

Technical validation of novel 325 RNA predictive biomarkers using gene expression data generated by Nanostring n-counter and Affymetrix microarray



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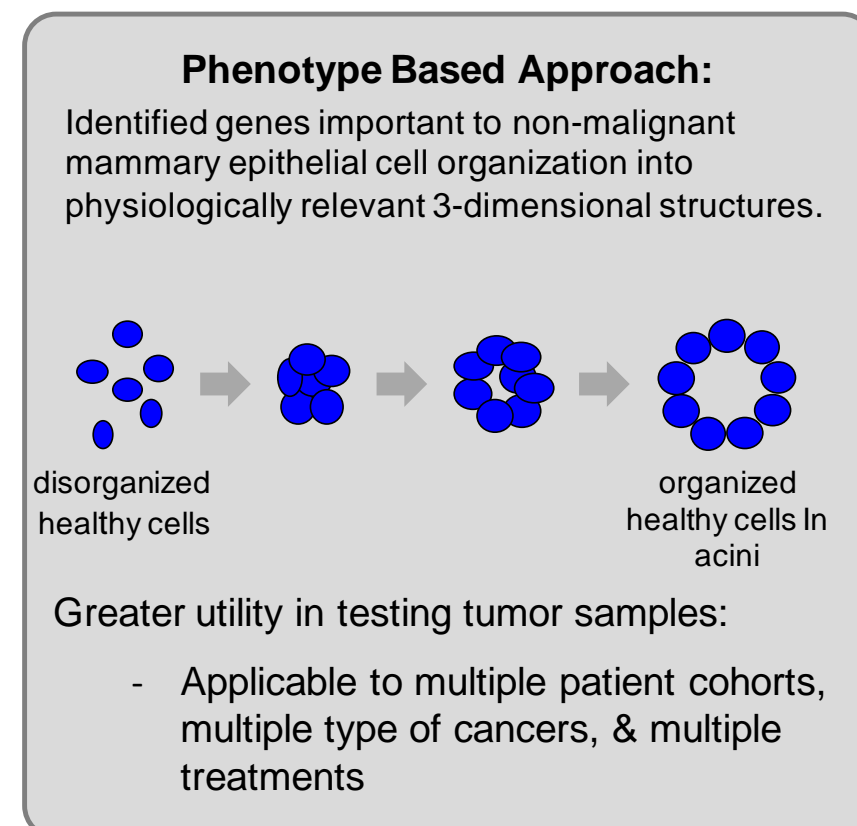
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BACKGROUND

With new developments of oncology drug combinations and targeted treatments, the ability to stratify categories of patient populations and to develop companion diagnostics has become increasingly important. A panel of 325 RNA-biomarkers was selected based on cancer-related biological processes of healthy cells and gene expression changes over time during non-malignant epithelial cell organization. This "cancer in reverse" approach in combination with empirically derived algorithms resulted in a panel of biomarkers having little overlap with other widely-used commercial gene panels analyzed to date, providing a more in-depth and comprehensive view of gene expression profiles and key cellular signaling pathways beyond mutations in "driver-genes".

Unique Biomarkers Representing Organization Phenotypes Were Identified Using a Physiologically Relevant Model for Development



Minimal Overlap with Other Oncology Gene Panels and Tests

Oncology panels	Total number of genes	Overlapping genes	Percent overlap
FoundationOne	343	8	2%
NS_cancer_immune	770	15	2%
NS_cancer_path	770	31	4%
NS_cancer_prog	770	20	2.5%
Illum_PanCancer	1386	42	3%
OncotypeDX	21	3	14%
Prosigna	50	7	14%
Mammaprint	70	9	12%

NS: nanoString; Illum: Illumina

OBJECTIVES

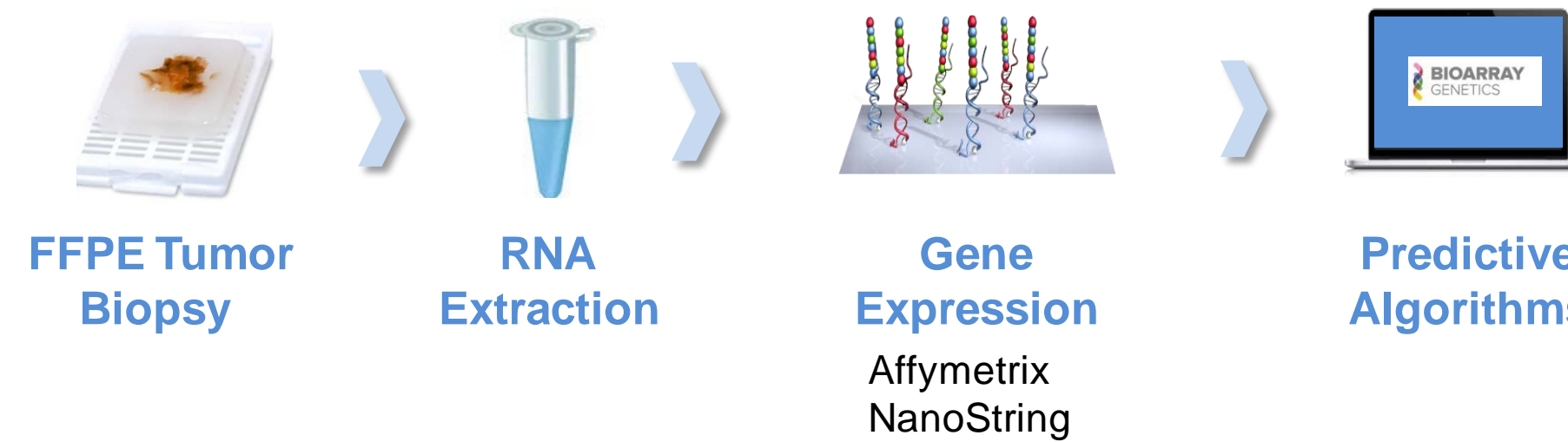
- 1) Technically validate an assay (BA325) for the 325 RNA-biomarkers with multiple days and users
- 2) Compare gene expression profiles side-by-side using two technology platforms to address the reproducibility of the assay

REFERENCES

Fournier et al. Cancer Res 2006; 66: (14). July 15, 2006
Fournier et al. Cancer Res 2009; 69: (10). May 15, 2009

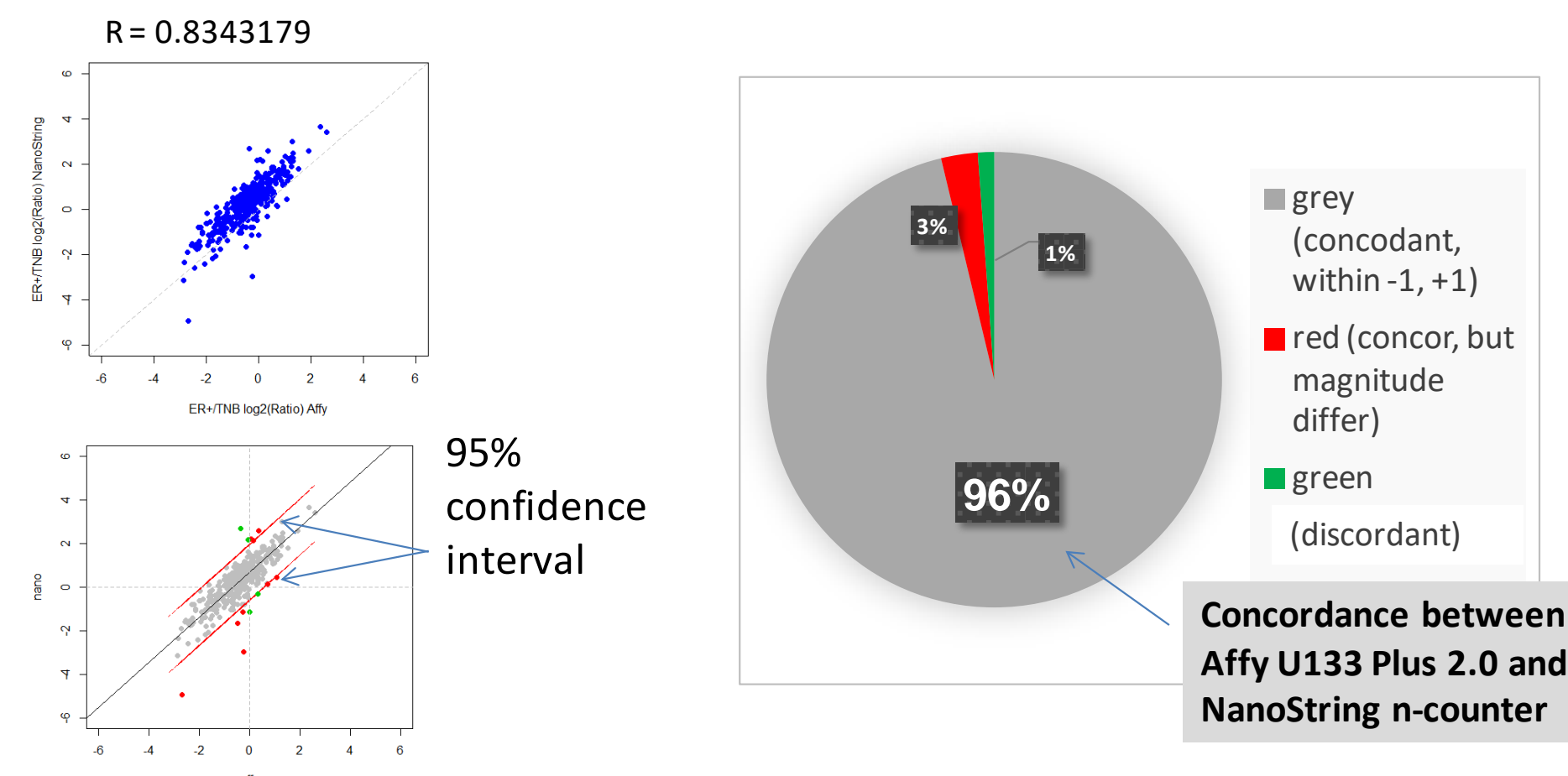
METHODS

Test Workflow



We have mapped the 325 RNA transcripts and 7 housekeeping genes in a custom NanoString n-Counter expression panel to be compared to all potential probe sets in the Affymetrix Human Genome U133 Plus 2.0. The experiments used 10 unique biological formalin-fixed paraffin-embedded (FFPE) breast tumor samples. Each laboratory site extracted RNA from four sections of 10-microns thick FFPE tissue over three different days by different users using an optimized standard operating procedure and quality control criteria. Samples were analyzed using mas5 in BioConductor and NanoStringNorm in R. The platforms were compared using relative expression fold changes and linear regression (lm). Concordant genes were defined to have gene expression levels within +/- 2 standard deviation (sd) of each other at 95% confidence interval, or to have greater than +/- 2 sd but have changes in the same direction in both platforms. The discordant genes were defined to have opposite direction of changes. By this definition, approximately 96% of the genes fell into the concordant category.

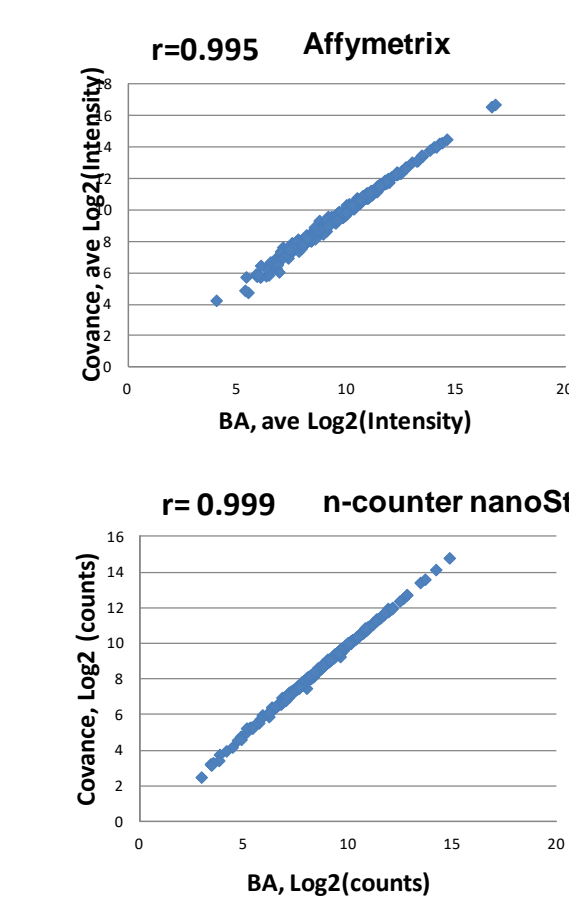
Correlation Between Affymetrix Data vs. Nanostring using Fold-Change (ER+/TNB)- Pooled with all the cases



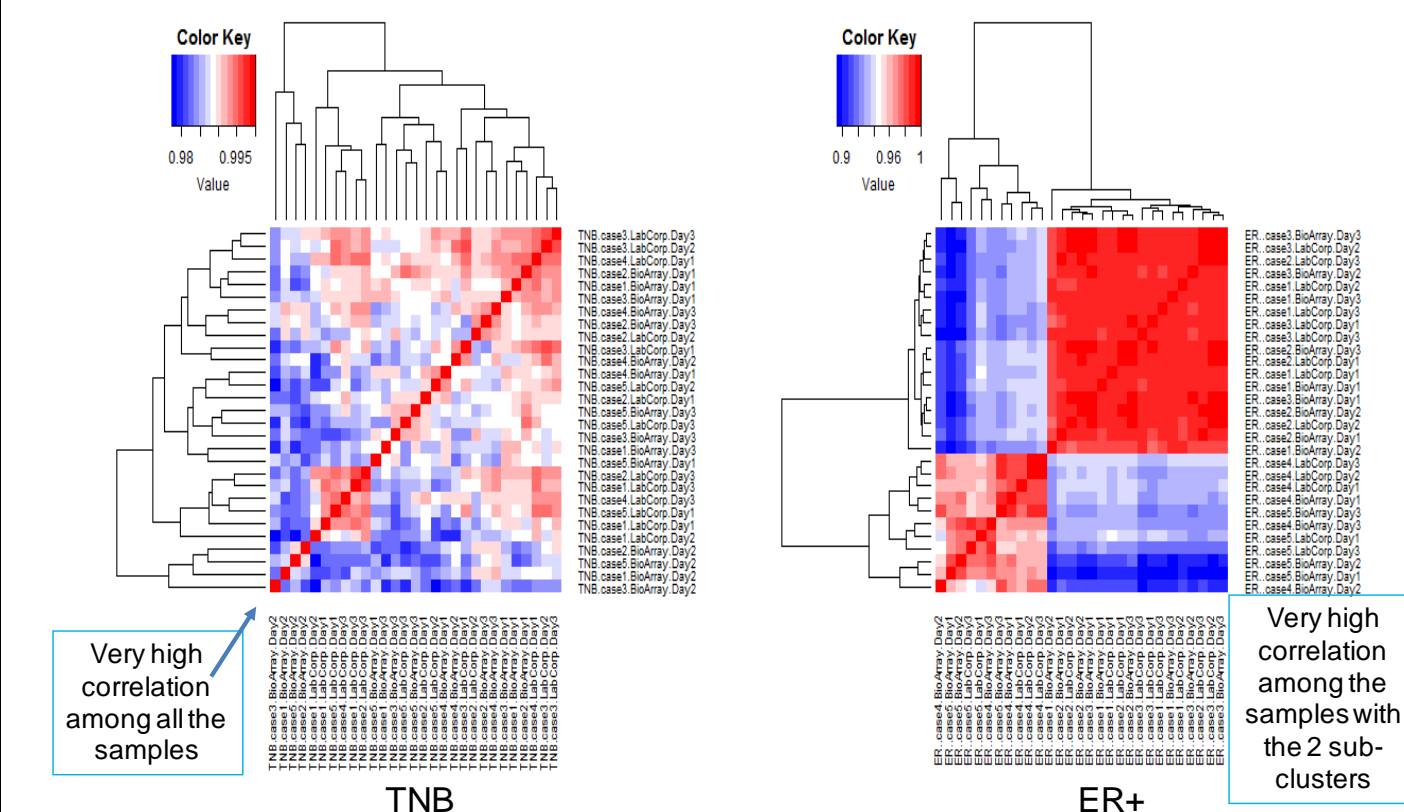
RESULTS

Pearson correlation showed reproducibility between sites for all 60 samples with r=0.995 for Affymetrix and r=0.999 for Nanostring.

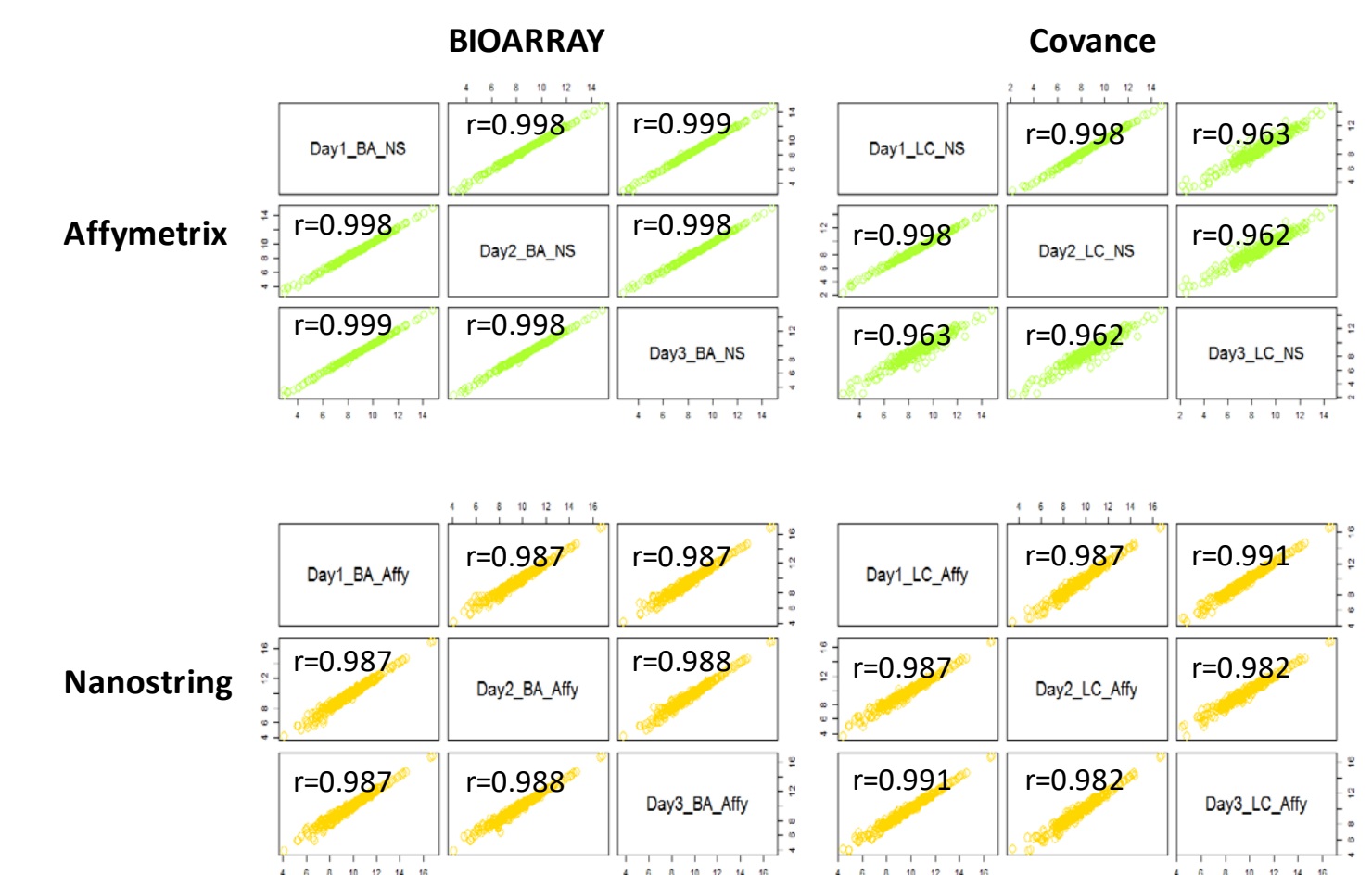
All 60 samples



Per subtype



Pearson Correlation in multiple days and multiple users were for Affymetrix r=(0.962-0.999) and for NanoString r=(0.982-0.991)



CONCLUSIONS

- 1) The BA325 assay was independently validated in multiple laboratories, multiple users, in multiple days with high reproducibility
- 2) The study showed high concordance between Affymetrix and a custom designed BA325 Nanostring assay
- 3) The study supports R&D use of the BA325 assay for oncology drug discovery and development