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 donor organs between anemic recipients within the first year after kidney transplantation (ESA group) and non anemic recipients (non ESA group).

2.1.3 Experiment factor

The expression of genes were investigated and compared between the two groups ESA vs. non ESA.

2.1.4 Hybridizations

Fifty hybridizations (25 replicates in group ESA, 25 in group non ESA).

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
ESA_shcm180	shcm180	non ESA_shcm181	shcm181
ESA_shcm182	shcm182	non ESA_shcm185	shcm185
ESA_shcm191	shcm191	non ESA_shdb204	shdb204
ESA_shcm192	shcm192	non ESA_shdb214	shdb214
ESA_shdb205	shdb205	non ESA_shdb218	shdb218
ESA_shdb215	shdb215	non ESA_shdb219	shdb219
ESA_shdb217	shdb217	non ESA_shdb220	shdb220
ESA_shdb221	shdb221	non ESA_shdb222	shdb222
ESA_shem206	shem206	non ESA_shdb223	shdb223
ESA_shem251	shem251	non ESA_shdb225	shdb225
ESA_shem252	shem252	non ESA_shem253	shem253
ESA_shem254	shem254	non ESA_sher180	sher180
ESA_sher182	sher182	non ESA_sher189	sher189
ESA_sher188	sher188	non ESA_sher198	sher198
ESA_sher190	sher190	non ESA_sher211	sher211
ESA_sher208	sher208	non ESA_sher221	sher221
ESA_sher209	sher209	non ESA_sher222	sher222
ESA_sher232	sher232	non ESA_sher231	sher231
ESA_sher244	sher244	non ESA_sher241	sher241
ESA_sher246	sher246	non ESA_sher242	sher242
ESA_shfr082	shfr082	non ESA_sher245	sher245
ESA_shfr094	shfr094	non ESA_shfr072	shfr072
ESA_shfr107	shfr107	non ESA_shfr085	shfr085
ESA_shfr119	shfr119	non ESA_shfr093	shfr093
ESA_shfr086	shfr086	non ESA_shfr096	shfr096

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 253	3.21	3.47	550	570	1	LocalFeature	85,457,1978,7019
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
shem 252	3.42	3.46	580	550	1	LocalFeature	103,457,1978,7011
sher 190	3.09	3.44	545	490	1	LocalFeature	162,397,2071,6891
sher 211	3.42	3.42	575	485	1	LocalFeature	138,455,2087,6915
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 180	3.02	3.64	655	510	1	LocalFeature	186,324,2051,6907
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 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
shcm 181	3.45	3.61	870	600	1	LocalFeature	88,648,2088,6272
shcm 185	3.37	3.61	940	560	1	LocalFeature	88,648,2088,6272
shcm 180	3.50	3.62	850	580	1	LocalFeature	88,648,2088,6272
shcm 182	3.49	3.61	880	580	1	LocalFeature	88,648,2088,6272
shcm 191	3.39	3.62	940	640	1	LocalFeature	100,616,2132,6432
shcm 192	3.44	3.61	940	560	1	LocalFeature	88,648,2088,6272
shdb 204	3.52	3.63	800	650	1	LocalFeature	112,628,2128,6276
shdb 205	3.45	3.63	810	650	1	LocalFeature	112,628,2128,6276
shdb 214	3.33	3.63	830	650	1	LocalFeature	112,628,2128,6276
shdb 215	3.42	3.65	830	650	1	LocalFeature	112,628,2128,6276
shdb 217	3.51	3.63	810	720	1	LocalFeature	112,628,2128,6276
shdb 218	3.44	3.63	840	720	1	LocalFeature	112,628,2128,6276
shdb 219	3.46	3.62	840	720	1	LocalFeature	112,628,2128,6276

Array	Laser power		PMT Gain		Lines Averaged	Background Subtraction	Scan region
	635 nm	532 nm	635 nm	532 nm			
shdb 220	3.59	3.63	870	640	1	LocalFeature	112,636,2100,6260
shdb 221	3.50	3.63	820	720	1	LocalFeature	112,628,2128,6276
shdb 222	3.61	3.63	870	640	1	LocalFeature	112,636,2100,6260
shdb 225	3.42	3.62	870	720	1	LocalFeature	112,628,2128,6276
shfr 072	3.28	3.48	535	560	1	LocalFeature	185,481,2056,6962
shfr 082	3.28	3.48	570	550	1	LocalFeature	155,475,2077,7118
shfr 085	3.38	3.52	640	570	1	LocalFeature	152,622,2096,6921
shfr 086	3.26	3.52	640	570	1	LocalFeature	152,622,2096,6946
shfr 093	3.30	3.49	620	550	1	LocalFeature	171,458,2102,6995
shfr 094	3.36	3.51	690	520	1	LocalFeature	163,232,2079,6988
shfr 096	3.23	3.49	640	560	1	LocalFeature	177,567,2041,6980
shfr 107	3.26	3.49	640	560	1	LocalFeature	177,449,2041,7011
shfr 119	3.30	3.48	595	550	1	LocalFeature	165,497,2046,6979

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 log2 (R/G normalized ratio [median]) was used. Additional quantile normalization procedure as implemented in the Bioconductor statistical software was applied to the dataset for data normalization after computation of missing values.

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 – Pearson Correlation

Significance analysis:
No adjustment for multiple testing was performed. Genes showing a fold change of at least 1.5 and a p-value (t-test) smaller than 0.05 were considered differentially expressed and used for further analysis.