Contents

1. Good Laboratory Practices **1**

Safety Aspects *1*
Laboratory Hazards *2*
Minimizing Laboratory Errors *2*
Preparing for the Laboratory *4*
Management and Handling of Biomedical Waste *4*

2. Equipment Used in Molecular Genetics Laboratory **21**

Autoclave *21*
Centrifuge *21*
Deep Freezers (–20°C and –70°C) *21*
Gel Electrophoresis Systems *22*
Incubator *22*
Rotamixer *22*
Sequencer *23*
Spectrophotometer *23*
SpeedVac *23*
Thermal Cycler *23*
UV Transilluminator with a Camera/Gel Documentation System *24*
Vortex Mixer *24*

3. Sample Collection **25**

4. Solutions **26**

5. DNA Extraction **27**

Phenol Chloroform Method *27*
Salt Method *28*
Quantification *28*
Extraction of DNA from Chorionic Villus Samples *28*
Extraction of DNA from Amniotic Fluid *29*

6. Checking Prenatal Samples for Maternal Contamination 30

Common Markers Used 30
Interpretation 31

7. Preparation of Gels and Staining 34

Agarose Gel Preparation 34
Electrophoresis 34
Polyacrylamide Gels 34
Silver Staining of Polyacrylamide Gels 35
Drying and Storage of Gels 36

8. Mutation Detection Methods 37

Single Strand Conformation Polymorphism (SSCP) Analysis 38
Sample Preparation 38
Gel Electrophoresis 38
Conformation-Sensitive Gel Electrophoresis (CSGE) 39
Linkage Analysis 40

9. Detection of Carriers 42

10. Autosomal Recessive Disorders 45

Chances of Inheriting a Trait 45

β-Thalassemia ***46***
Symptomatology 46
Molecular Genetics 46
Carrier Testing 47
Molecular Genetic Testing 47
XmnI Polymorphism 49

Hemoglobin E β-Thalassemia ***51***

Sickle Cell Anemia *51*
Symptomatology 51
Molecular Genetics 52
Molecular Genetic Testing 52

Hemoglobin D Variant ***52***
Molecular Genetic Testing 53

Spinal Muscular Atrophy 53
Symptomatology 53
Molecular Genetics 54
Molecular Genetic Testing 54

Cystic Fibrosis 55
Symptomatology 55
Molecular Genetics 55
Molecular Genetic Testing 56
Prenatal Diagnosis by Polymorphic Markers 58

Megalencephalic Leukodystrophy with Subcortical Cysts (MLC) 59
Symptomatology 59
Molecular Genetics 60
Molecular Genetic Testing 60

Metachromatic Leukodystrophy (MLD) 61
Symptomatology 61
Molecular Genetics 62
Molecular Genetic Testing 62
Detection of Pseudodeficiency Alleles 63

Congenital Adrenal Hyperplasia (CAH) 66
Symptomatology 66
Molecular Genetics 66
Molecular Genetic Testing 67

Gaucher Disease 71
Symptomatology 71
Molecular Genetics 71
Molecular Genetic Testing 71

Non-syndromic Hearing Loss 74
Symptomatology 74
Molecular Genetics 74
Hearing Loss Research—Indian Scenario 75
Molecular Genetic Testing 75
PCR Condition for Connexin-26 Screening 77

Albinism 77
Molecular Genetics 77
Molecular Genetic Testing 77

11. X-Linked Disorders 80

Duchenne/Becker Muscular Dystrophy ***80***
Symptomatology 81
Molecular Genetics 82
Molecular Genetic Testing 83
Deletion Study 83
CA Repeat Analysis for Carrier Status Analysis 85
DMD Analysis Using Multiplex Ligation-Dependent Probe Amplification (MLPA) 87

Fragile X Syndrome ***89***
Transmission of the Fragile X 89
Symptomatology 90
Molecular Genetics 90
Molecular Genetic Testing 91
CGG Repeat Sizing in Fragile X Syndrome 92

Rett Syndrome ***96***
Symptomatology 96
Molecular Genetics 96
Molecular Genetic Testing 97
Analysis of Common MECP2 Mutations 97

Hemophilia A ***101***
Symptomatology 101
Molecular Genetics 101
Molecular Genetic Testing 101

Hemophilia B ***105***
Molecular Genetics 105
Molecular Genetic Testing 105

Ectodermal Dysplasia ***107***
Symptomatology 107
Molecular Genetics 108
Molecular Genetic Testing 108

12. Autosomal Dominant Disorders 112

Achondroplasia (ACH) ***113***
Symptomatology 113
Molecular Genetics 113
Molecular Genetic Testing 113

13. Methylenetetrahydrofolate Reductase (MTHFR) 115

Molecular Genetics 115
Molecular Genetic Testing 115

14. Sequencing Protocol 117

Preparing Template for Sequencing 117
Cycle Sequencing Reactions v 3.1 117
Performing Cycle Sequencing for Big Dye Terminator v 3.1 Chemistries 118
Purifying the Cycle Sequencing Product 118
Sequencing Data Analysis 119
Primary Analysis 119
Basic Local Alignment Search Tool (BLAST) 120

Annexure 122
Index 129