

WHAT IS CLAIMED IS:

1. A human embryonic kidney (HEK) cell line which does not express functional endogenous di-hydrofolate reductase (DHFR) and glutamine synthetase (GS).
2. The HEK cell line of claim 1, stably or transiently transfected with a first heterologous nucleic acid sequence, and optionally stably or transiently transfected with a second heterologous nucleic acid sequence.
3. The HEK cell line of claim 1, stably or transiently transfected with a first heterologous nucleic acid sequence and a first selectable marker, and optionally stably or transiently transfected with a second heterologous nucleic acid sequence and a second selectable marker.
4. The HEK cell line of claim 2 or 3, wherein the first heterologous nucleic acid sequence encodes a therapeutic protein or polynucleotide sequence and optional second heterologous nucleic acid sequence encodes a therapeutic protein or polynucleotide sequence.
5. The HEK cell line of claim 3, wherein the first or second selectable marker does not provide resistance to an antibiotic.
6. The HEK cell line of claim 3, wherein the first or second selectable marker provides a means to amplify the first and/or second heterologous nucleic acid sequence(s).
7. The HEK cell line of claim 3, wherein the first or second selectable marker comprises a nucleic acid encoding a protein having DHFR function.
8. The HEK cell line of claim 3, wherein the first or second selectable marker comprises a nucleic acid encoding a protein having GS function.
9. The HEK cell line of claim 2 to 10, wherein the first heterologous nucleic acid sequence comprises a first vector, and the optional second heterologous nucleic acid sequence comprises a second vector.
10. The HEK cell line of claim 9, wherein the first vector comprises a first viral vector and optional second vector comprises a second viral vector.

11. The HEK cell line of claim 9, wherein the first or second viral vector comprises an AAV vector genome.
12. The HEK cell line of claim 9, comprising first and second viral vectors, wherein the viral vectors each comprise an AAV vector genome, wherein the AAV vector genome(s) comprises one or two AAV ITRs that flank the 5' and/or 3' ends of the heterologous nucleic acid sequence.
13. The HEK cell line of any of claims 3-12, wherein copy number of the heterologous nucleic acid sequence(s) and/or vector(s) and/or viral vector(s) and/or AAV vector genome(s) in the HEK cell line is 1-5 copies per cell, 5-10 copies per cell, 10-50 copies/cell, 50-100 copies per cell, 100-250 copies per cell, 250-500 copies per cell, 500-1,000 copies per cell, 1,000-2,000 copies per cell, or about or greater than 2,000, 3,000, 4,000 or 5,000 copies per cell, and optionally wherein copy number appears stable over many passages, e.g., at least or greater than 5, 10, 15 or more passages.
14. The HEK cell line of any of claims 11-13, wherein copy number of the AAV vector genome(s) in the HEK cell line is at least 1,000 copies per cell, and rAAV vector particle yield optionally is at least 1×10^8 vg/ml, or at least 1×10^9 vg/ml, or at least 1×10^{10} vg/ml, or at least 1×10^{11} vg/ml or at least 2×10^{11} vg/ml from a Roller Bottle of the HEK cell line.
15. The HEK cell line of any of claims 1-14, further comprising AAV rep and/or cap sequences.
16. The HEK cell line of any of claims 1-15, wherein the HEK cell line is HEK 293.
17. The HEK cell line of any of claims 1-16, in a culture or growth medium, or in a medium suitable for long-term storage.
18. The HEK cell line of any of claims 7-17, in a culture medium or growth comprising methotrexate (MTX) and/or methionine sulfoxamine (MSX).
19. The HEK cell line of any of claims 7-18, wherein the HEK cell line produces rAAV vector particles having packaged therein the heterologous nucleic acid sequence(s).
20. The HEK cell line of claim 19, wherein the rAAV vector particles are produced in greater amounts than amounts produced by HEK293 cells that express functional

endogenous DHFR and/or GS and transiently transfected with AAV vector genomes having the heterologous nucleic acid sequence.

21. The HEK cell line of claim 19, wherein the AAV vector particles produced contain less quantities of rAAV empty capsids and/or less quantities of rAAV particles that have packaged contaminating DNA than amounts of AAV empty capsid and/or rAAV particles that have packaged contaminating DNA produced by HEK293 cells that express functional endogenous DHFR and/or GS and transiently transfected with rAAV vector genomes having the heterologous nucleic acid sequence.

22. The HEK cell line of any of claims 2-21, wherein the heterologous nucleic acid sequence(s) encodes a therapeutic protein(s) or an inhibitory nucleic acid sequence(s).

23. A method of producing a therapeutic protein(s), viral vector(s) or rAAV vector particles comprising culturing the HEK cell line of any of claims 4-22, under conditions allowing production and/or secretion of the therapeutic protein(s), viral vector(s) or rAAV vector particles and isolating or purifying the therapeutic protein(s), viral vector(s) or rAAV vector particles from the cell culture, culture medium, or cell culture and culture medium.

24. The HEK cell line or method according to any of claims 2-23, wherein said first and/or second heterologous nucleic acid sequence 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 (TGF α), platelet-derived growth factor (PDGF), insulin growth factors I and II (IGF-I and IGF-II), TGF β , 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.

25. The HEK cell line or method according to any of claims 19-21 or 23, wherein said rAAV vector particles comprise the first and/or the second heterologous nucleic acid sequence encoding 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 (TGF α), platelet-derived growth factor (PDGF), insulin growth factors I and II (IGF-I and IGF-II), TGF β , 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.

26. The HEK cell line or method according to any of claims 2-23, wherein said first and/or second heterologous nucleic acid sequence 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.

27. The HEK cell line or method according to any of claims 19-21 or 23, wherein said rAAV vector particles comprise the first and/or the second heterologous nucleic acid sequence encoding 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.

28. The HEK cell line or method according to any of claims 2-23, wherein said first and/or second heterologous nucleic acid sequence encodes a protein useful for correction of in born errors selected from the group consisting of carbamoyl synthetase I, ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase, fumarylacetate 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.

29. The HEK cell line or method according to any of claims 19-21 or 23, wherein said rAAV vector particles comprise the first and/or the second heterologous nucleic acid sequence encoding encodes a protein useful for correction of in born errors selected from the group consisting of carbamoyl synthetase I, ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase, fumarylacetate 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.

30. A method of producing a human embryonic kidney (HEK) cell line which does not express functional endogenous di-hydrofolate reductase (DHFR) and glutamine synthetase (GS), comprising mutating or knocking out the endogenous DHFR gene and GS gene.