WHAT IS CLAIMED IS:

1. A method for purifying recombinant adeno-associated (rAAV) vector particles said method comprising the steps of:

(a) harvesting cells and cell culture supernatant comprising rAAV vector particles to produce a harvest;

(b) optionally concentrating said harvest produced in step (a) to produce a concentrated harvest;

(c) lysing said harvest produced in step (a) or said concentrated harvest produced in step (b) to produce a lysate;

(d) treating the lysate produced in step (c) to reduce contaminating nucleic acid in the lysate thereby producing a nucleic acid reduced lysate;

(e) filtering said nucleic acid reduced lysate produced in step (d) to produce a clarified lysate, and optionally diluting said clarified lysate to produce a diluted clarified lysate;

(f) subjecting said clarified lysate or diluted clarified lysate produced in step (e) to anion exchange column chromatography to produce a column eluate comprised of rAAV vector particles, and optionally concentrating said column eluate to produce a concentrated column eluate;

(g) subjecting said column eluate or said concentrated column eluate produced in step (f) to size exclusion column chromatography to produce a second column eluate comprised of rAAV vector particles thereby separating rAAV vector particles from protein impurities, and optionally diluting said second column eluate to produce a diluted second column eluate;

(h) subjecting said second column eluate or said diluted second column eluate produced in step (g) to cation exchange column chromatography to produce a third column eluate comprised of rAAV vector particles thereby separating rAAV vector particles from protein or other production impurities, and optionally concentrating said third column eluate to produce a concentrated third column eluate; and

(i) filtering said third column eluate or said concentrated third column eluate produced in step (h) thereby producing purified rAAV vector particles.

2. A method according to claim 1, wherein said concentrating of step (b) and/or step (f) and/or step (h) is via is by ultrafiltration/diafiltration, optionally by tangential flow filtration.

3. A method according to claim 1, wherein said concentrating of step (b) reduces the volume of said harvested cells and cell culture supernatant by about 2-10 fold.

4. A method according to claim 1, wherein said concentrating of step (f) reduces the volume of said column eluate by about 5-20 fold.

5. A method according to claim 1, wherein said lysing of said harvest produced in step (a) or said concentrated harvest produced in step (b) is by microfluidization.

6. A method according to claim 1, wherein step (d) comprises treating with a nuclease thereby reducing contaminating nucleic acid.

7. A method according to claim 6, wherein the nuclease comprises benzonase.

8. A method according to claim 1, wherein said filtering said clarified lysate or said diluted clarified lysate of step (e) is via a filter having a pore diameter of between about 0.1 and 0.8 microns, inclusive.

9. A method according to claim 1, wherein said diluting said clarified lysate or said diluted clarified lysate of step (e) is with an aqueous buffered acetate solution.

10. A method according to claim 1, wherein said diluting of said second column eluate of step (g) is with an aqueous buffered acetate solution

11. A method according to claim 10, wherein said aqueous buffered acetate solution has a pH of between about 4.0 and 7.0, inclusive.

12. A method according to claim 1, wherein said rAAV vector particles resulting from step (i) and/or step (i) are formulated with a surfactant to produce an AAV vector formulation;

13. A method according to claim 1, wherein said anion exchange column chromatography of step (f) comprises polyethylene glycol (PEG) modulated column chromatography.

14. A method according to claim 13, wherein said anion exchange column chromatography of step (f) comprises washing said column with a PEG solution prior to elution of said rAAV vector particles from the column.

15. A method according to claim 14, wherein the PEG in said PEG solution has an average molecular weight in a range of about 1,000 to 50,000, inclusive.

16. A method according to claim 1, wherein said anion exchange column of step (f) comprises washing said column with an aqueous surfactant solution prior to elution of said rAAV vector particles from the column.

17. A method according to claim 1, wherein said cation exchange column of step (h) comprises washing said column with a surfactant solution prior to elution of said rAAV vector particles from the column.

18. A method according to any of claims 14-17, wherein said PEG solution and/or said surfactant solution comprises an aqueous Tris-Cl/NaCl buffer or an aqueous phosphate/NaCl buffer.

19. A method according to claim 18, wherein said NaCl buffer comprises between about 20-250 mM NaCl, inclusive, or between about 50-200 mM NaCl, inclusive.

20. A method according to claim 1, wherein said rAAV vector particles are eluted from said anion exchange column of step (f) in an aqueous Tris-Cl/NaCl buffer.

21. A method according to claim 20, wherein said Tris-Cl/NaCl buffer comprises 50-200 mM NaCl.

22. A method according to claim 1, wherein said rAAV vector particles are eluted from said cation exchange column of step (h) in an aqueous phosphate/NaCl buffer.

23. A method according to claim 22, wherein said phosphate/NaCl buffer comprises between about 100-500 mM NaCl, inclusive.

24. A method according to claim 1, wherein said anion exchange column of step (f) comprises a quarternary ammonium functional group such as quaternized polythyleneimine.

25. A method according to claim 1, wherein said size exclusion column of step (g) has a separation rage (Molecular weight) between about 10,000 and 600,000, inclusive.

26. A method according to claim 1, wherein said cation exchange column of step (h) comprises a sulfonic acid or functional group such as sulphopropyl.

27. A method according to any of claims 1-26, wherein the method excludes a step of cesium chloride gradient ultracentrifugation.

28. A method according to any of claims 1-27, wherein said rAAV vector particles comprise a transgene that encodes a nucleic acid selected from the group consisting of a siRNA, an antisense molecule, miRNA a ribozyme and a shRNA.

29. A method according to any of claims 1-28, wherein said rAAV vector particles comprise a transgene that encodes a gene product selected from the group consisting of insulin, glucagon, growth hormone (GH), parathyroid hormone (PTH), growth hormone releasing factor (GRF), follicle stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotropin (hCG), vascular endothelial growth factor (VEGF), angiopoietins, angiostatin, granulocyte colony stimulating factor (GCSF), erythropoietin (EPO), connective tissue growth factor (CTGF), basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), epidermal growth factor (EGF), transforming growth factor α (TGFα), platelet-derived growth factor (PDGF), insulin growth factors I and II (IGF-I and IGF-II), TGFβ, activins, inhibins, bone morphogenic protein (BMP), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophins NT-3 and NT4/5, ciliary neurotrophic factor (CNTF), glial cell line derived neurotrophic factor (GDNF), neurturin,

agrin, netrin-1 and netrin-2, hepatocyte growth factor (HGF), ephrins, noggin, sonic hedgehog and tyrosine hydroxylase.

30. A method according to any of claims 1-28, wherein said rAAV vector particles comprise a transgene that encodes a gene product selected from the group consisting of thrombopoietin (TPO), interleukins (IL1 through IL-17), monocyte chemoattractant protein, leukemia inhibitory factor, granulocyte-macrophage colony stimulating factor, Fas ligand, tumor necrosis factors α and β, interferons α, β, and γ, stem cell factor, flk-2/flt3 ligand, IgG, IgM, IgA, IgD and IgE, chimeric immunoglobulins, humanized antibodies, single chain antibodies, T cell receptors, chimeric T cell receptors, single chain T cell receptors, class I and class II MHC molecules.

31. A method according to any of claims 1-28, wherein said rAAV vector particles comprise a transgene encoding a protein useful for correction of in born errors of metabolism selected from the group consisting of carbamoyl synthetase I, ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase, fumarylacetacetate hydrolase, phenylalanine hydroxylase, alpha- 1 antitrypsin, glucose-6-phosphatase, porphobilinogen deaminase, factor V, factor VIII, factor IX, cystathione beta-synthase, branched chain ketoacid decarboxylase, albumin, isovaleryl-coA dehydrogenase, propionyl CoA carboxylase, methyl malonyl CoA mutase, glutaryl CoA dehydrogenase, insulin, beta-glucosidase, pyruvate carboxylate, hepatic phosphorylase, phosphorylase kinase, glycine decarboxylase, RPE65, H-protein, T-protein, a cystic fibrosis transmembrane regulator (CFTR) sequence, and a dystrophin cDNA sequence.

32. A method according to any of claims 1-28, wherein said rAAV vector particles comprise a transgene that encodes Factor VIII or Factor IX.

33. A method according to any of claims 1-32, wherein the method recovers approximately 40-70% of the total rAAV vector particles from the harvest produced in step (a) or said concentrated harvest produced in step (b).

34. A method according to any of claims 1-33, wherein the method produces rAAV vector particles having a greater purity than rAAV vector particles produced or purified by AAV affinity column purification.

35. A method according to any of claims 1-34, wherein the method produces rAAV vector particles having a greater purity than rAAV vector particles produced or purified by an AAV affinity column combined with an anion exchange column purification.

36. A method according to any of claims 1-34, wherein the method produces rAAV vector particles having a greater purity than rAAV vector particles produced or purified by an

AAV affinity column combined with an anion exchange column and a cation exchange purification.

37. A method according to any of claims 1-37, wherein said rAAV vector particles are derived from an AAV selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9 and AAV10.

38. A method for purifying recombinant adeno-associated (rAAV) vector particles said method comprising the steps of:

(a) harvesting cells and cell culture supernatant comprising rAAV vector particles to produce a harvest;

(b) optionally concentrating said harvest produced in step (a) to produce a concentrated harvest;

(c) lysing said harvest produced in step (a) or said concentrated harvest produced in step (b) to produce a lysate;

(d) treating the lysate produced in step (c) to reduce contaminating nucleic acid in the lysate thereby producing a nucleic acid reduced lysate;

(e) filtering said nucleic acid reduced lysate produced in step (d) to produce a clarified lysate, and optionally diluting said clarified lysate to produce a diluted clarified lysate;

(f) subjecting said clarified lysate or diluted clarified lysate produced in step (e) to cation exchange column chromatography to produce a column eluate comprised of rAAV vector particles thereby separating rAAV vector particles from protein or other production impurities, and optionally concentrating said column eluate to produce a concentrated column eluate;

(g) subjecting said column eluate or said concentrated column eluate produced in step (f) to size exclusion column chromatography to produce a second column eluate comprised of rAAV vector particles thereby separating rAAV vector particles from protein impurities, and optionally diluting said second column eluate to produce a diluted second column eluate;

(h) subjecting said second column eluate or said diluted second column eluate produced in step (g) to anion exchange column chromatography to produce a third column eluate comprised of rAAV vector particles, and optionally concentrating said third column eluate to produce a concentrated third column eluate; and

(i) filtering said third column eluate or said concentrated third column eluate produced in step (h) thereby producing purified rAAV vector particles.

39. A method according to claim 38, wherein said concentrating of step (b) and/or step (f) and/or step (h) is via is by ultrafiltration/diafiltration, optionally by tangential flow filtration.

40. A method according to claim 38, wherein said concentrating of step (b) reduces the volume of said harvested cells and cell culture supernatant by about 2-10 fold.

41. A method according to claim 38, wherein said concentrating of step (f) reduces the volume of said column eluate by about 5-20 fold.

42. A method according to claim 38, wherein said lysing of said harvest produced in step (a) or said concentrated harvest produced in step (b) is by microfluidization.

43. A method according to claim 38, wherein step (d) comprises treating with a nuclease thereby reducing contaminating nucleic acid.

44. A method according to claim 43, wherein the nuclease comprises benzonase.

45. A method according to claim 38, wherein said filtering said clarified lysate or said diluted clarified lysate of step (e) is via a filter having a pore diameter of between about 0.1 and 0.8 microns, inclusive.

46. A method according to claim 38, wherein said diluting said clarified lysate or said diluted clarified lysate of step (e) is with an aqueous buffered acetate solution.

47. A method according to claim 38, wherein said diluting of said second column eluate of step (g) is with an aqueous buffered acetate solution

48. A method according to claim 47, wherein said aqueous buffered acetate solution has a pH of between about 4.0 and 7.0, inclusive.

49. A method according to claim 38, wherein said rAAV vector particles resulting from step (i) are formulated with a surfactant to produce an AAV vector formulation;

50. A method according to claim 38, wherein said anion exchange column chromatography of step (h) comprises polyethylene glycol (PEG) modulated column chromatography.

51. A method according to claim 38, wherein said anion exchange column chromatography of step (h) comprises washing said column with a PEG solution prior to elution of said rAAV vector particles from the column.

52. A method according to claim 51, wherein the PEG in said PEG solution has an average molecular weight in a range of about 1,000 to 50,000, inclusive.

53. A method according to claim 38, wherein said anion exchange column of step (h) comprises washing said column with an aqueous surfactant solution prior to elution of said rAAV vector particles from the column.

54. A method according to claim 38, wherein said cation exchange column of step (f) comprises washing said column with a surfactant solution prior to elution of said rAAV vector particles from the column.

55. A method according to any of claims 50-54, wherein said PEG solution and/or said surfactant solution comprises an aqueous Tris-Cl/NaCl buffer or an aqueous phosphate/NaCl buffer.

56. A method according to claim 55, wherein said NaCl buffer comprises between about 20-250 mM NaCl, inclusive, or between about 50-200 mM NaCl, inclusive.

57. A method according to claim 38, wherein said rAAV vector particles are eluted from said anion exchange column of step (h) in an aqueous Tris-Cl/NaCl buffer.

58. A method according to claim 57, wherein said Tris-Cl/NaCl buffer comprises 50-200 mM NaCl.

59. A method according to claim 38, wherein said rAAV vector particles are eluted from said cation exchange column of step (f) in an aqueous phosphate/NaCl buffer.

60. A method according to claim 59, wherein said phosphate/NaCl buffer comprises between about 100-500 mM NaCl, inclusive.

61. A method according to claim 38, wherein said anion exchange column of step (h) comprises a quaternized polythyleneimine functional group.

62. A method according to claim 38, wherein said size exclusion column of step (g) has a separation rage (Molecular weight) between about 10,000 and 600,000, inclusive.

63. A method according to claim 38, wherein said cation exchange column of step (f) comprises a sulphopropyl functional group.

64. A method according to any of claims 38-63, wherein the method excludes a step of cesium chloride gradient ultracentrifugation.

65. A method according to any of claims 38-64, wherein said rAAV vector particles comprise a transgene that encodes a nucleic acid selected from the group consisting of a siRNA, an antisense molecule, miRNA a ribozyme and a shRNA.

66. A method according to any of claims 38-64, wherein said rAAV vector particles comprise a transgene that encodes a gene product selected from the group consisting of insulin, glucagon, growth hormone (GH), parathyroid hormone (PTH), growth hormone releasing factor (GRF), follicle stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotropin (hCG), vascular endothelial growth factor (VEGF), angiopoietins, angiostatin, granulocyte colony stimulating factor (GCSF), erythropoietin (EPO), connective tissue growth factor (CTGF), basic fibroblast growth factor (bFGF), acidic

fibroblast growth factor (aFGF), epidermal growth factor (EGF), transforming growth factor α (TGFα), platelet-derived growth factor (PDGF), insulin growth factors I and II (IGF-I and IGF-II), TGFβ, activins, inhibins, bone morphogenic protein (BMP), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophins NT-3 and NT4/5, ciliary neurotrophic factor (CNTF), glial cell line derived neurotrophic factor (GDNF), neurturin, agrin, netrin-1 and netrin-2, hepatocyte growth factor (HGF), ephrins, noggin, sonic hedgehog and tyrosine hydroxylase.

67. A method according to any of claims 38-64, wherein said rAAV vector particles comprise a transgene that encodes a gene product selected from the group consisting of thrombopoietin (TPO), interleukins (IL1 through IL-17), monocyte chemoattractant protein, leukemia inhibitory factor, granulocyte-macrophage colony stimulating factor, Fas ligand, tumor necrosis factors α and β, interferons α, β, and γ, stem cell factor, flk-2/flt3 ligand, IgG, IgM, IgA, IgD and IgE, chimeric immunoglobulins, humanized antibodies, single chain antibodies, T cell receptors, chimeric T cell receptors, single chain T cell receptors, class I and class II MHC molecules.

68. A method according to any of claims 38-64, wherein said rAAV vector particles comprise a transgene encoding a protein useful for correction of in born errors of metabolism selected from the group consisting of carbamoyl synthetase I, ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase, fumarylacetacetate hydrolase, phenylalanine hydroxylase, alpha- 1 antitrypsin, glucose-6-phosphatase, porphobilinogen deaminase, factor V, factor VIII, factor IX, cystathione beta-synthase, branched chain ketoacid decarboxylase, albumin, isovaleryl-coA dehydrogenase, propionyl CoA carboxylase, methyl malonyl CoA mutase, glutaryl CoA dehydrogenase, insulin, beta-glucosidase, pyruvate carboxylate, hepatic phosphorylase, phosphorylase kinase, glycine decarboxylase, RPE65, H-protein, T-protein, a cystic fibrosis transmembrane regulator (CFTR) sequence, and a dystrophin cDNA sequence.

69. A method according to any of claims 38-64, wherein said rAAV vector particles comprise a transgene that encodes Factor VIII or Factor IX.

70. A method according to any of claims 38-64, wherein the method recovers approximately 40-70% of the total rAAV vector particles from the harvest produced in step (a) or said concentrated harvest produced in step (b).

71. A method according to any of claims 38-64, wherein the method produces rAAV vector particles having a greater purity than rAAV vector particles produced or purified by AAV affinity column purification.

72. A method according to any of claims 38-64, wherein the method produces rAAV vector particles having a greater purity than rAAV vector particles produced or purified by an AAV affinity column combined with an anion exchange column purification.

73. A method according to any of claims 38-64, wherein the method produces rAAV vector particles having a greater purity than rAAV vector particles produced or purified by an AAV affinity column combined with an anion exchange column and a cation exchange purification.

74. A method according to any of claims 38-64, wherein said rAAV vector particles are derived from an AAV selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9 and AAV10.