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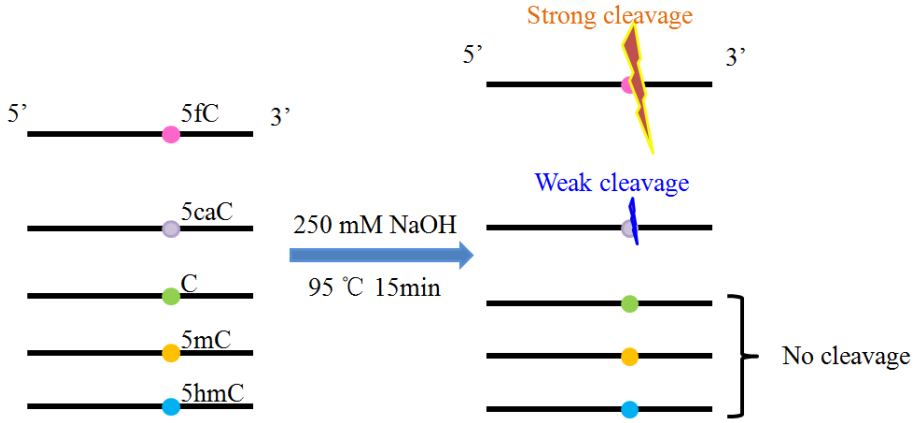
((Catch Phrase))

Systematic investigation of DNAs with modified cytosines under alkali-hot treatment



We have first conducted a systematic investigation of DNAs with modified cytosines, including 5-methylcytosine (5mC), 5-hydroxymethylcysosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), under alkali-hot treatment. The results demonstrated that DNAs could be selectively cleaved at the sites of 5fC and 5caC without any interference resulting from C, 5mC or 5hmC.

Supporting Information

Systematic investigation of DNAs with modified cytosines under alkali-hot treatment

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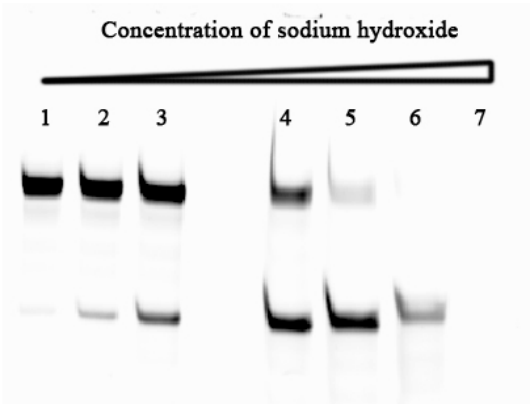
General information: All the oligonucleotides were purchased from Takara Co. (Dalian, China). Thermostable TDG Protein was purchased from Trevigen Inc. Sodium hydroxide was purchased from Sigma Inc. PAGE analyses were performed with denaturing 20% (19:1) polyacrylamide gel in presence of 8 M urea.

DNAs with modified cytosines under alkali-hot treatment: The reaction mixtures (10 μL) consisting of different concentrations of NaOH and 100 nM DNAs with modified cytosines were incubated at 95 ^{o}C for 15 mins. After cooling down to 4 ^{o}C, the reactions were stopped by adding 30 μL 80% aqueous formamide(deionized). The solutions were analyzed by denaturing 20% PAGE.

Table S1 Sequences of oligomers used for hot alkali-treatment.

Oligomer	Sequence(from 5'to 3')
FAM-31mer-C17	5' FAM labeled TAATTGCGGTCAATTG**C**GGATCATG**C**GTATA
FAM-31mer-mC17	5' FAM labeled TAATTGCGGTCAATTG(5m**C**)GGATCATG**C**GTATA
FAM-31mer-hmC17	5' FAM labeled TAATTGCGGTCAATTG(5hm**C**)GGATCATG**C**GTATA
FAM-31mer-fC17	5' FAM labeled TAATTGCGGTCAATTG(5f**C**)GGATCATG**C**GTATA
FAM-31mer-fC26	5' FAM labeled TAATTGCGGTCAATTG**C**GGATCATG(5f**C**)GTATA
FAM-31mer-fC17-fC26	5' FAM labeled TAATTGCGGTCAATTG(5fC)GGATCATG(5f**C**)GTATA
FAM-31mer-mC17-hmC26	5' FAM labeled TAATTGCGGTCAATTG(5m**C**)GGATCATG(5hm**C**)GTATA
FAM-14-mer	5' FAM labeled TAATTGCGGTCAAT
FAM-19-mer	5' FAM labeled TAATTGCGGTCAATTG**C**GG
FAM-24-mer	5' FAM labeled TAATTGCGGTCAATTG**C**GGATCAT
14-3-3-fC52	FAM-ATGGAGAGAGCCAGTCTGATCCAGAAGGCCAAGCTGGCAGAGCAGGCCGAA(5fC)GCTATGAGGACATGGCAGCCTTCATGAAAGGCGCCGTGGAGAAGG
14-3-3-caC52	FAM-ATGGAGAGAGCCAGTCTGATCCAGAAGGCCAAGCTGGCAGAGCAGGCCGAA(5caC)GCTATGAGGACATGGCAGCCTTCATGAAAGGCGCCGTGGAGAAGG
FAM-97-mer	FAM-ATGGAGAGAGCCAGTCTGATCCAGAAGGCCAAGCTGGCAGAGCAGGCCGAACGCTATGAGGACATGGCAGCCTTCATGAAAGGCGCCGTGGAGAAGG
C14-3-3	CCTTCTCCACGGCGCCTTTCATGAAGGCTGCCATGTCCTCATAGCGTTCGGCCTGCTCTGCCAGCTTGGCCTTCTGGATCAGACTGGCTCTCTCCAT
FAM-51-mer	FAM-ATGGAGAGAGCCAGTCTGATCCAGAAGGCCAAGCTGGCAGAGCAGGCCGAA
FAM-55-mer	FAM-ATGGAGAGAGCCAGTCTGATCCAGAAGGCCAAGCTGGCAGAGCAGGCCGAACGCT

A



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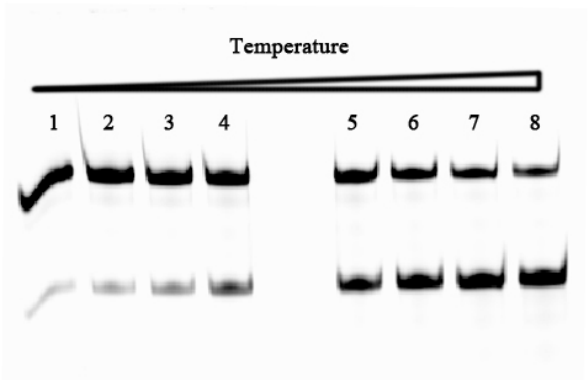


C

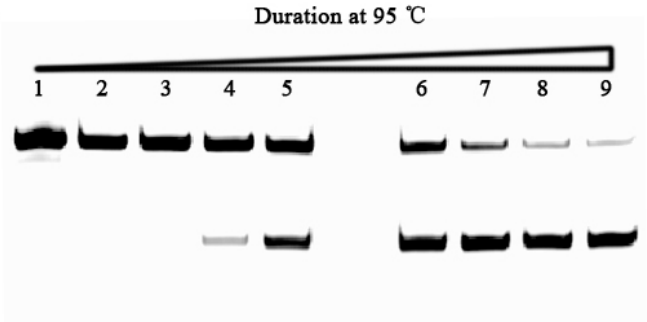
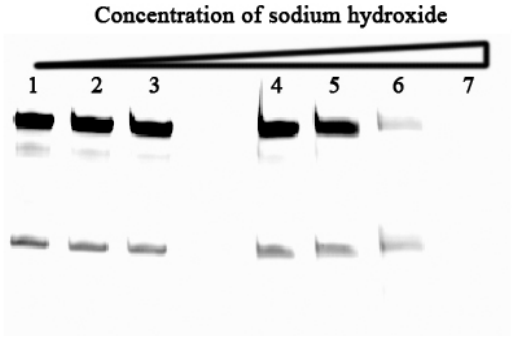


Figure S1. Comparative studies of influences on cleavage efficiencies of FAM-31mer-fC17. A: Varied NaOH concentrations on cleavage efficiencies. The cleavage reaction was performed at 95 ^{o}C for 15 mins, with a varied concentration of NaOH. Lane 1, 30 mM NaOH; lane 2, 60 mM NaOH; lane 3, 125 mM NaOH; lane 4, 250 mM NaOH; lane 5, 500 mM NaOH; lane 6, 1.0 M NaOH; lane 7, 2.0 M NaOH. B: Varied temperatures on cleavage efficiencies. The cleavage reaction was performed in presence of 250 mM NaOH for 15 mins, with a varied temperature. Lane1, 75.0 ℃; lane 2, 77.1 ℃; lane 3, 80.2 ℃; lane 4, 84.0 ℃; lane 5, 89.3 ℃; lane 6, 95.3 ℃; lane 7, 98.4 ℃; lane 8, 100 ℃. C: Varied heating duration on the cleavage reaction. The cleavage reaction was performed in presence of 250 mM NaOH at 95 ^{o}C, with a varied heating duration. Lane 1, no heating; lane 2, 1 min of heating; lane 3, 2 mins; lane 4, 4 mins; lane 5, 8 mins; lane 6, 15 mins; lane 7, 30 mins; lane 8, 45 mins; lane 9, 60 mins.

A



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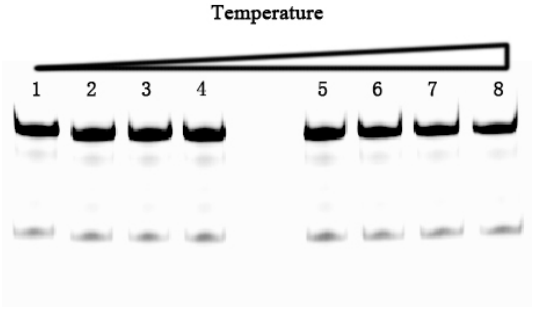

C

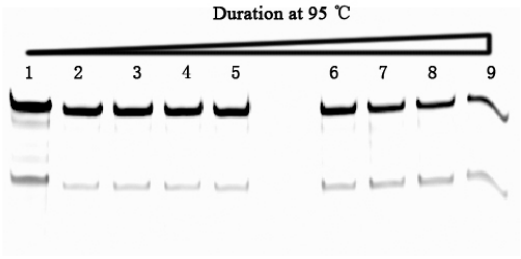

Figure S2. Comparative studies of influences on cleavage efficiencies of 14-3-3-caC52. A: Varied NaOH concentrations on cleavage efficiencies. The cleavage reaction was performed at 95 ^{o}C for 15 mins, with a varied concentration of NaOH. Lane 1, 30 mM NaOH; lane 2, 60 mM NaOH; lane 3, 125 mM NaOH; lane 4, 250 mM NaOH; lane 5, 500 mM NaOH; lane 6, 1.0 M NaOH; lane 7, 2.0 M NaOH. B: Varied temperatures on cleavage efficiencies. The cleavage reaction was performed in presence of 250 mM NaOH for 15 mins, with a varied temperature. Lane1, 75.0 ℃; lane 2, 77.1 ℃; lane 3, 80.2 ℃; lane 4, 84.0 ℃; lane 5, 89.3 ℃; lane 6, 95.3 ℃; lane 7, 98.4 ℃; lane 8, 100 ℃. C: Varied heating duration on the cleavage reaction. The cleavage reaction was performed in presence of 250 mM NaOH at 95 ^{o}C, with a varied heating duration. Lane 1, no heating; lane 2, 1 min of heating; lane 3, 2 mins; lane 4, 4 mins; lane 5, 8 mins; lane 6, 15 mins; lane 7, 30 mins; lane 8, 45 mins; lane 9, 60 mins.

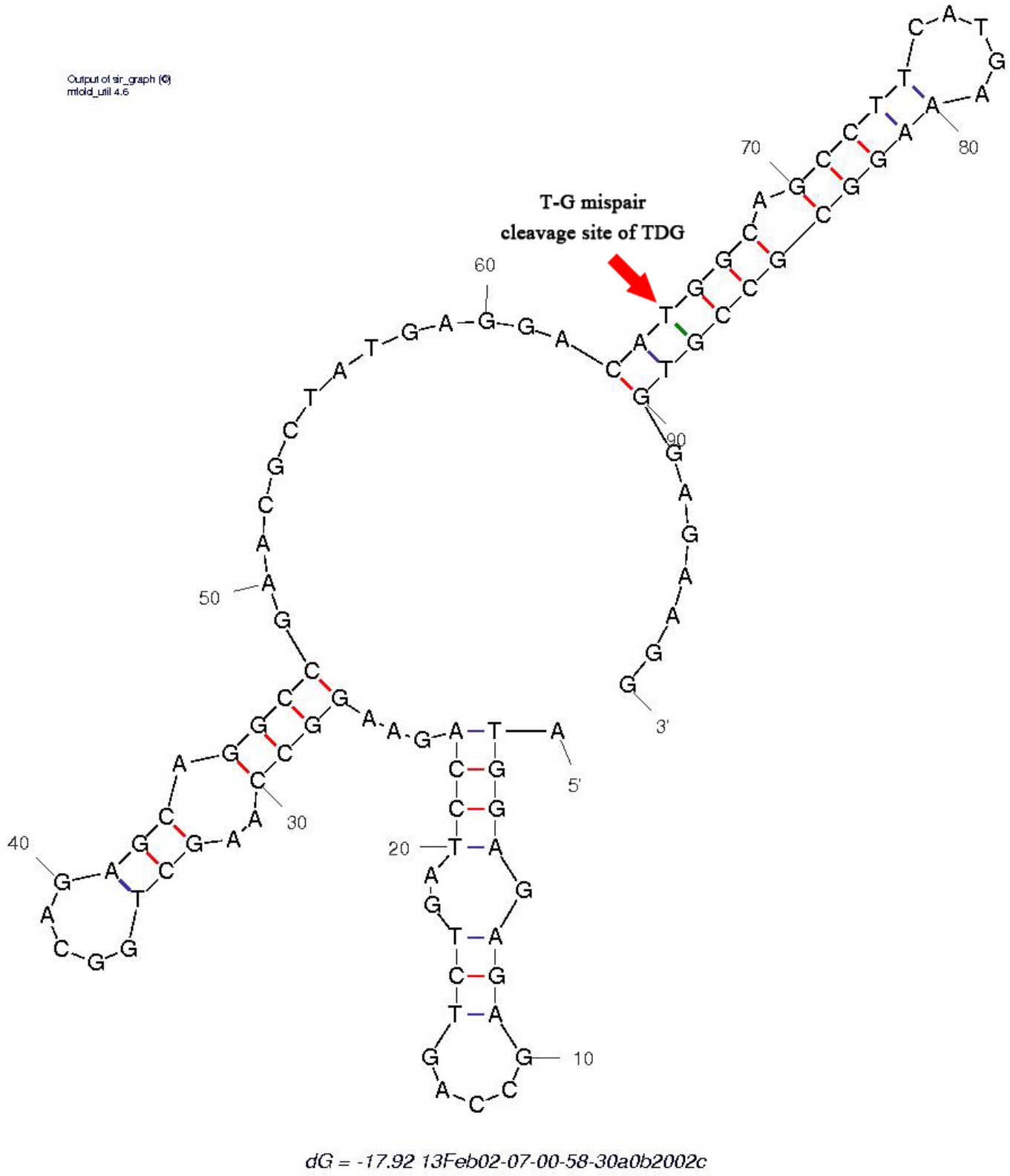

Figure S3. The postulated structure of the 14-3-3-fC52 and 14-3-3-caC52 from 14-3-3 sequence. The strand could be cleaved at T65 position of the sequence by TDG proteins.

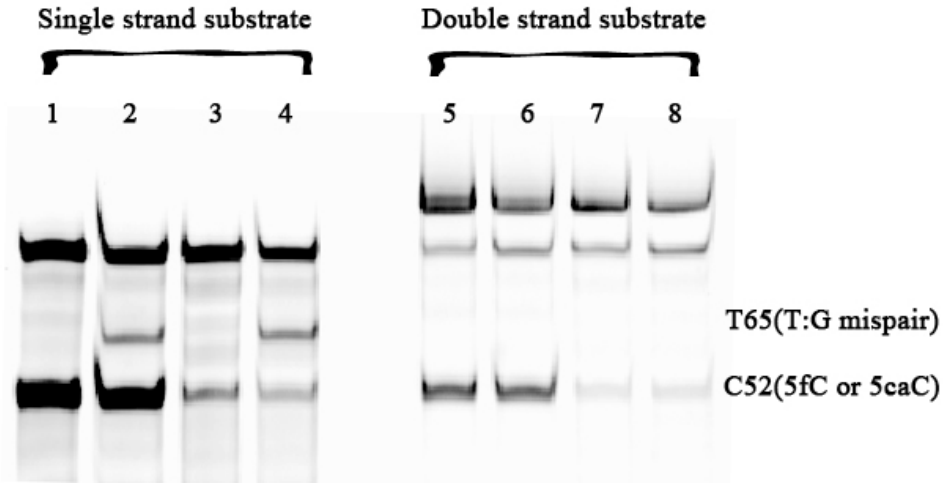

Figure S4. Denatured PAGE gel analysis of single stranded and double srranded templates upon alkaline treatment and/or TDG pretreatment. The reaction was performed in 1 X REC™ Buffer 4(10 mM HEPES-KOH (pH 7.4), 100 mM KCl, and 10 mM EDTA) and 0.5 µL of TDG enzyme or water in a 10 µl reaction volume are incubated for 1 hour at 65°C. Stock solution of 3 M sodium hydroxide was added to make a final concentration of 250 mM, followed by an incubation at 95 °C for 15 mins. Lane 1, 14-3-3-fC52 without TDG treatment; lane 2, 14-3-3-fC52 with TDG pretreatment; lane 3, 14-3-3-caC52 without TDG treatment; lane 4, 14-3-3-caC52 with TDG pretreatment; lane 5, duplex of 14-3-3-fC52 and C14-3-3 without TDG treatment; lane 6, duplex of 14-3-3-fC52 and C14-3-3 with TDG pretreatment; lane 7, duplex of 14-3-3-caC52 and C14-3-3 without TDG treatment; lane 8, duplex of 14-3-3-caC52 and C14-3-3 with TDG pretreatment.