Chapter V

Conclusions

1. Eight microsatellite loci; CSCUPmo1, CSCUPmo2, CSCUPmo3, CSCUPmo4, CSCUPmo6, CSCUPmo7, CSCUPmo9 and CSCUPmo11 were shown to be highly polymorphic with the number of alleles at each locus of 29, 27, 27, 21, 29, 22, 33 and 10 alleles, respectively. Analysis of these microsatellite using *P. monodon* from Trad revealed heterozygosities between 0.21-0.90 and size ranges of alleles between 132-380 bp. Six of these microsatellite loci, were chosen for further improve of detection methods because CSCUPmo3 and CSCUPmo7 loci gave ambiguous allelic patterns and highly scored as homozygous genotypes.

2. An alkaline extraction was the most appropriate DNA isolation method for use in DNA typing of *P. monodon*, particularly, when dealing with a large number of specimens. This method is rapid, cheap and yielded good enough in DNA quality for microsatellite amplification.

3. Multiplex analyses were developed for 1 set of a multiplex PCR (CSCUPmo1+2) and two sets of single loading of combined amplified products of different microsatellite loci (CSCUPmo4+ CSCUPmo9 and CSCUPmo6+CSCUPmo11).

4. Application of a multiplex PCR of CSCUPmo1+2 and single loading of combined CSCUPmo4+CSCUPmo9 and CSCUPmo6+CSCUPmo11 products were analyzed against three families of *P. monodon*. Result from microsatellite analysis suggested successful development of rapid DNA typing method in *P. monodon*. Nevertheless, care must be taken during preparation of pedigree samples otherwise genotype results could not be concluded.

5. For non-isotopic detection, 8% denaturing polyacrylamide sequencing gel electrophoresis was appropriate to separate microsatellite alleles and detected by silver staining. The locus CSCUPmo11 had allelic ladder constructed for size estimation of its alleles. The M13 mp18 sequencing ladder constructed with non-isotopic method should be used for size estimation of alleles of other loci.