CLAIMS

What is claimed is:

1. A system for recombinant cell-free expression comprising:

- a core recombinant protein mixture having at least the following components:
- a plurality of initiation factors (IFs);
- a plurality of elongation factors (EFs);
- a plurality of peptide release factors (RFs);
- at least one ribosome recycling factor (RRF);
- a plurality of aminoacyl-tRNA-synthetases (RSs); and
- at least one methionyl-tRNA transformylase (MTF);
- at least one nucleic acid synthesis template;
- a reaction mixture having cell-free reaction components necessary for in vitro macromolecule synthesis;
- wherein the above components are situated in a bioreactor configured for cell-free expression of macromolecules; and

- wherein at least one of the components of said core recombinant protein mixture is derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria.

2. The system of claim 1, wherein said Geobacillus thermophilic bacteria is selected from the group consisting of: *Geobacillus subterraneus*, and *Geobacillus stearothermophilus*.

3. The system of claim 1, wherein said core recombinant protein mixture derived from bacteria comprises a core recombinant protein mixture wherein at least one components is derived from a non-thermophilic bacteria.

4. The system of claim 3, wherein said non-thermophilic bacteria comprise *Escherichia coli*.

5. The system of claim 1, wherein said plurality of initiation factors (IFs) comprises a plurality of initiation factors derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic.

6. The system of any one of claims 1, and 5, wherein said plurality of initiation factors comprise IF1, IF2, IF3.

7. The system of any one of claims 1, 5, and 6, wherein the plurality of initiation factors are selected from the group of amino acid sequences consisting of: SEQ ID NOs. 2, 4, 6, 70, 72, and 74, or a sequence having at least 90% sequence identity.

8. The system of claim 1, wherein said plurality of elongation factors (EFs) comprises a plurality of elongation factors derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria.

9. The system of any one of claims 1, and 8, wherein said plurality of elongation factors comprise EF-G; EF-Tu; EF-Ts; EF-4; EF-P.

10. The system of any one of claims 1, 8, and 9, wherein the plurality of elongation factors are selected from the group of amino acid sequences consisting of: SEQ ID NOs. 8, 10, 12, 14, 16, 76, 78, 80, 82, and 84, or a sequence having at least 90% sequence identity.

11. The system of claim 1, wherein said plurality of peptide release factors (RFs) comprises a plurality of peptide release factors is derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria or a *Bacillus* bacteria.

12. The system of any one of claims 1, and 11, wherein said plurality of peptide release factors comprise RF1, RF2, and RF3.

13. The system of any one of claims 1, 11, and 12, wherein the plurality of peptide release factors are selected from the group of amino acid sequences consisting of: SEQ ID NOs. 18, 20, 22, 86, 88, or a sequence having at least 90% sequence identity.

14. The system of claim 1, wherein said ribosome recycling factor (RRF) comprises a ribosome recycling factor derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria .

15. The system of any one of claims 1, and 14, wherein said ribosome recycling factor is derived from *Geobacillus*.

16. The system of any one of claims 1, 14, and 15, wherein the ribosome recycling factor comprises a ribosome recycling factor according to amino acid sequences SEQ ID NOs. 14, and 90, or a sequence having at least 90% sequence identity.

17. The system of claim 1, wherein said plurality of aminoacyl-tRNA-synthetases (RSs) comprises a plurality of aminoacyl-tRNA-synthetases derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria , or E. Coli.

18. The system of any one of claims 1, and 17, wherein the plurality of aminoacyl-tRNA-synthetases comprises AlaRS; ArgRS; AsnRS; AspRS; CysRS; GlnRS; GluRS; GlyRS; HisRS; IleRS; LeuRS; LysRS; MetRS; PheRS (a); PheRS (b); ProRS; SerRS; ThrRS; TrpRS; TyrRS; and ValRS.

19. The system of any one of claims 1, 17, and 18, wherein said plurality of aminoacyl-tRNA-synthetases are selected from the group of amino acid sequences consisting of: SEQ ID NOs. 26, 28. 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, and 130, or a sequence having at least 90% sequence identity

20. The system of claim 1, wherein said methionyl-tRNA transformylase (MTF) comprises a methionyl-tRNA transformylase derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria .

21. The system of claim 1, and 20, wherein said methionyl-tRNA transformylase is derived from *Geobacillus*.

22. The system of any one of claims 1, 20, and 21, wherein the methionyl-tRNA transformylase comprises a methionyl-tRNA transformylase according to amino acid sequences SEQ ID NOs. 68, and 132, or a sequence having at least 90% sequence identity.

23. The system of claim 1, wherein said nucleic acid synthesis template comprises a DNA template.

24. The system of claim 23, wherein said DNA template comprises a linear DNA template having:

- at least one target sequence operably linked to a promoter, and wherein said target sequence may optionally be codon optimized;
- at least one ribosome binding site (RBS);
- at least one expression product cleavage site; and
- at least one tag.

25. The system of claim 1, wherein said nucleic acid synthesis template comprises an RNA template.

26. The system of claim 1, wherein said reaction mixture comprises one or more of the following components:

- a quantity of ribosomes, and optionally a quantity of ribosomes derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria;
- a quantity of RNase inhibitor;
- a quantity of RNA polymerase;
- a quantity of tRNAs, and optionally a quantity of tRNAs derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria ;
- a buffer; and
- a quantity of amino acids.

27. The system of claim 26, wherein said reaction mixture further comprises one or more of the following components:

- Tris-Acetate;
- Mg(OAc)2;
- K+-glutamate;
- amino-acetate;
- NaCl;
- KC1;
- MgCl2;
- DTT;
- octyl-b-glycoside;
- NAD;

- NADP;
- sorbitol;
- FADH;
- CoA;
- PLP; and
- SAM.

28. The system of any of claims 1, and 26, and further comprising an energy source.

29. The system of claim 28, wherein said energy source comprises a quantity of nucleotide tri-phosphates (NTPs).

30. The system of claim 29, wherein said nucleotide tri-phosphates comprise one or more of the nucleotide tri-phosphates selected from the group consisting of: adenine triphosphate (ATP); Guanosine triphosphate (GTP), Uridine triphosphate UTP, and Cytidine triphosphate (CTP).

31. The system of any of claims 28, 29 and 30, wherein said energy source comprises an inorganic polyphosphate-based energy regeneration system.

32. The system of claim 31, wherein said inorganic polyphosphate-based energy regeneration system comprises:

- a cellular adenosine triphosphate (ATP) energy regeneration system comprising:
- a quantity of Adenosyl Kinase (Gst AdK) enzyme;
- a quantity of Polyphosphate Kinase (Taq PPK) enzyme;
- a quantity of inorganic polyphosphate (PPi); and
- a quantity of adenosine monophosphate (AMP);

- wherein said AdK and PPK enzymes work synergistically to regenerate cellular ATP energy from PPi and AMP.

33. The system of claim 1, wherein said bioreactor comprises a continuous flow bioreactor.

34. A recombinant cell-free expression reaction mixture comprising:

- a plurality of initiation factors (IFs);
- a plurality of elongation factors (EF);
- a plurality of release factors (RF)
- at least one ribosome recycling factor (RRF);
- a plurality of aminoacyl-tRNA-synthetases (RSs);

wherein at least one of the components of said reaction mixture is derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria.

35. The system of claim 34, wherein said plurality of initiation factors (IFs) comprise a plurality of initiation factors derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria .

36. The system of any one of claims 34, and 35, wherein said plurality of initiation factors derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria comprise IF1, IF2, IF3.

37. The system of any one of claims 34, 35 and 36, wherein the plurality of initiation factors are selected from the group of amino acid sequences consisting of: SEQ ID NOs. 2, 4, 6, 70, 72, and 74, or a sequence having at least 90% sequence identity.

38. The system of claim 34, wherein said plurality of elongation factors (EFs) comprise a plurality of elongation factors derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria .

39. The system of any one of claims 34, and 38, wherein said plurality of elongation factors derived from a thermophilic bacteria comprises EF-G, EF-Tu, EF-Ts, EF-4, EF-P.

40. The system of any one of claims 34, 38, and 39, wherein the plurality of elongation factors are selected from the group of amino acid sequences consisting of: SEQ ID NOs. 8, 10, 12, 14, 16, 76, 78, 80, 82, and 84, or a sequence having at least 90% sequence identity.

41. The system of claim 34, wherein said plurality of peptide release factors (RFs) comprise a plurality of release factors derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria , or a *Bacillus* sp. bacteria.

42. The system of any one of claims 34, and 41, wherein the plurality of peptide release factors comprises RF1, RF2, and RF3.

43. The system of any one of claims 34, 41, and 42, wherein the plurality of peptide release factors are selected from the group of amino acid sequences consisting of: SEQ ID NOs. 18, 20, 22, 86, 88, or a sequence having at least 90% sequence identity.

44. The system of claim 34, wherein said ribosome recycling factor (RRF) comprise a ribosome recycling factor derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria .

45. The system of any one of claims 34, and 44, wherein said ribosome recycling factor is derived from *Geobacillus*.

46. The system of any one of claims 34, 44 and 45, wherein the ribosome recycling factor comprise a ribosome recycling factor according to amino acid sequence SEQ ID NOs. 14, and 90, or a sequence having at least 90% sequence identity.

47. The system of claim 34, wherein said plurality of aminoacyl-tRNA-synthetases (RSs) comprise a plurality of aminoacyl-tRNA-synthetases wherein at least one is derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria.

48. The system of any one of claims 34, and 47, wherein the plurality of aminoacyl-tRNA-synthetases comprise AlaRS; ArgRS; AsnRS; AspRS; CysRS; GlnRS; GluRS; GlyRS; HisRS; IleRS; LeuRS; LysRS; MetRS; PheRS (a); PheRS (b); ProRS; SerRS; ThrRS; TrpRS; TyrRS; and ValRS.

49. The system of any one of claims 34, 47 and 48, wherein said plurality of aminoacyl-tRNA-synthetases are selected from the group of amino acid sequences consisting of: SEQ ID NOs. 26, 28. 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, and 130, or a sequence having at least 90% sequence identity

50. The system of any one of claims 34, wherein said methionyl-tRNA transformylase (MTF) comprises a methionyl-tRNA transformylase derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria .

51. The system of any one of claims 34, and 50, wherein said methionyl-tRNA transformylase derived from *Geobacillus*.

52. The system of any one of claims 34, 50, and 51, wherein the methionyl-tRNA transformylase comprises a methionyl-tRNA transformylase according to amino acid sequence SEQ ID NOs. 68, and 132, or a sequence having at least 90% sequence identity.