Gene Copy Number analysis using semi-quantitative multiplex PCR-based assay on capillary electrophoresis systems

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INTRODUCTION

Large genomic rearrangements such as duplications and deletions have been recognized as pathogenic mutations for many diseases. These types of mutations are thought to represent 5.5% of reported mutations(1). However, given that mutation scans have not included searches for deletions and duplications, it seems likely that these figures are an underestimate of the actual number(1). Detection of genomic rearrangements is technically challenging and is typically done using techniques such as Southern blot analysis or Fluorescent In Situ Hybridization (FISH). These techniques often require high quantities of DNA or can be time-consuming and laborious, therefore limiting the efficiency of molecular screening. To better facilitate the detection of such Copy Number Variations (CNVs), researchers have developed simple, semi-quantitative methods using a multiplex PCR-based assay of short fluorescent fragments(1,2) on Applied Biosystems capillary electrophoresis (CE) platforms. In this poster, we highlight this technique on the Applied Biosystems 3730/3730xl DNA Analyzers.

MATERIALS AND METHODS

CNV assays consist of the simultaneous amplification and fluorescent labeling of short, specific DNA loci, using a limited number of cycles to allow accurate quantitation in the exponential amplification range. Each multiplex PCR will yield a pattern composed of fluorescent peaks, with each peak corresponding to a specific DNA locus. The comparison of fluorescence is done between the same peaks generated from different samples, controls and suspect samples (Figure 1).

Targeted loci for BRCA1, BRCA2, MLH1/MSH2 genes and 9p21 region were amplified using dye-labelled primers from DNA that had been isolated from blood. Amplified samples were then run on an Applied Biosystems capillary electrophoresis platform, and the data was analyzed using GeneMapper® Software version 4.0.

GeneMapper® Software version 4.0 provides new features especially useful for Copy Number analysis:

1. AFLP® Analysis Method - for sample normalization by sum of signal (Figure 2)

2. Report Manager - for sample to sample ratio calculation, and to append a "normal", "deletion" or "duplication" result. Vertical calculation divides the peak height of the sample amplicon by that of the same amplicon in the control. An example is shown in Figure 3. Note that the number of rows will depend on the number of samples that are being assayed. Also, the row location of the control sample needs to be specified as indicated in Figure 3. In the example shown, the control sample was the first sample in a set of 8.
Set up an analysis, as shown in Figure 4, by setting an appropriate threshold to identify candidate samples with deletions or duplications in a specific genomic region.

3. Dye Scale - for graphic normalization, preferred for low-throughput analysis or for some specific signal adjustment.

Figure 1. Example of deletions after control amplicon normalization. Reduction of the peak heights corresponds to the deleted regions, while an increase of the peak heights suggests a partial duplication of a given region. The peaks are being displayed using the custom plot colors feature in GeneMapper® Software.

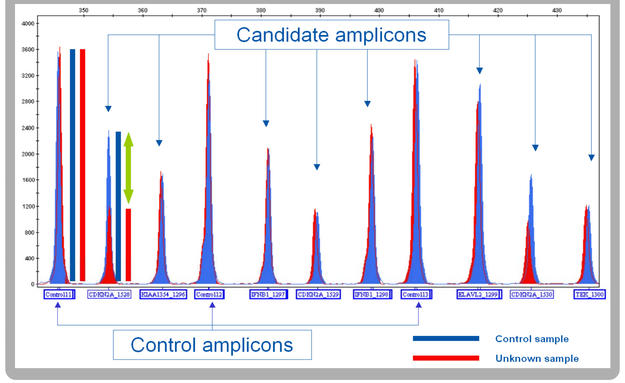


Figure 2. AFLP analysis method used to normalize samples by sum of signal.

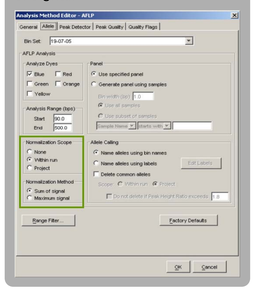


Figure 3. The Calculations options let the user specify custom calculations, such as ratio, averages, sums or Control Row.

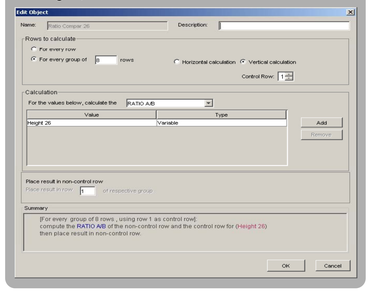
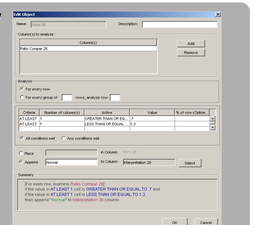


Figure 4. Final analysis can be performed to identify duplications or deletions using thresholds.

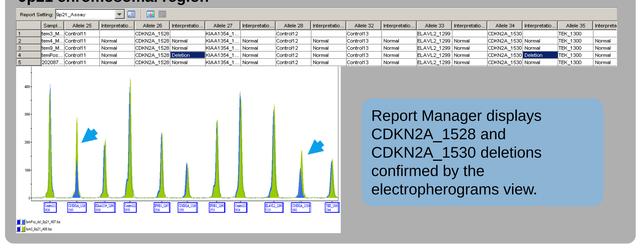
Typical Thresholds used:

Deletion <0.7
0.7=< Normal =< 1.3
Duplication > 1.3



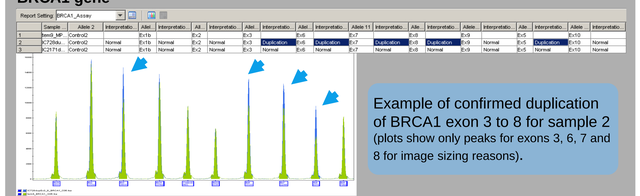
RESULTS

9p21 chromosomal region



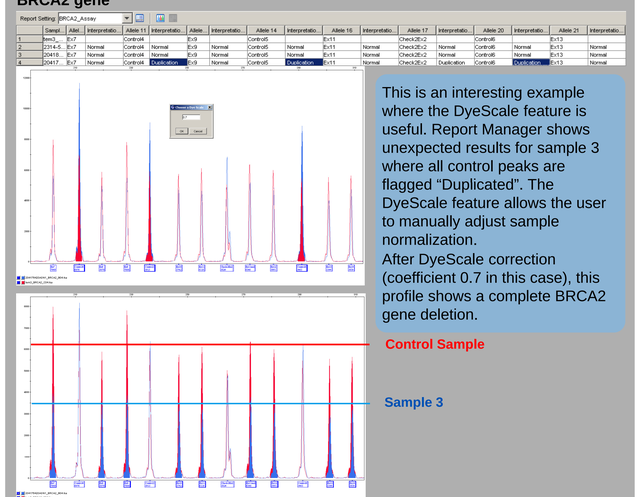
Report Manager displays CDKN2A_1528 and CDKN2A_1530 deletions confirmed by the electropherograms view.

BRCA1 gene



Example of confirmed duplication of BRCA1 exon 3 to 8 for sample 2 (plots show only peaks for exons 3, 6, 7 and 8 for image sizing reasons).

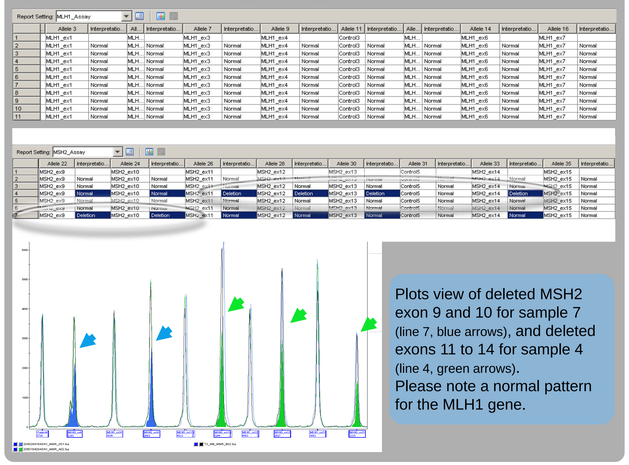
BRCA2 gene



This is an interesting example where the DyeScale feature is useful. Report Manager shows unexpected results for sample 3 where all control peaks are flagged "Duplicated". The DyeScale feature allows the user to manually adjust sample normalization.
After DyeScale correction (coefficient 0.7 in this case), this profile shows a complete BRCA2 gene deletion.

MLH1 and MSH2 genes

This assay amplifies MLH1 and MSH2 genes in the same reaction. The Report Manager feature allows the user to edit MLH1 and MLH2 results in two separate reports by a single click on the Report Setting menu.



Plots view of deleted MSH2 exon 9 and 10 for sample 7 (line 7, blue arrows), and deleted exons 11 to 14 for sample 4 (line 4, green arrows).
Please note a normal pattern for the MLH1 gene.

CONCLUSIONS

Copy Number Variation analysis for 50 DNA samples has been successfully demonstrated using multiplex PCR-based assays on the Applied Biosystems Genetic Analyzers and GeneMapper® Software version 4.0 . One sample report for each gene or region has been generated for rapid detection of candidate samples with deletion(s) or duplication(s). The fast, accurate, and automated workflow demonstrated in this poster allows the user to easily detect large genomic rearrangements in unknown samples.

REFERENCES/ AKNOWLEDGEMENTS

(1) J.A.L. Armour, D.E. Barton, D.J. Cockburn, and G.R. Taylor.
The Detection of Large Deletions or Duplications in Genomic DNA. HUMAN MUTATION 20:325-337, 2002.
(2) Frans B. L. Hogervorst and all.
Large Genomic Deletions and Duplications in the BRCA1 Gene Identified by a Novel Quantitative Method. CANCER RESEARCH 63, 1449-1453, April 2003

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TRADEMARKS/LICENSING