Electronic Supplementary Information (ESI)

Nanoporous scaffold for DNA polymerase: Pore-size optimisation of mesoporous silica for DNA amplification

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Experimental Procedures

Preparation of mesoporous silica

We prepared different mesoporous silica capsules for *Taq* DNA polymerase: two types of folded-sheet material (FSM; FSM2.6 and FSM4.2 with pore diameters of 2.6 and 4.2 nm, respectively) and five types of Santa Barbara amorphous (SBA; SBA5.4, SBA7.1, SBA10.6, SBA18.5, and SBA24.5, with pore diameters of 5.4, 7.1, 10.6, 18.5, and 24.5 nm, respectively).

FSM2.6 and FSM4.2 were synthesised from kanemite [$NaHSi_2O_5{\cdot}3H_2O$] using hexadecyltrimethylammonium chloride (HDTMACl) [$C_{16}H_{33}N(CH_3)_3Cl$] and docosyltrimethylammonium chloride (DCTMACl) [$C_{22}H_{45}N(CH_3)_3Cl$], respectively, according to a method reported previously.[13b,13d] Briefly, 3.2 g of HDTMACl or 4.2 g of DCTMACl was added to 100 mL of water at 70 °C, and the mixtures were vigorously stirred for 30 min at 70°C. Next, 5 g of kanemite was added to each mixture, followed by stirring for 3 h at 70 °C. The pH of these FSM mixtures was adjusted to 8.5 by slowly adding 2 $mol{\cdot}L^{-1}$ HCl aqueous solution during stirring. After the suspensions had been stirred for 3 h at 70 °C, the solid products were isolated by filtration, washed three times with 400 mL of distilled water at 70 °C, and dried at 45 °C. The samples were then calcined at 550 °C for 6 h in air.

SBA5.4, SBA7.1, and SBA10.6 were synthesised from tetraethyl orthosilicate (TEOS) using the triblock copolymer poly(ethyleneglycol)-block-poly(propylene glycol)-block-poly(ethyleneglycol) with the composition $EO_{20}PO_{70}EO_{20}$ (Pluronic P123).[13a,13d] During a typical synthesis, 10 g of Pluronic P123 was dissolved in 300 mL of water, and the resultant mixture was stirred overnight at 35 °C. Then, 21.9 g of 12 N HCl and 21.3 g of TEOS were added and the mixture was stirred for 20 h at 35 °C. After stirring, the mixture was aged for 24 h at 35, 80, and 130 °C for SBA5.4, SBA7.1, and SBA10.6, respectively. The solid products from the SBA series were filtered, washed three times with 400 mL of distilled water at 80 °C, and dried at 45 °C. The samples were calcined in air at a heating rate of 105 °C/h and held at 550 °C for 10 h.

SBA18.5 and SBA24.5 were prepared according to a method reported previously,[13c] with some modifications. Briefly, SBA18.5 and SBA24.5 were synthesised from TEOS using Pluronic P123 and mesitylene. Four grams of Pluronic P123 was added to 120 mL of water, followed by 6.1 g of KCl; this mixture was stirred at room temperature until it became translucent. Next, 23.6 g of 12 N HCl and 3 g of mesitylene were added, and the mixture was stirred for 2 h at room temperature. After adding 8.5 g of TEOS to the above solution, the mixture was stirred vigorously for 10 min at room temperature, aged for 24 h at 35 °C and subsequently aged for an additional 24 h at 100 and 130 °C for SBA18.5 and SBA24.5, respectively. The solid products from the SBA series were filtered, washed three times with 400 mL of distilled water at 80 °C, and dried at 45 °C. The samples were calcined in air at a heating rate of 60 °C/h and held at 500 °C for 6 h.

Characterization of mesoporous silica

Nitrogen adsorption and desorption measurements were performed at –196 °C on BELSORP-max gas adsorption apparatus (BEL Japan Inc., Osaka, Japan) to evaluate the pore diameters, pore volumes, and specific surface areas of the calcined mesoporous silica. The pore-size distributions were determined by analysing the adsorption branch according to the Barrett-Joyner-Halenda (BJH) method (E. Barrett, L. Joyner and P. Halenda, *J. Am. Chem. Soc.*, 1951, **73**, 373–380.). The specific surface areas were calculated using the Brunauer-Emmett-Teller (BET) method (S. Brunauer, P. Emmett and E. Teller, *J. Am. Chem. Soc.*, 1938, **60**, 309–319.), with adsorption data ranging from $P/P_0 = 0.05$ to $P/P_0 = 0.25$.

To confirm the particle and pore morphologies of the mesoporous silica particles, scanning electron microscopy (SEM) was performed. Micrographs of the mesoporous silica were obtained using a field-emission scanning electron microscope (S-4800; Hitachi High-Technologies Corp., Tokyo, Japan) with an acceleration voltage of 1 kV.

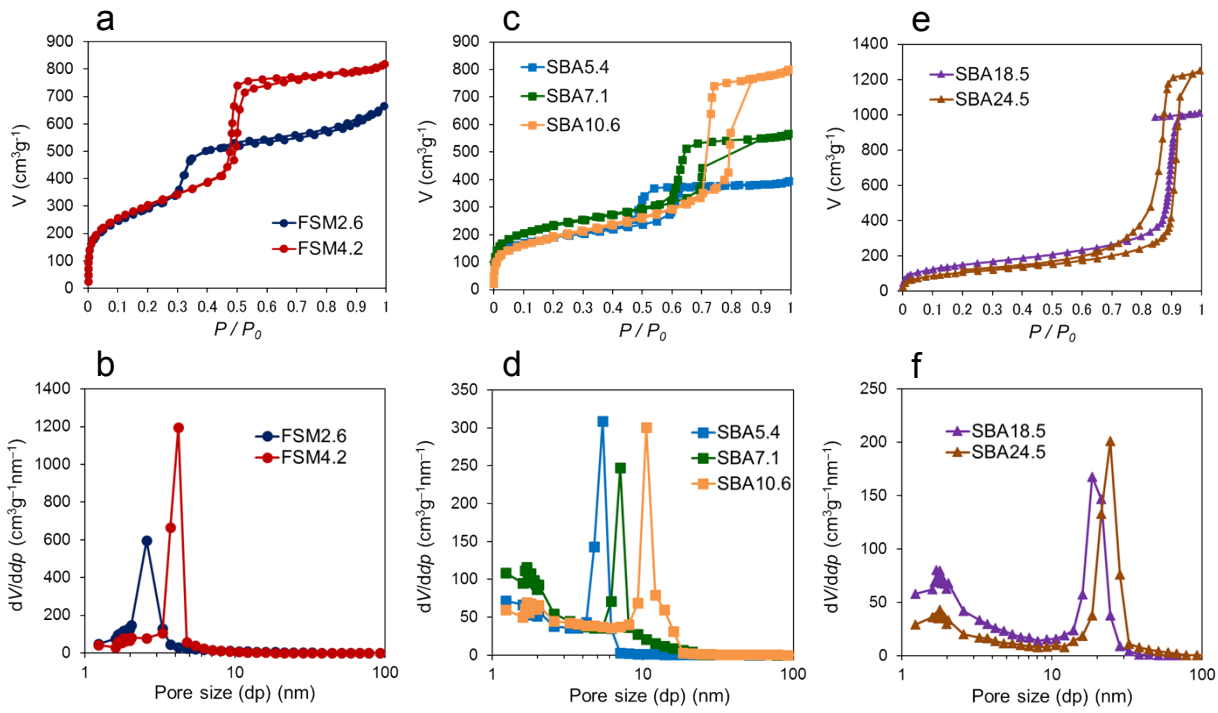

Fig. S1 Nitrogen adsorption-desorption isotherms and the corresponding pore-size distribution curves for the mesoporous silicas: (a, b) FSM2.6, FSM4.2, (c, d) SBA5.4, SBA7.1, SBA10.6, and (e, f) SBA18.5, SBA24.5.

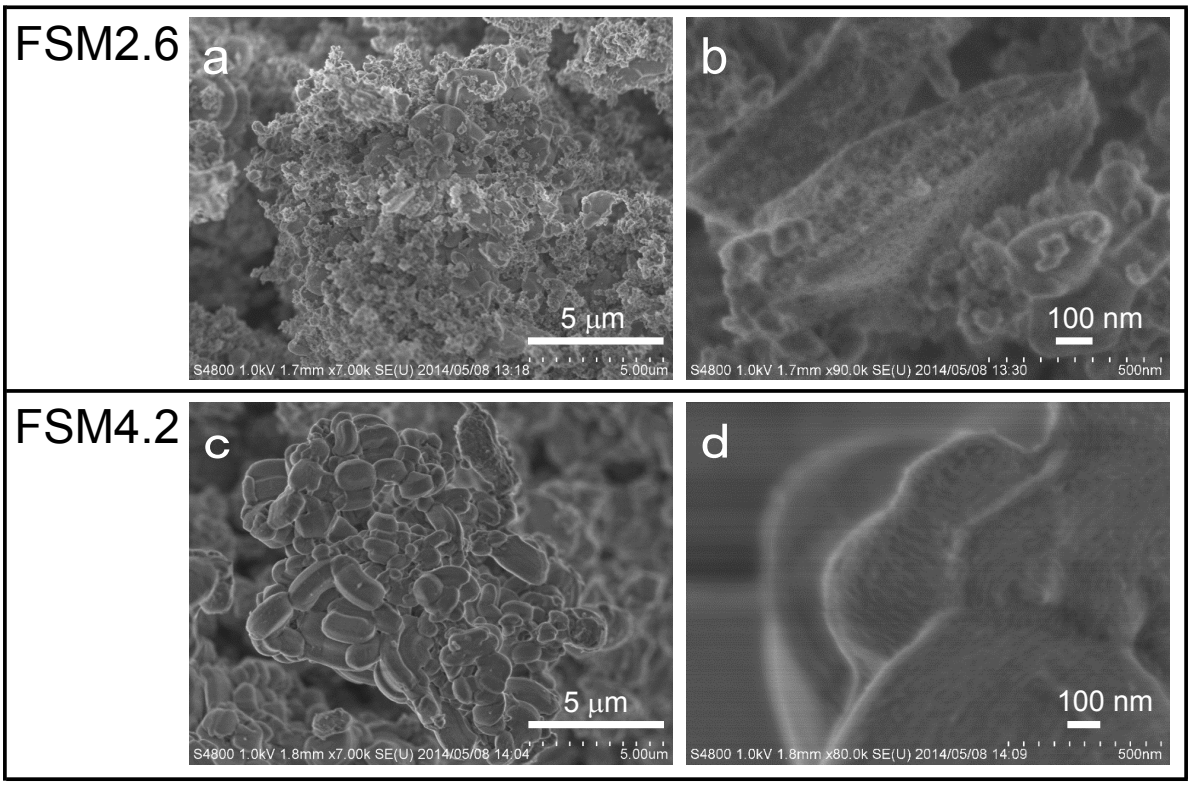

Fig. S2 SEM images of the mesoporous silicas: (a, b) FSM2.6 and (c, d) FSM4.2.

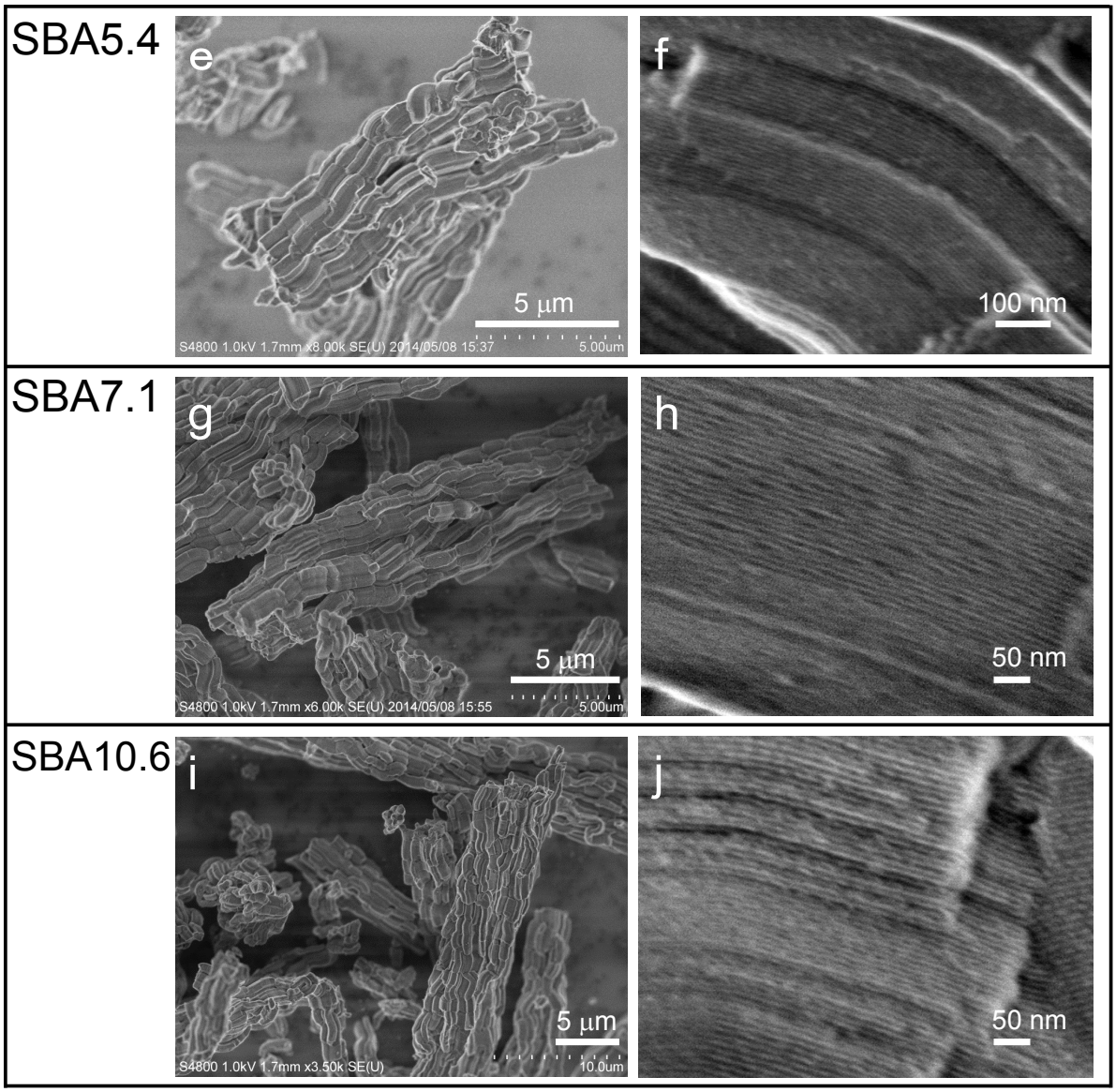

Fig. S3 SEM images of the mesoporous silicas: (e, f) SBA5.4, (g, h) SBA7.1, and (i, j) SBA10.6.

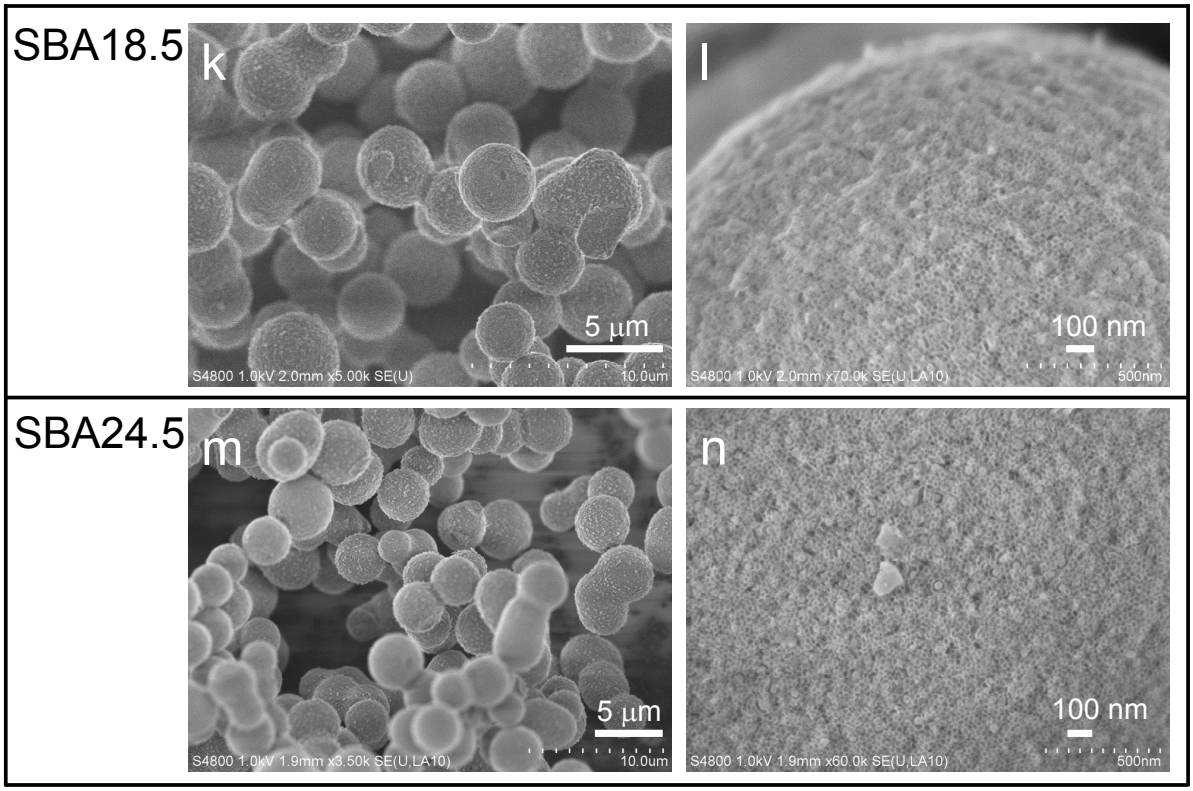

Fig. S4 SEM images of the mesoporous silicas: (k, l) SBA18.5 and (m, n) SBA24.5.

Preparation of substrate DNA

Template DNA derived from the polylinker region of the pcDNA3 plasmid was obtained as a synthetic single-stranded DNA from Life Technologies (Carlsbad, CA, USA). The T7 and SP6 promoter primers (Life Technologies) were used to amplify the 100-bp double-stranded DNA as the substrate DNA. The sequences of the template DNA and primers used are as follows:[9]

Template single-stranded DNA, 5′-TAATACGACTCACTATAGGGAGACCCAAGCTTG GTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTCCTATAGT GTCACCTAAATC-3′; T7 promoter primer (20-mer), 5′-TAATACGACTCACTATAGGG-3′; SP6 promoter primer (19-mer), 5′-GATTTAGGTGACACTATAG-3′.

To prepare the 100-bp double-stranded substrate DNA, PCR amplification was performed using the above template DNA and primers. The reaction mixture (50 μL) contained 0.5 mg mesoporous silica, 5 U *Taq* DNA polymerase (TaKaRa Ex *Taq*), 5 ng template DNA (100-base single-stranded DNA), 0.4 mM each dNTP, 0.75 μM T7 promoter primer, 0.75 μM SP6 promoter primer, and Ex *Taq* buffer containing BSA. PCR amplification was then performed using the following cycling conditions: 1 min at 94 °C; 50 cycles of 30 s at 94 °C, 60 s at 50 °C, and 30 s at 72 °C; followed by 5 min at 72 °C. PCR products were then separated on a 2% low melting point agarose gel and stained with ethidium bromide (Bio-Rad Laboratories, Inc. Hercules, CA, USA). The PCR product (*i.e.*, 100-bp substrate DNA) was then extracted from the gel and purified using Wizard SV Gel and PCR Clean-Up System (Promega Corp., Madison, WI, USA).

PCR amplification using DNA polymerase-mesoporous silica composites and evaluation of the adsorption of Taq DNA polymerase to mesopores after the PCR amplification

A batch adsorption experiment was performed by combining 0.5 mg of each mesoporous silica powder with 42.5 μL of *Ex Taq* buffer solution (pH 8.5) containing 5 U *Taq* DNA polymerase (TaKaRa Ex *Taq*), 5 ng substrate DNA (100-bp dsDNA), and 0.4 mM each dNTP. The *Taq* DNA polymerase was adsorbed onto mesoporous silica by mixing the two together, and then PCR amplification was started by the addition of 7.5 μL of each primer (T7 and SP6 promoter primers, 0.75 μM each). The PCR amplification conditions were as follows: 1 min at 94 °C, 30 cycles of 30 s at 94 °C, 60 s at 50 °C, and 30 s at 72 °C followed by 5 min at 72 °C. After amplification, the immobilisation state of *Taq* DNA polymerase on the pores of the mesoporous silica was evaluated using SDS-PAGE (Fig. S5). Each sample was separated on 4–15% SDS-polyacrylamide gels and stained with Oriole Fluorescent Gel Stain (Bio-Rad Laboratories, Inc.).

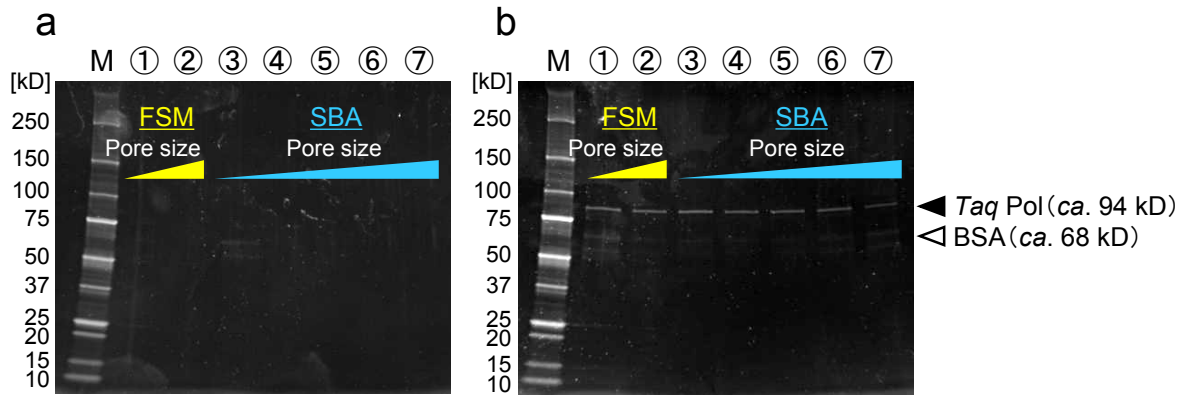

Fig. S5 Analyses of immobilised *Taq* DNA polymerase after PCR amplification using SDS-PAGE. (a) Supernatants after PCR amplification, and (b) supernatants after incubating the pellets from (a) with SDS-sample buffer for 10 min at 95 °C. The numbers above the gels correspond to each mesoporous silica as follows: 1, FSM2.6; 2, FSM4.2; 3, SBA5.4; 4, SBA7.1; 5, SBA10.6; 6, SBA18.5; 7, SBA24.5; M, protein molecular weight marker. Black and white arrowheads indicate the position of *Taq* Pol and BSA (*ca*. 94 and 68 kDa), respectively. See Fig. 1 for SDS-PAGE images of immobilised *Taq* Pol before PCR amplification.

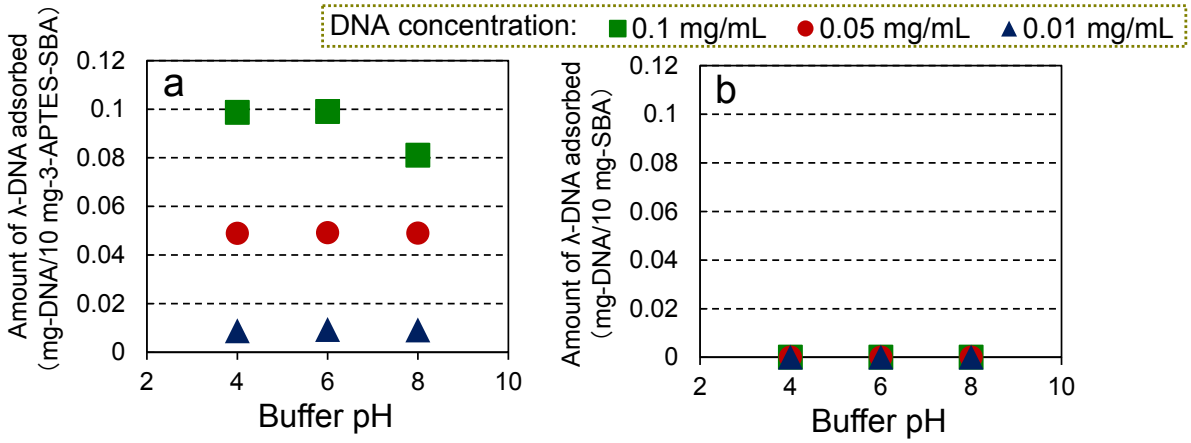

Fig. S6 Evaluation of adsorption of DNA molecules onto mesopores. (a) 3-aminopropyltriethoxysilane-modified SBA-type mesoporous silica (pore size of 6.2 nm), and (b) unmodified SBA-type mesoporous silica (pore size of 7.1 nm).