Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

for

Fluorescence Turn-on Hairpin-Probe PCR

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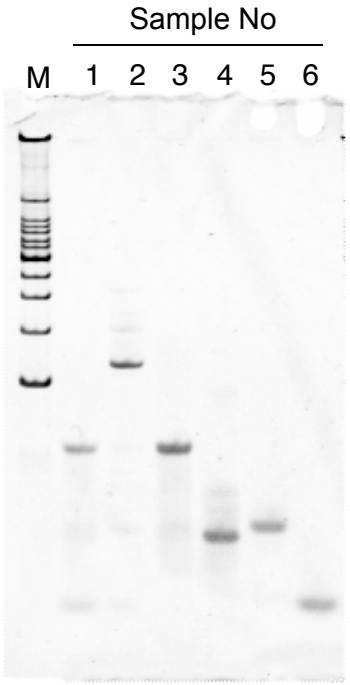

Fig. S1 Native PAGE analysis of PCR solutions using Hpro w/wo tag(0). Sample No 1: 0 cycle of PCR solution, No 2: 40 cycle of PCR solution. No 3 to No 6 were solutions of primers w/wo Hpro. No 3: Hpro with tag(0), No 4: tag(0), No 5: Hpro. No 6: M13M3. The PCR was done Taq PCR master Mix Kit (QIAGEN).

Experimental Section

Measurement of melting temperature (T_m)

All melting temperatures (T_m) of the Hpro (5 μM, final duplex concentration) w/wo tag-primer (5 μM, final concentration) were determined in 10 mM cacodylate (pH 7.0) containing 100 mM NaCl with DANP (20 μM). Absorbance profiles were measured at 260 nm using a Shimadzu UV-2550 spectrometer equipped with a temperature controller using a 1-cm path length cell. The absorbance of the samples was monitored at 260 nm from 2 to 90 °C with a heating rate of 1 °C/min.

PCR on pUC18 plasmid

PCR on the pUC18 plasmid was performed with a forward primer, M13M3 (5'-d(GTT GTA AAA CGA CGG CCA GT)-3'), and a reverse primer with tag and Hpro. The amplification reaction was carried out with 0.5 μM of each primer, 0.5 μM of probe, 5 μM of DANP, Taq PCR Master Mix Kit (QIAGEN), and pUC18 (100 pg/μL) in a final volume of 40 μL. The amplification protocol was 95 °C for 1 min, and 40 cycles of 95 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s on a TP600 PCR Thermal Cycler (TAKARA). All PCR products were analyzed by native PAGE (8% polyacrylamide gel stained with SYBR gold). Fluorescence intensities of PCR solutions after certain cycles were measured on a BERTHOLD Mithras LB 940 microplate reader with 400 nm excitation and 450 nm emission filters.