



# **Cost Action 853**

## **Agricultural Biomarkers for Array Technology**

**Management Committee Meeting  
and combined  
Meeting of Working Group 4**

**September 24 – 26, 2003**

### **Meeting Report**

**Centre of Applied Gensensorik (CAG), University of Bremen, Germany  
in the Centre of Environmental Research and Technology (UFT)**

# Draft Minutes of the Management Committee Meeting

## 1. Welcome to participants

The Management Committee Meeting was opened by Vice-Chairman Günter Adam and Working Group 4 leader and host of this meeting, Dietmar Blohm, on Wednesday afternoon, September 24 2003. 30 minutes later (because of delayed arrival time of his flight), Chairman Jürg E. Frey welcomed all Management Committee Members and MC Delegates representing 18 out of 21 participating countries: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Netherlands, Norway, Poland, Portugal, Spain, Switzerland and the United Kingdom. Representatives from Hungary did not reply to any of the previous e-mail or phone calls, and Lithuania and Slovenia did not communicate any MC Members yet.

In commemoration of Piero Roggero, dear colleague and MC Member of Italy, who passed away in June 2003 during his holidays, a minute's silence was held.

## 2. Adoption of agenda

The draft agenda for the meeting in Bremen was approved unanimously.

A list of additional topics to be discussed during this meeting was collected together with all participants (see point 15)

## 3. Minutes of last meeting

The minutes of the second MCM meeting held in Wädenswil on September, 26 – 28, 2002 were approved with no further requests.

## 4. Report from the Scientific Officer

News from the Commission: John Williams informed on the problems with financing of COST actions in 2003. Because of the reorganization including the European Science Foundation (ESF) as third party, delays in payments were observed. 5 Mio Euro from Framework Program 5 could be invested into COST actions, which was the reason why this meeting could take place. From January, 1<sup>st</sup>, 2004, money will be managed by ESF (with the idea of making administration more straightforward). It was also discussed that the budgets should be handed over to the individual COST actions, i.e. in their own responsibility. The trade-off between more responsibility and higher administrative work (ca. 15% of the budget) must be given a second thought. If COST 853 MCMembers have an opinion on how the budget should be organized, they should summarize their feelings in a report and give it to John Williams who will send it to ESF.

Status of the action: The status of the action can be accessed on the internet under <http://www.COST853.ch>

Number of signatories: Until the day of the meeting, 21 out of 33 COST countries as well as one associated country, had signed the action (see <http://www.COST853.ch>)

Budget status, budget allocation process: see below, 5. Budget planning 2004

Information on the proceeding with new countries: The formal application to the chairman is followed by confirmation of agreement by all MCMembers. Then, Brussels is informed about the decision and the collaboration confirmed.

John Williams also informed about his personal future plans. Unfortunately, for us, he will finish his job as a Scientific Officer of COST actions and work for ESF from next year on. We thank him sincerely for his great support and wish him and his family all the best for the future!

## 5. Budget planning 2004

Applications for meetings in 2004 should be sent in as soon as possible. The requirements for meeting and STSM reimbursement will probably change with the new organization of ESF.

## 6. STSM status, applications

For the rest of 2003, there is only a small chance for STSM applications. However, the contract for Short Term Scientific Missions is already signed, and STSM money will be available for 2004. Applications should be formulated soon and be sent to the chairman or the vice-chairman, in case, some money is left from 2003. Günter Adam agrees to put together a proposal to apply for the STSM money. A committee of 5 to 6 members within COST853 will be set up to evaluate the applications; application forms and proposals will be available, protected by a password, on the ftp-site of the University of Hamburg (Günter Adam). The approximate amount of money available per STSM is 1500 Euro per month for a maximum of 6 STSMs (=max STSM period).

## 7. Publications, annual report

Information on the proceedings within the countries will be published in the *Annual Report* of COST 853. Additionally, the cost-homepage [www.COST853.ch](http://www.COST853.ch) will open the planned site on publications of related work, including publications from members of this action. John Williams, the scientific secretary, informed the participants about the option to apply for money from COST to be used for publications, such as presentations of results from a workshop or for a special number in a scientific journal.

## 8. Evaluations

To get a comprehensive overview over all working groups in all 21 countries, it was agreed that every national delegate will fill out a progress report on topics formulated by chairman Jürg E. Frey by mid-November. This report will not only include results from the labs of the delegate, but also other important work at the front in their countries. The results will be summarized and presented in the *Annual Report*.

Peter Bonants offers to establish a database including methodical information of the involved scientific groups in COST853. Topics include extraction methods, amplification methods, probe design, target genes, available spotting facilities etc. A questionnaire will be sent to MCMembers and collected information will most probably be put on a password protected ftp-server. Included in this questionnaire will also be an evaluation of the interest to organize combined probe-orders of, or combined chips for probe validation.

## 9. Request for new members

No request for a new membership is open.

## 10. Non-COST participations

No requests for a new membership of non-COST countries are open.

## 11. Web news <http://www.COST853.ch>

The COST853 homepage (<http://www.COST853.ch>) is now running for more than one year. As statistics show, the visits are remarkable. From January 2003 to October 2003 a total of 1080 visitors were counted, 837 of them visited the site only once, the rest of them repeatedly. Besides the entering page, the most popular pages were the MCMember page, the Participating Scientists page and the WorkingGroup Info page. Downloaded files included presentations that were given at the Wädenswil Meeting in September 2002 as well as the agenda of Wädenswil, whereby the presentation downloads ranged from 523 to 25 for each presentation.

The homepage will be extended by the following pages:

- **Publications:** This page is depending on the collaboration of the participating scientists, informing chairman Jürg Frey or homepage administrator Moni Pfunder about new publications from their side. Additionally, any important paper in the field of microarray technology, especially with a focus on diagnostics, can be proposed for a separate list of interesting reading.

All existing pages will be actualised among others including:

- The assortment of talks and posters from this meeting in Bremen, 2003
- the addresses of MCMembers and Participating Scientists
- the poster for general use to all participants to inform about our COST853 action on conferences and meetings.

## **12. Progress report of working groups**

On Friday, September 26, each representative of the working groups presented their progress and their focus of work during the year 2003. Working Group 4, the host of this meeting, organized a whole day (Thursday, September 25) of interesting talks and discussions (Meeting Report WG4, next chapter). More information is included in the *Annual Report 2003*.

## **13. Long-term planning**

The MCMembers agreed to refrain from an application for extension of the COST853 action for the time being, as progress is consistent with the time plan of the action, however, to keep the option in mind to be re-evaluated next year.

## **14. Time and place of next meeting**

The Management Committee Members agreed to meet again next year, this time in Finland. The date and place is still to be decided by the host country and his representative, Jari Valkonen. In addition, the MCMembers agreed to organize a workshop for young scientists (level PhD, postdoc) to exchange “hands-on-experience”. The financing of this workshop will be done on STSM account.

## **15. Additional topics**

1. Probe design
2. Amplification of targets
3. Labelling
4. Avoiding PCR
5. Hybridization platforms
6. New microarray surface materials
7. Quantification (normalization and standardization)
8. Protein arrays
9. Target genes
10. Taxa list (what's on the chip)
11. Referencing 853
12. Confidentiality
13. Extension of the action
14. List of topics for information form (activities within countries)
15. EU-FP6 projects

### 15.1. Probe design

It is agreed that designed and validated probes are of high value. Different ways to distribute this useful information, including patenting as well as publication, are discussed. COST would not finance a patent and a joint venture in patenting is, at this point, not yet wished. However, D. Blohm offers to support and help groups within our COST853 who are interested in sending in a patent.

The idea is accepted to provide a board on the pdf-server of Hamburg, where participants of COST853 can collect their designed and validated probes for a corporate publication, for example in the "Journal of Molecular Probes".

### 15.2. Amplification of targets / 4. Avoiding PCR

The WG3 Group of Prof. Blohm presents a nice all in one flow through system allowing to use more volume, and therefore offering the possibility to use more DNA in less concentrated volume. Levente Bodrossy proposes the approach of using magnetic beads to concentrate DNA. Ian Barker informs on his success on direct labeling of viruses and promises to send references. Jürg Frey mentions the possibility of "rolling-circle-amplification" for whole genome amplification.

### 15.3. Labeling

Nobody knows about label-free solutions. Labeling after hybridisation (SybrGreen) was discussed, and seems to be possible with certain chip-surfaces (Niels Ramsing).

### 15.5-15.8, 15.10 Different methodic approaches

The discussion of these points are agreed to be postponed to the next meeting, as time runs out.

### 15.9. Target genes

Target genes depend on the organism(s) that should be diagnosed. However, within groups, such as viruses, microorganisms or eukaryotes, it might be efficient to concentrate on a few reliable genes. It is agreed to share the experiences from the different scientific groups involved in COST853 on the different tested genes in a combined database (see Chapter 8, Evaluations).

### 15.11. References

COST 853 activity will be measured by productivity. All participants agreed to reflect before each publication whether membership in COST853 had an influence on the results and would justify a reference in the acknowledgements. Moreover, all publications that could be interesting for COST853 should be sent to M. Pfunder for integration in the [www.cost853.ch](http://www.cost853.ch) homepage.

### 15.12. Confidentiality

The Management Committee decides to give interested persons and companies the possibility to visit the part of the meeting, where official talks will be given. However, they are excluded from the MCMmeeting itself.

### 15.13. Extension of the action

See Chapter 13. Long Term Planning

### 15.14. List of topics for information form

See Chapter 8. Evaluations

## Meeting of WG4 - Report

This year's combined management committee and Working Group 4 meeting was held in Bremen, home of the Center for Applied Sensorik (CAG) and organized by Dietmar Blohm and his collaborators. The WG4 meeting filled the whole of Thursday, September 25, including many interesting talks as well as a guided visit of the chip production as well as analysis laboratories (see Agenda).

The WG4 day started with **Dietmar Blohm** who presented the "Bremen Sensorik Project". After an introduction into the status of the technology, he gave insight into the idea of the Sensorik-project, the development away from isolated modules (spotter, hybridizer, reader etc) to an integrated all-in-one flow through system with an online reading facility, label-free readout, automation, reusable chips, PCR-independent and being usable for proteins as well as DNA. This project is interdisciplinary, involving scientists from the field of biotechnology, bio-informatics, chemistry, micro- and nanotechnology.

From the integrated system of high complexity, the invited expert **Niels Ramsing** from Exiqon Company in Denmark led the participants back to the basic components of DNA-MAs: the probes and primers. He presented convincing results on Locked Nucleic Acids, an analogue to DNA with enhanced affinity and specificity. The higher melting temperature stability allows more tolerance in primer/probe design. In addition, the destabilization through mismatches is enhanced which supports higher discrimination. Niels Ramsing reported on the design of a universal LNA array, using 7mer random oligonucleotides producing random but highly reproducible patterns. An online-reader, scanning during hybridization, should gather more information on probe/primer behavior at different hybridization temperatures.

**Uta Bohnebeck** from the "Bremerhavener Institut für biologische Informationssysteme" (BIBIS) followed up from the informatics side, giving an overview on the status of bioinformatics, and reported on a program for the determination of target sequences. The program includes taxonomy based selection of target sequences, the relative arrangement of the selected sequences, alignment, text views as well as combined search queries. The program should help to compute capture oligos with consideration of the criteria sensitivity, specificity, melting temperature (length, GC, salt concentration of the buffer), secondary structure of the capture probes and target sequences. The product is a hierarchical oligo library. But the BIBIS group also approaches automated DNA chip interpretation, interpretation of complex hybridization patterns, problems of unspecific hybridization as well as of false positive and negative signals.

**Frank Oliver Glöckner** from the Max-Planck-Institute for Marine Microbiology (MPI) presented the use of ARB (Latin, "arbor"=tree) software for the identification of microorganisms. The project bases on the fact that only about 1% of all microorganisms are culturable, therefore the molecular diagnosis is of high interest. ARB allows the automated design of gene probes useful for the investigation of microbial community structure, including phylogenetic information.

Pitfalls and solutions for target quantification by microarray analysis were then presented by **Jörg Peplies** from the UFT, Biotechnology and Molecular Genetics in Bremen. He illustrated different factors that can add to the goal of a perfect tool: the highly sensitive, specific, parallel and quantitative robust marker. Problems to be solved include inhomogeneous surfaces, unequal quantities of target DNA (PCR is not a quantitative method), differential secondary structures of probes, surface-mediated steric hindrance etc. Spacers, so he showed, can affect efficiency through higher distance to surface and helpers involved in secondary structure can increase signal intensity. According to Peplies, the methodology is not yet developed at this point to allow using signal intensities for gaining quantitative information on specific organisms from mixed cultures.

After these results from the bench-front the participants were guided through a mechanical and technical approach, the construction of a flow-through microarray hybridization chamber by **Marin Gheorghe** from the Institute for Microsensors, Actuators and Systems (IMSAS). The silicon-chamber, developed to use normal glass-slides, includes temperature control and online reading of signals. The

volume consists of maximal 200 ul, temperature can be controlled between 25 and 75 °C (points of measurement 1°C) with a heating power of 15W. The combination of hybridization at different temperatures with online reading is of great potential for the validation of probes.

A further practical insight gave **U. Steller**, Director of Research of PicoRapid Technology GmbH in Bremen. From the manufacturers point of view, he introduced different products such as the chemically activated PicoSlides (covalent attachment of aminomodified DNA), PiezoPipettes (volume of 500fl to 700pl), TopSpot (Imtek, Freiburg; micropipetting in parallel with 24, 96 or 384 pins). He presented the technology of CCD-camera based controls during spotting process, as applied in the PicoRapid facility. After Lunch, the participants had the opportunity to visit the PicoRapid facility and to throw a glance behind the scenes of array production.

In the afternoon, **Christiane Glöckner** from UFT, Biotechnology and Molecular Genetics, gave insight into the application of microarray technology in milk industry. The problem of unsatisfying acidification can be monitored with DNA-chips, which allow parallel detection of different organisms and genes. Tests can be done from starter-cultures, milk or milk products. Three to four 16S targeted probes are designed per organism. A test of different extraction kits leads to a preference for "Agowa", which is fast (less than 1 hour), gives high yields of DNA, and for which desalting prior to fragmentation is not necessary. PCR is not needed, labeling is conducted with Psoralen-Biotin and Streptavidin-Cy5. Including extraction, fragmentation and 2 hours of hybridization, results can be gained within 6 hours.

Another application of microarrays was presented by **Nina Silkenbeumer**, also from the UFT group. She showed her (far advanced) progress in developing a chip for the identification of fish eggs and larvae. The identification of fish eggs and embryos is important for biodiversity monitoring projects, as it is easier to calculate the spawning stock biomass than to find the adults all over the ocean. First, she had to select genes, testing them from DNA of adult and identified fish species. She found cytochrome b to be too variable among individuals, while COI seemed more convenient. Her probes showed very different performances on the chip, which supports the experience of other COST853 participants in probe development. Near future work will include the evaluation of increased and equalized hybridization efficiency, evaluation of other target genes and expansion to other species and more individuals.

The WG4 meeting was completed with a visit in the labs of the UFT group, being guided by very well prepared UFT collaborators. Not only the picture of the chip, reading "COST853", will be spotted onto the memory-chip of the participants of the Bremen Meeting 2003! Thanks for the superb organisation of a very productive and very interesting meeting.

Moni Pfunder

# Agenda

## Wednesday, September 24

13:30 Registration and Welcome

14:00 Management Committee Meeting, Part 1

1. Welcome to participants
2. Adoption of agenda
3. Minutes of last meeting
4. Report from the Scientific Officer
  - News from the Commission
  - Status of Action
  - Number of Signatories
  - Budget Status, budget allocation process
5. Budget planning 2003/04
6. STSM status, applications
7. Publications, annual report

15:30 – 16:00 Coffee Break

8. Evaluations
9. Request for new members
10. Non-COST participations
11. Web news

17:30 End of Management Committee Meeting, Part 1

**Thursday, September 25**

- 08:30 – 08.45 Welcome address  
D. Blohm,  
Centre of Applied Gensensorik (CAG)
- 08:45 – 09.30 “The Bremen Gensensorik Project”  
D. Blohm, Speaker of the Centre of Applied Gensensorik (CAG)
- 09:30 – 10.00 “New opportunities with LNA-based microarrays”  
N. Ramsing, Director for New Technologies, Exiqon A/S, Denmark (guest)

**10:00 – 10:30 Coffee Break**

- 10:30 – 10:50 “Bioinformatics tools for choosing the right capture sequences”  
U. Bohnebeck et al., Bremerhaven Institute of Biological Information (BIBIS)
- 10:50 – 11:10 “Using the ARB-tool for identification of microorganisms”  
O. Glöckner et al., Max-Planck-Institute for Marine Microbiology (MPI)
- 11:10 – 11:30 “Pitfalls and solutions for target quantification by microarray analysis”  
J. Peplies et al., UFT, Biotechnology and Molecular Genetics
- 11:30 – 11:50 “Constructing a flow-through microarray hybridisation chamber”  
M. Gheorghe et al., Institute for Microsensors, Actors and Systems (IMSAS)
- 11:50 – 12:10 “Advanced technologies for mass production of reliable microarrays”  
U. Steller et al., Director of Research, PicoRapid Technology GmbH, Bremen
- 12:10 – 13.30 Lunch at Cafeteria

**Room: Facility of PicoRapid Technology GmbH, Fahrenheitstr. 1**

- 13.45 - 14.15 Production of microarrays – a demonstration

**Room: UFT-1790**

- 14.30 - 14.50 “Applying microarray technology in milk industry”  
Ch. Glöckner et al., UFT, Biotechnology and Molecular Genetics
- 14.50 - 15.10 “On the way to identify fish eggs and larvae by the help of microarrays”  
N. Silkenbeumer et al., UFT, Biotechnology and Molecular Genetics

**15.10 - 15.40 Coffee Break****UFT, first floor, CAG-Laboratories**

- 15.45 – 17.45 Poster session  
and parallel
- 15.45 - 17.45 Demonstration of microarray handling

**20 h : Dinner at "Haus am Walde"**

**Friday, September 26**

08:00 – 12:00 Management Committee Meeting, Part 2

12. Report of the WG-Coordiators on their activities since our last meeting
  - WG1. Nucleic-Acid Based Microarrays, Peter Bonants
  - WG2. Protein-Based Microarrays, Ian Barker
  - WG3. Bio-Informatics and Information Dissemination, Peter von Rohr
  - WG4. see Thirsday, September 25
  - WG5. Microarray Technology for Environmental Monitoring, Xavier Nesme

10:00 – 10:30 Coffee Break

13. Long-term planning
15. part I AOB

12:00 End of Management Committee Meeting, Part 2

12:00 – 14:00 Lunch

14:00 – 17:00 Management Committee Meeting, Part 3

14. Time and place of next meeting
15. part II AOB

17:00 End of Management Committee Meeting

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