CHAPTER VI
RESULTS AND DISCUSSION OF STUDY WITH bt2-7503 ALLELE

6.1 Cloning of bt2-7503 cDNA

To identify the molecular basis of the spontaneous mutant bt2-7503 allele, a cDNA library of this genotype was synthesized and screened. From approximately 65,000 plaques, two clones were detected using the 1.7kb Bt2 cDNA as probe. The larger clone, containing a 1.0kb insert was subcloned into pUC19 and sequenced.

6.2 PCR Amplification of Insertion Region

About 810bp of sequence of 3' bt2-7503 mRNA was obtained by sequencing the cloned 1.0kb bt2-7503 cDNA (The clone contains a very long >150bp poly(A)+ tail). The cDNA sequencing revealed no difference from wild type except a single base pair substitution at nucleotide #1370. This was discussed in 5.3. About 900bp of 5' bt2-7503 mRNA sequence including the 5' untranslated region was obtained by direct mRNA sequencing. This revealed two populations of transcripts, each with two insertions relative to wild type. The two insertions occur between nucleotide #720 and #721 and between #900 and #901. Since mRNA sequencing failed to