A Comparative Study of the CYTAG® CGH and CYTAG® SuperCGH DNA Labeling Kits to Detect CNVs With Low Amounts of DNA

Mosca-Boidron Anne-Laure[1], Marle Nathalie[1], Payet Muriel[1], Ragon Clémence[1], Szczepaniak Justine[1], Poulleau Marlène[1], Callier Patrick[1]
[1]Laboratoire de Génétique Chromosomique et Moléculaire, Plateau technique de biologie,
Centre Hospitalier Universitaire Dijon, 21079 Dijon, France

INTRODUCTION

Array comparative genomic hybridization (aCGH) is a powerful clinical diagnostic tool that can be used to evaluate copy number variation (CNV) changes in the genome. The detection of CNVs depends on the quality and quantity of DNA, especially for prenatal or oncological samples. The principle of aCGH is based on the quantitative comparison between a test DNA and a reference DNA, which depends on the labeling of DNA. Until now, aCGH in diagnosis has been done using more than 1 µg of DNA, as recommended by several manufacturers. Here, we present a new aCGH labeling kit called CYTAG® SuperCGH Labeling Kit that accurately detects CNVs with less than 100ng of DNA and compare this new kit with the one previously used in our laboratory, namely CYTAG® CGH Labeling Kit.

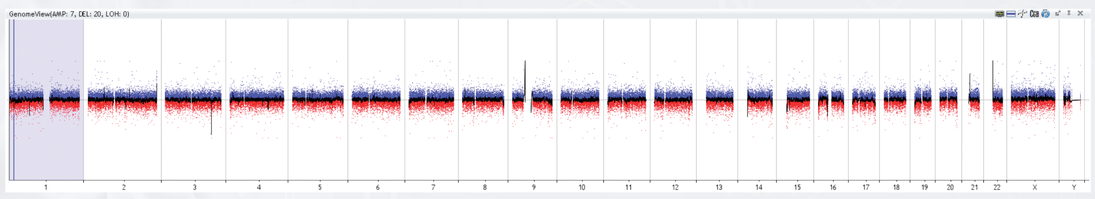
METHODS AND RESULTS

Validation of the labeling procedure with this new kit included a comparison between the two commercial kits on less than 100 ng of seven different samples with DNA isolated from cultured amniotic fluid (prenatal; 90 ng), blood (postnatal; 90 ng, 50 ng, and 30 ng), and frozen tissue from three separate glioma cases (96 ng, 32 ng, and 8ng). CNVs in prenatal, postnatal and glioma samples were compared. QC metrics were compared for samples labeled separately using each kit.

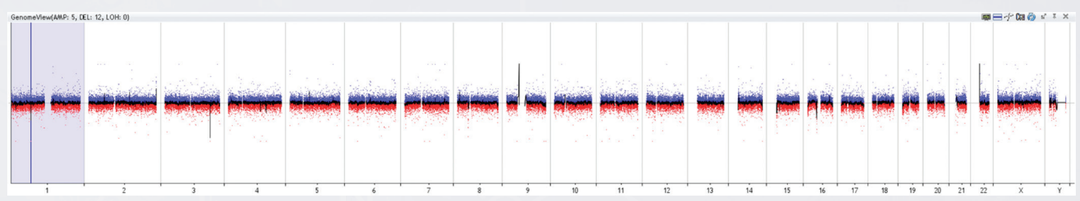
For the prenatal case, DLRS were 0.21 and 0.16 with the traditional and the new labeling kit, respectively. For the postnatal case, DLRS were 0.14 vs 0.12 (90 ng), 0.17 vs 0.13 (50 ng), 0.21 and 0.14 (30 ng). Finally, DLRS were 0.43 vs 0.34 and 0.26 vs 0.21 for two of the three glioma cases. The glioma sample with only 8 ng of DNA gave a DLRS of 0.19 with the new kit but no comparison was possible as there was not enough DNA for the other kit. Altogether, these results indicate that the two kits allowed the detection of CNVs but with significantly better DLR spread values and higher signal-to-noise ratios on low-input DNA samples when using the new CYTAG® SuperCGH Labeling Kit.

A

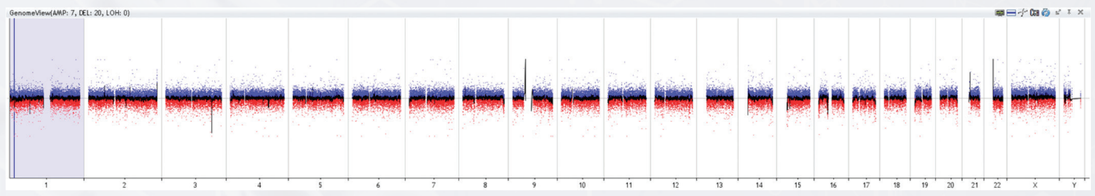
Prenatal (90 ng); CYTAG® CGH Labeling Kit; DLRS: 0.21



Prenatal (90 ng); CYTAG® SuperCGH Labeling Kit; DLRS: 0.16



Postnatal (90 ng); CYTAG® CGH Labeling Kit; DLRS: 0.14



Postnatal (90 ng); CYTAG® SuperCGH Labeling Kit; DLRS: 0.12

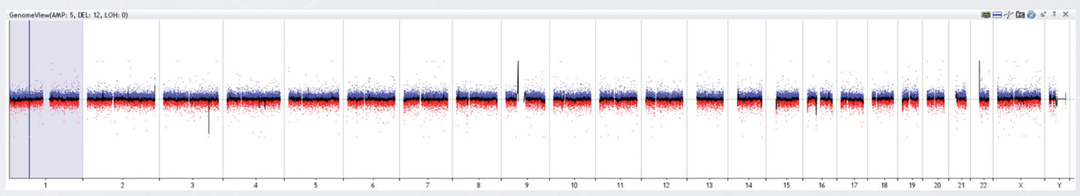
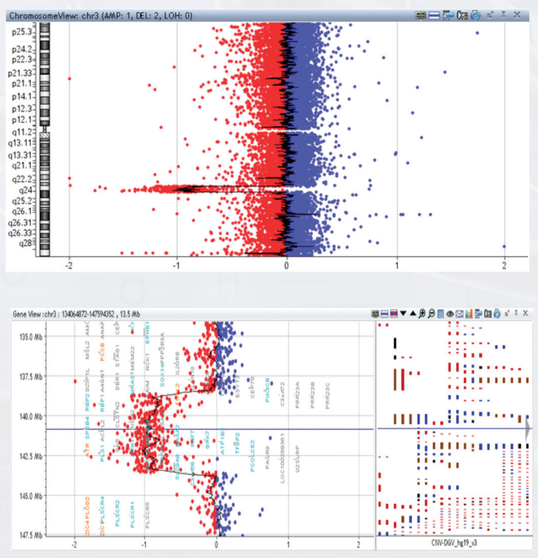


Figure A: aCGH profiles showing higher signal-to-noise ratios when labeling 90 ng of DNA isolated from either cultured amniotic fluid (prenatal) or blood (postnatal) with the new CYTAG® SuperCGH Labeling Kit.

B

Postnatal (30 ng); CYTAG® CGH Labeling Kit; DLRS: 0.21



Postnatal (30 ng); CYTAG® SuperCGH Labeling Kit; DLRS: 0.14

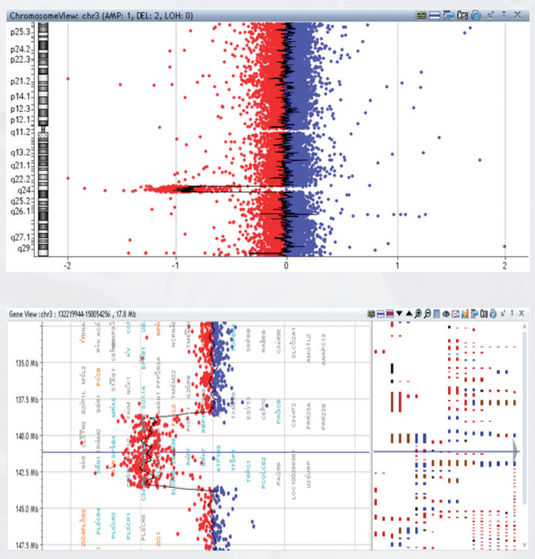


Figure B: Oligonucleotide microarray (Agilent 4x180k) demonstrating a 3q22.3q24 deletion of 4.9 Mb in a postnatal case using 30 ng of DNA.

C

Glioma (8 ng); CYTAG® SuperCGH Labeling Kit; DLRS: 0.19

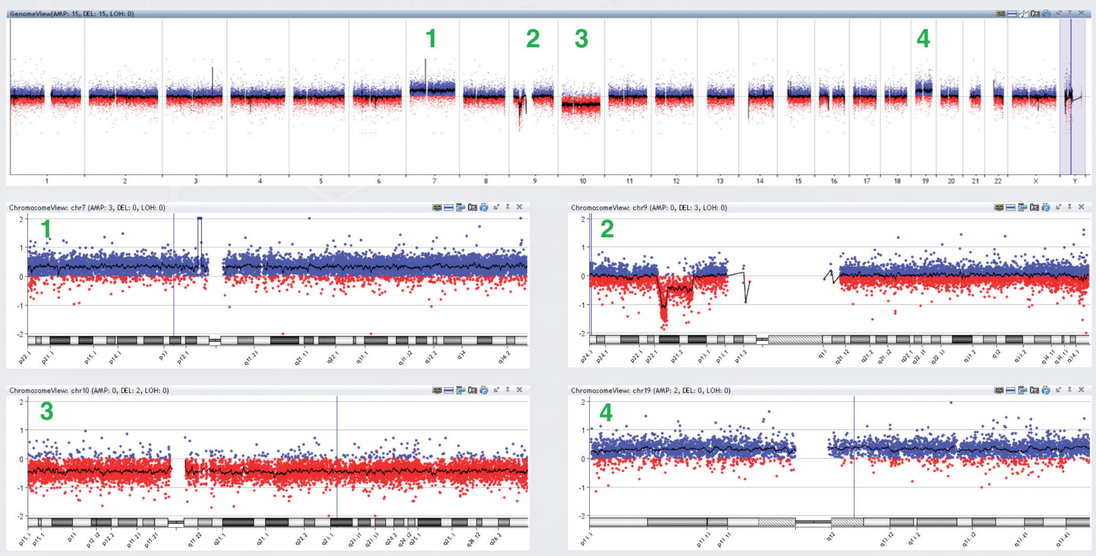


Figure C: Oligonucleotide microarray (Agilent 4x180k) demonstrating multiple genomic aberrations at chromosomes 7, 9, 10, and 19 (panels 1, 2, 3, and 4, respectively) in a glioma case using 8 ng of DNA.

DISCUSSION AND CONCLUSION

Requirements for validation were achieved successfully and the comparative study showed that the labeling efficiency of the CYTAG® SuperCGH Labeling Kit was higher than that achieved with the CYTAG® CGH Labeling Kit. This study showed that the CYTAG® SuperCGH Labeling Kit was especially useful for CGH experiments using prenatal, postnatal and tumor samples. Moreover, we showed that CNVs in glioma samples could be detected in as little as 8 ng of DNA. This technology is particularly useful in diagnosis where small amounts of DNA are available, and offers new possibilities for aCGH analysis in prenatal, postnatal or oncology, where the samples are poor and precious.

REFERENCES

A.L. Mosca-Boidron, *et al.* An improved method to extract DNA from 1ml of uncultured amniotic fluid from patients at less than 16 weeks' gestation. PLoS One. (2013) 8: e59956.

J. Aury-Landas, *et al.* Germline copy number variation of genes involved in chromatin remodelling in families suggestive of Li-Fraumeni syndrome with brain tumour. Eur. J. Hum. Genet. (2013) 21: 1369.

J.W. Ahn, *et al.* A new direction for prenatal chromosome microarray testing: software-targeting for detection of clinically significant chromosome imbalance without equivocal findings. Peer. J. (2014) 2: e354.

J.W. Ahn, *et al.* Validation and implementation of array comparative genomic hybridisation as a first line test in place of postnatal karyotyping for genome imbalance. Mol. Cytogenet. (2010) 3: 9.