CLAIMS

What is claimed is:

1. A method of fluorescence activated cell sorting (FACS) to batch select producer cells expressing a target polypeptide without use of methotrexate (MTX) as an amplification agent, the method comprising:

(a) providing a heterogeneous population of producer cells, wherein the producer cells in the population express varying levels of a FACS selectable polypeptide and a target polypeptide that are encoded by the same multicistronic mRNA,

(b) selecting from the heterogeneous population of producer cells a first heterogeneous sub-population of producer cells using FACS, wherein the producer cells in the first heterogeneous sub-population express the FACS selectable polypeptide at a level that is higher than the level of at least 80% of the producer cells in the heterogeneous population in (a), and

(c) expanding the first heterogeneous sub-population of producer cells in drug-selection-free medium, thereby producing an expanded first heterogeneous sub-population of producer cells.

2. The method of claim 1, further comprising:

(d) selecting from the expanded first heterogeneous sub-population of producer cells in (c) a second heterogeneous sub-population of producer cells using FACS, wherein the producer cells in the second sub-population express the FACS selectable polypeptide at a level that is higher than the level of at least 80% of the producer cells in the expanded first heterogeneous sub-population of producer cells in (c), and

(e) expanding the second heterogeneous sub-population of producer cells in a drug-selection-free medium, thereby producing an expanded second heterogeneous sub-population of producer cells, optionally further comprising isolating the target polypeptide from the expanded first or second heterogeneous sub-population, and/or optionally further comprising

(f) isolating one or more single producer cells from the expanded first or second heterogeneous sub-population and individually culturing the one or more single producer cells to produce clonal populations of the one or more single producer cells.

3. The method of claim 1 or 2, wherein the expanded first heterogeneous sub-population of producer cells yields a 1.2- to 5-fold improvement in production of the target polypeptide compared to that of the heterogeneous population of producer cells in (a).

4. The method of claim 2 or 3, wherein the expanded second heterogeneous sub-population of producer cells yields a 1.2- to 2.5-fold improvement in production of the target polypeptide compared to that of the expanded first heterogeneous sub-population of producer cells in (c).

5. The method of any one of claims 2 to 4, wherein at least one of the clonal populations of the one or more single cells yields a 5- to 30-fold improvement in production of the target polypeptide compared to that of the heterogeneous population of producer cells in (a).

6. The method of any one of claims 1 to 5, wherein the expanding in c) is for between 7-14 days.

7. The method of any one of claims 2 to 6, wherein the expanding in e) is for between 7-14 days.

8. The method of any one of claims 1 to 7, wherein the drug-selection-free medium is methionine sulphoximine-free medium.

9. The method of any one of claims 1 to 8, wherein the heterogeneous population of producer cells in (a) is produced by transfecting cells with a vector that encodes the multicistronic mRNA and subjecting the transfected cells to one round of medium-based selection to select cells expressing varying levels of the multicistronic mRNA.

10. The method of claim 9, wherein the vector further contains a dihydrofolate reductase (DHFR) gene, optionally wherein the medium-based selection is nucleotide-deficient medium.

11. The method of any one of claims 1 to 8, wherein the heterogeneous population of producer cells in (a) is produced by transfecting cells with a vector that encodes the

multicistronic mRNA and subjecting the transfected cells to FACS to select cells expressing varying levels of the multicistronic mRNA.

12. The method of any one of claims 1 to 11, wherein the multicistronic mRNA comprises a first open reading frame (ORF) that encodes the FACS selectable polypeptide and a second ORF that encodes the target polypeptide, wherein the first ORF is 5' to the second ORF, optionally wherein

the first ORF has a non-AUG start codon, optionally wherein

the second ORF has an AUG start codon, optionally wherein

the non-AUG start codon is a UUG, GUG or CUG in a Kozak consensus sequence.

13. The method of claim 12, wherein the ORF that encodes the FACS selectable polypeptide is devoid of any AUG sequences, and/or the FACS selectable polypeptide is CD52 or CD59.

14. The method of claim 12, wherein the ORF that encodes the FACS selectable polypeptide is devoid of any AUG sequences, and the FACS selectable polypeptide is CD52.

15. The method of any one of claims 1 to 14, wherein the target polypeptide is:

(i) a therapeutic agent,

(ii) a secreted protein, and/or

(iii) an antibody or an Fc fusion protein.