

Supporting Information

DNA Sensor's Selectivity Enhancement and Protection from Contaminating Nuclease due to Hydrated Ionic Liquid

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Materials. All oligodeoxynucleotides used in this study were high-performance liquid chromatography grade (Japan Bio Service). Single-strand concentrations of DNA oligonucleotides were determined from absorbance at 260 nm at 80 °C and single-strand extinction coefficients calculated from the mononucleotide and dinucleotide data according to the nearest-neighbor approximation model. The absorbance was measured using a Shimadzu 1700 spectrophotometer connected to a thermoprogrammer. The hydrated ionic liquid, choline dhp, was purchased from Ionic Liquids Technologies Co. Ltd. and used without further purification. The pH values of solution containing choline dhp and NaCl were controlled by 50 mM MES (pH 5.0 - 6.0) or Tris (pH 7.0). The pH of 4 M choline dihydrogen phosphate (dhp) solution without pH control is approximately 6.0 and it lowers to pH 7.0. As the amount of water was approximately 20 wt% in the 4 M choline dhp solution, the definition of pH might change between 4 M choline dhp solution and aqueous solution. We tried to control the pH using Tris and MES, and the solution pH was determined by a pH meter.

Melting Temperature Analysis. Ultraviolet (UV) absorbance was measured on a Shimadzu 1700 spectrophotometer equipped with a temperature controller. Melting curves at 260 and 295 nm were measured in buffers containing 50 mM Tris (pH 7.0), 1 mM Na₂EDTA, and 1 or 4 M NaCl or choline dhp. Samples were heated at a rate of 0.5 °C min⁻¹ after ensuring that melting curves were unaffected by heating rates between 0.2 and 0.5 °C min⁻¹ (data not shown). Before the measurements, the DNA samples were heated to 80 °C, cooled to 0 °C at a rate of -0.5 °C min⁻¹, and incubated at 0 °C for 30 min.

Fluorescence Measurements. Fluorescence spectra were collected on a JASCO FP-6500 fluorescence spectrophotometer at 1 to 2 μM probe DNA and different concentrations of the target duplex in buffers containing 50 mM Tris (pH 7.0), 1 mM Na₂EDTA, and 4 M NaCl or 4 M choline dhp. The spectra at 45 °C were obtained from 500 to 600 nm in a cuvette with a pathlength of 0.1 cm. All measurements were carried out with excitation at 494 nm, the excitation wavelength of 6-FAM (6-carboxyfluorescein). The temperature of the cell holder was regulated by a JASCO EHC-573 temperature controller. Before the measurement, the sample was heated to 80 °C, cooled at a rate of -2 °C min⁻¹ to 4 °C, and incubated at 4 °C for 30 min.

The fluorescence intensity is strongly dependent on solution pH. The experiments were performed in a solution containing 4 M choline dhp and 1 mM Na₂EDTA without pH control because we wanted to investigate DNA stability in a pure hydrated liquid. Consequently, the fluorescence intensity was dramatically decreased because of low pH values. Thus, our condition (solution in choline dhp was controlled at pH 7.0 by a pH meter) is suitable for the sensing system.

Measurements of Fluorescence correlation spectroscopy (FCS). Diffusion time of fluorescent oligonucleotides were measured by a Hamamatsu C9413-01 compact FCS unit incorporated with 473 nm LD-pumped solid state laser. The instrument is equipped with a water-immersion type Olympus objective lens and a detector having pulse pair resolution of 70 ns. For the FCS measurements, oligonucleotides were modified with Alexa Fluor 488 moiety to the 5' end. Experiments were carried out at room temperature for 10 nM probe (fluorescent oligonucleotides) in the absence or presence of the

target in 50 mM Tris (pH 7.0), 1 mM Na₂EDTA and 2 M choline dhp. To study the detection, target concentrations were varied in a range of 10 nM to 1000 μM for a fixed concentration of probe (10 nM). Obtained autocorrelation curves were fitted with an appropriate model function using the data analysis software to extract the characteristic time scale of the system.

Table S1. DNA sequences

Sequence name	sequence (5' to 3')
probe DNA1	F-CGAATCTTTTCTTTTCCCCCTGATTCG-B ^a
Ss1	CACTTTTATAGGGGGAAAAGAAAACTGGAA
Ss2	CACTTTTATAGGGGGAGAAGAAAACTGGAA
Ds1	CACTTTTAAAAGAAAAGGGGGGACTGGAA/ TTCCAGTCCCCCCTTTTCTTTTAAAAGTG ^b
Ds2	CACTTTTAAAAGAAGAGGGGGGACTGGAA/ TTCCAGTCCCCCCTCTTCTTTTAAAAGTG ^b
probe DNA2	F-TTTTCTTTTCCCCCT ^a

^a F and B indicated 5' fluorophore (6-carboxylfluorescein) and a 3' quencher (Black Hole Quencher 1), respectively.

^b Two DNAs form a full-matched DNA duplex.

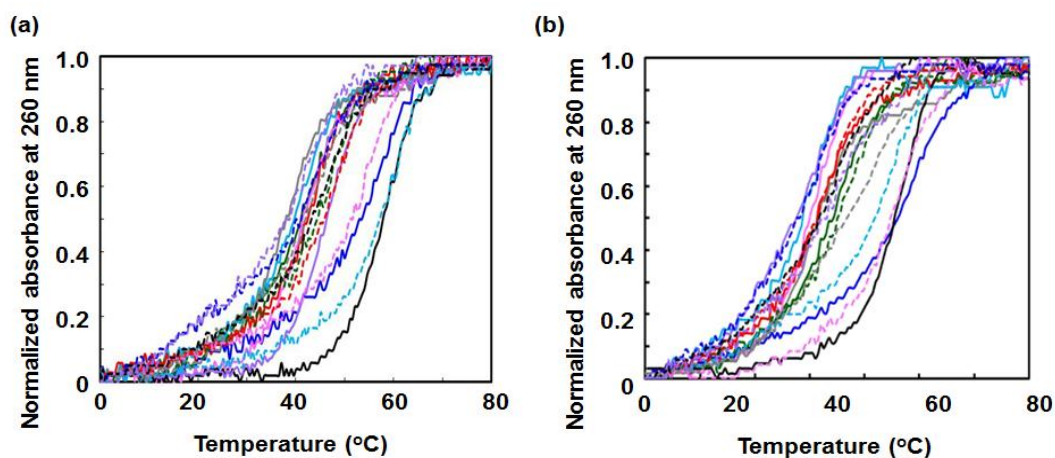


Fig. S1 Normalized UV melting curves for 20 μ M DNA duplexes with A-A (solid green), A-C (solid light blue), A-G (solid red), A-T (solid blue), C-A (solid pink), C-C (solid gray), C-G (solid black), C-T (solid purple), G-A (dashed green), G-C (dashed light blue), G-G (dashed red), G-T (dashed blue), T-A (dashed pink), T-C (dashed gray), T-G (dashed black), and T-T (dashed purple) base pairs in a buffer solution containing 50 mM Tris (pH 7.0), 1 mM Na_2EDTA and (a) 4 M NaCl or (b) 4 M choline dhp.

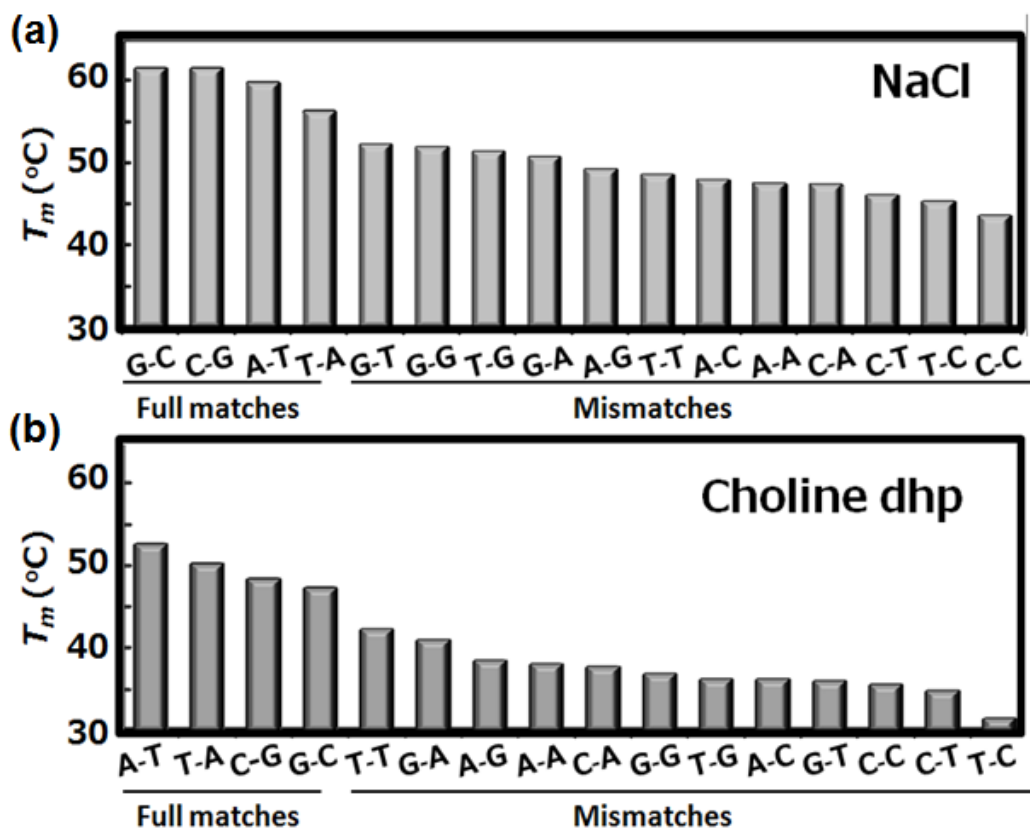


Fig. S2 (a) The T_m values for 20 μ M DNA duplexes in the solution containing 50 mM MES (pH 6.0), 1 mM Na_2EDTA , and 4 M NaCl. (b) The T_m values in 50 mM MES (pH 6.0), 1 mM Na_2EDTA , and 4 M choline dhp. The T_m values for the duplexes were arranged from the highest to the lowest.

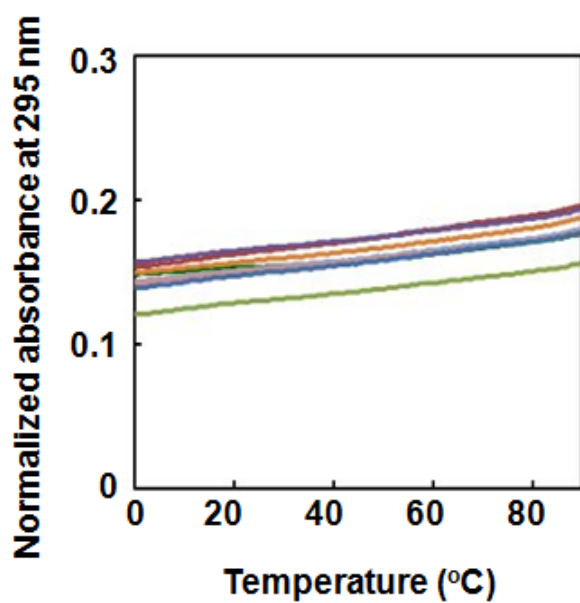


Fig. S3 Original UV melting curves at 295 nm for A*A (light blue), C*A (orange), G*A (pink), T*A (green), A*G (light green), C*G (red), G*G (purple), and T*G (blue) in a solution containing 50 mM Tris (pH 7.0), 1 mM Na₂EDTA, and 4 M NaCl. DNA strand concentration was 40 μ M.

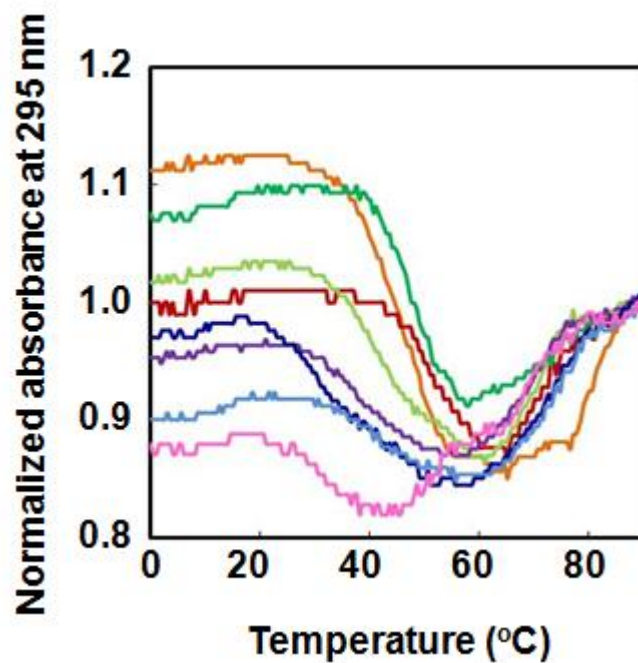


Fig. S4 Normalized UV melting curves at 295 nm for A*A (light blue), C*A (orange), G*A (pink), T*A (green), A*G (light green), C*G (red), G*G (purple), and T*G (blue) in a solution containing 50 mM Tris (pH 7.0), 1 mM Na₂EDTA, and 4 M choline dhp. DNA strand concentration was 40 μ M.

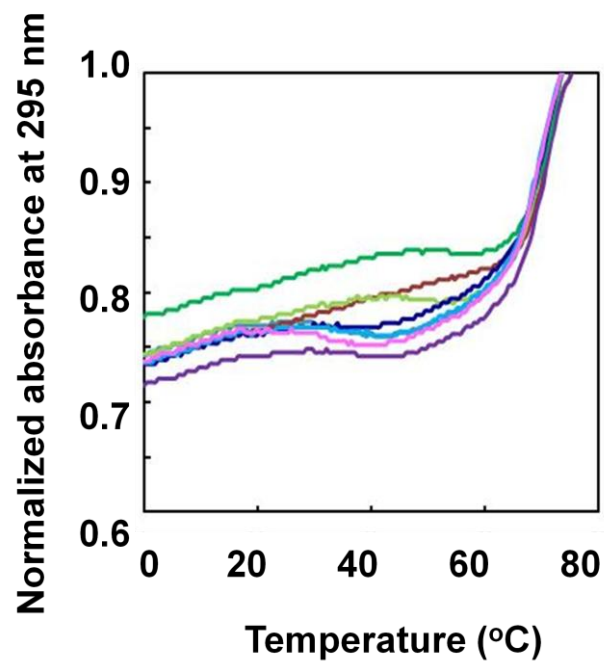


Fig. S5 Normalized UV melting curves at 295 nm for A*A (light blue), C*A (orange), G*A (pink), T*A (green), A*G (light green), C*G (red), G*G (purple), and T*G (blue) in a solution containing 50 mM MES (pH 5.0), 1 mM Na₂EDTA, and 100 mM NaCl. DNA strand concentration was 40 μ M.



Fig. S6 Sequences and sequence names of target strand (Ss1 or Ss2) with or without single mutation of base pairs (red letter) in the middle of the DNA.

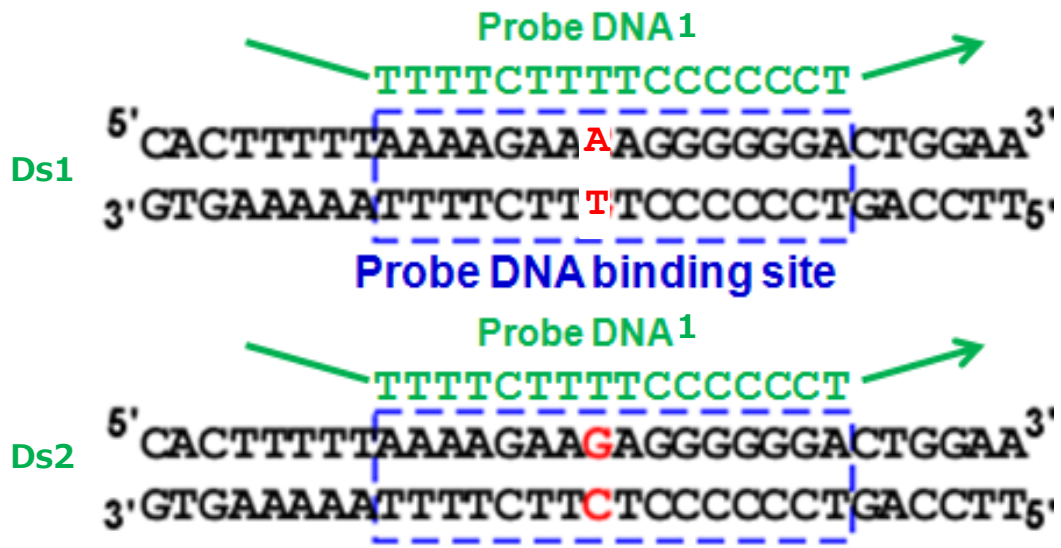


Fig. S7 Sequences and sequence names for the target duplexes (Ds1 or Ds2) with and without a single mutation of a base pair (red letters) in the middle of the duplex.

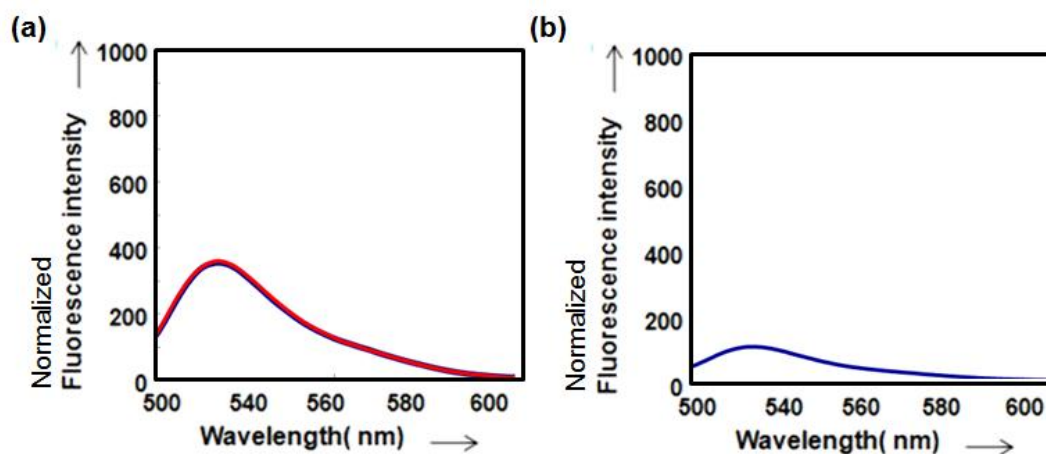


Fig. S8 Normalized emission spectra recorded at 45°C for 1 μ M probe in the absence (black) and presence of 2 μ M fully matched target sequence (blue) and with mismatched target (red). Probe DNA1 with (a) Ss1 or Ss2 and (b) Ds1 and Ds2 in a solution containing 50 mM Tris (pH 7.0), 1 mM Na₂EDTA, and 100 mM NaCl.

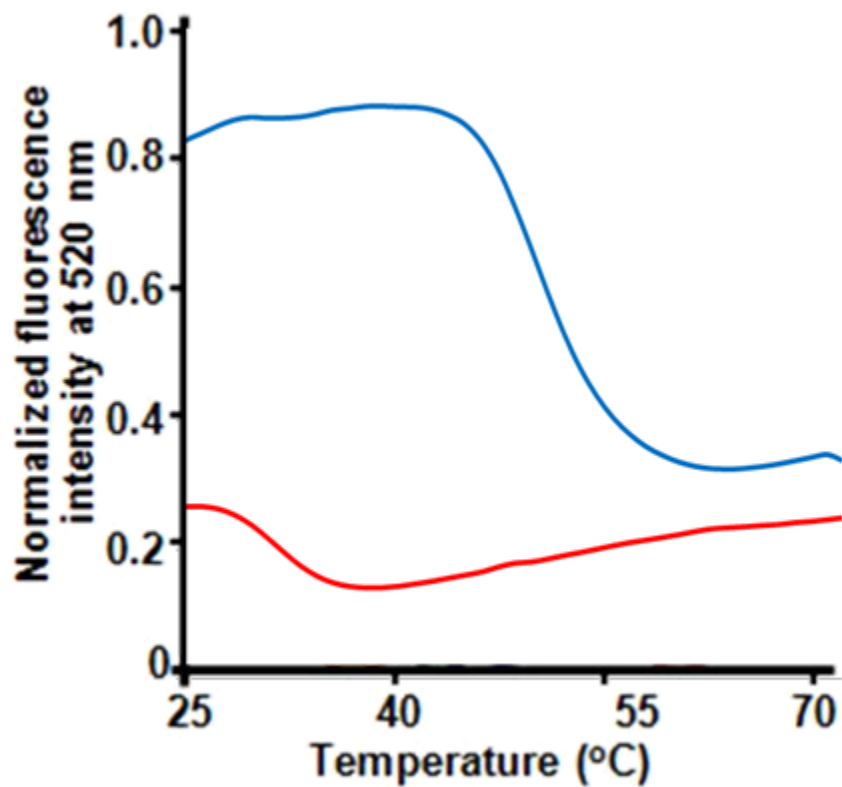


Fig. S9 Normalized fluorescence melting curves for the DNA triplexes of probe DNA 2 and Ds1 (blue) and Ds2 (red) in a solution containing 50 mM Tris (pH 7.0), 1 mM Na₂EDTA and 4 M choline dhp.

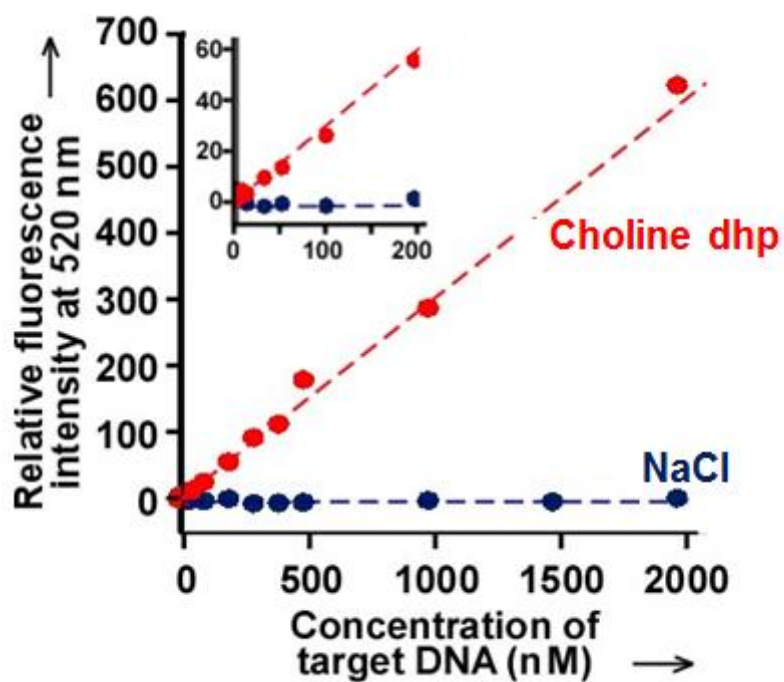


Fig. S10 Dependence of relative fluorescence intensities of 2 μ M probe DNA1 at 520 nm on the target duplex (Ds1) concentration in NaCl (blue) and choline dhp (red) solutions. Relative fluorescence at 520 nm was calculated by subtracting the fluorescence intensity at 520 nm obtained in the absence of the target duplex from that in the presence of the target duplex. Inset: Enlarged view of the plot for relative fluorescence intensity vs. concentration of target DNA at low concentrations. Buffer contained 50 mM Tris (pH 7.0), 1 mM Na₂EDTA and 4 M NaCl or 4 M choline dhp.

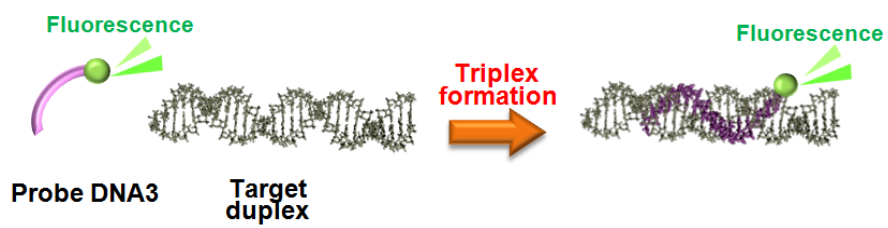


Fig. S11 Schematic of target duplex sensing using fluorescence correlation spectroscopy and probe DNA2 as the labelling probe.

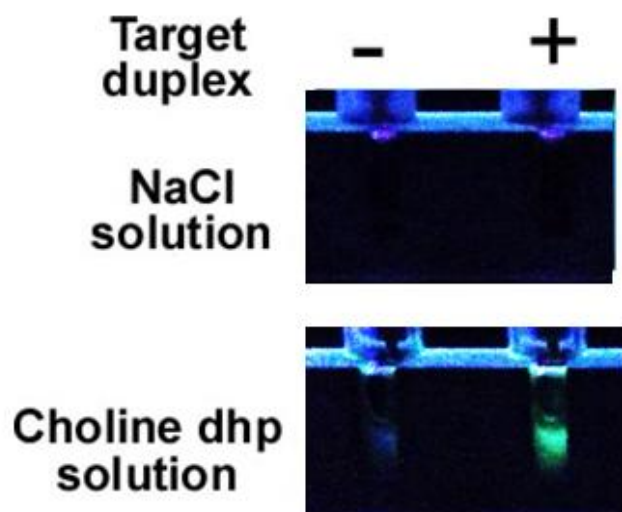


Fig. S12 (a) Photograph of 2 μM probe DNA2 fluorescence images in the absence (left) and presence (right) of 2 μM target duplex Ds1 in 4 M NaCl (upper panel) and choline dihydrogen phosphate (choline dhp) (lower panel) at 25°C. Buffer was 50 mM Tris (pH 7.0), 1 mM Na_2EDTA and 4 M NaCl or 4 M choline dhp.