Structure and unzipping behavior of dumbbell and hairpin DNA revealed by real-time nanopore sensing

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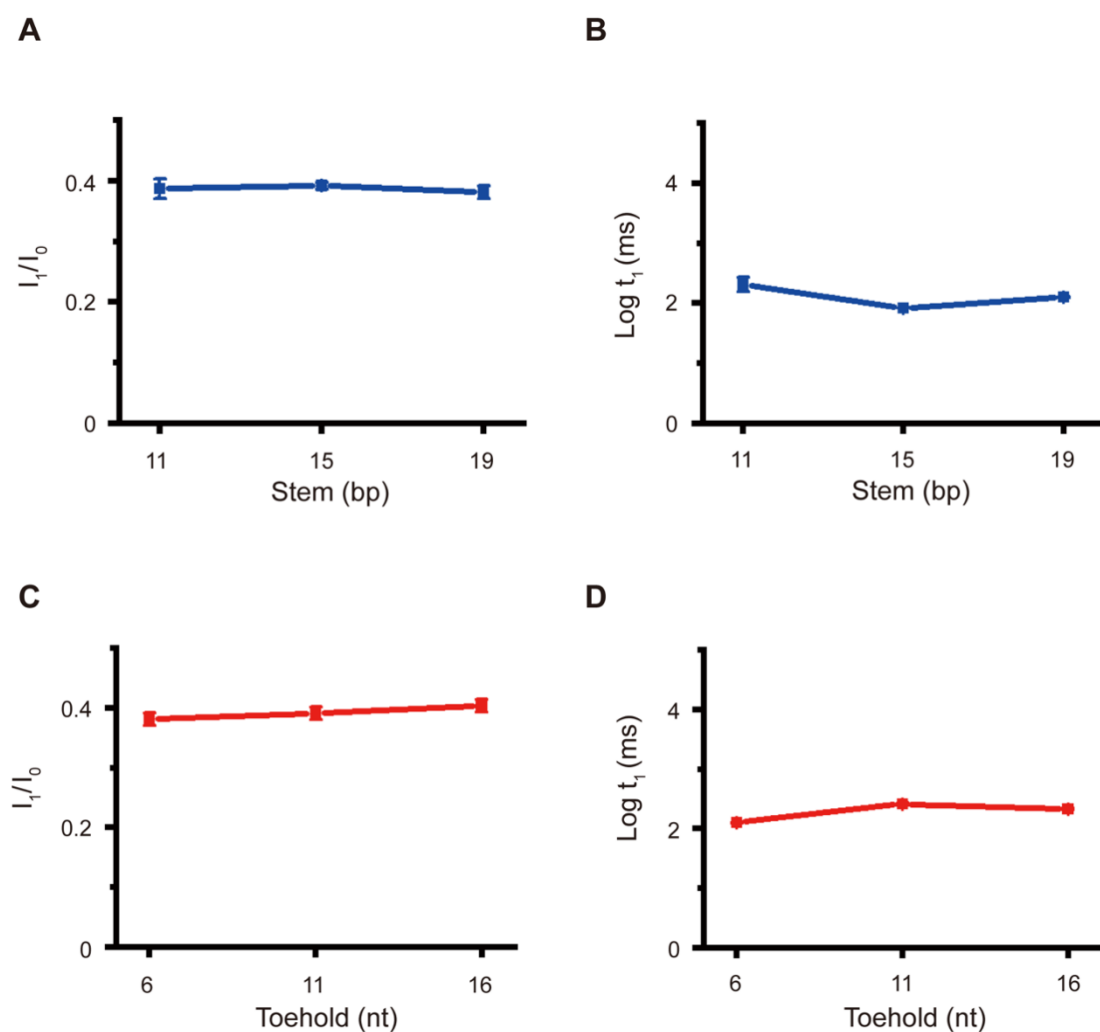
Fig. S1. Analysis of hairpin DNA with different stem and toehold length in the MspA nanopore. (A) (C) line chart of current blockage events (n=3). (B) (D) line chart of dwell time (n=3). Error bar equals standard deviation. The recording voltage was 120 mV with the buffer condition: *cis*: 0.5 M NaCl, 10 mM Tris, pH 8.0; *trans*: 3 M NaCl, 10 mM Tris pH 8.0.
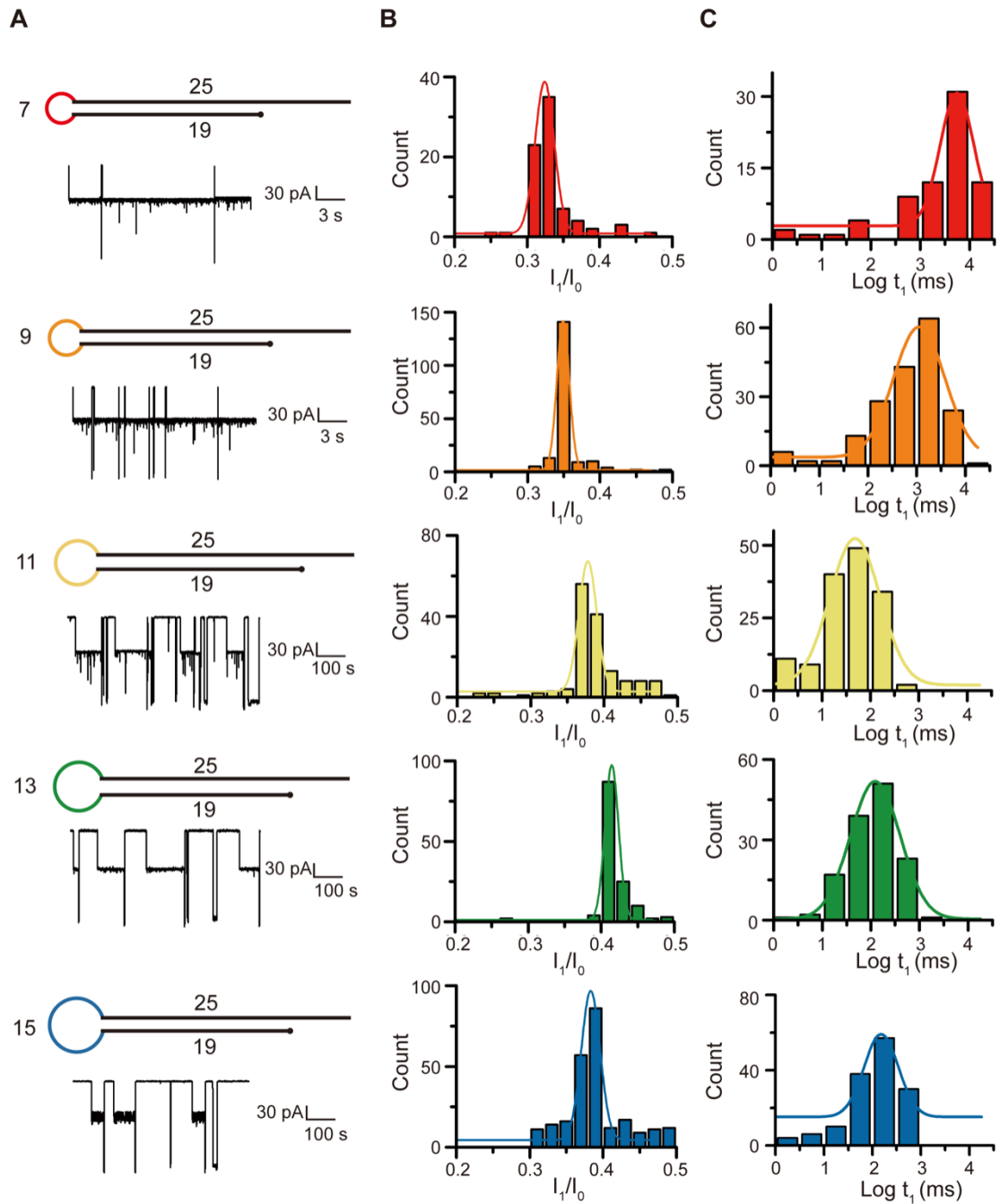
Fig. S2. Detection of hairpin DNA with different loop through the MspA nanopore (level 1). (A) The structure and current trace of hairpin DNA. (B) Histograms of current blockage. (C) Histograms of dwell time. The recording voltage was 120 mV with the buffer condition: *cis*: 0.5 M NaCl, 10 mM Tris, pH 8.0; *trans*: 3 M NaCl, 10 mM Tris pH 8.0.
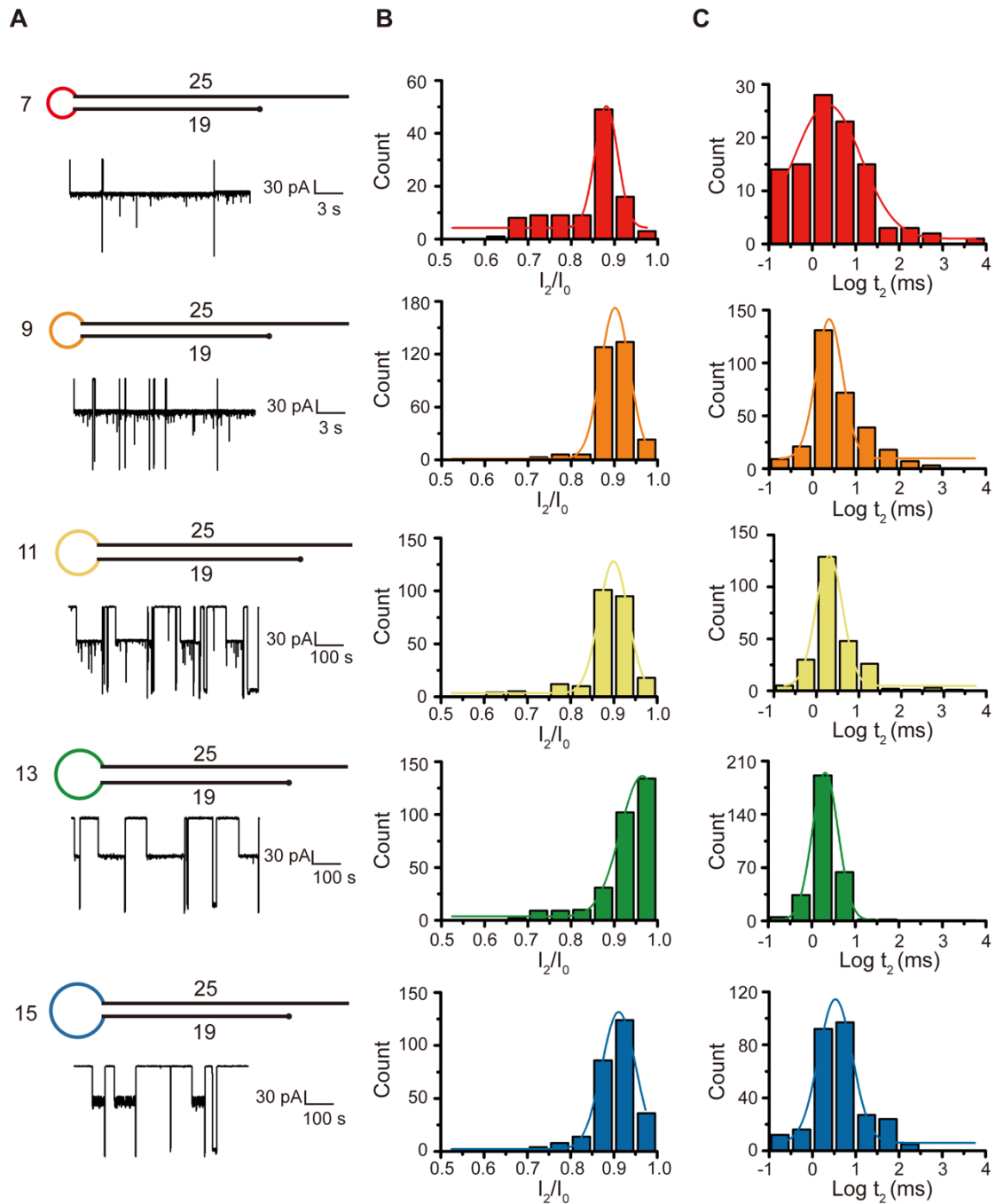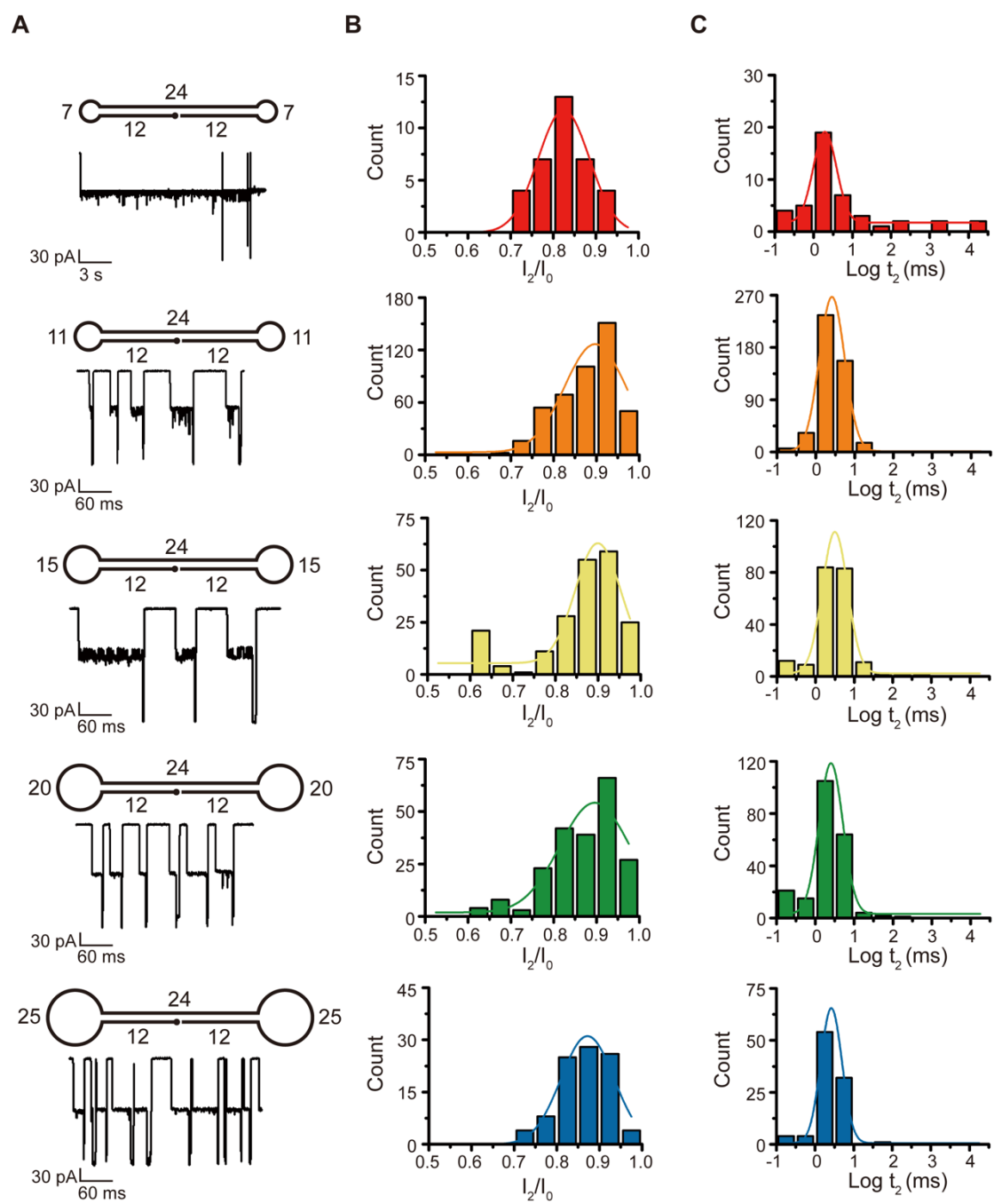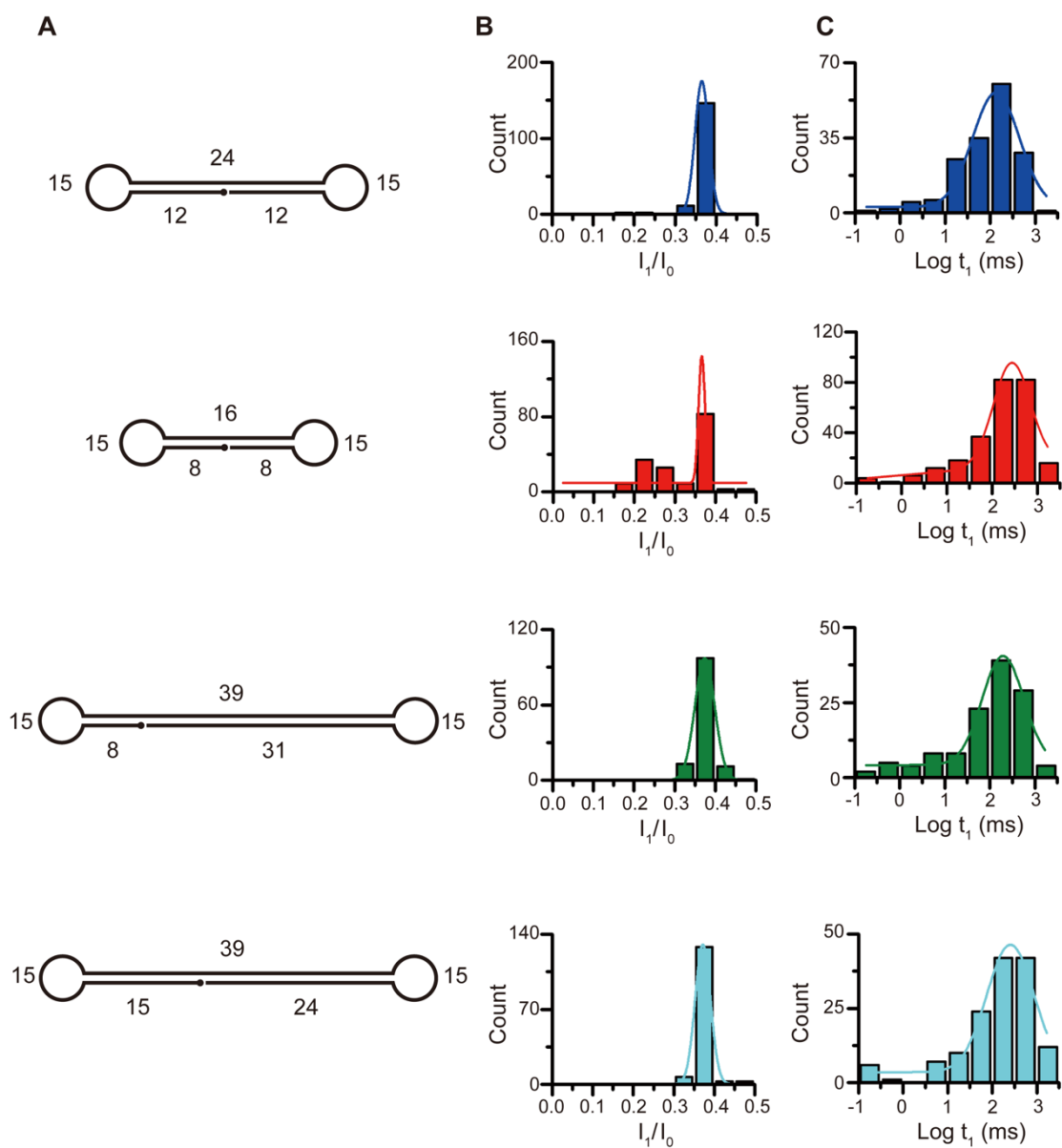
Fig. S3. Detection of hairpin DNA with different loop through the MspA nanopore (level 2). (A) The structure and current trace of hairpin DNA. (B) Histograms of current blockage. (C) Histograms of dwell time. The recording voltage was 120 mV with the buffer condition: *cis*: 0.5 M NaCl, 10 mM Tris, pH 8.0; *trans*: 3 M NaCl, 10 mM Tris pH 8.0.
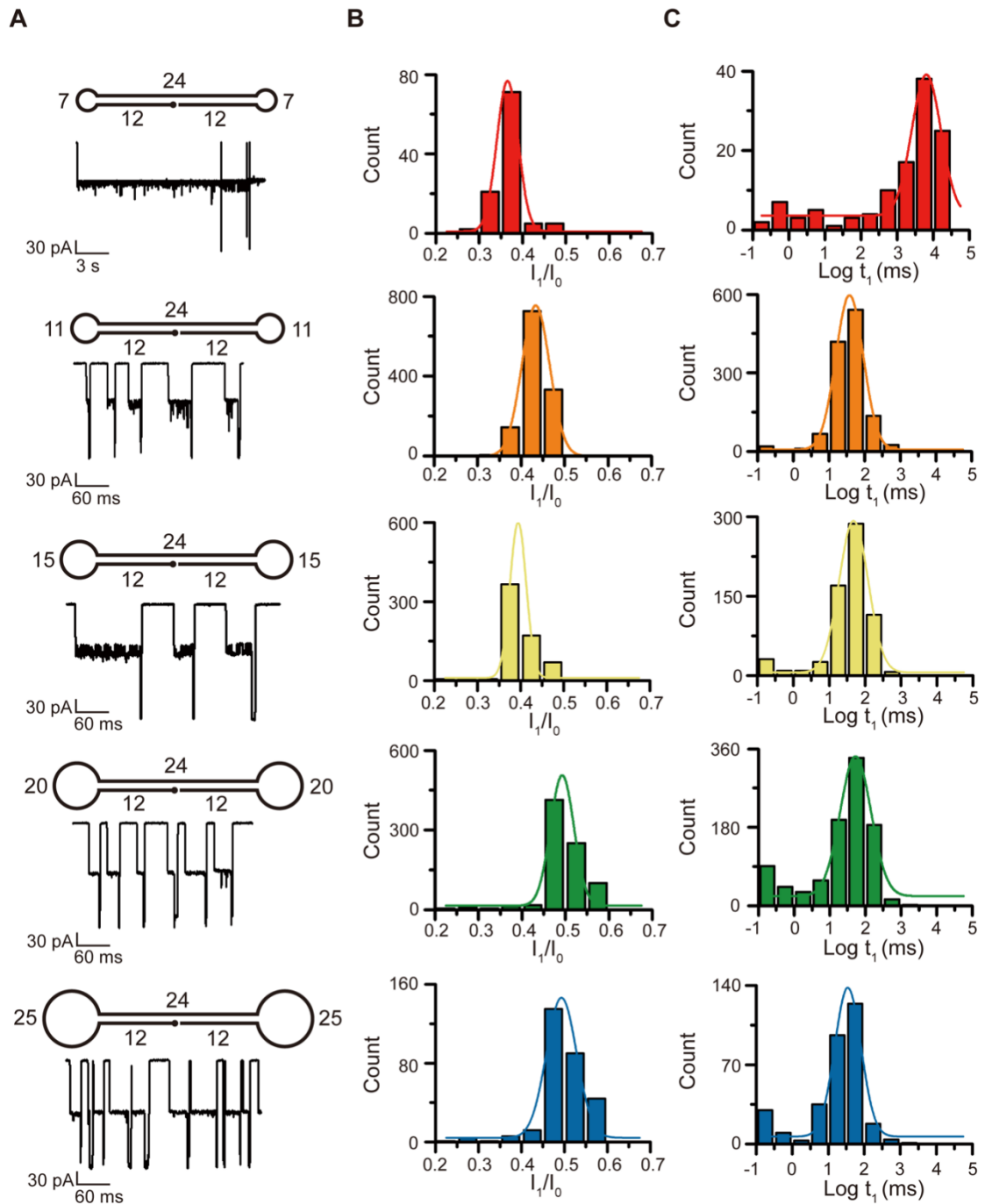
Fig. S4. Detection of dumbbell structure DNA with different loop through the MspA nanopore (level 1). (A) The structure and current trace of dumbbell DNA. (B) Histograms of current blockage. (C) Histograms of dwell time. The recording voltage was 120 mV with the buffer condition: *cis*: 0.5 M NaCl, 10 mM Tris, pH 8.0; *trans*: 3 M NaCl, 10 mM Tris pH 8.0.

Fig. S5. Detection of dumbbell structures DNA with different loop through the MspA nanopore (level 2). (A) The structure and current trace of dumbbell DNA. (B) Histograms of current blockage. (C) Histograms of dwell time. The recording voltage was 120 mV with the buffer condition: *cis*: 0.5 M NaCl, 10 mM Tris, pH 8.0; *trans*: 3 M NaCl, 10 mM Tris pH 8.0.
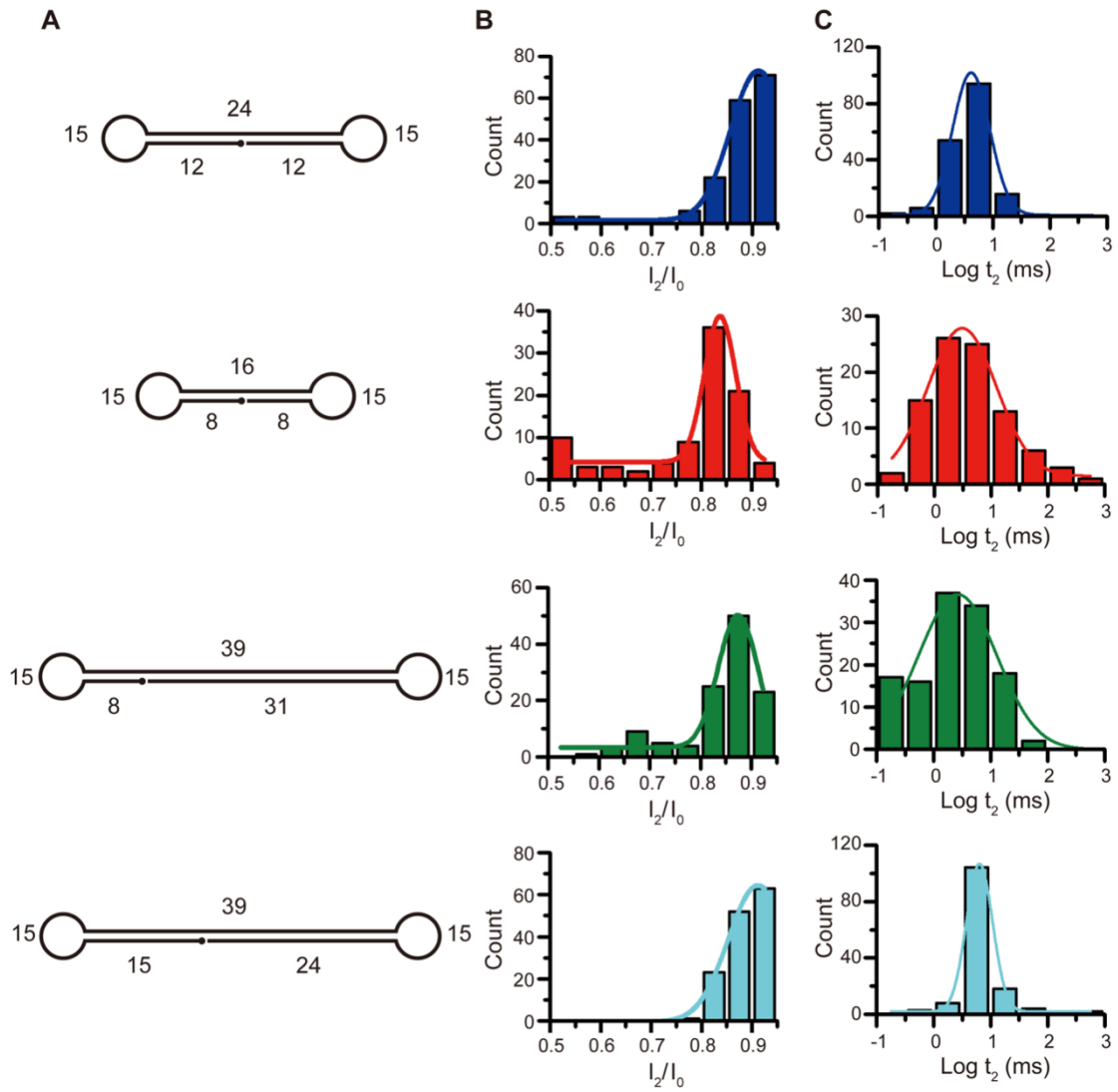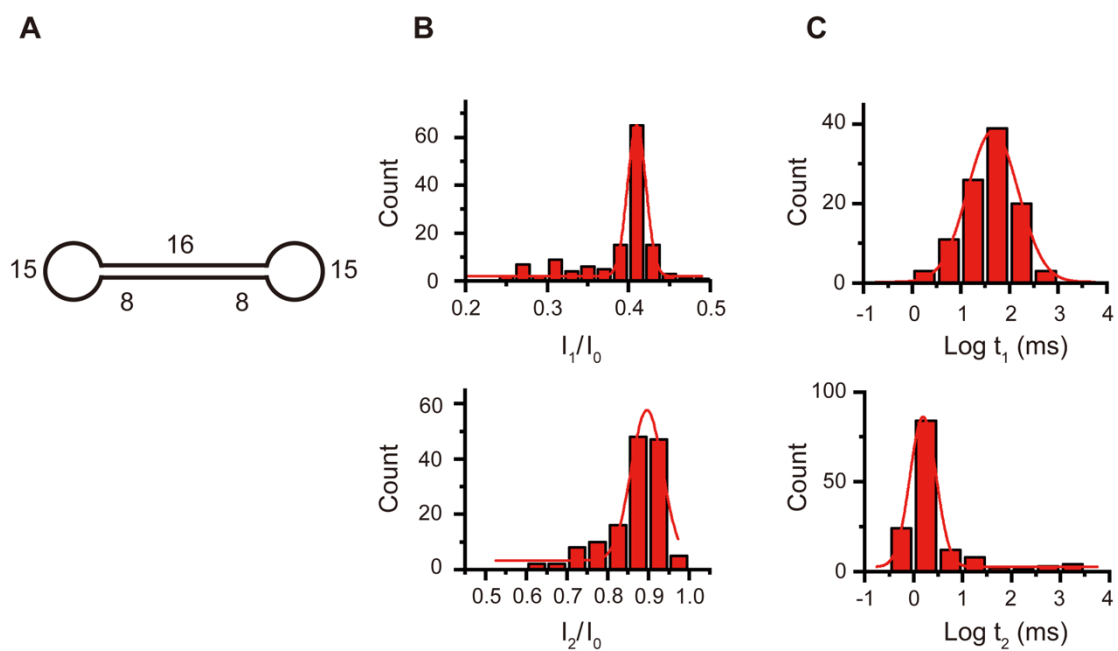
Fig. S6. Detection of dumbbell structure DNA with different stem length through the MspA nanopore (level 1). (A) The structure of dumbbell DNA. (B) Histograms of current blockage. (C) Histograms of dwell time. The recording voltage was 120 mV with the buffer condition: *cis*: 0.5 M NaCl, 10 mM Tris, pH 8.0; *trans*: 3 M NaCl, 10 mM Tris pH 8.0.

Fig. S7. Detection of dumbbell structures DNA with different stem through the MspA nanopore (level 2). (A) The structure of dumbbell DNA. (B) Histograms of current blockage. (C) Histograms of dwell time. The recording voltage was 120 mV with the buffer condition: *cis*: 0.5 M NaCl, 10 mM Tris, pH 8.0; *trans*: 3 M NaCl, 10 mM Tris pH 8.0.

Fig. S8. Events of ligated dumbbell DNA with different stem length through the MspA nanopore. (A) The structure of ligated dumbbell DNA. (B) Histograms of current blockage. (C) Histograms of dwell time. The recording voltage was 120 mV with the buffer condition: *cis*: 0.5 M NaCl, 10 mM Tris, pH 8.0; *trans*: 3 M NaCl, 10 mM Tris pH 8.0.
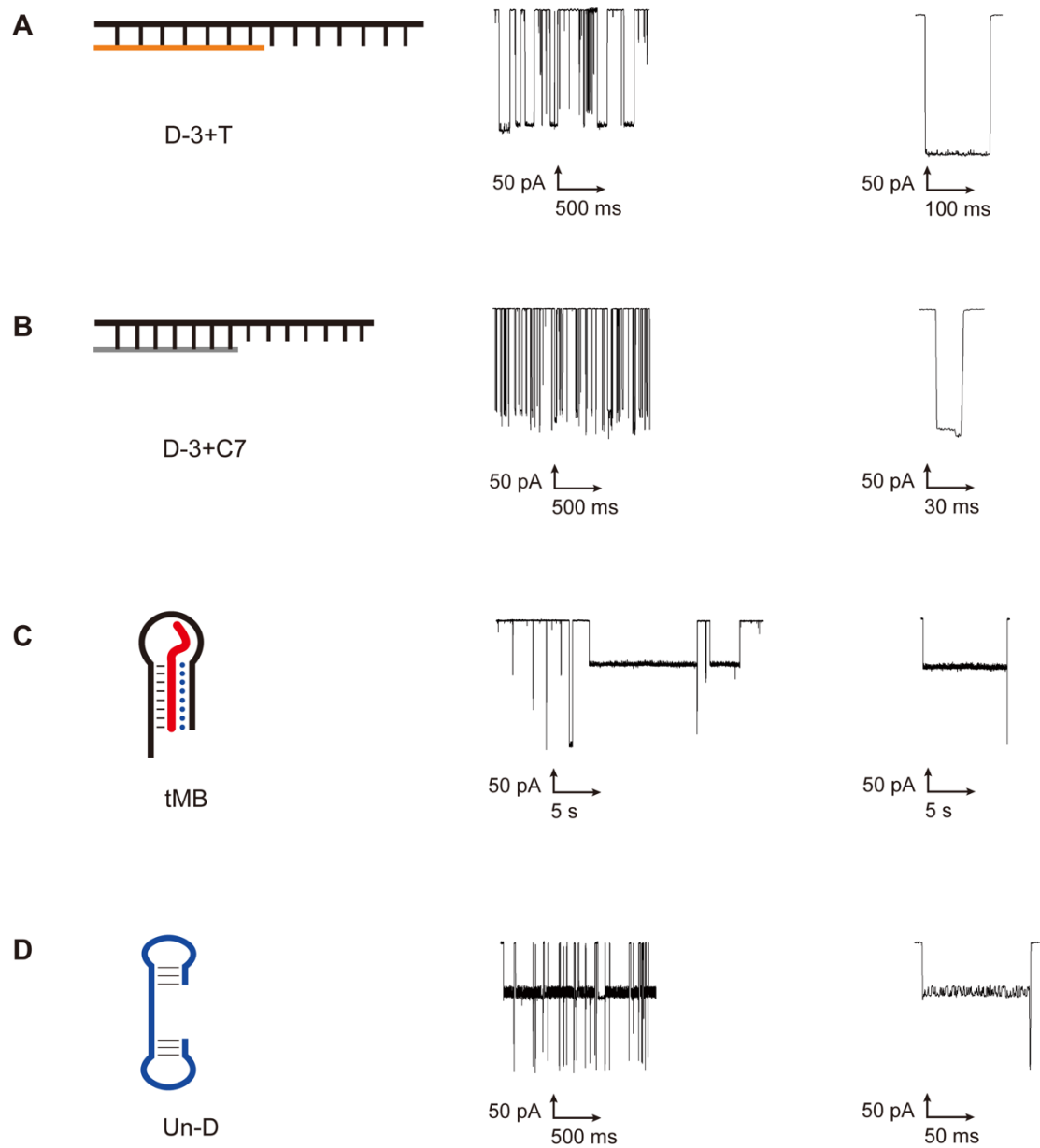
Fig. S9. Different structure of DNA translocation through MspA nanopore. (A) D-3+T. (B) D-3+C7. (C) tMB. (D) Un-dumbbell DNA. The recording voltage was 120 mV with the buffer condition: *cis*: 0.5 M NaCl, 10 mM Tris-HCl, pH 8.0; *trans*: 3 M NaCl, 10 mM Tris-HCl, pH 8.0.
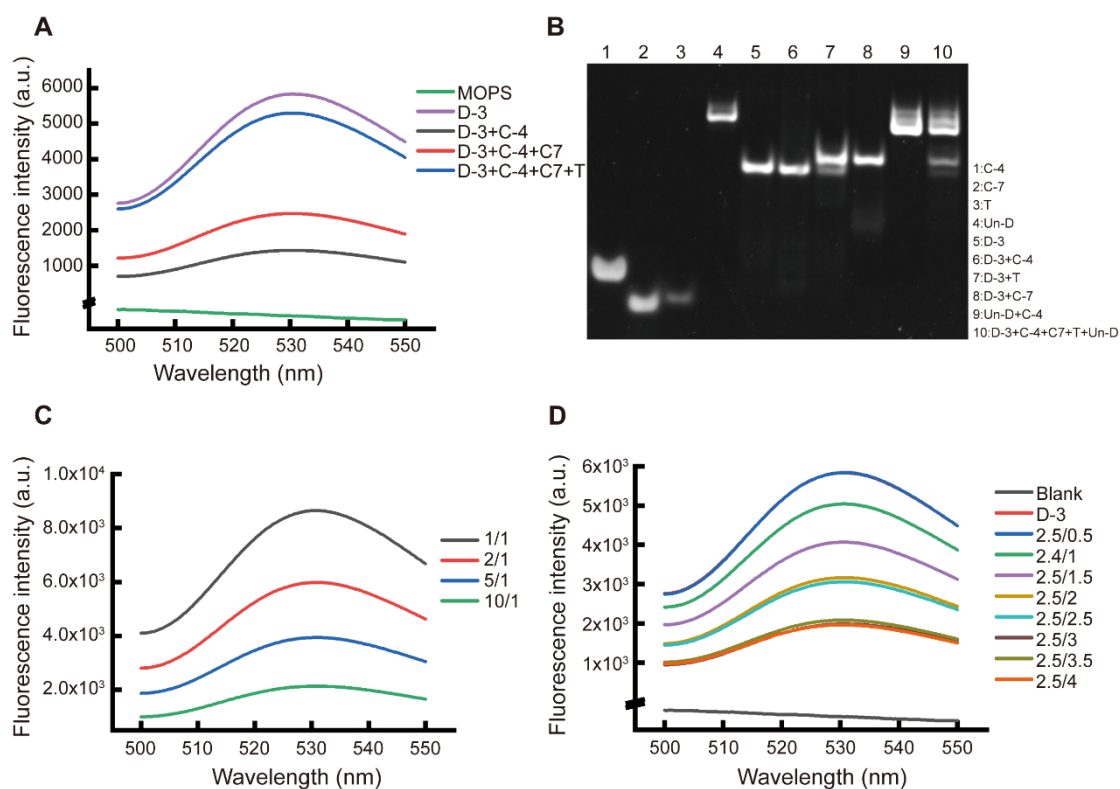
Fig. S10. Feasibility and optimization of the experimental condition in SNP detection. (A) UV−vis absorption of tMB. (B) Native polyacrylamide gel electrophoresis analysis of dumbbell DNA. (C) Fluorescence emission spectra of the method using different concentrations ration of C7 and B-D-3+C-4. (D) Fluorescence emission spectra of the method using different concentrations of B- D-3 and C-4.

Table S1. The sequences of hairpin and dumbbell DNA used in this study

Name (Structure-loop-stem-toehold)	Sequence (5'-3')
H-15-19-6	GTATGAGCGCTCATACGTTCATTCATTTCTCAGCAACGTATGAGCGCTCATACGTTCAT
H-13-19-6	GTATGAGCGCTCATACGTTCATTCATCTCAGCAACGTATGAGCGCTCATACGTTCAT
H-11-19-6	GTATGAGCGCTCATACGTTCATTCCTCAGCAACGTATGAGCGCTCATACGTTCAT
H-9-19-6	GTATGAGCGCTCATACGTTCATTTCAGCAACGTATGAGCGCTCATACGTTCAT
H-7-19-6	GTATGAGCGCTCATACGTTCATCAGCAACGTATGAGCGCTCATACGTTCAT
H-15-19-11	GTATGAGCGCTCATACGTTCATTCATTTCTCAGCAACGTATGAGCGCTCATACGTTCATTTTTT
H-15-19-16	GTATGAGCGCTCATACGTTCATTCATTTCTCAGCAACGTATGAGCGCTCATACGTTCATTTTTTTTTTT
H-15-15-6	GTATGAGCGCTCATACATTCATTTCTCAGCTATGAGCGCTCATACGTTCAT
H-15-11-6	GTATGAGCGCTCATTCATTTCTCAGCAGCGCTCATACGTTCAT
D-15-8-16	CATACGTTCATTCATTTCTCAGCAACGTATGCTCATACCCATTCATTTCTCAGCGGTATGAG
D-7-12-24	CGCTCATACGTTCATCAGCAACGTATGAGCGCACGTATGAGCGCATCAGCCGCTCATACGTG
D-11-12-24	CGCTCATACGTTCATTCCTCAGCAACGTATGAGCGCACGTATGAGCGCATTCCTCAGCCGCTCATACGTG

D-15-12-24	CGCTCATACGTTCATTCATTTCTCAGCAACGTATGAGCGCAC GTATGAGCGCATTCATTTCTCAGCCGCTCATACGTG
D-20-12-24	CGCTCATACGTTCATTCATTTTTTTCTCAGCAACGTATGAGC GCACGTATGAGCGCATTCATTTTTTTCTCAGCCGCTCATACG TG
D-25-12-24	CGCTCATACGTTCATTCATTTTTTTTTTTTTCTCAGCAACGTAT GAGCGCACGTATGAGCGCATTCATTTTTTTTTTTTTCTCAGCC GCTCATACGTG
D-30-12-24	CGCTCATACGTTCATTCATTTTTTTTTTTTTTTTTCTCAGCAA CGTATGAGCGCACGTATGAGCGCATTCATTTTTTTTTTTTTTTT TTCTCAGCCGCTCATACGTG
D-15-8-39	CATACGTTCATTCATTTCTCAGCAACGTATGTAAAGTGCTTAT AGTGCAGGTAGAACGTATGCATTCATTTCTCAGCCATACGTTC TACCTGCACTATAAGCACTTTA
D-15-15-39	TGAAGTGCATACGTTCATTCATTTCTCAGCAACGTATGCACTT CACTTATAGTGCAGGTAGAACGTATGCATTCATTTCTCAGCCA TACGTTCTACCTGCACTATAAG
Un-15-8-23	CATACGTTCATTCATTTCTCAGCAACGTATGTAAAGTGCTTAT AGTGCAGGTAGAACGTATGCATTCATTTCTCAGCCATACGTT
Un-15-15-9	TGAAGTGCATACGTTCATTCATTTCTCAGCAACGTATGCACTT CACTTATAGTGCAGGTAGAACGTATGCATTCATTTCTCAGCCA TACGTTCTACCTG
T	GCTCCCCGCACCGGCCCATC
TM1	GCT**G**CCCGCACCGGCCCATC
TM2	**C**CTCCCCGCACCGGCCCATC
TM3	GCTCCCCGCA**G**CGGCCCATC
TM4	GCTCCCCGCACCGGCC**G**ATC
TM5	GCTCCCCGCACCGGCCCAT**G**
C-4	AAAAAAAAAGGGGGGGGGGGGGG
D-3	AGCTTTTTTTT TTGATGGGCCGGTGCGGGGAGCTTT TTTTTT

B-D-3	FAM-AGCTTTTTTT TTGATGGGCCGGTGCGGGGAGCTTT TTTTTT-BHQ
C7	AAAAAGCTCCCCGCACCGGC
Un-D	CATACGTTCATTCATTTCTCAGCAACGTATGCCC CCCCCC CCCCTTTTTT TTTAACGTATGCATTCAT TTCTCAGCCATACGTT