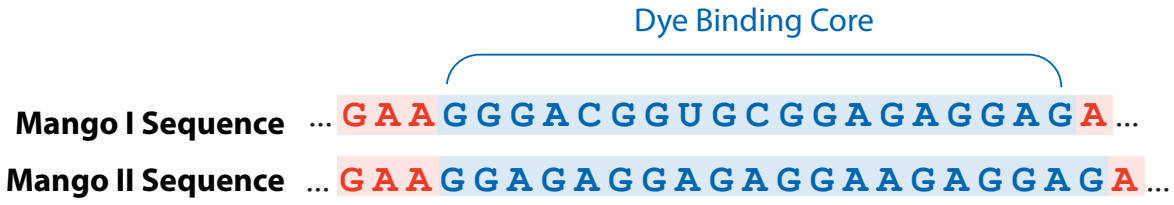
RNA Mango Aptamer Insertion in Cellular Transcripts

1. Non-coding RNA (ncRNA) insertion

Many ncRNA contain stem loop sequences that can be exploited to insert RNA Mango without causing any modifications to the remaining structure of the RNA of interest. The dye binding cores (blue) of the RNA Mango I and II aptamers are flanked by a GNRA tetraloop-like motif (red). These dye binding cores can be inserted into the ncRNA of interest by simply removing the loop of an RNA of interest and inserting the sequences below:



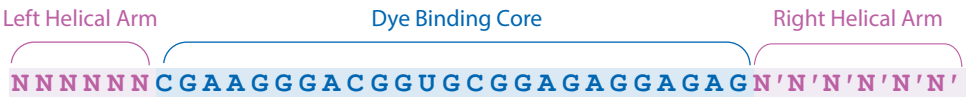
A bacterial transcription regulating RNA (6S RNA) and spliceosome RNA in yeast (U1 snRNA) have been successfully modified and functionalized with RNA Mango by this method:

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5602116/

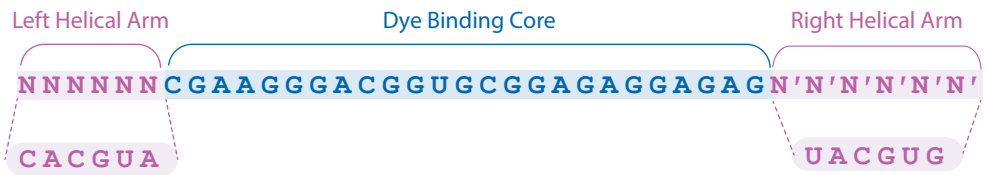
2. mRNA Insertion

a. RNA Duplex Stem

Example sequence:



To achieve proper folding of the RNA Mango aptamer, it is important that the sequences flanking the dye binding core of the Mango aptamer (binding core shown in blue) are able to form an RNA duplex. The left helical arm (N sequence) must be able to hybridize with the right helical arm (N'). Any sequence may be used on the left or right helical arms, but once the sequence is decided for one of the arms (for example, the N helical arm sequence), the other arm must be constructed so as to be complementary to the other (in this case, N' must be complemented to N). You may use the following sequences as a simple example of correctly constructed complementary helical arms:



b. Bacterial and Eukaryotic UTR Insertions

In general, RNA Mango aptamers can be easily inserted in the 5´ and 3´ UTR of a target mRNA:

i. For a 5'UTR insertion, use caution when inserting in this region. Ensure that the RNA Mango aptamer being inserted does not:

- interrupt any known existing structural RNA elements
- contain the start codon
- interrupt the Shine-Dalgarno sequence in bacteria
- disrupt eukaryotic ribosomal scanning

ii. For a 3' UTR insertion, add a Mango in any frame. Mango II aptamer arrays have been inserted immediately or shortly after the stop codon in a gene of interest to successfully track RNA:

- https://www.nature.com/articles/s41467-020-14932-7

c. ORF Insertions

For ORF insertions, Mango aptamers can be added in any frame and will result in the alteration of a local region of the protein sequence. You may use the alignments below to determine how these insertions can change the protein that will be translated. **Conveniently, the dye binding cores of Mango I, II, and III/IIIA10U aptamers do not contain stop codons in any frame.** To keep the aptamers and the remainder of the peptide sequence in frame, simply add nucleotides (+ 1 or + 2) as illustrated in the examples below.

	Reading Frame 1	Reading Frame 2	Reading Frame 3	(X dependent on stem chosen)
Mango I	NNN NNN	CGA AGG GAC GGU GCG GAG AGG AGA GN'N'	R R D G A E R R X	N'N'N' N' +2
	NN NNN	NCG AAG GGA CGG UGC GGA GAG GAG AGN'	X K G R C G E E X	N'N'N' N'N' +2
	N NNN	NNC GAA GGG ACG GUG CGG AGA GGA GAG	X E G T V R R G E	N'N'N' N'N'N' +2
Mango II	NNN NNN	CGA AGG AGA GGA GAG GAA GAG GAG AGN'	R R R G E E E E X	N'N'N' N'N' +1
	NN NNN	NCG AAG GAG AGG AGA GGA AGA GGA GAG	X K E R R G R G E	N'N'N' N'N'N' +1
	N NNN	NNC GAA GGA GAG GAG AGG AAG AGG AGA GN'N'	X E G E E R K R R X	N'N'N' N' +1
Mango III	NNN NNN	AGG AUU GGU AUG UGG UAU AUN'	R I G M W Y X	N'N'N' N'N' +1
	NN NNN	NAG GAU UGG UAU GUG GUA UAU	X D W Y V V Y	N'N'N' N'N'N' +1
	N NNN	NNA GGA UUG GUA UGU GGU AUA UN'N'	X G L V C G I X	N'N'N' N' +1
Mango III (A10U)	NNN NNN	AGG UUU GGU AUG UGG UAU AUN'	R F G M W Y X	N'N'N' N'N' +1
	NN NNN	NAG GUU UGG UAU GUG GUA UAU	X V W Y V V Y	N'N'N' N'N'N' +1
	N NNN	NNA GGU UUG GUA UGU GGU AUA UN'N'	X G L V C G I X	N'N'N' N' +1