

Supporting Information

The Effect of Microwave Irradiation on DNA Hybridization

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PROTOCOLS

General. All hybridization experiments were performed in a CEM Discover microwave reactor equipped with a Coolmate system, which circulated chilled ($-60\text{ }^{\circ}\text{C}$), microwave transparent fluid through a jacket reaction vessel to allow the maintenance of low temperatures during microwave irradiation. The CEM Discover system is a single-mode cavity allowing for focused delivery of microwave irradiation (Figure S1). All reactions were conducted at a constant frequency of 2450 MHz.



Figure S1. Left: CEM Discover single-mode microwave cavity. The sample is located in the center of the cavity and focused microwaves are generated and used to irradiate the sample. Right: CEM Discover equipped with a Coolmate. Images obtained from www.cem.com.

Initial Hybridizations. Dabcyl- and fluorescein-modified DNA oligomers were purchased lyophilized from Alpha DNA and re-suspended in distilled water to a stock concentration of $200\mu\text{M}$. Initial hybridization reactions contained $7\mu\text{M}$ of each labeled oligomer in $20\mu\text{L}$ of 10X hybridization buffer (100mM Tris-HCl, pH 8.3, 500mM KCl, 15mM MgCl_2) and distilled water to a final volume of $200\mu\text{L}$. Reactions were heated in an Eppendorf Mastercycler to 95°C for 5 minutes, 37°C for 30 minutes, and then cooled to 4°C to ensure complete hybridization.

Microwave Hybridizations. Non-labeled complementary DNA oligomers were purchased lyophilized from IDT DNA and re-suspended in distilled water to a stock concentration of 200 μ M. 40 μ L of the initial hybridization reactions were mixed with 7 μ M of each complement oligomer in 50 μ L of 10X hybridization buffer and distilled water to a final volume of 500 μ L. Each reaction was transferred to a microwave reaction vial containing a stir bar. Samples were cooled to -20 $^{\circ}$ C, irradiated at the appropriate power until the temperature of the reaction reached 20 $^{\circ}$ C, then cooled to 0 $^{\circ}$ C. In order to ensure that no single-stranded DNA breaks occurred and that alkali labile sites were not created during microwave irradiation, samples were run on a 13% denaturing PAGE gel. As expected, only one band was present for each power; no streaking or smears were evident, indicating that the aforementioned damage did not occur (Figure S2). We were unable to determine, however, whether the DNA sustained damage to individual bases.

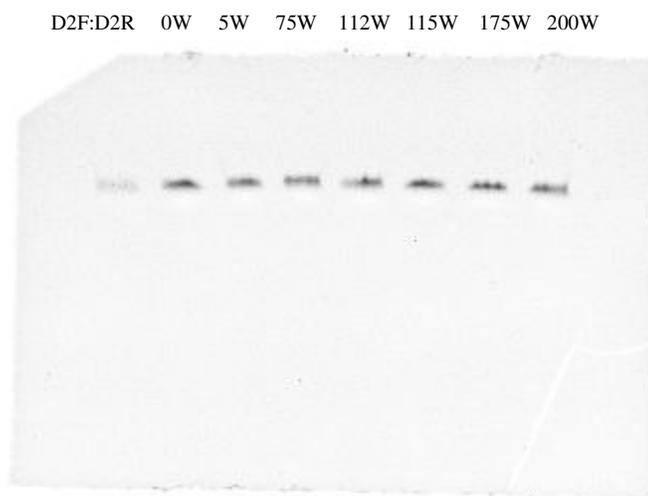


Figure S2. Microwaved DNA sustains no damage as detected with denaturing PAGE. Aliquots of D2F*:D2R*:D2F:D2R hybridizations were subjected to microwave irradiation and analyzed by PAGE through ethidium bromide staining. Only a single band is visible in each lane.

Thermal Hybridizations. Following initial hybridizations, thermal hybridization reactions were set up with non-labeled DNA complements as described for microwave hybridizations, then returned to the Eppendorf Mastercycler and heated to 95 $^{\circ}$ C for 5 minutes, 37 $^{\circ}$ C for 30 minutes, and then cooled to 4 $^{\circ}$ C to ensure complete hybridization.

Control Hybridizations. Control hybridizations were performed to mimic the temperature profile of the microwave experiment. Initial hybridizations were performed, and then complement oligomers were added as described above. Measuring the temperature with a thermocouple probe, reactions were chilled in a dry ice/ethanol bath to -20 $^{\circ}$ C, then placed in warm water until 20 $^{\circ}$ C, then returned to the dry ice/ethanol bath until 0 $^{\circ}$ C.

Determining Melting Temperatures. Actual T_m s were calculated for the fluorescein-labeled:dabcyl-labeled oligo pairs using a BioRad MyiQ RT-PCR thermocycler and performing three heating and cooling cycles (a total volume of 25 μ L; 30 $^{\circ}$ C to 80 $^{\circ}$ C with a 0.5 $^{\circ}$ C/min ramp). All samples were measured in triplicate and averaged to determine the T_m . The fluorescence increase due to DNA melting and the subsequent dequenching of fluorescein was measured to afford a standard melting curve. To confirm this data, melting curves were also obtained with the non-modified oligonucleotides (10 μ M of both DNA and complementary DNA with 12.5 μ L iQ SYBR Green Supermix to a total

volume of 25 μ L, and melting temperatures were found to be identical to those obtained using the fluorescently modified oligomers (within the error margin of the experiment).

REPRESENTATIVE POWER/TEMPERATURE/PRESSURE PROFILES

