



Positional Cloning by Exon Trapping and cDNA Selection (EMBO)

Bernhard Korn, Marie-Laure Yaspo, Hans Lehrach, Annemarie Poustka

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Tremendous strides in decoding the human genome have been made over the last ten years. Large, chromosome-scale, genome-scale EST-sequencing and mapping techniques have dramatically expanded the base of identified genes and sequenced DNA. Gene isolation and cloning techniques that emerged earlier on, however, still remain effective mainstays for constructing dense transcript maps to isolate coding sequences and disease-associated genes contained within a specific chromosomal region. Positional Cloning by Exon Trapping and cDNA Selection examines two powerful methods for locating the coding region of a given gene by isolating gene fragments from individual clones or pools of genomic clones. This comprehensive guide details the exon-trapping and cDNA selection processes step by step—from isolating genomic templates and nuclear splicing, to verifying generated clones and sequenced data, to analyzing exon libraries and cDNA sublibraries. Procedures covered include: Exon-trapping systems and descriptions of pSPL1 and pSPL3 vectors. Exon amplification protocols for preparing vectors for cloning; subcloning genomic DNA into vectors; transformation, analysis, and transfection of sublibraries; RNA transcription; and PCR amplification and PCR product cloning. Evaluation of exon libraries by PCR colony testing, identifying artifactual clones using Southern blot, and sequencing and mapping back candidate exons. Isolation of genomic templates, such as COSMID-, P1-, PAC, and YAC DNA. cDNA selection experiment protocols including cDNA screening, hybridization, and biotinylation; preparation of genomic and cDNA sources; and PCR cloning. Clone analysis and analysis by hybridization. Positional Cloning by Exon Trapping and cDNA Selection offers researchers, scientists, and graduate students an invaluable tool for probing gene distribution and molecular organization. Most importantly, it provides a critical approach to isolating specific disease genes within a targeted genomic area.



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