

- A. Validation of array data by RT-PCR for four significant genes derived from the array experiments (BF, CR2, GRK5 and STAT1). RT-PCR showed the same separation of h_GFR and l_GFR groups as the microarray experiment (see table below). However the absolute values of PCR and microarray derived data are not comparable since for PCR the specific primer is customized and the hybridization conditions optimized. Furthermore the sequence of cDNA probes and RT-PCR primers are different and additionally RT-PCR quantification was done by the standardized delta-delta C_T method using a reference gene (β -actin). In the array experiments, the probe competes with the standard RNA and normalization was performed by total intensity normalization (Stanford microarray database, <http://genome-www5.stanford.edu>) and not by using β -actin as reference. Thus not only the absolute expression data but also the sign of the expression value is irrelevant for the comparison, only the relative difference of expression values between the two groups may be used (1-3).

	group	Mean				Standard deviation			
		BF	CR2	GRK5	STAT1	BF	CR2	GRK5	STAT1
PCR	H	4,37	2,97	2,31	5,34	2,08	1,96	0,47	1,55
	L	5,43	4,19	1,72	5,89	1,00	2,01	0,90	1,40
Array	H	1,24	-0,21	-0,17	1,51	1,06	0,64	2,17	1,55
	L	2,28	1,05	-0,04	2,62	1,39	2,01	1,98	0,86

1. Hegde PS, White IR, Debouck C. Interplay of transcriptomics and proteomics. *Current Opinion in Biotechnology* 2003; 14 (6): 647.
2. Liew KJ, Chow VT. Microarray and real-time RT-PCR analyses of a novel set of differentially expressed human genes in ECV304 endothelial-like cells infected with dengue virus type 2. *J Virol Methods* 2006; 131 (1): 47.
3. Rajeevan MS, Vernon SD, Taysavang N, Unger ER. Validation of array-based gene expression profiles by real-time (kinetic) RT-PCR. *J Mol Diagn* 2001; 3 (1): 26.

B. Prospective validation was performed using biopsies from recipients with currently six months of follow up because no biopsies from matched recipients (immunosuppression, BCAR, DGF) with one year of follow-up are available. In this prospective validation, the same pattern of relative expression between h_GFR and l_GFR was derived however the differences are smaller (only 6 mo follow-up). The same caveat as above (Webtable 1A) applies when comparing (the non-comparable) expression values of PCR and array experiments.

	group	Mean				Standarddeviation			
		BF	CR2	GRK5	STAT1	BF	CR2	GRK5	STAT1
PCR	H	3,77	2,78	0,96	4,10	1,08	2,76	0,67	0,66
	L	3,45	4,09	0,25	4,14	0,66	2,62	0,77	0,51