

Legend to gene expression raw data files.

In the following the *in vitro* experiments from primary BEC and LEC cultures analyzed in the manuscript, Transcriptomal mapping of human dermal lymphatic endothelial cells *ex vivo* and *in vitro*. Wick et al., submitted to XXX, are described. Please, find the corresponding information on *ex vivo* experiments in the separate file ¹.

MIAME (Minimal Information About Microarray Experiments) criteria were fulfilled as described ². Briefly, the following experimental characteristics should be mentioned:

1. Experimental design

Type of the experiment: Labeled cRNA deriving from 10µg total RNA from dermal BEC and LEC primary cultures was amplified in a one round protocol. The samples were independently hybridized to separate Affymetrix GeneChips U133A deriving from the same lot.

The aim was investigation of BEC and LEC transcriptomes and analysis of the cell culture effect on endothelial transcriptomes.

The experiments are described in the following manuscript: Transcriptomal mapping of human dermal lymphatic endothelial cells *ex vivo* and *in vitro*. Wick et al., submitted to XXX.

2. DNA-chip design

The design and characteristics of Affymetrix GeneChips U133A is described at www.affymetrix.com.

3. Samples

Blood and lymphatic microvascular endothelial cells (BECs, LECs) were separated by magnetic bead sorting from commercially available crude dermal endothelial cell preparations (HDMECs; Promocell GmbH, Germany). Total RNA of sorted BECs and LECs was isolated using an RNeasy Mini Kit (Quiagen, no. 74104). Ten µg total RNA was used for one round amplification protocol. Reverse transcription using oligo(dT)-T7 primers with SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen, no. 11917-010) was followed by IVT using Bio Array High Yield RNA Transcript Labeling Kit (Enzo Life Sciences Inc, no. 42655-10). Labeled cRNA samples were fragmented by heat (95°C, 35 min.) and salt (20mM Tris-acetat (pH 8.1), 500mM KOAc, 150mM MgOAc). Prior to hybridization external control transcripts (GeneChip Eukaryotic Hybridization Control Kit, Affymetrix) were added.

4. Hybridization

The hybridization solution contained 0.5 mg/ml acetylated BSA, 0.5mg/ml herring sperm DNA in 2xMES buffer. Five μ g of fragmented cRNA were resuspended in 38 μ l volume and added to obtain a 230 μ l hybridization solution. The solution was hybridized to GeneChips Human Genome U133A for 16 hours at 45°C using a rotating device fixed in an incubator case. Washing was done with 6xSSPE and MES buffers. Detection was done by streptavidin-phycoerythrin (SAPE) principle.

5. Scanning

Scanning was performed on a GeneChip Scanner. The hybridization scan raw data were generated as .tif files. Fluorescence intensities scaled in arbitrary units were saved as .cel files and reopened as .txt files. They can be downloaded as .txt tab-delimited files. Data were normalized using Microarray Analysis Suite 5.0 software (Affymetrix Inc., CA) as described ¹ and can be downloaded as .txt tab-delimited files at <http://www.meduniwien.ac.at/complex-systems/supplementary2005/>. A master file containing fluorescence values of all experiments after normalization can be viewed in alleCsnorm.xls.

6. Controls

Normalization was against the optimized method of normalization to a selected housekeeping gene subset using Matlab software ¹. In addition to background subtraction inherent to MAS 5.0, BioB fluorescence intensity was used as the threshold for designation as being expressed.

7. References:

1. Wick N, Bruck J, Gurnhofer E, et al. Nonuniform hybridization: a potential source of error in oligonucleotide-chip experiments with low amounts of starting material. *Diagn Mol Pathol*. 2004; 13:151-159.
2. Brazma A, Hingamp P, Quackenbush J, et al. Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. *Nat Genet*. 2001; 29:365-371.