

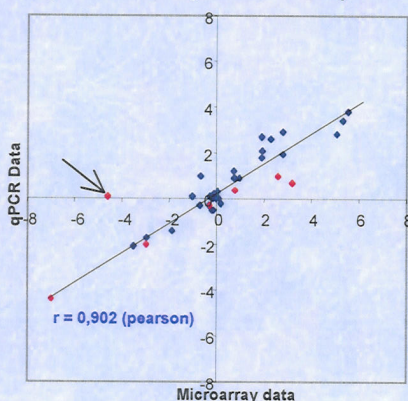
Validation of a bacterial gene array by qPCR using the human probe library

Mads Bennedsen, Christel Garrigues and Martin B. Pedersen Chr Hansen,
Boege Allé 10-12, DK-2970 Hoersholm, Denmark. mads.bennedsen@dk.chr-hansen.com

Introduction:

An in-house genome array based on the complete published *Lactococcus lactis* sequence was set up in our laboratory. In the present work, a good correlation to results obtained by qPCR on 36 of the genes from the array is shown. It is also demonstrated, that the human "Probe Library" (Exiqon/Roche) is a widely applicable and flexible tool, even when working with a gram-positive bacterium.

Fig 2: Microarray data versus qPCR data



Relative gene expression levels of 36 genes in *Lactococcus lactis* in static versus respiratory fermentation. Six relatively low expressed genes are shown in red. For one gene (*aspB*, arrow) bad correlation between the assays is seen.

Materials and methods

Cell culture and mRNA purification: CHCC2862 (Chr. Hansen Culture Collection) was grown at 30°C in M17+0.5% lactose without agitation (static culture) and with 5mg/L of hemin and agitation (respiratory culture). Cells were harvested at OD600=1.0 in RNeasy Protect (QIAGEN) and total RNA was isolated using RNeasy (QIAGEN).

Microarray procedure: Spotted 65-75mer whole genome arrays of practically all 2268 putative genes of *Lactococcus lactis* were set up in-house (Pedersen *et al.* 2005). Briefly, oligos were spotted in 4 replicates on UltraGAPS slides (Corning). Ten µg of RNA from static culture or from respiratory culture was copied into cDNA and labelled with either Cy3 or Cy5 (CyScribe Post-Labeling Kit, Amersham). The cDNA's were mixed and hybridized. The arrays were washed and scanned (GenePix 4100A with GenePix Pro 6.0, Axon) and ratio-normalized (Acuity 4.0, Axon). The experiment was repeated six times.

qPCR procedure: qPCR assay was designed using the "probelibrary.com" homepage. mRNA was copied into cDNA (Reverse transcriptase core kit, Eurogentec). Four independent RT steps of each sample were performed. cDNA were pooled in two pools of each two samples and qPCR assays were performed in triplicates on an Applied Biosystems 7500 system using qPCR MasterMix Plus Low Rox (Eurogentec) with the standard conditions of 300 nM primer and 100 nM probes, hotstart and 40 cycles 15 sec melting at 95°C and 60 sec of annealing/extension at 60°. Standard conditions were based on primer optimization of ten assays. PCR efficiencies of individual wells were checked using LinRegPCR software (Ramakers *et al.* 2003).

References:

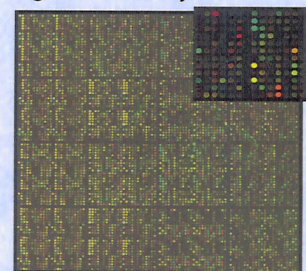
- Pedersen, M.B., Iversen, S.L., Sørensen, K.L., and E. Johansen. (2005): The long and winding road from the research laboratory to industrial applications of lactic acid bacteria. *FEMS Microbiology Reviews* 29:611-624.
- Ramakers C, Ruijter JM, Deprez RH, Moorman AF. (2003): Assumption-free analysis of quantitative real-time PCR data analysis. *Neurosci Letters* 339(1): 62-66.

Results and discussion:

Assay Design and qPCR: DNA sequences were submitted to "Probelibrary Design Center" and 39 of the 42 genes (93%) could be covered by assays based on the human probe library. 37 of these assays were then used for qPCR analysis of which 36 worked, giving a total rate of success of 90%. The assay not working might be due to sequence differences between the published sequence and our strain. The PCR efficiency was generally high, for most genes in the range of 1,8-2,0 and consistent in intra-assay comparisons. These data show, that the Probe Library is widely applicable, also when working with gram-positive bacteria.

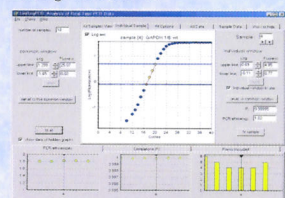
Comparison of micro-array and qPCR: Six independent repetitions were carried out on micro-arrays. The data were highly reproducible with standard deviations of typically 0,1-0,3 on the log₂ of gene expression changes. The data were generally in good correlation with the data acquired from qPCR experiments. A Pearson correlation showed a correlation coefficient of 0,902 (figure 2). This holds true also for low-expressed genes (red color). One gene (*aspB*) however showed strong downregulation by microarray and virtually no regulation by qPCR. Further analysis of the array data showed strong downregulation of a gene upstream and a gene downstream for *aspB* in the same operon, pointing to a problem with the qPCR assay. Generally there is a tendency towards lower differential expression in the qPCR data as compared to microarray data.

Fig. 1. Microarray



A whole genome array of all 2268 putative genes in *Lactococcus lactis* in static versus respiratory fermentation.

LinRegPCR Software



Assay design using probe library

