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Some Pertinent Findings from MAQC Related to Reproducibility of Gene Expression

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Peer Review of Draft NTP Approach to Genomic Dose-Response
Modeling Expert Panel Meeting
October 23rd -25th, 2017
NIEHS, RTP, NC

Outline

- Brief overview of MAQC/SEQC
- How to assess reproducibility?
- A few ways MAQC explored reproducibility
  - Between sites
  - Data processing platform
  - Across platforms
  - Transcriptional response dependency
- Take home messages

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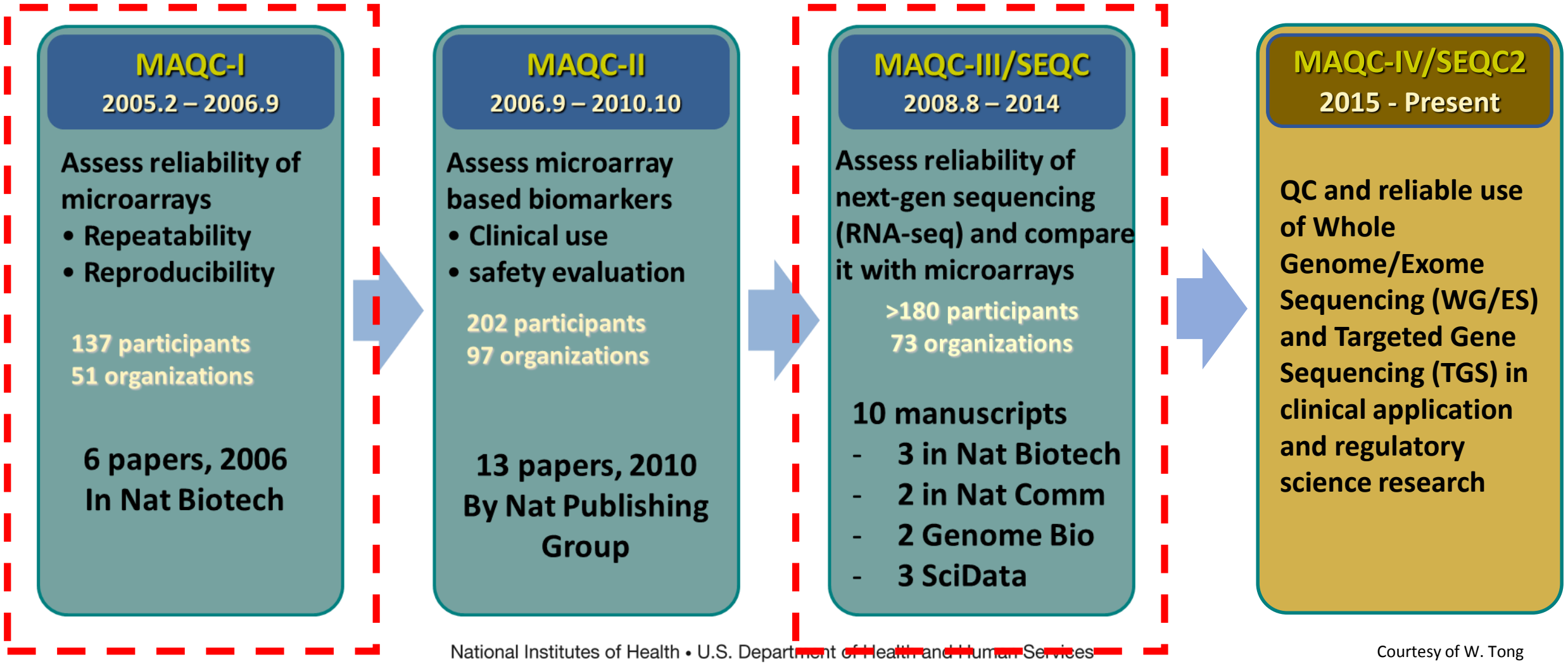
MicroArray Quality Control (MAQC) Consortium

An FDA-led community wide crowd-sourced effort to assess technical performance and application of genomics technologies (microarrays, GWAS and next-gen sequencing) in clinic and safety evaluation.



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MAQC Leadership

Previously: Dr. Leming Shi, Professor
Fudan University in Shanghai, China
(formally with NCTR)

Currently: Dr. Weida Tong, Director
Division of Bioinformatics and Biostatistics
NCTR

Javier Santoyo-Lopez, Laure Sambourg, Elia Stupka and Yiming Zhou

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What Do We Mean by Reproducibility?

Under the same (or close to) conditions, study design, protocols and research tools, produce the same results from a previous experiment

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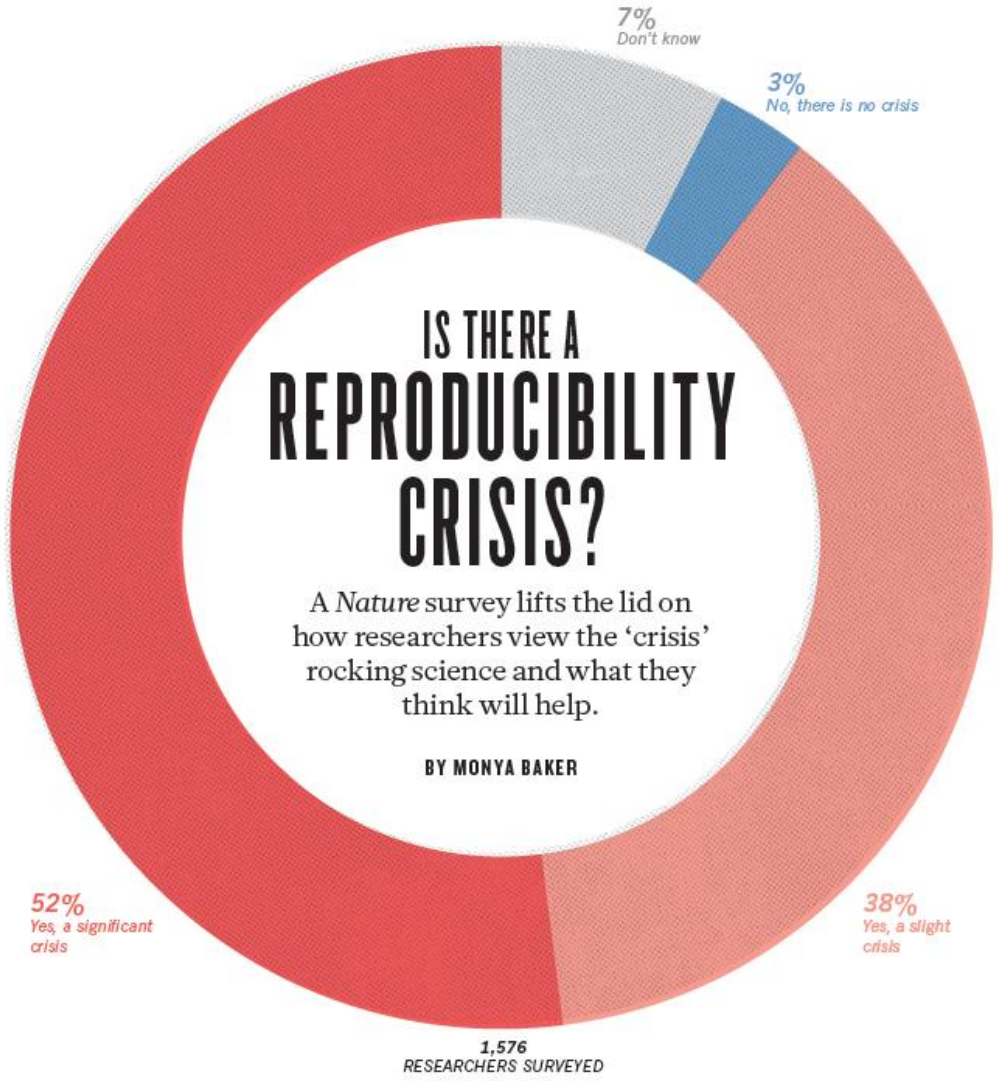
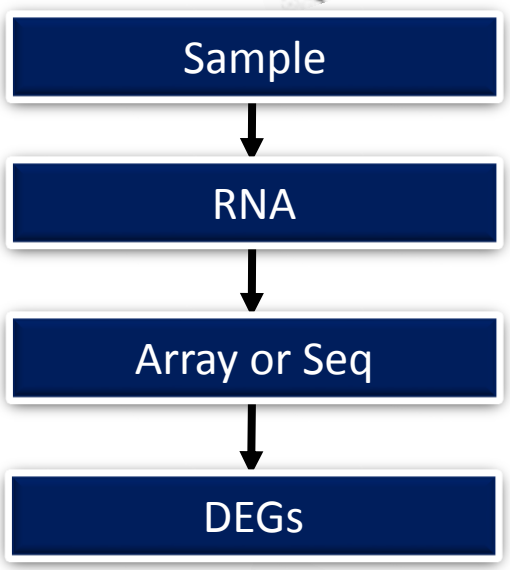
2016 Nature Survey on Reproducible Science



More than 70% of researchers have tried and failed to reproduce another scientist's experiments

Gene Expression Reproducibility in the Context of MAQC/Sequence Quality Control

Experiment 1…N



Can we obtain the same DEGs?

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Some Factors Evaluated in Relation to Reproducibility in a Toxicogenomics Study

- Study design
- Platform
- Between and within study sites
- Data processing/Normalization
- Treatment effect

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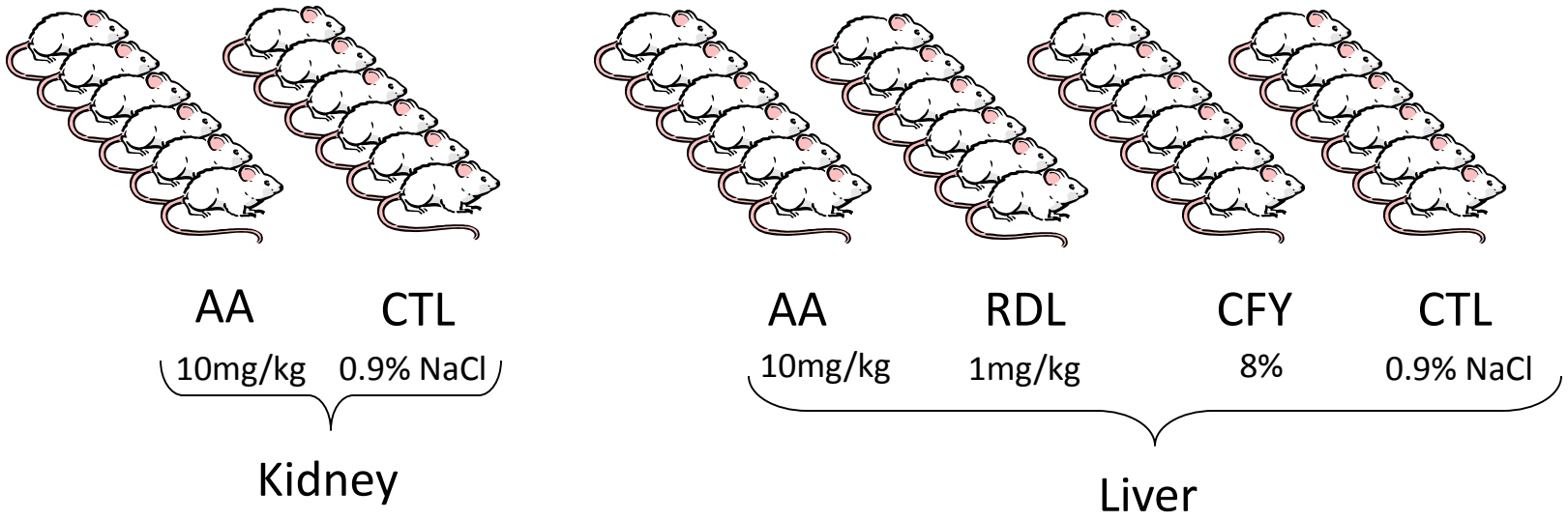
MAQC-I: Rat Toxicogenomics Study

6-week-old
Big Blue
Fisher 344
male rats
12 weeks
exposure



AA – Aristolochic acid; RDL – Riddelliine; CFY – Comfrey; CTR – Control

- Microarrays from Applied Biosystems, **Affymetrix (2 sites)**, Agilent, and GE Healthcare.

- Results are summarized in
  - Guo *et al.*, *Nat. Biotechnol*. 24, 1162-1169 (2006)
  - Tong *et al.*, *Nat. Biotechnol*. 24, 1132-1139 (2006)

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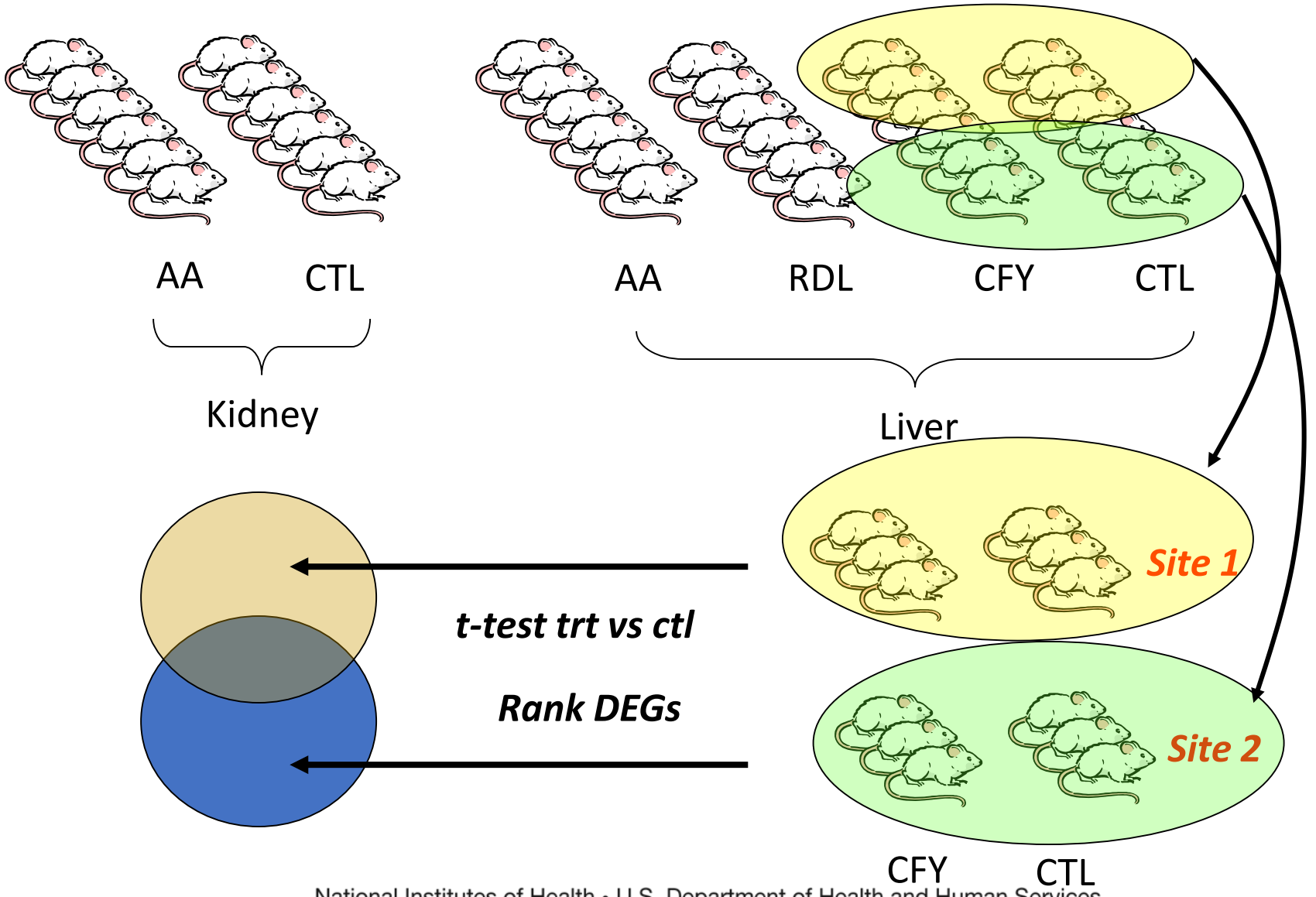
Between Sites Reproducibility - Rat TGx Study



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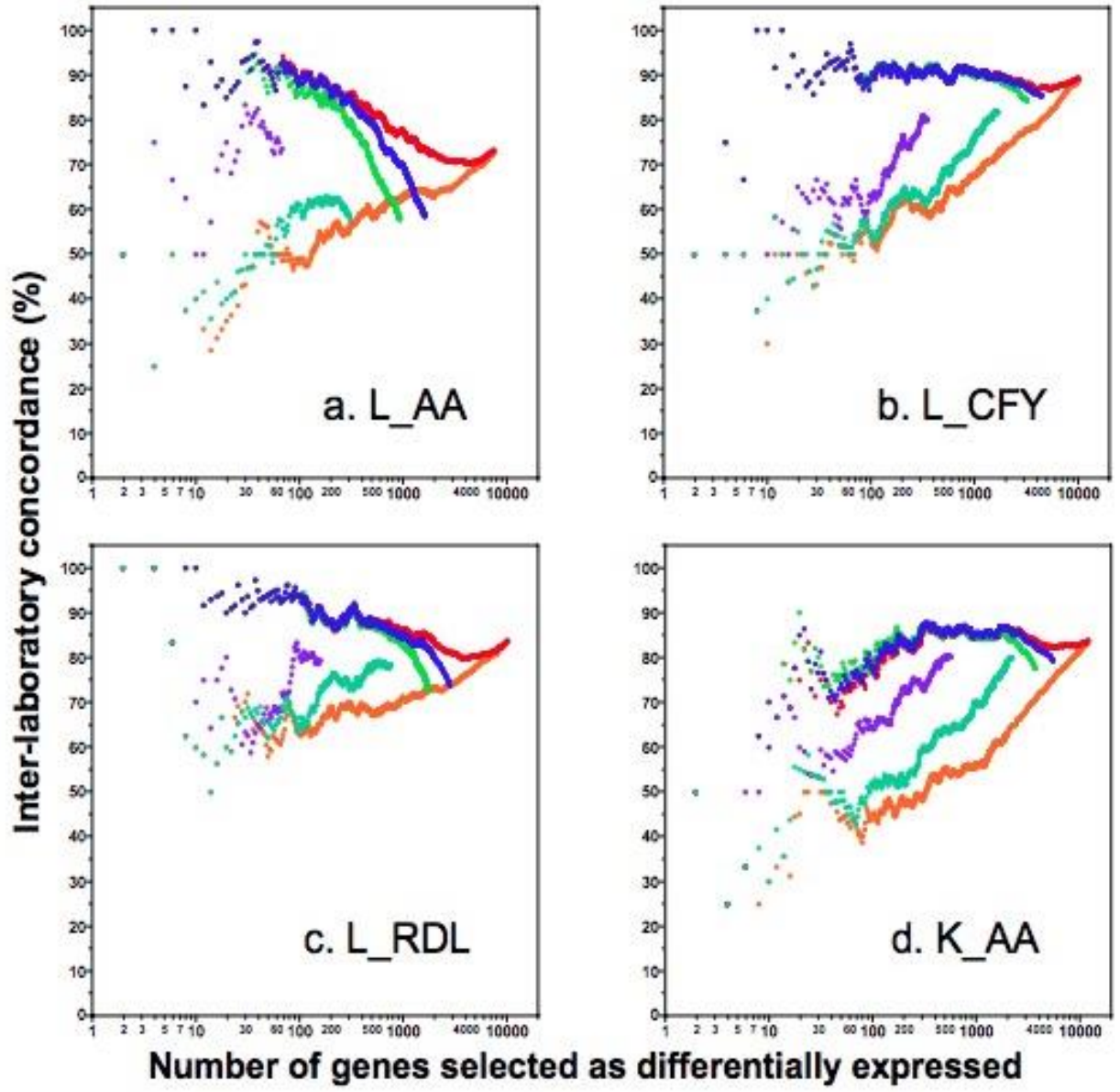
Concordance of DEGs Between Two Study Sites

$$\text{Percent of overlapping Genes (POG)} = \frac{2 \times intersect(DEGs_{Site\ 1}, DEGs_{Site\ 2})}{DEGs_{Site\ 1} + DEGs_{Site\ 2}} \text{ X } 100$$

Percentage of Overlapping Genes



- FC ranking
- FC + *P*<0.05
- FC + *P*<0.01

- *P* + FC >2.0
- *P* + FC >1.4
- *P* Ranking

Courtesy of W. Tong

Guo L *et al.*, *Nature Biotechnology*, **24**(9), 1162-9 (2006).

MAQC-I: Coupling p-value with Fold Change Improved Reproducibility

- Within site
- Between sites
- Between microarray platforms

MAQC-III/SEQC1: Let's Up the Ante Regarding Between Platforms Reproducibility

Are the DEGs detected on the microarray platform detected on the mRNA-Seq platform?

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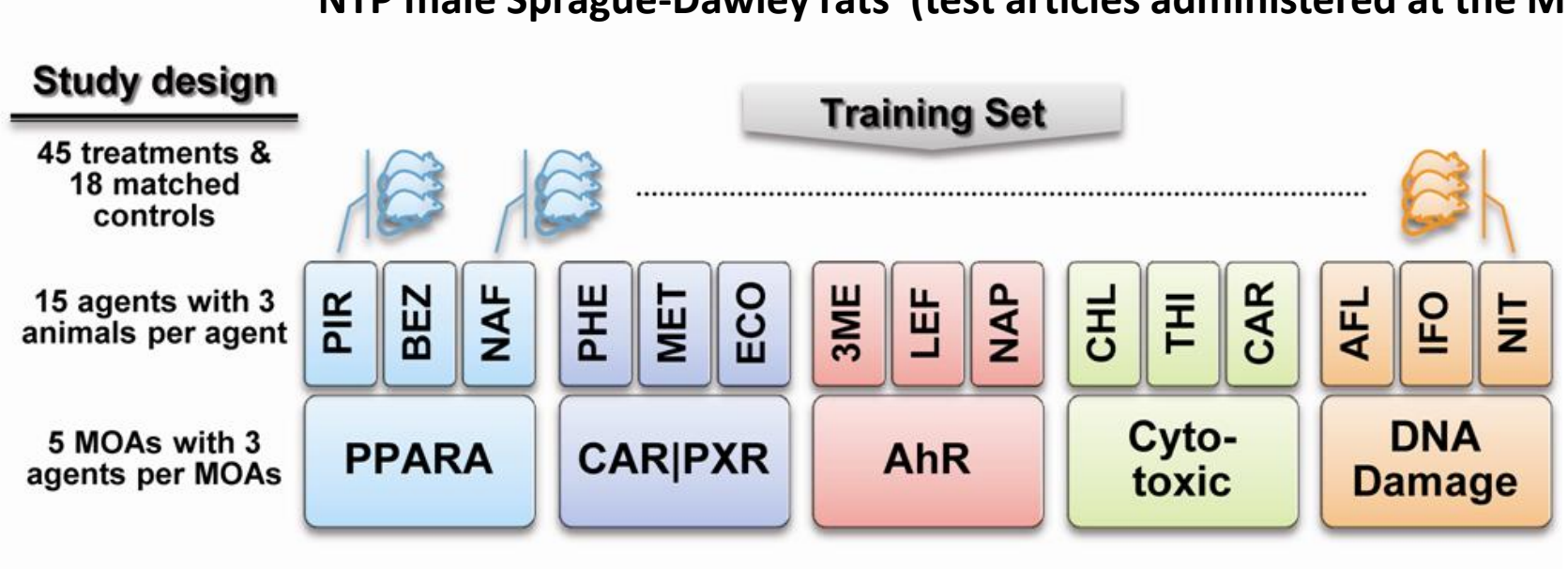
MAQC-III/SEQC1: Toxicogenomics Study Design

NTP male Sprague-Dawley rats (test articles administered at the MTD)



Agents
Pirinixic acid (PIR)
Bezafibrate (BEZ)
Nafenopin (NAF)
Phenobarbital (PHE)
Methimazole (MET)
Econazole (ECO)
3-Methylcholanthrene (3ME)
Leflunomide (LEF)
beta-Naphthoflavone (NAP)
Chloroform (CHO)
Thioacetamide (THI)
Carbon tetrachloride (CAR)
Aflatoxin B1 (AFL)
Ifosfamide (IFO)
N-Nitrosodimethylamine (NIT)

Samples: Total RNA, then poly-A selection
Affy chip: Rat 230_2.0, MAS5 and RMA normalizations
RNA-Seq: Illumina HiScanSQ or HiSeq2000 , 100bp PE
Depth of 23-25 M reads
6 Bioinformatics pipelines

Results published in Wang et al. (2014) Nature Biotechnology

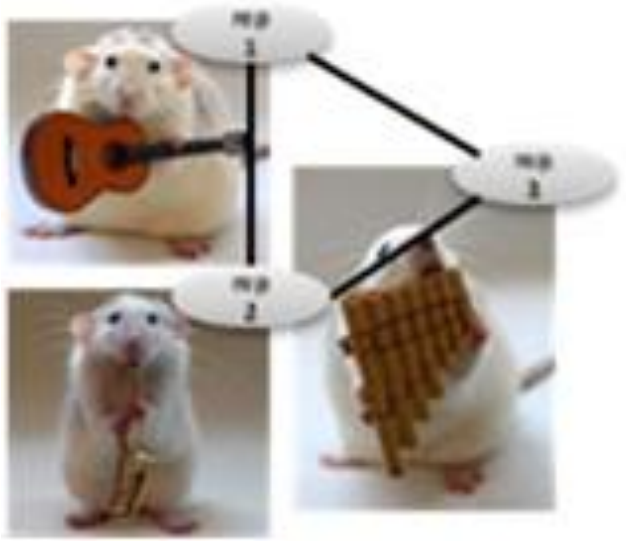
Courtesy of W. Tong

Root Mean Squared Distance

Measures the overall gene expression distance/deviation between pairs of samples *i* and *j*

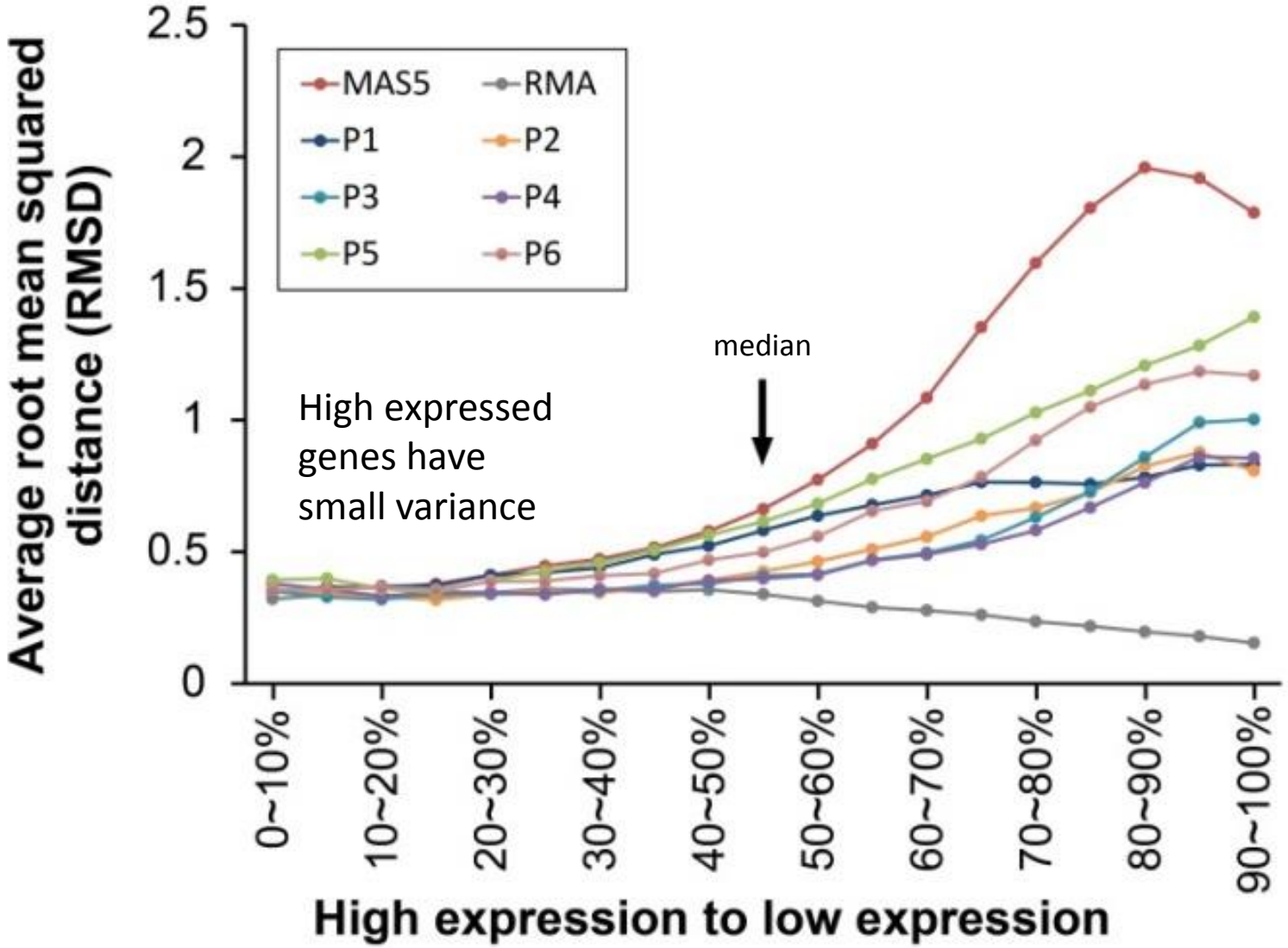
$$RMSD_{ij} = \sqrt{\frac{\sum_g (I_{ig} - I_{jg})^2}{N_g}}$$

I_g is the log2 transformed expression level of gene g in the corresponding sample and N_g is the number of genes in the set

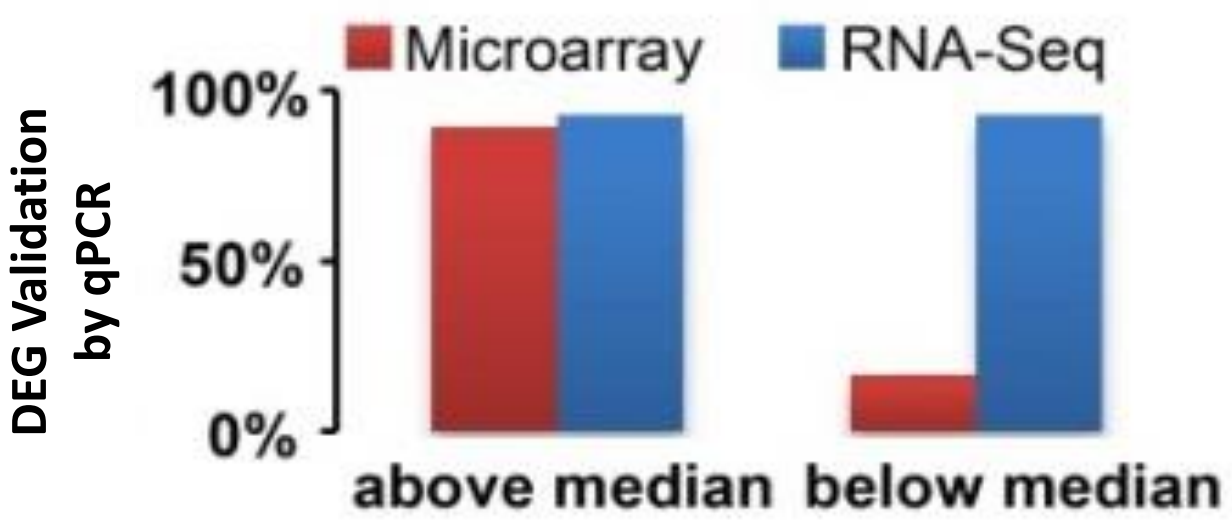


Compute the average RMSD for all pairs of replicates and compound treatments

Variability of Expressed Genes

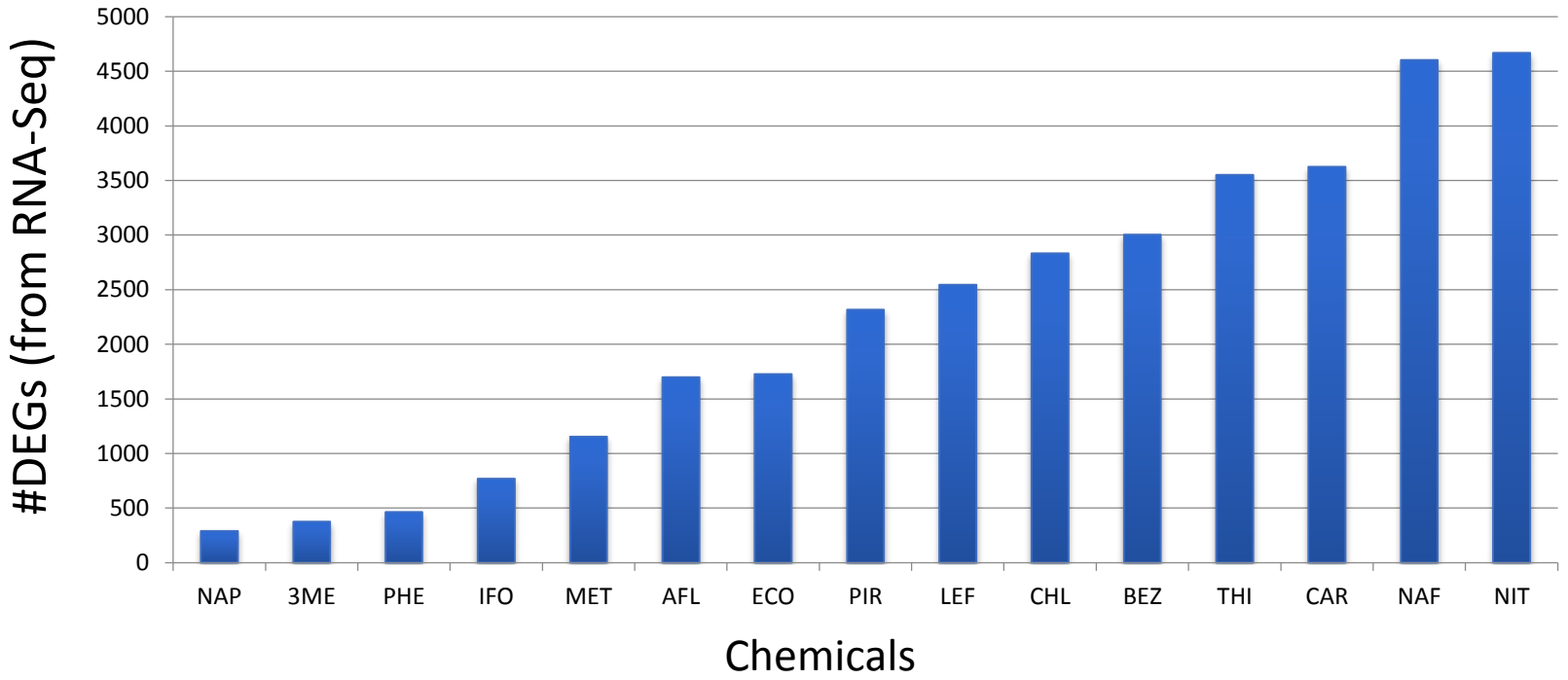


qPCR Validation



The Chemicals Elicited a Wide Range of DEGs

limma Treated vs control, FC > |1.5| and p-value < 0.05



How does the strength of the perturbation affect the agreement between the two platforms?

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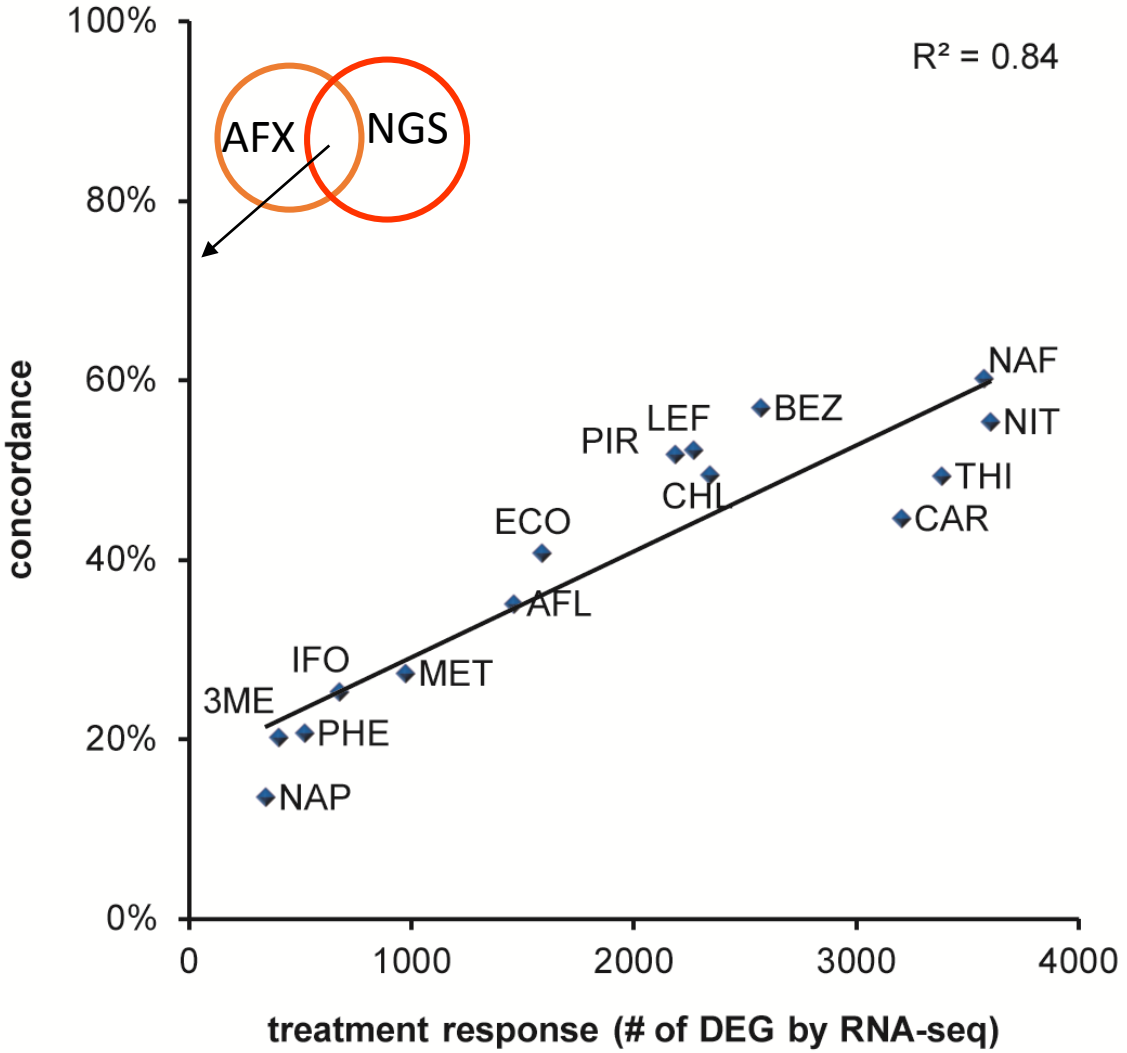
Concordance Linearly Correlates with the Treatment Response



The stronger the system is perturbed, the higher the concordance

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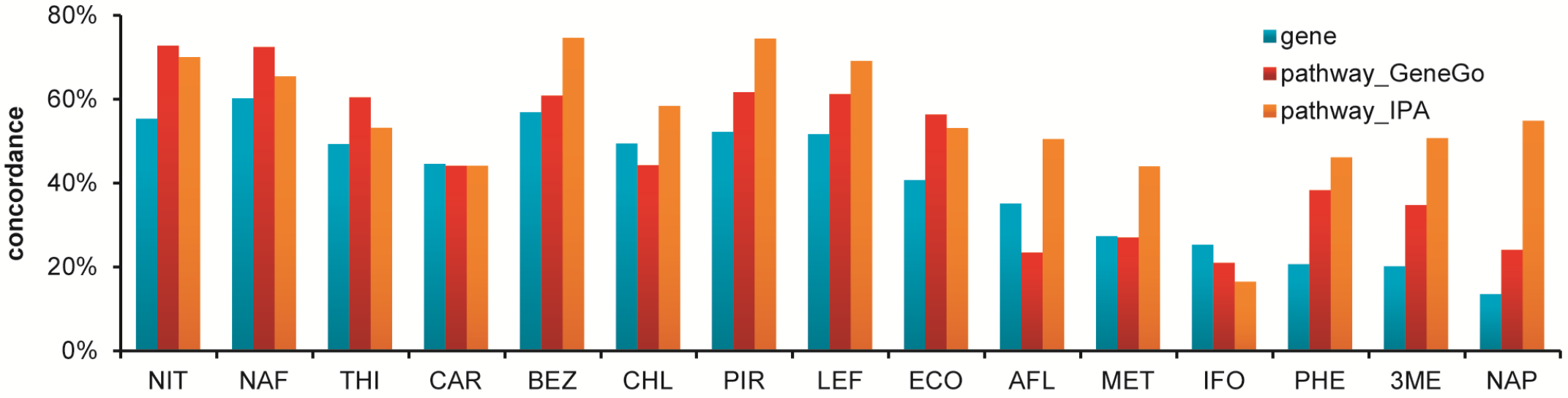
Concordance Increased at the Pathway Level

DEGs were mapped to GeneGo and IPA pathways
Concordance is the percentage of enriched pathways shared by the two platforms



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Take Home Messages

- Use fold change threshold coupled with a p-value cut off
- Filter out low expressed genes (primarily for microarray)
- Know your chemical's transcriptional strength (if at all possible)
- Pathways perform better than genes individually
- It is of interest if some of these findings can be extended to other transcriptomics platforms such as Tempo-Seq

Acknowledgements

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