5.1 Cloning of bt2-C cDNA

The spontaneous mutant bt2-C allele produces wild type size transcript and the steady state transcript level is elevated in comparison to wild-type (Figure 2). Since the strongly-hybridizing Bt2 transcript is totally abolished in some bt2 mutants (i.e., bt2-7315 and bt2-B, Figure 2) the chance that the transcript in bt2-C comes from a non-allelic gene needs two changes; one to abolish the Bt2 transcript and one to turn on the non-allelic gene in the endosperm. This will be extremely rare. Thus the wild type-sized transcript in bt2-C is most likely from Bt2 gene. This also suggests that the mutation is not in the regulatory region of the gene. Thus, to elucidate the molecular basis of bt2-C allele, the cDNA was cloned and sequenced. Initially 30 clones were isolated from a maize developing seed cDNA library using 1.7kb Bt2 cDNA as probe. The longest 1.2 kb insert was subcloned into pUC19. Purified inserts were used as double-stranded templates for sequencing directly to identify the cloned fragment.