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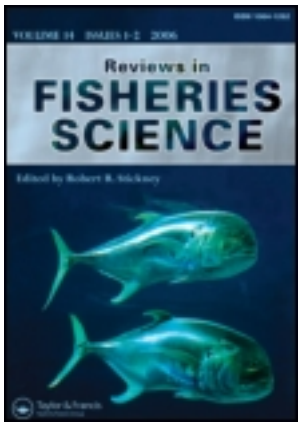

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Developmentally Utilizing Molecular Biological Techniques into Aquaculture

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Developmentally Utilizing Molecular Biological Techniques into Aquaculture

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Molecular biology techniques have been applied in aquatic areas with great success and have largely replaced traditional methods as a fishery management tool. Molecular biology techniques are focused on the cultivation of high-yield and stress-resistant varieties, detecting and preventing diseases as well as the development of new types of better breeding technology. The aim of this article is to introduce applications of molecular biology and techniques into these areas.

Keywords molecular biology and techniques, environmental management, aquaculture, disease diagnosis, disease prevention

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INTRODUCTION

In recent years, one of the most prominent features in the life sciences is the rapid development of molecular biology techniques. At present, the molecular biology theory and technology have been widely used in the improvement and identification of plant and animal species, diagnosis and treatment of human diseases, and other fields. Now molecular biology techniques have been applied in aquatic areas with great values. Molecular biology plays a very important role in solving problems in aquaculture technology, opening up new areas, and transforming the traditional model of industry. Many countries have researched and developed aquaculture-related molecular biology techniques, focusing on the development of new types of good breeding, the cultivation of high-yield stress-resistant varieties as well as new technologies and methods in detecting and preventing diseases. Therefore, there are still a lot to develop in the application of molecular biology techniques into the improvement of aquaculture species and prevention of diseases.

In the detection of pathogens in plants and animals, methodology develops towards a more specific, faster, more convenient, more secure, integrated, micro-oriented, quantitative, low-cost direction after the stages of biological culture, microscopy, biochemical testing, immunization, and molecular biology. Traditional pathogen detection analyzes the peripheral or serum characteristics of the pathogens cultivated in mediums or cells, or analyzes the impact of pathogens on the host organs, using histological detection (Adams et al., 1996). Because of time-consuming research and the low specificity and sensitivity, these methods are far from meeting the needs of daily rapid detection work. Immunological method can shorten the inspection time to a certain extent. In sensitivity, the enzyme-linked immunosorbent assay (ELISA) does better than immunofluorescence, reverse immune gel electrophoresis, and immunodiffusion, and it is the most widely used method in pathogens immunological detection (Meyers et al., 1993). However, immunological methods have been greatly limited because of much cross-reactivity between pathogens. In the past ten years, the rapid development

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of molecular biology techniques has greatly improved the level of disease diagnosis in aquaculture. Nucleic acid-based molecular diagnostic techniques rely on the specific DNA or RNA sequences contained in each pathogen; therefore, even when the *in vitro* culture medium or the method has not been established, molecular biology techniques can exclude these intermediate links, directly access to the core of the test, and greatly enhance the detection sensitivity. According to the study scope and purpose, molecular biology techniques can identify pathogenic microorganisms to sub-species or strains. Molecular diagnostic techniques include polymerase chain reaction (PCR), probe hybridization, restriction enzyme digestion, nucleotide sequencing, the first two of which are most widely used. At present, molecular diagnostic technology has been successfully applied to the detection and identification of bacterial, viral, parasitic, and fungal pathogens in fish, shrimp, and shellfish (Cunningham, 2002; Karunasagar et al., 1996; Lightner and Redman, 1998).

APPLICATION OF MOLECULAR BIOLOGICAL TECHNIQUES IN DISEASE DIAGNOSIS

Applications of PCR Technique in Disease Diagnosis

PCR is a method of enzymatic synthesis and amplification of specific DNA fragments *in vitro.* The typical reaction system includes templates, primers, polymerase, and deoxy-nucleoside triphosphate and appropriate buffer. Each PCR thermal cycle includes denaturation, annealing, and extension. As the PCR is affected by many factors, further verification tests are needed (such as restriction enzyme digestion, probe hybridization, or nucleotide sequencing). Many scholars have developed a number of PCR-based pathogen detection methods. For instance, Beaz-Hidalgo et al. (2008) amplified *fst*A gene by PCR to detect Aeromonas in salmon and Altinok et al. (2008) developed a multi-PCR, which can simultaneously detect five kinds of bacterial pathogens in fish. Xie et al. (2001) used nested PCR to detect White Spot Syndrome Virus (WSSV) of Penaeus monodon.

In recent years, many new PCR technologies have been developed, such as immunization PCR, nested primer PCR, and real-time quantitative fluorescent PCR. Please see detailed information in Table 1.

Table 1 List of three new types of PCR technology

Category	Advantages	Limitations
Immune PCR	High sensitivity, mass concentration of antigen material is as low as 2 ng/L	Affected by many factors, needed to explore the conditions
Nested-PCR	Specificity, sensitivity is 100 times higher than conventional PCR, rapid	Complex in operation, likely to cause pollution
Real-time quantitative fluorescent PCR	Specificity, effective in elimination of PCR contamination	Expensive

Applications of In Situ Nucleic Acid Hybridization in Disease Diagnosis

In situ nucleic acid hybridization technique, bind known base sequences with marked nucleic acid probes with nucleic acid bases in organization or cells that are to be detected to form hybrids, according to the principle of base pairing. With the corresponding markers detection system, the hybridization signals with a color are formed *in situ* through histochemistry or immunohistochemical staining methods. The technology is of high sensitivity, specificity, and practicability. For example, Lei et al. (2001) used *in situ* hybridization to detect WSSV in Chinese shrimp, showing that this technique has a high application value in the diagnosis of epidemic outbreaks of shrimp. Venkateswaran et al. (1998) used the gyrase B gene (*gyr*B) as a molecular diagnostic probe. Virus (Vibrio, Parahaemolyticus) is detected in 27 artificial inoculated shrimp samples, providing us with a fast, reliable, and sensitive method for V. parahaemolyticus detection in shrimp. Mari et al. (1998) extracted ssRNA gene from the purified Taura Syndrome Virus (TSV), transcribed it into double-stranded cDNA and constructed cDNA library, and then filtered two specific probes, pP15 and pQ1, by plasmid vectors reorganization. Through the Northern blotting and dot blotting, the two probes can specifically hybridize with extracted RNA-TSV gene, TSV and TSV-infected shrimp tissue fluid.

Applications of Restriction Detection in Disease Diagnosis

Restriction enzyme recognizes short sequences of DNA and cuts DNA in the recognition sites. Single nucleotide change can lead to an increase or missing in restriction enzyme sites. As a result, the number of restriction fragments, the so-called restriction fragment length polymorphisms (RFLP), changes. Followed by gel electrophoresis, DNA fragments are separated according to their sizes in the gel. Different fragments can be observed for variation analysis after staining. With restriction, the total DNA of different samples can generate enzyme polymorphism. Detecting these polymorphism differences, however, needs to use labeled specific DNA probes for confirmation. Isotope labeling is shown by autoradiography, while the non-isotope-labeled (such as an enzyme marker) can be shown by color technology; in this way, DNA polymorphism can be revealed. Another method does not require DNA probe hybridization, and the first step is restriction of DNA fragments. With specific DNA fragments as the target, restriction fragment length polymorphism can be shown through PCR amplification, which is called amplification fragment length polymorphisms. Such a diagnosis for pathogenic microorganisms is a very useful and direct tool (Austin et al., 1997; Borrell et al., 2000; Talaat et al., 1997). It can also be used to identify specific parasites (Cunningham,

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1997; Cunningham et al., 1995). For example, Einer-Jensen et al. (2005) analyzed genotypes of rod-like virus and heme septicemia virus in fish by RFLP. Chang et al. (2001) confirmed the existence of WSSV by diagnosing Callinectes sapiduses collected from three different coastal water bodies in the United States with PCR and *in situ* hybridization analysis. They also amplified a 1,156 bp fragment of the WSSV rrl-specific RsaI by RFLP method to distinguish the WSSV isolated from Callinectes sapidus in New Jersey and elsewhere.

Applications of Immunological Techniques in Disease Diagnosis

Enzyme-linked immunosorbent assay technique (ELISA) absorbs the antigen or antibody on the surface of a solid phase carrier, so that antigen-antibody reaction takes place on it. For example, Milne et al. (2006) detected infectious pancreatic necrosis virus (IPNV) in co-cultured rainbow trout by RT-PCR ELISA method. Fan et al. (2006) detected Litopenaeus vannamei red body disease pathogen by indirect ELISA with satisfying results. Some examples of applications are shown in Table 2.

Based on highly specific antigen-antibody reaction, fluorescent antibody technique uses the fluorescence as antigen marker to inspect fluorescence-specific antigen complex and its existing site with fluorescence microscope, which is also applied in the detection of aquatic animal pathogens. For example, Yan et al. (2006) detected flounder *in vivo* Vibrio with fluorescent antibody technique. The main advantage of this technology is specific, fast, and highly-sensitive, but there are also disadvantages, such as the problems concerning non-specific staining, the cumbersome operating procedures, the requirement for special equipment (fluorescence microscope), and inability of preserving the stained specimen for a long term.

Monoclonal antibody (McAb) is a hybrid cell of an antibody cell and a bone marrow cell, with characteristics of strong specificity and consistent affinity. It is also able to identify characteristics of a single antigenic determinant. Since 1975 when Kohler and Milstein successfully produced monoclonal antibody, the technology of antibody specificity detection and microorganism's identification has been developed rapidly. For example, Maddison et al. (2005) made monoclonal antibody of rainbow trout prion, which plays an important role in the detection of genetic spongiform encephalopathy in economic fish.

Table 2 ELISA detection methods and applications

Detection methods	Applications
Indirect ELISA	Gill-rot disease
	Infectious pancreatic necrosis virus
	Reovi virus
	Aeromonas hydrophila
	Turbot hemorrhagic virus
	Lateolabrax vibrio anguillarum
Sandwich ELISA	Grass carp hemorrhage virus
	Rainbow trout IPNV
	Infectious hematopoietic necrosis virus
Dot-ELISA	Aeromonas septicemia
	Bacterial fish disease
mAbs ELISA	VHSV
	WSSV
BAS-ELIS	Vibrio parahaemolyticus

Cell Culture Technology

Studies of aquatic animal cell culture first began in the area of fish. Now, more fish cell lines have been established since the first time RTG2 cell line of rainbow trout gonad was established in 1963. For example, Christianson-Heiska and Isomaa (2008) established primary cultured cell lines RTH-149 from the liver of brown trout. China has also established many fish cell lines, such as ZC7901, CAB80, CIK cell lines, rainbow trout macrophage cell line, and so on. Ryan et al. (2008) has studied the radiation-induced adaptive response of fish cell lines. Salinas et al. (2008) made studies on cell apoptosis through analyzing pre-cytoplasmic extracts of fish cell lines. Cell culture researches of shellfish, shrimp, and other aquatic animals have reported less comparatively.

APPLICATIONS OF MOLECULAR BIOLOGICAL TECHNIQUES IN DISEASE PREVENTION

In addition to strengthening pathogen detection, it is more important to cultivate aquaculture species with strong resistibility and resistance to disease, and to develop new disease prevention techniques. In recent years, practice has shown that molecular biology techniques are of great potentiality in these areas, such as using recombinant DNA technology to inject disease-resistant genes to get disease-resistant transgenic aquatic animals and genetic engineering vaccine.

Application of Transgenic Technology in Disease Prevention

The use of recombinant DNA technology for putting stress-resistant or disease-resistant genes into a living body in order to enhance their disease resistance, has become one of the effective ways for aquatic animal disease prevention and control. For example, through transferring antibacterial peptide gene into aquatic animals, aquatic animals will have strong immune systems to resist a variety of bacteria and virus infection. It is expected that with an in-depth study of genetic engineering, in the near future, new aquaculture animal species with the abilities of resistibility and resistance will be cultivated.

Application of Genetic Engineering Vaccine in Disease Prevention

With genetic engineering technology, we can combine antigen genes of immunity separated from the bacteria or virus with

vectors, and then transfer this recombinant DNA into an *in vitro* expression system, such as E. coli or yeast, to manufacture antigen proteins that are used as vaccine. Animals injected with this antigen protein generate antiserum with the ability of resisting the very kind of bacteria or viruses. Genetic engineering vaccine is antigen-specific and can be applied to fermentation technology for mass production.

Currently, fish pathogens, such as Vibrio, Aeromonas bacteria, and Edwardsiella, have been successfully made into whole-cell inactivated vaccine, and with LPS extracted from the biomass, extracellular proteins and other effective antigens, monoclonal antibodies, and other bio-engineering vaccines have been prepared under commercial production. Vaughan et al. (2007) found that DNA vaccine for dolphin morbillivirus has immunogenicity against bottlenose dolphin. But, as for the fish vaccines (especially virus vaccine), there are still many problems, such as high cost and lack of reasonable and effective ways of medication and evaluation. There are also some difficulties in vaccine preparation and actual production.

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BREEDING OF IMPROVED VARIETY

Improvement of Disease Resistance of Fish

Diseases are the main cause of economic loss in the fish farming industry. The application of transgenic technology can effectively improve the disease resistance of fish to substantially reduce economic losses. Dunham et al. (2002) transferred cecropin B gene into catfish body by electroporation with F1 generation of catfish showing significant disease resistance.

In addition, genetic engineering techniques can be used to combine antigen genes of immunity with the carriers. After being transferred into receptors, such as E. coli or yeast, this recombinant DNA can be used in mass production of antigen protein as vaccine. This vaccine, compared with conventional vaccines, is antigen-specific and is available for mass production by fermentation technology. At the present, many countries have prepared engineered vaccines, and applied them in practical production. In aquaculture, a kind of carbohydrate antigen with strong immunogenicity has been obtained, which can effectively prevent infection by infectious sepsis.

Improvement of Anti-Freezing Ability of Fish

The low water temperature in winter can cause considerable stimulation to many fish, for example, most fish cannot tolerate temperatures as low as -1.4 to -19°C in the Arctic Ocean sea water. Fishes adaptive to warm water may all be dead in the face of rarely-confronted low temperatures, which is a serious problem in the aquaculture industry. Over the years, people have always expected to raise cold-resistant fishes in order to make some fine varieties adaptive to larger waters and capable of growing in lower temperatures. The emergence of transgenic technology has brought hope for solving such problems.

In earlier years, scientists conducted researches about transferring antifreeze protein into fertilized eggs of Atlantic salmons. In recent years, injection of antifreeze proteins into goldfishes (1995) or to tilapias or juvenile fishes of lactaria (1998), can improve their ability to withstand freezing. Fletcher et al. (1998) transferred antifreeze protein genes of flounder into Atlantic salmons, so salmons have a certain degree of resistance to low temperatures. Zhu et al. (1997) has done a preliminary study of *Cirrhina molitorellas* with transferred antifreeze protein genes, showing that their ability to withstand freezing was improved.

Improvement of Growth Rate of Fish

Fish is an important source of animal protein for humans. Currently, the world's output of fish products is far from the needs of humans, so it is of great significance to cultivate fast-growing fish to satisfy the growing needs of the people. Transgenic technology can be used for transferring growth hormone genes into the fish in order to obtain fast-growing, high-yield "super-fish." At present, a variety of fast-growing fishes with transferred growth hormone genes have been obtained, whose rate of growth is obvious. Du et al. (1992) recombined the growth hormone cDNA of king salmons with antifreeze protein promoters of rock skippers, then transferred them into Atlantic salmons, and finally obtained fast-growing transgenic fishes successfully. Maclean et al. (2002) transferred growth hormone of salmons into fertilized eggs of tilapia, making them gain weight faster. Martínez et al. (2000) detected that tilapias containing transgenic homologous growth hormone genes utilize food more thoroughly. Fishes with transgenic growth hormone genes obviously grow fast with high adaptability. The establishment of fine varieties of transgenic fish strains will bring huge economic benefits for the fishing industry. Transgenic fishes are most apt to be in mass production.

Application of Transgenic Technology in Other Aquatic Animals

As early as 1985, there were reports of researches on transgenic sea urchins, and later on transgenic shrimps, shellfishes, crabs, and other aquatic animals. Pimentel et al. (1996) transferred lacZ genes into fertilized eggs of Litopenaeus schmitts, and got transient expression. Sun et al. (2005) cloned muscle actin promoters of *Litopenaeus vannamei*, transferred them into prawns, and thus, obtained transgenic *Litopenaeus vannamei*. He also applied for a patent on it. The Institute of Oceanology, Chinese Academy of Sciences successfully transferred exogenous DNA into penaeus, and obtained transgenic shrimp (Du et al., 1992). Growth hormone genes of sheep have been successfully transferred into fertilized eggs of prawns, and

transgenic shrimp are obtained (Zhang et al., 2006; Meng et al., 2005). Hu and Yu (2000) mixed sperms of *Pinctada maximas* with linearized DNA fragments of targeted genes. With the continuous in-depth researches of transgenic technology, gene modification (GM) technology will be applied to researches of other aquatic animals.

WATER ENVIRONMENT PROTECTION AND BIOREMEDIATION

In addition to the above-mentioned application, molecular biology techniques have been widely applied in protection and restoration of environment, which is mainly reflected in the applications of enzyme engineering technology. The enzyme engineering technology uses catalytic effect of enzymes, organelles, or cells to transform the corresponding raw materials into needed products in certain bio-reactors. Enzyme engineering technology includes enzyme immobilization technology, cell immobilization technology, enzyme modification technology, enzyme reaction technology, and so on. Scientists can develop a variety of engineering bacteria by enzyme engineering technology, which has effective degradation to oil and alkyl halides in sea culture environments. Thus, the purification capacity of water body can be increased, and ecological environments are protected.

In addition, enzymes that are produced by enzyme engineering technology added to the feed can eliminate the harmful effects of anti-nutritional factors, destruct plant cell walls, and promote digestion and absorption level of nutrients so as to improve the health of aquatic animals. Such enzymes can also reduce the emissions of the breeding industry, thus protecting the ecological environment. Good ecological environment is the basis for prevention of aquatic animal diseases (Chi et al., 2009).

PROSPECTS

The late 1980s and 1990s saw the rapid development of molecular biology, which initially supplemented and now has largely replaced traditional methods as a fishery management tool. In addition to bringing greater power of resolution and the capability of non-lethal sampling to traditional fishery genetics questions, DNA methods have opened up a vast new range of applications in modern marine biology—from ecosystem assessment and ocean observation to seafood safety. The molecular revolution in biology is just getting underway—changing how we conduct researches and developing new approaches and tools for management of natural marine resources and large marine ecosystems. The increasing capacity and reduced costs for high-throughput DNA sequencing, comparative genomics, detecting changes in gene expression, and bioinformatics is fueling rapid changes in marine science (Chen et al., 2009; Rao et al., 2009).

With the increased investment, molecular biology technologies will play a more important role in cultivation of good strains, germ plasm identification, pathogen detection and disease prevention, and will produce a far-reaching impact on aquaculture all over the world (Thellin et al., 2009).

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REFERENCES

Adams, A., K. D. Thompson, H. McEwan, S. C. Chen, and R. H. Richards. Development of monoclonal antibodies to *Mycobacterium* spp. isolated from Chevron snakehead and Siamese fighting fish. *J. Aquat. Anim. Health.*, **8:** 208–215 (1996).

Altinok, I., E. Capkin, and S. Kayis. Development of multiplex PCR assay for simultaneous detection of five bacterial fish pathogens. *Vet. Microbiol.*, **131:** 332–338 (2008).

Austin, B., D. A. Austin, A. R. Blanch, M. Cerda, F. Grimont, P. Grimont, J. Jofre, S. Koblavi, and M. J. Larsen. A comparison of methods for the typing of fish-pathogenic Vibrio spp. *Syst. Appl. Microbial.*, **20:** 89–101 (1997).

Beaz-Hidalgo, R., G. E. Magi, S. Balboa, J. L. Barja, and J. L. Romalde. Development of a PCR protocol for the detection of Aeromonas salmonicida in fish by amplification of the fstA (ferric siderophore receptor) gene. *Vet. Microbiol.*, **128:** 386–394 (2008).

Borrell, N., S. G. Acinas, M. J. Figueras, and A. J. Martínez-Murcia. Identification of Aeromonas clinical isolates by restriction fragment length polymorphism of PCR-amplified 16S rRNA genes. *J. Clin. Microbiol.*, **38:** 2023–2025 (2000).

Chang, Y. S., S. E. Peng, H. C. Wang, H. C. Hsu, C. H. Ho, C. H. Wang, S. Y. Wang, C. F. Lo, and G. H. Kou. Sequencing and amplified restriction fragment length polymorphism analysis of ribonucleotide reductase large subunit gene of the white spot syndrome virus in blue crab (*Callinectes sapidus*) from American coastal waters. *Mar. Biotechnol.*, **3:** 162–171 (2001).

Chen, P., H. B. Shao, D. Xu, and S. Qin. Progress in Gracilaria biology and developmental utilization: Main issues and prospective. *Rev. Fisheries Sci.,* **17:** 494–504(2009).

Chi, Z. M., Z. Chi, T. Zhang, G. L. Liu, J. Li, and X. H. Wang. Production, characterization and gene cloning of the extracellular enzymes from the marine-derived yeasts and their potential applications. *Biotech. Adv.*, **27:** 236–255 (2009).

Christianson-Heiska, I., and B. Isomaa. The use of primary hepatocytes from brown trout (Salmo trutta lacustris) and the fish cell lines RTH-149 and ZF-L for in vitro screening of (anti)estrogenic activity of wood extractives. *Toxicol. In Vitro.*, **22:** 589–597 (2008).

Cunningham, C. O. Molecular diagnosis of fish and shellfish diseases: Present status and potential use in disease control. *Aquaculture*, **206:** 19–55 (2002).

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Cunningham, C. O. Species variation within the internal transcribed spacer (ITS) region of Gyrodactylus (Monogenea: Gyrodactylidae) ribosomal RNA genes. *J. Parasitol.*, **83:** 215–219 (1997).

Cunningham, C. O., D. M. Mcgillivray, K. Mackenzie, and W. T. Melvin. Identification of Gyrodactylus (Monogenea) species parasitizing salmonid fish using DNA probes. *J. Fish. Dis.*, **18:** 539–544 (1995).

Du, S. J., Z. Y. Gong, G. L. Fletcher, M. A. Shears, M. J. King, D. R. Idler, and C. L. Hew. Growth enhancement in transgenic Atlantic salmon by the use of an "All Fish" chimeric growth hormone gene construct. *Bio/Technology*, **10:** 176–181 (1992).

Dunham, R. A., G. W. Warr, A. Nichols, P. L. Duncan, B. Argue, D. Middleton, and H. Kucuktas. Enhanced bacterial disease resistance of transgenic channel catfish ictalurus punctatus possessing cecropin genes. *Mar. Biotechnol.*, **4:** 338–344 (2002).

Einer-Jensen, K., J. Winton, and N. Lorenzen. Genotyping of the fish rhabdovirus, viral haemorrhagic septicaemia virus, by restriction fragment length polymorphisms. *Vet. Microbiol.*, **106:** 167–178 (2005).

Fan, J. F., Y. B. Liang, L. C. Song, B. Wang, H. M. Zang, and W. Z. Li. Indirect ELISA method for detecting the pathogenic bacteria of Litopenaeus vannamei red body disease. *J. Fish. China*, **30:** 114–l17 (2006).

Fletcher, G. L., M. A. Shears, M. J. King, P. L. Davies, and C. L. Hew. Evidence for antifreeze protein gene transfer in Atlantic salmon (Salmo salar). *Can. J. Fish. Aquat. Sci.*, **45:** 352–357 (1998).

Hu, W., and D. H. Yu. Preliminary study on transgenic mudcarp of antifreeze protein gene. *J. Biotechnol. Chinese*, **16:** 165–168 (2000).

Karunasagar, I., G. Sugumar, I. Karunasagar, and J. A. Reillyp. Rapid polymerase chain reaction method for detection of Kanagawa positive vibrio parahaemolyticus in seafood. *Int. J. Food. Microbiol.*, **31:** 317–323 (1996).

Lei, Z. W., C. Y. Shi, J. Huang, B. Yang, K. K. Yu, and W. B. Zhan. Development of digoxigenin labeled probe for detection of white spot syndrome by dot-blot hybridization. *Vet. Microbiol.*, **31:** 201–204 (2001).

Lightner, D. V., and R. M. Redman. Shrimp diseases and current diagnostic methods. *Aquaculture*, **164:** 201–220 (1998).

Maclean, N., M. Rahman, F. Sohm, G. Hwang, A. Iyengar, H. Ayad, A. Smith, and H. Farahmand. Transgenic tilapia and the tilapia genome. *Gene*, **295:** 265–277 (2002).

Maddison, B. C., S. Patel, R. F. James, H. E. Conlon, B. Oidtmann, M. Baier, G. C. Whitelam, and K. C. Gough. Generation and characterisation of monoclonal antibodies to rainbow trout (Oncorhynchus mykiss) prion protein. *J. Immunol. Methods*, **306:** 202–210 (2005).

Mari, J., J. R. Bonami, and D. V. Lightner. Taura syndrome of penaeid shrimp: Cloning of viral genome fragments and development of specific gene probes. *Dis. Aquat. Org.*, **33:** 11–17 (1998).

Martínez, R., J. Juncal, C. Zaldívar, A. Arenal, I. Guillén, V. Morera, O. Carrillo, M. Estrada, A. Morales, and M. P. Estrada. Growth efficiency in transgenic tilapia (Oreochromis sp.) carrying a single copy of a homologous cDNA growth hormone. *Biochem. Bioph. Res. Co.*, **267:** 466–472 (2000).

Meng, X. H., J. Kong, P. Liu, C. Y. Ma, and Y. Li. Screen of white-spot-syndrome-virus (WSSV)-resistance molecular markers in Fenneropenaeus chinensis. *J. Fish. Sci. China*, **12:** 35–38 (2005).

Meyers, T. R., S. Short, C. Farrington, K. Lipson, H. J. Geiger, and R. Gates. Comparison of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody test (FAT) for measuring the prevalence and levels of Renibacterium salmoninarum in wild and hatchery stocks of salmonid fishers in Alaska, USA. *Dis. Aquat. Organ.*, **16:** 181–189 (1993).

Milne, S. A., S. Gallacher, P. Cash, and A. J. Porter. A reliable RT-PCR-ELISA method for the detection of infectious pancreatic necrosis virus (IPNV) in farmed rainbow trout. *J. Virol. Methods.*, **132:** 92–96 (2006).

Pimentel, R., E. Cabrera, O. Hernández, B. Alvarez, C. Canino, Z. Abad, J. C. Piña, V. Sánchez, R. Lleonart, and J. de la Fuente. Transient expression of a lacZ transgene in shrimp (P. schmitti) using two different gene transfer methods. *Biotechnol. Apl.*, **13:** 26–37 (1996).

Rao, A. Q., A. Bakhsh, S. Kiani, K. Shahzad, A. A. Shahid, H. Tayyab, and S. Riazuddin. The myth of plant transformation. *Biotech. Adv.*, **27:** 753–763 (2009).

Ryan, L. A., C. B. Seymour, A. O'Neill-Mehlenbacher, and C. E. Mothersill. Radiation-induced adaptive response in fish cell lines. *J. Environ. Radioactiv.*, **99:** 739–747 (2008).

Salinas, I., J. Meseguer, and M. A. Esteban. Antiproliferative effects and apoptosis induction by probiotic cytoplasmic extracts in fish cell line. *Vet. Microbiol.*, **126:** 287–294 (2008).

Sun, P. S., N. C. Venzon, F. R. O. Calderon, and D. M. Esaki. Evaluation of methods for DNA delivery into shrimp zygotes of Penaeus (Litopenaeus) vannamei. *Aquaculture*, **243:** 19–26 (2005).

Talaat, A. M., R. Reimschuessel, and M. Trucksis. Identification of mycobacteria infecting fish to the species level using polymerase chain reaction and restriction enzyme analysis. *Vet. Microbiol.*, **58:** 229–237 (1997).

Thellin, O., B. El Moualij, E. Heinen, W. Zorzi. A decade of improvements in quantification of gene expression and internal standard selection. *Biotech. Adv.*, **27:** 323–333 (2009).

Vaughan, K., J. Del Crew, G. Hermanson, M. K. Wloch, R. H. Riffenburgh, C. R. Smith, and W. G. Van Bonn. A DNA vaccine against dolphin morbillivirus is immunogenic in bottlenose dolphins. *Vet. Immunol. Immunopathol.*, **120:** 260–266 (2007).

Venkateswaran, K., N. Dohmoto, and S. Harayama. Cloning and nucleotide sequence of the gyrB gene of vibrio parahaemolyticus and its application in detection of this pathogen in shrimp. *Appl. Environ. Microbiol.*, **64:** 681–687 (1998).

Xie, S. T., J. G. He, X. M. Yang, L. Lv, and J. B. Jiang. Detection of white spot syndrome virus (WSSV) in penaeus monodon using nested PCR. *J. Ocean. Univ. Qingdao.*, **31:** 201–204 (2001).

Yan, Q. P., W. Z. Zou, R. X. Ji, Z. S. Zhuang, and X. R. Wang. Detection of vibrio fluviails in paralichthys olivaceus using indirect fluorescent antibody technique. *Mar. Sci.*, **30:** 16–19 (2006).

Zhang, T. S., P. Liu, J. Li, J. Kong, and Y. Q. Wang. Preliminary study on specific microsatellites markers related to growth trait in Fenneropenaeus chinensis. *Mar. Fish. Res.*, **27:** 139–142 (2006).

Zhu, X. P., S. L. Xia, Y. Zhang, and J. Z. Liu. Preliminary study on transgenic mud carp of antifreeze protein gene. *J. Fish. Sci. China*, **4:** 79–80 (1997).