What is Claimed is:

1. A composition comprising a plasmid/PEI mixture, said plasmid/PEI mixture comprising components:

(a) one or more plasmids comprising nucleic acids encoding AAV packaging proteins and/or nucleic acids encoding helper proteins;

(b) a plasmid comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest; and

(c) a polyethylenimine (PEI) solution,

wherein said plasmids are in a molar ratio range of about 1:0.01 to about 1:100, or are in a molar ratio range of about 100:1 to about 1:0.01, and wherein the mixture of said components (a), (b) and (c) has optionally been incubated for a period of time from about 10 seconds to about 4 hours.

2. A composition according to Claim 1, further comprising cells.

3. A composition according to Claim 2, wherein said cells are in contact with said plasmid/PEI mixture of said components (a), (b) and (c).

4. A composition according to Claim 3, further comprising Free PEI.

5 A composition according to Claim 4, wherein said cells are in contact with said Free PEI.

6. A composition according to Claim 3, wherein said cells have been in contact with the mixture of said components (a), (b) and (c) for at least about 4 hours.

7. A composition according to Claim 3, wherein said cells have been in contact with the mixture of said components (a), (b) and (c) for a period of time in the range of about 4 hours to about 140 hours.

8. A composition according to Claim 3, wherein said cells have been in contact with the mixture of said components (a), (b) and (c) for a period of time in the range of about 4 hours to about 96 hours.

9. A composition according to Claim 5, wherein said cells have been in contact with the mixture of said components (a), (b) and (c) and said Free PEI for at least about 4 hours.

10. A composition according to Claim 5, wherein said cells have been in contact with the mixture of said components (a), (b) and (c) for a period of time in the range of about 4 hours to about 140 hours.

11. A composition according to Claim 5, wherein said cells have been in contact with the mixture of said components (a), (b) and (c) for a period of time in the range of about 4 hours to about 96 hours.

12. A composition according to any of Claims 6 to 11, wherein said cells produce recombinant AAV vector comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest.

13. A composition according to any of Claims 1 to 12, wherein said composition comprises a container, said container optionally comprising a flask, plate, bag, or bioreactor, said container optionally sterile, and/or said container optionally suitable for maintaining cell viability or growth.

14. A composition according to Claim 1, further comprising a plasmid comprising nucleic acids that encode AAV capsid proteins.

15. A composition according to Claim 14, further comprising cells in contact with said plasmid/PEI mixture of said components (a), (b) and (c) and said plasmid comprising nucleic acids that encode AAV capsid proteins.

16. A composition according to Claim 15, further comprising Free PEI, wherein said cells are in contact with said Free PEI.

17. A composition according to Claim 16, wherein said cells have been in contact with the mixture of said components (a), (b) and (c), said plasmid comprising nucleic acids that encode AAV capsid proteins, and said Free PEI for at least about 4 hours.

18. A composition according to Claim 16, wherein said cells have been in contact with the mixture of said components (a), (b) and (c), said plasmid comprising nucleic acids that encode AAV capsid proteins, and said Free PEI for a period of time in the range of about 4 hours to about 140 hours.

19. A composition according to Claim 16, wherein said cells have been in contact with the mixture of said components (a), (b) and (c), said plasmid comprising nucleic acids that encode AAV capsid proteins, and said Free PEI for a period of time in the range of about 4 hours to about 96 hours.

20. A method for producing transfected cells, said method comprising:

(a) providing a plasmid;

(b) providing a solution comprising polyethylenimine (PEI);

(c) mixing the plasmid of (a) with the PEI solution of (b), to produce a plasmid/PEI mixture, and optionally incubating said plasmid/PEI mixture for a period in the range of about 10 seconds to about 4 hours;

(d) contacting cells with said plasmid/PEI mixture of step (c), to produce a plasmid/PEI cell culture;

(e) adding Free PEI to said nucleic acid/PEI cell culture produced in step (d) to produce a Free PEI/plasmid/PEI cell culture; and

(f) incubating said Free PEI/plasmid/PEI cell culture of step (e) for at least about 4 hours, thereby producing transfected cells.

21. A method according to Claim 20, wherein said plasmid comprises a nucleic acid that encodes a protein or is transcribed into a transcript of interest.

22. A method for producing transfected cells that produce recombinant AAV vector comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest, said method comprising:

(a) providing one or more plasmids comprising nucleic acids encoding AAV packaging proteins and/or nucleic acids encoding helper proteins;

(b) providing a plasmid comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest;

(c) providing a solution comprising polyethylenimine (PEI);

(d) mixing the plasmids of steps (a) and (b) with the PEI solution of step (c), wherein said plasmids are in a molar ratio range of about 1:0.01 to about 1:100, or are in a molar ratio range of about 100:1 to about 1:0.01, to produce a plasmid/PEI mixture, and optionally incubating said plasmid/PEI mixture for a period in the range of about 10 seconds to about 4 hours;

(e) contacting cells with said plasmid/PEI mixture of step (d), to produce a plasmid/PEI cell culture;

(f) adding Free PEI to said plasmid/PEI cell culture produced in step (e) to produce a Free PEI/plasmid/PEI cell culture; and

(g) incubating said Free PEI/plasmid/PEI cell culture of step (f) for at least about 4 hours, thereby producing transfected cells that produce recombinant AAV vector comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest.

23. A method according to Claim 20 or 22, further comprising the step of harvesting said transfected cells produced in steps (f) or (g) and/or culture medium from said transfected cells produced in steps (f) or (g) to produce a cell and/or culture medium harvest.

24. A method according to Claim 22, further comprising isolating and/or purifying recombinant AAV vector from said cell and/or culture medium harvest of step (g) thereby producing recombinant AAV vector comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest.

25. A method for producing recombinant AAV vector comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest, said method comprising the steps:

(a) providing one or more plasmids comprising nucleic acids encoding AAV packaging proteins and/or nucleic acids encoding helper proteins;

(b) providing a plasmid comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest;

(c) providing a solution comprising polyethylenimine (PEI);

(d) mixing said plasmids of steps (a) and (b) with said PEI solution of step (c), wherein said plasmids are in a molar ratio range of about 1:0.01 to about 1:100, or are in a molar ratio range of about 100:1 to about 1:0.01, to produce a plasmid/PEI mixture, and optionally incubating the plasmid/PEI mixture for a period of time in the range of about 10 seconds to about 4 hours;

(e) contacting cells with said plasmid/PEI mixture produced in step (d), to produce a plasmid/PEI cell culture;

(f) adding Free PEI to said plasmid/PEI cell culture produced in step (e) to produce a Free PEI/plasmid/PEI cell culture;

(g) incubating said plasmid/PEI cell culture of step (e) or said Free PEI/plasmid/PEI cell culture of step (f) for at least about 4 hours to produce transfected cells;

(h) harvesting said transfected cells produced in step (g) and/or culture medium from the transfected cells produced in step (g) to produce a cell and/or culture medium harvest; and

(i) isolating and/or purifying recombinant AAV vector from said cell and/or culture medium harvest produced in step (h) thereby producing recombinant AAV vector comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest.

26. A method for producing transfected cells that produce recombinant AAV vector comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest, said method comprising the steps:

(a) providing a mixture of components (i), (ii) and (iii):

(i) one or more plasmids comprising nucleic acids encoding AAV packaging proteins and/or nucleic acids encoding helper proteins;

(ii) a plasmid comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest; and

(iii) a polyethylenimine (PEI) solution,

(b) mixing said plasmids (i) and (ii) with said PEI solution (iii) so that said plasmids are in a molar ratio range of about 1:0.01 to about 1:100, or in a molar ratio range of

about 100:1 to about 1:0.01, to produce a plasmid/PEI mixture, and optionally incubating said plasmid/PEI mixture for a period of time in the range of about 10 seconds to about 4 hours;

(c) contacting cells with said plasmid/PEI mixture produced in step (b) to produce a plasmid/PEI cell culture;

(d) adding Free PEI to said plasmid/PEI cell culture produced in step (c) to produce a Free PEI/plasmid/PEI cell culture;

(e) incubating said plasmid/PEI cell culture of step (c) or said Free PEI/plasmid/PEI cell culture of step (d) for at least about 4 hours to produce transfected cells that produce recombinant AAV vector comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest.

27. A method according to Claim 25 or 26, further comprising the step of harvesting the transfected cells produced in step (e) and/or culture medium from the transfected cells produced in step (e) to produce a cell and/or culture medium harvest; and/or isolating and/or purifying recombinant AAV vector from the cell and/or culture medium harvest of step (e), thereby producing recombinant AAV vector comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest.

28. A method for producing recombinant AAV vector comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest, comprising the step:

(a) providing a mixture of components (i), (ii) and (iii):

(i) one or more plasmids comprising nucleic acids encoding AAV packaging proteins and/or nucleic acids encoding helper proteins;

(ii) a plasmid comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest; and

(iii) a polyethylenimine (PEI) solution,

(b) mixing said plasmids (i) and (ii) with said PEI solution (iii) so that said plasmids are in a molar ratio range of about 1:0.01 to about 1:100, or are in a molar ratio range of about 100:1 to about 1:0.01, to produce a plasmid/PEI mixture, and optionally incubating said plasmid/PEI mixture for a period of time from about 10 seconds to about 4 hours;

(c) contacting cells with said plasmid/PEI mixture produced in step (b) to produce a plasmid/PEI cell culture;

(d) adding Free PEI to said plasmid/PEI cell culture produced in step (c) to produce a Free PEI/plasmid/PEI cell culture;

(e) incubating said plasmid/PEI cell culture of step (c) or said Free PEI/plasmid/PEI cell culture of step (d) for at least about 4 hours to produce transfected cells;

(f) harvesting said transfected cells produced in step (e) and/or culture medium from the transfected cells produced in step (e) to produce a cell and/or culture medium harvest; and

(g) isolating and/or purifying recombinant AAV vector from the cell and/or culture medium harvest produced in step (f), thereby producing recombinant AAV vector comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest.

29. A method for producing recombinant AAV vector comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest, comprising the steps:

(a) providing a mixture of components (i), (ii) and (iii):

(i) one or more plasmids comprising nucleic acids encoding AAV packaging proteins and/or nucleic acids encoding helper proteins;

(ii) a plasmid comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest; and

(iii) a polyethylenimine (PEI) solution,

wherein said plasmids (i) and (ii) are in a molar ratio range of about 1:0.01 to about 1:100, or are in a molar ratio range of about 100:1 to about 1:0.01, and wherein the mixture of components (i), (ii) and (iii) has optionally been incubated for a period of time from about 10 seconds to about 4 hours.

(b) contacting cells with the mixture produced in step (a) to produce a plasmid/PEI cell culture;

(c) adding Free PEI to said plasmid/PEI cell culture produced in step (b) to produce a Free PEI/plasmid/PEI cell culture;

(d) incubating said plasmid/PEI cell culture of step (b) or said Free PEI/plasmid/PEI cell culture of step (c) for at least about 4 hours to produce transfected cells;

(e) harvesting said transfected cells produced in step (d) and/or culture medium from the transfected cells produced in step (d) to produce a cell and/or culture medium harvest; and

(f) isolating and/or purifying recombinant AAV vector from the cell and/or culture medium harvest produced in step (e), thereby producing recombinant AAV vector comprising a nucleic acid that encodes a protein or is transcribed into a transcript of interest.

30. A composition or method according to any of Claims 3 to 29, wherein said plasmid/PEI cell culture, or said or said Free PEI/plasmid/PEI cell culture, or said nucleic acid/PEI cell culture is incubated for a period of time in the range of about 4 hours to about 140 hours.

31. A composition or method according to any of Claims 3 to 30, wherein said plasmid/PEI cell culture, or said or said Free PEI/plasmid/PEI cell culture, is incubated for a period of time in the range of about 4 hours to about 96 hours.

32. A composition or method according to any of Claims 1 to 31, wherein said plasmid/PEI mixture has a PEI:plasmid weight ratio in the range of about 0.1:1 to about 5:1, or has a PEI:plasmid weight ratio in the range of about 5:1 to about 0.1:1, or wherein said Free PEI/plasmid/PEI cell culture has a PEI:plasmid weight ratio in the range of about 0.1:1 to about 5:1, or has a PEI:plasmid weight ratio in the range of about 5:1 to about 0.1:1.

33. A composition or method according to any of Claims 1 to 32, wherein said plasmid/PEI mixture has a PEI:plasmid weight ratio in the range of about 1:1 to about 5:1, or has a PEI:plasmid weight ratio in the range of about 5:1 to about 1:1; or wherein said Free PEI/plasmid/PEI cell culture has a PEI:plasmid weight ratio in the range of about 1:1 to about 5:1, or has a PEI:plasmid weight ratio in the range of about 5:1 to about 1:1.

34. A composition or method according to any of claims 1 to 33, wherein the PEI of said plasmid/PEI mixture and/or the Free PEI comprises a hydrolyzed linear polyethylenimine.

35. A composition or method according to any of claims 1 to 34, wherein the PEI of said plasmid/PEI mixture and/or said Free PEI comprises a hydrolyzed linear polyethylenimine with a molecular weight in the range of about 4,000 to about 160,000 and/or in the range of about 2,500 to about 250,000 molecular weight in free base form.

36. A composition or method according to any of claims 1 to 35, wherein the PEI of said plasmid/PEI mixture and/or said Free PEI comprises a hydrolyzed linear polyethylenimine with a molecular weight of about 40,000 and/or about 25,000 molecular weight in free base form.

37. A composition or method according to Claims 1 to 36, wherein the molar ratio of nitrogen (N) in Total PEI to phosphate (P) in plasmid is in the range of about 1:1 to about 50:1 (N:P) in said Free PEI/plasmid/PEI cell culture.

38. A composition or method according to Claims 1 to 37, wherein the molar ratio of nitrogen (N) in Total PEI to phosphate (P) in plasmid is about 5:1, 6:1, 7:1, 8:1, 9:1, or 10:1 (N:P) in said Free PEI/plasmid/PEI cell culture.

39. A composition or method according to any of Claims 1 to 38, wherein said plasmid/PEI mixture is incubated for a period of time in the range of about 30 seconds to about 4 hours.

40. A composition or method according to any of Claims 1 to 39, wherein said plasmid/PEI mixture is incubated for a period of time in the range of about 1 minute to about 30 minutes.

41. A composition or method according to any of Claims 1 to 40, wherein the amount of Free PEI is in the range of about 10% to about 90% of Total PEI.

42. A composition or method according to any of Claims 1 to 41, wherein the amount of Free PEI is in the range of about 25% to about 75% of Total PEI.

43. A composition or method according to any of Claims 1 to 42, wherein the amount of Free PEI is about 50% of Total PEI.

44. A composition or method according to any of Claims 4, 5 and 9-43, wherein said Free PEI is added to said cells before, at the same time as, or after said plasmid/PEI mixture is contacted with said cells.

45. A composition or method according to any of Claims 2 to 44, wherein said cells are in suspension culture.

46. A composition or method according to any of Claims 2 to 44, wherein said cells are grown or maintained in a serum-free culture medium.

47. A composition or method according to any of Claims 2 to 46, wherein said cells are at a density in the range of about 1×10^5 cells/mL to about 1×10^8 cells/mL when contacted with said plasmid/PEI mixture and/or when contacted with said Free PEI.

48. A composition or method according to any of Claims 2 to 47, wherein viability of said cells when contacted with said plasmid/PEI mixture or with said Free PEI is about 60% or greater than 60%, or wherein said cells are in log phase growth when contacted with said plasmid/PEI mixture.

49. A composition or method according to any of Claims 2 to 48, wherein viability of said cells when contacted with said plasmid/PEI mixture or with said Free PEI is about 90% or greater than 90%, or wherein said cells are in log phase growth when contacted with said plasmid/PEI mixture or with said Free PEI.

50. A composition or method according to any of Claims 1 to 49, wherein the encoded AAV packaging proteins comprise AAV rep and/or AAV cap.

51. A composition or method according to Claim 50, wherein the encoded AAV packaging proteins comprise AAV rep and/or AAV cap proteins of any AAV serotype.

52. A composition or method according to any of Claims 1 to 51, wherein the encoded helper proteins comprise adenovirus E2 and/or E4, VARNA proteins, and/or non-AAV helper proteins

53. A composition or method according to any of Claims 2 to 52, wherein said cells are mammalian cells.

54. A composition or method according to any of Claims 2 to 52, wherein said cells are HEK 293E or HEK 293F cells.

55. A composition or method according to any of Claims 2 to 54, wherein the total amount of plasmid comprising the nucleic acid that encodes a protein or is transcribed into a transcript of interest and the one or more plasmids comprising nucleic acids encoding AAV packaging proteins and/or nucleic acids encoding helper proteins is in the range of about 0.1 μg to about 15 μg per mL of cells.

56. A composition or method according to any of Claims 1 to 55, wherein the molar ratio of the plasmid comprising the nucleic acid that encodes a protein or is transcribed into a transcript of interest to the one or more plasmids comprising nucleic acids encoding AAV packaging proteins and/or nucleic acids encoding helper proteins is in the range of about 1:5 to about 1:1, or is in the range of about 1:1 to about 5:1.

57. A composition or method according to any of Claims 1 to 56, wherein said one or more plasmids comprises a first plasmid comprising the nucleic acids encoding AAV packaging proteins and a second plasmid comprising the nucleic acids encoding helper proteins.

58. A composition or method according to Claims 57, wherein the molar ratio of the plasmid comprising the nucleic acid that encodes a protein or is transcribed into a transcript of interest to the first plasmid comprising the nucleic acids encoding AAV packaging proteins to the second plasmid comprising the nucleic acids encoding helper proteins is in the range of about 1-5:1:1, or 1:1-5:1, or 1:1:1-5.

59. A composition or method according to any of Claims 1-58, wherein the recombinant AAV vector comprises any of AAV serotypes 1-12, an AAV VP1, VP2 and/or VP3 capsid protein, or a modified or variant AAV VP1, VP2 and/or VP3 capsid protein, or wild-type AAV VP1, VP2 and/or VP3 capsid protein.

60. A composition or method according to any of Claims 1-59, wherein the AAV vector comprises an AAV serotype or an AAV pseudotype, wherein said AAV pseudotype comprises an AAV capsid serotype different from an ITR serotype.

61. A composition or method according to any of Claims 1-60, wherein the AAV vector further comprises an intron, an expression control element, one or more adeno-associated virus (AAV) inverted terminal repeats (ITRs) and/or a filler polynucleotide sequence.

62. A composition or method according to Claim 61, wherein the intron is within or flanks the nucleic acid that encodes a protein or is transcribed into a transcript of interest, or wherein the expression control element is operably linked to nucleic acid that encodes a protein or is transcribed into a transcript of interest, or wherein the AAV ITR(s) flanks the 5' or 3' terminus of nucleic acid that encodes a protein or is transcribed into a transcript of interest, or wherein the filler polynucleotide sequence flanks the 5' or 3'terminus of nucleic acid that encodes a protein or is transcribed into a transcript of interest.

63. A composition or method according to Claim 61, wherein the expression control element comprises a constitutive or regulatable control element, or a tissue-specific expression control element or promoter.

64. A composition or method according to Claim 61, wherein the expression control element comprises an element that confers expression in liver.

65. A composition or method according to Claim 61, wherein the ITR comprises one or more ITRs of any of: AAV2 or AAV6 serotypes, or a combination thereof.

66. A composition or method according to any of Claims 1-65, wherein the AAV vector comprises a VP1, VP2 and/or VP3 capsid protein having 75% or more sequence identity to any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV10, AAV11, or AAV-2i8 VP1, VP2 and/or VP3 capsid proteins, or comprises a modified or variant VP1, VP2 and/or VP3 capsid protein selected from any of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV10, AAV11, and AAV-2i8 AAV serotypes.

67. A composition or method according to any of Claims 3-65, wherein the cells are subcultured to a reduced cell density prior to contact with said plasmid/PEI mixture.

68. A composition or method according to any of Claims 3-65, wherein the cells are subcultured to a cell density in the range of about 0.1×10^6 cells/ml to about 5.0×10^6 cells/ml prior to contact with said plasmid/PEI mixture.

69. A composition or method according to any of Claims 67-68, wherein the cells are contacted with said plasmid/PEI mixture between a period of 2 days to 5 days after subculture.

70. A composition or method according to any of Claims 67-68, wherein the cells are contacted with said plasmid/PEI mixture between a period of 3 days to 4 days after subculture.

71. A method according to Claim 20, wherein the amount of plasmid introduced into said transfected cells is at least 50% greater with the step of adding Free PEI to the plasmid/PEI cell culture compared to without adding Free PEI to the plasmid/PEI cell culture.

72. A method according to any of Claims 22-71, wherein the amount of recombinant AAV vector produced is at least 50% or greater with the step of adding Free PEI to the plasmid/PEI cell culture compared to without adding Free PEI to the plasmid/PEI cell culture.

73. A method according to any of Claims 22-71, wherein the amount of recombinant AAV vector produced is 1-5, 5-10 or 10-20 fold greater with the step of adding Free PEI to the plasmid/PEI cell culture compared to without adding Free PEI to the plasmid/PEI cell culture.