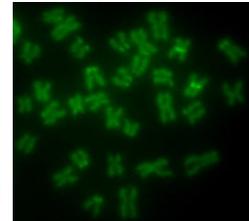
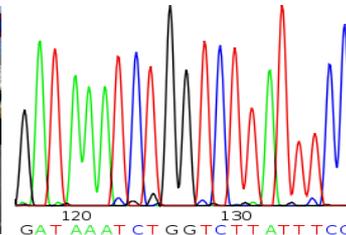
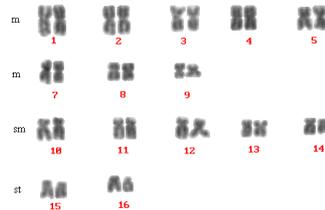


FRM-111 (1+2) "Taxonomy of Finfish"

Introduction to Modern Taxonomic Tools

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Fisheries Resource Management
College of Fisheries, Kishanganj
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Taxonomic Tools

1. Morphometrics & meristics: Morphological

2. Karyotaxonomy: Cytogenetic

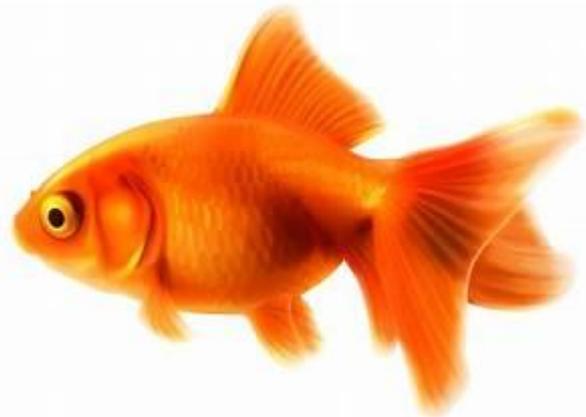
3. Protein Markers: Biochemical

4. DNA Barcoding: Molecular

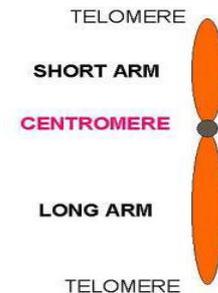
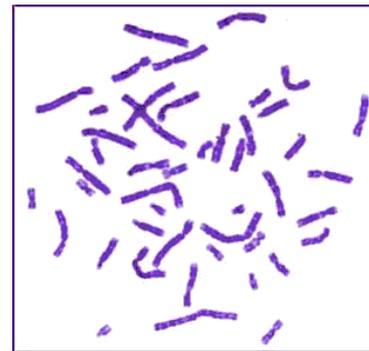
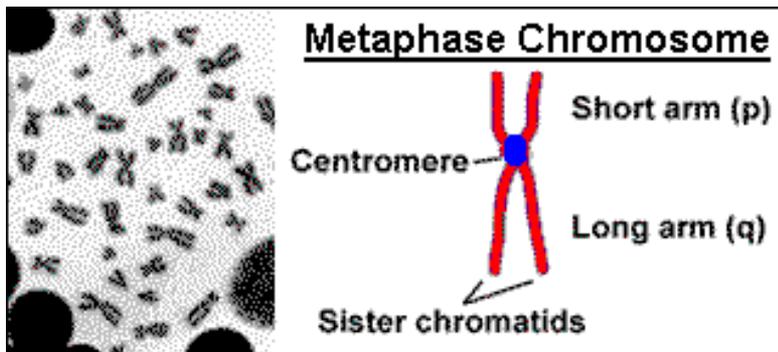
5. DNA Markers: Molecular



1. Morphologic Tool: Species Identification by morphological comparison

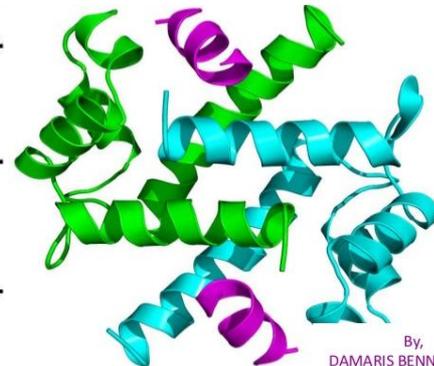
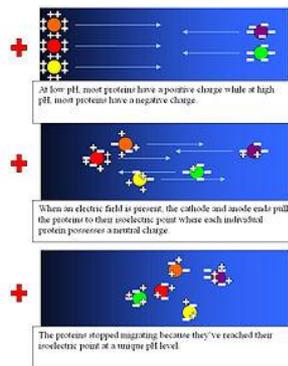
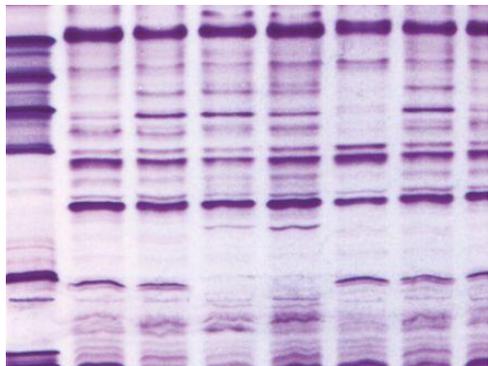


2. Karyotaxonomy: Species Identification by karyotyping



4. Protein Marker for taxonomy

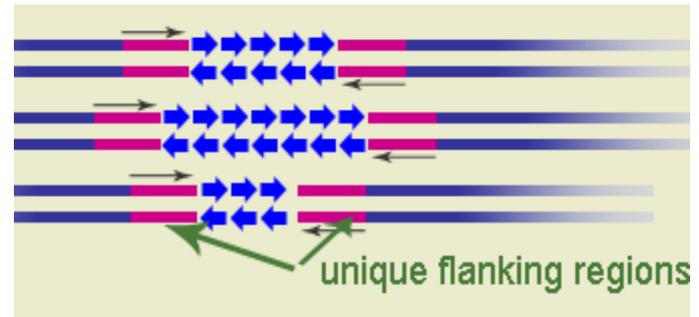
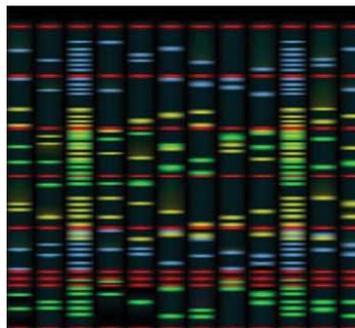
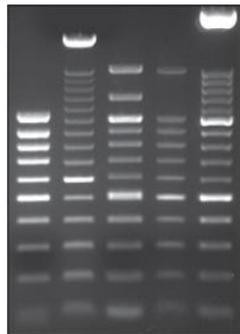
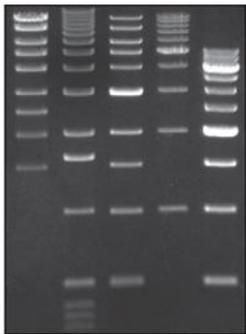
Species/stock identification by analysis of protein/enzyme variants



By,
DAMARIS BENNY DANIEL

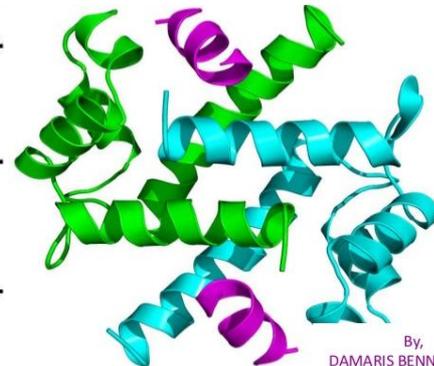
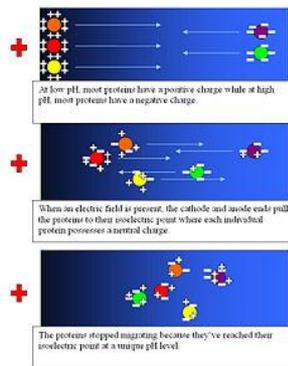
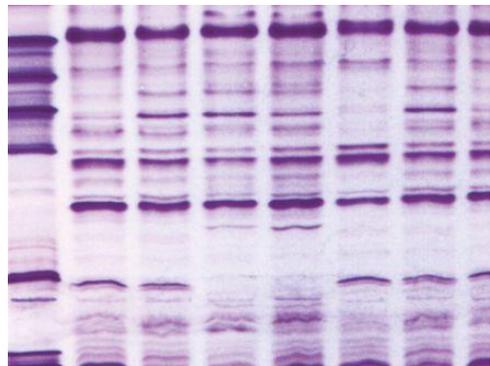
5. DNA Marker for taxonomy

Species/stock identification based on variations present in DNA



4. Protein Marker for taxonomy

Species/stock identification based on protein variants



By,
DAMARIS BENNY DANIEL

What is Marker?

- **Marker- that mark, tag or identify**
- Morphological marker (size, shape, color etc.),
- Biochemical markers: isozymes, proteins
- Molecular markers (DNA level): an allelic difference / variation at a given locus in the genome that can be observed at the level of morphology



Properties of Marker

- **Polymorphic**
- **Reproducible**
- **Easy to use and economical**
- **High-throughput**
 - **automation**
 - **combination of different markers in one reaction**



Biochemical Markers

- Biochemical markers are proteins produced by gene expression.
- Isozymes, allozymes, proteins and secondary metabolites are successful biochemical markers.

Common Protein Markers

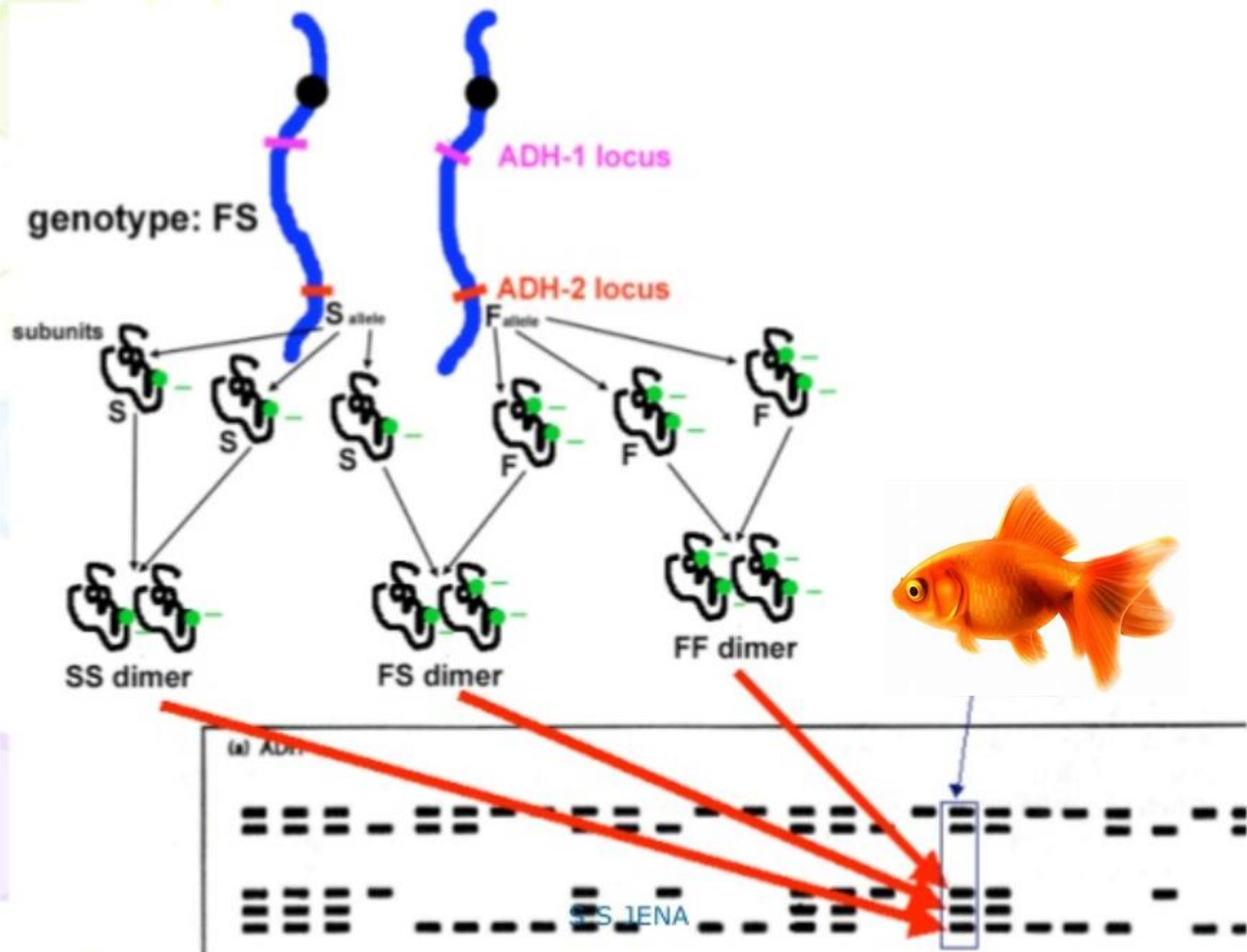
Allozyme

Isozyme

Isozymes

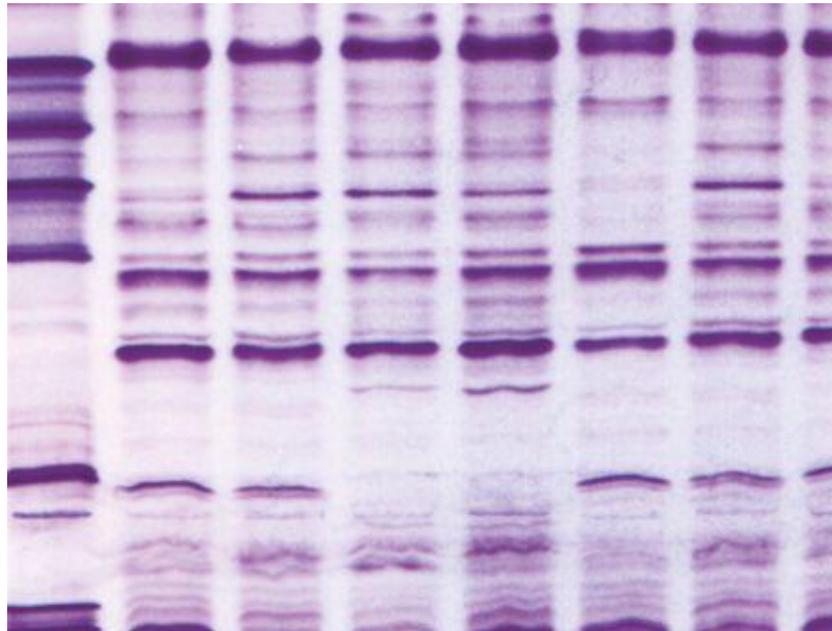
-  Multiple forms of the same enzyme sharing a catalytic activity derived from a tissue of single organism.
-  Major functions of isozymes are controlling metabolic activities of the organism.
-  Coded by same allele at **more than one gene locus.**
(gene duplication; gene families)

Isozymes were first described by **R. L. Hunter** and **Clement Markert** (1957) who defined them as different variants of the same enzyme having identical functions and present in the same individual.



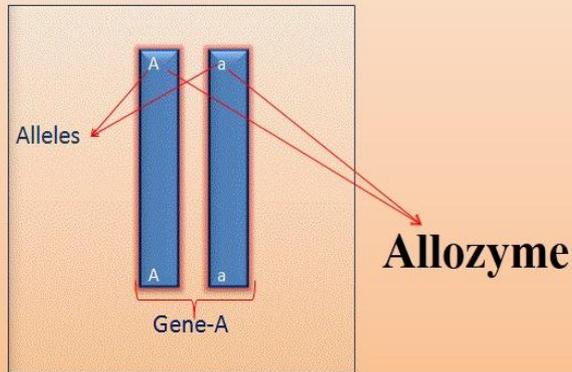
Allozyme

The term allozyme refers to only those genetically different forms of an enzyme that are produced by different alleles at the locus and often detected by protein electrophoresis.



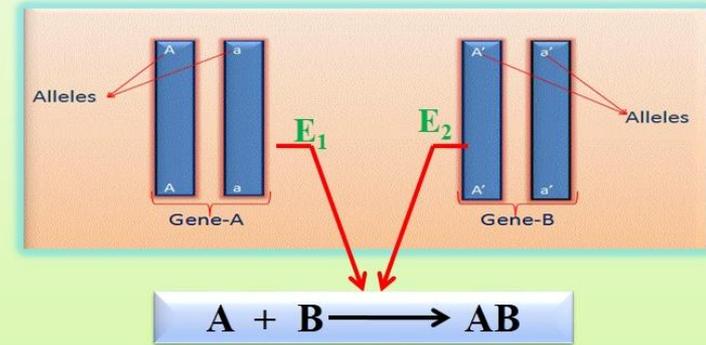
Allozyme

It represents the enzymes which is produced by the different alleles of the same gene



Isozyme/Isoenzyme

- It represent the enzymes from different genes that process or catalyzed the same reaction
- Differs in amino acid sequence



.....then E_1 & E_2 will be Isozymes

Allozymes represent enzymes from different alleles of the same gene, and Isozymes represent enzymes from different genes that process or catalyse the same reaction.

Since allelic variation reflected in an enzyme may result in different properties, it is possible to identify different alleles by electrophoresis; tissue extracts are applied to a gel and an electrical current is applied.

Different allelic variants of an enzyme may then migrate through the gel at a rate determined by the net charge and conformation of the enzyme.

Finally, enzyme specific histochemical staining is used to visualise specific enzymes, and different alleles are identified from different banding patterns.

- The general method for detecting allozyme variation includes extraction, electrophoresis and detection.

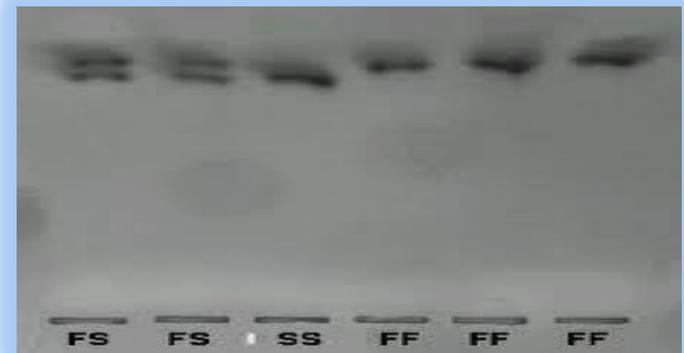
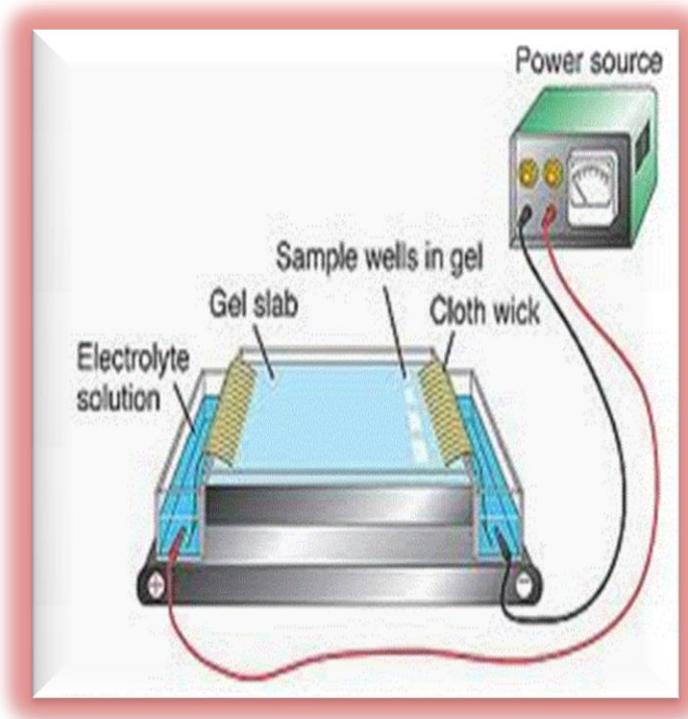
- Electrophoresis is a separation technique based on the motion of charged particles in an electric field. Tissue extracts are introduced into a solid support medium (a gel) at the Origin.

- As matrix (gel) for electrophoresis various media can be used such as acrylamide, cellulose acetate and hydrolyzed potato starch.

- Acrylamide typically has the best resolving power, however it is the most difficult to handle and also toxic.

When an electrical field is applied, most proteins have a net negative charge and will migrate from the Origin at the cathode ("negative") end towards the anode ("positive") end of the field.

The positions of the protein products are detected either directly, or by coupled enzymatic reactions. For a monomeric enzyme, individuals that are homozygous each show a single band; those that are heterozygous show two bands.



Advantage

Simple, fast, relatively low cost.

Any source of soluble proteins, from bacterial cultures to animal fluids, is suitable for allozyme analysis.

Electrophoretic separation and staining are easy and adjustable from species to species.

The genetic interpretation of allozyme profiles is also straightforward.

It allows for screening a large number of loci, often more than 30-40.

Disadvantage

Inability to read genotypes from **small quantities of tissue**, which makes allozymes inapplicable for small organisms (e.g. larvae).

One disadvantage that appears to be difficult to overcome is that **only a small fraction of enzyme loci appear to be allozymically polymorphic in many species.**

There are important demands concerning the **freshness of tissue samples** and **many loci exhibit tissue-specific expression** (e.g. some loci are only expressed in heart tissue). Thus the invasive tissue sampling method, which requires sacrificing the fish and the **need for cryogenic storage to preserve enzyme activity** are serious constraints.

Disadvantage

In addition a given change in nucleotide sequence may not result in a change in amino acid at all, and thus would not be detected by protein electrophoresis

		Second base					
		U	C	A	G		
First base	U	UUU } Phenyl-alanine F UUC } UUA } Leucine L UUG }	UCU } Serine S UCC } UCA } UCG }	UAU } Tyrosine Y UAC } UAA } Stop codon UAG } Stop codon	UGU } Cysteine C UGC } UGA } Stop codon UGG } Tryptophan W	U C A G	
	C	CUU } Leucine L CUC } CUA } CUG }	CCU } Proline P CCC } CCA } CCG }	CAU } Histidine H CAC } CAA } Glutamine Q CAG }	CGU } Arginine R CGC } CGA } CGG }	U C A G	
	A	AUU } Isoleucine I AUC } AUA } AUG } Methionine start codon M	ACU } Threonine T ACC } ACA } ACG }	AAU } Asparagine N AAC } AAA } Lysine K AAG }	AGU } Serine S AGC } AGA } Arginine R AGG }	U C A G	
	G	GUU } Valine V GUC } GUA } GUG }	GCU } Alanine A GCC } GCA } GCG }	GAU } Aspartic acid D GAC } GAA } Glutamic acid E GAG }	GGU } Glycine G GGC } GGA } GGG }	U C A G	

Furthermore, a change in the DNA that results in a change in an amino acid may not result in a change in the overall charge of the protein and, therefore, would also not be detected.