CLAIMS

WHAT IS CLAIMED IS:

1. A method for cell-free polypeptide synthesis comprising:

(a) lysing a cell to produce a cellular lysate;

(b) passing the cellular lysate through a filter to produce a cellular extract;

(c) contacting the cellular extract with a nucleic acid encoding a polypeptide to produce a reaction mixture; and

(d) incubating the reaction mixture to produce the polypeptide.

2. The method of claim 1, wherein the cellular lysate is not subject to clarification through centrifugation.

3. The method of claim 1, wherein the cellular extract is not subject to clarification through centrifugation.

4. The method of claim 1, wherein the reaction mixture is not subject to clarification through centrifugation.

5. The method of claim 1, further comprising flocculating the cellular lysate before the passing through the filter.

6. The method of claim 1, further comprising precipitating the cellular lysate before the passing through the filter.

7. The method of claim 1, wherein the filter comprises a surface filter.

8. The method of claim 1, wherein the filter comprises a depth filter.

9. The method of claim 1, wherein the passing comprises one or more selected from the group comprising cross-flow filtration, belt filtration, microfiltration, ultrafiltration, microporous filtration, vacuum-drum filtration, sieving, membrane filtration, sand filtration, and screen filtration.

10. The method of claim 1, wherein the filter comprises a pore that retains or rejects a particle with a size between about 0.1 micron and about 100 microns, or about 0.4 micro and about 15 microns.

11. The method of claim 1, wherein the filter comprises a pore that retains or rejects a particle with a size between about 0.2 micro and about 0.4 micron.

12. The method of claim 1, wherein the filter comprises a pore that retains or rejects a particle with a size of about 0.2 micron.

13. The method of claim 1, wherein the filter comprises at least two filters that are arranged in series or in parallel, wherein the filter comprises:

(a) a first filter comprising a pore that retains or rejects a particle with a size between about 0.5 micron and about 15 microns;

(b) a second filter comprising a pore that retains or rejects a particle with a size between about 0.2 micron and about 0.5 micron; and

(c) a third filter comprising a pore that retains or rejects a particle with a size of about 0.2 micron.

14. The method of claim 8, wherein the depth filter comprises a lenticular filter, a deep bed filter, a pad filter, a panel filter, a cartridge-type depth filter, or a combination thereof.

15. The method of claim 14, wherein the filter comprises:

(a) a first depth filter comprising a pore that retains or rejects a particle with a size between about 0.5 micron and about 15 microns;

(b) a second depth filter comprising a pore that retains or rejects a particle with a size between about 0.2 micron and about 0.5 micron; and

(c) a third filter comprising a sterilization-grade membrane and a pore that retains or reject a particle with a size of about 0.2 micron,

wherein the cellular lysate is subject to filtration through the first depth filter, then, the second depth filter, and, last, the third filter.

16. The method of claim 1, further comprising obtaining a cell from a cell culture.

17. The method of claim 16, wherein the cell is obtained by hollow fiber membrane separation.

18. The method of claim 1, wherein the lysing comprises one or more selected from the group comprising sonication, homogenization, nitrogen cavitation, freeze-thawing, syringing, chemical lysis, enzymatic lysis, osmotic lysis, French press lysis, bead-beating, and a combination thereof.

19. The method of claim 1, further comprising freezing or freeze-drying the cellular extract.

20. The method of claim 16, wherein the cell comprises a transgenic cell, a mammalian cell, a bacterial cell, a plant cell, a yeast cell, an insect cell, a fungal cell, an algal cell, or a combination thereof.

21. The method of claim 1, wherein the nucleic acid comprises a DNA,

wherein the DNA comprises a promoter for a DNA-dependent RNA polymerase, and wherein the open reading frame is operatively linked to the promoter, and

wherein the DNA comprises a terminator located downstream of the 3' end of the open reading frame.

22. The method of claim 21, wherein the promoter comprises a T7 promoter.

23. The method of claim 22, wherein the T7 promoter comprises a sequence having at least 95% sequence identity with SEQ ID NO: 1.

24. The method of claim 21, wherein the terminator comprises a T7 terminator comprising a sequence having at least 95% sequence identity with SEQ ID NO: 2.

25. The method of claim 1, wherein the reaction mixture comprises a divalent cation.

26. The method of claim 1, wherein the reaction mixture comprises an amino acid, a nucleotide, a salt, a cofactor, an energy source, a translation template, or a combination thereof.

27. The method of claim 26, wherein the energy source comprises a phosphate group or a non-phosphorylated energy group.

28. The method of claim 26, wherein the energy source comprises glutamate and is present at a concentration between about 10 mM and about 400 mM, or
wherein the energy source comprises pyruvate and is present at a concentration between about 10 mM and about 400 mM, or
wherein the energy source comprises glucose at a concentration between about 10 and about 400 mM.

29. The method of claim 26, wherein the salt comprises potassium at a concentration between about 50 mM and about 500 mM, or
wherein the salt comprises magnesium at a concentration between about 1 mM and about 30 mM, or
wherein the salt comprises ammonium at a concentration between about 1 mM and about 400 mM, or
wherein the salt comprises:

(a) potassium at a concentration between about 10 mM and about 400 mM; and

(b) magnesium at a concentration between about 1 mM and 30 mM.

30. The method of claim 1, further comprising isolating the polypeptide from the reaction mixture.

31. The method of claim 26, wherein the nucleotide comprises a nucleoside monophosphate (NMP), a nucleoside diphosphate (NDP), a nucleoside triphosphate (NTP), or a combination thereof.