

# 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

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#### 2.1.2 Type of experiment

Comparison of unilateral nephrectomy (UN) and unilateral ureteric obstruction (UUO) in rats at three time points (12, 24 and 72 hours) after contralateral injury.

#### 2.1.3 Experiment factors

The expression and time course of genes were investigated and compared between the two groups UN vs. UUO and to sham rat control kidneys.

#### 2.1.4 Hybridizations

Twenty-four hybridizations (4 replicates for each timepoint and type of contralateral nonfunction).

### 2.1.5 Reference

Sham operated rat kidneys were 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: *Rattus norvegicus*

Body weight and blood creatinine of rats 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

At time point 0 rats were subjected to unilateral ureteric obstruction or unilateral nephrectomy. After 12, 24 or 48 hours the rats were sacrificed and the kidneys removed 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
UUO 12_1	12682030_MMAB	UN 12_1	12671177_MMAB
UUO 12_2	12665968_MMAB	UN 12_2	12671176_MMAB
UUO 12_3	12682028_MMAB	UN 12_3	12665967_MMAB
UUO 12_4	12671465_MMAB	UN 12_4	12671097_MMAB
UUO 24_1	12671102_MMAB	UN 24_1	12671174_MMAB
UUO 24_2	12671488_MMAB	UN 24_2	12671464_MMAB
UUO 24_3	12671198_MMAB	UN 24_4	12671489_MMAB
UUO 24_4	12671096_MMAB	UN 24_5	12671110_MMAB
UUO 72_1	12671112_MMAB	UN 72_1	12682031_MMAB
UUO 72_3	12682029_MMAB	UN 72_2	12682032_MMAB
UUO 72_4	12671466_MMAB	UN 72_3	12665966_MMAB
UUO 72_5	12671175_MMAB	UN 72_5	12665969_MMAB

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			
12682030 MMAB	2.97	3.45	660	670	2	LocalFeature	147,209,2100,6941
12665968 MMAB	3.27	3.50	735	668	2	LocalFeature	147,286,2069,6895
12682028 MMAB	2.98	3.49	650	690	2	LocalFeature	92,147,2107,6949
12671465 MMAB	2.92	3.43	629	640	2	LocalFeature	97,201,2127,6918
12671102 MMAB	2.92	3.46	740	620	2	LocalFeature	97,255,2127,6980
12671488 MMAB	2.95	3.44	697	650	2	LocalFeature	96,263,2126,6910
12671198 MMAB	3.22	3.46	722	650	2	LocalFeature	178,364,2069,6964
12671096 MMAB	2.97	3.46	670	630	2	LocalFeature	105,162,2087,6972
12671112 MMAB	2.97	3.45	715	620	2	LocalFeature	97,286,2127,6949
12682029 MMAB	2.99	3.54	650	650	2	LocalFeature	131,232,2069,6964
12671466 MMAB	3.19	3.45	730	640	2	LocalFeature	97,403,2127,6957
12671175 MMAB	2.98	3.47	703	680	2	LocalFeature	147,457,2069,6871
12671177 MMAB	2.96	3.46	670	630	2	LocalFeature	105,129,2087,6980
12671176 MMAB	2.94	3.45	675	620	2	LocalFeature	105,194,2087,6899
12665967 MMAB	3.01	3.46	660	670	2	LocalFeature	116,131,2099,7026
12671097 MMAB	2.98	3.48	680	650	2	LocalFeature	77,201,2115,7026
12671174 MMAB	2.97	3.45	680	620	2	LocalFeature	154,226,2030,6859
12671464 MMAB	2.97	3.45	690	620	1	LocalFeature	105,226,2062,6932
12671489 MMAB	2.99	3.50	650	640	2	LocalFeature	97,217,2127,6980
12671110 MMAB	3.18	3.47	720	630	2	LocalFeature	97,457,2127,7050
12682031 MMAB	2.95	3.45	735	620	2	LocalFeature	97,302,2127,6910
12682032 MMAB	2.97	3.45	740	630	2	LocalFeature	97,453,2127,6996
12665966 MMAB	3.23	3.54	660	650	2	LocalFeature	131,201,2069,6926
12665969 MMAB	3.22	3.47	703	660	2	LocalFeature	154,356,2069,6918

## 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:

Normalization was done through the default computed normalization by SMD (see [http://genome-www5.stanford.edu/help/results\\_normalization.shtml](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/PACKAGES.html#EMV>) 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.tigr.org/software/tm4/mev.html>).

Linkage rule: Average linkage

Distance measure: 1 – Pearson r

**Significance analysis:**

No adjustment for multiple testing was performed. We predefined a  $p < 0.001$  as statistically significant.