

Additional file 1: Materials and methods of the DNA microarray analysis.

DNA amplification was carried out in 25 μ l PCR reaction, including 1x master mix and MolTaq polymerase (Molzym16S Basic, Molzym, Bremen, Germany), one universal primer pair (forward 784 and reverse 1386 primer, each 0,12 μ M) and 1 ng per μ l of DNA extract. PCR cycling included an initial denaturation step at 95°C, 5 min, followed by 40 cycles of 95°C for 1 min, 60°C for 1 min, 72°C for 1 min, and a termination step at 72°C for 10 min using a GeneAmp® PCR System 2700 thermal cycler (Life Technologies, Vienna, Austria). Successful amplification was confirmed by analyzing the PCR products on a 1.5 % agarose gel with Sybr Safe in 1x TBE buffer (Sigma Aldrich, St Louis, MO, USA). For labeling, the primer extension method was used and a final primer extension reaction mixture of 40 μ l containing 7 μ l of PCR product, 0.9 μ M forward primer 784, 0.08 U Vent (exo⁻) DNA polymerase (New England Bio Labs, Ipswich, UK), 1x PCR-buffer, 1 mM MgSO₄, 50 μ M dGTP, dATP, dTTP, 25 μ M dCTP (Roche Life Science, Vienna, Austria) and 0.06 U 5'-Propagylamino-dCTP-Atto532 (MoBiTec, Goettingen, Germany). PCR cycling conditions consisted of 95°C for 3 min, followed 25 cycles at 95°C, 58°C and 72°C each 20 sec and a final extension step for 3 min at 72°C. Prior to hybridization, the spotted slides were blocked with 3 M urea and 0.1% (w/v) SDS for 30 min at room temperature, washed, dried by centrifugation and covered with HybriWell™ (Grace Bio Labs, Blend, OR, USA). Hybridization mixture, containing 40 μ l prewarmed Express Hyb hybridization solution (Clontech, Mountain View, CA, USA) and 40 μ l labeled DNA product and a hybridization control (0.125 μ M Bsrev-Cy5-AAGCTCACTGGCCGTCGTTTTAAA) was denaturated at 95°C for 3 min, prior transferring 50 μ l of the suspension to the pre-treated microarray surface. Hybridization was carried out at 65°C for 1 h in a humidity chamber. Afterwards, the hybridwells were removed, and the slides were washed and dried.