Mutation Detection 2001

VI International Symposium on Mutations in the Human Genome

Bled, Slovenia

3rd-7th May, 2001

Mutation Detection 2001

VI International Symposium on Mutations in the Human Genome

Organizing and scientific committee:

Richard G.H. Cotton
Damjan Glavač
Metka Ravnik-Glavač
Ann-Christine Syvänen

Edited by:	Damjan Glavač and Metka Ravnik-Glavač
Technical editor:	Aleš Novak
Cover page design:	Aleš Novak
Printed by:	Littera picta d.o.o.
Number of copies:	250

CIP - Kataložni zapis o publikaciji
Narodna in univerzitetna knjižnica, Ljubljana

575.224 (063)

INTERNATIONAL Symposium on Mutations in the Human Genome (6 ; 2001 ; Bled)
Mutation Detection 2001 / VI International Symposium on Mutations in the Human Genome, Bled, Slovenia, 3rd - 7th May, 2001 ; [edited by Damjan Glavač and Metka Ravnik-Glavač]. - Ljubljana : Human Genome Organization, 2001

1. Gl. stv. nasl. 2. Glavač, Damjan
112240128

Published by the Human Genome Organization
Ljubljana, May, 2001

Acknowledgements

The Organizing Committee wishes to express its gratitude to Cathy Pole and Frank Wright (HUGO-London) and to the following public and private institutions and companies for their support to the VI Symposium on »Mutations in the Human Genome«.

Republic of Slovenia, Ministry of Education, Science and Sport, Office of Science
The Human Genome Organization

MAJOR SPONSOR
Varian, Inc.

SPONSORS
Perkin Elmer Life Sciences
Gemini Genomics Ltd
Transgenomic, Inc.
Sequenom, Inc.

DONORS
Luminex Corporation
Genset Oligos
Genset Pacific Pty. Ltd
Pyrosequencing AB
Orchid Biosciences, Inc.
Ambion, Inc.

SUPPORTERS
Affymetrix
Nanogen, Inc.

EXHIBITORS
Amersham Pharmacia
Bruker Saxonia Analytik GmbH
Gemini Genomics Ltd
Genset Oligos
Genset Pacific Pty. Ltd
Luminex
Nanogen, Inc.
Orchid Biosciences, Inc.
Perkin Elmer Life Sciences
Sequenom, Inc.
Transgenomic, Inc.
Varian, Inc.

LOCAL SUPPORTERS
Krka d.d.
Lek d.d.
Roche Diagnostics
Doc. J.Cholewa Fundation
Mediline d.o.o.

MUTATION DETECTION 2001

The year 2001 has already been marked by outstanding achievements in human genome research. Now the completed sequence of the human genome and the discovery of more than 2 million common genetic variants represent a challenge for efficient mutation detection and require new strategies and methods. For example, large-scale pharmacogenetics and complex disease association studies will require typing of thousands of single-nucleotide polymorphisms (SNPs) in thousands of individuals. Therefore high-throughput aspect and the cost effectiveness are becoming increasingly important for practical mutation detection. After Oxford (UK), Orta (Italy), Visby (Sweden), Brno (Czech Republic) and Vicoforte (Italy) the VI International Symposium on Mutations in the Human Genome will take place in the attractive mountain lake resort Bled, Slovenia from 3rd - 7th May, 2001. Alpine scenery, points of historical interest and an advantageous geographical location south of the Alps make Bled an ideal place for this meeting. It will host the inventors and prominent users of methods based on this subject who will review progress, identify current problems and propose solutions. We trust that the scientific and social programmes and the beautiful scenery of Bled will provide the atmosphere of friendly and fruitful exchange of recent and relevant knowledge, and create the settings for establishing new personal contacts.

We are pleased to welcome you to Bled, Slovenia.

Dobrodošli na Bledu !

Richard Cotton,
Damjan Glavač,
Metka Ravnik-Glavač,
Ann-Christine Syvanen

Table of contents

Programme

Thursday, May 3, 2001

12:00-20:00 ***Registration***

16:30-17:00 ***Opening ceremony and cultural program***
Professor Katja Breskvar on behalf of the Chancellor of the University of Ljubljana
Professor Boštjan Žekš on behalf of the Presidency of the Slovenian Academy of Sciences and Arts

Chair persons: Richard Cotton, Damjan Glavač, Ann-Christine Syvänen

17:00-17:20 Introduction and Overviews
Richard Cotton and Ann-Christine Syvänen

17:20-18:00 **Keynote lecture:**
Ed Southern
DNA arrays: making them and using them

18:00-20:00 ***Welcome reception and dinner***

20:00-22:00 **Poster viewing I accompanied by a glass of champagne**

Friday, May 4, 2001

Registration desk will be open from 8 a.m. to 8 p.m.

8:30-10:15 **Identification of new SNPs and mutations I**
(Session sponsored by Varian)
Chairpersons: Ed Southern, Michael Dean

8:30-9:00 **Kenshi Hayashi**
SNP characterization: Allele-frequency estimation by SSCP and genotyping by multiplex sequencing

9:00-9:30 **Eric Gerber** (Varian, Sponsor)
New advancements in mutation detection by DHPLC

9:30-10:00 **Arupa Ganguly**
High throughput mutation analysis of cancer susceptibility genes by conformation sensitive gel electrophoresis

10:00-10:15 **Metka Ravnik-Glavač**
Optimization of DHPLC method for analysis of *CFTR* gene mutations

10:15-10:45 ***Coffee break***

10:45-12:30 **Identification of new SNPs and mutations II**
Chairpersons: Sanjay Tyagi, Kenshi Hayashi

10:45-11:15 **Michael Dean**
Use of DHPLC to identify genes for complex diseases

11:15-11:45 **Damjan Glavač**
Capillary Electrophoresis Single-Stranded Conformational Analysis (CE-SSCA) - possibilities and limitations

11:45-12:15 **Niels Storm** (Sequenom, Sponsor)
Mining the genome with an integrated strategy based on SNP discovery, SNP scoring and MassARRAY MALDI-TOF MS

12:15-12:30 **Habibul Ahsan**
Evaluating appropriateness and suitability of mutation-detection methods in large-scale epidemiology studies examining genetic associations

12:30-13:30 ***Lunch break***

13:30-16:00 **Clinical applications I**
Chairpersons: Jean Amos, Graham Taylor

13:30-14:00 **Jean Amos**
Genotyping in the clinic

14:00-14:30 **Johan den Dunnen**
The DMD gene - looking for a needle in a 2,400,000 piece haystack

14:30-15:00 **Jeffrey Wine**
Natural animal models of genetic diseases

15:00-15:30 **Michael Krawczak**
Phenotypic consequences of pathological missense mutations

15:30-15:45 **Joan Bentzen**
Apolipoprotein B haplotypes and their impact on blood lipids and insulin

15:45-16:00 **Zbigniew Rudzki**
Mutation detection for Hereditary Non-Polyposis Colon Cancer (HNPCC) – the value of MSI and antibody staining in screening or mutations

16:00-19:00 ***Visit the Bled Island and St. Catherine Church including concert***

19:00-20:00 ***Dinner***

20:00-22:00 **Poster viewing II accompanied by a glass of wine**

Saturday, May 5, 2001

Registration desk will be open from 8 a.m. to 8 p.m.

8:30-10:00 **Methods for SNP scoring and mutation detection I**
(Session sponsored by Perkin Elmer)
Chairpersons: Ann-Christine Syvänen, Jing Cheng

8:30-9:00 **Mats Nilsson**
Padlock probes for multiplex genotyping and for in situ analysis of mitochondrial mutations

9:00-9:30 **Allan Asp** (Pyrosequencing, Sponsor)
The use of pyrosequencing in applied genomic analysis

9:30-9:45 **Ivo Gut**
High-throughput SNP genotyping by the GOOD Assay

9:45-10:00 **Joseph McClay**
High-throughput, cost-effective SNP genotyping by competitive allele-specific polymerase chain reaction (SNiPTag)

10:00-10:30 ***Coffee break***

10:30-12:30 **Methods for SNP scoring and mutation detection II**
Chairpersons: Pui Kwok, Lloyd Smith

10:30-11:00 **Ann-Christine Syvänen**
High-throughput analysis of human genomic sequence variation by primer extension on microarrays

11:00-11:30 **Janet Warrington**
New developments in variation detection using high density microarrays

11:30-12:00 **Kenneth Livak**
High throughput SNP analysis on the ABI PRISM® 7900HT Sequence Detection System

12:00-12:15 **Henrik Vissing**
EURAY: a platform for high-throughput multiplex SNP analysis

12:15-12:30 **Martin Schalling**
Genotyping of single nucleotide polymorphisms by Pyrosequencing™, validation against the 5' nuclease (TaqMan®) assay, and analysis of pools of DNA samples

12:30-14:00 ***Lunch break***

14:00-15:30 **Clinical applications II**
Chairpersons: Johan den Dunnen, Mats Nilsson

14:00-14:30 **Graham Taylor**
Scanning for point mutations and deletion/duplications

14:30-15:00 **Teresa Bromidge** (Speaker sponsored by Ambion)
Screening of the entire coding region of *p53* using the non-isotopic RNase cleavage assay

15:00-15:15 **Mario Tosi**
Fluorescent multiplex PCR: a powerful and simple method for the detection of genomic rearrangements

15:15-15:30 **Silvia Paracchini**
Y-chromosomal haplotyping to test testicular and prostate cancer predisposition using high-throughput MALDI-TOF mass spectrometry for SNP genotyping

15:30-18:00 ***Visit of the Bled Castle and Museum with snacks and drinks***

18:00-20:30 **Methods for SNP scoring and mutation detection III**
Chairpersons: Heikki Lehväslaiho, Eric Gerber

18:00-18:30 **Sanjay Tyagi**
Genotyping single-nucleotide variations with molecular beacons

18:30-19:00 **Michael Phillips**
Determination of SNP allele frequencies in three defined populations and CEPH pedigrees using primer extension technology, SNP-IT, on multiple platforms

19:00-19:30 **Pui Kwok**
High density SNP map of the human genome

19:30-20:00 **Carrie Shawber**
Using ligation detection reactions combined with a programmable universal DNA microarray to detect mutations, SNPs and CpG methylation

20:00-20:15 **Scott White**
High-throughput SNP scoring with GAMMArrays: Genomic Analysis using Multiplexed Microsphere Arrays

20:15-20:30 **Ross Hawkins**
High throughput genetic analysis of type 2 diabetes: A candidate gene approach

20:30-22:00 ***Dinner***

Sunday, May 6, 2001

8:30-10:30 **Bioinformatics, statistics and databases**
(Session sponsored by Gemini Genomics)
Chairpersons: Mark Perlin, Michael Krawczak

8:30-9:00 **Richard Cotton**
Mutation databases promise and problems

9:00-9:30 **Heikki Lehväslaiho**
SNPs and databases

9:30-10:00 **Mark Perlin**
Determining sequence length or content in zero, one, and two dimensions

10:00-10:30 **Joseph Terwilliger**
Population genetic epidemiology: Theoretical background and study design issues

10:30-11:00 ***Coffee break***

11:00-12:45 **New technologies for mutation detection I**
Chairpersons: Andrei Mirzabekov, Janet Warrington

11:00-11:30 **Lloyd Smith**
The surface invader assay for high-parallel SNP analysis

11:30-12:00 **Robert Kwiatkowski**
The Invader® Assay for direct detection and quantitation of nucleic acids

12:00-12:30 **Michael Heller**
Microelectronic DNA array devices for molecular diagnostic, pharmacogenomic and drug discovery applications

12:30-12:45 **Cinzia Pera**
DNA microchips technology for HLA-typing

12:45-14:00 ***Lunch break***

14:00-15:30 **New technologies for mutation detection II**
Chairpersons: Michael Heller, Arupa Ganguly

14:00-14:30 **Jing Cheng**
Microchips and chip-based systems

14:30-15:00 **Michael Daniels (Transgenomics, Sponsor)**
A novel, high throughput, method for the detection of truncating mutations utilising methylase inactivation with the Transgenomic WAVE® Nucleic Acid Fragment Analysis System

15:00-15:15 **Joanne Walter**
Evaluation of PCR enzyme related parameters affecting denaturing high performance liquid chromatography

15:15-15:30 **Johannes Zschocke**
Nucleic acid crosslinking probes for mutation screening

15:30-16:00 ***Coffee break***

16:00-17:15 **New technologies for mutation detection III**
Chairpersons: Joseph Terwilliger, Jeffrey Wine

16:00-16:30 **Andrei Mirzabekov**
Application of MAGIChips in analysis of polymorphisms and mutations

16:30-17:00 **James Jacobson (Luminex, Sponsor)**
High-throughput profiling using suspension Arrays™

17:00-17:15 **Andrzej Kilian**
Diversity Arrays: a solid state technology for sequence information independent genotyping

17:15-17:30 **Ants Kurg**
APEX-based resequencing assay of *p53* tumor suppressor gene

17:30-17:45 **Conclusions**

Coffee break

17:45-22:30 **Farewell party in Škofja Loka and »Kulinarika Jezeršek« with a dinner of typical Slovenian dishes and dancing.**

Monday, May 7, 2001

7:00-16:00 **POST-CONFERENCE TOUR**
BLED-LJUBLJANA-PREDJAMA-POSTOJNA-BLED

Lectures

Keynote Lecture

DNA arrays: making them and using them

E.M. Southern
University of Oxford, UK

DNA microarrays are used to analyse known sequence variants, for resequencing genes to look for mutations and for expression profiling. Arrays are made by spotting presynthesised nucleic acid probes, or by synthesizing them in situ using light directed methods or ink-jet printing. I will review the different technologies and their various applications.

About Ed Southern

Professor Ed Southern is globally recognised for his key insight 25 years ago that labelled nucleic acid molecules could be used to interrogate genetic material to determine DNA sequences. His techniques and their derivatives have led to vast research programmes in many different fields of biology, diagnostics and medicine including early work in the human genome project.

In the late eighties and early nineties, with his co-workers, he developed methods of synthesising customised short sequences of nucleic acids on glass surfaces, which would be capable of extreme miniaturisation. Coupled with bioengineering, these techniques allow very powerful experimental tools, DNA microarrays, to be built to investigate gene sequences and behaviour. It is widely accepted that the commercial potential for such techniques is large. Professor Southern has been concerned that the commercial potential is balanced with the needs of potential customers including academic researchers and the advancement of science. His techniques are seen as building blocks for or complementary to the skills of researchers in biology and medicine and OGT is looking to build constructive collaborative relationships with them.

Session sponsored by Varian

Identification of new SNPs and mutations

SNP characterization: Allele-frequency estimation by SSCP and genotyping by multiplex sequencing

K. Hayashi, K. Higasa, Y. Kukita, S. Baba and T. Tahira
Division of Genome Analysis, Institute of Genetic Information, Kyushu University, Japan

World-wide large scale effort to find SNPs have achieved collection of millions of them, and they are available in the public or private sectors. Using the SNPs identified throughout the genome as markers, genes responsible for polygenic traits are supposed to be pinpointed by the whole-genome association studies. However, hundreds to thousands of individuals may have to be genotyped in the association study, and tens of thousands or more of loci must be interrogated for each individual. Studies of this scale may be done by limited facilities that are equipped with ultra-high-throughput machines and are funded for full-time operation of the machines. A more realistic and perhaps democratic usage of SNPs should be in the candidate gene approach, which requires examination of much less loci. Such studies still demand genotype information of many individuals, and improvement in the throughput of the analysis using available reliable technology is needed. Here we show two relevant new methods, one for estimation of allele-frequencies of SNPs by SSCP analysis of pooled DNA using capillary-array, the other for genotyping of individuals by sequencing of polymerized small STSs each carrying a SNP.

1. Precise estimation of SNP-allele frequencies by PLACE-SSCP analysis of pooled DNA.

Virtually all methods for detection of SNPs rely on the PCR amplification of STSs harboring the SNPs. We have developed a cost-effective and streamlined technique of SNP-allele separation, in which the PCR products of genomic DNA are fluorescently labelled after the amplification, and directly

applied to capillary array electrophoresis for SSCP-separation of alleles. A system for data processing is being developed so that SNP-allele frequencies in the pooled DNA are precisely estimated from the heights of peaks of alleles, in an semi-automated manner.

2. SNP-genotyping by sequencing of ordered concatemers of SNP-containing micro-STSs.

A group of small STSs (micro-STSs) each containing a SNP are amplified using primers carrying short extra-segments at their 5' ends that serve as cohesive ends. The products are then combined and concatenated in a predefined order by the second thermal cyclings. Genotypes of all SNPs within the group are unambiguously determined by one direct sequencing of the concatemer and PolyPhred interpretation. With a newly developed dedicated software, we could define amplification/catenation primers of shorter than 25mer for more than 90% of arbitrarily chosen reference SNPs available in the public database (dbSNP), and genotypes thus obtained were perfectly concordant with other independent SNP genotyping results.

New advancements in mutation detection by DHPLC

E. Gerber
Varian, Inc.

In the last few years, DHPLC has become a widely employed method for the discovery of sequence variants in DNA. Although DHPLC has significant cost and automation advantages over other techniques, many scientists have expressed their desire for further improvements in automation and are looking for increased capacity beyond the current 200 samples per day per instrument level. Efforts to improve the throughput of the Varian Helix System for DHPLC have focused both on sample throughput and work flow efficiency. New run methodology allows for up to a four fold increase in throughput without loss of detection sensitivity. New oven technology, being introduced at the Mutation Detection '01 Conference, provides ballistic heating and open air cooling for a 3000% reduction in thermal equilibration times; a key concern of DHPLC scientists. Post run sample processing has also been improved with the introduction at the Mutation Detection '01 Conference of the new Helix Review automated scoring software for the fast and accurate processing of DHPLC results. Combined, these improvements represent a significant improvement in automation and throughput and directly address the concerns of many scientists involved in mutation detection.

High throughput mutation analysis of cancer susceptibility genes by conformation sensitive gel electrophoresis

A. Ganguly
University of Pennsylvania, School of Medicine, Department of Genetics, Philadelphia, USA

Conformation Sensitive Gel Electrophoresis (CSGE) was developed about eight years ago as a method of heteroduplex analysis to screen large multi-exon genes for sequence alteration. The method is highly specific, sensitive and cost effective as compared to direct sequencing. The novelty of the method is in the use of a non-proprietary acrylamide gel matrix that uses 1,4-bis (acrolyl) piperazine (Fluka) as a cross linker and Ethylene glycol and Formamide as mildly denaturing solvents. The denaturing environment helps to enhance the conformation polymorphism present in heteroduplexes of DNA amplicons containing as small as single nucleotide variations. The method has been widely used for many large multi exon genes. It has also been adapted for use on a fluorescent platform (F-CSGE) that resulted in higher throughput and sensitivity.

Variation in sensitivity of CSGE as a function of the exact nature of the mismatch, size of the amplicon or location of the mismatch in the amplicon has been studied. The results demonstrate that the nature of the mismatched base in a defined sequence context has the most profound effect on the conformation of the heteroduplex in the following order G:G=G:T=T:G>G:A=A:G=T:T>A:A>C:T>C:C=C:A=A:C=T:C. Also, the size of the amplicon as well as the location of the mismatch with respect to the ends are two important parameters that determine the resolution of the mismatch containing heteroduplexes. We have now adapted the CSGE gel matrix for analysis of denatured amplicons (D-CSGE). This provides us with information about the conformation of single stranded molecules complimentary to that obtained by non-denaturing CSGE(ND-CSGE) in the same gel format.

We routinely scan a large number of multi-exon cancer susceptibility genes including *BRCA1*, *BRCA2*, *hMLH1*, *hMSH2*, *APC*, *TP53*, *RB* as well as *Factor VIII* gene using CSGE. For specific genes, presence of a large number of polymorphisms in the coding sequences can be confounding. In the case of *BRCA1* and *BRCA2* genes, which harbor multiple single nucleotide polymorphisms, the sequencing load is large. There is no theory to predict how close two sequence variants can be and still give rise to unique migration aberrations. In contrast, *Factor VIII* gene is very poor in polymorphisms and is a great candidate for scanning based on CSGE followed by limited sequencing. Detailed results covering these aspects will be presented.

In conclusion, CSGE scanning provides a powerful cost efficient way to scan genes with high sensitivity and specificity. Adaptation to fluorescent platform has allowed for multiplexing and increased throughput.

Use of DHPLC to identify genes for complex diseases

M. Dean[1], B. Gerrard[2], R. Allikmets[3], and M. Carrington[1]
[1] Laboratory of Genomic Diversity,
[2] Intramural Research Support Program, SAIC Frederick, National Cancer Institute-Frederick, Frederick, MD 21702-1201
[3] Departments of Ophthalmology and Pathology, Columbia University, New York, NY

The identification of sequence variants in candidate genes represents a valuable approach to the study of complex diseases. This approach bypasses the use of linkage or linkage disequilibrium-based approaches, which can have poor power to detect genes of low relative risk. Genes that play even a minor role in a disease can provide a valuable link to the identification of critical pathways for further research. Using the direct gene analysis approach we have identified several alleles of chemokine receptors that play a role in resistance to HIV infection, and progression to AIDS. The *CCR5D32* allele encodes for a truncated and non-functional CCR5 protein and is found at a frequency of 10% in Caucasians. Analysis of multiple HIV high-risk cohorts demonstrates that *CCR5D32* homozygotes are highly protected from HIV infection. However no difference is seen in allele

frequencies between HIV- and HIV+ groups indicating that allele pooling strategies would have been unsuccessful at identifying this effect. Despite the recent origin of this allele (700-2000 years), *CCR5D32* occurred on the most common *CCR5* haplotype and surrounding SNPs would not have been informative to detect the effect of this allele. The *CCR5D32* allele, *CCR5* promoter variants, and an allele of the nearby *CCR2* gene all affect the progression of HIV infected individuals to AIDS. Despite the complex nature of HIV disease progression, survival analysis has proven to be a powerful approach to identify gene effects. The *ABCA4* gene is responsible for the juvenile recessive retinal degeneration Stargardt disease (STGD), we observed pedigrees in which obligate carriers were affected with age-related macular degeneration (AMD), a late-onset condition with phenotypic similarities to STGD. AMD is the most common cause of vision loss in the elderly and is a highly complex disease in which environmental and genetic factors have been implicated. Analysis of the *ABCR* gene in 167 AMD patients revealed a high percentage of missense alleles that were not found in a set of 220 controls. Analysis of two *ABCA4* alleles (*G1961E* and *D2177N*) in an international cohort of 1218 AMD cases and 1258 controls demonstrate that these alleles are significantly associated with disease and together account for about 6% of the risk of this common complex disease.

Capillary Electrophoresis Single Stranded Conformational Analysis (CE-SSCA)- possibilities and limitations

D. Glavač
Laboratory of Molecular Genetics, Institute of Pathology, Medical faculty, Ljubljana, Slovenia

Single-strand conformation analysis (SSCA) is one of the most popular methods for detection of mutations. Unprecedented by any other mutation detection technique to date, more than 7000 papers were published using either original SSCP method discovered in 1989 or different variations of the original technique. The reason for this popularity of the SSCA was its technical simplicity and its relatively high sensitivity for the detection of sequence variation. Disadvantages of SSCA are limitation of a fragment size and absence of theory for prediction of mobility shift and therefore sensitivity in general. Consequently multiple conditions were necessary to detect all mutations and apparently SSCA is less applicable for DNA analysis with unknown sequence. Recently, automated capillary electrophoresis (CE) systems have been developed and used for SSCA instead of conventional slab gel electrophoresis. SSCA in combination with CE offer very rapid, simple, sensitive and when used in a 96-capillary array electrophoresis system, also very high-throughput mutation screening tool. As a typical migration time in CE analysis for single- stranded DNA conformation of fragment size between 100 and 500 bp is between 10 min and 60 minutes. One could easily analyze several thousand samples for SNPs and mutations in a single day. Its additional advantage is that it is expected for CE to be more reproducible and certainly easy to automate.

However, development of methods and standards for CE-SSCA also requires the optimization of experimental conditions and comparison to previous established method. To address these questions we have studied with CE-SSCA more than 150 mutations of several disease genes *ß-globin*, *CFTR* gene, *p-53*, *VHL*, *mlh1* and *msh2* on two different capillary systems. Fluorescent - labeled PCR samples were prepared for electrophoresis on either Beckman P/ACE™ Model 5510 instrument or/and Perkin Elmer Applied Biosystems PRISM™ 310 Genetic Analyzer. Effect of type of polymer and polymer concentration, electric field and temperature were studied. Moreover, in an attempt to predict the stability of single strand conformations in non-denaturing polyacrylamide electrophoretic gels and capillary electrophoresis, Zuker's Mfold 2 program was used for calculations of free energies and other thermodynamic parameters. Results demonstrate that for at least some alterations in single-stranded DNA conformations there is a correlation between free enery of conformation compared to wild type and migration time of mutation and wild type. The relative differences between the free enery of wild type and mutation were higher or lower for the mutations which produce biger difference in migration time as compared to wild type. Therefore the combination of theoretical predictions of stability of single-stranded DNA conformations in folding and electrophoretic behaviour in capillary electrophoresis suggest that computer models can be used in order to predict the sensitivity of CE-SSCA.

Mining the genome with an integrated strategy based on SNP discovery, SNP scoring and MassARRAY MALDI-TOF MS

N. Storm and D. van den Boom
SEQUENOM GmbH, Mendelssohnstr. 15d, D-22761 Hamburg

The completion of the draft Human Genome sequence is now a reality. Analysis of genetic variations on an individual as well as a population level receives an ever increasing importance. A mass spectrometry-based system will be presented which provides error-free results for SNP analysis in high-throughput environments. In addition to being able to determine thousands of genotypes per day, this method can be used to determine allelic frequencies in defined DNA-pools (e.g., patient populations), which significantly shifts the paradigm for doing candidate gene association studies and pharmacogenomics. The described approach allows researchers to follow allelic frequencies as a function of quantitative or qualitative traits, gender, race, age, disease, drug response, or other criteria. Examples of such studies will be presented along with the underlying technology.

Clinical Aplication

Genotyping in the clinic

J. Amos
Molecular Genetics, Specialty Laboratories Inc., Santa Monica, USA

Molecular diagnosis is the detection of pathogenic mutations in DNA and RNA samples and the subsequent interpretation of results in the clinical and family context of the at-risk patient. Principles underlying DNA-based diagnosis originate from localization, identification, and characterization of genes responsible for human disease. Clinical molecular genetics is now part of the mainstream of medical care in the United States. All commercial clinical reference

laboratories now have a molecular genetic diagnostic unit, many of which are in contractual agreement with third party payors to provide services.

The broad range of mutation spectrum and type performed in the clinical laboratory require the use of multiple technologies rather than a single typing platform. Platform choice depends on such diverse factors as local expertise, test volume, economies of scale, R&D budget, and royalties. Test validation is a major hurdle and positive control samples are often not readily available.

Indications for clinical mutation analysis include diagnosis of disease for symptomatic patients, carrier risk revision for adults for the purpose of reproductive decision making, prenatal diagnosis for at-risk pregnancies, presymptomatic diagnosis for asymptomatic patients at risk for later onset genetic disorders, and population screening. Results are interpreted in the context of the indication for testing, the family history and the ethnic background of the patient. Illustrative cases will be presented.

Oversight and the regulatory environment for clinical molecular genetics laboratories in the United States are evolving rapidly. A synthesis of the current recommendations and pending requirements *vis a vis* CLIA, FDA, CDC, SACGT, CAP, ACMG, and the individuals states will be discussed.

The *DMD* gene - looking for a needle in a 2,400,000 piece haystack

J.T. den Dunnen[1], R.H.A.M. Vossen[1], S. White[1], M.H. Breuning[1], E. Bakker[1], H.B. Ginjaar[1], R. Hofstra[2] and G.J.B. van Ommen[1]

[1] Human and Clinical Genetics, Leiden University Medical Center, LEIDEN, Nederland

[2] Medical Genetics, University Groningen, GRONINGEN, Nederland

Duchenne and Becker muscular dystrophy (DMD/BMD) are X-linked recessive diseases caused by mutations in the human dystrophin gene. Reading frame disrupting mutations cause DMD, mutations which leave the reading frame in tact cause BMD. The dystrophin gene's complexity, its unprecedented size, 2.4 Mb, and the high new mutation rate make mutation-based diagnosis a daunting task. To increase the yield of mutations detected, we constantly try to improve the technology applied.

Two-thirds of mutations are deletions/duplications affecting large segments of the gene. In male patients, 95% of the deletions can be easily detected using an 18-exon multiplex PCR method. Detection of duplications, deletions in females and of the exact borders of the rearrangement requires a laborious Southern blot analysis using a set of 7 cDNA segments. We have now developed a versatile MAPH-based (Multiplex Amplifiable Probe Hybridisation) deletion/duplication screen facilitating the simultaneous analysis of 96 samples using capillary electrophoresis. The method is sensitive, yields reliable quantitative data, can be performed within 48 hours and detected several mutations missed by multiplex PCR and Southern blot analysis. These mutations include small deletions/duplications in carriers and one-exon duplications in patients. The analysis confirmed the unexpected finding recently reported by Hoffman and co-workers, that a significant set of patients have an exon-2 duplication which was missed by other diagnostic methods for nearly 14 years.

Mutation detection in the remaining 1/3 of cases turned out to be very difficult. The size of the gene, an 11.5 kb open reading frame spread over 79 exons, and the complexity of the mutations encountered made an immunohistochemical dystrophin-staining on muscle biopsy samples the preferred tool. However, this tool may fail in BMD cases and when no patient material is available. Since only the mutation itself provides absolute certainty regarding the diagnosis, we have tried and developed additional mutation detection methods. The Protein Truncation Test (PTT) was developed to zoom in on the majority of DMD point mutations, i.e. those disrupting the reading frame. Although technically demanding, methodological changes, amongst which protein tagging and fluorescent labelling, significantly simplified the protocol. PTT, was very effective and resolved a large set of mutations altering RNA-processing (mainly splicing). Still, PTT proved difficult to apply on female samples where non-sense mediated mRNA-decay and non-random X-inactivation often gave large allelic differences in expression.

A DGGE-based screen was developed which covers the *DMD*-gene in 95 amplicons. After PCR, before electrophoresis, 5-7 products with different melting profiles are mixed. Applied to dystrophin-negative DMD-patients, DGGE resolves mutations in over 85%. Nearly all mutations disrupt the reading frame. Missense mutations are very rare and when detected, difficult to link directly to the disease. The success rate in BMD-patients and carrier females is significantly lower. The mutations in the remaining fraction of cases remains elusive, although the direct gene flanking sequences and even more the 2.4 Mb of intronic sequences provide obvious candidates.

Although the success rate for DMD now exceeds 95%, several mutations still go undetected leaving patients and families with uncertainty regarding diagnosis and carrier status. To resolve exceptional cases, we regularly apply *in vitro* induced muscle differentiation using the *myoD*-gene. Especially for prenatal diagnosis, this tool can be extremely helpful.

References

http://www.dmd.nl

Natural animal models of genetic diseases

J. J. Wine[1], M. Dean[2] and D. Glavač[3]

[1] Cystic Fibrosis Research Laboratory, Stanford University, Stanford, CA, USA

[2] Human Genetics Section, Laboratory of Genomic Diversity, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD, USA

[3] Laboratory of Molecular Pathology, University of Ljubljana, Ljubljana, Slovenia

We introduce a strategy for developing natural animal models of human recessive genetic diseases. The approach uses rapid mutation screening of genomic DNA to detect unaffected carriers of disease genes. Because carrier frequencies of recessive genetic diseases are much higher than the incidence of disease (1/500 carriers for a disease with 1/1,000,000 incidence), the method works even for rare diseases. The strategy is practical with present methods for screening genomic DNA for unknown mutations, and will be increasingly cost-effective as more efficient methods for mutation detection are perfected. To establish feasibility, we used single strand conformation polymorphism and heteroduplex analysis (SSCP/HA) to screen genomic DNA of 1,500 non-human primates for mutations in *CFTR*, the gene responsible for cystic fibrosis. We detected >40 different amino acid changes in the coding region, and approximately half of these had properties that caused us to con-

sider them as candidate missense mutations. Physiological assays indicated that at least 4 of the candidate mutations have reduced function. A selective breeding program has begun to determine if homozygous animals have a distinctive phenotype. Continuing advances in mammalian cloning will permit rapid expansion of homozygous offspring if a useful phenotype is generated.

Phenotypic consequences of pathological missense mutations

M. Krawczak
Institute of Medical Genetics, University of Wales College of Medicine, Cardiff, UK

The inclusion of mutations into a pathology-based database like the Human Gene Mutation Database (HGMD), Cardiff, is a two-stage process. First, the mutation in question has to occur at the DNA level, then it has to pass a number of selective stages in order to cause a detectable disease state. The likelihood of the latter steps combined, called the "Relative Clinical Observation Likelihood" (RCOL), can be modelled as a function of the structural/functional consequences of a mutation at the protein level. Following this approach, we assessed 26 different biochemical/biophysical parameters of amino acid replacements that could potentially arise from a single base-pair substitution in one of five human genes: *ARSA*, *AT3*, *PAH*, *PROC* and *TTR*. A total of 9795 mutant structures were modelled *in silico* and analysed using the *WhatIf* software. The five genes were chosen since (i) a crystallographic structure was available of their protein products, and (ii) a sufficiently large number of amino acid replacements had been logged in HGMD. The modelling data allowed maximum likelihood estimates of RCOLs to be obtained for each gene as a function of the parameters employed, and a comparison of these RCOL estimates between genes. Several parameters (including energy difference between wild-type and mutant structure, accessibility of the mutated residue, distance from binding/active site) exhibited biologically meaningful RCOL profiles. Furthermore, although some profiles were found to vary between genes, others were applicable to all five proteins.

Scanning for point mutations and gene dosage

G.R. Taylor[1,2]
[1] ICRF Mutation Detection Facility and [2] Regional DNA Laboratory, Ashley Wing, St James's University Hospital, Leeds LS9 7TF, UK

Mutations in DNA can be defined broadly as any change of sequence when compared to a reference sequence. The changes may be point mutations (base substitutions), small insertions or deletions (indels) or larger re-arrangements of exons, genes or chromosomes. No one method can scan for all of these types of mutations-or can it?

Whilst methods for the detection of point mutations and small insertions or deletions are well established, the detection of larger (>100bp) genomic duplications or deletions can be more difficult. Most mutation scanning methods use PCR as a first step, but the subsequent analyses are usually qualitative rather than quantitative. Gene dosage methods based on PCR need to be quantitative (i.e. report molar quantities of starting material) or semi-quantitative (i.e. report gene dosage relative to an internal standard). Without some sort of quantitation, heterozygous deletions may be overlooked and therefore be under-ascertained. Gene dosage methods would provide the additional benefit of reporting allele drop-out in the PCR. Available methods for gene dosage analysis and for point/indel mutation scanning will be reviewed and preliminary experiments looking at combined approach will be discussed.

Screening of the entire coding region of *p53* using the Non-Isotopic RNase Cleavage Assay

T. Bromidge and D. Howe
Leukaemia Research Unit, Taunton and Somerset NHS Trust, UK

The tumour supressor gene *p53* has an established role in human malignancies and its mutation has been linked with a poor prognosis in various diseases, including haematological malignancies. Although the majority of mutations have been identified within exons 5-8 of the *p53* gene and most studies are limited to this region, mutations do occur elsewhere. Ideally, a method for screening this gene should identify mutations throughout the entire coding region of this gene but this has been difficult to achieve without sequencing. The non-isotopic RNase cleavage assay (NIRCA), based on the fact that RNase cleaves both strands of duplex RNA at mismatches, has been applied to *p53* mutation screening. However, this was again limited to exons 4-11.

We have developed a rapid method, based on NIRCA, for screening the entire coding region (exons 2-11- 1179bp) of *p53* in a single reaction. This method uses novel primer sequences for RT-PCR and an adaptation of the MutationScreener™ method (Ambion, USA). Two PCR reactions are performed for each patient using two sets of primers. Each primer set has one primer with a T7 promoter attached, but differ in that the T7 promoters are on opposing strands. This limits transcription to opposing strands of wild type and test samples and reduces the number of matched hybrids formed. The modification results in an increase in sensitivity over the original MutationScreener™ method and enables detection of a mutant clone in 20% of a sample.

We have utilised this method to screen 200 patients with a low grade lymphoproliferative disorder. Mutations were confirmed by sequencing in those patients showing a positive NIRCA. In addition to identification of point mutations the assay also provided information on a common polymorphism, present at codon 72, due to an easily recognisable cleavage pattern and highlighted a patient exhibiting abnormal splicing of the *p53* gene.

This NIRCA based method is a reliable technique for screening for mutations and can allow the rapid identification of polymorphisms by the presence of common cleavage patterns. It can be applied to any sequence for which primers can be designed.

Session sponsored by Perkin Elmer

Methods for SNP scoring and mutation detection

Padlock probes for multiplex genotyping and for in situ analysis of mitochondrial mutations

M. Nilsson
The Beijer Laboratory, Department of Genetics and Pathology, Uppsala University, Sweden

We are developing tools for genetic and protein analysis. My talk will be focused on recent developments in the padlock probe technology with emphasis on two unique advantages with these circularisable oligonucleotide probes. First, for localised detection of DNA sequence variants, and second, for highly multiplexed genotyping.

Padlock probes are well suited for localised detection of DNA variants due to the topological link that is formed between a circularised probe and it's target sequence. Further, padlock probes only becomes circularised if both probe arms form correct hybrids with the target sequence. This is due to the strict substrate requirement of the ligase used to join the ends of the probe. We have previously shown that by obtaining quantitative fluorescence results, padlock probes can be applied for *in situ* genotyping of centromeric repeat sequences. We have now, together with Ton Raap's group in Leiden, applied padlock probes for *in situ* genotyping of mitochondrial point mutations. We studied cybrid cell lines carrying different levels of the MELAS (npA3243G) and MERRF (npA8344G) mutations. In both cases the applied padlock probes could clearly distinguish mutant from wild type mitochondrial DNA (mtDNA). We found a very high variability in heteroplasmy level among cells in heteroplasmic cell lines, indicating a rapid random drift from the initial heteroplasmy level of the founding cell. Moreover, the two mtDNA variants apparently cluster together, which may explain the observed rapid drift.

We are also applying padlock probes for highly multiplexed genotyping of DNA in solution. Here the virtue of using circularisable probes is that the detection reaction is intra-molecular, thus in principle not sensitive to the number of probes added to a reaction. We add probes for multiple mutations and polymorphisms to one ligation reaction, amplify circularised probes in a PCR, using general primers, and finally, hybridise the amplification products to a zip-code array in presence of product-complementary fluorescence labelled oligonucleotide probes. The position on the array determines locus identity, and the colour of the fluorescence tag determines which alleles are present at that locus. We are currently exploring alternative means of amplifying the probes, and we have also recently shown that a similar strategy can be used for sensitive RNA detection.

The use of Pyrosequencing in applied genomic analysis

A. Asp
Pyrosequencing AB, Uppsala, Sweden

Late in 1999, Pyrosequencing AB launched its first instrument, PSQ 96, an automated DNA sequence analysis instrument based on the innovative Pyrosequencing technology. Reliable chemistry and a simple, robust detection mechanism eliminate the need for gels, dyes or specific labels and allows real time detection of sequencing events. Pyrosequencing uses an enzyme-cascade system, consisting of four enzymes and specific substrates, to produce light whenever a nucleotide forms a base-pair with the complementary base in a template strand. This light is detected, the base registered, and the next nucleotide added. If the nucleotide added is not incorporated, no light will be detected.

Focusing on SNP determination as first application, numerous other applications have now been developed e.g. allele frequency estimations and sequence analysis of typically 20-30 bases of DNA, all of which will be presented. Furthermore, SNP data from a new 384 platform instrument will also be presented.

High-throughput analysis of human genomic sequence variation by primer extension on microarrays

A. C. Syvänen
Department of Medical Sciences, Uppsala University, Sweden

DNA-polymerase-assisted primer extension reactions allow robust and highly specific discrimination between heterozygous and homozygous genotypes in the diploid human genome. Primer extension has consequently been applied to genotyping single nucleotide polymorphisms (SNPs) using a variety of assay formats and detection principles.

The high sequence specificity of the "minisequencing" single nucleotide primer extension reaction makes it a useful tool for sensitive, quantitative detection of SNPs. We are routinely using quantitative analysis of pooled DNA samples by minisequencing to determine allele frequencies of SNPs to be included in multiplex detection panels. We are able to quantitatively detect alleles with a frequency of less than 1% in a pooled DNA sample. For multiplex, high-throughput genotyping we have developed a microarray system based on minisequenicng or allele specific primer extension reactions and fluorescence detection. In this system detection primers are covalently immobilized through contact printing on a microscope slide in a pattern compatible with miniaturised reaction chambers in a 384-microtiter well format. This assay format allows the analysis of 80 samples with 240 primers per single microscope slide. Thus our genotyping capacity is mainly limited by the ability of multiplexing the PCR.

Using minisequencing single nucleotide primer extension reaction on microarrays we have screened a panel of 25 Y-chromosmal SNPs in a unique collection of samples representing five Finno-Ugric populations. The genetic diversity in the Finno-Ugric populations was assessed from more than 10,000 genotypes. Using allele-specific primer extension reactions on the microarrays we have monitored the prevalence of 31 recessive disease mutations underlying 27 clinical phenotypes in a large population-based study sample. In this

study over 140,000 genotypes were generated, evidencing for the high through-put of our system. Recently, we have established a minisequencing system for the analysis of about 80 SNPs in candidate genes regulating hypertension in a pharmacogenetic study. Because all equipment and reagents required are generally available, the microarray system is easy to establish "in house". It is universally applicable for genotyping any SNP or mutation.

New developments in variation detection using high density microarrays

J. A. Warrington, N. A. Shah, C. Liu, Z. Zhang, M. DeGuzman, D. Watts, J. Baid, J. Snyder, J. Saunders, M. E. Zwick, D. J. Cutler, E. E. Eichler and A. Chakravarti
Affymetrix Inc., Santa Clara CA, Johns Hopkins School of Medicine,Baltimore MD

We analyzed 8.2 Mb of the human genome and identified 15,682 SNPs using high density variation detection arrays (VDA, microarray). DNA from forty unrelated individuals of three different ethnic origins was amplified, labeled and hybridized to arrays designed with probes representing genomic, coding and regulatory regions. Over the two-year course of this study a number of improvements in sample preparation methods, hybridization assay, array handling and analysis method were developed and implemented. Protocol improvements including the use of long PCR and semi-automation reduced labeling and fragmentation costs by 33%. Automation improvements include the development of a scanner autoloader for arrays, a faster array wash station, and a linked laboratory tracking and data management system. Validation of a smaller feature size, 20 x 24 microns, allows the simultaneous screening of 30 kb sense and 30 kb antisense DNA on each microarray, increasing throughput to 1.4 Mb per day per two researchers. A new analysis method, Abacus(tm), greatly improves reproducibility, accuracy and throughput. Automated SNP calling and assignment of a quality score eliminates the need for each call to be individually reviewed and evaluated thus significantly reducing analysis time. The biggest challenge continues to lie in making heterozygote calls. Abacus (tm) identifies those bases for which reliable calls can be made and assigns a quality score. A sufficiently high quality score was achieved for 402 Kb of X-linked sequence screened in 40 individuals, 13.6 Mb of 16.1 Mb (85%) and for 1.6 Mb autosomal sequence 51.5 Mb/64.5 Mb (80%). Replicate experiments were carried out to assess genotyping accuracy. At present we have confirmed 1515 out of 1515 homozygous calls (100%), and 423 out of 426 heterozygous genotype calls (99.30%). To assess the accuracy of haploid genotype calls, we confirmed, through 6X sequencing coverage of a single individual, 17876 bases identical to the array calls, 0 bases different. To assess the accuracy of SNP discovery from the arrays using the new algorithm, we are confirming SNPs using both sequencing and data base information when it's available. To date we have confirmed 125 out of 125 SNPs tested. This is composed of 108 SNP identified experimentally and 17 SNPs found in dbSNP. Thus our SNP detection accuracy, so far, is 100%. In addition to SNP discovery we have developed a method, SNPmetric(tm), to identify SNPs whose state (homozygous/ heterozygous/ wildtype) segregates with gene expression data. This metric ranks genomic variants in order to identify candidates that may have biological relevance.

High throughput SNP analysis on the ABI PRISM® 7900HT Sequence Detection System

J. Stevens, J. Ma, C. Lee, W. Bi, and **K. J. Livak**
Applied Biosystems PCR SDS R&D

The ABI PRISM® 7900 HT Sequence Detection System features a real-time PCR instrument with 384-well-plate compatibility and robotic loading. The 7900 HT Automation Accessory loads up to eighty-four 384-well plates into the instrument without user intervention. Hand-held and integrated bar code readers simplify sample tracking, while continuous wavelength detection enables the use of multiple fluorophores in a single reaction. Sample tubes remain closed throughout the PCR and detection process to control contamination. The probe-based, fluorogenic 5' nuclease assay provides homogeneous detection of any SNP using universal reaction conditions. By using fluorogenic probes with a Minor Groove Binder (MGB) attached to the 3' end, robust discrimination is achieved even for SNP's in AT-rich DNA. These TaqMan® MGB probes also utilize a non-fluorescent quencher, which enables the detection of four reporter dyes, making it possible to multiplex TaqMan SNP analysis. These features coupled with developments to reduce reaction volume and automate probe manufacturing enable large scale and cost effective SNP analysis. Used as an endpoint reader, the ABI PRISM® 7900 HT Sequence Detection System can make up to 300,000 genotype determinations per 24-hr day.

Genotyping single-nucleotide variations with molecular beacons

S. Tyagi
Public Health Research Institute, 455 First Avenue, New York, NY 10016

Single-nucleotide variations represent the largest source of diversity in the human genome. We have developed a simple method to accurately genotype a large number of individuals for single-nucleotide variations at particular sites. This method utilizes hairpin-shaped hybridization probes called molecular beacons. Molecular beacons can report the presence of specific nucleic acids by undergoing reorganization in their conformation that switches on the fluorescence of an internally quenched fluorophore. In this method the region of genome that harbors the mutation is amplified in presence of a number of different molecular beacons that are each specific to a different haplotype and are labeled with a spectrally distinguishable fluorophore. In sealed amplification tubes molecular beacons wait for the concentration of the amplicon to reach a certain critical threshold and then respond by turning on their characteristic fluorescence. For a bi-allelic target region, the appearance of a green fluorescence indicates homozygous wild-type, red fluorescence indicates homozygous mutants and both green and red fluorescence indicate heterozygotes. These assays can also be performed accurately for two closely spaced SNP's where four possible alleles and ten possible genotypes exist. We have demonstrated the utility of this approach for drug resistance mutations in the *Mycobacterium tuberculosis*, for mutations in human methylenetetrahydrofolate reductase gene, in human chemokine receptor gene, and in human genes that are associated with Tay Sachs disease, Gauchers disease, sickle cell anemia and cystic fibrosis.

Molecular beacons can not only be used to track the synthesis of nucleic acids in amplification reactions, they can also be used to monitor the expression of specific genes in living cells. When introduced into living cells by microinjection or via liposomes, they bind to their target mRNAs and become fluorescent. Exciting results that demonstrate imaging of the gene expression in living cells will be described.

Determination of SNP allele frequencies in three defined populations and CEPH pedigrees using primer extension technology, SNP-IT, on multiple platforms

M. S. Phillips, W. M. Ankener, M. G. Pohl, and M. T. Boyce-Jacino
Orchid BioSciences, Inc., Princeton, NJ 08540 USA

A key component of the next phase of the Human Genome Project is the identification, localization and population frequency determination of single nucleotide polymorphisms (SNPs). In collaboration with The SNP Consortium (TSC), Orchid BioSciences is determining SNP allelic frequencies in samples from three defined populations, and selected CEPH pedigrees. Forty individuals from each of the Caucasian, African American and Asian populations are being analyzed to estimate the frequencies of more than 60,000 TSC SNPs distributed equally across the genome. The 60,000 TSC SNPs are also being analyzed on 10 CEPH pedigrees (80 individuals) for the determination of both SNP allelic frequency and phase. Well over 7 million genotypes will be determined over the life time of this project using Orchid's core high throughput single-base primer extension biochemistry, SNP-IT. The SNP-IT technology will be performed on multiple platforms. One of the key platforms being utilized is Orchid's SNPstream 25K platform, which is based on an automated, single-well assay formatted in 384-well microtiter plates. Another platform that is being utilized for this project is Orchid's SNPcode, which uses the Affymetrix universal tag array chip. This platform can type 500-1000 SNPs per chip and uses a homogenous primer extension reaction with multiplexed PCR reactions. Lastly, several other SNP-IT platforms currently being integrated into the project will be highlighted. Initial results from the analysis of the TSC SNPs will be presented. The culmination of these studies will form the basis for the first defined human SNP map, but more importantly, these studies will provide the resources necessary to conduct genome-wide linkage/association studies using SNPs.

High density SNP map of the human genome

P. Y. Kwok and the International SNP Map Working Group
Washington University School of Medicine, St. Louis, MO 63110, USA

Recently, a map of 1.42 million single nucleotide polymorphisms (SNPs) distributed throughput the human genome was published. This map provides a set of markers at an average density of on SNP every 1.9 kb. The SNPs were primarily discovered through two large projects: The SNP Consortium and the Human Genome Project's analysis of clone overlaps. The map integrates all publicly available SNPs with described genes and other features of the genomic landscape. We estimate that 60,000 SNPs fall within exons, and 85% of exons are within 5 kb of the nearest SNP.

Although an estimated 90-95% of the SNPs are validated, they have not been characterized as to their allele frequencies in the general population. In a pilot study, >1,200 SNPs were tested against population pools from the Caucasians, Africans, and Asians. We found that about 17% of the SNPs, only one allele was seen when the pooled DNA samples were sequenced. 27% of the SNPs were common (with minor allele frequencies of >=20%) in all 3 ethnic samples. When one ethnic group is considered, about 50% of the SNPs are found to be common.

Work is under way to characterize >200,000 SNPs and the data will be released to the public on a regular basis. The characterized SNPs will be an important public resource for defining haplotype variation across the genome, and should speed the identification of biomedically important genes.

Using ligation detection reactions combined with a programmable universal DNA microarray to detect mutations, SNPs and CpG methylation

C. J. Shawber, R. Favis, N. P. Gerry, N. E. Witowski and F. Barany
Weill Medical College Cornell University, Department of Microbiology and Immunology, New York, NY 10021

The ligase detection reaction (LDR) which our lab has developed takes advantage of the thermostable *Tth*, DNA ligase which has a high specificity for discriminating base variations in DNA. Combined with multiplex PCR, LDR has the ability to simultaneously detect dozens of genomic alterations in a single-tube reaction. To enable us to rapidly assay large numbers of samples, we have linked this mutation detection scheme with analysis on a Universal DNA microarray. We have successfully applied this approach to characterize *K-ras* and *p53* mutations in DNA derived from un-dissected colon tumors. The sensitivity of the assay has also facilitated detection of low frequency germline insertions and deletions in *BRCA1* and *BRCA2* in pooled samples of DNA, thus PCR / LDR can rapidly screen large numbers of DNA samples required for population studies. Additionally, this technology has been used to determine heterozygosity of SNPs in DNA repair genes and in the hypertension related genes, adducin, AGT and Factor V Leiden. Finally, the methylation status of CpGs within gene promoters can be determined using a combination of standard sodium bisulfite treatment of the DNA followed by PCR/LDR/array analysis. Our PCR/LDR technology with the use of the universal DNA microarray allows for large-scale multiplexing, provides for the rapid and robust detection of mutations in a high background of normal sequences, allows for the detection of closely-clustered mutations, and is amenable to automation.

Session sponsored by Gemini Genomics

Bioinformatics, statistics and databases

Mutation databases promise and problems

R. G. H. Cotton and O. Horaitis
Genomic Disorders Research Centre, St. Vincent's Hospital, 35 Victoria Parade, Fitzroy, VIC 3065, Australia

Tens of millions of sequence variations are likely to be found in the human genome. These are comprised of variations causing single gene disorders that are termed "mutations" in the field of clinical genetics and harmless variations termed "polymorphisms", and presumably a continuum in between. Polymorphisms that are single base changes have been termed single nucleotide polymorphisms (SNPs) and databases of these have been compiled through drug company activity, NIH funding and analysis of those parts of the genome that have been sequenced in DNA from multiple individuals. Collection of genomic variation that causes single gene disorders is a special problem due to the fact that not all mutations are published. This is for reasons such as many are found in diagnostic laboratories and not published and also that it is difficult to publish the 654th mutation in a gene.

The HUGO Mutation Database Initiative was founded to attempt to collect and distribute all such variation. The key to this activity is the curator of these databases who often collects mutations not only from the literature but also from consortia in the form of unpublished mutations.

Funding of such individuals is sporadic and the software available is not tailor-made. In recent years the HUGO MDI has made considerable progress towards a worldwide collection and distribution system. However, funding has remained a problem. Currently, a consortium of members is attempting to attract funds to produce a system to document refereed mutations and assist the curators by way of software. An initiative is beginning to collect variation from diagnostic laboratories.

The HUGO MDI website is at:
www.genomic.unimelb.edu.au/mdi/

SNPs and databases

H. Lehväslaiho
EMBL Outstation, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambs. CB10 1SD, UK

I will give an overview of publicly available programs, web sites and data bases used to analyse and organize sequence variations. This will be based on work attaching vaiations to annotated genomic sequences in and Ensembl (*http://www.ensembl.org/*), and data exchange between HGBASE (*http://hgbase.cgr.ki.se/*) and dbSNP (*http://www.ncbi.nlm.nih.gov/SNP/*)

The problem can be

From informatics point of view there are two distict approaches to sequence variations: genic and genomic, which can be treated separately but are closely linked. I will describe efforts to

1. Create a framework represent an eukaryotic transcribed and translated gene in such a way that the effects of (multiple) sequence changes can easily be traced up and down DNA, RNA, and AA levels.

2. Store that information in exhangeabe format and compute additional descriptive attributes.

3. Place variations into genomic context using their location in genomic clones.

The work is done using BioPerl objects (*http://bio.perl.org/*) genome annotation system.

Determining sequence length or content in zero, one, and two dimensions

M. W. Perlin
Cybergenetics, Pittsburgh, PA, USA

High-throughput assays are essential for the practical application of mutation detection in medicine and research. Moreover, such assays should produce informative data of high quality that have a low error rate and a low cost. Unfortunately, this is not currently the case. Instead, we typically witness legions of people reviewing imperfect data at astronomical expense yielding uncertain results.

To address this problem, for the past decade we have been developing methods that exploit the inherent quantitative nature of DNA experiments. By generating high quality data, careful DNA signal quantification permits robust analysis for determining true alleles and certainty measures. We will explore several assays and methods.

In a one dimensional readout, short tandem repeat (STR) data displays interesting artifacts. Even with high quality data, PCR artifacts such as stutter and relative amplification can confound correct or automated scoring. However, by appropriate mathematical analysis, these artifacts can be essentially removed from the data. The result is fully automated data scoring, quality assessment, and new types of DNA analysis. For example, such highly restored data enables the accurate analysis of pooled DNA samples, for both genetic and forensic applications.

On a two dimensional surface (comprised of zero dimensional spots) one can perform assays of extremely high throughput at low cost. The question is how to determine DNA sequence length or content from nonelectrophoretic intensity data. Here again, mathematical analysis of highly quantitative data provides a solution. We will discuss new lab assays that can produce data containing such information; mathematical transformation then determines DNA length or content.

Population genetic epidemiology: Theoretical background and study design issues

J. D. Terwilliger
Columbia University, Columbia Genome Center, and New York State Psychiatric Institute

Study designs for genetic studies have traditionally focused on single data structures, and oversimplified epidemiological models for which simple data structures can be informative. If the true state of nature is even slightly more complex, as it appears to be, then these simple methods will fail almost surely. To this end, it may be important to restructure our thinking in terms of what sorts of etiological models would be consistent with what we know about evolution, about temporal changes in phenotypes, and about the overall complexity and ephemeral nature of the genotype-phenotype relationships. Study design for segregation analysis attempts to make predictions about the penetrance functions, or P(Phenotype| Genotype) while gene mapping strategies are based on using study design strategies to maximize the predictive value, P(Genotype|Phenotype; Ascertainment), such that neither can be predicted meaningfully from the other in general, at least not if study design is optimized for one or the other of these questions. Popular misconceptions about the "world-changing effects" of the genome project have lead even serious scientists to ignore inconvenient scientific arguments in an effort to justify expenditures of large sums of money on mapping strategies that are critically dependent on sets of difficult-to-justify assumptions.

Combining data from different study designs will certainly increase the number of degrees of freedom available for estimation of parameters and testing of hypotheses in a given dataset. Of course the size of the dataset also needs to be large, but such approaches can be shown to be more informative about the true state of nature. Each conventional study design focuses on one specific aspect of variation. Twin studies focus on relative effects of genes and shared environmental factors in a household. Adoption studies focus on inter-household variation in environmental exposure. Migrant studies focus on intercultural variation in environmental exposures, and family studies focus on genetic correlations within larger kindreds. The components of variance emphasized by each study are radically different, and it is clear that a study combining elements of each would certainly be more informative about the relative contributions of nature, nurture, and interactions between them on trait etiology. Similarly, this information can be used to assist in mapping strategies, and genotype-phenotype correlation studies. We are currently developing such a study using populations of the Korean diaspora, composed of families of Koreans in Kazakstan, Korea, and the USA, twins, and internationally adopted Koreans in the USA and Europe. Preliminary data from this study and some sketch of the currently ongoing research will be described in brief as well.

New technologies for mutation detection

The surface invader assay for high parallel SNP analysis

L. M. Smith[1,4], M. Lu[1], M. Shortreed[1], L. Wang[1], P. Wilkins Stevens[2,3], V. Lyamichev[4], J. G. Hall[4], D. R. Kelso[2], B. P. Neri[4] and R. Kwiatkowski[4]
[1] Department of Chemistry, University of Wisconsin-Madison, 1101 University Avenue, Madison, Wisconsin 53706-1396
[2] Department of Biomedical Engineering, Robert R. McCormick School of Engineering and Applied Science, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208-3107
[3] Biosciences Division, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois 60439-4844
[4] Third Wave Technologies, 502 South Rosa Road, Madison, Wisconsin 53719-1256

In the aftermath of publication of the first draft human genome sequence, much attention is being directed to the problem of deciphering the functions and interactions encoded within the sequence. As has been the case for the last century, arguably the most powerful tool to this end is Genetics, which relies on detecting and understanding correlations between genotype and phenotype. Single nucleotide polymorphisms, or SNPS, as the most frequent type of genetic variation found in the genome, are the focus of attention for such genetic analyses of genome function. The analysis of SNPs has two natural phases: the discovery phase, in which the SNPs occurring within the population are discovered and archived in databases for use in genetic studies; and the scoring of SNPs in populations and patient cohorts, to make correlations between SNP genotype and individual phenotype. The former problem is being addressed effectively by a variety of public and private efforts such as the SNP Consortium; the scope of the latter problem is quite significant, as in many cases it may be desirable and necessary to score hundreds of thousands of SNPs on hundreds of thousands of individuals, corresponding to as many as billions of genotypes per study.

Recent work has described a SNP scoring technology referred to as the "Invader", which has a number of desirable characteristics: these include the ability to directly analyze SNPs from nanogram amounts of genomic DNA without a prior target amplification procedure, high accuracy, and a homogeneous assay format. One attractive approach to very large scale SNP genotyping would be to combine this assay platform with a surface array format in which hundreds of thousands of SNPs could be scored in parallel from a single sample of human genomic DNA. In this talk the basic principles of such an approach to large-scale SNP genotyping will be presented, along with the technical challenges presented by its implementation and results obtained to date.

The Invader® Assay for direct detection and quantitation of nucleic acids

R. Kwiatkowski
Assay Development, Third Wave Technologies, WI, USA

The Invader technology has been developed for the detection of nucleic acids directly from genomic DNA, or total

RNA, without prior target amplification. It is a signal amplification system that is able to accurately quantify DNA and RNA targets with high sensitivity. Exquisite specificity is achieved by combining hybridization with enzyme substrate recognition, which provides the ability to discriminate single base changes in mixed populations. The technology is isothermal, flexible, and incorporates a homogeneous fluorescence readout. It is therefore readily scalable and has been adapted for use in clinical reference laboratories as well as high throughput applications using 96 and 384 well microtiter plate formats. The molecular mechanism of the system and specific applications will be described.

Microelectronic DNA array devices for molecular diagnostic, pharmacogenomic and drug discovery applications

M. J. Heller
Nanogen, 10398 Pacific Center CT, San Diego, CA 92121, USA

Active microelectronic array devices have been developed for applications in DNA diagnostics and pharmacogenomic research. These microarray devices allow charged reagent and analyte molecules, including DNA, RNA, oligonucleotide probes, amplicons antibodies, proteins, enzymes, nanostructures, cells, and even semiconductor structures to be moved to or from any of the microscopic test sites on the device surface. A research laboratory system (Molecular Biology Workstation and 100 test site NanoChip™) has been designed to provide the end-user with "make your own chip" capabilities. A fluorescent reader and controller instrument system is designed to carry out both rapid and sensitive multiplex DNA hybridization analysis. More recently, newer versions of electronic hybridization systems are being designed for high throughput and general laboratory applications. Electronic hybridization analyses of amplified samples can be carried out with minimal preparation in reverse dot blot, dot blot and sandwich assay formats. Electronic hybridization techniques provide considerable advantages for carrying out rapid SNP, point mutation and STR analysis with extremely high accuracy and reliability. Results from over three thousand test samples have shown that electronic chip hybridization provides higher reliability for homozygous and heterozygous calls than conventional "gold standard" methods (DNA sequencing, passive array, RFLP, etc.). Electronic hybridization allows highly reliable results to be obtained for problematic SNP's, which include SNP's with associated secondary structure, SNP's within high G/C sequences and multiple SNP's in close proximity. High reliability minimizes the potential for false positives or false negatives, which will be an important performance criterion for any genotype panel that would be used for actual clinical diagnostic applications. Nanogen is also developing electronic techniques for carrying out gene expression analysis and direct on-chip amplification of target DNA sequences. Additionally, Nanogen is investigating the use of active electronic devices for high throughput screening assays (kinase, phosphatase, protease enzyme inhibitors). This technology will prove useful for pharmacogenomic and drug discovery applications.

Michael J. Heller received his Ph.D. in Biochemistry from Colorado State University in 1973. He was an NIH Postdoctoral Fellow at Northwestern University from 1973 to 1976. Dr. Heller was supervisor of the DNA Technology Group at Amoco Corporation from 1976 to 1984, and then the Director of Molecular Biology at Molecular Biosystems, Inc., from 1984 to 1987. He then went on to Integrated DNA Technologies, where he served as President and Chief Operating Officer from 1987 to 1989. Presently, he is the Chief Technical Officer at Nanogen, Inc., located in San Diego, California. Dr. Heller has extensive industrial experience in biotechnology, with particular expertise in the areas of DNA probe diagnostics, DNA synthesis, and fluorescent based detection technologies. He has been the founder of several new high technology companies. Nanogen Inc., the most recently formed is directed at the development of novel microelectronic DNA array technology. He has numerous patents and publications in the biotechnology and medical diagnostics areas.

Microchips and chip-based systems

J. Cheng[1,2,3]
[1] Aviva Biosciences Corporation, San Diego, CA 92121, USA
[2] Beijing National Biochip Research and Engineering Center, Beijing 100084, China
[3] Department of Biological Sciences and Biotechnology, Tsinghua University, 100084, Beijing China

Any typical analytical system usually consists of three classical steps, i.e. sample preparation, chemical reaction and detection. The total integration of these three steps has been the dream for many years for both academic researchers and entrepreneur. The marriage between molecular biology and the semiconductor industry for the first time brings hope to the scientific community. This presentation will cover the efforts made towards the construction of a laboratory-on-a-chip system with emphasis on the development of the front-end sample processing technology. Progress made on micro-scale separation and isolation of cells, DNA and RNA molecules, transportation of cells and molecules through the combined use of the active multiple-force chips and specially engineered microbeads with diversified properties, nucleic acid amplification in microchips, and on-chip detection through chip-based capillary electrophoresis and affinity binding will be described. Different aspects of biochip technology including system integration and its broad range of applications will be introduced as well.

References

Cheng, J., et al.: Isolation of cultured cervical carcinoma cells mixed with peripheral blood cells on a bioelectronic chip. Analytical Chemistry, 70:2321-2326, 1998.

Cheng, J., et al: Sample Preparation in Microstructured Devices. Manz, A. and Becker, H. (eds.) "Microsystems Technology in Chemistry and Life Science", in Topics in Current Chemistry, 194: 215-231, Springer-Verlag, Heidelberg, Germany. 1998.

Cheng, J. et al.: Chip PCR. II. Investigation of different PCR amplification systems in microfabricated silicon-glass chips. Nucleic Acids Research, 22: 380-385, 1996.

Cheng, J., et al.: Degenerate oligonucleotide primed-polymerase chain reaction and capillary electrophoretic analysis of human DNA on microchip-based devices. Analytical Biochemistry, 257: 101-105, 1998.

Cheng, J., et al.: Preparation and hybridization analysis of DNA/RNA from E.coli *on microfabricated bioelectronic chips. Nature Biotechnology, 16:541-546,1998.*

Xie, W., Yang, R., XU, J., Zhang, L., Xing, W and Cheng, J.: Microchip-based electrophoresis systems", Mitchelson, K.R. and Cheng, J. (eds.) "Capillary Electrophoresis of Nucleic Acids", in Methods in Molecular Biology, 162, in press, 2000.

Cheng, J., Wu, L., Swanson, P. O'Connell, J.P.: Biochip-based portable laboratory. Cheng, J. and Kricka, L.J. (eds.) Biochip Technology, Harwood Academic Publishers, New York, pp269-289, 2001.

A novel, high throughput, method for the detection of truncating mutations utilising methylase inactivation with the Transgenomic WAVE® Nucleic Acid Fragment Analysis System.

M. Matin[1], **M. J. Daniels**[2], C. Lowes[3] and D. Hornby[1]
[1] Transgenomic Ltd, The Krebbs Institute, University of Sheffield, Sheffield, UK
[2] Transgenomic Ltd, The Quadrangle, Crewe Hall, Crewe, Cheshire, UK
[3] Transgenomic Ltd, Cramlington, Northumberland, UK

Truncating mutations are common causes of genetic disease, representing up to 95% of disease causing mutations in Familial Adenomatous Polyposis (Van der Luijt et al. 1994), 90% in Breast and Ovarian Cancer (Hogervorst et al. 1995, Lancaster et al. 1996), 95% in Duchenne Muscular Dystrophy (Roest et al. 1993, Gardner et al. 1995) and 70% in Non-Polyposis Colorectal Cancer (Papadopoulos, 1994). Identification of genes containing truncating mutations has previously been carried out using the well established method of *in vitro* translation of a target gene, followed by analysis of the size of the protein product to establish if full length or truncating product is translated.

We describe here a novel method for the identification of truncating mutations through the utilisation of methylase inactivation followed by WAVE ® System analysis. Target sequences are amplified by PCR and then cloned, in frame, into a specially modified methylase gene (*MSPRI*) encoded on a plasmid vector (pSPRX). Suitable *E. coli* host cells are transformed with pSPRX and then incubated at 37°C for a minimum of 8 hours. Plasmid is then harvested from these cells and digested using the *Hae*III restriction endonuclease. pSPRX containing wild type target sequences express an active MSPRI methylase which protects pSPRX from digestion with *Hae*III. pSPRX containing target sequences having truncating mutations express an inactive MSPRI methylase which is unable to protect *Hae*III restriction sites. *Hae*III restriction digests of re-purified pSPRX, followed by analysis using the WAVE System reveal peak patterns indicative of the presence or absence of truncating mutations.

We describe work to investigate the suitability of this method for the analysis of large DNA fragments between 1 and 3Kb and to identify the presence of truncating mutations in pooled samples from 10 or more patients. In addition, the methylase inactivation method detects truncations of any size, avoids artefacts due to mistranslation and does not require the use of radioactivity.

Application of MAGIChips in analysis of polymorphisms and mutations

A. D. Mirzabekov
Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilova 32, Moscow 119991, Russia

MicroArrays of Gel Immobilized Compounds on a chip (MAGIChip) provide a higher capacity for immobilization within the 3-D structure of gel pads, a clear discrimination between perfect and mismatched duplexes, and offer a possibility to carry out reactions in parallel in many individual microchip gel pads. The developed research-grade fluorescence microscope and simple inexpensive analyzer (1) are convenient for monitoring and documentation of different processes on microchips by using fluorescently labeled compounds. Oligonucleotide microchip bearing 15-20 nucleotide-long immobilized probes is an efficient tool for identification of various known mutations (2). Generic microchip containing all possible 4,096 hexamers enables one to analyze DNA sequences and identify new mutations in different genes (3). Contiguous stacking hybridization and its monitoring with MALDI mass spectrometry is a sensitive approach for identification of mutations (4). Different enzymatic reactions have been performed with MAGIChips. On-chip single-base extension has been applied for analysis of human mutations (5). On-chip PCR and allele-specific PCR amplifications can be performed in nanoliter gel pads of the MAGIC chip, each pad containing a specific set of primers (6). The use of ligase detection reaction increases the sensitivity and allows to detect as little as 1% of a mutant in wild type DNA. Clinical trials of the MAGIChip applications will be discussed.

References
1. Bavykin, S.G., et al. (2001) Appl. & Environ. Microbiology, 67, 922-928.
2. LaForge, K.S., et al.(2000), Am. J. Med. Genet., *Neuropsyciatric Genetics Section, 96 (5), 604-615.*
3. Proudnikov, D., et al. (2000) Biologicals*, 28, 57-66.*
4. Stomakhin, A., et al. (2000) Nucl. Acids Res*. 28 (5), 1193-1198.*
5. Dubiley, S.,et al. (1999) Nucl. Acids Res*. 27, e19.*
6. Strizhkov, B.N., et al. (2000) BioTechniques 29, No4, 844-857.

High-throughput profiling using suspension Arrays™

C. Johnson and **J. W. Jacobson**
Luminex Corporation, 12212 Technology Blvd, Austin, TX 78727

Discovery and application of linkage associations between disease states and genetic polymorphisms such as SNPs require high-throughput screening capabilities that are both sensitive and specific. At the same time, these methods must be flexible, widely accessible and of economic scale to allow practical usage. While a wide variety of SNP microarray technologies are available, few achieve the key adoption criteria of easy access and low cost. The Luminex LabMap™ system is available in a low-cost bench top analyzer that provides for concurrent, rapid, sensitive, and specific analyses of tens, hundreds and even thousands of nucleic acid targets. The method utilizes unique microsphere sets that act as carriers for any ligand-binding reaction, including antigen-antibody, enzyme-substrate, and nucleic acids. Incorporating precise intensities of multiple fluorescent dyes into the core of polystyrene microspheres imparts defining fluorescent signatures to distinct microsphere sets. Assays are quantified with a separate fluorescent reporter molecule reacting at the microsphere surface. Suspension arrays are read in real time using a cost-effective benchtop analyzer. By moving microarray design control into the customer's laboratory, Luminex offers easy, economical access to a high-throughput screening technology capable of analyzing nearly 400,000 data points per day. LabMAP Suspension Arrays used to rapidly detect genetic polymorphisms associated with clinically relevant markers will be described and results of multiplexed profiling studies will be presented.

Posters

Poster No: 1 Short Presentation

Evaluating appropriateness and suitability of mutation-detection methods in large - scale epidemiology studies examining genetic associations

H. Ahsan, J.Cuomo and R. M. Santella
Divisions of Epidemiology and Environmental Health Sciences of Mailman School of Public Health and Herbert Irving Comprehensive Cancer Center, Columbia University, New York, N.Y. 10032, USA

As a result of the sequence of the human genome becoming known and high-throughput methods for detecting genomic sequence variations becoming available and affordable, more and more large-scale epidemiologic studies are being conducted to investigate the associations between variations in the human genome and disease risk. Accordingly, there will be a great demand for epidemiologists to understand the rationale, principles, techniques, costs, advantages and limitations of various available high-throughput mutation detection methods and their relevance to study design, analysis and interpretation of results. We have been conducting several large-scale population-based and family-based genetic epidemiologic association studies to identify and characterize genetic variants that are related to the risk of breast, skin, brain and other cancers. As we are going through the process of using several mutation detection methods in our own projects, we are faced with the need for a systematic evaluation of these methods to establish a set of epidemiologic criteria (in addition to the standard criteria that are important for method developers, manufacturers and basic science researchers). We are currently in the process of examining and developing a list of epidemiologic criteria based on the sensitivity, specificity, costs, validity, broadness, automation, requirement of technical know-how/ manpower, etc. which will be useful to epidemiologists for planning and implementing large-scale genetic association studies requiring rapid, cost-effective, and reliable mutation detection. These criteria, which have implications on the design, analysis and interpretation of study findings, can be helpful for investigators in evaluating the suitability and appropriateness of specific methods for large - scale epidemiology projects. We will present our experiences and recommendations in this regard in the meeting.

Poster No: 2

DNA copy number analysis at the *PMP22* gene

S. M. Akrami and J. A. L. Armour
Institute of Genetics, University of Nottingham, UK

Micro-deletions or micro-duplications are known to underlie an increasing number of human disorders. DNA copy number alterations are responsible for several categories of diseases and syndromes. These alterations can range in scale from a chromosome or chromosomal region to just one exon of a gene. These changes can be detected by cytogenetic studies when there is involvement of several kilobases or megabases of DNA. Examination of sub-microscopic changes is possible by using short probes flanked by the same primer pairs. These probes can be recovered and amplified quantitatively following hybridization to a genomic target. Multiplex Amplifiable Probe Hybridization (MAPH) is a simple, high resolution method by which alterations of several hundred base pairs are detectable. Charcot-Marie-Tooth (CMT) syndrome is the most common inherited peripheral neuropathy with a prevalence of about 1/2500. By a gene dosage mechanism, commonly trisomic overexpression of *PMP22* (17p11.2-12) results in CMT1A whereas its monosomic underexpression causes hereditary neuropathy with liability to pressure palsies (HNPP). We are applying MAPH to measure copy number of exons of the *PMP22* gene. Initial work on genomic DNA of normal people shows reproducible results approximating to a normal distribution. Moreover, it can distinguish genomic DNA of CMT1A/ HNPP patients from unaffected controls.

Poster No: 3

From linkage analysis to gene identification: advantages of a central facility

N. Avidan, E. Ben-Asher, Z. Olender, M. Khen, A. Adato, J. Beckman and D. Lancet
Department of Molecular Genetics and The Crown Human Genome Center, The Weizmann Institute of Science, Rehovot 76100, Israel

The genome's first draft is most advantageous to researchers in Israel. This is so because of the unique genetic composition of the Israeli population, Jewish as well as non-Jewish. Such populations consist of various defined ethnic groups and genetic isolates, which are highly informative for genetic studies. Linkage analysis of a particular disease among members of affected families, originating from a single ethnic group, usually enables defining a refined genomic interval for the disease-causing mutation. For a monogenic disease, once a particular disease has been thus mapped to a chromosomal interval, the study is ripe for an attempt to identify the specific gene. The Genome Center at the Weizmann Institute of Science plays a central role in the transit from linkage mapping to disease causing mutation, by providing genome-related practical know-how as well as computer-intensive tools with regards to DNA sequence and variation analyses. This applies to the entire academic and technological community of Israel. Several specific projects are currently under study, aimed to identify genes which are involve in monogenic diseases with one success so far. ML-IV -

mucolipidosis type IV, in collaboration with Prof. Gideon Bach (Nat Genet. 26(1):118-23 (2000)). More projects, such as USH3 - Usher syndrome type-III, in collaboration with Prof. Batsheva Bonne-Tamir, HIBM - Hereditary Inclusion Body Myopathy, headed by Prof. Stella Mitrani-Rosenbaum, CDA1- Congenital dyserythropoietic anemia type 1, headed by Prof. Hanna Tamary, and PVT - Polymorphic Ventricular Tachycadia, headed by Prof. Elon Pras, are in progress. Bioinformatics software, in particular our in-house developed tools as GESTALT (Bioinformatics16:482-483 (2000), *http://bioinformatics.weizmann.ac.il/GESTALT*) and TAPI (a Tool for Annotation of Pac phase I, (unpublished)), GeneCards (Bioinformatics 14:656-664 (1998), *http://bioinformatics.weizmann.ac.il/cards*) and the Unified DataBase (UDB, *http://bioinformatics.weizmann.ac.il/udb*) are employed and allow the mapping, annotation, and integration of information of large genomic region quickly and efficiently. These greatly facilitate the process of finding and re-sequencing candidate genes. A combination of sequence analysis dexterity and careful genetic analysis allow distinguishing between bona fide mutations from common polymorphism or sequencing errors.

Poster No: 4 Short Presentation

Apolipoprotein B haplotypes and their impact on blood lipids and insulin

J. Bentzen[1], T. Jřrgensen[2] and M. Fenger[1]
[1] Department of Clinical Biochemistry, Copenhagen University Hospital Hvidovre, Denmark
[2] Centre for Preventive Medicine, Copenhagen County Hospital Glostrup, Denmark

Apolipoprotein B (ApoB) is the non-exchangeable protein component of Very Low Density Lipoprotein (VLDL) and Low Density Lipoprotein (LDL). VLDL and LDL act as shuttles for cholesterol and triglyceride molecules between the liver and peripheral tissues. Changes in ApoB structure affect binding of the lipoprotein particles to their receptors and thereby the amounts of lipoproteins in the blood stream, which is a risk factor for arteriosclerosis and cardiovascular disease.

Several genetic polymorphisms in the *ApoB* gene have been identified and correlated to elevated levels of plasma lipids. Determination of single nucleotide polymorphisms (SNPs) is uncomplicated and is widely used for research purposes. From a physiological point of view, however, SNPs are not particularly informative. Haplotypes (the set of SNP alleles borne on each chromosome) are the determinants of the amino acid composition in the mature protein.

We have studied the effect of unequivocally determined haplotypes on fasting levels of lipoprotein, lipid and insulin in 1839 Danes aged 40-71 years. The haplotypes in the present study were composed of two SNPs in exon 26 in the ApoB gene. One was the silent SNP in codon 2488 and one was the functional SNP in codon 2712. Both SNPs have been shown to influence parameters of lipid and glucose metabolism.

Haplotypes had a significant influence on fasting levels of triglyceride (p=0.003), VLDL-cholesterol (p=0.003), insulin (p=0.01), total cholesterol (p=0.02) and LDL-cholesterol (p=0.05). The frequency of haplotypes varied from 0.02 (73 chromosomes of 3,678) to 0.44 (1,619 chromosomes of 3,678).

The observed effect of haplotypes on lipid parameters was also seen with SNPs alone while the effect on insulin was unique to haplotypes. This stresses the relevance of haplotype testing.

Poster No: 5

Shear stress response elements in the 5' regulatory region of the Von Willebrand gene

E. Berber[1], C. Cameron[2], C. Hough[2], S. Hande Caglayan[1] and D. Lillicrap[2]
[1] Department of Molecular Biology and Genetics, Bogazici University. Istanbul, Turkey
[2] Department of Pathology, Queen's University, Kingston, Ontario, Canada

Von Willebrand Factor (vWF) is a large multimeric adhesive glycoprotein synthesized exclusively in endothelial cells and megakaryocytes. ABO blood group, age, pregnancy, ethnicity, and the acute phase contribute to a 6-fold variability in normal plasma levels of VWF. Expression of the protein is regulated by the binding of a combination of tissue-specific and ubiquitous trans-acting factors to both transcriptional activator and repressor elements in the 5' upstream region of the gene. Moreover, studies revealed that *vWF* gene expression is associated with the hemodynamic forces like fluid shear stress (FSS), produced by the blood flow on the endothelial cell surface and 4 shear stress response elements (SSRE) have been identified within the 5' region of the *vWF* gene (-2081 bp, -2045 bp, -111 bp, +578 bp). In this study, in order to explore the molecular mechanism involved in *vWF* gene expression regulation by the fluid shear stress, functional role of SSRE in *vWF* gene expression is analysed in cultured endothelial cells under resting and shear stress conditions. Passage 3, bovine aortic endothelial cells (BAECs) were transfected with a luciferase cDNA reporter plasmid (pGL3B) into which ~2.5 kb of *vWF* promoter sequence (nt -1800 to nt +660) containing SSRE at -111 and + 578 positions had been cloned. A promoterless PGL3B plasmid served as the negative control, cells were cotransfected with ß-glactosidase plasmid to evaluate transfection efficiency. Cells were shear stimulated by applying 15 dynes/cm^2 laminar shear stress for 6 hours in a parallel plate flow chamber. In addition, nuclear factor binding to the analysed SSRE under resting and shear stress stimulated conditions was assessed by performing Electrophoretic Mobility Shift Assays (EMSAs). Preliminary results demonstrated that the 2.5 kb region analysed is responsive to fluid shear stress and there is at least 4-fold increase in transcriptional activity with this regulatory sequence. Furthermore, EMSAs revealed that nuclear proteins interact with SSREs and the interaction is affected with the flow stimulation of the cells. Furthermore, SSRE located nt -111 might be an active shear SSRE and shear stress responsiveness of *vWF* gene expression might be mediated by this element.

Poster No: 6

DHPLC-based germline mutation screening in the analysis of the *VHL* tumor suppressor gene: usefulness and limitations

B. Klein[1], G. Weirich[2] and **H. Brauch**[1]
[1] Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology Stuttgart and Institute of Pathology, Technical University Munich, Germany

Von Hippel-Lindau disease (VHL) is an autosomal dominant multi tumor syndrom, that includes tumors in the eyes, brain, spinal cord, kidney, pancreas, adrenal gland, epididymis and inner ear. The underlying genetic defects are germline mutations in the *VHL* tumor suppressor gene. So far, more than 160 different *VHL* germline mutations have been identified. Individuals at risk for VHL, i.e. members of families with a known family history and also isolated cases with incomplete phenotype will benefit from molecular testing by one of the following specifications: (1) confirmation of clinical diagnosis, (2) presymptomatic diagnosis, and (3) exclusion from being a gene carrier of a specific *VHL* mutation. Although at present there is no cure, molecular diagnosis of VHL had a major impact on clinical management resulting in the reduction of blindness, neurological impairment and metastatic disease in affected patients. This improvement in patient care and prevention relies on early determination of gene carrier status and subsequent organ specific regular clinical screening for lesions in *VHL* mutation carriers. Thus, good clinical practise in the management of VHL disease must include germline mutation analysis.

In order to evaluate sensitivity and specificity of the recently introduced high-throughput method DHPLC (denaturing high performance liquid chromatography) for mutation screening of the *VHL* gene, we subjected DNA from 43 unrelated VHL patients with previously sequenced *VHL* germline mutations to this method. In addition 36 genomic DNAs of unrelated individuals suspected of being *VHL* carriers but with unknown germline status were analyzed by DHPLC and sequencing. Aims of the present study were to compare mutation results obtained by direct sequencing and DHPLC, and the comparison of two different DHPLC systems. The sensitivity of DHPLC was tested with two commercial devices and protocols i.e. the Varian-Helix™-System and the Wave® Nucleic Acid Fragment Analysis System. Both resolved all but one mutation in exons 2 and 3 of the *VHL* gene. In contrast, the GC rich exon 1 showed discrepancies in the rate of mutation detection. Whereas the Varian Helix™-System detected 10/15 (67%) mutations of the known mutations, the Wave® Nucleic acid Fragment Analysis System detected 13/14 (93%). All three mutations of samples with unknown mutation status were called by both systems raising the mutation detection rate to 72% and 94%, respectively. Cases with different substitutions at the same nucleotide showed different elution profiles but similar elution profiles could be obtained from different mutations. The Wave® Nucleic Acid Fragment Analysis System detected most *VHL* mutations, however when 100% detection rate is needed sequencing is still required and must therefore be the standard *VHL* mutation detection procedure. Once a family specific mutation has been established, DHPLC may be suitable for a rapid and cost-effective determination of *VHL* carrier status in family members.

Poster No: 7

Genetic association as a tool for the study of Alzheimer's disease pathogenesis

M. J. Bullido, J. Aldudo, M.C. Ramos, I. Sastre and F. Valdivieso
Centro de Biologiá Molecular Severo Ochoa , Facultad de Ciencias, Campus de Cantoblanco, Madrid, Spain

In a small proportion of cases, Alzheimer's disease (AD) presents as an autosomally transmitted genetic trait, caused by mutations in *APP* and presenilin (*PSEN*) genes. Many different mutations causative of Alzheimer's disease (AD) have been found in *PSEN1* gene and are associated with the most aggressive forms of the disease. With the aim of screening for *PSEN1* genetic variations, we have developed a method based on denaturing gradient gel electrophoresis (DGGE) which allows the mutational analysis of all the coding exons and the proximal promoter of *PS-1* in a fast and highly reproducible way; by this method, we have detected several novel mutations in the coding and promoter region of the gene. Since *PSEN1* pathogenic mutations are located throughout the whole coding region, DGGE is a very useful tool for the screening of novel mutations.

In the high majority of cases, AD appears as a complex trait resulting from the interaction between several genetic and non-genetic factors. Identifying the genes involved in the sporadic form of AD is necessary for the understanding of its pathogenic mechanisms, which in turn will help to the development of therapeutic strategies. From the genes identified to date, the ε4 allele of apolipoprotein E (*APOE*, gene; ApoE, protein) is the single most important genetic determinant for late-onset AD; furthermore, more recent evidences indicating that ApoE levels correlate with AD risk reinforce *APOE* involvement in AD. However, *APOE* is neither necessary nor sufficient to cause the disease. Actually, current data strongly indicates that multiple pathways and mechanisms (apoptosis, inflammation, amyloid production and clearance, etc.) are involved in the pathogenesis of sporadic AD. Inside this complexity, to find out the individual contribution of each gene or protein to AD pathogenesis and the interactions between the different risk factors requires the use of different complementary approaches.

In order to get insight into the mechanisms involved in AD pathogenesis, we are using an approach that combines genetic epidemiology with cellular and animal models. Candidate genes are grouped into functional modules and the risk associated with each of them and with their combinations are studied in case-control samples, complemented with studies in transfected cells and transgenic mice. We have performed an association study of a genetic module composed by ApoE, the lipoprotein receptor related protein (LRP) and alpha-2 macroglobulin (α2-M), which combined effect could produce a decrease in Aß clearance through LRP, resulting in Aß deposition and toxicity; this study indicated that *APOE* promoter variants modulate the risk associated with ApoE4 allele, suggesting that the combined effect of Apoe structure and level is relevant in AD; besides, the study suggested that gender modulates the risk associated with *APOE* and *LRP* polymorphism, raising the possibility that the effect posed by these loci could be, at least in part, responsible for the lower AD risk in males compared to females.

This approach is also allowing us to reveal potential interactions relevant to AD, as the one between ApoE4 and tau, among others. In summary, our data suggest that data derived from association studies of functional genetic modules could be very valuable as indicators of pathways involved in the pathogenesis of the "complex" form of Alzheimer's disease.

Poster No: 8

Multi-Temture Automated Capillary Electrophoresis Fluorescent Single Strand Conformation Polymorphism Analysis for sensitive high throughput mutation scanning

D. Cuthbert-Heavens[1], S.A. Bass[1], **C.F. Taylor**[1], R.F. Charlton[2] and G.R. Taylor[1,2]
[1] ICRF Mutation Detection Facility, [2] Regional DNA Laboratory, Ashley Wing, St James's University Hospital, Leeds LS9 7TF, UK

Single Stranded Conformational Polymorphism (SSCP) is the most widespread mutation detection scanning method due to its simplicity and use of everyday laboratory equipment. Various modifications have been reported but it is generally accepted that for 100% detection analysis at five different running conditions is required. Whilst improving sensitivity this also increases complexity and reduces throughputs. Here we report the suitability of multi-temperature automated capillary electrophoresis fluorescent-SSCP (MACEF-SSCP) on an ABI3100 for use in high throughput mutation scanning.

Poster No: 9

Genetic polymorphism of xenobiotic metabolising enzymes and susceptibility for chemical carcinogenesis

V. Dolžan[1], M. Ravnik-Glavač[1,2], Z. Rudolf[3] and K. Breskvar[1]
[1] Inst. of Biochemistry, Faculty of Medicine, Ljubljana, Slovenia
[2] Lab. of Molecular Genetics, Inst. of Pathology, Faculty of Medicine, Ljubljana, Slovenia
[3] Inst. of Oncology, Ljubljana, Slovenia

A significant proportion of human cancers is initiated by environmental and dietary carcinogens. A large number of enzymes, most of which are polymorphic, participate in activation and inactivation of xenobiotics such as drugs and carcinogens. Phase I drug metabolising enzymes (DMEs), mostly cytochromes P450, metabolically activate procarcinogens to reactive electrophilic DNA-damaging forms. Phase II DMEs, such as glutathione S-transferases (GSTs) and N-acetyl transferases (NATs), catalyse conjugation reactions to form inactive water-soluble metabolites. Inter-individual variability in metabolism of carcinogens is known to exist due to genetic polymorphisms of DMEs. This variability may be used to explain the differences in susceptibility of humans to chemical carcinogens. Our aim was to look for inherited metabolic susceptibility for chemical carcinogenesis in Slovenian population. DNA samples from 200 lung cancer, 137 melanoma and 162 colorectal cancer (CRC) patients as well as 109 healthy controls were analysed. PCR-based genotyping approach was used to determine the frequencies of polymorphic alleles of two phase I enzymes, CYP2D6 and CYP1A1, and three phase II enzymes, GST M1, T1 and P1. PCR followed by Bst*NI* restriction was used to distinguish between the wild type (*CYP2D6*1*) and polymorphic *CYP2D6*4* allele. Allele specific PCR was used to identify *CYP2D6*3* allele. The polymorphism in 3'-flanking region of *CYP1A1* gene was analysed by PCR followed by Msp*I* restriction. Triplex PCR with conserved beta globin primers as internal control was used to analyse for GST M1 and GST T1 null alleles. PCR amplification of exon 5 and exon 6 of *GST P1* gene followed by restriction with Bsm*AI* and *Cac8I* were used to identify Ile105Val and Ala114Val substitutions, respectively. The frequency of poor metabolizers carrying two *CYP2D6* deficiency alleles (PMs) was decreased in lung cancer patients when compared with normal controls ($p = 0.056$), indicating that poor metabolizers may be less susceptible to lung cancer than extensive metabolizers. Enhanced metabolic activation of carcinogens by CYP1A1 enzymes may also be one of the factors determining genetic susceptibility to lung cancer ($p= 0.07$), especially squamous cell carcinoma ($p = 0.032$). Genetic polymorphism of *CYP1A1* also seems to influence susceptibility for melanoma, but not for CRC. The decreased efficiency of phase II detoxification reactions due to genetic polymorphism of *GST M1* seems to modify the risk for the pulmonary adenocarcinoma ($p = 0.02$), while GST P1 polymorphism seems to modify the risk for CRC. Decreased frequency of GST P1 *A/*A genotype, reported to result in high conjugating activity, and increased frequencies of GST P1*A/*B and *B/*B genotypes, reported to result in lower conjugating activity, were observed in CRC patients when compared to controls ($p = 0.0096$, $m = 1$). Although cancer is a polygenic disease it is likely that one of the factors contributing to susceptibility for chemical carcinogenesis is the genetic polymorphism of DMEs. Genotyping individuals for DMEs polymorphisms may have important implications for risk assessment and prevention of human cancer caused by chemical carcinogenesis.

Poster No: 10

DNA sequence similarity between human and equine casein cDNAs support potential use of mare milk in diets for children with cows' milk allergy

T. Lenasi, I. Rogelj and **P. Dovč**
Department of Animal Science, Biotechnical Faculty, Groblje 3, SI-1230 Domzale, Slovenia

Caseins comprise about 50% of the total milk proteins in mare milk and their most important role is providing the major supply of amino acids, calcium and phosphorus to the suckling. They can be divided into two classes, so called calcium-sensitive caseins (α_{S1}-, α_{S2}- and ß-casein) and κ-casein. The important role of κ-casein is in determining the micelle structure and maintaining the calcium-sensitive caseins in suspension. In addition, κ-casein is responsible for clotting of milk in the gut. Most of the research has been done on bovine casein genes, followed

by casein genes of some other traditional dairy species. The aim of our study was to characterize equine casein mRNAs because of the possible use of mares' milk for children with cows' milk allergy. This allergy is caused by protein components of cows' milk; therefore we compared equine casein genes with human and bovine casein counterparts. We prepared cDNA library from equine mammary gland tissue and sequenced coding regions for equine α_{S1}-, ß- and κ-casein. For α_{S1}-casein we obtained two mRNAs, differing in length for 33 nucleotides. From DNA sequences we deduced that both mRNAs are processed from the same pre-mRNA and that the shorter mRNA probably lacks the whole exon, which is skipped during the splicing process. Interspecies comparison of cDNA sequences coding caseins showed considerably higher sequence identity between equine and human casein mRNAs then of corresponding human and bovine mRNAs. Similar chemical composition of human and mare milk together with high homology of human and equine casein mRNAs offer the possibility for successful use of mares' milk for children, sensitive to cows' milk proteins.

Poster No: 11

Polymorphism of the dopamine transporter gene (*DAT1*) in patients with opiate drug addiction

A. Gareeva, A. Galeyeva and E. Khusnutdinova
Institute of Biochemistry and Genetics of Ufa Scientific Center, Russian Academy of Sciences, Laboratory of Human Molecular Genetics, Russia

Analysis of VNTR polymorphism in 3' - untranslated area of the dopamine transporter gene (*DAT1*) was performed for allelic association in 118 patients (male) at the age of 13-40 years old with the 2nd stage of the opiate drug addiction (ODA). Five genotypes were determined, the most frequent were 10/10 and 9/10 (0,65 and 0,33 for the control sample; 0,66 and 0,27 for patients). The veracious differences were determined in the in the genotypes frequencies allocation between patients and the control sample (X^2 = 10,19; P = 0,013), first of all specified by the 9/9 genotype absence in the control sample, when it's frequency in patients was 0,06 (x^ = 8,04; P = 0,005). The relational disease risk (RR) in 9/9 genotype patients became very high - 5,15.

The analysis of the allelic fraction allocation of *DAT1* locus doesn't show any veracious differences between investigated groups. There is a 10 allele prevalence (0,79 and 0,83 accordingly) among patients and in the control group. The most seldom were 11 and 8 repeated alleles, which frequency was not higher than 0,004 in the control group and it was absent in patients.

The series of the researches devoted to the genetics of the psychoactive substances indicate for the association between allelic variants of the genes - markers of the catecholaminic system and the disease onset age. That is why we endeavored to divide patients into to age subgroups. The first subgroup included patients with ODA narcotization onset in the age of 16 years old and younger ("early ODA"), the second subgroup-the rest patients. For the age subgroups and for all patients either the more higher were gomozygotic frequencies with 9 - repeated allele at P < 0,03 (accordingly 0,08 and 0,06 in males with the early and more later disease onset). There were no veracious differences between age subgroups. The analysis in the allelic frequency allocation in the gene *DAT1* hasn't shown any veracious differences between investigated groups.

Analysis of VNTR polymorphism of the *DAT1* depending on narcotization experience duration was performed. The genotype frequencies allocation analysis indicated the difference between patience with the narcotization experience more than 4 years and the control sample (X^2 = 15,25; P = 0,001). These differences specified by quite high genotype 9/9 frequency in patients - 0,12 (X^2 = 15,23; P = 0,001) on the background of an authentic geterozygotic fraction descent 10/9 (X^2 = 4,79; P = 0,035) compare to the control sample, in which these genotypes fractions are 0,00 and 0,34 accordingly (X^ = 4,77; P = 0,041). The allocation genotype frequency way in patients with 4 and less years narcotization experience duration was coincide to the same in the control sample and authentically differentiated from patients with more than 4 years narcotization experience duration. The differences in the allelic frequency allocation in the polymorphic locus of gene *DAT1* in all investigated samples were statistically not significant.

Thus, the results certificate that the dopamine transporter gene is taking part in drug addiction formation and points at a possible association of the gomozygotic gene varieties 9/9 with the drug addiction onset and more extended drug addiction experience. The comparative risk of the opiate addiction in genotype 9/9 carries equals 5,15.

Poster No: 12

A two-tiered approach to mutation screening for the *COL4A5* gene in Alport syndrome patients

K. King, F. A. Flinter and **P. M. Green**
Division of Medical and Molecular Genetics, GKT School of Medicine, Guy's Hospital, London, UK

Alport syndrome (AS) is an hereditary nephritis caused by alterations in the type IV collagens that are structural components of the basement membrane. The X-linked form of the disease, affecting about 1 in 10,000, is due to mutations in the *COL4A5* gene, the product of which contributes one of the three collagen polypeptides that make up the the type IV triple helix macromolecule. The gene for COL4A5 comprises 51 exons and spans 250kb of genomic DNA in Xq22.

Until recently, mutation studies on the large and complex *COL4A5* gene yielded only about 50% success rate. This, it turns out, was due to a number of reasons: before the full genomic sequence of the gene was known, primer positions for amplifying the exons were suboptimal (many were too close or even within the exon sequence thereby excluding identification of a proportion of splice site mutations); some patients (estimated at 10-15% of all Alport Syndrome patients) have mutations in one of the other two type IV collagen genes that result in the autosomal recessive AS; a minority of patients have an ambiguous diagnosis; Some mutations are intronic and therefore not screened by conventional procedures.

Early mutation studies using ectopic RNA from peripheral blood lymphocytes were not very robust, presumable due to very low levels of mRNA and alternative splicing.

We have used hair roots as a source of RNA to circumvent this problem: hair roots are ideally suited to such study as the RNA can be extracted after 10 days at room temperature with no loss of quality, and the isolation procedure is straightforward. Furthermore, the COL4A5 transcript can be amplified more reliably than from lymphocyte RNA. Therefore we have designed 4 overlapping nested PCRs covering the entire coding sequence of *COL4A5* gene and screened them by fluorescent chemical cleavage of mismatch. Using this strategy we have detected mutations in 87% male probands.

As this is an X linked dominant disorder, many probands are, in fact, female. Inevitably there is a problem screening for heterozygous mutations in the RNA: the mutant allele may not be equally transcribed or as stable as the normal message. Therefore, we have endeavoured to set up a mutation screening protocol based upon genomic PCR of individual exons and Denaturing High Performance Liquid Chromatography (DHPLC). One difficulty with this approach has been the unusually GC-rich exonic sequence compared to the flanking intronic sequence resulting in different melting domains requiring several different melting temperatures in order to screen all regions of a given PCR product. However, we believe this two step approach to be highly effective at detecting mutations for the *COL4A5* gene.

Poster No: 13 Short Presentation

High-throughput SNP genotyping by the GOOD Assay

I. G. Gut, D. Lechner, F. Matsuda and M. Lathrop
Centre National de Génotypage, 2 rue Gaston Crémieux, 91057 Evry Cedex, France

The GOOD assay is a procedure for SNP genotyping by MALDI mass spectrometry. It requires only reagent additions and incubations for five reaction steps (PCR, shrimp alkaline phosphatase digestion, primer extension, phosphodiesterase II digestion and alkylation). In contrast to all other published SNP genotyping methods using mass spectrometric detection, it does not require any sample purification at all. It operates with the smallest volumes manageable by standard liquid handling robotics, making it very economical in terms of reagent consumption. The CNG applies the GOOD assay for high-throughput SNP genotyping with according integration of automatic sample preparation, data acquisition and analysis. The integrated robotic platform will be described. Currently it produces 20.000 SNP genotypes per day and is targeted to generate 100.000 SNP genotypes daily in the near future. Data quality is assessed by a number of control measures. These include comparison of a subset of samples with sequencing results, control within CEPH families, Hardy-Weinberg analysis of each set of 384 SNP genotypes and haplotype analysis.

Upstream of the SNP genotyping is DNA banking and SNP discovery. SNP discovery focuses on SNPs in target genes. Variation is identified by sequencing pooled DNA samples.

A lot of attention has been paid to organisational means in terms of LIMS tracking and automatic data assessment. These have been put in place for the DNA banking, SNP discovery and SNP genotyping platforms and ensure fluent operation.

The CNG genotyping platform is being put to work in a range of projects, from association studies on cardiovascular and auto-immune target genes in cohorts with close to 10.000 individual DNAs, to agro-alimentary applications, like for generating genetic fingerprints of cattle for traceability and SNP studies in plants.

Improvements of the GOOD assay are continually being integrated for high-throughput. Mainly these are the direct, physical measurement of the phase of several SNPs (haplotyping) and the simplification of the GOOD assay to a three-step procedure, comprising only of PCR, primer extension and phosphodiesterase II digestion.

Poster No: 14 Short Presentation

High throughput genetic analysis of type 2 diabetes: A candidate gene approach

R. Hawkins, P. Weller, N. Anwar, M. Palmer, P. Swarbrick, R. Cubberley, A. Ambler, J. Greenham, I. Barroso, A. Schafer and T. Weaver
Incyte Genomics, #214 Cambridge Science Park, Milton Road, Cambridge CB4 0WA, UK

Diabetes is a complex disorder resulting from failure to appropriately control sugar levels in the body. In the United States, diabetes affects 4% of the entire population, and 95% of these cases are attributed to non-insulin dependent or "type 2" diabetes. The impact of this disease on human health is staggering, it is recognized as one of the leading causes of death and disability and contributes to the incidence of blindness, heart disease, strokes, kidney failure, amputations, and nerve damage. In addition to human suffering, type 2 diabetes presents a huge burden on a nation's health service, with an estimated $100B per year for direct and indirect costs in the USA alone. Family studies indicate that in common with many other complex diseases, type 2 diabetes has a significant genetic component. Using existing knowledge of the cell biology and biochemistry of glucose regulation, it is possible to nominate candidate genes likely to be involved in the aetiology of type 2 diabetes.

Our work has focused on applying genomic and population genetic technologies to identify and validate potential drug targets or therapeutic proteins for combating type 2 diabetes. Incyte's target identification process begins with selecting relevant genes from the literature, and through consultation with experts in the field. Expansion of this seed list is accomplished by applying bioinformatic techniques, including homology screens and guilt by association (Walker M.G. et al. Genome Res 12:1198-203; 1999), as well as directed microarray expression studies using Incyte's Human GEM arrays (representing >50K non-redundant human ESTs). These strategies result in the identification of novel genes from Inycte's LifeSeqGold database as well as known genes previously not thought to be involved in type 2 diabetes. Once identified, candidate genes are comprehensively assessed for the presence of single nucleotide polymorphisms (SNPs) by a streamlined fSSCP process. Each candidate gene is covered on average by 15 PCR products ranging in size up to 300 bp and minimally tiled. Amplimers are multiplexed by size and color as sets of 6 during the fSSCP screening on

ABI 377s. Current capacity allows 3200 amplimers screened per month (approximately 200 gene equivalents). A normal human population (n=47) of mixed ethnicity is screened in order to identify all common SNPs (>3% allele freq, approx 1 SNP every 500 bp). A second panel of Severe Insulin Resistance patients (n=130) composed of a collection of rare monozygotic disorders characterized by severe insulin resistance are also screened in order to discover any causative mutations for these disorders (Barroso I. et al. Nature 402:880-3; 1999). High priority SNPs are then nominated for typing across independent case/control (n=1034) and QTL (n=1128) populations. Stringent quality-control of the DNA samples to be tested is essential for the production of high quality genotypes. In addition to Pico-Green quantitation, all genomic samples are subjected to a functional assay to ensure minimal variance in amplification rates, eliminating the potential for allele bias in genotyping. The genotyping method used is "FP-TDI", a homogeneous endpoint primer extension assay with detection by fluorescence polarization (Chen X, Levine L, Kwok PY Genome Res 5:492-8; 1999). Modifications include using a two-dye system in 384-well plate format employing Hamilton liquid delivery robots, barcoding and complete LIMS data tracking, resulting in 5K typing/day capacity. FP-TDI cluster plot data is viewed using the Spotfire graphing program and genotypes are called using custom-made software termed Adaptive Ridge Cluster Analysis (ARCA) which also assigns a confidence value to each data point. Low confidence genotypes are automatically eliminated. Current status and results from this pipeline will be presented.

Poster No: 15

Identification of genes within the 500-kb deletion encompassing the *high growth* (*hg*) mutation in mice

H. Motaln[1], B. A. Freking[2], J. F. Medrano[3] and **S. Horvat**[1]
[1]University of Ljubljana, Zootechnical Dept., Biotechnical Faculty, 1230 Domzale, Slovenia
[2] U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933, USA
[3] Department of Animal Science, University of California, Davis, CA 95616-8521, USA

The high growth (*hg*) mutation in mouse is characterized by a 30-50% increase in weight gain and mature body size. High growth (HG) mice grow more efficiently, are not obese, exhibit muscle fiber hyperplasia and a proportional increase of organs and skeleton. Our previous genetic and physical mapping studies have shown that *hg* maps to a deletion of ~500-kb (*hg* region) on mouse chromosome 10. Using positional cloning strategy, we have recently identified that the molecular cause for the high growth phenotype is lack of *Socs2* expression, a gene that is disrupted by one of the deletion breakpoints in the *hg* region. To identify other genes that lie within the *hg* region, we have employed comparative mapping, exon trapping, and direct sequencing. For comparative mapping, 56 human expressed sequence tag (EST) clones from a region on human chromosome 12 that is homologous to the mouse *hg* region were selected and hybridized to five mouse BAC probes spanning the *hg* region. 10 candidate human ESTs were identified, which now need to be studied further using sequence and expression analyses to identify true orthologs. A second approach of exon trapping involved isolation of potential exon sequences within the mouse BAC clones. For RT-PCR analysis, 49 exon trap clones were tested in various HG or control tissues. A total of 24 exon traps gave positive RT-PCR signal in at least one of the studied tissues. Sequence homology analyses showed that some exon traps belong to one of the three known genes within the *hg* region, *Vespr*, *Raidd*, and *Socs2,* while some may present novel transcription units. Further Northern analysis should confirm, which exon traps are true expressed sequences. Cloning of complete mouse transcripts using exon traps as probes should help to determine, which exon traps belong to new transcription units and which ones to known genes (*i.e., Vespr, Raidd or Socs2*) but to novel exons or alternative splice variants of these genes. A third approach of direct sequencing and searching for genes with computer-based approaches (BLAST, GRAIL) is also underway. Using the above three approaches it should be possible to characterize all the genes within the 500-kb deletion to permit further functional characterization studies of the *hg* deletion.

Poster No: 16

Association between genes of the serotonergic system and depressive symptoms in the elderly

M. Jansson[1,2], M.Schalling[2] and N. Pedersen[1]
[1.]Institute for Medical epidemiology at Karolinska Institute, Stockholm
[2.]Institute for Molecular Medicine at Karolinska Hospital, Stockholm

The life time risk of major depression is as high as 10-20 percent. Major depression tends to run in families, such that the more closely "blood-related" a person is to someone who has major depression, the more likely that person is to develop the disorder. However a genetic predisposition to depression itself does not seem to be enough to cause depression. The role of genetic factors is indicated by difference in concordance between monozygotic (MZ) and dizygotic (DZ) twins as well as the correlation between adopted persons and their biologic relatives. The concordance for major depression in MZ twins is substantially higher than it is in DZ twins, with broad diagnostic criteria 66 and 34 percent respectively. To identify the genetic effect and genes influencing depression, the population-based Swedish Twin Registry has been used. In the study we have 550 DZ and 300 MZ twin pairs. An association study has been conducted based on candidate genes for mood disorders where we have used a newly developed method for SNP detection, the Pyrosequencer. The genes evaluateted are the seretonin transporter (5-HTT) and the promotor of the serotonin receptor 2A gene. There is no association between these polymorphisms, in the serotonin transporter (5HTT) and the serotonin receptor 2A promoter (5HTR2Aprom), and depressed symptoms.

Poster No: 17

TP53 status in human carcinomas is associated with DNA copy number abnormalities

A-L. Bŕrresen-Dale[1], **H. Johnsen[1], P. Vu[1]**, K. Chin[2] and J.W. Gray[2]
[1] Dept. of Genetics, Norwegian Radium Hospital
[2] Cancer Center, University of California, San Francisco, CA 94143

We have over the years screened for *TP53* mutations in breast carcinomas from different series of patients (>500 cases). *TP5*3 mutation screening was performed using Constant Denaturant Gel Electrophoresis (CDGE) or Temporal Temperature Gel Electrophoresis (TTGE). Automated DNA sequencing (ABI 373) was used to verify the mutations.

The assess whether *TP53* mutations status by itself or specific mutations in *TP53* gene contribute to genomic instability, we selected 52 cases with known *TP53* status (28 *TP5*3 ^{mut} cases and 24 *TP53* ^{wt}) and performed genome copy number analysis using Comparative Genomic Hybridization (CGH).

The total number of genome copy number abnormalities (CNAs) was significantly higher in *TP53*^{mut} tumors than *TP53*^{wt} tumors (p=0.0001). We also identified a significant relationship between *TP53* status and abnormalities at specific loci. Both gain at 8q24 and loss at 5q15-23 were significantly linked with mutant *TP53*. On the other hand, the type of *TP53* mutation had no influence on the spectrum of genome copy number abnormalities.

Poster No: 18

BRCA1/2 gene mutations and single nucleotide polymorphisms under familial predisposition to breast/ovarian cancer

A.V. Karpukhin[1], N.I. Pospekhova[1], L.N. Lubchenko[2], A.N. Loginova[1], E.V. Khomich[1], A.V. Budilov[3], V.M. Zakharyev[3], R.F. Garkavtseva[2] and E.K. Ginter[1]
[1] Research Centre For Medical Genetics, Russian Academy of Medical Sciences, Moscow, Russia (email: karpuhin@orc.ru)
[2] Cancer Research Centre, Russian Academy of Medical Sciences, Moscow, Russia
[3] Engelgardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia

Investigation of single nucleotide polymorphisms (SNPs) simultaneously with mutation analysis is essential for more understanding of gene structure and genotype/phenotype correlation. In present study the entire coding region of *BRCA1/2* genes in a sample of 46 patients and 13 members of proband families (Moscow region predominantly) was screened by conformation sensitive gel electrophoresis (CSGE). All structural variants of amplified DNA fragments found by CSGE were sequenced on both strands.

There were 17 mutations of *BRCA1* and 8 mutations of *BRCA2* gene among the patients. 2 mutations of the *BRCA2* gene are revealed for the first time. Mutation spectrum of *BRCA1* is characterized by predominance of 5382insC (76,4% of 17 mutations). Such high frequency (the most of known one) may suggest on geographic region of the mutation origin. *BRCA1* mutations were found predominantly in breast/ovarian families and the *BRCA2* mutations were identified in breast cancer families. Germ-line variations of the *BRCA1* gene were found as a frequently occurred set of predominantly 8 SNPs that is inherited as a whole. Both the mutation and SNP set were on the same chromosome when these gene alterations were investigated in available families. So frequent and extensive set of SNPs that was typical for *BRCA1* gene in Russia was not found for *BRCA2*. High frequency of *BRCA2* some variances was shown, including that are seldom in other populations. It is interesting that 7 of 8 mutations of *BRCA2* are correlative to SNP set of *BRCA1* gene. This may suggest on association between *BRCA2* mutation and SNP set of *BRCA1*. The presence of the SNP set of BRCA1 among patients with and without *BRCA1* mutations was different (29% and 72%, respectively; $P < 0,01$). Under *BRCA1* mutation the ratio of breast to ovarian cancer in families was higher when the gene had SNP set also ($P < 0,01$). An inheritance of breast cancer was found in 68% of families if proband had the SNP set whereas only 25% of probands had these variations if ovarian cancer occurred in families ($P < 0,01$).

Poster No: 19 Short Presentation

Diversity Arrays: A solid state technology for sequence information independent genotyping

D. Jaccoud[1], K. Peng[1], D. Feinstein[2], D. Kudrna[3] and **A. Kilian**[1]
[1] Center for the Application of the Molecular Biology to International Agriculture (CAMBIA) Canberra, ACT, Australia
[2] Analytic Animations, Bend, Oregon, USA
[3] Washington State University, Pullman, USA

We present the successful application of the microarray technology platform to the analyses of DNA polymorphisms. Using the rice genome as a model, we demonstrate the potential of a high throughput genome analysis method called Diversity-Array Technology - DArT™. In the format presented here the technology is assaying for the presence (or amount) of a specific DNA fragment in a subgenomic sample derived from the total genomic DNA of an organism or a population of organisms. Two different approaches are presented: the first involves contrasting two subgenomic samples on a single array while the second approach involves contrasting a subgenomic sample with reference DNA fragment common to all elements of the array. The technology was positively tested on two larger genomes: mouse and barley. Polymorphisms detected using DArTs behave in Mendelian fashion and mapped with high LOD scores on the genetic maps. The Diversity panels created using this method allow genetic fingerprinting of any organism or a group of organisms belonging to the genepool from which the panel was developed. Diversity Arrays enable rapid and economical application of a highly parallel, solid state genotyping technology to any genome or complex genomic mixtures.

Poster No: 20

Genetic aspects of Papillon Lefčvre syndrom in Slovenia

R. Kosem[1] , K. Trebušak[2], A. Kansky[3], T. Battelino[2] and U. Skalerič[4]
[1] Dept. of Paediatric and Preventive Dentistry, Faculty of Medicine, University of Ljubljana, Slovenia
[2] Dept. of Paediatrics, Faculty of Medicine, University of Ljubljana, Slovenia
[3] Dept. of Dermatology, Faculty of Medicine, University of Ljubljana, Slovenia
[4] Dept. of Periodontology, Faculty of Medicine, University of Ljubljana, Slovenia

Papillon Lefčvre syndrom (PLS) is an autosomal recessive disorder characterised by palmoplantar hyperkeratosis and severe early onset periodontitis that results in the premature loss of the deciduous and permanent dentitions. Due to severe alveolar bone destruction and early loss of teeth the alveolar ridges of the patients are thin and atrophic making a successful prosthetic rehabilitation very difficult. Besides palms and soles the keratosis also affects other sites such as elbows and knees. The skin symptoms may appear psoriasiform.

The syndrom is estimated to have frequency of 1 to 4 per million. In Slovenia among 2 million population 13 PLS patients were detected in 7 families. No parental consanguinity was identified. 7 PLS patients and 10 family members of 5 families were clinically examined. DNA was isolated from peripheral blood samples from all available members of these 5 families. PCR primers were designed to cover the cathepsin C gene aiming to find mutations in the region of its 7 exons and 6 introns using sequence analysis of PCR products. Since clinical presentations vary among investigated families we expect interesting genotype - phenotype correlation.

Poster No: 21

Genetic status of cell cycle regulators in squamous cell carcinoma of esophagus: the *CDKN2A (p16^{INK4a} and p14^{ARF})* and *p53* genes are major inactivation targets

J. Smeds, P. Berggren, X. Ma, K. Hemminki and **R. Kumar**
Department of Biosciences, Karolinska Institute, Novum, 141 57 Huddinge, Sweden

Alterations of the genes in the Rb and p53 pathways represent essential steps in the tumorigenic process in almost all cancer types. To investigate the role of the genes in these two pathways we studied the status of the *CDKN2A* (*p16^{INK4a}* & *p14^{ARF}*), *CDKN2B, CDKN2C*, *CDK4*, *p53* and *p53R2* genes in tumour tissues from a set of 21 cases of squamous cell carcinoma of esophagus from a high-risk area of China. In the *CDKN2A* gene, which encodes two cell cycle regulators *p16^{INK4a}* and *p14^{ARF}*, we determined mutations, hemizygous and homozygous deletions and promoter methylation for both p16^{INK4a} and p14^{ARF}. We detected frame-shift/ non-sense mutations, promoter hypermethylation and homozygous deletions of the *CDKN2A* involving *p16^{INK4a}* in 11 cases. One case with a frame-shift mutation involving *p16^{INK4a}* also had a novel mutation in the codon 51 of the *CDK4* gene causing isoleucine to methionine change in amino acid. One case with no mutation, homozygous deletion or promoter methylation showed over-amplification at the *CDKN2A* locus. In the *p53* gene 15 mutations were detected in 13 cases, whereas methylation in the promoter of *p14^{ARF}* was detected 10 cases with no correlation with *p53* mutations. The correlation between mutation or methylation in the *CDKN2A* gene and LOH at the *CDKN2A* locus was significant (Fischer exact test, P=0.001); no such correlation was detected between mutations in the *p53* and LOH at the locus. This study provides clear evidence to the involvement of the *CDKN2A* and *p53* genes in esophageal cancer. Furthermore, an independent role of the *p14^{ARF}* gene is indicated by the frequent methylation of its promoter, although no exclusive mutations were observed in exon 1ß. Other cell cycle regulators studied, *CDKN2B,* and *CDKN2C*, did not show important roles in the esophageal cancer cases. The *p53R2* gene which is *p53* induced and involved in DNA repair, did not show any mutation though we detected a novel polymorphism in the 5'UTR of the gene.

Poster No: 22 Short Presentation

APEX-based resequencing assay of *p53* tumor suppressor gene

A. Kurg[1], N. Tőnisson[1,2], J. Zernant[2], H. Roomere[1], A. Meiel[1], H. Pavel[2] and A. Metspalu[1,2]
[1] Institute of Molecular and Cell Biology, University of Tartu, 23 Riia St. Tartu 51010, Estonia
[2] Asper Biotech. Ltd., 3 Oru St. Tartu 51014, Estonia

After completion of the first draft of human genome sequence the resequencing assays will get more attention being in principle more economical than *de novo* DNA sequencing. Diagnostic sequencing of *p53* gene has been used to get mutation data of the *p53* gene, which have relevant prognostic and therapeutic value in several types of cancer. We have developed an APEX (Arrayed Primer Extension) - based test for the gene with goal of obtaining the full mutation data at both DNA strands in a single assay. A patient DNA sample is amplified, digested enzymatically, and annealed to arrayed primers, which promote sites for template-dependent DNA polymerase extension reactions using four fluorescently labeled dideoxy nucleotides. The Genorama™ imaging system and genotyping software are used for imaging and semiautomatic sequence analysis. The *p53* gene chip is scanning exons 2 to 9 plus introns 5 and 8 from both strands (total of 1218 bases). Average of 97.5% of the arrayed *p53* gene was sequenced from either sense or antisense strand and 81% simultaneously from both strands. In best cases, up to 99.8% and 95% of the sequence is readable. 100 normal DNA samples from the Estonian population were analyzed to obtain the overall performance data of the p53 APEX assay in large-scale studies. In addition, 11 tumor samples with known mutations were analyzed in a blind test. The *p53* gene chip could become a medium for accurate and efficient DNA sequence analysis of this or other frequently mutated genes.

Poster No: 23

TP53 gene mutations in Russian patients with breast cancer detected by chemical cleavage of mismatch and sequencing methods

A. Lambrinakos[1], **C. T. Bui**[1], M. Yakubovskaya[2], J. J. Babon[1], A. A. Neschastnova[2], Ya. V. Vishnevskaya[2], G. D' Cuhna[1] and R. G. H. Cotton[1]
[1] Genomic Disorders Research Centre, 7th Floor, Daly Wing, 41 Victoria Parade, Fitzroy, Melbourne, Victoria 3065, Australia
[2] Cancer Research Centre RAMS, Moscow, Russia

Mutations in the tumor-suppressor *TP53* gene are commonly found in most human tumors including breast cancer. A new solid-phase chemical cleavage of mismatch method (CCM) involving silica beads made it possible for rapid and efficient screening of 89 DNA samples extracted from tumors of Russian breast cancer patients.

Mutation analysis involved preparation of heteroduplexes DNA samples derived from exons five to nine of *TP53* gene. The DNA samples were immobilized on silica beads and then analyzed by the CCM method. The technique identified forty-eight signals including four signals in exon five, eleven signals in exon six, sixteen in exon seven, twelve in exon eight and five signals in exon nine.

The mutation identified in this study included: (i) three novel mutations (R213L, N200K and L201V) in this breast cancer cohort, (ii) three mutations that have been previously reported in other types of cancer (T253I, G244C and delT256) and (iii) one mutation that has been reported in breast cancer literature (H179Y) was also identified.

The combined method of solid-phase and CCM is very convenient, highly sensitive and reliable as no false positive signals have been detected in this investigation. Comparative study with the sequencing method will also be discussed

Poster No: 24

SNP-based genotyping using arrayed primer extension on the oligonucleotide array

E. Lõhmusaar[1], K. Kepp[1], N. Tõnisson[2], T. Nikopensius[1], I. Kask[1], A. Kurg[1] and A. Metspalu[1, 2]
[1] Institute of Molecular and Cell Biology University of Tartu, Estonian Biocenter
[2] Asper Biotech. Ltd., Tartu, Estonia

SNP-s offer a number of advantages with respect to population-based analysis of the human genome. We present Arrayed Primer Extension (APEX) technology for SNP scoring all over the genome. The method is based upon an array of oligonucleotides immobilized via 5'-end amino linker onto amino-silanized glass slide. Oligonucleotides are selected from the sense and antisense genomic sequence so that their 3'-end is one base upstream of the SNP. The amplified DNA template, containing the SNP, is digested enzymatically and then annealed to the immobilized primers, which promote sites for template-dependent DNA polymerase extension reactions. Four unique fluorescently labeled dye terminators are used to extend each primer by only one base. As a result of this reaction each primer identifies one base in the target sequence. The Genorama™ imaging system and software package is used for SNP scoring.

In the present study APEX technology was successfully used for SNP-based genotyping. We have selected 68 SNPs over the whole genome and estimated the allele frequencies and heterozygosities of these SNPs analyzing 120 individuals from Estonian population. From the 68 analyzed SNPs 58 were polymorphic according to the allele frequencies data. This current oligonucleotide array with 58 SNPs can be successfully applied for paternity testing and forensic analysis. Oligonucleotide design, quality, DNA Polymerase, dye terminators, template DNA quality and special software tools are all critical for the optimal results. APEX method is a reliable tool in SNP studies, which seem to have a great potential for large-scale genotyping in the near future.

Poster No: 25

Genotyping of Human Rotavirus by multiplex capture and type specific primer extension on microarrays

C. Fock[2], **L. Malmberg**[1], F. Espinoza[2], A.-C. Syvänen[1] and K. Bondesson[2]
[1] Molecular Medicine, Department of Medical Sciences, Uppsala University, Sweden
[2] Clinical Virology, Department of Medical Sciences, Uppsala University, Sweden

Human Rota Virus (HRV) is the major cause of gastroenteritis in infants and young children worldwide. In future vaccine-trials and generally, all interventions in microbal systems, stabile and high throughput methods will be needed to genotype circulating strains. When using strictly hybridisation based methods to genotype highly variable genomes there are problems finding stringent hybridisation conditions. In this pilot study we have, using HRV as a model system, developed a method for strain genotyping based on multiplex capture followed by type specific primer extension on microarrays. RT-PCR products derived from fecal samples were captured by genotype specific capture oligonucleotides immobilised on the microarray glass surface. The RT-PCR products are then used as templates for type specific extension of the capture oligonucleotides. During extension, fluorescent nucleotides are incorporated and the signal is detected by a microarray scanner. The results were interpreted using visual pattern recognition and we are currently working with development of numerical interpretation. 20 HRV samples were clearly genotyped by extension of 25 capture oligonucleotides. The genotypes are defined by two very variable HRV capsid protein genes. The microarray format gives a large capacity and due to its robustness and simplicity the method is likely to have wide applicability in genotyping of closely related organisms.

Poster No: 26

Development and validation of a method for SHOX mutation screening and SNP-based detection of whole SHOX gene deletions using DHPLC

P. Marttila[1], J. Ross[2], A. Zinn[3], S. Blakely[1], J. Sernberger[1] and M. Stene[1]
[1] Esoterix Endocrinology, Calabasas Hills, CA, United States
[2] Pediatrics, Thomas Jefferson University, Philadelphia, PA, United States
[3] University of Texas Southwestern Medical School, Dallas, TX, United States

SHOX (Short Stature Homeobox) gene defects cause impaired growth in some non-growth hormone deficient children, Turner syndrome (TS) and Léri-Weill dyschondrosteosis (LWD) and contribute to certain skeletal features of TS and LWD. Most SHOX defects, ~70%, are whole gene deletions but point mutations have also been reported. The phenotype varies within families affected by SHOX haploinsufficiency. We report here the development and validation of the SHOX-DNA-Dx test. SHOX-DNA-Dx is quick, sensitive and specific for identifying SHOX mutations, including whole gene deletions. It uses heteroduplex analysis. All SHOX encoding exons are examined for mutations, and intragenic SNPs are used to detect whole gene deletions. For SHOX SNP discovery, PCR products were designed throughout the SHOX gene and tested for SNP content using heteroduplex analysis by DHPLC (Denaturing High Performance Liquid Chromatography). Useful SNPs were identified by screening normal controls (n=52) and confirmed by DNA sequencing. Seven SNP-containing PCR amplicons were selected for the validation studies. For exon screening, exon primers were designed that span the exon-intron boundaries and have optimal melting characteristics for heteroduplex detection. Validation Studies: Normal samples (n=52) were negative for SHOX mutations when tested by SHOX-DNA-Dx™. Patient samples (n=18) previously evaluated by FISH (Fluorescent *in situ* hybridization) were tested in a "blinded" fashion by the SHOX-DNA-Dx test. DNA sequencing was used to confirm the SHOX mutations. Results: 6/18 patient samples tested showed whole gene deletions both in FISH-analysis and SHOX-DNA-Dx. 12/18 samples did not show whole gene deletions either in FISH- analysis or SHOX-DNA-Dx. Seven of these 12 samples showed point mutations by SHOX-DNA-Dx. Three of these point mutations were previously unknown. Conclusion: SHOX-DNA-Dx is a highly sensitive and specific test for SHOX-deficiency due to whole SHOX gene deletions, or known or previously undescribed SHOX point mutations.

Poster No: 27

Comparative Sequence Analysis (CSA): A new sequence based method for the identification and characterisation of mutations in DNA

C. Mattocks, P. Tarpey, M. Bobrow and J. Whittaker
Addenbrooke's Hospital, Cambridge, UK

Despite the numerous methods available for DNA mutation detection, direct sequencing remains the most informative. However since the calling of heterozygous mutations can be problematic using current software, time consuming visual inspection of sequencing electrophoretograms is required to give an unequivocal result. For this reason direct sequencing is largely used to confirm and characterise mutations previously detected by more rapid tests.

We have developed a method for the analysis of sequencing data, which allows simple and rapid visual analysis with the minimum of data manipulation, thus facilitating its use as a first screen for mutation detection. The technique was validated in a double blind assay for mutations in the von Hippel-Lindau tumour suppresser gene, which gave a sensitivity and specificity of 100%.

We have adapted this technique to allow simultaneous detection of both small mutations (point mutations, insertions, and deletions) and gross mutations (whole exon/gene duplications, and deletions) in a single assay. In addition we have designed single reaction assays, based on CSA, capable of screening two different amplicons; both direction of the same amplicon; or amplicons up to twice as long as normally analysed using conventional sequencing.

Preliminary results from software designed to automate CSA will be presented and the implications on sample throughput and detection sensitivity and specificity discussed.

Poster No: 28 Short Presentation

High-throughput, cost-effective SNP genotyping by competitive allele-specific polymerase chain reaction (SNiPTag)

J.L. McClay, K. Sugden., H.G. Koch, S. Higuchi and I.W. Craig
MRC SGDP Reseach Centre, Institute of Psychiatry, DeCrespigny Park, Denmark Hill, London, SE5 8AF, UK

Single Nucleotide Polymorphisms (SNPs) are becoming widely recognised as the new currency in gene mapping as increasing numbers are discovered. While many applications exist to facilitate rapid SNP genotyping in a high-throughput framework, these are often expensive and for many laboratories, require the purchase of new eqiupment. Here we outline a method for SNP analysis based on an allele-specific polymerase chain reaction (PCR), which employs a competitive approach, whereby both allele-specific primers are present in the same reaction and carry different fluorescent labels. This procedure is simple, reagents are readily available and analysis of PCR product is carried out on conventional automated sequencing machinery (e.g. Applied Biosystems 310,

377, 3100). It is further amenable to automation and robotic handling as no post-PCR modifications are required. Verification of the procedure was carried out by comparison of results derived from this method with those from restriction enzyme digestion of the ALDH2 exon 12 functional polymorphism (Glu-487-Lys) in 109 individuals. Additionally, we have examined all possible combinations of nucleotide substitutions and found them to be differentiated by this method. As proof of concept, several assays were combined and loaded on a single lane in order to substantially improve throughput. This was made possible by designing the PCR products to be of different lengths and no interference was observed between products only 6bp apart. We estimate that, with a sufficient number of thermal cyclers and loading 10 assays per lane, it would be possible to carry out 11500 genotypings per day on a single ABi 3700, at a cost of approximately 30 pence per genotype.

Poster No: 29

Haplotyping by high-throughput SNP analysis of the ATM locus using molecular beacons

M. M. Mhlanga[1,2], R. A. Gatti[3], F. Russell Kramer[1,2] and S. Tyagi[2]
[1] Department of Cell and Molecular Biology, The Sackler Institute of Graduate Biomedical Sciences, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA
[2] Department of Molecular Genetics, Public Health Research Institute, 455 First Avenue, New York, NY 10016, USA
[3] Department of Pathology, University of California at Los Angeles School of Medicine, Los Angeles, CA 90095, USA

Approximately one million single nucleotide polymorphisms (SNPs) have been identified in the human genome. SNPs are the most common form of human genetic variation. Every gene is decorated by SNPs that identify its haplotype. Genetic function is dependent on the two haplotypes that an individual inherits. Thus identification of an individual's haplotypes through the use of markers such as SNPs, can be used to predict health or disease. To exploit this resource the need for high-throughput methods of SNP typing is essential to a comprehensive examination of the role that genomic regions play in disease. Previously we reported the development of molecular beacons, highly specific nucleic acid probes that recognize and report the presence of nucleic acids. Further, we have exploited this capability for real-time hybridization monitoring in multiplex polymerase chain reactions (PCRs) to spectrally genotype human alleles. Recently, we turned this capability to high-throughput screening of SNPs in association studies to detect variants of ATM (mutations in thisgene cause ataxia-telangiectasia, an autosomal recessive disease) predesposing individuals to cancer. Our aim is to identify haplotypes for ATM, and once their efficacy is established in association studies, use them to screen families where cancer incidence data and ATM symptoms have been identified. The haplotypes are identified based on the use of ten biallelic neutral sequence variations that are found across the entire 150kb length of the ATM locus. Work by other groups has shown that there is extensive linkage disequilibrium and reduced recombination at the ATM locus in all ethnic groups examined. This indicates that the ATM haplotypes we are identifying will be useful in association studies to discern genetic backgrounds that contribute to some or all aspects of ataxia-telangiectasia. Of the several platforms available for SNP analysis, each presents a distinct set of advantages and disadvantages. Molecular beacons have demonstrated their efficacy in other studies in the detection of point mutations. Given their high specificity, low cost, and accessibility they should serve as an effective technology to robustly analyze SNPs with a high throughput.

Poster No: 30

HOX-B1 allelic variants in hindbrain malformations

A. Moroni[1], P. De Marco[1], **E. Merello**[1], L. Arata[1], A.Faiella[2], E. Boncinelli[2], A. Cama[1] and V. Capra[1]
[1] Servizio di Neurochirurgia, Istituto G. Gaslini, Genova-Italy
[2] DIBIT-S. Raffaele, Milano-Italy

Hindbrain malformations are a group of congenital developmental anomalies involving cerebellum, brain-stem, fourth ventricle, and cervical spinal cord. Chiari complex and the cystic malformations of the posterior fossa (Dandy-Walker syndrome and equivalent) are the most frequent and severe pathologies. Chiari complex is characterized by caudal cerebellar erniation, sometimes associated with lower brain stem dysmorphism, skull base and vertebral anomalies; Dandy-Walker anomaly is due to cerebellar vermis agenesis, hemispheric hypoplasia, cystic dilatation of the 4th ventricle and tentorial and venous sinus malposition. In both cases, morphological associated features may be hydrocephalus, deafness, external-middle ear malformation, occipital schisis, lower cranial nerves displacement and hemifacial hypoplasia. In vertebrates hindbrain, generation of regional diversity is achieved through a segmentation process that, during primary neurulation, leads to the formation of 7 transient methameric units, called rhombomeres. Each rhombomeric segment defines a cellular compartment that will go on to adopt a different identity respect to its immediate neighbours. As an integral part of this process, Hox genes display a key role in controlling and regulating neuronal migration and in mainteining cellular segmental identity. Among *labial* hortologous, Hoxb-1 gene seems to be the most interesting since it's the first to be activated in CNS; moreover, its expression domain is selectly restricted in rhombomere 4 and in the neural crest cells that from r4 migrate in the second branchial arch. Hoxa-1 cross-regolatory interactions and Hoxb-1 autoregolatory loop on its promoter region cause high expression levels just in r4 and allow a correct patterning of this region. In view of the fondamental role of *HOX-B1* gene in hindbrain patterning, we decided to evaluate a possible involvement of human homologous gene in the pathogenesis of hindbrain malformations by submitting 40 cases to mutational screening for *HOX-B1* gene. We identified two allelic variants: the first is a polymorphism characterized by the presence of 9 bp tandem duplication associated in cis to three transitions (two synonymous and one missense mutations), the second one, detected only in patients' families, carrying four nucleotide substitutions codifying for three synonymous and one missense mutation.

Poster No: 31

The Irish Cystic Fibrosis Mutation Database

D. E. Barton[1,2], **B. O'hIci**[1,2], C. LeMarechal[3], S. A. McQuaid[1], C. Joyce[1], A. J. Ward[1], F. Ryan[1], C. Clabby[1], T. M. McDevitt[1], D. J. Cockburn[1] and C. Férec[3].
[1] National Centre for Medical Genetics, and [2] Department of Medical Genetics, University College Dublin, Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Ireland
[3] Laboratoire de Genetique Moleculaire et d'Histocompatibilite, CHU Morvan, UBO, EFS-Bretagne, Brest, France

During the past five years (1995-2000) we have genotyped 452 Irish cystic fibrosis (CF) affected individuals (904 chromosomes) at the National Centre for Medical Genetics, Dublin, Ireland.

Through routine mutation testing and SSCP analysis, we have identified 20 different mutations, accounting for almost 95% of the CF alleles in the Irish population. The most common CF mutation, ΔF508, is found in 76.8% of Irish CF chromosomes, with G551D in 6.6%, R117H in 2.6%, R560T/K in 1.9% and 621+1G>T in 1.4%. These are the highest reported frequencies for the latter four mutations world-wide. A further 15 mutations were identified in 5.06% of CF chromosomes.

We have now embarked on a project to identify every CF mutation in the Irish population, using a combination of dHPLC analysis and direct sequencing. So far, one novel and 12 previously-reported mutations (or sequence variants) have been identified in 35?? patients analysed. One mutation, 3007delG, was seen in three apparently unrelated patients. Of interest is the finding of two instances of patients homozygous for rare CF mutations (1154insTC and 1471delA), although no consanguinity was suspected in either case. We have observed the sequence change 297-3C>T in a patient who also carries ΔF508 and the frameshift 4279insA. This finding makes it less likely that

297-3C>T, previously reported as a suspected mutation affecting splicing, is pathogenic.

Based on haplotype analysis of non-ΔF508 chromosomes, Cashman et al (1995) suggested that perhaps there is another fairly common CF mutation in Ireland. However, with less than 3.5% of CF mutations currently unidentified in the Irish population, this now seems unlikely.

Poster No: 32

Mutation hot spots in the *ED1* gene in patients with X-linked anhidrotic ectodermal dysplasia.

U. Orth and A. Gal
Institut für Humangenetik, Univ.-Krankenhaus Eppendorf, Hamburg, Germany

Anhidrotic ectodermal dysplasia (AED) is a rare genetically very heterogeneous congenital disorder due to abnormal morphogenesis of ectodermal structures. AED is characterized by hypohidrosis due to lack of sweat glands and hypo- or atrichosis, abnormally shaped and/or missing teeth. The disease locus for the X-linked form of the trait has been mapped to Xq12.1-q13. The corresponding gene (*ED1*) encodes a type II transmembrane protein of 391 aa with significant homology to proteins of the TNF ligand superfamily. The *ED1*-protein contains a protease recognition site as well as a collagen-like domain which may provide a novel mechanism for initiating or stabilizing a trimeric complex by triple-helix formation. The function of the protein remains still speculative, it is probably involved in epithelial-mesenchymal signalling, and may act as ligand for ectodysplasin-receptor (Eda R), a member of the TNF R family, recently shown to be encoded by a gene (*ED3*) whose mutations cause an autosomal type of AED (dominant and recessive).

In a total of 75 apparently unrelated patients with AED, including 19 females with carrier signs, we performed mutation screening by PCR-SSCP analysis and direct sequencing of 8 exons of *ED1* comprising 99.5% of the coding sequence. In case of X-linked disorders, most of the families have "private" mutations. Indeed, 40 different mutations were identified; 23 missense, 3 nonsense, 3 point mutations in splice consensus sequence, 7 smaller rearrangements (deletions or insertions of 1-50 bp) resulting in frame-shift and predicting premature termination of translation, 3 in frame deletions, and a 5 kb deletion starting upstream the 5'UTR and ending in the middle of exon 1. Cosegregation of the respective mutations with the disease phenotype and their absence on 50 control X chromosomes suggest that the sequence alterations found are indeed the primary genetic defects responsible for the disease. In about 10% of the male patients, the mutation occurred *de novo* in maternal meioses.

The 40 mutations identified account for a total of 48 disease alleles, for 5 mutations have been detected at least twice, in most likely unrelated patients. In addition, 8 of the 40 mutations (12 alleles in total) found in this study have already been described in patients from the USA and/or Northern Europe. The distribution of mutations identified to date in three independent studies does not seem to be random. There seems to be a clustering of point mutations in the middle part of exons 3 and 9, and a unique accumulation of larger deletions in exon 5.

Poster No: 33 Short Presentation

Y-chromosomal haplotyping to test testicular and prostate cancer predisposition using high-throughput MALDI-TOF mass spectrometry for SNP genotyping.

S. Paracchini, R. Chalk, B. Arredi and C. Tyler-Smith
CRC Chromosome Molecular Biology Group, Department of Biochemistry, University of Oxford, Oxford, UK

The absence of recombination along most of the length of the Y chromosome, facilitating interpretation of haplotypes, makes it a powerful tool to study human evolution and Y-associated diseases. Although determining Y sequence variation has been slow until recently, a large number of SNPs are now available. These, together with other biallelic and multiallelic markers define haplotypes stably transmitted from father to son. Y haplotypes and their distinct geographical distributions are well known.

Recent work has suggested the involvement of the Y chromosome in some forms of cancer and association studies provide a general way of investigating the contribution of Y genes. Since there is no recombination,

Y markers are completely linked to putative oncogenic mutations. The haplotype is determined for each individual, and then haplotypes frequencies are compared between patient and control populations. An increased frequency of a haplotype, or haplotype set, in the patients would suggest that the corresponding Y chromosomes carry a mutation predisposing towards cancer. Decreased frequency or absence of some haplotypes in patients would suggest an allele giving protection.

A high throughput genotyping method is required to score about 150 Y SNPs per individual. Here we use the *PinPoint* ® SNP kit, and MALDI-TOF mass spectrometry for allele detection (Haff and Smirnov, 1997. *Genome Res*. 7:378-388). A genotyping primer is annealed to a PCR target and extended at its 3' end by incorporation of a single ddNMP complementary to the polymorphic base. MALDI can readily detect the 9-40 Da mass difference between extended primers, thus determining which base was added. Pre-selected mass difference between unextended primers permits multiplex primer extension. All the steps are performed in 96-well format whilst a piezoelectric spotting robot prepares samples for mass spectrometry. Analysis is fully automatic, and genotypes are generated using onboard software.

The bialellic markers define a well-supported phylogenetic tree. A screening strategy has been designed making use of this information and multiplex PCR, in order to minimise the number of amplifications per sample. The first PCR defines the six basal branches of the tree and subsequent multiplexes subdivide each branch. Thus an individual's haplotype can be defined by two to five multiplexes. So far, it has been possible to type all the SNPs tested using this procedure.

Poster No: 34 Short Presentation

DNA microchips technology for HLA-Typing

C. Pera[1], L. Delfino[1], G. Salani[3], C. Consolandi[3], C. Battaglia[3], R. Cubeddu[4], P. Dario[5], L. Rossi Bernardi[3], G.L. De Bellis[2] and G.B. Ferrara[1]

[1] National Cancer Institute c/o Advanced Biotechnology Center, Genova, Italy
[2] ITBA CNR, Milano, Italy
[3] Universita' degli Studi di Milano, Dip di Scienze e Tecnologie Biomediche Avanzate Milano, Italy
[4] Politecnico, Milano, Italy
[5] Scuola Sup. S. Anna, Pisa, Italy

The possibility to use DNA microarray technology in biomedical research enable us to study and research polymorphisms spread in human genome. We have established a multidisciplinarity network consisting of one Biomedical Unit (IST/CBA), two Biochemical/Biotechnological Units (CNR-ITBA, UNIMI), two Technological Units (Politecnico di Milano, Scuola Sup di S. Anna, Pisa). Our first approach was to performe a HLA typing of patients for a correct vaccinotherapy (i.e. melanoma), with peptides derived from tumoral antigens of the recipient and to present T cells by different types or subtypes of the same HLA alleles .

A microchip defining antigens which cover many of known HLA specificities (HLA-A1, all -A2 subtypes detected so far, -A6802, A3, A3.1, A31, B44, Cw6, Cw16) can be used by tumor derived peptides to type patients for the HLA antigens bound by peptides for an efficient therapy. Furthermore a microchips for the analysis of the espression of the major melanoma-associated antigens (MAGE, BAGE, GAGE, MelanA/MART1 etc) is going to set up. In this study we present a typing technology that can be performed in few hours.

Our method consists of an extension and /or a ligation reaction performed on a glass surface, of 1mmx1mm, where synthetic oligos , used as primers and as probes, are covalently bound; in such reactions we utilized both dNTPs labeled with fluorescent molecule cy-3 and fluorescently labelled probes . Using an opportune Custom Scanning Lasersysthem, it was possible to determine where real positive reaction occured and obtain the desired information regarding the genotype of the samples. The reading is made by Cw/Laser Scanning and analyzed with software processing.

Poster No: 35

Megencephaly, identification of a molecular cause

S. Petersson[1], **A.-S. Persson**[1], **J. Johansen**[1], M. Diez[2], T. Hökfelt[2], M. Schalling[1] and C. Lavebratt[1]

[1] Dept of Molecular Medicine, [2] Dept of Neuroscience, Karolinska Institute, Sweden

Megencephaly, enlarged brain, is a major sign in several human neurological diseases. The mouse model for megencephaly, *mceph/mceph*, has an enlarged brain and exhibits neurological and motor disturbances with seizure-like activity. The brain enlargment results from hypertrophy of the brain cells, rather than hyperplasia. No structural abnormalities, edema or increased myelination have been found. Identification of the molecular cause of increased brain growth in the megencephaly mouse would provide important knowledge about mechanisms governing development and/or degeneration of the central nervous system.

We have found that the *mceph/mceph* mouse displays dramatic expression disturbances of several growth regulating molecules, such as hormones of the insulin-like growth factor (IGF) system as well as the neuropeptides cholecystokinin, enkephalin, galanin and neuropeptide Y. The changes were confined to discrete brain areas, mainly the associated regions of the hippocampus, piriform and entorhinal cortex and the parietal cortex. Whether these expression changes contribute to the excessive growth of the brain or represent a consequence of this growth and/or of the neurological and motor disturbances remains to be elucidated.

To identify the *mceph* gene we have utilized a positional cloning approach. Mapping of the mutation was achieved by a two-generation crossing of mutant and wild type mice. A DNA marker, which co-segregates with the phenotype among the progeny, is close to the mutation. We have produced a breeding panel corresponding to a total of 2026 meioses from intercrosses between BALB/cByJ-*mceph*/+ and CAST/Ei and C57BL/6 respectively. We have exhausted all public micro satellite markers in the region, and in addition developed our own markers. Thereby, we have narrowed the *mceph* region to approximately 0.8 cM. We have isolated BAC clones and constructed a contig and physical map of the *mceph* region. The physical distance between the closest flanking

markers is estimated to be approximately 300 kb and full coverage is provided by a single BAC. Five genes have been found in the region. We are presently screening these genes for mutations and expression differences.

Poster No: 36

Identification of Hereditary Non-Polyposis Colorectal Cancer patients among newly diagnosed colorectal cancers using microsatellite instability analysis and mutational analysis of mismatch repair genes

U. Potočnik[1], D. Glavač[1], R. Golouh[2] and M. Ravnik-Glavač[1,3]

[1] Laboratory of Molecular Genetics, Institute of Pathology, [2] Institute of Oncology, [3] Institute of Biochemistry, Medical Faculty; Ljubljana, Slovenia

Hereditary Non-Polyposis Colorectal Cancer (HNPCC), the common form of hereditary colorectal cancer (CRC) is caused by germline mutations in *hMLH1* and *hMSH2* genes, the major mismatch repair genes (MMR). There is a lack of characteristic diagnostic features in order to distinguish hereditary form of CRC from sporadic one, since there are no clear phenotypical differences between both forms. In addition, mutations in both major MMR genes are distributed throughout whole coding region therefore no efficient mutational strategy for HNPCC screening could be designed at the moment. Microsatellite instability (MSI) is characteristic of more than 90% of tumors from (HNPCC) patients and to less extend of sporadic CRC patients and could be a valuable marker in HNPCC screening.

We performed MSI analysis using highly informative microsatellite markers in 345 unselected primary colorectal cancers (CRC). Thirty-five (10%) tumors were classified as high MSI (MSI-H), 28 (8%) as low MSI (MSI-L) and 289 (82%) as microsatellite stabile (MSS) tumors. To examine possible causes of MSI, we studied several mechanisms of inactivation of major MMR genes. Mutational analysis of *hMLH1* and *hMSH2* genes was performed using non-isotopic conformation analysis and silver staining. For loss of heterozygosity (LOH) studies we used intragenic *hMLH1* and *hMSH2* polymorphisms in addition to microsatellite markers (3p, 2p). The methylation status of *hMLH1* promoter was assessed by methylation specific PCR (MSP) and sequencing of bisulphite modified DNA. We identified 6 (17%) MSI-H tumors with germline MMR mutations (tumors from patients with hereditary non-polyposis colorectal cancer (HNPCC tumors)) and 29 (88%) MSI-H tumors without germline MMR mutations (sporadic MSI-H tumors). Since we found 6 HNPCC tumors (1.7%) among 345 unselected newly diagnosed primary CRC, the incidence of HNPCC syndrome in Slovenian population could be estimated. If we consider 19,000 newborns annually and 850 newly diagnosed colorectal cancer cases annually (of which 1.7% have germ-line MMR gene mutation) the HNPCC incidence in a Slovenian population is approximately 1 in 1300. In our study we identified two germline mutations, a deletion of codons 188-190 in *hMSH2* and nonsense mutation (Q562X) in *hMLH1*, that are so far specific for Slovenian population. Hypermethylation of *hMLH1* promoter was found in 26/29 (90%) sporadic MSI-H tumors but only in 1/6 (17%) HNPCC tumors (p=0,014). These results together indicate different way of inactivation of MMR genes in sporadic MSI-H tumors versus MSI-H tumors from HNPCC patients. Moreover, in countries like Slovenia, where no access to well organized cancer registries containing family data is currently available, we propose, that MSI analysis of newly diagnosed primary CRC and subsequent methylation analysis of *hMLH1* promoter followed by mutational analysis of MMR genes in MSI-H tumors lacking *hMLH1* promoter methylation, might be an efficient molecular genetic approach for HNPCC screening and could help to initiate building of national HNPCC register. LOH analysis at MMR loci could suggest which gene to screen first and thus further improve the efficiency of mutational screening.

Poster No: 37 Short Presentation

Optimization of DHPLC method for analysis of *CFTR* gene mutations

M. Ravnik-Glavač[1], A. Atkinson[2], D. Glavač[1] and M. Dean[2]

[1] Laboratory of Molecular Genetics, Institute of Pathology, Medical Faculty, Ljubljana, Slovenia

[2] Laboratory of Genomic Diversity, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, Maryland, USA

It is of fundamental importance to efficiently and accurately detect gene sequence variations in genes of common genetic diseases. Denaturing high-performance liquid chromatography (DHPLC) is a technique, which depends on the detection of heteroduplexes in PCR products by ion-pair reversed phase HPLC under partially denaturing conditions. With this study we evaluated the sensitivity and optimized the conditions for DHPLC scanning of *CFTR* gene mutations, which are associated with the common lethal disease cystic fibrosis.

We studied 73 different mutations in 10 exons (3, 4, 7, 10, 11, 13, 17b, 19, 20 and 21) of the *CFTR* gene. The majority (53 mutations) was single basepair substitutions, 16 were small deletions and 4 were small insertions. All DNA samples were gathered from CF patients heterozygous for the mutation. Ten DNA samples of wild-type homozygous individuals of each analyzed exon (100 samples) were used as negative controls.

We amplified all DNA samples using a step-down PCR program and formed heteroduplexes by denaturation and gradual reannealing. For DHPLC analysis, we used WAVE Nucleic Acid Fragment Analysis System and WAVEMaker Utility Software (Transgenomic, Inc.)

In the DHPLC system, the operating temperature must be carefully optimized in order to achieve partial denaturation of heteroduplexes while leaving homoduplexes intact. In the first part of our study, we used WAVEMaker software that calculates the melting temperature (Tm) and % GC content of the analyzed DNA fragment and recommends the running temperatures. At the recommended temperature (RTm) and acetonitrile gradient conditions we were able to detect 66 of 73 (90.4%) studied *CFTR* gene mutations. Interestingly, we observed differential sensitivity for detecting transversion and transition mutations. Among seven undetected mutations there were five transitions, one single base insertion and one transversion. Additional analysis at Tm one or two degrees above the

recommended temperature increased the sensitivity to 100%. Based on the results of this study we can conclude that the accuracy of the DHPLC method is sufficient for *CFTR* mutation scanning and diagnostics.

Poster No: 38 Short Presentation

Mutation detection for Hereditary Non Polyposis Colon Cancer (HNPCC) - the value of MSI and antibody staining in screening or mutations.

Z. Rudzki[1], G. Bennett[1], S. McDougall[1], A. Gordon[1], S. Siah[1], A. Ruszkiewicz[2] and G. Suthers[3]
[1] Division of Molecular Pathology, The Institute of Medical and Veterinary Science
[2] Division of Tissue Pathology, The Institute of Medical and Veterinary Science
[3] Familial Cancer Unit-South Australian Clinical Genetics Service

Mutations in the mismatch repair (MMR) genes are a cause of HNPCC. While mutations have been demonstrated in six MMR genes, the great majority are in *MLH1* and *MSH2*. As HNPCC has a variable phenotype, many patients undergo genetic studies without a mutation being found. Mutations in the MMR genes also result in (i) microsatellite instability (MSI) and (ii) absence of the MMR gene products in tumour tissue. We have investigated the use of MSI and immunohistochemistry (IHC) using antibodies against MMR gene products to select patients for mutation detection.

A cohort of 127 patients with colorectal cancer have been tested for MSI and IHC (MSH2, MLH1 and MSH6 gene products). All patients met at least one of the modified Bethseda criteria. Paraffin embedded tumour tissue was used for IHC staining and to make DNA for MSI. Normal tissue for MSI was either peripheral blood or normal histological tissue from the section. Mutation detection utilised PCR amplification of each exon and direct automated sequencing.

MSI was detected in 29 patients and 28 of these had abnormal IHC for at least one of the gene products. Patients with MSI were then referred for counselling and informed consent obtained before mutation analysis. Three of the 29 patients were lost to follow up while a further 6 patients are in the process of being tested. The remaining patients had their MMR genes screened. The IHC results were valuable in determining which gene was screened first. Fourteen mutations were found. No mutations were detected in the remaining 6 patients, even though they had abnormal IHC (3 X MLH1, 2 X MSH6 and 1 X MSH2). The MLH1 genes are likely to be silenced by methylation but the reason for the other results is not clear.

MSI was detected in 23% of patients who met the Bethesda criteria. MSI negative patients were not tested further. Of the 20 patients who have undergone mutation detection, mutations have been found in 14 (70%). We conclude that MSI and IHC are efficient screening techniques for identifying patients likely to be carrying germline mutations.

Poster No: 39

D variants and weak D in Slovenian population

R. Rupreht[1], M. Hemker[2], V. Čurin Šerbec[1], G. Kosec[1], K. Pretnar Hartman[1], V. Galvani[1] and C. E. van der Schoot[2]
[1] Blood Transfusion Centre of Slovenia, Šlajmerjeva 6, SI-1105 Ljubljana, Slovenia
[2] Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Plasmanlaan 125, 1067 CX Amsterdam, The Netherlands

Rhesus (Rh) system is a highly complex red cell blood group system, the most important Rh polymorphism from the clinical aspect is the D antigen. Eighteen types of *RHD* genes carrying point mutations were described recently, resulting in weak D antigens.

The aim of the present work was to design and optimise allele specific ASO-PCR methods to genotype the donors carrying weak D antigens (type 1, 2, 3 and 14) in Slovenian population.

The 94 weak D donors were selected by two stage routine serological testing out of 20000 donors. The donors were tested with anti-D monoclonal IgM Pasteur by direct agglutination test, if negative the antiglobulin test with anti-D polyclonal was performed. Red cells carrying only weak D antigens are negative in the first and positive in the second serological test. Genotypes were determined by *RHD* Multiplex PCR and novel ASO-PCR methods for the weak D type 1, 2, 3 and 14. 3' ends of the ASO-PCR primers were designed in a way to anneal only with weak D type specific mutations of *RHD* gene (type 1 G809, type 2 C1154, type 3 G8, type 14 G602 and A544).

Out of 94 donors with weak D phenotype we found 1 partial $D^{VI\ typeI}$, 3 partial $D^{VI\ typeII}$ and 1 partial D^{DFR}. 42 donors were typed as weak D type1, 18 as weak D type 2, 20 as weak D type 3 and one donor as weak D type 14. Estimated frequencies for the weak D types 1 and 3 in the Slovenian population were statistically significantly different from the frequencies in the German population ($p<0.05$). The simultaneous application of the said innovative ASPA PCR methods and *RHD* Multiplex PCR provides a way of a faster and more accurate characterisation of the weak D donors.

Poster No: 40

Genetic susceptibility to tuberculosis: study of candidate QTL regions in congenic mice derived from A/Sn and I/St

F. Sánchez[1], A. Apt[2], E. Schurr[3], M. Schalling[1] and C. Lavebratt[1]
[1] Neurogenetics Unit, Department of Molecular Medicine, Karolinska Institute, Stockholm, Sweden
[2] Department of Immunology, Central Institute for Tuberculosis
[3] McGill Center for the Study of Host Resistance, Montreal General Hospital, Montreal, Canada

Genetic variability underlies the susceptibility to develop disease under numerous environmental conditions. Infections have applied strong selective pressure on exposed populations throughout evolution. Malaria, tubercu-

losis and HIV, among many other infections, continue to claim millions of lives every year. Genetic factors play a role in susceptibility to infection as suggested by epidemiological and genetic data. Murine models of infection are one source of such evidence. They have the advantage that the phenotypic and genotypic characterization can be well controlled. Mice from the strains A/Sn and I/St develop disease after intravenous exposure to *Mycobacterium tuberculosis.* However, I/St mice lose weight and dye twice as fast as compared to A/Sn. A wide genome screening in this model indicated that several chromosomes are involved in the expression of such phenotypes. Particularly strong signals were observed on chromosome 9 and 3. These candidate regions are currently being explored in F2 animals and additional crosses. Congenic strains are being established in order to narrow down the size of the regions of interest. Access to new markers and faster genotyping technologies, such as the Pyrosequencing® method, could positively affect our search for tuberculosis susceptibility genes in this model.

Poster No: 41 Short Presentation

Genotyping of single nucleotide polymorphisms by Pyrosequencing™, validation against the 5' nuclease (TaqMan®) assay, and analysis of pools of DNA samples

M. Schalling[1], L. Nordfors[1], M. Jansson[1], G. Sandberg[1], S. Sengul[1], P. Arner[2] and C. Lavebratt[1]
[1] Karolinska Institute at the Neurogenetics Unit, Department of Molecular Medicine, Karolinska Hospital, Stockholm, Sweden
[2] Karolinska Institute at the Department of Medicine and the Research Center, Huddinge University Hospital, Stockholm, Sweden

During the last couple of years, analysis of single nucleotide polymorphisms (SNPs), has become a powerful tool for studying genetic variation. The need to investigate the human genome to decipher the molecular background of multifactorial diseases has accelerated the development of enhanced, high throughput methodologies. As the number of identified and mapped SNPs increase, there is a growing need for robust and effective SNP genotyping technologies to allow screening of large patient materials and populations, for example in mutation analysis and in association or linkage disequilibrium studies. Pyrosequencing™ is a straight-forward sequencing method, based on real-time pyrophosphate detection, which in several aspects may be advantageous compared to already existing methodologies. Here we present a large-scale effort at SNP genotyping using Pyrosequencing™, where six SNPs at a locus on chromosome 10 were genotyped in a large Swedish sample. In addition, the Pyrosequencing™ method and the PSQ96™ Instrument was evaluated in comparison to the 5'nuclease (TaqMan®) assay, by duplicate analysis of 1022 genotypes using both instruments. Identical genotyping results were obtained for both methods. Large scale genotyping would greatly benefit from the possibility to analyze pooled samples. In the present study we have analyzed pooled samples with the PSQ96™ Instrument and evaluated the accuracy in the SNP allele frequencies generated. Genomic DNA from 500 intercross and backcross mice were concentration determined using fluorometry (fluorochrome Hoechst 33258). Individual genomic DNAs were pooled in groups of 10, 20, 50 100, 200 and 500. As a separate experiment, the pooling was instead done after the PCR amplification step preceding the Pyrosequencing™. All individual and pooled samples were analyzed for 2 mouse SNPs. The SNP allele frequencies in pooled samples were compared to the allele frequencies calculated from genotypes of the respective individual samples. We conclude that Pyrosequencing™ is highly efficient and accurate in the analysis of SNPs and represents a promising solution to high throughput genotyping.

Poster No: 42

A cloning strategy for identification of genes containing trinucleotide repeat expansion mutations

M. Schalling[1], Q. P. Yuan[1], K. Lindblad-Toh[1,3], C. Zander[1,2], C. Burgess[1,4] and A. Durr[2]
[1] Neurogenetics Unit, Department of Molecular Medicine, Karolinska Institute, 17176 Stockholm, Sweden
[2] INSERM U289, Hôpital Pitié-Salpętričre, 47 bd de l'Hôpital, 75013 Paris, France
[3] Current address: Whitehead Institute Center for Genome Research, Whitehead Institute, One Kendall Square, Bdlg 300, Cambridge, 02139, MA, USA
[4] Current address: CuraGen Corporation, 555 Long Wharf Drive, New Haven, CT, USA

Until today, nineteen trinucleotide repeat expansions larger than forty repeat copies have been found in the human genome. Of these, the CAG/CTG repeat is predominant motif with twelve loci identified, ten of which have been associated with the development of neurodegenerative diseases. We have developed a cloning approach which isolates disease genes containing trinucleotide repeat expansions. The method is based on size separation of genomic fragments, followed by subcloning and library hybridization with an oligonucleotide probe. Fractions and clones containing expanded repeats are identified by the repeat expansion detection (RED) method throughout the cloning procedure. Large family materials are not required and as little as 10 mg genomic DNA from a single individual is sufficient for this method. Using this strategy we have cloned two DNA fragments containing expanded repeats from two unrelated patients with a clinical diagnosis of cerebellar ataxia. Sequencing of the two fragments showed sequence identities with two disease genes, the Huntingtin gene and the ataxin 3 gene, respectively. The method should be adaptable to the cloning of any long repeat motif in any species. Furthermore the experimental steps can be performed in one-tenth the time of a positional cloning approach, making it very effective and time efficient to disease gene identification.

Poster No: 43

A novel mutation in the *G4.5* gene responsible for Barth syndrome

M. Stopar-Obreza[1], K. Trebušak[1], S. Vesel[2], J. Jazbec[3], T. Battelino[4] and T. Podnar[2]
[1] Centre for Medical Genetics, University Children's Hospital, Ljubljana, Slovenia
[2] Cardiology Unit, University Children's Hospital, Ljubljana, Slovenia
[3] Hematology and Oncology Unit, University Children's Hospital, Ljubljana, Slovenia
[4] Department of Pediatric Endocrinology, Diabetology and Metabolism, University Children's Hospital, Ljubljana, Slovenia

Barth syndrome is a rare X-linked recessive disorder characterized by dilated cardiomyopathy and the variable expression of additional clinical and laboratory manifestations: cyclic neutropenia and organic aciduria, skeletal myopathy and short stature. Affected boys usually present with congestive cardiac failure and/or sepsis in infancy or early childhood. In surviving patients, the later clinical course is usually more benign. The *G4.5* gene, also called the TAZ gene, located in the Xq28 region, has been identified to be responsible for Barth syndrome. It encodes tafazzins, a family of proteins, specifically involved in the remodelling of cardiolipin and phosphatidylglycerol which are the major polyglycerophospholipids in mammalian tissues. Although exact phenotype-genotype correlation has not yet been established, altogether 23 unique mutations of the *G4.5* gene have been found in different pedigrees so far. Variable phenotype, possible episodic appearance of crucial diagnostic findings and the usually favourable prognosis in patients surviving infancy emphasize the need for a reliable diagnostic method.

In the present study, an eleven-year-old boy, with a clinical picture, personal and family history consistent with Barth syndrome, and his family (mother, mother's uncle, both aunts and grandmother) were analysed. A detailed pedigree of the family was constructed. Genomic DNA from peripheral blood leukocytes of the patient and his relatives was used for polymerase chain reaction (PCR). The exon regions and exon/intron boundaries of *G4.5* gene were PCR amplified in six fragments. PCR products were purified and directly sequenced for mutation detection using the same primers as for PCR. Obtained sequences were compared to the *G4.5* gene normal sequence (GeneBank Accession Numbers X92763 and X92764).

Inheritance in the presented pedigree was consistent with an X-linked recessive pattern. A novel mutation in exon 6 of the *G4.5* gene was identified. In the patient a hemizygous deletion of one base pair in exon 6 was detected. This so far unreported deletion of a C nucleotide in codon 179 caused a frame shift and introduced a stop codon four codons downstream the mutant one. Thus the last five exons of *G4.5* gene were not translated at all. The patient's mother and grandmother were confirmed to be heterozygous for this particular mutation. All other investigated relatives had normal sequence of *G4.5* gene on both alleles. Except those, that due to the alternative splicing normally lack exon 7, the majority of defective gene protein products were severely truncated because of this mutation. The great portion of the high conserved exposed hydrophilic domain believed to act as an exposed loop interacting with other proteins was lacking. The high conserved character of this protein domain suggests that it is a vital functional part of the protein. As seen also with other mutations in this region, changes of this domain result in clear (and mostly severe) clinical picture of Barth syndrome. Thus, the novel mutation identified in the *G4.5* gene of the investigated kindred was the likely cause of the clinical manifestations. According to the nature of the disease the analysis of the *G4.5* gene is currently not only the most reliable way of confirming the diagnosis of Barth syndrome, but it also identifies female carriers and therefore allows exact genetic counselling. In addition, precise analysis of the gene mutation offers an opportunity for prenatal diagnosis in fetuses at risk and may offer an immense relief for the family.

Poster No: 44

Developement of a bi-directional allele-specific amplification system for genotyping the platelet glycoprotein IIIa A1/A2 polymorphism

M. Szőke, C. Jeney, L. Hernold and Z. Holló
H-Med Diagnostic and Research Laboratory, 1134 Budapest, Róbert Károly krt. 44

The presence of an intact fibrinogen receptor is a prerequisite for platelet-aggregation, which is a crucial step of the coagulation cascade. This receptor is composed of two glycoproteins: GPIIb, and GPIIIa. Point-mutations of the gene encoding the receptor-proteins result in disorders of platelet-binding, attesting the role of detecting these mutations. Certain mutations appear to be independent risk-factors for vascular thrombosis.

We investigated the T196C polymorphism of GPIIIa (gene localisation: 17q21.32), resulting in a leucine to proline substitution at position 33 from the NH2 terminus of the protein, by different genotyping methods.

The most prevalent method for identifying point-mutations is PCR-RFLP. In our system the 106bp PCR product is digested by MspI. The normal allele is not cut by the enzyme, however, the mutation creates a restriction site for this enzyme, and the result of digestion is a 39 bp and a 67 bp fragment, respectively. In case of PCR-RFLP, if the enzyme does not digest the given product for any reason, or the digestion is too sensitive and uncalled-for, the result can be misinterpreted.

To eliminate this source of misinterpretation, we developed another method: bi-directional allele-specific amplification. The major advantage of bi-directional ASA over traditional ASA is the single-tube genotyping of the two different alleles. The two PCR products can be easily separated by agarose gel-electrophoresis. In this system the allele-discrimination is achieved by a size difference between the PCR fragments corresponding to the normal and the mutant allele. In our system the normal allele-specific product is 152 bp, and the mutant one has a length of 97 bp. A 212 bp control PCR-fragment is also formed in the reaction, proving the succes of the reaction. This control fragment is formed between the two external primers, the ones opposite to the allele-specific primers starting from the mutation's point.

In certain cases the allele-specific primer can be elongated even for the mismatching allele, or the priming efficiency of the two allele-specific primers might be different.

To adjust the optimal high-stringency conditions for bi-directional ASA, we compared the method with the PCR-RFLP used before.

To investigate the efficiency of the formation of the allele-specific products, and for automated genotyping purposes the primers of bi-directional ASA were fluorescently labeled by FAM and the products were separated by fluorescent capillary gel electrophoresis. This way the efficiency of the formation of different products can be compared as well. Since this high-resolution separation method can discriminate 2-3 bp length-differences, the size-difference between the two allele-specific products can be significantly decreased, eliminating another factor which might negatively influence the efficiency of the formation of PCR-products.

Poster No: 45

Application of Non-Isotopic Single-Strand Conformation Analysis (SSCA) for mutations detection in *COL4A5* gene

M. Šlajpah, M. Ravnik-Glavač and D. Glavač

Laboratory of Molecular Genetics, Institute of Pathology, Medical faculty, Ljubljana, Slovenia.

We used non-isotopic high resolution conformation analysis technique, a combination of non-radioactive SSCP, HA and DSCA, for detection of sequence variation in DNA fragment which under controlled and optimized electrophoretical conditions enables to detect: single-stranded conformations (SSC), double stranded conformations (DSC) and/or heteroduplexes (HDX) for mutational analysis of *COL4A5* gene. We used thin gel matrix fixed on one of the glass plate and very sensitive silver staining protocol for DNA visualization that makes it possible to load less PCR product and improves the resolution of single-stranded and double-stranded DNA. Selection of an appropriate electrophoretical condition, temperature control and variation of concentration and crosslinking of acrylamide enables practically every DNA conformation formed due to mutation in the PCR fragment to be detected. In a silver staining protocol gel was fixed for 10 min. in 20% Trichloro-acetic acid at 55°C, followed by three consecutive washings with 50% EtOH, 10% AcOH and finally 10% EtOH for two min. and 10 min. at 55°C, respectively. Next, gel was pretreated with 6% glutaraldehyde for 6 min at 55°C and than washed in three consecutive washings, in first two with 10% EtOH, 5% AcOH for 3 min at 55°C and finally with water for two min at 55°C. Than the gel was impregnated with 0.5% $AgNO_3$ for 10 min at 55°C. After washings two times with distilled water, development was achieved with 2.5% Na_2CO_3, 0.4%HCHO in two steps. First for 0.5 min, following by additional 3 to 5 min with fresh developer. Development was stopped with 5% AcOH for 2 min, and to reduce background, gels were additionally washed in 50 nmol EDTA (pH 8.0) before final washing with distilled water. Non-isotopic SSCA was used for systematic mutation screening of all 51 exons with boundary intronic sequences of *COL4A5* gene in 16 Alport syndrome suspected families and in 124 keratoconus patients in Slovene population. PCR-SSCA of the *COL4A5* gene of eight Alport families revealed the presence of an aberrant band. In all samples mutations were identified by cycle sequencing. Among them six were novel and not previously described. In keratoconus patients several polymorphisms were identified.

Poster No: 46

Evaluation of base excision sequence scanning

C. Taylor[1], S. Bass[1], R. Charlton[2], D. Cuthbert-Heavens[1] and G.R. Taylor[1,2]

[1]ICRF Mutation Detection Facility, [2] Regional DNA Laboratory, Ashley Wing, St. James's University Hospital, Leeds LS9 7TF, UK

Base excision sequence scanning T- track (BESS-T) utilises cleavage of DNA molecules at modified T residues to detect mutations. In principle, BESS-T allows detection of all insertion or deletion mutations and any substitution involving a T residue on either strand. Importantly, it also allows localisation of the mutation within the fragment and absolute identification in many cases. However, although the technique has been available commercially for several years, it has not been widely used.

We have updated BESS-T to make use of fluorescent labelling technologies and have evaluated BESS-T for use in both research and diagnostic mutation detection environments. We conclude that while F-BESS-T is simple and effective, it is not a suitable technique for routine mutation scanning. It is, however, of value for sequence-specific confirmation of mutations.

Poster No: 47 Short Presentation

Fluorescent multiplex PCR: a powerful and simple method for the detection of genomic rearrangements

M. Tosi, G. Raux, F. Charbonnier, C. Di Rocco, C. Duponchel and T. Frebourg

INSERM EMI 9906, Faculté de Médecine et Pharmacie, Université de Rouen, France

The rapid and faithful detection of heterozygous exon deletions or duplications or complete gene deletions represents a challenge in several areas of medical genetics. In spite of the variety of traditional and of recent techniques that can be used (based on various formats of hybridisation or on PCR with either real time or end-point quantitation) each method has advantages and disadvantages. We have previously described a method designated Multiplex Polymerase Chain Reaction of Short Fluorescent Fragments, which provides a rapid semi-quantitative analysis based on the comparison of fluorence profiles of multiplexes obtained from different samples. This method is able to scan rapidly large genes like the Mismatch Repair (MMR) genes involved in hereditary non polyposis colorectal cancer (HNPCC) and allowed us to detect new exon deletions and the first case of partial duplication of *MSH2* (Charbonnier et al., Cancer Res. 2000, 60:2760-3). The fluorescent multiplex PCR method has also been used in our laboratory for the detection of exon deletions

at other loci, e.g. *BRCA1*, *C1NH* and *SMN1* (P Saugier-Veber, 2001, J Med. Genet., in press). The simplicity and rapidity of the method allows the analysis of large numbers of samples. We recently applied this method to a series of 63 HNPCC families, either fulfilling Amsterdam criteria or including multiple primary cancers belonging to the HNPCC spectrum, without point mutations in *MSH2* and *MHL1*. Eleven distinct rearrangements were found in the *MSH2* gene in 14 of these families (22%), only 2 deletions were found in *MHL1* and none in *MSH6*. These results indicate that screening of the *MSH2* gene by this method should be considered as an early step in the molecular diagnosis of HNPCC families. We have also evaluated a number of parameters that affect the reproducibility of multiplex PCR, particularly protocols for DNA extraction and modified PCR conditions. We have also optimised criteria for the design of primers in order to guide the construction of multiplexes containing 8 to 12 amplicons in the size range of 100 to 300 bp. Use of universal 5'extensions of 10 nt, i.e. shorter than those that we have described for multiplexes covering a wider size-range (Duponchel et al. Hum Mutat. 2001, 17:61-70) and use of modified PCR conditions yielded reproducible profiles with homogenous peak height. These optimisations resulted in a protocol that is applicable for gene dosage at large numbers of loci in genomic stretches larger than one megabase.

Poster No: 48

Mutational analyses of nine patients with Autoimmune Polyglandular Syndrome type 1 (APS-1)

K. Trebušak, B. Repič-Lampret, C. Kržišnik and T. Battelino
University Children's Hospital, Ljubljana, Slovenia

Autoimmune polyglandular syndrome type 1 (APS-1) is a rare autosomal recessive disease. It is characterized by presence of two of the three major clinical symptoms: hypoparathyroidism, Addison's disease and mucoutaneous candidiasis. Several other disorders may be present, such as ectodermal dystrophy, gonadal failure, IDDM, hypothyroidism. The disease usually occurs in childhood, but new tissue-specific symptoms may appear throughout life. Although APS-1 cases are reported worldwide, the incidence of the disease is higher in some genetically isolated populations (Finland 1/25000, Iranian Jews 1/9000, Sardinia 1/14400). The aethiology of APS-1 is associated with mutations in the *AIRE* (autoimmune regulator) gene encoding protein that contains motifs suggesting a role as a transcriptional regulator. We present mutational analyses of the *AIRE* gene in nine APS patients from eight families, their parents and healthy siblings from five families. The aim of this study was to confirm the diagnosis of the APS-1 patients, to determine whether healthy brothers and sisters of the patients will get APS-1 and to determine phenotype-genotype correlation in APS-1 patients.

Genomic DNA was extracted from peripheral blood lymphocytes by salting out procedure. All exons and the exon/intron boundaries of the *AIRE* gene were individually PCR amplified and directly sequenced. Nucleotide sequences were compared to the sequence of the normal gene (GeneBank Access No. AB006684). Where mutation eliminates *Taq*1 restriction-enzyme site, *Taq*1 restriction digestion and RFLP (restriction fragment length polymorphism) analyses was performed.

Six of nine patients were homozygous for nucleotide substitution C→T at codon 257 in exon 6 , that changes an arginine codon (CGA) to a stop codon (TGA). The mutation R257X results in 288 amino acids shorter and probably unfunctional protein AIRE. Parents and four analyzed siblings of two patients were heterozygous for R257X. One patient was found to be compound heterozygous for R257X and duplication of 23 bp in exon 1 between nucleotides 156 and 179 of AIRE cDNA (Acc.No. AB006682). This duplication is a novel mutation and changes the reading frame causing premature truncation at the beginning of the coding region.

Six of nine patients are homozygous for mutation R257X, all parents and 4 siblings from two families are heterozygous. Since siblings are heterozygous, they will not get APS-1. One patient is compound heterozygote for R257 and novel 156-179ins23bp. Two of nine analyses patients do not have mutation in coding region and intron/exon boundaries, therefore their diagnosis was not confirmed. Mutations may exist in promoter region or in intron regions that were not analyzed. Although six patients have the same mutation the clinical presentation varies markedly from patient to patient, even between two patients that are brothers. No phenotype-genotype correlation was found. Other genetic and/or environmental factors must influence the phenotype and disease progression of individual APS-1 patient.

Poster No: 49 Short Presentation

EURAY: A platform for high-throughput multiplex SNP analysis

H. Vissing, A.T. Nielsen, M Nř rholm, P. Mouritzen, P. E. Hř jby, S. Petersen, Y. Choleva and L. Kongsbak
Euray, Exiqon A/S, Bygstubben 9, DK-2950 Vedbaek, Denmark

We have developed a new approach for nucleic acid detection by taking advantage of three novel technologies: 1) LNA (Locked Nucleic Acids) 2) anthraquinones for photoactivated cross-linking and 3) a new polymer microarray platform. LNA, a bicyclic DNA analogue, has proven very useful due to the exceptional high specificity and affinity in hybridizations (1). Short chimeras of LNA and DNA with the length of 10-12 nucleotides allow discrimination of single base mutations in microarray based hybridizations. Furthermore, the anthraquinone phosphoramidites (2) facilitate an efficient and robust polarised coupling of the capture probes or target to our polymer platform. The polymer platform supports high quality hybridisation spots with a diameter of approximately 50 mm and homogeneous distribution and immobilisation of the capture probe within the spot. We have applied these technologies in the design of microarrays for multiplex SNP detection as well as high through-put patient SNP analysis. We will demonstrate a microarray targeting more than 70 SNP's which corresponds to more than 140 different capture probes monitoring polymorphism within various diseases. An advanced software package integrating microarray design, microarray database and a neural network has been developed to optimise the design of new microarrays. The microarray platform is currently

being adapted to a microfluidics device supporting automated high throughput genotyping.

References

1. Jesper Wengel, Acc. Chem. Res., 1999, 32, 301-310,
2. Troels Koch et al., Bioconjugate Chem, 2000, 11, 474-483

Poster No: 50 Short Presentation

Evaluation of PCR enzyme related parameters affecting Denaturing High Performance Liquid Chromatography

J. Walter, H. Lamb, C. Griffiths, K. Hecker, M. Robinson, P. Krause, M. Daniels, D. Gjerde and P. Taylor
Transgenomic Ltd. The Quadrangle, Crewe Hall, Weston Road, Crewe, Cheshire, CW1 6UZ, UK

Denaturing High Performance Liquid Chromatography (DHPLC) has gained significant popularity as a method for indetifying mutations. Routinely PCR from genomic DNA samples followed by heteroduplex formation in the presence of a mutation enables resolution of homoduplex and heteroduplex species using Ion Paired Reverse Phase HPLC and DNASep® Cartridge technology (Transgenomic Inc, Ca). A variety of PCR ezymes have been used in conjunction with the DHPLC application to successfully locate both unknown mutations and develop diagnostic genotyping applications. The same principle enabling the discovery of sequence changes in DNA using the DNASep® Cartridge technology and the WAVE® Nucleic Acid Fragment Analysis System allows analysis of the differing PCR enzyme related parameters affecting the quality of DHPLC results. Primarily the application specific PCR enzyme misincorporation rate can be compared between enzymes as individual misincorporation events throughout the PCR amplification process will be present during subsequent DHPLC sample analysis as a random low level heteroduplex the level of which can be comparatively quantified. Secondarily the high throughput nature for DHPLC related applications (SNP mapping and diagnostic genotyping for example) requires high level of reproducibility which can be enhanced by PCR component compatibility. One of the major contributors to sample to sample variability lies in the compatibility of PCR buffer constituents in relation to the DNASep® matrix. These two main factors affecting DHPLC related methodologies are examined here for a variety of routinely used PCR enzyme preparations.

Poster No: 51

High-throughput mutation screening of candidate tumour suppressor genes in prostate cancer

B. Weber, C. Pilarsky, C. Wissman, S. Kaiser, G. Kristiansen, B. Hinzmann and A. Rosenthal
metaGen, Gesellschaft für Genomforschung mbH, Ihnestrasse 63, 14 195 Berlin, Germany

The rapidly expanding public and proprietary databases represent a valuable source for the *in silico* identification of suitable targets for cancer therapy and diagnosis. Analysing four million ESTs, we identified 759 transcripts which are highly differentially expressed in human tumours of breast, ovary, endometrium, prostate, bladder and pancreas.

Software tools for the efficient and systematic analysis of EST libraries were developed in house (Schmitt, A. et al. 1999, Nucl. Acids Res. 27, 4251-4260). The analysis started with a single seed EST and built a contig of maximal length by iterative sequence comparison and extension. Contigs, representing putative genes, were then analysed by "electronic Northern blot" (eNorthern) that compared the sequences of the assembled cDNA contigs against pooled EST libraries from 16 different tissue pairs (normal and tumour). Using this approach we were able to detect both tissue specific expression and differential expression in tumours. Of the 759 contigs showing a statistically significant differential expression, 218 were downregulated in tumour tissue representing a source for the identification of tumour suppressor genes.

Microdissected tissue samples of prostate tumours at various stages were used for RNA expression profiling on a custom Affimetrix chip representing the candidate genes with differential expression as seen by the bioinformatic approach. Genes, whose downregulation in cell lines or tumour tissue could be confirmed in these hybridisation experiments are evaluated by high-throughput mutation analysis.

Sample DNA is amplified in fragments of up to 300bp length, representing exons plus the flanking intronic regions, post-PCR labelled with R6G nucleotides and subjected to fluorescent SSCP. Usually multiplexed samples are analysed at three different gel temperatures to maximise the detection rate. Thus far we screened seven genes in up to ninety samples of prostate tumours. Although a variety of polymorphisms were identified, no mutations were detected. Since downregulation of expression is not necessarily due to loss of the allele or mutations, we are currently establishing assays to test the promoter regions of these genes for hypermethylation.

Poster No: 52 Short Presentation

High-throughput SNP scoring with GAMMArrays: Genomic Analysis Using Multiplexed Microsphere Arrays.

P. S. White[1], H. Cai[1], D. C. Torney[2] and J. P. Nolan[1]
[1] Life Sciences Division and [2] Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM 87545 USA

We have developed a platform for the discovery and scoring of SNPs that is capable of meeting greatly increasing demands for high throughput and low cost assays. Called GAMMArrays, or Genomic Analysis using Multiplexed Microsphere Arrays, the basic platform consists of fluorescently labeled DNA fragments bound to microspheres, which are analyzed using flow cytometry. The platform provides no-wash assays that can be analyzed in less than 1 minute per sample, with sensitivities far superior to other approaches. SNP scoring is performed using minisequencing primers and fluorescently labeled dideoxynucleotide terminators. Furthermore, by using commercially available sets of multiplexed microspheres it is possible to score dozens to hundreds of SNPs simultaneously. Multiplexing, when coupled with the high throughput rates possible with this platform makes it

possible to score several million SNPs per day at costs that are a fraction of competing technologies.

GAMMArrays are enhanced by the use of universal oligonucleotide tags. These tags consist of carefully designed, unique DNA tails, or capture tags, incorporated into each minisequencing primer, that are complementary to an address tag attached to a discrete population of microspheres in a multiplexed set. This enables simultaneous minisequencing of large numbers of SNPs in solution, followed by capture onto the appropriate microsphere for multiplexed analysis by flow cytometry.

We present results from multiplexed SNP analyses of bacterial pathogens, and human mitochondrial DNA and HLA genetic variation. These analyses are performed on a small number of relatively large PCR amplicons, each containing numerous SNPs that are scored simultaneously. In addition, these assays are easily integrated into conventional liquid handling automation systems, and require no unique instrumentation for setup and analysis. Very high signal-to noise ratios, ease of setup, flexibility in format and scale, as well as low cost of these assays make them highly versatile and extremely valuable tools for a wide variety of studies where SNP scoring is needed.

Poster No: 53

MAPH detection of deletions/duplications in Duchenne/Becker muscular dystrophy: An alternative to quantitative Southern blotting

S. White, M. Kalf, M. Villerius, M. Breuning, G. J. van Ommen and J. den Dunnen
Department of Human and Clinical Genetics, Leiden University Medical Center, Wassenaarseweg 72, 2333AL Leiden, Nederland

Currently most genetic diagnostic protocols are PCR-based and do not readily yield quantitative data. As a consequence, potential deletions and duplications of the regions analyzed go undetected unless specific methods are applied. Southern Blotting is the most commonly used method but is time consuming and laborious. Recently an alternative method was published, called Multiplex Amplifiable Probe Hybridization (MAPH). In this technique a series of short DNA fragments are individually cloned in such a way that all can be PCR-amplified using one pair of primers. These probes are hybridized to genomic DNA immobilized on nylon filters, and after stringent washing the probes are recovered off the filters and PCR- amplified in a quantitative manner.

We have applied this technique to the diagnosis of Duchenne/Becker Muscular Dystrophy (DMD/BMD), diseases caused by mutations in the dystrophin gene. This gene is the largest known, covering 2.4 megabases and containing 79 exons. In approximately 65% of cases the mutation is known to be a deletion or duplication of one or more of the exons. Frame-shift mutations cause the lethal DMD, whereas maintenance of the reading frame leads to the less severe BMD. For this reason it is important to know the exact boundaries of the rearrangements, a potentially arduous task. A technique allowing simultaneous analysis of all exons would greatly simplify this procedure.

We cloned all 79 exons individually into the same vector and divided the resultant PCR products into 2 pools. Following hybridization the secondary PCR amplification was performed using a fluorescently labeled primer, allowing the products to be analyzed on a 96 capillary sequencer. This allows parallel analysis of 96 samples in ~48 hours. The number of copies of each exon could be determined by comparing the appropriate peaks between controls and patients. Using this technique we were able to detect exon deletions or duplications missed using current methods.

The excellent results and simplicity of analysis prompted us to investigate automation of the technique in both the hybridization and subsequent data analysis. Further probe sets are being developed to cover other areas of interest such as breast cancer and microdeletion syndromes, and the possibility of analyzing many more regions simultaneously using microarrays is being investigated.

Poster No: 54

Population structure and evolution trends in dalmatians and istrian shorthaired hounds as revealed by polymorphic microsatellites

I. Zajc[1] and M. Kus[2]
[1] National Institute of Biology, Večna pot 111, Ljubljana, Slovenia
[2] Independent Researcher, Hrastje 105, Kranj, Slovenia

We have examined the population structure of Dalmatians and Istrian shorthaired hounds and estimated their phylogenetic relationships to some other breeds. Despite the numerous efforts, the origin of Dalmatians that is still not unequivocally resolved. Istrian shorthaired hounds were chosen for a comparative breed, because they represent an ingenious, widespread hound's breed in Slovenia and proved to be the closest relatives of Dalmatians in our pilot study. In this project, canine specific microsatellites were amplified by polymerase chain reaction, analyzed by S-PLUS computer program and the populations evaluated by hierarchical clustering method with complete linkage. Their evolutionary relationship were estimated from pairwise genetic distances.

The population tree of Dalmatians represents the country of origin of individual dogs well, since the microsatellite alleles shared among individuals correspond to geographical distribution of their ancestors. This was not the case with Istrian shorthaired hounds. Their highly inbred population is small in number and geographically limited to Slovenia, what also explains much lower within-breed variability compared to Dalmatians. Genetic distance among Dalmatians, Istrian shorthaired hounds and Greyhounds is relatively low, in contrast to the distance between these three breeds and German shepherds. Our results indicated that the "shepherd" lineage and the "hound" lineage separated at a very early stage of canine domestication, whereas the hound lineage gave rise to the separate breeds of Greyhounds, Istrian shorthaired hounds and Dalmatians much more recently. Although Dalmatians have been long used as companion dogs and watch dogs, their hound origin is still obvious from their surprisingly strong hunting instinct.

The "real" phylogenetic relationships might differ slightly from these that are based on relatively short pieces of genome, but the present research should contribute to the better knowledge of Dalmatian and Istrian shorthaired

hound populations and their evolutionary relationships with some other breeds.

Poster No: 55 Short Presentation

Nucleic acid crosslinking probes for mutations screening

J. Zschocke[1], R. Peoples[2], R.B. Vanatta[2] and M.L. Wood[2]
[1] Institute of Human Genetics, Heidelberg University, Germany
[2] NAXCOR, Menlo Park, California. USA

We report on a novel, non-enzymatic method for mutation detection developed by NAXCOR, Menlo Park, USA. The technology is based on irreversible photo-crosslinking of oligonucleotide probes, containing a photoactive coumarin agent, to target DNA. High sensitivity and specificity is achieved through the use of wild-type or mutation specific biotinylated capture probes in combination with several hapten-modified reporter probes complementary to adjacent DNA sequences. Briefly, DNA is incubated with the reporter probes and one of the specific capture probes and exposed to near-UV irradiation at a controlled temperature. The crosslinked probe-target complex is then captured on streptavidin-coated magnetic beads, and unhybridised probes are removed through stringent washing. The probe-target complex is visualised by addition of an antibody-enzyme conjugate which, after removal of unbound conjugate, is used to develop the fluorescent signal of an enzyme substrate.

The proprietary crosslinker-containing probes are prepared using standard automated DNA synthesis methods. They are stable, require no special handling, are cost-effective, and perform with high efficiency. Crosslink formation occurs specifically within hybridized probe/target duplexes. Comparison of the crosslinking reaction for fully complementary probe/target hybrids with those having a single mismatch reveals a greater than 10-fold difference in the yield of crosslinked product. The method does not require PCR amplification of target DNA and has been successfully applied to the detection of the haemochromatosis mutations C282Y and H63D and the factor V Leiden mutation. Further work is required to assess the possible impact of other DNA variants in the target region on the reliability of the assay, and to develop multiplex assays for the parallel detection of different alleles or mutations in the same sample well.

Poster No: 56

The MegaSNPatron: An Ultra-High Throughput Genotyping Platform

R. B. Derbyshire, C. A. Gelfand, M. Donaldson, J. Tarantino, M. Pohl, G. Schnerr, and M. Boyce-Jacino
Orchid BioSciences, Inc. 303 College Road East, Princeton, NJ 08540

The availability of the completed human genome and the availability of more than 1,000,000 SNP's in the public domain has fuelled the demands for rapid, low-cost and flexible methods for single nucleotide polymorphism (SNP) genotyping. There is an emerging opportunity to understand how drug response, adverse drug effects, and genetic predisposition to disease of individual patients can be correlated to specific SNPs on a genome-wide scale. Finding these correlations requires that an ever increasing number of SNP's be analysed against a very large number of patient samples, and has driven a requirement toward vast genotyping capacity in order to make these key pharmacogenetic correlations. Orchid has been a leader in high throughput genotyping with its SNPstream 25K, a robotic platform performing Orchid's SNP-IT primer extension biochemistry. The next generation product, internally referred to as the "MegaSNPatron," is designed as a series of modules, each capable of routine 100K SNP/day operation with the ability to perform at higher throughputs.

To achieve the ultra-high throughputs of the MegaSNPatron, the core primer extension genotyping biochemistry, SNP-IT, has been adapted to a novel fluidics platform device, which draws from the inherent scalability and automatability of the standard microtitre plate format. Direct fluorescence read-out in a novel array-reading detection system, and PCR and genotyping multiplexing support in an array-based platform maintain the processing flexibility features of the SNPstream system while enabling significant per analysis reagent reduction.

Results of multiplexed PCR and multiplexed SNP genotyping on this and other array platforms will be presented.

Department of Genetics, University of Melbourne
Parkville, Victoria, Australia

April 5, 2001

The XIX International Congress of Genetics will be held in Melbourne Australia from July 6 to 12, 2003. The attached briefing document provides a detailed overview of our vision for the Congress. One critical element of this vision is that the extremely broad Congress program should be complemented by workshops and satellite meetings that would provide an in depth coverage of research in key areas of Genetics. Given the completion/impending completion of many genome sequences, no area could be considered more important than 'Mutation Detection'. It would therefore seem logical that a satellite 'Mutation Detection' meeting be held in conjunction with the Congress. I am keenly aware of the quality of the 'HUGO International Symposia on Mutations in the Human Genome'. Rather than 'reinventing the wheel' and trying to develop our own mutation detection satellite meeting, a far better alternative would be to bid to hold the HUGO meeting in Australia, just before or after the Congress. I detail this proposal below.

I would propose that the HUGO meeting be staged in Cairns, Australia on the Great Barrier Reef. Aside from the extraordinary natural beauty of the tropical rain forests and the reef, Cairns offers a wide range of superb conference facilities. There are other benefits linked with this proposal:

1. The International Congress of Genetics 2003 (ICG2003) has engaged the services of Australia's best professional conference organizers, 'The Meeting Planners'. We are therefore able to offer as much or as little management backup as is required. By 'management' I refer to booking of venues, receipt of registrations, bookkeeping etc. Of course, you may choose to run all or part of this through the HUGO office.
2. ICG2003 has a multi-layered marketing plan that involves providing detailed information to all known Genetics and Molecular Societies in the world for dissemination to their members and direct contact with individuals through our web site, mailouts/emailouts and promotions at major Genetics conferences. As a satellite of ICG2003, the HUGO Symposium would be promoted through all of these channels. Of course we would value reciprocal marketing support.
3. ICG2003 is devoting an enormous amount of work into gaining sponsorship support. A coordinated approach to sponsors by ICG2003 and the HUGO meeting should provide greater leverage with sponsors.
4. It is certain that there will be an ICG2003 symposium or symposia that would incorporate Mutation Detection. I can therefore pledge that we would be willing to pay return international airfares to Cairns and between Cairns and Melbourne for at least three speakers who would address the HUGO meeting and ICG2003. While the Program Committee of ICG2003 will want to be involved in the selection of these speakers, I doubt that it will be difficult to reach agreement on this. Dick, it is my hope that you will agree to be on the Program Committee of ICG2003. Your involvement in both meetings would facilitate the best possible coordination.

In making this proposal I am certain that both meetings could benefit through cooperation. I am hopeful that if the proposal is adopted, many HUGO meeting registrants would also choose to register for ICG2003. I realize that in order for this to happen the ICG2003 program will need to have a significant amount of human genetics content. This will certainly be the case. The Human Genetics Society of Australasia have been actively involved in the planning of ICG2003. More importantly, the National and International Program Committees will both have many human geneticists as part of their membership.

I wish you every success with the meeting in Bled, Slovenia. I hope that the 2003 'International Symposium on Mutations in the Human Genome' will be held in Cairns, Australia.

Dr. Philip Batterham
Secretary General
XIX International Congress of Genetics

Author index

List of participants

A

Habibul Ahsan
Columbia University,
Division of Epidemiology
PH-18, 622 W 168th Street
New York 10032, USA
HA37@columbia.edu

Seyed Mohammed Akrami
Institute of Genetics, Queen's Medical Centre,
Universitiy of Nottingham
NG7 2UH Nottingham, UK
mrxsma@nottingham.ac.uk

Jean Amos
Specialty Laboratories Inc.
2211 Michigan Avenue
Santa Monica, CA 90404, USA
Jamos@specialtylabs.com

Allan Asp
Pyrosequencing AB
Vallongatan 1
Uppsala, Sweden
allan.asp@pyrosequencing.com

Nili Avidan
Weizmann Institute
Herzel ST.
Rehovot 76100, Israel
nili.avidan@weizmann.ac.il

B

Joan Bentzen
Copenhagen University Hospital Hvidovre
Department of Clinical Biochemistry
Kettegaard alle 30
DK-2650 Hvidovre, Denmark
joan.bentzen@hh.hosp.dk

Ergul Berber
Department of Molecular Biology and Genetics,
Bogazici University
80815 Bebek-Istanbul, Turkey
berbere@boun.edu.tr

Hiltrud Brauch
Dr Margarete-Bosch-Institute of Clinical Pharmacology
Auerbachstrasse 112
Stuttgart, Germany
hiltrud.brauch@ikp-stuttgart.de

Teresa Bromidge
Dept. of Haematology,
Leukaemia Research Unit,Taunton and Somerset NHS Trust
Musgrove Park Hospital Taunton, UK
teresa.bromidge@tauntonsom-tr.swest.nhs.uk

Chinh Bui
Genomic Disorders Research Centre,
7th Floor, Daly Wing, 41 Victoria Parade Fitzroy
Melbourne, Victoria 3065, Australia
chinhbui@mail.medstv.unimelb.edu.au

Maria Bullido
Centro de Biologia Molecular Severo Ochoa (CSIC-UAM),
Faculdad de Ciencias, Campus de Cantoblanco
28049 Madrid, Spain
mjbullido@cbm.uam.es

C

Etienne Calvet
Genset Oligos
1 rue Robert & Sonia Delaunay
75545 Paris Cedex 11, France

Jing Cheng
Tsinghuy University, Biochip R&D Centre
10084 Beijing, China
jcheng@tsinghua.edu.cn

Richard Cotton
Mutation Research Centre, St Vincents Hospital
7th Floor Daly Wing, 41 Victoria Parade
Fitzroy 3065, VIC 3065, Australia
cotton@ariel.ucs.unimelb.edu.au

Darren Cuthbert-Heavens
Imperial Cancer Research Fund, Mutation Detection Facility,
St James's University Hospital
Beckett Street, Leeds, LS9 7TF, UK
d.cuthbert-heavens@icrf.icnet.uk

D

Michael Daniels
Transgenomic Ltd.
The Quadrangle, Crewe Hall, Weston Road, Crewe
Cheshire, CW1 6UZ, UK
mdaniels@transgenomic.co.uk

Michael Dean
National cancer Institute, NCI-Frederick
MD 21702-1201, USA
dean@ncifcrf.gov

Johan den Dunnen
Human and Clinical Genetics,
Leiden University Ledical Center
Leiden, NL
ddunnen@lumc.nl

Vita Dolžan
Institute of Biochemistry
Faculty of Medicine
Vrazov trg 2, 1000 Ljubljana, Slovenia
dolzan@ibmi.mf.uni-lj.si

Peter Dovč
University of Ljubljana
Zootechnical Dept., Biotechnical Faculty,
Groblje 3, 1230 Domzale, Slovenia
peter.dovc@bfro.uni-lj.si

Tadeja Dovč
Blood Transfusion Centre of Slovenia
Slajmerjeva 6, Ljubljana, Slovenia
tadeja.dovc@mf.uni-lj.si

G

Arupa Ganguly
University of Pensylvania
School of Medicine, Department of Genetics
415 Curie Boulevard, CRB 450

Philadelphia, PA 19104, USA
ganguly@mail.med.upenn.edu

Anna Gareeva
Institute of Biochemistry and Genetics of UFA Scientific Center
Russian Academy of Sciences, Laboratory of Human Molecular
Genetics, K. Marx srt. 6, Ufa, Russia
ganna@anrb.ru

Aleš Gasparič
KRKA Novo mesto
Šmarješka c. 6
8000 Novo mesto, Slovenia
ales.gasparic@mf.uni-lj.si

Eric Gerber
Varian Inc.
2700 Mitchell Dr, Walnut Creek
CA 94598, USA
eric.gerber@varianinc.com

Damjan Glavač
Laboratory of Molecular Genetics,
Medical Faculty Ljubljana, Institute of Pathology
Korytkova 2, 1000 Ljubljana, Slovenia
damjan.glavac@mf.uni-lj.si

Peter Green
GKT School of Medicine
London, UK
peter.green@kcl.ac.uk

Ivo Glynne Gut
Centre National de Genotypage
2 Rue Gaston Cremieux
Evry Cedex 91057, France
ivogut@cng.fr

H

Peter Halkjaer-Knudsen
Nanogen bv
20 Johannevej
2970Horsholm, Denmark
phalkjaer@Nanogen.com

Ross Hawkins
Incyte Genomics Ltd
214 Science Park, Milton Rd
Cambridge, UK
ross.hawkins@incyte.com

Kenshi Hayashi
Division of Genome Analysis, Institute of Genetic Information
Kyushu University, Maidashi 3-1-1, Higashi-ku
Fukuoka 812-8582, Japan
khayashi@gen.kyushu-u.ac.jp

Michael Heller
Nanogen
10398 Pacific Center CT
San Diego, CA 92121, USA
mheller@nanogen.com

Simon Horvat
University of Ljubljana,
Zootechnical Dept., Biotechnical Faculty
Groblje 3, 1230 Domžale, Slovenia
simon.horvat@bfro.uni-lj.si

J

James Jacobson
LUMINEX Corporation
12212Technology Blvd
Austin, TX 78727; USA
jjacobson@luminexcorp.com

Marten Jansson
Department of Molecular Medicine, Karolinska Hospital
CMM L8:00
17176 Stockholm, Sweden
marten.jansson@cmm.ki.se

Jeanette Johansen
Department of Molecular Medicine, Karolinska Hospital
CMM L8:00
17176 Stockholm, Sweden
jeanette.johansen@cmm.ki.se

Hilde Johnsen
Department of Genetics,
Norwegian Radium Hospital, Norway
hilde.johnsen@labmed.uio.no

Dirk Juergensen
Transgenomic
Furstendamm 5
Heidelberg, Germany
djuergensen@transgenomic.co.uk

K

Alexander Karpukhin
Research Centre for Medical Genetics
Russian Academy of Medical Sciences
ul. Moskorechie 1
Moscow 115478,
socrates@orc.ru

Andrzej Kilian
CAMBIA
Clunies Ross Street
Canberra, ACT 2601, Australia
a.kilian@cambia.org.au

Janko Kos
KRKA d.d.
Research and Development Division,
Department F Biochemical Research and Drug design
1000 Ljubljana, Slovenia
janko.kos@krka.si

Katriina Kontu
Perkin Elmer life sciences
Katriina.Kontu@perkinelmer.com

Rok Kosem
Department of Pediatric and Preventive Dentistry
Faculty of Medicine, University of Ljubljana,
Sl - Hrvatski trg 6, 1000 Ljubljana, Slovenia
rok.kosem@mf.uni-lj.si

Michael Krawczak
Institute of Medical Genetics, University of Wales,
College of Medicine
Heath Park, Cardiff CF14 4XN, UK
Krawczak@Cardiff.ac.uk

Rajiv Kumar
Karolinska Institute
Center for Nutrition & Toxicology
Novum, 14157 Huddinge, Sweden
rajiv.kumar@cnt.ki.se

Ants Kurg
Institute of Molecular and Cell Biology,
University of Tartu
23 Riia St., Tartu 51010, Estonia
akurg@ebc.ee

Robert Kwiatkowski
Third Wave Technologies Inc.
502 South Rosa Road
Madison 53719, Wiscounsin, USA
bkwiatkowski@twt.com

Pui-Yan Kwok
Washington University, School of Medicine,
Division of Dermatology
P.O. Box 8123, 660 S Euclid Street
St Louis, MO 63110, USA
kwok@im.wustl.edu

L

Andreana Lambrinakos
Genomic Disorders Research Centre
7th Floor, Daly Wing, 41 Victoria Parade
Fitzroy,Melbourne, Victoria 3065, Australia
andyl@ariel.ucs.unimelb.edu.au

Heikki Lehväslaiho
EMBL Outstation, European Bioinformatics Institute,
Wellcome Trust Genome Campus
Hinxton, Cambs., CB10 1SD, UK
heikki@ebi.ac.uk

Kenneth Livak
Applied Biosystems
850 Lincoln Centre Drive
CA 94404, USA
livakkn@appliedbiosystems.com

Elin Lőhmussaar
Institute of Molecular and Cell Biology,
University of Tartu
23 Riia St., 51010 Tartu, Estonia
elin@ebc.ee

M

Martina Macht
Bruker Saxonia Analytik GmbH
Permoserstr. 15
04318 Leipzig, Germany
mm@bsax.de

Mohammed Makrelouf
Labonatoire Central CHU BAB-EL-OUED
Algiers, Algeria
mmakrelouf@hotmail.com

Lovisa Malmberg
Dept. of Medical Sciences
Uppsala University, Uppsala, Sweden
loma0102@student.uu.se

Alicia Marchlewska
Department of Molecular Medicine
Karolinska Hospital
CMM L8:00
17176 Stockholm, Sweden
tillalicia@hotmail.com

Chris Mattocks
Addenbrookes Hospital, Molecular Genetics Laboratory
Level 1, Box 158, Hills Road
Cambridge, CB2 2QQ, UK
CJM599@hotmail.com

Gerry Mayer
Varian Deutschland GmbH
Alsfelder Strasse 6
64289 Darmstadt, Germany
gerry.mayer@varianinc.com

Joseph McClay
SGDP Research Centre, Institute of Psychiatry
DeCrespigny Park, Camberwell
London SE5 8AF, UK
j.mmclay@iop.kcl.ac.uk

Elisa Merello
Servizio di Neurochirurgia, Instituto G. Gaslini
Genova, Italy
capraval@tin.it

Musa Mhlanga
New York School of Medicine, PHRI
455 First Avenue, Rm 973
New York 10021, USA
mhlanga@phri.nyu.edu

Andrei Mirzabekov
Engelhardt Institute of Molecular Biology
Russian Academy od Sciences
Vavilova 32, Moscow 119991, Russia
amir@genome.eimb.relarn.ru

Anna Moroni
Servizio di Neurochirurgia
Instituto G. Gaslini, Genova, Italy
capraval@tin.it

Helena Motaln
University of Ljubljana
Zootechnical Dept., Biotechnical Faculty
Groblje 3, 1230 Domzale, Slovenia

Peter Mouritzen
Exiqon A/S
Bygstubben 9
2950 Vedbaek, Denmark
mouritzen@exiqon.com

Edvin Munk
SEQUENOM GmbH
Mendelssohnstr. 15d
22761 Hamburg, Germany
emunk@seguenom.de

N

Mats Nilsson
Medical Genetics, Uppsala University
Box 589, Biomedical Centre
Uppsala S-75123, Sweden
mats.nilsson@genpat.uu.se

Mario Noyer-Weidner
Transgenomic
Ebersstrasse 36/1
10827 Berlin, Germany
mnoyer_weidner@transgenomic.com

O

Bronagh O'hlci
National Centre for Medical Genetics
Our Lady's Hospital for Sick Children
12 Dublin, Ireland
bronagh.ohici@olhsc.ie

Ulrike Orth
Institut fur Humagenetik,
Universitaetsklinikum Hamburg Eppendorf
Butenfeld 42, D - 22529 Hamburg, Germany
uorth@uke.uni-hamburg.de

P

Alstair Pagnamenta
AP Biotech
APB White Lion Road, Amersham
Buckinghamshire HP7 9LL, UK
alistair.pagnamenta@eu.apbiotech.com

Silvia Paracchini
Dept of Biochemistry, University of Oxford
South Parks Road
Oxford OX1 3QU, UK
silvia@bioch.ox.ac.uk

Cinzia Pera
National Research Cancer Institute
Largo Rosanna Benzi 10
Genova 16132, Italy
pera@vega.cba.unige.it

Mark Perlin
Cybergenetics
160 North Craig Street, Suite 210
Pittsburgh, PA 15213, USA
perlin@cybgen.com

Ann-Sophie Persson
Department of Molecular Medicine
Karolinska Hospital
CMM L8:00
17176 Stockholm, Sweden
annsopersson@hotmail.com

Susanna Petersson
Department of Molecular Medicine
Karolinska Hospital
CMM L8:00
17176 Stockholm, Sweden
susanna.petersson@cmm.ki.se

Michael Philips
Orchid Biosciences , Inc.
303A College Rd E, Princeton
NJ 08540, USA
mphillips@orchid.com

Uroš Potočnik
Laboratory of Molecular Genetics
Medical faculty Ljubljana, Institute of Pathology,
Korytkova 2, 1000 Ljubljana, Slovenia
uros.potocnik@mf.uni-lj.si

R

Metka Ravnik-Glavač
Laboratory of Molecular Genetics
Medical faculty Ljubljana, Institute of Pathology,
Korytkova 2
1000 Ljubljana, Slovenia

Rod Rodman
Varian Inc.
2700 Mitchell Dr.
94598 Walnut Creek, USA
rod.rodman@varianinc.com

Zbigniew Rudzki
Institute of Medical and Veterinary Science
Molecular Pathology Unit
PO Box 14 Rundli Mall
Adelaide, South Australia 5000, Australia
zbigniew.rudzki@imvs.sa.gov.au

Ruth Rupreht
Blood Transfusion Centre of Slovenia
Šlajmerjeva 6
1000 Ljubljana, Slovenia
rrupreht@mf.uni-lj.si

S

Fabio Sánchez
Department of Molecular Medicine
Karolinska Hospital
CMM L8:00
17176 Stockholm, Sweden
fabio.sanchez@cmm.ki.se

Martin Schalling
Department of Molecular Medicine
Karolinska Hospital
CMM L8:00
17176 Stockholm, Sweden
martin.schalling@cmm.ki.se

Selim Sengul
Department of Molecular Medicine
Karolinska Hospital
CMM L8:00
17176 Stockholm, Sweden
selim.sengul@cmm.ki.se

Carrie Shawber
Weill Medical College Cornell University
Dept of Microbiology and immunology
1300 York Avenue, B-406
NY 10025, UK
cjs2005@med.cornell.edu

Steven Sheardown
Glaxo Smithkline
NFSP (N) Third Avenue, Harlow
Essex CM19 5AW, UK
steven_a_sheardown@sbphrd.com

Asgeir Sigurdsson
Decode Genetics
Lynghals 1
Reykjavek, Iceland
asgeirs@decode.is

Lloyd Smith
University of Wisconsin-Madison
Chemistry; Analytical Division
1101 University Avenue
Madison, WI 53706-1396, USA
smith@chem.wisc.edu

Ed Southern
University of Oxford
Depart. Of Biochemistry
South Parks Road
Oxford, OX1 3QU, UK
ems@bioch.ox.ac.uk

Mirjam Stopar-Obreza
University Children's Hospital Ljubljana
Vrazov trg 1
1000 Ljubljana, Slovenia
mirjam.stopar@mf.uni-lj.si

Niels Storm
Sequenom Gmbh
Mendlssohnstr. 15d
22761 Hamburg, Germany
nstorm@sequenom.de

Ann-Christine Syvänen
Department of Medical Sciences, Molecular Medicine
Uppsala University Hospital
Ing 70, 3tr, Faud 2
75185 Uppsala, Sweden
Ann-Christine.Syvanen@medsci.uu.se

Melinda Szőke
H-Med Diagnostic and Research Laboratory
Robert Karoly krt. 44
1134 Budapest, Hungary
mszoke@matavnet.hu

Sylvie Szpiro-Tapina
Laboratoire Pasteur Cerba
95066 Cergy Pontoise CX09
Cergy Pontoise, France
sstapia@pasteur-cerba.com

Š

Maja Šlajpah
Laboratory of Molecular Genetics
Medical faculty Ljubljana, Institute of Pathology
Korytkova 2, 1000 Ljubljana, Slovenia
maja.slajpah@mf.uni-lj.si

T

Claire Taylor
ICFR Mutation Detection Facility
James's University Hospital
Leeds, UK
c.taylor@icrf.icnet.uk

Graham Taylor
Regional DNA Laboratory, Ashley Wing
St. James's University Hospital
Leeds, LS9 7TF, UK
gtaylor@hgmp.mrc.ac.uk

Joseph Terwilliger
Columbia University
Columbia Genome Center
1150 St Nicholas Avenue, Room 530C
NY 10032, USA
jdt3@columbia.edu

Mario Tosi
Inserm EMI 9906
Faculte de Medecine
22 Boulevard Gambetta
Rouen, France
mario.tosi@univ-rouen.fr

Wolfgang Trautwein
Nanogen bv
20 Johannevej
2970Horsholm, Denmark
wtrautwein@Nanogen.com

Katarina Trebušak
University Children's Hospital Ljubljana
Vrazov trg 1
1000 Ljubljana, Slovenia
Katarina.trebusak@kclj.si

Sanjay Tyagi
The Public Health Research Institute
Room 973, 455 First Avenue
NY 10016, USA
sanjay@phri.nyu.edu

V

Dirk van den Boom
SEQUENOM GmbH
Mendelssohnstr. 15d
22761 Hamburg, Germany
dvandenboom@sequenom.de

Maruška Vidovič
Institute of Public Health of the Republic of Slovenia
Trubarjeva 2
1000 Ljubljana, Slovenia
maruska.vidovic@lvz.si

Henrik Vissing
Exiqon A/S
Bygstubben 9
2950 Vedbaek, Denmark
vissing@exiqon.com

Phuong Vu
Department of Genetics
Norwegian Radium Hospital, Norway
phuong.ngoc@labmed.uio.no

W

Joanne Walter
Transgenomic Ltd.
The Quadrangle, Crewe Hall
Weston Road, Crewe
Cheshire, CW1 6UZ, UK
jwalter@transgenomic.co.uk

Janet Warrington
Affymetrix Inc.
3380 Central Expressway
Santa Clara, CA 95051, USA
Janet_Warrington@affymetrix.com

Birgit Weber
metaGen
Gesellschaft fur Genomforschung mbH
Ihnstrasse 63
14195 Berlin, Germany
birgit.weber@metagen.de

Gregory Werner
Genset Oligos
1 rue Robert & Sonia Delaunay
75545 Paris Cedex 11, France

Scott White
Los Alamos National Laboratory
B-N1, Mailstop M888, Bioscience Division
Los Alamos, NM, B7545, USA
scott_white@lanl.gov

Stefan White
Department of Human and Clinical Genetics
Leiden University Medical Cenetr
Wassenaarsewag 72
2333AL Leiden, The Netherlands
s.white@lumc.nl

Jeffery Wine
Cystic Fibrosis Research Lab.
Stanford University
Bldg 420, Jordan Hall, 450 Serra Mall
Stanford, CA 94305-2130, USA
wine@stanford.edu

Z

Irena Zajc
National Institute of Biology
Vecna pot 111
1000 Ljubljana, Slovenia
irena.zajc@uni-lj.si

Akila Zenati
Labo Central de Patologie
CHU de Bab El Oued
BP Said Touati, Algeria
mmakrelouf@hotmail.com

Johannes Zschocke
nstitute of Human Genetics
I Im Neuenheimer Feld 344a
69120 Heidelberg, Germany
johannes_zschocke@med.uni-heidelberg.de