Supplementary Data

Labeling of unique sequences in double-stranded DNA at sites of vicinal nicks generated by nicking endonucleases

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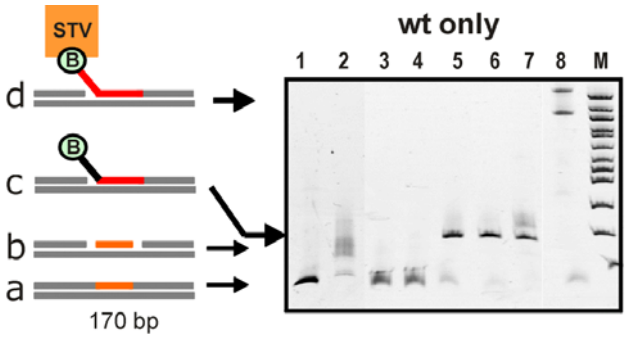
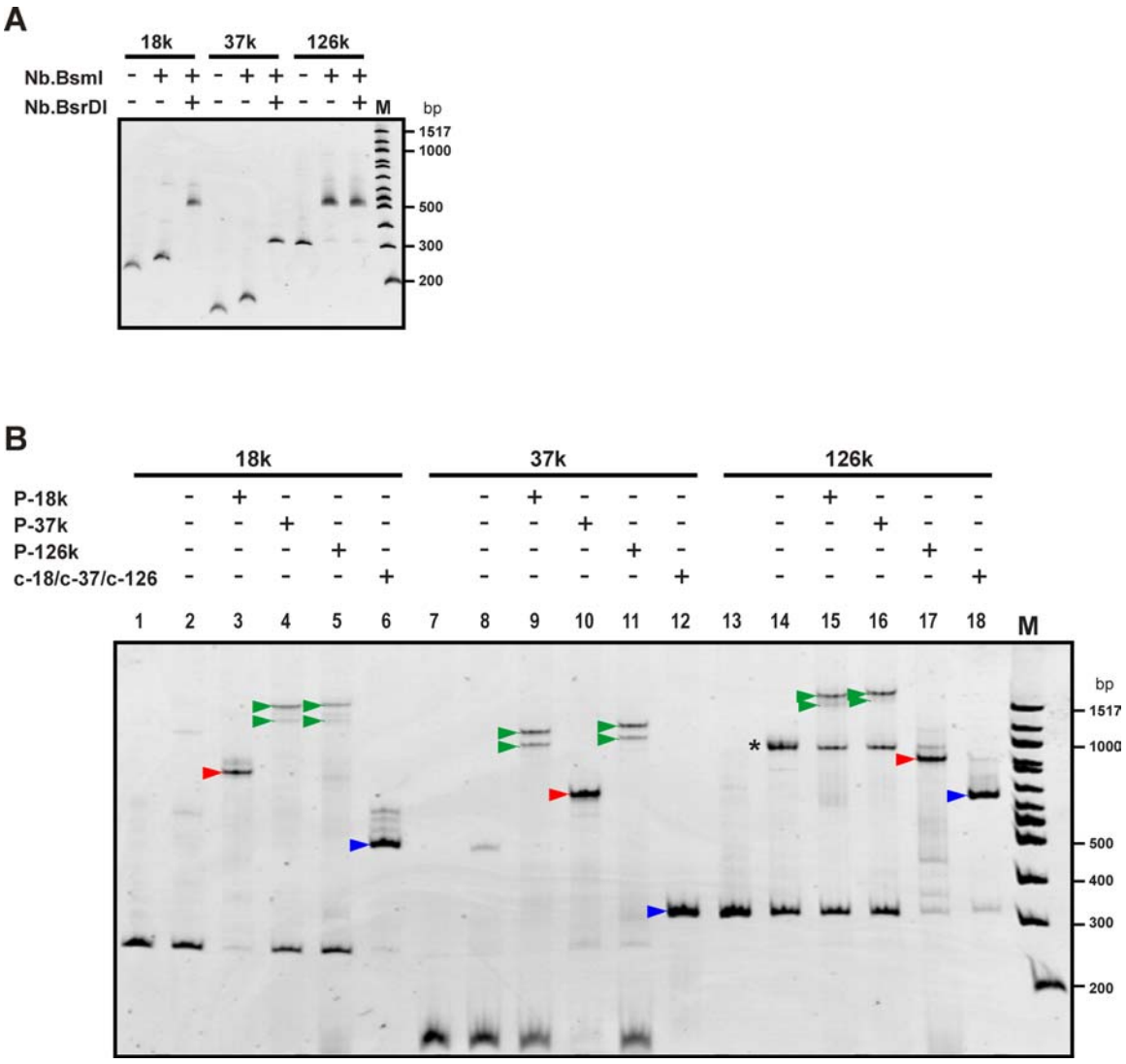


Supplementary Figure 1. Probe hybridization and ligation with a dsDNA fragment containing wt λ-DNA sequence. Lane 1: intact dsDNA; lane 2: dsDNA after incubation with nicking endonuclease Nt.BstNBI; lanes 3 to 7: nicked dsDNA after incubation with excess probe oligonucleotide P-45k-5'F (5'-biotin-TTTTTATCGTGAAGAGTCGGCG-3') at 30°C, 40°C, 50°C, 60°C, or 70°C, respectively, followed by ligation with T4 DNA ligase at 16°C; lane 8: an aliquot of sample shown in lane 5 after incubation with streptavidin.



Supplementary Figure 2. (A) Monitoring of nicking reactions with dsDNA fragments containing either HHV-6B target site at 18 k (225 bp), 37 k (152 bp), or 126 k (304 bp). Samples were analyzed by 7.5 % PAGE containing 7 M urea. (B) Analysis of correct and incorrect probe ligation products. Ligation reactions with each dsDNA fragment, nicked by Nb.BsmI and Nb.BsrDI, were performed for 16 h at 6 ^{o}C using 10 units of T4 DNA ligase. Correct and incorrect probe ligation products are marked by red and green arrowheads, respectively. Gapped DNA fragments, obtained by incubation of doubly-nicked fragments with complementary capture oligonucleotides, are marked by blue arrowheads. Note that in the control with the 126k target fragment (lane 14), a new band with lower mobility appeared (asterisk).

Supplementary Table 1. Oligonucleotides employed for PCR and ssDNA circle preparation

Oligo	Sequence[a] (5'→3')
A	GAGCGGTTGTAGTCTTCGTAAAACGACGGCCAGTGAACATGTCCCAACATGTTGATGAGATGAAG
B-wt	CATCACAGTCGCACCCTCGCCGACTCTTCACGATTATCGACTCCTTCATCTCATCAACA
B-m1	CATCACAGTCGCACCCTCGCCGACTCTTCACGATTATCGACT**G**CTTCATCTCATCAACA
B-m2	CATCACAGTCGCACCCTCGCC**C**ACTCTTCACGATTATCGACTCCTTCATCTCATCAACA
C	GGTGCGACTGTGATGATTAATATAGGGATATCCACACCAAACGTCA
D	CAGAGCAACTTGTCTTCCAGGAAACAGCTATGACGTTTGGTGTGG
F1-18k	TTCCACCAACGATTTCCTGCGA
R1-18k	CCGAGGAGTGCAATACAGAAGCC
F1-37k	ACGTCTTTCGTCTCTTGTTCCACA
R1-37k	CCGCCGTCCTATTCAGAGGTGC
F1-126k	TCGGGAATAAACCTGTATTTGCACAA
R1-126k	CTCAAACGCCGTGTTTCACCAAG
CO-1	P-AAGGCTAGGAATGCGTCTCTCACTGCTTA*CTTGTTCACTCTGCTGTCTG*AAGGCTAGGAATCGT-CTCTCACTGCTTACTACT

[a]Complementary overlaps between oligonucleotides A-D are marked in color. For oligonucleotide CO-1, the underlined sequences are identical with the sequence of the PNA beacon, while the italized sequence is complementary to the priming segment of probe P*-45k-3'F.