

# Contents

## Part I

### Fundamentals

<b>Introduction . . . . .</b>	<b>1</b>
<b>1 Electrophoresis . . . . .</b>	<b>7</b>
1.0 General . . . . .	7
1.1 Electrophoresis in non-restrictive gels . . . . .	15
1.1.1 Agarose gel electrophoresis . . . . .	15
1.1.2 Polyacrylamide gel electrophoresis of low-molecular weight substances . . . . .	18
1.2 Electrophoresis in restrictive gels . . . . .	19
1.2.1 The Ferguson plot . . . . .	19
1.2.2 Agarose gel electrophoresis . . . . .	20
1.2.3 Polyacrylamide gel electrophoresis of nucleic acids . . . . .	20
1.2.4 Polyacrylamide gel electrophoresis of proteins . . . . .	32
<b>2 Isotachopheresis . . . . .</b>	<b>41</b>
2.1 Migration with the same speed . . . . .	41
2.2 "Ion train" separation . . . . .	41
2.3 Zone sharpening effect . . . . .	42
2.4 Concentration regulation effect . . . . .	42
<b>3 Isoelectric focusing . . . . .</b>	<b>45</b>
3.1 Principles . . . . .	45
3.2 Gels for IEF . . . . .	47
3.3 Temperature . . . . .	48
3.4 Controlling the pH gradient . . . . .	48
3.5 The kinds of pH gradients . . . . .	48
3.5.1 Free carrier ampholytes . . . . .	48
3.5.2 Immobilized pH gradients . . . . .	52
3.6 Preparative isoelectric focusing . . . . .	55
3.7 Titration curve analysis . . . . .	56
<b>4 Blotting . . . . .</b>	<b>59</b>
4.1 Principle . . . . .	59
4.2 Transfer methods . . . . .	59
4.3 Blotting membranes . . . . .	63
4.4 Buffers for electrophoretic transfers . . . . .	64
4.5 General staining . . . . .	66
4.6 Blocking . . . . .	66
4.7 Specific detection . . . . .	67

4.8	Protein sequencing	69
4.9	Transfer problems .	69
<b>5</b>	<b>Interpretation of electropherograms</b>	<b>71</b>
5.1	Introduction . . . . .	71
5.1.1	Purity control . . . . .	71
5.1.2	Quantification prerequisites . . . . .	71
5.2	Image analysis . . . . .	73
5.2.1	Hardware for image analysis . . . . .	74
5.2.2	Software for image analysis . . . . .	75
<b>6</b>	<b>Proteome Analysis . . . . .</b>	<b>81</b>
6.1	General . . . . .	81
6.2	Sample preparation . . . . .	84
6.3	Two-dimensional electrophoresis . . . . .	85
6.4	Detection techniques . . . . .	88
6.5	Image analysis . . . . .	90
6.6	Protein spot identification . . . . .	90
6.6.1	Mass spectrometry methods . . . . .	92
6.6.2	Peptide mass fingerprinting . . . . .	97
6.6.3	Protein characterization . . . . .	99
6.7	Bioinformatics . . . . .	99
6.8	Functional proteomics . . . . .	100
<b>7</b>	<b>Instrumentation . . . . .</b>	<b>101</b>
7.1	Current and voltage conditions . . . . .	101
7.2	Power supply . . . . .	103
7.3	Separation chambers . . . . .	103
7.3.1	Vertical apparatus . . . . .	103
7.3.2	Horizontal apparatus . . . . .	104
7.4	Staining apparatus for gels and blots . . . . .	107
	Automated electrophoresis . . . . .	107
7.6	Instruments for 2-D electrophoresis . . . . .	109
7.6.1	Isoelectric focusing apparatus . . . . .	109
7.6.2	Multiple slab gel apparatus . . . . .	110
	Safety measures . . . . .	110
7.8	Environmental aspects . . . . .	
	<b>Equipment for Part II</b>	<b>113</b>
	Instrumentation . . . . .	113
	Laboratory equipment . . . . .	115
	<i>Consumables</i> . . . . .	116
	Chemicals . . . . .	

## Part II

### Methods

<b>Method 1: PAGE of dyes</b> . . . . .	123
1 Sample preparation . . . . .	123
2 Stock solutions . . . . .	123
3 Preparing the casting cassette . . . . .	123
4 Casting the ultrathin-layer gels . . . . .	126
5 Electrophoretic separation . . . . .	126
<b>Method 2: Agarose and immuno electrophoresis</b> . . . . .	129
1 Sample preparation . . . . .	129
2 Stock solutions . . . . .	129
3 Preparing the gels . . . . .	130
4 Electrophoresis . . . . .	134
5 Protein detection . . . . .	137
<b>Method 3: Titration curve analysis</b> . . . . .	141
1 Sample preparation . . . . .	141
2 Stock solutions . . . . .	141
3 Preparing the blank gels . . . . .	142
4 Titration curve analysis . . . . .	145
5 Coomassie and silver staining . . . . .	147
6 Interpreting the curves . . . . .	149
<b>Method 4: Native PAGE in amphoteric buffers</b> . . . . .	151
1 Sample preparation . . . . .	152
2 Stock solutions . . . . .	152
3 Preparing the empty gels . . . . .	155
4 Electrophoresis . . . . .	157
5 Coomassie and silver staining . . . . .	160
<b>Method 5: Agarose IEF</b> . . . . .	163
1 Sample preparation . . . . .	163
2 Preparing the agarose gel . . . . .	164
3 Isoelectric focusing . . . . .	167
5 Protein detection . . . . .	169
<b>Method 6: PAGIEF in rehydrated gels</b> . . . . .	171
1 Sample preparation . . . . .	171
2 Stock solutions . . . . .	172
3 Preparing the blank gels . . . . .	172
4 Isoelectric focusing . . . . .	175
5 Coomassie and silver staining . . . . .	177
6 Perspectives . . . . .	181

<b>Method 7: Horizontal SDS-PAGE</b> . . . . .	183
1 Sample preparation . . . . .	183
2 Stock solutions for the preparation of gels . . . . .	187
3 Preparing the casting cassette . . . . .	187
4 Gradient gel . . . . .	189
5 Electrophoresis . . . . .	193
6 Protein detection . . . . .	195
7 Blotting . . . . .	198
8 Perspectives . . . . .	198
<b>Method 8: Vertical PAGE</b> . . . . .	201
1 Sample preparation . . . . .	202
2 Stock solutions . . . . .	202
3 Single gel casting . . . . .	203
4 Multiple gel casting . . . . .	207
5 Electrophoresis . . . . .	210
6 SDS electrophoresis of small peptides . . . . .	211
7 Two-dimensional electrophoresis . . . . .	213
8 DNA electrophoresis . . . . .	213
9 Long shelflife gels . . . . .	214
10 Protein detection . . . . .	214
<b>Method 9: Semi-dry blotting of proteins</b> . . . . .	215
1 Transfer buffers . . . . .	216
2 Technical procedure . . . . .	217
3 Staining of blotting membranes . . . . .	221
<b>Method 10: IEF in immobilized pH gradients</b> . . . . .	223
1 Sample preparation . . . . .	224
2 Stock solutions . . . . .	224
3 Immobiline recipes . . . . .	225
4 Preparing the casting cassette . . . . .	228
5 Preparing the pH gradient gels . . . . .	229
6 Isoelectric focusing . . . . .	234
7 Coomassie and silver staining . . . . .	236
8 Strategies for IPG focusing . . . . .	238
<b>Method 11: High-resolution 2D electrophoresis</b> . . . . .	239
1 Sample preparation . . . . .	240
2 Stock solutions . . . . .	243
3 Preparing the gels . . . . .	244
4 Separation conditions . . . . .	248
5 Staining procedures . . . . .	256
<b>Method 12: PAGE of double stranded DNA</b> . . . . .	261
1 Stock solutions . . . . .	262
2 Preparing the gels . . . . .	263

3	Sample preparation	266
4	Electrophoresis . . .	267
5	Silver staining . . .	271

**Method 13: Native PAGE of single stranded DNA** 273

1	Sample treatment . . . . .	275
2	Gel properties . . . . .	276
3	Buffers and additives . . . . .	276
4	Conditions for electrophoresis . . . . .	277
5	Strategy for SSCP analysis . . . . .	278

**Method 14: Denaturing gradient gel electrophoresis** 279

1	Sample preparation . . . . .	280
2	Rehydration solutions . . . . .	280
3	Preparing the rehydration cassette . . . . .	280
4	Rehydration . . . . .	282
5	Electrophoresis . . . . .	284
6	Silver staining . . . . .	286

**Method 15: Denaturing PAGE of DNA** 287

1	Sample preparation . . . . .	288
2	Solutions . . . . .	288
3	Rehydration . . . . .	289
4	Electrophoresis . . . . .	289
5	Silver staining . . . . .	292

**Appendix**

<b>A</b>	<b>Trouble-shooting guide</b> . . . . .	293
A1	Isoelectric focusing . . . . .	293
A1.1	PAGIEF with carrier ampholytes . . . . .	293
A1.2	Agarose IEF with carrier ampholytes . . . . .	301
A1.3	Immobilized pH gradients . . . . .	304
A2	SDS electrophoresis . . . . .	310
A3	Vertical PAGE . . . . .	318
A4	Semi-dry blotting . . . . .	320
A5	2-D electrophoresis . . . . .	326
A6	DNA electrophoresis . . . . .	330

**B References** 333

**Index** 341