

# Affymetrix and Illumina, a toxicogenomic based interplatform comparison

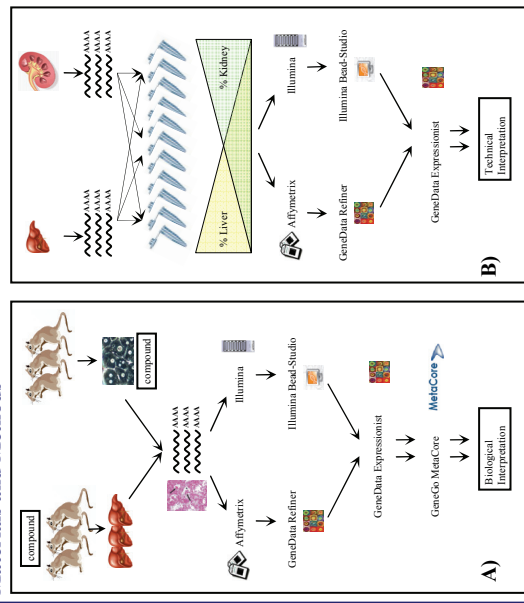
J. Hrach<sup>1</sup>, J.H. Harleman, P. G. Hewitt<sup>1</sup>  
<sup>1</sup>Molecular Toxicology, Institute of Toxicology, Merck Serono Research, 64271 Darmstadt, Germany  
 \*e-mail: philip.hewitt@merck.de



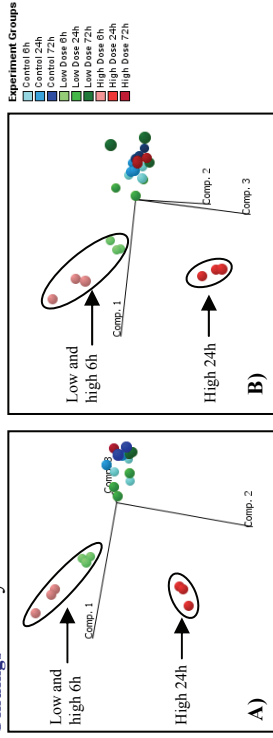
## Background and Aim

The analysis of genomic data is becoming more and more important in modern toxicology and drug development, enabling researchers to identify changes in global gene expression as well as specifically affected pathways. One big challenge in genomics today is the analysis and comparison of datasets generated with different platforms. As many manufacturers use different technologies, there is always the question of how comparable and reliable the achieved results are. Variability can be caused by the type of probes, the probe selection and design, the number of probes, different labelling methods etc. In this comparative study we compared a dilution series of rat liver and kidney samples and samples from a rat toxicogenomic studies using Tetracycline (a model compound which causes steatosis in the liver) *in vivo* and *in vitro* and two different whole genome expression platforms (Affymetrix Rat Genome 230 2.0 and Illumina RatRef-12 Expression BeadChip arrays). We tested two different doses and three time points, 6h, 24h and 72h. This study design gave us the option to analyze the comparability of both platforms on a technical as well as biological basis.

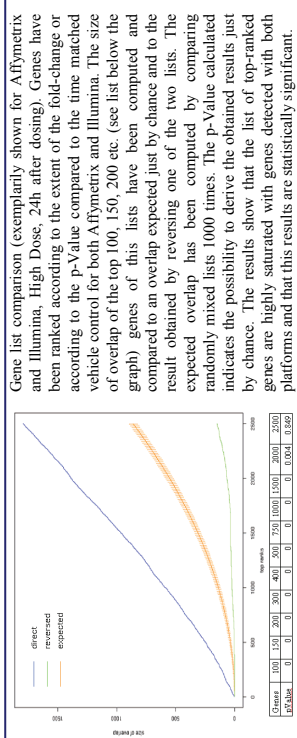
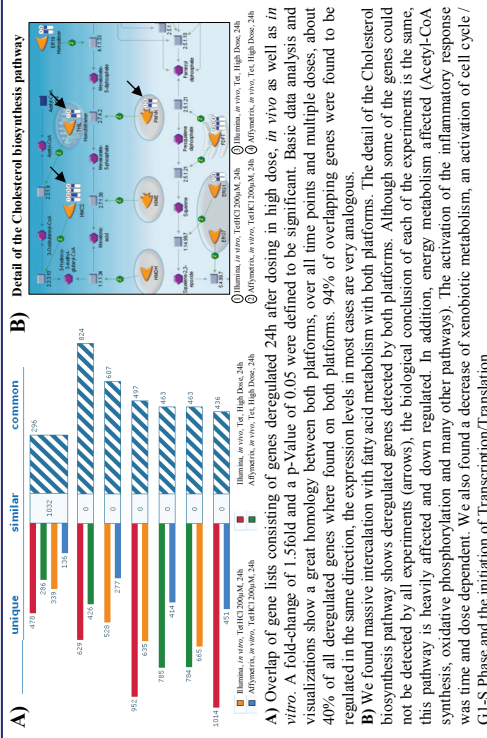
## Materials and Methods



## Findings



Principal components analysis of the same Dataset (Tetracycline *in vivo* study) measured A) Rat Genome 230 2.0 with Affymetrix and B) Illumina RatRef-12 Expression BeadChip arrays, separating the experiments according to the variances of the datasets. Each sphere constitutes one experiment. The two plots show clearly that there is a distinct, concordant behavior of the data, regardless which platform was used.



Liver and kidney dilution series, RNA of both tissues were mixed resulting in 11 samples containing different ratios of Liver and kidney RNA ranging from pure liver RNA in steps of 10% to pure Kidney RNA. Samples were subsequently labeled separately and hybridized to either Affymetrix Rat Genome 230 2.0 Chips or Illumina RatRef-12 Expression BeadChips. The intensities of the 44 most tissue specific platform overlapping genes are plotted. The gene list has been clustered hierarchically for Illumina and genes have been arranged in the same order. The intensity scale had to be adjusted for each platform separately due to intrinsic intensity differences. The tissue concentration responses for high and medium expressed genes could be detected with high correlation with both platforms, genes with expression values close to the background tended to oscillate and showed no clear tissue concentration dependency. Many of the genes found to be tissue specific could be confirmed by literature search.

## CONCLUSIONS

**Biological comparison**  
 The need to be able to compare data generated from different platform with existing gene expression databases is high and will even increase in future. Therefore, it is essential to find methods and algorithms to overcome the problems caused by their different nature. Essential for this comparison is a gene mapping to find probes valid for both platforms.

We tested samples from tetracycline *in vivo* and *in vitro* studies on two different platforms. Although many genes were found by only one platform, the biological interpretation of the results was the same.

Ranked gene lists generated have been found to be highly saturated with genes, detected by both platforms, whereas setting a cut off value (fold change 1.5/ p-Value 0.05) resulted in gene lists which have about 45% overlap.

Gene expression changes found with both platforms *in vivo* and *in vitro* match known effects of Tetracycline and give new insights in its molecular mode of action. Both platforms seem to be well suited to find global gene expression changes, although some differences could be detected.

Many genes affected *in vivo* could as well be detected as affected *in vitro*. Therefore, cell culture of primary hepatocytes has the potential to be used as an early screening tool for hepatotoxicity and thus help to reduce the extent of animal testing.

## Technical comparison

From all deregulated genes (fold change 1.5/ p-Value 0.05), 94% of genes detected were deregulated in the same direction showing a high concordance between both platforms, although the degree of change was not always constant.

Genes showing a linear tissue concentration dependency between Liver and Kidney samples could clearly be detected with both platforms.

261 Genes were detected as not expressed in either liver or kidney samples with both platforms, most of them showed a linear dependency to the mixing ratio. Very high expressed genes showed saturation effects, low expressed genes on a level close to the background had a high variation in both platforms.

## Acknowledgements

The authors wish to thank members of the Institute of Toxicology of Merck Serono Research for critical discussion.