Supplementary Material for:

Quantitative and Discriminative Analysis of Nucleic Acid Samples Using Luminometric Nonspecific Nanoparticle Methods

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Labeling of Amino-modified Polystyrene Nanoparticles with Eu^{3+} Chelate

Amino-modified polystyrene nanoparticles were labeled with 9-dentate Eu^{3+} chelate, {2,2',2'',2'''-{[4'-(4'''-isothiocyanatophenyl)-2,2':6,6''-terpyridine-6,6''-diyl]bis(methylenenitrilo)}tetrakis-(acetato)} europium. Before labeling, the nanoparticles were washed by centrifugation (19,000g for 5 min) four times. The 9-dentate (60 nmol) Eu^{3+} chelate was added to 120 µL of 40 mM carbonate buffer, pH 10, containing 82 fmol of nanoparticles. After overnight incubation, the nanoparticles were washed in water by centrifugation (19,000g for 5 min) several times. The number of Eu^{3+} chelates per nanoparticle (66 000 per nanoparticle) was determined with DELFIA enhancement solution, as recommended by the manufacturer, except that the europium ions were first dissociated from the chelates in 1 M hydrochloric acid before mixing and enhancing with DELFIA.

Optimization of Nucleic Acid Binding Protein for Quenching Assay

The optimal protein was chosen in two tests. In the first test, different proteins (BSA, γ-gammaglobulin, histone, lysozyme, and thyroglobulin) were tested as described in the article. For the second test, see supporting information for the details. In the second test, nucleic acid binding proteins were investigated to obtain efficient adsorption to nanoparticles, high signal-to-background ratio, and sensitive detection of both DNA and RNA. Therefore, 70 μL of 0, 5, or 500 μg/L DNA sodium salt from calf thymus (dsDNA) or RNA from yeast (approximately 85 nucleotides, ssRNA) in 3 mM acetate buffer, pH 5.0, and 5.0 μL of the Eu^{3+} surface-labeled nanoparticles (5.5 amol) were mixed. Finally, 7.0 μL of 140 μg/L one of the different proteins (protamine or arginine rich, lysine rich, or unfractionated histone) and 10 μL of 1.0 mM $NiCl_2$ were added.

Ratiometric Measurement of DNA and RNA with Quenching Assay

The quantification of DNA in the presence of RNA was demonstrated. RNase A was applied instead of histone to degrade RNA and simultaneously adsorb DNA. In the demonstration, different concentrations of DNA sodium salt from calf thymus (dsDNA), RNA from yeast, or their 1:1 mixture in 3 mM acetate buffer, pH 5.0, and the Eu^{3+} surface-labeled nanoparticles were mixed in 75 μL. After mixing, 7.0 μL of 400 μg/L RNase A and 10 μL of 1.0 mM $NiCl_2$ were added.

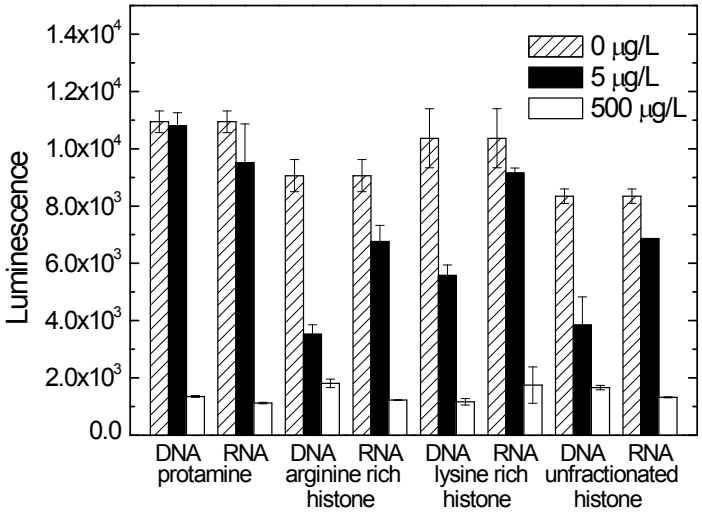

Figure S1. Effect of adsorbing nucleic acid binding proteins on the quenching-based assay performance and detection sensitivity. The europium luminescence in the assay with protamine and different histone fractions in the presence of varying concentration of sample DNA or RNA.

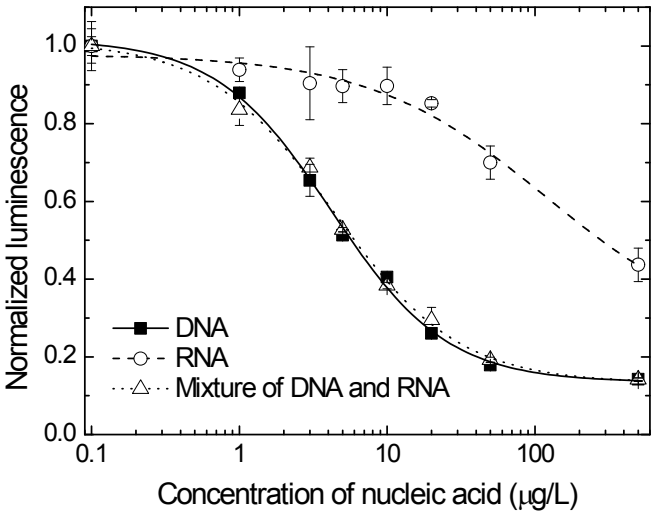

Figure S2. Calibration curves of the DNA selective quantification assay measured for DNA, RNA, and their 1:1 mixture with the quenching-based DNA quantification assay, in which RNase A is applied instead of histone. The data were fitted to a logistic function.