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Altri autori (Persone)	FieldKatharine G ReamWalt
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Nota di contenuto	Front Cover; Molecular Biology Techniques: An Intensive Laboratory Course; Copyright Page; Contents; Preface; Course Synopsis; Introduction; Safety Precautions; Daily Schedule; Acknowledgments; Exercises I. DNA Preparation, Polymerase Chain Reaction, and Molecular Cloning; A. Cesium chloride-ethidium bromide density gradient centrifugation; B. PCR to synthesize virD2 flanked with restriction sites; C. Restriction digests of plasmid pGEX2 and PCR products; D. Purification of DNA fragments from agarose; E. Ligation of PCR product to pGEX2 vector F. Transformation of E. coli with the ligated plasmidG. Small-scale preparation of plasmid DNA by the alkaline lysis method; H. Restriction analysis; Study questions; Exercises II. Protein Expression, Purification, and Analysis; A. Expression and purification of a fusion protein; B. SDS-polyacrylamide gel electrophoresis; C. Silver stain detection of proteins; D. Western blot (immunoblot) detection of proteins; Study questions; Exercises III. Oligonucleotide-Directed Mutagenesis; A. Restriction digests of virD2 (in pCS64) and pUC119; B. Purification of DNA fragments from agarose C. Ligation of restriction fragment and vectorD. Transformation of E.

coli with the ligated plasmid and recovery of clones; E. Small-scale preparation of plasmid DNA from broth cultures; F. Restriction digest of DNAs: Examination to confirm insert; G. Preparation of single-stranded DNA template; H. Phosphorylation of oligonucleotide; I. Annealing mutant oligonucleotide to template; J. In vitro DNA synthesis by primer extension; K. Transform synthesis reaction into E. coli DH5a; L. Small-scale preparation of plasmid DNA; M. Confirmation of mutants by restriction analysis; Study questions
Exercises IV. DNA SequencingA. Polyacrylamide sequencing gel electrophoresis; B. Dideoxy sequencing; C. Automated sequencing; D. Introduction to databases and gene sequence analysis; Study questions; Exercises V. Southern Blot Detection of DNA; A. Preparation of genomic DNA from Agrobacterium tumefaciens; B. Restriction digestion of genomic DNA; C. Agarose gel electrophoresis of restriction fragments; D. Southern blot: Denaturation and blotting of DNA; E. Preparation of probe by nick translation; F. Hybridization and washing of Southern blots; Study questions
Exercises VI. Northern Blot Detection of mRNAA. Preparation of RNA from tobacco leaves; B. Agarose-formaldehyde gel electrophoresis; C. Northern blot: Denaturation and blotting of RNA; D. Probe preparation; E. Hybridization and washing of Northern blots; Study questions; Exercises VII. Protein Interaction Analysis in Yeast; A. Yeast transformation; B. Filter ß-galactosidase assay; Index

Sommario/riassunto

This manual is designed as an intensive introduction to the various tools of molecular biology. It introduces all the basic methods of molecular biology including cloning, PCR, Southern (DNA) blotting, Northern (RNA) blotting, Western blotting, DNA sequencing, oligo-directed mutagenesis, and protein expression.Key Features* Provides well-tested experimental protocols for each technique* Lists the reagents and preparation of each experiment separately* Contains a complete schedule of experiments and the preparation required* Includes study questions at the end of each ch