# Using CYTAG™ TotalCGH Labeling Kit for CGH + SNP Array

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## CYTAG™ TotalCGH Labeling Kit (ENZ-42674)

### INTRODUCTION

Array CGH is a well-established method for investigating copy number variations. It is used to detect a variety of genomic gains and losses ranging from copy number variants, duplications and deletions, unbalanced translocations, and aneuploidies.

In an array CGH experiment, test DNA and reference DNA are labeled with two different fluorophores. Equal amounts are combined and hybridized to a microarray containing DNA or oligonucleotide probes representing small sections of chromosomes. This holistic approach allows the rapid screening of the entire genome while also enabling the investigation of changes in sequence of a particular section of a chromosome.

In addition, single nucleotide polymorphism (SNP), variation at a single nucleotide in a genome, can also be examined on a CGH+SNP array. A CGH+SNP array enables detection of genomic aberrations associated with copy number variations, but in addition, it can also detect copy neutral aberrations, such as loss of heterozygosity (LOH) and uniparental disomy (UPD), on the same array.

The main objective of this study was to demonstrate the superior labeling capability of the CYTAG™ TotalCGH Labeling Kit in preparing and labeling DNA for a CGH+SNP microarray, and further, to analyze the chip on the Innopsys InnoScan 910.

### 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, 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 fully 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

- SureTag DNA Labeling Kit (Agilent, 5190-4240)
- CYTAG™ TotalCGH labeling kit (Enzo, ENZ-42674) which includes:
  - CYTAG™ CGH Labeling kit (Enzo ENZ-42671)
  - AluI (Enzo, ENZ-GEN108)
  - RsaI (Enzo, ENZ-GEN109)
  - PCR & Gel Clean-up Columns (Enzo, ENZ-GEN100)
- Hybridization and blocking buffer (Agilent, 5188-5220)
- SurePrint G3 ISCA CGH+SNP Microarray Kit, 4x180K (Agilent, G4890)
- 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)

## METHODS & RESULTS

### Labeling

Labeled DNA was prepared by first digesting the DNA with *Alu*I and *Rsa*I, followed by the incorporation of cyanine-labeled nucleotides according to the procedure described below using the Enzo CYTAG™ CGH labeling kit and summarized in Table 2. 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 are swapped in a parallel or subsequent experiment.

| Step | Component/Condition | Amount |
|---|---|---|
| 1. Add | DNA (1 µg) | up to 16.34 µL |
| 2. Add | 10X *Alu*I/*Rsa*I buffer | 1.9 µL |
| 3. Add | *Alu*I (10 ū/µL) | 0.38 µL |
| 4. Add | *Rsa*I (10 ū/µL) | 0.38 µL |
| 5. Add | Nuclease-free water (Vial W) | up to 16.34 µL |
| 6. Incubate | 37°C, 2 hours | |
| 7. Incubate | 65°, 15 minutes | |
| 8. Add | Primers/Reaction buffer (Vial 1) | 20 µL |
| 9. Incubate | 99°C, 20 minutes | |
| 10. Incubate | Ice, 5 minutes | |
| 11. Add | Nucleotide mix (Vial 2 or 3) | 10 µL |
| 12. Add | Klenow Exo$^-$ DNA polymerase (Vial 4) | 1 µL |
| 13. Incubate | 37°C, 4 hours | |
| 14. Add | Stop buffer (Vial 5) | 5 µL |
| 15. Incubate | 65°C, 10 minutes and place on ice | |

**Table 1: Procedure overview for DNA labeling reaction**

*Cleaving DNA with AluI and RsaI*

1000 ng of genomic DNA was combined with 1.9 µL of the restriction enzyme buffer (10X), and the reaction mixture was brought up to 18.24 µL with nuclease free water (Vial W). 0.38 µL of *Rsa*I and 0.38 µL of *Alu*I were added, this was mixed, and the contents centrifuged briefly, and incubated at 37°C for 2 hours. The restriction enzymes were inactivated at 65°C for 15 minutes.

*Denaturing DNA and annealing random primers*

The restricted DNA was combined with 20 µL of Primers/Reaction buffer (Vial 1). The DNA was denatured at 99°C for 20 minutes and placed on ice for 5 minutes. After a brief centrifugation, samples were returned on 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 centrifuge and incubated at 65°C for 10 minutes and placed on ice.

*Purifying the labeled DNA*

Each labeling reaction should be purified separately. Enzo PCR & Gel clean-up columns from Enzo Life Sciences were used and the following 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 after 1 minute at room temperature were centrifuged at 11,000 x g for 30 seconds, and the flow-through was discarded. This wash was repeated one time. The columns were placed back in the collection tube and spun at 11,000 x g for 2 minutes to remove all traces of liquid. The columns were transferred to clean 1.5 mL micro-centrifuge tubes. 25 µL of Elution Buffer pre-warmed to 50°-65°C was added to each column, after one 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 25 µL of elution buffer to remove all remaining DNA from the column, for a total elution volume of around 50 µL.

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 SureTag® kit*

1 µg of either Prader-Willi male DNA (Coriell Institute for Medical Research) or the single source control male DNA (Agilent) was labeled as recommended by the manufacturer of the SureTag® kit (Agilent).

*Determining DNA yield and dye incorporation*

A NanoDrop® ND-1000 UV-VIS Spectrophotometer from Thermo 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 SureTag® DNA labeling kit from Agilent.

### Hybridization & Scanning

*Preparing the labeled DNA for hybridization on Agilent Oligonucleotide CGH+SNP 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 and Mapix software. By Selecting the Oligo 4 x 180K slide configuration together with the autogain, the scan was done under the optimal gain settings to get appropriate signals in both channels automatically. The final image is fully compatible with the Agilent's image analysis software Feature Extraction. 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 Enzo's CGH labeling kit with 1 µg input yielded greater than 6 µg DNA (Fig. 1A). The SureTag kit yielded slightly more DNA (Fig. 1B). Dye incorporation was, however, significantly different, especially with Cy5 ($p<0.001$, using the T-test). Specific activity for Cy3 was 66 pmole dye/µg DNA for the Enzo kit and 28 pmole dye/µg DNA for the SureTag kit. (Fig. 1B). Cy5 also had a significant difference ($p<0.001$, using the T-test) in Dye incorporation between the two kits, with the Enzo kit yielding 41 pmole dye/µg DNA and the SureTag kit yielding 27 pmole dye/µg DNA.

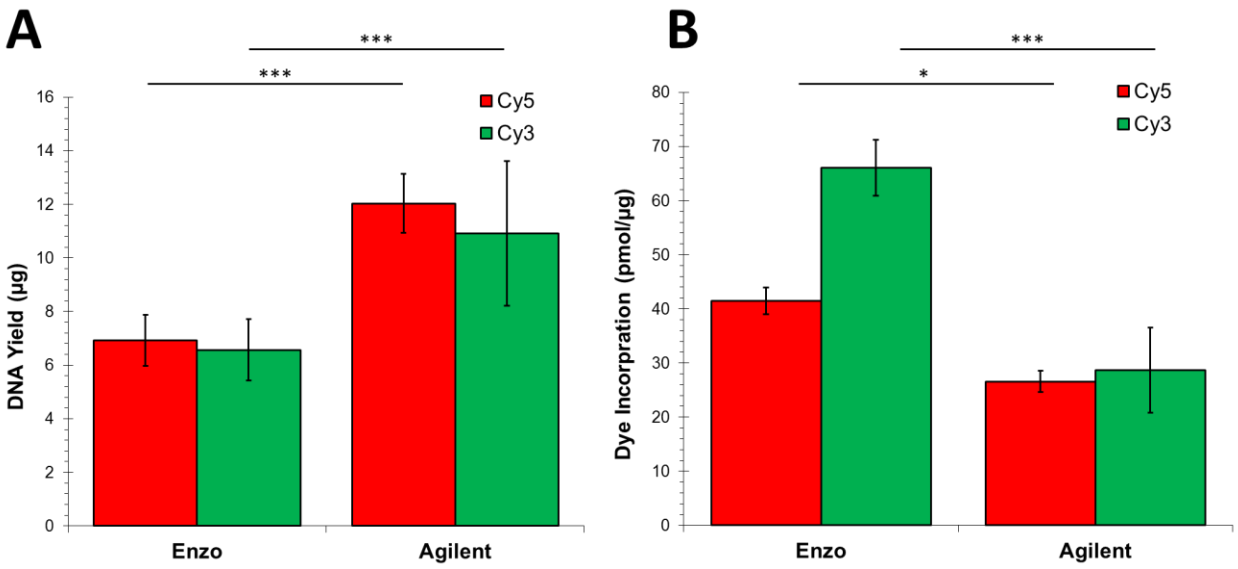


**Figure 1 DNA yield and specific activity comparative analysis.** DNA yield (A) and specific activity (B) after labeling with CGH labeling kit from Enzo Life Sciences or SureTag® DNA labeling kit from Agilent and measurement using NanoDrop ND-1000 spectrophotometer. Statistical significance was determined using the T-Test (*p<0.05; ***p<0.001 with n >= 6 samples).

*Comparative analysis of derivative log ratio (DLRs)*

Low derivative log ratios (DLRs) reduce the need for experimental repeats. Derivative log ratios were lower using Enzo's CGH labeling kit with DLRs of 0.11 when compared with SureTag's DNA labeling kit having DLRs of 0.25 (see figure 2).

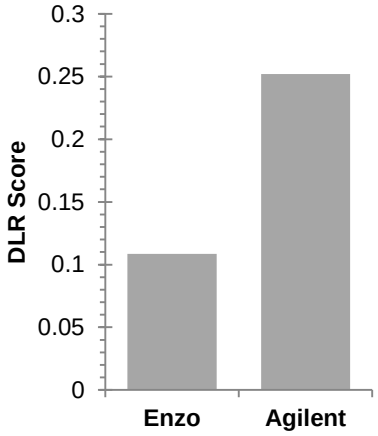


**Figure 2: Derivative log ratio comparative analysis.** Measurement of derivative log ratio (DLR) after labeling with the CYTAG™ TotalCGH labeling kit from Enzo Life Sciences or SureTag® DNA labeling kit from Agilent, hybridization on a SurePrint G3 ISCA CGH+SNP Microarray Kit, 4x180K microarray and scanning.

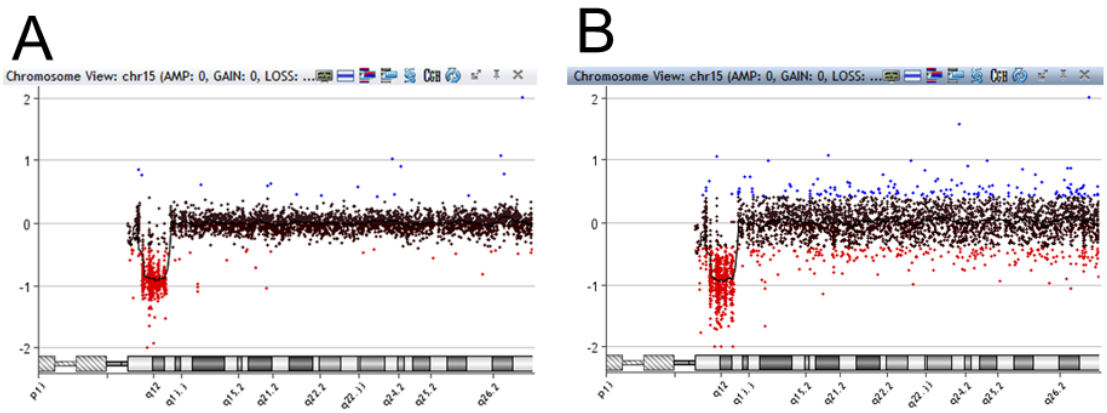


**Figure 3: Comparative analysis of chromosomes 15**

Upon scanning, the quality of the labeling in DNA samples was visually inspected to demonstrate detection of known deletion in the Prader-Willi DNA. This demonstrates the superior labeling obtained with the CGH labeling kit from Enzo Life Sciences (A) when compared with the SureTag® kit (B).

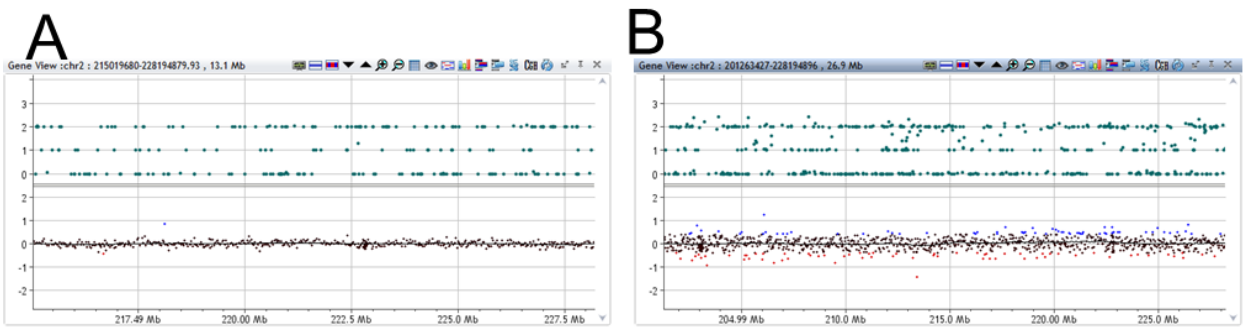


**Figure 4: SNP analysis of a segment of chromosome 2**

Scanning of DNA labeled with the Enzo kit (A) or the SureTag kit (B).

Figure 4A shows that the Enzo kit cleanly identifies the sequence at single nucleotide polymorphisms (SNPs). Quantification of the SNP analysis of DNA labeled with the SureTag labeling system (Fig 4B) are not as clean.

## CONCLUSION

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

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

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

- References
- Cited Samples
- Other Application Notes

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