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International Workshop on Chromosome 3

Final Report

for Period April 15, 1991 - April 14, 1992

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Prepared for

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NOTICE

Second International Workshop on Chromosome 3

The Second International Workshop on Human Chromosome 3, sponsored by the DOE Human Genome Program, the NIH National Center for Human Genome Research, and the Eleanor Roosevelt Institute, was held April 4–5 in Denver, Colorado. With 43 participants representing 8 nations, the workshop focused on whole-chromosome resources including probes, polymorphic markers, hybrids, yeast artificial chromosomes (YACs), and genes, and on efforts in genetic linkage and region-specific physical mapping. Two major workshop goals were to identify sets of common reference DNA markers and somatic cell hybrids.

Whole-Chromosome Resources

Cloned DNA markers were described by David Smith (Wayne State University), Harry Drabkin (University of Colorado Health Science Center, Denver), Eugene Zabarovsky (Karolinska Institute, Stockholm, and Engelhart Institute of Molecular Biology, Moscow), Lakshmi Atchison (Fox Chase Cancer Center), and Kazuhiro Yamakawa (Cancer Institute, Tokyo). The total number of clones isolated in their respective laboratories exceeds 12,000, of which about 1400 have been regionally localized.

Localization of DNA markers by fluorescence in situ hybridization analysis was reported by Yamakawa, William Modi (PRI/Dyncorp), and Pamela Rabbitts (Medical Research Council, Cambridge, U.K.).

Somatic cell hybrids useful for localizing DNA markers or isolating specific chromosomal regions were reported by Drabkin, Atchison, Susan Naylor (University of Texas Health Science Center, San Antonio), and Tom Glover (University of Michigan, Ann Arbor).

Isolation of YAC clones specific for chromosome 3 was reported by Mike Mendez (Eleanor Roosevelt Institute). Phyllis McAlpine (University of Manitoba, Winnipeg) described a chromosome 3–specific YAC library.

Manfred Zorn (Lawrence Berkeley Laboratory) demonstrated the Chromosome Information System, which is computer software for entering and storing mapping data.

Genetic Linkage Maps

Polymorphic loci including C-A repeat clones and primer sets for polymerase chain reaction–based allele assays were reported by several groups, including those of Drabkin, Rabbitts, and Naylor.

Several genetic linkage maps with various degrees of coverage were described. The linkage map reported by Yamakawa contained 41 continuously linked markers and extended for a sex-averaged distance of 314 cM. Margaret Pericak-Vance (Duke University Medical Center) and Jonathan Haines (Massachusetts General Hospital) described a 43-loci map that extended 156 cM in males and 203 cM in females. Kalman Tory [NCI-Frederick Cancer Research Facility (FCRF)] reported on a map that included 20 3p loci covering a sex-averaged distance of 130 cM. Vince Stanton (Massachusetts Institute of Technology) described the use of denaturing gradient gel electrophoresis

A more detailed report of resources and mapping information presented at the workshop will be published in *Cytogenetics and Cell Genetics*.

Chromosome 5 Update

Colon Cancer Gene Discovered

New Diagnostic Test Possible

Investigators have found the gene responsible for familial adenomatous polyposis (FAP), an inherited form of colon cancer. The gene, called APC for adenomatous polyposis coli, is believed to act as a tumor suppressor. However, individuals who inherit a damaged version of the gene may develop hundreds of thousands of tiny colon polyps that very often become malignant before the person reaches the age of 30. About 1 in 5000 people suffer from FAP.

"This is a gene discovery that can have direct impact for the patient. Early diagnosis and removal of polyps can prevent colon cancer," said investigator Raymond White, whose group reported its findings in the August 9 issue of *Cell*. Similar results were reported in the August 9 issue of *Science* by Bert Vogelstein of Johns Hopkins University and Yusake Nakamura of the Cancer Institute in Tokyo. Nakamura contributed to earlier polyposis research in White's laboratory at the University of Utah. The work was funded in part by the NIH National Center For Human Genome Research.

Discovery of the polyposis gene not only allows early detection of colon tumors but has enabled the development of a simple blood test to identify family members who have inherited the mutant gene. Affected individuals can be watched closely, and intervention may become possible at an earlier stage of the disease.

Colorectal cancer is second only to lung cancer as a cause of death by malignancy. Over 150,000 people will contract the disease this year, and over 60,000 will die of it. At least 20% of these cases, perhaps many more, are attributed to genetic causes. ◇

First International Chromosome 4 Conference

A 2-day conference incorporating three meetings on the genetic and physical maps of human chromosome 4 was held June 22–23 in Philadelphia. Some 25 participants from 5 countries attended the conference, which was sponsored by the Centre d'Etude du Polymorphisme Humain (CEPH), NIH, and the Fox Chase Cancer Center.

The CEPH consortium group developing the genetic linkage map of chromosome 4 discussed marker inclusion, map development, significance criteria, and checks for genotyping errors. From CEPH data, Ken Buetow (Fox Chase Cancer Center) generated a preliminary 33-point multilocus map that encompassed all of chromosome 4 at an average resolution of about 8 cM.

Preliminary 33-point map generated with average resolution of 8 cM.

The facioscapulohumeral muscular dystrophy consortium discussed recent data pooling for linkage analysis of this disorder, which has been mapped to 4q35-4qter.

In the plenary meeting, Peter Pearson (Johns Hopkins University) gave an overview of consensus vs component maps; he made the Genome Data Base (GDB) available to investigators during the conference. A number of individual presentations described chromosome 4 genetic and physical maps and specific loci or genes:

- mouse homologies and human DNA variants associated with the *c-kit* oncogene and pigmentary abnormalities [Maja Bucan (University of Pennsylvania) and Richard Spritz (University of Wisconsin)];
- evolution of the glycophorin gene family and an extensive physical map based on cosmid walking [Shinichi Kudo (La Jolla Cancer Research Foundation)]; and
- identification of cDNAs in the 4p16.3 region [Olaf Riess (University of British Columbia), Leon Carlock (Wayne State University), Gert-Jan Van Ommen (Leiden University, Netherlands), and Richard Myers (University of California, San Francisco {USCF})].

Discussions included human chromosome 4 mapping resources now being developed and made available both by individual investigators and through the human genome center headed by Myers at USCF. These resources will help to generate high-resolution maps of regions near identified chromosome 4 genes and to develop chromosome 4 maps (radiation, genetic, and clone-based) not based solely on genes and genetic disorders.

Participants discussed organization of future workshops around GDB. The second chromosome 4 conference will be held June 13–14, 1992, in Leiden, Netherlands. ◇

Reported by Jeffrey C. Murray
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For complete proceedings of the conference, contact

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Biotechnology Resource Lists Researcher Information

Biotechnology Research Faculty Profile, a new database of up-to-date information on more than 4300 U.S. biotechnology researchers, was compiled from a survey conducted this year by the North Carolina Biotechnology Center, a nonprofit corporation funded by the N.C. General Assembly. [For survey announcement, see *HGN* 2(6), 6 (March 1991)].

The database, which can be purchased on computer disks, contains respondents' contact information, institutional affiliation, and area of scientific interest; it also includes their laboratory's research technique expertise, organisms most frequently used, funding sources, and biotechnology research being conducted. The data are available in printed form as the *Biotechnology Research Directory: 4000 Faculty Profiles.*

The database and directory are expected to have a wide variety of uses, especially in technology transfer or in interactions between industry and researchers. Sales proceeds will be used to support information collection on an annual basis; future editions will include researchers at federal laboratories and private research institutions.

- Disks, $1200; printed directory, $125. Database and directory information: Biotechnology Information Division; North Carolina Biotechnology Center; P.O. Box 13547; Research Triangle Park, NC 17709; 919/541-9366. ◇

Abstract

The need for a chromosome 3 workshop became acute because of the large expansion of markers on chromosome 3. At HGM9 there were 47 genes and 29 DNA markers on chromosome 3, whereas at HGM10 there were 59 genes and 114 DNA markers. However, at least 1500 unreported markers are in various stages of publication. Therefore, the objective of the Second Interantional Workshop on Chromosome 3 was to develop physical, genetic linkage, and radiation maps for chromosome 3 by bringing together the laboratories working on this chromosome. Since most of the work on chromosome 3 appeared to be directed towards the short arm of the chromosome, it was also hoped that interest could be generated in developing markers for the long arm.

Final Report

The Second Workshop on Human Chromosome 3 was held on April 4-5, 1991 at Denver, Colorado. There were 43 participants representing 8 nations. The workshop participants reviewed the current state of the chromosome 3 map, both physical and genetic, and prepared lists of markers and cell lines to be made commonly available. These markers and cell lines should be incorporated into the mapping efforts of diverse groups to permit the integration of data and development of consensus maps at future workshops. Region specific efforts were described for sections of the chromosome harboring genes thought to be involved in certain diseases including Von Hippel-Lindau disease, 3p- syndrome, lung cancer and renal cancer. A full report of the workshop was published in Cytogenetics and Cell Genetics (57:p161-166, 1991) and a copy is enclosed. The Third workshop was proposed for Boston, MA to be held in March 1992. However, after this decision was made, much further discussion took place by letter. This led ultimately to a second vote by the participants to decide a location. Tokyo was chosen with Yusuke Nakamura as host. The difficulties experienced in deciding the next workshop location should hopefully not recur given the new guidelines developed by HUGO for the single chromosome workshops. Enclosed with this report is a copy of the published workshop report, an agenda, a list of participants and the collection of abstracts submitted for the workshop.

AGENDA

INTERNATIONAL CHROMOSOME 3 WORKSHOP

April 4-5, 1991
Brown Palace Hotel
Denver, Colorado

Thursday - April 4, 1991

DNA PROBES, GENES, YACS, HYBRIDS, PHYSICAL MAPPING

2:00 - 2:10p	Introduction	
2:10 - 2:30p	David Smith	
2:30 - 2:50p	Harry Drabkin	
2:50 - 3:10p	Eugene Zabarovsky	
3:10 - 3:30p	Lakshmi Atchison	
3:30 - 3:45p	BREAK	
3:45 - 4:05p	Kazuhiro Yamakawa	
4:05 - 4:25p	Mike Mendez	
4:25 - 4:45p	Pamela Rabbitts	
4:45 - 5:05p	Phyllis McAlpine	

INFORMATICS

5:05 - 5:30p	Manfred Zorn	
5:30 - 6:00p	Discussion	Harry Drabkin/Carol Jones Moderators

Somatic Cell hybrids for distribution, identification and mechanism of distribution, etc.

7:00p Bus to Mr. Cooper's house for dinner (Casual attire)

Friday - April 5, 1991

GENETIC LINKAGE MAPS - GENERAL

8:30 - 8:50a	Susan Naylor
8:50 - 9:10a	Kalman Tory
9:10 - 9:30a	Margaret Pericak-Vance/Jonathan Haines
9:30 - 9:50a	Kazuhiro Yamakawa
9:50 - 10:10a	Vince Stanton
10:10 - 10:30a	BREAK

REGION SPECIFIC EFFORTS/DISEASE LOCI/FAMILY RESOURCES

3p24-pter

10:30 - 10:50a	Eamonn Maher
10:50 - 11:10a	Berton Zbar/William Modi
11:10 - 11:30a	Bernd Seizinger
11:35 - 1:00p	LUNCH

3p13-p23

1:00 - 1:20p	Kazuhiro Yamakawa
1:20 - 1:40p	Charles Buys
1:40 - 2:00p	Bob Gemmill/Harry Drabkin
2:00 - 2:20p	Gyula Kovacs
2:20 - 2:30p	BREAK
2:30 - 2:45p	David Smith
2:45 - 3:45p	General Discussion on Reference Markers, YAC Screening (Slides/Overheads encouraged)
3:45 -	Draft Summary Statement for Workshop Report Additional talks not listed above

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APRIL 4-5, 1991

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CHARACTERIZATION OF HUMAN CHROMOSOMAL BAND 3P21 AND THE SEARCH FOR GENES

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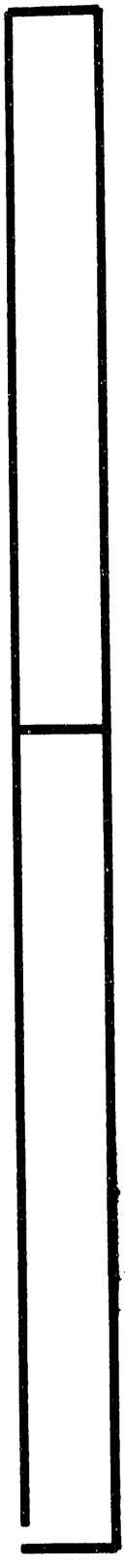
Specific rearrangements involving chromosome 3p have been described for several malignant disorders, most notably for small-cell lung cancer and renal carcinoma. Deletion of DNA sequences within chromosomal region 3p21 has been found in all major types of lung cancer and many renal cell carciomas examined. This evidence suggests that at least one tumor suppressor gene must reside within 3p21. Our total resource of chromosome 3-specific recombinants now numbers greater than 7,000. Included within this are 5,700 cosmids, 1,000 lambda clones, 243 Not1 boundary clones, and 131 small-insert flow sorted clones. We localized 1,048 (15%) of the clones and now have approximately 128 clones representing 3.4 Mb of sequences from within 3p21. We are currently performing cosmid based walking from multiple start-points within this region to increase our representation of 3p21 sequences. In conjunction with physical mapping strategies, we have also concentrated efforts on identifying large numbers of genes from this region which could subsequently be tested as tumor suppressor gene candidates. Several different strategies have been employed to identify gene sequences present in the recombinants localized to this region; screening recombinants for HTF islands, testing fragments derived from the recombinants for evolutionary sequence conservation, and several additional strategies are currently being tested. Fragments derived from many of the 3p21 gene candidate recombinants were then hybridized to Northern's and those which hybridized to transcripts were used to screen cDNA libraries. We have already isolated and partialy sequenced four new 3p21 genes. One of the cosmids was found to contain all of the gene corresponding to DNF15S2 (D3S48E) and this gene was 92% homologous to rat acyl peptide hydrolase. Detailed characterization of a sub-region of 3p21 (3p21.1) has shown that this region is extremely gene rich. For example, we have isolated a total of 250 kB from an 800 kB region between ACY-1 and cA199 and there are at least 5 genes present within the cloned DNA. We have narrowed down the 3;6 translocation breakpoint to a 500 bp region within one of our gene candidate cosmids and are currently sequencing the 500 bp as well as searching for all genes within 200 kB of this breakpoint. All the genes isolated from this region are also being tested at both the DNA and RNA levels to determine if any alterations of these genes can be detected in small cell lung and renal carcinomas as well as a variety of other tumors.



p26 lambda 64 — MspI RFLP; in situ localized to 3p26-pter

p25 cA479 — BglI RFLP, (CA)n repeats; genetic linkage to VHL

p24

p23

p22

p21 cA476 (DNF15S2) — multiple CG islands; acyl peptide hydrolase?; multiple RFLP's; additional genes in vicinity

cA199 cA84 — multiple CG islands; precise PFG data on positions; cDNA's already isolated and sequenced

p14

p13

p12

q13 stefin A (STF1)

q21

q22

q23

q24

q25

q26

q27

cA34 — multiple CG islands

q28 kinenogen (KNG)

q29

Harry Drabkin

Eugene Zabarovsky

A new strategy for mapping of the human genome based on novel vectors and procedures for constructing linking and jumping libraries.

Eugene R. Zabarovsky[1,2], Ferenc Boldog[1,4], Rikard Erlandsson[1], Vladimir I. Kashuba[1], Rando L. Allikmets[1], Gösta Winberg[1], Olga V. Turina[2], Malik K. Nurbekov[2], Zoltan Marcsec[1], Arina Yu. Grigorieva[2], Eric J. Stanbridge[3], Lev L. Kisselev[2], Janos Sumegi[1,4] and George Klein[1].

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New vectors for construction of representative gene libraries have been designed. The vectors can be subdivided either according to their properties or considering the purpose for which they were made (creation of genomic, jumping, linking or cDNA libraries). The following new vectors were constructed and characterised: regular lambda phages (SK3, SK6, SK11 etc.), phasmids (SK2, SK2A), hyphages-hybrid phages-M13 with the cos-region of phage lambda (MC18, MC19), diphasmids (i.e. vectors wich can exist as a plasmid and be packaged in lambda and M13 phage particles). The diphasmids are either incapable of lytic growth (SK18) or competetent to replicate as a phage (SK9, SK15, SK21, SK23, SK28 etc.). These diphasmid vectors have the advantages of contemporary vectors: genetic selection against non-recombinant phages and possibility to reduce the generation of wild phages without isolation of the phage arms but with the help of concomitant cutting phage DNA with two enzymes (biochemical selection). They have rich polylinkers with many restriction sites, allowing specific labelling of 5' or 3' ends of inserts. This is suitable for chromosome walking, physical mapping of inserts and efficient transmission of the inserts into the plasmid and SS-form. In addition they have unique features that open new opportunities in gene cloning. Distinctive features of the vectors for cDNA cloning is that false recombinants, containing only linker sequences are incapable of generating viable phages. In the same vector one may construct either expressed or non-expressed libraries, this depends on the E. coli strains, so expression

is controlled either by ochre or amber codon. SK28 allows construction of oriented cDNA libraries using SfiI-NotI and expression in all three reading frames.

A novel procedure for constructing linking and jumping libraries have been developed. The essential features of this procedure are as follows: 1) two diphasmid vectors (lambda SK17 and SK22) are simultaneosly used in the library construction to improve representativity, 2) a partial filling-in reaction is used to prevent cloning of the artificial jumping clones and to obviate the need for selectable marker. Using this approach chromosome 3 specific linking libraries have been constructed with NotI (170.000 recombinant clones), SalI ($1.5x10^6$ recombinant clones) and XhoI ($1.8x10^6$ recombinant clones) restriction enzymes, using DNA from a human chromosome 3/mouse microcell hybrid cell line, MCH 903.1. The procedure has been used to construct a representative human NotI-jumping library from the lymphoblastoid cell line CBMI-Ral-STO which features a low level of methylation (220.000 independent recombinant clones) and human chromosome 3 specific NotI-jumping library from MCH 903.1 (500.000 independent recombinant clones). Of these recombinant clones 50-80% represent jumps to the neighboring NotI site. This new procedure makes a new genome mapping strategy feasible. This strategy includes the determination of tagging sequences adjacent to NotI sites in random linking and jumping clones. The special feature of the lambda SK17 and SK22 facilitate such sequencing. The STS information obtained will be assembled with assistance of a computer into a map of the linear order of the NotI sites for a chromosome or for the entire genome. The strategy facilitates the isolation of chromosome translocation breakpoints and borders of deletions. It obviates need for cloning linking or jumping clones, because information about a NotI site of interest can be obtained directly from computerised data and necessary clones can be obtained using PCR or from the panel of original clones. A new approach was developed, which allows the construction of libraries using any restriction enzyme with 5' protruding ends. The method was applied to the construction of a chromosome 3 specific MluI linking library. All these libraries can be transferred into SS- and plasmid form, which was verified with some of the libraries. Biotin-avidin selection using such linking libraries in plasmid form have been done to get NotI linking clones from a region deleted in renal cell carcinoma. Large regions of chromosomes can best be mapped by means of jumping and linking libraries constructed using these new strategies in the vectors SK4, SK10, SK16, SK17, SK20, SK22, and SK25.

Lakshmi Atchison

Title: Further analysis of Radiation Hybrids (RH), rare-site sublibraries and single-copy DNA clones.

A) Identification of new candidate single-copy clones from chromosome 3 library

B) Characterization of 72 radiation hybrids in CHO background.

(i) PCR primers from single copy sequences for RH analysis
(ii) Sub-regional identification of RH lines using <u>in situ</u> mapped clones.

C) Characterization of Not I and Sac II clones from chromosome 3 linking libraries.

<u>Please Note</u>:
The single copy candidate clones are just recently isolated and they are not characterized for their informativeness at this time.

Similarly, the Radiation Hybrids are isolated and are being characterized. The presence of a defined and specific subregion in these cellular clones is currently being done by PCR-primers and <u>In situ</u> mapped clones.

Kazuhiro Yamakawa

Construction of a physical map and a linkage map of human chromosome 3, and application to the analysis of renal cell carcinoma

Kazuhiro Yamakawa[1], Ei-ichi Takahashi[2], and Yusuke Nakamura[1] ([1]Cancer Institute, Tokyo, Japan; [2]Dept. of Genetics, National Inst. of Radiological Sciences)

Abstract

6000 cosmid clones containing human inserts were isolated from a cosmid library constructed from human-mouse somatic hybrid cell which contained human chromosome 3 as the only human material. By screening 600 clones of them, we have isolated 265 new RFLP markers.

Using these markers, we have constructed a physical map of 194 markers including 123 RFLP markers by fluorescence in-situ hybridization (FISH). These markers were scattered throughout the chromosome, but they were concentrated in the R-positive bands. We have also constructed a genetic linkage map with 41 continuously linked markers using 40 CEPH families. This map covers 314 cM on sex-averaged recombination rates; 261 cM in males and 413 cM in female. This linkage map demonstrated a high consistency with physical data obtained by FISH and covered the whole chromosome.

In addition, we constructed a detailed deletion map of the short arm of chromosome 3 in 38 renal cell carcinomas using 30 RFLP markers. These maps revealed that two commonly deleted regions existed at 3p12-14 and at 3p21.3. Furthermore, we have characterized the breakpoint of t(3;8) chromosomes which were contained in patients with hereditary renal cell carcinoma by FISH.

Mike Mendez

RAPID SCREENING OF A YAC LIBRARY BY PULSED FIELD GEL SOUTHERN BLOT ANALYSIS OF POOLED YAC CLONES

M. J. Mendez[1], S. Klapholz[2], B. H. Brownstein[3] and R. M. Gemmill[1,4]
[1]Eleanor Roosevelt Institute, Denver, CO; [2]Cell Genesys, Inc., Foster City, CA; [3]Center for Genetics in Medicine, Washington Univ. School of Medicine, St. Louis, MO.

We describe an alternative method for YAC library screening that is both rapid and low-cost. Individual YACs were pooled into groups of 384 clones and prepared as samples suitable for pulsed field gel electrophoresis. A five-hit human YAC library containing approximately 60,000 clones was condensed into 150 such pools. Chromosomal DNAs in each sample were separated on three pulsed field gels containing 50 samples each. Resulting Southern blots were hybridized with probes of interest to identify pools containing homologous YACs. Further purification was performed using standard colony hybridization procedures. Twenty four probes used thus far have identified 60 positive pools and corresponding YACs have been purified from 36 of these. Isolated YACs ranged in size from 50 to over 1300 kb in size. Useful probes derived from plasmid and phage clones or PCR amplified sequences ranged from under 500 bp to over 5 kb. Some significant advantages of this method include avoidance of DNA sequence analysis and primer generation prior to YAC screening and the ability to handle the entire library on three filters. PFG separation provides an array of additional information such as size estimation and allows targeted isolation. The screening approach described here permits rapid isolation of YACs corresponding to un-sequenced loci and will accelerate establishment of YAC contigs for large chromosomal segments. Application of this method to YAC isolation for chromosome 3 will be presented.

Pamela Rabbitts

ABSTRACT (P Rabbitts)

1) I would like to present some physical mapping of readily available probes localised by FISH.

2) I would also like to present the use of double colour in situ hybridization which provides both physical location and marker order. The probes used in this study are not cosmids. All are small and one is less than 1 Kb. There is no specific chromosome segment that I want to present information on.

Oligonucleotides for Genotyping 11 RFLP's on Chromosome 3p by PCR

P Ganly and P Rabbitts.
MRC Clinical Oncology Unit, MRC Centre, Hills Road, Cambridge, CB32 2QH UK

Locus	RFLP Enzyme	Oligonucleotide Primers	Alleles (bp) 1	2
D3S3	Msp1	5'-CAGAAGGACATATTCCCATTTG-3' 5'-GCAGTTTCCTCTAGCTTTTACT-3'	828;	442+386
D3S30	Msp1	5'-AAAGACTTCCTTCTGAGATGGG-3' 5'-CAGTCTGCTGTGGTTTCAGTCT-3'	293;	224+69
D3S6	Msp1	5'-CCTGTCCACATTGAACTCCT-3' 5'-GAACCACTGCTTCTGAAATG-3'	510;	255+255
D3S2	Msp1	5'-CCAAGTGGCAGAGCTACTTA-3' 5'-ACTATCTTGCCCTAGAGCAA-3'	473;	237+236
D3S32	Rsa1	5'-TTGGCGGTGGGAGCCTGAAGTGT-3' 5'-GGCTGGAAGTGGAGGAGTGCATG-3'	421;	211+210
D3F15S2	Hind111	5'-TAGATCTGAGCCCGGTGCCTGGGCCACGAA-3' 5'-GAGTCCATGGCAGCAGTTGGCAACCATGGC-3'	535;	383+182
THRB	Hind111	5'-ACGTTAGTGGCTCATATGAG-3' 5'-TCATTCGAGTTAGTGCAAAG-3'	432;	221+211
THRB	Msp1	5'-AACGTTGGACCTCAAGCCCAT-3' 5'-CAGGGTTCCTTCTATAAACATG-3'	683; 657;	560+123 (Msp1) 405+252 (Dra1)
THRB	EcoR1	5'-GCTAATCTAGAAATGTATTTACTATAGG-3' 5'-TTTACTTCATGAAGCTGGCACG-3'	367;	298+69
THRB	BamH1	5'-AACATCCAAGATGGCTGGAGTT-3' 5'-CCCTGAAAATGCAGAGGACTCT-3'	364;	182+182

PCR amplification was performed in a volume of 15 µl containing 0.3 µg human DNA, 0.2 µM each primer, 200 µM each dNTP, 1.5mM $MgCl_2$, 50mM KCl, 10mM Tris pH 8.3, 0.01% gelatin, 1% Triton X100 and 0.4 units Taq polymerase (Promega). 30 cycles of PCR were carried out, each cycle consisting of 95C for 0.5 minute, 55C for 1 minute (except for D3F15S2 -65C for 1 minute and D3S6 -58C for 1 minute) and 72C for 1 minute. The entire PCR product was electrophoresed on a 4% NuSieve 3:1 gel after overnight digestion with the appropriate enzyme using the manufacturers reccommended conditions.

Phyllis McAlpine

[1]Casey, G., [2]Allderdice, P.W., [1]McAlpine, P.J.

[1]Department of Human Genetics, University of Manitoba, Winnipeg, Canada, [2]Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

We have been analysing genetic markers in a very extensive kindred segregating an inv(3)(p25q21) with the view to contributing to the development of the genetic map of chromosome 3. Using a combination of linkage analysis, in situ hybridization and the analysis of somatic cell hybrids, TF and PCCB have been both found to lie outside the inversion with the order being 3q21:PCCB:TF. These new data reduce the regional localisation of PCCB to 3q21-q22. In collaboration with B. LaDu and O. Lockridge, we have found a new RFLP for BCHE, mapped it 3q26.2 and estimated that it maps approximately 11 map units distal to TF. D3S4 maps close to the centromere of inversion and normal chromosome 3's. Segregation from one nuclear family places D3S4 between the centromere and D3F15S2.

Butyrylcholinesterase

Supported by MRC Canada (MT6112:PJM, MT6725:PWA) and Childrens Hospital of Winnipeg Research Foundation Inc.

[1]Woods, R.A., [1]Coopland, G.R., [2]Allderdice, P.W. and [1]McAlpine, P.J.
[1]Department of Human Genetics, University of Manitoba, Winnipeg, Canada, [2]Faculty of Medicine, Memorial University of Newfoundland.

DNA from a somatic cell hybrid containing chromosome 3 as its only human chromosome (Firnhaber et al. Amer. J. Hum. Genet. 37:A28, 1985) was partially digested with EcoRI, size fractionated by CHEF electrophoresis, ligated to pYAC4 arms and transformed into yeast strain AB1380. 47 of the 3500 transformants isolated on selective medium and colony hybridized to total human DNA and total hamster (CHO) DNA had inserts of human origin. These inserts of human DNA ranged in size from 75 - 450Kb, with the average size being approximately 125Kb. DNA from one YAC, (pYACG68) appears to represent a segment of DNA from the 3p24.2-q21 region on the basis of its hybridization pattern on dot blots of DNA of a series of somatic cell hybrids, each containing a structurally rearranged chromosome 3. Regional localisation of additional YAC's and screening their inserts for hybridization to known cloned gene sequences is continuing.

Supported by MRC Canada (MT6112:PJM, MT6725:PWA) and Childrens Hospital of Winnipeg Research Foundation Inc.

Manfred Zorn

Harry Drabkin/Carol Jones

Susan Naylor

PCR based markers for chromosome 3.

S.L. Naylor, D. Garcia, M.E. Wolf, L. Schantz, W. Huang, R-H. Xiang, N. Ghosh-Choudhury, R. Leach, and S. Sherman

To make a definitive order of the genes on chromosome 3, we have developed PCR primers for 40 genes on chromosome 3. In addition, primers for CA repeat polymorphisms have been developed by Jim Weber and our laboratory. All these primers have been used to assay radiation hybrids. Radiation hybrids made with 2500, 4500, and 6000 rads and microcell hybrids with spontaneous deletions were used to generate a gene order for chromosome 3. The data were analyzed by David Cox's programs on the statistical analysis of radiation hybrids. With this number of markers, it is not possible to obtain distances nor is it possible to produce a definitive gene order in the regions of p21 and q21. However, the order for the remainder of chromosome 3 is consistent with known data and all types of hybrids.

We have screened a pBS library constructed from a flow sorted chromosome 3 (kindly given by Joe Gray) for CA repeat polymorphisms. The insert size of these clones are such that they could be sequenced directly with double strand sequencing. Three highly polymorphic CA repeats were found: 3GTA8, 3GTB9, and 3GTE2. They range in heterozygosity from 0.7 to 0.85 and have 7 to 9 alleles. 3GTA8 and 3GTB9 are located near ACAA and D3S11 on the radiation hybrid map while 3GTE2 is near D3S30. Linkage studies with the CEPH families also place the markers at the same location.

Kalman Tory

A genetic map of chromosome 3p

Kalman Tory, Farida Latif, Berton Zbar and Michael I. Lerman, Laboratory of Immunobiology, National Cancer Institute-Frederick Cancer Research Facility

Two thousand single copy probes were isolated from two chromosome 3 specific (small insert) bacteriophage libraries. The probes were localized to the p and q arms of chromosome 3 with a simple mapping panel and 3p probes were regionally localized with a somatic cell hybrid panel. About 800 3p probes were tested for ability to detect restriction fragment length polymorphisms. Polymorphic probes were screened on heads of CEPH families and probes with the highest degree of information were mapped on informative CEPH families. Results were entered into a database provided by CEPH and converted with LNKTOMAP for analysis with the CRIMAP program. Errors were detected using a male specific probe, VNTR probes, by use of CHROMPIC and by comparison of autoradiographs with the genotypes in the CEPH database.

Initial maps were constructed with the minimum requirement of 1000:1 for accepting an order as correct. A second map was constructed with a minimum requirement for correct order being 100:1 odds.

Twenty 3p probes have been ordered at 1000:1 odds. The genetic map of 3p covers a distance (sex averaged) of 130 cM. The average frequency of heterozygosity was 49%. Framework markers were CRI-L892, LIB 46-9, RAF1, LIB 12-69, THRB, EFD145, LIB 4a-7d and LIB 37-42".

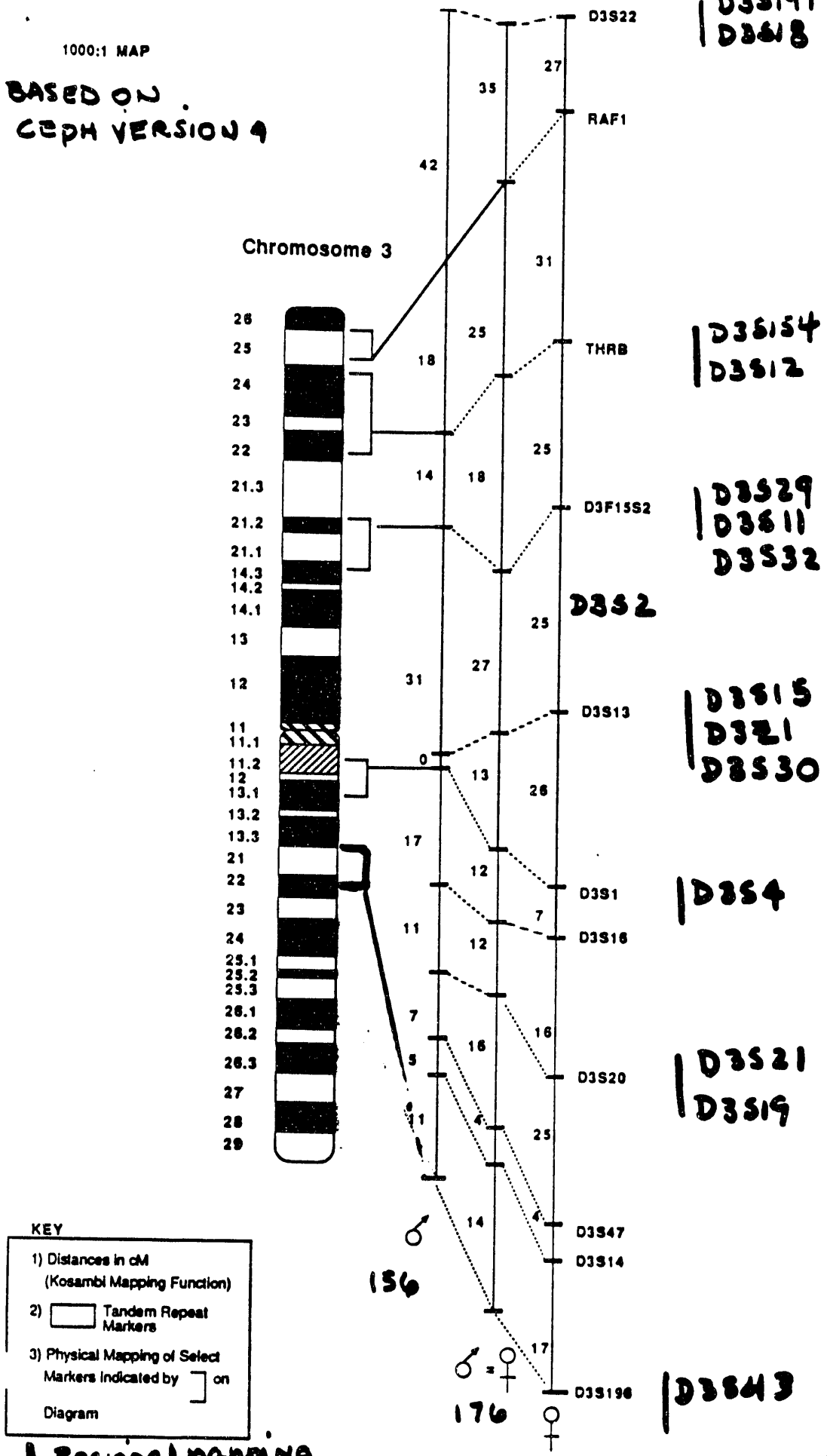
Margaret Pericak-Vance/Jonathan Haines

Updated genetic linkage maps of chromosome 3 in the CEPH and Venezuelan Reference Pedigrees. Margaret A. Pericak-Vance and Jonathan Haines.

We will present two genetic linkage maps constructed from the CEPH version 4.0 database, and on the Venezuelan reference pedigree. This CEPH version 4 database contains 51 chromosome 3 markers. Fifteen of these markers represent multiple enzyme cuts of the same locus; thus a total of 43 independent loci were available for analysis. Framework and comprehensive maps will be presented. The Venezuelan database contains ten chromosome 3 markers. Several markers overlap with the CEPH markers in the region of the Von Hippel-Lindau disease locus on 3p. These include the loci THRB, cRAF, D3F1582 (H3H2) and D3S30 (pYNZ86.1). We will attempt to integrate the maps from the two resources as well as extend the map to include distal 3p probes typed exclusively in the Venezuelan reference pedigree. The integration of the two resources should allow for an extended genetic maps of chromosome 3, indicate areas needing additional genetic mapping studies, and provide information on which markers might best be designated as index markers.

1000:1 MAP

BASED ON CEPH VERSION 4



D3S191
D3S18

D3S154
D3S12

D3S29
D3S11
D3S32

D3S2

D3S15
D3S21
D3S30

D3S4

D3S21
D3S19

D3S43

Regional mapping 1000:1

Bericak-Vance/
Haines

Kazuhiro Yamakawa

Vince Stanton

Eamonn Maher

1. The loci I would like designated as anchor loci are D3S18, RAF1, THRB, D3F15S2, D3S3 and D3S2.
2. I would like too present my data to the disease linkage forum.

GENETIC LINKAGE ANALYSIS OF VON HIPPEL-LINDAU DISEASE

Von Hippel-Lindau disease is a dominantly inherited cancer syndrome with variable expression. Major complications include retinal, cerebellar and spinal haemangioblastomas, renal cell carcinoma and phaeochromocytoma. We have performed a genetic linkage study of 36 VHL families using polymorphic DNA markers from chromosome 3p24-p26. Age-at-onset corrections were made with data from segregation analysis (Maher et al. J Med Genet in press). Significant linkage was detected with RAF1 (Zmax=6.36 at theta=0.06, CI 0.01-0.15) and D3S18 (Zmax=11.70 at theta=0.00, CI 0.0-0.04). Multipoint linkage analysis gave the following order: THRB--RAF1--(VHL,D3S18)--D3S191. There was no evidence of locus heterogeneity.

Berton Zbar

A genetic map in the region of the von Hippel-Lindau disease (VHL) locus
Berton Zbar, Farida Latif, Kalman Tory, William Modi and Michael I. Lerman
Laboratory of Immunobiology, National Cancer Institute-Frederick Cancer Research Facility

Flanking markers have been identified for the VHL disease locus. Our laboratory has reported that VHL is located in a 6-8 cM interval between RAF1 and D3S18. D3S18 was localized to 3p26 by in situ hybridization. Seizinger and coworkers have reported that VHL is located in the interval between RAF1 and 64E2.

We have expanded the NCI VHL family panel from 25 to 41 families (this total includes 2 families studied by other workers). Evidence for disease (clinical) heterogeneity was detected; no evidence for genetic heterogeneity was found.

Probes identified to flank the disease gene were used in a prospective trial to compare the result of RFLP analysis with a comprehensive clinical examination. 48 asymptomatic individuals at risk of developing von Hippel-Lindau disease were tested with probes located close to, and on either side of, the VHL gene. We were able to predict risk in 42/48 (88%) of tested individuals. We found agreement between the results of the DNA test and the clinical screening examination in 41/42 informative individuals.

We have constructed a more detailed genetic map in the region of VHL with the 56 families provided by CEPH.

William Modi

Physical mapping of 3p and deletion analysis in the 3p- syndrome. WS Modi* (1), F Latif (2), MI Lerman (2), PM Morey (3), MJ Chorney (3), B Ebar (2), PH Rabbitts (4), N Maher (5), RL Ladda (3). (1) PRI/Dyncorp, Frederick, MD, USA (2) National Cancer Institute, Frederick, MD, USA (3) Hershey Medical Center, Hershey, PA, USA (4) MRC, Cambridge, UK (5) Cambridge University, UK.

Fluorescent in situ hybridisation has been utilised to physically map 15 polymorphic genomic lambda or cosmid clones to the short arm of chromosome 3. These clones are also being used for genetic mapping on the CEPH and VHL pedigrees. Six of these clones recognise VNTR polymorphisms with heterozygosities ranging from 0.4 to 0.6 . The remaining nine markers identify RFLP polymorphisms with heterozygosities ranging from 0.3 to 0.5 . Several of these clones were also used in studying patients with the 3p- syndrome. Approximately 15 cases have been reported of patients with a deletion of 3p25 -> 3pter. We are interested in mapping the breakpoint by determining the presence or absence of certain genes and anonymous DNA segments using fluorescent in situ hybridisation and Southern blotting analyses. Available are EBV-transformed cell lines from three patients and their parents and about 15 genomic and cDNA clones that are known to be located in or near the deleted region. The in situ hybridisation indicated that five clones that had mapped to p26 were deleted, while one clone assigned to p25.3 was present. Southern blotting of two informative RFLP clones revealed that the maternal alleles were missing in both cases. Three of the genomic clones that were deleted hybridize to homologous sequences in rodents and recognize messages in brain mRNA.

Toward the cloning of the VHL gene on chromosome 3p25-p26

- **Bernd Seizinger/Update on VHL Linkage-Overview of Approach**

- **Sabine Klauck/Isolation of (GT)n repeats in the VHL region**

- **Jean Whaley/PFGE in individuals with VHL**

- **Jochen Decker/In-situ fluorescence hybridization with markers in the VHL region**

Kazuhiro Yamakawa

Charles Buys

Bob Gemmill

PROGRESS TOWARDS A COMPREHENSIVE PHYSICAL MAP COVERING THE p14 TO p21 REGION OF HUMAN CHROMOSOME 3

[1]R. Gemmill, [1]M. Mendez, [1]M. Garcia, [2]D. Smith, [2]W. Golembieski, [3]P. Rabbitts, [4]P. Erickson, [1,4]H. Drabkin. [1]Eleanor Roosevelt Institute, Denver, CO; [2]Wayne State Univ., Detroit, MI; [3]MRC Clinical Oncology and Radiotherapeutics Unit, Cambridge, England; [4]Univ. of Colorado Health Sciences Center, Denver, CO.

Deletions and rearrangements of the 3p14 to p23 region are consistently observed in a variety of human cancers including renal cell carcinoma (RCC) and small cell lung carcinoma (SCLC). We are constructing a comprehensive physical map, consisting of macro-restriction maps coupled with YAC contigs, that will cover a major portion of this region. Six translocation breakpoints retained in somatic cell hybrids and one internal deletion have defined 6 sub-regions within 3p14.1 to p21.1, estimated to contain 20 to 30 Mb. Sixty four cloned DNA probes have been mapped into these intervals to date; mapping for an additional 52 is in progress. Pulsed field gel analysis has generated macro-restriction map data accounting for 21 Mb of this region; six groups of linked probes cover 10 Mb. Five loci, including ACY1 and the polymorphic locus D3S2, are linked over a 2.5 Mb region surrounding the t(3;7)(p21.1;p13) breakpoint associated with Greig cephalopolysyndactyly syndrome. Six other markers, including D3S3, have been lost in the SCLC cell line U2020, which contains a sub-microscopic, homozygous deletion estimated to extend 4 Mb. PFG analysis has linked 5 of these probes together. A gene critical to development of SCLC probably resides within this deleted region. Nine markers that map within the U2020 deletion or flank the nearby familial RCC t(3;8)(p14.2;q24.1) breakpoint have been converted into 27 YACs using a screening method based upon pulsed field gel separated YAC pools. Walking from a YAC end has established a contig surrounding D3S3 that covers over 600 kb. Hybridization of YAC end-probes to PFG blots of human chromosomal DNA has permitted comparison of DNA structure, as represented in the YACs, to that observed in the genome. Such comparisons should provide a powerful method for checking the integrity of YAC contigs developed in conjunction with the Genome Initiative.

Harry Drabkin

Gyula Kovacs

David Smith

Ferenc Boldog

IN-SITU CYTOGENETIC AND MOLECULAR CHARACTERIZATION OF CHROMOSOME 3 SPECIFIC MONOCHROMOSOMAL AND SUBCHROMOSOMAL MICRO-CELL HYBRIDS.

Ferenc Boldog (1,2); David Scanlon (3); Teryl Thomson (3); Rando Allikmets (2); Rikard Erlandsson (2); Eugene R. Zabarovsky (2); Zoltan Marcsek (2); Javier S. Castresana (2); Catharina Larsson (4); Eric J. Stanbridge (3); George Klein (2) and Janos Sumegi (1)

1: Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68 198 USA.

2: Department of Tumor Biology Karolinska Institute, Box 60 400, S-104 01 Stockholm, Sweden.

3: Department of Microbiology and Molecular Genetics, California College of Medicine, Irvine, CA 92 717 USA.

4: Department of Clinical Genetics Karolinska Hospital, Stockholm, Sweden.

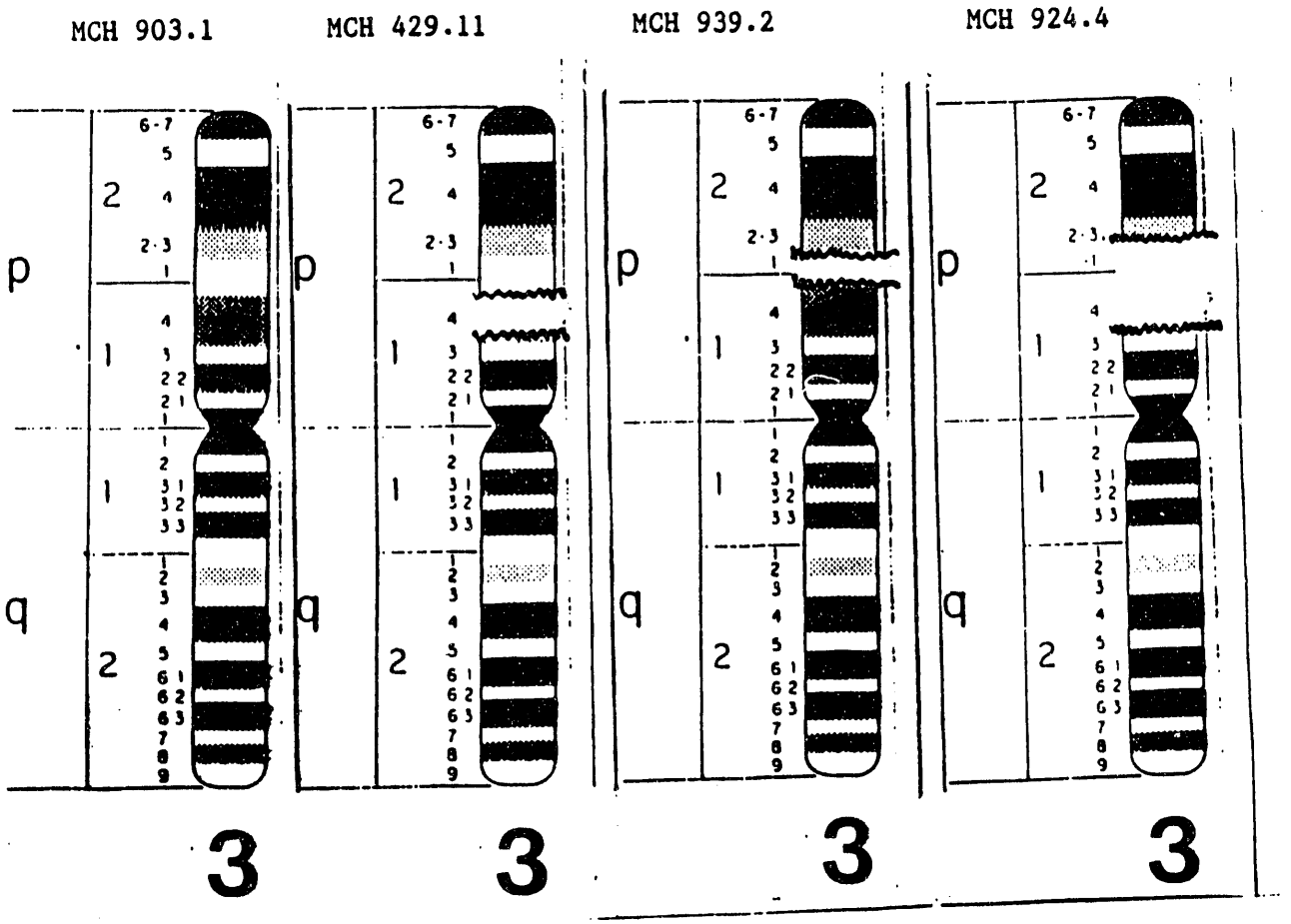
Mouse-human microcell hybrids containing the intact human chromosome 3 (MCH903.1) or chromosome 3 with deletions (MCH429.11, MCH939.2, MCH924.4) were constructed. To map the deletions the following chromosome 3 specific probes, with known map location, were used as hybridization probes; c-raf (3p25), RarB (3p24), D3F15S2 (3p21.1-2), D3S2 (3p21), D3S3(3p14),NRSL26 (3p12-14) D3S4 (pter-q21) and PCCB (3q13-22) were used. DNA from MCH429.11 cells failed to hybridize with D3S3. DNA sequences around and between D3S2 and D3F15S2 were not present in the MCH939.2 derived DNA. The third hybrid cell line MCH924.4 did not hybridized with probes NRSL26, D3S3, D3S2 and D3F15S2. These hybrids are used to construct region specific libraries, and isolate chromosome 3 specific DNA probes using Alu mediated PCR technique.

The NRSL26 a 3p12-p14 specific DNA probe isolated from a chr. 3 specific NotI restriction site library shows highly polymorphic character with TaqI, EcoRI, HindIII, NotI and SfiI. It will be useful to map disease locus on chromosome 3 in tumor derived DNA.

Boldog et al

Probe, localization	HYBRIDS			
	MCH 903.1	MCH 429.11	MCH 939.2	MCH 924.4
c-raf (3p25)	+	+	+	+
RarB (3p24)	+	+	+	+
D3F15S2 (3p21.1-2)	+	+		
D3S2 (3p21)	+	+		
D3S3 (3p14)	+		+	
NRSL 26 (3p12-14)	+	+	+	
D3S4 (pter-q21)	+	+	+	+
PCCB (3q13-22)	+	+	+	+

Bobby et al.



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