MIAME CHECKLIST

1. Array Design Description

A brief description of the array design, feature location, information on the cDNA collection and the spotting protocols can be found on the producer´s website at
http://www.microarray.org/sfgf/jsp/home.jsp
Protocols for the pre-hybridisation procedures (post-processing) of the arrays can be downloaded from our website at http://www.microarray.at

2. Experiment Description

2.1. Experimental design

2.1.1. Laboratory, authors, contact

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2.1.2. Type of experiment

Transcriptional comparison of:

(a) unstimulated PMN

(b) PMN stimulated with cuprophan and polysulphone dialysis fibers

(c) PMN stimulated with E. coli

2.1.3. Experiment factors

PMN were isolated from 5 independent healthy volunteers and incubated with (a) PBS/serum, (b) cuprophane dialysis fibers in the presence of serum, (c) polysulfone dialysis fibers in the presence of serum and (d) opsonized E. coli for 60 minutes. Thereafter total RNA was isolated using the Trizol protocol, and the RNA was subjected to one round of linear T7-based amplification.

2.1.4. Hybridizations

We performed 38 hybridizations: 11 unstimulated, 11 cuprophane, 11 polysulfone and 5 E. coli samples. The first three of the unstimulated, cuprophane and polysulfone samples were performed as technical triplicates, which showed a Pearson correlation of 0.99. For further analysis duplicates and triplicates were averaged.

2.1.5. Reference

Stratagene Universal Human Reference RNA was used as hybridization reference.

2.1.6. Quality controls

To test for reproducibility we calculated the intra-array variability of the triplicate arrays. Triplicate arrays were combined before statistical analysis.

2.2. Samples used, extract preperation, amplification and labelling

2.2.1. Bio-source properties

Organism: Homo sapiens. Patient and control characteristics can be found in the manuscript and on our website (http://www.microarray.at).

2.2.2. Biomaterial manipulations, amplification and labelling protocol

After informed consent heparinized whole blood from healthy volunteers was centrifuged on a Ficoll-Paque density gradient (Biochrom AG, Berlin, Germany), hypotonic lysis of red blood cells using an ammonium chloride buffer was performed, PMNs were collected by centrifugation at 4°C and washed with phosphate-buffered saline (PBS) without calcium and magnesium. The cells were resuspended in PBS with calcium and magnesium and kept on 6°C until use. PMNs were stimulated with 14mg of shredded hollow fibers of cuprophan or polysulfon (Fresenius, Bad Homburg, Germany) or opsonized E.coli (Miltenyi Biotec, Bergisch Gladbach, Germany) for 1 hour in 96-well, flat-bottomed microtiter plates. We used a ratio of 1 human PMN to 25 E.coli at 37°C in the presence of normal human serum (final concentration 25%). RNA of $5x10^6$ PMNs

was isolated using TRIzol reagent according to the protocol provided by the manufacturer (Sigma, St. Louis, MO, USA). The quality of the total RNA was assessed by performing reverse transcription and PCR for detection of the house-keeping gene β_2-microglobulin with a primer pair which was designed to span introns. Only samples which expressed β_2-microglobulin were subjected to amplification. We performed one round of linear RNA amplification using RiboAmp™ RNA Amplification Kit (Arcturus, Mountain View, CA). Reference RNA was as well amplified once. Using the CyScribe cDNA Post Labelling Kit (Amersham Biosciences, GE healthcare, UK) 1 µg of sample aRNA (Cy5, red) and 1µg of reference aRNA (Cy3 green) were labelled as described previously [Rudnicki et al Nephron Exp Nephrol 2004].

2.3. Hybridisation procedures and parameters

Protocols for RNA amplification, RNA labelling, hybridization and washing of microarrays can be downloaded from our website (http://www.microarray.at).

Quality of hybridization was tested using the ANOVA quality tool from Stanford Functional Genomics Facility. A sector ANOVA value of < 0.05 means no investigator bias during hybridizations. A printing-plate ANOVA of < 0.05 means no significant hybridization bias due to the printing process. During this project the mean sector ANOVA value was 0.0164 and the mean printing plate ANOVA was 0.0465 suggesting good hybridization conditions with no significant hybridization bias.

2.4. Measurement data and specification of data processing

2.4.1. Raw data description

Scan hardware: GenePix 4000 B (Axon Instruments, Union City, CA)

Scan software: GenePix Pro 4.1 (Axon Instruments, Union City, CA)

Raw data and array images can be found in the data section of our website (http://www.microarray.at).

Array-ID batch, number (Stanford)	bar code on array (Stanford)	Experiment Name user - defined	SMD-Array-color-tool Sector-ANOVA R-squared values	Printing-Plate-ANOVA R-squared values	GenePix Quality Report Median signal-to-background		Mean of median background		Median signal-to-noise	
					635	532	635	532	635	532
shfr198	12897276	CU 1.1	0,0156	0,0362	4,8	3,7	50	65	8,3	11,8
shfr236	12897681	CU 1.2	0,0053	0,0381	3,7	2,9	76	96	4,6	8,6
shfr254	12897635	CU 1.3	0,0060	0,0230	2,7	2,7	42	68	5,4	8,4
shfr184	12897243	CU 2.1	0,0169	0,0206	4,7	3,4	59	89	7,8	8,9
shfr220	12897517	CU 2.2	0,0130	0,0339	4,3	2,9	48	79	4,5	7,7
shfr41	12921674	CU 2.3	0,0133	0,0604	3,8	2,3	50	84	6,5	6,0
shfr183	12897400	CU 3.1	0,0141	0,0671	3,8	3,4	52	67	3,6	9,4
shfr199	12897274	CU 3.2	0,0054	0,0494	3,5	4,2	72	65	3,7	14,0
shfr251	12897638	CU 3.3	0,0065	0,0270	3,5	3,4	45	65	7,7	12,2
shfr30	12929003	CU 4	0,0240	0,0782	4,3	2,6	57	84	5,5	7,4
shfr53	12921638	CU 5	0,0216	0,0463	3,4	2,9	51	69	6,1	8,1
shfr38	12921645	EC 1	0,0065	0,0530	4,6	2,8	63	89	8,2	8,0
shfr31	12929004	EC 2	0,0392	0,0634	4,2	2,5	52	91	8,0	6,9
shfr39	12929012	EC 3	0,0347	0,0572	4,5	2,6	61	85	8,0	7,1
shfr52	12921639	EC 4	0,0125	0,0468	3,6	3,0	49	62	7,2	9,2
shfr19	12929013	EC 5	0,0148	0,0297	1,9	1,5	38	78	3,6	2,5
shfr182	12897916	PS 1.1	0,0208	0,0479	5,4	3,7	69	69	8,3	11,6
shfr224	12897457	PS 1.2	0,0249	0,0528	3,8	3,3	70	77	3,6	9,6
shfr54	12921637	PS 1.3	0,0179	0,0451	3,7	2,9	45	65	7,9	8,6
shfr196	12897278	PS 2.1	0,0616	0,0463	4,3	3,7	74	70	6,9	10,7
shfr235	12897802	PS 2.2	0,0113	0,0276	3,6	3,0	64	88	3,7	8,4
shfr42	12921673	PS 2.3	0,0139	0,0665	3,2	2,0	47	80	5,3	4,4
shfr197	12897277	PS 3.1	0,0273	0,0628	6,7	4,3	61	73	9,7	12,7
shfr221	12897516	PS 3.2	0,0130	0,0598	3,8	3,0	67	84	3,2	8,0
shfr20	12921605	PS 3.3	0,0061	0,0441	3,3	2,5	61	85	3,9	7,1
shfr40	12921604	PS 4	0,0117	0,0776	3,2	2,2	67	87	5,6	5,5
shfr252	12897637	PS 5	0,0184	0,0387	3,6	3,5	62	65	5,3	12,2
shfr209	12897326	UN 1.1	0,0297	0,0441	7,8	3,7	57	103	15,5	12,1
shfr223	12897514	UN 1.2	0,0074	0,0300	4,5	3,6	58	84	6,7	10,6
shfr250	12897569	UN 1.3	0,0143	0,0309	4,2	3,9	46	53	8,0	14,6
shfr222	12897515	UN 2.1	0,0091	0,0412	4,4	3,2	55	83	4,7	8,9
shfr237	12897680	UN 2.2	0,0042	0,0261	3,8	3,4	78	93	4,7	10,8
shfr253	12897636	UN 2.3	0,0135	0,0320	4,2	3,4	47	73	7,4	11,3
shfr181	12897915	UN 3.1	0,0172	0,0527	4,2	4,1	54	63	4,6	12,5
shfr238	12897679	UN 3.2	0,0066	0,0505	3,4	2,4	50	80	3,5	6,3
shfr29	12929002	UN 3.3	0,0233	0,0514	4,7	2,7	62	96	7,2	7,4
shfr239	12897678	UN 4	0,0122	0,0613	3,1	2,3	48	71	4,6	6,3
shfr32	12929005	UN 5	0,0109	0,0461	2,2	1,6	44	88	3,9	3,1

2.4.2. Image analysis and quantitation

Image gridding and calculation of spot intensity was performed with GenePix Pro 4.1 software

2.4.3. Normalized and summarized data

Normalization was done through the default computed normalization by SMD (at http://genome-www5.stanford.edu/help/results_normalization.shtml). For data retrieval the log2 red/green normalized ratio was used.

Missing values were obtained through computation of k-nearest neighbour (k=10) with the EMV module (http://cran.at.r-project.org/src/contrib/PACKAGES.html#EMV) of the R software package (http://cran.r-project.org).

Significance analysis was performed with the maxT algorithm which is available in the Bioconductor module (http://www.bioconductor.org) of the R software package as well as with the SAM software (significance analysis of microarrays) which is available at http://www-stat.stanford.edu/~tibs/SAM/.