Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary information

DNA modification and visualization on an origami-based enzyme nano-factory

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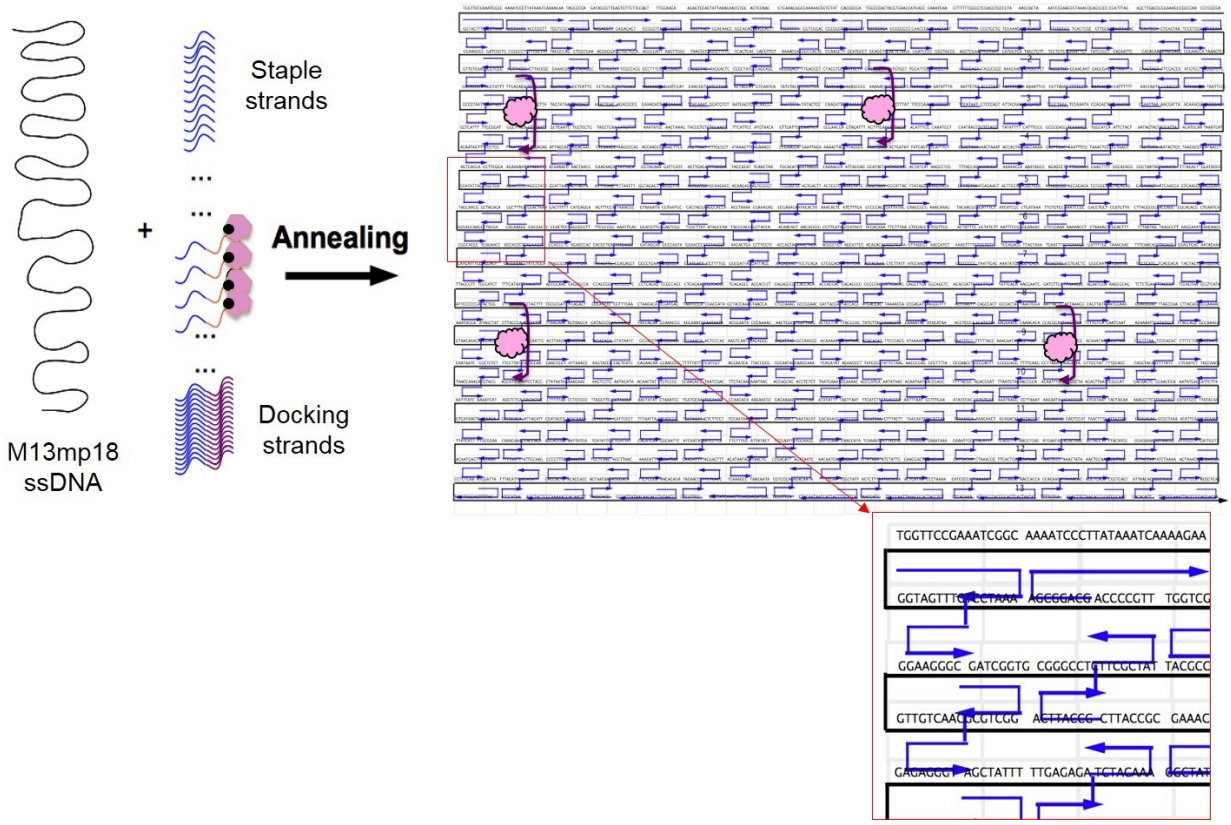

Figure S1. Construction of the origami to host DNA modifying enzymes shown in pink and DNA substrate in purple: Schematic representation showing the folding of M13mp18 single-stranded (ss) plasmid in rectangular shape to form desired origami with a dimension of 92 nm by 52 nm. Four docking strands (extended staple strands) bind four ODN-modified enzymes through hybridization and eight docking strands are designed to host target DNA in close proximity of the four enzymes bound to the DNA origami surface. 221 staple strands are used to fold the origami in this shape.

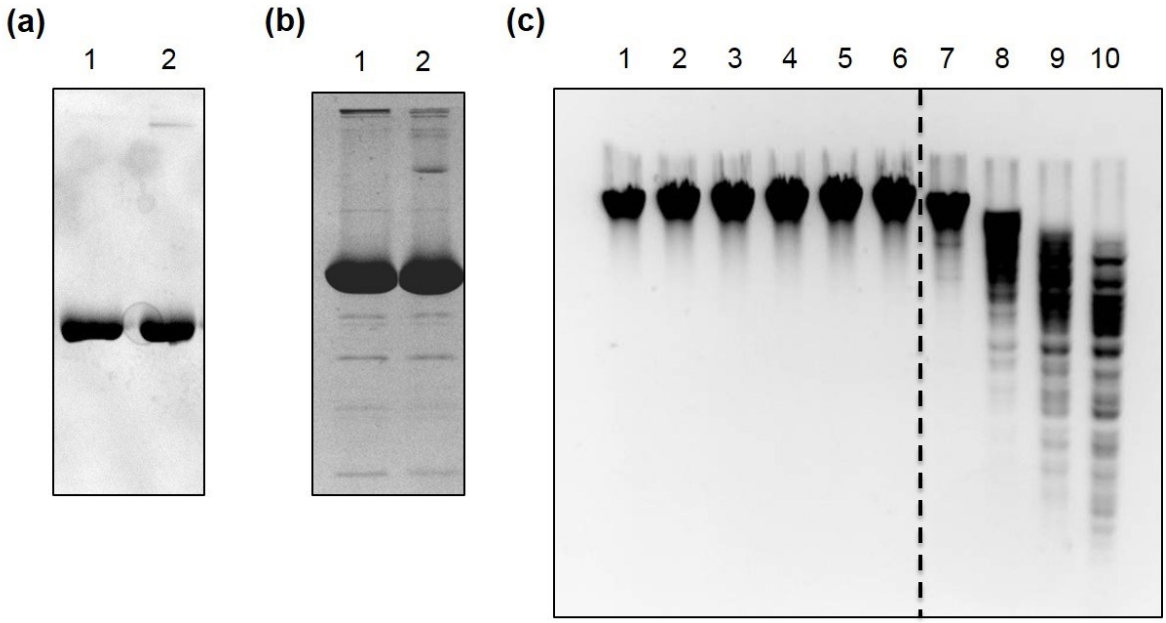

Figure S2. Purification of M.TaqI double mutant C48S422C and activity test with the natural cofactor *S*-adenosyl-L-methionine (AdoMet, SAM): **(a)** SDS-PAGE analysis of purified wild type M.TaqI with (lane 1) or without (lane 2) β-mercaptoethanol in the SDS loading buffer; **(b)** Purified M.TaqI double mutant; numbers of lanes represent the same as in (a); **(c)** Activity test of M.TaqI double mutant with AdoMet and λ DNA. The amount of M.TaqI mutant in lane 1 is 10 ng and halved from lane to lane. After incubation at 65°C for 1 h the DNA is challenged by the cognate restriction endonuclease R.TaqI (same recognition sequence as M.TaqI) and the degree of protection analysed by agarose gel electrophoresis. One enzymatic unit is defined as the amount of enzyme that fully protects the DNA against fragmentation (0.3125 ng in lane 6, dashed line). The specific activity of the mutant (3200 units/μg) was found to be identical to the specific activity of the wild type enzyme.

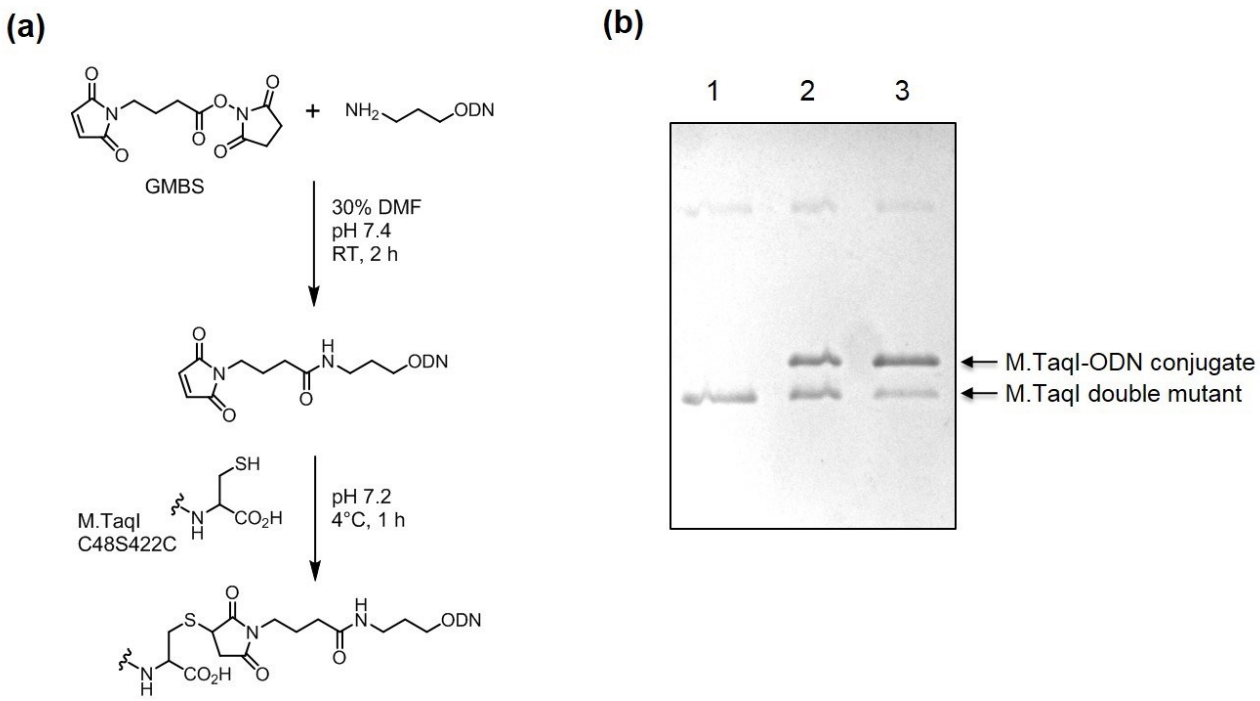

Figure S3. Coupling of amine-modified ODN and M.TaqI double mutant with the hetero-bifunctional crosslinker *N*-(γ-maleimidobutyryloxy)succinimide (GMBS): **(a)** Scheme for the synthesis of M.TaqI-ODN conjugate; **(b)** SDS-PAGE analysis of M.TaqI double mutant (lane 1) and coupling of M.TaqI double mutant with 1 eq. (lane 2) and 10 eq. maleimide-modified ODN (lane 3).

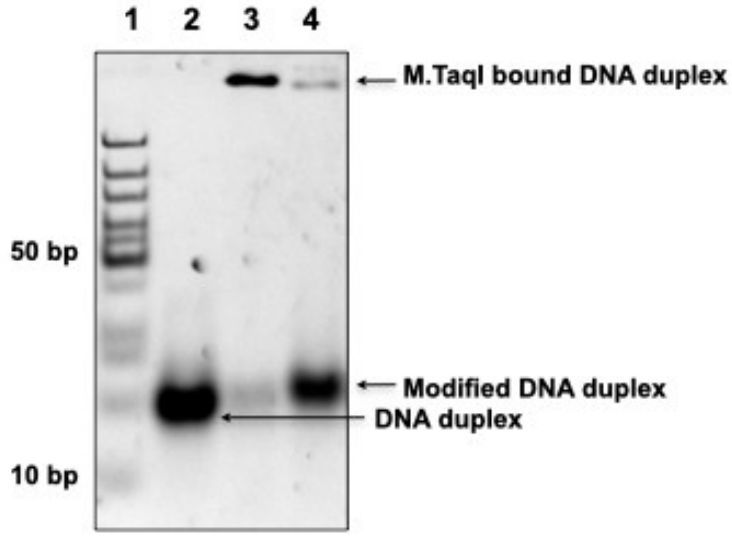

Figure S4.

Labelling short duplex DNA with M.TaqI and 6BAz in solution analysed by non-denaturing polyacrylamide gel electrophoresis. Lane 1: 10 base pair DNA ladder marker; lane 2: 14 base pair duplex DNA (with target sequence); lane 3: M.TaqI bound to 6BAz-modified DNA duplex; lane 4: Release of 6BAz-modified product DNA from the DNA-M.TaqI-complex by fragmentation with Proteinase K.

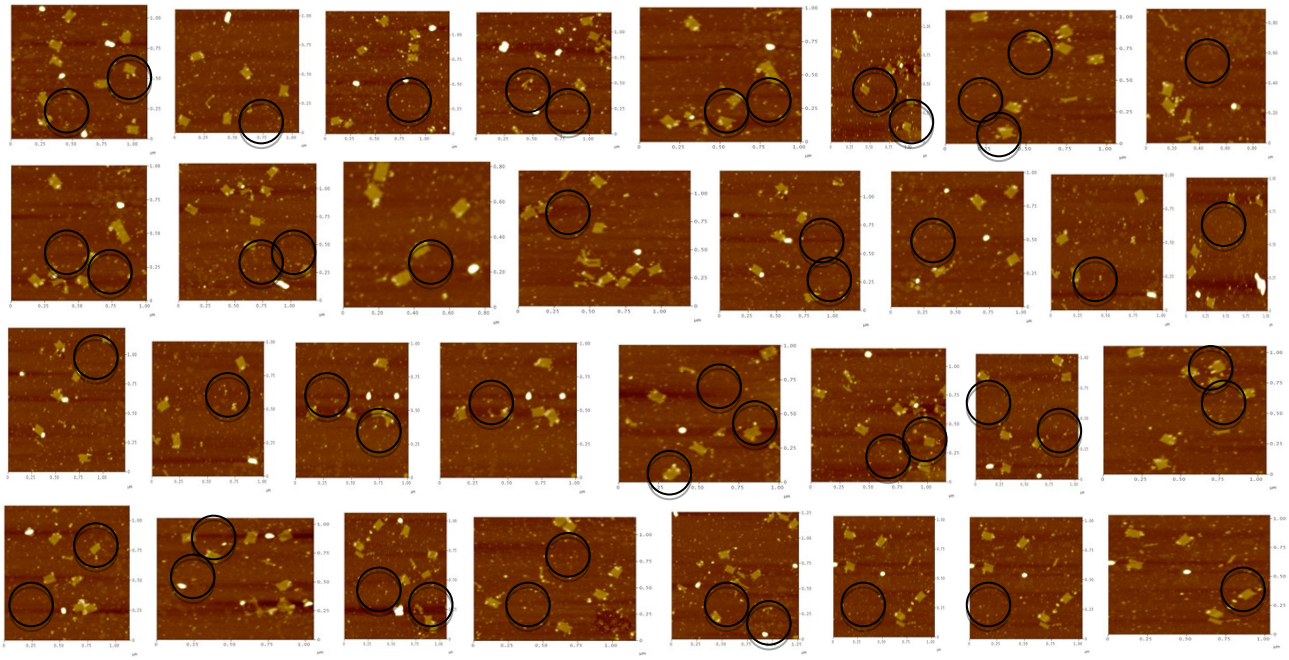

Figure S5. Representative AFM images for calculating the efficiency of modification as described in Table 1. Twin spots of 5 nm each or 10 nm high spots are marked with black circle.

Addition/ Reagent	(I) Origami	(II) GMBS-DNA	(III) M.TaqI C48S/422C	(IV) Target DNA duplex	(V) 6BAz	(VI) Streptavidin	(VII) Undocking strands
Stoichiometry	Staple strands 50:1, docking strands 200:1 over M13mp18	10:1 over docking strands	1:1 with GMBS-DNA	1:1 with M.TaqI mutant	2.5:1 over target DNA	(on surface)	10:1 over target DNA
Time (h)	0	24	24	36	48	50	74
Specific details and final concentrations	0.4 nM M13mp18 + 20 nM staple strands + 80 nM docking strands thermocycled in origami buffer overnight, ultrafiltration with 50 kDa cutoff	8 µM GMBS-coupled DNA + 8 µM M.TaqI mutant incubated in conjugation buffer at 4°C for 1 h (Fig. S3); 800 nM M.TaqI-ODN added to (**I**) and incubated at 4°C overnight		800 nM pre-annealed target DNA duplex added to (**I+II+III**) and incubated at 4°C overnight	2 µM 6BAz added to (**I+II+III+IV**) and incubated at 4°C for 2 h	Deposition of (**I+II+III+IV+V**) on mica, washed with 50 µL origami buffer (5x) and drying; then 2 µM streptavidin added, incubated for 5 min, washed with 50 µL origami buffer (5x) and dried	8 µM of each undocking strand added to (**I+II+III+IV+V**) and incubated at 4°C overnight
AFM check time (h)	24	48		48	50	51	74

Table S1: Details of stepwise experimental conditions of full enzyme nano-factory cycle.

List of Sequences [from 5' to 3']

1 TTTTTGGTTCCGAAATCGGCAAATCCTGTTTGATGGTTTTGGAAGGGC

2 GATCGGTGGGCTGCGCAACTGTTGTTTTGAGAGGGT

3 AGCTATTTTGATAAATTAATGCCGTTTTGGTCATTT

4 TTGCGGATGCTCCTTTTGATAAGATTTTAGTCAGGA

5 CGTTGGGAACTGGCTCATTATACCTTTTTAGCAACG

6 GCTACAGAAGCATCGGAACGAGGGTTTTCGCCACCC

7 TCAGAACCTCAGGAGGTTTAGTACTTTTTTAGCGTT

8 TGCCATCTGGTCATAGCCCCCTTATTTTAAATAGCA

9 ATAGCTATAGAGCAAGAAACAATGTTTTCAATAATC

10 GGCTGTCTGCATGTAGAAACCAATTTTTAATTTATC

11 AAAATCATAGAAGAGTCAATAGTGTTTTTTATCATT

12 TTGCGGAAAAAAGTTTGAGTAACATTTTCGTCTGAA

13 ATGGATTACATTTTGACGCTCAATTTTT

14 GCAGGCGAAAAATCCCTTATAAATCAAAAGAA

15 CTGCCGCTACCGACGATAAGTCGCCATTCACGGGCCTCTTCGCTATTTGCCCCA

16 GTTCTAGCTTGAGAGATCTACAAACGCCATTC

17 CTTTAATTGGCTTAGAGCTTAATTATTCAACCGACTTATCGTCGGGAGCG

18 TTTTAAGAAGAAAAATCTACGTTAGAGAGTACCTGCCGCTACCGACGATAAGTC

19 CGAAAGACGGCTTTGAGGACTAAATTATGCGA

20 CACCGTACGCCACCCTCAGAACCGCTCAGCAG

21 CTGCCGCTACCGACGATAAGTCGCATTTTCTTTCATAATCAAAATCAGGTGTAT

22 CAATAATACTTACCGAAGCCCTTTTTTCATCG

23 ATTTACGATTCCTTATCATTCCAAGTTAAGCCGACTTATCGTCGGGAGCG

24 AGACGCTGAGGTCTGAGAGACTACCCATCCTACTGCCGCTACCGACGATAAGTC

25 TTAATTTTCAAAGAAACCACCAGATTAGATTA

26 AATACCTATTTACATTGGCAGATTGAACGTTA

27 TAGCCCGAAGCGGTCCACGCTGGTTACGCCAG

28 CTGGCGAACGGAAACCAGGCAAAGGGCTATCA

29 GGTCATTGTCACCATCAATATGATGCTGAATC

30 TGGTGCTGCAACAGGTCAGGATTAATAAAACG

31 AACTAACGATCATTGTGAATTACCGACTTTTT

32 CATGAGGATTGCGGGATCGTCACCCCACCCTC

33 AGAGCCACAAGTATAGCCCGGAATACCGGAAC

34 CAGAGCCATCAGACTGTAGCGCGTTTAAGAAA

35 AGTAAGCAAACCCACAAGAATTGAGAACGGGT
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38 AATTATCAATTAAATCCTTTGCCCCACCAGTC
39 ACACGACCTGCAACAGGAAAAACGCTCATGGA
40 TTGCAGCAGATAGGGTTGAGTGTTGTTCCAGT
41 TCTGGTGCAGGGGGATGTGCTGCATGAGAGAG
42 CAGTCAAACCTGAGAGTCTGGAGCGCACCGCT
43 GCAAACTCTAGCTCAACATGTTTTGCCGGAGA
44 CAACTTTAGAACAACATTATTACAGACCGGAA
45 GGCCGCTTAGTTTCCATTAAACGGTTTAATTT
46 GTTGATATCACCCTCATTTTCAGGGAGTTAAA
47 CTTTAGCGCCACCGGAGTGCTGCATGAGAGAG
48 AGAGAGATGATAGCCGAACAAAGTAAGTTTGC
49 GTCCTGAAAAGTACCGCACTCATCCTAATATC
50 AATCCTTGTTAGGTTGGGTTATATATAGATAA
51 CAACTCGTTCATATTCCTGATTATTCCCTTAG
52 CCAGCCATAGTAATAAAAGGGACACAATTCGA
53 TTGGAACACTTCACCGCCTGGCCCAGGCGATT
54 AAGTTGGGTCCAGCCAGCTTTCCGAAACAAGA
55 GAATCGATCAAAAGGGTGAGAAAGAAATATGC
56 AACTAAAGGCTTCAAAGCGAACCAGGTAGAAA
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64 GGCAATTCTATTAGACTTTACAAATTCTGGCC
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68 TAAAGATTGAACGGTAATCGTAAACTCAGGAA
69 TAATTCGATACGGTGTCTGGAAGTTGTGTAGGGACTTATCGTCGGGAGCG

70 AGTAGTAAAGTTGAGATTTAGGAATCGCGTTTCTGCCGCTACCGACGATAAGTC
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72 TCAGTACCGGAACCCATGTACCGTAACCGATA
73 ACCGTAATCTCAGAACCGCCACCCGGTTTTGC
74 ACAAAGTCAGGAAACGCAATAATAATAGCAGC
75 GCAGAACGTTTTTATTTTCATCGTCACCCTGA
76 TAAATCGTTGATGCAAATCCAATCCAGCTAAT
77 TTTAGAAGATCAATATAATCCTGATGCTTCTG
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82 ATGTAACACGAAAGACTTCAAATATACCACAT
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207 AAGCATCAGGAGAAACAATAACGGAAATCGCG
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209 CGTGGCGATTTTAAAGCCTGGGGTGCCTCGGAAGCA

210 TAAAGTGTTTTTGTCTGGCCTTCCTGTAATCAAAAA

211 TAATTCGCTTTTACAGGCAAGGCAAAGAACATCCAA

212 TAAATCATTTTTTCCAATACTGCGGAATTTTAGACT

213 GGATAGCGTTTTTAAGGGAACCGAACTGCGCAGACG

214 GTCAATCATTTTGGAACAACTAAAGGAAGGAGTGAG

215 AATAGAAATTTTCATGGCTTTTGATGATTCCAGTAA

216 GCGTCATATTTTCAAAAGGGCGACATTCTTACCAGC

217 GCCAAAGATTTTCTAACGAGCGTCTTTCCTGAATCT

218 TACCAACGTTTTATTCTTACCAGTATAACATATGCG

219 TTATACAATTTTTGAATACCAAGTTACAATTCGCCT

220 GATTGCTTTTTTAGCCAGCAGCAAATGAACAGTGCC

221 ACGCTGAGTTTTAACAGGAGGCCGATTAAAGGGATT

222. NH_2-*CGA CGA TAA GTC*

[M.TaqI binding ODN (**X**), partially complementary to overhang strands = 17, 23, 69 &199].

223. CGC TCC *CGA CGA TAA GTC*

[Full complement of overhang strands = 17, 23, 69 & 199 to remove M.TaqI-ODN-conjugate from origami (**X'**)].

224. GACTTATC*GTCGGTAGCGGCAG*

[Full complement of overhangs of (15, 18), (21, 24), (67, 70) & (197, 200) to remove modified target from origami surface (**Y'**)].

225. *GTCGGTAGCGGCAG*TTTTTTTTGCCGATCGATGCCGTTTTTTTTT*GTCGGTAGCGGCAG*

[Target DNA partially complementary to overhangs of (15, 18), (21, 24), (67, 70) & (197, 200) (**Y**)].

226. *CGG CAT CGA TCG GC*

[Complementary DNA to form duplex with target DNA sequence around the binding site for M.TaqI (225)].