

Contents

Part I Archive Tissues

1 Archive Tissues	3
Giorgio Stanta	
2 Preanalytical Time Interval (PATI) and Fixation	5
Lorenzo Daniele, Giuseppe D'Armento, and Gianni Bussolati	
3 Formalin-Free Fixatives	13
Isabella Dotti, Serena Bonin, Giorgio Basili, and Valentina Faoro	

Part II Dissection of Tissue Components

4 Manual Microdissection	21
Valentina Faoro and Giorgio Stanta	
5 Tissue Microarray (TMA)	23
Valentina Faoro and Anna Sapino	
6 Laser Capture Microdissection (LCM)	27
Elvira Stacher, Hannelore Kothmaier, Iris Halbwedl, and Helmut H. Popper	

Part III Extraction, Purification, Quantification and Quality Assessment of Nucleic Acids

7 DNA Extraction from Formalin-Fixed Paraffin-Embedded (FFPE) Tissues	33
Serena Bonin, Patricia J.T.A. Groenen, Iris Halbwedl, and Helmut H. Popper	
8 DNA Extraction from Formalin-Fixed Paraffin-Embedded Tissues (FFPE) (from Small Fragments of Tissues or Microdissected Cells)	37
Serena Bonin, Patricia J.T.A. Groenen, Iris Halbwedl, and Helmut H. Popper	

9	Fast Protocol for DNA Extraction from Formalin-Fixed Paraffin-Embedded Tissues	41
	Falk Hlubek and Andreas Jung	
10	DNA Extraction from Blood and Forensic Samples	45
	Solange Sorçaburu Cigliero, Elisabetta Edalucci, and Paolo Fattorini	
11	Restoration and Reconstruction of DNA Length	55
	Serena Bonin and Federica Tavano	
12	RNA Extraction from Formalin-Fixed Paraffin-Embedded Tissues	57
	Serena Bonin and Giorgio Stanta	
13	RNA Extraction from Decalcified and Non-decalcified Formalin-Fixed Paraffin-Embedded Tissues	63
	Marco Alberghini, Stefania Benini, Gabriella Gamberi, Stefania Cocchi, and Licciana Zanella	
14	RNA Temperature Demodification	67
	Serena Bonin and Giorgio Stanta	
15	MicroRNA Extraction from Formalin-Fixed Paraffin-Embedded Tissues	71
	Roberto Cirombella and Andrea Vecchione	
16	Quantification of Nucleic Acids	75
	Isabella Dotti and Serena Bonin	
17	Integrity Assessment of Nucleic Acids	81
	Isabella Dotti and Serena Bonin	
18	DNase Treatment of RNA	87
	Isabella Dotti and Serena Bonin	
 Part IV Reverse Transcription (RT)		
19	Reverse Transcription	93
	Isabella Dotti, Serena Bonin, and Ermanno Nardon	
 Part V Nucleic Acid Amplification		
20	General Protocol for End-Point PCR	101
	Serena Bonin and Isabella Dotti	
21	Quantitative End-Point PCR	105
	Serena Bonin and Isabella Dotti	

22	Nested-PCR	111
	Serena Bonin and Isabella Dotti	
23	General Protocol for Dot-Blot	115
	Serena Bonin and Isabella Dotti	
24	PCR-ELISA	117
	Christiane Schewe, Wilko Weichert, and Manfred Dietel	
25	Quantitative Real-Time RT-PCR	121
	Isabella Dotti, Ermanno Nardon, Danae Pracella, and Serena Bonin	
 Part VI DNA Sequencing		
26	DNA Sequencing from PCR Products	135
	Giorgio Basili, Serena Bonin, and Isabella Dotti	
27	Pyrosequencing Analysis for Detection of KRAS Mutation	143
	Gerlinde Winter and Gerald Höfler	
 Part VII Microsatellite Analysis		
28	Microsatellite Instability (MSI) Detection in DNA from FFPE Tissues	155
	Damjan Glavač and Ermanno Nardon	
29	General Protocol for Loss of Heterozygosity Detection	171
	Damjan Glavač and Ermanno Nardon	
 Part VIII Methylation Analysis		
30	Qualitative Methylation Status Assessment	181
	Damjan Glavač and Ermanno Nardon	
31	Quantitative Methylation Status Assessment in DNA from FFPE Tissues with Bisulfite Modification and Real-Time Quantitative MSP	193
	Ermanno Nardon	
 Part IX Analysis of Copy Number Variations		
32	Comparative Genomic Hybridisation (CGH)	203
	Hannelore Kothmaier, Elvira Stacher, Iris Halbwedl, and Helmut H. Popper	
33	Multiplex Ligation-dependent Probe Amplification (MLPA)	215
	Ana Pilar Berbegall, Eva Villamón, Samuel Navarro, and Rosa Noguera	

- 34 Fluorescence In Situ Hybridization (FISH) on Formalin-Fixed Paraffin-Embedded (FFPE) Tissue Sections** 225
Marta Piqueras, Manish Mani Subramaniam, Samuel Navarro,
Nina Gale, and Rosa Noguera

Part X Microarrays

- 35 Introduction on Genome-wide Expression Profiling from Formalin-Fixed Paraffin-Embedded Tissues Using Microarrays** 233
Manfred Dietel, Jan Budczies, Wilko Weichert, and Carsten Denkert

Part XI Protein Extraction in Archive Tissues

- 36 Introduction** 241
Valentina Faoro, Karl-Friedrich Becker, and Giorgio Stanta
- 37 Protein Extraction from Formalin-Fixed Paraffin-Embedded Tissues** 245
Karl-Friedrich Becker and Christina Schott
- 38 Rapid Protein Extraction from Formalin-Fixed Paraffin-Embedded Tissue Samples** 249
Valentina Faoro and Giorgio Stanta
- 39 Protein Extraction Protocol from Tissues Treated with Formalin-Free Fixatives** 253
Valentina Faoro and Giorgio Stanta
- 40 Trichloroacetic Acid (TCA) Precipitation of Proteins** 257
Valentina Faoro and Giorgio Stanta

Part XII Protein Electrophoretic Separation

- 41 One-Dimensional Sodium-Dodecyl-Sulfate (SDS) Polyacrylamide Gel Electrophoresis** 261
Valentina Faoro, Karl-Friedrich Becker, and Giorgio Stanta
- 42 Visualization of Proteins** 265
Valentina Faoro, Karl-Friedrich Becker, and Giorgio Stanta

Part XIII Protein Immunodetection

- 43 Western Blotting** 271
Valentina Faoro, Karl-Friedrich Becker, and Giorgio Stanta
- 44 Dot Blot** 275
Valentina Faoro and Giorgio Stanta

45	Membrane Stripping	277
	Valentina Faoro and Giorgio Stanta	
46	Reverse-Phase Protein Microarrays.	279
	Christina Schott and Karl-Friedrich Becker	
 Part XIV Mass Spectrometry		
47	Introduction	285
	Valentina Faoro, Giorgio Stanta, and Alessandro Vindigni	
48	Sample Preparation and In Gel Tryptic Digestion for Mass Spectrometry Experiments	289
	Valentina Faoro and Giorgio Stanta	
49	MALDI Imaging Mass Spectrometry on Formalin-Fixed Paraffin-Embedded Tissues	293
	Axel Walch, Sandra Rauser, and Heinz Höfler	
 Part XV Elements of Good Laboratory Practice		
50	Introduction	299
	Giorgio Stanta, Ermanno Nardon, and Renzo Barbazza	
51	Internal Quality Control (IQC) Organization.	303
	Giorgio Stanta, Ermanno Nardon, and Renzo Barbazza	
52	Standards for Equipment and Reagents	309
	Ermanno Nardon and Renzo Barbazza	
	Appendices	315
	Index	319