

Supporting Information

Effect of thiazole orange double-labeled thymidine on DNA duplex formation

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This work was supported by research grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) for RIKEN Omics Science Center to YH, strategic programs for R&D (President's Discretionary Fund) of RIKEN, and the Grant-in-Aid for Scientific Research for A.L.

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Table S1. Observed thermodynamic parameters derived from melting curve experiments with absorbance measurement.

ECHO sequence (5' to 3')	ΔH° [kcal mol ⁻¹]	ΔS° [cal mol ⁻¹ K ⁻¹]	ΔG°_{37} [kcal mol ⁻¹]	ΔG°_{60} [kcal mol ⁻¹]	T_M [°C]	$\Delta\Delta H^\circ$ [kcal mol ⁻¹]	$\Delta\Delta S^\circ$ [cal mol ⁻¹ K ⁻¹]	$\Delta\Delta G^\circ_{37}$ [kcal mol ⁻¹]	$\Delta\Delta G^\circ_{60}$ [kcal mol ⁻¹]	ΔT_M [°C]
11-mer full-match										
CCAAT ^E ACTACC	-73.5 ± 9.0	-196 ± 27.4	-12.7 ± 0.52	-8.2 ± 0.52	51.6 ± 0.18	4.9	23.4	-2.3	-2.8	10.6
CCAAT ^E CCTACC	-77.2 ± 5.7	-204 ± 17.1	-13.9 ± 0.41	-9.2 ± 0.41	56.2 ± 0.19	1.8	13.4	-2.4	-2.7	10.6
CCAAT ^E GCTACC	-75.9 ± 7.4	-199 ± 21.9	-14.3 ± 0.60	-9.7 ± 0.60	58.3 ± 0.15	5.2	23.8	-2.2	-2.8	10.7
CCAAT ^E TCTACC	-81.8 ± 7.3	-220 ± 22.0	-13.5 ± 0.44	-8.4 ± 0.44	53.4 ± 0.09	-2.9	-0.4	-2.8	-2.8	11.2
CCACT ^E ACTACC	-86.1 ± 8.0	-228 ± 23.2	-15.3 ± 0.84	-10.1 ± 0.84	60.1 ± 0.32	-6.6	-7.9	-4.1	-4.0	15.7
CCACT ^E CCTACC	-66.4 ± 3.2	-169 ± 9.6	-14.0 ± 0.26	-10.1 ± 0.26	60.3 ± 0.12	13.7	49.5	-1.7	-2.8	11.3
CCACT ^E GCTACC	-69.3 ± 3.0	-176 ± 9.5	-14.8 ± 0.22	-10.8 ± 0.22	63.5 ± 0.26	12.9	47.7	-1.9	-3.0	12.6
CCACT ^E TCTACC	-87.5 ± 3.3	-234 ± 9.3	-15.0 ± 0.47	-9.7 ± 0.47	58.5 ± 0.31	-7.5	-12.7	-3.6	-3.3	13.1
CCAGT ^E ACTACC	-80.0 ± 3.9	-212 ± 11.4	-14.2 ± 0.34	-9.3 ± 0.34	57.0 ± 0.08	-0.5	8.2	-3.0	-3.2	12.6
CCAGT ^E CCTACC	-79.5 ± 8.2	-209 ± 24.7	-14.8 ± 0.62	-10.0 ± 0.62	59.8 ± 0.22	0.6	9.9	-2.5	-2.7	10.9
CCAGT ^E GCTACC	-69.5 ± 5.4	-178 ± 16.2	-14.3 ± 0.38	-10.2 ± 0.38	60.9 ± 0.07	12.7	45.7	-1.5	-2.5	10.0
CCAGT ^E TCTACC	-63.9 ± 2.5	-166 ± 7.9	-12.5 ± 0.20	-8.6 ± 0.20	52.8 ± 0.27	16.1	55.0	-1.0	-2.2	7.4
CCATT ^E ACTACC	-74.4 ± 6.0	-199 ± 18.2	-12.6 ± 0.40	-8.0 ± 0.40	51.1 ± 0.08	4.0	20.0	-2.2	-2.7	10.1
CCATT ^E CCTACC	-83.3 ± 7.4	-221 ± 21.9	-14.7 ± 0.64	-9.6 ± 0.64	58.1 ± 0.20	-4.3	-3.7	-3.2	-3.1	12.5
CCATT ^E GCTACC	-73.5 ± 2.1	-191 ± 5.9	-14.2 ± 0.25	-9.8 ± 0.25	58.7 ± 0.13	7.6	31.6	-2.1	-2.9	11.2
CCATT ^E TCTACC	-74.3 ± 9.1	-197 ± 27.4	-13.3 ± 0.57	-8.8 ± 0.57	54.3 ± 0.06	4.6	23.3	-2.6	-3.2	12.1
11-mer mis-match T ^E •C										
CCAAT ^E ACTACC	-57.3 ± 5.2	-156 ± 15.9	-8.9 ± 0.29	-5.3 ± 0.29	34.5 ± 0.08	6.7	27.1	-1.7	-2.3	7.6
CCAAT ^E CCTACC	-67.6 ± 2.2	-186 ± 7.0	-9.9 ± 0.05	-5.6 ± 0.05	39.5 ± 0.15	-5.1	-10.3	-2.0	-1.7	9.3
CCAAT ^E GCTACC	-54.2 ± 3.5	-143 ± 10.8	-9.9 ± 0.19	-6.6 ± 0.19	40.1 ± 0.07	13.2	47.4	-1.5	-2.6	7.6
CCAAT ^E TCTACC	-73.0 ± 7.6	-204 ± 24.0	-9.8 ± 0.11	-5.1 ± 0.11	38.9 ± 0.44	-8.7	-20.2	-2.4	-2.0	11.1
CCACT ^E ACTACC	-74.4 ± 1.1	-205 ± 2.3	-10.8 ± 0.46	-6.1 ± 0.46	43.2 ± 0.41	-9.6	-21.8	-2.9	-2.3	12.7
CCACT ^E CCTACC	-75.6 ± 7.7	-206 ± 23.1	-11.7 ± 0.50	-6.9 ± 0.50	46.7 ± 0.05	-12.3	-30.0	-2.9	-2.3	12.8
CCACT ^E GCTACC	-67.9 ± 12.4	-182 ± 37.6	-11.4 ± 0.71	-7.2 ± 0.71	46.6 ± 0.21	0.3	8.3	-2.3	-2.5	10.6
CCACT ^E TCTACC	-75.6 ± 10.3	-208 ± 30.7	-11.0 ± 0.84	-6.2 ± 0.84	43.8 ± 0.28	-10.5	-24.6	-2.9	-2.3	12.3
CCAGT ^E ACTACC	-64.4 ± 6.9	-175 ± 22.3	-10.1 ± 0.08	-6.0 ± 0.08	40.5 ± 0.50	-6.9	-13.1	-2.8	-2.5	14.5
CCAGT ^E CCTACC	-70.1 ± 12.0	-190 ± 36.4	-11.1 ± 0.69	-6.7 ± 0.69	44.7 ± 0.16	-14.1	-35.4	-3.1	-2.3	15.1
CCAGT ^E GCTACC	-63.8 ± 9.9	-169 ± 30.6	-11.3 ± 0.43	-7.4 ± 0.43	46.5 ± 0.31	-2.9	-0.1	-2.9	-2.9	14.4
CCAGT ^E TCTACC	-61.0 ± 10.6	-165 ± 33.6	-9.8 ± 0.23	-6.0 ± 0.23	38.9 ± 0.57	-3.2	-2.8	-2.3	-2.3	12.0
CCATT ^E ACTACC	-65.5 ± 4.0	-178 ± 13.0	-10.2 ± 0.28	-6.1 ± 0.28	41.2 ± 0.44	-4.4	-4.0	-3.2	-3.1	15.5
CCATT ^E CCTACC	-78.9 ± 8.7	-217 ± 26.6	-11.6 ± 0.43	-6.6 ± 0.43	46.0 ± 0.22	-19.3	-50.1	-3.8	-2.6	16.8
CCATT ^E GCTACC	-66.2 ± 9.4	-176 ± 28.8	-11.6 ± 0.44	-7.5 ± 0.44	47.5 ± 0.24	-1.7	5.1	-3.3	-3.5	15.9
CCATT ^E TCTACC	-71.5 ± 6.9	-197 ± 21.5	-10.5 ± 0.28	-6.0 ± 0.28	42.0 ± 0.22	-10.1	-21.9	-3.3	-2.7	15.3
11-mer mis-match T ^E •G										
CCAAT ^E ACTACC	-55.5 ± 0.7	-147 ± 2.1	-9.9 ± 0.11	-6.5 ± 0.11	40.0 ± 0.08	11.1	40.7	-1.5	-2.4	7.3
CCAAT ^E CCTACC	-73.4 ± 3.3	-200 ± 10.0	-11.3 ± 0.18	-6.7 ± 0.18	45.4 ± 0.10	-10.6	-27.4	-2.1	-1.5	9.4
CCAAT ^E GCTACC	-73.0 ± 6.9	-198 ± 20.8	-11.6 ± 0.45	-7.1 ± 0.45	46.9 ± 0.04	-1.0	1.8	-1.5	-1.6	6.6
CCAAT ^E TCTACC	-75.5 ± 3.0	-209 ± 9.1	-10.5 ± 0.23	-5.7 ± 0.23	41.8 ± 0.11	-10.2	-24.6	-2.5	-2.0	11.2
CCACT ^E ACTACC	-72.3 ± 10.5	-192 ± 31.1	-12.6 ± 0.83	-8.2 ± 0.83	51.2 ± 0.17	-4.9	-5.0	-3.3	-3.2	14.8
CCACT ^E CCTACC	-80.6 ± 2.9	-218 ± 8.4	-13.0 ± 0.34	-8.0 ± 0.34	51.8 ± 0.20	-17.0	-44.9	-3.1	-2.0	11.7
CCACT ^E GCTACC	-65.0 ± 3.7	-168 ± 10.2	-12.8 ± 0.51	-9.0 ± 0.51	54.4 ± 0.27	7.8	31.1	-1.9	-2.6	10.6
CCACT ^E TCTACC	-69.3 ± 11.0	-187 ± 33.8	-11.4 ± 0.57	-7.1 ± 0.57	46.2 ± 0.21	-3.2	-1.9	-2.6	-2.5	11.8
CCAGT ^E ACTACC	-74.9 ± 5.1	-204 ± 16.3	-11.8 ± 0.02	-7.1 ± 0.02	47.5 ± 0.36	-6.5	-13.1	-2.5	-2.2	10.5
CCAGT ^E CCTACC	-74.4 ± 7.6	-200 ± 23.1	-12.3 ± 0.41	-7.7 ± 0.41	49.8 ± 0.16	-9.8	-24.5	-2.2	-1.7	9.2
CCAGT ^E GCTACC	-74.3 ± 8.5	-198 ± 24.9	-12.8 ± 0.74	-8.2 ± 0.74	51.9 ± 0.21	-0.5	4.0	-1.7	-1.8	7.7
CCAGT ^E TCTACC	-69.1 ± 2.0	-189 ± 5.9	-10.6 ± 0.39	-6.2 ± 0.39	42.6 ± 0.36	-2.0	-1.2	-1.7	-1.6	7.6
CCATT ^E ACTACC	-66.1 ± 4.3	-180 ± 13.5	-10.2 ± 0.19	-6.1 ± 0.19	41.1 ± 0.19	-1.4	2.6	-2.2	-2.3	10.5
CCATT ^E CCTACC	-69.1 ± 5.8	-185 ± 17.1	-11.6 ± 0.46	-7.4 ± 0.46	47.5 ± 0.08	-8.2	-17.2	-2.9	-2.5	13.5
CCATT ^E GCTACC	-68.6 ± 11.0	-183 ± 33.6	-11.9 ± 0.65	-7.7 ± 0.65	48.6 ± 0.20	1.5	11.8	-2.1	-2.4	10.1
CCATT ^E TCTACC	-75.7 ± 6.1	-210 ± 17.3	-10.5 ± 0.79	-5.6 ± 0.79	41.4 ± 0.44	-12.3	-30.5	-2.9	-2.2	12.8
11-mer mis-match T ^E •T										
CCAAT ^E ACTACC	-55.6 ± 3.5	-146 ± 11.0	-10.2 ± 0.27	-6.9 ± 0.27	41.9 ± 0.30	10.9	43.7	-2.7	-3.7	13.2
CCAAT ^E CCTACC	-72.0 ± 3.8	-197 ± 12.4	-11.0 ± 0.14	-6.5 ± 0.14	44.3 ± 0.33	-3.6	-2.5	-2.8	-2.8	12.6
CCAAT ^E GCTACC	-69.2 ± 7.4	-186 ± 22.3	-11.6 ± 0.51	-7.3 ± 0.51	47.3 ± 0.02	3.9	20.4	-2.5	-2.9	11.3
CCAAT ^E TCTACC	-74.0 ± 1.9	-205 ± 5.8	-10.6 ± 0.12	-5.9 ± 0.12	42.1 ± 0.06	-4.9	-5.6	-3.2	-3.0	13.7
CCACT ^E ACTACC	-81.5 ± 8.5	-220 ± 26.0	-13.1 ± 0.47	-8.0 ± 0.47	51.8 ± 0.16	-12.2	-25.4	-4.3	-3.7	17.4
CCACT ^E CCTACC	-73.4 ± 2.8	-196 ± 7.7	-12.7 ± 0.41	-8.3 ± 0.41	52.0 ± 0.26	-2.1	4.0	-3.3	-3.4	14.7
CCACT ^E GCTACC	-73.2 ± 2.9	-194 ± 9.1	-13.0 ± 0.10	-8.5 ± 0.10	53.2 ± 0.11	2.7	17.1	-2.6	-3.0	12.0
CCACT ^E TCTACC	-80.0 ± 6.7	-219 ± 20.4	-11.9 ± 0.35	-6.8 ± 0.35	47.1 ± 0.12	-8.0	-15.2	-3.2	-2.9	13.2
CCAGT ^E ACTACC	-67.2 ± 8.0	-180 ± 24.6	-11.3 ± 0.35	-7.2 ± 0.35	46.2 ± 0.26	-1.3	6.4	-3.2	-3.4	15.2
CCAGT ^E CCTACC	-74.6 ± 8.1	-202 ± 24.5	-11.9 ± 0.53	-7.3 ± 0.53	48.0 ± 0.15	-6.8	-11.5	-3.2	-3.0	14.0
CCAGT ^E GCTACC	-71.6 ± 9.6	-191 ± 29.2	-12.4 ± 0.58	-8.0 ± 0.58	50.6 ± 0.10	0.9	11.7	-2.7	-3.0	12.3
CCAGT ^E TCTACC	-60.3 ± 3.7	-162 ± 12.0	-10.2 ± 0.07	-6.5 ± 0.07	41.3 ± 0.33	8.2	33.8	-2.3	-3.0	10.7
CCATT ^E ACTACC	-75.0 ± 13.0	-206 ± 40.1	-11.2 ± 0.59	-6.5 ± 0.59	44.6 ± 0.38	-12.1	-26.9	-3.8	-3.1	16.8
CCATT ^E CCTACC	-73.0 ± 5.3	-197 ± 16.7	-11.9 ± 0.10	-7.4 ± 0.10	48.2 ± 0.29	-8.1	-13.6	-3.8	-3.5	17.3
CCATT ^E GCTACC	-75.6 ± 12.7	-204 ± 38.4	-12.4 ± 0.84	-7.7 ± 0.84	49.9 ± 0.06	-6.1	-8.9	-3.4	-3.2	14.4
CCATT ^E TCTACC	-76.3 ± 3.6	-213 ± 12.8	-10.3 ± 0.46	-5.4 ± 0.46	40.9 ± 0.73	-10.7	-24.8	-3.0	-2.4	13.5
20-mer full-match										
AATATGCGGAC T ^E AAATATAC	-132.7 ± 5.0	-355 ± 15.9	-22.5 ± 0.18	-14.3 ± 0.18	71.0 ± 0.34	15.9	59.3	-2.5	-3.8	10.1
TTATGTGTGGT ^E CGCTTAATA	-135.7 ± 5.5	-359 ± 16.1	-24.3 ± 0.53	-16.0 ± 0.53	75.2 ± 0.17	14.9	53.5	-1.7	-2.9	8.5
ATTATGCTCCA AT ^E CATGTCG	-155.9 ± 3.9	-419 ± 11.0	-26.1 ± 0.53	-16.5 ± 0.53	74.4 ± 0.28	-3.3	0.1	-3.4	-3.4	7.5

Table S2. Observed thermodynamic parameters derived from melting curve experiments with fluorescence measurement.

ECHO sequence (5' to 3')	ΔH° [kcal mol ⁻¹]	ΔS° [cal mol ⁻¹ K ⁻¹]	ΔG_{37}° [kcal mol ⁻¹]	ΔG_{60}° [kcal mol ⁻¹]	T_M [°C]	$\Delta\Delta H^\circ$ [kcal mol ⁻¹]	$\Delta\Delta S^\circ$ [cal mol ⁻¹ K ⁻¹]	$\Delta\Delta G_{37}^\circ$ [kcal mol ⁻¹]	$\Delta\Delta G_{60}^\circ$ [kcal mol ⁻¹]	ΔT_M [°C]
11-mer full-match										
CCAAT ^E ACTACC	-92.2 ± 1.2	-251 ± 3.5	-14.4 ± 0.09	-8.7 ± 0.09	55.1 ± 0.01	-13.8	-31.3	-4.1	-3.4	14.1
CCAAT ^E CCTACC	-96.7 ± 1.2	-261 ± 3.4	-15.9 ± 0.11	-9.9 ± 0.11	59.5 ± 0.03	-17.7	-43.0	-4.4	-3.4	13.9
CCAAT ^E GCTACC	-95.5 ± 3.5	-256 ± 10.3	-16.2 ± 0.34	-10.3 ± 0.34	60.8 ± 0.10	-14.4	-33.2	-4.1	-3.4	13.2
CCAAT ^E TCTACC	-86.6 ± 1.1	-234 ± 3.4	-14.0 ± 0.10	-8.6 ± 0.10	54.4 ± 0.02	-7.7	-14.1	-3.3	-3.0	12.3
CCACT ^E ACTACC	-96.1 ± 1.2	-257 ± 3.5	-16.5 ± 0.10	-10.6 ± 0.10	61.9 ± 0.04	-16.6	-36.5	-5.3	-4.5	17.5
CCACT ^E CCTACC	-95.7 ± 1.9	-253 ± 5.5	-17.3 ± 0.21	-11.5 ± 0.21	65.2 ± 0.09	-15.6	-34.1	-5.0	-4.2	16.2
CCACT ^E GCTACC	-93.4 ± 0.2	-244 ± 0.6	-17.8 ± 0.02	-12.2 ± 0.02	67.7 ± 0.03	-11.2	-20.5	-4.9	-4.4	16.8
CCACT ^E TCTACC	-91.2 ± 1.0	-243 ± 3.0	-15.7 ± 0.09	-10.1 ± 0.09	60.3 ± 0.02	-11.2	-22.3	-4.2	-3.7	14.9
CCAGT ^E ACTACC	-95.3 ± 1.2	-257 ± 3.5	-15.8 ± 0.10	-9.9 ± 0.10	59.3 ± 0.02	-15.8	-36.3	-4.6	-3.7	15.0
CCAGT ^E CCTACC	-92.5 ± 0.7	-245 ± 2.1	-16.4 ± 0.06	-10.7 ± 0.06	62.5 ± 0.04	-12.4	-26.8	-4.1	-3.4	13.6
CCAGT ^E GCTACC	-93.5 ± 1.4	-246 ± 4.1	-17.0 ± 0.13	-11.4 ± 0.13	64.8 ± 0.04	-11.3	-22.9	-4.2	-3.6	13.9
CCAGT ^E TCTACC	-80.4 ± 1.3	-214 ± 4.0	-14.0 ± 0.11	-9.1 ± 0.11	56.1 ± 0.02	-0.4	6.9	-2.5	-2.7	10.6
CCATT ^E ACTACC	-88.6 ± 1.8	-240 ± 5.3	-14.2 ± 0.12	-8.7 ± 0.12	54.9 ± 0.04	-10.2	-20.4	-3.8	-3.4	13.9
CCATT ^E CCTACC	-89.6 ± 3.3	-240 ± 9.5	-15.0 ± 0.29	-9.5 ± 0.29	57.8 ± 0.07	-10.6	-22.8	-3.5	-3.0	12.2
CCATT ^E GCTACC	-98.7 ± 1.9	-264 ± 5.7	-16.8 ± 0.17	-10.8 ± 0.17	62.4 ± 0.05	-17.6	-41.3	-4.8	-3.8	14.8
CCATT ^E TCTACC	-85.1 ± 2.1	-229 ± 6.1	-14.0 ± 0.18	-8.8 ± 0.18	55.0 ± 0.04	-6.2	-8.9	-3.4	-3.2	12.9
11-mer mis-match T ^E •C										
CCAAT ^E ACTACC	-71.5 ± 0.8	-199 ± 2.3	-9.8 ± 0.05	-5.2 ± 0.05	39.0 ± 0.04	-7.5	-15.7	-2.6	-2.2	12.1
CCAAT ^E CCTACC	-72.4 ± 3.9	-199 ± 12.0	-10.7 ± 0.21	-6.1 ± 0.21	42.8 ± 0.07	-9.9	-22.9	-2.7	-2.2	12.6
CCAAT ^E GCTACC	-71.8 ± 10.8	-194 ± 32.1	-11.5 ± 0.91	-7.1 ± 0.91	46.4 ± 0.26	-4.4	-4.0	-3.2	-3.1	13.9
CCAAT ^E TCTACC	-63.3 ± 3.2	-174 ± 9.9	-9.3 ± 0.13	-5.3 ± 0.13	36.7 ± 0.12	1.0	9.3	-1.9	-2.1	8.9
CCACT ^E ACTACC	-80.8 ± 4.0	-222 ± 12.1	-12.0 ± 0.25	-6.9 ± 0.25	47.5 ± 0.06	-16.0	-38.6	-4.0	-3.2	17.0
CCACT ^E CCTACC	-84.6 ± 3.8	-233 ± 11.7	-12.5 ± 0.21	-7.2 ± 0.21	49.0 ± 0.06	-21.3	-56.6	-3.8	-2.5	15.1
CCACT ^E GCTACC	-82.1 ± 5.2	-223 ± 15.6	-12.9 ± 0.39	-7.7 ± 0.39	50.7 ± 0.05	-13.9	-32.9	-3.7	-3.0	14.8
CCACT ^E TCTACC	-77.8 ± 4.1	-213 ± 12.2	-11.7 ± 0.27	-6.8 ± 0.27	46.7 ± 0.07	-12.7	-29.4	-3.6	-2.9	15.3
CCAGT ^E ACTACC	-73.6 ± 3.5	-200 ± 10.4	-11.8 ± 0.29	-7.2 ± 0.29	47.4 ± 0.05	-16.1	-37.5	-4.5	-3.7	21.4
CCAGT ^E CCTACC	-69.8 ± 0.7	-186 ± 1.9	-12.1 ± 0.07	-7.8 ± 0.07	49.7 ± 0.04	-13.8	-31.2	-4.1	-3.4	20.2
CCAGT ^E GCTACC	-79.7 ± 6.3	-214 ± 18.8	-13.2 ± 0.48	-8.3 ± 0.48	52.6 ± 0.18	-18.8	-45.0	-4.8	-3.8	20.5
CCAGT ^E TCTACC	-65.3 ± 0.5	-175 ± 1.5	-11.0 ± 0.14	-7.0 ± 0.14	45.1 ± 0.14	-7.5	-12.5	-3.6	-3.3	18.1
CCATT ^E ACTACC	-71.1 ± 8.9	-194 ± 27.2	-10.8 ± 0.48	-6.4 ± 0.48	43.4 ± 0.18	-10.0	-20.0	-3.8	-3.3	17.6
CCATT ^E CCTACC	-71.7 ± 1.7	-195 ± 5.1	-11.4 ± 0.20	-6.9 ± 0.20	46.0 ± 0.12	-12.1	-27.6	-3.6	-2.9	16.8
CCATT ^E GCTACC	-80.0 ± 2.4	-215 ± 8.0	-13.2 ± 0.08	-8.3 ± 0.08	52.8 ± 0.26	-15.5	-33.7	-5.0	-4.3	21.3
CCATT ^E TCTACC	-70.0 ± 2.0	-192 ± 6.3	-10.5 ± 0.07	-6.1 ± 0.07	42.1 ± 0.09	-8.6	-17.3	-3.2	-2.8	15.4
11-mer mis-match T ^E •G										
CCAAT ^E ACTACC	-81.0 ± 4.8	-224 ± 14.6	-11.5 ± 0.22	-6.4 ± 0.22	45.5 ± 0.11	-14.4	-36.3	-3.1	-2.3	12.9
CCAAT ^E CCTACC	-82.5 ± 0.9	-226 ± 2.9	-12.4 ± 0.05	-7.2 ± 0.05	49.0 ± 0.03	-19.7	-53.1	-3.3	-2.0	13.0
CCAAT ^E GCTACC	-87.6 ± 2.2	-240 ± 6.5	-13.1 ± 0.15	-7.6 ± 0.15	51.0 ± 0.00	-15.6	-40.6	-3.0	-2.1	10.7
CCAAT ^E TCTACC	-77.7 ± 0.9	-216 ± 2.9	-10.8 ± 0.04	-5.8 ± 0.04	42.8 ± 0.04	-12.4	-31.0	-2.8	-2.1	12.2
CCACT ^E ACTACC	-90.8 ± 0.2	-245 ± 0.6	-14.8 ± 0.02	-9.2 ± 0.02	56.7 ± 0.02	-23.4	-57.5	-5.6	-4.2	20.3
CCACT ^E CCTACC	-88.4 ± 1.0	-239 ± 3.0	-14.3 ± 0.07	-8.8 ± 0.07	55.2 ± 0.00	-24.8	-66.3	-4.3	-2.7	15.1
CCACT ^E GCTACC	-100.1 ± 4.2	-271 ± 12.3	-16.0 ± 0.35	-9.7 ± 0.35	58.9 ± 0.07	-27.3	-71.8	-5.0	-3.4	15.1
CCACT ^E TCTACC	-84.8 ± 1.4	-231 ± 4.2	-13.1 ± 0.08	-7.8 ± 0.08	51.3 ± 0.03	-18.7	-46.6	-4.3	-3.2	16.8
CCAGT ^E ACTACC	-83.9 ± 1.4	-229 ± 4.0	-12.9 ± 0.12	-7.7 ± 0.12	50.8 ± 0.04	-15.5	-38.3	-3.6	-2.7	13.9
CCAGT ^E CCTACC	-81.1 ± 0.1	-218 ± 0.3	-13.4 ± 0.00	-8.4 ± 0.00	53.3 ± 0.01	-16.5	-42.4	-3.3	-2.3	12.7
CCAGT ^E GCTACC	-92.5 ± 1.7	-251 ± 5.0	-14.6 ± 0.13	-8.9 ± 0.13	55.8 ± 0.02	-18.7	-48.6	-3.6	-2.5	11.5
CCAGT ^E TCTACC	-72.8 ± 2.2	-198 ± 6.5	-11.5 ± 0.14	-6.9 ± 0.14	46.1 ± 0.02	-5.7	-10.2	-2.5	-2.3	11.1
CCATT ^E ACTACC	-81.6 ± 2.3	-225 ± 7.1	-11.7 ± 0.08	-6.5 ± 0.08	46.1 ± 0.09	-16.9	-42.6	-3.7	-2.7	15.5
CCATT ^E CCTACC	-77.8 ± 1.6	-211 ± 4.9	-12.3 ± 0.07	-7.5 ± 0.07	49.3 ± 0.04	-16.9	-43.0	-3.6	-2.6	15.3
CCATT ^E GCTACC	-88.6 ± 0.6	-242 ± 1.8	-13.6 ± 0.04	-8.1 ± 0.04	52.8 ± 0.05	-18.5	-47.0	-3.9	-2.8	14.2
CCATT ^E TCTACC	-77.3 ± 0.8	-214 ± 2.4	-10.9 ± 0.03	-6.0 ± 0.03	43.2 ± 0.04	-13.9	-34.3	-3.3	-2.5	14.6
11-mer mis-match T ^E •T										
CCAAT ^E ACTACC	-86.5 ± 0.8	-240 ± 2.3	-12.0 ± 0.06	-6.5 ± 0.06	46.8 ± 0.03	-20.0	-50.2	-4.5	-3.3	18.1
CCAAT ^E CCTACC	-82.0 ± 3.4	-225 ± 10.3	-12.1 ± 0.19	-6.9 ± 0.19	47.8 ± 0.06	-13.6	-31.0	-4.0	-3.2	16.1
CCAAT ^E GCTACC	-90.9 ± 1.7	-250 ± 4.9	-13.4 ± 0.15	-7.7 ± 0.15	51.6 ± 0.05	-17.8	-43.6	-4.3	-3.3	15.5
CCAAT ^E TCTACC	-75.8 ± 3.5	-210 ± 10.9	-10.8 ± 0.17	-5.9 ± 0.17	42.8 ± 0.08	-6.7	-10.9	-3.4	-3.1	14.4
CCACT ^E ACTACC	-93.2 ± 0.3	-255 ± 0.9	-14.3 ± 0.05	-8.4 ± 0.05	54.3 ± 0.04	-23.9	-59.4	-5.5	-4.1	19.9
CCACT ^E CCTACC	-91.9 ± 0.6	-250 ± 1.8	-14.5 ± 0.06	-8.7 ± 0.06	55.3 ± 0.02	-20.6	-50.0	-5.1	-3.9	18.1
CCACT ^E GCTACC	-94.5 ± 1.8	-256 ± 5.4	-15.0 ± 0.17	-9.1 ± 0.17	56.7 ± 0.05	-18.6	-45.1	-4.6	-3.6	15.5
CCACT ^E TCTACC	-83.9 ± 2.1	-230 ± 6.1	-12.6 ± 0.16	-7.3 ± 0.16	49.5 ± 0.05	-11.9	-25.6	-4.0	-3.4	15.6
CCAGT ^E ACTACC	-82.5 ± 3.0	-225 ± 8.8	-12.7 ± 0.25	-7.5 ± 0.25	50.0 ± 0.07	-16.6	-38.6	-4.6	-3.7	19.0
CCAGT ^E CCTACC	-79.2 ± 1.7	-214 ± 5.2	-13.0 ± 0.13	-8.1 ± 0.13	51.9 ± 0.07	-11.4	-23.0	-4.3	-3.8	18.0
CCAGT ^E GCTACC	-85.5 ± 1.2	-231 ± 3.3	-13.9 ± 0.15	-8.6 ± 0.15	54.2 ± 0.08	-13.0	-28.4	-4.2	-3.6	16.0
CCAGT ^E TCTACC	-70.5 ± 2.9	-191 ± 8.6	-11.3 ± 0.25	-6.9 ± 0.25	45.6 ± 0.07	-2.0	4.4	-3.4	-3.5	15.1
CCATT ^E ACTACC	-80.3 ± 3.7	-220 ± 11.4	-12.1 ± 0.19	-7.0 ± 0.19	47.8 ± 0.08	-17.4	-41.2	-4.6	-3.7	20.1
CCATT ^E CCTACC	-78.6 ± 4.2	-214 ± 12.7	-12.4 ± 0.30	-7.5 ± 0.30	49.4 ± 0.08	-13.7	-30.4	-4.3	-3.6	18.5
CCATT ^E GCTACC	-88.4 ± 0.4	-241 ± 1.1	-13.7 ± 0.08	-8.2 ± 0.08	53.0 ± 0.07	-18.9	-46.0	-4.7	-3.6	17.5
CCATT ^E TCTACC	-78.5 ± 5.9	-216 ± 17.9	-11.6 ± 0.32	-6.7 ± 0.32	46.2 ± 0.09	-12.9	-27.7	-4.3	-3.7	18.7

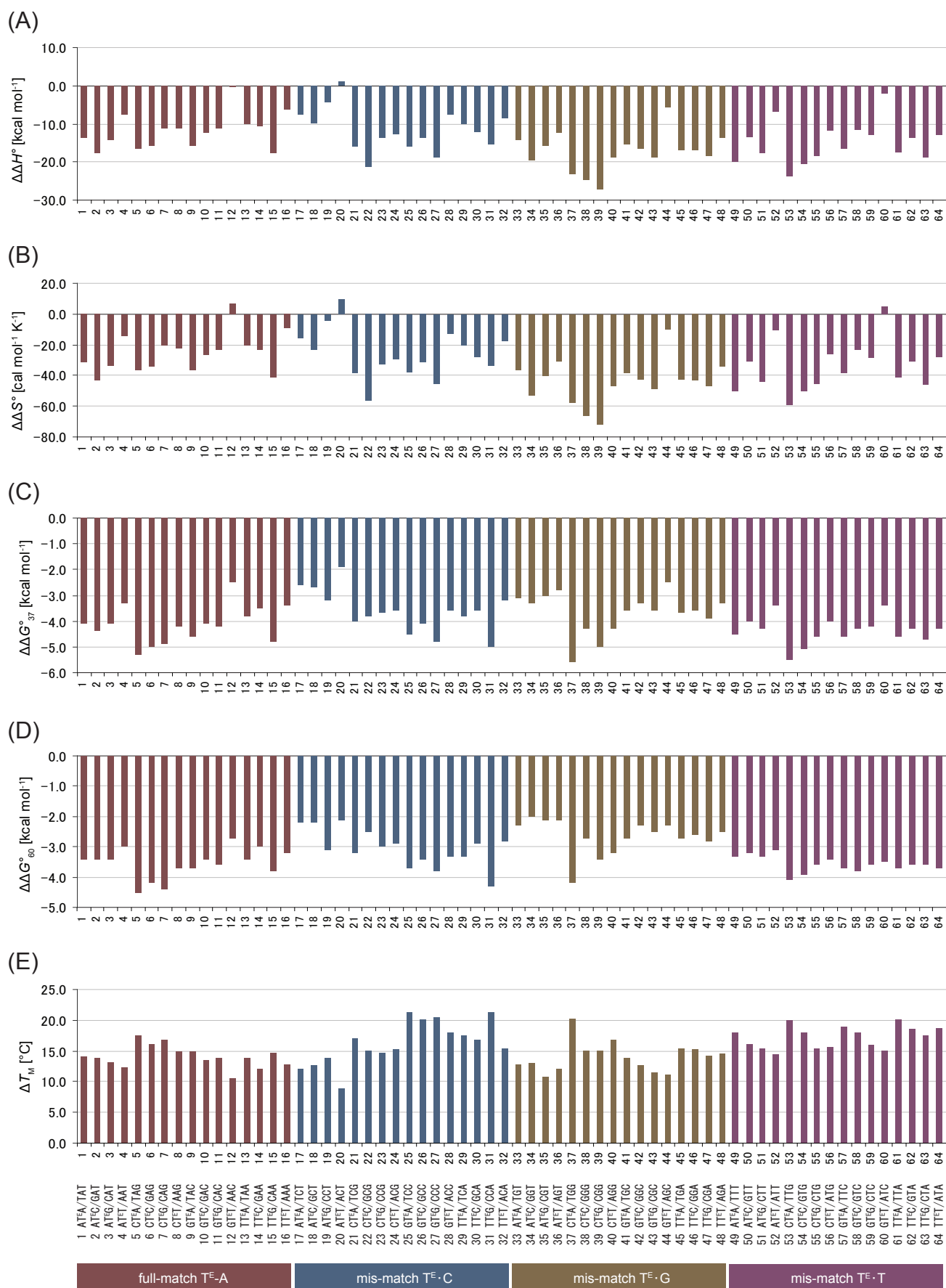


Figure S1.
Incremental thermodynamic effect of T^E obtained from fluorescence measurements. (A) $\Delta\Delta H^\circ$. (B) $\Delta\Delta S^\circ$. (C) $\Delta\Delta G^\circ_{37}$. (D) $\Delta\Delta G^\circ_{60}$. (E) ΔT_m .
Trinucleotide sequences around T^E are indicated in the figure.

Table S3. Average thermodynamic parameters derived from fluorescence measurements.

group	ΔH° [kcal mol ⁻¹]	ΔS° [cal mol ⁻¹ K ⁻¹]	ΔG°_{37} [kcal mol ⁻¹]	ΔG°_{60} [kcal mol ⁻¹]	T_M [°C]	$\Delta\Delta H^\circ$ [kcal mol ⁻¹]	$\Delta\Delta S^\circ$ [cal mol ⁻¹ K ⁻¹]	$\Delta\Delta G^\circ_{37}$ [kcal mol ⁻¹]	$\Delta\Delta G^\circ_{60}$ [kcal mol ⁻¹]	ΔT_M [°C]
full-match										
T ^E -A	-91.9 ± 4.9	-246 ± 12.8	-15.7 ± 1.3	-10.0 ± 1.1	59.8 ± 4.1	-12.0 ± 4.6	-25.5 ± 12.9	-4.1 ± 0.7	-3.5 ± 0.5	14.1 ± 1.8
mis-match										
T ^E •C	-74.1 ± 6.1	-202 ± 16.9	-11.5 ± 1.1	-6.9 ± 0.9	46.1 ± 4.5	-11.7 ± 5.6	-26.0 ± 16.1	-3.6 ± 0.8	-3.0 ± 0.6	16.3 ± 3.5
T ^E •G	-84.3 ± 6.8	-230 ± 17.8	-12.9 ± 1.5	-7.6 ± 1.2	50.5 ± 4.8	-17.4 ± 5.1	-44.3 ± 14.3	-3.7 ± 0.8	-2.7 ± 0.6	14.0 ± 2.4
T ^E •T	-83.9 ± 6.8	-229 ± 18.4	-12.8 ± 1.2	-7.6 ± 0.9	50.2 ± 3.9	-15.0 ± 5.5	-34.2 ± 16.1	-4.4 ± 0.6	-3.6 ± 0.3	17.2 ± 1.8
all										
T ^E •N	-83.6 ± 8.8	-227 ± 22.8	-13.2 ± 2.0	-8.0 ± 1.6	51.7 ± 6.6	-14.0 ± 5.6	-32.5 ± 16.5	-4.0 ± 0.8	-3.2 ± 0.6	15.4 ± 2.8

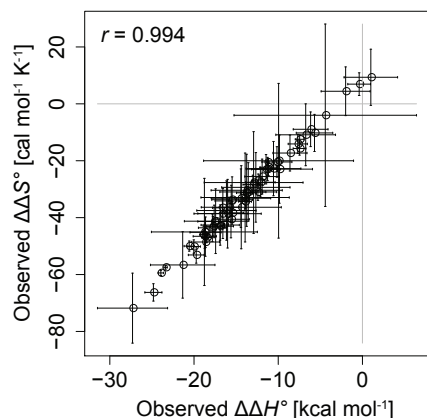


Figure S2.
Relationship of $\Delta\Delta H^\circ$ versus $\Delta\Delta S^\circ$ obtained from fluorescence measurements.

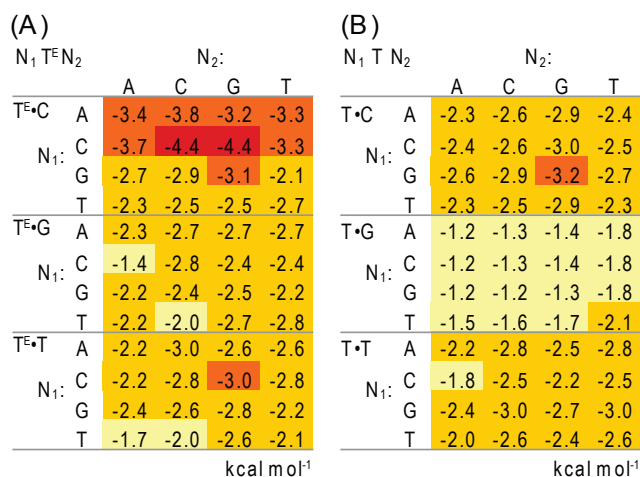


Figure S3.
Effect of mismatches on ΔG°_{60} . Differences in the free energies at 60 °C ($\Delta_{\text{full/mis}} \Delta G^\circ_{60}$) between full-match T^E-A and mismatches T^E•C, T^E•G, T^E•T were calculated and visualized in heat maps based on fluorescence measurements. (A) ECHO/DNA duplexes. (B) DNA/DNA duplexes. Red indicates larger changes whereas yellow indicates smaller effects on $\Delta_{\text{full/mis}} \Delta G^\circ_{60}$.

Table S4. Nearest neighbor parameters derived from fluorescence measurements.

nearest neighbor (5' to 3' / 5' to 3')	$\Delta\Delta H^\circ$ [kcal mol ⁻¹]	$\Delta\Delta S^\circ$ [cal mol ⁻¹ K ⁻¹]	$\Delta\Delta G_{37}^\circ$ [kcal mol ⁻¹]	$\Delta\Delta G_{60}^\circ$ [kcal mol ⁻¹]
full-match				
AT ^E /AT	-6.9 ± 0.8	-16.1 ± 1.3	-1.9 ± 0.2	-1.5 ± 0.1
CT ^E /AG	-6.9 ± 0.6	-13.4 ± 1.0	-2.7 ± 0.2	-2.4 ± 0.1
GT ^E /AC	-3.9 ± 0.7	-7.0 ± 1.2	-1.8 ± 0.2	-1.6 ± 0.1
TT ^E /AA	-5.7 ± 0.9	-12.5 ± 1.6	-1.8 ± 0.3	-1.6 ± 0.1
T ^E A/TA	-8.9 ± 0.7	-20.7 ± 1.3	-2.4 ± 0.2	-1.9 ± 0.1
T ^E C/GA	-8.9 ± 0.7	-21.3 ± 1.3	-2.3 ± 0.2	-1.8 ± 0.1
T ^E G/CA	-4.5 ± 0.6	-7.5 ± 1.0	-2.2 ± 0.2	-2.0 ± 0.1
T ^E T/AA	-1.2 ± 0.7	0.5 ± 1.2	-1.3 ± 0.2	-1.3 ± 0.1
mis-match T ^E •C				
AT ^E /CT	0.0 ± 1.0	2.5 ± 1.8	-0.8 ± 0.2	-0.8 ± 0.2
CT ^E /CG	-10.7 ± 1.3	-27.7 ± 2.3	-2.1 ± 0.3	-1.4 ± 0.2
GT ^E /CC	-6.7 ± 0.8	-13.8 ± 1.3	-2.5 ± 0.3	-2.1 ± 0.2
TT ^E /CA	-6.4 ± 1.0	-13.6 ± 1.8	-2.1 ± 0.2	-1.6 ± 0.2
T ^E A/TC	-7.5 ± 1.0	-18.1 ± 1.8	-1.8 ± 0.2	-1.6 ± 0.2
T ^E G/CC	-8.4 ± 1.3	-18.2 ± 2.4	-2.8 ± 0.3	-1.6 ± 0.2
T ^E C/GC	-7.1 ± 0.8	-17.4 ± 1.4	-1.7 ± 0.3	-1.3 ± 0.2
T ^E T/AC	-0.8 ± 0.8	1.1 ± 1.4	-1.1 ± 0.2	-1.4 ± 0.2
mis-match T ^E •G				
AT ^E /GT	-7.9 ± 0.7	-20.9 ± 1.3	-1.4 ± 0.2	-0.9 ± 0.1
CT ^E /GG	-14.0 ± 0.6	-35.6 ± 1.1	-3.0 ± 0.2	-1.8 ± 0.1
GT ^E /GC	-5.6 ± 0.5	-13.0 ± 0.9	-1.6 ± 0.1	-1.3 ± 0.1
TT ^E /GA	-8.2 ± 0.7	-20.4 ± 1.2	-1.8 ± 0.1	-1.5 ± 0.1
T ^E A/TG	-9.4 ± 0.6	-21.9 ± 1.1	-2.5 ± 0.2	-2.1 ± 0.1
T ^E C/GG	-10.9 ± 0.5	-29.4 ± 0.9	-1.7 ± 0.1	-1.0 ± 0.1
T ^E G/CG	-10.5 ± 0.7	-27.3 ± 1.3	-2.1 ± 0.2	-1.2 ± 0.1
T ^E T/AG	-4.9 ± 0.7	-11.2 ± 1.2	-1.4 ± 0.1	-1.1 ± 0.1
mis-match T ^E •T				
AT ^E /TT	-7.5 ± 0.8	-18.5 ± 1.3	-1.8 ± 0.2	-1.4 ± 0.1
CT ^E /TG	-11.5 ± 0.6	-28.2 ± 1.0	-2.7 ± 0.2	-2.0 ± 0.1
GT ^E /TC	-3.0 ± 0.9	-3.6 ± 1.5	-1.9 ± 0.3	-1.8 ± 0.2
TT ^E /TA	-9.1 ± 0.8	-21.7 ± 1.3	-2.3 ± 0.3	-1.9 ± 0.2
T ^E A/TT	-12.4 ± 0.6	-31.3 ± 1.1	-2.7 ± 0.2	-2.0 ± 0.1
T ^E C/GT	-8.8 ± 0.7	-21.1 ± 1.3	-2.3 ± 0.2	-1.9 ± 0.1
T ^E G/CT	-9.8 ± 0.7	-24.1 ± 1.2	-2.3 ± 0.2	-1.7 ± 0.2
T ^E T/AT	-0.1 ± 1.1	4.4 ± 1.9	-1.5 ± 0.3	-1.6 ± 0.2