Claims

1. An isolated mammalian cell comprising an exogenously introduced nucleic acid that comprises

a mammalian tunicamycin (Tn)-resistance gene encoding a protein having at least 93% identity to the amino acid sequence of SEQ ID NO: 3, and

a first gene of interest (GOI) encoding an antibody light chain or antigen-binding fragment thereof, an antibody heavy chain or antigen-binding fragment thereof, or an Fc-fusion protein,

wherein the Tn-resistance gene is operably linked to the first GOI.

2. The isolated cell of claim 1, wherein the Tn-resistance gene comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17.

3. The isolated cell of claim 1, wherein the first GOI is operably linked to at least one regulatory element selected from the group consisting of a promoter, a ribosome-binding site, and an enhancer.

4. The isolated cell of claim 1, further comprising a second exogenously-added gene of interest (GOI).

5. The isolated cell of claim 4, wherein the second GOI encodes a glycoprotein.

6. The isolated cell of claim 5, wherein the glycoprotein is selected from an antibody light chain or antigen-binding fragment thereof, an antibody heavy chain or antigen-binding fragment thereof, an Fc-fusion protein, a ligand, and a receptor or ligand-binding fragment thereof.

7. The isolated cell of claim 1, wherein the cell is selected from the group consisting of CHO, COS-7, HEK293, tumor cell, lymphocyte, retinal cell, and stem cell.

8. The isolated cell of claim 1, wherein the cell is a CHO cell.

9. A vector comprising a nucleic acid, wherein the nucleic acid comprises

a mammalian tunicamycin (Tn)-resistance gene encoding a protein having at least 93% identity to the amino acid sequence of SEQ ID NO: 3, and

a first gene of interest (GOI) encoding an antibody light chain or antigen-binding fragment thereof, an antibody heavy chain or antigen-binding fragment thereof, or an Fc-fusion protein,

wherein the Tn-resistance gene is operably linked to the first GOI.

10. The vector of claim 9, wherein the Tn-resistance gene comprises the nucleic acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 and SEQ ID NO: 17.

11. The vector of claim 9, wherein the first GOI is operably linked to at least one regulatory element selected from the group consisting of a promoter, a ribosome-binding site, and an enhancer.

12. The vector of claim 9, further comprising a second gene of interest (GOI).

13. The vector of claim 12, wherein the second GOI encodes a glycoprotein.

14. The vector of claim 13, wherein said glycoprotein is selected from an antibody light chain or antigen-binding fragment thereof, an antibody heavy chain or antigen-binding fragment thereof, an Fc-fusion protein, a ligand, and a receptor or ligand-binding fragment thereof.

15. A method of employing tunicamycin (Tn) as a selection marker in mammalian cell culture, comprising

(a) providing a mammalian host cell population,

(b) introducing a vector according to claim 9 into the cell population of step (a) by transfection;

(c) culturing the cell population of step (b) in the presence of Tn, thereby obtaining a cell transfectant comprising said nucleic acid of said vector, wherein said first GOI of said vector encodes a first protein of interest (POI).

16. The method of claim 15, wherein the Tn is at a concentration of at least 1 µg/mL.

17. The method of claim 15, wherein the Tn is at a concentration of 1 µg/mL, 2.5 µg/mL, or 5

μg/mL.

18. The method of claim 15, wherein the cell transfectant is obtained by culturing the cell population of step (b) in the presence of sequentially increasing concentrations of Tn.

19. The method of claim 18, wherein said culturing comprising culturing at a first Tn concentration of 1 μg/mL, followed by culturing at a second Tn concentration of 2.5 μg/mL or 5 μg/mL.

20. The method of claim 15, further comprising culturing the obtained cell transfectant, expressing said first POI from said first GOI in the cell transfectant, and isolating said first POI from the cultured cell transfectant.

21. The method of claim 18, further comprising culturing the obtained cell transfectant, expressing said first POI from said first GOI in the cell transfectant, and isolating said first POI expressed from the cultured cell transfectant.

22. The method of claim 15, wherein the mammalian host cell is selected from the group consisting of CHO, COS-7, HEK293, tumor cell, lymphocyte, retinal cell, and stem cell.

23. The method of claim 15, wherein the mammalian host cell is a CHO cell.

24. The method of claim 15, wherein the vector comprises a second GOI which encodes a protein selected from the group consisting of an antibody light chain or antigen-binding fragment thereof, an antibody heavy chain or antigen-binding fragment thereof, an Fc-fusion protein, a ligand, and a receptor or ligand-binding fragment thereof.

25. A method of producing a recombinant protein of interest (POI), wherein the method comprises:

a. providing a mammalian host cell comprising an exogenous nucleic acid, wherein the nucleic acid comprises (i) a mammalian tunicamycin (Tn)-resistance gene encoding a protein having at least 93% identity to the amino acid sequence of SEQ ID NO: 3, (ii) a first a gene of interest (GOI), and (iii) at least one regulatory element, wherein the Tn-resistance gene is operably linked to the first GOI and said at least one regulatory element, and wherein said first GOI encodes a first

POI, and wherein the first POI is selected from the group consisting of an antibody light chain or antigen-binding fragment thereof, an antibody heavy chain or antigen-binding fragment thereof, and an Fc-fusion protein; and

b. culturing the cell in the presence of Tn to express said first POI.

26. The method of claim 25, wherein the Tn is at a concentration of at least 1 µg/mL.

27. The method of claim 25, wherein the Tn is at a concentration of 1 µg/mL, 2.5 µg/mL, or 5 µg/mL.

28. The method of claim 25, wherein said culturing comprising culturing in the presence of sequentially increasing concentrations of Tn.

29. The method of claim 28, wherein said culturing comprising culturing at a first Tn concentration of 1 µg/mL, followed by culturing at a second concentration of 2.5 µg/mL, or 5 µg/mL.

30. The method of claim 25, wherein the mammalian host cell is selected from the group consisting of CHO, COS-7, HEK293, tumor cell, lymphocyte, retinal cell, and stem cell.

31. The method of claim 25, wherein the mammalian host cell is a CHO cell.

32. The method of claim 25, wherein the exogenous nucleic acid further comprises a second GOI which encodes a protein selected from the group consisting of an antibody light chain or antigen-binding fragment thereof, an antibody heavy chain or antigen-binding fragment thereof, an Fc-fusion protein, a ligand, and a receptor or ligand-binding fragment thereof.

33. A method of employing tunicamycin (Tn) as a selection marker in mammalian cell culture, comprising

(a) providing a mammalian host cell population,

(b) introducing into the cell population of step (a) a nucleic acid by transfection, wherein the nucleic acid comprises (i) a mammalian tunicamycin (Tn)-resistance gene encoding a protein having at least 93% identity to the amino acid sequence of SEQ ID NO: 3, and (ii) a first a gene of interest (GOI) encoding a first protein of interest (POI), wherein the Tn-resistance gene is

operably linked to the first GOI;

(c) culturing the cell population of step (b) in the presence of sequentially increasing concentrations of Tn, thereby selecting a cell transfectant that comprises said nucleic acid; and

(d) culturing the selected cell transfectant, expressing said first POI from said first GOI in the selected cell transfectant, and isolating said first POI from the cell culture.

34. The method of claim 33, wherein the culturing in step (c) comprises culturing at a first Tn concentration of 1 μg/mL, followed by culturing at a second Tn concentration of 2.5 μg/mL or 5 μg/mL.

35. The method of claim 33, wherein the Tn-resistance gene comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17.

36. The method of claim 33, wherein the mammalian host cell is selected from the group consisting of CHO, COS-7, HEK293, tumor cell, lymphocyte, retinal cell, and stem cell.

37. The method of claim 33, wherein the mammalian host cell is a CHO cell.

38. A method of producing a recombinant protein of interest (POI), wherein the method comprises:

(a) providing a mammalian host cell comprising an exogenous nucleic acid, wherein the exogenous nucleic acid comprises (i) a mammalian tunicamycin (Tn)-resistance gene encoding a protein having at least 93% identity to the amino acid sequence of SEQ ID NO: 3, and (ii) a first a gene of interest (GOI) encoding a first POI, wherein the Tn-resistance gene is operably linked to the first GOI;

(b) culturing the cell in the presence of Tn to express said first POI; and

(c) isolating said first POI from the cell culture.

39. The method of claim 38, wherein the Tn is at a concentration of 1 μg/mL, 2.5 μg/mL, or 5 μg/mL.

40. The method of claim 38, wherein said culturing comprising culturing in the presence of sequentially increasing concentrations of Tn.

41. The method of claim 40, wherein said culturing comprising culturing at a first Tn concentration of 1 µg/mL, followed by culturing at a second concentration of 2.5 µg/mL or 5 µg/mL.

42. The method of claim 38, wherein the Tn-resistance gene comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17.

43. The method of claim 38, wherein the mammalian host cell is selected from the group consisting of CHO, COS-7, HEK293, tumor cell, lymphocyte, retinal cell, and stem cell.

44. The method of claim 38, wherein the mammalian host cell is a CHO cell.