Effects of Biospecimen Integrity, Intratumoral Heterogeneity, and Analytical Variance on Microarray-Based Pharmacogenomics Tests of Breast Cancer

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NCI/BRN Research Contract

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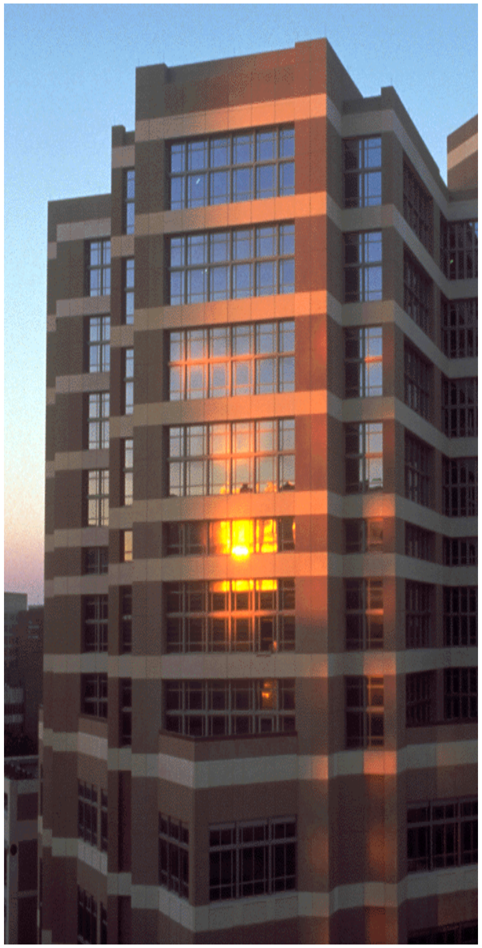
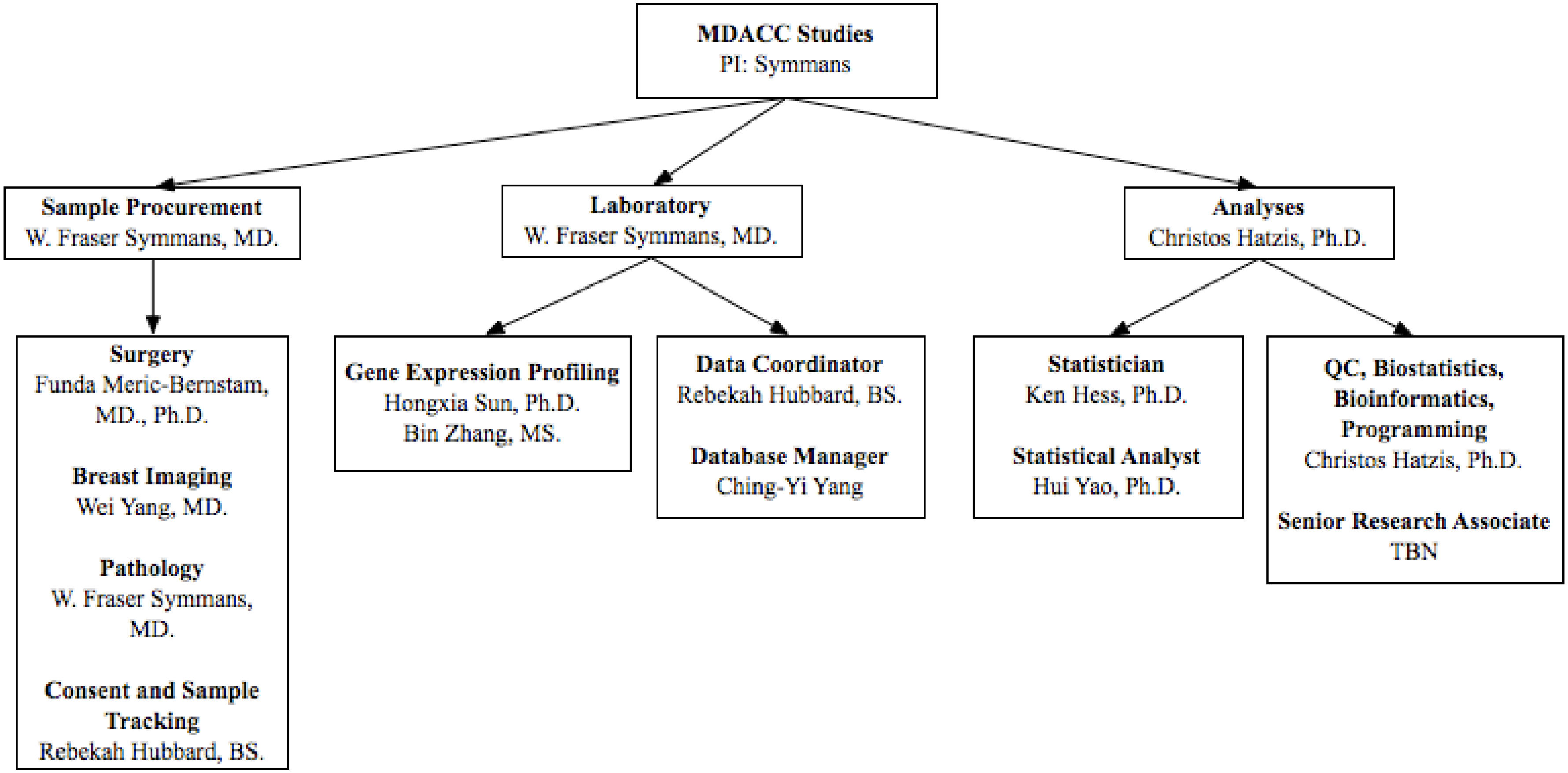
The overall objective is to establish an annotated, publicly available source of genomic data that investigators can use to assess specific contributions of pre-analytical and analytical factors to the measurement of the expression of any single gene, or any multi-gene signature, measured from a commercially available microarray platform when applied to any clinical breast cancer sample.



THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER

Organization of The Project Team



MDACC Studies
PI: Symmans

Sample Procurement
W. Fraser Symmans, MD.

Surgery
Funda Meric-Bernstam, MD., Ph.D.

Breast Imaging
Wei Yang, MD.

Pathology
W. Fraser Symmans, MD.

Consent and Sample Tracking
Rebekah Hubbard, BS.

Laboratory
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Gene Expression Profiling
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Bin Zhang, MS.

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Database Manager
Ching-Yi Yang

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Statistician
Ken Hess, Ph.D.

Statistical Analyst
Hui Yao, Ph.D.

QC, Biostatistics, Bioinformatics, Programming
Christos Hatzis, Ph.D.

Senior Research Associate
TBN

Evaluation of Pre-Analytical Factors

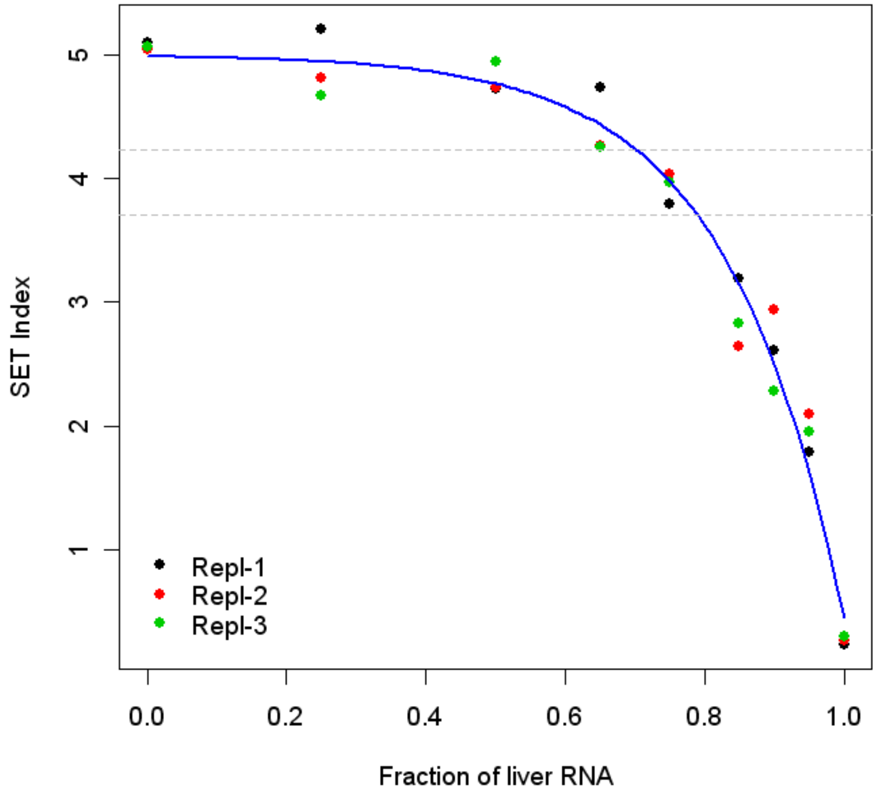
A. Integrity and Purity of Tumor Samples

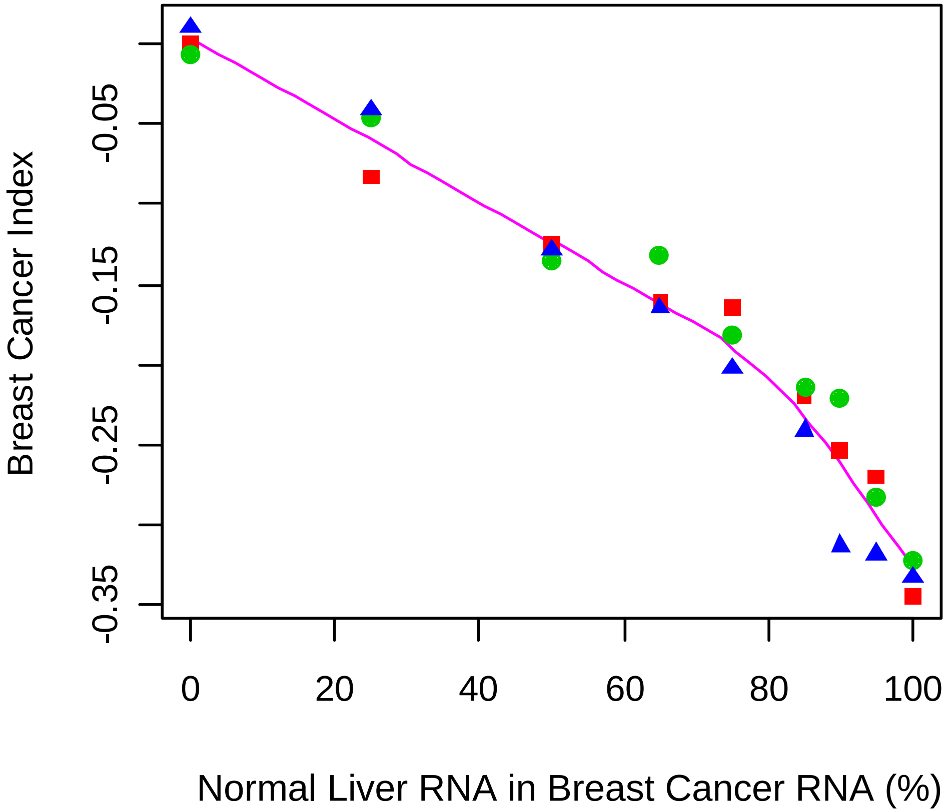
1. Controlled decay of RNA integrity to simulate effects of delayed *ex vivo* sample collection.

2. Dilution of breast cancer RNA with normal tissue RNA to simulate the effect of tumor RNA variation in clinical samples.

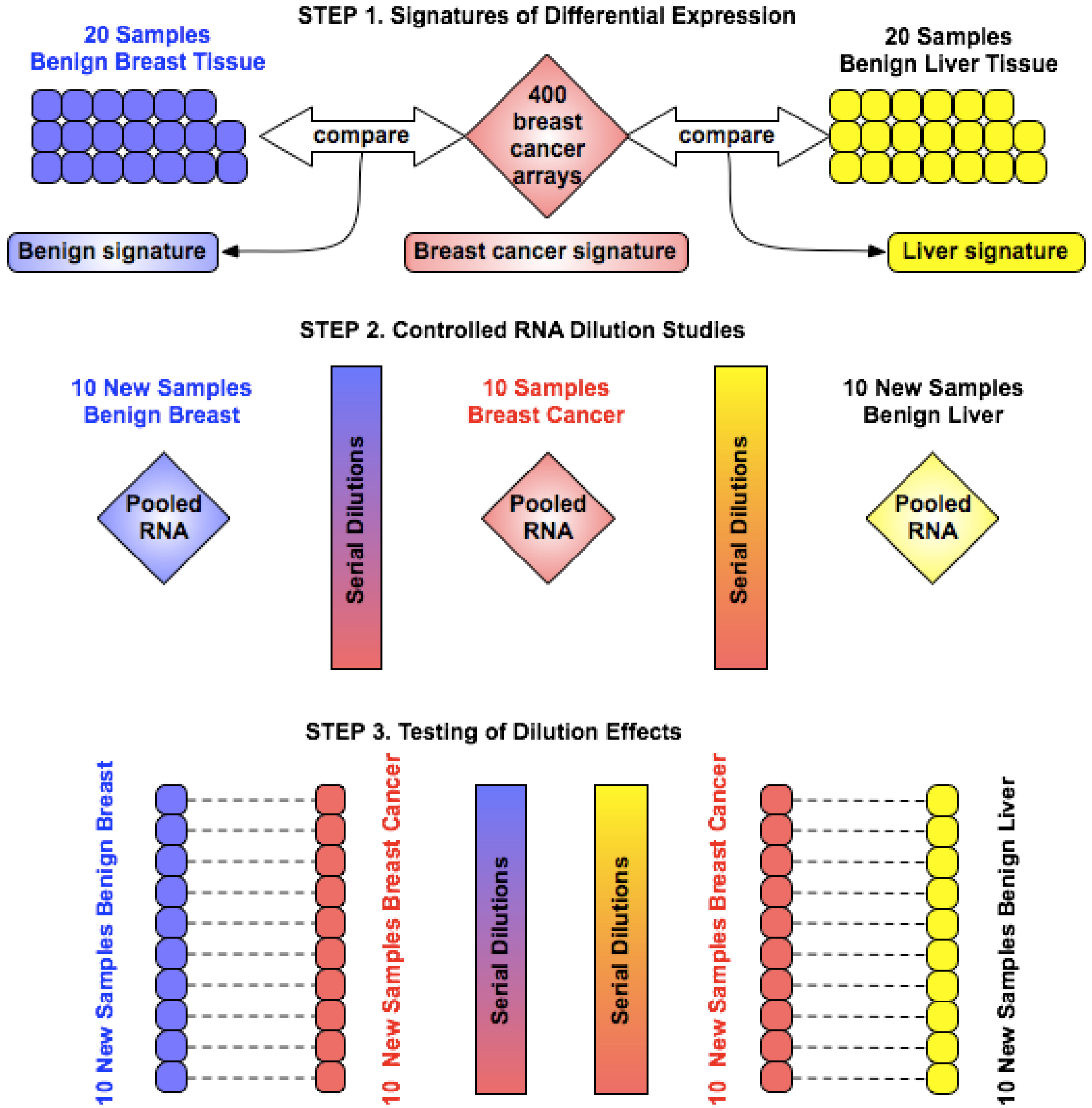
3. Dilution of tumor RNA with normal liver RNA to simulate the effect of host organ contamination in samples at metastatic site.

Preliminary Data: Effects of Liver RNA Dilution





Study Schema to Evaluate Effects of Dilution



STEP 1. Signatures of Differential Expression

20 Samples
Benign Breast Tissue

400 breast cancer arrays

20 Samples
Benign Liver Tissue

compare

compare

Benign signature

Breast cancer signature

Liver signature

STEP 2. Controlled RNA Dilution Studies

10 New Samples
Benign Breast

Pooled RNA

Serial Dilutions

10 Samples
Breast Cancer

Pooled RNA

Serial Dilutions

10 New Samples
Benign Liver

Pooled RNA

STEP 3. Testing of Dilution Effects

10 New Samples Benign Breast

10 New Samples Breast Cancer

Serial Dilutions

Serial Dilutions

10 New Samples Breast Cancer

10 New Samples Benign Liver

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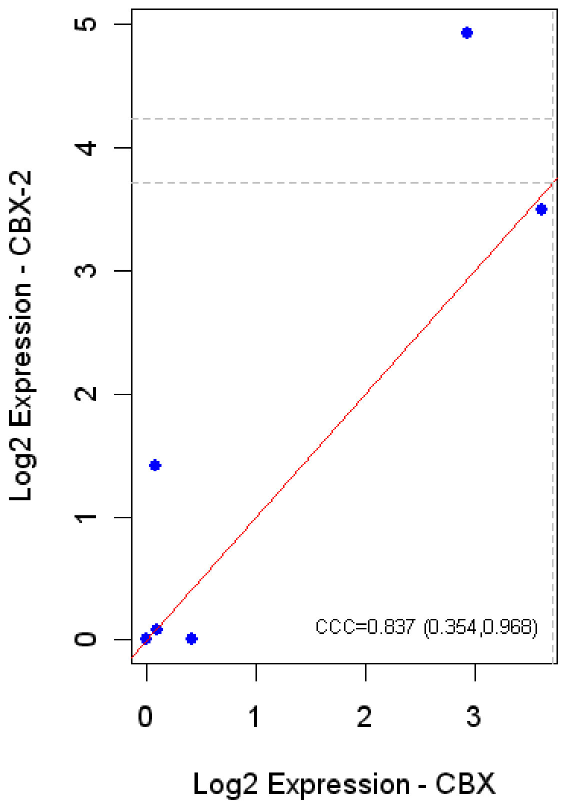
Evaluation of Pre-Analytical Factors

B. Intratumoral Heterogeneity and Sampling Practices

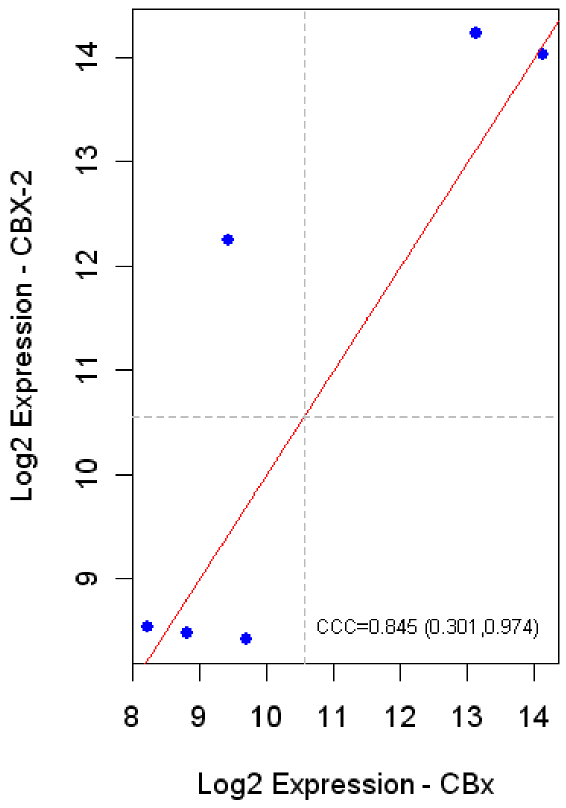
1. Obtain punch biopsy samples of freshly resected breast cancer tissue from 3 different sites within the tumor.
2. Determine whether pooling of more than one samples could help reduce intratumoral variance in genomic measurements.

Concordance in Paired Core Biopsies (N=6)

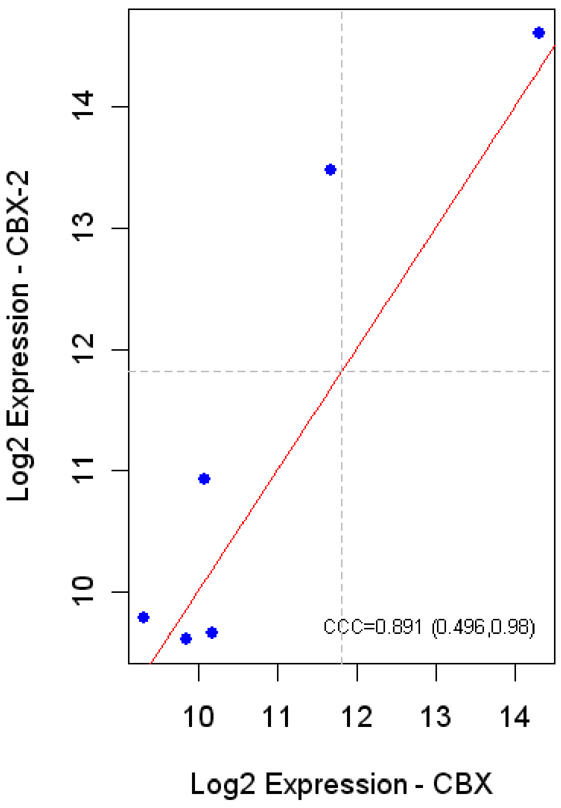
Concordance of SET Index



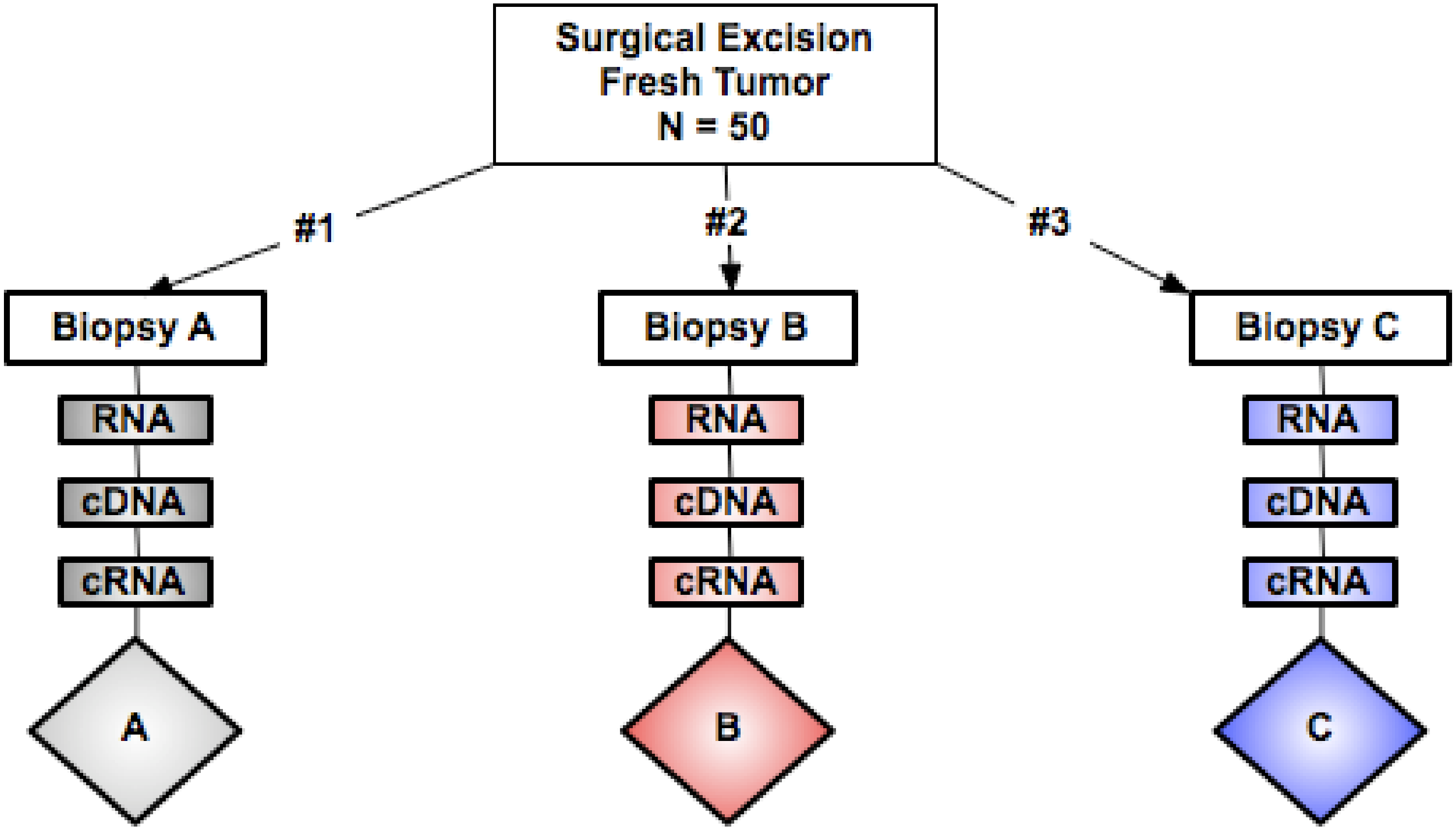
Concordance of ESR1



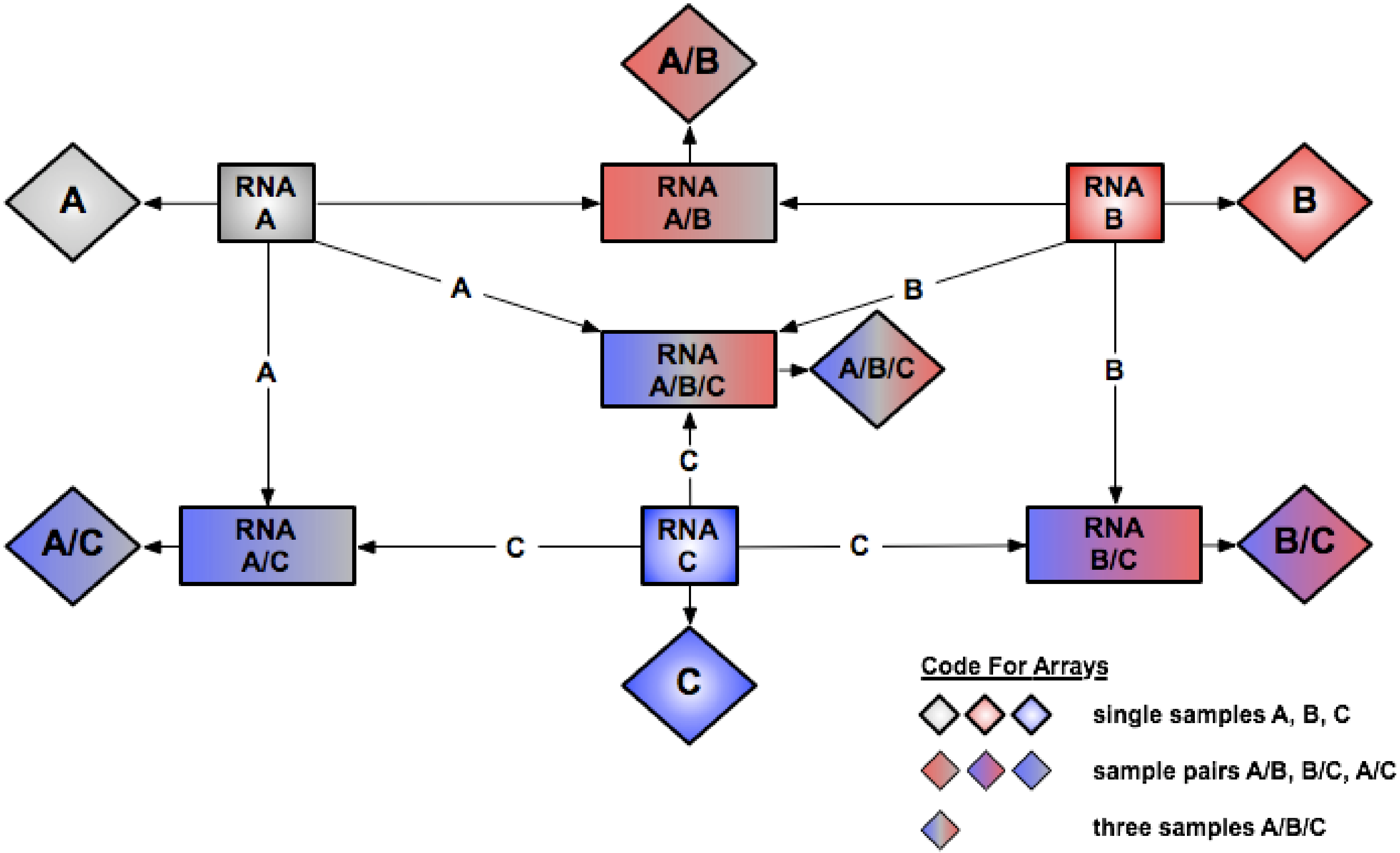
Concordance of ERBB2



Study Schema for Intratumoral Heterogeneity



Study Schema for Effect of Pooling



Evaluation of Pre-Analytical Factors

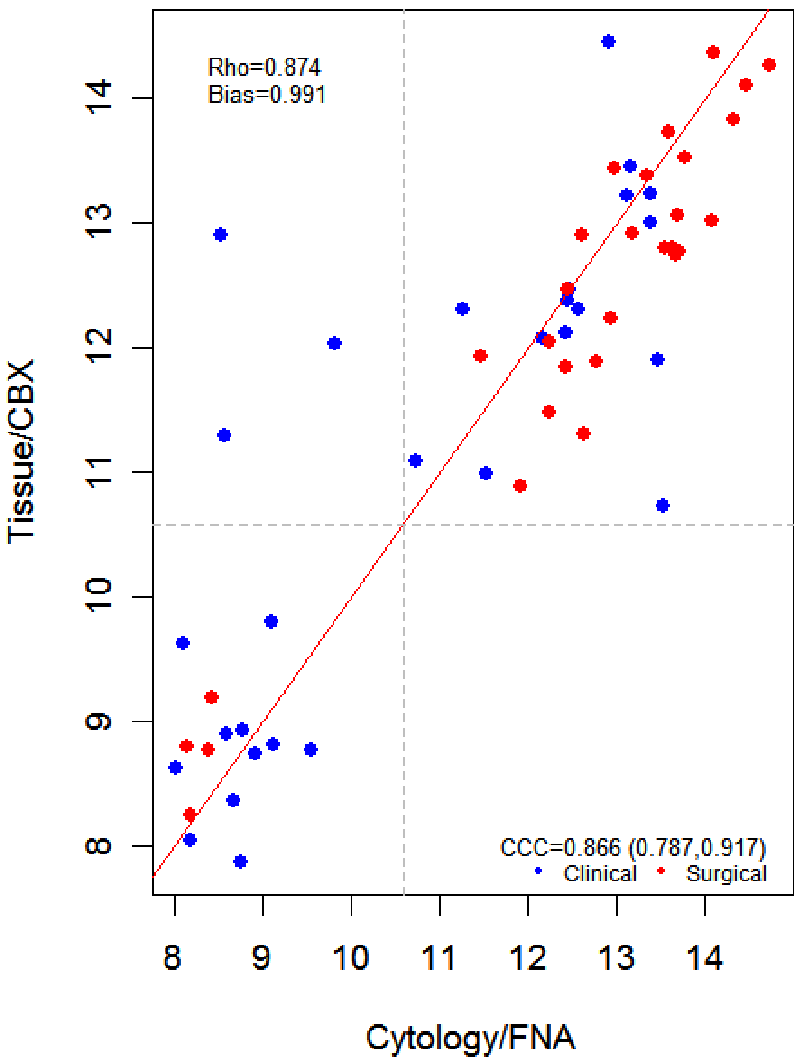
C. Similarity of Clinical Sample Types

1. Compare genomic test results obtained from matched pairs of cytologic tumor surface scrape preparation and tumor tissue (in collaboration with FDA's MAQC-II Consortium).

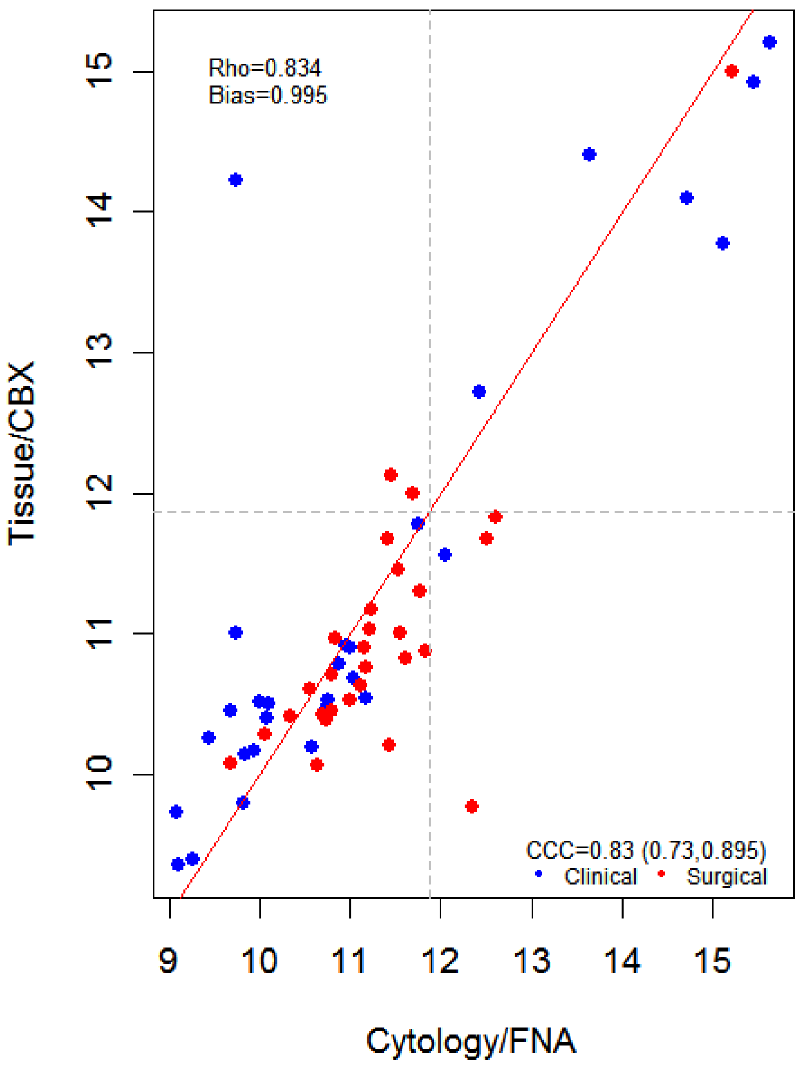
2. Use a 2x2 factorial design to compare data from cytologic (FNA) and tissue (core biopsy) samples obtained from ultrasound-guided needle biopsies and from tumor tissue obtained at the time of subsequent surgical resection (without intervening treatment).

Preliminary Results: Effect of Sample Type

ESR1

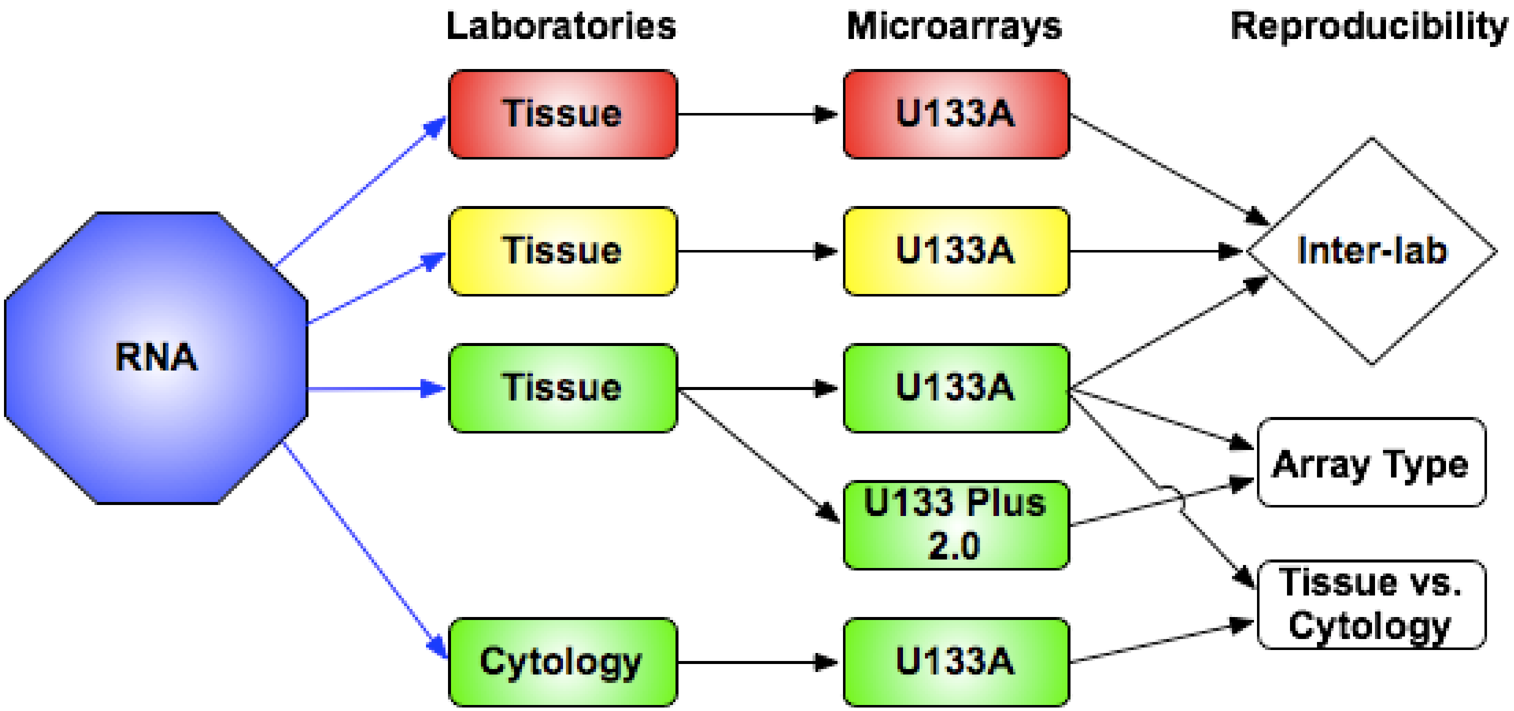


ERBB2



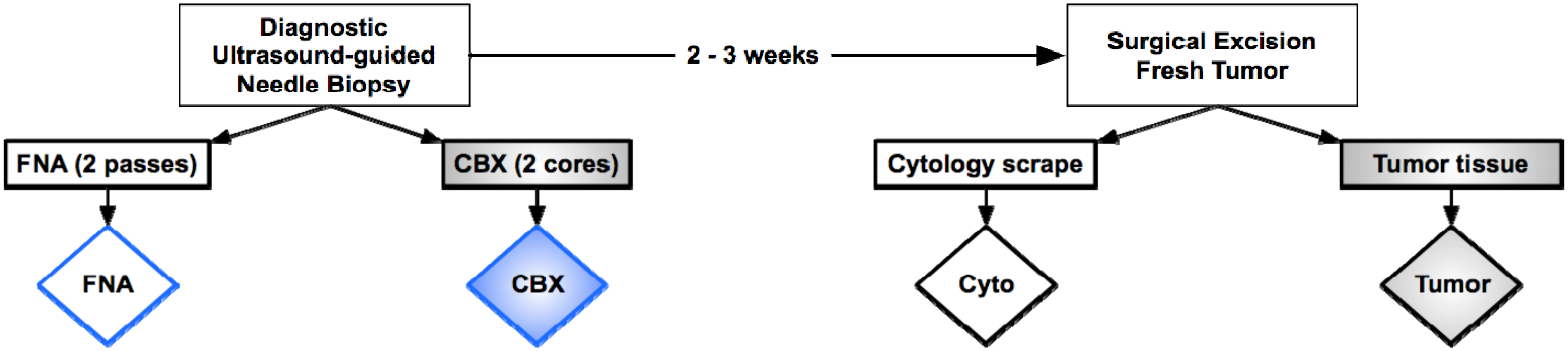
Study Schema for Evaluating Reproducibility Between Laboratories and Sample Types (MAQC-II)

N = 125 patients



Study Schema to Evaluate Variance from Type and Timing of Clinical Samples

N = 20 patients

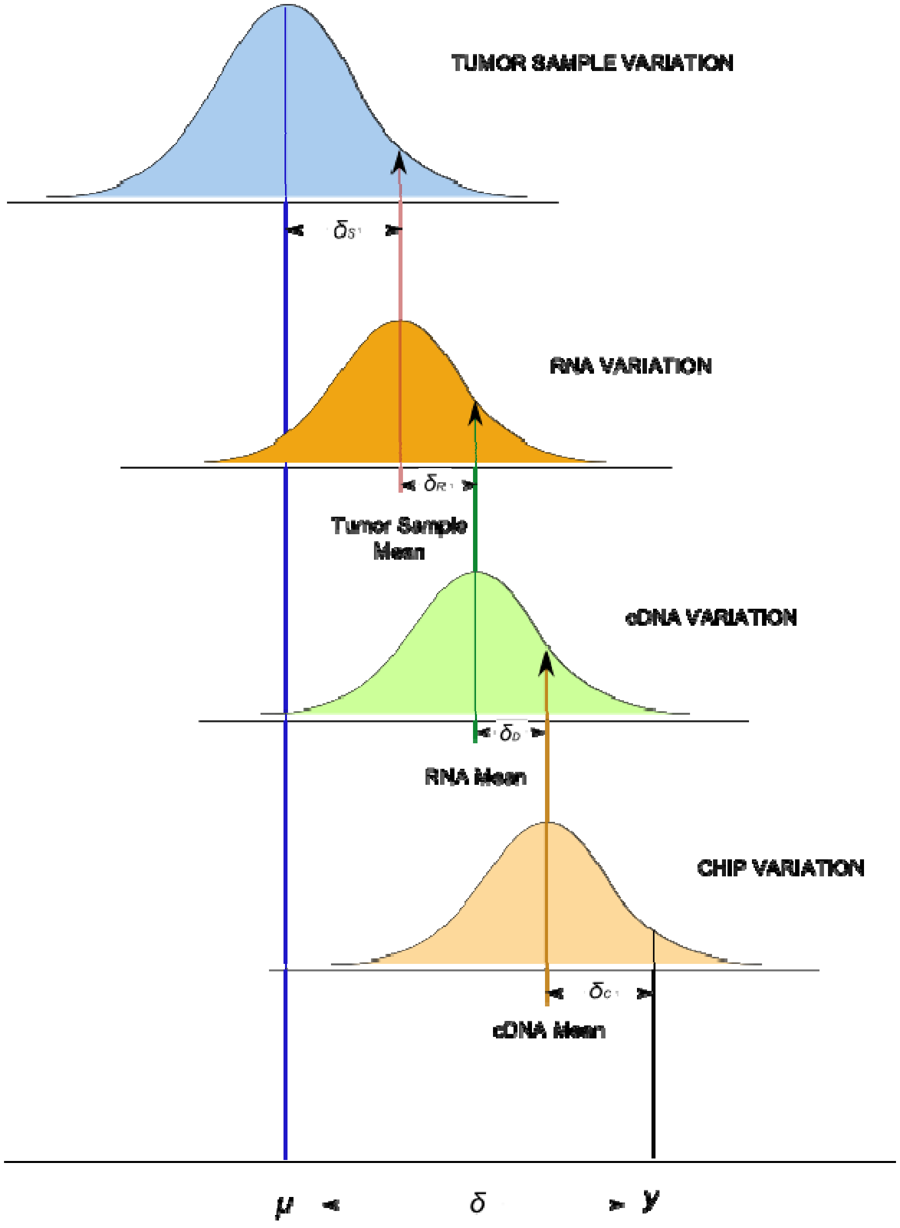


Evaluation of Analytical Variability

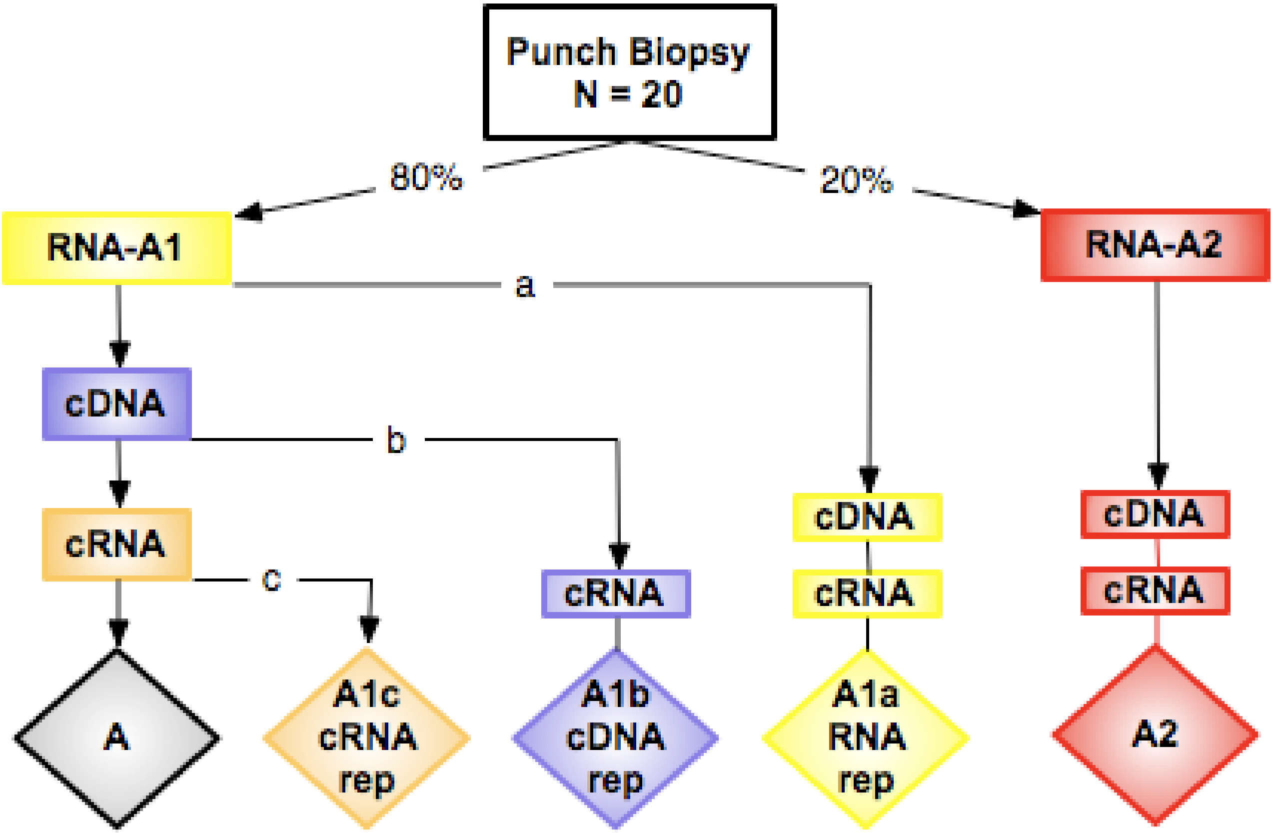
D. Contribution of Analytical Factors to Microarray Test Measurement Variance

1. Employ a nested study design of samples from 20 breast cancers to measure the variability of test results at each stage of the gene expression microarray assay. Perform replicate microarray experiments starting at the stages of RNA extraction, reverse transcription, formation of second DNA strand and in vitro transcription, and hybridization to a microarray, and scanning of the microarray

Sources of Variance in Genomic Measurements



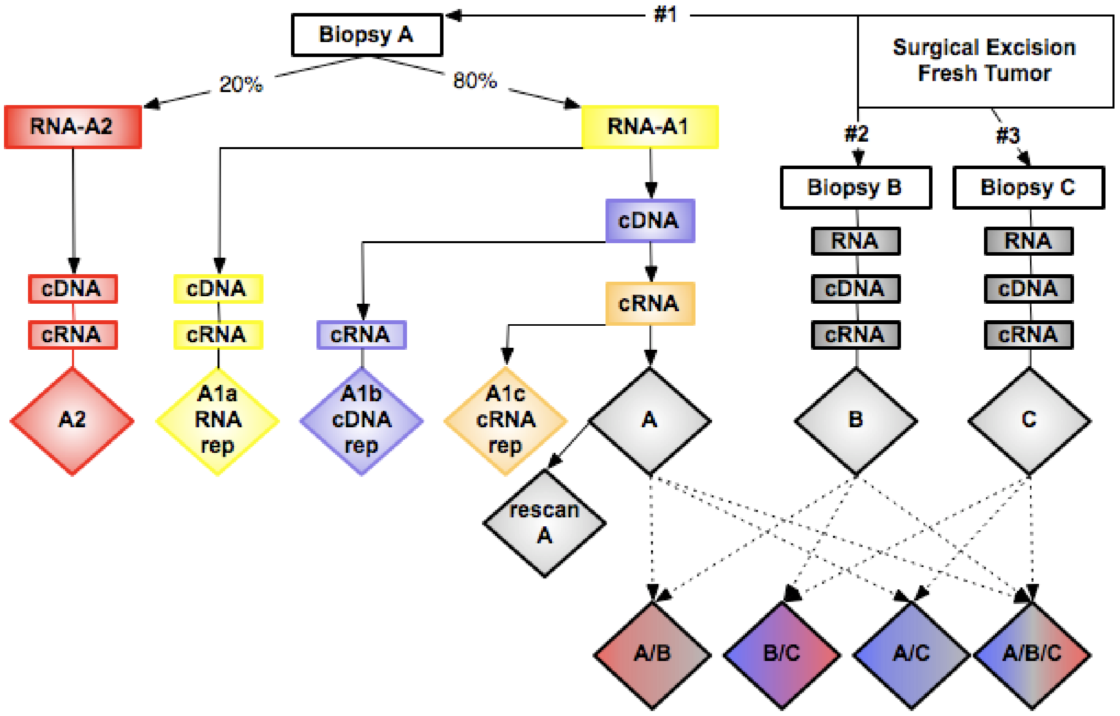
Study Schema for Analytical Variability


Punch Biopsy
N = 20
80%
20%
RNA-A1
RNA-A2
a
cDNA
b
cRNA
c
cDNA
cRNA
cDNA
cRNA
cRNA
A
A1c
cRNA
rep
A1b
cDNA
rep
A1a
RNA
rep
A2

Data Generation and Deliverables

- Sample collection table
  - Date and time of removal, put in RNAlater, put in freezer
- Clinical case report table
  - Patient and clinicopathological tumor characteristics
- Sample processing table
  - Date and time sample was removed from freezer, date and time for all subsequent processing steps
  - RNA quality metrics and microarray QC
- Experimental series table
  - Link each sample ID to experimental design condition
- Microarray data
  - CEL files
  - Normalized datasets (MAS5, invariantly scaled)

Estimation of Genomic Assay Variance Components



Surgical Excision Fresh Tumor

#1

Biopsy A

20%

80%

RNA-A2

RNA-A1

cDNA

cRNA

cDNA

cRNA

cRNA

cDNA

cRNA

A2

A1a RNA rep

A1b cDNA rep

A1c cRNA rep

A

rescan A

#2

#3

Biopsy B

RNA

cDNA

cRNA

B

Biopsy C

RNA

cDNA

cRNA

C

A/B

B/C

A/C

A/B/C

Code For Arrays

◇◇◇◇◇ analytical variability (section B.2.1, N = 20)

◇◇◇ intratumoral variability (section B.3.1, N = 50)

◆◆◆◆ pooled biopsy samples (section B.3.2, N = 50)

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