



BRIEF COMMUNICATIONS

Cross Comparison of DNA Microarray Platforms

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Abstract: A variety of approaches have been taken in applying DNA microarray technology to the measurement of transcript levels in cells. The Alliance for Cellular Signaling (AfCS) has tested a few of these approaches to determine which ones might be most suitable for our large-scale experiments, with particular emphasis on the reliability of transcript measurements. This study compared the reproducibility and sensitivity of several microarray platforms, including the Affymetrix GeneChip, custom cDNA arrays, and custom oligo arrays. We also examined different methods for DNA microarray target preparation. In general, transcript measurements with all three array systems showed a high correlation with transcript levels measured using real-time quantitative reverse transcription-polymerase chain reaction (QRT-PCR) analysis. Important factors for maintaining these reliable measurements were identified, including the printing method and source of commercial oligos. Interestingly, the reliability of measurements was not affected by performing a double amplification of RNA or by normalizing data with a commercially available universal reference RNA.

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Introduction

DNA microarray technology has been widely used to simultaneously determine the expression levels of thousands of genes (1,2). In addition to the Affymetrix GeneChip platform, various other arrays generated by spotting cDNAs or oligonucleotides have been introduced. The existence of alternative array platforms, which may differ in probe preparation methods and array surface chemistry, raises the question of cross-platform agreement in gene expression measurements (3). In our cross-platform comparison, we compared three different microarray platforms, including the Affymetrix Murine Genome U74Av2 (MG-U74Av2) Array; a custom cDNA array; and custom oligo arrays printed with oligonucleotides from three different sources. We have also developed and tested a double amplification method, which requires only 100 ng of total RNA. All experiments were carried out in triplicate, and real-time quantitative reverse transcription-polymerase chain reaction (QRT-PCR) analysis was performed on selected genes to confirm the results.

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Experimental design: Mouse liver and spleen total RNA purchased from Clontech were used as a common starting material for the Affymetrix, cDNA, and oligo arrays. Mouse spleen was used as the reference, labeled with cyanine 3 (Cy3; green) dye, while the liver was labeled with cyanine 5 (Cy5; red) dye for cDNA and oligo experiments. For Affymetrix arrays, mouse spleen was also used as the reference. All experiments were carried out in triplicate, and real-time QRT-PCR analysis was performed on selected genes to confirm the results.

Microarray fabrication: The PCR-amplified cDNAs from the mouse RIKEN FANTOM library (<http://fantom.gsc.riken.go.jp/>) and mouse oligonucleotides purchased from Operon Technologies (70-mer) and Compugen-Sigma-Genosys (65-mer) were inkjet printed by Agilent Technologies. A high-precision robot (Amersham-Pharmacia Generation III spotter; Molecular Dynamics) was utilized to spot oligos onto the glass slides (Corning CMT-GAPS coated slides) to make pin-spotted oligo arrays. The Affymetrix GeneChip Murine Genome U74 Set Version 2 (MG-U74Av2) was purchased from Affymetrix.

Labeling and hybridization: Mouse liver and spleen total RNAs were purchased from Clontech. Affymetrix GeneChip experiments were carried out as described by Affymetrix. All oligo array experiments were performed using the Agilent Fluorescent Linear Amplification Kit (product no. G2554A). For the cDNA array, the T7 RNA polymerase promoter sequence was introduced into the antisense primer. cRNAs were then produced from the double-strand cDNA templates by in vitro transcription using the MEGAscript kit (Ambion). These synthetic cRNAs were labeled with either Cy5 or Cy3 (Amersham Biosciences) when reverse transcribed into cDNA. After hybridization and washing, cDNA and oligo arrays were scanned by Agilent Scanner G2505A, while the Affymetrix GeneChip was scanned by the Agilent GeneArray Scanner. The double amplification was performed by first- (using random primers) and second- (using T7-oligo dT primers) strand cDNA synthesis from the cRNA produced at first cycle. Details of the T7 amplification microarray experiment are described in the AfCS protocol [PP00000019](#).

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Quantitative real-time RT-PCR: Quantitative RT-coupled PCR was performed using the GeneAmp 5700 Sequence Detector System (Applied Biosystems). The measurement was normalized to an 18S ribosomal RNA control. To measure the copy number of each transcript, a PCR-amplified segment of each gene was cloned into pGEM-Teasy (Promega Corp.), and then cRNA was linearly amplified from *NdeI*-digested plasmid using the MEGAscript T7 kit (Ambion). cRNA was measured with the spectrophotometer DU640 (Beckman Coulter, Inc.) and used to perform QRT-PCR. All QRT-PCR measurements were replicated for each experiment and the values were averaged.

Data analysis: The Affymetrix array information was extracted, and data were computationally compared using Affymetrix Microarray Suite Version 5.0 software. Genes flagged as not changed/marginal increase/marginal decrease (NC/MI/MD) were removed. Genes with two or more replicate values were averaged and used for the analysis. The oligo and cDNA array information was extracted using Agilent G2566AA Extraction Software Version A.6.1.1. Several criteria were used to filter the oligo and cDNA array data. Genes that were saturated, nonuniform, and not significantly above background (below 2.6 x SD of background) in either channel were removed. After removing these spots from each replicate, a triplicate filter was applied to the data set. This filter involved the removal of genes that did not have at least two or more replicate values, genes for which the replicate values differed in signs and had a standard deviation above 0.5 (in \log_2 scale), and genes for which one replicate value showed more than twofold change while the other two replicate values showed less than twofold change (unless the standard deviation was less than 0.5 in \log_2). The remaining values were averaged and used for the analysis. To compare each platform, genes with the same UniGene ID were matched, and the \log_2 ratios were used to calculate the Pearson correlation coefficient (r). In an alternate comparison, genes with the same overlapping probe sequences among the platforms were matched, and the \log_2 ratios were used to calculate the Pearson correlation coefficient.

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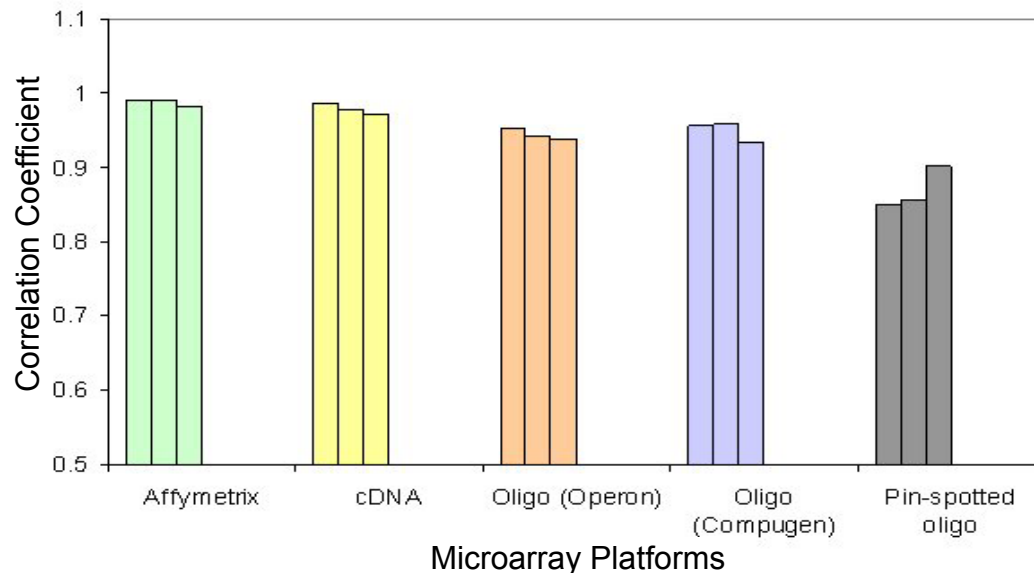
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Correlation Between Replicates

Affymetrix, cDNA, and oligo microarray experiments (spleen vs. liver) were performed in triplicate, and the Pearson correlation coefficients between all combinations of replicate pairs within each platform were calculated. Each bar in the figure below represents the correlation coefficient between two replicate pairs, providing a total of three bars for each platform. All replicates were highly reproducible, showing correlations of 0.93 to 0.99, with the exception of the pin-spotted arrays. For the oligo platform, we used probes from two different companies (Operon Technologies and Compugen Inc.). To ensure quality from the two sources, probes from Operon and Compugen were compared separately. The lower precision of the pin-spotted array was expected, since the inkjet method is known to be more consistent. The cDNA and oligo arrays were printed by the Agilent inkjet method and the Affymetrix GeneChip was synthesized in situ. These approaches apparently provide less spot to spot and chip to chip variability than pin-spotting methods.

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Correlation Between Platforms on Unigene ID Matched Genes

To make comparisons among different platforms, genes with the same UniGene ID across all platforms were used. Correlations in \log_2 ratio of the Affymetrix versus cDNA and Affymetrix versus Operon oligo data were high, 0.81 and 0.85, respectively, while the Compugen oligo data was less correlated with the Affymetrix or cDNA data. Overall, the Affymetrix and Operon oligo data sets retained the most similarity, with a correlation coefficient of 0.85. In the figure below, the superimposed number represents the number of genes used to calculate each respective correlation coefficient.

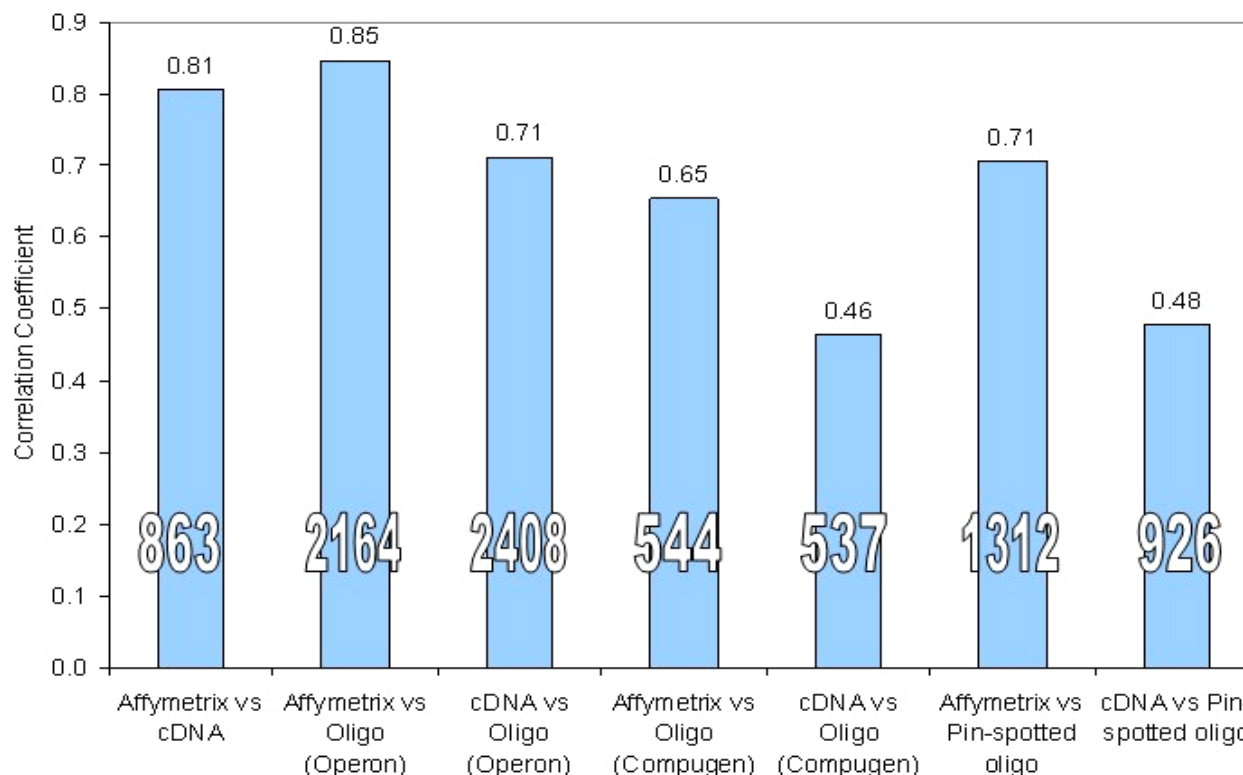


Table 2

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Gene Name	UniGene	cDNA				Affymetrix				Oilgo					RT-PCR						
		GenBank ID	Log Ratio	Liver Intensity	Spleen Intensity	GenBank ID	Log Ratio	Liver Intensity	Spleen Intensity	Source	GenBank ID	Log Ratio	Liver Intensity	Spleen Intensity	Log Ratio	Liver Copy # (2.5ng)	Spleen Copy # (2.5ng)	Liver Transcript Per Cell		Spleen Transcript Per Cell	
																		From	To	From	To
Alpha 1 microglobulin/bikunin	Mm.2197	AK004907	5.10	26502.8	782.1	X68680	8.38	26911.6	77.0	Operon	D28812	4.42	47559.2	2412.3	9.61	2127917	2723	5107	8512	7	11
Diazepam binding inhibitor	Mm.2785	AK018720	3.90	28451.6	1904.3	X61431	3.60	14198.0	958.0	Operon	X61431	3.14	26591.0	3013.7	3.88	2756746	187666	6616	11027	450	751
		AK008576	4.14	63135.4	3600.5																
Riken cDNA 0710008N11 gene	Mm.29141	AK009660	2.52	46894.0	8187.7	AA674669	2.18	7312.8	1647.4	Operon	NM_023374	1.98	35208.5	8904.9	2.58	142586	23846	342	570	57	95
		AK003052	2.57	14050.9	2364.8																
Glutathione S-transferase, theta 1	Mm.2746	AK002338	3.99	16525.3	1045.6	X98055	3.60	4389.3	327.4	Compugen	NM_008185	-0.16	344.1	380.9	4.51	100616	4428	241	402	11	18
										Operon	X98055	2.61	3798.3	613.7							
Riken cDNA 1810009A06 gene	Mm.29135	AK007389	-0.04	6897.6	7032.9	AI837853	-0.53	2326.9	3590.6	Operon	AK007389	-0.36	15916.9	20526.6	-0.99	50259	99816	121	201	240	399
BCL2/adenovirus E1B 19 kDa-interacting protein 1, NIP3	Mm.2159	AK014223	4.14	2861.0	165.0	AF041054	3.05	1048.9	125.9	Compugen	NM_009760	3.91	30813.1	2210.2	4.72	400750	15208	962	1603	36	61
										Operon	AF041054	4.29	28705.6	1316.2							
Glutathione S-transferase, alpha 4	Mm.2662	AK019271	3.82	53665.0	3846.9	L06047	4.90	657.0	27.2	Operon	L06047	2.78	8619.0	1166.1	3.38	65629	6310	158	263	15	25
		AK008189	3.82	53368.1	3791.1																
		AK008490	3.85	31636.8	2215.1																
		AK011177	4.02	48693.2	3000.4																
		AK010098	4.07	48687.2	2905.2																
		AK008400	4.07	56724.2	3408.7																
		AK011841	4.08	19011.0	1125.6																
		AK008193	4.20	64587.9	3552.9																
Suppressor of Ty 4 homolog (S. cerevisiae)	Mm.622	AK002990	-0.39	5201.7	6847.7	U96810	-1.33	266.7	620.5	Compugen	NM_009296	-0.19	802.1	914.2	-0.99	30687	60875	74	123	146	244
Deiodinase, iodothyronine, type I	Mm.2774	AK002549	3.13	939.4	112.7	U49861	3.18	125.1	15.4	Operon	U49861	2.97	2585.5	296.9	6.84	31591	276	76	126	1	1
Lectin, galactose binding, soluble 3	Mm.2970	AK008593	-2.05	3479.2	14487.1	X16834	-2.43	109.8	835.8	Compugen	X16834	-1.25	1129.2	2682.2	-3.22	2372	22133	6	9	53	89
										Operon	X16834	-0.59	566.0	857.2							
DNA-damage inducible transcript 3	Mm.7549	AV070098	-0.18	2301.3	2634.1	X67083	-3.10	48.9	399.1	Operon	X67083	-0.65	245.1	389.8	-2.31	4074	20269	10	16	49	81