

1. Record Nr.	UNINA9910784643203321
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Titolo	RNA methodologies [[electronic resource]] : a laboratory guide for isolation and characterization // Robert E. Farrell, Jr
Pubbl/distr/stampa	Amsterdam ; ; Boston, : Elsevier/Academic Press, c2005
ISBN	1-280-62859-6 9786610628599 0-08-045476-3
Edizione	[3rd ed.]
Descrizione fisica	1 online resource (794 p.)
Disciplina	572.8/8
Soggetti	RNA - Analysis Nucleic acids - Analysis
Lingua di pubblicazione	Inglese
Formato	Materiale a stampa
Livello bibliografico	Monografia
Note generali	Description based upon print version of record.
Nota di bibliografia	Includes bibliographical references and index.
Nota di contenuto	Front cover; Title page; Copyright page; Dedication; Table of contents; Preface; 1 RNA and the Cellular Biochemistry Revisited; Why Study RNA?; What Is RNA?; Assembly of Polynucleotides; Types of RNA; Stringency: Conditions That Influence Nucleic Acid Structure; Types of Double-Stranded Molecules; References and Suggested Reading; 2 Transcription and the Organization of Eukaryotic Genes; Transcription and the Central Dogma; Gene Organization; RNA Polymerases and the Products of Transcription; References; 3 Messenger RNA; Topology of a Typical mRNA Molecule; Stability in the Cytoplasm Levels of Regulation References; 4 Resilient Ribonucleases; Rationale; Elimination of Ribonuclease Activity; Types of Ribonuclease Inhibitors; Preparation of Equipment and Reagents; Other Reagents Used to Control Nuclease Activity; Protocol: Synthesis of Vanadyl Ribonucleoside Complexes; References; 5 RNA Isolation Strategies; Rationale; Goals in the Purification of RNA; Lysis Buffer Formulations; Isolation of RNA with Guanidinium Buffers; Guanidinium-Acid-Phenol Extraction Techniques; Density Gradient Centrifugation; Simultaneous Isolation of RNA and DNA; The Word on Kits; Other Methods Short- and Long-Term Storage of Purified RNAs References; 6 The Truth About Tissues; Rationale; Tissue Culture or Tissue?; Homogenization Methods; RNA Isolation Strategies for Various Organs and Tissues;

Protocol: LiCl-Urea Method for RNA Isolation from Tissue; Protocol: RNA Isolation from Lipid-Enriched Tissue; Purification of Polysome-Engaged mRNA; Collecting Samples in the Field; RNA "Clean-Up" Methods; References; 7 Isolation of Polyadenylated RNA; Rationale; Polyadenylation; The Poly(A) Caveat; Selection of Polyadenylated Molecules; Magnetic Bead Technology for Poly(A)+ Purification Oligo(dT)-Cellulose Column ChromatographyRapid, Non-Column Poly (A)+ Purification; References; 8 Quality Control for RNA Preparations; Rationale; Quality Control Technique 1: Electrophoretic Profile of the RNA; Quality Control Technique 2: Ultraviolet Spectrophotometry and Absorption Ratios; Quality Control Technique 3: Sample Capacity to Support RT-PCR; Quality Control Technique 4: Northern Analysis; Quality Control Technique 5: Sample Capacity to Support In Vitro Translation; References; 9 Dot Blot Analysis; Rationale; Advantages and Disadvantages; Appropriate Positive and Negative Controls Limitations of the DataReferences; 10 Electrophoresis of RNA; Rationale; Normalization of Nucleic Acids; RNA Denaturing Systems for Agarose Gel Electrophoresis; Molecular Weight Standards; Gel Staining Techniques; Safety Considerations in Electrophoresis; Maintenance of Electrophoresis Equipment; Running Agarose Gels for the First Time: A Few Tips; References; 11 Photodocumentation and Image Analysis; Rationale; Photodocumentation; Digital Image Analysis; Suggested Reading; 12 Northern Analysis; Rationale; Choice of Filter Membrane; Handling and Filter Preparation Northern Transfer Techniques

#### Sommario/riassunto

This laboratory guide represents a growing collection of tried, tested and optimized laboratory protocols for the isolation and characterization of eukaryotic RNA, with lesser emphasis on the characterization of prokaryotic transcripts. Collectively the chapters work together to embellish the RNA story, each presenting clear take-home lessons, liberally incorporating flow charts, tables and graphs to facilitate learning and assist in the planning and implementation phases of a project. RNA Methodologies, 3rd edition includes approximately 30% new material, including chapters on