Chapter V

Conclusions

1. Among 9 microsatellite repeat types, $(GAA)_n$ and $(GATA)_n$ were abundant while $(CACC)_n$, $(TCAG)_n$ and $(CATA)_n$ were not existent in *P. monodon* genome.
2. Three hundred and ninety-four microsatellite loci were isolated from conventional and enriched libraries. The predominant category of microsatellite loci was perfect repeats. Among non-corresponding microsatellites to probes, $(GA)_n$ and $(TAA)_n$ were the highest proportion for di- and trinucleotide repeats, found in this study implying that these microsatellite repeats were abundant in *P. monodon* genome.
3. Genomic DNA of *P. monodon* containes long arrays of highly repetitive DNA. Microsatellites of *P. monodon* were often separated among each locus by the short tracts of non-tandem repeat arrays or degenerate microsatellite-like motifs. Therefore, the flanking sequences of many clones were unsatifactory for primer designs.
4. The highest efficiency of microsatellite marker development was achieved from the conventional library established from *Hin*c II/*Alu* I digested fragments.
5. In this study, microsatellite enrichment based on selection of DNA fragments from the genomic DNA was not successful for development of microsatellites when compared to the conventional library. Out of 8 designed primers, 7 failed to amplify *P. monodon* genomic DNA.
6. Twenty-one polymorphic markers were developed, 18 loci from conventional libraries and 3 loci from enrichment libraries. In general, microsatellites exhibited

high levels of polymorphism. The average number of alleles per locus, PIC value, observed and expected heterozygosities were 17.80, 0.82, 0.70 and 0.84, respectively. Among 21 polymorphic loci, 18 were usable loci which produced reliable allelic patterns.

7. Four multiplex PCR sets were developed. These included 1 set of tetraplex, 2 sets of triplex and 1 set of duplex. The annealing and extension temperature and time, KCl concentrations have the effect on amplification of microsatellite loci in the multiplex system.
8. Non-isotopic detection system based on a silver staining method can be used to determine genotypes of microsatellite loci.
9. Seven microsatellite loci were mapped into 9 linkage groups. Each locus of CUPmo 1 and CUPmo 9 were mapped in to 2 linkage groups, male and female linkage groups.