Across Platform Comparisons

Collaborators

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- Ernest Kawasaki, David Petersen (NIH/NCI)
- John Quackenbush (TIGR)
- Shui Qing Ye, Skip Garcia (Hopgenes)
- Shyam Biswal, Hannah Lee (JHBSPH)
- Mike Wilson (NIH/NIAID)
- Constance Griffin, Laura Morsberger (JHMI)

Previous Work

Disagreement in Results

WP Kuo et al (2002) Analysis of mRNA measurements from two different microarray technologies. *Bioinformatics* 18(3):405 (Stanford cDNA vs Affy)

R Kothapalli et al (2002) Microarray results: how accurate are they? *BMC Bioinformatics* 3:22 (Incyte cDNA vs Affy)

J Li et al (2002) Differential gene expression patterns revealed by oligo-nucleotide versus long cDNA arrays. *Toxicological Sciences* 69:383 (Affy vs Incyte cDNA: semi-disagreement)

PK Tan et al (2003) Evaluation of gene expression measurements from commercial platforms. *Nucleic Acids Res* 31(19):5676 (Agilent cDNA, Affy, Amersham 30mer)

AT Rogojina et al (2003) Comparing the use of Affymetrix to spotted oligonucleotide microarrays using two retinal pigment epithelium cell lines. *Molec Vision* 9:482 (Affy vs Clontech long oligo)

This slide and the next are courtesy of Ernest Kawasaki

Previous Work

Agreement in Results

MD Kane et al (2000) Assessment of the sensitivity and specificity of oligonucleotide (50mer) microarrays. *Nucleic Acid Res* 28(22):4552 (Operon 50mer vs cDNA)

TR Hughes et al (2001) Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. *Nature Biotech* 19:342 (Rosetta oligo vs cDNA)

T Yuen et al (2002) Accuracy and calibration of commercial oligonucleotide and custom cDNA microarrays. *Nucleic Acid Res* 30(10):e48 (Affy vs custom cDNA)

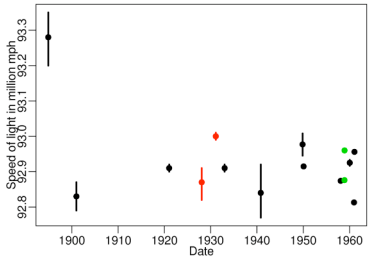
A Barczak et al (2003) Spotted long oligonucleotide arrays for human gene expression analysis. *Genome Res* 13:1775 (Affy vs Operon 70mer)

MG Carter et al (2003) In situ-synthesized novel microarray optimized for mouse stem cell and early developmental expression profiling. *Genome Res* 13:1011 (Agilent 60mer vs custom cDNA)

H-Y Wang et al (2003) Assessing unmodified 70-mer oligonucleotide performance on glass-slide microarrays. *Genome Biol* 4:R5 (custom oligo and cDNAs)

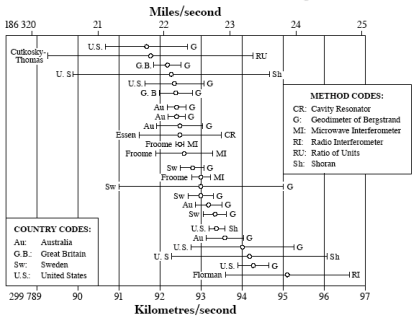
JK Lee, et al (2003) Comparing cDNA & oligonucleotide array data: concordance of gene expression across platforms for the NCI-60 cancer cells. *Genome Biol* 4:R82

Speed of Light Estimates with "Confidence Intervals" (1900-1960)

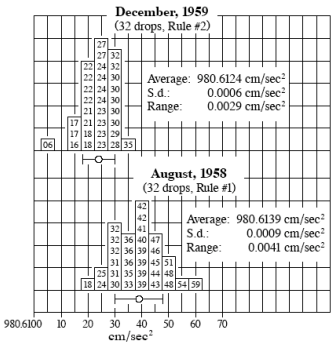


Youden, WJ (**1972**), "**EnduringValues**," **Technometrics** , 14, 1-11.

Speed of light (recent measurments)



Acceleration due to gravity (Ottawa)



Problems

Previous studies have at least one of these problems:

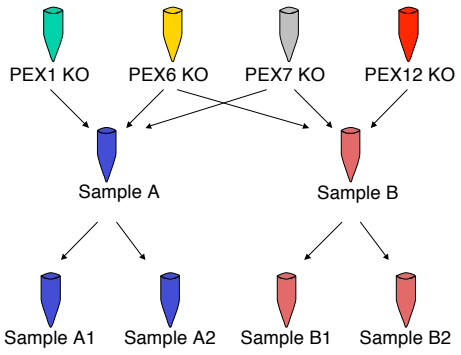
- **Precision/Accuracy not properly assessed**
  - **Sensitivity/Specificity trade-off not considered**
  - **Assessment based on validation of few genes**
- **No a-priori expectation of truth**
  - **RT-PCR is considered gold standard**
- **Effect of preprocessing not explored**
- **Lab effect not explored**

Experimental Recommendations

Our Approach

- Obtain results from at least two labs from each platform
- We used only labs in the DC/Baltimore area
  - 5 Affymetrix oligo
  - 3 two-color cDNA
  - 2 two-color oligo
- Send them same RNA
- Include tech reps (assess precision)
- Try do induce a-priori knowledge of DE
- Compare to RT-PCR (16 genes)

Sample Preparation



Sample Preparation

- Sample A = 1/2 PEX1 + 2/5 PEX6 + 1/10 PEX7
- Sample B = 1/10 PEX6 + 2/5 PEX7 + 1/2 PEX12

Gene	A	B	Fold change
PEX1	5/10	10/10	2.0
PEX6	6/10	9/10	1.5
PEX7	9/10	6/10	1.5 (-)
PEX12	10/10	5/10	2.0 (-)

Statistical Recommendations

Data

Basic unit of measurement

$$M_1=log_2(B_1 / A_1) \qquad M_2=log_2(B_2 / A_2)$$

It is important to look at relative expression as opposed to expression

Why? The probe effect

Why relative expression?

- A simple random effect model is

$$M_{ij} = d + t_i + e_{ij},$$

i represents lab and *j* replicates

- Correlation within technologies:

$$Corr(M_1,M_2) = (v_d+v_t)/(v_d+v_t+v_e)$$

- Correlation across technologies:

$$Corr(M_1,M_2) = v_d/(v_d+v_t+v_e)$$

Table 2 | Correlation and s.d. measurements computed for absolute and relative measurements of expression

	Correlation		s.d.	
	Absolute	Relative	Absolute	Relative
Affymetrix oligo versus Affymetrix oligo	0.98	0.79	0.16	0.15
Two-color cDNA versus two-color cDNA	0.91	0.65	0.29	0.23
Affymetrix oligo versus two-color cDNA	0.40	0.44	0.91	0.25

Affymetrix oligo lab 4 and two-color cDNA lab 1 were used for this comparison.

Precision/Accuracy

- Precision: SD of $M_1 - M_2$
- Accuracy: Slope of *M*s regressed against nominal log fold change

How do we match?

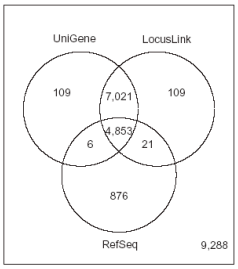


Figure 3 | Venn diagram illustrating agreement between annotation databases. For each mapping (UniGene, LocusLink and RefSeq) we obtained a different set of genes that had identifiers for each platform. This Venn diagram shows the agreement between these three different lists.

Results

Platform	Lab ID	Precision		Accuracy	Proportion of Agreement		
		Correlation	SD	Signal (SE)	25	50	100
Affy oligo	1	0.48	0.32	0.62 (0.05)	0.72	0.56	0.54
Affy oligo	2	0.76	0.17	0.64 (0.05)	0.80	0.70	0.70
Affy oligo	3	0.67	0.24	0.66 (0.05)	0.68	0.66	0.60
Affy oligo	4	0.79	0.15	0.59 (0.04)	0.80	0.70	0.65
Affy oligo	5	0.59	0.25	0.58 (0.05)	0.64	0.68	0.55
two-color cDNA	1	0.65	0.23	0.41 (0.12)	0.68	0.64	0.65
two-color cDNA	2	0.68	0.21	0.13 (0.04)	0.28	0.30	0.38
two-color cDNA	3	0.46	0.23	0.54 (0.09)	0.72	0.68	0.50
two-color oligo	1	0.68	0.51	0.21 (0.09)	0.40	0.36	0.33
two-color oligo	2	0.90	0.10	0.76 (0.13)	0.44	0.72	0.81

Precision

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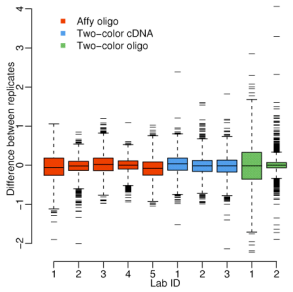
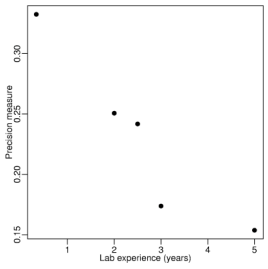
Precision



Experience seems important



Acuracy

Platform	Lab ID	Precision Correlation	Precision SD	Accuracy Signal (SE)	Proportion of Agreement 25	50	100
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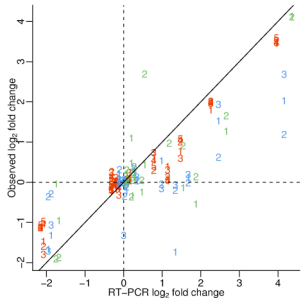
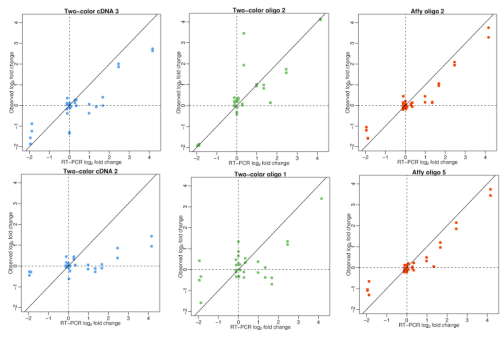
Accuracy



Accuracy



Results

Table 1 | Assessment measures for all ten labs

		Precision			Proportion of agreement		
Platform	Lab number	Correlation	s.d.	Accuracy signal (s.e.m.)	25	50	100
Affymetrix oligo	1	0.48	0.32	0.62 (0.05)	0.72	0.56	0.54
Affymetrix oligo	2	0.76	0.17	0.64 (0.05)	0.80	0.70	0.70
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To summarize precision we used the correlation across replicate $\log_2$-fold change measurements and standard deviation (s.d.) of the difference between replicate $\log_2$-fold change measurements. To quantify accuracy we regressed the observed $\log_2$-fold changes of 16 genes against nominal $\log_2$-fold changes obtained using RT-PCR. The slope of the regression line defines what we refer to as accuracy signal. The proportion of agreement in interesting genes lists—ranked by fold change—of sizes 25, 50 and 100, created with replicate $\log_2$-fold change measurements, are also used to assess precision.

Should we use all genes to assess agreement?

If no differential expression

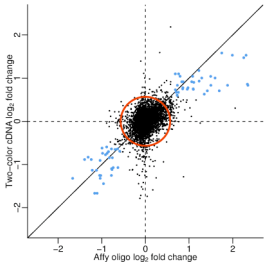
- A simple model is

$$M_{ij} = d + t_i + e_{ij}$$

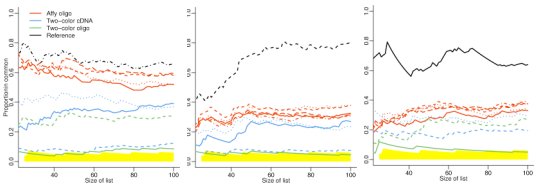
- But $d=0$, so correlation across technologies should be:

$$Corr(M_1, M_2) = 0$$

All genes not important



CAT plots



Affy cDNA Oligo

Here, we use the best performing labs for each platform as references

CAT plots

- **Can also be used to assess precision**
- **The precision assessment gives us an idea of what to expect:**
  - **Is 40% concordance good?**

Precision II

Platform	Lab ID	Precision Correlation	Precision SD	Accuracy Signal (SE)	Proportion of Agreement 25	50	100
Affy oligo	1	0.48	0.32	0.62 (0.05)	0.72	0.56	0.54
Affy oligo	2	0.76	0.17	0.64 (0.05)	0.80	0.70	0.70
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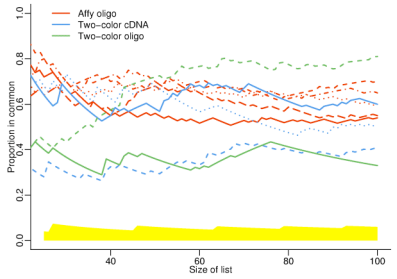
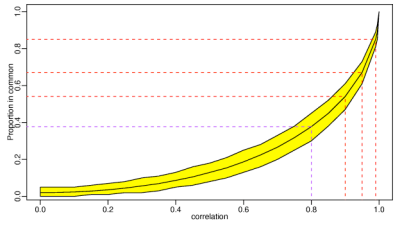
Precision II



Is 40% concordance good?

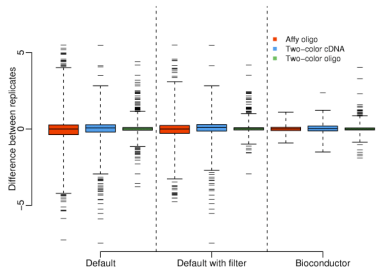


Conclusions

- **Important to assess accuracy/precision together**
- **Lab effect is big**
- **2 color oligo provided one of the best and worst**
- **Affymetrix more consistent across labs**

Supplemental Slides

Pre-processing



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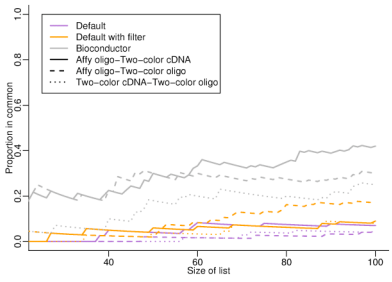
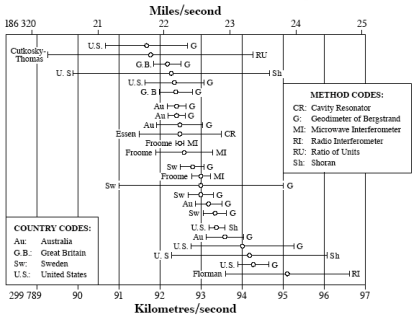


Speed of light (recent measurments)



Acceleration due to gravity (Ottawa)