

# **GENE MAPPING, MARKER-ASSISTED SELECTION, GENE CLONING, GENETIC ENGINEERING AND INTEGRATED GENETIC IMPROVEMENT PROGRAMS AT AUBURN UNIVERSITY**

**Z. J. Liu**

*Fish Molecular Genetics and Biotechnology Laboratory  
Department of Fisheries and Allied Aquaculture  
Auburn University, Alabama 36848, USA*

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## **ABSTRACT**

Auburn University's fish genetics program has four major research projects in aquaculture genetics, namely: (1) selective breeding, (2) genomic mapping and marker-assisted selection (MAS), (3) gene cloning and (4) transgenic fish production, all with the long-term goal to produce fish broodstocks with improved economic traits. In addition to aquaculture interest, the university has a population genetics program with the goal of proper management and preservation of natural fisheries resources. However, this program will not be discussed in this report.

The aquaculture genetics projects were developed based on long-term efforts of selective breeding programs. These projects are dependent upon one another so that the maximum benefits will be obtained by using the integrated genetic improvement approaches. For instance, gene mapping will produce more economically important genes for cloning and application in biotechnology or gene-assisted selection (GAS), and produce markers that are linked to important performance traits for MAS. Biotechnology should use strains that are already developed through selective breeding to obtain the best performance traits. Further genetic improvements by gene transfer into an already superior strain provide the best "starting point" for genetic engineering. Regardless of the selection approaches to be used, traditional or marker-assisted, selection of one trait should not take the expense of another. Thus, a comprehensive selection index should be developed based on relative economic importance of each trait. This report focuses on genomic mapping, marker-assisted selection and genetic engineering. Auburn University has a strong selective breeding program and the genetics team works collaboratively in all projects, directed by a philosophy of integrated genetic improvement. For the most part, this paper will only discuss some of the progress on the work with catfish and, to a less extent, tilapia.

## **Current Status of Catfish and Tilapia Aquaculture**

Catfish is the most important cultured fish in the US and accounts for over 50% of all US aquaculture production. The catfish industry is valued at over US\$2 billion and production in 1999 should exceed 750 million pounds. It is the only agricultural sector with a steady annual growth rate of over 8%. In Mississippi, Alabama, Arkansas, Louisiana, Georgia and several other southern states, catfish is one of the top agricultural commodities. Tilapia culture is relatively small in the country, although ranked as the second or third largest cultured finfish.

Despite the development of the aquaculture and catfish industry in the US, a large trade deficit, US\$3-6 billion, exists annually for seafood products. This deficit is still increasing. Aquaculture appears to be more and more important, especially considering the collapsing natural fisheries (Anon. 1996, 1997). According to the US Department of Agriculture (DA) estimates, the demand for seafood is increasing steadily and wild fisheries will be able to supply only 25-30% of the additional demand (USDA ERS 1995). Future projections predict a steadily widening gap between the world's demand for fish and the ability of the oceans to meet it (Anon. 1996). Development of a profitable, productive, environmentally sound and sustainable aquaculture industry, therefore, provides an alternative to the already overexploited, collapsing natural fisheries.

Despite its importance, the catfish industry is still a young industry and suffers from various production problems. A recent survey by Auburn University Extension Systems indicated that disease problems are ranked as the top concern of the catfish industry. "To keep my fish alive" is the greatest wish of all farmers (Jensen, unpubl.). Disease problems cause over 30% loss of the industry each year, equivalent to a loss of several hundred million dollars annually. Other concerns include traits in growth, feed conversion efficiency, carcass and fillet yields, tolerance to low dissolved oxygen, tolerance to poor water quality, reproductive success and harvestability. Broodstocks with enhanced culture traits are urgently needed for a sustainable catfish aquaculture. However, most, if not all, economic targets are polygenic and quantitative and controlled by quantitative trait loci (QTL). Understanding genomic organization and genetic linkage and QTL mapping is required for marker-

assisted selection, improvements of economic traits through biotechnology and retrogression of beneficial genes from channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*). Auburn University's research addresses all three areas.

The research goal is to provide necessary scientific and technological information for improving the catfish broodstocks by MAS, introgression of important QTLs from channel catfish and blue catfish and genetic engineering using beneficial genes. To reach this goal, initial steps are to construct genetic linkage maps using various polymorphic markers and establish linkages of QTLs with markers. This linkage information should be useful for MAS programs. Development of the catfish genetic linkage map has been identified as one of the most important research for aquaculture by the USDA Aquaculture Steering Committee and it, among mapping of four other aquaculture species, was just approved by USDA as a regional project (NE-186) (Liu and Dunham 1998; Waldbieser et al. 1998).

Second, physical maps of catfish will be constructed so that major genes for important QTLs can be eventually isolated and used for improving broodstocks through the more effective GAS or biotechnology. Beneficial genes from various sources have been used to improve genetic stocks of catfish and develop technology needed for production of improved stocks through genetic engineering (Hallerman et al. 1990; Yoon et al. 1990a, 1990b; Hayat et al. 1991; Chen et al. 1992, 1993; Dunham et al. 1992; Gross et al. 1992; Powers et al. 1992; Chatakondi et al. 1994, 1998a, 1998b; and Dunham 1995).

Third, linkage analysis using polymorphic DNA markers and channel catfish x blue catfish interspecific hybrids should produce information on QTLs that can be introgressed from both species to generate synthetic breeds that can grow faster, have higher feed conversion efficiencies and higher carcass yields, and are more resistant to diseases and other environmental stresses.

In spite of dramatic development in many livestock animals, genomic mapping of aquaculture species is still at its infancy (Liu and Dunham 1998; Waldbieser et al. 1998). However, gene mapping in catfish is timely since well-developed, efficient marker systems are now available. The plan is to generate a catfish gene map with a resolution of 2-5 cM from the USDA-supported project, which would have been virtually

impossible, or required much more resources several years ago. In addition, background research concerning genetics of economic trait loci (ETL) has been completed in the university's laboratory and elsewhere (Dunham and Smitherman 1983a, 1983b, 1987; Tiersch et al. 1990; Dunham et al. 1993b; Tiersch and Goudi 1993; Wolters and Johnson 1994; Dunham 1996; Wolters et al. 1996; Liu et al. 1998a, 1998b, 1998c). The catfish genetics research and breeding programs, heritability studies and availability of large numbers of markers, make mapping of important ETLs in catfish immediately feasible. Development of linkage maps is certainly important, but QTLs will also be mapped for four important economic traits that will be immediately useful for MAS programs in catfish, in addition to generating a genetic linkage map of catfish.

Genetic improvement of catfish is a proven method of addressing production problems such as diseases. Previous research in the areas of traditional selective breeding and molecular genetics has resulted in genetically improved catfish (Dunham et al. 1983a, 1983b, 1987, 1990, 1992; Dunham 1996) and four releases of it to the industry. In addition to disease resistance, growth, feed conversion efficiency and processing yields are also addressed. Faster growth rate would allow for more crops per unit of time, thus saving management cost while increasing productivity. Feed accounts for 60% of the variable costs of catfish farming and, therefore, any improvement would significantly improve profit margins; processing yields are important since a 1% increase translates into US\$20-30 million net income annually.

## **Research Progress in Genomic Mapping**

### **Economic traits, genetic variation and heritability**

Domestic channel and blue catfish exhibit significant phenotypic and genetic variation for economic traits such as disease resistance, growth rate, feed conversion efficiency (found highly correlated with growth), environmental stress tolerance, carcass yield, seinability and reproduction (Dunham et al. 1982, 1983, 1984, 1985, 1987a, 1990, 1993; Dunham and Smitherman 1983a, 1983b, 1987; Bondari 1984; Hallerman et al. 1986; Wolters and Johnson 1994; Dunham 1996; Wolters et al. 1996). Auburn University established a catfish genetics research program in 1969 to evaluate

traditional selective breeding and molecular genetics for improving these quantitative traits. Growth rate and feed conversion efficiency have been improved by as much as 50% through selection (Bondari 1983; Dunham and Smitherman 1983a, 1987; Rezk 1993; Padi 1995); intraspecific crossbreeding (Bondari 1983, 1984; Dunham and Smitherman 1983b); interspecific hybridization (Dunham et al. 1990; Dunham 1996); and genetic engineering (Dunham et al. 1992, 1995; Dunham 1996). Disease resistance has also been improved primarily through interspecific hybridization (Dunham et al. 1990; Argue 1996; Dunham 1996; Wolters et al. 1996), intraspecific crossbreeding and strain selection (Wolters and Johnson 1994). Tolerance to low oxygen was enhanced primarily by interspecific hybridization (Dunham et al. 1983; Dunham 1996); seinability, by interspecific hybridization (Dunham 1996) and strain selection; and carcass yield by strain selection (Dunham et al. 1984), hybridization (Dunham 1996; Argue and Dunham 1998) and indirect selection (Rezk 1993; Dunham et al. 1985). Heritabilities and genetic correlations have been calculated (Argue 1996; Argue and Dunham 1998). The rationale for creating genetic maps of catfish is to increase the efficiency of selection (Waldbieser et al. 1998). Breeders wish to find molecular markers correlated with genetic loci controlling economic traits and use these markers to select superior broodstocks. Because traits, such as growth rate, are relatively easy to measure with traditional selection, a genetic map will be more useful to select fish for traits for which measurement is difficult or expensive (e.g., disease resistance) and is lethal to broodstocks (e.g., carcass composition). Such map will also be useful for introgression of alleles into channel catfish from other species with which hybrid production is feasible such as blue catfish.

### **Polymorphic marker development and evaluation**

In the last three years, various polymorphic markers were evaluated for their usefulness in catfish gene mapping. These markers include allozymes, restriction fragment length polymorphism (RFLP), expressed sequence tags (EST), random amplified polymorphic DNA (RAPD), microsatellites, amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNPs). Allozyme markers are type I markers and should be highly useful as anchorage points for comparative mapping. Total numbers of polymorphic loci are small and polymorphic rates are

low at each locus. RFLP markers are codominant markers. However, previous knowledge is required, such as probes for Southern analysis or sequences for polymerase chain reaction (PCR) facilitated RFLP analysis. Only a few RFLP markers are available for use in catfish mapping. We do not anticipate major increases in numbers of RFLPs because developing RFLP markers is slow and costly. Our results using EST from a pituitary cDNA library indicated low polymorphism between channel and blue catfish (about 10%). After sequencing and PCR analysis of 100 cDNA clones, 11 EST markers were obtained (Karsi et al. 1998; Liu et al. 1999e). RAPD markers were evaluated (Welsh and McClelland 1990; Williams et al. 1990) for their application in catfish gene mapping. Polymorphic rates are low among strains of channel catfish, but high between channel and blue catfish (Liu et al. 1999a). Therefore, RAPD should be only useful for mapping using the interspecific hybrid system. Inheritance of RAPD markers was normal following Mendelian expectations (Liu et al. 1998b). To date, 142 primers have been tested and 682 polymorphic markers have been identified using the channel catfish x blue catfish hybrids (Liu et al. 1998b, 1999a). Reproducible results with RAPD markers of sizes 200-1 500 bp were obtained. These markers should be useful for gene mapping analysis in the future.

Microsatellites are abundant, highly polymorphic, easy for genotyping with PCR and are reliable because of their codominance. The progress on developing microsatellite markers from channel catfish is summarized in Table 1, and efforts on this are being continued. Six microsatellite-enriched, small-insert libraries were constructed with channel catfish genomic DNA using the procedure of Orstrander et al. (1992). Two of the six libraries were screened using radioactively labeled (CA)15 and (GA)15 oligonucleotide primers. A total of 1 530 microsatellite-containing clones (900 CA and 630 GA) were purified. Plasmid DNA containing microsatellites was prepared from 890 clones (500 [CA], 390 [GA]). To date, 590 clones have been sequenced, of which 403 generated enough flanking sequences to design PCR primers (Liu et al. 1999d; Tan et al. 1999, in review). In more than 100 clones, microsatellite sequences were immediately at the cloning site making them useless. There were 403 pairs of primers for their performance in amplifying channel catfish genomic DNA. Of this total, 257 pairs successfully amplified genomic DNA and generated PCR products of expected sizes. High levels of heterozygosity were observed.

As will be discussed below, the channel catfish x blue catfish hybrid system offers great advantages for gene mapping. However, one requirement for using microsatellite markers with the interspecific system is that the microsatellite flanking sequences must be conserved between the two species. This issue was addressed by determination of amplifiability of blue catfish DNA using PCR primers designed from channel catfish microsatellite sequences. Over 90% loci were amplified from both species using channel catfish primers. In fact, most of the microsatellite flanking sequences have been conserved across the genus borders of Ictalurid catfish (Liu et al. 1999d). This indicates that more than 230 microsatellites will be useful for mapping with the catfish interspecific system. The conservation of microsatellite loci between channel and blue catfish is important for practical applications. This will allow a comprehensive linkage map to be constructed using the interspecific hybrid system and various types of markers: allozymes, RFLP, EST, RAPD, AFLP, microsatellites and SNPs.

In addition to type II microsatellite markers, type I microsatellite markers were also developed. Type I markers represent genes of known functions, thus, these are more useful for comparative gene mapping. A pituitary cDNA library was enriched for microsatellite-containing clones. Fifty clones of cDNAs containing microsatellite CA or GA repeats were obtained, which belong to 15 unique cDNAs after sequencing. Most clones harbor microsatellite sequences at their 3' nontranslated regions (NTR) or 5'-NTR, but two clones harbor CCA and CA repeats in their coding regions (Liu et al., in review).

AFLP markers (Vos et al. 1995) combine the strengths of RFLP and RAPD markers and overcome their problems. The approach is PCR-based and requires no probe or previous sequence information as needed by RFLP. It is reliable because of high stringent PCR in contrast to RAPD's problem of low reproducibility. The weakness is that they are dominant markers thus on average half of which are useful for a given backcross reference family. For gene mapping in

**Table 1. Summary of progress on microsatellite marker development from *I. punctatus*.**

Microsatellite-enriched library	6
Microsatellite clones purified	1530
Clones plasmid DNA prepared	890
Clones sequenced	590
Loci primers ordered	403
Loci PCR worked	257

catfish, AFLP polymorphic markers are highly abundant between channel catfish and blue catfish. They are inherited in the interspecific hybrids as dominant markers and segregated normally according to Mendelian ratios (Liu et al. 1998c). Some 64 primer combinations were tested and over 3 000 AFLP markers suitable for genetic mapping of catfish were produced (Liu et al. 1999c).

SNuP markers were evaluated several months ago. To date, the university has sequenced 100 each of cDNAs from the channel and blue catfish muscle cDNA libraries. Preliminary results indicate that SNuPs (type 1 in this case since they are cDNAs) are abundant using the catfish interspecific hybrid system.

In summary, feasibility of using seven types of polymorphic markers in gene mapping of catfish was evaluated. Microsatellite and AFLP are the two most useful types of markers for catfish mapping. Mapping the available microsatellite and AFLP markers should generate a map with less than 2 cM resolution.

### **Resource/reference families**

Although channel catfish is the major cultured catfish, channel catfish x blue catfish hybrid system offers great advantages. The F<sub>1</sub> hybrid is fertile and in fact has produced F<sub>2</sub>, F<sub>3</sub> and various backcrosses (Argue 1996; Liu et al. 1997; Dunham and Argue 1998a, 1998b, 1998c, in press). They are a major resource for QTL mapping and for MAS. Backcross progeny designed for this QTL mapping project was successfully produced. Sixteen backcross families (eight from channel and eight from blue backcrossed with heterozygous F<sub>1</sub>) were produced. These families are reared in 0.1 acre ponds ready for QTL evaluation. The interspecific hybrid system for gene mapping of catfish is advantageous because: (1) high rates of polymorphic markers are assured to exist between blue and channel catfish; (2) several important ETLs are possessed by blue catfish, mainly, the disease resistance gene(s) to enteric septicemia of catfish (ESC), carcass yield genes and genes controlling better seinability (Dunham et al. 1993); mapping these important genes is of great importance by itself; (3) mapping ETLs in blue catfish is important to selective breeding programs using backcrossing to introgress beneficial genes from blue catfish into channel catfish; and (4) drastic phenotypic variation of the hybrid system offers tools to be exploited for easy QTL evaluation and segregation.

### **Genotyping**

Genotyping, using AFLP and microsatellite markers, is in progress now. It is reasonable to assume that in about a year, a linkage map for catfish should be produced.

### **Research Progress in QTL Mapping and Marker-assisted Selection**

Two types of experiments toward marker-assisted selection have been initiated. The first is within the efforts for genetic linkage mapping. Phenotypes were evaluated for all the reference families to be used for mapping with the following traits: growth, body conformation (including body length, width and depth; head length, width and depth; caudal width and depth), disease resistance to ESC and to columnaris. The selective genotyping approach is used to have a strong phenotype selection pressure on the potential genotype differences. The top and bottom 12.5% of the individuals in each trait will be genotyped. It is reasonable to expect that markers linked to QTLs controlling these traits will be differential between the best and the worst performers.

The second approach is a whole-genome QTL scan from outcrossing populations. Again, if markers are linked to a specific QTL, they should harbor variable alleles between the best and the worst performers. To date, markers that are linked to QTLs controlling growth and QTLs controlling feed conversion efficiency have been identified. These identified markers, upon confirmation, will be highly useful for marker-assisted selection programs. Equally important, they will be useful for checking the success of traditional selective breeding programs. Selection for growth, for instance, should produce populations that are highly enriched with alleles linked with fast-growing QTLs.

### **Research Progress in Gene Cloning Efforts and Aquaculture Genomics Other than Mapping**

Genomic research has two ultimate applied goals: marker-assisted selection and cloning genes of economic importance. The Auburn University laboratory has been active in isolating channel catfish genes involved in growth, development and reproduction (Tang et al. 1993; Liu et al. 1997; Karsi et al. 1998).

In the last four years, genes for gonadotropin alpha-subunit (Liu et al. 1997), gonadotropin beta-subunit I and beta-subunit II (Liu et al. 2001) were cloned. Two classes of Tc1-like transposable elements named IpTc1 and IpTc2 (Liu et al., in press) and a class of nonautonomous transposons from channel catfish were isolated and characterized (Liu et al. 1999b). Another class of highly repetitive elements, named as *Xba* elements, was characterized, accounting for about 5% of the catfish genome (Liu et al. 1998a). Over 300 cDNA clones were sequenced for EST marker development. These efforts are being continued, and in the near future, it can be expected that over 1 000 catfish genes will be cloned and partially sequenced.

## **Research Progress in Transgenic Fish**

The Auburn University laboratory was one of the pioneers in the world to transfer foreign genes in fish (Dunham et al. 1987b; Dunham 1990; Liu et al. 1990a, 1990b, 1991). It has demonstrated the effects of the transfer of growth hormone genes into warmwater fish in terms of growth, protein content, carcass yield, oxygen tolerance, disease resistance, fat reduction, and enhancement of flavor and texture (Zhang et al. 1990; Hayat et al. 1991; Dunham 1992; Dunham et al. 1992; Powers et al. 1992; Chen et al. 1993; Chatakondi et al. 1994). The laboratory has also demonstrated that transgenic catfish does not pose an environmental risk in regards to growth without supplemental feed, predator avoidance and reproduction (Dunham 1995, 1996). There is greater potential for genetic improvement through the use of genes identified from Auburn's mapping project, which applied traditional selective breeding and molecular genetics. Maximum progress will likely be made by combining the tools of selective breeding with genetic engineering (Dunham 1992), using information obtained from genomic research.

Auburn University is currently working on homologous transfer of growth hormone gene from channel catfish into channel catfish, with promoters of various kinds, including metallothioneine, beta-actin, elongation factor 1 and histone H3 (Moav et al. 1991, 1992a, 1992b). Also ongoing is work on gene transfer of antibacterial peptide genes to fish to enhance resistance against bacterial pathogens.

The two most significant problems in transgenic studies are high levels of mosaicism and inactivation of transgene expression after gene transfer. The

university is developing expression vectors using the border elements to prevent gene inactivation, and using the Sleeping Beauty transposase system to enhance temporal integration. Upon development, these technologies should circumvent much of the current problems in transgenic fish production. As discussed above, the gene mapping projects will identify and essentially clone more economically important genes, including disease-resistant ones.

## **Genetic Enhancement of Tilapia in Africa**

In collaboration with the International Center for Living Aquatic Resources Management, a tilapia project entitled "Genetic Enhancement of Tilapia in Africa by Combined Selection, QTL Mapping and Marker-assisted Selection" is being undertaken. The goal is to genetically enhance Nile tilapia (*Oreochromis niloticus*), the most widespread farmed tilapia in sub-Saharan Africa, to increase production, efficiency, profit margins and quality of life of the poor fish farmers and consumers. Thus, the project will also meet the needs for food security in Africa and initiate decreasing child and infant malnutrition. The specific objectives of this research are:(1) determination of heritabilities and genetic correlations for important performance traits such as growth, feed conversion efficiency, stress tolerance and sexual maturation; (2) development of selection indices; (3) gene mapping of important QTL; (4) initial evaluation of the feasibility of marker-assisted selection; and (5) training of African scientists in quantitative and molecular genetic techniques. This three-year project is beginning.

## **Future Perspectives**

### **Radiation hybrid mapping**

Although the idea of irradiation and fusion gene transfer was published over 20 years ago (Goss and Harris 1975), the technology was underutilized until resurrected by Cox et al. (1990) for applications in genomic research by combining it with efficient genotyping using PCR. Because of its extreme power for gene mapping, radiation hybrid (RH) panels are recognized as a milestone in human genomic research and is now regarded as the ultimate tool for correction of marker orders for linkage mapping in mammals (Womack et al. 1997). Extremely fine chromosome maps have been constructed in humans

(e.g., Schuler et al. 1996, reviewed by McCarthy 1996), mouse (McCarthy et al. 1997), and bovine (Womak et al. 1997). Recently, RH panels have been reported for rat, baboon, dog (Priat et al. 1998), pig (Yerle et al. 1998), chicken and zebrafish (Chevrette et al. 1997; Kwok et al. 1998). No RH panels are available for aquaculture species yet. In catfish, RH panels are desperately needed as an initial genetic linkage map is nearing. Undoubtedly, the development of RH in catfish will accelerate its genomic mapping and progress in genetic improvement of catfish broodstocks. A tilapia RH panel is under development elsewhere.

### **Physical mapping using large insert libraries**

The second step after construction of a genetic linkage map is to focus on chromosomes that contain important QTLs controlling disease resistance, growth, feed conversion efficiency and processing yields. Catfish have 29 pairs of homogenous chromosomes (LeGrande et al. 1984) which make it extremely difficult to microdissect for development of chromosome-specific markers. Therefore, the strategy is to first identify the chromosomes containing important QTLs of interest. Then, large insert libraries, such as BAC library, will be used to tag markers linked to specific QTLs to specific BAC clones. Once the anchorage points are found, contigs of the BAC clones can be established rapidly surrounding the linked markers. This would allow development of chromosome-specific or regional markers for fine mapping of QTLs from the associated BAC clones. The work on physical mapping started in late 1999.

### **Research Teams and Facilities**

A major strength of Auburn University's research is that it combines the expertise of two complementary research teams specialized in molecular genetics for molecular marker development, evaluation, and genotyping, gene cloning, sequencing and expression vector development, and in catfish aquaculture for strain selection, phenotypic evaluation, fish culture and maintenance, QTL measurement and evaluation, population and quantitative genetic analysis, transgenic fish production and rearing, and evaluation of environmental impact of transgenic fish.

Auburn University has the best facility in the nation for simulated commercial conditions for catfish genetic research such as QTL mapping. A large earthen pond

facility, with 128 experimental research ponds, is available for evaluation of performance under closely simulated commercial conditions. This is important since genotype-environment interactions can occur in genetic evaluations of catfish requiring pond experiments to obtain realistic results.

Auburn University's Fish Genetics Research Unit also has the best facility to develop transgenic fish for aquaculture application. A large earthen pond facility with 28 ponds, secured with nets and filters approved by USDA for transgenic fish studies, is available for evaluation of performance of transgenic fish. This is the only government-approved outdoor testing facility for transgenic fish in the world. Its features include concrete stabilized walls, chain-link fence and barbed wire surrounding the complex, bird netting, common drain line, quadruple filtration, static conditions and French drain located in a seepage pond. The ponds are located 35 feet above the 100-year flood level, and a 17-acre barrier pond is located below these ponds.

A 6 500 ft<sup>2</sup> hatchery contains 300 tanks for indoor spawning, incubation and initial QTL evaluations.

The 6 500 ft<sup>2</sup> Molecular Genetics and Biotechnology Laboratory includes a computer room, main laboratory (radioactive safety approved), refrigerated room, dark room and tissue culture room. Two offices are attached to the laboratory. The recently acquired automated DNA sequencer (LI-COR IR<sup>2</sup> system) is equipped with genotyping apparatus for both microsatellite and AFLP analysis and genotyping software. The laboratory also has all the equipment for molecular biology: molecular cloning, PCR, restriction analysis, gel electrophoresis, DNA sequencing, blotting, hybridization, gene expression studies of RNA and proteins, radioimmunoassays (RIA), ELISA and Western blotting. Other major equipment include: four PCR cyclers, a microinjection system with Brinkman MM33 micromanipulator controlled by a Medical Systems Corporation pico-injector Model PLI-100, a Backeon Model 2000 for electroporation, a Beckman DU20 spectrophotometer, a Sorvall RC-6B clinical centrifuge, a Beckman J2-21 centrifuge, a Beckman GS-15 centrifuge, five microfuges, computerized low and high voltage power supplies for electrophoresis, two 37°C CO<sub>2</sub>/humidified incubators, two 80°C freezers, one -20°C freezer, one analytical balance, one 37°C shaker incubator, one 37°C incubator for dish culture of *E. coli*, UV and visible transilluminators with camera, a Leitz microscope, two dissecting microscopes, one ice

maker, autoclave, three biosafety cabinets, one of which is devoted as a radioactive work station, CAT assay tanks and a scintillation counter. The following computer hardware are available - Power Mac 7100, Power Book Mac and two IBM computers, with two laser printers. Available are DNA analysis software packages, such as DNASTar and DNASIS and free access to GenBank, EMBO and other databases, and to GCG software packages with minimal cost. Auburn University also has excellent core facilities for oligo synthesis, sequencing, TaqMan PCR, confocal microscope and EM facilities with minimal costs.

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