Supplementary Material

Tuning the cell-free protein synthesis system for biomanufacturing of monomeric human filaggrin

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ATGCAAGTCAGCACACACGAGCAGTCGGAAAGTTCTCATGGTTGGACAGGTCCATCAACTCGTGGTCGCCAAGGATCACGTCACGAGCAAGCACAAGATTCTAGCCGCCATTCTGCGAGCCAGGATGGCCAGGATACGATTCGCGGGCATCCTGGTAGCTCGCGTGGTGGACGTCAAGGTTATCACCACGAGCACAGTGTTGATTCGTCGGGGCACTCAGGTTCGCACCATAGCCACACGACGTCACAGGGACGCTCGGACGCGAGCCGTGGACAGTCGGGGTCTCGTTCAGCATCACGTACAACTCGTAATGAAGAGCAATCTGGCGATGGCAGTCGTCATAGCGGCAGTCGCCACCATGAGGCCTCAACTCACGCAGACATCTCTCGCCATTCTCAAGCGGTTCAAGGACAATCGGAAGGTAGCCGCCGCAGTCGCCGCCAAGGATCTAGCGTTTCCCAGGACTCAGATTCAGAGGGACATTCAGAAGACTCCGAACGTTGGTCTGGTAGTGCGTCCCGCAATCACCATGGCTCCGCCCAGGAGCAACTTCGCGACGGGTCACGCCATCCACGCAGTCACCAGGAAGATCGCGCGGGTCATGGACATTCAGCGGATAGTTCTCGCCAGAGCGGGACTCGTCATACGCAGACCTCGTCGGGAGGTCAGGCAGCTTCAAGTCATGAGCAAGCACGTAGCAGTGCGGGAGAGCGTCACGGCTCGCATCATCAGCAGAGTGCAGACAGCTCTCGTCACTCGGGGATCGGGCATGGTCAAGCTAGTTCGGCTGTTCGTGATTCCGGGCATCGTGGGTACTCGGGCAGCCAAGCGTCGGACAACGAGGGACATTCGGAAGACAGTGATACACAGTCAGTGAGCGCGCACGGGCAGGCTGGTTCCCACCAACAATCGCACCAAGAGTCGGCTCGCGGGCGCTCTGGTGAAACAAGCGGACATTCGGGATCCTTTTTATACTAATAA

**Supplementary Figure 1.** Gene sequence of *E. coli* codon-optimized Filaggrin 8th repeat (981 bp, including the start codon (ATG) and two stop codons (TAATAA)).

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Description automatically generated**Supplementary Figure 2.** **Preparation of expression vectors.** **(A)** a schematic cloning workflow for expression vectors preparation. **(B)** the PCR-amplified gene fragments from the expression vectors. The size of wild-type and codon-optimized FLG gene fragment is identical. The size of the PCR-amplified fragment, including the overlapping sequences with linearized vectors, vary. The gene sizes are 1389 bp from pETBlue-1 and 1174 bp for pJL1-based recombinant plasmids.

**Supplementary Figure 3.** **The effect of the supplemental IPTG in the CFPS reaction (ROS extract).** The arrows indicate the synthesized FLG in the CFPS. Lane 1: protein size marker, lane 2: negative control without DNA, lane 3: pJL1-FLG WT addition, lane 4: pJL1-FLG Codon Opt addition, lane 5-8: pET-FLG WT addition (IPTG supplement concentration: 0, 0.5, 0.75, and 1 mM), lane 9-12: pET-FLG Codon Opt addition (IPTG supplement concentration: 0, 0.5, 0.75, and 1 mM). All CFPS reactions were performed at 30 °C for 20 hours.

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Description automatically generatedSupplementary Figure 5. Solubility of cell-free synthesized sfGFP and FLG, and optimization of Mg2+ and reaction time.** The green arrows indicate sfGFP and red arrows indicate FLG. **(A)** the sfGFP and FLG expression in CFPS at different incubation temperatures. **(B)** a side-by-side comparison of sfGFP and FLG expression at different Mg2+ concentrations. **(C)** the effect of magnesium ions concentration in CFPS. Lane 1: protein size marker, lane 2: negative control without DNA, lane 3-7: sfGFP expression at 4, 8, 12, 16, and 20 mM of Mg2+ concentration, lane 8-12: FLG expression at 4, 8, 12, 16, and 20 mM of Mg2+ concentration. **(D)** protein expression by incubation time. Lane 1: protein size marker, lane 2: negative control, lane 3-7: sfGFP expression at different reaction time (8, 12, 16, 20, and 24 hours), lane 8-12: FLG expression at reaction time (8, 12, 16, 20, and 24 hours).