Metrics for Universal Standard Quantitative RT-PCR

Context for Standardization of Quantitative RT-PCR
John Sninsky

Practical Application of Quantitative RT-PCR for In Vitro Human Diagnostics: normalization of mRNA levels
Sheng-Yung Chang

Quantification Standards for 5' Nuclease Gene Expression Assays
Manohar Furtado

A Reference RNA for QPCR Assay Standardization
Reinhold Mueller

Is There Anything Left to Discuss? Some Thoughts on Real-time PCR and Microarray Normalization and Reference Standards
Mickey Williams

Panel Discussion

Context for Standardization of Quantitative RT-PCR

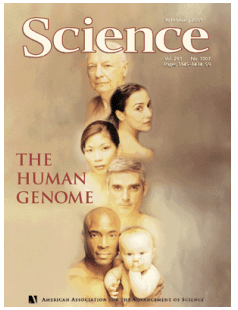
John Sninsky

Celera Diagnostics

Metrology and Standards Needs for Gene Expression Technologies: Universal RNA Standards
Palo Alto
March 28-29, 2003

What We Learned From Human Genome Projects

- Fewer genes than expected (~30,000)
- More genes that are spliced than expected
- More splice variants per gene than expected
- Nearly half of predicted genes have no known function
- 5% of our genome represents segmental duplication



What does this mean? There is much biology that we have yet to learn. Biological and disease complexity are based on sequence variation and expression differences.

Context Consideration for Standards

- Historical
- Methodological
- Process control
- Analyte
- Application

perspective

Medical applications of microarray technologies: a regulatory science perspective

Emanuel F. Petricoin III[1], Joseph L. Hackett[2], Lawrence J. Lesko[3], Raj K. Puri[4], Steven I. Gutman[5], Konstantin Chumakov[6], Janet Woodcock[7], David W. Feigal, Jr. [8], Kathryn C. Zoon[9] & Frank D. Sistare[10]

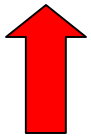
doi:10.1038/ng1029

The potential medical applications of microarrays have generated much excitement, and some skepticism, within the biomedical community. Some researchers have suggested that within the decade microarrays will be routinely used in the selection, assessment, and quality control of the best drugs for pharmaceutical development, as well as for disease diagnosis and for monitoring desired and adverse outcomes of therapeutic interventions. Realizing this potential will be a challenge for the whole scientific community, as breakthroughs that show great promise at the bench often fail to meet the requirements of clinicians and regulatory scientists. The development of a cooperative framework among regulators, product sponsors, and technology experts will be essential for realizing the revolutionary promise that microarrays hold for drug development, regulatory science, medical practice and public health.

Petricoin *et al. Nature Genetics* supplement 32, 474 (2002).

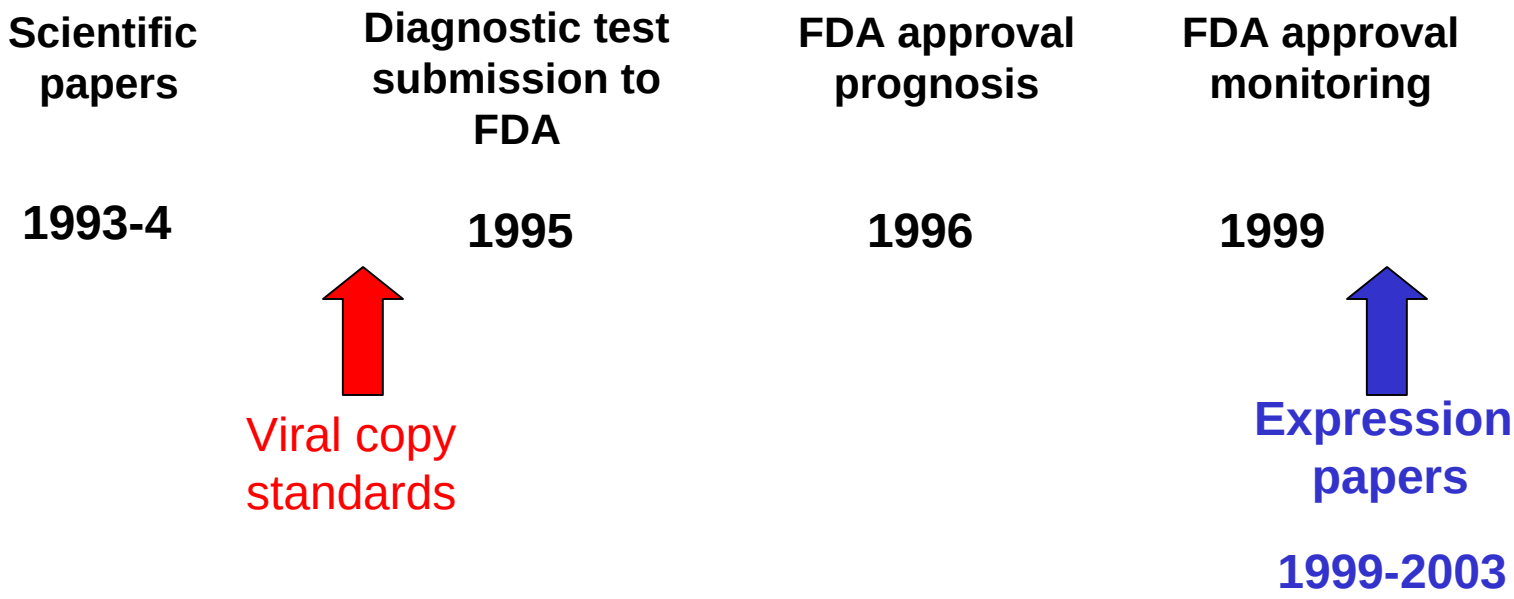
Historical Precedent: HIV

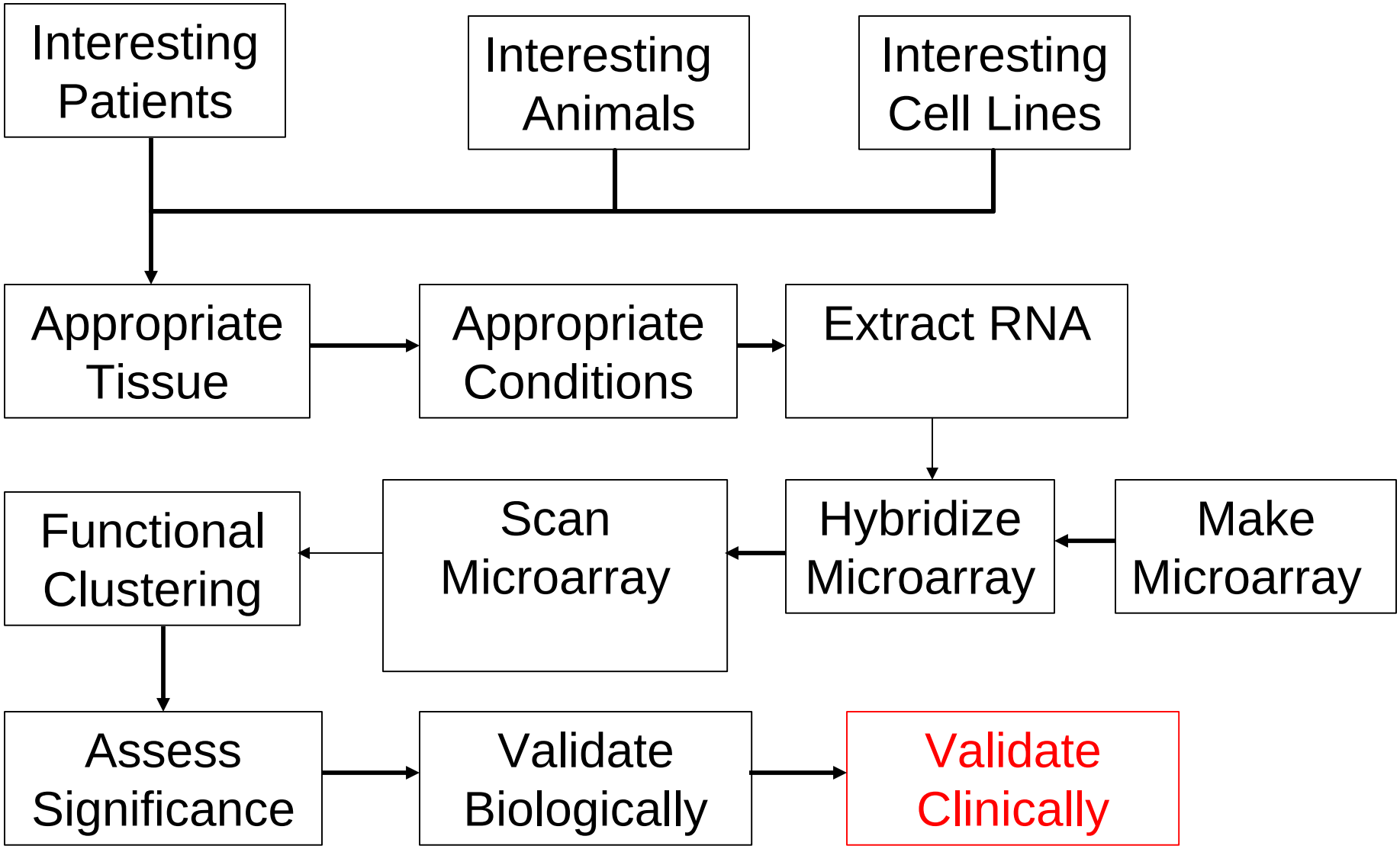
Scientific papers	Diagnostic test submission to FDA	FDA approval prognosis	FDA approval monitoring
1993-4	1995	1996	1999



Viral copy standards

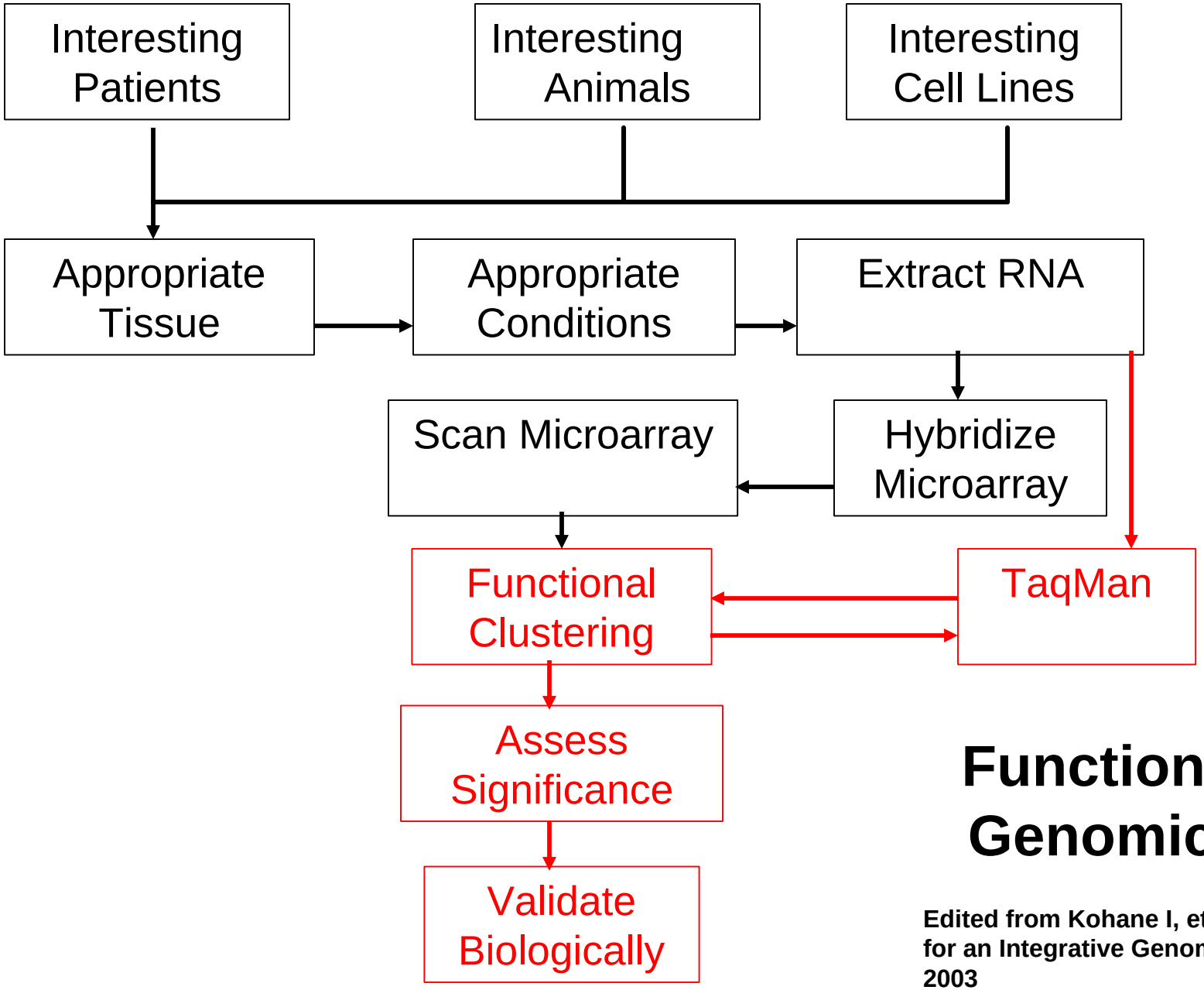
Historical Precedent: HIV





Functional Genomics

Kohane I, et al., "Microarrays for an Integrative Genomics" MIT Press 2003



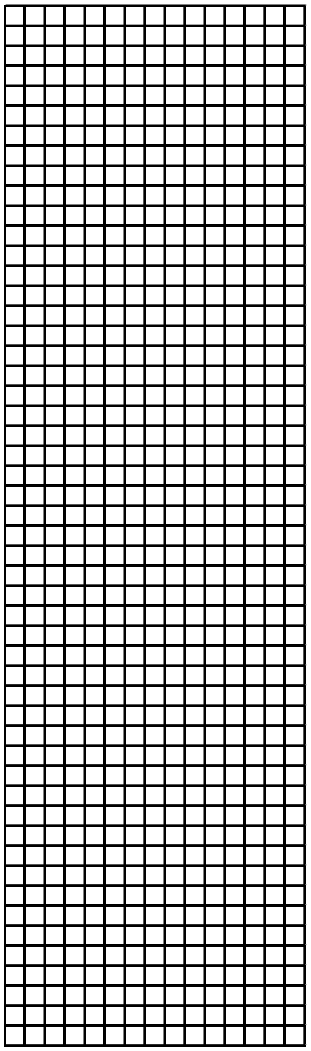
Functional Genomics

Edited from Kohane I, et al., "Microarrays for an Integrative Genomics" MIT Press 2003

A typical clinical study

Variables (10's – 100's)

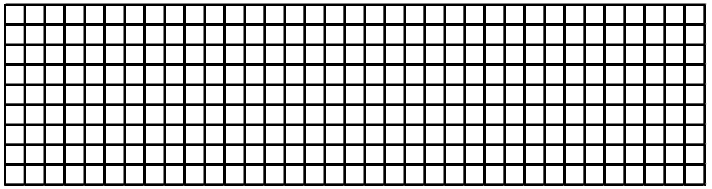
Cases (1000's – 1,000,000's)



A typical genomic study

Variables (10,000's – 100,000's)

Cases (10's – 100's)



A major difference between classic clinical studies and microarray analyses. The high dimensionality of genomic data in contrast to the relatively small number of samples typically obtained results in a highly underdetermined system.

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A typical clinical study

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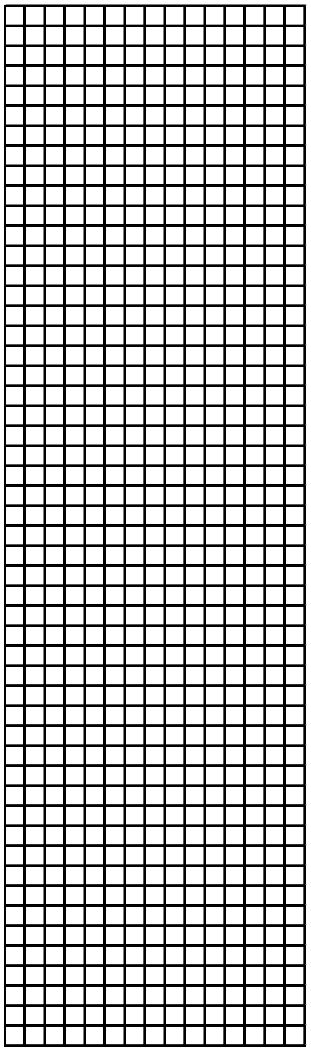

Cases (1000's – 1,000,000's)

A typical genomic study

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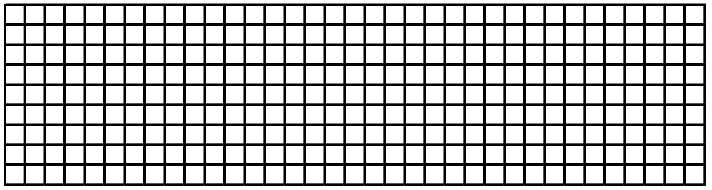

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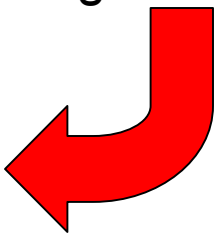


following survey studies, move to targeted studies to gather data on a larger number of patients

A major difference between classic clinical studies and microarray analyses. The high dimensionality of genomic data in contrast to the relatively small number of samples typically obtained results in a highly underdetermined system.

edited from Kohane I, et al., "Microarrays for an Integrative Genomics" MIT Press 2003

Consideration of Standards is Application-Specific

Academic Research

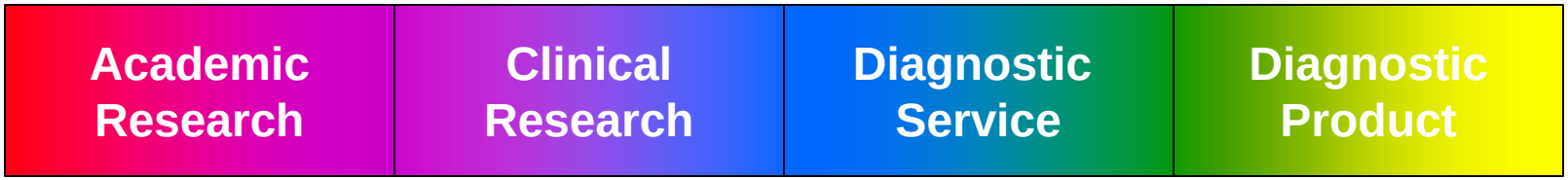
Clinical Research

Diagnostic Service

Diagnostic Product

Platform-specific standards
Analyte-specific standards
Sample-specific standards

Consideration of Standards is Application-Specific



Significant overlap exists and will need to be considered when establishing performance criteria

Consideration of Standards is Application-Specific

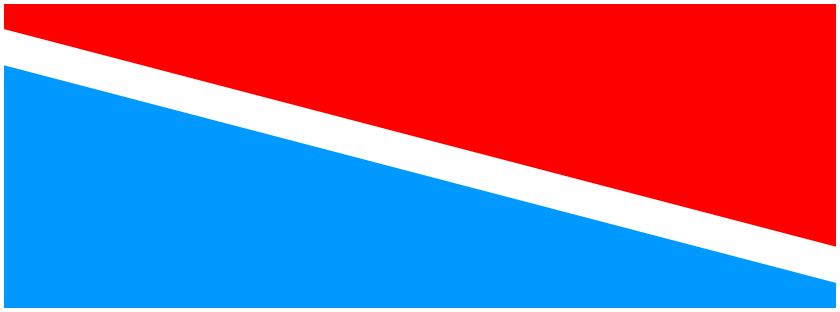
Discovery

Clinical Research

Diagnostic Service

Diagnostic Product

Survey platforms



Targeted platforms

Center for Drug Evaluation and Research (CDER)

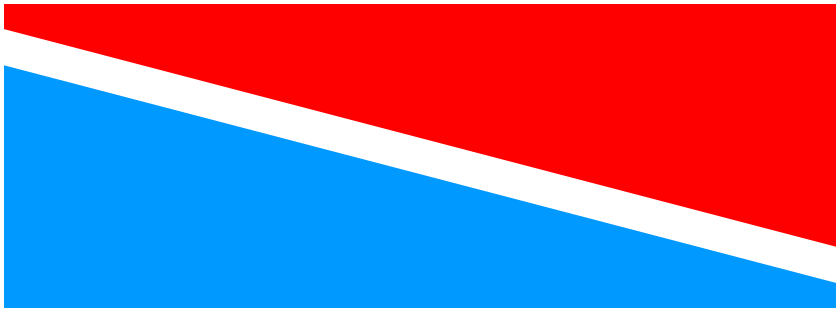
CLIA

Center for Devices and Radiological Health (CDRH)

Consideration of Standards is Application-Specific

Discovery | Clinical Research | Diagnostic Service | Diagnostic Product

Magnitude



Characterization

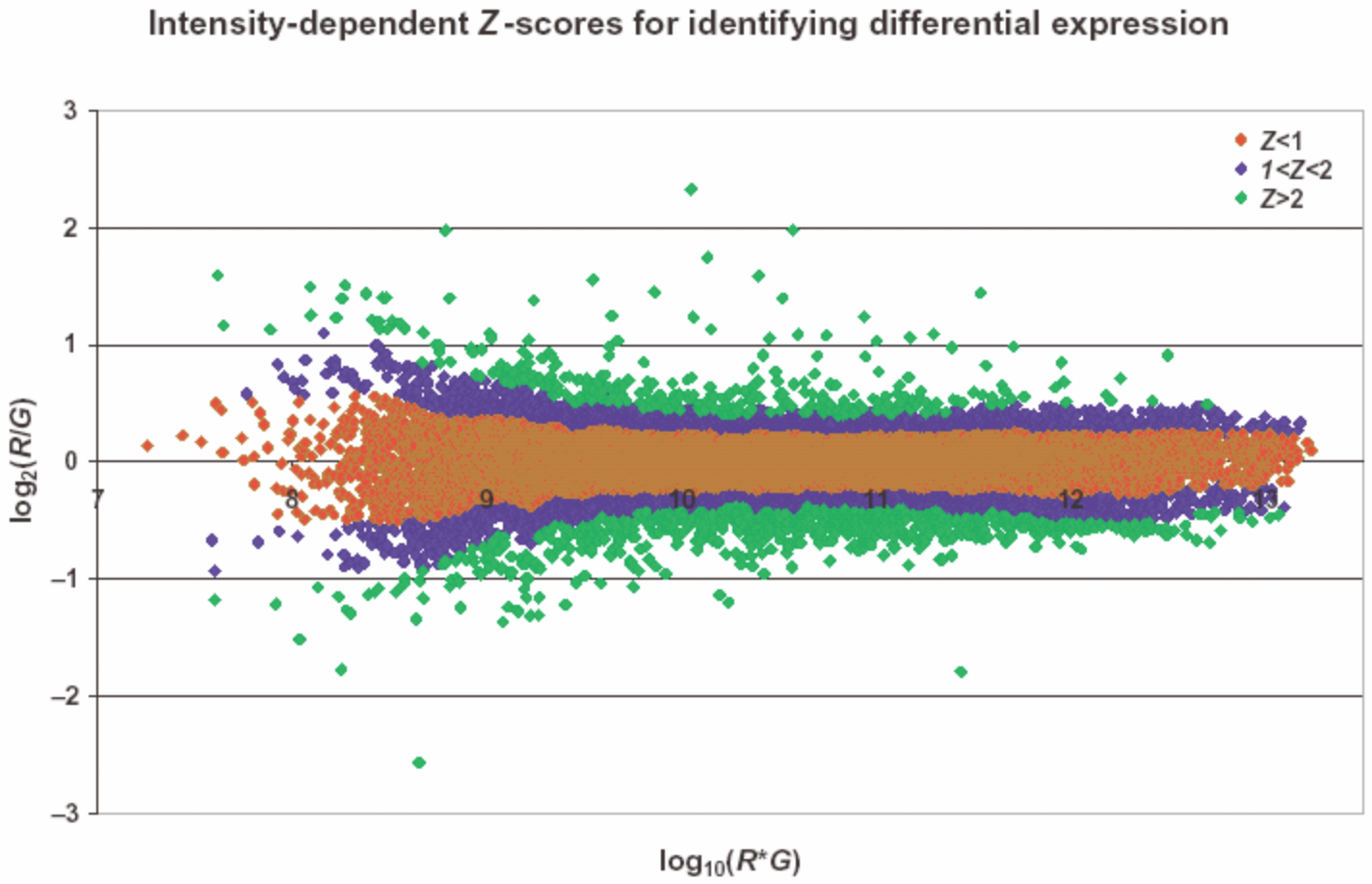
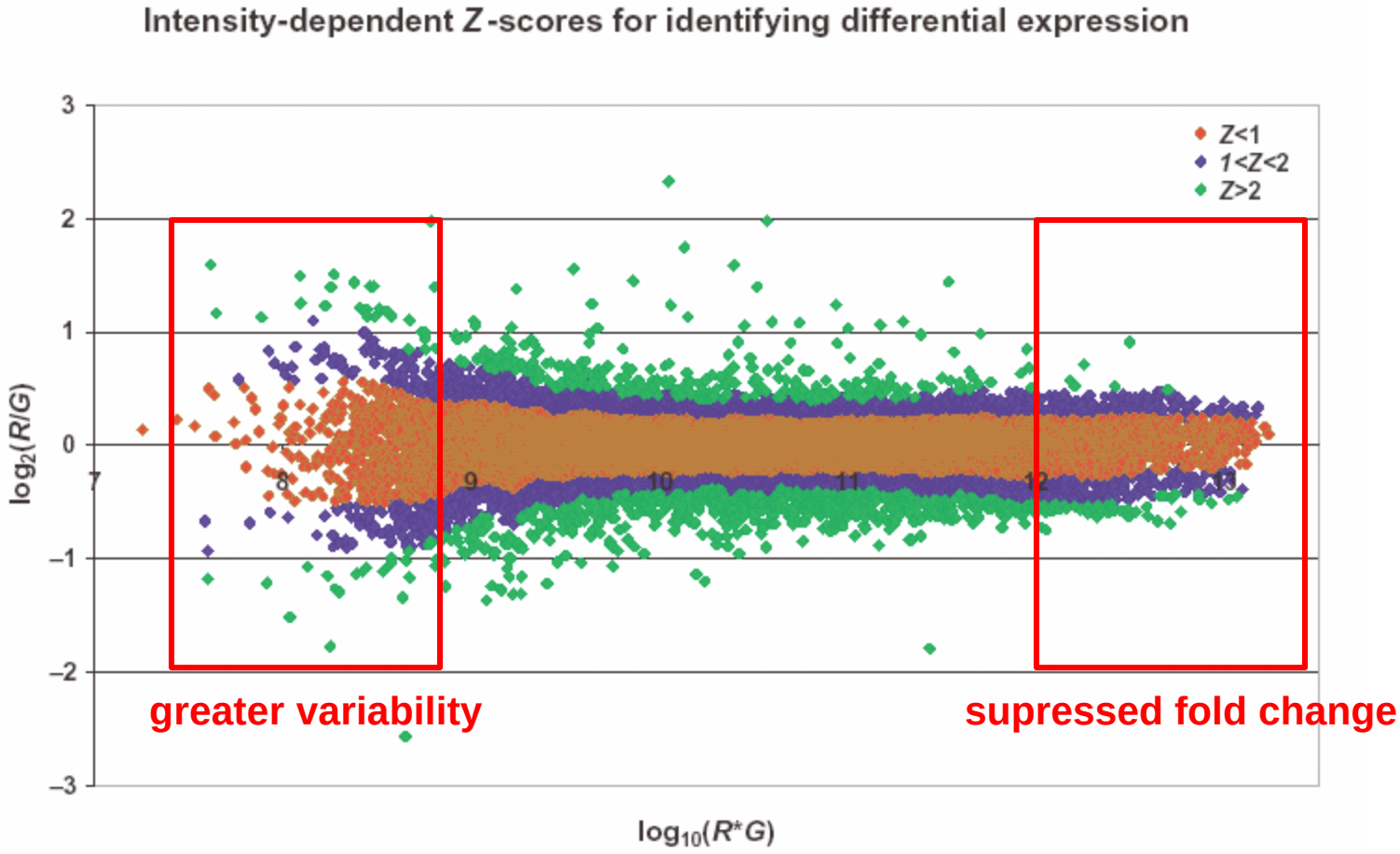


Fig. 4 Local variation as a function of intensity can be used to identify differentially expressed genes by calculating an intensity-dependent *Z*-score. In this R-I plot, array elements are color-coded depending on whether they are less than one standard deviation from the mean (blue), between one and two standard deviations (red), or more than two standard deviations from the mean (green).

Quackenbush *Nature Genetics* The Chipping Forecast 2002.



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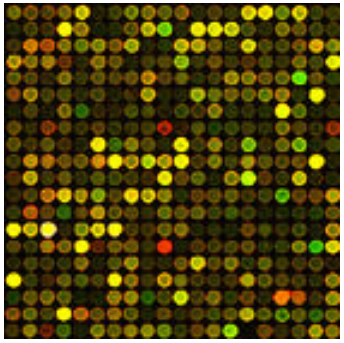
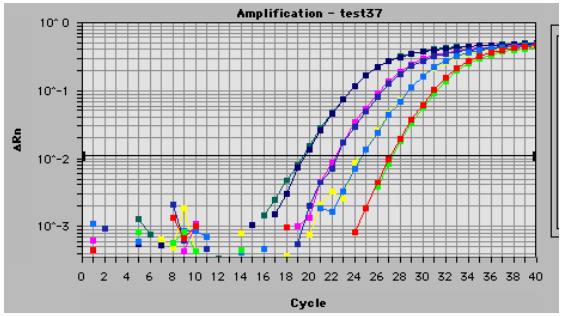
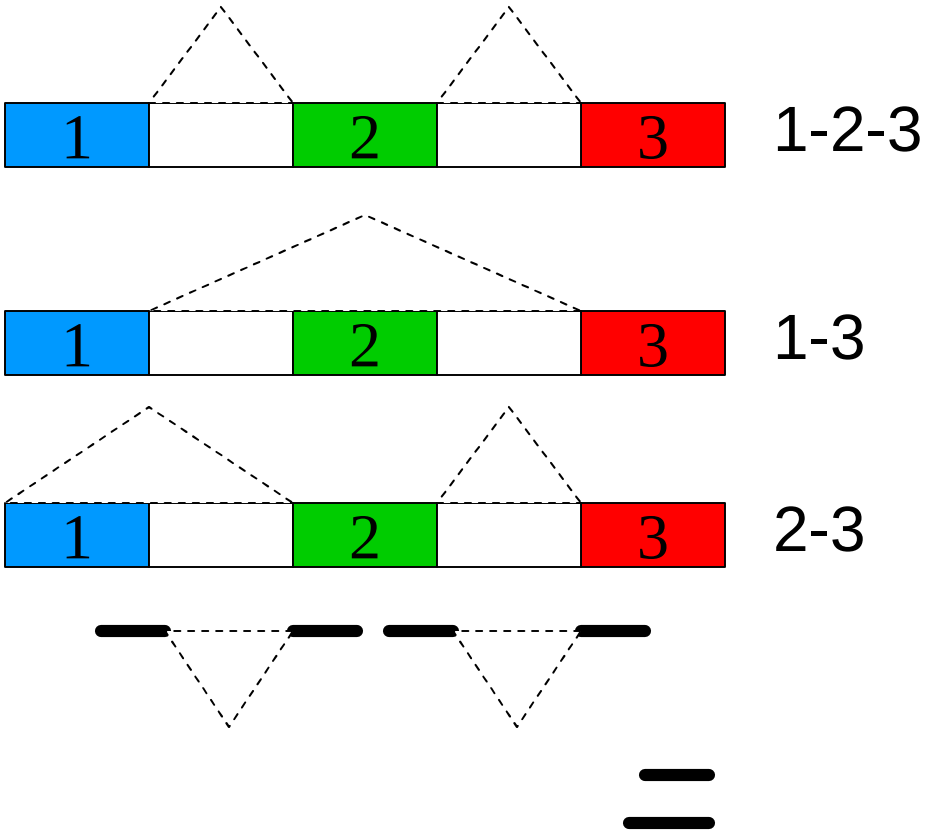
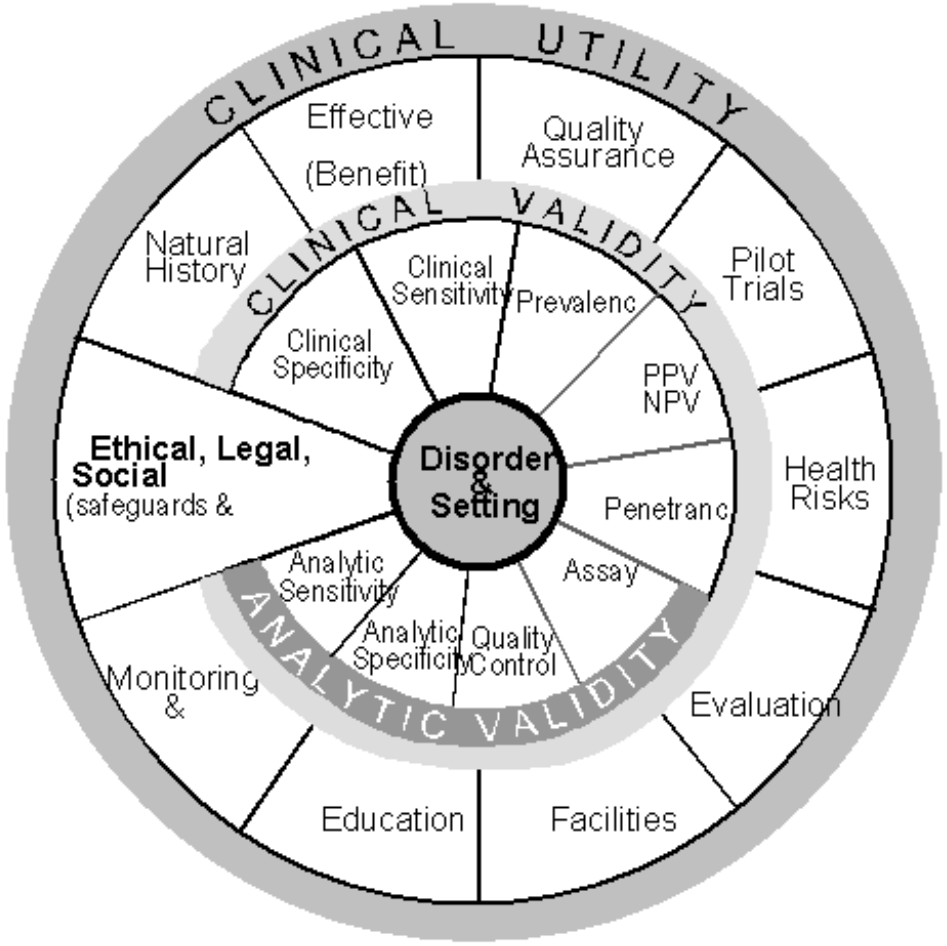
Are the Platforms Measuring the Same Analyte







Determine Precedent that May Be Informative



The ACCE Evaluation Process for Genetic Testing:

takes its name from the four components of evaluation - analytic validity, clinical validity, clinical utility and ethical, legal and social implications - is a model process for evaluating data on emerging genetic tests.

builds on a methodology originally described by Wald and Cuckle Br J Obstet Gynaecol 96,389 (1989).

http://www.cdc.gov/genomics/info/perspectives/files/testACCE.htm

Representative Questions That Can Be Considered

How many copies of mRNA can be detected?

How often is the test positive when an mRNA is present?

How often is test negative when an mRNA is not present?

Is an internal QC program defined and externally monitored?

Have repeated measurement been made on specimens?

What is the within and between laboratory precision?

If appropriate, how is confirmatory testing performed to resolve false positive results in a timely manner?

What range of patient specimens have been tested?

What criteria should be used for and how often does the test fail to give a usable result?

How similar are results in multiple laboratories using the same, or different technology?

Summary

- Standards discussion is timely
- Establish definitions for common terminology
- Review all applications where standards will contribute
- Establish context-dependent standards criteria
- Select a subset of applications for prioritized focus
- Review precedent for molecular standards
- Caution needs to be used in cross over studies