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MarBEF



# Marine Biodiversity and Ecosystem Functioning

*EU Network of Excellence*

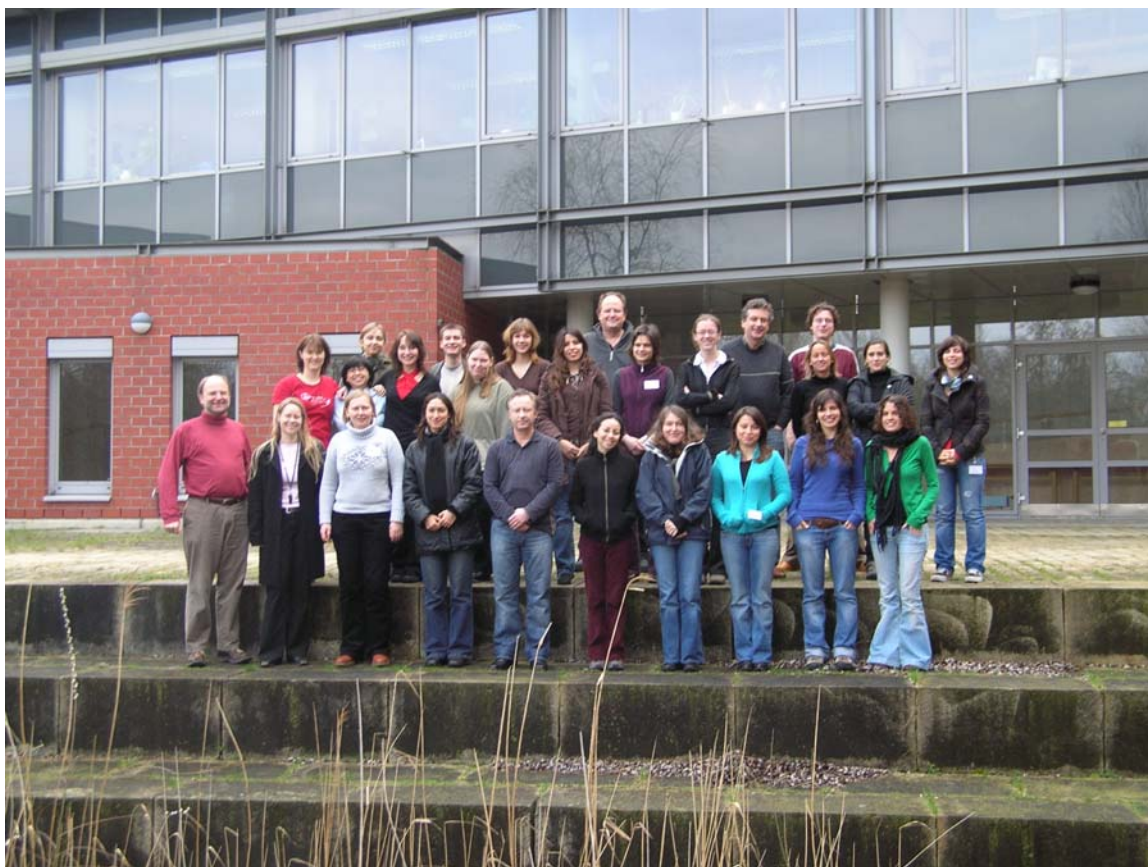
*Sustainable development, global change and ecosystems*

## Report on the MarBEF training course GENETIC FINGERPRINTS IN BIODIVERSITY RESEARCH MARCH %\_), 2007

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Max-Planck-Institute for Marine Microbiology



Max-Planck Institute for Marine Microbiology



Report on the MARBEF Training Course

# “GENETIC FINGERPRINTS IN BIODIVERSITY RESEARCH”

Max-Planck-Institute for Marine Microbiology, Bremen, Germany,  
March 5-9, 2007.



Framework Programme (2002-2006)

### Dissemination Level

<b>PU</b>	Public	X
<b>PP</b>	Restricted to other programme participants (including the Commission Services)	
<b>RE</b>	Restricted to a group specified by the consortium (including the Commission Services)	
<b>CO</b>	Confidential, only for members of the consortium (including the Commission Services)	

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local organization: Jens Harder, Judith Klatt, Maya Shovitri, Christina Probian

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Delft University of Technology, The Netherlands

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**Nyree West**

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**Alban Ramette**

Max-Planck-Institute for Marine Microbiology,  
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## Participants

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## **Summary**

The training course “Genetic fingerprints in biodiversity research” was performed at the Max-Planck-Institute for Marine Microbiology in Bremen, Germany, March, 5–9, 2007. A total number of twenty PhD students and Postdoctoral researcher were selected for participation.

The course covered the application of diverse fingerprinting methods while focusing on biodiversity characterization in marine habitats. In the morning, lectures covering the different fields were presented by experts of the RMP Microbiology. In the afternoon, the participants were split into twelve beginners and eight advanced students to meet the different demands. The beginners analyzed their own samples or local sediment samples by T-RFLP analysis (Terminal Restriction Fragment Polymorphism Analysis) and were also

introduced to practical DGGE (Denaturing Gradient Gel Electrophoresis). In the advanced student's workshop the actual research was presented and discussed.

## **Lectures**

After the reception of the participants, an introductory lecture giving an overview on fingerprinting methods was presented by Klaus Jürgens from the Baltic Sea Research Institute Warnemünde, Germany. Jens Harder (MPI-MM) gave lectures on elementary steps in fingerprint research, DNA preparation and PCR amplification. He introduced the concept of terminal restriction fragment length polymorphism (T-RFLP) analysis and explained the selection procedure for restriction endonucleases to be applied.

On Tuesday, Jens Harder covered capillary electrophoresis of the fragments and the interpretation of the profiles obtained. A case study was presented concerning the community analysis in deep sea sediment samples. Alban Ramette (MPI-MM) gave a lecture on ARISA (Automated rRNA Intergenic Spacer Analysis), the amplification of the 16S-23S intergenic spacer of the rRNA operon from total community DNA resulting in distinct patterns of PCR-product lengths.

An essential in the interpretation of fingerprinting patterns, Gunnar Gerdt (Alfred Wegener Institute for Polar Research, Helgoland, Germany) gave a presentation concerning multivariate statistics and the practical approaches to link biological information with environmental observations. Gerald Muyzer from the Delft University of Technology, Netherlands, explained in two lectures the principles and applications of DGGE (denaturing gradient gel electrophoresis) as fingerprint method.

On Thursday Arantxa Lopez-Lopez (University of the Balearic Islands, Spain) gave a lecture on PFGE (Pulsed Field Gel Electrophoresis) as a method achieving a non-PCR biased genetic fingerprint of microbial strains. SSCP analysis (Single Strand Conformational Polymorphism Analysis) was presented by Nyree West (Laboratoire de Océanologie de Banyuls, France). She focused on the CE-SSCP analysis based on a capillary electrophoresis. Lucas Stal gave a lecture on "Diversity and quantification of functional gene expression in microbial

mats” and discussed the question if representative results of clone library sequencing are possible despite a high “microdiversity” in a community structure in biological mats. He provided examples how the underexplored biosphere could become more accessible using quantitative PCR or Fluorescence *in-situ* Hybridization (CARD-FISH and TSA-FISH) and gave a case study dealing with the analysis of diatom populations along the Westerschelde estuary.

Rudi Amman focused in his lecture on the evolution and improvement of different molecular methods. Thereby he concentrated on fingerprinting techniques and FISH, but emphasized the importance of classical approaches and also the increasing significance of the interdisciplinary approach towards habitat descriptions. Finally the participants summed up in an open discussion the advantages of the individual methods.

### **Practical course for beginners**

The participants of the lab course for beginners were composed of PhD students with diverse scientific background. Therefore the practical work was centered on T-RFLP to teach the necessary laboratory techniques from DNA extraction, PCR and restriction fragment generation to data analysis. Objective for the four days was a complete T-RFLP analysis.

March, 5<sup>th</sup> 2007

- DNA extraction from sediments sample
- Quantification and qualification of extracted DNA using Nanodrop UV-VIS spectrophotometer
- PCR with bacterial primer

March, 6<sup>th</sup> 2007

- Desalting of PCR products using Sephadex microcolumns
- Quantification of PCR products using Nanodrop UV-VIS spectrophotometer
- Enzyme restriction of PCR products

March, 7<sup>th</sup> 2007

- Desalting of T-restriction fragments (T-RFs) using Sephadex microcolumn
- Preparation of T-RFs for capillary electrophoresis (CE)

- CE analysis overnight on the ABI sequence analyzer

March, 8<sup>th</sup> 2007

- Data analysis using Gene Mapper, binning with R program and presentation using PAST
- Practical demonstration of critical DGGE steps





MarBEF training course GENETIC FINGERPRINTS IN BIODIVERSITY RESEARCH

Lecture Hall, Max-Planck-Institute for Marine Microbiology, Bremen, Germany, March, 5-9, 2007

Time	Monday	Tuesday	Wednesday	Thursday	Friday
9.00 – 10.00	Klaus Jürgens Overview on fingerprinting techniques	Jens Harder Capillary electrophoresis and Genemapper software	Gunnar Gerdts Multivariate analysis (room 4021)	Arantxa Lopez-Lopez Pulsed field gel electrophoresis: genomic macrorestrictions and its applications	Rudi Amann Fingerprinting and fluorescence <i>in situ</i> hybridization (FISH)
10.15 – 11.15	Jens Harder DNA isolation, primer design and PCR	Jens Harder T-RFLP studies on deep-sea sediments	Gerard Muyzer Denaturing gradient gel electrophoresis (DGGE)	Nyree West Single strand conformation polymorphism (SSCP)	10.30-11.00 Course evaluation questionnaire
11.30 – 12.30	Jens Harder DNA quantification and restriction digest	Alban Ramette Automated rRNA intergenic spacer analysis (ARISA)	Gerard Muyzer Denaturing gradient gel electrophoresis (DGGE)	Lucas Stal Diversity and quantification of functional gene expression in microbial mats	11.00-12.30 All participants and Jens Harder (chairmen) Open discussion: the future place of genetic fingerprints in biodiversity research
	Lunch	Lunch	Lunch	Lunch	Lunch
14.00 – 18.00	Laboratory course: DNA isolation and PCR	Laboratory course: DNA quantification and restriction digest	Laboratory course: capillary electrophoresis	Laboratory course: data analysis, DGGE demonstration	
14.00 – 18.00	Advanced student's workshop	Advanced student's workshop	Advanced student's workshop	Advanced student's workshop	
	Town visit		16.00: Social event		

**Klaus Jürgens** Baltic Sea Research Institute    **Gunnar Gerdts** Alfred Wegener Institute for Polar Research    **Gerard Muyzer** Delft University of Technology    **Arantxa Lopez-Lopez** University of the Balearic Islands    **Nyree West** Laboratoire de Océanologie de Banyuls    **Lucas Stal** NIOO-KNAW Centre for Estuarine and Marine Ecology    **Rudi Amann, Alban Ramette, Jens Harder** Max-Planck-Institute for Marine Microbiology

## **Participants' Evaluation Summary**

After the last lecture a questionnaire was handed out to the participants of the course. Overall the course was evaluated as very good to excellent. Especially the local organization and the competence of the instructors were complimented.

Students identifying themselves as beginners in molecular methods desired a longer practical part of the course with a less concentrated program. The comments revealed a broad interest for additional training courses dealing with more experimental details of fingerprinting methods.

The expectations of the participants on the training course were on average met with 85 %.

Some comments after the course:

“In general the course was really satisfying. As a beginner it met a lot of my expectations. The lab sessions were well organized and Maya, Christina and Judith altogether did a lot for us. They really exerted themselves for us to understand, practice and etc. Well, thank them for everything. The lectures were clear enough for me. I think I got the basic of this technique. It was also good that the lectures give case studies and advise papers for detailed and different studies. And most of the lecturers are really funny. Being at the MPI was also nice. We found the chance to see what about and how you are actually doing your job. Thanks for your effort and kindness. Finally, food was really good.”

“In general the content of the course for the limited time of a week was really good. It would be preferable to have more time of practice laboratory work, in order to get really into the application of all these techniques and tools that we’ve learned about.”

“A very good insight into the methods currently used in molecular microbiology. The courses offered me the background to start thinking how to organize my own research.

The time spent on how to analyze all data was not completely sufficient, but I think to do both (practical part and using the appropriate software) during four days of laboratory course was not possible. Maybe there could be a continuation on data analysis (a part B training course).”