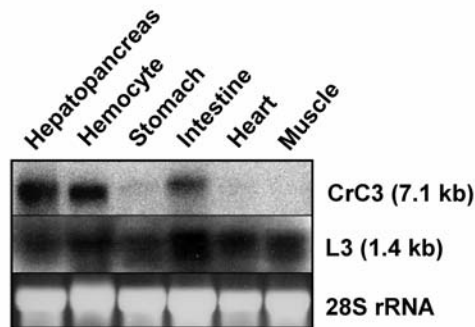
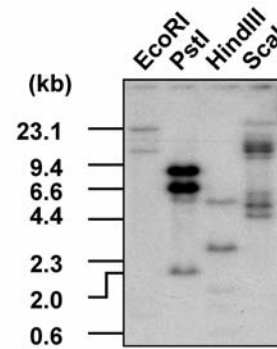


A Tissue expression profile**B Genomic DNA analysis**

Supplementary Fig. 3. Northern and Southern blot analysis of CrC3. A fragment of ~1 kb (398-1485 bp in the CrC3 cDNA sequence), released by EcoRI digestion from a cloned product of final nested 5'- RACE, was ³²P-labeled using RediPrime™ kit (Amersham Biosciences). The probe was used for both Northern and Southern blot hybridization, following standard procedures. Positively charged Nylon membrane was used for both analyses. Total RNA samples of 10 µg from each tissue were resolved in 1.0 % formaldehyde agarose gel for the Northern blot analysis. Ten microgram aliquots of genomic DNA were digested singly by different restriction enzymes, resolved in 0.6 % agarose gel for Southern blot analysis. **(A)** Northern blot hybridization shows a wide tissue-specific expression spectrum of CrC3. The intensities of 28S rRNA band in the EtBr-stained gel approximates almost same loading amount of RNA samples. The signals were also controlled by re-hybridization of the blot with a ribosome subunit (L3) probe. **(B)** Southern genomic blot analysis detected multiple bands in the genomic DNA digested singly with several restriction enzymes. The probe region of of ~1 kb in cDNA does not contain any of the four restriction enzyme sites. Thus, it indicates that either the CrC3 gene in the small probed region contains intron(s) having sites for all the restriction enzymes or more likely, there are multiple copies or isoforms of CrC3 gene present in the horseshoe crab genome.