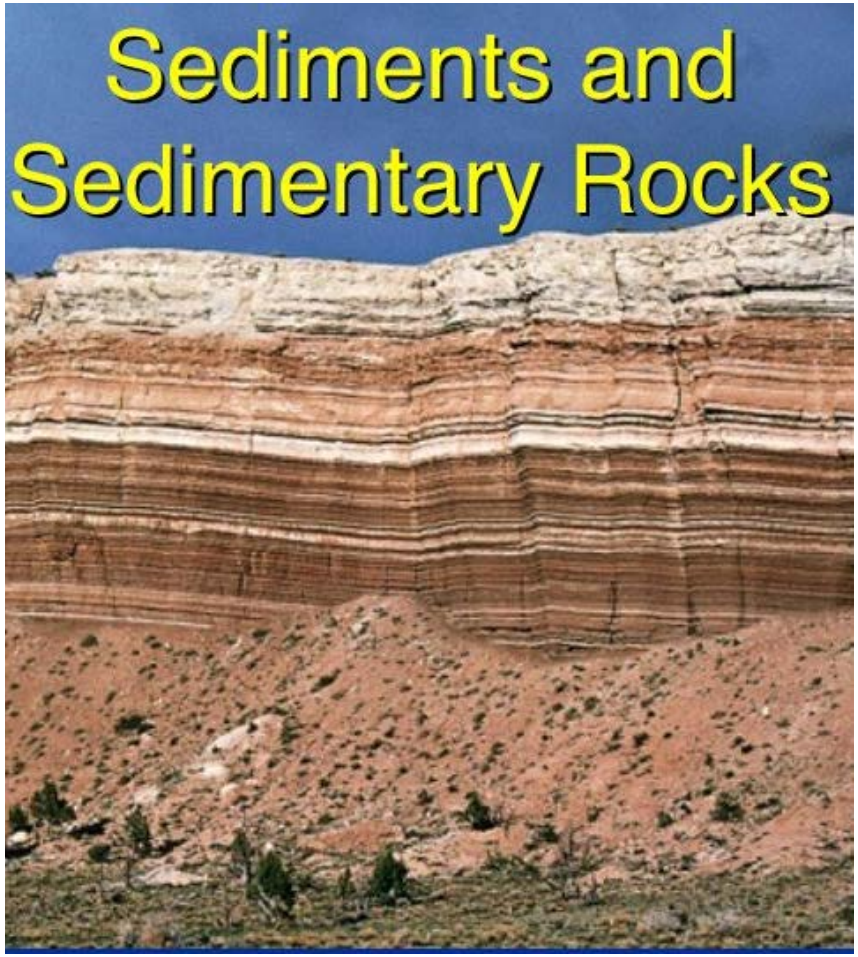
Steps towards metrology for quantitative biology

Marc Salit and David Duewer
Materials Measurement Laboratory

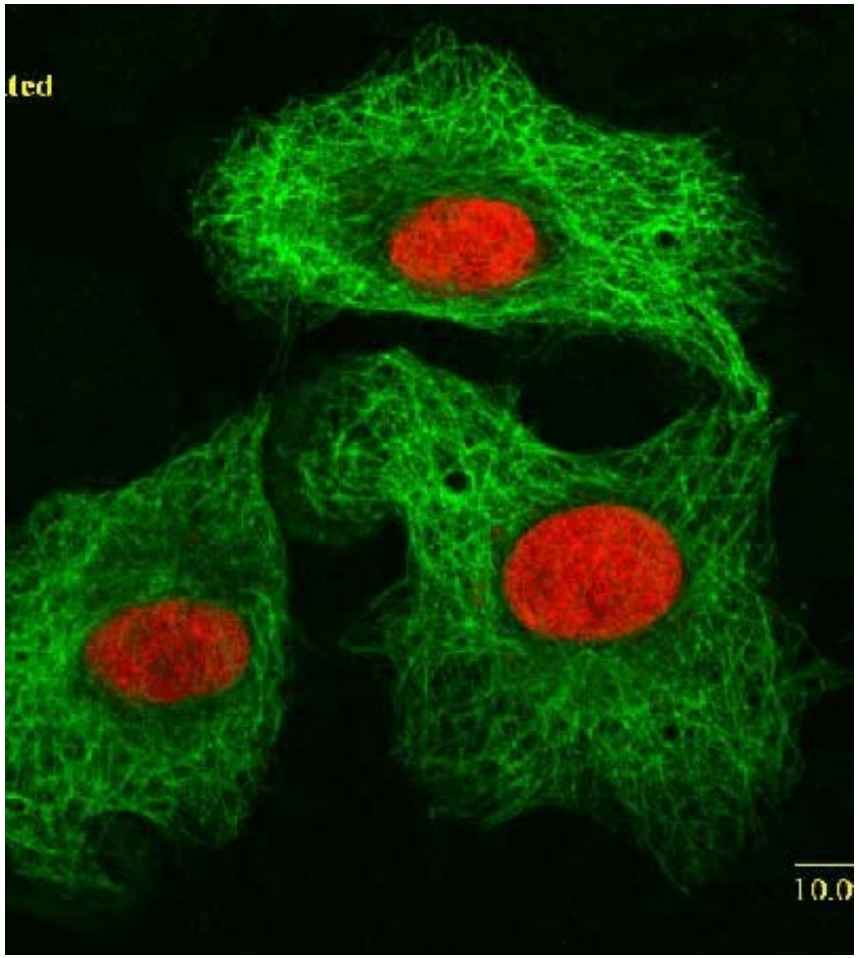
SIM Metrology Course

National Institute of Standards and Technology
October 2013

Metrology for Physical and Chemical Properties…



Measuring Biological Properties…



What makes Metrology in Biology different?

What is Biology?

- Biology is a natural science concerned with the study of life and living organisms, including their structure, function, growth, evolution, distribution, and taxonomy.

From Wikipedia entry for "Biology"

What do we measure?

- Properties over many length scales
  - ~10^9 range
- Dynamic properties over many time scales
  - 10^{10} range
- Many properties at once
  - genomes can have ~10^9 elements
- Abundances of many things
  - proteomes can have ~10^{15} dynamic range

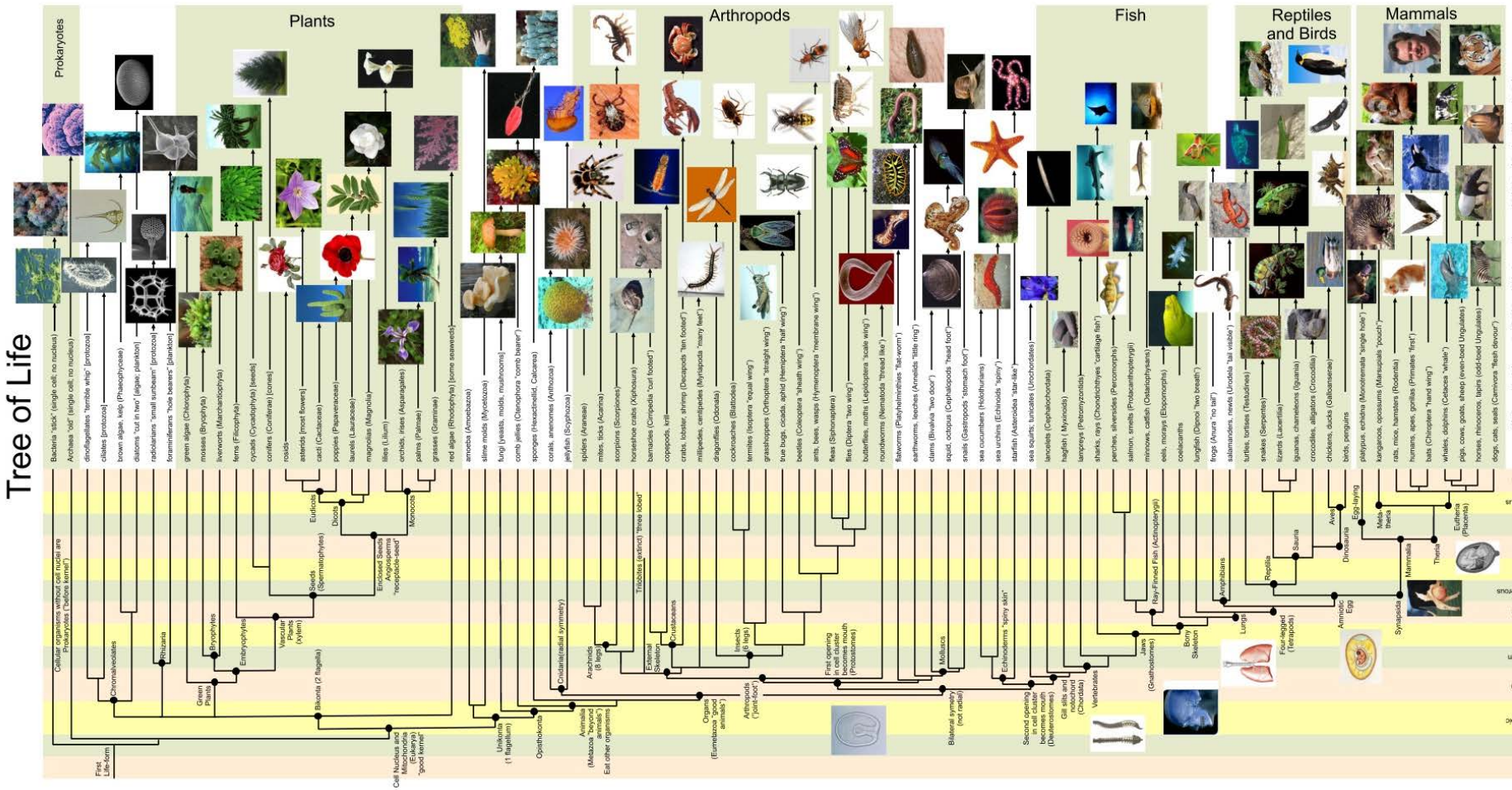
What Properties?

Property Classes

- Morphology
- Physical Activity
- Metabolic Activity
  - dynamic biochemical activity
- Interactions with other entities
- Amount
- Identity
  - from organism taxonomy to nucleotide identity to sequence identity…

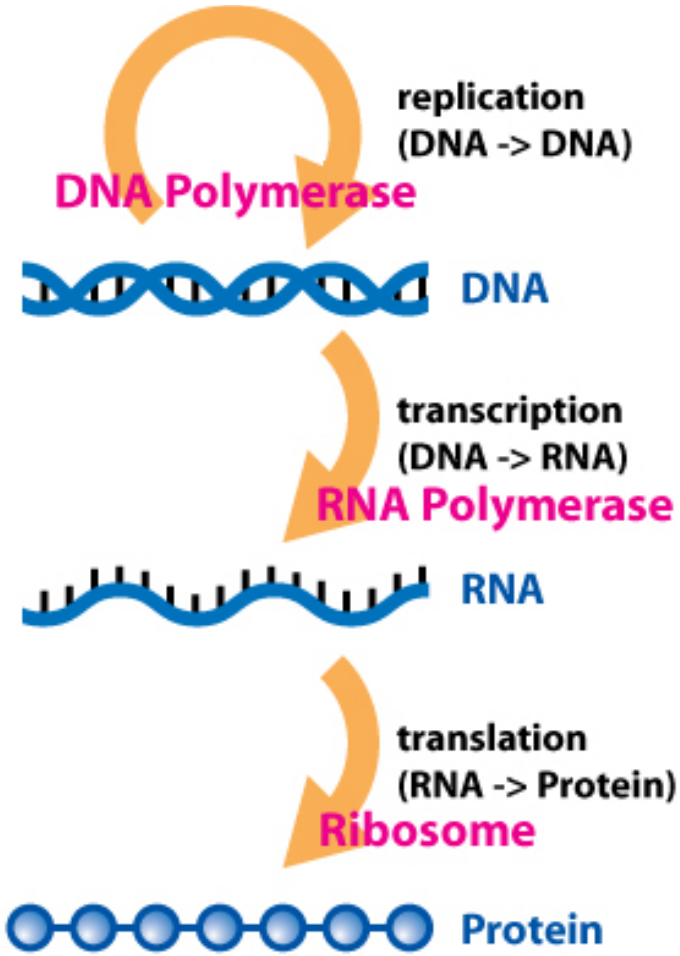
Over what space??

Complex Space for Metrology



Where do we start?

- One starting point is *Molecular Biology*
  - molecular basis of biological activity
- Interactions amongst systems in cells
- Can we measure key elements in the molecular "plan" underlying biological phenomena?



"Central Dogma" of molecular biology

'Omics

Omics aims at the collective characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of an organism or organisms.

Wikipedia entry for Omics accessed 31 Oct 2013

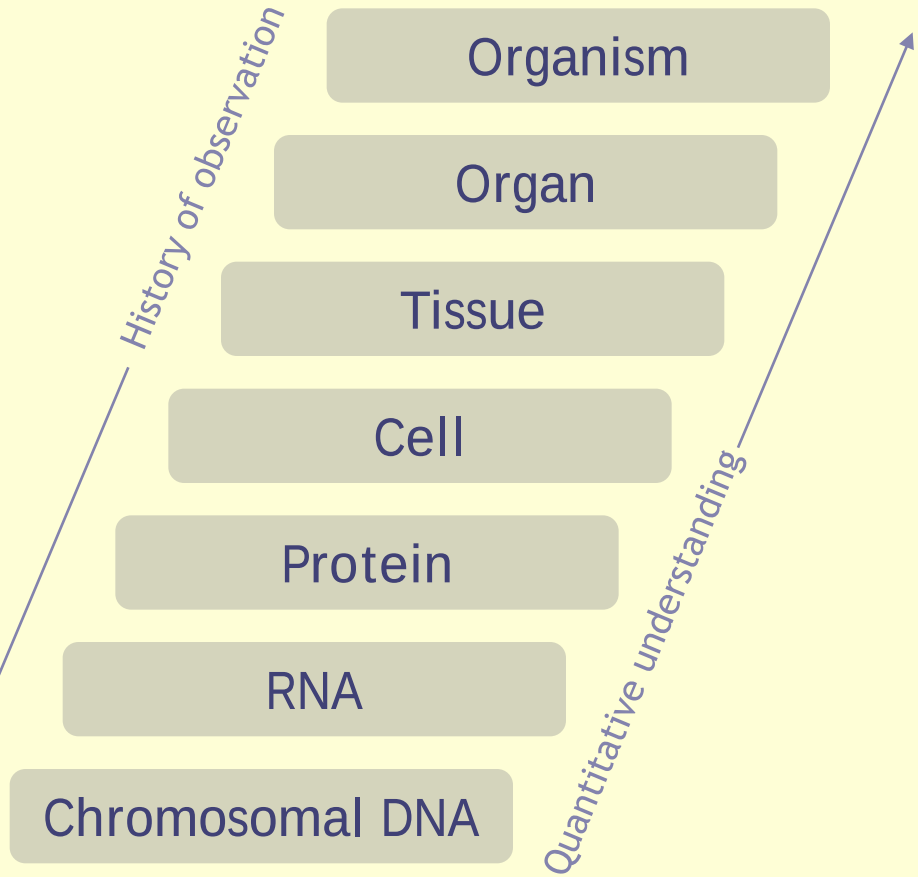
- typically involves measurement of a "complete" cohort of biomolecules to characterize a biological state
- genomes began to be reported first
  - "templates" for living matter
- transcriptomes began to be measured
  - "gene expression"

What is Gene Expression?

- A caterpillar and a butterfly have identical genomes!
  - The difference between them arises from which genes are expressed, and when and where they are expressed.
  - From a genome of billions of base pairs and tens of thousands of genes, the subset expressed in a cell confer its nature.
- Quantitative biology in the post-genomic world

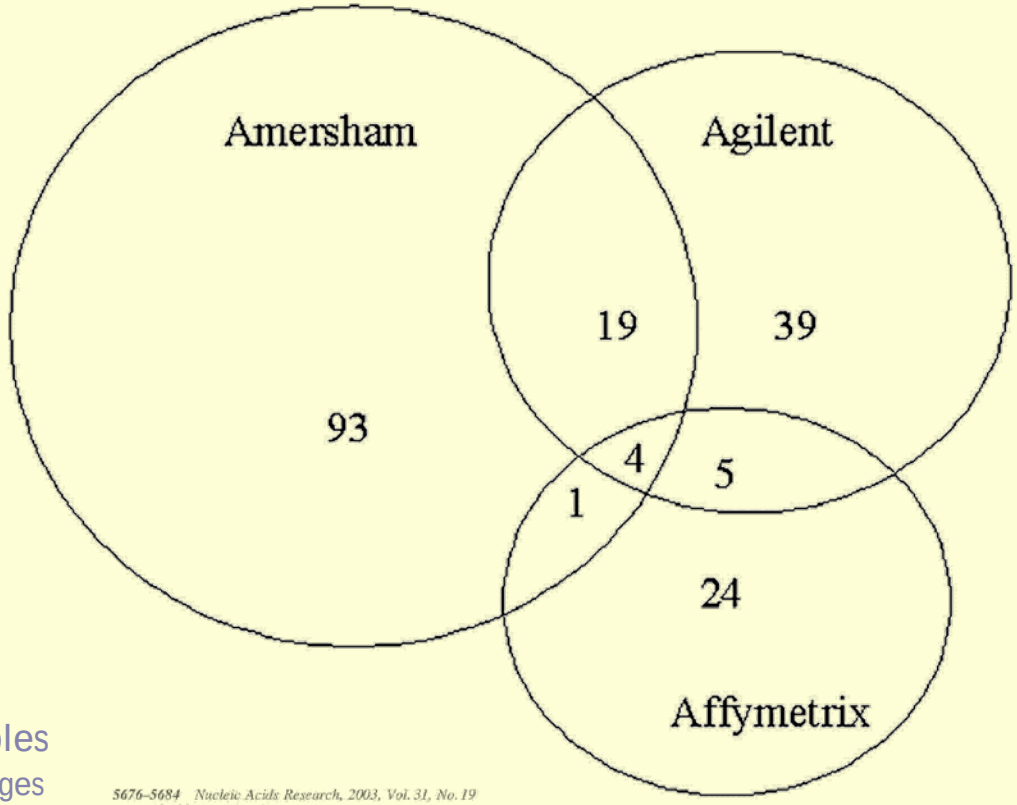
How is Gene Expression profiling used?

- Systems Biology
  - research phase
- Toxicogenomics
  - appearing in clinical trials
  - environmental application
- Pharmacogenomics
  - appearing in clinical trials
- Clinical Diagnostics
  - devices being developed
- Genomic Medicine
  - expected to be an underlying technology



Measurement Problems

- Comparability of results
  - Traceability
  - Validation
  - Uncertainty
- Management of variability
  - Across platforms
- Truth
  - quantitative accuracy
  - specificity
- Informatics
  - High dimensionality
    - Many more genes than samples
      - presents statistics challenges
  - statistics alone are insufficient to deliver biological interpretation



5676–5684 *Nucleic Acids Research, 2003, Vol. 31, No. 19*
DOI: 10.1093/nar/gkg763

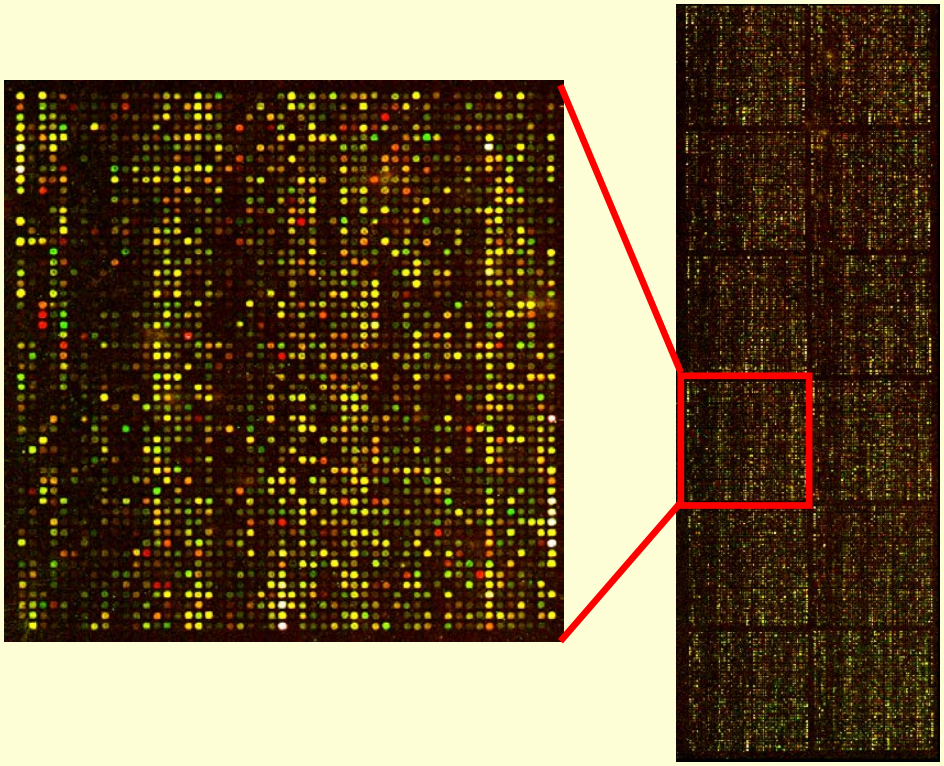
Evaluation of gene expression measurements from commercial microarray platforms

Paul K. Tan, Thomas J. Downey[1], Edward L. Spitznagel Jr[2], Pin Xu, Dadin Fu, Dimiter S. Dimitrov[3], Richard A. Lempicki[4], Bruce M. Raaka[5] and Margaret C. Cam*

Microarray Core Laboratory, National Institute of Diabetes and Digestive and Kidney Disorders (NIDDK), National Institutes of Health, [1]Partek Incorporated, [2]Department of Mathematics, Washington University, [3]Laboratory of Experimental and Computational Biology (LECB), National Cancer Institute, NIH, [4]National Institute of Allergy and Infectious Diseases (NIAID), NIH, SAIC-Frederick, Inc., [5]Clinical Endocrinology Branch, NIDDK, NIH, USA

Measurement Strategies

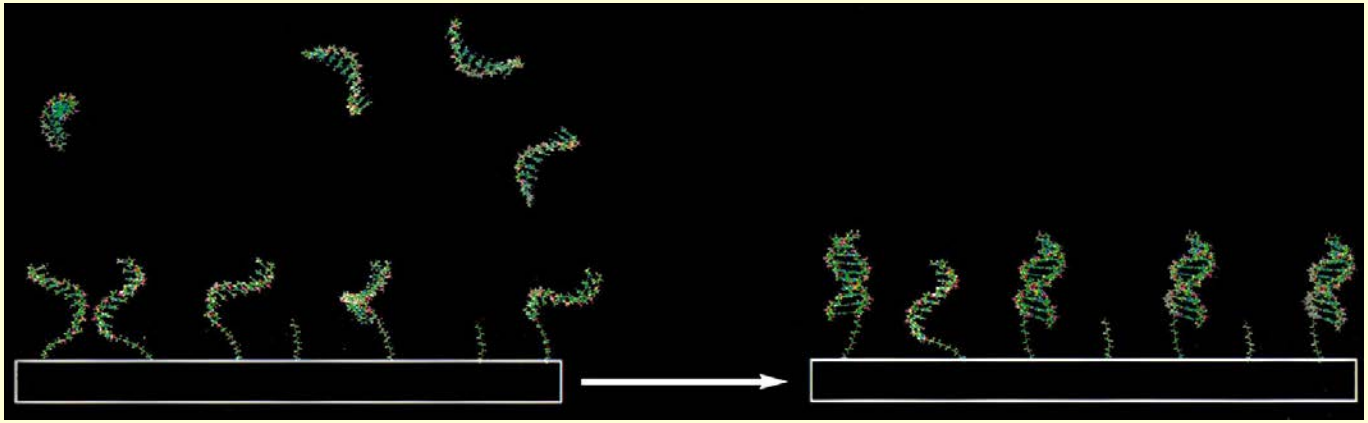
- QRT-PCR
  - only a few RNA transcripts at a time
- Microarrays
  - proposed NIST focus
  - massively multiplexed RNA measurements
    - high-throughput, combinatorial approach
    - “bio-nano-informatics”
    - develop, deploy infrastructure
  - technology also applies to
    - DNA sequencing
    - proteomics/protein sequencing



TIGR 32,000 element Human array

Microarrays

- Affinity hybridization
- Spectroscopic detection
- Image analysis
- Statistical analysis of massively multiplexed data
  - To identify gene expression patterns of interest



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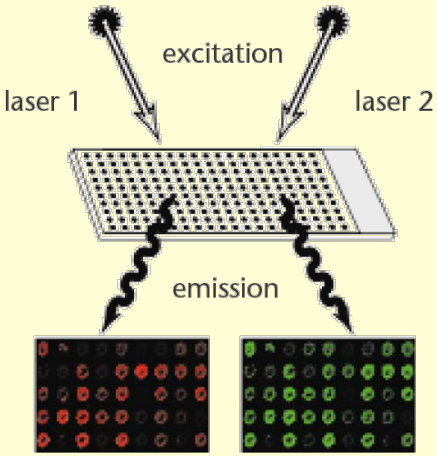

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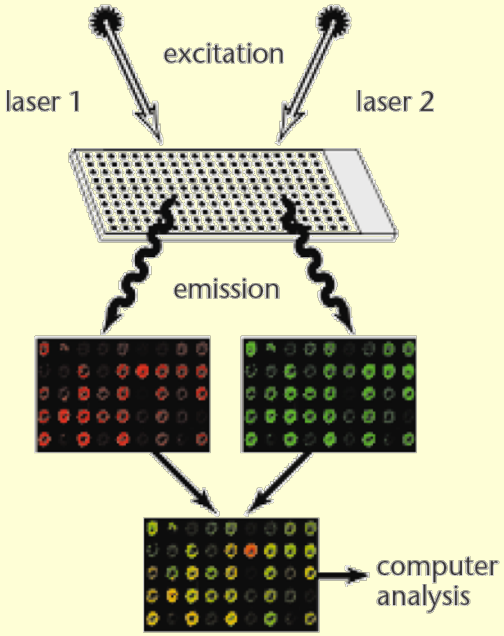

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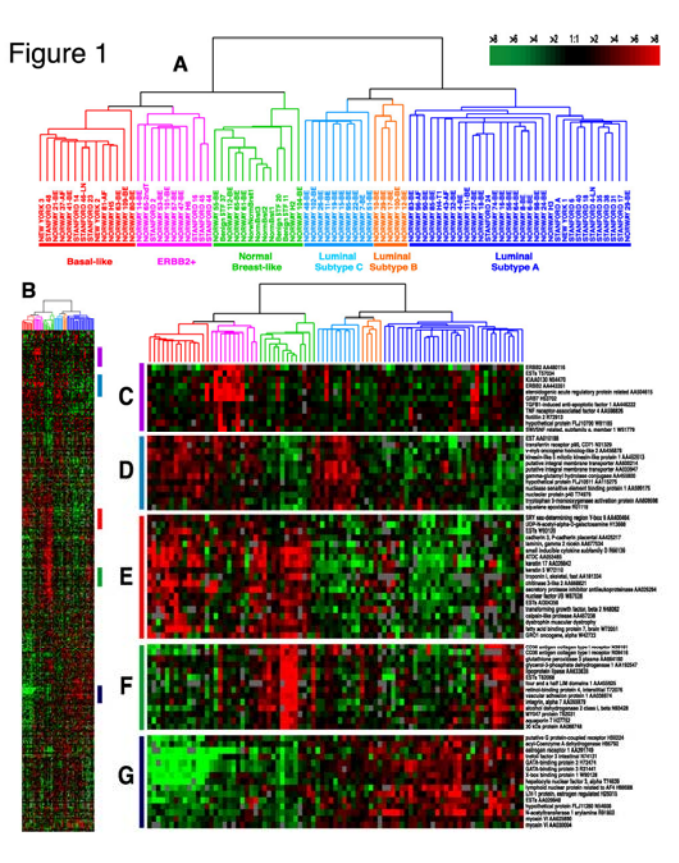
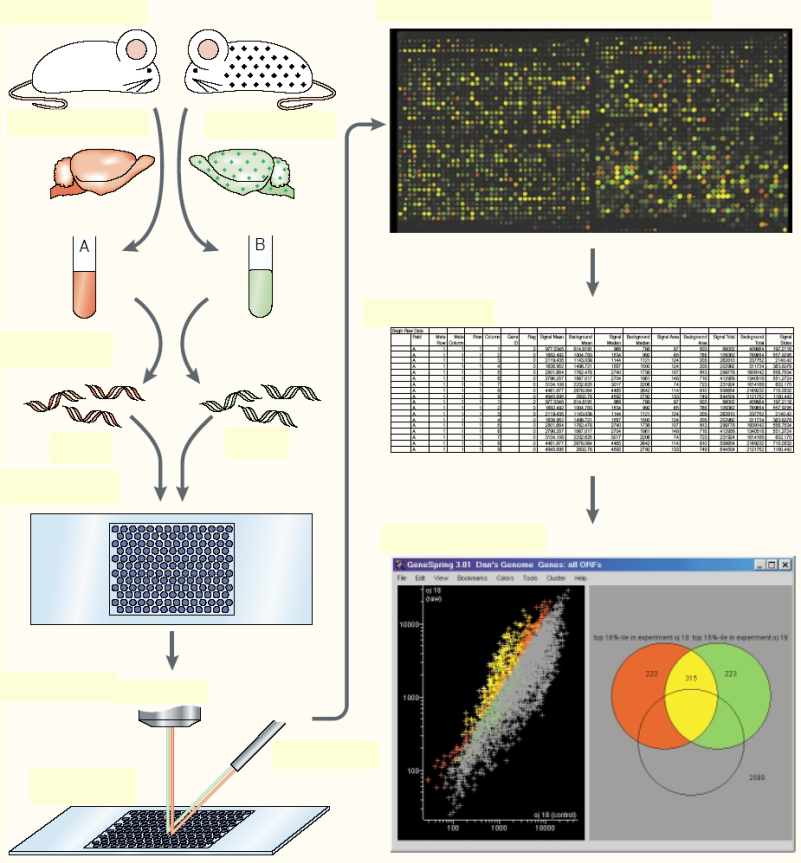


What does a microarray experiment look like?

- RNA isolation
- Clean-up, labeling, hybridization
- Imaging of spatially arrayed signals
- Image feature extraction
- Informatics to assign gene expression measures
- Bioinformatics to elucidate biological insight



Worldview

- Three legged stool of metrology
  - Traceability
  - Measurement Uncertainty
  - Method Validation

Traceability

- tying results to a common reference
  - usually realized with calibration
  - enables comparison of results amongst those using the common reference
    - across space and time
- biological measurement results are often traceable to the *experimental control*
  - enrichment of a molecular signal

VIM 2 Defintion

Traceability

- *"property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties."*

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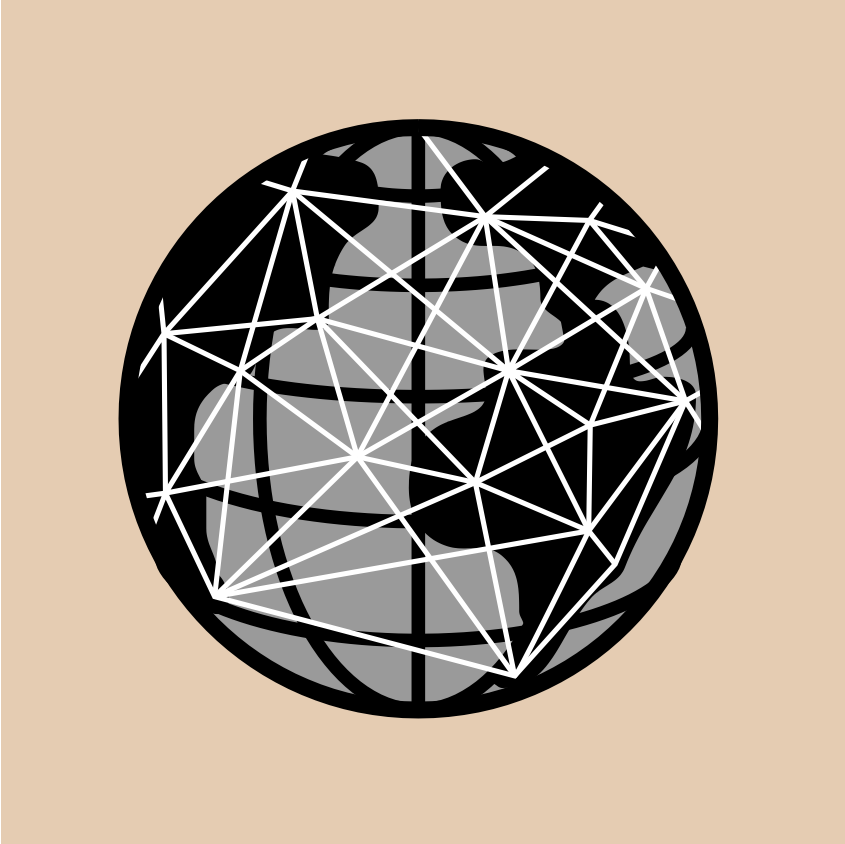
Comparing results

- results are only useful when compared
  - to other results
    - e.g., to observe a trend
  - to limits
    - e.g., a threshold for action
  - different results in different places or measured at different times…
    - “comparability over space-and-time”

Comparability of results

- Whole and sole goal of traceability.
  - *raison d'être!*
- results linked to a common reference can be compared
- scope of reference defines scope of comparability
  - global network
    - SI



Comparability of results

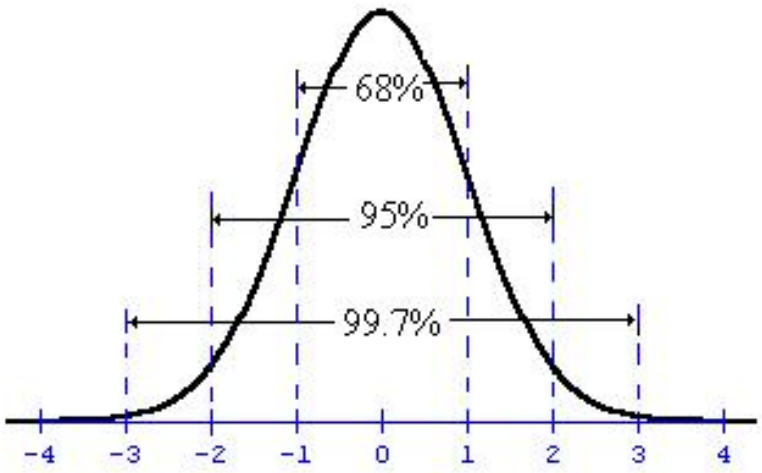
- Whole and sole goal of traceability.
  - raison d'être!
- In biology, we use a ‘control’ as our reference, and compare against that control to “measure” biological effects
  - experiment design establishes traceability
  - scope of traceability is to the *control*

Emerging Challenges in Metrology for Biological Measurements

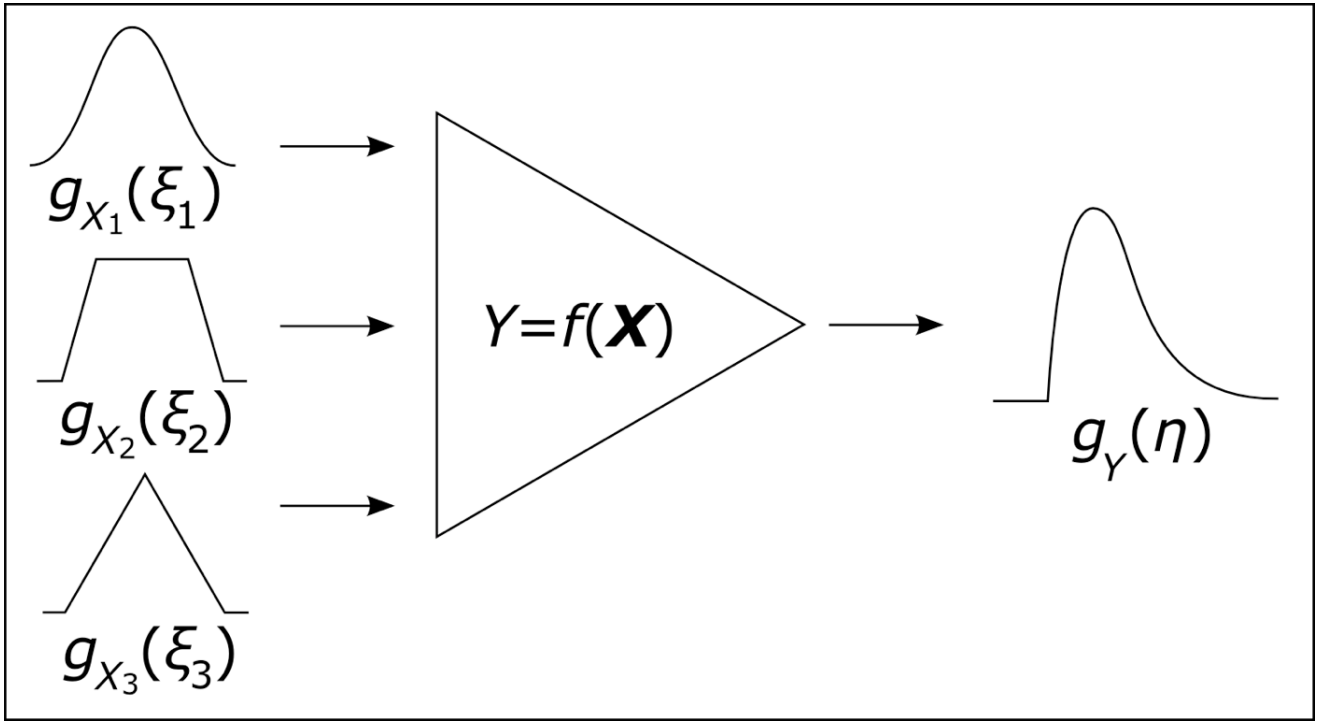
- Commutability
  - measurand “mismatch”
  - typically caused by bias arising from matrix
- Speciation
  - specificity matters
- Unstable measurands & dynamic processes
- Complex and multiplexed measurands
  - artifacts, biostatistics
- Qualitative properties
  - traceability?
  - measurement uncertainty?
- Measurement of stochastic processes
  - population studies poorly, misleadingly representative
- *in vivo* measurement
  - imaging-based
  - molecular sensors
- *in vivo* sensor logic
  - detect condition in engineered cells
  - trigger action
    - switch metabolic pathway

Measured Data are Samples from Distributions

- The thing we're measuring has some distribution
  - we sample it
  - from our samples we infer the underlying distribution
  - our sampling is fraught with extra dispersion and biases
  - a lot of what we try to do is to separate out the underlying stuff that gives rise to the distribution of our measured data



Basics of Measurement Uncertainty Calculation



PDF‘s of the influence quantities X_1, X_2, X_3

equation of the measurand

measurand Y

How does this work for Genomics?

- Quantitative results
  - e.g., *Gene Expression*
- Qualitative results
  - e.g., *absence/presence, identity, mixtures*
    - variant calling
- Hybrids
  - e.g., *gene specific methylation*

- Critical Elements
  - too many measurands to calibrate
  - no existing metrology for "nominal" properties
  - *variation arising from bioinformatics*
- Traceability ill-posed
  - traceable to A, C, G, T?
- MU needs extension for qualitative results
  - A ± ?

Approaches for Genomics

Quantitative Results

- Use external "Spike-in" controls to validate *in situ*
  - *External RNA Controls*
- Use mixed tissue samples to validate/monitor performance of measurement process
  - *FDA-developed Gene Expression controls*
  - *Pilot project at UMMC*

Qualitative Results

- Research to develop strategy to create and apply *in situ* controls
  - *ERCC Controls for Systematic Sequencing Errors*
- Initially, establish strategies to use shared, well-characterized samples to demonstrate measurement performance
  - *Controls for Whole-genome Sequencing*

Approaches for Genomics

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Method Validation

- Demonstration by provision of objective evidence…
  - that what I'm measuring is what I intend to be measuring
  - to prove I'm not just reporting artifacts

Measurement Uncertainty

- estimated value that gives me reasonable expectation of dispersion around my result
  - given my measurement system
  - use data from validation experiment to develop estimates

Standards for Genome-scale Measurements

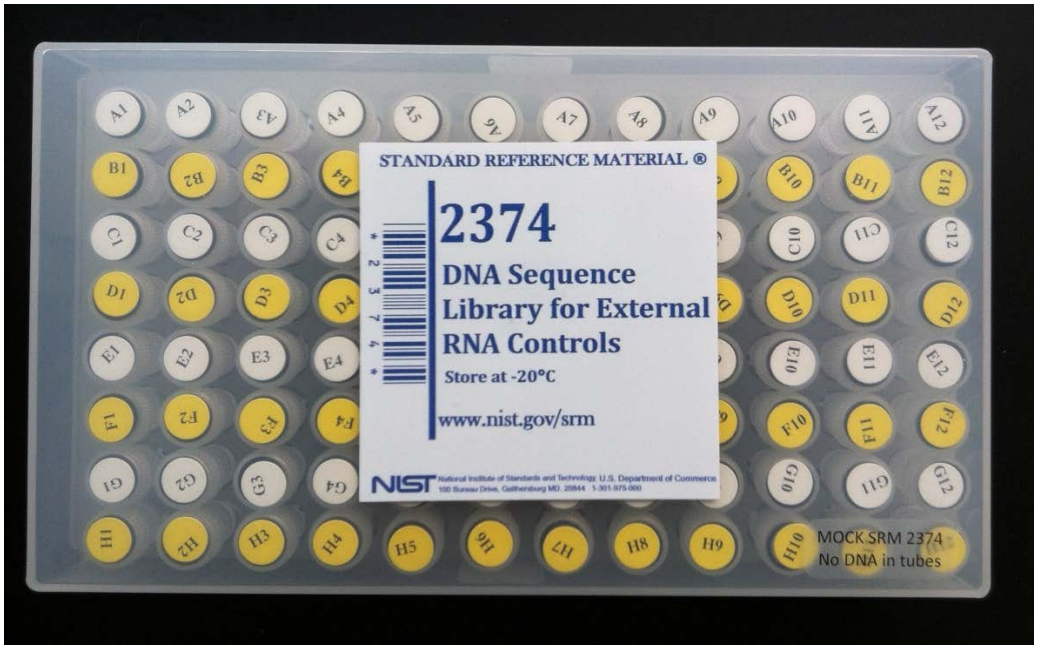
Sarah A. Munro, Ph.D.
Marc Salit, Ph.D.
Biosystems and Biomaterials Division

NIST
National Institute of Standards and Technology
U.S. Department of Commerce

MATERIAL MEASUREMENT LABORATORY

What does NIST do in Biology?
Example: Genome-scale Reference Materials

- Whole Human Genome Reference Materials
  - Cell line DNA
  - Genome in a Bottle Consortium
- Standard Reference Material (SRM) 2374
  - DNA Sequence Library for External RNA Controls
  - External RNA Control Consortium



ERCC Collaborative Study to Create SRM 2374

- ERCC members contributed candidate sequences
  - Collaborative study to test RNA controls on variety of platforms
  - Selected well-performing sequences for SRM
- SRM contains 96 unique control sequences in plasmid DNA
  - *In vitro* transcription to make RNA controls
  - Intended to mimic mammalian mRNA

BMC Genomics

BioMed Central

Methodology article

Open Access

Proposed methods for testing and selecting the ERCC external RNA controls

External RNA Controls Consortium*[1,2]

Address: [1]Members of the External RNA Controls Consortium are listed in Appendix 1 and [2]Send correspondance to Laura H Reid

Email: External RNA Controls Consortium* - Lreid@expressionanalysis.com

* Corresponding author

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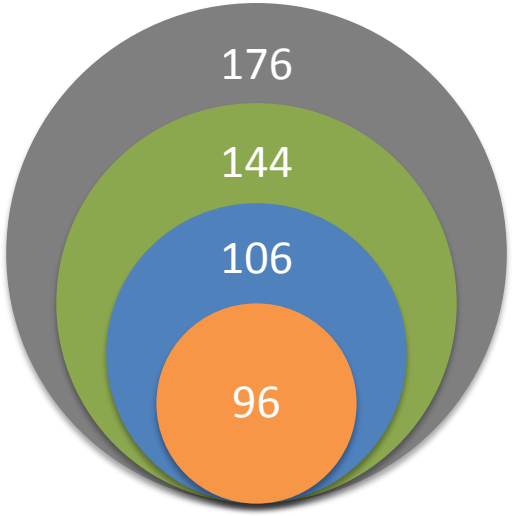
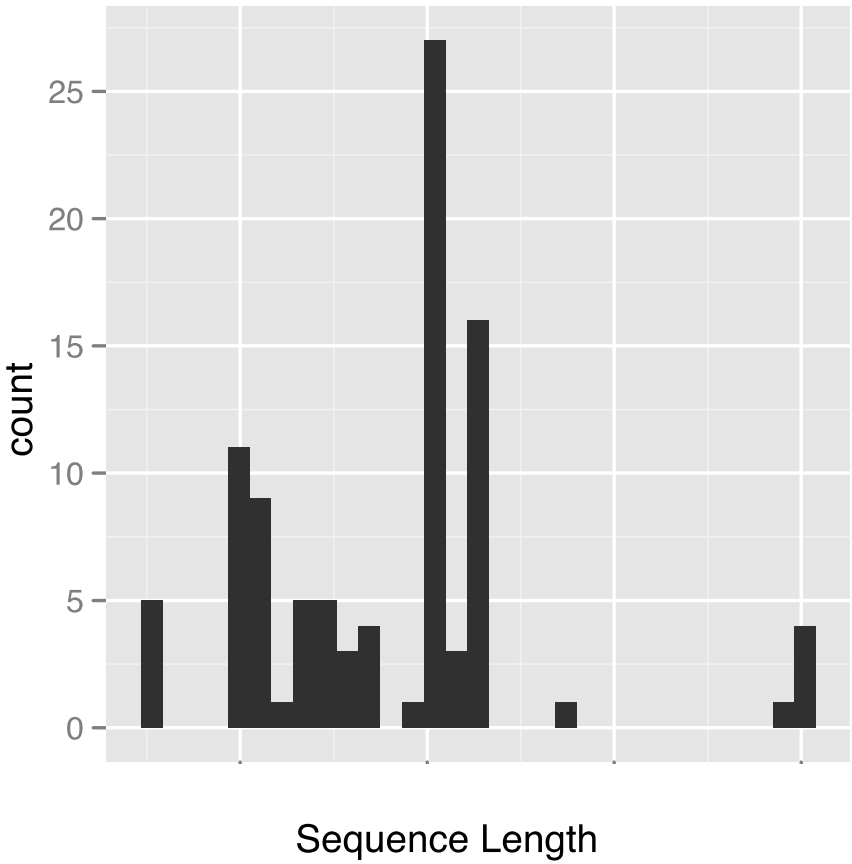
Abstract

The External RNA Control Consortium (ERCC) is an ad-hoc group with approximately 70 members from private, public, and academic organizations. The group is developing a set of



Library of 96 Controls in SRM 2374

Sequence Lengths



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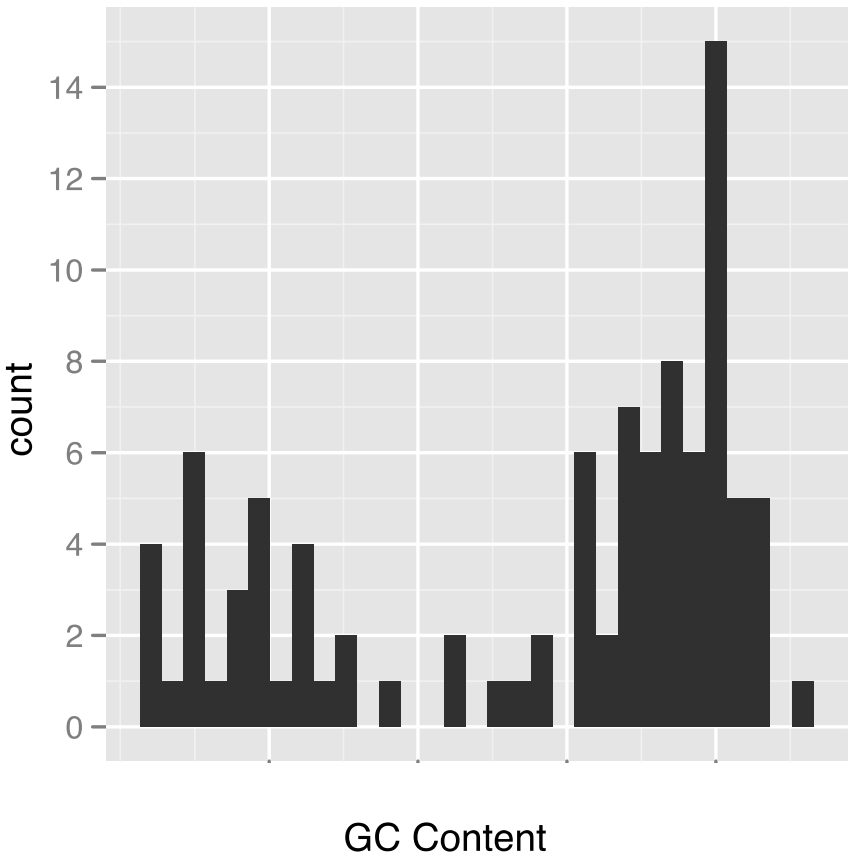
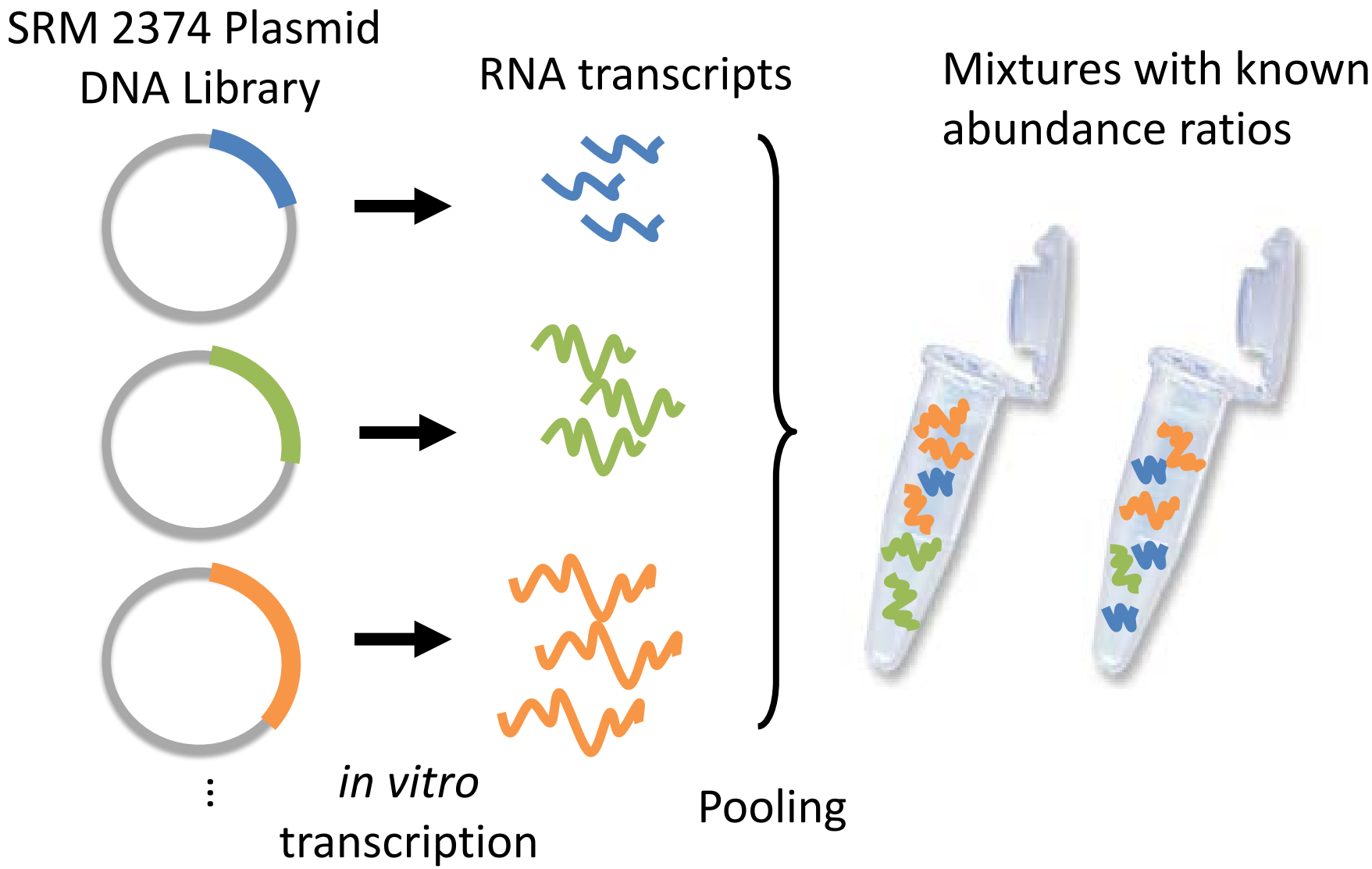
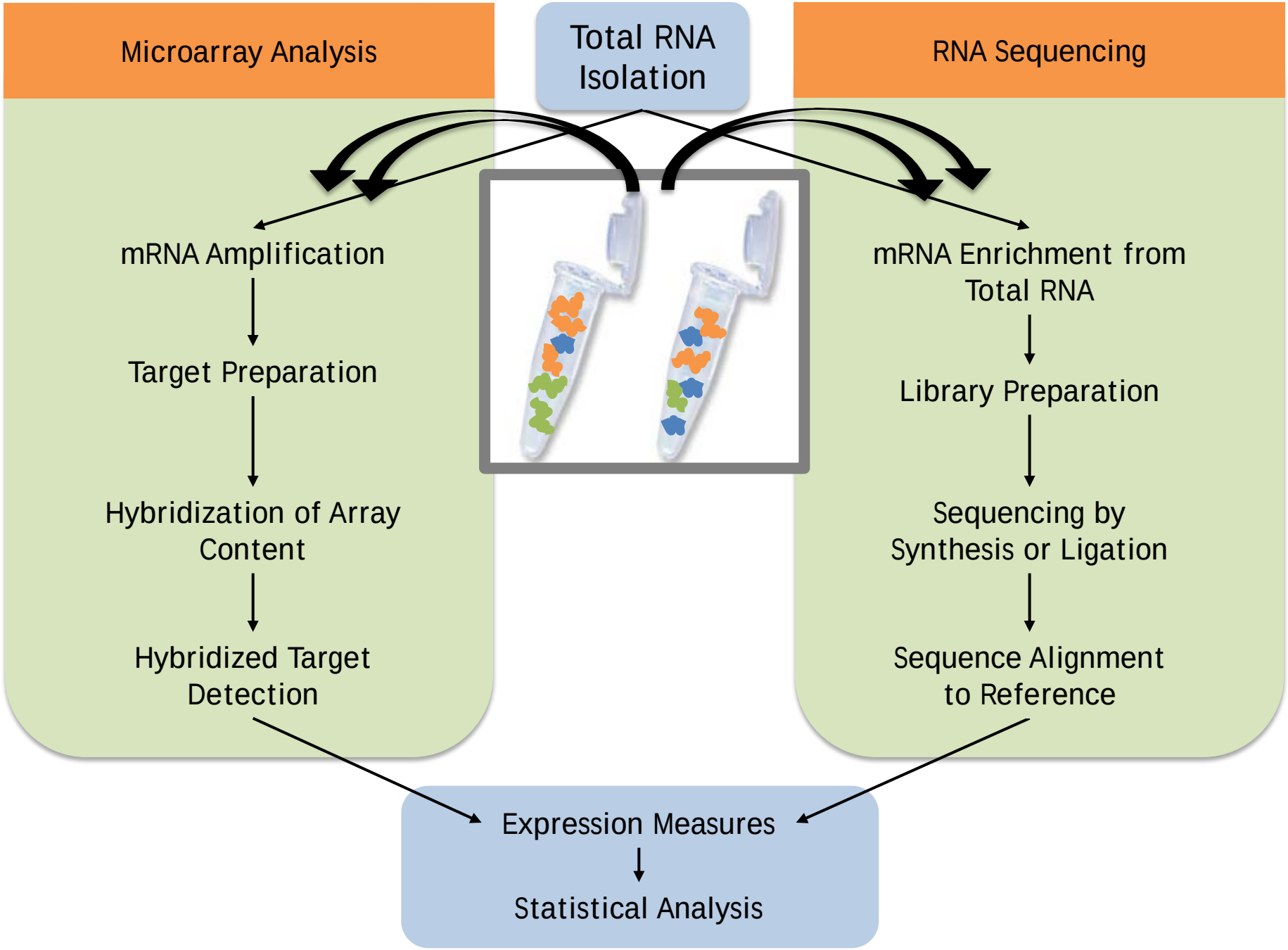


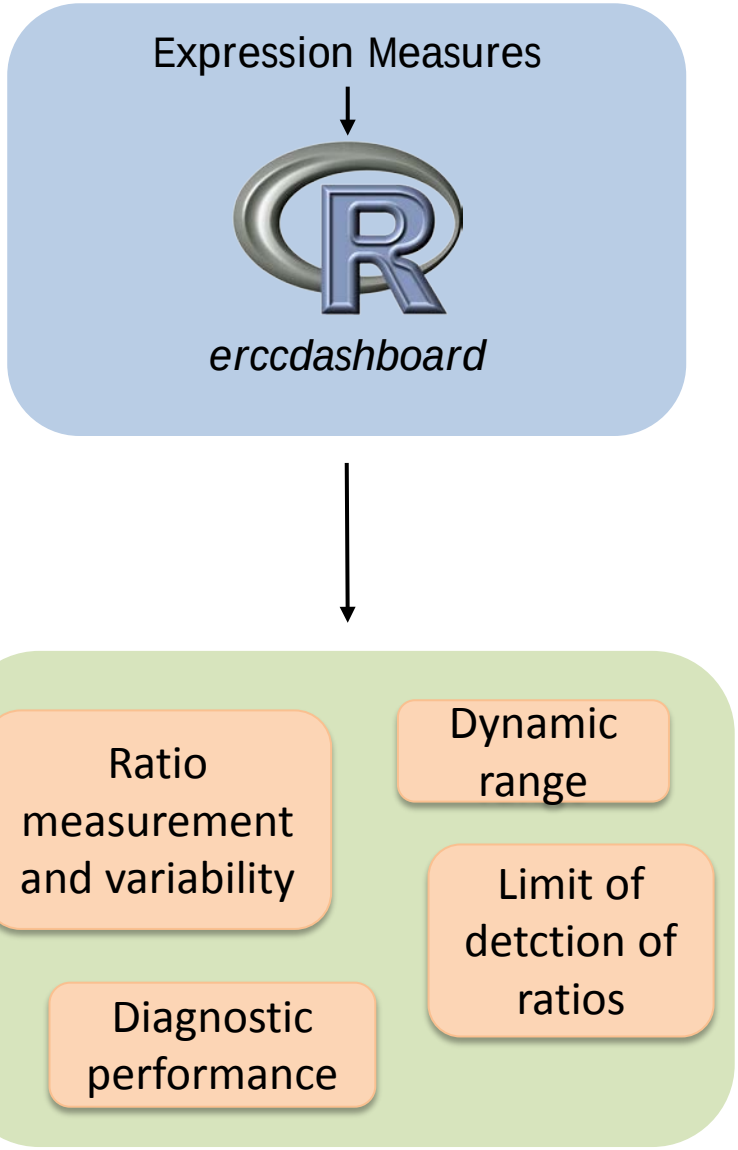
Creating Spike-in Mixtures from SRM 2374




Microarray Analysis
Total RNA Isolation
RNA Sequencing
mRNA Amplification
Target Preparation
Hybridization of Array Content
Hybridized Target Detection
mRNA Enrichment from Total RNA
Library Preparation
Sequencing by Synthesis or Ligation
Sequence Alignment to Reference
Expression Measures
Statistical Analysis

Method Validation with the *erccdashboard*

- Open-source R package - *erccdashboard*
- Developed as part of FDA-led SEQC interlaboratory study
- Assess technical performance of a gene expression experiment
- Compare results
  - Within a single laboratory
  - Between laboratories



Ambion
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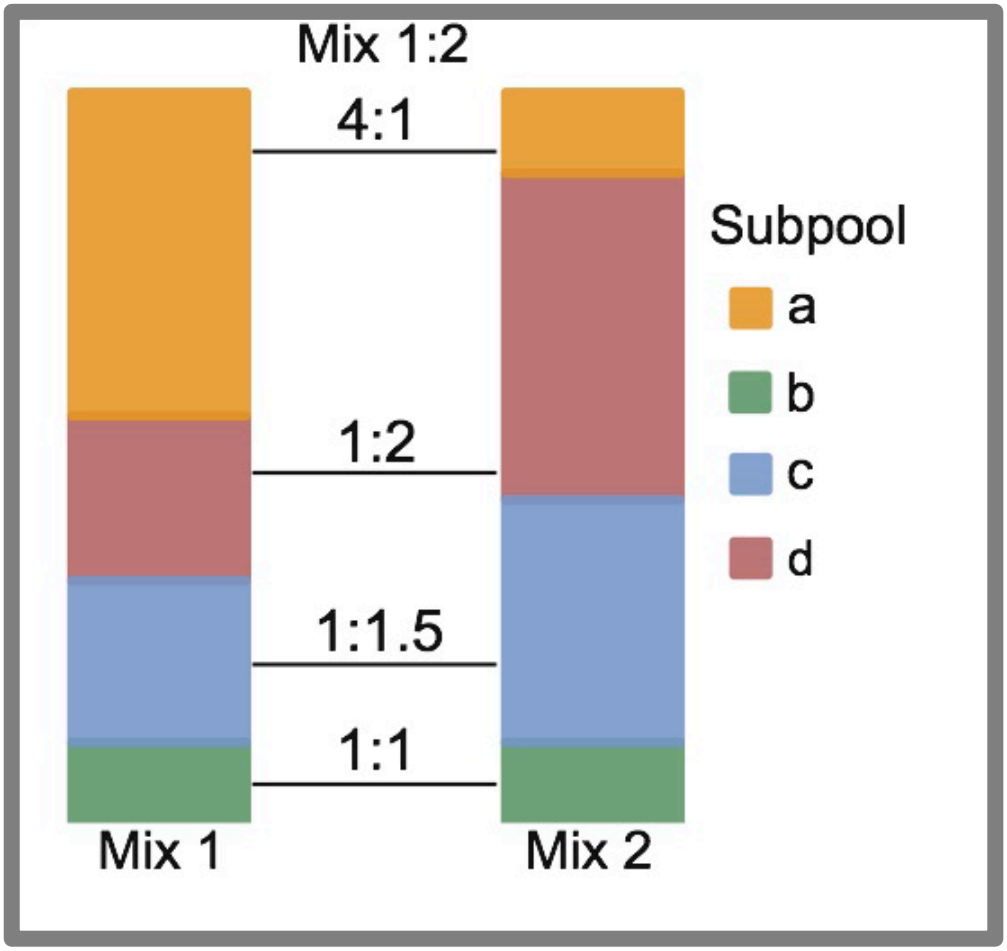
P/N: 4456739
Lot# 0801001
MADE IN USA
ERCC ExFold RNA
Spike-In Mixes
Store at -20°C
100 rxn
READ MSDS
-20C/D
4455864$0801001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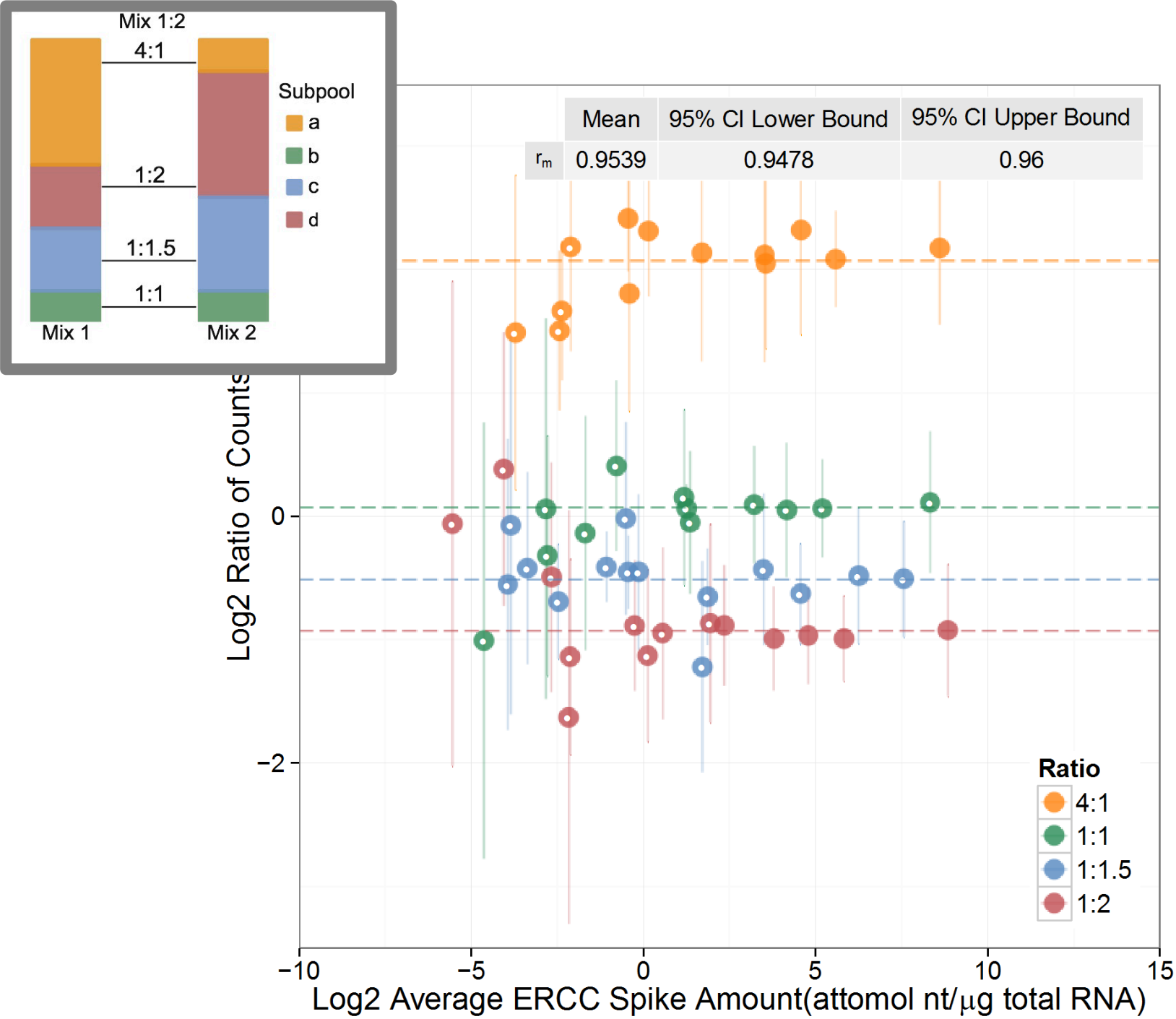
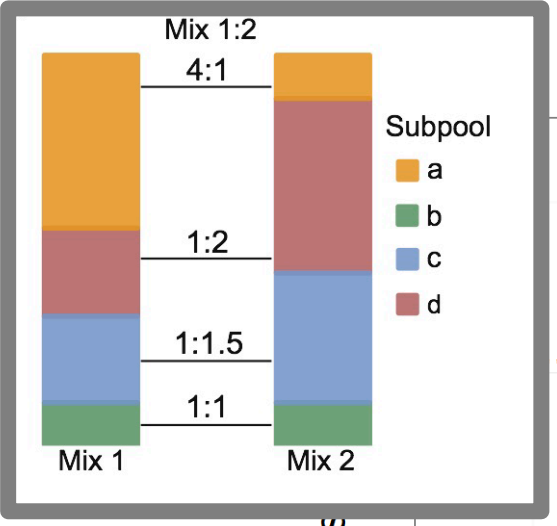
4:1
1:2
1:1.5
1:1
Mix 1
Mix 2
Subpool
a
b
c
d


Mix 1:2
4:1
1:2
1:1.5
1:1
Mix 1
Mix 2
Subpool
a
b
c
d
Mean
95% CI Lower Bound
95% CI Upper Bound
r_m
0.9539
0.9478
0.96
Log2 Ratio of Counts
0
−2
−10
−5
0
5
10
15
Log2 Average ERCC Spike Amount(attomol nt/μg total RNA)
Ratio
4:1
1:1
1:1.5
1:2



	Mean	95% CI Lower Bound	95% CI Upper Bound
r_m	0.9539	0.9478	0.96

Signal Ratio vs. Spike-in Abundance

Ratio
4:1
1:1
1:1.5
1:2

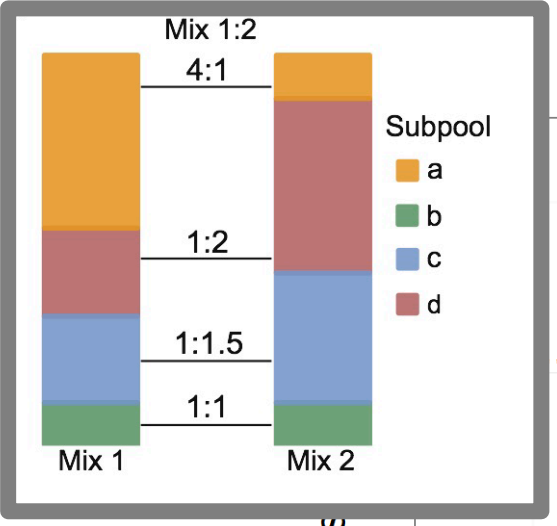
Log2 Ratio of Counts

0

−2

−10 −5 0 5 10 15

Log2 Average ERCC Spike Amount(attomol nt/μg total RNA)



	Mean	95% CI Lower Bound	95% CI Upper Bound
r_m	0.9539	0.9478	0.96

Bias in ratios
mRNA fraction?
mRNA enrichment?

Signal Ratio vs.
Spike-in Abundance

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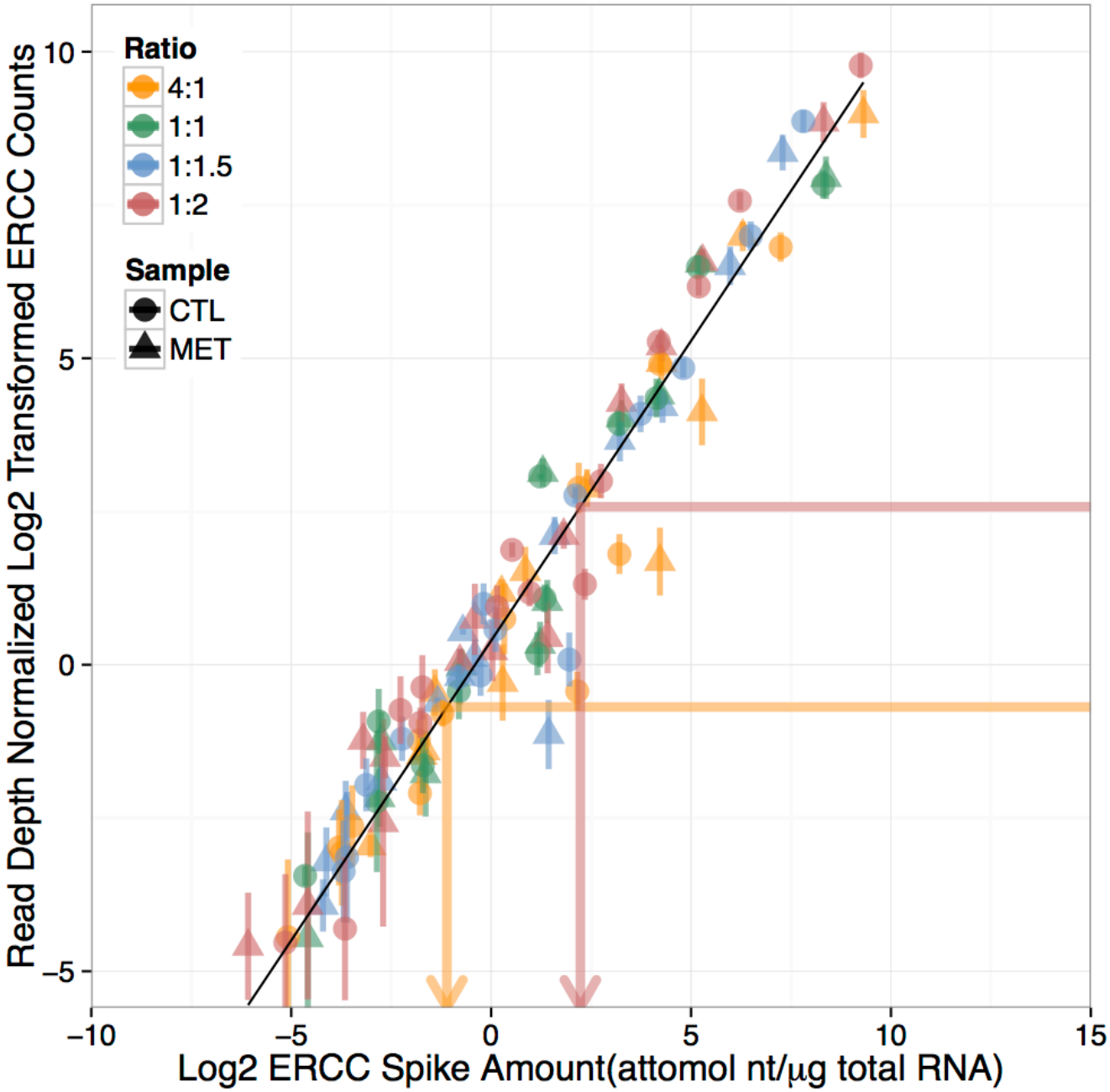
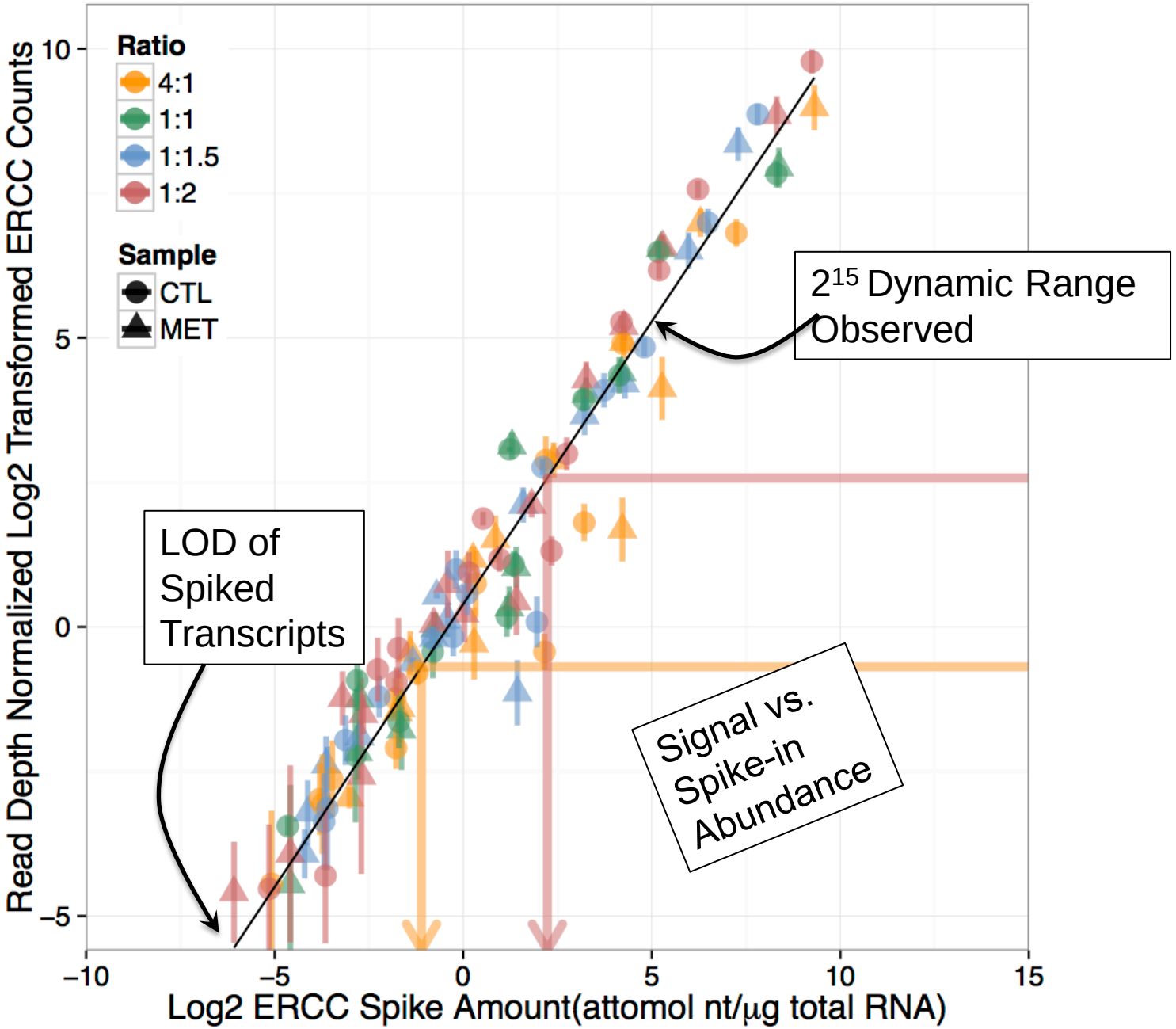

Ratio
4:1
1:1
1:1.5
1:2
Sample
CTL
MET
Read Depth Normalized Log2 Transformed ERCC Counts
Log2 ERCC Spike Amount(attomol nt/μg total RNA)
10
5
0
−5
−10
−5
0
5
10
15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Sample
CTL
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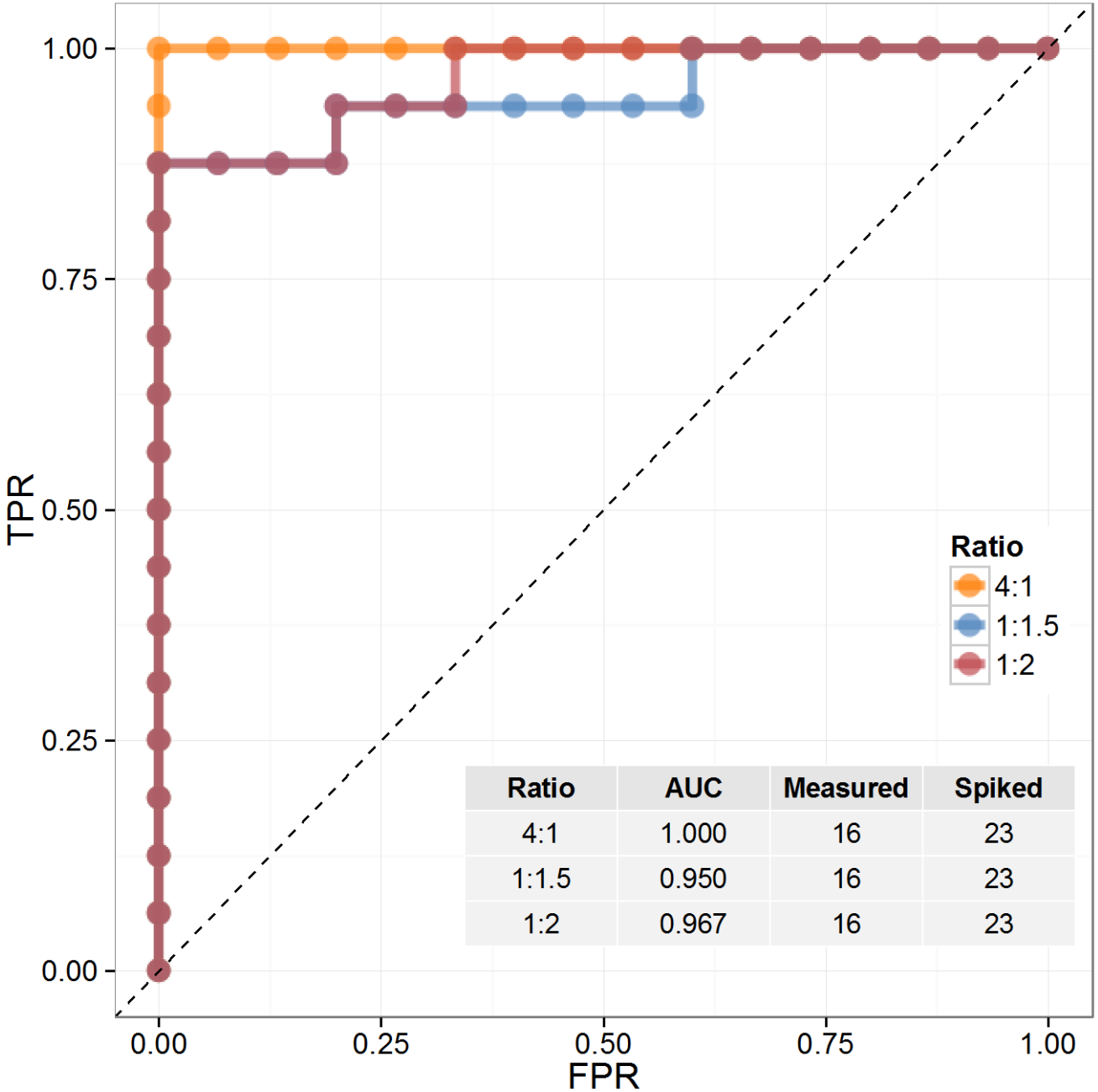
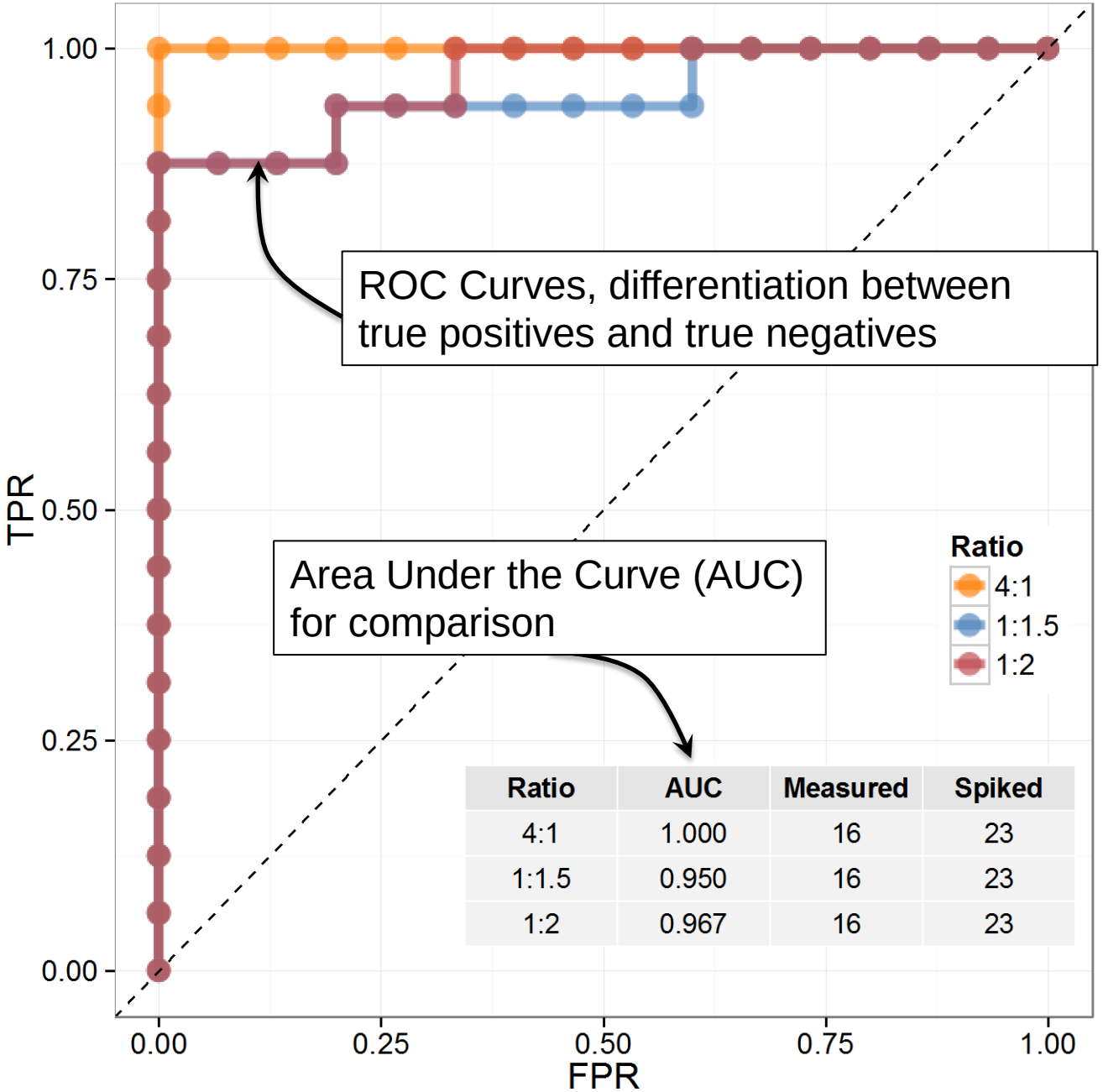
Read Depth Normalized Log2 Transformed ERCC Counts
Log2 ERCC Spike Amount(attomol nt/µg total RNA)
10
5
0
−5
−10
−5
0
5
10
15
2^{15} Dynamic Range Observed
LOD of Spiked Transcripts
Signal vs. Spike-in Abundance



Ratio	AUC	Measured	Spiked
4:1	1.000	16	23
1:1.5	0.950	16	23
1:2	0.967	16	23



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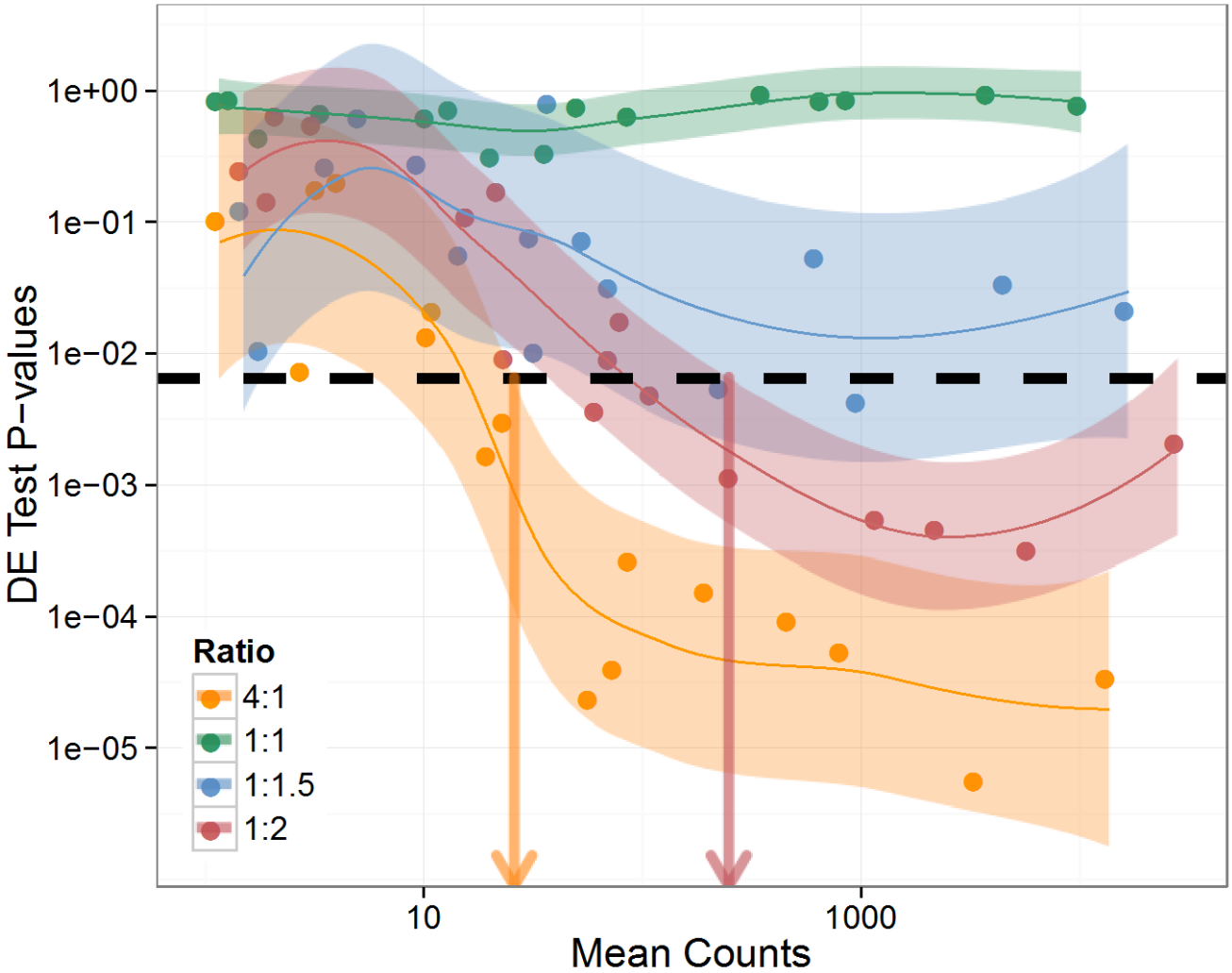
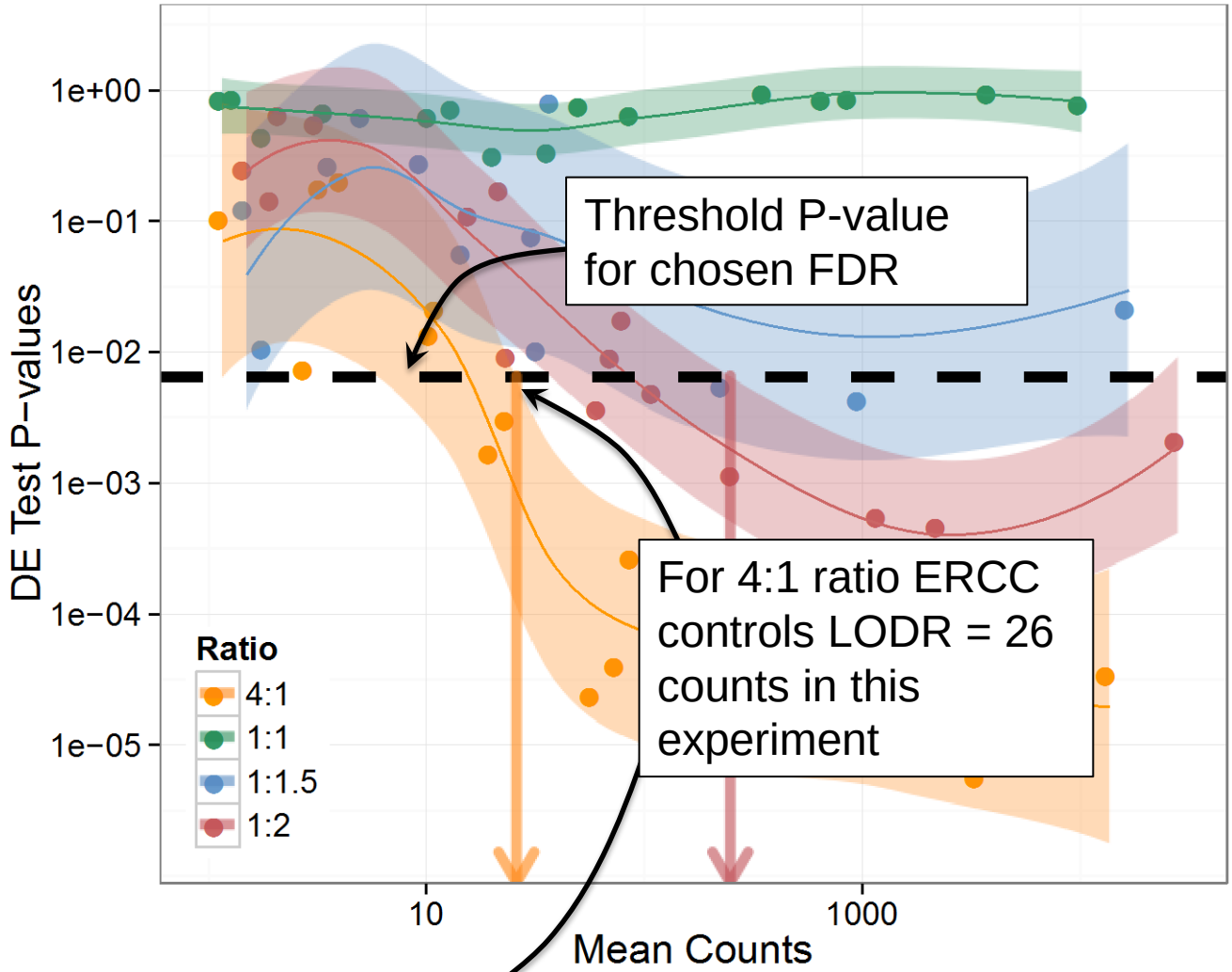
1:2	0.967	16	23



Ratio	LODR Estimate	90% CI Lower Bound	90% CI Upper Bound
4:1	26	19	32
1:1.5	Inf	NA	NA
1:2	250	120	350



Ratio	LODR Estimate	90% CI Lower Bound	90% CI Upper Bound
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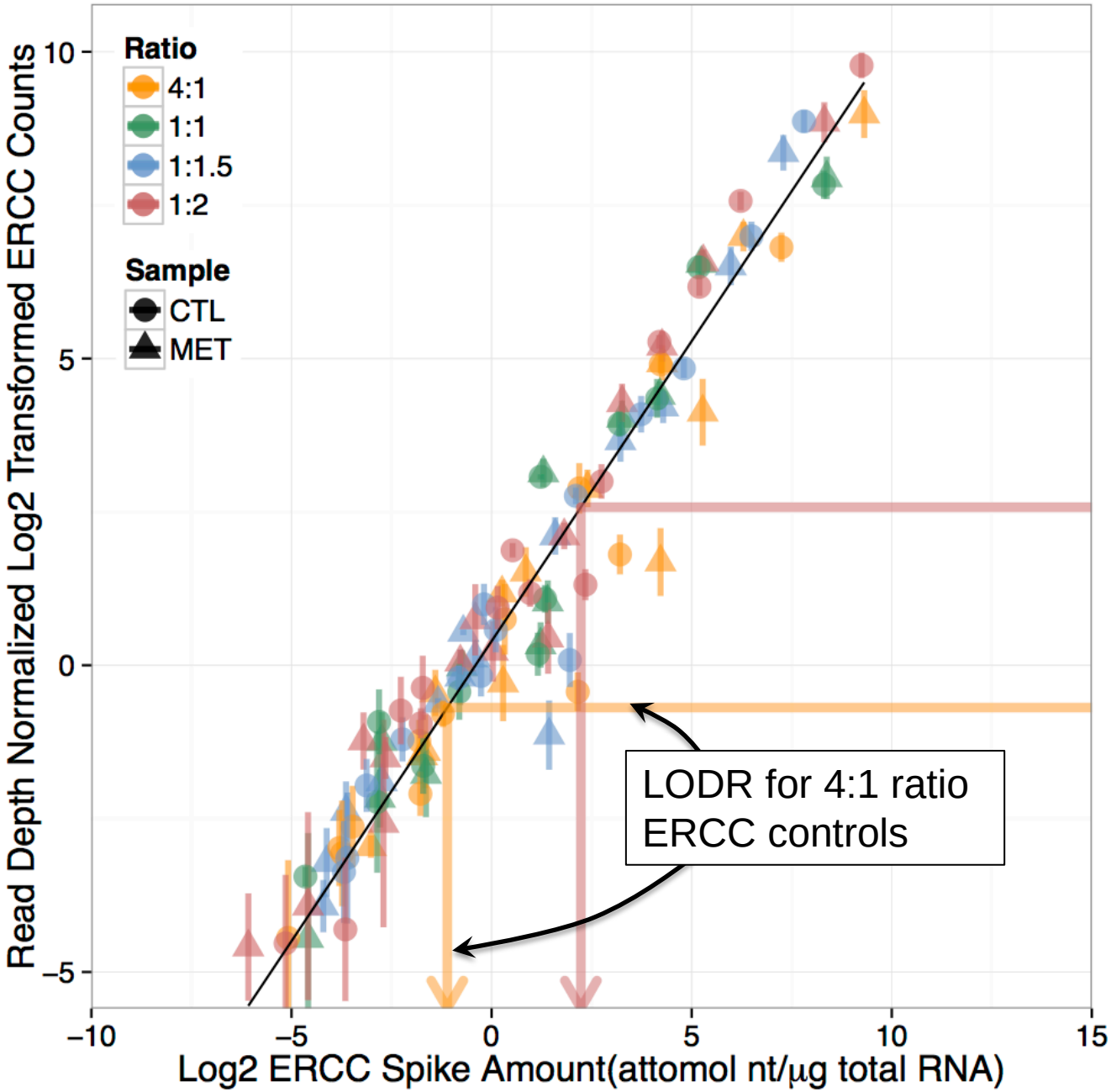
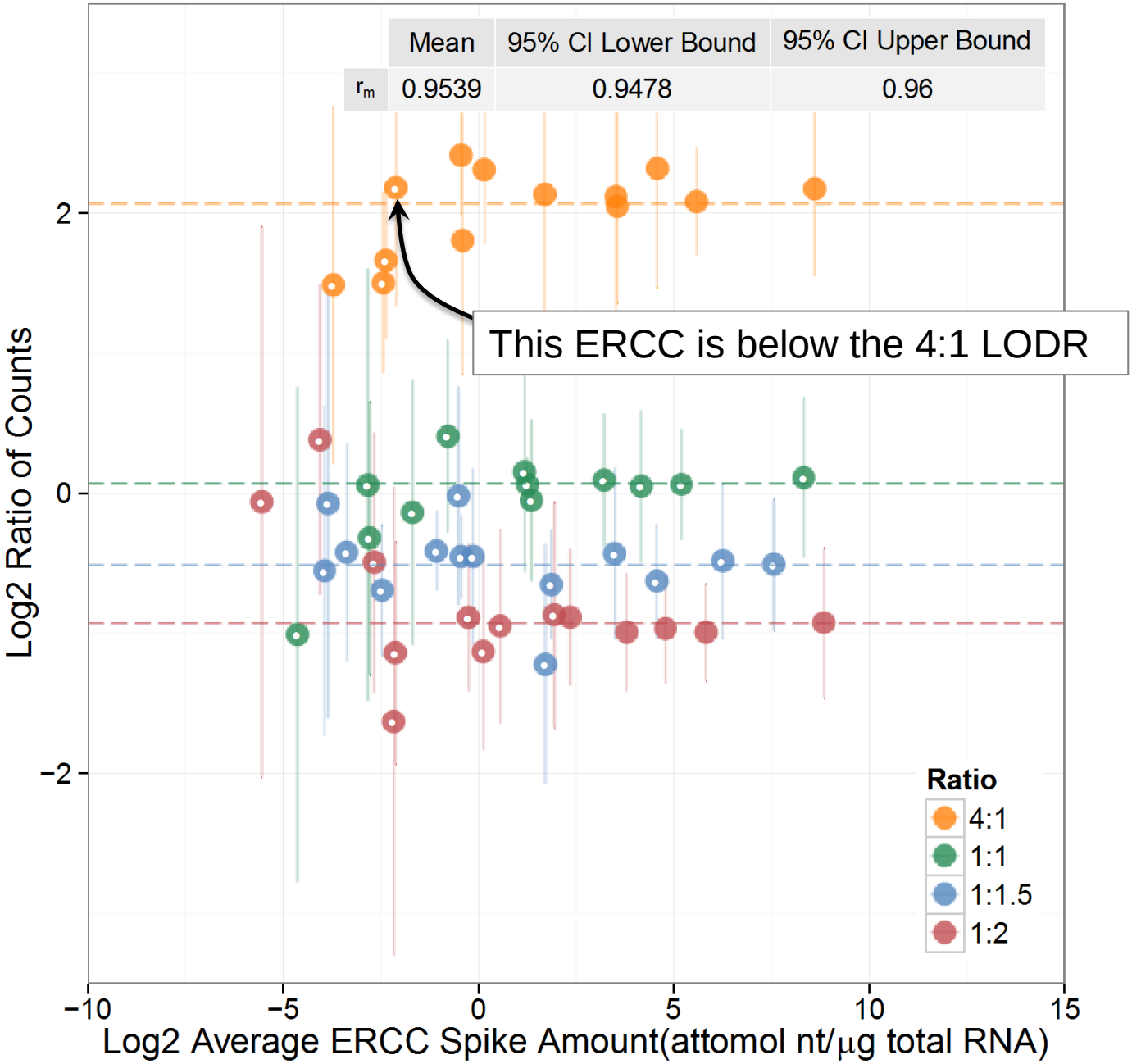
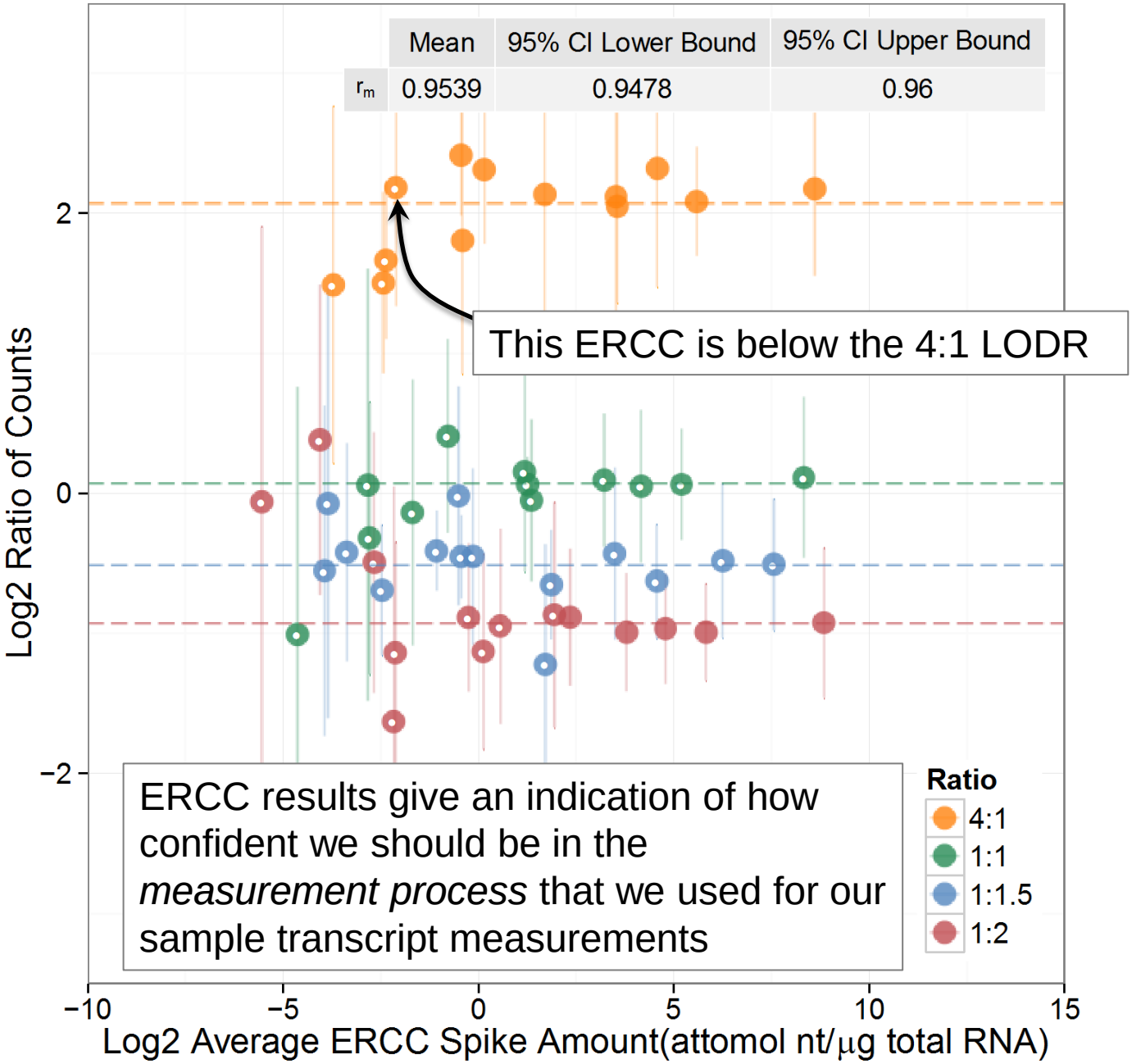

Ratio
4:1
1:1
1:1.5
1:2
Sample
CTL
MET
Read Depth Normalized Log2 Transformed ERCC Counts
10
5
0
−5
−10
−5
0
5
10
15
Log2 ERCC Spike Amount(attomol nt/μg total RNA)
LODR for 4:1 ratio
ERCC controls


Mean
95% CI Lower Bound
95% CI Upper Bound
r_m
0.9539
0.9478
0.96
This ERCC is below the 4:1 LODR
Log2 Ratio of Counts
Log2 Average ERCC Spike Amount(attomol nt/μg total RNA)
Ratio
4:1
1:1
1:1.5
1:2


Mean
95% CI Lower Bound
95% CI Upper Bound
r_m
0.9539
0.9478
0.96
This ERCC is below the 4:1 LODR
ERCC results give an indication of how confident we should be in the *measurement process* that we used for our sample transcript measurements
Ratio
4:1
1:1
1:1.5
1:2
Log2 Ratio of Counts
2
0
−2
−10
−5
0
5
10
15
Log2 Average ERCC Spike Amount(attomol nt/μg total RNA)

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  - Margaret Kline
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- FDA SEQC Study Participants

Postdoc Opportunities at NIST in Gaithersburg, MD and Stanford University, CA

- New joint research program *"Advances in Biomedical Measurement Science"* (ABMS) at Stanford

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