F829

Allele-specific hybridization of tandem repeats

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The human genome contains a large number of tandemly repeated sequences occurring sporadically throughout the genome. The number of repetition as well as the size of the repeat sequence vary widely presumably because the tandem repeats do not encode anything. These hypervariable genetic loci due to the variable tandem repeats show high heterozygosity and frequently two distinct bands are observed upon gel electrophoresis when an indivisual's genomic DNA is examined after PCR. We noticed that the alleles of the tandem repeats segregate from each other even though the two alleles are almost identical in sequence through the denaturation and renaturation cycles of PCR. Taking advantage of such segregation, the alleles of tandem repeats could be easily identified without resorting to gel electrophoresis but by reverse dot-blotting in which the standard DNAs of each allele are fixed on the membrane and the amplified sample is applied for the selective hybridization.
[Supported by a grant from the Inje Foundation]

F830

Screening of Candidate Imprinted Genes through Isolation of Embryonic cDNA Subtracted by Parthenogenic Driver cDNA

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A subtraction procedure was desigined to isolated genes which are expressed only in normal mouse embryos while silent in mouse parthenogenons. Parthegenons are supposed not to express maternally imprinted genes, or if any at a very low level because their genome consists of only maternal haploid genomes. Total RNA was isolated from the parthenogenic embryos and normal embryos respectively. The RNA was then served as template for cDNA synthesis. This cDNA was amplified by PCR. Partheongenic cDNA was used as driver DNA which eliminate cDNA common in both embryos from normal embryonic cDNA pool. Subracted DNA was then rescreened by differential hybridization method. Seven clones were selected and two of them were sequenced. These two sequences were absent in public databases. Instead, the sequence of one clone named *Nepl* showed, partial homologies whth an EST sequence which was sequenced from cDNA library of human leg tissues. Northern blot analysis indicated that *Nepl* is expressed specifically in testis. We are now trying differential methyl-sensitive Southern blot analysis with the probe of *Nepl* and its full length cDNA is under screening procedures with mouse testis cDNA library.