

[Supporting Information to Accompany Manuscript]

Achieving Room Temperature DNA Amplification by Dialling in Destabilization

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Methods and Materials

Oligonucleotides. Applied Biosystems Model 392 DNA/RNA Synthesizer was used for the synthesis of all oligonucleotides using standard phosphoramidite reagents and CPGs (Glen Research, Sterling, VA). In DNA strands that contain special modifications, fluorescein was added using Fluorescein-dT phosphoramidite (Glen Research, Sterling, VA) while an abasic group was incorporated using a dSpacer CE phosphoramidite (Glen Research, Sterling, VA). Furthermore, Chemical Phosphorylation Reagent II (Glen Research, Sterling, VA) was used for the phosphorylation of the 5'-end of appropriate oligonucleotides. The synthesis was performed under DMT-on option and upon completion of the synthesis, strands were incubated in concentrated ammonium hydroxide (from Fisher Sci. cat. # A669500) at room temperature to remove blocking groups and the solid support. The purification was done following the manufacturer's instruction using Glen-Pak cartridges. DNA strand concentrations and thermal dissociation profiles were determined using an HP 8453 diode-array spectrophotometer equipped with a HP 89090A Peltier temperature controller. The strands were characterized by MALDI-TOF using a Voyager Elite (applied Biosystems, Foster City, CA) time of flight-mass spectrometer following our previous work.¹ A linear negative mode was employed. To confirm the purity of all the oligonucleotides, they were visualized with StainsAll reagent (Aldrich cat #E9379) after it has been run on a polyacrylamide denaturing gel using the ImageQuant RT ECL

Imager from GE Healthcare Life Science (with fluorescein Filter). Polyacrylamide gel electrophoresis was also used to monitor the kinetics of cross catalysis. 10% PAGE gels were made using urea (99%, Fisher, # BP169212) and tris base (Fisher, BP1521), TEMED from fisher Bioreagent (Fisher, BP15020), 40% acrylamide/bis solution 19:1 (Bio-Rad, 161-0144) and ammonium persulphate (BioShop, AMP 001). Ultrapure water was obtained from a Milli-Q Ultra-Pure Water System, which was autoclaved and then used for all experiments.

Table S1. DNA sequences.

Temperature Tuning Reaction			
DNA-I	5'-TTGTTAAATATTGATAAG-3'		
F-DNA-I	5'-T _F TGTTAAATATTGATAAG-3'		
DNA-IIa	5'-p(Ab)ATTTAACAA-3'	F-DNA-Ia	5'-T _F TGTTAAAT-3'
DNA-IIb(T)	5'-CTTATCAA-3'	DNA-Ib	5'-pATTGATAAG-3'
DNA-IIb(G)	5'-CTTAGCAA-3'		
DNA-IIb(C)	5'-CTTACCAA-3'		
DNA-IIb(A)	5'-CTTAACAA-3'		
DNA-IIb(Ab)	5'-CTTA(Ab)CAA-3'		
F-DNA-IIb(T)	5'-T _F CTTATCAA-3'		
F-DNA-IIb(G)	5'-T _F CTTAGCAA-3'		
F-DNA-IIb(C)	5'-T _F CTTACCAA-3'		

MALDI characterization

DNA-I: calculated mass, 5552; measured, 5552. **F-DNA-Ia** (fluorescein modified): calculated mass, 3240; measured, 3241. **DNA-Ib** (5'-phosphate): calculated mass, 2842; measured, 2842. **DNA-IIa** (abasic group, 5'-phosphate): calculated mass, 2966; measured, 2966. **DNA-IIb(T)**: calculated mass, 2369; measured, 2368. **F-DNA-IIb(T)** (fluorescein modified): calculated mass, 3185; measured, 3187. **DNA-II(G)**: calculated mass, 2394; measured, 2394. **DNA-IIb(C)**:

calculated mass, 2354; measured, 2354. **DNA-IIb(A)**: calculated mass, 2378; measured, 2379.

DNA-IIb(Ab): calculated mass, 2244; measured, 2243.

DNA ligation equipment, materials and procedure

To maintain a constant temperature for all the ligation reactions, a Torrey Pines Scientific Ecotherm Chilling/Heating Plate was used. The course of the reaction was monitored using polyacrylamide gel electrophoresis (PAGE) and was analyzed using ImageQuant RT ECL Imager using UV transillumination. The enzyme T4 DNA ligase (2,000,000 cohesive end units/mL, catalog #M020T) and the corresponding ligase buffer that was used for the experiments were bought from New England Biolabs. The DNA cross-catalysis ligation experiments were performed as described in our earlier work for 15 μ L volume reactions and utilized 2,000 cohesive end units per 15 μ L reaction.^{1,2} Briefly, the final concentration of the limiting fluorescent probe was 1.4 μ M while the other probes were 2.8 μ M except for the template concentration variation studies where the fluorescent probe was 0.7 μ M while the other probes were 1.4 μ M. For the single turnover experiments, the enzyme T4 DNA ligase was purchased from Invitrogen (1 WEISS unit/L, catalogue # 15224-017) was used with a final concentration of 1.4 μ M of limiting fluorescently labelled probe and 2.8 μ M of the destabilizing probe.

Quantifying ligation yields and turnover numbers (TON)

The data in each figure represent the average of at least two ligation reactions and the error bars represent the standard deviation. The percent yield (percent conversion) for every ligation reaction was calculated from polyacrylamide gel images obtained after electrophoretic separation of the ligation reaction according to our previous work.^{1,2} The following equation was used to calculate the % yield of the product at each data point:

$$\% \text{ Yield} = \frac{\text{Product Band}}{\text{Product Band} + \text{Reactant Band}} \times 100\%$$

By multiplying this percentage by the concentration of the fluorescently labeled probe (the limiting fragment = 1.4 μM), the concentration of the product was obtained. The turnover was calculated according to the equation below.

$$\text{TON} = \frac{\Delta[\text{F} - \text{DNA} - \text{I}]}{[\text{Template}]}$$

The maximum difference of **F-DNA-I** is calculated as follows:

$$\Delta[\text{F} - \text{DNA} - \text{I}] = [\text{F} - \text{DNA} - \text{I}(\text{Template})] - [\text{F} - \text{DNA} - \text{I}(\text{Control})]$$

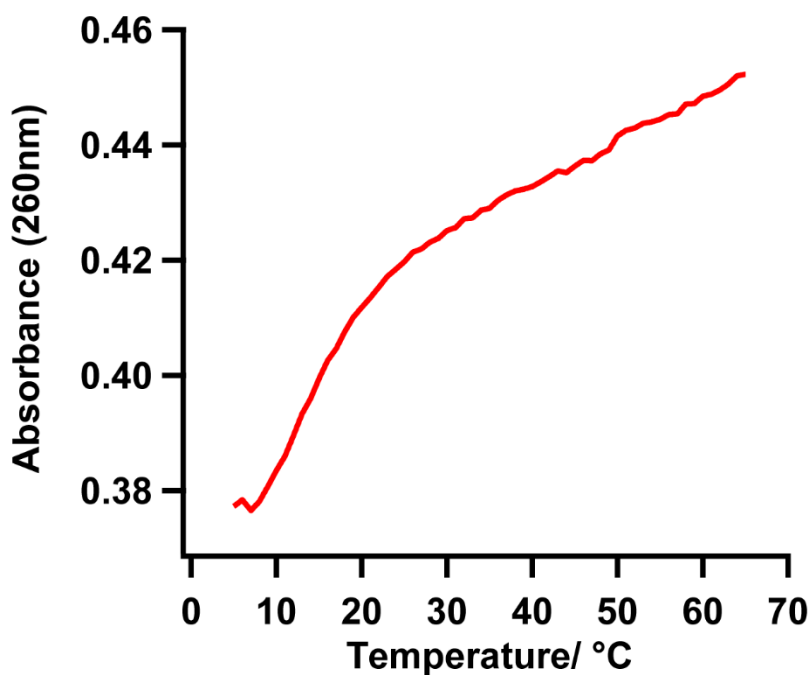


Figure S1: Melting profile of the nicked duplex corresponding to **DNA-I: DNA-IIa: DNA-IIb(G)**. A melting temperature of 11.8 °C was observed. *Experimental conditions:* 1.3 μM of each DNA strand in 10 mM MgCl_2 , 10 mM PBS, pH 7.

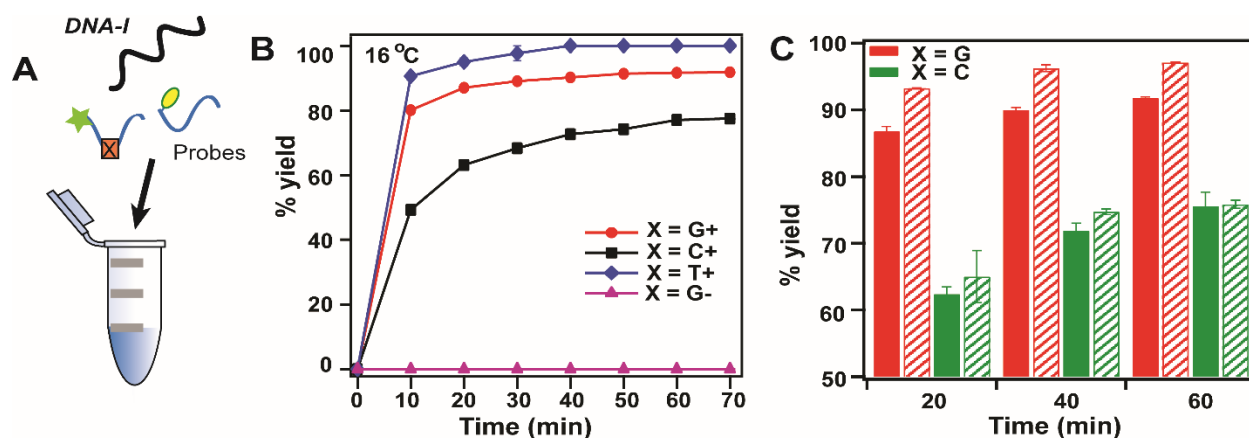


Figure S2: A) Scheme illustrating the four components in the single-turnover experiment (abasic = oval; X = base across from A on the **DNA-I** template). B) The formation of **F-DNA-II(X)** at 16 °C for ligations initiated by 1.4 μM (+) or 0 μM (-) **DNA-I**. C) The % yield of **F-DNA-II(X)** formed at 16 °C (solid) and 12 °C (striped). The percent yield increased with decreasing temperature, which indicates that the amount of product formed is proportional to the amount of nicked duplex present at that temperature. As the association constants increase with decreasing temperature, the % yield of ligation is expected to increase. These results suggest that the intrinsic rate of ligation is similar for the different mismatches, so the rate is most affected by the amount of nicked duplexes present, which in turn depends on the value of their equilibrium constant at a given temperature. *Experimental conditions:* 1.4 μM **DNA-I**, 1.4 μM **F-DNA-IIb(X)**, 2.8 μM **F-DNA-IIa**, 1 Weiss Unit T4 DNA ligase, 10 mM MgCl₂, pH 7.

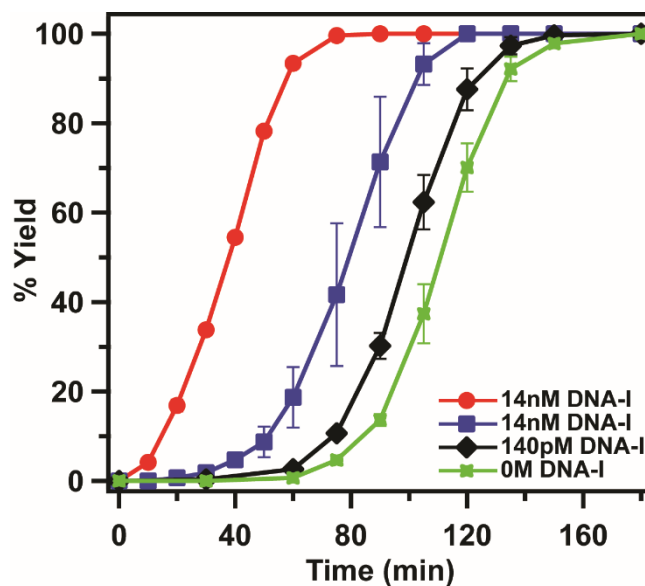


Figure S3: The % yield of **F-DNA-I** formed as a function of time with different concentrations of initial **DNA-I** template and lower replicator concentrations than standard conditions. Experimental conditions: 14 nM, 1.4 nM, 140 pM or 0 nM **DNA-I**; 0.7 μ M **F-DNA-Ia**; 1.4 μ M **DNA-Ib**; 1.4 μ M **DNA-IIa** (M = Ab); 1.4 μ M **DNA-IIb(G)**; 26 $^{\circ}$ C.

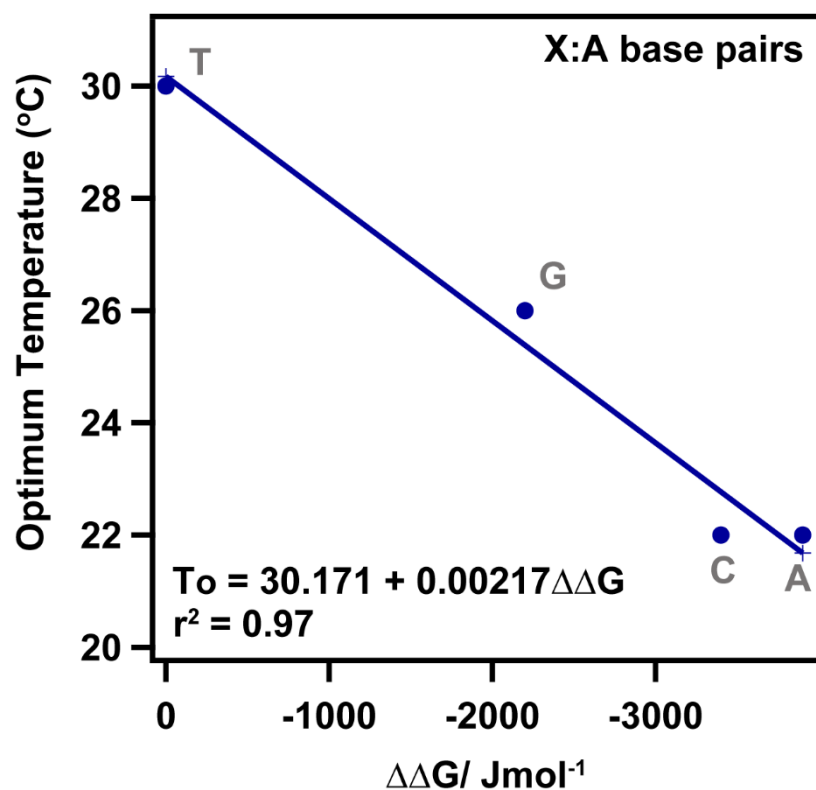


Figure S4: Linear fit of $\Delta\Delta G$ against the optimum replication temperature for the corresponding matched or mismatched base. The ΔG of the matched and mismatched systems were obtained from the work of Gaffney and Jones. $\Delta\Delta G$ is the difference between the corresponding ΔG values of the matched and the mismatched system for a series of A:X mismatches.³

Gel images

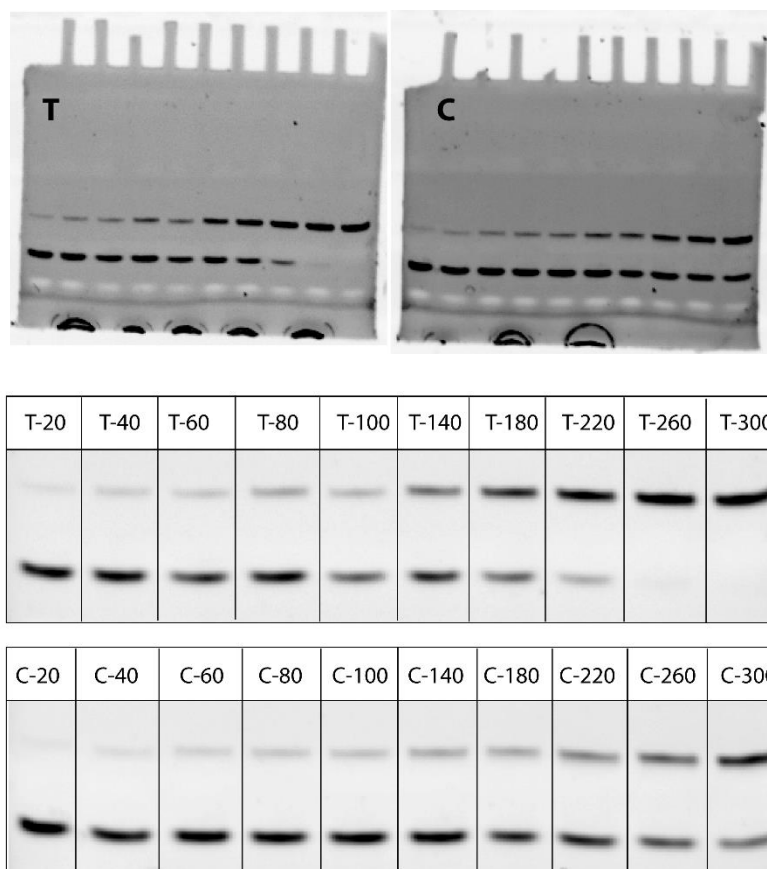


Figure S5: Gel images representing the ligation reaction using destabilizing probes (Ab and no mismatch) as a function of time in minutes (shown in Fig. 2, Ab + X = T, black trace). T and C refers to the presence or absence of 14 nM of initial target **DNA-I**, respectively. *Top band: F-DNA-I; bottom band: F-DNA-Ia.* *Experimental conditions:* 14 or 0 nM **DNA-I**; 1.4 μ M **F-DNA-Ia**; 2.8 μ M **DNA-Ib**; 2.8 μ M **DNA-IIa**; 2.8 μ M **DNA-IIb(T)**; 26°C.

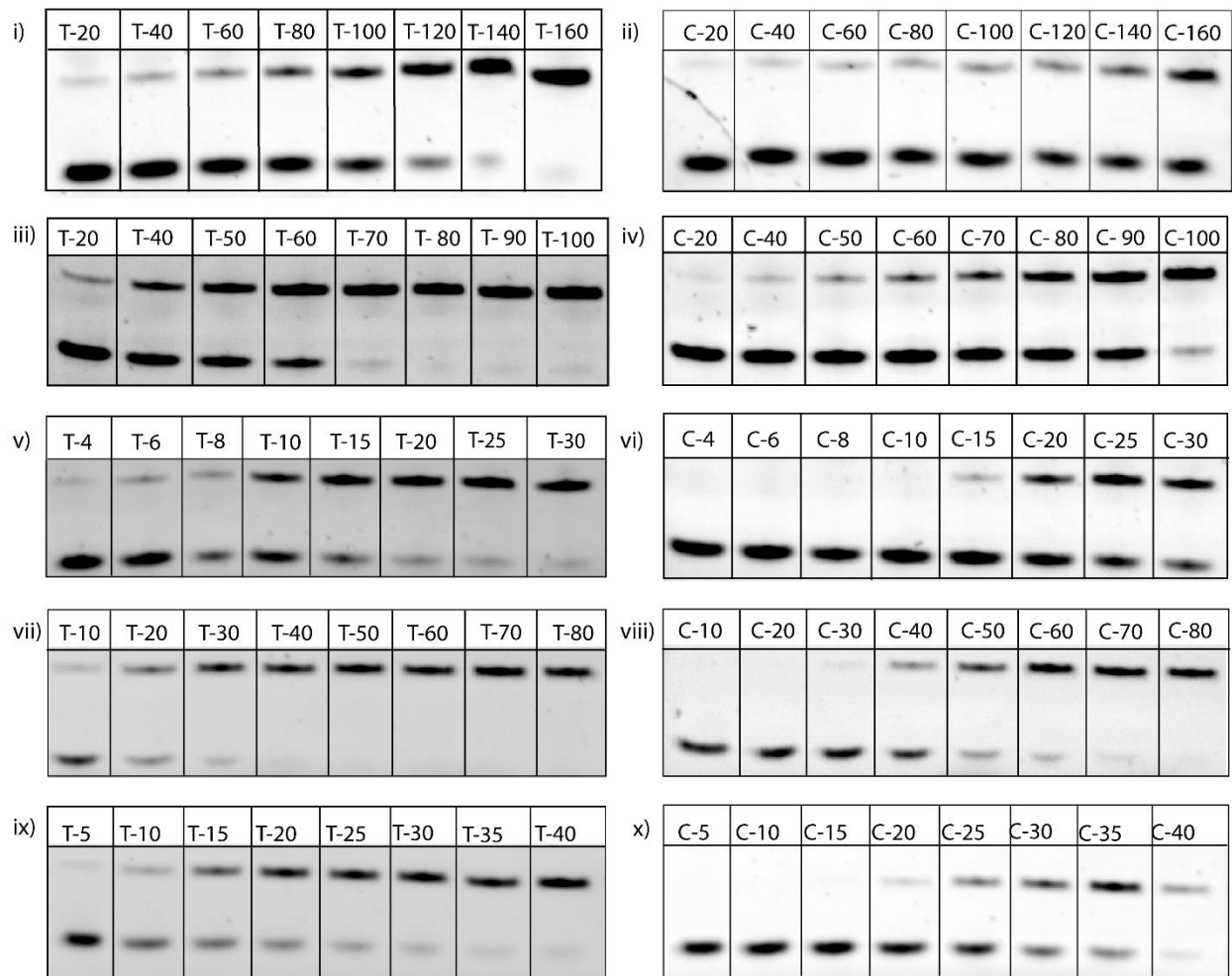


Figure S6: Gel images representing ligation reactions using destabilizing probes containing the abasic and mismatch as a function of time and at different temperatures. These data are exhibited in Figures 2 and 3 ($Ab + X = G$). Experiments shown in i) iii) v) vii) ix) were done in the presence of initial target (14 nM **DNA-I**) and ii) iv) vi) viii) x) were performed with no initial target (0 nM **DNA-I**). *Top band: F-DNA-I; bottom band: F-DNA-Ia.* *Experimental conditions:* 14 or 0 nM **DNA-I**; 1.4 μ M **F-DNA-Ia**; 2.8 μ M **DNA-Ib**; 2.8 μ M **DNA-IIa**; 2.8 μ M **DNA-IIb(G)**; i) and ii) 22°C; iii) and iv) 24°C; v) and vi) 26°C; vii) and viii) 28°C; ix) and x) 30°C.

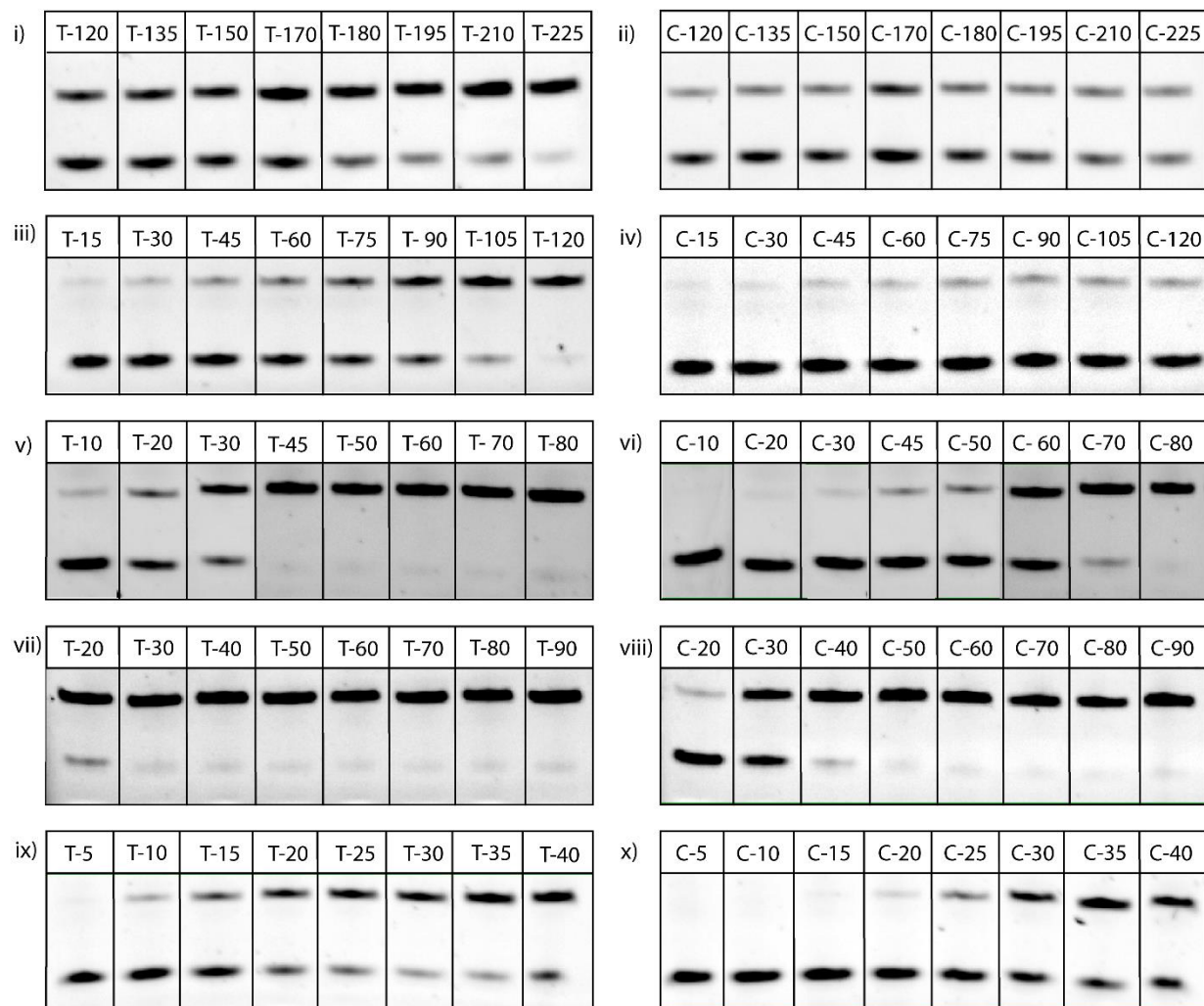


Figure S7: Gel images representing ligation reactions using destabilizing probes containing the abasic and mismatch as a function of time and at different temperatures. These data are exhibited in Figures 3 ($Ab + X = C$). Experiments shown in i) iii) v) vii) ix) were done in the presence of initial target (14 nM **DNA-I**) and ii) iv) vi) viii) x) were performed with no initial target (0 nM **DNA-I**). *Top band: F-DNA-I; bottom band: F-DNA-Ia.* *Experimental conditions:* 14 or 0 nM **DNA-I**; 1.4 μ M **F-DNA-Ia**; 2.8 μ M **DNA-Ib**; 2.8 μ M **DNA-IIa**; 2.8 μ M **DNA-IIb(C)**; i) and ii) 18°C; iii) and iv) 20°C; v) and vi) 22°C; vii) and viii) 24°C; ix) and x) 26°C.

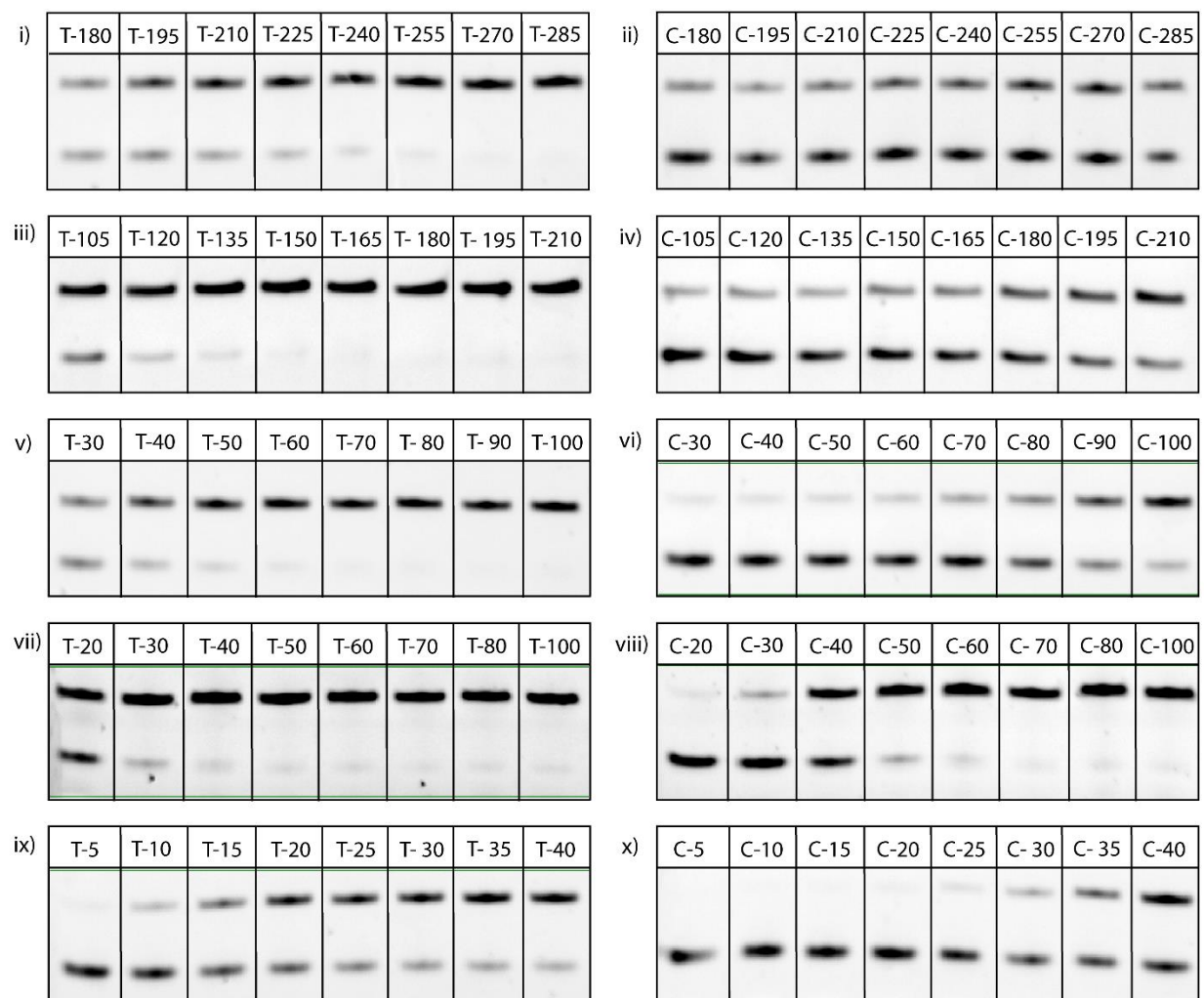


Figure S8: Gel images representing ligation reactions using destabilizing probes containing the abasic and mismatch as a function of time and at different temperatures. These data are exhibited in Figures 3 ($Ab + X = A$). Experiments shown in i) iii) v) vii) ix) were done in the presence of initial target (14 nM **DNA-I**) and ii) iv) vi) viii) x) were performed with no initial target (0 nM **DNA-I**). *Top band: F-DNA-I; bottom band: F-DNA-Ia.* *Experimental conditions:* 14 or 0 nM **DNA-I**; 1.4 μ M **F-DNA-Ia**; 2.8 μ M **DNA-Ib**; 2.8 μ M **DNA-IIa**; 2.8 μ M **DNA-IIb(A)**; i) and ii) 18°C; iii) and iv) 20°C; v) and vi) 22°C; vii) and viii) 24°C; ix) and x) 26°C.

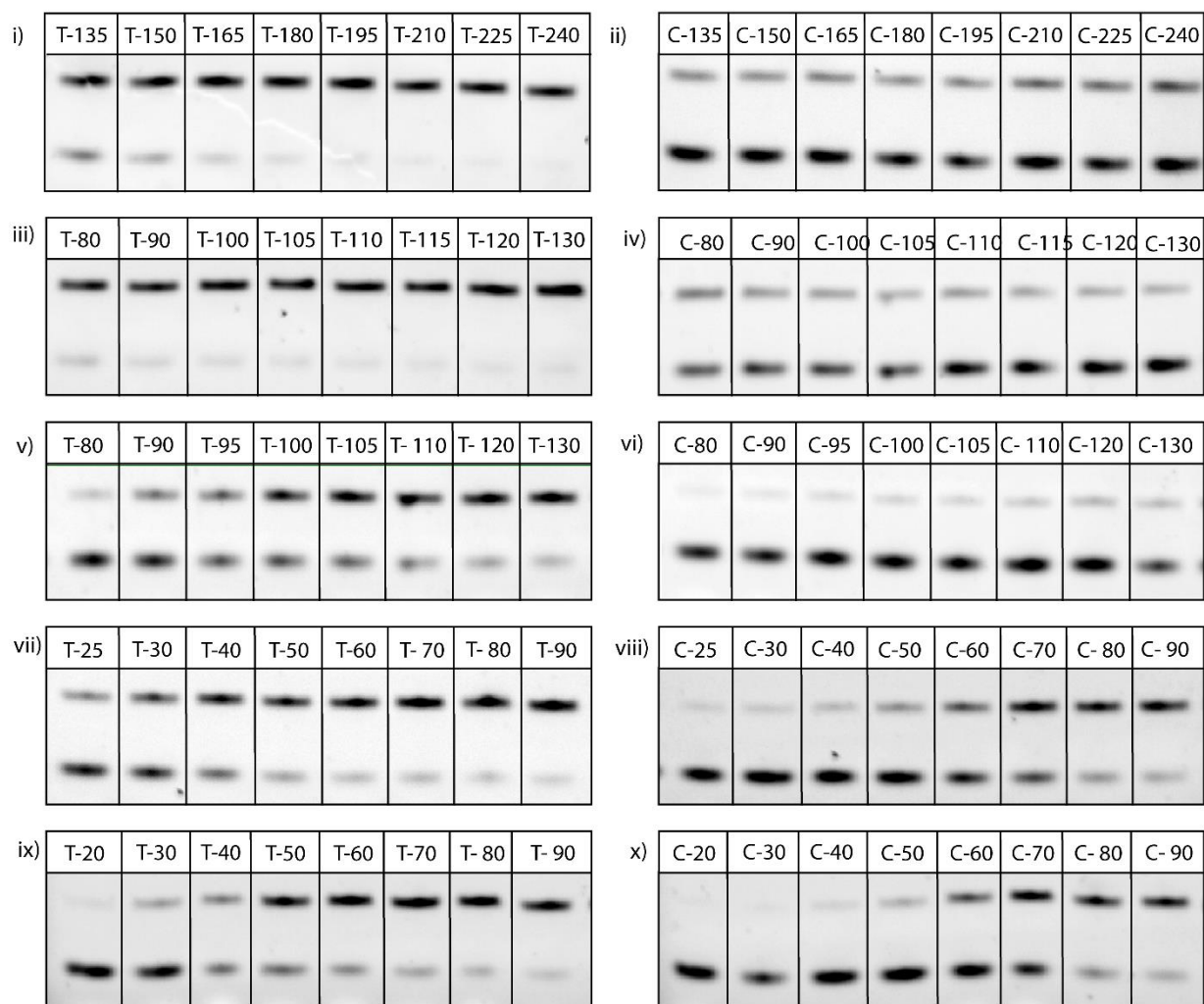


Figure S9: Gel images representing ligation reactions using destabilizing probes containing the abasic and mismatch as a function of time and at different temperatures. These data are exhibited in Figures 3 ($Ab + X = Ab$). Experiments shown in i) iii) v) vii) ix) were done in the presence of initial target (14 nM **DNA-I**) and ii) iv) vi) viii) x) were performed with no initial target (0 nM **DNA-I**). *Top band: F-DNA-I; bottom band: F-DNA-Ia.* *Experimental conditions:* 14 or 0 nM **DNA-I**; 1.4 μ M **F-DNA-Ia**; 2.8 μ M **DNA-Ib**; 2.8 μ M **DNA-IIa**; 2.8 μ M **DNA-IIb**(**Ab**); i) and ii) 16°C; iii) and iv) 18°C; v) and vi) 20°C; vii) and viii) 22°C; ix) and x) 24°C.

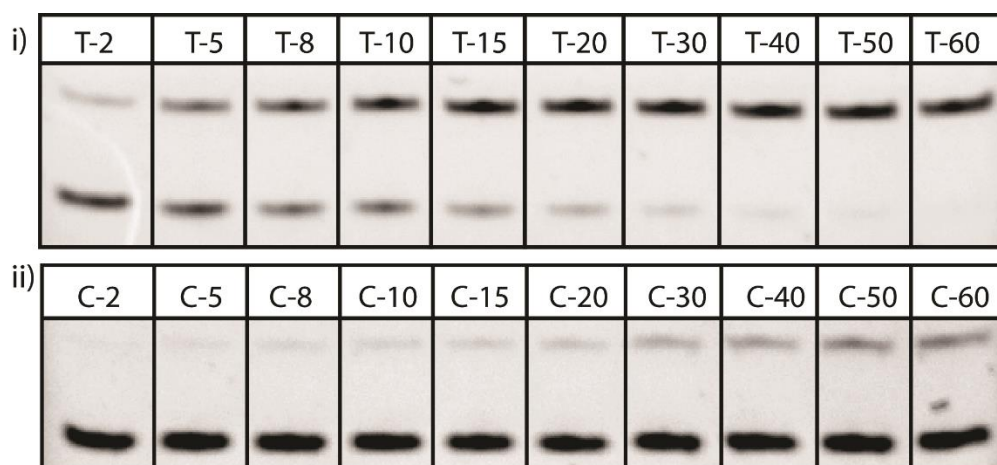


Figure S10: Gel images representing the ligation reaction done on the bench top using destabilizing probes containing the abasic and mismatch as a function of time in minutes and at room temperature (shown in Figure 4). T and C refers to the presence or absence of 14 nM of initial target **DNA-I**, respectively. *Top band: F-DNA-I; bottom band: F-DNA-Ia.* *Experimental conditions:* 14 or 0 nM **DNA-I**; 1.4 μ M **F-DNA-Ia**; 2.8 μ M **DNA-Ib**; 2.8 μ M **DNA-IIa**; 2.8 μ M **DNA-IIb(C)**; 21°C.

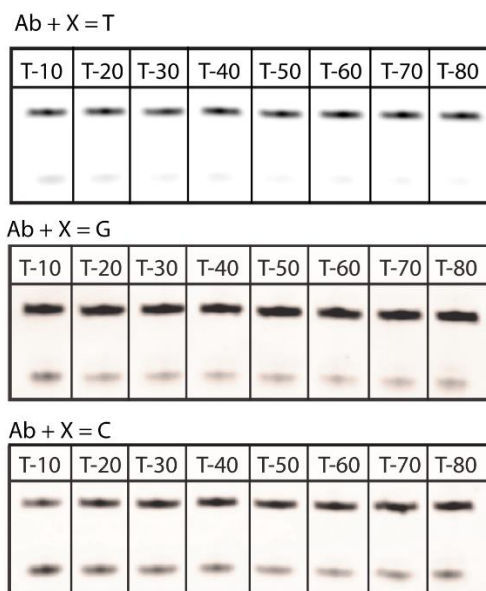


Figure S11: Gel images representing the single turnover ligation reaction using destabilizing probes (Ab and X=T/G/C mismatch) as a function of time in minutes (shown in Figure S1) T refers to the presence of initial of 14 μ M target **DNA-I**. *Top band: F-DNA-II; bottom band: F-DNA-IIb(X).* *Experimental conditions:* 14 μ M **DNA-I**; 1.4 μ M **F-DNA-IIb(X)**; 2.8 μ M **DNA-IIa**; 16°C.

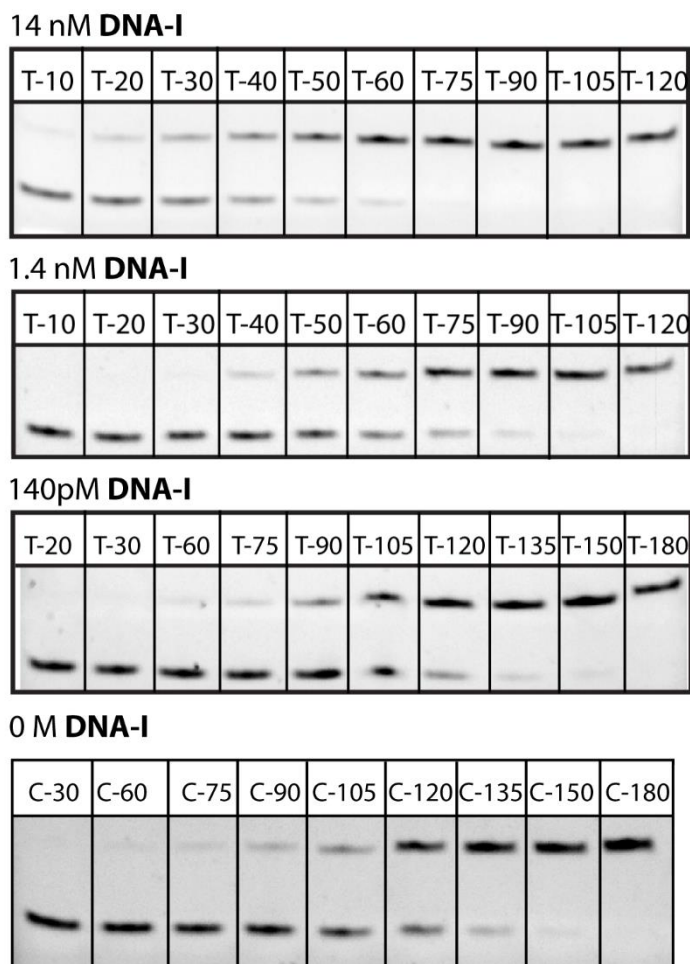


Figure S12: The concentration of **F-DNA-I** formed as a function of time with different concentrations of initial DNA-I template and lower replicator concentrations than standard conditions (shown in Figure S2). T and C refers to the presence or absence of initial target **DNA-I**, respectively. *Top band: F-DNA-I; bottom band: F-DNA-Ia.* *Experimental conditions:* 14 nM, 1.4 nM, 140 pM or 0 nM **DNA-I**; 0.7 μ M **F-DNA-Ia**; 1.4 μ M **DNA-Ib**; 1.4 μ M **DNA-IIa**; 1.4 μ M **DNA-IIb(T)**; 26°C.

- (1) Kausar, A.; McKay, R. D.; Lam, J.; Bhogal, R. S.; Tang, A. Y.; Gibbs-Davis, J. M. *Angew. Chem. Int. Edit.* **2011**, 50, 8922.
- (2) Kausar, A.; Mitran, C. J.; Li, Y.; Gibbs-Davis, J. M. *Angew. Chem. Int. Edit.* **2013**, 52, 10577.
- (3) Gaffney, B. L.; Jones, R. A. *Biochemistry* **1989**, 28, 5881.