Pooling Data Across Microarray Experiments Using Different Versions of Affymetrix Oligonucleotide Arrays

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Combining Information across Microarray Studies

- Many publically available microarray data sets
- Can combine information across studies to:

1. Validate results from individual studies
   - Find intersection of differentially expressed genes
   - Build model using one study, validate using another
2. Discover new biological insights by analyses pooling data across studies.
   - Potential for increased statistical power
   - Important since many individual studies are underpowered.

Pooling Data across Studies

- **Challenge**: In general, microarray data from different studies not comparable
- Clinical differences
  - Different study populations
- Technical differences
  - Laboratory differences: sample collection and storage, microarray protocol
  - Different platforms: cDNA/oligo, different versions of same technology (e.g. Affy chips)

Pooling Data across Studies

- Approaches in Existing Literature:

1. Include study effects in model
   - Gene-specific study effects
   - SVD, Distance-weighted Discrimination

   Drawback: First-order corrections not enough
2. Model unitless summary measures
   - standardized log fold-change
   - t-statistics
   - probabilities of +/0/- expression

   Drawback: Implicit assumptions about comparability of clinical populations across studies

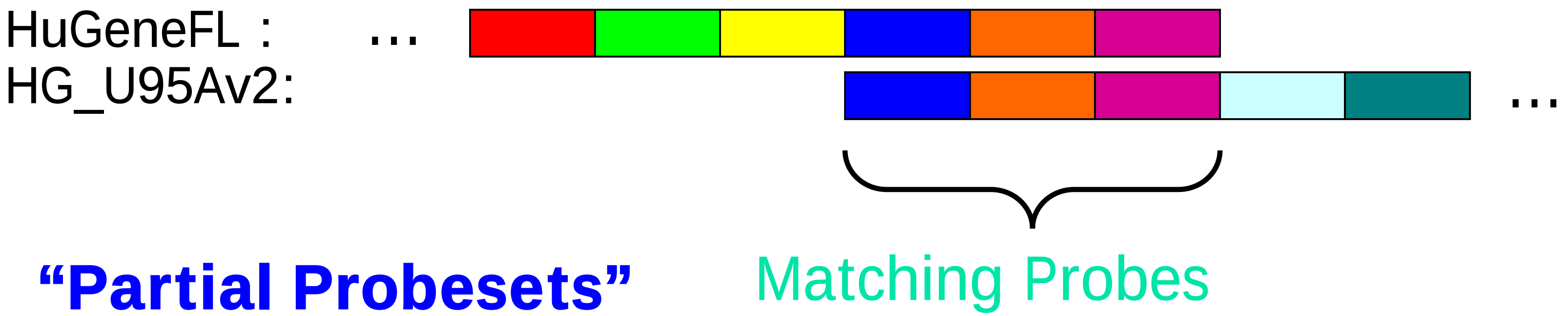
Pooling Data across Studies

- Sequence-related reasons for incomparability of raw expression levels across platforms:
  - Cross-hybridization
  - RNA degradation (near 5’ end)
  - Probe validity – map to RefSeq?
  - Alternative splicing
- It may be possible that, by taking these into account, we can obtain more comparable raw expression levels to use in pooled analyses
- **Our focus:** combining information across different versions of Affymetrix genechips

Overview of Affymetrix GeneChips

- **Probes:** 25-base sequences from gene of interest
- **Probesets**: set of probes corresponding to same gene.
  - Obtained from current sequence information in GenBank, Unigene, RefSeq
- Generations of human chips:
  - HuGeneFL: 5600 genes, 20 probes/gene
  - U95Av2: 10,000 genes, 16 probes/gene
  - U133A: 14,500 genes, 11 probes/gene

"Partial Probeset" Method



"Partial Probesets"

1. Identify "**matching probes**"
2. **Recombine** into new probesets based on UNIGENE clusters, which we refer to as "partial probesets"
3. **Eliminate** any probesets containing just one or two probes

- Note: Any quantification method can subsequently be used (MAS, dChip, RMA, PDNN)

Example: Lung Cancer Data

- **Two studies** relating gene expression data to survival in lung cancer patients

1. **Harvard** (Bhattacharjee, et al. 2001)
   - 124 lung adenocarcinoma samples
2. **Michigan** (Beer, et al. 2002)
   - 86 lung adenocarcinoma samples

- **GOAL:** Pool data across studies to identify prognostic genes for lung cancer.

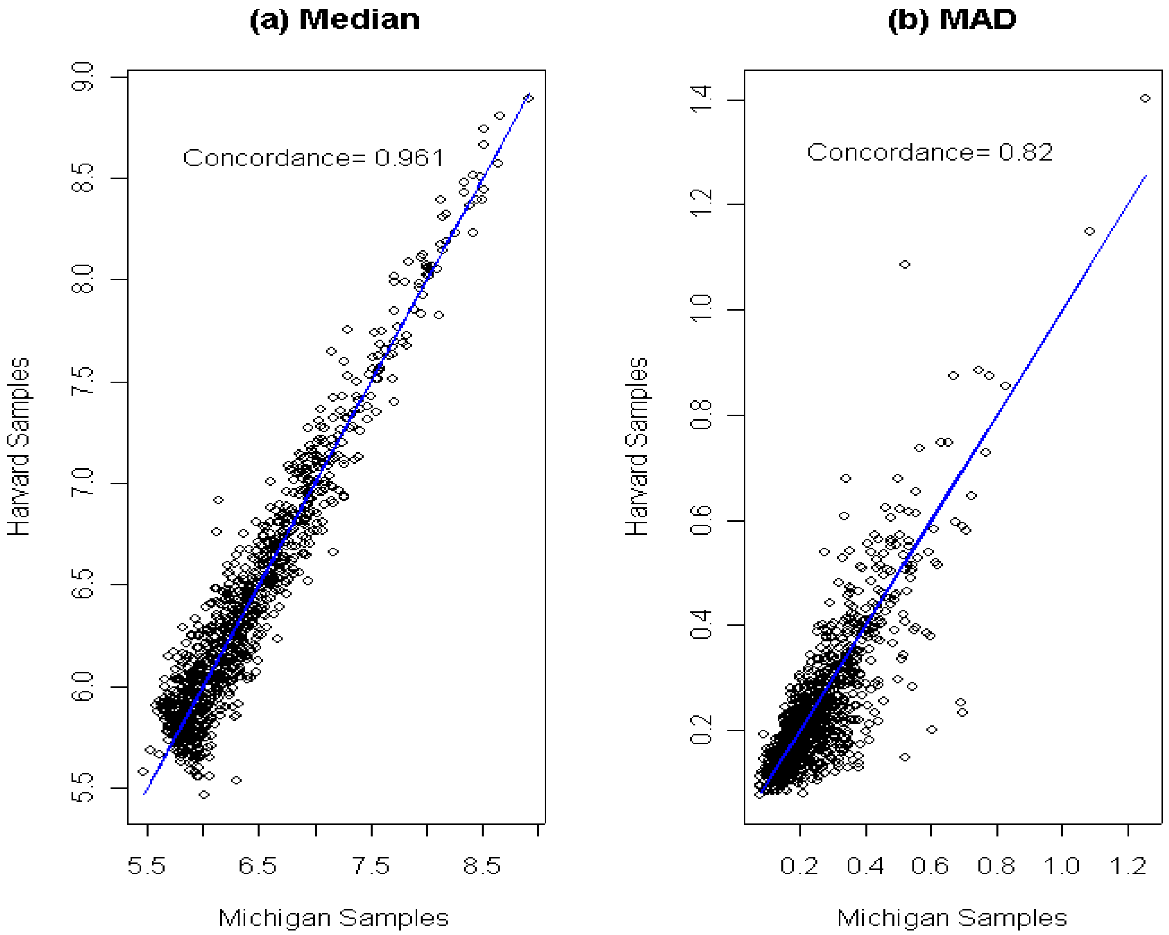
Example: CAMDA 2003 Data

- Two studies used different chip types:
  - **Michigan**: **HuGeneFL**

    ~130,000 probes in 6,633 probesets
  - **Harvard**: **U95Av2**

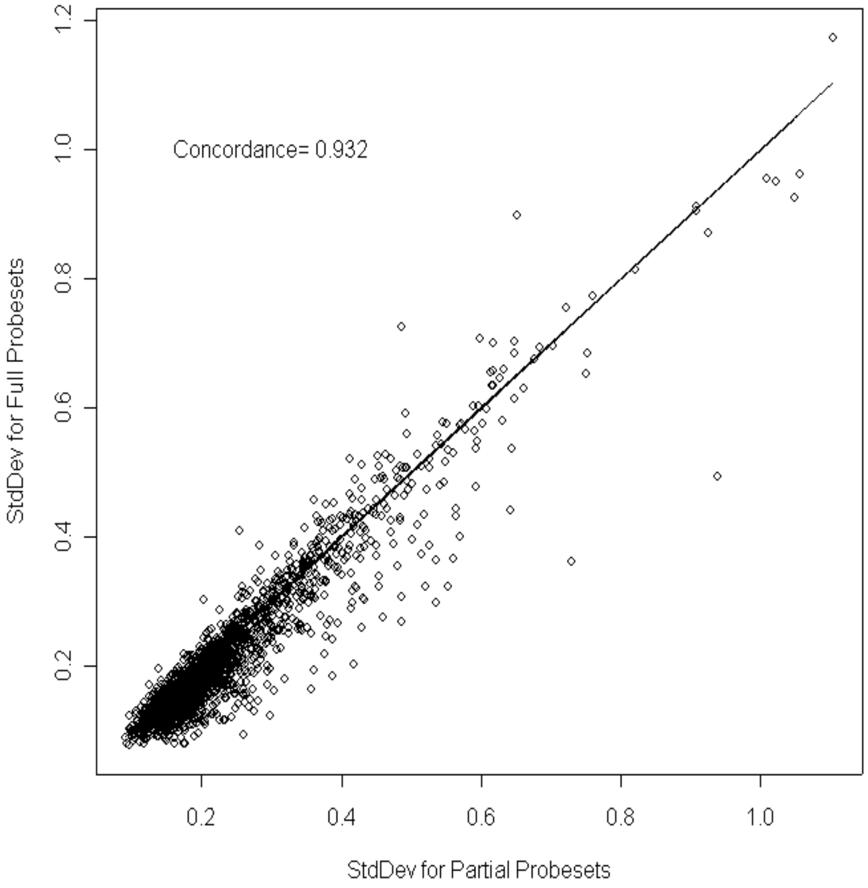
    ~200,000 probes in 12,625 probesets
- 34,428 “matching probes” combined into 4,101 partial probesets
- After preprocessing, 1036 probesets considered in subsequent analyses
- We used PDNN (Zhang et al. 2003 *Nature Biotech*) method for quantification

Assessing Our Method for Combining Information Across Chip Types



- “Partial Probeset” method appears to give **comparable expression levels** across chip types.

Assessing our Method for Combining Information across Chip Types



- Median “partial probeset” size is 7, vs. 16 or 20

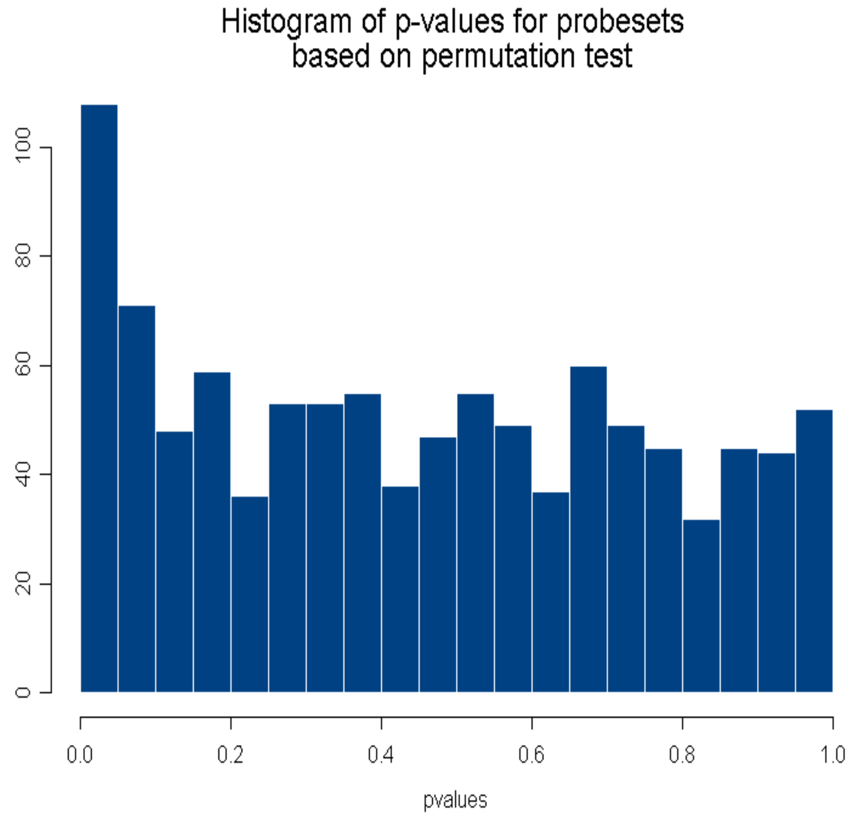
  Loss of precision?

- No evidence of significant precision loss
- Also, relative ordering of samples well preserved (median r=0.95, using Spearman correlation)

Identifying Prognostic Genes

- Series of 1036 **multivariable Cox models** fit to identify prognostic genes. Each model contained:
  - Study (Michigan=-1, Harvard=1).
  - Age (continuous factor).
  - Stage (early=0/late=1).
  - Probeset (log intensity value as continuous factor).
- Exact p-values for each probeset computed using **permutation approach**
- By using multivariate modeling, we search for genes offering prognostic information **beyond** clinical predictors

Results



- Histogram suggests there are some **significant probesets**
- FDR=0.20 corresponds pval cutoff of 0.0024 (BUM, Pounds and Morris 2003)
- **26** probesets flagged as significant

Selected Flagged Genes

Rank	Gene	β	p	Function
1	FCGRT	-2.07	<0.00001	Induced by IF-γ in treating SCLC
2	ENO2	1.46	0.00001	Marker of NSCLC
4	RRM1	1.81	0.00002	Linked to survival in NSCLC
8	CHKL	-1.43	0.00010	Marker of NSCLC
11	CPE	0.72	0.00031	Marker of SCLC
12	ADRBK1	-2.20	0.00044	Co-expressed with Cox-2 in lung ADC
16	CLU	-0.52	0.00109	Marker of SCLC
20	SEPW1	-1.29	0.00145	↓ H202 cytotox. in NSCLC cell lines
21	FSCN1	0.66	0.00150	Marker of invasiveness in Stg 1 NSCLC
25	BTG2	-0.75	0.00232	Induced by p53 in SCLC cell lines

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Results

- Our gene list has almost no overlap with other publications of these data. Reasons:

1. We addressed a **different research question**
   - **Us**: ID Genes offering prognostic info beyond clinical
   - **Michigan**: Univariate Cox models fit; results used to construct dichotomous “risk index”
   - **Harvard**: Cluster analysis done; clusters linked to survival; found genes driving the clustering
2. Pooling across studies yielded **significant gains in statistical power**.
   - Most genes (17/26) in our study are not flagged if we analyze 2 data sets separately (i.e. no pooling)

Limitations of Partial Probeset Method

- Worked well for combining across HuGeneFL/ U95Av2
  - ~25% probes from HuGeneFL on U95Av2, with 4,101 probesets
- Not enough matching probes for use with U95Av2/U133A
  - ~6% of probes from U95Av2 also on U133A, with only 628 probesets
- Requiring matching probes strong criterion, maybe weaker criterion would suffice?

Alternative Splicing

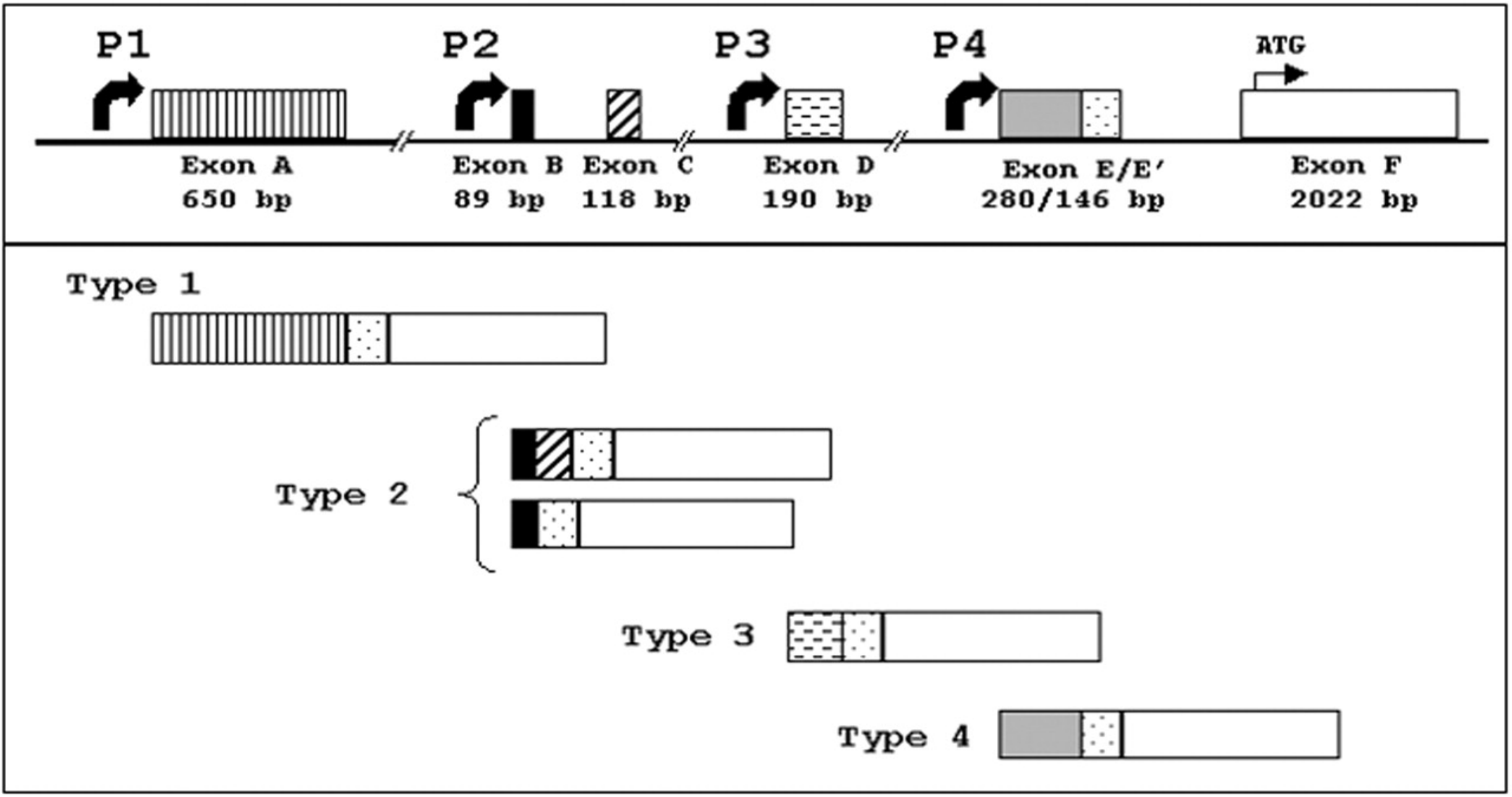


Diagram of C2GnT I gene organization and different mRNA variants of this gene that are **differentially expressed across tissue types**. From Falkenberg, et al. (2003) *Glycobiology* 13(6), 411-418.

Full-Length Transcript Based Probesets

- New probeset definition (FLTBP): probes match the same set of full-length mRNA sequences
- Procedure

1. Construct comprehensive library of full-length mRNA transcript sequences from RefSeq and HinvDB
2. For each probe, identify all matching full-length transcripts using Blast program

   U95Av2: 15% matched no sequence, 33% matched multiple seq.

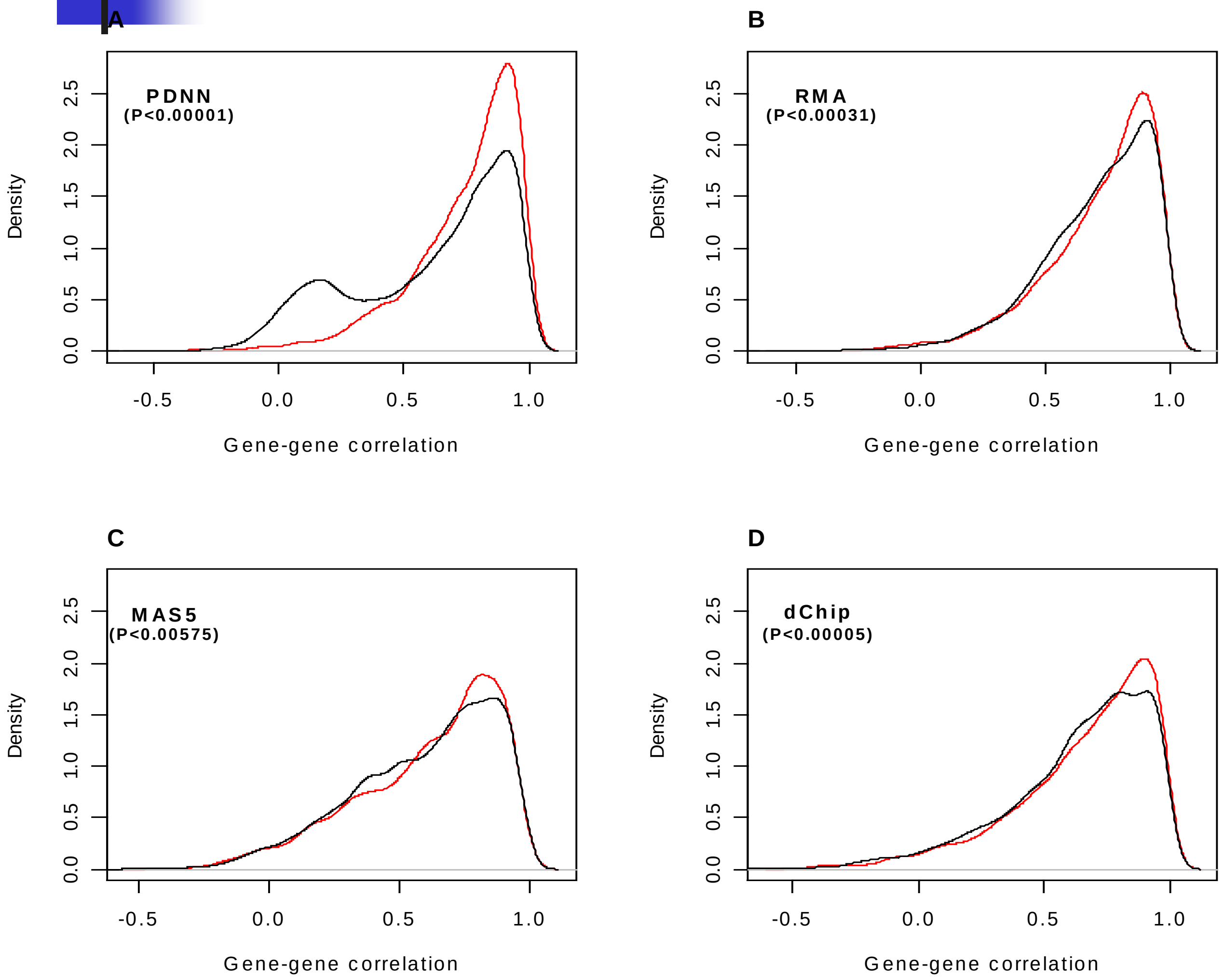
   U133A: 18% matched no sequence, 38% matched multiple seq.
3. Group probes with same matched target lists (FLTBPs)

   U95Av2: 23,972 probesets, U133A: 14,148 probesets

Full-Length Transcript Based Probesets

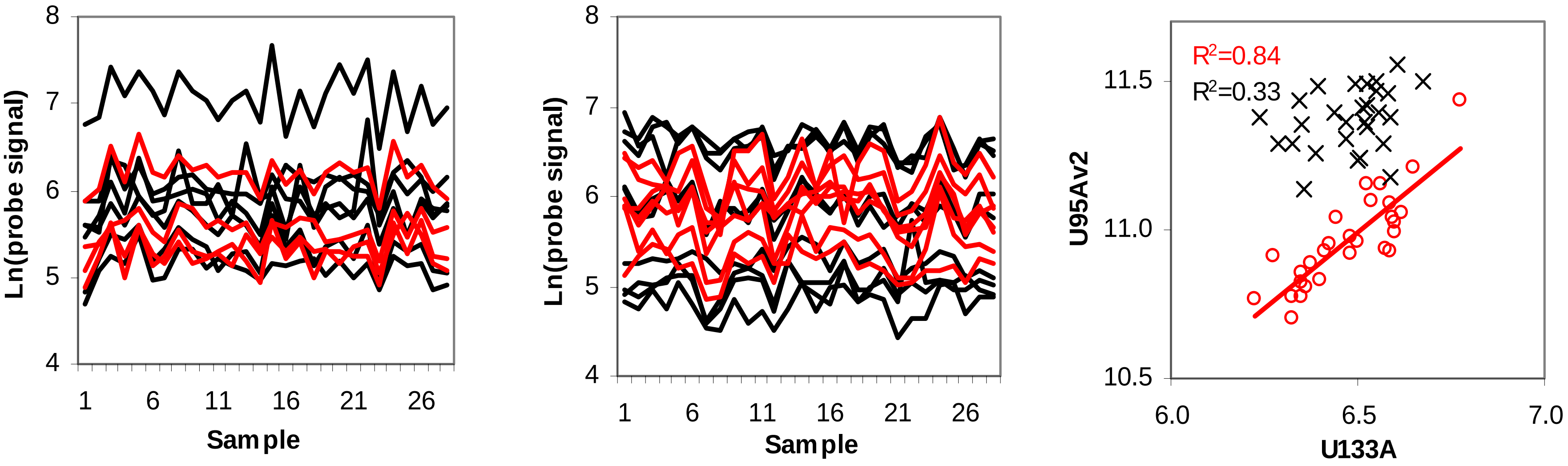
- Matching across chip types:
  - 9,642 FLTBPs match across U95Av2 and U133A
  - Affymetrix has their own method for mapping their probesets across arrays – 9,480 pairs of probesets (only about ½ map the same way as FLTBPs)
- Example: Lung cancer cell line data
  - 28 cell lines, each hybridized onto both U95Av2 and U133A arrays.
  - Paired design suggests any differences between paired measurements due to technical, not biological, sources.
  - Different quantification methods (PDNN, RMA, MAS, dChip)

Results



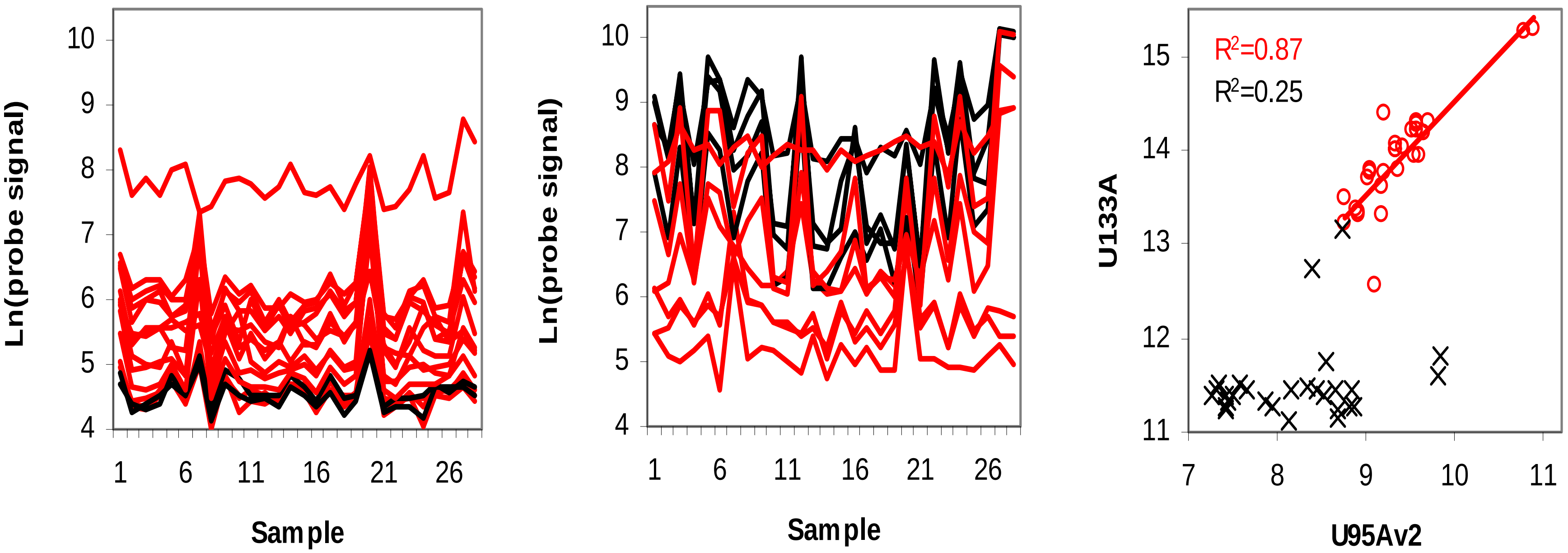
- Density estimate of chip-to-chip correlations for each gene
- Positive shift for FLTBP suggests better correlations
- Improvement greatest for PDNN
- Correlation still not perfect

Example: Sample Gene 1



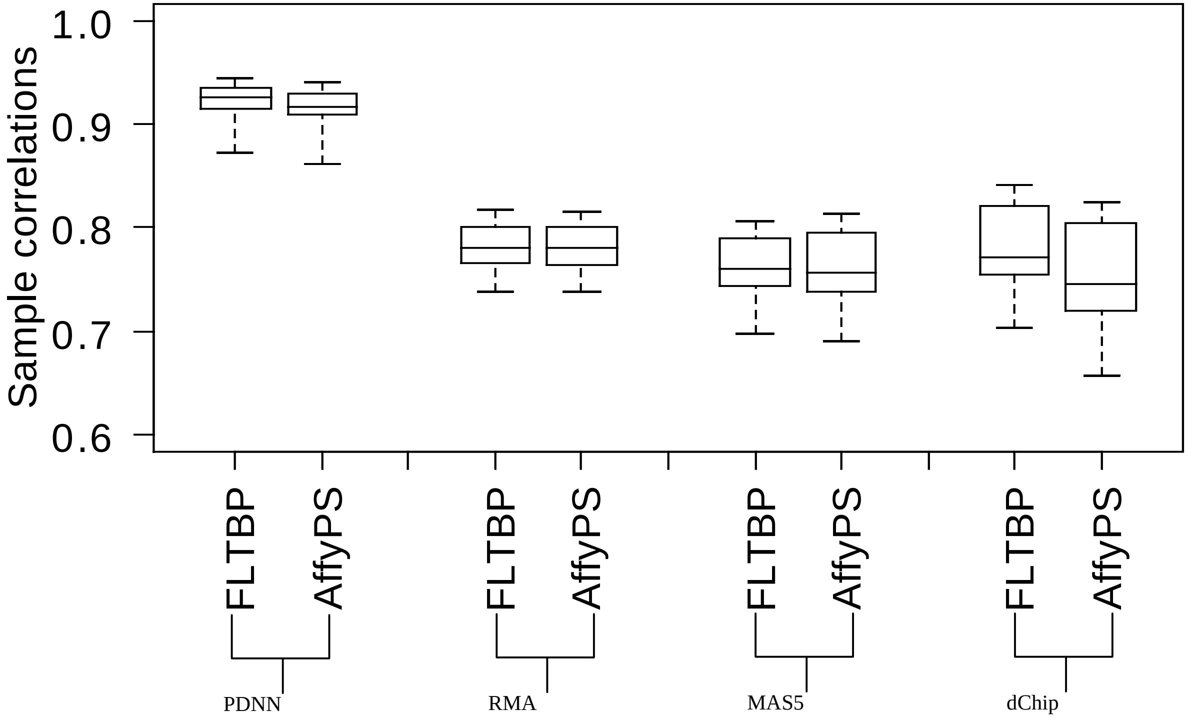
- Plot of probe signals for two chip types (Red=FLTBP)
- Scatterplot of log-expression values for each sample across the two chip types (Black=all probes, Red=FLTBP)
- Correlation across chips significantly improved with FLTBP

Example: Sample Gene 2



- Again, significantly higher correlation using FLTBP than using Affymetrix’ definition

Results



- Boxplot of chip-to-chip correlations (over genes) for each sample
- PDNN resulted in higher correlations

Conclusions

- New method for pooling info across studies using different versions of Affymetrix chips.
  - Recombine **matched probes** into **new probesets** using Unigene clusters.
  - Method appears to obtain **comparable** expression levels across chips without sacrificing much **precision** or significantly altering the **relative ordering** of the samples.
  - Worked well combining information across HuGeneFL/U95Av2, but not U95Av2/U133A

Conclusions

- Discussed new probeset definition based on full-length transcript sequences.
  - Removes effect of known alternative splicing
  - Yields stronger between-chip correlations than Affymetrix standard definitions
- Pooling information across studies is difficult – there is still more work to be done – but worth the effort.

References

- Morris JS, Yin G, Baggerly KA, Wu C, and Zhang L (2005). Pooling Information Across Different Studies and Oligonucleotide Microarray Chip Types to Identify Prognostic Genes for Lung Cancer. *Methods of Microarray Data Analysis IV,* eds. JS Shoemaker and SM Lin, pp. 51-66, New York: Springer-Verlag.

- Wu C, Morris JS, Baggerly KA, Coombes KR, Minna JD, and Zhang L (2005). A probe-to-transcripts mapping method for cross-platform comparisons of microarray data taking into account the effects of alternative splicing. Under review.

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