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Nota di contenuto	Cell-free Protein Synthesis; Contents; Preface; List of Contributors; 1 Cell-free Protein Synthesis Systems: Historical Landmarks, Classification, and General Methods; 1.1 Introduction: Historical Landmarks; 1.1.1 Discovery of Protein Synthesis in Cell Extracts; 1.1.2 Translation of Exogenous Messages; 1.1.3 Coupled Transcription-translation in Bacterial Extracts; 1.1.4 Combined Transcription-translation Systems; 1.1.5 Continuous Flow/Continuous Exchange Principle; 1.2 Prokaryotic and Eukaryotic Types of Cell-free Expression Systems; 1.2.1 Cell Extracts; 1.2.1.1 E. coli extract (ECE) 1.2.1.2 Wheat Germ Extract (WGE)1.2.1.3 Rabbit Reticulocyte Lysate (RRL); 1.2.2 Genetic Constructs (Expression Vectors); 1.2.2.1 Prokaryotic Systems; 1.2.2.2 Eukaryotic Systems; 1.3 Preparing Cell Extracts; 1.3.1 E. coli Extracts; 1.3.1.1 Genetics; 1.3.1.2 Cell Growth; 1.3.1.3 Extract Preparation; 1.3.2 Wheat Germ Extracts; 1.4 Designing Reaction Composition; 1.4.1 Mg(2+) and Phosphate; 1.4.2 Other Salts; 1.4.3 Nucleotides and Amino Acids; 1.4.4 Stabilization Reagents; 1.4.5 Other Factors; 1.5 Providing Energy; 1.5.1 Direct Nucleotide

Regeneration; 1.5.2 Indirect Nucleotide Regeneration
1.6 Enhancing Protein Folding
1.6.1 Temperature Effects; 1.6.2 Cell Extract Concentration; 1.6.3 Effects of Folding Ligands; 1.6.4 Effects of Chaperones and Foldases; 1.6.5 Effects of Detergents; 2 The Constructive Approach for Cell-free Translation; 2.1 Introduction; 2.2 The Process of Protein Synthesis; 2.2.1 Polypeptide Synthesis; 2.2.2 Protein Maturation; 2.3 A Constructive Approach to Protein Synthesis; 2.3.1 In Vitro Reconstitution of Polypeptide Synthesis; 2.3.2 Protocol of Protein Synthesis using PURE System; 2.3.3 Addition of Protein Folding Machinery to the PURE System
2.3.4 Integration of a Membrane Targeting System with the PURE system
2.3.5 Protein Synthesis using the PURE System containing Molecular Chaperones; 2.4 Conclusion; 3 Functional Genomic Analysis using Sequential Cell-free Protein Synthesis; 3.1 Introduction; 3.1.1 The Post-genomic Era; 3.1.2 Cell-free Protein Synthesis (CFPS) as a Functional Proteomic Tool; 3.2 Developing an enabling Technology for Sequential Expression Analysis; 3.2.1 Improving Linear Template Stability; 3.2.2 Improving PCR Reactions for generating Genomic Linear Templates
3.2.3 Optimizing Cofactor Concentrations for Enzyme Activation
3.3 Demonstrating Functional Genomic Analysis with CFPS; 3.3.1 Isolation and Expression of Genomic Targets; 3.3.2 Effects of Sample Library on -Lactamase Expression and Activity; 3.4 Conclusions and Projections; 4 Cell-free Technology for Rapid Production of Patient-specific Fusion Protein Vaccines; 4.1 Introduction; 4.1.1 Lymphoma and Fusion Protein Vaccine Treatments; 4.1.2 Comparing Cell-free and In Vivo Production Systems; 4.2 Developing the Fusion Protein Construct and the Cell-free Production Process
4.2.1 Fusion-protein Production in the Cell-free System

Sommario/riassunto

With its detailed description of membrane protein expression, high-throughput and genomic-scale expression studies, both on the analytical and the preparative scale, this book covers the latest advances in the field. The step-by-step protocols and practical examples given for each method constitute practical advice for beginners and experts alike.
