A Comparison of Agilent Quick Amp and Low RNA Input Linear Amplification Kits

In an effort to enhance product stability and reproducibility, Agilent has introduced the Quick Amp Labeling Kits for gene expression profiling and other downstream analyses. A comparison of the Low RNA Input Linear Amplification Kits (LRILAK) and the new Quick Amp Labeling Kits shows that both kits are equivalent when used consistently through an entire one-color or two-color gene expression microarray experiment. The Quick Amp kits have also proven to be more reliable in this study, with zero labeling failures observed. This is likely due to minor reformulations and the ISO 13485 compliant manufacturing environment.

Study Purpose and Methodology

The purpose of this study was to demonstrate that the Quick Amp Labeling kit and the LRILAK kit provide equivalent functionality.

The experiments analyzed the factors in each study group in all possible combinations[1] and used four replicates. Experimental design details are shown in **Table 1***. In **Table 1**, MAQC A and MAQC B[2] refer to the RNA samples used in the MicroArray Quality Control (MAQC) Consortium. MAQC A is the Stratagene Universal Human RNA Reference and MAQC B is the Ambion Human Brain Reference RNA. Labeling reactions, array hybridizations and array data extraction were all performed on Agilent Whole Human Genome Microarrays, 4x44K (PN G4112F), using current Agilent procedures and reagents. Arrays were scanned using an Agilent DNA Microarray Scanner equipped with extended dynamic range (XDR) software.

Factorial designs and generalized linear modeling were performed using Minitab 15 software (Minitab, Inc.). Additional statistical analyses were performed using Agilent's GeneSpring GX 9.0 and JMP 6 software (SAS Institute, Inc.).

Measurements	*Factors*	*Levels*
1-Color Labeling, Arrays	Sample	MAQC A, MAQC B
	Kit	LRILAK, Quick Amp
	Kit Lot	1, 2
2-Color Labeling	Sample	MAQC A, MAQC B
	Kit	LRILAK, Quick Amp
	Kit Lot	1, 2
	Label	Cy3, Cy5
2-Color Arrays	Dye Swap	+1, -1
	Kit	LRILAK, Quick Amp
	Kit Lot	1, 2

*4-fold replication across all study groups using full-factorial design

Table 1. Experimental Design Detail

Results and Discussion

In the two-color study, the Quick Amp kits were superior to the LRILAK kits, in cRNA yield and total dye incorporation consistency. While the ratio of incorporated dye to cRNA yield (specific activity) was statistically lower for the new kits, this effect was negligible. Both one-color and two-color studies showed that overall, the LRILAK and Quick Amp kits produced equivalent correlations with the TaqMan reference method. Clustering analysis showed that experimental samples constitute the principal differentiating factor for the data. The effects of using the different labeling kits are minor in comparison **(Figure 1)**.

Researchers are encouraged not to use both kits within the same study, as subtle variations between the kits may lessen the ability to observe small differences in gene expression.

Conclusion

This study showed that while the LRILAK and Quick Amp kits are functionally equivalent for both one- and two-color gene expression experiments, the Quick Amp Labeling Kits are more reliable. The ISO 13485 compliant manufacturing facility and minor reformulations of the Quick Amp Labeling Kits are most likely responsible for the improved reliability.

References

1. Box, G. E. P., W. G. Hunter, *et al*. (1978). *Statistics for Experimenters*. New York, John Wiley & Sons.
2. Shi, L., L. H. Reid, et al. (2006). "The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements." *Nat Biotechnol* **24**(9): 1151-61.

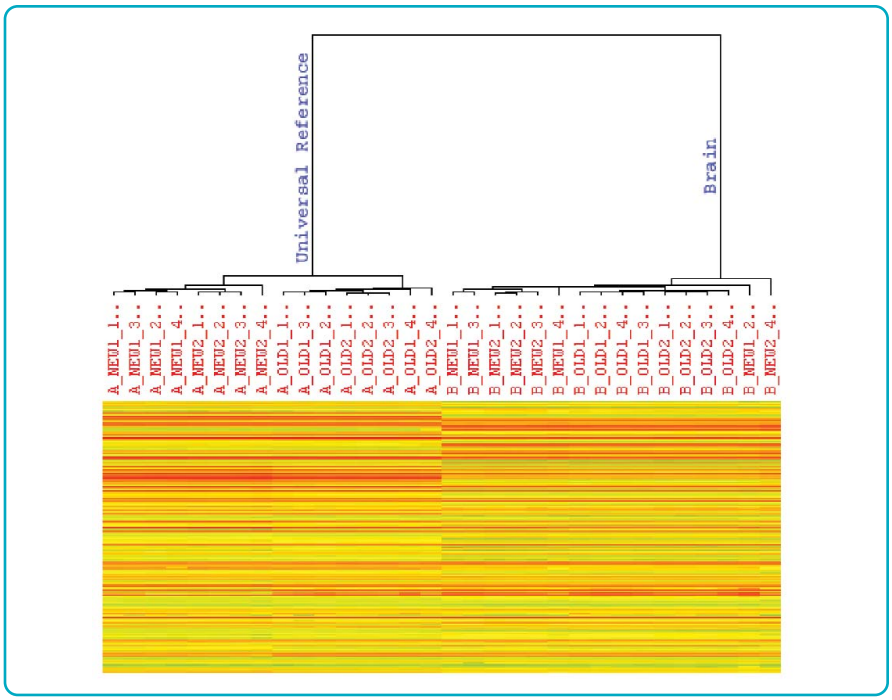

Figure 1: Illustration of one-color data clustering primarily (long branches) according to sample. Secondary clustering (short branches) shows minor differentiation depending on the kit used. Quadruplicate replicates were prepared using two lots of either the "old" LRILAK or the "new" Quick Amp labeling kits and hybridized to four different arrays. GeneSpring GX 9.0 software was used to generate 75th percentile normalized signals for all non-control genes. These were hierarchically clustered using Pearson Correlation.

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