

## 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´s website at

<http://www.microarray.org/sfgf/jsp/home.jsp>

Protocols for the pre-hybridisation procedures (post-processing) of the arrays can be downloaded from our website at <http://www.microarray.at>

## 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 human renal tubule cells from patients with proteinuric nephropathies with those of healthy controls.

### **2.1.3. Experiment factors**

The cells of interest were laser-capture microdissected from frozen sections from archived kidney biopsy material. Unaffected parts of tumor nephrectomies served as "healthy controls". These samples were as well embedded in TissueTek, cryocut in 5µm slices and stored at -80°C.

### **2.1.4. Hybridizations**

We performed 48 hybridizations. 19 patient samples and 5 control samples were hybridized in technical duplicates.

### **2.1.5. Reference**

Stratagene Universal Human Reference RNA was used as hybridization reference.

### **2.1.6. Quality controls**

To test for reproducibility we calculated the intra-array variability of the duplicate arrays. Duplicate arrays were combined before statistical analysis.

## ***2.2. Samples used, extract preparation, amplification and labelling***

### **2.2.1. Bio-source properties**

Organism: Homo sapiens. Patient and control characteristics can be found in the manuscript and on our website (<http://www.microarray.at>).

### **2.2.2. Biomaterial manipulations, amplification and labelling protocol**

Frozen kidney biopsies were stained for alkaline phosphatase, then the tubule cells were laser-capture microdissected using the PixCell II™ Laser Capture Microdissection System and CapSure™ LCM Caps. RNA was isolated using Pico Pure™ RNA Isolation Kit (all Arcturus, Mountain View, CA). We performed two rounds of linear RNA amplification using RiboAmp™

RNA Amplification Kit (Arcturus, Mountain View, CA). Reference RNA was as well amplified twice.

Protocols for RNA amplification, RNA labelling, hybridization and washing of microarrays can be downloaded from our website (<http://www.microarray.at>).

### ***2.3. Hybridisation procedures and parameters***

F = FSGS = focal-segmental glomerulosclerosis

I = IGAN = immunoglobuline A nephritis

M = MCD = minimal change disease

KO = CO = controls

| <b>Experiment Name</b> | <b>Array-ID batch, no.</b> | <b>Experiment Name</b> | <b>Array-ID batch, no.</b> |
|------------------------|----------------------------|------------------------|----------------------------|
| F1-T neu 1             | shep169                    | I4-T neu 1             | shep93                     |
| F1-T neu 2             | shep66                     | I4-T neu 2             | shep191                    |
| F2-T neu 1             | shep119                    | I7-T neu 1             | shep220                    |
| F2-T neu 2             | shfr23                     | I7-T neu 2             | shep144                    |
| F4-T neu 2             | shep95                     | M2-T neu 1             | shfa144                    |
| F4-T neu 4             | shep219                    | M2-T neu 2             | shep246                    |
| F5-T neu 2             | shfr185                    | M4-T neu 1             | shep192                    |
| F5-T neu 3             | shep117                    | M4-T neu 2             | shep166                    |
| F7-T neu 1             | shep167                    | M5-T neu 2             | shep248                    |
| F7-T neu 2             | shep69                     | M5-T neu 3             | shep142                    |
| F8-T neu 2             | shep195                    | M6-T neu 1             | shep168                    |
| F8-T neu 3             | shep70                     | M6-T neu 2             | shep221                    |
| I11-T neu 1            | shep120                    | M8-T neu 1             | shep11a                    |
| I11-T neu 2            | shep250                    | M8-T neu 2             | shep249                    |
| I14-T neu 1            | shep141                    | KO5-T 1                | shfd23                     |
| I14-T neu 2            | shep36                     | KO5-T 2                | shfr167                    |
| I15-T neu 1            | shep145a                   | KO6-T 1                | shfd24                     |
| I15-T neu 2            | shep218                    | KO6-T 2                | shfr168                    |
| I16-T neu 2            | shep116                    | KO7-T 2                | shfr169                    |
| I16-T neu 3            | shep92                     | KO7-T 3                | shfd143                    |
| I18-T neu 1            | shep13                     | KO8-T 1                | shfd66                     |
| I18-T neu 3            | shep91                     | KO8-T 2                | shfr170                    |
| I1-T neu 1             | shep217                    | KO9-T 2                | shfd64                     |
| I1-T neu 2             | shep35                     | KO9-T 3                | shfd144                    |

### ***2.4. Measurement data and specification of data processing***

#### **2.4.1. Raw data description**

Scan hardware: GenePix 4000 B (Axon Instruments, Union City, CA)

Scan software: GenePix Pro 4.1 (Axon Instruments, Union City, CA)

Raw data and array images can be found in the data section of our website (<http://www.microarray.at>).

| Array-ID   | bar code   | Experiment     | Laser  | Power  | PMT    | Gain   | Lines    | Background   |
|------------|------------|----------------|--------|--------|--------|--------|----------|--------------|
| batch, no. | on array   | Name           | 635 nm | 532 nm | 635 nm | 532 nm | Averaged | Substraction |
| (Stanford) | (Stanford) | user - defined | nm     | nm     | nm     | nm     |          |              |
| shep169    | 12667937   | F1-T neu 1     | 4,4    | 3,8    | 650    | 500    | 1        | LocalFeature |
| shep66     | 12667815   | F1-T neu 2     | 4,2    | 3,8    | 650    | 500    | 1        | LocalFeature |
| shep119    | 12667741   | F2-T neu 1     | 4,1    | 3,8    | 630    | 500    | 1        | LocalFeature |
| shfr23     | 12921608   | F2-T neu 2     | 4,3    | 3,8    | 620    | 500    | 1        | LocalFeature |
| shep95     | 12667755   | F4-T neu 2     | 4,3    | 3,8    | 690    | 500    | 1        | LocalFeature |
| shep219    | 12663958   | F4-T neu 4     | 4      | 3,8    | 675    | 500    | 1        | LocalFeature |
| shfr185    | 12897241   | F5-T neu 2     | 4,2    | 3,8    | 670    | 500    | 1        | LocalFeature |
| shep117    | 12667743   | F5-T neu 3     | 4,2    | 3,8    | 650    | 500    | 1        | LocalFeature |
| shep167    | 12667935   | F7-T neu 1     | 4,2    | 3,8    | 650    | 500    | 1        | LocalFeature |
| shep69     | 12668210   | F7-T neu 2     | 4,3    | 3,8    | 670    | 500    | 1        | LocalFeature |
| shep195    | 12663932   | F8-T neu 2     | 4,2    | 3,8    | 750    | 500    | 1        | LocalFeature |
| shep70     | 12668212   | F8-T neu 3     | 4,2    | 3,8    | 710    | 500    | 1        | LocalFeature |
| shep120    | 12667740   | I11-T neu 1    | 4,2    | 3,8    | 675    | 500    | 1        | LocalFeature |
| shep250    | 12664349   | I11-T neu 2    | 4,2    | 3,8    | 670    | 500    | 1        | LocalFeature |
| shep141    | 12667924   | I14-T neu 1    | 4,4    | 3,8    | 690    | 500    | 1        | LocalFeature |
| shep36     | 12667835   | I14-T neu 2    | 4,3    | 3,8    | 690    | 500    | 1        | LocalFeature |
| shep145a   | 12667919   | I15-T neu 1    | 4,4    | 3,8    | 700    | 500    | 1        | LocalFeature |
| shep218    | 12663957   | I15-T neu 2    | 4,3    | 3,8    | 680    | 500    | 1        | LocalFeature |
| shep116    | 12667744   | I16-T neu 2    | 4,3    | 3,8    | 640    | 500    | 1        | LocalFeature |
| shep92     | 12667752   | I16-T neu 3    | 4,2    | 3,8    | 710    | 500    | 1        | LocalFeature |
| shep13     | 12664313   | I18-T neu 1    | 4,3    | 3,8    | 690    | 500    | 1        | LocalFeature |
| shep91     | 12667751   | I18-T neu 3    | 4,1    | 3,8    | 680    | 500    | 1        | LocalFeature |
| shep217    | 12663956   | I1-T neu 1     | 4,3    | 3,8    | 660    | 500    | 1        | LocalFeature |
| shep35     | 12667836   | I1-T neu 2     | 4,3    | 3,8    | 600    | 510    | 1        | LocalFeature |
| shep93     | 12667753   | I4-T neu 1     | 4,3    | 3,8    | 650    | 500    | 1        | LocalFeature |
| shep191    | 12663937   | I4-T neu 2     | 4,2    | 3,8    | 650    | 500    | 1        | LocalFeature |
| shep220    | 12663959   | I7-T neu 1     | 4,3    | 3,8    | 700    | 500    | 1        | LocalFeature |
| shep144    | 12667921   | I7-T neu 2     | 4,2    | 3,8    | 710    | 500    | 1        | LocalFeature |
| shfa144    | 12795928   | M2-T neu 1     | 4,1    | 3,8    | 670    | 500    | 1        | LocalFeature |
| shep246    | 12664345   | M2-T neu 2     | 4,4    | 3,8    | 630    | 500    | 1        | LocalFeature |
| shep192    | 12663934   | M4-T neu 1     | 4,3    | 3,8    | 630    | 500    | 1        | LocalFeature |
| shep166    | 12667934   | M4-T neu 2     | 4,2    | 3,8    | 620    | 500    | 1        | LocalFeature |
| shep248    | 12664347   | M5-T neu 2     | 4,2    | 3,8    | 650    | 500    | 1        | LocalFeature |
| shep142    | 12667923   | M5-T neu 3     | 4,2    | 3,8    | 650    | 500    | 1        | LocalFeature |
| shep168    | 12667936   | M6-T neu 1     | 4,3    | 3,8    | 670    | 500    | 1        | LocalFeature |
| shep221    | 12663960   | M6-T neu 2     | 4,3    | 3,8    | 670    | 500    | 1        | LocalFeature |
| shep11a    | 12664308   | M8-T neu 1     | 4,4    | 3,8    | 600    | 520    | 1        | LocalFeature |
| shep249    | 12664348   | M8-T neu 2     | 4,3    | 3,7    | 600    | 520    | 1        | LocalFeature |
| shfd23     | 12789329   | KO5-T 1        | 4,2    | 3,8    | 720    | 500    | 1        | LocalFeature |
| shfr167    | 12897305   | KO5-T 2        | 4,2    | 3,8    | 690    | 500    | 1        | LocalFeature |
| shfd24     | 12789328   | KO6-T 1        | 4,2    | 3,8    | 700    | 500    | 1        | LocalFeature |
| shfr168    | 12897756   | KO6-T 2        | 4,2    | 3,8    | 660    | 500    | 1        | LocalFeature |
| shfr169    | 12897755   | KO7-T 2        | 4,2    | 3,8    | 750    | 500    | 1        | LocalFeature |
| shfd143    | 12789557   | KO7-T 3        | 4,1    | 3,8    | 720    | 510    | 1        | LocalFeature |

|         |          |         |     |     |     |     |   |              |
|---------|----------|---------|-----|-----|-----|-----|---|--------------|
| shfd66  | 12794746 | KO8-T 1 | 4,2 | 3,8 | 680 | 500 | 1 | LocalFeature |
| shfr170 | 12897759 | KO8-T 2 | 4,2 | 3,8 | 640 | 500 | 1 | LocalFeature |
| shfd64  | 12794748 | KO9-T 2 | 4,2 | 3,8 | 770 | 500 | 1 | LocalFeature |
| shfd144 | 12789556 | KO9-T 3 | 4,2 | 3,9 | 860 | 510 | 1 | LocalFeature |

## 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 was done through the default computed normalization by SMD (at [http://genome-www5.stanford.edu/help/results\\_normalization.shtml](http://genome-www5.stanford.edu/help/results_normalization.shtml)). For data retrieval the log2 red/green normalized ratio was used.

Missing values were obtained through computation of k-nearest neighbour (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>).

Significance analysis was performed with the maxT algorithm which is available in the Bioconductor module (<http://www.bioconductor.org>) of the R software package as well as with the SAM software (significance analysis of microarrays) which is available at <http://www-stat.stanford.edu/~tibs/SAM/>.