

Cloning, Gene Expression and Protein Purification



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出版者:

出版时间:2001-3

装帧:

isbn:9780195132946

The central purpose of this combination lecture/laboratory manual is to present detailed protocols for the multi-step process involved in isolating a gene, cloning and

characterizing it, expressing the encoded protein, purifying the protein, and characterizing rudimentary aspects of its basic physical properties. The manual includes 20 experiments designed to train students to prepare, manipulate, and analyse plasmids, to produce fusion proteins in bacteria, and to purify these proteins based on unique chemical properties or substrate affinities. Electrophoresis, Southern and Western blotting and combinatorial techniques are emphasized. Radioisotopes, fluorophores and solvent effects on protein structure are discussed. (No radioisotopes are required.) The experiments included have been selected because of their high success rate and because they emphasize a project-oriented approach. Sufficient detail and background are provided such that the intended audience includes advanced undergraduate students, beginning graduates, and practising professionals engaged in a broad range of pursuits. Each experiment is accompanied by detailed process rationale. Where appropriate, alternate methods are also discussed. Maximizing its utility for both students and instructors, this unique manual precedes each lab experiment with the necessary background theory and principles, which culminate in a detailed process rationale and, ultimately, the experimental techniques themselves. While many lab manuals list background references, this innovative book combines both theory and procedure into one manageable volume. In addition to this theoretical background material, new innovations and insights are provided along with carefully selected primary source research literature.

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