Electronic Supplementary Material (ESI) for Nanoscale.

Electronic Supplementary Information

Identification of four single-stranded DNA homopolymers with a solid-state nanopore using alkaline CsCl solution

Yusuke Goto*, Itaru Yanagi, Kazuma Matsui, Takahide Yokoi and Ken-ichi Takeda

Center for Technology Innovation - Healthcare, Research & Development Group,

Hitachi Ltd., 1-280 Higashi-Koigakubo, Kokubunji, Tokyo 185-8601, Japan

The supporting information includes the following:

SI-1. Dwell time of translocation for ssDNA in neutral CsCl solution

Figure S1 presents a histogram of the dwell times of poly(dA)$_{60}$ translocation in neutral (pH 7.5) 1 M CsCl solution buffered with 10 mM Tris-HCl. All data were digitally filtered with a 100-kHz low-pass filter (LPF). The characteristic peak of the dwell time was 3.3 μs/base at 0.2 V or 0.8 μs/base at 0.4 V. This translocation speed was much slower than the typical value (< 0.1 μs/base) in a conventional KCl solution.

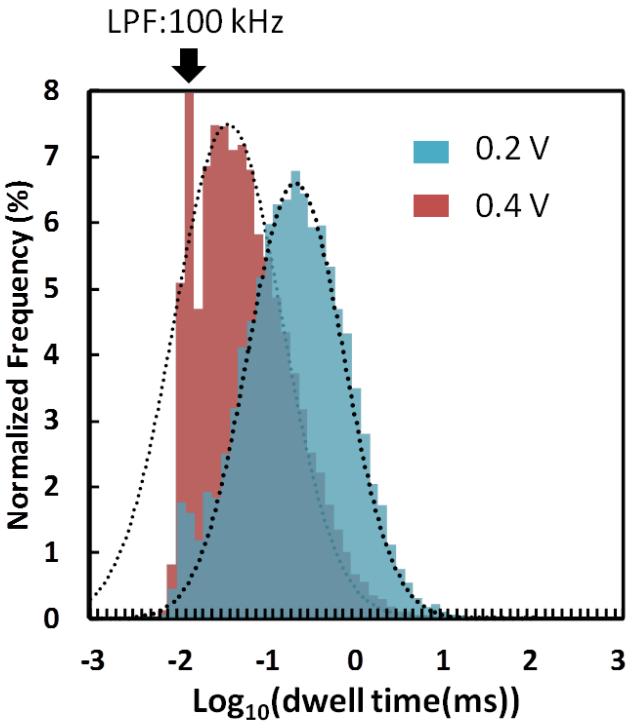


Figure S1. Histograms of translocation time for poly(dA)$_{60}$ passing through the nanopore in 1 M CsCl aqueous solution at pH 7.5. The ssDNA translocation events were measured in 1 M CsCl solution at pH 7.5. The applied voltages were 0.2 V (blue, N = 13357) and 0.4 V (red, N = 51564) (filtered at 100 kHz). The dashed lines are the curves fitted by a log-normal distribution.

SI-2. Dwell time of translocation for four types of DNA homopolymer in alkaline CsCl solution

Figure S2 shows histograms of the dwell time estimated from DNA translocation data in an alkaline CsCl solution. The dwell times of poly(dC)$_{60}$, poly(dG)$_{20}$ and poly(dA)$_{45}$ translocation ranged from 10 μs to 1000 μs. The most frequent events were faster than the low-pass cutoff frequency. The translocation speed of adenine DNA homopolymer in an alkaline CsCl solution was faster than that in a neutral pH solution. This pH dependence can be explained by the increase in the negative charge of adenine (pKa = 9.8) at alkaline pH. Unexpectedly, only the dwell time of the thymine DNA homopolymer exhibited a prolonged distribution; the homopolymer's peak dwell time was approximately 200 μs (corresponding to 3.3 μs/base). The translocation speed of the T homopolymer was 10-fold slower than those of the other nucleotide homopolymers. Although the mechanism is still unclear, the increase in Tris concentration might cause this slowing effect.

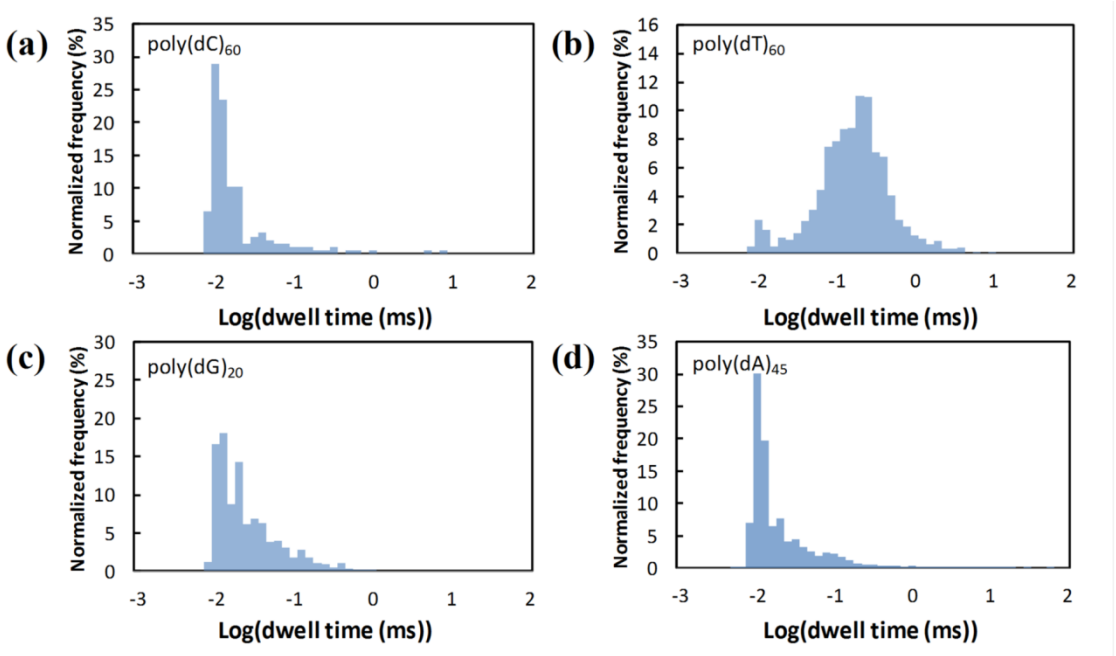


Figure S2. Histograms of dwell time for each DNA homopolymer. Histograms of dwell time at the nanopore for (a) poly(dC)$_{60}$, (b) poly(dT)$_{60}$, (c) poly(dG)$_{20}$ and (d) poly(dA)$_{45}$ using 1 M CsCl with 0.1 M Tris solution with a 5-nm-thick nanopore. All events were measured at 0.5 V and low-pass filtered at 100 kHz.

SI-3. Effect of Cs^+ cation on the blockade current of DNA homopolymers

Figure S3a shows histograms of the normalized blockade current for poly$(dA)_{33}(dC)_{33}(dA)_{33}$ measured in neutral 1 M KCl solution, pH 7.5 (red), and in neutral 1 M CsCl solution, pH 7.5 (blue), by using 10-nm-thick substrates. Only a single peak (0.53 ± 0.034 nA / 0.1 V) was observed in the blockade histogram using the K^+ cation. In contrast, surprisingly, two peaks (Peak 1: 0.38 ± 0.065 nA / 0.1 V, Peak 2: 0.24 ± 0.055 nA / 0.1 V) were clearly confirmed using the Cs^+ cation. This peak splitting implies that two types (A and T) of homopolymer in poly$(dA)_{33}(dC)_{33}(dA)_{33}$ can be distinguished using a solid-state nanopore. Furthermore, Figure S3b depicts normalized histograms of a normalized blockade current for A-homopolymer and C-homopolymer using the neutral 1 M CsCl solution. Notably, each characteristic peak (A-homopolymer: 0.35 ± 0.079 nA / 0.1 V, C-homopolymer: 0.20 ± 0.13 nA / 0.1 V) is consistent with the corresponding individual peak in poly$(dA)_{33}(dC)_{33}(dA)_{33}$. This consistency strongly suggests that Cs^+ ions can significantly improve the signal difference between the A and C bases.

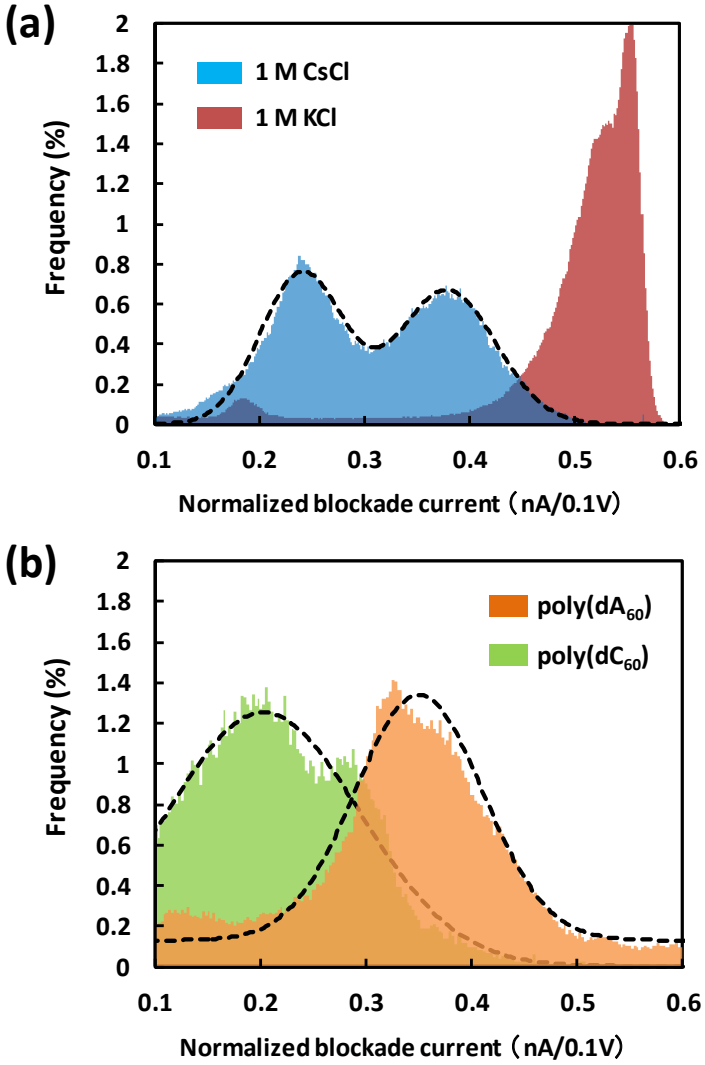


Figure S3. Histograms of blockade current for homopolymers using K^+ and Cs^+ cations. (a) Histograms of normalized blockade current for poly(dA)$_{33}$(dC)$_{33}$(dA)$_{33}$ using K^+ and Cs^+ cations. All data were measured at 0.5 V in neutral 1 M KCl solution, pH 7.5 (red), and in neutral 1 M CsCl solution, pH 7.5 (blue), using a 10-nm-thick substrate. Nanopore diameters were estimated to be 1.9 nm (1 M KCl) and 1.9 nm (1 M CsCl), and DNA translocation events (1 M KCl: N = 1838, 1 M CsCl: N = 1217) were measured. (b) Normalized histogram of blockade current for poly(dA)$_{60}$ (orange) and poly(dC)$_{60}$ (green) in neutral 1 M CsCl solution using the same substrate. The nanopore diameter was estimated to be 2.7 nm, and DNA translocation events (poly(dA)$_{60}$: N = 1014, poly(dC)$_{60}$: N = 1330) were measured. The blockade current was normalized to 0.1 V. The mean values and errors were calculated from the Gaussian fits (dashed line) to the histograms.

SI-4. Effect of membrane thickness on the blockade current

Figure S4 presents the histograms of the normalized blockade current for poly(dA)$_{33}$(dC)$_{33}$(dA)$_{33}$ measured in neutral 1 M CsCl solution using 10-nm- and 5-nm-thick nanopores. Both histograms split into two peaks (10 nm thick: A 0.38 ± 0.065 nA / 0.1 V, C 0.24 ± 0.055 nA / 0.1 V, 5 nm thick: A 0.55 ± 0.049 nA / 0.1 V, C 0.27 ± 0.061). The signal difference between A and C was clearly higher with the 5-nm-thick nanopore. The nanopore with the thinner membrane markedly enhanced the signal difference between the nucleotides.

Similarly, Figure S5 shows histograms of the normalized blockade current for poly(dA)$_{54}$(dC)$_{33}$(dT)$_{33}$ measured in neutral 1 M CsCl solution using the 5-nm-thick nanopore. Surprisingly, the histogram still contains the three peaks corresponding to each (A, C and T) homopolymer. The histogram was well fitted by a trimodal Gaussian with peaks of 0.63 ± 0.026 nA / 0.1 V (A), 0.48 ± 0.062 nA / 0.1 V (T) and 0.31 ± 0.066 nA / 0.1 V (C). This result is consistent with the results for poly(dA)$_{54}$(dC)$_{33}$(dT)$_{33}$ and poly(dG)$_{33}$(dA)$_{54}$(dT)$_{33}$ described in the main text.

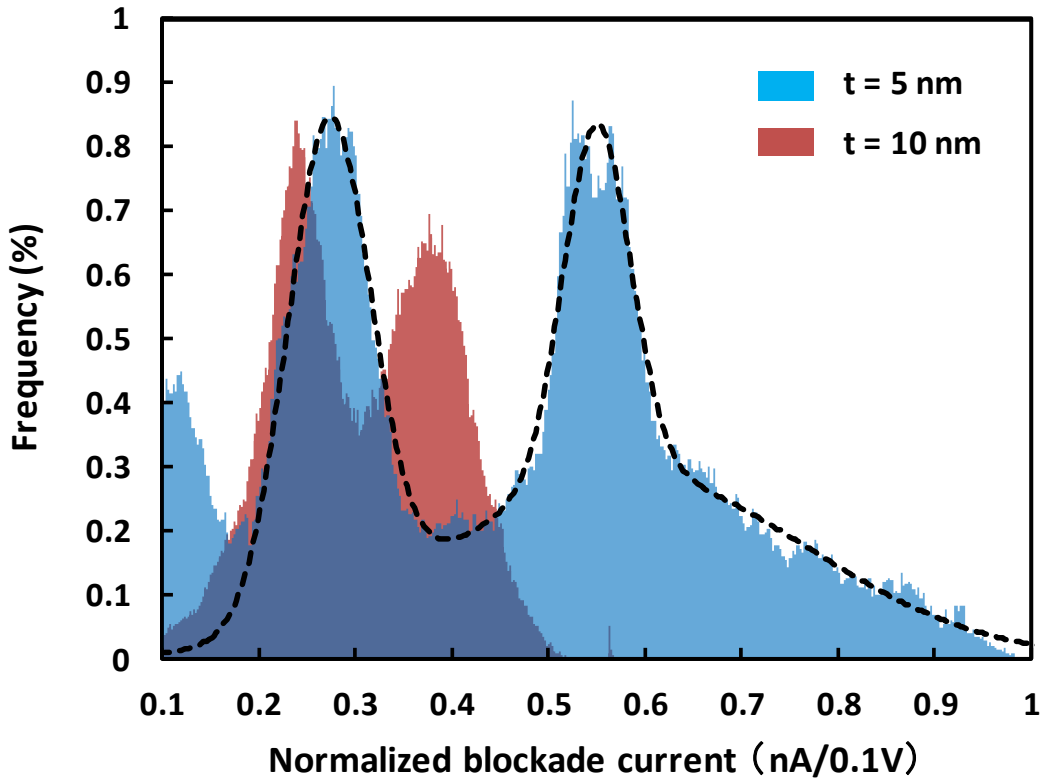


Figure S4. Histograms of normalized blockade current for poly($dA_{33}dC_{33}dA_{33}$) using 10-nm- and 5-nm-thick SiN nanopores. Normalized histogram of the blockade current for poly($dA_{33}dC_{33}dA_{33}$) with a 10-nm-thick substrate (red) and a 5 nm-thick substrate (blue). The nanopore diameters were estimated to be 1.9 nm (10 nm thick) and 1.5 nm (5 nm thick). DNA translocation events (10 nm thick: N = 1203, 5 nm thick: N = 1605) were measured in neutral 1 M CsCl solution. The applied voltage was 0.3 V (filtered at 30 kHz). The mean values and errors were calculated from Gaussian fits (dashed line) to the histograms.

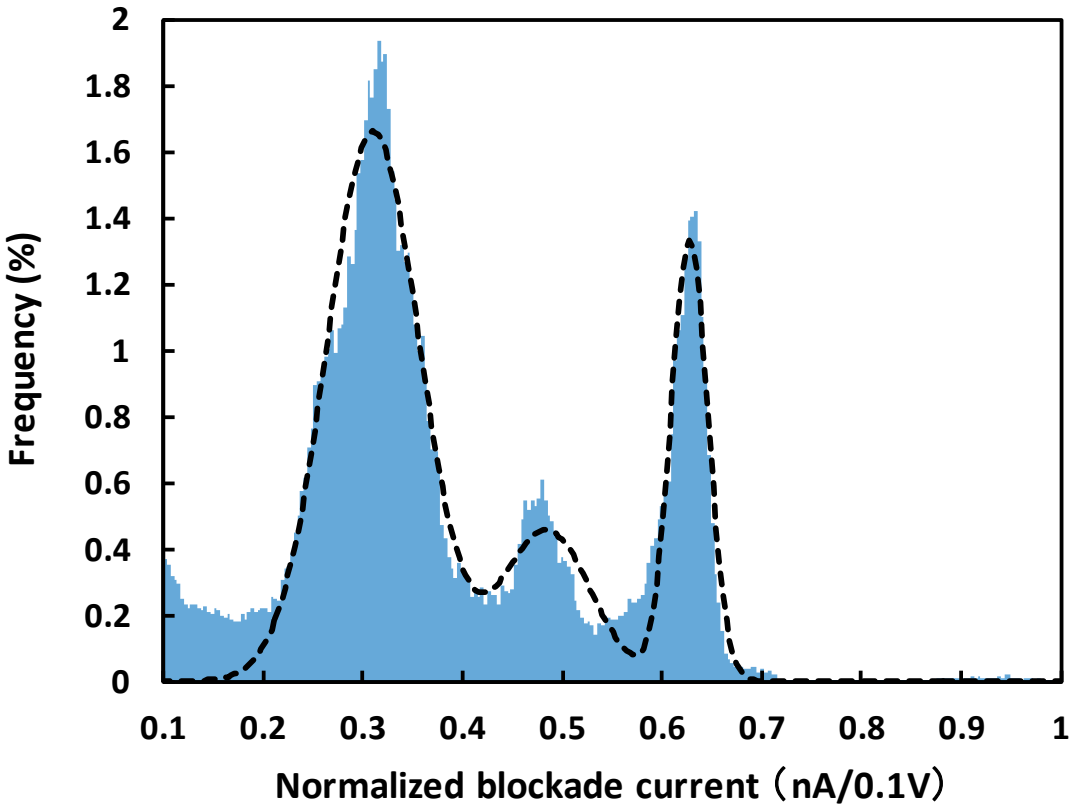


Figure S5. Histograms of normalized blockade current for poly(dA)$_{54}$(dC)$_{33}$(dT)$_{33}$ with a 5-nm-thick SiN nanopore. Normalized histogram of the blockade current for poly(dA)$_{54}$(dC)$_{33}$(dT)$_{33}$ with a 5-nm-thick substrate (blue) in neutral 1 M CsCl solution. The nanopore diameter was estimated to be 3.2 nm. DNA translocation events (N = 1602) were measured at 1 V (filtered at 30 kHz). Mean values and errors were calculated from Gaussian fits (dashed line) to the histogram.

SI-5. Data reproducibility for the measurement of four ssDNA homopolymers

Figure S6 presents the remaining histograms of the normalized blockade current for different ssDNA homopolymers and poly$(dG)_{33}(dA)_{54}(dT)_{33}$ with different nanopores with diameters of 1.8 nm ~ 2.2 nm. As discussed in the main text, the blockade current levels of ssDNA homopolymers are highly sensitive to the identity of the nucleotides. The magnitude of the blockade current for each nucleotide (C < T < G < A) is consistent with the results shown in Figure 4 in the main text. Additionally, the blockade current of the triblock copolymer poly$(dG)_{33}(dA)_{54}(dT)_{33}$ clearly represents a trimodal Gaussian distribution whose blockade current peaks are well matched with those of the homopolymers. These results strongly indicate that the blockade currents of the four nucleotide homopolymers are reproducible in alkaline CsCl solution.

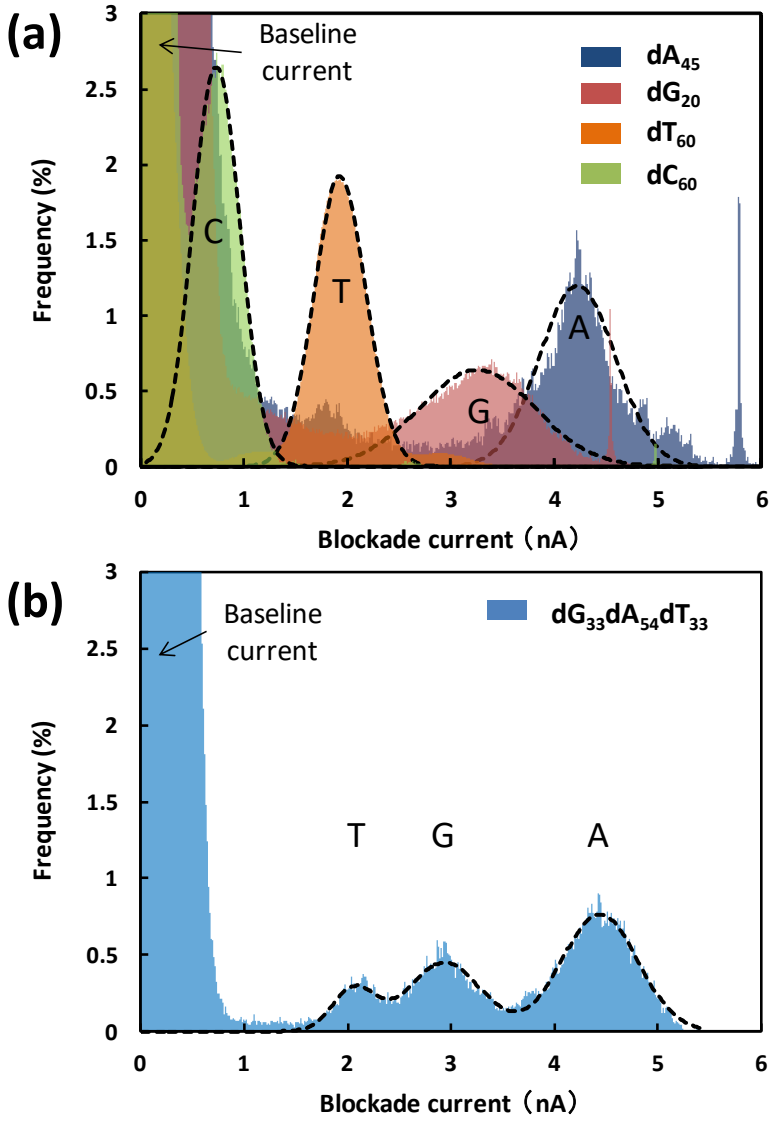
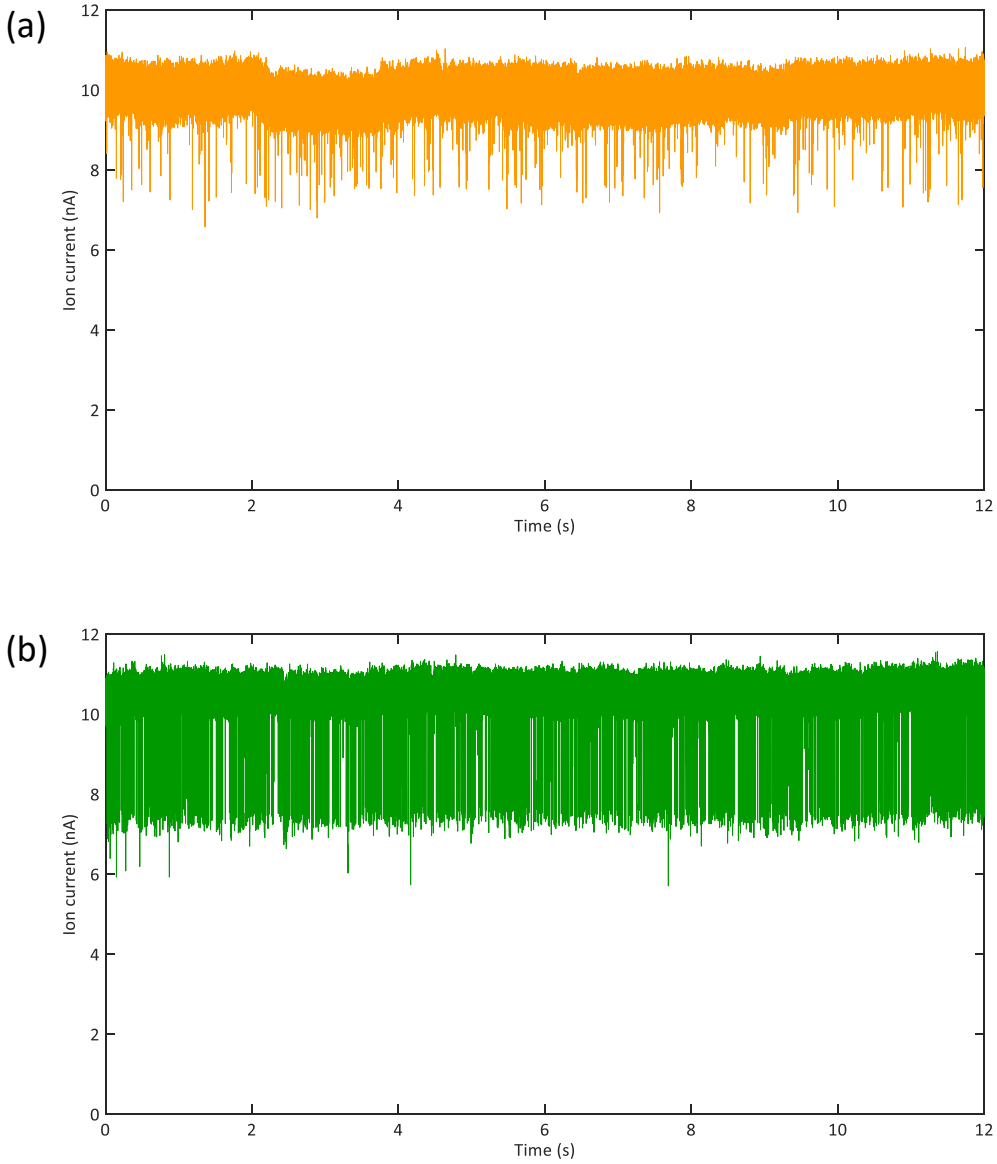


Figure S6. The remaining histograms of normalized blockade current for ssDNA homopolymers of all four nucleotides and for poly(dG)$_{33}$(dA)$_{54}$(dT)$_{33}$ with the 5-nm-thick SiN nanopore. The remaining normalized histograms of blockade current for (a) the four ssDNA homopolymers and (b) poly(dG)$_{33}$(dA)$_{54}$(dT)$_{33}$ with a 5-nm-thick substrate (blue) in alkaline 1 M CsCl 0.1 M Tris solution. The data were obtained using different nanopores (poly(dA$_{45}$): 2.1 nm, poly(dG$_{20}$): 1.8 nm, poly(dC$_{60}$): 2.1 nm, poly(dT$_{60}$): 2.0 nm and poly(dG)$_{33}$(dA)$_{54}$(dT)$_{33}$: 2.2 nm). DNA translocation events were measured at 0.5 V (filtered at 100 kHz). The mean values and errors were calculated from the Gaussian fits (dashed line) to the histogram.

SI-6. Time traces for each ssDNA homopolymer

Figure S7 presents typical raw time traces for the ssDNA homopolymers (poly(dC)$_{60}$, poly(dT)$_{60}$, poly(dG)$_{20}$ and poly(dA)$_{45}$) shown in Figure 3 and 4(a). Each ssDNA homopolymer translocates smoothly across the nanopore without stacking, similarly to the G-quadruplex shown in Figure S9(a)(b).



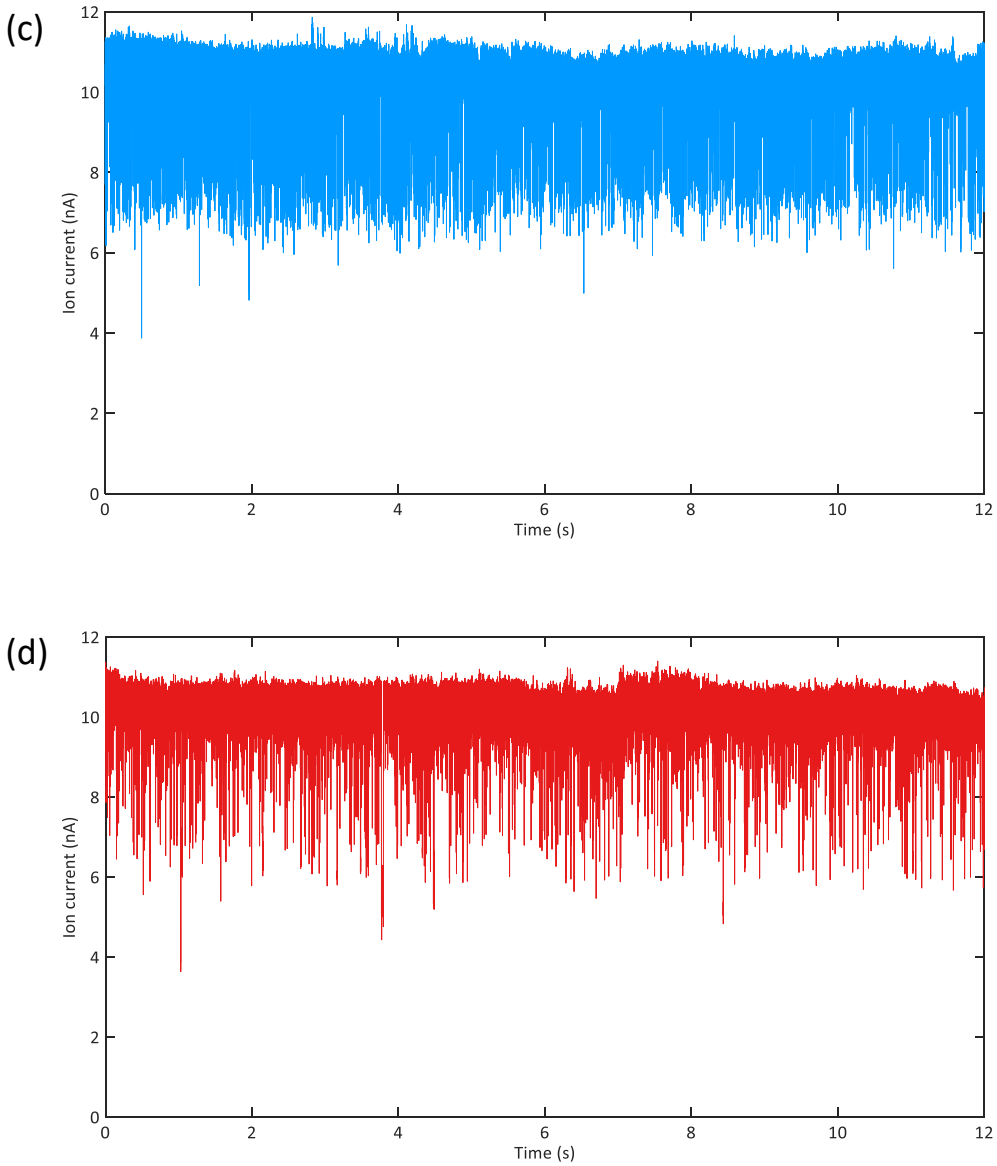


Figure S7. The typical time trace for each ssDNA homopolymer. The typical time trace for the (a) poly(dC)$_{60}$, (b) poly(dT)$_{60}$, (c) poly(dG)$_{20}$ and (d) poly(dA)$_{45}$ homopolymers with a 5-nm-thick substrate in alkaline 1 M CsCl 0.1 M Tris solution. The data were obtained using nanopores measuring 3.2 nm. DNA translocation events were measured at 0.5 V (filtered at 100 kHz).

SI-7. Blockade current analysis for each ssDNA homopolymer by picking up blockade events

We performed a blockade current analysis by picking up the blockade events shown in Figure S7. The pick-up procedure was performed using the open-source software OpenNanopore [1]. To avoid picking up attenuated events, the lower limit of duration time for picking up events was set to 100 μs. Figure S8 shows a histogram of the blockade current for each ssDNA homopolymer by picking up events. All histograms exhibit a single Gaussian distribution; poly(dC)$_{60}$ yields a mean value of 2.0 ± 0.39 nA; poly(dT)$_{60}$ yields a mean value of 2.6 ± 0.17 nA; poly(dG)$_{20}$ yields a mean value of 3.0 ± 0.38 nA; and poly(dA)$_{45}$ yields a mean value of 3.5 ± 0.41 nA. These values are consistent with the values obtained from the all-point histograms shown in Figure 4(a).

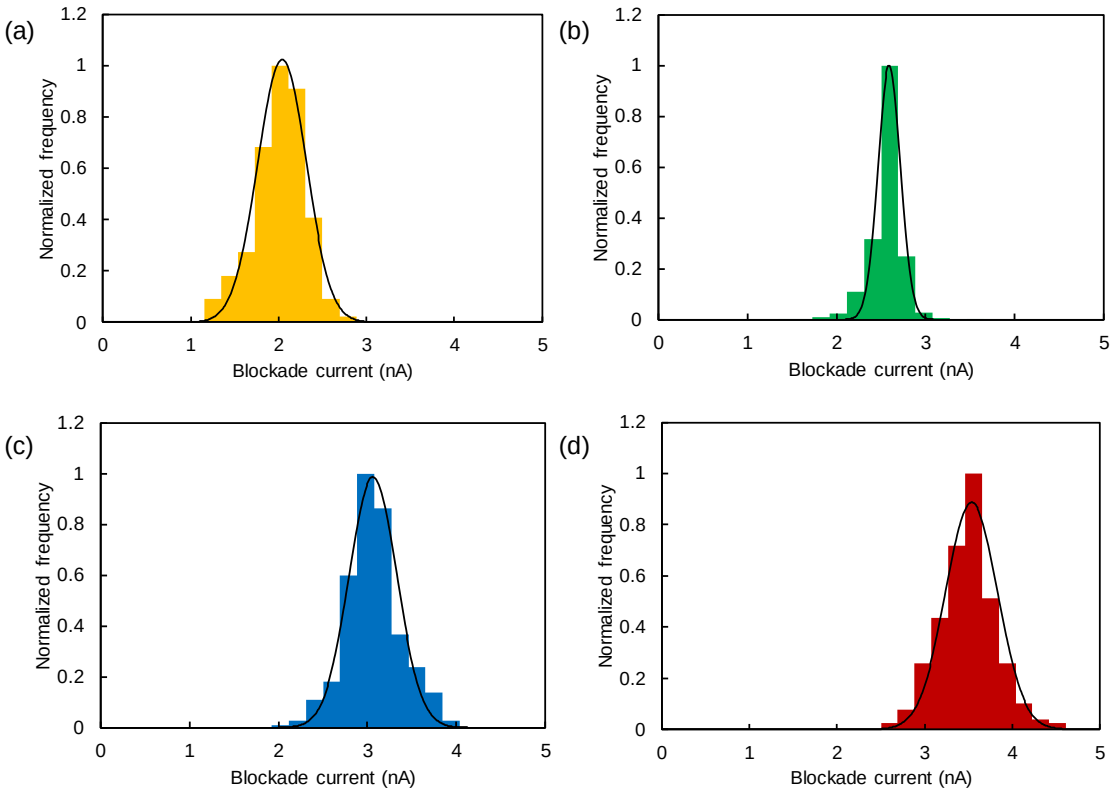


Figure S8. Normalized histograms of blockade current for each ssDNA homopolymer by picking up blockade events. The normalized histograms of blockade current for (a) poly(dC)$_{60}$ (N = 1320), (b) poly(dT)$_{60}$ (N = 1486), (c) poly(dG)$_{20}$ (N = 1362) and (d) poly(dA)$_{45}$ (N = 1180) homopolymers with a 5-nm-thick substrate in alkaline 1 M CsCl 0.1 M Tris solution. The events were picked up using the open-source software OpenNanopore [1]. The data were obtained using nanopores measuring 3.2 nm. DNA translocation events were measured at 0.5 V (filtered at 100 kHz).

SI-8. Poly(dG)$_{20}$ homopolymer translocation experiment

Figure S9 shows typical time traces of poly(dG)$_{20}$ homopolymer in a conventional 1 M KCl solution, pH 7.5, and an alkaline 1 M CsCl solution, pH 10.8. Figure S9(a)(b) shows that nanopores are immediately and strongly clogged with the G-quadruplex even after applying “zapping” pulses, and poly(dG)$_{20}$ homopolymer cannot translocate smoothly across the nanopores. Under this condition, a stable blockade current cannot be evaluated. This clogging phenomenon is evidently different from the nanopore blocking phenomenon caused by normal DNA stacking because DNA stacking is easily removed after applying “zapping” pulses. In contrast, Figure S9(c) shows that poly(dG)$_{20}$ homopolymer can translocate across a nanopore without clogging under alkaline CsCl conditions. These results indicate that the alkaline CsCl condition induced the unfolding of the G-quadruplex.

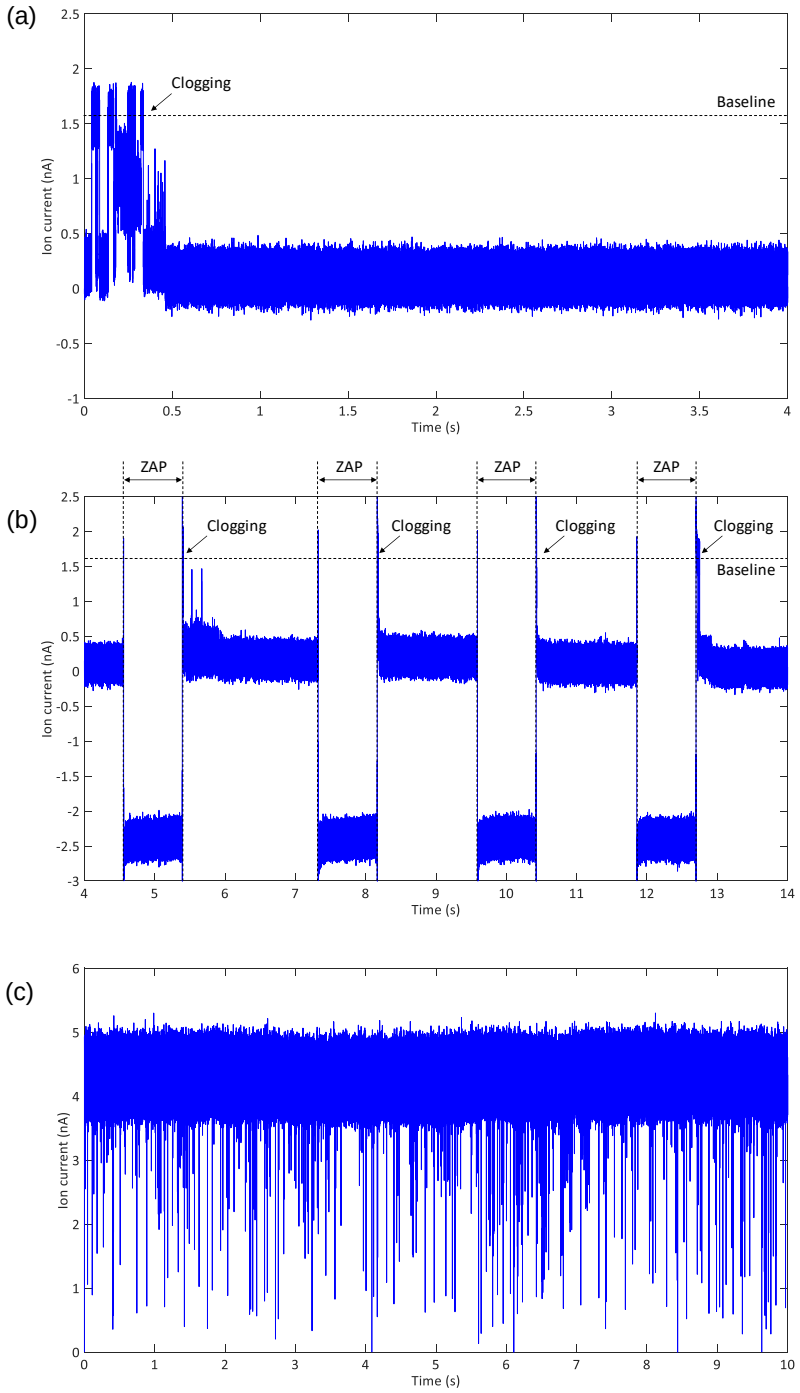


Figure S9. The typical time trace for poly(dG)$_{20}$ homopolymer. The typical time trace for poly(dG)$_{20}$ homopolymer with a 5-nm-thick substrate in (a)(b) conventional 1 M KCl solution (pH 7.5) and (c) alkaline 1 M CsCl 0.1 M Tris solution (pH 10.8). Figure SX(b) is the continued time trace after obtaining the measurement shown in Figure S8(a). The data were obtained using nanopores measuring 1.7 nm. DNA translocation events were measured at (a)(b) 0.2 V and (c) 0.5 V (filtered at 100 kHz).

SI-9. Time traces of mixed homopolymer translocation events

Figures S10 and S11 show typical time traces of blockade events for triblock copolymers poly(dA)$_{54}$(dC)$_{33}$(dT)$_{33}$ and poly(dG)$_{33}$(dA)$_{54}$(dT)$_{33}$. These mixed homopolymers can also translocate smoothly across a nanopore. Unfortunately, attenuated fast events show only impulse-like structures and account for approximately 70% of all events (N = 4420, 4120). Except for the attenuated events, the residual events (N = 1326, 1153) have a relatively long duration and a three-level event structure.

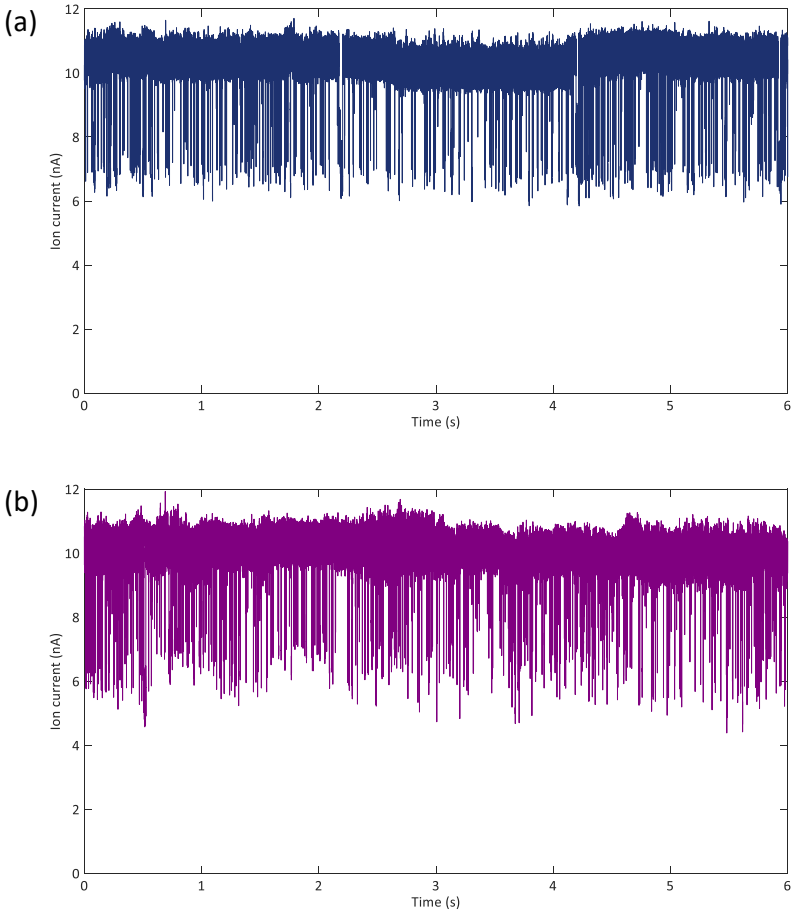


Figure S10. Typical raw time traces for mixed homopolymer measurement.

Typical raw time traces of blockade events for (a) poly(dA)$_{54}$(dC)$_{33}$(dT)$_{33}$ and (b) poly(dG)$_{33}$(dA)$_{54}$(dT)$_{33}$ homopolymers with a 5-nm-thick substrate in alkaline 1 M CsCl 0.1 M Tris solution. The data were obtained using a nanopore measuring 3.2 nm. DNA translocation events were measured at 0.5 V (filtered at 100 kHz).

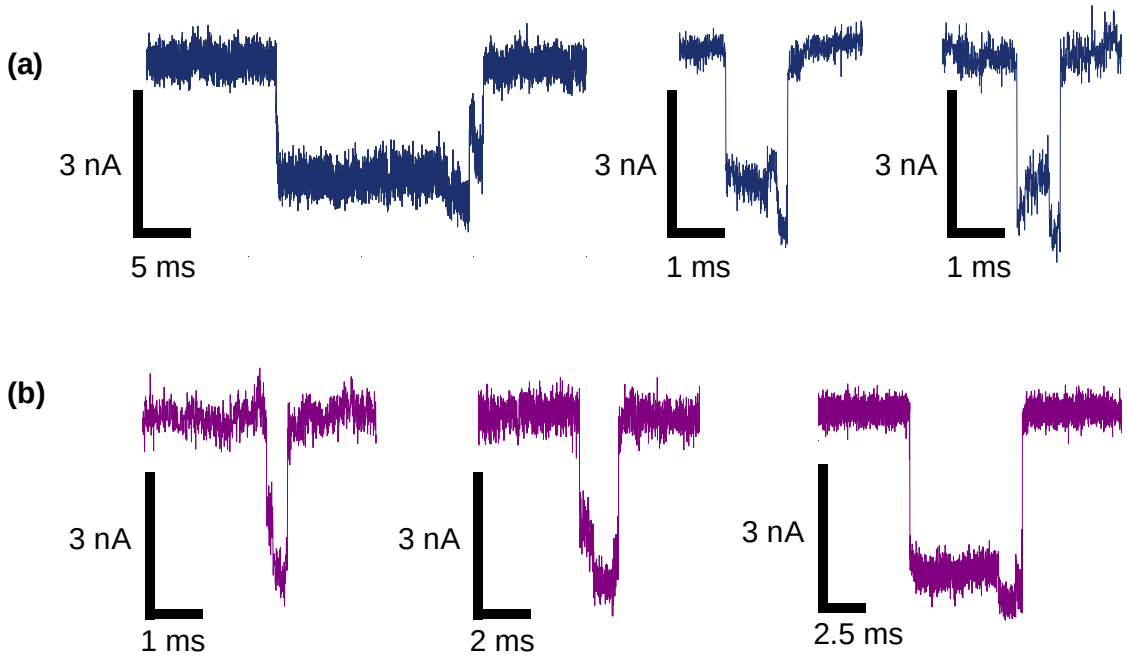


Figure S11. Typical magnified time traces of blockade events for mixed homopolymers. The magnified typical time traces of blockade events for (a) poly(dA)$_{54}$(dC)$_{33}$(dT)$_{33}$ and (b) poly(dG)$_{33}$(dA)$_{54}$(dT)$_{33}$ homopolymers shown in Figure S10.

SI-10. TEM observation of a nanopore fabricated by dielectric breakdown

A substrate membrane was observed using TEM (JEM-2100F, JEOL, Ltd., Tokyo, Japan) at an accelerating voltage of 200 kV. Before observation, the device was washed with pure water to remove any residual salts from the solution. Figure S12 shows a typical TEM image of a nanopore created by dielectric breakdown under alkaline CsCl conditions. Figure S13 indicates that the measured diameter (2.8 nm) precisely predicts I-V characteristics based on Equation (1). The dielectric breakdown process with the alkaline CsCl solution can be harnessed to fabricate a single stable nanopore, similarly to that performed in conventional KCl solution [2,3].

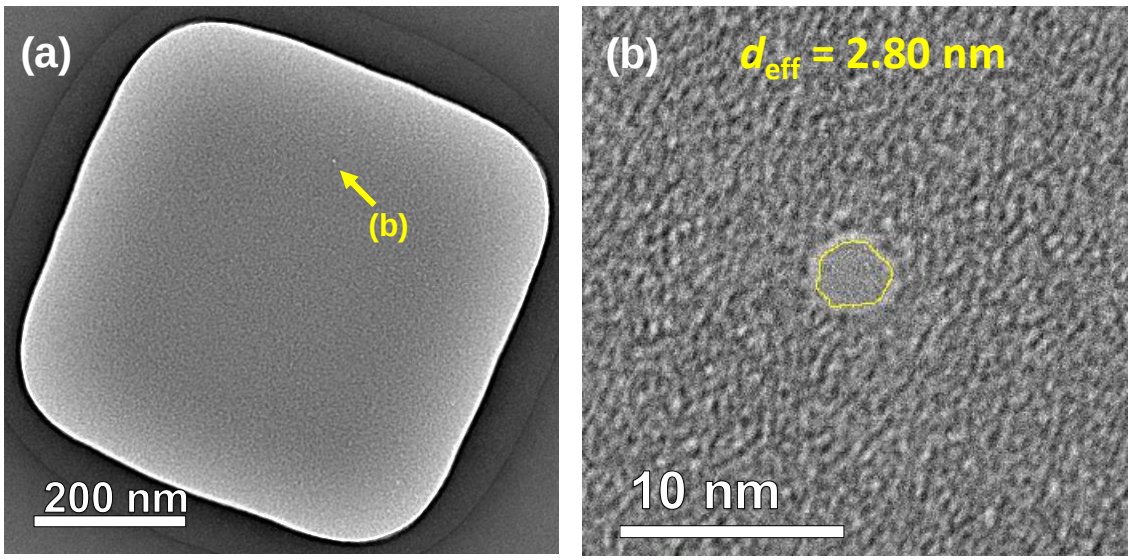


Figure S12. Typical TEM image of a nanopore fabricated by dielectric breakdown with the alkaline CsCl solution. (a) Top-view TEM image of the entire area of the 5-nm-thick SiN membrane after dielectric breakdown and (b) magnified view of the nanopore shown in (a). The area of the nanopore was surrounded by a yellow line using image processing software (ImageJ, National

Institutes of Health, Bethesda, MD, USA). TEM images confirmed that a nanopore with a diameter of 2.8 nm was fabricated.

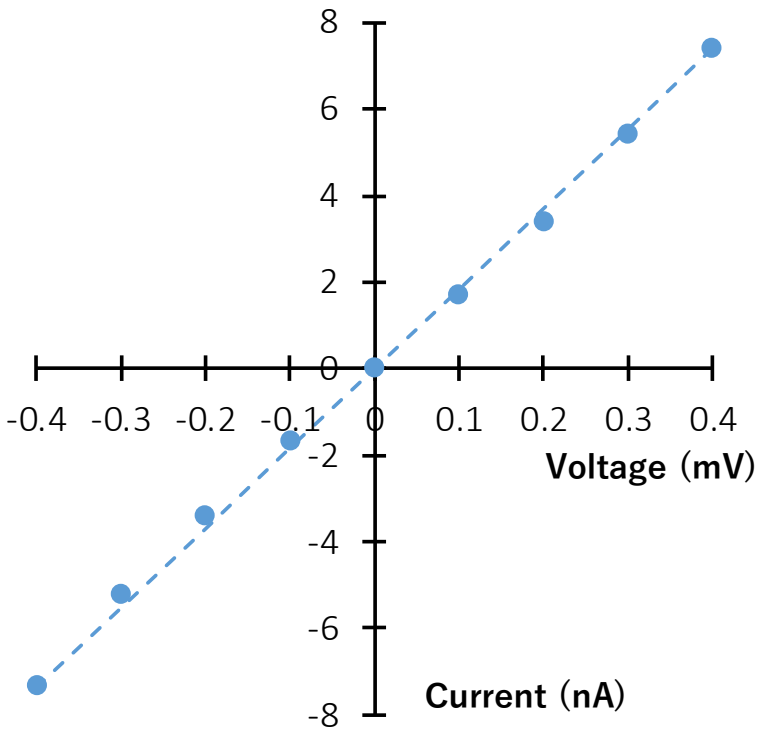


Figure S13. I-V characteristics of the nanopore fabricated by dielectric breakdown with the alkaline CsCl solution. I-V characteristics of the nanopore shown in Figure S12. Plotted points are the current values measured at each voltage. The dashed line is the I-V line derived from Equation (1) using the diameter (2.8 nm) measured from the TEM image shown in Figure S12.

SI-11. “Unknown” homopolymer measurement

We performed the measurement for the “unknown” poly(dT)$_{60}$ sample (unknown to an experimenter) as a blind test using the alkaline CsCl condition. Figure S14 shows a typical time trace and the all-point histogram of the blockade current for the “unknown” homopolymer. The histogram exhibits a single Gaussian distribution and yields a mean blockade current of 2.5 ± 0.32 nA. This value indicates that the “unknown” homopolymer sample can be correctly classified as “poly(dT)”.

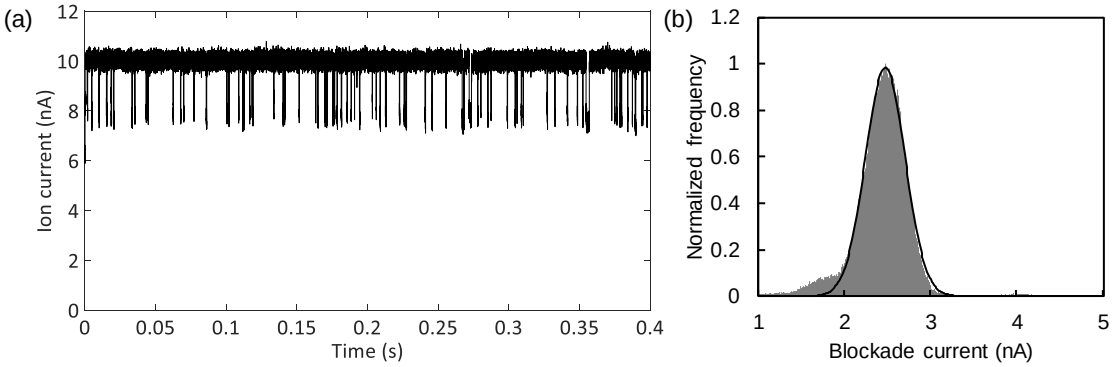


Figure S14. A typical time trace and the all-point histogram of blockade current for “unknown” poly(dT)$_{60}$ homopolymers. (a) A typical time trace and (b) the all-point histogram of blockade current measured for “unknown” poly(dT)$_{60}$ homopolymer with a 5-nm-thick substrate in alkaline 1 M CsCl 0.1 M Tris solution. The data were obtained using a nanopore measuring 3.1 nm. DNA translocation events were measured at 0.5 V (filtered at 100 kHz). The mean values and errors were calculated from the Gaussian fit (solid line) to the histogram.

SI-12. Gel electrophoresis of poly(dG)$_{20}$

Figure S15 shows gel electrophoresis results obtained for poly(dG)$_{20}$ dissolved in conventional KCl solution, or alkaline CsCl solution. The poly(dG)$_{20}$ dissolved in alkaline solution migrated faster than the homopolymers dissolved in the conventional KCl solution. This result also suggests that the alkaline CsCl condition contributes to the unfolding of the G-quadruplex.

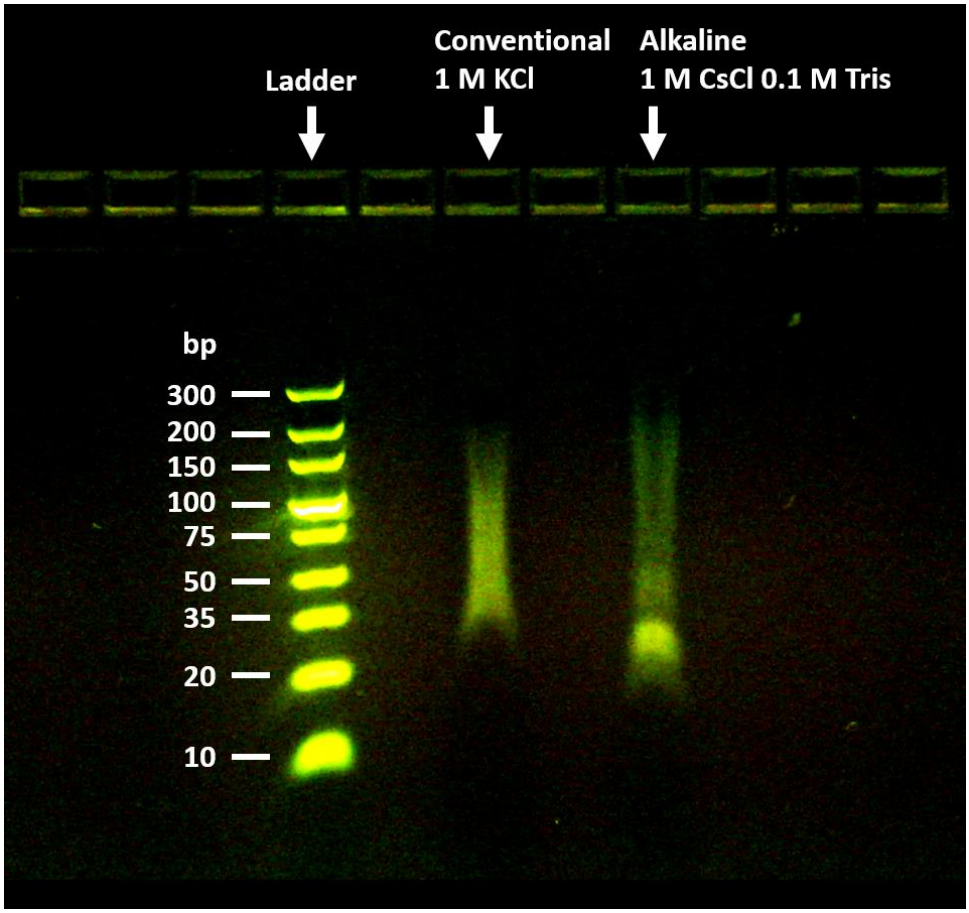


Figure S15. Gel electrophoresis of poly(dG)$_{20}$. Gel electrophoresis was performed with an E-Gel power snap electrophoresis device (Invitrogen) using E-Gel EX 4 %. Poly(dG)$_{20}$ was modified with 6-FAM at the 5' end for fluorescence observation. Migration time: 8 min, Lane left: DNA ladder, Lane middle: poly(dG)$_{20}$ in conventional 1 M KCl TE buffer, Lane 5: poly(dG)$_{20}$ in alkaline 1 M CsCl 0.1 M Tris solution.

References:

[1] Raillon C. P. et al. Fast and automatic processing of multilevel events in nanopore translocation experiments. *Nanoscale* **4**, 4916–4924 (2012).

[2] Kwok, H. et al. Nanopore fabrication by controlled dielectric breakdown. *PLOS ONE.* **9**, e92880 (2014).

[3] Yanagi, I. et al. Fabricating nanopores with diameters of sub-1 nm to 3 nm using multilevel pulse-voltage injection. *Sci. Rep.* **4**, 5000 (2014).