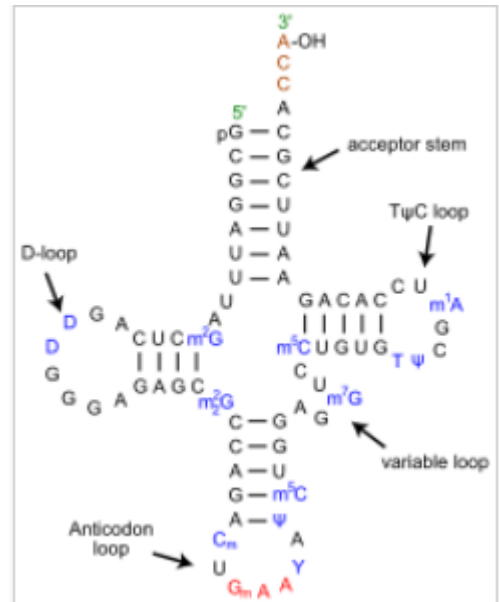


The Transfer RNA Puzzle

Each of you will create an Eterna puzzle based on a specific tRNA from any species.

1. Locate a tRNA sequence. Try starting with the tRNA database at Leipzig: <http://trna.bioinf.uni-leipzig.de/DataOutput/>
2. Your sequence will probably be written in DNA, i.e. with T instead of U. Copy your sequence into a text file, remove spaces between letters and then use the search and replace function to convert all of the T to U.
3. Open the puzzle maker at Eterna. <https://eternagame.org/game/puzzlemaker/>
4. You will see a default puzzle that is a simple stem and loop. At the bottom of the page will be something that looks like this:((((((((.....)))))))). This is the code that defines the stem and loop shape. Each dot represents an unpaired base, while each open or closed parenthesis (or) represents a member of a paired base.
5. The structural code for a tRNA would look something like this (based on the yeast tRNA-Phe in the figure at the right (Wikipedia)). ((((((((((((.....)))))).((((.....))))))......((((.....)))))))).
 - Acceptor stem 5'
 - **D stem and loop**
 - Anticodon stem and loop
 - Variable
 - **TYC stem and loop**
 - Acceptor stem 3'
 - Extra dots at 3' end are for adjusting stems and loops.
6. Click on the sequence button to open a sequence entry box. Then copy your tRNA sequence into the sequence box. Your base sequence will then replace all of the As in your puzzle with the bases from the sequence.
7. Your puzzle maker has the same Target and Natural buttons that you see in a finished puzzle. Click on the Natural button to see how your RNA folds. Does it assume the shape you expected? Take a screen shot or a snip of the shape that it does assume. Why do you think your sequence folds into this shape?
8. Play with the pattern code and try to improve the fit between your sequence and the pattern. Make these changes carefully; the “undo” only works on pattern changes up to a point. In a normal tRNA “cloverleaf” there should be matching base pairs at the beginning and end of the sequence that form the stem of the molecule and additional pairing regions for the 3 stem and loop structures. N.b. In eukaryotes, the CCA at the 3' end of tRNAs is added post-transcriptionally.
9. Your challenge is this: can you adjust the structural code of the puzzle to optimize base pairing in your tRNA sequence? Remember tRNA has a number of modified bases, so look for the parent base in each case. Might



it be necessary to change some of the bases to achieve the conventional folding pattern?

10. As you adjust your tRNA pattern to your sequence, look for the conserved UUC in the TYC loop, the conserved UU in the D loop and the appropriate anticodon in the anticodon loop. A table of anticodons can be found here: <http://waynesword.palomar.edu/codons.htm>

11. When you have arrived at a stable tRNA pattern with appropriate features, give your puzzle a name and publish it!

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SAMPLE tRNA PUZZLE

1. RNA sequence: *Drosophila melanogaster* tRNA-Thr
GGCGCCGUGGCCUAGUUGGUUAAAGCGCCUGUCUAGUAAACAGGAGAUCGUGAGUUCGAAUCUCGCCGGGGCCU
2. I began with the tRNA structural pattern from #7 above, and then pasted in the RNA sequence.
(((((((.....))))).((((.....)))).....((((.....)))))).....
3. The resulting target sequence (Figure 1) showed multiple mismatches in the stem regions. The white lines in the image show how base pairing might be improved with a shift in the positioning of the bases in the stems. As might be expected the progress image indicates that this structure is completely unstable.
4. The structural pattern was adjusted as follows to optimize the base pair fit and the extra dots were trimmed from the 3' end. Note the addition of the dot to the D loop section (in red). This was the only change necessary to get good base pairing for this sequence.
(((((((.....))))).((((.....)))).....((((.....)))))).....
5. The adjusted target sequence then looked like Figure 2. Note that all of the bases in the stem regions are now paired, except for a GG mismatch in the acceptor stem. In addition, the conserved bases for the D loop and the TYC loop are present in the loops. An appropriate anticodon for threonine is also at the apex of the anticodon loop. Nevertheless only the TYC loop (UUC) is stable.
6. Two base changes were needed to stabilize the structure: the addition of a C at #70 to correct the GG mismatch, and the replacement of base pair GU at #51/65 with an AU pair. Replacement of #51/65 with a GC base pair also stabilized the target structure. The stabilized structure looked like Figure 3.
7. Reflection: Why do you think that this tRNA sequence failed to assume a stable configuration even after adjustments in the pattern?

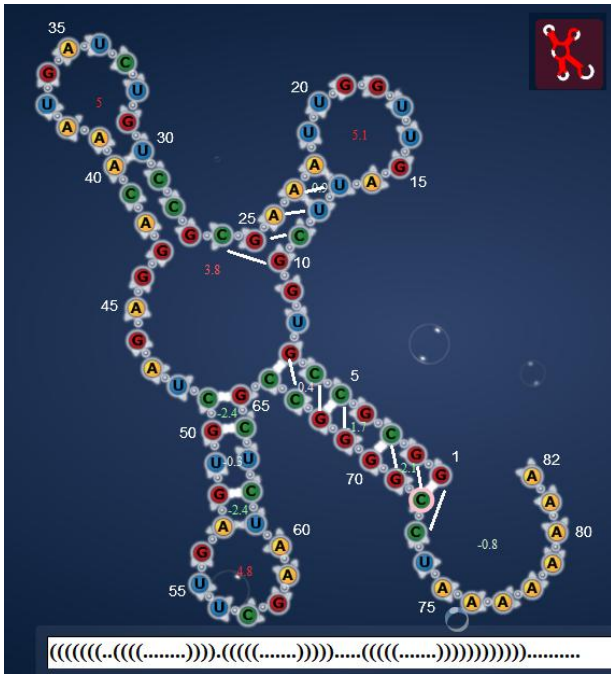


Figure 1 (left). Original pattern code and sequence. There is extensive mispairing and the structure is unstable throughout. White lines indicate possible improvements in base pairing by an adjustment in the position of the bases in the stems.

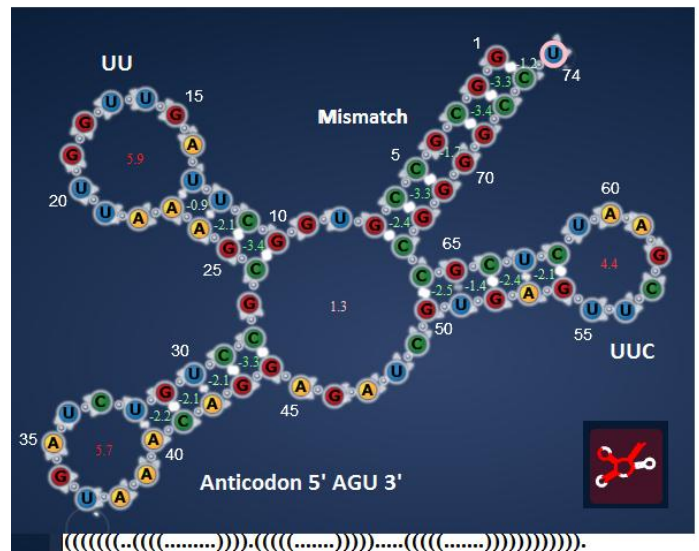


Figure 2 (right). Improved base pairing due to an adjustment in the puzzle pattern. Note that all three loops have appropriate base content. All base pairs are appropriate except for the GG mismatch at #4/70. However, only the TYC stem and loop (UUC) section is stable.

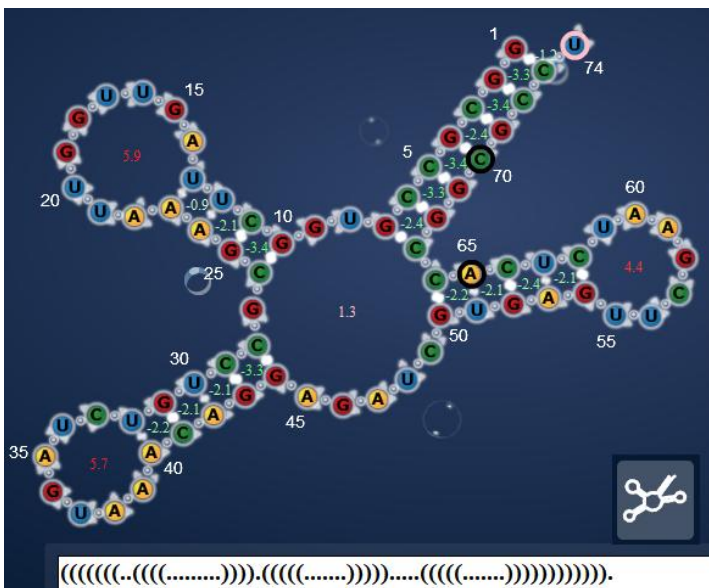


Figure 3 (left). Puzzle stabilization by selected base changes (circled in black). The G formerly at #70 was changed to a C and the GU pair at #51/65 was replaced with an AU pair. Replacing the GU pair with a GC pair was also effective in stabilizing the puzzle. This puzzle was published on the Eterna site under the name Dm tRNA-Thr 2.