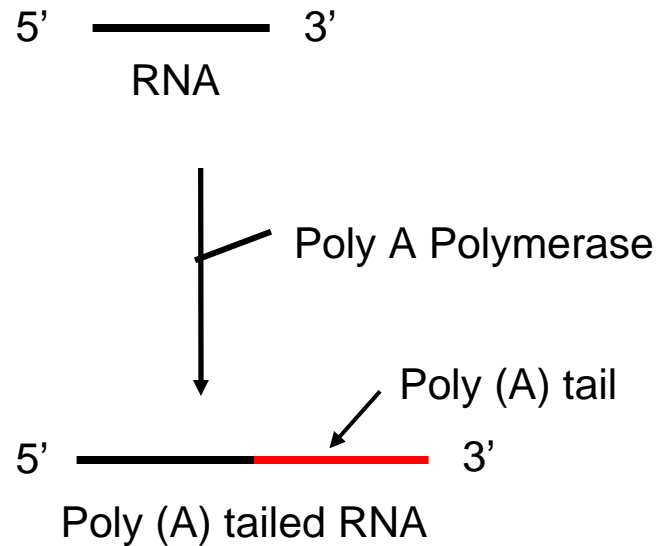
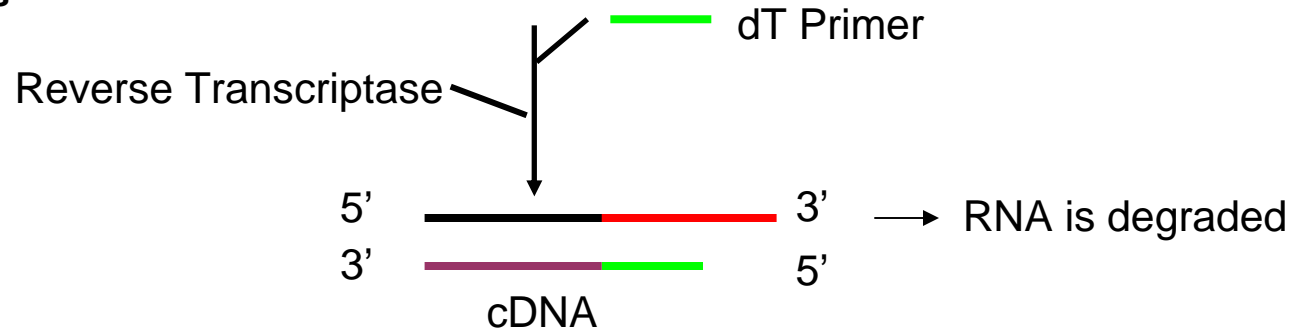


# Amplification of LMW RNA using SenseAmp Plus: Procedure Overview

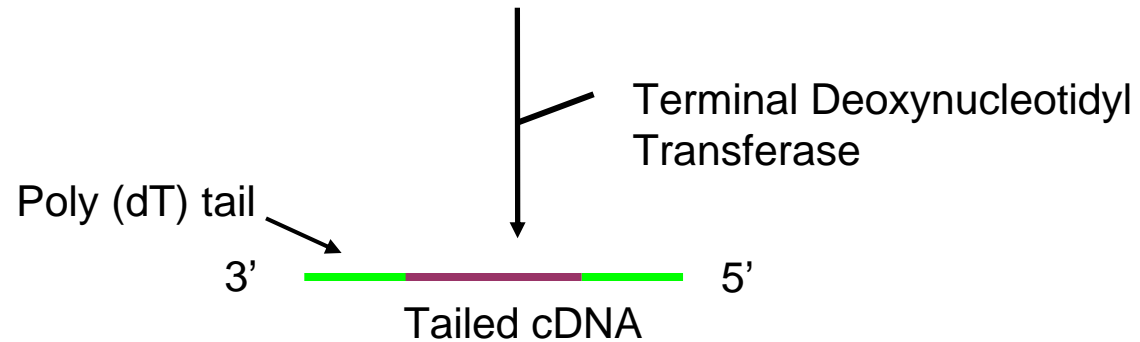
## ① Poly (A) Tailing



## ② First Strand cDNA Synthesis

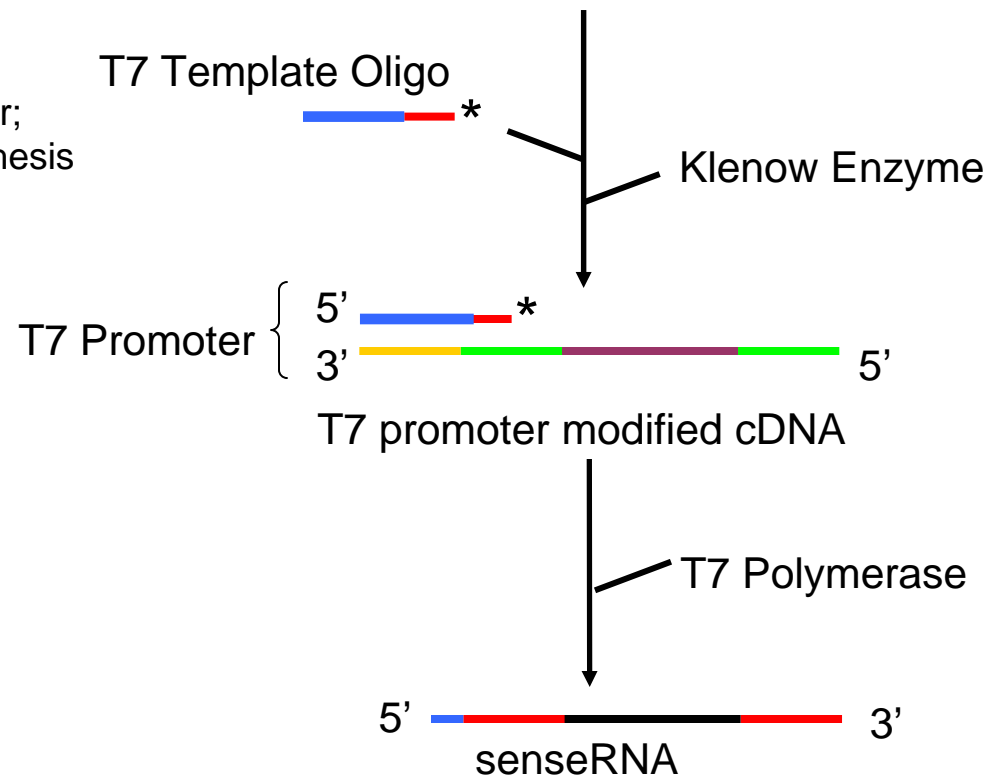


## ③ Tailing of First Strand cDNA



#### ④ T7 Promoter Synthesis

\* = DNA Polymerase blocker;  
prevents second strand synthesis



#### ⑤ *In Vitro* Transcription

① **Poly (A) Tailing:** Poly (A) tails are generated on all RNA molecules.

② **First Strand cDNA Synthesis:** RNA is primed using an Oligo (dT) primer to produce single-stranded cDNA.

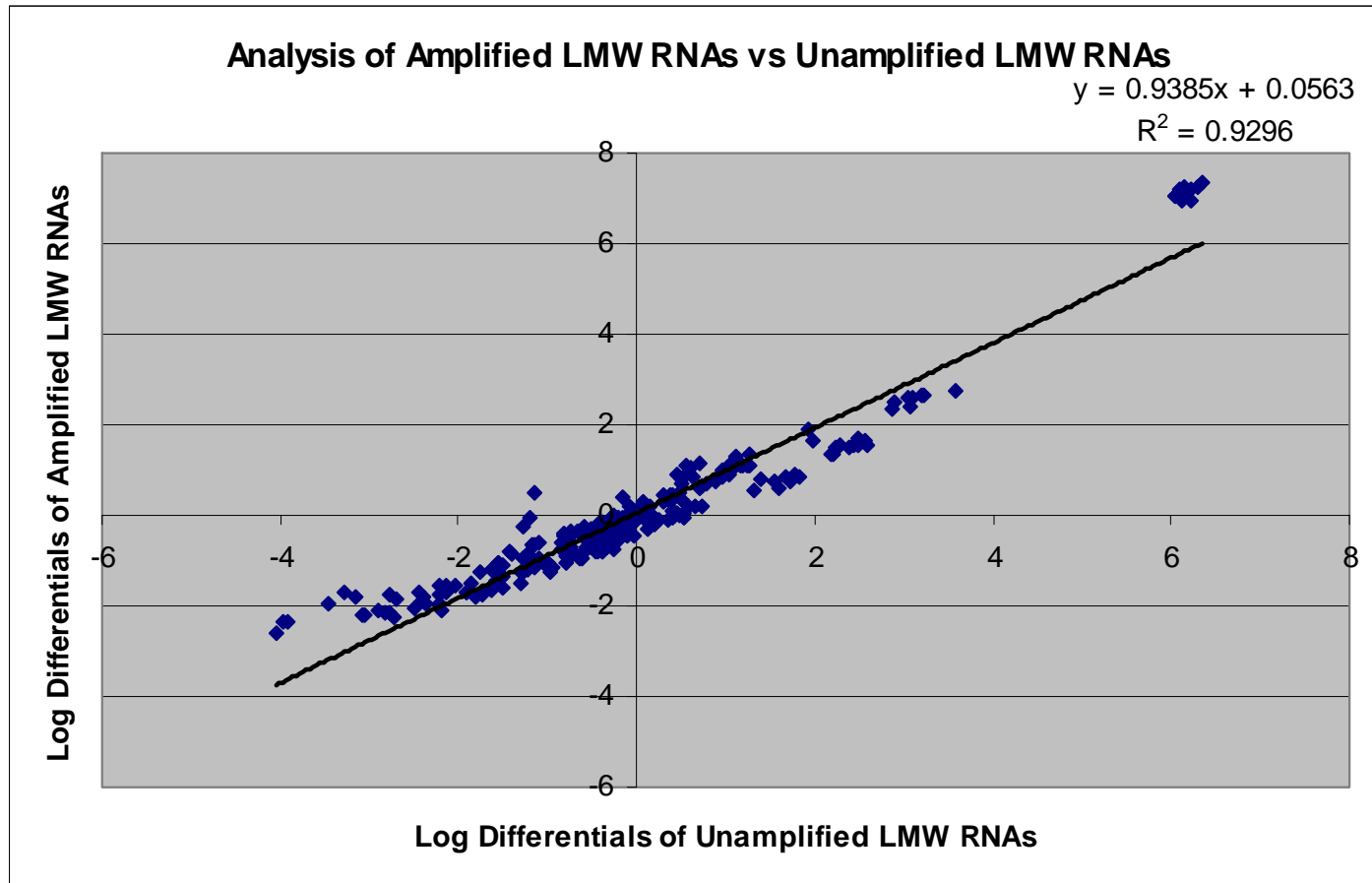
③ **Tailing of First Strand cDNA:** First strand cDNA is tailed with dTTP using Terminal Deoxynucleotidyl Transferase.

④ **T7 Promoter Synthesis:** The T7 Template is annealed to the 3' tail of the cDNA. Klenow enzyme fills in the 3' end of first strand cDNA to produce a double-stranded T7 promoter. The T7 Template contains a blocker to prevent second strand synthesis.

⑤ ***In Vitro* Transcription:** senseRNA copies of the original RNA molecules are generated.

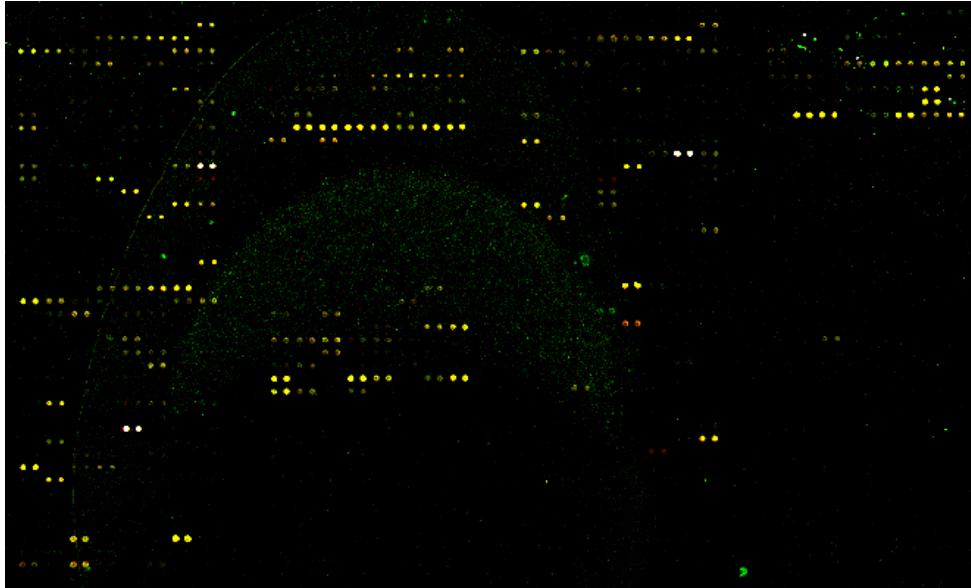
# FlashTag Labeling of senseRNA

Rat Brain and Liver Total RNA were enriched for LMW RNAs with YM100 columns. The LMW RNA enriched from 1ug total was amplified with the SenseAMP Plus LMW kit (Genisphere). After amplification, 17ug senseRNA was obtained for the Brain sample, and 13ug senseRNA was obtained for the Liver sample. 250ng of these amplified RNAs were labeled with FlashTag, and hybridized to NCode microarrays. Parallel FlashTag labeling reactions and hybridizations were run with the LMW RNA from 0.75ug Total RNA (no RNA amplification). The Brain/Liver differentials were compared.



# SenseAmp Plus Amplification of LMW RNA

Amp. 1 (Cy3) vs Amp. 2 (Cy5)

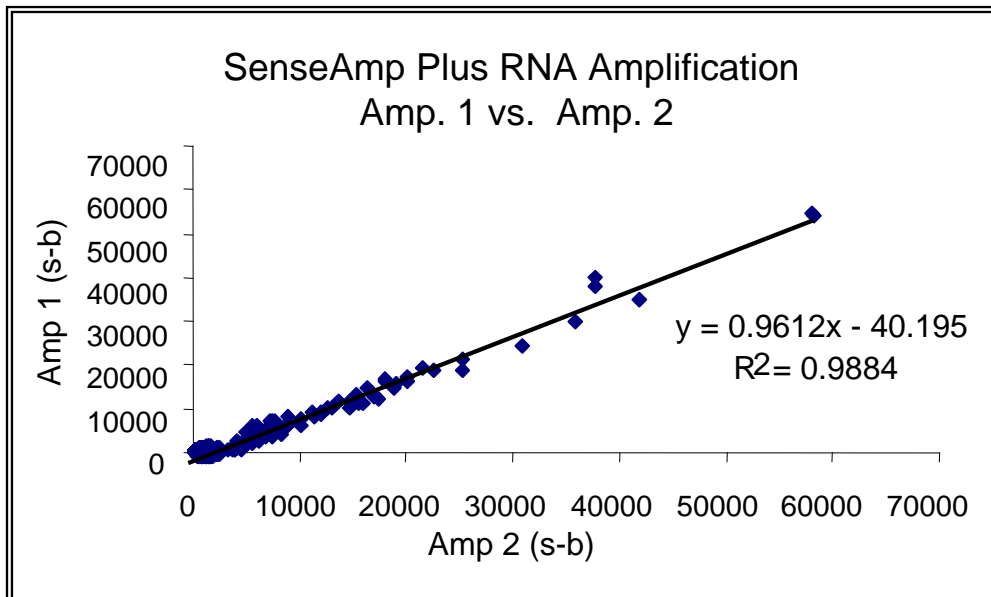


## Reproducibility of Amplification

Experimental Design:

500 picograms of enriched LMW RNA was amplified in duplicate using the SenseAmp Plus amplification protocol for LMW RNA ([www.genisphere.com](http://www.genisphere.com)).

Equal amounts (750ng) of both amplifications were labeled with either Cy3 or Cy5, using the Array 900miRNA DIRECT kit. The labeled senseRNAs were hybridized to a miRMAX array for analysis. The reproducibility of the amplification and labeling was determined by comparing the signal intensities of each of the features in both channels.

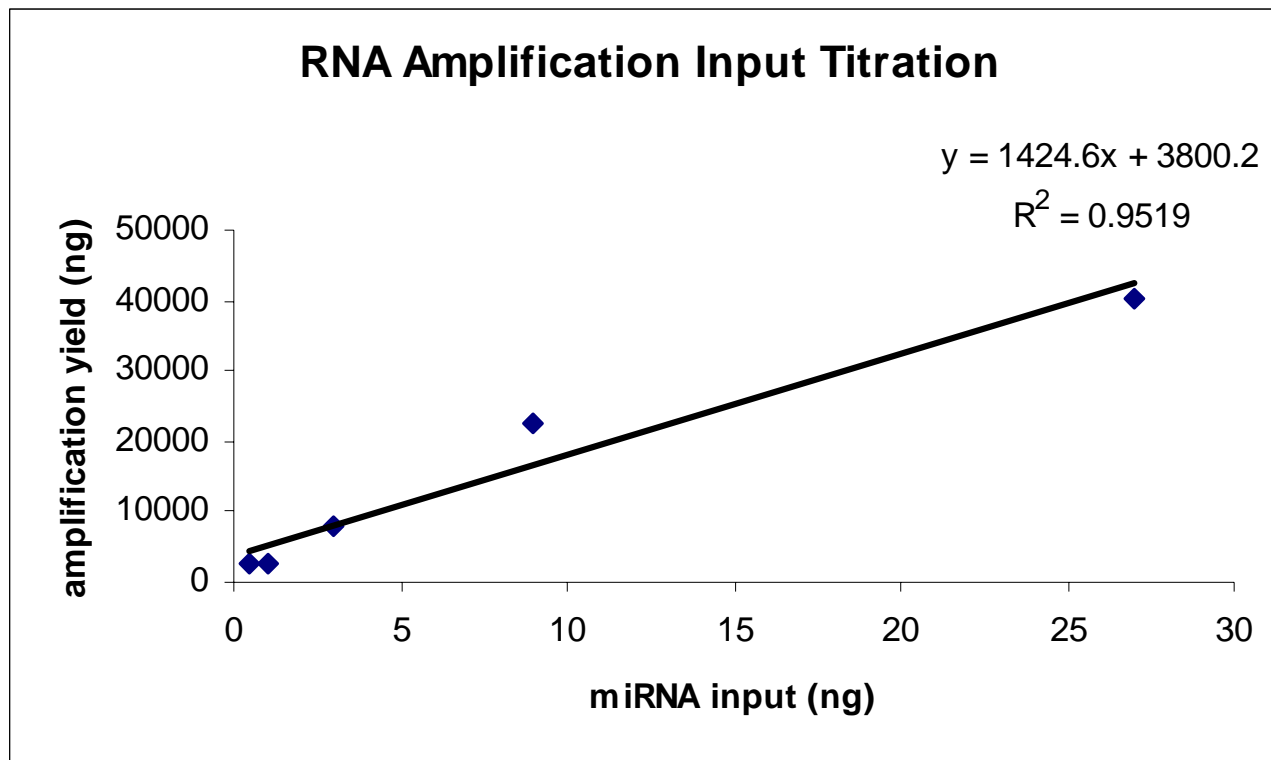


# SenseAmp Plus Amplification of LMW RNA

## Input Titration

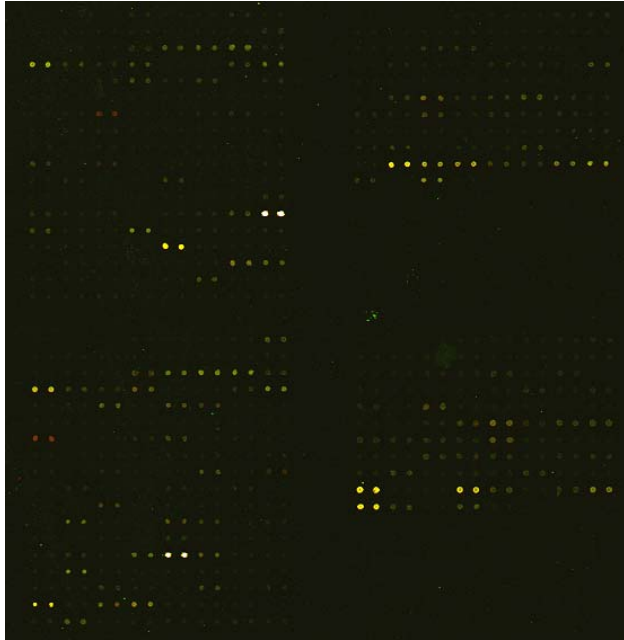
Experimental Design:

Five different input amounts of human LMW RNA were used in the SenseAmp Plus amplification protocol for LMW RNA. The resulting senseRNAs were quantitated using the RiboGreen RNA Quantitation Kit (Molecular Probes).



<i>RNA input (ng)</i>	<i>Yield (ng)</i>
0.5	2664
1	2791
3	8165
9	22736
27	40340

9ng amp input (Cy3) vs 3ng amp input (Cy5)



# SenseAmp Plus Amplification of LMW RNA

## Input Titration - Array Comparison

Experimental design:

Equal portions (1000ng) of the senseRNA resulting from amplification of 1ng, 3ng, and 9ng of human LMW RNA were directly labeled using the Array 900miRNA DIRECT kit. The miRMAX array data were analyzed to compare each respective amplification reaction.

1ng amp input (Cy3) vs 3ng amp input (Cy5)

