

Insilico analysis of mRNA of menthe

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Abstract- Insilico refer to the analysis which is carried out in computer environment rather than in laboratory. Plant micro RNA are small non coding RNAs that have a length of 21 of 24 nucleotides which have role in growth and defense process. Many plants mRNAs are evolutionary conserved from species to species. MiRNA precursor display high variability their mature sequence display expensive sequence complementarity to their target mRNA sequence MiRNA play very important role in plant post transcriptional gene regulation by target mRNA cleavage or repressing translation. MiRNA are involved in plant development, signals transduction, proteins degradation, response to environment stress and pathogen invasion and regulate their Owen biogenesis. MiRNA regulate the expression of many important genes a majority of these genes are transcriptional factors. So here we use homology method meaning searching for mRNA sequence which is involved in inti oxidant. The species that we selected is mentha canadensis

PROCEDURE

Following are steps involved in the procedure

Data Source

Data source is very important factor when it comes to insilico analysis, here we have two public data library for retrieving sequence information.

1 <https://www.ncbi.nlm.nih.gov/>

2 <https://www.mirbase.org/>

Search for ESTs in NCBI

To find a novel unique miRNA 224 EST sequence were screened for identifying miRNAs of lowest E value. The criterion of lowest E value was 0.01-0.099. Mirbase is an online search engine used to get the micro RNAs of our specie, at the end of screening all the ESTs only 1 miRNA were found that meets the criteria 0f required E value. Following are the ESTs from which the E values are derived.

Serial no	EST Sequence	No of E value
1	>KY527367.1 Mentha canadensis AT5G40820 mRNA, partial sequence AAAACAAATCCTCAAATTAAGCGTA	

TCTATGACCAATCGCAAGGTAAAAT ACCTGAAGATGAAATGCTGA AGACAAAATCTGCCAATGTTCCCT CCAGCTTCCACAAATGGTTTCTAAA CACATTCTCAGAACCAGC TATGTGGTTTAGGGCCGCATAGCCT ATGCACATACTACTGCTGTGGTCA ATGGTTGGTCATATTGTT GGACTTGGAGATAGGCATGGCGAAA ACATCCTCTTTGATTCTACTACTGGG GATTGTGTGCATGTTGATT TTAGTTGCTTGTGTTGATAAAGGCTG CAGTTGGAGAAGCCAGAGTTGGTGC CTTTCAGGCTGACACAGAA CATGATTGATGGGCTTGGCCTACTG GCTATGAAGCACCTACCTTAGGGTT TCTGAGATCACACTTCA ATTCTGAGGGCCACAAGGAGACAC TAATGAGTGTCTGGAACTTTTATC CATGACCCTCTTGTGCAAT GGACTAAAATCTCACAATCTAGTGG AGTAGAAGTACAAAATCCACATGCC CAGCGAGCCATAAGCAACAT AGAAGCACGTCTTCAAGGAGTTGTT GTTGGTGTAGGAGCTGCGCTTCTTT GCCTCTAGCTGTGGAGGGA CAAGCACGCGTCTAATTTCTGAAGC AGTTTCTCTCAAAAACCTTGGAAAAA TGTATATATGGTGATGC CTTGGTTC
--

E VALUE SEQUENCE

The E value sequence which is obtained from miRbase

S #	Accession #	Sequence	E value
1	MIMAT0025799	UUUUCAGUCUGACA CAGAACAU	0.052

Structural Annotation

Mfold

The next step is structural annotation of these microRNAs to identifies their structures through an online software called Mfold that use these sequence to determine the best possible secondary structure and energies of these miro RNAs .

<http://unfold.rna.albany.edu/?q=mfold/RNA-Folding-Form2.3>

Micro RNA Structure

>gga-miR-6693-3p MIMAT0025799

Energy =0.20

6 of boxplot.ng (C)
Lutil 4.7

Created Tue Jul 3 04:35:38

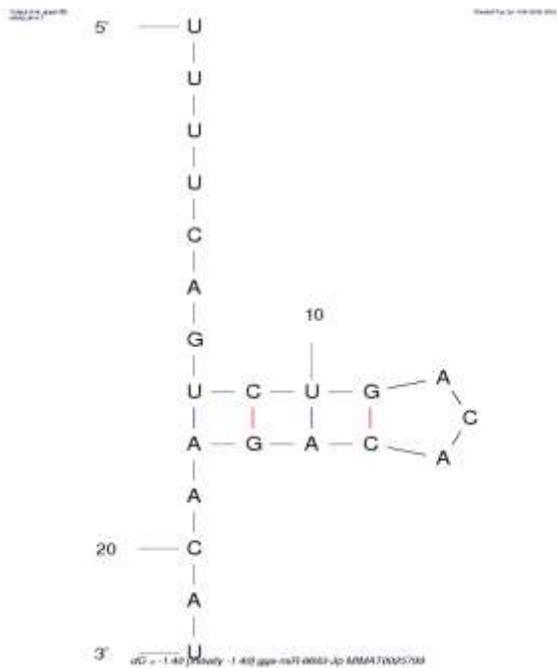
Fold of gga-miR-6693-3p MIMAT0025799 at 37 C.

deltaG in Plot File = 0.2 kcal/mol



Lower Triangle Shows
Optimal Energy
Upper Triangle
Base pairs Plotted: 6

Optimal energy: -1.4
-1.4 < energy <= -1.4
-1.3 < energy <= -1.3
-1.2 < energy <= -1.2



Linear RNA folding at 5%, window = 0, max folds = 50

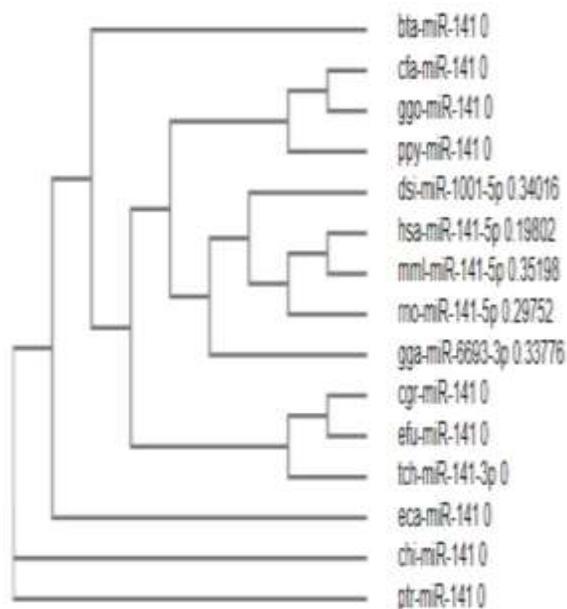
7 A's, 5 C's, 3 G's, 7 U/T's and 0 N's
UUUUCAGUCU GACACAGAAC AU

Align Sequence

bta-miR-141	UA--ACACUG-UCUGGU-A-A-AGAUG-G
cfa-miR-141	AA--CACU-G-UCUGGU-A-A-AGAUG-G
cgr-miR-141	UA--ACACUG-UCUGGU-A-A-AGAUGGC
chi-miR-141	UA--ACACUG-UCUGGU-A-A-AGAUG-G
dsi-miR-1001-5p	UGGUUA-A-CUCCAA-G-G-AUCAG-G
eca-miR-141	UA--ACACUG-UCUGGU-A-A-AGAUG-G
efu-miR-141	UA--ACACUG-UCUGGU-A-A-AGAUGGC
gga-miR-6693-3p	UU--UUCA-G-UCUGAC-A-CAGAACA-U
ggo-miR-141	AA--CACU-G-UCUGGU-A-A-AGAUG-G
hsa-miR-141-5p	CA--UCU-U-C-CAGUACAGU-GUUGG-A
mml-miR-141-5p	UC--UUC-C-A-GUACA-G-U-GUUGG-A
ppy-miR-141	AA--CACU-G-UCUGGU-A-A-AGAUG-G
ptr-miR-141	UA--ACACUG-UCUGGU-A-A-AGAUG-G
rno-miR-141-5p	UC--CAUCUUC-CAGUG-C-A-GUGUU-G
tch-miR-141-3p	UA--ACACUG-UCUGGU-A-A-AGAUGGC

PHYLOGENETIC ANALYSIS

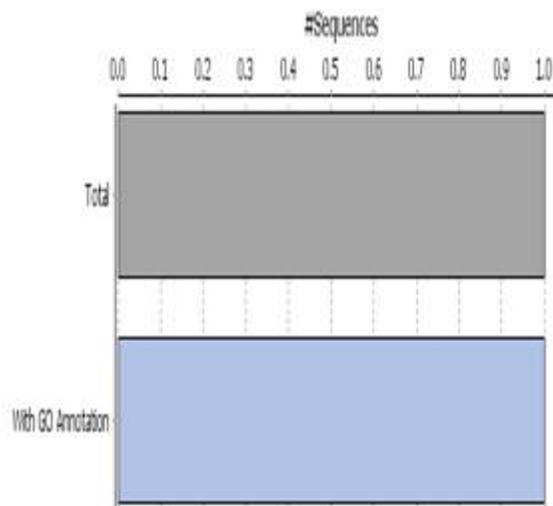
Phylogenetic as also called evolutionary tree are to find out the relation among present sequence and their root sequences so here we have a *Mentha canadensis* species consist of 224 EST that have only one E-value sequence so there for we are not able to construct the phylogenetic tree but however we align that sequence with model organism *Arabidopsis*.



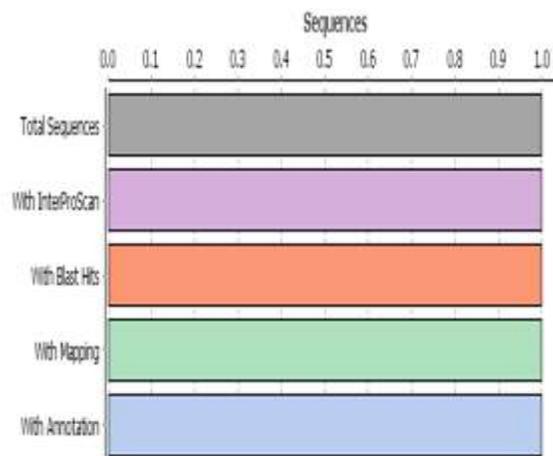
DATA DISTRIBUTION GRAPH

Blast2go helps us to know about our data distribution that how much sequences were annotated with gene ontology.

Data distribution graph



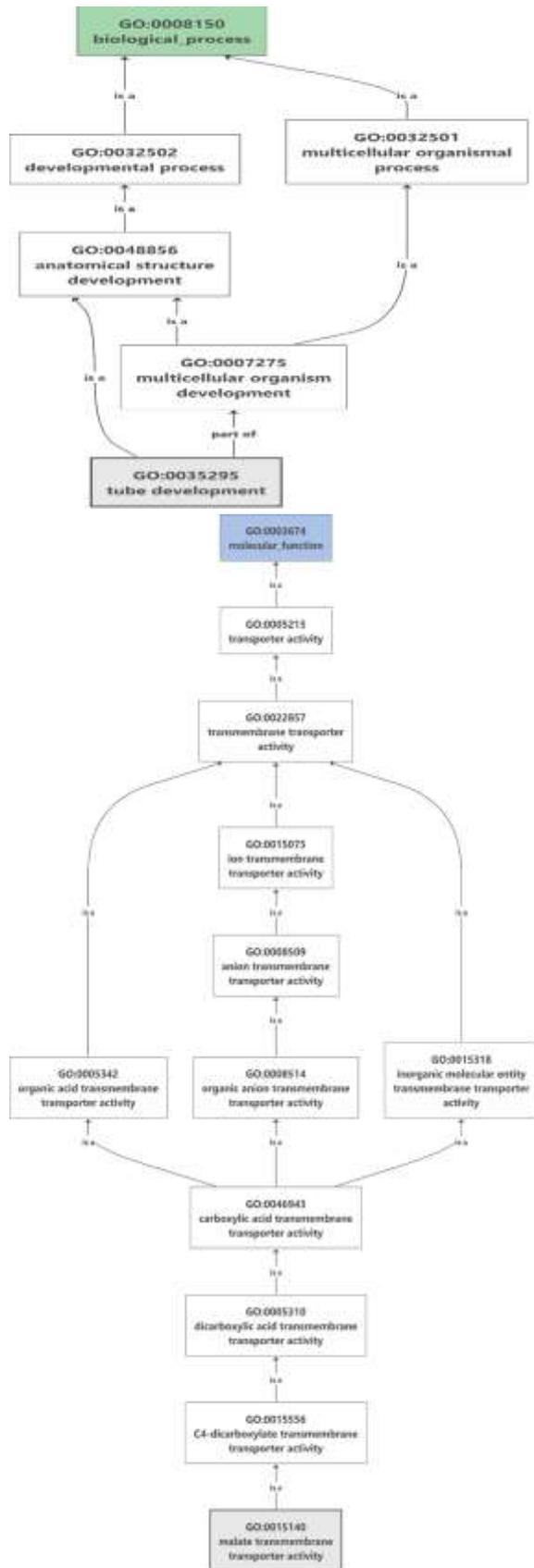
DATA LENGTH SEQUENCE



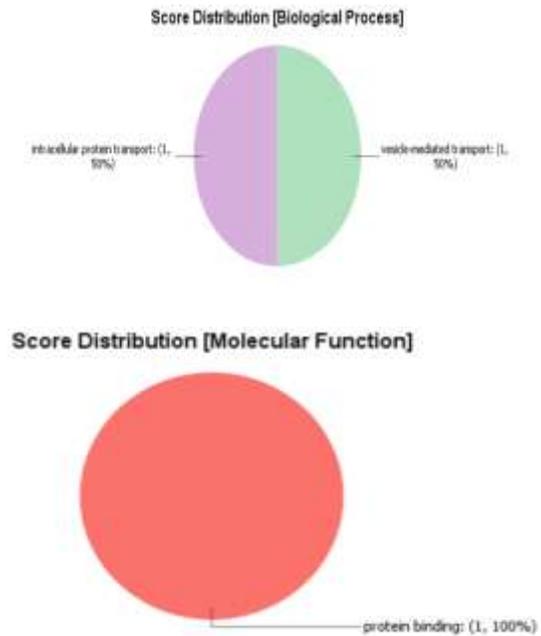
FUNCTIONS OF SEQUENCE

The one sequence has roles in biological process and at molecular level as well. We will discuss in following discussion.

Canadensis mRNA	Function	Go term annotation biological process	Go term annotation molecular function
>gga-miR-6693-3p MIMAT0025799 UUUUCAGUCUGACACAGA ACA	Intracellular proteins transport Visual mediated transport, binding proteins	Multicellular process Go:0008150	Go:0001552 Transporter activity Go:0022857 Transmembrane transporter activity



GRAPHICAL REPRESENTATION



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