# Detection of Tumor-associated Antigen Gene Expression in Melanoma Tissues

## A fast method to enrich rare mRNA templates for Reverse Transcription-PCR (RT-PCR)

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## Introduction

The possibility of generating significant immune responses directed against melanoma-associated antigens has long been suspected and is currently under investigation. In hopes of developing clinically useful vaccination protocols, the analysis of a number of tumor-associated antigens (TAA) led to the identification of several HLA class I-restricted TAA epitopes that can serve as targets for cytotoxic T lymphocytes (CTL) (1–4). Almost all melanomas express these genes, albeit to a varying extent, as shown by RT-PCR and immunohistochemistry.

Our aim was to analyze this TAA antigen expression using RT-PCR on mRNA isolated from tumor tissue. To obtain high RT-PCR sensitivity and to avoid inhibiting factors present in the tissue (e.g., some contaminating peripheral blood) or in highly concentrated total RNA, we took advantage of the poly(A)-mRNA purification procedure employed by Roche Molecular Biochemicals' mRNA Isolation Kit for Tissue. To prove applicability of the procedure in life science research studies for detecting tumor cells in the tissue, we used the poly(A)-mRNA fraction in combination with the highly sensitive RT-PCR procedure (5–7). The sensitivity of the technique was tested on the melanoma tissues of six patients.

## Results and Discussion

In order to control the sensitivity of the assay, we added varying amounts of tumor cells of the melanoma cell line HBL to normal mucosal tissue samples. As shown in **Figure 2**, the purification procedure, coupled with our RT-PCR protocol, was sensitive enough to detect the expression of the tumor-associated antigen MART-1/Melan-A in as few as 20 cells per 5 mg colon tissue, within the background of the mRNA of 0.5 x $10^6$ normal mucosal cells. For gp100, the detection limit was two cells. Tyrosinase gene expression was detected down to 200 cells under these conditions. The results were obtained with a one-step PCR reaction (60 cycles), and the qualities of the different RNA preparations proved to be positive for β-actin (35 cycles).

The described procedure provides a fast, convenient poly(A)-mRNA purification procedure for large numbers of tissue samples. It also enables mRNA to be concentrated in very small eluates, which is a prerequisite for a highly sensitive subsequent RT-PCR.

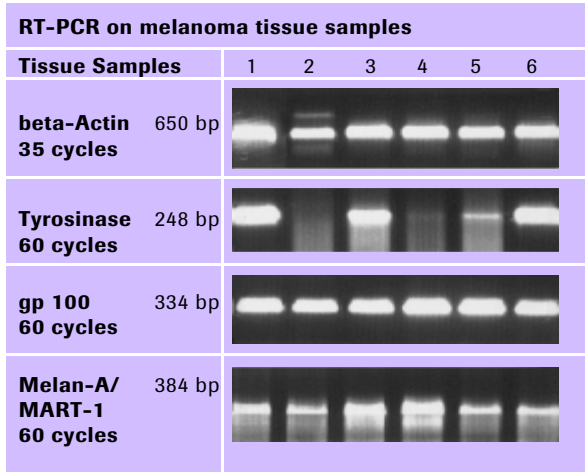


**Figure 1: RT-PCR amplification products of three TAAs**, *such as tyrosinase, gp100, and MART-1/Melan-A from 5 mg melanoma tissue from 6 different patients (control: β-actin).*

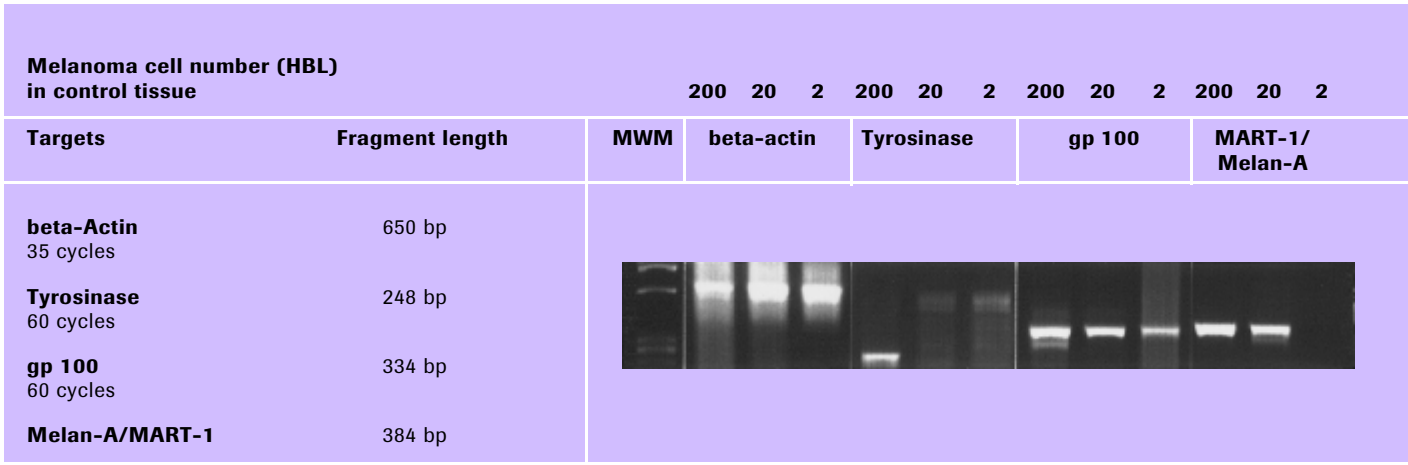


**Figure 2: RT-PCR on mucosal control tissue spiked with different amounts of melanoma cells (HBL).** *The cDNA of 0.5 x $10^6$ mucosal cells containing 200, 20, or 2 tumor cells were cycled in a single PCR and analyzed for β-actin, tyrosinase, gp 100, and MART-1/Melan-A.*



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