Fish genomics and analytical genetic technologies, with examples of their potential applications in management of fish genetic resources

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1. SUMMARY

The successful completion of the human genome project marked the start of a genomics revolution, which has the potential to impact aquaculture and fisheries production and has implications for the future management of fish genetic resources (FiGR). Aquaculture has the potential to compensate for dwindling capture fisheries, but biological and production hurdles must be overcome in order to develop cost-effective and sustainable aquaculture. Diseases are major threats to sustainability, and therefore the disease problems should be given high priority. In addition, important production and performance traits (such as growth rate, feed conversion efficiency, body conformation and fillet yield) must be improved in order to make aquaculture more productive and profitable. Genetic enhancement of farmed fish is needed not only to meet the demands of fish production, but also to ensure profitability.

The analytical genetic technologies most relevant to aquaculture and capture fisheries include: DNA markers, genome mapping, microarrays, and sequencing. DNA marker technologies are not only the basis for genetic linkage mapping, but also for the analysis of genetic resources, strain differentiation, species differentiation, parentage identification, and preservation of genetic diversity and conservation of genetic integrity.

The application of genomics in aquaculture is still at the early stages. For many important species of farmed fish, molecular markers have been developed allowing genetic analysis for FiGR conservation and genetic enhancement of farmed fish. Linkage and physical maps have been developed allowing elucidation of genes responsible for important performance and production traits; genome reagents such as expressed sequence tags have been produced providing material basis for the development of microarray technology.

Studies of the genomes of farmed and fished aquatic species have shown both common and unique characteristics that provide both advantages and challenges. In most cases, the genomes of farmed aquatic species are smaller than or comparable to the human genome. Many farmed aquatic species have high fecundity that provides large full-sib and half-sib families, and this greatly facilitates quantitative trait loci (QTL) mapping. However, the large number of farmed aquatic species tends to dilute genomic research efforts.

The genomics revolution and its impacts on aquaculture are expected to contribute to resolving problems such as diseases, environmental impacts, and low profit margins. The major potential applications of genome technologies, primarily in aquaculture but also to some extents in capture fisheries include: marker-assisted selection (MAS) for genetic enhancement; environmetal improvements through enhanced productivity as well as the development of novel technologies for environment monitoring, development of effective vaccines and their delivery technologies; monitoring antibiotic resistance; diagnosis for fish diseases and for the safety of aquatic produce; accurate identification of fish stocks for capture fisheries management and for their use as FiGR in aquaculture; conservation of FiGR, including protection of endangered species, in response to fish production strategies and consumer interests; and the development and application of transgenic fish technology including, for example, sterilization technology to address concerns about their possible environmental impacts .

A great challenge for aquaculture and capture fisheries is the long-term conservation of FiGR. Genome technologies provide new tools for genetic analysis. Innovative DNA marker technologies have opened a broad avenue for the analysis of genetic diversity based on genotypes. Some aquaculture operations still use wild fish seed. For these and for future fish breeding programs, conservation of wild FiGR is important.

The applications of genomics in aquaculture and capture fisheries raise ethical, economic, environmental, legal, and social concerns. The most prominent of these at present relate to the development and use of genetically modified organisms. More research is needed not only to resolve issues related to safety of using transgenic fish, but also to produce novel technologies allowing safe use of transgenic technology.

Public education about genomics and its applications is a key issue. **The public is relatively naïve and ill-informed about genomics. Conversely, genomics researchers may not understand the practical needs of aquaculture and capture fisheries or of fish consumers. While information dissemination about genomics to the public is very important, better exchanges of information between genome researchers and aquaculture and fisheries professionals are also essential.**
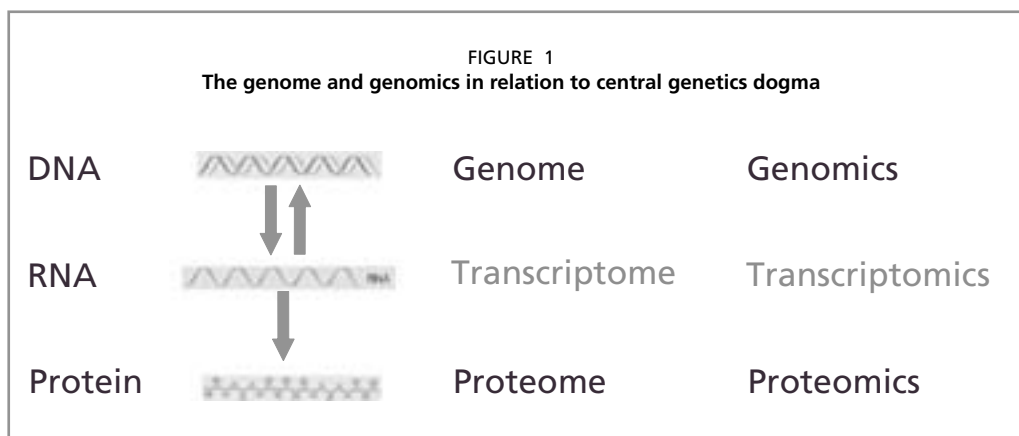
Fish genomics and analytical genetic technologies are reviewed here, with some examples of their implications for FiGR management. Genomics is a highly dynamic research field, currently dominated by human genomics but rapid developments in genomics can afford new opportunities for applications in aquaculture and capture fisheries, particularly in the areas of FiGR conservation and genetic enhancement.

2. BACKGROUND

Genomics began to receive substantial attention as a result of the Human Genome Project. The Human Genome Project faced the tasks of decoding the three billion base pairs of the human genome. Genomics always generates large data sets and these demand new ways of data management. Genomics draws data from cytogenetics, molecular genetics, quantitative genetics, and population genetics, and has led to the development of bioinformatics, through which raw genome information links to meaningful biological information. Genomics comprises the study of genome structure, organization, expression, evolution, and functions. Many sub-branches of genomics are emerging, including aquaculture genomics (http://www.genomicglossaries.com).

Genomes and genomics

The term genome refers to the complete genetic material of an organism. This includes the nuclear and mitochondrial genomes for plant and animals, and also chloroplast genomes for plants. Mitochondrial and chloroplast genomes are small and contain only a limited number of genes. The focus of most genome research is on the nuclear genome,

FIGURE 1
The genome and genomics in relation to central genetics dogma

DNA   Genome   Genomics

RNA   Transcriptome   Transcriptomics

Protein   Proteome   Proteomics

though mitochondrial genomes have been extremely useful for the identification of fish species and populations. Genomics is the science that studies the genome.

The genetic information stored in DNA cannot be used without being transcribed into RNA which then, with very few exceptions, must be translated into proteins in order to have biological functions. The term genomics often is used to cover not only this narrow sense genomics, but also transcriptomics, and in many cases proteomics as well. As Figure 1 shows, the entire DNA content of an organism (the genome) is transcribed into RNA (the entire RNA content of the organism is called the transcriptome), and the RNA is translated into proteins (the proteome). Genomics, transcriptomics, and proteomics are sciences that study the genome, transcriptome, and proteome, respectively. Genomics can be divided into structural genomics, which studies the structures, organization, and evolution of genomes, and functional genomics, which studies expression and functions of the genomes.

Genetic diversity at the genome level

Through the long process of evolution, many mutations and other genetic changes have accumulated. Accumulation of different mutations in reproductive isolated populations and individuals, as a result of their environment, is the fundamental basis of fish genetic diversity. The basic idea behind fish population genetic analysis is to unravel such differences and their inheritance among populations.

Whereas the genome is relatively stable in an organism, the transcriptome is highly dynamic. The types of transcripts and their relative levels of expression are highly regulated by tissue specificity, developmental stage, physiological state, and the environment. For instance, an organism might have 25 000 genes, but not all are expressed in every type of cell. Those genes required for the basic cell structure and functions are probably expressed in all tissues, organs, and cell types; whereas each cell type expresses a subset of the genes specific for those cell types. Many genes are expressed throughout the life history of an organism, but certain genes are expressed only at a specific developmental stage. The environment can insert its effect on gene expression in multiple dimensions. Temperature, pH, water quality, stress, dissolved oxygen, and many other environmental factors can induce or suppress expression of a large number of genes. Environmental pollution can lead to activation and suppression of expression of many genes in both the types of genes being expressed, and the levels of gene expression. Consequently, genome technologies have much to do with the environment, as well as the genome. It is now widely believed that the complexity of the transcriptome is much larger than the genome, because of alternatively processed transcripts. The information stored in the genome is amplified and diversified once at the RNA level, and is further amplified and diversified at the protein level by post-translational glycosylation, acetylation, phosphorylation, and other modifications leading to drastically different biological functions.

3. THE GENOMICS REVOLUTION AND ITS EMERGING TRENDS

Francis Collins, the Director of the National Human Genome Research Institute (NHGRI), with inputs from 600 scientists, described the "three-floor house" for the future of genomics (Box 1).

From this three-floor house plan of genomics, the following trends can be deduced:
- Genomics goes functional
- Genomics goes global
- Genomics will continue to be dominated by human genomes and human health concerns
- Genomics goes environmental
- Genomics moves towards systems biology, metagenomics, and predictive biology
- Advances in genomics will accelerate; some significant discoveries and their impacts will probably be unintentional

Genomics goes functional

The first and the overwhelming trend of genomics is that it is going functional. Although having the human genome sequenced still seems new, genomics research is rushing ahead to the next step, functional genomics. What functional genomics covers depends largely on who is being asked, but many scientists agree that the scope of functional

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BOX 1
The three floor house of future genomics

First floor: genomics to biology
- o   Define the structure of genetic variations with tools like the HapMap.
- o   Decode many additional genomes.
- o   Reduce the cost of decoding an individual genome from $30 million today to $1,000.
- o   Identify the functional elements of the human genome.
- o   Identify all proteins of the cell and their interactions.
- o   Build a computational model of a human cell and extend it to many types of human cells.

Second floor: genomics to health
- o   Identify genetic and environmental risk factors for common diseases.
- o   Build "sentinel" systems to detect diseases before they become advanced.
- o   Get academic researchers to embrace modern drug discovery techniques to create promising compounds.
- o   Gather and study genotypes from a 500,000-person segment of the U.S. population for 7 to 10 years.
- o   Figure out modern-day health disparities such as why some groups are afflicted with specific diseases more than others.
- o   Use genomics outside our borders to combat malaria, tuberculosis, and other diseases.

Third floor: genomics to society
- o   Enhance genetic privacy and protection via legislation.
- o   Understand genomics with respect to race and ethnicity. Be wary of demagogues who would exploit genomics for political purposes.
- o   Understand genomics' impact on human characteristics such as intelligence and sexual orientation.
- o   Define areas that should not be exploited, such as designing characteristics of future generations.

Source: Francis Collins: http://www.bio-itworld.com/archive/041503/collins-sidebar/

genomics ranges from expression profiling, the relationship between genome expression and functions, discovery of gene functions and their interrelationships, understanding networking among genes in relation to carrying out their functions, to proteomics and protein-protein interactions. Potential application areas include clinical diagnostics, agro biotechnology, environmental biotechnology, and pharmacogenomics. Although functional genomics remains young enough that people argue over their definition, few squabble over the value of this field. Advances in areas from gene expression to proteomics promise to push ahead basic research, biotechnology, and medicine. In fact, some experts predict an annual compound growth rate of 28% for the next six years in commercial sectors of functional genomics. As functional genomics moves forward, it will provide many options for applications in aquaculture and capture fisheries.

Genomics goes global

Genomics is going yet more global as many countries have an interest in participation, and no one wants to fall behind. Genome science is so big that no single individual or single laboratory can do it alone. It requires collaborations, team work, and international cooperation. Not only is international cooperation important for genome research because many of the genetic resources are shared by the international communities, collaboration among the private sector is another trend. The complexity concomitant with genomics and proteomics has had two key organizational impacts for large pharmaceutical companies (Arlington and Peakman, 2001). First, it has created a situation wherein the industry no longer has the resources to cover every technology, disease and therapeutic area. The second impact of the genomics revolution is to lower the entry barriers to new competitors who might be much more nimble in finding and validating targets and leads using virtual networks. These changes are per se healthy from the perspective that more opportunities are created for new players, while existing giant players have to ask the hard question about how to maintain a competitive edge in the genomics era with explosive growth with the amount of available information. Companies and national programs need to consider making adequate investments in education and capacity building to provide the human resources needed to take genomics forward.

More consortia are likely to be established to address the big questions that genomics can answer, but such questions are too big and too risky for companies to tackle on an individual competitive basis (Arlington and Peakman, 2001). Aquaculture research communities are already working collaboratively by forming various genome consortia including Salmonids Genome Consortium, Oyster Genome Consortium, and Catfish Genome Consortium etc. (e.g., http://web.uvic.ca/cbr/grasp/). The Animal Genome Project in the United States of America is organized under a National Project of NRSP-8, in which aquaculture genome is a component. Under NRSP-8, each species has a coordinator (http://www.animalgenome.org/).

Genomics will continue to be dominated by human genomes and human health concerns

Human genomics and human health concerns will continue to dominate genomics, even though many other areas, such as agricultural genomics and environmental genomics may be equally important because they affect human health. All of the genomics information and genetic technologies developed to date will be exploited to the maximal extent in human health and pharmaceutical developments. Genome technologies and genomic information allow genetic testing to be performed with a much greater precision for the prediction of predisposition to disease and ailments, carrier status, and prenatal testing. Such capabilities likely will lead to a trend of using genome technologies for pre-symptom predictions of diseases. Genome information,

genome resource, and genetic technologies also assist development of pharmaceutical products, including genes and gene products.

Genomics goes environmental

Public concern about environment changes and environmental quality is high and there are driving forces to address this in government, NGOs, and the public at large, with modern technologies available for the benefit of the environment (Gracey and Cossins, 2003; Cossins and Crawford, 2005; Almeida *et al.* 2005). Such technologies can be grouped into two general categories: those that enhance agricultural production with the same or less input, and those that can provide novel sentinels for environmental monitoring. For instance, broodstocks can be selected for better feed conversion efficiencies using gene-assisted technologies allowing greater yields with less feed, reducing environmental problems from agricultural production including aquaculture; microarrays can provide precise information on environmental pollution and its impact on the organisms involved in the system. Functional genomics is expected to contribute information for defining environmental issues, as well as technology for environmental monitoring and environment-friendly technologies for agriculture, aquaculture, and natural resource utilization.

Genomics moves towards metagenomics, systems biology, and predictive biology

Genomics is moving toward systems biology, metagenomics, and predictive biology. Genomics, once wholly described by single-organism sequencing efforts, is poised to fulfill its scientific promise in a number of different ways as sequence information is transformed into biological meaning by evolving technologies, theoretical frameworks and practical goals. Systems biology and metagenomics are two of the most ambitious of these emerging genomic sciences, concerned with 'total' understanding of cellular and ecological systems. Metagenomics is also referred to as environmental genomics or community genomics. It is the culture-independent genomic analysis of microbial communities (Eyers *et al.*, 2004; Galperin, 2004; Riesenfeld *et al.*, 2004; Rodriguez-Valera, 2004; Schloss and Handelsman, 2003). Systems biology aims to reconcile the exponentially growing amount of data about macromolecules, cells, tissues, organisms, populations, and ecosystems into coherent and systemic views of organization (Ge *et al.*, 2003; Kitano *et al.*, 2002). The genomics era has led to a much greater understanding of physiology and pathology at the molecular level and is enabling scientists to begin to unravel cellular processes as the result of the interplay of networks of genes. The publication of the human genome sequence and the use of expression databases and sophisticated bioinformatics software to find and characterize new genes and gene families have identified a huge number of potential and actual targets in a wide range of diseases. Further, the understanding of genome variation and the impact this has on health and disease will significantly improve the development and delivery of new medicines. On top of systems biology, modern genome sciences should generate information concerning expression of genomes as to "when this happens, then that happens". Predictive biology will provide insights as to whether and when certain conditions, such as disease epidemics, may or may not happen.

Advances in genomics will accelerate: some significant discoveries and their impacts will probably be unintentional

With the great expectations from the human genome project and the potentially revolutionary advances of sequencing technology, it is likely that genomics will make new rapid leaps forward. While sequencing a single genome was regarded as utopia 20 years ago, sequencing thousands of human genomes is now possible. Such sequencing

capacities and efficiency, when coupled to the ability to analyse the genomics data and to disseminate them through bioinformatics, suggest that there will be great advances in genomics. However, some discoveries and their impacts will probably be unintentional. Computerized analysis of complex genomics data can bring discoveries that are not related to the main purposes for which they were collected.

4. EXAMPLES OF FISH GENOMICS AND ANALYTICAL GENETIC TECHNOLOGIES

Since the completion of the Human Genome Project, major progress has been made in genome research, including the genomics of some farmed fish. The first Workshop on Aquaculture Species Genome Mapping was held in May 1997 in Dartmouth, Massachusetts, United States of America. Thus it was decided to focus on five species groups in the United States of America: catfish, tilapia, salmon/trout, shrimps, and oysters. In 2003, an Aquaculture Genome Project joined USDA project NRSP-8, as a part of the National Animal Genome Project, with the addition of striped bass (*Morone saxatilis*) as the sixth aquaculture species of focus in the United States of America.

The most potentially useful genomic and analytical genetic technologies for application in aquaculture and capture fisheries are: DNA markers, genome mapping, and microarrays (see Annex 1). DNA marker technologies include various techniques and methods for the analysis of genetic variation at the individual, population, or species levels. They are not only the basis for genetic linkage mapping, but also for the analysis of genetic resources, strain differentiation, species differentiation, parentage identification, and preservation of genetic diversity and conservation of genetic integrity.

Examples of major aquaculture genome projects

The US NRSP-8 project was initiated in 1998 and it is now in its second five-year phase (2003-2008). The major objective in the first phase was to develop molecular markers, and construction of genetic linkage, physical, and radiation hybrid maps. The project has three objectives in its current phase: 1) enhance and integrate genetic and physical maps of agriculturally important animals for cross species comparisons and sequence annotation; 2) facilitate integration of genomic, transcriptional, proteomic and metabolomic approaches toward better understanding of biological mechanisms underlying economically important traits; and 3) facilitate and implement bioinformatic tools to extract, analyze, store and disseminate information (http://www.animalgenome. org/).

The Genome Research on Atlantic Salmon Project (GRASP) has been conducted in Canada, where Atlantic salmon (*Salmo salar*) is an important farmed fish. In this project, genetic linkage maps and physical maps have been constructed for the Atlantic salmon genome. Genome reagents and tools have been prepared, including large numbers of expressed sequence tags (ESTs), bacterial artificial chromosome (BAC) end sequences (BES), and microarray platforms. This project has been renewed and re-termed as the cGRASP project (Consortium for Genomic Research on All Salmonids Project; http://web.uvic.ca/cbr/grasp/).

Several major genome projects have been conducted and initiated in Europe, including the SalMap project for mapping the Atlantic salmon genome, the BASSMAP project for mapping the genome of the European sea bass (*Dicentrarchus labrax*), the BRIDGEMAP project for genome of sea bream (*Sparus aurata*), and a metagenomics project focused on evolution of genome components coping with environmental stresses. The BRIDGEMAP project is a collaborative project funded by the EU initiated in 2001 with three major objectives: 1) construction of a framework genetic linkage map of sea bream for aquaculture as well as for population monitoring for fisheries; 2) To develop basic genome tools and reagents from sea bream for use in comparative genomics across species; and 3) Construction of physical maps using

bacterial artificial chromosome (BAC) libraries and radiation hybrid mapping panels (http://www.bridgemap.tuc.gr/).

In China, the Grass Carp Genome Project was initiated in 2005 and officially announced in the 13th International Congress On Genes, Gene Families And Isozymes (http://www.cafs.ac.cn/page/cafs/guanggao/jiyin/show1eng.htm). This project involves multiple institutions in China and is aimed at producing genome reagents like ESTs, BAC libraries, BAC end sequences, physical maps, linkage maps, before eventually sequencing the entire genome of the grass carp *(Ctenopharyngodon idella)*. Genome studies of shrimps have been conducted for several years in China and Thailand and ESTs and microarrays have been produced; linkage maps have also been constructed (http://pmonodon.biotec.or.th/; Wang *et al.*, 2006). In Japan, genome projects have been conducted with Japanese flounder, yellow tail, shrimps, and oysters. Fish genome studies are in progress in many parts of world, but the major efforts are located in the United States of America, Canada, Europe, China, and Japan. Genome projects are expensive and many developing countries cannot afford them.

Major achievements of aquaculture genomics
Framework genetic linkage maps have been established in salmon, trout, tilapia, catfish, shrimp, oysters, and many other species (Table 1). Large numbers of molecular markers have been developed and efforts for mapping more markers are increasing. Basic genome reagents have been or are now being established for farmed finfish, crustaceans and molluscs. Large-insert DNA libraries, such as BAC libraries, are available for Atlantic salmon, rainbow trout *(Oncorhynchus mykiss)*, tilapia *(Oreochromis* spp.), channel catfish *(Ictalurus punctatus)*, and several other finfish species. Two BAC libraries have been constructed for oysters and those for shrimps are being constructed. Physical maps have been constructed in Atlantic salmon (Ng *et al.*, 2005), Nile tilapia *(Oreochromis niloticus)* (Katagiri *et al.*, 2005), and channel catfish (Xu *et al.*, 2007). Gene discovery efforts through sequencing ESTs are increasing. A total of almost one million ESTs are now available from farmed aquatic species, of which a large percentage is from Atlantic salmon, rainbow trout, and channel catfish. The Joint Genome Institute (JGI) of the US Department of Energy (DOE) has initiated large EST projects for channel catfish (to produce 600 000 ESTs, John Liu of Auburn University serves as the principal investigator), oysters (to produce 600 000 ESTs, Dennis Hedgecock of the University of Southern California serves as the principal investigator), and genome survey project in tilapia-related species (to sequence a total of 10% genome coverage from five tilapia-related species, Thomas Kocher of the University of New Hampshire serves as the principal investigator). Descriptions of these JGI sequencing projects can be found at http://www.jgi.doe.gov/News/news_5_12_05.html. cDNA microarray technologies have been developed and used in Atlantic salmon, shrimps, oysters, and channel catfish. Although farmed fish genome research had a late start, this allowed researchers to learn lessons from scientists working with other species, and more advanced genome technology also provided greater efficiency.

Research on the genomes of farmed fish has focused on performance and production traits such as growth rate, feed conversion efficiency, disease resistance, tolerance to environmental stresses such as high ammonia, low dissolved oxygen, tolerance to cold temperature and to various salinities. In most cases, the genomes of farmed aquatic species are smaller than, or comparable to the human genome. Many farmed fish species have high fecundity, which provides opportunities to create large resource and reference families that allow great selection pressure to be applied at the phenotypic level for the analysis of quantitative trait loci (QTL). Experiments can be repeated many times as the related expense is relatively small. The large size of resource families allows accurate mapping of important genes responsible for traits. However,

TABLE 1
Genetic linkage maps, constructed using various marker systems, for various fish, shrimp, and molluscs species

Species	Common name	Marker system used	References
Salmonids			
Oncorhynchus mykiss	Rainbow trout	AFLP; RAPD Microsatellites	Young *et al.*, 1998; Nichols *et al.*, 2003b; Felip *et al.*, 2005; Sakamoto *et al.*, 2000; Rogers *et al.*, 2003; Danzmann *et al.*, 2005
Salmo salar	Atlantic salmon	AFLP, Microsatellites	Moen *et al.*, 2004c; Gilbey *et al.*, 2004
Salmo trutta	Brown trout	Microsatellites	Gharbi *et al.*, 2006
Salvelinus alpinus	Arctic char	Microsatellites	Woram *et al.*, 2004
Tilapia			
Oreochromis spp.	Tilapia	AFLP, Microsatellites	Kocher *et al.*, 1998; Agresti *et al.*, 2000; McConnell *et al.*, 2000; Lee *et al.*, 2005
Catfish			
Ictalurus punctatus	Channel catfish	AFLP; Microsatellites	Liu *et al.*, 2003; Waldbieser *et al.*, 2001
Clarias macrocephalus	Walking catfish	AFLP	Poompuang and Na-Nakorn, 2004
Carp			
Cyprinus carpio	Common carp	RAPD, Microsatellites	Sun and Liang, 2004
Other fish species			
Dicentrarchus labrax	European sea bass	Microsatellites	Chistiakov *et al.*, 2005
Seriola quinqueradiata and Seriola lalandi	Yellowtails	Microsatellites	Ohara et al., 2005
Paralichthys olivaceus	Japanese flounder	AFLP, Microsatellites	Coimbra *et al.*, 2003
Plecoglossus altivelis	Ayu	AFLP, Microsatellites	Watanabe *et al.*, 2004
Astyanax mexicanus	Cave fish	RAPD	Borowsky *et al.*, 2002
Oryzias latipes	Medaka	RAPD, Microsatellites	Ohtsuka *et al.*, 1999; Naruse *et al.*, 2004
Poecilia reticulata [	Guppy	RAPD	Khoo *et al.*, 2003
Danio rerio	Zebrafish	RAPD, Microsatellites	Postlethwait *et al.*, 1994; Mohideen *et al.*, 2000; Knapik *et al.*, 1998; Shimoda *et al.*, 1999; Woods *et al.*, 2000, 2005
Xiphophorus sp.		RAPD, Microsatellites	Kazianis *et al.*, 1996; Walter *et al.*, 2004
Shrimp			
Penaeus monodon	Black tiger shrimp	AFLP	Wilson *et al.*, 2002
Penaeus vannamei	White shrimp	AFLP	Pérez *et al.*, 2004
Penaeus japonicus	Kuruma prawn	AFLP	Li *et al.*, 2003
Penaeus chinensis	Chinese shrimp	AFLP	Li *et al.*, 2006
Molluscs			
Crassostrea virginica	Eastern oyster	Microsatellites	Yu and Guo, 2003
Crassostrea gigas	Pacific oyster	AFLP, Microsatellites	Li and Guo, 2004; Hubert and Hedgecock, 2004
Chlamys farreri	Zhikong scallop	AFLP	Li *et al.*, 2005
Haliotis discus hannae	Pacific abalone	AFLP, RAPD, microsatellites	Liu *et al.*, 2006; Sekino and Hara, 2007
Other			
Stongylocentrotus nudus	Sea urchin	AFLP	Zhou *et al.*, 2006

farmed fish genomics faces great challenges because there are many farmed fish species and funding levels for aquaculture genomics have been low. Technically, labeling of individual fish in research is often a daunting task, unless intrusive procedures are used; the aquatic environment also makes real time observations extremely difficult; genome duplication is widespread in teleost fishes, which poses challenges related to complexities for the analysis of gene arrangement, expression and function.

Sequencing farmed fish genomes

In spite of the large effort in genome sequencing of the zebrafish (*Danio rerio*) (http://www.sanger.ac.uk/Projects/D_rerio/), green spotted pufferfish (*Tetraodon nigroviridis*) (http://www.genoscope.cns.fr/externe/English/Projets/Projet_C/), Japanese pufferfish (*Fugu rubripes)* (http://www.genomenewsnetwork.org/articles/11_01/Pufferfish_sequenced.shtml*)* and medaka (*Oryzias latipes*) (http://dolphin.lab.nig.ac.jp/medaka/), no genomes of aquaculture species have been sequenced. White papers have been generated advocating sequencing a few genomes of important aquaculture species including those for rainbow trout (http://www.animalgenome.org/aquaculture/salmonids/RainbowProposal.pdf) and oysters (http://findarticles.com/p/articles/mi_m0QPU/is_2_24/ai_n15390229). The major constraints for sequencing the fish genomes are financial. In the overall genomics revolution, there has been little attention so far to the genomics of farmed fish, even for the most important species. Plant and livestock genomes have been given priority. Of the major agricultural animals, bovine, swine, and chicken genomes are being sequenced. The entire genome sequences would provide research and application advantages for the involved species. Taking USDA funding priorities as an example, only species whose genome is sequenced with a draft sequence over five fold of the genome coverage (i.e., the total base pairs sequenced should be greater than five times of the genome size of the species) is eligible for funding from the Functional Genomics Program. Obviously, no species used in aquaculture meets the criteria. All aquaculture species are thus currently excluded from its funding. However, emerging sequencing technologies might enable genomes to be sequenced more cheaply and efficiently and then it should be possible to sequence important farmed fish genomes. With or without this, fish genomics should focus on FiGR management (use and conservation), genetic enhancement, and the environmental aspects of fish genomes. Among these, genetic enhancement often takes the priority, but it is very important to note that FiGR management, and environmental genetic issues are often directly related to genetic enhancement, and should be given much greater attention.

5. ACTUAL AND POTENTIAL APPLICATIONS OF GENOMICS AND ANALYTICAL GENETIC TECHNOLOGIES IN FISH GENETIC RESOURCES MANAGEMENT

Aquaculture production is growing rapidly to provide food fish for the world's rapidly growing population and now provides approximately 40% of food fish consumed by humans (FAO, 2006). Many capture fisheries are currently harvested at or above maximum sustainable levels, and are in global decline because of over-harvesting and habitat degradation. Wild fish genetic resources (FiGR) are being depleted and some are facing extinction. Some aquaculture operations still depend on wild FiGR for seed and broodstock and wild fish populations are important resources for fish genetics research, including breeding programs and genomics.

The genomics revolution and genetic analytical technologies have many actual and potential applications for capture fisheries and aquaculture, including FiGR management. Their practical applications in aquaculture include, *inter alia*, marker-assisted selection (MAS), environment protection, genetic management of broodstocks, and genetic improvement of framed fish. Analytical genetic technologies will contribute

to lessening the adverse environmental impacts of aquaculture as well as to resolution of the disease problems through genetic enhancement, development of effective vaccines and their delivery systems, and development of rapid and accurate diagnostic tools. Future applications also include the safe use of transgenic technologies. Annex 1 summarizes major genomics and other methods of genetic analysis for application to natural and farmed aquatic species.

Diseases are major threats to sustainable aquaculture, in crustacean farming, especially shrimp farming, and in the farming of some mollucs, especially oysters. Diseases also affect the farming of many types of finfish, including carps, catfish and salmonids**.** Countermeasures to ensure the health and survival of farmed fish, including genetic technologies, are much needed. Superior broodstocks resistant to major diseases are needed. Although rich genetic resources must exist among aquaculture species for resistance to major fish diseases, for fast growth and for efficient feed conversion, genomic research is required to identify and then utilize these. Resistance-linked markers are especially needed for marker-assisted selection. Direct selection of disease resistance has proven to be very difficult in aquaculture. Genome based-technologies could provide solutions to meet some of the challenges presented by economically important pathogens. Genetic technologies for increasing and identifying disease resistance have proven safe, reliable, and environmentally sound for livestock. Mapping of large numbers of markers will pave the way for seeking QTLs for disease resistance in fish. This will add a new dimension to the new generation of technology for genetic improvement of disease resistance through marker-assisted selection in aquaculture. Genome research should facilitate marker-assisted selection for genetic improvement in many production traits of farmed fish. Some recent QTL studies are listed in Table 2.

Genetic improvement through marker-assisted selection

Marker-assisted selection is a major potential application that is used as an argument for expanding research on the genomics of farmed fish. DNA marker technologies are already used routinely for stock identification is routine in some farmed fish species (Beacham *et al*., 2000; 2005, Duchesne and Bernatchez, 2007). A few markers linked with performance and production traits have been identified (Moen *et al*., 2004a; 2004b; Martyniuk *et al*., 2003; O'Malley *et al*., 2003; Reid *et al*., 2005; Nichols *et al.,* 2003a; Palti *et al.,* 2001; Cnaani *et al.,* 2003). However, despite a great deal of promise, only a handful of cases demonstrating practical usefulness of MAS in reducing frequencies of recessive alleles causing genetic diseases, determining simple Mendelian traits and improvement in a few species have been reported so far (Dentine, 1999; Dekkers, 2004). In livestock, commercial implementation of MAS related to improvement of quantitative traits has been employed for removal of deleterious major genes, growth rate, meat quality, disease resistance and reproductive traits in pigs and in other species such as cattle where markers are used routinely for improvement of protein percentage in milk and marbling and tenderness in beef cattle. Dekkers (2004) has summarized the progress and use of markers as "the current attitude toward MAS is … cautious optimism". Fine QTL mapping will allow traits-linked markers to be identified and used for MAS, which should not replace traditional selective breeding, but should complement to ensure accurate and effective selection and to contirbute indicators at the molecular level that phenotypically selected fish truly contain the genes that breeders believe they do. Specific studies related to performance and production traits for aquaculture species must be accomplished before applying MAS in aquaculture. With low levels of funding, it is anticipated that such research may still requires years of effort, and actual MAS in aquaculture may still need at least five years.

TABLE 2
Recent QTL studies conducted in various farmed fish species

Common name	Species name	Traits	References
Salmonids			
Atlantic salmon	*Salmo salar*	Body weight, condition factor, Disease resistance, sex	Reid *et al.*, 2005; Moen *et al.*, 2004; 2004c; Grimholt *et al.*, 2003 ; Artieri *et al.*, 2006
Rainbow trout	*Oncorhynchus mykiss*	Albinism, condition factor, disease resistance, growth rate, killer cell-like activity, meristic traits, pyloric caecae number, precocious maturation, spawning date, upper thermal tolerance	Danzmann *et al.*, 1999; Palti *et al.*, 1999; 2001; Sakamoto *et al.*, 1999; Nakamura *et al.*, 2001; Ozaki *et al.*, 2001; Perry *et al.*, 2001; 2005; Robison *et al.*, 2001; Martyniuk *et al.*, 2003; Nichols *et al.*, 2003a; O'Malley *et al.*, 2003; Somorjai *et al.*, 2003; Khoo *et al.*, 2004; Nichols *et al.*, 2004; Zimmerman *et al.*, 2004; 2005; Moen *et al.*, 2004b; Reid *et al.*, 2005; Rodriguez *et al.*, 2005
Coho salmon	*Oncorhynchus kisutch*	Flesh color	Arenada *et al.*, 2005
Arctic char	*Salvelinus alpinus*	Temperature tolerance, growth rate, condition factor	Somorjai *et al.*, 2003; Tao and Boulding, 2003; Reid *et al.*, 2005
Tilapia			
Tilapias	*Oreochromis* spp.	Body and peritoneum coloration, cold tolerance, disease resistance, growth rate, immune response prolactin expression level, survival sex determination, sex ratio, stress response	Shirak *et al.*, 2000; 2002; 2006 ; Streelman and Kocher 2002; Palti , 2002; Cnaani *et al.*, 2003; 2004 a; 2004b; 2004c; Lee *et al.*, 2003; 2004; 2005; Moen *et al.*, 2004a;
Carp			
Common carp	*Cyprinus carpio*	Cold tolerance	Sun and Liang, 2004
Molluscs			
Eastern oyster	*Crassostrea virginica*	Disease resistance	Yu *et al.*, 2006
Shrimp			
Kuruma prawn	*Penaeus japonicus*	Body weight, total length, and carapace length	Li *et al.*, 2006
Other			
Zebrafish	*Danio rerio*	Behavioral and morphological differentiation	Wright *et al.*, 2006

Protection of the environment, with increasing aquaculture production and profitability

Aquaculture has a great challenge ahead to address potential environmental impacts and genomics can make significant contributions. Genome research should focus on how to benefit aquaculture and at the same time on how to protect the environment. Through research on the gemones of farmed fish, new technologies can be developed for monitoring the aquatic environment using bioindicators, biomarkers and genome expression signatures (e.g. Almeida *et al.*, 2005; Gracey and Cossins, 2003; Cossins and Crawford, 2005). Environmental genomics is therefore an important focus for farmed fish genomics. Metagenomics and ecogenomics include the goal of using genome technologies to improve environmental quality. Environmental genomics is now a major driving force (e.g. Travis *et al.*, 2003; MacGregor, 2003; Frazier *et al.*, 2003; Almeida *et al.*, 2005).

Disease diagnosis, food safety, disease resistance, fish vaccines, drug-resistant pathogens

Genomics can contribute much to the accurate diagnosis of fish diseases and to ensuring the safety of aquatic produce. Existing technologies are practical and capable of delivering results immediately (Kerr and Cunningham, 2006; Adams and Thompson, 2006).

Genome research, through QTL mapping, MAS and transgenesis, provides potential avenues for addressing some of the disease problems that threaten aquaculture. Through QTL mapping, it is possible to locate major genes responsible for disease resistance. Through MAS, brood stocks can be developed containing disease resistance genes (e.g. Palti *et al.*, 1999; Moen *et al.*, 2004b; Nichols *et al.*, 2003a) and conceptually, traditional hybridization can be used to allow their introgression (Liu *et al.*, 2003; Senanan *et al.*, 2004).

Vaccines should be developed for fish as for livestock, though their applications in aquaculture have some limitations (Lorenzen *et al.*, 2002; Evensen *et al.*, 2005). One major difference is the large number of individuals in populations of farmed fish species and their relatively low individual value. The aquatic environment also poses technical difficulties. Genome research may allow development of more effective vaccines, including DNA vaccines (Kurath, 2005). Effective vaccine delivery systems must also be developed. Genome technologies should also provide means for monitoring drug resistance in fish pathogens. This is a significant problem because countries that produce fish but have relaxed laws with respect to drug use in aquacutlure could contribute to increases in drug resistance in many pathogens, including some that affect humans (Graslund and Bengtsson, 2001; Cabello, 2006).

Genetic characterization

Genetic marking and identification of fish species, strains, lines, populations, and individuals is very important not only for aquaculture and hatchery operations, but also for capture fisheries management. Genome technologies have the capacity to provide "diagnostic kits" to identify many important species and populations, using DNA marker technologies (for reviews, see Liu and Cordes, 2004; Grant, 2007; Pullin, 2007; Smith, 2007).

DNA marker technologies should be adapted for wider use in the characterization of wild FiGR. This is particularly important for fish species and stocks that are captured by humans, because some fisheries might be depleting FiGR. For endangered species and stocks, genome technologies can characterize fish produce even after it has been cooked. This should provide greater levels of law enforcement. Consumers should also be protected to ensure that fish produce is labeled accurately at the point of sale (Maldini *et al.*, 2006).

Transgenic fish

Early attempts to develop transgenic fish were hindered by a lack of fish promoters and much of the early research was conducted with viral promoters (Dunham and Liu, 2006). Gene-based genetic improvements have now been well demonstrated in fish species using transgenic technologies. In spite of low public acceptance, transgenic work in salmon has demonstrated that growth rate can be enhanced over 10 times by transferring only a growth hormone gene (Du *et al.*, 1992; Roberts *et al.*, 2004; Devlin *et al.*, 2004), illustrating the plasticity of some fish genomes and their functions. Other transgenic fish have been developed with improved growth rate, color, disease resistance, survival in cold and body composition, and the ability to produce pharmaceutical proteins. Transgenic zebrafish with altered coloration have been commercialized and

applications are pending for commercialization of transgenic salmon, carp and tilapia transgenes with tranfered growth hormone genes (for examples of reviews, see Devlin *et al.*, 2006; Kapuscinski, 2005; Domergue *et al.*, 2005; Fu *et al.*, 2005; Napier *et al.*, 2004; Zbikowska, 2003; ; FAO, 2000; Maclean, 2000; Zhu and Sun, 2000; Iyengar *et al.*, 1996; Chen *et al.*, 1996; Gong and Hew, 1995; Hew *et al.*, 1992; Houdebine and Chourrout, 1991; Chen and Powers, 1990).

To minimize environmental risks, additional technologies such as transgenic sterilization need to be developed (Dunham and Liu, 2006). Genomic research has produced an abundance of molecular genetic information including many genes for consideration for gene transfer, highly regulated gene promoters, and knowledge about their expression and function. Functional genomics analysis should be applied in the future to enhance the capacity and versatility of transgenic technology, and to facilitate assessment of the biosaftey aspects of development and use of transgenic fish.

Increased research will be needed for determining environmental risk, measuring the fitness of transgenic fish and for determining the safety of aquatic produce derived from them. The future success and application of transgenic fish will be dictated by successful demonstration of acceptable environmental risk, assurance of food safety, appropriate government regulation and labeling, public education and opinion, and development of genetic sterilization for transgenic fish. Where commercial production of transgenic food fish is the objective, fish promoters should be used. Advances in genomics will provide these as well as important genes for gene transfer that could have greater public acceptance.

Some important commercial traits of farmed fish - such as resistance to diseases, feed conversion efficiency, tolerance to poor water quality, harvestability, carcass yield, increased reproduction and improved utilization of plant resources have yet to be addressed by transgenic technology. Basic information from genomic research may be the starting point to address effetively genetic enhancement of these traits. One of the greatest future potential benefits of gene transfer in fish could be enhancement of disease resistance in fish. Transgenic fish with enhanced disease resistance would increase profitability, production, efficiency and the welfare of the cultured fish. Preliminary research (Dunham *et al.*, 2002; Chiou *et al.*, 2002; Sarmasik *et al.*, 2002) indicates great promise for success of this approach for enhancing disease resistance.

The use of transgenic fish in recreational fisheries could involve release of transgenic fish into open waters or into more confined, urban environments. Public opinion will vary in regards to this application and the use of transgenic fish in aquaculture of food fish and ornamental fish will likely occur much earlier than their use in recreational fisheries. In the ornamental fish trade, a transgenic petfish named Glofish has already been marketed (Gong *et al.*, 2002, 2003).

Combining genetic technologies
Transgenic technology is no silver bullet; neither are genome technologies or traditional selective breeding. Genomics and combined genetic technologies are expected to lead to a much larger scope of genetically improved farmed fish. Partly, this is because the history of domestication and selective breeding of many farmed fish species has been short and great potentials for genetic improvement have yet to be realized. Continued selection plus the application of MAS will likely overcome many of the challenges faced by traditional selection alone, and provide faster and more effective results. Markers for complex traits are more difficult to be identified, and usually complex traits are controlled by multiple genes. Nonetheless, MAS has great potential to fulfill the promises made by agricultural genomics.

6. ENVIRONMENTAL AND SOCIAL ISSUES

A number of governmental and non-governmental organizations (NGO) have started discussions on issues of genomics related to ethics, environment, economy, law, and society (GE₃LS). Genome Canada (http://www.genomecanada.ca/) has conducted annual GE₃LS symposium for several years focusing on conflicting worldviews, social cohesion, ownership, and the democratic deficit. These themes were explored in relation to the application of genomics and proteomics to the fields of agriculture, environment, fisheries and forestry. In the United States of America, the Department of Energy (DOE) and the National Institutes of Health (NIH) Genome Programs set aside 3% to 5% of their respective annual Human Genome Project budgets for the study of the project's ethical, legal, and social issues (ELSI) (http://www.ornl.gov/sci/techresources/Human_Genome/elsi/elsi.shtml). Many of the issues and concerns discussed in this section were obtained from this website because published papers are scarce in this area. As the GE₃LS issues started to emerge with human genome related issues, many of the similar concerns related to genomics will emerge in aquaculture and fisheries related areas.

GMOs in aquaculture

An important issue in aquaculture and fisheries is the application of GMOs, the genetically modified organisms produced through the use of genetic engineering. Other issues include genetic impact of farmed and ornamental fish on wild populations, the ownership of and access to FiGR, and imbalances of genome technologies and capacities in various parts of the world.

The linkage of genomics to biotechnology and transgenic technology is its ability to rapidly discover, identify and characterize genes of economic importance. Such genes can be used for biotechnology, pharmaceutical purposes, or transgenics. A number of controversies exist concerning the use of genetically engineered organisms. The supporting forces come from the benefits GMO's can bring to the society. The fundamental argument for the development of GMOs is the increased food production efficiency for growing human populations.

Researchers at the University of Guelph have developed a new breed of Yorkshire pigs trademarked Enviropig™ that use plant phosphorus more efficiency (Golovan *et al.*, 2001a, 2001b). Non-transgenic pigs are unable to use an indigestible form of phosphorus called phytate present in the cereal grain diet. Therefore producers add supplemental phosphate or phytase enzyme to the diet in order to meet the phosphorus requirement for optimal growth and development. The novel trait of the Enviropig™ enables it to degrade the indigestible phytate and absorb the phosphate eliminating the need to supplement the diet with readily available phosphate, and as a consequence the phosphorus content of the manure is reduced by as much as 60%. Digestion of the phytate also leads to improvements in digestion of minerals in the diet. Clearly, potential use of such transgenic animals would have positive impact to the environment, and such research may inspire similar research in fish.

Consumer choice

Informing consumers of fish products about their identity and origin, by accurate labeling, facilitates consumer choice and product development and marketing. However, economic interests and international trade politics may seriously constrain product labeling. Labeling is not yet mandatory in some countries (e.g. United States of America). Another consideration is consumer awareness or public education. A survey conducted in New Jersey (http://www.nal.usda.gov/bic/Pubpercep/) found that most residents (91%) felt they had an "adequate" or "very good" understanding of how food is grown and produced. However, much of the public was unfamiliar

TABLE 3
Existing genomic reagents and tools for important aquaculture and fisheries species. All information was obtained from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) by using Entrez, the Life Sciences Search Engine as of 6 December 2006. Expressed sequence tags represent sequence reads from single pass sequencing of transcribed sequences, while total DNA sequences are number of sequence reads obtained from both expressed sequences and genomic sequences

Common name	Scientific name	Importance in aquaculture, capture Fisheries, or both	Number of expressed sequence tags (ESTs)	Total DNA sequences	Characterized proteins
Atlantic salmon	*Salmo salar*	Both	430 340	434 380	1 380
Rainbow trout	*Oncorhynchus mykiss*	Aquaculture	262 256	265 613	2 727
Catfish	*Ictalurus spp.*	Aquaculture	57 084	79 108	1 641
Common carp	*Cyprinus carpio*	Aquaculture	19 344	20 555	1 099
Eastern oyster	*Crassostrea virginica*	Aquaculture	9 018	9 125	73
Tilapia	*Oreochromis spp.*	Both	676	6 688	519
Pacific oyster	*Crassostrea gigas*	Both	4 201	5 259	284
Scallop	*Chlamys farreri*	Both	3 466	3598	208
Striped bass	*Morone saxatilis*	Aquaculture	1 055	1055	201
Grass carp	*Ctenopharyngodon idella*	Aquaculture	534	809	227
Skipjack tuna	*Katsuwonus pelamis*	Fisheries	0	323	77
Japanese anchovy	*Engraulis japonicus*	Fisheries	0	238	127
Alaska Pollock	*Theragra chalcogramma*	Fisheries	0	161	252
Silver carp	*Hypophthalmichthys molitrix*	Aquaculture	0	84	19
Bighead carp	*Aristichthys Nobilis*	Aquaculture	0	70	21
Indian carp	*Labeo rohita*	Aquaculture	0	48	10
Atlantic herring	*Clupea harengus*	Fisheries	0	45	35
Capelin	*Mallotus villosus*	Fisheries	0	39	8
Crucian carp	*Carassius auratus*	Aquaculture	0	29	10
Blue whiting	*Micromesistius poutassou*	Fisheries	0	24	9
Chilean jack mackerel	*Trachurus symmetricus*	Fisheries	0	11	1
Constricted tagelus	*Tagelus dombeii*	Aquaculture	0	9	0
Anchoveta	*Engraulis ringens*	Fisheries	0	8	7
Largehead hairtail	*Trichiurus lepturus*	Fisheries	0	4	0

with traditional methods for producing hybrid plants and animals. While over half the population (54%) acknowledged that they had heard of cross-fertilization or cross-breeding, only 28 percent said they had eaten a fruit or vegetable produced by this method. This is surprising because most commercially available fruit and vegetables are now hybrids. Even more surprising, 17% of the population interviewed believed that they have eaten a fruit or vegetable produced by genetic engineering, though no such product is yet available. This demonstrates the great need for improving public education and awareness about applications of genetics in food production.

Geographical distribution of fish production and fish genomics research
Developed countries will likely play leading roles in the development of farmed fish genomics and genetic technologies that in turn will enhance aquaculture production. However, most of the world's farmed fish production, comes from developing countries

(Pullin, 2007 in this volume; FAO, 2006). How the farmed fish genome technologies will be disseminated is a major concern. Efforts should be made to promote international cooperation and collaboration in genome research and utilization of results and products.

Genetic maps have yet to be developed for many important farmed fish and for many important species that are targeted by capture fisheries. Some of the available genome reagents and resources of fish genomic research so far are summarized in Table 3, from which it is clear that the major genome research activities have been focused on farmed fish species. Much more international collaboration is needed in order to enhance genomics research efforts on major farmed fish and capture fisheries species of the world. Microsatellites are needed for genetic linkage mapping and mapping of QTL, as well as for population genetic studies. ESTs are needed for analysis of gene expression, and also ESTs serve as rich sources for polymorphic markers, and serve as material basis for the development of microarrays unless otherwise the genome sequence is available. Genome sequence surveys (GSS) allow sampling of the genome for the assessment of the genome composition, repeat structure, as well as for polymorphic marker identification.

Need for globally accessible information
There are currently no comprehensive globally accessible databases on FiGR. It will be important to gather information on intraspecific genetic diversity for major capture fisheries species and major farmed fish species (Pullin, 2007). Databases of DNA fingerprints will help in species identification and also in the interest of protection of endangered species and the consumer's interest (Smith, 2007). Law enforcement agencies have trouble in identification of fish in markets and served in restaurants. In many cases, endangered species are involved, but unless more effective genome technologies are developed to provide rapid and accurate identification using fingerprinting techniques, it is difficult to provide effective means for the protection of endangered species. In some cases, inferior fish products are mislabeled as having come from more expensive species. Genome research on major farmed fish species has generated molecular markers allowing population studies and genetic resource analysis. In contrast, little genome information exists for most capture fisheries species and this is limiting the application of genome technologies in assessment of the status and conservation of wild FiGR.

Public education
The public is generally ill-informed and naïve about biotechnology in food production, including the pros and cons of transgenics, genomics and genetic technologies. Public education should be considered an important issue. Many professionals in capture fisheries and aquaculture also do not understand well the potentials and implications of genomics and genetic technologies. While information dissemination from genomics to the public is very important, better exchanges of information between genome researchers and aquaculture and fisheries professionals are also essential. As noted by Dr. Alex Mackenzie, Vice President of Research at Genome Canada, "in order for us to conduct ourselves optimally as a society, I think an informed populace is our most potent weapon" (http://www.iog.ca/about_us.asp?pageid=28).

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ANNEX 1

Genetic marker, mapping and other technologies

Allozyme markers

Allozymes", or "allelic isozymes", are the different allelic forms of the same enzymes encoded at the same locus (Parker *et al.*, 1998). The most common use of allozyme electrophoresis is to detect genetic variation in natural populations. In the last 30 years, large amounts of allelic frequency data were collected from many fish species for management purposes. Although use of allozyme data in aquaculture appears to be limited compared to its use in capture fisheries population studies, aquaculture has utilized this information for its development because aquaculture and fisheries sometimes cannot be separated from each other (Dunham 2004). Allozyme electrophoresis in aquaculture is used for stock identification, parentage analysis, hybrid identification, inbreeding analysis and limited genetic mapping (Liu and Cordes 2004). Although allozyme studies have not found common application in marker assisted selection, correlations between certain allozyme markers and performance traits has been reported (Hallerman *et al.*, 1986). Similarly, due to the limited number of polymorphic loci available, use of these markers in linkage mapping in fish is limited.

The major drawback of allozyme analysis is the necessity for a large amount of fresh or frozen tissue samples. This often requires lethal sampling, especially if a full array of allozyme markers is to be studied. Furthermore, although allozymes represent actual gene products, they often measure a very small portion of the genomic variation because a limited number of loci are involved (Utter *et al.*, 1987). Although cheap and technically easy, numbers of allozyme loci and polymorphisms are low (Agnèse *et al.*, 1997). These drawbacks seriously limit the applications of allozymes for genome studies.

Mitochondrial DNA markers

The mitochondrial genome evolves more rapidly than the nuclear genome. The rapid evolution of the mtDNA makes it highly polymorphic within a given species. Mitochondrial DNA is maternally inherited for the most part, but there are reports of paternal leakage during fertilization (Birky *et al.*, 1989). Mitochondrial DNA analysis is actually a restriction fragment length polymorphism (RFLP) analysis except that the target molecule is mtDNA rather than nuclear genomic DNA (Liu and Cordes 2004). The high levels of polymorphism, the maternal inheritance and the relatively small size of mtDNA make the RFLP analysis using mtDNA one of the easiest methods for many population studies (Okumus and Ciftci, 2003; Liu and Cordes, 2004, May, 2003; Billington, 2003). Mitochondrial DNA markers have been used extensively to analyze genetic variation in several different aquaculture species including striped bass (Wirgin *et al.*, 1991; Garber and Sullivan, 2006), channel catfish (Waldbieser *et al.*, 2003), walleye (Merker and Woodroff, 1996), salmonids (Nilesen *et al.*, 1998, Crespi and Fulton 2004), red snapper (Pruett *et al.*, 2005), and bluegill (Chapman, 1989). There are two major drawbacks of mtDNA markers. One is the non-Mendelian inheritance of mtDNA; and the second is the proportion of the total genomic variation one can observe with mtDNA alone. These characteristics place limitations to the validity of using mtDNA for genetic studies.

RFLP markers

Restriction fragment length polymorphism (RFLP) was the most popular approach for analysis of genetic variation during the 1980's. As indicated by its name, RFLP is based on DNA fragment length differences after digesting genomic DNA with one or more restriction enzymes. In spite of its earlier popularity, RFLP is able to

detect only large shifts in DNA fragment sizes. It is unable to detect the vast majority of point mutations. As a result, polymorphic rates are low at most loci. The efforts involved in RFLP marker development have been enormous. RFLP attempts to detect genetic variation one locus at a time. The low polymorphic rates, when coupled with expensive and laborious processes, have made application of RFLP limited. It should be particularly noted that RFLP requires previous genetic information, such as the availability of probes or sequence information, information often not available for many fish or other aquaculture species. Future use of RFLP will focus on analysis of Single Nucleotide Polymorphisms (SNP) residing within restriction sites.

RAPD markers

Random amplified polymorphic DNA (RAPD) is a PCR-based multilocus DNA fingerprinting technique (Welsh and McClelland, 1990; Williams *et al.*, 1990). RAPD markers are inherited as Mendelian markers in a dominant fashion. RAPDs have all the advantages of a PCR-based marker, with the added benefit that primers are commercially available and do not require prior knowledge of the target DNA sequence or gene organization. Other advantages of RAPDs are the ease with which a large number of loci and individuals can be screened. The major weakness of RAPD is its low reproducibility due to the use of low annealing temperatures, and its dominant mode of inheritance. RAPD makers have been widely used for species and strain identification in fishes (Partis and Wells, 1996; Liu *et al.*, 1998; 1999) and mollusks (Klinbunga *et al.*, 2000; Crossland *et al.*, 1993), analysis of population structure in black tiger shrimp (Tassanakajon *et al.*, 1998) and marine algae (Van Oppen *et al.*, 1996), analysis of genetic impact of environmental stressors (Bagley *et al.*, 2001), and analysis of genetic diversity (Wolfus *et al.*, 1997; Hirschfeld *et al.*, 1999; Yue *et al.*, 2002). RAPD markers have also been used for linkage mapping in fish species (Table 1). However, as more efficient and reliable marker systems such as AFLP emerged, the use of RAPD markers in genome research declined rapidly. However, it is a very useful marker system for rapid hybrid identification, strain identification, and population studies in fisheries species where other genomic information may be lacking.

AFLP markers

Amplified fragment length polymorphism (AFLP) is a PCR-based DNA fingerprinting technique that provides robust analysis of the genome variations. AFLP markers are inherited in a Mendelian fashion as dominant markers. Several major strengths make AFLP markers of choice in many situations. First AFLP requires no prior molecular information for application to the species of interest. This is particularly useful for aquaculture and fisheries species where molecular information is often not available. Second, AFLP is highly robust allowing generation of a large number of polymorphic markers with limited efforts and resources. Third, it is highly reproducible and reliable. The major weakness of AFLP markers is their dominant nature of inheritance. AFLP is more technically demanding, requiring special equipment such as automated DNA sequencers for optimal operations. AFLP is well adapted for many types of genetic analysis such as analysis of genetic diversity, population structures, migration, hybrid identification, strain identification, parentage identification, genetic resource analysis, reproduction contribution, endangered species protection, marker-assisted selection, and genome mapping. Despite the advantages of AFLP, published literature on its application for the analysis of genetic variation of fish population genetic studies is still limited due to technical difficulties and requirement for special equipment (Seki *et al.*, 1999; Jorde *et al.*, 1999; Sun *et al.*, 1999; Chong *et al.*, 2000; Kai *et al.*, 2002; Mickett *et al.*, 2003; Whitehead *et al.*, 2003; Mock *et al.*, 2004; Campbell and Bernatchez, 2004; Simmons *et al.*, 2006). Many AFLP analyses in fish so far have been limited to genetic linkage analysis (Table 1), and analysis of parental genetic contribution involving

interspecific hybridization (Youngson *et al.*, 2001) and meiogynogenesis (Felip *et al.* 2005). In a recent study of the black rockfish (*Sebastes inermis*), Kai *et al.* (2002) used AFLP to distinguish three color morphotypes, in which diagnostic AFLP loci were identified as well as loci with significant frequency differences. In such reproductive isolated populations, it is likely that "fixed markers" of AFLP can be identified to serve as diagnostic markers. Fixed markers are associated most often with relatively less migratory, reproductive isolated populations. With highly migratory fish species, fixed markers may not be available. However, distinct populations are readily differentiated by difference in allele frequencies. For instance, Chong *et al.* (2000) used AFLP for the analysis of five geographical populations of Malaysian river catfish (*Mystus nemurus*) and found that AFLP was more efficient for the differentiation of sub-populations and for the identification of genotypes within the populations than RAPD although similar clusters of the populations were concluded with either analysis. Genetic resource diversity have been assessed using AFLP (Micketti, 2003), and a comparison of the aquacultured catfish with wild populations suggested that the domestic fish had much narrower genetic diversity (Simmons *et al.*, 2006). The impact of the aquaculture catfish on wild catfish populations was found to be little, if any (Simmons *et al.*, 2006).

Microsatellite markers
Microsatellites are tandemly arranged simple sequence repeats (Tautz and Renz, 1984; 1989). Microsatellites are highly abundant in various eukaryotic genomes including all aquaculture species studied to date. Generally speaking, more compact genomes tend to contain smaller proportion of repeats including simple sequence repeats. For example, the highly compact genome of Japanese pufferfish contains 1.29% microsatellites (Crollius *et al.*, 2000). During a genomic sequencing survey of channel catfish, microsatellites were found to represent 2.58% of the catfish genome (Xu *et al.*, 2006). In most fish species, dinucleotide $(AC)_n$ repeats are the most abundant forms of microsatellites. Microsatellites are highly polymorphic such that they are suitable for differentiation of individuals, as well as populations, and species. Microsatellites are inherited in a Mendelian fashion as co-dominant markers. As microsatellites have the greatest differentiating power, they have been widely used in aquaculture and fisheries in areas including linkage mapping (Table 1, Liu and Cordes, 2004; Chistiakov *et al.*, 2006), analysis of genetic diversity, population genetics and conservation genetic analysis, parentage analysis, molecular epidemiology and pathology, QTL mapping (Chistiakov *et al.*, 2006). Microsatellites are highly adaptable for marker-assisted selection, but have not been applied in aquaculture yet because the linkage maps and QTL analysis for important traits are still lacking.

SNP markers
Single nucleotide polymorphisms (SNPs) are alternative bases at a given nucleotide position. Such sequence differences due to base substitutions have been well characterized since the beginning of DNA sequencing in 1977, but the ability to genotype SNPs rapidly in large numbers of samples was not possible until in the late 1990s. SNPs are becoming a focal point in molecular marker development since they are the most abundant polymorphism in any organism, adaptable to automation, and reveal hidden polymorphism not detected with other markers and methods. Theoretically, a SNP within a locus can produce as many as four alleles, however, most SNPs are usually restricted to one of two alleles and have been regarded as bi-allelic. SNP markers are inherited as co-dominant markers. Several approaches have been used for SNP discovery including SSCP analysis (Hecker *et al.*, 1999), heteroduplex analysis (Sorrentino *et al.*, 1992), and direct DNA sequencing. DNA sequencing has been the most accurate and most-used approach for SNP discovery. Random shotgun

sequencing, amplicon sequencing using PCR, and comparative EST analysis are among the most popular sequencing methods for SNP discovery.

Despite technological advances, SNP genotyping is still a challenging endeavor and requires specialized equipment. Traditional methods available for SNP genotyping include: direct sequencing, single base sequencing (reviewed by Cotton, 1993), allele-specific oligonucleotide (ASO, Malmgren *et al.,* 1996), denaturing gradient gel electrophoresis (DGGE, Cariello *et al.,* 1988), single strand conformational polymorphism assays (SSCP, Suzuki *et al.,* 1990), and ligation chain reaction (LCR, Kalin *et al.,* 1992). Each approach has its advantages and limitations, but all are still useful for SNP genotyping, especially in small laboratories limited by budget and labor constraints. Large-scale analysis of SNP markers, however, depends on the availability of expensive, cutting-edge equipment. Several options are available for efficient genotyping using state of the art equipment. Particularly popular are methods involving MALDI-TOF (Matrix-assisted laser desorption ionization - time of flight) mass spectrometry (Ross *et al.,* 1998; Storm *et al.,* 2003), pyrosequencing (Ahmadian *et a.,,* 2000; Alderborn *et al.,* 2000; He *et al.,* 2003), Taqman allelic discrimination (Li *et al.,* 2004), real-time (quantitative) PCR (Nurmi *et al.,* 2001), and the use of microarray or gene chips (Hacia *et al.,* 1999). Mass spectrometry and microarray technologies require a large investment in equipment. The equipment for pyrosequencing and quantitative PCR is generally under $100 000, and should be more affordable to many laboratories working in the area of aquaculture genetics. Another consideration is the expense of genotyping in relation to sample sizes. Microarray (gene chip) technology and quantitative PCR are particularly useful in medical and clinical settings where large numbers of samples (thousands of individuals per locus) are involved and that can justify the cost involved in the development of the gene chips and hybridization probes. Mass spectroscopy and pyrosequencing are relatively cost-effective (after acquisition of the equipment) when working with relatively small sample sizes (e.g., hundreds of individuals per locus), as is most likely the case with aquaculture and fisheries species.

SNPs can be genotyped with a wide range of techniques and instrumentations, from small-scale, low-budget to expensive high-throughput systems. For SNP genotyping, the greatest determinants of the genotyping platform depend on the availability of equipment. Given the availability of the equipment, considerations can be made based on budget, number of markers, number of individuals, and the requirement for robustness. In spite of its low levels of application in aquaculture and fisheries genome research, SNP markers should gain popularity as more and more sequence information becomes available in aquaculture species. Equally important, once the genetic linkage maps are well constructed, genome scans for QTLs are expected to follow to study traits important to aquaculture, which then depends on the use of well-defined association analysis. As SNP markers are great markers for the analysis of trait-genotype associations, their increased application in aquaculture and fisheries is assured.

Microarray technology
In addition to DNA marker technologies and genome mapping technologies, microarray technology is very important for genome scale analysis of gene expression. This is particularly important for environment-related issues. While microarrays utilize several recent technological innovations, they are, at their core, simply a high density dot blot where DNA samples are applied to a solid support in the form of very small dots, and hybridized to specific DNA probes. Microarrays achieve higher gene feature densities and, therefore, greater power for expression analysis by applying new tools to this old process. High-density spotting robots and photolithography allow each feature to be placed accurately within nanometers of the next feature on a glass slide,

clearly an impossible task with the human hand. Furthermore, fluorescence-based probe labeling provides a cleaner and clearer signal than the radiation traditionally used in blotting. Finally, laser scanners facilitate the resolution of such tremendous feature densities and provide accurate fluorescent signal quantification. Microarray technology allows the changes of gene expression with a specific treatment to be determined at the entire genome scale. For instance, Ju *et al.*, (2002) used microarray technology to determine which genes were up or down regulators in the brain of catfish after treating the fish with cold temperature, and found that 61 genes were significant up-regulators and 12 were down-regulators.

Potential applications of microarray technology in aquaculture and fisheries are wide open. As a genome expression analysis technology, it can be used for analysis of gene expression after any treatment. The first microarray experiment was conducted in catfish for the analysis of cold acclimation (Ju *et al.*, 2002; Kocabas *et al.*, 2004). The microarray created by the GRASP project is widely used in the aquaculture community (Rise *et al.*, 2004a; von Schalburg *et al.*, 2005) for gene expression profiling after infection and vaccination, and stress (Rise *et al.*, 2004b; Purcell *et al.*, 2006; Ewart *et al.*, 2005; Sarropoulou *et al.*, 2005). It is expected that microarrays will find great applications in aquaculture and fisheries.

Gene mapping technologies

Although the term gene mapping is widely used in the scientific community, it really refers to several different types of mapping approaches including genetic linkage mapping, physical mapping for the construction of BAC contigs, radiation hybrid mapping, QTL mapping, cytological mapping by FISH (fluorescent in situ hybridization), and comparative mapping. The goal of linkage mapping is to conduct mapping using polymorphic DNA markers in a segregating population (usually F2 population or backcross progenies). Physically linked DNA markers co-segregate. The greater the marker distance, the more likely the recombination during meiosis. Based on co-segregation, markers are placed into the same linkage groups; based on recombinant frequency, marker distances are assigned. Linkage mapping is the basis for genome analysis, and linkage maps have been constructed in many aquaculture and a few fisheries species. The quality of linkage maps are measured by marker density. All aquaculture linkage maps are framework maps or intermediate density maps.

Quantitative trait loci (QTL) mapping technology

The goal of QTL mapping is to locate the positions of quantitative trait loci. Most, if not all, performance and production traits of aquaculture are controlled by multiple genes and therefore are inherited as quantitative traits. These genes segregate along with linked DNA markers. By measuring association of trait segregation patterns with marker segregation patterns, it is possible to place trait (or genes responsible for the trait) on linkage maps. QTL studies have been conducted mainly in farmed fish species; for example, rainbow trout. It is expected that QTL mapping will be the key to genetic improvements using marker-assisted selection.

Physical mapping technology

Although several approaches are available for physical mapping, the most popular is the BAC-based approach (Bacterial Artificial Chromosome). This approach is based on restriction fingerprinting. Adjacent overlapping DNA segments should share fingerprints that allow large insert BAC clones to be lined up in a linear fashion reflective of their position in the genome.

BAC-based physical mapping has been conducted in several fish species, but mostly with model species. BAC-based physical maps have been only conducted in a few farmed fish; for example, Nile tilapia (*Oreochromis niloticus*) (Katagiri *et al.*, 2005),

Atlantic salmon (*Salmo salar*) (Ng *et al.*, 2005), and channel catfish (Xu *et al.*, 2007). BAC libraries have been constructed for more farmed species including rainbow trout, Pacific and eastern oysters, and penaeid shrimps. Because physical maps are required for position-based gene cloning, it is expected that physical maps will be constructed soon for many important farmed fish species.

Other mapping technologies

Cytological approaches have been used to map genes to chromosomes of some farmed fish species, but because of its relatively low resolution, this mapping strategy is used only as a complementary strategy for the purpose of chromosome marking and related purposes. Radiation hybrid mapping panels have been only established in zebrafish (*Danio rerio*) and European sea bass (*Dicentrarchus labrax*) (Senger *et al.* 2006). Although this approach has been extremely popular in mammalian species, its application in fish is limited. The major reason is that BAC-based physical mapping provides greater levels of resolution and is also more cost effective. The goal of comparative mapping is to use known information from a map-rich species for genome studies of a map-poor species. Knowing the location of genes in a well studied species such as a related model species like zebrafish, one can ask if the genes are arranged similarly in the same chromosomal locations. Comparative mapping is still at its infancy stage in aquaculture species, but hold great promises for the identification of candidate genes responsible for important economic traits.