



Barcoding of fish cell lines - the origin of cell lines is not always what we believe

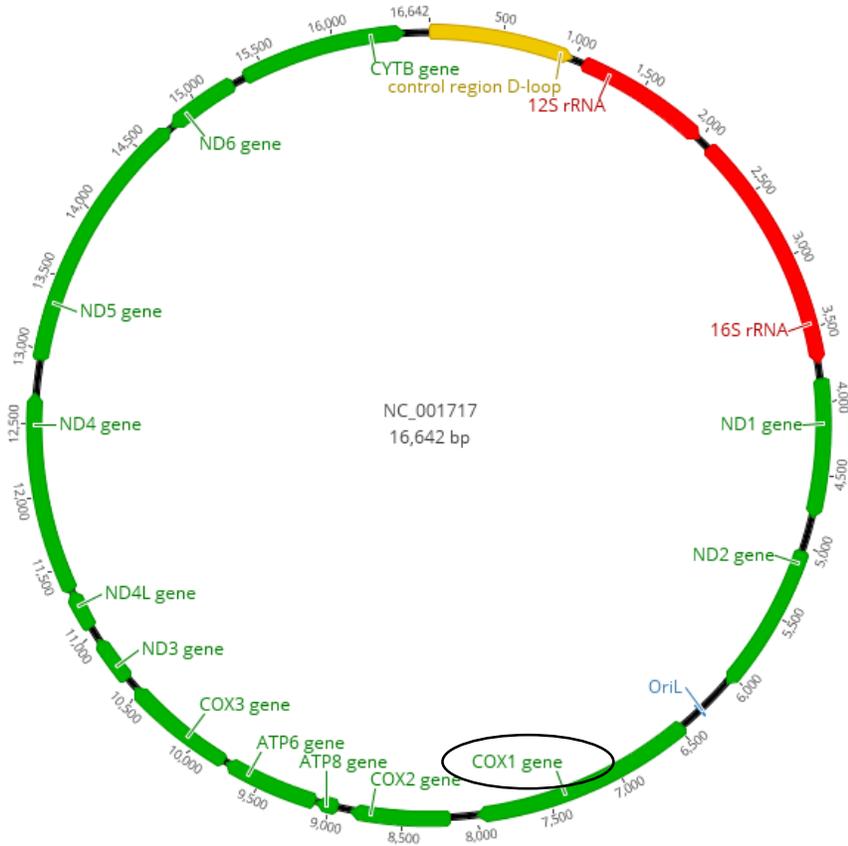
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*21st Annual Workshop of the National Reference Laboratories for Fish Diseases,
Kgs. Lyngby, Denmark,*

Introduction

- For several years rumors that some cell lines do not have the expected origin.
- e.g. EPC "Epithelioma Papullosum Carpio" = FHM (Fathead minnow) Cells.
Both in the *cyprinid* family
- Who cares?
- Correlates to the fish species
- All cell lines shipped from the EURL will have a barcode of origin
- Provide a SOP for COI barcoding cell lines.

Rainbow trout mitochondrion



COX1 or COI

Code for cytochrome c oxidase subunit I

Part of the cytochrome c oxidase enzyme complex (Respiratory complex IV), a transmembrane protein found in bacteria and in the mitochondrion of eukaryotes.

Respiratory complex IV consists of 3 catalytic subunits encoded by mitochondrial genes (COXI, COXII, COXIII) and multiple structural subunits encoded by nuclear genes.

COXI is widely used in DNA barcoding in animals,

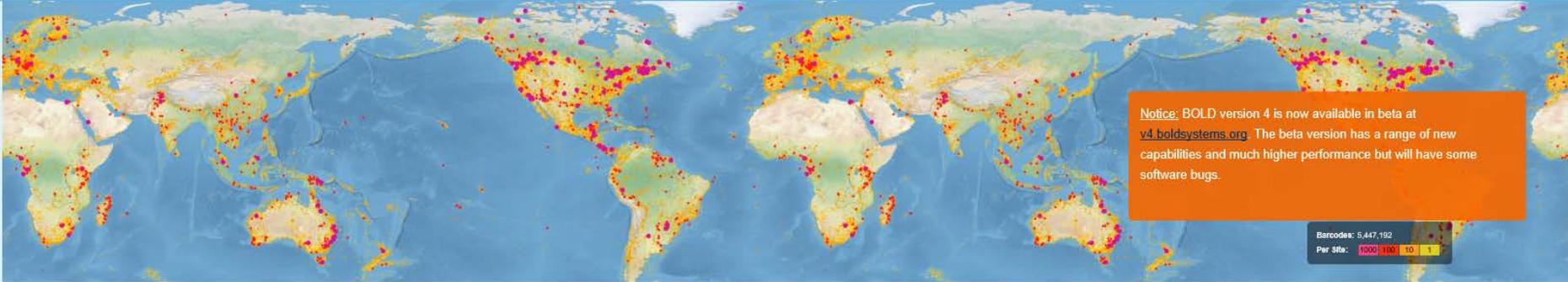
- it is variable enough to distinguish closely related species,
- well conserved among co-specific individuals

Barcoding of Life Project

- The Barcode of Life is a project to create a public collection of reference sequences from vouchered specimens of all species of life.
- A Barcode sequence is a short nucleotide sequence from a standard genetic locus for use in species identification.
- For animals, this standard genetic locus is a 650 base pair region on the 5' end of the mitochondrial cytochrome oxidase subunit I (COI) gene.

Barcoding of life database

BOLDSYSTEMS | Databases | Taxonomy | Identification | Workbench | Resources



Notice: BOLD version 4 is now available in beta at v4.boldsystems.org. The beta version has a range of new capabilities and much higher performance but will have some software bugs.

Barcodes: 5,447,192
Per Site: 1000 100 10 1

Taxonomy Search



Public Data Portal:

A data retrieval interface that allows for searching over 1.7M public records in BOLD using multiple search criteria including, but not limited to, geography, taxonomy, and depository.



Barcode Index Numbers:

A searchable database of Barcode Index Numbers (BINs), sequence clusters that closely approximate species.



DNA Barcode Education Portal:

A custom platform for educators and students to explore barcode data and contribute novel barcodes to the BOLD database.



Workbench:

An integrated data collection and analysis environment that securely supports the assembly and validation of DNA barcodes and ancillary sequences.

The Barcode of Life Data Systems is designed to support the generation and application of DNA barcode data. The platform consists of four main modules: a data portal, a database of barcode clusters, an educational portal, and a data collection workbench.

Sequence statistics

| | |
|-------------------------------------|-----------|
| Barcode clusters for animals (BINs) | 506,773 |
| All Sequences | 6,297,285 |
| Barcode Sequences | 5,447,192 |

Species coverage (formally described)

| | |
|--------------------|---------|
| Animals | 178,312 |
| Plants | 65,765 |
| Fungi & Other Life | 20,901 |

Barcoding of Life database- BoLD

Records for bony fishes

▼ Taxon Description (Wikipedia)

The **Actinopterygii** / æktɪn ɒptəˈrɪdʒi.əɪ/, or **ray-finned fishes**, constitute a class or subclass of the bony fishes. The ray-finned fishes are so called because they possess lepidotrichia or \"fin rays\", their fins being webs of skin supported by bony or horny spines (\"rays\"), as opposed to the fleshy,lobed fins that characterize the class Sarcopterygii which also, however, possess lepidotrichia. These actinopterygianfin rays attach directly to the proximal or basal skeletal elements, the radials, which represent the link or connectionbetween these fins and the internal skeleton (e.g., pelvic and pectoral girdles). [full article at Wikipedia](#)

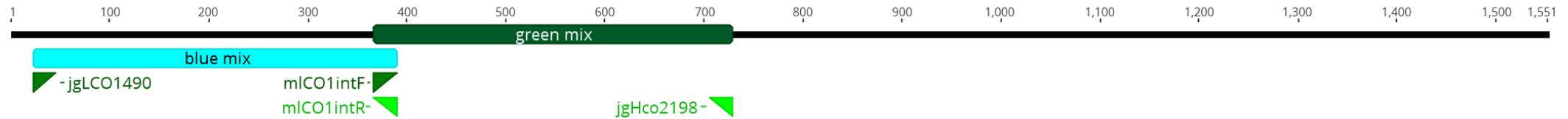
▼ BOLD Stats

| | | | |
|---------------------------|---------|-----------------|---------|
| Specimen Records: | 268,390 | Public Records: | 141,676 |
| Specimens with Sequences: | 221,426 | Public Species: | 11,328 |
| Specimens with Barcodes: | 213,892 | Public BINs: | 14,914 |
| Species: | 18,551 | | |
| Species With Barcodes: | 17,173 | | |

[Species List - Progress](#)

[Access Published & Released Data](#)

http://www.barcodinglife.com/index.php/Taxbrowser_Taxonpage?taxid=77



The figure shows the full COI for rainbow trout and the primer binding sites. The whole region (blue + green) is the barcoding region, that can be amplified with the primers jgLCO1490 and jgHCo2198

Two internal primers were designed to amplify a range of metazoans mlCO1int forward and reverse.

Each reaction (blue and green) amplifies ~330 nt

Currently only the green region is needed to barcode fish cell lines

Primer set jgLCO1490 + jgHco2198 (Folmer et al. 1994 *Mol Mar Biol Biotechnol*)

Internal primers mlCO1intF and mlCO1intR (Leray et al. 2013. *Frontiers in Zoology*)

Results

| Cell line | Navn | Species of origin | RESULTS | identity |
|-----------------|-------------------------------|---|--|----------|
| EPC* | Epithelioma Papullosum Carpio | <i>Cyprinus carpio</i> (common carp) | <i>Pimephales promelas</i> (fathead minnow) | 100% |
| BF-2 | Bluegill Fry | <i>Lepomis macrochirus</i> (bluegill) | <i>Lepomis</i> | 100% |
| CHSE-214 | Chinook Salmon Embryo | <i>Oncorhynchus tshawytscha</i> (Chinook Salmon) | <i>Oncorhynchus tshawytscha</i> (Chinook Salmon) | 100% |
| RTG-2 | Rainbow trout gonad | <i>Oncorhynchus mykiss</i> (rainbow trout) | <i>Oncorhynchus mykiss</i> (rainbow trout) | 100% |
| FHM | Fat Head Minnow | <i>Pimephales promelas</i> (Fat Head Minnow) | <i>Pimephales promelas</i> (Fat Head Minnow) | 100% |
| CCO | Channel Catfish Ovary | <i>Ictalurus punctatus</i> (Channel catfish) | <i>Ameiurus nebulosus</i> (Brown bullhead) | 100% |
| EK-1 | Eel Kidney | <i>Anguilla anguilla</i> or <i>A. obscura</i> | <i>Anguilla japonica</i> | 100% |
| ASK | Atlantic Salmon Kidney | <i>Salmo salar</i> (Atlantic salmon) | <i>Oncorhynchus mykiss</i> (Rainbow trout) | 100% |
| CCB | Common Carp Brain | <i>Cyprinus carpio</i> (common carp) | <i>Cyprinus carpio</i> (common carp) | 100% |
| SBL | Sea Bass Lymphoid | <i>Dicentrarchus labrax</i> (European seabass) | <i>Oncorhynchus tshawytscha</i> (Chinook Salmon) | 100% |
| WSSK | White Sturgeon SKin-1 | <i>Acipenser transmontanus</i> | <i>Acipenser transmontanus</i> | 100% |

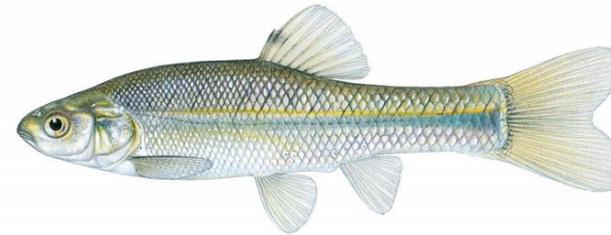
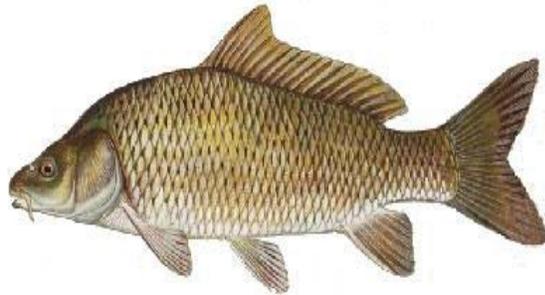
*two different passages (287 and 295), same result

EPC Epithelioma Papullosum Carpio cells = FHM cells

Cyprinus carpio
(common carp)



Pimephales promelas
(fathead minnow)



History of origin : (Tomasec & Fijan, 1971; Fijan et al., 1983), "Epithelioma Papullosum Carpio" Tomasec, J. and Fijan, N. Virusne bolesti riba (viral diseases of fish) (1971). Final report on research under a part of project 6n/1966, 1-29. Zagreb. Most likely mistake at production as Fathead is only observed in Americas

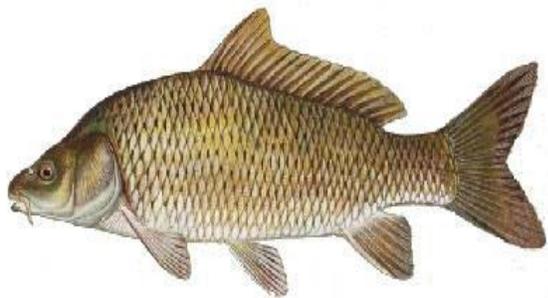
Identification Summary:

From BoLD

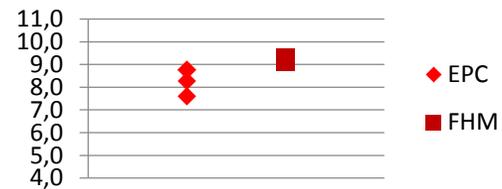
| Taxonomic Level | Taxon Assignment | Probability of Placement (%) |
|-----------------|---------------------|------------------------------|
| Phylum | Chordata | 100 |
| Class | Actinopterygii | 100 |
| Order | Cypriniformes | 100 |
| Family | Cyprinidae | 100 |
| Genus | Pimephales | 100 |
| Species | Pimephales promelas | 100 |

EPC and FHM: Most likely same origin but different appearance and sensitivity

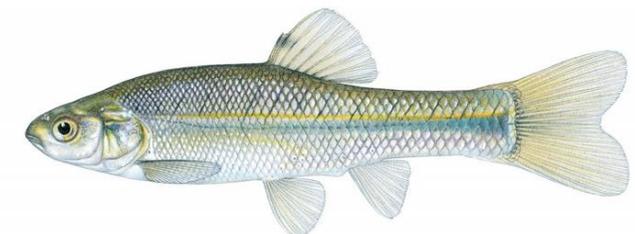
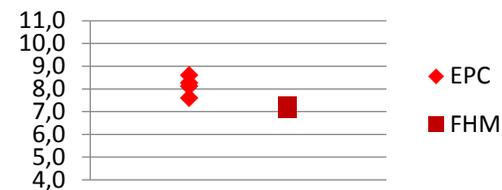
EPC = FHM



SVCV

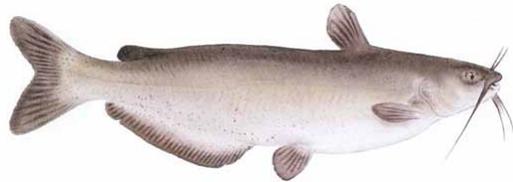


IHNV



CCO Channel Catfish Ovary: related species but not Channel catfish

Ictalurus punctatus
(Channel catfish)



Ameiurus nebulosus
(Brown bullhead)



Identification Summary:

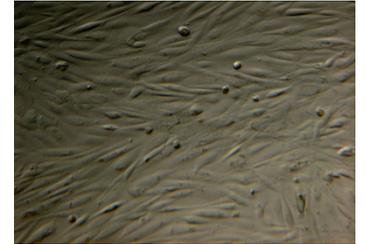
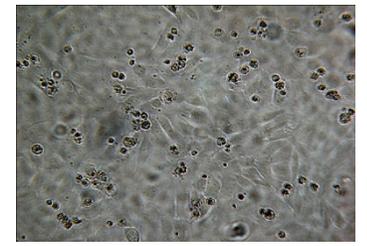
| Taxonomic Level | Taxon Assignment | Probability of Placement (%) |
|-----------------|--------------------|------------------------------|
| Phylum | Chordata | 100 |
| Class | Actinopterygii | 100 |
| Order | Siluriformes | 100 |
| Family | Ictaluridae | 100 |
| Genus | Ameiurus | 100 |
| Species | Ameiurus nebulosus | 99.6 |

ASK Atlantic Salmon Kidney from Rainbow trout!?

Salmo salar
(Atlantic salmon)



Oncorhynchus mykiss
(Rainbow trout)



Search Result:

A species level match could not be made, the queried specimen is likely to be one of the following:

[Oncorhynchus mykiss](#)
[Oncorhynchus sp.](#)

For a hierarchical placement - a neighbor-joining tree is provided: [Tree Based Identification](#)

Identification Summary:

| Taxonomic Level | Taxon Assignment | Probability of Placement (%) |
|-----------------|------------------|------------------------------|
| Phylum | Chordata | 100 |
| Class | Actinopterygii | 100 |
| Order | Salmoniformes | 100 |
| Family | Salmonidae | 100 |
| Genus | Oncorhynchus | 100 |

Most susceptible cell line for ISAV
RTG-2 cells?

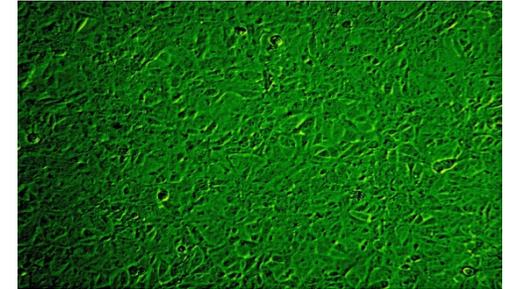
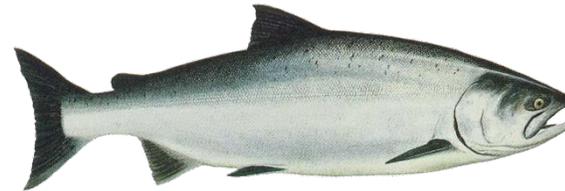
Originally derivate from TO cells (NOT trout
Ovary but the name of the technician who
produced them from A. salmon: Tove!
To be tested with cell from VI, Oslo

SBL Sea Bass Lymphoid = CHSE-214 from Chinook??

Dicentrarchus labrax
(European seabass)



Oncorhynchus tshawytscha
(Chinook Salmon)



Original mistake or cross contamination
in our laboratory?
To be tested with original cells from
ANSES, Brest

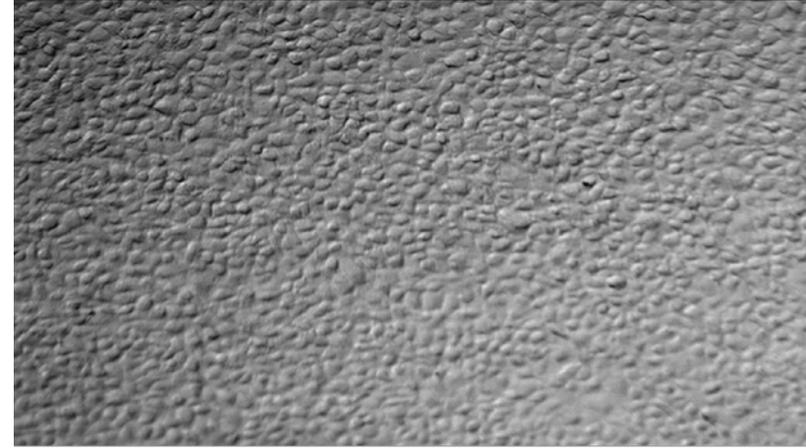
Identification Summary:

| Taxonomic Level | Taxon Assignment | Probability of Placement (%) |
|-----------------|--------------------------|------------------------------|
| Phylum | Chordata | 100 |
| Class | Actinopterygii | 100 |
| Order | Salmoniformes | 100 |
| Family | Salmonidae | 100 |
| Genus | Oncorhynchus | 100 |
| Species | Oncorhynchus tshawytscha | 100 |

A number of cell lines in our repository still to be tested

| | | | | |
|--------------|---|-----------------------|--|------------|
| SSN-1 | Striped Snakehead Nephron | Striped Snakehead | <i>Channa striata</i> | |
| CCB | Common Carp Brain | Common Carp | <i>Cyprinus carpio</i> | |
| CIK | <i>Ctenopharyngodon</i> <i>idella</i> Kidney | Grass carp | <i>Ctenopharyngodon</i> <i>idella</i> | |
| SBL | Sea Bass Lymphoid | European seabass | <i>Dicentrarchus</i> <i>labrax</i> | |
| CLC | Carp leucocyte cells | Common Carp | <i>Cyprinus carpio</i> | |
| R-KF (=KF-1) | Koi carp fin | Koi carp | <i>Cyprinus carpio</i> | |
| SHK-1 | Salmon Head Kidney | Atlantic Salmon | <i>Salmo salar</i> | fibroblast |
| GF | Grunt fin | blue striped Grunt | <i>Haemulon sciurus</i> | |
| GFF | Goldfish Fin | Goldfish | <i>Carassius auratus</i> | |

Conclusion



- **Barcoding of fish cell lines using COI efficient and simple method**
- **SOP produced and can be provided**
- **Continuous risk of cross contamination of cell lines and they should therefore be tested regularly**
- **Barcoding of all cell line shipped from the EURL**
- **Next step: NGS and further genetic characterization of our cell lines for use in virology, immunology, toxicology etc.**

Acknowledgement



- Betina Lynnerup, Didde Hedegaard Sørensen, Troels Secher Rundqvist and the rest of the DTU team
- Morten Limborg Ucp, Susie S. Mikkelsen

- Thank you for your attention