

High Throughput Technology (htpt) Group



Head:

Dr. Richard Reinhardt

Phone: +49 (0)30-8413 1226

Fax: +49 (0)30-8413 1365

Email: reinhardt@molgen.mpg.de

Secretary:

Tamara Safari

Phone: +49 (0)30-8413 1126

Fax: +49 (0)30-8413 1365

Email: safari@molgen.mpg.de

Scientists:

Carola Burgtorf

Michael Kube

Giannino Patone

Sascha Sauer

Undergraduate student:

Heiner Kuhl

Technicians/TFH:

Stephen Gelling

Verena Gimmel

Thomas Honeck

Oliver Ruffer

Ilka Slosarek (part-time)

Anett Smyra

Gregor Wozniak

Technicians:

Beatrice Baumann

Gabriele Bläß (part-time)

Susan Böhm

Christin Bunk

Daniela Gröger

Pamela Kepper

Ines Müller

Ines Pickersgill

Mario Sontag

Janina Thiel

Scientific overview

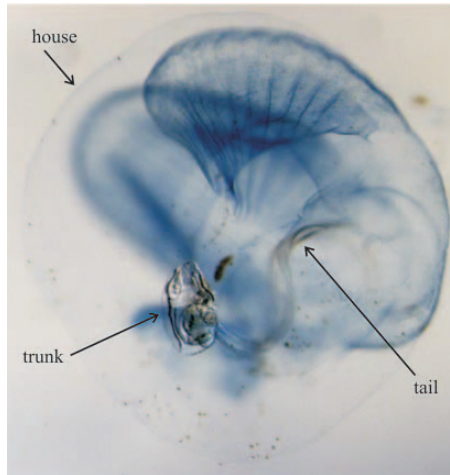
The recent publication of the completed human genome sequence was the ultimate success of our major effort within the last 6 years period. Important steps on this successful way are also the sequencing and final analysis of chromosome 21, the second finished human chromosome, still one of the most accurately analysed one, and our contribution to several regions of the human chromosomes 1, 3, 17, and X. Our latest finalised project along this line is the completed elucidation of chimpanzee chromosome 22, the ortholog to human chromosome 21. The whole project was organised by an German-Asian consortium, wherein MPIMG was responsible for the German part. These results, being presently summarised, are not only important because chimpanzee is our closest relative, it is the first time that a large genomic arrangement, two complete chromosomes of man and chimpanzee, are comparatively analysed. Therefore, not only genes and variations within the coding elements are comparable, but also intronic regions and even more important, promoter elements are accessible for any comparative analysis and elucidation.

In addition, we are involved in the analysis of model organisms such as mouse (chromosome 2 and 6), rhesus MHC and the complete analysis of the rat MHC (RT1) complex, which plays an important role in infectious diseases. The MHC region belongs to the most densely packed, gene rich regions and although it spans only over a 4 Mbases area, we have identified 220 genes, nearly as many as in the human chromosome 21 region, which is about 34 Mbases large.



Other launched projects concern contributions to the final sequence of chimpanzee chromosomes X and Y, with special interest to Xq28 and regions associated with mental retardation. We are also involved in national and international projects, from bacterial genomes to model organisms (Michael Kube) like the urochordate *Oikopleura*, as listed below (project grants and MPG projects).

The early scientific interest related to *Oikopleura dioica* was focused on questions of systematics, the phenomenon of “marine snow” and of bioluminescence, research in *Oikopleura*'s nervous system, and ecological questions, like the influence on picoplankton. With a genome size of only around 75 Mbases (estimated number of 15.000 genes), smaller than *C. elegans*, and less than half that of *D. melanogaster*, the genome of *Oikopleura dioica* gives the chance for a closer look inside an early chordate genome. In addition, this organism has also other interesting features, making it a key system to understand the functions of human/ vertebrate genome.



Oikopleura in its house (E. Thompson, Norway)

Today we have a good molecular base for completing the genome sequence, as our norwegian partners at SARS established a culture line for *Oikopleura*. More than 170'000 reads from a whole genome shotgun were assembled into 45'000 contigs with a total contig length of more than 41Mb at Berlin. These sequences cover more than half of the genome. The sequencing of more than 50 selected BACs from a BAC library, covering the whole genome 10fold are finalized or near finished status. The determination of all BAC-end-sequences, nearly 10.000 BACs, is in progress and will be finished about the end of year 2003.

The **htpt**-group (which is completely externally funded, except for 30% of RR and TS) is also a co-operation partner for all departments within the institute and has established a good infrastructure for large-scale genomic analysis projects such as sequencing, mutation analysis and mass spectrometry. In addition, we are also managing several projects for disease gene identification and systematic re-sequencing of candidate genes or genomic regions of interest (<http://www.resequencing.mpg.de/>). Finished genomic sequence data are submitted to public data bases and presented on our project Web pages.

Furthermore our work (Sascha Sauer) is focused on technology development for detection of DNA variations and protein-ligand interaction by mass spectrometry. Sample preparation methods for genotyping of SNPs by MALDI-MS, termed GOOD assays, were modified to significantly reduce costs and adapted to our automated liquid handling. With the current equipment at MPIMG we could maintain a routine throughput of ca. 10,000 genotypes per day. Moreover, a novel approach for molecular haplotyping (haplotypes are the phase of several marker alleles on a single chromosome) by means of pooled fosmid/cosmid libraries and seamless analysis by mass spectrometry was elaborated. Due to our recent adoption of photocleavable linker and charge-tag technology we are able to make use of the multichannel detection capability of mass spectrometers and apply this approach for a novel, parallel oligonucleotide fingerprinting approach (ONF) by MALDI-MS to provide pre-selection of cDNA and shotgun clones from large libraries.

We will focus on HT-genotyping in collaboration with NGFN and Génoplante and will develop procedures for DNA analysis with partners as the Centre National de Génotypage and Bruker Daltonik. An issue of prior importance, to detect binding of ligands (natural or chemical compounds) with proteins, is soft ionisation of non-covalent interactions by ESI-MS (together with Protein Structure Factory and Analyticon Discovery).

General information

Selected Publications 1998-2003

Glöckner FO, Kube M, Bauer M, Teeling H, Lombardot T, Ludwig W, Gade D, Beck A, Rabus R, Schlesner H, Amann R & Reinhardt R (2003). *Complete genome sequence of the marine planctomycete *Pirellula* sp. strain 1*. PNAS 100(14): 8298 – 8303

Olbrich H, Fliegau M, Hoefele J, Kispert A, Otto E, Volz A, Wolf MT, Sasmaz G, Trauer U, Reinhardt R, Sudbrak R, Antignac C, Gretz N, Walz G, Schermer B, Benzing T, Hildebrandt F & Omran H (2003). *Mutations in a novel gene, *NPHP3*, cause adolescent nephronophthisis, tapeto-retinal degeneration and hepatic fibrosis*. Nature Genetics 34:455-459

Sauer S, Lehrach H & Reinhardt R (2003). *MALDI mass spectrometry analysis of single nucleotide polymorphisms by photocleavage and charge-tagging*. Nucleic Acids Res 31: e63

Olbrich H, Häffner K, Kispert A, Völkel A, Volz A, Sasmaz G, Reinhardt R, Hennig S, Lehrach H, Konietzko N, Zariwala M, Noone PG, Knowles M, Mitchison HM, Meeks M, Chung EMK, Hildebrandt F, Sudbrak R & Omran H (2002). *Mutations in *DNAH5* cause primary ciliary dyskinesia and randomization of left-right asymmetry*. Nature Genetics 30:143-144

The Schizosaccharomyces pombe sequencing consortium (MPI contributors: Borzym K, Langer I, Beck A, Lehrach H & Reinhardt R) (2002). *The genome sequence of Schizosaccharomyces pombe*. Nature 415:871-880

Seo HC, Kube M, Edvardsen RB, Jensen MF, Beck A, Spriet E, Gorsky G, Thompson EM, Lehrach H, Reinhardt R & Chourrout D (2001). *Miniature Genome in the Marine Chordate *Oikopleura dioica**. Science 294:2506

The International Human Genome Mapping Consortium (MPI contributors: Ramser J, Reinhardt R & Lehrach H) (2001). *A physical map of the human genome*. Nature 409(6822):934-941

The International Human Genome Sequencing Consortium (MPI contributors: Ramser J, Lehrach H & Reinhardt R) (2001). *Initial sequencing and analysis of the human genome*. Nature 409:860-921

Paulsen M, El-Maarri O, Engemann S, Strodicke M, Franck O, Davies K, Reinhardt R, Reik W & Walter J (2000). *Sequence conservation and variability of imprinting in the Beckwith-Wiedemann syndrome gene cluster in human and mouse*. Hum Mol Genetics 9(12):1829-1841

The chromosome 21 consortium (MPI contributors: Ramser J, Beck A, Klages S, Hennig S, Riesselmann L, Dagand E, Haaf T, Wehrmeyer S, Borzym K, Francis F, Lehrach H, Reinhardt R & Yaspo ML) (2000). *The DNA sequence of human chromosome 21*. Nature 405(6784):311-319

Duitman EH, Hamoen LW, Rembold M, Venema G, Seitz H, Saenger W, Bernhard F, Reinhardt R, Schmidt M, Ullrich C, Stein T, Leenders E & Vater J (1999). *The mycosubtilin synthetase of *Bacillus subtilis* ATCC6633: A multifunctional hybrid between a peptide synthetase, an amino transferase, and a fatty acid synthase*. PNAS USA 96(23):13294 – 13299

Radelof U, Hennig S, Seranski P, Steinfath M, Ramser J, Reinhardt R, Poustka A, Francis F & Lehrach H (1998). *Preselection of shotgun clones by oligonucleotide fingerprinting: an efficient and high throughput strategy to reduce redundancy in large-scale sequencing projects*. Nucl Acids Res 26(23): 5358-5364

Theses

Michael Kube, *Sequenzierung und Struktur von *Pirellula* sp. Stamm 1*, PhD Thesis, University of Bremen, 7/2003

Heiner Kuhl, *Multiplex PCR Screening einer genomischen BAC Library des Organismus *Oikopleura dioica* zur Identifikation von mHC verwandten Genen*, Students Thesis, Technical University of Berlin, 11/2001

External funding

EU BIO4-CT 96-0159: *European schizosaccharomyces genome sequencing project*, 12/1996-12/2000

BMBF 01KW97065: *Genomic sequence analysis of human chromosome 21 and selected regions of the human genome*, 5/1997 - 6/2001

EU BIO-98-0079: *European Leishmania major Friedlin genome sequencing project*, 12/1998 – 11/2001

BMBF 03F0279C: *Funktionelle Genomanalyse umweltrelevanter mariner Bakterien*, 4/2000 – 3/2002

Magnanomed EU CT00-00375: *Magnetic nanoparticles for medical and biological diagnostics and devices*, 1/2001 – 4/2003

BMBF 01KW0001: *Disease gene-oriented genomic sequence analysis of medically important regions of the human genome and homologous regions of the mouse genome*, 1/01 – 6/04



BMBF 01GR0105, Plattform 1.1: *NGFN Core Area Genomic sequencing and mapping*, 3/2001 – 10/2004

BMBF 01GR0105, Plattform 6.2: *NGFN Core Area Wave-technology*, 3/2001 – 10/2004

BMBF 01GR0105, Plattform 6.8.2: *NGFN Core Area Comparative candidate gene sequencing*, 6/2002 – 06/2004

BMBF Uni Göttingen 031U113A / U213A: *Netzwerk GenoMik Göttingen, Genomanalyse noch nicht kultivierter mariner und terrestrischer methanoxidierender Mikroorganismen und Konsortien*, 7/2001 – 6/2004

Joint projects with the MPI for Marine Microbiology, Bremen

Pirellula, 7,15 Mbases, finished, cosmid verification

EBN1, ca. 4,8 Mbases, finishing

Magnetospirillum, ca. 4,5 Mbases, finishing

Five other bacterial projects of similar size are currently in data collection or data assembling status (GhdN, HxN, MxyN, ToN1 and Pcy) (2001-2002).

Oikopleura (joint project between MPG & SARS Bergen), ca. 75 Mbases, working draft BAC-ends

Patents

Kalkum M, Müller M, Nordhoff E, Reinhardt R, Eickhoff H, Rauth H. *Method and Device for processing extremely small substance quantities*, PCT/EP99/02964 / *Verfahren und Vorrichtung zum Prozessieren von Kleinstsubstanzenmengen*, DE 198 23 719 A1

Rauth H, Reinhardt R, Nordhoff E. *Verfahren zum Anbinden von Nukleinsäuren an eine Festphase*. PCT/EP00/08807, DE, EU, US

Rauth H, Reinhardt R, Nordhoff E. *Verfahren zur Umkehrphasenaufreinigung und -konzentrierung von Peptiden und Proteinen an magnetischen Partikeln*. PCT Nr. 60/175,958

Rauth H, Reinhardt R, Starke A. *Verfahren und Vorrichtung zur Gelelektrophorese*. DE 199 26 985.8

Selected academic co-operations

Varies bacterial genomic and metagenomic projects, with MPI for Marine Microbiology, Bremen

German-Deep Phylogeny-Consortium, with Prof. J. W. Waagele, Ruhr-University, Bochum

Development of tailored magn. nanoparticles, with Prof. H. Hofmann, EPFL-Laboratoire de Technologie des Poudres, CH-Lausanne

NoE Marine Genomics consortium (member of scientific steering committee)

MolTools Consortium (IP of EU 6th framework)

SNP-technology, with Centre National de Génotypage

Selected industrial co-operations

Bruker Daltonik, Bremen and Leipzig
micromod Partikeltechnologie GmbH, Rostock

Organization of scientific events

Chimpanzee Chromosome 22 consortium meeting, Harnack-Haus, Berlin-Dahlem, 16.-17.7.2002

MAGNANOMED exploitation workshop and satellite meetings, Harnack-Haus, Berlin-Dahlem, 3.-5.12.2002