High Resolution Microarray Scanner used to Compare CGH Labeling Methods

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CYTAG™ CGH Labeling Kit (ENZ-42671)

INTRODUCTION

Array-based comparative genomic hybridization (aCGH) is a powerful tool for detecting copy number variations. It is a well-established method used to analyze genomic gains and losses ranging from duplications, deletions, unbalanced translocations, and aneuploidies.

Array CGH is a universal approach that enables the rapid screening of the entire genome. Test DNA and reference DNA are labeled with two different fluorophores and equal amounts are hybridized to a microarray. The microarrays contain either DNA or oligonucleotide probes representing small sections of chromosomes. Array CGH looks at chromosomal imbalances between the test DNA and the reference DNA and is capable of giving a holistic comparison of the genome.

The main objective of this study was to utilize the high resolution Innopsys InnoScan 910 to demonstrate the superior performance of the CYTAG™ CGH Labeling Kit in labeling DNA for CGH analysis on a 4x180 whole genome microarray.

INSTRUMENTATION

The InnoScan 910 is a high resolution microarray scanner developed by Innopsys for the analysis of high density arrays. It scans at 532 nm and 635 nm and has a resolution range of 1-40 µm/pixel and the capability to scan at an extended dynamic range (up to 6 log). In addition to its ease of use and sensitivity, the IS 910 provides advantages in terms of speed (3.5 min for a whole slide at 10 µm/pixel) and pricing.

This scanner is completely compatible to scan Agilent arrays and to use with the Agilent CytoGenomics software. In order to easily obtain great data from the Agilent High Density oligo arrays, the Mapix image acquisition software offers pre-loaded scan configurations with optimized parameters.

MATERIALS

- BioPrime® DNA Labeling kit (Thermo Fisher, 18097-011)
- CYTAG™ CGH labeling kit (Enzo, ENZ-42671)
- Hybridization and blocking buffer (Agilent, 5188-5220)
- 4x180K SurePrint G3 Human CGH Microarray Kit (Agilent, G4449A)
- Wash buffers (Agilent, 5188-5226)
- Cot DNA (Agilent, 5190-3393)
- Hybridization gasket slide kit (Agilent, G2534-60012)
- Hybridization Chamber (Agilent, G2534A)
- DNA, Prader-Willi (Coriell, NA09024)
- DNA, Pooled Female (Promega, G1521)
- Microarray Oven (SciGene, model 777)
- Nanodrop ND-1000 (Thermo Fisher)

METHODS

Labeling

Labeled DNA was prepared by the incorporation of cyanine-labeled nucleotides according to the procedure described below using Enzo's CYTAG™ CGH labeling kit and summarized in Table 1. For each pair of genomic DNAs to be compared, one sample was labeled with cyanine 3 and the other with cyanine 5. For further validation, the labels were swapped in a parallel or subsequent experiment.

Step	Component/Condition	Amount
1. Add	DNA (1 µg)	up to 19 µL
2. Add	Primers/Reaction buffer (Vial 1)	20 µL
3. Add	Nuclease-free water (Vial W)	to 39 µL
4. Incubate	99°C, 20 minutes	
5. Incubate	Ice, 5 minutes	
6. Add	Nucleotide mix (Vial 2 or 3)	10 µL
7. Add	Klenow Exo- DNA polymerase (Vial 4)	1 µL
8. Incubate	37°C, 4 hours	
9. Add	Stop buffer (Vial 5)	5 µL
10. Incubate	65°C, 10 minutes and place on ice	

Table 1: Procedure overview for DNA labeling reaction

Denaturing DNA and annealing random primers
1000 ng of genomic DNA was combined with 20 µl of Primers/Reaction buffer (Vial 1). The reaction mixture was then brought to 39 µL with Nuclease-free water (Vial W). The DNA was denatured at 99°C for 20 minutes and placed on ice for 5 minutes. After a brief centrifugation, samples were returned to ice.

Extending primers with Klenow Exo- DNA polymerase
While on ice, 10 µl of the appropriate cyanine dye-labeled Nucleotide mix (Vial 2 or 3) and 1 µl of Klenow Exo- DNA polymerase (Vial 4) were added to the primer-annealed DNA sample. Tubes were flicked gently to mix contents, briefly centrifuge and incubated at 37°C for 4 hours. 5 µL of Stop Buffer was added to the DNA samples. Tubes were mixed gently, briefly centrifuged and incubated at 65°C for 10 minutes and placed on ice.

Purifying the labeled DNA
Each labeling reaction should be purified separately. PCR and Gel clean-up columns from Enzo Life Sciences were used and the manufacturer's protocol was followed. Samples were mixed with 110 µL Binding Buffer, and then applied to the columns and centrifuged at 11,000 x g for 30 seconds. 700 µL of Wash Buffer was added to the columns and incubated for 1 minute at room temperature. Columns were centrifuged at 11,000 x g for 30 seconds and flow-through was discarded. This wash was repeated one time. The columns were placed back in the collection tube and spun at 11,000 g for 2 minutes to remove all traces of liquid. The columns were transferred to clean 1.5mL microcentrifuge tubes. 25 µL of Elution Buffer, pre-warmed to 50°-65°C was added to each column and incubated for 1 minute at room temperature. The DNA was eluted by centrifugation at 11,000 x g for one minute. The elution step was repeated with a second 25µL of Elution Buffer to remove all remaining DNA from the column, for a total volume of around 50µL.

Depending on the requirements of the hybridization platform, volume reduction may be required. A SpeedVac concentrator was used to bring the sample volume down. Genomic DNA should not be excessively dried as pellets might become difficult to resuspend.

Labeling and Purification using the BioPrime® kit
1 µg of either Prader-Willi male DNA (Coriell Institute for Medical Research) or pooled human female DNA (Promega) was labeled as recommended by the manufacturer of the BioPrime kit (ThermoFisher).

Determining DNA yield and dye incorporation
A NanoDrop® ND-1000 UV-VIS Spectrophotometer from ThermoFisher was used in the Microarray Measurement Mode to determine yield and dye incorporation in 1.5 µl from each sample. For a typical labeling reaction, with an input of 1000 ng of high quality genomic DNA, the expected yield should be at least 5 µg. This DNA should contain either at least 300 pmol of incorporated Cyanine 3 or at least 200 pmol of incorporated Cyanine 5. DNA yield and dye incorporation obtained with CGH labeling kit for oligo arrays from Enzo Life Sciences were compared with DNA yield and dye incorporation achieved with BioPrime® DNA labeling kit from ThermoFisher Scientific.

Hybridization & Scanning

Preparing the labeled DNA for hybridization on Agilent Oligonucleotide Array

Cyanine 3- and cyanine 5-labeled DNA eluates were combined and the volume brought up to the volume specified by Agilent for SurePrint® G3 human CGH 4x180K microarrays. DNA preparation, pre-hybridization, hybridization and array washing were performed as indicated for 4x180K microarrays in the Agilent genomic DNA analysis protocol for oligonucleotide array-based CGH (version 7.3, March 2014).

The hybridized arrays were scanned within 2 hours of the end of hybridization and washing, using an Innopsys InnoScan 910 scanner using the Mapix software. The autogain feature was used to obtain the optimal gain setting. Selecting the tab for 4x 180K arrays automatically set the scan area and scan resolution.

Scans were analyzed using the Agilent CytoGenomics software (3.0.4.1).

RESULTS

Comparative analysis of DNA yield and specific activity

Measurement of DNA yield and dye incorporation allows the evaluation of the quality of the DNA labeling for CGH analysis. High dye incorporation also correlates with increased accuracy of variant detection and minimized manual data analysis. Using either Enzo's CYTAG™ CGH labeling kit or ThermoFisher's BioPrime® DNA labeling kit with 1 µg input yielded greater than 6 µg DNA (Fig. 1A). Dye incorporation was, however, significantly different, especially with Cy5 ($p<0.001$, using the Mann-Whitney U-test). Specific activity for Cy3 was 65.7 pmole dye/µg DNA for the Enzo CYTAG™ kit and 16.6 pmole dye/µg DNA for the BioPrime® kit. (Fig.1B). Cy5 also had a significant difference in Dye incorporation between the two kits ($p<0.01$), with the CYTAG™ kit yielding 44.2 pmole dye/µg DNA and the BioPrime® kit yielding 23.5 pmole dye/µg DNA.

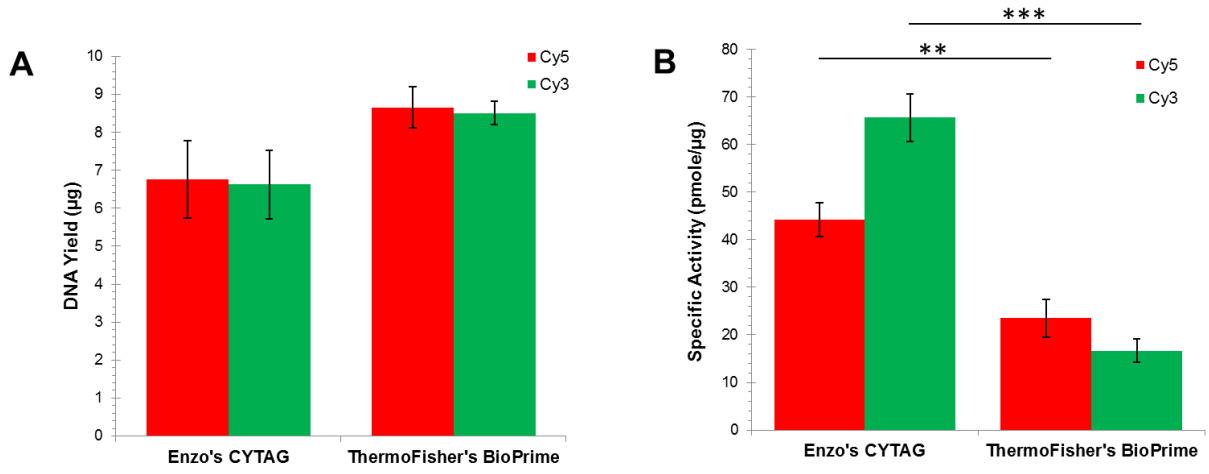

Figure 1 DNA yield and specific activity comparative analysis. DNA yield (A) and specific activity (B) after labeling with CYTAG™ CGH labeling kit from Enzo Life Sciences or BioPrime® DNA labeling kit from ThermoFisher and measurement using NanoDrop ND-1000 spectrophotometer. Statistical significance was determined using the Mann-Whitney U-test (**$p<0.01$; ***$p<0.001$ with $n >= 13$ samples).

Comparative analysis of derivative log ratio (DLRS)

Low derivative log ratios (DLRs) reduce the need for experimental repeats. Derivative log ratios were significantly lower ($p<0.05$) using Enzo's CYTAG™ CGH labeling kit with DLRs around 0.12 when compared with ThermoFisher's BioPrime® DNA labeling kit having DLRs around 0.2 (see figure 2).

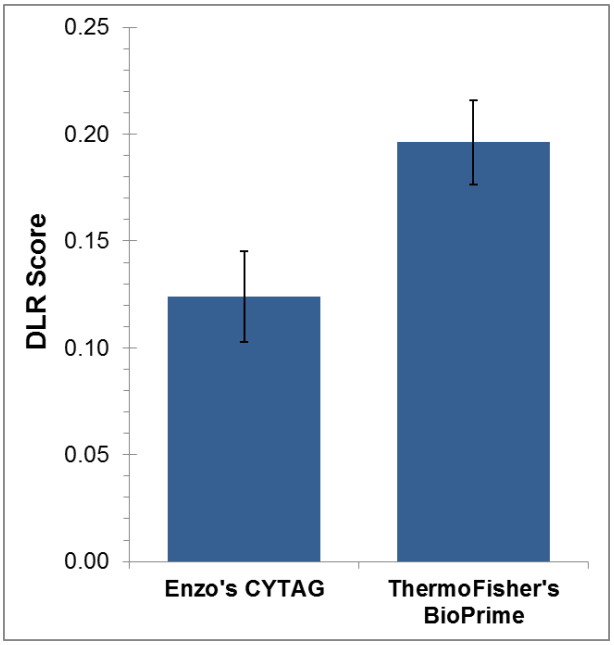

Figure 2: Derivative log ratio comparative analysis. Measurement of derivative log ratio (DLRS) after labeling with CYTAG™ CGH labeling kit from Enzo Life Sciences or BioPrime® DNA labeling kit from ThermoFisher, hybridization on a SurePrint® G3 human CGH 4x180K microarray and scanning.

Superior labeling technology results in more uniform dye incorporation so that comparisons between genomes is done at higher resolution and with improved signal-to-noise ratios. High quality data provides fewer errors (false positive or false negative) and less time with manual analysis of the data, thereby increasing efficiency. Analysis of segments of chromosomes 15, 1 and all of chromosome 3 of Prader-Willi DNA samples were compared to pooled female DNA labeled with Enzo's CYTAG™ CGH labeling kit (Fig. 3A, 3C and 3E, respectively) or ThermoFisher's BioPrime® DNA labeling kit (Fig.3B, 3D and 3F, respectively). The results demonstrated dramatically improved signal-to-noise ratio, higher efficiency with fewer false positives and false negatives as well as a higher resolution when using Enzo's CYTAG™ CGH labeling kit.

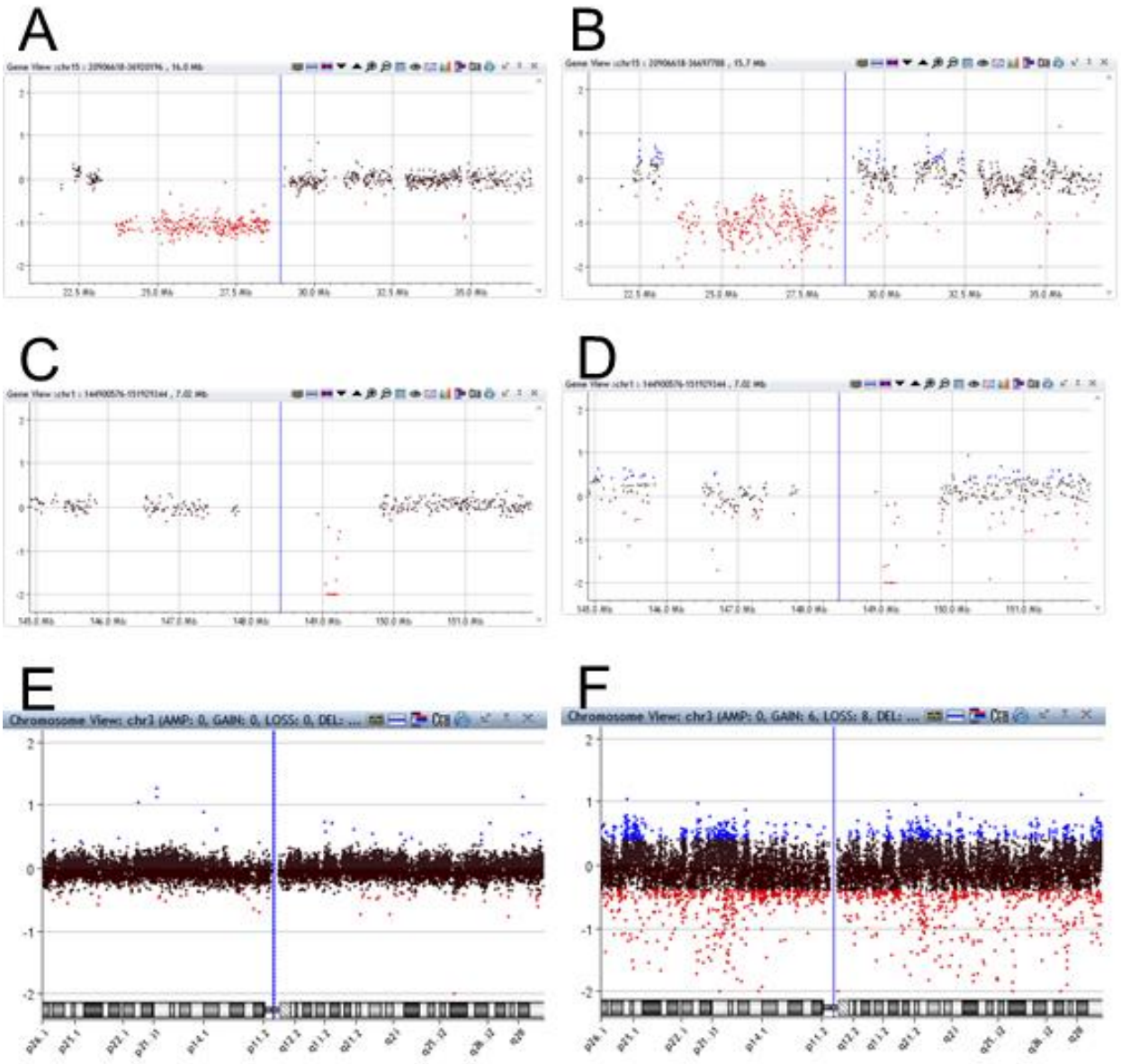

Figure 3: Comparative analysis of segments of chromosomes 15 and 1, and all of chromosome 3 in Prader-Willi DNA sample. Upon scanning, the quality of the labeling in DNA samples was visually inspected. Segments of chromosomes 15 (A and B) and 1 (C and D) were selected to demonstrate detection of known deletions and the superior labeling obtained with the CYTAG™ CGH labeling kit from Enzo Life Sciences (A, C and E) when compared with the BioPrime® kit from ThermoFisher (B, D and F). Chromosome 3 (E and F) demonstrates clean results with no significant deletions.

CONCLUSION

The aim of this work was to demonstrate the superiority, in terms of specificity, of the Enzo's CYTAG™ CGH labeling kit compared to the BioPrime® kit from ThermoFisher. The results demonstrate that although the BioPrime® kit produces more DNA, the CYTAG™ kit incorporates more label. The results after hybridization to an Agilent SurePrint® G3 human CGH 4x180K microarray show that the Enzo CYTAG™ kit yields lower DLR scores, and the plots are much easier to interpret.

The high specific activity and low DLR scores make the Enzo's CYTAG™ CGH labeling kit an ideal choice for labeling precious samples for CGH array analysis.

Visit www.enzolifesciences.com/cgh for more information about our CYTAG™ CGH Labeling Kit including:

- References
- Cited Samples
- Other Application Notes

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