A Comparison of the Genomic DNA Enzymatic Labeling Kit and the Genomic DNA Labeling PLUS Kit

In an effort to enhance product stability and reproducibility, Agilent has introduced the Agilent Genomic DNA Enzymatic Labeling Kit for the labeling of genomic DNA for studies using comparative genomic hybridization (CGH) microarrays. Replacing the Genomic DNA Labeling PLUS Kit, the new kit is produced in Agilent Stratagene's ISO 13485-compliant manufacturing environment. While prior kits have proven to be reliable and of high quality, Agilent continues to guarantee the quality, reliability, and availability of this kit to ensure the best possible performance in a CGH study.

Study Purpose and Methodology

The purpose of this study was to demonstrate whether the Genomic DNA Labeling PLUS Kit and the Genomic DNA Enzymatic Labeling Kit (5190-0449) provide equivalent quality data when performing CGH experiments using Agilent CGH arrays.

A quantity of 500 ng of DNA was used for each sample. A standard pool of normal males was compared to a standard pool of normal females, and a male tumor sample (Burkitt Lymphoma) was compared to a standard pool of normal females. Comparisons were performed using technical replicates plus dye swaps on the 4x44K arrays as well as the 1x244K arrays.

Both the assay and the hybridizations were performed according to protocol (Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis version 5). Arrays were scanned using an Agilent DNA Microarray Scanner (G2565BA).

Log_2 ratios and QC metrics were determined using the Agilent Feature Extraction software version 9.5. Copy number profiles were determined using Agilent's DNA Analytics 4.0 software.

Results and Discussion

To determine whether the new genomic labeling kit performs with the same reliability as the previous kit, QC metrics were compared for samples labeled separately using each kit. **Table 1** provides average scores for a sampling of these QC metrics.

The two kits provided equivalent results, with excellent DLRSpread values and high signal-to-noise ratios. This indicated that the new kit had equal efficiency in labeling the DNA samples and that probe-to-probe noise remained low. Further, both kits provided the same values for the Area Under the ROC Curve (a measure of false positive versus true positive rates) and equivalent responses to copy number changes (displayed as MedianDiff).

	DLRSpread	*Signal-To-Noise Green*	*Signal-To-Noise Red*	*Area Under ROC*	*MedianDiff*
New Kit	0.118 ± 0.004	112 ± 13	138 ± 12	0.97 ± 0.01	0.83 ± 0.03
Old Kit	0.126 ± 0.002	132 ± 9	158 ± 13	0.97 ± 0.01	0.83 ± 0.02

Table 1: QC results for the Genomic DNA Enzymatic Labeling Kit (new) and the Genomic DNA Labeling Kit PLUS (old). Average scores are provided along with standard deviations across six normal female samples hybridized against normal male samples labeled with either the old or the new kit and hybridized to the 4x44K CGH arrays.

The ability to differentiate between single copy number changes using the new Genomic DNA Enzymatic Labeling Kit was further examined. **Figure 1** displays $\log_2$ ratios for all probes from an experiment in which the test sample was a pool of males and the reference sample was a pool of females. The clear separation between probes on the autosomes and the X chromosome indicates that the new labeling kit maintains the ability to differentiate between single copy number changes. Response to copy number is also maintained, as the peak of the autosomal $\log_2$ ratios is positioned at the expected value near 0, and the peak of the X-chromosomal $\log_2$ ratios lands at the expected value near –1.

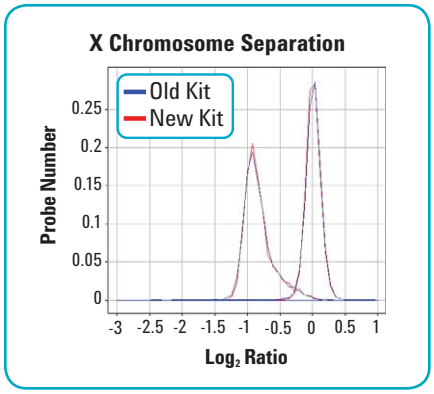

Figure 1: Hybridization of DNA labeled with the Genomic DNA Enzymatic Labeling Kit (new) and the DNA Labeling PLUS Kit (old) using sex-mismatched test and reference DNA. For X-chromosomal probes, where the ratio or XY/XX provides a theoretical value of 0.5, the $\log_2$ ratio is expected to be –1. For autosomal probes, the expected $\log_2$ ratio is 0. Both labeling kits resulted in similar array performance in which $\log_2$ ratios of the expected values were observed.

To visualize the appropriateness of the new kit for copy number studies, a Burkitt Lymphoma sample was included in this study. **Figure 2** provides a snapshot of an approximately 100 kb homozygous deletion on chromosome 2. The same copy number profiles were observed whether using the old or new labeling kit on the 1x244K array. The consistency in array performance regardless of kit used further supports the suitability of replacing the old kit with the Genomic DNA Enzymatic Labeling Kit for future CGH studies.

Conclusion

This study showed that the Genomic DNA Labeling PLUS Kits and the Genomic DNA Enzymatic Labeling Kits are functionally equivalent for CGH experiments using either normal or tumor samples. These results enable transition to the Genomic DNA Enzymatic Labeling Kit with high confidence of obtaining the same quality data that the Agilent CGH arrays have provided in the past. By transitioning, the researcher benefits from the strict guidelines adhered to at Agilent Stratagene's ISO 13485-compliant manufacturing facility. Agilent can now further guarantee the quality, reliability, and availability of the labeling kit for CGH studies.

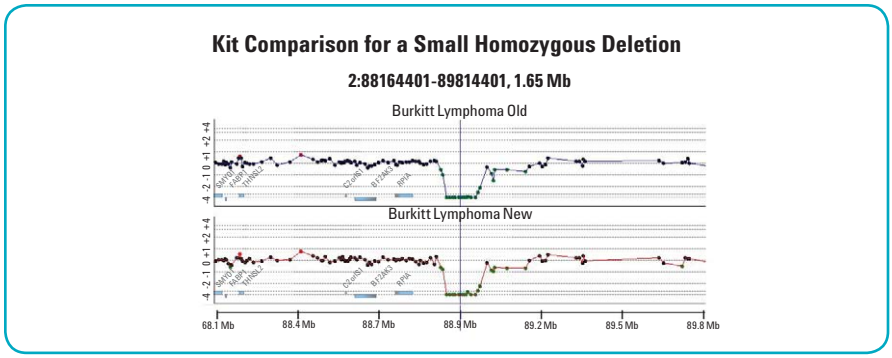

Figure 2: Comparison of Copy Number Profiles Using DNA Labeled by the Genomic DNA Enzymatic Labeling Kit (new) and the DNA Labeling PLUS Kit (old). A homozygous deletion of about 100 kb was observed on chromosome 2 using either kit on the 1x244K array. Raw $\log_2$ ratio values are displayed in DNA Analytics without any averaging (X-axis = genomic position; Y-axis = $\log_2$ ratio).

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