



# **COST Action 853**

## **Agricultural Biomarkers for Array Technology**

**Management Committee Meeting  
and combined  
Meeting of Working Groups 1 and 2**

**Gdansk  
June 20 – 21, 2005**

**Draft Minutes**

**Centre of Excellence in Bio-safety and Molecular  
Biomedicine "BioMoBiL", Gdansk**

# Draft Minutes of the Management Committee Meeting

## 1. Welcome to participants

The MC meeting is opened by Juerg E. Frey, the chairman of the action, at 16.10 on Monday 20.6.2005.

## 2. Adoption of agenda

The draft agenda for the Meeting in Gdansk is approved by all MCMembers. No additional requests are mentioned.

## 3. Minutes of last meeting

The minutes of the third MCMeeting held in Helsinki in 2004 are approved with the exception of one change (an address-change) that reached the Chairman via e-mail.

## 4. Report from the Scientific Officer

*News from the COST office:* Chairman J. Frey informs the Working Group leaders via e-mail about the letter of the scientific officer Bouktje Stol from April, where she pointed out that all available STSM money from the central STSM fond for 2005 has already expired and that there are no further requests accepted this year.

*Status of the action:* The actual status of the action is available for all interested participants and collaborating scientists 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, have signed the action (see <http://www.COST853.ch>). This is the same status as at the last MCMeeting.

*Budget status, budget allocation process:* see below, 5. Budget planning 2005

## 5. Budget planning 2005

Budget planning was performed at a dedicated meeting in November 2004 in Brussels, attended by the chairpersons and all working group leaders as well as B. Stol (scientific secretary) and Chr. Peeters (scientific secretary assistant). At that time it was decided that the WG4/5 meeting in November in Lyon will be paid from the 2006 budget. However, in April 2005 the information came from the side of Brussels that the Lyon-Meeting will have to be financed by 2005 budget.

After the MCMeeting, J. Frey discussed the topic with John Williams, earlier and new Scientific Officer that will replace B. Stol. The meeting in Lyon was confirmed with 40 total participants, 32 eligible for reimbursement. The meeting in Zurich on 16th December for a total of 20 participants of whom up to 16 will be eligible for reimbursement is provisionally planned, however, the number of participants will depend on the final level of reimbursements from the Gdansk meeting.

Via e-mail, J. Frey will inform the working group leaders about the decision.

## 6. STSM status, applications

As reported by Neil Boonham during the meeting, the second STSM workshop in York in October 2004 was very successful. The question, whether we should plan for other workshops were discussed. Jürg Frey informed the MCMembers that the participants at both STSM workshops would have been interested to go deeper into the topics of probe design and data handling.

Neil Bonham offers to do another workshop in York, UK. J. Frey suggests to combine the workshop, by including hands-on experience on slides with bioinformatics from the produced data. It was discussed that the workshop could be organized as a ring test, which was also planned for York 2004.

The idea of such a workshop was supported by the MC members and it was decided that there should be another STSM workshop in 2006 if this is approved by COST Brussels. There will be a decision at the next meeting in Lyon after having had the opportunity to discuss the topic with the scientific officer in Brussels. As there is another WG3 (Bioinformatics) meeting in Switzerland, the participants of that meeting may take the occasion and prepare an outline of the organization of a STSM meeting on bioinformatics in Switzerland. To organize such a workshop, J. Frey asks the members to inform him via e-mail on the probe design program they use as well as on what the advantages and disadvantages of these programs are.

J. Frey points out that, independent of the workshops, individual STSM are still possible but will have to be delayed to 2006.

### **7. Publications, annual report, mid-term evaluation**

At our last meeting from August 16-17 in Helsinki, Finland, Jørgen Jacobsen from the Danish Institute of Agricultural Sciences was attending as rapporteur for the Technical Committee for Agriculture, Biotechnology and Food Sciences to perform the mid-term evaluation of our action. As he told us at our social dinner he was positively impressed and he indicated that his report will be favourable.

For the next annual report (deadline end of 2005), the working group leaders are asked to send again a short information on the proceedings in their field.

As in the earlier meetings, the participants were encouraged to send their publications that relate to COST 853 to be listed on our homepage.

J. Frey thanked for the authors that acknowledged COST853 in their publications. He stresses that this is an important feature as references for potential follow up projects.

### **8. Evaluation**

Peter Bonants, working group leader of WG1, nucleic acid based microarrays, informed on the process of 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. An earlier E-mail request for information from P. Bonants had had an unsatisfactory feedback. Only about 10 persons responded and it may already be necessary to be updated. Therefore, there will be one further attempt with the **deadline of September 1<sup>st</sup>, 2005**. From October 1<sup>st</sup> the information will be put onto the web.

### **9. Request for new members**

No request for a new membership is open.

### **10. Non-COST participations**

No request for a new membership of non-COST countries is open.

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

Jürg Frey makes the yearly call for information that can be put on the web, including links, presentation, interesting meetings etc.

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

As already mentioned in point 7, a written statement will be expected by the working group leaders by the end of the year. The chairman will send a reminder, **December 6, 2005 is deadline!** The working group leaders can delegate the task to the single labs of the country to collect information. There would also be the possibility to ask country delegates to collect information on the progress in their country.

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

G. Adam initiated a cross-COST Action meeting in 13./14. October 2005 in Hamburg to evaluate possibilities of collaboration among the different groups. This meeting will also be used as the COST Action 853 year 2006 organisation meeting. Therefore, the WG leaders were asked to participate. MCMembers were asked to submit their ideas and inputs to the corresponding WG leaders.

The MCMembers discuss about a possible follow up of this COST action that will end in 2007, either as another COST, or as a FW7 project. A follow-up COST could include a scientific rather than a developmental topic. Topics discussed are “soil health”, “harmonization and quality of ring testing” EPPO may strongly support such an action.

Chairman J. Frey suggests to think about collecting material to summarize our activity and experience, as COST offers the opportunity to publish books or papers. It is suggested that each active microarray scientist of the group could write 4-5 pages and the work is collected in a booklet.

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

Peter Bonants from the Netherlands offers to host a combined MCM and WG1/2 meeting in 2006. The offer is thankfully accepted. The date may be around end of May and mid June.

### **15. Varia**

P. Bonants informs about the 2008 ICPP meeting in Turin. As he is invited to address a part in the field of diagnostics and potential speakers, he asks MCMs to send any input and ideas to him.

The meeting is closed at 17:30.

# Meeting of WG1 and WG2 - Report

This year's combined management committee and Working Group 1 and 2 meeting was held in Gdansk, Poland. It was organized by Ewa Lojkowska and her team from the Centre of Excellence in Biosafety and Molecular Biomedicine "BioMoBiL".

The meeting included 6 talks from experts as well as 12 seminar presentations. The opportunity to give a five minute oral introduction to a poster was taken by 3 of the 11 poster presenters. More information on the talks and posters are available on [www.COST853.ch/Meetings853.htm](http://www.COST853.ch/Meetings853.htm)

On **Sunday**, the day of arrival for most participants, the organizers invited everybody to a grill party. At this informal gathering in the open, including a large campfire and many grilled delicacies, people had the opportunity to chat, exchange news and to strengthen the network.

On **Monday**, the meeting was opened by the Chairman Jürg Frey, the local organizer Ewa Lojkowska and Dr. Jacek Bigda, the dean of the Intercollegiate Faculty of Biotechnology. The meeting started with 6 talks of expert in the morning session. In the afternoon, the first batch of seminar talks followed. From 16:10 – 17:30 the Management Committee Meeting took place. A workshop dinner with infinite amounts of Polish specialities rounded the first day off.

**Tuesday** was organized into seminar talks in the morning and a poster session as well as important informations on ERANET and funding opportunities in FP7 by Ian Barker the afternoon. The meeting was closed at 13:00 with a final word from Peter Bonants as well as from Jürg Frey, both expressing their satisfaction with this successful meeting and giving sincere thanks to the host Ewa Lojkowska and her hard-working team of co-organisers.

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## Talks, Posters and Discussions

### Monday, 20.6.2005

**A. Mienko, J. Odkowinski, L. Handschuch, W. Wloszczak, M.M. Sikorski, A.B. Legocki, M. Figlerowicz**

*The application of microarray technology in the studies of plant-microbe interactions.*

Introduction of CENAT (Center of Excellence for Nucleic Acid-based technologies), that was established 2004. The Regional Genomic Center, Part of CENAT, is prepared for microarrays and has obtained all technological instruments.

#### Plant – pathogen interactions:

Study of *Arabidopsis thaliana* resistance using microarrays for the classification of pathogenesis-related genes. General approach is to isolate RNA from healthy and stressed, pathogen infiltrated, elicitor-treated and hormone-treated plants. cDNA is then hybridized to a plant specific microarray.

#### Production of microarray:

Per gene they use 28bases long probes, with a 8-fold replication per slide. Probes are printed on SpotArray24, PerkinElmer. Hybridization at 45°C, 16h with RNA. Scanned with ScanArray Express.

#### Sample preparation:

Biotinylated cRNA after RT-PCR followed by IVT, or Cy3 labelled cDNA.

#### First results:

High expression levels of pr-10 genes, while low level of csbp and ferritins families.

Some of the results were confirmed by RT-PCR, others were contrary (subclass of Llpr-10.2).

Optimization needed.

#### Plant infection-pathogen infiltration assay

Microarray hybridization experiments with RNA isolated from pathogen-infected tissues.

Comparison of pathogenesis-related genes expression patterns in different pathogenetic situations.

Applications of microarray technology in plant disease diagnosis.

Example of *Lupinus angustifolia*: infected by *Bradyrhizobium* sp.  
cDNA of marker genes: actin, 1S, 26S. SNF: eg. SXMRk, ENOD40.  
Stress: e.g. R10, Pathogen induced oxygenase.  
768 clones from SNF 5dpi were analysed with membrane arrays of density 10 clones per cm<sup>2</sup>.  
Several clones found with enhanced expression. 79 candidate clones were found and confirmed with macroarray technology.

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### **Piotr Formanowicz**

*On some principles of DNA microarrays design and data analysis*

Introduction to the different microarray types, and the basic features of probes (complementarity, specificity, melting temperature). Probe types are probes specific to single genes, probes specific to a group of genes or reference probes. Probe features: substrings not longer than 25%, GC between 30 and 70%, no secondary structure. Overall complementarity to non-target genes should be less than 75%.

T<sub>m</sub>: minimal difference between melting temperature of duplexes created with target and non-target template DNA. Nearest Neighbour model. Models are designed for duplexes in solutions and may be inappropriate for oligonucleotides attached to a solid surface.

Data analysis: used for either hypothesis testing or knowledge discovery.

Hypothesis testing:

#### A) Top-down process

Starts with hypothesis that is verified or not.

3 main stages:

1. design and execution of MA experiment
2. Construction of a mathematical model
3. Evaluation to verify the model

#### B) Bottom-up process

No prior assumption of the data. Let the data suggest new hypothesis. Can be directed or undirected.

In first case we try to explain patterns, in second we try to find some patterns.

Algorithms are used to either classify or cluster data.

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### **Jan Sadowski**

*DNA microarrays in studies of plant responses to abiotic stresses*

Study of the genetic bases of a response of a plant to environmental stress. The process is complex and includes many different genes and gene complexes. Only 35% of plant genes are unique. Therefore, we study many homologues of genes when we study plant genomes.

#### Experiment:

Goal: analyse plant stress response upon ozone- and drought stress. Analyse the regulation of ethylene and abscisic acid pathway.

Role of ABA in ozone induced response. Identify responses with selected multigene families. Used *Arabidopsis thaliana* ecotype Columbia and mutant *abi1-t-salk*.

#### Microarray:

Affymetrix technology.

#### Analysis:

Check for outliers, using dCHIP outlier detection. Principal component analysis (PCA) was used to check the chips for similarity in profile. Groups could be observed for the two stress and the control chips. Normalization was done using the robust multi array analysis (RMA). Statistical analysis of the data was done by Genespring.

Verification of microarray accuracy was conducted by quantitative realtime PCR, analysing the different ratios with regression analysis.

Analysis of drought stress: variance analysis (2-way ANOVA). Genes that changed transcription level more than 2-fold at least at one time point of the stress.

Conclusions of the study:

ABA is required for ABI1 regulation

ABA signalling requires phosphorylated components

ABI1 is a negative regulator of ABA signalling.

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**Cor Schoen**

*Diagnostic application of padlock probes - multiplex detection of plant pathogens using universal microarrays*

Targets to be detected: Phytopathogens important to agroecosystems

Multiplex detection of different DNA in two colors.

Padlock Probes

Ca. 100bp length

Target complementary sequence on 3' and 5' end. Hybridization forms a circle (using a ligase). If there is a mismatch between the probe and the target, then the probe is not circularized.

Experiment were conducted with model padlock and oligonucleotides targets, using padlocks with pointmutations on the ends or also in the middle of the probes. Result: 5' end is longer than 3' end, ca. 30 bp long, functioning as an anker, while the 3' end is the real probe, ca. 14–18 bp. That way a mismatch is most destabilizing.

Test with different pathogens.

Zip-Code part (next to probe on 3' end) was designed by Affymetrix. 20 nt oligo, nonsense sequence, universal melting temperature. Each padlock contains ZipCode which is unique for the targeted pathogen.

Universal Microarray (Epoxycoated slides) with 16 different arrays on one slide. cZipCodes spotted on the slide. PP with corresponding ZipCode will hybridize onto the array.

No targets: if you don't add target you get non-specific amplifications! => always add a non-target control labelling with Cy5, using targets with Cy3, so they can correct for these amplifications using them as background for normalization!

The dynamic range of the detection still works with 0.5pg of pathogenic DNA.

Approach is not quantitative.

Applications:

Array for 33 pathogens (Fungi 22, Bacteria 5, Nematodes 4, viruses 2).

Detection planned for Soil, Air, Quarantine organisms, recirculation water, irrigation water.

Multiplex experiments were done by spiking in water and soil.

Quantitative multiplex detection is strongly wished for further studies. What are the possibilities with Padlocks?

Multiplex ligation, singleplex amplification in a nano-liter Realtime PCR Array, using unique primer pairs and universal TaqMan probe (collaboration with another company).

48 different arrays prespotted with different primer combinations, so one can follow different amplification in an array of 96 wells.

Advantages:

Quantitative

Target recognition and amplification is independent

Universal TaqMan conditions

High throughput

Low background

Disadvantages:

design more complicated than regular padlock probes

Possible low copy numbers of ligated PLPs in nanoliter system

Instrumentation:

BioTrove Thru-Hole array – nanoliter PCR arrays

Multichamber traditional glass microarray (Schott Nexterion)

Nucleic Acids Research, 2005, Vol 33, No 8 e70

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## **Markus Templin**

*Protein microarrays: assays and systems*

Solid phase assay systems: major disadvantage is the small size of spots, therefore the number of detectable material is limited. Advantages are that it is highly parallel, sensitive etc.

DNA-Chips: differential gene expression.

Protein- Microarrays: What is different?

- Proteins are complex with low stability
- No synthetic capture molecules
- No amplification

Types of Protein arrays:

Capture Arrays: antibody on Chip-labeled sample

μELISA on Arrays: miniaturized sandwich assays

reversed Phase Arrays: multitude of arrayed samples

Interaction arrays: Proteins (Proteome) on Chip

Sample labelling Antibody arrays:

Commercial arrays with 200-500 Antibodies

Sample labelling introduces detectable label, but you need very specific antibodies. What you normally capture are multiprotein complexes.

Pro: specificity, multiplex

Contra: crossreactivity, sensitivity, not quantitative, very expensive (500-1000 Euro per array).

μELISA Antibody Arrays:

arrayed antibodies, captured analyte detected by matched detection antibody

Pro: sensitivity,

Contra: low multiplex (10-40 analytes)

Reversed Phase Arrays

Immobilize sample => capture molecules are added => detection

Immobilised samples dozens to hundreds of samples in parallel. Complete protein content of a cell.

Hundreds of spots even from laser capture microdissection

One antibody per analyte is enough.

Pro: specificity, multiplex samples, quantitative, Antibodies used also work in western blot, so there are already antibodies around that might work.

Contra: sensitivity cross reactivity

### Protein Arrays

Array proteins directly (not antibodies).

Purification of proteins is heavy work. But there are companies that sell them.

### Technologies:

Zeptosens.

Planar Waveguide Technology: Light of the laser travels “through the slide” just below the molecules. Therefore, things in elution are not measured. Sensitivity is very high (detection of 2-3000 molecules in a spot). High reproducibility, semi automation.

### Arrays on beads:

Based on established Cytometry Technology, Simple liquid handling, standard lab equipment, robust assay performance, good reproducibility. Instead of working with slides you can work as with liquid.

LUMINEX xMAP- BD FACSarray

Beads vs. Planar arrays:

Planar	bead
Up to 2000 analytes	max. 100 analytes
Up to 100 samples a day	thousands of samples a day
Image processing necessary	no image processing
Automation under development	adapted to the MTP format

### Areas of research:

Biomarker screening: Sepsis study (not much material of patients, test less than 50 ul plasma)

Tumour characterisation: Tumour marker identification, look at the interesting proteins with reverse phase arrays (8 spots per sample in duplicate). Example with  $\beta$ -Catenin expression in tumor tissue.

Cell signalling: Reverse Phase Array used for the study of Phosphorylation: Some proteins in signalling pathways get phosphorylated.

Diagnostics: autoimmune diseases

Human kinase substrate profiling array (invitrogen).

More information on:

[www.biochipnet.com](http://www.biochipnet.com)

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### **Xavier Nesme**

*Pangenomic microarrays at the turning point of bacterial systematic and more: investigating the Ralstonia solanacearum diversity*

There are different phylotypes of the *R. solanacearum*. Bacterial species concept: Genomic clusters are ecotypes, adapted to a special niche. Microarray was produced to differentiate the 4 different phylotypes. Presence or absence of genes can give phylogenetic signal as well as ecological informations.

*R. solanacearum* develops a competent stage, where it is open to accept DNA from the outside. This was shown in vitro as well as in the plant. What is the level of transformation ability of the strains?

Can be measured by arrays.

Pangenomic microarrays can be used for systematic investigations:

- Looking for strain clusters
- Looking for core genome e.g. house-keeping genes
- Looking for Accessory genome e.g. ecologically relevant genes
- Looking for genome similarity e.g. large horizontal gene transfers

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## Neil Boonham

*COST 853 plant virus nucleic acid microarray: ringtesting and results*

Review on the COST853 workshop in York 25-29<sup>th</sup> October 2004:

1. Array of 12 viruses of Petunia. Species and genus specific oligos. Testing vegetative propagation material. Currentling used: ELISA. Aim was training and oligo testing.

Always working with pairs of inoculated vs. non-inoculated healthy plants.

24 slides were done. Controls worked on 2, good detection of TuMV, weak detection of TMT

=> as the probes have never been tested before, the results are good.

Control spots used are generally a gene from the host plant

2. Spherical virus array:

Generally used: biological tests or electron microscopy. Array with 22 different viruses. Test an unknown virus in Digitalis. By ELISA all tests negative. With array they found Broad bean wilt virus 2, a virus that is not listed for Digitalis, but was on the chip.

Future work

Need for amplification methods

Currently have multiple arrays – a bag of chips.

Better formats?

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## Jenny Tomlinson

*DIAG CHIP update: ring testing comparisons of slide chemistries*

Array of all EU quarantine potato pathogens. 12 viruses, 2 bacteria 1 fungi, 6 invertebrates, etc. 50mers, amino-allyl post labelling protocol for viruses.

Epoxy-slides: Schott Nexterion ca 10 E

Amine slides: CEL associates Amino ca 1E

Aldehyde: CEL associates Aldehyde ca 1.2 E

Post -spotting

Epoxy-slides: high humidity

Amine slides: UV crosslink

Aldehyde: low humidity

Amino modification of oligos

For this size of oligos no need for aminolinkers.

Experiment: with and without aminolinkers and triton added to spotting buffer

Tested with RNA extracted from tobacco infected with PVX, indirect label with dUT during rev. transcription. All hybrid. And washing conditions with same conditions.

Result: Epoxi have smaller spotsizes. Triton increases spotsize a lot.

Modified vs unmodified oligos: There is generally not much difference. Crossreactions have been observed with either with one or the other type for different spots.  
Choice to take Aldehyde slides which have nicest spots and highest signal. Can be used with unmodified oligos.

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### **J. van der Wolf**

*Multiplex detection of viral and bacterial pathogens with the Luminex technology, a fluid microarray system*

Principle of Luminex multiplex immunoassay:

Very simple sample preparation. Extracts are transferred to 96er microplates. Beads are added, 5µm diameter, mixture of red and infrared fluorophores. Like a sandwich-ELISA. 100 different colors can be used, changing the ratio of the two colors red and infrared. Antibodies (green color) are attached to the beads with the proteins. Colored beads are measured floating through a laser with red and green color. 100beads /7seconds.

5plex assay in PBS. Comparing luminex with ELISA showed that there is a very good correlation. Only one negative MA that was positive in ELISA out of more than 90 tests. Assays were also tested with leaf and seed extracts. Reproducibility test with 5 replicates and different treatment (blocking or not). Without blocking, the standard deviations were much larger, for blocked arrays the results were highly reproducible.

Multiplex is possible. Sensitivity comparable with ELISA, can be done in 96 wells format, using non-wash procedure, within 2h, a minimum reagens. Sometimes clogging of needle. Sometimes crossreactions that have to be solved.

Follow up: enrichment techniques , detection of plant proteins in milk products.

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### **M. Ravnikar**

*Nucleic acid microarray application to potato and grapevine pathogen interaction studies*

Main rsearch area: Plant-pathogen and plant-pest interaction. Development of high troughput

Fields: Potato virus

Colorado potato beetle

Grape-vine- phytoplasma interaction.

Long term aim to identify the genes, proteins and signalling molecules or these interactions.

PVY<sup>nm</sup> interaction with potato: Microarray - Time course of differential gene expression after PVY infection. Use of realtime PCR for confirmation.

Different cultivars, resistant, tolerant and sensitive plants.

Studies of plant hormones/enzymes/immunolocalisation of proeinases. Found that virus infection is related to changes of cytokinins. Others are Jasmonic acid and salicylic acid, photosynthetic pigments, peroxidases and proteinase inhibitors, and changes in morphology on cellular level.

Use of cDNA microarrays to find what happens during infection. Sequenced 400 clones and prepared slide. Identification of 175 genes. Expression after different infection levels.

Strongest changes in expression at systemic infections. Different sets of genes are activated during development. Over the whole time about 50% of the genes responded somehow.

Conclusion:

MA were very sensitive and useful for early stages of infection compared to ELISA and elektromicroscopy.

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Gene silencing potato genes: using Tigr microarrays in combination with RT PCR to evaluate resistance in plants with inserted viral coat protein.

Using transgenic plants resistant to PVY.

Colorado beetle: studied time course of the adaptation of plant defense mechanism.

Grape-vine-phytoplasma: oligo microarrays with Genoplant.

Diagnostics of viruses, bacteria and phytoplasmas, Real time PCR, ring testing of microarrays.

GMO detection. CO EXTRA project development of microarrays.

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### **L. Belbahri**

*A DNA Oligo Array for identification of Phytophthora spp. to the species level.*

Development of an Arabidopsis-Phytophthora pathosystem. *Phytophthora brassicae* as a study organism to study the interaction between host-pathogen interaction with Brassica. Preparation of transformed *P. brassicae*. A stage species EST libraries of 8000 Ests. Gene silencing experiments.

Gateway based vectors to enhance efficiency of longer fragments. Genbank data for Phytophthora: ca. 500 sequences (mostly ITS).

Diagnostics: Realtime PCR based on ITS. Microarray. SNP development.

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### **Miroslav Sip**

*Oligonucleotide-based microarray detection of plant viruses*

ssRNA viruses of potato. Propagation by insects, generally found in mixed infections.

Main approaches used: RT-PCR, multiplex RT-PCR, Elisa, Macroarray

Poly-L-lysine coated slides for amino and non-modified DNA. Additional weak (electrostatic) interactions between Slide and DNA. UV-crosslinking improves binding but reduces hybridization accessibility. Although it makes a better (irreversible) binding, UV crosslinking also results in DNA damage, photodimers, strand breaks, multiple crosslinking, less hybridization accessibility and hybridization mismatches.

Hybridization: Known to influence sequence, temp solution composition etc.

Unknown parameters: interaction with support, cross linking and DNA damage, influence of tethers, influence of fluorescence tags, interactions between targets (mixed infections).

Short oligonucleotides approach (40nt). Length 40nt, Tm 60-65°C,  $\Delta G < -65 \text{ kcal/mol}$  (Vector NTI Suite, InforMax). Min 4 probes per virus species.

Results: chip detects different viruses in single and mixed infections.

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### **O. Lenz**

*Parallel detection of fruit-trees viruses by oligonucleotides microarrays*

Investigated are ssRNA viruses. Two groups, with a poly-A-tail ASPV, ASGV, ACLSV, PPV and without poly-A tail (ApMV, PNRSV, PDV). Kane et al 2000:  $\geq 85\%$  similarity, and  $\geq 15\text{nt}$  homologous region. More probes, more specificity.

Probe design:

$\Delta G \leq -60,5 \text{ kcal/mol}$ , Tm 60-65°C, minimum of dimers and hairpins ( $\Delta G \geq -1 \text{ kcal/mol}$ ), length 40 nt. 3 redundant probes.

Used 30ug of total RNA, tested by RT-PCR, and labeled by incorporating modified dUTP.

Slides: Greiner Bio-One, plastic support, commercially printed  
10 arrays per slide, fragmented by frames (the plastic slide is highly sensitive to dust).  
Hybridization: 1-2h vs. overnight (42°C). Scanning by Typhoon Scanner (Amersham).

Results:

cDNA from plant in sufficient concentration  
Control oligos work  
no cross-hybridization of labeled plant cDNA  
viral cDNA is not detectable.  
Detection of PCR product worked.  
No cross-hybridization of labeled cDNA (PCR Product)  
There was no difference between overnight and 2 hours hybridization.

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**Cor Schoen**

*Thru-hole open array technology*

Collaboration with BioTrove (Boston, USA).  
Quantification: using multiplex PCR is inherently problematic to quantify.  
Available qPCR systems:  
ABI7900 (microfluid card),  
BioTrove: Thru-Hole OpenArray system.

BioTrove: slide based realtime PCR with 3072 assays per plate, 3 slides can be run at the same time (OpenArray). Holes that can be filled with fluid (33nl), the hole is hydrophilic, so the fluid stays in there.

Primer combinations can be ordered, they do amplicons of 100-120 bp.  
Array-in-Array loading device for flexible pattern.  
Compared to microplate: less material (sample and reagent) needed, more samples per time possible.  
High flexibility for loading samples  
Pates are preloaded, so researchers only have to add samples.  
Can also be used for expression analysis.

Prices:

System: 100000kE  
Slides: 350 Euro  
Per sample: 7 Euro  
Per single target: 7 Eurocent

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**J. Lewosz**

*Conductive synthetic polymers and metal nanoparticles as structural components of microarrays*

Gold nanoparticles are coated with DNA particles or proteins. Organic metals: forms of polyaniline polymerized on glass plates by oxidation with ammonium persulfate. Aniline can be copolymerized or doped with various organic and inorganic compounds giving functional groups of binding biomolecules and may serve as indicator or transducer of electric signal. Polyaniline can be put on a electrode and here enzymes can be attached. Reactions can be measured by the electrodes. Array of interdigitated electrodes treatment of polyaniline during polymerization allows to get a small polymer micelle (small beads of some molecules) of polymer of 20nm diameter. This was then used to make metallic gold nanostructures, putting the gold particles onto these polyaniline beads. Particles can be

used for dielectric separation (changing the current of energy to keep particles “floating”). Particles can now be attached to bacteria. Dead bacteria behave differently in the electronic field than alive ones, so they can be separated. Detection of DNA on IDE: oligos attached to electrodes. Portable 8-channel galvanostat-potentiostat. This portable instrument allows the measurement of maximal 8 tests (macroarray).

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## **J. Kinkorova**

### *The importance of nonstandard methods in systematic entomology*

Research in classical systematic entomology is based on standard keys, based on anatomical and both external and internal morphological characters, as well as newly also morphometry and advanced statistical methods. The above-mentioned tools work for the determination of most species. However, certain species within species complexes as well as individuals of different populations cannot or hardly be distinguished using morphological markers.

Example for population studies: *Urophora quadrifasciata* (Diptera: Tephritidae) a species of fruit flies which was introduced to the USA and Canada to act as biological control agent against serious and introduced (from Europe) knapweeds. There were introduced two populations of the fly: first introduction came from Greece and was originally released to British Columbia in Canada in 1972 and the second was released later and originated from different European locality to eastern states of the USA. We would like to compare the two populations and their development in the new conditions, but standard methods of the systematic entomology has not powerful tools to do it seriously, and at this moment the molecular methods help us without any doubt. In this special case we are approaching the question with *microsatellites* or *AFLP* methods.

A second example is the differentiation of different fruit fly species, many of which are agronomically relevant pest organisms. For example, species within the complex of *Bactrocera dorsalis* or the two species *Rhagoletis cingulata* and *R. indifferens* can only be distinguished morphologically if they can be found on their host plant. These species all belong to quarantine organisms in Europe. Molecular tools would greatly help to identify these species if they occur in traded fruit on the border and to initiate the appropriate quarantine measures. Here, a diagnostic microarray is an interesting approach.

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## **Ian Barker**

### *Plant Health ERANET proposal and funding opportunities in FP7*

Ian Barker is involved in FP7 agricultural projects concerning pathogens

#### **FP6:**

- Area 8: portcheck , Sigma (GMO detection), Rapra (risc studies)
- Area 5: co-extra (Food area) (microarrays?)

New calls ? Not clear

Phytosanitary ERA-Net – introduction

Is being implemented right now. Officers of plant health services are supporting it (COPHS)

Was initiated by this group in Jan 05

Scope:

**ERA-net** for research policy development and implementation in the area of Statuary and merging pests diseases and invasive species (but not GMOs).

Objectives:

- Manage and coordinate ERA-Net
- Gather and analyze information
- develop instruments,
- mechanisms and processes to joint trans-national activities and for the funding of trans-national phytosanitary research programs
- Trial evaluate and refine instruments
- Develop a common research area and long term study
- Communicate the aims and process

Right now finding commission. Till now 13 countries are involved. Proposal submitted in October 2005 to FW6.

Relation to FP7: once the network is established would want the network to be a key point of contact with the commission on issues relating to FP7!

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**FP7:**

Plant Health appears in FP7 program draft document:

FP7 lobbying on behalf of LUX/UK/NL to include calls on (only suggested topics from these countries)

- plant health diagnostic capabilities, methods and expertise
- plant health diagnostic infrastructures
- plant health risk analysis
- plant health invention (management, control of quarantine organisms)

**Poster presentation**

**Complete list see in the Agenda**

**Oral presentations by:**

Polona Kogovsek  
J. Lewosz  
P. Bonants

**Closing remarks by Peter Bonants**

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