

FlashTag Biotin

RNA labeling kit

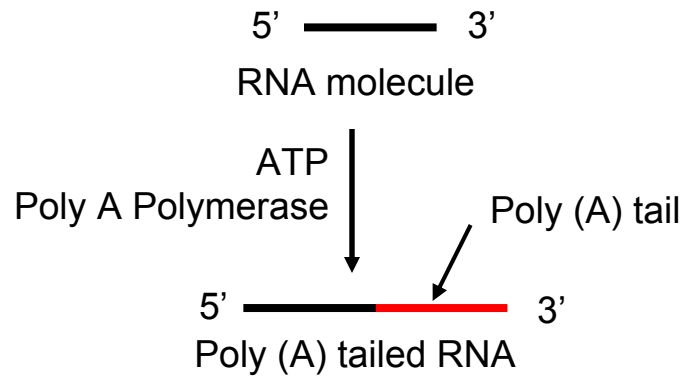
# Summary of FlashTag

- Fast and Simple:
  - Directly label RNA in 45 minutes
  - No purification steps
- Accurate and Reproducible:
  - Replicate analysis:  $R^2 = 0.99$
  - Replicate differential analysis:  $R^2 = 0.96$
- Sensitive and Flexible:
  - Total RNA (0.5-3 $\mu$ g)
  - LMW RNA enriched from 0.5-3 $\mu$ g Total RNA
  - senseRNA (250ng)
  - Compatible with commercially available miRNA microarrays, ELOSA assays, Luminex bead-flow assays (Marligen Bioscience, Inc), and other platforms

# FlashTag: Procedure Overview

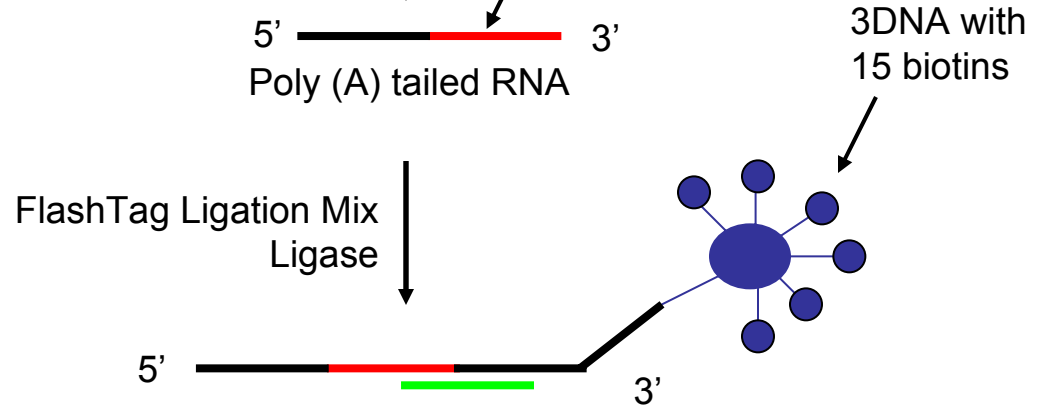
## 1 Poly (A) Tailing

(15 minutes)

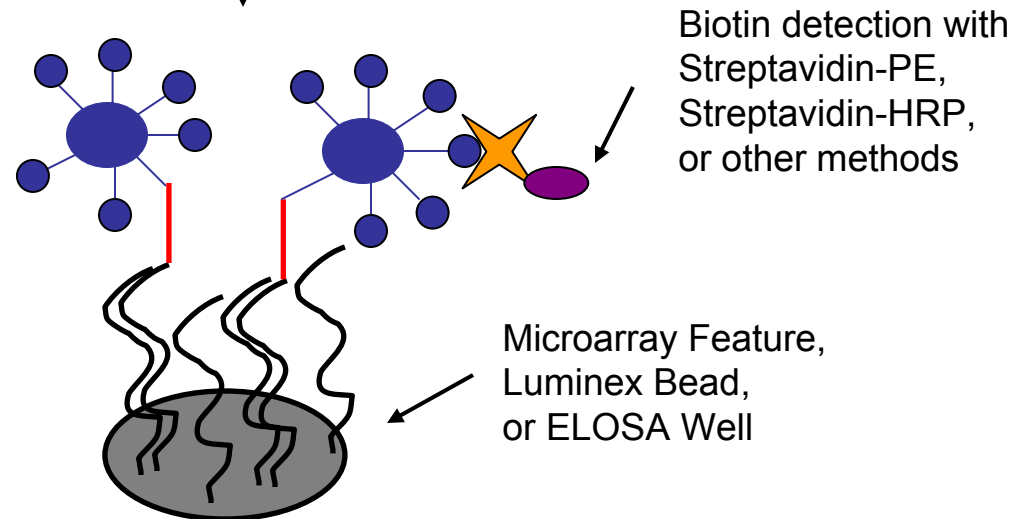


## 2 Ligation

(30 minutes)



## 3 Analysis

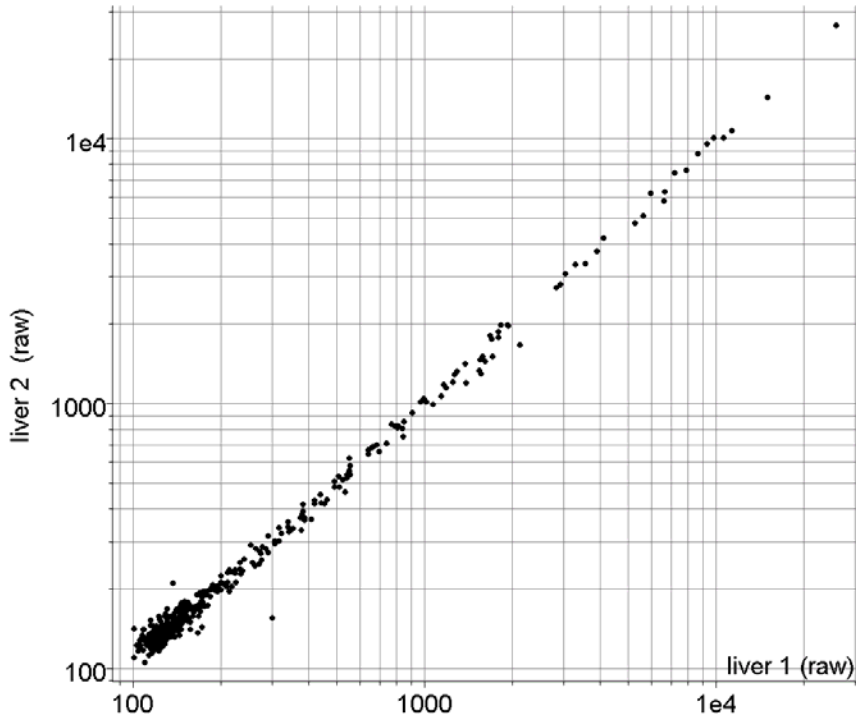


# Overview of Methods

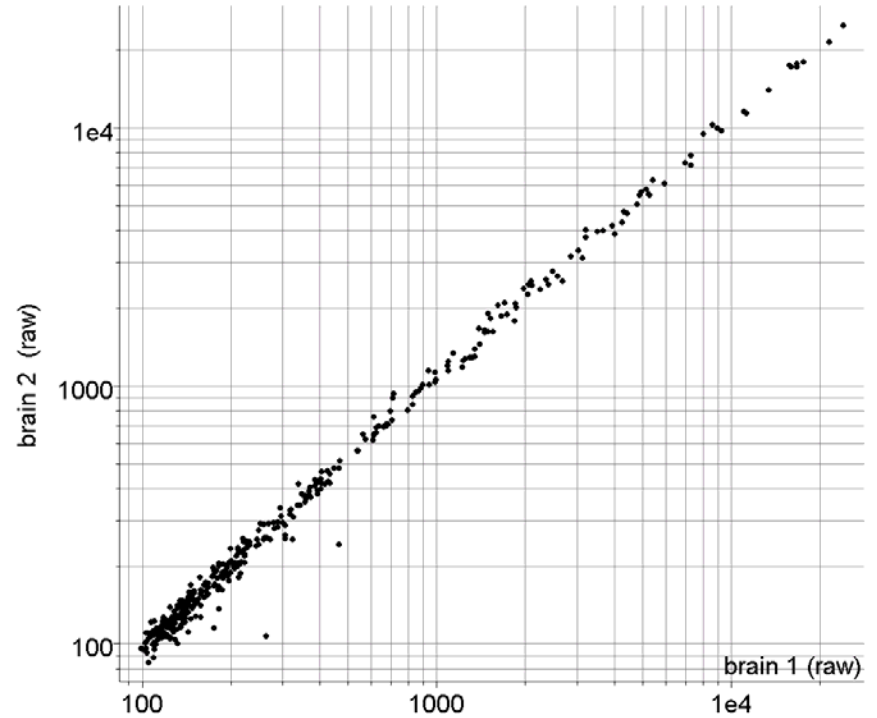
- Complete protocols are available in the product manual for FlashTag Biotin ([www.genisphere.com](http://www.genisphere.com)).
  - For Geniom Biochip microRNA microarrays, the labeled RNA hybridized overnight at 42°C. The Biochip was washed and hybridized with Streptavidin-PE. PE signal was measured using the appropriate filter set.
  - For ELOSA experiments, a microtiter plate was coated with oligonucleotides specific for miRNA species. The plate was washed, blocked, and hybridized with labeled RNA. The plate was washed and hybridized with Streptavidin-HRP. TMB Substrate was added to develop the signal. The reactions were stopped and the absorbance was read at 450.
- Additional protocols for bead-flow assays are available from Marligen Bioscience, Inc.

# Technical duplicates demonstrate the reproducibility of the microRNA microarray

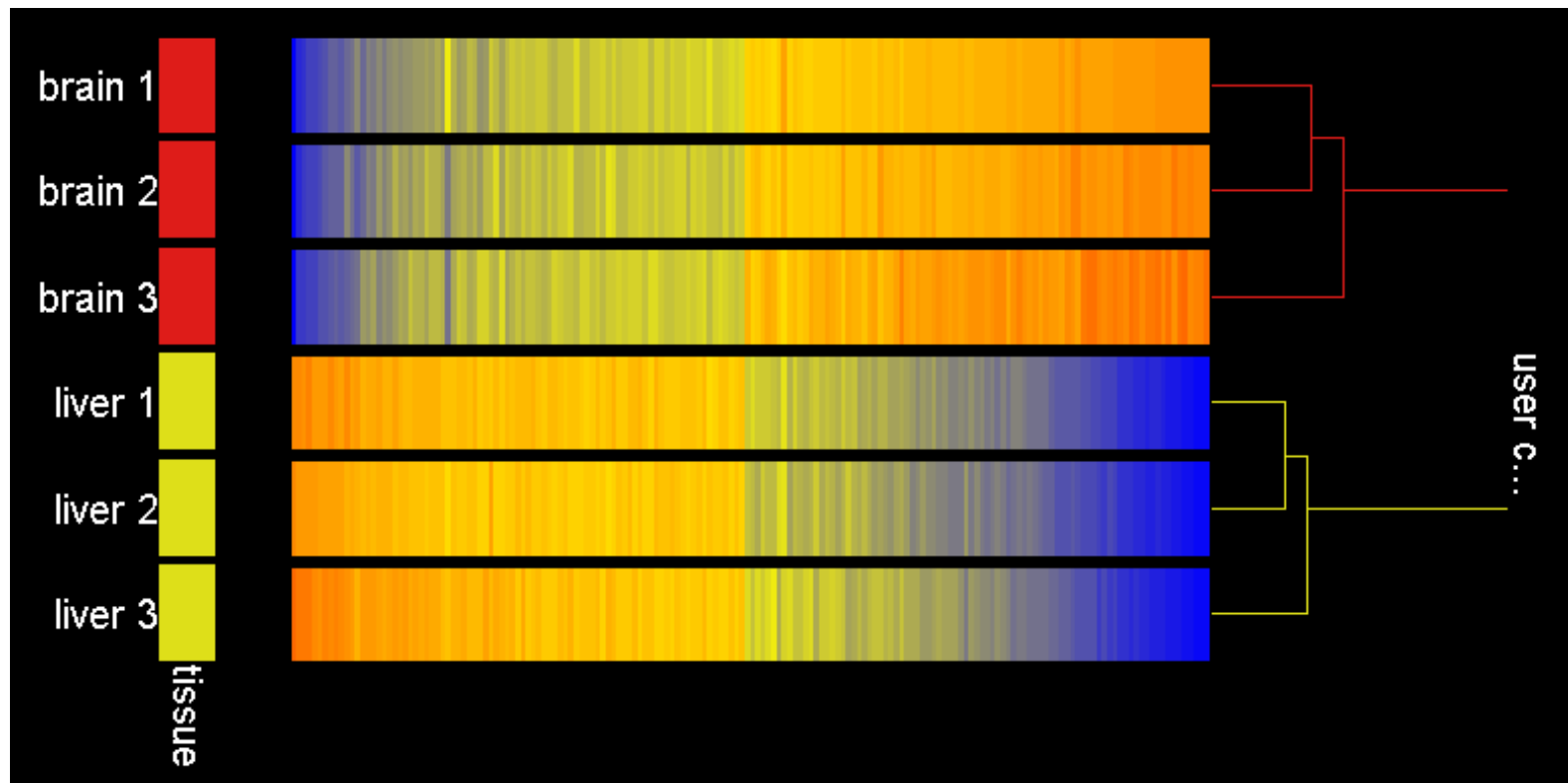
Mouse Liver



Mouse Brain

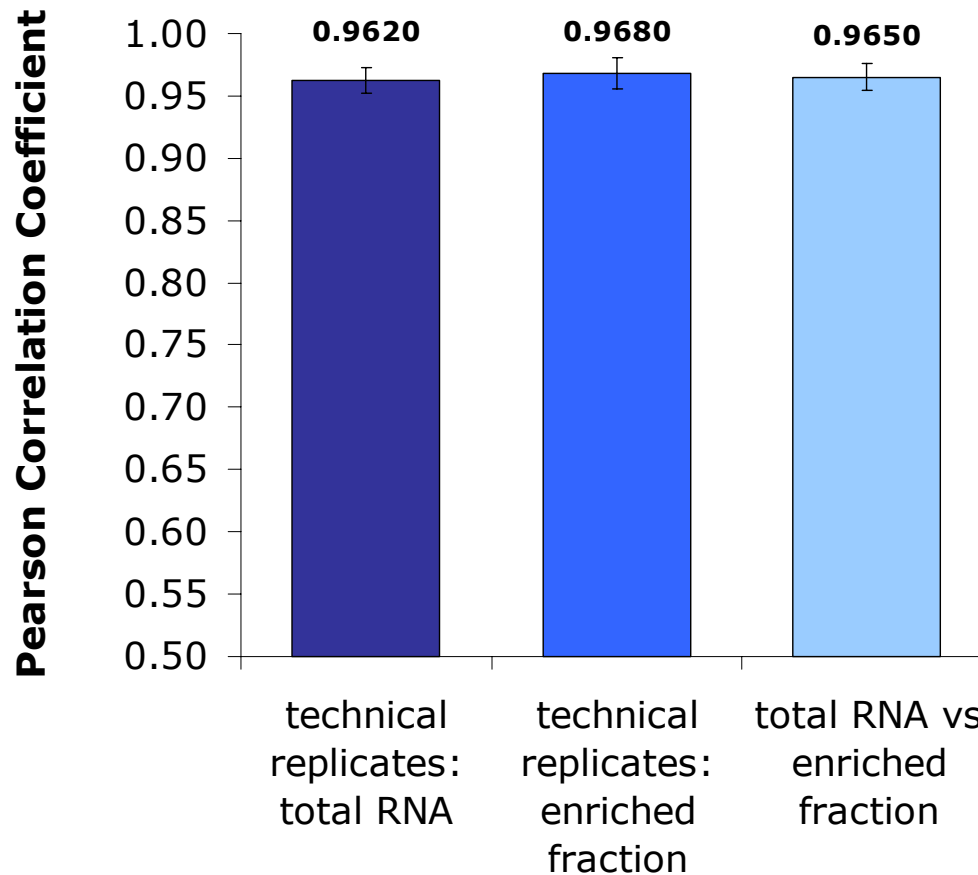


# Differential analysis of mouse liver and brain samples (three technical replicates)



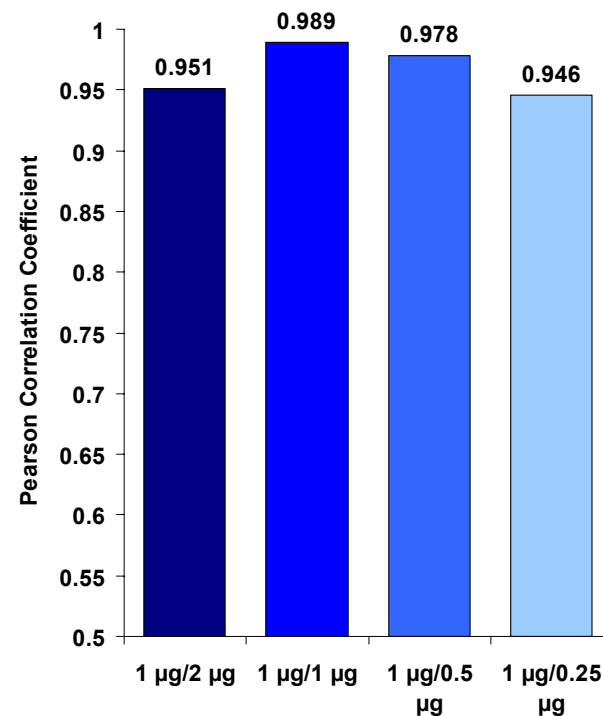
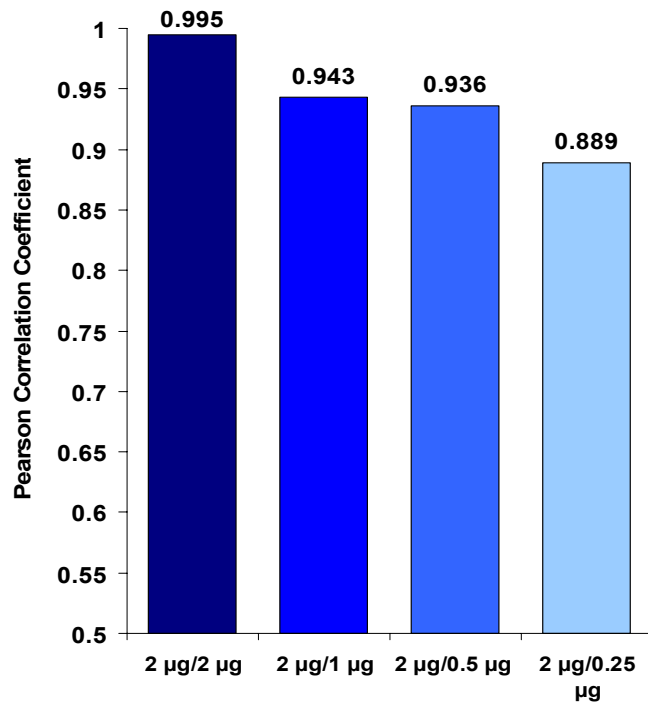
## Small/miRNA enrichment is not necessary

**Comparison total RNA vs RNA enriched for  
small molecules**

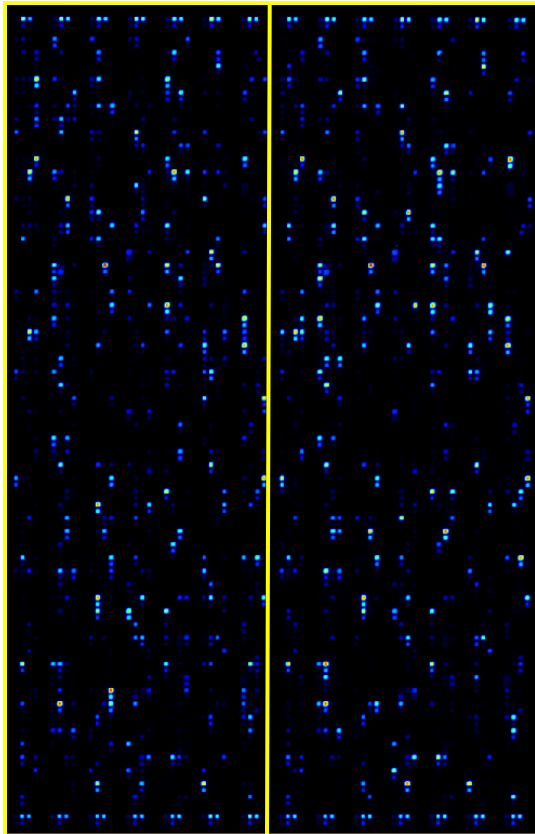


## Titration of input total RNA

- Amounts of starting material compared: 2 $\mu$ g, 1 $\mu$ g, 0.5 $\mu$ g, 0.25 $\mu$ g
- Data was obtained from different exposure times (to adjust for different signal intensities)
- Even amounts as low as 0.25 $\mu$ g total RNA provide meaningful data



## Geniom Microarray Data Is Confirmed By Real-Time PCR

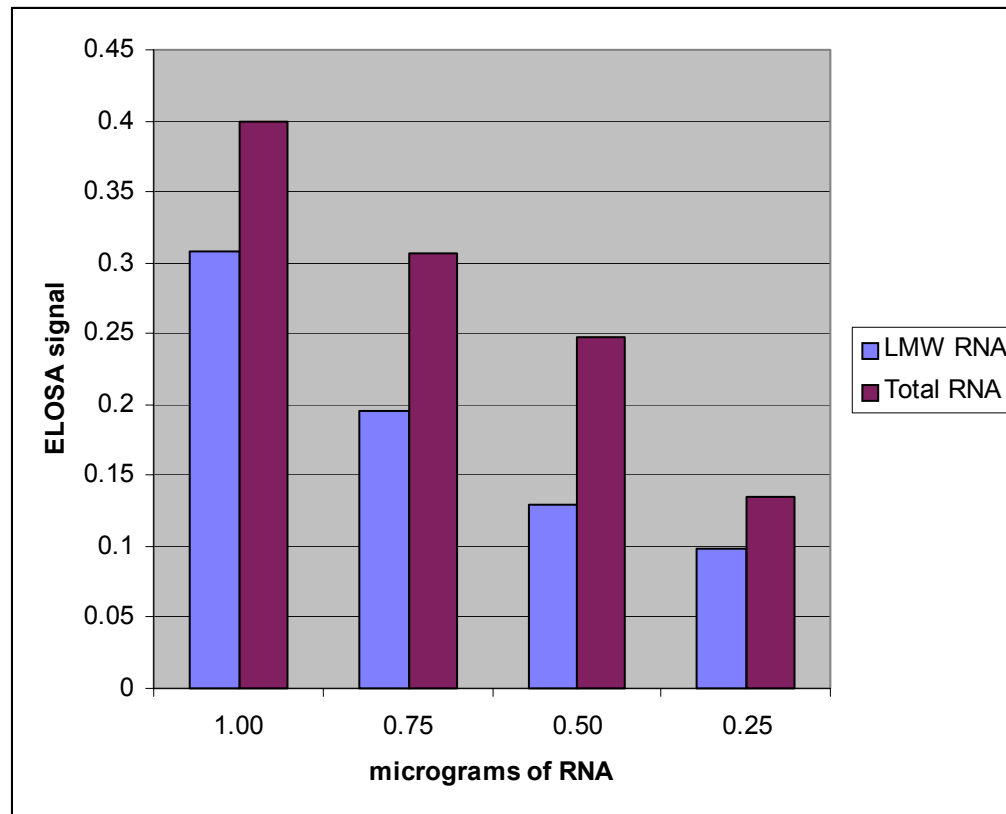


human brain    human heart

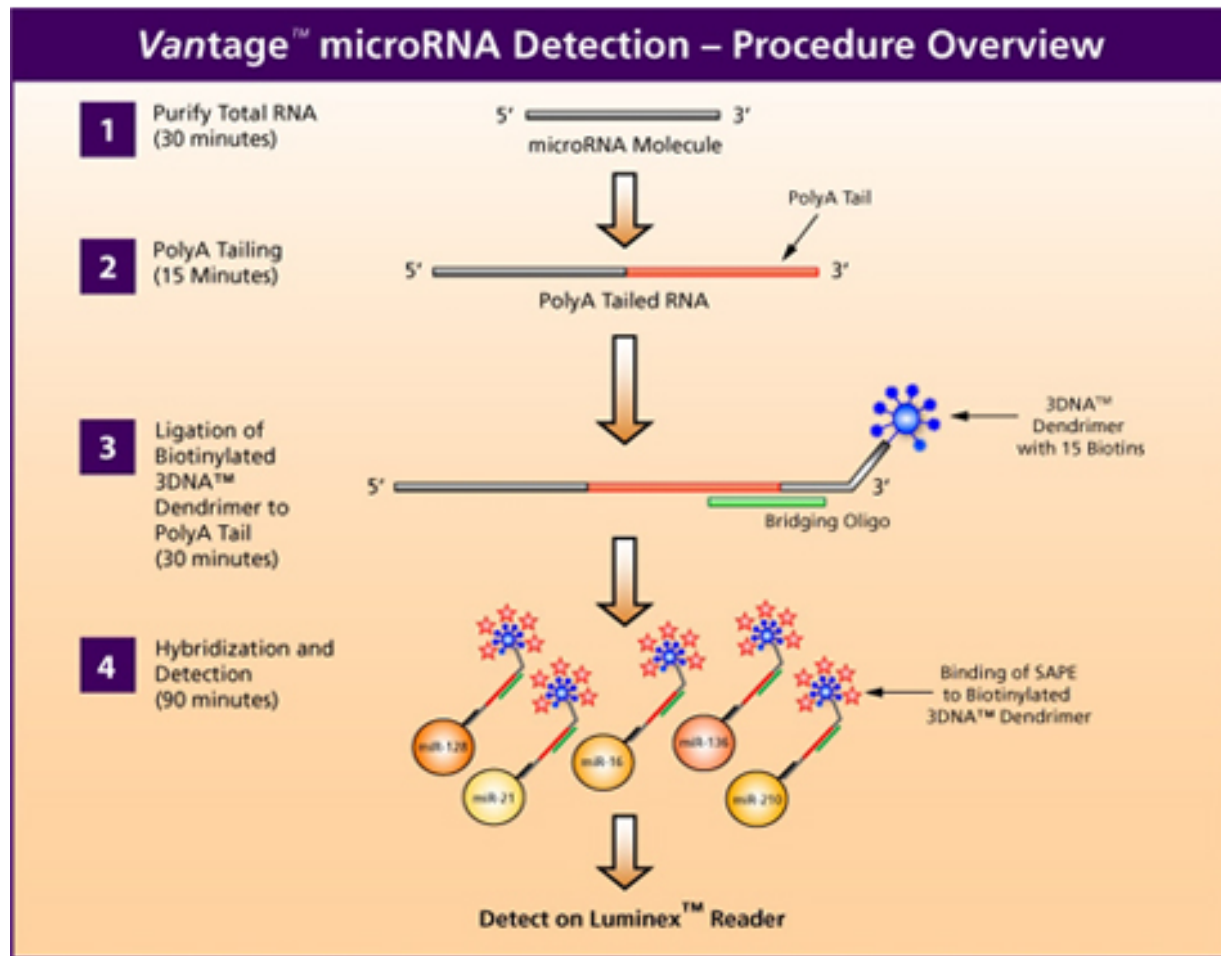
miRNA	brain/heart ratio febit biochip	brain/heart ratio ABI TaqMan PCR
hsa-miR-16	0.94	1.028
hsa-mir-124	2265	12682
hsa-mir-134	2.6	4.92
hsa-mir-422b	0.0395	0.0574

## Comparison of LMW RNA and Total RNA in a miR122 ELOSA assay

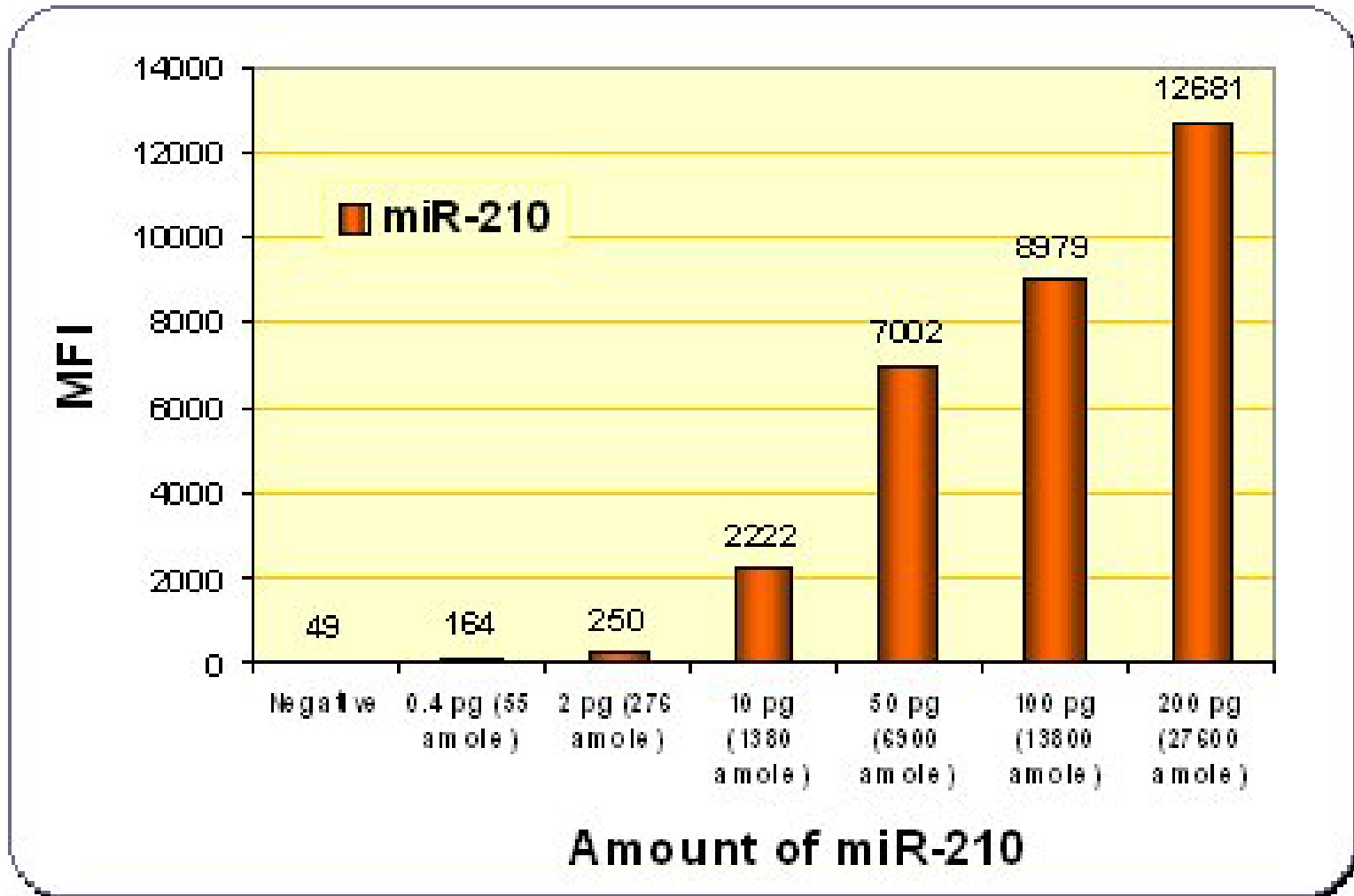
A microtiter plate was coated with miR122 oligo. A portion of rat liver total RNA was enriched for LMW RNA using YM-100 columns (Millipore). Both total RNA and LMW RNA were labeled with FlashTag Biotin, and used in the ELOSA assay.



Marligen's Vantage™ products offer researchers a comprehensive solution for microRNA analysis. Total or low molecular weight RNAs can be isolated using our simple, rapid purification method. The Vantage™ Labeling Kit directly labels total or enriched samples with multiple biotins to give the utmost sensitivity in profiling microRNA expression. Our disease-specific Multiplex Detection Panels are formatted on the Luminex xMAP® platform to rapidly and accurately profile the expression levels of microRNAs. The full process from purification to detection can be completed in less than 4 hours and more than a hundred samples can be processed in just one day.

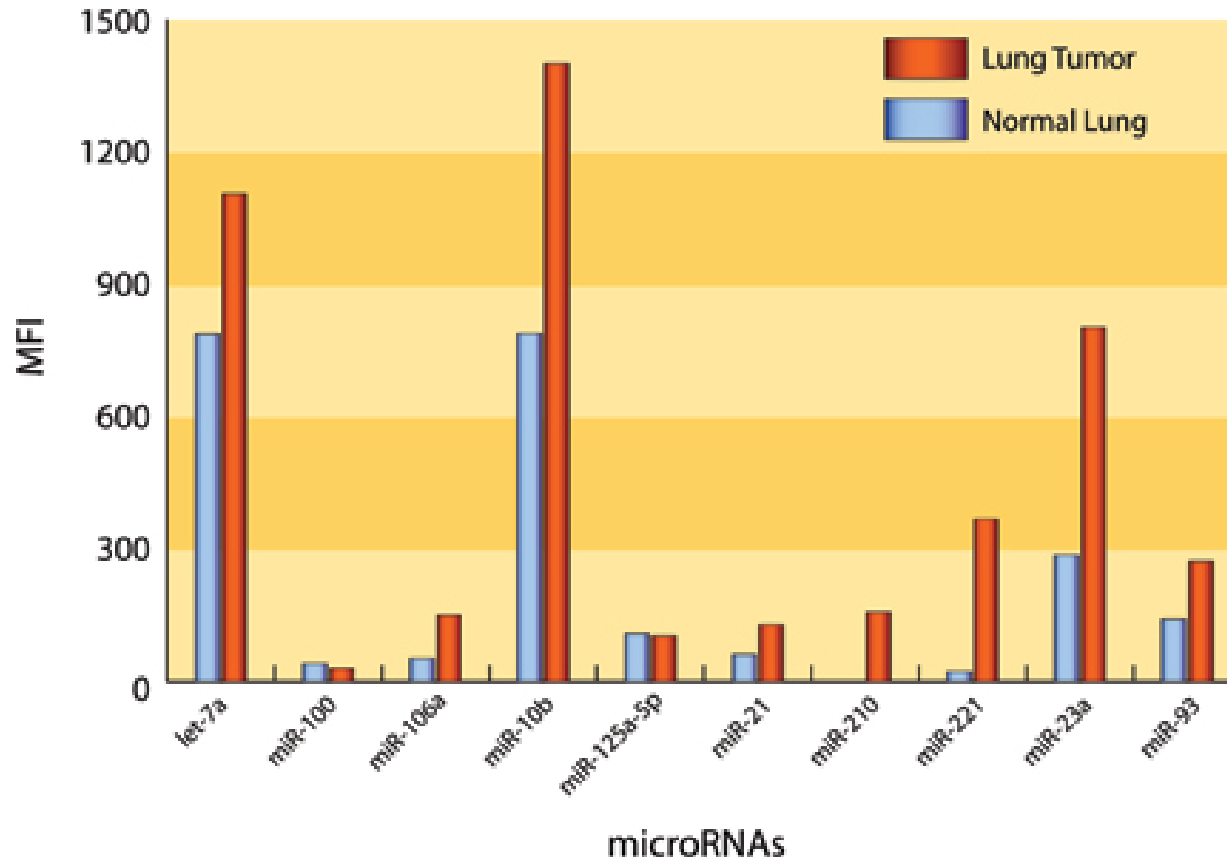


## Highly sensitive labeling method requires little input RNA



Marligen's Vantage™ microRNA labeling and detection kits can detect <1pg (or <250 attomoles) synthetic microRNA.

## Labels degraded RNA



Total RNA was extracted from normal and tumor lung. The total RNA from both samples was determined to be degraded when analyzed using Agilent LabChips®. The RNA samples were labeled and detected using Marligen's Vantage™ microRNA Labeling Kit and Vantage™ Oncology Detection Panel 1 (Cat. No. 11831-050). A subset of microRNAs detected by the Vantage™ Oncology Panel 1 is shown here. The data shows that expression profiles from degraded RNA can be analyzed using the Marligen Vantage™ labeling and detection method.