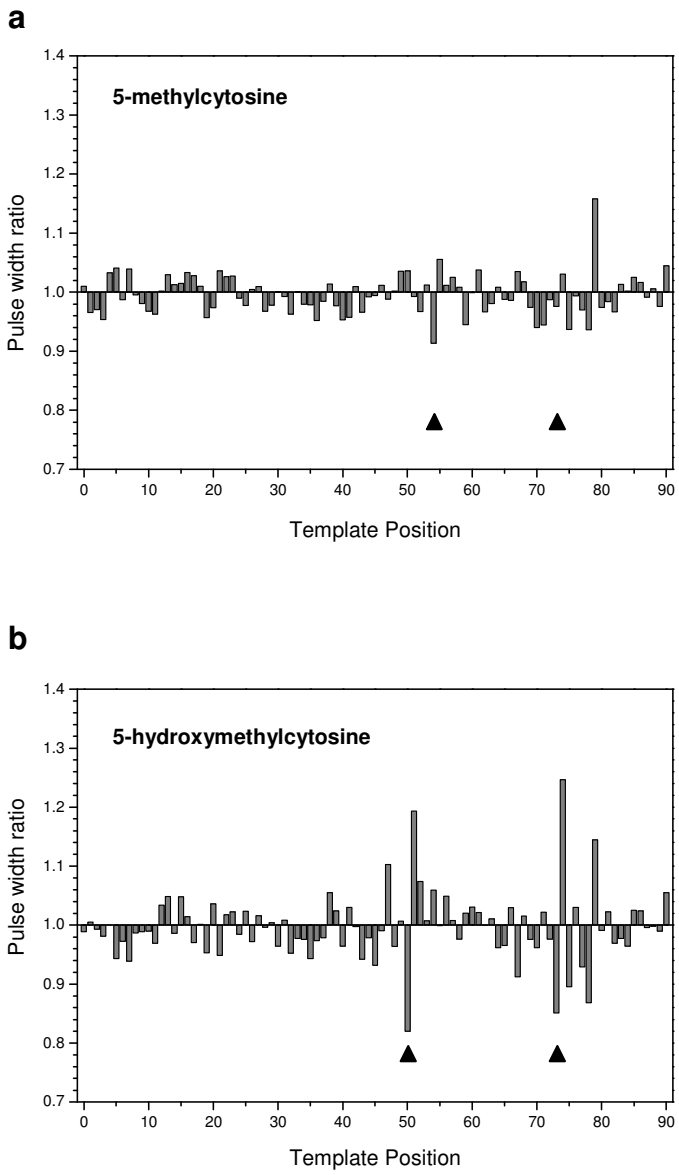
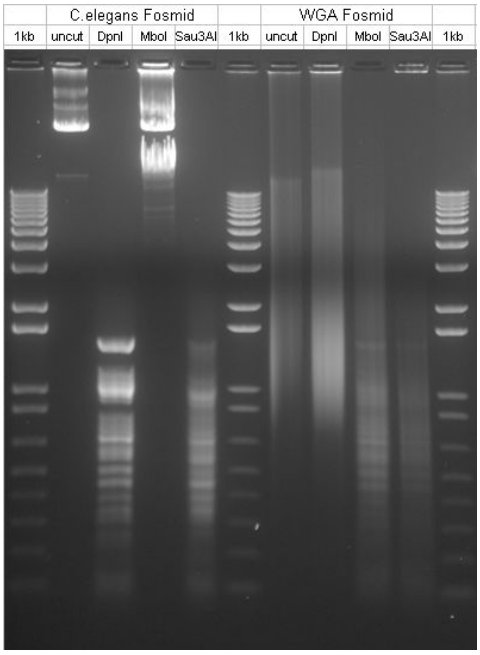
Supplementary Figure 1: Pulse width ratios for mC and hmC



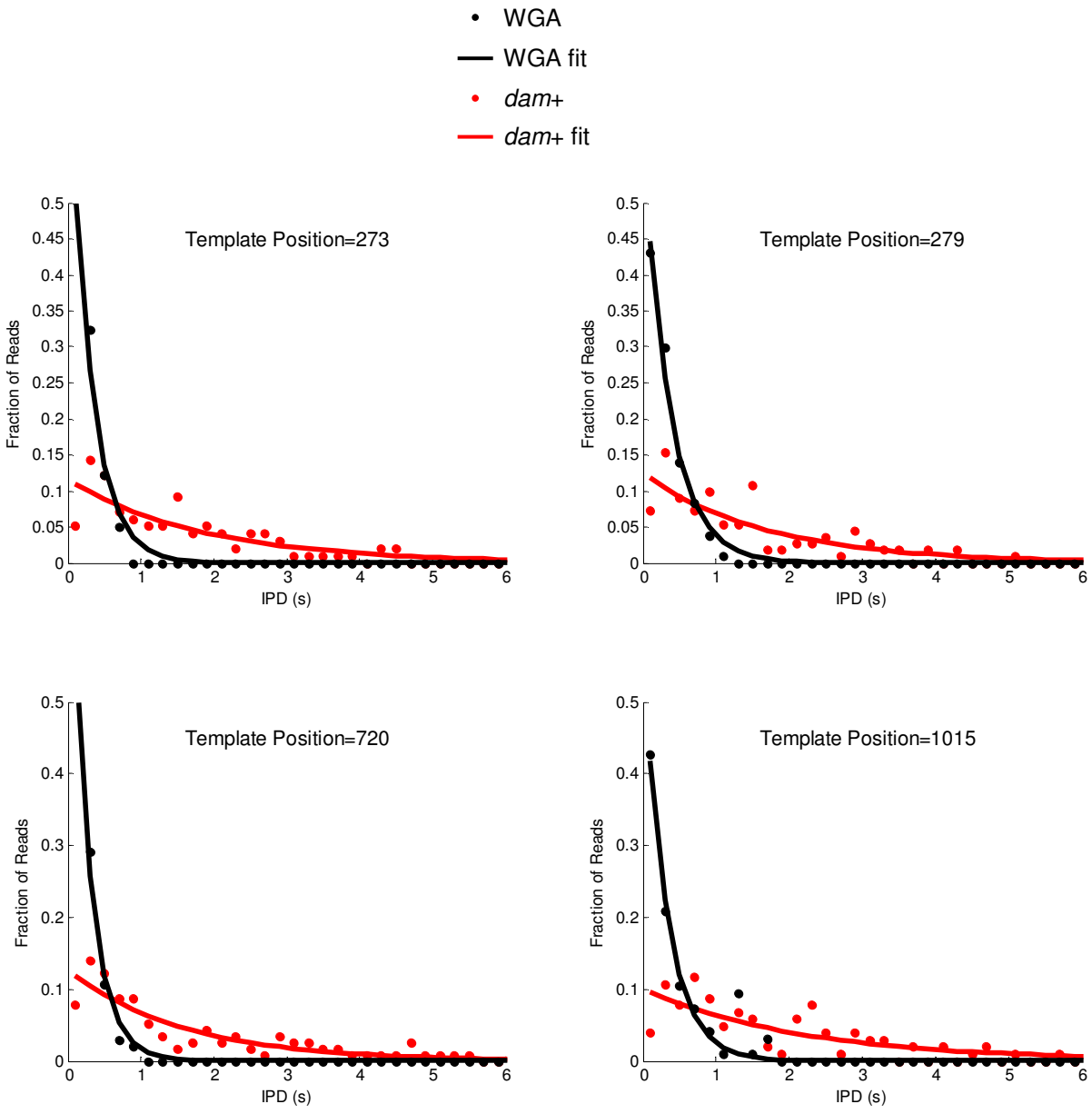
Supplementary Figure 1: Pulse width ratios for (**a**) mC (**b**) hmC. Both panels show the ratio of the average PW in the methylated template to the average PW in the control template, plotted versus DNA template position. In the region shown, the two templates are identical except at the two positions marked by triangles. These positions of differential methylation are (a) 55 and 74 (mC), and (b) 51 and 74 (hmC).

Supplementary Figure 2: Restriction digest of *dam*+ and WGA fosmid samples

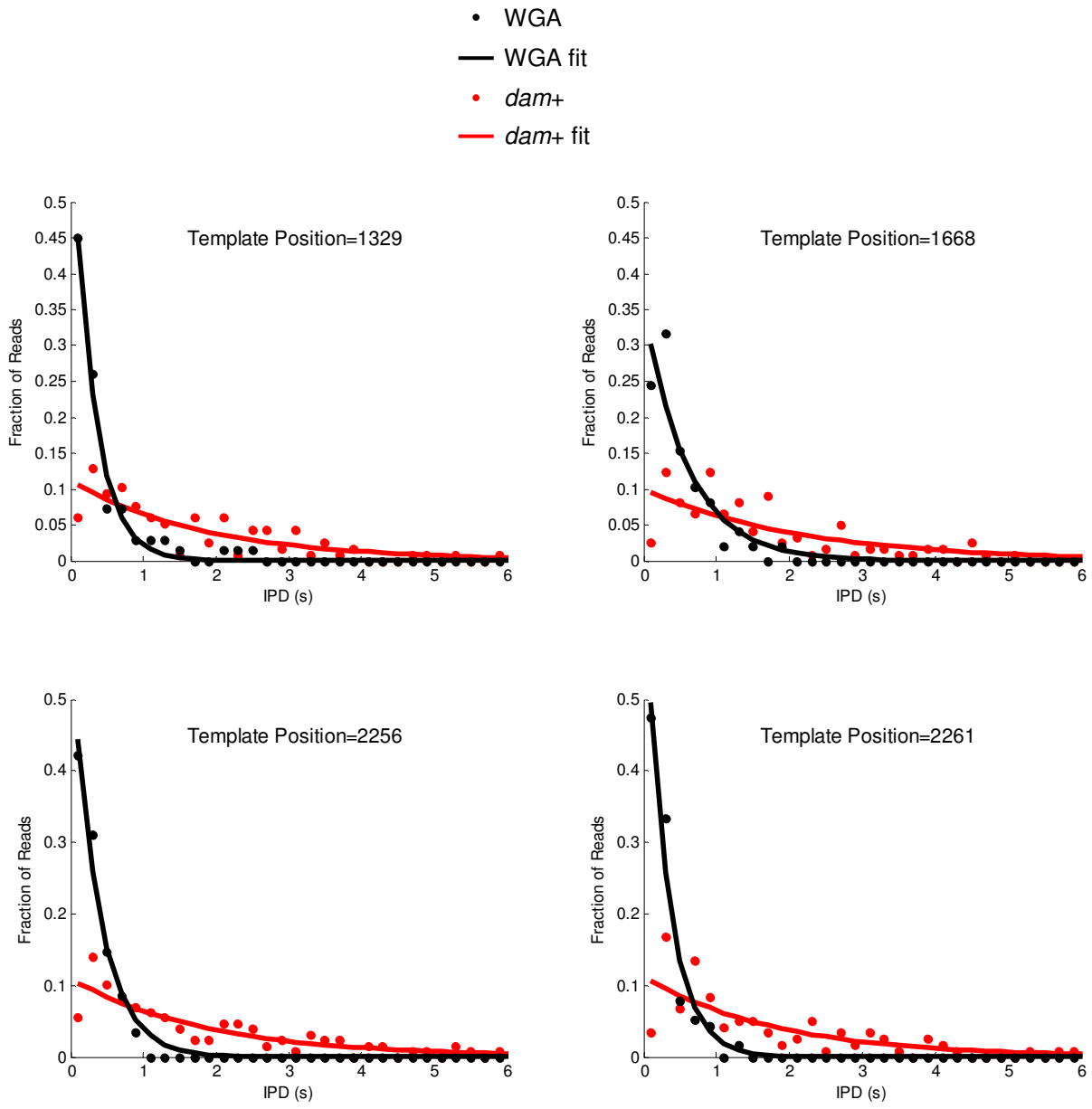


Supplementary Figure 2: GATC adenosine methylation of unamplified and WGA-amplified fosmid samples. To estimate bulk levels of adenosine methylation, DNA samples were digested with a series of restriction enzymes: DpnI (cleaves only methylated GATC), MboI (cleaves only unmethylated GATC), and Sau3AI (insensitive to methylation state). Digested DNAs were separated on 1.2% TAE agarose gel alongside appropriate size markers (1kb Plus Ladder, Invitrogen, Carlsbad, CA) and uncut DNA. The unamplified fosmid shows virtually complete cleavage by DpnI and almost no cleavage by MboI, supporting the assertion that a very high fraction of GATC sites are methylated in this sample. Conversely, the WGA-amplified fosmid DNA does not show appreciable cleavage by DpnI, while MboI produces the same banding pattern as the Sau3AI control.

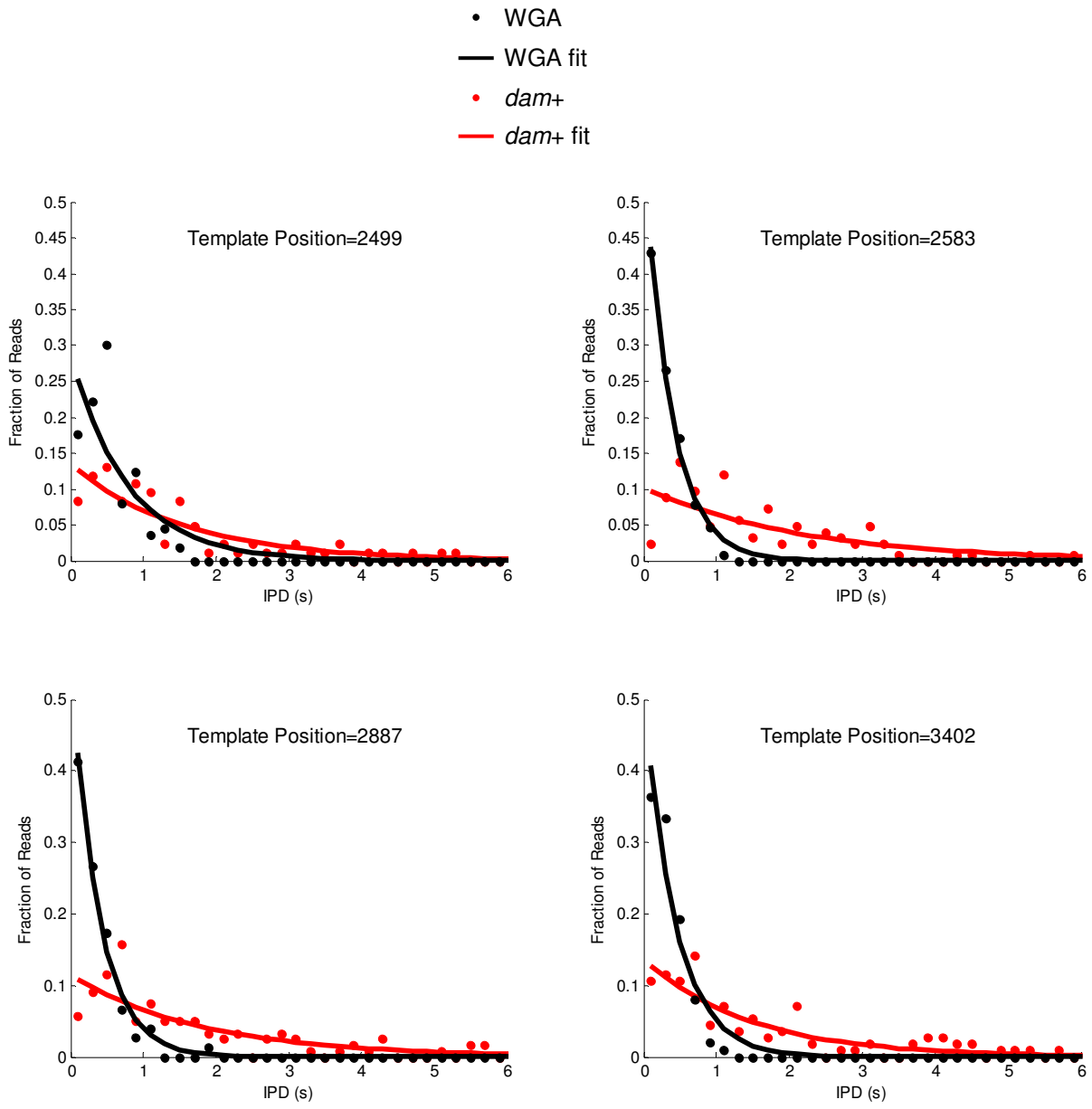
Supplementary Figure 3: IPD histograms at fosmid GATC positions



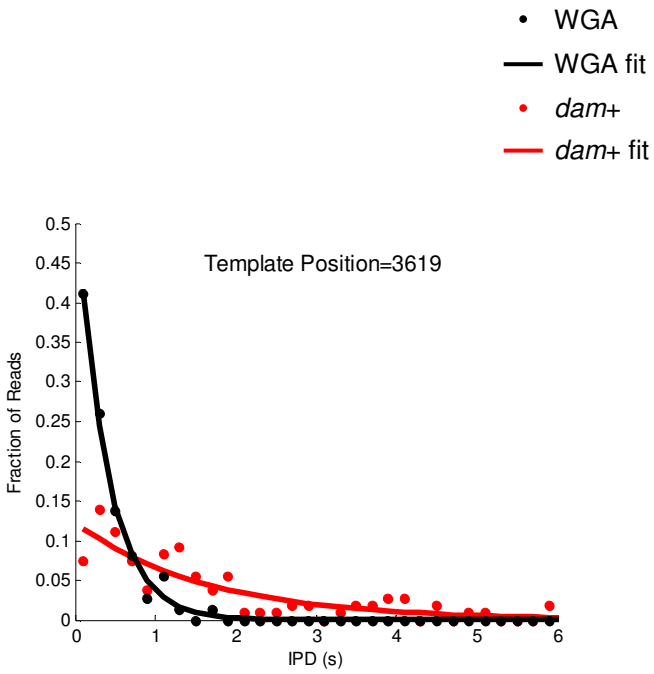
Supplementary Figure 3: IPD histograms at fosmid GATC positions (continued)



Supplementary Figure 3: IPD histograms at fosmid GATC positions (continued)

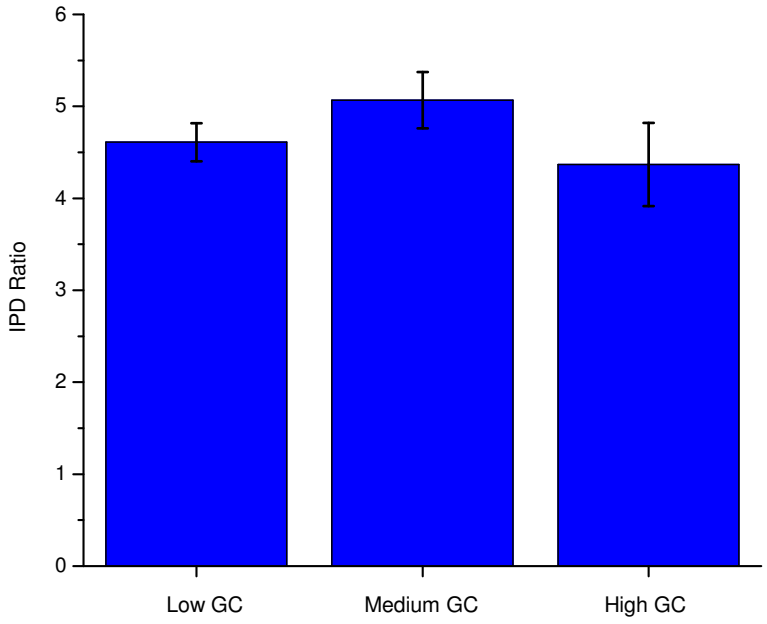


Supplementary Figure 3: IPD histograms at fosmid GATC positions (continued)



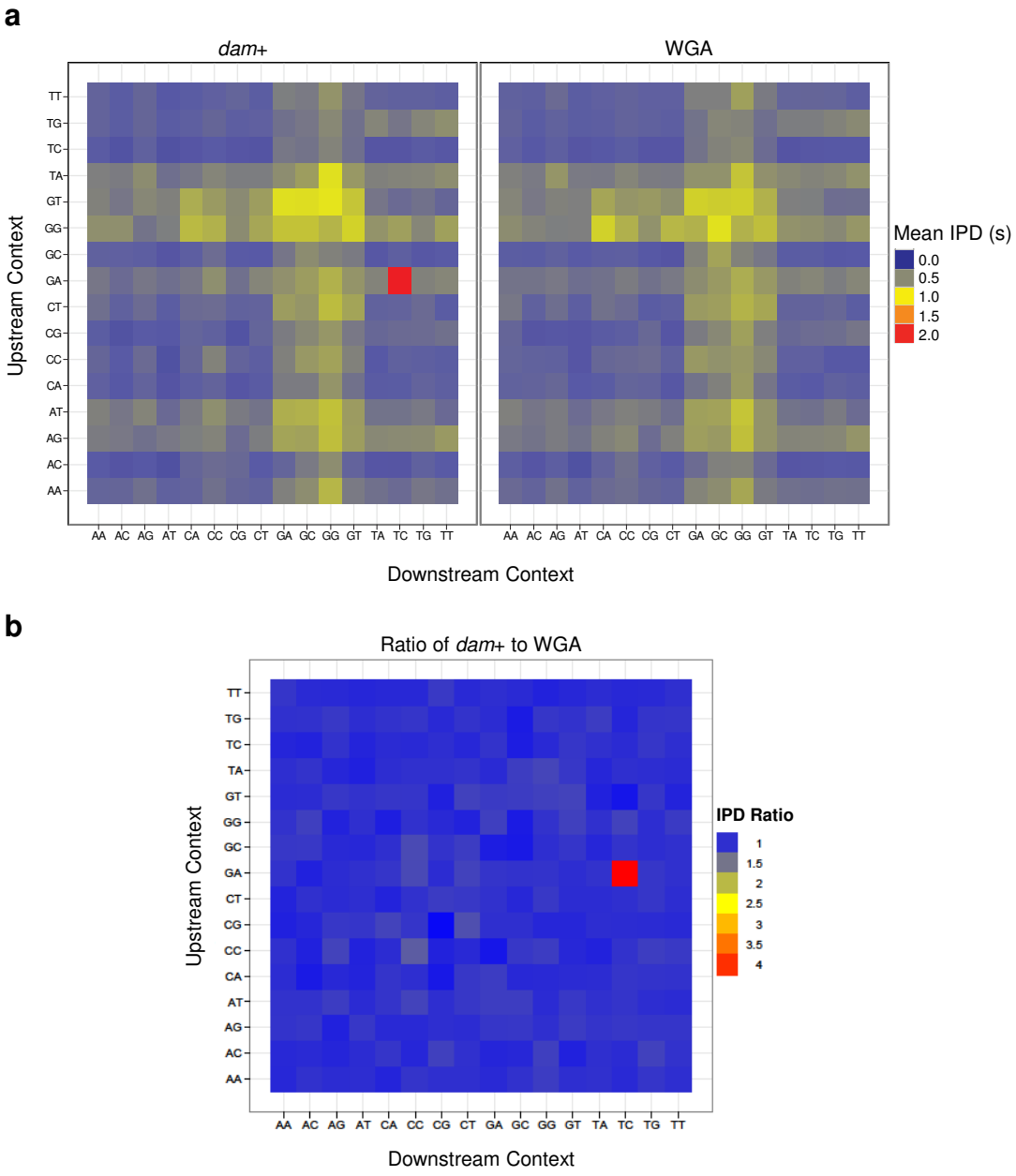
Supplementary Figure 3: Histograms of IPD measurements at each of the 13 GATC positions in the 3.7-kb subregion of the fosmid. Solid circles are the IPD histogram values for the WGA (black) and *dam*+ (red) and samples. Lines are exponential fits to these *dam*+ histogram values. In each plot, the template position at which the histograms were generated is indicated.

Supplementary Figure 4: Fosmid IPD ratios grouped by GC-content



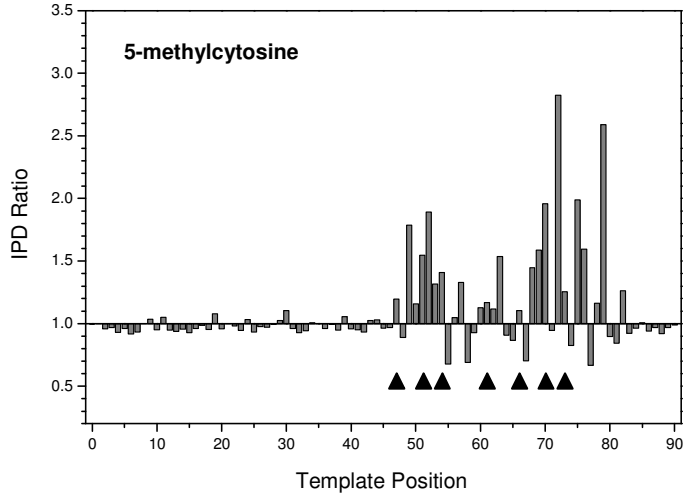
Supplementary Figure 4: IPD ratios at GATC sequence motifs are similar within different GC-content regions. Each bar shows the mean ± standard deviation of the IPD ratios for 35 representative GATC contexts (all 13 from the fosmid shown in Fig. 5 along with others from the surrounding *E. coli* vector) that fall within that GC-content category. Low GC is defined as 20-45% GC-content, medium GC as 45-55% GC-content, and high GC as 55-75% GC-content, all computed within a 50-bp window.

Supplementary Figure 5: Sequence context dependence of IPDs in fosmid samples



Supplementary Figure 5: (**a**) Heat maps of average IPD broken down by 4mer sequence context for *dam*+ *E. coli* DNA samples, and for the same samples after WGA, which erases any methylation signature. The left-axis labels show the two bases detected (on the strand being synthesized) before the IPD, and the bottom-axis labels show the two bases detected after the IPD. The GATC context is the only one out of all 256 contexts in which the mean IPD is considerably shifted after WGA. There were 136 instances of GATC thoughout the entire fosmid and its associated *E. coli* vector, all of which were included in this analysis. Note that the *dam* methylation template motif is 5'-GATC-3', and, because the polymerase moves along the template in the 3'→5' direction, the order of bases detected at a methylation site (complementary to the template) is also GATC. (**b**) Heat map showing the ratio of the left panel in (a) to the right panel in (a). The IPD ratio is ~4 for the GATC context, while it is <1.5 for all other contexts.

Supplementary Figure 6: IPD ratios at an mC cluster



Supplementary Figure 6: SMRT sequencing of a template containing a cluster of mC bases. The plot shows the ratio of the average IPD in the methylated template to the average IPD in the control template, plotted versus DNA template position. In the region shown, the two templates are identical except at the seven positions marked by triangles, which are differentially methylated and correspond to positions 47, 51, 54, 61, 66, 70, and 73. Polymerase synthesis proceeds in the direction of increasing template position number.

Supplementary Table 1: Principal component analysis weightings

Parameter	PC1	PC2
IPD_{-2}	0.16	-0.37
IPD_{-1}	-0.25	-0.18
IPD_{0}	0.24	-0.21
IPD_{1}	0.26	0.14
IPD_{2}	0.27	0.09
IPD_{3}	0.27	-0.05
IPD_{4}	0.22	0.27
IPD_{5}	0.06	0.44
IPD_{6}	0.27	-0.10
PW_{-2}	-0.23	-0.25
PW_{-1}	0.26	-0.17
PW_{0}	-0.27	-0.12
PW_{1}	0.27	0.11
PW_{2}	-0.25	0.18
PW_{3}	-0.19	-0.33
PW_{4}	0.19	-0.32
PW_{5}	0.28	-0.04
PW_{6}	0.17	-0.35

Supplementary Table 1: Weightings for the first two principal components for position 73 in the control, mC, and hmC templates (where position 0 is the base at the 3' end of the template). Subscripts for the kinetic parameters refer to the template position with respect to position 73. Each principal component is normalized such that the sum of the squares of all its weightings equals one.

Supplementary Note: Sequences of synthetic DNA templates

The sequences of the four synthetic DNA templates (control, mA, mC, and hmC) described in **Figs. 1-4** and **Supplementary Fig. 1** were as follows:

5'-TCCTCCTCCTCCGTTGTTGTTGTTGAGAGAGAGAAGTGCACGGTCGATCAAGTACAGATCATG
CGTTGCACGGTCGATCAAGTACAGATCATGCGTCGGGCTCGGAACGAAAGTTCCGAGCCCGAC
GCATGATCTGTACTTGATCGACCGTGCAACGCATGATCTGTACTTGATCGACCGTGCACTTCTCT
CTCTCTTT-3';

5'-TCCTCCTCCTCCGTTGTTGTTGTTGAGAGAGAGAAGTGCACGGTCGATCAAGTACAGATCATG
CGTTGCACGGTCG(mA)TCAAGTACAG(mA)TCATGCGTCGGGCTCGGAACGAAAGTTCCGAGCC
CGACGCATG(mA)TCTGTACTTG(mA)TCGACCGTGCAACGCATGATCTGTACTTGATCGACCGTG
CACTTCTCTCTCTCTTT-3';

5'-TCCTCCTCCTCCGTTGTTGTTGTTGAGAGAGAGAAGTGCACGGTCGATCAAGTACAGATCATG
CGTTGCACGGTCGATCAAGTACAGATCATGCGTCGGGCTCGGAACGAAAGTTCCGAGCCCGA(m
C)GCATGATCTGTACTTGAT(mC)GACCGTGCAACGCATGATCTGTACTTGATCGACCGTGCACTT
CTCTCTCTCTTT-3';

5'-TCCTCCTCCTCCGTTGTTGTTGTTGAGAGAGAGAAGTGCACGGTCGATCAAGTACAGATCATG
CGTTGCACGGTCGATCAAGTACAGATCATGCGTCGGGCTCGGAACGAAAGTTCCGAGCCCGA(h
mC)GCATGATCTGTACTTGATCGAC(hmC)GTGCAACGCATGATCTGTACTTGATCGACCGTGCAC
TTCTCTCTCTCTTT-3'.

The sequences of the synthetic DNA templates (control, mC) used in the experiment with multiple neighboring mC bases (**Supplementary Fig. 6**) were as follows:

5'-TCCTCCTCCTCCGTTGTTGTTGTTGAGAGAGAGAAGTCGTACGACGTTATTCGTTTCGTCCGA
CGATCGTACGACGTTATTCGTTTCGTCCGACGACGGGCTCGGAACGAAAGTTCCGAGCCCGTCG
TCGGACGAAACGAATAACGTCGTACGATCGTCGGACGAAACGAATAACGTCGTACGACTTCTC
TCTCTCTTT-3';

5'-TCCTCCTCCTCCGTTGTTGTTGTTGAGAGAGAGAAGTCGTACGACGTTATTCGTTTCGTCCGA
CGAT(mC)GTA(mC)GA(mC)GTTATT(mC)GTTT(mC)GTC(mC)GA(mC)GACGGGCTCGGAACGAAA
GTTCCGAGCCCGT(mC)GT(mC)GGA(mC)GAAA(mC)GAATAA(mC)GT(mC)GTA(mC)GATCGTCGG
ACGAAACGAATAACGTCGTACGACTTCTCTCTCTCTTT-3'.

All templates consisted of a central 84-bp double-stranded region with single-stranded loops at each end, comprising a total of 199 bases. The primer, annealed to the single-stranded loop region of one of the hairpin adaptors, had the sequence 5'-GGAGGAGGAGGA-3'.