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FishmiRNA: An evolutionarily supported microRNA annotation and expression database for ray-finned fishes

Thomas Desvignes ^{1#}, Philippe Bardou ², Jérôme Montfort ³, Jason Sydes ¹, Cervin Guyomar ², Simon George ⁴, John H. Postlethwait ¹, Julien Bobe ^{3#}

1. Institute of Neuroscience, University of Oregon, Eugene OR 97403, USA
2. Sigénae, GenPhySE, Université de Toulouse, INRAE, ENVT, F-31326, Castanet Tolosan, France
3. INRAE, LPGP, 35000, Rennes, France
4. MGX - Montpellier GenomiX, Univ. Montpellier, CNRS, INSERM, Montpellier France

Correspondence to tdesvign@uoregon.edu and julien.bobe@inrae.fr

Email addresses:

Thomas Desvignes: tdesvign@uoregon.edu

Philippe Bardou: philippe.bardou@inrae.fr

Jérôme Montfort: jerome.montfort@inrae.fr

Jason Sydes: sydes@uoregon.edu

Cervin Guyomar: cervin.guyomar@inrae.fr

Simon George: simon.george@mgx.cnrs.fr

John H. Postlethwait: jpostle@uoregon.edu

Julien Bobe: julien.bobe@inrae.fr

ORCID:

Thomas Desvignes: 0000-0001-5126-8785

Philippe Bardou: 0000-0002-0004-0251

Jason Sydes: 0000-0003-4187-2828

Cervin Guyomar: 0000-0003-2707-2541

John H. Postlethwait: 0000-0002-5476-2137

Julien Bobe: 0000-0002-9355-8227

Running title: FishmiRNA, the fish microRNA annotation and expression database

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Abstract

microRNAs are important post-transcriptional regulators of gene expression involved in countless biological processes and are widely studied across metazoans. While miRNA research continues to grow, the large community of fish miRNA researchers lacks exhaustive resources consistent among species. To fill this gap, we developed FishmiRNA, an evolutionarily supported microRNA annotation and expression database for ray-finned fishes: www.fishmirna.org. The self-explanatory database contains detailed, manually-curated miRNA annotations with orthology relationships rigorously established by sequence similarity and conserved syntenies, and expression data provided for each detected mature miRNA. In just few clicks, users can download the annotation and expression database in several convenient formats either in its entirety or a subset. Simple filters and BLAST search options also permit the simultaneous exploration and visual comparison of expression data for up to any ten mature miRNAs across species and organs. FishmiRNA was specifically designed for ease of use to reach a wide audience.

Keywords

Teleost, Holostei, actinopterygian, non-coding RNA, Whole Genome Duplication, bowfin *Amia calva*

Introduction

microRNAs (miRNAs) have emerged as key post-transcriptional regulators of gene expression that act by binding to the 3'-untranslated region of target messenger RNAs when incorporated into the RNA-induced silencing complex (Jonas and Izaurralde 2015; Bartel 2018). Regulatory functions of miRNAs have now been implicated in countless biological processes, including cell differentiation and proliferation, organ development and physiology, pathologies and diseases (Mendell and Olson 2012; Sun and Lai 2013), and genetic noise buffering, especially in stressful conditions (Schmiedel et al. 2015; Liufu et al. 2017). Furthermore, miRNAs evolve in lineage- and environment-specific manners and can modulate alternative developmental and physiological pathways that may influence adaptation, diversification, and speciation (Loh et al. 2011; Li and Zhang 2013; Quah et al. 2015).

Although several databases and bioinformatic tools facilitate the study of miRNAs in cellular, developmental, physiological, and pathological contexts, they cover mostly humans and main laboratory model organisms, so researchers studying other species often lack appropriate resources to accurately facilitate miRNA studies in their systems. This situation is exacerbated in ray-finned fishes, actinopterygians. While several valuable miRNA annotation databases already exist, miRNA annotations in fish species are often unavailable, incomplete, or inconsistent across fish phylogeny, inhibiting the study of miRNAs in half of all vertebrate species. The legacy database miRBase was

created in 2006 but has not been updated since 2018 (Kozomara et al. 2019). miRBase contains partial miRNA annotations for several fish species, but none are exhaustive or curated and many new or improved annotations have not been incorporated. In 2015, MirGeneDB was created and covers a breadth of metazoan species, but this database provides miRNA annotation for only one fish species, zebrafish (Fromm et al. 2020). miRNAs, however, are of great interest in fish research with studies ranging from genomic evolution (Xiong et al. 2019; Desvignes et al. 2021), development (Giraldez et al. 2006; Kasper et al. 2017; Gay et al. 2018), medical models (Hsu et al. 2017), aquaculture (Herkenhoff et al. 2018; Blödorn et al. 2021; Cardona et al. 2021), pathology (Andreassen and Høyheim 2017; Wang et al. 2018), toxicology (Goodale et al. 2019; Ahkin Chin Tai and Freeman 2020), to adaptation and speciation (Franchini et al. 2019; Kelley et al. 2021). In most fish miRNA studies, the lack of consistent annotation resources leads to using, by default, combinations of annotations existing in other, often distantly-related species, or to *de novo* prediction of miRNAs using different algorithms in different research groups, leading to inconsistent results among species. Therefore, while miRNA research continues to grow rapidly, the large community of fish miRNA researchers is plagued by the lack of exhaustive and phylogenetically supported resources.

The complexity of studying miRNAs in fish emerges from two main sources. First, a whole-genome duplication, called the Teleost Genome Duplication or TGD, initially duplicated every miRNA gene (Amores et al. 1998; Taylor et al. 2003; Braasch et al. 2016). This genome duplication was followed by lineage-specific gene resolution events that confound orthology assignment among species (Postlethwait 2007). Second, because fish represent more than half of living vertebrate species and inhabit virtually all aquatic habitats on the planet, they harbor dramatic variations in gene repertoires, which makes miRNA gene and mature miRNA computational predictions and annotations unreliable without expert manual curation.

To fill this resource gap, we developed FishmiRNA: An evolutionarily supported microRNA annotation and expression database for ray-finned fishes.

New approaches

FishmiRNA integrates several new approaches that provide accurate, consistent, and exhaustive annotations of evolutionarily-conserved miRNAs in ray-finned fishes, as well as the innovative ability to explore expression datasets across species and organs in a user-friendly interface.

Two novelties of FishmiRNA for achieving miRNA annotation consistency among species are the full integration of the teleost genome duplication (TGD) in gene orthology assignments and a broad phylogenetic context. For that purpose, we built on our recent establishment of genome-wide synteny-verified miRNA gene orthologies among several fish species, including the spotted gar

Lepisosteus oculatus whose lineage, the Holostei, diverged before the TGD. This work allowed us to infer the miRNA gene repertoire of the hypothetical Teleost-Holostei last common ancestor (TH-LCA) (Desvignes et al. 2021). In addition, FishmiRNA annotations rely on small RNA sequencing expression data to first, identify miRNA gene loci and second, to detect the most abundantly expressed mature miRNAs, leading to data-supported annotation of both the 5p and 3p strands for the majority of genes (76% across the database). The novel annotation of four teleost species and of the bowfin, *Amia calva*, a second holostean outgroup to the teleosts (Thompson et al. 2021), further increased confidence in the inferred TH-LCA miRNA gene repertoire and in intermediate teleost ancestors. This ancestral reconstruction approach, a cornerstone of the FishmiRNA database, allows the retracing of gene evolution across lineages. Each miRNA gene annotated in FishmiRNA is thus linked to its orthologs among other teleosts and with the TH-LCA. So far, FishmiRNA contains miRNA gene and mature miRNA annotations for 10 actinopterygian species, including eight teleost species (zebrafish, catfish, panga, medaka, molly, perch, stickleback, and icefish) and two Holostei species that diverged before the TGD (gar and bowfin) (Table 1). These species were selected based on their broad phylogenetic distribution within ray-finned fishes, on their importance in evolutionary, aquacultural, and biomedical research, and on the availability of high-quality genome sequences.

A unique approach of FishmiRNA unavailable in any other annotation database is to provide expression data for mature miRNAs. The consistent re-analysis of expression data for each species using the smallRNA-seq software *Prost!* (Desvignes et al. 2019) coupled with a graphical module enables users to compare the expression patterns of up to ten miRNAs from any species in the database, thus offering an innovative opportunity to incorporate in a study the evolutionary conservation of expression of any miRNA across species.

Finally, an important novel approach of FishmiRNA is the simplicity and efficient design of its user-friendly website. Contrary to other databases that incorporate hundreds of webpages, FishmiRNA database relies on a single webpage and two spreadsheets: an annotation table and an expression table, both of which can be interactively and quickly filtered, searched, and exported, in full or in part. This novel approach eases access to miRNA annotations and miRNA expression data and significantly increases the diversity of users reached by providing accessibility to miRNA data to anyone, with or without bioinformatic skills.

Database features

Graphic information

A summary of the annotation database is displayed at the top of the page in a phylogenetic context (Rabosky et al. 2018), including graphical representations of general statistics for each

species (Fig. 1). These immediate visual representations help users determine which species' annotation would be the most appropriate to apply to their experimental question or species of interest. For example, if FishmiRNA lacks a species, a user might select the phylogenetically most closely related species with the largest miRNA annotation. The phylogenetic coverage of FishmiRNA will grow as new species become annotated.

The FishmiRNA annotation database

The FishmiRNA annotation database consists of a single table containing 38 columns and as many lines as there are miRNA genes in the database, currently 3028 genes. Four columns present the taxonomy of the species and its reference genome assembly; six columns describe the miRNA gene with a name, potential previous names, a unique gene identifier, links to miRBase, Ensembl, and to other databases when available; six columns describe the miRNA hairpin with a name, its sequence, position, and strand in the genome assembly; two columns report gene orthology among teleosts and with the Teleost-Holostei last common ancestor (TH-LCA); three columns inform on gene clustering; one column summarizes 5p and 3p strand annotations, each detailed in eight columns providing the mature strand name, a unique mature identifier, a reference sequence, its position in the genome, and whether this mature miRNA can be produced by more than one miRNA gene. All of this information can be simultaneously visualized for each gene in an "individual gene ID card" by clicking on the magnifier button associated with the miRNA gene name in the annotation table (Fig. 2).

For rendering purposes, only nine columns are displayed by default, but users can select their own column preferences or display the entire table (Fig. 2). Users can also filter the database by species, by FishmiRNA Gene ID, hairpin and mature miRNA names, and by orthology relationships among teleosts and with the TH-LCA. In addition, users can search the database by genomic location or by a BLAST search of sequences on hairpins or mature miRNA sequences (Fig. 2). The entirety or filtered parts of the annotation database can be immediately exported as a spreadsheet or a GFF file. Hairpin and mature miRNA sequences can also be exported in a click in FASTA format for ready use with smallRNA-seq analysis software or other purposes (Fig. 2).

The filtering of miRNA genes reduces the portion of the annotation table displayed to only genes of interest, which can then be individually selected using tick boxes to conveniently filter their respective mature miRNAs and explore their expression patterns in a single click (Fig. 2).

The FishmiRNA expression database

The FishmiRNA expression database consists of a single table containing 23 columns and as many lines as mature miRNAs detected in the smallRNA-seq libraries analyzed. Three columns provide information on the mature miRNA name, its unique mature identifier, and the sequence of

the most-highly expressed isomiR; and 10 columns that contain the raw counts and 10 columns that contain normalized read counts (reads per million, RPM) for each mature miRNA in a selection of major organs (brain, gills, heart ventricle, skeletal muscle, intestine, liver, ovary, testis, hematopoietic kidney, and spleen). Not all species have expression data for all of the selected organs; the entry “No_Data” signals these cases. For rendering purposes, only the mature miRNA name, its sequence, and normalized read counts are displayed by default. Similar to the annotation table, users can configure columns shown in the expression table, filter by species, mature miRNA name, unique identifier, or explore expression data by BLAST. The entire expression database or filtered parts of it can also be immediately exported (Fig. 3).

One of the most useful features of the FishmiRNA expression database is its graphical module. The expression profile of each mature miRNA can be visualized by a click on the blue histogram button associated with its name (Fig. 3). This link opens a modal box displaying expression data in a histogram with the organ of highest expression in red and the mean expression across organs marked by a horizontal red line. Furthermore, by selecting mature miRNAs using tick boxes next to the mature miRNA name, users can instantly plot on the same graph the expression profiles of up to any 10 mature miRNAs in the database. All graphs generated can also be exported in various image formats using the dropdown menu located at the top-right corner of the graph (Fig. 3).

[Quick download links and origin of analyzed data](#)

To facilitate the dissemination, re-use, and transparency of data provided in FishmiRNA, the download section of the FishmiRNA database provides quick links for downloading, per species or for all species, annotation files in FASTA format, and raw output files from the smallRNA-seq software *Prost!* used for the annotation and expression analyses (Desvignes et al. 2019) (Fig. 4). Furthermore, links to the publication of each original annotation are provided along with links to the NCBI BioProject of the expression data analyzed and the article that published these original data (Fig. 4).

Materials and Methods

Small RNA sequencing data presented in FishmiRNA are all publicly available in NCBI (Table 1). Organ sampling and library preparation protocols as well as sequencing platforms may differ between species and therefore expression patterns may not be fully comparable. All species were, however, re-analyzed the same way and for each species, Illumina sequencing libraries were simultaneously analyzed using *Prost!* (Desvignes et al. 2019), selecting for read length from 17 to 25 nucleotides with a minimum of five identical reads. Publicly available genome assemblies were used for each species (Table 1). Gene and mature miRNA annotations were performed as previously described (Desvignes et al. 2019), using orthology and ohnology relationships established across

species (Desvignes et al. 2021), and following nomenclature rules established for zebrafish (Desvignes et al. 2015; Ruzicka et al. 2019; Desvignes et al. 2020). FishmiRNA was developed based on the RumimiR web interface (Bourdon et al. 2019).

Authors' contributions

Study concept and design: TD, JHP, and JB

Acquisition of data: TD, SG, and JB

Analysis and interpretation of data: TD, JM, JS, CG, JHP, and JB

Web interface development: PB

Wrote the manuscript: TD and JB

Critical revision of the manuscript: TD, PB, CG, JHP, and JB

Obtained funding: JHP, TD, PB, and JB

Study supervision: TD, JHP, and JB

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Tables

Table 1: Summary of annotations and sequencing data included in FishmiRNA

Species	Genome Assembly	Annotation Citation	Sequencing Data NCBI Acc #	Expression Data Citation
Spotted gar, <i>Lepisosteus oculatus</i>	LepOcu1 (GCA_000242695.1)	(Braasch et al. 2016)	PRJNA296503	(Braasch et al. 2016)
Bowfin, <i>Amia calva</i>	AmiCal1 (GCA_017591415.1)	Present Study	PRJNA255850	Present Study
Black bullhead, <i>Ameiurus melas</i>	AMELA_1.0 (GCA_012411365.1)	Present Study	PRJNA730692	Present Study
Striped catfish, <i>Pangasianodon hypophthalmus</i>	GENO_Phyp_1.0 (GCF_009078355.1)	Present Study	PRJNA256963	Present Study
Zebrafish, <i>Danio rerio</i>	GRCz11 (GCA_000002035.4)	(Desvignes et al. 2014)	PRJNA240316	(Desvignes et al. 2014)
Japanese medaka, <i>Oryzias latipes</i>	ASM223467v1 (GCA_002234675.1)	(Desvignes et al. 2021)	PRJNA477647	(Gay et al. 2018)
Molly, <i>Poecilia mexicana</i>	P_mexicana-1.0 (GCA_001443325.1)	(Kelley et al. 2021)	PRJNA471100	(Kelley et al. 2021)
European perch, <i>Perca flavescens</i>	GENO_Pfluv_1.0 (GCA_010015445.1)	Present Study	PRJNA256973	Present Study
Three-spined stickleback, <i>Gasterosteus aculeatus</i>	BROAD S1	(Desvignes et al. 2019)	PRJNA486149	(Desvignes et al. 2019)
Blasckfin icefish, <i>Chaenocephalus aceratus</i>	cace 20180227a pilon1	(Kim et al. 2019)	PRJNA310135	(Desvignes et al. 2016)

Figure legends

Fig. 1. General statistics of the FishmiRNA database.

Screen shot of the home page. On the left, the species present in FishmiRNA database are displayed in their phylogenetic context (Rabosky et al. 2018). On the right, the miRNA gene (in blue) and mature miRNA (in red) annotation statistics are given for each species.

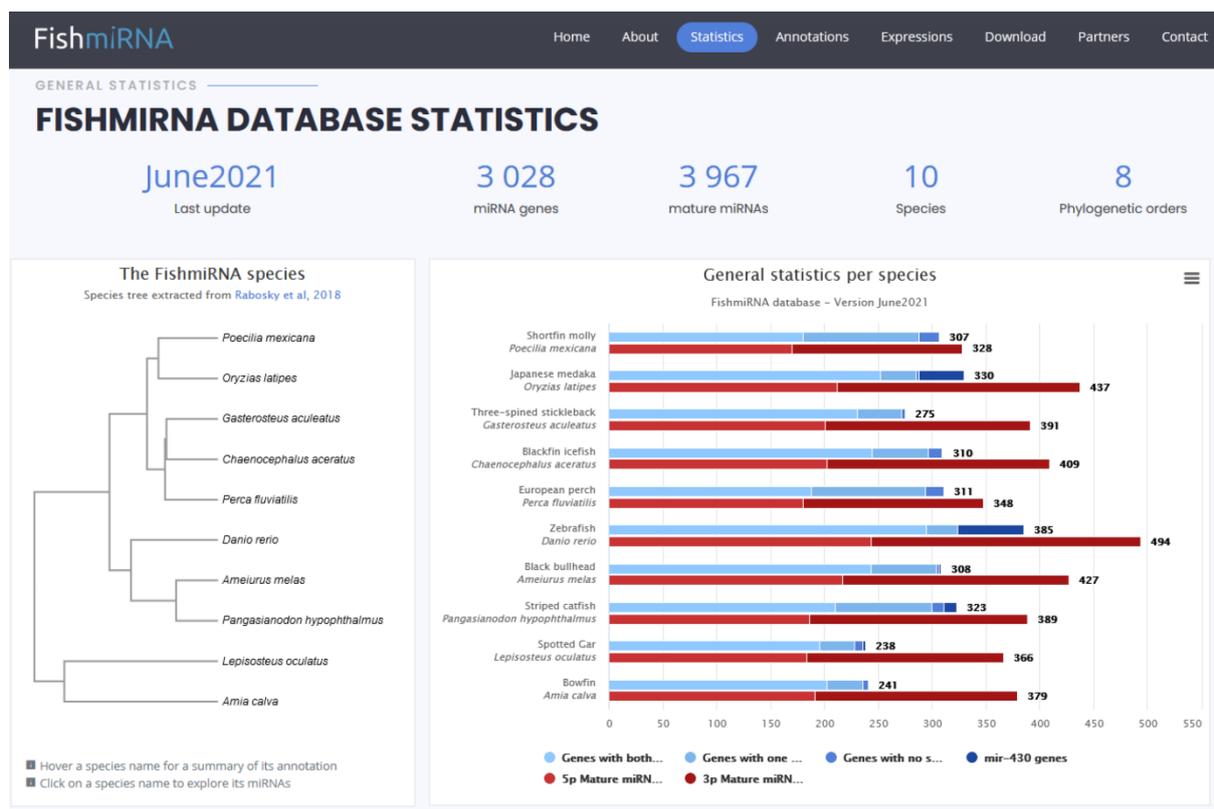


Fig. 2. Exploring the FishmiRNA Annotation database.

Screen shot of the Annotation section of the database. Users can choose to add or remove columns in the display and can filter and search the database by species, miRNA gene and mature miRNA names, and orthology among species. The entire annotation database, or subsets of it, can be exported in various convenient formats. The magnifier icon opens the corresponding gene identification card that contains all the FishmiRNA information related to this gene. Selecting genes using tick boxes on the left allows the filtering of the FishmiRNA expression database for mature products of selected genes.

EXPLORE FISHMIRNA DATABASE

Show/Hide columns
Configure the table header by selecting column name(s)

Search
Filter by source and/or by position
Search by BLAST against hairpin and/or mature sequences

Export
For the filtered row(s) export data, sequences or annotations

9 items selected
Number of visible columns: 9/38
All Reset
'All' columns or 'Reset to default'.
Remove all filters
'Remove all filters' in the search box.

By source By position By BLAST

Species Nothing selected Species Nothing selected
Select species common name(s). Select species scientific name(s).

FishmiRNA
Filter by FishmiRNA Gene ID.

Hairpin mir-144 Mature
Filter by hairpin name. Filter by mature miRNA name.

Teleost TH-LCA*
Filter by orthology among teleosts. Filter by orthology with the TH LCA*.

Copy Excel CSV
The data.
Hairpin
A FASTA file of the hairpin sequence(s).
All 5p 3p
A FASTA file of the mature sequence(s).
GFF
A GFF file of the miRNA(s).

Show 10 entries

Display the gene ID Card

	Species common name	Species scientific name	Hairpin name	Orthology among teleosts	Orthology with Teleost-Holostei LCA	5p miRNA name	5p miRNA sequence	3p miRNA name	3p miRNA sequence
<input type="checkbox"/>	Black bullhead	<i>Ameiurus melas</i>	ame-mir-144	mir144	TH-LCA-mir144	ame-miR-144-5p	GGATATCATCATATACTGTAAGT	ame-miR-144-3p	CTACAGTATAGATGATGTA
<input checked="" type="checkbox"/>	Blackfin icefish	<i>Chaenocephalus aceratus</i>	cac-mir-144	mir144	TH-LCA-mir144	cac-miR-144-5p	AGGATATCATCTTATACTGTA	cac-miR-144-3p	CTACAGTATAGATGATGTA
<input type="checkbox"/>	Bowfin	<i>Amia calva</i>	aca-mir-144	mir144	TH-LCA-mir144	aca-miR-144-5p	GGATATCATCATATACTGTAAGT	aca-miR-144-3p	TACAGTATAGATGATGTA
<input type="checkbox"/>	European perch	<i>Perca fluviatilis</i>	pfl-mir-144	mir144	TH-LCA-mir144	pfl-miR-144-5p	GGATATCATCTTATACTGTAAGT	pfl-miR-144-3p	CTACAGTATAGATGATGTA
<input checked="" type="checkbox"/>	Japanese medaka	<i>Oryzias latipes</i>	ola-mir-144	mir144	TH-LCA-mir144	ola-miR-144-5p	GGATATCATCTTATACTGTAAGT	ola-miR-144-3p	CTACAGTATAGATGATGTA
<input type="checkbox"/>	Shortfin Molly	<i>Poecilia mexicana</i>	pme-mir-144	mir144	TH-LCA-mir144	pme-miR-144-5p	GGATATCATCTTATACTGTAAG	pme-miR-144-3p	CTACAGTATAGATGATGTA
<input checked="" type="checkbox"/>	Spotted Gar	<i>Lepisosteus oculatus</i>	loc-mir-144	mir144	TH-LCA-mir144	loc-miR-144-5p	GGATATCATCATATACTGTAAGT	loc-miR-144-3p	CTACAGTATAGATGATGTA
<input type="checkbox"/>	Striped catfish	<i>Pangasianodon hypophthalmus</i>	phy-mir-144	mir144	TH-LCA-mir144	phy-miR-144-5p	GGATATCATCATATACTGTAAGT	phy-miR-144-3p	TACAGTATAGATGATGTA
<input type="checkbox"/>	Three-spined stickleback	<i>Gasterosteus aculeatus</i>	gac-mir-144	mir144	TH-LCA-mir144	gac-miR-144-5p	GGATATCATCTTATACTGTAAGT	gac-miR-144-3p	CTACAGTATAGATGATGTA
<input checked="" type="checkbox"/>	Zebrafish	<i>Danio rerio</i>	dre-mir-144	mir144	TH-LCA-mir144	dre-miR-144-5p	GGATATCATCGTATACTGTAAGT	dre-miR-144-3p	CTACAGTATAGATGATGTA

Showing 10 of 10 entries (filtered from 3,028 total entries) 4 rows selected

Select all Deselect all For the selected row(s): Explore expression

Select miRNA genes and explore the expression of their mature products

Fig. 3. Exploring the FishmiRNA Expression database.

Screenshot of the Expression section of the database. Like the Annotation section, users can choose to add or remove columns in the display and can filter and search the database by species, mature miRNA name and unique identifier. The entire expression database, or subsets of it, can also be exported in convenient table formats. The histogram icon displays the corresponding mature miRNA expression pattern in a variety of organs. Users can also select up to any 10 mature miRNAs using tick boxes on the left to display their expression patterns on the same graph.

FishmiRNA Home About Statistics Annotations Expressions Download Partners Contact

EXPRESSIONS

EXPLORE EXPRESSION DATA

Show/Hide columns

Configure the table below by selecting column name(s)

12 items selected

Number of visible columns: 12/23.

All Reset

'All' columns or 'Reset to default'.

'Remove all filters' in the search box.

Search

Search by BLAST against mature sequences

By source: **By BLAST**

Species: Nothing selected

Select one or more species scientific name.

Mature: ame-miR-202-5p,aca-miR-202-5p,pfl-miR-202-5p,ola-miR-202-5p,

Filter by miRNA name.

FishmiRNA

Filter by FishmiRNA Mature ID.

Export

For the filtered row(s) export data, sequences or annotations

The data.

Show 10 entries **Display individual miRNA expression** Search:

miRNA name	miRNA sequence	Brain RPM	Gills RPM	Heart RPM	Muscle RPM	Intestine RPM	Liver RPM	Ovary RPM	Testis RPM	Head-kidney RPM	Spleen RPM
<input checked="" type="checkbox"/> aca-miR-202-5p	TTCTATGCATATACCTCTTT	190.0	149.5	321.3	119.7	276.2	369.7	92808.3	192186.1	310.1	No_Data
<input type="checkbox"/> ame-miR-202-5p	TTCTATGCATATACCTCTTT	186.2	70.9	60.7	9.5	29.4	5.6	297898.2	84492.1	No_Data	No_Data
<input checked="" type="checkbox"/> dre-miR-202-5p	TTCTATGCATATACCTCTTTGA	8.6	No_Data	29.9	No_Data	No_Data	No_Data	39266.5	26612.8	No_Data	No_Data
<input type="checkbox"/> gac-miR-202-5p	TTCTATGCATATACCTTTTC	54.0	No_Data	40.6	No_Data	No_Data	No_Data	54368.3	33247.3	No_Data	No_Data
<input checked="" type="checkbox"/> loc-miR-202-5p	TTCTATGCATATACCTCTTT	28.5	No_Data	94.1	No_Data	No_Data	No_Data	12020.4	64826.5	No_Data	No_Data
<input checked="" type="checkbox"/> ola-miR-202-5p	TTCTATGCATATACCTCTTT	62.5	294.8	133.2	29.7	31.0	24.6	207245.4	215827.7	68.0	No_Data
<input type="checkbox"/> pfl-miR-202-5p	TTCTATGCATATACCTTTTC	20.9	8.0	6.9	1.9	13.1	2.8	33102.9	69471.5	No_Data	No_Data
<input checked="" type="checkbox"/> phy-miR-202-5p	TTCTATGCATATACCTCTTT	23.6	10.8	5.3	8.2	13.0	41.0	119844.6	186988.8	No_Data	No_Data

Showing 1 to 8 of 8 entries (filtered from 3,855 total entries) 5 rows selected

Select all Deselect all For the selected row(s):

Select up to 10 miRNAs to compare their expressions

miRNAs names, unique identifier, and sequences

miRNA name	aca-miR-202-5p	FishmiRNA Mature ID	FMAMICALM0094	miRNA sequence	TTCTATGCATATACCTCTTT
miRNA name	dre-miR-202-5p	FishmiRNA Mature ID	FMDANRER0112	miRNA sequence	TTCTATGCATATACCTCTTTGA
miRNA name	loc-miR-202-5p	FishmiRNA Mature ID	FMLEPOCUM0093	miRNA sequence	TTCTATGCATATACCTCTTT
miRNA name	ola-miR-202-5p	FishmiRNA Mature ID	FMORYLATM0100	miRNA sequence	TTCTATGCATATACCTCTTT
miRNA name	phy-miR-202-5p	FishmiRNA Mature ID	FMPANHYPM0094	miRNA sequence	TTCTATGCATATACCTCTTT

Interactive expression data

Export Graph

Expression profile comparison
FishmiRNA database - Version June2021

Reads per million reads (RPM)

Brain Gills Heart Muscle Intestine Liver Ovary Testis Head-kidney Spleen

● aca-miR-202-5p ● dre-miR-202-5p ● loc-miR-202-5p ● ola-miR-202-5p ● phy-miR-202-5p

Ovary
 ● aca-miR-202-5p: 92808.3
 ● dre-miR-202-5p: 39266.5
 ● loc-miR-202-5p: 12020.4
 ● ola-miR-202-5p: 207245.4
 ● phy-miR-202-5p: 119844.6

Fig. 4. Downloads and links.

Screenshot of the Download section of the database. Quick links for downloading, per species or for all species, annotations files in FASTA format and raw output files from the smallRNA-seq software *Prost!* used for the annotation and expression analyses. Links to the publication of each original annotation are also provided along with links to the NCBI Project of the expression data analyzed and the article that published these original data.

FishmiRNA
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DOWNLOAD
DOWNLOAD DATA

If you make use of the data presented in FishmiRNA, please cite the FishmiRNA paper but also the original articles for the specific annotations and/or sequencing data used in your study.

Data and citations are available for FishmiRNA as a whole or by species:

HAIRPIN FASTA:

MATURE FASTA:

PROST! FILES:

ANNOTATION CITATION: [MOLECULAR BIOLOGY AND EVOLUTION](#)

SEQUENCING DATA SRA: [PRJNA477647](#)

EXPRESSION DATA CITATION: [PLOS GENETICS](#)

Japanese medaka — *Oryzias latipes*

**miRNA sequences
 Expression data
 analyzed with *Prost!***

Species-specific citations

Expression data in NCBI