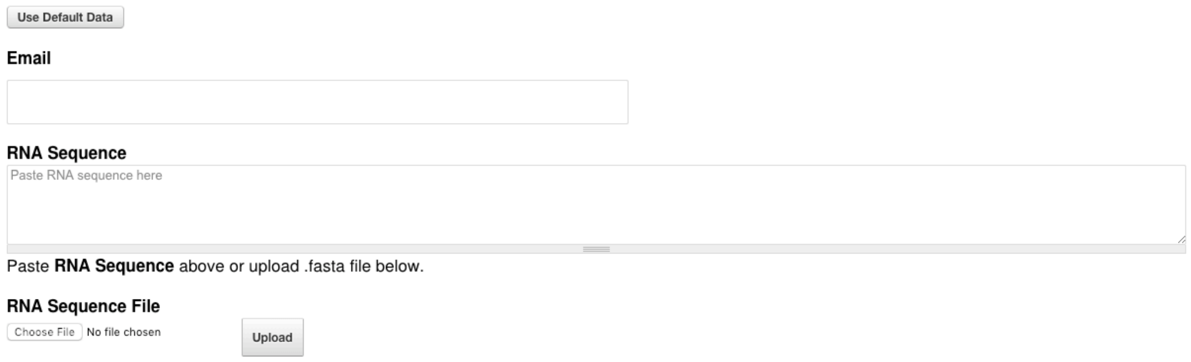
ScanFold Tutorial

1. ScanFold

The ScanFold webserver allows users to identify potentially functional RNA structures given an input RNA/DNA sequence. The ScanFold pipeline (which can be separated into “scanning” and “folding” steps) is described in detail in the manuscript where it was introduced (https://peerj.com/articles/6136).

Step 1.
Go to https://mosslabtools.bb.iastate.edu/scanfold



Use Default Data

Email

RNA Sequence
Paste RNA sequence here

Paste **RNA Sequence** above or upload .fasta file below.

RNA Sequence File
Choose File No file chosen
Upload

Step 2.
Input RNA sequence (must be in FASTA format; can be DNA, RNA, uppercase or lowercase)
Provide your email address (optional); an e-mail will be sent here when your job has finished.

Step 3.
Click on Submit

Your sequence will now be analyzed using the ScanFold pipeline which is described below.

1.1. ScanFold-Scan

In the initial step (i.e. ScanFold-*Scan*) a scanning window approach is used whereby the input sequence is fragmented into windows which are individually analyzed for the propensity to form structures. For the ScanFold-Scan approach implemented we calculate four metrics per window:

1.2. ScanFold-Scan Metrics

a) **Minimum Free Energy**

Each window is first analyzed with an RNA folding algorithm to predict the most stable structure it could theoretically adopt.

The **Minimum Free Energy (MFE)** is the free energy value of this most stable structure in kcal/mol. The more negative the value, the more stable the structure.

b) **Z-score**

It was found that, in general, structured RNA molecules adopted more stable (lower MFE) structures than shuffled versions of the same sequence (as described by Clote et. Al., in **Structural RNA has lower folding energy than random RNA of the same dinucleotide frequency https://www.ncbi.nlm.nih.gov/pubmed/15840812)**. This was a phenomenon that would then be used to **detect regions of RNA which** *could potentially* **be structured for a functional role**: as inferred by the thermodynamic **z-score (adapted from Clote et al.)**:

$$\boldsymbol{z-score} = \frac{MFE_{native} - \overline{MFE}_{random}}{\sigma_{all\ MFE}}$$

The z-score is calculated for each window of the input sequence. We then have two sets of sequences: native and randomized. For each set, MFE values were calculated. If the native sequence always has a much lower MFE than the average of scrambled versions this will lead to a negative z-score (if the native sequence MFE is always more positive, i.e., less stable, then the z-score will be positive). The equation normalizes the value by dividing by the standard deviation between all MFEs. The magnitude of the z-score then, states the number of standard deviations the native (window) MFE is from the random MFEs.

Negative z-score indicates a window generates a more stable structure than the sequence content would typically produce.

Positive z-score indicates a window generates a less stable structure than the sequence content would typically produce.

c) **P-value**

This value is directly related to the z-score. It is simply the fraction of random sequences which were more stable (more negative, or less than) the original sequence. This can be used as a quality check for the z-score and is calculated via the following equation:

$$\boldsymbol{p-value} = \frac{\#of MFE_{random} < MFE_{native}}{\#of Randomizaitons}$$

Therefore, a value of “1” indicates that all of the random sequences were actually more stable than the native and a value of “0” indicates the native was more

stable than all random sequences.

d) **Ensemble Diversity**

When calculating an MFE structure/value for a particular sequence, we are always finding a *single* result: the *minimum* free energy structure/value. However, theoretically, there are many possible structures/values. The ensemble diversity is a metric which attempts to describe the *variety* of possible structures. How is this determined? All possible structures are first calculated. The probability of the RNA adopting one of these structures is calculated using the partition function. This partition function can then be used to measure the “diversity” of possible structures. **If the structures are very similar (different by only a few base pairs) the ensemble diversity will be low, however, if there are a wide variety of structures possible, the ensemble diversity will be high.**

1.3. ScanFold-Scan Input

a) Sequence (required)

Must be in Fasta format (input as text or uploaded). Can be in DNA or RNA format. This is the minimal input and is required for ScanFold-Scan.

b) Name (No spaces or characters allowed; default “UserInput” | optional)
c) E-mail (optional)

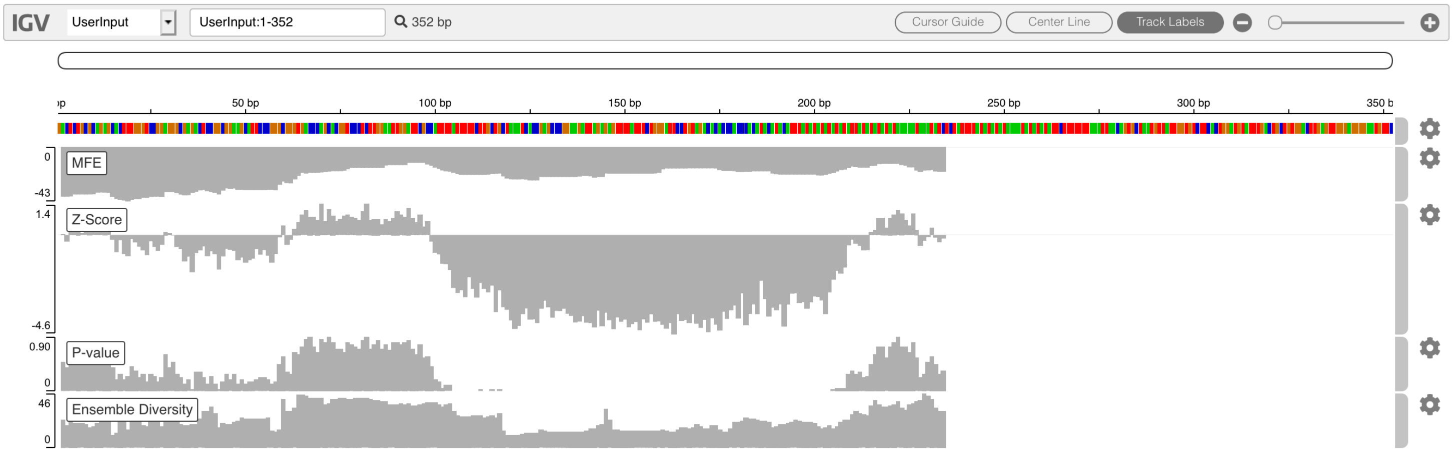
The following input parameters are set to default values which will be optimal for most users, but can be changed by user input.

d) Temperature (in Celsius; default 37C)
e) Window Size (length in nucleotides; default 120 nt)
f) Step Size (length in nucleotides; default 10 nt)
g) Randomizations (number of random sequences which will be generated during the calculation of z-score; default 30x)
h) Randomization type (randomization method | mononucleotide OR dinucleotide; default mononucleotide shuffle)

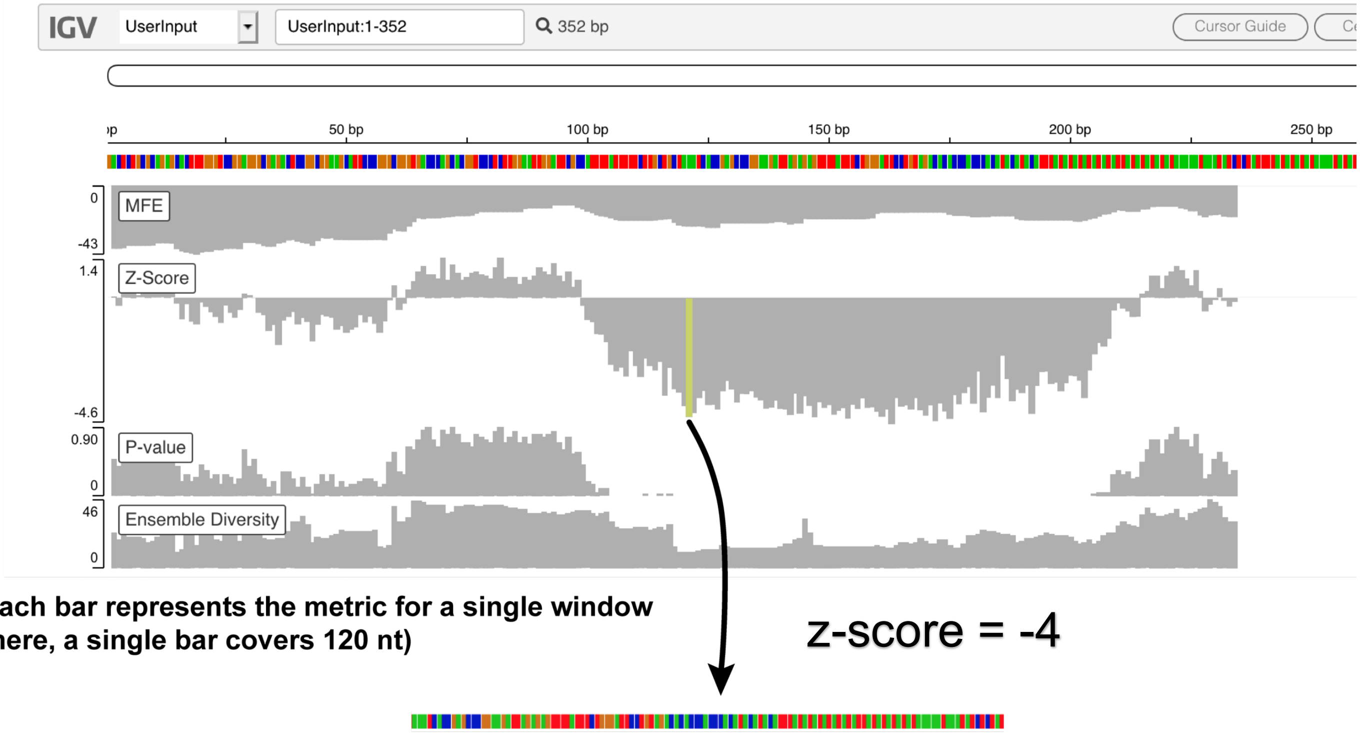
1.4. ScanFold-Scan Output

The results of ScanFold-Scan are shown in an IGV web browser and output to downloadable text files.

IGV output (ScanFold-Scan)



Each metric has its own track on the IGV.js browser. Here the bars correspond to the metric's value. The axis values are shown on the left (all values are unit-less except for MFE; kcal/mol).



Each bar represents the metric for a single window (here, a single bar covers 120 nt)

z-score = -4

z-score of the highlighted bar refers to the z-score calculated for the corresponding 120 nt window sequence

Above we use a step size of 1 nt (default is 10 nt; which takes ~10x less computation time). You may want to use smaller or larger step sizes depending on how large your sequence is. It is likely that potentially functional structures are not bound by the chosen window size. Therefore, you may want to adjust the window size (max 600 nt).

In the example above, we see a large stretch of highly negative z-scores (indicating that a region from nt 100 to ~320 contains some RNA secondary structure which is unusually stable for its particular nucleotide composition). In this example case, we have used a region from the human genome known to contain a riboswitch: the VEGFA 3'UTR.

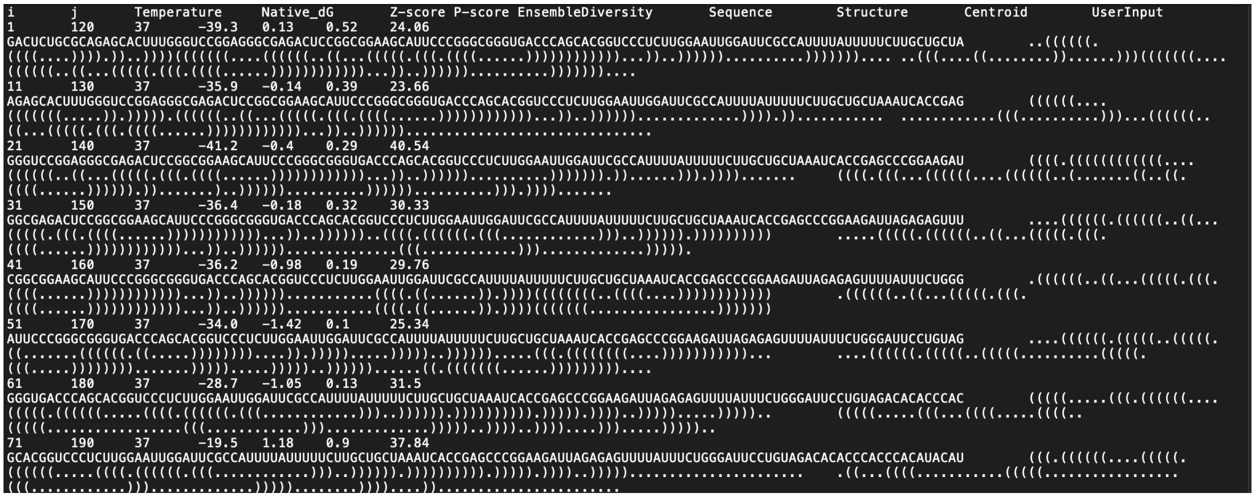
Indeed, the region with low z-scores correspond with the known riboswitch sequence. Using this method can allow for a quick search for low z-score regions in a given sequence. If your sequence has highly negative regions you may want to find more details. In any case, we also provide detailed text output of the scan results.

Text output (ScanFold-Scan)

The metrics depicted in IGV.js browser, can also be found in the text output. The output is in the tab separated value (.tsv) file. This simply means that each column is separated by a tab value. Our .tsv files contain 10 columns (shown in table below).

i	j	Temp	MFE	z-score	p-value	ED	Sequence	Structure	Centroid
1	120	37C	-12.34 kcal/mol	-2.34	0.1	23	GGGGUUCCCC	((((…))))	((((…))))

The .tsv files can be opened in a text editor…



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i       j       Temperature     Native_dG       Z-score P-score EnsembleDiversity       Sequence        Structure       Centroid        UserInput
1       120     37      -39.3   0.13    0.52    24.06
GACUCUGCGCAGAGCACUUUGGGUCCGGAGGGCGAGACUCCGGCGGAAGCAUUCCCGGGCGGGUGACCCAGCACGGUCCCUCUUGGAAUUGGAUUCGCCAUUUUAUUUUUCUUGCUGCUA        ..((((((.
((((....)))).))..))))(((((((....((((((..((...(((((.(((.((((......)))))))))))))...))..))))))..........))))))).... ..(((....((........))......)))(((((((....
((((((..((...(((((.(((.((((......))))))))))))...))..))))))..........)))))))....
11      130     37      -35.9   -0.14   0.39    23.66
AGAGCACUUUGGGUCCGGAGGGCGAGACUCCGGCGGAAGCAUUCCCGGGCGGGUGACCCAGCACGGUCCCUCUUGGAAUUGGAUUCGCCAUUUUAUUUUUCUUGCUGCUAAAUCACCGAG        ((((((....
((((((((.....)).))))).((((((..((...(((((.(((.((((......))))))))))))...))..))))))............)))).)).........  ............(((..........)))...((((((..
((...(((((.(((.((((......))))))))))))...))..))))))...............................
21      140     37      -41.2   -0.4    0.29    40.54
GGGUCCGGAGGGCGAGACUCCGGCGGAAGCAUUCCCGGGCGGGUGACCCAGCACGGUCCCUCUUGGAAUUGGAUUCGCCAUUUUAUUUUUCUUGCUGCUAAAUCACCGAGCCCGGAAGAU        ((((.((((((((((((....
((((((..((...(((((.(((.((((......))))))))))))...))..))))))..........)))))))).))......))).))))........      ((((.(((...((((((....((((((..(........((..((.
((((......)))))).))........)..))))))..........)))))).........))).)))).......
31      150     37      -36.4   -0.18   0.32    30.33
GGCGAGACUCCGGCGGAAGCAUUCCCGGGCGGGUGACCCAGCACGGUCCCUCUUGGAAUUGGAUUCGCCAUUUUAUUUUUCUUGCUGCUAAAUCACCGAGCCCGGAAGAUUAGAGAGUUU        ....((((((.((((((..((...
(((((.(((.((((......))))))))))))...))..))))))..((((.((((((.(((.............)))..)))))).))))))))))        .....(((((.((((((..((...(((((.(((.
((((......))))))))))))...))..))))))..............(((.............)))..............))))).
41      160     37      -36.2   -0.98   0.19    29.76
CGGCGGAAGCAUUCCCGGGCGGGUGACCCAGCACGGUCCCUCUUGGAAUUGGAUUCGCCAUUUUAUUUUUCUUGCUGCUAAAUCACCGAGCCCGGAAGAUUAGAGAGUUUUAUUUCUGGG        .((((((..((...(((((.(((.
((((......))))))))))))...))..))))))...........((((.((......)).))))((((((((..((((....))))))))))))        .((((((..((...(((((.(((.
((((......))))))))))))...))..))))))...........((((.((......)).))))((((((((...............)))))))
51      170     37      -34.0   -1.42   0.1     25.34
AUUCCCGGGCGGGUGACCCAGCACGGUCCCUCUUGGAAUUGGAUUCGCCAUUUUAUUUUUCUUGCUGCUAAAUCACCGAGCCCGGAAGAUUAGAGAGUUUUAUUUCUGGGAUUCCUGUAG        ....((((((.(((((..(((((.
((.......((((((.((.....))))))))....)).))))).....)))))..))))))).....(((.((((((((....)))))))))))...        ....((((((.(((((..(((((..........(((((.
(((.....)))))))).......))))).....)))))..))))))......((.(((((((......)))))))))....
61      180     37      -28.7   -1.05   0.13    31.5
GGGUGACCCAGCACGGUCCCUCUUGGAAUUGGAUUCGCCAUUUUAUUUUUCUUGCUGCUAAAUCACCGAGCCCGGAAGAUUAGAGAGUUUUAUUUCUGGGAUUCCUGUAGACACACCCAC        (((((.....(((.((((((....
(((((.((((((.....((((.((((((.(((.............)))..)))))).))))))))))).))))).)))))..)))))).....)))))..        (((((.....(((...((((.....((((..
(((((..................(((............))).............)))))..))))..))))....))).....)))))..
71      190     37      -19.5   1.18    0.9     37.84
GCACGGUCCCUCUUGGAAUUGGAUUCGCCAUUUUAUUUUUCUUGCUGCUAAAUCACCGAGCCCGGAAGAUUAGAGAGUUUUAUUUCUGGGAUUCCUGUAGACACACCCACCCACAUACAU        (((.((((((....(((((.
((((((.....((((.((((((.(((.............)))..)))))).))))))))))).))))).)))))..))))).....................    .((...((((...........(((((.................
(((.............)))...............)))))........))))....)).......................
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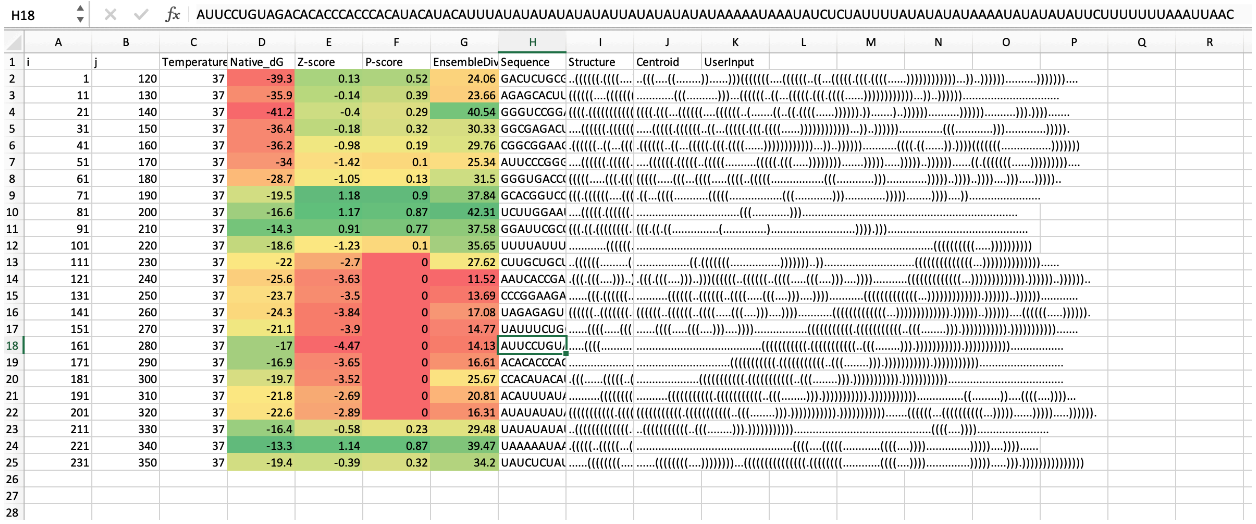
…or in a spreadsheet program (such as Excel).



H18 fx AUUCCUGUAGACACACCCACCCACAUACAUACAUUUAUAUAUAUAUAUAUUAUAUAUAUAUAAAAAUAAAUAUCUCUAUUUUAUAUAUAUAAAAUAUAUAUAUUCUUUUUUAAAUUAAC

	A	B	C	D	E	F	G	H	I	J	K
1	i	j	Temperature	Native_dG	Z-score	P-score	EnsembleDiv	Sequence	Structure	Centroid	UserInput
2	1	120	37	-39.3	0.13	0.52	24.06	GACUCUGCG	..((((((.((((....	..(((....((........))......)))(((((((....((((((..((...(((((.(((.((((......)))))))))))...))..))))))..........)))))))....	
3	11	130	37	-35.9	-0.14	0.39	23.66	AGAGCACUU	((((((....((((((	............(((..........)))...((((((..((...(((((.(((.((((......)))))))))))...))..))))))...........................	
4	21	140	37	-41.2	-0.4	0.29	40.54	GGGUCCGGA	((((.((((((((((	((((.(((...((((((....((((((..(.......((..((.((((......)))))).)).......)..))))))..........))))))..........))).))))......	
5	31	150	37	-36.4	-0.18	0.32	30.33	GGCGAGACU	....((((((.((((((	.....(((((.((((((..((...(((((.(((.((((......)))))))))))...))..))))))............(((...........)))............))))).	
6	41	160	37	-36.2	-0.98	0.19	29.76	CGGCGGAAC	.((((((..((...(((	.((((((..((...(((((.(((.((((......)))))))))))...))..))))))..........((((.((......)).))))(((((((...............)))))))	
7	51	170	37	-34	-1.42	0.1	25.34	AUUCCCGGG	....((((((.(((((.	....((((((.(((((..(((((..........(((((.(((.....))))))))).......))))).....))))).))))))......((.(((((((......)))))))))....	
8	61	180	37	-28.7	-1.05	0.13	31.5	GGGUGACCC	(((((.....(((.(((	(((((.....(((...((((.....((((..(((((................(((...........)))............))))).)))).))))....))).....)))))..	
9	71	190	37	-19.5	1.18	0.9	37.84	GCACGGUCC	(((.((((((....(((	.((...((((...........(((((................(((...........)))............)))))........))))....))......................	
10	81	200	37	-16.6	1.17	0.87	42.31	UCUUGGAAU	....(((((.((((((.	..............................(((...........)))...	
11	91	210	37	-14.3	0.91	0.77	37.58	GGAUUCGCC	(((.((.((((((((.	(((.((.((...............(..............).........................)))).)))..	
12	101	220	37	-18.6	-1.23	0.1	35.65	UUUUAUUU	............(((((.	....((((((((((.....))))))))))	
13	111	230	37	-22	-2.7	0	27.62	CUUGCUGCU	..((((((.........(	.................((.(((((((................)))))))..))........................((((((((((((((...))))))))))))))......	
14	121	240	37	-25.6	-3.63	0	11.52	AAUCACCGA	.(((.(((....)))..)	.(((.(((....)))..)))((((((..((((((..((((.....(((....)))....))))...........((((((((((((...))))))))))))).))))))..))))))..	
15	131	250	37	-23.7	-3.5	0	13.69	CCCGGAAGA	......(((.((((((..	..........((((((..((((((..((((.....(((....)))....))))...........((((((((((((...))))))))))))).))))))..))))))............	
16	141	260	37	-24.3	-3.84	0	17.08	UAGAGAGU	((((((..(((((((.	((((((..((((((..((((.....(((....)))....))))...........((((((((((((...))))))))))))).))))))..))))))....((((((.....)))))).	
17	151	270	37	-21.1	-3.9	0	14.77	UAUUUCUGU	......((((.....(((.	......((((.....(((....)))....))))................((((((((((.((((((((((..(((........))).)))))))))))).))))))))))......	
18	161	280	37	-17	-4.47	0	14.13	AUUCCUGUA	.....((((.........	...................................((((((((((.((((((((((..(((........))).))))))))))).))))))))))................	
19	171	290	37	-16.9	-3.65	0	16.61	ACACACCCAC	....	..........................((((((((((.((((((((((..(((........))).))))))))))).))))))))))........................	
20	181	300	37	-19.7	-3.52	0	25.67	CCACAUACAU	.(((......(((((..(	...................((((((((((.((((((((((..(((........))).))))))))))).))))))))))..................................	
21	191	310	37	-21.8	-2.69	0	20.81	ACAUUUAUA	..........(((((((((	..........((((((((((.((((((((((..(((........))).))))))))))).))))))))))...............((.........))....((((....))))...	
22	201	320	37	-22.6	-2.89	0	16.31	AUAUAUAUA	(((((((((((.((((	(((((((((((.((((((((((..(((........))).))))))))))).)))))))))))........((((((...(((((((((...)))))......))))).....)))))).	
23	211	330	37	-16.4	-0.58	0.23	29.48	UAUAUAUAU	..((((((((((((.	..((((((((((..(((........))).))))))))))..((((....))))....................	
24	221	340	37	-13.3	1.14	0.87	39.47	UAAAAAUAA	.(((((..(((((...(	....((((....(((((...........((((....))))............)))))....))))......	
25	231	350	37	-19.4	-0.39	0.32	34.2	UAUCUCUAU	......((((((((....	......((((((((....))))))))...((((((((((((((.(((((((...........((((....))))............)))))....))).)))))))))))))))	
26											
27											
28											

Here, you can use program features or simple browsing to hone in on windows with the most interesting metrics. However the .tsv file is also be used as an input for ScanFold-Fold.

1.5. ScanFold-Fold

Using the default ScanFold pipeline will automatically push your ScanFold-Scan output file directly into the ScanFold-Fold program.

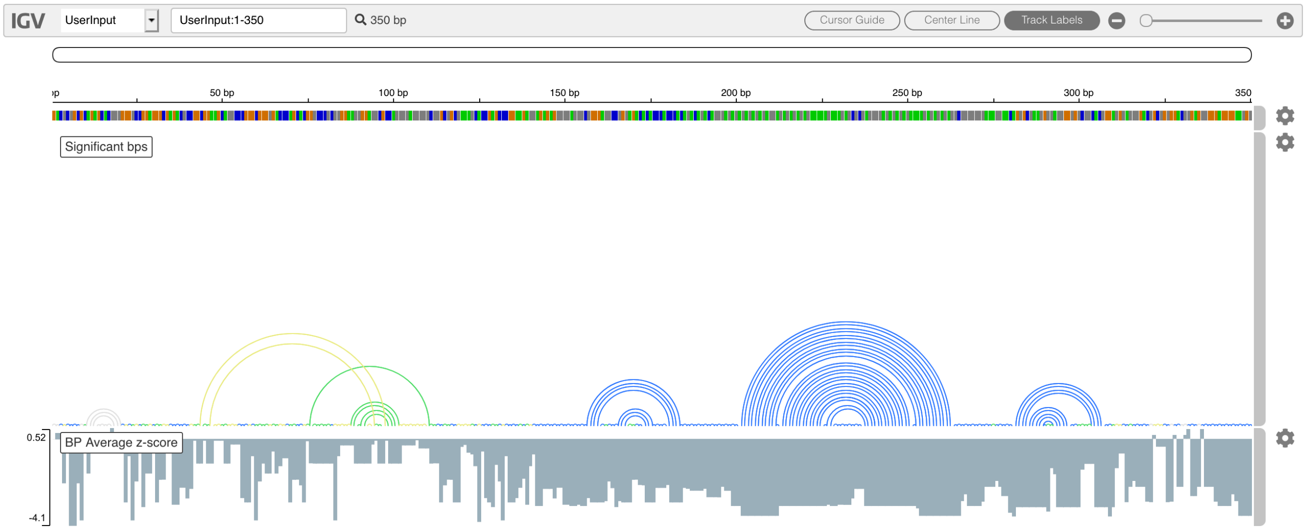
The ScanFold-Fold program attempts to extract the base pairs which consistently contributed to low z-score generating structures; this essentially condenses the results of the scan (potentially hundreds of windows) into a single list of base pairs (as well as their average metrics throughout the scan), greatly reducing the dependence on lengthy and subjective manual data processing.

In the above example then, we can determine which base pairs throughout all windows which were most likely generating that stretch of low z-scores.

1.6. ScanFold-Fold Output

The basic output of ScanFold-Fold is a list of base pairs (and nucleotides which are likely not to base pair) which have been extracted from the windows of the ScanFold-Scan results. Again, we output these results to IGV and text files.

IGV output (ScanFold-Fold)



Here we can see the base pairs predicted to form low z-score generating structures depicted as arcs (in the “Significant bps” track). The arcs are colored based on their z-scores (where colored arcs are negative: yellow is 0 to -1, green is -1 to -2, and blue is < -2).

Below we also show a wig track (BP Average z-score) which reports the value of the average z-score which corresponds to the above base pair (or unpaired) arrangement.

However, the ScanFold-Fold results depicted in the IGV browser only depict the “best” results based on the z-score; full data resulting from the ScanFold-Fold algorithm can be found in the text outputs.

Text output (ScanFold-Fold)

Connectivity Files

Three connectivity table (CT) files are produced which can be loaded directly into RNA structure viewers (such as VARNA or RNAStructure). The base pairs (or unpaired nucleotides) reported in each are selected based on their average z-scores (Z_{avg}), whereby only base pairs which had Z_{avg} less than a filter value are reported. We produce three CT files:

1. No Filter (all base pairs reported – even with positive z-scores)
2. $Z_{avg} < -1$
3. $Z_{avg} < -2$

Text files (logs and details)

We also generate two log files. The first is simply a list of the final base pair (and unpaired nucleotide) arrangements and their corresponding average metrics. The second is a detailed report of all the different base pairing arrangements which a nucleotide formed throughout the scan.

1. Final Partners:

i	*i*	*j*	MFE$_{avg}$	Z$_{avg}$	ED$_{avg}$
1099*	1104	1104	-31.31	-0.57	26.24
1100	1100	1100	-32.9	-1.27	23.94
1101	1101	1101	-32.1	-1	25.77
1102	1102	1102	-31.48	-0.75	27.21
1103	1103	1103	-31.67	-0.82	25.13
1104	1104	1104	-31.31	-0.57	26.24
1105	1105	1105	-31.26	-0.55	26.76
1106	1106	1106	-31.13	-0.57	26.92
1107	1107	1107	-30.99	-0.47	27.4
1108:	1108	1108	-31.31	-0.65	27.11

2. Log File

i	*j*	nt	W$_{i\text{-}j}$	MFE$_{avg}$	Z$_{avg}$	ED$_{avg}$	Z$_{sum}$	Z$_{norm}$
1099	1099	U	7	-30.63	-0.16	31.91	-1.09	-0.01
1099	1095	A	9	-28.82	0.37	36.79	3.34	0.03
1099	1032	A	24	-29	0.28	30.65	6.74	0.06
1099	1015	A	4	-27.75	0.7	37.61	2.8	0.02
1099	1106	A	3	-30.53	0.08	21.54	0.23	0
1099	1140	G	7	-27.79	0.68	33.15	4.79	0.04
1099	1137	G	5	-29.06	0.22	34.73	1.09	0.01
1099	1087	A	5	-30.48	-0.44	34.69	-2.21	-0.02
1099	1042	A	1	-31.6	-0.42	34.99	-0.42	0
1099	1122	A	13	-32.87	-1.14	20.31	-14.83	-0.12
1099	1055	A	1	-34.7	-1.45	27.95	-1.45	-0.01
1099	1082	A	11	-33.64	-1.71	27.5	-18.82	-0.16
1099	1104	A	16	-33.33	-1.68	23.25	-26.82	-0.22
1099	1177	A	9	-31.43	-0.89	21.01	-8.03	-0.07
1099	1080	G	2	-29.6	-0.6	25.53	-1.2	-0.01
1099	1189	A	2	-28.3	-0.41	28.36	-0.82	-0.01
1099	1182	G	1	-31.6	-0.67	25.74	-0.67	-0.01