Supplementary Materials

A unique m6A-dependent restriction endonuclease from an archaeal virus

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Table S1. The primers for amplification of the DNA fragments from pBR322 plasmid, T7 phage genome or λ DNA.

Name	Primers (F/R 5′-3′)	Template	Size
F1	GCAGGCCATGCTGTCCAGGCAGGTAGATGACGACCATCAG	pBR322 plasmid	3 kb
	AGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAA		
F2	AGAAGCAGGCCATTATCGCCGGC	pBR322 plasmid	300 bp
	CGGCGCCTACAATCCATGCCAACCC		
F3	CGTGCTCCTGTCGTTGAGGACCCGGCTAGG	pBR322 plasmid	600 bp
	CGGTAAAGCTCATCAGCGTGGTCG		
F4	CTCCACGCGGTGCAATCGTTGCCGATAAGACCAACATG	T7 phage genome	177 bp
	CCCCGCAGTGGAACTTAGTGACGCTCTCTAAGAGGG		
λ DNA-1	GGGCGGCGACCTCGCGGGTTTTCGCTATTTATG	λ DNA	549 bp
	CGCGACAGCACGAAAGTACAGAATGCGGTTTC		
λ DNA-2	CCGCATTTTATGCGTTTTCATGTTGCCTGCCCG	λ DNA	572 bp
	CAGCTTTCCTCACCCGGCCCCCATCCCCATACGC		
λ DNA-3	ACGGGAGGCGCTGTGGCTGATTTCGATAACC	λ DNA	570 bp
	TGGCCCTTTTCAGCCTGGCCCTTTCCTTTACCAG		
λ DNA-4	GATAGTGCGGGTGTTGAATGATTTCCAGTTGC	λ DNA	589 bp
	ACTGGAGGCAGGAAGACAAACACAGAGCTC		
λ DNA-5	AAGCCATGAATGTAACGTAACGGAATTATCAC	λ DNA	530 bp
	TGCAGACGTAACCAATATTCGAATTGAAGAAC		
λ DNA-6	CCAACAAGCCGTAAACGCCTTCATCAGAG	λ DNA	568 bp
	GCCATCAATTTTTTCGTAATAGCGCATCTC		
λ DNA-7	TACCTACAAAGCCCAGCGCGACAAAAATGCC	λ DNA	573 bp
	CTTCGTTTCTGGAATTGGGCAGAAGAAAAC		
T7 DNA-8	TCAAGCGAGACGGTACTGTGGAGGCAGGAC	T7 phage genome	1018 bp
	CACCGTCTACTTTGGCAATCCAGTAGCCAG		
T7 DNA-9	TTCGCAACGGTAAGGCGACTATGGTTTACCGCTG	T7 phage genome	1100 bp
	ATCTCGCCTAAGCGATAACCCCACGCCTCCAAAGC		
T7 DNA-10	CTGAGACTTTCAGAAACCAAGCGGAGGGC	T7 phage genome	941 bp
	CTGTGAAACAGTCACACTTACCCCACCGCC		
T7-3 kb	CTCTTTCGTTACGTGAACGAATCCGTGAGCACCTA	T7 phage genome	3 kb
	TTAAACACAACATGTTCAACTGGGGTGTAAGGAG		
T7-2 kb	GTGGTATCGGCTCTTTCGTTACGTGAACGAATCCG	T7 phage genome	2 kb
	TCATATTGATTTCTCCTATTGATTATCGTGAC		
T7-1 kb	TCGTTTCTGACATCGAAGCTAAC	T7 phage genome	1 kb
	TTAAACACAACATGTTCAACTGGGGTGTAAGGAG		

pBR-1.2 kb	AGTCCAACCCGGTAAGACACGAC AGTTGGGTGCACGAGTGGGTTACATCGAAC	pBR322 plasmid	1.2 kb

Figure S1. SDS-PAGE analysis of the purified HHPV4I and its mutants. Related to Figure 3.

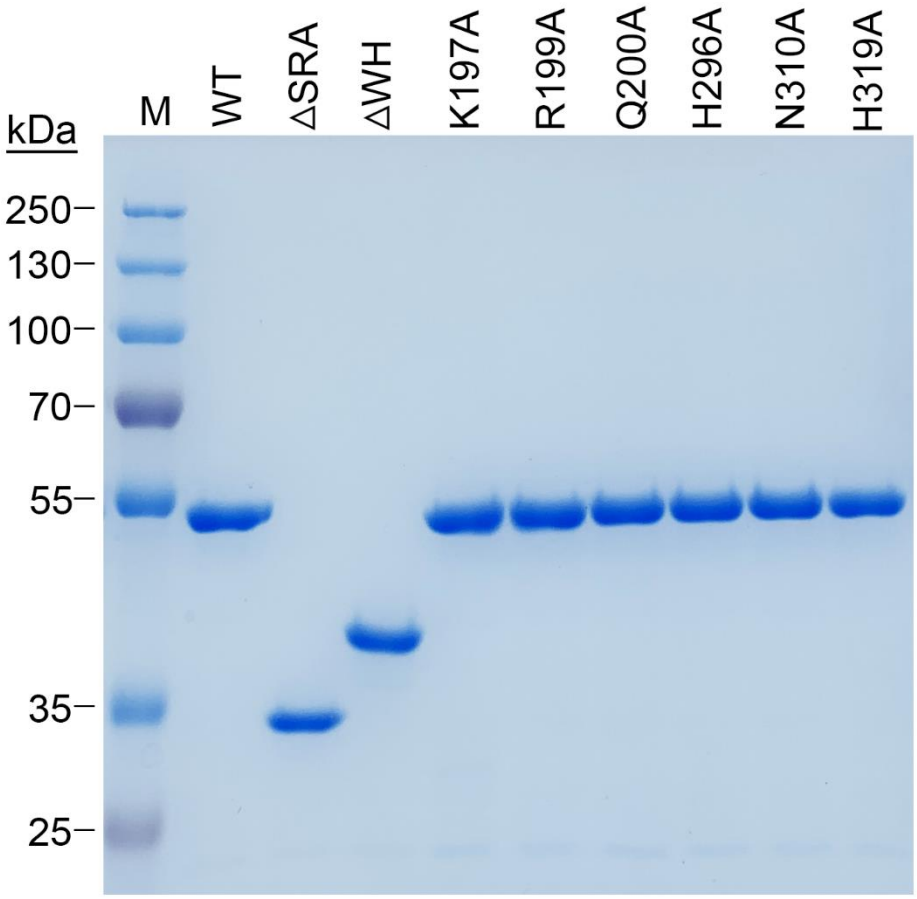


Figure S2. Determination of the△SRA-generated cleavage sites by run-off sequencing. (A) Agarose gel showing that the Dam-methylated DNA F3 digested by △SRA for 15 min, 30 min and 60 min. (B–D) The Dam-methylated DNA F3 was digested by HHPV4I for 15 min (B), 30 min (C) and 60 min (D), and then the generated cleavage sites were determined by using run-off sequencing.

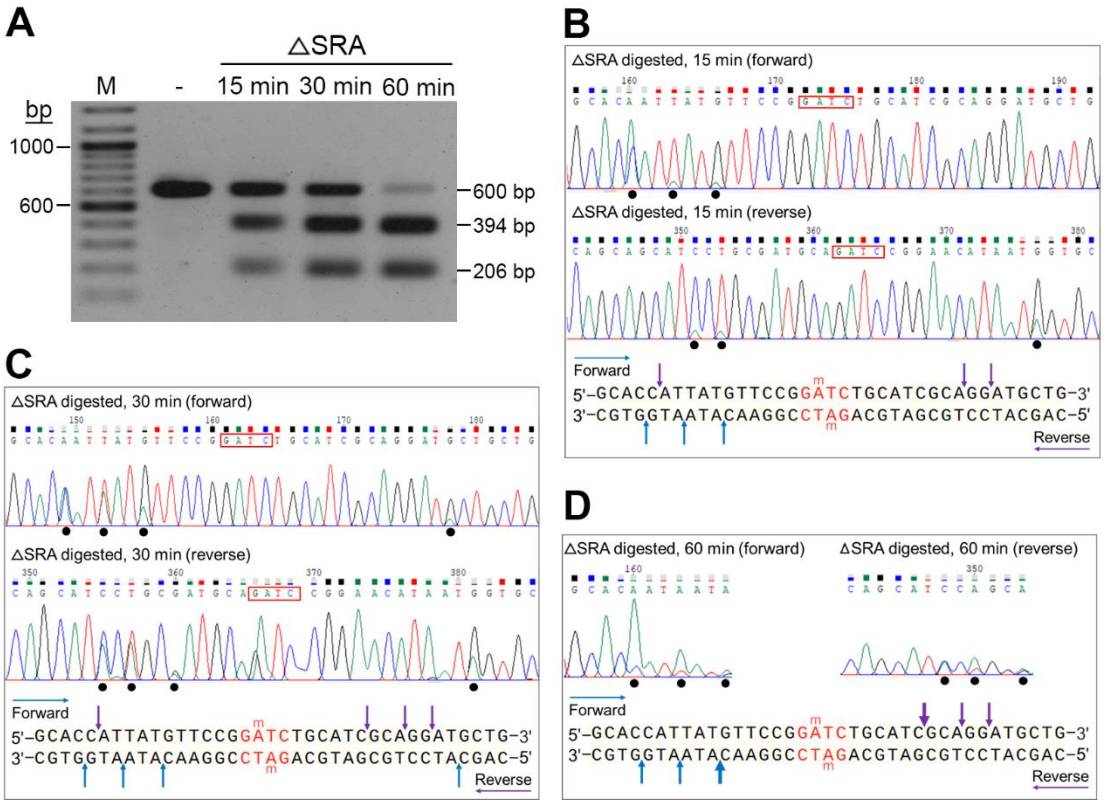
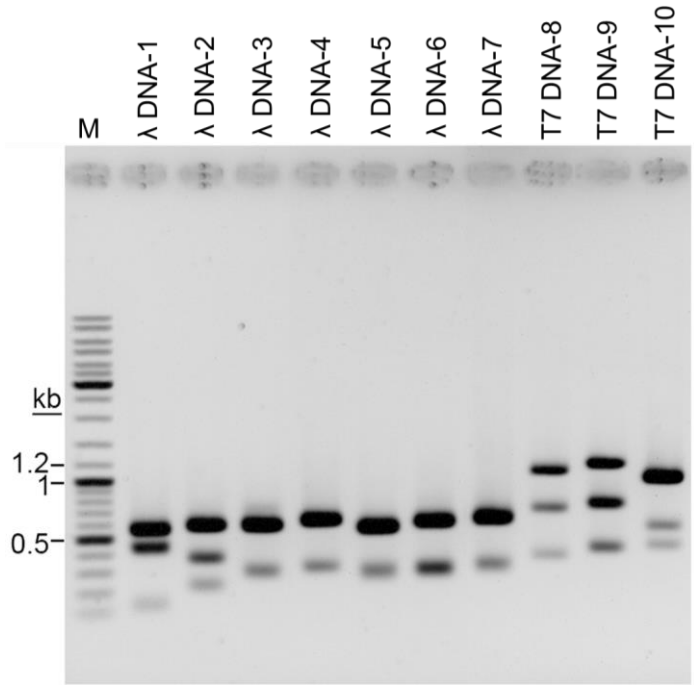
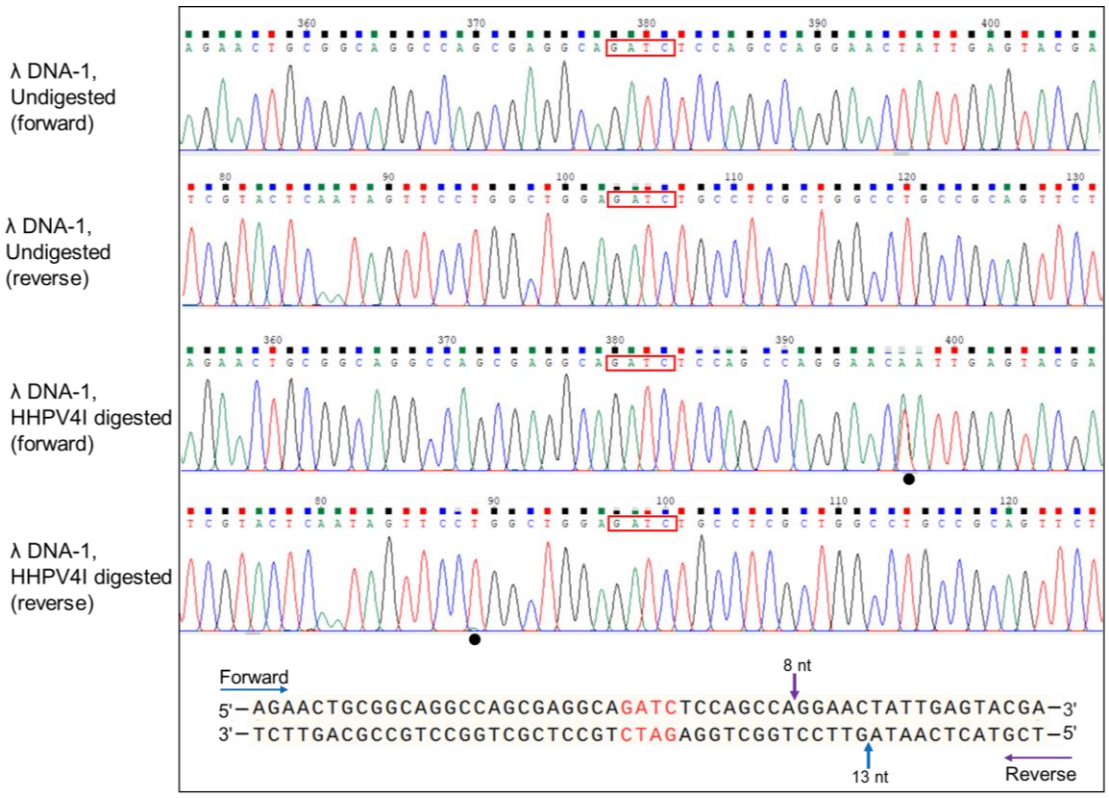


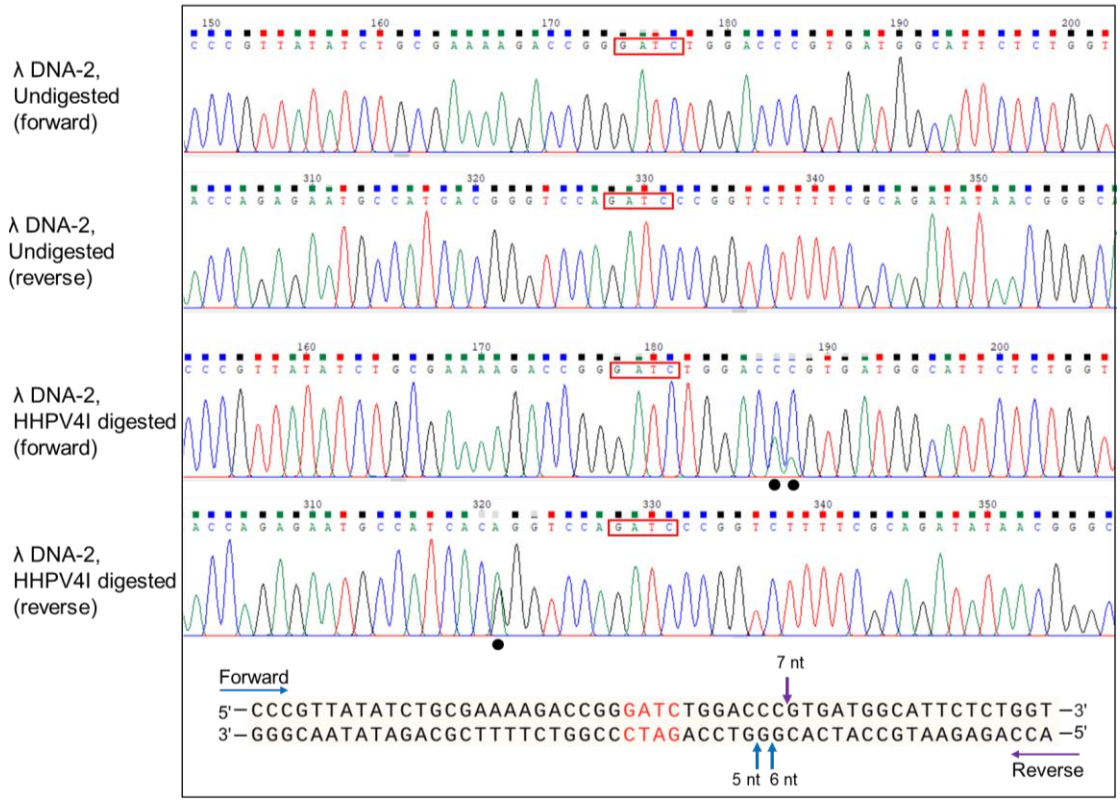
Figure S3. Determination of the cleavage sites generated by HHPV4I digestion for 15 min. (A) Different Dam-methylated DNA fragments were subjected to HHPV4I digestion for 15 min. After digestion, the DNA fragments were purified by using Qiaquick PCR purification kit (Qiagen), and then analyzed by agarose gel electrophoresis. (B) The purified DNA fragments were subjected to run-off sequencing.

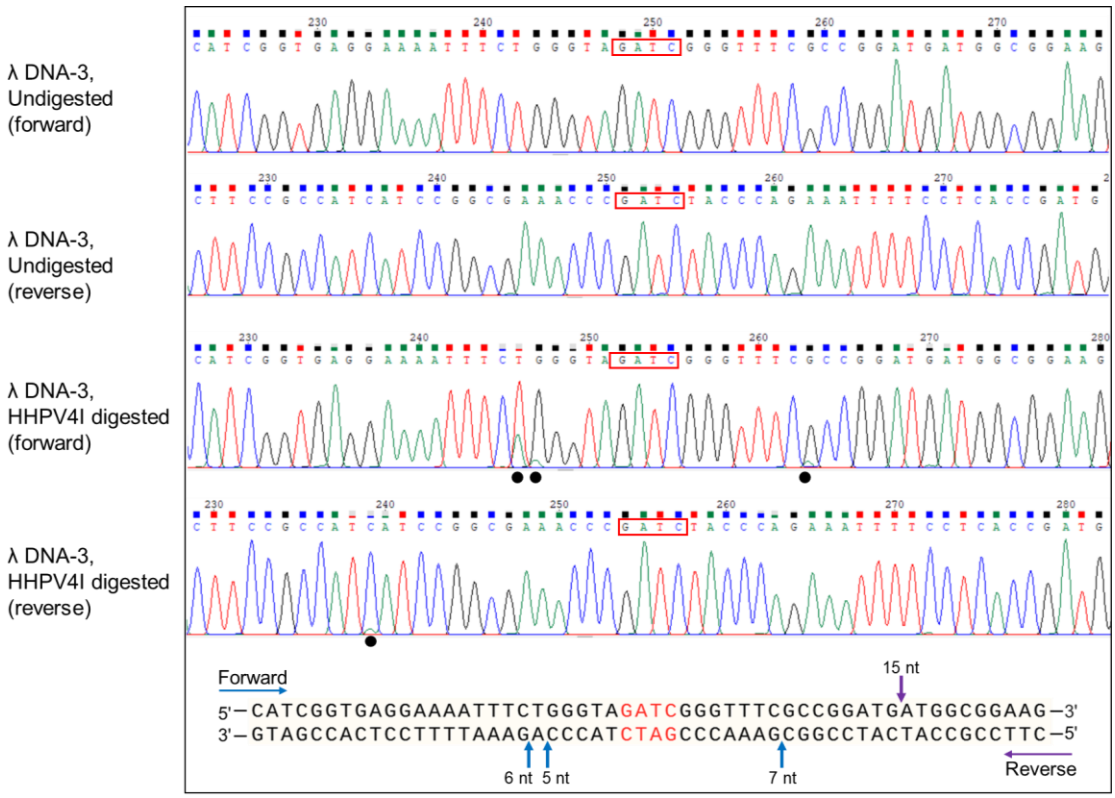
A

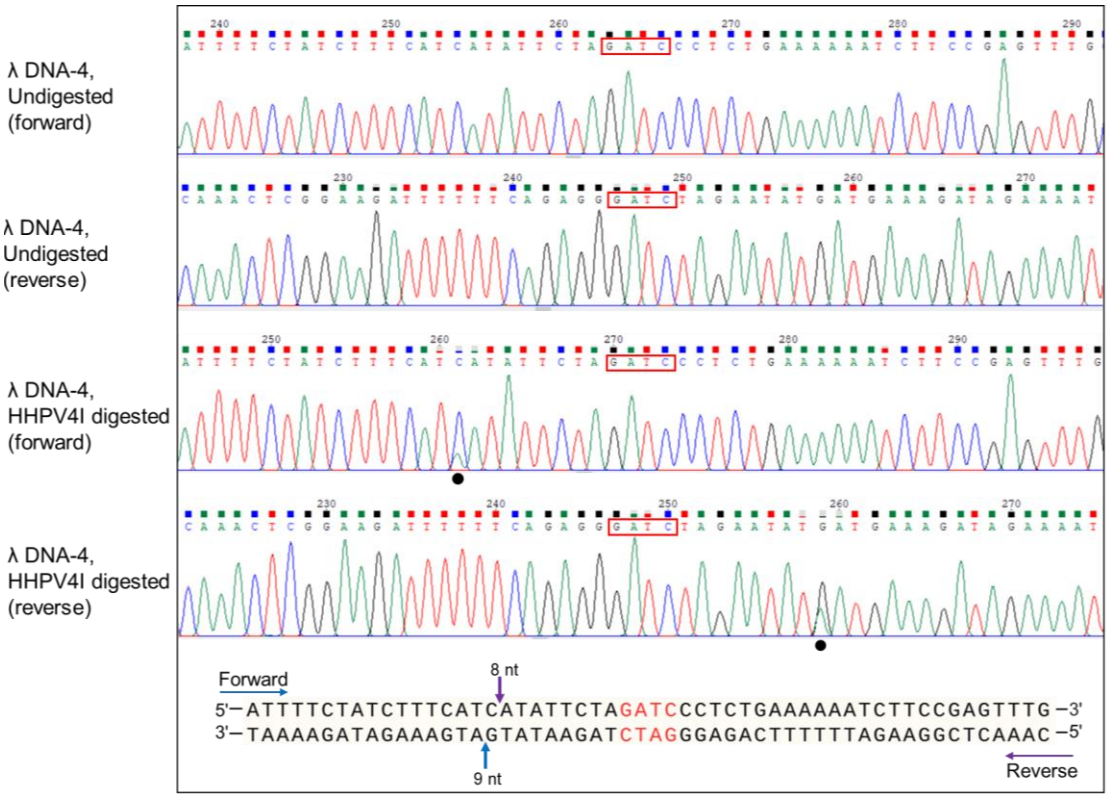


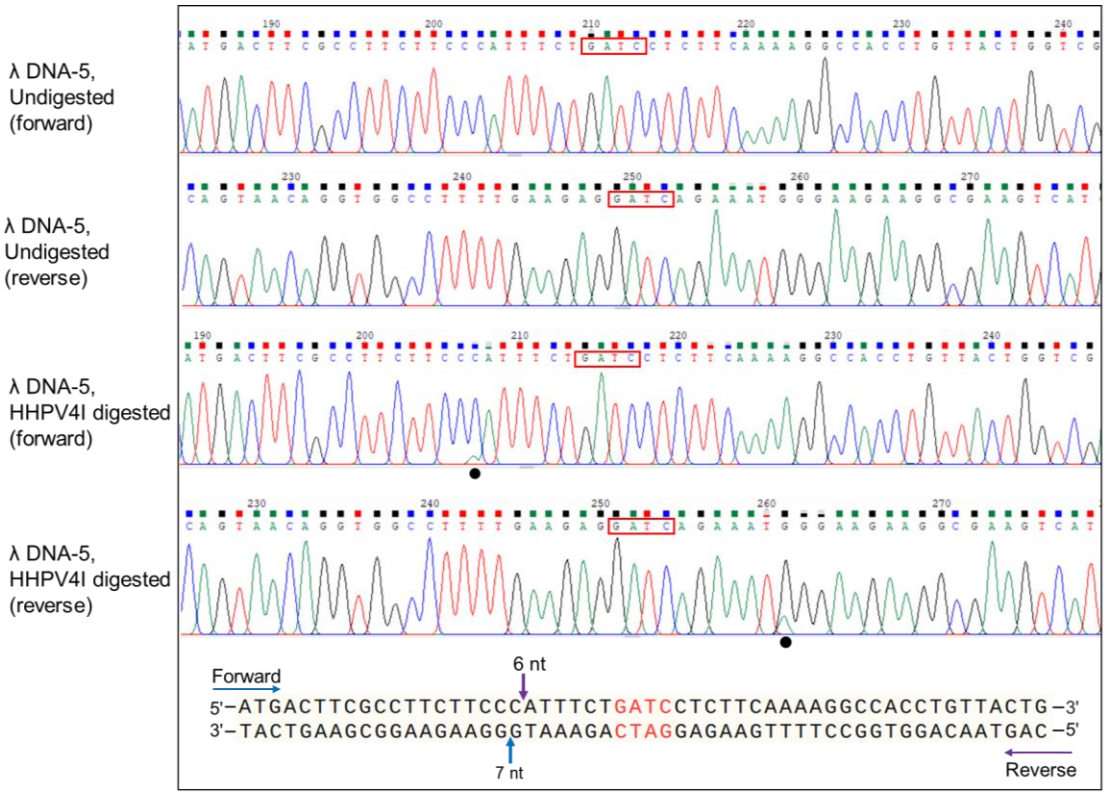
B

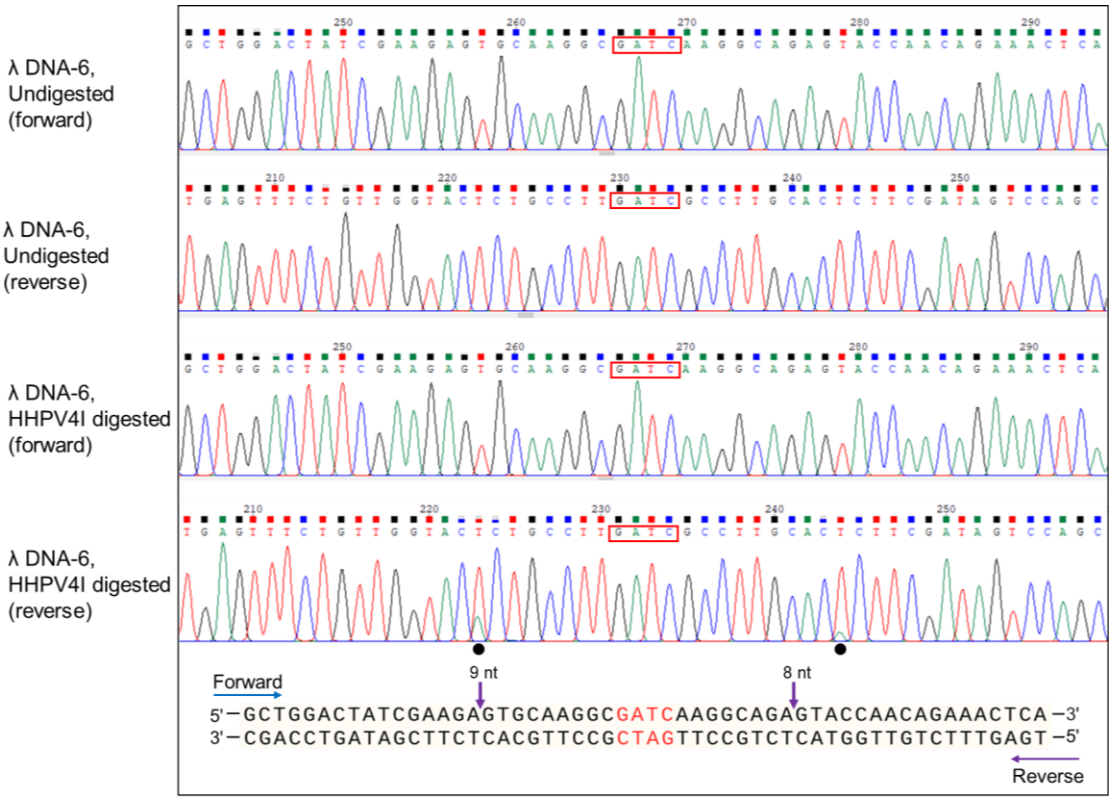

λ DNA-1, Undigested (forward)
λ DNA-1, Undigested (reverse)
λ DNA-1, HHPV4I digested (forward)
λ DNA-1, HHPV4I digested (reverse)
Forward
8 nt
5'—AGAACTGCGGCAGGCCAGCGAGGCAGATCTCCAGCCAGGAACTATTGAGTACGA—3'
3'—TCTTGACGCCGTCCGGTCGCTCCGTCTAGAGGTCGGTCCTTGATAACTCATGCT—5'
13 nt
Reverse

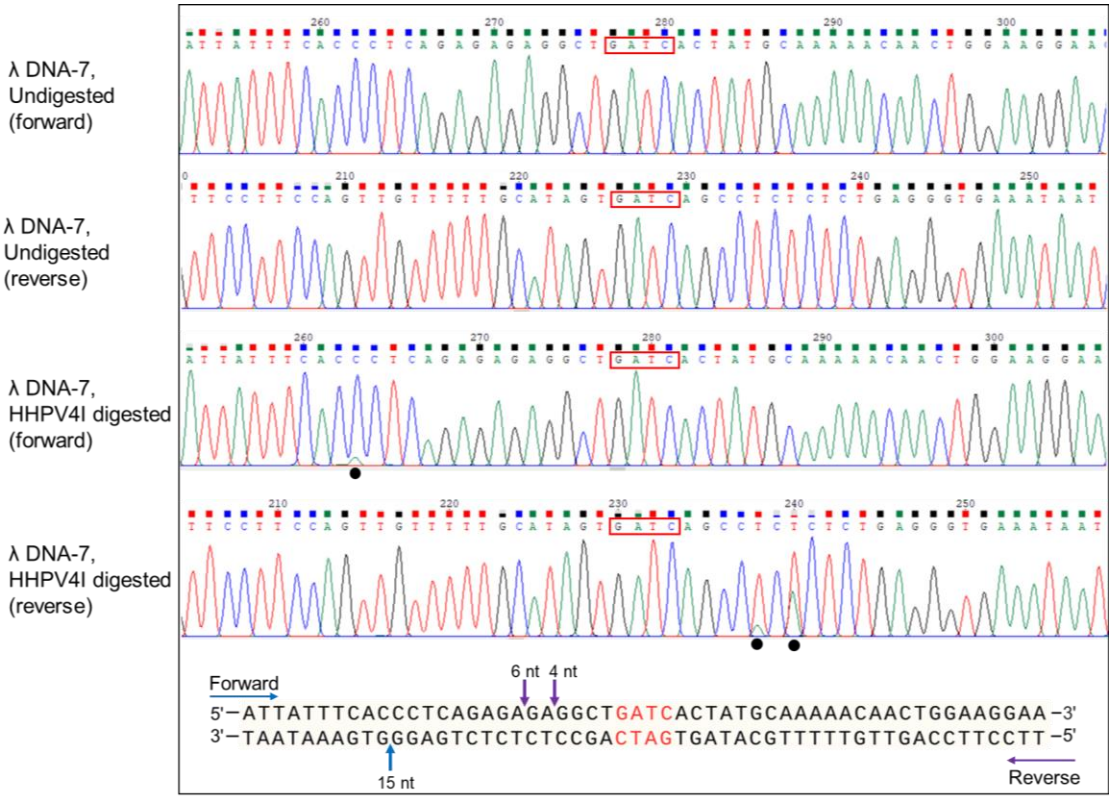

λ DNA-2, Undigested (forward)
λ DNA-2, Undigested (reverse)
λ DNA-2, HHPV4I digested (forward)
λ DNA-2, HHPV4I digested (reverse)
Forward
7 nt
5'—CCCGTTATATCTGCGAAAAGACCGGGATCTGGACCCGTGATGGCATTCTCTGGT—3'
3'—GGGCAATATAGACGCTTTTCTGGCCCTAGACCTGGGCACTACCGTAAGAGACCA—5'
5 nt
6 nt
Reverse

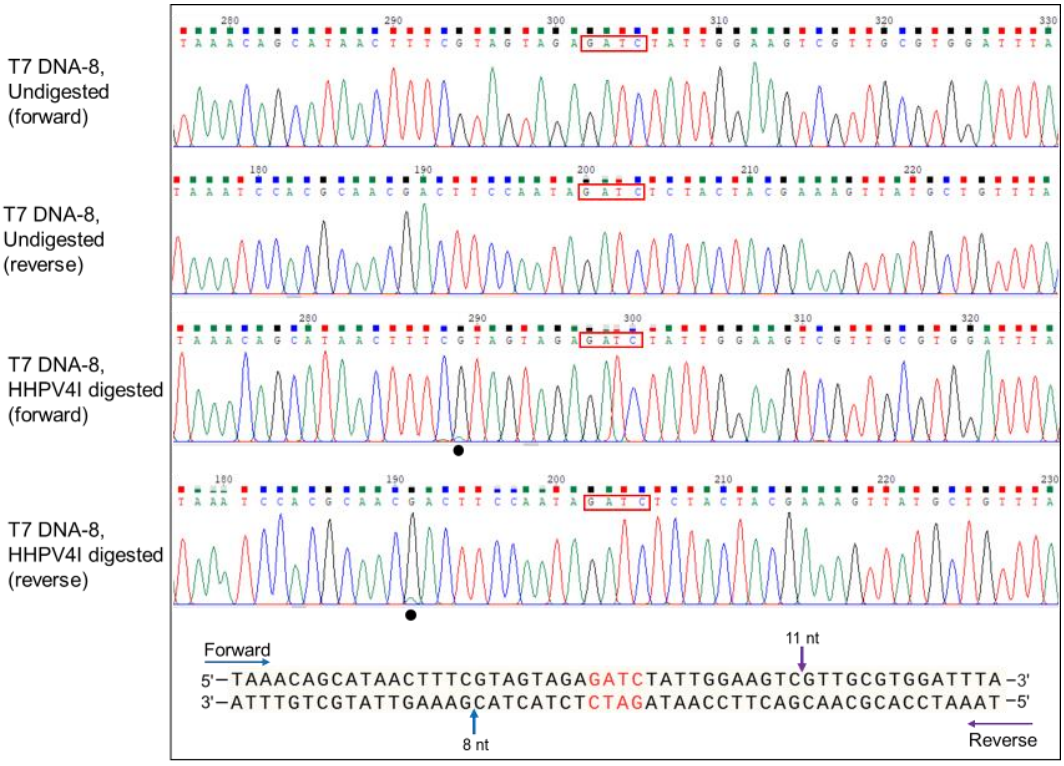

λ DNA-3, Undigested (forward)
λ DNA-3, Undigested (reverse)
λ DNA-3, HHPV4I digested (forward)
λ DNA-3, HHPV4I digested (reverse)
Forward
15 nt
5'–CATCGGTGAGGAAAATTTCTGGGTAGATCGGGTTTCGCCGGATGATGGCGGAAG–3'
3'–GTAGCCACTCCTTTTAAAGACCCATCTAGCCCAAAGCGGCCTACTACCGCCTTC–5'
6 nt
5 nt
7 nt
Reverse

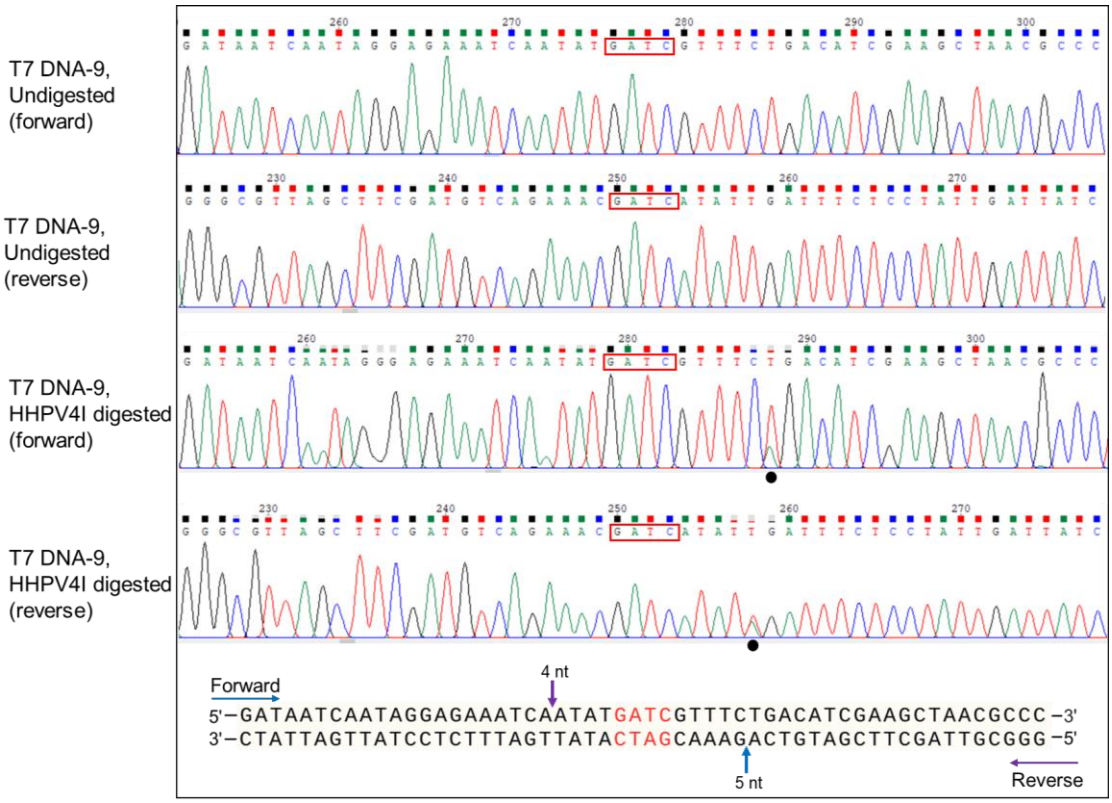

λ DNA-4, Undigested (forward)
λ DNA-4, Undigested (reverse)
λ DNA-4, HHPV4I digested (forward)
λ DNA-4, HHPV4I digested (reverse)
Forward
8 nt
5'–ATTTTCTATCTTTCATCATATTCTAGATCCCTCTGAAAAAATCTTCCGAGTTTG–3'
3'–TAAAAGATAGAAAGTAGTATAAGATCTAGGGAGACTTTTTTAGAAGGCTCAAAC–5'
9 nt
Reverse


λ DNA-5, Undigested (forward)
λ DNA-5, Undigested (reverse)
λ DNA-5, HHPV4I digested (forward)
λ DNA-5, HHPV4I digested (reverse)
Forward
6 nt
5'–ATGACTTCGCCTTCTTCCCATTTCTGATCCTCTTCAAAAGGCCACCTGTTACTG–3'
3'–TACTGAAGCGGAAGAAGGGTAAAGACTAGGAGAAGTTTTCCGGTGGACAATGAC–5'
7 nt
Reverse


λ DNA-6, Undigested (forward)
λ DNA-6, Undigested (reverse)
λ DNA-6, HHPV4I digested (forward)
λ DNA-6, HHPV4I digested (reverse)
Forward
9 nt
8 nt
5'–GCTGGACTATCGAAGAGTGCAAGGCGATCAAGGCAGAGTACCAACAGAAACTCA–3'
3'–CGACCTGATAGCTTCTCACGTTCCGCTAGTTCCGTCTCATGGTTGTCTTTGAGT–5'
Reverse


λ DNA-7,
Undigested
(forward)
λ DNA-7,
Undigested
(reverse)
λ DNA-7,
HHPV4I digested
(forward)
λ DNA-7,
HHPV4I digested
(reverse)
Forward
6 nt
4 nt
5'–ATTATTTCACCCTCAGAGAGAGGCTGATCACTATGCAAAAACAACTGGAAGGAA–3'
3'–TAATAAAGTGGGAGTCTCTCTCCGACTAGTGATACGTTTTTGTTGACCTTCCTT–5'
15 nt
Reverse


T7 DNA-8,
Undigested
(forward)
T7 DNA-8,
Undigested
(reverse)
T7 DNA-8,
HHPV4I digested
(forward)
T7 DNA-8,
HHPV4I digested
(reverse)
Forward
11 nt
5'–TAAACAGCATAACTTTCGTAGTAGAGATCTATTGGAAGTCGTTGCGTGGATTTA–3'
3'–ATTTGTCGTATTGAAAGCATCATCTCTAGATAACCTTCAGCAACGCACCTAAAT–5'
8 nt
Reverse


T7 DNA-9, Undigested (forward)
T7 DNA-9, Undigested (reverse)
T7 DNA-9, HHPV4I digested (forward)
T7 DNA-9, HHPV4I digested (reverse)
Forward
4 nt
5'–GATAATCAATAGGAGAAATCAATATGATCGTTTCTGACATCGAAGCTAACGCCC–3'
3'–CTATTAGTTATCCTCTTTAGTTATACTAGCAAAGACTGTAGCTTCGATTGCGGG–5'
5 nt
Reverse

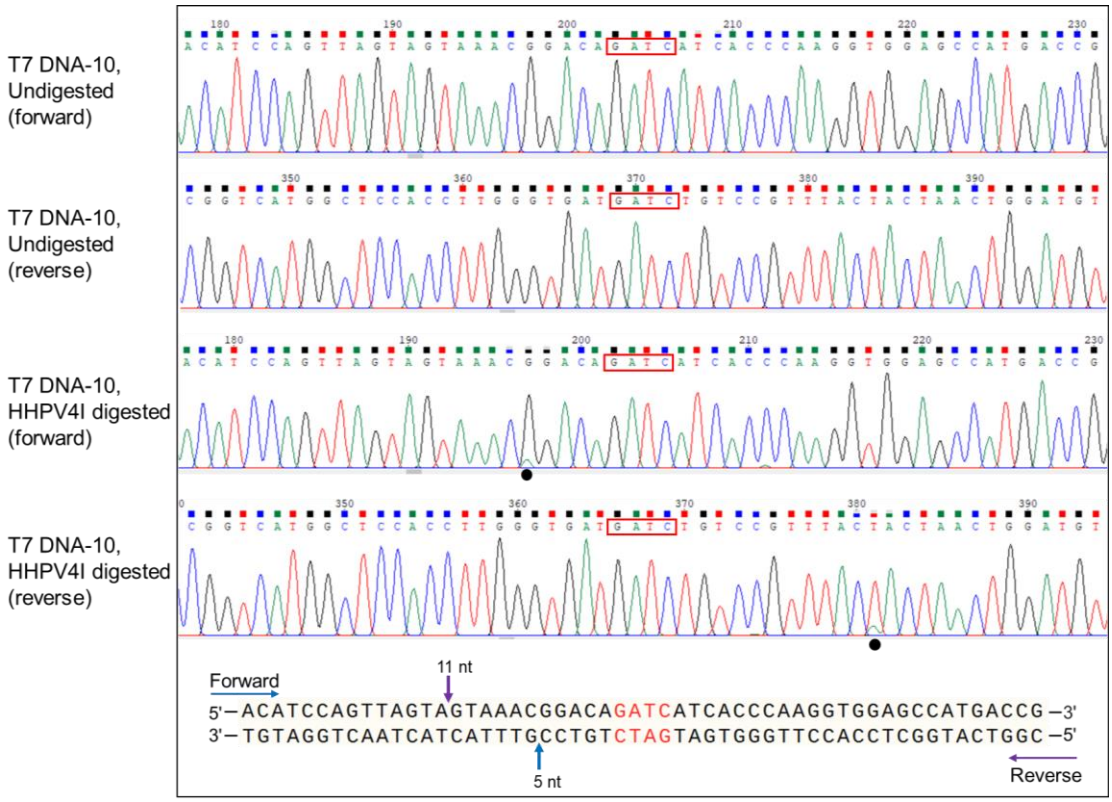
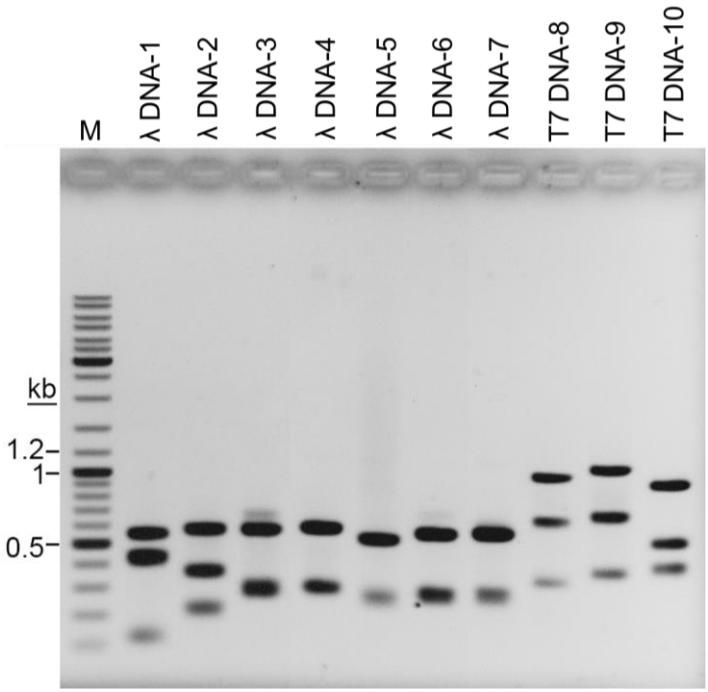
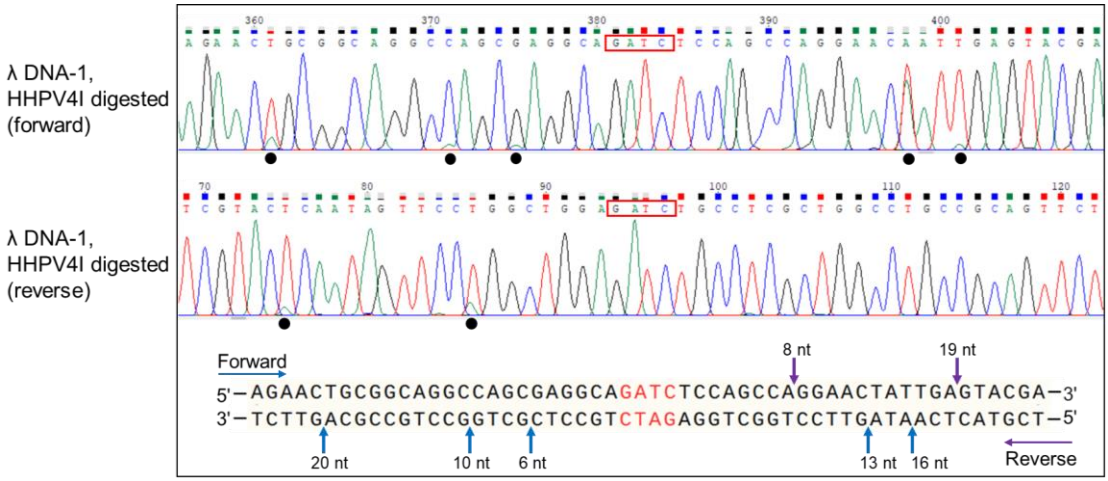

T7 DNA-10, Undigested (forward)
T7 DNA-10, Undigested (reverse)
T7 DNA-10, HHPV4I digested (forward)
T7 DNA-10, HHPV4I digested (reverse)
Forward
11 nt
5'–ACATCCAGTTAGTAGTAAACGGACAGATCATCACCCAAGGTGGAGCCATGACCG–3'
3'–TGTAGGTCAATCATCATTTGCCTGTCTAGTAGTGGGTTCCACCTCGGTACTGGC–5'
5 nt
Reverse

Figure S4. Determination of the cleavage sites generated by HHPV4I digestion for 30 min. (A) Different Dam-methylated DNA fragments were digested by HHPV4I for 30 min. After digestion, the DNA fragments were purified by using Qiaquick PCR purification kit (Qiagen), and then were analyzed by agarose gel electrophoresis. (B) The purified DNA fragments were subjected to run-off sequencing.

A

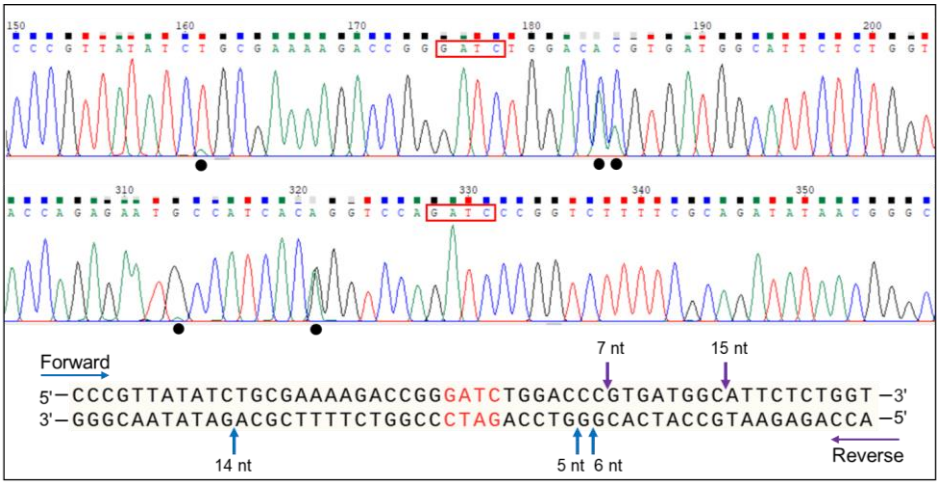


B



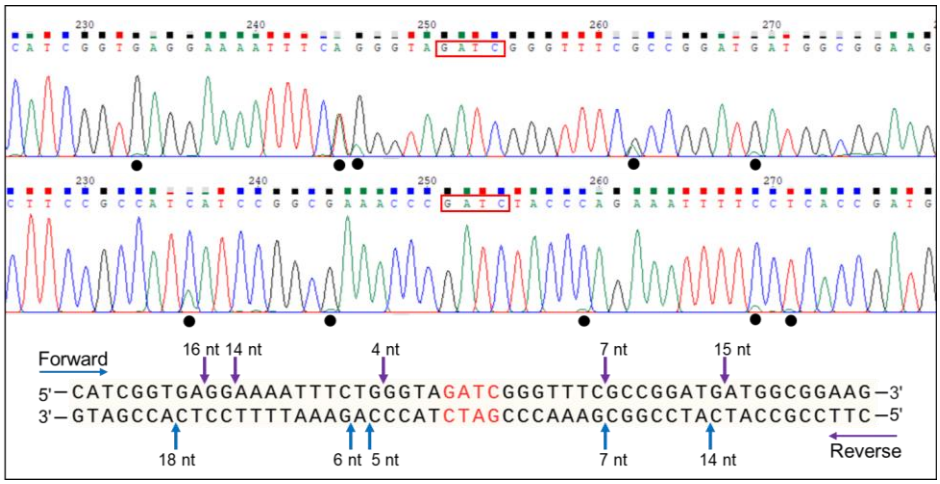
λ DNA-2,
HHPV4I digested
(forward)

λ DNA-2,
HHPV4I digested
(reverse)



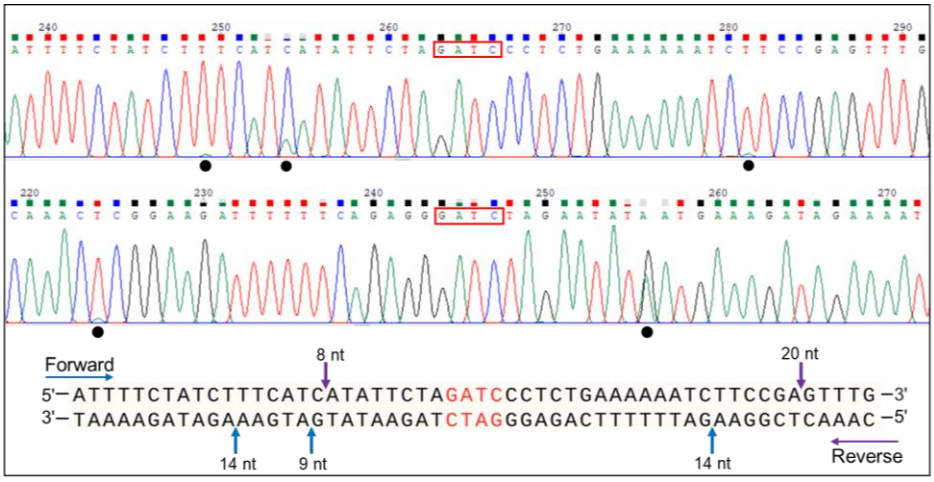
λ DNA-3,
HHPV4I digested
(forward)

λ DNA-3,
HHPV4I digested
(reverse)



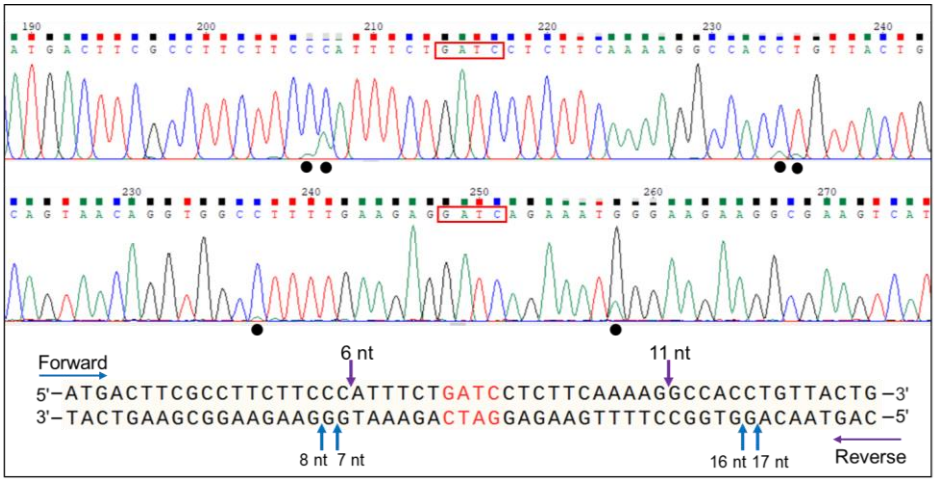
λ DNA-4,
HHPV4I digested
(forward)

λ DNA-4,
HHPV4I digested
(reverse)



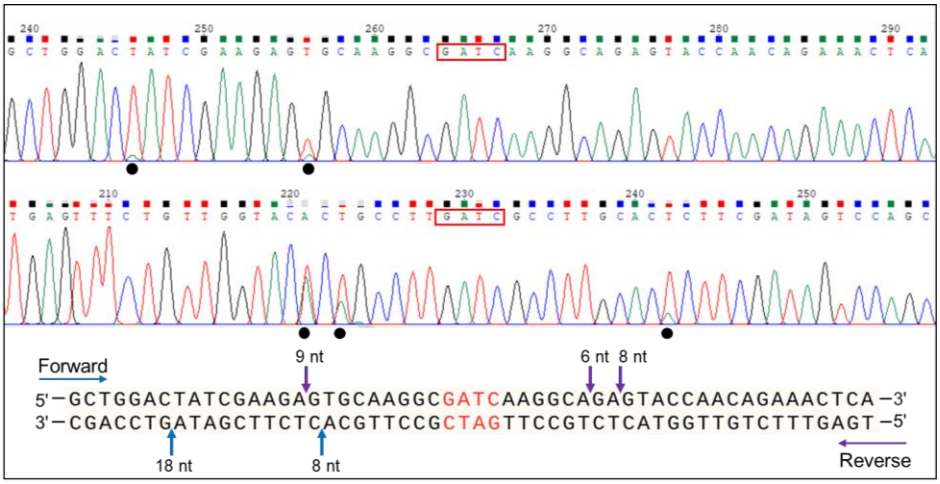
λ DNA-5,
HHPV4I digested
(forward)

λ DNA-5,
HHPV4I digested
(reverse)



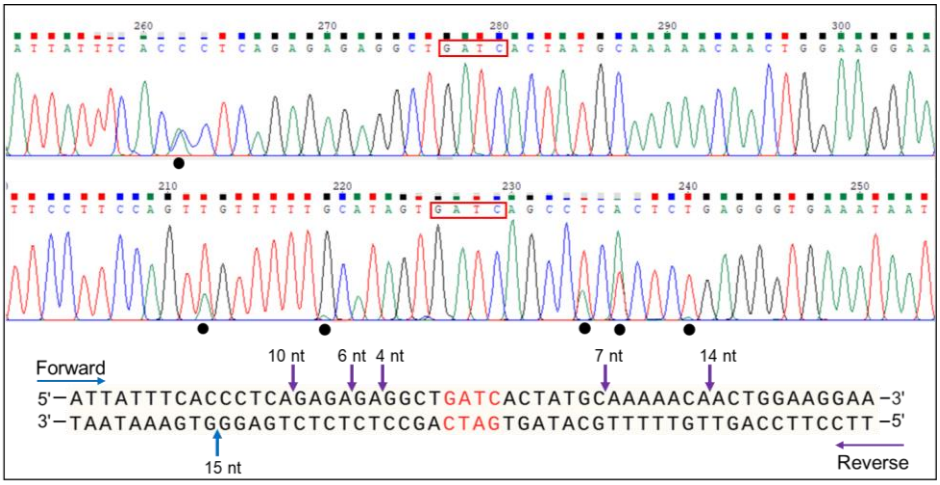
λ DNA-6,
HHPV4I digested
(forward)

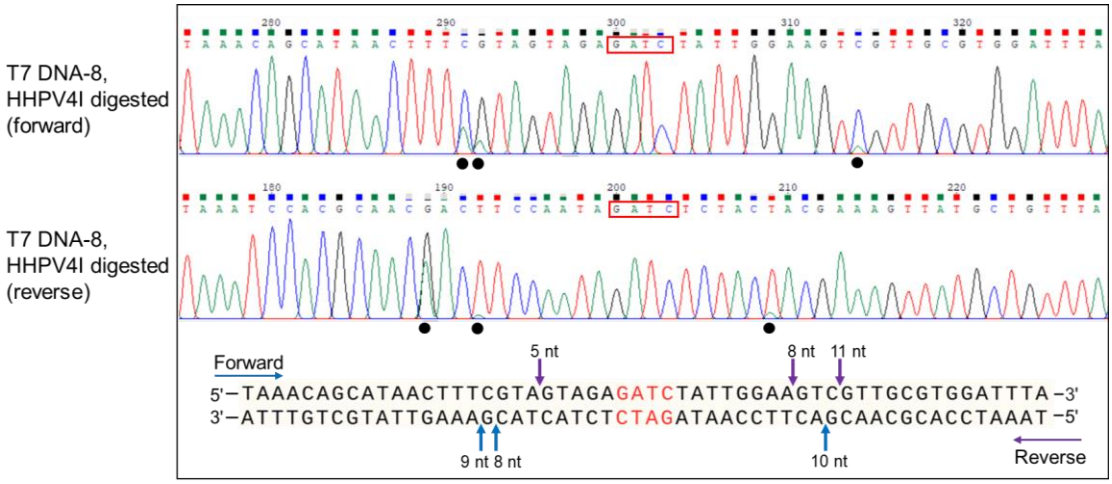
λ DNA-6,
HHPV4I digested
(reverse)

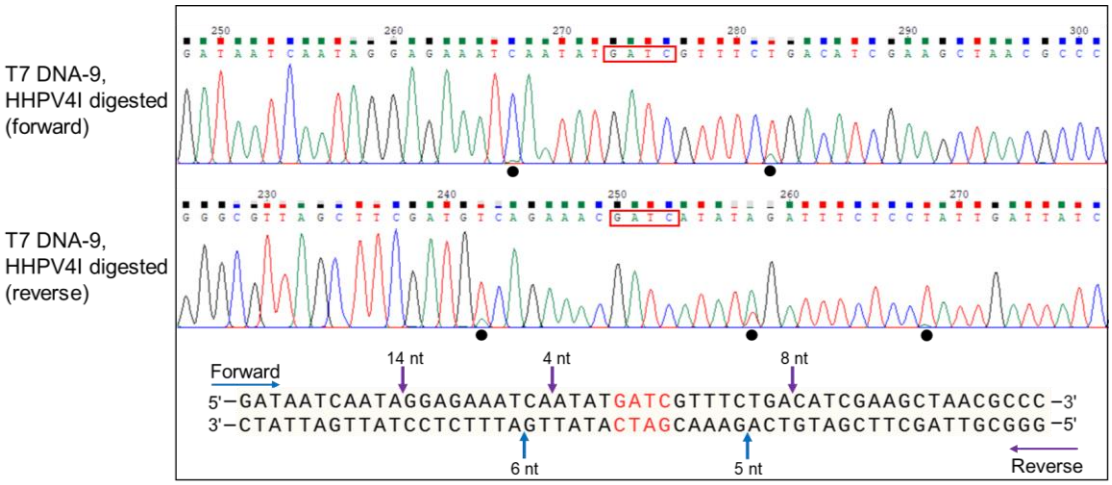


λ DNA-7,
HHPV4I digested
(forward)

λ DNA-7,
HHPV4I digested
(reverse)




T7 DNA-8,
HHPV4I digested
(forward)
T7 DNA-8,
HHPV4I digested
(reverse)
Forward
5'–TAAACAGCATAACTTTCGTAGTAGAGATCTATTGGAAGTCGTTGCGTGGATTTA–3'
3'–ATTTGTCGTATTGAAAGCATCATCTCTAGATAACCTTCAGCAACGCACCTAAAT–5'
5 nt
8 nt
11 nt
9 nt
8 nt
10 nt
Reverse


T7 DNA-9,
HHPV4I digested
(forward)
T7 DNA-9,
HHPV4I digested
(reverse)
Forward
5'–GATAATCAATAGGAGAAATCAATATGATCGTTTCTGACATCGAAGCTAACGCCC–3'
3'–CTATTAGTTATCCTCTTTAGTTATACTAGCAAAGACTGTAGCTTCGATTGCGGG–5'
14 nt
4 nt
8 nt
6 nt
5 nt
Reverse

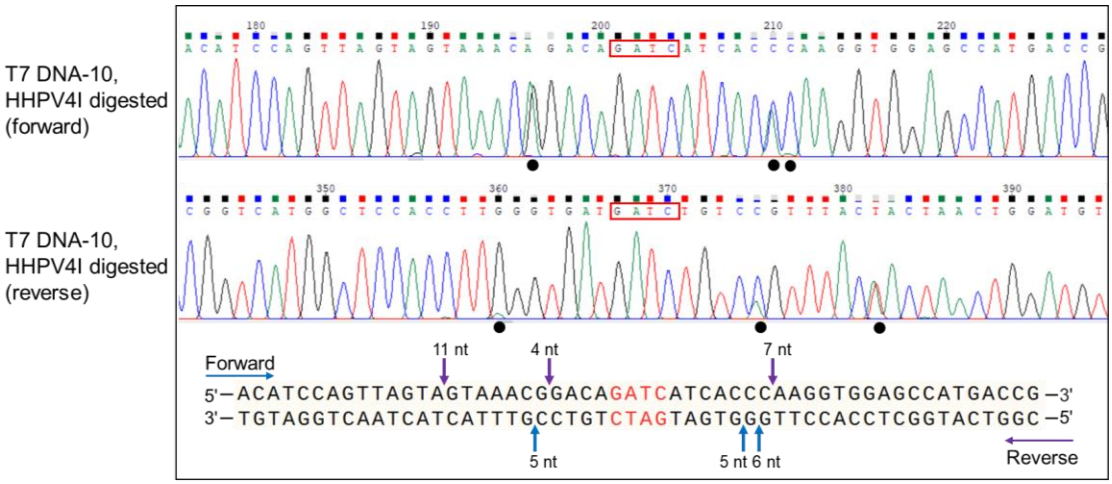
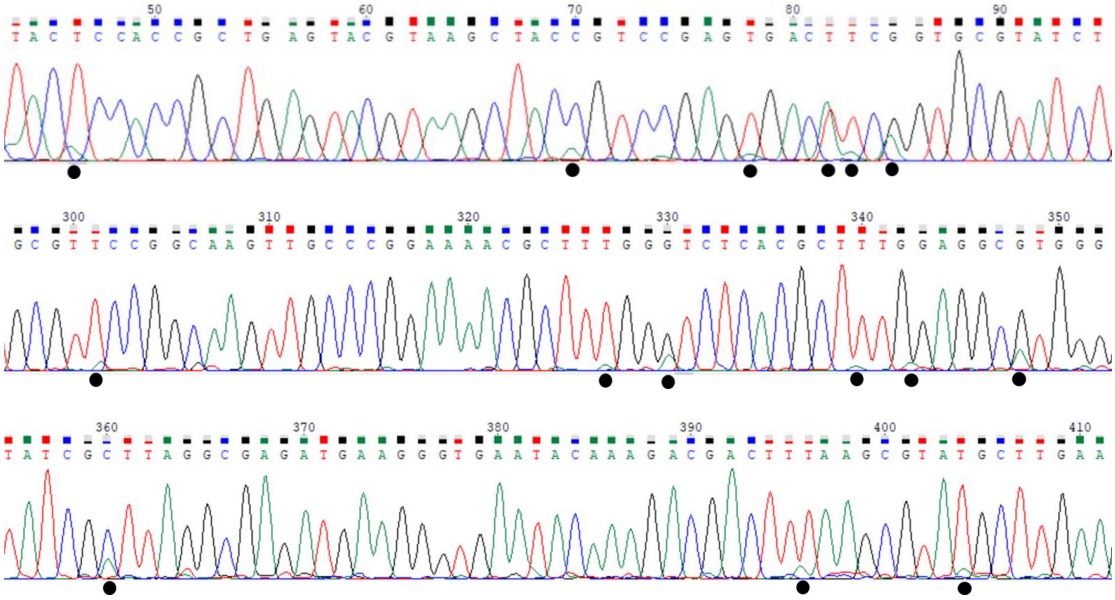

T7 DNA-10,
HHPV4I digested
(forward)
T7 DNA-10,
HHPV4I digested
(reverse)
Forward
5'–ACATCCAGTTAGTAGTAAACGGACAGATCATCACCCAAGGTGGAGCCATGACCG–3'
3'–TGTAGGTCAATCATCATTTGCCTGTCTAGTAGTGGGTTCCACCTCGGTACTGGC–5'
11 nt
4 nt
7 nt
5 nt
5 nt
6 nt
Reverse

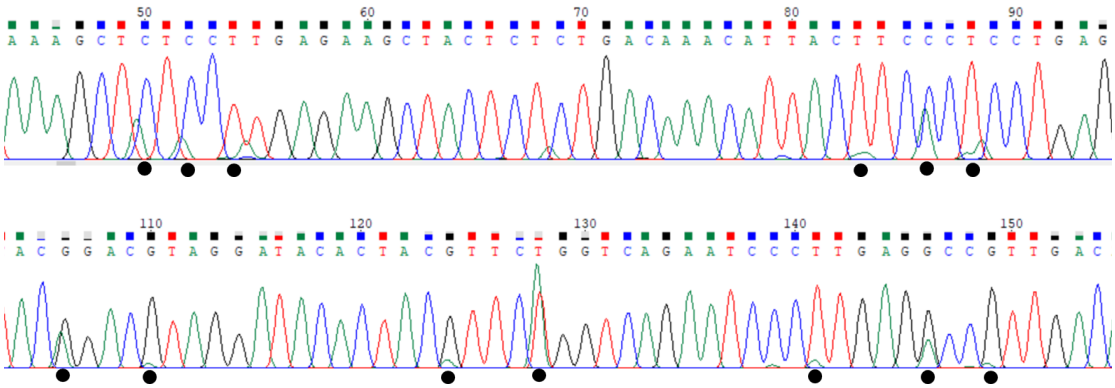
Figure S5. Determination of the cleavage sites generated by K197A digestion. (A) M. EcoGII-methylated T7-1 kb DNA was digested by K197A for 30 min. After digestion, the DNA fragments were purified by using Qiaquick PCR purification kit (Qiagen), and then the purified DNA fragments were subjected to run-off sequencing. Four primers (Forward primer 1, Forward primer 2, Reverse primer 1, and Reverse primer 2) are used for sequencing of the full-length of the two DNA strands. (B) Summary of the positions of the cleavage sites on the T7-1 kb DNA. Green arrows and red arrows indicate the positions of the cleavage sites on the forward DNA strand and reverse DNA strand, respectively. (C) Summary of the cleavage site sequences.

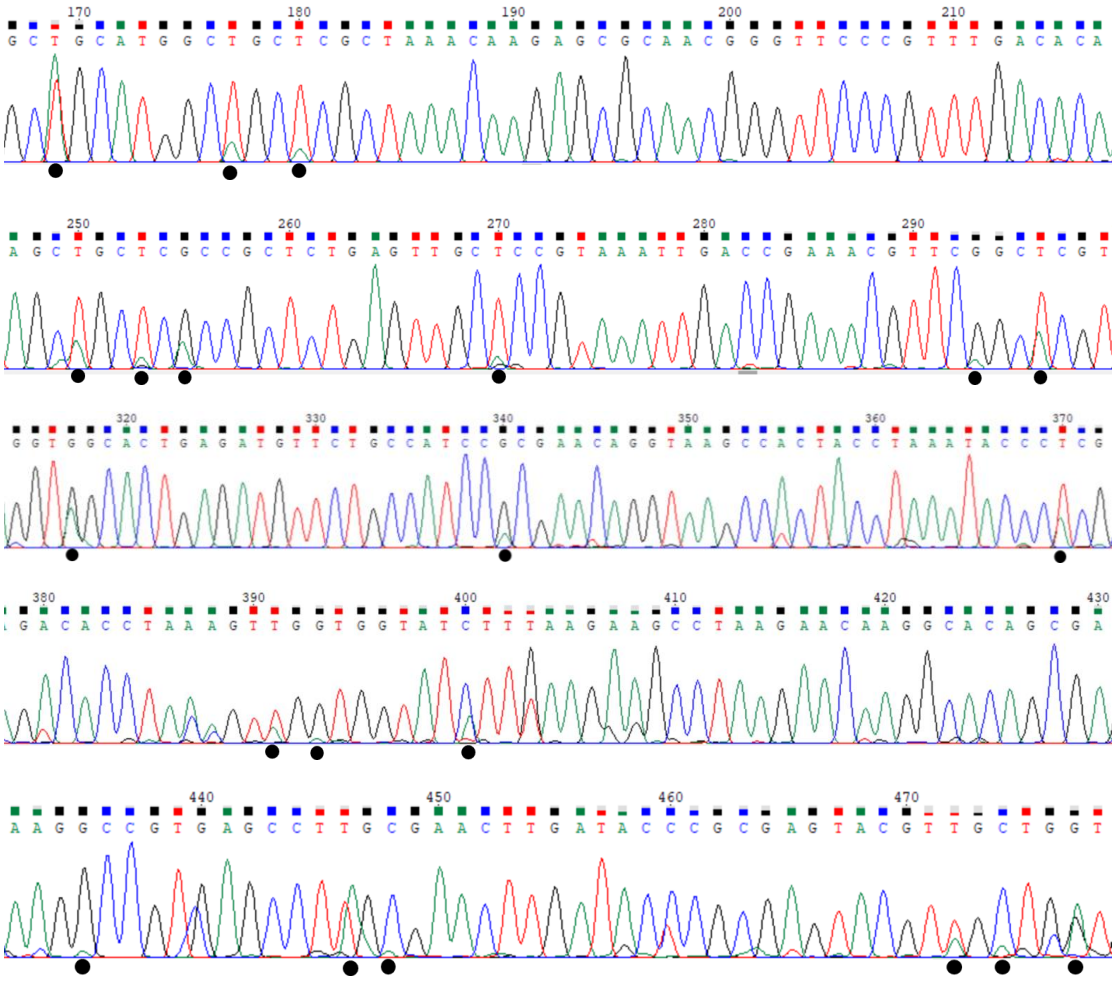
A

(Forward primer 1)

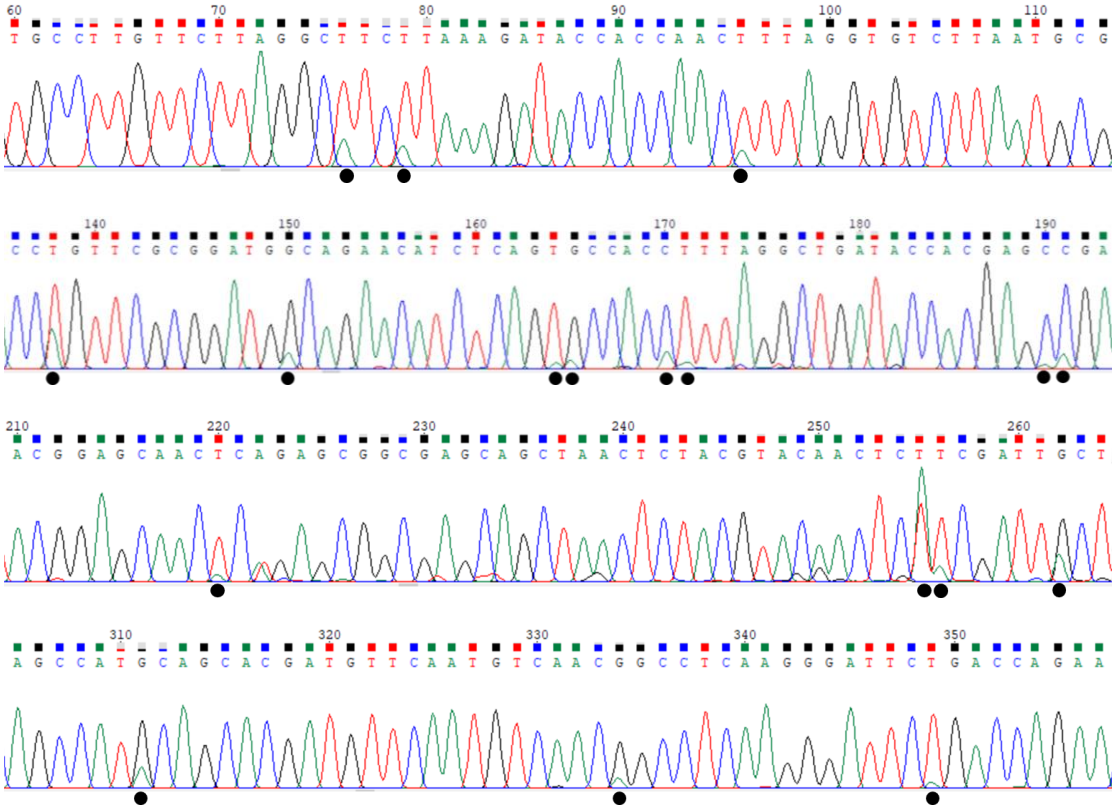


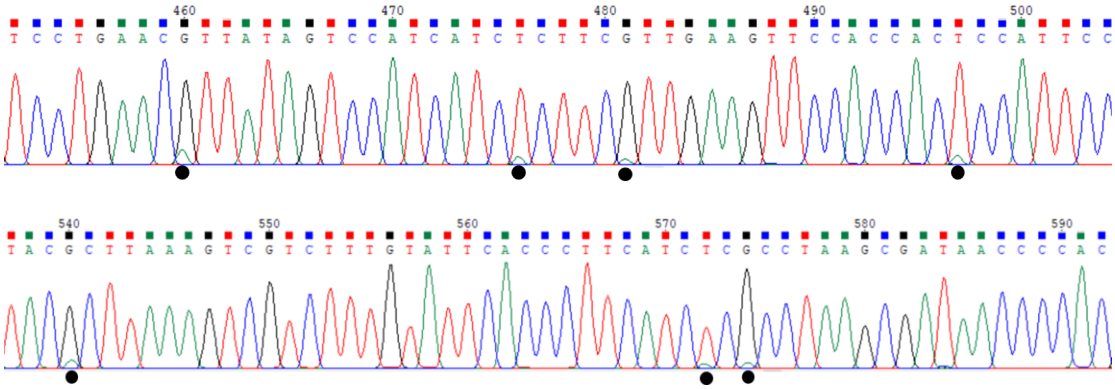
(Forward primer 2)



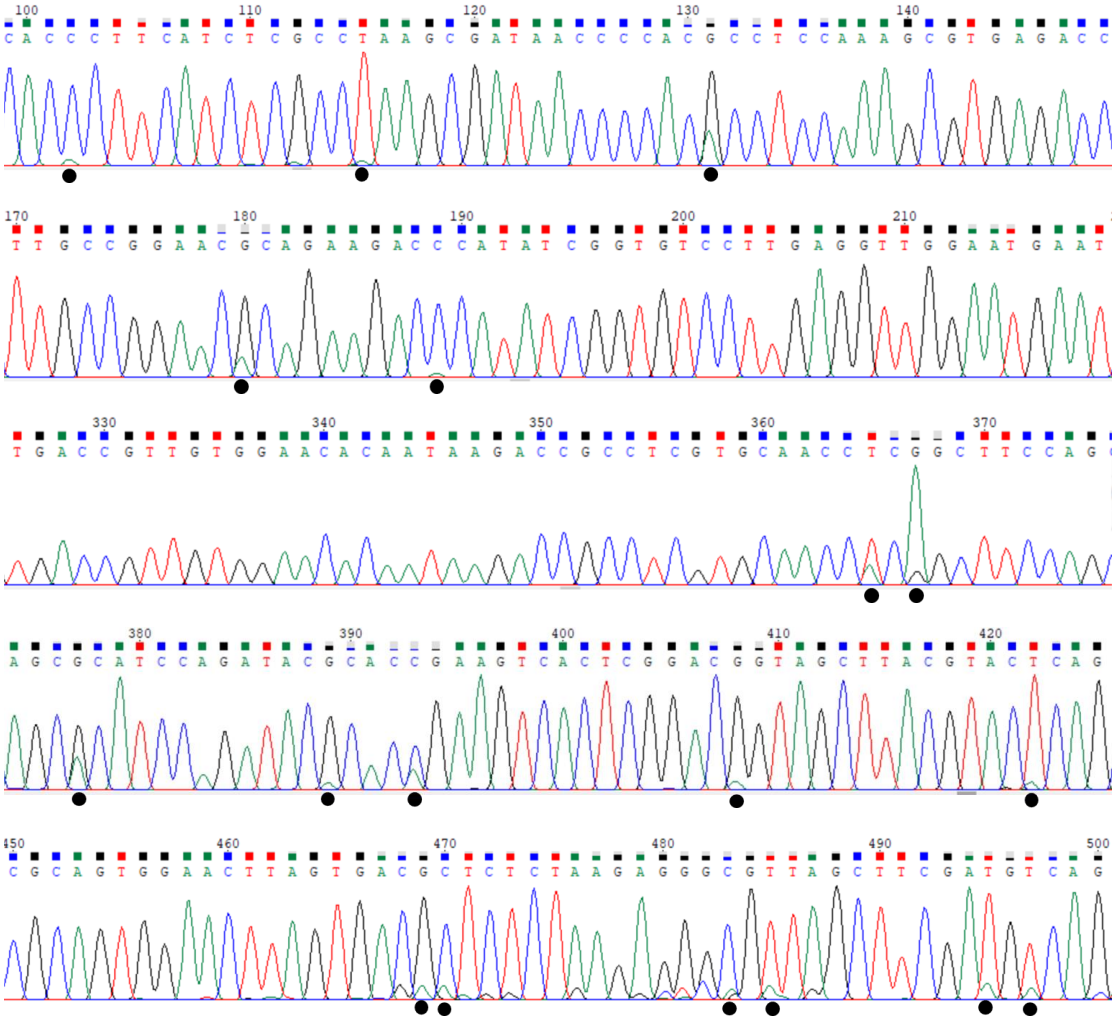


(Reverse primer 1)

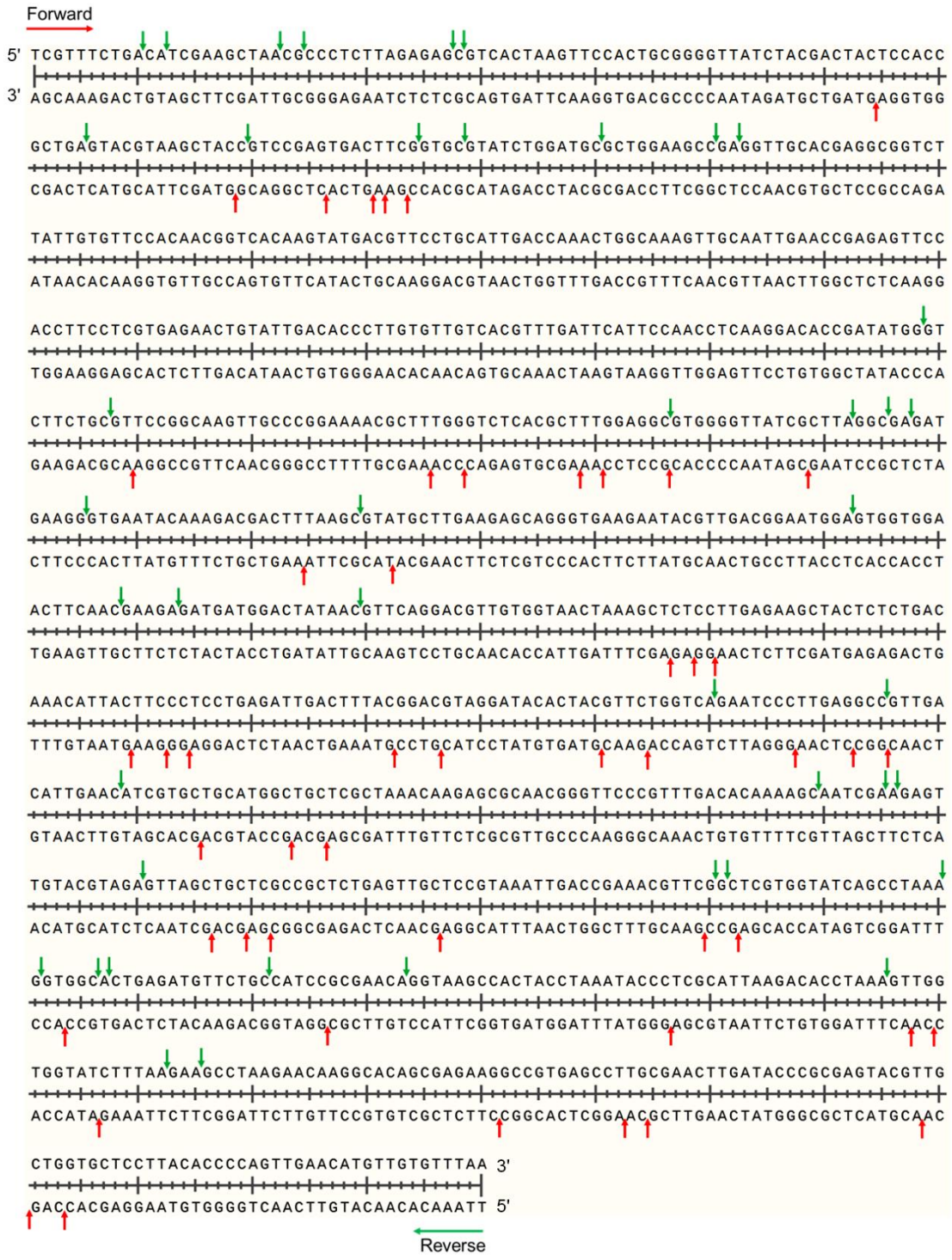




(Reverse primer 2)



B


Forward
5' TCGTTTCTGACATCGAAGCTAACGCCCTCTTAGAGAGCGTCACTAAGTTCCACTGCGGGGTTATCTACGACTACTCCACC
3' AGCAAAGACTGTAGCTTCGATTGCGGGAGAATCTCTCGCAGTGATTCAAGGTGACGCCCCAATAGATGCTGATGAGGTGG
GCTGAGTACGTAAGCTACCGTCCGAGTGACTTCGGTGCGTATCTGGATGCGCTGGAAGCCGAGGTTGCACGAGGCGGTCT
CGACTCATGCATTCGATGGCAGGCTCACTGAAGCCACGCATAGACCTACGCGACCTTCGGCTCCAACGTGCTCCGCCAGA
TATTGTGTTCCACAACGGTCACAAGTATGACGTTCCTGCATTGACCAAACTGGCAAAGTTGCAATTGAACCGAGAGTTCC
ATAACACAAGGTGTTGCCAGTGTTCATACTGCAAGGACGTAACTGGTTTGACCGTTTCAACGTTAACTTGGCTCTCAAGG
ACCTTCCTCGTGAGAACTGTATTGACACCCTTGTGTTGTCACGTTTGATTCATTCCAACCTCAAGGACACCGATATGGGT
TGGAAGGAGCACTCTTGACATAACTGTGGGAACACAACAGTGCAAACTAAGTAAGGTTGGAGTTCCTGTGGCTATACCCA
CTTCTGCGTTCCGGCAAGTTGCCCGGAAAACGCTTTGGGTCTCACGCTTTGGAGGCGTGGGGTTATCGCTTAGGCGAGAT
GAAGACGCAAGGCCGTTCAACGGGCCTTTTGCGAAACCCAGAGTGCGAAACCTCCGCACCCCAATAGCGAATCCGCTCTA
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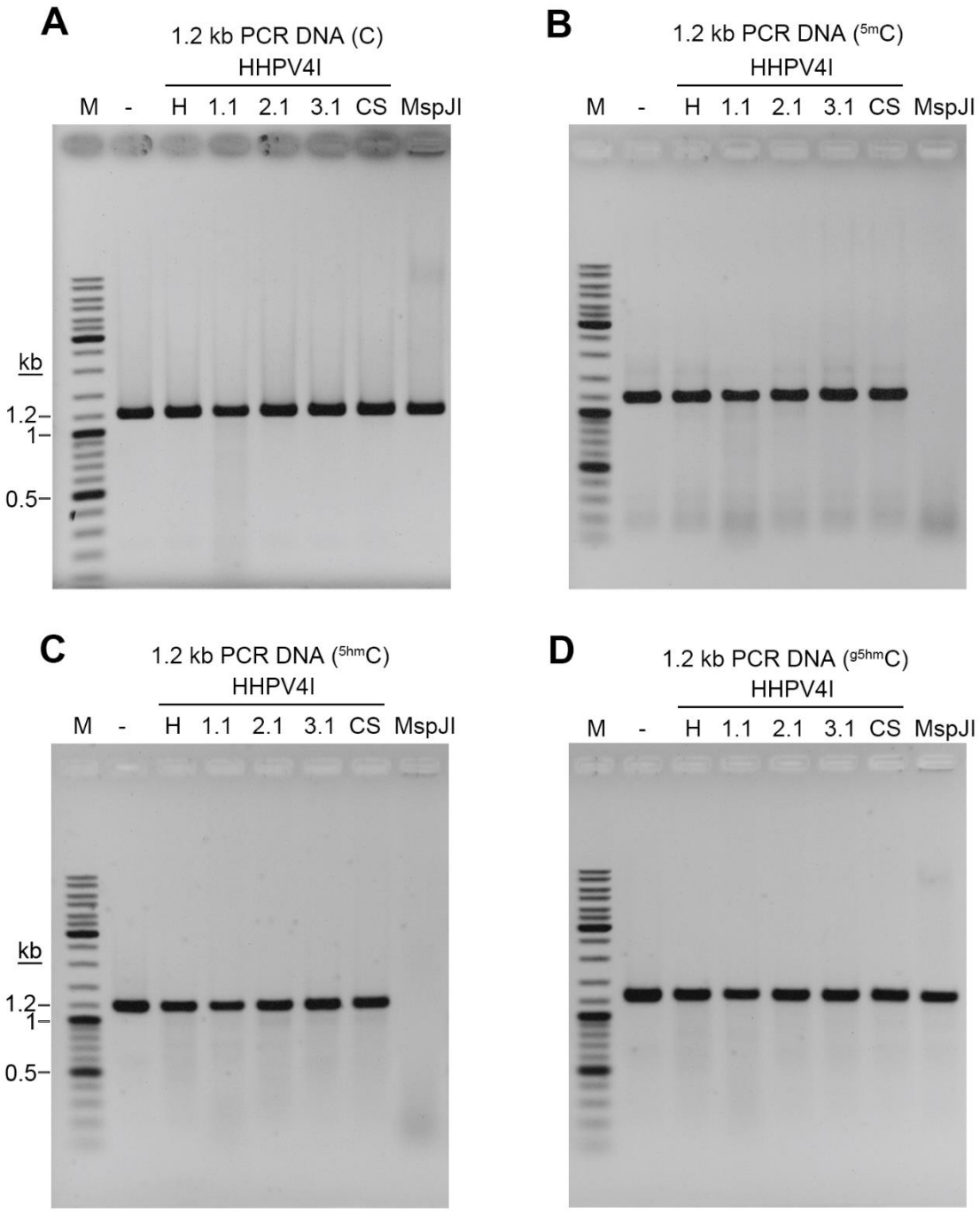
TGGTATCTTTAAGAAGCCTAAGAACAAGGCACAGCGAGAAGGCCGTGAGCCTTGCGAACTTGATACCCGCGAGTACGTTG
ACCATAGAAATTCTTCGGATTCTTGTTCCGTGTCGCTCTTCCGGCACTCGGAACGCTTGAACTATGGGCGCTCATGCAAC
CTGGTGCTCCTTACACCCCAGTTGAACATGTTGTGTTTAA 3'
GACCACGAGGAATGTGGGGTCAACTTGTACAACACAAATT 5'
Reverse

C

Figure S6. Examination of the HHPV4I cleavage activity towards DNA with different cytosine modifications. (A) cytosine, (B) 5mC or (C) 5hmC-containing 1.2 kb DNA fragments were amplified from pBR322 plasmid (pBR-1.2 kb, Table S3). The 5hmC-containing DNA fragment was glycosylated by using T4 phage β-glucosyltransferase (New England Biolabs) to generate the g5hmC-containg DNA fragment (D). For examining the cleavage activity of HHPV4I, different reaction buffers were used, as shown below. H: the HHPV4I reaction buffer (50 mM Tris-HCl, 100 mM NaCl, 5 mM $MnCl_2$, pH 7.9); 1.1: NEBuffer 1.1 (10 mM bis-Tris-propane-HCl, 10 mM $MgCl_2$, 0.1 mg/ml BSA, pH 7.0) supplemented with 5 mM $MnCl_2$; 2.1: NEBuffer 2.1 (10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, 0.1 mg/ml BSA, pH 7.9) supplemented with 5 mM $MnCl_2$; NEBuffer 3.1 (50 mM Tris-HCl, 100 mM NaCl, 10 mM $MgCl_2$, 0.1 mg/ml BSA, pH 7.9) supplemented with 5 mM $MnCl_2$; CS: NEB CutSmart buffer (20 mM Tris-Ac, 50 mM KAc, 10 mM $Mg(Ac)_2$, 0.1 mg/ml BSA, pH 7.9) supplemented with 5 mM $MnCl_2$. The reactions (10 μl) contained 100 ng/μl of the indicated DNA substrate and 500 nM of HHPV4I and were incubated at 37°C for 1 h. MspJI was used as a control.


A
1.2 kb PCR DNA (C)
HHPV4I
M - H 1.1 2.1 3.1 CS MspJI
kb
1.2
1
0.5
B
1.2 kb PCR DNA (5mC)
HHPV4I
M - H 1.1 2.1 3.1 CS MspJI
C
1.2 kb PCR DNA (5hmC)
HHPV4I
M - H 1.1 2.1 3.1 CS MspJI
kb
1.2
1
0.5
D
1.2 kb PCR DNA (g5hmC)
HHPV4I
M - H 1.1 2.1 3.1 CS MspJI