

Contents

HEALTH WARNING

ABBREVIATIONS

1. GEL ELECTROPHORESIS OF RNA

Don Grierson

Introduction

Polyacrylamide Tube Gel Electrophoresis

Apparatus

Preparation of gels

Setting up the electrophoresis apparatus

Sample preparation and loading of the gels

Electrophoresis of gels

Scanning the gels

Slicing of gels

Measurement of radioactivity in gel slices

Elution and recovery of RNA

Modifications to the Basic Tube Gel Method

Removal of DNA prior to electrophoresis

Denaturation of RNA before loading

Running gels at non-ambient temperatures

Gradient gels

Solubilisable polyacrylamide gels

Agarose-acrylamide composite gels

Other electrophoresis buffers

Denaturing Gels

Formamide gels

Urea gels

Methyl mercuric hydroxide gels

Molecular Weight Estimations

Non-denaturing gels

Denaturing gels

Electrophoresis in Polyacrylamide Slab Gels

Apparatus

Recipes for gels

Casting the gels

Electrophoresis

Detection and recovery of RNA in slab gels

Transfer of RNA to diazobenzoyloxymethyl-paper for hybridisation analysis

Problems of Polyacrylamide Gel Electrophoresis and their Remedies

Purification of Histone Messenger RNA: A Case Study

References

1

1

2

2

2

3

4

6

8

9

10

10

12

12

13

13

14

14

14

14

15

16

17

17

17

17

19

19

19

21

24

24

24

29

31

36

38

2. ELECTROPHORESIS OF DNA	39
Paul G. Sealey and Ed M. Southern	
Introduction	39
Basic Techniques and Their Application	39
Equipment	40
Buffers for non-denaturing gels	41
Buffers for denaturing gels	43
Gel preparation	43
Sample preparation and loading of the gels	46
Size markers for gels	48
Electrophoresis conditions	49
Analysis of gels after electrophoresis	49
Recovery of DNA fragments from agarose gels	53
Analysis of DNA fragments using hybridisation	55
Large Scale Preparative Gel Electrophoresis of DNA	59
The annular preparative apparatus	59
The linear preparative apparatus	67
Examples of fractionations	73
Acknowledgements	75
References	75
3. TWO-DIMENSIONAL GEL ELECTROPHORESIS OF NUCLEIC ACIDS	77
Rupert De Wachter and Walter Fiers	
Introduction to Two-dimensional Gel Electrophoresis of RNA	77
Factors governing the electrophoretic mobility of RNA on polyacrylamide gels	77
Main types of two-dimensional gel systems	79
Apparatus and Experimental Procedure for Two-dimensional Separations of RNA	81
Separation in the first dimension	81
Separation in the second dimension	86
Variations and modifications of the procedure	88
Temperature control	89
Auto-radiography	89
Detection of non-radioactive RNA	90
Measurement of radioactivity in excised slices	90
Recovery of RNA from the gel	91
Examples of Two-dimensional Separations of RNA	92
Separation of oligonucleotides	92
Separation of RNA fragments	95
Separation of small RNA molecules	98
Separation of mRNAs	101

Introduction to Two-dimensional Gel Electrophoresis of DNA	105
Separation of DNA Restriction Fragments on Conventional Gels	106
Basis of the separation	106
Separation in the first dimension	106
Separation in the second dimension	108
Examples of the application of the technique: separation of viral DNA restriction fragments	109
Separation of DNA Restriction Fragments on Denaturing Gradient Gels	110
Basis of the separation	110
Apparatus	111
Separation in the first dimension	112
Separation in the second dimension	112
Staining and autoradiography of gels	115
Examples of application of the technique: separation of bacterial DNA fragments	115
Acknowledgements	115
References	115
4. DNA SEQUENCING	117
R. Wayne Davies	
Introduction	117
DNA Sequencing by the Chain Terminator Method	120
Equipment and materials	120
Manipulation of small volumes of liquid	120
Characteristics of primer fragments	123
Isolation of DNA fragments	123
Preparation of single-stranded template DNA	128
Annealing of primer and template	130
The primed DNA synthesis reaction	132
Ribosubstitution: an alternative way of removing primer fragments	136
Application of the chain terminator method to the determination of DNA sequences <i>within</i> DNA fragments	137
Making Polyacrylamide Gels for DNA Sequencing	139
Equipment	139
Materials	141
Preparing the gel mould	141
Stock solutions	142
Pouring the gel	143
Preparation of the gel for electrophoresis	144
Loading the samples	144
Electrophoresis	145
Preparing the gel for autoradiography	145
Autoradiography	146
Reading and Interpreting Autoradiographs of Chain Terminator Sequencing Experiments	146

Common problems in interpreting gel autoradiographs	148
The M13 Sequencing System	149
Characteristics of M13 bacteriophage	149
The M13 cloning system	150
Using the M13 cloning system	151
Chain terminator sequencing with the M13 system	155
DNA Sequencing by the Chemical Method	159
The basic steps of the procedure	159
Equipment and materials	159
Input and purification of DNA fragments	161
End-labelling DNA fragments	161
Isolation of DNA fragments labelled at one end only	166
The base-specific cleavage reactions	166
Electrophoresis of the reaction products	171
Reading and interpreting autoradiographs of Maxam-Gilbert sequencing gels	171
Use of Computers	171
References	172
5. RNA SEQUENCING	173
James M. D'Alessio	
Introduction	173
The Isolation of RNA for Sequencing	174
Handling Microgram Quantities of RNA	175
Precautions against ribonuclease	175
Reaction vessels	176
Ethanol precipitation of RNA	176
General Comments on Terminally-labelled RNA Molecules for Sequencing	176
Labelling 5' termini	176
Labelling 3' termini	177
Preparative gel electrophoresis	177
Preparation of Terminally-labelled RNA for Sequencing	178
Removal of the cap structure	178
Labelling the 5' terminus with [γ - 32 P]ATP and T4 polynucleotide kinase	179
Labelling the 3' terminus with [5'- 32 P]pCp and T4 RNA ligase	180
Purification of 32 P-RNA by polyacrylamide gel electrophoresis	181
Elution of 32 P-RNA from polyacrylamide gels	182
Specific Cleavage of RNA for Sequencing	183
Fragmentation strategy using ribonuclease H	183
Site specific cleavage of RNA	184
Base Specific Cleavage of RNA: Enzymatic Sequencing	185
General comments	185
Procedures for the enzymatic sequencing of RNA	185
Alkaline hydrolysis of RNA	186

Base Specific Cleavage of RNA: Chemical Sequencing	187
General comments	187
Procedures for the chemical sequencing of RNA	188
RNA Sequencing Gels	191
Important considerations	191
Casting a thin sequencing gel	192
Autoradiography of sequencing gels	193
Interpretation of the autoradiographs	194
References	196
6. ELECTROPHORESIS OF NUCLEOPROTEINS	199
Graham H. Goodwin and Albert E. Dahlberg	
Electrophoresis of Nucleosomes	199
Introduction	199
Equipment	199
Stock solutions	201
Preparation of the sample	202
Preparation of the polyacrylamide gel slab	202
Sample loading and electrophoresis	204
Detection of the nucleosomal bands	204
Extraction of nucleosomal particles from the gels	206
Recovery and analysis of the protein and DNA of the extracted nucleosomes	207
Possible problems and their remedies	209
Two-dimensional electrophoresis of nucleosomes	210
Electrophoresis of Ribosomes and Polysomes	213
Introduction	213
Equipment	214
Stock solutions	214
Preparation of the sample	215
Preparation of agarose-acrylamide composite gels	215
Sample loading and electrophoresis	217
Gel staining	217
Two-dimensional gel electrophoresis	218
Applications of different composite gels	218
Possible problems and their remedies	224
References	224
APPENDIX I. Nucleic Acid Molecular Weight Markers	227
APPENDIX II. Suppliers of Specialist Items for Electrophoresis	233
INDEX	237