**Datenerfassungsformular hybridisierter Chips**

Experiment\_Title  \_agilent\_AK\_181005\_Dace3\_vs\_DaceEDpycE2.3\_Chip3

Experiment\_Summary  Comparison of DaceE and DaceEDpyc mutant on transcriptional level under standard conditions (CGXII+4%Glucose+1.5%Acetate) in exponential phase

Experiment\_Overall\_Design 

Experiment\_Contributors  Aileen Krüger (Frunzke Lab)

Sample\_Description  RNA from ATCC13032\_DaceEDpyc cultivated in CGXII+4%Glucose+1.5%Acetate extracted from approximately 20 ml cell culture at OD600 = 12, 19.5h after inoculation

Sample\_Organism  C.glutamicum ATCC 13032\_DaceEDpyc

Sample\_Characteristics  C. glutamicum ATCC 13032 possessing a deletion in the aceE gene and pyc gene, cultivated in minimal medium (CGXII+4%Glucose+1.5%Acetate)

Sample\_Molecule  Total RNA: 2.83 µg/µl in 80 µl, 20 µg used

Sample\_Label  sample RNA is red-labeled, Cy5

Control\_Description  RNA from ATCC13032\_DaceE cultivated in CGXII+4%Glucose+1.5%Acetate extracted from approximately 20 ml cell culture at OD600 = 12, 10h after inoculation

Control\_Organism  C.glutamicum ATCC13032\_DaceE

Control\_Characteristics  C. glutamicum ATCC 13032 possessing a deletion in the aceE gene, cultivated in minimal medium (CGXII+4%Glucose+1.5%Acetate)

Control\_Molecule  Total RNA: 3.69 µg/µl for DaceE in 80 µl, 20 µg used

Control\_Label  control RNA is green-labeled, Cy3

Growth\_Protocol  1. Pre-culture: 5 ml BHI+0.5%Ac, 30°C, over day ~8h, 170 rpm

2. Pre-culture: 20 ml CGXII+1.5%Ac, 30°C, over night ~16h, 120 rpm

Main culture: Start OD600=1, 50 ml CGXII+1.5%Acetate+4%Glucose, 120 rpm

Harvest: ~20 ml at OD600=12

Treatment\_Protocol 

Extract\_\_Protocol  RNA extraction using C.g.-standard-method for RNA Extraktion: RNeasy Mini-Kit with DNAse digestion on column. Elution in 80 µl H2O.

Label\_Protocol  cDNA synthesis according to lab protocol using random hexamer primer. For each sample 20 µg RNA were used. Purification using amicon ultra columns.

Hyb\_Protocol  according to manufacturers protocol (Agilent). 65°C over night (17 h).

Scan\_Protocol  according to lab protocol. Scan-PMT green: 515; Scan-PMT red: 460

Data\_processing  data processing and Lowess-normalisation using R packages marray and limma; protocol from Tino Polen

Value\_Definition  relative RNA level = Ratio of Medians (sample RNA / control RNA) = 635nm/532nm (Genepix)

Diagnostic\_Plots 

Platform\_Title  4plex, design ID 085072

Platform\_Technology  synthesized 60mer oligonucleotides

Platform\_Distribution  custom-made

Platform\_Organism  C.glutamicum ATCC13032

Platform\_Manufacturer  Agilent

Platform\_Manuf\_Protocol  on-chip synthesis of 60-mer oligonucleotides