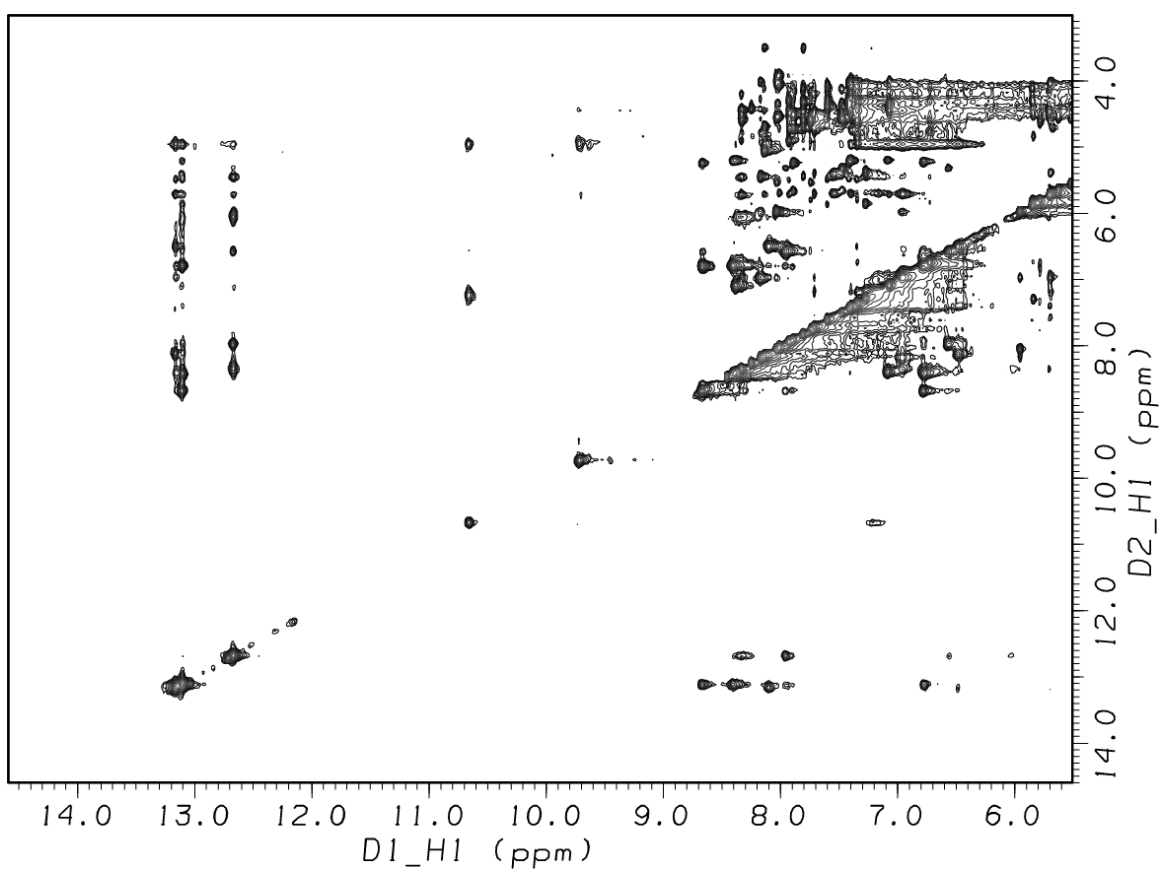


# Factors Affecting Thermodynamic Stabilities of RNA $3 \times 3$ Internal Loops<sup>†</sup>

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**Figure S1.** SNOESY spectrum of  $\begin{matrix} \text{CGCGAAGGC} \\ \text{GCGAGGCCG} \end{matrix}$  at 10 °C. The sample was dissolved in 90:10 (v:v) H<sub>2</sub>O:D<sub>2</sub>O and recorded with a 150 ms mixing time. The spectrum was acquired with 4k points and a spectral width of 11 kHz and processed with the Felix (2000) software package (Molecular Simulations Inc.).



**Table S1.** Thermodynamic Parameters for Single Strand Duplex Formation

Sequence	$-\Delta H^\circ$ (kcal/mol)	$-\Delta S^\circ$ (eu)	$-\Delta G^\circ_{37}$ (kcal/mol)	$T_m$ ( $^\circ\text{C}$ )	$(C_T)$ (M)
CGCAAAGGC	no transition with $C_T \sim 10\text{E-}5$ M				
CGACCGAGCAG	37.2	106.9	4.01	20.7	5.26E-5
CGACGCAGCAG	63.5	184.6	6.27	38.2	5.57E-5
	50.3	143.5	5.75	28.0	7.67E-6
CGCAGAGGC	no transition with $C_T \sim 10\text{E-}5$ M				
CGCAUAGGC	no transition with $C_T \sim 10\text{E-}5$ M				
CGCGAAGGC	47.0	131.1	6.33	30.9	7.48E-6
	46.6	130.6	6.12	31.8	1.34E-5
	43.5	120.5	6.14	34.0	2.36E-5
CGCGCAGGC	57.7	156.6	9.13	50.6	1.86E-5
	53.1	143.2	8.73	52.3	4.09E-5
CGCUCUGGC	no transition with $C_T \sim 10\text{E-}5$ M				
CGCUUUGGC	no transition with $C_T \sim 10\text{E-}5$ M				
CGGAAGCGC	32.9	87.8	5.69	30.6	3.17E-5
CUGCAAAGUCG	39.6	109.9	5.54	33.2	5.54E-5
CUGCGAAGUCG	no transition with $C_T \sim 10\text{E-}5$ M				
GAGCAAACGAC	no transition with $C_T \sim 10\text{E-}5$ M				
GAGCAGACGAC	no transition with $C_T \sim 10\text{E-}5$ M				
GAGCCGAC <sup>a</sup>	44.3	124.9	5.62	40.2	2.28E-4
	39.1	107.4	5.77	30.5	2.20E-5
GAGCCGACGAC	47.8	136.3	5.49	34.7	7.60E-5
	51.4	148.6	5.34	26.9	1.04E-5
GAGCGAACGAC	33.2	92.5	4.52	23.8	5.95E-5
	39.2	108.4	5.56	27.0	1.46E-5
GAGCGAGCGAC	63.9	177.5	8.89	51.4	5.36E-5
	58.7	162.8	8.18	43.9	1.36E-5
GAGCGGACGAC	62.2	179.3	6.55	38.6	4.15E-5
	50.1	141.8	6.13	31.2	1.08E-5
GAGCGUACGAC	66.8	184.5	9.57	55.9	8.99E-5
	62.9	172.4	9.47	53.3	3.48E-5
GAGCUGACGAC	53.5	156.5	5.00	32.4	8.04E-5
	42.6	120.4	5.28	28.1	2.49E-5
GAGCUGCCGAC	61.0	172.3	7.53	46.0	7.99E-5
	63.1	178.3	7.77	41.6	1.48E-5
GAGCUGUCGAC	77.5	224.0	8.03	46.4	8.69E-5
	70.6	203.4	7.52	40.7	1.95E-5
GAGUAAAUGAC	no transition with $C_T \sim 10\text{E-}5$ M				
GAGUGAAUGAC	no transition with $C_T \sim 10\text{E-}5$ M				
GAGUUGAC <sup>a</sup>	47.8	144.6	2.92	24.5	3.39E-4
	35.9	104.4	3.5	14.3	3.41E-5
GCCAAAGCG	45.2	128.3	5.45	27.8	1.56E-5
	35.9	99.4	5.07	25.6	3.11E-5

GCCCAAGCG	no transition with $C_T \sim 10E-5$ M				
GCCGAAGCG <sup>b</sup>	64.4±5.6	185.6±17.9	6.81±0.14	42.6	
	61.7±7.5	176.8±24.0	6.86±0.17	43.0	
GCCGGAGCG	45.4	121.0	7.83	37.1	3.09E-6
	34.8	86.9	7.81	40.1	5.52E-6
	31.2	76.0	7.64	43.4	1.14E-5
GCCGUAGCG	51.6	153.1	4.12	26.6	6.77E-5
GCCUCUGCG	no transition with $C_T \sim 10E-5$ M				
GCCUUUGCG	no transition with $C_T \sim 10E-5$ M				
GCGAAGCCG	65.1	198.6	3.54	23.9	2.96E-5
GGUGGA	22.3	52.1	6.08	31.6	2.72E-5
GUCAACUC	no transition with $C_T \sim 10E-5$ M				
GUCAAGAACUC	no transition with $C_T \sim 10E-5$ M				
GUCGAAAGCUC	no transition with $C_T \sim 10E-5$ M				
GUCGAAUGCUC	no transition with $C_T \sim 10E-5$ M				
GUCGAGAGCUC	41.6	109.5	7.63	49.9	6.26E-5
	30.9	76.6	7.15	40.5	1.59E-5
GUCGAUAGCUC	63.4	182.0	6.96	40.6	4.08E-5
	59.8	170.7	6.88	35.9	1.01E-5
GUCGGAAGCUC	no transition with $C_T \sim 10E-5$ M				
GUCGGCUC <sup>a</sup>	69.9	197.9	8.51	52.2	2.02E-4
	65.6	185.2	8.22	41.7	8.01E-6
GUCGGUAGCUC	no transition with $C_T \sim 10E-5$ M				
GUCGUAUGCUC	61.9	183.0	5.15	33.5	7.53E-5
	51.8	150.0	5.32	27.7	1.32E-5
UCCGCC	no transition with $C_T \sim 10E-5$ M				

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<sup>a</sup> Data from ref 78; <sup>b</sup> Data on top line are the thermodynamics derived from  $T_M^{-1}$  vs  $\ln(C_T)$  plots, data on bottom line are the thermodynamics from average of melt curve fits.

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**Table S2.** Phylogenetic analysis

Closing bp	CG	GC	UG	AU	UA	GU
Number	1054	370	274	182	179	99
Percentage	48.8	17.1	12.7	8.4	8.3	4.6

Middle pair	AA	AU	GA	AC	GG	UU	CG	CU	GU	CC
Number	457	243	212	64	42	26	12	10	7	6
Percentage	42.4	22.5	19.6	5.9	3.9	2.4	1.1	0.9	0.6	0.6

Terminal pair	GA	UU	UA	AA	CA	CC	GG	UG	UC	AC	AG	GC	CU	GU	CG	AU
Number	863	223	156	149	148	146	92	71	62	50	45	39	38	30	28	18
Percentage	40.0	10.3	7.2	6.9	6.9	6.8	4.3	3.3	2.9	2.3	2.1	1.8	1.8	1.4	1.3	0.8