A vertical strip on the left side of the slide features a blurred, light blue double helix structure, likely representing DNA or RNA, against a white-to-blue gradient background.

Sensation

RNA Amplification Kit

Genisphere[®]

SIGNAL + SAMPLE AMPLIFICATION PRODUCTS

Sensation: Procedure Overview

First Strand cDNA Synthesis

1 hour, then 1 hour for purification

Total RNA is reverse transcribed using random 9mer primer and dT12V primer, dNTP mix, and Reverse Transcriptase. The cDNA is purified with RNAClean® XP.

Promoter Synthesis

1 hour

The cDNA is poly d(A) tailed on the 3' end using dATP and Terminal Deoxynucleotidyl Transferase. A T7 dT oligo with a 3' blocking group is hybridized to the 3' dA tail of the cDNA. Klenow and dNTP mix generate a double stranded T7 promoter region on the single stranded cDNA.

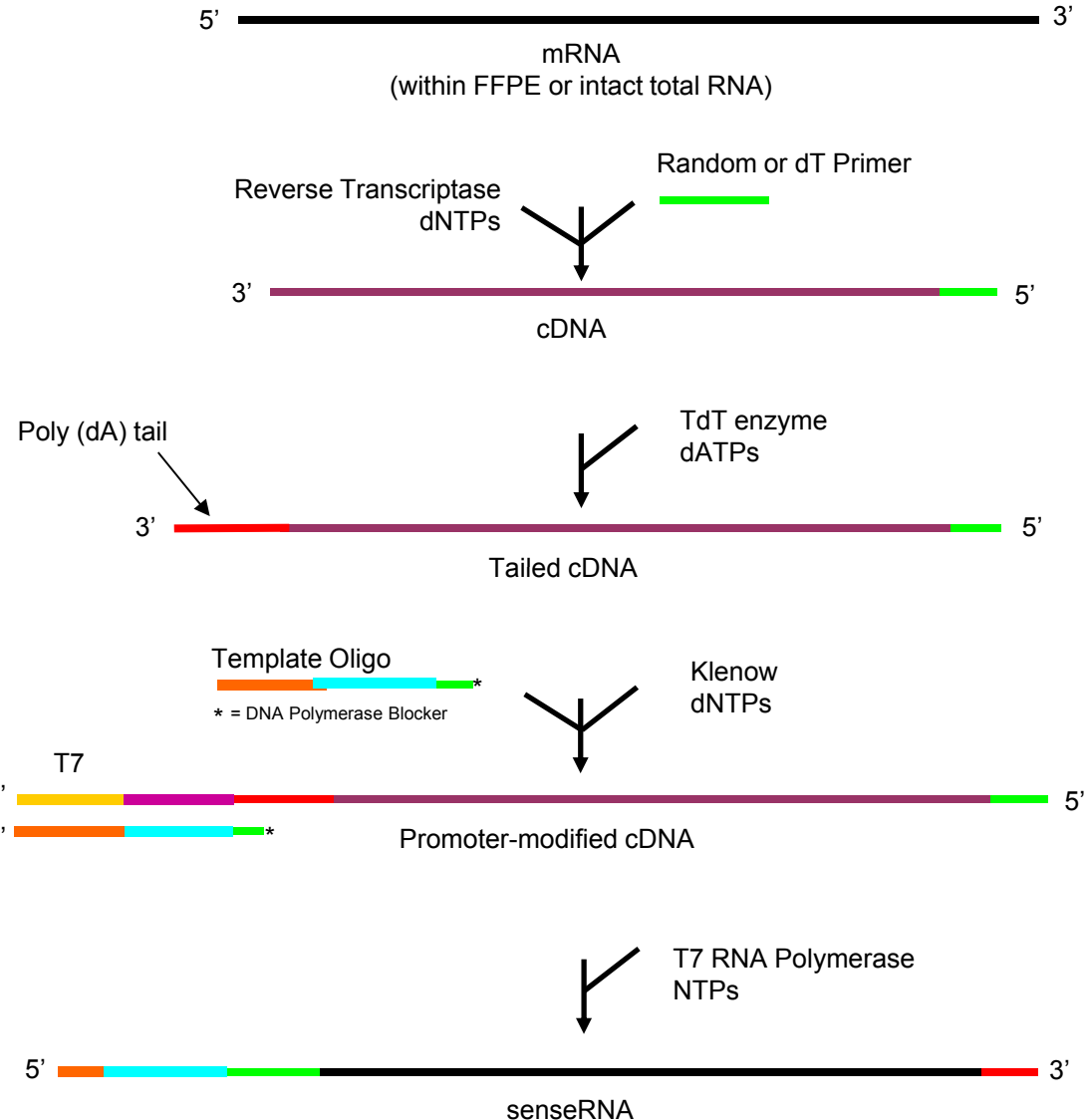
In Vitro Transcription

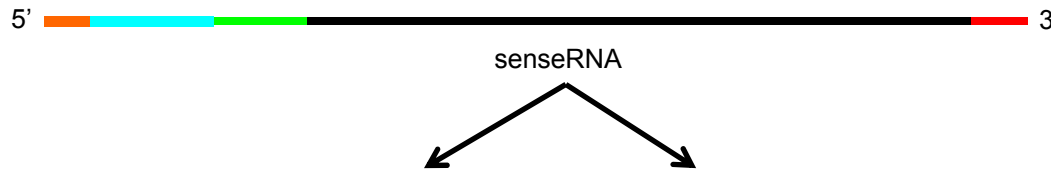
Overnight (16-18 hours), then 1 hour for purification

The promoter-modified cDNA is in vitro transcribed using T7 RNA Polymerase and NTP Mix. The senseRNA is purified with RNAClean XP.

Analysis of senseRNA

The senseRNA is ready for gene expression applications such as qRT-PCR or biotin-labeling for Affymetrix® Arrays (see next page).



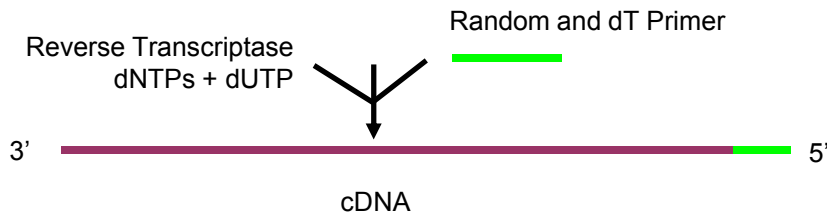


Biotin Labeling Kit for 3' Arrays

cDNA Synthesis

2.5 hours, then 1 hour for purification

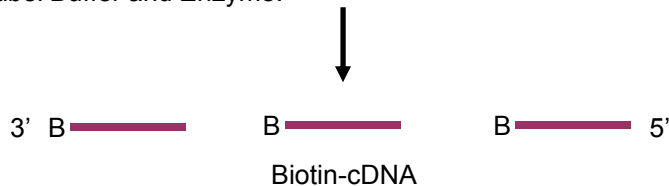
SenseRNA is reverse transcribed using random and dT primer, dNTP mix + biotin-dUTP, and Reverse Transcriptase. The cDNA is purified with RNAClean XP.



Terminal Labeling of cDNA

1 hour

cDNA is fragmented and end-labeled with 1-Step Fragment and Label Buffer and Enzyme.



Analysis of Biotin-cDNA

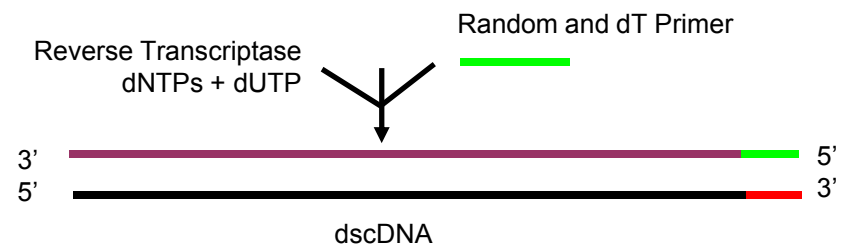
The biotin-cDNA is ready for hybridization to Affymetrix 3' IVT Expression Arrays.

Biotin Labeling Kit for ST/Exon Arrays

Double Stranded cDNA Synthesis

3.5 hours, then 1 hour for purification

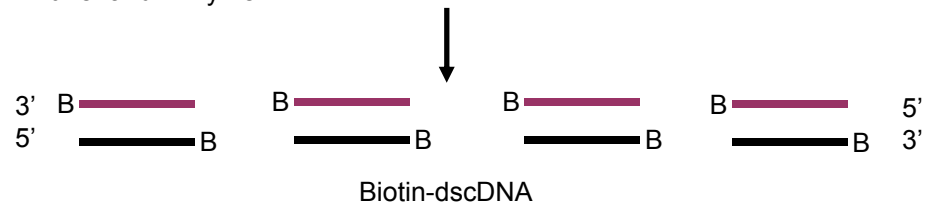
SenseRNA is reverse transcribed using random and dT primer, dNTP mix + dUTP, and Reverse Transcriptase. Second Strand cDNA is made with DNA Polymerase I and RNase H. The ds-cDNA is purified with RNAClean XP.



Terminal Labeling of double stranded cDNA

1 hour

dscDNA is fragmented and end-labeled with 1-Step Fragment and Label Buffer and Enzyme.



Analysis of Biotin-dscDNA

The biotin-dscDNA is ready for hybridization to Affymetrix Whole-Transcript Expression Arrays.

- Random and dT primers used in first step, reverse transcription (RT)
 - Whole Transcriptome amplification
 - Same procedure whether RNA is intact or FFPE
- No 5' extension on primers
 - No bias during RT; true random primers
 - No Template (no RNA) produces minimal amplification products
- RT from 3' end then amplify from 5' end
 - No bias to either 3' or 5' end of mRNAs
 - Accurate sense-strand amplification products
- Labeling options for 3' arrays and ST/exon arrays

Pillai R, et al. Validation and Reproducibility of a Microarray-Based Gene Expression Test for Tumor Identification in Formalin-Fixed, Paraffin-Embedded Specimens. *Journal of Molecular Diagnostics* January 2011, Vol. 13, No. 1.

Roberts L, et al. Identification of methods for use of formalin-fixed, paraffin-embedded tissue samples in RNA expression profiling. *Genomics* 2009, 94(5):341-8.

Koh SS, et al. Molecular classification of melanomas and nevi using gene expression microarray signatures and formalin-fixed and paraffin-embedded tissue. *Mod Pathol.* 2009 Apr;22(4):538-46.

Goff LA, et al. Evaluation of Sense-Strand mRNA Amplification by Comparative Quantitative PCR. *BMC Genomics*, 5:76, 2004.

1. Acquire Matched FFPE and Fresh Frozen Samples from **Colon**
2. Purify FFPE samples using **Ambion RecoverAll™ kit** and FF samples using **Qiagen® miRNeasy Mini kit**
3. Follow target preparation protocols from Genisphere
100ng input FFPE total RNA
50ng input FF total RNA
4. Hybridize biotin-labeled targets to Affymetrix **U133plus2.0** and **GeneST** Arrays

Colon Samples from 2005

FF	3 Reps	RIN = 8.5
FFPE	3 Reps	RIN = 2.2

sensation™



U133plus2.0 Array Metrics

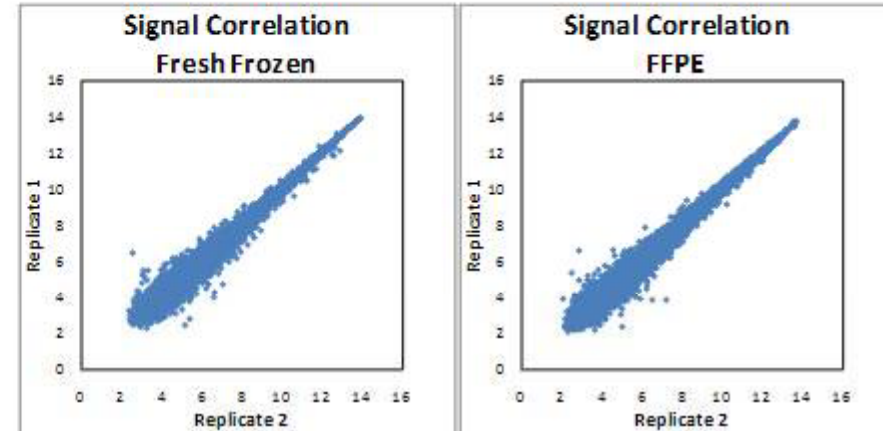
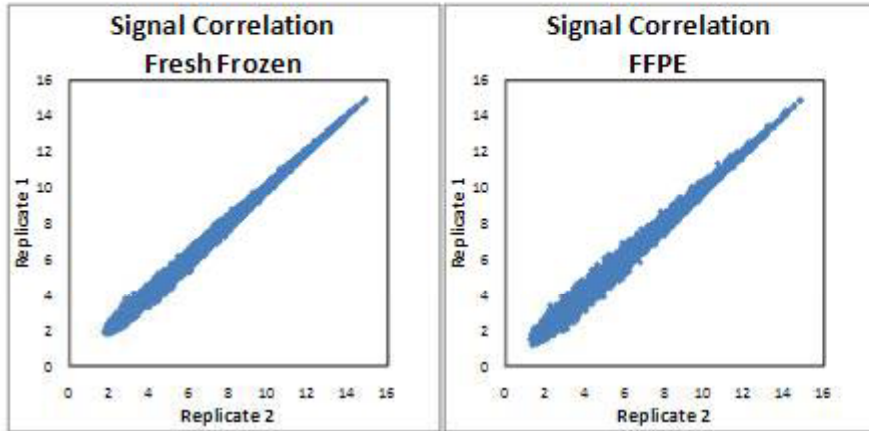
Sample	SF	%P	Actin 3'/5'	GAPDH 3'/5'
FFPE Colon -1	5.9	53.4	1.2	1.6
FFPE Colon-2	6.7	52.0	1.2	1.5
FFPE Colon -3	8.3	52.2	1.2	1.5
Frozen Colon-1	4.2	64.1	1.2	1.1
Frozen Colon-2	4.3	63.9	1.2	1.0
Frozen Colon-3	4.4	64.0	1.2	1.0

HG U133 Plus 2.0 Arrays

HG 1.0 ST Arrays

	Fresh Frozen	FFPE
Median CV, Gene Level	4.71%	7.30%
Probesets Used	38202	38202

	Fresh Frozen	FFPE
Median CV, Gene Level	5.46%	6.61%
Probesets Used	33297	33297



Pearson = 0.997

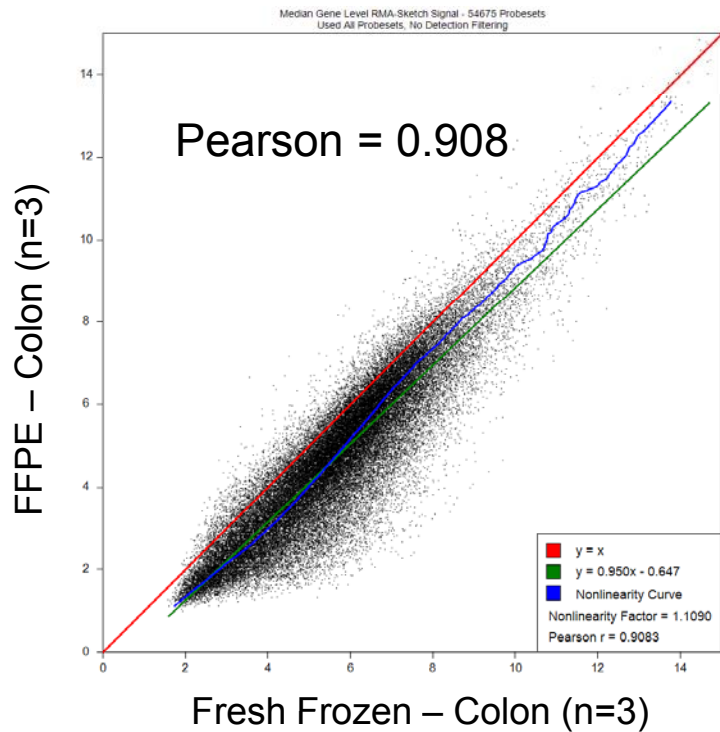
Pearson = 0.995

Pearson = 0.994

Pearson = 0.992

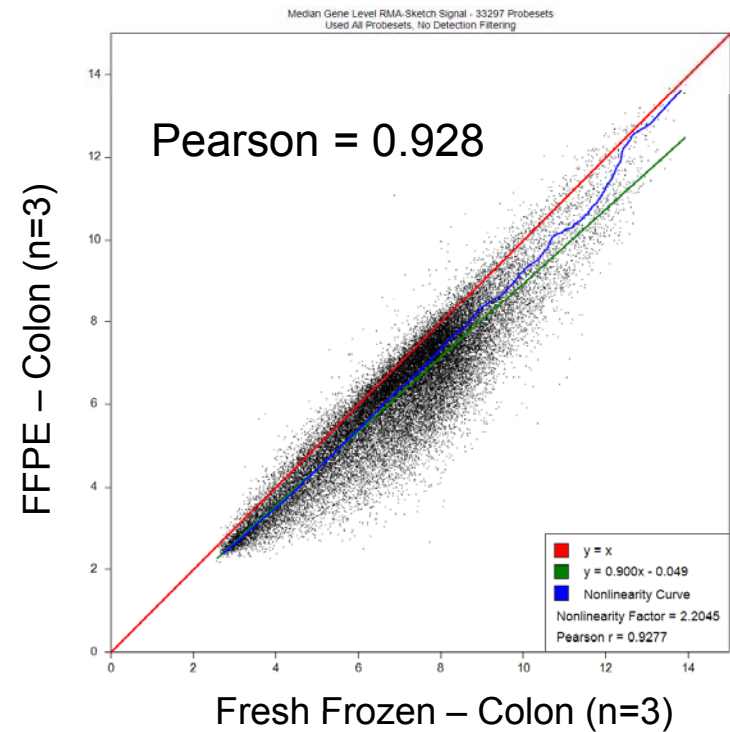
HG U133 Plus 2.0 Arrays

Signal Correlation - Fresh Frozen vs. FFPE



HG 1.0 ST Arrays

Signal Correlation - Fresh Frozen vs. FFPE



1. Acquire FFPE Samples from a **variety of tissues**
2. Purify FFPE samples using **Agencourt® FormaPure kit**
3. Follow target preparation protocols from Genisphere:
30ng input FFPE total RNA
4. Hybridize biotin-labeled targets to Affymetrix **U133A 2.0** Arrays

sensation™



FFPE Sample	SF	BG Avg	%P	Signal(P)	Actin 3'/5'	GAPDH 3'/5'
Pancreas -1	4.2	34.8	62.1	1130.7	1.30	2.01
Pancreas -2	3.5	34.4	67.3	1025.6	1.31	2.08
Lymph	3.8	35.0	58.5	1185.3	1.31	1.65
Bladder	2.8	40.1	63.3	1117.6	1.26	1.66
Liver	9.3	33.7	48.3	1513.4	1.76	1.85
Lung -1	5.6	34.6	53.4	1374.3	1.46	1.68
Lung -2	1.8	38.4	68.5	970.7	1.21	1.53
Kidney -1	2.4	36.7	65.5	1081.4	1.20	1.52
Kidney -2	5.1	34.1	49.3	1432.6	1.24	1.52
Ovary -1	8.2	33.9	48.3	1497.2	1.33	1.77
Ovary -2	2.6	37.9	68.0	989.8	1.38	1.74

1. Acquire Matched FFPE and Fresh Frozen Samples from **Breast**
2. Purify FFPE samples using **Ambion RecoverAll™ kit** and FF samples using **Qiagen® miRNeasy Mini kit**
3. Follow target preparation protocols from Genisphere
10ng and 20ng input FFPE total RNA
10ng and 20ng input FF total RNA
4. Hybridize biotin-labeled targets to Affymetrix **GeneST** Arrays

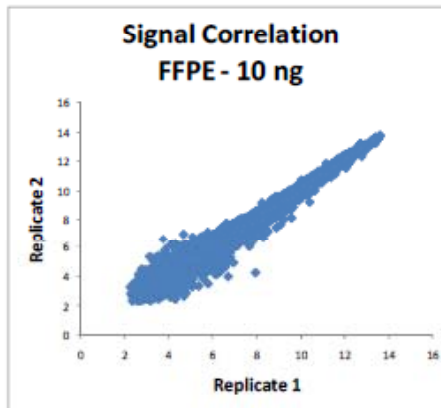
The logo for 'sensation™' features the word 'sensation' in a lowercase, sans-serif font. Above the 'a' and 'i' are three blue circles of varying sizes, and a blue dot is positioned above the 's'. A trademark symbol (TM) is located at the top right of the word.

HG 1.0 ST Arrays – 10 ng

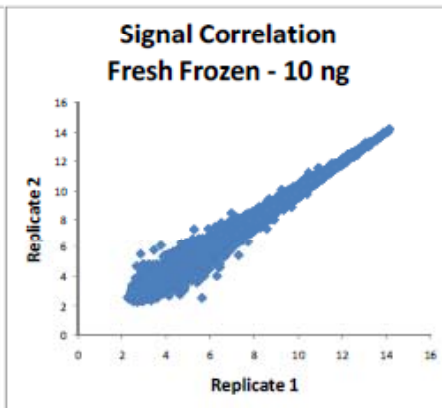
	Fresh Frozen	FFPE
Median CV, Gene Level	6.67%	7.09%
Probesets Used	33297	33297

HG 1.0 ST Arrays – 20 ng

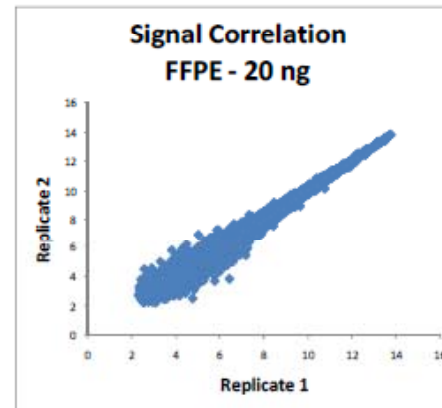
	Fresh Frozen	FFPE
Median CV, Gene Level	6.24%	5.85%
Probesets Used	33297	33297



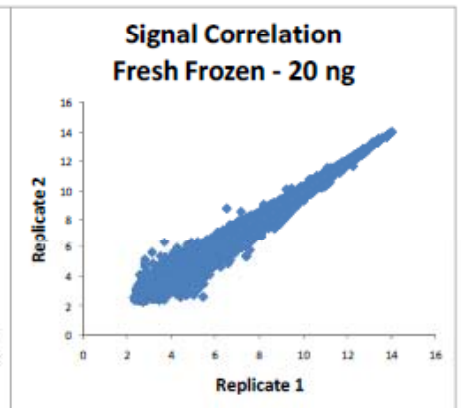
Pearson = 0.992



Pearson = 0.993



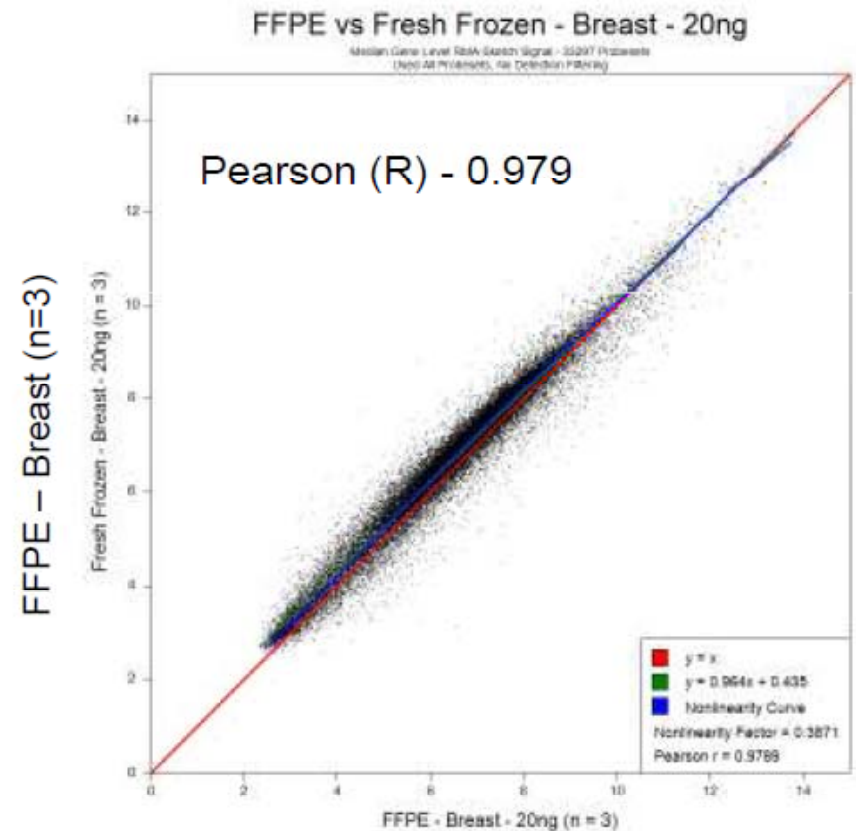
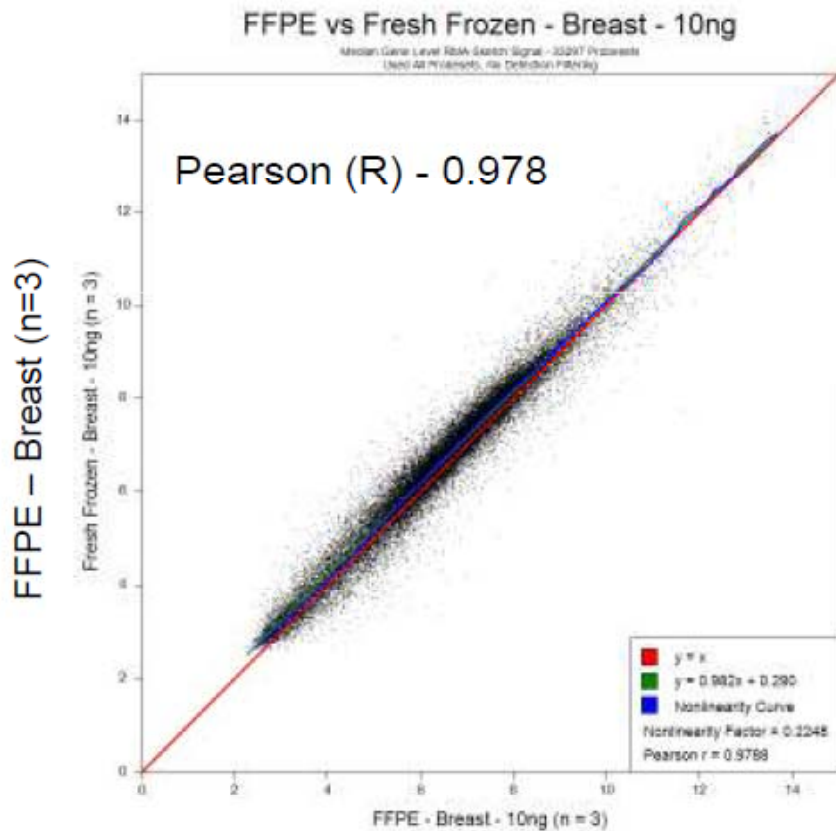
Pearson = 0.994



Pearson = 0.993

HG 1.0 ST Arrays – (10ng)

HG 1.0 ST Arrays – (20ng)



Fresh Frozen – Breast (n=3)

Fresh Frozen – Breast (n=3)

Part Number	Product	Description
Amplification Kit		
SNSAT	Sensation	12 assays
Supporting Products		
SS3BIO12	Biotin Labeling Kit for 3' Arrays	12 assays
STBIO12	Biotin Labeling Kit for ST/Exon Arrays	12 assays

- Customer Service
 - Email: info@genisphere.com
 - Phone: 1-877-888-3362 or 215-996-3002
- Technical Support
 - Email: techsupport@genisphere.com
 - Phone: 1-877-888-3362 or 215-996-3040

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