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

Comparison of human renal tubule cells from patients with proteinuric nephropathies with those of healthy controls.

2.1.3. Experiment factors

The cells of interest were laser-capture microdissected from frozen sections from archived kidney biopsy material. Unaffected parts of tumor nephrectomies served as “healthy controls”. These samples were as well embedded in TissueTek, cryocut in 5µm slices and stored at -80°C.

2.1.4. Hybridizations

We performed 48 hybridizations. 19 patient samples and 5 control samples were hybridized in technical duplicates.

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 duplicate arrays. Duplicate 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

Frozen kidney biopsies were stained for alkaline phospatase, then the tubule cells were laser-capture microdissected using the PixCell II™ Laser Capture Microdissection System and CapSure™ LCM Caps. RNA was isolated using Pico Pure™ RNA Isolation Kit (all Arcturus, Mountain View, CA). We performed two rounds of linear RNA amplification using RiboAmp™

RNA Amplification Kit (Arcturus, Mountain View, CA). Reference RNA was as well amplified twice.

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

2.3. Hybridisation procedures and parameters

F = FSGS = focal-segmental glomerulosclerosis

I = IGAN = immunglobuline A nephritis

M = MCD = minimal change disease

KO = CO = controls

Experiment Name	**Array-ID** batch, no.	**Experiment Name**	**Array-ID** batch, no.
F1-T neu 1	shep169	I4-T neu 1	shep93
F1-T neu 2	shep66	I4-T neu 2	shep191
F2-T neu 1	shep119	I7-T neu 1	shep220
F2-T neu 2	shfr23	I7-T neu 2	shep144
F4-T neu 2	shep95	M2-T neu 1	shfa144
F4-T neu 4	shep219	M2-T neu 2	shep246
F5-T neu 2	shfr185	M4-T neu 1	shep192
F5-T neu 3	shep117	M4-T neu 2	shep166
F7-T neu 1	shep167	M5-T neu 2	shep248
F7-T neu 2	shep69	M5-T neu 3	shep142
F8-T neu 2	shep195	M6-T neu 1	shep168
F8-T neu 3	shep70	M6-T neu 2	shep221
I11-T neu 1	shep120	M8-T neu 1	shep11a
I11-T neu 2	shep250	M8-T neu 2	shep249
I14-T neu 1	shep141	KO5-T 1	shfd23
I14-T neu 2	shep36	KO5-T 2	shfr167
I15-T neu 1	shep145a	KO6-T 1	shfd24
I15-T neu 2	shep218	KO6-T 2	shfr168
I16-T neu 2	shep116	KO7-T 2	shfr169
I16-T neu 3	shep92	KO7-T 3	shfd143
I18-T neu 1	shep13	KO8-T 1	shfd66
I18-T neu 3	shep91	KO8-T 2	shfr170
I1-T neu 1	shep217	KO9-T 2	shfd64
I1-T neu 2	shep35	KO9-T 3	shfd144

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	bar code	Experiment	Laser	Power	PMT	Gain	Lines	Background
batch, no.	on array	Name					Averaged	Substraction
(Stanford)	(Stanford)	user - defined	635 nm	532 nm	635 nm	532 nm		
shep169	12667937	F1-T neu 1	4,4	3,8	650	500	1	LocalFeature
shep66	12667815	F1-T neu 2	4,2	3,8	650	500	1	LocalFeature
shep119	12667741	F2-T neu 1	4,1	3,8	630	500	1	LocalFeature
shfr23	12921608	F2-T neu 2	4,3	3,8	620	500	1	LocalFeature
shep95	12667755	F4-T neu 2	4,3	3,8	690	500	1	LocalFeature
shep219	12663958	F4-T neu 4	4	3,8	675	500	1	LocalFeature
shfr185	12897241	F5-T neu 2	4,2	3,8	670	500	1	LocalFeature
shep117	12667743	F5-T neu 3	4,2	3,8	650	500	1	LocalFeature
shep167	12667935	F7-T neu 1	4,2	3,8	650	500	1	LocalFeature
shep69	12668210	F7-T neu 2	4,3	3,8	670	500	1	LocalFeature
shep195	12663932	F8-T neu 2	4,2	3,8	750	500	1	LocalFeature
shep70	12668212	F8-T neu 3	4,2	3,8	710	500	1	LocalFeature
shep120	12667740	I11-T neu 1	4,2	3,8	675	500	1	LocalFeature
shep250	12664349	I11-T neu 2	4,2	3,8	670	500	1	LocalFeature
shep141	12667924	I14-T neu 1	4,4	3,8	690	500	1	LocalFeature
shep36	12667835	I14-T neu 2	4,3	3,8	690	500	1	LocalFeature
shep145a	12667919	I15-T neu 1	4,4	3,8	700	500	1	LocalFeature
shep218	12663957	I15-T neu 2	4,3	3,8	680	500	1	LocalFeature
shep116	12667744	I16-T neu 2	4,3	3,8	640	500	1	LocalFeature
shep92	12667752	I16-T neu 3	4,2	3,8	710	500	1	LocalFeature
shep13	12664313	I18-T neu 1	4,3	3,8	690	500	1	LocalFeature
shep91	12667751	I18-T neu 3	4,1	3,8	680	500	1	LocalFeature
shep217	12663956	I1-T neu 1	4,3	3,8	660	500	1	LocalFeature
shep35	12667836	I1-T neu 2	4,3	3,8	600	510	1	LocalFeature
shep93	12667753	I4-T neu 1	4,3	3,8	650	500	1	LocalFeature
shep191	12663937	I4-T neu 2	4,2	3,8	650	500	1	LocalFeature
shep220	12663959	I7-T neu 1	4,3	3,8	700	500	1	LocalFeature
shep144	12667921	I7-T neu 2	4,2	3,8	710	500	1	LocalFeature
shfa144	12795928	M2-T neu 1	4,1	3,8	670	500	1	LocalFeature
shep246	12664345	M2-T neu 2	4,4	3,8	630	500	1	LocalFeature
shep192	12663934	M4-T neu 1	4,3	3,8	630	500	1	LocalFeature
shep166	12667934	M4-T neu 2	4,2	3,8	620	500	1	LocalFeature
shep248	12664347	M5-T neu 2	4,2	3,8	650	500	1	LocalFeature
shep142	12667923	M5-T neu 3	4,2	3,8	650	500	1	LocalFeature
shep168	12667936	M6-T neu 1	4,3	3,8	670	500	1	LocalFeature
shep221	12663960	M6-T neu 2	4,3	3,8	670	500	1	LocalFeature
shep11a	12664308	M8-T neu 1	4,4	3,8	600	520	1	LocalFeature
shep249	12664348	M8-T neu 2	4,3	3,7	600	520	1	LocalFeature
shfd23	12789329	KO5-T 1	4,2	3,8	720	500	1	LocalFeature
shfr167	12897305	KO5-T 2	4,2	3,8	690	500	1	LocalFeature
shfd24	12789328	KO6-T 1	4,2	3,8	700	500	1	LocalFeature
shfr168	12897756	KO6-T 2	4,2	3,8	660	500	1	LocalFeature
shfr169	12897755	KO7-T 2	4,2	3,8	750	500	1	LocalFeature
shfd143	12789557	KO7-T 3	4,1	3,8	720	510	1	LocalFeature

shfd66	12794746	KO8-T 1	4,2	3,8	680	500	1	LocalFeature
shfr170	12897759	KO8-T 2	4,2	3,8	640	500	1	LocalFeature
shfd64	12794748	KO9-T 2	4,2	3,8	770	500	1	LocalFeature
shfd144	12789556	KO9-T 3	4,2	3,9	860	510	1	LocalFeature

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/.