CHAPTER IV

DISCUSSION

This study aimed to isolate and characterize the dinucleotide microsatellite loci because the dinucleotide microsatellite DNA are most abundant and found in many species. There are four type of dinucleoticle microsatellites, GT, AT, CT and GC. The work chose to study GT, CT and AT dinucleotide microsatellite because of their abundance in many species both vertebrate and invertebrate. Three probes, (GT)15, (CT)15 and (AT)20 were used to select microsatellite DNA in *P. monodon* genome. Sixty positive clones when used (CT)15 probe were selected from a total of 5,250 clones (1.1%) and 225 positive clones of (GT)15 from 6,390 clones (3.5%). For Library B, one hundred and twenty-one positive clones when used (GT)15 probe were selected from 2,400 clones (5.0%), the (AT)20 probe can not be used because AT repeats may bind to themselves causing no hybridization to shrimp genome.

Four restriction enzymes were used to construct Library A according to Tiptawonnukul, (1997). Who had found 2 microsatellite markers that can be used in selective breeding. Two restriction enzymes were used to construct Library B, hoping to find more microsatellite DNA markers. The competent hosts were changed from DH5-α to XL-1 Blue because XL-1 Blue was more competency than DH5-α. This study aimed to find more markers however, due to the large repeat arrays found in *P. monodon* microsatellites only one usable marker was obtained.

A rough estimation of the average distance between (CT/AG)n and (GT/AC)n arrays in the genome of giant tiger prawn was calculated by dividing

the total length of the screened DNA by the number of isolated microsatellites, with the assumption that (CT/AG)n and (GT/AC)n sequences were distributed evenly throughout the prawn genome. The average distance between neighboring (CT)n and (GT)n microsatellites in *P. monodon* genome was 164 kb, and 42 kb in Library A and 30 kb in Library B, respectively. Table 3.10 compared our results to those reported in various invertebrate species, fish and mammals. It appeared that the density of (CT)n microsatellites was less abundant than (GT)n microsatellites repeat in *P. monodon* and (GT)n repeats in this study was more abundant than that reported by Tiptawonnukul (every 92.8 kb). This many result from the differences in the selection of positive clones. In the previous study only strong positive clones were picked whereas in this study weaker positive clones were also picked and more microsatellite clones were found. The frequency of $(GT)_n$ in P. monodon was comparable to those reported in mammals (Weber, J.L., 1990) and in fish (Booker et al., 1994).

Most common size classes of $(CT)_n$ and $(GT)_n$ in Library A were 12-17 and 36-45 repeats, respectively and $(GT)_n$ in Library B was 46-55 repeats, $(GT)_n$ in Library A and B had about the same size. $(CT)_n$ repeat number (n) of Library A relatively short (6-29 repeats) whereas that of $(GT)_n$ was long repeats (26-75 repeats). On the contrary the $(GT)_n$ repeat number in Library B was shorter (16-55 repeats) than in Library A. Most of the $(CT/AG)_n$ microsatellites were imperfect microsatellite DNA both in Library A; (CT)n 75%, $(GT)_n$ 39.5% and in Library B; $(GT)_n$ 42.5%. More than 90% of microsatellite loci had at least one cloning site located adjacent to microsatellite sequence some clones contained two or more microsatellite regions that had flanking regions too short to be used for primer design. Therefore, PCR primers were only designed from the unique flanking sequences of 6 perfect microsatellites, 1 pairs for $(GTT)_n$ and 5 pairs for $(GT)_n$ microsatellites. By testing unrelated individual prawns, it was found that 2 out of 6 microsatellite primer pairs, namely, Pmo195 and Pmo519, produced polymorphic

fragments and amplification pattern whose sized were in the expected range while the others gave nonspecific amplifications.

The results of nonspecific amplifications, which were presented as multiple accessory bands, could still be detected at locus Pmo 131, Pmo142, Pmo 517 and Pmo 524 after several optimization experiments by varying annealing temperatures or components of the PCR conditions. The occurrence of this problematic nonspecific bands could be explained as reasoned by O' Reilly and Wright (1995) that microsatellites frequently locate within or near large dispersed repetitive elements (Beckmann and Weber, 1992). Therefore, if one or both of the PCR primers annealed to the repetitive DNA, the pattern of nonspecific amplification and lower yields of the expected PCR products resulted.

In this present study, two polymorphic microsatellite loci, namely, Pmo195 and Pmo519, exhibited the expected PCR products. However, due to the nonspecific amplification, the alleles were ambiguously scored. The polymorphism alleles of these loci was due to variation in the copy numbers of dinucleotide repeat motif. The appearance of shadow or stutter bands in those loci, which were visible as a ladder of bands, usually occurred in most dinucleotide microsatellite alleles, rather than a single discrete PCR product. Stutter bands were generally thought to be due to slippage strand mispairing between Taq polymerase with template during PCR (Litt and Lutty, 1989; Tautz, 1989; Luty et al., 1990). However, the alleles could be determined by analysing the number and relative intensity of bands in the stutter bands on the film (O' Reilly and Wright, 1995).

From the results of Pmo195 locus, 5 out of 24 unrelated individual templates were heterozygous, 8 out of 24 gave no amplified products and11 out of 24 were homozygous. The results could be explained by two possible reasons. First, the low heterozygosity of *P. monodon* was presented in this locus from random samples. Second, as occurred in many studies of other species, there

may be an occurrence of null alleles, the existing alleles in this microsatellite locus that are not observed using standard assays. Null alleles were caused by a mutation occurred in one of the priming sites (Koorey et al., 1993; Callen et al., 1993, cited in Pemberton et al., 1995).

Allelic patterns of both Pmo195 and Pmo519 exhibited polymorphism at both loci although some alleles were ambigously scored. The extentof polymorphism varies greatly amongst loci in many species reported previously (O'Reilly and Wright, 1995). However, in *P.monodon*, most microsatellite loci exhibited≥ 20 observed alleles (Tassanakajon et al., 1998, Supangul, 1998). The more variable microsatellite loci are ideal for determination of parentage in aquaculture genetics and pedigree analysis. However, the use of extremly polymorphic DNA markers may cause incorrect conclusions. Jeffreys et al. (1988) reported that polymorphic minisatellite loci in human exhibited mutation rates on the order of 0.005 to 0.05 per gamete can limit the use of these markers in paternity assesment by leading to false exclusions. In *P.monodon*, microsatellite mutation rate has not yet been determined but similar conclusion may be drawn. Therefore, microsatellite loci exhibited high levels of heterozygosity may lead to confound analyes and should be avoided.