



# RNA Amplification Solutions for Affymetrix® 3' Expression Arrays

Genisphere®

SIGNAL + SAMPLE AMPLIFICATION PRODUCTS

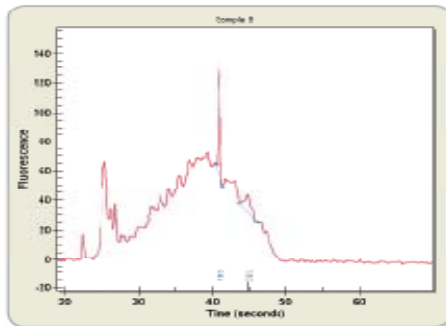
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# Goals for 3' Expression Arrays

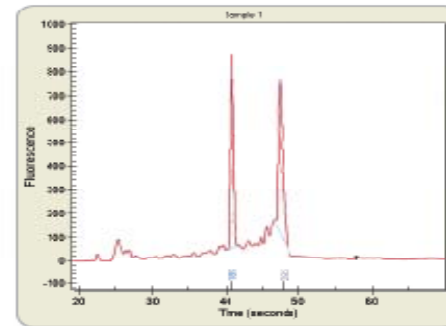
Genisphere's kits for RNA amplification were designed to achieve the following goals:

- Enable profiling of 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

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 kits. The biotinylated cDNA was hybridized to U133A GeneChips. A Pearson correlation of 0.9023 was obtained after analyzing the GeneChip data.



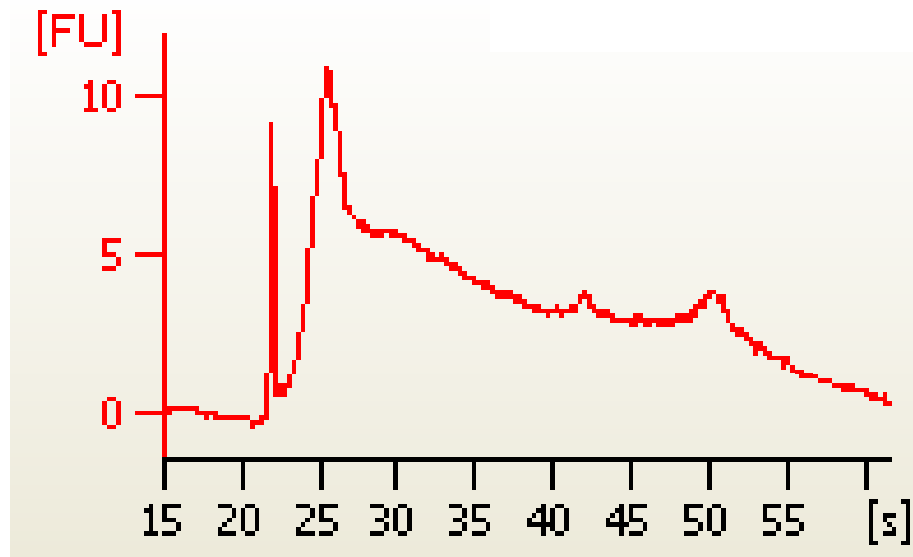
RNA isolated from 5 year old Formalin-Fixed Paraffin Embedded (FFPE) colon cancer tissue sample.



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

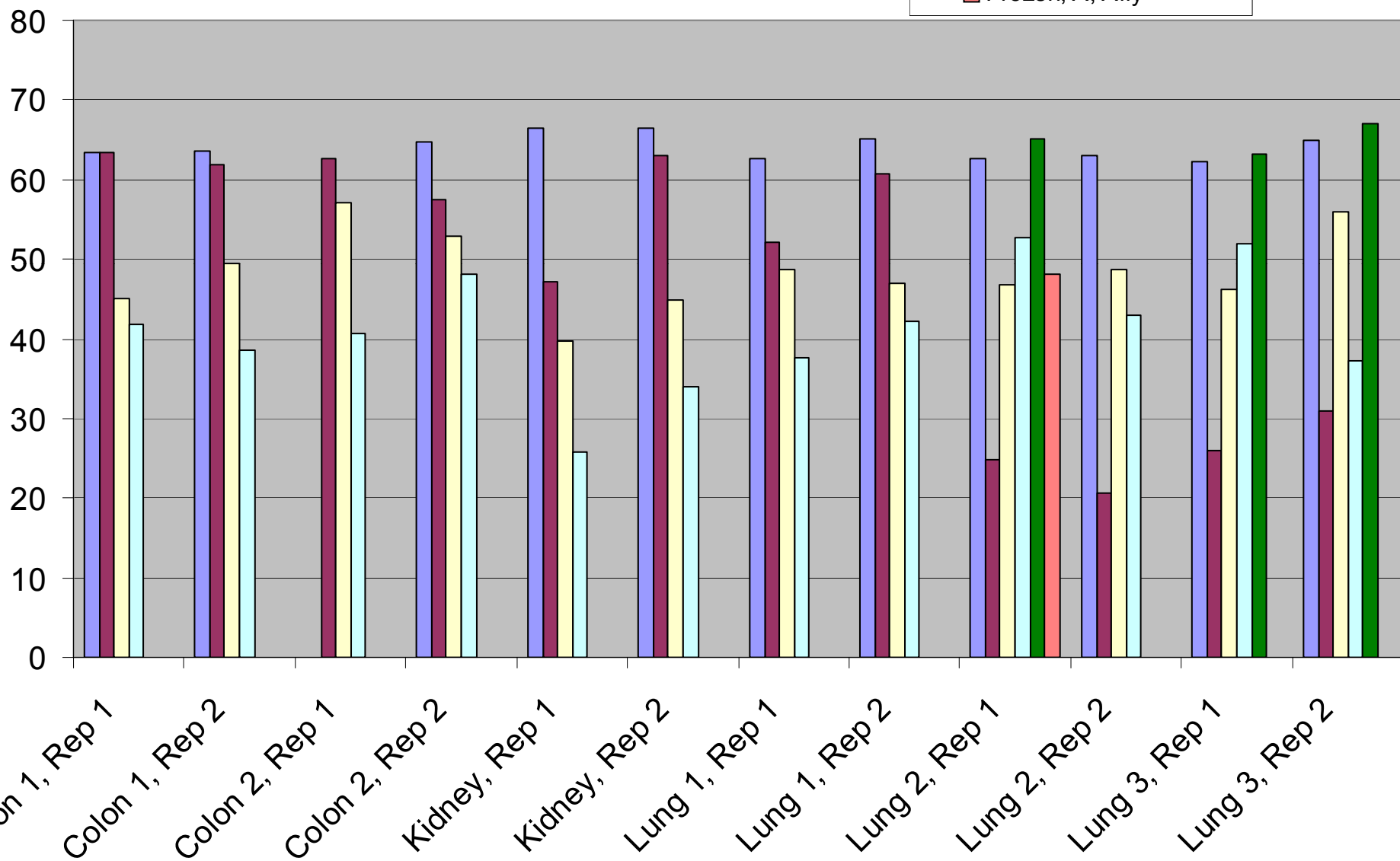
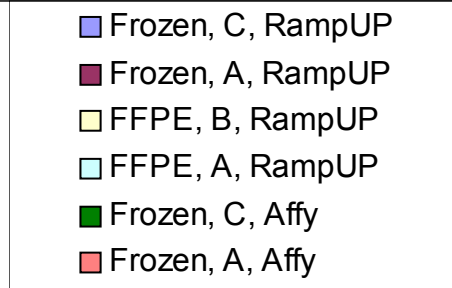
- To expand the last study, 6 FFPE RNA samples were obtained. The specimens were tumor samples from colon, kidney and lung, between 1-5 years old. A biologically similar fresh frozen sample was obtained for each of the 6 FFPE RNAs.
- Each of the 12 RNAs were isolated/purified in duplicate using two different 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 kit. 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



Further analysis of the array data is described in:

Roberts L, Bowers J, Sensinger K, Lisowski A, Getts R, Anderson MG. Identification of methods for use of formalin-fixed, paraffin-embedded tissue samples in RNA expression profiling. *Genomics* 2009, 94(5):341-8.

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) under RNA Amplification data (Development of a streamlined, high fidelity, two round sense RNA amplification method).

<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

## 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	NuGEN	Affymetrix
Sensitivity	100-250ng	1-30ng	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. Contains reagents designed to convert senseRNA from into labeled cDNA, for subsequent gene expression analysis on Affymetrix 3' expression arrays.</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 SenseAMP kit</p>	<p>Contains reagents designed to convert senseRNA from SenseAMP kits into labeled cDNA, for subsequent gene expression analysis on Affymetrix 3' expression arrays.</p>	<p>10 assays</p>	<p>CDNAMOD</p>