**CLAIMS**

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) derived from thermophilic *Bacillaceae* bacteria or *Geobacillus* thermophilic bacteria;
- a plurality of elongation factors (EFs) derived from thermophilic *Geobacillus* bacteria;
- a plurality of peptide release factors (RFs) wherein at least one or more of the IFs are derived from *Bacillaceae* bacteria or thermophilic *Geobacillus* bacteria;
- at least one ribosome recycling factor (RRF) derived from thermophilic *Geobacillus* bacteria;
- a plurality of aminoacyl-tRNA-synthetases (RSs) wherein at least one or more of the IFs are derived from thermophilic *Geobacillus* bacteria, thermophilic *Bacillaceae* or *E. Coli* bacteria; and
- at least one methionyl-tRNA transformylase (MTF) derived from thermophilic *Geobacillus bacteria*;
- 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.

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 claims1, wherein said plurality of initiation factors comprise IF1, IF2, IF3.

4. The system of any one of claims 1, and 3, 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.

5. The system of claim 1, wherein said plurality of elongation factors comprise EF-G; EF-Tu; EF-Ts; EF-4; EF-P.

6. The system of any one of claims 1, and 5, 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.

7. The system of claim 1, wherein said plurality of peptide release factors comprise RF1, RF2, and RF3.

8. The system of any one of claims 1, and 7, 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.

9. The system of claim 1, wherein said ribosome recycling factor is derived from *Geobacillus*.

10. The system of any one of claims 1, and 9, 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.

11. The system of claim 1, 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.

12. The system of any one of claims 1, and 11, 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

13. The system of claim 1, 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.

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

15. The system of claim 14, wherein said DNA template comprises a linear DNA template having:

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

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

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

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

18. The system of claim 17, wherein said reaction mixture further comprises one or more of the following components:

a. Tris-Acetate;

b. Mg(OAc)2;
c. K+-glutamate;
d. amino-acetate;
e. NaCl;
f. KC1;
g. MgCl2;
h. DTT;
i. octyl-b-glycoside;
j. NAD;
k. NADP;
l. sorbitol;
m. FADH;
n. CoA;
o. PLP; and
p. SAM.

19. The system of claim 1, and further comprising an energy source.

20. The system of claim 19, wherein said energy source comprises a quantity of nucleotide tri-phosphates (NTPs).

21. The system of claim 20, 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).

22. The system of any of claims 19, 20 and21, wherein said energy source comprises an inorganic polyphosphate-based energy regeneration system.

23. The system of claim 22, wherein said inorganic polyphosphate-based energy regeneration system comprises:

a. a cellular adenosine triphosphate (ATP) energy regeneration system comprising:

b. a quantity of Adenosyl Kinase (Gst AdK) enzyme;
c. a quantity of Polyphosphate Kinase (Taq PPK) enzyme;
d. a quantity of inorganic polyphosphate (PPi); and
e. a quantity of adenosine monophosphate (AMP);

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

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

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

a. a plurality of initiation factors (IFs) selected from the group consisting of SEQ ID NOs. 2, 4, 6, 70, 72, and 74, or an IF having at least 90% sequence identity with any of SEQ ID NOs. 2, 4, 6, 70, 72, and 74;
b. a plurality of elongation factors (EF) selected from the group consisting of SEQ ID NOs. 8, 10, 12, 14, 16, 76, 78, 80, 82, and 84, or an EF having at least 90% sequence identity with any of SEQ ID NOs. 8, 10, 12, 14, 16, 76, 78, 80, 82, and 84;
c. a plurality of release factors (RF) selected from the group consisting of SEQ ID NOs. 18, 20, 22, 86, and 88, or an RF having at least 90% sequence identity with any of SEQ ID NOs. 18, 20, 22, 86, and 88.
d. at least one ribosome recycling factor (RRF) selected from the group consisting of SEQ ID NOs. 14, and 90, or a RRF having at least 90% sequence identity with any of SEQ ID NOs. 14, and 90;
e. a plurality of aminoacyl-tRNA-synthetases (RSs) selected from the group 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 RSs having at least 90% sequence identity with any 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;
f. at least one methionyl-tRNA transformylase (MTF) selected from the group consisting of SEQ ID NOs. 68, 132, or a MTF having at least 90% sequence identity with any of SEQ ID NOs. 68, and 132; and

g. a nucleic acid synthesis template.