1 Array design description

Description of the array design of the RNA6000 LabChip® kit (Agilent, Palo Alto, CA, USA), including description of features, can be found on the manufacturers website. http://www.affymetrix.com/products/arrays/specific/rat230.affx

Protocols for the post processing procedure can be found on the *Center for Array Technologies* website. http://ra.microslu.washington.edu/samplePrep.php

2 Experiment description

2.1 Experimental design

2.1.1 Laboratory, authors, contact

Peter Viktor Hauser [1†]	peter.hauser@gmail.com
Paul Perco [3,4,5]	paul.perco@emergentec.com
Irmgard Mühlberger [5]	irmgard.muehlberger@emergentec.com
Jeffrey Pippin [1]	scoobie@u.washington.edu
Mary Blonsky [1]	blonskim@u.washington.edu
Bernd Mayer [5]	bernd.mayer@emergentec.com
Charles E. Alpers [2]	calp@u.washington.edu
Rainer Oberbauer [3,4]	rainer.oberbauer@meduniwien.ac.at
Stuart J Shankland [1]	stuartjs@washington.edu

[1] Division of Nephrology, University of Washington, Seattle, WA, USA.

[2] Department of Pathology, University of Washington, Seattle, WA, USA.

[3] Department of Nephrology, KH Elisabethinen, Linz, Austria

[4] Department of Nephrology, Medical University of Vienna, Austria

[5] emergentec biodevelopment GmbH

[†] currently Center for Molecular Biotechnology, University Torino, Torino, Italy

2.1.2 Type of experiment

Gene expression study of renal glomeruli from rats with experimental membranous nephropathy (Passive Heymann Nephritis) at two time points was performed (before and after the onset of proteinuria).

2.1.3 Experiment factors

Expression and time course of genes in the isolated glomeruli before (day 3) and after (day 6) the onset of proteinuria was compared to control animals. Two experiment factors were used: time and diseased versus control state.

2.1.4 Hybridizations

Twenty hybridizations were conducted (five biological replicates of experimental and control animals at each time point).

2.1.5 Reference

Gene expression was hybridized against RNA harvested from control animals.

2.1.6 Quality control

Quality of the isolated total RNA was checked by gel electrophoresis and with Agilent Bioanalyzer using a RNA6000 LabChip® kit (Agilent, Palo Alto, CA, USA). Quantity and OD 260/280 of total RNA and cRNA was assessed by UV spectrophotometer. Quality of hybridizations and overall chip performance was determined by visual inspection of the raw scanned data.

2.2. Samples used, extract preparation and labeling

2.2.1 Bio-source properties

Organism: *Rattus norvegicus*
Sprague Dawley (Simson, Gilroy, CA, USA)
Gender: male
Weight: 180-200g

2.2.2 Biomaterial manipulations, hybridization and labelling protocol

At timepoints day3 and day 6, glomeruli were isolated by differential sieving. Kidney cortices were removed, minced, and pressed through a series of sieves with 180µm,

106µm, and 75µm-sized mesh. Glomeruli were collected on the 75µm-sized mesh, removed from the sieve and pelleted in phosphate buffered saline by centrifugation. Glomerular purity was typically 90-100%. Isolation of total RNA was performed by TRIZOL® method (Invitrogene Corp, Carlsbad, CA, USA). RNA quality was checked using the Agilent Bioanalyzer in combination with the RNA6000 LabChip® kit (Agilent, Palo Alto, CA, USA).

Protocols for the post processing procedure can be found on the *Center for Array Technologies* website. http://ra.microslu.washington.edu/samplePrep.php

2.3 Hybridization procedures and parameters

Hybridization protocol can be found on
http://ra.microslu.washington.edu/samplePrep.php

The following twenty hybridizations were performed:

FileName_new	control	treatment	day	Noise	SF	NF	Background	Corner -	% P	Average Signal(All)
RAE230A_d3_S1		X	3	5.97	0.771	1	91.90	8049	63.60%	310.3
RAE230A_d3_S2		X	3	6.30	0.915	1	94.54	8887	62.30%	312.3
RAE230A_d3_C3	X		3	7.08	0.744	1	105.16	9594	61.90%	306.8
RAE230A_d3_C4	X		3	7.61	0.844	1	106.01	8876	57.90%	304.9
RAE230A_d3_S5		X	3	8.90	1.283	1	111.85	9621	53.90%	316.1
RAE230A_d3_S6		X	3	4.36	0.747	1	67.80	9300	63.80%	310.6
RAE230A_d3_S7		X	3	7.37	0.664	1	105.70	8464	62.90%	304.6
RAE230A_d3_C8	X		3	6.49	0.859	1	96.36	8281	61.70%	309.1
RAE230A_d3_C9	X		3	6.24	0.862	1	94.72	7821	60.80%	312.4
RAE230A_d3_C10	X		3	7.97	0.853	1	114.64	8680	59.50%	311.7
RAE230A_d6_C1	X		6	6.45	0.652	1	85.22	11689	60.60%	306.5
RAE230A_d6_C2	X		6	5.27	0.781	1	83.79	10039	60.40%	313.1
RAE230A_d6_C3	X		6	5.64	0.694	1	83.61	10928	60.00%	307.7
RAE230A_d6_C4	X		6	6.11	0.802	1	94.60	11647	59.00%	314.0
RAE230A_d6_C5	X		6	5.65	0.701	1	84.34	12819	62.10%	312.0
RAE230A_d6_S6		X	6	5.07	0.811	1	77.76	11487	62.40%	311.0
RAE230A_d6_S7		X	6	5.69	0.895	1	86.54	13784	60.20%	313.4
RAE230A_d6_S8		X	6	6.67	0.625	1	99.84	13076	63.00%	308.9
RAE230A_d6_S9		X	6	7.44	0.693	1	121.70	15024	60.00%	312.2
RAE230A_d6_S10		X	6	5.75	0.767	1	86.24	12185	62.30%	313.3

2.4 Measurement data and specification of data processing

2.4.1 Raw data description

Raw data files (CEL files) and the gene expression data matrix are available on http://www.meduniwien.ac.at/nephrogene - data - “Gene expression in Passive Heymann Nephritis”.

2.4.2 Image analysis and quantitation

Image processing and expression analysis was performed using Affymetrix MAS 5.0 software.

2.4.3. Normalized and summarized data

Data preprocessing

Pre-processing of microarray raw data including probe-specific background correction, summarization of probe set values, and normalization was carried out with the CARMAweb (comprehensive R- and bioconductor-based web service for microarray data analysis) tool, using the robust multi-chip analysis (RMA) approach.

Significance analysis

The significance analysis of microarrays (SAM) method was used to identify differentially expressed genes (DEGs) between immunized rats and controls setting the false discovery rate to < 5%.