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 pretreated donor organs (1g methylprednisolone) between delayed graft function (DGF) and primary function (PF).

2.1.3 Experiment factor

The expression of genes were investigated and compared between the two groups DGF and PF.

2.1.4 Hybridizations

Twenty hybridizations (10 replicates in group DGF, 10 in group PF).

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

Steroid samples	Array
PF-C124R	SHEO20
PF-C134L	SHEO68
PF-C145R	SHEO67
PF-C157L	SHEO66
PF-C184L	SHEO65
PF-C124L	SHEO142
PF-C139R	SHEO190
PF-C386L	SHEO189
PF-C139L	SHEO168
PF-C389L	SHEO167
DGF-C106R	SHEO21
DGF-C198R	SHEO165
DGF-C113L	SHEO214
DGF-C113R	SHEO213
DGF-C168R	SHEO139
DGF-C198L	SHEO143
DGF-C172R	SHEO243
DGF-C501R	SHEO242
DGF-C195R	SHEO241
DGF-C101R	SHEO139

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			
SHEO139	3.42	3.48	485	495	1	LocalFeature	62,537,2013,7049
SHEO21	3.45	3.54	600	520	1	LocalFeature	155,458,2060,6979
SHEO214	3.50	3.44	530	530	1	LocalFeature	101,454,1977,7057
SHEO213	3.42	3.43	485	495	1	LocalFeature	101,477,1977,7002
SHEO139	3.41	3.52	555	490	1	LocalFeature	122,474,2021,7069
SHEO243	3.71	3.54	580	550	1	LocalFeature	88,454,1966,7017
SHEO241	3.52	3.55	550	580	1	LocalFeature	86,469,1994,7057
SHEO143	3.43	3.49	670	430	1	LocalFeature	122,474,2003,7101
SHEO165	3.55	3.56	580	530	1	LocalFeature	130,474,2011,7036
SHEO242	3.46	3.51	470	460	1	LocalFeature	86,477,1994,7049
SHEO142	3.45	3.53	580	510	1	LocalFeature	122,458,2021,7069
SHEO20	3.50	3.57	600	450	1	LocalFeature	179,458,2070,7020
SHEO68	3.31	3.45	565	520	1	LocalFeature	139,475,2070,6946
SHEO168	3.46	3.52	565	500	1	LocalFeature	130,474,2011,7061
SHEO190	3.39	3.52	580	450	1	LocalFeature	122,474,2008,7061
SHEO67	3.29	3.46	580	520	1	LocalFeature	163,335,2043,6847
SHEO66	3.29	3.42	560	525	1	LocalFeature	154,507,2046,6987
SHEO65	3.27	3.46	580	525	1	LocalFeature	170,507,2062,7003
SHEO189	3.42	3.52	555	460	1	LocalFeature	127,474,2008,7085
SHEO167	3.54	3.52	600	480	1	LocalFeature	122,474,2003,7069

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://smd.stanford.edu/help/results_normalization.shtml). For data retrieval the log2 (R/G normalized ratio [mean]) was used.

Computation of missing values:
Missing values were obtained through computation of k-nearest-neighbor (k=10) with the EMV module (version 1.3.1.1) (http://cran.r-project.org/web/packages/EMV/index.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:
We used the significance analysis of microarrays (SAM) and Student's t-test ($p<0.05$, fold change > 2) to determine significant differentially expressed genes (DEGs) between DGF and PF group. The number of permutations in the SAM method was set to twenty-thousand and a false discovery rate of 2.5% was selected. Software used for SAM analysis was TIGR Multi Experiment Viewer 3.0.3 (http://www.tm4.org/mev.html).