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| Nota di bibliografia | Includes bibliographical references and index. |
| Nota di contenuto | Cover; Title Page; Copyright; Contents; Preface; List of Contributors; Part I RNA Synthesis and Detection; Chapter 1 Enzymatic RNA Synthesis Using Bacteriophage T7 RNA Polymerase; 1.1 Introduction; 1.2 Description of Method - T7 Transcription In vitro; 1.2.1 Templates; 1.2.1.1 Strategy (i): Insertion into a Plasmid; 1.2.1.2 Strategy (ii): Direct Use of Templates Generated by PCR; 1.2.1.3 Strategy (iii): Annealing of a T7 Promoter DNA Oligonucleotide to a Single-Stranded Template; 1.2.2 Special Demands on the RNA Product<br>1.2.2.1 Homogeneous 5' and 3' Ends, Small RNAs, Functional Groups at the 5' End1.2.2.2 Modified Substrates; 1.3 Transcription Protocols; 1.3.1 Transcription with Unmodified Nucleotides; 1.3.2 Transcription with 2' -Fluoro-Modified Nucleotides; 1.3.3 T7 Transcripts with 5' - Cap Structures; 1.3.4 Purification; 1.4 Troubleshooting; 1.4.1 Low or No Product Yield; 1.5 Rapid Preparation of T7 RNA Polymerase; 1.5.1 Required Material; 1.5.1.1 Medium; 1.5.1.2 Buffers and Solutions; 1.5.1.3 Electrophoresis and Chromatography; 1.5.2 Procedure<br>1.5.2.1 Cell Growth, Induction, and Test for Expression of T7 RNAP1. 5.2.2 Purification of T7 RNAP; 1.5.3 Notes and Troubleshooting; References; Chapter 2 Production of RNAs with Homogeneous 5' - and |

3' -Ends; 2.1 Introduction; 2.2 Description of Approach; 2.2.1 Cis-Cleaving Autocatalytic Ribozyme Cassettes; 2.2.1.1 The 5' -Cassette; 2.2.1.2 The 3' -Cassette; 2.2.1.3 Purification of Released RNA Product and Conversion of End Groups; 2.2.2 Trans-Cleaving Ribozymes for the Generation of Homogeneous 3' Ends; 2.2.3 Further Strategies toward Homogeneous Ends
2.3 Critical Experimental Steps, Changeable Parameters, Troubleshooting2.3.1 Construction of Cis-Cleaving 5'- and 3'-Cassettes; 2.4 PCR Protocols; 2.5 Potential Problems; References; Chapter 3 RNA Ligation; 3.1 General Introduction; 3.1.1 T4 Polynucleotide Ligases; 3.1.2 Reaction Mechanism; 3.1.3 Advantages of T4 DNA Ligase for RNA Ligation; 3.1.4 Chapter Structure; 3.2 RNA Ligation Using T4 DNA Ligase (T4 Dnl); 3.2.1 Overview of the RNA Ligation Method Using the T4 DNA Ligase (T4 Dnl); 3.2.2 Large-Scale Transcription and Purification of RNAs
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3.4.2.2 Substrate Specificity and Reaction Conditions

Sommario/riassunto

The second edition of a highly acclaimed handbook and ready reference.  Unmatched in its breadth and quality, around 100 specialists from all over the world share their up-to-date expertise and experiences, including hundreds of protocols, complete with explanations, and hitherto unpublished troubleshooting hints. They cover all modern techniques for the handling, analysis and modification of RNAs and their complexes with proteins. Throughout, they bear the practising bench scientist in mind, providing quick and reliable access to a plethora of solutions for practical questions of RNA research