Supporting Information

for

Location analysis of 8-oxo-7,8-dihydroguanine in DNA by polymerase-mediated differential coding

Feng Tang,[1,†] Shan Liu,[1,†] Qiao-Ying Li,[1] Jun Yuan,[2] Lin Li,[2] Yinsheng Wang,[2] Bi-Feng Yuan,[1,*] Yu-Qi Feng[1]

[1] Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, P.R. China

[2] Department of Chemistry and Environmental Toxicology Graduate Program, University of California, Riverside, CA, 92521-0403, USA.

[†] These authors contributed equally to this work.

* To whom correspondence should be addressed. Tel.:+86-27-68755595; fax: +86-27-68755595. E-mail address: bfyuan@whu.edu.cn.

The Supporting Information includes following items:

Page S3	**Table S1.** The sequences of the oligodeoxynucleotides used in this study.
Page S4	**Table S2.** PCR primers used for the single-base resolution analysis of OG in HeLa genomic DNA by sequencing.
Page S5	**Table S3.** Steady-state kinetics for primer extension with dATP or dCTP opposite G- or OG-containing DNA template using different DNA polymerases.
Page S6	**Figure S1.** Steady-state kinetics for primer extension with dATP opposite G- or OG-containing DNA template using different DNA polymerases.
Page S7	**Figure S2.** Steady-state kinetics for primer extension with dATP or dCTP opposite G- or OG-containing DNA template using different DNA polymerases.
Page S8	**Figure S3**. Four DNA templates (DNA-A, DNA-T, DNA-G and DNA-C) with a single site that has different nucleobases (A, T, G and C) were used to perform the primer extension assay by *Bsu* polymerase using dATP as the substrate.
Page S9	**Figure S4**. Four DNA templates (DNA-A, DNA-T, DNA-G and DNA-C) with a single site that has different nucleobases (A, T, G and C) were used to perform the primer extension assay by *Tth* polymerase using dATP as the substrate.
Page S10	**Figure S5**. Single nucleotide primer extension reaction by *Tth* polymerase using the mixture of DNA-G and DNA-T with the percentage of DNA-T ranging from 0% to 10%.
Page S11	**Figure S6**. The single nucleotide primer extension reaction by *Tth* polymerase using 5 μg HeLa DNA and different fluorophores-labeled primers (5 pmol for each) that target different guanosine sites in telomeric DNA.
Page S12	**Figure S7**. Single-base resolution analysis of multiple OG sites in DNA by sequencing. The primer extension products by *Bsu* Pol with DNA templates of L-DNA-G, L-DNA-OG1 (one OG site), L-DNA-OG2 (two OG sites), or L-DNA-OG3 (three OG sites).
Page S13	**Figure S8**. Single-base resolution analysis of multiple OG sites in DNA by sequencing. The primer extension products by *Tth* Pol with DNA templates of L-DNA-G, L-DNA-OG1 (one OG site), L-DNA-OG2 (two OG sites), or L-DNA-OG3 (three OG sites).
Page S14	**Figure S9.** Quantitative evaluation of the level of OG in DNA using various ratios of L-DNA-OG1/L-DNA-G (0%, 0.1%. 1%, 10%, 20%) as the DNA template by high-throughput sequencing.
Page S15	**Figure S10.** Schematic illustration of the procedure for the library construction.
Page S16	**Figure S11**. Schematic illustration of the analysis of OG in genomic DNA of HeLa cells.

Table S1. The sequences of the oligodeoxynucleotides used in this study.

ODNs	**Sequence (from 5'to 3')**
FAM-primer-1	5'-FAM-CGCATAACCCTAACC-3'
DNA-G	5'-GCGTATTGGGATTGG**G**ATTGACACG-3'
DNA-T	5'-GCGTATTGGGATTGG**T**ATTGACACG-3'
DNA-A	5'-GCGTATTGGGATTGG**A**ATTGACACG-3'
DNA-C	5'-GCGTATTGGGATTGG**C**ATTGACACG-3'
DNA-OG	5'-GCGTATTGGGATTGG**(OG)**ATTGACACG-3'
Cy3-primer	5'-Cy3-TAACCCTAACCCTAACC-3'
Cy5-primer	5'-Cy5-CCTAACCCTAACCCTAAC-3'
FAM-primer-2	5'-FAM-CCCTAACCCTAACCCTAA-3'
T-DNA-1	5'-GTTA**GGG**TTAGGGTTAGGGTTAGGG-3'
T-DNA-2	5'-GTTA**(OG)GG**TTAGGGTTAGGGTTAGGG-3'
T-DNA-3	5'-GTTA**G(OG)G**TTAGGGTTAGGGTTAGGG-3'
T-DNA-4	5'-GTTA**GG(OG)**TTAGGGTTAGGGTTAGGG-3'
L-DNA-G	5'-*GCCCAAGTGCTGAGGCTGATAA*TAATCGGGGCGGCGATCAGACAGCCCCGGTGTGGGAAATCGTCCGCCCGGTCTCCCTAAGTCCCCGAAGTCGCCTCCCACTTTTGGT**G**ACT*GCTTGTTTATTTACATGCAGinvertedT*-3'
L-DNA-OG1	5'-*GCCCAAGTGCTGAGGCTGATAA*TAATCGGGGCGGCGATCAGACAGCCCCGGTGTGGGAAATCGTCCGCCCGGTCTCCCTAAGTCCCCGAAGTCGCCTCCCACTTTTGGT**(OG)**ACT*GCTTGTTTATTTACATGCAGinvertedT*-3'
L-DNA-OG2	5'-*GCCCAAGTGCTGAGGCTGATAA*TAATCGGGGCGGCGATCAGACAGCCCCGGTGTGGGAAATCGTCCGCCCGGTCTCCCTAAGTCCCCGAAGTC**(OG)**CCTCCCACTTTTGGT**(OG)**ACT*GCTTGTTTATTTACATGCAGinvertedT*-3'
L-DNA-OG3	5'-*GCCCAAGTGCTGAGGCTGATAA*TAATCGGGGCGGCGATCAGACAGCCCCGGTGTGGGAAATCGTCCGCCCGGTCTCCCTAAGTCCCC**(OG)**AAGTC**(OG)**CCTCCCACTTTTGGT**(OG)**ACT*GCTTGTTTATTTACATGCAGinvertedT*-3'
L-primer	5'-TGAGCAGATGTGTGACGGCTACACTGCATGTAAATAAACAAGC-3'
PCR reverse primer	5'-GCCCAAGTGCTGAGGCTGATAA-3'
PCR forward primer	5'-TGAGCAGATGTGTGACGGCTAC-3'
NN-OG-NN	5'-*CCAGATGACGACTGGCAC*TAATGNN**(OG)**NNTGCTG*CGTGGATCGACATCGAGinvertedC*-3'
NN-L-Primer	5'-TGAGCAGATGTGTGACGGCTACGCTCGATGTCGATCCACG-3'
NN-F-Primer	5'-CCAGATGACGACTGGCAC-3'
NN-R-Primer	5'-TGAGCAGATGTGTGACGGCTAC-3'

Table S2. PCR primers used for the single-base resolution analysis of OG in HeLa genomic DNA by sequencing.

Gene / Amplified region	**Sequence (from 5' to 3')**
VEGFA / chr6: 43772151-43772344	*VEGFA*-L (Primer-L): TGAGCAGATGTGTGACCACCGCCACGACTTCTGACAGTGA *VEGFA*-F: TCTCCAGACCCTACCTCTGC *VEGFA*-R: TGAGCAGATGTGTGACCACC
TP53 / chr17: 7686438 -7686641	*TP53*-L (Primer-L): GAGCAGATGTGTGACGGCTAGCAGCTCACTATTCACCCGA *TP53*-F: TGGGGCACACCATTCAAAGA *TP53*-R: GAGCAGATGTGTGACGGCTA
KRAS / chr12: 25248470 - 25248719	*KRAS*-L (Primer-L): GGTTAGAGCAGATGTGACGCCCTCCCAGCCCATGATCTTC *KRAS*-F: GAGGGGTCGTTAAGGCCAAA *KRAS*-R: GGTTAGAGCAGATGTGACGC

Table S3. Steady-state kinetics for primer extension with dATP or dCTP opposite G- or OG-containing DNA template using different DNA polymerases.

DNA polymerase	dNTP	DNA template	V_{max} (% min^{-1})	K_M (μM)	V_{max}/K_M ($min^{-1}\mu M^{-1}$)
Bsu	dATP	DNA-G	14.5 ± 2.3	178.6 ± 4.9	0.8×10^{-3}
	dATP	DNA-OG	25.1 ± 2.4	9.2 ± 0.6	27.3×10^{-3}
	dCTP	DNA-G	42.2 ± 0.6	1.8 ± 0.1	234.4×10^{-3}
	dCTP	DNA-OG	27.8 ± 2.3	107.5 ± 6.3	2.6×10^{-3}
Tth	dATP	DNA-G	5.4 ± 0.5	228.3 ± 15.4	0.2×10^{-3}
	dATP	DNA-OG	6.2 ± 0.6	275.8 ± 18.2	0.2×10^{-3}
	dCTP	DNA-G	50.4 ± 2.6	2.5 ± 0.3	201.6×10^{-3}
	dCTP	DNA-OG	29.7 ± 1.4	174.3 ± 23.5	1.7×10^{-3}

V_{max}, the maximum rate of the enzyme reaction.

K_M, the Michaelis constant.

Figure S1. Steady-state kinetics for primer extension with dATP opposite G- or OG-containing DNA template using different DNA polymerases.

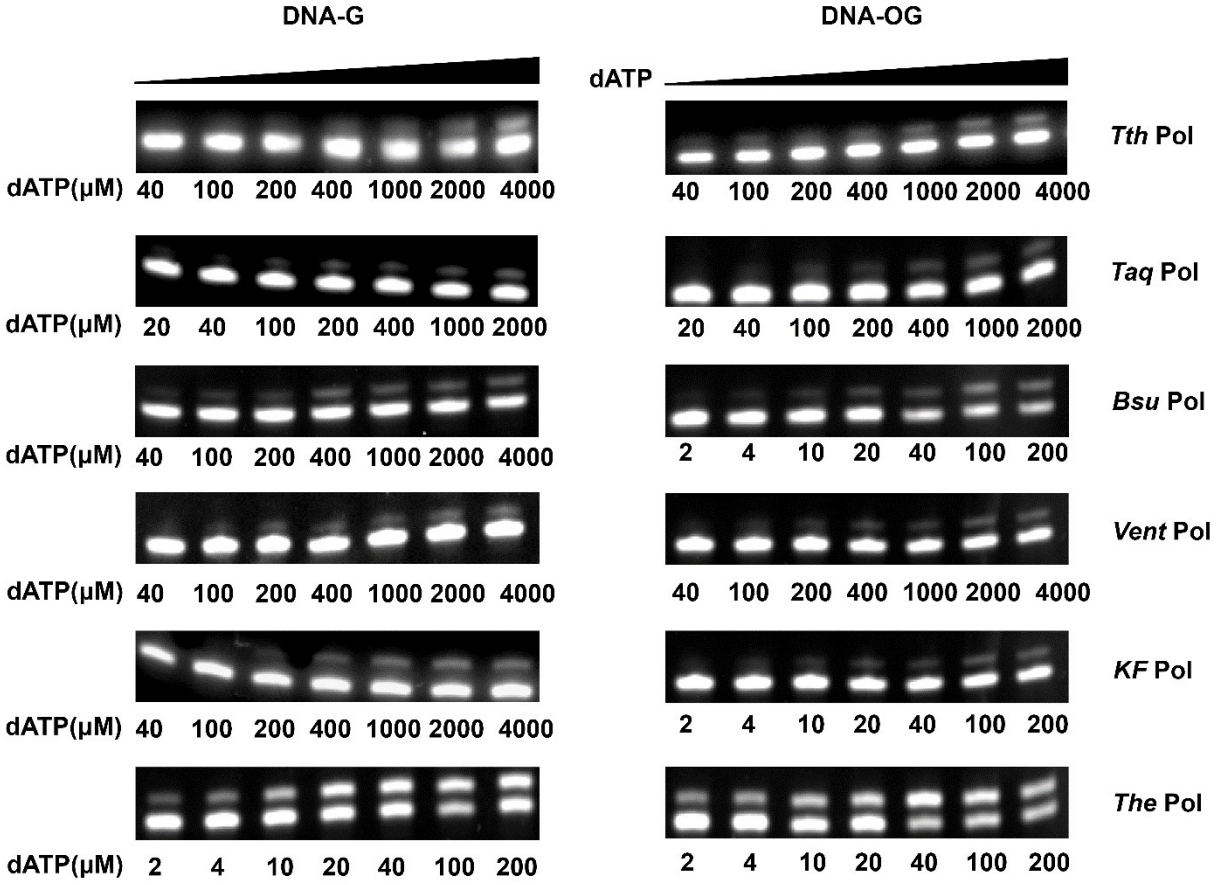

Figure S2. Steady-state kinetics for primer extension with dATP or dCTP opposite G- or OG-containing DNA template using different DNA polymerases.

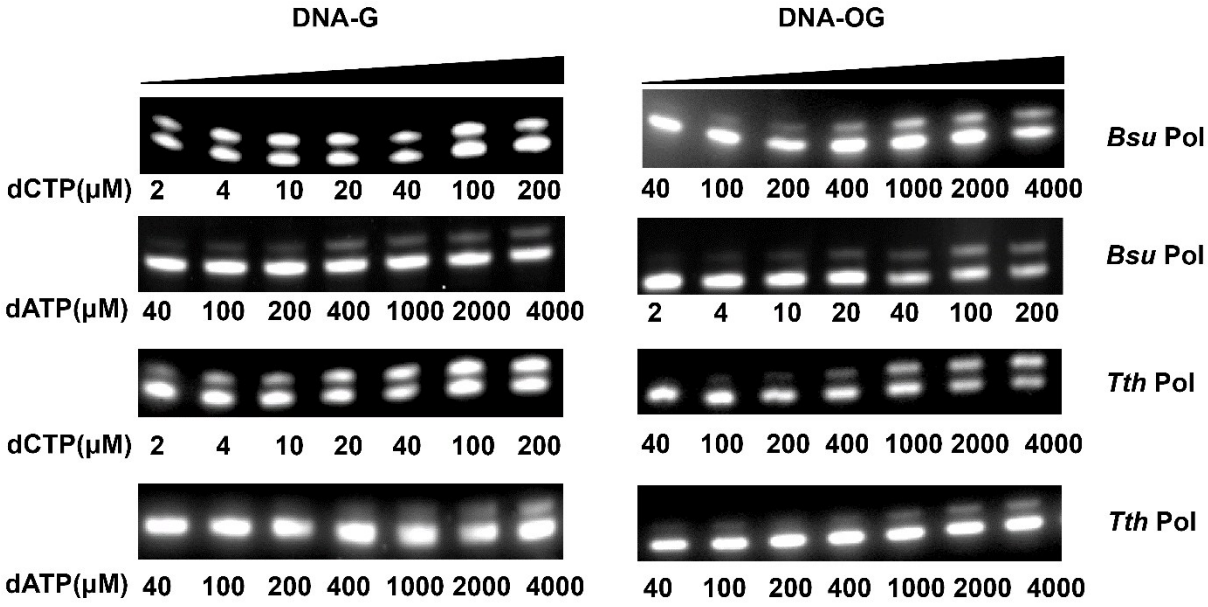

Figure S3. Four DNA templates (DNA-A, DNA-T, DNA-G and DNA-C, detailed sequence information can be found in Table S1 in Supporting Information) with a single site that has different nucleobases (A, T, G or C) were used to perform the primer extension assay by *Bsu* polymerase using dATP as the substrate. The reaction products were analyzed by 20% denaturing PAGE.

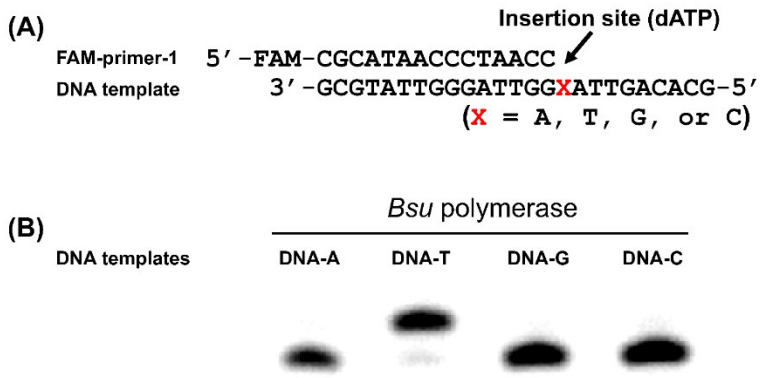

Figure S4. Four DNA templates (DNA-A, DNA-T, DNA-G and DNA-C, detailed sequence information can be found in Table S1 in Supporting Information) with a single site that has different nucleobases (A, T, G or C) were used to perform the primer extension assay by *Tth* polymerase using dATP as the substrate. The reaction products were analyzed by 20% denaturing PAGE.

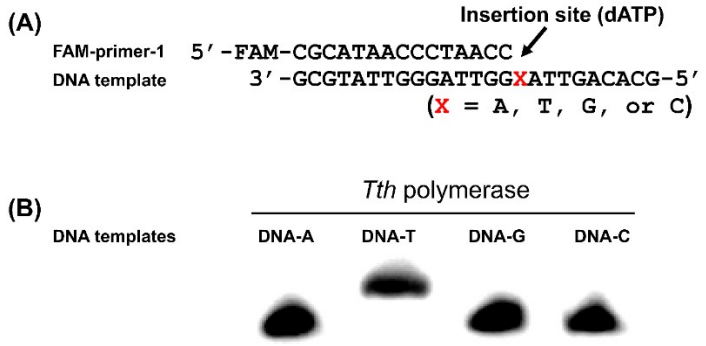

Figure S5. Single nucleotide primer extension reaction by *Tth* polymerase using the mixture of DNA-G and DNA-T with the percentage of DNA-T ranging from 0% to 10%.

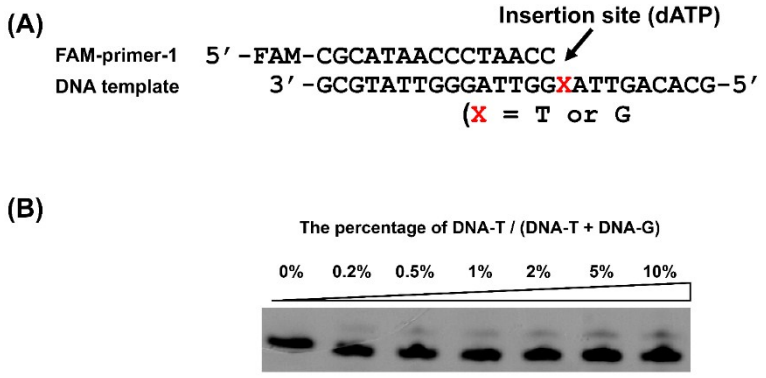

Figure S6. The single nucleotide primer extension reaction by *Tth* polymerase using 5 μg HeLa DNA and different fluorophores-labeled primers (5 pmol for each) that target different guanosine sites in telomeric DNA.

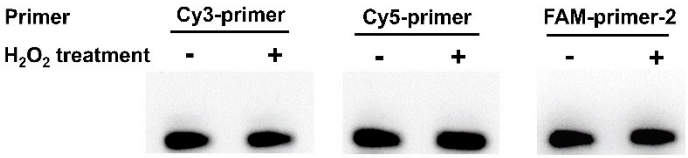

Figure S7. Single-base resolution analysis of multiple OG sites in DNA by sequencing. The primer extension products by *Bsu* Pol with DNA templates of L-DNA-G, L-DNA-OG1 (one OG site), L-DNA-OG2 (two OG sites), or L-DNA-OG3 (three OG sites). The detailed sequence information of DNA templates can be found in Table S1 in Supporting Information.

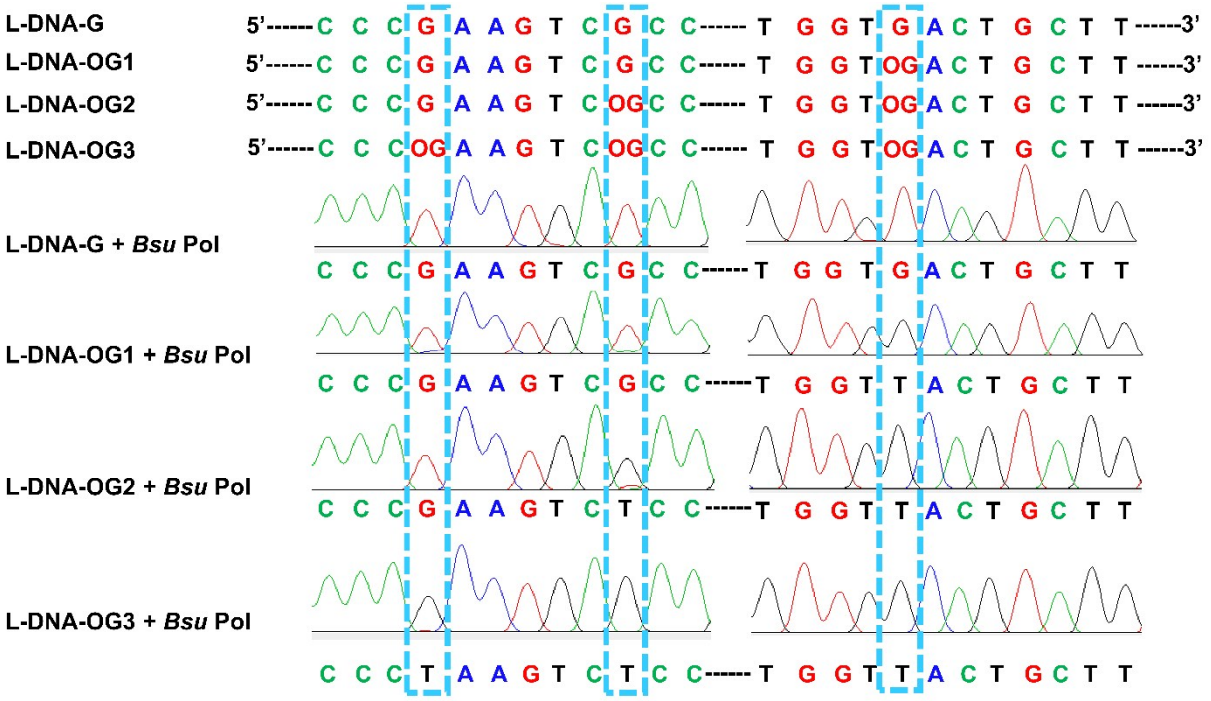

Figure S8. Single-base resolution analysis of multiple OG sites in DNA by sequencing. The primer extension products by *Tth* Pol with DNA templates of L-DNA-G, L-DNA-OG1 (one OG site), L-DNA-OG2 (two OG sites), or L-DNA-OG3 (three OG sites). The detailed sequence information of DNA templates can be found in Table S1 in Supporting Information.

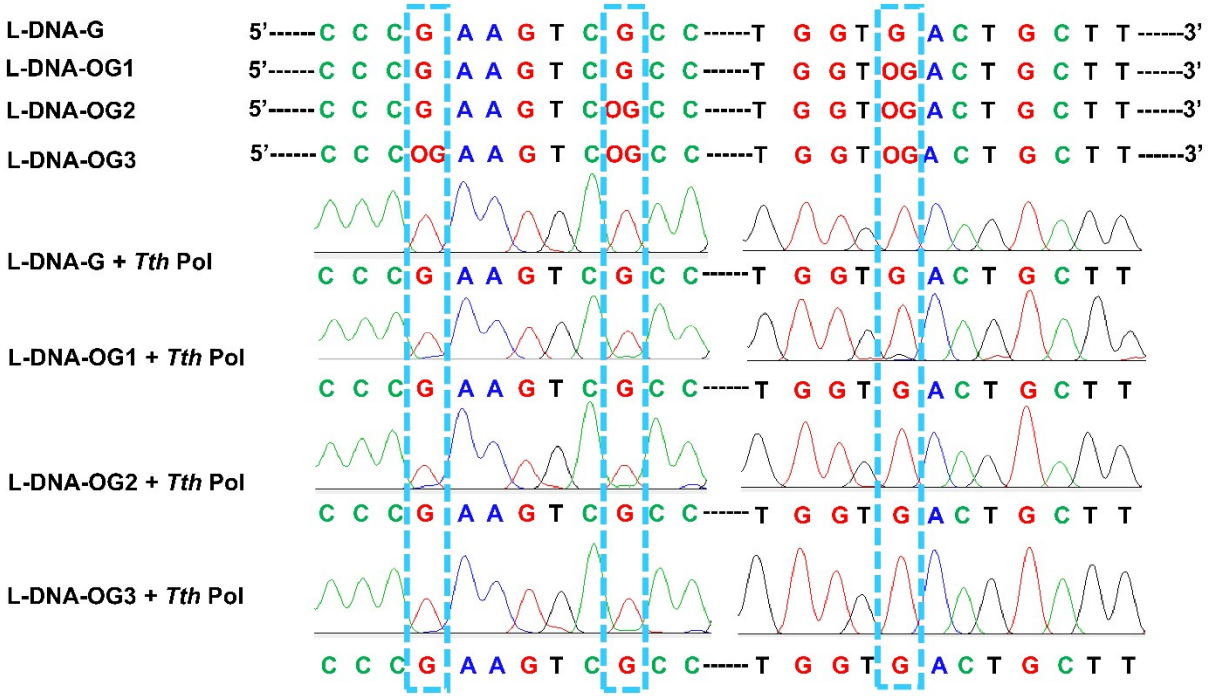

Figure S9. Quantitative evaluation of the level of OG in DNA using various ratios of L-DNA-OG1/L-DNA-G (0%, 0.1%. 1%, 10%, 20%) as the DNA template by high-throughput sequencing.

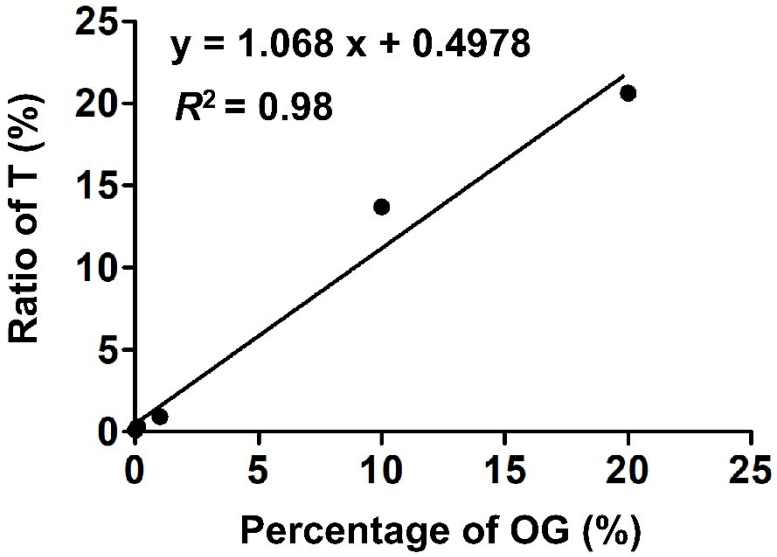

Figure S10. Schematic illustration of the procedure for the library construction.

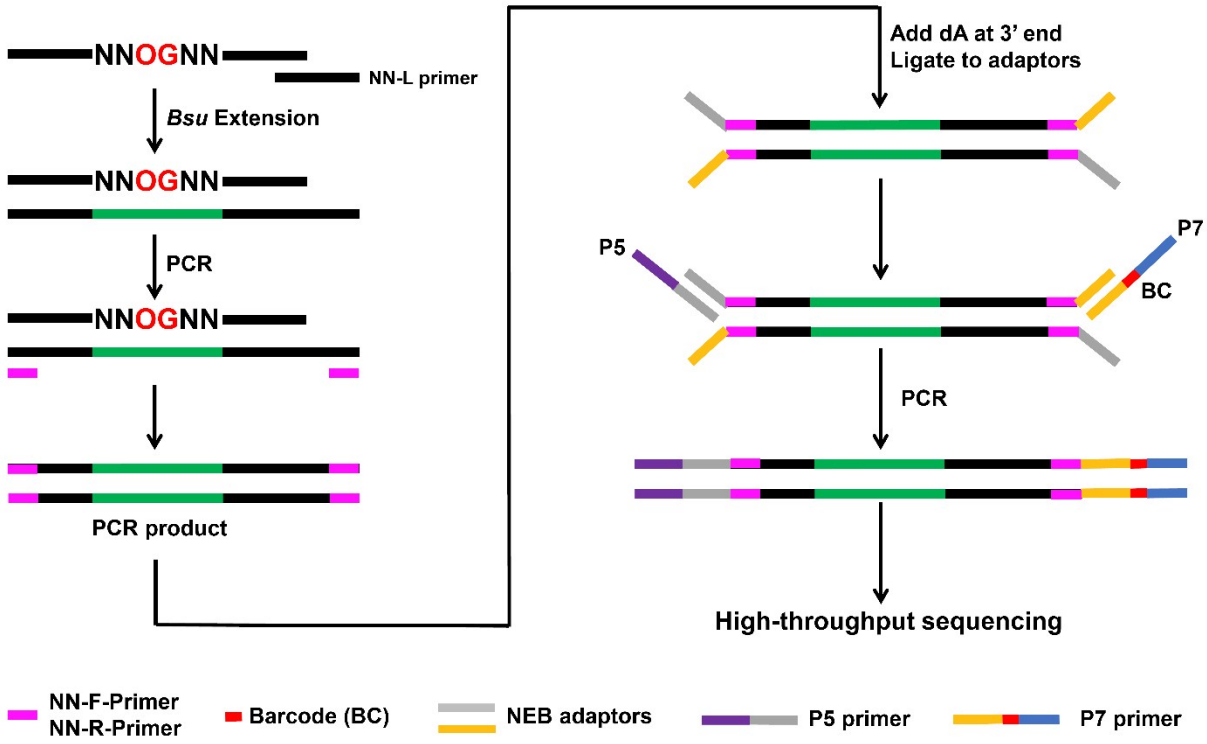

Figure S11. Schematic illustration of the analysis of OG in genomic DNA of HeLa cells. HeLa genomic DNA was denatured and annealed with the corresponding extension primers (Primer-L, Table S2 in Supporting Information). *Bsu* Pol or *Tth* pol was added for the primer extension. The extension product was purified as template for PCR amplification and then subjected to Sanger sequencing.