
Correlation of microarray and quantitative real-time PCR results
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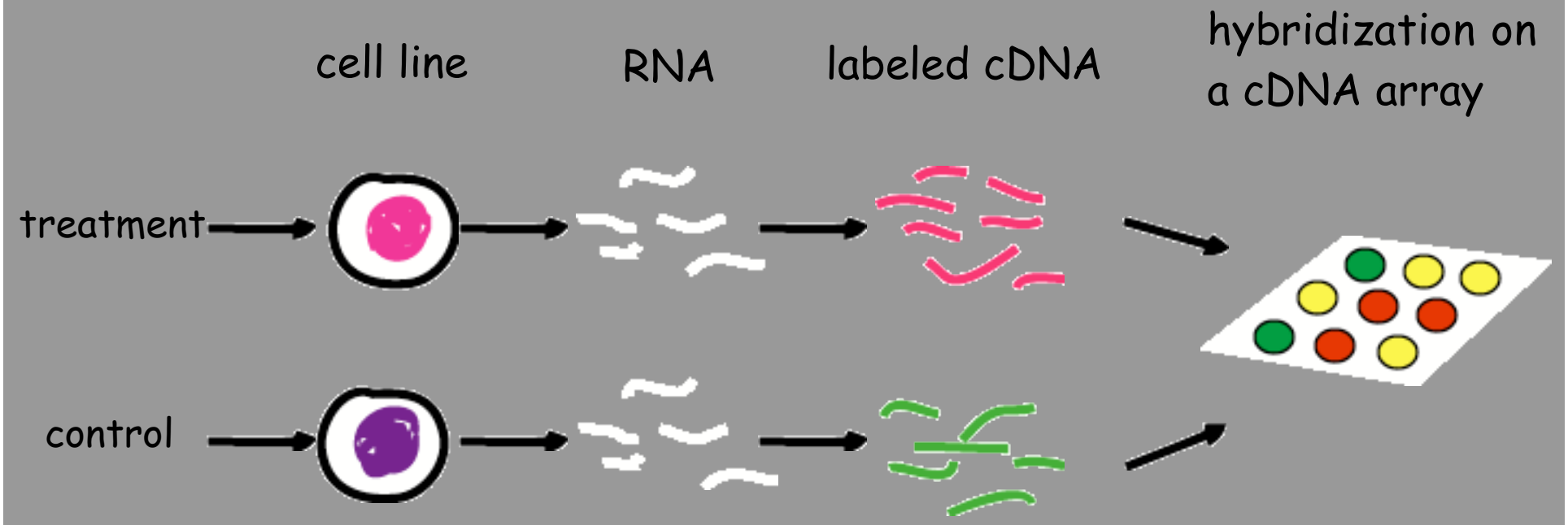
Microarray techniques

Oligo-array: Affymetrix, Codelink, spotted oligo-arrays (60-70mers)

cDNA array: purified PCR products, spotted in triplicates, chosen genes

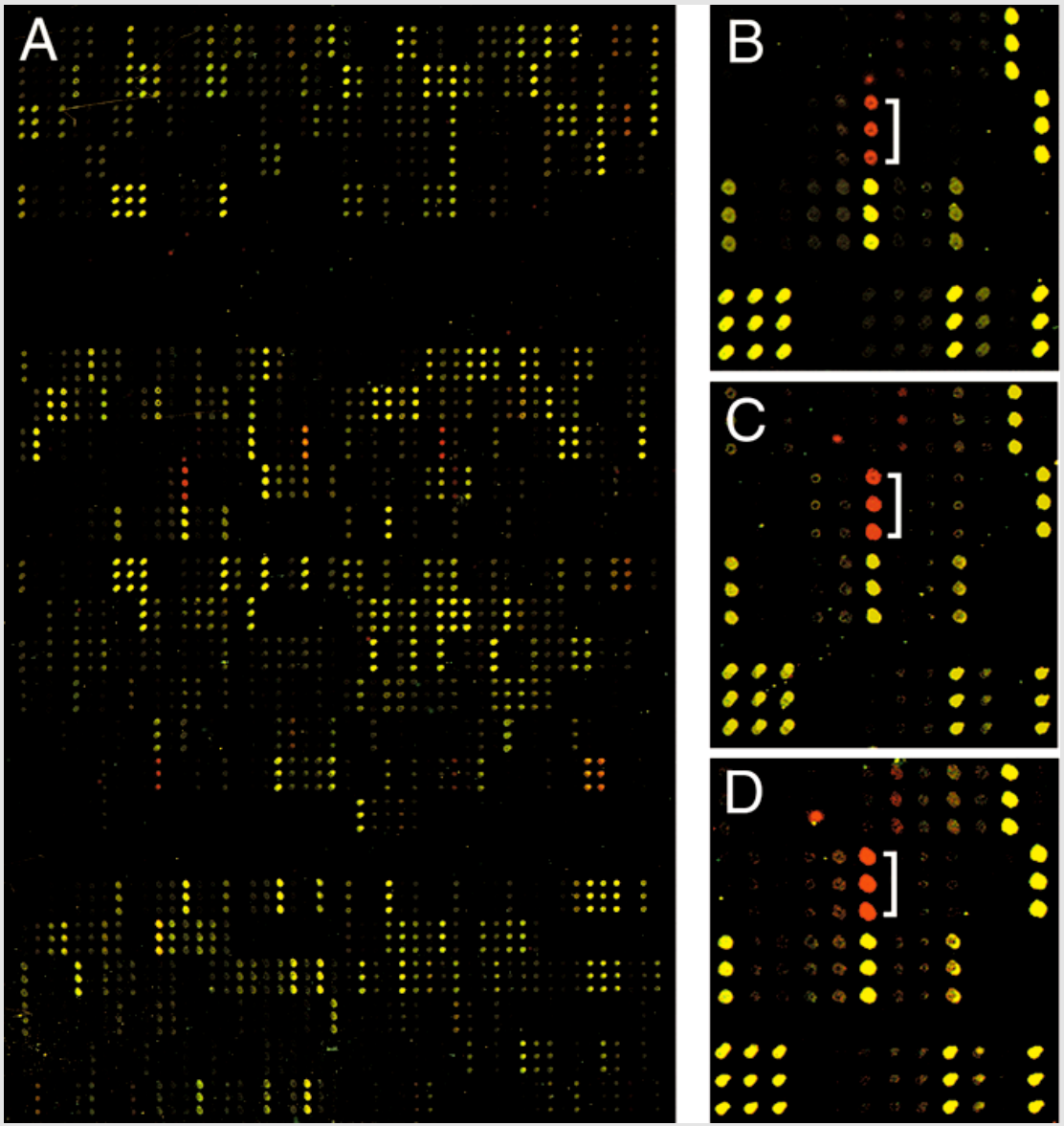
- Number of genes, density
- Verification of genes
- Concentration of probe
- Region of gene represented

Experimental design microarray

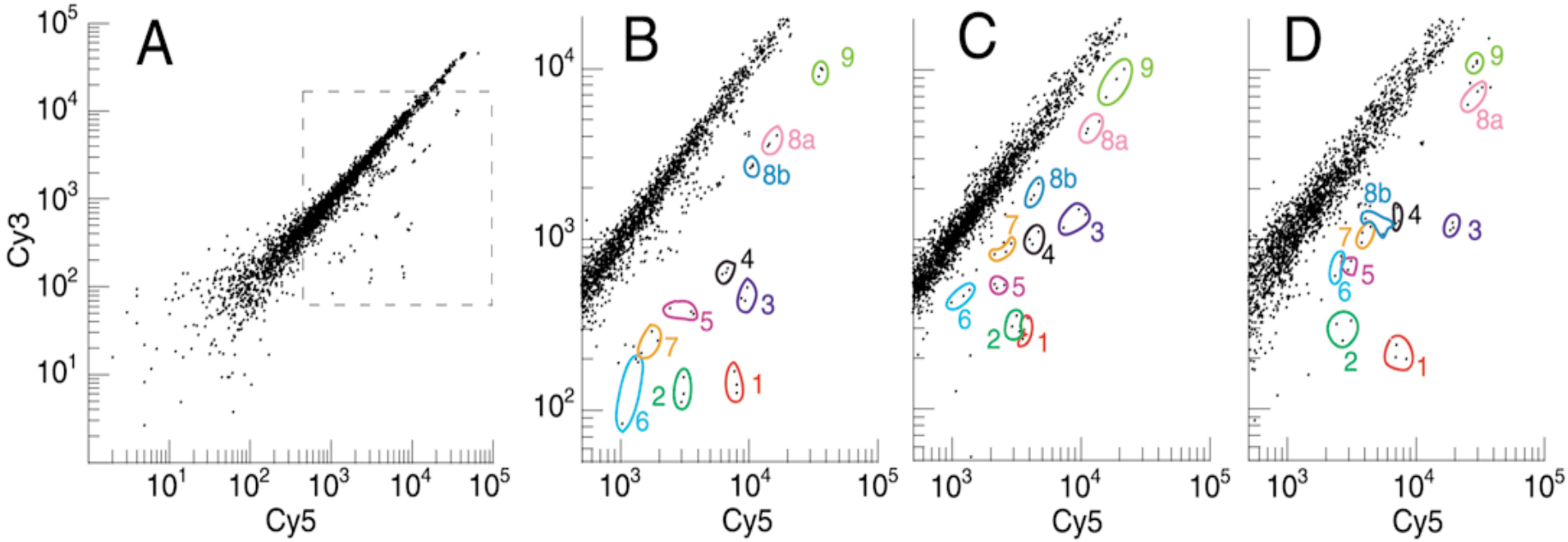


Custom made cDNA microarray: 956 genes spotted in triplicates

Wurmbach et al.: 2001, JBC, 276, 50, 47195-202



Reproducibility of the results generated by the cDNA microarray



Wurmbach et al.: 2001, JBC, 276, 50, 47195-202

Hybridization on microarrays and during PCR

Microarray

System: tethered-liquid

- direct or indirect
- concentration of spotted probe
- steric hinderance

PCR

liquid-liquid

- direct
- probe is accessible

- cDNA array: long probes
- Affymetrix: RNA-RNA, RNA-DNA hybrids
  oligo hybridization
- CodeLink: Gel

Comparison of the results from a cDNA microarray with QRT-PCR

Gene Name	Microarray (n=3) 1h	SYBR PCR (n=9) 1h
LRG 21	31.2 ± 16.6	96.8 ± 15.7
Egr1	12.9 ± 3.8	390.6 ± 83.9
c-fos	12.5 ± 4.0	52.0 ± 5.8
Nr4a1/nur77	5.9 ± 1.5	49.4 ± 10.1
Ier2/Pip92	5.2 ± 1.2	17.1 ± 2.4
Rgs2	4.0 ± 1.3	8.7 ± 0.4
c-jun	3.8 ± 0.6	6.6 ± 0.9
TSC22	3.4 ± 0.4	3.3 ± 0.2
γ-actin	2.7 ± 0.3	3.9 ± 0.4
Klf-like EST	2.3 ± 0.3	2.5 ± 0.2
Period1	2.1 ± 0.1	4.9 ± 1.1
β-actin	2.0 ± 0.5	1.6 ± 0.1
PRL1	2.0 ± 0.4	2.1 ± 0.1
IκB	1.7 ± 0.2	2.6 ± 0.2
Klf4	1.7 ± 0.3	2.9 ± 0.3
Gem	1.7 ± 0.2	4.3 ± 0.3
gly96	1.7 ± 0.6	3.8 ± 0.5
junD	1.6 ± 0.2	1.2 ± 0.2
Egr2	1.5 ± 0.2	294.7 ± 35.6
transgelin	1.4 ± 0.1	1.8 ± 0.2
NMDMC	1.4 ± 0.3	1.6 ± 0.3
Stat3B	1.4 ± 0.2	1.2 ± 0.1
MKP1/3CH134	1.4 ± 0.1	3.4 ± 0.4
Nrf2	1.3 ± 0.2	1.5 ± 0.1
HSP30	1.3 ± 0.2	1.9 ± 0.2
STY-Kinase	1.3 ± 0.04	1.7 ± 0.2
glucose transport protein	1.1 ± 0.4	2.2 ± 0.4
SCL	1 ± 0.2	2.4 ± 0.2
Gata2	0.7 ± 0.1	1 ± 0.1

- **Microarray**

28 up-regulated genes (1.3 - 31.2 fold changes)

3 down-regulated genes (1.3 - 1.4 fold changes)

- **QRT-PCR:**

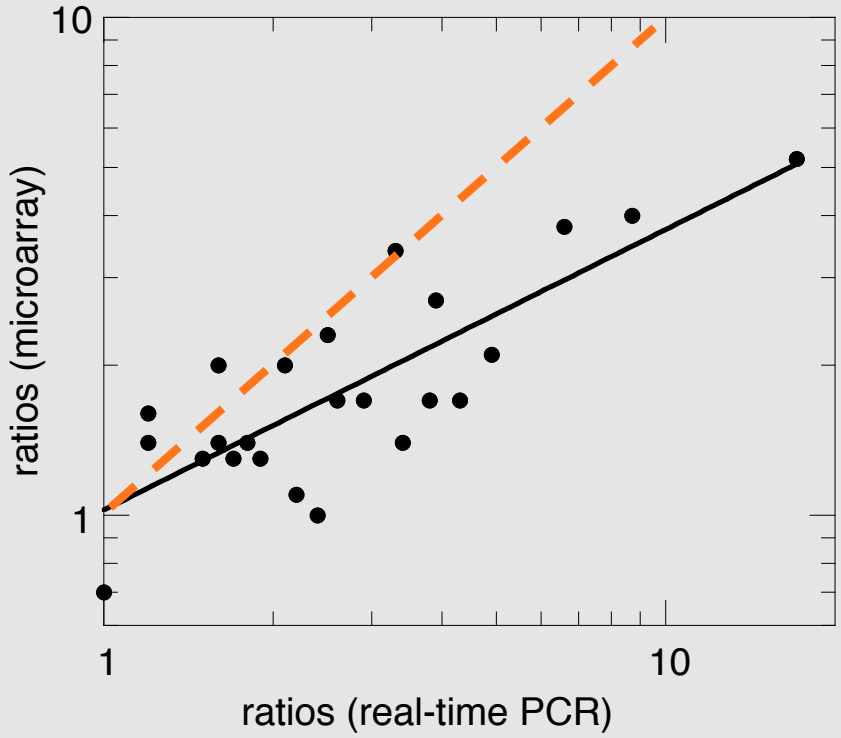
confirmation of 26 genes

100% (17/17) for gene changes > 1.6-fold

66% (6/9) for gene changes between 1.3 and 1.6

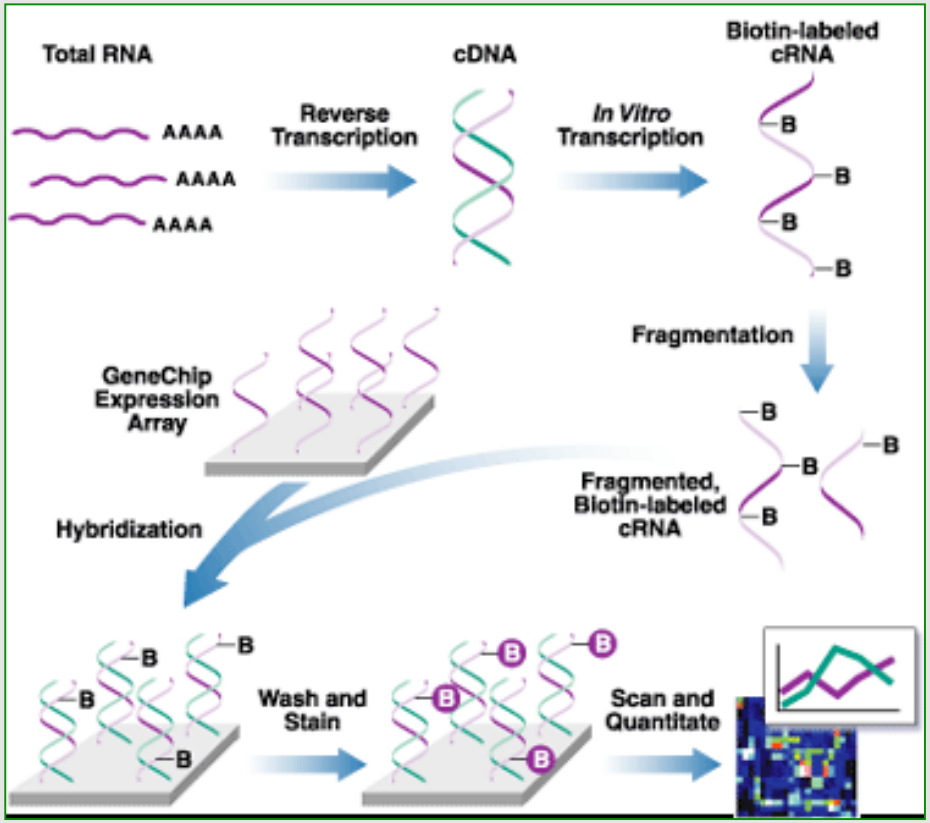
Wurmbach et al.: 2001, JBC, 276, 50, 47195-202

Correlation of fold change measurements obtained by cDNA-microarray and QRT-PCR



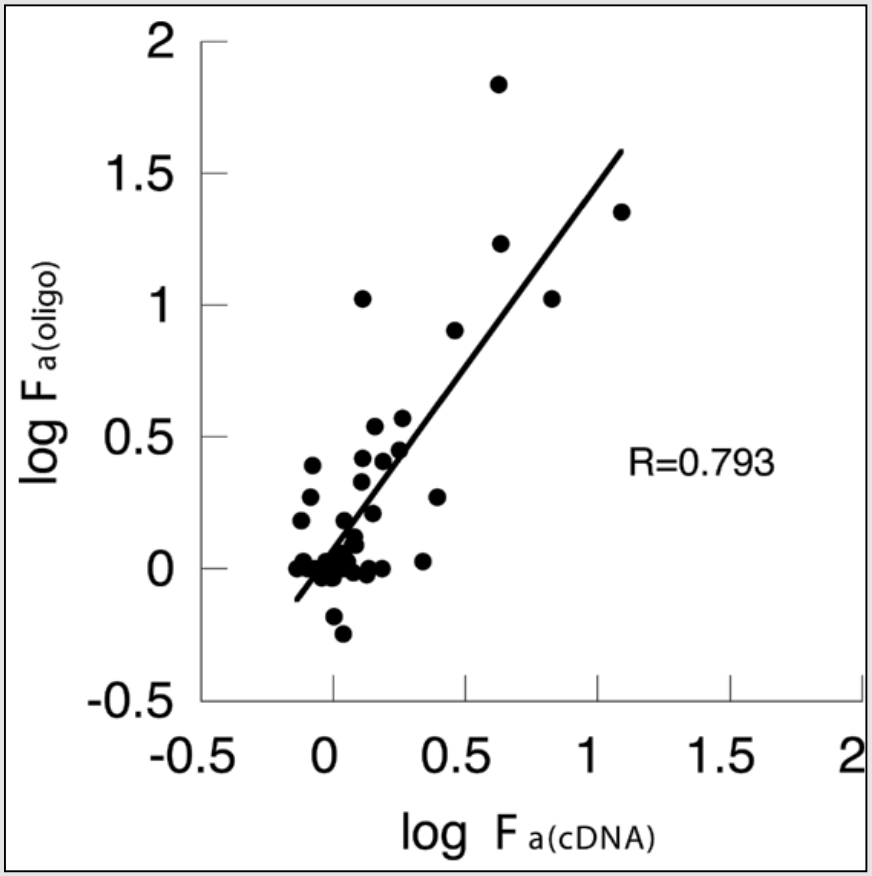
Genes showing less than 20-fold changes by QRT-PCR are highly correlated to cDNA-microarray fold changes (r=0.87).

Affymetrix: Gene expression analysis



Differential expression is measured by the comparison of two or more data-sets

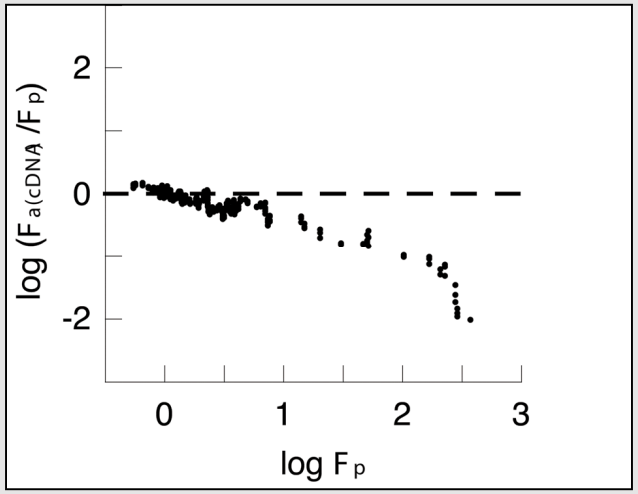
Comparison of the Affymetrix platform to a cDNA microarray



Yuen et al.,2002, Nucleic Acids Research, 30,10,e48

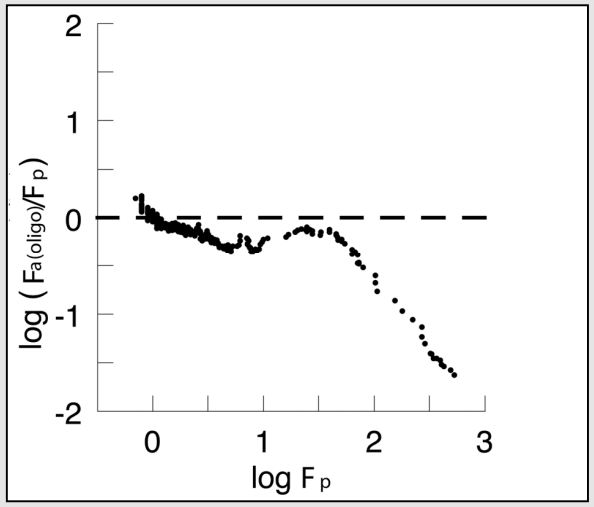
Fold change measurements obtained by cDNA and oligo-microarray compared to QRT-PCR

cDNA array



- bias to underestimate
- predictable correlation of the bias error
- saturation effect

Affymetrix



- bias to underestimate
- no predictable correlation of the bias error

Yuen et al.,2002, Nucleic Acids Research, 30,10,e48

Complex tissues

Biological variation is increased:

- increase the number of replicates
  - pooling individual samples

Heterogeneous cell population (dilution effect):

- treatment affect only a subset of cells
- the gene of interest is only expressed in a subset of cells

Complex tissues

Hypothalamus:

Hypoglycemia-associated autonomic failure

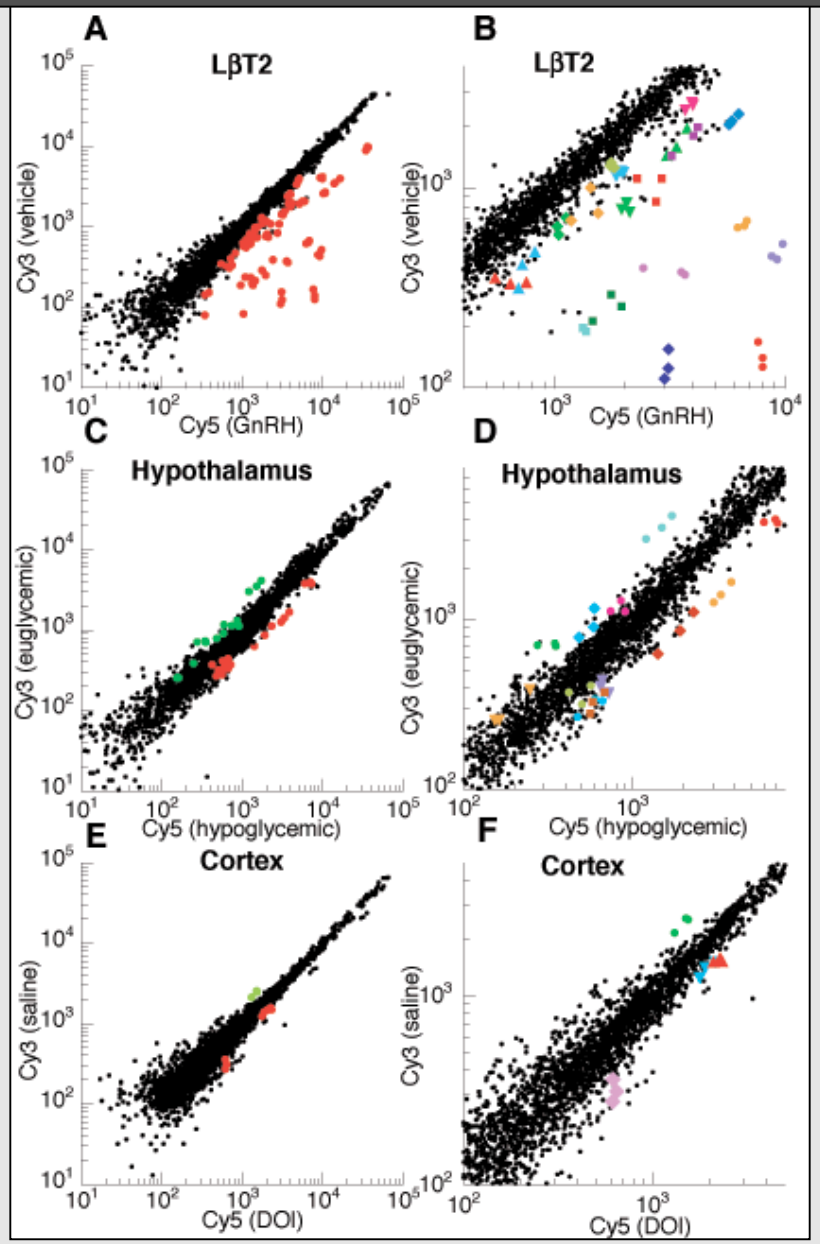
(Mice were fasted for 48h and given an insulin injection. After 3h the animals were killed by CO_2 and the RNA was extracted from their hypothalami).

Cortex:

Serotonergic hallucinogens

(Mice were treated with DOI, a hallucinogenic chemical. After 1h the animals were killed and the RNA was extracted).

Complex tissues



Wurmbach *et al.*, 2002,
Neurochem. Res. **27**, 1027-1033

Complex tissues

source	arrays used	gene candidates tested by PCR	**PCR confirmed genes**		
			all candidates	FC >1.6 on the array	FC <1.6 and >1.3
LßT2 cells	**3**	26	**23 (88.5%)**	17/17 (100%)	6/9 (66.7%)
hypothalamus	**5**	16	**12 (75%)**	9/12 (75%)	3/4 (75%)
cortex	**7**	14	**4 (28.6%)**	3/7 (42.9%)	1/7 (14.3%)

Wurmbach *et al.*, 2002, *Neurochem. Res.* **27**, 1027-1033

Normalization

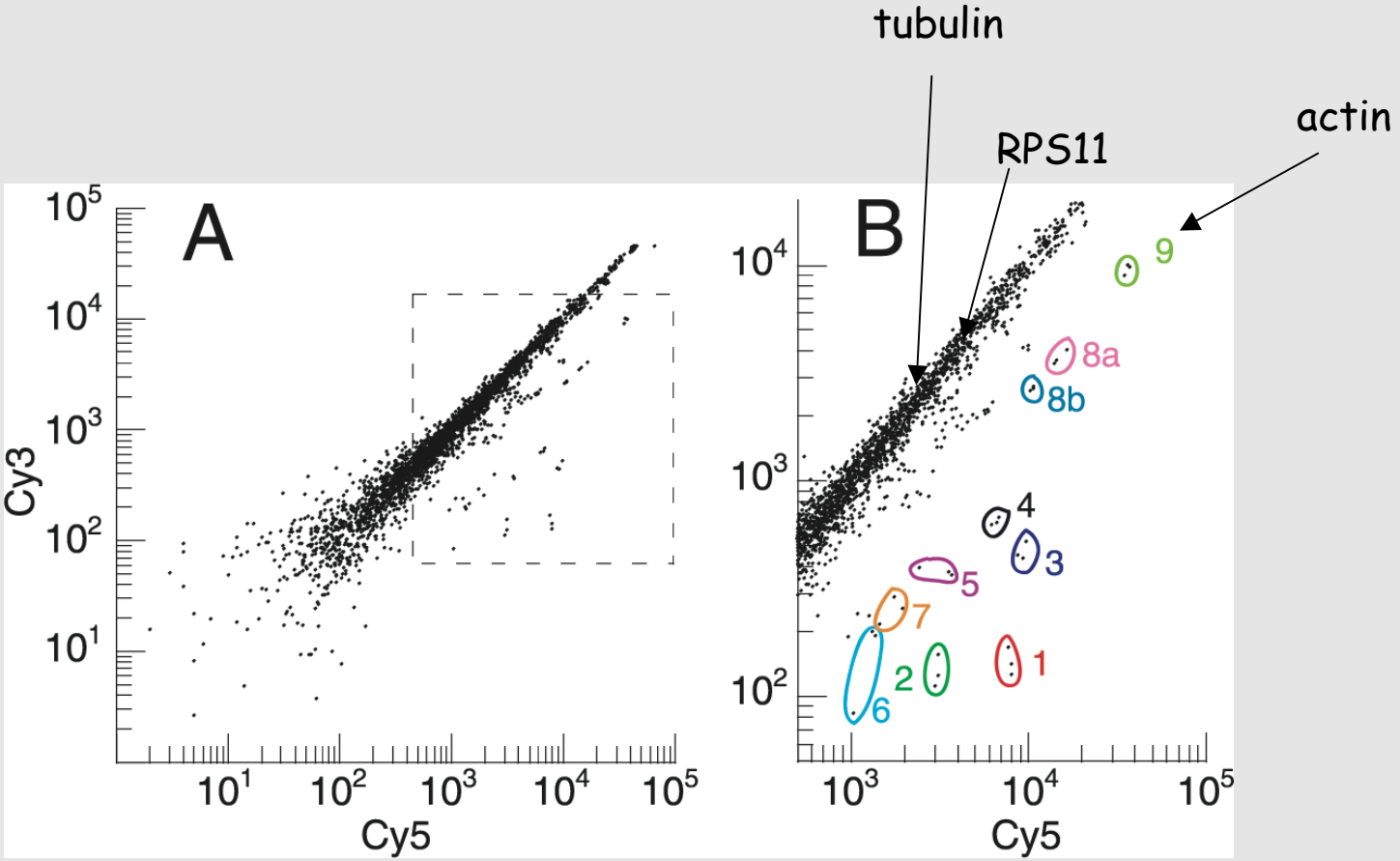
To adjust for experimental differences

- RNA quantity and quality
- overall transcriptional activity
- cDNA synthesis and PCR efficiency

→internal endogenous control: reference, housekeeping gene

- Constant expression in cells/tissue under investigation.
- No response to experimental treatment.

Normalization



Normalization

Using one "housekeeping gene" can lead to misinterpretation or even make interpretation impossible:

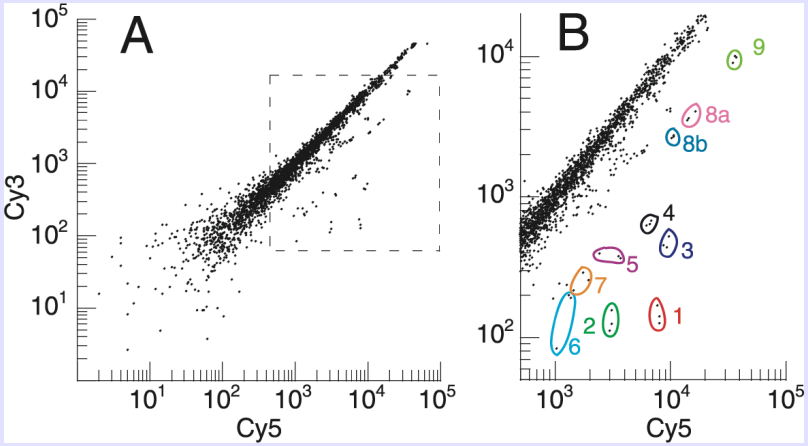
→ in slightly regulated genes: main information of the data may be inversed.
→ in strongly regulated genes: the regulation may be under- or overestimated.

Microarray: Experimental Design

Comparison

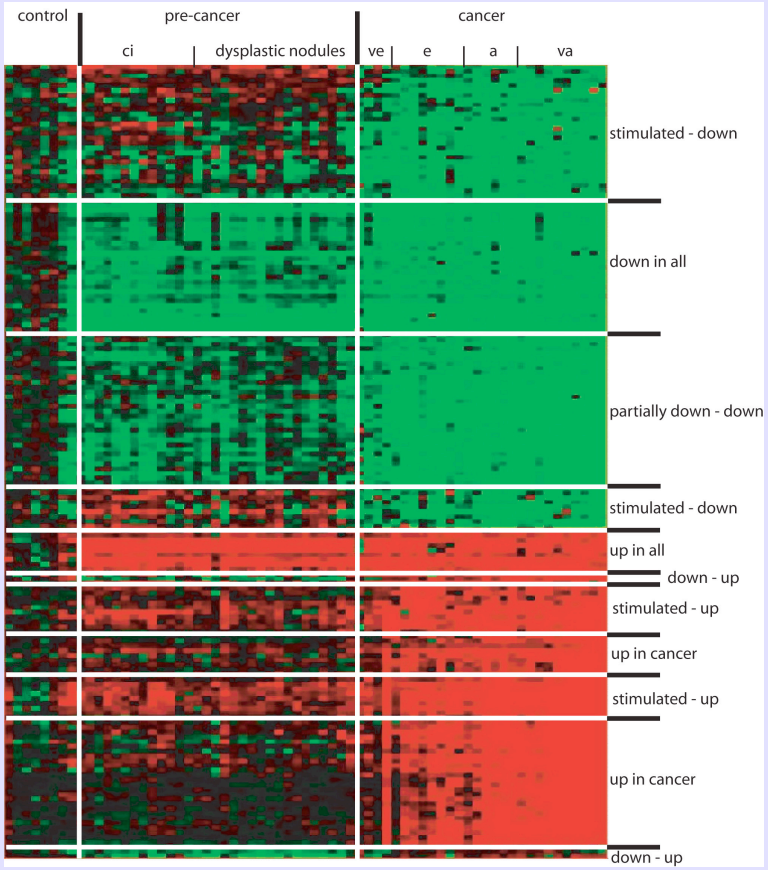
Treatment vs control

→ Confirmation of regulated genes



Matrix

Time course experiments
Cancer studies (diff. stages)



Normalization

323 genes

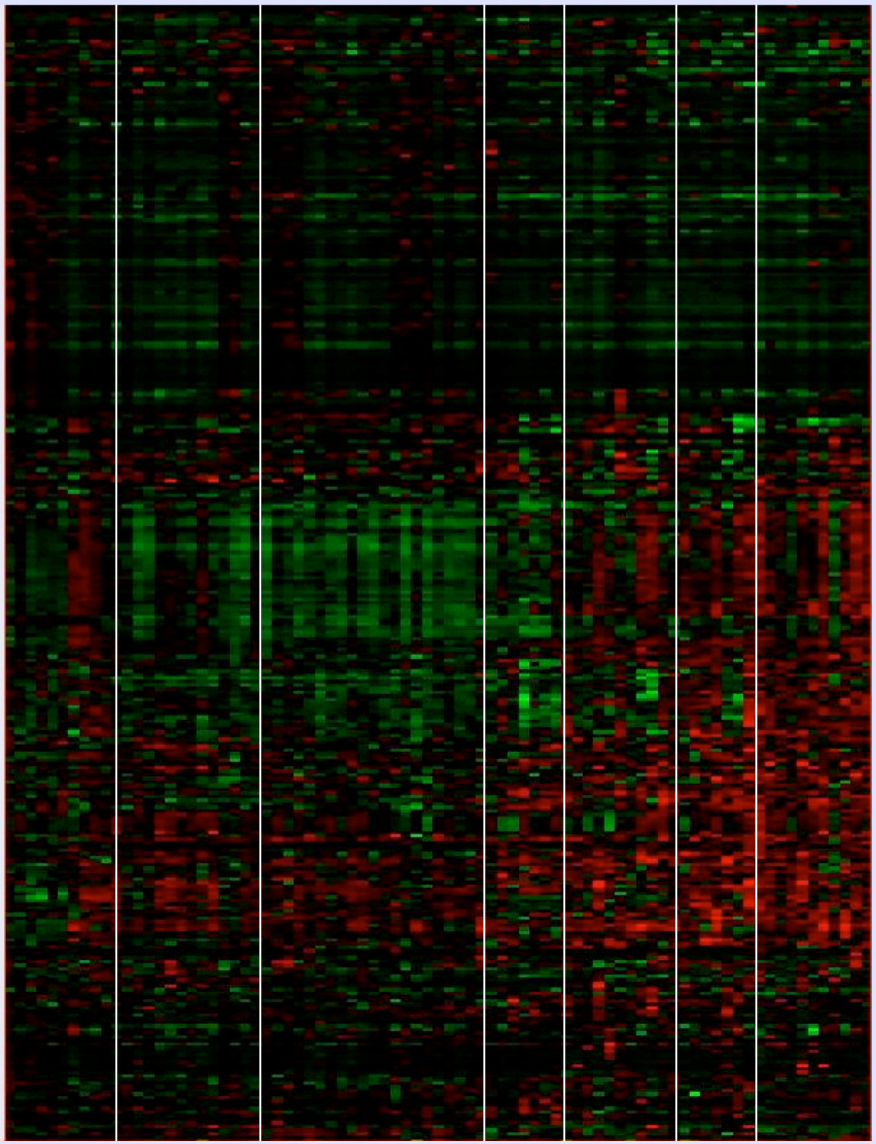
Metabolism:

- Sugar
- Nucleotide
- Lipid
- Amino acid
- Energy

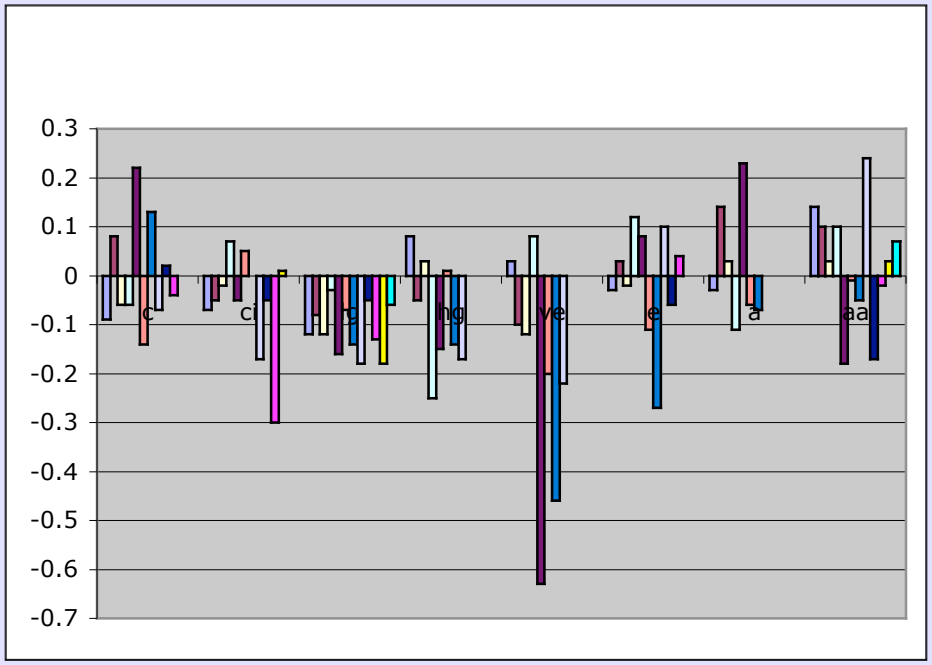
Ribosomal genes

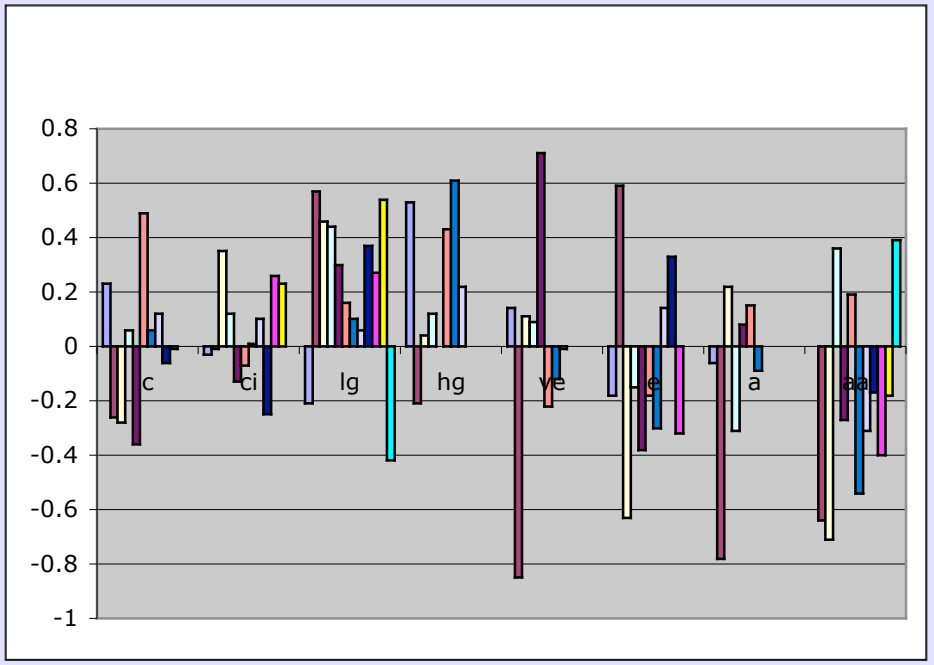
Cytoskeleton

Basal TF

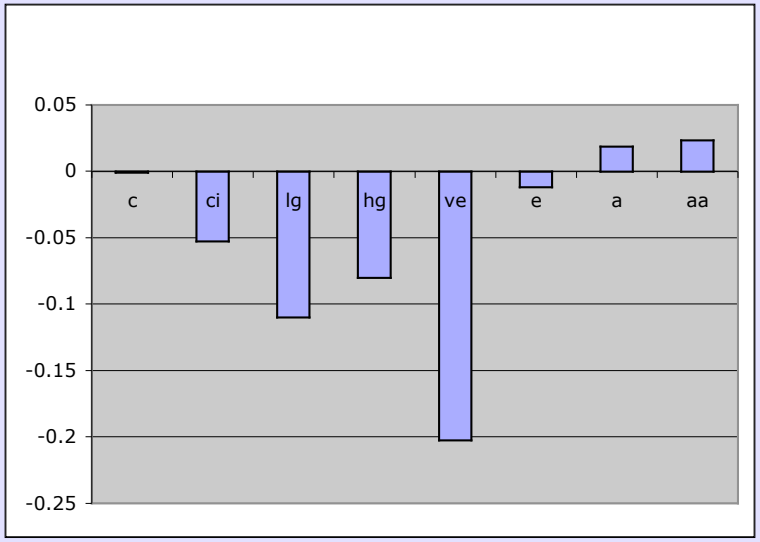


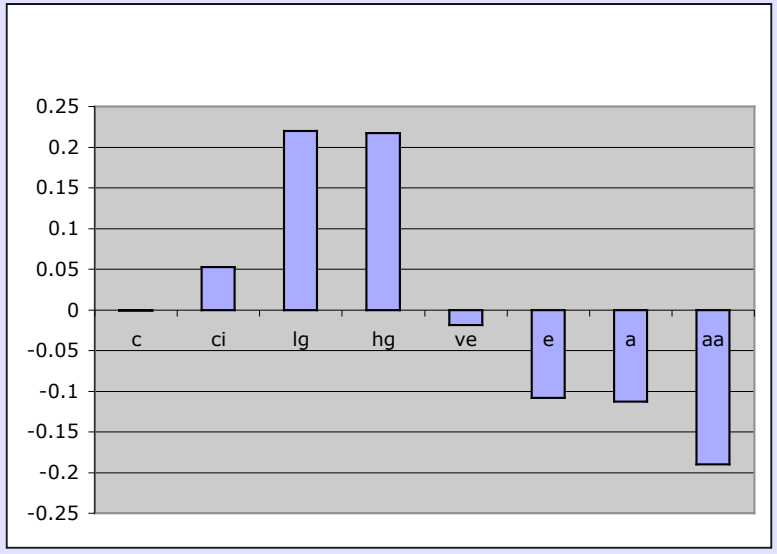
Normalization

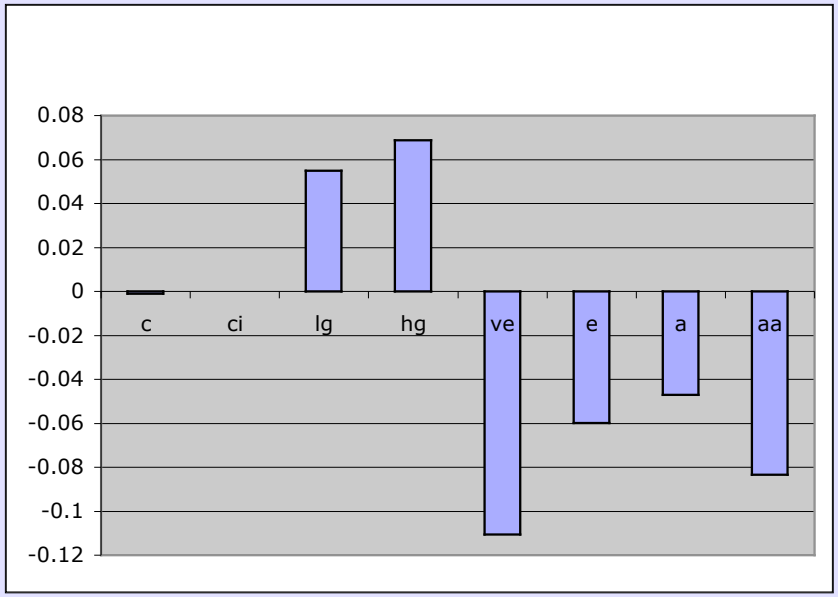




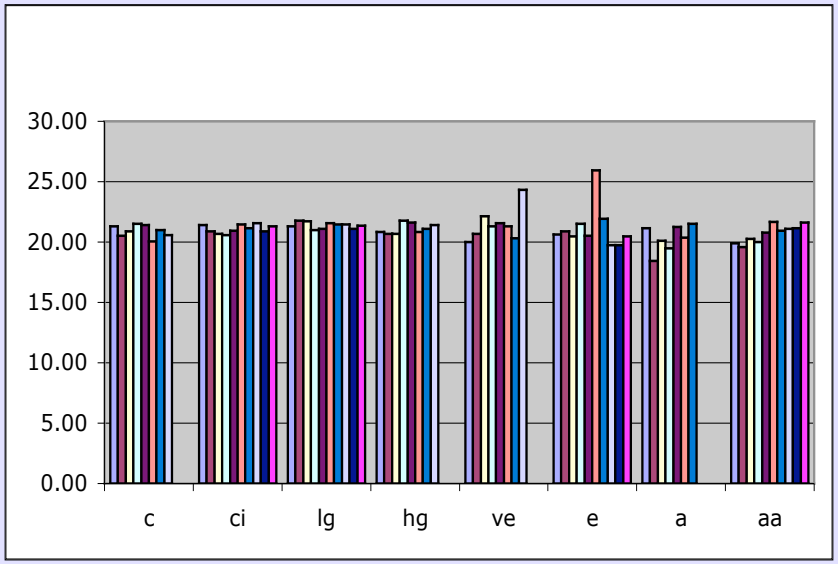
Normalization (microarray)

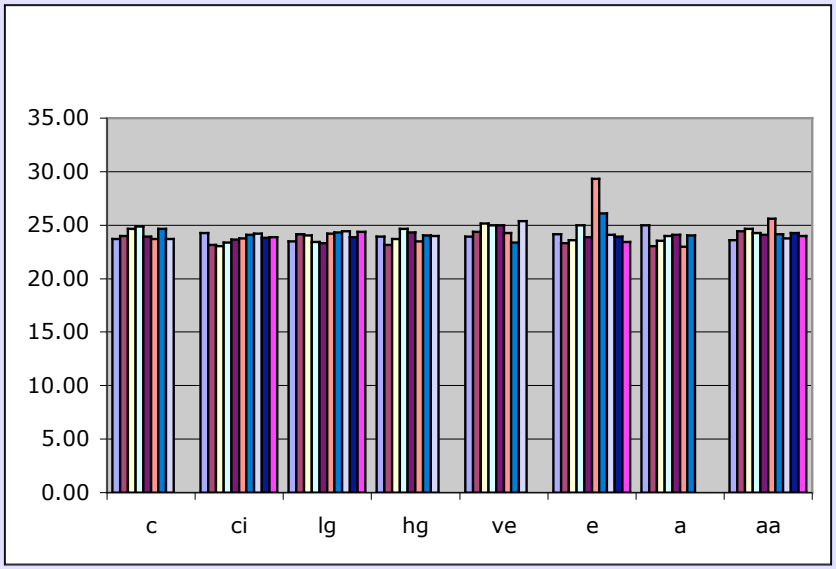


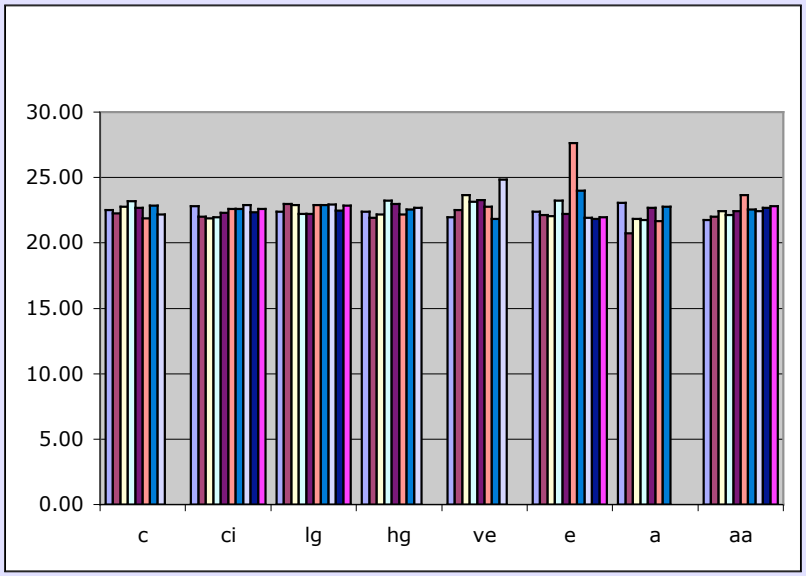




Normalization (QRT-PCR)

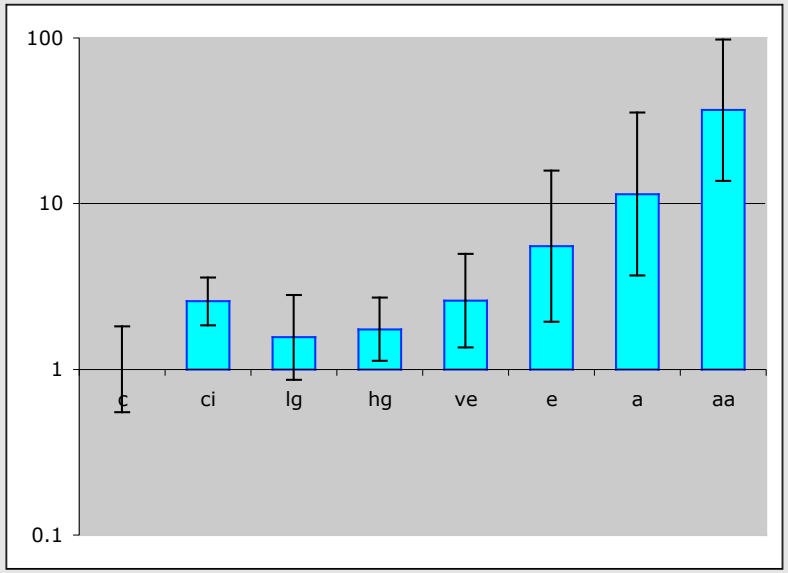




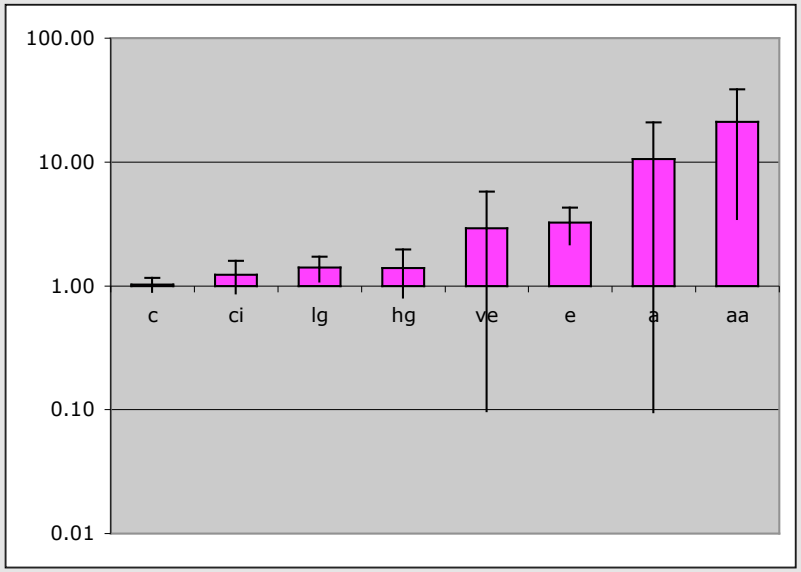


Correlation of QRT-PCR (TaqMan) and microarray (GeneChip)

TaqMan

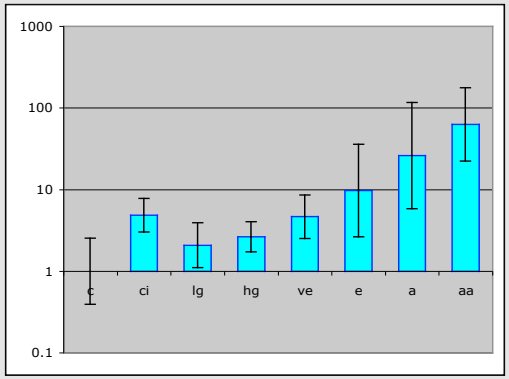


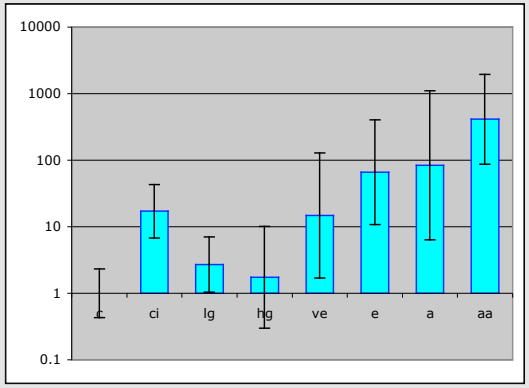
GeneChip

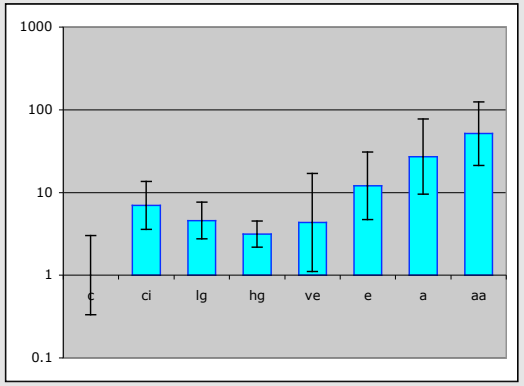


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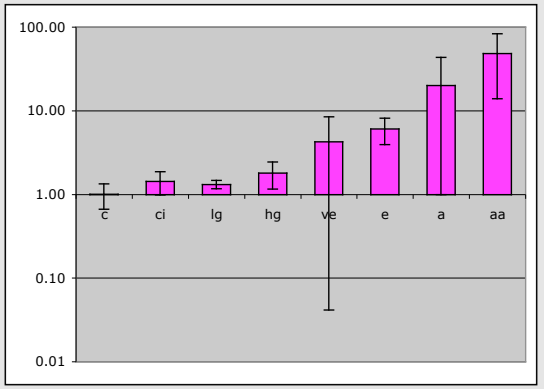
TaqMan

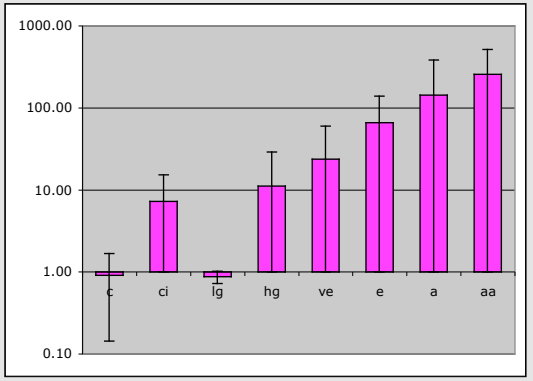


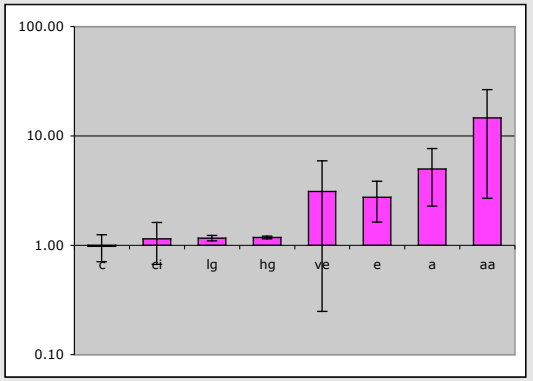




GeneChip

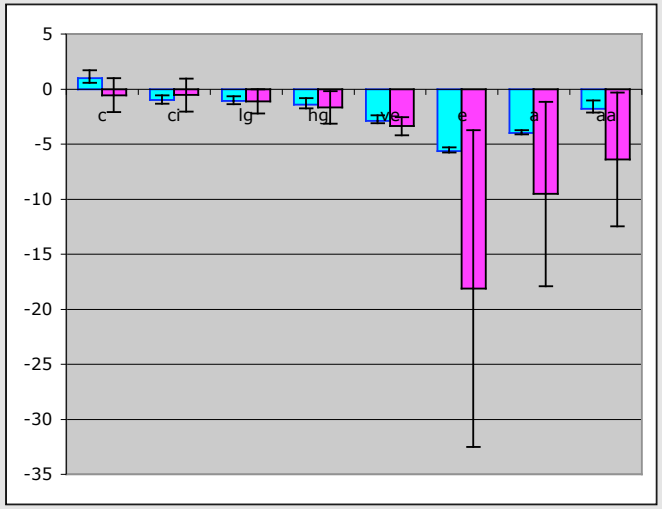


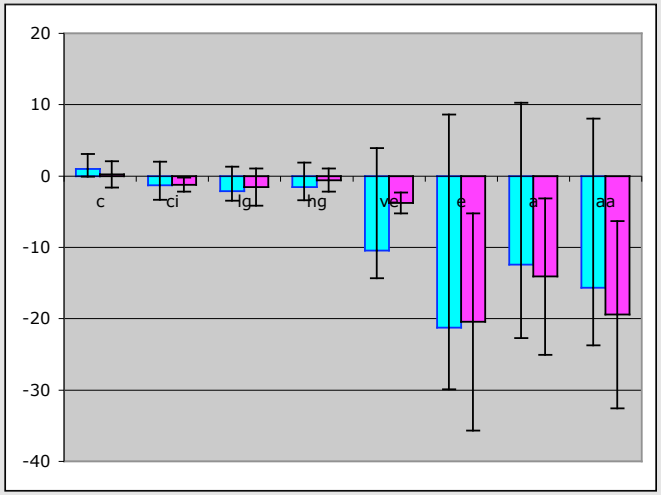




Correlation of QRT-PCR (TaqMan) and microarray (GeneChip)

down-regulated genes



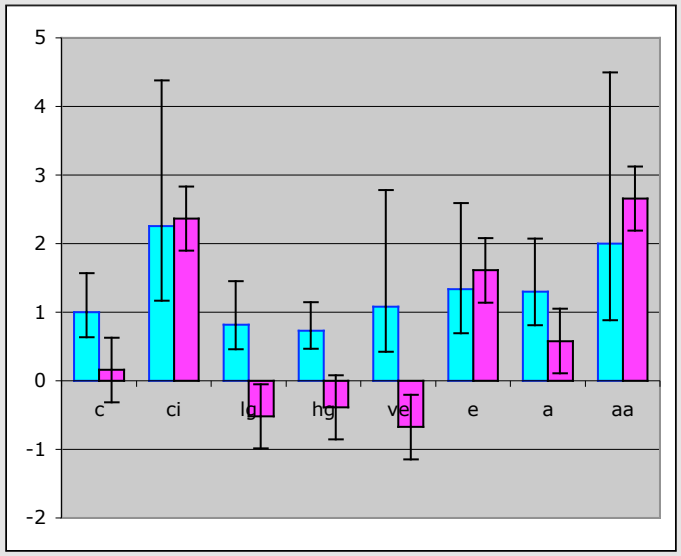


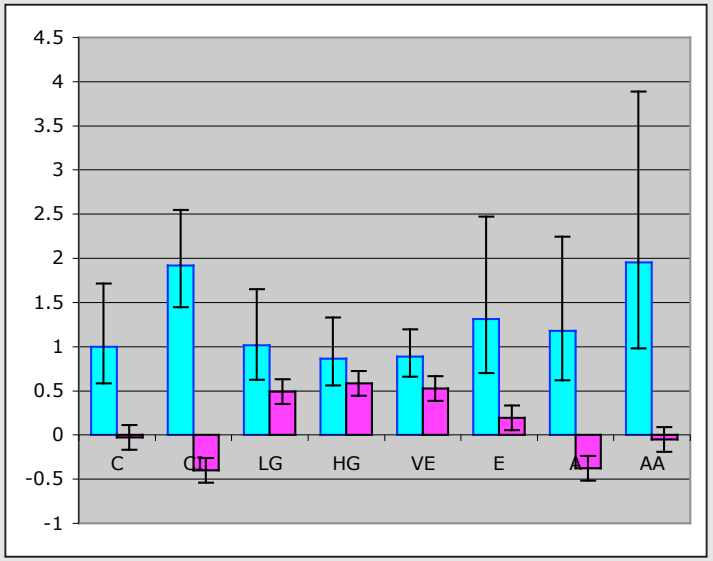
QRT-PCR

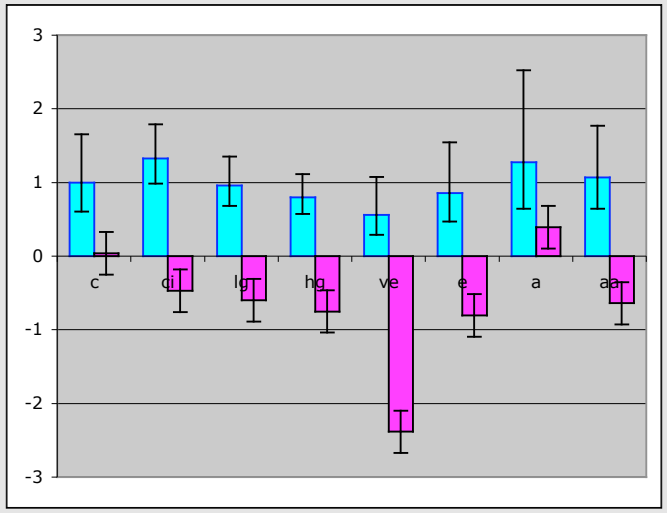
Affymetrix

Correlation of QRT-PCR (TaqMan) and microarray (GeneChip)

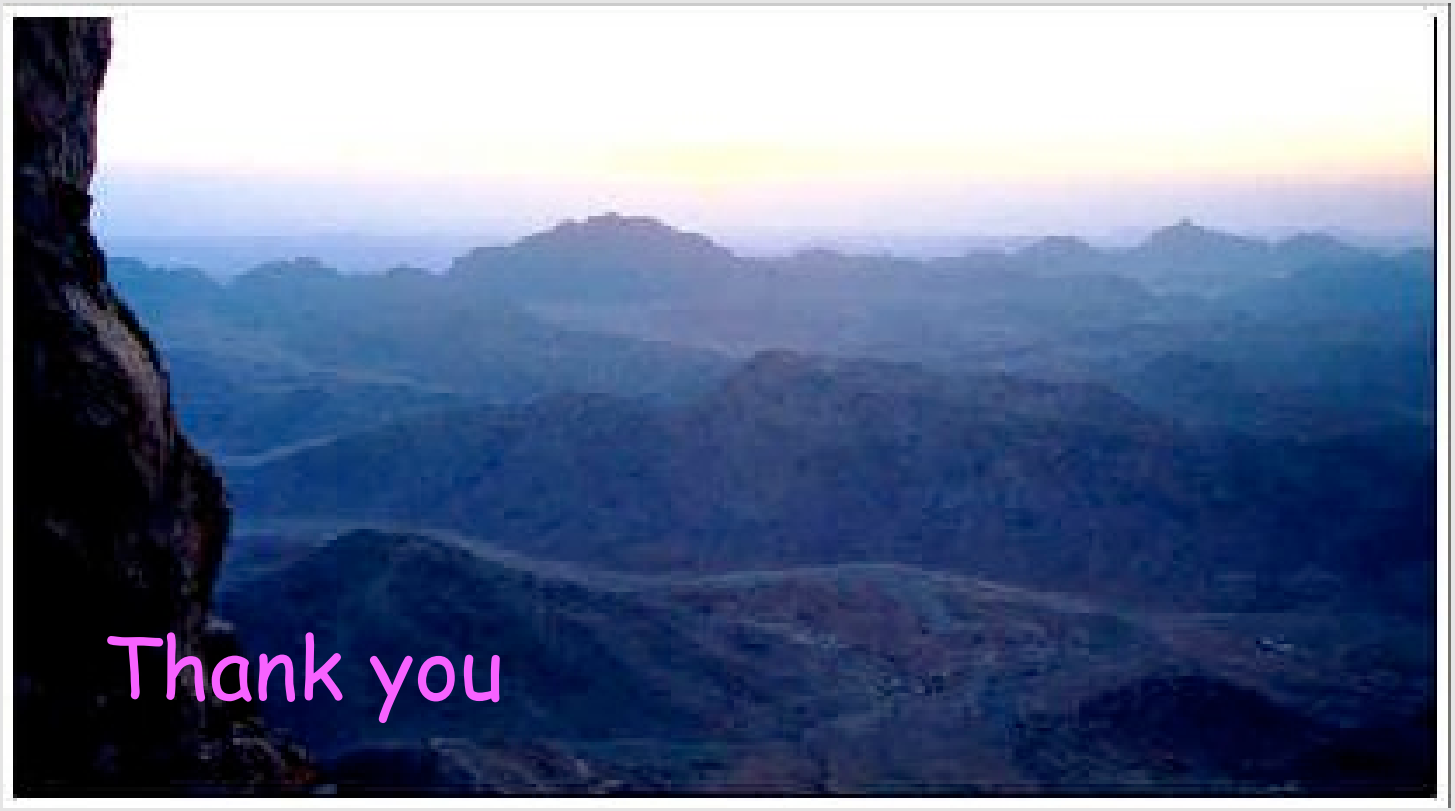
not-regulated genes







QRT-PCR
Affymetrix



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