Chapter Two

Review of Literature
Biodiversity or biological diversity is the diversity of life which refers to variation at all level of biological organization (Kevin and Spicer, 2004). Diversity includes diversity within species and among species and comparative diversity among ecosystems. The foundation for biodiversity and organic evolution is the genetic variation within the species. In small isolated populations genetic variability can be substantially reduced through genetic drift and inbreeding and such reduction may result in the reduced fitness and eventual extinction. In conjunction with other evolutionary forces like selection and genetic drift, genetic variation that arises between individuals eventually leads to differentiation at the level of population, species and higher order taxonomic groups. Molecular genetic markers are powerful tools to detect genetic variation between of individuals, which can be inferred as differentiation at that level of populations or species (Avise, 1994; Linda and Paul, 1995). These markers have revolutionized the analytical power, necessary to explore the genetic diversity (Hillis et al., 1996). With DNA markers, it is theoretically possible to observe and exploit genetic variation in the entire genome (Lakra et al., 2006) Since Markert and Moller(1959) first discovered the existence of multiple molecular forms of enzymes, called isozymes, various classes of markers have been developed that have revolutionized the field of screening genetic diversity (Hillis et al., 1996). Mitochondrial DNA and micro/mini satellites have been used
for various objectives ranging from species specific markers, population structures and detection of genetic bottlenecks. The conclusion from genetic diversity data has varied application in research on evolution, conservation and management of natural resources, genetic improvement programmes etc (Ferguson et al., 1995; Neff and Gross, 2001; Jehle and Arntzen, 2002; Wasko et al., 2003; Liu and Cordes, 2004; Morin et al., 2004).

Genetic markers are important tools for the study of fish populations. The development of molecular methods has permitted genomic analysis and made it possible to analyze existing variation, both in regions that encode genes, as well as in regions with unknown functions (Regitano, 2001). Genetic monitoring is ideal for use in a reproduction program with the aim of genetic conservation (i.e., stocking). Molecular markers are a realistic and useful tool for the investigation and monitoring of genetic conditions both in native populations and in captive lots (Alam and Islam, 2005). Molecular markers are classified into two categories: Type I markers are associated with genes of known function, while Type II markers are associated with anonymous genomic segments (O'Brien, 1991). Microsatellite markers are type II markers unless they are associated with genes of known function. Type I markers have utility in studies of comparative genomics, genome evolution, candidate gene identification, and enhanced communication among laboratories for example, Allozymes. Type I markers serve as a bridge for comparison and transfer of genomic information from a map rich species into a
relatively map-poor species. In general, *Type II* markers such as RAPDs, microsatellites, and AFLPs are considered to be non-coding and, therefore, selectively neutral. Such markers have found widespread use in population genetic studies whose characterizations of genetic diversity and divergence within and among populations are based on assumptions of Hardy–Weinberg equilibrium and selective neutrality of the markers employed (Brown and Epifanio, 2003). For the most frequently used microsatellite markers, such comparative studies depend on conservation of the flanking sequences used for the design of PCR primers. *Type II* markers also have proven useful in aquaculture genetics for species, strain and hybrid identification, in breeding studies, and more recently as markers linked to QTL. Such markers have found widespread use in population genetic studies to characterize genetic divergence within and among the populations or species (Brown and Epifanio, 2003).

The potential of molecular markers to fisheries management has long been recognized (Utter, 1991). Early studies on the molecular phenotypes used blood group polymorphisms to discriminate between spatially discrete populations of fish (Sick, 1961). Because of the problem of interpretation, research turned to specific histochemical stain procedures (Hunter and Markert, 1957). Staining for specific proteins was used in association with starch gel electrophoresis and permitted the detection of allozyme variation (Harris and Hopkinson, 1976). In the late 1970s, workers start investigating of DNA sequences and first focused on mitochondrial
DNA (mtDNA) molecule. However, more recent marker types that are finding service in this field include, restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite, single nucleotide polymorphism (SNP) and expressed sequence tag (EST) markers.

2.1 Types of molecular markers

1. Allozymes

Allelic form of an enzyme that can be distinguished by gel electrophoresis is known as an Allozyme or Isozyme. They can be used to observe genetic variation from gene products. Since 1960s, the starch gel electrophoresis of allozymes has been the most commonly employed molecular method in fishery genetics using protein-coding loci (Ryman and Utter, 1987; Hillis et al., 1996). Allozymes were among the earliest markers used in aquaculture genetics (May et al., 1980; Seeb and Seeb, 1986; Johnson et al., 1987; Liu et al., 1992, 1996; Morizot et al., 1994). Allozyme found use in aquaculture for tracking inbreeding, stock identification, and parentage analysis. In a few cases, correlations existed between certain allozyme markers and performance traits (Hallerman et al., 1986; McGoldrick and Hedgecock, 1997). Their use in linkage mapping has been demonstrated in studies of salmoids (Pasdar et al., 1984; May and Johnson, 1993) and poeciliids (Morizot et al., 1991). Murphy et al (1990) list 75 enzymes systems coded by several
genetics loci that may potentially be analyzed in fishes. They have been successful in determining genetic variation in a large no of species like, *Labeo dero* (Anshumala *et al.* 2005), *Labeo rohita* (Khan *et al.* 2006), *Cirrhinus mrigala* (Mohindra *et al.* 2007), *Channa punctatus* (Haniffa *et al.* 2007), *Gadus morhua* (Pogson *et al.* 2008), *Chitala chitala* (Mandal *et al.* 2009) and *Labeo dussumeri* (Gopalakrishnan *et al.* 2009). Disadvantage associated with the allozymes include null (enzymetically inactive) alleles and the amount of tissue sample required. Some DNA sequence changes are masked at the protein level, reducing the level of detectable variation and low level of genetic variation revealed in many allozyme studies of marine fish population (Siddell *et al.*, 1980; Mork *et al.*, 1985; Crawford *et al.*, 1989). However, in spite of their strength as co dominant Type I markers, ease of use and low cost their use in aquaculture has become limited.

2. Random amplified polymorphic DNA (RAPD)

RAPD procedures were first developed in 1990 (Welsh and McClelland, 1990; Williams *et al.*, 1990) using PCR to randomly amplify anonymous segments of nuclear DNA with an identical pair of primers 8-10 bp in length. Genetic variation and divergence within and between the taxa of interest are assessed by the presence or absence of each product, which is dictated by changes in the DNA sequence at each locus. RAPD polymorphisms can occur due to base substitutions at the primer binding sites or to indels in the regions
between the sites. The potential power is relatively high for detection of polymorphism. RAPD analysis has been successfully used for genetic variation in genus Tor (Mohindra et al., 2007), Acanthopagrus schlegeli (Liu et al., 2007), Oncorhynchus mykiss, Salmo trutta (Diaz et al., 2007), Mystus vittatus (Garg et al., 2009) and Labeo dussumeri (Gopalakrishnan et al., 2009).

Shortcomings of this type of marker include the difficulty of demonstrating Mendelian inheritance of the loci and the inability to distinguish between homozygotes and heterozygotes. Analysis follows the assumption that populations under study follow Hardy-Weinberg expectations. In addition, the presence of paralogous PCR product (different DNA regions which have the same lengths and thus appear to be a single locus), low reproducibility due to the low annealing temperature used in the PCR amplification, have limited the application of this marker in fisheries science (Wirgin and Waldman, 1994).

3. Amplified fragment length polymorphism (AFLP)

AFLP is a PCR-based, multi-locus fingerprinting technique that combines the strengths and overcomes the weaknesses of the RFLP and RAPD methods. Like RFLPs, the molecular basis of AFLP polymorphisms includes indels between restriction sites and base substitutions at restriction sites. Like RAPD, it also includes base substitutions at PCR primer binding sites. The unique feature of the technique is the addition of adaptors of known sequence to DNA
fragments generated by digestion of whole genomic DNA. This allows for the subsequent PCR amplification of a subset of the total fragments for ease of separation by gel electrophoresis. Its primary target of genetic variation is the same as RFLP, but instead of analyzing one locus at a time, it allows for the analysis of many loci simultaneously.

The power of AFLP analysis is tremendously high for revealing genomic polymorphisms. For instance, Young *et al.* (2001) used the AFLP technique that generated 133 polymorphic markers, 23 of which were diagnostic in distinguished rainbow trout, coastal cutthroat trout, and their hybrids. AFLP markers also have used for analysis of meogynogens and androgens (Young *et al.*, 1996; Felip *et al.*, 2000). They have been successfully used in fishes like *Ictalurus punctatus* (Liu *et al.*, 2003), *Oncorhynchus tshawytscha* (Smith *et al.*, 2005) *Scomberomorus niphonius* (Shui *et al.*, 2008), *Brachymystax lenok* (Wang *et al.*, 2009).

### 4. Single nucleotide polymorphism (SNP)

Single nucleotide polymorphism (SNP) describes polymorphisms caused by point mutations that give rise to different alleles containing alternative bases at a given nucleotide position within a locus. Such sequence differences due to base substitutions have been well characterized since the beginning of DNA sequencing in 1977. However, the ability to genotype SNPs rapidly in large numbers of samples was not possible until the application of gene
chip technology in the late 1990s. SNPs are again becoming a focal point in molecular marker development since they are the most abundant polymorphic markers in any organism, adaptable to automation, and reveal hidden polymorphism not detected with other markers and methods. In fishes SNP analysis has been applied to *Salmo salar* (Primmer et al., 2006), *Gadus morhua* (O'Leary et al., 2006), genus *Trachurus* and genus *Mullus* (Apostolidis et al., 2008). Despite technological advances, SNP genotyping is still a challenging endeavour and requires specialized equipment. Traditional methods available for SNP genotyping include direct sequencing, single base sequencing (Cotton, 1993), allele specific oligonucleotide (Malmgren et al., 1996), denaturing gradient gel electrophoresis (Cariello et al., 1988), single strand conformational polymorphism assays (Suzuki et al., 1990) and ligation chain reaction (Kalin et al., 1992). Each approach has its advantages and limitations.

5. **Expressed sequence tags (ESTs)**

Expressed sequence tags (ESTs) are single-pass sequences generated from random sequencing of cDNA clones (Adams et al., 1991). The EST approach is an efficient way to identify genes and analyze their expression by means of expression profiling (Franco et al., 1995; Azam et al., 1996; Lee et al., 2000). It offers a rapid and valuable first look at genes expressed in specific tissue, types, under specific physiological conditions or during specific developmental stages. ESTs are useful for the development of cDNA microarray that
allow analysis of differentially expressed genes to be determined in a systematic way (Schena et al., 1996; Wang et al., 1999), in addition to their great value in genome mapping (Boguski and Schuler, 1995; Hudson et al., 1995; Schuler et al., 1996). For genome mapping, ESTs are most useful for linkage mapping and physical mapping in animal genomics such as those of cattle and swine, were radiation hybrid panels are available for mapping non polymorphic DNA marker (Cox et al., 1990). Much progress has been made in fisheries for analysis of ESTs. Tissue analysis of ESTs and expression profiling has been conducted in channel catfish (Ju et al., 2000; Cao et al., 2001; Kocabas et al., 2002 Karsi et al., 1998, 2002).

6. Mitochondrial DNA:

By the early 1980s, examination of the gene became possible by determining directly or indirectly difference in the nucleotide sequence of DNA molecule. One of the finding that arose from early studies was that the DNA of mitochondria (mtDNA) is characterized by high level of sequencing diversity at the species or infra-species level despite great conservation of gene function and arrangement (Wirgin and Waldam, 1994). Mitochondrial DNA became a very popular marker and dominated genetic studies designed to answer questions of phylogeny and population structure in fish for more than a decade. Three properties of mtDNA set apart from nuclear DNA: it occurs in multiple copies in each cell (in contrast to two copies to a "single copy" nuclear locus), it is transmitted uniparentally, and it
does not recombine. Moreover, it evolves much faster than coding regions of DNA (Brown et al., 1982; Attardi, 1985; Moritz et al., 1987; Avise, 1994). One consequence of uniparental transmission is that the effective population size for mtDNA is smaller than that of nuclear DNA (Moritz et al., 1987). Hence mtDNA variation is a more sensitive indicator of population phenomena such as bottlenecks and hybridizations. Mitochondrial DNA analysis is increasingly being used in recent population and phylogenetic surveys. Mitochondrial DNA analysis has proven useful in clarifying relationships among closely related species. Different parts of mt-gene are known to evolve at different rates (Meyer, 1993). The 16s rRNA gene in mitochondrial genome is one of the slowest evolving genes (Meyer, 1993) whereas rapidly evolved region includes control region (Chow et al., 1997; Gold et al., 1997). With regard to empirical data on mt DNA sequence divergence in fishes, highly varying levels of divergence have been disclosed. Analyses of mtDNA markers have been used extensively to investigate stock structure in a variety of vertebrates including fishes (Avise et al., 1986; Graves et al., 1992; Chow et al., 1993; Gold et al., 1993; Heist and Gold, 1999), birds (Baker and Marshall, 1997; Greenberg et al., 1998; Mila et al., 2000; Zink et al., 2000), mammals (Menotti-Raymond and O'Brien, 1993) and reptiles (Avise et al., 1998; Serb et al., 2001; Riberson et al., 2002; Shanker et al., 2004). Some sympatric species complex of freshwater fishes show very small mt DNA sequence divergence levels (Meyers et al., 1990), while some show divergence as large as 15% (Billington and Hebert, 1991). Some
marine species show even higher levels of intra-specific divergence (Becker et al., 1988) while some congeneric species have small, moderate or very large divergence (Billington and Hebert 1991). Large variations in mtDNA sequences among species can be utilized to produce species specific markers. Since the structures of mitochondrial RNA genes (tRNA and rRNA) and the functional molecule of 16s rRNA are highly conserved among animal taxa that are related even distantly (Meyer, 1993; Orti et al., 1996). Change of even few nucleotides in such a gene between closely related taxa might indicate a substantial degree of genetic divergence (Suneetha et al., 2000). In addition to protein and nuclear DNA markers, different mtDNA gene sequences have been used to determine variation at interspecific and intraspecific levels in fishes. The fast rate of mtDNA evolution coupled with maternal inheritance have made mtDNA an extremely useful genetic system for studying gene flow, hybrid zones, population structure and other population related questions. Even conservative protein coding genes like Cytochrome b tend to show intraspecific variation mainly in 3rd position of codon which can be used to identify stocks. Variation in mtDNA Cytochrome b gene has been used for population studies in fishes across taxonomic orders such as, Clupeiformes (Lecomte et al., 2004); Acipenceriformes (Fontana et al., 2007; Pages et al., 2009); Squaliformes (Murray et al., 2008); Salmoniformes (Bouza et al., 2008); Anguilliformes (Deamon et al., 2001); Cypriniformes (Fayazi et al., 2006) and Siluriformes (So et al., 2006) and Perciformes, (Brown et al., 2008). In addition, ATPase8
and ATPase6 genes of mtDNA are generally variable in vertebrates (Zardoya & Meyer, 1996). These genes have been consistently found to have high evolutionary rate (1.3% per million years) in fishes (Bermingham et al., 1997). ATPase 8 and ATPase 6 regions have been successfully analysed for both phylogeny as well as phylogeography in several fish species, (Chow et al., 2004; Dammannagoda et al., 2008; Vergara-Chen et al., 2009). The well characterized COI gene has proved to be a robust evolutionary marker for the analysis of intraspecific and interspecific relationships in many marine fish and shellfish. The 16S rRNA mitochondrial gene has also been shown to be a good marker to differentiate fish species and has been used in comparative intergeneric and interspecific studies in several families of Perciformes. Tautz et al. (2002, 2003) made the case for a DNA based taxonomic system. DNA sequence analysis has been used for 30 years to assist species identifications, but different sequences have been used for different taxonomic groups. Hebert et al. (2003) proposed that a single gene sequence would be sufficient to differentiate all, or at least the vast majority of, animal species, and proposed the use of mitochondrial DNA gene Cytochrome oxidase sub unit I (coxI) as a global bio identification system for animals. The sequence was linked to a barcode, with species being delineated by particular sequence or by tight cluster of very similar sequences. Whether bar-coding can be used to discriminate fish species or not was examined by Ward et al. (2004). Bar-coding discriminated all of the fish species examined by them. It was concluded that it would be
clearly capable of unambiguously identifying individually isolated fish eggs, larvae, fillets and fins from species. According to Pegg et al. (2006) DNA barcode approach for fish identification appears valid and that while HVR1 or cox1 mt DNA sequence data both appear useful for this purpose, cox1 should be used in future studies as marker of choice since a large internal database for fish identification needs to be constructed.

7. Tandemly Repeated DNA

Tandemly repeated blocks of DNA of identical or similar sequence dispersed throughout the genome of most if not all, eukaryotic genome (O'Reilly and Wright, 1995). Three different class of this repetitive and highly polymorphic DNA have been distinguished traditionally, based on the size of the repeat unit.

7.1 Major Satellite arrays

Satellite DNA in which a single repeat sequence family can constitute several percent of the total genome, and can occur in individual repeat area as 5Mb. Satellites are often preferentially associated with centromeres. Major satellites are only infrequently used to genotype individuals, but have been useful in human genome mapping in providing genetic markers anchored at centromeres. They can be typed either by Southern blot/hybridization (Mahtani and Willard, 1990; Oakey and Tyler-Smith, 1990), or using restriction
digests or PCR primers which detect locus specific repeat unit variants (Warburton and Willard, 1996).

7.2 Minisatellites

Minisatellite or variable Number of tandem repeat (VNTR) DNA (Warburton and Willard, 1996) may be present at hundreds or thousands of different loci per genome. The repeat unit sequence is long enough (> 10 bp) to be locus specific and is gives repeat blocks of intermediate size (0.5-30 kb). The term "DNA fingerprinting" was originally associated with the approach of Jeffreys et al. (1985), in which Southern blot/hybridization assays of minisatellite regions of DNA (after restriction digestion of individual genomic DNA) reveal multi locus gel banding profiles that distinguish most or all individuals within a sexually reproducing species (Avise, 1994). The original Jeffrey's probes, which hybridized to conserved core sequences, 10-15 bp long, were isolated from a myoglobin intron in humans, but it was found that they also cross-hybridized in many other species, including fishes (Baker et al., 1992). These limitations render multi locus fingerprinting rather unsuited for population level applications (Wright, 1993). Moreover, quite often the results of multi-locus fingerprinting protocols are not reproducible. As a result, the development of single-locus profiling techniques was sought, in which allelic variation is surveyed at individual VNTR loci. Single-locus approaches obviate most of the problems associated with multi-locus methods; nevertheless some limitations still exist, mainly
because often alleles do not differ from one another by discrete, integral increases or decreases in the number of repeat copies (Jeffreys et al., 1988). This makes the comparison of allele sizes between gels difficult, and has necessitated the binning of alleles into defined size classes (O'Reilly and Wright, 1995).

7.3 Microsatellites

Microsatellites are becoming a powerful DNA marker for quantifying genetic code and genetic variations within and between populations of species. Microsatellites have been shown to be highly polymorphic in fishes (Brocker et al., 1994). Microsatellite markers are type II markers unless they are associated with genes of known function. They are also considered as non-coding and neutral. In India, microsatellite markers have been developed or thirteen fish species, using primers from related species and have been found effective for population structure analysis (Gopalakrishnan et al., 2004, Lai et al., 2004, Mohindra et al., 2004, 2005).

Microsatellites consist of multiple copies of tandemly arranged simple sequence repeats (SSRs) that range in size from 1 to 6 base pairs e.g. ACA or GATA (Litt and Luty, 1989; Tautz, 1989). Abundant in all species studied to date, microsatellites have been estimated to occur as often as once every 10 kb in fishes (Wright, 1993). Microsatellites tend to be evenly distributed in the genome on all chromosomes and all regions of the chromosome. They have been found inside gene coding regions (Liu et al., 2001c), introns, and in
the non-gene sequence. The best known examples of microsatellites within coding regions are those causing genetic diseases in humans, such as the CAG repeats that encode polyglutamine tract, resulting in mental retardation. Most microsatellite loci are relatively small, ranging from a few to a few hundred repeats.

Database analyses of tandem repeats in genomic sequences by Beckmann and Weber (1992) showed that CA/TG repeats are the most common dinucleotide repeats, occurring about twice as frequently as AT repeats and three times as often as AG/TC repeats. Microsatellite polymorphism is based on size differences due to varying numbers of repeat units contained by alleles at a given locus. Microsatellite mutation rates have been reported as high as $10^{-2}$ per generation (Weber and Wong, 1993; Crawford and Cuthbertson, 1996), and are believed to be caused by polymerase slippage during DNA replication, resulting in differences in the number of repeat units (Levinson and Gutman, 1987; Tautz, 1989). Direct studies of human families have shown that new microsatellite mutations usually differed from the parental allele by only one or two repeats (Weber and Wong, 1993), favoring a stepwise mutation model (Estoup and Cornuet, 1999). However, in a few fish species, we have observed alleles with very large differences in repeat numbers, predictive of an infinite allele model (Balloux et al., 2002).

Microsatellites are inherited in a Mendelian fashion as codominant marker; this is the strength of microsatellite markers in addition to their abundance, even genomic distribution, small locus
size, and high polymorphism. However, use of microsatellite markers involves a large amount of upfront investment and effort. Each microsatellite locus has to be identified and its flanking region sequenced for the design of PCR primers. Because of PCR based technique significant increases in the number of samples that can be typed in a day have been achieved by using automated fluorescent sequencers coupled with computer imaging systems (O'Reilly and Wright, 1995).

Microsatellites enriched libraries have been constructed for many organisms, including fishes. The high frequency of tandem repeats in fish genomes provides a good opportunity to obtain libraries significantly enriched in microsatellites. For example, libraries containing 74%, 95% and 96% clones with (CA)$_n$ repeats have been developed for the Mediterranean angler fish *Lophius* sp. (Garoia et al., 2003), gilthead sea bream *Sparus aurata* (Zane et al., 2002), and *Nile tilapia* (Carleton et al., 2002), respectively. A library usually contains recombinant clones. Screening of these clones typically yields 10-15% unique polymorphic SSRs, resulting in the production of 100-500 non-redundant variable microsatellites from a single library (Zane et al., 2002).

Evolutionary analyses of microsatellites have shown a wide variety of degrees of conservation. Microsatellite primers from one species can be used for amplification of polymorphic microsatellite loci from species of same family or cross species. Studies have shown that cross species amplification using primers designed from related
species generate polymorphic loci to identify different populations and their genetic viabilities (Welsh et al., 1990; Williams et al., 1990).

The presence of highly conserved flanking regions has been reported for some microsatellite loci in cetaceans (Schlotliferer et al., 1991), turtles (FitzSimmons et al., 1995) and fish (Rico et al., 1996), allowing cross-amplification from species that diverged as long as 470 million years ago. It should be noted that during the isolation procedure, loci are selected from the upper end of the repeat length distribution in the genome, the fraction which is known to harbour the most polymorphic markers (Primmer et al., 1995). Such bias in loci isolation is likely result in a lower level of polymorphism when orthologous loci are tested in other species (Ellegren et al., 1995). Therefore, high polymorphism observed in a species does not guarantee that similar polymorphism will be found in related species especially when increasing the evolutionary distance (Rubinsztein et al., 1995; Morin et al., 1998). This becomes a major hindrance to study genetic variation and hence required isolation of microsatellite loci development of suitable primers for study of variations. Phylogeographical applications of microsatellites are eminently suitable, where population structure is observed over a large geographical scale (Koskinen et al., 2002; Gum et al., 2005). The latter study on grayling Thymallus thymallus shows that there is strong admixture among major lineages in contact zones between drainages zones.
Microsatellites are even more revealing over shorter geographical distances, where a few cases of panmixia (Dannewitz et al., 2005) and numerous cases of isolation by distance patterns (Ruzzante et al., 1999; O'Reilly et al., 2004), clinal variation (Nielsen et al., 2004), fragmentation (Lemaire et al., 2005), hybridisation (Gum et al., 2005) and cryptic speciation (Fillatre et al., 2003) have been identified. In those cases, differences in the microsatellite allelic composition of populations are converted into evolutionary distances. Microsatellite genotypes are particularly helpful to detect structure in closely related populations, regardless of whether they are in evolutionary equilibrium.

DNA libraries, like conventional libraries, are used to collect and store information. In DNA libraries, the information is stored as a set of DNA molecules, each of which contains biological sequences that can be used for a variety of applications. All DNA libraries are collections of DNA fragments that represent a particular biological system of interest. By analyzing the DNA from a particular organism or tissue, researchers can answer a variety of important questions. The two most common uses for these DNA collections are DNA sequencing and gene cloning. DNA libraries are created by generating a set of DNA fragments of the desired size and then attaching those fragments to the appropriate vector sequence. For genomic DNA, the fragments are normally generated by either enzymatic digestion or simple mechanical shearing of all the DNA of the genome, including non coding sequences. Fragments are then enzymatically attached to
the vector sequences, in a reaction known as ligation. The collected fragments, now attached to vector sequences, are then moved into the appropriate host organism for growth and evaluation. Conditions are chosen so that only one fragment enters each organism, which can then be grown up into a colony whose individuals all carry the same fragment.

Conservation and fisheries genetics focus on the effects of inbreeding, demography, contemporary genetic structuring and adaptation on the long-term survival of a species. The examination of genetic markers has had major impacts on three fisheries areas in particular: stock structure analysis, aquaculture and taxonomy/systematics. There have also been studies on the genetics of introduced species, the effects of fishing and pollutants on genetic diversity, and the genetics of rare or endangered species. Stock identification is a big issue (Ferguson et al., 1995), helping wildlife managers to protect biodiversity by identifying series of conservation units such as evolutionarily significant units (ESUs), management units (MUs) and action units (AUs) (Wan et al., 2004). Mitochondrial and microsatellite DNA markers revealed four genetically differentiated lineages of European grayling (*Thymallus thymallus*) in central and northern Europe, which evolved in geographical isolation during the Pleistocene and could be recognized as the ESUs (Gum et al., 2005). Conservation strategies depend on neither paternal nor maternal variation, but focus on using biparental polymorphism of nuclear DNA to reflect characteristics needed to cope with
environmental conditions (Zhang and Hewitt, 2003). Measuring genetic diversity in wild fish populations or aquaculture stocks is essential for interpretation, understanding and effective management of these populations or stocks. Genetic diversity has been measured indirectly and inferentially through controlled breeding and performances studies or by classical systematic analysis of phenotypic traits. Ecological, tagging, parasite distribution, physiological and behavioural traits, morphometrics and meristics, calcified structures, cytogenetics, immunogenetics and blood pigments are among the diverse characteristics and methods used to analyze stock structure in fish populations (Ihssen et al., 1981).

The assessment of the phylogeography of a species and the identification of genetically divergent areas is a fundamental step for the success of any conservation effort. Genetic variability is widely recognized as a component of natural biodiversity, several national and international conventions and laws claiming the necessity of its preservation and protection. Phylogeography hence is a powerful tool for inferring the processes that affect the genetic composition of species or species groups. It can also be helpful in elucidating historical events, such as habitat fragmentation and range expansion, which have influenced the population structure of a species or have caused speciation.
**Fig 5:** Basic Structure of Fish Mitochondrial DNA.