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Fast-FISH using repeat sequence-depleted painting probes from microdissected DNA. *H. He[1], W. Huang[1], L. Scheller-Malin[1], X.Y. Guan[2].* 1) American Lab Technologies, Inc, Rockville, MD; 2) Department of Clinical Oncology, University of Hong Kong, Hong Kong, China.

There is currently an increasing demand by researchers and clinicians for high quality FISH painting probes that aid in the diagnosis of cancer and hereditary diseases. We have designed a novel method of removing repetitive sequences from microdissected probes resulting in products that are more specific and are easier to use. We named our repetitive sequence-depleted probes "ReSeD Probes". We tested our ReSeD probes of 5p, 9q, 12p, 15q and a few band specific probes in Fast-FISH. When used on metaphase chromosomes and interphase cells, the ReSeD probes produced strong, uniform, and specific hybridization signals with little background staining in only 30 minutes of hybridization. Dual-color Fast-FISH also produced comparable results. These new probes will make Fast-FISH a useful tool for the research and clinical community and allow faster turn around time for individual FISH cases.

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Molecular and cytogenetic studies in Ambras syndrome. *M. Tadin[1], E.P. Braverman[5], C-Y. Yu[1], J.A. Frank[1], F.A.M. Baumeister[3], S. Cianfarani[4], A.M. Christiano[1,2], D. Warburton[1].* 1) Department of Genetics and Development, Columbia University, New York, NY; 2) Department of Dermatology, Columbia University, New York, NY; 3) Dr. v. Haunersches Kinderspital der Universität München; 4) Department of Pediatrics and Public Health, University 'Tor Vergata', Rome, Italy; 5) School of Allied Health, University of Connecticut.

Ambras syndrome (AMS) is a unique form of congenital universal hypertrichosis. In patients with this syndrome the whole body is covered with fine, long vellus hair, except for areas where no hair normally grows. There is accompanying facial dismorphism and abnormalities of the teeth, including retarded first and second dentition, and absence of teeth. No metabolic or endocrine defect has been detected. Multiple affected family members suggest a genetic basis for the syndrome. An inversion of chromosome 8 has been found in two isolated cases. These inversions have a breakpoint in band q22 in common, suggesting that this region (8q22) contains the gene which causes AMS. Using chromosomal FISH analysis we have narrowed the breakpoint interval to about 500 kb in one of the patients. We also performed loss of heterozygosity (LOH) analysis to check for possible microdeletions that are not evident at the cytogenetic level. A deletion of about 1 Mb was detected in the other patient in the vicinity of the previously defined 500 kb inversion interval. We are currently saturating the region with additional microsatellite markers to determine the borders of the deleted interval. We anticipate that these combined approaches will further narrow the interval and lead to the identification of the Ambras syndrome gene.

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A case with an extra large inverted duplicated chromosome 15. *S.-D. Cheng[1], S.-P. Lin[2].* 1) Department of Anatomy, College of Medicine, Chang Gung University, Kweishan, Taiwan; 2) Division of Genetics, Department of Pediatrics, MacKay Memorial Hospital, Taipei, Taiwan.

The proximal region in the long arm of chromosome 15 (15q11q13) has been known to be related to the Angelman syndrome (AS) and Prader-Willi syndrome (PWS). The increase of the copy number of the region, depending on the size, may also create abnormalities to the phenotype. With fluorescence *in situ* hybridization (FISH) method combined with quantitative Southern analyses, we detected a proband with an extra large inverted duplicated chromosome 15 (inv dup(15)). Under DAPI staining an extraordinarily long segment is revealed between two centromeres of the inv dup(15). We tested with FISH with lambda clones of 34-10 (D15S9), λ16 (GABRB3) and IR10-1-45 (D15S12) individually. Each of the three probes revealed four copies of the signals on each sister chromatid of the inv dup(15). Together with two copies of each locus on the normal chromosomes 15, the proband has six copies totally in her genome. The quantitative Southern analyses with PhosphorImager indicated that the copy number of p3-21 (D15S10) locus of proband is 2.27 times, and that of p189-1 (D15S13) is two-fold than that of a normal female after normalization with probe 87-15 (DXS164). The proband at her age of 12 featured short stature, hypertelorism, upward slant of palpebral fissures, thin upper lips, mildly dysplastic ears, and simian crease of right hand. She had developmental delay with mental retardation and poor language ability. Other than emotional instability and hyperactivity, she also had neurofibromatosis type I, which is inherited from her mother.

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An *In Situ* Hybridization-Based Strategy to Detect Neuronal Patterns of Genomic Imprinting in the Autism Candidate Region of Chromosome 15q11-q13. *R.A. Kesterson[1], E.L. Nurmi[1], D.K. Johnson[2], J.S. Sutcliffe[1].* 1) Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN; 2) Mammalian Genetics and Development Section, Oak Ridge National Laboratory, Oak Ridge, TN.

Reciprocal patterns of genomic imprinting are involved in the etiology of genomic disorders associated with human chromosome 15q11-q13. Paternal deletions of 15q11-q13 are associated with Prader-Willi syndrome, while maternal deletions of a common region are associated with Angelman syndrome. Interstitial duplications or triplications using similar homologous breakpoints, or "duplicons", and inverted duplications utilizing more distal breakpoints are associated with autism-spectrum phenotypes, possibly in a maternal-specific manner. An autism candidate region has been suggested based on the duplications, as well as linkage and linkage disequilibrium data in a common area. Given the possible involvement of genomic imprinting in the etiology of autism susceptibility in the chromosome 15 region, we are assessing positional and functional candidate genes in this region for neuronal patterns of genomic imprinting using an *in situ* hybridization-based strategy for their murine orthologs. Mice with large deletions encompassing the p locus are hemizygous for genes within the autism candidate region. Using mice with reciprocal maternal or paternal deletions, or controls, we hybridized brain sections with probes corresponding to a cluster of $GABA_A$ receptor subunit genes (*Gabrb3*, *Gabra5*, and *Gabrg3*), and the *p*locus. Our initial studies have revealed that these genes do not show complete allele-specific silencing within the brain. We are currently exploring the possibility of more subtle allelic differences in expression. It is feasible that genes within this region may show complex imprinting effects as evidenced by maternal-biased expression of *Ube3a* within certain brain regions. This experimental strategy provides greater sensitivity to detect genomic imprinting in this region compared to northern-based approaches which may not detect subtle patterns of parental-specific expression.

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Molecular analysis of Severe Mental Retardation associated 16p-chromosomal breakpoint. *K. Bhalla, H. Eyre, A. Gardner, G. Kremmidiotis, G.R. Sutherland, D.F. Callen.* Department of Cytogenetics and Molecular Genetics, The University of Adelaide, Women's & Children's Hospital, Adelaide, Australia.

Identification of families with mentally retarded males has led to the mapping and cloning of a number of X-linked recessive loci and relevant genes for mental retardation. However, there has been little progress in the identification of autosomal genes for mental retardation. We have exploited a *de novo* balanced translocation, 46,XY,t(1;16)(q12;p13.3) in a patient with severe intellectual retardation. We have generated a somatic cell hybrid containing the der(16) of this translocation, designated as CY196. Since the chromosome 1 breakpoint was shown to be within 1q heterochromatin, we propose that this translocation either disrupts a gene on chromosome 16 or alters expression of a gene in the near vicinity by a position effect of the chromosome 1 heterochromatin. Prelimnary work in the vicinity of the breakpoint identified *C16orf5*, a novel proline rich gene, highly expressed in the brain. The YAC pulse field map of the region placed this gene distal to CY196. A PAC/BAC contig was then constructed across the chromosome 16 breakpoint. FISH analysis with these clones to the metaphase chromosome identified a PAC clone spannning the breakpoint. The PAC was sequenced and analysis indicates that the region surrounding the breakpoint is repetitive with very few matches to expressed sequences (ESTs) or computer predicted exons. *C16orf5* is the closest gene to the breakpoint and is highly expressed in the brain. Quantitative RT-PCR analysis suggests that the expression of the *C16orf5* transcript is reduced in the patient. We propose that the mental retardation of the translocation patient is the reult of reudced expression of this brain specific transcript due to the position effect of the adjacent translocated chromomsome 1 heterochromatin. Mutation analysis in the candidate gene will now be carried out to show its involvement in mental retardation.

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Simultaneous Detection of Three Different Chromosomes in Multi-PRINS Using only two types of labeled nucleotides *J. Yan, M. Bronsard, R. Drouin.* Dept Medical Biol, Laval Univ, CHUQ Hop.St-Francois d'Assise, Quebec, Quebec, Canada.

In multi-PRINS technique, the blocking step using ddNTP incorporated by a DNA polymerase is an important procedure that can block the free 3-end generated in the last PRINS reaction, thus avoiding the next PRINS reaction to use it as a primer to perform a spurious elongation at non-desired sites. However, in our tests, omission of the blocking step never affected the correct identification of two chromosomes because the signals from the second PRINS reaction site always showed the pure original color. Taking advantage of the color mixing and creating a new color, we successfully performed a multi-PRINS technique to obtain a third color for simultaneous detection of three chromosomes in the same cell. By arranging the incorporation order of the labeled nucleotides either in bio-dig-bio or in dig-bio-dig, two most common labeled nucleotides, biotin- and digoxigenin-dUTP, were alternatively incorporated in the newly synthesized DNA strand during three sequential PRINS reactions. Two detection systems were coupled with the incorporation order: 1) avidin-fluorescein mixed with anti-dig-rhodamine was used for bio-dig-bio incorporation order and 2) anti-dig-fluorescein mixed with avidin-rhodamine was used for dig-bio-dig incorporation order. Three different chromosomes can be identified as yellow, red and green color signals, respectively. We measured the relative intensity of the two original red and green color elements. The results showed that a red signal can contain up to 38% of green color and still appears red, whereas a fluorescent signal must contain at least 43% of green color to generate the yellow color. A green signal can contain up to 12% of red color and still retain its green color. The entire procedure could be completed in less than 90 minutes because the blocking step is omitted. We believe that this multi-PRINS technique is a practical and efficient way to simultaneously detect three or more chromosomes.