

Chantapim Sukkornong 2009: Characterization of EST-linked Microsatellites and Centromere Mapping in Günther's Walking Catfish *Clarias macrocephalus*. Master of Science (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Supawadee Poompuang, Ph.D. 93 pages.

Expressed sequence tag (EST) databases potentially are a valuable source for developing gene-associated microsatellite markers. EST sequences of fish tissues are found to contain microsatellite repeats, particularly in the untranslated regions (UTRs). A total of 2,029 *Clarias macrocephalus* EST sequences were screened for di-, tri-, tetra-, and pentanucleotide repeat, 113 of which contained microsatellite repeats. Forty-three loci (37.7%) contained dinucleotide repeats, 44 loci (38.5%) contained trinucleotide repeats, 22 loci (19.3%) contained tetranucleotide repeat motifs and four loci (3.5%) contained for pentanucleotide repeats. EST sequences of known genes indicated that 32 microsatellite loci were found in the 3' UTR region, seven loci in the 5' UTR region and 13 loci in the open reading frame (ORF) of known genes, e.g., vitellogenin, myosin light chain, troponin, and parvalbumin. Primers were designed and synthesized for 41 loci. Fourteen loci were polymorphic with the number of alleles ranging from 2-15 alleles per locus and the observed and expected heterozygosities ranging from 0.47 to 1.0 and from 0.427 to 0.8819 per locus respectively. Eleven microsatellites contained dinucleotide core sequences, two loci contained trinucleotide repeat motifs and one locus contained a pentanucleotide repeat motif. Cross-species amplifications of ten primer pairs were observed in African catfish *Clarias gariepinus*, five in striped catfish *Pangasius hypophthalmus* and black ear catfish *P. larnaudii* and four in Mekong giant catfish *Pangasianodon gigas*.

Eleven EST-linked microsatellites and 33 microsatellites derived from genomic DNA were mapped in relation to their centromeres in two gynogenetic diploid families of walking catfish. Twenty-six loci showed high microsatellite-centromere recombination with a frequency greater than 0.67 and three loci displayed recombination frequencies greater than 0.9. The recombination frequency data suggested that microsatellites were randomly distributed within genome of walking catfish and supported the use of these markers for constructing linkage maps. Gene-centromere distances calculated under the assumption of complete interference, ranged from 2.15 cM to 46.8 cM with average distance of 31 cM. Mapping information obtained in this study proves useful for improving the initial linkage map of walking catfish.

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Student's signature

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Thesis Advisor's signature