

RNA Amplification Solutions  
for Affymetrix® 3' Expression Arrays

Genisphere®

SIGNAL + SAMPLE AMPLIFICATION PRODUCTS

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# Goals for 3' Expression Arrays

Genisphere's kits for RNA amplification were designed to achieve the following goals:

- Enable profiling of 1-20 nanogram amounts of FFPE RNA samples. Specifically, must produce data from FFPE RNAs that are similar to data from fresh frozen RNAs.
- Provide ultimate accuracy, as measured by qRT-PCR of amplified and unamplified RNAs
- Highly reproducible procedures
- Low cost

## 3' Expression Arrays

1. Amplify RNA
  - Use SenseAMP for 100-250ng total RNA
  - Use RampUP for 1-20ng total RNA
2. Use the cDNA Synthesis Kit to reverse transcribe the senseRNA, incorporating biotin-dUTP
3. Hybridize biotin-cDNA to the array

## RampUP RNA Amplification

### ROUND 1

Round 1 of RampUP is the same procedure as SenseAMP

#### First strand cDNA synthesis

80 minutes

#### Purification of cDNA

40 minutes

#### Tailing of cDNA

30 minutes

#### Annealing of T7/T3 Template Oligo

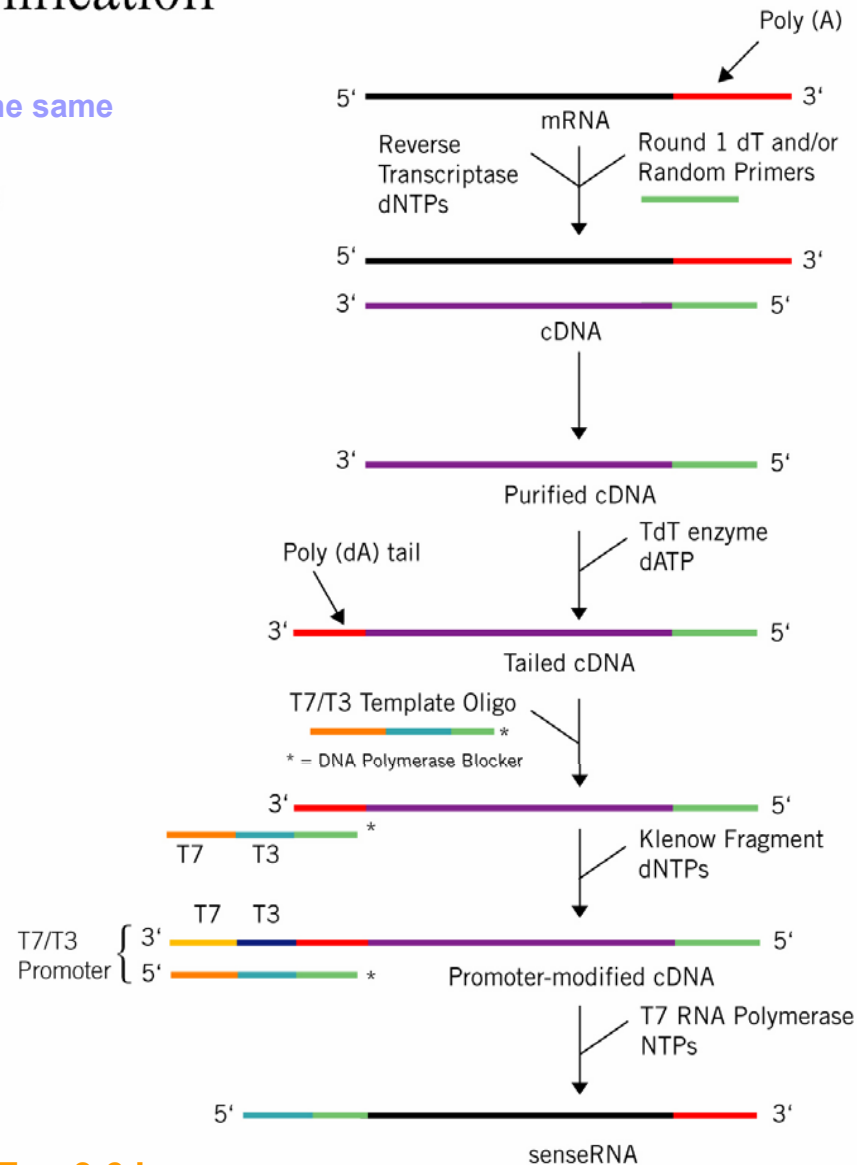
15 minutes

#### T7/T3 Promoter Synthesis

50 minutes

#### T7 In Vitro Transcription

Overnight



**Total Time to Round 1 IVT: ~3.6 hours**

## ROUND 2

### Reverse Transcription

90 minutes

### RNase H degradation of senseRNA

60 minutes

### Annealing of T3 Oligo

10 minutes

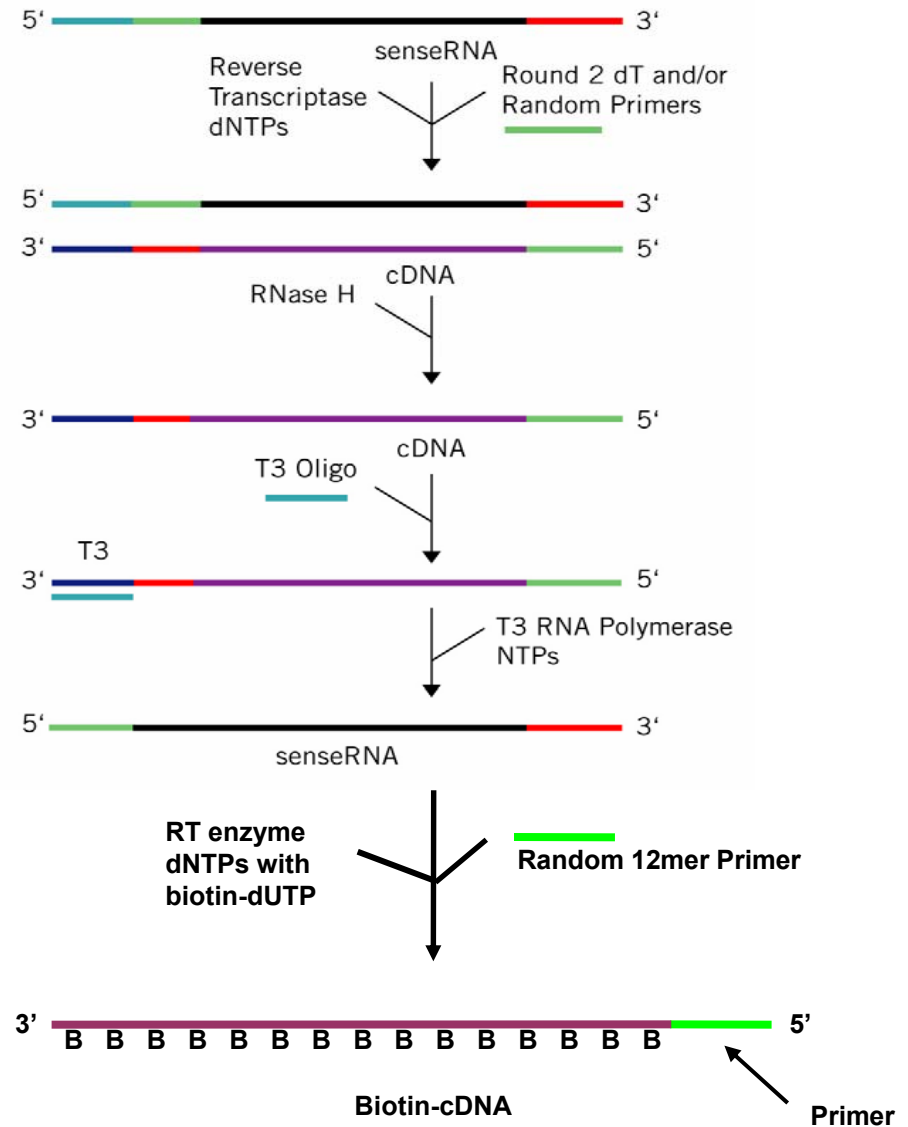
### T3 In Vitro Transcription

Overnight

**Total Time to Round 2 IVT: ~2.7 hours**

### cDNA Synthesis

3 hours

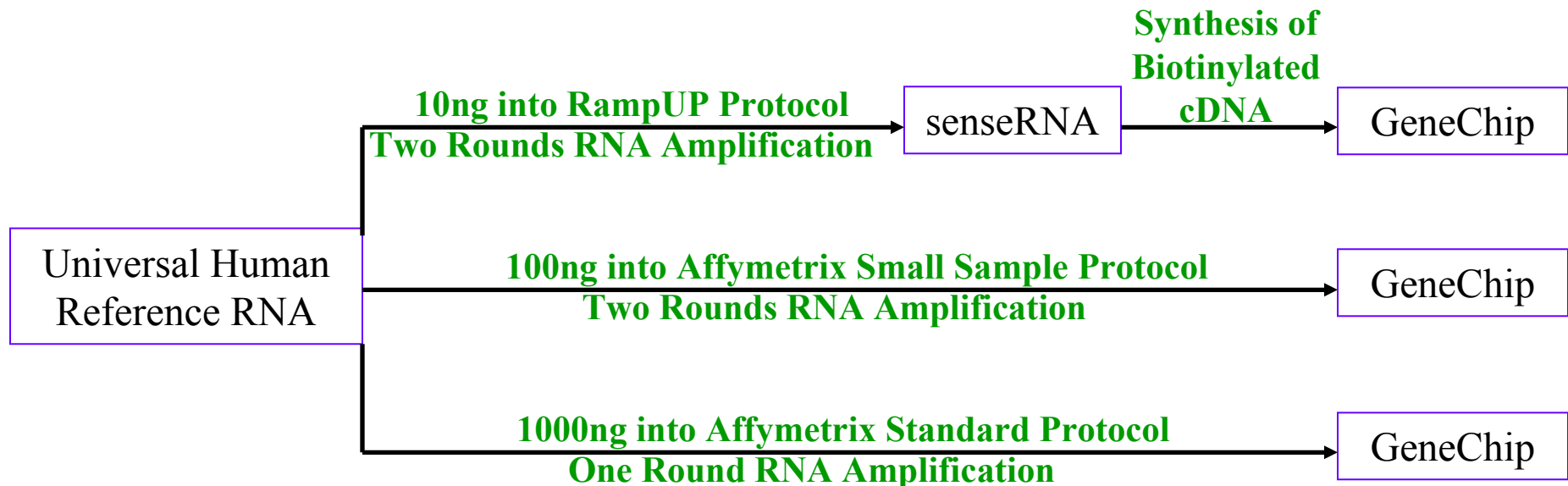


Intact and degraded universal human reference RNA samples were used in four RNA amplification kits designed for use with degraded RNA, according to the protocol of the manufacturer, in order to assess the relative fidelity of the RampUP amplification process. The amplified RNAs and unamplified RNAs were then analyzed with qRT-PCR as validated. The Fold Amplification Bias was calculated for each gene by comparing the Ct values between amplified and unamplified samples. A negative Fold Amplification Bias means that the amplified RNA sample has underrepresented amounts of the gene. A positive Fold Amplification Bias means that the amplified RNA sample has over-represented amounts of the gene. Ideally, the smaller the Fold Amplification Bias, the more accurate the amplification. An overall grouped summary of these experiments is presented in the table below. Individual qRT-PCR data for all samples is available on [www.genisphere.com](http://www.genisphere.com).

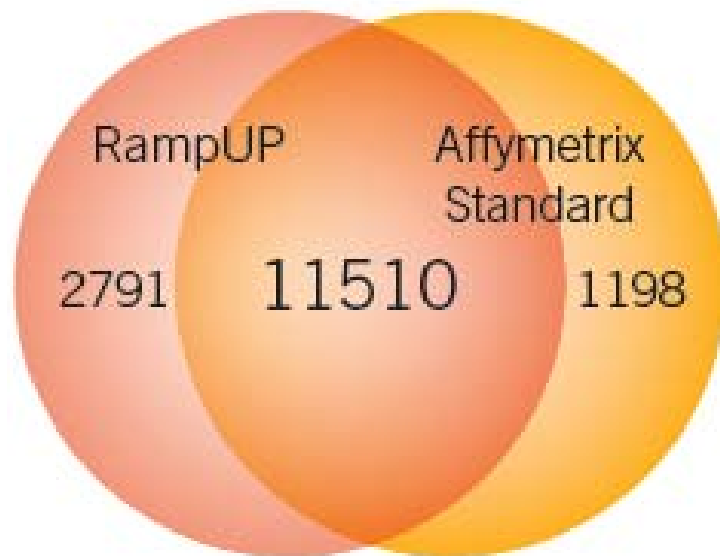
<b>RNA Amplification Kit</b>	<b>Input RNA</b>	<b>Range of Fold Amplification Bias</b>	<b>Number of Lost Transcripts</b>
Full Spectrum RNA Amplification (Systems Biosciences)	100ng Intact	-192 to 43.7	0 out of 16
Full Spectrum RNA Amplification (Systems Biosciences)	100ng Degraded	-8.8 to 25	4 out of 16
Paradise (Arcturus)	100ng Intact	-18.3 to 274.9	5 out of 16
Paradise (Arcturus)	100ng Degraded	-37.2 to 22.2	9 out of 16
SenseAmp (Genisphere)	100ng Intact	-0.8 to 6.0	3 out of 16
SenseAmp (Genisphere)	100ng Degraded	-2.1 to 3.6	3 out of 16
RampUP (Genisphere)	10ng Intact	-3.53 to 2.69	0 out of 21
RampUP (Genisphere)	10ng Degraded	-1.49 to 0.93	0 out of 21
RampUP (Genisphere)	1ng Intact	-3.14 to 4.51	0 out of 21
RampUP (Genisphere)	1ng Degraded	-1.01 to 1.91	1 out of 21
RampUP (Genisphere)	0.1ng Intact	-3.61 to 9.08	0 out of 21
RampUP (Genisphere)	0.1ng Degraded	-0.88 to 4.19	3 out of 21

# Compatibility with 3' Expression Arrays

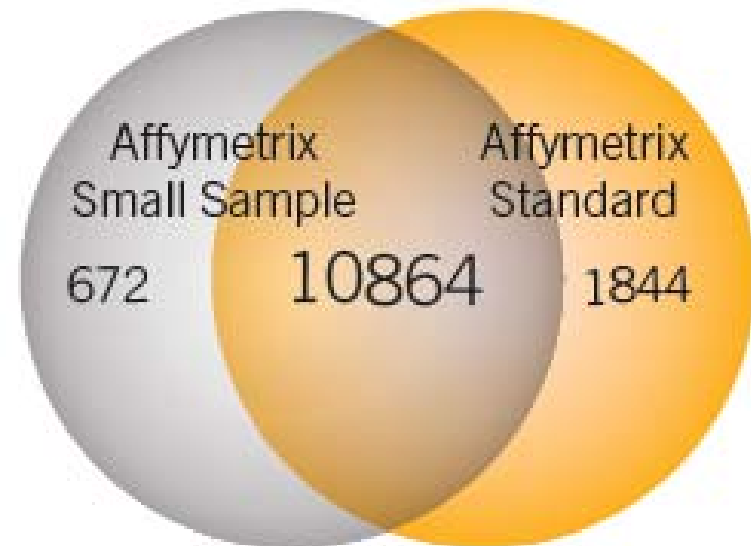
To demonstrate the compatibility of Genisphere kits with the Affymetrix platform, 10ng of Universal Human Reference RNA (Stratagene, cat. no. 740000) was used in the RampUP and cDNA Synthesis Kit protocols. The same Reference RNA was also used in the Affymetrix standard protocol and Affymetrix small sample protocol. All 3 samples were hybridized to Human Genome U133A arrays (Affymetrix, cat. no. 900366).



	Affymetrix Standard	Affymetrix Small Sample	RampUP
Input Total RNA	1000ng	100ng	10ng
Scale Factor	1.53	1.10	0.66
% Present	55	52	64.2
GAPDH 3'/5'	2.20	2.50	1.39
Actin 3'/5'	2.80	22.4	2.73
Correlation to Standard Labeling	N/A	0.90	0.87

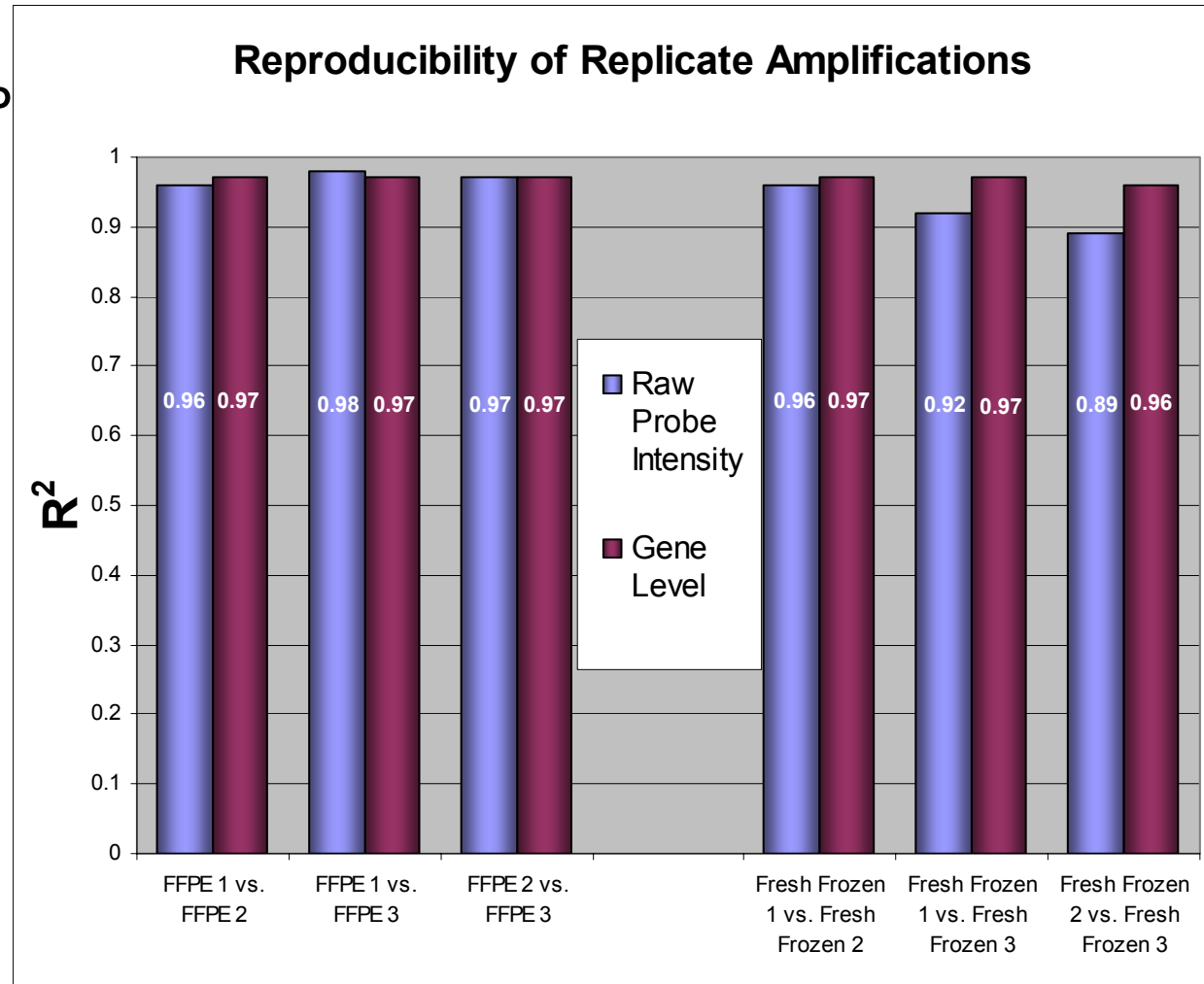


91% genes common  
between RampUP and  
Affymetrix Standard

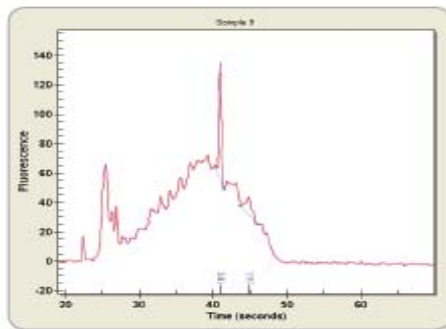


85% genes common  
between Affymetrix Small Sample  
and Affymetrix Standard

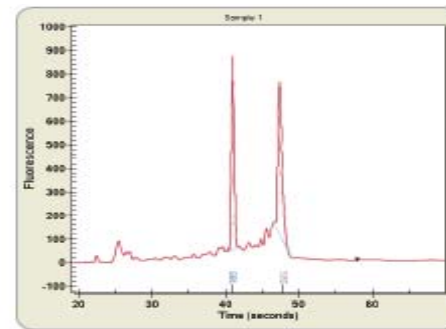
An experiment was run to test the fidelity of RampUP amplification, using both intact and FFPE RNA samples. Three parallel amplifications were performed with a FFPE sample and a matched Fresh Frozen sample. Analysis of the six U133A arrays shows high reproducibility for both FFPE and Fresh Frozen samples.



A pilot experiment was run with two RNA samples: an FFPE-derived cancer sample, and a biologically similar fresh frozen sample. After running both RNA samples on the Bio-RAD Experion, 10ng of each sample was used in the RampUP and cDNA Synthesis Kits. The biotinylated cDNA was hybridized to U133A GeneChips. A Pearson correlation of 0.9023 was obtained after analyzing the GeneChip data.



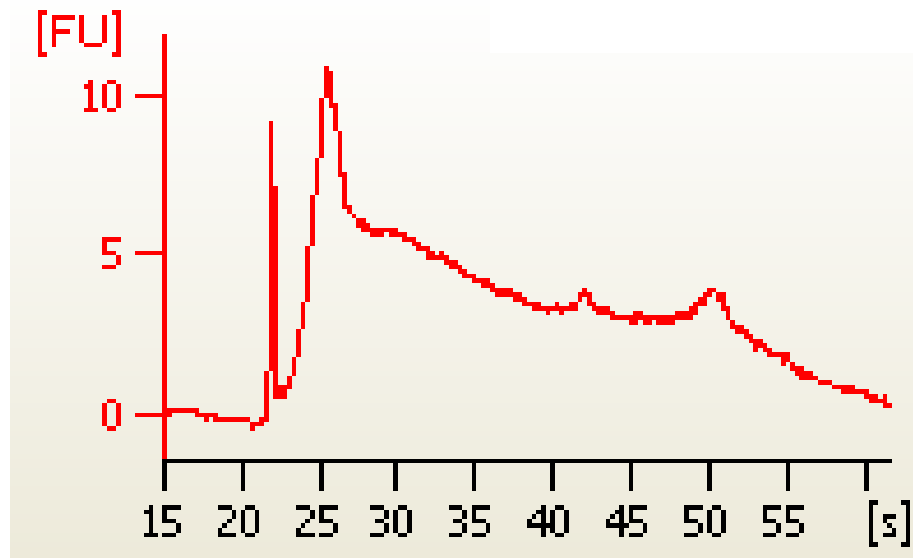
RNA isolated from 5 year old Formalin-Fixed Paraffin Embedded (FFPE) colon cancer tissue sample.



RNA isolated from a biologically similar fresh frozen colon cancer sample.

QC Report	Frozen	FFPE
Scale Factor	0.668	0.975
Average Background	33.73	33.21
% Present	54.04	38.92
Average Signal (P)	415.42	487.24
GAPDH 3'/5'	1.67	3.94
Actin 3'/5'	2.93	6.31

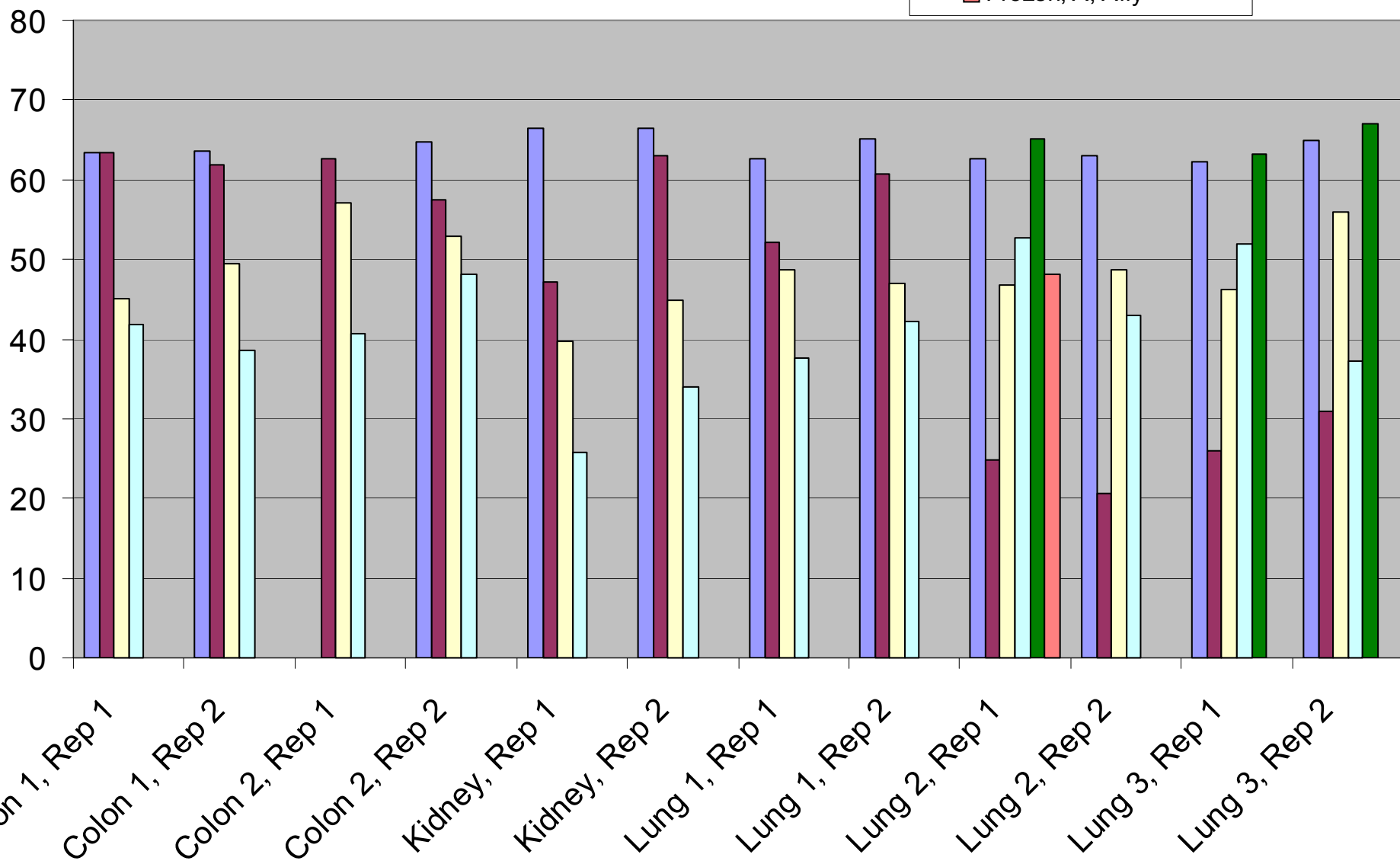
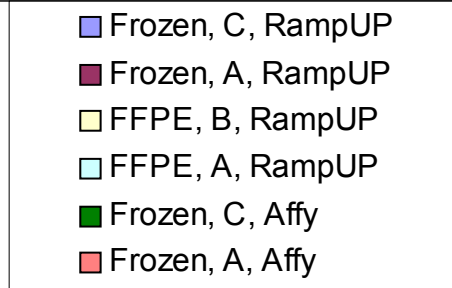
- To expand the last study, 6 FFPE RNA samples were obtained. The specimens were tumor samples from colon, kidney and lung, between 1-5 years old. A biologically similar fresh frozen sample was obtained for each of the 6 FFPE RNAs.
- Each of the 12 RNAs were isolated/purified in duplicate using two different methods. Methods A and B were used for the FFPE samples. Methods A and C were used for the frozen samples. All of the purified RNAs were run on the Agilent Bioanalyzer. A typical Bioanalyzer profile of the FFPE samples is shown:



- A portion (20ng) of each of the 48 isolation/purifications were used in the RampUP and cDNA Synthesis Kits. Another portion (1ug) of 4 of the purifications were used in amplification and labeling kits from Affymetrix.
- All 52 samples were hybridized to U133A 2.0 GeneChips.
- Although each FFPE and matched fresh frozen sample are not identical samples, they were grouped together for data analysis. There are 6 groups of FFPE/frozen samples:
  1. Colon 1
  2. Colon 2
  3. Kidney
  4. Lung 1
  5. Lung 2
  6. Lung 3

Rep 1 and Rep 2 refer to the parallel RNA isolation technique.

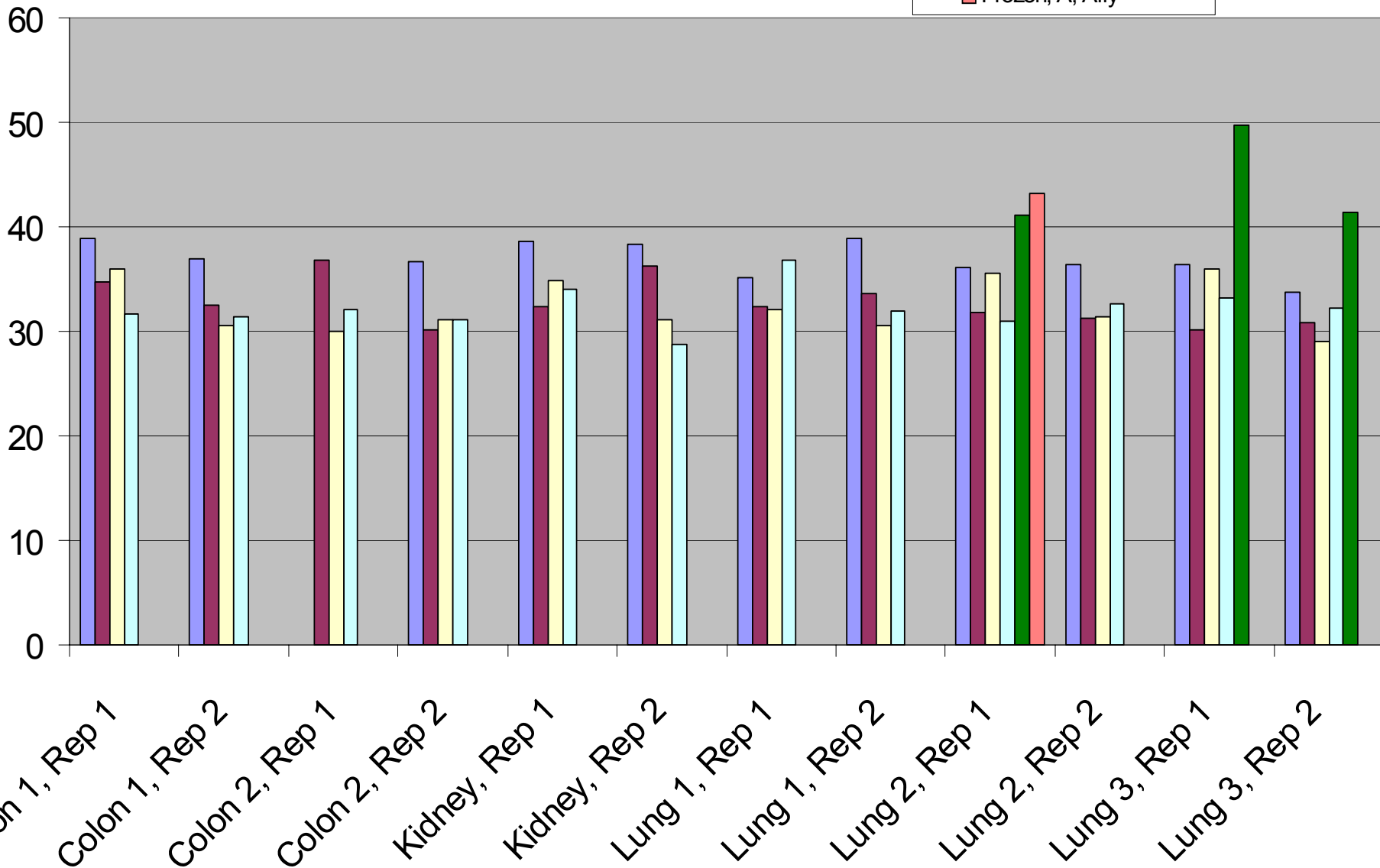
## % Probesets Present Matched FFPE and Frozen



# Background

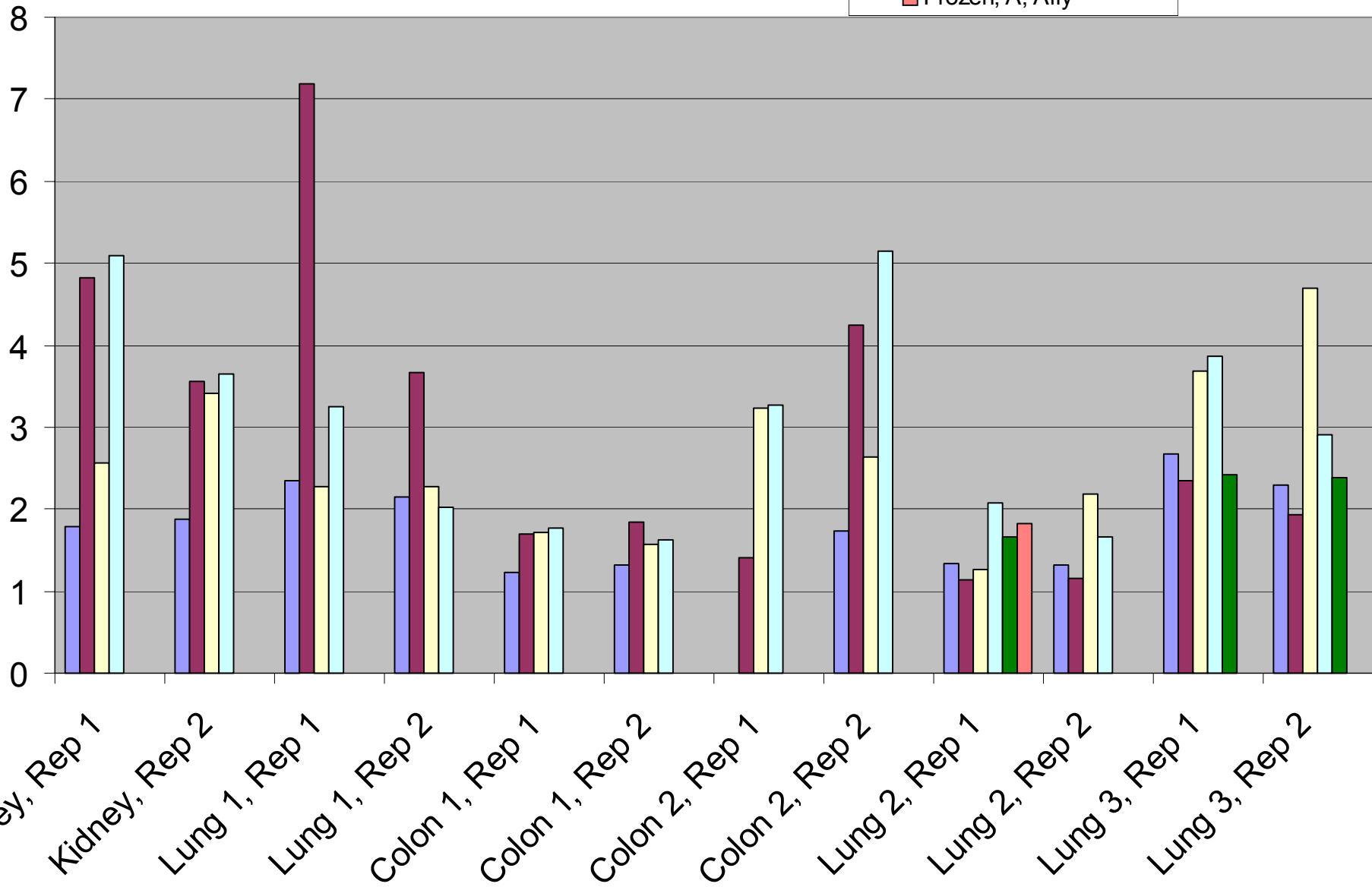
## Matched FFPE and Frozen

- Frozen, C, RampUP
- Frozen, A, RampUP
- FFPE, B, RampUP
- FFPE, A, RampUP
- Frozen, C, Affy
- Frozen, A, Affy



# GAPDH 3' / 5' Ratio Matched FFPE and Frozen

- Frozen, C, RampUP
- Frozen, A, RampUP
- FFPE, B, RampUP
- FFPE, A, RampUP
- Frozen, C, Affy
- Frozen, A, Affy



## Summary of Solutions for 3' Expression Arrays

- RampUP can be used to amplify and label both FFPE and Frozen RNAs. For Frozen RNAs, RampUP data is similar to Affy data.
- RampUP enables the ability to resolve similar data from FFPE samples compared with similar fresh frozen samples.
- In addition to microarrays, senseRNA can be used directly in qRT-PCR methods.
- qRT-PCR analysis of unamplified RNA and senseRNA demonstrates the ultimate accuracy of Genisphere's amplification process.

	SenseAMP and cDNA Synthesis	RampUP and cDNA Synthesis	NuGEN	Affymetrix
Sensitivity	100-250ng	1-20ng	50ng	Not compatible with FFPE
Protocol	1.5 days	2.5 days	1.5 days	
Price per sample (all reagents, columns, etc.)	\$99	\$155	\$150-\$250	

<p><b>RampUP</b> Two-Round RNA Amplification Kit</p>	<p>Two-round linear RNA amplification kit that produces sense strand RNA representing the entire transcript for further use in expression analysis experiments or in other relevant RNA-based applications. Can be used with intact or degraded, eukaryotic or prokaryotic RNA samples.</p>	<p>10 assays</p>	<p>RAMPUP10</p>
<p><b>SenseAMP</b> One-Round RNA Amplification Kit</p>	<p>One-round linear RNA amplification kit that produces sense strand RNA for further use in expression analysis experiments or in other relevant RNA-based applications. Can be used with intact or degraded, eukaryotic or prokaryotic RNA samples.</p>	<p>10 assays 20 assays</p>	<p>SENSEAMP10 SENSEAMP20</p>
<p><b>cDNA Synthesis Kit</b> For reverse transcription of senseRNA from RampUP and SenseAMP</p>	<p>Contains reagents designed to convert senseRNA from SenseAMP and RampUP kits into labeled cDNA, for subsequent gene expression analysis on Affymetrix 3' expression arrays. Not needed for Affymetrix exon arrays.</p>	<p>10 assays</p>	<p>CDNAMOD</p>