

Electronic supplementary information

Enzymatic formation of consecutive thymine-Hg^{II}-thymine base pairs by DNA polymerases†

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Table of Contents

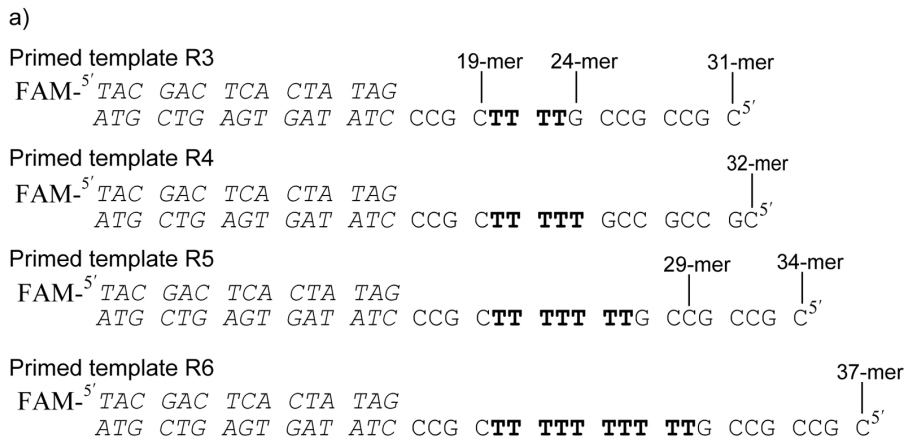
1. Experimental.....	S2
1-1. General methods	S2
1-2. Primer extension reaction	S2
1-3. UV melting experiments	S2
2. Figure S1. Hg ^{II} -mediated primer extension reactions on primed templates containing consecutive T residues catalyzed by Klenow fragment	S3
3. Figure S2. Primer extension reactions catalyzed by Klenow fragment in the presence of Mn ^{II} ions	S4
4. Table S1. Melting temperatures (T_m) of duplexes containing consecutive T-T mismatched base pairs in the presence or absence of various amounts of Hg ^{II} ions.....	S5
5. Figure S3. Melting profiles of duplexes containing consecutive T-T mismatched base pairs in the presence or absence of various amounts of Hg ^{II} ions.....	S6
6. Figure S4. Relationship between number of T-T mismatched base pairs and ΔT_m values of duplexes containing consecutive T-T mismatched base pairs	S6

1. Experimental

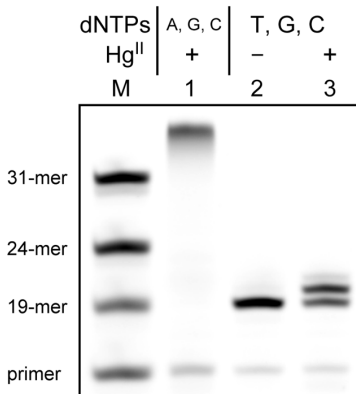
1-1. General methods: Commercially available reagents were used without further purification. ODNs for UV melting experiments were purchased from Nihon Gene Research Laboratories, Inc. (Miyagi, Japan). 5'-FAM-labeled primers and templates used in the primer extension reactions were purchased from Japan Bio Service Co., Ltd. (Saitama, Japan). PAGE was carried out with denaturing 20% (19:1) polyacrylamide gel containing 8 M urea. Gels were analyzed with LAS-3000 (FUJIFILM) at 460 nm excitation. The Klenow fragment (KF), 3'→5' exonuclease-deficient Klenow fragment (KF exo-), and Terminator DNA polymerase (Th) were purchased from New England Biolabs, Inc. (Ipswich, MA).

1-2. Primer extension reaction: All components of the reaction mixture except DNA polymerases, dNTPs, and metal ions were combined and heated at 90 °C. After gradual cooling to room temperature, dNTPs, metal ions, and DNA polymerases were added. The total reaction volume was 20 µL and the final concentrations of all components were as follows: 100 nM 5'-labeled primer, 150 nM template, 10, 100 or 500 µM dNTPs, 30 µM Hg^{II} ion, 50 mM NaCl, 10 mM Tris-HCl (pH 7.9), 10 mM MgCl₂, and 8 µM dithiothreitol (DTT). In experiments employing 1 mM MnCl₂, reactions were carried out under the same conditions except that MgCl₂ is not used. Commercially available polymerase solutions usually contain 1 mM DTT. DTT concentration was set at 8 µM to standardize the concentrations in the reaction mixtures. The mixtures were incubated at 37 or 70 °C for 1 hr. In some cases, after the 1 hr incubation, 500 µM each of dGTP and dCTP was added and further incubation was carried out at 37 °C for 30 min. In the case of the reactions with Th, after the 1 hr incubation, 100 µM dNTPs and 5.0 units of KF exo- were added and the reaction mixtures were further incubated at 37 °C for 30 min. The reactions were quenched by adding 0.5 µL of 100 mM DTT and a loading solution (5 µL) containing 8 M urea, 70 % sucrose, and 0.2 % bromophenol blue, and the mixtures were immediately heated at 90 °C for 10 min. After cooling, the mixtures were analyzed with denaturing 20% polyacrylamide gel containing 8 M urea and gel images were recorded with LAS-3000 by excitation of 5'-labeled FAM at 460 nm.

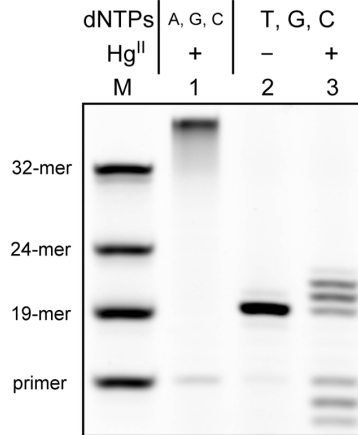
1-3. UV melting experiments: Duplex solution (2 µM) in 100 mM NaClO₄ and 10 mM 3-(N-morpholino)propanesulfonic acid (MOPS) (pH 7.1) containing appropriate metal ions was heated at 90 °C and cooled gradually to room temperature. Melting curves were measured at least twice at 260 nm on a JASCO V-660 spectrophotometer equipped with a programmable temperature control unit. The temperature was raised at the rate of 0.5 °C/min. *T_m* values were obtained from the first-derivative plots of the melting curves.



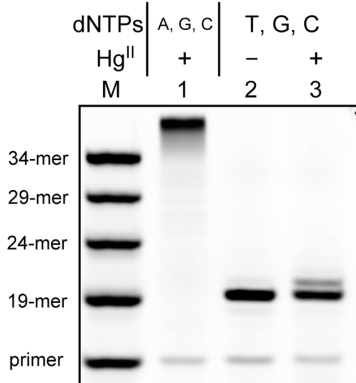
b) Primed template R3



c) Primed template R4



d) Primed template R5



e) Primed template R6

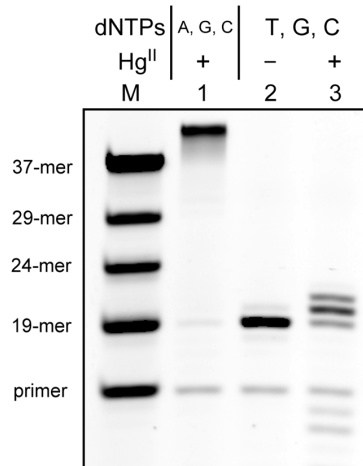


Figure S1. Hg^{II}-mediated primer extension reactions on the primed templates containing consecutive T residues catalyzed by Klenow fragment. a) Sequences of FAM-labeled primed templates. b–e) PAGE analysis of reactions using the primed templates containing b) four, c) five, d) seven and e) ten consecutive T residues shown in a). Reaction mixtures containing 10 μ M dNTPs, 0.2 unit of KF, and 30 μ M Hg(ClO₄)₂ were incubated at 37 °C for 1 hr. M indicates markers. Other experimental details are described in Experimental section.

a)

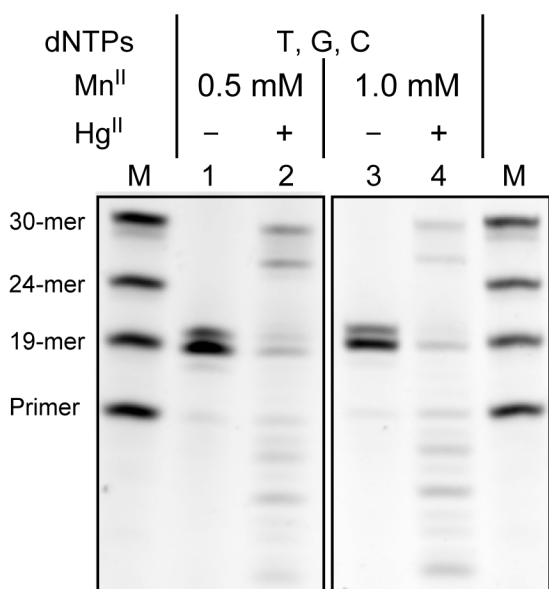
Primed template R2



Primed template R4



b) Primed template R2



c) Primed template R4

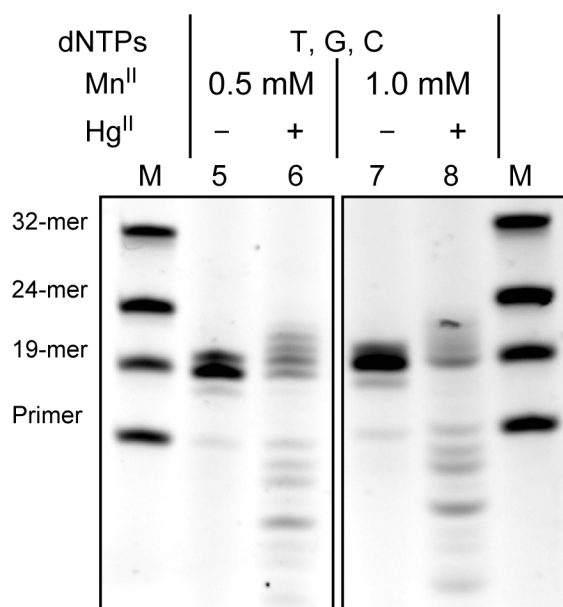


Figure S2. Primer extension reactions catalyzed by Klenow fragment in the presence of Mn^{II} ions. a) Sequences of FAM-labeled primed templates. b, c) PAGE analysis of reactions using the primed templates containing b) three and c) five consecutive T residues shown in a). Reaction mixtures containing b–c) 10 μM dNTPs, 0.2 unit KF, 30 μM Hg(ClO₄)₂, and 0.5 or 1 mM MnCl₂ were incubated at 37 °C for 1 hr. M indicates markers. Other experimental details are described in Experimental section.

Table S1 Melting temperatures (T_m) of duplexes containing consecutive T-T mismatched base pairs in the presence or absence of various amounts of Hg^{II} ions.^a

duplex	number of T-T mispairs	T_m (°C)											
		+ Hg^{II}											
		0 eq.	1 eq.	2 eq.	3 eq.	4 eq.	5 eq.	6 eq.	7 eq.	8 eq.	9 eq.	10 eq.	11 eq.
duplex 1 5' CAT TGG ATT GAC GCT ^{3'} 3' GTA ACC TTA CTG CGA ^{5'}	1	46.5	55.4	55.4	—	—	—	—	—	—	—	—	—
duplex 2 5' CAT TGG ATT TGA CGC T ^{3'} 3' GTA ACC TTT ACT GCG A ^{5'}	2	41.8	44.7 (54.2) ^b	54.3	54.6	—	—	—	—	—	—	—	—
duplex 3 5' CAT TGG ATT TTG ACG CT ^{3'} 3' GTA ACC TTT TAC TGC GA ^{5'}	3	39.2	39.6	(41.4) ^b 54.1	55.2	55.5	—	—	—	—	—	—	—
duplex 4 5' CAT TGG ATT TTT TGA CGC T ^{3'} 3' GTA ACC TTT TTT ACT GCG A ^{5'}	5	32.2	34.1	36.1 (52.4) ^b	(39.6) ^b 54.8	55.7	56.7	58.0	—	—	—	—	—
duplex 5 5' CAT TGG ATT TTT TTT GAC GCT ^{3'} 3' GTA ACC TTT TTT TTA CTG CGA ^{5'}	7	29.0	30.3	29.5	32.7 (54.2) ^b	(42.0) ^b 56.9	58.4	59.6	60.7	62.2	—	—	—
duplex 6 5' CAT TGG ATT TTT TTT TTT GAC GCT ^{3'} 3' GTA ACC TTT TTT TTT TTA CTG CGA ^{5'}	10	23.4	24.8	27.5	28.4 (50.6) ^b	29.1 (53.6) ^b	(31.2) ^b 55.3	(31.9) ^b 56.2	58.9	60.5	62.0	65.4	66.7

^a Samples contained 2 μ M duplex, 100 mM $NaClO_4$, 10 mM MOPS (pH 7.1), and various amounts of Hg^{II} ions. T_m values were obtained from first-derivative plots of melting curves and values are averages of at least two measurements. ^b Two maxima were observed in the first-derivative plots. The lower maximum is shown in parentheses.

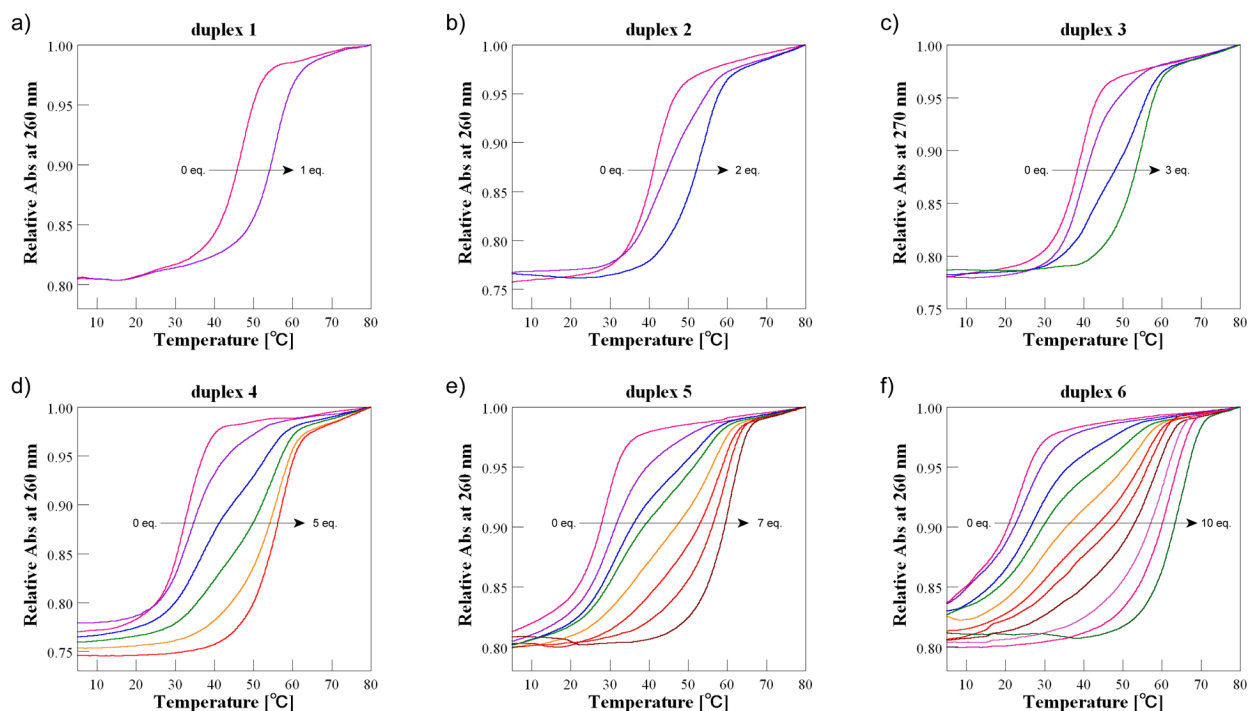


Figure S3. Melting profiles of duplexes containing consecutive T-T mismatched base pairs in the presence or absence of various amounts of Hg^{II} ions. Melting curves of duplexes containing a) one, b) two, c) three, d) five, e) seven, and f) ten consecutive T-T mismatched base pairs were measured in the presence or absence of various amounts of Hg^{II} ions at 260 nm. Samples contained 2 μM duplex, 100 mM NaClO_4 , and 10 mM MOPS (pH 7.1).

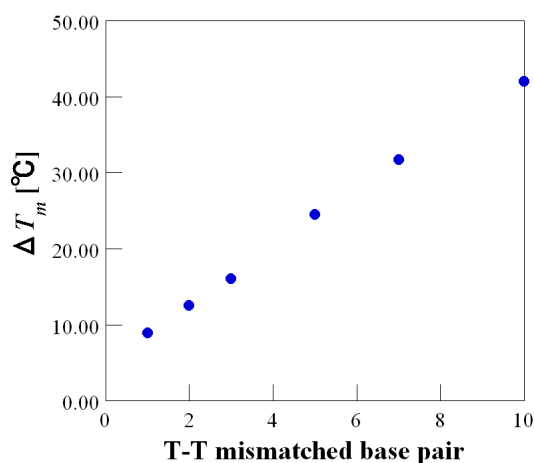


Figure S4 Relationship between number of T-T mismatched base pairs and ΔT_m values of duplexes containing consecutive T-T mismatched base pairs. The ΔT_m values were calculated by subtracting T_m values in the absence of Hg^{II} ions from T_m values in the presence of equimolar amounts of Hg^{II} ions for a T-T mismatched base pair.