Chromosome microdissection and cloning : a practical guide /

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Monografía

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Tipo Audiovisual: Chromosomes Analysis

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Contenido: Introduction to chromosome microdissection -- Chromosome organization -- Cloning DNA from chromosome fragments -- Preparation of chromosomes for microdissection -- Critical aspects of chromosome preparation -- Enrichment of metaphase spreads -- Hypotonic treatment -- Chromosome fixing and spreading -- Aging, storing, and staining of metaphase spreads -- Reagents -- Equipment -- Protocols -- Protocol 1. Preparation of chromosomes from peripheral blood T lymphocytes (whole blood microculture method) -- Protocol 2. Preparation of chromosomes from monolayer tissue culture cell lines -- Protocol 3. Preparation of chromosomes from monolayer cells grown on coverslips -- Protocol 4. Preparation of chromosomes from dipteran salivary glands -- Protocol 5. Solid staining and GTG banding of metaphase chromosomes -- Methods of chromosome microdissection -- Methods -- Video microscope method -- Oil chamber method -- Laser microdissection method -- Summary of chromosome microdissection and collection for DNA cloning -- Reagents and equipment -- Protocol (cont) Molecular cloning of microdissected chromosal DNA -- Cloning of DNA from microdissected chromosomal DNA fragments -- Method 1. Direct cloning of DNA from microdissected chromosomal fragments -- Direct cloning into [gamma] phage -- Method 2. Ligation of microdissected chromosomal DNA with plasmid vector or linker-adaptor and PCR amplification -- Protocol 2.1. Ligation of microdissected DNA with plasmid vector, PCR amplification, and cloning -- Protocol 2.2. Ligation of microdissected DNA with linker-adaptor, PCR amplification, and cloning -- Method 3. PCR amplification of microdissected chromosomal DNA fragments followed by probing

a complete recombinant library -- Protocol 3.1. Preparation of chromosomal DNA for amplification -- Protocol 3.2. PCR amplification of microdissected chromosomal DNA using "universal" primers -- Protocol 3.3. PCR amplification using human Alu sequence-based primers -- Analysis of recombinant clones derived from microdissected chromosomal DNA -- Determination of DNA insert size range -- Determination of the percentage of recombinant clones containing repeat and unique sequences (cont) Protocol 4.1. Assay for repeat sequences -- Protocol 4.2. Assay for unique sequences -- Calculation of the percentage of total microdissected DNA cloned -- Determination of potential structural gene sequences -- Localization of recombinant clones using in situ hybridization -- Protocol 5.1. DNA probe labeling for fluorescence in situ hybridization -- Protocol 5.2. Fluorescent in situ hybridization to metaphase chromosome spreads -- Applications of chromosome microdissection -- Direct analysis of the PCR product of microdissected chromosome fragments -- Gene mapping -- Mapping sites of chromosome rearrangement and deletions -- Determination of coupling phase -- Recombinant DNA libraries generated from microdissected chromosome fragments -- Genetic analysis of specialized chromosome structures -- Applications in genomic sequencing projects -- Characterization of disease-related genetic loci -- Study of chromosome abnormalities in cancer cells -- Gene transfer using chromosome fragments

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