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 website (http://www.microarray.org/sfgf/jsp/home.jsp).

Protocols for the post processing procedure can be found on our website (http://www.meduniwien.ac.at/nephrogene/).

2 Experiment description

2.1 Experimental design

2.1.1 Laboratory, authors, contact

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2.1.2 Type of experiment

Random cross-over design: Comparison of PBMCs from hemodialysis (HD) patients between two different HD membranes (semi-synthetic and full-synthetic membrane).

2.1.3 Experiment factor

The expression of genes were investigated and compared between the two treatments semi-synthetic and full-synthetic membrane.

2.1.4 Hybridizations

Fourteen hybridizations (three replicates before treatment with semi-synthetic membrane, three replicates before treatment with full-synthetic membrane, four replicates after treatment with semi-synthetic membrane, four replicates after treatment with full-synthetic membrane).

2.1.5 Reference

Universal Human reference RNA (Stratagene®) was used as hybridization reference.

2.1.6 Quality control

To test for reproducibility of sample processing, RNA of three specimen was reprocessed twice.

2.2 Samples used, extract preparation and labeling

2.2.1 Bio-source properties

Organism: *Homo sapiens*

2.2.2 Biomaterial manipulations, hybridization and labeling protocol

Protocols for PBMC isolation, RNA extraction, RNA labeling, hybridization and washing of microarrays can be found on our website (http://www.meduniwien.ac.at/nephrogene/).

2.3 Hybridization procedures and parameters

Patient-ID	Measurement time-point	HD-Membrane	Array name	Batch-ID
A	pre-treatment	semi-synthetic (M1)	ApreHD_M1	shem52
A	post-treatment	semi-synthetic (M1)	ApostHD_M1	shem53
A	pre-treatment	full-synthetic (M2)	ApreHD_M2	shem106
A	post-treatment	full-synthetic (M2)	ApostHD_M2	shem103
B	pre-treatment	semi-synthetic (M1)	BpreHD_M1	shem154
B	post-treatment	semi-synthetic (M1)	BpostHD_M1	shem56
B	pre-treatment	full-synthetic (M2)	BpreHD_M2	shem153
B	post-treatment	full-synthetic (M2)	BpostHD_M2	shem104
C	pre-treatment	semi-synthetic (M1)	CpreHD_M1	shem54
C	post-treatment	semi-synthetic (M1)	CpostHD_M1	shem102
C	pre-treatment	full-synthetic (M2)	CpreHD_M2	shem55
C	post-treatment	full-synthetic (M2)	CpostHD_M2	shem151
D	post-treatment	semi-synthetic (M1)	DpostHD_M1	shfr120
D	post-treatment	full-synthetic (M2)	DpostHD_M2	shfr116

The hybridization protocol can be found on our website (http://www.meduniwien.ac.at/nephrogene/).

2.4 Measurement data and specification of data processing

2.4.1 Raw data description

Scan hardware: GenePix Personal 4100 A

Scan software: GenePix Pro 6.0

Raw data images can be found in the data section of our website (http://www.meduniwien.ac.at/nephrogene/).

Array	Laser power		PMT Gain		Lines Averaged	Background Subtraction	Scan region
	635 nm	532 nm	635 nm	532 nm			
shem53	3.34	3.48	530	495	1	LocalFeature	153,474,2046,7085
shem103	3.34	3.48	650	560	1	LocalFeature	136,474,2029,7036
shem52	3.43	3.51	600	450	1	LocalFeature	145,474,2038,7061
shem106	3.45	3.50	580	480	1	LocalFeature	136,474,2029,7020
shem56	3.39	3.48	610	460	1	LocalFeature	180,474,2054,7052
shem104	3.33	3.48	645	590	1	LocalFeature	136,474,2029,7085
shem154	3.27	3.49	580	510	1	LocalFeature	154,450,2015,7052
shem153	3.27	3.48	570	535	1	LocalFeature	152,483,2013,6979
shem102	3.34	3.49	650	560	1	LocalFeature	136,474,2029,7028
shem151	3.39	3.46	635	450	1	LocalFeature	136,474,2014,7044
shem54	3.37	3.49	550	560	1	LocalFeature	145,474,2038,7085
shem55	3.39	3.50	580	480	1	LocalFeature	145,466,2038,7077
shfr120	3.73	3.50	560	545	1	LocalFeature	186,458,2010,6987
shfr116	3.64	3.48	550	570	1	LocalFeature	172,462,2003,7033

2.4.2 Image analysis and quantitation

Image griding and calculation of spot intensity was performed with GenePix Pro 6.0 software.

2.4.3 Normalized and summarized data

Normalization:
Normalization was done through the default computed normalization by SMD (see http://genome-www5.stanford.edu/help/results_normalization.shtml). For data retrieval the $\log_2$ (R/G normalized ratio [median]) was used.

Computation of missing values:
No missing values were computed.

Cluster analysis:
Before cluster analysis different standard deviation filters were applied (program Cluster ©1998-9 (http://rana.lbl.gov/EisenSoftware.htm). Software used for cluster analysis was TIGR Multi Experiment Viewer 3.0.3 (http://www.tm4.org/mev.html).

Linkage rule: Complete linkage
Distance measure: 1 – Pearson Correlation

Significance analysis:
No adjustment for multiple testing was performed. Genes showing a fold change of at least two between the two treatments were considered differentially expressed and used for further analysis. Pre-treatment values acted as baseline.