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DNA testing for ornamental fish

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With the rapid development of PCR (1) and DNA sequencing technology, DNA testing has been extensively used in humans, for example, in forensic investigations and in diagnostics of human genetic diseases.

In economically important animals and food fish species (e.g. pig, cattle, sheep, salmon, tilapia and rainbow trout), DNA testing techniques have also been used for identifying superior animals which grow faster, produce high-quality products, show strong disease resistance and other preferred phenotypes (2). However, the application of DNA testing technology in the ornamental fish industry is just beginning. Here I briefly introduce the very complicated subject of DNA testing techniques and their applications in the ornamental fish industry.

What is DNA testing?

DNA testing is a type of genetic test that looks for changes/differences in the DNA molecule among individuals. The changes/differences, scientifically called mutations, may be associated with biological characteristics such as body mass, colour, sex and body shape.

What is DNA?

DNA (deoxyribonucleic acid) is the genetic blueprint that determines an individual's biological characteristics: eye colour, hair colour, stature, bone density, sex and many other traits. DNA is a long but narrow string-like structure. A one-foot (30-cm) long string of DNA is normally packed into a space roughly equal to a cube 2.5/millionth of a centimetre on each side. Animal body cells each contain a complete sample of DNA (Figure 1). A strand of DNA is made up of tiny building blocks.

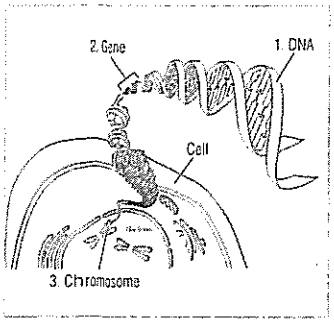


Figure 1. DNA in a cell

There are only four different basic building blocks. Scientists usually refer to these using four letters: A (Adenine), T (Thymine), G (Guanine) and C (Cytosine). DNA is passed through the sperm and egg to the offspring. A single sperm cell contains 0.4-3 billion bases consisting of A, T, G and C that follow each other in a well-defined sequence along the strand of DNA. DNA may vary from one individual to another and it is these DNA variations that form the basis of DNA testing.

How does DNA testing work?

There are basically two types of DNA testing: RFLP (3) analysis and PCR-based analysis. Generally, RFLP analysis requires larger amounts and high quality of DNA. PCR-based DNA testing often requires less DNA than RFLP testing and the DNA may be partially degraded. PCR tests are extremely sensitive; DNA from single small fish scale is enough for 100 DNA tests. Therefore, currently PCR-based DNA testing techniques are currently preferred.

PCR-based DNA testing contains the following steps (Figure 2):

1) Isolate DNA from tissues.
2) Select or develop DNA markers (DNA fragments show difference among individuals)
3) Perform PCR
4) Check PCR products by gel electrophoresis (4)
5) Analyse data with bioinformatic tools (5)

Since the development of DNA markers is a very complicated issue, I shall not discuss it here. Readers who are interested in developing DNA markers, can consult the scientific literature for more rigorous details.

What can DNA testing do for ornamental fish industry?

Identification of species, varieties and individuals

Although traditional morphological taxonomy has been successful in distinguishing species/varieties for many fish groups, this approach can be problematic due to genetic and phenotypic variation in characters used for identification. Furthermore, successful use of many morphologically-based keys requires significant expertise and many keys are specific to certain genders or life stages. DNA testing provides a solution to these limitations in species/varieties identification.

For example, the dragon fish is one of the most expensive ornamental fish in the word. There are three natural colour varieties, namely the green, golden and red. However, the colour of young fish (up to several months of age) is hardly distinguishable. Temasek Life Sciences Lab

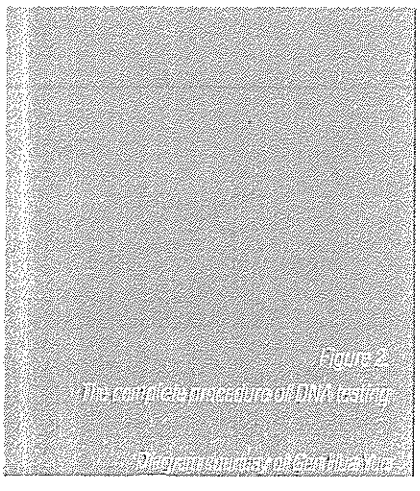
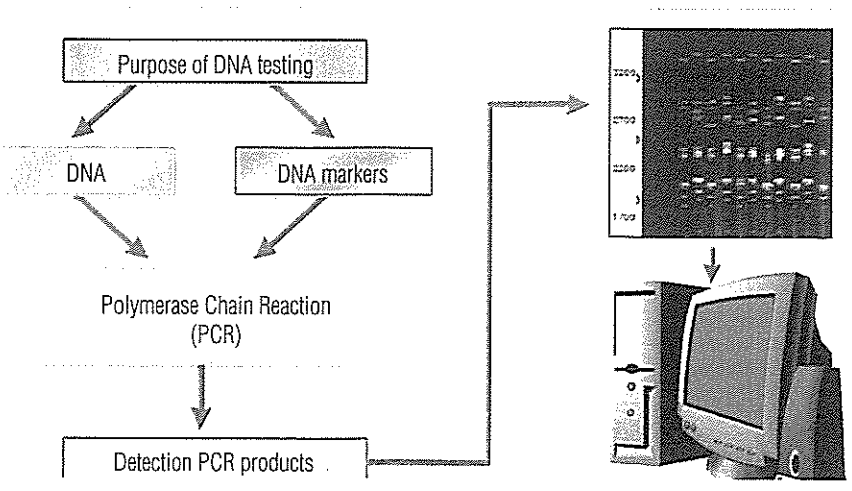


Figure 2. The complete procedure of DNA testing. Diagram courtesy of Gen Hua Yue



(Singapore) has developed a PCR-based DNA testing method for differentiating golden from red and green dragon fish. On two per cent agarose gel (6), individuals showing one band are golden; two bands are shown either by green or red dragon fish (Figure 3). The accuracy of DNA testing is 100 per cent. The DNA testing procedure lasts ca. 4 hours. However, the DNA testing method cannot differentiate between green and red individuals.

For identification of species, the DNA sequence in the COX1 gene of the maternally-inherited mtDNA is a good choice. Currently, an international consortium is trying to barcode most of the species on earth using DNA sequences. DNA testing for identification of interesting traits is very complicated, but it is feasible. With the large number of DNA markers, and high-density linkage maps, DNA markers can be identified and used for identifying interesting traits. However, the development of a large number of DNA markers and construction of high-density linkage maps is very costly.

2. *Identification of sex*

Although many ornamental fish species have been raised in captivity for many years, experienced farmers are still unable to ascertain the gender of each fish in some of these species. This is a major obstacle in any selective breeding programmes for ornamental fish. Temasek Life Sciences Lab has developed a number of DNA makers, which can be used for identifying sex in several fish species. One PCR-based DNA testing method was developed for identifying the sex of green dragon fish (the efficiency of the method is 82.6 per cent) but the sex-associated DNA marker did not work in the more expensive golden or red varieties. Molecular sexing in fish is much more difficult than in mammals and, so far, no single universal sex-associated DNA marker has been identified for any fish species, presumably due to the complex nature of sex determination systems in fish.

3. *Estimation of genetic diversity*

Genetic diversity is the basis of genetic improvement. Experiences from livestock breeding and food fish breeding clearly show that loss of genetic diversity and inbreeding cause a reduction in production and low fitness. In ornamental fish species, their genetic diversity is difficult to identify without DNA testing methods, due to the fact that fish produce a lot of eggs, are difficult to tag, and pedigree information is (usually) either not available or incomplete. For example, right now, dragon fish farmers place 20 to 30 fish into the ponds without knowing the relationship of fish, and hope to see some babies every few months.

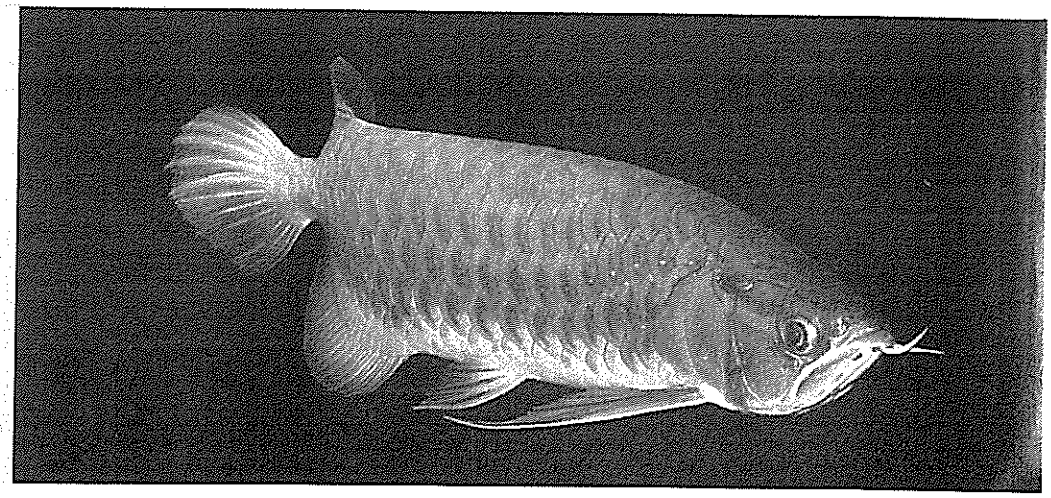
With a tiny scale from each fish and polymorphic DNA markers (e.g. microsatellites (7)), DNA testing can be easily conducted to give information about the relationship among fish, their genetic diversity and inbreeding. With this information, farmers can properly design mating plans to avoid inbreeding, maintain high genetic diversity and produce superior fish over other farmers who do not have such information. The Temasek Life Sciences Lab has developed a number of polymorphic DNA markers (microsatellites, mtDNA, RAPD, AFLP (8)) for ornamental fish species (dragon fish, guppy, pufferfish, swordtail and koi). It has already conducted DNA testing on captive-bred dragon fish populations for some fish farms.

4. *Reconstruction of pedigree*

One major problem in conducting selective breeding programmes in fish is the maintenance of pedigree information. Offspring from different families have to be isolated and reared under the same environmental conditions until they are big enough to be tagged. This traditional approach is not only space-intensive, but also labour-intensive.

Genetic profiles from polymorphic DNA markers would allow retrospective assignment of offspring to family groups (their parents) even when all offspring are reared in same place.

Experience in food fish has shown that using one set of 8-18 polymorphic DNA markers, all offspring can be assigned to their parents with 99.999 per cent certainty. The reconstructed pedigree information can then be used for selective breeding, setting up proper mating among individuals to produce superior offspring.



A magnificent mature red dragon fish

5. *Identification of individual fish: Genetic ID card*
Traditionally, fish can be tagged by an electrical tag (e.g. PIT tag) containing a digital number. However, the electrical tag can only be implanted in relatively large fish. Furthermore, it is easily lost, removed and exchanged. Therefore, it is not the most reliable method for tagging fish.

Cells/tissues of each fish naturally contain a unique 'fingerprint' embedded in its DNA, which could be used as the basis for labelling each individual. The same DNA in each fish is maintained throughout its whole lifespan and cannot be removed or changed. Therefore, by selecting the most variable parts of the DNA, it is possible to differentiate each individual with certainty.

One of the most variable parts of DNA is a special class of repetitive DNA called a microsatellite. Microsatellites consist of variable numbers of tandem repeats, ranging from two to six base pairs (9) in length, which are distributed evenly and widely in the genomes (10) of most vertebrate species. As microsatellite patterns are highly polymorphic (11) between individuals within a species, they can be used to generate genetic profiles or 'fingerprints' unique to the individual animal, using PCR technology and high-resolution gel electrophoresis or capillary electrophoresis (12). A genetic ID card looks like a human ID card, but contains details about genetic information. The Temasek Life Sciences Lab is developing a genetic card for expensive ornamental fish species (e.g. dragon fish).

What are the benefits of DNA testing to the ornamental fish industry?

- Allows for knowledge-based decisions to be made for selective breeding programmes
- Increases performances of fish to ensure product quality
- Increases production efficiency to increase return on investment
- Accelerates development of new species, new strains and new varieties
- Protects intellectual property
- Conserves precious genetic materials more effectively

How much does a DNA testing cost ?

The cost of DNA testing could vary from $8 to $200 depending on how many DNA markers are used and how high the certainty of test is.

Pictures courtesy of Gen Hua Yue

Definitions of some terms used

1) PCR (Polymerase Chain Reaction) *is a molecular biological technique for amplifying (creating multiple copies of) DNA without using a living organism.*

2) Phenotype: The phenotype *of an individual organism is either its total physical appearance and constitution, or a specific manifestation of a trait, such as size or eye colour, that varies between individuals.*

3) RFLP (Restriction Fragment Length Polymorphism) *is a method used for detection of DNA polymorphism, where DNA is cut with restriction enzymes. The cut DNA is separated by size by gel electrophoresis. Slight differences in homologous fragments may exist between individuals. These length polymorphisms are referred to as RFLP.*

4) Gel Electrophoresis *is a group of techniques used by scientists to separate molecules based on physical characteristics such as size and shape.*

5) Bioinformatics *- also called computational biology - is the use of techniques from applied mathematics, informatics, statistics and computer science to solve biological problems.*

6) Agarose *is a polysaccharide polymer material, generally extracted from seaweed.*

7) A microsatellite *is a short DNA sequence (a **Tandemly Repetitive DNA Sequence**) that is repeated many times within the genome of an organism.*

8) mtDNA *is the abbreviation for mitochondrial DNA, which differs from that contained within the nucleus. mtDNA contains genes necessary for cell life, such as the genes coding for tRNA, indispensable for protein synthesis.*

8) RAPD *stands for **Random Amplification of Polymorphic DNA.** It is a type of PCR reaction, but the segments of DNA that are amplified are random.*

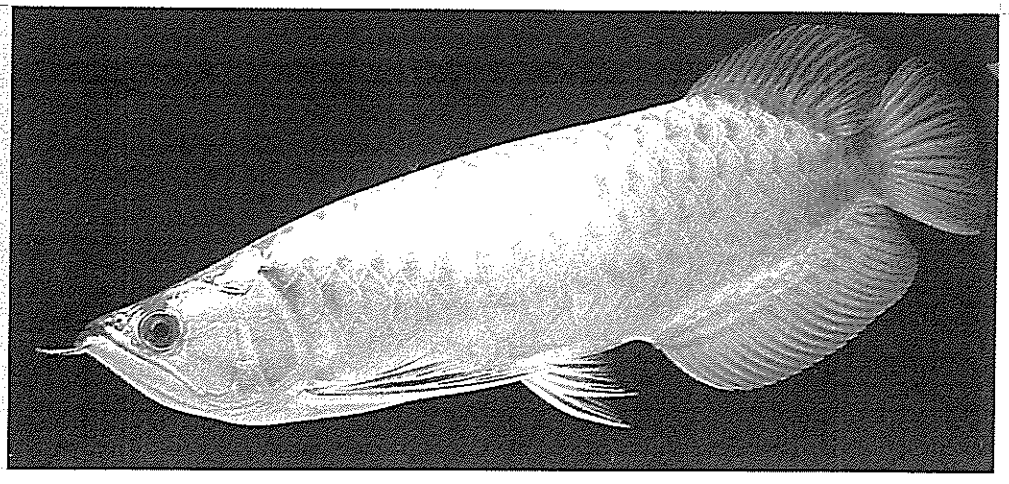
8) AFLP (Amplified Fragment Length Polymorphism) *is a highly sensitive method for detecting polymorphisms in DNA. Following restriction enzyme digestion of DNA, a subset of DNA fragments is selected for PCR amplification and visualisation.*

9) Base pair: *in genetics, two nucleotides on opposite complementary DNA or RNA strands that are connected via hydrogen bonds are called a base pair (often abbreviated as bp)*

10) Genome: *in biology, the genome of an organism is the whole hereditary information of an organism that is encoded in the DNA.*

11) Polymorphic: *in general, polymorphism describes multiple possible states for a single property (it is said to be polymorphic)*

12) Capillary Electrophoresis (CE) *can be used to separate ionic species by their electrical charge and frictional forces. In traditional electrophoresis, electrically charged substances (analytes) move in a conductive liquid medium under the influence of an electric field. Introduced in the 1960's, the technique of **Capillary Electrophoresis (CE)** was designed to separate species based on their size to charge ratio in the interior of a small capillary filled with an electrolyte.*



Adult snow dragon fish