DETERMINATION OF GENE COPY NUMBER VARIATIONS: USEFULNESS OF MULTIPLEX PCR IN COMBINATION WITH GEL ELECTROPHORESIS-MASS SPECTROMETRY (GE-ICP-MS).

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DNA copy number variations (CNVs), defined as genomic structural variations caused by gain (duplications) or loss (deletions) of specific genome regions, are known to be involved in cancer as well as in chemotherapy response like cisplatin resistance. Their determination is performed mainly by real-time PCR and digital PCR, in single or in multiplex reactions. Both qPCR and digital PCR require fluorescent labels that limit their uses. Therefore, new methodologies enabling CNVs determination without labeling are needed. In this work we have studied the potential of the combination between end-point multiplex PCR and gel electrophoretic separations (GE) coupled with inductively coupled plasma detection (ICP-MS), monitoring the P present in the DNA backbone. The quantitative dimension can be obtained by using inorganic phosphate standards with known concentration as well as the calibration of the GE system in terms of the separation as function of the base pair number of the fragments. We have applied this methodology to study CNV in human ovary cancer cell lines. We have chosen three genes that are thought to be related to cisplatin chemotherapy response such as *CCNE1*, *ERBB2* and *GSTM1*, using *ACTB* as reference gene. We have found that the OVCAR-3 cell line presented around three more copies of *CCNE1* than the reference gene. We have also observed that both OVCAR-3 and A2780 cell lines may be heterozygous for the null *GSTM1* mutant allele.