*Pollack, Jonathan R.*

## Genome-wide analysis of DNA copy number variation in breast cancer using DNA microarrays

**Jonathan R. Pollack[1], Charles M. Perou[1], Therese Sorlie[2], Ash A. Alizadeh[1], Christian Rees[1], Michael B. Eise[1], Alexander Pergamenschikov[1], Cheryl F. Williams[1], Matt van de Rijn[1], Stefanie S. Jeffrey[1], Hilde Johnsen[2], Per E. Lonning[2], Stephanie Geisler[2], Turid Aas[2], Anne-Lise Borresen-Dale[2], David Botstein[1] & Patrick O. Brown[1]**

[1]Stanford Medical Center, Stanford, California, USA
[2]The Norwegian Radium Hospital, Motebello Oslo, Norway, and the Haukeland University Hospital, Bergen, Norway

Gene amplifications and deletions frequently have pathogenetic roles in cancer. 30,000 radiation-hybrid mapped cDNAs provide a genomic resource to map these lesions with high resolution. We developed a cDNA microarray-based comparative genomic hybridisation method for analysing DNA copy number changes across thousands of genes simultaneously. Using this procedure, we could reliably detect DNA copy number alterations of twofold or less. In breast cancer cell lines, we have mapped regions of DNA copy number variation at high resolution, revealing previously unrecognised genomic amplifications and deletions, and new complexities of amplicon structure. Recurrent regions of DNA amplification, which may harbour novel oncogenes, were readily identified. Alterations of DNA copy number and gene expression could be compared and correlated in parallel analyses.

We have now collected genome-wide DNA copy number information on a set of 9 breast cancer cell lines and over 35 primary breast tumours. For the breast tumours, DNA copy number information is being compared and correlated with data already collected on p53 status, microarray gene expression profiles, and treatment response and clinical outcome. The results of this analysis will be presented.

*Porkka, Kati*

## Detection of differentially expressed genes by combining suppression subtractive hybridization and cDNA library array

**Kati Porkka & Tapio Visakorpi**

Laboratory of Cancer Genetics, Institute of Medical Technology, University of Tampere and Tampere University Hospital, Tampere, Finland

Differential expression can be used to identify genes that are likely to be involved in the development and progression of cancer. To detect genes whose expression is decreased in prostate cancer, we have combined two methods: suppression subtractive hybridization (SSH) and cDNA library array. Although both methods are potentially powerful, so far they have been inadequately validated. Here we have studied the enrichment of differentially expressed sequences by subtraction and investigated the sensitivity, linearity and reliability of cDNA array hybridization. A subtracted cDNA library was constructed using benign prostatic hyperplasia (BPH) as a tester and a prostate cancer cell line (PC-3) as a driver. Inserts from 386 individual clones were PCR amplified and arrayed on nylon membranes, which were subsequently hybridized with radioactively labelled first-strand cDNA preparations from BPH, PC-3 and several other cancer cell lines. To demonstrate the enrichment of differentially expressed sequences, the number of clones from prostate-specific antigen (PSA) was studied in the subtracted library and in unsubtracted library. Northern analysis was used to confirm the results of cDNA array hybridization. Array hybridization sensitivity and linearity were studied by hybridizing the membranes with cDNA probes containing various amounts of PSA cDNA. The number of PSA was 17-fold higher in subtracted cDNA library compared with unsubtracted cDNA library, demonstrating the enrichment of differentially expressed sequences by the SSH. The detection limit of the array hybridization was found to be 50 pg, corresponding 0.01% of the total poly(A)$^+$ RNA. cDNA array hybridization was linear from 50 pg to 1,000 pg. Of the 386 subtracted cDNA clones analysed, 111 were classified as differentially expressed between BPH and PC-3 by array hybridization. In conclusion, by combining SSH and cDNA array hybridization we were able to detect sequences that are differentially expressed in BPH and PC-3. Further studies will be carried out to determine whether some of them are truly involved in tumorigenesis of prostate cancer.

*Raitio, Mirja*

## Y chromosomal SNP genotyping using oligonucleotide microarrays: comparison of different oligonucleotide immobilization chemistries

**M. Raitio[1], K. Lindroos[1], L. Kauppi[2], T. Pastinen[1], P. Niini[3], A. Sajantila[2] & A-C. Syvänen[1, 4]**

[1] Department of Human Molecular Genetics, National Public Health Institute
[2] Department of Forensic Medicine, University of Helsinki, Helsinki, Finland
[3] Technical Research Center of Finland, Finland
[4] Department of Medical Sciences, Uppsala University, Academic Hospital, Finland

Y-chromosomal single-nucleotide polymorphic (SNP)-markers are of particular importance in studies on population genetics and human evolution, because they represent slowly evolving mutations and may be regarded as unique events during evolution. We are currently investigating paternal lineages from different Finno-Ugric speaking populations. An oligonucleotide microarray screening system for analysing 28 published Y chromosomal SNPs has been set up. The method is based on the single nucleotide primer extension reaction[1] and allows direct multiplex determination of SNPs at known sequence positions. The microarrays are prepared using a custom-built robot to deliver the oligonucleotide primers onto a support. We have investigated three different oligonucleotide attachment chemistries. 5' NH2-modified oligonucleotides have been attached onto epoxysilanized[2] or isothiocyanate activated glass[3] and 5' succinylated oligos have been attached by amide bonds to aminopropyl silanized glass[4, 5]. We show that the signals obtained in the mini-sequencing reactions on the array are greatly affected by the chemistry used for attaching the oligonucleotides.

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