

GST1 gene deletion determined by polymerase chain reaction

Kenine E.Comstock, Barbara J.S.Sanderson, Ginger Claflin and W.David Henner
Division of Hematology and Medical Oncology, Oregon Health Sciences University, Portland, OR 97201, USA

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The human glutathione transferase (GST) isozyme GST μ is frequently deficient (31–61%) in individuals (1). The absence of GST μ has been ascribed to a homozygous deletion of the gene GST1 (2) and correlates with an increased risk of lung cancer among smokers (3). We developed a simple assay based on PCR technology to determine the presence or absence of GST1, which may be useful in studies of the role of GST μ in lung cancer risk and drug resistance. Primers were designed based on the cDNA sequence for human GST μ (2) and on the intron/exon boundaries of the related rat gene Yb2 (4). The primers hybridize to the 5' region of exon 4 (5'-CTGCCCTACTTGATTGATGGG-3') and the 3' region of exon 5 (5'-CTGGATTGTAGCAGATC-ATGC-3') of GST1. Genomic DNA was prepared from human blood lymphocytes by standard procedures. A commercial PCR kit containing TAQ DNA polymerase (GeneAmp, US Biochemical Corp.) was used with 1 μ g DNA. Reactions were heated for 2 min at 94°C, 1 min at 55°C and 1.5 min at 72°C for 35 cycles in an Ericomp Thermal Cycler. PCR products were electrophoresed on a 2.1% agarose gel. The 273 bp product, present in positive reactions, was identified by sequence analysis as GST1 intron 4, a previously unsequenced region (Fig. 1), flanked by exon 4 and exon 5 (EMBL Accession no. X51451).

The presence or absence of GST μ was determined in 10 cell lysates by Western blotting and ELISA (Mukit, Medlabs, Dublin, Ireland). All Western blot/Mukit positive samples contained the GST1 gene when analyzed by PCR assay (4/4). Western blot/Mukit negative samples lacked the gene (6/6) (Fig. 2). Eighteen samples of human breast carcinoma also had a one to one correspondence between the presence of the 273 bp PCR product and the presence of GST μ by Western blotting (not shown). This assay is an efficient alternative to other methods for determining GST μ status.

REFERENCES

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1  GTG AGT GTG GGT GGC TGC AAT GTG TAG GGG GAA GGT 36
37 GGC CTC CTC CTT GGC TGG GCT GTG ATG CTG AGA TTG 72
73 AGT CTG TGT TTT GTG GGT GGC AG

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Figure 1. Nucleotide sequence of GST1 intron 4 (splice site at nucleotide 276 of the human cDNA (2)).

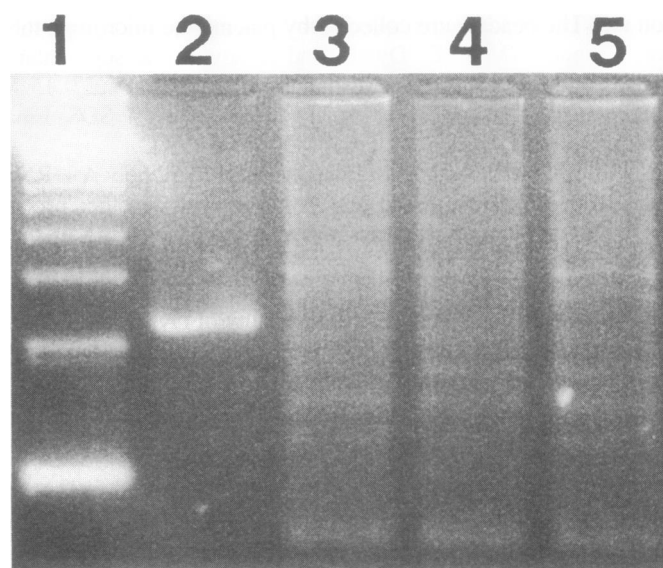


Figure 2. Lane 1: 123 bp Ladder, 2: Positive sample, 3–5: Negative samples.