labeled primer, 3 ul 5X M-MLV reverse transcriptase buffer, 10 units of RNAsin, and 9.5 ul of distilled water were added. After 10 minutes incubation at 85°C, primer annealing was done for various times (from 20 minutes to 5 hours) at 37°C. 1 ul of 100 mM DTT and 200 units of M-MLV reverse transcriptase were added to the primer annealed template mixture. Then the mixture was divided into four A, C, G, and T tubes which contained 1 ul of A-mix, C-mix, G-min and T-mix respectively. The composition of chain-terminating nucleotide stock mixture was as used in the first procedure. Sequencing reaction was done for 15 minutes at 45°C. In some instances, another 200 units of M-MLV reverse transcriptase was added after 15 minute of incubation and the incubation was continued for additional 15 minutes. At this point the same procedure used in the first procedure was followed.

3.9 PCR Amplification and Sequencing

An oligonucleotide corresponding to Bt2 cDNA residues #923 - #940 (primer B) was used to reverse transcribe the mRNA of bt2-7503 and bt2-C. The ethanol precipitates of 25 ug bt2-7503 poly(A)⁺RNA and 20ug bt2-C poly(A)⁺RNA were dissolved in the hybridization buffer [4 ul 5X M-MLV reverse transcriptase buffer, 4 ul (5ng/ul) T₄ gene 32 protein (Boehringer Mannheim), 1 pmole Bt2 specefic primer(1 ul) and 7.5 ul distilled water]. After denaturation for 10 minutes at 85°C, the annealing was done for 10 minutes at 50°C. Then the first