: 16 is A; the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N; the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S; the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 590 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G or Y; the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M; the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K; the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C; or, the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G.

35. The rAAV of claim 34, wherein:

(a) the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G, and the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G;

(b) the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H, the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M;

(c) the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R;

(d) the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A, and the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; or

(e) the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C.

36. The rAAV of claim 33, wherein the capsid protein comprises the amino acid sequence of amino acids 138-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

37. The rAAV of any one of the preceding claims, wherein the AAV capsid protein comprises an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of amino acids 1-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

38. The rAAV of claim 37, wherein: the amino acid in the capsid protein corresponding to amino acid 2 of SEQ ID NO: 16 is T; the amino acid in the capsid protein corresponding to amino acid 65 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 68 of SEQ ID NO: 16 is V; the amino acid in the capsid protein corresponding to amino acid 77 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 119 of SEQ ID NO: 16 is L; the amino acid in the capsid protein corresponding to amino acid 151 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 160 of SEQ ID NO: 16 is D; the amino acid in the capsid protein corresponding to amino acid 206 of SEQ ID NO: 16 is C; the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H; the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q; the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A; the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N; the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S; the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I; the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 590 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G or Y; the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M; the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R; the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K; the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C; or, the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G.

39. The rAAV of claim 38, wherein:

(a) the amino acid in the capsid protein corresponding to amino acid 2 of SEQ ID NO: 16 is T, and the amino acid in the capsid protein corresponding to amino acid 312 of SEQ ID NO: 16 is Q;

(b) the amino acid in the capsid protein corresponding to amino acid 65 of SEQ ID NO: 16 is I, and the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is Y;

(c) the amino acid in the capsid protein corresponding to amino acid 77 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 690 of SEQ ID NO: 16 is K;

(d) the amino acid in the capsid protein corresponding to amino acid 119 of SEQ ID NO: 16 is L, and the amino acid in the capsid protein corresponding to amino acid 468 of SEQ ID NO: 16 is S;

(e) the amino acid in the capsid protein corresponding to amino acid 626 of SEQ ID NO: 16 is G, and the amino acid in the capsid protein corresponding to amino acid 718 of SEQ ID NO: 16 is G;

(f) the amino acid in the capsid protein corresponding to amino acid 296 of SEQ ID NO: 16 is H, the amino acid in the capsid protein corresponding to amino acid 464 of SEQ ID NO: 16 is N, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 681 of SEQ ID NO: 16 is M;

(g) the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 687 of SEQ ID NO: 16 is R;

(h) the amino acid in the capsid protein corresponding to amino acid 346 of SEQ ID NO: 16 is A, and the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R; or

(i) the amino acid in the capsid protein corresponding to amino acid 501 of SEQ ID NO: 16 is I, the amino acid in the capsid protein corresponding to amino acid 505 of SEQ ID NO: 16 is R, and the amino acid in the capsid protein corresponding to amino acid 706 of SEQ ID NO: 16 is C.

40. The rAAV of claim 37, wherein the capsid protein comprises the amino acid sequence of amino acids 1-736 of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, or 17.

41. A method for expressing an iduronate-2-sulfatase (IDS) polypeptide in a cell, the method comprising transducing the cell with a recombinant adeno-associated virus (rAAV) of any one of the preceding claims.
42. The method of claim 41, wherein the cell is a cell of the central nervous system.
43. The method of claim 41, wherein the cell is a cell of the central nervous system region selected from the group consisting of the spinal cord, the motor cortex, the sensory cortex, the hippocampus, the putamen, the cerebellum optionally the cerebellar nuclei, and any combination thereof.
44. The method of claim 41, wherein the cell is a neuron or a glial cell, optionally wherein the cell is a neuron or a glial cell of the central nervous system or the peripheral nervous system.
45. The method of claim 41, wherein the cell is a cell selected from the group consisting of a motor neuron, an astrocyte, an oligodendrocyte, a cell of the cerebral cortex in the central nervous system, a sensory neuron of the peripheral nervous system, a Schwann cell, and any combination thereof.
46. The method of claim 41, wherein the cell is a cell of the liver, the heart, the lung, the kidney, or the spleen.
47. The method of any one of claims 41-46, wherein the cell is in a mammalian subject and the rAAV is administered to the subject in an amount effective to transduce the cell in the subject.
48. A pharmaceutical composition comprising an rAAV of any one of claims 1-40.
49. A method for treating a subject having Hunter Syndrome (HS), the method comprising administering to the subject an effective amount of the rAAV of any one of claims 1-40 or the pharmaceutical composition of claim 48.
50. The method of claim 49, wherein the rAAV or pharmaceutical composition is administered intravenously.

51. The method of claim 49 or 50, wherein Hunter Syndrome (HS) is associated with an iduronate-2-sulfatase (IDS) gene mutation.
52. The method of any one of claims 49-51, wherein the subject is a human subject.
53. A packaging system for preparation of an rAAV, wherein the packaging system comprises:
- (a) a first nucleotide sequence encoding one or more AAV Rep proteins;
 - (b) a second nucleotide sequence encoding a capsid protein of the rAAV of any one of claims 1-40; and
 - (c) a third nucleotide sequence comprising an rAAV genome sequence of the rAAV of any one of claims 1-40.
54. The packaging system of claim 53, wherein the packaging system comprises a first vector comprising the first nucleotide sequence and the second nucleotide sequence, and a second vector comprising the third nucleotide sequence.
55. The packaging system of claim 53 or 54, further comprising a fourth nucleotide sequence comprising one or more helper virus genes.
56. The packaging system of claim 55, wherein the fourth nucleotide sequence is comprised within a third vector.
57. The packaging system of claim 55 or 56, wherein the fourth nucleotide sequence comprises one or more genes from a virus selected from the group consisting of adenovirus, herpes virus, vaccinia virus, and cytomegalovirus (CMV).
58. The packaging system of any one of claims 53-57, wherein the first vector, second vector, and/or the third vector is a plasmid.
59. A method for recombinant preparation of an rAAV, the method comprising introducing the packaging system of any one of claims 53-58 into a cell under conditions whereby the rAAV is produced.

60. A polynucleotide comprising a nucleic acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 25, 26, 27, 37, 38, 43, 50, 52, 53, 54, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, or 78, optionally wherein the polynucleotide is comprised within a viral vector or plasmid vector.

61. A recombinant cell comprising the polynucleotide of claim 60.

62. The rAAV of any one of claims 1-40, the pharmaceutical composition of claim 48, the polynucleotide of claim 60, or the recombinant cell of claim 61, for use as a medicament.

63. The rAAV of any one of claims 1-40, the pharmaceutical composition of claim 48, the polynucleotide of claim 60, or the recombinant cell of claim 61, for use in the treatment of Hunter Syndrome (HS).

64. The rAAV of any one of claims 1-40, the pharmaceutical composition of claim 48, the polynucleotide of claim 60, or the recombinant cell of claim 61, for use in a method of treating a subject having Hunter Syndrome (HS), the method comprising administering to the subject an effective amount of the rAAV, the pharmaceutical composition, the polynucleotide, or the cell.