National Advisory Board for
Management of Genetic Resources

Guidelines for Management of Fish Genetic Resources in India

2016

ICAR-National Bureau of Fish Genetic Resources
(Indian Council of Agricultural Research)
Lucknow-226 002, India

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This document is outcome of recommendations of the National Advisory Board for Management of Genetic Resources (NABMGR), a high level committee constituted by the Indian Council of Agricultural Research (ICAR), New Delhi, in 2010. The NABMGR has been constituted to advise on issues related to efficient management of all genetic resources (GR) including plants, animals, fishes, insects and agriculturally important microbes. It is mandated to recommend national policies on conservation, management and sustainable use of GR, to provide guidance for related issues emerging at international fora and to assess and guide on actions related to GR management.

Technical Core Committee Constituted by NABMGR for Developing Guidelines for Genetic Resources Management

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Contents

List of Annexures

त्रिलोचन महापात्र, पीएच.डी.
एफ एन ए, एफ एन ए एस सी, एफ एन ए ए एस
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Message

Sustainable management of fish genetic resources is of paramount importance and of great relevance to overcoming the challenges of hunger and malnutrition, for which appropriate management guidelines and policy framework are warranted. It is timely that the National Bureau of Fish Genetic Resources (NBFGR). Lucknow is bringing out the 'Guidelines for Management of Fish Genetic Resources in India' under the guidance of National Advisory Board for Management of Genetic Resources, highlighting best practices in fisheries that would be helpful for all stakeholders to efficiently manage fish genetic resources.

I congratulate the scientists of NBFGR for their effort in bringing out this useful document.

(T. MOHAPATRA)

Dated the 19th October, 2016
New Delhi

Foreword

Genetic resources represent highly variable genetic diversity, being a result of survival of the fittest theory in nature. Genetic variability in organisms is an outcome of selection and breeding as evolutionary processes spread over the millions of years. Genetic resources are also associated with rich local and traditional knowledge and their value in terms of cultural heritage. During the last two decades, we have witnessed increased focus of UN and national governments to establish policy frameworks for sustainable management of genetic resources. Convention on Biological Diversity (CBD), being an important instrument, has facilitated the process of sovereign rights of nations and communities on their natural biological wealth.

The provisions in CBD have ensured the rights of country of origin and have further provided options for facilitated access and benefit sharing (ABS) mechanisms. This has resulted in both national and international developments on regulated processes for enhanced use of genetic resources while ensuring the rights of breeders/farmers/fisherfolks. As a result, a number of treaties, laws, and conventions have changed the way genetic resources are now collected, evaluated, conserved, utilized and exchanged.

With the enactment of Biological Diversity Act, 2002 and Biological Diversity Rules, 2004 resulting in the establishment of National Biodiversity Authority, India too has ushered into a new era of managing available rich biological diversity. All these policy instruments have catalysed a number of countries to enhance their capabilities for scientific documentation of the genetic resources as well as the traditional knowledge they possess. India is among few who took the lead to accelerate scientific research, evaluation, assessment and conservation of valuable fish diversity by establishing a National Bureau of Fish Genetic Resources (NBFGR) in1983. The institute has since then made valuable contributions and has become a focal point for technical support on fish genetic resources in the country.

It is also quite encouraging that FAO Commission on Genetic Resources for Food and Agriculture (CGRFA) is planning to complete country-wise status reports on aquatic genetic resources by 2017. In view of several national and international regulatory developments, focus is now needed on the sustainable management of genetic resources by initiating systematic science-based efforts, from their exploration, use and conservation. It is in this context that the present document on 'Guidelines for Management of Fish Genetic Resources in India' was conceived to be a comprehensive account of all the national regulations and best scientific practices that need to be adopted in India for efficient management of fish genetic resources. I am pleased to see the efforts made by the leadership of the institute to bring out these guidelines. I do hope that these guidelines would serve all stakeholders in understanding the processes and legal procedures necessary for an efficient management and conservation of our valuable fish genetic resources.

(R.S. Paroda)
Former Chairman
National Advisory Board for Management of Genetic Resources (NABMGR),
Indian Council of Agricultural Research and
Chairman, Trust for Advancement of Agricultural Research (TAAS), New Delhi

Preface

Genetic resources (GR) are receiving considerable attention globally from the perspective of sustainable utilization to ensure livelihood and nutritional security for the growing populations. The fish genetic resources (FGR) have received importance for their role in direct consumption, providing new species for aquaculture diversification, genetic diversity to improve domesticated species, utilization for ornamental trade and also for the products of commercial value. The availability of genetic diversity, within and between species, is of utmost significance in mitigating the impacts of environmental changes, including climate change.

FGR management in India is now a well-institutionalized activity. Besides exploring, collecting, conserving and utilizing FGR for increasing aquaculture productivity, genetic resources management today encompasses a multilevel domain of scientific, policy, social, ecological, legal and financial aspects. Various stakeholders are included in this process of FGR management viz., fisherfolks, fish farmers, communities, breeders, researchers, managers and policy makers. Moreover, FGR management involves interaction at global level with the countries and inter-governmental platforms like the Food and Agriculture Organization of the United Nations (FAO), Network of Aquaculture Centers in Asia-Pacific (NACA), Secretariat of the 'Convention on Biological Diversity' (CBD, 1993), 'Commission on Genetic Resources for Food and Agriculture' (CGRFA), FAO 'Code of Conduct for Responsible Fisheries' (CCRF, 1995), 'FAO Committee on Fisheries' (COFI) and so on. Several international treaties such as the CBD, World Trade Organization (WTO) Agreement, and national laws such as Biological Diversity Act, 2002, are the most important ones. The various Central Acts are supported by a number of state laws/statutes, policies and strategies directly relevant to FGR.

The Indian Council of Agricultural Research (ICAR) plays an important role in the management of genetic resources for food and agriculture in the country through its network of institutions. With a vision of assessment and conservation of fish genetic resources for sustainable utilization, posterity and intellectual property protection of the country, ICAR-National Bureau of Fish Genetic Resources (NBFGR) has taken up various research programmes to generate empirical information relevant to sustainable management and

conservation of FGR. In 2010, the ICAR constituted a high level 'National Advisory Board on Management of Genetic Resources (NABMGR)' to advise on issues related to efficient management of GR. The Board was mandated to recommend a national policy on conservation, management and sustainable use of GR, to provide guidance for related issues emerging at international fora and to assess and guide on actions related to GR management. The NABMGR recommended that all the Bureaux prepare 'Guidelines document for management of genetic resources' applicable at a national level. The present document is the outcome of this recommendation. The guidelines address different aspects of FGR management like exploration, characterization, conservation, utilization, exchange, quarantine, registration, and policy issues.

This document is the outcome of the experience and contribution of several scientists of ICAR-NBFGR and has been reviewed by eminent scientists. It is envisaged that it will be widely used by all the stakeholders of FGR.

Editors

Acknowledgements

The present guidelines on Fish Genetic Resources (FGR) management are an outcome of a recommendation of the National Advisory Board on Management of Genetic Resources (NABMGR). We are extremely grateful to all the honorable members of NABMGR for their valuable guidance. Special mention is made of Dr R.S. Paroda, Chairman and Dr S. Ayyappan, Co-Chairman for their vision and constant encouragement.

We express our gratitude to Dr. Trilochan Mohapatra, Secretary, Department of Agricultural Research and Education, Ministry of Agriculture and Farmers Welfare, Government of India and Director General, Indian Council of Agricultural Research, New Delhi for continuous encouragement, guidance and support. We are thankful to Dr. B. Meena Kumari, Former Deputy Director General (Fisheries), ICAR for her support.

The NABMGR constituted a technical committee to finalize guideline documents for all genetic resources, who are gratefully acknowledged for their supervision, advice and support. Sincere thanks are expressed to Dr Bhag Mal, Chairman of the Technical Core Committee constituted by NABMGR, for providing leadership to accomplish the important task to collate, synthesize and harmonize the guidelines on GR, and sparing his valuable time for meetings and critical comments on the FGR guidelines.

The document was vetted by eminent experts on FGR management and their critical inputs have helped immensely in improving its quality. Each one of the experts is gratefully acknowledged.

We express sincere thanks to Dr R.K. Tyagi, Member-Secretary, Expert Committee & Head, Division of Germplasm Conservation, ICAR-NBPGR, New Delhi for his guidance in the preparation of the document.

We thankfully acknowledge the technical support and critical inputs provided by a Committee of ICAR-NBFGR Scientists, for preparation of this document. Editors provide the names of members of different committees, reviewers and contributors in the succeeding pages as an expression of their sincere gratitude to all of them.

Thanks are due to all the present and former staff members of ICAR-NBFGR, Lucknow who directly or indirectly contributed in preparation of this document.

Editors

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[Constituted by President, Indian Council of Agricultural Research (ICAR), vide F.No. 8(2)/2011-Cdn. (Tech.) (Part) dated October 14, 2011 and February 21, 2014]

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Glossary

access	Acquisition (right to use) of biological resources and/or knowledge associated thereof, and of their derivatives or, as applicable, intangible components, for purposes of research, conservation, biological prospecting and industrial application or commercial use, among others
accession	A distinct, uniquely identifiable sample of fish, blood, tissue of fish representing a species, stock, breeding line or a population, which is maintained in a genebank for conservation and use
accession number	A unique identifier assigned to an accession, when it is registered with a genebank. This number is never assigned again to another accession even after loss of the accession
alleles	One of several alternative forms of a gene occupying the same locus on a particular chromosome. When the alternates exceed two, the alleles form a multiple allelic series. Multiple alleles arise by repeated mutations of a gene, each with different effects
allelic frequency	A measure of commonness of an allele in a population. It is used to describe the frequencies of polymorphic genes statistically correlated with temporal variation of environmental factors
benefit sharing	Means sharing of benefits arising from use of biological resources and associated knowledge based on prior informed consent and mutually agreed terms, with contracting party providing such resources
biochemical markers	Biochemical markers are the genetic markers and can be classified on the basis of protein/isozymes
biological diversity	The variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part. It includes diversity within species, between species and of ecosystems.
collecting	Includes identifying, locating, acquiring, organizing, cataloguing, post collection handling the germplasm of importance as FGR
collection	A group of germplasm accessions maintained under defined conditions for a specific purpose
collector	A specialist who explores, surveys and collects germplasm in the form of fish speciemens, blood or tissue accessions, milt accessions, etc., and records related information on diversity distribution, use, environmental features, etc.
conspecifics	Members of the same species
country of origin (of a consignment of fish)	Country where the fish has been collected or cultured
cryopreservation	Storage of cells, tissues, organs or organisms, in a viable condition, at ultra-low temperature/cryogenic conditions (-196 °C under LN_2)
database	An organized set of interrelated data assembled for a specific purpose and held in one or more storage media

descriptor	An easily identifiable and measurable trait or characteristic of a fish species observed in an accession, which is used to facilitate data classification, storage, retrieval and use
distribution	The process of supplying samples of germplasm accessions to breeders, researchers, curators and other bonafide users
documentation	Presentation of recorded data on germplasm accessions in a standard format that describes structure, purpose, operation, maintenance and other requirements
domestication	Domestication is the process by which fishes, plants, animals or microbes selected from the wild adapt to a special habitat created for them by humans, bringing a wild species under human management. In a genetic context, the process in which changes in gene frequencies and performance arise from a new set of selection pressure exerted on a population (http://cmsdata.iucn.org)
donor	An institution or individual responsible for donating germplasm
ecosystem	A dynamic complex of plant, animal and microrganism communities and their abiotic environment interacting as a functional unit
ecotype	A population of a species that survives as a distinct group through environmental selection and isolation, maintains its genetic identity and usually is linked to one or more environmental factors in which it is favoured (mostly with reference to wild species)
endangered	When used in the context of the IUCN Red List, a taxon is classified as 'Endangered' when there is very high risk of extinction in the wild in the immediate future (IUCN 2001)
endemic	Native to, and restricted to, a particular geographical region. Highly endemic species, those with very restricted natural ranges, are especially vulnerable to extinction if their natural habitat is eliminated or significantly disturbed
evaluation	The recording of quantitative traits whose expression is often influenced by environmental factors; it provides an assessment of the potential of germplasm for use in breeding/research
ex situ	Outside the original habitat of the organism
fish	From agro biodiversity point of view fish represent the aquatic organisms including shellfish
fisherfolks	People associated with fishing or fish farming activities
gene	The basic unit of heredity transmitted from generation to generation during sexual or asexual reproduction. Generally, the term is used in relation to the transmission and inheritance of particular identifiable trait
gene flow	The movement of genes from one population to another. The exchange of genes (in one or both directions) at a low rate between two populations, due to the dispersal of gamentes or of individuals from one population to another, also called migration
genebank	Is a facility where genetic resources (genetic material) are conserved under suitable conditions to prolong their lives
genepool	The sum total of all the genes and combination of the genes that occur in a population of organisms of the same species

genetic conservation	The collection, maintenance, storage and sustainable management of genetic resources aimed at ensuring their continued existence, evolution and availability for future generations. Also referred as 'gene conservation' and 'genepool conservation'
genetic diversity	The genetic variability (variety of genetic traits) within a population or a species, arising due to number and relative abundance of alleles. It can be assessed at three levels: (a) diversity within breeding populations, (b) diversity between breeding populations; and (c) diversity within the species. Genetic diversity occurs at gene level (the molecular level), the individual level, the population level, the species level, and the ecosystem level
genetic drift	Random change in allele frequencies in a population from one generation to the next because of small population size, and not due to selection, migration, or mutation. The smaller the population, the greater the genetic drift, with the result that some alleles are lost, and genetic diversity is reduced. Minimization of genetic drift is an important consideration for conservation of genetic resources
genetic material	Any material of plant, animal, microbe or other origin containing functional units of heredity
genetic resources	Genetic material of actual or potential economic, scientific or societal value contained within and among species. In a domesticated species, it is the sum of all the genetic combinations produced in the process of evolution. The term includes modern cultivars and breeds; traditional cultivars and breeds; special genetic stocks (breeding lines, mutants, etc); wild relatives of domesticated species; and genetic variants of wild species
genetic shift	Change in the performance of the accession/ genotype if grown over a long period in areas outside their adaptation
genetic stock	Reproductively isolated group of individuals of the same species
genetic stocks	Broadly defined as plants or populations generated and/or selected for genetic studies represent a unique and growing class of extremely valuable germplasm
genetic variation	The occurrence of differences among individuals of the same species, arising due to variation in alleles, genes or genotype. Genetic variation is brought about by a change in genes, as distinct from differences due to environmental factors
genome	All the genetic material (DNA sequences) in a single (haploid) set of chromosomes of an organism. The genetic material inherited from either parent
genomics	Genomics is the study of the genomes of organisms includes intensive efforts to determine the entire DNA sequence and genetic mapping
genotype	The genetic constitution of an individual plant or organism as distinguished from its appearance or phenotype. The genotype interacts with the environment to produce the phenotype
genotyping	The process of identifying the genetic make-up of an organism by using molecular methods (DNA sequence level)
germplasm	The genetic material which forms the physical basis of heredity and which is transmitted from one generation to the next by means of germ cells. Often synonymous with genetic material

germplasm exchange	Mutual give and take of germplasm or plant genetic resources from all available sources
habitat	Part of an ecosystem with conditions in which an organism naturally occurs or can establish
holotype	The single specimen on which the taxon was based or the single specimen designated as the name-bearing (or primary) specimen
ICZN	International Commission on Zoological Nomenclature; the judicial body empowered to enforce and interpret the International Code of Zoological Nomenclature
import permit	An official document authorizing importation of a commodity/resource in accordance with specified phytosanitary requirements, into the country from outside
in situ	In the original habitat of the organism
indigenous	A species that is assumed to be intrinsically part of the ecosystem of a country owing to having developed there having arrived in the area long before record of such matters was kept having arrived by natural means (unaided by human action) etc.
intellectual property rights	Intellectual Property Rights (IPR) are legal rights that are conferred to the owner of an intellectual creation. The IPR are granted by means of protection through appropriate legislation based on the type of creation that generally include patents, copyright, trademark, industrial designs, geographical indications, trade secrets, protection of layout design of integrated circuits and protection of new varieties. The Intellectual Property Right protection entitles the owner of the Intellectual Property or his assignee the exclusive right to fully utilize the invention/creation for commercial gain generally for a fixed period of time
locus	A segment of DNA or position of gene in genome
material transfer agreement (MTA)	A contractual arrangement (can be legally binding) in commercial and academic research partnerships involving the transfer of tangible biological materials (such as germplasm, microorganisms and cell cultures) from a provider to a recipient. The MTA sets conditions for use, commercialization and sharing of benefits derived from the use of the materials provided
meristic data	Counting quantitative features of fish
microsatellites or SSRs	Repeat sequences in genome
milt	White fluid with male gametes, a term analogous to animal semen
molecular markers	Molecular markers are the DNA based markers used to describe an individual
morphological data	Measurements to define structure and appearance or shape of fish
mtDNA	Mitochondrial DNA
novel and unique trait	A novel, unique, distinct and stable trait(s) which has been the basis for registration of germplasm
neotype	The specimen designated as the name-bearing type of a nominal species or subspecies for which no holotype, or lectotype, or syntype, or prior neotype is believed to exist

oocytes	Female haploid gametes
original description	The description of a nominal taxon when first established
passport data	Basic information about the origin of an accession, such as details recorded at the collecting site, pedigree or other relevant information that assists in the identification of an accession
paralectotype	The type specimens remaining after a lectotype is designated
paratype	Specimens of the type series other than the holotype
pathogen	A living microorganism such as a virus, bacterium or fungus that causes disease in another organism
phytosanitary certificate	An official paper document or its official electronic equivalent, issued by an authorized officer at the country of origin of consignment or re-export, consistent with the model certificates of the IPPC, attesting that a consignment meets phytosanitary import requirements
phytosanitary measure	Any legislation, regulation or official procedure having the purpose to prevent the introduction or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests
polymorphism	Occurrence of two or more clearly different phenotypes or genotypes for a trait exist in the same population of a species
quarantine	Official confinement of regulated articles (introduced germplasm) for observation and research or for further inspection, testing or treatment to ensure that it does not carry diseases or pests injurious to the importing country
spermatozoa	Male haploid gamete
synonym	Each of two or more scientific names of the same rank used to denote the same taxon. Typically, two names for the same taxon, however, only one is considered valid; they are said to be synonyms
syntype	Each specimen of a type series (of equal rank) when no holotype or lectotype has been named
systematics	The classification and study of organisms with regard to their natural relationships
taxon (taxa)	A taxonomic unit, such as a species, subgenus, genus, family
taxonomy	The study and practice of naming and classifying organisms, as done by taxonomists
threatened	Any species which is likely to become endangered within the foreseeable future in all or a significant portion of its range
trait	A recognizable quality or attribute resulting from interaction of a gene or a group of genes with the environment
truss network	A network between homologous landmarks on the body of fish for shape analysis
voucher specimen	A specimen archived in a permanent collection (usually in a museum) as representative specimen of the species for referral identification

Acronyms

AAQF	Aquatic animal quarantine facility
AFL	Amplified fragment length polymorphism
ANOVA	Analysis of variance
BAC	Bacterial artificial chromosome
BDA	Biological Diversity Act
CBD	Convention on Biological Diversity
CGIAR	Consultative Group on International Agricultural Research
CGRFA	Commission on Genetic Resources for Food and Agriculture
CIBA	Central Institute of Brackishwater Aquaculture
CIFA	Central Institute of Freshwater Aquaculture
CMFRI	Central Marine Fisheries Research Institute
DADF	Department of Animal Husbandry, Dairying and Fisheries
DARE	Department of Agricultural Research and Education
DBT	Department of Biotechnology
DFA	discriminant function analysis
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked lmmunosorbent Assay
FAO	Food and Agriculture Organization of the United Nations
FGR	Fish Genetic Resources
FGRC	Fish Germplasm Registration Committee
FWB	Fresh weight basis
GBS	Genotyping-by-sequencing
GIS	Geographical information system
GMO	Genetically modified organism
GOI	Government of India
GPS	Global positioning system

GR	Genetic resources
GRFA	Genetic resources for food and agriculture
HWE	Hardy-Weinberg Equilibrium
ICAR	Indian Council of Agricultural Research
IP	Import permit
IPR	Intellectual property rights
ITK	Indigenous technical knowledge
MAS	Marker assisted selection
MoU	Memorandum of Understanding
MTA	Material transfer agreement
NABMGR	National Advisory Board for Management of Genetic Resources
NARS	National Agricultural Research System
NBA	National Biodiversity Authority
NBFGR	National Bureau of Fish Genetic Resources
OIE	Office International des Epizooties
PAGE	Polyacrylamide gel electrophoresis
PC	Phytosanitary certificate
PCA	Principal component analysis
PCR	Polymerase chain reaction
PVA	Polyvinyl alcohol
RNA	Ribonucleic acid
SAU	State Agricultural University
SL	Standard length
SMD	Subject Matter Division (Under ICAR)
SNP	Single nucleotide polymorphism
SSRs	Simple sequence repeats
TEK	Traditional ecological knowledge
TRIPs	Trade related aspects of intellectual property rights
WTO	World Trade Organization

INTRODUCTION

India possesses rich aquatic biodiversity spread across different ecosystems. The term 'fish' is used here in a broader sense and includes all the aquatic organisms viz., finfishes, shellfishes, molluscs, crustaceans and echinoderms. Of the 32,042 finfish species reported globally, about 9.2% are found in Indian waters. Apart from finfish resources, nearly 2934 species of crustaceans, 5070 species of molluscs and 765 species of echinoderms also contribute to India's rich aquatic genetic resources. Natural aquatic genetic resources are important as majority of the genetic resources for food still come from the wild due to low domestication level in fisheries sector. In other words, capture fisheries is as important as aquaculture for nutritional security across the globe. This is in contrast to the livestock farming and agriculture where only domesticated varieties contribute to the food basket. Therefore, management of the fisheries resources draw parallel to that followed in wildlife and forestry besides the agriculture. While it is true that certain aspects of biodiversity and genetic resources policy can apply equally to plants, animals, or fish, however, significant differences need to be taken into account as well. Besides being a source of food, aquatic germplasm resources are also an important source of various products of commercial value and to sustain other related trades like ornamental fishes. The challenge is to secure the IPRs related to aquatic germplasm so that the country is able to maintain its stake on its natural wealth and its potential benefits. There is a need to develop repositories of genetic resources that store the registered germplasm accessions. In view of the multiple kinds of accessions, there is a need for integration of information for the varied types of accessions. The repositories will also store the accessions of genetic stocks discovered/varieties developed. Such repositories will maintain accessions for future use to retrieve information as well as the whole or part of genome, if the species is not found in nature. The documented information with those registered germplasm can also serve as a means to protect the traditional knowledge. To harness the potential of biotechnological innovations, it will be essential that DNA, whole and modified such as gene constructs, also become the part of these repositories. Besides, protecting IPR, these will also provide material for future research.

The species level accessions can be secured through building DNA and tissue bank as a fast mode to store the material for long-term. This can be used to retrieve the genetic information and for genetic manipulation in future with technological advancement. Sperm/cells and live gene bank accessions can be made only for prioritized and selected fish species. In case of animals, the strains/breeds of natural populations have been defined, in addition to the improved domesticated breeds. However, in fisheries, there is still a knowledge gap and efforts are in place to characterize genetic stocks/races in wild populations of cultivable fish species of the country using molecular markers, morphological and production traits. One of the important characteristic difference of fisheries accessions with respect to the *ex situ* conservation is that the species revival is only possible through live germplasm resource centers. Such centers are resource intensive, as these involve holding of effective breeding population of live animals. Therefore, these can be feasible only for the prioritized species. Real time working models of

such centers are, however, very limited globally. Another tool is cryopreservation of gametes. Though cryopreservation of sperm has been successful, yet it is limited to a few species due to necessity of species-specific protocols. Successful protocol for cryopreservation of embryo or egg of any fish has not been possible till date. *In situ* conservation approach also has wide implications in conservation of fish germplasm resources. It has been practiced in various forms including marine reserves/marine protected areas, freshwater sanctuaries, temple tanks, water bodies under wildlife protected areas, etc.

The guidelines concerning management of fish genetic resources will be useful in adoption of uniform approach related to their collection, documentation, characterization, registration, conservation, distribution and exchange. The guidelines also provide for standard procedures and modalities for quarantine and health related aspects while dealing with the fish genetic resources in the country.

1 EXPLORATION AND COLLECTION

Explorations for fish germplasm and the knowledge therein, is one of the primary activities of the organizations undertaking research on genetic resources. The fish germplasm collection is required for varied purposes such as species or biodiversity description, finding genetic subpopulations. Such strategies will be required for domesticated species at farms, their wild conspecifics or other fish diversity present in natural ecosystems. Therefore, the exploration mission and strategies will largely depend upon prioritization of species, the areas and also the purpose or issue to be addressed. These guidelines have been framed to help the explorers in maintaining standard methods and procedures in collecting fish genetic resources (FGR).

1.1 Planning of exploration program and strategies

1.1.1 Purpose

Exploration plans need objectivity to get precise information with optimization of resources and efforts. The purpose of the exploration must be clearly defined before planning. The exploration programmes can be multipurpose. The variety of purposes that can guide the exploration programmes are given (*Box i*).

Box i

Sl. No.	Purpose	Definition of Purpose	Prioritization Need
1	Fish Diversity Documentation	Description of organisms in the aquatic habitat, including validation using molecular tools is the first level of description of resources. This is a macro-level process and could lead to identification of germplasm resources not described till now and addressing taxonomic ambiguity arising from time to time in fish genetic resources.	Area, Habitats, Climatic conditions, Geological Information
2	Intra-specific Variation or Genetic Stock Documentation	Exploration for documenting within species variation for population genetics data from native distribution of species, for neutral markers or genomic levels, bioprospecting of genes and alleles for specific traits or adaptive divergence. This will lead to identification of genetic stocks that need conservation or have specific utility for breeding programmes, document genomic resources from native aquatic biodiversity for utilization in improvement programmes using biotechnological applications.	Species, Distribution range and Target locality.

Box i

Sl. No.	Purpose	Definition of Purpose	Prioritization Need
3	Captive Breeding Population	Acquisition of live brood fish or juveniles for growing in Live Germplasm Centres to develop effective breeding population. Specific collection and husbandry requirements as per the prioritised fish species. Such centres can be developed only for fish or identified genetic stocks with conservation value or proven elite aquaculture traits. Breeding population is also required for developing sperm cryopreservation protocols and for ranching purposes in endangered species.	Species, Target area and Fishing method, Knowledge on Biology
4	Traditional Ecological Knowledge	The art and science of fishing have been evolved by fisherfolks using several location-specific indigenous methods and passed on from generation to generation. With their rich location-specific knowledge, traditional ecological knowledge of fisherfolks can also contribute in germplasm exploration, documentation and broodstock collection for fish diversity conservation. Thus, there is a strong need to explore, facilitate and strengthen traditional ecological knowledge of fisherfolk communities for sustainable management of fish germplasm resources.	Area, Anthropological information of local tribes

1.1.2 Prioritization of Species and Areas

- The areas to be explored and fish species to be collected should be prioritized after thorough gap analysis, based on the information available from different sources.
- The literature should be thoroughly scanned to understand native distribution range and type of habitats preferred by the prioritized species.
- Similarly, for description of diversity of area, knowledge should be obtained for the length of survey stretch of river/aquatic habitats, type of habitats, likely fishing tribes in the area. Scanning the river stretch/wetlands through Google maps can be extremely useful in prior planning logistics of the mission.
- The explorers should be versed with the nature and extent of diversity and biological aspects, especially breeding cycle of the fish species to be collected, and plan to facilitate the preparations of the proposed missions.
- For live fish transport, likely collaborators in SAUs, line departments or other suitable organizations should be tied up before hand.

1.1.3 Finalization of Germplasm Exploration and Collection Plan

- **Types of survey:** Coarse grid survey should be conducted in unexplored areas to capture the overall variability, while fine grid survey is carried out to build-up more collections for specific trait(s) known to exist in identified pockets in previously explored areas. These two types of surveys are applicable for both general diversity and within species diversity documentation.

- **Multi-species/species-specific explorations:** Multi-species exploration is carried out to collect the diversity of a given region (also referred as region-specific exploration). Species-specific exploration should be undertaken to collect the variability within the particular species and its genepool. The samples collected must be representative of the diversity that exists within each species/taxonomic groups in a given area.
- **Permission for collection in protected/restricted areas:** Prior permission should be obtained from the concerned authorities for undertaking explorations in protected (biosphere reserves, sanctuaries, national parks) and restricted areas (border areas/some states in Northeast India).
- **Period of collection:** Duration of explorations should be planned as per the area to be covered and abundance of the particular species or the nature of work. Exploration period of 10-12 days is normally adequate. Flooding periods are not suitable for collections from rivers, and closed fishing season should be avoided or due permission of authorities for experimental fishing can be taken, if necessary.
- **Domestic quarantine:** All precautions including need-based domestic quarantine should be followed for disease-free collection and its transportation, especially in live fish transport.
- **Team composition:** The collecting team should be familiar with the fish genetic resources, collection procedures and use of taxonomic information to meet the objective of the mission. Team comprising 3-5 members along with a collaborator and need-based local-aid is formed with a fisheries scientist as leader. It is preferred that two scientists are available in the team. Depending upon the catch availability and to cover multiple locations at the same time, team can be subdivided during operation.
- **Area and route of exploration:** This should be fine-tuned in consultation with the subject experts of local bodies, keeping in view the targeted species and areas of the proposed mission using the appropriate maps of the area and river course.
- **Equipments and other items required:** As per the nature of the germplasm to be collected and the area(s) to be explored, the required equipments and other items have been listed in Annexure I.

1.2 Collection Methodology

1.2.1 Sampling Sites and Methods

Foundation of precise genetic analysis is laid from the time of exploration and sampling itself. When the target species for the study are wild populations, the researcher has to be extra careful about the source of the fish. There can be two commonly encountered points of apprehension during field explorations for collection of fish germplasm:

- The specimens (commercial species) are not mixed with those from farmed stocks. This may not be a problem for the species which are not cultured.
- The fish for sale may not be actually from the same location, but could have been transported from other nearby locations or even from distance. e.g. large size catla from Punjab (Indus river) are sent to Guwahati for fetching higher price. Such situation will give false picture after analysis.

The following guidelines can help in acquisition of fish samples from the wild:

- Repeated cross-questioning to more than one fisherman/auctioneer.
- Reaching the actual site at the time of fishing.
- Recording GPS coordinates of the selected site for future reference.
- Use of Google maps to narrow down the location, where the fishermen claim to have captured.
- The heap of fishes with differential size range of the target fish or with heterogeneous compositions including non-cultured species is more likely to be from wild than from the farms.
- Collecting the samples from landing centre close to fishing site, where fishermen bring the catch directly for first auction.
- Normally, traders who buy the catch, they may sell directly or pool the fish from other sources to send them to larger markets in cities. Hence, the markets, especially in cities need to be avoided.
- After source of specimen is identified, decision needs to be made whether it is fit for sampling. A live or recently dead fish can be an optimum specimen to collect samples. However, in larger water bodies, this is not always possible. Reservoirs and river fishing is done through gill nets and fish size is normally large. Most of the non air-breathing fish species will reach dead at the time of landing. The decision whether the specimen is worth sampling or not, is very crucial. Besides conventional checks like redness of gills etc., first trial may be made to draw blood. If blood is available, it may be worth to sample other tissues too. The blood can be drawn from caudal vein from the live fish or dead fish up to 5-6 hrs of dealth.
- The samples required for population differentiation are collected from different geographical locations from far off places, where repeated visits are not possible. All types of tissues, i.e. blood, muscle, gill, fin clip, etc. may be collected simultaneously from the same fish at each location to make it cost as well as labour effective.
- To record reproductive data, fish should be dissected to record the reproductive status. A fish with distinct visible oocytes in the ovary and milt oozing from the cut testis, should be considered mature fish (stage 3 & above). The detailed collection protocols are given with tissue banking section and documentation format for field information.

1.2.2 Establishing Taxonomic Identity

- For material with ambiguous identity, vernacular name(s) should be recorded along with specimen and photographs for authentication.
- Normally 4-5 individual animals having representation of all body parts, especially scales, should be collected for preparing voucher museum specimens. Locality, date of collection, field notes and passport data should be recorded.

1.2.3 Type of Material

- For DNA analysis, the tissues, preferably blood, muscle or fin clips should be collected and kept in 95% ethanol.
- For protein or allozyme scoring, frozen tissue such as muscle, liver etc. to be collected.
- Target tissue from euthanized animal, for transcriptome analysis, using RNAlater as storage medium or snap frozen for storing under dry ice or LN_2.
- For cytogenetic analysis, slides to be prepared on site or cell suspensions in culture conditions.
- Cryopreserved sperm to be stored in LN_2.
- Voucher specimens to be preserved in 5% formalin.

1.2.4 Transportation

- The collected samples should be transported in cool and dark conditions to the laboratory for further appropriate storage and use.

1.3 Recording Information

1.3.1 Passport Data

Passport data are important source for the enhanced utilization of FGR and studying the variation in distributional pattern with respect to ecological and socio-economic factors. It is advisable to record information on both the essential and optional fields in the passport data sheet at the site of the collection itself by the explorer (Annexure Ib). However, under any circumstances, the explorer should not leave the information blank on essential fields namely sample labeling (name of organization(s) and collectors, collectors' no., date and type of material); sample identification (Zoological identity, vernacular name, its biological status); sampling information (sampling type, method and source) and collection site localization (state, district, village with latitude, longitude and altitude).

1.3.1 Related Information: Traditional Ecological Knowledge

There is a strong need to explore, facilitate and strengthen traditional ecological knowledge of fisherfolk communities for sustainable management of fish germplasm resources. Such knowledge can be explored and recorded as and when explorer encounters the knowledgeable or ethnic tribes associated with fisheries related activities, especially aged fishermen and farmers. The information collection format is given as Annexure II.

1.4 Post Collection Handling

- The samples collected should be sorted out species-wise.
- Relabel wherever necessary.
- Samples from the specimens of ambiguous identity should be kept separately with specific codes till ambiguity is resolved.

- Exchange formalin twice a week upto fortnight, followed by shifting to iso-propanol.
- Details of the sample to be made in repository records.
- The data need handling and processing for analysis depending upon objective and purpose. General biodiversity data should be placed under GIS information tools and analysed (*Box ii*)

Box ii

Biodiversity and spatial data processing: Geographical Information Systems

Geographical Information Systems (GIS) and remote sensing are important tools for mapping and visualizing fish distribution and their abundance. Species distribution modeling evolves from the relationship between species distributions and their habitats, and is a valuable tool for managing and conserving aquatic resources. These spatial planning tools can also help in simplifying the process of zoning and site selection for *in situ* conservation.

The GPS coordinates of the sampling/collection points recorded during exploration are used for GIS mapping and analysis. ESRI's ARCGIS 10 and PCI's Geomatica 10 can be used to develop the GIS based base map covering administrative district boundaries, water bodies and basin boundaries of the area explored.

The data on fish explorations should be analyzed on various parameters viz., species richness, species abundance and species diversity (Shanon-Weiner diversity index) using the available software for the purpose.

The point vector layers are created both for fish and physiochemical parameters of water using the GPS data of collection points and these layers are arranged on the base map.

1.5 Report Writing

After completing the mission of germplasm exploration and collection from a target area and processing of the collected material, it is important to write a comprehensive report to fulfil the mission's objectives. This helps in follow-up collection(s) and the users to know the availability of the germplasm. The information of the samples collected can be entered into database for its access to users. The broad details desired in the report regarding the exploration and collection, are given (*Box iii*)

Box iii

- Name of the organisation
- Name of the scientist(s)/person(s) involved
- Collaborating organisation(s)
- Objectives of the exploration mission

Box iii

- A description of the environment of the target area
- An account of the logistics and scientific planning
- Details of the execution of the mission (timing, itinerary, sampling strategy and collection techniques)
- A summary of the results (areas surveyed along with route maps, germplasm and voucher specimens collected, indigenous knowledge documented, and extent and magnitude of diversity collected, taxonomic ambiguity, evolutionary significance and inhabitation in unconventional area, if any)
- Photographs
- An account on loss of germplasm/species, if any
- Difficulties encountered during collection mission
- Recommendations for follow-up action(s)
- Important contact persons
- Acknowledgements

1.6 Do's and Don'ts

In addition to above guidelines for exploration and germplasm collection, the collector(s) should observe a well-defined code of conduct as well as take necessary precautionary measures for its smooth execution as given (*Box iv*)

Box iv

Do's

- Always keep a route map of the target area with list of important places and the distance covered during travel to facilitate report writing.
- Before entering forest or any protected area, take the help of forest guards to have forehand knowledge of possible dangers in the target area. If needed, help of an authorised gunman may be taken during survey in dense forest.
- Explain the purpose and get consent from the farmers for collecting germplasm.
- Keep important telephone numbers of concerned officers including district authorities, hospitals, dispensaries and police stations.
- Keep your identity card and a certificate from Head of Organization for proposed mission.
- Honour social customs of local inhabitants of the target area.
- While talking and discussing with ladies, be polite and respectful.

Box iv

- After day's collection and before completing the day, have a glance at your equipments, data, collected material and remarks for the collected samples for need-based updating.

Don'ts

- Do not provide lift to strangers in your vehicle under any circumstances.
- Do not indulge in unnecessary discussion related to politics, religion and local beliefs with the local people.
- Do not make false promises with donors.
- Do not plan the exploration programme during important festivals and peak election campaign in the target area.

1.7 Precautions and safety of researchers

The planning of exploration programmes not only requires care and precautions for samples but also must consider convenience and safety of researchers. The activity is operated at both field and laboratory; and therefore explorer should follow the standard precautions (*Box v*)

Box v

In field

- It is desirable that a four-wheeler is used for undertaking exploratory missions along with safety equipments depending on the regions/areas.
- For forest areas and damp areas close to rivers, which are known habitats for leeches; one should carry salt or anti-leech material (socks/lotion), etc.
- The hazardous chemicals such as formalin should be used in isolated place with use of appropriate facemask and gloves.
- Proper clothing (woolen cloth/jacket/raincoat) are to be carried when exploration mission is planned to cover mountainous region/hot and humid climatic zones.
- The waste such as syringes, needles and gloves should not be discarded in the forest for the safety of local people, grazers, cattle etc., but should be carried back in a bag to appropriate disposal place. Needles should be disposed of using syringe cum needle destroyer.

In laboratory

- Failure in power supply can result in loss of collected material especially frozen material. The freezers should have adequate backup system, including power generators or carbon dioxide.

Box v

- Hazardous chemicals, such as formalin should be used under fume hood during processing and masks should be used during process.
- Fire extinguishers and other fire fighting equipments should be provided in the premises.

1.8 Suggested readings

Jayaram, K. C., 2010. The Freshwater Fishes of the Indian Region. Narendra Publishing House, New Delhi.

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Talwar, P. K. and A. Jhingran, 1991. Inland fishes of India and adjacent countries. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, 2 vol. xix+1158.

Carocci, F., B. Gabriella, E. Paul and G. Meaden, 2009. Geographic information systems to support the ecosystem approach to fisheries: Status, opportunities and challenges. *FAO Technical Paper 532*, Rome, p. 101.

Meaden, G. J. and J. Aguilar-Manjarrez, 2013. Advances in geographic information systems and remote sensing for fisheries and aquaculture. *FAO Technical Paper 532*, p. 395.

□ □ □

2 CHARACTERIZATION OF GERMPLASM

Characterization is the description of germplasm. In fishes, it can be done with the objective to determine taxonomic validity, genetic stock structure, baseline genetic variation, traits of aquaculture importance or adaptive significance and heritability of traits as a part of selective breeding programme. It determines the expression of highly heritable characters based on morphological, physiological or quality parameters and use of molecular markers. Characterization should be based on standardized and calibrated measuring formats and characterization data analysis should follow international/national norms. Characterization should be carried out at initial stage of conservation, i.e. *in situ* or *ex situ*, to add value to the collection. It is essential that the germplasm being conserved, is known and described to the maximum extent as possible to add value to the collection and to assure its maximum use by the decision maker for conservation, culturists or breeders. Use of standardized internationally or nationally accepted minimum set of markers and sample size for use of molecular or morphological descriptors is recommended. Use of internationally/nationally agreed standards of techniques for characterization and data analysis increases the usefulness of the published data. Characterization will be useful for documenting intra-specific diversity. Appropriate sampling strategies may be necessary for ensuring to capture the genetic variation that is representative of natural diversity. Documentation of field observations is also extremely important for germplasm utilization.

2.1 Pre-requisites

- Collection of accessions and characterization is time, effort and resource intensive; hence possibility of multiple collections from a particular region may be explored. Necessary replicates of the samples should be used for characterization.
- Data recording needs to be carried out by the trained staff, using calibrated and standardized procedures. The data should be generated for widely used taxonomic and diagnostic characters through use of standard procedures of estimation and appropriate molecular markers. Combination of microsatellite markers (15 to 20) and mtDNA genes (2 to 3) are considered to be quite useful. However, these should be supported by adequate sample size as per the requirement of the markers. It is also recognized that reference collections (voucher specimens, photographs) play an essential role in validated identification of the accession.
- With the advancement in biotechnology, molecular markers and genomic tools are increasingly used in combination with phenotypic observations for characterization because they have advantages in the estimation of uniqueness of a source of variation within or among accessions. Data obtained from characterized germplasm using molecular techniques have advantages over phenotypic data, as the former are largely neutral to environmental influences. Although several markers and techniques are available, only

well-established and repeatable markers such as microsatellite markers and SNP markers (where available) are found to be quite relevant for characterization. There may be a need to develop species-specific markers or look for their availability in related species. The cross species utility may be successful in most of the closely related species (up to subfamily); however, it is necessary to be tested for polymorphism and suitability for genetic variation detection. Cross species amplifications should be confirmed for the presence of repeat motifs through direct sequencing. The cross species amplicon without repeat region, consistent and scorable polymorphism or high molecular weight should be rejected. The inclusion of reference accessions in molecular characterization also plays an essential role for comparison among different genebanks.

2.2 . Collection of specimen images, tissue accessions and storage

- Identify the fish on the basis of taxonomic characters and simultaneously fill the work sheet for every fish collected for all the parameters given in it.
- Collect the blood from individual fish with heparinized syringe (1 or 2 ml) and needle from caudal vein and store in 95% ethanol.
- Weigh the fish and note down in the work sheet.
- Take a photograph of the fish on graph paper by placing the code number along with the fish, so that it can be identified easily on a later date.
- Collect tissues from each fish, ie., small white muscle piece or fin clips and store the tissue in 95% ethanol (1ml) in 2 ml tubes.
- Label each tube with specimen code and store in refrigerator.

2.3. Truss landmark based morphometry (size-free landmark based morpho-meristic traits)

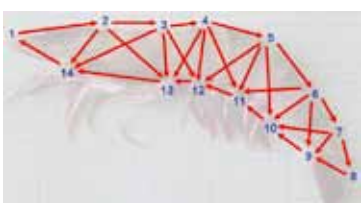
2.3.1 Digitization of specimens

- Specimens should be placed on a flat platform on graph paper as a background having mouth towards the left side and labeled with a specific code for identification. From the top, a digitized image should be captured and downloaded in the computer.

2.3.2 Generation of truss data

The extraction of the truss distances from the digital images of specimens can be generated using a linear combination of three software platforms, tpsUtil, tpsDig2 and PAST.

Truss landmarks *Catla catla* (Finfish)



Truss landmarks *Penaeus monodon* (Shrimp)

- Two–dimensional Cartesian coordinates of multiple landmarks to be recorded on the left view of each specimen and truss networks to be constructed by interconnecting the landmarks.
- Extract multiple inter-landmark morphometric characters by measuring the truss distances.

2.3.3 Statistical analysis

- The truss data generated by the software 'PAST' should be first log-transformed and second is to eliminate size effect when comparing fish of different sizes. Standard length (SL) should be excluded from the final analysis because SL is used as a basis for transformation to eliminate size effect.
- To identify whether there are any statistically significant differences between the populations for each character, a one-way analysis of variance (ANOVA) should be performed on each variable and significant variables should be retained and subjected to principal component analysis (PCA), linear discriminant function analysis (DFA) and cluster analysis.

2.4. Development and application of molecular markers

2.4.1 Genomic DNA extraction

- Total DNA can be extracted from fresh, frozen or fixed blood, fresh/frozen liver, muscle, sperm, kidney and any other tissue of fish.
- Most frequently used method of DNA isolation is the phenol-chloroform method, which removes proteins and other cellular components from nucleic acids and relatively pure DNA can be obtained for further analysis. Extract the total genomic DNA from ethanol-fixed blood and muscle using the modified phenol-chloroform method, re-suspend in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0).
- After RNAase treatment, determine quality and concentration of DNA isolated through serial dilutions on Spectrophotometer (such as nanodrop/picodrop, etc.) and on 0.7% agarose gels.

2.4.2 Mitochondrial DNA Markers

2.4.2.1 Species discriminating markers for taxonomic validation

At least 5 samples of each species from at least two locations are required. For species discriminating marker**,** partial sequence (655 bp) of mitochondrial cytochrome c oxidase I (COI) is used**.**

- For PCR amplification, primers used are universal COI primers (Ward et al. 2005)
- Set up PCR reaction in 50 µl reaction containing Buffer (1x), dNTPs 200mM each, primers 5-10 pmol and DNA 50-100 ng
- Run PCR in a thermal cycler with annealing temperature of 50-55^{0}C
- Visualize the PCR products on 2% agarose gel in 1X TAE

- Purify the PCR products with commercial PCR purification kit, following manufacturer's protocols.
- For bidirectional sequencing of the PCR product, set up two sequencing reactions per sample using same individual primer as PCR reaction using sequencing kit, provided by the company of the Automated sequencer, following manufacturer's protocols.
- Load the samples in Automated sequencer and run the machine, following manufacturer's protocols.
- Align the sequences, generated by forward and reverse reactions, using online software Clustalw, to generate the consensus sequence.

2.4.2.2 For Intra-specific genetic stock structure

At least 20 samples from each location are analysed with mitochondrial markers, cytochrome b and ATPase6/8. However, it should be checked if COI gives enough phylogenetic sign to discriminate. Preferably additional genes like 16sRNA or cytochrome b is also included.

- For PCR amplification, primers used are universal primers for cytochrome b and ATPase6/8 for fish.
- Follow the above steps for setting up PCR reaction, purification and sequencing of PCR product and generating consensus sequences.
- Analyse the sequences generated for composition of sequences, total haplotypes in populations, haplotype and nucleotide diversity and genetic divergence using softwares DNASP, MEGA and Arlequin.

2.4.3 Microsatellite DNA markers

- **Development of Species-specific novel microsatellite markers:** Develop the species-specific microsatellites markers through construction of microsatellite-enriched libraries or through cross priming with related species
- **Primer designing:** Design the primers from the unique flanking regions of the repeat sequences through software available online (e.g. Primer3 for genotyping of individual samples)
- **Amplification and visualization:** Amplify samples (n=5) of given species for validating the utility of the primers. If cross priming tests are used, amplicons should be sequenced to ensure the presence of microsatellite repeats. For visualization, the amplicons are run on Polyacrylamide Gel Electrophoresis (8-12% gel) followed by Silver staining.
- **Testing of locus polymorphism:** Test respective primers to assess the polymorphic informativeness by amplifying n=8 samples from at least three distant (geographical) localities.
- **The selected (validated) primers** are fluorescently labelled at 5' end of the forward primer. The dyes are FAM, NED, TET for Applied Biosystems (use sequencing machine specific dyes for labeling).

- **Amplify** individual samples using labelled microsatellite primers (about 15-20) from various riverine localities with approximately 50-70 samples per site
- **Purify** amplicons and genotype on automated genotyping system (Applied Biosystems)
- **Individual genotype data** is used to calculate various population genetics parameters. Quantify the allele frequencies, Hardy-Weinberg Equillibrium (HWE), presence of private/ rare alleles, genetic differentiation (Fst), inbreeding coefficient (Fis) using softwares (e.g. Genetix, GenAlex, Genepop, etc.).

Standard primer sequences for microsatellite loci for Indian major carps are given in Annexure IV

2.5 Contingency planning

- Quality of data might vary among data collectors, if they are not well-trained and experienced. Therefore, trained technical staff in the field of fish genetic resources should be available to record and document characterization data. Access to expertise in taxonomy, reproductive biology and molecular markers during the process of characterization is desirable.
- Characterization is very labor-intensive and requires sufficient funding to ensure good quality data.
- The contamination and cross contamination can happen especially in DNA samples or PCR products.

2.6 Suggested readings

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Lakra, W. S., V. Mohindra and K. K. Lal, 2007. Fish genetics and conservation research in India: Status and perspectives. *Fish Physiology and Biochemistry*, 33(4): 475-487.

3 EVALUATION OF GERMPLASM

Evaluation is the analysis and documentation of characteristics of fish germplasm. It involves the systematic recording of data on morphological, husbandry and disease resistance, life history traits in captivity, through appropriately designed experimental trials. Such expression of traits can be often influenced by evolutionary process, environmental factors and traits that are genetically inherited. This type of information provides focused identification of trait-specific individuals or populations or group of individuals or pedigree to meet culturists' needs. These data sets can be extremely useful for breeders to incorporate traits into breeding programs and to enhance utilization of resources. Reliable evaluation of data, that are easily retrievable by researchers, facilitates accessions in germplasm resource centers for aquaculture and conservation. Germplasm should be systematically evaluated through a network/ coordinated approach under different agro-climatic zones. Evaluation in fisheries needs holding of live animals under captive conditions. Hence, it needs specialized establishments of live germplasm resource centers. However, such working models are scarcely available, even globally. Generation of evaluation data is more time and resource intensive than obtaining characterization data. Therefore, this can be done for prioritized fish species and molecular characterization can be part of developing effective breeding population.

3.1 Technical aspects

- Prioritization of species and working partners at regional level is the first step.
- Development of broodstock through collection from wild sources, to establish effective breeding population of species, at least 200 to 300 breeding pairs with near equal sex-ratio. However, number can vary depending upon biology of fish species.
- Inventory of data pertaining to life history traits/biological parameters and molecular genotyping of tagged individuals in captivity should be maintained.
- Evaluation data on gene bank accessions should be done for targeted traits that are of importance for aquaculture or any known adaptation against biotic or abiotic stress.
- Evaluation data should be obtained for as many accessions as practically possible. Evaluation trials should be carried out in at least three to four environmentally diverse locations and data should be collected and collated.
- Technological intervention for breeding of captive broodstock and husbandry management practices will need optimization.
- Assessment for grow-out in monoculture and/or polyculture, economic advantage and feasibility analysis and establishment of domesticated broodstock are required.
- With the advancement of techniques, molecular markers and genomics are increasingly being used for evaluation of germplasm. The most commonly used molecular markers in

germplasm characterization and evaluation include Microsatellites and Single Nucleotide Polymorphisms (SNP). Also advances in next generation sequencing and the accompanying reduction in costs have resulted in the increasing use of sequencing based assays such as the sequencing of coding and non-coding regions and genotyping-by-sequencing (GBS) in germplasm evaluation.

- Use of quantitative PCR can also help in evaluation, if expression of specific gene known for adaptive trait is to be evaluated. This is possible if knowledge of genes involved in adaptation is available.
- Knowledge of markers (microsatellite and SNP) will also be used to screen populations or group of animals, if these markers have been found to be associated or linked to specific genes controlling a favorable phenotype.
- The introduction of material to the germplasm center should pass through quarantine procedures.
- A pathologist must be involved to keep watch on emergence of any disease.

3.2 Contingency planning

- The accessions collected from wild or in the process of acclimatization are prone to losses through mortality or other hazards.
- The evaluation of germplasm is very labor-intensive and requires adequate levels of sustained funding to collect reliable high quality data.
- Community evaluation using tagged populations should be undertaken to avoid differences due to pond environment effect. Variations in the biotic and abiotic stresses and the fluctuations in environmental and climatic condition in the field affect the accuracy of data.
- The captive populations sometimes may not complete life history cycles due to different biological demands.

3.3 Suggested readings

Lal, K. K., S. Raizada, and J. K. Jena, 2012. Species prioritization for *ex situ* conservation and freshwater aquaculture. *Theme paper and Proceedings of the National Consultation on Species Prioritization for Ex situ Conservation and Freshwater Aquaculture*, Lucknow, 17-18 September, 2011.

Ponniah, A. G. and K. K. Lal, 1998. Reproductive biology estimators for conservation and culture of fish. *In:* Ponniah, A. G. and U. K. Sarkar, (eds.), *Proceedings of the Workshop on Germplasm Inventorisation and Gene Banking of Freshwater Fishes*, Cochin, 12-13 October, 1998, pp. 4-6.

Ponniah, A. G. and K. K. Lal, 1998. Documentating life history parameters for utilising in conservation and genetic upgradation programmes. *In:* Ponniah, A. G. and A. Gopalkrishnan, (eds.), *Proceedings of the Workshop on Germplasm Inventorisation and Gene Banking of Freshwater Fishes,* Cochin, 12-13 October, 1998, pp. 16-18.

4 QUARANTINE OF GERMPLASM

Quarantine of aquatic animals for research or trade or aquaculture is the measure to prevent the introduction of exotic pathogens along with fish consignments into the country. It plays an important role in preventing spread of alien disease into aquaculture system.

4.1 International Exchange

The Department of Animal Husbandry, Dairying and Fisheries (DADF), Ministry of Agriculture, Govt. of India is the nodal organization to permit introduction of exotic fish germplasm in the country. The Department receives technical backstopping on various issues like disease diagnosis, quarantine guidelines and other biological information related to exchange from the research organizations, mainly NBFGR, Lucknow; CMFRI, Kochi; CIFA, Bhubaneswar and CIBA, Chennai. The introductions are permitted after approval of National Committee on Introduction of Exotic Aquatic Species in Indian Waters. The quarantine facilities operational at Chennai are administratively controlled by DADF.

4.2 Pre-entry quarantine requirements

- Import Permit is a statutory requirement from the country of import and the conditions/ additional declarations laid on it need to be fulfilled by the exporting country.
- Phytosanitary Certificate is also a statutory requirement and is a proof that the consignment has been examined according to the requirements of the importing country and found to be free from the specified pathogens.

4.3 Post-entry quarantine requirements

- At present, only airport allowed for import of fish germplasm is Chennai.
- Screening at Aquatic Quarantine Facility, Chennai - the examination is carried out to make sure that phytosanitary conditions laid down in the import permit have been taken care of and consignment is free from exotic pathogens and diseases especially listed under OIE notification.
- Examination of the material after entry as per guidelines & procedures laid.

4.4 Standards for transporting aquatic germplasm to India

- All aquatic animals in the consignment must be packaged in leak-proof bags, each bag containing only one species. The bag must be colorless and sufficiently transparent to enable proper inspection and identification of the aquatic animals and must not contain any extraneous matter, unapproved material, or unauthorized species. The use of outer bags of opaque materials or half-black bags to provide a dark shipping environment is acceptable, provided the contents of the bag can be properly inspected to the satisfaction of competent authority.

- The inclusion of inert material such as zeolite, activated carbon, shredded plastic or dried terrestrial plants is permitted provided the contents of the bag can be properly inspected to the satisfaction of competent authority and the material is disinfected or destroyed as directed by competent authority.
- Each bag must be of a size and weight that will allow inspection to the satisfaction of competent authority.
- Each bag must be placed within polystyrene boxes or cartons fitted with a plastic lining. Each box or carton must be clearly identified as part of a shipment/consignment and be individually identified.
- The consignment must be accompanied by documents that include the identification number of each box or carton, and the scientific name and number of the imported aquatic animals. It is recommended that the common names of the aquatic animals are also mentioned on the papers.
- The aquatic animals in each bag must be stocked at a density that will facilitate inspection and hence must not be overcrowded. When packed for export, aquatic animals must be placed in clean water. The use of pH indicator in the water is permissible, provided it does not interfere with inspection.
- Each consignment of aquatic animals must be accompanied by a health certificate issued by the Competent Authority of the exporting country, signed by an individual with an appropriate knowledge of aquatic animal health and the export premises.

4.5 Inspection and procedures to be adopted on arrival

- Each consignment of aquatic animals entering India (including packaging) will be examined on entry by competent inspection authority that will evaluate the health of the aquatic animals, check that all documentation is in order, and only approved species are included, and that no material of quarantine concern is present. Any prohibited species or materials of quarantine concern must be re-exported, destroyed or treated to the satisfaction of competent authority, all at the importer's expense.
- After inspection, all aquatic animals will be ordered into quarantine at a place approved by competent authority as aquatic animals quarantine facility (AAQF). On arrival at the quarantine premises, the aquatic animals will be transferred by net to new water in the quarantine facility. All bags, polystyrene boxes and cartons that have been used for importing exotic aquatic organisms must be either incinerated or effectively disinfected in an approved method prior to disposal. All water imported with consignment must be disinfected as per standard methods prior to disposal.

4.6 Quarantine Procedures

- All aquatic animals must be kept in units i.e. tanks or other competent authority approved containers. Units must be kept clean at all times. Units must be free of gravel, sand, plants, soil or shell grit and only sterilisable materials (e.g. polypropylene) may be used in the unit. Tanks must be fitted with lids (or approved equivalent) to prevent aquatic animals jumping

out of the tanks and to minimize splash contamination. Each unit must contain only a single aquatic animal species.

- Where separate consignments of aquatic animals share a water recirculation system, aquatic animals may only be approved for release from quarantine when the last consignment of aquatic animals to enter the system has satisfactorily completed its quarantine requirements. All aquatic animals sharing the system may be subject to quarantine risk management measures (*e.g.* destruction, treatment or detention beyond the normal quarantine period) if any aquatic animals in the system are suspected to carry disease agents or pests of quarantine concern. In deciding regarding the need for measures to be applied to all aquatic animals sharing a recirculation system, the water sterilization systems (*e.g.* ozonation or ultraviolet irradiation) should be used.
- The quality of water used in the quarantine unit should be monitored at regular intervals to ensure that mortality in the quarantine population is not due to environmental conditions but rather to disease agents.
- Only authorized persons should be allowed entry in the quarantine facility. Entrance to the quarantine facility is restricted to the importer, his nominated employees and competent authority officers, or other persons approved by competent authority. Once a person has entered a Quarantine facility, such person will not on same day enter any other place where live aquatic organisms are kept. A proper record of persons entering quarantine facilities needs to be maintained.
- Any unusual levels of mortality or unusual signs of disease/pests (levels of mortality or illness above that normally observed in imported aquatic animals) must be reported to competent authority immediately. If the quarantine unit suffers a disease outbreak that cannot be controlled, the diseased stocks must be destroyed and disposed off after sterilization in an approved manner, but not before notification of the appropriate government authority. The quarantine unit or the particular module and associated items must be disinfected prior to its reuse
- The importer must ensure that no aquatic animals leave the quarantine facility under any circumstances without approval of competent authority, except dead aquatic animals moved to a nearby refrigerator or freezer. Aquatic animals may not be released from quarantine until completion of the following quarantine detention periods and fulfillment of all quarantine requirements to the satisfaction of competent authority.
- Approval of requests for prophylactic or therapeutic treatments will be considered by competent authority, taking into account the need to ensure that exotic disease agents are not inadvertently released from quarantine. Any treatments may result in the extension of quarantine detention period or other measures as deemed necessary by competent authority.
- Where competent authority has reason to believe at the end of the quarantine detention period that the aquatic animals still present an unacceptable risk of disease or pest introduction, they may be kept in quarantine detention for further investigation, observation, treatment, testing or for any other purpose appropriate to the circumstances. If the risk cannot be effectively managed, destruction of the aquatic animals will be ordered. The costs associated

with any of these measures will be borne by the importer.

- Permission may be granted by competent authority for healthy aquatic animals to be held in the quarantine facility after completion of quarantine period. Further, on completion of quarantine, aquatic animals are to be transferred by net into clean water prior to removal from the quarantine facility.

4.7 Documentation

- A proper record of aquatic animals kept in quarantine should be maintained.. Unit record sheets must be legible and available for inspection by competent authority officers during the quarantine period and for 12 months thereafter. A water treatment record should be maintained for all water treatments. All drug/chemical treatment of aquatic animals must have competent authority approval and be recorded on unit record sheets.

4.8 Disinfection of material

- All nets, equipments, used water, effluents, dead organisms or any other material shared between units must be disinfected in the quarantine facility by a method approved by competent authority before being used for other consignment of aquatic animals or prior to removal from the quarantine facility.
- Water sterilization and equipment disinfection should be effective against the resistant aquatic animal pathogens or pests. Disinfection/sterilization protocols should reduce pathogen titers to levels below that likely to cause infection when exposed to a susceptible host. The following disinfection/sterilization protocols provide an indication of the level of disinfection and/or sterilization required by competent authority. Alternative methods that provide equal or greater level of quarantine security may also be used. But it needs the approval of competent authority before itself.
- Chlorine is very toxic; hence hypochlorite powders and concentrated hypochlorite solutions should be kept in properly sealed containers in well-ventilated area outside the quarantine facility.
- All water to be treated must pass though a filter, capable of removing suspended organic material, prior to hypochlorite treatment.
- All water to be treated must pass to a retention vessel where sufficient hypochlorite must be added to achieve a final concentration of 200 ppm. Sodium hypochlorite (bleach) should be used at 1.6 milliliters of hypochlorite solution (12.5% available chlorine) per litre of water, while calcium hypochlorite powder (65-70% available chlorine) should be used at 0.3 g of powder per litre of water.
- Following addition of hypochlorite, wastewater must be agitated for a period of not less than 10 minutes to ensure thorough mixing of hypochlorite and retained for a period of not less than 1 hour.
- After the one-hour retention period, the chlorine in the wastewater may be neutralized by adding sodium thiosulphate (hypo) at a rate of 1.25 g (2.5 ml of 50% sodium thiosulphate

solution) per litre of treated wastewater and then agitated for not less than 10 minutes before discharge.

4.9 Disinfection of units, equipments

- Units and unit equipment to be disinfected must be thoroughly cleaned and treated with hypochlorite solution at 200 ppm concentration for 5 minutes or with an iodophore solution containing 0.5% available iodine for 5 minutes or by other approved disinfection methods.
- Hands should be thoroughly washed with soap and water to remove any contaminant material, prior to exiting the quarantine facility.
- If footwear is to be removed from the quarantine facility, it should be clean and the soles and lower portion of the footwear must be disinfected by immersion of the exterior surface in an approved disinfectant such as a 5% solution of Betadine.
- All dead aquatic animals or eggs can be kept in a solution of 10% formalin for a minimum of 5 days before disposing of such fish or fish eggs. The ratio of dead fish or fish eggs volume to solution volume shall not be less than 1:5.

4.10 Suggested readings

Ponniah, A. G., V. K. Unnithan and N. Sood, 2002. National Strategic Plan for Aquatic Exotics and Quarantine. *NBFGR Special Publication No. 3*, NBFGR, Lucknow, p. 119.

Ponniah, A. G. and N. Sood, 2002. Aquatic Exotics and Quarantine Guidelines. *NBFGR Special Publication No. 4*, NBFGR, Lucknow, p. 97

5 REGISTRATION OF GERMPLASM

5.1 Fish Germplasm Registration Committee

i) The Fish Germplasm Registration Committee (FGRC) is constituted under the Chairmanship of Deputy Director General (Fisheries Science), Indian Council of Agricultural Research.

ii) It would include Director, ICAR-NBFGR as a permanent member and a senior level scientist from ICAR-NBFGR to function as Member-Secretary, which would be identified by the Chairman, FGRC. The other members will be co-opted as per the advice of the Chairman.

iii) It will have provision for adoption of need-based resource specialists with reference to the material under consideration, with the approval of the Chairman.

5.1.2 Nodal Agency

i) ICAR-NBFGR, Lucknow is the nodal agency for registration of germplasm. The application should be addressed to the Director, ICAR-NBFGR, along with the details of the material.

ii) The Member-Secretary, FGRC will duly acknowledge the receipt of the application with date, application number and the national identity.

iii) ICAR-NBFGR will maintain a permanent register and database listing the germplasm materials approved by FGRC with details of related information.

5.1.3 Application Form

Application shall be made on the prescribed Proforma available on (http://www.nbfgr.res.in/RegofAGP.aspxForm A, Annexure II).

5.1.4 Eligibility Criteria for Registration

Germplasm or genetic stocks of aquatic organisms, including finfish and shellfish species, species of aquaculture importance for food or ornamental purpose which are unique, uniform and stable and have potential attributes of academic, scientific or commercial value shall be registered. The above attributes should be proven with evidence from standard morphological data, molecular data or experimental data that prove unique adaptation, genomic expression. The organisms under consideration can be from wild, domesticated stocks or improved strain out of human intervention.

All claims concerning the material submitted for registration should accompany scientific evidence for uniqueness, reproducibility and value in the form of:

- Publication in standard peer reviewed journal (a copy of reprint to be submitted).

  AND/OR

- Publication of information on potential value of proposed germplasm in institute annual report or any other reports.

AND/OR

- Certificate of the validation test of the claimed attribute by any institution as per the advice of Member-Secretary.

5.1.5 Germplasm Not Eligible for Registration

- Germplasm or genetic stock without accompanying documentary evidence for the claim made in the application.
- Germplasm or genetic stock that does not contain complete passport data, including authenticated taxonomic identity, parentage data (improved strain), institutional or national identity, geographical location of origin and all such information related to the development and contribution, if any, to the uniqueness of the germplasm.
- Exotic germplasm *per se,* with no evidence of local human intervention in its improvement.
- Strains of common knowledge or selection from aquaculture farms without prior approval from the concerned farmers.
- Germplasm of any genera or species, which involves any technology, which is injurious to the life or health of human being, animals or plants.
- Material for which any form of IPR protection has been sought elsewhere.

5.1.6 Screening of Application(s) and their Consideration by the FGRC

- The Member-Secretary, FGRC, will screen the proposal(s) submitted on prescribed Proforma, as per the guidelines at ICAR-NBFGR.
- After initial screening, the incomplete applications would be advised for appropriate revision.
- Each proposal will be forwarded to the concerned Director for validation of information, particularly on uniqueness and novelty of the proposed germplasm.
- The application in which the validation of the data is felt necessary, the applicant would be asked to produce a validation report from an appropriate institute, advised by the Member-Secretary. The revised application should accompany such report duly endorsed by the competent authority of the institute, advised for the validation.
- The proposals complete in all respects along with the comments of concerned Director, PD or PC will be put up to the FGRC for consideration.
- The FGRC will consider the proposal as early as possible and not later than one year.
- The decision of the FGRC will be final.

5.1.7 Validity of Registration

The period for validity of registration shall be applicable to only the strains improved developed through human interventions. This period will be 15 years for the species which attain maturity within 1 year and validity will be 25 years for the species attaining maturity after more than 1 year. After the validity period, the genetic stocks that are collected from wild with unique traits and submitted for registration will be treated as national sovereign property.

5.1.8 Publication of Registered Germplasm

All germplasm material approved for registration would be officially communicated to the applicants along with Registration Number. A certificate to this effect will also be issued to the applicant. A brief description of not more than one page would be published in the ensuing issue of appropriate periodicals, such as:

- Indian Journal of Animal Sciences or Indian Journal of Fisheries.
- NBFGR Newsletter.
- NBFGR Website http://nbfgr.res.in

5.1.9 Conservation, Maintenance and Sustainable Utilization of Registered Germplasm

- Registered germplasm accessions will be stored in repository at NBFGR for future reference.
- All the registered germplasm needs to be present as live material at the institute/organization or individual breeder farm claiming registration. The concerned institute will be mandated to maintenance of working stock of germplasm for supply to bonafide users. The declaration to this effect will be given by the concerned organization.

5.1.10 De-registration

A registration may be repealed by the FGRC in case of false claim(s). Appeal for counter claim, if any, should reach the FGRC within a period of three months of the publication.

5.2 Procedure for Submission of Proposal

5.2.1 Submission of Application and Material

- All fish germplasm proposed to be registered should be submitted to the following address:

  The Director
  ICAR-National Bureau of Fish Genetic Resources
  Canal Ring Road, P.O. Dilkusha, Lucknow, UP 226002
  Phone: 0522-2442440, 2441735; Fax: 0522-2442403
  E-mail: director@nbfgr.res.in

- The material must be accompanied with properly filled Form–A (http://www.nbfgr.res.in/RegofAGP.aspx) and duly signed by the applicant and the Head of Institution with official seal (15 copies, each with attached documentary evidence to be submitted).

The Form-A must be accompanied by complete description of the germplasm material using standard descriptors.

An undertaking to the effect that working-stock of improved strain for supply to users would be maintained by the institution which is associated with the development of the improved strain.

5.2.2 Genetic Stocks from Wild Populations

- The germplasm accessions discovered as distinct genetic stocks will be registered upon request of the applicant and submission accompanied with relevant documentary evidence and data.
- The genetic stock is a group of individuals reproductively isolated from other group of conspecific individuals.
- The distinct genetic stock will be considered for registration if there is sufficient evidence that allele/haplotype frequencies significantly differ from another neighboring sub-population at least at one locus.
- The genetic stock, thus considered distinct, must be submitted with standard morpho-meristic data and production traits also, if claimed for the production value.
- The genetic stocks differences based on only morpho-meristic characteristics will be considered, if data is supported with sufficient sample size (>50) per location and done through use of standard methodologies & parameters.
- The appropriate proforma, to derive information from applicant for registration of genetic stock will be developed as Form 1B. Form 1C will be used for production traits, if some genetic stock is claimed for superior production value with respect to trait.
- The desired information will include sample size (>50) per location; only co-dominant & mtDNA markers will be allowed; no. of markers (loci) used; Results to prove that pairs of loci do not suffer from linkage disequilibrium and are neutral; results to prove that the allele frequencies at locus that differ from the nearest neighbors examined with same set or parameters and other details of analysis.
- Both marker data and morpho-meristic characters including Truss Network Analysis will be used as descriptors.
- For genetic stocks, besides species descriptors, stock descriptor based on individual genotype data using molecular markers or morphological/biological descriptors for stock/variety (wherever available) will be used. To discriminate stock, at least at one locus in genotype data or haplotypes or with respect to one trait, significant differences must be proven.

5.2.3 Improved Strains Developed for Aquaculture

- The improved strains will be a group of fish of common origin and of one species, genetically similar and with known economic and biological properties and morphological characteristics, demanding similar requirement as to natural and production conditions and capable of reproduction.
- Commercial exploitation of varieties will be for culture purpose for food or ornamental trade developed through selective breeding/hybridization.
- Under no circumstances, these will be meant for releasing in open waters.
- Appropriate proforma to derive the information from applicant will be developed so as to precisely know the verification for the claim. The "applicant" shall mean a breeder who is entitled to file an application for the protection of an improved strain, the applicant shall be

entitled to file such an application only if the variety or breed has been described, developed or created by his own breeding research, in his own name and on his own account. An applicant may be represented by several breeders provided that the variety or the breed has been discovered, developed or created by his material support.

- Each improved strains should be labeled by a name which is its general name.
- The applicant shall be granted, on the basis of his application, a Registration Certificate in respect of variety or a breed as framed in definition.
- Registration Certificate shall certify: i) The creation of improved strains; ii) The name of the improved strains, with an indication of the species and genus; iii) The holder of the Registration Certificate by stating the improved strains trade name, only for Indian citizens, farmers or companies registered in India and iv) The dates of commencement and termination of the protection of the rights in respect of the variety.

5.2.4 Conditions for granting Breeder's Certificate for Fish Strain

The conditions for the granting of the Certificate for Fish Strain would ensure if the germplasm is:

- Different in one or more assessment characteristics from any other strain, whose existence is a matter of common knowledge at the date of priority.
- Uniform to a level that is adequate to the biological properties of the species concerned.
- Stable in its major traits, while respecting the peculiarities ensuing from the environment in which the fish are bred.
- It is new and sufficiently large in number for reproduction.
- The strain shall be deemed reproducible if its assessment characteristics remain unchanged through several generations.
- The denomination must, at the date of priority, enable the strain to be identified.
- Should not be liable to mislead. It must be different from the denomination of an existing strain of the same or a closely related fish species and its use must not be contrary to public policy or morality.
- The created or discovered and developed, germplasm, lines or hybrids shall be liable for registration.

5.2.5 Type of Accessions

The different types of accessions that can be considered for registration of fish germplasm are given in (*Box vi*)

Box vi

S. No.	Type of Accession	Specific Mode of Accession Submission
1	Species	Voucher specimen Tissue samples DNA
2	Genetic Stock/ Elite/ adapted Germplasm	Cryopreserved sperm; Cell culture and Cell Lines; Live Animal
3	Developed Variety	Cryopreserved sperm; Cell culture and Cell Lines; Live Animal
4	Modified DNA Material	DNA material
5	Live Fish	Cryopreserved sperm; Cell culture and Cell Lines; Live Animal
6	Fish Samples under Germplasm Exchange	Voucher specimen Tissue samples DNA

5.2.6 Descriptors for Registration of Fish Germplasm

- Both morpho-meristic characters data and genetic marker data to be used as descriptors together or individually.
- The distinct genetic stock to be considered for registration, if there is sufficient evidence that allele/haplotype frequencies significantly differ from another neighboring subpopulation at least at one locus.
- The genetic stock considered to be distinct must be submitted with standard morpho-meristic data and production traits, if claimed for the production value.
- The genetic stocks differences based on only morpho-meristic characteristics will also be considered, if data is supported with sufficient sample size (>50) per location and done through use of standard methodologies & parameters.
- Appropriate proforma to derive information from applicant for registration of genetic stock to be used for production traits, if some genetic stock is claimed for superior production value with respect to trait.
- The desired information will include sample size (>50) per location; only co-dominant & mtDNA markers will be allowed; no. of markers (loci) used; Results to prove that pairs of loci did not suffer from linkage disequilibrium and are neutral; results to prove that the allele frequencies at a locus that differ from the nearest neighbors are examined with same set of samples and parameters.
- Under all types of accessions, voucher specimen and tissue samples to be essentially submitted along with photograph and other details/documents required as per Form 1A. As far as possible, the total DNA can be provided. However, if the submitting institute does not have facility, it is necessary that the repository makes arrangement.

- For identified genetic stocks/strains from natural populations, proven elite germplasm or improved variety from domesticated stocks of aquaculture species, in addition to Voucher specimen, tissue samples & DNA isolates, specific modes of accession will be used.
- For modified DNA material, voucher specimens/tissue/total DNA of the fish which is source of gene should be provided along with the DNA material submitted as accession. For every species, at least there should be 3 samples/isolates for which corresponding voucher specimens are also submitted. Holotype or paratype or other type should be deposited (2-3 specimens).
- For the germplasm under exchange for farming; at least tissue samples or DNA of 50 specimens will be deposited with 3 voucher specimens. If the germplasm is collected location-wise, the sample size should be considered as per location.

6 DISTRIBUTION AND EXCHANGE OF GERMPLASM

The genetic resource exchange is likely to occur for culture or domestication for future propagation in importing country. Under this, fish may be farmed ones, improved varieties or wild stocks. Even feral populations of the exotic species can be in demand for using the adaptive significance in genetic improvement programmes. Since the domestication level in the fish is low, many of the exchange, especially export for ornamental trade, may need harvest from natural resources. In addition, germplasm exchange is also likely to be done by academic institutes for museum purpose or to solve taxonomic and evolutionary ambiguity or even for specific model species for research. Overall, type of material may include developing eggs, animal at various stages of life history, cryopreserved gametes or cell lines, DNA or tissue material or whole preserved specimens. The number and quality may vary as per objective. Till now, germplasm exchange for research has not been significant but is likely to increase in future. Hence, in this backdrop, there is a need for regulatory mechanism and appropriate guidelines to protect country's stake on its natural fish diversity.

6.1 Procedure for Germplasm Exchange

The details of procedures pertaining to 'Germplasm Exchange' under collaborative mode and other categories are as reproduced below:

6.1.1 Request by Indenter to NBFGR

- The indenter seeking export of germplasm will send request to NBFGR on the prescribed Proforma along with necessary documents and information.

6.1.2 Pre-examination by NBFGR

6.1.2.1 Determination of "Approved" status of the collaborative research project and other than collaborative research project

- In case of any ambiguity or discrepancy, views of SMD i.e. Fisheries Division with respect to collaborative research projects involving the fish germplasm of aquatic animals should be obtained and recorded.

6.1.2.2 The consent of concerned Subject Matter Division (SMD) of ICAR

- Specific views/recommendations of SMD, if needed, in relation to any ambiguity or discrepancy; or no objection to the proposed transfer of germplasm.

6.1.3 Material Transfer Agreement

- MTA approved by DARE, GOI/NBA to be used for export of all Indian germplasm (Annexure III)

- MTA of exporting country to be used for import of designated germplasm, as applicable.

6.1.4 Recommendation of the Germplasm Export Facilitation Committee

- Recommendation should be explicit and self-explanatory.
- Proforma for Collaborative Research Project as per section 5 of BDA (clause 1-14) based on which recommendation is made should be properly and not merely filled. Clarity in terms of name and designation of the person authorized by the Department for sending germplasm should be clearly reflected in clause 11. The Bureau will generally perform the single window transfer of germplasm, unless otherwise warranted/approved/authorized. For the categories other than collaborative research projects, details in similar format would be provided.

6.1.5 Forwarding the request to DARE for approval through SMD

6.1.6 Compliance by NBFGR

Execution of Germplasm exchange under MTA, necessary communication and maintenance of database of approved transactions.*

Box vii

*NBA has conveyed to DARE on 10th October, 2009 that collaborative projects are exempted from obtaining approval of NBA. NBA also informed in the above mentioned letter that in case of any violation of the Act/Rules (BDA), NBA will not take any responsibility, and the Indian collaborator will have to explain and defend the action. The gazette notification issued by Govt. of India, S.O.no. 1911 (E) dt. 08.11.2006 provides the necessary guidelines.

6.2 General Guidelines for Issue of Export/Import Permit for Fish Germplasm

- To get 'Permit' for export/import, the proformas prepared by the NBFGR for submitting proposals should be duly filled and submitted to NBFGR at least one month in advance. A fee shall be charged for scrutinizing the proposals.
- The request for fish germplasm export will be submitted to NBFGR with an Indian interface, if the material/species is not captive propagated/needs to be collected from natural resources. The interface can be any public sector research institute, university including SAU or any other academic institute and will act as resource organization. The consent of resource organization will be mandatory and needs to be appended with application
- Exporter/Importer should prepare the details as per format (Form IV rule 19 BDA rules see *box vii*) of concerned germplasm based upon available biological information and submit along with request to NBFGR.
- The import/export permit shall be valid for six months from the date of issue. The issuing Authority may, on request, extend the period of validity for a further period of six months after charging 'Revalidation fee' provided such request for extension of validity is made to

the issuing authority before the expiry of the permit with adequate reasons to be recorded in writing.

- The import/export permit issued shall not be transferable and no amendments to the permit shall be issued.
- The issue of permit may be refused or withheld by the Issuing Authority.
- The export/import of germplasm will be taken up on case-by-case basis.
- The non-threatened, native species can be exported after proper documentation, to protect the IPRs. For such species, decision can be taken by NBFGR.
- Regarding the species falling under restricted export, the decision to be taken at the level of Secretary, DARE, Govt. of India and DG, ICAR (with inputs from Fisheries Division, ICAR and ICAR-NBFGR).
- Decision making regarding import/export to be based on categorization of indigenous and exotic fishes.
- A National Accession Number will be given by NBFGR to each species to be exported/ imported. Repeat export of the same species will be allotted sub-accession number under the species accession number.
- From export/import consignment, reference sample from each species will be submitted to NBFGR for gene bank accessions. The details of type and sample size will be provided along with issue of permit. However, DARE, GOI would need to authorize Director, ICAR-NBFGR for the same.
- Records of importers/exporters must be maintained in the form of a database, to enable tracing of the movement of fish germplasm.
- Import and export of fish germplasm will be done through designated airports, which have quarantine facilities available, established by Ministry of Agriculture and Farmers Welfare.

Box viii

No.	**BDA Rule 19. Procedures for third party transfer under sub-section (2) of section 20.** (for reference only as per : http://nbaindia.org/content/17/20/1/rules.html)
1	The persons, who have been granted approval for access to biological resources and associated knowledge, intend to transfer the accessed biological resource or knowledge to any other person or organization, shall make an application to the Authority in Form IV.
2	Every application under sub-rule (1) shall be accompanied by a fee of rupees ten thousand only in the form of Bank draft or cheque drawn in favour of the Authority.
3	After collecting any additional information, the Authority shall decide upon the application as far as possible within a period of six months of receipt of the same.
4	On being satisfied that the applicant has fulfilled all the necessary requirements, the Authority may grant approval for third party transfer subject to such terms and conditions it may deem fit to impose in each case.

Box viii	
5	The approval may be granted under sub-rule (4) in the form of a written agreement duly signed by an authorized officer of the Authority and the applicant.
6	The form of the agreement shall be decided by the Authority.
7	The Authority, may for reasons to be recorded in writing, reject the application if it considers that the request cannot be acceded to, provided that no application shall be rejected unless the applicant has been given an opportunity of being heard.

6.3 Export of Germplasm from India: Germplasm for research/ experimental purposes under the provisions of Biological Diversity Act, 2002

Under the provisions of the Convention on Biological Diversity (CBD), Government of India enacted legislation called Biological Diversity Act (BDA), 2002 and also notified the Biological Diversity Rules, 2004. As per section 3 of the Act, no person from outside India or a corporate body, association, organization incorporated or registered in India having non-Indian participation in its share capital or management, can access any biological resources or knowledge associated, for research, commercial utilization, bio-prospecting or bio-utilization, without prior approval of National Biodiversity Authority (NBA).

The persons who shall be required to take the approval of the NBA, are the following, namely: (a) a person who is not a citizen of India; (b) a citizen of India, who is a nonresident Indian as defined in clause (30) of section 2 of the Income tax Act, 1961; (c) a body corporate, association or organization (i) not incorporated or registered in India; or (ii) incorporated or registered in India under any law for the time being in force which has any non-Indian participation in its share capital or management. All such persons or organizations seeking approval of the Authority (NBA) for access to biological resources and associated knowledge for research or for commercial utilization shall apply in the prescribed form (www.nbaindia.org).

As per Section 5 of BDA, 2002, exchange of germplasm for collaborative research under the bilateral agreements/collaborative projects is, however, exempted which conform to the policy guidelines issued by the Central Government or approved by the Central Government.

6.4 Suggested reading

Bartley, D. M., J. A. H. Benzie, R. E. Brummett, F. B. Davy, S. De Silva, A. E. Eknath, X. Guo, M. Halwart, B. Harvey, Z. Jeney, J. Zhu, U. Na-Nakorn, T. T. T. Nguyen and I. I. Solar, 2009. The use and exchange of aquatic genetic resources for food and agriculture. Commission on Genetic Resources for Food and Agriculture. *FAO Background Study Paper No. 45*, Rome, p. 40.

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7 CONSERVATION OF GERMPLASM

7.1 *Ex situ* Conservation and Genebank

- The foremost aim of an *ex situ* genebank is to conserve the material under hygienic conditions for reference, research and utilization wherever, feasible. Two forms of *in vitro* conservation are discussed here;
  - **Non-Retrievable Form:** Tissue Bank and Voucher Specimen Repository
  - **Retrievable Form:** Cryopreservation Bank and Live Germplasm Resource Centers

7.1.1 Tissue bank: Collection of Tissue Accessions & Storage

- Identify the fish on the basis of taxonomic characters and simultaneously fill the work sheet (list I) for every fish collected for all the parameters given in it.
- Draw the blood with syringe and needle and store in 95% ethanol (protocol given below). Use clean forceps and surgical blade for every fish for collection of muscle/gill/fin clip.
- Weigh the fish and note down in the work sheet for this purpose.
- Take a photograph of the fish by placing the code number along with the fish, so that it can be identified easily on a later date.
- Cryovials with screw caps and 'O' ring can be effective method of storage without necessity for parafilm sealing.
- Label the tube and keep the tube with tissues in a cryobox serially and store in refrigerator, till the same is sent to the repository.
- Wherever refrigerator is not available especially under field conditions, it is possible to hold sealed tubes of tissues at room temperature till these are brought to laboratory. Exposure to heat and sunlight should be avoided.

7.1.2. Voucher Specimen Repository

- Essentially required as a first information base of the fish repository due to possible taxonomic ambiguities in fisheries and changes in systematic position of the species. The voucher specimens are required in the repository not only to refer the specimens but also for taxonomic research.
- Minimum of three fish of each species to be preserved for depositing along with tissue samples. In case species exhibit sexual dimorphism, two each of male and female to be preserved.
- For identification, make a folded aluminum foil with label and insert deep in the mouth, so that it does not come out.
- The specimens can be preserved in formalin solution (5%) in plastic jars or of appropriate size.

- Different species can be put in one jar which should be numbered and name of centre should be pasted on it.

7.1.3 Sperm Cryopreservation

- Sperm cryopreservation is the only technique available for long-term storage of fish genome that can be used to retrieve the endangered species. ICAR-NBFGR, Lucknow has been associated with research in this area and expertise is available for development of fish sperm cryopreservation protocols for various applications. The basic setup and technique has been customized to avoid need for sophisticated equipment, for easy adaptability. The technique, both application and protocol development, has been tested successfully under difficult locations. The setup is also useful in research to develop species-specific protocols, as proven with success achieved for fish species belonging to different taxonomic groups, including the non-domesticated too. The important requirements are source of male broodstock, cryocans (depending upon storage capacity) and reliable source of liquid nitrogen, other minor chemicals and lab material only.
- Injecting the male fish with Ovaprim as per body weight 6-8 hrs before collection of milt.
- The technique for obtaining milt depends upon type of fish.
- In case of fish that do not ooze milt, the testis are used directly to get sperm suspension through stripping like in catfishes.
- The fish that ooze milt such as carps, the milt is obtained in clean dry plastic containers using gentle pressure on belly.
- In catfish, clove oil may be applied as anesthestic as dip treatment before dissecting the fish after 6-8 hrs of Ovaprim administration and testis taken out carefully. The testis are weighed to nearest miligram and macerated in a mortar pestle over boiling silk cloth, using 0.9% NaCl which is added drop by drop. The middle milky layer contains milt. Further, it needs to be diluted in 0.9% NaCl in ratio 1:4 (milt: NaCl) and to be kept in ice.
- Solution of milt, extender and DMSO to be made in the ratio of 1:3.5:0.5, which needs to be kept on the ice.
- The milt solution to be filled in 0.25cc French medium straws by suction.
- The terminal sides of the straw to be sealed by PVA powder and the straws to be transferred on ice and left for 10 minutes.
- Now the straws can be transferred to LN_2 vapour filled chamber and kept again for 10 minutes.
- Subsequently these straws can be stored in LN_2 for future use. For using cryopreserved sperms for fertility trials, rapid thawing must be done immediately by removing straws from LN_2 and dipping them in 37°C water for 20 minutes.
- After 20 minutes, the straws to be taken out, wiped, cut at one end and the milt is poured over the eggs (obtained by stripping) kept in the petri-dish.
- The eggs and sperms are gently mixed for 3 minutes by slight shaking (manually).

- About 100 µl of pond water is added and mixed manually for 3-5 minutes.
- The pond water to be changed 2 times and kept for 2 hrs.
- Now this can be transferred to the incubation pools.

7.1.4. Live Germplasm Resource Centers

- Prioritize species and working partners at regional level.
- Identification of research gaps and technology intervention for individual species to develop propagation practices.
- Baseline surveys to identify abundance of wild fish, capture statistics, relative contribution to capture (quantity & value), locality for brood collection and rehabilitation programme.
- Development of broodstock through wild collection to establish effective breeding population of species. Approximately 200-300 broodstocks comprising equal numbers of males and females to be raised; number can vary depending upon biology of fish species.
- Inventory of data pertaining to life history traits/biological parameters and molecular genotyping of tagged individuals in captivity.
- Technological intervention for breeding of captive broodstock.
- Rearing protocols (Nutritional requirement, wherever not known) from larvae to fry to fingerling will need optimization.
- Conservation: Selection of Protected Sites for propagation assisted rehabilitation and stock enhancement.
- Protocols for cryopreservation of sperm and embryonic cells for long-term storage of germplasm or utilization in breeding programmes and utility for enhancing breeding population.

7.1.5 Suggested Readings

Diwan, A. D., S. Ayyappan, K. K. Lal and W. S. Lakra, 2010. Cryopreservation of Fish Gametes and Embryos. *Indian Journal of Animal Sciences*, 80(4)Supplement 1: 109-124.

8 MANAGEMENT OF GENOMIC RESOURCES

With the advancement in molecular marker and other technologies, nucleic acids are now routinely extracted, purified and used in PCR and non-PCR based assays. These advancements coupled with risks of loss of the significant genetic resources throughout the world, have led to the need of a detour for conservation of the genetic resources in the form of nucleic acids popularly referred as "DNA Banking".

DNA banking is an emerging technique in genetic resources conservation. Extracted DNA, DNA or RNA samples are considered as genetic resources and they are now routinely extracted and conserved in DNA banks. It is often argued that DNA banking is an unrealistic option for germplasm conservation given that DNA cannot resurrect extinct species. DNA storage should still be regarded as an insurance policy rather than a replacement for conventional modes for germplasm storage. One of the most frequent forms of germplasm requested from Gene banks is DNA material (de Vicente, 2004). If such is a need for provision of DNA material for molecular genetic research, then DNA banks have their raison d'ẽtre.

8.1 Genomic resources

Article 2 of the CBD defines the term "genetic resources" to mean "any material of actual or potential value of plant, animal, microbial or other origin containing functional units of heredity". In the same logic, Genomic Resources are whole or parts of the genome (DNA) or its functional units (RNA) of actual or potential value. Whereas, genetic resources can actually give rise to whole organisms, genomic resources can at best recover traits (directly or indirectly) of these organisms.

8.2 Need to conserve genomic resources

Current research (both cloning experiments and genome sequencing projects) generates a lot of genomic resources. These genomic resources are indispensable tools for post-genomic research, be it physiological and morphological characterization of a species or functional analysis of genes or comparative genomics or marker-assisted selection. Therefore, it is necessary to maintain an efficient system for conservation and management of spin-off DNA materials.

8.3 Deposition of Genomic Resources

Types of genomic resources that can be deposited:

- Whole genomic DNA
- Stored tissues or cryopreserved cells, as source of DNA
- Cloning vectors, expression vectors

- Cloned genes, promoters fused to reporter genes
- Sub-genomic, cDNA, EST Clones,
- Microsatellite/Repeat enriched libraries
- BAC Libraries
- Cloned DNA exclusively for the repository
- Recombinant vaccines
- Gene construct for which expression has been demonstrated
- Probes for specific/important genes

8.4 Depositor

Only the Principal Investigator of the project can deposit the material (i.e. co-investigators and research associates need PI's consent; A research student needs the consent of the supervisor/ guide). The Repository shall not be party to any dispute once the material is deposited. Disputed material shall be removed from the distribution pipeline/repository if deemed necessary.

8.5 Storage Methodologies

- Total DNA 1–2 years at 4°C; 4–7 years at -20°C and more than 5 years when stored at -70°C
- ESTs, full-length cDNAs, BACs are maintained in 96-well or 384 well microplates at -80°C
- cDNA clones as plasmid DNA at -20°C
- Lyophilized DNA for long-term storage
- Ambient temperature storage
- **Tissue**: Frozen at –80°C, Ethanol preserved stored at 4°C.
- **Genomic Libraries (Amplified: genomic/cDNA):** Stored as Glycerol stock at –80°C in different aliquots.
- **Total DNA isolate**
  - Long term: in ethanol at –20°C
  - Short term: buffer at –20°C
- Clones with specific gene/DNA fragments
  - Long term: Recombinant plasmid in host cell in culture
  - Medium term: with glycerol at –80°C
  - Short term: buffer at –20°C
- **DNA material:** Lyophilized & stored at –20°C
- **Cells lines / gametes:** Cryopreserved as per specific protocol.
- **Voucher specimen:** Formalin/Isopropyl Alcohol

8.6 Quality and quantity of the material:

- Genomic DNA
  - A_{260}/A_{280}=1.7-2.0; A_{260}/A_{230}>1.5
  - Agarose gel electrophoresis image with l marker
  - >100 µg genomic DNA [MINIMUM, at which it is used only for repository and not for distribution]
- Library
  - <5% empty vectors
  - Free from all sorts of contamination
  - 384 well plate (BACs), 96 well plate (cDNA, shotgun, EST)
  - 1 library @192 plates or @ one filter (36,884 spots)
  - Amplified library @10^{10} pfu/ml; minimum 10^{6} pfu/ml

8.7. Accompanying data/Voucher information

- Name of the fish, species, common name, Genus, Family, TaxID, Diagnostic characters, Genebank Accession No.
- Photograph or Voucher specimen.
- Extraction procedure, DNA dissolved in buffer used for dissolving nucleic acid & concentration in ng per microL
- Importance of the fish
- Reasons for choice of the accession
- Source of the biological material
- MTA form for Fish material
- Any information on ITK
- Any publications (pl. attach a copy)
- IP rights/patents, etc.

8.8 Intellectual property

- Even after submission of the material, the intellectual property rights (if any) shall remain with the depositor.
- The Repository is FREE to distribute the material to anyone it may deem appropriate
- The recipient will be made aware about all the clauses put forth by the depositor, for the use of material.
- Intellectual property rights over any significant modifications of the distributed material rest with the subsequent researchers and not with the original depositor; however, the IPR on the original submitted material shall remain with the depositor.

- In case of any commercial exploitation, the agreement shall be between the depositor and the actual user (who has generated the said commercial potential) for equitable benefit sharing as governed by the rules and regulations of the respective organizations of the researchers under the framework of the Biological Diversity Act, 2002 and Biological Diversity Rules, 2004. The Repository shall only be a facilitator of the storage and distribution of the material UNLESS specified otherwise.

8.9 Material Transfer Agreement

The deposition of the material is incomplete without signing an MTA.

8.10. Documentation and database

Every successful deposition gets a unique accession number. The number shall remain constant any downstream distribution and during the storage. It is by this number, a genomic material deposited shall be accessed, viewed, requested for, and distributed.

8.11 Suggested readings

Fears, R., 2007. Genomics and genetic resources for food and agriculture. Commission on Genetic Resources for Food and Agriculture. *FAO Background Study Paper No. 34*, p. 47.

de Vicente, M. C. and M. S. Andersson, 2006. DNA banks- providing novel options for genebanks? *Topical Reviews in Agricultural Biodiversity*, International Plant Genetic Resources Institute, Rome, p. 84.

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9 SECURITY AND SAFETY IN GERMPLASM REPOSITORIES

During the genebank or laboratory handling, procedures should have a risk management strategy in place which includes *inter alia* measures against power cut, fire, flooding and earthquakes. A genebank should follow the local Occupational Safety and Health requirements and protocols where applicable. A genebank should employ the requisite staff to fulfill all the routine responsibilities to ensure that the genebank can acquire, conserve and distribute germplasm according to the standards. Achieving a gene bank's goal of acquisition, conservation and distribution of germplasm requires that adequate procedures and equipments for germplasm handling are in place, and properly trained staff is employed to carry out the required work and to guarantee the security of the genebank. Gene bank planners and operators must aim to minimize installation and operational costs without jeopardizing cold room safety laws and codes of practice. Frequent surveys and alarm system tests are recommended.

9.1 Power backup

- Failure in power supply can result in complete loss of genebank accessions. Consideration should be given to the provision of a backup generator that automatically starts when the main power supply fails. This will require stockpiling adequate amounts of fuel to run the generator during power cuts.
- Lighting must be available at all times, with light switches located within the cold room area. Both normal and emergency – mains and low voltage (battery) – circuits are recommended. A single low wattage safety light (battery powered) can be kept on permanently in each cold room and positioned above the entrance, the wiring to be done with fire resistant cable. Emergency lighting must also be provided in the machinery room to allow emergency repair work to be undertaken.
- All electrical fittings and electrical motors are potential fire hazards and must be designed for low temperature service and ideally, protected by separate fuses. An exterior red warning lamp, wired in parallel with the cold room lighting circuit, should be fitted to indicate when the cold room is occupied.
- Hand lamps, portable power tools and frost protection equipment must be low voltage (50 V) and all metal work should be properly earthed.

9.2 Training

- Active genebank management requires well-trained staff, and it is crucial to allocate responsibilities to suitable competent employees.
- A genebank should, therefore, have a plan or strategy in place for personnel, and a corresponding budget so as to guarantee that a minimum of properly trained personnel are available to fulfill the responsibilities of ensuring that the genebank can acquire, conserve

and distribute germplasm. Access to specialists in a range of subject areas is desirable, depending on the mandate and objectives of each individual genebank. However, staff competence and training will depend on specific circumstances.

- Staff should have adequate training acquired through certified training and/or on-the-job training. Capacity development needs should be assessed from time to time to update with new advances. Genebank personnel should be aware of and trained in safety procedures to minimize risks to the germplasm.

9.3 Security system

- Storage facilities should be protected with standard security facilities such as fences, alarm systems, security doors and any other system that helps to shield the genebank from burglars and other intruders.
- An audible personnel alarm, low voltage type, should be provided for cold room staff. Fire alarm switches should be also located near fire exit points so that a person can raise the alarm but still have a direct escape route.
- The alarm, a gong or siren, must have a distinctive tone and should be located near security guard room. The alarm system should be controlled by a key-switch in the charge of a senior security officer.
- It is recommended that this communication link to a central office or security guard be used to record when staff enters or leaves a cold room facility.

9.4 Protective clothing

- Protective clothing should be provided and used in the storage area. The in-store working period should be kept to a minimum.

9.5 Environment

- The genebank facilities should be constructed so as to withstand natural disasters, such as hurricanes, cyclones, earthquakes, or floods that are known to occur in the location where the genebank has been built.
- A genebank should therefore, implement and promote systematic risk management that addresses the physical and biological risks in the every-day environment to which the collections and related information are exposed.
- Additional ventilation must be provided if the cold room respired carbon dioxide gas concentration exceeds 0.5%.
- The gas detection apparatus may be installed for direct and indirect refrigerants. For safety, and security, only authorized personnel should be allowed within the cold store.

9.6 System of refrigeration

- Refrigeration will be reliant on electrical power and it is therefore, necessary that the power supply is adequate and reliable. The power generators should be controlled with AMF panels.

- Ultrafreezers should be supported by adequate carbon dioxide backup system to maintain low temperature in case of machinery failures, such as freezer or power generators.

9.7 Monitoring devices

- Fire alarm is required in the genebank. Most fires begin from faulty electrical circuits and periodic checks should be made on the electrical circuitry to ensure compliance with safety standards.

9.8 Firefighting system

- It includes extinguishers and fire blankets. For areas affected by thunderstorms, a lightning rod should be fitted to the genebank. The selection and use of 'first stage' fire lighting equipment should be made in consultation with local fire brigades and staff instructed accordingly. In particular it should be noted that synthetic foams (within the cold room walls, ceiling and floor) will emit both toxic vapours and fumes, and thus such a fire should only be tackled by trained personnel wearing breathing apparatus. Water reservoirs may be required in rural areas. Fire extinguishers are essential but must be selected cautiously.
- Danger exists with water (electrocution) and carbon dioxide (asphyxiation), whereas certain chemicals are poisonous. Dry chemical powder extinguishers are to be recommended and must be positioned near access and emergency doors. Fire-proof blankets and buckets of dry sand can be used to smother small fires, while automatic water sprinkler systems outside the cold room can protect the cold room exterior.
- A major gene bank should have one set of breathing apparatus, with a self-contained compressed air supply, and arrangements must be made for expert training and fire drills. In addition, attention to methods of escape from cold rooms is more important than in most other types of building, because of their construction and low temperature. In large stores, emergency doors should be installed to give the widest field of escape via at least two routes. The machinery room should have an alternative exit point through a fire resistant external door.
- Emergency exits should, whenever possible, lead directly to the outside at ground level. External handles can be removed to improve security, with doors secured on the inside for quick release. The main cold room door should have a safety latch designed to prevent staff from being locked in.
- If possible it might be worth considering the possibility of giving accessions a priority rating in case rapid removal from a cold room is necessary, where a thought should be given to the position of priority accessions within the store and type of storage container used for them. Alternatively, temporary low temperature storage arrangements may be made.

9.9 Hazardous chemicals and safety precautions

The laboratories that use techniques of molecular biology, sample and specimen storage, often use chemicals which are hazardous in their native or product form. These chemicals can be potential human risk in long and short term. Therefore research personnel should be aware of such hazards and should follow safety precautions (*Box ix*).

Box ix

Chemicals

Phenol - Phenol is highly corrosive to the skin and readily absorbed. It can pose a severe health hazard and should be handled with extreme caution. It can create severe burns. Phenol should always be used in a fume hood

Acrylamide - It is potential neurotoxin and should be handled with care. In its powder form, the monomer is extremely dangerous because the dust can easily become airborne and enter the respiratory system.

Ethidium bromide - It is a potent mutagen/carcinogen. Ethidium bromide has also been used extensively to reduce mitochondrial DNA copy number in proliferating cells. Always wear gloves when using potentially hazardous chemicals and never mouth-pipette them.

If you accidentally splash any of these chemicals on your skin, immediately rinse the area thoroughly with water and inform the instructor. Discard the waste in appropriate containers.

Ultraviolet Light

Exposure to ultraviolet light can cause acute eye irritation. Since the retina cannot detect UV light, you can have serious eye damage and not realize it until 30 min to 24 hours after exposure. Therefore, always wear appropriate eye protection when using UV lamps.

Disposal of Buffers and Chemicals

1. Any uncontaminated, solidified agar or agarose should be discarded in the trash, not in the sink, and the bottles should be rinsed well.
2. Any media that becomes contaminated should be promptly autoclaved before discarding it. Petri dishes and other biological waste should be discarded in Biohazard containers which will be autoclaved prior to disposal.
3. Organic reagents, e.g. phenol, should be used in a fume hood and all organic waste should be disposed of in a labeled container, not in the trash or the sink.
4. Ethidium bromide is a mutagenic substance that should be treated before disposal and should be handled only with gloves. Ethidium bromide should be disposed of in a labeled container.
5. Dirty glassware should be rinsed, all traces of agar or other substance that do not get cleaned in a dishwasher should be removed, all labels should be removed (if possible), and the glassware should be placed in the dirty dish bin. Bottle caps, stir bars and spatulas should not be placed in the bins but should be washed with hot soapy water, rinsed well with hot water, and rinsed three times with distilled water.
6. Do not use plastic or polycarbonate containers, test tubes, pipettes, etc., with phenol and or chloroform. Instead use polypropylene or glass with these organic compounds. Make sure to use gloves, goggles and lab coats when handling these chemicals.

9.10 General

- Infrastructure related standards:
  - Monitoring devices: Environment data sensors for freezers and cold rooms
  - Security system: CCTV surveillance and restricted entry systems
  - Environment/Complaint system of refrigeration: Non-electric backup systems for ultrafreezers
  - Incinerators: For waste disposal
  - Fume Chambers with appropriate scrubbers
  - For every project handling acquisition of animal germplasm, necessary ethical clearance must be taken.
- Devices to insulate against water infiltration from the outside should be installed
- The effectiveness of the cooling system can be monitored by weighing the condensate melted during successive defrost cycles. The amount of moisture entering a cold room fitted with a dehumidified air-lock should be small. It is recommended that the defrost cycle be time switch initiated and stopped by a thermostat to keep the heating period to a minimum.
- High and low temperature alarm thermostats are also desirable.
- Each cold room should be provided with two independent refrigeration systems. Allowance must be made for any additional refrigeration load-such as sorption-type air dehumidifiers. The independent refrigeration systems should be run alternately, for monthly periods, to ensure they remain in good working order.
- All the genebank staff should be trained not only for technical issues but also for safeguarding genebank during any disasters, natural or manmade.
- Unauthorized entry to genebank facilities can result in direct loss of material, and also jeopardize the collections through inadvertent introduction of pests and diseases and interfere in management systems.

9.11 Suggested readings

Engels, J. M. M. and L. Visser, 2003. A guide to effective management of germplasm collections. *IPGRI Handbooks for Genebanks No. 6*, IPGRI, Rome, p. 172.

Annexure Ia

List of items and equipments for exploration and collection of FGR

Survey / collecting items	Global Positioning System (GPS), digital camera with additional memory card, binocular, magnifying glasses with LED lights, handheld microscope, digital vernier caliper and portable balance, Electrofishing equipment. Insulated boxes, Dry ice or local ice filling, Haversack/kitbag, envelopes for scales, cloth bags, polythene bags, aluminum & tag labels, rubber bands, packing tape, Jute or cotton thread (thick and thin), secateurs, scissors, knife, torch light, measuring tape, passport data book, field note book, pencil, ballpoint pen and permanent marker, cards for tags, tagging gun with tags, Extra dry batteries, Graph paper sheets, measuring tape. Formalin, MS-222, Cryobox with cryovials prefilled with 95% ethanol, Disposable syringe (2ml). Life Jackets, when going for sampling/surveys in deep rivers/water currents Others: Stapler, candle, matchbox, water bottle, hunter shoes, hand gloves, waist pouch, rain suit (shirts, trousers), rucksacks, sunglasses, etc.
Reference material	Lap-top and accessories with Internet facility, political/geographical-map, vegetation/climate map, list of rest-houses/lodges, hotels, resting/ stay places and list of local contacts (phone, fax, e-mail).
First aid box	Anti-malaria pills, anti-allergen tablets, pain killers, anti- amoebic and anti-diarrhoeal tablets, mosquito repellent, antifungal/antibacterial/antiseptic creams or lotions, cotton-packs, band-aid, dettol, dressing gauze, water-purifying tablets, etc.

Annexure Ib

Survey of Fish Genetic Resources

Date:…… Collector:……..

Locality/Area:…… Block:…… District:…… State:……

Latitude:…… Longitude:…… Altitude:……

I Habitat Parameters:

1. Physical (Annexure Ia):

1.	**Name of the river /Stream/reservoir/ wetland**				
2.	**Name of the site/ sub site code**				
3.	**Altitude (MSL)**				
4.	**Habitat type**	Mid Channel	Shoreline		Others
5.	**Width of wet area**				
6.	**Depth (M)**				
7.	**Substrate (%)**	100-80%	80-60%	40-20%	20% or less
(ı) (ıı) (ııı) (ıϖ) (ϖ) (ϖı)	**Boulders (5)** **Cobbles (4)** **Pebbles (3)** **Gravels (2)** **Sand (1)** **Silt & Clay (0)**				
8.	**Water Colour (Green/Clear/Brown/Other)**				
9.	**Aquatic vegetation**	100-80%	80-60%	40-20%	20% or less
(ı) (ıı) (ııı) (ıϖ)	**Floating** **Submerged** **Emerged** **Other**				
10.	**Riparian Vegetation (Abundance)**	100-80%	80-60%	40-20%	20% or less
11.	**Water Velocity (m/s)***				

* use simple float and stop watch.

2. Physico-chemical (Annexure II)

Parameters	Site 1	Site 2	Site 3
(ι) Temp (A)° C			
(ιι) Temp (W)° C			
(ιιι) pH			
(ιϖ) Turbidity			

II. Fish Biodiversity (macro-level information)

1. Primary information:

Name of sampling site: ……..…… Date of collection: ……..……

River/ Stream name……..……..

Collection source: ……..…… (Landing Center/Experimental Fishing)

Sampling Area covered…….....m^2/km^2 Duration of Fishing (Hrs).....

Time of fishing …… Total catch (kg).....

No. of species ……..…..

Name of Fish (Genus and species)	Local name(s)	Order/ Family/ Subfamily	Total Number	Approx. Wt. (kg)	Proportion to total Catch	Gear used

For exotic/alien Fish Species

Name of Fish (Genus and species)	Local name(s)	Order/ Family/ Subfamily	Total Number	Approx. Wt. (kg)	Proportion to total Catch	Gear used

2. Secondary information:

Name of the fish	Largest size know(cm)	Breeding Information (month)	Known Economic Importance (Food/Ornamental/ game)	Local value	Commercial value (Rs/ Kg)

III. Fish Species Identification Sheet (Using systematic keys)

1. Key characters diagnosis:

Name of Fish	Species 1	Species 2	Species 3	Species 4	Species 5
Order					
Family					
Genus					
Taxonomic Key Followed					
Page number/Diagnostic Observed					
Any Observed Deviation					

2. DIAGNOSTIC TAXONOMIC CHARACTER (DESCRIPTORS)

Morphological and Meristic Characters

Finfish

A. Morphometric Descriptors of Species

No		A	B	C	D	E
I	Specimen code no.					
II	Sample Field ID					
III	Morphometric Characters					
1	Total length (TL)					
2	Total body weight (g)					
3	Standard length (SL)					
4	Head length (HL) (Lateral)					
	Head Length (Dorsal)					
5	Head depth at eye					
	Head depth at occiput					
6	Head width (HW) (widest)					
	Head width (HW) at eye					
7	Snout length (tip of snout to nostril)					
8	Inter nostril width					
9	Inter orbital width					
10	Eye diameter (ED)					
11	Body depth (BD) at dorsal fin origin					
	Body depth at anal fin origin					
12	Mouth gape width (For *Garra* – Disc width length)					
13	Body Colour					
14	Mouth position					
	(i) Terminal					
	(ii) Sub terminal					
	(ii) Inferior					
15	Lips					
	(i) Thick					
	(ii) Thin					

No		A	B	C	D	E
16	Lateral line complete/ incomplete					
17	Lateral line scale					
18	Circumferential scales					
19	Circumpendicular scales					
20	Pre-dorsal scales					
21	No. of Dorsal fins D1/D2					
22	Dorsal fin base length					
23	Dorsal fin height D1					
24	Dorsal fin height D2					
25	Pectoral fin length					
26	Pelvic fin length					
27	Anal fin length					
28	Caudal fin peduncle length					
29	Caudal fin peduncle depth					
30	Length of origin of dorsal fin to caudal base					
31	Pre dorsal length					
32	Pre pectoral length					
33	Pre pelvic length					
34	Pre anal length					
35	Body shape Elong / comp (E/C)					
36	Caudal fin shape					
37	Any Other observation					

B. Meristic parameters

1	Dorsal Fin (First dorsal fin ray oceous, spinous or simple, serrated posterior or anterior)					
	(i) Simple					
	(ii) Branched					
2	Pectoral Fin					
	(i) Spine					
	(ii) Rays					

3	Pelvic Fin					
	(i) Spine					
	(ii) Rays					
4	Anal Fin					
	(i) Spine					
	(ii) Rays					
5	Caudal Fin					
	(i) Spine					
	(ii) Rays					
6	Gill rakers Ascending + Descending					
7	Dentition No. of patches & vomerine/ palate Villiform/molariform					

Shrimp/Prawn

A. Morphological descriptors of species:

No.	Attribute	A	B	C	D	E
	Species name and Specimen code no					
	Sample Field ID					
1.	Shape of the body and colouration					
2.	Carapace/Cephalothorax- shape and structures					
3.	Abdominal segments or somites (number and shape)					
4	Tail fan (telson + uropod) structure					
5	Mouth parts					
6	Number, type and structure of appendages and sexual dimorphism					
7	Rostrum shape, structure, rostral teeth					
8	Appendix masculine in male shrimps on the endopods of the first pair of pleopods- its structure					
9	Structure of thelycum in females					
10	Appendix interna					
11	Petasma- types					
12	Dactyl of the third maxilliped- its length compared to the length of the propodus					
13	Larval stages- types (Nauplius/Protozea/Mysis/Zoea/ Ahina/ Phyllosoma/Puerulus/Megalopa) and number per stage					

No.	Attribute	A	B	C	D	E
14	"Berried" condition (females carrying eggs) or spawning females					
15	Structure of spermatophore in shrimps with open and closed thelycum					

Molluscan Descriptors

Sl. No.	Descriptor	Details
1	Shell	Present/Absent
2	Shell	Outside/inside the body
3	No. of Shell pieces	One/two valves/8broad plates
4	Shape of the shell	Flat/cup-shaped/coiled/coiled with complex septa and sutures/conical
5	If coiled, dextral/sinistral	
6	Colour of Shell	
7	Texture of the shell	
8	Shape of the Umbo of shell	
9	Shape of the hinge ligament of shell	
10	Hinge teeth number	
11	Inner surface of the shell in bivalves	Coarse/smooth/pearl-like (lustrous)/dull
12	Operculum	Present/absent
13	Movement	Free-swimming/sedentary/slow-moving/attached
14	Body Symmetry	Bilaterally symmetrical/asymmetrical
15	Body	Had undergone torsion/detorsion & coiling
16	Position of mouth and arms	
17	Byssus threads	Present/Absent
18	Nature of mantle	
19	Foot	Present/Absent
20	Position of Foot	
21	Shape and size of foot	
22	Foot is	Muscular/Flat (creeping sole)
23	Distinct Head region	Present/Absent
24	Eyes	Present/Absent
25	Position of the eyes	Base of the tentacle/Tip of the tentacle
26	Tentacles (small as in gastropods)	Present/Absent
27	Arms & tentacles (modified foot)	Present/Absent

Sl. No.	Descriptor	Details
28	Number of tentacles	
29	Suckers in tentacles	Present/Absent
30	Shape & size of Arms & tentacles	
31	Ctenidium/Gills	Present/Absent
32	Gills	Monopectinate/Bipectinate
33	Secondary gills	Present/Absent
34	Secondary gills	Pallial gills/pulmonary sac/Nuchal lobes/Pseudepipodia
35	Osphradium	Present/Absent
36	Scraping radula (lingual ribbon)	Present/Absent
37	Number of teeth in each transverse row on radula	Seven/three/two
38	Proboscis	Absent/Present & well developed
39	Adductor muscles	Present/Absent
40	Position of attachment of adductor muscles on the shell	
41	Ganglions/aggregation of neurons	Developed/not developed
42	Poison gland	Present/Absent
43	Reproductive System	Dioecious (sexes separate)/ hermaphrodite
44	Sexual dimorphism	Present/Absent
45	Copulatory organs	Present/Absent
46	Hectocotylus arm	Present/Absent
47	Ink sac	Present/Absent
48	Siphon	Present/Absent
49	Lateral fins (parapodium)	Present/Absent
50	Fertilization	External/internal
51	Eggs	Calcareous/gelatinous & soft/microscopic/elongated/large
52	Larval stages	Trochophoe/Veliger/Pediveliger/Platigrade/D-shaped/Glochidium
53	Larval movement	Free-swimming/planktonic
54	Larval cycle	Free-living/Parasitic
55	Habitat	Marine/brackish water/freshwater/terrestrial/ amphibious

Annexure Ic.

1. Stream/ River Habitat Assessment:

Components of Physical Habitat Characterization

A. Habitat type	Description
Shoreline	A shore or shoreline is the fringe of land at the edge of a large body of water, such as an river, stream, lake or reservoir.
Pool	Still water, low velocity, smooth, glassy surface, usually deep compared to other parts of the channel.
Riffle	Riffles - Fast, turbulent flow with exposed substrate material. Moderate slope at points of channel constriction
Others	Dry Channel (DR) : No water in the channel Falls (FA): Free falling water over a vertical or near vertical drop into plunge, water turbulent and white over high falls. Cascade (CA): Water movement rapid and very turbulent over steep channel bottom. Most of the water surface is broken in short, irregular plunges, mostly whitewater. Rapid (RA): Water movement rapid and turbulent, surface with intermittent whitewater with breaking waves.
B. Stream type (Sinuosity index)	Sinuosity or sinuosity index (SI) is a measure of deviation of a path length from the shortest possible path. For this reason, bedrock streams that flow directly down slope have a sinuosity index of 1, and meandering streams have a sinuosity index that is greater than 1.it is given by the ratio of: SI = Actual channel length / shortest channel length
C. Substrate	Substrate refers to the bottom material of a water body. The composition of the substrate determines the roughness of stream channels, and roughness has a large influence on channel hydraulics (water depth, width, and current velocity) of stream habitat.
Boulders	Particle size > 256 mm
Cobbles	Particle size 64- 256mm
Pebbles	Particle size 16- 63 mm
Gravels	Particle size 2- 15 mm
Silt & Clay	Particle size < 0.059 mm
Sand	Particle size 0.06- 1mm
D. Cover	Cover includes boulders and logs, aquatic vegetation, water turbulence, and concealing water depth (Armantrout, 1998). It can be examined by the water and the banks within the 10-m segment of stream/ river for the following features: filamentous

Annexure II

Format for Documentation of the Indigenous Knowledge

Date ..... Principal Investigator ..... Field Investigator .....

Locality/Area/Village: ..... River/ stream .....

Block/Taluka: ..... District: ..... State: .....

Latitude: ..... Longitude: ..... Altitude: .....

Indigenous knowledge of fishing communities about:

I. Traditional gears/Indigenous Fishing practices

- Name of gear/devise:
- Basic design and material used for making:
- Specific rationale/purpose behind its use:
- Used by the fishermen/community since when:
- Used in any particular season or throughout the year:
- Used in any particular type or stretch of river/water body or used in all parts of water body:
- Used in any particular locality/village or on a wider geographical scale in the state/driver basin:
- Used by selected/restricted group/communities or widely used by fishermen in the area:
- Good quality photographs from 2-3 angles:
- Any other relevant information/experience provided/narrated by the fishermen/women:

II. Availability of fish diversity:

Location–specific Indigenous knowledge on specific locations for:

- Availability/presence of specific species at specific locations of a river/ stream
- Distribution of various fish species in various stretches
- Spawning grounds/aggregations of various/selected species
- Juvenile habitats of various/selected species
- Good quality photographs from 2-3 angles of the specific location, etc..
- Any other relevant info/ experience provided/narrated by the fishermen/women:

II. Population level knowledge on biology of fish species

Location–specific Indigenous knowledge on specific biological processes like:

If yes,

- Natural fluctuations in population size of locally important species

  If yes, details on:

  In which species

  since when

  any perceived reasons, etc.

- Habitat specificity of locally important species

  If yes, details on:

  which species

  which specific habitat

  GPS coordinates of specific habitat

  brief description of the habitat

  any perceived reasons, etc.

- Dietary preferences of locally important species

  If yes, details on:

  In which species

  what kind of specific dietary preference

  brief description of the diet as told/ seen

- Any insights on spawning and mating behaviour of any species

  If yes, details on:

  In which species

  under what specific conditions

  a brief description as told/seen

- Good quality photographs wherever possible
- Any other relevant info/ experience provided/narrated by the fishermen/women:

III. Ecological processes/ environmental linkages

Location–specific Indigenous knowledge due to long-term association with a specific area

- Migratory movements of fishes in the selected rivers/ streams

  If yes, details on:

  In which species

  in which stream/river

  under what specific conditions

  a brief description as told/seen

- Any specific knowledge about timing of a host of significant biological events/processes. (i.e. any rare/unique knowledge about biological and ecological processes/events related to aquatic life across time scale)
- A brief description as told/seen
- Any specific knowledge about linkages between multiple species between ecological process
- A brief description as told seen
- Good quality photographs wherever possible
- Any other relevant info/experience provided/narrated by the fishermen/women

IV. Folk Taxonomy and Systematic

- Any specific Indigenous/traditional naming systems of fish species
- Any pattern of how the different types of fishes are grouped in folklore
- Fish species in which indigenous naming system is more advanced (i.e. specific names given for various stages of a species).
- People's rationale behind the indigenous names
- Any other relevant information/experience provided/narrated by the fishermen/women:

V. Traditional/Customary resource management system/practices

Any specific/ unique indigenous system by which local people use, allocate, transfer, and manage their fishery resources in the area/ locality

1. Name of system:
2. Specific rationale/ purpose behind its use:
3. Used by the fishermen/community since when:
4. Used in any particular season or throughout the year:
5. Used in any particular type or stretch of river/water body or used in all parts of water body:
6. Used in any particular locality/village or on a wider geographical scale in the state/driver basin:
7. Used by selected/restricted group/communities or widely used by fishermen in the area:

Annexure III

Indian Council of Agricultural Research

MATERIAL TRANSFER AGREEMENT

For Multilateral/Bilateral Exchange of Germplasm for Research.

We (name of person and institution) .....of (address)..... requested for germplasm MATERIAL, of the following fish specified below from/through the .... (indicate focal point of respective country).

Fish Species Names : Germplasm Identity Number

Name of Fish : **(Accession Number) and Permit Information**

Scientific Name : .....

Common Nsme : .....

Local Name : ..... (language).

We agree to abide by the following terms of the MTA:

i. That the transfer of GERMPLASM is strictly for (a) research, captive propagation for research or training purpose.

ii. Not to claim ownership and Intellectual Property Right (IPR) over the MATERIAL(S) received/accessed including its related information.

iii. Not to use the MATERIAL(S) for commercial purposes or profit making whatsoever.

iv. Not to distribute or transfer sample(s) of the MATERIAL(S) to any other party, except those directly engaged in research without prior written approval of the Department of Agricultural Research and Education. Government of India.

v. To handle the derivatives of the material received/accessed as per IPR laws of the exporting/ importing Nations. Nevertheless, the benefits accruing from commercialization of derivative(s) will be shared appropriately with the provider country.

vi. To acknowledge explicitly the name/original identity and source of the MATERIAL(S), if used directly or indirectly in all research publication(s) and to send one copy of each publication to the provider/source of the MATERIAL(S).

vii. Importer/Indenting country's bio-safety and any other related hazards due to release of Genetic Material is entirely responsibility of Importing/Indenting Organization/ Indentor.

a. Export of Germplasm from India can be permitted provided the importer gives assurance that The material will not be used for any other purpose besides the specified one as the objective of import.

b. Research on gene manipulation/ selective breeding programme for genetic improvement and development of commercial product thereof, will not be undertaken without consent of Indian authorities and modalities of undertaking such work alone or with Indian agency will be worked out case by case basis.

c. The live or dead/ DNA or tissue or any other form that can be used to retrieve whole DNA/ fragment or sequence or any other genetic information, will not be shared with third country without consent of India

viii. If any third party will be associated with any commercial development arising out of the germplasm exported they should be made to sign the MTA with India.

ix. Concept Note of Research Project in which the material will be used and the manner in which to be used, to be provided by importer/recipient.

x. In case of any dispute between the parties to this MTA, the dispute shall be amicably settled between the parties. In case of non agreement the dispute shall be referred to the Sole Arbitrator to be appointed by the Secretary, DARE, Government of India. The Decision of the Sole Arbitrator shall be final and binding on the Parties. The Arbitration proceedings shall be governed by the Arbitration and Conciliation Act, 1996. The Arbitration proceedings shall be in New Delhi.

Annexure IV

Primers used for amplification of genes from mitochondrial DNA and microsatellite DNA markers in Fish species.

Microsatellite markers: Primer sequences for 26 polymorphic loci in three Indian major carp species *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla*

Locus	Primer sequence	Repeat motif/ Size range/No. of alleles in *L. rohita*	Repeat motif/Size range/No. of alleles in *C. mrigala*	Repeat motif/ Size range/ No. of alleles in *C. catla*
Lr27	GGCAAGCGCTGATTGTTT TACAGTCCATTGGCTCCAGA	(AC)12 N6 (AC)6	$(AC)_{14}$ 90-104(6)	-
Lr28	AAAGGAAACAGACTCACATCAGC CGCTAGCACTTTAATTTCACAGAG	(AC)18 151-189(13)	$(AC)_{13}$ 90-116(10)	-
Lr29	CCCACGCAAACTCCTGTT GGAACAAGGCCAGAGCTTTA	(AC)10	$(AC)_{6}$ 130-162(7)	-
Lr35	CAAGCTCTCAGTGACGAACA ACAGGTACCCAGGACACACA	(CA)13	$(CA)_{8}$ 100-118(7)	$(CA)_{10}$ 114-130 (6)
Lr36	CTTGTTCACTGCACAGACACC AAGGTTCAGATTGCCTCCTG	(CA)10	$(CA)_{12}$ 100-118(6)	-
Lr37	CCGAGTTCAGCATGTTCCTT TATTAGGGAGCGTCGAGTGG	(CA)23 100-156(20)	$(GA)_{15}$ $(TG)_{10}$ 135-163(14)	-
Lr41	TCCAGTCACCACATGCGTTTG GTCGATTTCATCGTGAGGCTC	(TG)16 189-217(10)	-	-
Lr45	AGGCGCAGTATTGTTTAGGC AACGCAGCCAACCTAACGTA	(CA)28 91-149(10)	-	$(CA)_{21}$ 140-162 (8)
Lro12	CAGCGCTGGACCGACACCA TGCTGCGGGTCATTAGTATTCATC	(CA)3(CT)1 (CA)15 81-151(18)	-	-
Lro14	GATCATTGCTGGGGAGTGTT CTCCGGAGACTGCTTACAGTT	(GT)12 90-130(13)	-	$(TG)_{15}$ N_{18} $(TG)_{3}$ 123-143 (10)
Lro23	GCACTCGCACACACATTCAC CAGCCGCCTGTCAGTAATCT	(CA)5(TGTA) 1(CA)6(CGCT) 1(CA)5 90-148(7)	-	-
Lro25	TCTCCAAGTGGCTGACACAC GTTCTGCAACTACTGCAACCTG	(GT)14 72-148 (19)	-	(TG)16N7 (TC)3 88-130 (13)

Locus	Primer sequence	Repeat motif/ Size range/No. of alleles in *L. rohita*	Repeat motif/Size range/No. of alleles in *C. mrigala*	Repeat motif/ Size range/ No. of alleles in *C. catla*
Lro26	GATCATTGCTGGGGAGTGTT CATCAGACGCACACACAGAG	(GT)2(C)1(GT) 17(AT)1(GT)5	(GT)25 135-171 (12)	-
Lro31	CATAATAGCAGTGGCGAGCAG AACCACCAGCACACCTTTCAC	(GT)22 151-211 (17)	-	-
Lro32	TCTCCAAGTGGCTGACACAC CCCTGTTTCTCTGTAGCTCTCTC	(GT)17	(GT)16 94-140 (8)	-
Lro33	GTCTGGACATTTCTGCACTGAG CCATCTAGAAGTCTGTTTACGG	(GT)18 100-178 (16)	-	(TG)8 131-143 (8)
Lro34	TCATGCATCTGGAATGTGAA ACGGGTTTACGAGAGAATCG	(AT)6(GTAT)2 (AT)2(GT)18 83-165 (17)	(AT)24(GT)8 133-161 (12)	-
Lro36	GTCTGGACATTTCTGCACTGAG CTTTGGTGAGCCATTGACAG	(GT)13	(GT)15 136-186 (16)	-
Lro37	TGAGATGTTCAGCAGGAGCTC GAGCGTCGAGTGGCGTTTC	(CA)3(CG)1(CA) 9(CT)2(T)1(CT)3(GA)1(CA)4 127-169 (17)	-	(CA)12 (CT)3 (AC)5 130-146 (8)
Lro39	AAGGCAGGAATGTCGCTCT TCGTCATCCTCCTCTTCCTC	(GT)14	(GT)8 101-135 (6)	-
Lro40	TCAGCCATCACACACACTCA TCGATAGTCAACCCTGTGACC	(CA)5(TA)2(CA)5	(CA)5(TA)2(CA)5 142-162 (6)	-
Lro41	CTGCAGTCCTGGTTTCTGTG GTCCACTTTGTCCAACTGAGTC	(TG)8(TC)7	(TG)11(TC)18	-
Lro43	TCTCTGCGCCTGTCTACCT TGTTTATTAAAGCACTTTCCTCA	(GA)39G (CT)2(GT)8 121-199 (16)	-	(AG)21N11 (GT)12 125-149 (10)
Lro44	CATAATAGCAGTGGCGAGCA GAACGAGGAGAGGACGAATG	(GT)16 82-148 (16)	(GT)14 123-171 (17)	-
Lro49	GCACTCGCACACACATTCAC GAGTGGCCGAAATTAGCTGT	(GT)5(AT)1(GT)4 (AT)1(GT)4(AT)1 (GT)14 102-184 (18)	(GT)10 96-120 (8)	-
Lro50	AGCCTAAACGCTGCCTTG AGTTACTGGGAGGGTGTTGC	(GT)4AT(GT)9	-	(TG)8 129-145 (9)

Mitochondrial DNA : Primers used for PCR amplification in Fish

Cytochrome b (307 bp)

L14841-5'AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA3'

H15149-5'AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA3'

(Meyer, A., 1993. Evolution of mitochondrial DNA in fishes. In: **Molecular Biology Frontiers, Biochemistry and Molecular Biology of Fishes, Volume 2, Hochachka, P. W. and T. P. Mommsen (eds.), Elsevier, London)**

Cytochrome c oxidase I (655 bp)

FishF1-5'TCAACCAACCACAAAGACATTGGCAC3',

FishR1-5'TAGACTTCTGGGTGGCCAAAGAATCA3'

(Ward, R. D., T. S. Zemlak, B. H. Innes, P. R. Last and P. D. N. Hebert, 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences,* 360: 1847-1857)

ATPase 6/8 (842bp)

L 8331-5'AAAGCRTYRGCCTTTTAAGC3'

H9236-5'GTTAGTGGTCAKGGGCTTGGRTC3'

(Lovette, I. J., E. Bermingham, G. Seutin and R. Ricklest, 1998. Evolutionary differentiation in three endemic West Indian warblers. *Auk,* 115: 890–903)

ICAR-National Bureau of Fish Genetic Resources
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