CLAIMS

What is claimed is:

1. A method of producing a population of producer cells expressing a target polypeptide, the method comprising:

(a) transfecting host cells with one or more vectors that encode one or more mRNAs, wherein the one or more mRNAs encode a selectable polypeptide and the target polypeptide;

(b) isolating from the transfected host cells, within 2 to 5 days of transfection, a sub-population of early-expressing transfected host cells which express the selectable polypeptide wherein the isolating employs magnetic activated cell sorting (MACS) or fluorescence activated cell sorting (FACS) on the selectable polypeptide; and

(c) expanding the isolated sub-population of transfected host cells, thereby producing a population of producer cells expressing the target polypeptide, wherein steps (b) and (c) are each performed in drug-selection-free medium.

2. The method of claim 1, further comprising:

(i) isolating the target polypeptide from the population of producer cells; or

(ii) isolating one or more single transfected host cells from the expanded sub-population and culturing the one or more single transfected host cells to produce clonal populations of the one or more single transfected host cells.

3. The method of claim 2, wherein at least one of the clonal populations of the one or more single transfected host cells yields a 2- to 30-fold improvement in production of the target polypeptide compared to that of a stable pool of transfected but uncloned host cells obtained at step (c).

4. The method of any one of claims 1 to 3, wherein the isolating in step (b) is:

(i) performed between two and four days after transfection;
(ii) performed two days after transfection; or
(iii) performed three days after transfection.

5. The method of any one of claims 1 to 4, wherein the expanding in step (c) is for between 4-31 days.

6. The method of any one of the preceding claims, wherein the selectable polypeptide is a FACS selectable polypeptide and the isolating in step (b) employs FACS.

7. The method of any one of claims 1 to 6, wherein the target polypeptide and the selectable polypeptide are encoded by a single multicistronic mRNA.

8. The method of claim 7, wherein the multicistronic mRNA comprises a first open reading frame (ORF) that encodes the selectable polypeptide and a second ORF that encodes the target polypeptide, wherein the first ORF is 5' to the second ORF.

9. The method of claim 7 or 8, wherein the first ORF has a non-AUG start codon.

10. The method of claim 9, wherein the non-AUG start codon is a UUG, GUG, or CUG in a Kozak consensus sequence.

11. The method of any one of claims 7 to 10, wherein the second ORF has an AUG start codon.

12. The method of any one of claims 7 to 11, wherein the ORF that encodes the selectable polypeptide is devoid of any AUG sequences.

13. The method of any one of claims 1 to 12, wherein the target polypeptide is a therapeutic agent.

14. The method of any one of claims 1 to 13, wherein the target polypeptide is a secreted protein.

15. The method of any one of claims 1 to 14, wherein the target polypeptide is an antibody or an Fc fusion protein.