

## **Application of Microsatellite Markers for Stock Identification and Improvement of the Malaysian River Catfish *Mystus nemurus***

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### **Introduction**

The river catfish *Mystus nemurus* is widely distributed in both mainland as well as archipelago Southeast Asia. Due to its good flesh quality it is a popular fresh water food fish throughout the region and is therefore of interest to aquaculturists. Earlier we typed five populations of river catfish from Peninsular Malaysia (Kedah, Perak, Selangor, Johor, Terengganu) for 16 loci coding for nine enzymes and sarcoplasmic protein. These populations were also typed for DNA markers by using the Random Amplified Polymorphic DNA (RAPD) and the Amplified Fragment Length Polymorphism (AFLP) techniques. Another class of DNA markers called microsatellites are very powerful genetic tools. They are codominant and have become increasingly popular as genetic markers for use in a variety of applications. Our aim is to isolate and characterize microsatellite markers for this commercially viable species.

### **Materials and Methods**

River catfish samples from Perak, Kedah, Johor, Selangor and Sarawak were collected and brought back to our laboratory. For microsatellite isolation only one DNA sample was required. Microsatellite loci were isolated using two techniques. The first technique involved using RAPD primers and cloning of the PCR products followed by screening of the clones for microsatellites. This procedure is called RAHMs. The second procedure called Random Amplified Microsatellite (RAM) is a 5' anchored PCR technique used to isolate microsatellites. Both these procedures are shortcuts to the conventional method which have proven to be efficient and economical. The microsatellite loci that we developed are being used in a follow up project on gene mapping of this species. Primers were designed for each microsatellite locus and tested for polymorphism. Direct Amplification of Length Polymorphisms (DALP) was also used to obtain codominant DNA markers for this species in the absence of DNA sequence data. Six primers have been assessed for the level of polymorphisms among the five populations.

### **Results and Discussion**

Microsatellites allow accurate genetic assessment of population differentiation. They are the most frequently used type of markers in linkage mapping. They are codominant markers and are known to be associated with quantitative trait loci. This would prove beneficial for the aquaculture industry as the breeders can then make informed decisions on their brood stocks. The use of RAHMs and RAMs together resulted in the isolation of over 100 microsatellite loci in *M. nemurus*. A total of 26 simple sequence repeats were identified using RAHMs. Five of these have been characterized. The average number of alleles per locus was 3.2. Primers have also been designed for another 56 loci and are being tested for polymorphisms. Till now we have found 30 polymorphic markers. These markers can be tested for possible associations with quantitative traits of economic importance.

### **Conclusions**

Codominant microsatellites are powerful genetic markers which are useful for the precise characterization of the species that can help in choosing the right stocks for breeding programmes. The microsatellite loci which were developed during the course of this project are now being mapped in a follow up project on gene mapping entitled Population Characterization and Linkage mapping in Ikan Baung, *Mystus nemurus*, using Microsatellite Markers which is part of a Malaysia-Thailand Bilateral Biotechnology Project on Genome Mapping of Freshwater Catfish.

### **Benefits from the study**

Knowledge as reported in scientific publications which includes journal, conference and seminar papers as well as theses of undergraduate and postgraduate students. This project started in 1996 and was extended with additional funding in 1999.

### **Patent(s), if applicable:**

Nil-

### **Stage of Commercialization, if applicable:**

Nil-

### Project Publications in Refereed Journals

1. Chong LK, Tan SG, Yusoff K, Siraj SS. 2000 Identification and characterization of Malaysian river catfish, *Mystus nemurus* (C & V): RAPD and AFLP analysis. *Biochemical Genetics* 38: 63-75
2. Chong LK, Tan SG, Siraj SS, Christianus A and Yusoff K. 1999. Mendelian inheritance of random amplified polymorphic DNA (RAPD) markers in the river catfish. *Malaysian Applied Biology* 28: 79-84.
3. Usmani, S., Tan, S. G., Siraj, S. S. & Yusoff, K, (2001) Isolation and characterization of microsatellites in the Southeast Asian river catfish *Mystus nemurus*. *Molecular Ecology Notes* 1: 264-266.
4. Usmani, S., Tan, S.G., Siraj, S.S., Yusoff, K. (2003). Population structure of the Southeast Asian river catfish *Mystus nemurus* as revealed by twenty microsatellite markers. *Animal Genetics* (In press).

### Project Publications in Conference Proceedings

1. Bhassu, S., Yusoff, K., Panandam, J.M., Embong, W.K., Sivarajasingam, S., Oyyan, S., Tan, S.G. (1999). Microsatellite analysis of *Oreochromis spp.* In: Proceedings Malaysian Science and Technology Congress '99. Confederation of Scientific and Technological Associations in Malaysia (COSTAM). Kuala Lumpur. p 62-68.
2. Usmani, S., Tan, S.G. (1999). Capture of microsatellites using direct amplification of length polymorphisms. In: Proceedings Malaysian Science and Technology Congress '99. Confederation of Scientific and Technological Associations in Malaysian (COSTAM). Kuala Lumpur. p. 439-444.
3. Usmani, S., Chua, B.E., Tan, S.G., Siraj, S.S., Yusoff, K.M. (2000). A study of three populations of the river catfish *Mystus nemurus* using random hybridising microsatellites. In: Saad et al. (Eds). Genetic Manipulations: Challenges and Advances. Genetics Society of Malaysia. Bangi. p. 88-97.
4. Usmani, S., Tan, S.G., Siraj, S.S., Yusoff, K.M. (2001). Isolation of microsatellites in *Mystus nemurus* using 5' anchored PCR. In: Proceedings of The 6<sup>th</sup> Symposium of the Malaysian Society of Applied Biology, 12-13 February 2001 at Monash University Malaysia. Published on CD. Malaysian Society of Applied Biology, Bangi.
5. Hoh, B.P., Tan, S.G., Siraj, S.S., Yusoff, K. (2003). Identification of microsatellite loci in *Mystus nemurus* using RAPD markers. In : M.K. Thong (ed.) From Peas to Chips: The Globalisation of Genetics. The Genetics Society of Malaysian. Bangi. p. 45-46.

### Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Chong Lee Kim	Development of PCR-based DNA markers to identify and characterize Malaysian river catfish, <i>Mystus nemurus</i> (C & V): RAPD and AFLP.	Genetics	MS	1999
Sahar Usmani	Isolation, characterization and application of microsatellite markers in the Southeast Asian river catfish (Baung) <i>Mystus nemurus</i> (C & V).	Genetics	PhD	2002
Subha Bhassu	Applications of DNA microsatellite markers in tilapia culture.	Genetics	PhD	2002

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**Project Leader** Tan Soon Guan