

HIV-1 frameshift efficiency is primarily determined by the stability of base pairs positioned at the mRNA entrance channel of the ribosome

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Supplementary Data

Supplementary Materials & Methods

Error determination for equation 4

$$\Delta G = \ln K * (-RT) \quad [4]$$

Where, $K = e^{-\left(\frac{\Delta H}{RT}\right) * \left(1 - \frac{T}{T_m}\right) + \frac{\Delta Cp}{R} \left(\frac{T_m}{T} - 1 + \ln \frac{T}{T_m}\right)}$

The **error in Equation 4** is calculated the following way:

$$\Delta G = A \left(1 - \frac{T}{B}\right), \text{ where } A = \Delta H \text{ and } B = T_m$$

If $\Delta G = A - ATB^{-1}$, then the error in ΔG is the following:

$$\sigma_{\Delta G}^2 = \sigma_A^2 \left(\frac{d\Delta G}{dA}\right)^2 + \sigma_B^2 \left(\frac{d\Delta G}{dB}\right)^2$$

Where,

$$\sigma_A^2 = (\sigma \Delta H)^2, \sigma_B^2 = (\sigma T_m)^2, \frac{d\Delta G}{dA} = 1 - \frac{T}{B} = 1 - \frac{T}{T_m}, \text{ and } \frac{d\Delta G}{dB} = ATB^{-2} = \frac{\Delta H * T}{T_m^2}.$$

Supplementary Table 1. Sequences inserted into the p2luc vectors for plasmid construction

Construct	Sequence (5'- 3')
WT ^φ	GATCCTTTTTTAGGGAAGATCTGGCCTTCCCACAAGGGAAGGCCAGGGAATTTTCT TCAGAGCAGACCAGAGCCAACAGCCGCACCGAGCT
MS1	GATCCTTTTTTAGGGAAGATGGGGCCTTCCCACAAGGGAAGGCCCCGGAATTTTCT TTCAGAGCAGACCAGAGCCAACAGCCGCACCGAGCT
MS2	GATCCTTTTTTAGGGAAGATGGGGCCTTCTTACAAAGGAAGGCCCCGGAATTTTCT TCAGAGCAGACCAGAGCCAACAGCCGCACCGAGCT
MS3	GATCCTTTTTTAGGGAAGATGGCGCCTTCTTACAAAAGAAGGCCCCGGAATTTTCT TCAGAGCAGACCAGAGCCAACAGCCGCACCGAGCT
MS4	GATCCTTTTTTAGGGAAGATGGCGCCTTACAAAAGGCCGCCGGAATTTTCTTCAGAG CAGACCAGAGCCAACAGCCGCACCGAGCT
MS5	GATCCTTTTTTAGGGAAGATGCGTTCCTTGCTTCGGCAAGGAGCGCGGAATTTTCT TCAGAGCAGACCAGAGCCAACAGCCGCACCGAGCT
MS6	GATCCTTTTTTAGGGAAGATCTGGCCTTCTTACAAAGGAAGGCCAGGGAATTTTCT TCAGAGCAGACCAGAGCCAACAGCCGCACCGAGCT
MS7	GATCCTTTTTTAGGGAAGATCTGGCCTTCTTACAAAAGAAGGCCAGGGAATTTTCTT CAGAGCAGACCAGAGCCAACAGCCGCACCGAGCT
MS8	GATCCTTTTTTAGGGAAGATCTGGCACAAGCCAGGGAATTTTCTTCAGAGCAGACC AGAGCCAACAGCCGCACCGAGCT
MS9	GATCCTTTTTTAGGGAAGATCTCTGGGGCCCACAAGGGCCCCAGAGGGAATTTTCT TTCAGAGCAGACCAGAGCCAACAGCCGCACCGAGCT
MS10	GATCCTTTTTTAGGGAAGATATTGCCTTCCCACAAGGGAAGGCCGGTGAATTTTCT TCAGAGCAGACCAGAGCCAACAGCCGCACCGAGCT
MS11	GATCCTTTTTTAGGGAAGATATTGGGCCCCCACAAGGGCCCCGGTGAATTTTCT TTCAGAGCAGACCAGAGCCAACAGCCGCACCGAGCT
MS12	GATCCTTTTTTAGGGAAGATATTGCCTTCTTACAAAAGAAGGCCGGTGAATTTTCTT CAGAGCAGACCAGAGCCAACAGCCGCACCGAGCT
MS13	GATCCTTTTTTAGGGAAAGATCTGGCCTTCCCACAAGGGAAGGCCAGGGAATTTTCT TTCAGAGCAGACCAGAGCCAACAGCCGCACCGAGCT
MS14	GATCCTTTTTTAGGGAGATCTGGCCTTCCCACAAGGGAAGGCCAGGGAATTTTCTT CAGAGCAGACCAGAGCCAACAGCCGCACCAAGAGCT
MS15	GATCCTTTTTTAGGGGATCTGGCCTTCCCACAAGGGAAGGCCAGGGAATTTTCTTC AGAGCAGACCAGAGCCAACAGCCGCACCAAGAGCT
MS16	GATCCTTTTTTAGGGGATCTCTGCTTCCCACAAGGGAAGCAGAGGGAATTTTCTTC AGAGCAGACCAGAGCCAACAGCCGCACCAAGAGCT
MS17	GATCCTTTTTTAGGGGATCTCTGGGGCCCACAAGGGCCCCAGAGGGAATTTTCTT CAGAGCAGACCAGAGCCAACAGCCGCACCAAGAGCT
3HJ WT	GATCCGGGCTGTTGGAAATGTGGAAGGAAGGACACCAAATGAAAGATTGTAAGT AGAGACAGGCTAATTTTTTAGGGAAGATCTGGCCTTCCCACAAGGGAAGGCCAGG GAATTTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACCGAGCT
3HJ Mut	GATCCCGCATAATGAAATGTGGAAGGTTCTTACCAAATCCTTCTTGTACTGA GAGACAGGCTAATTTTTTAGGGAAGATCTGGCCTTCCCACAAGGGAAGGCCAGGG AATTTTCTTCAGGCCACCAGAGCCAACAGCCCCACCGAGCT
3HJ MS1	GATCCGGGCTGTTGGAAATGTGGAAGGAAGGACACCAAATGAAAGATTGTAAGT AGAGACAGGCTAATTTTTTAGGGAAGATGGGGCCTTCCCACAAGGGAAGGCCCCG GAATTTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACCGAGCT

^φ The wild-type HIV-1 sequence represents a naturally occurring HIV-1 M subtype B sequence (<http://www.hiv.lanl.gov>, accession number AB078005).

Supplementary Table 2. Overall and local thermodynamic stabilities (kcal/mol) at 37 °C.

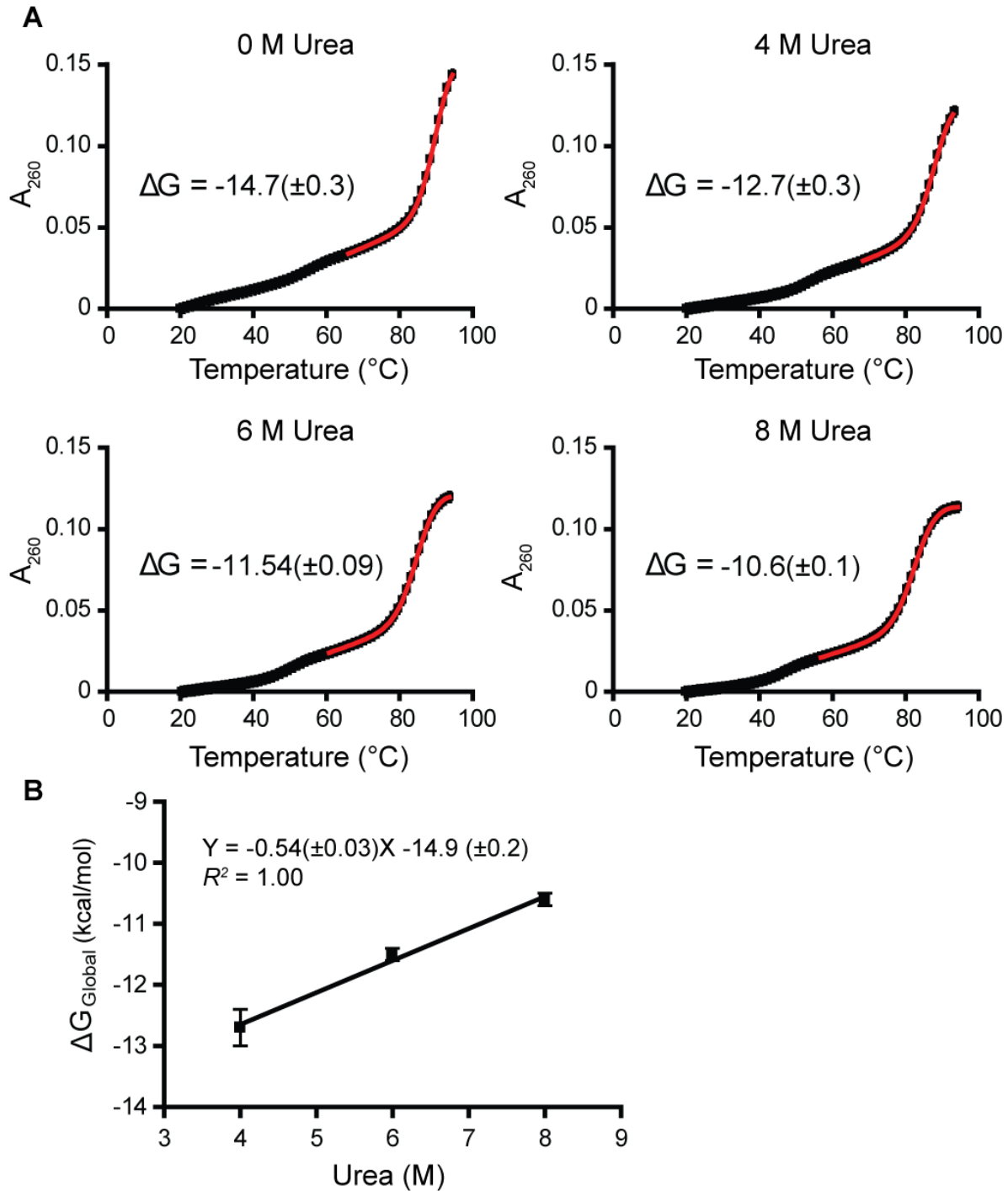
Salt	10 mM Potassium Phosphate			1 M NaCl	
	Measured ΔG_{Global}	Extrapolated ΔG_{Global}	Predicted ΔG_{Global}	Predicted ΔG_{Global}	ΔG_{Local} (3bp)
WT	-14.7 (0.3)	-14.9 (0.2)	-15.5 (1.8)	-21.8 (0.2)	-7.5 (0.1)
MS1	-16.6 (1.7)	-16.8 (0.5)	-17.8 (1.8)	-24.1 (0.2)	-9.8 (0.1)
MS2	-14.7 (0.5)	-14.3 (0.02)	-15.7 (1.8)	-22.0 (0.2)	-9.8 (0.1)
MS3	-	-	-12.8 (1.8)	-19.1 (0.2)	-9.0 (0.1)
MS4	-	-	-9.0 (1.4)	-13.7 (0.2)	-9.0 (0.1)
MS5	-10.9 (0.1)	-	-12.3 (1.8)	-18.6 (0.2)	-7.9 (0.2)
MS6	-12.5 (0.3)	-12.4 (0.4)	-13.3 (1.8)	-19.6 (0.2)	-7.5 (0.1)
MS7	-10.4 (0.1)	-	-11.0 (1.8)	-17.3 (0.2)	-7.5 (0.1)
MS8	-	-	-3.7 (0.9)	-6.8 (0.1)	-7.5 (0.1)
MS9	-	-	-18.2 (1.8)	-24.5 (0.2)	-6.4 (0.1)
MS10	-11.1 (0.4)	-11.2 (0.1)	-10.0 (1.8)	-16.3 (0.3)	-2.0 (0.2)
MS11	-	-	-14.5 (1.8)	-20.8 (0.3)	-2.0 (0.2)
MS12	-6.8 (0.1)	-	-5.5 (1.8)	-11.8 (0.3)	-2.0 (0.2)
MS13	-	-	-16.8 (2.0)	-23.6 (0.2)	-6.0 (0.1)
MS14	-	-	-13.5 (1.7)	-19.3 (0.2)	-8.3 (0.1)
MS15	-	-	-12.4 (1.5)	-17.6 (0.2)	-9.9 (0.1)
MS16	-	-	-10.1 (1.5)	-15.3 (0.2)	-7.5 (0.1)
MS17	-	-	-14.6 (1.5)	-19.8 (0.2)	-7.2 (0.1)

Standard errors shown in parenthesis.
- Not determined.
Extrapolated = determined using a urea titration (Materials & Methods).
 ΔG_{Local} is shown for three base pairs.

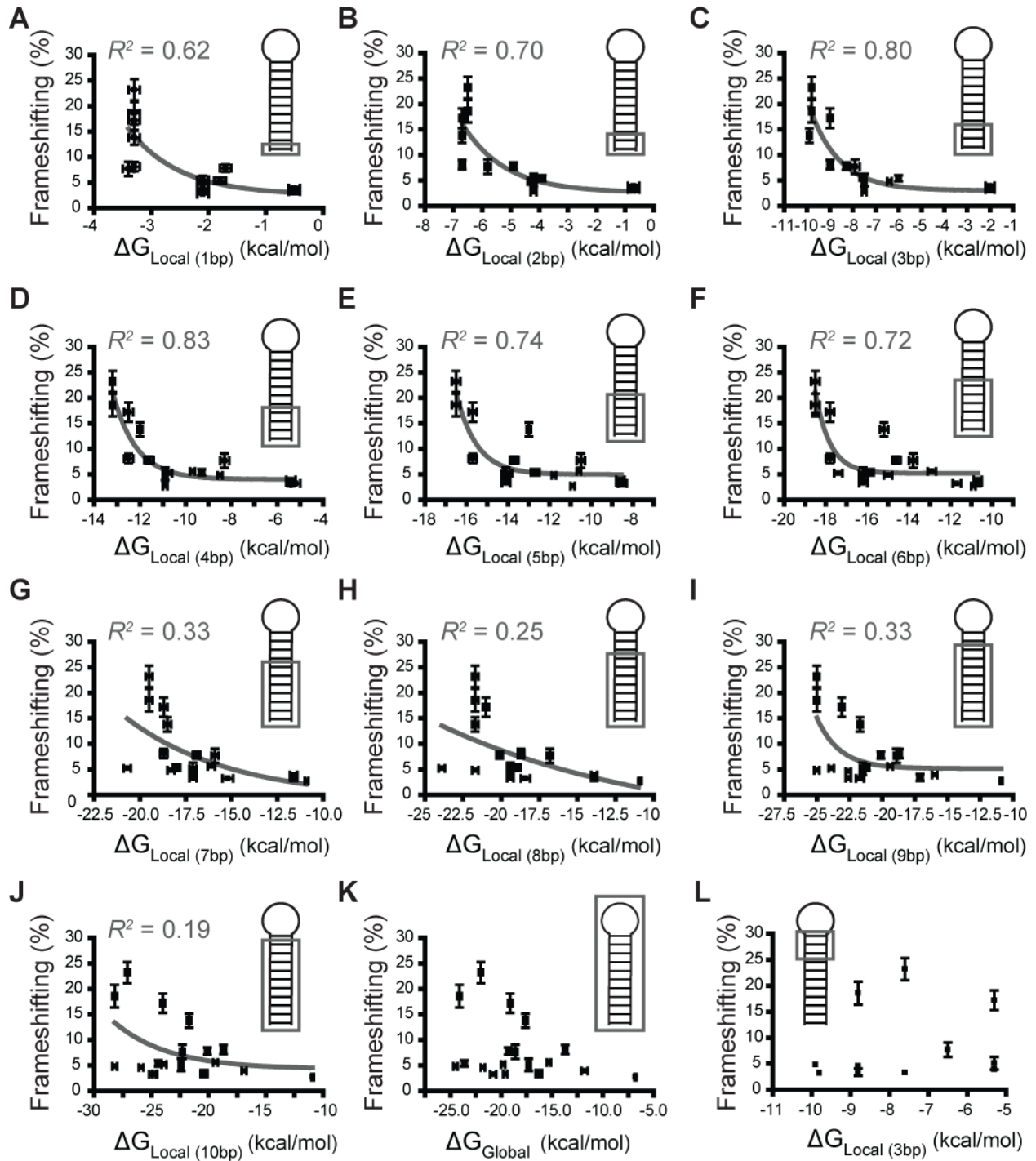
Supplementary Table 3. Correlations between $\Delta G_{\text{Local (N bp)}}$ and frameshift efficiency for the WT and MS1-12 RNAs

N (base pairs)	R^2
1	0.61
2	0.70
3	0.88
4	0.86
5	0.87
6	0.87
7	0.84
8	0.55
9	0.44
10	0.25
Correlations examined using a one-phase exponential decay equation.	

Supplementary Figure 1. A) Representative thermal denaturation experiments in 10 mM potassium phosphate, pH 7.0, with various concentrations of urea. Red lines are indicative of the fit. The calculated ΔG_{Global} is shown for each. B) ΔG_{Global} at 0 M urea was extrapolated using the linear regression fit of ΔG_{Global} at 4, 6, and 8 M urea.



Supplementary Figure 2. Defining the relationship between stem-loop local stability and frameshift efficiency. A-J) Using a one-phase exponential decay equation, correlations between local stability and frameshift efficiency for WT and MS1-17 were examined. Local stability was calculated using nearest-neighbor parameters for the indicated number of base pairs. K) Global stability vs. frameshift efficiency for WT and MS1-17. L) Local stability of the top of the stem-loop does not correlate to frameshift efficiency. A representative plot is shown.



Supplementary Figure 3. A) MS RNA secondary structures redrawn in the context of a 7 nt spacer.

Local stabilities are shown for 3 to 6 base pairs. B) Local stability vs. frameshift efficiency. A single exponential decay function was fit to the data. The number of base pairs used to calculate local stability is indicated.

A

	WT	MS1	MS2	MS3	MS4	MS5
	C A	C A	C A	C A	C A	U C
	A A	A A	A A	A A	A A	U C
	C-G	C-G	U-A	U-A	C-G	G-C
	C-G	C-G	C-G	U-A	C-G	C-G
	C-G	C-G	C-G	C-G	A A	U-A
	U-A	U-A	U-A	U-A	U-A	C-G
	U-A	U-A	U-A	U-A	C-G	G-C
	C-G	C-G	C-G	C-G	C-G	U-A
	C-G	C-G	C-G	C-G	C-G	G-C
	G-C	G-C	G-C	G-C	G-C	U-G
	G-C	G-C	G-C	C-G	C-G	G-C
	U-A	G-C	G-C	G-C	G-C	C-G
	C-G	G-C	G-C	G-C	G-C	G-C
	U G	U G	U G	U G	U G	U G
ΔG_{Local}						
(3bp)	-6.0 (0.1)	-7.8 (0.1)	-7.8 (0.1)	-7.9 (0.2)	-7.9 (0.2)	-7.0 (0.2)
(4bp)	-9.3 (0.1)	-11.0 (0.1)	-11.0 (0.1)	-10.3 (0.2)	-10.3 (0.2)	-9.2 (0.2)
(5bp)	-12.7 (0.2)	-14.5 (0.2)	-14.5 (0.2)	-13.7 (0.2)	-13.7 (0.2)	-9.5 (0.2)
(6bp)	-15.9 (0.2)	-17.7 (0.2)	-17.7 (0.2)	-17.0 (0.2)	-17.0 (0.2)	-11.8 (0.2)

	MS6	MS7	MS8	MS9	MS10	MS11
	C A	C A	C A	C A	C A	C A
	A A	A A	A A	A A	A A	A A
	U-A	U-A	U-A	C-G	C-G	C-G
	C-G	C-G	C-G	C-G	C-G	C-G
	C-G	C-G	C-G	C-G	C-G	C-G
	U-A	U-A	U-A	U-A	U-A	C-G
	U-A	U-A	U-A	U-A	U-A	G-C
	C-G	C-G	C-G	C-G	C-G	G-C
	C-G	C-G	C-G	C-G	C-G	G-C
	G-C	G-C	G-C	G-C	G-C	U-G
	G-C	G-C	G-C	G-C	G-C	U-G
	U-A	U-A	U-A	U-A	U-A	U-G
	C-G	C-G	C-G	C-G	A-U	A-U
	U G	U G	U G	U G	U-G	U-G
ΔG_{Local}						
(3bp)	-6.0 (0.1)	-6.0 (0.1)	-6.0 (0.1)	-6.1 (0.1)	-1.5 (0.2)	-1.5 (0.2)
(4bp)	-9.3 (0.1)	-9.3 (0.1)	-9.3 (0.1)	-8.2 (0.1)	-2.8 (0.2)	-2.8 (0.2)
(5bp)	-12.7 (0.2)	-12.7 (0.2)	-12.7 (0.2)	-10.3 (0.1)	-6.2 (0.2)	-6.1 (0.2)
(6bp)	-15.9 (0.2)	-15.9 (0.2)	-12.7 (0.2)	-13.6 (0.2)	-9.5 (0.2)	-9.3 (0.2)

	MS12	MS13	MS14	MS15	MS16	MS17
	C A	C A	C A	C A	C A	C A
	A A	A A	A A	A A	A A	A A
	U-A	C-G	C-G	C-G	C-G	C-G
	U-A	C-G	C-G	C-G	C-G	C-G
	C-G	C-G	C-G	C-G	C-G	C-G
	U-A	U-A	U-A	U-A	U-A	C-G
	U-A	U-A	U-A	U-A	U-A	C-G
	C-G	C-G	C-G	C-G	C-G	C-G
	C-G	C-G	C-G	C-G	C-G	C-G
	G-C	G-C	G-C	G-C	G-C	G-C
	G-C	G-C	G-C	G-C	G-C	G-C
	U-G	U-A	U-A	U-A	U-A	U-A
	U-G	U-G	U-A	G-C	C-G	C-G
	A-U	U-G	C-G	G-C	U-A	U-A
	U-G	A G	C-G	U-A	U-A	U-A
ΔG_{Local}						
(3bp)	-1.5 (0.2)	-4.2 (0.1)	-7.0 (0.1)	-8.8 (0.1)	-6.0 (0.1)	-6.0 (0.1)
(4bp)	-2.8 (0.2)	-6.3 (0.1)	-10.9 (0.2)	-12.1 (0.1)	-9.5 (0.1)	-9.3 (0.1)
(5bp)	-6.2 (0.2)	-9.6 (0.1)	-14.1 (0.2)	-14.1 (0.2)	-11.5 (0.1)	-12.6 (0.1)
(6bp)	-9.5 (0.2)	-13.0 (0.2)	-16.2 (0.2)	-15.1 (0.2)	-12.5 (0.2)	-15.8 (0.2)

