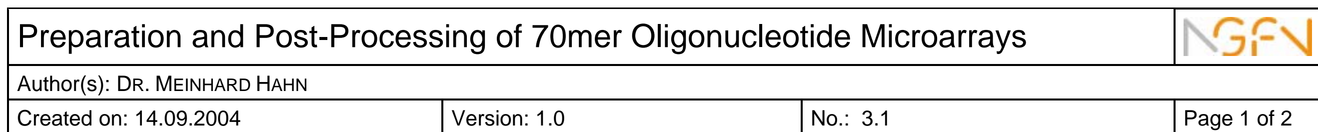

General

Synthetic 70mer oligonucleotides ("Human Genome Oligo Set Version 2.1"; consisting of 21,329 oligonucleotides representing human genes and transcripts plus 24 controls, as well as "Human Genome Oligo Set Version 2.1 Upgrade", consisiting of 5,462 human 70mer probes) were purchased from Operon Technologies (Cologne, Germany) and dissolved in FBNC spotting buffer (formamide, betaine, nitrocellulose; Wrobel et al., NAR 2003, 31(12), e67) at 40 µM, using a MiniTrak robotic liquid handling system (Perkin Elmer, Rodgau-Juegesheim, Germany). DNA spotting was performed in duplicates on QMT epoxysilane coated slides (Quantifoil Micro Tools, Jena, Germany) using an OmniGrid Microarrayer (GeneMachines, San Carlos, USA) equipped with Stealth SMP3 Micro Spotting Pins (Telechem, Sunnyvale, USA). Spot centers were 129 µm apart. DNA adhesion to the glass surface was accomplished by 1 h incubation at 60 °C, followed by UV irradiation (2x 120 mJ/cm2 at 254 nm) in a Stratalinker Model 2400 UV illuminator (Stratagene). Just prior to hybridization, slides were washed for 2 min in 0.2% SDS (w/v), 2 min in ddH2O at room temperature and 2 min in boiling ddH2O (95 °C), followed by 3 min centrifugation at 2,000 rpm (867 x g).

Microarray Hybridization

Following completion of the labeling reactions, corresponding cDNA samples were combined and purified on Microcon YM-30 filter columns (Millipore, Eschborn, Germany), as previously described[29]. For blocking of repetitive sequence elements, 25 µg Cot-1 DNA (Roche Diagnostics), 25 µg poly-A RNA (Sigma) and 75 µg yeast tRNA (Sigma) were added before the final washing step. Purified, dye-labeled cDNA was mixed with 120 µl UltraHyb hybridization buffer (Ambion), agitated for 30-60 min at 60 °C, then for 10 min at 70 °C on a thermo mixer and subsequently applied to pre-heated (60 °C) microarrays mounted in a GeneTAC Hybridization Station (Genomic Solutions, Ann Arbor, USA). Hybridizations were performed for 16 h at 42 °C with gentle agitation. Thereafter, the arrays were automatically washed at 36 °C with (i) 0.5x SSC, 0.1% (w/v) SDS for 5 min; (ii) 0.05x SSC, 0.1% (w/v) SDS for 3 min; (iii) 0.05x SSC for 2 min. Flow time was set to 40 sec, respectively. Immediately after completion of the final washing step, the arrays were unmounted, immersed in 0.05x SSC, 0.1% (w/v) Tween 20 and dried by centrifugation in 50 ml Falcon tubes (30 sec at 500, 1000 and 1500 rpm, respectively, followed by a final step of 90 sec at 2000 rpm; 54 x g, 216 x g, 488 x g, 867 x g).

Data Acquisition, Processing and Analysis

Hybridized microarrays were scanned at 5 µm resolution and variable PMT voltage to obtain maximal signal intensities with <0.1% probe saturation, a count ratio of 0.8-1.2 (Cy5 / Cy3) and maximal congruence of histogram curves, using a GenePix 4000B microarray scanner (Axon Instruments, Union City, USA). Subsequent image analysis was performed with the corresponding software GenePix Pro 5.0. Spots not recognized by the software were excluded from further considerations. Result files containing all relevant scan data were further processed using the statistical programming language R (http://www.r-project.org) together with packages of the Bioconductor project (http://www.bioconductor.org). For each hybridization, raw fluorescence intensities were normalized applying the variance stabilization method developed by Huber *et al.* (Bioinformatics. 2002;18 Suppl 1:S96-104). To eliminate low quality data, the data points were ranked according to spot homogeneity, as assayed by the ratio of median to mean fluorescence

intensity, the ratio of spot to local background intensity and the standard deviation of the logarithmic ratios ($\log_2$ Cy5 / Cy3) between spot replicates. Those data points ranked among the lower 20% were removed from the data set. Genes that could not be quantified in more than 33% of all experiments after filtering were excluded as well. To combine the data of dye swap experiments, the $\log_2$-transformed intensity ratios of one array were inverted and averaged with the corresponding values of the other array.

Version	Tracking of changes	Name	Date