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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 Chromatography Rapid, 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
