Electronic Supplementary Material (ESI) for Analyst.


## Supporting Information

# Technical Aspects of Nicking Enzyme Assisted Amplification

Liu Wang, Cheng Qian, Hui Wu, Wenjuan Qian, Rui Wang, and Jian Wu*

College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China.

* (J.Wu) E-mail: wujian69@zju.edu.cn

Tel.: 0086-571-88982180

Fax: 0086-571-88982180

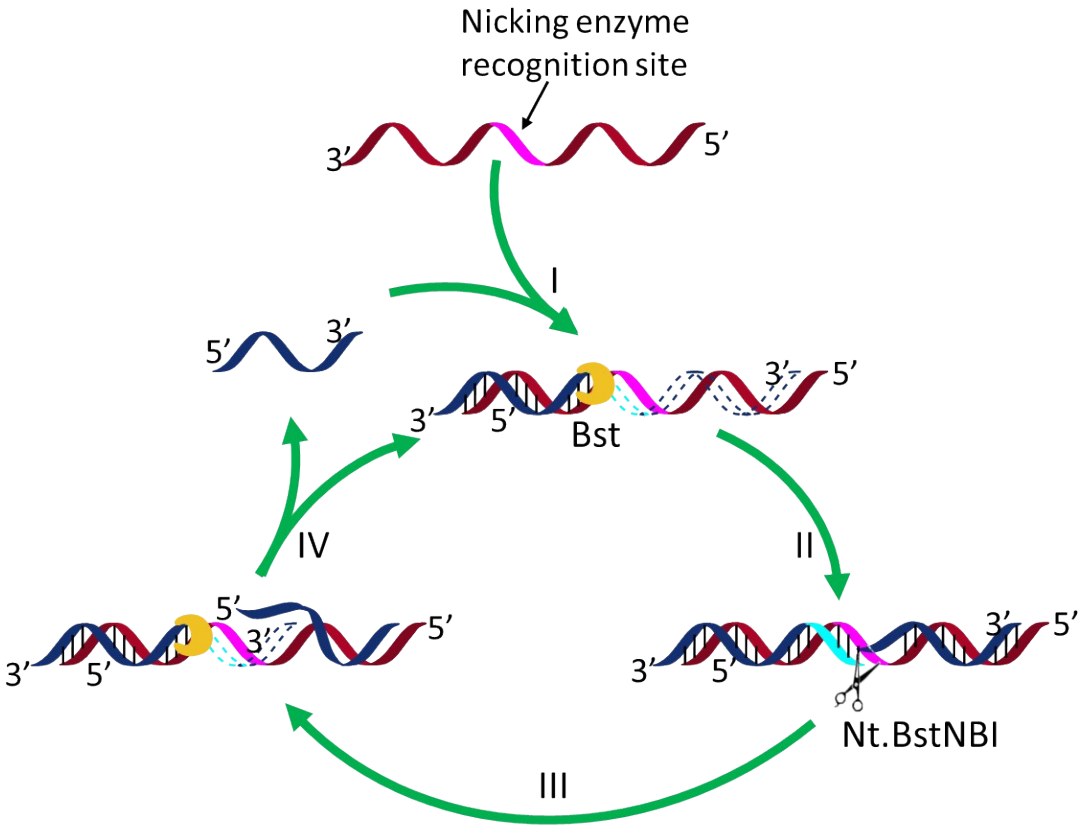


**Figure S1.** Schematic of exponential amplification reaction (EXPAR). The sequences separated by the restriction site (GAGTCNNNN) are the same, which is very different from NEAA.

**Table S1. Oligonucleotide sequences employed in this work.**

| Oligonucleotides | Sequence (5'-3') [a,b] |
|---|---|
| Nicking primer 1F | ATTTGTAGCTGAGTCCCAC\GACCCTTCCTCTAT |
| Nicking primer 10F | GTGTGCTTCTGAGTCTTGT\GATGCCTCTGCC |
| Nicking primer 12F | GTGTTCGTTCGAGTCATCT\GGCTATCGTTCA |
| Nicking primer 1R | CTTATTGTCCGAGTCTTAT\TGTCAGCGTGTCCT |
| Nicking primer 10R-1 | GTGTGCTTCTGAGTCCCAT\TTTCCACGATGC |
| Nicking primer 10R-2 | GTGTGCTTCTGAGTCTTGT\TCCTCGTGGGT |
| Bumper primer BF1 | ATCCCACTATCCTTCG |
| 49 nt oligonucleotide | GATGCCTCTGCCGACAGTGGTCCCAAAGATGGA CCCCCACCCACGAGGA |
| 53 nt oligonucleotide | TGTCAGCGTGTCCTCTCCAAATGAAATGAACTTC CTTATATAGAGGAAGGGTC |
| 62nt oligonucleotide (9A-short) | GGCTATCGTTCAAGATGCCTCTGCCGACAGTGG TCCCAAAGATGGACCCCGCATCGTGGAAA |
| 62nt oligonucleotide (9A-4) | GGCTATCGTTCAAGATGCCTCTGCCGACAGTGG TCCCAAAGATGGACCCCCACCCACGAGGA |
| 73 nt oligonucleotide | TGTCAGCGTGTCCTCTCCAAATGAAACCGATAA CTCCAGATAACAATGAACTTCCTTATATAGAGG AAGGGTC |
| 74 nt oligonucleotide (9A) | GGCTATCGTTCAAGATGCCTCTGCCGACAGTGG TCCCAAAGATGGACCCCCACCCACGAGGAGCAT CGTGGAAA |
| 79 nt oligonucleotide | TGTCAGCGTGTCCTCTCCAAATGAAATGAACTTC CTTATATAGAGGAAGGGTCTTGCGAAGGATAGT GGGATTGTGCGT |
| 113 nt oligonucleotide | TGTCAGCGTGTCCTCTCCAAATGAAACCGATAA CTCCCAGCACAGACCTAAACCTCAACGACAGCC AAGATAAAGACAGATAACAATGAACTTCCTTAT ATAGAGGAAGGGTC |

[a] The underlined letters indicate the restriction site of nicking enzyme Nt.BstNBI.

[b] The backslashes indicate the nicking position of Nt.BstNBI.

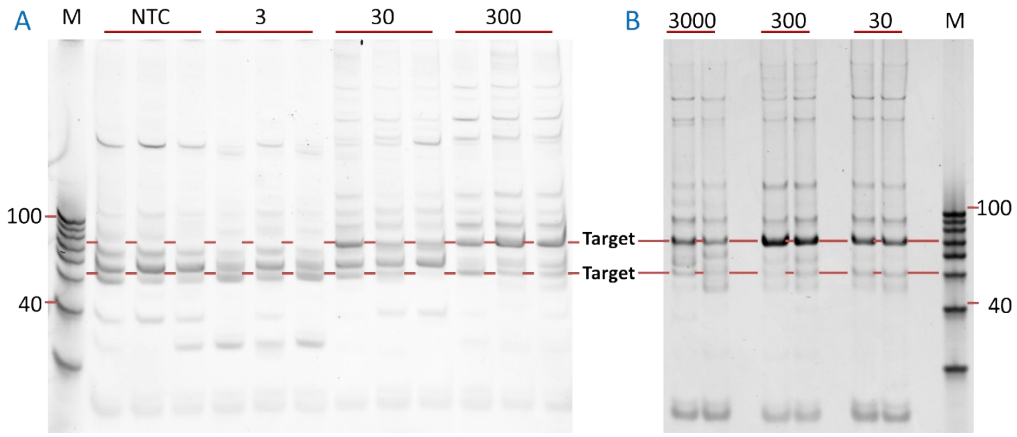


**Figure S2.** Detection of 3, 30, 300, and 3000 copies of genomic DNA in GTS 40-3-2 (A) without outer primer; and (B) with 100 nM outer primer BF1. 4.8 U Bst 3.0 DNA polymerase, 0.75 U Nt.BstNBI, 400 nM 1F, 100 nM 1R, 2 mM $Mg^{2+}$, 40 mM Tris-Cl (pH 8.8), 150 mM KCl, 10 mM $(NH_4)_2SO_4$, 50 mM NaCl, and 320 μM dNTP were used for amplification at 56 °C for 10 min. NTC: no template control, with 2.5 μL TE buffer replacing 2.5 μL template. Refer to Table S1 in Supplementary Information for DNA sequences. M: 10 bp DNA ladder.

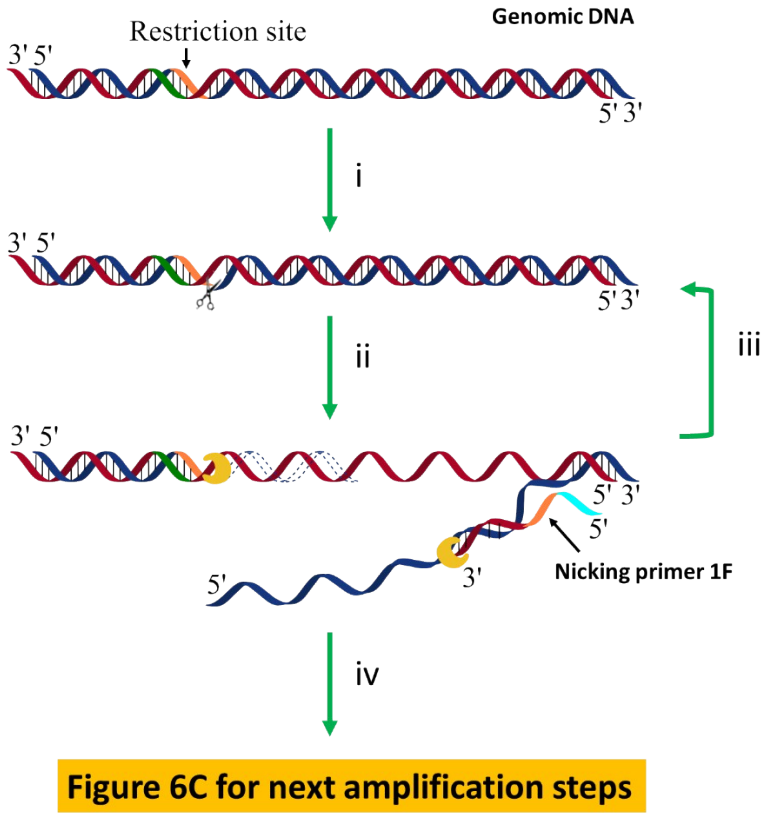


**Figure S3.** Scheme of amplification of genomic DNA adjacent to the restriction site without the presence of outer primer. (i) the restriction site on genomic DNA is recognized and nicked by the nicking enzyme Nt.BstNBI. (ii) The sequence downstream of the nicking site is dissociated from the genomic DNA and the nicking site is extended by the Bst 3.0 DNA polymerase. (iii) Repeated nicking and extension at the nicking site on the genomic DNA. (iv) The nicking primer primes the dissociated ssDNA and the amplification progresses as shown in Figure 6C and then Figure 1. The sequence upstream of the restriction site on the genomic DNA acts like a natural nicking primer and produces a great deal of dissociated ssDNA so that the amplification has a high sensitivity even without the outer primer.

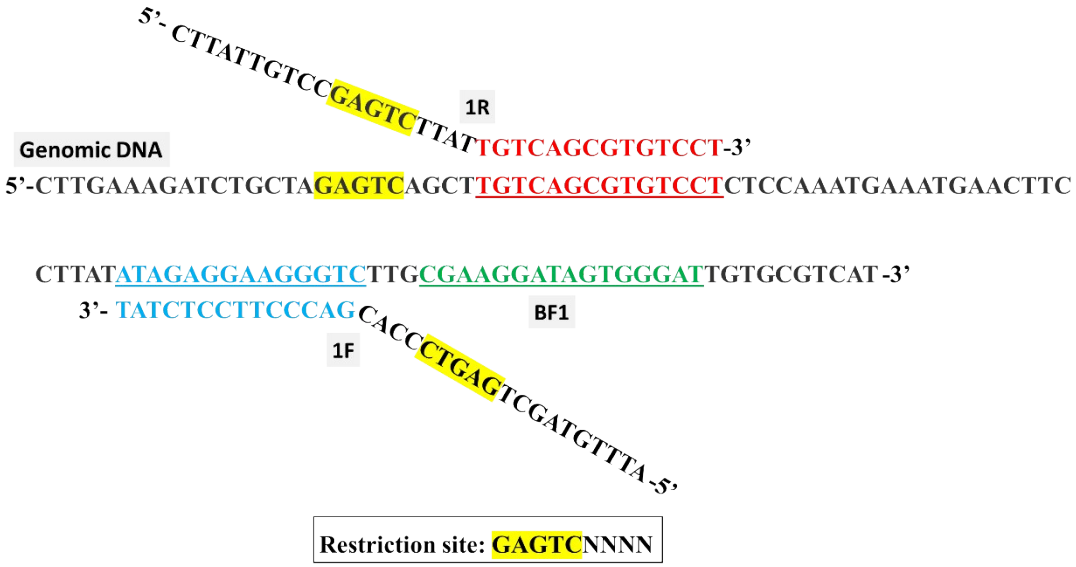


**Figure S4.** Target sequence and primer locations for real sample analysis. Nucleotide positions are based on the reverse complementary sequence of *CaMV 35S* and *CP4 epsps* gene on genomic DNA in transgenic soya GTS 40-3-2 (GenBank accession number AY596948).