Electronic supplementary information

Sequence-selective extraction of single-stranded DNA using DNA-functionalized reverse micelles

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Experimental

Dilauroyl-sn-glycero-3-phosphocholine (DLPC) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Synthesized DNA oligonucleotides were purchased from Tsukuba Oligo Service Co., Ltd (Ibaraki, Japan). Fluorophore-labeled DNAs were purified by reversed-phase HPLC. The DNA manufacturer guaranteed that the fluorophore-labeled DNA did not contain a free fluorophore (unconjugated to DNA). Other chemicals were of analytical grade.

5'-aminated DNA oligonucleotides (0.5 mM) in phosphate buffer (50 mM, 10 μl) were mixed with dimethylsulfoxide solution (54 μl) containing oleic acid N-hydroxysuccinimide ester (1 mM). Following incubation of the mixtures at 40ºC for 24 h, the synthesized 5'-oleoyl DNA oligonucleotides (DNA surfactants) were purified by HPLC using an ODS column, meaning that the unmodified DNA was not present in the purified DNA-surfactant. The synthesis of each DNA surfactant was confirmed by MALDI-TOF-MS analysis.

The liquid/liquid extraction of single-stranded DNA using reverse micelles coupled with the DNA-surfactant was typically carried out as follows. The aqueous phase was a Tris-HCl buffer (pH 8, 10 mM) containing EDTA (1 mM), MgCl2 (10 mM), a fluorophore-labeled target DNA (25 nM) and DNA-surfactant (25 nM). The organic phase was 2,2,4-trimethyl pentane containing dilauroylphosphatidylecholine (DLPC, 10 mM) and 1-hexanol (3 vol%), where DLPC was used as a zwitterion surfactant and 1-hexanol was employed as a co-surfactant. One ml of the organic phase was added by pouring to 1 ml of the aqueous phase. The two phases were gently stirred at 25ºC for 3 h not to disrupt the interface between two phases. After a while, each phase was separately withdrawn using a
syringe and transferred to a 0.7ml-quartz cuvette. The fluorescence of a target DNA in each phase was measured using and a luminescence spectrometer LS 50B (Perkin Elmer) at 25 °C to determine the concentration of a target DNA. Excitation and emission wavelengths were 494 nm and 520 nm for FITC, 559 nm and 586 nm for TAMRA, 649 nm and 670 nm for Cy5, respectively. Karl-Fisher analyses revealed that the organic phase contained 25 g/l water. All experiments were carried out in triplicate and error bars in figures represent standard deviations.

The extraction of non-labeled DNA using reverse micelles was carried out as follows. The aqueous phase was Tris-HCl buffer (pH 8, 10 mM), EDTA (1 mM), MgCl₂ (10 mM), a non-labeled target DNA (22-nt, 25 nM) and DNA-surfactant 1 (25 nM). The sequence of the non-labeled DNA is identical to that of Target 1a. The organic phase was 2,2,4-trimethyl pentane containing DLPC (10 mM) and 1-hexanol (3 vol%). One ml of the organic phase was added by pouring to 1 ml of the aqueous phase. After the two phases were gently stirred at room temperature for 3 h, the organic phase (0.8 ml) was taken from the mixture and then added by SYBR Green I stock solution (0.1 μl). After gently stirring for 1 h, the fluorescence due to SYBR Green I (Ex. 494 nm) was measured in the organic phase at room temperature.

The unextracted DNA in the aqueous phase was mixed with its complementary strand (25 pmol) and the resultant duplex was stained by SYBR Green I, followed by fluorescence measurements (Ex. 494 nm and Em. 528 nm) to determine the percent extraction.