



## IN SILICO IDENTIFICATION AND CHARACTERIZATION OF miRNA IN *TURSIOPS TRUNCATUS*

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### ABSTRACT

MicroRNAs (miRNAs) belong to class of single-stranded RNA molecules having length of 22 nucleotides. They take part in regulation of gene expression in numerous organisms at post-transcriptional repression level of messenger RNA. In the current research, the EST databases of *Tursiops truncatus* were explored for the prediction and analysis of potential miRNAs and their target genes with the aid of various computational and bioinformatics tools. In *Tursiops truncates*, the identification of miRNAs and their targets were carried out on linux operating system featured with Standalone BLAST, CD-Hit, Clustalw, RNAhybrid and imiRNA-SSF. Initially 24 potential miRNAs were identified among them two of them are paralogous with 100% identity. These 24 miRNAs were subjected for analysis of paralogous sequences, secondary structures, true and pseudo miRNAs and phylogenetic studies using CD-HIT, RNAhybrid, imiRNA-SSF, clustalw tools respectively. Finally 2 miRNA with id ttr- mir-147 and ttr- miR-4680 were predicted as mature and real miRNA. The present study suggested that there is an sample of miRNAs in this specie playing some crucial role in the development and physiology of *Tursiops truncatus* and can further explored for functional studies. These predicted miRNAs will be helpful to accelerate the pace miRNA discovery that eventually lead to an improved and better understanding of miRNA role in the course of development and physiology of *Tursiops truncatus*.

**KEYWORDS:** miRNA, *Tursiops truncatus*, Insilico analysis



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## INTRODUCTION

MicroRNAs (miRNAs) are small noncoding RNA sequences usually consisting of 22 nucleotides. Its precursor is known as miRNA precursor or pre-miRNAs which is a stem-loop hairpin with length of 80 nucleotides. These precursor molecules are firstly transcribed as long RNA molecules.<sup>1</sup>This precursor is then cleaved with two different RNAase III enzyme in subsequent manner namely Drosha and Dicer into mature miRNA. MicroRNA genes are among the most common and abundant class of regulatory genes in kingdom Animalia. It is supposed that miRNAs may involve in large number of regulatory functions yet not explored because of their high percentages in predicted genes of several organisms.<sup>6-10</sup> The role of miRNAs in various regulatory processes in class of mammals is still not known. It may be due to their involvement in the course of tissue or developmental stage-specific expression processes or may because of evolutionary homology with other mammals or even with invertebrates.<sup>7,8,10-18</sup> Experimental approach of miRNAs and their targets identification is time consuming and problematic. Therefore, various bioinformatics based methodologies have been established and employed for fast and reliable miRNAs prediction. Phylogenetic sequence conservation approach is the most successful scheme among the various in silico miRNAs prediction methodologies.<sup>19, 20</sup> The computational programs like miRscan<sup>10</sup>, miRseeker<sup>7</sup> and RNAhybrid<sup>21</sup>, work on the basis of comparative genomics have been used for the successful prediction of miRNAs. *Tursiops truncatus*, commonly known as bottlenose dolphin, is among one of the eminent and well-characterized mammal species of marine world. Its natural habitat is tropical water with moderate temperature conditions throughout the world. Molecular understanding of bottlenose dolphin is still in primitive stage despite of its familiarity<sup>22-24</sup>. Therefore, there is a need to explore its molecular aspect of various biological functions and their regulations including information regarding miRNAs found in bottlenose dolphin. In this present study, with the aid of available bioinformatics based tools we have predicted miRNA from whole express sequence tags (ESTs) of *Tursiops truncatus*. This study will be helpful in better understanding of various cellular and development of *Tursiops truncatus*.

## MATERIALS AND METHODS

### Retrieval of Dataset for miRNAs prediction

A total of 8,045 express sequence tags (ESTs) of *Tursiops truncatus* available at NCBI (<http://www.ncbi.nlm.nih.gov/nucest/?term=tursiops%20truncatus>) and miRNA sequences from miRBase database<sup>25</sup> (<http://www.mirbase.org/ftp.shtml>) were retrieved as a data set for blastn.

### Homology search for prediction of potential miRNAs

Blastn of complete ESTs of bottlenose dolphin was run against the data set of miRBase database using standalone blast version 2.2.29<sup>26</sup>. Default parameters with e-value of 0.01 were used for blastn search for the prediction of potential miRNAs.

### Removal of duplications among predicted miRNAs

In order to remove duplications among the predicted potential miRNAs, Clustering tool CD-HIT ([cd-hit.org/](http://cd-hit.org/))<sup>27</sup> will be run against newly identified miRNAs.

### Secondary structure prediction and validation

In predicted miRNA, nearly 80 nucleotides upstream and downstream of selected and then using sliding window of about 100 nucleotides with increment of 10 nucleotides in moving were used as pre-miRNA sequences. These precursor sequences were then subjected to web based software RNAhybrid version 2.2<sup>28</sup> ([bibiserv.techfak.uni-bielefeld.de/rnahybrid/](http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/)) for precursor secondary structure prediction.

### Conservative analysis of miRNA

*Tursiops truncatus* conservation with their orthologs was analyzed with other eukaryotic species and will be align using ClustalW<sup>29</sup> ([www.ebi.ac.uk/Tools/msa/clustalw2/](http://www.ebi.ac.uk/Tools/msa/clustalw2/)).

### Phylogenetic analysis

Results of ClustalW will be analyzed by aligning selected miRNA sequences with CLUSTALW ([www.ebi.ac.uk/Tools/msa/clustalw2/](http://www.ebi.ac.uk/Tools/msa/clustalw2/)).

## RESULTS

### Computational prediction of miRNA in *Tursiops truncatus*

In kingdom animalia, most of miRNAs are highly conserved across different species and this characteristic can be explored for the prediction of novel miRNAs based on sequence alignments strategy. Therefore, we carried out Basic Local Alignment Search Tool BLAST searches of known animal miRNAs against the ESTs database of targeted organism in order to identify potential miRNAs. From 24 predicted different precursor miRNAs, two new and potential miRNAs in *Tursiops truncatus* were identified on the basis of their secondary structures; free energy; and true and pseudo content analysis. In miRBase database, two 2 datasets are available that is mature miRNA dataset and hairpin miRNA dataset respectively. Standalone BLAST was performed against mature miRNA datasets which resulted in no significant hits. It was then followed by BLAST search against hairpin miRNA dataset which results in 24 hits. The result summary of identified potential 24 microRNAs with their precursors, paralogous sequences and true microRNAs are listed in Table 1. The comprehensive information including names, sequence of hairpin miRNAs, MFE, and sources of novel *Tursiops truncatus* miRNA genes are also listed in Table 2. The length of 24 predicted miRNAs ranged from 20 to 21 nucleotides, while the predicted precursor sequences ranged in length from 77 to 300 nucleotides. Among 24 newly identified miRNAs, Paralogous sequences were observed in two predicted miRNA (mir-147 and mir-147b) (miR-1244-4 and miR 1244-4b). These sequences were perfectly (100%) matched with each other. The secondary structures of remaining 22 miRNAs were identified using RNAhybrid software. Among these 22 miRNAs, 6 miRNAs have higher minimal free energies which is greater than -24 kcal/mol. These 6 structures were excluded and finally 16

miRNAs were selected which are listed in Table 3. The 16 identified *Tursiops truncatus* miRNAs have MFEs ranging from -0.47 to -27.8 kcal/mol, with an average of about -54.6 kcal/mol. All the MFEs of hairpin structures of newly identified miRNAs were above 0.60 that used to discriminate miRNAs from other ones.<sup>30</sup> All the predicted miRNAs have typical stem-loop in their structure and their expected secondary structures are shown in Table 3. The variation among the sizes of the predicted miRNAs may be due to their different

precursors that might be responsible for unique functions in miRNA biogenesis or gene regulation.<sup>30</sup> According to study of Zhang, the probability of occurrence of single miRNA in a length of about 100,000 ESTs is 1 miRNA indicating a rough frequency of miRNA occurrence is 0.1% in entire EST sequences of species.<sup>30</sup> Therefore, from the above assumption *Tursiops truncatus* having 8,045 ESTs should have 0.8045 miRNAs.

Total number of <i>Tursiops truncatus</i> EST sequences	<b>8,045</b>
Total number of microRNAs identified in <i>Tursiops truncatus</i>	<b>24</b>
Prologues sequences found in 24 microRNAs	2 24-2=22
Real miRNA sequences found	2 ttr-mir 147; ttr- miR-4680

**Table 1**  
**Result summary of microRNAs in *Tursiops truncatus***

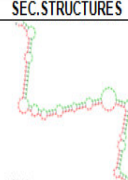



S#	Genbank IDs	MicroRNAsID	Sequence of microRNAs	Precursor sequence
1.	Gi74261219	Equus caballus miR-1261	TGCTCTGGATTAGGCTTTGGCTTAAG GGAATGTTGTGGCTGATTTGATCTTCT ATCCAGACCTTTAAAGCGTTCTCCATA TTAGC	CCCTTAAACCTCACGGGCTAGTCTCTGCT AGCTTCAAACCTTTCTTCTGCAGCTTCTTC ACCTCTCTCAGCCTTACAGAATTGAAGA GAGTTAGGGCCTTGCCTGGATTAGGCT TTGGCTTAAGGGAATGTTGTGGCTGATTT GATCTTCTATCCAGACCTTTAAAGCGTTC TCCATATTAGCAATACAGCTATTTCACTTT TTTATCATCCATGTACTCAATGGAGTAGC ACTTTTAATTTCTTCCAGGAACCTTTCTTT GCATTCACAACCTTGGCTGCTG
2	Gi74261902	Homo sapiens miR-1244-4	aTCTTATTCCGAGCATTCCAGTAACTtttt tGTGTATGTACTTAGCTGTACTATAAGT AGTTGGTTTGTATGAGATGGTTAAAAA	GTCTTACTTCTGACTTTACTTTGTGGTGTG ACCATGTTCAATTATAATCTCAAAGGAGAA AAAAAACCTTGTAATAAAAGCAAAAACAA CAACAAAAACAATCTTATCCGAGCAT TCCAGTAACTTTTTTTGTGTATGACTTAG CTGACTATAAGTAGTTGGTTTGTATGAG ATGGTTAAAAAGGCCGAAGATAAAAGGTT TCTTTTTTTTCTTTTTTTGTCTATGAAGTT GCTGTTTATTTTTTTGGCCTGTTTGTATGT ATGTGTGAAACAATGTTGTCC
3.	Gi77992081	Capra hircus miR-147	TACTCTATGAATCTAGTGAAACACTT CTGCACAACTAGATTATGGATACCAG TGTGCGGAAATGCTTCTGCTACATTTT TAGGGC	AAGCTTATAACAATCAACCAAGAATGGAA GCCTATTGAAGAGTTGCAGCAGGTCCGA AAGGCAACCAGGTGACCAGTCTTCGCCA TTTTCTTCCAAAGAGTACTCTATGAATCTA GTGGAACACTTCTGCACAACTAGATTA TGGATACCAGTGTGCGGAAATGCTTCTG CTACATTTTTAGGGCTTGCCTACGTTTTTC AGATTCTGGATAAAGAATTATAAAGGTGG TGCAACAATAACCACGTAGTCCCAAATA AGATTCTATGTTTATTTCTTGTGTAA
4.	Gi77992096	Capra hircus miR-147b	TACTCTATGAATCTAGTGAAACACTT CTGCACAACTAGATTATGGATACCAG TGTGCGGAAATGCTTCTGCTACATTTT TAGGGC	AAGCTTATAACAATCAACCAAGAATGGAA GCCTATTGAAGAGTTGCAGCAGGTCCGA AAGGCAACCAGGTGACCAGTCTTCGCCA TTTTCTTCCAAAGAGTACTCTATGAATCTA GTGGAACACTTCTGCACAACTAGATTA TGGATACCAGTGTGCGGAAATGCTTCTG CTACATTTTTAGGGCTTGCCTACGTTTTTC AGATTCTGGATAAAGAATTATAAAGGTGG TGCAACAATAACCACGTAGTCCCAAATA AGATTCTATGTTTATTTCTTGTGTAA
5.	Gi77992533	Xenopus laevis miR-703	AGACTGGTTAATGATAACAATGCATCG TAAAACCTTCAGAAGGAAAGGAGAAT GTGTTGTGGACCATTTGTTTTGTGTGT GGCAGTTTTAAGTTATTAGTTTTAAAA TCAGT	GAATATTATCCCCAATACCTGCCACCCCG GTCTTAATCAGTGGTGAAGAAGCGGTCT CAGAAGTGTCTCAATTGGCCATTTA AGTTTAATAGCAAAGACTGGTTAATGAT ACAATGCATCGTAAAACCTTCAGAAGGA AAGGAGAATGTGTTGTGGACATTTGTTT TGTGTGTGGCAGTTTTAAGTTATTAGTTTT TAAAATCAGTACTTTGCAATAGAAACAAC TTGACCAAAAATCTGTACAGAATTTGAG ACTCCTTAAAAAAGTTAATGAGGGGGAA AAAAAAAAGGAATCTGTTATGTA




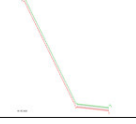
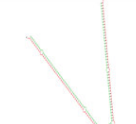

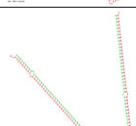

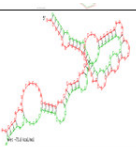



6.	Gi259803777	Xenopuslaevis miR-703	ACTGATTTTAAAACTAATAACTTAAAA CTGCCACACACAAAACAAATGGTCCA CAACACATTCTCCTTTCTCTGAAGG TTTTACAATGCATTGTTATCATTAACCA GTCT	ATTTCTTAAGGAGGGCTTTAACCAATTA GTTATTAACCTTTTTTAATGGGTCTCAAAT TCTGTGACAAATTTTTGGTCAAGTTGTTTC CATTAAAAAGTACTGATTTTAAAACTAAT AACTTAAAACTGCCACACACAAAACAAAT GGTCCACAACACATTCTCCTTTCTCTG AAGGTTTTACAATGCATTGTTATCATTAAC CAGTCTTTTGTATTAACTTAAATGGCC AATTGAAACAAACAGTTCTGAAACCGTTC TTCCACCCTGATTAACCGGGGTGGC AGGTATTGGGGATAATTC
7.	Gi259802500	Eptesicusfuscus miR-9366	ACTGAGAGATCATGCTGATAGTAACAT TGTTATCACGCTTGTGGGCAATAAGA GTGATTTGCGCATCTCAGGGCGGTT CCTACAGATGAAGCAAGAGCTTTTGC AGAAAAGAATGGTTTGTCTTTCATTGA GACATCTGCTCTAG	TATAACATCAGCATACTATCGTGGAGCTG TAGGTGCCTTATTGGTTTATGACATTGCT AAACATCTCACATATGAAAATGTAGAGCG ATGGCTGAAAGAATGAGAGATCATGCT GATAGTAACATTGTTATCAGCTGTGGG CAATAAGAGTGATTTGCGCCATCTCAGG GCGGTTCTACAGATGAAGCAAGAGCTT TTGCAGAAAAGAATGGTTTGTCAATTCATT GAGACATCTGCTCTAGACTCTACAAATGT AGAAGCTGCTTTTTCAGACAATCTTGACAG AGATATACCGCATTGTTCCAGAAAGCAA ATGTCAGACAGACGTGAAAATGACATGTC
8.	Gi259802594	Eptesicusfuscus miR-9366	CTAGAGCAGATGTCTCAATGAATGACA AACCATTCTTTTCTGCAAAAGCTCTTG CTTCATCTGTAAGAACCGCCCTGAGAT GGCGCAAATCACTTATTGCCACAA GCGTGATAACAA TGTTACTATCAGCATGATCTCTCAGT	GACATGTCATTTTACGCTCTGTGACAT TTGCTTCTGGGAAACAATGCGGTATATCT CTGTCAAGATTGTCTGAAAAGCAGCTTCT ACATTTGTAGAGTCTAGAGCAGATGTCTC AATGAATGACAAACCATTCTTTTCTGCAA AAGCTTTGCTTCATCTGTAAGAACCGCC CTGAGATGGCGCAAATCACTCTTATTGCC CACAAGCGTGATAACAATGTTACTATCAG CATGATCTCTCAGTTCTTTAGCCATCGC TCTACATTTTCAATGTGAGATGTTTAGCA ATGTCATAAACCAATAAGCCAATCAGTTA AATAAATCTTGGTCTAAAGAGATTAC
9.	Gi259802835	Mus musculus miR-684-1	GTAGGGCAATCTGTCTTTAAGTAGGG ATAAATTACTCTAAAAGAAA- TGAATCCTAGATAGTTTTCCCTTCAA TCAAGCGTCTTG	TTCTTGCATACCTTGTTCGATTGGAGAAT TTAATGTTTTTCATTTATCATTGTAACAA CATGGACAATTTTTATAACTTTTTGTACGT AGCTGTTACATGTAGGGCAATCTGTCTTT AAGTAGGGATAAATTACTCTAAAAGAAAT GAATCCTAGATAGTTTTCCCTTCAAATCA AGCGTCTTGTGTTTAAATAAACTTCTTGT TTAAAATGAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAGAAAAAAAAAAAAAA
10.	Gi259802942	Xenopuslaevis miR-703	ACTGATTTTAAAACTAATAACTTAAAA CTGCCACACACAAAACAAATGGTCCA CAACACATTCTCCTTTCTCTGAAGG TTTTACAATGCATTGTTATCATTAACCA GTCT	GTTTTCTCTGTTGACTGTGGGCTCCAGGA TGAATGTAGTTTCCAATGGCTTAATCCAAA TCAGGAGATAATTTCTTAAGGAGGGCTT TAACCAATTAGGTACTGATTTTAAAACTA ATAACTTAAAAGTCCACACACAAAACAA ATGGTCCACACACATTCTCCTTTCTTC TGAAGGTTTTACAATGCATTGTTATCATT ACCAGTCTTTTGTATTAACTTAAATGG CCAATTGAAACAAACAGTTCTGAAACCGT TCTTCCACCCTGATTAACCGGGGTG GCAGGTATTGGGGATAATATTC
11.	Gi259803027	Xenopuslaevis miR-703	ACTGATTTTAAAACTAATAACTTAAAA CTGCCACACACAAAACAAATGGCCCA CAACACATTCCCCTTTCTCTGAAGG TTTTACAATGCATTGTTATCATTAACCA GTCT	TAAGAGATTTGGGAGGATTGTTTTCTCA TTAAGCTTTGTTTTATCATGGGTCTCAAAT TCTGTGACAAATTTTTGGTCAAGTTGTTTC CATTAAAAAGTACTGATTTTAAAACTAAT AACTTAAAACTGCCACACACAAAACAAAT GGCCACAACACATTCCCCTTTCTCTG AAGGTTTTACAATGCATTGTTATCATTAAC CAGTCTTCTGCTATTAACTTAAATGGCC AATTGAAACAAACAGTTCTAAACCGTTC TTCCACCCTGATTAACCGGGGTG AGGTATTGGGAATAATTC
12.	Gi259803318	Homo sapiens miR-1244-4	TTTTAACCATCTCATACAAACCAACTA CTTATAGTACAGCTAAGTACATACACa aaaaaGTTACTGGAATGCTCGGAATAA	GGACAACATTGTTTTCACACATACATCAA CAGGCCAAAAAAATAAACAGCAACTTCA TAAACAAAAAAGGAAAAAAAAAAAAACCT TTTACCTTTGGCCTTTTAAACCATCTCATA CAAACCACTACTTATAGTACAGCTAAGT ACATACACAAAAAAGTTACTGGAATGCT CGGAATAAAATTGTTTTTTGTTGTTGTT TTGCTTTTTTTTACAAGGTTTTTTTTCTC CTTTGAAATTATAATGAACATGGCCACAC CACAAGTAAAGTCAAAGT
13.	Gi259803425	Homo sapiens miR-1244-4	aTCTTATTCCGAGCATTCCAGTAACttttt tGTGTATGACTTAGCTGTACTATAAGT AGTTGGTTTGTATGAGATGGTTAAAAA	CCTACTTCTGACTTTACTTGTGGTGTGAC CATGTTTATTATAATCTCAAAGGAGAAAA AAAACCTTGTAATAAAAAAGCAAAAAACAA CAACAAAAAACAATCTTATCCGAGCAT TCCAGTAACTTTTTTGTGTATGACTTAG

				CTGACTATAAGTAGTTGGTTTGTATGAG ATGGTTAAAAAGGCCAAAGGTAAGGTT TCTTTTTTTCCTTTTTTGTCTATGAAGTT GCTGTTATTTTTTGGCCTGTTTGATGT ATGTGTGAAACAATGTTGTCC
14.	Gi259803533	Homo sapiens miR-1244-4b	aTCTTATTCCGAGCATTCCAGTAACttttt tGTGTATGTACTTAGCTGTACTATAAGT AGTTGGTTTGTATGAGATGGTTAAAAA	CCTACTTCTGACTTTACTTGTGGTGTGAC CATGTTTATTATAATCTCAAAGGAGAAAA AAAACCTTGTAAAAAAAGCAAAAAACA CAACAAAAACAATCTTATTCCGAGCAT TCCAGTAACTTTTTTGTGTATGTACTTAG CTGACTATAAGTAGTTGGTTTGTATGAG ATGGTTAAAAAGGCCAAAGGTAAGGTT TCTTTTTTTCCTTTTTTGTCTATGAAGTT GCTGTTATTTTTTGGCCTGTTTGATGT ATGTGTGAAACAATGTTGTCC
15.	Gi259803694	Xenopuslaevis miR-703	ACTGATTTTAAAACTAATAACTTAAAA CTGCCACACACAAAACAATGGTCCA CAACACATTCTCCTTTCTTCTGAAGG TTTTACAATGCATTGTTATCATTAACCA GTCT	TTTCCTTAAGGAGGGCTTTAACCAATTAG CTCATTAAACTTTTTTAAATGGGTCTCAA TCTGTGACAAATTTTTGGTCAAGTTGTTT CATTAAAAAGTACTGATTTTTAAAACTAAT AACTTAAACTGCCACACAAAAACAAT GGTCCACAACACATTCTCCTTTCTTCTG AAGGTTTTACAATGCATTGTTATCATTAA CAGTCTTTTGTATTAACTTAAATGGCC AATTGAAACAAACAGTTCTGAAACCGTTC TTCCACCACTGATTAACCGGGGTGGC AGGTATTGGGGATAATATTC
16.	Gi259805583	Xenopuslaevis miR-703	ACTGATTTTAAAACTAATAACTTAAAA CTGCCACACACAAAACAATGGTCCA CAACACATTCTCCTTTCTTCTGAAGG TTTTACAATGCATTGTTATCATTAACCA GTCT	TTTCCTTAAGGAGGGCTTTAACCAATTAGT TCTCATTAACTTTTTTAAATGGGTCTCAA TTCTGTGACAAATTTTTGGTCAAGTTGTTT CCATTAAAAAGTACTGATTTTTAAAACTAA TAACTTAAACTGCCACACAAAAACA TGGTCCACAACACATTCTCCTTTCTTCT GAAGGTTTTACAATGCATTGTTATCATTAA CCAGTCTTTTGTATTAACTTAAATGGC CAATTGAAACAAACAGTTCTGAAACCGTTC CTTCCACCACTGATTAACCGGGGTGG CAGGTATTGGGGATAATATTC
17.	Gi259805668	Xenopuslaevis miR-703	ACTGATTTTAAAACTAATAACTTAAAA CTGCCACACACAAAACAATGGTCCA CAACACATTCTCCTTTCTTCTGAAGG TTTTACAATGCATTGTTATCATTAACCA GTCT	TTTCCTTAAGGAGGGCTTTAACCAATTAGT TCTCATTAACTTTTTTAAATGGGTCTCAA TTCTGTGACAAATTTTTGGTCAAGTTGTTT CCATTAAAAAGTACTGATTTTTAAAACTAA TAACTTAAACTGCCACACAAAAACA TGGTCCACAACACATTCTCCTTTCTTCT GAAGGTTTTACAATGCATTGTTATCATTAA CCAGTCTTTTGTATTAACTTAAATGGC CAATTGAAACAAACAGTTCTGAAACCGTTC CTTCCACCACTGATTAACCGGGGTGG CAGGTATTGGGGATAATATTC
18.	Gi259805867	Xenopuslaevis miR-703	ACTGATTTTAAAACTAATAACTTAAAA CTGCCACACACAAAACAATGGTCCA CAACACATTCTCCTTTCTTCTGAAGG TTTTACAATGCATTGTTATCATTAACCA GTCT	TCCTTAAGGAGGGCTTTAACCAATTAGT CTCATTAACTTTTTTAAATGGGTCTCAA TTCTGTGACAAATTTTTGGTCAAGTTGTTT CATTAAAAAGTACTGATTTTTAAAACTAAT AACTTAAACTGCCACACAAAAACAAT GGTCCACAACACATTCTCCTTTCTTCTG AAGGTTTTACAATGCATTGTTATCATTAA CAGTCTTTTGTATTAACTTAAATGGCC AATTGAAACAAACAGTTCTGAAACCGTTC TTCCACCACTGATTAACCGGGGTGGC AGGTATTGGGGATAATATTC
19.	Gi259805909	Homo sapiens miR-1244-4	TTTTTAACCATCTCATACAAACCAACTA CTTATAGTACAGCTAAGTACATACACa aaaaaGTTACTGGAATGCTCGGAATAA	GGACAACATTGTTTTCACACATACATCAAA CAGGCCAAAAAATAAACAGCAACTTCA TAAACAAAAAAGGAAAAAATAAACCT TTTACCTTTGGCCTTTTTAACCATCTCATA CAAACCACTACTTATAGTACAGCTAAGT ACATACACAAAAAAGTACTGGAATGCT CGGAATAAATTGTTTTTGTGTTGTTT TTGCTTTTTTTTACAAGGTTTTTTTCTC CTTTGAAATTATAATGAACATGGTCACAC CACAAGTAAAGTCAAAAGT
20.	Gi259805914	Xenopuslaevis miR-703	ACTGATTTTAAAACTAATAACTTAAAA CTGCCACACACAAAACAATGGTCCA CAACACATTCTCCTTTCTTCTGAAGG TTTTACAATGCATTGTTATCATTAACCA GTCT	TTTTTTTTTTTTTTTTTTTTTTTTTACTC ATTAACCTTTTTTAAATGGGTCTCAAATTCT GTGACAAATTTTTGGTCAAGTTGTTTCCA TAAAAAGTACTGATTTTTAAAACTAATA CTTAAACTGCCACACAAAAACAATGG TCCACAACACATTCTCCTTTCTTCTGAA GGTTTTACAATGCATTGTTATCATTAAACCA GTCTTTTGTATTAACTTAAATGGCCAAT TGAACAAACAGTTCTGAAACCGTTCCTC CACCCTGATTAACCGGGGTGGCAGG TATTGGGGATAATATTC
21.	Gi259804379	Bosstaurus miR-2904-3	ACGGCAGCGCCGCGGGAGCCTCGGT TGGCCTCGGATAGCCGGTCCCCACC	TGGTGTATGCTTACCCTGAGGAGCCAA TGGGGCAGAGCTACCATCTGTGGGATTA

			GTCC	TGACTGAACGCCTCTAAGTCAGAATCCC GCCAGGCGGAACGATACGGCAGCGCC GCGGAGCCTCGGTTGGCCTCGGATAG CCGGTCCCCACCGTCCACACCATTCC AACCTCATAGGAGCCGATGTATTTATTTT CCTTGAGTTTTTATTTATGCTGTAAAATGT ACCAAGCGATGGTTAAAGGGGACGTCAG AC
22.	Gi259805383	Xenopuslaevis miR-703	ACTGATTTTAAAACTAATAACTTAAAA CTGCCACACACAAAACAAATGGTCCA CAACACATTCTCCTTTCTTCTGAAGG TTTTACAATGCATTGTTATCATTAACCA GTCT	TTCCTTAAGGAGGGCTTTAACCAATTAGT CTCATTAAACTTTTTTAATGGGTCTCAAAT TCTGTGACAAATTTTTGGTCAAGTTGTTTC CATTAAAAAGTACTGATTTTAAAAACTAAT AACTTAAAACTGCCACACAAAAACAAAT GGTCCACAACACATTCTCCTTTCTTCTG AAGGTTTTACAATGCATTGTTATCATTAAC CAGTCTTTTGTATTAACCTTAAATGGCC AATTGAAAACAAACAGTTCTGAAACCGTTC TTCCACCCTGATTAACCGGGGTGGC AGGTATTGGGGATAATATTC
23.	Gi259803963	Xenopuslaevis miR-703	ACTGATTTTAAAACTAATAACTTAAAA CTGCCACACACAAAACAAATGGTCCA CAACACATTCTCCTTTCTTCTGAAGG TTTTACGATGCATTGTTATCATTAACCA GTCT	TTTTTTTTTTTTTTTTTTTTTTTTTTTTGTCC ATTAACCTTTTTTAATGGGTCTCAAATTCT GTGACAGATTTTTGGTCAAGTTGTCTCCA TTAAAAAGTACTGATTTTAAAAACTAATAA CTTAAAACTGCCACACAAAAACAAATGG TCCACAACACATTCTCCTTTCTTCTGAA GGTTTTACGATGCATTGTTATCATTAACC AGTCTTTTGTATTAACCTTAAATGGCCAA TTGAGACAAACAGTTCTGAAACCGTTC CCACCCTGATTAAGACCGGGGTGGCAG GTATTGGGGATAATATTC
24.	Gi259802663	Bostaurus miR-4680	CTGAATATAAGAACTCTTGCAGTCTTA GATGTTATAAAAAATATATATCTGAA TTGTAAGAGTTGTTAGCACAG	GAATTTGTATCTGTTTTCATGTTTGTGTTT TTTTATTGCAGGGGCAGAAAACGTTTTG TAAGTGAAGGAGATGGAGGTCGTCTTAA ACCAGAGAGCTACTGAATATAAGAACTCT TGCAGTCTTAGATGTTATAAAAAATATATA TATCTGAATTGTAAGAGTTGTTAGCACAG TTTTTTGGTTTTTTTTTTTTTAGCACTTGT TTTGGGTACAAGACATTTTTGAAATTTTGT AACTTACATTTAAGGGAAATTTTTAAAGT AAGTGT

**Table 2**  
**Sequences of 24 identified miRNA and precursor**

S/No	Gi No.	Length of RNA	MFE	GC%	MFEI	SEC. STRUCTURES
1	74261219	285	-56.8	41.75	-0.477	
2	74261902	286	-48.3	31.46	-0.53	
3	77992533	313	-62.5	35.46	-0.563	
4	259803777	313	-165.0	33.546	-1.57	

5	259802500	345	-98.7	40.869	-0.70	
6	259802835	285	-49	22.456	-0.76	
7	259803027	313	-159.6	35.143	-1.45	
8	259803318	283	-140.2	29.68	-1.66	
9	259803694	313	-165	34.185	-1.542	
10	259805583	314	-165	34.076	-1.542	
11	259805867	313	-165	34.185	-1.542	
12	259805914	312	-165	30.448	-1.736	
13	259804379	255	-73.6	53.725	-0.537	
14	259805383	313	-165	34.185	-1.542	
15	259803963	313	-170.9	35.5878	-1.53	
16	77992081	287	-69.5	39.024	-0.620	

**Table 3**  
**Sequence details and sec. structures of miRNA**

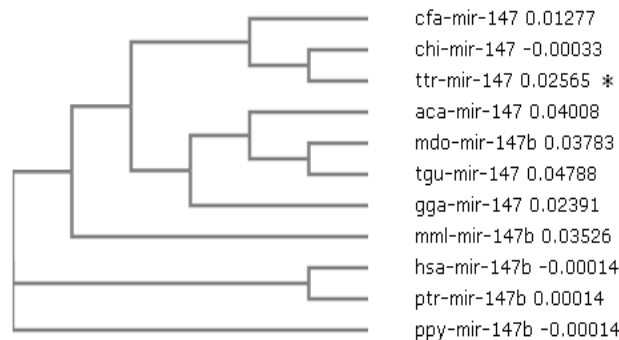
**Conservation of *Tursiops truncatus* miRNAs**

The conservation search of pre-miRNA (ttr-mir-147) of *Tursiops truncatus* against orthologous species was carried out and suggested sequence conservation of miRNA with other animals with same physiological functions. This outcome strongly validate that the newly identified miRNAs is a potential candidate of miRNAs in

*Tursiops truncatus*. The sequence conservation results are shown in Figure:1. Phylogenetic analysis of the miRNA (ttr-mir-147) sequence shown in Figure: 2 suggest that the *Tursiops truncatus* is more closely related to *Capra hircus* and *Canis familiares* indicating its evolutionary conservation during the course of development.



**Figure 1**  
Sequence variation among ttr-mir-147 and other miRNAs using Clustalw



**Figure 2**  
Phylogenetic tree showing evolutionary relatedness of ttr-mir-147

**CONCLUSION**

By seeking the publicly accessible *Tursiops truncatus* ESTs with those bioinformatics method, an aggregate number for 2 possibility miRNAs were distinguished. We anticipate that seeing of the part of miRNAs in the metabolic drought resistant in the animal will assistance clarify the methodology from a post transcriptional level.

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Those discoveries about miRNAs clinched alongside *Tursiops truncatus* will clear those manners to understanding the function and processing of *Tursiops truncatus* small RNAs in future.

**CONFLICT OF INTEREST**

Conflict of interest declared none.

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