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

Comparison of longterm outcome between transplanted kidneys of patients with high (group h_GFR) or low (group l_GFR) calculated glomerular filtration rate after one year of transplantation.

2.1.3 Experiment factors

The expression of genes were investigated and compared between the two groups h_GFR vs. l_GFR.

2.1.4 Hybridizations

Thirty-one hybridizations (15 replicates in group h_GFR, 16 in group l_GFR).

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*
Demographic data of kidney donors can be found in the manuscript and on our website (http://www.meduniwien.ac.at/nephrogene/).

2.2.2 Biomaterial manipulations, hybridization and labeling protocol

Kidney wedge biopsies were obtained immediately before implantation of the grafts and submerged into RNAlater™ to preserve RNA.

Protocols for 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

Sample	Array	Sample	Array
h_GFR 121	SHEM 251	l_GFR 123	SHER 232
h_GFR 130	SHER 180	l_GFR 138	SHER 240
h_GFR 153	SHER 211	l_GFR 147	SHER 190
h_GFR 155	SHER 189	l_GFR 162	SHER 228
h_GFR 156	SHER 208	l_GFR 167	SHEM 205
h_GFR 160	SHER 210	l_GFR 190	SHER 209
h_GFR 169	SHER 239	l_GFR 195	SHEM 253
h_GFR 172	SHER 198	l_GFR 206	SHER 182
h_GFR 200	SHER 230	l_GFR 207	SHER 188
h_GFR 201	SHEM 206	l_GFR 216	SHER 238
h_GFR 211	SHER 246	l_GFR 229	SHER 231
h_GFR 213	SHER 222	l_GFR 232	SHER 221
h_GFR 214	SHER 241	l_GFR 255	SHER 244
h_GFR 227	SHER 242	l_GFR 258	SHER 245
h_GFR 244	SHER 243	l_GFR 260	SHEM 252
		l_GFR 262	SHEM 254

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 4.1

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			
shem 251	3.17	3.46	580	560	1	LocalFeature	93,449,1986,7003
shem 205	3.46	3.48	580	550	1	LocalFeature	92,488,1999,6926
shem 253	3.21	3.47	550	570	1	LocalFeature	85,457,1978,7019
shem 252	3.42	3.46	580	550	1	LocalFeature	103,457,1978,7011
shem 206	3.28	3.47	550	545	1	LocalFeature	92,504,1999,7019
shem 254	3.36	3.46	580	560	1	LocalFeature	86,465,1979,7003
sher 240	3.13	3.45	590	490	1	LocalFeature	137,518,2053,7045
sher 239	3.16	3.45	590	490	1	LocalFeature	156,435,2082,6995
sher 190	3.09	3.44	545	490	1	LocalFeature	162,397,2071,6891
sher 238	3.21	3.47	590	490	1	LocalFeature	138,462,2047,6956
sher 211	3.42	3.42	575	485	1	LocalFeature	138,455,2087,6915
sher 210	3.38	3.46	590	480	1	LocalFeature	121,502,2118,6972
sher 209	3.49	3.47	585	480	1	LocalFeature	169,496,2040,6915
sher 208	3.16	3.4	590	485	1	LocalFeature	140,526,2029,6922
sher 228	3.15	3.47	555	450	1	LocalFeature	164,445,2072,6883
sher 180	3.02	3.64	655	510	1	LocalFeature	186,324,2051,6907
sher 230	3.17	3.46	600	470	1	LocalFeature	186,494,2062,6956
sher 182	3.03	3.45	625	480	1	LocalFeature	166,478,2079,6915
sher 232	3.46	3.47	590	550	1	LocalFeature	173,494,2066,7029
sher 231	3.29	3.47	590	530	1	LocalFeature	150,478,2082,6940
sher 189	3.14	3.48	630	480	1	LocalFeature	165,575,2059,6907
sher 188	3.09	3.48	450	420	1	LocalFeature	146,470,2062,6745
sher 244	3.13	3.45	560	545	1	LocalFeature	154,504,2053,6964
sher 245	3.15	3.44	555	545	1	LocalFeature	139,535,2038,6957
sher 198	3.11	3.45	555	500	1	LocalFeature	159,494,2042,6907
sher 246	3.16	3.5	570	540	1	LocalFeature	145,457,2061,6964
sher 241	3.34	3.43	575	500	1	LocalFeature	178,403,2037,6941
sher 222	3.48	3.48	625	490	1	LocalFeature	169,512,2046,6584
sher 243	3.13	3.46	585	505	1	LocalFeature	154,418,2077,6949
sher 242	3.42	3.44	600	590	1	LocalFeature	139,412,2039,6926
sher 221	3.13	3.47	555	500	1	LocalFeature	159,535,2063,6907

2.4.2 Image analysis and quantitation

Image griding and calculation of spot intensity was performed with GenePix Pro 4.1 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:
Missing values were obtained through computation of k-nearest-neighbor (k=10) with the EMV module (http://cran.at.r-project.org/src/contrib/Descriptions/EMV.html) of the R software package (http://cran.r-project.org).

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 – Cosine Correlation

Significance analysis:
No adjustment for multiple testing was performed. Genes showing a fold change of at least two and a p-value (t-test) smaller than 0.05 were considered differentially expressed and used for further analysis.