Supplemental Information

Supplementary Figure 1: Control Experiments
Events from experiments taken with ‘Block Homopolymer’ DNA, see Supplementary Table 1 for sequence. (a) Example events without phi29 DNAP present. Two classes of events were observed, (1) short events with a blockage current ($I_b$) of about 0.70 of the open pore current ($I_o$) that correspond to DNA entering the pore vestibule but never passing through the constriction and (2) those with $I_b/I_o < 0.50$ that correspond to DNA passing through MspA after the blocking oligomer and primer sequentially unzip from the DNA template strand. For both classes, the translocation time is too fast to observe nucleotide specific current levels. (b) Example event with phi29 DNAP but without divalent cations and dNTPs. The phi29 DNAP binds to DNA in the cis volume and the bound complex is then captured in MspA (Fig. 1). The blocking oligomer unzips from the template with the phi29 DNAP’s force-activated strand displacement function. As the DNA moves through the pore, a single high peak (designated with an ‘X’) is observed when the two abasic residues in the template strand pass through MspA’s constriction. Since phi29 DNAP is unable to synthesize in the absence of divalent cations and dNTPs, it eventually either falls off allowing the DNA primer to cooperatively unzip from the template, or continues unzipping the primer strand with phi29 DNAP’s force-activated strand displacement function (as is shown in the given trace). In either case, the template strand passes through the pore to the trans side and the current returns to $I_o$. (c) Expanded view the region around the abasic peak.
Supplementary Figure 2: Similarity of ‘Block Homopolymer’ DNA Current Pattern

(a) Current traces of two example events with the ‘Block Homopolymer’ DNA. This DNA template first moves into MspA as the blocking oligomer unzips, and then moves out of MspA as phi29 DNAP extends the primer strand. The reversal of DNA motion is indicated by the vertical blue dashed line. Solid black lines indicate the average current of levels found by automated analysis (see Methods). Regions where the phi29 DNAP fails to incorporate a base result in toggles of the current and are indicated by regions with *. Blue arrows assist in identifying a few corresponding current levels in the two events. The two current traces, Event A and Event B, were recorded in two separate experiments with different pores and several days apart. Levels were found using a computer algorithm (see Methods).

(b) Using a “dot plot”, we compared every level found in Event A with every level found in Event B. The greyscale indicated how much a level in Event A differs from a level in Event B. Two levels with average current differing by less than 0.5% are indicated with a black square (‘dot’) and for those differing by less than 1% a grey dot was entered. For example, the dot found at (6,4) represents the similarity between the 6th observed level in Event A and the 4th observed level in Event B. The shading of this point reveals that the two levels differ by less than 1% of Io. The diagonal line of dots that emerges (upper left to lower right) indicates that the levels of each event follow the same time-ordered sequence. A second perpendicular line of dots, demonstrates that levels occurring during blocking oligomer unzipping have the opposite time-ordering of current levels observed during synthesis. Similar results were found with each DNA sequence examined (Supplementary Figures 7, 9, 12 and 15).

Supplementary Figure 3: Reproducibility of 'Block Homopolymer' DNA Current Pattern
(a) Average current levels extracted from events with 'Block Homopolymer' DNA. Levels for n=24 events collected on N=2 pores were found using a level detection algorithm (see Methods). A consensus level sequence of currents was constructed to serve as a reference. The levels of each event were then automatically aligned to this consensus level sequence to extract average currents associated with each level (see Methods). The average current levels of each event are overlaid (one color for each event). The symmetry of the plot across the blue dashed line indicates that the DNA template changes direction allowing nucleotides to pass through the pore twice, first during unzipping of the blocking oligomer and again during synthesis. Each of these passes produces a similar current level. (b) The standard deviation of the spread in each of the overlaid levels in (a). The average 1-sigma fluctuation of all levels is ~0.6% of I_o. (c) The probability, P_{obs}, of finding a given level in the proper order. Levels with P_{obs}=1, such as level 4, are found in all events. Some levels such as level -18 and level 18 are not efficiently detected due to their similarity in current to neighboring levels. Levels near the DNA template turn around point (blue dashed line) are often too fast to be detected, resulting in low P_{obs}.
Supplementary Figure 4: Exponential Behavior of Level Durations

Normalized histograms of level durations for levels found during the unzipping phase (red) and during the synthesis phase (black) for (a) ‘Block Homopolymer’ DNA, (b) ‘Heteromer DNA 1,’ (c) ‘Heteromer DNA 2,’ (d) ‘Heteromer DNA 3,’ and (e) ‘Heteromer DNA 4.’ Both unzipping (red) and synthesis (black) distributions are well-described by an exponential distribution suggesting that stochastic processes govern both unzipping and synthesis (see figures below for exponential time constants). The level duration distribution for synthesis has a tail due to a few levels of very long duration. We note that these time constant values differ slightly, which is likely due to differences in the sequences. Several levels during the unzipping are much longer so that they dominate the length the unzipping process, but do not significantly skew the duration time constants.

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Supplementary Figure 5: Location of Synthesis Site

Using 'Block Homopolymer' DNA we determine the nucleotide-distance between the phi29 DNAP synthesis site and the constriction of MspA. We performed experiments with either no dCTP or with 100-fold reduced concentration (~1 μM) of dCTP but with normal concentrations (~100 μM) of dATP, dGTP and dTTP. When no dCTP was present, blocking oligomer could be unzipped from the DNA template but the phi29 DNAP could not synthesize nucleotides beyond a dG within the template strand (data not shown). With the 100-fold reduced [dCTP ] the phi29 DNAP was eventually able to synthesize beyond a dG of the template strand but took longer when compared to experiments run with normal dCTP concentration. This figure shows two schematic representations of the location of the DNA as the phi29 DNAP unsuccessfully attempted to synthesize a dG along the template strand. (a) After the blocking oligomer unzipped from the template (first 9.5 seconds not shown) the polymerase extended 9 nucleotides and then back-stepped to the 8th nt. The current continued to toggle between these levels for ~30 seconds, as indicated by region with the * before synthesis continued past the dG. (b) The phi29 DNAP synthesized past the first dG of the template strand allowing the abasic residues in the template strand to pass through MspA. When another dG entered the phi29 DNAP synthesis site the toggling was observed for the remainder of the event. We expect that these toggles are due to failed attempts to insert a dCTP into the growing primer stand. Comparing the ion current levels to those in Figure 2, the traces in (a) and (b) each individually indicate there are 14-15 nt between MspA's constriction and the phi29 DNAP synthesis site.
Supplementary Figure 6: Example Event for ‘Heteromer DNA 1’
Example current trace taken with ‘Heteromer DNA 1’ annealed to a 23 nt primer and a 15 nt ‘blocking oligomer’. The single-stranded 5′ end of the DNA template enters an M2-NNN MspA pore, see Supplementary Table 1 for sequences and number of events observed. (a) The current spontaneously drops from the open pore level (I₀) to <0.5 I₀ when DNA enters the pore. During the unzipping of the blocking oligomer there are occasional long pauses. When the blocking oligomer is completely removed, as indicated by the vertical blue dashed line, the phi29 DNAP begins synthesis and the DNA motion changes direction. The region of synthesis (blue box) is expanded in (b). Solid black lines indicate current levels observed in many events. The levels observed during blocking oligomer removal contain a reverse time-ordered subset of the levels during the synthesis phase. (b) Expansion of the event during phi29 DNAP synthesis shows individual current levels, numbered for convenience. Toggles in current levels are marked with an *. (c) Currents from extracted levels in (b) are plotted with the matching sequence of the DNA template (3′ to 5′). The nucleotides aligning to the right and left of each level most influence the current for that level. It is suspected that levels 8 and 26 each contain two levels of indistinguishable current value. This is indicated with dashes inserted to account for levels that are not observed but are expected. Two abasic residues at the end of the sequence cause a high current (level 30) and are used to indicate the end of a successful read.
Supplementary Figure 7: Example Event for ‘Heteromer DNA 1’
Same as Supplementary Figure 2 but with the ‘Heteromer DNA 1’ See Supplementary Table 1 for sequences.
Supplementary Figure 8: Example Event for ‘Heteromer DNA 2’

Same as Supplementary Figure 6 but with the ‘Heteromer DNA 2.’ See Supplementary Table 1 for sequences.
Supplementary Figure 9: Similarity of ‘Heteromer DNA 2’ Current Pattern

Same as Supplementary Figure 2 but with the ‘Heteromer DNA 2.’ See Supplementary Table 1 for the sequence. The region marked with a * indicates an area where the current levels toggled between multiple values, suggesting a phi29 DNAP backstep.
Supplementary Figure 10: Reproducibility of ‘Heteromer DNA 2’ Current Pattern
Caption as for Supplemental Figure 3 but for ‘Heteromer DNA 2.’ Time ordered levels for n=64 events collected on N=2 pore.
Supplementary Figure 11: Example Event for ‘Heteromer DNA 3’ Sequence
Same as Supplementary Figure 6 but with the ‘Heteromer DNA 3’ template. The dashed lines between levels 24 and 25 are two levels observed in other events but are absent in this example trace. See Supplementary Table 1 for sequences.
Supplementary Figure 12: Similarity of ‘Heteromer DNA 3’ Current Pattern
Same as Supplementary Figure 2 but with the ‘Heteromer DNA 3.’
See Supplementary Table 1 for the sequence.
Supplementary Figure 13: Reproducibility of ‘Heteromer DNA 3’ Current Pattern

Caption as for Supplemental Figure 3 but for ‘Heteromer DNA 3.’ Time ordered levels for n=32 events collected on N=1 pore. Note the series of levels after the abasic peak during the synthesis phase. This series of levels reproduces the levels observed in CAT DNA (Figure 3), demonstrating that despite differing DNA sequence within MspA’s lumen, level progressions are reproducible.
Supplementary Figure 14: Example Event for 'Heteromer DNA 4'
Same as Supplementary Figure 6 but with the 'Heteromer DNA 4.' See Supplementary Table 1 for sequences.
Supplementary Figure 15: Similarity of ‘Heteromer DNA 4‘ Current Pattern
Same as Supplementary Figure 2 but with the ‘Heteromer DNA 4.’
See Supplementary Table 1 for sequences.
Supplemental Figure 16: Reproducibility of ‘Heteromer DNA 4’ Current Pattern
Caption as for Supplemental Figure 3 but for ‘Heteromer DNA 4.’ Time ordered levels for n=62 events collected on N=3 pores.
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Notes:
1. Primer/Template duplex region is 23 nt long and is indicated by brackets [ ]
2. Blocking Oligo / Template duplex region is indicated by underline
3. The same primer sequence is used for all DNA Hybrid Strands.
4. An abasic residue is indicated by an 'X' and a 3 Carbon Spacer is indicated by a 'Z'
5. The primer contains a hairpin structure on it's 5' end and indicated by underline