From gene expression signature to diagnostic test: Challenges in applying genomic technology to molecular diagnostics

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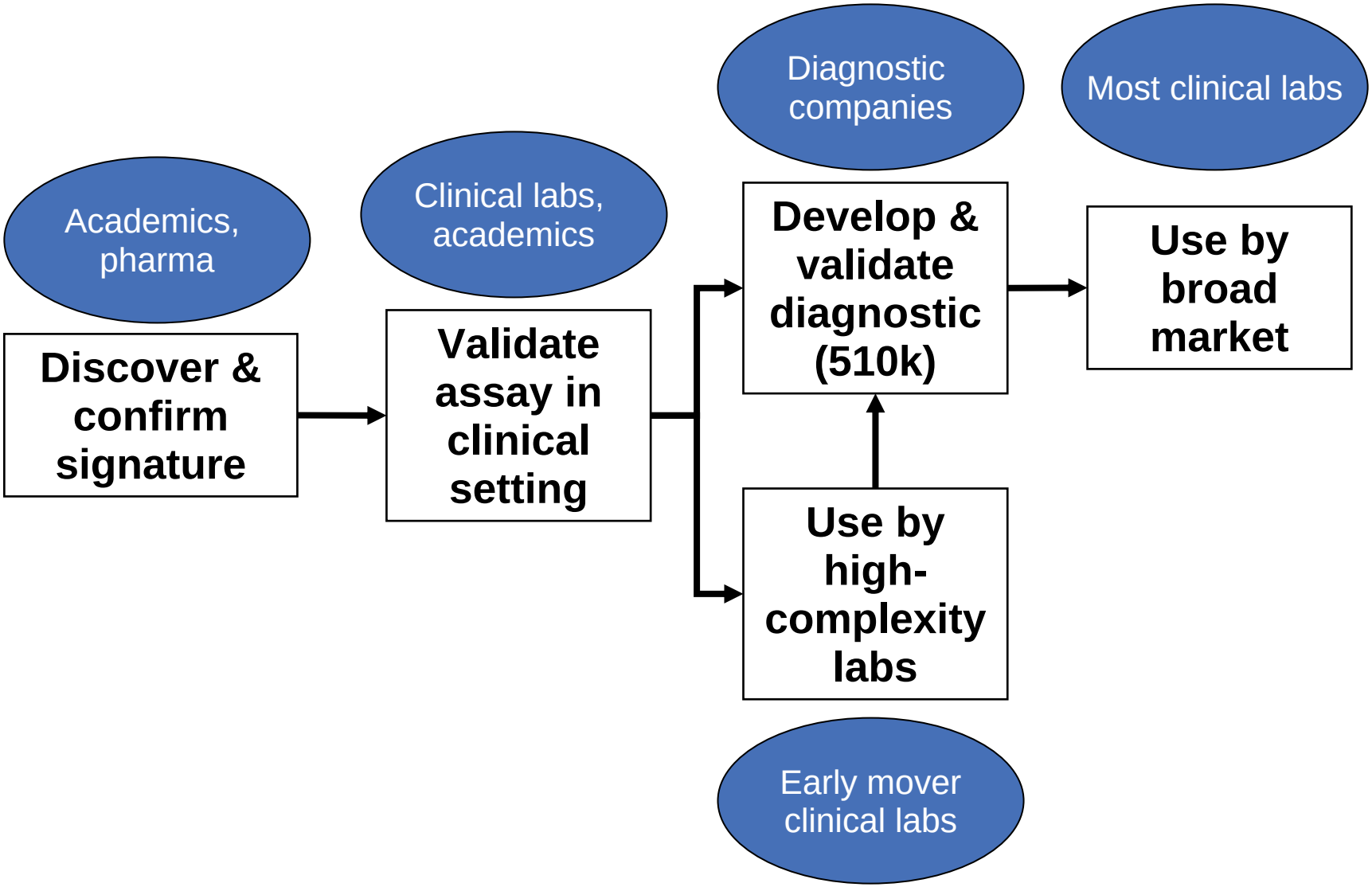
Talk content

- Background
- Current challenges
- Standard controls and best practices
  - ERCC, MAQC, CLGGS, AHIC
- Summary

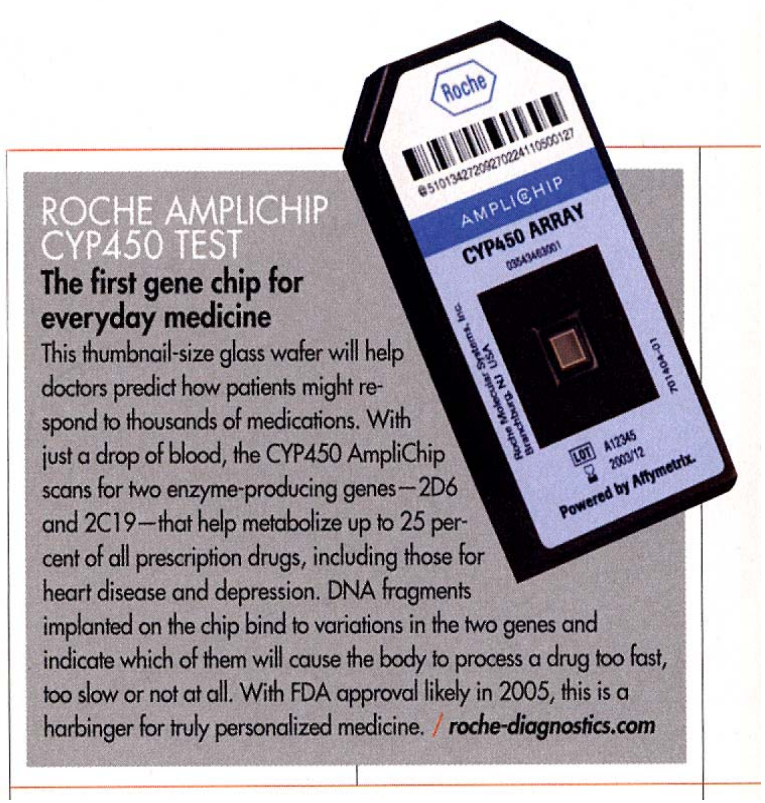
How does a signature become a diagnostic?



Genomic technology in the clinic – It's here now

Therapy Selection & Clinical Trials

- AmpliChip CYP 450 - Drug Response
- Oncotype Dx – Breast Cancer
- MammaPrint - Breast Cancer
- HercepTest – Breast Cancer
- Gleevec – Chronic Myelogenous Leukemia
- Iressa – Non-Small Lung Cell Carcinoma



Popular Science: 100 Coolest Inventions

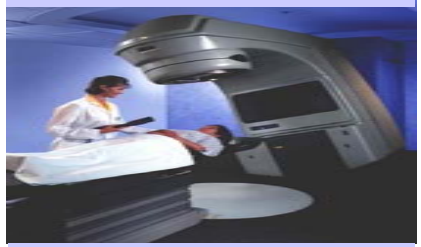
…and more is on the way…

- > 20 assays in development
- > 30 partners *creating signatures*
- 10 partners *creating products*
- Clinical validation of microarray assays
- First 1 M SNP assay for whole genome association studies and copy number

Positive trends

- Regulatory leadership at FDA
- SRM and guideline development
- Developments in reimbursement
- National EMR initiative

ONCOLOGY



COMPLEX DISEASES

INHERITED DISEASE

PHARMACOGENETICS

There are still monsters in the closet

- Study design quality
  - Requires good scientific method
  - Adequate sample number and quality
  - Informed selection of data processing and analysis methods
- Data quantity anxiety
  - *"How is more data better ?"*
  - Too complex
- Privacy concerns
  - Legislation exists to protect privacy
  - Technology exists to protect information
- Reimbursement
  - What is covered
  - Who pays
  - Who is reimbursed

Thousands of publications

- Some are good, some not so good
- Many supporting reagents and s/w tools have been developed in parallel
  - Information/methods may be outdated by the time a paper is published

Study design

Michiels S. et al., Lancet 2005; 365: 488–492

"Five of the seven studies did not classify patients better than chance."

Study reference	Cancer type	Clinical endpoint	Sample size	Number of events (%
2	Non-Hodgkin lymphoma	Survival	240	138 (58%)
3	Acute lymphocytic leukaemia	Relapse-free survival	233	32 (14%)
4	Breast cancer	5-year metastasis-free survival	97	46 (47%)
5	Lung adenocarcinoma	Survival	86	24 (28%)
6,7	Lung adenocarcinoma	4-year survival	62†	31 (50%)
8	Medulloblastoma	Survival	60	21 (35%)
9	Hepatocellular carcinoma	1-year recurrence-free survival	60	20 (33%)

*For the data of van 't Veer and colleagues,[4] the same filter was used as in the original publication. For other studies, genes with little variation years after surgical resection were analysed.[7]

Table: Description of eligible studies ordered by sample size

This paper is about a novel method

Thousands of samples are needed to generate a robust gene list for predicting outcome in cancer

Liat Ein-Dor[†], Or Zuk[†], and Eytan Domany[‡]

Department of Physics of Complex Systems, The Weizmann Institute of Science, Rehovot 76100, Israel

Communicated by Leo Sachs, The Weizmann Institute of Science, Rehovot, Israel, February 15, 2006 (received for review February 1, 2006)

PNAS | **April 11, 2006** | vol. 103 | no. 15 | **5923–5928**

- Created a mathematical method for predicting number of samples required.
- No independent confirmation of the method reported.
- Assumptions buried in supplemental material deserve scrutiny.
- Why not title paper, A novel method for predicting sample number required in gene expression experiments?

EDITORIALS

Development and Evaluation of Therapeutically Relevant Predictive Classifiers Using Gene Expression Profiling

Richard Simon

Journal of the National Cancer Institute, Vol. 98, No. 17, September 6, 2006

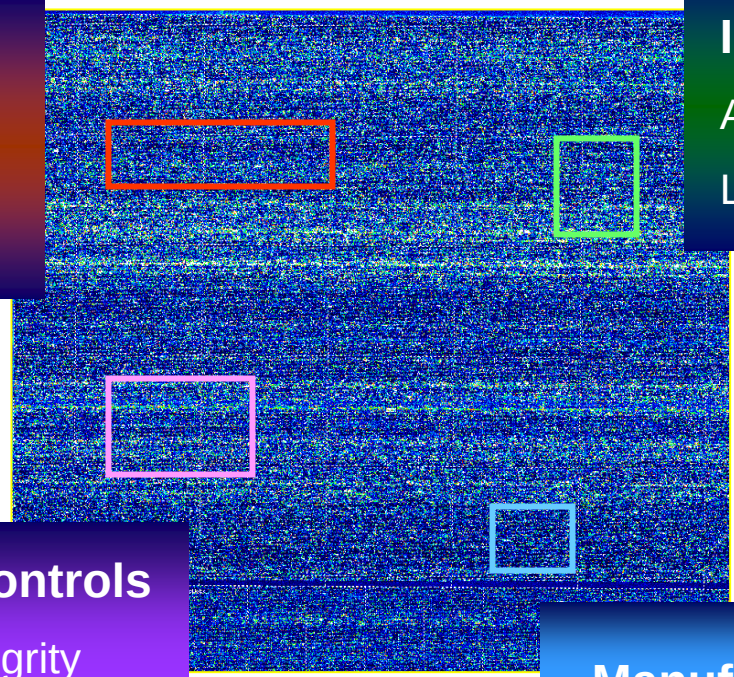
"…a field that is filled with misinformation, hype, and inappropriate cynicism, …"

How is more information better?
Leverage content to monitor performance in real time

External controls

Technical performance

Labeling efficiency



Instrument controls

Alignment

Linear performance

Analyte controls

Sample integrity

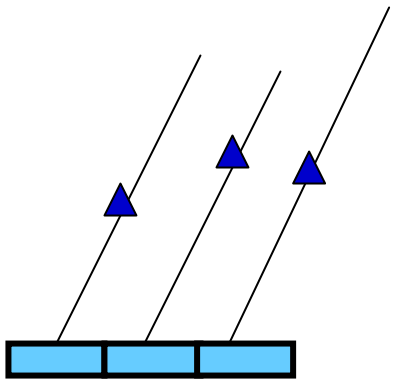
Reference sample

Manufacturing controls

Utilize content to optimize accuracy and statistical confidence

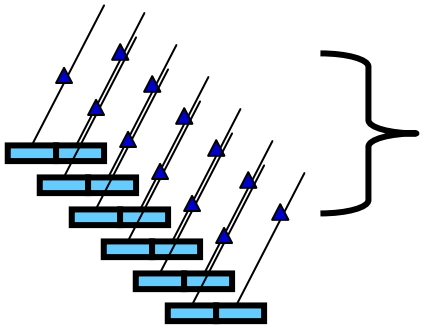
Ask the same question different ways

Multiple query design includes shifted query position



- $n_{12}\ G_{13}\ n_{25}$
- $n_{16}\ G_{17} n_{25}$
- $n_{14}\ G_{15}\ n_{30}$

From millions of data points data to simplified output and decision making information



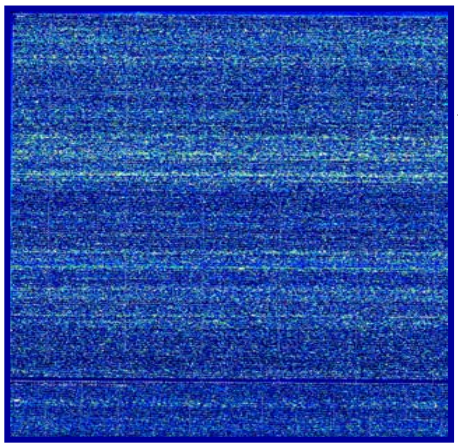
Multiple data points per unit of information

↓ **algorithm**

Single piece of information

↓ times many

Millions of data points



↓ **algorithm**

Output: Many types of information in simple format

- Assay performance
- Data quality
- Categorical output
- Confidence

Talk content

- Background
- Current challenges
- Standards controls and best practices
  - CLSI, NCI/OBBR, ERCC, MAQC, CLGGS, AHIC
- Summary

Standard controls and guidelines accelerate uptake of technology in clinical applications

- Provide a tool for bringing new technology into the existing regulatory and reporting framework
- Provide a technological reference point for advancing research and development
- Provide accepted tools for quality management and proficiency testing in the laboratory
- Aid regulatory decision-making

What are “standards”?

Serve as a benchmark for comparison and reference

Serve as community accepted tools for quality management

- Standard reference materials
- Best practices
- Guidelines

The international prototype of the kilogram in Sèvres. (Credit: Image courtesy of BIPM)

Desirable characteristics of a standard material

- Known provenance
- Well-characterized
- Fit for purpose
- Interoperable
- Renewable
- Accessible
- Accepted and used across industry

Physicist Richard Steiner adjusts the electronic kilogram, an experimental apparatus for defining mass in terms of the basic properties of nature.

Best Practices for Tissue Specimen Handling Clinical and Laboratory Standards institute

- CLSI H18-A3 Procedures for the Handling and Processing of Blood Specimens
- CLSI MM13-A Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods
- CLSI ILA18-A2 Specifications for Immunological Testing for Infectious Diseases

www.clsi.org

Initiatives of the NCI Office of Biorepository and Biospecimen Research

- Cancer Biomedical Information Grid (caBIG)
  - Create infrastructure to facilitate data exchange, access to informatics tools to support collection, annotation, storage, dissemination of high quality biospecimens
  - Uniform nomenclature
  - Tracking procedures
  - IT interoperability across networks
- Development of evaluation tools, quality assessment of biospecimen resources
- Launched Biospecimen Research network
  - DNA, RNA, protein biospecimen data
  - Developing evidence based standards for collection, processing and storage

Initiatives of the NCI Office of Biorepository and Biospecimen Research

- Best Practices for Biospecimen Resources
  - Published June 2007
  - 2+ year effort of public and private sectors
  - Biospecimen collection, processing, storage, retrieval and dissemination
  - Good laboratory practices for consistency and standardization

http://biospecimens.cancer.gov./practices
OR
www.nci.gov

The External RNA Controls Consortium

- 180 members, 95 organizations, 14 countries
  - Government, regulatory, academic, biotechnology, pharmaceutical and diagnostic companies
- Goals
  - Develop well-characterized interoperable standard controls for multiple genomic technology platforms e.g. microarray, RT-PCR
  - Develop protocols for clinical use
- Deliverables
  - Guideline for use of external RNA controls in expression assays
  - Standard control material

The External RNA Controls Consortium Progress Report

- Guideline for Use of External RNA Controls
  - published August 2006, CLSI MM16
- Testing of donated controls complete in 2007
- NIST/Marc Salit advancing ERCC controls to establish SRM
- SRM 2374 will be available from NIST Q2 2008 via NIST Standards Program

www.nist.gov

The MAQC Study

- Initiated by NCTR/FDA – Dr. Leming Shi
- 137 participants, 51 organizations from public and private sectors
- Carry out controlled study to measure intra-site and inter-site reproducibility of microarray expression assays.
- Invited 3 sites per platform.
- Gene expression levels were measured from two high-quality independent RNA samples along with two mixtures from these samples.
- Study design did not account for content complexity and reported performance based on only 12,000 genes.
- A number of analytical methods were used to assess performance.

September 2006

Expression Platforms in the MAQC Study

The only microarray platform which did not remove or replace arrays.

Manufacturer	Code	Protocol	Platform	Number of probes[a]	Number of test sites	Number of samples	Number of replicates	Total number of microarrays[b]
Applied Biosystems	ABI	One-color microarray	Human Genome Survey Microarray v2.0	32,878	3	4	5	58
Affymetrix	AFX	One-color microarray	HG-U133 Plus 2.0 GeneChip	54,675	3	4	5	60
Agilent	AGL	Two-color microarray[c]	Whole Human Genome Oligo Microarray, G4112A	43,931	3	2	10	56
	AG1	One-color microarray	Whole Human Genome Oligo Microarray, G4112A	43,931	3	4	5	56
Eppendorf	EPP	One-color microarray	DualChip Microarray	294	3	4	5	60
GE Healthcare	GEH	One-color microarray	CodeLink Human Whole Genome, 300026	54,359	3	4	5	60
Illumina	ILM	One-color microarray	Human-6 BeadChip, 48K v1.0	47,293	3	4	5	59
NCI_Operon	NCI	Two-color microarray	Operon Human Oligo Set v3	37,632	2	4	5	33
Applied Biosystems	TAQ	TaqMan assays	>200,000 assays available	1,004	1	4	4	N/A
Panomics	QGN	QuantiGene assays	~2,600 assays available	245	1	4	3	N/A
Gene Express	GEX	StaRT-PCR assays	~1,000 assays available	207	1	4	3	N/A
							Total	442

[a]A global definition of probes is used to include individual probes, probe sets or primer pairs depending on the gene expression platform. The numbers listed in this table are derived from product literature and may include some platform duplication.

[b]Maximum number of microarrays per one-color protocol is 60 (3 sites x 4 sample types x 5 replicates). Replacement hybridizations but not outlier hybridizations are included in the main study data analysis.

Table 1: Shi *et al., Nat. Biotechnol.* 24, 1151-1161 (2006)

Repeatability of Expression Signals

Best repeatability within each test site (median CV 5%)

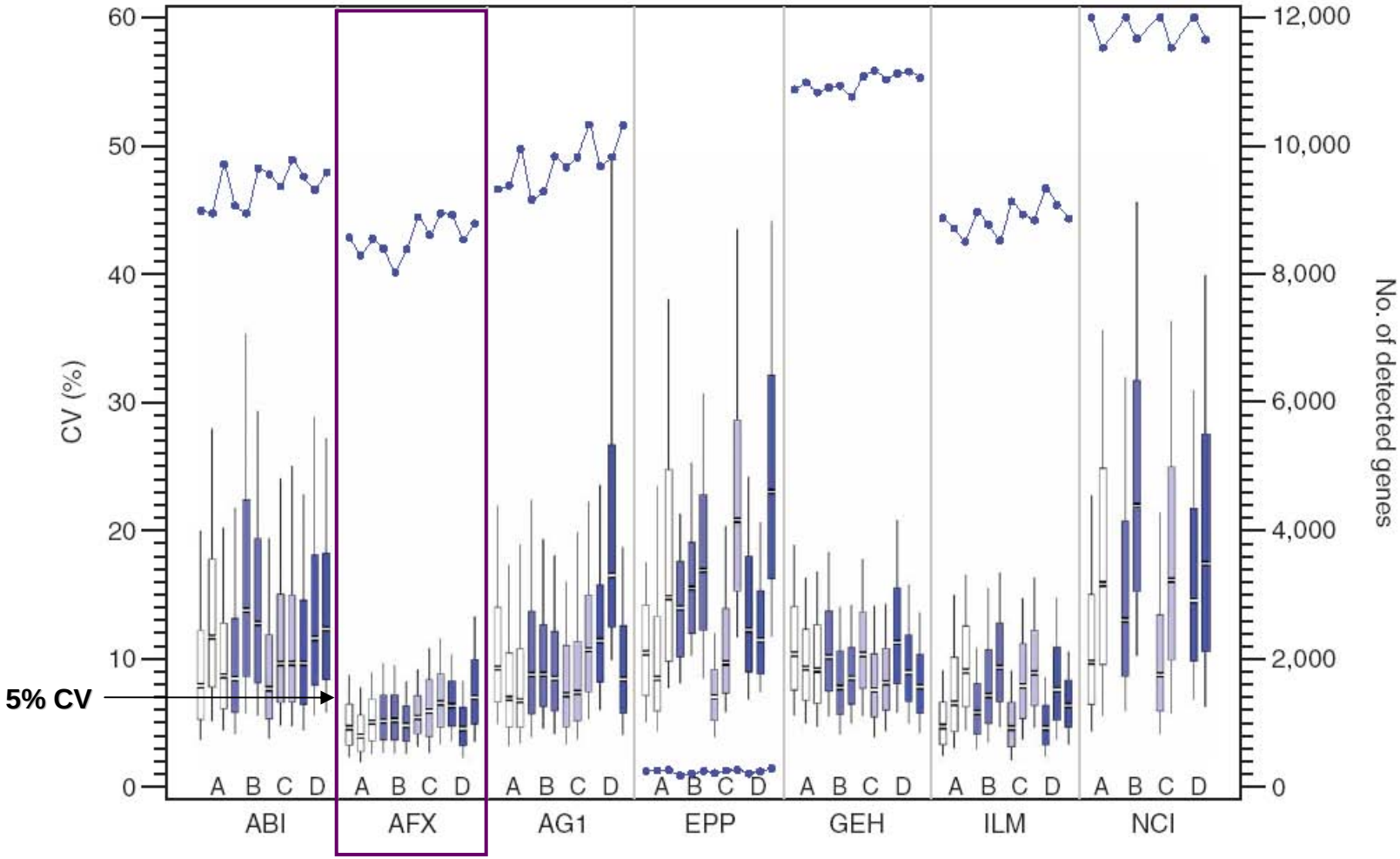
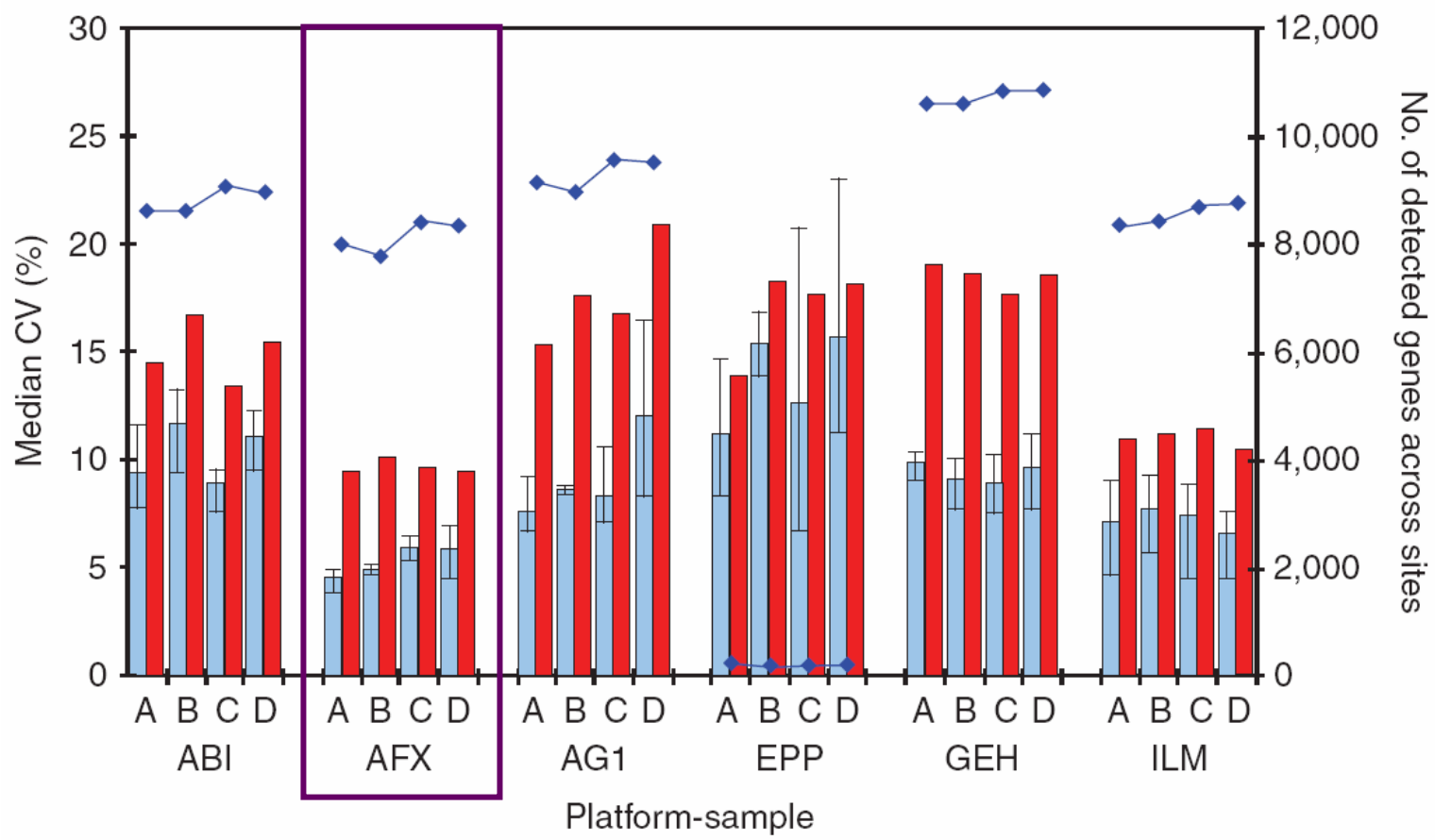


Figure 1: Shi *et al., Nat. Biotechnol.* 24, 1151-1161 (2006)

Reproducibility of Expression Signals

Best repeatability and reproducibility



- The average repeatability (within laboratory) is represented as blue bars.
- The average reproducibility (across laboratories) is represented as the red bars.

Figure 2: Shi *et al., Nat. Biotechnol.* 24, 1151-1161 (2006)

Microarray Correlations Relative to TaqMan

Highest correlation to TaqMan across all three test sites.

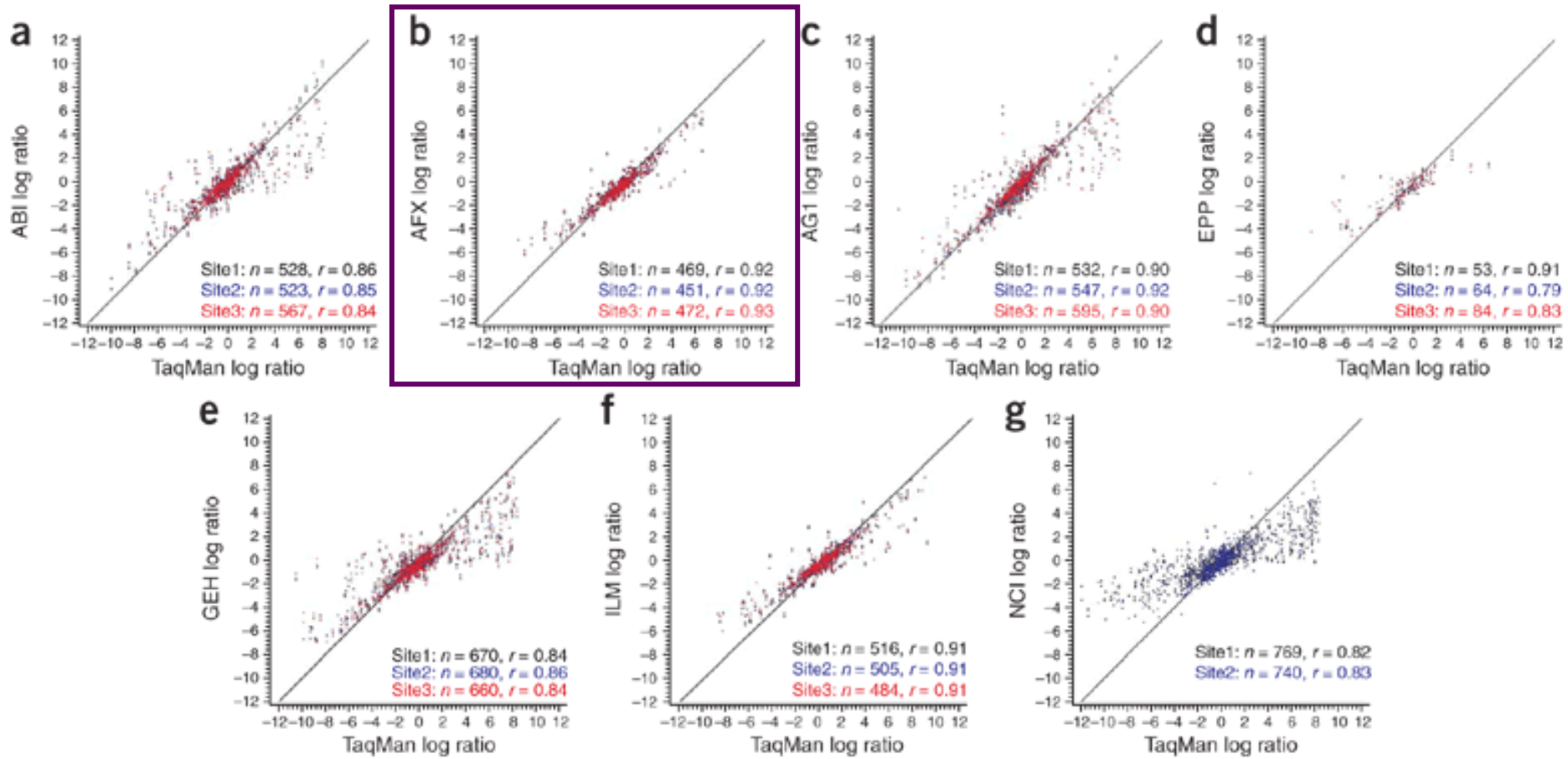
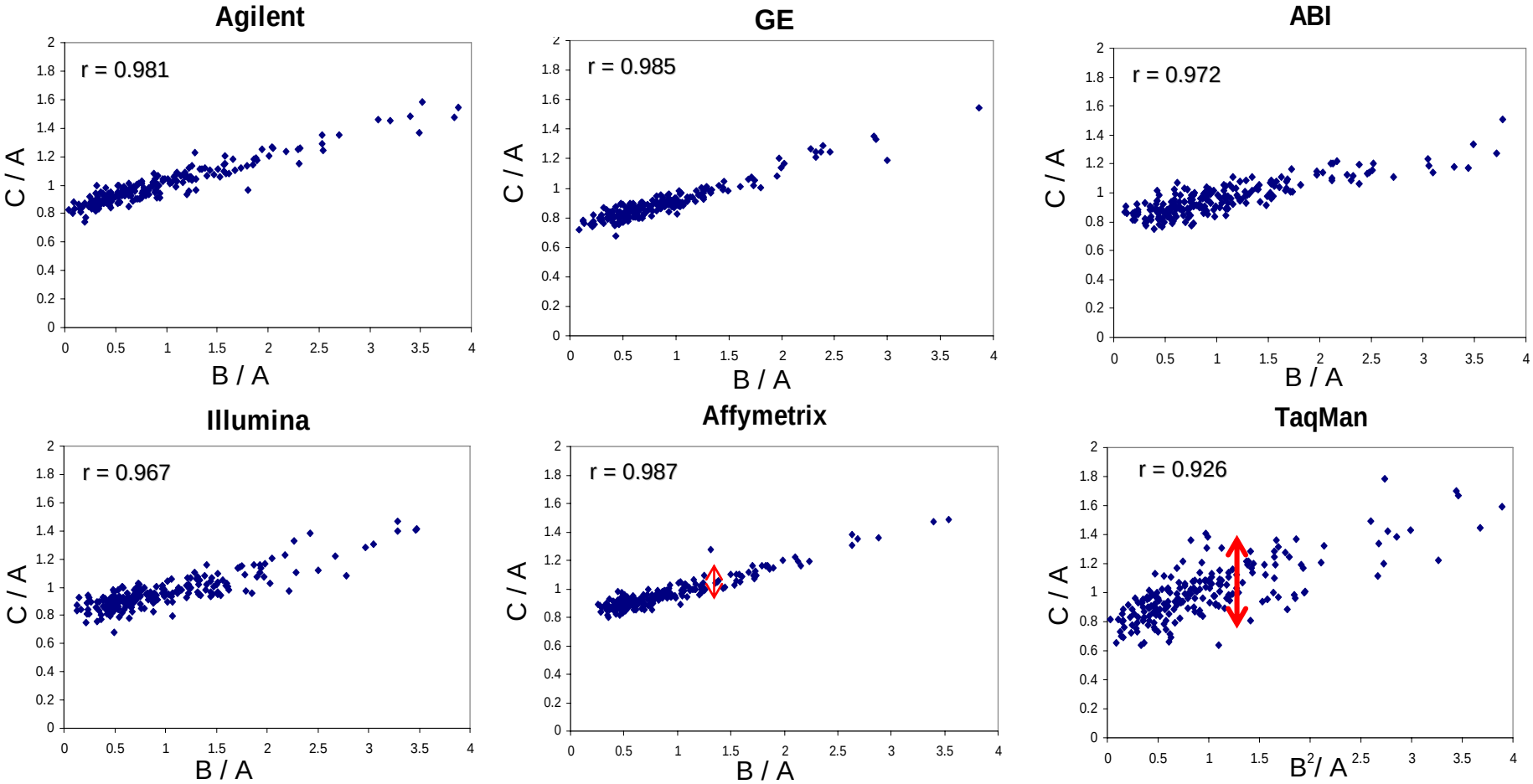


Figure 6: Shi *et al.*, *Nat. Biotechnol.* 24, 1151-1161 (2006)

Microarrays were more accurate than Taqman in resolving small expression changes

Illustrated by the difference in width ⇕ for the scatter plots



Titration samples provided a method for assessing accuracy and discrimination of signature patterns.
C/A is plotted versus B/A and we expect C/A = 0.75 + 0.25 B/A as a result of known titration ratios.

Clinical and Laboratory Genetic and Genomic Standards Meeting – DIA December 2007

- Forum for discussion of use of SRMs and guidelines in development, regulatory and clinical end user implementation
- Further the discussion on regulatory submissions and validation of algorithms
- International perspective
  - 3 meetings: 3 continents
- Washington DC, December 13,14, 2007
- DIA and FDA sponsorship

DIA FDA CLGGS December 12 and 13 Agenda

- Collaborations for the Development of Standard Controls and Protocols for Microarray Assays
- The Advantage and Utility of Standard Reference Materials and Guidelines in Submissions of Microarray Data to Regulatory Agencies
- Translating Microarray Data into Clinical Applications
- What do laboratories need to achieve clinically meaningful results that test developers can provide?
- A Regulatory Perspective on the Incorporation of Standards for Test Development
- Standards in Algorithm Development and Validation: Clinical Decision-Making Algorithms
- Standardization of Electronic Medical Records: Current Practice, Standards Initiatives, and the Future.

US Department of Health and Human Services
American Health Information Community (AHIC)

- Consider means to establish standards for reporting and incorporation of common medical genomic tests data into electronic health records
- Provide incentives for adoption across the country including federal government agencies
- Case study development

www.ahic.gov

Summary: Embrace the complexity

- Good scientific method, study design and data analysis are important to success of moving from biomarker discovery to test development
- Standard materials and best practices will accelerate development, acceptance and use
- Changing the culture takes time, idea exchange, education and legislation
- Stakeholders must work together to ensure success
- Integrated and interoperable databases will help accelerate building the knowledge base for clinical decision making
- The challenges are opportunities to do it right

Thank you

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Integration of genomic technologies in clinical practice requires commitment and working together

Patients & Families
Participation in trials
Productive engagement
Articulate need

R&D
Public private partnerships
Clinical utility
Quality

Care Providers
Public private partnerships
Productive engagement
Articulate need

Government
GINA
Reimbursement strategy
Supportive infrastructure
Stakeholder incentives