UNIVERSITY OF NORDLAND

MASTER THESIS

Characterization and expression analysis of microRNAs during embryonic development of Siberian sturgeon (*Acipenser baerii*)

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List of acronyms and abbreviations

RNA- Ribonucleic acid

miRNA- microRNA

GDE- Genome duplication events

mRNA- messenger RNA

rRNA- ribosomal RNA

tRNA- transfer RNA

snoRNA- small nucleolar RNA

EST- Expressed sequence tag

pri-miRNA- Primary-microRNA

pre-miRNA- Precursor-microRNA

RISC- RNA induced silencing complexes

RIN- RNA integrity number

dNTPs- Deoxyribo nucleoside triphosphates

PCR- Polymerase chain reaction

TBE- Tris-borate-EDTA

PAGE- Polyacrylamide gel electrophoresis

MZT- Maternal-to-zygotic transition

Hox-genes- Homeobox-genes

MRF- Myogenic regulatory factor

bHLH- Basic helix-loop-helix protein family

myf5- Myogenic factor 5

myoD- Myogenic differentiation 1

Abstract

Siberian sturgeon is a member of the ancient, and critically endangered Acipenseridae family, also known as sturgeons. Throughout history, the consumption of sturgeon has been both shunned and praised, with the latter having the biggest consequence for the sturgeon populations. As the efforts for protecting and restoring the populations grow, the opportunities and incentives to do scientific work with these ancient creatures, grow along with it. microRNAs are short RNA sequences (~22 nt) that have been shown to be an important factor in the post-transcriptional regulation of gene expression. By being able to target specific sequences at the 3' untranslated regions of mRNA, a single miRNA gene is able to silence possibly hundreds of different mRNAs. As a post-transcriptional regulators, miRNAs are very important during embryogenesis and in the process of tissue differentiation. There has been very little research done on the topic of miRNA expression in sturgeons, and no previous studies on miRNA expression during embryonal development. The objective of this study was to charactrize and analyze the miRNA expression throughout the embryonic development of Siberian sturgeon. 414 unique miRNAs were identified across 172 miRNA families. Amonst the identified miRNAs were several isomiRs of miR-430, a miRNA responsible for the clearance of maternally expressed mRNAs during early embryonic development in teleost, suggesting that the clearance of maternal mRNA during the maternal-to-zygotic transition in sturgeon is mediated through miRNAs. Other miRNAs identified include miR-1, a regulator impacting actin-related proteins in zebrafish, which is expressed slightly later in sturgeon, and miR-10, which has been shown to possibly target Hox genes, which have and important role in the development of the anterior-posterior axis.

Sammendrag

Sibirsk stør er en fisk i den eldgamle størfamilien (*Acipenseridae*). Gjennom historien har stør vært en fisk som har vekket både appetitter og avsky, hvorav den førstnevnte har skapt de største problemene for de globale stør populasjonene. Gjennom de siste 60 årene har den globale stør populasjonen blitt beregnet til å ha sunket med så mye som 80% grunnet faktorer som overfiske, tømming og demming av sjøer og vassdrag, og forurensning. Grunnet de innsatsene som blir lagt I beskyttelse og gjenoppretting av størpopulasjonene, har mulighetene og interessen for forskning på disse spennende og primitive artene økt. MicroRNAer er korte RNA (ca. 22 nucleotider) som har vist seg å være viktige faktorer i posttranskripsjonell genregulering. Ved å kunne binde seg til spesifikke sekvenser i 3' utranslerte regionen på mRNA har et enkelt microRNA-gen mulighet til å binde seg til opptil hundrevis av ulike mRNAer. Som en posttranskripsjonell regulator er microRNA meget viktige under embryogenese og under differensiering. Det har blitt gjort lite forskning innen microRNA utrykkelse i stør, og ingen forskning innen microRNA utrykkelse i under den embryologiske utviklingen. Målene i denne oppgaven var å karakterisere og analysere utrykkelsen av microRNA under den embryologiske utviklingen i Sibirsk stør. RNA-prøver fra 11 ulike stadier i den embryologiske utviklingen ble sekvensert ved bruk av Illumina NextSeq plattformen og analysert bioinformatisk. Under kvalitets analysene av sekvensene ble det bestemt at ut fra 11 prøver fra ulike stadier i utviklingen, hadde en av prøvene (32-celler stadiet) for få brukbare sekvenser og ble forkastet fra videre analyse. 414 ulike microRNA ble identifisert i 172 ulike familier. Blant de identifiserte microRNAene var flere isomiRer av miR-430, en microRNA som kan binde seg til maternalt utrykket mRNA sekvenser i embryoet hos egentlige beinfisker. Dette tyder på at stør har i likhet med egentlige beinfisker microRNAer som viktige faktorer i klareringen av maternale mRNAer under den maternal-zygotiske overgangen. Andre microRNA som ble identifisert var miR-1, som har vist seg å være viktig i reguleringen av actin-beslektede proteiner hos zebrafisk, som ser ut til å utrykkes litt senere i Sibirsk stør, og miR-10, som har blitt vist å være en mulig regulator av Hox-gener, som har en viktig rolle i utviklingen av lengde aksen i zebrafisk.

1. Introduction

1.1. Ecology and evolutionary history of sturgeons

Siberian sturgeon (*Acipenser baerii*) belongs to the Superclass: Osteichthyes, Class: Actinopterygii, Subclass: Chondrostei, Order: Acipenseriformes, Family: Acipenseridae. Sturgeons have been threatened during the past century due to anthropogenic effects, such as the destruction of their natural habitats by damming the rivers, irrigation, pollution, and overfishing. The latter is mostly due to the sturgeon caviar, which is expensive and desired. Over the past 60 years it is estimated that the global population of Siberian sturgeon has declined by up to 80% (Ruban and Bin, 2010). Sturgeons have a cartilaginous skeleton, although their ancestors had a bone-based skeleton, with the only remnants of this being the bone plates along their dorsal side (Ruban and Bin, 2010). They have a rather long generation time: Siberian sturgeon are not sexually mature until they reach 9-20 years of age depending on sex and location, in addition to a spawning periodicity of 2-5 years depending on sex (Ruban and Bin, 2010).

Sturgeons are a widespread family, composed of both cosmopolitan and endemic species, living in temperate and sub-arctic waters in the northern hemisphere, from China and Russia to western United States, in saltwater as well as freshwater. The Acipenseridae is divided in four genera with a total of 24 different species: 17 species belong to the genus Acipenser (sturgeons), 2 species in the genus Huso (giant sturgeons), two species in the genus Scaphirhynchus (shovel-nosed sturgeons) and three species in the genus Pseudoscaphirhyncus, Aral shovel-nosed sturgeons (Dettlaff et al., 1993). The diet of sturgeons depends on the species and habitat, and may vary from mollusks and crustaceans to scavenging dead fish (Ruban and Bin, 2010).

Sturgeons have some commercial applications, mainly for its roe, which is salt-cured and sold as caviar. Although caviar can be sold for a very high price, it takes a very long time for sturgeons to become sexually mature (in some species, such as beluga, *Huso huso*, up to 20 years). The best caviar is produced from older individuals, and since the fish usually is slaughtered in order to collect the roe, it makes a very costly and time-consuming endeavor. Recently, the Norwegian government has given approval for the construction and operation of a land based aquaculture facility for the farming of Siberian- and Russian sturgeons (*Acipenser gueldenstaedtii)* (Thonhaugen, 2015).

The cartilaginous bone structure of sturgeons had earlier been seen as being a primitive trait, but now we know that even though the sturgeons have a cartilaginous skeleton, they devolved from ancestors having bony skeletons. Yet they do have some characteristics that imply a differentiation from what would later become known as the Subclass Neopterygii . These differences are amongst other a longer upper lobe in their tail fins (McPhail, 2007).

The sturgeons have what look to be decorations of rows of bony plates along their back, mid lateral sides and lower sides. These plates, called scutes, are the only remnants of their bony-skeleton ancestors. The scutes are sharp and spiked and are used as a defense against predators by juvenile sturgeons, but become less obtrusive and dull as the animals grows in to adults. (McPhail, 2007).

1.2. The genetics and transcriptomics of *Acipenseridae* family

There has not been many genetic studies published on sturgeons. Sturgeons are highly polyploidic due to genome duplication events (GDE), meaning that they have many copies of each chromosome, in contrast to most chordates, who have two copies of each chromosome (Ludwig et al., 2001). It is widely agreed that the diploidic common ancestor for all *Acipenseriformes* had a karyotype 60 chromosomes (Ludwig et al., 2001). This common ancestor would then go through a GDE, which would then be the ancestor of all *Acipenseridae*, with other GDE happening further on in evolutionary time within the sturgeon clade, causing some ancestral species to be octaploidic. As time has passed, some of these chromosomes might be lost due to evolutionary pressure, meaning that the ploidity of some species would be less than octaploid but more than tetraploid (Ludwig et al., 2001). This factor becomes even more complex due to the fact that sturgeons hybridize quite easily, and hybrids have sometimes reproductively capable offspring. If the two parental species have different number of chromosomes, the number of chromosomes the offspring would have would be the average number of the parents, resulting in homologous chromosomes appearing in different numbers. This complicates mapping of the genome, in addition the sheer size of the genome, which in lake sturgeon (*Acipenser fulcescens*), is about 5 times the size of the human genome of 3.2 billion bp (Flicek et al., 2014; Hale et al., 2009).

There are very few studies on the transcriptome of sturgeons. In analysis of the gonadal transcriptome of lake sturgeon (*Acipenser fulvescens*) . More than 5,000 Expressed Sequence Tags (EST) were identified (Hale et al., 2009). These sequences could be used in the further investigation of miRNA targets in the sturgeon transcriptome. Vidotto et al. (2013), published a study where they were able to sequence and assemble 55,000 high quality ESTs from gonads and brain from one male and of one female Adriatic sturgeon (*Acipenser naccacarii).* Recently Yuan et al. (2014) published a study of the miRNA transcriptome of 5 different tissues (liver, spleen, muscle, heart and brain) of juvenile Amur sturgeon (*Acipenser schrenckii*). In this study, they were able to identify a total of 103 miRNAs expressed across all analyzed tissues.

1.3. MicroRNA: biogenesis and mechanisms for action

MicroRNAs (miRNAs) are short (~22 nucleotide) non-coding RNAs, most notably responsible for gene-regulation in plants and animals. The first miRNA, lin-4, was discovered in the early 90's in the nematode *Caenorhabditis elegans* (Lee et al., 1993; Wightman et al., 1993).
Due to the absence of miRNAs in fungi (although they do have miRNA-like small non-coding RNAs (Shabalina and Koonin, 2008), it appears that miRNA have evolved twice in two different lineages, plants and animals, in an example of convergent evolution, where two similar traits or strategy evolve individually in two different lineages (Shabalina and Koonin, 2008). In both cases, miRNA appear to have evolved from the RNA-interference pathway (Shabalina and Koonin, 2008).

miRNAs have several biogenesis pathways. Most understood is shown in Fig. 1: Primary-miRNA (pri-miRNA) are transcribed by RNA-polymerase II, adding a poly-A tail on the 3' end and a 5' cap. The pri-miRNA forms a series of hairpin-shaped secondary structures which are cut and processed by a protein complex called Drosha. The processed hairpin structures are now called precursor-miRNA (pre-miRNA), which are exported into the cytoplasm by Exportin-5 protein, and the Dicer protein complex finally slice the pre-miRNA into miRNA-duplex (Tarver et al., 2013). One strand is then loaded to an Argonaute protein. This structure is better known as the RNA-induced-silencing-complex (RISC). miRNAs can also be produced from introns (miRNAs are then called mirtrons), avoiding being processed by the Drosha protein complex (Ruby et al., 2007).
The primary function of miRNA is to down regulate the amount of mRNA expressed in cells by binding to matching sequences in mRNA (most often at the 3' UTR). This is done through the RNA-induced silencing complex (RISC). A near-perfect match between miRNA bound to the Argonaute protein and mRNA would cause a chain of proteins to attach themselves to the RISC ending with deadenylase (Eulalio et al., 2009), which removes the mRNA strand to degrade while a near perfect match would cause translational repression (Eulalio et al., 2009). The RISC structure works as a

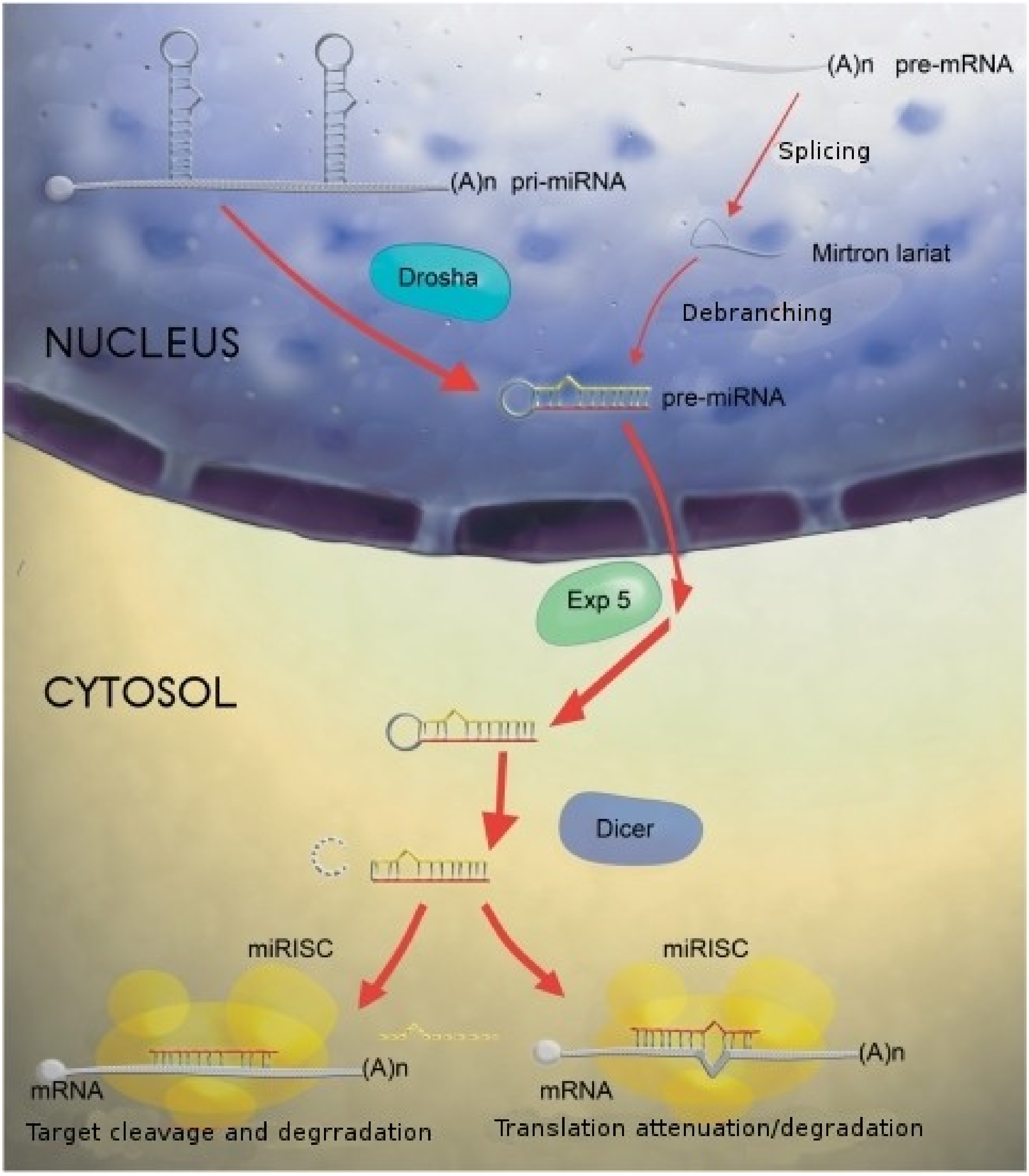


Fig 1. *Canonical pathway for miRNA biosynthesis.*
Primary-microRNA (pri-miRNA) is trasncribed from the gene coding for the miRNA. The pri-miRNA folds and forms a hairpin formed secondary structure. The hairpins are processed by Drosha, a ribonucleic enzyme, forming precursor-microRNA (pre-miRNA) and then exported to cytosol by the exportin-5 protein. The pre-miRNA is then processed by Dicer, which removes the hairpin-loop forming a double-stranded miRNA called a miRNA duplex. One of the strands is then attached to a Argonaute protein , which along with a few other proteins form the RNA-induced silencing complex (RISC). Figure from Mendes et al., 2009 licensed under creative commons license CC BY-NC 2.0 UK.

promoter for deadenylase protein complex which would in turn start deadelylate the stabilizing polyA-tail of the mRNA molecule (Giraldez, 2006).

miRNAs have a highly conserved region called the seed, the 2nd to 8th nucleotides at the 5' region, which most miRNAs in any a single family share (Bartel, 2009). The seed is so well conserved due to it being the most important part for target recognition (Bartel, 2009).

1.4. Embryology of sturgeons and the differences with embryonic development in teleost

Studies on sturgeon embryology started as early as 1881 with the first major scientific study done by Salensky (Salensky, 1881) where he described the development of sterlet (*Acipenser ruthenus*) from an unfertilized egg until hatching. The interest in sturgeon started due to sturgeons being seen as primitive fish which at the time could help explain features in the embryonic development of other fishes such as teleosts and sharks. The pattern of embryonic development of sturgeons has a number of similarities with that of amphibians such as the cleavage pattern, known as holoblastic cleavage, but also the amount of yolk and how it is distributed in the egg (Kilarski and Grodziński, 1969). The clearest difference between the embryonic development of sturgeon and teleost, apart from the yolk distribution, is the cleavage pattern during early development. While in teleost the cleavage only occurs in the blastodisc, a yolk-free region of cytoplasm at the animal pole, in sturgeons the cleavages occurs throughout the entire cell, albeit slower and fewer divisions occur at the vegetal pole (Fig.2) (Gilbert, 2000; Dettlaff et al., 1993)

In chordates, the transcriptome of a newly fertilized egg is expressed from the maternal genome, thus maternally controlled. In teleost, the zygotic transcriptome is first expressed around the 1000-cell stage, at this point miR-430, a microRNA responsible for the silencing and degradation of maternal mRNA, is expressed (Giraldez, 2006).

There is no prior research done on the microRNA transcriptome of sturgeon during early development, and only one study published on sturgeon miRNA is on the tissue-specific transcriptome in juvenile Amur sturgeon (Yuan et al., 2014). It is still unknown whether miR-430 is also responsible for maternal clearance in the *Acipenseridae*-family, and since there are some significant differences between the embryonic development of sturgeon and teleost fishes, there might be different miRNAs having a similar role as miR-430.

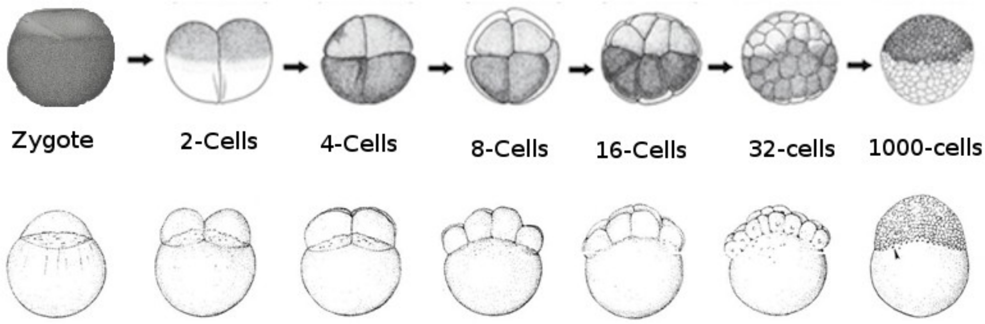


***Fig. 2**. Comparison between holoblastic cleavage, typical cleavage pattern in sturgeon and amphibians (Top), and meroblastic cleavage, typical cleavage in teleost (bottom). The zygote phase is quite different between the two patterns, as in the holoblastic cleavage the transition from the animal pole to the vegetal pole is gradual whilst in meroblastic cleavage, the transition is much sharper. The main difference between the two patterns is observable at the 2-cell stage, holoblastic cleavage divides the entire egg cell in two parts, albeit the vegetal pole at a slower rate than the animal pole, whilst in meroblastic cleavage only the blastodisc goes through cell-division. These patterns can be seen throughout early development. Figure modified from: Institute of Molecular and Cell Biology, A* Research, 2012; Dettlaff et al., 1993; Tan et al., 2013*

1.5. Objectives of this study

There has been very few studies done on the transcriptomics of the *Acipenseridae* family, a family whose most members are critically endangered species living in habitats that get more and more polluted as time goes by. Understanding the regulatory system in the embryos of a critically endangered species with a long generation- and spawning time could help a lot in the effort to protect these ancient creatures. In addition, the study of miRNAs in a family of animals that stretches back millions of years in evolutionary time could give an insight to the evolution of miRNAs, to the role they play during embryogenesis in fishes, and the similarities in the cleavage pattern when comparing sturgeon- and amphibian embryology.

The main objective of this study was to characterize the microRNA transcriptome and to analyze the expression pattern of microRNAs during embryonal development in Siberian sturgeon in order to give some possible insight in the evolution of miRNA and their role in embryonal development in fishes.

The specific objectives in this study were

- To sequence and annotate the microRNA transcriptome of Siberian sturgeon embryogenesis using the Illumina sequencing technology.
- To analyze the miRNA expression patterns during embryogenesis and determine possible parallels with the miRNA expression in the embryogenesis of teleosts.

2. Methods

2.1. Material and sampling

The material was obtained from the Department of Sturgeon Fish Breeding in the Inland Fisheries Institute, Pieczarki, Poland.
Broodstock of Siberian sturgeon, was reared in ground ponds at 4-6º C, and later moved to an indoor facility where the water temperature gradually was elevated to 14-15º C within the two following weeks. The maturation of the eggs was assessed by measuring the diameter of the oocytes and by observing the germinal vesicle migration in ovarian biopsies. The female was induced to spawn by two injections of 6 mg/kg of body weight of *Cyprinus carpio* L. pituitary extracts and then transferred to a all-female recirculating water system held at 15º C. The males were induced to spermiate by a single injection of 1g/kg bodyweight of Ovopel. Ovulated oocytes were obtained by stripping females within 20-24 h post injection. The sperm was collected from males by stripping and placed into syringes and kept at 4º C. Sperm motility check was performed by activating the sperm using freshwater, and then observed under a light microscope. The material for this study was derived from a cross of one female and four males The eggs were then fertilized artificially by pooling the sperm from the four males together before fertilizing the eggs. The embryos were incubated in Weiss jar flow-through incubators at an average temperature of 15º C with a dissolved oxygen concentration of approximately 10 mg/l and a pH of approximately 7.5. The water flow rate ranged from 0.03 to 0.07 l/s, depending on the development of the eggs (Szczepkowski and Kolman).

The developmental stages used for this study were based on staging by Park et al., 2013, with verification of each stage under light microscope and were as following:

Table 1: List of stages and notes of sampling, time of sampling is noted as hours post fertilization (HPF)

Stage	Note
Unfertilized eg	from one single Siberian sturgeon female.
Sperm	pooled from 4 male Siberian sturgeon.
2-cell	sampled approximately 2 HPF
32-cell	sampled approximately 6 HPF.
64-512 cells	sampled approximately 7 HPF
mid phase of b	sampled approximately 10 HPF
early gastrulat	sampled approximately 19 HPF
50% epiboly	sampled approximately 23 HPF
Onset of neuru	sampled approximately 33 HPF
10 Somites	sampled approximately 50 HPF
Hatched	sampled approximately 130 HPF

The samples were taken in duplicates of ca. 100 embryos for each stage, washed twice in Phosphate-buffered saline, wrapped in aluminum foil and plunged in liquid nitrogen (-196º C). The samples were then transported by courier shipment to the University of Nordland on dry ice (-79º C).

2.2. RNA extraction

Total RNA was extracted from 5-7 eggs/larvae from each sample. Total RNA from the sperm samples was extracted using 1 ml sperm for each of the four males. The extracted RNA from the sperm samples was then pooled together for sequencing. Extraction was done using a modified TRIzol extraction protocol (the full protocol can be seen in the Appendix 4). The original TRIzol extraction protocol was based on the one-step extraction method first developed by Chomczynski and Sacchi (Chomczynski, 1993; Chomczynski and Sacchi, 1987). From each sample0.1 g was homogenized in lysis matrices (D-type lysing matrix, MP Biomedicals, Santa Ana, California, USA) along with 1 ml of TRIzol Reagent (Invitrogen, Waltgam, Massachusets, USA) in Precellys 24 homogenizer (Bertin technologies, Montigny-le-Bretonneux, France) at 5,000 rpm for 2 x 15 s. The homogenate was incubated at room temperature for 5 min in order to dissociate protein complexes. Due to high amount of insoluble materials in the homogenate, the samples were centrifuged at 12,000 x g for 10 min at 4º C before transferring to a new tube and adding chloroform. For precipitation of RNA, the aqueous phase was transferred to a new tube and 0.75 ml of 100% ethanol was added along with 2 µl of 5 mg/ml glycogen and sodium acetate (5 mM), which was equivalent to 10% of the aqueous phase recovered. The samples were mixed well and precipitated over night at -80º C. The samples were then centrifuged at 12,000 x g for 30 min. at 4º C. Pellets were washed twice with 1 ml 75% ethanol, air-dried and resuspended in nuclease-free deionized water. RNA samples were stored at -80º C. RNA quality was checked by 2% agarose gel electrophoresis, Qubit fluorometer (Invitrogen, Waltham, Massachusets, USA) and Bioanalyzer (Agilent Technologies, Santa Clara, California, USA). All samples had a 260/230 score above 1.8 and an RNA integrity number (RIN) above 7.5 (Table 2).

Table 2: RNA concentration and RIN of Siberian sturgeon samples for sequencing.

Sample ID	Developmental stage	Concentration ng/µl	RIN
N28	Unfertilized egg	3264	N/A
N29	2-Cells	2359	7,7
N30	32-Cells	2194	7,7
N31	64-512 Cells	2358	7,5
N32	Mid blastula	1468	7,6
N33	Start of gastrulation	2915	7,7
N34	50% Epiboly	2967	8,5
N35	Neurulation	2500	8,1
N36	10 Somites	3510	8,4
N37	Hatch	12061	7,8

2.3. Library preparation and sequencing

Libraries preparations and sequencing were performed at the University of Oregon, Genomics and Cell Characterization Core Facility, Eugene, USA, as a part of the FishmiR project sequencing. For library preparation,NextFlex Small RNA Sequencing Kit v2 for Illumina (Bioo Scientific, Austin, Texas, USA) was used, and the libraries were prepared follwoing the manufacturer's protocol. Adapters used had four random nucleotides (randomer) at the ends in order to decrease any bias that might arise during library preparation (Jayaprakash et al., 2011).
The first step in the protocol was to ligate the 3' adapters, which include randomers and an adenylated 3' end. Each sample was mix along with ligase, ligase buffer, 50% polyethylene glycol and a non-specified RNase inhibitor, and incubated for 2 h at 22º C.
Then the excess 3' adapters was removed by mixing the incubated samples along with 40 µl AMPure XP beads and letting the mix incubate for 5 min. The mix was then placed on a magnetic rack until the solution became clear. The supernatant was then again purified using AMPure XP beads, but now with the addition of isopropanol and adapter depletion solution, then mixed and incubated for 5 min. The samples were then magnetized until the samples were clear and the supernatant was discarded, and the samples were washed twice with 80% ethanol. The beads were resuspended in a resuspension buffer, and the supernatant in each sample was transferred to a new well and mixed with adapter depletion solution, AMPure XP beads and isopropanol before incubating for 5 min. The samples were then magnetized, the supernatant removed, and the beads again, washed twice with 80% ethanol, and the supernatant discarded. The beads are then resuspended in nuclease-free water, then magnetized and the supernatant transferred to a new well.

The ligation of 5' adapters along with the randomers was performed almost identically as for ligation of 3' adapters, except that in addition to ligase, 50% polyethylene glycol, RNase inhibitor and ligase buffer, ATP was added to the mix before incubation at 20º C for 1 h.

Reverse transcription was performed using M-MuLV Reverse Transcriptase on a mixture of sample that has had the adapters ligated, nuclease-free water, 10X M-MuLV Buffer, and deoxyribo nucleoside triphosphates (dNTPs). The samples were incubated at 44º C for 1 h followed by 10 at 90º C.

The samples were then cleaned up using similar cleanup protocol as the cleanup after the 3' adapter ligation, using AMPure XP beads, followed up by polymerase chain reaction amplification (PCR) for 12-18 cycles.

After PCR amplification, the samples were run on a TBE-PAGE gel for size selection. Following size selection, the samples were again cleaned using AMPure XP beads, with the protocol being very similar as previously described.

Sequencing was performed using a Illumina NextSeq 500 (Illumina, San Diego, California, USA) using a half flow cell per sample. Technical replicates were also sequenced from 64-512 cells, mid-blastula and early gastrulation.
The raw sequenced data were downloaded from Genomics and Cell Characterization Core Facility server in fastq format for further analysis at the University of Nordland.

2.4. Quality control and bioinformatical analysis

After removal of adapter sequences and randomers using CutAdapt (Martin, 2011), quality of the sequences were checked using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) with default settings.
Sequences with read length between 15 and 30 were annotated against all vertebrate mature miRNAs from miRBase, Release 21 (Kozomara and Griffiths-Jones, 2014) with one additional or missing nucleotide upstream, 3 additional or missing nucleotides downstream and 2 alignment mismatches on CLC Genomics Workbench Version 7.0.3 (http://www.clcbio.com). Sequences were then grouped according to mature miRNA and according to families. The data were then normalized as number of reads of each mature miRNA per million reads in each sample. The normalized data were later plotted as a heatmap using R statistical software (R Core Team, 2014) with the heatmap.2 function from the gplots package (Warnes et al., 2015). The technical replicates were normalized in the same manner and plotted with the original samples as a correlation plot, and tested for correlation using Pearson's product-moment correlation test. The correlation plot and -test were done in R.

3. Results

3.1 Quality of RNA samples before preparation

The overview of quality and concentration of total RNA samples is given in Table 2.

3.2 Technical replicates and correlation-test

A significant positive correlation was found between the two technical replicates of the three samples using Pearson's product-moment correlation coefficient (64-512 cells: R= 0.9144017, p-value < 0.001; Mid-blastula: R= 0.9082662, p-value < 0.001; Early gastrulation: R= 0.905885, p-value < 0.001). Correlation graphs of the replicates are shown as Figures in Appendix 2-4.

3.3 Next generation sequencing using Illumina NextSeq

The total read count of sequences was 142 409 023 for all samples, of which 11 225 838 were annotated as miRNAs (Table 3). In it, 414 unique mature miRNAs and 172 miRNA families were identified above the set threshold (minimum 30 normalized reads at any stage except 32-cells). All annotated reads are available in the appendix (Appendix 5: Absolute reads, Appendix 6: Normalized reads as reads per million)

Table 3: Total number of reads for each stage sampled

Stage	Total reads	Annotated MiRNAs	Percentage of Annotated reads	Number of MiRNA's
Sperm	15150066	3820173	25.22%	370
Unfertilized egg	4458030	35551	0.80%	244
2-cells	9233737	44646	0.48%	270
32-cells	8955230	17513	0.20%	85
64-512 cells	15512837	263202	1.70%	323
Mid-blastula	11871322	493518	4.16%	313
Early gastrulation	13997789	441931	3.16%	344
50% epiboly	24015110	1334219	5.56%	354
neurulation	14313326	1289215	9.01%	389
10 somites	8095880	963785	11.90%	302
Hatch	16805696	2522085	15.01%	367

Most of the reads, as shown in Fig. 3, were between 11 and 25 bp in length. Reads mapped against available ESTs (6088), rRNAs (169) and tRNAs (22 mitochondrial, 2 nuclear) from the *Acipenseridae* family compared to the mapping of reads against all vertebrate miRNAs from miRBase 21 are shown in Tables 5, 6 and 7. As shown in Fig. 4 there is a very low number of reads from samples from 2-cell and 32-cell stages that were mapped to miRBase.

Table 4: Number of mapped reads against all ESTs registered on NCBI from the family Acipenceridae.

EST	Total	Exact matches	Mutant variants
Sperm	1702046	1503130	198916
Unfert egg	387189	328497	58692
2-cell	1129687	938227	191460
32-cells	664228	523093	141135
64-cells	1715831	1385194	330637
Mid-blastula	1384386	1158084	226302
Early gastrulation	1106365	871556	234809
50% epiboly	1763957	1299860	464097
Neurulation	903957	642663	261294
10 Somites	573142	413595	159547
Hatched	1623885	1326828	297057

Table 5: Number of mapped reads against all rRNA sequences available on the SILVA rRNA database from the family Acipenceridae.

rRNA	Total	Exact matches	Mutant variants
Sperm	1171898	829449	342449
Unfert egg	149407	105086	44321
2-cell	1079543	606113	473430
32-cells	1960413	999924	960489
64-cells	2053120	1172184	880936
Mid-blastula	904905	520893	384012
Early gastrulation	1197381	634597	562784
50% epiboly	3555943	1826878	1729065
Neurulation	857933	465071	392862
10 Somites	500447	287945	212502
Hatched	1328730	927660	401070

Table 6: Number of mapped reads against all tRNA sequences available on NCBI and on the genomic tRNA database from the family Acipenceridae.

tRNA	Total	Exact matches	Mutant variants
Sperm	78723	64292	14431
Unfert egg	1094	941	153
2-cell	2838	2233	605
32-cells	2257	2202	55
64-cells	8976	7403	1573
Mid-blastula	7663	6347	1316
Early gastrulation	5564	4667	897
50% epiboly	9395	7777	1618
Neurulation	6110	4907	1203
10 Somites	1808	1432	376
Hatched	9722	8496	1226

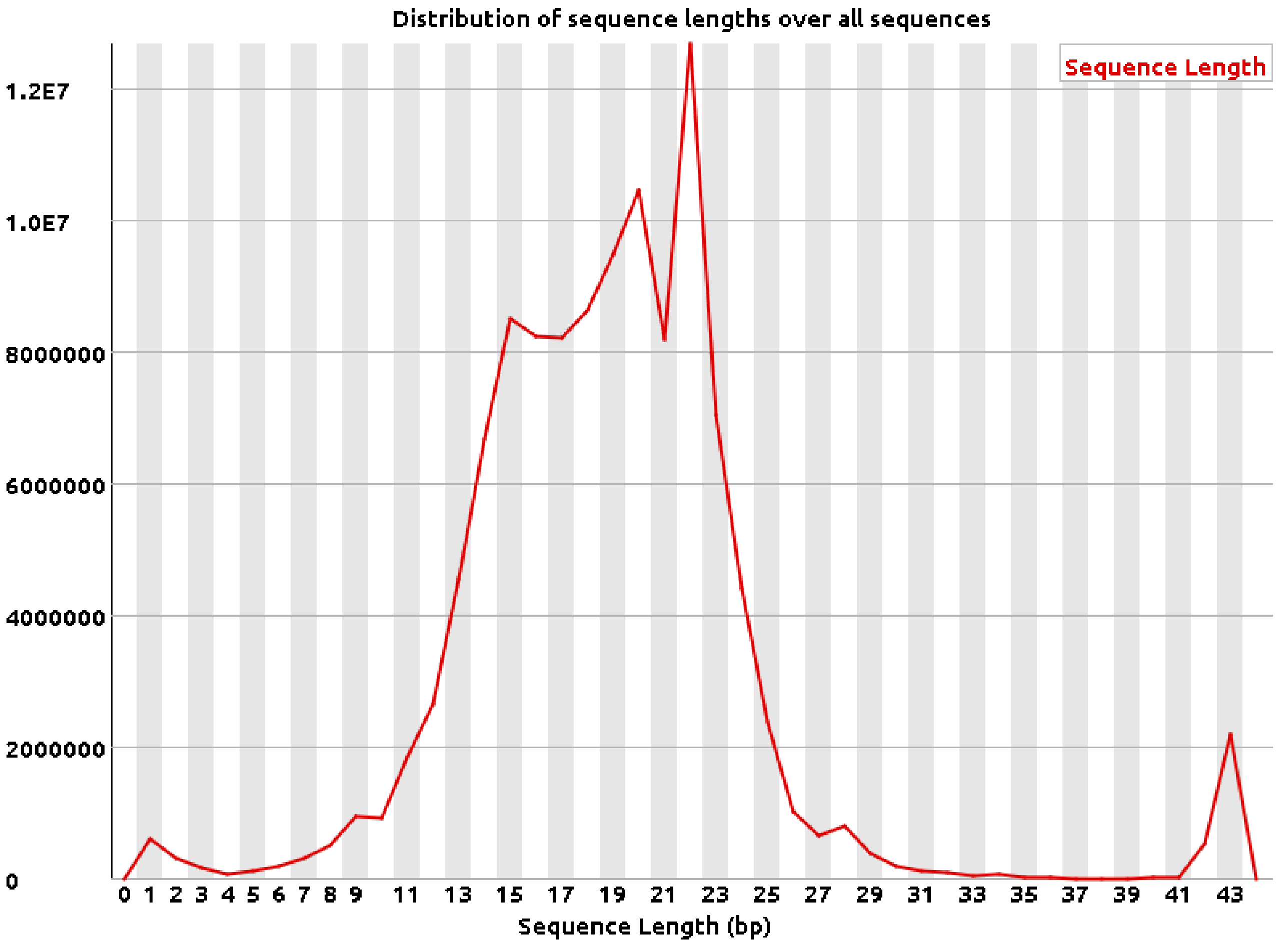
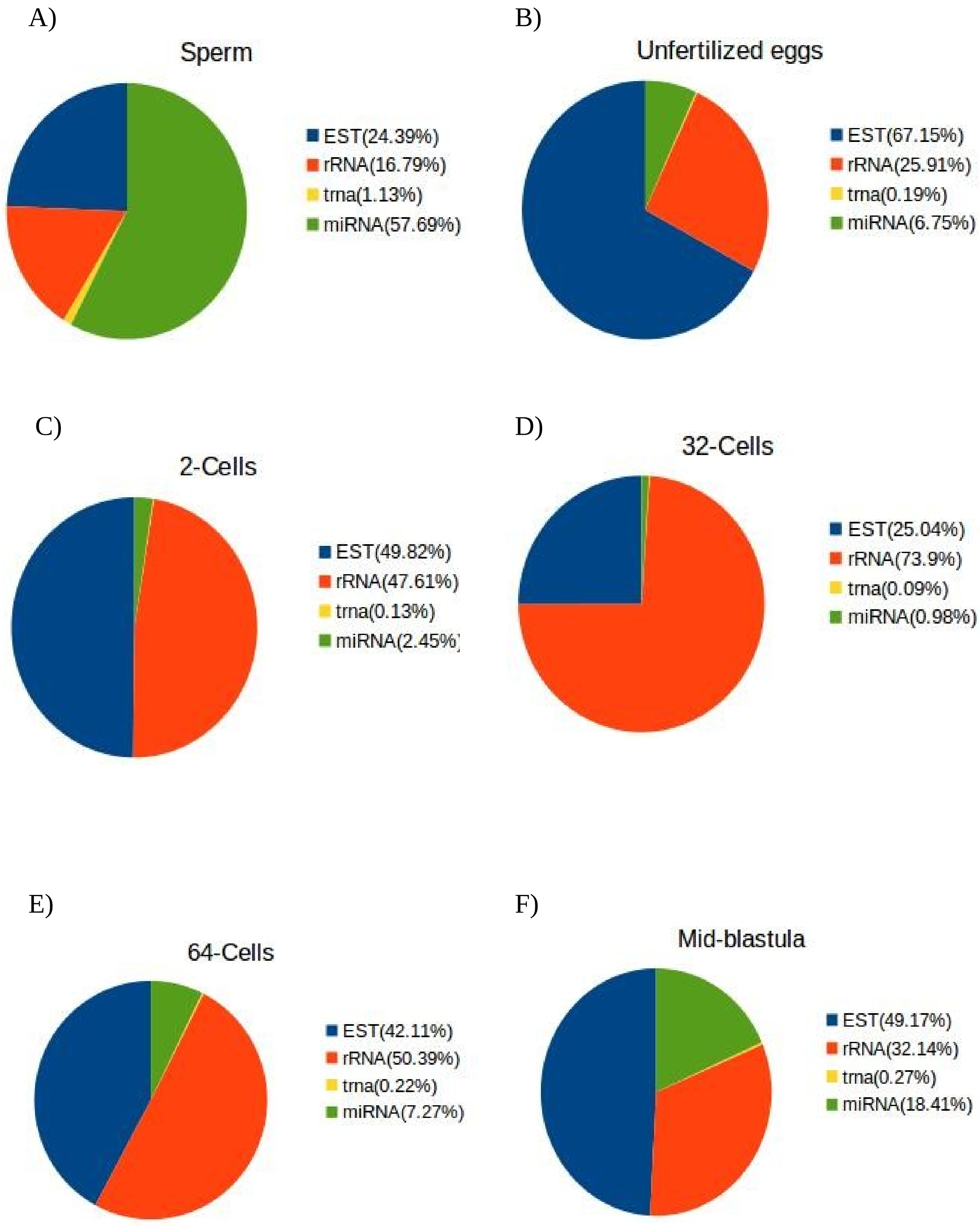
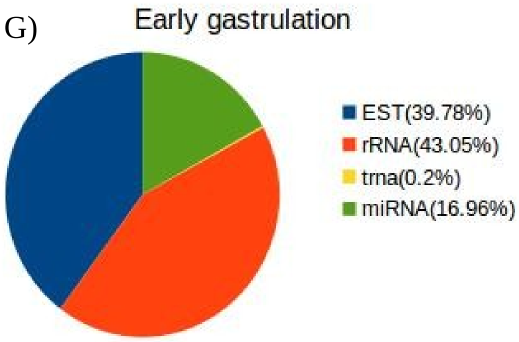


Fig. 3. *Length distribution for sequences for all samples*
The broader bump (11-25 bp) is corresponding to miRNA, although it is expected that a significant part of the sharp peak at 22 bp are miRNAs with siRNA possibly contributing. Past 37 bp we can observe possible fragments of mRNA, rRNA, snoRNA or long non-coding RNAs.

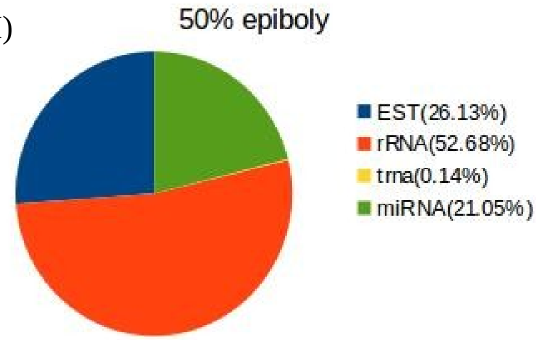


A)
Sperm
EST(24.39%)
rRNA(16.79%)
trna(1.13%)
miRNA(57.69%)
B)
Unfertilized eggs
EST(67.15%)
rRNA(25.91%)
trna(0.19%)
miRNA(6.75%)
C)
2-Cells
EST(49.82%)
rRNA(47.61%)
trna(0.13%)
miRNA(2.45%)
D)
32-Cells
EST(25.04%)
rRNA(73.9%)
trna(0.09%)
miRNA(0.98%)
E)
64-Cells
EST(42.11%)
rRNA(50.39%)
trna(0.22%)
miRNA(7.27%)
F)
Mid-blastula
EST(49.17%)
rRNA(32.14%)
trna(0.27%)
miRNA(18.41%)

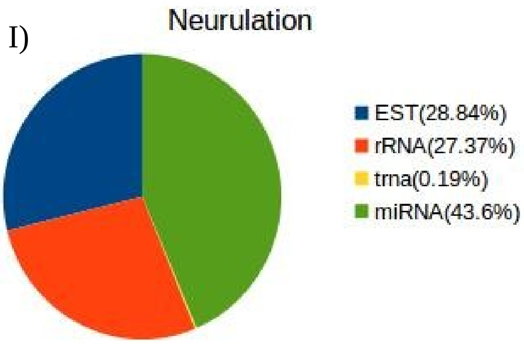
G)



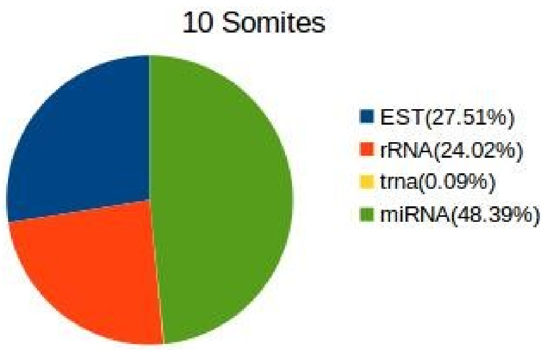
H)



I)



J)



K)

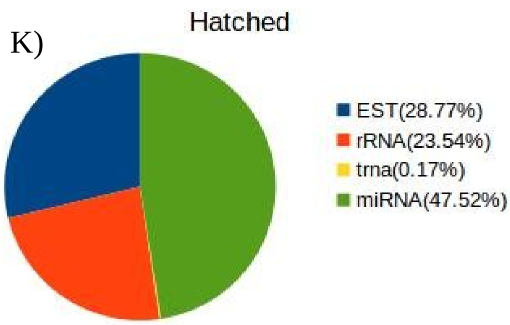


Fig. 4. *Mapping statistics of each sample. The pie charts show the percentage of mapped reads that were mapped to each RNA class.*

3.4. Characterization of miRNAs during the development of Siberian sturgeon

The number of sequences annotated as miRNAs varied vastly among samples, with some samples having 25% of the sequenced reads annotated and others having less than 1%. As the number of annotated miRNAs in the 32-cell stage was considerably low, it was removed from the further analysis as it could potentially affect the results after normalization. The potential misrepresentation could be observed as this was also the sample with the least variety in numbers of miRNAs represented with only 85 miRNAs in comparison to the second lowest sample, 2-cells, with 270 different miRNAs. There was a relation between the number of miRNAs annotated and the number of different miRNAs in each sample (Fig. 5)

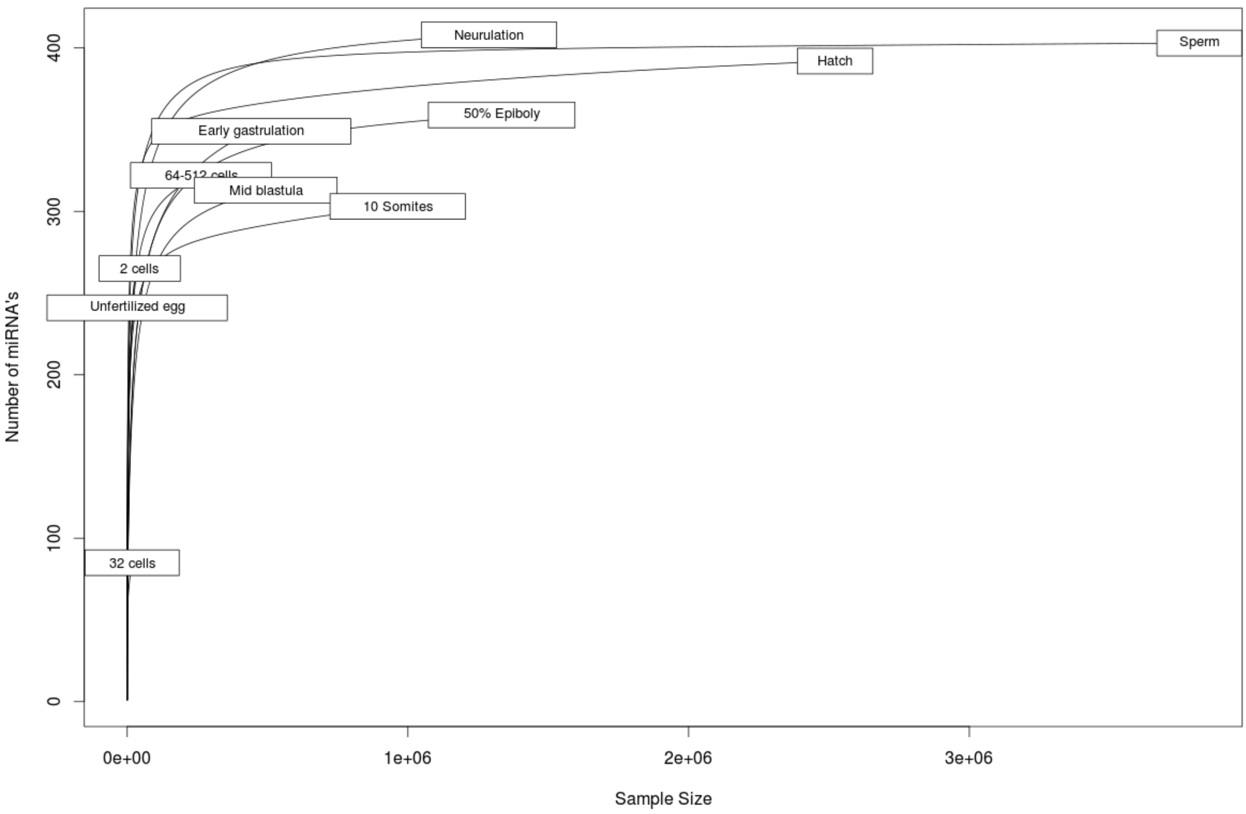


Fig. 5. *Rarefaction curve showing the number of miRNAs sequenced as a function of reads. This type of curve is used to determine if the sampling or depth of sequencing was sufficient to give a representative result. The number of miRNAs reach a plateau at between 300 and 400 miRNAs, with the exception of Unfertilized egg, 2-cell and 32-cell, which stop while the curve is still increasing.*

The miRNA expression between 64-512 cells stage and 50% epiboly stage dominated by variants of miR-430, whilst gamete samples and 2-cells samples had much higher variation in of highly expressed miRNAs (Fig. 6).

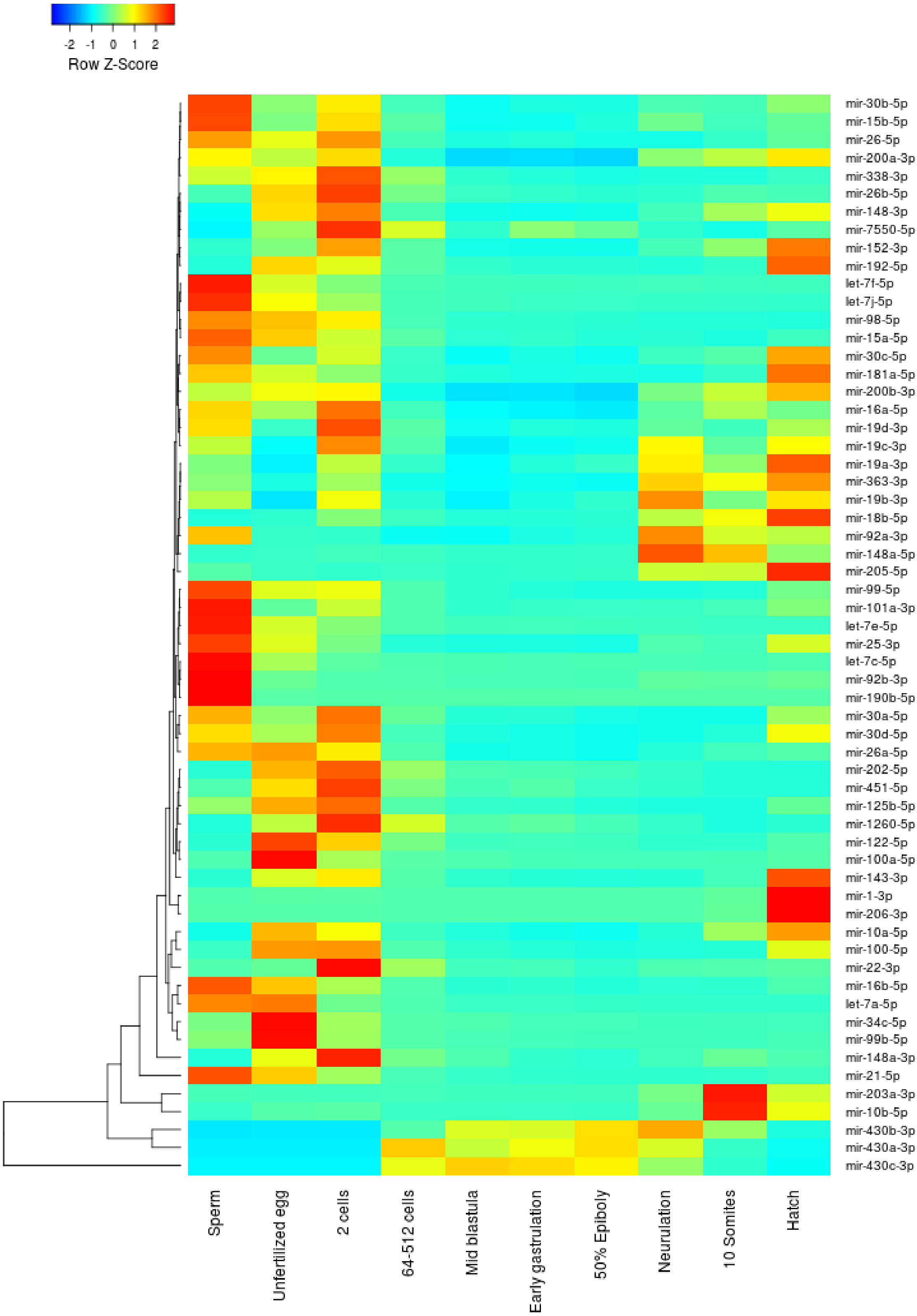


Fig. 6. *Heatmap of the 60 most numerous miRNAs, plotted as the log of the normalized reads (per million) for each miRNA.*

3.5. Patterns of miRNA expression during development

miRNA expression profiles were clustered in the three groups (Fig. 6): Group one is gametes and early development (gametes and 2 cells), group two is mid development (64-cells, mid blastula, early gastrulation and 50% epiboly), and group three is late development/hatched (neurulation, 10 somites and hatch). Quite a few different miRNAs showed a gradual decrease in expression, starting with high expression at unfertilized eggs, such as miR-21-5p, miR-34c-5p, let-7a-5p, miR-99b-5p, miR-202-5p, miR-451-5p, miR-1260-5p, let-7e-5p, let-7f-5p, let-7j-5p and miR-98-5p (Fig. 7 A, Fig 8 A and D). At the 2-cell stage, there were some miRNAs showing an increase in expression in comparison to the unfertilized egg samples and then decrease in expression, such as miR-148a-3p, miR-22-3p, miR-30a-5p, miR-30c-5p, miR-30d-5p, miR-30b-5p, miR-1260-5p,miR-152-3p and miR-26b-5p (Fig 7 D, Fig 8 E). With the exception of miR-1260-5p, expression of these miRNAs was increasing during later stages of development (neurulation and onward). At the 64-512 cells stage, miR-430a-, b- and c-3p became highly expressed and remained highly expressed until neurulation, when the transcript level started to decrease in prevalence (Fig 7 B, Fig 8 F). Lastly, some miRNAs reach peak of expression at later embryonic stages, or even after hatching, such as miR-203-a-3p, miR-10b-5p, miR-1-3p, miR-206-3p, miR-205, miR-148a-5p and miR-92a-3p (Fig 7 C, Fig 8 B and C).

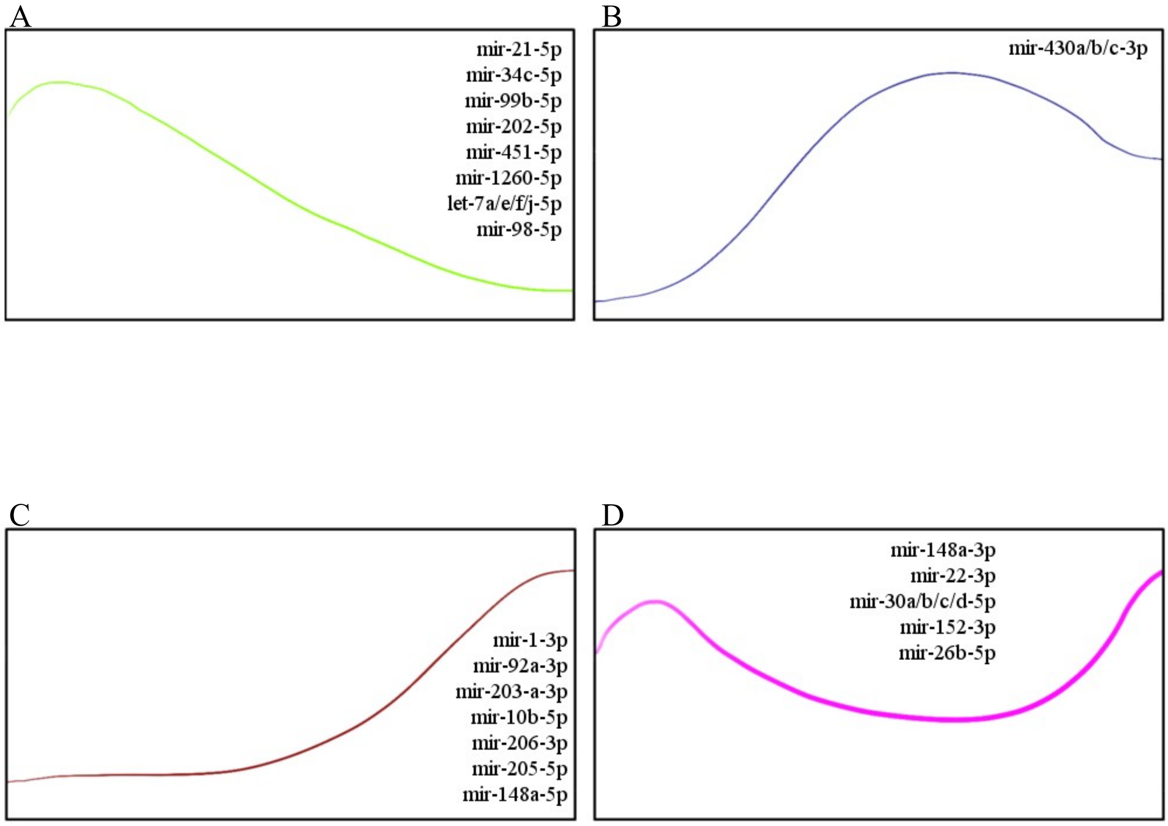


Fig. 7 *Schematic outline of the expression patterns of miRNAs throughout embryonic development of Siberian sturgeon. A. High expression during earlier stages with a gradual decrease. B. Low expression in early stages of development, followed by a gradual increase with peak during late-mid development. C. Low expression in early stages of development followed by an increased expression during late development. D. High expression in early development, followed by an decrease in expression during mid development, and again an increase during late development.*

Fig. 8

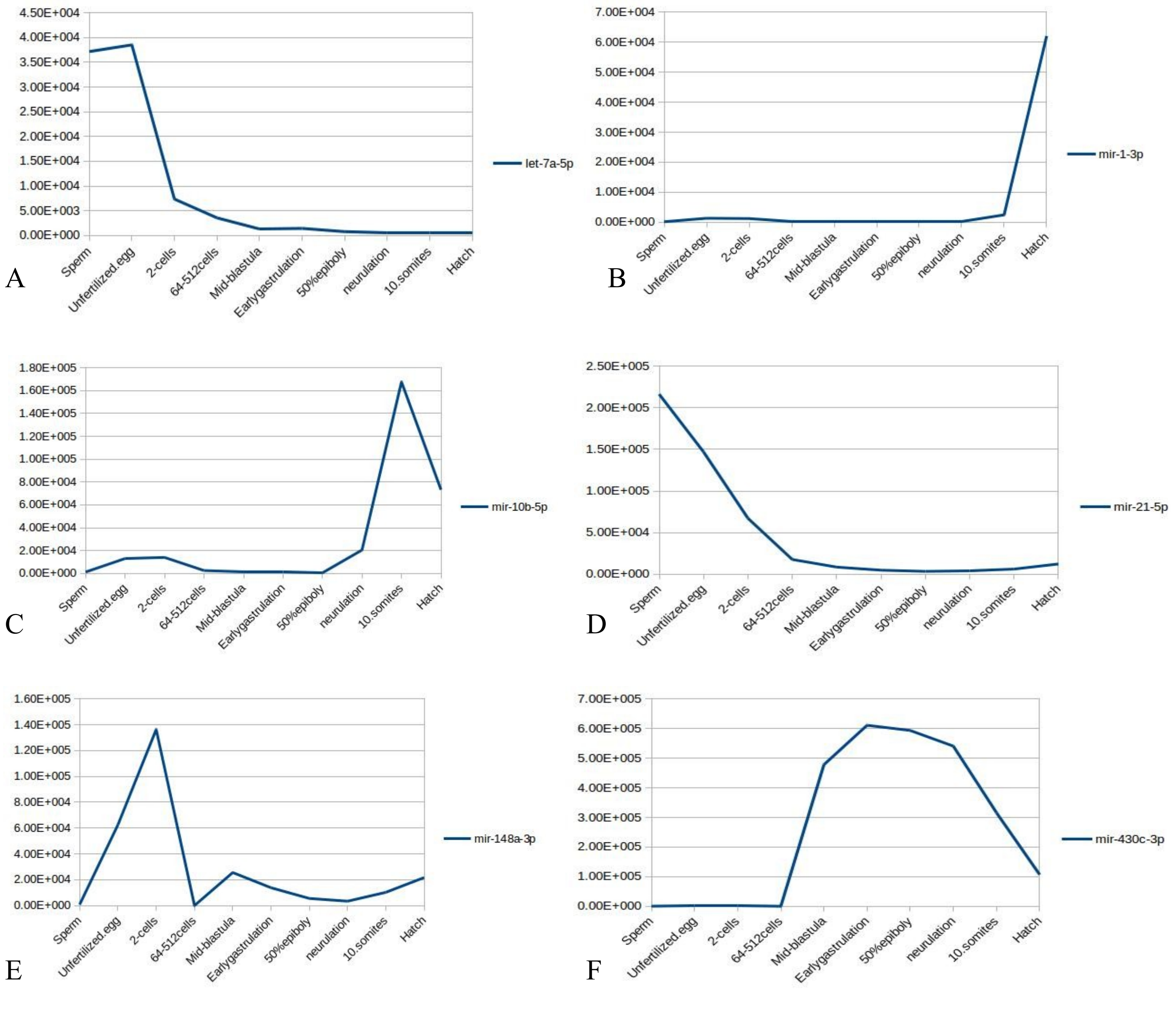


Fig. 8. *Expression profiles of six different miRNAs with different expression patterns.*
A: let-7a-5p B: miR-1-3p C: miR-10b-5p D: miR-21-5p E: miR-148a-3p F: miR-430c-3p.

4. Discussion

4.1. MicroRNA diversity during embryonic development of Siberian sturgeon

Due to the low percentage of reads annotated from the unfertilized eggs, 2-cells and 32-cells, the number of mature miRNAs in the rarefaction plot had yet not leveled off in to a plateau, thus indicating that the sequenced reads are not a full representation of the miRNA diversity in these samples. The cause for this might be that the sequencing depth might not been adequate. When determining the methods for sequencing, it is important to determine what will be prioritized: the depth of sequencing of a sample, thus getting even the lesser expressed miRNAs, or differential sequencing, sequencing several biological replicates. Earlier research on early development in teleost fish, such as in Atlantic halibut, *Hippoglossus hippoglossus* (Bizuayehu et al., 2012a), Atlantic cod, *Gadus morhua* (Bizuayehu et al., 2015) and zebrafish, *Danio rerio* (Chen et al., 2005), have shown that there is a low diversity of miRNAs during this period

The sperm samples were collected by stripping and contained seminal fluid. There was a possibility of contamination of samples with somatic cells. It might be an explanation to why there is such a high diversity of miRNAs in sperm, though there might be a biological reason for the high diversity.

miRNAs play an important role in the differentiation of tissues and cells, such as miR-21 in the formation of the heart valve (Banjo et al., 2013), or miR-122 which is a liver-specific miRNA (Girard et al., 2008). Yet these two miRNAs are highly expressed in unfertilized egg and sperm respectively, indicating that they are there either to target transcripts during early zygotic development, or are there as a bi-product from the production of gametes.

4.2. miR-430 as a regulator during maternal-to-zygotic transition

One of the most vital points during early development is the maternal-to-zygotic transition (MZT). During early development, all processes are entirely controlled by the maternal mRNA, proteins and other vital components which are stored during oogenesis. Early observation of cell division without using chromatin during the initial 8 cell divisions of sea urchin suggested that early embryogenesis was maternally controlled (Harvey, 1936). In the time since then, our knowledge about early embryogenesis has developed and grown, yet there is need for further investigation in the role and organization of maternally stocked transcripts and proteins, the processes involving and regulating the transition from maternal-to-zygotic controlled transcription. It has been suggested that MZT is a two step process (Tadros and Lipshitz, 2009), first the maternally stocked transcripts are removed, followed by the activation of zygotic transcription. This process is similar, if not identical, in all metazoa, although the time and scale of these processes may vary among different species and taxa. For instance in *Drosophila melanogaster* the continuous cycle of cellular division in a fertilized egg increases the proportional size of the nucleus (nucleocytoplasmic ratio) in each cell until it reaches a threshold and activates MZT (Pritchard and Schubiger, 1996). This process has also been suggested in zebrafish (Dekens et al., 2003). Among these regulatory elements during early embryogenesis, miRNA have been implicated in the clearance of maternally stocked transcripts. In zebrafish, the miRNA of importance during the clearance of maternal transcripts have zygotic transcription (Giraldez, 2006), marking the end of the maternal control of embryonic development.

Among early zygotic transcripts the miRNA family miR-430 has been shown to be an important cleaning factors for maternal mRNAs (Giraldez, 2006). This clearance is done by the reducing translation, destabilizing and degradation of maternal mRNAs. Although not validated experimentally, the miRNA family miR-430 were characterized in different teleost during early embryonic development in medaka (Tani et al., 2010), Atlantic halibut (Bizuayehu et al., 2012a) and Atlantic cod (Bizuayehu et al., 2015) targeting possibly hundreds of different maternal mRNAs. Although not necessary for all types of specifications in developing embryos, they do facilitate the further development (Schier and Giraldez, 2006). This was shown by Schier and Giraldez by using zebrafish mutants that were lacking the protein Dicer, an essential component in the miRNA biosynthesis assembly line. Half of the mutants were observed as they developed, and the other half were injected during the one cell stage with the miRNA duplex of miR-430a and miR-430b. They showed that miRNAs are not necessary for cell differentiation, although the embryo would develop defects in retina, brain trunk and tail. The embryos that were injected with processed miR-430 did develop physiological defects during morphogenesis but at a much later stage than their non injected counterpart. By promoting the decay of maternal mRNAs, which might inhibit or delay development, prior to the zygotic genome activation using the mass-targeting approach of miRNAs, the zygotic mRNAs can be expressed unobstructed and efficiently. This same process has been shown in *Xenopus laevis,* but mediated by a different miRNA, miR-427 (Lund et al., 2009). Although miR-427 is the main miRNA responsible for targeting maternal mRNAs for deadenylation, Lund et al. (2009) showed that some maternal mRNAs can also be targeted by miRNAs in the let-7 family. Whether this is the case in Siberian sturgeon is yet unknown. Furthermore, these miRNAs have roles in cell fate specification, cell migration and primordial germ cell development.

The expression of the miR-430 family in Siberian sturgeon appears to have a similar pattern to that of teleost, as there is a sharp increase in the 64-512 sample. Although it is difficult to determine when the transcription started somewhere between 2-cells and 64-512, as there is not good enough resolution during this period. The results show that the expression of the most prevalent miRNAs in the miR-430 family have slightly different expression patterns with peaks at different points during development, which suggests they might have different sets of targets even though they have similar roles in an overlapping period of time. Previously, miRNAs of the same family, even with same seed sequence, have been shown to target different sets of mRNAs, target sites and having opposing roles. Taken together, the conserved role of the miR-430 family in MZT and different early developmental processes can be speculated for Siberian sturgeon, as the sequencing data showed similar expression pattern at comparable developmental stages with that of teleost.

4.3. let-7 and possible sources

The expression of miRNAs in the let-7 family has been studied in Atlantic halibut where it has been shown to be highly expressed in the gonads of adults (Bizuayehu et al., 2012b). In that study the authors looked for sexually dimorphic expression of miRNAs in brain and gonads of Atlantic halibut males, females and masculinized genetic females of three different age groups. They found that there is a significant up-regulation of let-7 in mature male gonads compared to the gonads of immature female of the same age. It has also been shown that let-7 is significantly higher expressed in adult zebrafish than in developing embryos (Soares et al., 2009) and has been shown to be the most abundant miRNA present in the unfertilized eggs of rainbow trout (Ma et al., 2012), accounting for 24% of the reads from known miRNAs. The expression of let-7 has also been studied in mammalian testis where it has been shown to be important in spermatogenesis (Tong et al., 2011). The high presence of let-7 in both sperm and unfertilized eggs of Siberian sturgeon, and then low to no presence during embryonic development stages might be an indication of contaminated samples. Although, as mentioned earlier, miRNAs in the let-7 family have been shown to target maternal mRNA in *xenopus laevis* (Lund et al., 2009), which might give some biological meaning to the high presence of this miRNA. The miRNA might be in the sample due to flaws during sampling, or as a bi-product of spermatogenesis/oogenesis.

4.4. miR-10 possibly regulating Hox-genes in Siberian sturgeon

It has been suggested that some miRNAs in the miRNA family miR-10 target Homeobox-genes (Hox-genes), which play an important role in the development of anterior-posterior axis in metazoans such as fruitfly (Enright et al., 2003) and zebrafish (Woltering and Durston, 2008) although the research on the link between miR-10 and Hox genes in fruit fly have been disputed (Lemons et al., 2012).
Interestingly, in their 2008 paper, Woltering and Durston presented evidence showing miR-10c is located in the same primary transcript as one of the target sequences HoxB4a, then the miRNA is spliced out from the transcript which is then processed in to a mature mRNA. Mir-10c is when matured able to target the primary transcript from which it was spliced out from, functioning as a self regulating mechanism, as well as regulating the expression of HoxB4a.

The expression pattern of miR-10 in sturgeon appears to be similar to the expression of other teleost, such as zebrafish (Chen et al., 2005) and cod (Bizuayehu et al., 2015). Though it is difficult to assess a more precise image as the sampling resolution were not optimal for comparisons.

4.5. Expression differences in miR-1 suggest possible differences in muscle development.

During late development, muscle cells start to differentiate. One very important miRNA in this process is miR-1, which has been shown that the deletion of this miRNA causes several dysfunctions in myocytes in mice (Zhao et al., 2007) and zebrafish (Mishima et al., 2009). The expression of miR-1 along with miR-206 and miR-133 is initiated by myogenic regulatory factors (MRF) in the bHLH protein family such as Myf5 and Myogenin. Another MRF in the bHLH family, MyoD, although it is not an initiation factor, it can upregulate the expression of miR-1 (Sweetman et al., 2008). miR-1 and miR-133 have been shown to be regulators of actin-related proteins, such as actin binding proteins and actin structure in sarcomeres in zebrafish (Mishima et al., 2009). Regarding the expression of miR-133a and miR-133b, both miRNAs have been shown to be expressed in zebrafish during the segmentation period, while miR-1 is detectable as expressed during pharyngula and miR-133c is not detected before hatching (Chen et al., 2005). In sturgeon we have observed that the expression of miR-133a-3p and miR-1-3p (Fig. 6) is only shown in abundance at the hatched stage. This is later than what has been shown in zebrafish, suggesting slightly different muscle development in these two species.

4.6. Conclusion

The aim of this study was to sequence the microRNA transcriptome of Siberian sturgeon during embryonic development, something that has never been done before in a Chondrostei fish.

414 unique miRNAs were identified across 172 miRNA families were identified.

The presence of several isomiRs of miR-430 in Siberian sturgeon was confirmed with expression pattern coinciding with what is observed in teleost fishes during the maternal-to-zygotic transition.

The expression pattern of many prevalent miRNAs were classified and compared to homologous sequences in teleost. Most notably the expression of miR-430 showed possible clearance pathways for maternal mRNAs.

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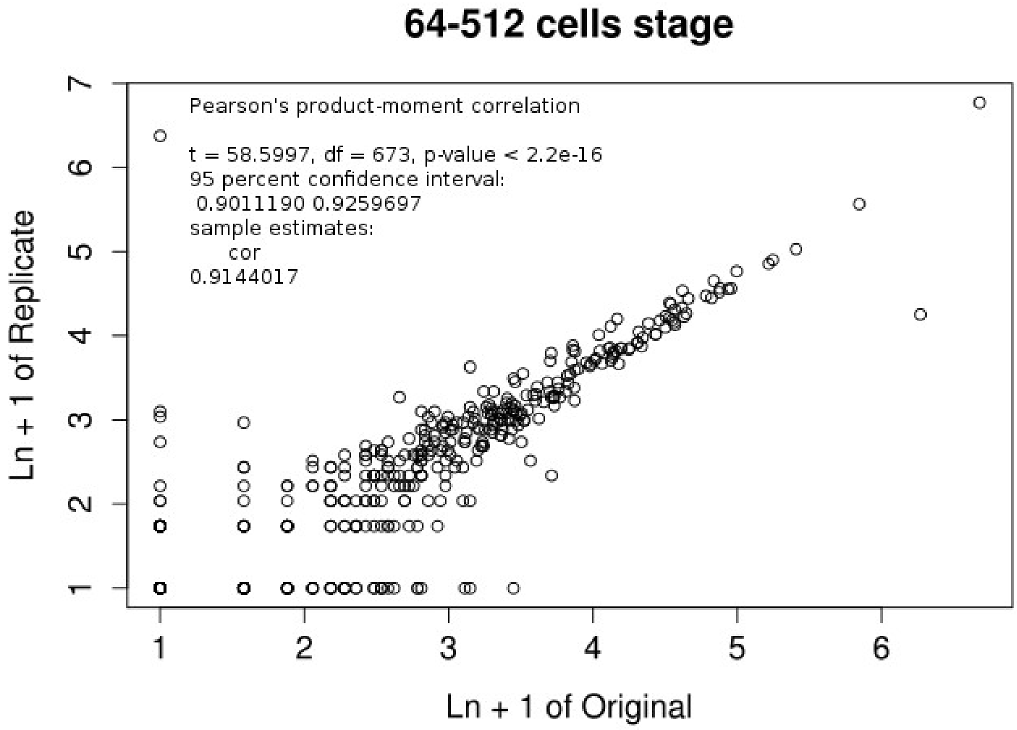
Appendix 1

RNA extraction protocol

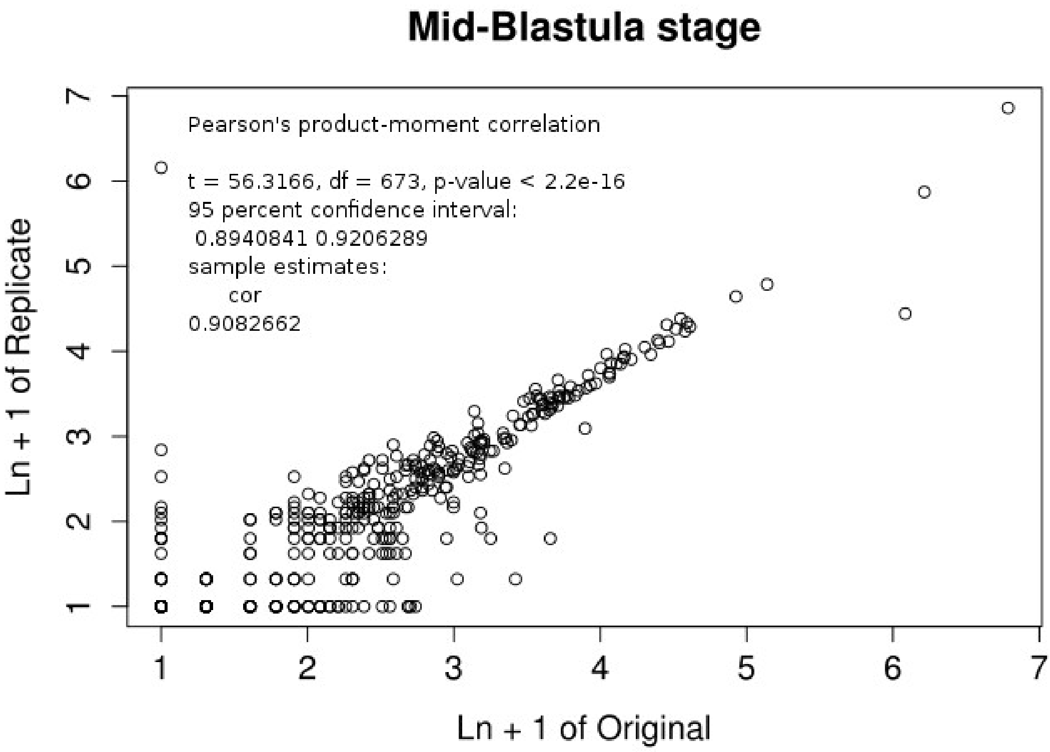
Homogenized 50 – 100 mg sample per 1 ml TRIzol. Use 5000 rpm for 15 seconds twice.
Keep the homogenated samples at room temperature for 5 min, but not more than 10 min.
If a lot of insoluble material exists after homogenization and 5 minute room temperature incubation, remove by centrifugation at 12,000 x g for 10 min at 4°C before adding chloroform.
Transfer the clear homogeneity to a new 1.5 ml tube. NOTE: If fatty layer is visible on top avoid it during transfer. Add 200 µl of chloroform per ml TRIzol, mix well (vortex for 15 sec) and incubate at room temperature for 2-3 min. Centrifuge samples for 15 min. at 12,000 x g at 4°C.
Transfer the aqueous phase to a fresh tube. The aqueous phase is the colorless upper phase that corresponds to ~60% of the volume of TRIzol used. Precipitate the RNA by mixing with 0.75 ml of ethanol (100 %) per ml of TRIzol. Add Sodium acetate (10 % of the recovered aqueous phase volume) and 2 µl of glycogen (5 mg/ml) or linear acrylamide. Mix well and incubate overnight at –70 °C. Centrifuge samples for 30 min. at 12,000 x g at 4 °C. Remove the supernatant. Wash pellet with 1 ml 75% ethanol for every 1 ml of TRIzol used. Mix sample by flicking and inverting the tube or vortexing and centrifuge at 7500 x g for 5 min. at 4°C. Repeat the above step, to remove any remaining salt. Air dry RNA. NOTE: Be careful not to over dry it. A dried RNA pellets are usually white or may have a clear jelly-like appearance. Resuspend RNA with RNAase free water on ice. The volume of water may vary depending on pellet size.

Appendix 2



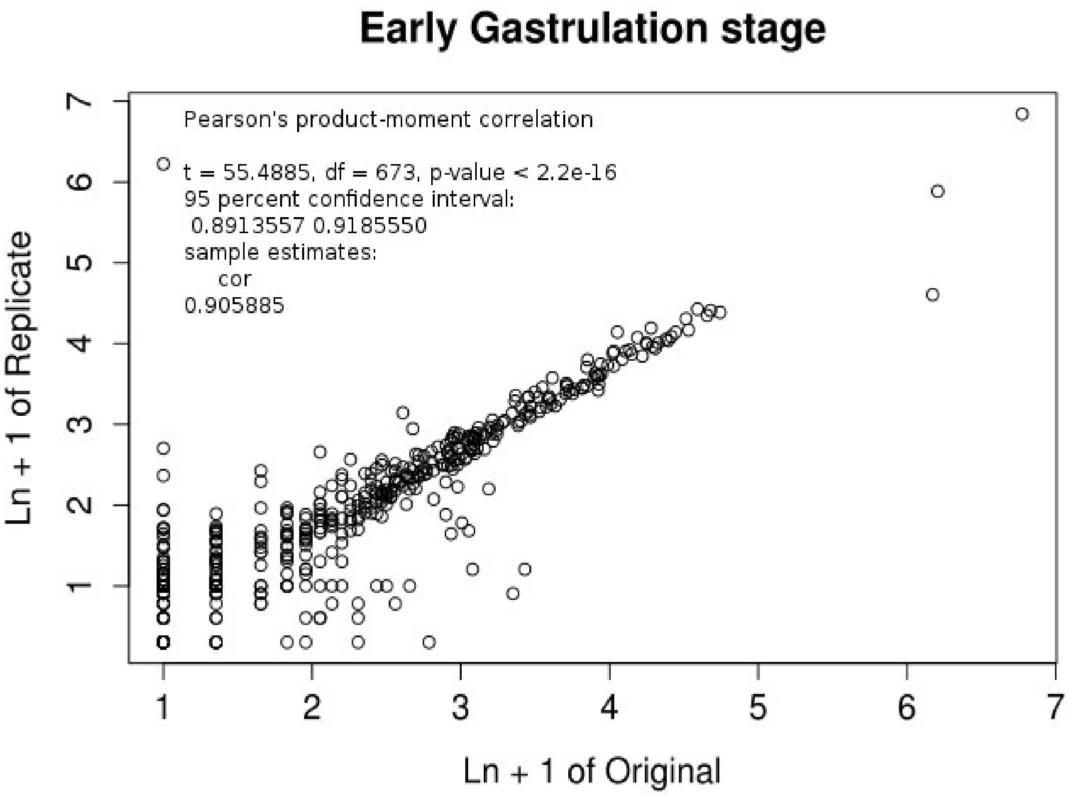
Correlation graph of sample and technical replicate for 64-512 stage

Appendix 3



Correlation graph of sample and technical replicate for mid blastula stage

Appendix 4



Correlation graph of sample and technical replicate for early gastrulation stage

Appendix 5

miRNA	Sperm	Unfertilized eggs	2 Cells	32 Cells	64-512 Cells	Mid blastula	Early gastrulation	50% Epiboly	Neurulation	10 Somites	Hatch	Sum
mir-430c-3p	92	64	47	8	125859	301645	262356	721368	407008	102760	59659	1980866
mir-430b-3p	33	36	29	10	18456	80907	71015	278774	318150	114819	85175	967404
mir-21-5p	826787	5228	3001	1329	4639	4170	2107	4483	5175	5871	30844	893634
mir-430a-3p	33	8	9	3	48897	59891	65696	231731	171061	37348	36831	651508
mir-203a-3p	20669	98	97	302	834	389	370	1878	48045	281283	250683	604648
mir-10b-5p	4390	461	623	197	638	680	471	641	26358	161641	184759	380859
mir-16b-5p	199357	1342	865	426	1989	1221	1227	1986	3403	2770	16781	231367
mir-10a-5p	6159	1590	1521	608	1975	1929	859	1330	5804	20658	122530	164963
mir-1-3p	477	46	53	2	54	47	38	289	226	2328	156464	160024
let-7a-5p	142067	1370	328	150	934	642	623	1022	691	419	1347	149593
mir-25-3p	98860	441	283	39	362	397	366	721	4953	2977	30126	139525
mir-26a-5p	91659	917	891	308	1597	994	938	1958	4212	5032	16562	125068
mir-30c-5p	69067	194	473	224	839	409	778	1520	4394	4325	42159	124382
mir-190b-5p	119220	13	3	0	27	27	14	17	39	85	400	119845
mir-30a-5p	83105	338	1158	275	1746	1253	1147	2010	1571	1134	26324	120061
mir-101a-3p	99256	117	419	418	582	229	364	1178	1266	1653	13350	118832
mir-206-3p	1952	22	25	0	19	110	45	157	394	1970	110502	115196
mir-100-5p	19575	1676	2136	1648	2275	1875	1054	1852	2197	2525	72972	109785
mir-200b-3p	40560	452	599	149	894	716	679	1503	9896	10396	40444	106288
let-7e-5p	103471	350	221	79	337	235	229	407	219	44	101	105693
mir-148a-3p	2932	2222	6087	1026	6724	6809	2443	4525	13205	20868	33568	100409
mir-92b-3p	86926	66	27	0	150	64	63	104	1801	1086	4534	94821
let-7c-5p	92300	194	54	2	127	75	43	167	101	1	706	93770
mir-99-5p	78048	351	473	115	716	569	297	487	524	638	11242	93460
mir-30d-5p	53577	294	829	570	888	805	537	975	1343	1785	30107	91710
mir-205-5p	8676	27	13	269	171	74	107	646	11731	8634	60157	90505
mir-181a-5p	44331	269	220	0	381	291	130	561	358	1301	37645	85487
mir-143-3p	2510	478	780	186	974	459	205	347	528	2513	72347	81327
mir-19c-3p	29005	62	584	2	1096	546	839	2868	12443	4382	23982	75809
mir-19d-3p	40428	90	696	960	969	496	789	2181	5165	2665	16574	71013
mir-16a-5p	40976	237	623	433	911	730	464	1119	5507	6585	12647	70232
mir-22-3p	23761	311	2696	751	4343	2027	2000	2828	7001	5890	18325	69933
mir-92a-3p	32851	59	70	190	133	188	429	1652	12910	5637	13277	67396
let-7f-5p	62422	219	131	15	191	162	139	347	555	232	870	65283
mir-125b-5p	33917	781	1180	448	1169	1095	898	1446	928	737	14194	56793
let-7j-5p	54419	244	166	0	175	230	98	217	120	30	60	55759
mir-34c-5p	40959	2707	803	372	1087	1622	603	1635	703	757	2178	53426
mir-223-3p	50781	30	36	0	53	39	54	160	78	3	628	51862
mir-9-5p	12617	35	11	0	20	44	43	284	846	803	34037	48740
mir-30b-5p	35021	107	256	58	413	207	348	987	2180	1550	7638	48765
mir-148a-5p	71	10	33	0	140	44	35	143	23202	12414	11571	47663
mir-19a-3p	11297	6	191	6	387	181	499	2003	7511	3136	22345	47562
mir-142-3p	41445	34	120	1	195	87	69	210	175	263	4880	47479
mir-200a-3p	21109	156	267	186	472	300	289	694	4522	4162	14436	46593
mir-363-3p	11958	33	161	1	198	222	310	763	8275	5138	19286	46345
mir-19b-3p	14857	7	223	3	349	196	471	1961	9331	2684	14313	44395
mir-99b-5p	34932	1891	541	175	766	714	384	784	775	394	3035	44391
mir-15b-5p	26367	82	210	1	466	333	312	1284	2763	1401	5010	38229
mir-18b-5p	804	16	112	0	208	122	120	483	4897	4950	24674	36386
mir-98-5p	34279	270	281	0	276	193	173	347	198	145	166	36328
mir-30e-5p	21010	7	182	93	304	167	224	530	548	505	12517	36087
mir-15a-5p	31216	211	171	67	364	222	254	463	534	169	2065	35736
mir-146a-5p	32658	154	253	1	289	254	156	322	252	380	770	35489
mir-457b-5p	30637	31	15	0	53	27	54	121	896	354	3020	35208
mir-26-5p	26635	162	317	0	350	253	289	517	465	948	4670	34606
mir-152-3p	3720	107	376	1	539	197	166	443	2019	3251	23658	34477
mir-132-3p	32355	0	0	0	4	0	0	0	3	2	161	32525
mir-130b-3p	2211	14	2	0	74	49	53	337	3467	2630	22810	31647
mir-338-3p	22423	270	554	190	1125	572	376	786	988	732	3719	31735
mir-219a-5p	25	0	0	0	7	2	0	24	764	3053	25196	29071
mir-181a-3p	872	23	31	0	61	49	25	220	1175	2195	23830	28481
mir-208a-3p	4620	6	0	0	1	1	1	11	8	242	22856	27746
mir-181b-5p	11413	70	10	0	101	49	38	213	318	865	13451	26528
mir-133a-3p	937	1	0	1	4	5	5	26	49	137	25113	26278
mir-451-5p	13436	667	1366	445	1812	1405	1723	2351	1828	459	967	26459
mir-192-5p	676	224	209	1	360	257	130	401	227	516	22690	25691
mir-499-5p	3482	4	11	0	17	33	22	47	21	212	21427	25276
mir-20b-5p	9686	28	27	0	21	72	126	778	5230	1765	7396	25129
mir-16-5p	22858	216	263	0	191	178	182	267	336	93	131	24715
mir-429a-3p	10532	49	61	1	84	75	36	242	2226	3222	8113	24641
mir-1329-5p	21128	46	14	0	61	18	38	58	160	431	2211	24165
mir-128-3p	19361	48	69	0	142	53	71	158	650	382	2554	23488
mir-203b-5p	171	0	0	0	76	18	13	97	12965	6792	2999	23131
mir-1388-5p	17700	45	91	0	67	68	43	60	205	1214	1907	21400
mir-101b-3p	3526	11	325	343	714	139	249	705	3124	1581	10690	21407
mir-221-5p	11073	26	8	1	40	79	29	56	1304	1465	6914	20995
mir-20a-5p	5538	15	33	0	86	114	125	510	4481	1710	8269	20881
let-7i-5p	20250	22	11	0	39	20	6	26	49	0	18	20441
mir-196a-5p	12083	34	7	0	26	20	23	58	263	394	6865	19773
mir-16c-5p	13909	114	294	0	294	260	228	458	803	705	2049	19114
mir-99a-5p	17473	85	105	0	106	112	36	57	43	69	271	18357
mir-199a-3p	4189	31	23	0	71	40	17	126	57	223	12638	17415
mir-106a-5p	4806	14	42	163	91	26	125	427	4173	1301	6170	17338
mir-148-3p	650	176	293	3	345	203	147	542	1563	2772	10483	17177
mir-34a-5p	2999	77	64	93	136	76	68	253	531	1176	11648	17121
mir-126-3p	7473	8	51	0	80	20	38	135	54	386	8595	16840
mir-184-3p	331	51	37	0	53	62	32	83	238	771	14986	16644
mir-1260-5p	2196	285	935	552	2387	1444	1500	3019	1841	434	2529	17122
mir-148b-5p	455	12	31	0	34	18	27	195	3977	6367	5412	16528
mir-202-5p	2358	875	1424	96	2606	1747	1442	3519	1980	275	242	16564
mir-216b-5p	4632	4	0	0	17	4	0	30	175	145	11297	16304
mir-142a-3p	13894	8	41	0	120	24	25	43	96	0	1005	15256

Absolute reads

miRNA	Sperm	Unfertilized eggs	2 Cells	32 Cells	64-512 Cells	Mid blastula	Early gastrulation	50% Epiboly	Neurulation	10 Somites	Hatch	Sum
mir-218a-5p	1684	8	5	0	47	10	20	92	782	897	11622	15167
mir-29a-3p	13314	34	36	0	183	48	112	307	371	79	215	14699
mir-150-5p	13906	22	23	0	69	35	23	30	47	0	9	14164
mir-222-3p	7167	41	49	0	42	12	45	46	291	277	6044	14014
mir-7550-5p	1385	97	324	595	960	569	1093	2659	1434	762	4413	14291
mir-7a-5p	4076	121	187	454	135	148	103	314	1756	2638	3473	13405
mir-140-3p	7177	21	15	0	25	75	16	49	365	451	4603	12797
mir-142-5p	12048	19	54	13	66	19	58	79	55	77	321	12809
mir-148b-3p	3185	205	165	0	251	172	191	341	1010	730	5779	12029
mir-182-5p	2596	31	5	0	26	0	16	49	410	1337	7370	11840
mir-101-3p	9209	14	175	2	254	71	128	287	345	163	1180	11828
mir-122-5p	37	834	682	390	1205	584	568	1626	407	233	5640	12206
mir-17-5p	3764	5	12	4	68	39	72	324	2108	760	4628	11784
mir-208b-3p	9587	0	0	0	0	0	0	6	1	146	1826	11566
mir-133a-5p	17	0	0	0	0	0	1	17	44	57	11149	11285
mir-736-3p	10251	0	0	0	11	1	0	2	9	8	958	11240
mir-27b-3p	4686	146	61	0	155	92	77	291	103	184	5366	11161
mir-4286-5p	7833	0	2	0	8	9	38	198	449	54	2218	10809
mir-133-3p	101	0	0	0	0	5	1	8	14	123	10215	10467
mir-24-3p	3390	36	78	0	178	159	77	91	142	280	5929	10360
mir-18a-5p	831	39	150	0	187	125	102	453	1045	1199	6097	10228
mir-20a-3p	2864	2	0	0	10	67	179	1018	2236	1409	2320	10105
mir-142a-5p	8666	8	28	0	74	23	52	58	164	52	926	10051
mir-130a-3p	3555	17	93	0	77	38	35	138	666	854	4560	10033
mir-210-5p	9141	18	18	0	17	14	21	24	10	1	738	10002
mir-100a-5p	3035	1050	314	0	411	423	170	338	347	308	3495	9891
mir-26b-5p	3620	220	431	62	546	309	226	391	486	1110	2354	9755
mir-130c-5p	5530	15	11	1	52	53	71	387	2808	407	336	9671
mir-103-3p	7161	12	6	0	39	11	23	68	177	285	1861	9643
mir-458-3p	8954	6	15	0	2	2	3	6	12	1	600	9601
mir-429-3p	5084	25	36	0	46	19	56	94	660	509	3038	9567
mir-222a-5p	5352	0	2	0	14	8	18	46	1036	1792	1090	9358
mir-130c-3p	103	6	10	0	17	17	28	195	2480	1224	5201	9281
mir-30e-3p	2762	22	23	0	19	16	55	47	927	414	4586	8871
mir-93a-5p	4274	5	19	0	57	49	35	124	906	642	2606	8717
mir-130a-5p	134	22	0	0	15	30	51	286	2071	858	5194	8661
mir-196-5p	2040	49	5	0	14	4	13	32	209	826	5463	8655
mir-190-5p	7532	4	40	0	45	27	20	57	45	31	785	8586
mir-92a-5p	1624	6	1	0	23	37	237	1182	1843	694	2806	8453
mir-20-5p	1557	9	11	0	50	22	50	317	2432	997	2743	8188
mir-144-5p	2508	23	67	21	48	34	52	67	259	1666	3348	8093
mir-222a-3p	2826	0	12	1	18	6	1	16	1637	1201	2237	7955
mir-9-3p	760	2	8	0	3	7	12	168	322	303	6217	7802
mir-199-3p	2278	1	1	0	24	9	3	36	10	1	5434	7797
mir-737-5p	5221	42	78	0	237	227	174	475	449	149	715	7767
mir-183-5p	690	15	8	0	3	26	6	48	381	560	5859	7596
mir-454b-3p	216	4	11	0	4	13	5	56	366	451	6278	7404
let-7g-5p	6822	30	21	0	58	31	16	66	17	0	5	7066
mir-7977-5p	1795	37	180	154	572	350	373	716	1363	719	915	7174
mir-367-3p	14	0	0	0	74	72	314	877	4387	1033	169	6940
mir-22a-5p	3128	2	24	0	49	34	55	208	1108	445	1566	6619
mir-17a-5p	1841	0	15	0	42	20	42	146	1525	546	2422	6599
mir-132-5p	6247	0	0	0	0	2	1	7	13	1	251	6522
mir-462a-5p	6406	0	15	0	1	8	4	0	8	0	2	6444
mir-449a-5p	284	47	6	0	62	44	48	183	123	125	5486	6408
mir-725-3p	1882	4	0	0	46	16	51	51	448	363	3471	6332
mir-212-3p	6153	0	0	1	1	0	0	0	5	1	1	6162
mir-726-3p	6046	0	9	0	1	0	0	0	7	0	2	6065
mir-217-5p	3622	1	12	0	18	23	10	4	82	98	2156	6026
mir-222b-3p	4792	19	2	0	13	12	15	47	19	144	720	5783
mir-9226-3p	91	0	0	0	197	107	354	1679	924	937	1426	5715
mir-23a-3p	3486	20	56	0	63	39	57	67	120	106	1699	5713
mir-210-3p	4500	60	25	0	59	27	36	119	56	0	783	5665
mir-29b-3p	4821	20	46	0	108	61	49	107	138	72	177	5599
mir-18-5p	267	0	2	0	25	18	29	119	1314	627	3159	5560
mir-9a-3p	340	4	0	0	1	6	7	11	75	85	4912	5441
mir-462-5p	5267	3	1	0	0	18	0	9	13	1	3	5315
mir-125a-3p	3021	0	0	0	11	23	6	21	14	1	2178	5275
mir-219-3p	27	4	0	0	6	1	3	69	1225	1182	2671	5188
mir-126a-5p	1498	0	7	1	16	7	7	34	19	519	3060	5168
mir-21a-5p	4868	20	41	43	51	29	14	42	40	1	7	5156
mir-21b-3p	1111	0	21	0	37	2	11	7	1489	884	1464	5026
mir-454-3p	63	0	1	0	7	5	15	35	950	773	3138	4987
mir-1388-3p	4065	19	0	0	1	0	14	1	40	92	734	4966
mir-124-3p	6	0	0	0	0	0	0	29	181	262	4318	4796
mir-301a-3p	1569	3	20	0	14	13	7	34	275	163	2683	4781
mir-199-5p	1611	15	18	63	21	10	8	26	41	22	2857	4692
mir-457a-5p	4221	3	6	0	41	19	16	17	41	5	215	4584
mir-140-5p	1512	14	36	0	28	14	11	40	265	229	2425	4574
mir-1692-5p	0	0	138	0	397	278	733	1997	713	148	0	4404
mir-3168-5p	1255	266	287	1089	532	259	390	378	248	72	654	5430
mir-212b-5p	4242	2	0	0	0	7	1	0	10	73	3	4338
mir-96-5p	273	40	14	0	17	25	10	52	808	650	2297	4186
mir-7-5p	1231	90	74	0	70	141	73	133	130	206	1953	4101
mir-194a-5p	353	24	57	0	87	61	42	31	64	112	3266	4097
let-7b-5p	3633	84	37	1	66	31	42	72	39	22	1	4028
mir-200a-5p	296	12	10	0	9	1	1	34	1723	974	806	3866
mir-203-3p	145	0	0	0	9	3	8	16	322	1760	1594	3857
mir-301d-3p	273	0	26	1	17	3	12	100	52	63	3221	3768
mir-222b-5p	3180	0	9	0	0	10	14	43	157	148	154	3715
mir-215-5p	130	33	35	65	33	12	21	47	20	115	3220	3731
mir-456-3p	2334	0	5	0	23	9	3	10	104	0	1051	3539
mir-216a-5p	652	0	7	0	9	12	3	5	21	64	2716	3489
mir-133b-3p	55	4	0	0	0	0	0	3	20	4	3351	3437

Absolute reads

miRNA	Sperm	Unfertilized eggs	2 Cells	32 Cells	64-512 Cells	Mid blastula	Early gastrulation	50% Epiboly	Neurulation	10 Somites	Hatch	Sum
mir-124-5p	0	0	0	0	0	0	8	10	366	726	2291	3401
mir-737-3p	1322	21	174	240	135	108	84	173	376	234	724	3591
mir-133c-3p	78	0	0	0	0	0	2	5	4	186	3045	3320
mir-7641-5p	2538	3	18	0	196	51	61	148	64	74	108	3261
mir-194-5p	668	41	23	0	36	38	22	95	32	146	2148	3249
mir-22b-3p	1139	17	202	0	269	169	149	175	173	301	648	3242
mir-449b-5p	19	0	4	0	8	1	2	21	21	203	2879	3158
mir-93-5p	1534	1	19	51	37	16	18	94	250	148	980	3148
mir-200b-5p	409	0	3	0	22	0	3	27	1340	323	954	3081
mir-106b-3p	680	0	0	0	6	3	25	208	984	468	700	3074
mir-139-5p	2264	15	0	0	19	11	7	26	60	1	636	3039
mir-144-3p	340	28	77	0	105	45	24	41	260	13	2016	2949
mir-34a-3p	2196	53	10	0	35	36	31	93	54	0	388	2896
mir-181b-3p	1261	0	0	0	0	6	3	2	77	29	1492	2870
mir-204-5p	381	11	0	0	19	18	10	45	171	63	2131	2849
mir-153-3p	998	0	0	0	0	3	3	11	53	114	1539	2721
mir-141-3p	1769	94	50	2	74	88	50	25	77	15	474	2718
mir-30b-3p	1940	7	0	0	4	5	14	2	55	92	453	2572
mir-10a-3p	36	0	12	0	33	1	4	0	430	189	1843	2548
mir-27a-3p	942	25	23	0	93	39	38	54	108	0	1221	2543
mir-15b-3p	2244	4	2	0	1	4	2	36	82	12	132	2519
mir-2184-5p	2127	13	8	0	84	30	15	51	45	11	114	2498
let-7d-5p	2393	37	9	1	10	4	1	11	1	0	3	2470
mir-203-5p	36	0	0	0	16	2	2	26	1298	619	455	2454
mir-301-3p	19	0	6	0	0	0	0	13	98	139	2132	2407
mir-142b-5p	2010	3	0	0	11	0	5	5	5	0	318	2357
mir-30a-3p	428	0	3	0	16	2	5	20	749	198	768	2189
mir-2478-3p	1166	8	21	0	88	73	107	334	199	78	104	2178
mir-145-3p	51	22	15	0	17	7	5	13	94	517	1358	2099
mir-1386-5p	453	14	15	0	72	18	64	45	223	163	1000	2067
mir-10b-3p	0	0	0	0	3	4	5	1	836	510	661	2020
mir-130b-5p	1325	1	3	0	9	0	5	31	59	64	498	1995
mir-153a-3p	1010	2	3	0	34	13	14	16	68	34	779	1973
mir-18b-3p	39	0	0	0	0	17	53	323	1206	211	105	1954
mir-23b-3p	1083	22	27	0	61	30	39	46	48	59	508	1923
mir-130d-3p	39	0	0	0	9	1	4	8	82	250	1524	1917
mir-125a-5p	199	51	69	0	142	65	54	111	81	16	1077	1865
mir-489-3p	1699	0	0	0	4	13	0	5	14	57	31	1823
mir-2188-5p	565	68	67	0	141	75	152	224	154	0	357	1803
mir-155-5p	1771	3	0	0	0	2	0	2	10	0	1	1789
mir-34b-5p	789	115	28	1	76	68	58	139	80	37	382	1773
mir-133b-5p	0	0	0	0	0	1	1	0	33	138	1590	1763
mir-212-5p	1695	0	0	0	0	0	0	2	12	0	17	1726
mir-1c-3p	0	0	15	0	0	10	1	0	52	58	1563	1699
mir-216-5p	347	0	0	0	0	0	0	0	23	152	1162	1684
mir-125-3p	923	0	0	0	0	0	1	18	11	0	713	1666
mir-7147-5p	827	3	25	0	10	1	4	1	50	273	472	1666
mir-190a-5p	512	0	5	0	13	7	11	60	64	68	796	1536
mir-29c-3p	1364	4	10	0	28	33	11	23	17	0	1	1491
mir-7-3p	412	0	1	0	17	15	16	110	583	230	102	1486
mir-24a-5p	208	8	2	0	37	12	6	2	48	47	1113	1483
mir-10c-3p	16	2	9	0	7	0	0	0	242	58	1141	1475
let-7c-3p	1177	3	0	0	0	0	0	23	2	0	260	1465
mir-7132a-5p	1391	0	0	0	0	0	0	0	27	0	29	1447
mir-143-5p	0	0	0	0	0	1	4	0	3	0	1409	1417
mir-375-3p	414	12	8	0	7	15	28	53	122	16	691	1366
mir-199a-5p	169	15	8	0	45	23	19	44	18	2	996	1339
mir-455-5p	294	1	52	0	54	7	12	51	75	0	759	1305
mir-138-5p	483	0	0	0	24	3	0	24	126	130	480	1270
mir-33a-5p	412	3	23	0	77	15	12	15	76	0	634	1267
mir-4492-3p	361	36	34	0	152	63	112	80	49	1	368	1256
mir-1335-3p	874	26	42	0	60	33	47	65	30	0	57	1234
mir-6412-3p	1071	0	3	0	5	0	13	60	52	1	1	1206
mir-24-5p	95	0	0	0	0	0	4	0	48	173	868	1188
mir-17-3p	77	0	0	0	4	10	55	223	712	17	78	1176
mir-19a-5p	0	0	0	0	1	18	87	293	723	0	35	1157
let-7d-3p	1141	0	0	0	0	0	3	0	9	0	0	1153
mir-181c-5p	152	0	0	0	0	0	0	0	0	1	977	1130
mir-126a-3p	244	10	12	0	17	13	5	18	29	0	721	1069
mir-203a-5p	10	0	0	0	8	1	0	5	607	202	223	1056
mir-19b-5p	43	0	1	0	1	20	39	374	344	166	65	1053
mir-135a-5p	45	9	14	0	12	12	13	32	53	22	824	1036
mir-203b-3p	11	0	1	0	37	225	119	6	21	58	475	953
mir-124a-3p	0	0	0	0	0	0	1	0	5	39	908	953
mir-27c-3p	313	17	15	0	8	10	2	8	55	20	503	951
mir-101-5p	373	0	0	0	12	11	11	140	267	15	106	935
mir-145-5p	282	10	38	0	132	76	40	53	63	25	208	927
mir-15c-3p	520	22	0	0	1	9	21	28	211	29	69	910
mir-22a-3p	224	6	37	8	111	75	42	103	61	54	194	915
mir-455-3p	519	25	5	0	22	11	17	41	52	24	161	877
mir-1260a-5p	277	4	8	6	18	13	40	150	128	40	190	874
mir-731-5p	861	0	0	0	0	1	1	0	0	0	0	863
mir-128b-3p	839	0	0	0	5	3	1	0	2	0	1	851
mir-499-3p	81	0	0	0	0	0	0	9	1	1	750	842
mir-5119-3p	0	0	18	0	72	79	78	336	222	15	2	822
mir-139-3p	395	0	5	0	10	0	0	4	100	38	248	800
mir-125c-5p	515	11	8	90	9	30	8	19	12	1	182	885
mir-219-5p	0	0	0	0	0	0	1	0	25	33	736	795
mir-15c-5p	715	3	9	0	12	1	5	22	3	3	14	787
mir-214-5p	0	0	8	0	5	0	6	20	0	8	728	775
mir-281a-3p	147	0	0	0	0	0	0	0	0	0	623	770
mir-466i-5p	441	0	3	0	33	19	7	101	64	0	101	769
mir-722-3p	485	1	13	7	30	19	7	48	24	10	126	770
mir-219a-3p	0	0	0	0	5	4	5	8	4	220	512	758

Absolute reads

miRNA	Sperm	Unfertilized eggs	2 Cells	32 Cells	64-512 Cells	Mid blastula	Early gastrulation	50% Epiboly	Neurulation	10 Somites	Hatch	Sum
mir-31a-5p	558	3	12	0	16	4	26	0	13	0	86	718
mir-430d-3p	21	0	0	0	97	130	99	195	102	10	44	698
mir-106-5p	270	0	1	0	7	4	2	35	85	39	253	696
mir-460-3p	47	0	0	0	0	0	0	1	1	386	234	669
mir-135c-5p	453	2	6	0	27	16	13	42	62	0	44	665
mir-126-5p	229	5	0	0	0	2	0	4	4	215	196	655
mir-31-5p	576	7	6	0	7	5	10	20	7	0	1	639
mir-214-3p	13	11	14	0	2	0	2	13	8	0	568	631
mir-3074-5p	608	0	0	0	2	0	0	0	0	0	0	610
let-7a-3p	422	13	7	0	25	10	13	31	19	0	62	602
mir-24a-3p	388	0	13	0	6	27	2	23	8	0	121	588
mir-218-3p	99	0	0	0	0	0	0	1	71	290	125	586
let-7b-3p	511	0	4	0	4	15	15	17	5	0	0	571
mir-27b-5p	230	0	1	0	0	0	3	0	26	0	311	571
mir-107-3p	124	0	0	0	0	0	0	21	14	10	400	569
mir-99a-3p	416	0	0	0	0	0	0	5	3	0	139	563
mir-31-3p	355	19	6	0	25	6	19	60	40	11	20	561
mir-137-3p	1	0	0	0	1	1	0	15	3	32	503	556
mir-146b-5p	476	0	1	0	10	4	6	51	2	1	3	554
mir-142b-3p	535	0	2	0	0	0	0	1	8	0	1	547
mir-460a-5p	191	0	0	0	0	0	1	1	0	217	120	530
mir-460b-5p	349	3	4	0	16	17	4	21	2	75	34	525
mir-301b-3p	23	4	0	0	1	0	1	14	82	7	389	521
let-7i-3p	461	0	0	0	10	2	4	20	11	0	0	508
mir-129-5p	92	10	0	0	25	3	7	69	22	0	271	499
mir-182-3p	9	0	0	0	0	0	0	12	35	0	427	483
mir-190b-3p	366	0	8	0	29	4	3	17	6	48	0	481
mir-8159-5p	171	2	9	0	5	4	1	6	1	60	208	467
mir-30d-3p	133	0	0	0	0	1	1	20	157	99	47	458
mir-29b-5p	264	6	2	0	8	10	18	14	33	21	72	448
mir-1338-3p	431	0	0	0	0	0	1	0	2	0	0	434
mir-103-5p	136	0	5	0	4	7	0	0	15	10	251	428
mir-193b-3p	140	0	15	0	3	0	4	0	52	0	207	421
mir-223-5p	413	0	0	0	0	0	1	0	0	0	0	414
mir-206-5p	0	0	0	0	0	3	0	0	24	0	387	414
mir-551-3p	137	0	0	0	8	9	2	2	15	0	240	413
mir-23a-5p	301	1	0	0	2	1	9	0	32	40	21	407
mir-100-3p	179	13	0	0	9	0	5	32	4	0	156	398
let-7g-3p	97	0	0	0	2	9	12	68	22	176	1	387
mir-221-3p	276	0	0	0	1	0	4	11	34	0	61	387
mir-194-3p	0	0	0	0	0	0	0	0	6	0	368	374
mir-128-5p	116	0	0	0	0	0	0	4	177	54	2	353
mir-1973-3p	0	3	15	0	83	43	23	63	23	95	1	349
mir-3123-3p	249	0	20	0	27	0	12	19	14	0	0	341
mir-135b-5p	45	15	7	0	24	7	26	43	22	0	149	338
mir-7704-5p	12	5	0	0	2	9	8	38	4	0	255	333
mir-9b-5p	218	0	0	0	12	0	6	3	6	1	86	332
mir-125b-3p	38	0	0	0	0	0	0	0	1	0	280	319
mir-147-3p	256	0	5	0	0	0	5	0	4	11	30	311
mir-10c-5p	0	0	1	0	2	22	9	20	20	136	98	308
mir-4286-3p	307	0	0	0	0	0	0	0	0	0	0	307
mir-4508-5p	127	0	0	0	30	0	13	39	5	0	80	294
mir-301-5p	148	0	0	0	0	0	1	0	19	115	1	284
mir-430c-5p	1	0	0	0	0	10	4	73	0	4	191	283
mir-27d-3p	205	3	0	0	13	0	3	35	14	0	2	275
mir-459-5p	120	0	0	0	0	0	0	1	21	79	47	268
mir-430a-5p	0	0	0	0	1	16	3	107	0	2	135	264
mir-107a-3p	36	0	0	0	0	2	1	16	2	0	198	255
mir-24b-3p	85	4	10	0	20	0	2	9	4	0	116	250
mir-17a-3p	55	0	0	0	0	10	9	52	115	0	8	249
mir-181e-5p	62	2	0	0	0	0	0	0	1	2	182	249
mir-365-3p	48	3	2	0	11	4	12	25	28	33	82	248
mir-30c-3p	12	0	0	0	0	2	0	7	68	0	159	248
mir-24b-5p	44	6	0	0	0	0	0	0	8	72	117	247
mir-122-3p	0	6	22	0	52	5	10	17	9	0	118	239
mir-4792-5p	76	0	39	0	13	12	9	39	24	0	5	217
mir-101a-5p	196	0	0	0	7	0	3	0	4	0	0	210
mir-9341-5p	0	0	0	0	43	24	13	3	54	7	60	204
mir-93a-3p	82	0	0	0	0	1	1	0	119	0	0	203
mir-193a-3p	0	0	0	0	34	2	5	4	38	0	118	201
mir-29a-5p	171	4	0	0	4	5	0	4	6	0	0	194
mir-190a-3p	5	6	5	0	44	11	20	76	25	0	0	192
mir-205b-5p	27	3	0	0	0	0	0	4	29	72	57	192
mir-183-3p	5	0	0	0	0	0	1	0	12	10	158	186
mir-96-3p	0	4	0	0	0	4	0	5	172	0	0	185
mir-106b-5p	52	0	1	0	2	0	1	6	48	16	58	184
mir-107b-3p	105	0	10	0	1	2	1	5	0	0	59	183
mir-33-5p	137	0	11	0	0	5	7	18	4	0	0	182
mir-4454-5p	118	0	0	0	34	8	1	9	12	0	0	182
mir-129-3p	27	0	0	0	8	0	34	42	10	0	60	181
mir-1260b-5p	3	7	5	0	7	19	40	53	41	2	0	177
mir-133-5p	0	0	0	0	0	0	1	0	0	0	172	173
mir-5124a-5p	0	0	20	0	21	36	21	42	31	0	0	171
mir-9277-3p	116	0	0	0	0	0	4	25	25	0	0	170
mir-1788-3p	8	0	16	0	0	0	0	0	0	1	144	169
mir-153c-5p	79	0	0	0	0	0	0	3	18	0	69	169
mir-204-3p	0	0	1	0	0	0	1	0	11	0	156	169
mir-196b-5p	67	1	0	0	2	24	9	1	3	4	55	166
mir-499a-5p	101	0	0	0	0	0	1	0	0	0	62	164
mir-18-3p	12	0	0	0	0	0	3	5	106	10	27	163
mir-3120-3p	139	0	2	0	17	0	0	1	1	0	0	160
mir-15a-3p	76	0	0	0	0	0	0	0	26	0	57	159
mir-100c-5p	155	0	0	0	0	0	0	0	0	0	0	155
mir-196a-3p	0	0	0	0	0	0	0	0	18	0	137	155

Absolute reads

miRNA	Sperm	Unfertilized eggs	2 Cells	32 Cells	64-512 Cells	Mid blastula	Early gastrulation	50% Epiboly	Neurulation	10 Somites	Hatch	Sum
mir-153a-5p	24	0	0	0	0	0	1	0	17	0	108	150
mir-10d-5p	1	0	1	0	0	0	0	5	8	41	91	147
mir-551b-3p	22	0	12	0	5	0	3	0	7	0	96	145
mir-1-5p	0	0	0	0	0	0	0	2	4	0	139	145
mir-2189-3p	1	0	0	0	10	52	20	10	0	2	48	143
mir-3968-3p	28	0	0	0	0	4	9	17	26	58	1	143
mir-1306-5p	117	0	0	0	5	0	0	8	10	0	0	140
mir-135a-3p	14	2	0	0	0	0	3	6	23	0	88	136
mir-4448-3p	0	0	6	0	0	6	33	61	27	0	0	133
mir-3618-3p	0	0	1	0	5	6	4	34	52	18	6	126
mir-2957-3p	0	0	21	0	17	5	8	7	68	0	0	126
mir-2187-5p	26	0	0	0	5	6	0	5	4	9	70	125
mir-5112-5p	2	0	0	0	8	9	10	56	39	0	0	124
mir-7a-3p	102	0	0	0	0	0	2	0	10	0	1	115
let-7e-3p	106	0	0	0	0	0	2	4	2	0	0	114
mir-16b-3p	87	0	0	0	0	0	0	0	24	0	0	111
mir-338-5p	28	0	1	0	0	0	0	1	9	67	1	107
mir-101c-3p	64	0	0	0	0	0	0	0	38	2	2	106
mir-723-3p	23	0	0	0	7	0	0	0	13	0	60	103
mir-135b-3p	0	0	0	0	0	0	1	46	34	19	0	100
mir-22b-5p	73	0	0	0	0	0	0	8	18	0	0	99
mir-192b-5p	0	0	0	0	0	0	0	0	2	0	97	99
mir-1329-3p	53	0	0	0	0	0	0	0	23	21	0	97
mir-1a-3p	0	0	0	0	0	0	0	0	0	0	91	91
mir-5108-3p	0	0	4	0	4	5	6	45	26	0	0	90
mir-92-3p	81	0	0	0	0	0	0	0	3	0	0	84
mir-4443-5p	6	9	0	0	4	0	8	27	30	0	0	84
mir-152b-3p	19	0	0	0	2	1	1	0	5	11	42	81
mir-23b-5p	10	0	0	0	0	0	0	0	0	38	32	80
mir-16a-3p	20	0	0	0	0	0	5	7	33	0	14	79
mir-724-5p	20	1	0	0	4	6	2	8	2	0	36	79
mir-205a-5p	57	0	0	0	0	0	0	0	20	0	2	79
mir-199b-5p	74	0	0	0	0	0	0	0	0	0	4	78
mir-301b-5p	52	0	0	0	0	0	2	2	14	0	7	77
mir-138b-5p	9	0	0	0	0	0	0	0	7	0	61	77
let-7f-3p	59	0	0	0	0	0	0	3	14	0	0	76
mir-124b-3p	0	0	0	0	0	0	0	0	0	0	74	74
mir-727-5p	66	0	0	0	0	0	0	5	1	0	1	73
mir-193-3p	0	0	0	0	4	4	3	0	0	12	50	73
mir-21c-5p	68	0	0	0	2	0	1	0	0	0	0	71
mir-365a-3p	0	0	0	0	0	0	4	0	14	0	52	70
mir-138-3p	51	0	0	0	0	0	0	5	10	0	0	66
mir-21b-5p	63	0	0	0	0	0	0	0	3	0	0	66
mir-27c-5p	0	0	0	0	1	0	1	0	6	0	55	63
mir-429-5p	0	0	0	0	0	14	7	0	37	0	4	62
mir-216b-3p	0	0	0	0	0	0	0	0	0	0	62	62
mir-2187-3p	50	0	0	0	0	0	1	0	10	0	0	61
mir-6651-5p	31	0	0	0	8	9	3	10	0	0	0	61
mir-218-5p	0	0	0	0	0	0	0	0	10	0	51	61
mir-208b-5p	26	0	0	0	0	0	0	0	0	0	33	59
mir-26d-5p	53	0	0	0	0	1	0	0	1	0	3	58
mir-551a-3p	8	4	0	0	0	0	0	12	33	0	0	57
mir-723-5p	0	0	0	0	0	0	0	0	18	38	0	56
mir-3620-5p	44	0	5	0	0	0	0	6	0	0	0	55
mir-2424-3p	43	0	4	0	0	1	1	2	1	0	0	52
mir-147b-3p	36	4	2	0	0	0	0	0	9	0	0	51
mir-3964-3p	45	0	0	0	0	0	0	0	0	0	1	46
mir-15e-5p	42	1	0	0	0	0	0	1	0	0	1	45
mir-365-5p	0	1	0	0	0	0	0	0	6	0	34	41
mir-3533-3p	0	0	3	0	0	0	0	0	6	0	31	40
mir-34c-3p	34	0	0	1	1	0	0	1	0	0	3	40
mir-551b-5p	4	0	0	0	0	0	0	0	4	0	31	39
mir-2881-5p	38	0	0	0	0	0	0	0	0	0	0	38
mir-1338-5p	33	0	0	0	2	0	0	0	0	0	2	37
mir-462b-5p	36	0	0	0	0	0	0	0	0	0	1	37
mir-7565-5p	36	0	0	0	0	0	0	0	0	0	0	36
mir-15d-5p	30	0	0	0	1	0	1	2	1	1	0	36
mir-7132b-5p	35	0	0	0	0	0	0	0	0	0	0	35
mir-29d-3p	31	0	0	0	1	1	0	1	0	0	0	34
mir-103b-3p	30	0	2	0	1	0	0	0	0	0	0	33
mir-4510-5p	31	0	0	0	0	0	0	0	0	0	0	31
mir-146d-5p	30	0	0	0	0	0	0	0	0	0	0	30

Appendix 6

miRNA	Sperm	Unfertilized eggs	2 Cells	32 Cells	64-512 Cells	Mid blastula	Early gastrulation	50% Epiboly	Neurulation	10 Somites	Hatch	Sum
mir-430c-3p	24	1800	1053	0	478184	611214	593658	540667	315702	106621	23655	2672578
mir-430b-3p	9	1013	650	0	70121	163939	160693	208942	246778	119133	33772	1005049
mir-430a-3p	9	225	202	1800	185777	121355	148657	173683	132686	38751	14603	817749
mir-21-5p	216427	147056	67218	1013	17625	8450	4768	3360	4014	6092	12230	488251
mir-203a-3p	5410	2757	2173	225	3169	788	837	1408	37267	291852	99395	445281
mir-10b-5p	1149	12967	13954	147056	2424	1378	1066	480	20445	167715	73256	441891
mir-148a-3p	768	62502	136339	0	25547	13797	5528	3391	10243	21652	13310	293076
mir-10a-5p	1612	44724	34068	12967	7504	3909	1944	997	4502	21434	48583	182244
mir-100-5p	5124	47144	47843	62502	8644	3799	2385	1388	1704	2620	28933	212086
mir-16b-5p	52185	37749	19375	44724	7557	2474	2776	1489	2640	2874	6654	180496
mir-22-3p	6220	8748	60386	47144	16501	4107	4526	2120	5430	6111	7266	168558
mir-34c-5p	10722	76144	17986	37749	4130	3287	1364	1225	545	785	864	154801
mir-26a-5p	23993	25794	19957	8748	6068	2014	2123	1468	3267	5221	6567	105219
let-7a-5p	37189	38536	7347	76144	3549	1301	1410	766	536	435	534	167746
mir-30a-5p	21754	9507	25937	25794	6634	2539	2595	1506	1219	1177	10437	109100
mir-99b-5p	9144	53191	12118	38536	2910	1447	869	588	601	409	1203	121016
mir-200b-3p	10617	12714	13417	9507	3397	1451	1536	1127	7676	10787	16036	88265
mir-202-5p	617	24613	31895	53191	9901	3540	3263	2637	1536	285	96	131575
mir-125b-5p	8878	21968	26430	12714	4441	2219	2032	1084	720	765	5628	86879
mir-451-5p	3517	18762	30596	24613	6884	2847	3899	1762	1418	476	383	95157
mir-143-3p	657	13445	17471	21968	3701	930	464	260	410	2607	28685	90599
mir-1-3p	125	1294	1187	18762	205	95	86	217	175	2415	62038	86599
mir-25-3p	25878	12405	6339	0	1375	804	828	540	3842	3089	11945	67046
mir-30c-5p	18080	5457	10594	0	3188	829	1760	1139	3408	4488	16716	65659
mir-30d-5p	14025	8270	18568	12405	3374	1631	1215	731	1042	1852	11937	75050
mir-19c-3p	7593	1744	13081	0	4164	1106	1898	2150	9652	4547	9509	55443
mir-16a-5p	10726	6666	13954	0	3461	1479	1050	839	4272	6832	5015	54294
mir-99-5p	20430	9873	10594	1744	2720	1153	672	365	406	662	4457	53078
mir-101a-3p	25982	3291	9385	0	2211	464	824	883	982	1715	5293	51030
mir-19d-3p	10583	2532	15589	9873	3682	1005	1785	1635	4006	2765	6572	60026
mir-1260-5p	575	8017	20943	3291	9069	2926	3394	2263	1428	450	1003	53358
mir-122-5p	10	23459	15276	2532	4578	1183	1285	1219	316	242	2236	52335
mir-206-3p	511	619	560	8017	72	223	102	118	306	2044	43814	56384
mir-205-5p	2271	759	291	0	650	150	242	484	9099	8958	23852	46758
let-7e-5p	27085	9845	4950	619	1280	476	518	305	170	46	40	45335
mir-181a-5p	11604	7567	4928	0	1448	590	294	420	278	1350	14926	43404
mir-100a-5p	794	29535	7033	0	1562	857	385	253	269	320	1386	42394
mir-148a-5p	19	281	739	7567	532	89	79	107	17997	12880	4588	44878
mir-92a-3p	8599	1660	1568	29535	505	381	971	1238	10014	5849	5264	65584
mir-338-3p	5870	7595	12409	0	4274	1159	851	589	766	760	1475	35747
mir-200a-3p	5526	4388	5980	1660	1793	608	654	520	3508	4318	5724	34679
mir-190b-5p	31208	366	67	0	103	55	32	13	30	88	159	32120
let-7c-5p	24161	5457	1210	0	483	152	97	125	78	1	280	32044
mir-92b-3p	22754	1856	605	366	570	130	143	78	1397	1127	1798	30823
mir-152-3p	974	3010	8422	0	2048	399	376	332	1566	3373	9380	29880
mir-19a-3p	2957	169	4278	1856	1470	367	1129	1501	5826	3254	8860	31668
mir-363-3p	3130	928	3606	0	752	450	701	572	6419	5331	7647	29537
mir-19b-3p	3889	197	4995	0	1326	397	1066	1470	7238	2785	5675	29037
let-7f-5p	16340	6160	2934	0	726	328	315	260	430	241	345	28079
mir-30b-5p	9167	3010	5734	0	1569	419	787	740	1691	1608	3028	27755
let-7j-5p	14245	6863	3718	6160	665	466	222	163	93	31	24	32650
mir-98-5p	8973	7595	6294	0	1049	391	391	260	154	150	66	25323
mir-26-5p	6972	4557	7100	6863	1330	513	654	387	361	984	1852	31573
mir-15b-5p	6902	2307	4704	7595	1771	675	706	962	2143	1454	1986	31204
mir-18b-5p	210	450	2509	4557	790	247	272	362	3798	5136	9783	28115
mir-192-5p	177	6301	4681	2307	1368	521	294	301	176	535	8997	25657
mir-7550-5p	363	2728	7257	0	3647	1153	2473	1993	1112	791	1750	23267
mir-26b-5p	948	6188	9654	0	2074	626	511	293	377	1152	933	22757
mir-148-3p	170	4951	6563	0	1311	411	333	406	1212	2876	4156	22390
mir-15a-5p	8171	5935	3830	6188	1383	450	575	347	414	175	819	28288
mir-146a-5p	8549	4332	5667	0	1098	515	353	241	195	394	305	21650
mir-101b-3p	923	309	7279	0	2713	282	563	528	2423	1640	4239	20900
mir-16-5p	5983	6076	5891	4332	726	361	412	200	261	96	52	24389
mir-9-5p	3303	985	246	0	76	89	97	213	656	833	13496	19994
mir-203b-5p	45	0	0	6076	289	36	29	73	10057	7047	1189	24841
mir-3168-5p	329	7482	6428	0	2021	525	882	283	192	75	259	18477
mir-16c-5p	3641	3207	6585	0	1117	527	516	343	623	731	812	18103
mir-30e-5p	5500	197	4077	7482	1155	338	507	397	425	524	4963	25565
mir-142-3p	10849	956	2688	3207	741	176	156	157	136	273	1935	21274
mir-130b-3p	579	394	45	0	281	99	120	253	2689	2729	9044	16232
mir-148b-3p	834	5766	3696	956	954	349	432	256	783	757	2291	17074
mir-223-3p	13293	844	806	394	201	79	122	120	61	3	249	16172
mir-7a-5p	1067	3404	4189	0	513	300	233	235	1362	2737	1377	15416
mir-181a-3p	228	647	694	844	232	99	57	165	911	2277	9449	15603
mir-429a-3p	2757	1378	1366	3404	319	152	81	181	1727	3343	3217	17926
mir-20b-5p	2535	788	605	647	80	146	285	583	4057	1831	2932	14489
mir-219a-5p	7	0	0	0	27	4	0	18	593	3168	9990	13806
mir-148b-5p	119	338	694	0	129	36	61	146	3085	6606	2146	13361
mir-181b-5p	2988	1969	224	0	384	99	86	160	247	898	5333	12387
mir-20a-5p	1450	422	739	338	327	231	283	382	3476	1774	3279	12700
mir-7977-5p	470	1041	4032	1969	2173	709	844	537	1057	746	363	13940
mir-457b-5p	8020	872	336	0	201	55	122	91	695	367	1197	11956
mir-34a-5p	785	2166	1433	1041	517	154	154	190	412	1220	4618	12690

Normalized reads

miRNA	Sperm	Unfertilized eggs	2 Cells	32 Cells	64-512 Cells	Mid blastula	Early gastrulation	50% Epiboly	Neurulation	10 Somites	Hatch	Sum
mir-128-3p	5068	1350	1545	872	540	107	161	118	504	396	1013	10714
mir-208a-3p	1209	169	0	0	4	2	2	8	6	251	9062	12031
mir-18a-5p	218	1097	3360	1350	710	253	231	340	811	1244	2417	10815
mir-1388-5p	4633	1266	2038	169	255	138	97	45	159	1260	756	0
mir-106a-5p	1258	394	941	1097	346	53	283	320	3237	1350	2446	11724
mir-133a-3p	245	28	0	0	15	10	11	19	38	142	9957	10467
mir-99a-5p	4574	2391	2352	394	403	227	81	43	33	72	107	10677
mir-27b-3p	1227	4107	1366	28	589	186	174	218	80	191	2128	10294
mir-499-5p	911	113	246	2391	65	67	50	35	16	220	8496	12610
mir-184-3p	87	1435	829	4107	201	126	72	62	185	800	5942	13845
mir-221-5p	2899	731	179	0	152	160	66	42	1011	1520	2741	9502
mir-101-3p	2411	394	3920	1435	965	144	290	215	268	169	468	10677
mir-1692-5p	0	0	3091	731	1508	563	1659	1497	553	154	0	9756
mir-1329-5p	5531	1294	314	394	232	36	86	43	124	447	877	9378
mir-132-3p	8470	0	0	0	15	0	0	0	2	2	64	8553
mir-199a-3p	1097	872	515	1294	270	81	38	94	44	231	5011	9548
mir-196a-5p	3163	956	157	0	99	41	52	43	204	409	2722	7846
mir-22b-3p	298	478	4524	872	1022	342	337	131	134	312	257	8709
mir-126-3p	1956	225	1142	956	304	41	86	101	42	401	3408	8662
mir-24-3p	887	1013	1747	478	676	322	174	68	110	291	2351	8118
mir-222-3p	1876	1153	1098	0	160	24	102	34	226	287	2396	7357
mir-130a-3p	931	478	2083	1013	293	77	79	103	517	886	1808	8267
mir-218a-5p	441	225	112	1153	179	20	45	69	607	931	4608	8390
mir-737-5p	1367	1181	1747	478	900	460	394	356	348	155	283	7670
mir-29a-3p	3485	956	806	0	695	97	253	230	288	82	85	6979
mir-737-3p	346	591	3897	1181	513	219	190	130	292	243	287	7888
mir-144-5p	657	647	1501	956	182	69	118	50	201	1729	1327	7437
mir-182-5p	680	872	112	0	99	0	36	37	318	1387	2922	6463
let-7i-5p	5301	619	246	0	148	41	14	19	38	0	7	6433
mir-7-5p	322	2532	1657	872	266	286	165	100	101	214	774	7289
mir-17-5p	985	141	269	619	258	79	163	243	1635	789	1835	7015
mir-367-3p	4	0	0	0	281	146	711	657	3403	1072	67	6340
mir-20a-3p	750	56	0	141	38	136	405	763	1734	1462	920	6405
mir-216b-5p	1213	113	0	0	65	8	0	22	136	150	4479	6186
mir-130c-3p	27	169	224	0	65	34	63	146	1924	1270	2062	5984
mir-142a-3p	3637	225	918	113	456	49	57	32	74	0	398	5959
mir-140-3p	1879	591	336	0	95	152	36	37	283	468	1825	5701
mir-130a-5p	35	619	0	0	57	61	115	214	1606	890	2059	5657
mir-142-5p	3154	534	1210	0	251	38	131	59	43	80	127	5627
mir-130c-5p	1448	422	246	0	198	107	161	290	2178	422	133	5605
mir-20-5p	408	253	246	0	190	45	113	238	1886	1034	1088	5501
mir-429-3p	1331	703	806	0	175	38	127	70	512	528	1205	5495
mir-92a-5p	425	169	22	0	87	75	536	886	1430	720	1113	5463
mir-196-5p	534	1378	112	703	53	8	29	24	162	857	2166	6027
mir-9226-3p	24	0	0	169	748	217	801	1258	717	972	565	5472
mir-150-5p	3640	619	515	0	262	71	52	22	36	0	4	5222
mir-30e-3p	723	619	515	0	72	32	124	35	719	430	1818	5088
mir-141-3p	463	2644	1120	619	281	178	113	19	60	16	188	5700
mir-2188-5p	148	1913	1501	0	536	152	344	168	119	0	142	5022
mir-34b-5p	207	3235	627	0	289	138	131	104	62	38	151	4982
mir-222a-5p	1401	0	45	0	53	16	41	34	804	1859	432	4685
let-7b-5p	951	2363	829	3235	251	63	95	54	30	23	0	7893
mir-93a-5p	1119	141	426	0	217	99	79	93	703	666	1033	4575
mir-133a-5p	4	0	0	0	0	0	2	13	34	59	4421	4533
mir-222a-3p	740	0	269	141	68	12	2	12	1270	1246	887	4647
mir-449a-5p	74	1322	134	0	236	89	109	137	95	130	2175	4502
mir-125a-5p	52	1435	1545	0	540	132	122	83	63	17	427	4415
mir-133-3p	26	0	0	1322	0	10	2	6	11	128	4050	5556
mir-210-3p	1178	1688	560	0	224	55	81	89	43	0	310	4229
mir-144-3p	89	788	1725	0	399	91	54	31	202	13	799	4191
mir-142a-5p	2268	225	627	1688	281	47	118	43	127	54	367	5846
mir-23a-3p	913	563	1254	788	239	79	129	50	93	110	674	4891
mir-183-5p	181	422	179	225	11	53	14	36	296	581	2323	4320
mir-194a-5p	92	675	1277	0	331	124	95	23	50	116	1295	4077
mir-17a-5p	482	0	336	422	160	41	95	109	1183	567	960	4354
mir-96-5p	71	1125	314	0	65	51	23	39	627	674	911	3899
mir-22a-5p	819	56	538	0	186	69	124	156	859	462	621	3890
mir-29b-3p	1262	563	1030	1125	410	124	111	80	107	75	70	4957
mir-103-3p	1875	338	134	0	148	22	52	51	137	296	738	3791
mir-210-5p	2393	506	403	563	65	28	48	18	8	1	293	4325
mir-454b-3p	57	113	246	0	15	26	11	42	284	468	2489	3751
mir-190-5p	1972	113	896	506	171	55	45	43	35	32	311	4178
mir-4286-5p	2050	0	45	0	30	18	86	148	348	56	879	3662
mir-9-3p	199	56	179	0	11	14	27	126	250	314	2465	3642
mir-21b-3p	291	0	470	0	141	4	25	5	1155	917	580	3589
let-7g-5p	1786	844	470	0	220	63	36	49	13	0	2	3484
mir-219-3p	7	113	0	0	23	2	7	52	950	1226	1059	3439
mir-208b-3p	2510	0	0	844	0	0	0	4	1	151	724	4234
mir-215-5p	34	928	784	0	125	24	48	35	16	119	1277	3390
mir-200a-5p	77	338	224	0	34	2	2	25	1336	1011	320	3370
mir-18-5p	70	0	45	0	95	36	66	89	1019	651	1253	3323
mir-194-5p	175	1153	515	0	137	77	50	71	25	151	852	3206
mir-140-5p	396	394	806	0	106	28	25	30	206	238	962	3190
mir-736-3p	2683	0	0	1153	42	2	0	1	7	8	380	4277
mir-458-3p	2344	169	336	0	8	4	7	4	9	1	238	3120

Normalized reads

miRNA	Sperm	Unfertilized eggs	2 Cells	32 Cells	64-512 Cells	Mid blastula	Early gastrulation	50% Epiboly	Neurulation	10 Somites	Hatch	Sum
mir-21a-5p	1274	563	918	0	194	59	32	31	31	1	3	3106
mir-4492-3p	94	1013	762	169	578	128	253	60	38	1	146	3241
mir-725-3p	493	113	0	0	175	32	115	38	347	377	1376	3066
mir-199-3p	596	28	22	0	91	18	7	27	8	1	2155	2953
mir-454-3p	16	0	22	0	27	10	34	26	737	802	1244	2919
mir-34a-3p	575	1491	224	0	133	73	70	70	42	0	154	2831
mir-203-3p	38	0	0	0	34	6	18	12	250	1826	632	2816
mir-27a-3p	247	703	515	0	353	79	86	40	84	0	484	2592
mir-199-5p	422	422	403	0	80	20	18	19	32	23	1133	2572
mir-301a-3p	411	84	448	0	53	26	16	25	213	169	1064	2510
mir-126a-5p	392	0	157	0	61	14	16	25	15	539	1213	2432
mir-222b-3p	1254	534	45	84	49	24	34	35	15	149	285	2511
mir-7641-5p	664	84	403	0	745	103	138	111	50	77	43	2418
mir-217-5p	948	28	269	534	68	47	23	3	64	102	855	2940
mir-1335-3p	229	731	941	0	228	67	106	49	23	0	23	2397
mir-9a-3p	89	113	0	28	4	12	16	8	58	88	1948	2364
mir-2478-3p	305	225	470	0	334	148	242	250	154	81	41	2252
mir-23b-3p	283	619	605	113	232	61	88	34	37	61	201	2335
mir-145-3p	13	619	336	0	65	14	11	10	73	536	538	2216
mir-301d-3p	71	0	582	0	65	6	27	75	40	65	1277	2209
mir-145-5p	74	281	851	619	502	154	91	40	49	26	82	2768
mir-124-3p	2	0	0	0	0	0	0	22	140	272	1712	2148
mir-1386-5p	119	394	336	0	274	36	145	34	173	169	396	2076
mir-1388-3p	1064	534	0	0	4	0	32	1	31	95	291	2052
mir-462a-5p	1677	0	336	0	4	16	9	0	6	0	1	2049
mir-200b-5p	107	0	67	0	84	0	7	20	1039	335	378	2038
mir-22a-3p	59	169	829	0	422	152	95	77	47	56	77	1982
mir-124-5p	0	0	0	0	0	0	18	7	284	753	908	1971
mir-106b-3p	178	0	0	0	23	6	57	156	763	486	278	1946
mir-203-5p	9	0	0	0	61	4	5	19	1007	642	180	1928
let-7d-5p	626	1041	202	0	38	8	2	8	1	0	1	1927
mir-455-5p	77	28	1165	0	205	14	27	38	58	0	301	1914
mir-93-5p	402	28	426	1041	141	32	41	70	194	154	389	2916
mir-726-3p	1583	0	202	0	4	0	0	0	5	0	1	1794
mir-125a-3p	791	0	0	0	42	47	14	16	11	1	864	1784
mir-132-5p	1635	0	0	0	0	4	2	5	10	1	100	1757
mir-457a-5p	1105	84	134	0	156	38	36	13	32	5	85	1689
mir-10a-3p	9	0	269	0	125	2	9	0	334	196	731	1675
mir-2184-5p	557	366	179	84	319	61	34	38	35	11	45	1730
mir-212-3p	1611	0	0	0	4	0	0	0	4	1	0	1620
mir-204-5p	100	309	0	0	72	36	23	34	133	65	845	1617
mir-18b-3p	10	0	0	0	0	34	120	242	935	219	42	1603
mir-216a-5p	171	0	157	0	34	24	7	4	16	66	1077	1556
mir-462-5p	1379	84	22	0	0	36	0	7	10	1	1	1541
mir-449b-5p	5	0	90	0	30	2	5	16	16	211	1142	1516
mir-133b-3p	14	113	0	0	0	0	0	2	16	4	1329	1477
mir-10b-3p	0	0	0	0	11	8	11	1	648	529	262	1471
mir-222b-5p	832	0	202	113	0	20	32	32	122	154	61	1567
mir-5119-3p	0	0	403	0	274	160	176	252	172	16	1	1454
mir-139-5p	593	422	0	0	72	22	16	19	47	1	252	1444
mir-133c-3p	20	0	0	0	0	0	5	4	3	193	1207	1432
mir-7147-5p	216	84	560	422	38	2	9	1	39	283	187	1842
mir-33a-5p	108	84	515	0	293	30	27	11	59	0	251	1379
mir-30a-3p	112	0	67	0	61	4	11	15	581	205	305	1361
mir-199a-5p	44	422	179	84	171	47	43	33	14	2	395	1434
mir-456-3p	611	0	112	0	87	18	7	7	81	0	417	1340
mir-212b-5p	1110	56	0	0	0	14	2	0	8	76	1	1268
mir-455-3p	136	703	112	0	84	22	38	31	40	25	64	1255
mir-27c-3p	82	478	336	0	30	20	5	6	43	21	199	1220
mir-301-3p	5	0	134	703	0	0	0	10	76	144	845	1918
mir-375-3p	108	338	179	0	27	30	63	40	95	17	274	1170
mir-203b-3p	3	0	22	0	141	456	269	4	16	60	188	1160
mir-430d-3p	5	0	0	338	369	263	224	146	79	10	17	1452
mir-135a-5p	12	253	314	0	46	24	29	24	41	23	327	1092
mir-30b-3p	508	197	0	0	15	10	32	1	43	95	180	1081
mir-1c-3p	0	0	336	0	0	20	2	0	40	60	620	1079
mir-7-3p	108	0	22	0	65	30	36	82	452	239	40	1075
mir-15c-3p	136	619	0	0	4	18	48	21	164	30	27	1067
mir-153-3p	261	0	0	0	0	6	7	8	41	118	610	1052
mir-1973-3p	0	84	336	0	315	87	52	47	18	99	0	1039
mir-126a-3p	64	281	269	0	65	26	11	13	22	0	286	1038
mir-4792-5p	20	0	874	0	49	24	20	29	19	0	2	1037
mir-181b-3p	330	0	0	0	0	12	7	1	60	30	592	1032
mir-24a-5p	54	225	45	0	141	24	14	1	37	49	441	1032
mir-19a-5p	0	0	0	0	4	36	197	220	561	0	14	1031
mir-31-3p	93	534	134	0	95	12	43	45	31	11	8	1007
mir-10c-3p	4	56	202	0	27	0	0	0	188	60	452	989
mir-130d-3p	10	0	0	0	34	2	9	6	64	259	604	989
mir-153a-3p	264	56	67	0	129	26	32	12	53	35	309	984
mir-122-3p	0	169	493	0	198	10	23	13	7	0	47	958
mir-17-3p	20	0	0	0	15	20	124	167	552	18	31	948
mir-29c-3p	357	113	224	0	106	67	25	17	13	0	0	923
mir-15b-3p	587	113	45	0	4	8	5	27	64	12	52	917
mir-19b-5p	11	0	22	113	4	41	88	280	267	172	26	1024
mir-214-3p	3	309	314	0	8	0	5	10	6	0	225	880

Normalized reads

miRNA	Sperm	Unfertilized eggs	2 Cells	32 Cells	64-512 Cells	Mid blastula	Early gastrulation	50% Epiboly	Neurulation	10 Somites	Hatch	Sum
mir-1260a-5p	73	113	179	0	68	26	91	112	99	42	75	878
mir-135b-5p	12	422	157	0	91	14	59	32	17	0	59	863
let-7a-3p	110	366	157	0	95	20	29	23	15	0	25	840
mir-125c-5p	135	309	179	0	34	61	18	14	9	1	72	833
mir-130b-5p	347	28	67	0	34	0	11	23	46	66	197	821
mir-190a-5p	134	0	112	0	49	14	25	45	50	71	316	815
mir-203a-5p	3	0	0	0	30	2	0	4	471	210	88	808
mir-133b-5p	0	0	0	0	0	2	2	0	26	143	630	804
mir-142b-5p	526	84	0	0	42	0	11	4	4	0	126	797
mir-722-3p	127	28	291	0	114	38	16	36	19	10	50	730
mir-216-5p	91	0	0	84	0	0	0	0	18	158	461	812
mir-5124a-5p	0	0	448	0	80	73	48	31	24	0	0	704
mir-31a-5p	146	84	269	0	61	8	59	0	10	0	34	671
mir-3123-3p	65	0	448	0	103	0	27	14	11	0	0	668
mir-138-5p	126	0	0	84	91	6	0	18	98	135	190	749
mir-2957-3p	0	0	470	0	65	10	18	5	53	0	0	621
mir-1957a-5p	0	506	22	0	4	28	14	21	13	0	0	609
mir-129-5p	24	281	0	0	95	6	16	52	17	0	107	599
mir-24-5p	25	0	0	0	0	0	9	0	37	180	344	595
mir-190a-3p	1	169	112	281	167	22	45	57	19	0	0	874
mir-489-3p	445	0	0	0	15	26	0	4	11	59	12	572
mir-143-5p	0	0	0	0	0	2	9	0	2	0	559	572
mir-135c-5p	119	56	134	0	103	32	29	31	48	0	17	571
mir-31-5p	151	197	134	0	27	10	23	15	5	0	0	562
mir-155-5p	464	84	0	0	0	4	0	1	8	0	0	562
mir-101-5p	98	0	0	0	46	22	25	105	207	16	42	560
mir-15c-5p	187	84	202	0	46	2	11	16	2	3	6	560
mir-125-3p	242	0	0	0	0	0	2	13	9	0	283	549
mir-100-3p	47	366	0	0	34	0	11	24	3	0	62	547
mir-24a-3p	102	0	291	0	23	55	5	17	6	0	48	546
mir-1260b-5p	1	197	112	366	27	38	91	40	32	2	0	905
mir-466i-5p	115	0	67	0	125	38	16	76	50	0	40	528
mir-214-5p	0	0	179	197	19	0	14	15	0	8	289	721
mir-193b-3p	37	0	336	0	11	0	9	0	40	0	82	515
let-7c-3p	308	84	0	0	0	0	0	17	2	0	103	514
mir-126-5p	60	141	0	0	0	4	0	3	3	223	78	512
mir-460-3p	12	0	0	0	0	0	0	1	1	401	93	507
mir-24b-3p	22	113	224	141	76	0	5	7	3	0	46	636
mir-6412-3p	280	0	67	0	19	0	29	45	40	1	0	483
mir-8159-5p	45	56	202	0	19	8	2	4	1	62	82	482
mir-219a-3p	0	0	0	0	19	8	11	6	3	228	203	479
mir-460b-5p	91	84	90	0	61	34	9	16	2	78	13	478
mir-139-3p	103	0	112	0	38	0	0	3	78	39	98	472
mir-190b-3p	96	0	179	0	110	8	7	13	5	50	0	467
mir-212-5p	444	0	0	0	0	0	0	1	9	0	7	461
mir-29b-5p	69	169	45	0	30	20	41	10	26	22	29	460
mir-218-3p	26	0	0	0	0	0	0	1	55	301	50	432
mir-181c-5p	40	0	0	169	0	0	0	0	0	1	387	597
mir-1788-3p	2	0	358	0	0	0	0	0	0	1	57	419
mir-124a-3p	0	0	0	0	0	0	2	0	4	40	360	407
mir-7132a-5p	364	0	0	0	0	0	0	0	21	0	11	397
mir-106-5p	71	0	22	0	27	8	5	26	66	40	100	365
mir-301b-3p	6	113	0	0	4	0	2	10	64	7	154	360
mir-219-5p	0	0	0	0	0	0	2	0	19	34	292	348
mir-551b-3p	6	0	269	113	19	0	7	0	5	0	38	456
mir-4443-5p	2	253	0	0	15	0	18	20	23	0	0	332
let-7g-3p	25	0	0	0	8	18	27	51	17	183	0	329
mir-499-3p	21	0	0	253	0	0	0	7	1	1	297	580
mir-365-3p	13	84	45	0	42	8	27	19	22	34	33	326
mir-460a-5p	50	0	0	0	0	0	2	1	0	225	48	326
mir-33-5p	36	0	246	84	0	10	16	13	3	0	0	409
mir-7704-5p	3	141	0	0	8	18	18	28	3	0	101	320
let-7b-3p	134	0	90	0	15	30	34	13	4	0	0	320
mir-9341-5p	0	0	0	0	163	49	29	2	42	7	24	317
let-7d-3p	299	0	0	0	0	0	7	0	7	0	0	312
mir-24b-5p	12	169	0	0	0	0	0	0	6	75	46	308
mir-10c-5p	0	0	22	0	8	45	20	15	16	141	39	305
mir-103-5p	36	0	112	0	15	14	0	0	12	10	100	299
mir-30d-3p	35	0	0	0	0	2	2	15	122	103	19	297
mir-107b-3p	27	0	224	0	4	4	2	4	0	0	23	289
mir-4448-3p	0	0	134	0	0	12	75	46	21	0	0	288
mir-281a-3p	38	0	0	0	0	0	0	0	0	0	247	285
mir-96-3p	0	113	0	0	0	8	0	4	133	0	0	258
mir-137-3p	0	0	0	0	4	2	0	11	2	33	199	252
mir-128b-3p	220	0	0	0	19	6	2	0	2	0	0	249
mir-146b-5p	125	0	22	0	38	8	14	38	2	1	1	249
mir-4508-5p	33	0	0	0	114	0	29	29	4	0	32	241
mir-27b-5p	60	0	22	0	0	0	7	0	20	0	123	233
mir-27d-3p	54	84	0	0	49	0	7	26	11	0	1	232
mir-489-5p	0	197	0	0	23	0	0	0	4	0	8	232
mir-731-5p	225	0	0	0	0	2	2	0	0	0	0	230
mir-107-3p	32	0	0	0	0	0	0	16	11	10	159	228
mir-128-5p	30	0	0	0	0	0	0	3	137	56	1	227
mir-193a-3p	0	0	0	0	129	4	11	3	29	0	47	224
mir-2189-3p	0	0	0	0	38	105	45	7	0	2	19	217

Normalized reads

miRNA	Sperm	Unfertilized eggs	2 Cells	32 Cells	64-512 Cells	Mid blastula	Early gastrulation	50% Epiboly	Neurulation	10 Somites	Hatch	Sum
mir-147-3p	67	0	112	0	0	0	11	0	3	11	12	217
mir-205b-5p	7	84	0	0	0	0	0	3	22	75	23	214
mir-23a-5p	79	28	0	0	8	2	20	0	25	42	8	212
mir-7b-5p	3	28	0	0	61	49	36	14	6	2	10	209
mir-182-3p	2	0	0	0	0	0	0	9	27	0	169	208
mir-551-3p	36	0	0	0	30	18	5	1	12	0	95	197
let-7i-3p	121	0	0	0	38	4	9	15	9	0	0	195
mir-4454-5p	31	0	0	0	129	16	2	7	9	0	0	195
mir-142b-3p	140	0	45	0	0	0	0	1	6	0	0	192
mir-29a-5p	45	113	0	0	15	10	0	3	5	0	0	190
mir-17a-3p	14	0	0	0	0	20	20	39	89	0	3	186
mir-5108-3p	0	0	90	113	15	10	14	34	20	0	0	295
mir-1279-5p	0	0	179	0	0	0	0	0	0	0	0	179
mir-430a-5p	0	0	0	0	4	32	7	80	0	2	54	179
mir-206-5p	0	0	0	0	0	6	0	0	19	0	153	178
mir-129-3p	7	0	0	0	30	0	77	31	8	0	24	177
mir-301-5p	39	0	0	0	0	0	2	0	15	119	0	175
mir-18c-5p	0	28	22	0	34	51	20	8	1	1	8	174
mir-147b-3p	9	113	45	0	0	0	0	0	7	0	0	174
mir-99a-3p	109	0	0	28	0	0	0	4	2	0	55	198
mir-3074-5p	159	0	0	0	8	0	0	0	0	0	0	167
mir-430c-5p	0	0	0	0	0	20	9	55	0	4	76	164
mir-9b-5p	57	0	0	0	46	0	14	2	5	1	34	158
mir-4485-3p	0	0	134	0	0	0	0	0	3	15	0	152
mir-196b-5p	18	28	0	0	8	49	20	1	2	4	22	151
mir-194-3p	0	0	0	0	0	0	0	0	5	0	146	151
mir-3618-3p	0	0	22	0	19	12	9	25	40	19	2	149
mir-551a-3p	2	113	0	0	0	0	0	9	26	0	0	149
mir-459-5p	31	0	0	0	0	0	0	1	16	82	19	149
mir-181e-5p	16	56	0	113	0	0	0	0	1	2	72	260
mir-3120-3p	36	0	45	0	65	0	0	1	1	0	0	147
mir-5112-5p	1	0	0	0	30	18	23	42	30	0	0	144
mir-221-3p	72	0	0	0	4	0	9	8	26	0	24	144
mir-429b-3p	5	56	0	0	19	0	43	4	9	0	1	137
mir-3968-3p	7	0	0	0	0	8	20	13	20	60	0	129
mir-30c-3p	3	0	0	56	0	4	0	5	53	0	63	184
mir-3620-5p	12	0	112	0	0	0	0	4	0	0	0	128
mir-106b-5p	14	0	22	0	8	0	2	4	37	17	23	127
mir-135a-3p	4	56	0	0	0	0	7	4	18	0	35	124
mir-125b-3p	10	0	0	0	0	0	0	0	1	0	111	122
mir-93a-3p	21	0	0	0	0	2	2	0	92	0	0	118
mir-18-3p	3	0	0	0	0	0	7	4	82	10	11	117
mir-1338-3p	113	0	0	0	0	0	2	0	2	0	0	117
mir-10d-5p	0	0	22	0	0	0	0	4	6	43	36	111
mir-223-5p	108	0	0	0	0	0	2	0	0	0	0	110
mir-107a-3p	9	0	0	0	0	4	2	12	2	0	79	108
mir-2424-3p	11	0	90	0	0	2	2	1	1	0	0	107
mir-338-5p	7	0	22	0	0	0	0	1	7	70	0	107
mir-5106-3p	0	0	0	0	57	22	11	5	5	3	0	104
mir-2428-5p	0	0	0	0	34	16	20	21	7	0	0	99
mir-204-3p	0	0	22	0	0	0	2	0	9	0	62	95
mir-101a-5p	51	0	0	0	27	0	7	0	3	0	0	88
mir-183-3p	1	0	0	0	0	0	2	0	9	10	63	86
mir-3533-3p	0	0	67	0	0	0	0	0	5	0	12	84
mir-135b-3p	0	0	0	0	0	0	2	34	26	20	0	83
mir-4286-3p	80	0	0	0	0	0	0	0	0	0	0	80
mir-9277-3p	30	0	0	0	0	0	9	19	19	0	0	78
mir-5100-3p	2	0	0	0	0	20	34	19	2	0	0	77
mir-6651-5p	8	0	0	0	30	18	7	7	0	0	0	71
mir-133-5p	0	0	0	0	0	0	2	0	0	0	68	70
mir-196a-3p	0	0	0	0	0	0	0	0	14	0	54	68
mir-153a-5p	6	0	0	0	0	0	2	0	13	0	43	65
mir-1306-5p	31	0	0	0	19	0	0	6	8	0	0	63
mir-1-5p	0	0	0	0	0	0	0	1	3	0	55	60
mir-103b-3p	8	0	45	0	4	0	0	0	0	0	0	56
mir-23b-5p	3	0	0	0	0	0	0	0	0	39	13	55
mir-722-5p	1	0	0	0	53	0	0	1	0	0	0	55
mir-723-5p	0	0	0	0	0	0	0	0	14	39	0	53
mir-100c-5p	41	0	0	0	0	0	0	0	0	0	0	41
mir-192b-5p	0	0	0	0	0	0	0	0	2	0	38	40
mir-7a-3p	27	0	0	0	0	0	5	0	8	0	0	39
mir-1582-3p	0	0	0	0	38	0	0	0	0	0	0	38
mir-1a-3p	0	0	0	0	0	0	0	0	0	0	36	36
mir-9b-3p	0	0	0	0	34	0	0	0	0	0	0	34
mir-467g-3p	3	0	0	0	30	0	0	0	0	0	0	33