Electronic Supplementary Material (ESI) for Analyst.

The Presence of a 5'-Abasic Lesion Enhances Discrimination of Single Nucleotide Polymorphisms While Inducing an Isothermal Ligase Chain Reaction

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Materials and instrumentation

T4 DNA ligase was purchased from New England Biolabs (2,000,000 cohesive end units/mL, catalogue #M0202T). The temperatures for DNA ligation experiments were maintained using a Torrey Pines Scientific Echotherm Chilling/Heating Plate Model IC22. The polyacrylamide gel electrophoreses (PAGE) gels were imaged using an ImageQuant RT ECL Imager from GE Healthcare Life Science using UV transillumination. The DNA was synthesized on an Applied Biosystems Model 392 DNA/RNA Synthesizer. Fluorescence spectra were measured in a Tecan Safire II plate reader using 384 well plates. Ultrapure water was obtained from a Milli-Q Ultra-Pure Water System, which was autoclaved and then used for all experiments.

DNA synthesis and purification

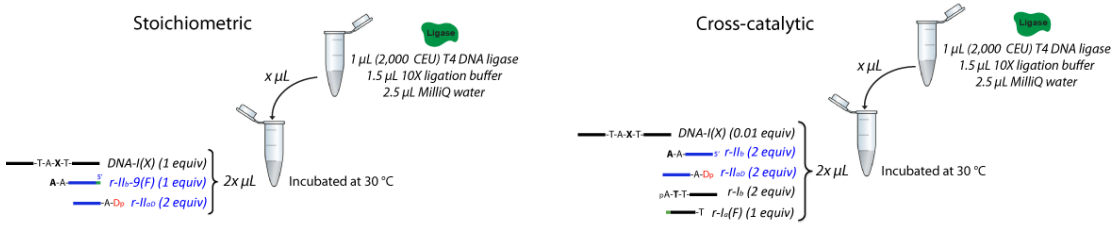
The sequences of the probes and targets are listed in Table S1. DNA was synthesized on an ABI Model 392 solid-phase synthesizer using Glen Research reagents. Strands were purified by Glen-Pak DNA Purification cartridges (cat. 60-5200-01) according to the DMT-On protocol. Standard nucleotide phosphoramidites and the following were used: fluorescein-dT phosphoramidite (Glen Research Cat. # 10-1056-95), dSpacer CE phosphoramidite (Glen Research Cat. # 10-1914-90) for abasic (**Ab**) group, and Cy5 phosphoramidite (Glen Research Cat. # 10-5915-95).

MALDI characterization

Synthesised DNA strands were characterized by MALDI-TOF using a matrix solution consisting of 2,4,6-trihydroxylacetophenone in 1:1 acetonitrile:water (20 mg/ mL) combined in a 9:1 ratio with aqueous ammonium citrate solution (50 mg/mL). Roughly 1 nmol of each desalted DNA sample, in 5 μL MilliQ water, was combined with 5 μL of the matrix/ammonium citrate solution. The DNA/matrix/ammonium citrate solution (0.6 to 1 μL) was spotted on a MALDI target and allowed to dry. MALDI-MS was then performed on a Voyager Elite (Applied BioSystems, Foster City, CA, USA) time of flight-mass spectrometer in linear positive mode. Bovine insulin and Bovine insulin chain B were used to calibrate the instrument.

DNA-I(G): calculated mass, 5576.7; measured, 5576.41. **DNA-I(C):** calculated mass, 5536.7; measured, 5536.97. **DNA-I(A):** calculated mass, 5560.7; measured, 5561.87. **r-Ia (F):** calculated mass, 3239.41; measured, 3340.53. **r-Ia(Cy5):** calculated mass, 3261.53; measured, 3262.75. **r-Ib (F):** calculated mass, 3353.39; measured, 3354.91. **r-IIb-9 (F):** calculated mass, 3184.31; measured, 3186.34. **r-IIb-13 (F)T:** calculated mass, 4392.11; measured, 4392.68. **r-IIb-13 (F)C:** calculated mass, 4377.11; measured, 4378.11.

PAGE-based ligation (stoichiometric/single cycle and cross-catalysis)



	Reaction volume (µL)			DNA (1 equiv)	
	DNA solution (2X)	Ligase master mix (X)	Total	Amount (mol)	Concentration (µM)
Stoichiometric	5	2.5	7.5	10.2	1.4
High conc. cross catalysis	10	5	15	20.3	1.4
Low conc. cross catalysis	5	2.5	7.5	2.54	0.34

DNA composition of single cycle experiments (1 equiv = 10.2 pmol): 2 equiv**. r-IIa$_D$**; 1 equiv. **r-IIb-9(F)**; 1 equiv. target **DNA-I(X) (X=T/G/C/A)** (final concentration of probes for 1 equiv = 1.4 µM, final ligation volume 7.5 µL). DNA composition of standard cross-catalysis reactions (1 equiv = 20.3 pmol): 1 equiv. **r-Ia(F)**; 2 equiv. **r-Ib(T)**; 2 equiv. **r-IIb**; 2 equiv. **r-IIa$_D$(D=Ab)** and 0.01 equiv of target **DNA-I(X) (X=T/G/C/A)** (final concentration of probes for 1 equiv = 1.4 µM, final ligation volume 15 µL). For cross-catalysis reactions performed at lower probe concentration as shown in Figure 1B, the same probe ratio as above was used, but one equivalent was reduced to 2.54 pmol (final concentration of probes for 1 equiv. = 340 nM, final ligation volume 7.5 µL). Additionally, the appropriate amount of target DNA was used to reach the desired final concentration. In a typical ligation, where 1 equiv = 1.4 µM, the appropriate amounts of DNA fragments and template were first combined in water in a 600 µL minicentrifuge tube to reach a final volume of 10 µL and incubated at 30 °C. While the DNA probe solutions were equilibrating the ligase master mix was prepared. For each ligation reaction, the master mix consisted of T4 DNA ligase (1 µL, 2,000 cohesive end unit μL^{-1}), ligation buffer (1.5 µL, 10X concentrated) and water (2.5 µL)}. After master mix preparation, this ligase mixture (5 µL per 10 µL DNA probe solution) was immediately added to each of the DNA solutions. The reactions were then placed in a covered thermal incubator at 30 °C. For the kinetic experiments, aliquots (3 µL) were removed from the bulk ligation mixture at various reaction times and placed in a separate microcentrifuge tube containing EDTA(aq) (1 µL, 0.5 M). To stop ligation, EDTA(aq) (1 µL, 0.5 M) was added to each aliquot. The amount ligated was

quantified by fluorescent imaging after separation by denaturing polyacrylamide gel electrophoresis (PAGE). For the experiments in Figure S2, r-Ib(A) and r-Ib(G) were used in place of r-Ib(T).

Table S1. DNA sequences used in this study

Sequence name	DNA sequence
DNA-I(X) ***(X=T/G/C/A)***	5'-TTGTTA AAT AXT GATAAG-3'
DNA-II$_Y$*(Y=D/T)*	5'-CTT ATC AAY ATT TAA CAA-3'
r-Ia	5'-TTGTTAAAT-3'
r-Ia(F)	5'-T_FTGTTAAAT-3'
r-Ia(Cy5)	5'-$_{Cy5}$TTGTTAAAT-3'
r-Ib (T)	5'-$_P$ATTGATAAG-3'
r-Ib (A)	5'-$_P$AATGATAAG-3'
r-Ib (G)	5'-$_P$AGTGATAAG-3'
r-Ib (F)	5'-$_P$ATT$_F$GATAAG-3'
r-IIa$_Y$(Y=*D/T*)	5'-$_P$YATTTAACAA-3'
r-IIb	5'-CTTATCAA-3'
r-IIb-9(F)	5'-T_FCTTATCAA-3'
r-IIb-13(F)T	5'-T_FTTTTCTTATCAT-3'
r-IIb-13(F)C	5'-T_FTTTTCTTATCAC-3'
	D = model abasic (dSpacer)
	T_F= Fluorescein-dT

Real-time FRET experiments (single cycle and cross-catalysis)

Real time FRET experiments utilized standard ligation mixtures very similar to those described above for the PAGE based experiments except the total ligation volume was reduced to 7.5 μL. DNA composition of standard cross-catalysis reactions (1 equiv = 10.5 pmol): 1 equiv. **r-Ia(Cy5)**; 1 equiv. **r-Ib(F)**; 2 equiv. **r-IIb**; 2 equiv. **r-IIa$_D$** and 0.01 equiv of target **DNA-I(X) (X=T/G/C/A)** (final concentration of probes for 1 equiv = 1.4 μM, final ligation volume 7.5 μL). At first, the DNA mixture and ligase mixture were also incubated at the desired ligation temperature for 10 minutes prior to mixing. During this time, the fluorescence spectrometer was turned on and set to the desired temperature (30 °C), and when the instrument reached this temperature, the ligase master mix mixture (2.5 μL, same composition as described above) was added to the DNA mixture (5.0 μL). The mixture was then vortexed thoroughly before being pipetted into one of the wells in the 384 well plate. The data were collected in the kinetic mode every 10 minutes for 85 minutes with the first data taken after 5 min of mixing the DNA solutions with the enzyme mixture. The following instrument settings were used: measurement mode fluorescence bottom; excitation λ = 485 nm; emission λ = 505-750 nm; excitation bandwidth = 10 nm; emission bandwidth = 10 nm; number of reads = 2; steps = 2 nm; gain = 60; integration = 500 μs.

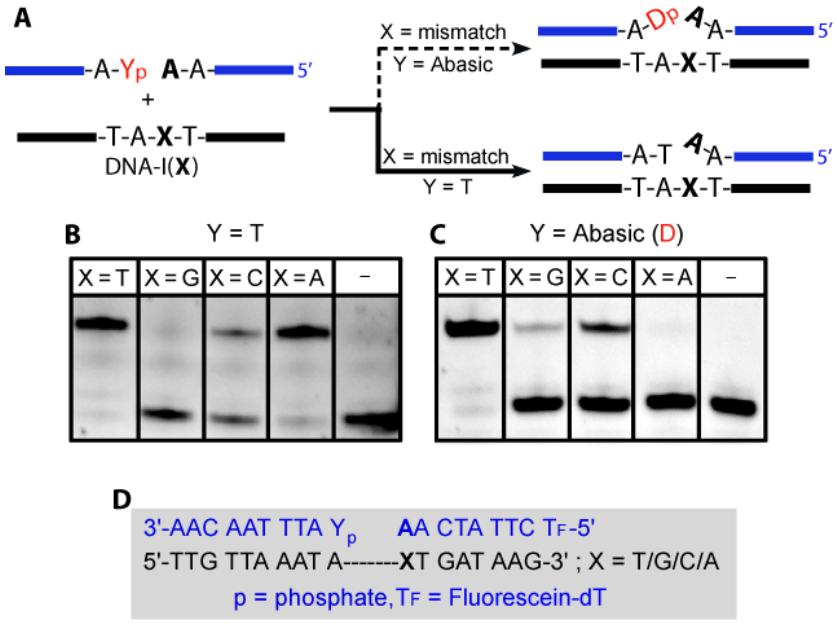


Figure S1: A) Schematic diagram of mismatch discrimination using probes with and without a destabilizing lesion (**D**). B) PAGE image of ligation after 1 hour, using a probe without a destabilizing group and different **DNA-I(X)** targets. The templated ligation is quantitative with matched target **(X=T)**, and there is evidence of template reaction in the presence of the mismatched targets **(X = G/C/A)**. C) PAGE image of ligation after 1 hour, using a probe with a destabilizing group and different **DNA-I(X)** targets. The templated ligation is again quantitative matched target **(X=T)**, whereas no product is observed in the presence of the mismatched targets **(X = G/C/A)**. The background-triggered process for both experiments is also shown (-, no initial template). D) DNA sequences used in this study. *Experimental conditions:* 1.4 μM **r-IIb (F)**. 2.8 μM **r-IIa$_Y$**, and 1.4 μM of target **DNA-I(X) (X=T/G/C/A)** or no target at 30 ºC, T4 DNA ligase 2000 cohesive end units (CEU) per 15 μL ligation volume.

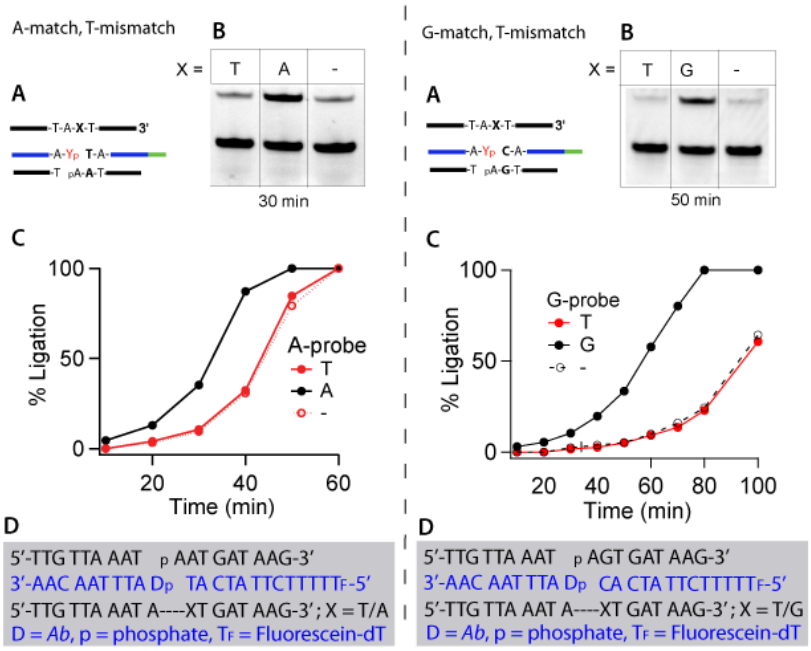


Figure S2: Mismatch discrimination with T4 DNA ligase using A-specific (left) and G-specific (right) probes. A) Schematic diagram of mismatch discrimination. B) PAGE images of ligation with match target, mismatch target and control ligation (-, no initial target) at 30 ºC. C) The kinetics of cross-catalytic amplification of **DNA-I(X)** initiated with 14 nM of different **DNA-I(X)** targets (matched or mismatched, or no initial template [-]). D) DNA sequences used, including **DNA-I(A)** and **DNA-I(G)**.

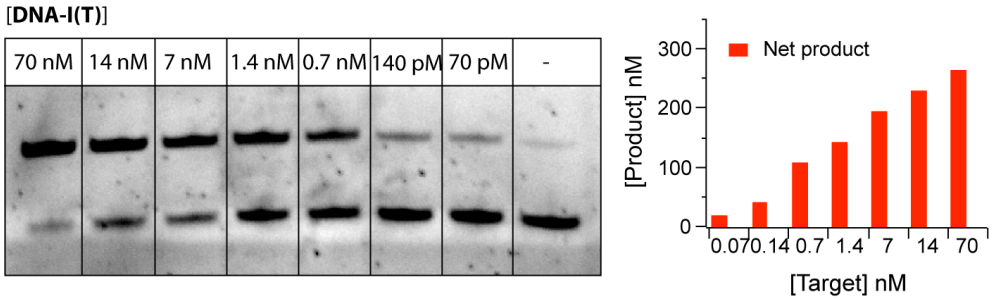


Figure S3: Left: Representative PAGE image of the cross-catalytic amplification shown in figure 2 with different concentrations of initial target **DNA-I(T)**. Right: Graph showing product yield. *Experimental conditions:* 340 nM **r-Ia(F)**, 680 nM **r-Ib(T)**, 680 nM **r-IIa$_{Ab}$**, 680 nM **r-IIb** and different concentration of target **DNA-I(T)** at 30 ºC, T4 DNA ligase 2000 CEU per 15 µL ligation volume.

FRET based detection

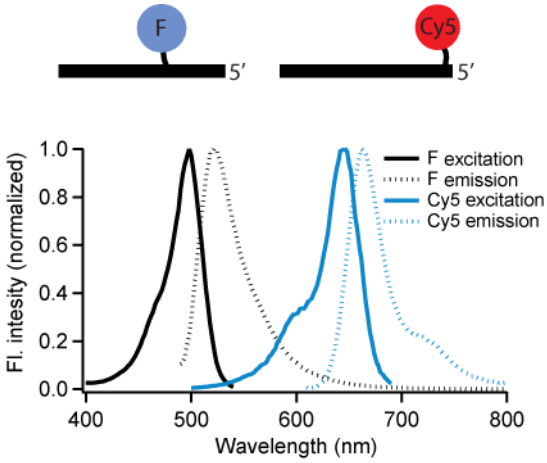


Figure S4: Normalized excitation and emission spectra of the fluorescein (F) and Cy5 labelled probes. For the F-probe, the λ_{em} was 521 nm and the λ_{ex} was 494 nm for the excitation and emission scans, respectively. For the Cy5-probe, the λ_{em} was 661 nm and the λ_{ex} was 645 nm for the excitation and emission scans, respectively.

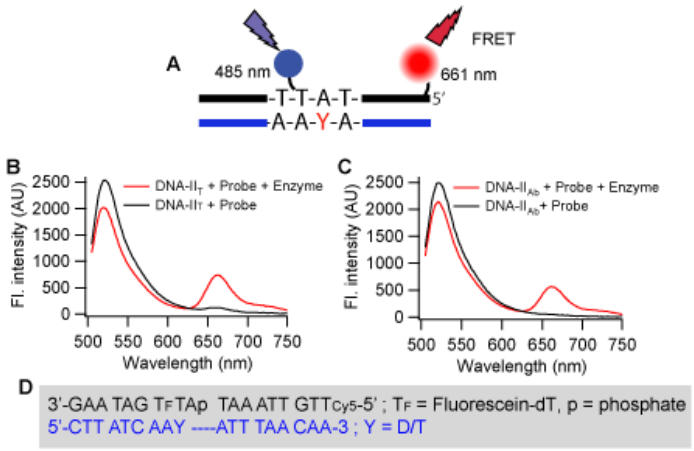


Figure S5: A) Schematic diagram of the FRET process. Upon excitation of the ligation mixture at 485 nm corresponding to F excitation, we observed the Cy5 emission at 661 nm. B) Fluorescent spectra of the DNA mixture containing the native template (D = T) and the FRET pair-labelled probes after 10 minutes in the absence and presence of ligation (no enzyme and with enzyme, respectively). C) Similar experiment to part B but with the destabilizing template **DNA-II$_{Ab}$.** *Experimental conditions*: 1.4 µM **r-Ia(Cy5)**; 1.4 µM **r-Ib(F)**; 1.4 µM **DNA-II$_D$ (D=T or Ab)**; 30 ºC, T4 DNA ligase 1000 CEU per 7.5 µL ligation volume.

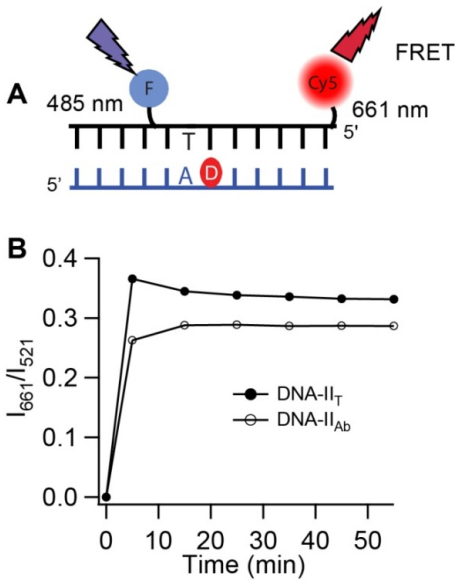


Figure S6: A) Schematic diagram of the FRET process. B) Plot of FRET efficiency (I_{661}/I_{521}) with respect to time for the single cycle ligation with 1.4 µM **r-Ia(Cy5)**; 1.4 µM **r-Ib(F)**; 1.4 µM **DNA-II$_D$ (D=T or Ab)**; 30 ºC, T4 DNA ligase 1000 CEU per 7.5 µL ligation volume.

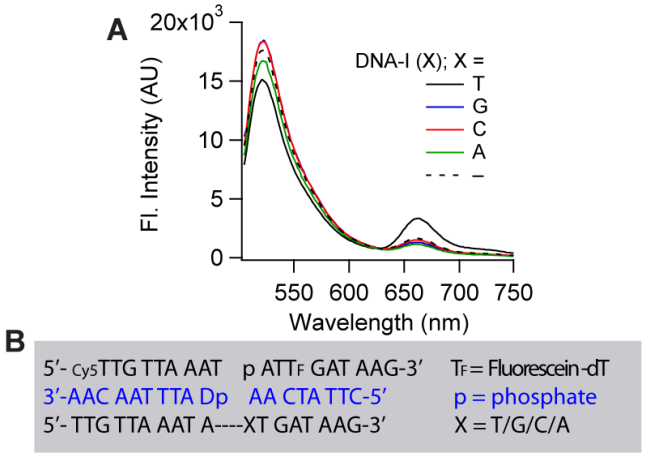


Figure S7: A) Difference in FRET signal with different mismatches at 30 ºC after 35 minutes of mixing. B) DNA sequences used in this study. *Experimental conditions:* 1.4 µM **r-Ia(Cy5)**, 1.4 µM **r-Ib (F)**, 2.8 µM **r-IIb**, 2.8 µM **r-IIa$_{Ab}$** and 14 nM of target **DNA-I(X), X= T/G/C/A** or no target (-), T4 DNA ligase 1000 CEU per 7.5 µL ligation volume.