provide reliable sequence of the insertions, PCR amplification of this region was carried out. An oligonucleotide corresponding to Bt2 cDNA sequence #923-#940 (primer B; 5'-CGATATCTTCCCAGTAAC-3') was used to reverse transcribe the bt2-7503 mRNA. To reduce formation of secondary structure, T4 gene 32 protein (Boehringer Manheim), single strand specific protein, was added to the reaction mixture. Inclusion of this protein aids in sequencing in regions with high secondary structure [Shimomaye and Salvato, 1989]. Also, primer annealing and first strand cDNA synthesis were performed at 50°C. Reaction temperature above 47°C was also reported to be the simplest way to overcome template secondary structure [Shimomaye and Salvato, 1989]. The second primer encompassed Bt2 cDNA sequence #666-#683 (primer A; 5'-GAGGATCATTGAGTTTGCC-3'). Primer A and B were used for PCR amplification.

In addition, the same region was amplified using the bt2-7503 genomic DNA and the first strand cDNA of bt2-C as templates with primer A and B. Figure 21 depicts the PCR amplified DNAs. Lane 1 is the products of bt2-7503 genomic DNA; lane 2 is the product of bt2-C first strand cDNA; lane 3 is the product of bt2-7503 first strand cDNA. The product in the lane 2 is the expected size of 270bp, which indicates the PCR amplification was performed with good fidelity. Lane 3 contained products of 700bp and 270bp. The smaller fragment of lane 3 possibly corresponds to wild type-sized transcript of which about 10% of bt2-7503 mRNA consists. On the Northern