

The influence of 5'*R* and 5'*S* cdA and cdG on the activity of BsmAI and SspI restriction enzymes

Supplementary Materials

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Table S1. Complete sequences of applied oligonucleotides. Abbreviations mean the following: SX - (5'S)-5'8-cyclo-2'-deoxyadenosine; RX - (5'R)-5'8-cyclo-2'-deoxyadenosine; SY - (5'S)-5'8-cyclo-2'-deoxyguanosine; RY - (5'R)-5'8-cyclo-2'-deoxyguanosine.

| Oligo | End | | End |
|-------------|-----|---|-----|
| Matrix 5' | 5' | CTCTTGTCAGGAATATTGTCTCTATGCTCCCACCAAAGGC | 3' |
| Matrix 3' | 3' | GAGAACAGTCCTTATAACAGAGATACGAGGGTGGTTTCCG | 5' |
| Sequence 1 | 5' | CTCTTGTCAGGAAT SX TTGTCTCTATGCTCCCACCAAAGGC | 3' |
| Sequence 2 | 5' | CTCTTGTCAGGAAT RX TTGTCTCTATGCTCCCACCAAAGGC | 3' |
| Sequence 3 | 5' | CTCTTGTCAGGAATATTGTCTCT SX TGCTCCCACCAAAGGC | 3' |
| Sequence 4 | 5' | CTCTTGTCAGGAATATTGTCTCT RX TGCTCCCACCAAAGGC | 3' |
| Sequence 5 | 3' | GAGAACAGTCCTT SX TAAACAGAGATACGAGGGTGGTTTCCG | 5' |
| Sequence 6 | 3' | GAGAACAGTCCTT RX TAAACAGAGATACGAGGGTGGTTTCCG | 5' |
| Sequence 7 | 3' | GAGAACAGTCCTTATAACAGAG SX TACGAGGGTGGTTTCCG | 5' |
| Sequence 8 | 3' | GAGAACAGTCCTTATAACAGAG RX TACGAGGGTGGTTTCCG | 5' |
| Sequence 9 | 3' | GAGAACAGTCCTTATAACAGAGATAC SY AGGGTGGTTTCCG | 5' |
| Sequence 10 | 3' | GAGAACAGTCCTTATAACAGAGATAC RY AGGGTGGTTTCCG | 5' |
| Sequence 11 | 5' | CTCTTGTCAGGA SX TATTGTCTCTATGCTCCCACCAAAGGC | 3' |
| Sequence 12 | 3' | GAGAACAGTCCTTATAACAG SY ATACGAGGGTGGTTTCCG | 5' |
| Sequence 13 | 5' | CTCTTGTCAGGA RX TATTGTCTCTATGCTCCCACCAAAGGC | 3' |
| Sequence 14 | 3' | GAGAACAGTCCTTATAACAG RY ATACGAGGGTGGTTTCCG | 5' |

Table S2. Calculated and found molecular masses [Da] of applied oligonucleotides.

| Oligonucleotide | Calculated mass | Found mass |
|-----------------|-----------------|------------|
| Matrix 5' | 12182.00 | 12182.42 |
| Matrix 3' | 12409.10 | 12409.82 |
| Sequence 1 | 12181.00 | 12180.10 |
| Sequence 2 | 12181.00 | 12180.20 |
| Sequence 3 | 12181.00 | 12180.10 |
| Sequence 4 | 12181.00 | 12180.20 |
| Sequence 5 | 12408.10 | 12407.30 |
| Sequence 6 | 12408.10 | 12407.30 |
| Sequence 7 | 12408.10 | 12407.30 |
| Sequence 8 | 12408.10 | 12407.30 |
| Sequence 9 | 12408.10 | 12407.30 |
| Sequence 10 | 12408.10 | 12407.50 |
| Sequence 11 | 12182.00 | 12180.20 |
| Sequence 12 | 12408.10 | 12407.20 |
| Sequence 13 | 12182.00 | 12180.10 |
| Sequence 14 | 12408.10 | 12407.40 |

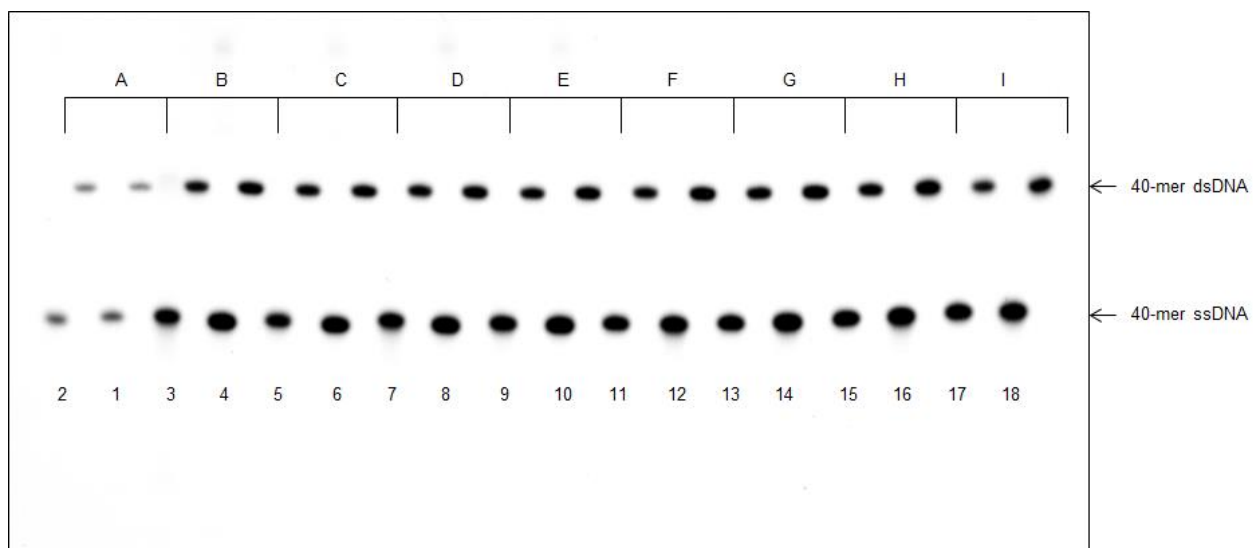


Figure S1. Visualization of efficient labeling (ssDNA) and hybridization (dsDNA) of oligonucleotides. Labeled strands 1-18 and obtained duplexes A-I are shown.

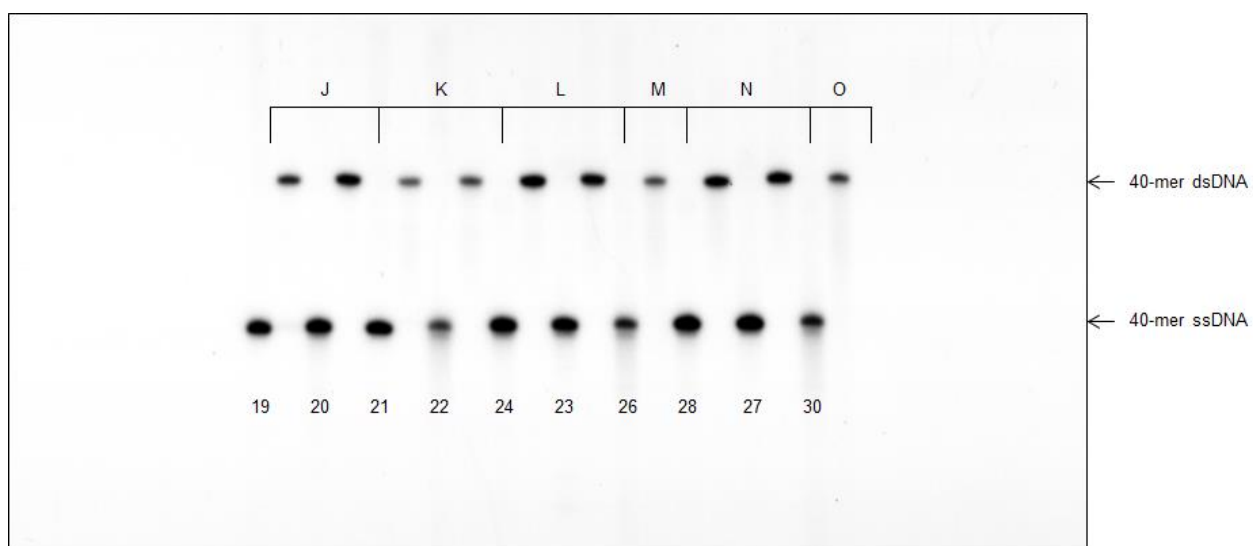


Figure S2. Visualization of efficient labeling (ssDNA) and hybridization (dsDNA) of oligonucleotides. Labeled strands 19-30 and obtained duplexes J-O are shown.

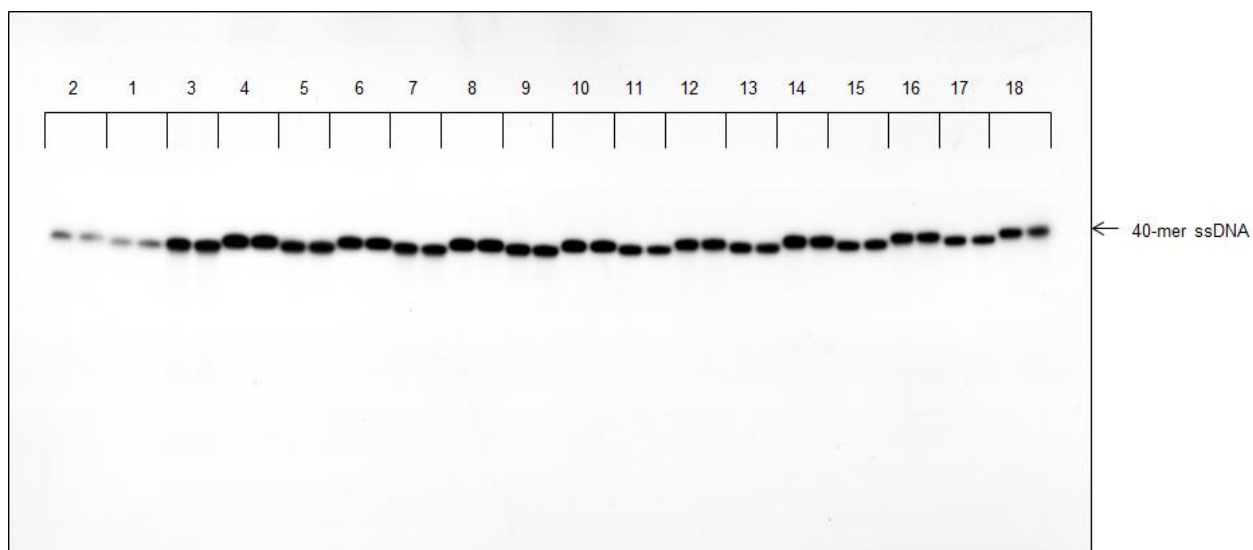


Figure S3. Control cleavage assay: cleavage of ssDNA (strands 1-18) by 0.5 U BsmAI for 0 min (left band) and 60 min (right band).

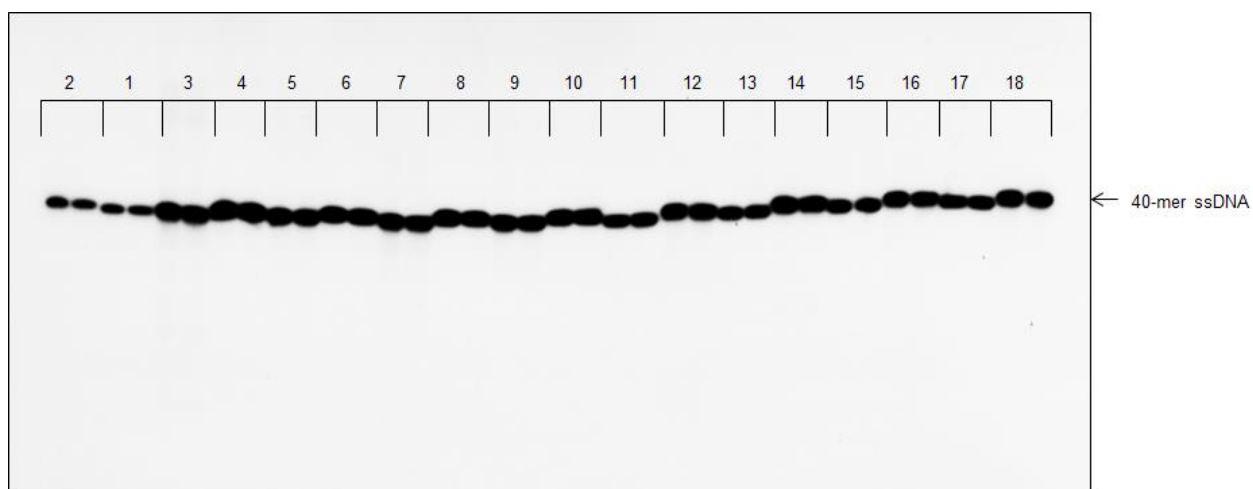


Figure S4. Control cleavage assay: cleavage of ssDNA (strands 1-18) by 1.5 U SspI for 0 min (left band) and 60 min (right band).

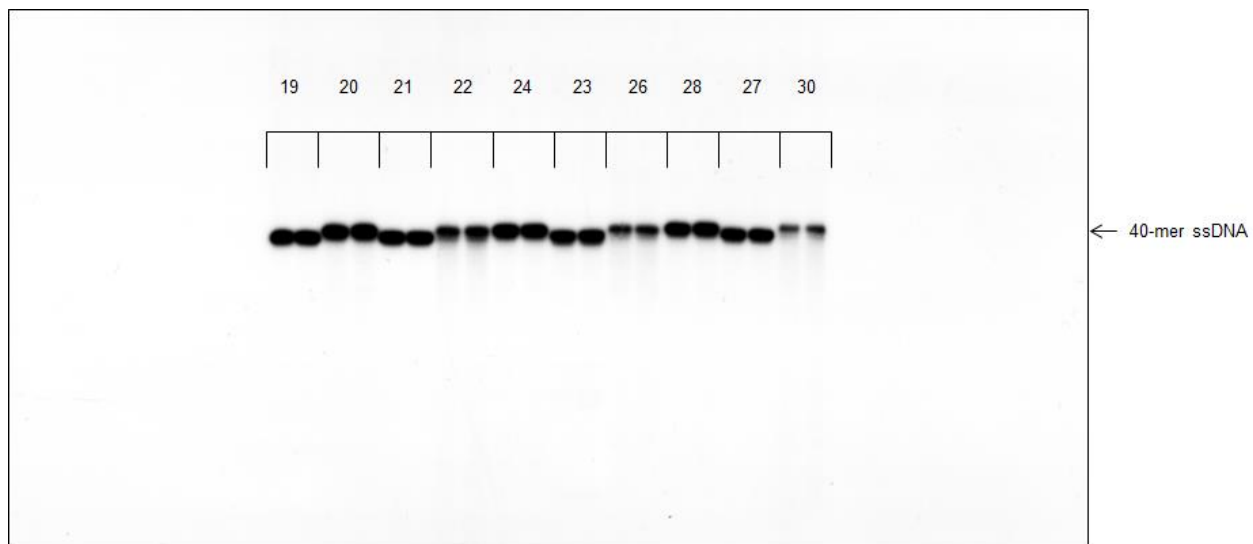


Figure S5. Control cleavage assay: cleavage of ssDNA (strands 19-30) by 0.5 U BsmAI for 0 min (left band) and 60 min (right band).

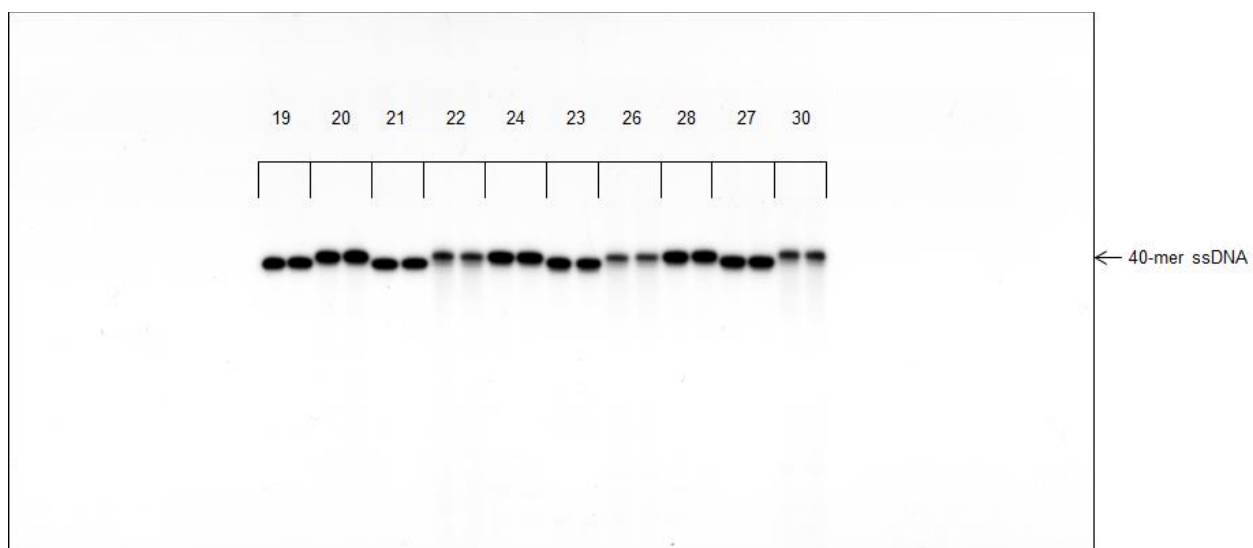


Figure S6. Control cleavage assay: cleavage of ssDNA (strands 19-30) by 1.5 U SspI for 0 min (left band) and 60 min (right band).

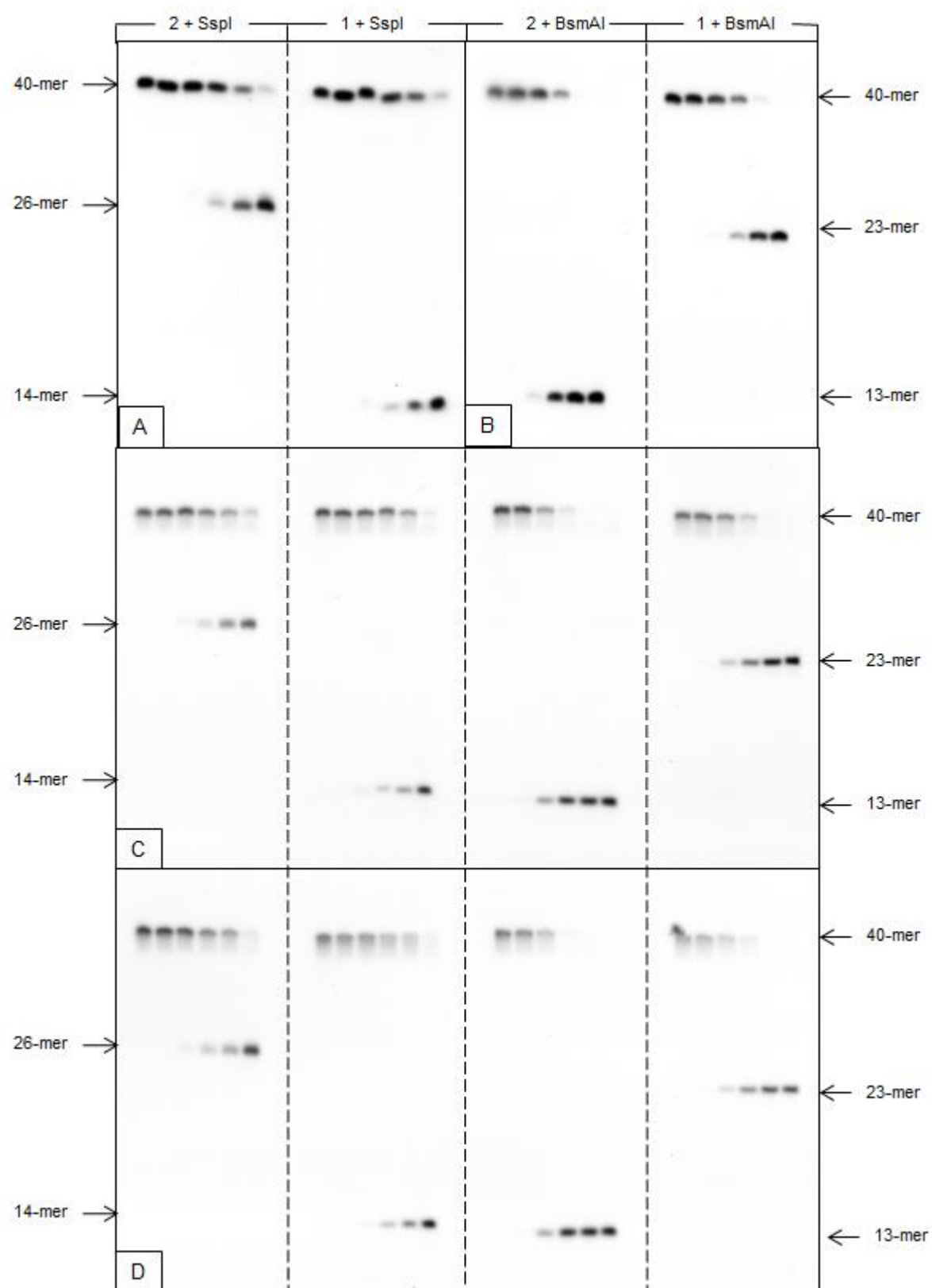


Figure S7. Native control assays for strands 1 and 2. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30 and 60 min starting from the left. (A) autoradiogram of repeat 1 for SsplI; (B) autoradiogram of

repeat 1 for BsmAI; (C) autoradiogram of repeat 2 for SspI and BsmAI; (D) autoradiogram of repeat 3 for SspI and BsmAI.

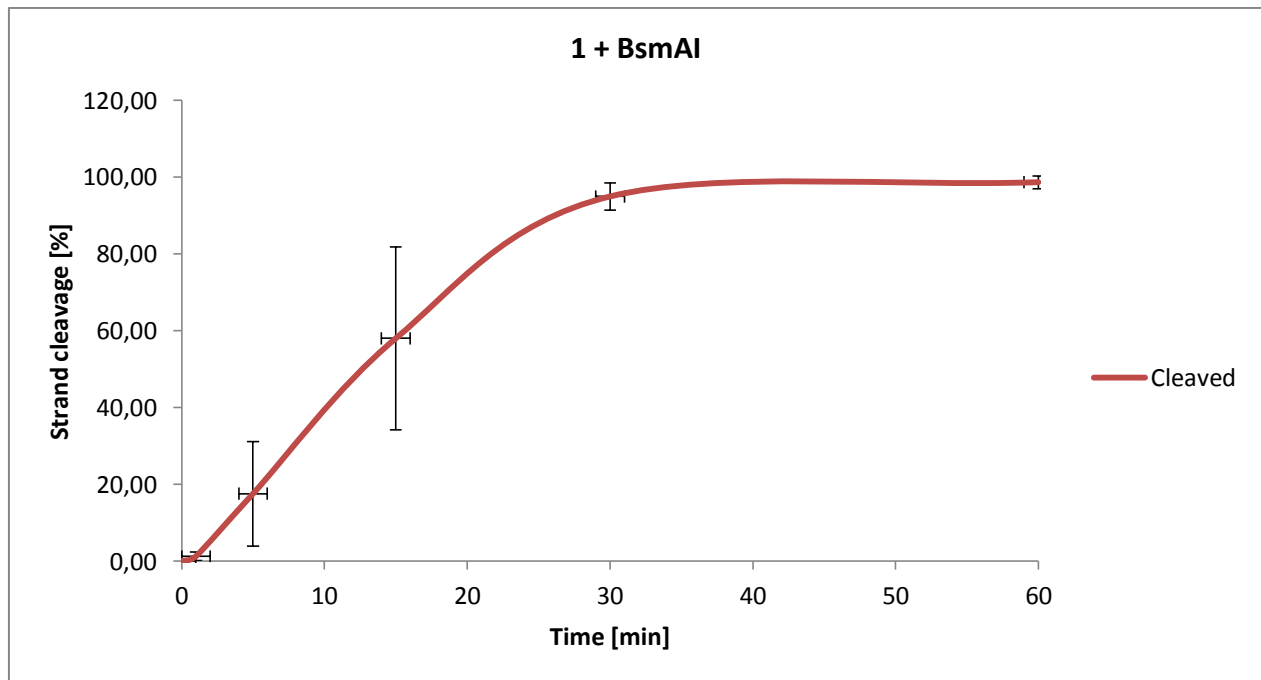


Figure S8. Cleavage of native control dsDNA (duplex A) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 1) is shown.

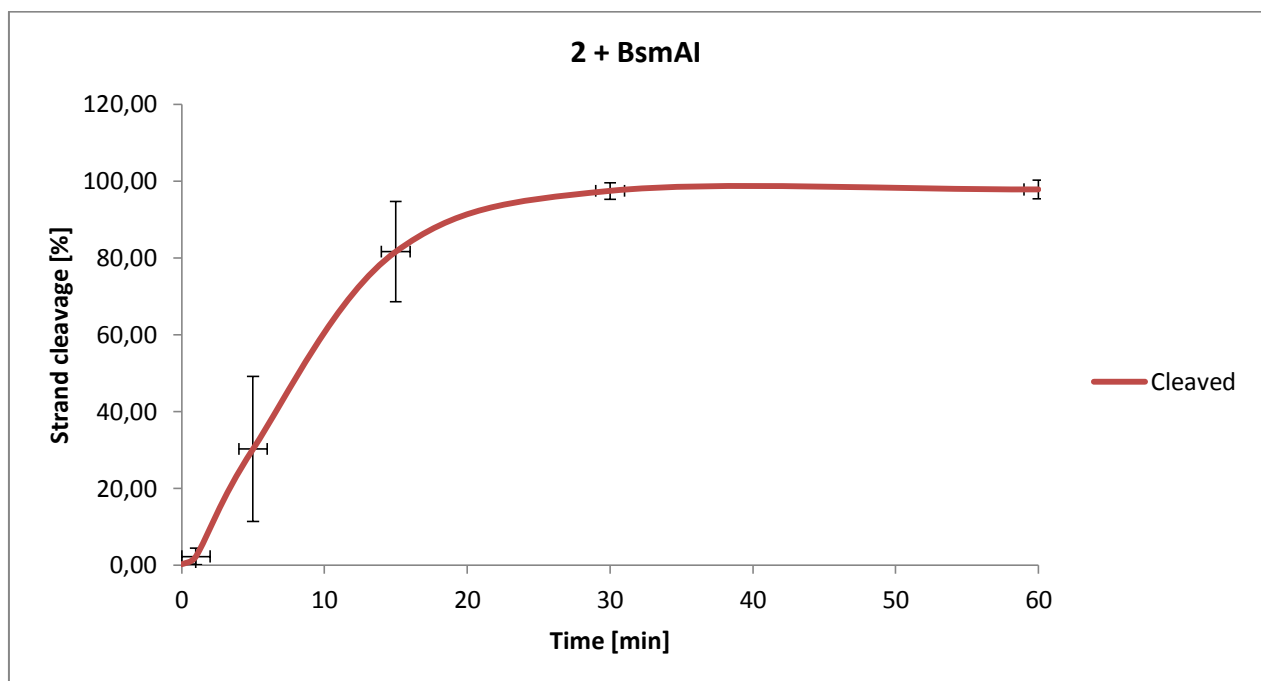


Figure S9. Cleavage of native control dsDNA (duplex A) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 2) is shown.

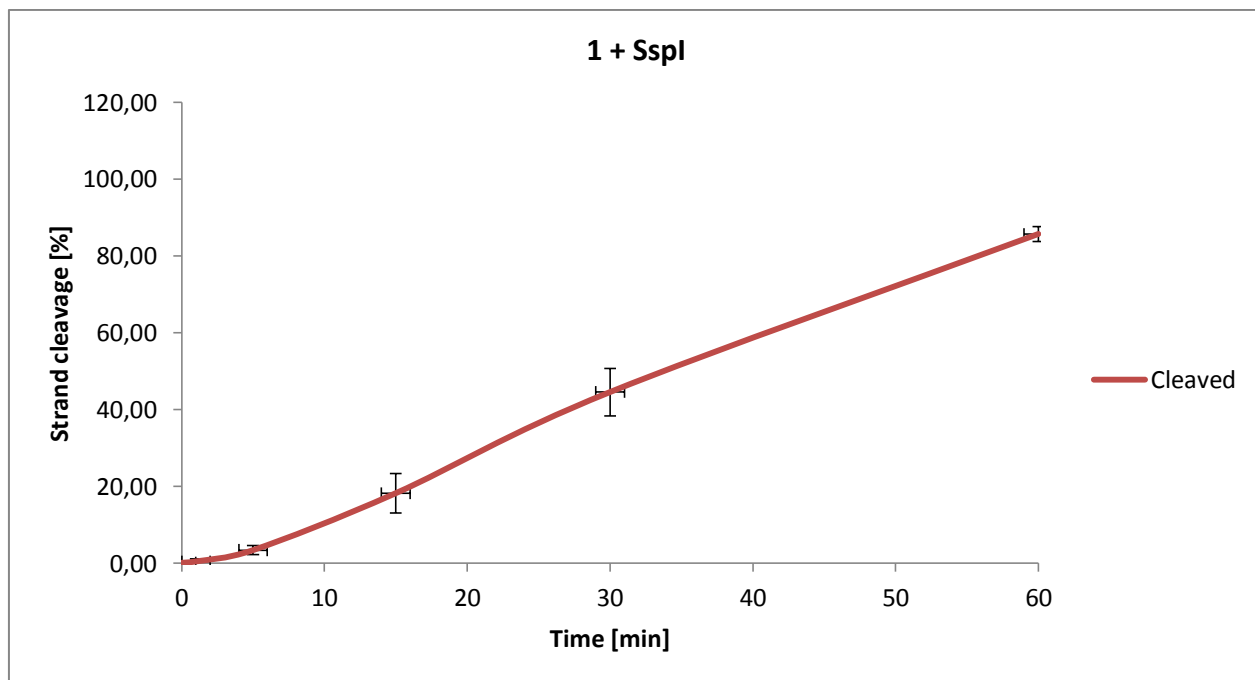


Figure S10. Cleavage of native control dsDNA (duplex A) by 1.5 U Sspl. Quantity increase of cleaved ssDNA (strand 1) is shown.

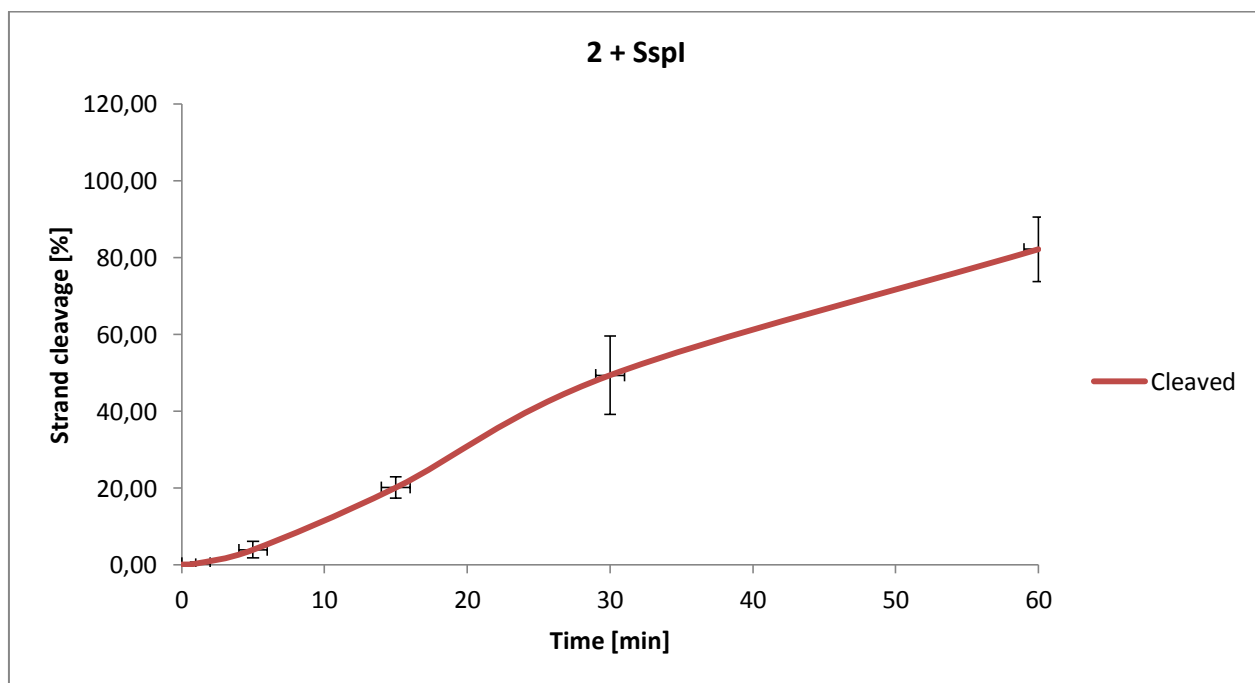


Figure S11. Cleavage of native control dsDNA (duplex A) by 1.5 U Sspl. Quantity increase of cleaved ssDNA (strand 2) is shown.

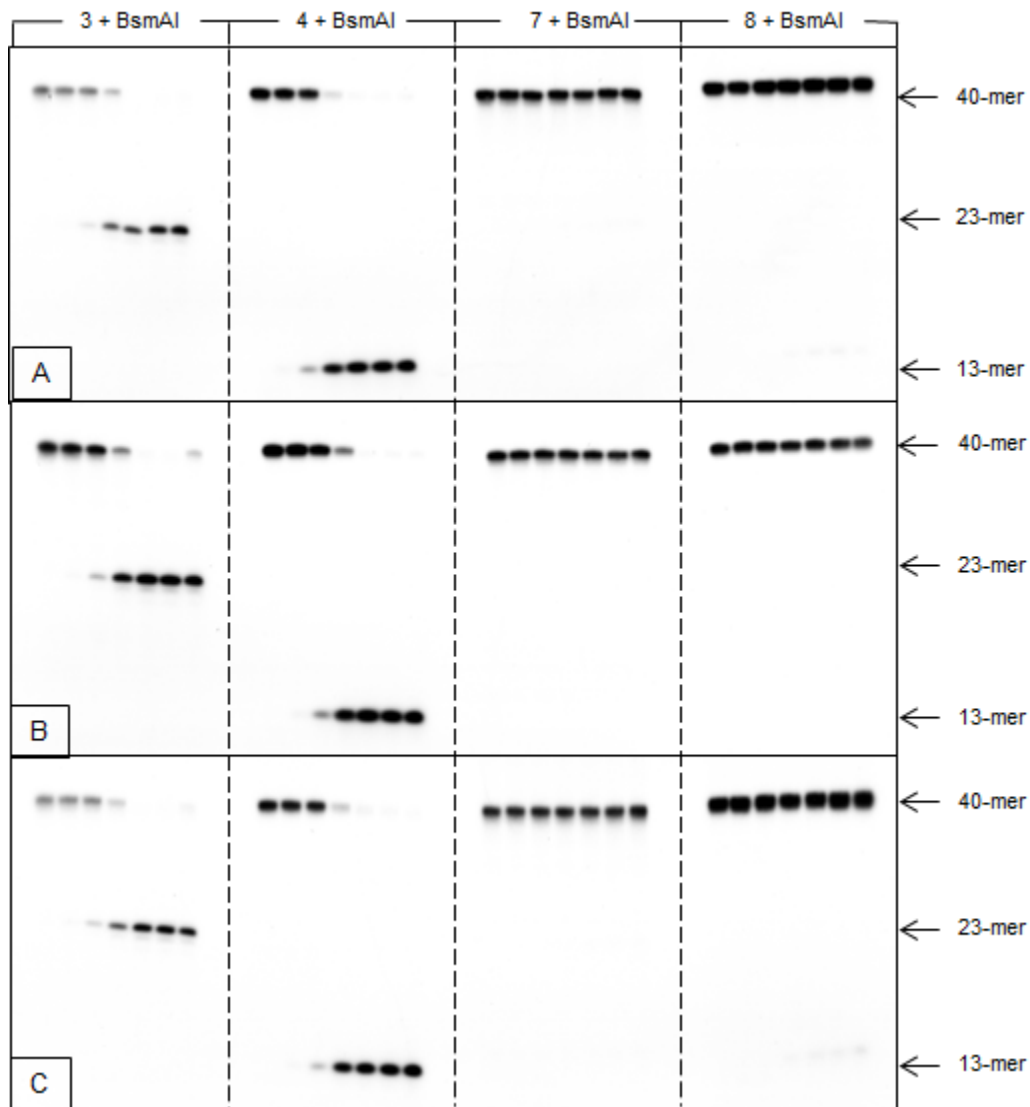


Figure S12. The autoradiograms of denaturing PAGE presenting cleavage of oligonucleotides by 0.5 U BsmAI – (A), (B) and (C) represent three repeats of assays. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30, 45 and 60 min starting from the left.

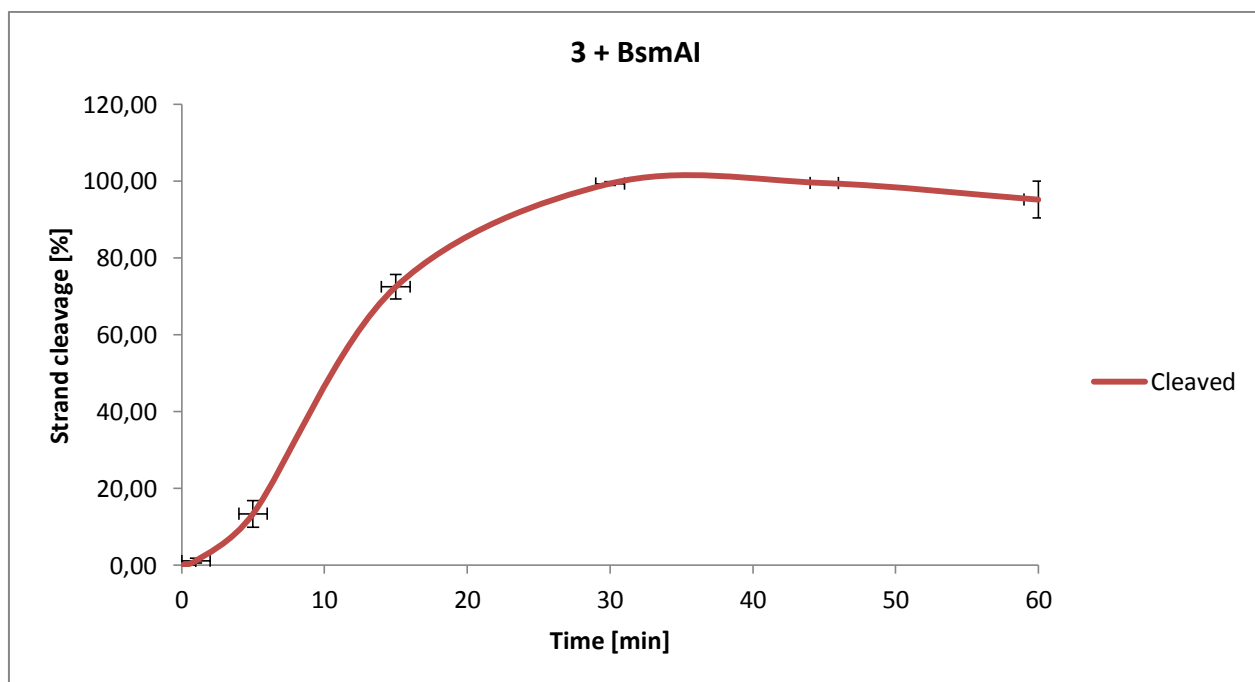


Figure S13. Cleavage of dsDNA (duplex B) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 3) is shown.

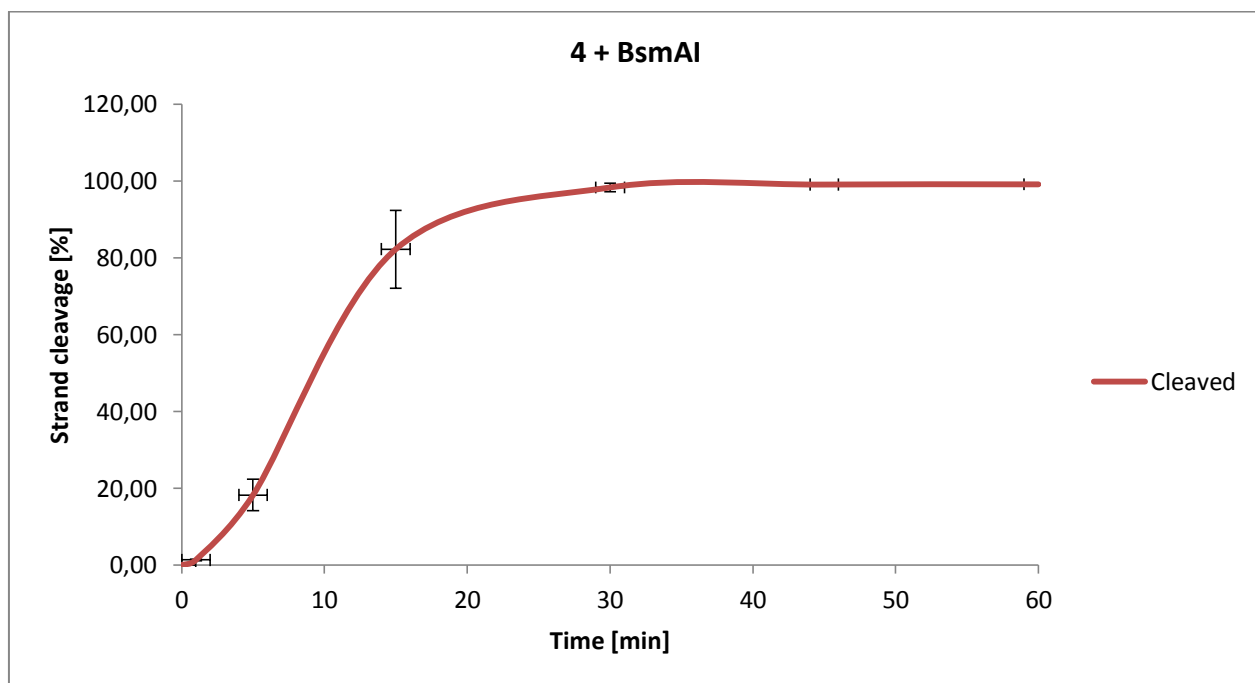


Figure S14. Cleavage of dsDNA (duplex B) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 4) is shown.

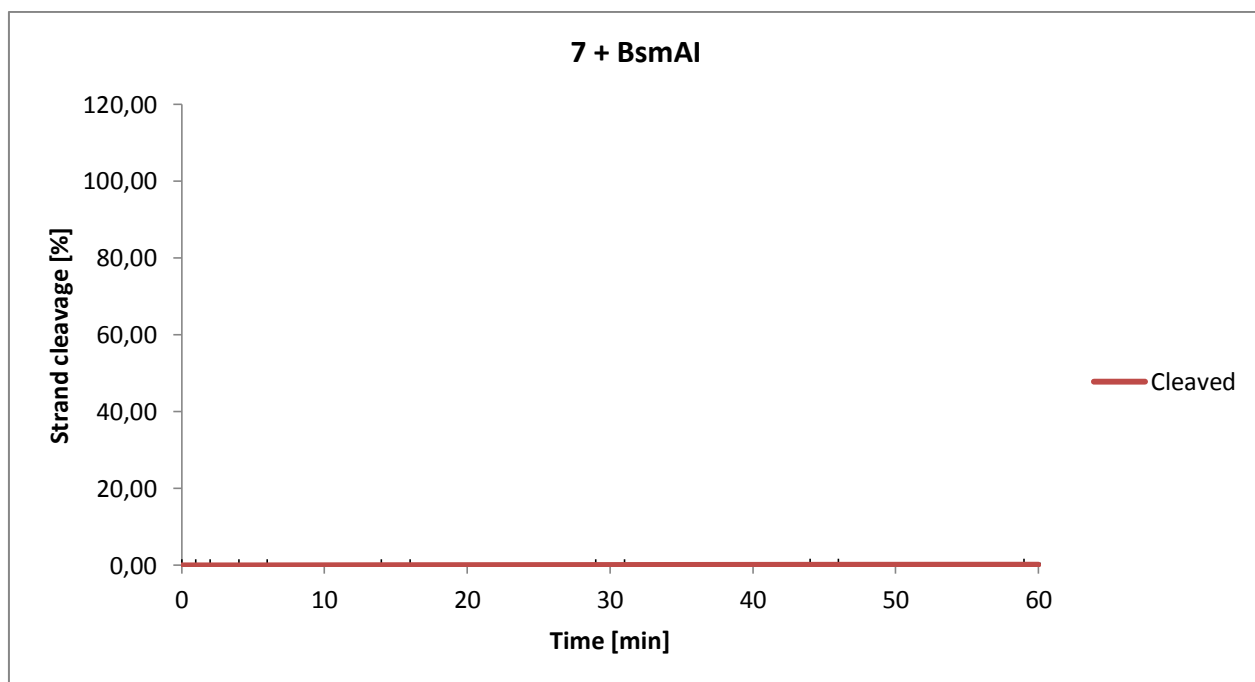


Figure S15. Cleavage of dsDNA (duplex D) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 7) is shown.

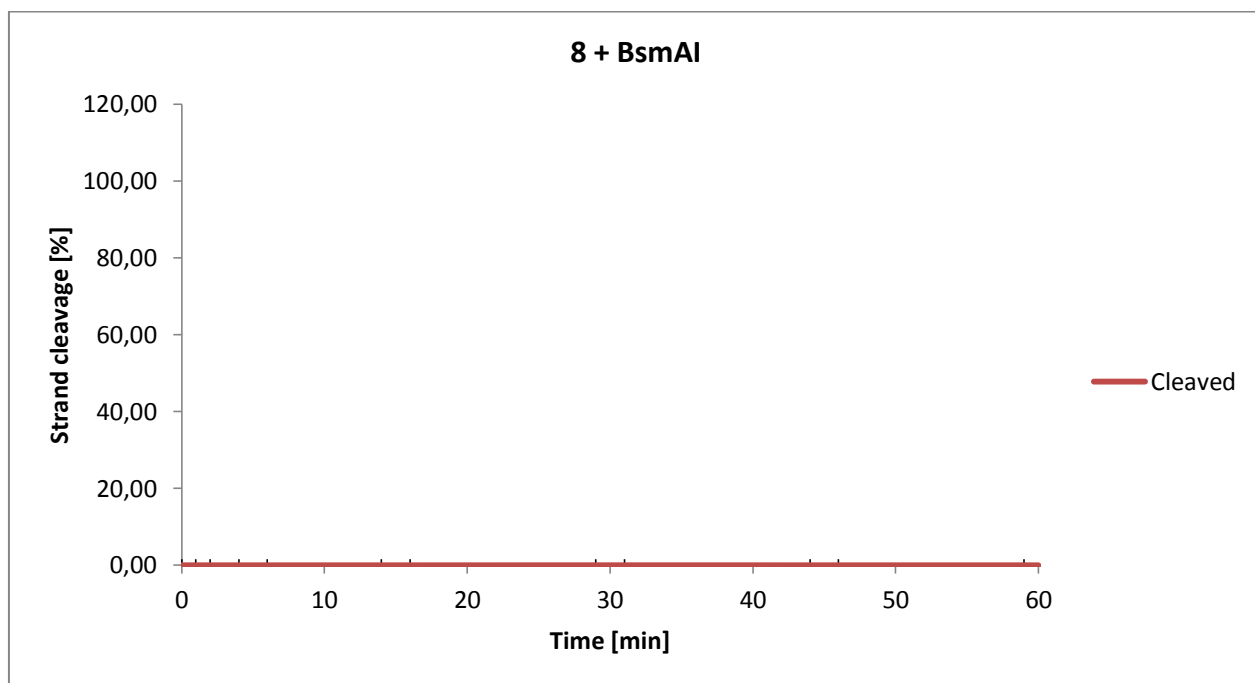


Figure S16. Cleavage of dsDNA (duplex D) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 8) is shown.

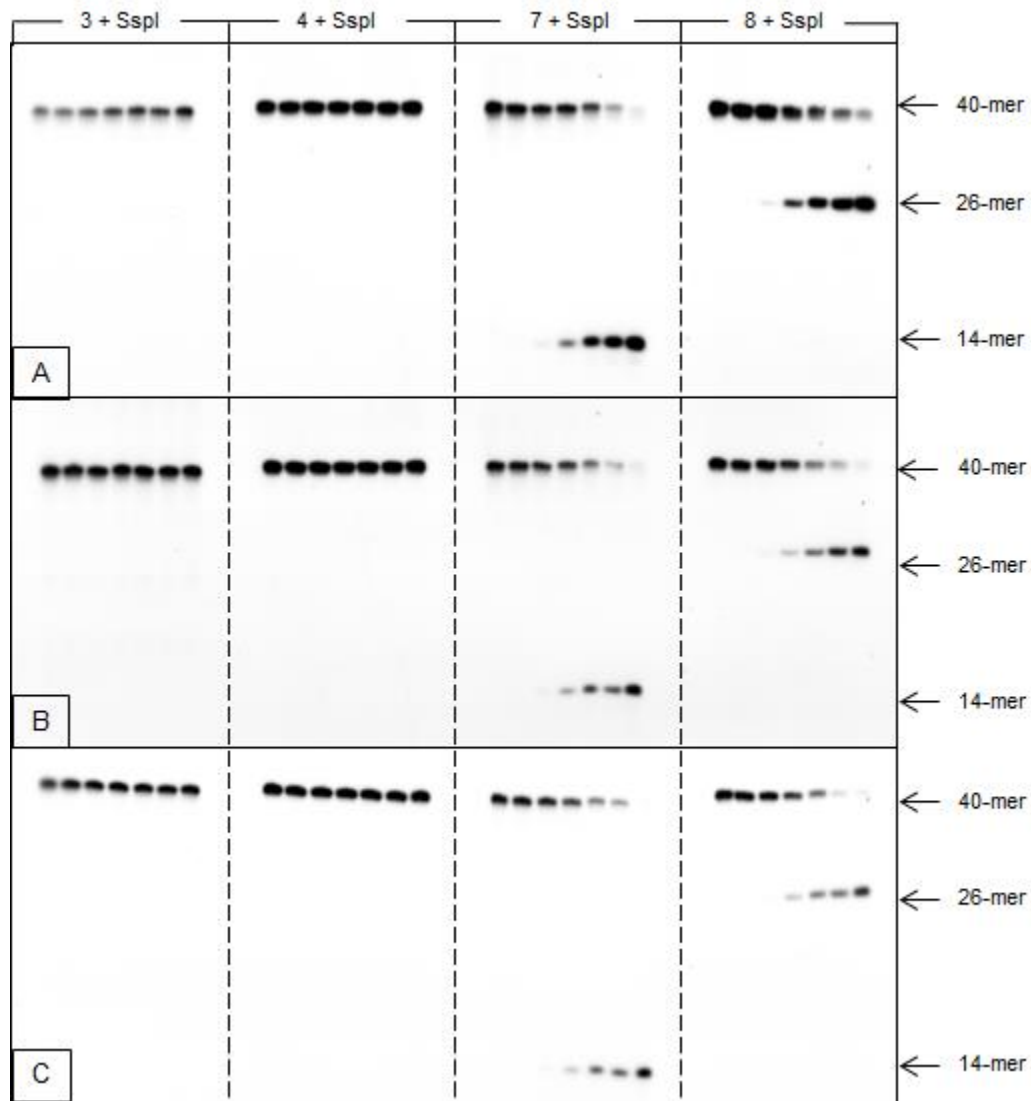


Figure S17. The autoradiograms of denaturing PAGE presenting cleavage of oligonucleotides by 1.5 U SspI – (A), (B) and (C) represent three repeats of assays. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30, 45 and 60 min starting from the left.

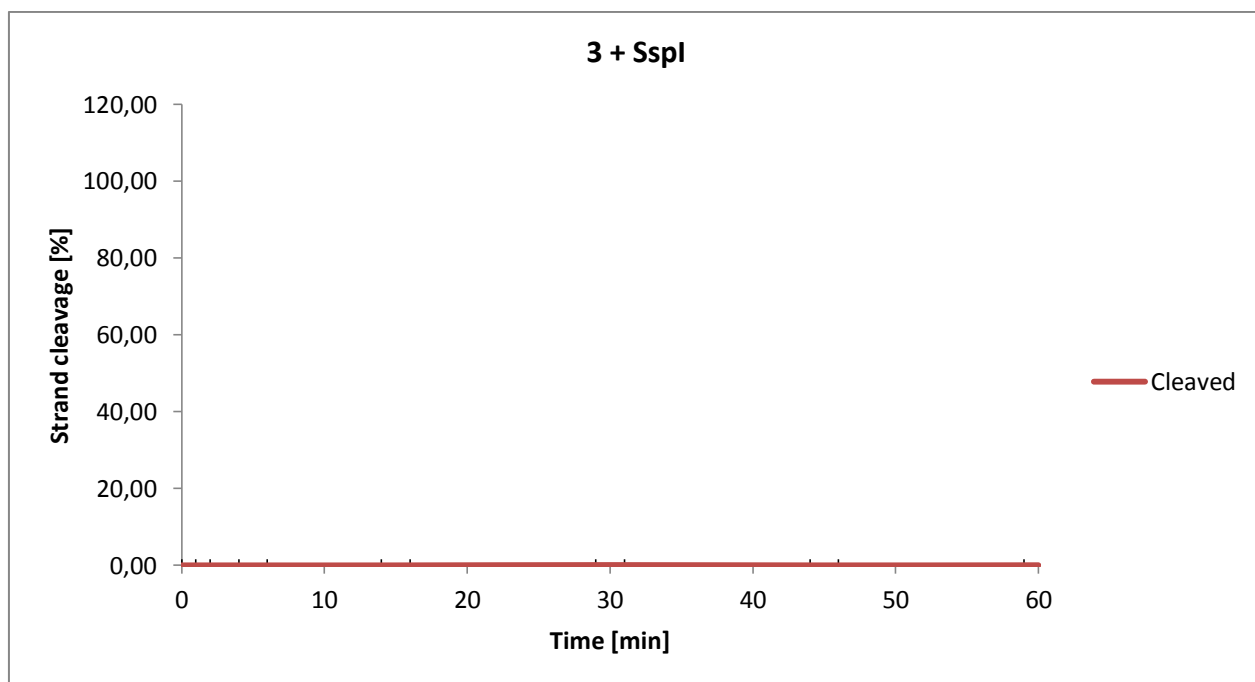


Figure S18. Cleavage of dsDNA (duplex B) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 3) is shown.

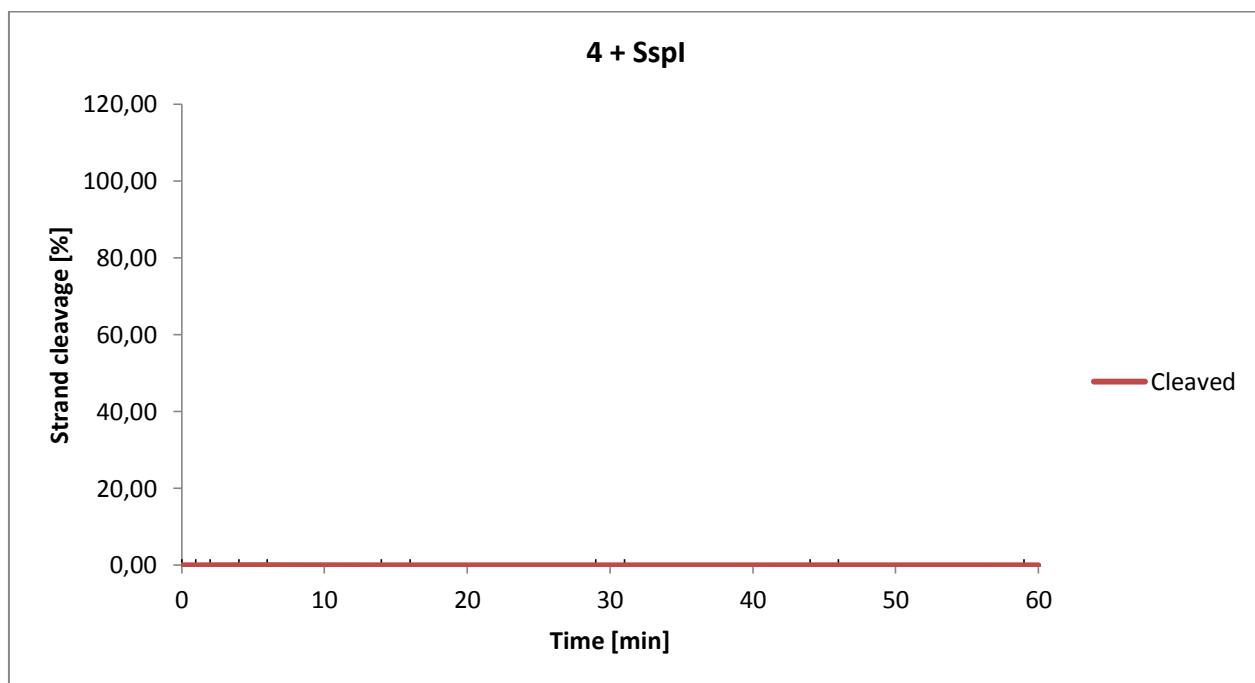


Figure S19. Cleavage of dsDNA (duplex B) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 4) is shown.

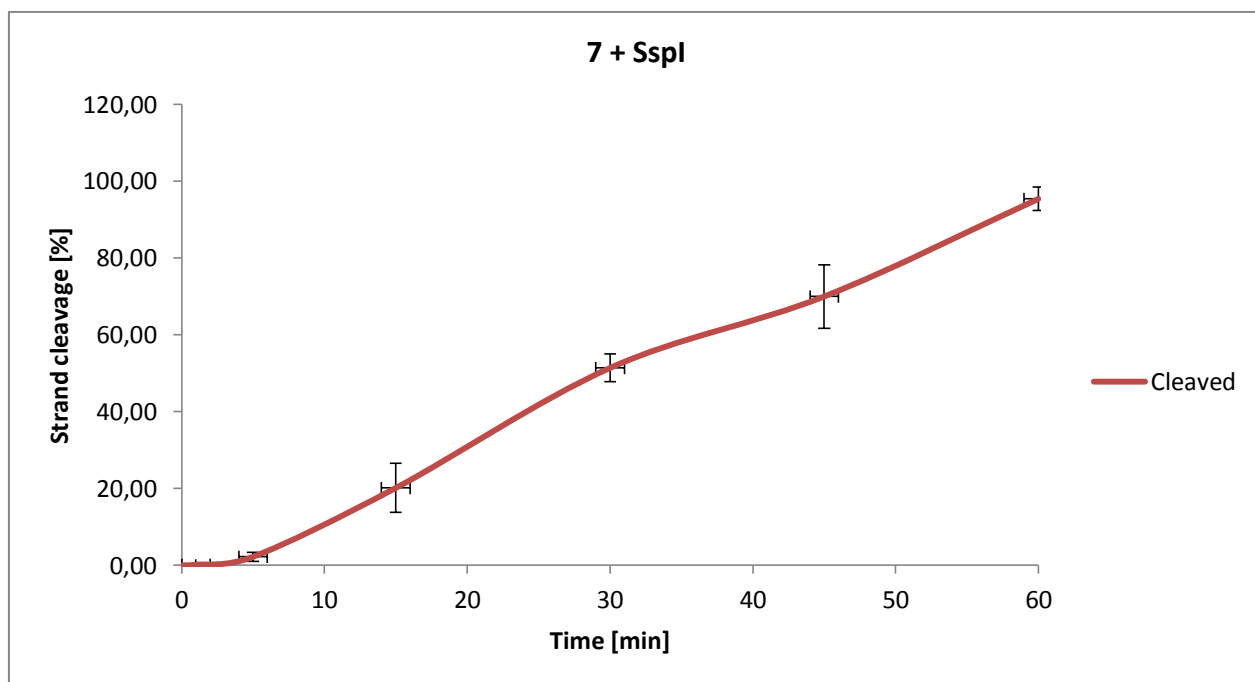


Figure S20. Cleavage of dsDNA (duplex D) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 7) is shown.

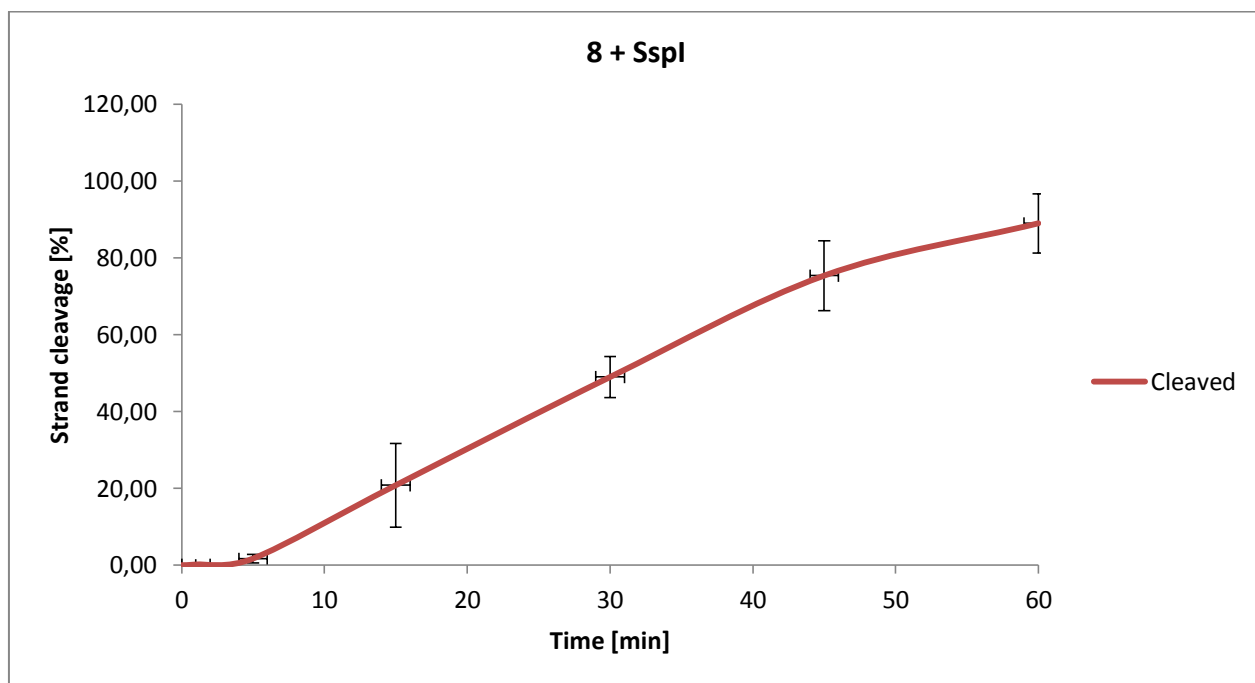


Figure S21. Cleavage of dsDNA (duplex D) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 8) is shown.

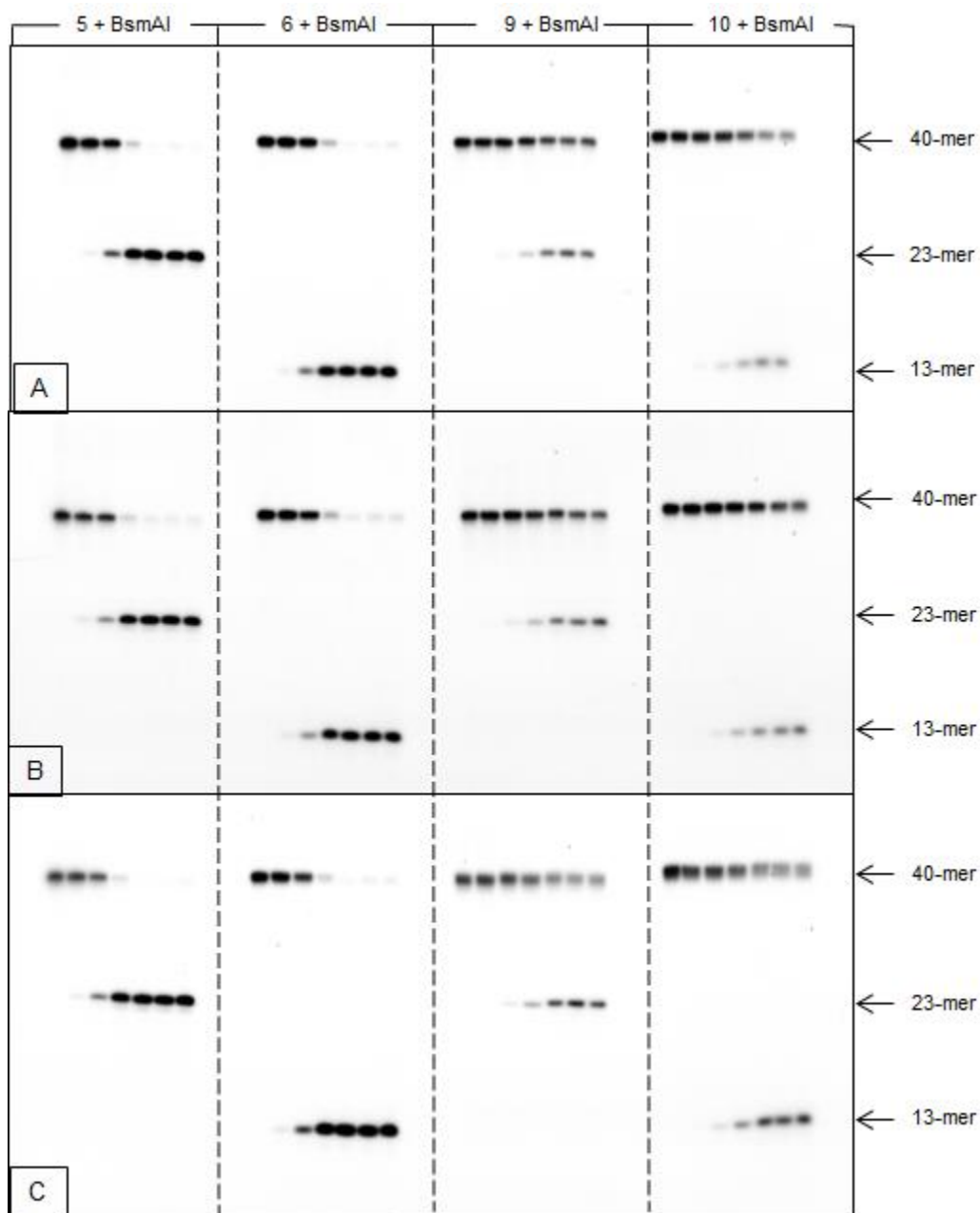


Figure S22. The autoradiograms of denaturing PAGE presenting cleavage of oligonucleotides by 0.5 U BsmAI – (A), (B) and (C) represent three repeats of assays. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30, 45 and 60 min starting from the left.

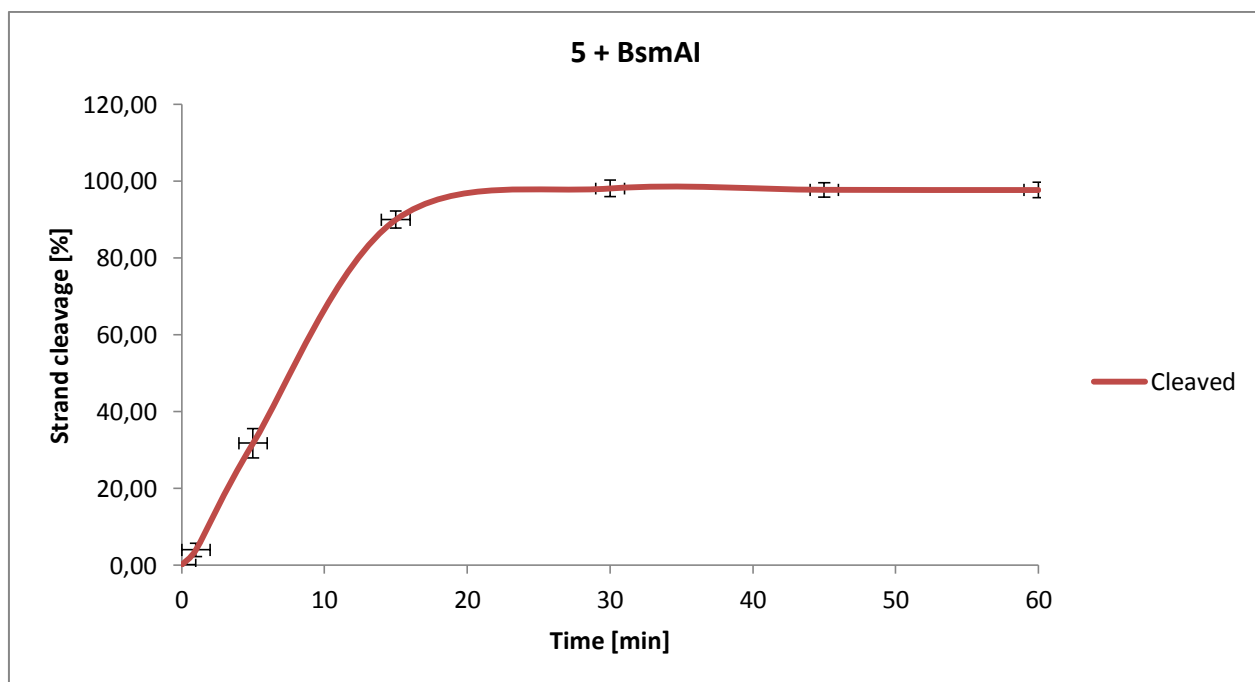


Figure S23. Cleavage of dsDNA (duplex C) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 5) is shown.

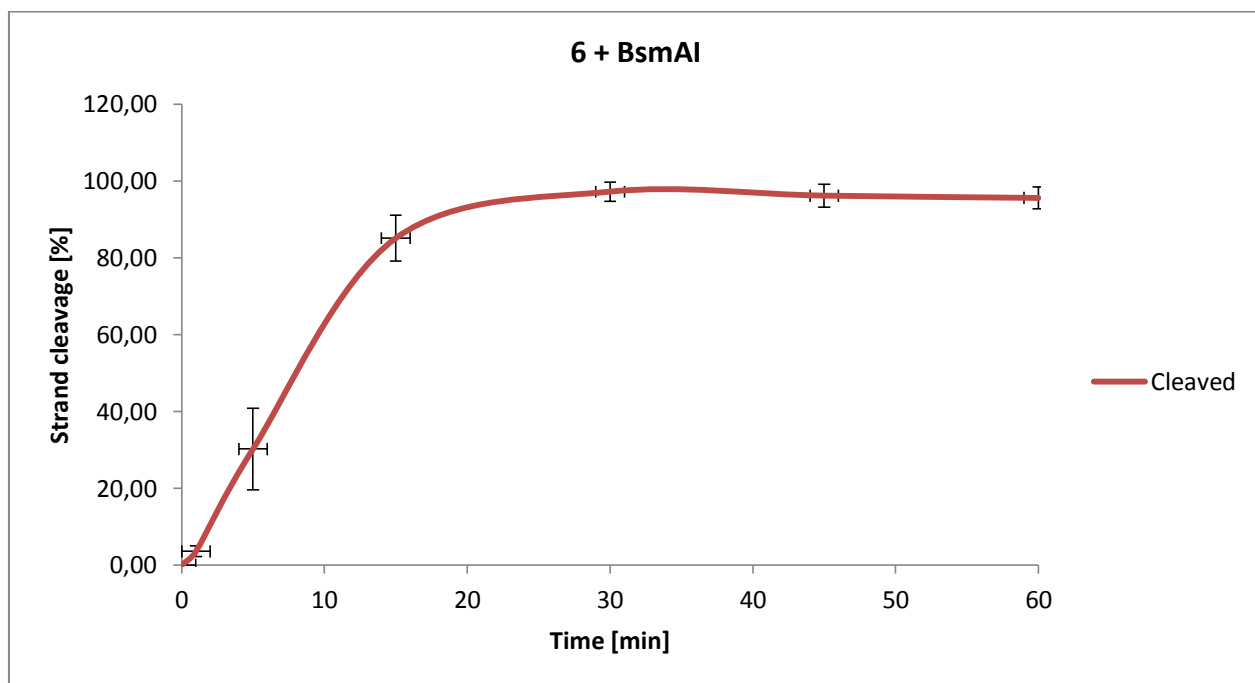


Figure S24. Cleavage of dsDNA (duplex C) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 6) is shown.

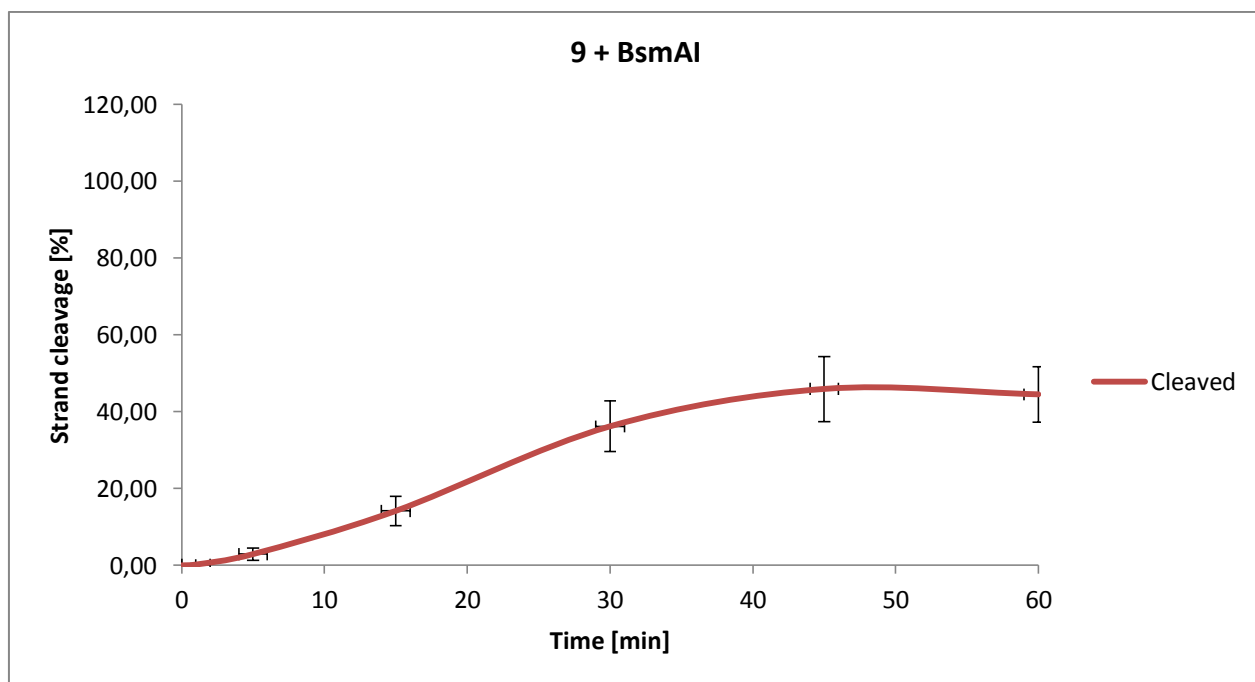


Figure S25. Cleavage of dsDNA (duplex E) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 9) is shown.

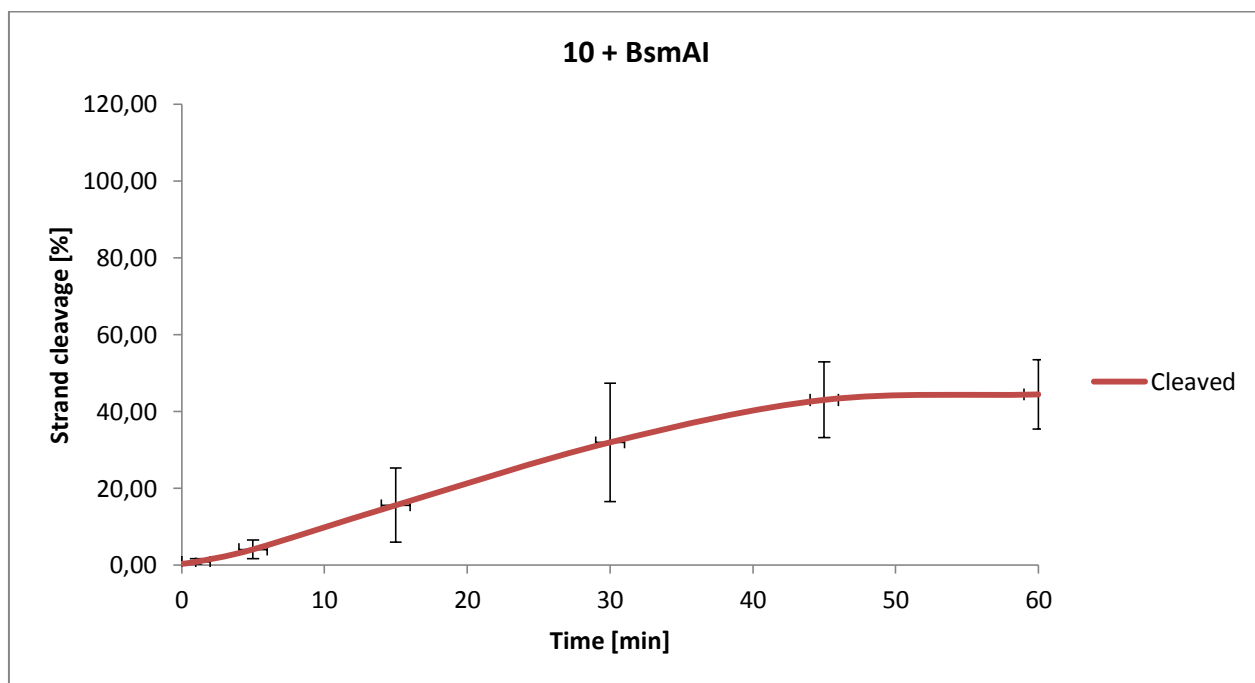


Figure S26. Cleavage of dsDNA (duplex E) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 10) is shown.

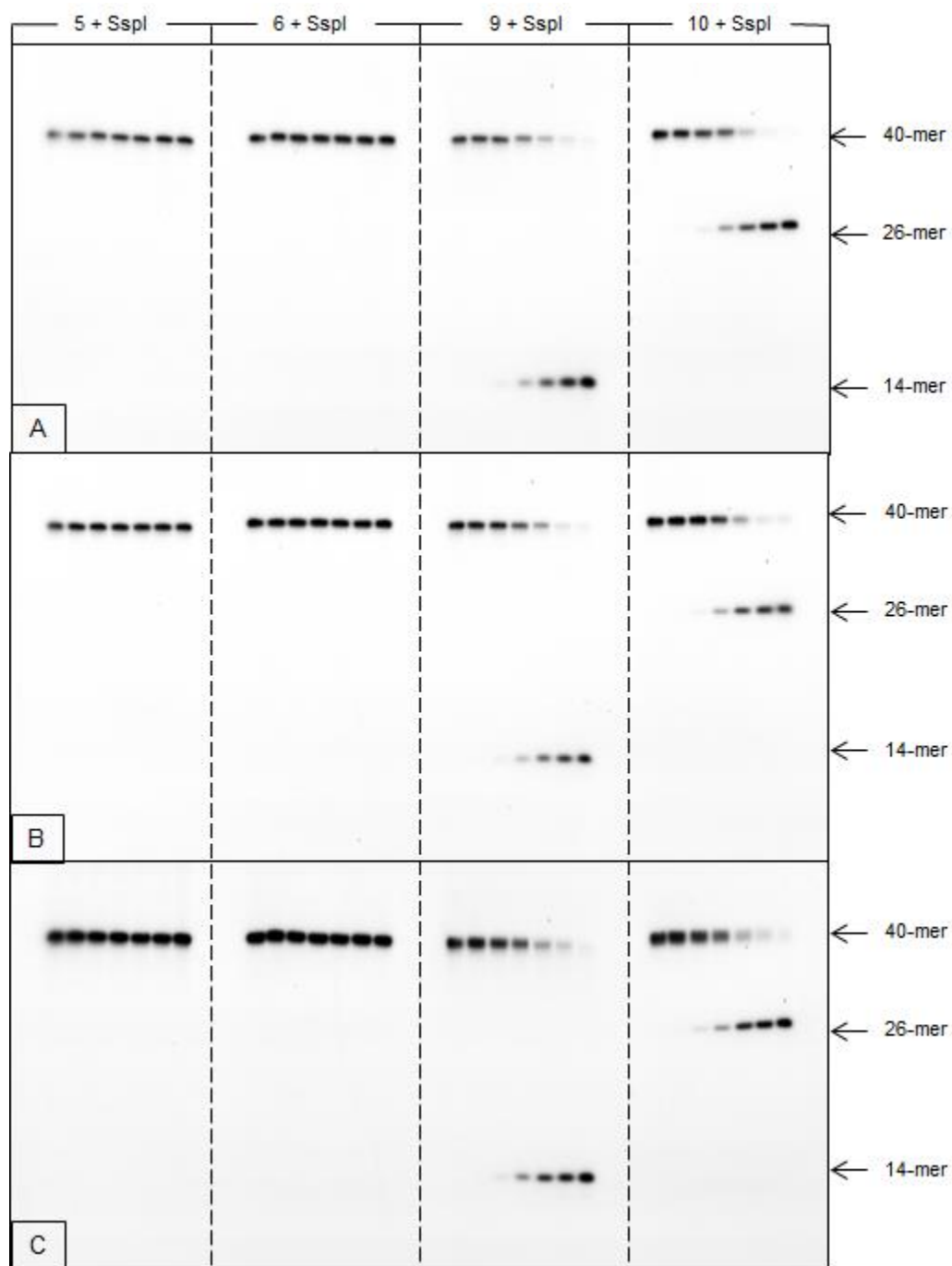


Figure S27. The autoradiograms of denaturing PAGE presenting cleavage of oligonucleotides by 1.5 U SspI – (A), (B) and (C) represent three repeats of assays. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30, 45 and 60 min starting from the left.

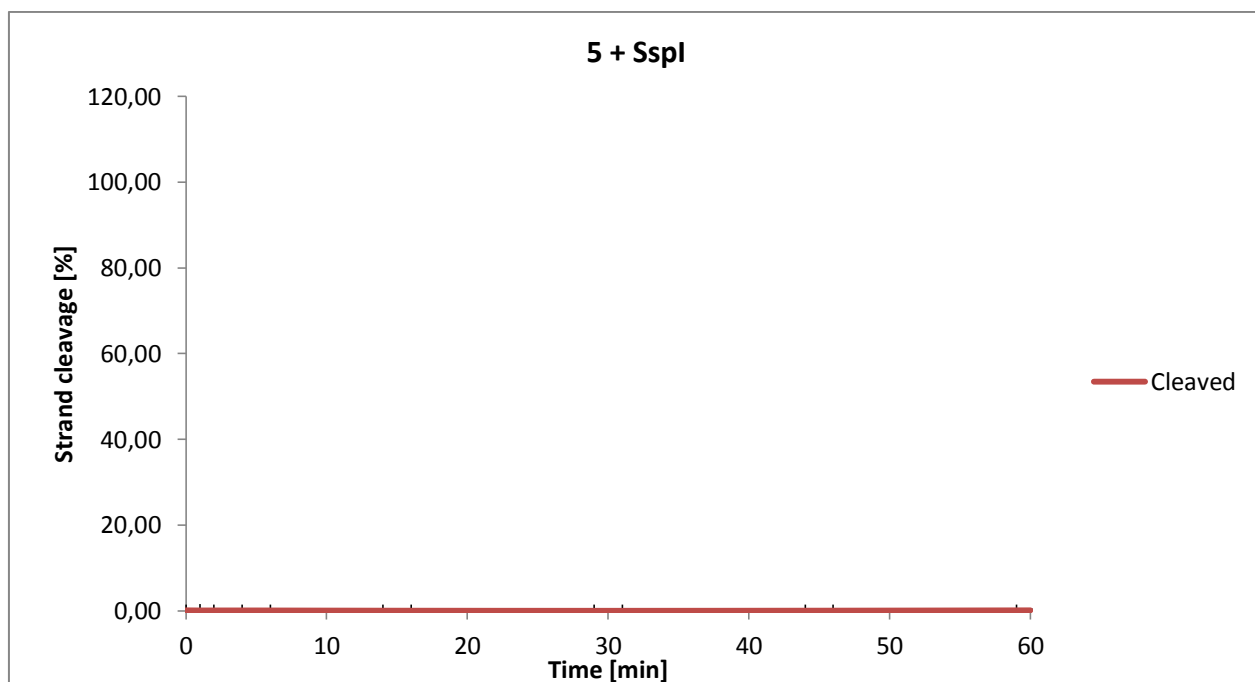


Figure S28. Cleavage of dsDNA (duplex C) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 5) is shown.

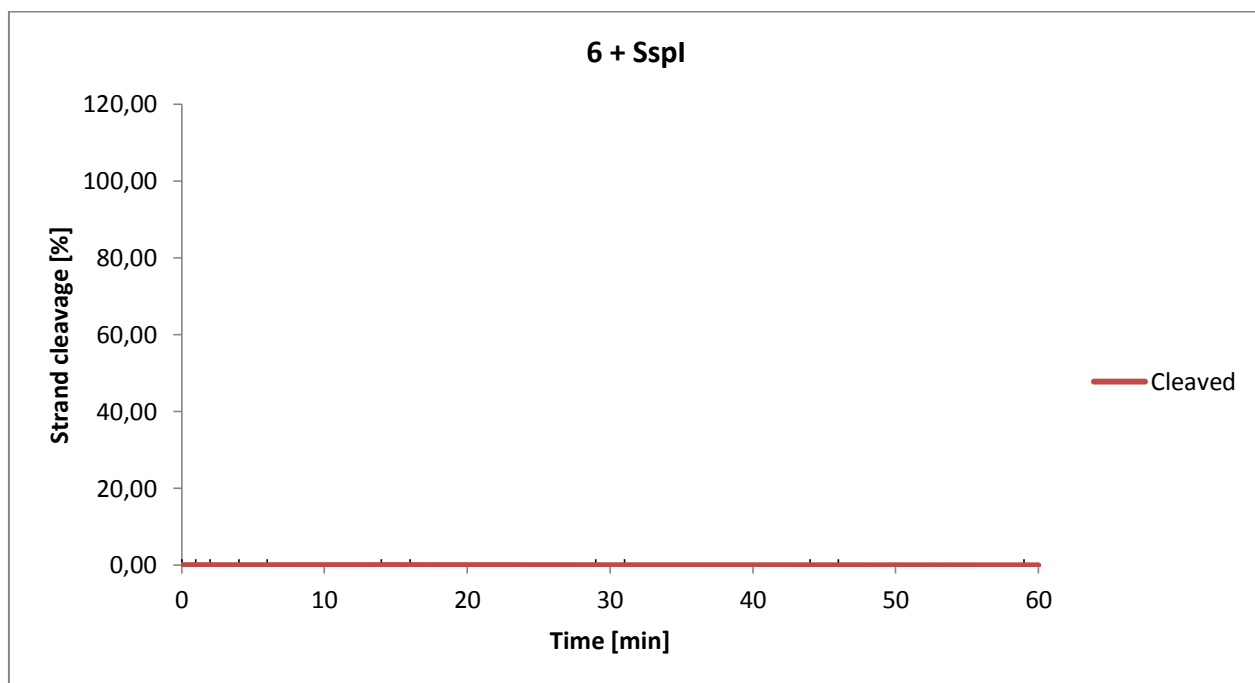


Figure S29. Cleavage of dsDNA (duplex C) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 6) is shown.

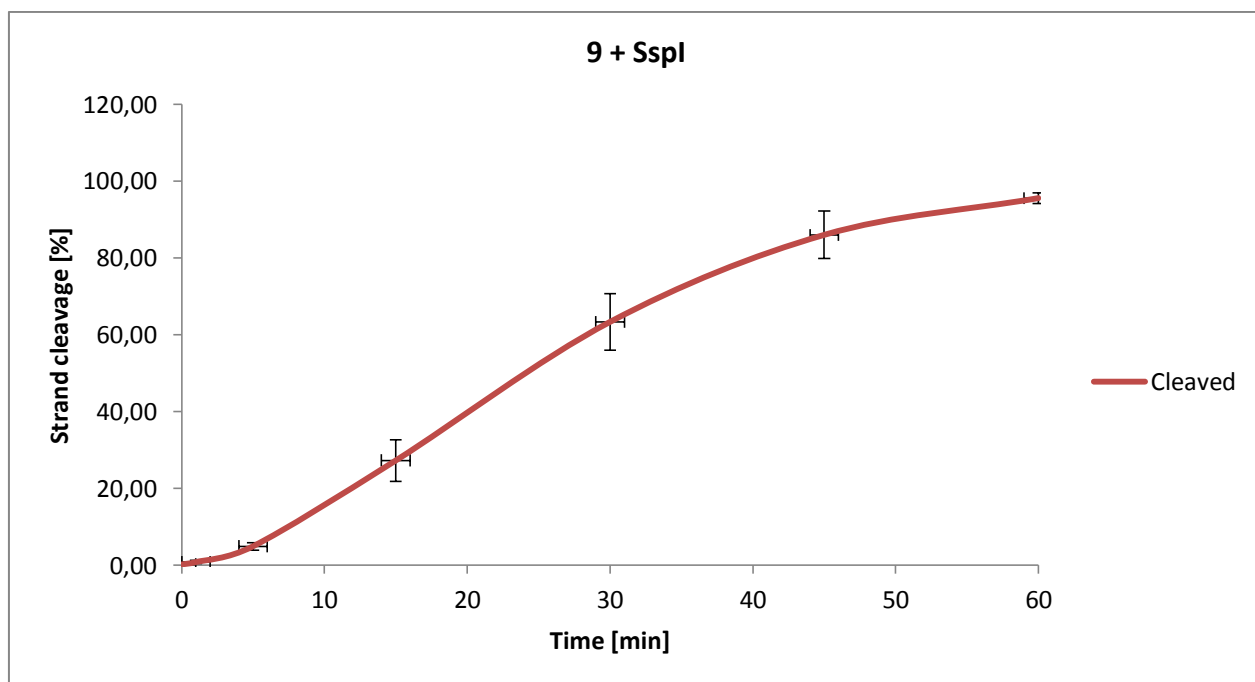


Figure S30. Cleavage of dsDNA (duplex E) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 9) is shown.

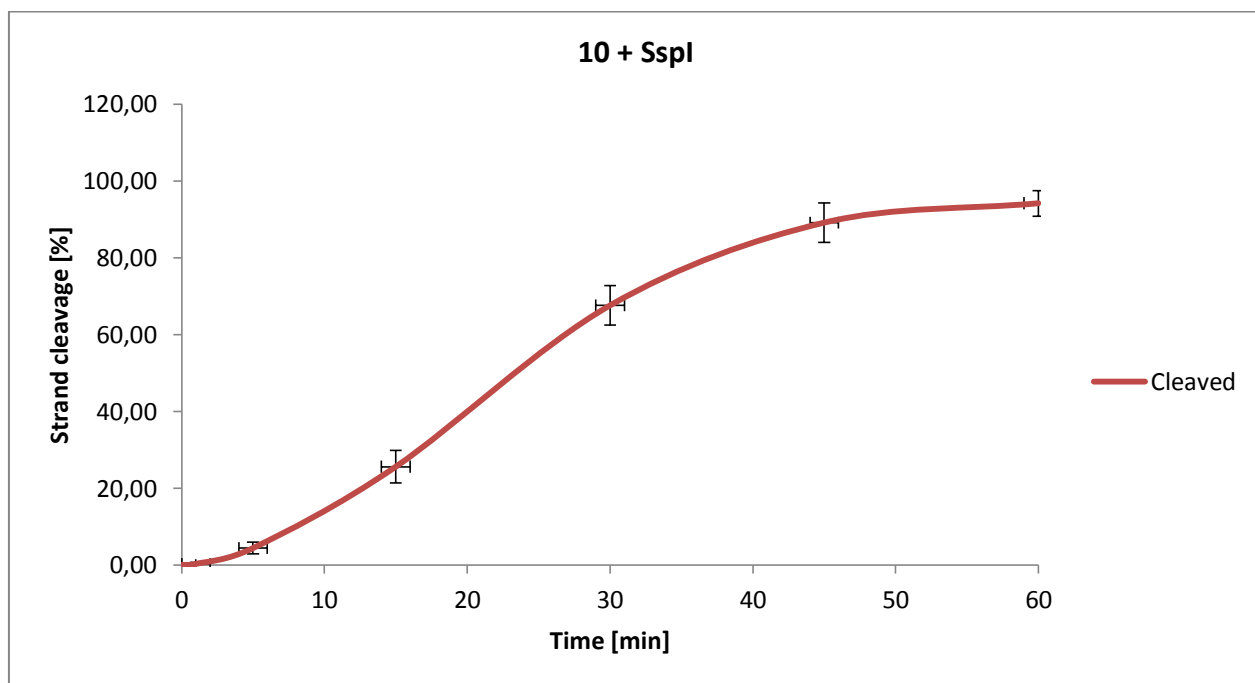


Figure S31. Cleavage of dsDNA (duplex E) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 10) is shown.

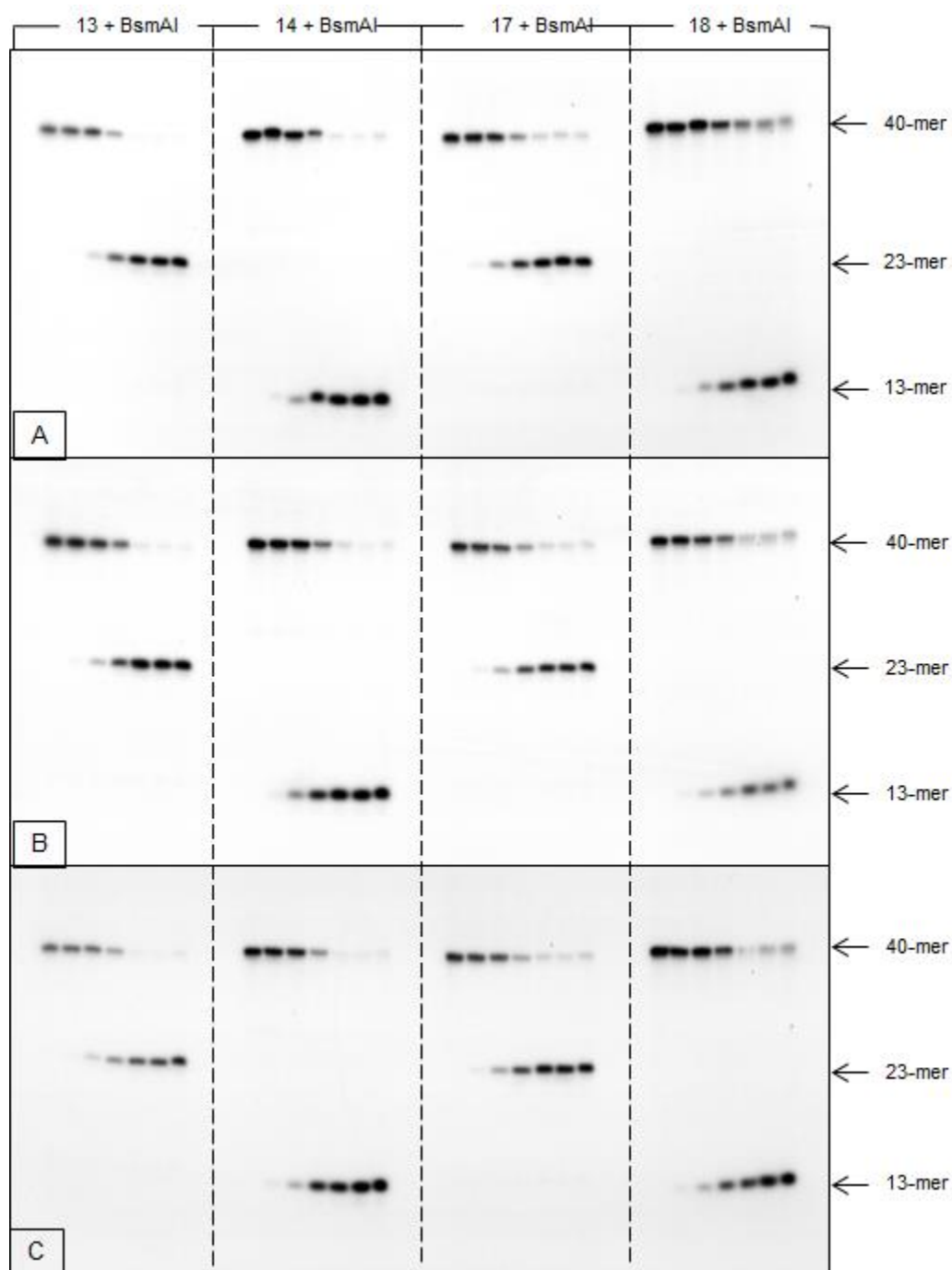


Figure S32. The autoradiograms of denaturing PAGE presenting cleavage of oligonucleotides by 0.5 U BsmAI – (A), (B) and (C) represent three repeats of assays. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30, 45 and 60 min starting from the left.

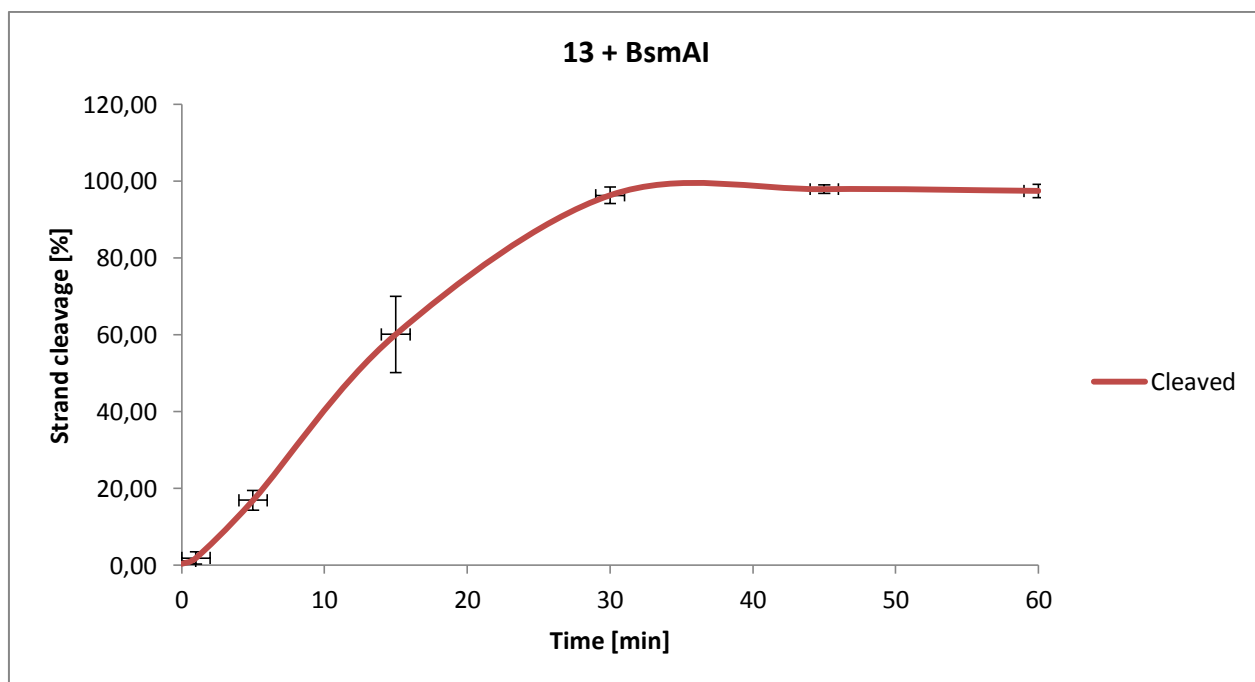


Figure S33. Cleavage of dsDNA (duplex G) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 13) is shown.

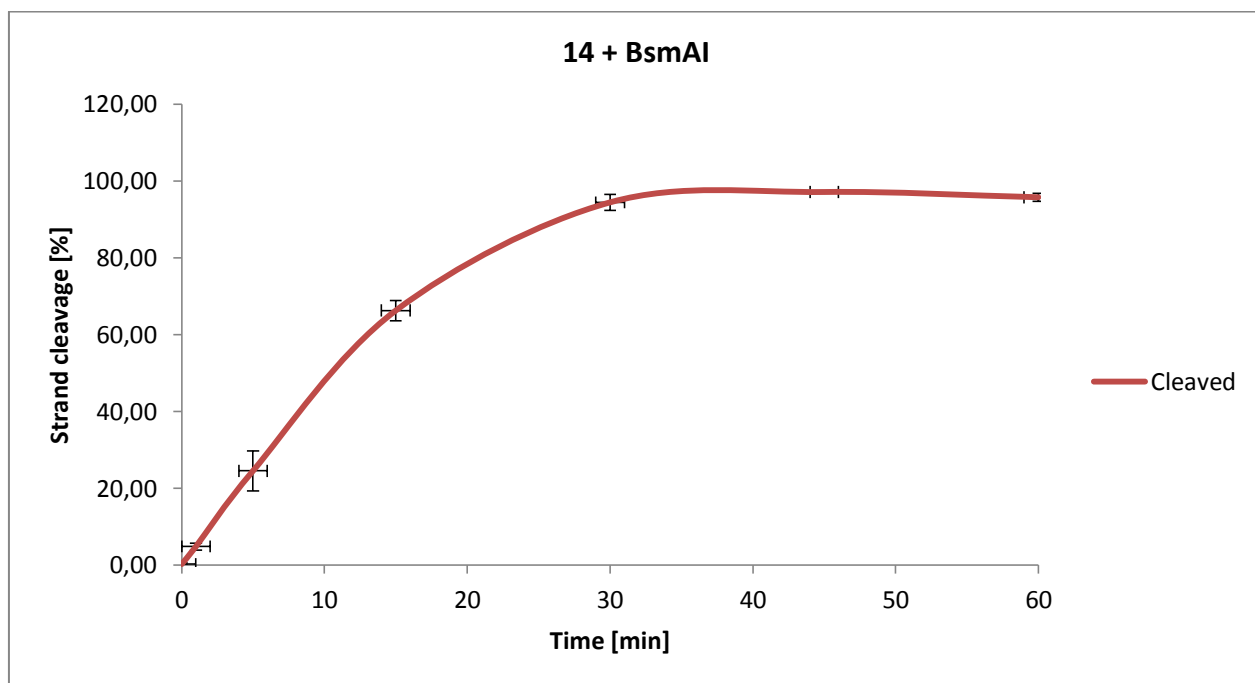


Figure S34. Cleavage of dsDNA (duplex G) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 14) is shown.

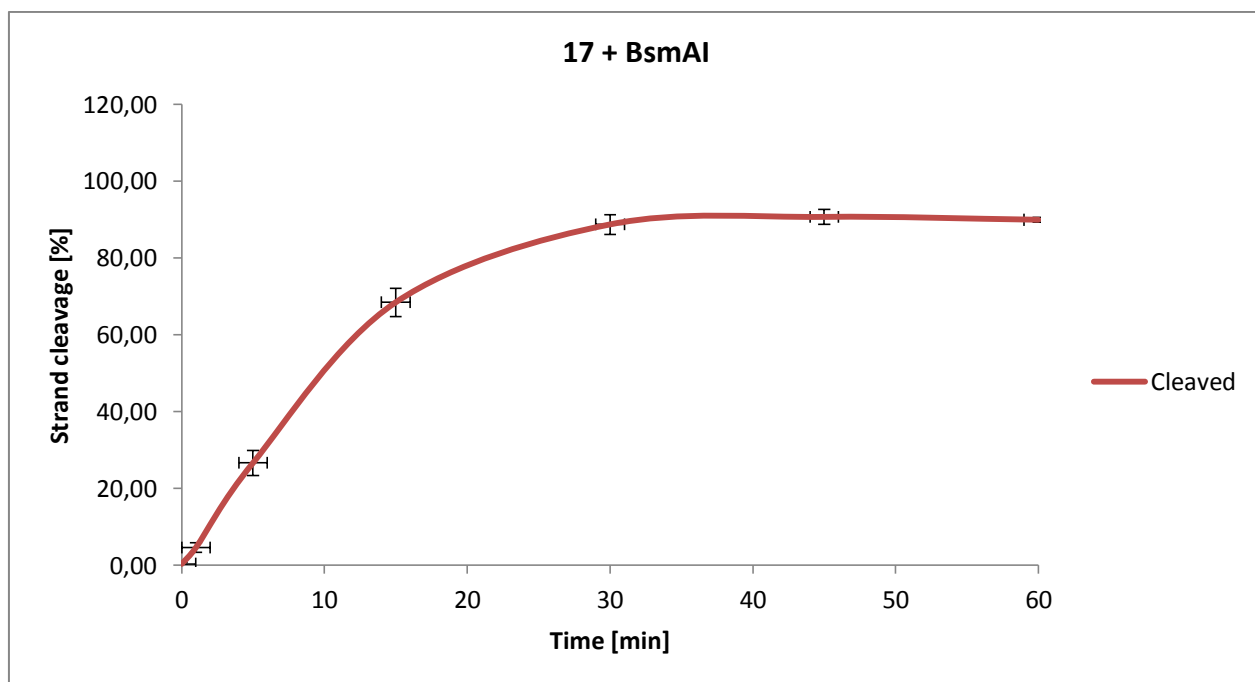


Figure S35. Cleavage of dsDNA (duplex I) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 17) is shown.

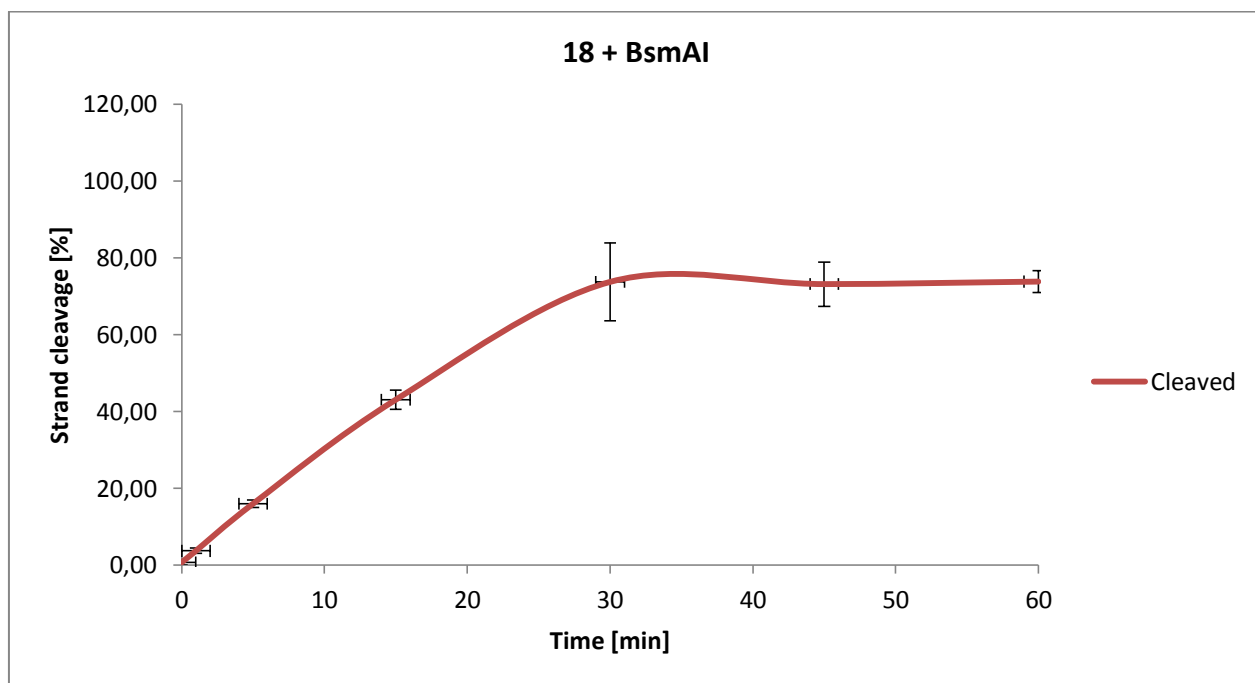


Figure S36. Cleavage of dsDNA (duplex I) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 18) is shown.

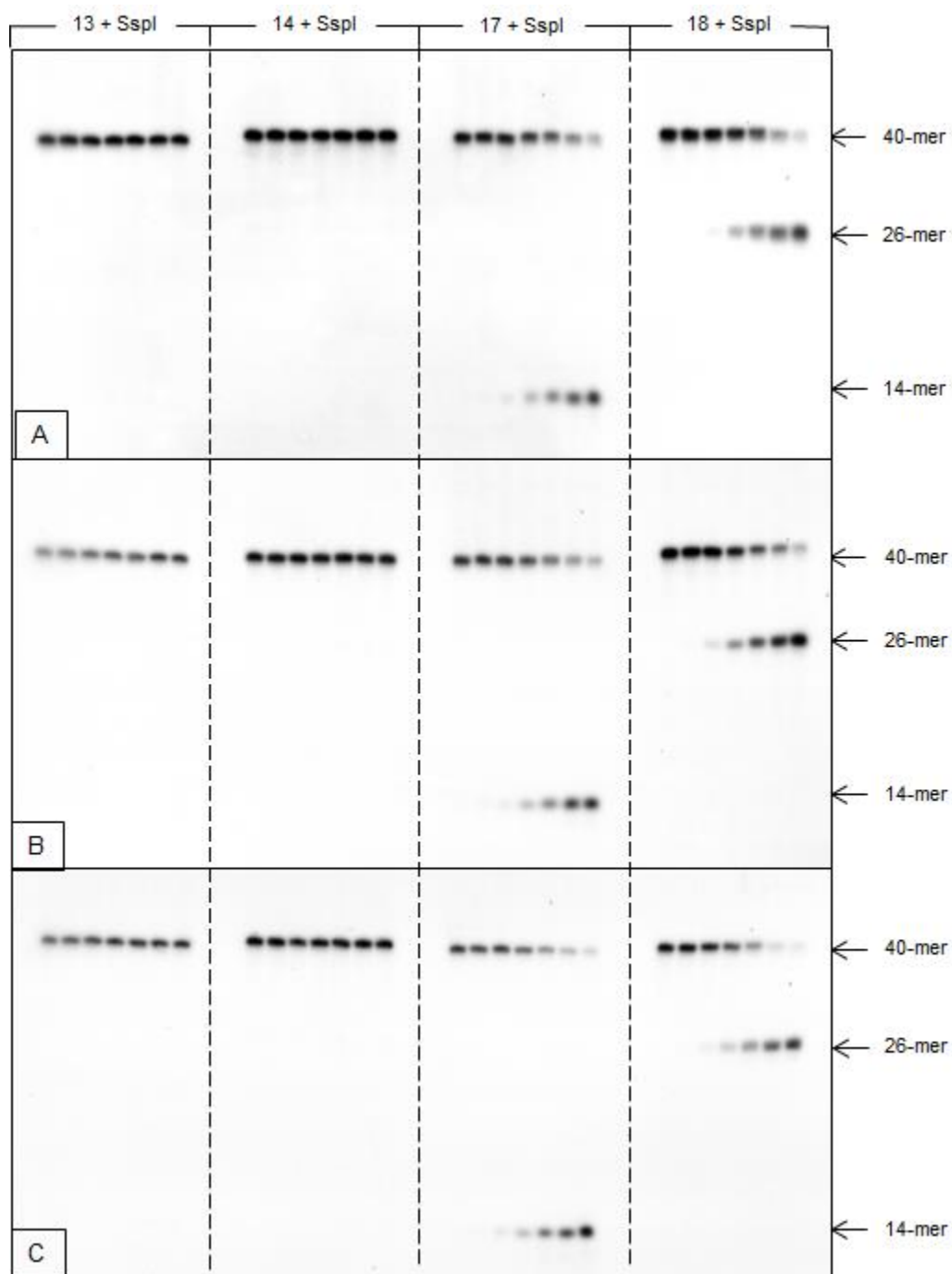


Figure S37. The autoradiograms of denaturing PAGE presenting cleavage of oligonucleotides by 1.5 U SspI – (A), (B) and (C) represent three repeats of assays. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30, 45 and 60 min starting from the left.

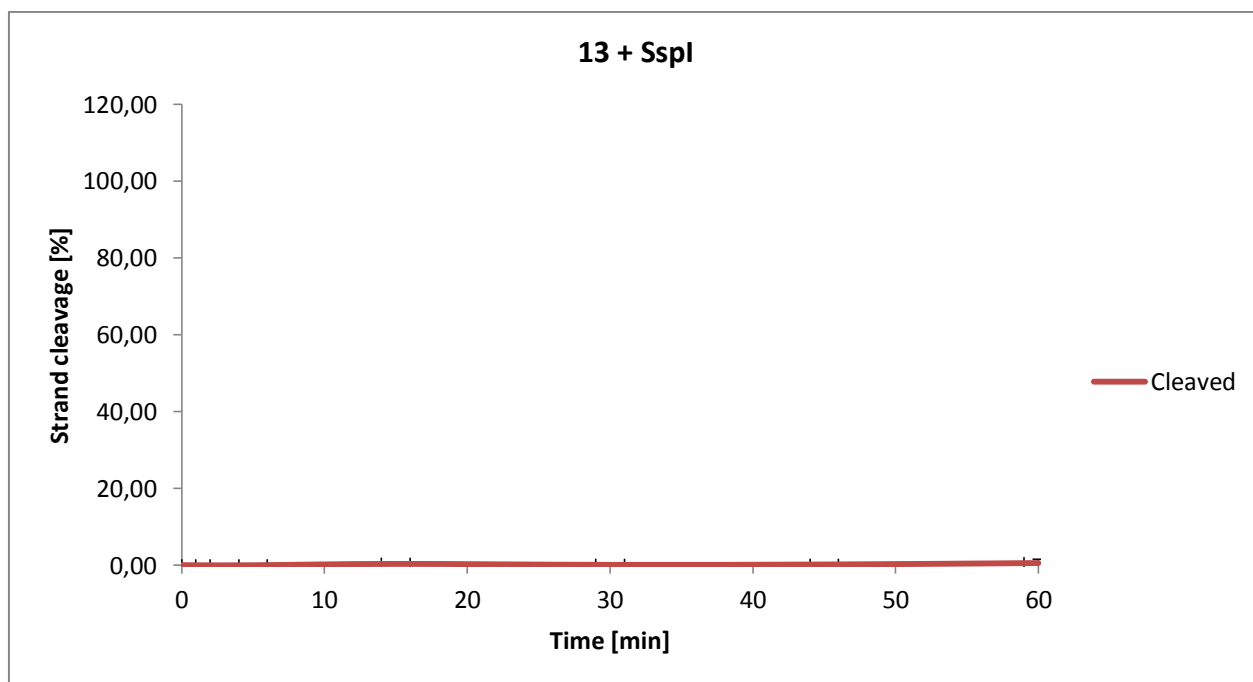


Figure S38. Cleavage of dsDNA (duplex G) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 13) is shown.

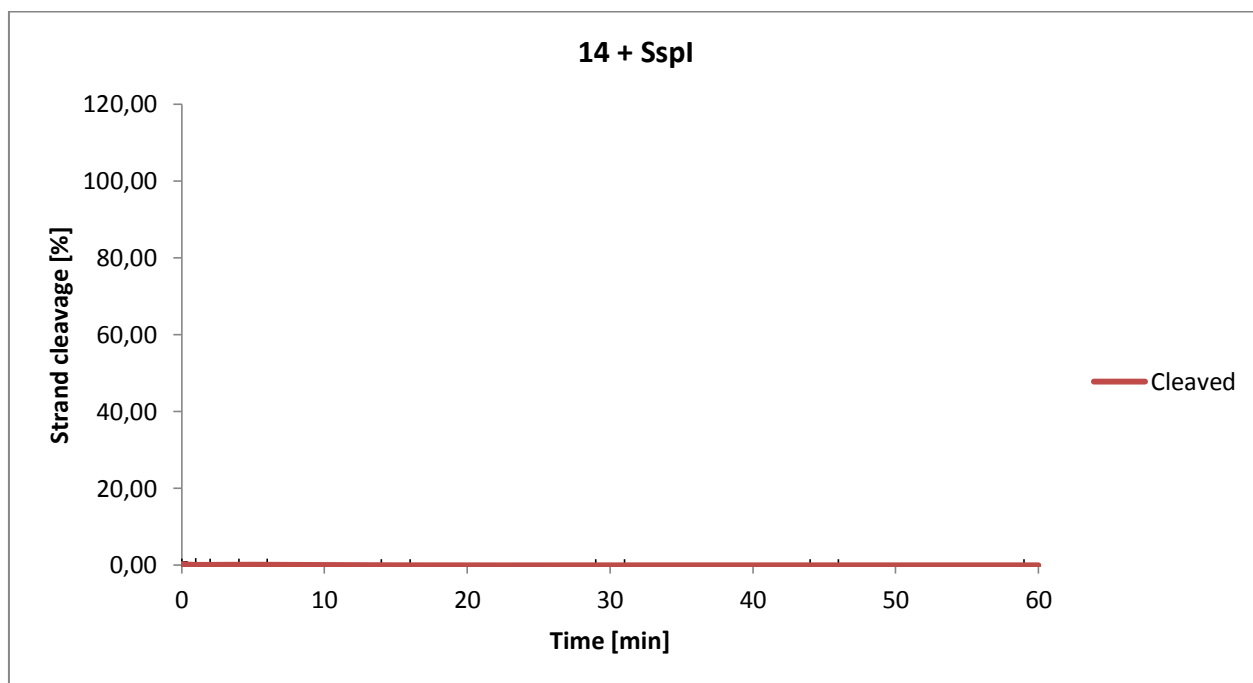


Figure S39. Cleavage of dsDNA (duplex G) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 14) is shown.

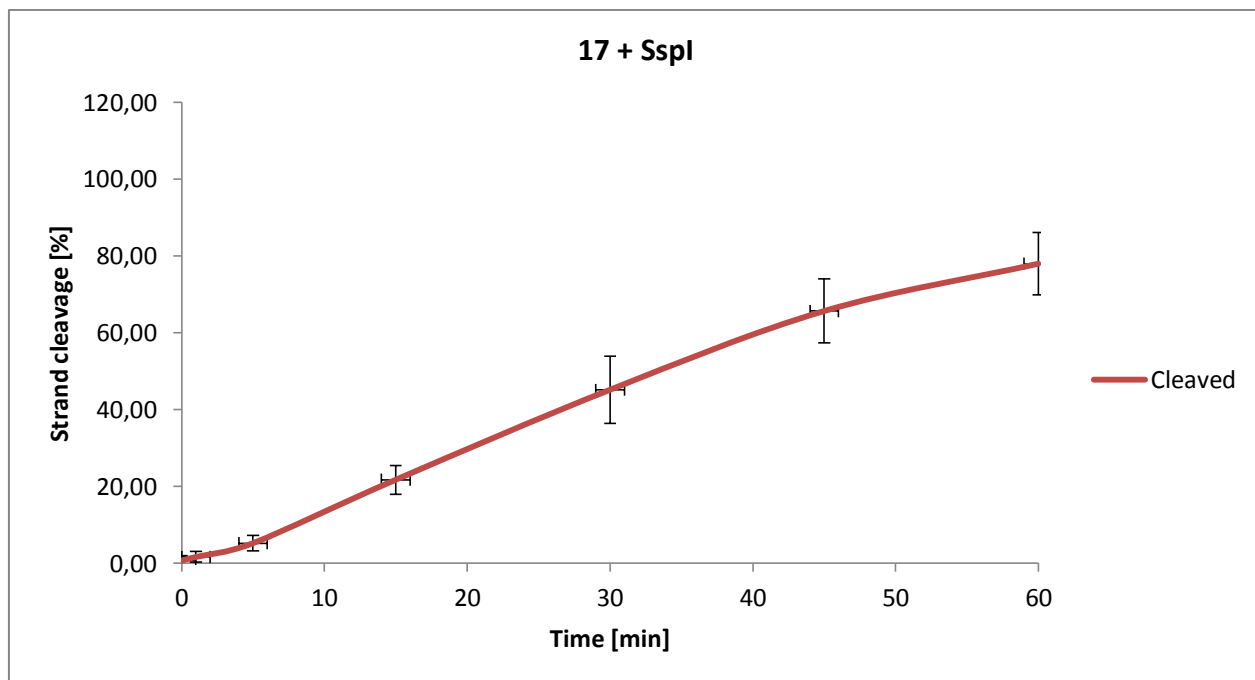


Figure S40. Cleavage of dsDNA (duplex I) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 17) is shown.

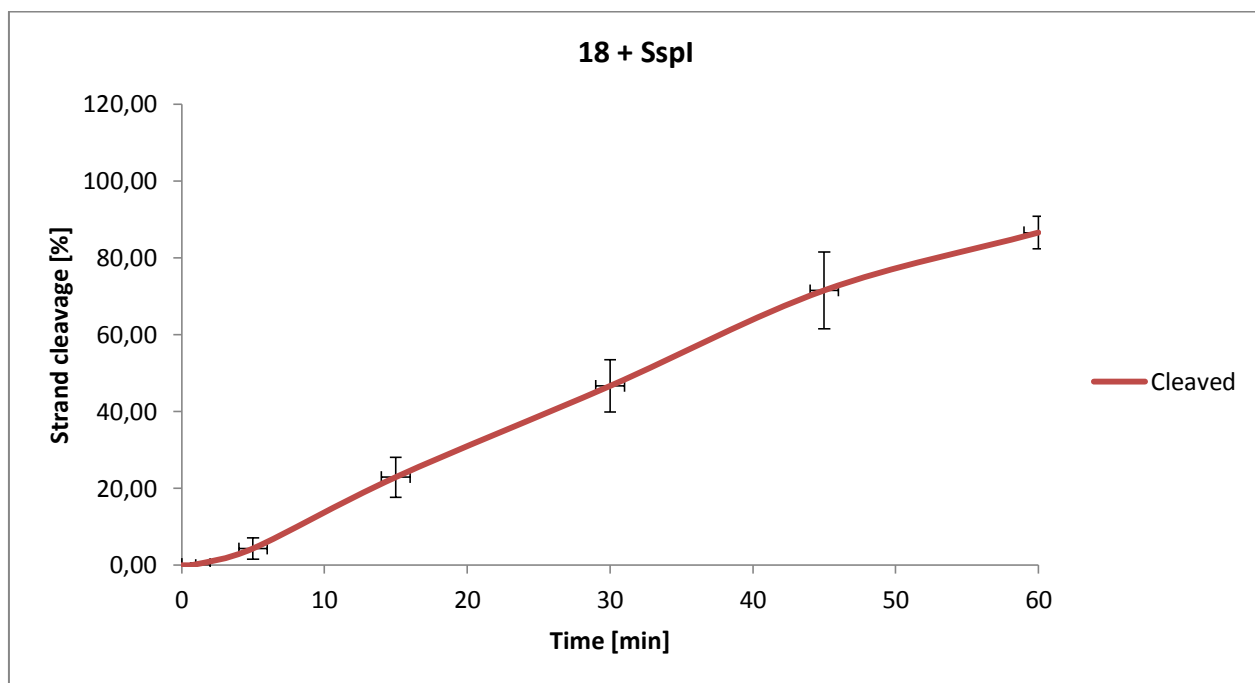


Figure S41. Cleavage of dsDNA (duplex I) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 18) is shown.

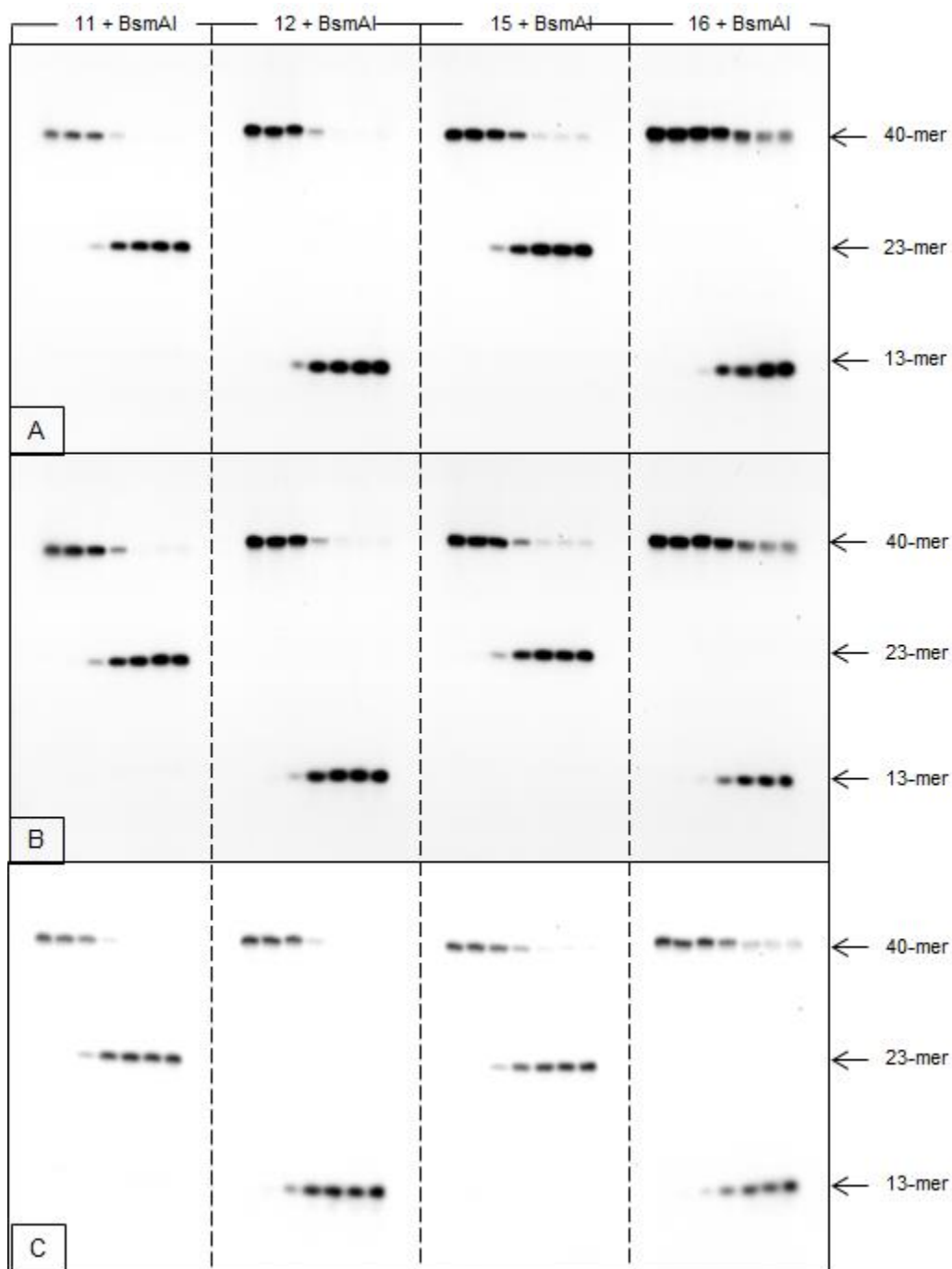


Figure S42. The autoradiograms of denaturing PAGE presenting cleavage of oligonucleotides by 0.5 U BsmAI – (A), (B) and (C) represent three repeats of assays. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30, 45 and 60 min starting from the left.

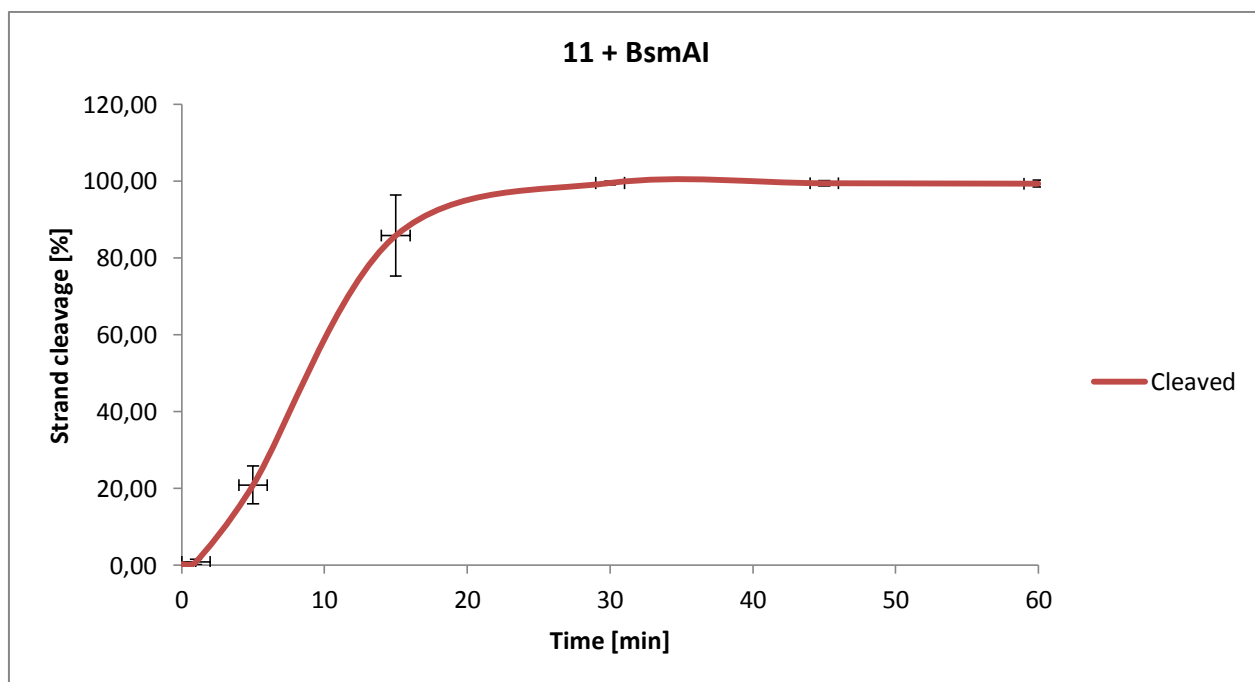


Figure S43. Cleavage of dsDNA (duplex F) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 11) is shown.

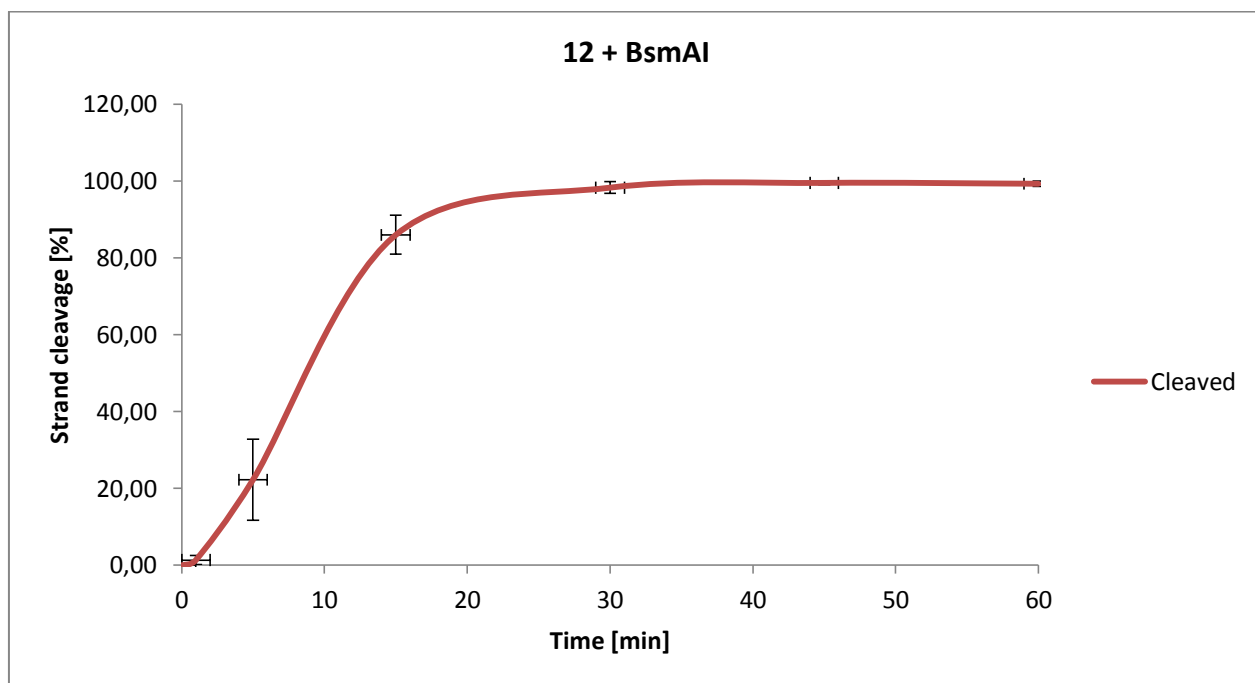


Figure S44. Cleavage of dsDNA (duplex F) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 12) is shown.

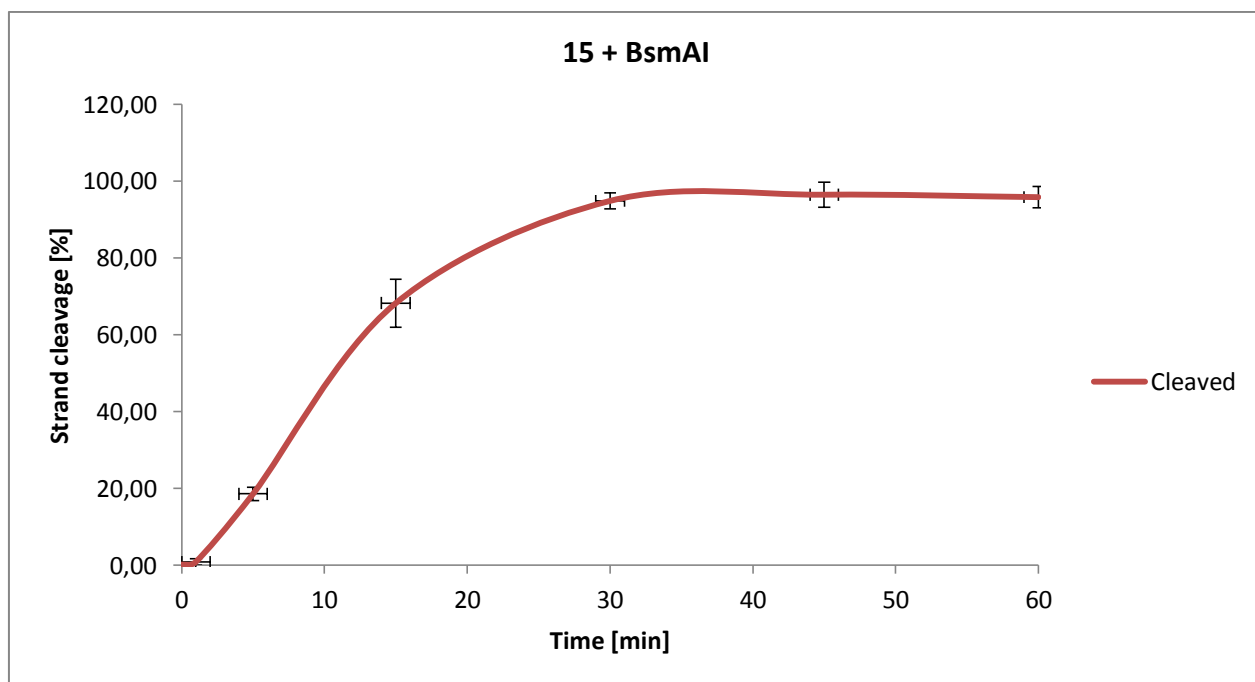


Figure S45. Cleavage of dsDNA (duplex H) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 15) is shown.

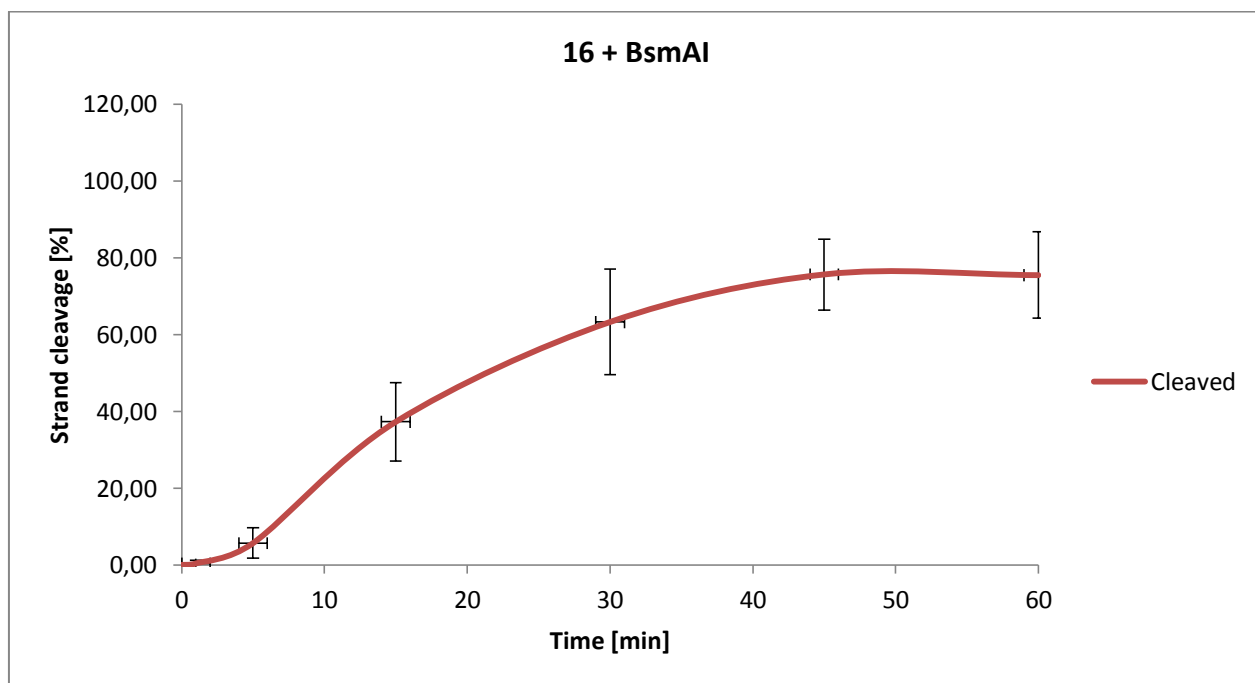


Figure S46. Cleavage of dsDNA (duplex H) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 16) is shown.

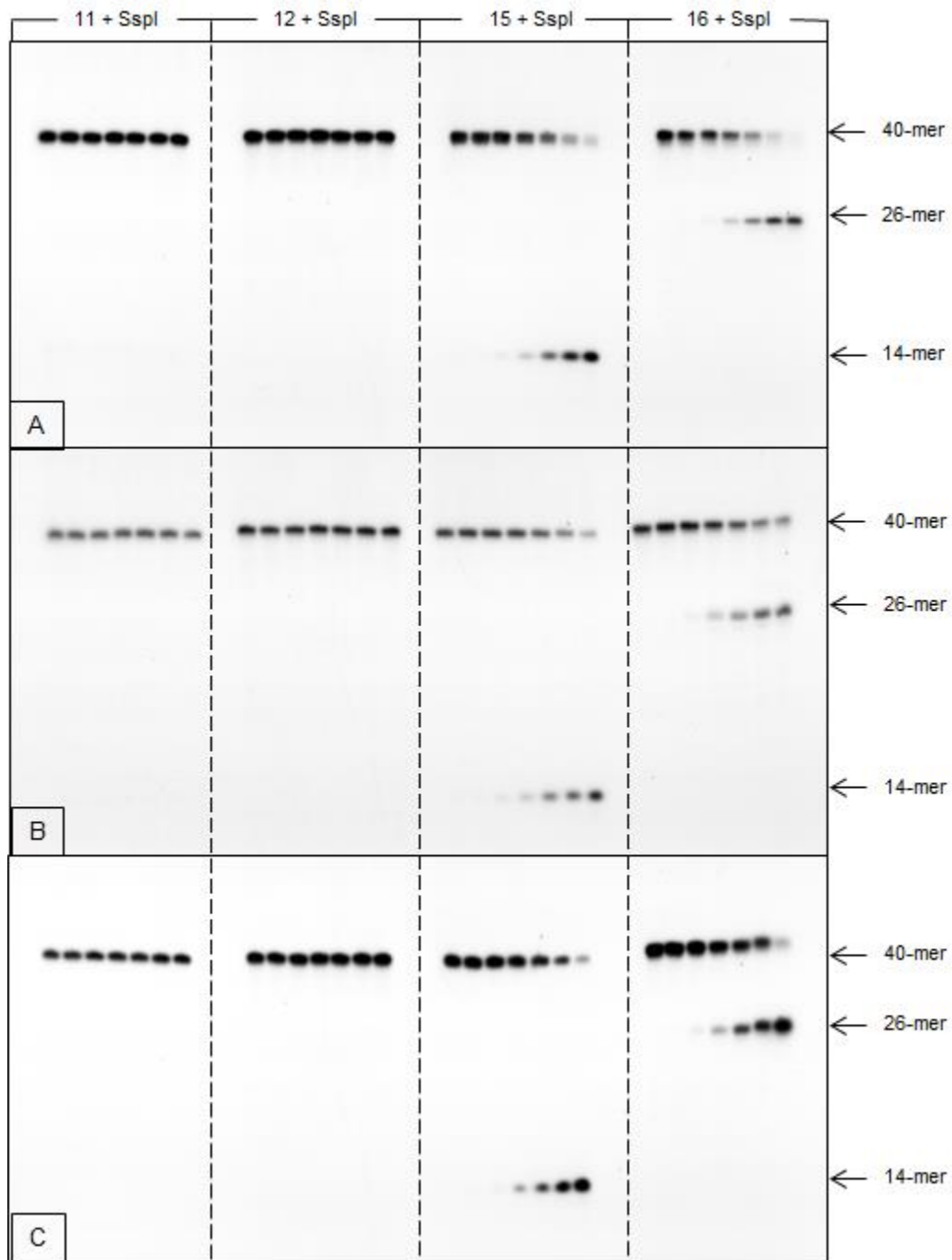


Figure S47. The autoradiograms of denaturing PAGE presenting cleavage of oligonucleotides by 1.5 U SspI – (A), (B) and (C) represent three repeats of assays. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30, 45 and 60 min starting from the left.

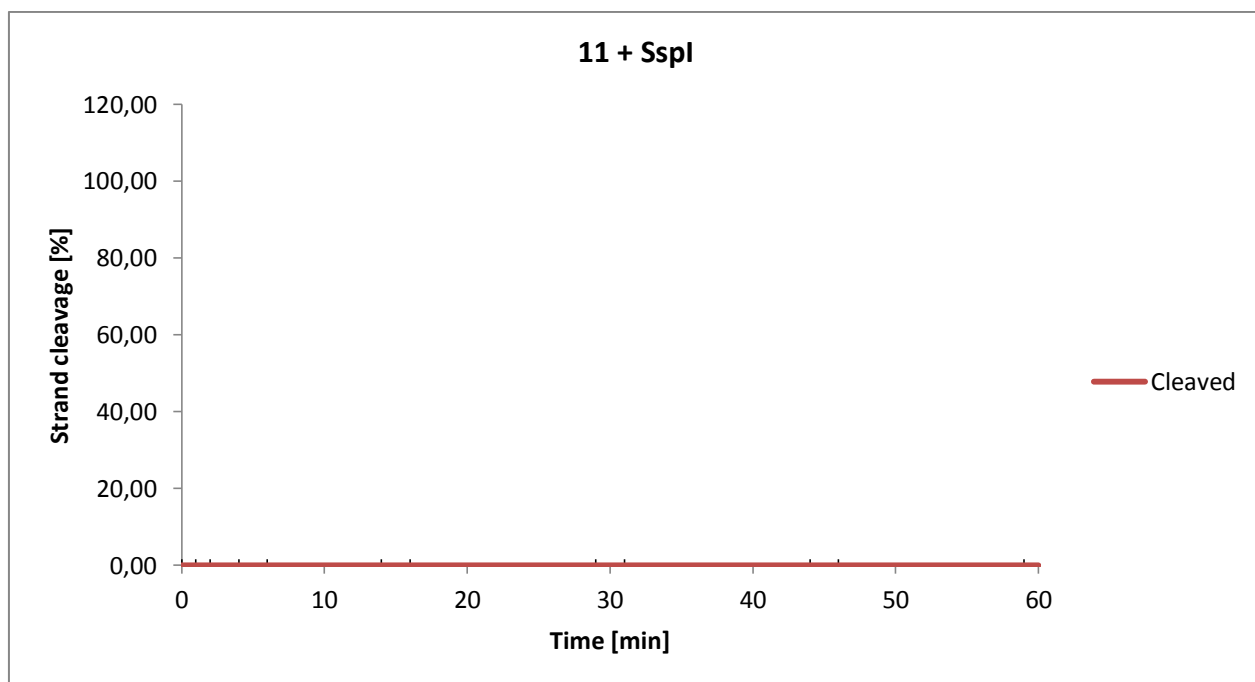


Figure S48. Cleavage of dsDNA (duplex F) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 11) is shown.

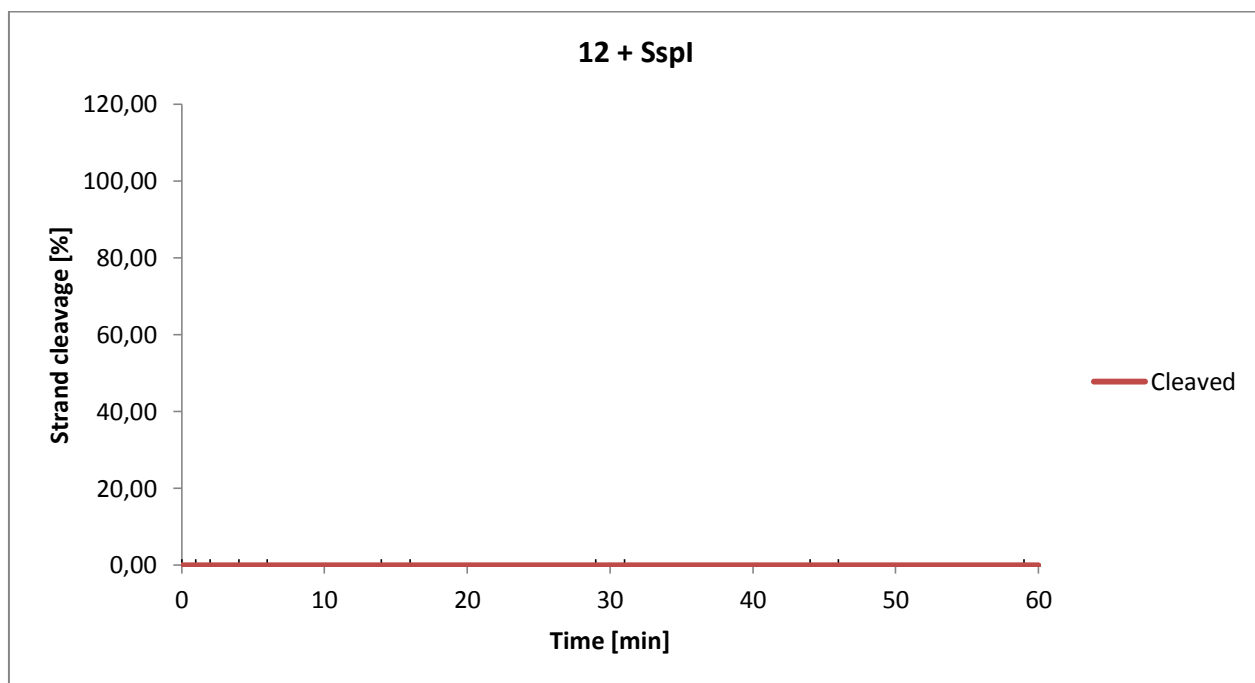


Figure S49. Cleavage of dsDNA (duplex F) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 12) is shown.

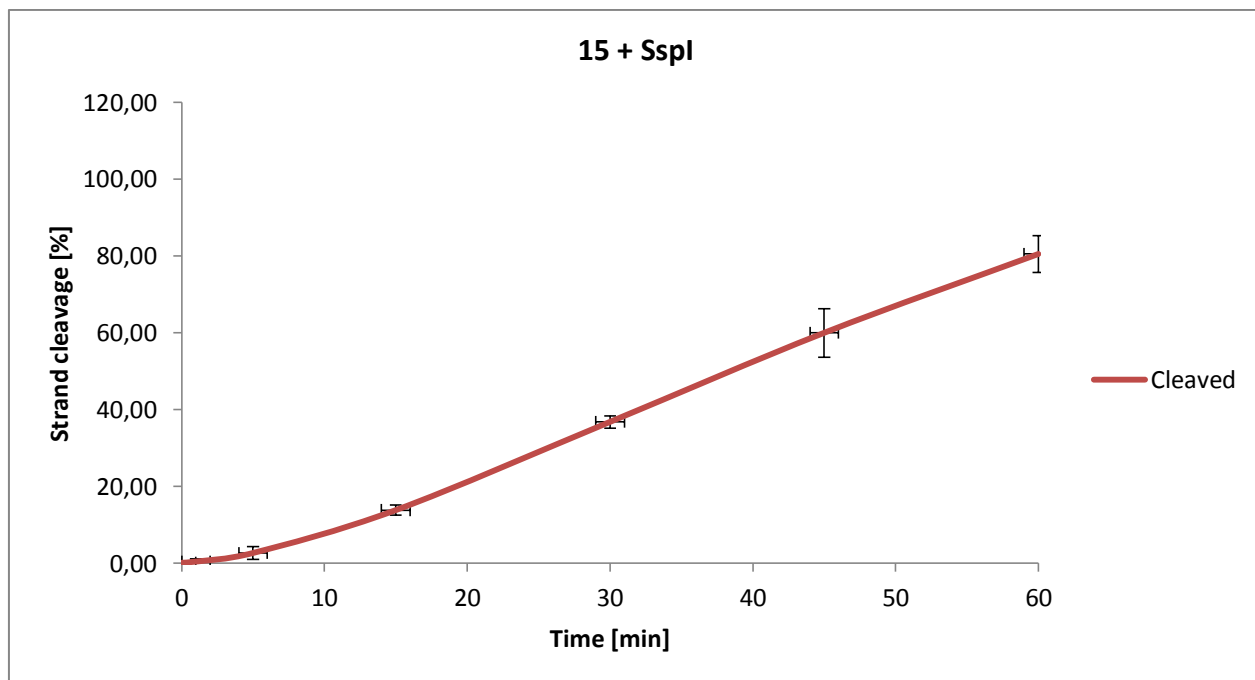


Figure S50. Cleavage of dsDNA (duplex H) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 15) is shown.

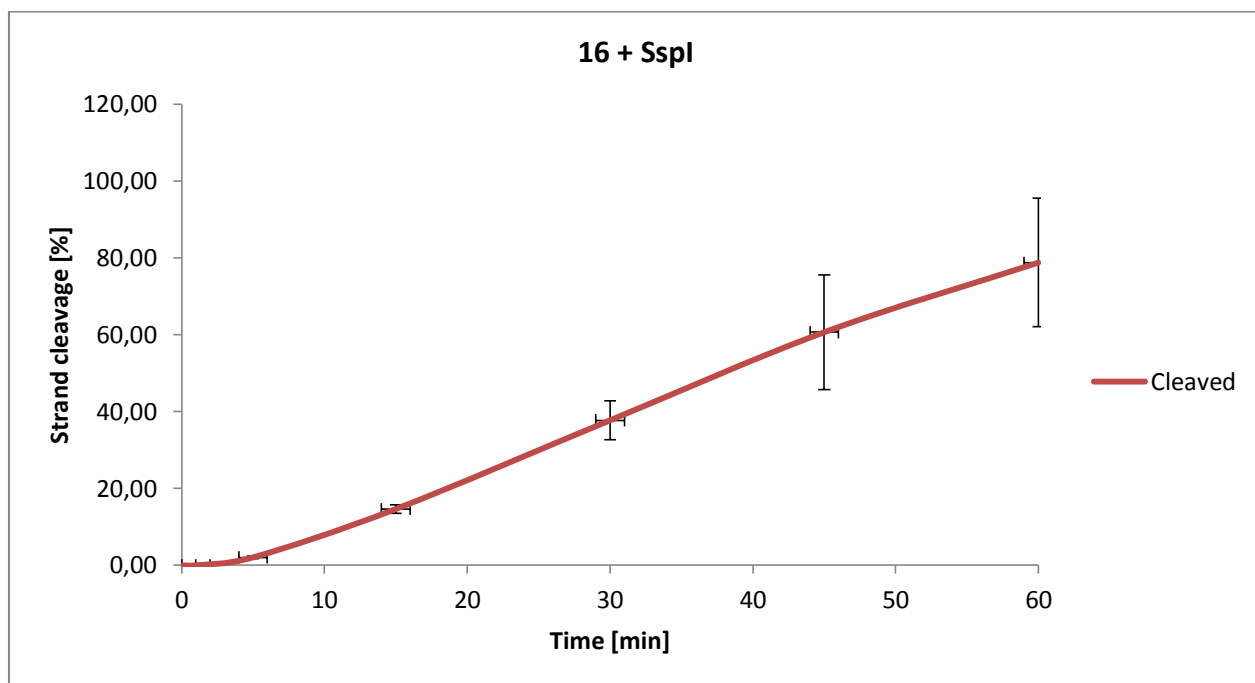


Figure S51. Cleavage of dsDNA (duplex H) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 16) is shown.

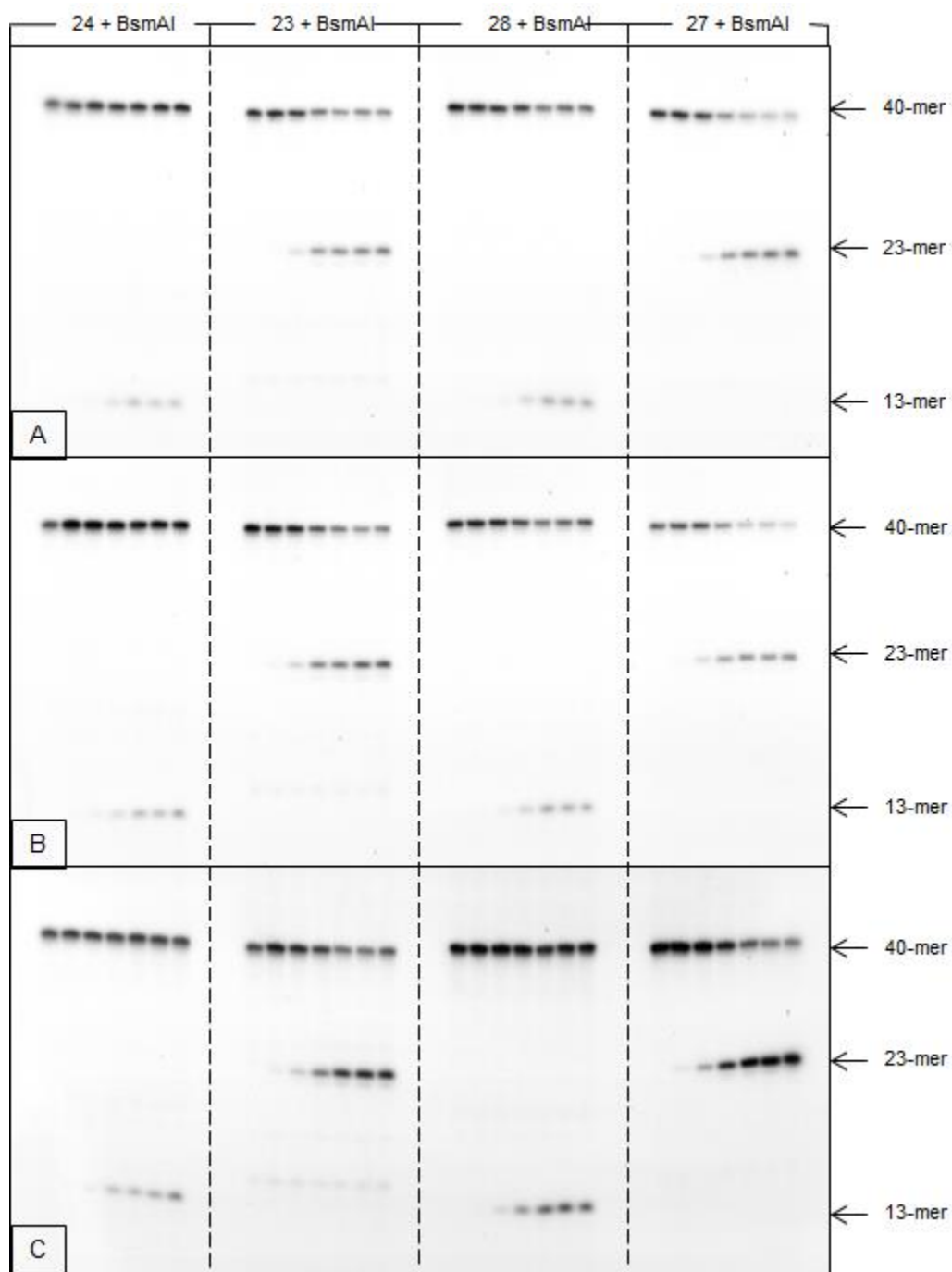


Figure S52. The autoradiograms of denaturing PAGE presenting cleavage of oligonucleotides by 0.5 U BsmAI – (A), (B) and (C) represent three repeats of assays. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30, 45 and 60 min starting from the left.

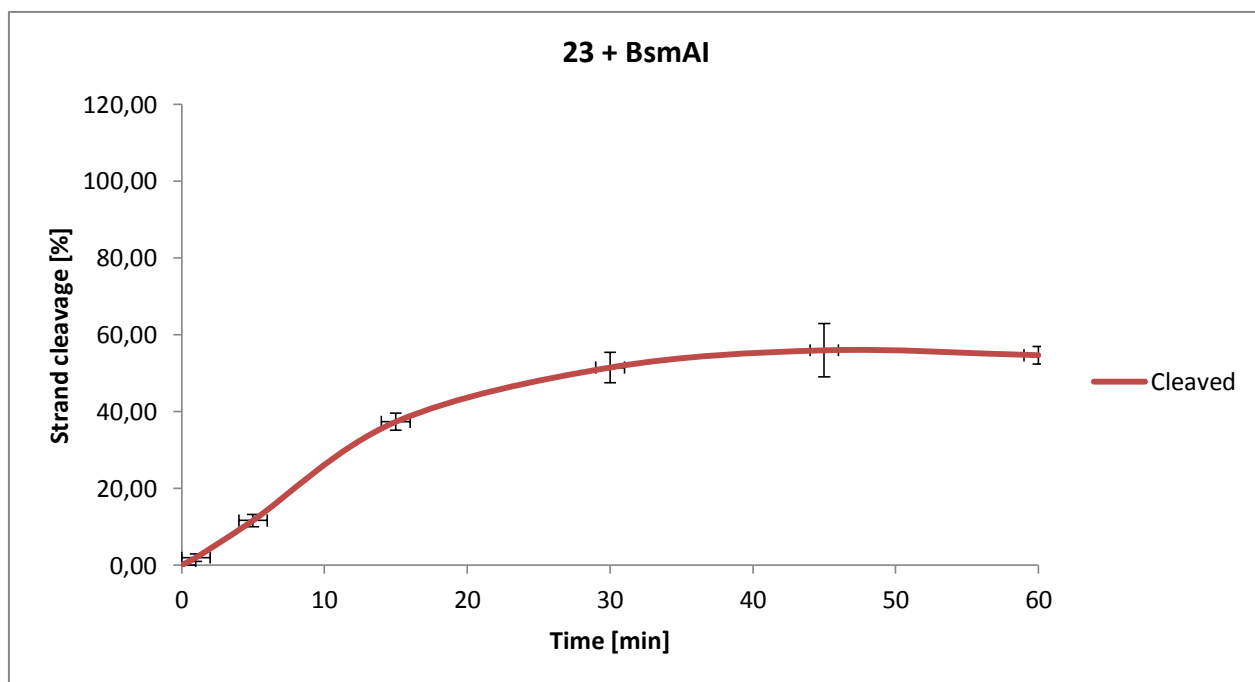


Figure S53. Cleavage of dsDNA (duplex L) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 23) is shown.

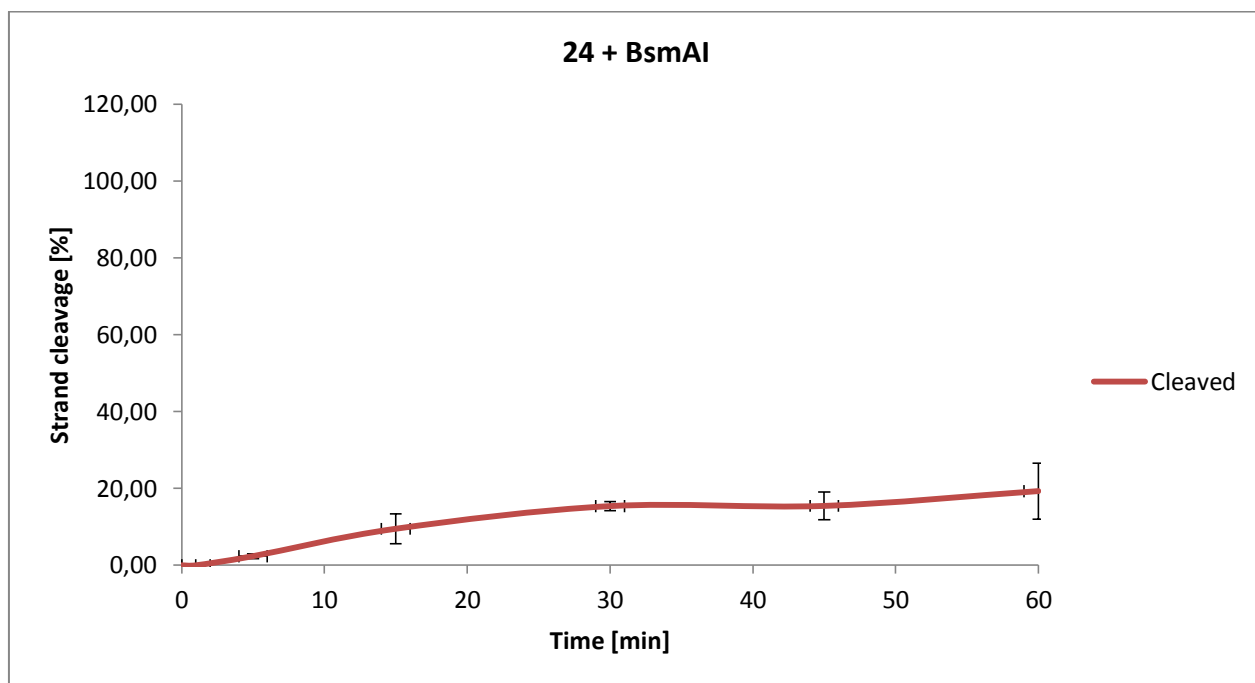


Figure S54. Cleavage of dsDNA (duplex L) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 24) is shown.

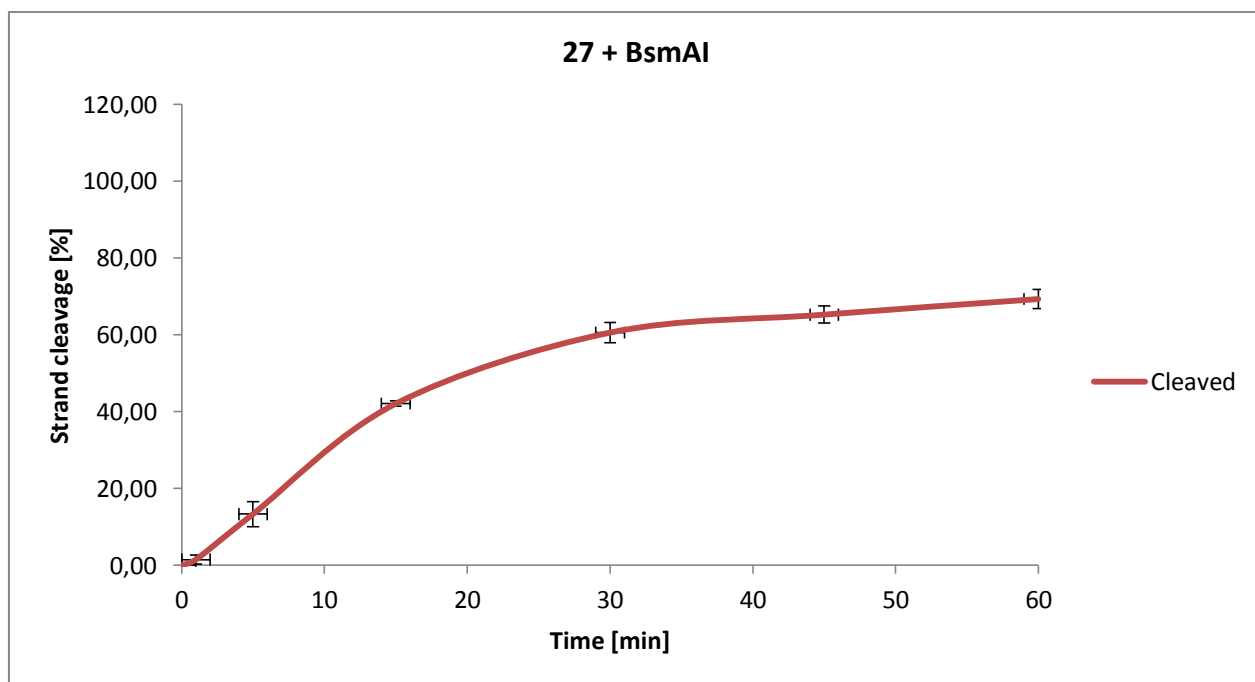


Figure S55. Cleavage of dsDNA (duplex N) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 27) is shown.

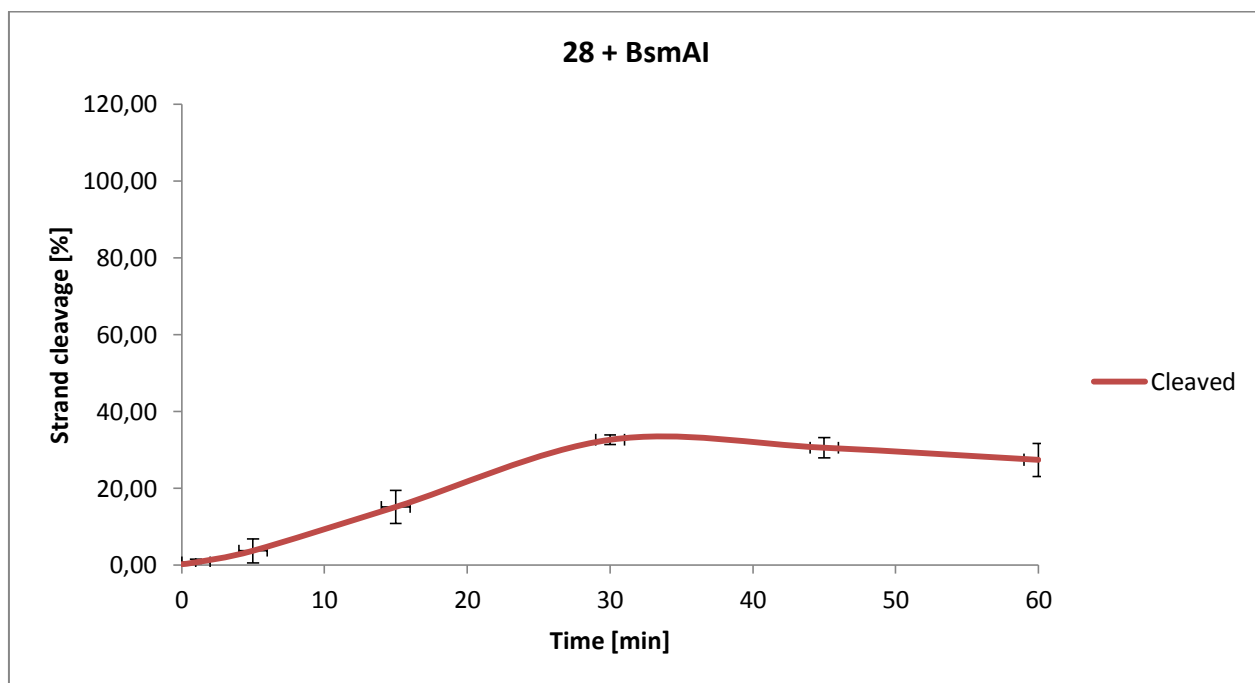


Figure S56. Cleavage of dsDNA (duplex N) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 28) is shown.

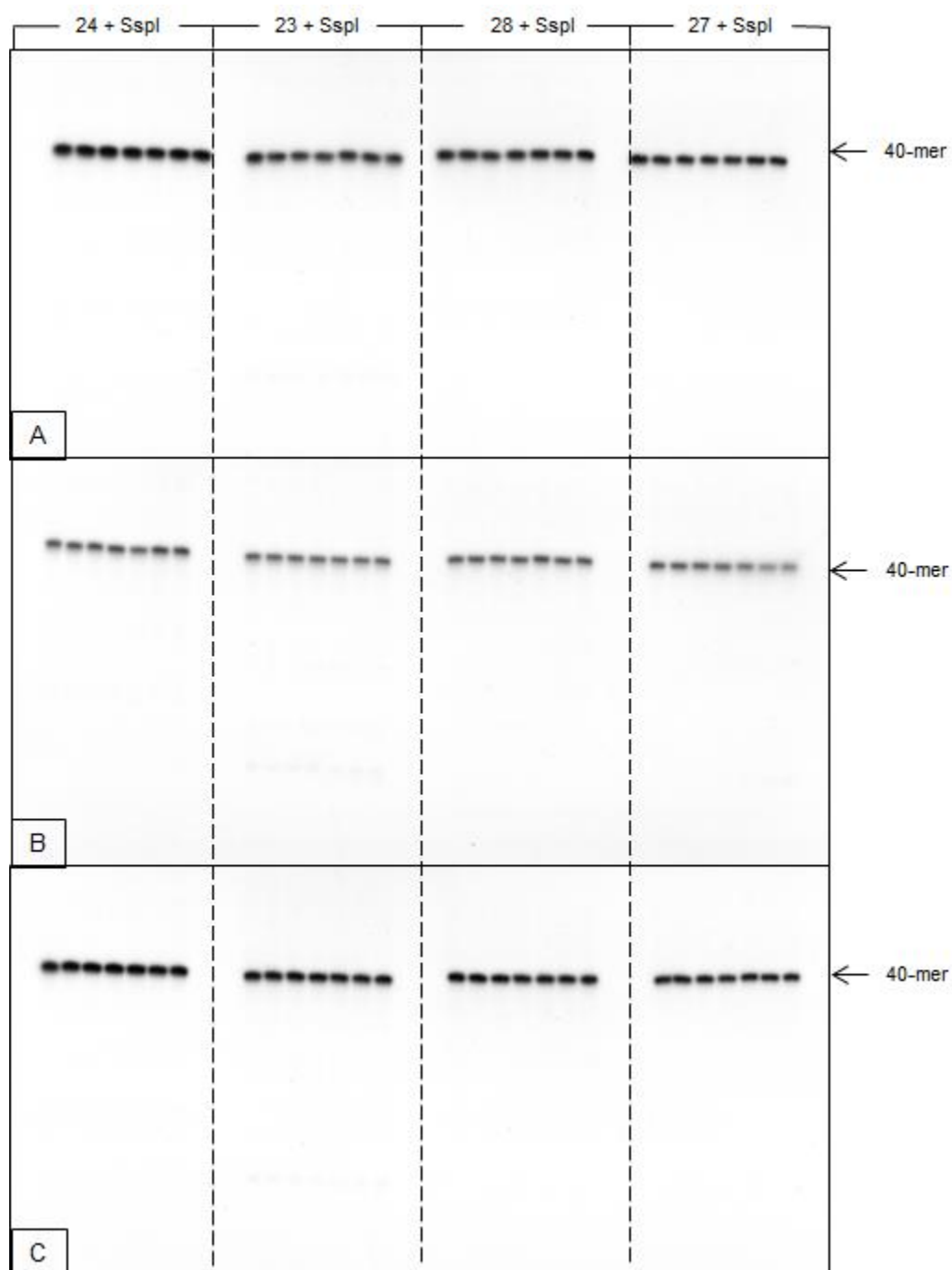


Figure S57. The autoradiograms of denaturing PAGE presenting cleavage of oligonucleotides by 1.5 U SspI – (A), (B) and (C) represent three repeats of assays. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30, 45 and 60 min starting from the left.

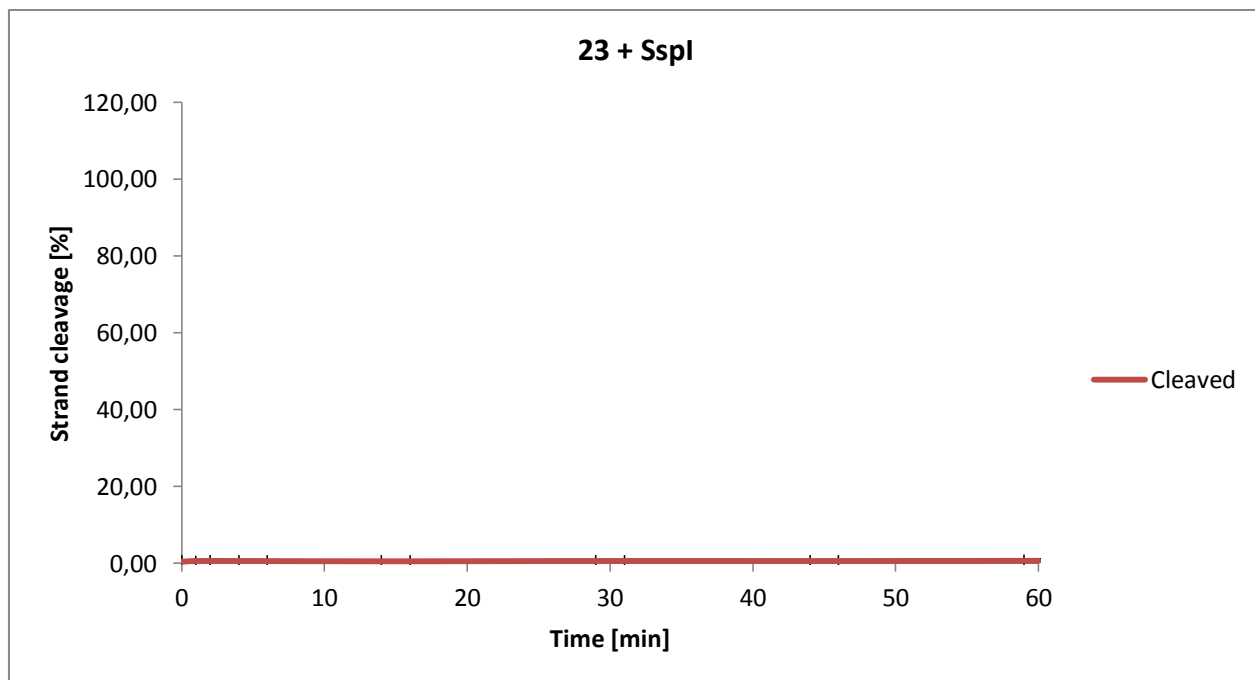


Figure S58. Cleavage of dsDNA (duplex L) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 23) is shown.

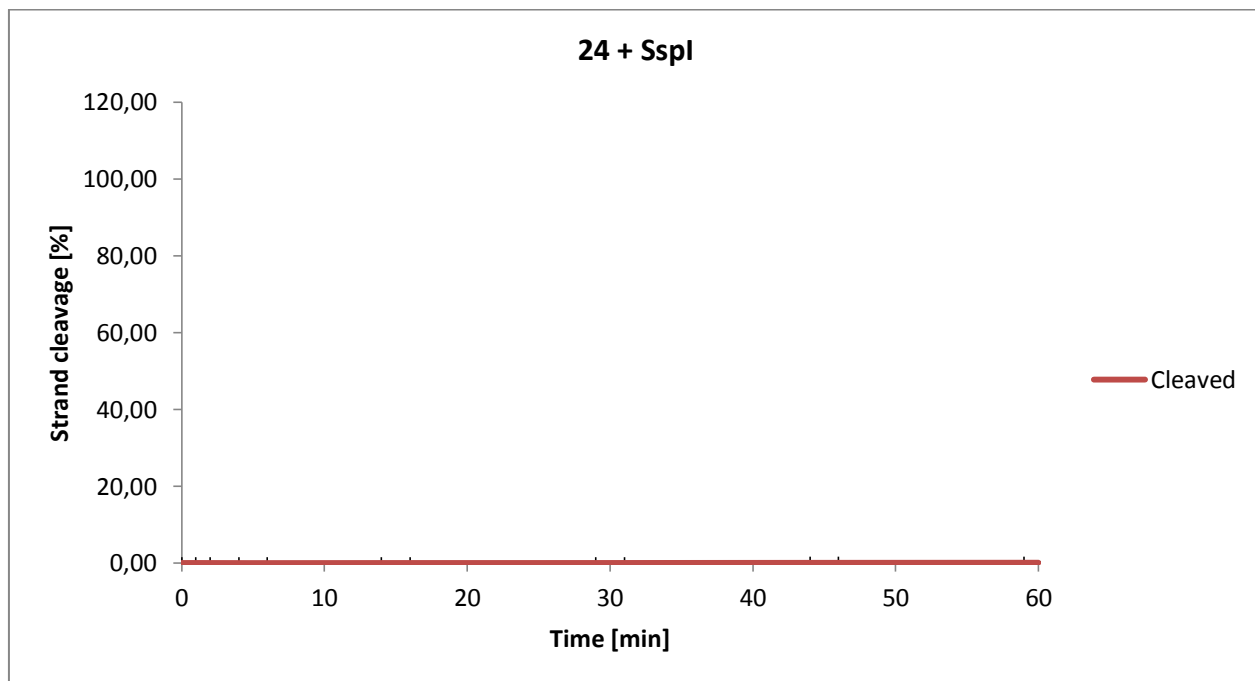


Figure S59. Cleavage of dsDNA (duplex L) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 24) is shown.

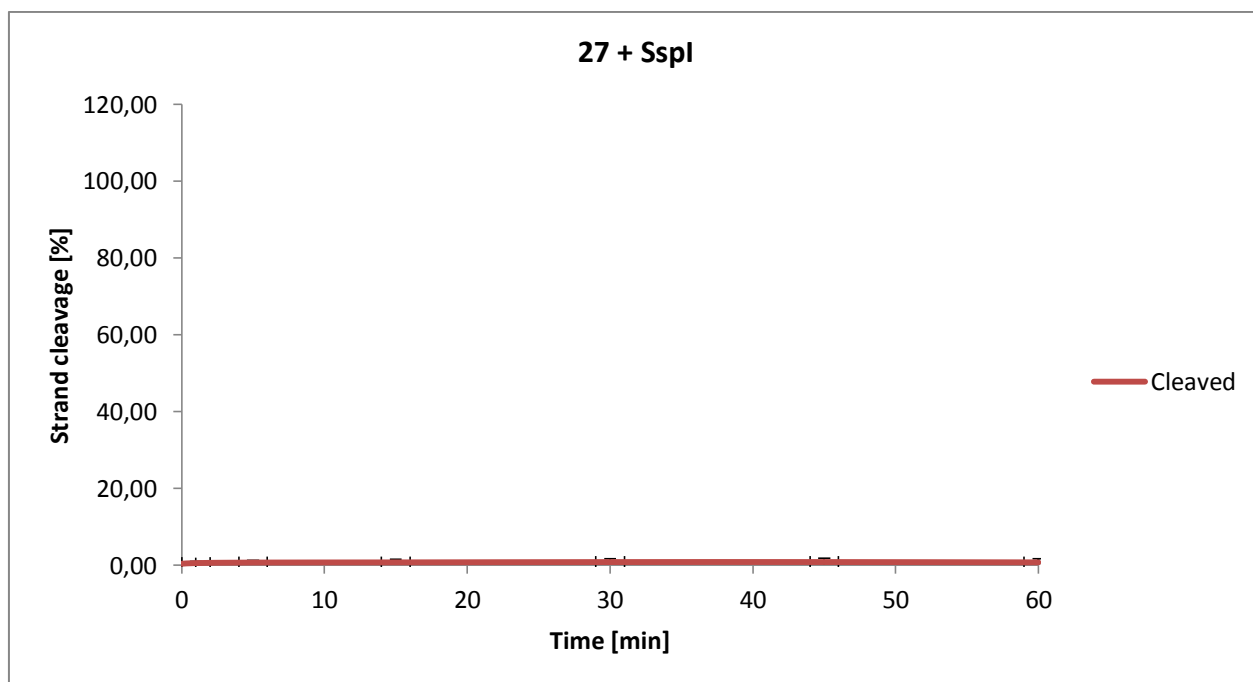


Figure S60. Cleavage of dsDNA (duplex N) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 27) is shown.

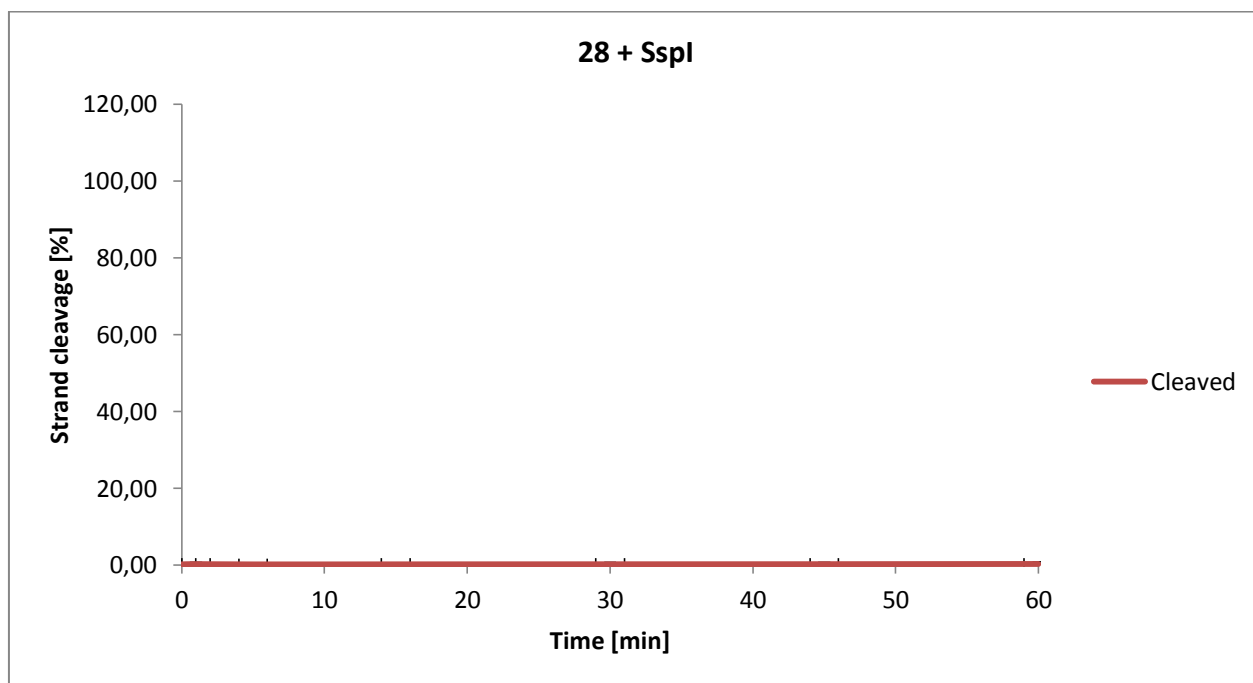


Figure S61. Cleavage of dsDNA (duplex N) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 28) is shown.

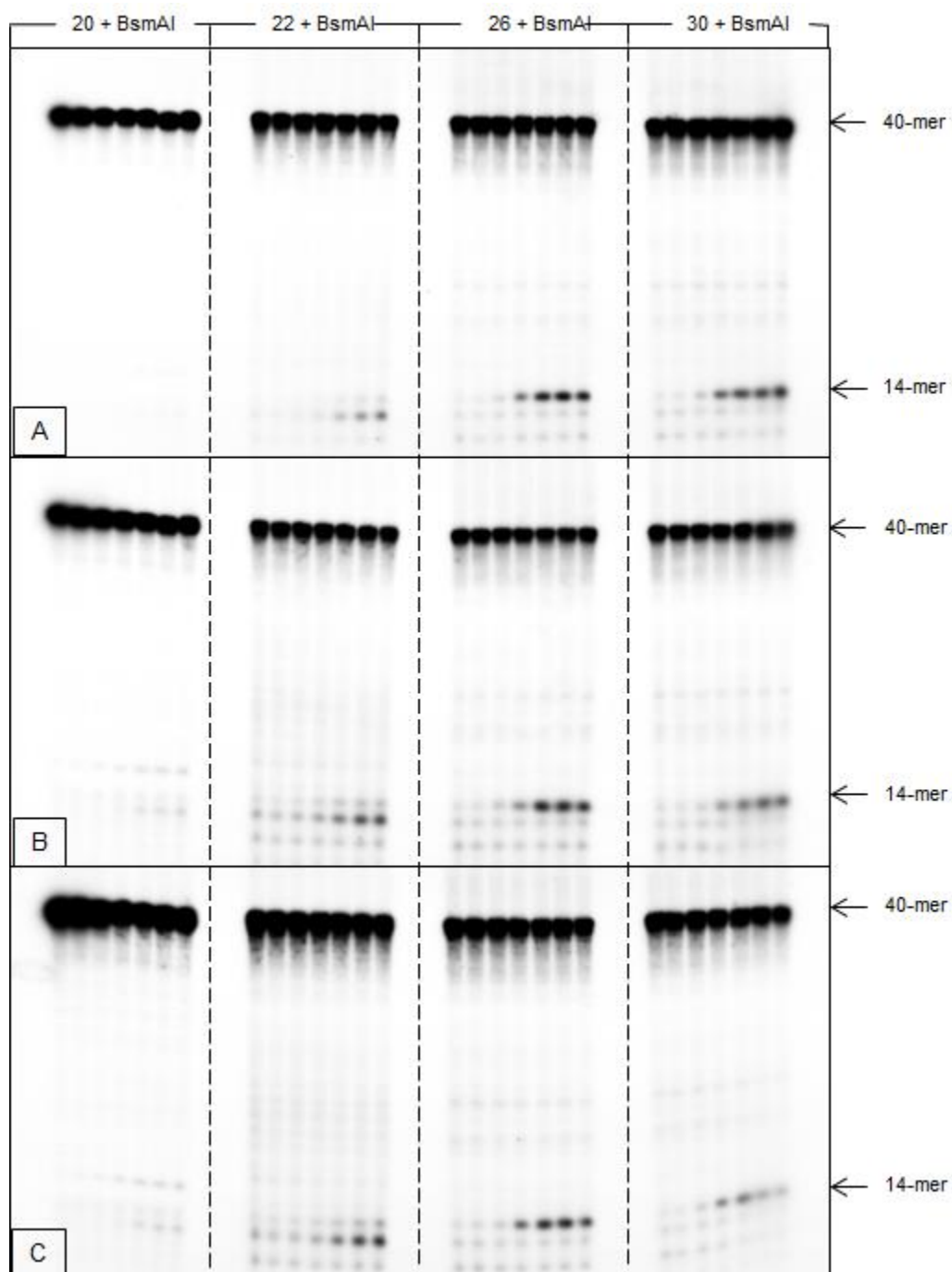


Figure S62. The autoradiograms of denaturing PAGE presenting cleavage of oligonucleotides by 0.5 U BsmAI – (A), (B) and (C) represent three repeats of assays. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30, 45 and 60 min starting from the left.

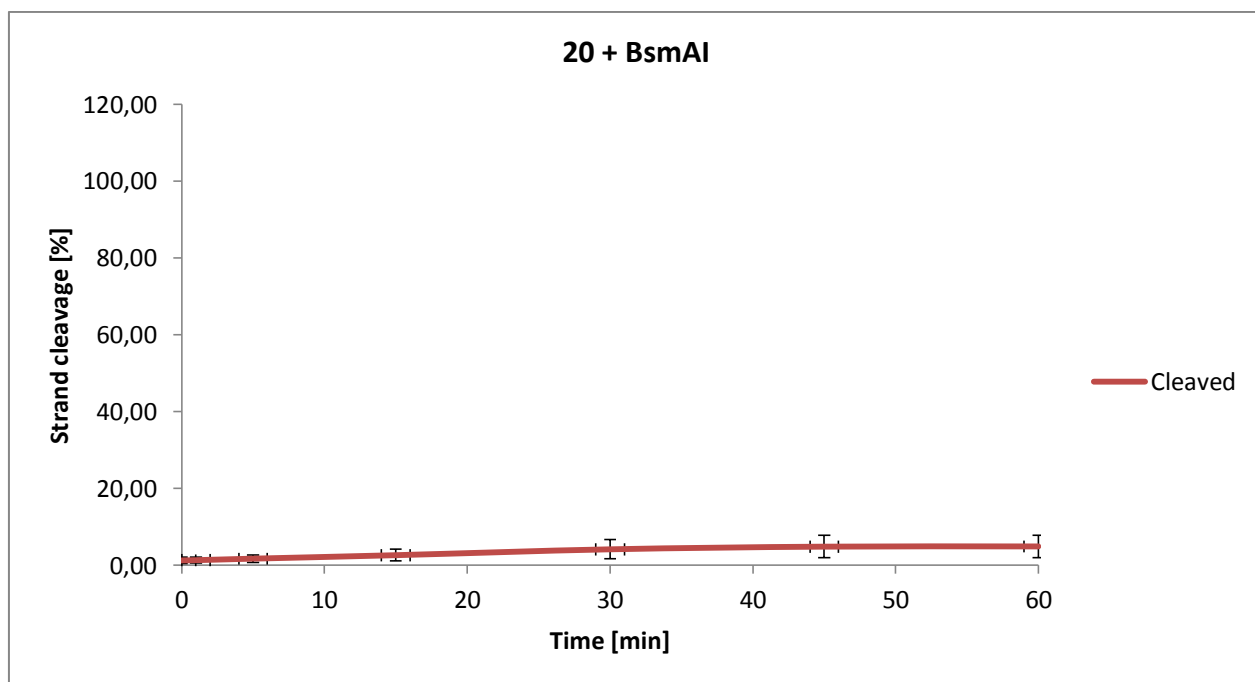


Figure S63. Cleavage of dsDNA (duplex J) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 20) is shown.

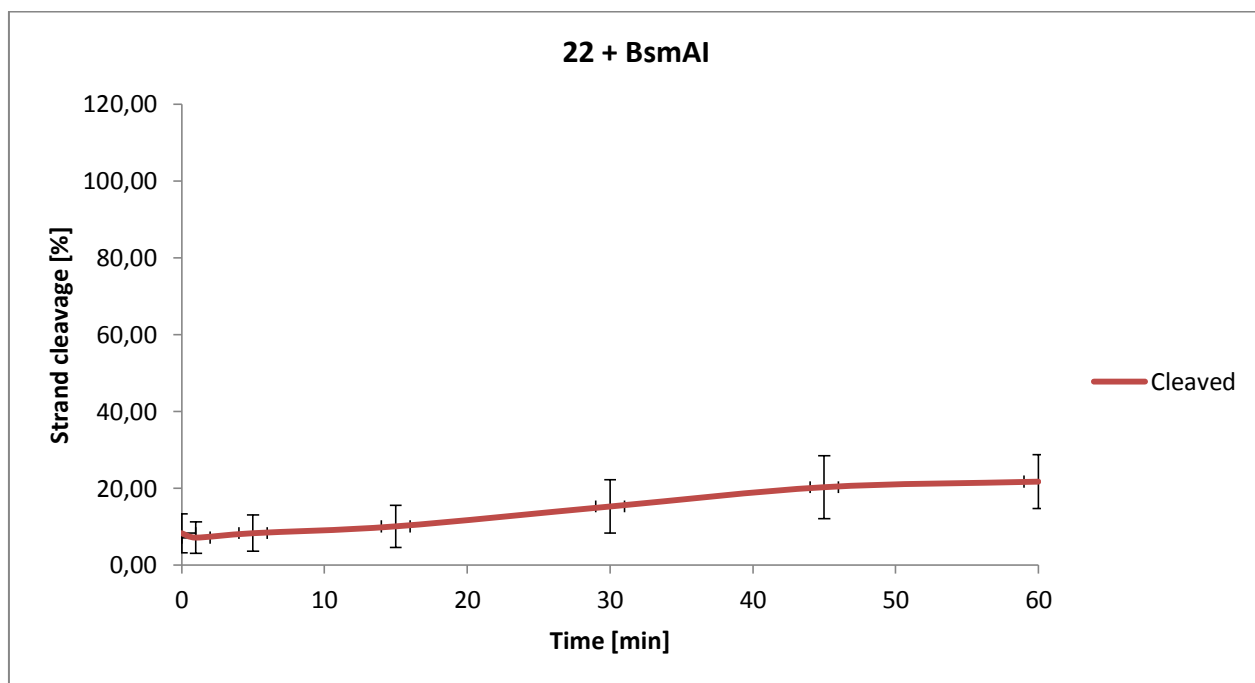


Figure S64. Cleavage of dsDNA (duplex K) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 22) is shown.

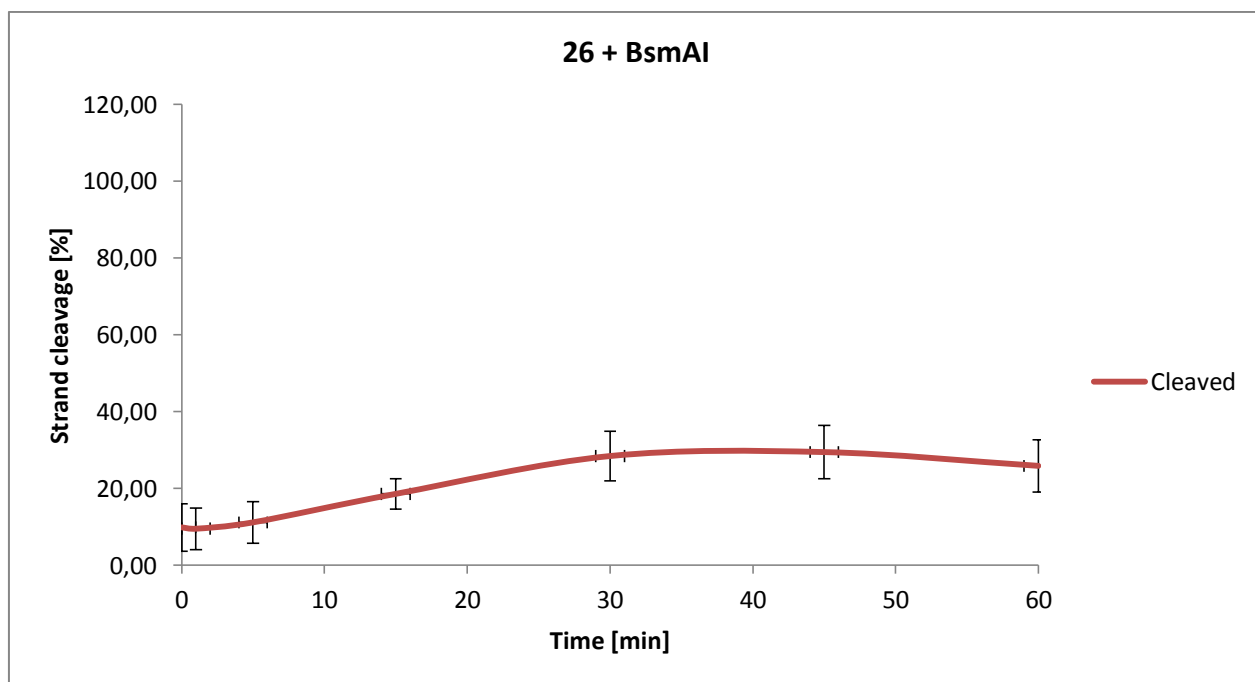


Figure S65. Cleavage of dsDNA (duplex M) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 26) is shown.

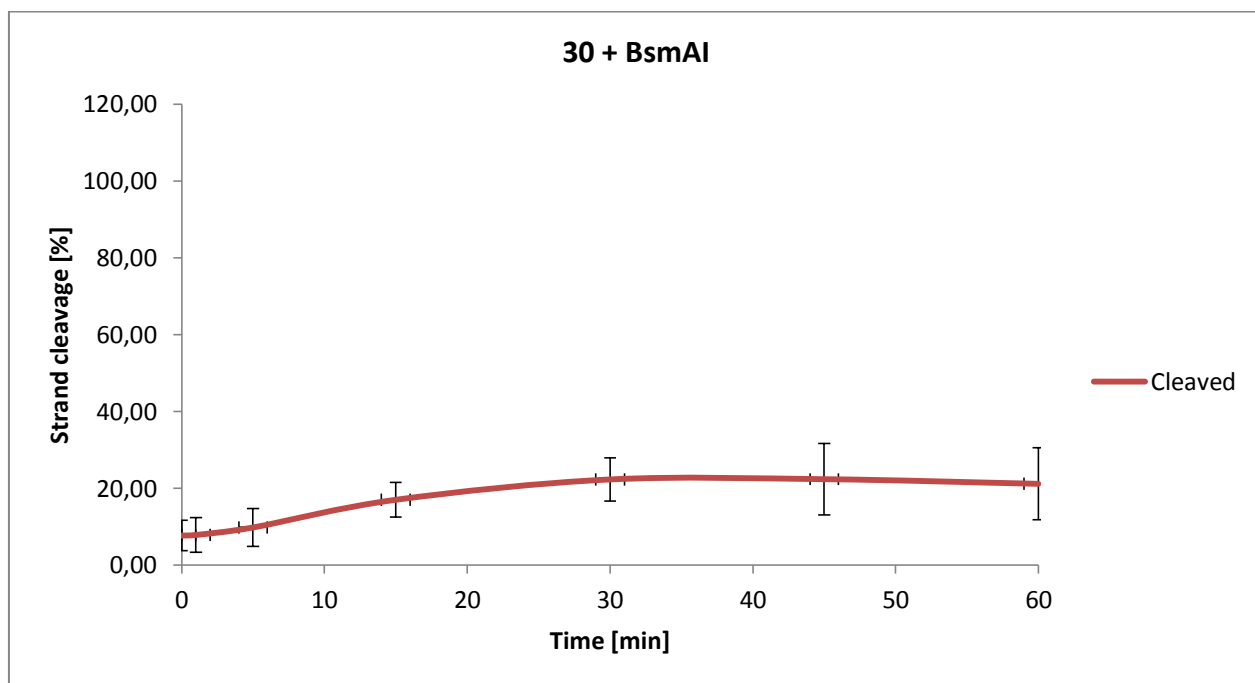


Figure S66. Cleavage of dsDNA (duplex O) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 30) is shown.

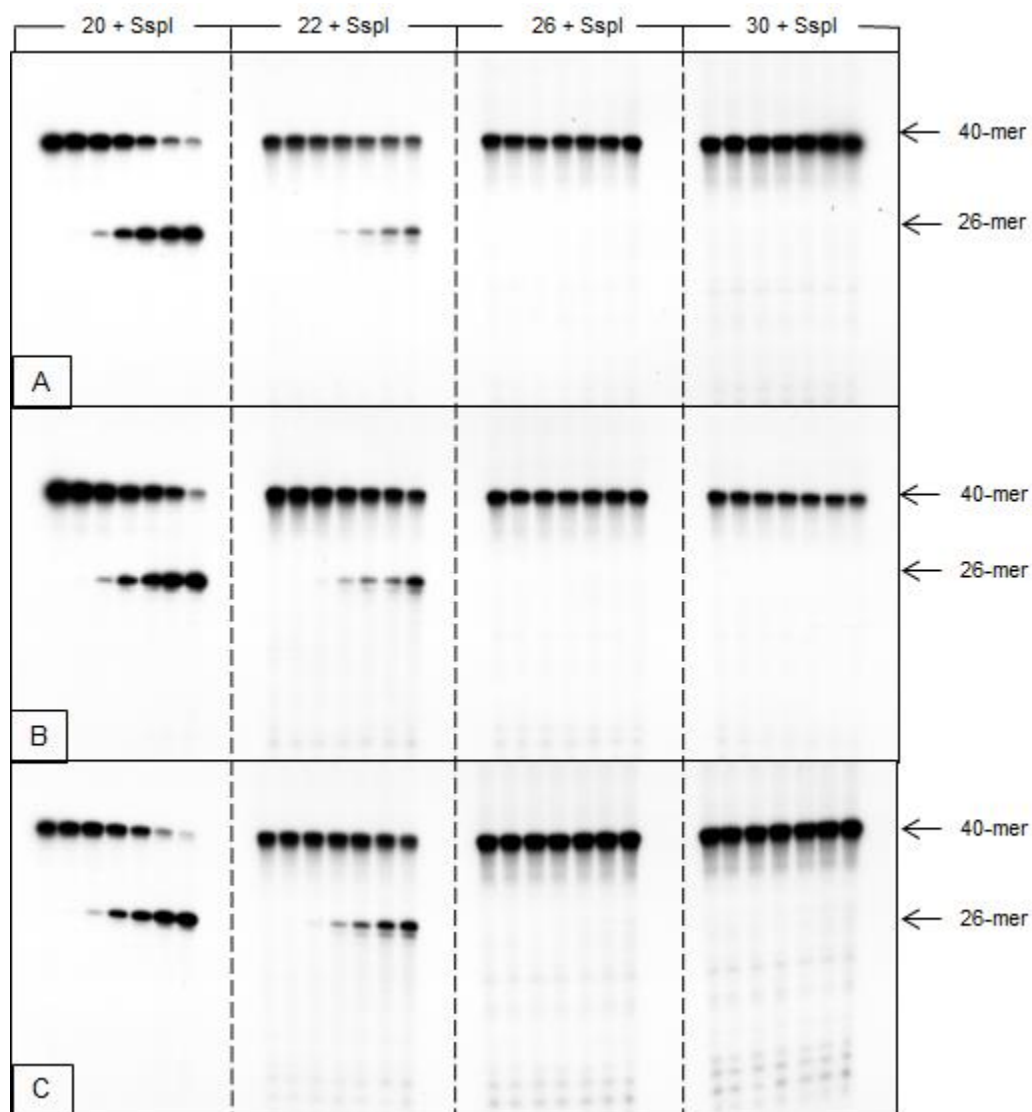


Figure S67. The autoradiograms of denaturing PAGE presenting cleavage of oligonucleotides by 1.5 U SspI – (A), (B) and (C) represent three repeats of assays. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30, 45 and 60 min starting from the left.

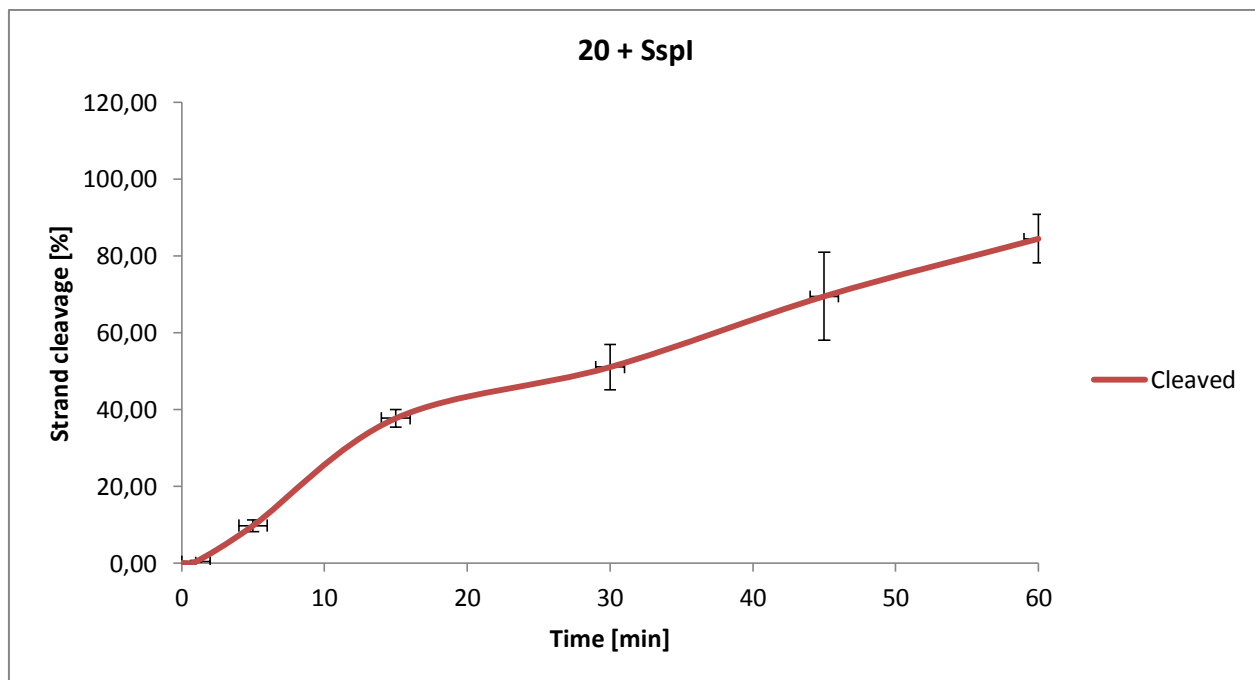


Figure S68. Cleavage of dsDNA (duplex J) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 20) is shown.

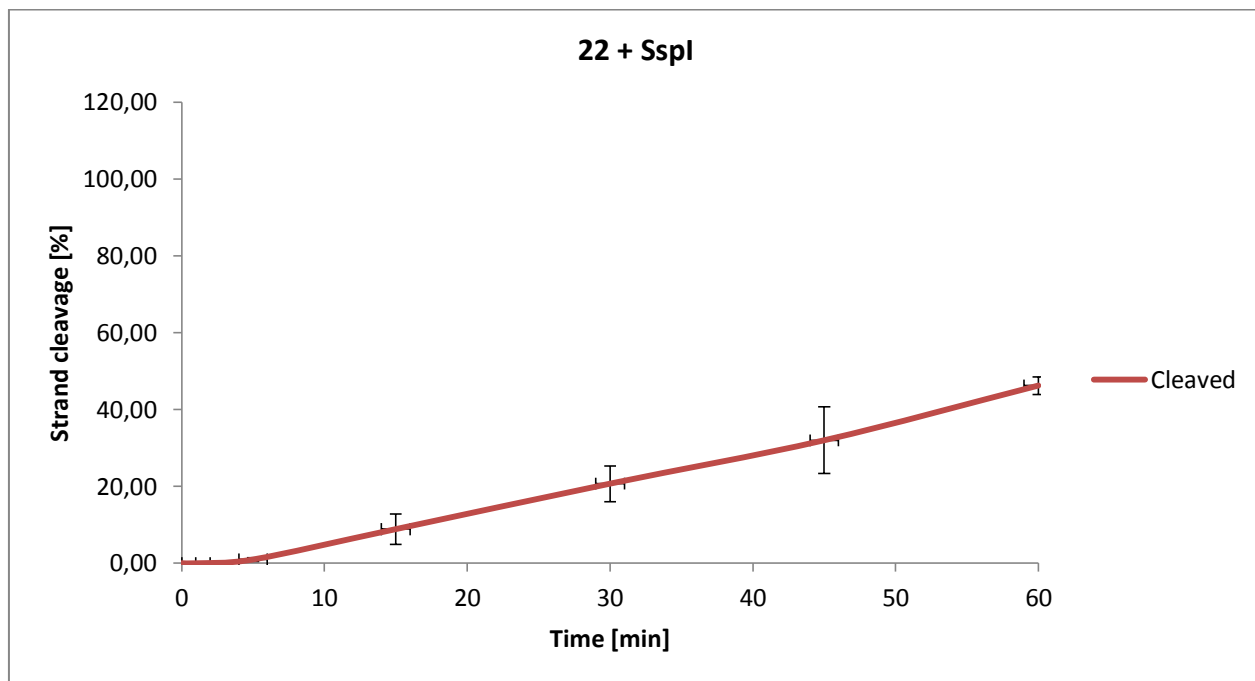


Figure S69. Cleavage of dsDNA (duplex K) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 22) is shown.

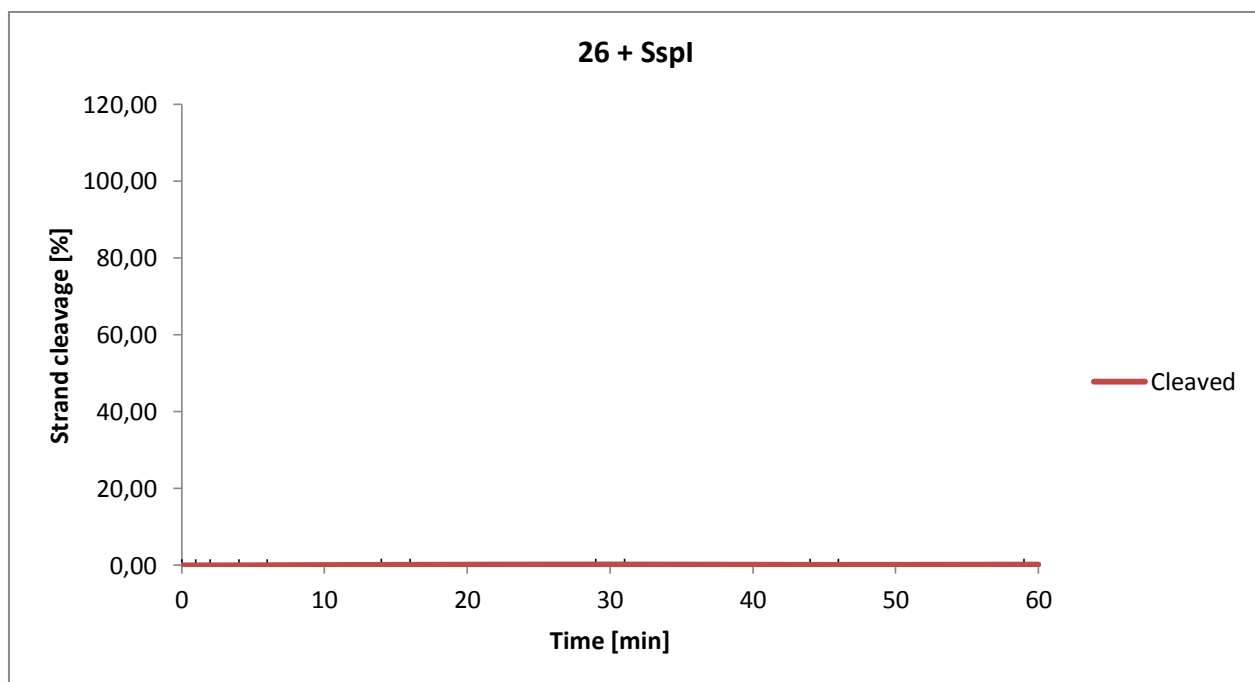


Figure S70. Cleavage of dsDNA (duplex M) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 26) is shown.

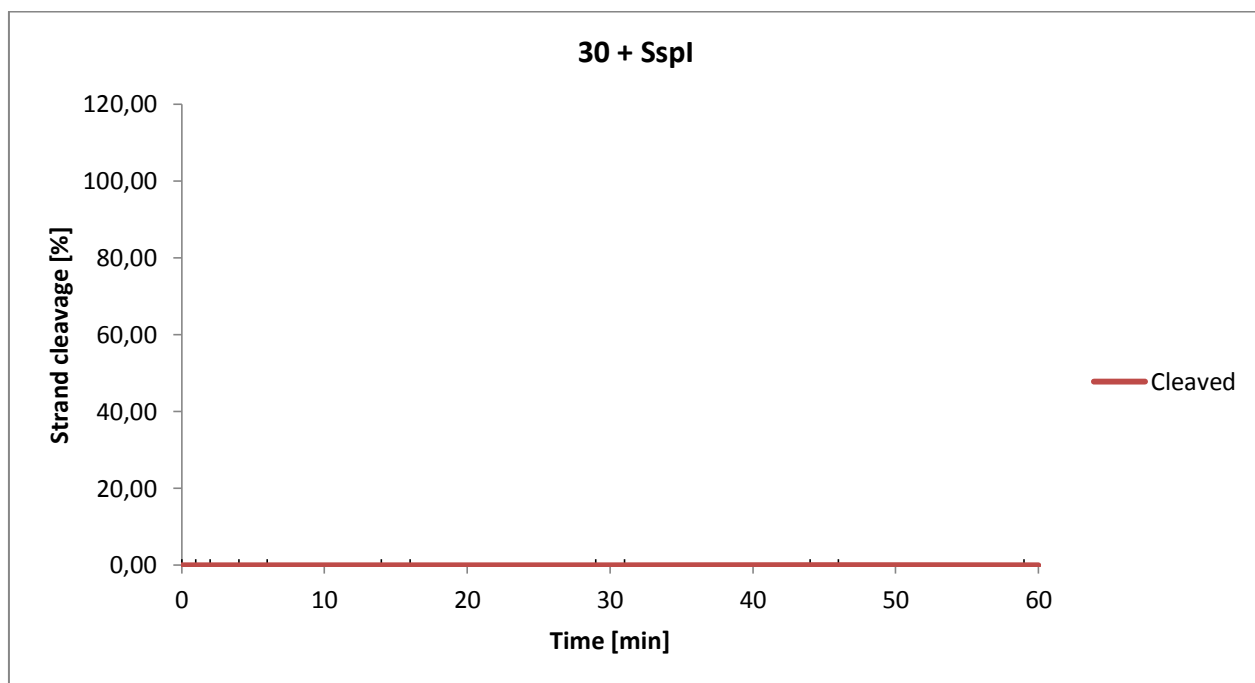


Figure S71. Cleavage of dsDNA (duplex O) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 30) is shown.

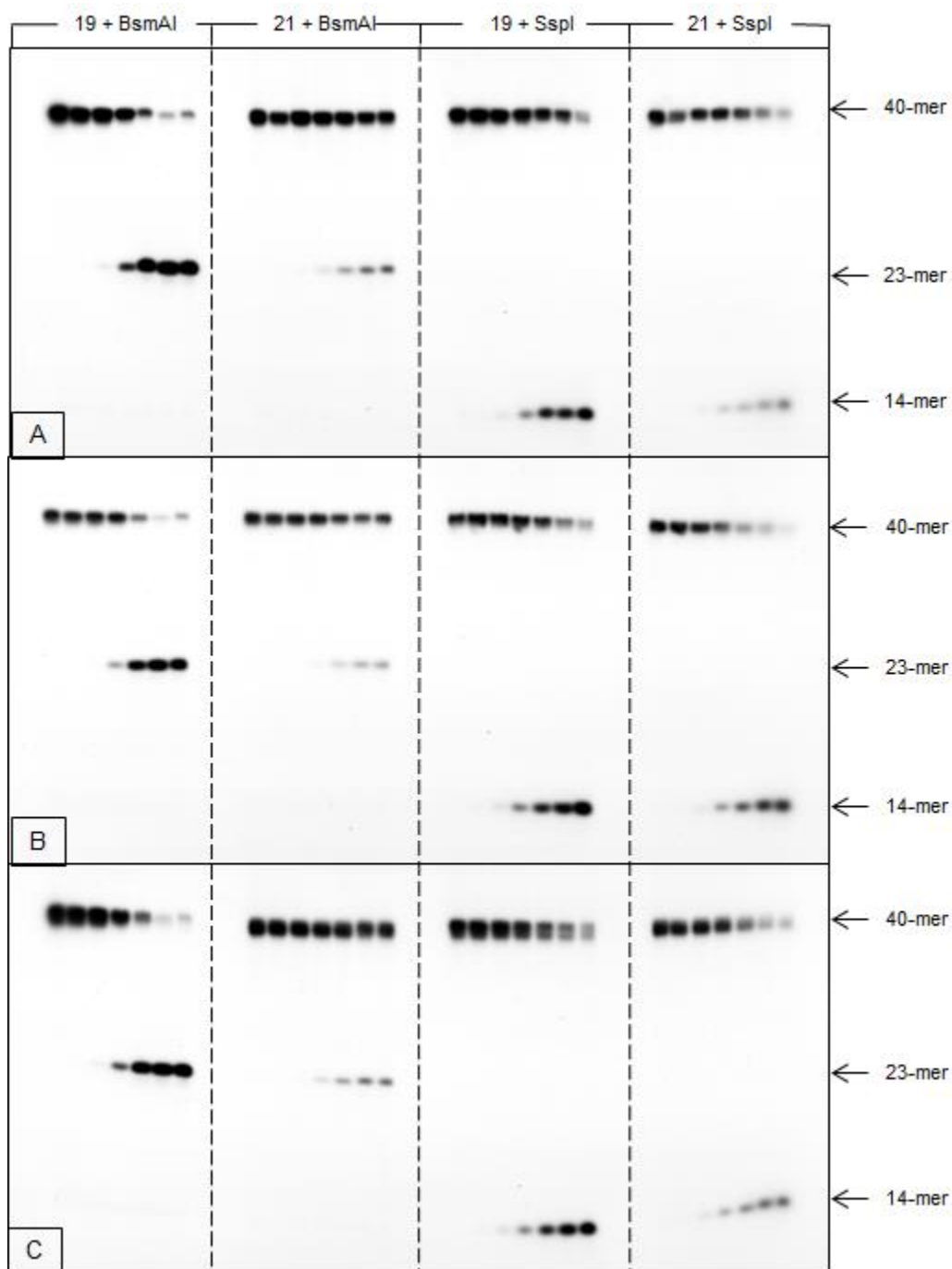


Figure S72. The autoradiograms of denaturing PAGE presenting cleavage of oligonucleotides by 0.5 U BsmAI or 1.5 U SspI – (A), (B) and (C) represent three repeats of assays. Lanes of bands represent following reaction times: 0, 1, 5, 15, 30, 45 and 60 min starting from the left.

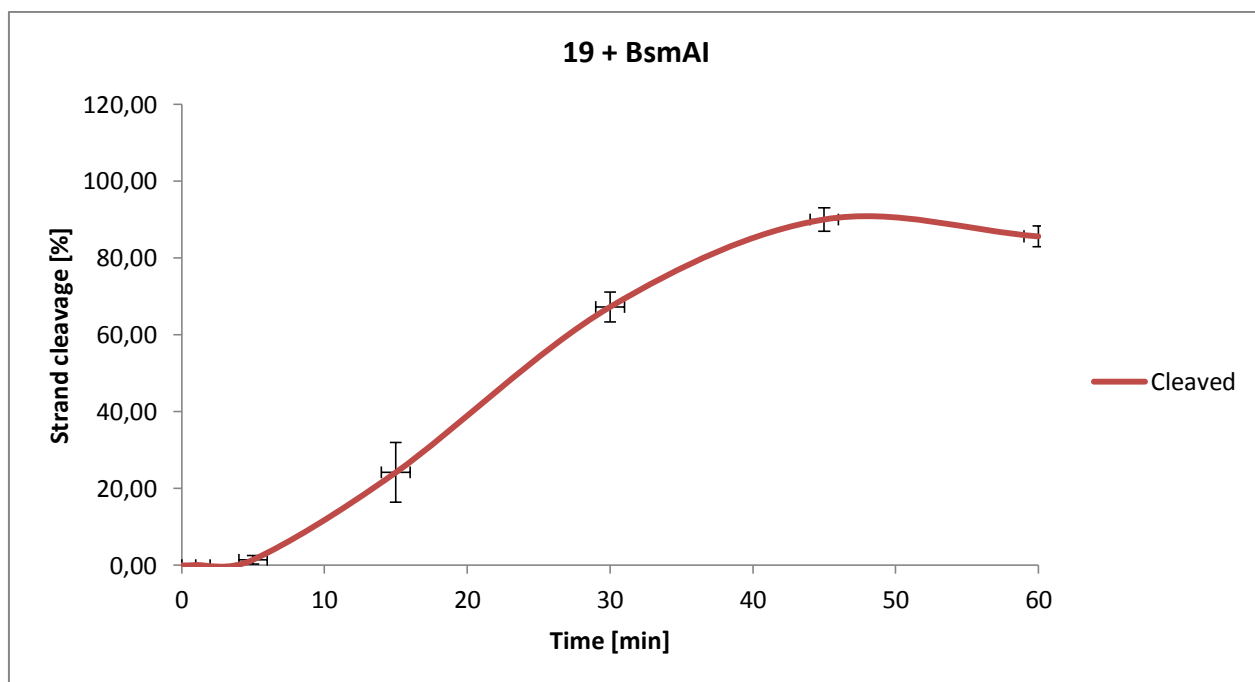


Figure S73. Cleavage of dsDNA (duplex J) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 19) is shown.

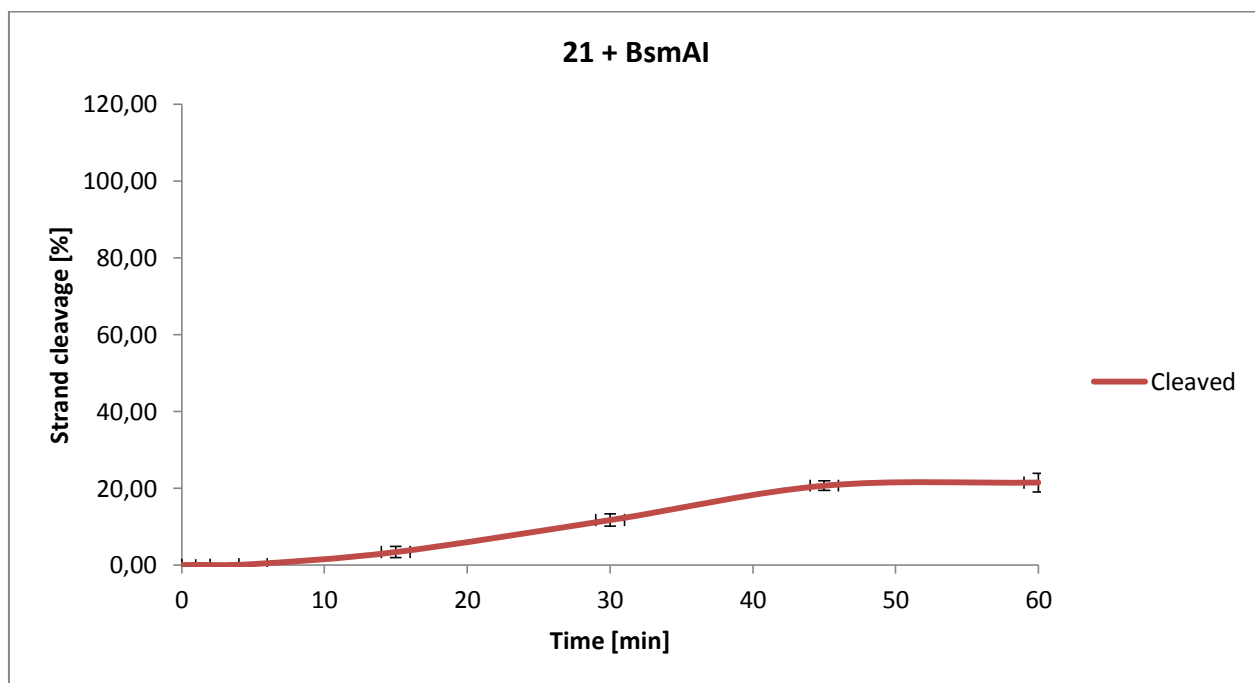


Figure S74. Cleavage of dsDNA (duplex K) by 0.5 U BsmAI. Quantity increase of cleaved ssDNA (strand 21) is shown.

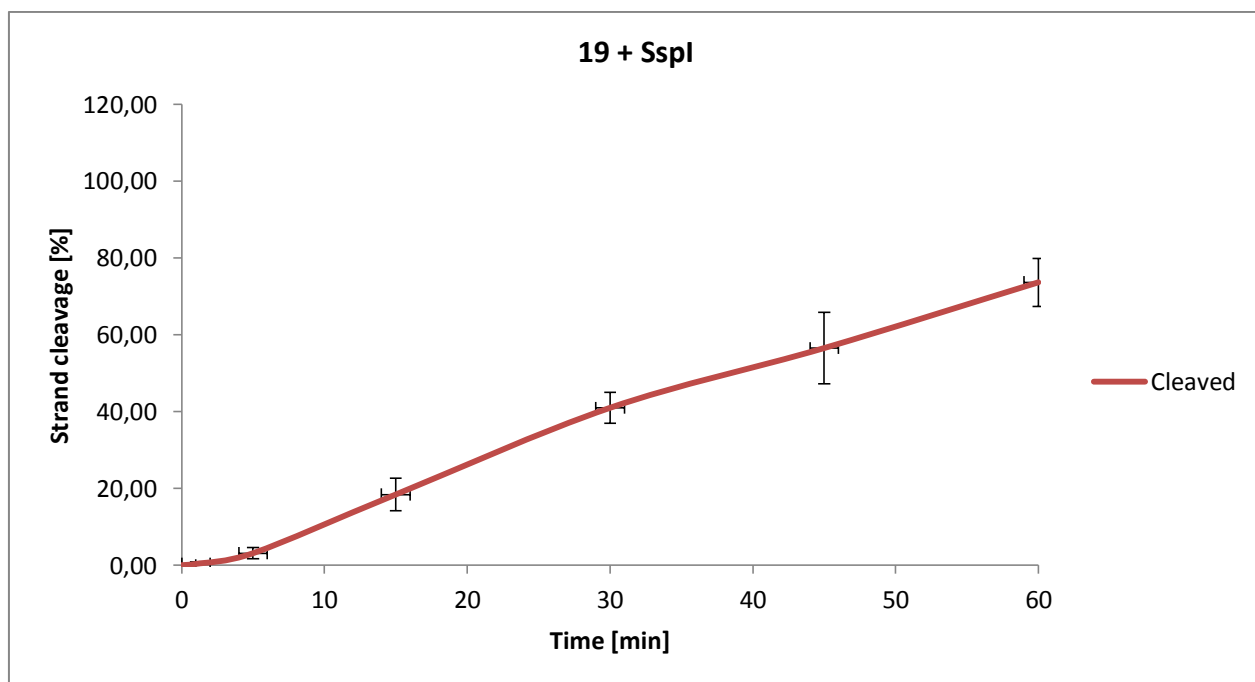


Figure S75. Cleavage of dsDNA (duplex J) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 19) is shown.

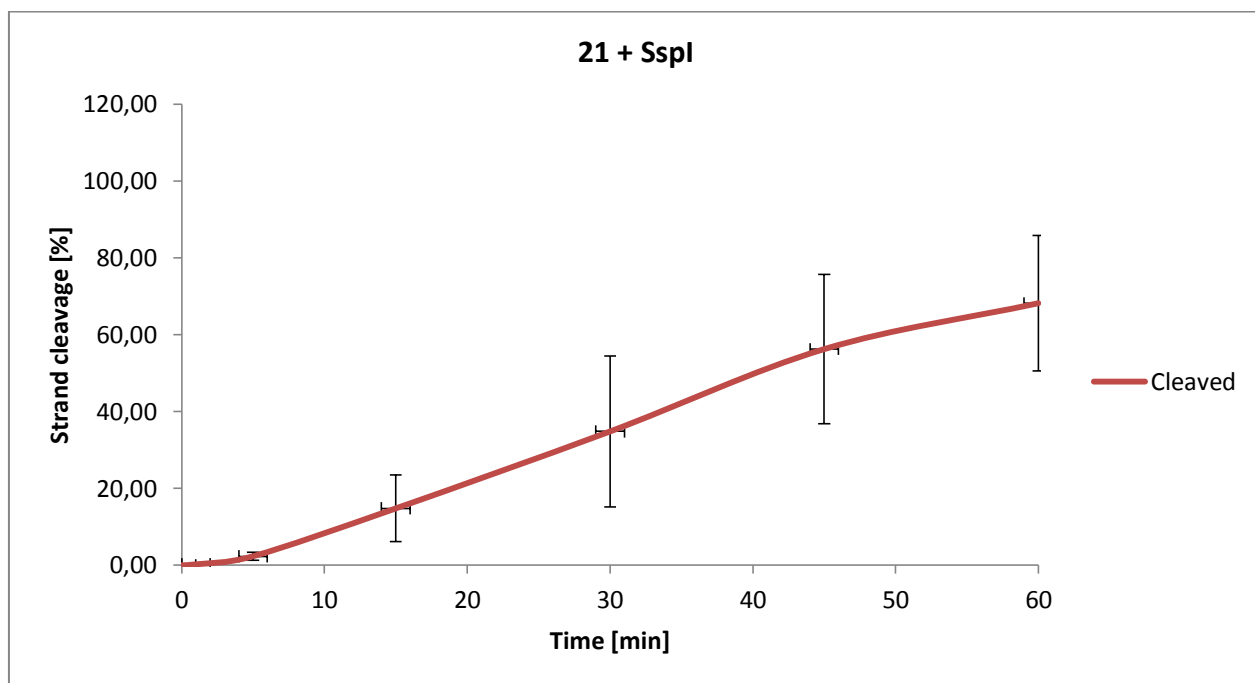


Figure S76. Cleavage of dsDNA (duplex K) by 1.5 U SspI. Quantity increase of cleaved ssDNA (strand 21) is shown.

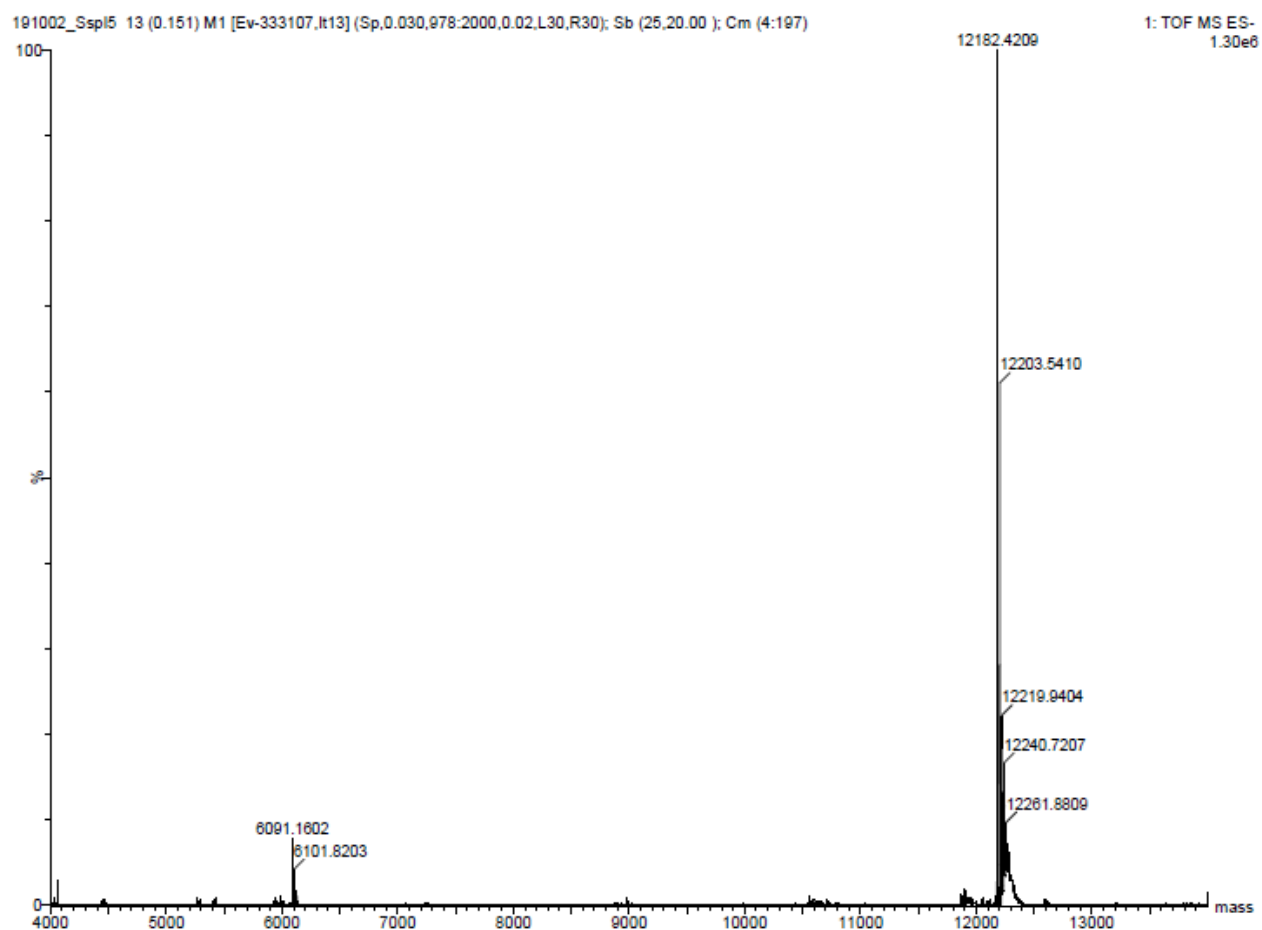


Figure S77. Mass spectrum of Matrix 5'.

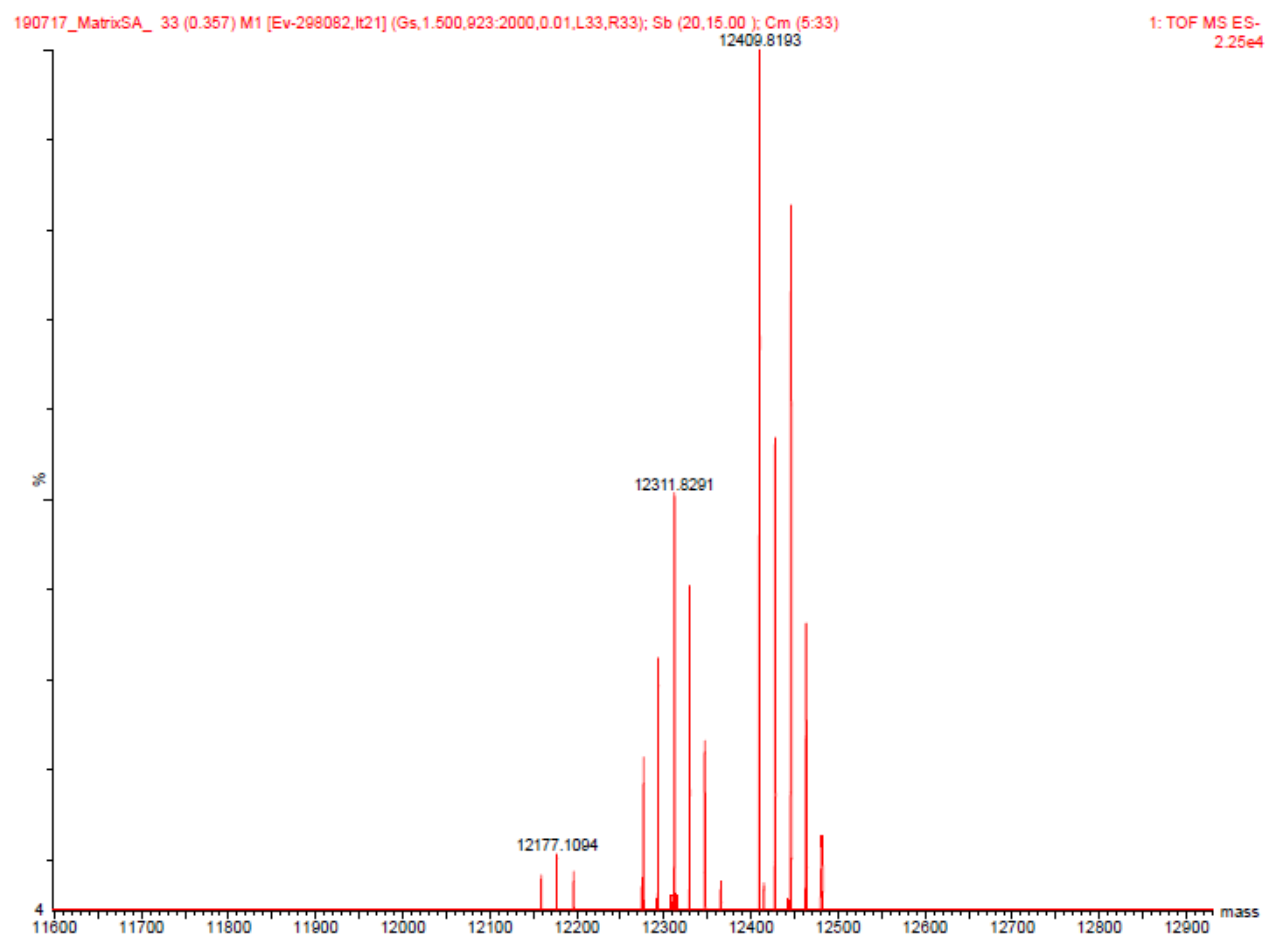


Figure S78. Mass spectrum of Matrix 3'.

210219_Sspl1_+1 105 (1.077) M1 [Ev-354696,l25] (Sp,0.030,794:1775,0.10,L33,R33); Cm (103:197)

1: TOF MS ES-
8.69e7

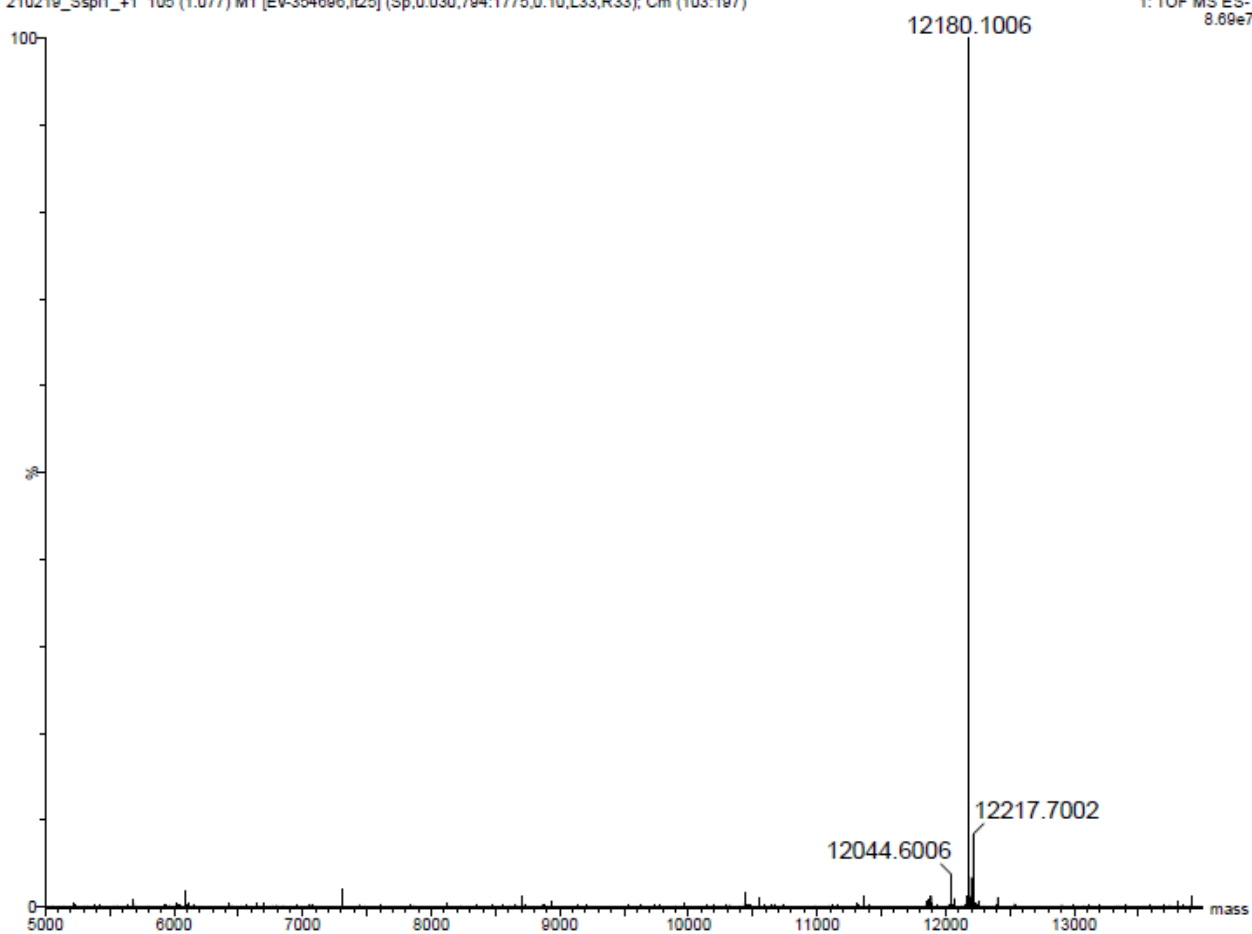


Figure S79. Mass spectrum of sequence 1.

210219_Sspl1_+1_R 81 (0.837) M1 [Ev-362611,lt25] (Sp,0.030,785:1779,0.10,L33,R33); Cm (77:197)

1: TOF MS ES-
1.16e8

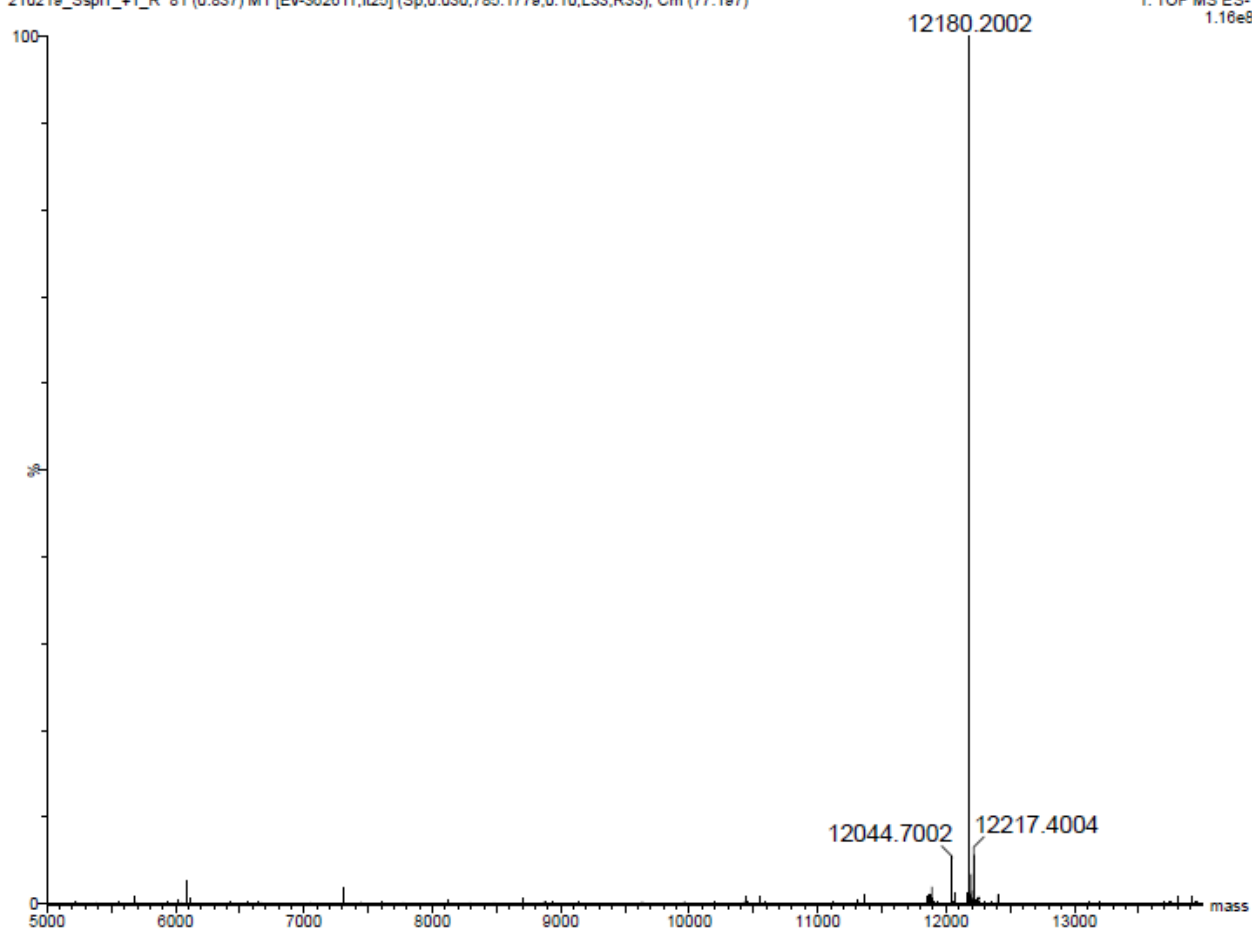


Figure S80. Mass spectrum of sequence 2.

210219_Bsmal_1cdA 113 (1.157) M1 [Ev-322359,It25] (Sp,0.030,854:1764,0.10,L33,R33); Cm (86:193)

1: TOF MS ES-
8.55e7

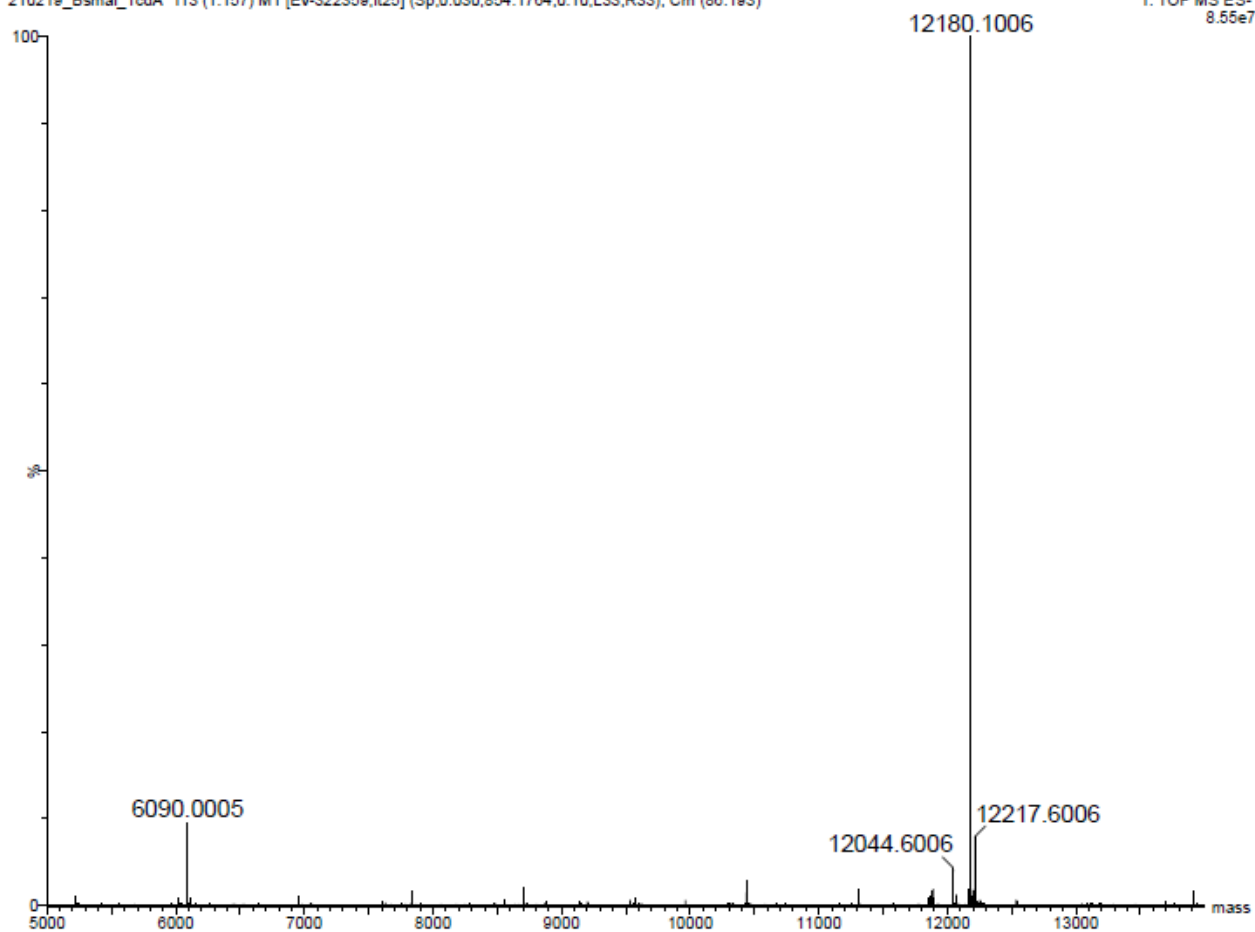


Figure S81. Mass spectrum of sequence 3.

210219_Bsmal_1cdA_R 115 (1.174) M1 [Ev-349760,lt25] (Sp,0.030,833:1812,0.10,L33,R33); Cm (67:197)

1: TOF MS ES-
8.21e7

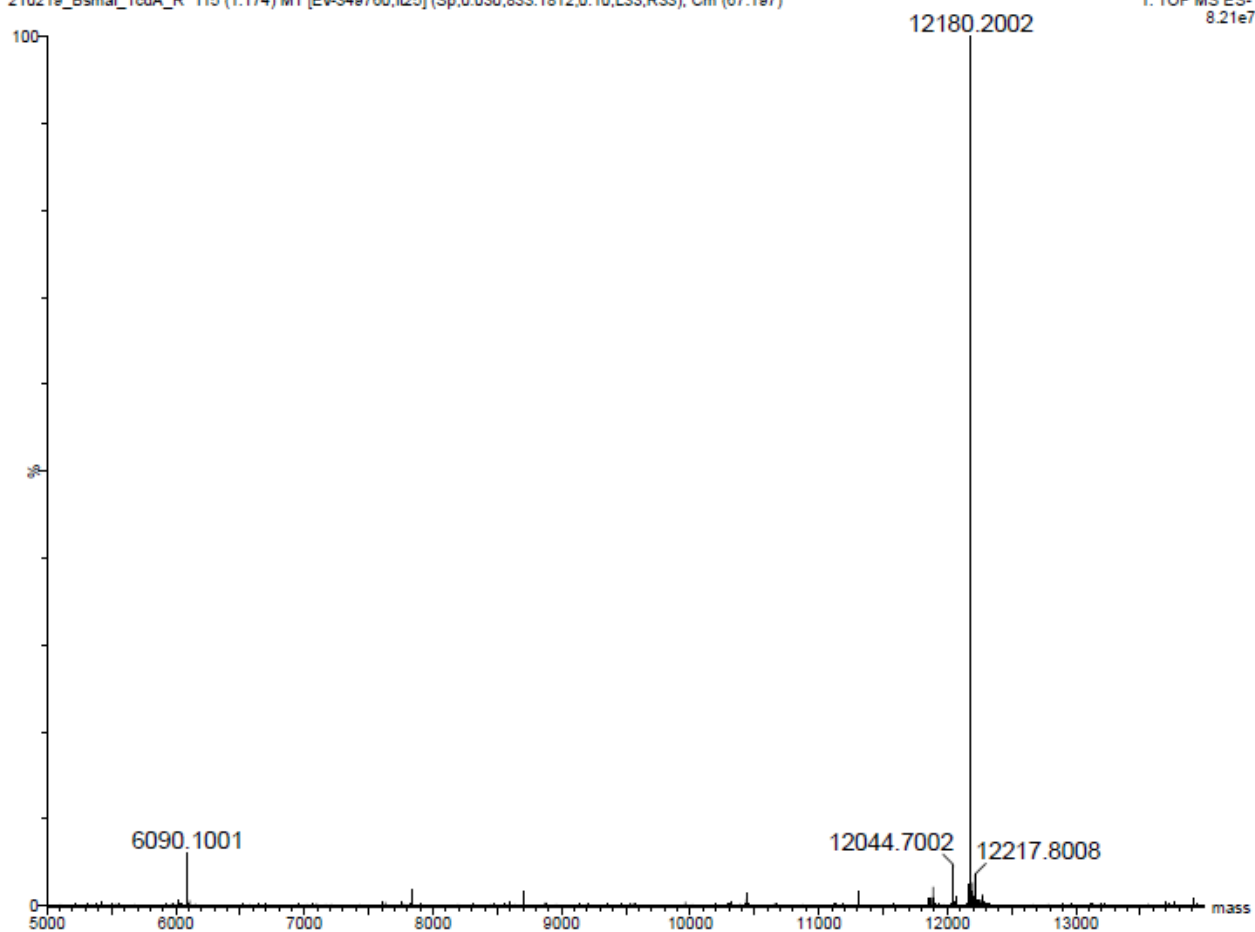


Figure S82. Mass spectrum of sequence 4.

210219_Ssp12_2 73 (0.757) M1 [Ev-380833,l25] (Sp,0.030,798:1823,0.10,L33,R33); Cm (54:197)

1: TOF MS ES-
1.54e8

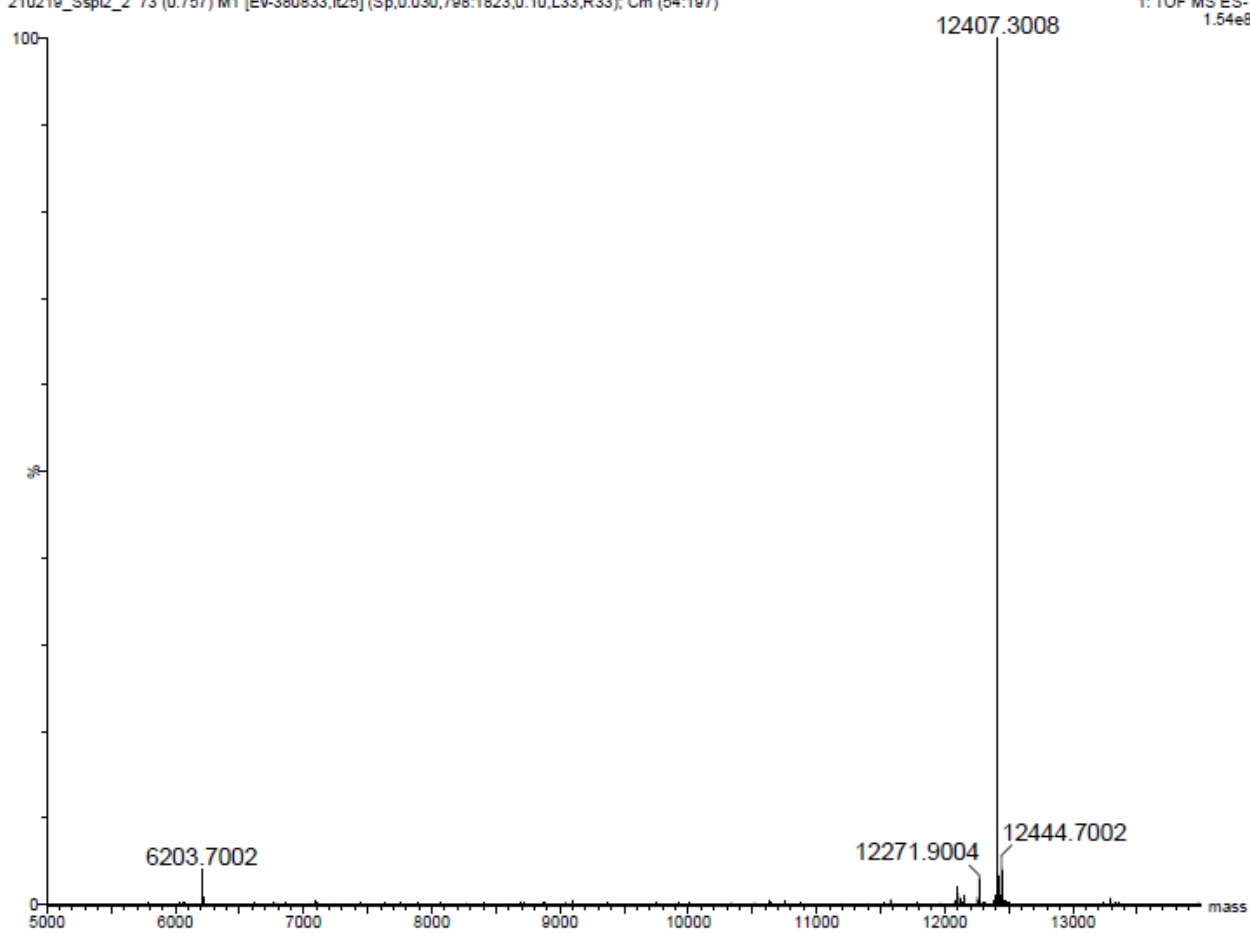


Figure S83. Mass spectrum of sequence 5.

210219_Sspl2_+2_R 83 (0.854) M1 [Ev-366419,lt25] (Sp,0.030,802:1818,0.10,L33,R33); Cm (65:197)

1: TOF MS ES-
1.23e8

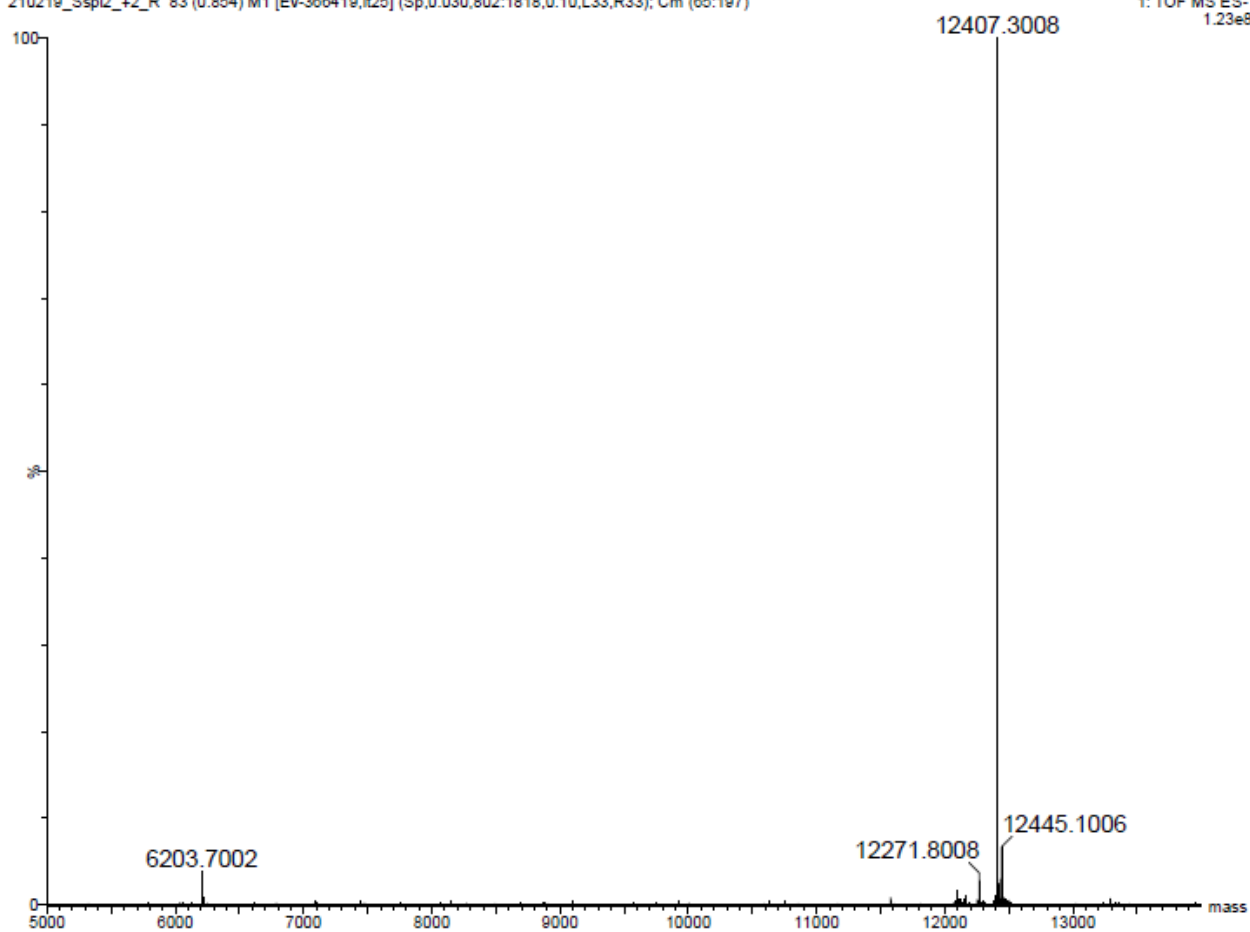


Figure S84. Mass spectrum of sequence 6.

210219_Bsmal_2cdA_ 137 (1.397) M1 [Ev-367698,lt25] (Sp,0.030,809:1803,0.10,L33,R33); Cm (90:196)

1: TOF MS ES-
7.53e7

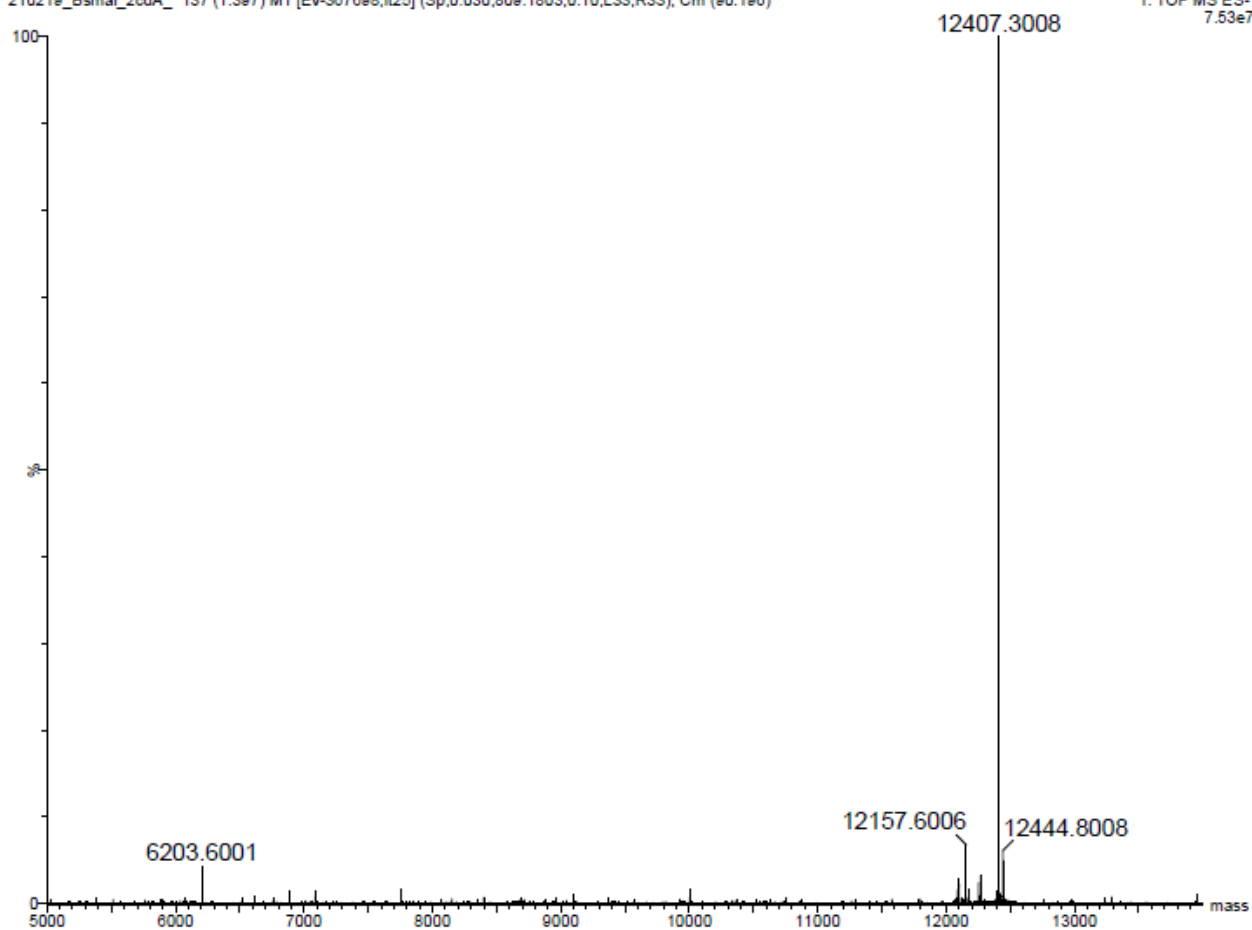


Figure S85. Mass spectrum of sequence 7.

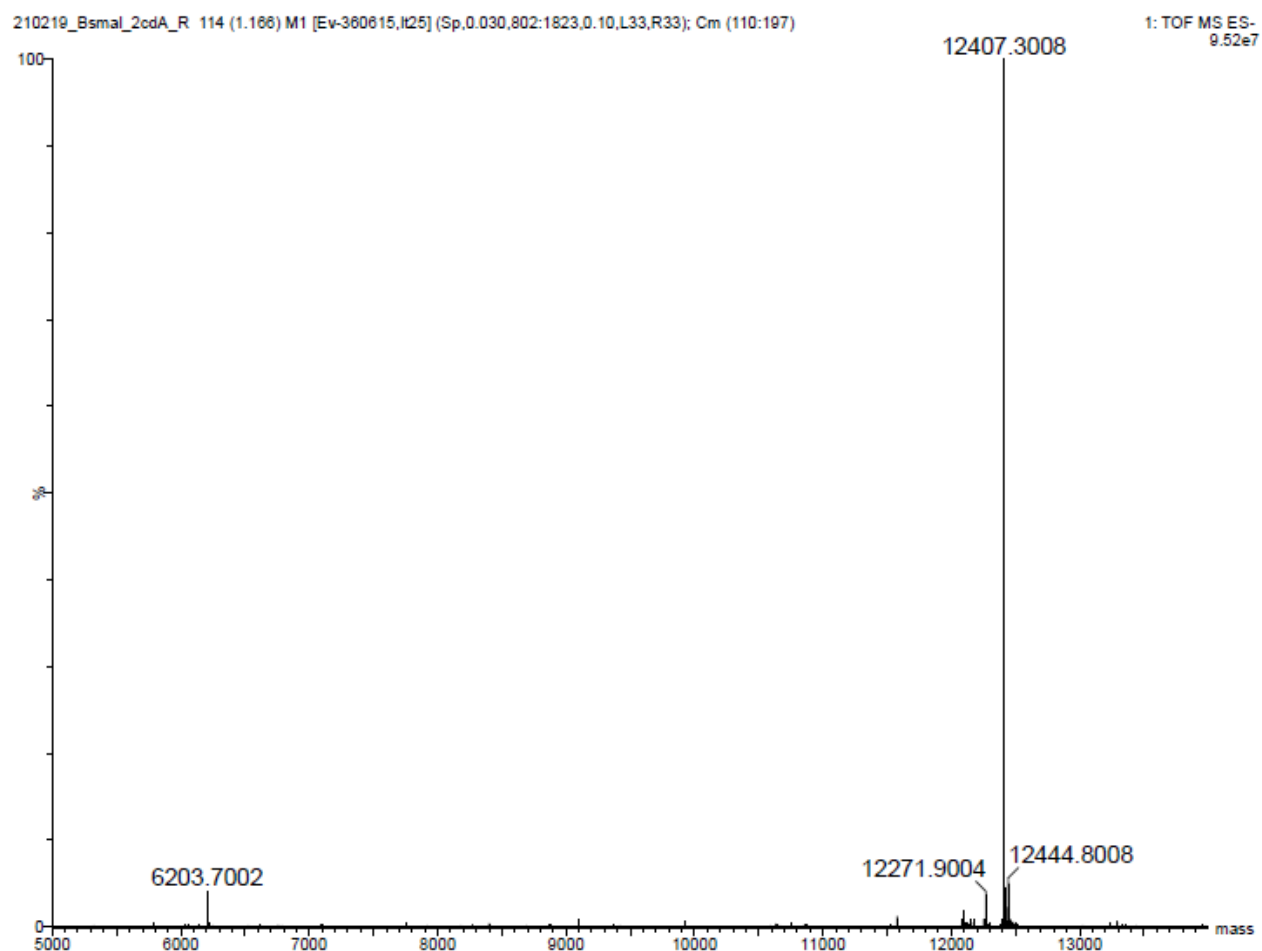


Figure S86. Mass spectrum of sequence 8.

210219_Bsmal_1cdG) 131 (1.334) M1 [Ev-284008,l25] (Sp,0.030,932:1801,0.10,L33,R33); Cm (121:197)

1: TOF MS ES-
4.37e7

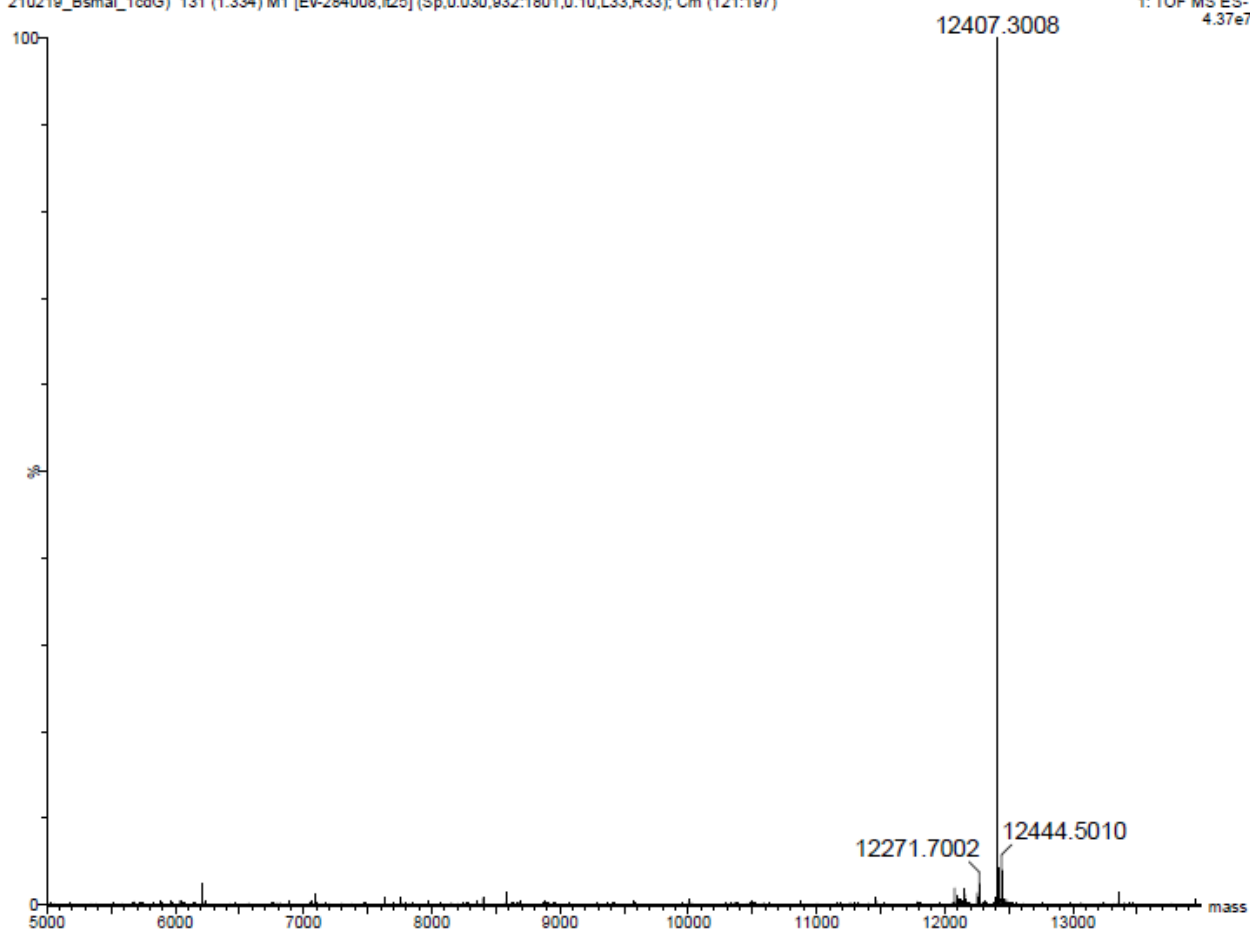


Figure S87. Mass spectrum of sequence 9.

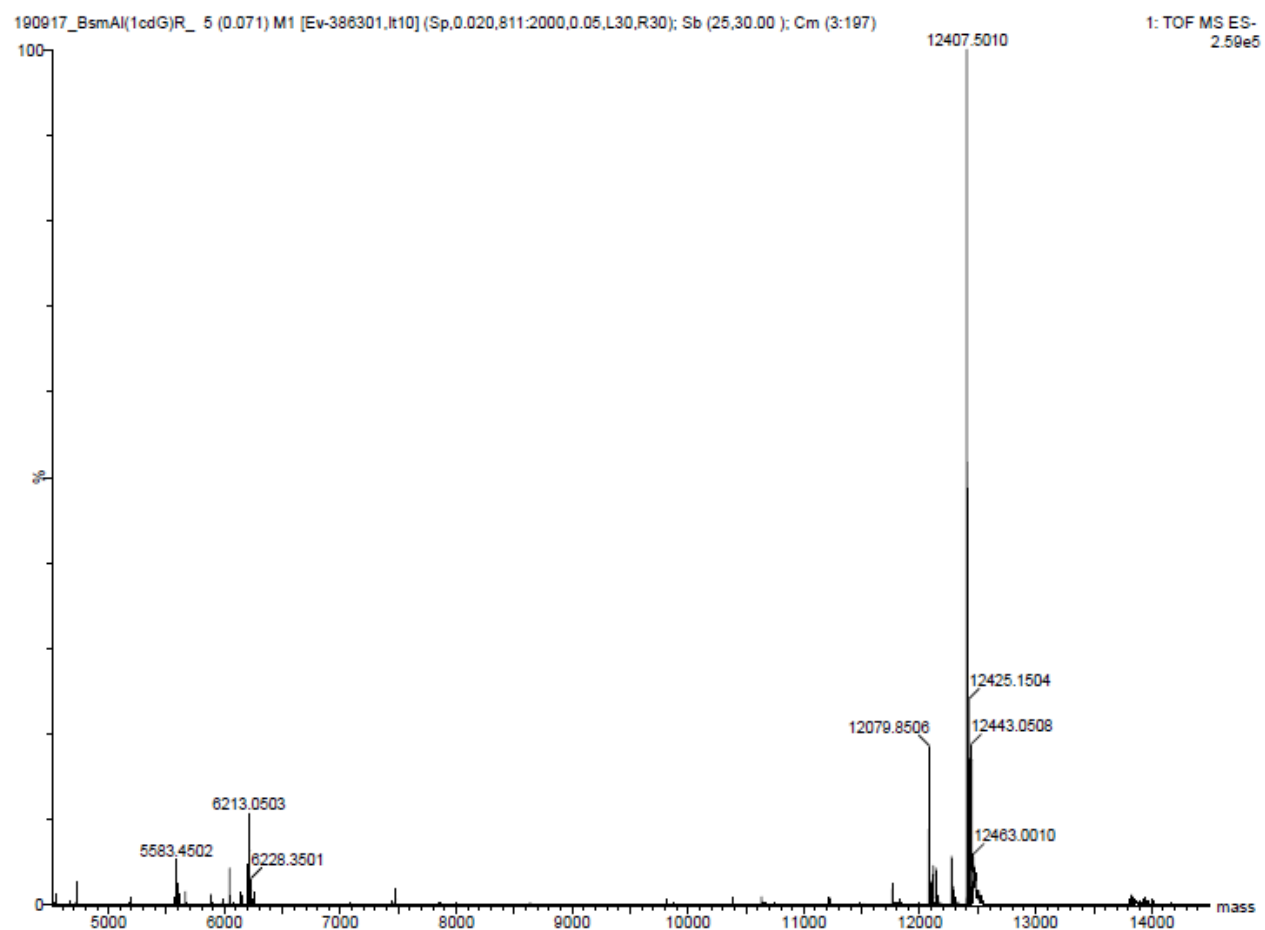


Figure S88. Mass spectrum of sequence 10.

210219_Bsmal_2cdG_sens 81 (0.837) M1 [Ev:406581,lt25] (Sp,0.030,777:1823,0.10,L33,R33); Cm (46:197)

1: TOF MS ES-
1.50e8

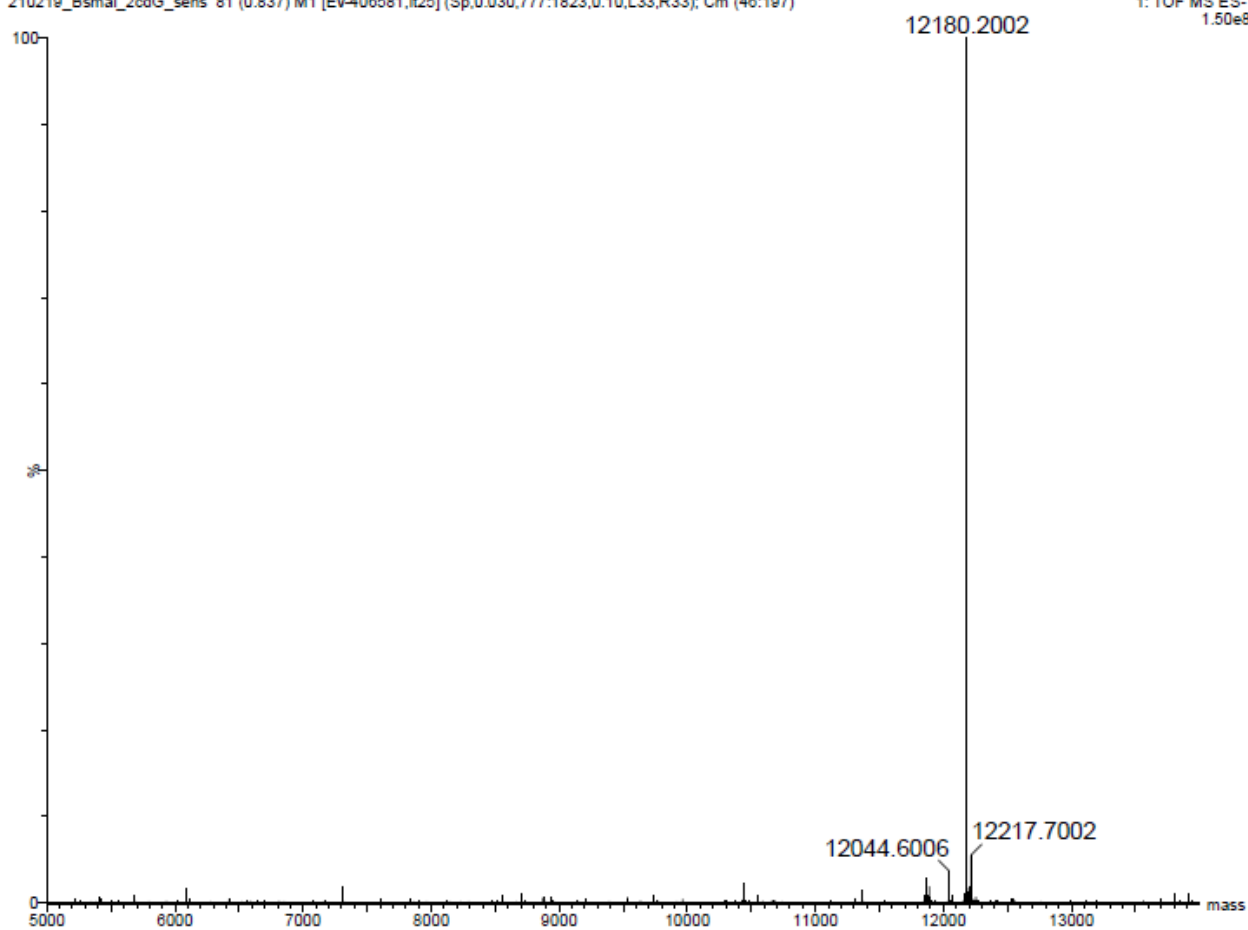


Figure S89. Mass spectrum of sequence 11.

210219_Bsmal_2cdG_antys 81 (0.837) M1 [Ev-336211,lt25] (Sp,0.030,852:1810,0.10,L33,R33); Cm (79:197)

1: TOF MS ES-
6.98e7

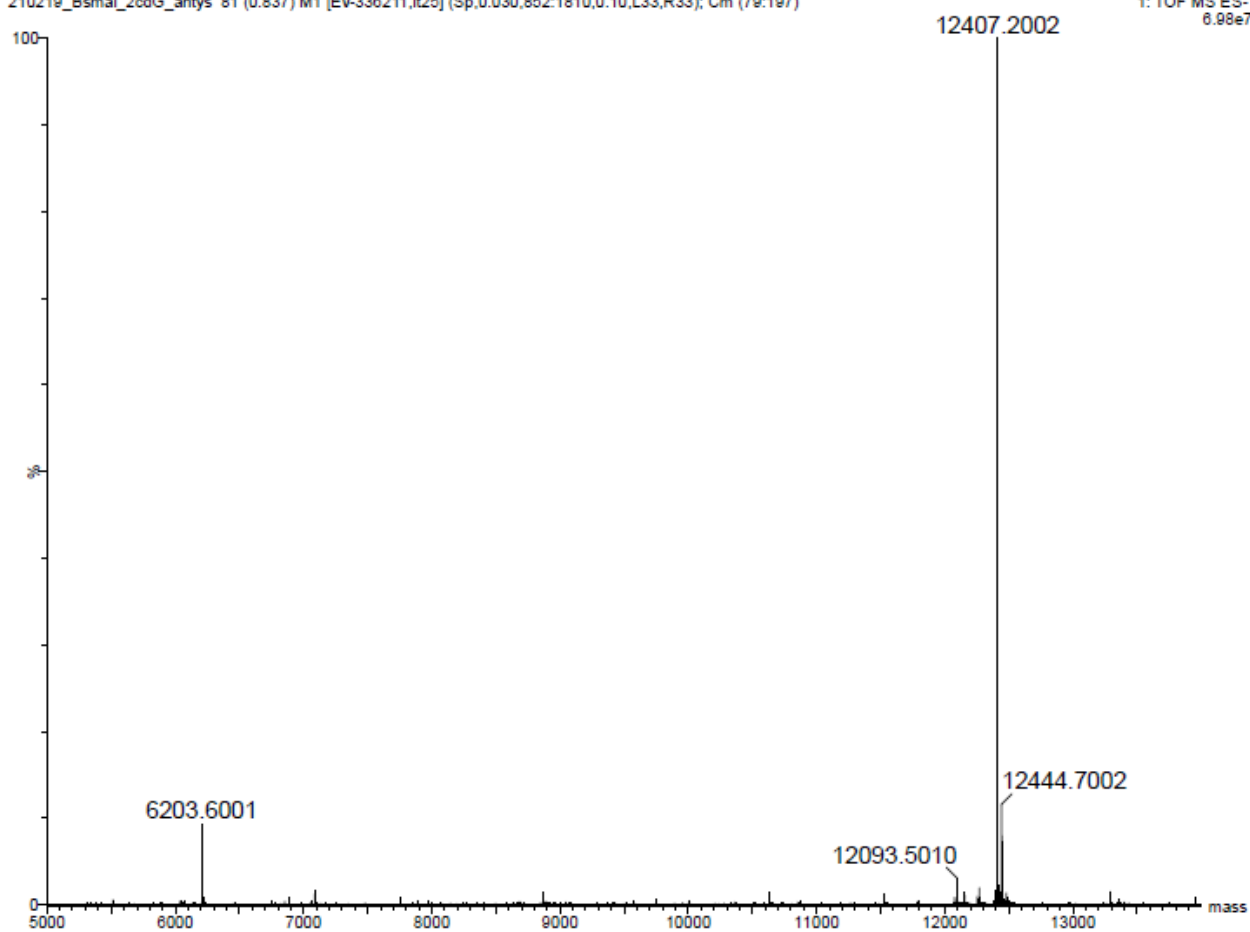


Figure S90. Mass spectrum of sequence 12.

210219_Bsmal_2odG_R_sens 41 (0.437) M1 [Ev-344664,lt25] (Sp,0.030,796:1795,0.10,L33,R33); Cm (30:88)

1: TOF MS ES-
5.71e7

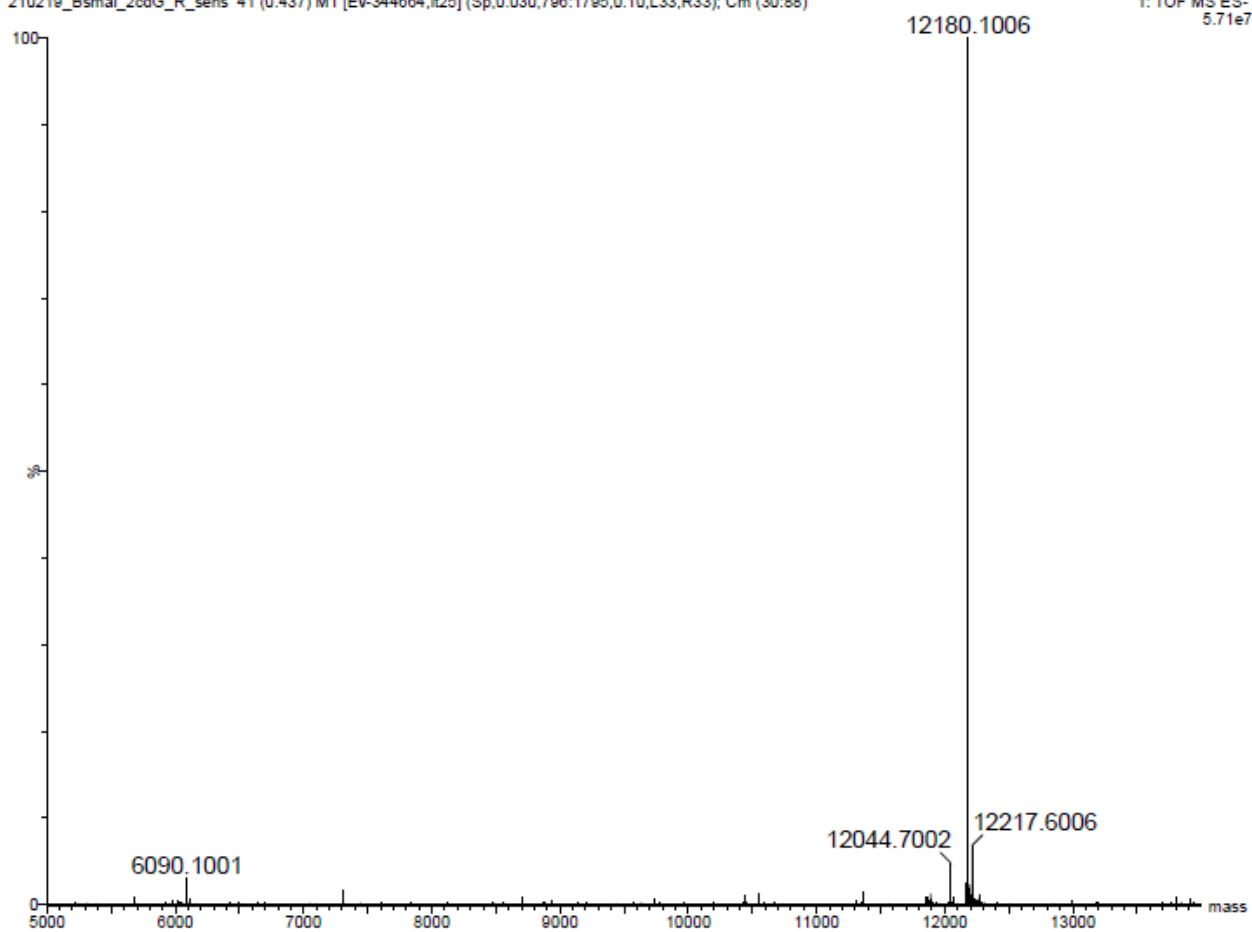


Figure S91. Mass spectrum of sequence 13.

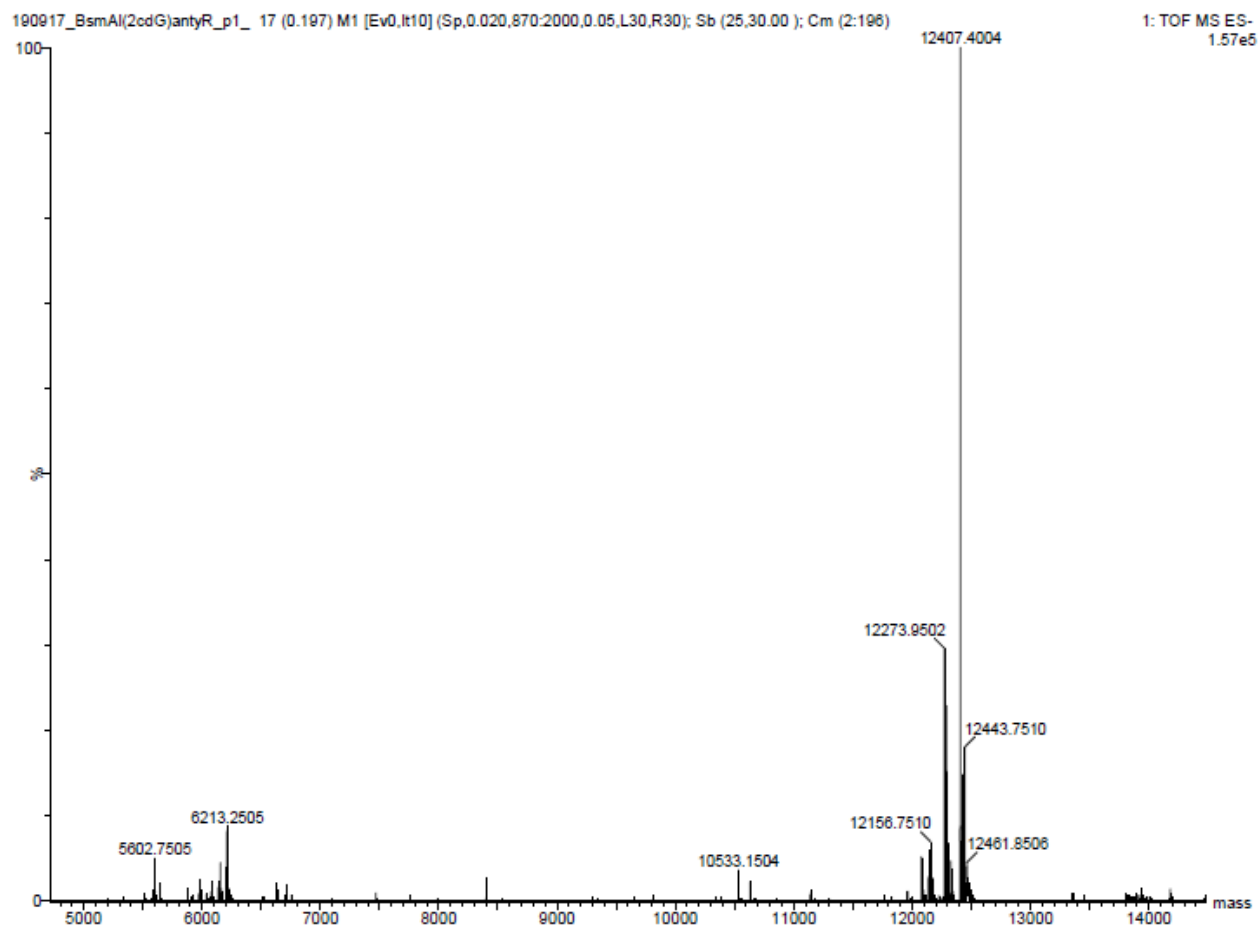


Figure S92. Mass spectrum of sequence 14.

Table S3. The influence of cdPus on the activity of BsmAI. Raw numerical data of bands intensity obtained from Quantity One software.

| BsmAI | | | Time [min] | | | | | | |
|--------|--------|----------|---------------------|------|-------|-------|-------|-------|--------|
| | | | 0 | 1 | 5 | 15 | 30 | 45 | 60 |
| Duplex | Strand | Data set | Strand cleavage [%] | | | | | | |
| A | 1 | 1. | 0,03 | 0,08 | 2,44 | 32,13 | 93,13 | | 99,32 |
| | | 2. | 0,00 | 2,30 | 21,70 | 63,20 | 92,75 | | 96,82 |
| | | 3. | 0,12 | 1,59 | 28,70 | 78,82 | 99,12 | | 100,00 |
| | | Avg | 0,05 | 1,32 | 17,62 | 58,05 | 95,00 | | 98,71 |
| | | SD | 0,06 | 1,13 | 13,60 | 23,77 | 3,57 | | 1,68 |
| | 2 | 1. | 0,00 | 0,00 | 8,61 | 68,13 | 98,67 | | 99,38 |
| | | 2. | 0,97 | 4,33 | 39,65 | 82,72 | 95,06 | | 95,07 |
| | | 3. | 0,00 | 2,71 | 42,67 | 94,24 | 98,86 | | 99,26 |
| | | Avg | 0,32 | 2,35 | 30,31 | 81,70 | 97,53 | | 97,90 |
| | | SD | 0,56 | 2,19 | 18,86 | 13,08 | 2,14 | | 2,45 |
| B | 3 | 1. | 0,08 | 1,03 | 10,18 | 72,40 | 99,78 | 99,70 | 99,44 |
| | | 2. | 0,00 | 0,70 | 13,01 | 69,39 | 98,91 | 99,53 | 90,07 |

| | | | | | | | | | |
|---|----|-----|------|------|-------|-------|-------|-------|-------|
| | | 3. | 0,00 | 1,89 | 17,12 | 75,81 | 99,50 | 99,38 | 96,15 |
| | | Avg | 0,03 | 1,21 | 13,44 | 72,53 | 99,40 | 99,54 | 95,22 |
| | | SD | 0,05 | 0,61 | 3,49 | 3,21 | 0,44 | 0,16 | 4,75 |
| | 4 | 1. | 0,00 | 1,32 | 18,48 | 90,86 | 98,99 | 99,39 | 99,05 |
| | | 2. | 0,00 | 1,22 | 22,26 | 71,10 | 99,06 | 99,24 | 99,13 |
| | | 3. | 0,25 | 1,70 | 14,14 | 84,96 | 97,07 | 98,76 | 99,36 |
| | | Avg | 0,08 | 1,41 | 18,30 | 82,31 | 98,37 | 99,13 | 99,18 |
| C | 5 | SD | 0,14 | 0,25 | 4,06 | 10,15 | 1,13 | 0,33 | 0,16 |
| | | 1. | 0,00 | 2,60 | 31,86 | 89,16 | 99,10 | 98,11 | 99,01 |
| | | 2. | 0,43 | 5,94 | 27,90 | 88,30 | 95,66 | 95,78 | 95,39 |
| | | 3. | 0,00 | 3,57 | 35,63 | 92,49 | 99,62 | 99,44 | 98,77 |
| | | Avg | 0,14 | 4,04 | 31,80 | 89,99 | 98,12 | 97,78 | 97,72 |
| | | SD | 0,25 | 1,72 | 3,87 | 2,21 | 2,15 | 1,85 | 2,03 |
| | 6 | 1. | 0,00 | 2,39 | 26,36 | 85,04 | 98,57 | 98,14 | 96,86 |
| | | 2. | 0,13 | 3,33 | 22,23 | 79,32 | 94,42 | 92,81 | 92,42 |
| | | 3. | 0,20 | 5,12 | 42,24 | 91,17 | 98,88 | 97,80 | 97,67 |
| | | Avg | 0,11 | 3,61 | 30,28 | 85,18 | 97,29 | 96,25 | 95,65 |
| | | SD | 0,10 | 1,39 | 10,57 | 5,93 | 2,49 | 2,98 | 2,82 |
| D | 7 | 1. | 0,01 | 0,00 | 0,00 | 0,15 | 0,29 | 0,46 | 0,54 |
| | | 2. | 0,00 | 0,01 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 3. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | Avg | 0,00 | 0,00 | 0,00 | 0,05 | 0,10 | 0,15 | 0,18 |
| | | SD | 0,00 | 0,01 | 0,00 | 0,09 | 0,17 | 0,26 | 0,31 |
| | 8 | 1. | 0,00 | 0,00 | 0,00 | 0,00 | 0,03 | 0,02 | 0,00 |
| | | 2. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 3. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | Avg | 0,00 | 0,00 | 0,00 | 0,00 | 0,01 | 0,01 | 0,00 |
| | | SD | 0,00 | 0,00 | 0,00 | 0,00 | 0,02 | 0,01 | 0,00 |
| E | 9 | 1. | 0,00 | 0,03 | 1,34 | 9,96 | 33,18 | 42,84 | 36,25 |
| | | 2. | 0,00 | 0,55 | 4,53 | 15,26 | 31,68 | 39,47 | 48,03 |
| | | 3. | 0,01 | 0,09 | 2,96 | 17,35 | 43,74 | 55,53 | 49,32 |
| | | Avg | 0,00 | 0,22 | 2,94 | 14,19 | 36,20 | 45,95 | 44,53 |
| | | SD | 0,01 | 0,28 | 1,59 | 3,81 | 6,57 | 8,47 | 7,20 |
| | 10 | 1. | 0,00 | 0,00 | 1,83 | 7,41 | 22,47 | 44,16 | 41,10 |
| | | 2. | 0,52 | 1,18 | 3,89 | 13,26 | 23,83 | 32,71 | 37,64 |
| | | 3. | 0,63 | 1,53 | 6,72 | 26,26 | 49,73 | 52,33 | 54,74 |
| | | Avg | 0,38 | 0,90 | 4,15 | 15,64 | 32,01 | 43,07 | 44,49 |
| | | SD | 0,34 | 0,80 | 2,45 | 9,65 | 15,36 | 9,85 | 9,04 |
| F | 11 | 1. | 0,92 | 1,46 | 19,19 | 89,49 | 99,70 | 99,84 | 99,84 |
| | | 2. | 0,09 | 1,03 | 17,08 | 73,97 | 99,02 | 98,66 | 98,35 |
| | | 3. | 0,00 | 0,19 | 26,48 | 94,16 | 99,96 | 99,97 | 99,92 |

| | | | | | | | | | |
|---|----|-----|------|------|-------|-------|-------|-------|-------|
| | | Avg | 0,34 | 0,89 | 20,92 | 85,87 | 99,56 | 99,49 | 99,37 |
| | | SD | 0,51 | 0,65 | 4,93 | 10,57 | 0,49 | 0,72 | 0,89 |
| | 12 | 1. | 0,00 | 0,50 | 22,35 | 84,63 | 98,49 | 99,67 | 99,51 |
| | | 2. | 0,00 | 0,92 | 11,74 | 81,80 | 96,75 | 99,08 | 98,61 |
| | | 3. | 0,00 | 2,73 | 32,82 | 91,65 | 99,75 | 99,93 | 99,90 |
| | | Avg | 0,00 | 1,38 | 22,31 | 86,02 | 98,33 | 99,56 | 99,34 |
| | | SD | 0,00 | 1,18 | 10,54 | 5,07 | 1,51 | 0,43 | 0,66 |
| | | | | | | | | | |
| G | 13 | 1. | 0,00 | 0,49 | 14,28 | 71,43 | 98,85 | 98,93 | 99,12 |
| | | 2. | 0,00 | 1,63 | 17,03 | 52,65 | 94,71 | 96,78 | 97,72 |
| | | 3. | 1,24 | 3,61 | 19,53 | 56,36 | 95,49 | 98,15 | 95,66 |
| | | Avg | 0,41 | 1,91 | 16,94 | 60,15 | 96,35 | 97,95 | 97,50 |
| | | SD | 0,72 | 1,58 | 2,63 | 9,95 | 2,20 | 1,09 | 1,74 |
| | 14 | 1. | 0,40 | 4,67 | 23,92 | 64,85 | 95,12 | 97,15 | 94,58 |
| | | 2. | 0,52 | 5,87 | 30,11 | 64,77 | 92,19 | 97,03 | 96,25 |
| | | 3. | 0,02 | 4,14 | 19,79 | 69,41 | 96,16 | 97,40 | 96,64 |
| | | Avg | 0,31 | 4,89 | 24,61 | 66,34 | 94,49 | 97,19 | 95,83 |
| | | SD | 0,26 | 0,88 | 5,19 | 2,66 | 2,06 | 0,19 | 1,09 |
| H | 15 | 1. | 0,13 | 0,77 | 18,92 | 61,18 | 93,62 | 96,78 | 94,55 |
| | | 2. | 0,56 | 1,86 | 16,68 | 70,88 | 93,74 | 93,17 | 94,02 |
| | | 3. | 0,00 | 0,13 | 20,23 | 72,77 | 97,30 | 99,58 | 99,04 |
| | | Avg | 0,23 | 0,92 | 18,61 | 68,28 | 94,89 | 96,51 | 95,87 |
| | | SD | 0,29 | 0,88 | 1,80 | 6,22 | 2,09 | 3,21 | 2,76 |
| | 16 | 1. | 0,00 | 0,00 | 4,81 | 38,53 | 54,82 | 74,45 | 74,14 |
| | | 2. | 0,00 | 0,00 | 2,38 | 26,67 | 55,98 | 67,21 | 65,06 |
| | | 3. | 0,31 | 1,47 | 10,15 | 46,88 | 79,22 | 85,53 | 87,41 |
| | | Avg | 0,10 | 0,49 | 5,78 | 37,36 | 63,34 | 75,73 | 75,54 |
| | | SD | 0,18 | 0,85 | 3,97 | 10,15 | 13,76 | 9,23 | 11,24 |
| I | 17 | 1. | 0,40 | 3,51 | 24,71 | 67,47 | 88,06 | 88,92 | 89,59 |
| | | 2. | 0,00 | 4,34 | 24,95 | 65,47 | 86,61 | 90,49 | 90,66 |
| | | 3. | 0,74 | 6,07 | 30,44 | 72,58 | 91,64 | 92,79 | 89,70 |
| | | Avg | 0,38 | 4,64 | 26,70 | 68,51 | 88,77 | 90,73 | 89,99 |
| | | SD | 0,37 | 1,31 | 3,24 | 3,67 | 2,59 | 1,94 | 0,59 |
| | 18 | 1. | 0,82 | 3,77 | 16,63 | 43,86 | 63,25 | 68,22 | 73,44 |
| | | 2. | 1,49 | 4,45 | 16,54 | 40,31 | 74,52 | 71,87 | 71,29 |
| | | 3. | 0,00 | 3,11 | 14,94 | 45,21 | 83,58 | 79,55 | 76,87 |
| | | Avg | 0,77 | 3,78 | 16,04 | 43,13 | 73,78 | 73,21 | 73,87 |
| | | SD | 0,75 | 0,67 | 0,95 | 2,53 | 10,18 | 5,78 | 2,81 |
| J | 19 | 1. | 0,00 | 0,12 | 2,48 | 31,98 | 69,29 | 87,67 | 82,97 |
| | | 2. | 0,00 | 0,00 | 0,35 | 16,44 | 69,66 | 93,46 | 85,55 |
| | | 3. | 0,00 | 0,14 | 1,52 | 24,17 | 62,86 | 89,04 | 88,41 |
| | | Avg | 0,00 | 0,09 | 1,45 | 24,20 | 67,27 | 90,06 | 85,65 |

| | | | | | | | | | |
|---|----|-----|-------|-------|-------|-------|-------|-------|-------|
| | | SD | 0,00 | 0,07 | 1,07 | 7,77 | 3,82 | 3,03 | 2,72 |
| | | 1. | 0,39 | 0,48 | 0,67 | 0,88 | 1,32 | 1,58 | 1,72 |
| | | 2. | 1,66 | 1,62 | 2,29 | 3,58 | 5,94 | 7,21 | 7,28 |
| | | 3. | 1,84 | 1,95 | 2,29 | 3,51 | 5,29 | 5,84 | 5,80 |
| | | Avg | 1,30 | 1,35 | 1,75 | 2,65 | 4,18 | 4,88 | 4,93 |
| | | SD | 0,79 | 0,77 | 0,94 | 1,54 | 2,50 | 2,94 | 2,88 |
| K | 21 | 1. | 0,00 | 0,07 | 0,49 | 4,10 | 13,23 | 21,79 | 24,12 |
| | | 2. | 0,00 | 0,00 | 0,00 | 1,73 | 10,06 | 20,97 | 21,18 |
| | | 3. | 0,16 | 0,25 | 0,46 | 4,54 | 12,04 | 19,40 | 19,35 |
| | | Avg | 0,05 | 0,11 | 0,32 | 3,46 | 11,78 | 20,72 | 21,55 |
| | | SD | 0,09 | 0,13 | 0,28 | 1,51 | 1,60 | 1,22 | 2,40 |
| | 22 | 1. | 2,72 | 2,81 | 3,38 | 4,38 | 8,24 | 11,77 | 14,29 |
| | | 2. | 12,54 | 10,78 | 12,75 | 15,29 | 22,03 | 28,11 | 28,14 |
| | | 3. | 9,81 | 8,06 | 8,96 | 10,78 | 15,67 | 21,13 | 22,93 |
| | | Avg | 8,36 | 7,22 | 8,36 | 10,15 | 15,31 | 20,33 | 21,78 |
| | | SD | 5,07 | 4,05 | 4,71 | 5,48 | 6,90 | 8,20 | 7,00 |
| L | 23 | 1. | 0,08 | 1,01 | 9,91 | 39,00 | 49,55 | 47,99 | 52,19 |
| | | 2. | 0,14 | 2,85 | 12,13 | 38,25 | 48,85 | 59,89 | 56,63 |
| | | 3. | 0,00 | 2,22 | 13,03 | 34,87 | 56,11 | 60,09 | 55,30 |
| | | Avg | 0,07 | 2,03 | 11,69 | 37,37 | 51,50 | 55,99 | 54,71 |
| | | SD | 0,07 | 0,94 | 1,61 | 2,20 | 4,01 | 6,93 | 2,28 |
| | 24 | 1. | 0,00 | 0,00 | 2,15 | 8,33 | 16,71 | 12,83 | 13,05 |
| | | 2. | 0,00 | 0,00 | 1,86 | 6,38 | 14,39 | 13,98 | 17,56 |
| | | 3. | 0,00 | 0,00 | 3,13 | 13,86 | 15,14 | 19,59 | 27,37 |
| | | Avg | 0,00 | 0,00 | 2,38 | 9,52 | 15,41 | 15,47 | 19,33 |
| | | SD | 0,00 | 0,00 | 0,67 | 3,88 | 1,19 | 3,62 | 7,32 |
| M | 26 | 1. | 3,90 | 3,88 | 6,07 | 15,08 | 25,00 | 26,85 | 23,91 |
| | | 2. | 16,22 | 14,73 | 16,87 | 22,92 | 35,97 | 37,36 | 33,50 |
| | | 3. | 9,59 | 10,04 | 10,65 | 17,87 | 24,43 | 24,28 | 20,34 |
| | | Avg | 9,90 | 9,55 | 11,20 | 18,62 | 28,47 | 29,50 | 25,92 |
| | | SD | 6,17 | 5,44 | 5,42 | 3,97 | 6,51 | 6,93 | 6,81 |
| N | 27 | 1. | 0,00 | 0,44 | 10,08 | 41,33 | 58,28 | 67,25 | 67,92 |
| | | 2. | 0,00 | 1,35 | 13,38 | 42,41 | 63,41 | 62,84 | 72,20 |
| | | 3. | 0,00 | 2,77 | 16,60 | 42,59 | 60,11 | 65,77 | 67,91 |
| | | Avg | 0,00 | 1,52 | 13,35 | 42,11 | 60,60 | 65,29 | 69,34 |
| | | SD | 0,00 | 1,18 | 3,26 | 0,68 | 2,60 | 2,25 | 2,48 |
| | 28 | 1. | 0,00 | 0,72 | 3,12 | 12,91 | 32,35 | 29,83 | 29,70 |
| | | 2. | 0,00 | 0,00 | 1,06 | 12,50 | 31,67 | 28,39 | 22,48 |
| | | 3. | 0,84 | 1,65 | 7,22 | 20,21 | 34,03 | 33,62 | 30,20 |
| | | Avg | 0,28 | 0,79 | 3,80 | 15,21 | 32,68 | 30,61 | 27,46 |
| | | SD | 0,48 | 0,83 | 3,14 | 4,33 | 1,21 | 2,70 | 4,32 |

| | | | | | | | | | |
|---|----|-----|-------|-------|-------|-------|-------|-------|-------|
| O | 30 | 1. | 4,02 | 4,43 | 6,37 | 16,48 | 22,70 | 22,61 | 23,20 |
| | | 2. | 11,90 | 13,03 | 15,48 | 21,79 | 27,81 | 31,66 | 29,41 |
| | | 3. | 7,32 | 6,25 | 7,71 | 12,91 | 16,57 | 12,99 | 11,03 |
| | | Avg | 7,74 | 7,91 | 9,86 | 17,06 | 22,36 | 22,42 | 21,21 |
| | | SD | 3,96 | 4,53 | 4,92 | 4,47 | 5,63 | 9,34 | 9,35 |

Table S4. The influence of cdPus on the activity of SspI. Raw numerical data of bands intensity obtained from Quantity One software.

| SspI | | | Time [min] | | | | | | |
|--------|--------|----------|---------------------|------|------|-------|-------|------|-------|
| | | | 0 | 1 | 5 | 15 | 30 | 45 | 60 |
| Duplex | Strand | Data set | Strand cleavage [%] | | | | | | |
| A | 1 | 1. | 0,08 | 0,36 | 2,73 | 14,48 | 46,87 | | 83,75 |
| | | 2. | 0,37 | 1,17 | 4,79 | 16,21 | 37,64 | | 85,93 |
| | | 3. | 0,00 | 0,00 | 2,84 | 24,16 | 49,31 | | 87,62 |
| | | Avg | 0,15 | 0,51 | 3,45 | 18,29 | 44,60 | | 85,77 |
| | | SD | 0,19 | 0,60 | 1,16 | 5,16 | 6,15 | | 1,94 |
| | 2 | 1. | 0,02 | 0,13 | 1,85 | 19,74 | 60,40 | | 88,89 |
| | | 2. | 0,00 | 0,16 | 3,94 | 17,60 | 47,57 | | 72,72 |
| | | 3. | 0,37 | 0,86 | 6,14 | 23,15 | 40,29 | | 85,02 |
| | | Avg | 0,13 | 0,39 | 3,97 | 20,17 | 49,42 | | 82,21 |
| | | SD | 0,21 | 0,41 | 2,15 | 2,80 | 10,18 | | 8,44 |
| B | 3 | 1. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 2. | 0,00 | 0,00 | 0,00 | 0,00 | 0,25 | 0,00 | 0,11 |
| | | 3. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | Avg | 0,00 | 0,00 | 0,00 | 0,00 | 0,08 | 0,00 | 0,04 |
| | | SD | 0,00 | 0,00 | 0,00 | 0,00 | 0,15 | 0,00 | 0,06 |
| | 4 | 1. | 0,00 | 0,00 | 0,13 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 2. | 0,07 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 3. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,02 | 0,02 |
| | | Avg | 0,02 | 0,00 | 0,04 | 0,00 | 0,00 | 0,01 | 0,01 |
| | | SD | 0,04 | 0,00 | 0,08 | 0,00 | 0,00 | 0,01 | 0,01 |
| C | 5 | 1. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 2. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 3. | 0,37 | 0,26 | 0,28 | 0,11 | 0,04 | 0,12 | 0,35 |
| | | Avg | 0,12 | 0,09 | 0,09 | 0,04 | 0,01 | 0,04 | 0,12 |
| | | SD | 0,21 | 0,15 | 0,16 | 0,06 | 0,02 | 0,07 | 0,20 |
| | 6 | 1. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 2. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 3. | 0,13 | 0,16 | 0,12 | 0,21 | 0,10 | 0,04 | 0,01 |
| | | Avg | 0,04 | 0,05 | 0,04 | 0,07 | 0,03 | 0,01 | 0,00 |
| | | SD | 0,08 | 0,09 | 0,07 | 0,12 | 0,06 | 0,02 | 0,00 |

| | | | | | | | | | |
|---|----|-----|------|------|------|-------|-------|-------|-------|
| D | 7 | 1. | 0,06 | 0,47 | 3,50 | 27,30 | 55,23 | 79,33 | 94,81 |
| | | 2. | 0,00 | 0,00 | 1,38 | 18,20 | 48,04 | 67,24 | 92,72 |
| | | 3. | 0,00 | 0,00 | 1,80 | 14,96 | 50,93 | 63,46 | 98,73 |
| | | Avg | 0,02 | 0,16 | 2,22 | 20,15 | 51,40 | 70,01 | 95,42 |
| | | SD | 0,04 | 0,27 | 1,12 | 6,40 | 3,62 | 8,29 | 3,05 |
| | 8 | 1. | 0,01 | 0,16 | 2,62 | 33,42 | 54,89 | 66,74 | 80,17 |
| | | 2. | 0,07 | 0,47 | 2,17 | 13,71 | 47,75 | 74,59 | 92,86 |
| | | 3. | 0,00 | 0,00 | 0,49 | 15,44 | 44,40 | 84,93 | 94,09 |
| | | Avg | 0,03 | 0,21 | 1,76 | 20,86 | 49,01 | 75,42 | 89,04 |
| | | SD | 0,04 | 0,24 | 1,12 | 10,91 | 5,35 | 9,13 | 7,71 |
| E | 9 | 1. | 0,00 | 0,63 | 5,99 | 32,31 | 71,12 | 91,25 | 97,23 |
| | | 2. | 0,74 | 1,37 | 4,07 | 21,52 | 56,38 | 87,71 | 94,68 |
| | | 3. | 0,00 | 0,59 | 4,86 | 28,14 | 62,66 | 79,19 | 94,94 |
| | | Avg | 0,25 | 0,86 | 4,97 | 27,32 | 63,39 | 86,05 | 95,62 |
| | | SD | 0,43 | 0,44 | 0,96 | 5,44 | 7,40 | 6,20 | 1,40 |
| | 10 | 1. | 0,00 | 0,06 | 4,86 | 30,40 | 72,92 | 94,44 | 97,96 |
| | | 2. | 0,00 | 0,28 | 2,78 | 21,99 | 62,68 | 89,16 | 91,44 |
| | | 3. | 0,25 | 0,79 | 5,83 | 24,66 | 67,39 | 84,05 | 93,40 |
| | | Avg | 0,08 | 0,37 | 4,49 | 25,68 | 67,66 | 89,22 | 94,27 |
| | | SD | 0,14 | 0,37 | 1,56 | 4,30 | 5,13 | 5,19 | 3,34 |
| F | 11 | 1. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 2. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 3. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | Avg | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | SD | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | 12 | 1. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 2. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 3. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | Avg | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | SD | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| G | 13 | 1. | 0,09 | 0,11 | 0,12 | 1,01 | 0,35 | 0,69 | 1,81 |
| | | 2. | 0,14 | 0,00 | 0,00 | 0,13 | 0,06 | 0,00 | 0,00 |
| | | 3. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | Avg | 0,08 | 0,04 | 0,04 | 0,38 | 0,14 | 0,23 | 0,60 |
| | | SD | 0,07 | 0,06 | 0,07 | 0,55 | 0,19 | 0,40 | 1,05 |
| | 14 | 1. | 1,02 | 0,31 | 0,49 | 0,06 | 0,00 | 0,00 | 0,00 |
| | | 2. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | 3. | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | | Avg | 0,34 | 0,10 | 0,16 | 0,02 | 0,00 | 0,00 | 0,00 |
| | | SD | 0,59 | 0,18 | 0,28 | 0,03 | 0,00 | 0,00 | 0,00 |
| H | 15 | 1. | 0,00 | 0,00 | 2,31 | 12,34 | 38,45 | 66,54 | 83,94 |

| | | | | | | | | | |
|--|--|-----|------|------|-------|-------|-------|-------|-------|
| | | 2. | 0,47 | 1,36 | 4,51 | 14,39 | 36,84 | 53,96 | 75,05 |
| | | 3. | 0,00 | 0,05 | 1,32 | 14,89 | 35,23 | 59,59 | 82,67 |
| | | Avg | 0,16 | 0,47 | 2,71 | 13,87 | 36,84 | 60,03 | 80,55 |
| | | SD | 0,27 | 0,77 | 1,63 | 1,35 | 1,61 | 6,30 | 4,81 |
| | | 16 | 1. | 0,00 | 0,00 | 1,72 | 13,34 | 42,17 | 93,28 |
| | | 2. | 0,00 | 0,00 | 2,17 | 15,22 | 32,27 | 53,84 | 60,49 |
| | | 3. | 0,00 | 0,00 | 2,33 | 15,43 | 38,80 | 50,35 | 82,63 |
| | | Avg | 0,00 | 0,00 | 2,07 | 14,66 | 37,74 | 60,68 | 78,80 |
| | | SD | 0,00 | 0,00 | 0,31 | 1,15 | 5,04 | 14,96 | 16,73 |
| | | I | 17 | 1. | 0,00 | 0,82 | 2,92 | 17,79 | 37,52 |
| | | 2. | 2,24 | 3,28 | 6,20 | 22,34 | 43,38 | 65,53 | 73,41 |
| | | 3. | 0,00 | 0,93 | 6,68 | 25,21 | 54,69 | 74,13 | 87,41 |
| | | Avg | 0,75 | 1,68 | 5,26 | 21,78 | 45,20 | 65,70 | 78,02 |
| | | SD | 1,29 | 1,39 | 2,05 | 3,74 | 8,73 | 8,34 | 8,14 |
| | | 18 | 1. | 0,00 | 0,03 | 1,79 | 19,21 | 38,94 | 65,55 |
| | | 2. | 0,00 | 0,75 | 7,30 | 28,89 | 51,60 | 66,14 | 82,84 |
| | | 3. | 0,00 | 0,00 | 4,08 | 20,79 | 49,54 | 83,06 | 91,21 |
| | | Avg | 0,00 | 0,26 | 4,39 | 22,96 | 46,70 | 71,58 | 86,63 |
| | | SD | 0,00 | 0,42 | 2,77 | 5,19 | 6,79 | 9,94 | 4,24 |
| | | J | 19 | 1. | 0,00 | 0,20 | 2,42 | 16,63 | 39,34 |
| | | 2. | 0,14 | 0,96 | 4,82 | 23,30 | 45,63 | 66,37 | 80,01 |
| | | 3. | 0,00 | 0,00 | 2,26 | 15,45 | 38,10 | 55,56 | 67,57 |
| | | Avg | 0,05 | 0,39 | 3,17 | 18,46 | 41,02 | 56,58 | 73,70 |
| | | SD | 0,08 | 0,51 | 1,44 | 4,23 | 4,04 | 9,32 | 6,22 |
| | | 20 | 1. | 0,04 | 0,46 | 11,22 | 39,73 | 56,74 | 73,61 |
| | | 2. | 0,00 | 0,17 | 10,08 | 35,21 | 44,93 | 56,57 | 78,79 |
| | | 3. | 0,30 | 0,73 | 8,18 | 38,37 | 51,62 | 78,38 | 91,28 |
| | | Avg | 0,11 | 0,45 | 9,83 | 37,77 | 51,10 | 69,52 | 84,51 |
| | | SD | 0,16 | 0,28 | 1,53 | 2,32 | 5,92 | 11,46 | 6,31 |
| | | K | 21 | 1. | 0,00 | 0,00 | 1,13 | 7,87 | 17,34 |
| | | 2. | 0,00 | 0,43 | 3,05 | 24,52 | 56,18 | 74,87 | 88,33 |
| | | 3. | 0,00 | 0,34 | 2,88 | 12,07 | 31,04 | 57,92 | 60,86 |
| | | Avg | 0,00 | 0,26 | 2,35 | 14,82 | 34,85 | 56,29 | 68,24 |
| | | SD | 0,00 | 0,23 | 1,06 | 8,66 | 19,69 | 19,46 | 17,61 |
| | | 22 | 1. | 0,10 | 0,04 | 0,57 | 4,78 | 17,09 | 33,38 |
| | | 2. | 0,00 | 0,00 | 0,86 | 9,35 | 19,13 | 22,79 | 45,87 |
| | | 3. | 0,00 | 0,00 | 1,56 | 12,57 | 25,99 | 39,94 | 48,76 |
| | | Avg | 0,03 | 0,01 | 1,00 | 8,90 | 20,74 | 32,04 | 46,28 |
| | | SD | 0,06 | 0,02 | 0,51 | 3,91 | 4,66 | 8,66 | 2,30 |
| | | L | 23 | 1. | 0,52 | 0,60 | 0,67 | 0,64 | 0,60 |
| | | 2. | 0,20 | 0,72 | 0,39 | 0,64 | 0,93 | 0,93 | 1,28 |

| | | End | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | End | Cleavage (60min) [%] | | | |
|---|----|-----|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|----------------------|-------|-------|-------|
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | BsmAI | SspI | | |
| A | 1 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | A | T | A | T | T | G | T | C | T | C | T | A | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | 98,71 | 85,71 | | |
| | 2 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | T | A | A | C | A | G | A | G | A | T | A | T | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 97,90 | 82,21 |
| B | 3 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | A | T | SA | T | T | G | T | C | T | C | T | A | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | 95,22 | 0,04 | | |
| | 4 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | T | A | A | C | A | G | A | G | A | T | A | T | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 99,18 | 0,01 |
| C | 5 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | A | T | RA | T | T | G | T | C | T | C | T | A | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | 97,72 | 0,12 | | |
| | 6 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | T | A | A | C | A | G | A | G | A | T | A | T | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 95,65 | 0,00 |
| D | 7 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | A | T | A | T | T | G | T | C | T | C | T | SA | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | 0,18 | 95,42 | | |
| | 8 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | T | A | A | C | A | G | A | G | A | T | A | T | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 0,00 | 89,04 |
| E | 9 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | A | T | A | T | T | G | T | C | T | C | T | RA | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | 44,53 | 95,62 | | |
| | 10 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | T | A | A | C | A | G | A | G | A | T | A | T | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 44,49 | 94,27 |
| F | 11 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | A | T | A | T | T | G | T | C | T | C | T | A | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | 99,37 | 0,00 | | |
| | 12 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | SA | T | A | A | C | A | G | A | G | A | T | A | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 99,34 | 0,00 |
| G | 13 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | A | T | A | T | T | G | T | C | T | C | T | A | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | 97,50 | 0,60 | | |
| | 14 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | RA | T | A | A | C | A | G | A | G | A | T | A | T | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 95,83 |
| H | 15 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | A | T | A | T | T | G | T | C | T | C | T | A | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | 95,87 | 80,55 | | |
| | 16 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | T | A | A | C | A | G | A | G | SA | T | A | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 75,54 | 78,80 | |
| I | 17 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | A | T | A | T | T | G | T | C | T | C | T | A | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | 89,99 | 78,02 | | |
| | 18 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | T | A | A | C | A | G | A | G | RA | T | A | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 73,87 | 86,63 | |
| J | 19 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | A | T | A | T | T | G | T | C | T | C | T | A | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | 85,65 | 73,70 | | |
| | 20 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | T | A | A | C | A | G | A | G | A | T | A | T | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 4,93 | 84,51 |
| K | 21 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | A | T | A | T | T | G | T | C | T | C | T | A | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | 21,55 | 68,24 | | |
| | 22 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | T | A | A | C | A | G | A | G | A | T | A | T | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 21,78 | 46,28 |
| L | 23 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | SA | T | A | T | T | G | T | C | T | C | T | A | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | 54,71 | 0,66 | | |
| | 24 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | T | A | A | C | A | G | A | SG | A | T | A | T | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 19,33 | 0,15 |
| M | 25 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | RA | T | A | T | T | G | T | C | T | C | T | A | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | N/A | | | |
| | 26 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | T | A | A | C | A | G | A | RG | A | T | A | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 25,92 | 0,21 | |
| N | 27 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | RA | T | A | T | T | G | T | C | T | C | T | A | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | 69,34 | 0,79 | | |
| | 28 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | T | A | A | C | A | G | A | SG | A | T | A | T | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 27,46 | 0,35 |
| O | 29 | 5' | C | T | C | T | T | G | T | C | A | G | G | A | SA | T | A | T | T | G | T | C | T | C | T | A | T | G | C | T | C | C | C | A | C | C | A | A | A | G | G | C | 3' | N/A | | | |
| | 30 | 3' | G | A | G | A | A | C | A | G | T | C | C | T | T | A | T | A | A | C | A | G | A | RG | A | T | A | T | C | G | A | T | C | G | G | T | G | G | T | T | T | C | C | G | 5' | 21,21 | 0,00 |

Figure S93. The sequence of substrate oligonucleotides containing 5',8-cyclo-2'-deoxypurines (cdPus). SX - (5'S)-5'8-cyclo-2'-deoxyadenosine; RX - (5'R)-5'8-cyclo-2'-deoxyadenosine; SY - (5'S)-5'8-cyclo-2'-deoxyguanosine; RY - (5'R)-5'8-cyclo-2'-deoxyguanosine. The cleavage value of each strand after 60 min by 0.5 U BsmAI and 1.5 U SspI is shown.