

**S2 Table. Thermodynamic parameters used for nearest neighbor parameters for mismatch next to labelled thymidine.**

Mismatch No	Trinucleotide type	ECHO sequence (5' to 3' / 5' to 3')	Antisense DNA sequence (5' to 3')	Observed thermodynamic parameters for ECHO/DNA					
				$\Delta H^\circ$ [kcal mol <sup>-1</sup> ]	$\Delta S^\circ$ [cal mol <sup>-1</sup> K <sup>-1</sup> ]	$\Delta G^\circ_{37}$ [kcal mol <sup>-1</sup> ]	$\Delta G^\circ_{60}$ [kcal mol <sup>-1</sup> ]	$T_M$ [°C]	
1	E16/17_A	GZA/TAA	TGTGZATCTTCTCTTCTC	5.91	-137.6 ± 0.86	-375.6 ± 2.53	-21.1 ± 0.07	-12.5 ± 0.02	65.9 ± 0.03
2	E16/17_G	GZA/TAG	TGTGZATCTTCTCTTCTC	5.91	-137.2 ± 1.07	-373.8 ± 3.19	-21.3 ± 0.09	-12.7 ± 0.03	66.4 ± 0.07
3	E16/17_T	GZA/TAT	TGTGZATCTTCTCTTCTC	5.91	-146.8 ± 3.65	-401.9 ± 10.79	-22.1 ± 0.31	-12.9 ± 0.08	66.5 ± 0.14
4	E14/15_A	AZC/GAA	TGTGTAZCTTCTCTTCTC	5.91	-124.9 ± 0.79	-337.9 ± 2.28	-20.1 ± 0.08	-12.4 ± 0.04	66.3 ± 0.07
5	E14/15_C	AZC/GAC	TGTGTAZCTTCTCTTCTC	5.91	-120.2 ± 1.82	-323.0 ± 5.31	-20.1 ± 0.17	-12.6 ± 0.06	67.3 ± 0.09
6	E14/15_G	AZC/GAG	TGTGTAZCTTCTCTTCTC	5.91	-127.6 ± 2.22	-343.7 ± 6.35	-21.0 ± 0.26	-13.1 ± 0.12	68.0 ± 0.23
7	E12/13_A	CZT/AAA	TGTGATCZTCTCTTCTC	5.91	-125.9 ± 0.24	-343.3 ± 0.75	-19.4 ± 0.01	-11.5 ± 0.00	63.8 ± 0.02
8	E12/13_C	CZT/AAC	TGTGATCZTCTCTTCTC	5.91	-104.7 ± 9.15	-282.1 ± 26.68	-17.2 ± 0.87	-10.7 ± 0.26	61.9 ± 0.69
9	E12/13_T	CZT/AAT	TGTGATCZTCTCTTCTC	5.91	-111.2 ± 8.35	-301.6 ± 24.36	-17.7 ± 0.79	-10.7 ± 0.23	61.9 ± 0.60
10	E10/11_C	TZC/GAC	TGTGATCTZCTCTTCTC	5.91	-127.2 ± 2.40	-344.9 ± 6.79	-20.2 ± 0.31	-12.3 ± 0.16	65.9 ± 0.36
11	E10/11_G	TZC/GAG	TGTGATCTZCTCTTCTC	5.91	-134.8 ± 1.88	-364.7 ± 5.25	-21.7 ± 0.25	-13.3 ± 0.14	68.1 ± 0.24
12	E10/11_T	TZC/GAT	TGTGATCTZCTCTTCTC	5.91	-134.0 ± 2.03	-364.2 ± 6.02	-21.1 ± 0.18	-12.7 ± 0.08	66.7 ± 0.19
13	E18/17_A	GZG/AAC	TGZGATCTTCTCTTCTC	5.91	-152.9 ± 7.43	-421.6 ± 21.61	-22.2 ± 0.73	-12.5 ± 0.24	65.4 ± 0.29
14	E18/17_G	GZG/GAC	TGZGATCTTCTCTTCTC	5.91	-145.1 ± 0.69	-398.6 ± 1.98	-21.5 ± 0.08	-12.3 ± 0.04	65.3 ± 0.09
15	E18/17_T	GZG/TAC	TGZGATCTTCTCTTCTC	5.91	-166.8 ± 14.36	-461.0 ± 41.97	-23.9 ± 1.34	-13.2 ± 0.38	66.5 ± 0.22
16	E16/15_A	GZA/AAC	TGTGATCTTCTCTTCTC	5.91	-123.3 ± 1.36	-333.2 ± 4.03	-20.0 ± 0.11	-12.3 ± 0.04	66.1 ± 0.10
17	E16/15_C	GZA/CAC	TGTGATCTTCTCTTCTC	5.91	-113.4 ± 0.61	-302.6 ± 1.93	-19.6 ± 0.08	-12.6 ± 0.08	67.7 ± 0.27
18	E16/15_G	GZA/GAC	TGTGATCTTCTCTTCTC	5.91	-131.3 ± 1.29	-354.8 ± 3.78	-21.3 ± 0.13	-13.1 ± 0.05	68.0 ± 0.09
19	E14/13_A	AZC/AAT	TGTGATZCTTCTCTTCTC	5.91	-116.8 ± 11.20	-318.0 ± 32.63	-18.2 ± 1.08	-10.8 ± 0.33	62.2 ± 0.79
20	E14/13_C	AZC/CAT	TGTGATZCTTCTCTTCTC	5.91	-90.1 ± 0.00	-237.4 ± 0.00	-16.4 ± 0.00	-11.0 ± 0.00	63.4 ± 0.00
21	E14/13_T	AZC/TAT	TGTGATZCTTCTCTTCTC	5.91	-121.2 ± 4.59	-329.4 ± 13.62	-19.1 ± 0.37	-11.5 ± 0.05	63.9 ± 0.00
22	E12/11_C	CZT/CAG	TGTGATCZTCTCTTCTC	5.91	-111.3 ± 1.70	-297.5 ± 5.17	-19.0 ± 0.10	-12.2 ± 0.03	66.5 ± 0.17
23	E12/11_G	CZT/GAG	TGTGATCZTCTCTTCTC	5.91	-129.8 ± 1.99	-351.4 ± 5.80	-20.8 ± 0.20	-12.7 ± 0.08	67.0 ± 0.14
24	E12/11_T	CZT/TAG	TGTGATCZTCTCTTCTC	5.91	-125.9 ± 1.62	-339.8 ± 4.74	-20.5 ± 0.15	-12.7 ± 0.05	67.2 ± 0.09

Mismatch No	Thermodynamic parameters of normal DNA					Positional effect				
	$\Delta H^\circ$ [kcal mol <sup>-1</sup> ]	$\Delta S^\circ$ [cal mol <sup>-1</sup> K <sup>-1</sup> ]	$\Delta G^\circ_{37}$ [kcal mol <sup>-1</sup> ]	$\Delta G^\circ_{60}$ [kcal mol <sup>-1</sup> ]	$\Delta T_M$ [°C]	$\Delta \Delta H^\circ$ [kcal mol <sup>-1</sup> ]	$\Delta \Delta S^\circ$ [cal mol <sup>-1</sup> K <sup>-1</sup> ]	$\Delta \Delta G^\circ_{37}$ [kcal mol <sup>-1</sup> ]	$\Delta \Delta G^\circ_{60}$ [kcal mol <sup>-1</sup> ]	
1	E16/17_A	-134.80	-379.46	-17.11	-8.38	55.91	17.3 ± 4.66	52.3 ± 13.55	1.1 ± 0.49	-0.1 ± 0.23
2	E16/17_G	-139.10	-391.49	-17.68	-8.68	56.72	19.6 ± 4.39	58.7 ± 12.64	1.4 ± 0.53	0.1 ± 0.31
3	E16/17_T	-139.80	-394.36	-17.49	-8.42	56.14	20.7 ± 3.52	62.8 ± 10.03	1.3 ± 0.46	-0.2 ± 0.29
4	E14/15_A	-133.90	-373.30	-18.12	-9.53	58.70	11.7 ± 9.41	31.2 ± 28.10	2.0 ± 0.72	1.3 ± 0.20
5	E14/15_C	-131.00	-365.21	-17.73	-9.33	58.16	11.2 ± 4.75	30.0 ± 14.02	1.9 ± 0.46	1.2 ± 0.23
6	E14/15_G	-139.70	-389.23	-18.98	-10.03	59.93	15.8 ± 7.79	45.5 ± 23.20	1.6 ± 0.64	0.6 ± 0.24
7	E12/13_A	-128.20	-361.47	-16.09	-7.78	54.17	5.2 ± 8.06	13.3 ± 24.19	1.1 ± 0.65	0.8 ± 0.35
8	E12/13_C	-132.00	-374.27	-15.92	-7.31	53.21	19.3 ± 19.12	55.1 ± 56.84	2.2 ± 1.55	1.0 ± 0.48
9	E12/13_T	-138.10	-391.75	-16.60	-7.59	54.15	10.7 ± 11.03	31.7 ± 33.00	0.9 ± 0.85	0.2 ± 0.31
10	E10/11_C	-136.60	-384.04	-17.49	-8.66	56.62	8.3 ± 8.71	24.3 ± 25.66	0.8 ± 0.78	0.2 ± 0.27
11	E10/11_G	-138.60	-389.39	-17.83	-8.87	57.18	12.1 ± 6.97	36.3 ± 20.92	0.8 ± 0.55	0.0 ± 0.26
12	E10/11_T	-140.80	-397.52	-17.51	-8.37	56.05	13.9 ± 9.39	42.5 ± 27.79	0.7 ± 0.80	-0.3 ± 0.26
13	E18/17_A	-134.80	-379.46	-17.11	-8.38	55.91	17.3 ± 4.66	52.3 ± 13.55	1.1 ± 0.49	-0.1 ± 0.23
14	E18/17_G	-139.10	-391.49	-17.68	-8.68	56.72	19.6 ± 4.39	58.7 ± 12.64	1.4 ± 0.53	0.1 ± 0.31
15	E18/17_T	-139.80	-394.36	-17.49	-8.42	56.14	20.7 ± 3.52	62.8 ± 10.03	1.3 ± 0.46	-0.2 ± 0.29
16	E16/15_A	-133.90	-373.30	-18.12	-9.53	58.70	11.7 ± 9.41	31.2 ± 28.10	2.0 ± 0.72	1.3 ± 0.20
17	E16/15_C	-131.00	-365.21	-17.73	-9.33	58.16	11.2 ± 4.75	30.0 ± 14.02	1.9 ± 0.46	1.2 ± 0.23
18	E16/15_G	-139.70	-389.23	-18.98	-10.03	59.93	15.8 ± 7.79	45.5 ± 23.20	1.6 ± 0.64	0.6 ± 0.24
19	E14/13_A	-128.20	-361.47	-16.09	-7.78	54.17	5.2 ± 8.06	13.3 ± 24.19	1.1 ± 0.65	0.8 ± 0.35
20	E14/13_C	-132.00	-374.27	-15.92	-7.31	53.21	19.3 ± 19.12	55.1 ± 56.84	2.2 ± 1.55	1.0 ± 0.48
21	E14/13_T	-138.10	-391.75	-16.60	-7.59	54.15	10.7 ± 11.03	31.7 ± 33.00	0.9 ± 0.85	0.2 ± 0.31
22	E12/11_C	-136.60	-384.04	-17.49	-8.66	56.62	8.3 ± 8.71	24.3 ± 25.66	0.8 ± 0.78	0.2 ± 0.27
23	E12/11_G	-138.60	-389.39	-17.83	-8.87	57.18	12.1 ± 6.97	36.3 ± 20.92	0.8 ± 0.55	0.0 ± 0.26
24	E12/11_T	-140.80	-397.52	-17.51	-8.37	56.05	13.9 ± 9.39	42.5 ± 27.79	0.7 ± 0.80	-0.3 ± 0.26

Mismatch No	Nearest Neighbor parameters of full-match					Nearest Neighbor parameters with mismatch					
	Nearest neighbor (5' to 3' / 5' to 3')	$\Delta \Delta H^\circ$ [kcal mol <sup>-1</sup> ]	$\Delta \Delta S^\circ$ [cal mol <sup>-1</sup> K <sup>-1</sup> ]	$\Delta \Delta G^\circ_{37}$ [kcal mol <sup>-1</sup> ]	$\Delta \Delta G^\circ_{60}$ [kcal mol <sup>-1</sup> ]	Nearest neighbor (5' to 3' / 5' to 3')	$\Delta \Delta H^\circ$ [kcal mol <sup>-1</sup> ]	$\Delta \Delta S^\circ$ [cal mol <sup>-1</sup> K <sup>-1</sup> ]	$\Delta \Delta G^\circ_{37}$ [kcal mol <sup>-1</sup> ]	$\Delta \Delta G^\circ_{60}$ [kcal mol <sup>-1</sup> ]	
1	E16/17_A	Z/A/TA	-8.9 ± 0.73	-20.7 ± 1.26	-2.4 ± 0.20	-1.9 ± 0.10	G/Z/AA	-11.2 ± 4.79	-27.7 ± 13.84	-2.6 ± 0.53	-2.0 ± 0.25
2	E16/17_G	Z/A/TA	-8.9 ± 0.73	-20.7 ± 1.26	-2.4 ± 0.20	-1.9 ± 0.10	G/Z/AG	-8.9 ± 4.58	-20.2 ± 13.10	-2.6 ± 0.57	-2.1 ± 0.33
3	E16/17_T	Z/A/TA	-8.9 ± 0.73	-20.7 ± 1.26	-2.4 ± 0.20	-1.9 ± 0.10	G/Z/AT	-18.9 ± 5.13	-49.7 ± 14.79	-3.5 ± 0.60	-2.3 ± 0.32
4	E14/15_A	Z/C/GA	-8.9 ± 0.73	-21.3 ± 1.25	-2.3 ± 0.22	-1.8 ± 0.14	A/Z/AA	6.2 ± 9.47	25.4 ± 28.22	-1.7 ± 0.76	-2.3 ± 0.24
5	E14/15_C	Z/C/GA	-8.9 ± 0.73	-21.3 ± 1.25	-2.3 ± 0.22	-1.8 ± 0.14	A/Z/AC	8.5 ± 5.14	33.6 ± 15.04	-1.9 ± 0.54	-2.7 ± 0.28
6	E14/15_G	Z/C/GA	-8.9 ± 0.73	-21.3 ± 1.25	-2.3 ± 0.22	-1.8 ± 0.14	A/Z/AG	5.3 ± 8.13	21.3 ± 24.08	-1.3 ± 0.72	-1.8 ± 0.30
7	E12/13_A	Z/T/AA	-1.2 ± 0.72	0.5 ± 1.24	-1.3 ± 0.21	-1.3 ± 0.11	C/Z/AA	-1.7 ± 8.10	4.4 ± 24.24	-3.0 ± 0.68	-3.1 ± 0.36
8	E12/13_C	Z/T/AA	-1.2 ± 0.72	0.5 ± 1.24	-1.3 ± 0.21	-1.3 ± 0.11	C/Z/AT	9.2 ± 21.21	36.6 ± 62.80	-2.1 ± 1.79	-3.0 ± 0.56
9	E12/13_T	Z/T/AA	-1.2 ± 0.72	0.5 ± 1.24	-1.3 ± 0.21	-1.3 ± 0.11	C/Z/AC	17.3 ± 13.85	58.0 ± 41.03	-0.6 ± 1.18	-2.0 ± 0.40
10	E10/11_C	Z/C/GA	-8.9 ± 0.73	-21.3 ± 1.25	-2.3 ± 0.22	-1.8 ± 0.14	T/Z/AC	10.0 ± 9.06	36.2 ± 26.58	-1.2 ± 0.87	-2.0 ± 0.35
11	E10/11_G	Z/C/GA	-8.9 ± 0.73	-21.3 ± 1.25	-2.3 ± 0.22	-1.8 ± 0.14	T/Z/AG	0.7 ± 7.26	9.7 ± 21.61	-2.3 ± 0.64	-2.6 ± 0.32
12	E10/11_T	Z/C/GA	-8.9 ± 0.73	-21.3 ± 1.25	-2.3 ± 0.22	-1.8 ± 0.14	T/Z/AT	1.8 ± 9.63	12.2 ± 28.46	-2.0 ± 0.85	-2.3 ± 0.31
13	E18/17_A	G/Z/AC	-3.9 ± 0.69	-7.0 ± 1.18	-1.8 ± 0.20	-1.6 ± 0.11	Z/G/AA	-31.5 ± 8.80	-87.4 ± 25.54	-4.4 ± 0.90	-2.4 ± 0.35
14	E18/17_G	G/Z/AC	-3.9 ± 0.69	-7.0 ± 1.18	-1.8 ± 0.20	-1.6 ± 0.11	Z/G/GA	-21.7 ± 4.50	-58.7 ± 12.85</		