We isolated a candidate choriocarcinoma suppressor gene from a PCR-based subtracted fragmentary cDNA library between normal placental villi and the choriocarcinoma cell line CC1. This gene comprises an open reading frame of 219 nt encoding 73 amino acids and contains a homeodomain as a consensus motif. This gene, designated NECC1 (not expressed in choriocarcinoma clone 1), is located on human chromosome 4q11-q12. NECC1 expression is ubiquitous in the brain, placenta, lung, smooth muscle, uterus, bladder, kidney, and spleen. Normal placental villi expressed NECC1, but all choriocarcinoma cell lines examined and most of the surgically removed choriocarcinoma tissue samples failed to express it. We transfected this gene into choriocarcinoma cell lines and observed remarkable alterations in cell morphology and suppression of in vivo tumorigenesis. Induction of CSH1 (chorionic somatomammotropin hormone 1) by NECC1 expression suggested differentiation of choriocarcinoma cells to syncytiotrophoblasts. Our results suggest that loss of NECC1 expression is involved in malignant conversion of placental trophoblasts.


http://www.sciencedirect.com/science/article/B6WG1-471W72M-4N/2/f7891522e50770bac39f3b422467aaad

A characteristic translocation t(2;13)(q35;q14) has been previously identified in the pediatric soft tissue tumor alveolar rhabdomyosarcoma. We have assembled a panel of lymphoblast, fibroblast, and somatic cell hybrid cell lines with deletions and unbalanced translocations involving chromosome 2 to develop a physical map of the distal 2q region. Twenty-two probes were localized on this physical map by Southern blot analysis of the mapping panel. The position of these probes with respect to the t(2;13) rhabdomyosarcoma breakpoint was then determined by
quantitative Southern blot analysis of an alveolar rhabdomyosarcoma cell line with two copies of the derivative chromosome 13 and one copy of the derivative chromosome 2 and by analysis of somatic cell hybrid clones derived from an alveolar rhabdomyosarcoma cell line. We demonstrate that the t(2;13) breakpoint is situated within a map interval delimited by the distal deletion breakpoint in fibroblast line GM09892 and the t(X;2) breakpoint in somatic cell hybrid GM11022. Furthermore, from a comparison of our data with the linkage map of the syntenic region on mouse chromosome 1, we conclude that the t(2;13) breakpoint is most closely flanked by loci INHA and ALPI within this map interval.


http://www.sciencedirect.com/science/article/B6WG1-471W7HX-7S/2/1d767bf258f3a2b9a62502beedd8200bb

Proteins with seven transmembrane segments (7TM) define a superfamily of receptors (7TM receptors) sharing the same topology: an extracellular N-terminus, three extramembranous loops on either side of the plasma membrane, and a cytoplasmic C-terminal tail. Upon ligand binding, cytoplasmic portions of the activated receptor interact with heterotrimeric G-coupled proteins to induce various second messengers. A small group, recently recognized on the basis of homologous primary amino acid sequences, comprises receptors to hormones of the secretin/vasoactive intestinal peptide/glucagon family, parathyroid hormone and parathyroid hormone-related peptides, growth hormone-releasing factor, corticotropin-releasing factor, and calcitonin. A cDNA, extracted from a neuroectodermal cDNA library, was predicted to encode a new 886-amino-acid protein with three distinct domains. The C-terminal third contains the seven hydrophobic segments and characteristic residues that allow the protein to be readily aligned with the various hormone receptors in the family. Six egf-like modules, at the N-terminus of the predicted mature protein, are separated from the transmembrane segments by a serine/threonine-rich domain, a feature reminiscent of mucin-like, single-span, integral membrane glycoproteins with adhesive properties. Because of its unique characteristics, this putative egf module-containing, mucin-like hormone receptor has been named EMR1. Southern analysis of a panel of somatic cell hybrids and fluorescence in situ hybridization have assigned the EMR1 gene to human chromosome 19p13.3.


http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-9V/2/60a1f6f0f6cd81e5fb841f9ad6363f6

An automated gridding procedure for the inoculation of yeast and bacterial clones in high-density arrays has been developed. A 96-pin inoculating tool compatible with the standard microtiter plate format and an eight-position tablet have been designed to fit the Biomek 1000 programmable robotic workstation (Beckman Instruments). The system is used to inoculate six copies of 80 x 120-mm filters representing a total of ~20,000 individual clones in approximately 3 h. High-density arrays of yeast artificial chromosome (YAC) and cosmid clones have been used for rapid large-scale hybridization screens of ordered libraries. In addition, an improved PCR library screening strategy has been developed using strips cut from the high-density arrays to prepare row and column DNA pools for PCR analysis. This strategy eliminates the final hybridization step and allows identification of a single clone by PCR in 2 days. The development of automated gridding technology will have a significant impact on the establishment of fully versatile screening of ordered library resources for genomic studies.
The ubiquitous nature of the Alu sequence throughout the human genome forms the basis of an assay we present here for analyzing the human chromosome content of human x rodent somatic cell hybrids. A human-specific Alu primer was used both to amplify sequences and to 32P label the products in a polymerase chain reaction (PCR) technique. Unlabeled inter-Alu PCR products from two series of human x rodent hybrids were used to prepare dot blots which were probed with labeled inter-Alu products prepared from between 103 and 104 hybrid cells. In the first series we demonstrate that a labeled inter-Alu probe from the hybrid DL18ts, containing a single chromosome 18, on a dot blot hybridized only with those inter-Alu products containing chromosome 18. Similar specificity for human chromosome 5 was shown when a Southern blot of the PCR products was hybridized with a probe made from the hybrid HHW 213, which contains only chromosome 5p. Using a dot blot from a second series of control hybrids, 15 of which contained single human chromosomes, hybridization of a labeled probe from the hybrid 18X4-1 was shown to react specifically with the controls that expressed chromosome 18. Application of the technique reported here allows simple and rapid characterization of the human chromosome content in human x rodent hybrids.

The human PDGFA gene, encoding the A chain of platelet-derived growth factor, has been previously cloned and characterized, but two conflicting chromosomal localizations have been presented. To resolve this controversy, we have now performed nonisotopic in situ hybridization using new genomic PDGFA subclones and analyzed somatic cell hybrid DNAs for the presence of human PDGFA by polymerase chain reaction. The results confirm our previous assignment of PDGFA to chromosome 7p22. New sequence data from the PDGFA locus have been obtained and analyzed. An unusual minisatellite, which includes an evolutionarily conserved protein-coding region of exon 4, was found within IVS4. The minisatellite includes an embedded polymorphic pentanucleotide microsatellite repeat. Analysis of this polymorphism and in situ hybridization both locate PDGFA outside the monosomic region in a patient with a de novo deletion of the short arm of chromosome 7 [del (7)(p22.1-pter)].

Four cosmid clones containing putative pseudogenes for human aldehyde dehydrogenase
(Aldose reductase) were isolated from libraries made to two individuals. These clones show different patterns on digestion with restriction endonucleases and probably represent distinct and separate loci. The DNA sequence of one of the putative pseudogenes (cosmid AR.F) was determined, and comparisons demonstrate 89.7% homology with the cDNA sequence of the functional aldose reductase gene. This pseudogene sequence contains no intronic sequences, whereas the functional aldose reductase has nine introns. In addition, the homology disappears in region 5' to the transcription start site for the cDNA, implying that regulatory elements such as the promoter are missing from this pseudogene. The pseudogene defined by cosmid AR.F has been mapped to chromosome 3 by polymerase chain reaction using amplimers specific for this pseudogene to amplify DNA from somatic cell hybrids.


http://www.sciencedirect.com/science/article/B6WG1-471W72M-4K/2/68b26433171dae6f8ca011c3934766e1


http://www.sciencedirect.com/science/article/B6WG1-471W72M-3J/2/c7906f446f88670feb49792e5ba90065

We constructed a yeast artificial chromosome (YAC) contig spanning the genes encoding Kit (Kit), the platelet-derived growth factor [alpha] receptor (Pdgfra), and fetal liver kinase 1 (Flk1), three members of a receptor tyrosine kinase gene family located in the central portion of mouse chromosome 5. The orientation of YAC clones and the extent of their overlap was determined by "probe content mapping," that is, hybridization analysis of YAC clones using the available gene probes and YAC end sequences. For four YAC clones, which constitute a minimal set spanning 1.8 Mb, a detailed restriction map was constructed. This map, in conjunction with the previously published long-range restriction map, indicates the order, the physical distances, and the relative transcriptional orientations of the Pdgfra, Kit, and Flk1 genes. The YAC clones and corresponding YAC end probes presented here provide an important resource for the molecular analysis of a cluster of developmental mutations, namely dominant white spotting (W), patch (Ph), recessive spotting (rs), and rump-white (Rw), associated with this chromosomal region.


http://www.sciencedirect.com/science/article/B6WG1-471W6W8-11/2/ffcc7468d836f93fbc46aeb5cad5bd2

We have cloned human genes that are encoded in the region 17q12-q23 and expressed in breast tissue using interspecific somatic cell hybrids and subtractive hybridization. Two mouse microcell hybrids containing fragments of human chromosome 17 with a nonoverlap region at 17q12-q23 were generated by microcell transfer. Radiolabeled cDNA was synthesized from the hybrid cell containing the 17q12-q23 interval and was subtracted with an excess of RNA from the hybrid cell lacking the interval. Resulting cDNA probes enriched for sequences from 17q12-q23 were used
to screen a human premenopausal breast cDNA library, and 60 cDNAs were identified. Three of these cDNAs mapped to the hybrid cell nonoverlap region. These cDNAs were expressed in mammary epithelial cell hybrids, although none appeared to be breast-specific. Sequence analysis of the cDNAs revealed that clone 93A represents a previously unidentified gene, clone 98C has homology to an expressed sequence tag from goat mammary tissue, and clone 200A is identical to the human homologue of the Drosophila melanogaster flightless-I gene. These genes map outside a 1-cM region linked to early onset familial breast cancer but may be useful genetic markers in the 17q12-q23 region.


http://www.sciencedirect.com/science/article/B6WG1-497H96X-2/2/c53b662063700b293eb20835107a8de

Human SSX was first identified as the gene involved in the t(X;18) translocation in synovial sarcoma. SSX is a multigene family, with 9 complete genes on chromosome Xp11. Normally expressed almost exclusively in testis, SSX mRNA is expressed in various human tumors, defining SSX as a cancer/testis antigen. We have now cloned the mouse ortholog of SSX. Mouse SSX genes can be divided into Ssxa and Ssxb subfamilies based on sequence homology. Ssxa has only one member, whereas 12 Ssxb genes, Ssxb1 to Ssxb12, were identified by cDNA cloning from mouse testis and mouse tumors. Both Ssxa and Ssxb are located on chromosome X and show tissue-restricted mRNA expression to testis among normal tissues. All putative human and mouse SSX proteins share conserved KRAB and SSX-RD domains. Mouse tumors were found to express some, but not all, Ssxb genes, similar to the SSX activation in human tumors.


http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-9J/2/1762078d2a2eef2ed35f8f88cc4c5161

A sequence tagged site (STS)-based approach has been used to construct a 2.6-Mb contig in yeast artificial chromosomes (YACs) spanning the human dystrophin gene. Twenty-seven STSs were used to identify and overlap 34 YAC clones. A DNA fingerprint of each clone produced by direct Alu-PCR amplification of YAC colonies and the isolation of YAC insert ends by vectorette PCR were used to detect overlaps in intron 1 (280 kb) where no DNA sequence data were available, thereby achieving closure of the map. This study has evaluated methods for mapping large regions of the X chromosome and provides a valuable resource of the dystrophin gene in cloned form for detailed analysis of gene structure and function in the future.


http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-6R/2/bba82d0d391c0c161076a04628b37f88

Human DNA segments from discrete chromosomal regions were generated by utilizing Alu-element-based polymerase chain reaction (Alu-PCR) of an irradiation-fusion hybrid containing
approximately 10 to 15 Mb of human DNA. Following cloning into a plasmid vector, a subset of
the clones was used to generate sequence tagged sites (STSs) de novo. By means of a panel of
hybrids containing portions of the human X chromosome, the STSs were shown to localize to two
chromosomal regions, Xq24-Xq26 and Xcen-Xq13, reflecting the presence in the irradiation-
fusion hybrid of two human chromosome fragments. These results demonstrate that high
densities of STSs can be rapidly and efficiently generated from defined regions of the human
genome using Alu-PCR.


http://www.sciencedirect.com/science/article/B6WG1-4DXB8F5-20/2/31524960512c989a971cbde2d2a74983

A CA repeat has been found on the human X chromosome within 16 kb of the gene encoding
properdin P factor (PFC) and has been shown to be a highly informative marker. Two more
polymorphic CA repeats were found in a cosmid containing DXS228. The CA repeats, and other
markers from proximal Xp, were mapped genetically in CEPH families and the likely order of
markers was established as Xpter-(DXS7, MAO-A, DXS228)-(PFC, DXS426)-(TIMP, OATL1)-
DXS255-Xcen. This places PFC in the region Xp11.3-Xp11.23, thus refining previous in situ
hybridization data. Two yeast artificial chromosomes (YACs) (440 and 390 kb) contain both PFC
and DXS426, and one of them (440 kb) also contains TIMP. This confirms the genetic order
TIMP-(PFC, DXS426). PFC and TIMP are located on the same 100-kb SalI/PvuI fragment of the
440-kb YAC. Given the genetic orientation of TIMP and (PFC, DXS426), this YAC can now serve
as a starting point for directional walking toward disease genes located in Xp11.3-Xp11.2 such as
retinitis pigmentosa (RP2) and Wiskott-Aldrich syndrome.


http://www.sciencedirect.com/science/article/B6WG1-4DXB8RP-37/2/3a0ce6a5c63f3be3029dca14780f8251

Forty-three sequences containing simple sequence repeats or microsatellites were generated
from an M13 library of total genomic mouse DNA. These sequences were analyzed for size
variation using the polymerase chain reaction and gel electrophoresis without the need for
radiolabeling. Seventy-two percent of the sequences showed allelic size variations between
different inbred strains of mouse and the wild mouse, Mus spretus; and 53% showed variation
between inbred strains. Thirty-seven percent were variant between B6/J and DBA/2J, and 81% of
these were resolved using minigel agarose electrophoresis alone. This approach is a useful way
of generating the large number of variants that are needed to create high resolution maps of the
mouse genome.

artificial chromosomes: A general method for generating highly polymorphic markers at chosen

http://www.sciencedirect.com/science/article/B6WG1-4DNHS2F-R6/2/f22bf99cda9fe1ba28974f53342cde8
The creation of a comprehensive genetic map in human has been limited by the lack of highly polymorphic markers spaced evenly throughout the human genome. We have utilized yeast artificial chromosomes (YAC) containing large human DNA inserts to help identify highly polymorphic (CA)n repeats at a chosen locus. The DNA of a YAC containing the locus was subcloned in M13 vectors, and the recombinants were screened at high stringency to detect preferentially long (CA)n repeats (n > 20). These repeats, which are the most likely to be highly polymorphic, were then studied to confirm both the level of polymorphism and their precise genetic location. This strategy has permitted the identification of a new, highly polymorphic CA repeat (77% heterozygosity) at the T cell receptor [alpha] chain (TCRA) locus on chromosome 14q. It provides a powerful marker for assessing the role of this locus in the susceptibility to autoimmune and infectious diseases. This approach should permit the development of highly polymorphic markers at any targeted locus and rapidly improve the current human genetic map.


http://www.sciencedirect.com/science/article/B6WG1-4DXB903-4W/2/f66a4ae413cea1efe9220786667e6993

A strategy for the isolation of DNA probes from small numbers of flow-sorted human chromosomes has been developed. A lymphoblastoid cell line carrying the 22q- derivative chromosome product of the constitutional t(11;22) translocation was used as the source of chromosomes. Synthetic oligonucleotide primers, based on the consensus Alu sequence, were used to amplify inter-Alu sequence from 500 flow-sorted 22q- derivative chromosomes. The amplified sequences were cloned into a plasmid vector by blunt end ligation, yielding clones with inserts in the range of 400 to 1000 bp. Approximately 70% of these clones hybridized to human DNA as single-copy probes. To identify clones derived from chromosome 11, the library was screened with a heterogeneous probe prepared by Alu-PCR amplification from the DNA of a somatic cell hybrid containing one homolog of chromosome 11. All the positive clones found were mapped to within the q23-q25 region of chromosome 11 known to be translocated onto the 22q- derivative chromosome. Further mapping studies showed that most of these probes (7/8) lay between the breakpoints for the t(4;11) translocation of acute lymphocytic leukemia and the t(11;22) of Ewing sarcoma. Thus, the use of Alu-PCR on the small derivative chromosome 22q- has provided a greatly enriched source of probes to region 11q23, a part of the genome that is currently of great interest. This approach will be particularly appropriate to small numbers of chromosomes when high specificity rather than total representation is required.


http://www.sciencedirect.com/science/article/B6WG1-4DNHPYR-C9/2/9a8e597b2feffb03a5a3c638348055759

A strategy for the rapid isolation from rodent hybrids of human chromosome-specific probes by enzymatic amplification is described. Synthetic oligonucleotide primers based on the consensus Alu sequence were used to amplify inter-Alu sequence from total human genomic DNA and from a somatic cell hybrid, PNTS-1, containing one homolog of chromosome 5 as its only human complement. Direct sequence analysis of the products from human genomic DNA confirmed their inter-Alu structure and provided a novel means for the examination of the 5' end of the Alu consensus. The amplified sequences from the somatic cell hybrid DNA were cloned into a plasmid vector by blunt-end ligation, yielding clones with inserts in the range 300 to 1000 bp. More than 80% of these clones carried inserts that behaved essentially as single-copy human
sequences. Hybridization of a selection of these clones to human DNA, hamster DNA, and the original hybrid DNA confirmed that they were derived from chromosome 5. Direct sequence analysis of the vector/insert boundaries in two clones confirmed that inter-Alu sequences had been cloned. This approach has significant advantages over other methods of isolating chromosome-specific probes from hybrid cells, enabling direct separation and cloning of human DNA probes that can be readily used for mapping studies.


http://www.sciencedirect.com/science/article/B6WG1-4DXK9Y3-3B/2/76b0ef9d0d47461b755b63946fda8fd

We have developed a rapid method of generating and simultaneously mapping interrepeat polymerase chain reaction products using DNA from interspecific backcross animals derived from mating C57BL/6J and Mus spretus mice. This method is based on the high degree of B1, B2, and L1 dispersed repeat position polymorphism found between these two species of mouse. We have mapped 13 new loci to 9 different chromosomes and have found no evidence of clustering among these loci. The advantages of this approach are that no prior knowledge of sequence is required, a single PCR reaction generates many markers which can be mapped simultaneously, and only 50 ng of each backcross DNA (a finite resource) is required. We anticipate that many more markers remain to be characterized in this valuable new source of polymorphism.


http://www.sciencedirect.com/science/article/B6WG1-48NX5NW-1/2/56e2ebf047d70f50a466bfcc324cb5e1d4

The linkage disequilibrium (LD) pattern within the adenosine deaminase (ADA) gene was analyzed by studying 13 polymorphic loci in 137 families from two European and three African populations. Evidence for the presence of a 12-kb meiotic crossover hot spot, spanning part of the first and the second intron and flanked by regions of reduced recombination activity, was obtained. Moreover, segregation analysis of 113 informative meioses revealed two recombination events that are internal or overlap the 12-kb region, thus suggesting a recombination rate for the hot-spot region about 50-fold higher than the mean rate across the human genome. Within the hot spot, a 144-bp palindromic sequence was also identified and its possible involvement in the recombination process is discussed. The 12-kb region characterized by the low degree of LD does not include the 3.2-kb region that is deleted, as a result of recurrent unequal homologous recombination between two Alu elements, in patients affected by autosomal severe combined immunodeficiency. This observation provides the first evidence for an absence of correlation between hot spots of equal and unequal homologous recombination.


http://www.sciencedirect.com/science/article/B6WG1-4FMHSSY-3/2/4473c5a56c9032893a61b52625c4e5f7
Monosomy 7 and deletions of 7q are recurring leukemia-associated cytogenetic abnormalities that correlate with adverse outcomes in children and adults. We describe a 2.52-Mb genomic DNA contig that spans a commonly deleted segment of chromosome band 7q22 identified in myeloid malignancies. This interval currently includes 14 genes, 19 predicted genes, and 5 predicted pseudogenes. We have extensively characterized the FBXL13, NAPE-PLD, and SVH genes as candidate myeloid tumor suppressors. FBXL13 encodes a novel F-box protein, SVH is a member of a gene family that contains Armadillo-like repeats, and NAPE-PLD encodes a phospholipase D-type phosphodiesterase. Analysis of a panel of leukemia specimens with monosomy 7 did not reveal mutations in these or in the candidate genes LRRC17, PRO1598, and SRPK2. This fully sequenced and annotated contig provides a resource for candidate myeloid tumor suppressor gene discovery.


http://www.sciencedirect.com/science/article/B6WG1-4DP5JHW-2D/2/3894e23d3b60dc4ed647c6f09afb4d55

The human amylase gene cluster includes a (CA)n repeat sequence immediately upstream of the [gamma]-actin pseudogene associated with the AMY2B gene. Analysis of this (CA)n repeat by PCR amplification of genomic DNA from the 40 families of the Centre d'Etude du Polymorphisme Humain (CEPH) reference panel revealed extensive polymorphism. A total of six alleles with (CA)n lengths of 16-21 repeats were found. The average heterozygosity for this polymorphism was 0.70. Multipoint linkage analysis showed that the amylase gene cluster is located distal to the nerve growth factor [beta]-subunit gene (NGFB) and is within 1 cM of the anonymous locus D1S10. The amylase (CA)n repeat provides a convenient marker for both the physical and the genetic maps of human chromosome 1p.


http://www.sciencedirect.com/science/article/B6WG1-471W704-2N/2/06b2dfafa3995acf1e549ae295425a

The enzymes of the 17[beta]-hydroxysteroid dehydrogenase (17[beta]-HSD) gene family are responsible for a key step in the formation and degradation of androgens and estrogens: catalyzing the interconversion of 17-ketosteroids and their active 17[beta]-hydroxysteroid counterparts. The structure of human type II 17[beta]-HSD cDNA was recently reported. This enzyme catalyzes the interconversion of [Delta]4-androstenedione and testosterone, androstanediol and dihydrotestosterone, and estrone and 17[beta]-estradiol, whereas type I 17[beta]-HSD catalyzes exclusively the interconversion of estrogens. To locate the HSD17B2 gene, the novel dinucleotide CA repeat sequence found 571 bp downstream from the end of exon 1 was genotyped into eight CEPH reference families by PCR. Two-point linkage analysis was performed between the latter polymorphism and the 2066 microsatellite markers of Genethon. The maximal pairwise lod score (Zmax = 33.3) with a maximal recombination fraction ([theta]max) of 0.008 was obtained with the marker D16S422 located on 16q24.1-q24.2. To define further the localization of the HSD17B2 gene, we constructed a high-resolution genetic map of the region flanking the polymorphic HSD17B2 gene including eight Genethon markers. The order of the HSD17B2 gene and markers is qter-D16S516 -- D16S512 -- D16S504 -- D16S507 -- D16S505 -- D16S511 -- [HSD17B2--D16S422]--D16S520--D16S413--tel.

http://www.sciencedirect.com/science/article/B6WG1-4DNHRHP-HX/2/377e1df023d83639664c5a229467e231

Trimeric and tetrameric short tandem repeats (STRs) represent a rich source of highly polymorphic markers in the human genome that may be studied with the polymerase chain reaction (PCR). We report the analysis of a multilocus genotype survey of 97-380 chromosomes in U.S. Black, White, Mexican-American, and Asian populations at five STR loci located on chromosomes 1, 4, 11, and X. The heterozygote frequencies of the loci ranged from 0.36 to 0.91 and the number of alleles from 6 to 20 for the 20 population and locus combinations. Relative allele frequencies exhibited differences between populations and unimodal, bimodal, and complex distributions. Although deviations were noted at some locus-population test combinations, genotype data from the loci were consistent overall with Hardy-Weinberg equilibrium by three tests. Population subheterogeneity within each ethnic group was not detected by two additional tests. No mutations were detected in a total of 860 meioses for two loci studied in the CEPH kindreds and five loci studied in other families. An indirect estimate of the mutation rates gave values from 2.3 x 10^{-5} to 15.9 x 10^{-5} for the five loci. Higher mutation rates appear to be associated with greater numbers of tandem repeats in the core motif. The most frequent genotype for all five loci combined appears to have a frequency of 7.59 x 10^{-4}. Together, these results suggest that trimeric and tetrameric STR loci are useful markers for the study of new mutations and genetic linkage analysis and for application to personal identification in the medical and forensic sciences.


http://www.sciencedirect.com/science/article/B6WG1-4DNHS2F-NX/2/c129dcef37105557e88b5718092f4255

We have used screening with the polymerase chain reaction and chemical mismatch detection of amplified cDNA to detect and characterize deletions and point mutations in six Hunter Syndrome patients. A high degree of mutational heterogeneity was observed. The first patient is completely deleted for the gene coding for [alpha]-iduronate sulfate sulfatase, while the second has a point mutation that creates a stop codon. The third patient shows a point mutation that creates a novel splice site that is preferentially utilized and results in partial loss of one exon in the RNA. Patients 4, 5, and 6 have point mutations resulting in single amino acid substitutions. Four of the six single-base changes observed in this study were examples of transitions of the highly mutable dinucleotide CpG to TpG. This study has demonstrated a procedure capable of detecting all types of mutation that affect the function of the IDS protein and should enable direct carrier and prenatal diagnosis for Hunter syndrome families.


http://www.sciencedirect.com/science/article/B6WG1-4DXB8RP-3J/2/59e6e560df74c722c4407579e7d08564
A porcine repetitive DNA sequence has been isolated from an intron of the glucose phosphate isomerase gene. The copy number of this and related sequences was estimated to be approximately 105 copies per genome. The sequence possesses all the characteristics of short interspersed elements (SINEs) described in other mammals: The repeat is 300 bp in length, has an poly(A) stretch, and contains insertion duplication sites. Homology to seven other porcine sequences, which also have the characteristics of SINEs, has been demonstrated. Primer oligonucleotides, based on conserved regions in the SINE sequences, have been synthesized. Using these primers, PCR-mediated specific amplification of porcine sequences was demonstrated from pig x mouse and pig x hamster hybrid cell lines. Cloning and sequencing of some amplified porcine sequences verify that the sites of priming are SINE sequences.


http://www.sciencedirect.com/science/article/B6WG1-471W6W8-14/2/bbf59b9c51822d6a89b42b9c009c3f1

In our effort to identify BRCA1, 22 genes were cloned from a 1-Mb region of chromosome 17q21 defined by meiotic recombinants in families with inherited breast and/or ovarian cancer. Subsequent discovery of another meiotic recombinant narrowed the region to ~650 kb. Genes were cloned from fibroblast and ovarian cDNA libraries by direct screening with YACs and cosmids. The more than 400 cDNA clones so identified were mapped to cosmids, YACs, and P1 clones and to a chromosome 17 somatic panel informative for the BRCA1 region. Clones that mapped back to the region were hybridized to each other and consolidated into clusters reflecting 22 genes. Ten genes were known human genes, 5 were human homologs of known genes, and 7 were novel. Each gene was sequenced, compared to genes in the databases to find homologies, and analyzed for mutations in BRCA1-linked families and tumors. Eight mutations were found in tumors or families and not in controls. In the gene encoding [alpha]-N-acetylglucosaminidase, ~100 kb proximal to the 650-kb linked region, somatic nonsense, missense, and splice junction mutations occurred in 3 breast tumors, but not in these patients' germline DNA nor in controls. In an ets-related oncogene in the linked region, a missense mutation cosegregated with breast cancer in one family and was not observed in controls. In a human homolog of a yeast pre-mRNA splicing factor, 3 different mutations cosegregated with breast cancer in 3 families and were not observed in controls. In these and the other genes in the region, 36 polymorphic variants were observed in both cases and controls.


http://www.sciencedirect.com/science/article/B6WG1-4DXB997-7X/2/0b8f0869b103b1c2cfb8f8360d1d0ad9

Dinucleotide repeats constitute so-called microsatellites of the human and other eukaryotic genomes. Microsatellite polymorphisms can be identified through the amplification of the microsatellite DNA using the polymerase chain reaction (PCR), followed by resolution of the amplified DNA fragments on a polyacrylamide sequencing gel. We performed a preliminary sequence database search to identify bovine sequences containing (CA)n, (AC)n, (GT)n, or (TG)n blocks, with n >= 6. This search yielded 10 sequences containing one or two of the specified repeat blocks and often additional dinucleotide repeat blocks. One of the microsatellite-containing regions has been sequenced twice from independent clones and the reported sequences showed variation in the number of repeats. PCR-amplified fragments of another
sequence, the gene for steroid 21-hydroxylase, ranged from 186 to 216 nucleotides in 43 unrelated animals. The database search, as well as the hypervariable microsatellite in the bovine steroid 21-hydroxylase gene, indicates that dinucleotide blocks may be an abundant source of DNA polymorphism in cattle.


http://www.sciencedirect.com/science/article/B6WG1-4DXB8RP-44/2/8951b4e39c1f855e6b99399ab150cbb8

Several mutations have been identified in the first nucleotide binding fold (NBF) of the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) gene. We have analyzed the DNA sequences of exons 10 and 11 in five different mammalian species, marmoset, mouse, cow, pig, and sheep; the amino acid conservation studied for nine disease mutations; and two "benign" mutations. For exon 10, 87% homology at the DNA level and 93.5% at the amino acid level were found for these species. For exon 11, the lowest homology (70%), as found in mouse and the highest in marmoset (93%), whereas the amino acid sequence conservation ranged from 82.5 to 100%. All codons involved in CF mutations are highly conserved throughout evolution.


http://www.sciencedirect.com/science/article/B6WG1-4DP5JB1-W/2/6631fb462c88a9396307ccbb23bb3982

The major mutation in the cystic fibrosis (CF) gene is a 3-bp deletion ([Δ]F508 in exon 10. About 50% of the CF chromosomes in Southern Europe carry this mutation, while other previously described mutations account for less than 4%. To identify other common mutations in CF patients from the Mediterranean area, we have sequenced, exon by exon, 16 chromosomes that did not show the [Δ]F508 deletion from a selected panel of eight unrelated CF patients. We describe here one missense and one nonsense mutation, and four sequence polymorphisms. We have also found two previously reported mutations in three chromosomes. Overall, these mutations may account for about 20% of CF alleles in the Italian and Spanish populations. No other mutations were detected in 10 out of 16 CF chromosomes after analyzing about 90% of the coding region of the CF gene, and 39 out of 54 intron/exon boundaries. Therefore, about 26% of CF mutations remain to be identified. In addition we provide the intron/exon boundary sequences for exons 4 to 9. These results together with previously reported linkage data suggest that in the Mediterranean populations further mutations may lie in the promoter region, or in intron sequences not yet analyzed.


http://www.sciencedirect.com/science/article/B6WG1-4DNHPYR-C6/2/bff476e69b2493a74e48844261bdd90d

The Lesch-Nyhan (LN) syndrome is a genetically lethal human neurological disease that results from mutations that inactivate the hypoxanthine phosphoribosyltransferase (HPRT) gene. The
elucidation of the complete DNA sequence of the human HPRT gene locus has enabled the construction of multiple oligonucleotide primer sets for the simultaneous in vitro amplification of all nine HPRT exons. The multiplex polymerase chain reaction provides a facile assay for the detection of HPRT exon deletions and the reaction products can be analyzed by direct automated fluorescent DNA sequencing to identify subtle alterations in the gene. Alterations have been identified in the HPRT genes from 15 independent LN cases, and 10 LN family studies were performed. The sequencing method uses solid supports and is sufficiently simple and sensitive to be a favored approach for LN diagnosis. LN heterozygotes can be diagnosed without reference to the affected male. In addition, these procedures will be useful for somatic mutagenesis studies.


http://www.sciencedirect.com/science/article/B6WG1-4DNHS2F-RP/2/00b323379c27113a5434bed747afaa85

Expressed sequence tags (ESTs) provide useful molecular landmarks for physical mapping and identify the position of an expressed region in the genome. The use of subtracted cDNA libraries enriched for tissue-specific genes as a source of ESTs should reduce the repetitive isolation of constitutively expressed sequences. We report here the sequence tags from the 3'-end region of 58 new directionally cloned cDNAs from a subtracted human retinal pigment epithelium (RPE) cell line library. Eight of the cDNAs have been assigned to human chromosomes using PCR-based EST assays. Chromosomal mapping of subtracted RPE cDNA clones may also help in identifying candidate genes for inherited eye diseases.


http://www.sciencedirect.com/science/article/B6WG1-471W6W8-S/2/81aa4e11b383875ab0086e6281a2b506

The paradigm of sequence-tagged site (STS)-content mapping involves the systematic assignment of STSs to individual cloned DNA segments. To date, yeast artificial chromosomes (YACs) represent the most commonly employed cloning system for constructing STS maps of large genomic intervals, such as whole human chromosomes. For developing a complete YAC-based STS-content map of human chromosome 7, we wished to utilize a limited set of YAC clones that were highly enriched for chromosome 7 DNA. Toward that end, we have assembled a human chromosome 7 YAC resource that consists of three major components: (1) a newly constructed library derived from a human--hamster hybrid cell line containing chromosome 7 as its only human DNA; (2) a chromosome 7-enriched sublibrary derived from the CEPH mega-YAC collection by Alu-polymerase chain reaction (Alu-PCR)-based hybridization; and (3) a set of YACs isolated from several total genomic libraries by screening for >125 chromosome 7 STSs. In particular, the hybrid cell line-derived YACs, which comprise the majority of the clones in the resource, have a relatively low chimera frequency (10-20%) based on mapping isolated insert ends to panels of human--hamster hybrid cell lines and analyzing individual clones by fluorescence in situ hybridization. An efficient strategy for polymerase chain reaction (PCR)-based screening of this YAC resource, which totals 4190 clones, has been developed and utilized to identify corresponding YACs for >600 STSs. The results of this initial screening effort indicate that the human chromosome 7 YAC resource provides an average of 6.9 positive clones per STS, a level of redundancy that should support the assembly of large YAC contigs and the construction of a high-resolution STS-content map of the chromosome.
Basic to the development of long-range physical maps of DNA are the detection and localization of landmarks within recombinant clones. Sequence-tagged sites (STSs), which are short stretches of DNA that can be specifically detected by the polymerase chain reaction (PCR), can be used as such landmarks. Our interest is to construct physical maps of whole human chromosomes by localizing STSs within yeast artificial chromosome (YAC) clones. Here we describe a generalized strategy for the systematic generation of large numbers of STSs specific for human chromosome 7. These STSs can be detected by PCR assays developed following the sequencing of anonymous pieces of chromosome 7 DNA, which was derived from flow-sorted chromosomes or from lambda clones made from DNA of a human-hamster hybrid cell line. Our approach for STS generation is tailored for the development of PCR assays capable of screening a large YAC library. In this study, we report the generation of 100 new STSs specific to human chromosome 7.

Somatic cell hybrids retaining the deleted chromosome 17 from 15 unrelated Smith-Magenis syndrome (SMS) del(17)(p11.2p11.2) patients were obtained by fusion of patient lymphoblasts with thymidine kinase-deficient rodent cell lines. Seventeen sequence-tagged sites (STSs) were developed from anonymous markers and cloned genes mapping to the short arm of chromosome 17. The STSs were used to determine the deletion status of these loci in these and four previously described human chromosome 17-retaining hybrids. Ten STSs were used to identify 28 yeast artificial chromosomes (YACs) from the St. Louis human genomic YAC library. Four of the 17 STSs identified simple repeat polymorphisms. The order and location of deletion breakpoints were confirmed and refined, and the regional assignment of several probes and cloned genes were determined. The cytogenetic band locations and relative order of six markers on 17p were established by fluorescence in situ hybridization mapping to metaphase chromosomes. The latter data confirmed and supplemented the somatic cell hybrid results. Most of the hybrids derived from [del(17)(p11.2p11.2)] patients demonstrated a similar pattern of deletion for the marker loci and were deleted for D17S446, D17S258, D17S29, D17S71, and D17S445. However, one of them demonstrated a unique pattern of deletion. This patient is deleted for several markers known to recognize a large DNA duplication associated with Charcot-Marie-Tooth (CMT) disease type 1A. These data suggest that the proximal junction of the CMT1A duplication is close to the distal breakpoint in [del(17)(p11.2p11.2)] patients.
We have determined the structure of the human CBFB gene, which encodes the [beta] subunit of the heterodimeric transcription factor core binding factor (CBF). This gene becomes fused to the MYH11 gene encoding smooth muscle myosin heavy chain by an inversion of chromosome 16 that occurs in the M4Eo subtype of acute myeloid leukemia. The CBFB gene contains 6 exons and spans 50 kb. The gene is highly conserved in animal species as distant as Drosophila, and the exon boundaries are in locations identical to those of the murine Cbfb homologue. The CBFB promoter region has typical features of a housekeeping gene, including high G+C content, high frequency of CpG dinucleotides, and lack of canonical TATA and CCAAT boxes. This gene has a single transcriptional start site, 345 nucleotides upstream of the beginning of the coding region. The human and mouse CBFB promoters show conservation of several transcriptional regulatory sequence motifs, including binding sites for Sp1, Ets family members, and Myc, but do not contain any CBF binding sites. The 5' end of the human CBFB gene also contains a highly polymorphic, transcribed CGG repeat that is not present in the murine homologue.


Interspersed repetitive sequence polymerase chain reaction (IRS-PCR) has become a powerful tool for the rapid generation of DNA probes from human chromosomes present in rodent somatic cell hybrids. We have constructed a somatic cell hybrid containing a major portion of the mouse X chromosome in a human background (clone 8.0). IRS-PCR was developed for the specific amplification of mouse DNA using either of two primers from the rodent-specific portion of the murine B1 repeat. Amplification was subsequently performed with clone 8.0 and a subclone, 8.1/1, which retains a small murine X-chromosomal fragment including Hprt and the Gdx locus. A total of 15-20 discrete PCR products ranging in size from 3000 bp were obtained from clone 8.0 with each primer. In clone 8.1/1, a subset of these bands plus some additional bands were observed. Nine bands amplified from clone 8.1/1 have been excised from gels and used as probes on Southern blots. All of the fragments behaved as single-copy probes and detected domesticus/spretus variation. They have been regionally mapped using an interspecific backcross. The probe locations are compatible with those of markers known to be present in clone 8.1/1. These results demonstrate the feasibility of this method as applied to the mouse genome and the high likelihood of generating useful DNA probes from a targeted region.


We report a comparative map of canine chromosome 1 (CFA1) incorporating single nucleotide polymorphisms (SNPs) and insertion/deletion (indel) polymorphisms, developed by using cross-species primers, radiation hybrid analysis, and pool-and-sequence identification of genetic variations. Fifty-five genes were chosen with relatively even spacing (approximately 3 Mb between the human homologues) and were mapped to CFA1, with 49 of these being new
Evolutionary chromosomal breakpoints between CFA1 and the corresponding human chromosomes (HSA6, HSA9, HSA18, and HSA19) were located within 1 to 5 Mb based upon the human genome sequence. The process of identifying the evolutionary chromosomal breakpoints between CFA1 and the relevant human chromosomes led to an improvement in the comparative maps of CFA7, CFA12, and CFA29 through the mapping of 21 additional genes. A manual pool-and-sequence method was used to identify 79 SNPs, 9 small indels, 7 simple tandem repeats, and 2 polymorphic SINE insertions within the genes mapped. The cross-species primers can also be used in the manner described here to improve the comparative maps for other mammalian species.


http://www.sciencedirect.com/science/article/B6WG1-471W704-2C/2/8918cfe56397b37a03172e69825e7fe4

Sterol regulatory element binding protein-1 (SREBP1) and SREBP2 are structurally related proteins that control cholesterol homeostasis by stimulating transcription of sterol-regulated genes, including those encoding the low-density lipoprotein (LDL) receptor and 3-hydroxy-3-methylglutaryl CoA synthase. SREBP1 and SREBP2 are 47% identical, and they share a novel structure comprising a transcriptionally active NH2-terminal basic helix--loop--helix--leucine zipper (bHLH-Zip) domain followed by a membrane attachment domain. Cleavage by a sterol-regulated protease frees the bHLH-Zip domain from the membrane and allows it to enter the nucleus. SREBP1 exists in several forms, possibly as a result of alternative splicing at both the 5' and the 3' ends of the mRNA. The genes for SREBP1 (SREBF1) and SREBP2 (SREBF2) have not been studied. In this paper we describe the cloning and characterization of the human SREBF1 gene. The gene is 26 kb in length and has 22 exons and 20 introns. The 5' and 3' sequences that differ between the two SREBP1 cDNAs are encoded by discrete exons, confirming the hypothesis that they result from alternative splicing. The chromosomal locations of human SREBF1 and SREBF2 were determined by analysis of human-rodent somatic cell hybrids and fluorescence in situ hybridization. The SREBF1 gene mapped to the proximal short arm of chromosome 17 (17p11.2), and the SREBF2 gene was localized to the long arm of chromosome 22 (22q13).


http://www.sciencedirect.com/science/article/B6WG1-4B667HD-1/2/6b31393608f34cc119d495ccf9a23394

Xcat mice display X-linked congenital cataracts and are a mouse model for the human X-linked cataract disease Nance Horan syndrome (NHS). The genetic defect in Xcat mice and NHS patients is not known. We isolated and sequenced a BAC contig representing a portion of the Xcat critical region. We combined our sequencing data with the most recent mouse sequence assemblies from both Celera and public databases. The sequence of the 2.2-Mb Xcat critical region was then analyzed for potential Xcat candidate genes. The coding regions of the seven known genes within this area (Rai2, Rbbp7, Ctps2, Calb3, Grpr, Reps2, and Syap1) were sequenced in Xcat mice and no mutations were detected. The expression of Rai2 was quantitatively identical in wild-type and Xcat mutant eyes. These results indicate that the Xcat mutation is within a novel, undiscovered gene.

http://www.sciencedirect.com/science/article/B6WG1-4DXK9Y3-2H/2/757def909f0cd302ec5c965ea83a8e89

A 680-kb yeast artificial chromosome (YAC) that contains a functional copy of the human hypoxanthine phosphoribosyltransferase (HPRT) gene has been isolated. This YAC, yHPRT, and another YAC, yXY837, which contains the 3' end of the HPRT gene, have been mapped with restriction enzymes that cleave human DNA infrequently. The HPRT gene lies near the center of yHPRT. Fusion of yHPRT-containing yeast spheroplasts with mouse L A-9 cells, which are HPRT-negative, gives rise to HPRT-positive colonies. These colonies contain the human HPRT gene and express human HPRT mRNA. Fusion of yeast with mammalian cells is an efficient way of testing the integrity and functionality of human DNA contained in YACs.


http://www.sciencedirect.com/science/article/B6WG1-4CVV6RK-1/2/fe70752c360778bef9227a50dceefcb1

Haplotype-based human genome research is important in identifying disease susceptibility genes efficiently. Although haplotype reconstruction by statistical methods is widely used, direct haplotype determination by molecular techniques has also been developed as a complementary method for statistical estimation. In this study, we demonstrate a molecular haplotyping method making use of single-strand conformation polymorphism (SSCP) gels. We identified 10 common SNPs and a dinucleotide insertion/deletion polymorphism within 2-kb region upstream of the transcription initiation site of MUC5B and determined haplotype structure, dividing the region into two DNA fragments. Real haplotypes were determined unambiguously by our SSCP-based analysis with fragments longer than 1 kb. Haplotypes reconstructed from diploid genotypes in the same region by the statistical methods including EM algorithm were also evaluated. Direct comparison between statistical estimation and direct determination of haplotypes revealed that major haplotypes containing multiple marker sites showing strong LD are estimated in great accuracy but that a variety of haplotypes reflecting weak LD are not reconstructed precisely enough. Our data can be helpful in implementing molecular haplotyping or statistical estimation, since usage of these methods may be determined depending on the haplotype structures.


http://www.sciencedirect.com/science/article/B6WG1-4FP1J0W-1/2/01996f6e737288ff0180fcf8b9e47fb

Holoprosencephaly (HPE) is the most common developmental field defect in patterning of the human prosencephalon and associated craniofacial structures. The genetics is complex, with 12 loci defined on 11 chromosomes. We defined a locus for HPE (HPE8) on human chromosome 14q13 between markers D14S49 and AFM205XG5, by mapping deletion intervals of affected subjects with proximal chromosome 14q interstitial cytogenetic deletions. A 35-BAC contig was built by chromosome walking. By annotation of the 2.82-Mb minimal critical region, we identified
28 possible genes. Seven genes were expressed in human fetal brain: NPAS3, SNX6, C14ORF11, C14ORF10, PAX9, NKX2.1, and C14ORF19, the last an apparent gene fragment. Molecular embryology, animal modeling, and human mutation studies were reported elsewhere for PAX9 and NKX2.1. We focused on three genes, SNX6, NPAS3, and C14ORF11, as potential candidates for HPE. Genomic structure, human expression patterns, protein cellular localization, and embryonic expression patterns of orthologous murine genes were determined, showing that the three genes have properties similar to those of known HPE genes.


http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-2N/2/f89e7e389e9de7b06263388dd5e9c986

We have developed the "shotgun polymerase chain reaction," a method for obtaining a large number of DNA markers specific to a giant DNA fragment, which facilitates analysis of a particular chromosomal region. We applied this method to a giant NotI fragment which carries the immunoglobulin lambda constant region on chromosome 22. NotI digests of chromosome 22 flow-sorted from human B-lymphoblastoid cell line GM130B were size fractionated by pulsed-field gel electrophoresis. Preliminary Southern hybridization analysis revealed that the immunoglobulin lambda constant region was conveyed on 1.4- and 1.3-Mb NotI fragments in this cell line. The agarose gel corresponding to 1.2 to 1.5 Mb in size was excised into slices and subjected to polymerase chain reaction to identify gel slices containing NotI fragments carrying Ke-Oz+, a subtype of the immunoglobulin lambda constant region. From the NotI fragment thus identified, a large number of small DNA segments were amplified through the ligation-mediated random polymerase chain reaction method. The amplified products were cloned and analyzed for chromosomal origin and localization to particular NotI fragments. Seven of eighteen clones originated from the 1.4-Mb NotI fragment of chromosome 22 in GM130B cells, which appears to be exactly the same as detected by a probe for the immunoglobulin lambda constant region.


http://www.sciencedirect.com/science/article/B6WG1-471W72M-4J/2/4ef10ff56ac6e73756529c4f554e3935


http://www.sciencedirect.com/science/article/B6WG1-471W7HX-89/2/741f76a52fca8065ba629f20af0dd1e

The phenol-preferring sulfotransferases aryl sulfotransferase IV and N-hydroxyarylamine sulfotransferase catalyze sulfate conjugation of N-hydroxy-2-acetyl-aminofluorene, a metabolite capable of causing hepatocarcinogenesis in rats. We utilized published cDNA sequences of these sulfotransferases to type the progeny of two multilocus crosses and determined that the genes, aryl sulfotransferase (Stp) and N-hydroxyaryl-amine sulfotransferase (Stp2), map to positions on mouse chromosomes 7 and 17.
The members of the carcinoembryonic antigen (CEA)/pregnancy-specific glycoprotein (PSG) gene family have a characteristic N-terminal domain that is homologous to the immunoglobulin variable region. We have estimated the size of the PSG subfamily by identification of N-domain exons from isolated genomic clones and from total genomic DNA through PCR amplification and DNA sequence determination. The PSG subfamily contains at least 11 different genes. For 7 of these, two DNA sequences differing from each other in 1 to 4 nucleotides were detected. Most likely, they represent different alleles. They are PSG1, PSG2, PSG3, PSG4, PSG5, PSG6, PSG7, PSG8, PSG11, PSG12, and PSG13. Six of the N-domain sequences described here are new. All of the PSGs except PSG1, PSG4, and PSG8 contained the arginine-glycine-aspartic acid sequence at position 93-95 corresponding to the complementarity determining region 3 of immunoglobulin. Parsimony analysis of 24 CEA and PSG sequences using 12 members of the immunoglobulin gene superfamily as out-groups to root the family free shows that the N-domain of the CEA group genes evolved in one major branch and the PSG group genes in the other.

The creation of a physical map of chromosome 18 will be useful for the eventual identification of specific chromosomal regions that are critical in the occurrence of Edwards syndrome, the 18q-syndrome, and the 18p-syndrome. To begin the investigation of these syndromes, a physical map has been constructed to order random DNA fragments to specific portions of chromosome 18. A set of somatic cell hybrids that retain deletions or translocations involving chromosome 18 has been isolated and characterized. Over 200 lambda phage from a chromosome 18-specific library have been localized to 11 distinct regions of chromosome 18 using the chromosomal breakpoints present in the somatic cell hybrids.
Heterogeneous nuclear ribonucleoprotein (hnRNP) A2 is a major nuclear protein and one of the major components of the hnRNP core complex in mammalian cells. We first determined the complete sequence of the human gene for hnRNP protein A2 (HNRPA2B1). The human HNRPA2B1 gene exists in a single copy over 9 kb in length. The gene was split into 12 exons, including a 36-nucleotide mini-exon, which was specific to the hnRNP protein B1, providing genetic evidence that the B1 mRNA was generated from the primary HNRPA2B1 transcript by alternative splicing. The 5’ region of HNRPA2B1 was GC-rich and contained several DNA motifs for the binding of several transcription factors, which included 2 CCAAT boxes and no TATA sequences. The 5’ ends of the mRNA were mapped to multiple positions. These structural features are characteristic of promoter regions of housekeeping genes. Northern blot and RT-PCR analyses of the HNRPA2B1 transcripts revealed levels of B1 mRNA from 2 to 5% of total A2/B1 transcripts and showed that both A2 and B1 mRNAs were transcribed in all human cell lines and mouse tissues studied. The structural and evolutionary characteristics of the A2 and A1 proteins as they relate to each other are discussed.


http://www.sciencedirect.com/science/article/B6WG1-4FBN76R-1/2/f20b45462cb5f407a98779d0713fe7f1

Nonsense-mediated mRNA decay (NMD) is a eukaryotic quality-control mechanism that detects and degrades aberrant transcripts prematurely terminating translation. NMD may be elicited by intergenic transcripts that contain premature termination codons (PTCs), but chimeric mRNAs of genes that have introns of identical phase would be predicted to lack PTCs and escape NMD. We examined intron phase I-containing HLA class II genes for the presence of intergenic mRNAs and found an extraordinary diversity of correctly spliced and polyadenylated intergenic transcripts. They lacked a significant homology at the chimeric joins and had no PTCs. Their expression levels were very low and positively correlated with the expression of natural transcripts. In contrast, pair-wise mixtures of separately transcribed plasmids carrying full-length HLA-DQB1, -DQA1, -DRB1, and -DRA cDNAs produced only hybrid molecules that lacked canonical exon boundaries, had homologous chimeric joins, and occasionally contained PTCs, implicating in vitro artifacts generated by template switching of Taq polymerase and reverse transcriptase. A differential exon structure of hybrid molecules observed in vitro and in cellular RNA preparations suggests that intergenic mRNAs with canonical exon boundaries arise in vivo during exon joining and/or transcription. Since the observed intergenic mRNAs may encode mixed class II heterodimers that were previously shown to present antigens it will be interesting to determine functional properties of such molecules in future studies.


http://www.sciencedirect.com/science/article/B6WG1-4DXB8RP-3D/2/727e29a21091f1da3423afbf1b7b884c56

Highly informative dinucleotide repeat polymorphisms were identified at the T-complex-associated-testes-expressed-1 (TCTE1) locus on human chromosome 6p. Electrophoresis of single-stranded DNA on native gels facilitated the analysis of the dinucleotide polymorphisms. Linkage mapping positions this marker midway between the centromere and HLA with recombination fractions as follows: D6Z1-0.21-TCTE1-0.24-HLA. Two-color fluorescence in situ hybridization places TCTE1 proximal to CRIL171 (D6S19). Together, linkage and in situ
hybridization indicate that the order of the loci is D6Z1-D6S4-D6S90-TCTE1-D6S19-D6S29-HLA-telomere. A sequence tagged site (STS) was established, and three yeast artificial chromosome (YAC) clones were identified for the TCTE1 locus.


http://www.sciencedirect.com/science/article/B6WG1-4DNHP53-32/2/0ebab682eaa6af6d2c42b6108e0f430ba

The systematic screening of yeast artificial-chromosome (YAC) libraries is the limiting step in many physical mapping projects. To improve the screening throughout for a human YAC library, we designed an automatable strategy to identify YAC clones containing a specific segment of DNA. Our approach combines amplification of the target sequence from pooled YAC DNA by the polymerase chain reaction (PCR) with detection of the sequence by an ELISA-based oligonucleotide-ligation assay (OLA). The PCR-OLA approach eliminates the use of radioactive isotopes and gel electrophoresis, two of the major obstacles to automated YAC screening. Furthermore, the use of the OLA to test for the presence of sequences internal to PCR primers provides an additional level of sensitivity and specificity in comparison to methods that rely solely on the PCR.


http://www.sciencedirect.com/science/article/B6WG1-471W76S-5H/2/48430e5e560db73f65977696591

Formation of tooth enamel is a poorly understood biological process. In this study we describe a 9-bp deletion in exon 2 of the amelogenin gene (AMGX) causing X-linked hypoplastic amelogenesis imperfecta, a disease characterized by defective enamel. The mutation results in the loss of 3 amino acids and exchange of 1 in the signal peptide of the amelogenin protein. This deletion in the signal peptide probably interferes with translocation of the amelogenin protein during synthesis, resulting in the thin enamel observed in affected members of the family. We compare this mutation to a previously reported mutation in the amelogenin gene that causes a different disease phenotype. The study illustrates that molecular analysis can help explain the various manifestations of a tooth disorder and thereby provide insights into the mechanisms of tooth enamel formation.


http://www.sciencedirect.com/science/article/B6WG1-4DXB8RP-3N/2/4b7c71241754541091e3c64c37a9c38

Amelogenesis imperfecta is characterized by the defective formation of tooth enamel. Here we present evidence that the X-linked form of this disorder (AlH1) is caused by a structural alteration in one of the predominant proteins in enamel, amelogenin. Southern blot analysis revealed a deletion extending over 5 kb of the amelogenin gene in males with the hypomineralization form of
the AIH1. Carrier females were heterozygous for the molecular defect. The deletion appears to include at least two exons of the amelogenin gene and the extent of the deletion was verified by PCR analysis. The mutation was shown to segregate with the disease among 15 analyzed individuals belonging to the same kindred. Our results link a defect in the amelogenin gene to the abnormal formation of enamel. We thus conclude that the amelogenin protein has a role in biomineralization of tooth enamel.


http://www.sciencedirect.com/science/article/B6WG1-4CWSVKC-2/2/160c7c84202c5453522e76357765691b

Sonic Hedgehog (SHH) plays a fundamental role in numerous developmental processes including morphogenesis of limbs, nervous system, and teeth. Using a Bayesian alignment algorithm for phylogenetic footprinting we analyzed [not, vert, similar]28 kb of noncoding DNA in the SHH locus of human and mouse. This showed that the length of conserved noncoding sequences (4196 nt) shared by these species was approximately 3 times larger than the SHH coding sequence (1386 nt). Most segments were located in introns (53%) or within 2-kb regions upstream (16%) or downstream (20%) of the first and last SHH codon. Even though regions more than 2 kb upstream or downstream of the first and last SHH codon represented 57% (16 kb) of the sequence compared, they accounted for only 11% (494 nt) of the total length of conserved noncoding segments. One region of 650 nt downstream of SHH was identified as a putative scaffold/matrix attachment region (SMAR). Human-mouse analysis was complemented by sequencing in apes, monkeys, rodents, and bats, thus further confirming the evolutionary conservation of some segments. Gel-shift assays indicated that conserved segments are targeted by nuclear proteins and showed differences between two cell types that expressed different levels of SHH, namely human endothelial cells and breast cancer cells. The relevance of these findings with respect to regulation of SHH expression during normal and pathologic development is discussed.


http://www.sciencedirect.com/science/article/B6WG1-4DNHP53-3C/2/178c28d1400e0f91aa5ac33c957e5374

Ninety-three phage clones identified by hybridization with a C2—H2 zinc finger sequence probe have been grouped into 23 genetic loci. Partial sequencing verified that each locus belonged to the zinc finger family. Oligonucleotide primer pairs were developed from these sequences to serve as STS markers for these loci. One or more clones from each locus was mapped onto human metaphase chromosomes by fluorescence in situ hybridization. Several loci map to identical chromosomal regions, indicating the possible presence of multigene clusters. Zinc finger loci were found to reside predominantly either in telomeric regions or in chromosomal bands known to exhibit chromosome fragility. Chromosome 19 carries a disproportionate fraction (10 of 23) of the mapped zinc finger loci.

Isolation of DNA segments adjacent to known sequences is a tedious task in genome-related research. We have developed an efficient PCR strategy that overcomes the shortcomings of existing methods and can be automated. This strategy, thermal asymmetric interlaced (TAIL)-PCR, utilizes nested sequence-specific primers together with a shorter arbitrary degenerate primer so that the relative amplification efficiencies of specific and nonspecific products can be thermally controlled. One low-stringency PCR cycle is carried out to create annealing site(s) adapted for the arbitrary primer within the unknown target sequence bordering the known segment. This sequence is then preferentially and geometrically amplified over nontarget ones by interspersion of high-stringency PCR cycles with reduced-stringency PCR cycles. We have exploited the efficiency of this method to expedite amplification and sequencing of insert end segments from P1 and YAC clones for chromosome walking. In this study we present protocols that are amenable to automation of amplification and sequencing of insert end sequences directly from cells of P1 and YAC clones.


The Duchenne muscular dystrophy locus is remarkable in that it shows a high mutation rate and the majority of mutations found are deletions. These deletions are generated as meiotic as well as mitotic events and occur preferentially in the central region of the gene. Nothing is known so far about the mechanisms involved. This paper reports the first sequencing of deletion junctions in the dystrophin gene. The data from a study of two patients with deletions in the central region of dystrophin show the breakpoints to lie in regions of introns in which stretches of dA-dT are seen. The relationship between these observations and possible mechanisms for the mutations is discussed.


Human carboxylesterases 1 and 2 (CES1 and CES2) catalyze the hydrolysis of many exogenous compounds. Alterations in carboxylesterase sequences could lead to variability in both the inactivation of drugs and the activation of prodrugs. We resequenced CES1 and CES2 in multiple populations (n = 120) to identify single-nucleotide polymorphisms and confirmed the novel SNPs in healthy European and African individuals (n = 190). Sixteen SNPs were found in CES1 (1 per 300 bp) and 11 in CES2 (1 per 630 bp) in at least one population. Allele frequencies and estimated haplotype frequencies varied significantly between African and European populations. No association between SNPs in CES1 or CES2 was found with respect to RNA expression in normal colonic mucosa; however, an intronic SNP (IVS10-88) in CES2 was associated with reduced CES2 mRNA expression in colorectal tumors. Functional analysis of the novel polymorphisms described in this study is now warranted to identify putative roles in drug
A compound imperfect dinucleotide repeat element, [CA]4TTTGT[CT]7[CA]9AA[CA]4CCACATA[CA]3, was found approximately 10 kb 3' to the human glucokinase gene (GCK) from analysis of contiguous genomic DNA obtained from a bacteriophage [lambda] chromosome walk. Direct human genomic sequencing revealed the source of polymorphism to be variable numbers of CT and CA repeats. Altogether six alleles that range in length from +10 to -15 nucleotides compared to the most common (Z) allele have been identified. Alleles Z, Z + 2, and Z + 4 were present in American Blacks, Pima Indians, and Caucasians, with somewhat varied frequencies among the group. Two alleles, Z + 10 and Z - 15, appear to be unique to American Blacks, while a Z + 6 allele was observed only in the Caucasian population studied. Observed heterozygosity of the polymorphism in the CEPH reference pedigree collection is 44% and the PIC 0.44. The polymorphism is assayed by PCR amplification and resolution of 32P-end-labeled products (ranging in length from 180 to 205 bp) on denaturing polyacrylamide sequencing gels. Using the PCR assay, the human glucokinase gene was physically localized to chromosome 7 in a panel of rodent/human somatic cell lines. Genetic analysis in CEPH pedigrees placed the dinucleotide repeat element, and thereby the human glucokinase gene, on chromosome 7p between TCRG and a RFLP locus D7S57. The glucokinase dinucleotide repeat genetic marker can now be used to assess the role of the glucokinase gene in diabetes by population association studies. In addition, this repeat marker and others flanking it on chromosome 7 can be used in linkage studies with families segregating the disorder.


A new high-resolution genetic linkage map for human chromosome 7p has been constructed. The map is composed of 47 loci (54 polymorphic systems), 19 of which are uniquely placed with odds of at least 1000:1. Four genes are represented, including glucokinase (GCK, ATP:-hexose-6-phosphotransferase, EC 2.7.1.2) which was mapped via a (CA)n dinucleotide repeat polymorphism. The sex-average map measures 94.4 cM and the male and female maps measure 73.2 and 116.1 cM, respectively. We believe that the genetic map extends nearly the full length of the short arm of chromosome 7 since a centromere marker has been incorporated, and the most distal marker, D7S21, has been cytogenetically localized by in situ hybridization to 7p22-pter. The average marker spacing is 2 cM, and the largest interval between uniquely placed markers is 13 cM (sex-average map). Overall, female recombination was observed to be about 1.5 times that of males, and a statistically significant sex-specific recombination frequency was found for a single interval. The map is based on genotyptic data gathered from 40 CEPH reference pedigrees and was constructed using the CRI-MAP program package. The map presented here represents a combined and substantially expanded dataset compared to previously published chromosome 7 maps, and it will serve as a "baseline" genetic map that should prove useful for future efforts to develop a 1-cM map and for construction of a contiguous clonebased physical map for this chromosome.
A contig of 36 overlapping yeast artificial chromosome (YAC) clones has been constructed for the complete Duchenne muscular dystrophy (DMD) gene in Xp21. The YACs were isolated from a human 48,XXXX YAC library using the DMD cDNA and brain promoter fragments as hybridization probes. The YAC clones were characterized for exon content using HindIII or EcoRI digests, hybridization of individual DMD cDNA probes, and polymerase chain reaction (PCR) amplification of specific exons near the 5' end of the gene. For comparison to the known long-range restriction map of the DMD gene, YAC clones were digested with SfiI and hybridized with DMD cDNA probes. The combined analysis of the exon content and the SfiI map allowed an approximately 3.2-Mb YAC contig to be constructed. The complete 2.4-Mb DMD gene could be represented in a minimum set of 7 overlapping YAC clones.

We have determined the genetic stability of three independent intragenic human HPRT gene duplications and the structure of each duplication at the nucleotide sequence level. Two of the duplications were isolated as spontaneous mutations from the HL60 human myeloid leukemia cell line, while the third was originally identified in a Lesch-Nyhan patient. All three duplications are genetically unstable and have a reversion rate approximately 100-fold higher than the rate of duplication formation. The molecular structures of these duplications are similar, with direct duplication of HPRT exons 2 and 3 and of 6.8 kb (HL 60 duplications) or 13.7 kb (Lesch-Nyhan duplication) of surrounding HPRT sequence. Nucleotide sequence analyses of duplication junctions revealed that the HL60-derived duplications were generated by unequal homologous recombination between clusters of Alu repeats contained in HPRT introns 1 and 3, while the Lesch-Nyhan duplication was generated by the nonhomologous insertion of duplicated HPRT DNA into HPRT intron 1. These results suggest that duplication substrates of different lengths can be generated from the human HPRT exon 2-3 region and can undergo either homologous or nonhomologous recombination with the HPRT locus to form gene duplications.

We have determined the nucleotide sequences of 10 intragenic human HPRT gene deletions isolated from thioguanine-resistant PSV811 Werner syndrome fibroblasts or from HL60 myeloid leukemia cells. Deletion junctions were located by fine structure blot hybridization mapping and then amplified with flanking oligonucleotide primer pairs for DNA sequence analysis. The junction region sequences from these 10 HPRT mutants contained 13 deletions ranging in
size from 57 bp to 19.3 kb. Three DNA inversions of 711, 368, and 20 bp were associated with tandem deletions in two mutants. Each mutant contained the deletion of one or more HPRT exon, thus explaining the thioguanine-resistant cellular phenotype. Deletion junction and donor nucleotide sequence alignments suggest that all of these HPRT gene rearrangements were generated by the nonhomologous recombination of donor DNA duplexes that share little nucleotide sequence dentity. This result is surprising, given the potential for homologous recombination between copies of repeated DNA sequences that constitute approximately a third of the human HPRT locus. No difference in deletion structure or complexity was observed between deletions isolated from Werner syndrome or from HL60 mutants. This suggests that the Werner syndrome deletion mutator uses deletion mutagenesis pathway(s) that are similar or identical to those used in other human somatic cells.


http://www.sciencedirect.com/science/article/B6WG1-49NRKG6-1/2/5bb65ac6f0b54bfd1285d8818c6ea9b7

Several mammalian sialidases have been cloned so far and here we describe the identification and expression of a new member of the human sialidase gene family. The NEU4 gene, identified by searching sequence databases for entries showing homologies to the human cytosolic sialidase NEU2, maps in 2q37 and encodes a 484-residue protein. The polypeptide contains all the typical sialidase amino acid motifs and, apart from an amino acid stretch that appears unique among mammalian sialidases, shows a high degree of homology for NEU2 and the plasma membrane-associated (NEU3) sialidases. RNA dot-blot analysis showed a low but wide expression pattern, with the highest level in liver. Transient transfection in COS7 cells allowed the detection of a sialidase activity toward the artificial substrate 4MU-NeuAc in the acidic range of pH. Immunofluorescence staining and Western blot analysis demonstrated the association of NEU4 with the inner cell membranes.


http://www.sciencedirect.com/science/article/B6WG1-4FDJ6VR-1/2/aaa2d8c75c81d642052dcdb84c92877

Four different transcripts of the Mecp2 gene can be distinguished by the length of the 3' untranslated region generated by usage of alternative polyadenylation sites. In situ hybridization analyses encompassing embryonic to 20-week postnatal age showed that transcripts are expressed in the central nervous system, with a progressive restriction during development culminating in localized strong expression in the cerebral cortex, olfactory bulb, hippocampal formation, and internal granule and Purkinje layer of the cerebellum. Real-time RT-PCR measurements of Mecp2 transcript levels showed variations with mouse age in two distinctive patterns that are unique to the central nervous system and the visceral organs, respectively. The 10-kb mRNA is the predominant form expressed in the brain in contrast to the shorter species expressed in the lung and liver. The developmental profile of Mecp2 mRNA highlights a potential tissue-specific function of the 3'UTR in the regulation of MeCP2 protein synthesis in response to the age-specific requirement of MeCP2 function during the life of the mouse.

http://www.sciencedirect.com/science/article/B6WG1-471W6W8-V/2/32e2c15b0a2486998edf44d9768fb724

Laminin 5 consists of three polypeptides, [alpha]3, [beta]3, and [gamma]2, encoded by the genes LAMA3, LAMB3, and LAMC2, respectively. In this study, we have elucidated the exon--intron organization of the human LAMB3 gene. Characterization of five overlapping [lambda] phage DNA clones revealed that the gene was approximately 29 kb in size. Subsequent sequence data revealed that the gene consisted of 23 exons that varied from 64 to 379 bp in size, accounting for the full-length cDNA with an open reading frame of 3516 bp encoding 1172 amino acids. Comparison of the LAMB3 gene structure with the previously characterized LAMB1 gene revealed that LAMB3 was considerably more compact. Knowledge of the exon--intron organization of the LAMB3 gene will facilitate elucidation of mutations in patients with the junctional forms of epidermolysis bullosa, some of which have been associated with mutations in the laminin 5 genes.


We have previously reported the isolation of a genomic clone encoding human liver-specific peroxisomal alanine:glyoxylate aminotransferase (AGT, EC 2.6.1.44), the deficient enzyme in primary hyperoxaluria type 1 (PH1) (P. E. Purdue, Y. Takada, and C. J. Danpure, J. Cell Biol. 111: 2341-2351, 1990). This clone has now been characterized, revealing that the coding sequence is distributed among 11 exons covering 10 kb. The nucleotide sequences of each exon have been determined, confirming that this clone corresponds to previously characterized AGT cDNA (Y. Takada, N. Kaneko, H. Esumi, P. E. Purdue, and C. J. Danpure, Biochem. J. 268: 517-520, 1990). In addition, to provide sequence data for the design of exon-specific PCR primers, the intron sequences immediately flanking each exon have been determined. Furthermore, in an attempt to identify putative transcriptional control sequences we have determined the sequence of 1.25 kb directly upstream of the cDNA 5' end. The results of genomic Southern blotting indicate that human AGT is probably encoded by a single copy gene, and a combination of in situ hybridization and PCR analysis of rodent/human somatic cell hybrids suggests that this gene is located on chromosome 2q36-q37. The gene symbol AGXT has been assigned for this locus.


http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-B4/2/dd8fdd6c0a15740491482e3b43a6def0

We report the molecular characterization of two novel galactosemia mutations that exhibit different molecular phenotypes. Both are of the missense type with low or no residual enzyme activity. The R148W mutation results in an unstable protein, although messenger RNA is still produced. In contrast, the L195P mutation produces stable but inactive immunoreactive protein. The R148W mutation alters an amino acid that is not evolutionarily conserved, while the L195P
mutation affects a well-conserved residue nine amino acids downstream from the putative active site nucleophile. These mutations provide evidence that different mechanisms can result in galactosemia: destabilizing mutations in any given area of the protein and missense mutations in conserved domains of the enzyme resulting in low or no activity. These two mutant alleles represent the fifth and sixth galactosemia mutations and confirm the hypothesis that galactosemia results from a multiplicity of mutations at the molecular level.


http://www.sciencedirect.com/science/article/B6WG1-49HSTP6-2/2/97337f6bf9ad77003aa3db6980aa38fb

cDNA-AFLP is a genome-wide expression analysis technology that does not require any prior knowledge of gene sequences. This PCR-based technique combines a high sensitivity with a high specificity, allowing detection of rarely expressed genes and distinguishing between homologous genes. In this report, we validated quantitative expression data of 110 cDNA-AFLP fragments in yeast with DNA microarrays and GeneChip data. The best correlation was found between cDNA-AFLP and GeneChip data. The cDNA-AFLP data revealed a low number of inconsistent profiles that could be explained by gel artifact, overexposure, or mismatch amplification. In addition, 18 cDNA-AFLP fragments displayed homology to genomic yeast DNA, but could not be linked unambiguously to any known ORF. These fragments were most probably derived from 5' or 3' noncoding sequences or might represent previously unidentified ORFs. Genes liable to cross hybridization showed identical results in cDNA-AFLP and GeneChip analysis. Three genes, which were readily detected with cDNA-AFLP, showed no significant expression in GeneChip experiments. We show that cDNA-AFLP is a very good alternative to microarrays and since no preexisting biological or sequence information is required, it is applicable to any species.


http://www.sciencedirect.com/science/article/B6WG1-4DXB88G-3/2/44e01632b9ec0f453c1d8393105de481

The highly polymorphic VNTR locus pYNZ32 has been more extensively characterized, and its analysis converted to a rapid PCR-based format. DNA sequencing in the areas within and flanking the repeated segment allowed the design of specific amplification primers. The repeated region of pYNZ32 consists of an imperfectly duplicated 27-bp motif, 16 bases of which are more highly conserved. Allelic products from PCR amplification were resolved into nine different size classes ranging from approximately 1400 to 2200 bp. Additional polymorphism was revealed when the amplified products were analyzed by restriction enzyme digestion. Both the overall size variation and the internal sequence polymorphism were used to determine a heterozygosity value of 86% for YNZ32 in 50 unrelated individuals. The rapid analysis and improved resolution of amplified alleles on agarose gels, and the internal variability within YNZ32, increase its diagnostic utility as a VNTR and as a linkage marker for the nearby Huntington disease gene.

Physical mapping of human chromosome 16 has been undertaken using somatic cell hybrid DNAs as templates for polymerase chain reaction (PCR) deletion analysis of sequence tagged sites (STSs). A panel of 29 somatic cell hybrids was analyzed, confirming and refining previous chromosome 16 breakpoint orders and distinguishing between the locations of breakpoints in new hybrids. Ten STS markers were coamplified in three multiplex reactions allowing the rapid, simultaneous deletion analysis of nine different loci. The locations of the protamine (PRM1), sialophorin (SPN), complement component receptor 3A (CR3A), NAD(P)H menadione oxidoreductase 1 (NMOR1), and calbindin (CALB2) genes were refined.


Focal nonepidermolytic palmoplantar keratoderma (NEPPK), or tylosis, is an autosomal, dominantly inherited disorder of the skin that manifests as focal thickening of the palmar and plantar surfaces. In three families studied, the skin disorder cosegregates with esophageal cancer and oral lesions. New haplotype analysis, presented here, places the tylosis esophageal cancer (TOC) locus between D17S1839 and D17S785. Envoplakin (EVPL) is a protein component of desmosomes and the cornified envelope that is expressed in epidermal and esophageal keratinocytes and has been localized to the TOC region. Mutation analysis of EVPL in the three affected families failed to show tylosis-specific mutations, and haplotype analysis of three intragenic sequence polymorphisms of the EVPL gene placed it proximal to D17S1839. Confirmation of the exclusion of EVPL as the TOC gene by location was obtained by integration of the genetic and physical mapping data using radiation hybrid, YAC, BAC, and PAC clones. This new physical map will allow further identification of candidate genes underlying NEPPK associated with esophageal cancer, which may also be implicated in the development of sporadic squamous cell esophageal carcinoma and Barrett's adenocarcinoma.


The structure of the 3' one-third of the dystrophin gene has not previously been established. We have used vectorette PCR on a yeast artificial chromosome containing part of the human dystrophin gene to determine that there are 20 exons in this region and to characterize adjacent intron sequences of each one. Combined with previous information on the remainder of the gene, this study shows that the coding sequence is distributed between 79 exons. We have used PCR between exons to measure the distances that separate the more closely clustered exons. Vectorette PCR products were used as probes on Southern blots to assign all the 3' exons to genomic HindIII fragments that are commonly detected in the analysis of dystrophin gene deletions. The results will be useful for determining the effect of genomic deletions on the
translational reading frame, for setting up genomic PCR assays to confirm point mutations, for analyzing splice site mutations, and for investigating potential cis-acting elements involved in tissue-specific alternative splicing. Vectorette PCR using primers derived from cDNA sequence represents an efficient and widely applicable method for establishing gene structure and obtaining intron sequence flanking exons, starting from a genomic clone and a cDNA sequence.


http://www.sciencedirect.com/science/article/B6WG1-4938JNP-1/2/75e2966a49ef4c264e7ff8742a9b5e3

We developed a simple method, based on the TaqMan technology, for fast genotyping of insertion/deletion polymorphisms of known location. The genotypes of 22 CEPH individuals, previously ascertained by conventional methods, were confirmed in the new assay without manual, time-consuming, post-PCR analysis. We propose to expand the application of TaqMan probes for population screening of insertion/deletion polymorphisms in which the exact endpoints of the insertion/deletion are known. The method can be applied to polymorphisms of any size and can be used for different applications such as diagnostics, genome variation, and species identification.


http://www.sciencedirect.com/science/article/B6WG1-4DXB88G-5/2/d3e588b67b023273dbeef16b56b1617d

The fine structure of the Chinese hamster hypoxanthine guanine phosphoribosyltransferase (HPRT) gene has been determined; the gene has nine exons and is dispersed over 36 kb DNA. Exons 2-9 are contained within overlapping [lambda] bacteriophage clones and exon 1 was obtained by an inverse polymerase chain reaction (PCR). All the exons have been sequenced, together with their immediate flanking regions, and these sequences compared to those of the mouse and human HPRT genes. Sequences immediately flanking all exons but the first show considerable homology between the different species but the region around exon 1 is less conserved, apart from the preserved location of putative functional elements. Oligonucleotide primers derived from sequences flanking the HPRT gene exons were used to amplify simultaneously seven exon-containing fragments in a multiplex PCR. This simple procedure was used to identify total and partial gene deletions among Chinese hamster HPRT-deficient mutants. The multiplex PCR is quicker to perform than Southern analysis, traditionally used to study such mutants, and also provides specific exon-containing fragments for further analysis. The Chinese hamster HPRT gene is often used as a target for mutation studies in vitro because of the ease of selection of forward and reverse mutants; the information presented here will enhance the means of investigating molecular defects within this gene.


http://www.sciencedirect.com/science/article/B6WG1-4F0PT9X-
The dopamine D2 receptor gene (gene symbol DRD2) is a candidate gene for schizophrenia because the potency of certain neuroleptics correlates with their affinity for this receptor. Seven regions of likely functional significance including the coding sequences and the splice junctions were fully sequenced in the dopamine D2 receptor of 14 schizophrenics (and partially in several others) meeting DSM-III-R diagnostic criteria and in four unaffected non-Caucasians (97 kb of total sequence). No structural changes were found, suggesting that alteration in the structure of the dopamine D2 receptor is not commonly involved in the etiology of schizophrenia. However, two common and one uncommon intragenic polymorphisms were found. At least one of the polymorphisms was informative for linkage in 70% of Caucasians and 78% of Koreans.


http://www.sciencedirect.com/science/article/B6WG1-48TM68C-3/2/5c9bda2d091f14900411f283a0c711c3

Zebrafish is one of the best model organisms for investigating gene functions in vertebrates. By 4,5',8-trimethylpsoralen mutagenesis, we isolated a zebrafish mutant, vibrato, with defects in the spontaneous contraction and touch response. Whole genome subtraction between the wild-type and the mutant genomes by representational difference analysis yielded polymorphic markers tightly linked to the vibrato locus. Using these markers, we constructed a high-resolution physical map and localized the vibrato locus within a genomic region of 720 kb. Direct cDNA selection with the contig led to the identification of a novel gene, solo, encoding a protein with SEC14 and spectrin repeat domains. These domains of Solo shared significant amino acid sequence identities with those of mammalian Trio and Karilin. In addition, we found the zebrafish orthologs for mammalian TTN, COL5A2, and CED-6 in the vibrato region. Mapping of these genes localized human chromosomal regions possibly involved in motor disorders. Our results suggest that representational difference analysis provides an efficient way to isolate mutated genomic regions in zebrafish.


http://www.sciencedirect.com/science/article/B6WG1-48TKF2F-7/2/a2a26d0d030d87c02893c5afa2015358

Here we report the identification of a novel transcript containing SNF2, PHD-finger, RING-finger, helicase, and linker histone domains mapping to the q24 band region of human chromosome 6. These domains are characteristic of several DNA repair proteins, transcription factors, and helicases. We have cloned both human and mouse homologs of this novel gene using interexon PCR and RACE technologies. The human cDNA, termed SHPRH, is 6018 bp and codes for a putative protein of 1683 amino acids. The mouse cDNA, termed Shprh, is 7225 bp and codes for a putative protein of 1616 amino acids. The deduced amino acid sequences of the two proteins share 86% identity. Both genes are expressed ubiquitously, with a transcript size of ~7.5 kb. Mapping of this gene to 6q24, a region reported to contain a tumor suppressor locus, prompted us to evaluate SHPRH by mutation analysis in tumor cell lines. We have identified one truncating and three missense mutations, thus suggesting SHPRH as a possible candidate for the tumor
suppressor gene.

http://www.sciencedirect.com/science/article/B6WG1-4DNHRXG-NJ/2/67be5165b93fc36e75c1bf14c5c3b480

http://www.sciencedirect.com/science/article/B6WG1-4DNHP53-4R/2/5690eedef6767d6c1fb06c879d5d6546

A strategy for the analysis of yeast artificial chromosome (YAC) clones that relies on polymerase chain reaction (PCR) amplification of small restriction fragments from isolated YACs following adapter ligation was developed. Using this method, termed YACadapt, we have amplified several YACs from a human Xq24-qter library and have used the PCR products for physical mapping by somatic cell hybrid deletion analysis and fluorescent in situ hybridization. One YAC, RS46, was mapped to band Xq27.3, near the fragile X mutation. The PCR product is an excellent renewable source of YAC DNA for analyses involving hybridization of YAC inserts to a variety of DNA/RNA sources.

http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-B3/2/4e845023a143685ac6da1968e59630db

Aspartylglucosaminuria (AGU) is a recessively inherited lysosomal disease caused by inadequate aspartylglucosaminidase (AGA) activity. The disease is prevalent in the genetically isolated Finnish population. We have used a new method, solid-phase minisequencing, to determine the frequency of two missense mutations in the AGA gene in this population. In samples from 70% of the Finnish AGU families, we found that the two nucleotide changes were always associated, and they were identified in 98% of the AGU alleles analyzed. Thus, the high prevalence of AGU in the Finnish population is the consequence of a founder effect of one ancient mutation. The identification of Asymptomatic carriers by the minisequencing test proved to be unequivocal. The method also allowed quantification of a mutated nucleotide sequence present in less than 1% of a sample. The frequency of AGU carriers in this population was 1/36 when estimated by quantifying the mutated AGU allele in a pooled leukocyte sample from 1350 normal Finnish individuals.

Annexin IV (placental anticoagulant protein II) is a member of the annexin or lipocortin family of calcium-dependent phospholipid-binding proteins. A cDNA for human annexin IV was isolated from a placental library that is 675 bases longer in the 3' untranslated region than previously reported, indicating the existence of alternative mRNA processing for this gene. Genomic Southern blotting with a cDNA probe indicated a gene size of 18-56 kb. Primers developed for polymerase chain reaction (PCR) allowed amplification of a 1.6-kb portion of the ANX4 gene. DNA sequence analysis showed that this PCR product contained a single intron with exon-intron boundaries in exactly the same position as in the mouse annexin I and annexin II genes. PCR analysis of a somatic cell hybrid panel mapped the ANX4 gene to chromosome 2, and in situ hybridization with a cDNA probe showed a unique locus for ANX4 at 2p13. This study provides further evidence that genes for the annexins are dispersed throughout the genome but are similar in size and exon-intron organization.


http://www.sciencedirect.com/science/article/B6WG1-48TKF2F-3/2/6df5538583c0bf9f2232edded5fcb6e87

Schizophrenia is a common neuropsychiatric disorder of uncertain etiology that is believed to result from the interaction of environmental factors and multiple genes. To identify new genes predisposing to schizophrenia, numerous groups have focused on CAG-repeat-containing genes. We previously reported a CAG repeat polymorphism that was shown to be associated with both the severity of the phenotype and the response to medication in schizophrenic patients. In this article, we now report the genomic structure of this gene, the retinoic acid inducible-1 gene (RAI1), and present its characterization. This gene, located on chromosome 17p11.2, comprises six exons coding for a 7.6-kb mRNA. The RAI1 gene is highly homologous to its mouse counterpart and it is expressed at high levels mainly in neuronal tissues.


http://www.sciencedirect.com/science/article/B6WG1-471W72M-4P/2/b6aa0e0b90f1717b060d76036dac5b58


http://www.sciencedirect.com/science/article/B6WG1-47TF6BT-4/2/19b6be69bbf0ed3aa0f61269644f1460

By microarray assay we identified ESTs (expressed sequence tags) whose expression was predominantly increased in the affected skin of patients with psoriasis vulgaris. Among them, a full-length cDNA sequence corresponding to one of those ESTs (AI829641) was isolated by screening of cultured human keratinocyte cDNA libraries. This cDNA encodes a novel member of the Ly-6/uPAR superfamily, designated SLURP-2 (secreted Ly-6/uPAR related protein 2). SLURP-2 has an open reading frame of 97 amino acids containing 10 conserved cysteine residues. SLURP-2 has a single functional copy within the LY6 superfamily gene cluster at...
chromosome 8q24.3. RT-PCR (reverse transcriptase-polymerase chain reaction) expression analysis revealed that SLURP-2 was expressed in multiple tissues, mainly in the epithelial cells including the skin and keratinocytes, but not in spleen or bone marrow. Comparison of the expression of this gene among the psoriatic lesional and nonlesional skin of patients and the normal skin of healthy individuals detected by quantitative real-time RT-PCR analysis disclosed that SLURP-2 was up-regulated threefold in psoriatic lesional skin. These findings suggest that SLURP-2 may be involved in the pathophysiology of psoriasis through its role in keratinocyte hyperproliferation and/or T cell differentiation/activation.


http://www.sciencedirect.com/science/article/B6WG1-4C0V55V-1/2/2398a7daa70c3b8446adbcd75d687e9

The Intersectin 1 (ITSN1) protein functions in clathrin-mediated endocytosis and in MAP kinase signaling. The complex domain structure comprises two EH and five SH3 domains in the short isoform, plus RhoGEF, pleckstrin, and putative calcium-interaction domains in the long isoform. Alternative splicing of exon 20, affecting the SH3A domain, has been shown in rat and that of exons 25 + 26, affecting the SH3C domain, has been shown in human and rat. Here we report 7 novel splice variants of the human and mouse ITSN1 genes and demonstrate conservation of alternative splicing affecting SH3A and SH3C in mouse. The novel variants encode transcripts with altered EH domain spacing and RhoGEF domain structure and possible targets of nonsense-mediated decay. Eight and 16 protein variants of the short and long ITSN1 isoforms, respectively, are predicted. These isoforms likely serve to modulate the many complex protein interactions and functions of ITSN1.


http://www.sciencedirect.com/science/article/B6WG1-4DNHRHP-JB/2/041950847b6868a39d1abe13da86344b

The role of genetic variation in isoenzyme gene families is often poorly appreciated. We report here on the determination of DNA sequences and typing of genetic variability in four creatine kinase B (CKB) gene loci in different inbred strains of mice. The unique functional murine CKB gene was found to be nearly identical to the previously characterised rat and human sequences in both size and exon-intron structure. In this gene, approximately 0.5% allelic nucleotide positions as well as the lengths of simple A-rich and [TG]n repetitive elements located at the 5’ and 3’ sides of the transcribed segment, differed between inbred strains of mice. Preliminary experiments suggest that this sequence divergence is of importance for design of gene targeting strategies involving homologous DNA recombination. The three additional CKB-like gene loci in mice all had the characteristics of processed pseudogenes. By Southern blot analysis we could demonstrate that both the type and number of pseudogenes differed between inbred strains. Analysis of the CKB gene sequences enabled us to speculate about the evolutionary history of this highly polymorphic subfamily of genes.

Human [beta] defensins contribute to the first line of defense against infection of the lung. Polymorphisms in these genes are therefore potential modifiers of the severity of lung disease in cystic fibrosis. Polymorphisms were sought in the human [beta]-defensin genes DEFB1, DEFB4, DEFB103A, and DEFB104 in healthy individuals and cystic fibrosis (CF) patients living in various European countries. DEFB1, DEFB4, and DEFB104 were very polymorphic, but DEFB103A was not. Within Europe, differences between control populations were found for some of the frequent polymorphisms in DEFB1, with significant differences between South-Italian and Czech populations. Moreover, frequent polymorphisms located in DEFB4 and DEFB104 were not in Hardy Weinberg equilibrium in all populations studied, while those in DEFB1 were in Hardy Weinberg equilibrium. Sequencing of a monochromosomal chromosome 8 mouse-human hybrid cell line revealed signals for multiple alleles for some loci in DEFB4 and DEFB104, but not for DEFB1. This indicated that more than one DEFB4 and DEFB104 gene was present on this chromosome 8, in agreement with recent findings that DEFB4 and DEFB104 are part of a repeat region. Individual DEFB4 and DEFB104 PCR amplification products of various samples were cloned and sequenced. The results showed that one DNA sample could contain more than two haplotypes, indicating that the various repeats on one chromosome were not identical. Given the higher complexity found in the genomic organization of the DEFB4 and DEFB104 genes, association studies with CF lung disease severity were performed only for frequent polymorphisms located in DEFB1. No association with the age of first infection by Pseudomonas aeruginosa or with the FEV1 percentage at the age of 11-13 years could be found.


http://www.sciencedirect.com/science/article/B6WG1-4CG2HPD-4/2/28447c7cb184f39581fef517a0e10277

The gene mutated in chorea-acanthocytosis (CHAC; approved gene symbol VPS13A) encodes chorein, a protein similar to yeast Vps13p. We detected several similar putative human proteins by BLAST analysis of chorein. We characterized the structure of three new genes encoding these CHAC-similar proteins, located on chromosomes 1p36, 8q22, and 15q21. The most similar gene in yeast to all four human genes is Vps13, and therefore the human genes were named VPS13A (CHAC, 9q21), VPS13B (8q22), VPS13C (15q21), and VPS13D (1p36). VPS13B has recently been reported as COH1, altered in Cohen syndrome. For each gene, we describe several alternative splicing variants; at least two transcripts per gene are major forms. The expression pattern of these genes is ubiquitous, with some tissue-specific differences between several transcript variants. Protein sequence comparisons suggest that intramolecular duplications have played an important role in the evolution of this gene family.


http://www.sciencedirect.com/science/article/B6WG1-4B0X4PV-2/2/315f165eaaedf33bc97f6f49d572a082

We have designed and evaluated a series of class-specific (Aves), order-specific (Rodentia), and species-specific (equine, canine, feline, rat, hamster, guinea pig, and rabbit) polymerase chain reaction (PCR)-based assays for the identification and quantitation of DNA using amplification of
genome-specific short and long interspersed elements. Using SYBR Green-based detection, the minimum effective quantitation levels of the assays ranged from 0.1 ng to 0.1 pg of starting DNA template. Background cross-amplification with DNA templates derived from sixteen other species was negligible prior to 30 cycles of PCR. The species-specificity of the PCR amplicons was further demonstrated by the ability of the assays to accurately detect known quantities of species-specific DNA from mixed (complex) sources. The 10 assays reported here will help facilitate the sensitive detection and quantitation of common domestic animal and bird species DNA from complex biomaterials.


http://www.sciencedirect.com/science/article/B6WG1-4F0PT9X-V/2/b11c0271a66ae35f17cda296683f248a

Treacher Collins syndrome is an autosomal dominant disorder of abnormal craniofacial development. Linkage analysis was performed in Treacher Collins families with restriction fragment length or microsatellite polymorphisms associated with eight loci previously mapped to 5q31->qter. Positive lod scores were obtained for four loci, D5S119, D5S207, D5S209, and D5S210, which map to 5q31.3->q33.3. The Treacher Collins syndrome locus was linked closest to locus D5S210, which is associated with microsatellite polymorphisms, with a maximum lod score of 8.65 at [theta] = 0.02. The Treacher Collins syndrome locus was excluded from locus ADRB2R, which maps to 5q31->q32, and loci D5S22, D5S61, and D5S43, which map to 5q34->qter. There was no evidence for genetic heterogeneity among eight families with variable expression of the condition.


http://www.sciencedirect.com/science/article/B6WG1-4DBCBNW-1/2/c95fb4280115112e7565739e611b3520

Infantile nephronophthisis is associated with cystic kidneys, situs inversus, and INVS mutations. The function of the INVS product, inversin, is unknown but evidence suggests there are multiple inversin isoforms with differing molecular weights, cellular localization patterns, and binding partners. We used Northern blots, RT-PCR, and sequence analysis to identify alternative INVS transcripts. Northern blots probed with Invs cDNA detected four bands in normal mouse kidney. RT-PCR of mouse kidney RNA revealed Invs transcripts with skipping of exon 5, 11, or 13. We sequenced canine (MDCK-II cells) INVS and determined that the corresponding full-length protein shares identity with mouse (74%) and human (84%) inversin. Canine INVS produces a transcript that skips exon 12. Exon skips cause loss of inversin protein motifs, including ankyrin repeats, IQ domains, destruction boxes, and nuclear localization signals. Identification of INVS splice variants will help us determine which inversin protein motifs contribute to left-right asymmetry and kidney development.


http://www.sciencedirect.com/science/article/B6WG1-4D5XCJP-
Amplicons are frequently found in human tumor genomes, but the mechanism of their generation is still poorly understood. We previously measured the replication timing of the genes along the entire length of human chromosomes 11q and 21q and found that many "disease-related" genes are located in timing-transition regions. In this study, further scrutiny of the updated replication-timing map of human chromosome 11q revealed that both amplicons on human chromosomal bands 11q13 and 11q22 are located in the early/late-switch regions of replication timing in two human cell lines (THP-1 and Jurkat). Moreover, examination of synteny in the human and mouse genomes revealed that synteny breakage in both genomes occurred primarily at the early/late-switch regions of replication timing that we had identified. In conclusion, we found that the early/late-switch regions of replication timing coincided with "unstable" regions of the genome.


http://www.sciencedirect.com/science/article/B6WG1-4DXB57P-8/2/85db262021c41c5b6ff7a1f8861325c1

Abundant human interspersed repetitive DNA sequences of the form (dC-dA)n. (dG-dT)n have been shown to exhibit length polymorphisms. Examination of over 100 human (dC-dA)n. (dG-dT)n sequences revealed that the sequences differed from each other both in numbers of repeats and in repeat sequence type. Using a set of precise classification rules, the sequences were divided into three categories: perfect repeat sequences without interruptions in the runs of CA or GT dinucleotides (64% of total), imperfect repeat sequences with one or more interruptions in the run of repeats (25%), and compound repeat sequences with adjacent tandem simple repeats of a different sequence (11%). Informativeness of (dC-dA)n. (dG-dT)n markers in the perfect sequence category was found to increase with increasing average numbers of repeats. PIC values ranged from 0 at about 10 or fewer repeats to above 0.8 for sequences with about 24 or more repeats. (dC-dA)n. (dG-dT)n polymorphisms in the imperfect sequence category showed lower informativeness than expected on the basis of the total numbers of repeats. The longest run of uninterrupted CA or GT repeats was found to be the best predictor of informativeness of (dC-dA)n. (dG-dT)n polymorphisms regardless of the repeat sequence category.


http://www.sciencedirect.com/science/article/B6WG1-4DXB5C5-1V/2/a7117b21bd7f5912b120741622b24f38

Thirteen moderately to highly informative microsatellite DNA polymorphisms based on (dC-dA)n. (dG-dT)n repeats were mapped to segments of human chromosome 5 using both linkage analysis and a panel of somatic cell hybrids which contained rearranged chromosomes. The markers were distributed throughout most of the length of the chromosome from the regions p15.3-p15.1 to q33.3-qter. Maps of the sites of meiotic recombination within the reference families proved particularly useful for the purpose of integrating new polymorphisms into the existing linkage map.

(NTRK1) to human chromosome 1q21-q22 by P1 clone selection, fluorescence in situ hybridization (FISH), and computer-assisted microscopy." Genomics 26(2): 390.

http://www.sciencedirect.com/science/article/B6WG1-471W7HX-82/2/8727cc75d255e548a733d2e1777ddc16

Physical mapping of small genomic DNA fragments or expressed sequences by in situ hybridization is typically limited by the size of the target DNA sequence. Isolation of large insert DNA clones from libraries containing the target DNA sequence facilitates physical mapping by fluorescence in situ hybridization and allows rapid assignment of genes to cytogenetic bands. Here, we demonstrate the scheme by mapping the human protooncogene trk (NTRK1), a tyrosine kinase receptor type I gene that has earlier been assigned to two different cytogenetic loci. Large DNA insert library screening was carried out by in vitro DNA amplification using oligonucleotide primers flanking exon 4 of trk. The scheme presented here can easily be generalized to map physically very small nonrepetitive genomic DNA fragments or incomplete cDNAs.


http://www.sciencedirect.com/science/article/B6WG1-4D10KHN-1/2/198682806ad797a174fb122f01bb8adf

Members of the human UDP-glucuronosyltransferase 2B family are located in a cluster on chromosome 4q13 and code for enzymes whose gene products are responsible for the normal catabolism of steroid hormones. Two members of this family, UGT2B15 and UGT2B17, share over 95% sequence identity. However, UGT2B17 exhibits broader substrate specificity due to a single amino acid difference. Using gene-specific primers to explore the genomic organization of these two genes, it was determined that UGT2B17 is absent in some human DNA samples. The gene-specific primers demonstrated the presence or absence of a 150 kb genomic interval spanning the entire UGT2B17 gene, revealing that UGT2B17 is present in the human genome as a deletion polymorphism linked to UGT2B15. Furthermore, it is shown that the UGT2B17 deletion polymorphism shows Mendelian segregation and allele frequencies that differ between African Americans and Caucasians.


http://www.sciencedirect.com/science/article/B6WG1-49HSTP6-4/2/db4e1c82b0306a884c75edf0c98fd27a

As a first step towards verifying the candidate status of DGAT1 as the causal gene for milk fat percentage in cattle, we constructed a bovine BAC contig spanning 576 kb of the chromosomal region containing DGAT1. High content of NotI sites (21 within the contig) indicated that the region is gene-rich. Twenty-three genes neighboring DGAT1 were mapped, including two bovine cDNA sequences that have no orthologous sequences within the NCBI sequence databases. On average, 2015 bp for each of the 23 neighboring genes were sequenced and entered into EMBL. Likewise, 10 new STS markers were established by BAC-end sequencing. Within the genes and STS markers, 55 polymorphisms were discovered. These will form the basis of future linkage disequilibrium studies to test whether any genes neighboring DGAT1 are associated with variation in milk fat percentage, thereby testing the candidate status of DGAT1.

http://www.sciencedirect.com/science/article/B6WG1-4C6TFXR-5/2/17b7e01a43dd9a7d3e7e3ad4db8d39c4

The telomeric region of chromosome 9p is paralogous to the pericentromeric regions of chromosome 9 as well as to 2q13, the site of an ancestral telomere-telomere fusion. These paralogous regions span approximately 200 kb and contain seven transcriptional units, including the previously identified CBWD, FOXD4, PGM5, F379, CXYorf1, and two human Unigene clusters, Hs.115173 and Hs.189160. Within these gene duplicates, the number of expressed paralogous loci varies, from one in PGM5 to all three in CBWD and Hs.115173. FOXD4 shows the most dramatic changes among its paralogs. Two independent insertion/deletion changes created four different carboxy ends of these intronless genes, two of which are within the 2q13 locus. A comparison of KA/KS values among functional paralogs shows these genes evolved rapidly in primates. This study shows the importance of paralogous regions in the generation of transcriptional diversity and highlights the significance that large-scale telomeric duplication may play in this process.


http://www.sciencedirect.com/science/article/B6WG1-482YXT2-1/2/673df987acffa3e46e7b807fd83f9da1

We have recently reported a new pathogen discovery approach, "computational subtraction". With this approach, non-human transcripts are detected by sequencing cDNA libraries from infected tissue and eliminating those transcripts that match the human genome. We show now that this method is experimentally feasible. We generated a cDNA library from a tissue sample of post-transplant lymphoproliferative disorder (PTLD). 27,840 independent cDNA sequences were filtered by computational subtraction against the known human sequence to identify 32 nonmatching transcripts. Of these, 22 (0.1%) were found to be amplifiable from both infected and noninfected samples and were inferred to be human DNA not yet contained in the available human genome sequence. The remaining 10 sequences could be amplified only from Epstein-Barr virus (EBV)-infected tissues. All 10 corresponded to the known EBV sequence. This proof-of-principle experiment demonstrates that computational subtraction can detect pathogenic microbes in primary human-diseased tissue.


http://www.sciencedirect.com/science/article/B6WG1-4B3NM01-2/2/055c7d3de394cebbdf6f30e7e1defa2

MEST is one of the imprinted genes in human. With the assistance of our integration map and the complete sequence in the registry, we mapped a total of 16 genes/transcripts at the 1.5-Mb MEST-flanking region at 7q32. This region has been suggested to form an imprinted gene cluster, because MEST and its three flanking genes/transcripts (MESTIT1, CPA4, and COPG2IT1) were reported to be imprinted, although two (TSGA14 and COPG2) were shown to escape imprinting.
In this study, 10 other genes/transcripts were examined for their imprinting status in human fetal tissues. The results indicated that 8 genes/transcripts (NRF1, UBE2H, HSPC216, KIAA0265, FLJ14803, CPA2, CPA1, and DKFZp667F0312) were expressed biallelically. The imprinting status of two (TSGA13 and CPA5) was not conclusive, because of their weak and/or tissue-specific expression and inconstant results. These findings provided evidence that only 4 of the 16 genes/transcripts located to the region show monoallelic expression, while others are not involved in imprinting. Therefore, it is less likely that the MEST-flanking 7q32 region forms a large imprinted domain.


http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-4F/2/f4c423de52dc4c492f0b5054

A method based on the differential screening of a chromosome-specific cosmid library with amplified inter-Alu sequences obtained from a set of somatic cell hybrids has been developed to target the isolation of probes from predefined subchromosomal regions. As a model system, we have used a chromosome 22-specific cosmid library and four cell hybrids containing different parts of this chromosome. The procedure has identified cosmids that demonstrate differential hybridization signals with Alu-PCR products from these cell hybrids. We show, by in situ hybridization or individual mapping, that their hybridization pattern is indicative of their sublocalization on chromosome 22, thus resulting in a large enrichment factor for the isolation of probes from specific small chromosome subregions. Depending on the local Alu-sequence density, from 3 to 10 independent loci per megabase of genome can thus be identified.