Electronic Supplementary Information

Fluorescence detection of polyadenylation reaction and single nucleotide polymorphisms using an oligonucleotide-based fluorogenic probe

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Experimental Section

Chemicals. Berberine chloride, palmatine chloride hydrate, coralyne chloride hydrate trisodium citrate, citric acid, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), hydrogen tetrachloroaurate (III) dehydrate, NaCl, Na_2CO_3, NaH_2PO_4, and Na_2HPO_4 were obtained from Sigma-Aldrich (St. Louis, MO, USA). All DNA samples were synthesized from Neogene Biomedicals Corporation (Taiwan). SYBR Green I (SG) was purchased from Molecular Probe Inc. (Portland, OR). AmpliScribe T7-Flash transcription kit and poly(A) polymerase tailing kit were ordered from Epicentre Biotechnologies (Wisconsin, USA). Milli-Q ultrapure water (Milli-pore, Hamburg, Germany) was used in all of the experiments.

Sample preparation. A solution of SG (10000×) was diluted to 250× with dimethyl sulfoxide. The resulting solution was diluted to 6.25× with ultrapure water in order to make a stock solution. Based on the previous study,[22] the concentration of a solution of 6.25× SG is calculated to be 1.23×10^{-5} M. We added berberine, palmatine or coralyne (1.0×10^{-5} M, 50 μL) to 450 μL of 100 mM HEPES solution (pH 7.0) containing 200 mM NaCl, 160 nM SG, and 1 μM A_{40}. To investigate the effect of the DNA length and sequence on our analytical system, we replaced A_{40} with A_5, A_{10}, A_{20}, A_{30}, A_{40}, A_{50}, or A_{60}, once at a time. The resulting solutions were equilibrated for the optimum incubation time. The fluorescence spectra of SG were measured using a

Hitachi F-4500 fluorometer (Hitachi, Tokyo, Japan) while the excitation wavelength was set to 494 nm.

Monitoring of polyadenylation: By using linearized plasmid DNA as a template, RNA was transcribed with an AmpliScribe T7-Flash transcription kit. Briefly, template DNA with appropriate promoter was added to a solution containing AmpliScribe T7-flash reaction buffer, RNase inhibitor, nucleoside triphosphate, dithiotheritol, and AmpliScribe T7-flash enzyme. The mixture was incubated at 37ºC for 1 h. After transcription, we treated the produced RNA with DNase I to remove DNA template. Polyadenylation of the 3´-end of the produced RNA was performed using poly(A) polymerase tailing kit. An aliquot of poly(A) polymerase (1 and 4 unit/μL; 1 μL) was incubated with a solution containing the produced RNA, adenosine triphosphate, and RNase inhibitor at 37°C. A portion of the mixture was added to a solution containing 1 μM coralyne, 160 nM SG, and 1 μM A_{40} at 10-min intervals over a period of 1 h. The fluorescence spectra of the resulting solutions were recorded after 10 min incubation.

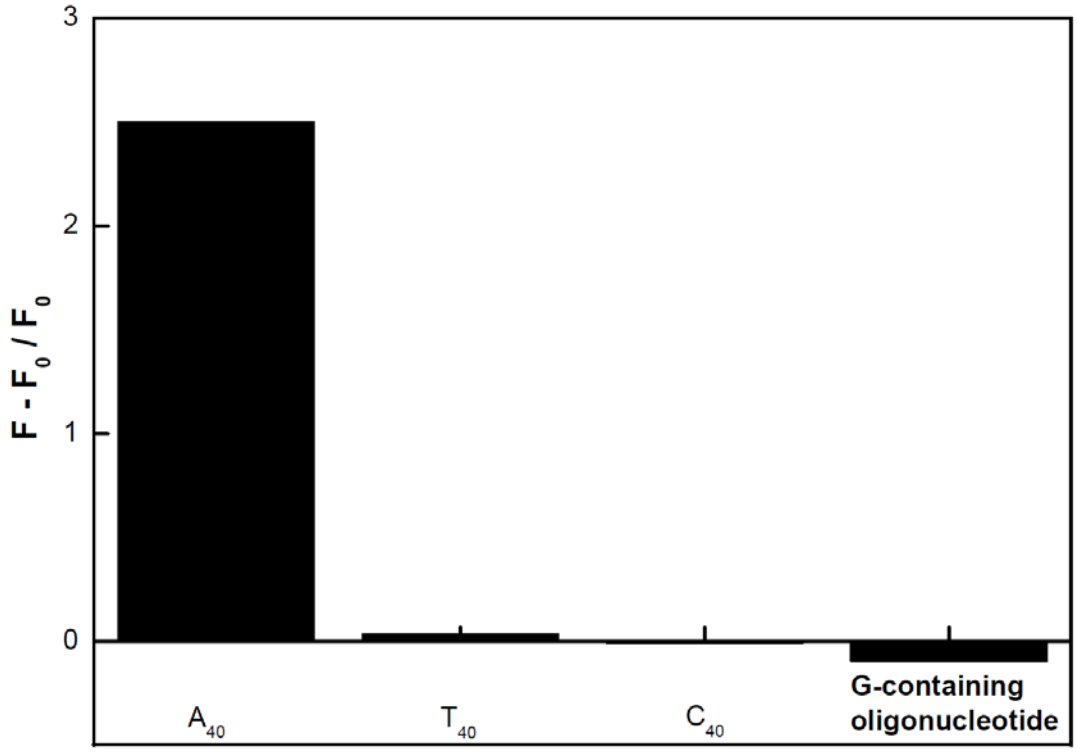

Fig. S1 Relative fluorescence increases [$(F - F_0)/F_0$] at 521 nm of a solution of SG and DNA samples—including A_{20}, T_{33}, C_{40}, and G-containing oligonucleotide (5′-GGG TTA GGG TTA GGG TTA GGG-3′)— after the addition of 1 μM coralyne. A mixture of 160 nM SG and 1 μM A_{40} was prepared in 100 mM HEPES and 200 mM NaCl at pH 7.0. The incubation time is 10 min. F_0 and F correspond to the fluorescence intensity of the SG and oligonucleotide solution in the absence and presence of coralyne, respectively.

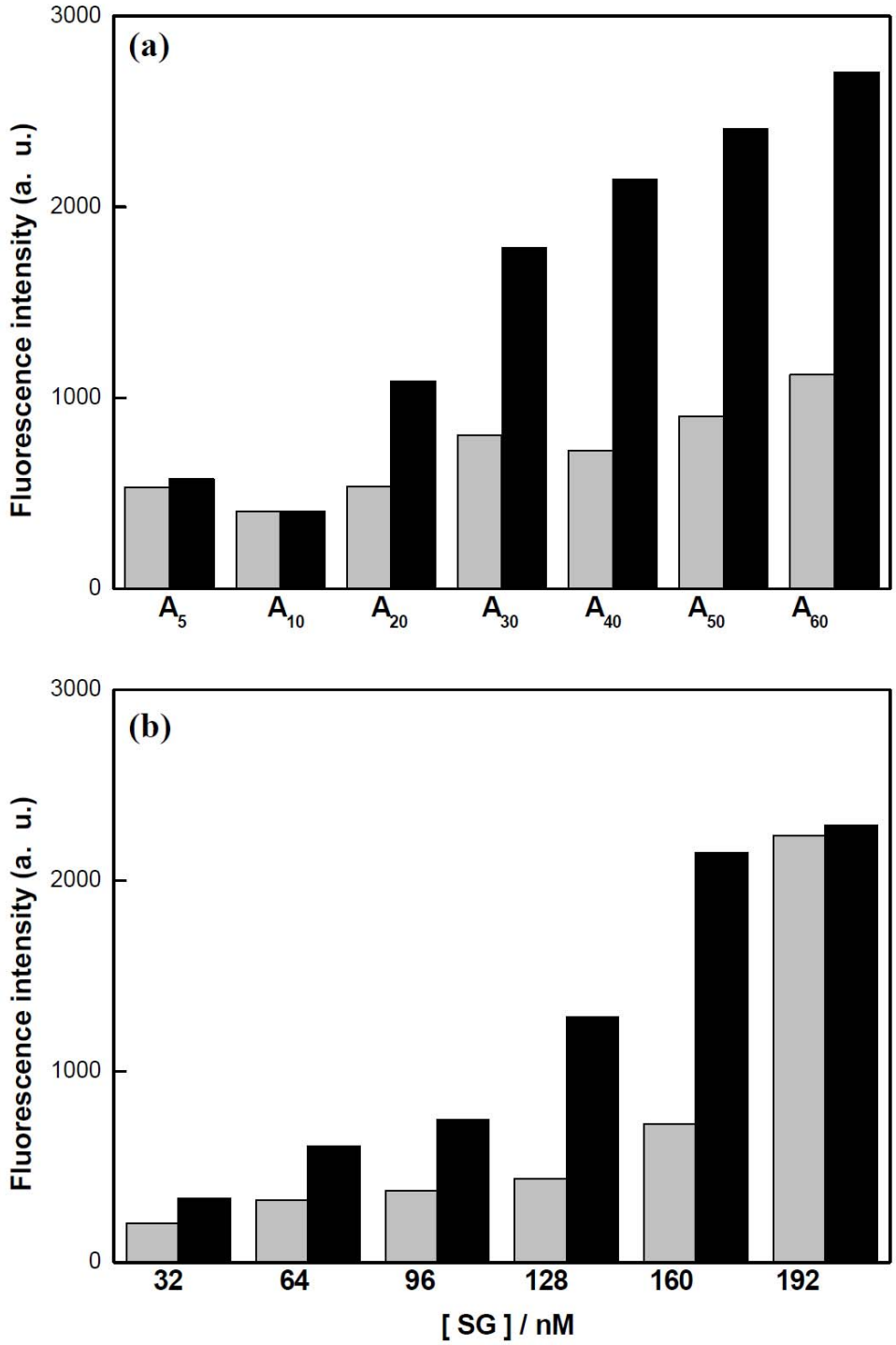

Fig. S2 Fluorescence intensity at 521 nm of solutions of (a) 160 nM SG and A_5-A_{60} (b) 32-192 nM SG and A_{40} in the absence (gray bar) and presence (black bar) of 1 μM coralyne. A mixture of SG and A_{40} was prepared in 100 mM HEPES and 200 mM NaCl at pH 7.0. The incubation time is 10 min.

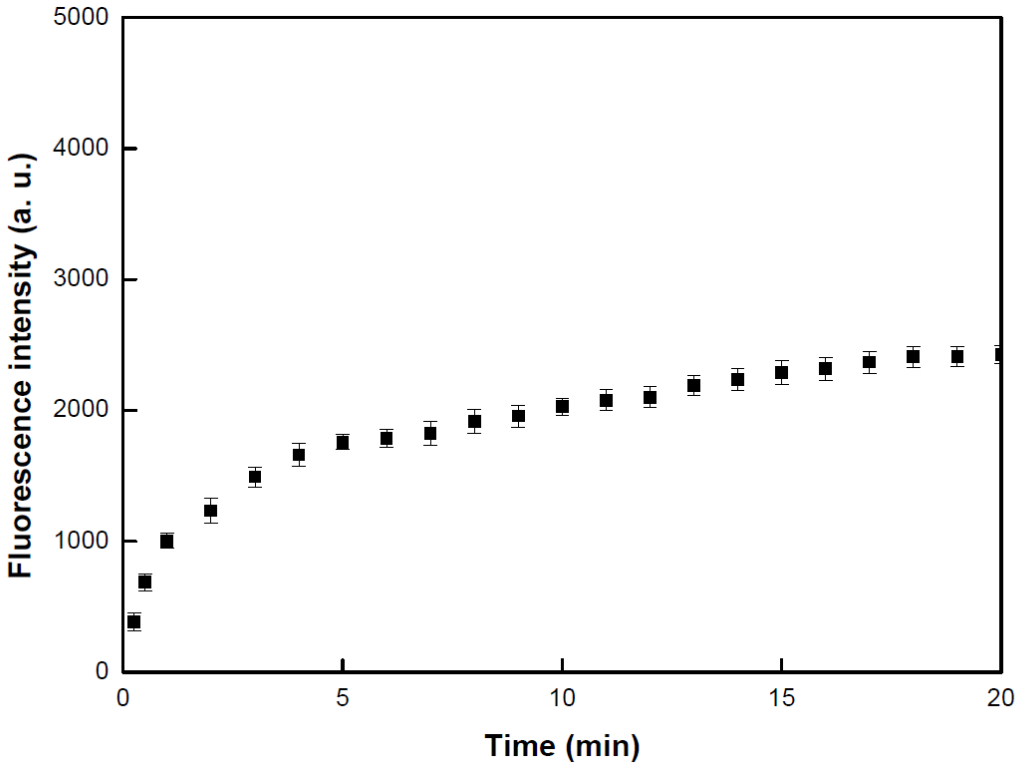

Fig. S3 Temporal change in the fluorescence intensity at 521 nm of a solution of SG and A_{40} on the addition of 1 μM coralyne. A mixture of 160 nM SG and 1 μM A_{40} was prepared in 100 mM HEPES and 200 mM NaCl at pH 7.0.

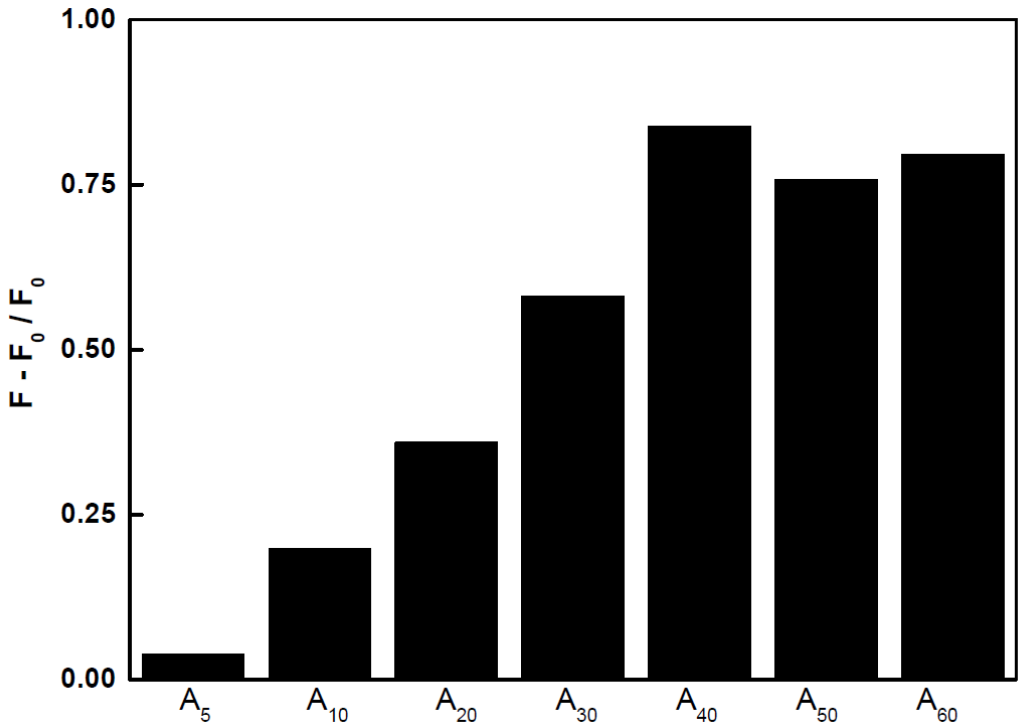

Fig. S4 The value of $(F - F_0)/F_0$ at 521 nm of a solution of 160 nM SG and 1 μM coralyne after the addition of different length of poly(A). A mixture of 160 nM SG and 1 μM coralyne was prepared in 100 mM HEPES and 200 mM NaCl at pH 7.0. The incubation time is 10 min. F_0 and F correspond to the fluorescence intensity of the SG and coralyne solution in the absence and presence of poly(A), respectively.