Supplementary Information

Assessment for melting temperature measurement of nucleic acid by HRM

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Supplementary Table S1 Comparison of reproducibility between HRM and UV melting method

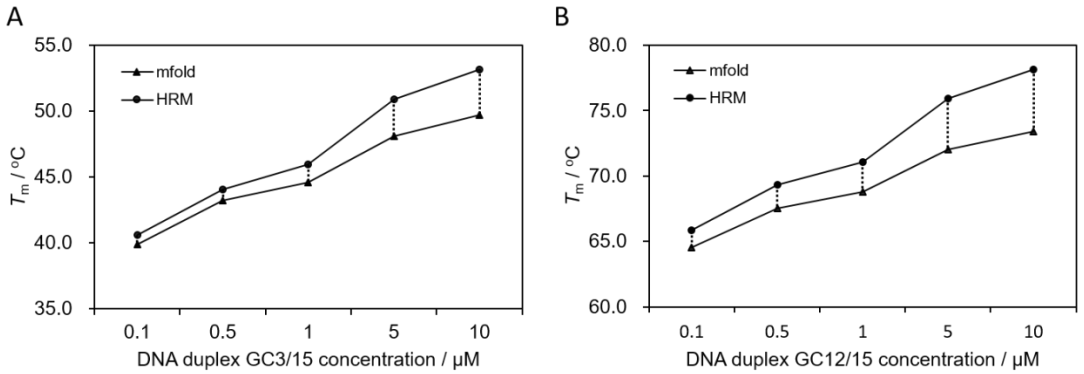
	HRM			UV melting method		
	Av. T_m (^oC)	S.D. (^oC)	CV (%)	Av. T_m (^oC)	S.D. (^oC)	CV (%)
GC0/15	34.6	0.5	1.6	31.7	0.6	1.9
GC1/15	37.9	0.5	1.2	33.4	0.2	0.6
GC2/15	42.7	0.3	0.8	38.8	0.2	0.6
GC3/15	45.4	0.2	0.5	42.9	0.7	1.7
GC4/15	48.3	0.3	0.6	46.0	0.1	0.3
GC5/15	50.5	0.3	0.6	48.3	0.2	0.4
GC6/15	54.1	0.2	0.4	52.5	0.6	1.1
GC7/15	55.9	0.3	0.5	53.5	0.3	0.6
GC8/15	59.3	0.3	0.4	56.9	0.5	1.0
GC9/15	61.0	0.3	0.5	59.4	0.6	1.0
GC10/15	65.2	0.2	0.3	63.5	0.4	0.7
GC11/15	69.9	0.1	0.2	68.9	0.2	0.2
GC12/15	70.6	0.3	0.5	69.7	1.0	1.4
GC13/15	72.1	0.2	0.3	71.4	1.0	1.5
GC14/15	75.5	0.2	0.3	74.6	0.5	0.6
GC15/15	78.7	0.4	0.5	77.4	0.2	0.3

Av. T_m, S.D. and CV denote the average T_m, standard deviation of the average T_m and coefficient of variation, respectively.

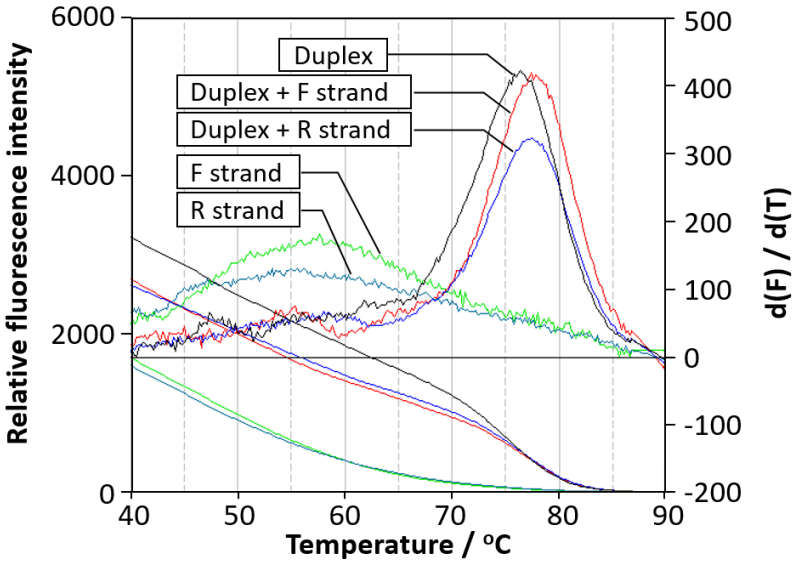
Supplementary Table S2 Comparison of UV T_m and HRM T_m of 20 bp DNA duplex

DNA duplex	UV T_m [a] (^oC)	mfold T_m [b] (^oC)	HRM T_m [c] (^oC)	T_m^{mod} [d] (^oC)	ΔT_m [e] (^oC)
GC0/20	39.7	43.5	42.8	39.7	-0.1
GC2/20	47.6	48.4	51.1	48.5	-0.9
GC4/20	50.4	53.3	53.5	51.1	0.6
GC6/20	56.3	56	58.1	56.1	-0.2
GC8/20	59.6	60.4	62.1	60.4	0.7
GC10/20	63.5	62.7	65.4	63.9	0.4
GC12/20	67.3	67.3	69	67.7	0.4
GC14/20	72.8	71.3	73.8	72.9	0.1
GC16/20	77.8	75.7	78.7	78.1	0.3
GC18/20	81.4	80.9	82.2	81.8	0.5
GC20/20	85.7	84.4	86.5	86.5	0.7
RMS[f]		1.7	2.1	0.5	

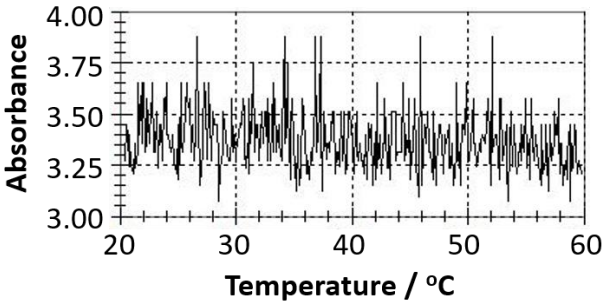
[a] UV T_m denotes T_m measured by UV melting method; [b] mfold T_m denotes T_m calculated in mfold web server; [c] HRM T_m denotes T_m measured by HRM; [d] T_m^{mod} denotes the corrected T_m, T_m^{mod} = 1.0724 * HRM T_m – 6.2673; [e] $\Delta T_m = T_m^{mod}$ - UV T_m; [f] RMSD denotes the root mean-squared deviation of HRM T_m or T_m^{mod} to UV T_m.



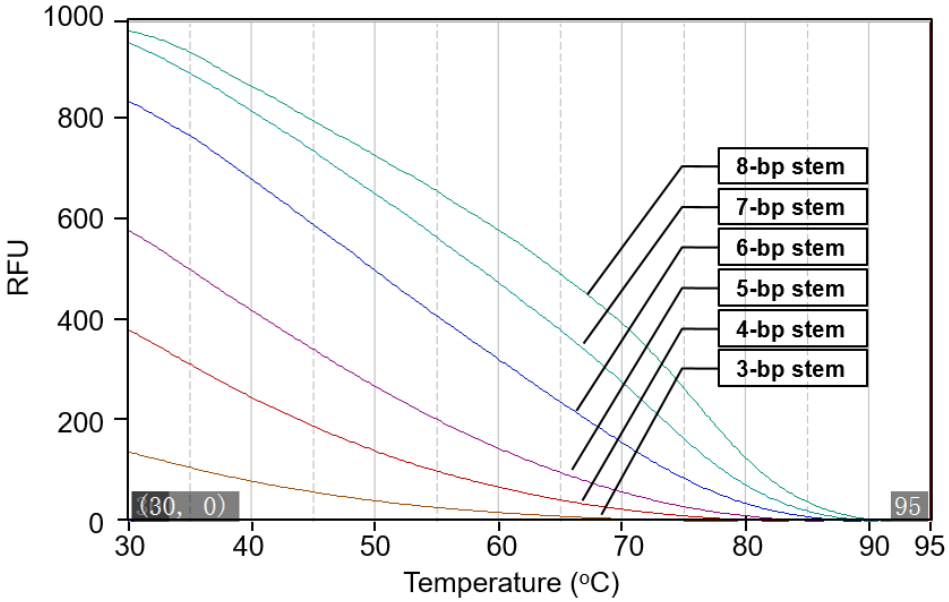
Supplementary Figure S1. T_ms of DNA duplexes at various strand concentrations. DNA duplexes **GC3/15** (A) or **GC12/15** (B) of 0.1, 0.5, 1, 5 or 10 μM was measured in the solution with 1× EvaGreen, 10 mM phosphate (pH 7.4) and 100 mM NaCl.



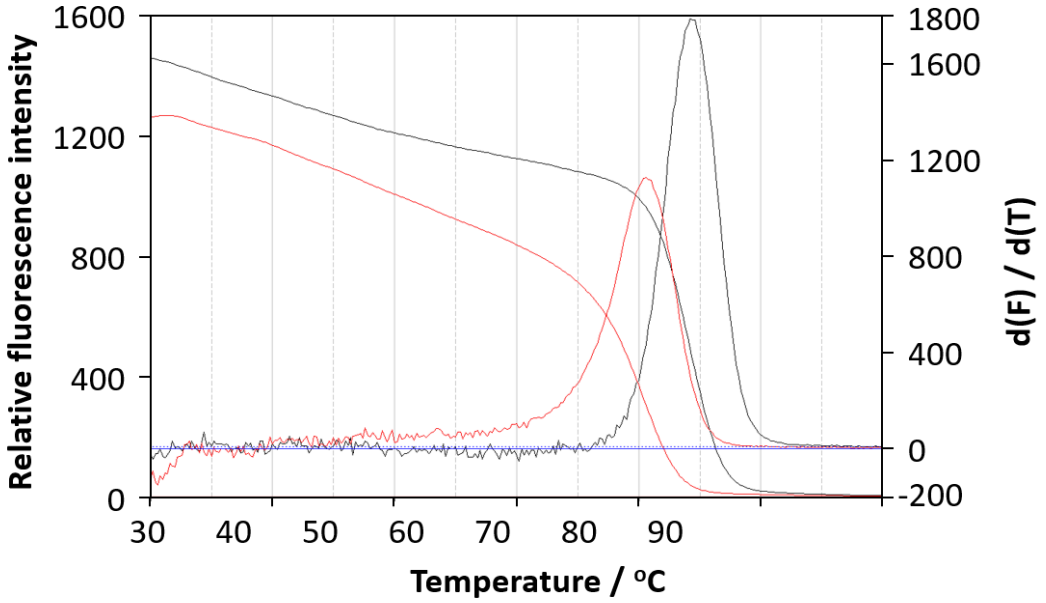
Supplementary Figure S2. Melting curves of **GC14/15** duplex, **GC14/15** duplex with additional F or R strand, and only F strand or R strand. In the assays for **GC14/15** or only F strand or R strand, the concentration of each strand was 1 μM; in the assays for **GC14/15** duplex with additional F or R strand, the concentration of **GC14/15** duplex or additional F or R strand was 1 μM.



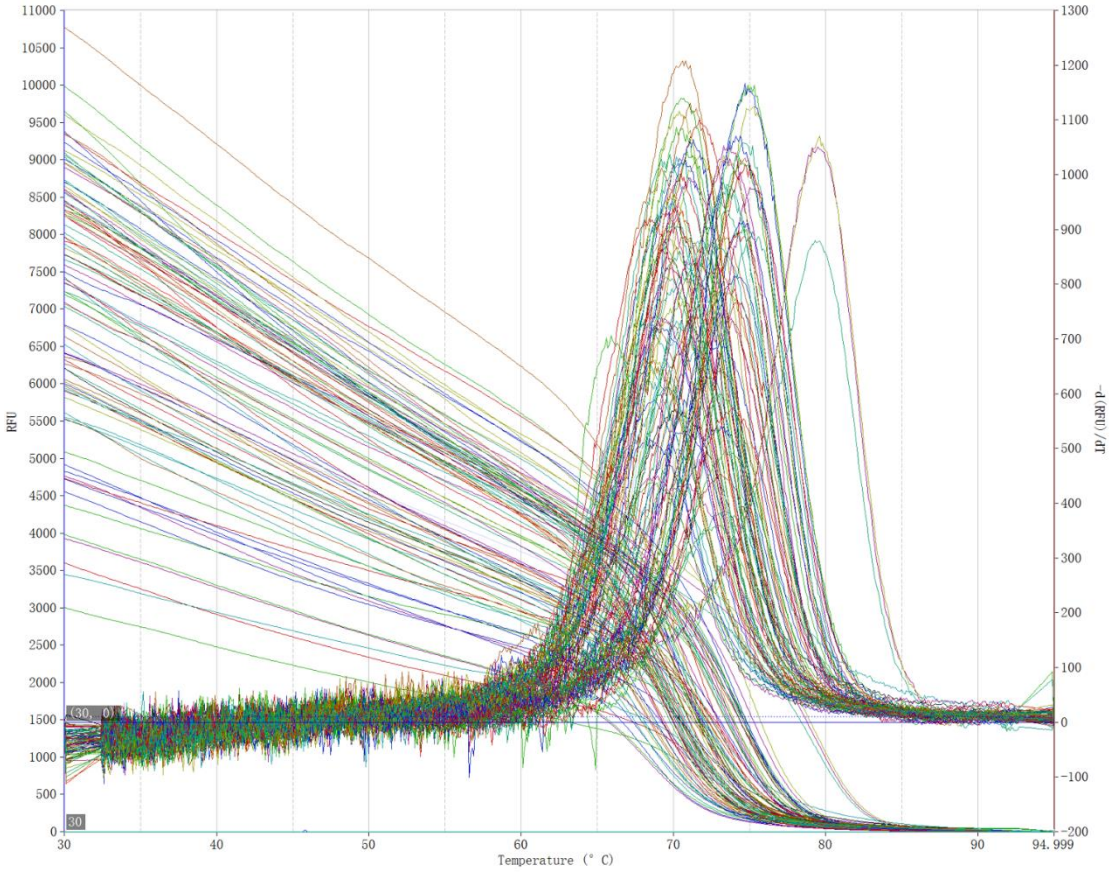
Supplementary Figure S3. Melting measurement of **GC3/15** mixed with 0.2 mM dNTPs by UV melting method.



Supplementary Figure S4. Melting curves and their first derivatives for hairpins with 3~8-bp stem obtained by HRM. The left Y-axis displays the detected relative fluorescence units (RFU), and the right Y-axis (-d(RFU)/dT) displays the negative of the first derivative of the melting curve.



Supplementary Figure S5. Fluorescence melting curves of 21-bp RNA duplex. The RNA duplex (**RR**, sequence in Table 1) was measured at 1 μM (red) and 10 μM (black) in a solution system with 1× EvaGreen, 10 mM phosphate buffer (pH 7.0), 0.5 mM Na_2-EDTA and 100 mM NaCl.



Supplementary Figure S6. Fluorescence melting curves and differential curves of 96 samples on a 96-well plate by HRM. All the data could be obtained in 1 to 2 h. The high throughput can be improved by using 384 well plates.