L.8.- Methods to detect central nervous DNA using specific DNA

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For the detection of non-muscle tissues in meat samples, to comply with BSE and QUID legislation, we have sought to develop a method in which DNA is extracted from a processed sample, modified by a chemical treatment process to preserve the methylation status of the DNA, then non-muscle tissue derived DNA is detected by PCR amplification. In order to differentiate between different tissue types we have exploited the fact that not all tissues express the same sets of genes and that such differences in expression can be controlled by site-specific methylation. The promoter regions of selected genes that are expressed in the target tissue of interest were isolated and key differences in the methylation patterns of CpG dinucleotides between these and those from the corresponding skeletal muscle identified. In this case we were looking for residues where the target tissue form (non-muscle) of the gene is unmethylated and the non-target (muscle) form is methylated. These differences were then used to design a PCR assay exploiting Methylation Specific PCR (MSP) to specifically amplify the target tissue derived (unmethylated) sequence and thereby identify the presence of that tissue in mixed samples. Examples of the various detection formats that have been utilised for these types of assays will be given.

Keywords :
PCR, methylation specific PCR (MSP), central nervous DNA