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Nota di contenuto	In Situ Hybridization; Contents; CHAPTER 1. Genomic In Situ Hybridization for Whole Chromosome and Genome Analysis; 1.1 Introduction; 1.2 Uses of Genomic In Situ Hybridization; 1.2.1 Genome Organization of Different Sequence Classes; 1.2.2 Species Identity, Differentiation and Relatedness; 1.2.3 Genomic Stability; 1.2.4 Spatial Organization of Chromosomes; 1.2.5 Chromosome Introgression and Rearrangements; 1.2.6 Chromosome Behaviour and Meiosis; 1.3 The Principle of Genomic In Situ Hybridization; 1.3.1 Genomic Probing; 1.3.2 Blocking and Competitive Hybridization; 1.4 Materials and Chemicals 1.4.1 Buffers and Reagents1.4.2 Chemicals; 1.4.3 Equipment; 1.5 Genomic In Situ Hybridization: Protocol; 1.5.1 Chromosome Preparations; 1.5.2 Probe DNA; 1.5.3 Blocking DNA; 1.5.4 In Situ Hybridization Procedure; 1.6 Troubleshooting; CHAPTER 2. Fluorescence In Situ Hybridization: Applications in Gene Mapping and Clinical Diagnostics; 2.1 Introduction; 2.2 Materials and Chemicals; 2.2.1 Buffers and Reagents; 2.2.2 Chemicals; 2.2.3 Equipment; 2.3 FISH Using Single Copy Cosmid Probes; 2.3.1 Preparation of Metaphase Chromosomes; 2.3.2 Preparation of Metaphase Chromosome Slides for

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2.3.3 Preparation and Labelling of Probes 2.3.4 Hybridization of Cosmid Probes; 2.4 Multicolor FISH Using Cosmid Probes; 2.4.1 Labelling of Probes and Hybridization Reactions; 2.5 Interphase FISH Analysis; 2.5.1 Preparation of Interphase Nuclear Slides from Short Term Cultures; 2.5.2 Direct Preparation of Interphase Spreads; 2.5.3 FISH; 2.6 FISH Analysis Using YAC Clones; 2.6.1 Preparation of Metaphase Chromosomes; 2.6.2 Isolation of YAC DNA; 2.6.3 Biotin Labelling of YACs; 2.6.4 FISH; 2.6.5 Microscopy; 2.7 Direct Visual In Situ Hybridization (DIRVISH); 2.7.1 Preparation of Stretched DNA 2.7.2 FISH Hybridization 2.7.3 Detection and Analysis; 2.7.4 Microscopy; 2.8 Troubleshooting; CHAPTER 3. Detection of Nucleic Acids (DNA and RNA) In Situ by Single and Cyclic Primed In Situ labelling (PRINS): Two Alternatives to Traditional In Situ Hybridization Methods; 3.1 Introduction; 3.2 Materials and Chemicals; 3.2.1 Buffers and Reagents; 3.2.2 Chemicals; 3.2.3 Equipment; 3.3 DNA-PRINS with Oligonucleotide Probes; 3.4 PRINS with Ddel Digested Cloned Probes; 3.4.1 Generation of Primers from Cloned Alpha Satellite DNA; 3.4.2 PRINS Reaction; 3.5 Multicolour-PRINS; 3.6 PRINS-painting 3.7 PRINS-PCR and Repeated-PRINS of DNA 3.7.1 Chromosome Spreads; 3.7.2 Pretreatment of Chromosome Spreads; 3.7.3 Labelling Reaction; 3.8 PRINS-PCR of mRNA; 3.9 Visualization of Hapten-Labelled Nucleotides; 3.9.1 Standard Slides; 3.9.1.1 Biotin; 3.9.1.2 Digoxigenin; 3.9.2 Micro-slides; 3.9.2.1 Detection of Labelled DNA; 3.9.2.2 Detection of Labelled RNA; 3.9.2.3 Alkaline Phosphatase Visualization; 3.9.2.4 Fluorescent Visualization; 3.10 Troubleshooting CHAPTER 4. Fluorescence Immunophenotyping and Interphase Cytogenetics as a Tool for Investigation of Neoplasms (FICTION): Combined In Situ Hybridization and Fluorescence Immunophenotyping

### Sommario/riassunto

In situ hybridization is a proven, powerful technique with applications in chromosome and genome analysis, as well as gene expression. Covering a carefully selected range of techniques with immediate and general applications in research and clinical diagnosis, the book starts with genome and DNA mapping, continues through gene expression localization in wholemount and tissue sections, and on to ultrastructural levels. The step-by-step protocols used reflect research in these areas and are all reproducible.