THE POLYMERASE CHAIN REACTION: METHODOLOGY AND APPLICATIONS

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Introduction

WH 001 QUANTITATIVE AMPLIFICATION OF mRNA USING POLYMERASE CHAIN REACTION, Gary Gilliland, Steven Perrin and H. Franklin Bunn, Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.

Polymerase chain reaction (PCR) has been a valuable tool to amplify mRNA, especially transcripts present in low copy number. Although large amounts of rare mRNA species can be produced, it has been very difficult to quantitate the amount of specific mRNA present in the starting sample. The main constraint in obtaining quantitative information is inherent in the amplification process: because amplification is (at least initially) a logarithmic process, very small differences in any of the variables which control rate of reaction will dramatically affect the amount of PCR product which is obtained. These variables include the concentrations of polymerase, Mg, dNTP, DNA and primers; annealing, extension and denaturing temperatures; cycle length and number of cycles; ramping times; rate of "primer-dimer" formation; and presence of contaminating DNA, among others.

We have devised a technique which obviates these problems and allows quantitation of specific mRNA species. The strategy involves co-amplification of a competitive template which uses the same primers as the target cDNA, but can be distinguished from target cDNA following amplification. The competitive template can be a mutant cDNA containing a new restriction site, or, if the primers are in separate exons and flank a small intron (100-200 bp), genomic plasmid DNA can be used. Target cDNA is co-amplified with a dilution series of competitive template of known concentration. Since a change in any of the variables listed above will affect yield of competitive template and target cDNA equally, relative ratios of the two will be preserved with amplification. Radiolabeled dNTP is used to quantitate the amount of competitive template and target cDNA after amplification, and because the starting concentration of competitor is known, the initial concentration of the target cDNA can be determined.

This method gives accurate quantitation of less than 1 pg of target cDNA from 1 ng of total starting mRNA, and can distinguish two-fold differences in mRNA concentration. The technique can be applied to quantitation of mRNA from as few as 10 cells, and is thus useful in screening colonies of cells or flow sorted cells for specific mRNA production under various conditions.

Genetic Disease Diagnosis

WH 002 Applications of the Polymerase Chain Reaction in the Analysis of Human Genetic Diseases Arising from New Mutations, Richard A. Gibbs, Jeffrey S. Chamberlain, Phi-Nga Nguyen, Joel E. Ranier, Chen-Chi Lee, [1]Lincoln J. McBride and C. Thomas Caskey, Institute for Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston TX 77030. [1]Applied Biosystems Inc., 850 Lincoln Center Boulevard, Foster City, CA. 94404.

The polymerase chain reaction (PCR) and related techniques have widespread application for the molecular analysis of human genetic diseases that arise from heterogeneous mutations. Subtle genetic changes leading to the disruption of the human hypoxanthine phosphoribosyltransferase (HPRT) gene can be detected *via* manual or automated direct DNA sequence analysis of PCR amplified HPRT cDNA. Gross genetic changes, such as DNA deletions leading to Duchenne muscular dystrophy, are rapidly identified by the presence or absence of an amplification product following PCR of an affected region. The DNA deletion assay can be multiplexed, with as many as nine separate amplification primer sets included in a single PCR, permitting the simultaneous analysis of many different regions of the genome.

The routine detection of single DNA base changes such as common disease alleles, DNA sequence polymorphisms and new mutations identified in affected families can be achieved by a variety of procedures following PCR amplification. As an alternative to the usual procedure of allele specific oligonucleotide (ASO) probing we have explored the preferential utilization of perfectly complementary oligonucleotide primers relative to those that differ by a single base when a DNA synthesis reaction is performed. Under appropriate conditions an oligonucleotide that is perfectly complementary to a DNA template will be incorporated during PCR with 100-fold higher efficiency than a primer with a single base difference. More complex mixtures of oligonucleotide primers have also been used as PCR primers to facilitate cDNA cloning from amino acid sequence information.

Disease Susceptibility and Cancer Diagnosis

WH 003 POLYMERASE CHAIN REACTION WITH SINGLE SIDED SPECIFICITY: ANALYSIS OF T CELL RECEPTOR δ CHAIN, Elwyn Loh†, Jonathan Hardy, Daniel Wettstein, Ian MacNeil, and Mark Davis§, Department of Immunology and Microbiology, †Department of Medicine, and the §Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305-5402.

In the polymerase chain reaction, generally two specific oligonucleotide primers are used to amplify the sequences between them. However this technique is not suitable for amplifying sequences where one does not know sequences that lie on both sides of the fragment of interest. The T-cell receptor and immunoglobulins are examples of where one may not know the 5' portion of the molecule due to unknown variable regions. We have devised a modification of the PCR technique, which we term anchored PCR (A-PCR), that overcomes this limitation (1). In A-PCR, one uses terminal deoxynucleotidyltransferase to add a homopolymer of dG to the 3' end of cDNA, and one then primes the second strand synthesis with a primer containing an anchor sequence attached to a homopolymer of dC. Subsequent amplification is done using a mixture of a specific 3' oligonucleotide as well as the nonspecific anchor primer. We are continuing to define the conditions to optimize this approach and to define its limitations.

We have applied this technique to the characterization of the δ chain diversity of populations of T cells. In man, there is evidence that a predominant V region [$V_{\delta}3$, or $V_{\delta}2$, in the terminology of (2)] is found in the peripheral blood and another ($V_{\delta}1$) that predominates in the thymus. We are continuing these studies to define the δ chain diversity of populations of mouse T-cells from the thymus, spleen, intestinal epithelium, and lung.

1. Loh, E., Elliott, J.F., Cwirla, S., Lanier, L.L., Davis, M.M., **Science** 243:217-220, 1989.

2. Hata, S., Clabby, M., Devlin, P., Spits, H., De Vries, J.E., Krangel, M., **J. of Exp. Med.** 160: 41-57, 1989.

WH 004 RAS GENE MUTATIONS AND HUMAN MALIGNANCIES. Johannes L. Bos, Laboratory for Molecular Carcinogenesis, Sylvius Laboratories, P.O.Box 9503, 2333AL Leiden, The Netherlands.

The presence of activated ras genes in various human malignancies was determined by means of synthetic oligonucleotide hybridization after in vitro amplification of relevant ras sequences (PCR). Of each type of malignancy 30-100 samples were screened for mutations in codons 12 and 61 (H-, K- and N-ras) and codon 13 (K- and N-ras). We have found mutated ras genes in colon carcinomas (50%, mostly K-ras), lung adenocarcinomas (30%, mostly K-ras), acute myeloid leukemias (20%, mostly N-ras), seminomas (20%), melanomas (20%), pancreas adenocarcinomas (85%, K-ras) and myelodysplastic syndromes (30%). No ras gene mutations were found in cervical cancer, ovary tumors, glioblastomas, CML and follicular cell lymphomas. These results demonstrate that activated ras genes do occur rather frequently in certain human malignancies, in particular in colon and pancreas tumors, whereas it is absent in others. Analysis of premalignant precursor tissues such as colon adenomas and myelodysplasia indicates that the mutational event can occur relatively early but also late in the course of tumorigenesis. Comparing the presence of a mutated ras gene and a variety of parameters does not reveal clear correlations with clinical or histo-pathological features of a tumor, but does reveal the involvement of mutagenic agents like tobacco smoke and UV in the induction of the ras mutation.

WH 005 DIRECT GENOMIC SEQUENCING OF ALLELES AT THE HUMAN RETINOBLASTOMA LOCUS: APPLICATION TO CANCER DIAGNOSIS AND RISK ESTIMATION, David W. Yandell, Siri H. Dayton, Tracey A. Campbell, Terri L. McGee, and Thaddeus P. Dryja, Department of Ophthalmology, Harvard Medical School and Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114

Mutations in the human retinoblastoma (RB) gene are known to cause both hereditary and nonhereditary forms of retinoblastoma. They may also play a role in the formation of several other tumor types including osteosarcoma, soft tissue sarcomas, and carcinomas of the breast, lung, and bladder. These mutations can occur somatically (causing the nonhereditary form of RB), or in the germ line (causing hereditary RB). DNA-based diagnosis can be used to distinguish between hereditary and nonhereditary forms, to identify unaffected carriers, and to allow the earliest possible clinical intervention for children who inherit the disease.

Until recently, it was difficult to identify mutations in this gene except for deletions large enough to visualize by Southern blotting. The nature of the mutations that cause 70-90% of all retinoblastomas was thus unknown. If no mutation can be found, molecular diagnosis must be based on analysis of restriction fragment length polymorphisms (RFLPs) linked to the mutant allele. This is of little value when there is no previous family history of the disease. We have used exon-specific polymerase chain reaction (PCR) amplification and direct genomic sequencing to find both naturally occurring DNA sequence polymorphisms and disease-causing mutations at the RB locus. Both forms of sequence variation are diagnostically useful. So far, four DNA sequence polymorphisms have been identified. None of these would have been detected by restriction enzyme-based screening. These new polymorphisms have allowed carrier status prediction in several families where RFLP analysis was useless. We have also used these techniques to identify disease-causing point mutations in 6 tumors, including four retinoblastomas, a small cell lung carcinoma, and a bladder tumor. Two of these are new germinal mutations. These techniques provide an extremely valuable complement to conventional methods for molecular diagnosis of hereditary retinoblastoma, as they allow us to base genetic counseling on direct assay of disease-causing mutations in a much larger percentage of families than can be reached by Southern blotting. In addition, they offer for the first time the possibility of differentiating between sporadic and hereditary forms of retinoblastoma in the majority of unilaterally affected retinoblastoma patients who have no previous family history of the disease.

Posters-I

WH 100 APPLICATION OF PCR TO THE DETECTION OF ENTERIC VIRUSES IN WATER. Leslie M Alexander, Malcolm A McCrae, Ray Morris and Edward B Pike, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, England.
Tissue culture isolation is the technique routinely used to detect enteric viruses in water. However, many of the enteric viruses that contaminate water supplies have fastidious growth requirements in tissue culture, making their detection by this method laborious and often impossible. PCR has significant potential for the diagnosis of enteric viral pathogens in water samples. An assay capable of simultaneously detecting both RNA and DNA viruses in water has been developed and has been used in the detection of rotavirus and poliovirus contamination of water concentrates from several sources. It offers a rapid and sensitive alternative to tissue culture isolation.

WH 101 DETECTION OF BKV AND JCV IN URINES AND BRAIN TISSUE BY POLYMERASE CHAIN REACTION, Ray R. Arthur, Sherry Dagostin, Keerti V. Shah, Department of Immunology and Infectious Diseases, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205
DNAs of the human polyomaviruses BKV and JCV were amplified by PCR using a single pair of 20-base oligonucleotide primers that were complementary for the same regions of both viruses. The sequences flanked by the primers were unique for each virus and could be differentiated by hybridization with 40-base ^{32}P-labeled oligonucleotide probes or by enzymatic cleavage with BamHI. The DNA fragments resulting from amplification were 176 and 173 np, respectively. The sensitivity of PCR for amplification of cloned BKV and JCV DNA was 10 copies. A total of 57 urines from three groups of subjects were processed by DNA extraction or boiling and tested by PCR. Urines collected from immunosuppressed patients (n=11) and previously documented to be positive for BKV and/or JCV, were positive by PCR. Ten per cent of urines from healthy adults (n=30) were positive for one or both viruses. Urines (n=16) from four BKV-seronegative bone marrow transplant recipients were uniformly negative for BKV. JCV was detected in deparaffinized brain tissue from a patient with progressive multifocal leukoencephalopathy. In addition to greater sensitivity than previously described techniques, results can be obtained by PCR on the day of specimen receipt thus making this method attractive for use in diagnosing polyomavirus infections.

WH 102 **THE RAPID CLONING OF HOMOLOGOUS cDNAs WITH PCR: THE USE OF HIGHLY DEGENERATE OLIGONUCLEOTIDES TO AMPLIFY TRYPSIN-LIKE SERINE PROTEASES FROM THE PACIFIC HAGFISH, *EPATRETUS STOUTI*.** David K. Banfield, and Ross T.A. MacGillivray, Department of Biochemistry, Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1W5. The proteins involved in coagulation are members of a family of trypsin-like serine proteases. These proteins also share various structural and functional domains including: Kringles, Epidermal Growth factor-like domains, and gamma-carboxyglutamic acid containing domains (Gla domains). To understand structural/functional relationships, and the evolutionary significance of these domains, we are using PCR to clone homologous cDNAs from the Pacific Hagfish. Using a 4,096 fold degenerate oligonucleotide synthesized to a highly conserved octapeptide (CEGDSGGP) in the active-site region, we have isolated partial cDNAs for two trypsin-like serine proteases. One has a 43.2% amino acid sequence identity to the protease domain of human plasminogen, and the other a 47.5% identity to the protease domain of human factor X. Sequence analysis of the ends of these clones revealed that amplification was a result of the active-site oligonucleotides priming both strands. Presently, we are using less degenerate active-site serine oligonucleotides in conjunction with oligonucleotides synthesized to the Gla (GNLEREC), and Kringle domains (ENYCRNP) to isolate additional trypsin-like serine proteases from the Hagfish.

WH 103 N- AND Ki-RAS MUTATIONS IN MYELODYSPLASTIC SYNDROMES (MDS), T-LYMPHOBLASTIC LEUKEMIA (T-ALL) AND COLON CANCERS: ANALYSIS BY PCR AND DIRECT SEQUENCING, Menashe Bar-Eli, Harish G. Ahuja, Andras G. Foti, Nestor Gonzalez-Cadavid and Martin J. Cline, Division of Hematology/ Oncology, University of California, Los Angeles, CA 90024-1678. Reported frequencies of mutations in codons 12 or 13 of N-RAS in MDS and acute myeloid leukemias vary between 5 and 50% depending on whether transfection, hybridization to small oligonucleotides or nuclease sensitivity is used in analysis. Each of these methods may have artifacts which affect sensitivity and reliability. Gene amplification by PCR and direct sequencing provides an unambiguous method of detecting those mutations which induce amino acid alterations. This procedure requires 48 hours from DNA isolation to sequencing. We analyzed 21 MDS cases and found N-RAS codon 12 mutations (GGT → GAT) in 4 and a codon 13 mutation (GGT → GCT) in one. In T-ALL, a novel mutation of the N-RAS gene was detected in one patient with threonine (ACA) for alanine (GCA) at codon 11. This mutation would have been overlooked by conventional probe hybridization techniques. A search for other mutations in N-RAS exon-1 in T-ALL revealed a codon 13 mutation (GGT → GAT) in one of 18 patients, but no detectable mutations at codon 12. Analysis of mutations in exon-1 of Ki-RAS colon cancers revealed a codon 12 mutation (GGT → TGT or GAT) in 2 of 12 patients and a codon 13 mutation (GGT → GAC) in a third. We conclude that N-RAS exon-1 mutations producing amino acid changes occur in about 20-25% of MDS cases and in about 10% of T-ALL cases. Ki-RAS exon-1 mutations occur in about 25% of colon cancers and may involve either codons 12 or 13.

WH 104 V-REGION SEQUENCE ANALYSIS OF ANTIBODIES FROM THE AUTOIMMUNE RESPONSE AGAINST THE ACETYLCHOLINE RECEPTOR USING PCR-AMPLIFIED cDNA, Joachim H. Bartels and Donard S. Dwyer, Neuropsychiatry Research Program, University of Alabama at Birmingham, Birmingham, AL 35294

A number of monoclonal antibodies have been obtained from the immune response to the nicotinic acetylcholine receptor (nAChR). Some of them bind directly to the nAChR, whereas others (anti-idiotypes) recognize anti-nAChR - antibodies or else are epibodies and bind to both the antigen (nAChR) and the anti-nAChR-antibodies. Because autoantibodies are the key pathogenic factor in the autoimmune disease, myasthenia gravis, it is important to determine the V-region sequences encoding the recognition site for binding to the nAChR or for binding to the idiotope of anti-nAChR-antibodies. Based on these sequences it may be possible to design synthetic idiotypic peptides for in vivo studies of regulation of autoimmunity.

The amino acid sequence of antibody V-regions was deduced from cDNA-sequences. cDNA was synthesized from hybridoma-RNA using reverse transcriptase and a specific primer. To obtain sufficient starting material for dideoxy-sequencing reactions, the cDNA was amplified using the primer for cDNA synthesis together with a second primer. The PCR-products were separated on a NuSieve agarose gel and the predominant band was used as template in a second (unbalanced) PCR-reaction to generate predominantly single stranded DNA template for sequencing. An aliquot of the amplified DNA, together with the *taq polymerase* as sequencing enzyme and one of the PCR-primers was used for sequencing by the Sanger chain terminator method. The PCR amplification of cDNA encoding antibody-V-regions makes it possible to start with very little hybridoma RNA and circumvents the need of cloning, thus saving time and material. Using this approach, additional antibodies will be sequenced to obtain a more complete picture of the structural basis of antibody recognition involved in autoimmune disease.

WH 105 APPLICATION OF THE PCR TO TISSUE TYPING: IMPROVEMENTS IN THE DETECTION OF HLA-DR POLYMORPHISM, Lee Ann Baxter-Lowe, Jack Gorski, and Jay Hunter, The Blood Center of Southeastern Wisconsin, Milwaukee Wisconsin 53233

The polymerase chain reaction (PCR) and sequence-specific oligonucleotide probe hybridization (SSOPH) were utilized to detect polymorphism in genes encoding HLA-DR β chains. Comprehensive analysis of HLA-DR polymorphism was accomplished by using a combination of locus-specific amplification, allele-specific amplification and SSOPH. This analysis discriminated between genes associated with 27 unique HLA-DR alleles. The use of this approach for detection of HLA-DR polymorphism provides several advantages over conventional typing methods that rely upon reactivity of the HLA-DR proteins with antibodies or alloreactive T-cells. Examples from clinical cases were utilized to illustrate the major advantages of the use of PCR/SSOPH for HLA typing, including (1) fine resolution of similar alleles (subtypes), (2) assessment of HLA-DR polymorphism when expression of HLA-DR proteins is abnormal, and (3) accurate analysis of alleles in heterozygous individuals expressing combinations of alleles that are difficult to discriminate using conventional methodologies. PCR/SSOPH analysis of HLA polymorphism offers substantial improvements in HLA typing for transplantation, identity testing and HLA-associated diseases.

WH 106 POLYMERASE CHAIN REACTION (PCR) AND GENE PROBES FOR ENVIRONMENTAL MONITORING. Asim K. Bej, Ronald M. Atlas, Robert J. Steffan, Meena Mahbubani, Richard D. Miller, Joseph DiCesare, and Lawrence Haff. University of Louisville, Louisville, KY and Perkin-Elmer Corp., Norwalk, CT.
The polymerase chain reaction (PCR) was adapted for use in the environmental monitoring of genetically engineered microorganisms (GEMs), coliform bacteria, and pathogens. Using primers flanking a 1.3 kb repeat sequence of the 2,4,5-T-degrading GEM Pseudomonas cepacia AC1100, as few as 1 bacterial cell per gram of soil was detected. Similarly lacZ and lamB target genes were detected in as little as 1 fg of E. coli DNA, and 1-4 cells of E. coli were detected in 1-100 ml water samples. The PCR method permitted simultaneous amplification of a 875 bp region of the lacZ gene and a 554 bp region of the lamB gene of E. coli. Amplification of the lacZ gene using a reannealing temperature of 50°C detected a strong signal for E. coli and Enterobacter cloacae, a very weak signal for Klebsiella pneumoniae, and no signal for Salmonella typhimurium, Pseudomonas putida, Citrobacter freundii, or Streptococcus lactis. Using a reannealing temperature of 50°C the lamB gene was amplified in both E. coli and S. typhimurium, but using a reannealing temperature of 60°C, the lamB gene was specific for E. coli. Biotin and trapping probes were used to achieve rapid and specific detection. Additional primers and probes were targeted at specific pathogens, including Legionella pneumophila. PCR and gene probes were able to detect either all Legionella species or specifically L. pneumophila. The sensitivity, specificity, and speed of this method makes it an attractive alternate methodology for environmental monitoring.

WH 107 **AMPLIFICATION OF A MULTIALLELIC LOCUS IN THE 3' FLANKING REGION OF THE INTERLEUKIN 6 (IL6) GENE,** Anne M. Bowcock (1), Anuradha Ray (2), Pravinkumar B. Sehgal (2), Henry A. Erlich (3); (1)Department of Genetics, Stanford University, Stanford, CA 94305; (2)The Rockefeller University, New York, NY 10021;(3)Cetus Corporation, Department of Human Genetics, 14300 Fifty-Third Street, Emeryville, CA 94608
We have previously determined that the 3' flanking region of IL6 is polymorphic with at least four alleles that are due to DNA insertions of variable length. We have determined the sequence of this region and shown that the polymorphic region contains approximately 475 base pairs which are highly AT rich. With knowledge of the DNA sequence flanking the AT rich locus we have synthesized oligonucleotides which amplify this region using the polymerase chain reaction (PCR). After analysis of the amplification products by agarose gel electrophoresis, fragments of the predicted size were detected. The fragments were between 0.7 and 0.83kb. DNA from Chimpanzee and Gorilla have also been amplified, and generated fragments of 0.5kb and 0.2kb respectively. We have determined the molecular nature of the different IL6 alleles by direct sequencing and demonstrated conservation of the AT rich sequence in the common chimpanzee. This system is a paradigm for analysis of VNTR polymorphisms by PCR.

WH 108 **ONE DAY PREPARATION FROM CELLS TO cDNA, PCR-AMPLIFIED FOR SEQUENCING,** Eleanor C. Brinson and Irene M. Jones, Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA 94550. A quick, one day procedure utilizes the polymerase chain reaction (PCR) for generating, from cells in small scale cultures, cDNA in a size ready for sequencing. Cells used are human T lymphocytes. The target gene is the hypoxanthine phosphoribosyltransferase gene, a single copy gene, hemizygously expressed in low copy number. Total, cytoplasmic RNA is extracted from 10^4 - 10^5 cells by gently lysing the cells with NP-40. Ribonucleoside vanadyl complex is used as RNAse inhibitor. A cDNA-RNA hybrid is produced using a sequence specific, oligonucleotide primer and the enzyme Moloney Murine Leukemia Virus Reverse Transcriptase. RNAsin is used as an RNAse inhibitor in this reaction. PCR amplified cDNA is generated using two sequence specific oligonucleotide primers and Taq Polymerase (Perkin Elmer Cetus). The reaction is carried out in the programmable Perkin Elmer Cetus DNA Thermal Cycler. By utilizing primers approximately 300 bp apart, amplified DNA in a size appropriate for sequencing is generated. Work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract number W-7405-ENG-48 and Interagency Agreement No. Y01-ES-80171 with NIEHS.

WH 109 PCR-MEDIATED AMPLIFICATION OF cDNA POPULATIONS: SCALING DOWN DIFFERENTIAL SCREENING TO THE LEVEL OF SINGLE IDENTIFIED NEURONS, Jean-Francois Brunet, Yuichi Iino, Paul Pfaffinger, Eric R. Kandel, Howard Hughes Medical Institute, Columbia University, New York, NY 10032.
A major problem in the differential screening of cDNA libraries is the need for a large and readily obtainable amount of probe, thus limiting this approach to abundant sources of mRNA. In an attempt to solve this problem, we are developing a novel technique for cDNA cloning based on a modification of PCR methodology. Single stranded, oligo-dT primed cDNA is synthesized from total RNA. It is subsequently dC-tailed and amplified with a set of two adaptor/primers that include a polyT and a polyG stretch, respectively. At this point, the cDNA can be either directionally cloned or labeled at high specific activity, thus providing the tools for the differential screening of cDNA libraries generated from a fraction of a microgram of total RNA. Preliminary evidence shows that such amplified cDNA populations are fairly representational, with no major inconsistency or distortion attributable to the amplification process. This methodology allows the production of an unlimited amount of probe, and therefore makes it now possible to apply differential screening, possibly coupled with subtraction, to single cells or very small anatomical structures. We are in the process of applying it to the study of transmitter-modulated and cell-specific genes in *Aplysia* nervous system.

WH 110 PCR ANALYSIS OF FUNGAL MITOCHONDRIAL RIBOSOMAL RNA GENES. Bruns, T. D., and Taylor J. W. Department of Botany, University of California, Berkeley, CA 94720
We have developed five pairs of oligonucleotide primers that are specific for fungal mitochondrial ribosomal RNA subunits. Use of these primers for PCR amplification and sequencing has enabled us to survey the distribution of two introns, examine the relative frequency of transitions, transversions, and insertion/deletion events, and infer phylogenetic relationships between species, genera, and families of fungi. Rapidly evolving regions of large subunit mitochondrial ribosomal RNA gene exist and appear to be useful targets for selection of oligonucleotide probes specific to fungal genera.

WH 111 RETROSPECTIVE ANALYSIS OF c-Ki-ras MUTATIONS IN COLON CANCER, Glenna C. Burmer, Bruce Kulander*, Katrina East and Lawrence A. Loeb, The Joseph Gottstein Memorial Cancer Res. Lab., Dept. Pathology SM-30, University of Washington, Seattle, WA 98195, *the Laboratory of Pathology of Seattle, 98104
Fresh and paraffin embedded colon carcinomas, adenomas and adjacent mucosa were analyzed for the presence of c-Ki-ras mutations by polymerase catalyzed chain reaction followed by direct DNA sequencing. Tissues treated in a variety of fixatives, including methyl carnoys, formalin, and Hollande's solution were suitable as sources of DNA for the PCR reaction. Enrichment for tumor cells was further obtained by sorting nuclei on the basis of DNA content differences. 67% of carcinomas and 33% of adenomas contained aneuploid cells. Mutations at the first base pair position of codon 12 were found in 65% of carcinomas and 75% of adenomas analyzed. After cell sorting, mutations were found in both the aneuploid and diploid subpopulations of carcinomas and adenomas. These results suggest that mutations in ras precede ploidy alterations in the progression of these neoplasms, and exist in diploid cells form which an aneuploid population arises.

WH 112 POLYMERASE CHAIN REACTION: QUANTITATION AND COMPARISON OF DNA AND RNA AS TARGET MOLECULES B.C. Byrne*, J.J. Li*, L. Zaumetzer*, B. Michaelis*, D. Mildvan•, M. Grieco▲, J.J. Sninsky+, S. Kwok+, and B.J. Poiesz*, *Department of Medicine, SUNY HSC at Syracuse, Syracuse NY 13210 •Division of Infectious Diseases, Beth Israel Medical Center, New York, NY, ▲Division of Allergy, Infectious Diseases and Epidemiology, St. Luke's Roosevelt Hospital Center, New York, NY 10019, and +Cetus Corporation, Emeryville, CA 94608. We explore the use of PCR using DNA or RNA substrates as useful measures of viremia in antiretroviral drug trials. In these studies we look both at RNA and DNA substrates, carefully considering the technical features of sample preparation and PCR protocol that affect PCR. We apply quantitative measures of PCR product as a measure of proviral load and compare these measures to other indications of viremia. Finally, we consider cut-off values for PCR positivity, methods to increase sensitivity, and methods to deal with apparent insensitivity (false negatives) or false positives (carryover DNA).

WH 113 HOMOLOGOUS RECOMBINATION INVOLVING SINGLE-STRANDED OLIGONUCLEOTIDE IN HUMAN CELLS, Colin Campbell, Wayne Keown, Linda Lowe, Deborah Kirschling and Raju Kucherlapati, University of Illinois, College of Medicine, Chicago, IL 60612

We have ascertained that synthetic single stranded oligodeoxynucleotides are capable of participating in homologous recombination in mammalian cells. Mixture of a plasmid carrying a mutant selectable gene (neomycin phosphotransferase) and a 40 nucleotide long single stranded oligomer containing the wild-type sequence and a silent mutation was introduced into human cells. We obtained G418 resistant colonies which result from the reconstruction of a wild-type neo gene at frequencies which are 20 fold higher than those obtained when a heterologous oligomer was used. A DNA fragment spanning the target sequence was amplified from the total cellular DNA of G418 resistant colonies. Hybridization of the amplification product with a probe specific for the silent mutation present in the oligonucleotide showed that the oligomer indeed participated in the recombination event. This approach may be useful in correcting or introducing single point mutations into the genomes of mammalian cells.

WH 114 DETECTION OF BACTEROIDES NODOSUS BY POLYMERASE CHAIN REACTION AMPLIFICATION OF PILIN GENE SEQUENCES, Jonathan Carlson, Gilbert John, and Robert Ellis, Department of Microbiology, Colorado State University, Fort Collins, CO 80523.

Bacteroides nodosus is a gram negative bacterium that is the causative agent for ovine foot rot. Current methods of identification are based on serological methods. Rapid diagnosis of B. nodosus infections is hampered by the slow growth characteristics of this fastidious anaerobe. There are at least eight major serotypes defined by antibodies that bind to pili on the surface of the bacteria. These pili are composed of pilin subunits of the mePhe type. The pilins are characterized by a highly conserved aminoterminal 32 amino acids while the remaining 120-125 amino acids vary with the serotype. We have shown that a single pair of primers made from the portion of the pilin gene that codes for the conserved aminoterminal amino acids can amplify a 100 bp fragment of DNA from all of the serotypes. Thus it is possible to diagnose B. nodosus infections regardless of serotype. Serotype specific primers that amplify the variable portions of the pilin genes are also being investigated.

WH 115 GENOMIC FOOTPRINTING BY AN AMPLIFIED PRIMER EXTENSION (APEX) TECHNIQUE, Iain L. Cartwright and Susan E. Kelly, Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267. We have developed a technique which we have dubbed APEX genomic footprinting that has allowed us to map DNA-protein interactions to single base resolution in vivo at a series of Drosophila small heat shock protein genes located at locus 67B1. In addition to thermal inducibility, these genes are developmentally regulated in a tissue-specific fashion by the steroid hormone ecdysterone. We have been analyzing hormone-induced changes in DNA-protein architecture at hsp22 and hsp27 in order to identify those DNA sequences that are likely to play a critical role in the hormonal response. After methylation of cultured Drosophila cells or brief nuclease (DNase I) digestion of isolated nuclei, genomic DNA is purified and, as required, sites of guanosine methylation cleaved by piperidine treatment. A synthetic 25-mer primer oligonucleotide that is complementary to sequences immediately abutting the region of interest is 5'-end labelled and hybridized in vast excess to genomic DNA. Repeated rounds of primer extension using a synthesis/denaturation/annealing cycle with Taq polymerase allows a linear amplification of extended DNA fragments that have one end defined by the site of 5'-labelling and the other end by the original sites of modification in chromosomal DNA. In this way it is possible to generate genomic footprints which can be analyzed directly on sequencing gels without the need for time-consuming and technically difficult blot/hybridization protocols. Technical aspects of the APEX protocol will be discussed, and representative data will be presented.

WH 116 DETECTION OF SPECIFIC DNA SEQUENCES IN GENOMIC DNA BY A COLOR COMPLEMENTATION ASSAY. Farid F. Chehab, Applied Biosystems, Foster City, CA 94404
A color complementation assay (CCA) that allows rapid screening of specific DNA sequences in genomic DNA was developed. It is based on the simultaneous amplification of 2 or more DNA segments with oligonucleotide primers conjugated to different fluorescent dyes, such that each amplified DNA segment emits a specific color when excited by standard UV light. For example, when the presence or absence of an amplified DNA segment is diagnostic, the amplification of an internal control DNA for the PCR reaction is also required. Thus, fluorescent amplification of the diagnostic and internal control DNA results respectively into a red and green color. However, when both fluorescent DNA segments are combined into solution and purified from the leftover fluorescent PCR primers, a yellow color results from the complementation of both red and green DNA, indicating that both DNA segments have been amplified. Therefore, the detection of DNA sequences by CCA is reduced to a PCR reaction, removal of the leftover primers by a 10 minute tabletop centrifugation, UV irradiation and eye visualization of the diagnostic color. No gel electrophoresis is required. In this context, CCA has been separately applied to the detection of a large deletion, a chromosomal translocation, an infectious disease agent as well as to the allele specific detection of a 4 basepair deletion and a point mutation in the human β-globin gene. CCA was also used to simultaneously detect 5 different loci in genomic DNA whereby amplification of each locus generates a different color. Thus, after removal of the fluorescent primers, and due to the limitation of the naked eye in visualising the complementation of many different colors, the emission of each fluorophore is scored on a fluorometer as an indication of a yes/no amplification at that DNA locus. CCA lends itself to automation and can be applied as a fast, colorimetric, non-radioactive detection/discrimination system in screening specific DNA sequences. CCA can be implemented in basic research, clinical and forensic settings where high sample throuhput, ease, cost and safety of the procedure are important factors.

WH 117 GENERAL TECHNIQUES WHICH BROADEN THE APPLICABILITY OF POLYMERASE CHAIN REACTION A.A. Christen and B. Montalbano, U.S.D.A.-S.R.R.C., New Orleans, LA 70179 Polymerase chain reactions (PCR) amplify defined regions of DNA and utilize two primers synthesized to complement a given sequence of each strand of DNA. In the present study procedures for the use of PCR without custom oligonucleotides were developed. Further applications resulted from the use of affinity beads in capturing DNA in procedures utilizing PCR. Homopolymer priming with poly d(A) was used to PCR cDNA containing a dT tract. Similarly, homopolymer tailing with TdT was utilized to PCR DNA fragments. Homopolymer priming techniques were then tested for potential amplification of total mRNA, via RT into cDNA, from small numbers of blood cells. Homopolymer priming was manipulated to produce DNA of varied lengths which were subsequently utilized as primers for specific gene regions. Avidin beads allowed the capture of template DNA for its reuse, and aided selection of DNA in gene isolation and analysis.

WH 118 A SUBSTITUTION OF CYSTEINE FOR GLYCINE 904 OF THE α1(I) CHAIN IN A PROBAND WITH LETHAL OSTEOGENESIS IMPERFECTA AND IN HIS ASYMPTOMATIC MOTHER, C.D. Constantinou, M.A. Pack, S.B. Young, and D.J. Prockop, Dept. of Biochemistry and Molecular Biology, Thomas Jefferson University, Phila., PA 19107, USA. Previous work has established that there is a mutation in codon 904 of one of the α1(I) collagen alleles in a proband with lethal osteogenesis imperfecta. The mutation is a transversion of G to T converting the codon for glycine to a codon for cysteine. The consequences were excessive over-modification, lower thermostability and impaired secretion of the abnormal molecules. Fibroblasts of the proband's mother synthesized a small amount of collagen that was also over-modified and unstable. In addition, at low passages they synthesized a small amount of disulfide linked α1(I) chains. Total RNA from the mother's fibroblasts was utilized for the synthesis of cDNA which was subsequently used for amplification of a relevant region in the α1(I) chain. Sequence analysis revealed the same G to T transversion in one of her α1(I) alleles. A similar approach using genomic DNA confirmed the mutation. Her clinical manifestations were limited to a slight blue discoloration of the sclerae, prominent frontal bossing and non-progressive scoliosis present since childhood. Collagens synthesized by fibroblasts from the proband's father, maternal grandparents and two living siblings did not show any abnormality. One possible explanation of the phenotypic variability seen in this family is that the proband, in addition to the mutation inherited from her mother, carried a second mutation which contributed to the phenotype. Another explanation is that the mother was mosaic. A sporadic mutation probably occurred early in her development in utero which affected both germinal and somatic lineages.

WH 119 DETECTION OF EBV IN LACRIMAL GLAND BIOPSIES OF SJOGREN SYNDROME PATIENTS. Cecelia A. Crouse, Stephen C. Pflugfelder, Stephen E. Demick and Sally S. Atherton. Departments of Ophthamology, University of Miami School of Medicine, Miami, Florida 33136 .
Sjogrens syndrome (SS) is a common autoimmune disease in which aqueous tear deficiency and dry mouth develop due to destruction of the lacrimal gland (LG) and salivary gland as a result of B-cell proliferation. However, the initial inciting factor(s) for this lymphoproliferation has(have) not been determined. There is increasing evidence that SS may result from persistant or reactivated Epstein-Barr virus (EBV) infection within these glands. Since EBV is a potent polyclonal B-cell activator, we hypothesized that the hyperglobulinemia, and the autoantibodies found in SS patients, may also result from EBV reactivation. The polymerase chain reaction (PCR) was used to determine whether EBV sequences were present in the DNA from LG specimens from normal and SS patients. A 240 bp sequence from the IR3 region of the EBV genome was amplified. DNA from a marmoset lymphocyte cell line (B95-8) was used as the EBV positive control and HSV-1 infected Vero cells as the negative control. The amplification procedure followed by Southern hybridization using an EBV specific probe resulted in the following: a) EBV IR3 sequences were not detected in three SS specimens and b) two of three normal LG tissues were negative. A weak positive signal in a third normal LG sample was detected only after a 48 hour exposure whereas the signal from the positive control was evident at 3 hours. These results suggest that EBV is not present in the LG of SS patients and therefore EBV reactivation may not be causally related to the onset of SS. The DNA from a larger sample of normal and SS LG tissue is currently being analyzed by PCR for EBV sequences to confirm our preliminary results.

WH 120 ANALYSIS OF GENETIC RECOMBINATION IN MURINE GERM CELLS, Mary K. Cullen, Joanne Trojnacki and Jan Geliebter, Howard Hughes Medical Institute, The Rockefeller University, New York, NY,10021
Products of murine histocompatibility (H-2) genes are responsible for transplant rejection and the presentation of cell associated antigens to the cellular immune system. These genes are part of the major histocompatibility complex class I multigene family whose other members are structurally similar but have unknown function. The extraordinary number and sequence diversity of H-2 gene alleles are thought to result from genetic recombination (gene conversion?) between H-2 and other class I genes. Skin graft studies of inbred mouse strains have indicated that these recombinations occur in germ cells at a frequency of 2×10^{-4} per gamete. The recombination process has been studied by the analysis of over a dozen recombinant mice whose H-2 genes differ from wild type by discrete clusters of 1 to 8 nucleotide substitutions. Further, several of these nucleotide substitutions have reoccurred, independently, in different mice. The accumulation of these substitutions, over time, is thought to account for sequence diversity among H-2 genes.

We are using the H-2 genes as a model system to study recombination in mammalian multigene families. Statistically, one recombinant gene should exist in the germ line of each female mouse. PCR amplification and cloning of H-2 genes from germ cells of normal mice provides a large and convenient source of genes that can be screened for recombination. Since recombinations reoccur, the detection of recombinant genes is facilitated by using oligonucleotide probes specific for recombination products that have previously been found to occur in nature. Thus, using PCR technology on genes from germ cells, we are able to analyze the equivalent of hundreds of thousands of mice. Studies are in progress to determine recombination frequencies of different H-2 genes in various mouse strains.

WH 121 THE USE OF POLYMERASE CHAIN REACTION TO IDENTIFY RECOMBINANT BACULOVIRUS AND TO MONITOR ITS PURIFICATIONS. Bruce Daugherty, Susan Zavodny, Albert Lenny, Melvin Silberklang, Jwu-Sheng Tung, Simon W. Law, George E. Mark III and Ronald W. Ellis. Department of Cellular and Molecular Biology, Merck Sharp and Dohme Research Laboratories, P.O. Box 2000, Rahway, NJ 07065. An accurate and time saving method is described to identify recombinant baculovirus and to monitor its plaque purification from transfected insect cells by utilizing the technique of polymerase chain reaction (PCR). Previously, recombinant plaques were identified by their refractive appearance under the light microscope. This kind of visual identification, requiring a trained eye, was still very unreliable. Plaques, so identified, contain varying amounts of wild type viruses whose removal will require multiple rounds of plaque purification. For recombinant identification, two twenty base long PCR primer sequences were synthesized. These primers were used to effect a PCR amplification of DNA templates extracted from individual plaques. Recombinant plaques were identified by the presence of the expected size DNA fragment following agarose gel analysis of the PCR products. The purity of recombinant virus stocks can be evaluated by substituting for the 3' primer mentioned above a primer derived from the region downstream of the cloning site of pVL941. In this case, two bands, one of the wild-type (smaller) and one of the recombinant (larger), are expected from a mixed viral stock, while a pure viral stock will show only one (larger) band.

WH 122 **CORRELATION STUDY INVOLVING THE DETECTION OF HIV-1 IN SAMPLES FROM AIDS PATIENTS USING CO-CULTURE, p24 ELISA, AND TAS- AND PCR-MEDIATED HYBRIDIZATION ASSAYS.** Geneva Davis, Kris Blumeyer, Kristina Whitfield, Hugh Chappelle, Luke DiMichele, Nanette Riggs, John Guatelli,[1] Douglas Richman[1], and Thomas Gingeras. The Salk Institute Biotechnology/Industrial Associates, Inc., 505 Coast Blvd. S., La Jolla, CA 92037. [1]Depts. of Medicine and Pathology, UCSD School of Medicine, San Diego, CA 92161. Co-culture, p24 ELISA, and nucleic acid hybridization assay formats represent three distinct monitors of HIV-1 infection. Each of these methods detects a specific characteristic of HIV-1. A collection of peripheral blood lymphocyte samples which were characterized as: 1) "+" for both co-culture and p24 assays; 2) "-" for both co-culture and p24 assays; 3) "+" for co-culture and "-" for p24 assays; or 4) "+" for p24 and "-" for co-culture assays was studied for the presence of HIV-1 nucleic acid sequences using TAS- and PCR-mediated hybridization assays. After amplification, detection of amplified products was carried out by a variety of methods, including bead-based sandwich hybridization, Northern and Southern blot hybridizations, and solution hybridization. Interestingly, sets of these samples were derived from the individual patients over the course of two years. Amplification-mediated hybridization assay results indicate increasing viral nucleic acid concentrations with time in some patients, whereas other assay results appear more variable. The capability to conduct quantitative measurements of HIV-1 sequence levels is discussed.

WH 123 SPECIFIC MULTIPRIMER PAIRS FOR THE DETECTION OF HTLV-I AND II
Barun K. De, M. Lairmore and A. Srinivasan.Retrovirus Diseases Branch,DVD,Centers for Disease Control, Atlanta,GA-30333.
Human T-cell lymphotropic viruses (HTLVs) are oncogenic, exogenous and replication competent retroviruses. The immunological and molecular techniques have revealed the presence of two distinct types of HTLVs. HTLV-I is associated with T cell malignancy whereas HTLV-II is derived from a benign T-cell variant of hairy cell leukemia. The antibody cross reactivity observed between HTLV-I and II makes it difficult to distinguish these retroviruses from routine serological surveys. To develop diagnostic tests specific for HTLV-I and II, we have performed the polymerase chain reaction using two sets of primer pairs (for HTLV-I : pol primer pair at position 3015-3034 sense strand and 3154-3134 antisense strand with probe at position 3050-3074; env primer pair at position at position 5627-5648 sense strand and 5792-5671 antisense strand with probe at position 5711-5735 and for HTLV-II : pol primer at position 2989-3010 sense strand and 3131-3110 antisense strand with probe 5711-5735). The amplified products corresponding to pol and env genes were analysed by using a 5% polyacrylamide gel and characterised by southern blot using viral specific probes. Our results indicate that the multiprimer pairs are specific for HTLV-I and II and should be useful for the detection and evaluation of structural changes in viral DNA.

WH 124 RAPID CARRIER DETECTION IN HEMOPHILIA B DURHAM BY USE OF PCR INDUCED RESTRICTION ANALYSIS Peter H. Denton[1], Howard M. Reisner[1,2], Departments of Microbiology and Immunology[1], and Pathology[2]. University of North Carolina, Chapel Hill, NC, 27599-7525. Tel. (919) 966-2949. We have previously characterized Hemophilia B Durham by PCR as a G to A transition which causes a highly conserved glycine in the Epidermal Growth Factor Domain of Factor IX to become a Serine. Blood samples were collected from a number of direct relatives of one of the two patients in whom the original lesion was found. The genomic DNA of the members of the pedigree was subject to PCR amplification using primers which have the property of complementing genomic sequences to create a restriction site if and only if the mutated base is present. In an exactly analogous manner a second separate amplification is performed with a primer which complements a restriction site if the wild-type is present. The reciprocal nature of this analysis allows positive identification of all three states: mutant, wild-type, and heterozygote. Even where the restriction enzyme chosen does not cut completely, an accurate diagnosis can be made. A standard coagulation assay was performed which was consistent with the observed pattern of allelic distribution. In addition, the DNA of all subjects was amplified in the region of interest and those areas cloned into pBS and sequenced by the Sanger method. An analysis of a variety of typical cases suggests that this method is of wide applicability.

WH 125 **USE OF PCR TO DETECT SINGLE BASE MUTATIONS IN THE HUMAN DIHYDROFOLATE REDUCTASE (DHFR) GENE.** A.P. Dicker, M. Volkenandt and J.R. Bertino, Cornell University Graduate School of Medical Sciences, Dept. of Molecular Pharmacology, Sloan-Kettering Institute, N.Y., N.Y. 10021.
In order to detect single base mutations in the DHFR gene giving rise to methotrexate (MTX) resistance from blast cells obtained from patients with leukemia, we investigated the PCR as a potential sensitive method for this purpose. To test the method, we examined a human colon adenocarcinoma cell line resistant to MTX (HCT/R), with a known single base mutation (J. Biol Chem, in press) elucidated by cDNA cloning and sequencing (position 91, (T to C). Using first strand cDNA synthesis followed by PCR total cellular RNA was isolated via the NP-40 lysis method and used for cDNA synthesis with reverse transcriptase, dNTPs and 10 pmoles of the 3′ primer that anneals outside the coding region of the human DHFR. The RNA:DNA hybrid was used as a template for PCR with the addition of the 5′ primer and Taq I DNA polymerase. These primers flank the coding region of the human DHFR and define a region of 650 bases. The PCR reaction was carried out for 40 cycles resulting in full length transcripts in microgram amounts clearly visible by ethidium bromide staining on agarose gels. DNA was isolated and double-stranded DNA was sequenced by the chain-termination method using Taq polymerase. A single point mutation was discovered at position 91 (T to C) resulting in a substitution of serine for phenylalanine at codon 31. Sequence analysis indicated that this base transition results in the loss of Eco RI and Xmn I sites which was confirmed by restriction digests. We are able to amplify the DHFR transcript from as little as 8 picograms of mRNA equivalent to 6 cells for mutational analysis. This technique now allows analysis of other MTX resistant cell lines as well as leukemia cells from patients who are refractory to this drug.

WH 126 ANALYSIS OF GENE EXPRESSION USING PCR, APPROACHES TO SPECIAL PROBLEMS, Dieffenbach, Carl W.[1] and Jacobsen, Helmut[2] Department of Pathology, Uniformed Services University of Health Sciences, Bethesda, MD 20814[1]; Institute of Virus Research, German Cancer Research Center, Heidelberg, FRG
The methodology of PCR can be applied easily to solve 2 problems commonly encountered when examining gene expression. (1) Is gene of interest really silent or is the level of mRNA produced below the limit of detection of the Northern blot? When investigating the nature of the antiviral activity induced by tumor necrosis factor, no interferon mRNAs were detectable by Northern blots. Using PCR we were able to demonstrate that at least a portion of the antiviral activity was due to tumor necrosis factor mediated induction of human interferon beta 1. (2) If there is differential splicing in the gene of interest, which mRNA is produced? In the case of human plasma and cytoplasmic gelsolin, both forms are encoded by mRNAs of approximately 2.8 KB but differ in only the first 150-200 nucleotides, the 5`- noncoding region and the leader and signal peptide sequences. Using PCR we were able to identify which type of mRNA was present and examine the induction of each species of gelsolin RNA.

WH 127 DETECTION OF THE PRODUCT OF THE POLYMERASE CHAIN REACTION USING ALKALINE PHOSPHATASE-LABELED OLIGONUCLEOTIDE PROBES, Richard M. Donovan, William E. Lippert, Ruth E. Dickover, Satya Dandekar, Elliot Goldstein, and Charlene E. Bush, Dept. Medicine, University of California, Davis, CA 95616, and MicroScan/Baxter Corp., Sacramento, CA.

We report a procedure for producing and using enzyme labeled oligonucleotides hybridization probes to detect the polymerase chain reaction (PCR) product. Oligonucleotides were synthesized with a 5' free amino group. An excess of disuccimidyl suberate was added and reacted for 20 minutes at 4°C. The solution was desalted three times using a Centricon filter and concentrated. A two molar excess amount of alkaline phosphatase was added and reacted for 48 hours at 4°C. The conjugate was purified using a BioRad P-60 column followed by a DEAE Sephadex anion exchange column. Twenty five microliters of the reaction product of PCR was blotted onto nitrocellulose paper, allowed to dry, then baked for 80°C for 2 hours. The membrane was prehybridized at 50°C with a mixture of 5xSSC, 0.5% BSA, 0.5% PVP and 1% SDS. Hybridization mix was the above mixture including the alkaline phosphatase labeled probe. Hybridization was at 50°C for 20 minutes, followed by a wash at room temperature and twice at 42°C. Hybridized probe was detected colorimetrically using a nitro blue tetrazolium substrate system. We conclude that these stable, enzyme-labeled probes are useful for the detection of the PCR reaction product.

WH 128 THE FIDELITY OF *Taq* DNA POLYMERASE, Kristin A. Eckert and Thomas A. Kunkel, Laboratory of Molecular Genetics, National Institute of Environmental Helath Sciences, Research Triangle Park, NC 27709

The thermostable DNA polymerase from *Thermus aquaticus* (*Taq*) has widespread use as a reagent in molecular biology, particularly in amplification of genetic information by the polymerase chain reaction (PCR). Highly accurate DNA synthesis throughout the amplification process is desirable for some uses of the final PCR products. We have previously analyzed the fidelity of synthesis by the *Taq* DNA polymerase using M13mp2-based mutation assays (Biochemistry 27, 6008,1988). At 70°C and using standard reaction conditions, this enzyme produces both base substitution and frameshift errors at a rate of 1/10,000 and 1/40,000, respectively. However, the processivity and the rate of *in vitro* DNA synthesis by other DNA polymerases are known to be affected by reaction conditions, including $MgCl_2$ concentration, pH, temperature and the absolute and relative concentrations of dNTPs. Since PCR reactions are performed using a variety of conditions, we are examining the effects of these parameters on base substitution and frameshift error rates. With this information, the contribution of *Taq* polymerase errors to the final mutation frequency within a population of amplified DNA molecules can be estimated as a function of specific PCR reaction conditions.

WH 129 A SIMPLE ASSAY FOR GENE TARGETING PROTOPLASTS BASED ON THE POLYMERASE CHAIN REACTION, Keith J. Edwards, Alison S. Bird and Wolfgang Schuch. Plant Biotechnology Section I.C.I. Seeds, Jealott's Hill Research Station, Bracknell, Berkshire, United Kingdom.

Gene targeting in mammalian cells has been shown to occur using a variety of loci, however it has yet to be demonstrated in a plant system using a naturally occurring gene. We have developed an assay based on P.C.R. in order to investigate gene targeting in *Nicotiana tabacum*. The assay is simple to perform and control experiments indicate that it is possible to detect a single homologous recombination event in 10^6 genomes. Experiments are now under way to detect gene targeting into a Chlorophyll a/b binding gene locus.

WH 130 SYNTHESIS OF CHIMERIC HEPATITIS A VIRUS/POLIOVIRUS SUBGENOMIC cDNA BY A PCR MUTAGENESIS SYSTEM, Stephen Feinstone, Czeslaw Wychowski, Suzanne Emerson and Jonathan Silver, Hepatitis Viruses Section, Laboratory of Infectious Diseases and The Laboratory of Molecular Microbiology, NIAID, Bethesda MD 20892. Using a modification of the Eckstein mutagenesis technique (Taylor et al., Nucleic Acids Res., 1985) and PCR we have created a subgenomic cDNA chimera of hepatitis A virus (HAV) in which the precise HAV VP4 coding region was replaced by the VP4 coding region of Sabin type 1 poliovirus (PV1). The method involved the use of PCR primers for the PV1 VP4 gene that had HAV VP4 flanking sequences on their 5' ends. Single stranded DNA was produced by using a limiting amount of one of the primers in a PCR reaction (Gyllenstein and Erlich, PNAS,1988). This single stranded DNA was used like a mutagenic oligonucleotide on a single stranded phagemid containing the first 2070 bases of the HAV genome and mutagenesis was carried out by the Eckstein method. Clones were isolated that had the PV1 VP4 substituted for the HAV VP4 as determined by DNA sequencing. This method is rapid, efficient, overcomes most of the difficulties of earlier methods and has wide application.

WH 131 DETECTION OF BCR.ABL RNA ON CRUDE CELL EXTRACT BY A MODIFIED POLYMRASE CHAIN REACTION. Francois Ferre and Fermin Garduno, Cytometrics Inc., Division of Specialty Laboratories, Inc., 11575 Sorrento Valley Road, San Diego, CA 92121. The Philadelphia chromosome (Ph^1 chr) is found in over 95% of patients with chronic myelogenous leukemia (CML) and in 15% to 25% of patients with acute lymphoblastic leukemia (ALL). The Ph^1 chr is the result of a translocation which move the C-ABL gene from chr 9 to chr 22, 3' to a region called the breakpoint cluster region (BCR). This gives rise to a fusion gene, BCR.ABL, which is transcribed into a mRNA of about 8kb for CML and 7kb for ALL. The splice site is 3' to either BCR exon "2" or exon "3" in CML or exon "1" in ALL, and 5' to ABL exon II. We have developed a modified polymerase chain reaction (PCR) that amplifies BCR.ABL RNA from crude blood cell extract. The blood cells are boiled in water + DEPC and centrifuged. An aliquot of the supernate is used in a reverse transcription assay. The resulting c-DNA is amplified exponentially by a thermostable DNA polymerase, using unique primers homologous to flanking sequences 5' and 3' to the BCR.ABL splice sites. Oligonucleotide probes complimentary to the three most common BCR.ABL splice sequences are then used to detect the amplified DNA by Southern blot analysis. This assay is extremely rapid, sensitive (as few as $5x10^5$ patient cells has been used) and can be used to diagnose Ph^1-positive patients.

WH 132 "USING PCR TO CLONE GENES FOR INSULIN-LIKE GROWTH FACTOR I AND ITS SERUM CARRIER PROTEIN", J. Flynn, D. Mascarenhas and C. Talkington-Verser, BioGrowth, Inc. 3065 Atlas Road, Richmond, CA 94806.
A cDNA clone encoding insulin-like growth factor I (IGF-I) was isolated from a lambda gt11 human heart cDNA library using a combination of PCR and traditional techniques. The use of PCR facilitated (a) the selection of a cDNA bank containing the desired clone and (b) the actual screening and characterization of positive isolates. In an independent study, degenerate oligonucleotides corresponding to the known amino-terminal protein sequence of the growth hormone-dependent IGF carrier protein (IGF-CP) from human serum were used to amplify an IGF-CP-specific cDNA fragment from human liver mRNA. This fragment was cloned and used as a probe for screening cDNA libraries for full-length IGF-CP cDNA clones.

WH 133 DETECTION OF PARVOVIRUS B19 DNA IN HUMAN SERUM BY POLYMERASE CHAIN REACTION, Norbert Frickhofen and Neal S. Young, NHLBI, Bethesda, MD 20892.
Persistent infection with human B19 parvovirus causes chronic anemia and neutropenia in immunocompromised patients. To investigate the incidence of chronic B19 infections in patients with chronic cytopenias we used the polymerase chain reaction (PCR) to amplify virus DNA from patient sera before dot blot hybridization with a ^{32}P-labeled cloned viral probe (pYT103c). Oligonucleotide primers (21-25mers) complementary to eight different regions of the published B19 sequence were used in 14 combinations, spanning from 102 to 1902 base pairs. With eight primer combinations there was at least a 100000-fold amplification of cloned pYT103c DNA and DNA isolated from reference sera, with an optimal annealing temperature of 55-60°C. High amplification was possible with primer pairs giving rise to PCR products as long as 1650 nucleotides; the inverted terminal repeat regions were not useful for amplification. Brief preheating of serum at 55-75°C prior to its addition to the PCR reaction mixture resulted in a virus specific signal of comparable intensity to extracted DNA of serum; virus DNA could not be amplified from unheated serum. Elimination of DNA extraction of serum avoids a time-consuming preparation step prone to contamination and should facilitate routine screening of sera for B19 by PCR. As an example of the clinical application of this method, we demonstrated viral DNA in serial serum specimens of a patient with chronic B19 infection during early remission, when he had been judged to be free of virus by standard dot blot methodology. Immunoglobulin therapy was continued until virus in blood and marrow was undetectable by PCR, and the patient is now in unmaintained clinical remission.

WH 134 THE USE OF POLYMERASE CHAIN REACTION TO DETECT EXPRESSION OF mdr-1 RNA IN HUMAN TUMORS, Suzanne A.W. Fuqua, Saundra D. Fitzgerald, David H. Kern, William L. McGuire, Department of Medicine/Oncology, University of Texas Health Science Center, San Antonio, TX 78284.
A major problem in the treatment of cancer is the frequent appearance of multidrug resistance, in which tumor cells simultaneously develop resistance to a number of the commonly used chemotherapeutic drugs. In tissue culture cell lines, multidrug resistance is associated with the 170 kDa P-glycoprotein, the product of the mdr-1 gene, which is thought to function as an energy-dependent efflux pump resulting in lower intracellular drug accumulation. In actual human tumors, however, the level of drug resistance needed to interfere with effective treatment is much lower, and the expression of mdr-1 is usually very low or undetectable using the standard techniques of RNA slot blot, Northern blot, or Western blot.

We have now successfully employed the polymerase chain reaction to detect mdr-1 RNA in human tumors. Using 0.1-1 µg of total RNA, we can easily detect mdr-1 RNA in most colon tumors, which are notoriously drug resistant. We are presently determining the presence of mdr-1 RNA in a number of other human tumors whose drug resistance or sensitivity has been determined by in vitro sensitivity assays, with the goal of evaluating whether detection of mdr-1 RNA by polymerase chain reaction will be effective for predicting multidrug resistance in human cancer.

WH 135 DETECTION OF HUMAN PAPILLOMAVIRAL DNA IN ORAL MUCOSA USING PCR. Denise A. Galloway, Audrey Christiansen, Christopher Maden, Janette Valentine, Steven A. Jenison and Anna Marie Beckmann. Fred Hutchinson Cancer Research Center, 1124 Columbia St., Seattle, WA. 98104.

There are many aspects of the natural history of human papillomavirus (HPV) infections of the genital tract that remain poorly understood. Estimates of the prevalence of HPV infections have been based on the detection of viral nucleic acids following sampling of various anogenital sites. The use of PCR has greatly increased the sensitivity of detection of HPV DNA and has contributed to the conclusion that infection with HPV types 6 and 16 is widespread. We have developed serologic assays which support this conclusion. As part of a study to determine whether HPV plays a role in the etiology of oral carcinomas we have obtained oral swabs from cases and from a control population obtained by random digit-dialling. DNA was prepared from the samples and analyzed for HPV 6 and HPV 16 DNA. PCR was performed using primer pairs (25 mers) from the E6/E7 region of the genome. Following 35 cycles of amplification the products were analyzed by electrophoresis in NuSieve agarose and by hybridization with a ^{32}P end-labelled internal oligonucleotide probe (40 mer). Of 40 samples analyzed to date 3 were positive for HPV 6 and 14 were positive for HPV 16, and the positives were distributed equally between cases and controls. Additional samples from this study are being analyzed, as well as samples from normal children. These results suggest that HPV types 6 and 16 are widespread in the population and may commonly infect oral mucosa, raising important questions about the mode of transmission.

WH 136 ASSESSMENT OF GENETIC DIVERSITY IN GORILLAS BY AMPLIFICATION OF MITOCHONDRIAL D-LOOP DNA. K.J. Garner and O.A. Ryder, Center for Reproduction of Endangered Species, Zoological Society of San Diego, San Diego, CA 92112.

Management of populations of endangered species requires an estimate of the level of genetic diversity present within and between populations. Also, pedigree information is needed for evaluation of the reproductive success of individual animals. The polymerase chain reaction (PCR) technique should prove extremely valuable for acquiring genetic information from wild animals in situations where hairs are the only biological samples readily obtainable.

In order to begin to develop primers that will be suitable for use with hair samples from wild gorillas, we have determined the nucleotide sequences of the mitochondrial D-loop region from seven western lowland gorillas. We have used primers located within the threonine tRNA and 12s rRNA coding regions (T.D. Kocher, et al. submitted manuscript) to amplify purified genomic DNA using PCR, and have determined nucleotide sequences after cloning into M13 vectors. Five distinct forms were found, all differing at multiple sites from a published gorilla sequence (D.R. Foran, et al., 1988). Pairwise comparisons of these six sequences over regions totalling 535 base pairs yielded nucleotide differences averaging 5.6% and ranging from 1.5% to 11.0%. Conserved and variable regions within the D-loop are becoming apparent. We are using this sequence information to select primer sequences for the amplification of DNA from hairs.

WH 137 ANALYSIS OF *pro*1 ACTIVITY DOMAIN BY DELETION MAPPING AND PCR-SITE DIRECTED MUTAGENESIS. Robert R. Garrity[1], John L. Seed[2] Glenn C. Hegamyer[2] and Nancy H. Colburn[2],[1]BCDP, PRI, NCI-FCRF, Frederick, MD 21701-1013; [2]Cell Biology Section, LVC, NCI-FCRF, Frederick, MD 21701-1013.

The murine gene *pro*1 has been cloned from JB6 epidermal cell lines which are sensitive to neoplastic transformation by tumor promoters. Insensitive JB6 variants acquire susceptibility to neoplastic transformation by promoters when transfected with *pro*1. Analysis of RNA from promotion sensitive cells revealed the presence of a small *pro*1-hybridizing transcript. Strand-specific RNA probes implicated an RNA polymerase III (RNAPIII) coding domain in *pro*1 as the source of this hybridization signal. Deletion of this domain from the probe eliminated the signal. RNase protection of gel-purified, *pro*1-hybridizing RNA from JB6 variant cell lines identified a 130 nucleotide transcript. The size of this transcript was corroborated in vitro by RNAPIII transcription of *pro*1. Deletion mapping of *pro*1 by exonuclease III demonstrated that the biologically active domain included the RNAPIII transcription unit. To confirm the functional significance of the *pro*1 RNAPIII transcript in the tumor promotion pathway, the intragenic RNAPIII B block consensus of *pro*1 was altered using PCR-site directed mutagenesis. These B block mutations inhibit RNAPIII transcription. The activity of these mutants in the tumor promotion pathway will be determined by transfection into promotion insensitive variants. To elucidate an activation mechanism, the active and inactive forms of the gene have been amplified and are being sequenced. Amplification and sequence analysis of *pro*1 RNA is also focused on *pro*1 activation. Understanding regulation of pro gene expression may provide the basis for preventing tumor promoter induced cancer induction.

Abstract Withdrawn

WH 139 ANALYSIS OF ALLELIC VARIATION AT THE HLA DQA, DPB LOCI IN SYSTEMIC LUPUS ERYTHEMATOSUS. Gilles, K., Newman, B., and King, M.C. University of California, School of Public Health, Berkeley, CA 94720

The genetics of SLE susceptibility are complex with genetic alterations related to immunologic function very likely to be involved. Therefore, we are analyzing the association between allelic and sequence variation in HLA genes and susceptibility to SLE. To determine the molecular basis for this disease we are examining the same gene segment in SLE patients and healthy controls by using the polymerase chain reaction and allele-specific oligonucleotide probes for HLA DQA and DPB. This will contribute to understanding the fundamental causes of SLE and to early identification of susceptible individuals.

WH 140 TRANSCRIPTION-BASED AMPLIFICATION SYSTEM AND THE DETECTION OF ITS RNA PRODUCTS BY A BEAD-BASED SANDWICH HYBRIDIZATION SYSTEM. Thomas Gingeras, Geneva Davis, Hugh Chappelle, Kristina Whitfield, Luke DiMichele, and Deborah Kwoh. Nucleic Acid Chemistry Dept., The Salk Institute Biotechnology/Industrial Associates, Inc., 505 Coast Blvd. S., La Jolla, CA 92037. The in vitro amplification of specific target nucleic acids (RNA or DNA) can proceed by means of an RNA transcription-based amplification system (TAS). In comparison to the PCR method of amplification, which amplifies by a DNA replication mechanism, TAS achieves similar levels of amplification, primarily by an RNA transcription mechanism. Increases in the copy number of 10^5- to 10^6-fold are routinely observed in 3 to 6 cycles of TAS. The specificity of the TAS amplification can be enhanced by the use of a bead-based sandwich hybridization system (BBSHS). The single-stranded nature of the RNA product fits well with this detection formation and permits quantitative detection of the amplified product. The efficiency, reproducibility, and shortcomings of both the TAS and BBSHS technologies will be discussed. Additionally, both technologies have been applied to the quantitative detection of HIV-1 sequences present in samples of AIDS patients. The results and conclusions from these studies will be reported.

WH 141 AMPLIFICATION OF cDNA BY THE POLYMERASE CHAIN REACTION : THE CLONING OF HOMOLOGOUS GENES USING DEGENERATE OLIGONUCLEOTIDES AND SIZE SELECTION. R.W. Graham and E.P.M. Candido, Dept. of Biochemistry, University of British Columbia, Vancouver, B.C., Canada. V6T 1W5

The isolation of cDNA clones from multiple organisms by conventional methods involves preparation of high quality cDNA libraries from each organism. This technique is time consuming and requires skilled manipulation of small quantities of nucleic acid. We are developing a method for the rapid cloning of genes utilizing the polymerase chain reaction. The substrate for amplification is homopolymer tailed first strand cDNA which has been primed with a modified oligodT. Single genes may be amplified using two gene-specific degenerate oligonucleotides or a single oligonucleotide and the appropriate 5' or 3' terminal oligonucleotide of the cDNA population. It is also possible to amplify the entire library using oligonucleotides directed to the sequences at the 5' and 3' ends of the cDNA population. Amplification of the bulk cDNA allows ready preparation of mini size-selected libraries which may be screened with DNA probes for homologous genes.

The polymerase chain reaction is thus a powerful tool for the rapid isolation of genes(especially those with rare transcripts) and avoids most of the complications associated with construction of conventional cDNA libraries in bacteria.

WH 142 GROUP SPECIFIC AMPLIFICATION OF HPV SEQUENCES, L Gregoire[1], M Arella[2], J Campione-Piccardo[3], WD Lancaster[4]. Univ. Ottawa, Ottawa, Ont., Canada[1], Inst. Armand Frappier, Laval, Quebec, Canada[2], LCDC, Ottawa, Ont., Canada[3], Wayne State Univ., Detroit, MI[4]. PCR has potential for detection of human papillomavirus (HPV) because of the small amount of virus nucleic acids present in clinical specimens. Furthermore, new HPV types for which no probes exist remain undetected by conventional hybridization techniques. Universal primers corresponding to highly conserved HPV sequences may be useful for detecting low amounts of viral DNA as well as these new HPV types. Here we analyze the specificity of a pair of universal primers for HPV sequence amplification using PCR. We reported previously the longest perfect homology among HPV sequences is a 12 mer (UNI) within the first exon of E1M. A region of conserved amino acids coded by the E1 ORF allowed the detection of another highly conserved region (WDO) about 850 bp downstream from UNI. Two 21 mers containing UNI and WDO were used to amplify sequences from all HPV types used as templates. The amplified DNA was shown to be specific for HPV sequences within the E1 ORF. PCR results correlated well with clinical specimens typed by Southern blot. PCR also detected positive samples which were previously negative by Southern blot. These reagents may be useful for demonstrating low concentrations of HPV sequences in clinical specimens as well as to be able to identify new HPV types.

WH 143 INTERCALATING DRUGS MARKEDLY AFFECT THE ABILITY OF THE E. coli RecA PROTEIN TO INSERT SMALL PRIMERS INTO HOMOLOGOUS DUPLEX DNA, Jack D. Griffith and Randy Thresher. Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, N.C. 27514. The RecA protein of E. coli binds single stranded DNA and then scans millions of base pairs along a double stranded DNA searching for a region of homology between the duplex DNA and the single stranded fragment (or oligonucleotide primer). Once homology is found, RecA protein will catalyze the insertion of the single stranded DNA into the duplex DNA to form a stable D-loop. In the presence of DNA polymerases and accessory proteins this D-loop should serve as a primer for extension of the single stranded primer. We are investigating the effects of intercalating drugs such as ethidium bromide, acridine orange, and m-AMSA in changing the properties of these reactions. We find that in the presence of m-AMSA and ethidium bromide a very stable complex of RecA protein and double stranded DNA forms in the absence of nucleotide cofactors and that this complex can carry out a search for homology in the absence of ATP hydrolysis. Other drugs such as adriamycin are potent inhibitors of single strand DNA binding while not apparently affecting double strand binding. It is possible that a combination of RecA protein and drugs could substantially promote initial amplifications in PCR reactions.

WH 144 **AN AMPLIFICATION-BASED STRATEGY FOR THE DETECTION OF SPECIFICALLY SPLICED mRNAs ENCODING REGULATORY GENES OF HIV-1.** John Guatelli,[1] Nanette Riggs,[2] Douglas Richman,[1] and Thomas Gingeras.[2] [1]Depts. of Medicine and Pathology, UCSD School of Medicine, San Diego, CA 92161. [2]The Salk Institute Biotechnology/Industrial Associates, Inc., 505 Coast Blvd. S., La Jolla, CA 92037. The differential detection of mRNAs encoding the HIV-1 regulatory genes tat, rev, and nef is complicated by the similar sizes of these transcripts and their large regions of shared nucleotide sequence. The presence of gene-specific splice events, however, can be detected by primer-directed nucleic acid amplification. For example, one primer is complementary to the sequence immediately 5' of the splice donor common to all processed RNAs (5' of the gag start codon). The second primer is complementary to the sequence at the 3' end of the tat and rev 5' coding exons. Between these two primers lie three splice acceptors whose use has been suggested to lead to specific mRNAs encoding tat, rev, and nef. Amplification should lead to products of 290, 108, and 92 base pairs in length, reflecting tat, rev, and nef mRNAs, respectively. Products of these lengths have been detected using specific oligonucleotide probes. Interestingly, several unexpected amplification products have also been detected. The characterization of these amplification products will be described.

WH 145 GENERATION OF SINGLE STRANDED DNA SEQUENCING TEMPLATES BY THE POLYMERASE CHAIN REACTION. Ulf B. Gyllensten. Department of Medical Genetics, University of Uppsala Biomedical Center, Box 589, S-751 23 Uppsala, Sweden. The polymerase chain reaction (PCR) provides a convenient method for generating suitable templates for direct sequencing by the chain termination method. Single stranded templates may be generated both from genomic and cloned templates by one of several methods: (A) Assymetric PCR, where the ratio of primers is initially unbalanced resulting in an excess of ssDNA of one specific strand at the end of the reaction, (B) Blocking PCR, where during the amplification the complement of one of the primers is introduced, resulting in priming of only the other strand in subsequent cycles, and (C) Primer extension on a purified PCR product using one of the PCR primers. Procedure (A) is normally preferred since it requires only a single PCR and no removal of excess oligonucleotides prior to sequencing.

WH 146 FACTORS INFLUENCING THE SPEED, EXTENT, AND VARIABILITY OF THE POLYMERASE CHAIN REACTION, Haff, L. and Katz, E., Biotechnology Department, Perkin-Elmer Corporation, Norwalk, CT 06859

Factors which influence the extent and reproducibility of amplification of a 500 base-pair product from lambda DNA were systematically examined. The effects of temperature overshoots and undershoots at annealing, extension, and denaturation steps were not equal. Temperature undershoots and overshoots at the denaturation step profoundly effected both amplification yield and variability. Total amplification time could be brought below 1 hour and the relative standard deviation of amplification lowered below 5% by incorporating the following changes to the standard PCR protocol: 1) combining the reannealing and extension steps 2) reducing plateau times to under 30 seconds 3)substituting higher concentrations of Taq DNA polymerase 4) adding autosegment extension 5) running fewer cycles.

WH 147 BIOPSY OF HUMAN PREIMPLANTATION EMBRYOS AND SEXING BY DNA AMPLIFICATION, A.H. Handyside(1), J.K. Pattinson(2), J.D.A. Delhanty(3), R.M.L. Winston(1) and E.G.D. Tuddenham(2). (1) Royal Postgraduate Medical School /Institute of Obstetrics & Gynaecology, Hammersmith Hospital, Du Cane Road, London W12 OHS. (2) Haemostasis Research Group, Clinical Research Centre, Watford Road, Harrow. (3) Galtron Laboratory, Department of Genetics and Biometry, University College, 4 Stephenson Way, London NW1 2HE. The detection of inherited diseases in very early preimplantation embryos would allow the selection and transfer of only healthy zygotes to the uterus. Following preimplantation diagnosis, couples with a high risk of a genetically defective baby could embark on a pregnancy, knowing it was free from a specific serious inherited disorder. These parents would avoid the dilema of a therapeutic abortion of a much wanted but affected fetus following prenatal diagnosis later in gestation. Following in vitro fertilization, thirty normally fertilized human preimplantation embryos were biopsied at the 6-10 cell cleavage stage three days after insemination. During biopsy, a single cell was removed through a hole made in the zona pellucida. A normal proportion of biopsied embryos (37%) hatched from the zona. Each male embryo was sexed from the DNA of the biopsy by amplifying a repeated sequence specific for the Y chromosome. In 15 normally fertilised embryos we subsequently confirmed the sex of the biopsied embryos in situ hybridisation with a Y specific probe and/or flourescent chromosome staining to detect metaphase Y chromosomes. In all of these embryos, the sex determined by Y specific DNA amplification of the biopsy was in agreement with that obtained using alternative methods. This approach may be valuable for couples at risk of transmitting X-linked disease.

WH 148 RNA ISOLATION FOR PCR OF BCR-ABL REARRANGEMENTS FROM CML SAMPLES WITH MINIMAL CELL NUMBERS, Susanna Hegewisch-Becker, Mitchel Smith, Arlene Redner, David Wisniewski, Bayard Clarkson and Michael Andreeff, Memorial Sloan- Kettering Cancer Center, New York, NY 10021.
DNA polymerase chain reaction (PCR) has the potential of detecting small numbers of cells with bcr-abl rearrangements characteristic of chronic myeloid leukemia (CML). The main limitation in the clinical application of PCR is the requirement of large total cell numbers (ca. 10^6-10^7 cells). We utilized the RNAzol™-method (Analy. Biochem. 162, 156-159, 1987) to extract total cellular RNA from K562 or patient cells, added t-RNA, synthesized c-DNA and performed PCR. One tenth of PCR amplified c-DNA was analyzed by Southern blot using probes for the bcr exon 2 or bcr exon 3/abl exon II junction sequences. Dilution experiments using bcr-abl negative HL-60 or Daudi cells with bcr-abl rearranged K562 or CML patient cells allowed detection of as few as 1:200.000 bcr-abl rearranged cells. Using decreasing total cell numbers, the bcr-abl rearrangement could be detected in one tenth of amplified c-DNA from as few as ten K562 cells. This makes possible the detection of rearranged bcr-abl in colonies of leukemic cells in-vitro, in bone marrow cells from patients after ablative therapy, bone marrow transplantation or after ex-vivo purging, and in FACS sorted subpopulations of CML cells.

WH 149 SENSITIVITY AND SPECIFICITY OF HIV-I GAG AND ENV GENE DETECTION IN PLASMA AND SERUM OF HIV-I INFECTED INDIVIDUALS BY THE POLYMERASE CHAIN REACTION: Indira K. Hewlett, C. Ann Hawthorne, Martin Ruta, Robert A. Gregg, Ronald E. Mayner Richard T. Schumacher, Jean-Pierre Allain, and Jay S. Epstein, Division of Blood and Blood Products, Food and Drug Administration, Bethesda, Md. 20892; Boston Biomedica Inc. Mansfield, MA; Abbott Laboratories, North Chicago, Illinois
HIV-I specific primer pairs have been assayed for their specificity and sensitivity using DNA extracted from plasma and serum of infected individuals in a polymerase chain reaction based detection system. DNA extracted from 300 sera and 250 plasma samples was analyzed by PCR using gag and env primers (Cetus) in the same reaction mixture (co-amplification). Sensitivity of detection using the two primer pairs was 100% for AIDS and ARCS patients while for healthy, asymptomatic, seropositive individuals it was 96% for both plasma and serum. In these reactions the gag primers were 96% specific whereas 100% specificity was observed for the env primers. The reaction wih the gag primers and prope was more efficient than that with the env. Samples assayed for specificity included sera from patients with viral infections such as HTLV-1, HTLV-2, EBV, CMV and HBV. One patient with EBV and one with HBV were cross-reactive with only the gag primers. Samples obtained from prospective bleedings will be tested to determine their HIV status by PCR since these individuals may belong to a high-risk group. Confirmatory testing of plasma and serum by PCR requires positive detection by atleast two primer pairs to different regions of the viral genome and this may be performed with accuracy and convenience in a co-amplification assay.

WH 150 NEW METHODS FOR LABELING NUCLEIC ACIDS WITH REPORTER GROUPS. George L Trainor, Frank W. Hobbs*, Kenneth J. Livak, K. Steve Kornher, Paul R. Johnson, Mark A. Jensen, and Peter N. Korolkoff, Central Research and Development Department, E. I. Dupont de Nemours and Co., Wilmington, DE, 19880. Technology for enzymatic and chemical labeling of nucleic acids will be presented with emphasis on applications involving PCR. Novel fluorescence-tagged and biotinylated nucleoside triphosphates have been prepared which are substrates for a variety of DNA polymerases including Taq polymerase. Nucleic acids, including those amplified by PCR, can be labeled with these substrates. The electrophoretic mobility of nucleic acids containing these modified nucleotides is perturbed by the presence of these reporters. An assay using the "mobility shift" induced by modified substrates has been developed which is capable of detecting a single base mutation *anywhere* in a region being amplified by PCR. A series of new phosphoramidites has been developed for automated synthesis of oligonucleotides bearing a 5'-fluorescent dye. Using these phosphoramidites, preparation of labeled primers for PCR is nearly as convenient as the preparation of conventional primers. The above chemical and enzymatic methods for labeling nucleic acids can be done with any one of a series of four, nearly identical but spectroscopically distinguishable, fluorescent dyes. The extreme similarity of this set of dyes makes them particularly useful for assays requiring more than one reporter.

WH 151 APPLICATION OF PCR/ASO ANALYSIS TO CLINICAL GENETIC TESTING.
Glenn Horn, Brenda Richards, Kathleen Hehir, Robert Rochelle, Barbara Handelin, and Katherine Klinger. Department of Human Genetics, Integrated Genetics, Framingham, MA.

The Genetic Reference Laboratory (GRL) at Integrated Genetics has provided RFLP-based clinical testing services since 1986. Recently, we have been investigating the application of the PCR DNA amplification method to clinical genetic testing.

A number of general issues have been considered, including the collection of patient samples, the extraction and storage of DNA, the accurate amplification and analysis of patient DNA samples and known controls, the prevention and detection of contamination, and the use of non-radioactive ASO probes. Of particular challenge has been the consideration of PCR tests for situations where it is impossible or impractical to directly test for the disease mutation(s). Here the capability of PCR/ASO tests to provide same-day results allows the application of a multi-stage analysis, where PCR/ASO is first used to screen for the more frequent disease mutations (or linked polymorphisms), and then only the less frequent mutations (or polymorphisms) are analyzed by PCR or on Southern blots.

More specifically, genetic polymorphisms linked to cystic fibrosis, Huntington's disease, Duchenne's muscular dystrophy, thalassemia, and hemophilia have been analyzed and converted to a PCR format. Genetic tests have been performed on DNA extracted from a variety of sources, including formaldehyde-fixed tissue sections. We will discuss these specific applications as well as the general aspects of genetic testing by PCR/ASO analysis.

WH 152 GENERATION OF RECOMBINANT DNA MOLECULES AND SITE-DIRECTED MUTANTS USING THE POLYMERASE CHAIN REACTION: GENE SPLICING BY OVERLAP EXTENSION.
Robert M. Horton, Henry D. Hunt, Steffan N. Ho, Zeling Cai, Jeffrey K. Pullen, and Larry R. Pease. Department of Immunology and Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN 55905.

Gene Splicing by Overlap Extension (SOE) is a novel way to modify and recombine DNA sequences using PCR. The key to this process is the "fusemer" primer which allows the product from a PCR reaction to itself be used as a primer in a subsequent 'SOE' reaction, forming the recombinant product. SOE has a significant advantage over current methodologies in that it is sequence-independent; no restriction enzyme sites are necessary. We have used 'gene SOEing' to perform site-directed mutagenesis as well as to generate a variety of tailor-made constructs, including genes for fusion proteins in which functional regions are switched among MHC molecules. This fast and technically easy technique should prove to be the method of choice for many genetic engineering projects, and we believe it represents the beginning of a new generation of recombinant DNA technology.

WH 153 MOLECULAR CHARACTERIZATION OF CHINESE β-THALASSEMIA MUTATIONS BY AMPLIFIED DNA SAMPLING FROM DRIED BLOOD,
Shu-zhen Huang, Xia-di Zhou, Hao Zhu, Zhao-rui Ren and Yi-tao Zeng, Laboratory of Medical Genetics, Shanghai Children's Hospital, Shanghai, P.R.China
A total of 170 β-thal genes originating from eastern China,southwestern China and southern China (Canton) were amplified directly from dried blood samples to be performed molecular characterization.The amplified sequences included nine Chinese β-thal mutation points. Nine sets of oligonucleotide probes specific for the known Chinese β-thal mutants were hybridized with the amplified sequence.The result revealed:(1)The different types of β-thal mutation were distributed in the different regions,the most commom types in southern China were codons 41-42 -4bp and IVS-II n.654 C→T,constituting nearly 2/3 of the total in this area; the most frequent types in southwestern China were codon 17 and IVS-II n.654 mutations, accounting for 60% of the frequency; and in eastern China,the most prominent mutations were frameshifts at codons 41-42 and 71-72,giving the frequencies as 29.7% and 25.0%,respectively.(2)All the mutants from Canton belonged to the known Chinese mutation types; while 14% of the mutants from eastern China remained unknown.The result is very useful to plan the prenatal diagnosis programs for β-thalassemia. DNA PCR amplification from dried blood provides a rapid and simple method for investigation of the β-thal mutations at large scale.

WH 154 **CHROMOSOME MAPPING BY PCR**, RD Iggo, DP Lane, A.Gough and NK Spurr, ICRF, Clare Hall, South Mimms,EN6 3LD, UK.

The human p68 protein is an RNA dependant ATPase and a putative RNA helicase. It is a member of a newly discovered gene family of proteins that includes the eukaryotic translation initiation factor eIF4-A, the drosophila maternal effect gene *vasa* and a yeast gene required for the splicing of mitchondrial transcripts. As p68 is very highly conserved in evolution and a member of a multigene family, conventional mapping by southern blotting proved cumbersome; therefore we have exploited PCR reactions across putative introns. This combined with the use of chromosome mediated gene transfer derived cell lines has allowed the rapid mapping of the chromosomal location of the human gene for p68. The use of PCR reactions to map human genes is of widespread application.

WH 155 CHARACTERIZATION OF FACTOR X DEFICIENCY MUTATION BY USE OF INEXPENSIVE PCR DEVICE, Pudur Jagadeeswaran, Kavala J. Rao and Zi-Qiang Zhou, Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284. Human factor X is a zymogen of serine protease which participates in the blood coagulation cascade. The deficiency of human factor X is called Stuart-Prower factor deficiency. At present nothing is known about the mutations causing the disease. Recently we have characterized the factor X gene structure. The gene is approximately 27 kb and contains 8 exons. In this abstract we present the characterization of the mutation in one of the alleles of the factor X deficient patient who has 14% Factor IX activity of the normal individuals. We have synthesized oligonucleotides corresponding to the exons of the factor X gene in opposing directions and used them in amplifying the exons of factor X gene by polymerase chain reaction. The amplification was done by a simple device which utilizes the principle of circulating water of different temperatures in a time dependent manner heating the aluminum eppendorf tube sitting in a plexiglass jacket in which the reaction vessel is placed. Two of the primers corresponding to the "h" exon of factor X gene gave a 500 bp fragment which was sequenced by the dideoxy sequencing method and a mutation CGC to TGC which alters Arg_{366} to Cys_{366} in the catalytic site of the molecule. This alteration also destroys HinP 1 site and creates Apa L1 site. We propose to name this mutation as Factor $X_{San\ Antonio}$ following the conventional nomenclature. Also this alteration leads us to propose a potential model for alteration of loop/kringle structures in the factor X protein which might alter interaction of factor X with other clotting factors.

WH 156 DETECTION OF HEPATITIS A VIRUS IN CLINICAL SPECIMENS BY IMMUNOAFFINITY CAPTURE FOLLOWED BY ENZYMATIC AMPLIFICATION OF NUCLEIC ACID: A "GENE CAPTURE ASSAY", Robert W. Jansen and Stanley M. Lemon, Department of Medicine, University of North Carolina, Chapel Hill, NC 27599-7030. We developed a single-tube immunoaffinity purification/gene amplification method for detection of hepatitis A virus (HAV), a medically important RNA-containing picornavirus. Virus present in clinical samples was captured by specific monoclonal antibody coating the walls of a reaction tube, and extraneous contaminants were removed by extensive washing. RNA was released from virions by heat denaturation in a buffer suitable for both reverse transcriptase-mediated cDNA synthesis and Taq polymerase-directed DNA primer-extension (polymerase chain reaction, PCR). Amplified transcripts were analyzed on ethidium-stained agarose gels, by Southern hybridization, and by direct dideoxynucleotide sequencing. When applied to fecal specimens from an outbreak of hepatitis A, the method proved much more sensitive than previous cDNA-RNA hybridization or immunoassay approaches. Viral RNA was detected in 19 of 20 specimens collected in the week following onset of symptoms, and in 5 of 6 specimens that were negative by hybridization. A single amplification product of the expected size was obtained from each positive sample, the absence of stochastic transcripts reflecting the relative purity of the target template and the additional level of specificity conferred by immunoaffinity capture. Direct DNA sequencing of the amplified transcripts (obtained from 50 μl of a 20% fecal suspension) identified sequence variations unique to the particular strain of HAV involved in this outbreak, suggesting that this approach may be very useful for molecular epidemiologic studies and for evaluation of vaccine recipients.

WH 157 cDNA CLONING STRATEGIES USING THE POLYMERASE CHAIN REACTION: LIBRARY SCREENING AND CLONING OF 5' ENDS BY PRIMER EXTENSION Ruud Jansen, Fred D. Ledley. Howard Hughes Medical Institute, Departments of Cell Biology and Pediatrics, Baylor College of Medicine, Houston, TX 77030.

We have employed several applications of PCR in the course of cloning the cDNA for human methylmalonyl CoA mutase (MCM). These include direct screening of cDNA libraries for "full length" clones and cloning of material generated by primer extension. Screening of a human liver library in the vector λgt11 was performed using oligonucleotides corresponding to the (antisense) sequence of an incomplete MCM cDNA clone and oligonucleotides to sequences in the lacZ gene of the λgt11 vector. Ten ul of a phage stock with a titer of $3x10^{11}$ was used as template and 30 cycles of the PCR were performed. MCM cDNA in the amplified material was visualized by southern blotting and cloned after digestion with EcoRI and a restriction site known to exist 5' to the oligonucleotide sequence in the cDNA. In order to authenticate the 5' extent of the cDNA clone, primer extension was performed on hybrid selected mRNA, followed by tailing with dG of the (-) strand cDNA using terminal transferase and the PCR was performed with an oligonucleotide consisting of oligo-dC with a XhoI linker and oligonucleotides in the known cDNA sequence. The resulting PCR products were cloned and sequenced. Library screening using PCR represents an extremely rapid method for identifying rare cDNA species, which enabled us to "screen" a total of $3x10^9$ phage representing approximately 2 million independent recombinants and identify a cDNA representing only $1x10^{-5}$ of all clones. The PCR-primer extension method enabled us to clone and sequence the "full length" of the MCM mRNA which is not possible with conventional primer extension and sequencing methods.

WH 158 ANALYSIS OF ESTROGEN RECEPTOR EXPRESSION IN RAT FRACTURE HEALING BY POLYMERASE CHAIN REACTION AMPLIFICATION Michael E. Joyce, Scott Boden, Barry Oliver, Ahlke Heydemann, and Mark E. Bolander, Orthopaedic Research Unit, NIAMS, NIH, Bethesda, Md. 20892

Osteoporosis, a disease characterized by significant loss of bone mass and an increased incidence of fracture, affects up to 25% of postmenopausal women. Since administration of exogenous estrogen slows the loss of bone mass in postmenopausal osteoporotic women, it is likely that estrogen is necessary to maintain normal bone density. Although a direct role of estrogen on bone metabolism, mediated via the estrogen receptor (ER), has been demonstrated *in vitro* and in pathologic bone *in vivo*, evidence of direct role for estrogen in a normal physiologic *in vivo* process such as fracture healing is lacking. Using traditional northern analysis it was possible to detect the ER message in uterus but not in fractured bone. Using reverse transcription followed by Polymerase Chain Reaction amplification (PCR) we were able to detect the estrogen receptor message in total RNA extracts from rat femur fractures at levels approximately 1/10 of that detected in the rat uterus. Verification of correctly amplified cDNA fragments was provided by restriction digestion. PCR, however, failed to detect ER message in total RNA extracts from unfractured bone, tendon, brain, and ear. These data demonstrate that the ER gene is expressed during fracture healing and suggest that estrogen may have a direct role, via its receptor, in bone formation and replacement in a physiologic *in vivo* system.

WH 159 APPLICATIONS OF THE DIGOXIGENIN-SYSTEM FOR SENSITIVE AND SPECIFIC NUCLEIC ACID DETECTION INCLUDING PCR, Christoph Kessler, Rudolf Seibl, Rüdiger Rüger and Gregor Sagner, Boehringer Mannheim GmbH, Biochemical Research Center, Nonnenwald 2, D-8122 Penzberg (FRG).

We applied the novel highly sensitive digoxigenin-system with digoxigenin-derivatised dUTP for Klenow-catalyzed enzymatic labeling in dot-, slot- or Southern-blots for specific detection of 0,1 pg DNA avoiding any significant background. With the enzymatic labeling system on the average every 36th nucleotide is modified. The high sensitivity of the digoxigenin-system has been applied for single-copy gene detection as well as virus detection in serum or in fixed cells by in-situ hybridization. We used the photoreactive digoxigenin-derivative photodigoxigenin for photochemical labeling of DNA and RNA too. With the photochemical labeling reaction on the average every 200th to 400th nucleotide is modified resulting in a sensitivity of detection of 1 pg. Because the size of the input DNA or RNA is not altered by digoxigenin modification, this labeling system is predestinated to be applied for synthesis of molecular weight markers. We also will present data on the application of the digoxigenin-system in the polymerase chain reaction (PCR). The digoxigenin-modified dUTP is accepted by Taq DNA polymerase as substrate for chain elongation; the intermediate digoxigenin-labeled DNA functions as template for the following PCR cycles. The resulting digoxigenin-labeled amplified DNA can be detected by the anti-digoxigenin ELISA reaction. Alternatively, the PCR cycles may occur in the absence of labeled nucleotides; in this case specific detection of amplified sequences is achieved by hybridization with digoxigenin-modified oligonucleotides and subsequent anti-digoxigenin ELISA reaction.

WH 160 SEQUENCE INDEPENDENT SINGLE PRIMER AMPLIFICATION (SISPA): UTILITY AND APPLICATION TO THE DETECTION OF ENTERICALLY TRANSMITTED NON-A,NON-B HEPATITIS (ET-NANBH), J. P. Kim, M. Purdy, C. Huang, K. Luk, K. E. Fry, L.Young ,D. Bradley and G. R. Reyes, Molecular Virology Department, Genelabs Incorporated, Redwood City, CA 94063 and Hepatitis Branch, Centers for Disease Control, Atlanta GA 30333

A technical modification to permit the amplification of heterogeneous low complexity DNA populations has been devised. Sequence Independent Single Primer Amplification (SISPA) utilizes a single primer (A) of a linker/primer pair (AB) to amplify all molecules to which AB has been ligated. The AB linker/primer is designed to directionally ligate onto blunt ended molecules. Self-ligated AB dimers reconstruct a rare cutting restriction endonuclease site which aids in their removal. SISPA was used to analyze ET-NANBH infected specimens to facilitate detection of ET-NANBH related sequences. DNA fragments of defined length were amplified from SISPA-cDNA samples prepared from different ET-NANBH infected specimens. This result suggests that the causative agent of ET-NANBH or water-borne hepatitis is a single common endemic agent. Our data indicate that SISPA is useful in the amplification and detection of rare sequences present in minute amounts in minimally complex biological specimens.

Posters-II

WH 200 PCR SEXING BOVINE EMBRYOS USING Y CHROMOSOME SPECIFIC SEQUENCES, Marek Kirszenbaum, Marcel Vaiman,CEA-IPSN-DPS-SPE-Laboratoire de Radiobiologie Appliquée, 78350 Jouy en Josas, France. Corinne Cotinot, INRA-Unité d'Endocrinologie Cellulaire et Moléculaire,78350 Jouy en Josas, France, Marc Fellous, Institut Pasteur-INSERM U 276, 75015 Paris, France. The sex determination of fresh or frozen bovine embryos before transfer would be of considerable economic advantage. From bovine plasmid library enriched for Y specific DNA sequences we have cloned and characterized the 50 bp male specific probe (pBC1.2). Hybridization pattern on Southern blot of bovine genomic DNA digested with different enzymes showed one strong male specific band. Interspecies somatic cells and chromosomal *In Situ* hybridization studies confirmed that BC1.2 sequence derived from bovine Y chromosome.Moreover this sequence has been found genus *BOS* specific, repeated at about 2000 copies in male genome and no transcribed. From BC1.2 sequence we have determined several 14 to 18 bases oligonucleotides primers and 18 to 20 bases probes. Because of degenerativity of repeated, non coding sequences different primers and probes were tested in the aim to obtain a satisfying amplification. In order to precise the optimal conditions for PCR amplification different parameters were also analysed: Mg^{2+} concentration, number of cycles, annealing and extension temperatures and hybridization conditions on dot or Southern blots. After 40 cycles of amplification a positif hybridization signal was observed only with male genomic DNA as less as 50 pg corresponding to about 10 cells. The results obtained with genomic DNA were applied to day 7 old bovine embryos. Ten embryo biopsies of about 50 cells were subjected to 40 cycles of amplification and a clear cut hybridization signal was observed in 4 samples (male) whereas no signal was observed in 6 others (female).

Abstract Withdrawn

WH 202 A SIMPLE STRATEGY FOR SEQUENCING DOUBLE STRANDED DNA, S. Koepf[1], R.A. Gibbs[2], P. Nguyen[2], W. Salser[3], P.E. Mayrand[1], M. Hunkapiller[1], M.N. Kronick[1], L.J. McBride[1], [1]Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, CA 94404, [2]Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, [3]Department of Biology, UCLA, Los Angeles, CA 90024
The fluorescence-based Sanger method of automated DNA sequence analysis has become a popular technology. Both single- and double-stranded template preparation and sequencing protocols exist. However, it is generally accepted that double strand sequencing can yield variable results. A promising solution is to convert double strand templates into single stranded form with the use of the polymerase chain reaction (Gyllensten and Erlich, 1988, Proc. Natl. Acad. Sci. USA, 85:7652-6). We have developed a simple sequencing procedure which employs direct Sanger sequencing of single-stranded polymerase chain reaction (PCR) products. Plasmid DNA, viral DNA, cDNA, and phage plaques all have been sequenced by a "universal protocol". We have sequenced PCR products directly without intermediate purification by the use of labeled sequencing primers. Furthermore, we have eliminated the need to synthesize template-specific sequencing primers by incorporating a universal primer sequence on the 5' end of the limiting PCR primer. This procedure should ultimately become both a general template preparation method as well as a sequencing strategy for many high throughput and/or repetitive DNA sequencing projects.

WH 203 **THE USE OF MOBILITY-SHIFTING NUCLEOTIDE ANALOGS TO DETECT DNA MUTATIONS AND POLYMORPHISMS**, J. Stephen Kornher and Kenneth J. Livak, Central Research & Development Dept., E. I. du Pont de Nemours & Co., Wilmington, DE 19880
We have devised a new, general procedure that will detect single nucleotide polymorphisms at any site in a DNA fragment and is particularly well-suited to the analysis of PCR-amplified templates. The method exploits the fact that the incorporation of certain nucleotide analogs into DNA causes a detectable shift in electrophoretic mobility. The method uses *Taq* polymerase to synthesize DNA strands of defined length, replacing one of the four dNTPs with a mobility-shifting analog. DNAs that are the same length but differ in the number of analog molecules per strand will exhibit different mobilities on a sequencing gel. This provides a rapid assay for distinguishing DNAs that may be identical in length but differ in base composition. To demonstrate this technique we have analyzed a nonsense mutation found in the gene encoding the human insulin receptor [Kadowaki *et al.*, *Science* **240**:787-790 (1988)]. Using PCR, a 140-bp segment containing the mutational site was amplified in genomic DNA isolated from a patient heterozygous for this mutation and from an homozygous normal individual. Each of the amplified DNAs was analyzed by primer extension using biotin-11-dUTP in place of TTP. On a sequencing gel, the primer extension product from the mutant allele migrates at a position one nucleotide slower than the product from the normal allele because the mutant product contains one more biotin-11-deoxyuridine residue than the normal product. This distinguishes heterozygote from homozygote. Similar analyses have been performed with an analog of dCTP.

WH 204 TISSUE SPECIFIC MUTATIONS IN THE HIV-1 EXTERNAL ENVELOPE V3 DOMAIN, Willy J.A. Krone*, Leon G. Epstein°*, Benjamin M. Blumberg°, Jacques de Jong*, Marjon Clement*, Leroy R. Sharer°, Jaap Goudsmit*; *Human Retrovirus Laboratory, AMC, Amsterdam, the Netherlands; °Department of Neurosciences, Pediatrics and Pathology, UMD-New Jersey, Medical School, USA.
The V3 domain (aa307-321) of the HIV-1 external envelope (gp120) is the binding site for viral strain specific neutralizing antibodies and has a conserved β-turn structure (GPGR) flanked on either side by 5 amino acid residues which are divergent between sequenced HIV-1 isolates. Due to this combination of conserved and variable regions the V3 domain was choosen to study in-vivo mutation in post-mortem brain and spleen tissue from 4 HIV-1 infected children. HIV-1 DNA was amplified using the polymerase chain reaction method with primers which bracket the complete V3 region of gp120. The amplified DNA was sequenced directly and in addition amplified DNA was cloned. Nucleotide sequences derived from cloned DNA confirmed the sequences obtained directly from the amplified DNA and revealed additional point mutations in the amino acids flanking the GPGR tetrad. Sequence variation was found between patients and in addition extensive variation was found within individual tissues. Specific mutations were found in brain which were not present in spleen of one patient.

WH 205 CHARACTERIZATION OF THE HIV MAJOR NEUTRALIZING EPITOPE SEQUENCE AND VARIATION, G. J. LaRosa*, K. Javaherian*, A. Profy*, J. Rusche*, A. Langlois**, T. Matthews**, D. Bolognesi**, and S. Putney*. *Repligen Corporation, Cambridge, MA 02139, **Duke University Medical School, Durham, NC.
Recombinant HIV vaccine candidates including the entire envelope, gp160, have been produced from several different HIV-1 isolates in E. coli and in insect cells using baculovirus expression vectors. The principle neutralizing epitope within gp160 has been mapped to a hypervariable region. Antibodies directed to this region neutralize free HIV and prevent fusion of infected and uninfected CD4 cells in an isolate-specific fashion. Antibodies bound to this epitope do not prevent binding of gp120 to CD4, but rather neutralize HIV via a post-binding step in the infection process. We are using the Polymerase Chain Reaction (PCR) to analyze the sequence of this isolate-specific neutralizing epitope from a large number of HIV isolates. This will allow us to better understand the extent and nature of the variability in the sequence of this region which will aid in our design of a vaccine, composed of a cocktail of immunogens, that will elicit protective immunity to a diverse array of virus isolates.

WH 206 OPTIMUM CONDITIONS FOR THE POLYMERASE CHAIN REACTION FOR INTRACELLULAR BACTERIA. Steven Larsen and David Welch, Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN 46223.
As an obligate intracellular parasite, Chlamydia trachomatis is an interesting candidate for detection by the polymerase chain reaction (PCR). Existing culture techniques require 2 to 3 days for bacterial outgrowth and must be done in tissue culture cells, besides. We have cloned a ten-copy plasmid from C. trachomatis to test for ideal PCR conditions. By determining the optimum times, temperatures, GC content, and size; we have been able to obtain a trillion-fold amplification in one and one-half hours easily. The reaction efficiency is 88% and has a high degree of specificity. Sample preparation is extremely simple and a single bacterium contains sufficient template material.

WH 207 THE CLONING AND EXPRESSION OF MULTIPLE cDNAs BY PCR. S.W. Law, L. O'Neill, S. Zavodny, B. Daugherty, J. Demartino, J.S. Tung, G. E. Mark and R.W. Ellis. Department of Cellular and Molecular Biology, Merck Sharp and Dohme Research Laboratories, P.O. Box 2000, Rahway, NJ 07065. Total phage library DNA and several subcloned plasmid DNAs representing a cDNA sequence isolated from this library, were subjected to PCR amplification utilizing primers complementary to regions defining relevant initiation and termination codons. The PCR products from the cDNA subclones were the size expected. However, the products from the total library DNA suggested the presence of heterogeneous transcripts. Furthermore, analyses of the nucleotide and predicted amino acid sequences of the cDNA subclones suggested that heterogeneity could be generated by the deletion of protein domains. New sets of primers representing the junctions of domains were then used to PCR the total library and three PCR products were obtained. These were found to represent the expected nondeleted species, as well as single domain-minus and double domain-minus species. Because of the heterogeneous source of RNA used for the library construction, the mechanism which generated the transcript heterogeneity was not obvious. Genomic DNA was isolated and Southern blotting was performed using cDNA probes. The result strongly suggested that the heterogeneous transcripts are generated from a single transcript (single gene) by post-transcriptional processing; ie alternative splicing. We have amplified the two alternatively processed transcripts, cloned them into expression vectors, and determined their DNA sequence. We have demonstrated that the conventional screening procedures could be bypassed to obtain clones from multiple related transcripts by PCR.

WH 208 HUMAN GENETIC MAPPING BY SPERM TYPING, Honghua Li, Xiangfeng Cui and Norman Arnheim, Department of Molecular Biology, SHS 172, University of Southern California, University Park, Los Angeles, CA 90089-1340. DNA regions containing allele-specific sequences at genetic loci in single human spermatozoa can be amplified by the polymerase chain reaction (PCR) to detectable levels. The PCR products are identified by hybridization to allele-specific oligomer (ASO) probes. Simultaneous amplification of the DNA sequences at two genetic loci has enabled us to measure the recombination rate between them. Approaches aimed at carrying out three point crosses and simplifying the sperm typing procedure will be presented.

WH 209 DETECTION OF HUMAN THYMIDINE KINASE mRNA PRECURSOR WITH RT-PCR, Kenneth E. Lipson and Renato Baserga, Department of Pathology and Fels Research Institute, Temple University Medical School, Philadelphia, PA 19140
We have been using PCR to detect unspliced RNA transcribed from the thymidine kinase (TK) gene of human diploid fibroblasts. This is accomplished by probing a Southern blot of amplification products of reverse transcribed total RNA with an oligonucleotide specific for the first intron of TK. The precursor of TK mRNA is maximally expressed early in S phase of the cell cycle. At this point, the precursor comprises 0.1 to 1% of the TK mRNA. A small amount of TK mRNA precursor can also be detected in quiescent cells. Experiments are in progress to determine whether this represents a small fraction of proliferating cells or a basal rate of TK transcription during G_0.

WH 210 GENETIC EPIDEMIOLOGY OF AIDS. Louie L, Gilles K, Newman B, Anderson L, and King MC. University of California, School of Public Health, Berkeley, California. 94720. We are integrating molecular genetic analysis with epidemiologic and virologic studies to test whether host genotype may contribute to susceptibility to HIV infection, likelihood of HIV transmission, and rate of disease progression. To address these questions, we are studying three cohorts with respect to genotypes of sequences potentially related to HIV infection and to immune response. The first is a study of prenatal/perinatal transmission from HIV-infected mothers to their newborn infants. The second is a heterosexual transmission study of couples in which the male partner has or has not transmitted HIV to his female partner. The third includes homo- and bi-sexual men at various stages of infection and disease. Candidate sequences include HLA B, DR, and DQ; CD4 and CD8 T-cell antigens; IL-2; IL-2 receptor; T-cell receptor alpha, beta and gamma chains; and interferon alpha, beta, beta-2, and gamma. We are currently using RFLP analysis to screen our subjects at these loci. Because blood from HIV-infected patients yields an extremely low amount of DNA due to reduced T-cell levels, PCR will allow us to screen all our subjects for the proposed sequences and others, as they arise. We will also incorporate PCR followed by hybridization to allele-specific oligonucleotides to enhance detection of polymorphisms, thereby increasing statistical power. If host genotype contributes to HIV susceptibility or transmissibility, identification of the genes involved would be important in understanding the pathogenesis of HIV and in development of therapeutic interventions.

WH 211 GENE DOSAGE BY PCR CO-AMPLIFICATION, Matthew B. Lubin and Hiroo Toyoda, Division of Medical Genetics, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA 90048. Many studies have recently investigated loss of heterozygosity or oncogene amplification in tumors. The PCR should be ideally suited for detecting gene dosage in small tissue samples. The PCR is exponential, satisfying the relationship $\log(N) = n \log(f) + \log(s)$, where N is the number of copies of sequence after n cycles, s is the number of copies at zero cycles, and f is the efficiency of the PCR such that $1 < f \leq 2$. We have observed that this remains true when two sequences are co-amplified in one reaction. Thus the relationship $\log(N_1/N_2) = n \log(f_1/f_2) + \log(s_1/s_2)$ obtains. Working with a dystrophin gene sequence (d) and using a beta globin gene sequence (b) as a reference we have investigated the ratio of s_d/s_b by co-amplifying these genes to various cycles and quantitating the amplified sequences after polyacrylamide gel electrophoresis. DNA from males and females has been distinguished. Further investigation will study s_d/s_b in DNA from X-chromosome aneuploid cell lines and eventually oncogene amplification in tumor tissue.

WH 212 LYMPHOKINE ABNORMALITIES OF AUTOIMMUNE MICE: ANALYSIS OF TRANSCRIPTS IN LYMPHOCYTE SUBSETS BY QUANTITATIVE PCR. R. Lee, L. Murray and C. Martens, DNAX Research Institute, Palo Alto, CA 94304. We are studying expression of lymphokine genes during development of autoimmune disease in the MRL/MPJ/*lpr* mouse, a model of systemic lupus erythematosus in which there is a massive accumulation of abnormal T lymphocytes which lack the maturation markers CD4 and CD8. These abnormal T cells, as well as the $CD4^+$ T cells, were reported to be defective in their ability to produce lymphokines, but those studies relied on detection of secreted factors after *in vitro* culture rather than analysis of cells taken directly from animals. We have analyzed gene expression in FACS-separated lymphoid subpopulations isolated from diseased animals. RNA from each cell type was reverse-transcribed and amplified using PCR primers specific for several lymphokine genes. Using serial dilutions of input cDNA, a limited number of amplification cycles, and ^{32}P-dCTP in the amplification reactions, radioactive PCR products were generated which could be quantitated by densitometric scanning of autoradiograms or by scintillation counting. The abnormal T cells in *lpr* mice were found to express high levels of IFNγ and TNFα genes, low levels of IL-3, and undetectable amounts of IL-1, IL-2, IL-4, IL-5 transcripts. The $CD4^+$ cells from these animals transcribed IFNγ, IL-2, IL-3, and IL-4, but, in contrast to IL-2 dependent $CD4^+$ T cell lines, they did not express IL-5 or TNFβ. The $CD8^+$ T cells transcribed IFNγ, IL-3, IL-4, and IL-5, but no TNFβ or IL-2. Thus the immunological abnormalities of the *lpr* mouse may be related to excessive production of IFNγ and TNFα, or defective expression of TNFβ or IL-2.

WH 213 EVALUATION OF THE POLYMERASE CHAIN REACTION FOR DETECTION OF HPV DNA IN CLINICAL SPECIMENS, James K. McDougall, Denise A. Galloway, Joe Chu, Beth A. Miller, Audrey E. Christiansen and Anna Marie Beckmann. Fred Hutchinson Cancer Research Center, Seattle, WA, 98104.

We have used both Southern filter hybridizations and the polymerase chain reaction (PCR) to detect HPV-6 or -16 DNA in cervical specimens from women attending three clinics. The study group is 195 women who had a cervical scrape (exfoliated cells) taken for Southern analysis. A Pap smear collected at the same visit was available from 144 women, and colposcopically-directed biopsies (formalin-fixed, paraffin-embedded) were taken from 40 women also at the same visit. Southern filter hybridizations were performed on total DNAs extracted from exfoliated cells. Southerns were carried out under stringent hybridization conditions with either ^{32}P-labelled HPV-6 or -16 DNA as the probe. PCRs were done with 25-mer primer pairs specific for the HPV-6 or -16 E6/E7 region with amplification for 35 cycles followed by Southern filter hybridizations of amplified DNA using an internal oligonucleotide (40 mer) endlabelled with γ ^{32}P-ATP as the probe. Pap smears were prepared for PCR by extraction of DNA from cells removed from the slides. Sections were deparaffinized and boiled, and the PCR was performed directly on that material. Preliminary analyses of specimens from 48 women showed a sensitivity of 66% when comparing Southerns on exfoliated cells to PCRs on Pap smears. When comparing Southerns to PCRs on either Pap smears or biopsies, the sensitivity was 39.5%. In each instance, the specificity of the PCR was 100%. Further PCRs on the remainder of the specimens are in progress and we are continuing the data analysis.

WH 214 Affinity Generation of Single Stranded DNA Following the Polymerase Chain Reaction: Application to Dideoxy Sequencing, Lloyd G. Mitchell and Carl R. Merril, NIMH, Laboratory of Biochemical Genetics, NIH, Bethesda, MD, 20892. We have developed a simple method to rapidly generate single stranded DNA for dideoxy sequencing following the polymerase chain reaction (PCR). The PCR is a rapid method for amplifying specified DNA regions; however, direct sequencing of PCR amplified DNA is difficult due to the relatively short, linear double stranded nature of the product DNA. By incorporating biotin in the 5' end of one amplification primer, we are able to physically separate the two DNA strands produced in the polymerase chain reaction. After amplification, the reaction mixture is passed through a column containing streptavidin agarose. The strand produced by the biotinylated primer is bound in this matrix. The unbiotinylated strand is subsequently eluted with 0.2 N NaOH and sequenced by the dideoxy method. This method was utilized to sequence mitochondrial DNA from crude genomic DNA and to determine the sequences of four clones containing human mitochondrial DNA as a test of its accuracy. The use of biotin facilitated separation permitted us to amplify and sequence DNA samples in a single day. This method should also prove useful in generating single stranded DNA for other applications such as in vitro mutagenesis and hybridization studies.

WH 215 APPLICATION OF THE PCR TO THE DETECTION OF RESIDUAL DISEASE AFTER BMT FOR CML, G.J. Morgan, J.M. Goldman, L.M. Wiedemann, Leukaemia Research Fund Centre, and Royal Postgraduate Medical School, London, U.K.
The presence of the Ph translocation in chronic myeloid leukaemia (CML) provides a specific genetic marker for leukaemic haemopoiesis. The ABL oncogene is translocated into the BCR gene locus giving rise to a hybrid BCR/ABL messenger RNA. Using oligonucleotide primers (one complementary to a region of ABL exon a3 and one representing a sequence corresponding to the b162 junction of the BCR gene) it is possible to specifically amplify the region encompassing the junction of the hybrid mRNA following synthesis of cDNA. The sensitivity of the test was assessed using serial dilutions of Ph^+ with Ph total RNA. Dilutions of 1 in 10; to $1:10^6$ were prepared. A positive signal could be detected down to 10^5 after autoradiography. This technique was then applied to 7 patients who were alive and in complete clinical, haematological and cytogenic remission 6 to 7 years after bone marrow transplantation (BMT) for CML. No BCR/ABL hybrid bands were seen in any of the patients' samples. To ensure the quality of RNA and success of the amplification reaction in the absence of a positive signal, half of the cDNA reaction mix was also amplified using a primer corresponding to a region of ABL exon a2 and the complementary a3 primer. Since ABL is ubiquitously expressed, this results in a band in all samples. Therefore, since no mRNA corresponding to the transcription product of the Ph chromosome was detected in these patients, we suggest that they may be cured of their disease.

WH 216 DETECTION OF TRYPANOSOMA CRUZI BY DNA AMPLIFICATION USING THE POLYMERASE CHAIN REACTION, David R. Moser, Louis V. Kirchhoff and John E. Donelson, Departments of Internal Medicine and Biochemistry, and Diabetes and Endocrinology Research Center, University of Iowa, and Veterans Administration Medical Center, Iowa City, IA, 52242. PCR amplification of a region of the major repetitive sequence of Trypanosoma cruzi provides an extremely sensitive and species-specific method of detecting the presence of T. cruzi parasites in both insect vectors and mammalian hosts. The method can detect a single parasite by the generation of a 188 bp specific amplification product, readily visualized as a band on an ethidium bromide-stained agarose gel. Hybridization of a radiolabelled probe to membrane-bound amplification products increases the sensitivity to a level where less than 1% of the DNA content of a single parasite can be detected. The method has been used to detect the presence of parasites in the abdominal contents and urine of laboratory-reared reduviid bugs and in the blood of infected mice. We are presently investigating the applicability of the PCR-based diagnostic method for detecting T. cruzi parasites in blood samples of patients with chronic Chagas' disease.

WH 217 IN VIVO FOOTPRINTING AT A DEVELOPMENTALLY REGULATED ENHANCER, Paul R. Mueller and Barbara Wold, Division of Biology, California Institute of Technology, Pasadena, CA 91125. Most polymerase chain reaction (PCR) applications require two defined ends for exponential amplification. We have developed a PCR methodology that initially requires only one defined end; the second end is added by ligation of a uniquely structured linker. This method should be generally applicable to any PCR problem in which only one end of the region to be amplified is known. We have applied it to the problem of *in vivo* footprinting which identifies sites of protein:DNA interactions that exist *in situ*. Despite the development of numerous genomic sequencing strategies, visualizing in vivo footprints in large genomes such as that of mammals is technically difficult due to a low signal to noise ratio. The methodology described here eliminates many of the problems associated with these techniques and provides the possibility of *in vivo* footprinting very small numbers of sorted cells or small amounts of dissected tissues (10^4 to 10^5 nuclei). Here, we have used the technique to study protein:DNA interactions at the muscle creatine kinase enhancer. MCK is expressed only in terminally differentiated myocytes, and we find evidence for numerous protein:DNA interactions at sequences similar to general transcription factor binding sites as well as to muscle specific elements in its enhancer. None of these interactions are observed in undifferentiated myoblasts (precursor cells of myocytes) where MCK is not expressed. The sites footprinted correspond to sequences which have been defined genetically as important for tissue specific MCK expression including the probable binding site of a myogenic regulatory protein, MyoD1.

WH 218 A MUTATIONAL ANALYSIS OF THE GENETIC REGULATION OF MAMMALIAN RECOMBINATION WITH Y-CHROMOSOME LINKED SEQUENCES, Ferez S. Nallaseth, Department of Biology, USC, Columbia, SC 29208. We have established that combinations of feral Y-chromosomes and genomes of inbred strains induce murine hybrid dysgenesis (Nallaseth and Whitney, 1988): which is a collection of phenotypic and genotypic traits that includes the loss of genetic control of recombination (Kidwell et al., 1977). Y-chromosome linked repeated sequence probes and combinations of reciprocally backcrossed inbred strain and feral Y-chromosomes have allowed the recovery and analysis of several distinct but reproducible aberrant recombination products. Because of their location in recombinationally suppresed loci and their male limited haploidy these Y-linked probes have allowed the unambiguous detection of traces of Y-chromosomal DNA in females. These sequences are tentatively identified as random interchromosomal gene conversion tracts. Alternative assays for the detection of aberrant recombination products are predicated on the co-ordinated rearrangement of most copies of the above Y-linked repeated sequence families in XY individuals. This class of aberrant recombination products included precise, co-ordinated rearrangement of fragments defining a multi-copy recombination breakpoint and an *Alu* sequence RFLP, on the Y-chromosome. Co-ordinated rearrangements occurred mostly during germline, but also during somatic development. The reproducibility and specificity of substrate-product relationships, the relative precision and high frequencies of these co-ordinated rearrangements in independent germlines, exceludes passive genetically non-directed non-homologous recombination as a possible mechanism(s). Distinct autosome or X-chromosome encoded trans activities inducing each of these aberrant recombination events are strongly suggested by the differences in dependence on distinct "genetic backgrounds" for their induction. References: Kidwell, M.G. et al. (1977). Genetics 86, 813-833. Nallaseth, F. S. and J. Barry Whitney III. (1988). XVIth International Congress on Genetics (Abstract No. 32.12.2).

WH 219 AUTOMATED DIRECT DIDEOXY SEQUENCING OF PCR AMPLIFIED SINGLE STRANDED DNA.
N.G. Cadavid, H. Neuwirth, R.A. Gatti, W. Salser, Depts. of Surgery, Pathology, and Biology, University of California, Los Angeles, CA 90024.
Automation of direct DNA sequencing from PCR generated fragments may provide a useful approach for screening point mutations. We have improved conditions for generating high yields of regions of the Ha-ras oncogene. Total genomic DNA (1 mcg) from the T24 cancer cell line was subjected to PCR using 28-mer primers containing an 8 base pair 5' Oliglow™ tail. An aliquot of this amplification subsequently underwent PCR using internal (i.e. nested) primers: 46-48 mers containing the M13 forward or reverse sequencing primers, restriction sites for subsequent cloning, and 20 bp of Ha-ras sequence. Nested PCR provided 4 mcg of a single band of either 652 (exons 1 and 2) or 204 bp (exon 1 alone). Manual dideoxy sequencing was performed on Elutip D purified ethanol precipitated dsDNA following electroelution from NuSieve™ gels. The sequences obtained were unambiguous in both directions. Aliquots from the first round of PCR were also subjected to 15-40 cycles of PCR using unbalanced sets of nested primers. The minor primer was end-labelled with ^{32}P and yields assessed as a function of the number of thermal cycles, Mg^{2+}, and primer ratios. Dried gel autoradiography showed that the peak of ssDNA yield occurred at 25-30 cycles with loss of ssDNA to high MW reaction products thereafter. The shorter sequence produced ssDNA more efficiently. Phenol extraction and Centricon 30 ultrafiltration of nested PCR products allowed manual or Applied Biosystems automated ssDNA sequencing using M13 forward or reverse primers labelled with ^{32}P or flourescent tags, respectively. This simplified amplification and purification regimen gave reproducible and unambiguous sequences with both methods and can be applied to large numbers of specimens run in parallel using the automated sequencer.

WH 220 MIS-SENSE MUTATION ASSOCIATED WITH RFLP HAPLOTYPES 1 AND 4 OF THE HUMAN PHENYLALANINE HYDROXYLASE GENE, Yoshiyuki Okano, Tao Wang, Randy C. Eisensmith, Richard Gitzelmann and Savio L.C. Woo, Howard Hughes Medical Institute, Department of Cell Biology and Institute of Molecular Genetics, Baylor College of Medicine, Houston, TX 77030
Phenylketonuria (PKU) is an autosomal recessive disorder caused by a deficiency of phenylalanine hydroxylase (PAH), and has a prevalence of about 1 in 10,000 births among Caucasians. The majority of mutant PAH alleles are associated with haplotypes 1-4 of the gene, and we have previously characterized two mutations tightly linked with mutant haplotypes 2 and 3 that are most prevalent in the Caucasian population. We now report the molecular lesions associated with haplotype 1 and 4 mutant alleles in the Swiss population. Exon-containing regions of the PAH gene were amplified by polymerase chain reaction from genomic DNA of a PKU patient bearing a haplotype 1 and a haplotype 4 mutant alleles. The amplified DNA fragments were subcloned into M13 for sequencing analysis. Mis-sense mutations were observed in exons 5 and 7, resulting in the replacement of Arg by Gln at residue 158 and 261 of the enzyme, respectively. Subsequent oligonucleotide hybridization analysis demonstrated that the exon 7 mutation is present in 13 out of 18 haplotype 1 mutant alleles, and the exon 5 mutation is present in 2 out of 6 haplotype 4 mutant alleles in the Swiss population. The results provide conclusive evidence of linkage disequilibrium and that haplotype 1 and 4 chromosomes bear multiple mutant PAH alleles in Caucasians.

WH 221 PCR AMPLIFICATION OF AN rDNA SEGMENT FROM TETRAHYMENA STARTING WITH INDIVIDUAL CELLS. Eduardo Orias*, Naoko Makita & Toru Higashinakagawa Mitsubishi-Kasei Institute of Life Sciences, Machida-shi, Tokyo, Japan (* permanent address: Dept. of Biol. Sci., UC Santa Barbara, CA 93106). The rDNA of the unicellular eukaryote Tetrahymena thermophila inbred B strain differs from that of inbred C3 strain in that the former has a SphI site and a deletion of 42 bp in the central spacer region. C3 rDNA replicates preferentially with respect to B rDNA in the macronucleus of B/C3 heterozygotes. To facilitate studies of this phenomenon, we attempted the detection of the polymorphic trait by PCR, starting with minute amounts of DNA. The targeted polymorphic region is 85% AT. The following conditions led to efficient in vitro amplification of the targeted segment: denaturation at 90C, primer annealing at 58C and primer extension at 68C. The identity of the amplified segments was confirmed by DNA sequencing. Digestion of the template DNA at restriction sites upstream and downstream of the targeted region increased the efficiency of amplification, presumably because the segment is near the center of symmetry in a palindromic molecule. Starting from total cell DNA corresponding to as little as 0.003 cells (about 30 rDNA molecules), the amplified band was observed after agarose gel electrophoresis and EtBr staining. The yield indicated more than 10 billion fold amplification. The amplified product was also detected in an EtBr stained gel when reactions were started with a single individual cell.

WH 222 DETECTION OF HIV INFECTION IN INFANTS BY THE POLYMERASE CHAIN REACTION, James B. Peter, and Karen K.Y. Young, Specialty Laboratories, Inc., Santa Monica, CA, 90404. Diagnosis of HIV infection in very young infants born to HIV-infected mothers is a difficult problem. Presence of HIV-specific antibody is not a good criterion for infection because maternal antibody can traverse the placenta. It is, however, important to identify infected infants because early treatment with azidothymidine (AZT) can minimize some of the neurological damage associated with HIV infections. It is equally important to rule out infection as AZT has serious side effects. We have tested peripheral blood mononuclear cells from 48 infants for the presence of HIV proviral DNA by the polymerase chain reaction (PCR). Twenty-seven of the infants were antibody-positive and the remaining 21 were antibody-negative. Seven of the antibody-positive infants were also positive for proviral DNA, the remaining 20 were HIV DNA negative. Four of the antibody-negative infants were positive for HIV proviral DNA. These four infants may have lost the maternally-transferred antibody and have not begun synthesis of their own antibody. The PCR gives promise of differentiating antibody-positive infants who are infected with HIV from those not infected. Furthermore, the PCR is able to detect HIV infection in infants that lack HIV-specific antibody.

WH 223 PCR BASED HOMOLOGY SCREENING AND CLONING OF A VOLTAGE GATED POTASSIUM CHANNEL FROM *APLYSIA* CENTRAL NERVOUS SYSTEM, Paul J. Pfaffinger, B. Zhao, Howard Hughes Medical Institute, Columbia University, New York, NY 10032. We are using PCR both for homology screening and for the cloning of full length potassium (K) channel clones directly from RNA. Homology screening is based on choosing regions of conserved amino acids. Oligonucleotide pools, in both sense and antisense orientations, are constructed based on these amino acid stretches. The signal for a positive screening is the amplification of a single band of the expected size when using two appropriate pools. For the potassium channel, amino acid stretches that are conserved between a *Drosophila* and a mouse K channel clone were chosen. Amplification with two appropriate oligonucleotide pools generated a single band of 180 bp, exactly the size predicted from the mouse and the *Drosophila* clones. Sequencing of this fragment showed >85 identity to the *Drosophila* and mouse clones. The complete coding region is being constructed by PCR out from this central sequence. The 3' end is produced by amplification between specific oligos generated from the 180 bp sequence and oligo dT + restriction site at the poly A tail of the message. We have generated several different 3' ends, all containing our original sequence but differing in the middle, suggesting that *Aplysia* potassium channels may have alternative splicing like the *Drosophila* K channel. The 5' end is being generated by homopolymer tailing of a primer extended cDNA and then amplification between the appropriate complementary homopolymer + restriction site and antisense oligonucleotides generated to the 180 bp sequence.

WH 224 POLYMERASE CHAIN REACTION AND THE DETECTION OF HUMAN RETROVIRUSES
B.J. Poiesz*, M.A. Abbott*, J.B. Glazer°, S.J. Greenberg▲, S. Bhagavati▼, J.J. Sninsky+, S. Kwok+, and G.D. Ehrlich*, *Department of Medicine, SUNY HSC at Syracuse, Syracuse NY 13210, °Division of Infectious Diseases, Staten Island Hospital, Staten Island, NY 10305, ▲Metabolism Branch, NCI, NIH, Bethesda, MD 20892, ▼Department of Neurology, SUNY HSC at Brooklyn, Brooklyn, NY 11203, and +Cetus Corporation, Emeryville, CA 94608.
We have used polymerase chain reaction (PCR) as a diagnostic tool for recognized retroviral etiologic agents (HIV-1 and AIDS; HTLV-I and Adult T-cell leukemia), to discern new relationships between these retroviruses and disease (HTLV-I and chronic progressive myelopathy) and to search for new sequences related to known retroviruses that may implicate as yet unrecognized agents with disease (multiple sclerosis). We present data here relevant to both epidemiologic and clinical applications and describe techniques of analysis of PCR products appropriate to different types of study.

WH 225 DETECTION OF MINIMAL RESIDUAL LEUKEMIA AND LYMPHOMA CELLS BY PCR AMPLIFICATION OF IMMUNOGLOBULIN HEAVY CHAIN GENES. Rechavi G, Brok-Simoni F, Amariglio N, Hakim I, Mandel M, Ben-Bassat I, and Ramot B. Institute of Hematology, The Chaim Sheba Medical Center Tel-Hashomer and Sackler School of Medicine, Tel-Aviv University, Israel.

Despite the high remission rate in patients with lymphatic malignancies, disease recurrence remains a major problem. The majority of the relapses are the result of the expansion of clinically undetectable residual disease. Even new techniques, such as flow cytometry and Southern blot analyses are limited at the level of detection of 1% of neoplastic cells, while patients in remission have residual cells that are below this level. In two hematologic malignancies, CML and follicular lymphoma, carrying the t(9;22) and t(14;18), respectively, the application of PCR using primers representing sequences from the chromosomal breakpoints enabled the detection of minimal residual disease. However, such translocations are detected only in the minority of lymphatic malignancies. We used immunoglobulin V_H and J_H specific sequences for the amplification of rearranged V_H-D-J_H genes in B-cell leukemias This method can serve as a tool for the detection of small clonal populations of malignant B-cells.

WH 226 THE DETECTION OF LATENT HCMV DNA USING THE PCR REACTION, J. Riolo, S. C. St Jeor, Cell and Molecular Biology Program and the Department of Microbiology, University of Nevada, Reno Nv. 89557 Human cytomegalovirus (HCMV) causes latent infections but the cell type(s) associated with latency had not been identified. We used the Polymerase Chain Reaction (PCR) for the detection of latent HCMV DNA. A region of the genomes of HCMV, HHV-6 and HSV-1 were identified that did not have DNA homology with either normal human DNA or with other latent herpes viruses. Optimal reaction conditions needed to detect a minimal amount of virus DNA in the presence of large amounts of cell DNA was determined. Primers from regions of the genomes of HCMV, HSV-1 and HHV-6 DNA were then used to examine DNA from 10 random autopsies for the presence of latent herpes virus DNA. Organs examined include brain, bone marrow, blood, lung, liver, muscle, kidney, and spleen. Analysis using the HCMV primers indicated that several organs contained sequences with homology to HCMV DNA. Although positive results have been obtained with nearly every organ; those that are most likely to contain positive HCMV DNA were bone marrow, lung and muscle. The data obtained from the PCR reaction correlated with serological results from the same patients. The results obtained using primers from HHV-6 and HSV-1 are in progress and will be compared to results obtained using the HCMV primers.

WH 227 MOLECULAR SYSTEMATICS OF SYMBIOTIC DINOFLAGELLATES.
Rob Rowan and Dennis A. Powers, Hopkins Marine Station, Pacific Grove, CA 93950.
Many marine invertebrates, including reef-building corals, contain photosynthetic dinoflagellates: the zooxanthellae. These symbioses appear to be excellent adaptations to nutrient-poor environments. Because of their problematic biology, there is no zooxanthella taxonomy, very limited data on zooxanthella diversity, and therefore nothing is known about the biological structure and evolution of these widespread symbioses. The PCR has removed the main obstacle to zooxanthella study: cell culturing. An analysis of PCR-amplified 17S ribosomal RNA genes reveals genetically distinct types of zooxanthellae. We are attempting to reconstruct the phylogeny of dinoflagellate symbioses, and we are also using PCR methods to study the ecology of coral symbioses in natural populations.

WH 228 DIRECT DETECTION OF HERPESVIRUS DNA SEQUENCES IN CLINICAL SAMPLES BY IN VITRO ENZYMATIC AMPLIFICATION. Anne H. Rowley, Steven M. Wolinsky Dept. of Peds and Med., Div. of Infectious Diseases, Northwestern U. Med. Sch., Children's Memorial Hosp. and Northwestern Memorial Hosp., Chicago, IL.
Rapid diagnosis of herpesvirus infections may be difficult due to slow growth of the virus in culture (e.g., CMV), inability to culture virus by standard techniques (e.g., EBV), the presence of small quantities of virus in body fluids, and the time required to develop a serologic response. We designed oligonucleotide primers and probes from a region of the DNA polymerase gene conserved among the family Herpesviridae for use in enzymatic amplification of the viral genome in clinical samples by the polymerase chain reaction technique. Clinical samples were lysed and treated with nonionic detergents and proteinase K. The proteinase K was heat-inactivated and an aliquot enzymatically amplified for 30 cycles of thermal denatur ation ($94^{o}C$), primer annealing ($55^{o}C$), and Taq polymerase-directed primer extension ($72^{o}C$). An aliquot of amplified product was hybridized in solution to a ^{32}P-labelled oligonucleotide probe from a conserved internal sequence. The target DNA:probe heteroduplex was resolved on a 6% polyacrylamide gel followed by autoradiography.
Generic primers are used to amplify HSV types 1 and 2, EBV and CMV DNA, and a type- specific probe uniquely identifies each virus. A different primer pair and probe specifically identifies VZV DNA. We have detected multiple strains of virus from infected cells, cell-free supernatant, and blood by this technique. Other body fluids are currently under study. We believe this method will be useful in the rapid diagnosis of herpesvirus infections, and in the detection of new human herpesviruses.

WH 229 CROSS-SPECIES PCR (CS-PCR) FOR EVOLUTIONARY STUDIES OF DEFINED GENOMIC SEGMENTS. Gualberto Ruano, Jeffrey Rogers and Kenneth K. Kidd. Dept. of Human Genetics, Yale University, New Haven CT 06510. We have PCR amplified a defined intergenic segment from genomic DNA of hominoids (human and gorilla) and Old World monkeys (baboon and rhesus). A 770 bp inter-Alu segment between $\psi\beta$ and δ globin genes has been amplified with primers 10 nucleotides away from each Alu according to the human sequence (Maeda *et al* 1988, *Mol Biol Evol* 5: 1). For this segment, human and rhesus differ by 5% whereas human differs from gorilla by less than 1% (Maeda et al, *op. cit*); the baboon sequence is unknown. Preliminary estimates show the baboon amplified fragment is within +/-25 bp of the length of the other three. In order to assess the species specificity of the amplified DNA, PCR products were radio-labelled to probe blots containing the 770 bp fragment amplified for each species. After high-stringency hybridization (42°, 50% formamide) and wash (70°, 0.5X SSC), the human probe gives a much stronger signal with the gorilla product than with either rhesus or baboon. The Old World monkey probes and products cross-hybridize with each other with a signal slightly fainter than is obtained for same-species hybridization. Both baboon and rhesus probes give weak but clear signal with hominoid product. We conclude that, for this sequence, baboon is >>95% similar to rhesus and about 5% different from human and gorilla. These data agree with known phylogeny for the baboon and demonstrate CS-PCR as a rapid method for comparing similarity of defined genes or DNA segments among related organisms. CS-PCR provides defined DNA segments specific to each species for direct sequencing, RFLP screens and probing libraries. [Supported by BNS-8813234 (NSF) and GM-40883-01 (NIH).]

WH 230 ALTERATIONS IN WOUND HEALING EXPLAINED UTILIZING THE PCR. A. Sank, T. Shima, M. Chi, G.R.Martin. NIH, LDBA, NIDR, Bethesda, MD 20892. Tissue repair is characterized by a cascade of molecular and cellular events. However, the complex mechanisms of altered skin healing are unclear. Current concepts suggest that epidermal and dermal gene expression play a central role in this repair process. These studies sought to determine the specific roles of keratin and collagen genes in cultured human cells as well as patient skin samples. We prepared cellular and tissue mRNA by standard techniques. Primers were selected from the coding region of human keratin 6 and collagen I genes. Results show the keratin gene (K6-epidermal hyperproliferation) is expressed by keratinocytes while the collagen I gene(dermis) is present in fibroblasts(5OIT) and endothelial cells (umbilical vein). In the human tissue samples, K6 was expressed in normal skin but not in keloid or hypertrophic scar tissue. Collagen I gene appeared to be more strongly expressed in keloids and hypertrophic scars than normal skin suggesting that collagen I gene overexpression may play a key role in patients with these clinical problems. These studies demonstrate important genetic differences in both cultured cells and human skin. The sensitive PCR will be employed to determine genetic mechanisms and formulate therapeutic treatments for patients suffering from impaired wound healing.

WH 231 USE OF NON-ISOTOPIC SEQUENCE-SPECIFIC OLIGONUCLEOTIDE PROBES FOR THE ANALYSIS OF PCR AMPLIFIED DNA. Stephen J. Scharf, Randall K. Saiki, Corey H. Levenson*, Chu-An Chang*, and Henry A. Erlich, Dept. of Human Genetics, * Dept. of Chemistry, Cetus Corporation, 1400 Fifty-Third Street, Emeryville, CA 94608. The polymerase chain reaction (PCR) readily provides sufficient quantities of a gene fragment such that detection and typing with non-isotopically labeled sequence-specific oligonucleotide (SSO) probes becomes feasible. This is particularly useful in the analysis of DNA from multi-allelic gene families. Horseradish peroxidase (HRP) conjugated to sequence-specific oligonucleotides as a reporter molecule provides a simple and rapid means for the analysis of amplified DNA. Specifically, amplified DNA is immobilized on solid supports and hybridized with the HRP-labelled SSO. HRP activity is detected in-situ using a chromogenic dye solution. Hybridization, washing, and color development with this system takes less than 1 hour. It is also possible to non-isotopically label PCR products by using biotinylated PCR primers. The biotinylated PCR product can be used to hybridize to SSO probes immobilized on solid supports, and the annealed PCR product can be detected in-situ by capture of a streptavidin-HRP conjugate. This approach is useful when the number of probes used in the analysis exceeds the number of amplified samples.

WH 232 USE OF PCR FOR QUANTITATIVE ANALYSES OF GENETIC AMPLIFICATION IN FORMALIN-FIXED ARCHIVAL TISSUE SPECIMENS, Gilbert Schreiber, Andy E. Sherrod and Louis Dubeau, USC Comprehensive Cancer Center, USC School of Medicine, Los Angeles, CA 90033. Amplification of specific genetic sequences is an important mechanism of oncogene activation and also plays a role in drug resistance. We wanted to determine if PCR technology could be used for quantitative genetic amplification studies because this would greatly increase the number of human tissue specimens suitable for such analyses. Our approach has been to perform PCR simultaneously and in a single reaction mix on 2 different DNA sequences of equal lengths, one from a gene with an unknown state of amplification and the other from a control unamplified gene located on the same chromosome. The ratio of the amounts of the 2 PCR products after the reaction indicates the relative number of copies of test genes compared to control genes in the initial genomic DNA samples. This technique proved to be as accurate as Southern blotting for quantitative determinations of genetic amplification and is readily applicable to studies using microsections of formalin-fixed, paraffin-embedded archival tissue specimens. We have used this approach to examine the state of amplification of the c-myc proto-oncogene in 29 different archival human ovarian carcinoma specimens. The results showed amplification of this locus in 7 (27%) of the specimens. Immunohistochemical staining of tissue sections of tumors containing amplified c-myc genes using a monoclonal antibody for the c-myc oncoprotein showed increased antigenicity of the tumor cells.

WH 233 IDENTIFICATION OF NOVEL HIV-1 mRNAs BY PCR AMPLIFICATION. Stefan Schwartz*, Barbara K. Felber**, E.M. Fenyö*, and George N. Pavlakis** **BRI-Basic Research Program, NCI-FCRF, Frederick, MD 21701 USA and *Karolinska Institute, Stockholm, Sweden.

We have analyzed the mRNA species produced by HIV-1 after infection of human lymphocytes and after transfections of proviral DNA clones into human cells. Viral mRNA was converted to cDNA and amplified by PCR. mRNAs of lengths up to 2 kilobases were successfully amplified by this procedure, allowing the study of all the multiply spliced mRNA species. It was found that HIV-1 produces more mRNA species than previously described. The complete characterization of these mRNAs is currently under investigation. A collection of HIV-1 isolates with variable growth and cytopathic in vitro properties were studied. DNA fragments of these isolates were also amplified successfully by PCR using appropriate primers. Fragments of lengths up to 4 kilobases have been amplified. This method allows the fast cloning of functional domains from different virus isolates and the introduction into appropriate expression vectors for further study. This methodology is expected to be useful for the understanding of virus-cell interactions. Research sponsored in part by the National Cancer Institute, DHHS, under contract No. NO1-CO-74101 with Bionetics Research, Inc.

WH 234 AN EFFICIENT METHOD OF DETECTING NUCLEOTIDE SEQUENCE POLYMORPHISMS OF HUMAN GENOME BY GEL ELECTROPHORESIS AS SINGLE STRAND CONFORMATION POLYMORPHISMS, Takao Sekiya, Masato Orita, Hiroyuki Iwahana, Hiroshi Kanazawa and Kenshi Hayashi, Oncogene Div. National Cancer Center Research Institute, Tokyo, Japan.

We developed a simple and efficient method of detecting DNA polymorphisms as single strand conformation polymorphisms (SSCPs). This method follows digestion of genomic DNA with restriction endonucleases, denaturation and neutral polyacrylamide gel electrophoresis of the digests. The mobility shift caused by a base substitution of a single-stranded DNA fragment, that might be due to a conformational change of the fragment, could be detected by hybridization with DNA or RNA probes. Like RFLPs, SSCPs should be useful genetic markers. Moreover, SSCP analysis has the advantage over RFLP analysis that it can detect single base substitutions at a variety of positions in DNA fragments. Grosser changes such as amplifications, deletions, insertions and rearrangements of particular genes can also be detected by SSCP analysis. We further improved the method by conjunction with the polymerase chain reaction (PCR). The PCR method could be performed using radioactively labeled primer(s) and the amplified segment was directly subjected to SSCP analysis. The PCR-SSCP analysis does not require digestion of DNAs and hybridization with probes and therefore it is the method of choice when genetic alterations known to be present in particular short regions are analyzed.

WH 235 DETECTION OF HIV-1 RNA BY POLYMERASE CHAIN REACTION, J. Sanders Sevall, and James B. Peter, Specialty Laboratories, Inc., Santa Monica, CA 90404. Detection of human immunodeficiency virus-1 (HIV-1) RNA from clinical samples is required to determine the infectious stage of the disease. The object of the study was to evaluate the feasibility of using the polymerase chain reaction for amplification of HIV-1 RNA from clinical blood samples stored at 24°C for 24 hours. RNA was purified from white blood cells by acid guanidinium thiocyanate-phenol-chloroform extraction. The integrity of the RNA was determined by the presence of the 28S and 18S ribosomal RNA bands in the cellular RNA on denaturing gel electrophoresis. The viral RNA was amplified with Taq DNA polymerase after reverse transcription from two sets of extension primers from the gag gene of HIV-1. The amplified product was detected and quantitated by liquid hybridization followed by polyacrylamide gel electrophoresis. Of 33 clinical samples tested, eight were positive only after reverse transcription of the RNA sample; three additional samples were positive without reverse transcription of the RNA (9% of the RNA samples show DNA contamination). Four DNA-positive samples were RNA-negative and one DNA-negative sample was RNA-positive. The RNA-positive, DNA-negative sample was also antibody-negative, whereas all DNA-positive samples were antibody-positive. The PCR technique can be used to amplify HIV-1 RNA from clinical specimens at least as long as one day after collection provided the cellular RNA is intact.

WH 236 RAPID AND SIMPLE AMPLIFICATION OF A SPECIFIC RNA SEQUENCE BY THE POLYMERASE CHAIN REACTION, David R. Sherman and George A.M. Cross, Laboratory of Molecular Parasitology, The Rockefeller University, New York, NY 10021. RNA substrates can be amplified by the polymerase chain reaction (PCR) providing that cDNA synthesis via reverse transcriptase is performed first. We have greatly simplified this procedure by eliminating all nucleic acid purification steps and employing a single buffer system throughout. Cell lysis, reverse transcription and DNA amplification by PCR are performed in one tube and, starting from live cells, amplified DNA is visualized within an ethidium bromide-stained gel in less than 8 hours. This method is extremely sensitive and should be applicable to virtually any cell or tissue which can be dispersed into single cell suspensions.

WH 237 DETECTION OF t(14;18) FROM FORMALIN FIXED, PARAFFIN EMBEDDED TISSUES, Darryl Shibata, Eddie Hu, Bharat N. Nathwani, Russell K. Brynes, and Norman Arnheim, Departments of Pathology and Biological Sciences, University of Southern California, Los Angeles, CA 90033
The DNA present in a single thin paraffin section can be extracted overnight in a Tris-EDTA-Proteinase K solution. Although much of the extracted DNA is size degraded (<12,000 bp), it is a suitable template for the PCR. Using primers and probes for the BCL2 region of chromosome 18 and the JH region of chromosome 14 as described by Crescenzi, et al (PNAS 1988;85:4869), paraffin embedded tissue sections from 65 cases were analyzed by the PCR. For cases with follicular patterns, t(14;18) was detected in 0/9 follicular hyperplasias and 11/27 (41%) follicular lymphomas (small cleaved 9/16, mixed 2/3, large cell 0/4, small non-cleaved 0/4). t(14;18) was detected in 1/29 (3%) diffuse lymphomas (lymphocytic 0/3, small cleaved 0/4, mixed 0/1, large cell 1/4, lymphoblastic 0/1, immunoblastic 0/9, small non-cleaved 0/7). The amplified t(14;18) fussion site products ranged in size from 120-300 bp. Since virtually all human tissues removed for therapy or diagnosis are fixed in formalin, embedded in paraffin, and stored for decades, this approach facilitates corelations between histology, clinical outcomes, and molecular genetic lymphoma analysis.

WH 238 A PROCEDURE FOR RAPID GENOME WALKING BY POLYMERASE CHAIN REACTION, Venkatakrishna Shyamala and Giovanna Ferro-Luzzi Ames, Department of Biochemistry, University of California, Berkeley, CA 94720. The polymerase chain reaction (PCR) is a powerful tool for molecular genetic studies with minute quantities of DNA or RNA. One of the limitations of PCR is that the sequence of the two extremeties of the DNA segment of interest be known. We have devised a strategy to overcome this limitation, and extend the use of PCR to amplify DNA even when sequence information at only one extremity is available. This technique allows rapid genome walking from known into unknown regions of the chromosome or amplification of genes for which a partial sequence is known.

The method involves restricting DNA, ligating the unknown end to any commonly used vector, and amplifying using one primer specific to the known region and a second primer specific to a segment of the vector DNA. The applicability of this method has been tested with the hisP gene of the S. typhimurium histidine transport operon. The gene was ligated into the multiple cloning site of M13, and amplified using a hisP specific primer and the M13 reverse primer. We are presently utilizing this technique for gene walking beyond the histidine transport operon and for the analysis of duplication mutations formed by recombination at REP sites.

WH 239 "INSIDE-OUT" PCR TO AMPLIFY CELLULAR DNA FLANKING INTEGRATED PROVIRUSES, Jonathan Silver and Vijaya Keerikatte, Laboratory of Molecular Microbiology, NIAID, NIH, Bethesda, MD 20892. PCR can be performed when one knows the sequence at only one end of a segment one wishes to amplify, by first circularizing a restriction fragment containing the segment, and then using oligos from the region of known sequence oriented such that their 5' -> 3' extension products proceed "around the circle" before reaching the complement of the other oligo. We will describe several variants of this circular permutation strategy which we have used to amplify cellular DNA flanking endogenous proviruses and proviruses in retrovirus-induced murine leukemias.

WH 240 USE OF PCR FOR QUANTITATIVE MEASUREMENT OF mRNA LEVELS OF X-LINKED GENES DURING SPERMATOGENESIS, AND FOR A MICRO ASSAY OF DNA METHYLATION, Judith Singer-Sam, *Murray Robinson, John J. Rossi, Robert L.Tanguay, *Mel Simon, and Arthur D. Riggs, Division of Biology, Beckman Research Institute, City of Hope, Duarte, CA 91010, and *Division of Biology, Caltech, Pasadena, CA 91125

We find that PCR can be used for a quantitative assay of mRNA levels over a 100-fold range. Using this PCR assay on total RNA from mouse cells at different stages of spermatogenesis, we find that the level of mRNA for X-linked PGK is greatly reduced relative to somatic cells by early meiotic prophase. HPRT mRNA is also reduced, but the rate of decrease is less than for PGK mRNA. We have also developed an assay to measure DNA methylation by use of PCR. The DNA is digested with Hpa II prior to PCR using primers which flank the site assayed; only if the site is methylated will the DNA remain a template for PCR. We are using the method to ascertain methylation differences between male and female mouse tissues, and are adapting it for use with a small number of cells.

WH 241 MOLECULAR HETEROGENEITY OF GAUCHER DISEASE, Frances I. Smith, Theresa Latham, Gregory A. Grabowski and Bimal Theophilus, Departments of Microbiology and Pediatrics, Mount Sinai School of Medicine, NYC, NY 10029.

Gaucher disease (GD), the most prevalent lysosomal storage disease, results from mutations in the acid ß-glucosidase gene. To date, four different allelic single base muations have been identified. Using PCR amplified sequences and allele-specific probes, the allelic status of only 23% of GD patients could be completely characterized. Since two alleles resulted from exon 9 mutations, this exon was screened for additional mutations in a group of incompletely characterized GD patients of diverse phenotype and ethnic heritage. Using primers from cDNA sequences for PCR, exon 9 flanking introns were cloned and sequenced. Intron primers were then used in the PCR to amplify genomic exon 9 from these GD patients. Screening of the PCR-amplified exon 9 products by chemical cleavage analyses of dsDNA heteroduplexes, or RNase A cleavage and denaturing gradient gel analysis of RNA-DNA heteroduplexes, identified new mutations. The comparative utility of the different methods for the detection of unknown point mutations will also be discussed.

WH 242 **THE USE OF P.C.R. AND SYNTHETIC PEPTIDES TO DEMONSTRATE A UNIQUE EPITOPE AT THE CARBOXY TERMINUS OF THE HIV-1 p18 PROTEIN GENERATED UPON CLEAVAGE OF P55, THE GAG POLYPROTEIN PRECURSOR.** **Paul Spence, Mike Jarvill and Peter Highfield. Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, England.**

The GAG polyprotein precursor (p55) is cleaved by a viral specified protease to generate the viral core protein (p24), a nucleic acid binding protein (p15) and a membrane associated protein (p18). During the course of a study of a panel of anti-p18 monoclonal antibodies one antibody (1D9) was identified which failed to react with a p18 recombinant protein expressed in E.coli in which the 13 carboxy amino acids were missing. A panel of synthetic peptides representing the carboxy terminus of p18 reacted with 1D9 in ELISA. These data indicated that the epitope for 1D9 fell at the carboxy terminus of p18. A recombinant protein representing p40 (the p18, p24 polyprotein precursor) failed to react with 1D9 on Western blots suggesting that its epitope was not present before cleavage. To confirm that the carboxy terminus amino acids of p18 did indeed form the epitope for 1D9 P.C.R. was used to generate a DNA fragment capable of expressing the exact carboxy amino acids of p18. This was achieved by making the 3' primer with in-frame stop codons following the codon for the carboxy terminus amino acid of p18. Both the 5' and 3' primers contained appropriate restriction enzyme sites for cloning into the E.coli expression vector used. The recombinant protein expressed from the P.C.R. product reacted specifically with 1D9. As E.coli expressed p18 is not myristilated and is therefore more easily crystalized than viral specified p18 this recombinant protein is now being purified by multi-step affinity chromatography to enable X-ray crystalographic analysis to be carried out. Data will also be presented that demonstrates, in detail, the nature of the association of p18 with viral and cellular membranes.

WH 243 DETECTION OF HUMAN CYTOMEGALOVIRUS DNA IN CLINICAL SAMPLES AND BLOOD DONOR MONONUCLEAR CELLS USING THE POLYMERASE CHAIN REACTION (PCR) Philip Stanier, *Debra L. Taylor and A. Stanley Tyms. Department of Molecular Genetics, St. Mary's Hospital Medical School, London W2. *MRC Collaborative Centre, Mill Hill, London NW7. Human cytomegalovirus (HCMV) infection becomes increasingly common with age although the majority of infections remain silent. Even so, this virus is a major pathogen of the fetus and newborn and of immunocompromised patients with particularly devastating effects in patients with AIDS. More often than not, diagnosis of HCMV is difficult, but early identification of infection is becoming important as methods for effective chemotherapy improve. Virus isolation still provides a major means of viral diagnosis but on average needs 2 weeks of incubation. The recent development of the polymerase chain reaction (PCR) allows specific amplification of both DNA and RNA sequences. In our study of clinical samples with low titre infectivity and fractionated mononuclear cells from donor blood products, the potential for rapid and sensitive detection of viral genomes is evident. We provide evidence that the use of PCR technology has an important application in the detection and prevention of disease due to HCMV.

WH 244 A METHOD TO CONTROL FOR INCOMPLETE RESTRICTION ENZYME DIGESTION IN PCR'S USED FOR PRENATAL DIAGNOSIS AND CARRIER DETECTION, Charles M. Strom, Eugene Pergament, Section of Reproductive and Medical Genetics, Illinois Masonic Medical Center, 836 Wellington Ave., Chicago, IL 60657
The PCR reaction is rapidly replacing the Southern blot as the method of choice for performing DNA linkage analysis for prenatal diagnosis and carrier detection. It is important to assure that restriction digestion has gone to completion, especially if crude DNA preparations from blood, chorionic villi, or mouth wash are used for the PCR reaction. We developed an internal control for completeness of restriction digestion that can be used for PCR linkage analysis of any polymorphic site. ^{32}P deoxynucleotides are added to the PCR mixture initially or after 15 cycles. A control reaction without isotope using DNA from an individual homozygous for the presence of the polymorphic site is performed simultaneously. After completion of the PCR, 1% of the labelled reaction mixture is added to 20% of the control mixture, and restriction digestion is performed. Following acrilamide gel electrophoresis, the DNA is visualized using ethidium bromide staining. If the digestion has gone to completion, the unlabeled control DNA will be completely digested. The patient's genotype is then determined by autoradiography of the gel for 20 min. - 2 hrs. If the PCR products are resistant to restriction digestion, longer digestion times can be attempted or the PCR products can be purified by phenol chloroform extraction and ethanol precipitation prior to digestion and analysis using the same technique.

WH 245 ISOLATED GROWTH HORMONE DEFICIENCY: SEARCH FOR THE MOLECULAR BASIS. Jennifer Swartz-Boyd, Fred Schaefer, Elaine Mardis, Sara Wiley, Don Wilson and Bruce Roe, Chapman Research Inst., Children's Med. Ctr., Tulsa, and, Univ. of Okla. Norman, OK.

Our laboratory is attempting to determine the molecular basis of isolated growth hormone deficiency (IGHD). In one of our studies, the incidence of IGHD1A (a deletion within the growth hormone gene) was studied by Southern techniques. To date, 52 patients from 51 different families from Arkansas, Oklahoma and Texas have been studied; no deletions of the hGH 3.8 kb band have been found. These results indicate that the incidence of IGHD 1A in midwest population is less than 5% ($p<0.05$) of the total IGHD population and 95% or more suffer from a defect which has not yet been described.

In search of another cause of IGHD, growth hormone releasing factor (GHRF) is also being studied. The DNA of IGHD patients and controls is being examined using polymerase chain reaction (PCR) techniques and direct sequencing. The 470 bp regulatory region of GHRF including the TATA and CAAT box, mRNA initiation site and 77bp first exon has been studied first. The DNA of 40 IGHD patients and 26 controls has been amplified and electrophoresed on a 2% agarose gel and no significant differences in size have been observed. To date, 27 IGHD Patients and 14 controls have been further evaluated by sequencing. There is a strong stop ocurring in part of the sequence; however, all the known regulatory elements are clearly sequenced and contain no point mutations.

These results indicate that neither major insertions or deletions nor point mutations of the regulatory region of GHRF are major causes of IGHD. Studies of the last 2 exons of GHRF are in progress, but do not yet detect a gross deletion or insertion. Studies to detect point mutations are underway.

WH 246 QUANTIFICATION OF POLYMERASE CHAIN REACTION PRODUCTS BY AFFINITY-BASED HYBRID COLLECTION, Ann-Christine Syvänen, Marina Bengtström, Jukka Tenhunen, Hans Söderlund, Orion Genetic Engineering Laboratory, Orion Corp. Ltd., Valimotie 7, 00380 Helsinki, Finland.
Identification of nucleic acid sequences by hybridization is a powerful tool for diagnosis. However, the sensitivity the hybridization methods is often insufficient. Our aim was to create a sensitive and convenient diagnostic test by combining the polymerase chain reaction and the affinity-based hybrid collection procedure (Syvänen, et al. 1986, Nucleic Acids Res. 14, 5037). We used oligonucleotides modified with biotin in their 5'-end as primers in the polymerase chain reaction. This results in the synthesis of 5'-biotinylated DNA molecules. These are detected by liquid hybridization to a labelled probe followed by collection of the formed hybrids on an avidin-matrix. The collected hybrids are measured and can be quantified with the aid of a hybridization standard curve prepared in parallell with varying amounts of biotinylated target DNA. Thus a quantitative measure of the DNA produced by the polymerase chain reaction is obtained. The procedure allows convenient identification of a very small number of DNA molecules of interest and using oligonucleotide probes mutations in the amplified DNA can be observed.

WH 247 PCR AMPLIFICATION OF CONSERVED GENES FROM NOVEL GENOMES WITH PEPTIDE-SPECIFIC PRIMERS, Martin F.J. Taylor and Martin E.Kreitman, Department of Biology, Princeton University, Princeton NJ08544. To date, the exploration of novel genomes has been tedious; entailing construction of gDNA or cDNA libraries, which are then screened for the recombinants of interest using a probe from a known genome. At least for highly conserved genes, it was thought possible to construct primers cocktails which are specific to known, conserved amino acid (AA) sequences, which should amplify the intervening coding regions from genomes of novel organisms. Such primers must have as low a cumulative genetic code redundancy as possible, effectively eliminating all sequences which included isoleucine, alanine, glycine, proline, threonine, valine, leucine, arginine, and serine. All AA sequences published in the NBRF database, and translated from Genbank, for ubiquitins, histones, cytochrome oxidase, and G3PDH were aligned using the FASTP program. Suitable pairs of oligopeptides were found for ubiquitin and cytochrome oxidase, and matching oligonucleotide primer cocktails were synthesised. Parts of coding regions for ubiquitin (200bp) and for cytochrome oxidase (1503bp between subunits I & II, and 1786bp between II and III), were amplified from total genomic DNA preparations of fruitfly, a butterfly, and 17-year cicada. The amplified products were blunt-end sub-cloned into BlueScribe plasmid, and the inserts sequenced with the Sequenase kit. This technique offers a drastic reduction in the time and labour involved in acquisition of sequence data for phylogenetic analysis of groups of novel organisms.

WH 248 USE OF PCR TO ANALYZE TRANSFECTION FREQUENCIES: APPLICATION TO HUMAN GENE THERAPY PROTOCOLS. F. Toneguzzo and A. Keating, EG&G Biomolecular Natick, MA 01760 and Toronto General Hospital, Toronto, Canada M5G 2C4. Bone marrow cells obtained from normal consenting donors were subjected to electroporation in the presence of selectable marker genes. To determine the optimal electroporation parameters for different cellular populations the cells were then plated for CFU-C, BFU-E and CFU-MIX in the presence and in the absence of selective media. The results show that when the cells were grown under conditions which ensured no breakthrough growth the transfection frequencies varied from 1 to 3%. Analysis by PCR of individual CFU-C colonies grown in non-selective media however showed that up to 15% of the colonies contained transfected DNA. PCR is being used to examine the events occurring within single hematopoietic progenitor cells in order to determine what is the correlation between the nature of the regulatory sequence, the site of DNA insertion and the levels of gene expression.

WH 249 HIV-1 and HTLV-1 Proviral Sequences Detected In Cells From Infected Rabbits Using PCR. Mary Ellen Truckenmiller, Henrietta Kulaga*, Ellen Gugel and Thomas J. Kindt. Laboratory of Immunogenetics, NIAID, NIH, Bethesda, MD 20892 and *Neuropsychiatry Branch, NIMH, Washington, D.C. Outbred rabbits were infected with HIV-1, HTLV-1 or a combination of both viruses, and infection was confirmed by PCR. Intravenous inoculation of rabbits was carried out using a continuous human T-cell line, A3.01, infected with HIV-1 and/or MT-2 cells which are a human HTLV-1-transformed T-cell line. Control animals received injections of uninfected A3.01 cells. HIV-1 proviral sequences were detected in peripheral blood lymphocytes (PBL) from infected rabbits after culturing in IL-2 (to stimulate T-cell proliferation) or after amplifying virus from PBL culture supernatants in the A3.01 indicator cell line. For these assays, two sets of oligonucleotide primer pairs were used which are complementary to conserved regions of the HIV-1 gag and env genes. HTLV-1 sequences were detected in DNA from PBL and in continuous T-cell lines derived from HTLV-1 infected rabbits. Three sets of primer pairs were used to amplify sequences in gag, env and tax HTLV-1 genes. The PCR products of predicted sizes were detected by gel electrophoresis and hybridization with specific probes. Further confirmation was obtained by analysis of the PCR products digested with specific restriction endonucleases. These data indicate that human retroviruses can be detected in rabbits in a sensitive and reproducible manner by use of PCR.

WH 250 APPROACHES TO SOLID-PHASE DNA TECHNOLOGY USING PCR. Mathias Uhlén, Thomas, Hultman and Johan Wahlberg, Department of Biochemistry, Royal Institute of Technology, S-10044 Stockholm, Sweden.

New approaches using solid-phase DNA technology have been developed involving the polymerase chain reaction (PCR). Biotin is selectively introduced into one of the strands of the amplified material during the PCR and this biotin is subsequently used for directed immobilization to magnetic beads coated with streptavidin. This basic concept has been used to develop new methods in (i) DNA sequencing; (ii) site-specific mutagenesis and (iii) DNA diagnostics. The sequencing approach is based on the Sanger method and selective elution of one strand is obtained with alkali and/or formamid. The *in vitro* mutagenesis approach takes advantage of the possibility to generate gap-duplex DNA with the mutated strand in a single stranded form. High yield of mutants is obtained without special host strains. The DNA diagnostics approach is based on incorporation of a non-isotope label into the strand not immobilized to the solid support. In addition, a method to avoid amplification of non-specific DNA will be described to allow direct DNA analysis without the necessity of electrophoresis for size separation of the amplified material.

WH 251 A RAPID METHOD FOR POLYMERASE CHAIN REACTION AMPLIFICATION OF HIV DNA IN CLINICAL SPECIMENS TO CONFIRM OR DETECT INFECTION, Pablo Vial, David Katzenstein, Sheila A. Grace, and Gerald V. Quinnan, Jr. Laboratory of Retrovirology Research, Center for Biologics Evaluation and Research, FDA, Bethesda, MD 20892

The variable number of peripheral blood mononuclear cells (PBMC) in clinical samples, contamination during processing, and loss of DNA are problems in the use of polymerase chain reaction (PCR) to detect HIV DNA. We have tested a simple rapid method that minimizes the DNA losses and risk of contamination. One million PBMC or serial dilutions of HIV infected and uninfected cells were aliquotted in 50mcl water. Prior to PCR, cells were boiled for 5' followed by chilling on ice for 5'. Duplicate cell pellets were subjected to phenol/chloroform extraction. DNA or cells were added to siliconized tubes containing PCR reagents and Taq polymerase (2.5u) (Perkin Elmer-Cetus) and one of three primer pairs (gag, pol, or env). After 2 minutes at 92°, 35 PCR cycles were performed (50°for 2 minutes, 70°for 2 minutes, 92°for 2 minutes). The amplification products were detected by gel electrophoresis and dot blot hybridization. HIV specific PCR products were detected in PBMC from 20/20 HIV seropositive subjects and 0/6 seronegative controls both in boiled cells and extracted DNA. With the infected and uninfected cell mixes, as few as 10 HIV infected cells yielded a positive result using boiled cells compared to 100 when DNA was extracted. We conclude that PCR amplification of boiled cells may enhance sensitivity and specificity of PCR by minimizing loss of DNA and decreasing manipulation that may cause contamination of clinical specimens.

WH 252 DETECTION OF HPV NUCLEIC ACIDS IN PAP SMEARS, J.M, Whitcomb, J.A. Zijlstra, I. Zbinden, J-F. Delaloye, H. Bossart and P.A. Cerutti, Dept of Carcinogenesis, ISREC and Dept of Obst and Gyn, CHUV, Lausanne, Switzerland. We are using PCR to directly assay for the presence of HPV DNA in pap smear material. The asasy is brief, inexpensive and requires no radioactivity. We are using the method to study the incidence of HPV infection in a large group of "normal" women, i.e. those visiting the gynecology clinic for routine consultations. Samples are obtained from the spatula used to perform the pap smear by vigorous shaking in PBS followed by washing in PBS, centrifugation and storage at -70. A two-temperature cycling protocol allows shorter cycling times and a stringent annealing temperature yields very low amplification of non-specific DNA sequences. The samples are prepared for amplification by sonication in buffer under paraffin for 15 secs. Amplimers for HPV 16 and 18 in the E6/E7 region and the human beta globin gene were utilized. The amplification products were analysed on an ethidium bromide-stained agarose gel and are differentiated by size. The amplimers chosen give rise to pieces 693, 544 and 447 bp for beta globin, HPV18 and HPV16 respectively. Hela(HPV18) and SiHa(HPV16) cells are used as controls. Reverse transcription before amplification allows detection of HPV RNA as well as DNA sequences. The position of the amplimers around the E6* splice site allows detection of the E6* RNA directly by size. We have tested 170 samples to date and have detected HPV 16 sequences in only four of the samples. We have detected no HPV 18. Of the 4 positive samples, 3 are from women with no signs of dysplasia. One has condyloma and type I dysplasia. The detection of HPV in women without dysplasia is an important goal of this project.

WH 253 PATTERNS OF INHERITANCE IN MAIZE: THE USE OF PCR AS AN ALTERNATIVE TO SOUTHERN BLOTTING IN RFLP ANALYSIS. Erik A. Whitehorn, David Zaitlin, Brad Hoo, and Phil Filner, Department of Molecular Biology, Sungene Technologies Corporation, 2050 Concourse Dr., San Jose, CA., 95131–1818. Restriction Fragment Length Polymorphisms (RFLPs) are being used extensively to evaluate genetic segregation in populations. Hundreds of probes have been developed which track specific loci and the technology has been applied to a number of plant species, notably maize, tomato, rice and Arabidopsis thaliana. This work has been primarily directed towards the establishment of linkage maps, which are becoming an important tool in selective plant breeding. We report here the monitoring of the genetic contribution of Wf9 during the development of a variety of Reid-type Maize inbreds, using both RFLP and PCR methods of analysis. The Wf9 background is represented by an introgression of DNA into chromosome 2 and has been characterized in our lab by the presence of a null allele at the locus detected by the RFLP probe, SGCR196. The PCR application uses oligonucleotides derived from SGCR196 sequences with partially purified plant DNA. The application results in a simple plus/minus assay which easily detects the genetic contribution of Wf9 in hybrid progeny. The conversion of RFLP probes to a PCR methodology expedites the assay and represents a significant savings in the cost of analysis especially when addressing the patterns of inheritance in large populations.

WH 254 A RAPID METHOD FOR DETECTION OF Y CHROMOSOMAL DNA FROM DRIED BLOOD SPECIMENS BY THE POLYMERASE CHAIN REACTION, Michal Witt, Robert P. Erickson, Department of Human Genetics and Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, MI 48109
Alphoid satellite family is the only repetitive DNA family showing chromosome specificity. We have developed a simple, rapid and reliable test for sex diagnosis based on detection of these sequences in undigested genomic DNA through the polymerase chain reaction. In our test, dried blood specimens were used as a source of DNA. When female DNA was used as a template for the reaction only a 130 bp X chromosome-specific fragment was detected, while on male DNA both a 170 bp Y chromosome-specific and X chromosome-specific fragments were detected. The Y chromosome specific fragment was identified and further characterized by restriction enzyme analysis. The Y fragment was detectable when DNA obtained from an equivalent of 10 μl of spotted blood was used in the reaction, while detection of the X fragment was possible with DNA from an equivalent of 5 μl of blood. Our test may find various applications in newborn screening and in forensic science.

WH 255 DETECTION OF HBV DNA IN HEPATITIS B PATIENT'S SERUM BY PCR---A PRELIMINARY REPORT, Xie Yan-bo and Lin Da, Molecular Biology Research Laboratory, Sun Yat-sen University of Medical Sciences, Guangzhou, 510080, China. By a computer analysis of all published HBV DNA sequences data, we found two regions which can serve as primers in PCR detection of HBV DNA. The sequence between these two primers is about 500 bp long. We synthesized these two primers (X3 and X4) by automated DNA synthesizer in collaboration with Prof. Wong of Fu-dan University, Shanghai. The sequences of X3 and X4 are: 5'ATCAGGATTCCTAGGACCCC3', 5'CCCAATACCACATCATCCAT3'. A PCR kit from Cetus using thermostable Taq enzyme was used to detect HBV plasmid AM6 which was cut by Bam HI. After 35 cycles the reaction was terminated and the mixture was analysed by 1.2% agarose gel electrophoresis. A clear band of about 500 bp was shown. This is the first time that a detection of HBV DNA by PCR is reported. Studies using these primers in PCR to detect HBV DNA in patient's sera are in progress.

WH 256 DETECTION OF VIRAL PATHOGENS IN PATHOLOGY SPECIMENS USING THE POLYMERASE CHAIN REACTION, T.S. Benedict Yen and Xiao Xiao, Department of Pathology, University of California, San Francisco, CA 94143-0506
It is often difficult to diagnose viral diseases in routinely processed tissue specimens submitted for pathologic examination. We have investigated whether it is possible to detect viral DNA in paraffin-embedded tissues using PCR and staining by ethidium bromide following gel electrophoresis. Using boiled deparaffinized material and 40 cycles of amplification, we have been able to detect several DNA viruses, including herpes simplex, varicella-zoster, human papilloma, and cytomegaloviruses. The sensitivity is much better than histopathology and immunocytochemistry, and seems comparable to culturing. No apparent false-positive results have been obtained, but negative specimens frequently give rise to weak non-specific bands. We are now comparing the sensitivity of this method with that of non-radioactive in situ hybridization. In conclusion, it appears that PCR may be a viable clinical method of diagnosing viral infections.

WH 257 DETECTION OF HIV PROVIRAL DNA IN PERIPHERAL BLOOD MONONUCLEAR CELLS BY THE POLYMERASE CHAIN REACTION (PCR), Karen K.Y. Young, Robert E. Winters, and James B. Peter, Specialty Laboratories, Inc., Santa Monica, CA 90404. The methods currently available for the detection of human immunodeficiency virus (HIV) infection rely on the detection of HIV-specific antibody, viral antigens, or direct detection of the virus by culture or nucleic acid hybridization. The detection of proviral nucleic acid by standard hybridization techniques is hampered by the fact that only a small proportion of mononuclear cells express viral RNA. This problem has been overcome by the in vitro amplification of a segment of the proviral DNA by the PCR. The PCR amplifies proviral DNA by repeated cycles of DNA synthesis using virus-specific oligonucleotide primers and DNA polymerase. We have examined the applicability of the PCR to a clinical setting in a cohort study of 225 antibody-negative, low-risk individuals; 29 antibody-negative, at-risk individuals; and 72 antibody-positive, at-risk individuals. All 225 antibody-negative, low-risk individuals studied had no detectable HIV proviral DNA. HIV proviral DNA was detected in 68 of 72 antibody-positive, at-risk individuals. HIV antigen was detectable in the plasma of only 12 of the antibody-positive individuals and all of these were also positive for proviral DNA in the peripheral blood mononuclear cells.

WH 258 USE OF AN RNA FOLDING ALGORITHM TO SELECT mRNA SEQUENCES FOR AMPLIFICATION BY THE POLYMERASE CHAIN REACTION. P.S. Zelenka, L.A. Pallansch, H.T. Beswick, and J.C. Talian, National Eye Institute, NIH, Bethesda, MD, 20892.
Low abundance mRNAs can be assayed by the polymerase chain reaction following synthesis of the necessary DNA template by reverse transcriptase. We have found, however, that certain sequences yield little or no full-length product when amplified from reverse-transcribed cDNA. The sequences which fail to amplify are not necessarily G:C rich, and can be amplified without difficulty using a cloned cDNA fragment. We, therefore, tested the possibility that secondary structure in the mRNA prevents reverse transcriptase from copying these sequences. We used an RNA folding program, FOLD, which employs the Zucker folding algorithm to identify sequences involved in secondary structures. This analysis revealed that the regions which had failed to amplify (4 regions from 3 different mRNAs) contained long, stable stem structures, with as many as 9 consecutive base-pairs. With the information provided by the folding analysis, we chose regions for amplification which contained little, if any, secondary structure. In every case, amplification by the polymerase chain reaction was successful. Using this method we have examined levels of c-myc, c-src, and glucose-3-phosphate dehydrogenase mRNA in the embryonic chicken lens.

WH 259 GENE DIAGNOSIS OF HEMOGLOBINOPATHY IN CHINESE BY AMPLIFIED DNA, Yi-tao Zeng, Shu-zhen Huang, Xia-di Zhou, Hao Zhu and Zhao-rui Ren, Laboratory of Medical Genetics, Shanghai Children's Hospital, Shanghai, the People's Republic of China

Here we report the application of DNA PCR amplification on the prenatal diagnosis of thalassemia syndromes and on detection of $_{\beta}$S and $_{\beta}$D Punjab genes.DNA amplification was performed on lysed amniotic fluid cells or chorionic villus samples and on DNA sampling from dried blood without prior DNA extraction. α-thalassemia was prenatally diagnosed by direct analysis of amplified fetal target sequences on gel electrophoresis; and ß-thalassemia was predicted by Hgi AI RLFP linkage analysis with amplified ß-globin DNA. Sickle cell gene was detected by Mst II mapping of the amplified ß-globin DNA directly on the electrophoretic gels. For identification of Hb D Punjab (ß121 $\frac{GAA\rightarrow CAA}{Glu\rightarrow Gln}$),the most commom variant in western China, the primers were designed and synthesized to amplify a 144bp fragment ß-globin gene, including codons 121 (GAA) and 122 (TTC). The 5'GAATTC-3' was just an Eco RI recognition site. So Hb D Punjab gene could be easily identified by Eco RI mapping of the 144bp amplified sequence,because of a single base change at codon 121. The analyses of the amplified DNA does not require radioactive DNA probe and southern hybridization. The total procedure can be completed within five hours. This simple method provides a powerful tool for gene diagnosis of hemoglobinopathy or other genetic diseases.

This abstract was inadvertently ommitted from Volume 13A, and pertains to Meeting B, GLYCOBIOLOGY

B 029 CANCER-ASSOCIATED CARBOHYDRATE ANTIGENS John L. Magnani*, WeiTong Wang*, Torgny Lundgren*, Peter S. Linsley+, Diane Horn+, & Joseph P. Brown , BioCarb Inc, Gaithersburg, MD 20898; *BioCarb AN, S23370 Lund, Sweden; +Oncogen, Seattle, WA 98121; and Genetic Systems, Seattle, WA 98121.

Many cancer-associated or differentiation antigens defined by monoclonal antibodies are carbohydrates. The fine structure of carbohydrate epitopes are systrematically determined by a progressive analysis of the antibody, beginning with a routine screen of purified glycolipids and neo-glycoconjugates followed by a detailed analysis of the native carbohydrate antigens using a variety of techniques including HPTLC immunostaining of glycolipids and high performance liquid affinity chromatography of oligosaccharides. For example, antibody M26, which is produced from a hybridoma obtained from a mouse immunized with the pleural effusion mucins from a breast cancer patient, binds a cancer associated carbohydrate antigen found on mucin-like molecules which are elevated in the serum of many patients with breast cancer (P.S. Linsley, et al., Cancer Res., 48:2133, 1988). The antibody binds weakly to high concentrations of sialylated lacto-N-fucopentaose III ceramide ($IV^3NeuAcIII^3FucnLc_4$); however, it binds with much higher affinity to a larger oligosaccharide sequence found in sialylated type 2 fucosylated polylactosamine structures. The techniques used for detailed epitope mapping of this oligosaccharide may also be used for other carbohydrate binding proteins such as toxins, lectins and bacterial or viral adhesins.