

CLAIMS

What is claimed is:

1. A system for recombinant expression of a target protein in eukaryotic cells, comprising one or more vectors, the one or more vectors comprising:
 - a) a first polynucleotide encoding the target protein; and
 - b) a second polynucleotide encoding an enhancer protein wherein:
 - i) the enhancer protein is an inhibitor of nucleocytoplasmic transport (NCT) and/or
 - ii) the enhancer protein is selected from the group consisting of a picornavirus leader (L) protein, a picornavirus 2A protease, a rhinovirus 3C protease, a herpes simplex virus (HSV) ICP27 protein, and a rhabdovirus matrix (M) protein,wherein the first polynucleotide and the second polynucleotide are operatively linked to one or more promoters.
2. The system of claim 1, wherein the enhancer protein is an inhibitor of nucleocytoplasmic transport (NCT).
3. The system of claim 2, wherein the NCT inhibitor is a viral protein.
4. The system of any one of claims 1 to 3, wherein the NCT inhibitor is selected from the group consisting of a picornavirus leader (L) protein, a picornavirus 2A protease, a rhinovirus 3C protease, a coronavirus ORF6 protein, an ebolavirus VP24 protein, a Venezuelan equine encephalitis virus (VEEV) capsid protein, a herpes simplex virus (HSV) ICP27 protein, and a rhabdovirus matrix (M) protein.
5. The system of claim 4, wherein the NCT inhibitor is a picornavirus leader (L) protein or a functional variant thereof.
6. The system of claim 4, wherein the NCT inhibitor is a picornavirus 2A protease or a functional variant thereof.
7. The system of claim 4, wherein the NCT inhibitor is a rhinovirus 3C protease or a functional variant thereof.
8. The system of claim 4, wherein the NCT inhibitor is a coronavirus ORF6 protein or a functional variant thereof.

9. The system of claim 4, wherein the NCT inhibitor is an ebolavirus VP24 protein or a functional variant thereof.
10. The system of claim 4, wherein the NCT inhibitor is a Venezuelan equine encephalitis virus (VEEV) capsid protein or a functional variant thereof.
11. The system of claim 4, wherein the NCT inhibitor is a herpes simplex virus (HSV) ICP27 protein or a functional variant thereof.
12. The system of claim 4, wherein the NCT inhibitor is a rhabdovirus matrix (M) protein or a functional variant thereof.
13. The system of claim 5, wherein the L protein is the L protein of Theiler's virus or a functional variant thereof.
14. The system of claim 5, wherein the L protein shares at least 90% identity to SEQ ID NO: 1.
15. The system of claim 5, wherein the L protein is the L protein of Encephalomyocarditis virus (EMCV) or a functional variant thereof.
16. The system of claim 5, wherein the L protein shares at least 90% identity to SEQ ID NO: 2.
17. The system of claim 5, wherein the L protein is selected from the group consisting of the L protein of poliovirus, the L protein of HRV16, the L protein of mengo virus, and the L protein of Saffold virus 2 or a functional variant thereof.
18. The system of any one of claims 1 to 17, wherein the system comprises a single vector comprising an expression cassette, the expression cassette comprising the first polynucleotide and the second polynucleotide.
19. The system of claim 18, wherein the expression cassette comprises a first promoter, operatively linked to the first polynucleotide; and a second promoter, operatively linked to the second polynucleotide.
20. The system of claim 18, wherein the expression cassette comprises a shared promoter operatively linked to both the first polynucleotide and the second polynucleotide.
21. The system of claim 20, wherein the expression cassette comprises a coding polynucleotide comprising the first polynucleotide and the second polynucleotide linked

by a polynucleotide encoding ribosome skipping site, the coding polynucleotide operatively linked to the shared promoter.

22. The system of claim 20, wherein the expression cassette comprises a coding polynucleotide, the coding polynucleotide encoding the enhancer protein and the target protein linked to by a ribosome skipping site, the coding polynucleotide operatively linked to the shared promoter.
23. The system of any one of claims 18 to 22, wherein the expression cassette is configured for transcription of a single messenger RNA encoding both the target protein and the enhancer protein, linked by a ribosome skipping site; wherein translation of the messenger RNA results in expression of the target protein and the L protein as distinct polypeptides.
24. The system of any one of claims 1 to 23, wherein the system comprises one vector.
25. The system of any one of claims 1 to 17, wherein the system comprises:
 - a) a first vector comprising the first polynucleotide, operatively linked to a first promoter; and
 - b) a second vector comprising the second polynucleotide, operatively linked to a second promoter.
26. The system of any one of claims 1 to 17 or claim 25, wherein the system comprises two vectors.
27. The system of any one of claims 1 to 26, wherein either the first polynucleotide or the second polynucleotide, or both, are operatively linked to an internal ribosome entry site (IRES).
28. The system of any one of claims 1 to 27, wherein at least one of the one or more vectors comprises a T7 promoter configured for transcription of either or both of the first polynucleotide and the second polynucleotide by a T7 RNA polymerase.
29. The system of any one of claims 1 to 28, wherein at least one of the one or more vectors comprises a polynucleotide sequence encoding a T7 RNA polymerase.
30. A vector for recombinant expression of a target protein in eukaryotic cells, comprising:
 - a) a first polynucleotide encoding the target protein; and
 - b) a second polynucleotide encoding an enhancer protein wherein:

- i) the enhancer protein is an inhibitor of nucleocytoplasmic transport (NCT) and/or
- ii) the enhancer protein is selected from the group consisting of a picornavirus leader (L) protein, a picornavirus 2A protease, a rhinovirus 3C protease, a coronavirus ORF6 protein, an ebolavirus VP24 protein, a Venezuelan equine encephalitis virus (VEEV) capsid protein, a herpes simplex virus (HSV) ICP27 protein, and a rhabdovirus matrix (M) protein.

wherein the first polynucleotide and the second polynucleotide are operatively linked to at least one promoter.

- 31. The vector of claim 30, wherein the expression cassette comprises a first promoter, operatively linked to the first polynucleotide; and a second promoter, operatively linked to the second polynucleotide.
- 32. The vector of claim 30, wherein the expression cassette comprises a shared promoter operatively linked to both the first polynucleotide and the second polynucleotide.
- 33. A eukaryotic cell for expression of a target protein, comprising an exogenous polynucleotide encoding an enhancer protein wherein:
 - a) the enhancer protein is an inhibitor of nucleocytoplasmic transport (NCT) and/or
 - b) the enhancer protein is selected from the group consisting of a picornavirus leader (L) protein, a picornavirus 2A protease, a rhinovirus 3C protease, a coronavirus ORF6 protein, an ebolavirus VP24 protein, a Venezuelan equine encephalitis virus (VEEV) capsid protein, a herpes simplex virus (HSV) ICP27 protein, and a rhabdovirus matrix (M) protein,

wherein the exogenous polynucleotide is operatively linked to a promoter

- 34. The eukaryotic cell of claim 33, wherein the polynucleotide is operatively linked to an internal ribosome entry site (IRES).
- 35. The eukaryotic cell of claim 33 or claim 34, wherein the promoter is an inducible promoter.
- 36. A method for recombinant expression of a target protein, comprising introducing a polynucleotide encoding the target protein, operatively linked to a promoter, into the cell of any one of claims 33 to 35.

37. A method for recombinant expression of a target protein, comprising introducing the system of any one of claims 1 to 29 or the vector of any one of claims 30 to 32 into eukaryotic cell.
38. The method of claim 36 or claim 37, wherein the target protein is a membrane protein
39. The method of any claim 38, wherein localization of the membrane protein to the cellular membrane is increased compared to the localization observed when the membrane protein is expressed without the enhancer protein.
40. A eukaryotic cell produced by introduction of the system of any one of claims 1 to 29, or the vector of any one of claims 30 to 32 into the eukaryotic cell.
41. A target protein expressed by introduction of the system of any one of claims 1 to 29 or the vector of any one of claims 30 to 32 into a eukaryotic cell.
42. A method for expressing a target protein in eukaryotic cells, comprising introducing a polynucleotide encoding the target protein, the polynucleotide operatively linked to a promoter, into the eukaryotic cells,
- wherein the method utilizes co-expression of an enhancer protein to enhance the expression level, solubility and/or activity of the target protein,
- wherein:
- a) the enhancer protein is an inhibitor of nucleocytoplasmic transport (NCT) and/or
 - b) the enhancer protein is selected from the group consisting of a picornavirus leader (L) protein, a picornavirus 2A protease, a rhinovirus 3C protease, a coronavirus ORF6 protein, an ebolavirus VP24 protein, a Venezuelan equine encephalitis virus (VEEV) capsid protein, a herpes simplex virus (HSV) ICP27 protein, and a rhabdovirus matrix (M) protein.
43. The method of claim 42, wherein the co-expression of enhancer protein comprises introducing into the eukaryotic cell a polynucleotide encoding the enhancer protein, operatively linked to a promoter.
44. The method of claim 42 or claim 43, wherein the introducing step or steps comprise transfection of the eukaryotic cells with one or more DNA molecules, transduction of the eukaryotic cells with a single viral vector, and/or transduction of the eukaryotic cells with two viral vectors.

45. The system of any one of claims 1 to 29, the vector of any one of claims 30 to 32, the eukaryotic cell of any one of claims 33 to 35, the method of any one of claims 36 to 39 and 42-44, the eukaryotic cell of claim 40, and the target protein of claim 41, wherein the target protein is a soluble protein.
46. The system of any one of claims 1 to 29, the vector of any one of claims 30 to 32, the cell of any one of claims 33 to 35, or the method of any one of claims 36 to 44, wherein the target protein is a secreted protein.
47. The system of any one of claims 1 to 29, the vector of any one of claims 30 to 32, the eukaryotic cell of any one of claims 33 to 35, the method of any one of claims 36 to 39 and 42-44, the eukaryotic cell of claim 40, and the target protein of claim 41, wherein the target protein is a membrane protein.
48. The system of any one of claims 1 to 29, the vector of any one of claims 30 to 32, the eukaryotic cell of any one of claims 33 to 35, the method of any one of claims 36 to 39 and 42-44, the eukaryotic cell of claim 40, and the target protein of claim 41, wherein the target protein is Dopamine receptor 1 (DRD1), optionally wherein the DRD1 comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 19.
49. The system of any one of claims 1 to 29, the vector of any one of claims 30 to 32, the eukaryotic cell of any one of claims 33 to 35, the method of any one of claims 36 to 39 and 42-44, the eukaryotic cell of claim 40, and the target protein of claim 41, wherein the target protein is Cystic fibrosis transmembrane conductance regulator (CFTR), optionally wherein the CFTR comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 18.
50. The system of any one of claims 1 to 29, the vector of any one of claims 30 to 32, the eukaryotic cell of any one of claims 33 to 35, the method of any one of claims 36 to 39 and 42-44, the eukaryotic cell of claim 40, and the target protein of claim 41, wherein the target protein is C1 esterase inhibitor (C1-Inh), optionally wherein the C1-Inh comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 16.
51. The system of any one of claims 1 to 29, the vector of any one of claims 30 to 32, the eukaryotic cell of any one of claims 33 to 35, the method of any one of claims 36 to 39 and 42-44, the eukaryotic cell of claim 40, and the target protein of claim 41, wherein the target protein is ITK, optionally wherein the ITK comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 15.

52. The system of any one of claims 1 to 29, the vector of any one of claims 30 to 32, the eukaryotic cell of any one of claims 33 to 35, the method of any one of claims 36 to 39 and 42-44, the eukaryotic cell of claim 40, and the target protein of claim 41, wherein the target protein is an NADase, optionally wherein the NADase comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 20.
53. A method for generating an antibody against a target protein, comprising immunizing a subject with the cell of any one of claims 33 to 35, the cell of claim 40, or the target protein of claim 41.
54. The method of claim 53, further comprising isolating one or more immune cells expressing an immunoglobulin protein specific for the target protein.
55. The method of claim 53 or claim 54, comprising generating one or more hybridomas from the one or more immune cells.
56. The method of any one of claims 53 to 55, comprising cloning one or more immunoglobulin genes from the one or more immune cells.
57. A method for antibody discovery by cell sorting, comprising providing a solution comprising:
- a) the cell of any one of claims 33 to 35, the eukaryotic cell of claim 40, or the target protein of claim 41, wherein the cell or target protein is labeled, and
 - b) a population of recombinant cells, wherein the recombinant cells express a library of polypeptides each comprising an antibody or antigen-binding fragment thereof; and
- isolating one or more recombinant cells from the solution by sorting for recombinant cells bound to the labeled cell or the labeled target protein.
58. A method for panning a phage-display library, comprising:
- a) mixing a phage-display library with the eukaryotic cell of any one of claims 33 to 35, the eukaryotic cell of claim 40, or the target protein of claim 41; and
 - b) purifying and/or enriching the members of the phage-display library that bind the cell or target protein.
59. The eukaryotic cell of any one of claims 33-35 and 40, wherein the eukaryotic cell is a human cell, an animal cell, an insect cell, a plant cell, or a fungal cell.

60. The eukaryotic cell of any one of claims 33-35, 40, and 59, wherein the eukaryotic cell is a eukaryotic cell line.
61. The eukaryotic cell of any one of claims 33-35, 40, 59 and 60, wherein the eukaryotic cell is Bc HROC277, COS, CHO, CHO-S, CHO-K1, CHO-DG44, CHO-DUXB11, CHO-DUKX, CHOK1SV, VERO, MDCK, WI38, V79, B14AF28-G3, BHK, HaK, NSO, 5P2/0-Ag14, HeLa, HEK293, HEK293-F, HEK293-H, HEK293-T, perC6 cell, Sf9 cell, a *Saccharomyces* cell, a *Pichia* cell or a *Schizosaccharomyces* cell.
62. The eukaryotic cell of claim 60, wherein the eukaryotic cell line is a stable cell line.
63. The system of any one of claims 1-29 and 45-52, wherein the one or more vectors is selected from the group consisting of adeno-associated virus (AAV) vector, a lentivirus vector, a retrovirus vector, a replication competent adenovirus vector, a replication deficient adenovirus vector, a herpes virus vector, a baculovirus vector or a non-viral plasmid.
64. The system of claim 63, wherein at least one of the one or more vectors is an AAV vector.
65. The vector of any one of claims 30-32, wherein the vector is an adeno-associated virus (AAV) vector, a lentivirus vector, a retrovirus vector, a replication competent adenovirus vector, a replication deficient adenovirus vector, a herpes virus vector, a baculovirus vector or a non-viral plasmid.
66. The vector of claim 65, wherein the vector is an AAV vector.
67. The system of claim 4, wherein the rhabdovirus matrix (M) protein is a M protein of Vesicular stomatitis virus (VSV).
68. The system of claim 67, wherein the M protein shares at least 90% identity to SEQ ID NO: 9.
69. A system for recombinant expression of a target protein in eukaryotic cells, comprising one or more vectors, the one or more vectors comprising:
- a) a first polynucleotide encoding the target protein; and
 - b) a second polynucleotide encoding an L protein of Encephalomyocarditis virus (EMCV), optionally wherein the L protein shares at least 90% identity to SEQ ID NO: 2, and

wherein the first polynucleotide and the second polynucleotide are operatively linked to one or more promoters.

70. A system for recombinant expression of a target protein in eukaryotic cells, comprising one or more vectors, the one or more vectors comprising:

- a) a first polynucleotide encoding the target protein; and
- b) a second polynucleotide encoding a L protein of Theiler's virus, optionally wherein the L protein shares at least 90% identity to SEQ ID NO: 1, and

wherein the first polynucleotide and the second polynucleotide are operatively linked to one or more promoters.

71. A system for recombinant expression of a target protein in eukaryotic cells, comprising one or more vectors, the one or more vectors comprising:

- a) a first polynucleotide encoding the target protein; and
- b) a second polynucleotide encoding a picornavirus 2A protease, optionally wherein the picornavirus 2A protease shares at least 90% identity to SEQ ID NO: 7, and

wherein the first polynucleotide and the second polynucleotide are operatively linked to one or more promoters.

72. A system for recombinant expression of a target protein in eukaryotic cells, comprising one or more vectors, the one or more vectors comprising:

- a) a first polynucleotide encoding the target protein; and
- b) a second polynucleotide encoding a M protein of Vesicular stomatitis virus (VSV), optionally wherein the M protein shares at least 90% identity to SEQ ID NO: 9, and

wherein the first polynucleotide and the second polynucleotide are operatively linked to one or more promoters.

73. The system of any one of claims 69-72, wherein the target protein is Dopamine receptor 1 (DRD1), optionally wherein the DRD1 comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 19.

74. The system of any one of claims 69-72, wherein the target protein is Cystic fibrosis transmembrane conductance regulator (CFTR), optionally wherein the CFTR comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 18.

75. The system of any one of claims 69-72, wherein the target protein is C1 esterase inhibitor (C1-Inh), optionally wherein the C1-Inh comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 16.
76. The system of any one of claims 69-72, wherein the target protein is ITK, optionally wherein the ITK comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 15.
77. The system of any one of claims 69-72, wherein the target protein is an NADase, optionally wherein the NADase comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 20.