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MEETING REPORT

DNA arrays: methods and applications: report on HUGO Meeting, Tartu, Estonia, 23–26 May, 1999

C Auffray[1], C Mundy[2] and A Metspalu[3]

[1]CNRS UPR 420, 19 rue Guy Mocquet, 94801 Villejuif cedex, France; [2]HGMP, Cambridge, UK; [3]Estonian Biocentre, riia 23, Tartu 51010, Estonia

In order to keep up with the rapid progress of the Human Genome Program, there is a pressing need for high-throughput technologies analysing DNA variations in genomes and RNA expression in the cells. One of the appropriate and obvious solutions to this is DNA microarray technology. To have an instant snapshot of the DNA array landscape taken from Europe, a DNA arrays workshop sponsored by HUGO was held recently in Tartu, Estonia.[a]

Mutation analysis and testing of biallelic markers

Mutation detection and testing of biallelic markers is as another expanding field where DNA oligonucleotide arrays have been used quite extensively. Nine speakers devoted 30–40 minutes to cover different aspects of the array methods. Russ Granzow (Orchid Biocomputers) presented impressive data about his company's approach to the SNP discovery. Currently, Orchid Biocomputers is holder of 50 relevant patents, in addition to nearly 100 still pending. The company controls IP on different modifications of the primer extension array, known as the GBA™ (Genetic Bit Analysis) and has set a target of making 10^8 genotypes per year, at a cost of less than 1 US cent per marker.

Laboratories specialising in developing primer extension type arrays were well represented at the meeting. Ann-Christine Syvänen (Uppsala/Helsinki), inventor of 'minisequencing' primer-guided nucleotide incorporation in 1990, gave an excellent overview of primer extension assays. Tomi Pastinen, a PhD graduate in A.C. Syvänen's Helsinki laboratory, presented actual minisequencing genotyping data on oligonucleotide arrays. In connection with the large-scale carrier screening program in Finland, they have managed to analyse more than 90 000 human genotypes. They use RNA reverse transcription to produce single-stranded template polynucleotide for primer extension.

[a] **HUGO/EU workshop *DNA Arrays — Methods and Applications*, 23–26 May 1999, Tartu, Estonia. (http://www.hugochip.ebc.ee)**
[b] **Currently with Corning Inc., USA.**

Andres Metspalu (Tartu) presented Arrayed Primer Extension (APEX) array technology designed for mutation detection and DNA resequencing. Ten of the most common β-thalassaemia mutations in Greece were analysed by using four-colour APEX array. A special four-colour CCD based fluorescence detector for APEX was designed in Tartu (www.asper.ee). Lisa White (Houston) presented a 52 SNP APEX Array for personal identification coupled to single tube 52-plex PCR. Comparative genomic hybridisation (CGH) was extended into matrix-CGH based on immobilisation of continuous genomic fragments of approximately 75 kb, to the glass slide (Peter Lichter, Heidelberg). By hybridising genomic DNA from B-CLL patients to this array, the resolution power of CGH in this case in this case was increased by two orders of magnitude with specificity of 95% compared with interphase cytogenetics. Michael Mindrinos (Palo Alto) reported on the usage of biallelic variant detection arrays (VDAs) combined with denaturing high performance liquid chromatography (DHPLC). This approach was used for the construction of the *Arabidopsis thaliana* genetic map in collaboration with Affymetrix Inc. To assess the feasibility of their SNP-based method for whole-genome genetic mapping, they confirmed the location of *eds* 16 mutation in a 7 cM interval at the bottom of the chromosome 1 of *Arabidopsis thaliana*.

Expression profiling

The emergence of microarray technology is enabling high-throughput approaches to gene expression profiling. Two key approaches are based on microarrays of oligonucleotides, synthesised *in situ* by a photolithographic process, and robotically-generated microarrays of PCR products derived from cDNA clones. Thomas Gingeras (Affymetrix) described the application of the former approach to the study of gene expression changes associated with viral infection (HCMV), and also to type bacterial isolates which differ in their pathogenicity. The Affymetrix system uses 10 perfect match

and 10 mismatched immobilised targets for each labelled probe species, and high-quality data were shown. Cost and flexibility seem to be the major perceived limitations to the use of this approach. François Chatelain (Protogene Laboratories) presented an approach to high-throughput solid-phase oligo synthesis based on accurate ink-jet printing of reagents on to hydrophilic spots on hydrophobic glass substrate. Hans Peter Saluz (Jena) described the use of tiny rotating sets of spongy stamps to print reagents on to glass. These methods may permit cheap and flexible chip manufacture. PNA arrays (Stefan Matysiak, Heidelberg) were also used for expression profiling.

Many presentations focused on the technical details of generating reproducible PCR product arrays; a good indication that in this case it is the current state of the art which is limiting. Printing on glass using metal pins was discussed in terms of optimum pin design; whether split, concave or flat-ended. Various labs have found different designs perform best; for example the pioneering work of the Stanford Laboratory (Mark Schena, USA) has been based on split pins. High-resolution photographs were shown to indicate the critical dependence of reproducible arrays on accurate pin fabrication. The pictures which showed complete evaporation of liquid from flat pins within 5 s (Holger Eickhoff, Berlin) emphasised the importance of humidity control.

Surface chemistry was seen as a major source of variability. Uwe Muller[b] (Vysis) described the use of chromium-coated glass. Tim Richardson (Amersham Pharmacia Biotech) referred to 'metal-coated' slides as the most recent development within the APB collaborative programme.

Amplification methods were discussed as a possible solution to limited mRNA availability; for example Ulf Landegren (Uppsala) described his 'padlock probes' yielding substrates for rolling circle replication of a probe hybridised to an immobilised oligo target. Bertrand Jordan's (Marseille) presentation of data using nylon membranes in microarray as well as macroarray format raised the key issue of required probe concentration and total probe quantity. Glass arrays require at least 10 times more probe than nylon arrays, but until now the benefits of double fluorescent labelling have not been realised on nylon. Gert-Jan von Ommen (Leiden) presented a new porous membrane product, PAM™ (developed by Organon Teknika) which allows fluorescent labelling, but it remains to be seen how it will perform.

Access to clones for PCR product deposition on micro-arrays was seen as the major current limitation to the use of this technology for expression profiling. Despite serious quality problems, the IMAGE clone bank was seen as the most important resource. Bernard Korn (Heidelberg) described the work at the German Genome Project resource centre (RZPD) to make available minimally redundant sequence-verified gene sets from human, mouse and rat. Protein expression libraries are also available from RZPD as macroarrays.

Future technologies

There were descriptions both of new uses of existing platform technologies, and of the application of new technologies to the field. Hans Peter Saluz (Jena) has isolated DNA sequences which bind protein *in vivo* by a combination of cross-linking, nuclease protection and protease digestion, and arrayed these sequences for hybridisation analysis with labelled cDNA. This enabled the study of the response of DNA binding proteins to induced cellular changes. The use of known inducers of c-Jun enabled the identification of new c-Jun binding elements. The piezo-electrically generated protein expression arrays described by Holger Eickhoff also offer an exciting view of the future.

Chris Mundy (Hinxton) addressed the future application of atomic force microscopy to the interrogation of nano-arrays, for the measurement of intermolecular interactions and other applications.

Data and knowledge bases

The large amount of data collected in expression profiling experiments using oligonucleotide or cDNA micro-arrays require the use of dedicated software and the development of data and knowledge bases.

Currently, multiple platforms are used by a growing number of groups, each set up with specific parameters and using different models and software packages to register, normalise and compare the hybridisation signals collected to study a wide variety of biological systems. The problem of radioactive spot recognition, quantitation and normalisation on macro-assays was discussed by Holger Eickhoff (Berlin) and Charles Auffray (Villejuif), whereas the use of two color fluorescence ratios was illustrated in the presentations by Mark Schena (Stanford) and Klaus Wilgenbus (Boehringer Ingelheim R & D) using cDNA micro-arrays, and by Shirley Horn-Saban (Rehovot) and Thomas Gingeras using oligo arrays. This included active discussions during the entire workshop pointing to the need of standardisation at all stages of the process, from image acquisition through knowledge extraction (*Nat Genet* 1999; **22**: July). It was felt very important to collect the data in a common format, a prerequisite for performing comparisons across platforms, laboratories and biological systems. This will be essential to validate the potential of gene expression profiling in monitoring modulations of entire transcriptomes, and their relevance to normal and pathological biological processes. Jaak Vilo (EBI, Hinxton) pointed to the fact that although there is a growing number of groups involved in the production of expression profiles, very limited data is currently publicly available on dedicated web sites, such as those produced by Pat Brown and David Botstein at Stanford and the Genexpress IMAGE Knowledge Base established by Charles Auffray at CNRS. He went on describing the plans to develop a public repository of gene expression data at EBI, based on input from the community of users.

Conclusion

The meeting provided a very good overview of microarray-based technologies, applied principally to expression profiling and mutation detection. In both cases many useful applications data were shown. The inherent reproducibility of the various mutation detection technologies is, however, generally better than that of the expression profiling technologies, and in the latter case technology development was the main emphasis. The need for standardisation of controls, and benchmarking to measure reproducibility between laboratories was discussed in detail.