Supporting Information

Highly sensitive detection of 6mA at single-base resolution based on A-C mismatch

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Experimental Methods:

General methods: Taq DNA ligase was purchased from New England Biolabs and phi29 DNA polymerase was purchased from thermo Fisher Scientific. The primers and templates used in primer extensions and RCA were purchased from TaKaRa Biotech (China) and their sequences were listed in Table S1. DNA concentrations were quantified by NanoDrop 2000c (thermo scientific, USA). Fluorescence signals were measured on PerkinElmer LS 55 (PerkinElmer, USA).

The formation of A or 6mA containing dsDNA templates. Mixed 1 μL of 1 μM A or 6mA containing templates DNA templates and 1 μL of 1 μM complementary strands uniformly in 10 mM Tris buffer in 10 μl volume system, the mixture was heated to 95 °C for 5 min and slowly cooled down to room temperature. Stored the double-stranded DNA templates at 4 ℃.

Verification the selectivity of Taq DNA ligase to A and 6mA by PAGE. The reactions were performed with 1 μL of 1 μM DNA templates carrying A or 6mA, 1 μL of 1 μM each padlock probe with PO_4^- at 5'end and A, T, C or G at 3' end, 2 μl of 4 U/μl Taq DNA ligase, 1 μL of 10X Taq DNA ligase reaction buffer and 5 μL of ddH_2O. To initiate the ligation, the mixture is denatured at 95 °C for 5 minutes. and ligation was performed at 70 °C for 60 min. Then the reactions were quenched by adding 10 μLof formamide. Then the mixtures were heated at 90 °C for 10 min immediately and cooled down to 4 °C before loaded to the 12% denaturing polyacrylamide gel.

Verification the selectivity of Taq DNA ligase to A and 6mA by RCA reaction. Mixed 1 μL of A or 6mA containing dsDNA templates and 1 μL of 10 μM each padlock DNA 2 μL of 4 U/μl Taq DNA ligase 1 μL of 10X Taq DNA ligase reaction buffer and 5 μl of ddH_2O to make the total reaction volume into 10 μl. Then the RCA reaction was performed at 30 °C for 2 h by adding 1 × phi 29 reaction buffer, 200 μM dNTP, 2 Unit phi 29 DNA polymerase in 20 μL volume. For the fluorescence measurement, the RCA products were mixted with 5 μL of 40 × SYBR Green I and diluted to a final volume of 200 μl. Perkin Elmer LS-55 Fuorescence Spectrometer was used to measure the fluorescence value of each sample, the excitation wavelength was set at 488 nm. For agarose gel electrophoresis analysis, 10 μL of the each RCA products and gel loading dye mixture was used to proceed this experiment.

The 6mA discrimination using this strategy for three different series of dsDNA. The DNA sequences of these three different DNA templates containing A/6mA and their corresponding padlock DNA sequences were listed in Table S2. Then the liagtion and RCA reactions were performed as previously described.

The quantification of 6mA in the mixed samples. Different ratios of 6mA containing dsDNA templates were mixed with A containing dsDNA templates to generate mixed samples. Then 1 nM mixed samples with different percentages of 6mA were used as templates in the ligation and RCA reaction.

Table S1 The sequences of DNA templates and primers used in this paper

Simulation Cases	Sequences
A/6mA-containing template	5’-TTTGTGCCTGTCCTGGGAGAGACA/6mACGCGCACAGAGGAAGAGAGAATCT-3’
Complementary strand	5’-AGATTCTCTTCCTCTGTGCGCGTGTCTCTCCCAGGACAGGCACAAA-3’
padlock probe A-C	5’-PO_4-GTCTCTCCCAGGACAGGCTTTTGATCACAGTTTACGGTTTAGCATAACTCTACTATCTTTCTCTTCCTCTGTGCGCGC-3’
padlock probe A-A	5’-PO_4-GTCTCTCCCAGGACAGGCTTTTGATCACAGTTTACGGTTTAGCATAACTCTACTATCTTTCTCTTCCTCTGTGCGCGA-3’
padlock probe A-T	5’-PO_4-GTCTCTCCCAGGACAGGCTTTTGATCACAGTTTACGGTTTAGCATAACTCTACTATCTTTCTCTTCCTCTGTGCGCGT-3’
padlock probe A-G	5’-PO_4-GTCTCTCCCAGGACAGGCTTTTGATCACAGTTTACGGTTTAGCATAACTCTACTATCTTTCTCTTCCTCTGTGCGCGG-3’

Table S2 The sequences of DNA templates and primers used in this paper

Simulation Cases	Sequences
A/6mA-containing template-2	5’-TGGCATTGAGGTGCTGGAGCGGA/6mATCCGGCAGGAGTGGCCCGAGCT-3’
A/6mA-complementary strand 2	5’-AGCTCGGGCCACTCCTGCCGGATCCGCTCCAGCACCTCAATGCCA-3’
padlock probe A-C-2	5’-PO_4-CCGCTCCAGCACCTCAATGGTTTTGATCACAGTTTACGGTTTAGCATAACTCTACTATCTGGGCCACTCCTGCCGGAC-3’
A/6mA-containing template-3	5’-TGGCTAGTGAGTCGTCCACGTCA/6mATGGCCTGGAGTGGCCCGAGCT-3’
A/6mA-complementary strand 3	5’-AGCTCGGGCCACTCCAGGCCATGACGTGGACGACTCACTAGCCA-3’
padlock probe A-	5’-PO_4-

C-3	GACGTGGACGACTCACTAGGTTTTGATCACAGTTTACGGTTTAGCATAACTCTA CTATCTGGGCCACTCCAGGCCAC-3’

Table S3 The reported methods for detecting 6mA at single-base resolution

Author	ssDNA or dsDNA	Detection limit
Hsin-Chih Yeh[1]	ssDNA	> 1nM
Xiang Zhou[2]	ssDNA	> 300 nM
Xiang Zhou[3]	dsDNA	> 0.5 nM
Yang Xiang[4]	ssDNA	> 44.427 pM

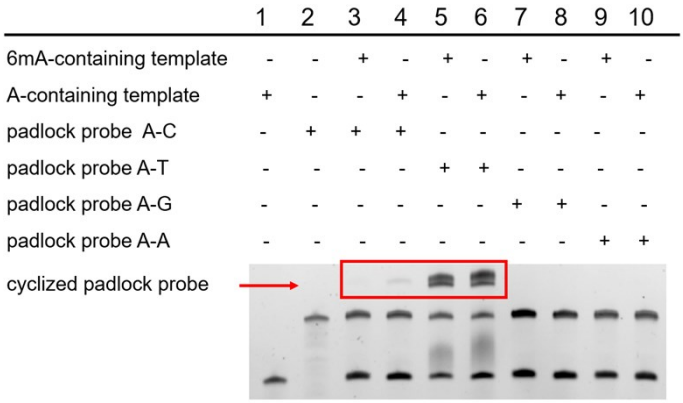

Figure S1. Discrimination of 6mA from A based on selective ligation by denaturing polyacrylamide gel electrophoresis. Difference in ligation yield of padlock probe bearing a base A, C, G or T at its 3’ end when single-stranded A or 6mA- containing template were used as templates. 100 nM single-stranded A or 6mA- containing template, 100 nM padlock probe.

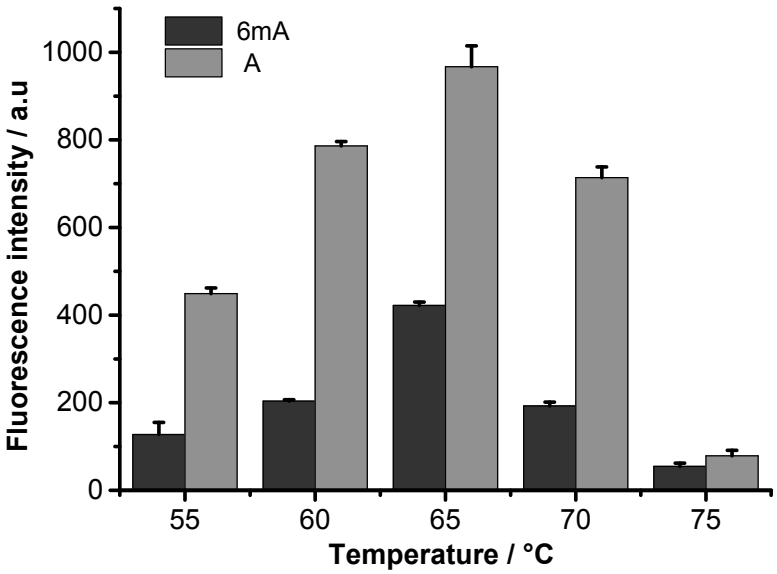

Figure S2. Fluorescent responses of input A or 6mA containing dsDNA templates in different ligation temperature. Ligation temperature

ranged from 55 °C to 75 °C. The concentration of A or 6mA containing dsDNA templates was 1 nM, the RCA reactions were performed at 30 °C for 2 h.

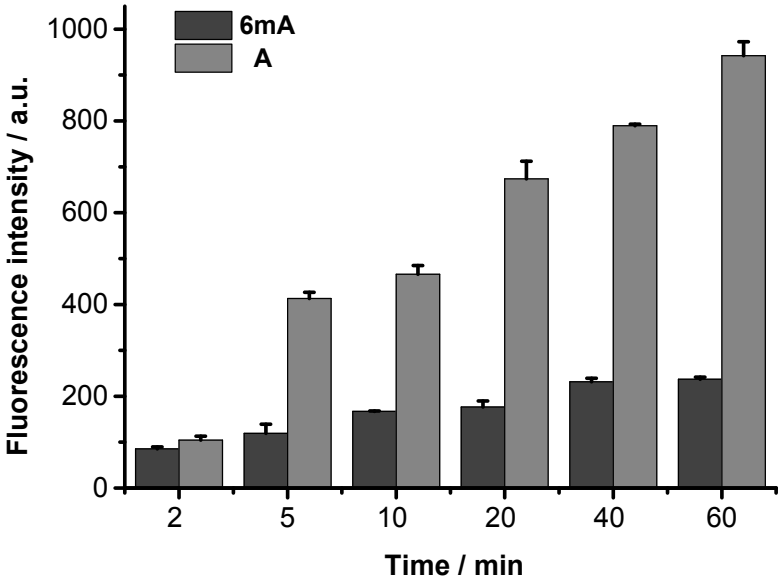

Figure S3. Fluorescent responses of input A/6mA-containing dsDNA templates for different ligation time. Ligation time ranged from 2 min to 60 min. The concentration of A or 6mA containing dsDNA templates was 1 nM, the RCA reactions were performed at 30 °C for 2 h.

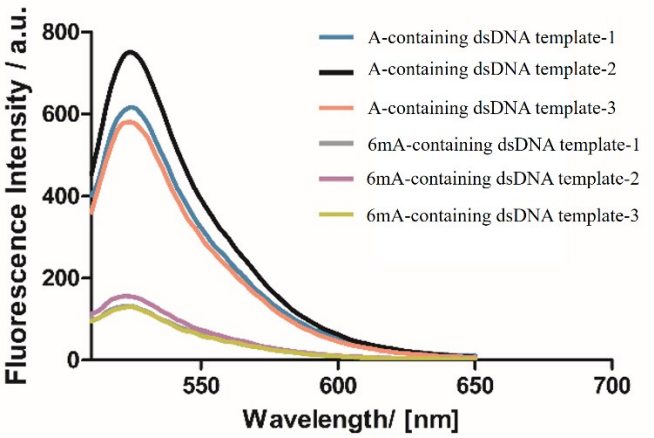

Figure S4. Fluorescent responses of input dsDNA carrying A or 6mA in three different series of dsDNA when A/6mA-C mispairs were formed in the presence of Taq DNA ligase. The concentration of A or 6mA containing dsDNA templates was 1 nM, the RCA reactions were performed at 30 °C for 2 h.

References

(1). Chen, Y.; Obliosca, J.; Liu, Y.; Liu, C.; Gwozdz, M..; Yeh, H. NanoCluster Beacons Enable Detection of a Single N (6)-Methyladenine. *J. Am. Chem. Soc.* **2015**, *137*, 10476-10479.
(2). Wang, S.; Wang, J.; Zhang, X.; Fu, B.; Song, Y.; Ma, P.; Gu, K.; Zhou, X.; Zhang, X.; Tian, T.; Zhou, X., N-6-Methyladenine hinders RNA- and DNA-directed DNA synthesis: application in human rRNA methylation analysis of clinical specimens. Chem. Sci. **2016**, *7*, 2, 1440-1446.
(3). Hong, T.; Yuan, Y.; Wang, T.; Ma, J.; Yao, Q.; Hua, X.; Xia, Y.; Zhou, X. Selective detection of N6-methyladenine in DNA via metal ion-mediated replication and rolling circle amplification. *Chem. Sci.* **2017**, *8*, 200-205.
(4). Wang, Y.; Zheng, J.; Duan, C.; Jiao, J.; Gong, Y.; Shi, H.; Xiang, Y. Detection of locus-specific N 6 -methyladenosine modification based on Ag^{+} -assisted ligation and supersandwich signal amplification. *Analyst*, **2021**, *146*, 1355-1360.