ANALYTICAL QUESTIONS & ANSWERS

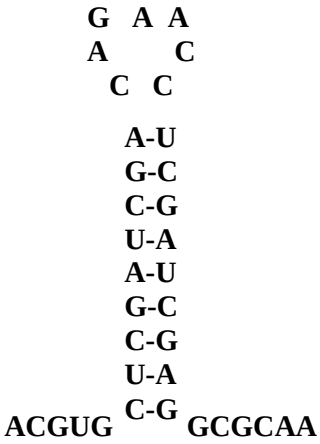
CHAPTER 3

1. Make up an RNA sequence that will form a hairpin with a 9 bp stem and a 7 bp loop. Draw both the primary structure and the secondary structure.

One of the many possible sequences that could be drawn is shown below:

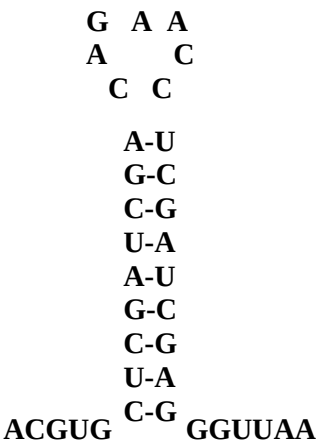
Primary structure: 5′-ACGUGCUCGAUCGACAGAACCUCGAUCGAGGCGCAA – 3′

Secondary structure:



2. What addition(s) would you need to make to the primary sequence in Question 1 to allow pseudoknot formation?

A pseudoknot motif forms when a single-stranded loop base pairs with a complementary sequence outside this loop. The sequence in (1) would need to be modified to allow complementary base pairing between the loop and the sequence 3′ of the loop. One possible example is shown below:



3. You suspect that a tetraloop is critical for folding of a ribozyme into its active form. Describe an experiment to demonstrate whether the RNA folds into a similar tertiary structure when the tetraloop is deleted.

Carry out a hydroxyl radical footprinting experiment, comparing the cleavage pattern of the RNA containing the tetraloop motif with the cleavage pattern of a mutant RNA lacking the tetraloop motif. Tertiary contacts within a folded RNA molecule result in local reductions in solvent accessibility. The hydroxyl radicals cannot react with the protected backbone sugar and hence there is a reduced cleavage of protected nucleotides, which can be visualized by electrophoresis. If the tetraloop motif is required for folding into the active form, then the mutant RNA would be predicted to be more accessible to solvent and to show enhanced cleavage.

4. You have discovered a small RNA involved in removal of a novel class of introns. Design an experiment to determine whether the small RNA functions as a catalytic RNA or RNP. Show sample positive results.

Prepare a labeled intron-containing precursor RNA (pre-RNA) by *in vitro* transcription from a DNA template. Incubate the pre-RNA with the small RNA either in the presence or absence of its associated proteins. Separate the samples by polyacrylamide gel electrophoresis and visualize the results by autoradiography. If the small RNA functions as a catalytic RNA, then the RNA alone will remove the intron from the pre-RNA. If the small RNA functions as a catalytic RNP, then it will remove the intron from the pre-RNA only when its associated proteins are present in the reaction. The proteins alone would not mediate intron removal in either case. Sample positive results are shown for the first scenario: