Analysis of *Caenorhabditis elegans* via Bioinformatics Approaches Basis on their Precursors Statistics Values

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**Abstract**

MicroRNA (miRNA) are a class of small regulatory non-coding RNAs. These are about 21 to 25 nucleotides in length. Analysis of miRNA is leading to new paradigms for control of gene expression during plants and animals. Most noncoding RNAs are characterized by a specific secondary structures that determine their function. In present study we determine the minimum free energy (MFE) of *C. elegans* precursor’s sequences. That retrieves from miRBASE.

**Keywords** MicroRNA; *C. elegans*; Minimum free energy (MFE); Noncoding; Transcriptional regulators; Ribonuclease III

**Introduction**

Micro RNA regulates gene expression. miRNAs are well conserved in both plants and animals, and are thought to be a vital and evolutionarily ancient component of genetic regulation [1]. Mature microRNAs (miRNAs) are a class of naturally occurring small non-coding RNA molecules; about 21 to 25 nucleotides in length. MicroRNAs are partially complementary to one or more messenger RNA (mRNA) molecules and their main function is to down-regulate gene expression in a variety of manners, including translational repression, mRNA cleavage and deadenylation [2]. miRNAs are a class of post-transcriptional regulators [3]. Most non-coding RNAs are characterized by a specific secondary and tertiary structure that determines their function.

Analysis of miRNAs is leading to new paradigms for control of gene expression during development in plants and animals. miRNAs arise from larger precursor molecules that can fold into a stable stem-loop structure [4-8]. Those structures are processed by ribonuclease III-like nuclease Dicer in animals and Dicer like in plants and all have a typical stem-loop shape [4-11].

In present study we predicted the minimum free energy (MFE) values of secondary structures of noncoding RNA sequences, such as microRNA precursors of *C. elegans* with the help of computational software miRBase.

**Methodology**

The precursors (pre-miRNA) sequences of *C. elegans* were retrieved from miRBase and then go for secondary structure with optimal minimum free energy [12]. Optimal minimum free energy was found out with the help of RNA fold web servers (http://rna.tbi.univie.ac.at) then retrieve the sequence of miRNA from miRBase. Present study which is exclusively based on in silico firstly retrieves precursor sequences from miRBase and then retrieve sequence is submitted in RNA fold web server for minimum free energy values and calculated the minimum free energy for objective analysis [13].

| S.No. | Precursor No. | Accession No. | Free energy of thermodynamics (kcal/mol) | Frequency of MFE structure | Ensembled diversity | Minimum free energy of secondary structure (kcal/mol) | Optimal secondary structure minimum free energy (kcal/mol) |
|-------|---------------|---------------|------------------------------------------|---------------------------|-------------------|-------------------------------------------------|-------------------------------------------------------|
| 1     | >cel-let-7    | M10000001     | -43.63                                   | 6.09%                     | 9.04              | -37.90 kcal/mol                                  | -41.90 kcal/mol                                       |
| 2     | >cel-lin-4    | M10000002     | -41.20                                   | 8.80%                     | 7.90              | -39.60 kcal/mol                                  | -39.70 kcal/mol                                       |
| 3     | >cel-mir-1    | M10000003     | -40.09                                   | 62.68%                    | 1.40              | -39.80 kcal/mol                                  | -39.80 kcal/mol                                       |
| 4     | >cel-mir-2    | M10000004     | -38.30                                   | 6.30%                     | 6.21              | -35.40 kcal/mol                                  | -36.60 kcal/mol                                       |
| 5     | >cel-mir-34   | M10000005     | -36.47                                   | 9.16%                     | 6.53              | -35.00 kcal/mol                                  | -35.00 kcal/mol                                       |
| 6     | >cel-mir-35   | M10000006     | -54.07                                   | 28.84%                    | 5.76              | -50.50 kcal/mol                                  | -53.30 kcal/mol                                       |
| 7     | >cel-mir-36   | M10000007     | -50.93                                   | 7.15%                     | 4.73              | -48.40 kcal/mol                                  | -49.30 kcal/mol                                       |
|   | Entry   | Precursor | Result 1 | Result 2 | Result 3 | Result 4 |
|---|---------|-----------|----------|----------|----------|----------|
| 8 | cel-mir-37 M0000008 | -43.70 kcal/mol | 16.70% | 3.06 | -42.60 kcal/mol | -42.60 kcal/mol |
| 9 | cel-mir-38 M0000009 | -49.47 kcal/mol | 4.06% | 7.27 | -47.50 kcal/mol | -47.50 kcal/mol |
| 10 | cel-mir-39 M0000010 | -44.12 kcal/mol | 16.28% | 3.45 | -43.00 kcal/mol | -43.00 kcal/mol |
| 11 | cel-mir-40 M0000011 | -46.35 kcal/mol | 29.44% | 2.61 | -45.60 kcal/mol | -45.60 kcal/mol |
| 12 | cel-mir-41 M0000012 | -41.95 kcal/mol | 29.48% | 9.07 | -41.20 kcal/mol | -41.20 kcal/mol |
| 13 | cel-mir-42 M0000013 | -41.91 kcal/mol | 14.00% | 4.63 | -41.50 kcal/mol | -41.50 kcal/mol |
| 14 | cel-mir-43 M0000014 | -47.24 kcal/mol | 18.65% | 4.27 | -46.20 kcal/mol | -46.20 kcal/mol |
| 15 | cel-mir-44 M0000015 | -44.32 kcal/mol | 5.18% | 8.74 | -43.10 kcal/mol | -43.10 kcal/mol |
| 16 | cel-mir-45 M0000016 | -42.70 kcal/mol | 37.73% | 4.26 | -42.10 kcal/mol | -42.10 kcal/mol |
| 17 | cel-mir-46 M0000017 | -40.52 kcal/mol | 10.03% | 12.08 | -39.10 kcal/mol | -39.10 kcal/mol |
| 18 | cel-mir-47 M0000018 | -42.03 kcal/mol | 41.98% | 4.90 | -41.50 kcal/mol | -41.50 kcal/mol |
| 19 | cel-mir-48 M0000019 | -36.68 kcal/mol | 14.65% | 5.36 | -33.10 kcal/mol | -33.50 kcal/mol |
| 20 | cel-mir-49 M0000020 | -36.97 kcal/mol | 17.61% | 4.69 | -37.70 kcal/mol | -37.90 kcal/mol |
| 21 | cel-mir-50 M0000021 | -61.30 kcal/mol | 8.75% | 5.84 | -59.80 kcal/mol | -59.80 kcal/mol |
| 22 | cel-mir-51 M0000022 | -34.90 kcal/mol | 0.40% | 5.3 | -33.30 kcal/mol | -31.50 kcal/mol |
| 23 | cel-mir-52 M0000023 | -26.80 kcal/mol | 2.82% | 7.58 | -24.50 kcal/mol | -24.60 kcal/mol |
| 24 | cel-mir-53 M0000024 | -31.59 kcal/mol | 10.46% | 6.92 | -30.20 kcal/mol | -30.20 kcal/mol |
| 25 | cel-mir-54 M0000025 | -35.03 kcal/mol | 6.00% | 8.56 | -31.00 kcal/mol | -33.30 kcal/mol |
| 26 | cel-mir-55 M0000026 | -35.63 kcal/mol | 15.91% | 7.79 | -34.50 kcal/mol | -34.50 kcal/mol |
| 27 | cel-mir-56 M0000027 | -62.07 kcal/mol | 15.09% | 5.96 | -58.80 kcal/mol | -59.90 kcal/mol |
| 28 | cel-mir-57 M0000028 | -32.39 kcal/mol | 27.97% | 5.52 | -31.60 kcal/mol | -31.60 kcal/mol |
| 29 | cel-mir-58a M0000029 | -37.29 kcal/mol | 10.42% | 8.52 | -35.60 kcal/mol | -35.90 kcal/mol |
| 30 | cel-mir-59 M0000030 | -36.94 kcal/mol | 15.65% | 7.37 | -33.30 kcal/mol | -35.80 kcal/mol |
| 31 | cel-mir-60 M0000031 | -36.59 kcal/mol | 3.38% | 9.31 | -33.30 kcal/mol | -34.50 kcal/mol |
| 32 | cel-mir-61 M0000032 | -52.19 kcal/mol | 6.45% | 5.23 | -50.50 kcal/mol | -50.50 kcal/mol |
| 33 | cel-mir-63 M0000034 | -38.66 kcal/mol | 24.84% | 5.78 | -37.80 kcal/mol | -37.80 kcal/mol |
| 34 | cel-mir-64 M0000035 | -37.69 kcal/mol | 6.45% | 9.76 | -31.40 kcal/mol | -36.00 kcal/mol |
| 35 | cel-mir-65 M0000036 | -42.68 kcal/mol | 33.35% | 2.72 | -42.00 kcal/mol | -42.00 kcal/mol |
| 36 | cel-mir-66 M0000037 | -39.16 kcal/mol | 34.08% | 2.53 | -38.50 kcal/mol | -38.50 kcal/mol |
| 37 | cel-mir-67 M0000038 | -32.72 kcal/mol | 8.45% | 5.96 | -31.20 kcal/mol | -31.20 kcal/mol |
| 38 | cel-mir-70 M0000041 | -32.86 kcal/mol | 17.40% | 5.12 | -31.10 kcal/mol | -31.80 kcal/mol |
| 39 | cel-mir-71 M0000042 | -37.50 kcal/mol | 19.59% | 5.77 | -36.50 kcal/mol | -36.50 kcal/mol |
| 40 | cel-mir-72 M0000043 | -43.59 kcal/mol | 16.92% | 4.07 | -42.50 kcal/mol | -42.50 kcal/mol |
| 41 | cel-mir-73 M0000044 | -36.16 kcal/mol | 13.05% | 6.62 | -34.90 kcal/mol | -34.90 kcal/mol |
| 42 | cel-mir-74 M0000045 | -38.57 kcal/mol | 24.51% | 4.22 | -37.70 kcal/mol | -37.70 kcal/mol |
| 43 | cel-mir-75 M0000046 | -34.87 kcal/mol | 7.86% | 5.79 | -33.30 kcal/mol | -33.30 kcal/mol |
| ID    | Name       | Energy | Percent | Value       | Comp Value  |
|-------|------------|--------|---------|-------------|-------------|
| 44    | cel-mir-77 | -35.74 kcal/mol | 6.97%   | 9.80        | -32.10 kcal/mol | -34.10 kcal/mol |
| 45    | cel-mir-79 | -33.50 kcal/mol | 19.60%  | 4.26        | -31.10 kcal/mol | -32.50 kcal/mol |
| 46    | cel-mir-80 | -29.47 kcal/mol | 6.64%   | 12.39       | -27.80 kcal/mol | -27.80 kcal/mol |
| 47    | cel-mir-81 | -42.01 kcal/mol | 5.32%   | 7.80        | -39.90 kcal/mol | -40.20 kcal/mol |
| 48    | cel-mir-82 | -34.56 kcal/mol | 27.98%  | 2.55        | -33.80 kcal/mol | -33.80 kcal/mol |
| 49    | cel-mir-83 | -28.16 kcal/mol | 10.94%  | 11.86       | -22.50 kcal/mol | -26.80 kcal/mol |
| 50    | cel-mir-84 | -24.11 kcal/mol | 14.01%  | 11.86       | -22.90 kcal/mol | -22.90 kcal/mol |
| 51    | cel-mir-85 | -40.70 kcal/mol | 7.44%   | 6.18        | -38.70 kcal/mol | -39.10 kcal/mol |
| 52    | cel-mir-86 | -43.84 kcal/mol | 15.83%  | 3.50        | -40.30 kcal/mol | -42.70 kcal/mol |
| 53    | cel-mir-87 | -46.54 kcal/mol | 1.63%   | 5.79        | -44.90 kcal/mol | -44.00 kcal/mol |
| 54    | cel-mir-88 | -44.62 kcal/mol | 10.02%  | 4.46        | -43.20 kcal/mol | -43.20 kcal/mol |
| 55    | cel-mir-89 | -39.59 kcal/mol | 14.58%  | 6.87        | -36.40 kcal/mol | -38.40 kcal/mol |
| 56    | cel-mir-90 | -44.86 kcal/mol | 15.23%  | 4.06        | -41.90 kcal/mol | -43.70 kcal/mol |
| 57    | cel-mir-91 | -55.67 kcal/mol | 1.54%   | 17.64       | -50.90 kcal/mol | -53.10 kcal/mol |
| 58    | cel-mir-92 | -39.42 kcal/mol | 5.19%   | 6.85        | -37.50 kcal/mol | -37.60 kcal/mol |
| 59    | cel-mir-93 | -33.40 kcal/mol | 5.40%   | 8.80        | -31.60 kcal/mol | -31.60 kcal/mol |
| 60    | cel-mir-94 | -37.20 kcal/mol | 16.90%  | 5.00        | -35.80 kcal/mol | -36.10 kcal/mol |
| 61    | cel-mir-95 | -38.92 kcal/mol | 8.53%   | 4.73        | -37.40 kcal/mol | -37.40 kcal/mol |
| 62    | cel-mir-96 | -27.04 kcal/mol | 8.26%   | 4.88        | -25.50 kcal/mol | -25.50 kcal/mol |
| 63    | cel-mir-97 | -31.29 kcal/mol | 1.76%   | 13.20       | -27.50 kcal/mol | -28.80 kcal/mol |
| 64    | cel-mir-98 | -38.76 kcal/mol | 3.55%   | 7.62        | -36.70 kcal/mol | -36.70 kcal/mol |
| 65    | cel-mir-99 | -37.15 kcal/mol | 3.57%   | 6.10        | -31.50 kcal/mol | -35.10 kcal/mol |
| 66    | cel-mir-100| -39.38 kcal/mol | 10.60%  | 6.22        | -37.70 kcal/mol | -38.00 kcal/mol |
| 67    | cel-mir-101| -33.64 kcal/mol | 13.28%  | 4.78        | -32.40 kcal/mol | -32.40 kcal/mol |
| 68    | cel-mir-102| -38.52 kcal/mol | 31.16%  | 2.88        | -37.80 kcal/mol | -37.80 kcal/mol |
| 69    | cel-mir-103| -22.86 kcal/mol | 5.74%   | 7.14        | -20.90 kcal/mol | -21.10 kcal/mol |
| 70    | cel-mir-104| -34.89 kcal/mol | 2.88%   | 6.77        | -32.20 kcal/mol | -32.70 kcal/mol |
| 71    | cel-mir-105| -36.99 kcal/mol | 20.12%  | 4.55        | -36.00 kcal/mol | -36.00 kcal/mol |
| 72    | cel-mir-106| -31.62 kcal/mol | 16.36%  | 8.64        | -27.00 kcal/mol | -30.50 kcal/mol |
| 73    | cel-mir-107| -24.77 kcal/mol | 12.66%  | 6.57        | -23.50 kcal/mol | -23.50 kcal/mol |
| 74    | cel-mir-108| -37.58 kcal/mol | 4.76%   | 8.19        | -35.70 kcal/mol | -35.70 kcal/mol |
| 75    | cel-mir-109| -39.30 kcal/mol | 14.37%  | 2.84        | -38.50 kcal/mol | -38.10 kcal/mol |
| 76    | cel-mir-110| -47.25 kcal/mol | 5.85%   | 5.22        | -45.30 kcal/mol | -45.50 kcal/mol |
| 77    | cel-mir-111| -28.55 kcal/mol | 9.54%   | 10.17       | -24.10 kcal/mol | -27.10 kcal/mol |
| 78    | cel-mir-112| -36.67 kcal/mol | 17.72%  | 5.05        | -35.60 kcal/mol | -35.60 kcal/mol |
| 79    | cel-mir-113| -37.24 kcal/mol | 2.26%   | 9.21        | -34.10 kcal/mol | -34.90 kcal/mol |
| 80  | cel-mir-356b MI0019158 | -20.09 kcal/mol | 6.45% | 6.52 | -15.30 kcal/mol | -18.40 kcal/mol |
| 81  | cel-mir-358 MI0000757 | -36.09 kcal/mol | 1.76% | 9.90 | -33.60 kcal/mol | -33.60 kcal/mol |
| 82  | cel-mir-392 MI0000819 | -38.44 kcal/mol | 35.39% | 3.11 | -37.80 kcal/mol | -37.80 kcal/mol |
| 83  | cel-mir-784 MI0005184 | -28.76 kcal/mol | 9.34% | 4.10 | -24.50 kcal/mol | -27.30 kcal/mol |
| 84  | cel-mir-786 MI0005186 | -36.92 kcal/mol | 7.28% | 6.03 | -35.30 kcal/mol | -35.30 kcal/mol |
| 85  | cel-mir-787 MI0005187 | -39.99 kcal/mol | 32.58% | 3.58 | -39.30 kcal/mol | -39.30 kcal/mol |
| 86  | cel-mir-788 MI0005188 | -33.38 kcal/mol | 33.34% | 1.90 | -32.70 kcal/mol | -32.70 kcal/mol |
| 87  | cel-mir-789-2 MI0005190 | -64.03 kcal/mol | 7.10% | 5.04 | -60.50 kcal/mol | -62.40 kcal/mol |
| 88  | cel-mir-790 MI0005191 | -34.37 kcal/mol | 20.68% | 2.47 | -33.30 kcal/mol | -33.40 kcal/mol |
| 89  | cel-mir-791 MI0005192 | -33.49 kcal/mol | 20.02% | 4.20 | -32.50 kcal/mol | -32.50 kcal/mol |
| 90  | cel-mir-794 MI0005195 | -27.49 kcal/mol | 27.76% | 2.51 | -26.70 kcal/mol | -26.70 kcal/mol |
| 91  | cel-mir-795 MI0005196 | -34.45 kcal/mol | 15.54% | 4.99 | -33.30 kcal/mol | -33.30 kcal/mol |
| 92  | cel-mir-797 MI0005198 | -28.50 kcal/mol | 2.81% | 11.22 | -26.10 kcal/mol | -26.30 kcal/mol |
| 93  | cel-mir-800 MI0005201 | -54.97 kcal/mol | 55.06% | 0.89 | -54.60 kcal/mol | -54.60 kcal/mol |
| 94  | cel-mir-1820 MI0007982 | -40.02 kcal/mol | 22.60% | 5.32 | -39.10 kcal/mol | -39.10 kcal/mol |
| 95  | cel-mir-1821 MI0007983 | -32.09 kcal/mol | 2.06% | 11.81 | -27.20 kcal/mol | -29.70 kcal/mol |
| 96  | cel-mir-1822 MI0007984 | -32.27 kcal/mol | 17.50% | 4.79 | -31.20 kcal/mol | -31.20 kcal/mol |
| 97  | cel-mir-1823 MI0007985 | -26.43 kcal/mol | 11.52% | 5.28 | -22.70 kcal/mol | -25.10 kcal/mol |
| 98  | cel-mir-1829b MI0008198 | -21.74 kcal/mol | 67.49% | 1.00 | -21.50 kcal/mol | -21.50 kcal/mol |
| 99  | cel-mir-1829c MI0008199 | -20.35 kcal/mol | 56.50% | 1.38 | -20.00 kcal/mol | -20.00 kcal/mol |
| 100 | cel-mir-1830 MI0008200 | -35.96 kcal/mol | 34.49% | 3.70 | -35.30 kcal/mol | -35.30 kcal/mol |
| 101 | cel-mir-1832a MI0008202 | -36.86 kcal/mol | 11.07% | 3.56 | -35.50 kcal/mol | -35.50 kcal/mol |
| 102 | cel-mir-1832b MI0010967 | -44.60 kcal/mol | 32.19% | 2.79 | -43.60 kcal/mol | -43.90 kcal/mol |
| 103 | cel-mir-2208a MI0010956 | -24.02 kcal/mol | 36.44% | 1.69 | -23.40 kcal/mol | -23.40 kcal/mol |
| 104 | cel-mir-2208b MI0010957 | -27.27 kcal/mol | 33.97% | 1.94 | -26.60 kcal/mol | -26.60 kcal/mol |
| 105 | cel-mir-2221 MI0010974 | -43.52 kcal/mol | 11.81% | 7.16 | -42.20 kcal/mol | -42.20 kcal/mol |
| 106 | cel-mir-4805 MI0017535 | -49.11 kcal/mol | 16.52% | 8.53 | -48.00 kcal/mol | -48.00 kcal/mol |
| 107 | cel-mir-4813 MI0017543 | -39.96 kcal/mol | 47.56% | 1.24 | -39.50 kcal/mol | -39.50 kcal/mol |
| 108 | cel-mir-4814 MI0017544 | -66.59 kcal/mol | 38.34% | 2.76 | -66.00 kcal/mol | -66.00 kcal/mol |
| 109 | cel-mir-4816 MI0017546 | -24.54 kcal/mol | 35.29% | 2.53 | -23.90 kcal/mol | -23.90 kcal/mol |
| 110 | cel-mir-5545 MI0019066 | -64.97 kcal/mol | 46.98% | 2.35 | -64.50 kcal/mol | -64.50 kcal/mol |
| 111 | cel-mir-5592-1 MI0019153 | -40.09 kcal/mol | 27.56% | 2.99 | -39.30 kcal/mol | -39.30 kcal/mol |
of the local free energy contributions suggests a linear relationship of centriod secondary structure. The free energy values.

For computational analysis of miRNA we always predict the MFE values from precursor statistics values, analyze the overall stability of an RNA structure by adding independent contributions of local free energy interactions due to adjacent base pairs and loop regions. In sequences with homogeneous nucleotide arrangements and compositions, the additive and independent nature of the local free energy contributions suggests a linear relationship between computed MFE and sequence length. Normalization by length, obtained by dividing MFE by the number of nucleotides, was introduced to exploit this linear relationship to directly compare the minimum free energies of RNAs of various lengths [14,15].

In Table 1 we have predicted Free energy of thermodynamics, Frequency of MFE structure, Ensemble diversity, Minimum free energy of centeriod secondary structure. The minimum free energy (MFE) of ribonucleic acids (RNAs) increases at an apparent linear rate with sequence length. Simple indices, obtained by dividing the MFE by the number of nucleotides, have been used for a direct comparison of the folding stability of RNAs of various sizes.

Conclusion

In this study entitled: Analysis of C. elegans via bioinformatics approaches basis on their precursors statistics values, analyze the statistical values of miRNA and their precursors. For computational analysis of miRNA we always predict the MFE values from precursor sequences which is already experimentally identified and this precursor sequences retrieves from miRBase, for miRNA targeted genes and other analysis also. This table explains all precursors and miRNAs mainly important values, these values always used in noncoding RNA analysis via the system biology. Our computational findings may be useful for researchers.

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Table 1: This table explains that miRBase precursors of C. elegans. These sequences pass from RNA fold web server for thermodynamics analysis with minimum free energy

Results and Discussion

Here we have analyzed optimal minimum free energy (MFE) of miRBase precursors of C. elegans. Present study is exclusively based on in silico. Firstly we retreives precursor sequences from miRBase and then this sequence is submitted in RNA fold web server for minimum free energy values.

The most common software programs, employed to predict the secondary RNA structures by MFE algorithms, make use of the so-called nearest-neighbor energy model. This model uses free energy rules based on empirical thermodynamic parameters and computes the overall stability of an RNA structure by adding independent contributions of local free energy interactions due to adjacent base pairs and loop regions. In sequences with homogeneous nucleotide arrangements and compositions, the additive and independent nature of the local free energy contributions suggests a linear relationship between computed MFE and sequence length. Normalization by length, obtained by dividing MFE by the number of nucleotides, was introduced to exploit this linear relationship to directly compare the minimum free energies of RNAs of various lengths [14,15].

| Precursor ID | MFE Value | Frequency | MFE/Length | MFE Value | MFE Value |
|--------------|------------|-----------|-------------|-----------|-----------|
| >cel-mir-5592-2 | -41.50 kcal/mol | 32.17% | 3.28 | -40.80 kcal/mol | -40.80 kcal/mol |
| >cel-mir-5594 | -26.98 kcal/mol | 14.80% | 3.10 | -25.80 kcal/mol | -25.80 kcal/mol |

Table 1: This table explains that miRBase precursors of C. elegans. These sequences pass from RNA fold web server for thermodynamics analysis with minimum free energy.