HELSINGIN YLIOPISTO — HELSINGFORS UNIVERSITET

Tiedekunta/Osasto — Fakultet/Sektion	Laitos — Institution
Mat.Luonn.tiet.tiedek./ Biotieteiden laitos	Perinnöllisyyst. osasto

Tekijä — Författare

MARIS LAAN

Työn nimi — Arbetets titel

Molecular cytogenetics in physical mapping and positional cloning

Oppiaine — Läroämne

Perinnöllisyystiede

Työn laji — Arbetets art	Aika — Datum	Sivumäärä — Sidoantal
Lisenssiaattitutkielma	13.04.1995	50+ 2 julkaisua

Tiivistelmä — Referat

Rapid developments in fluorescence in situ hybridization (FISH) have paved the way for the application of new visual high-resolution methods in physical mapping. The contribution of molecular cytogenetics in the construction of long-range maps of genomes and in projects aiming to positional cloning of disease genes, has increased significantly during the past few years.

The first half of this study was focused on the analysis of aspartylglucosaminuria (AGU) locus using more conventional molecular cytogenetic approaches. First, the chromosomal localization of the human AGA gene was refined to 4q34-35. In parallel, the corresponding mouse gene was mapped to 8B3, confirming the suggested homology between human 4q telomeric region and mouse chromosome 8 middle region. Second, the locus was studied as a model to estimate different approaches, FISH and solid-phase minisequencing, in detection of gene copy number. For the marker gene, AGA, copy number was analyzed in three patients with chromosomal alterations involving 4q. Interphase and metaphase FISH analysis indicated the loss of one AGA copy in two cases and the duplication of the gene in the third case. An alternative technique introduced for the detection of gene copy number, the PCR-based solid-phase minisequencing method, produced concordant results with the FISH analysis. A potential of the novel technique lies in the automation and large-scale analysis e.g. for screening trisomies or the loss of heterozygosity. FISH technique is required if subpopulations of cells in heterogenous samples, such as tumors, is studied.

The second half of the present work was performed within the frame of the larger positional cloning project striving to identify disease genes using high-resolution physical mapping as one of the main strategies. In this study, novel targets – mechanically stretched chromosomes, were studied for high-resolution FISH mapping. The result indicates that up to 10-fold higher degree of mapping resolution can be obtained compared to metaphase techniques. FISH to stretched chromosomes ideally bridges the resolution gap between the ranges covered by fiber FISH (1-300 kb) and metaphase analysis (2-3 Mb) and provides an accurate tool for ordering and orientating probes in the 200-3000 kb range. To illustrate the utility of this method, the gene for receptor tyrosine kinase TIE was located proximal to COL9A2, RLF, and L-MYC genes at 1p32.
Next, the study was concentrated on the physical mapping of the chromosomal area at 13q associated with the variant form of late infantile neuronal ceroid lipofuscinosis (vLINCL) disease with the main aim to identify the disease gene. Metaphase FISH analysis was used for the refinement of the vLINCL region to 13q22, exclusion of the candidate gene TPP and characterization of the loci of the YACs cloned for the establishment of a genomic contig over the critical region. Finally, a high-resolution physical map was constructed on the basis of the fiber-FISH analysis for the region found to be strongly associated with the vLINCL disease.

In conclusion, the field of this study involved the application and development of various FISH techniques for physical mapping purposes. In parallel with conventional metaphase FISH, new high-resolution techniques were studied and introduced. The study aimed to intensify and pave the way for bringing in the visual mapping techniques into routine usage in the projects concentrating on the genome mapping and the positional cloning of genes.

Avainsanat — Nyckelord

Physical mapping, FISH

Säilytyspaikka — Förvaringsställe

Muita tietoja — Övriga uppgifter