

Supporting Information for:

Replication Past the N⁵-Methyl-Formamidopyrimidine Lesion of Deoxyguanosine by DNA Polymerases and an Improved Procedure for Sequence Analysis of In Vitro Bypass Products by Mass Spectrometry.

Plamen P. Christov, Karen C. Angel, F. Peter Guengerich, Carmelo J. Rizzo*

Department of Chemistry and Biochemistry, Center in Molecular Toxicology, and Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN 37235-1822

Table of Contents

Figure S1.	Structure of the Biotinylated primer.....	S3
Figure S2.	MALDI -TOF spectrum of oligonucleotide 1	S3
Figure S3.	MALDI -TOF spectrum of oligonucleotide 2	S3
Figure S4.	Enzyme digestion of oligonucleotide 1	S4
Figure S5.	Enzyme digestion of oligonucleotide 2	S4
Figure S6.	Capillary gel electrophoretic analysis of oligonucleotide 1	S5
Figure S7.	Capillary gel electrophoretic analysis of oligonucleotide 2	S5
Figure S8.	Single and full-length incorporation of oligonucleotides 1 by Kf ⁻	S6
Figure S9.	Single and full-length incorporation of oligonucleotides 1 catalyzed by Dpo4.....	S6
Figure S10.	Single and full-length incorporation of oligonucleotides 2 catalyzed by Kf ⁻	S7
Figure S11.	Single and full-length incorporation of oligonucleotides 2 catalyzed by Dpo4.....	S7
Figure S12.	LC-ESI-MS/MS analysis of Dpo4 extension product from oligonucleotide 1	S8
Figure S13.	TIC spectrum of the <i>m/z</i> 1078.5 product form the Dpo4 extension of oligonucleotide 1	S8
Figure S14.	Calibration curve for 5'-pTCCATGA-3' vs. the internal standard 5'-pCTTACGAGCCCCC-3'	S9
Figure S15.	CID spectrum of the <i>m/z</i> 1078.5 product from the Dpo4 extension of oligonucleotide 1	S9
Figure S16.	LC-ESI-MS/MS analysis of Kf ⁻ extension product from oligonucleotide 10a	S10
Figure S17.	TIC spectrum of the Kf ⁻ extension products from oligonucleotide 10a	S10
Figure S18.	CID spectrum of the <i>m/z</i> 1247.7 product from the Kf ⁻ extension of oligonucleotide 2	S11
Table S1.	Observed and calculated CID fragmentation of 5'-pTGACACGA-3'	S11
Figure S19.	TIC and CID mass spectra of an authentic sample of 5'-pTGACACGA-3'	S12
Figure S20.	Calibration curve for 5'-pTGACACGA-3' vs. the internal standard 5'-pCTTACGAGCCCCC-3'	S12
Figure S21.	TIC spectrum of the Kf ⁻ extension products from oligonucleotide 2	S13

Figure S22.	CID spectrum of the m/z 1412.8 product form the Kf ⁻ extension of oligonucleotide 2	S13
Table S2.	Observed and calculated CID fragmentation of 5'-pTGACACGAG-3	S14
Figure S23.	TIC and CID mass spectra of an authentic sample of 5'-pTGACACGAG-3'	S14
Figure S24.	Calibration curve for 5'-pTGACACGAG-3' vs. the internal standard 5'-pCTTACGAGCCCCC-3'	S15
Figure S25.	TIC spectrum of the Kf ⁻ extension products from oligonucleotide 2	S15
Figure S26.	CID spectrum of the m/z 1259.2 product from the Kf ⁻ extension of oligonucleotide 2	S16
Table S3.	Observed and calculated CID fragmentation of 5'-pTGAAACGA-3	S16
Figure S27.	TIC and CID mass spectra of an authentic sample of 5'-pTGAAACGA-3'	S17
Figure S28.	Calibration curve for 5'-pTGAAACGA-3'vs. the internal standard 5'-pCTTACGAGCCCCC-3'	S17
Figure S29.	CID spectrum of the m/z 1091.42 product from the Kf ⁻ extension of oligonucleotide 2	S18
Figure S30.	TIC and CID mass spectra of an authentic sample of 5'-pTGACCGA-3'	S18
Figure S31.	Calibration curve for 5'-pTGACCGA-3' vs. the internal standard 5'-pCTTACGAGCCCCC-3'	S19
Figure S32.	TIC spectrum of the Kf ⁻ extension products from oligonucleotide 2	S19
Figure S33.	CID spectrum of the m/z 1236.3 product from the Kf ⁻ extension of oligonucleotide 2	S20
Table S4.	Observed and calculated CID fragmentation of 5'-pTGACCGAC-3	S20
Figure S34.	TIC and CID spectra of an authentic sample of 5'-pTGACCGAC-3'	S21
Figure S35.	Calibration curve for 5'-pTGACCGAC-3' vs. the internal standard 5'-pCTTACGAGCCCCC-3'	S21
Figure S36.	TIC spectrum of the Dpo4 extension products from oligonucleotide 2	S22
Figure S37.	CID spectrum of the m/z 1247.5 product from the Dpo4 extension of oligonucleotide 2	S22
Figure S38.	CID spectrum of the m/z 1260.0 product from the Dpo4 extension of oligonucleotide 2	S23
Figure S39.	TIC spectrum of the Dpo4 extension products from oligonucleotide 2	S23
Figure S40.	CID spectrum of the m/z 1091.5 product from the Dpo4 extension of oligonucleotide 2	S24

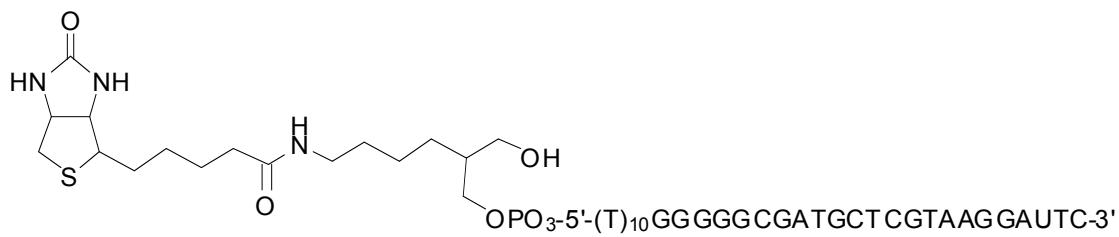


Figure S1. Biotinylated primer.

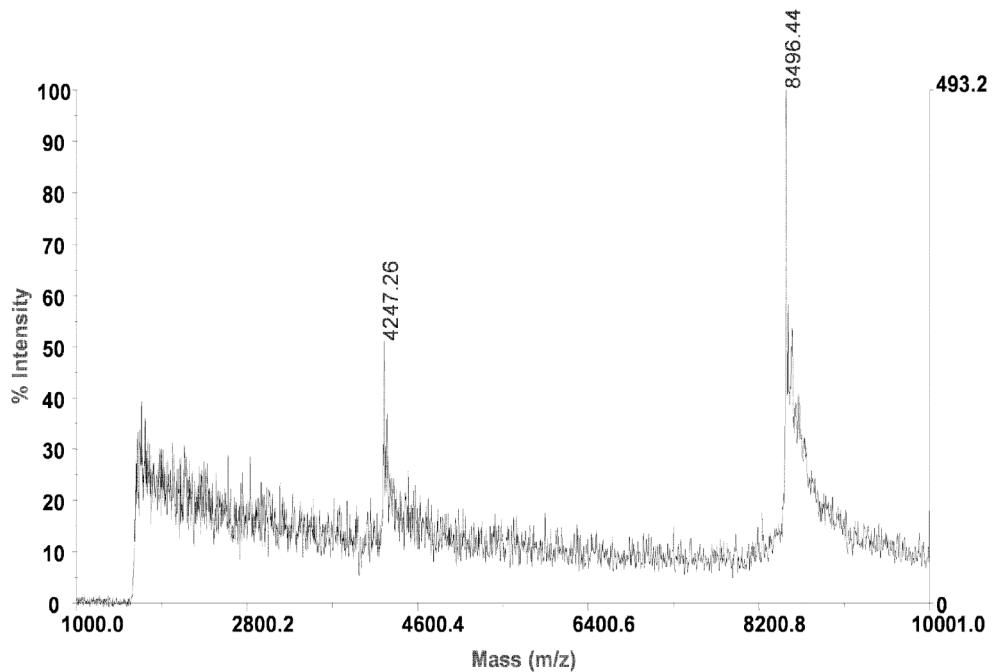


Figure S2. MALDI -TOF spectrum of oligonucleotide 1.

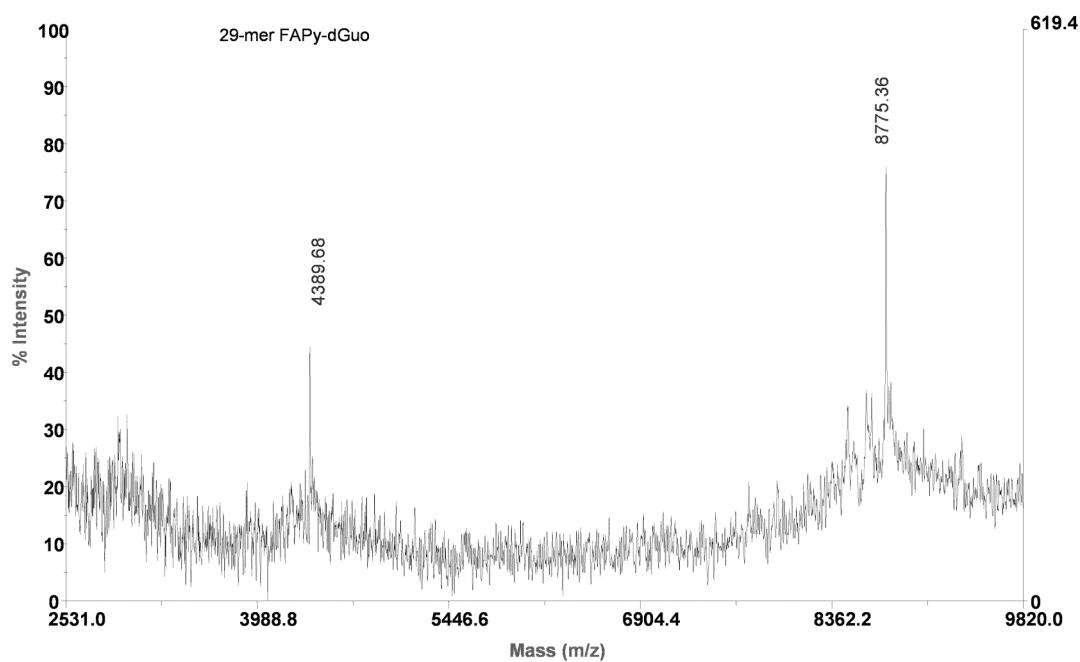


Figure S3. MALDI -TOF spectrum of oligonucleotide 2.

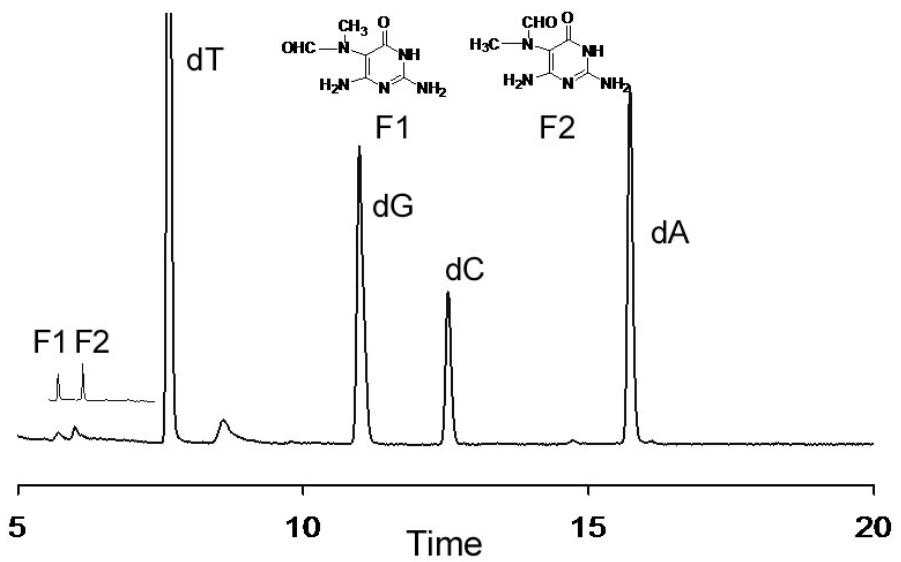


Figure S4. Enzyme digestion of oligonucleotide 1.

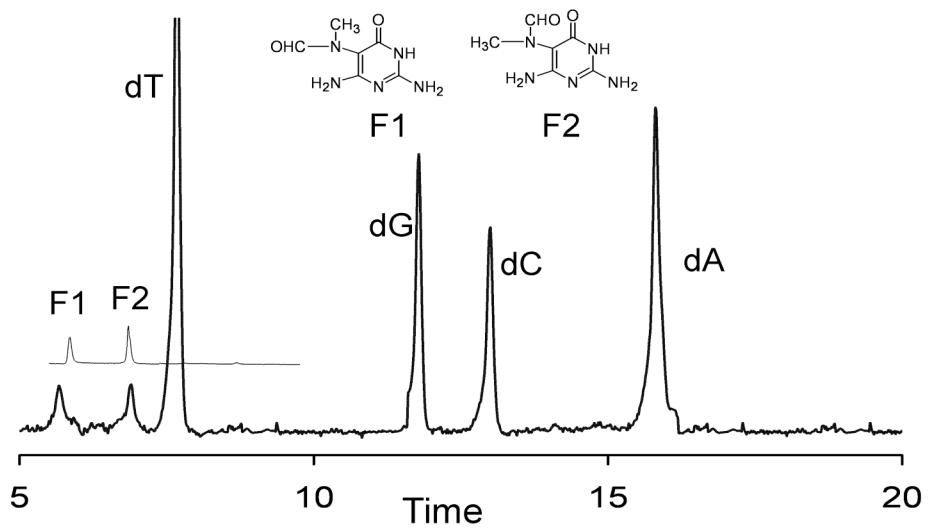


Figure S5. Enzyme digestion of oligonucleotide 2.

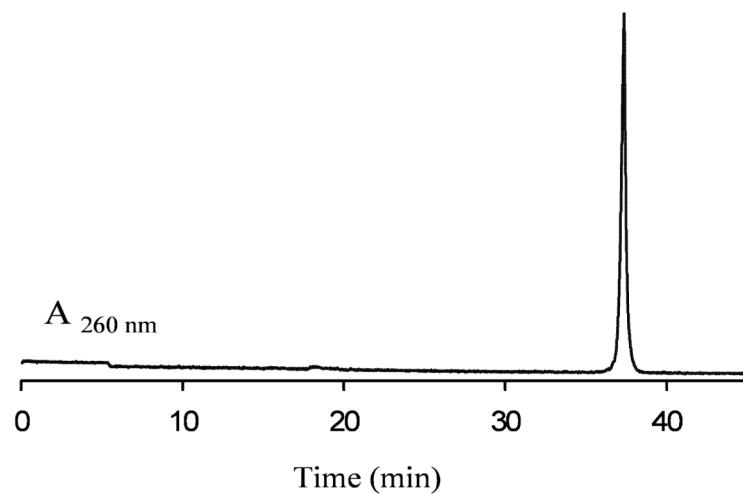


Figure S6. Capillary gel electrophoretic analysis of oligonucleotide **1**.

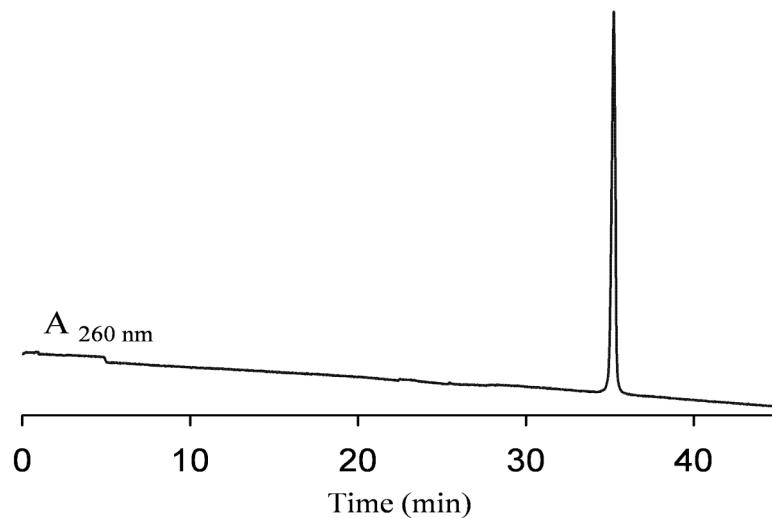


Figure S7. Capillary gel electrophoretic analysis of oligonucleotide **2**.



(X = MeFAPy-dGuo)

0 25 50 100 0 25 50 100 0 25 50 100 0 10 50 100

A

T

G

C

4 x dNTP's

(X = dGuo)

0 25 50 100 0 25 50 100 0 25 50 100 0 25 50 100 0 25 50 100

A

T

G

C

4 x dNTP's

Figure S8. Single and full-length incorporation of oligonucleotides **1** catalyzed by Kf⁻.



(X = MeFAPy-dGuo)

0 25 50 100 0 25 50 100 0 25 50 100 0 25 50 100 0 10 50 100

A

T

G

C

4 x dNTP's

(X = dGuo)

0 25 50 100 0 25 50 100 0 25 50 100 0 25 50 100 0 25 50 100

A

T

G

C

4 x dNTP's

Figure S9. Single and full-length incorporation of oligonucleotides **1** catalyzed by Dpo4.



Kf⁻

(X = MeFapy-dGuo)

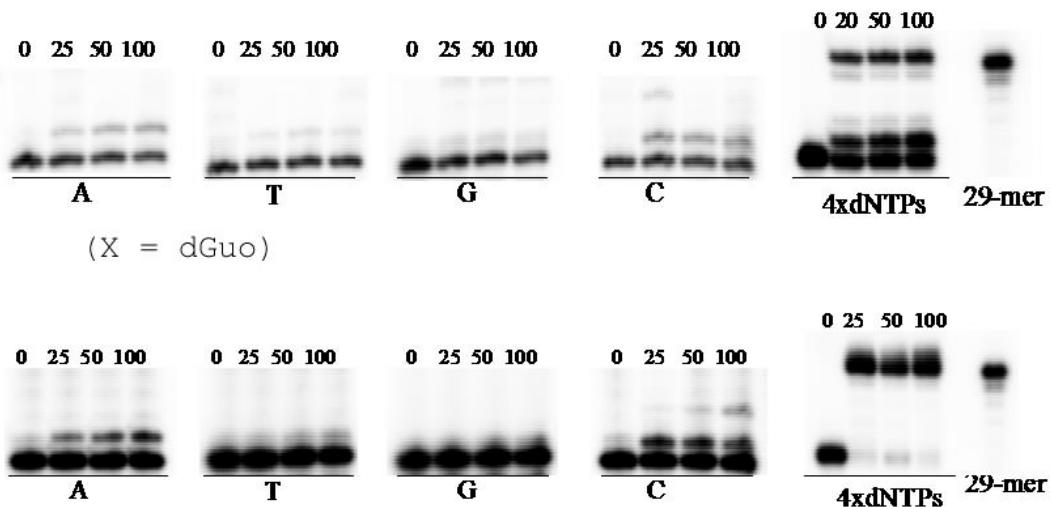


Figure S10. Single and full-length incorporation of oligonucleotides **2** catalyzed by Kf⁻.



2 (X = dGuo)

Dpo4

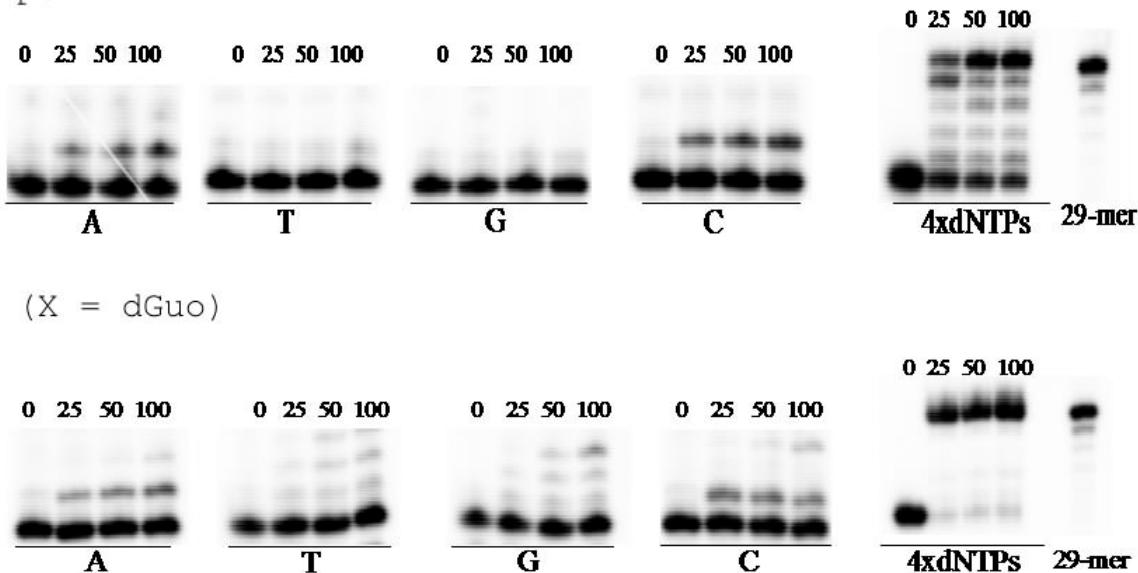


Figure S11. Single and full-length incorporation of oligonucleotides **2** catalyzed by Dpo4.

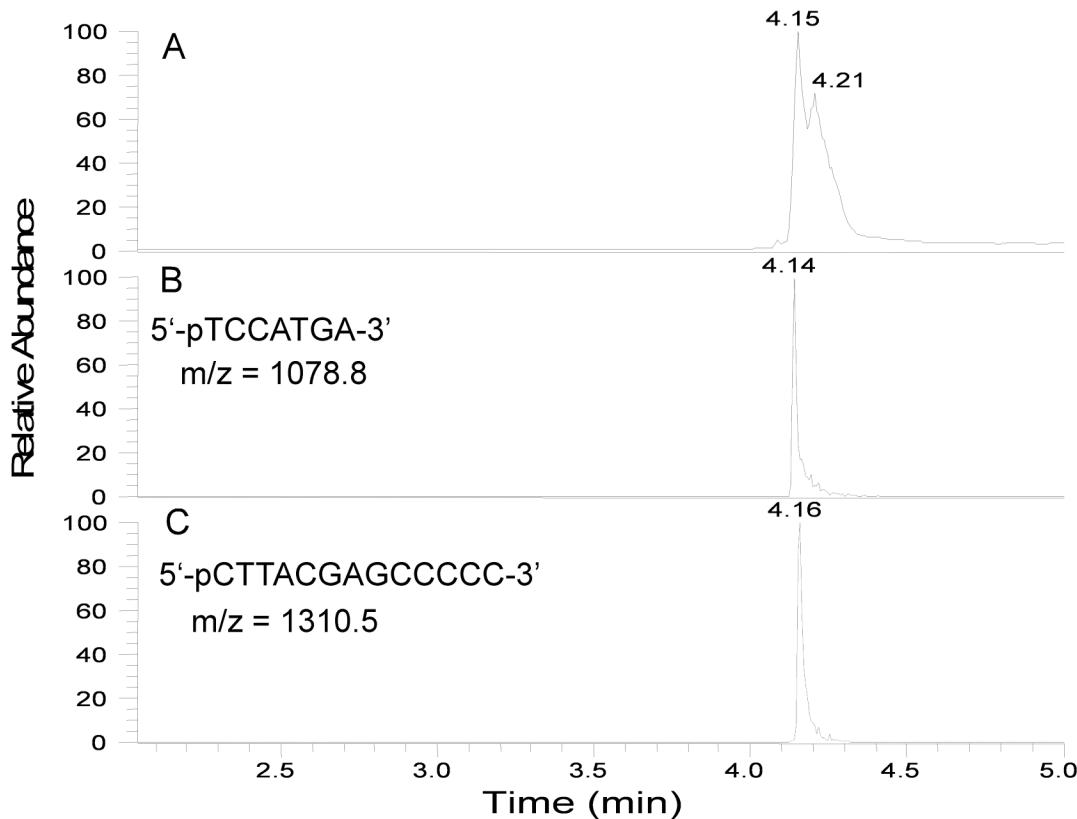


Figure S12. LC-ESI-MS/MS analysis of Dpo4 extension product from oligonucleotide **1**: TIC (A) and select ion profiles of product 5'-pTCCATGA-3' (B) and standard 5'-pCTTACGAGCCCC-3' (C).

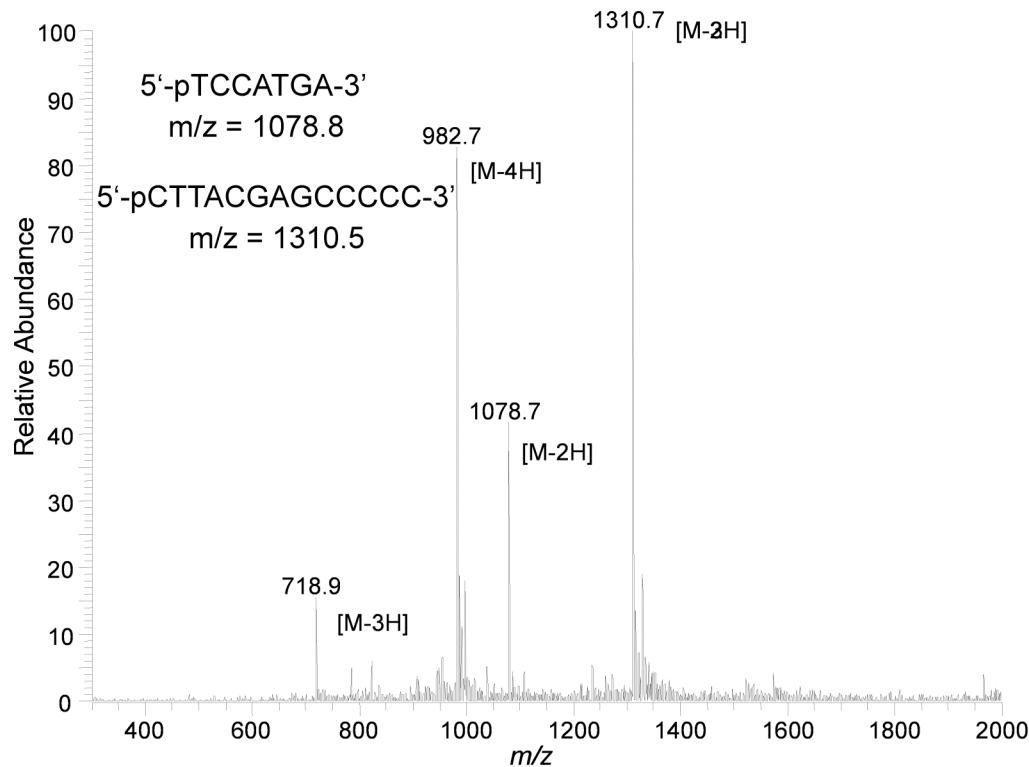


Figure S13. TIC spectrum of the m/z 1078.5 product from the Dpo4 extension of oligonucleotide **1**.

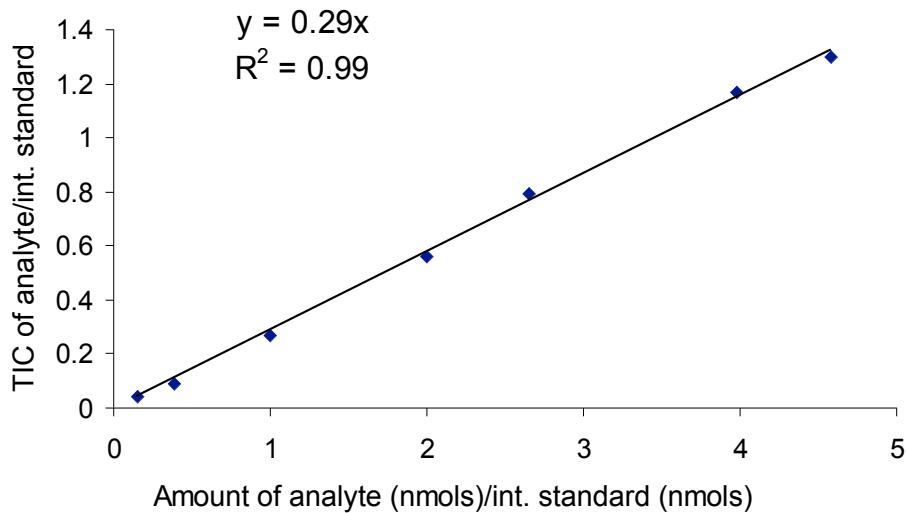


Figure S14. Calibration curve for 5'-pTCCATGA-3' vs. the internal standard 5'-pCTTACGAGCCCC-3'.

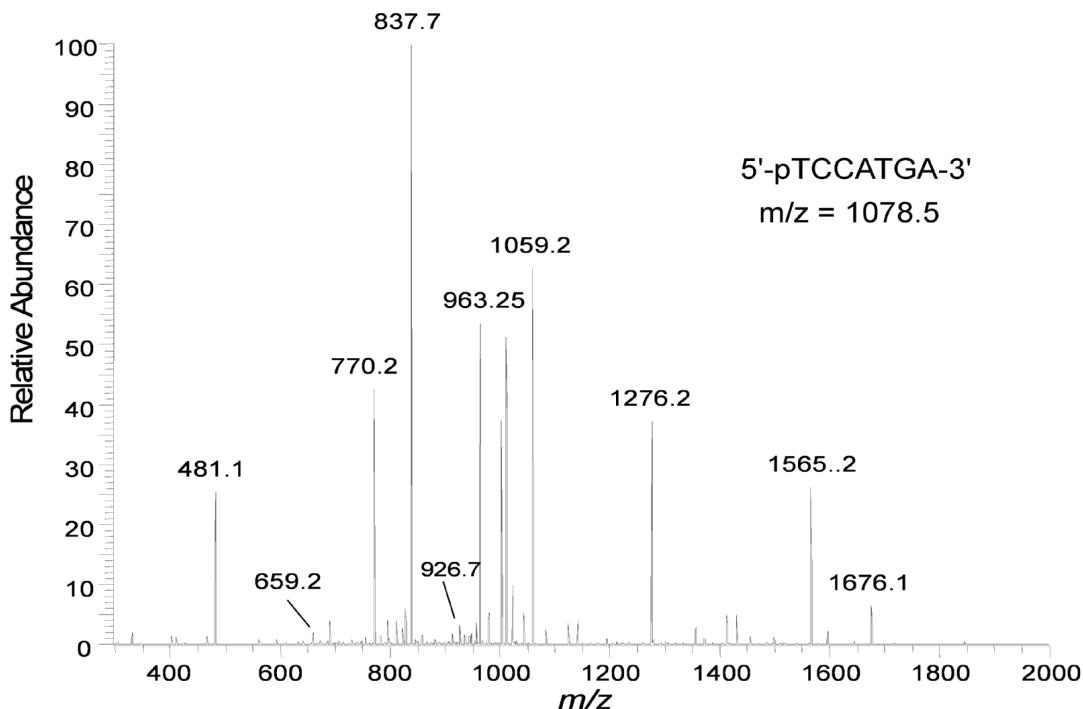


Figure S15. CID spectrum of the m/z 1078.5 product from the Dpo4 extension of oligonucleotide 1. See Table 3 for CID fragment assignments.

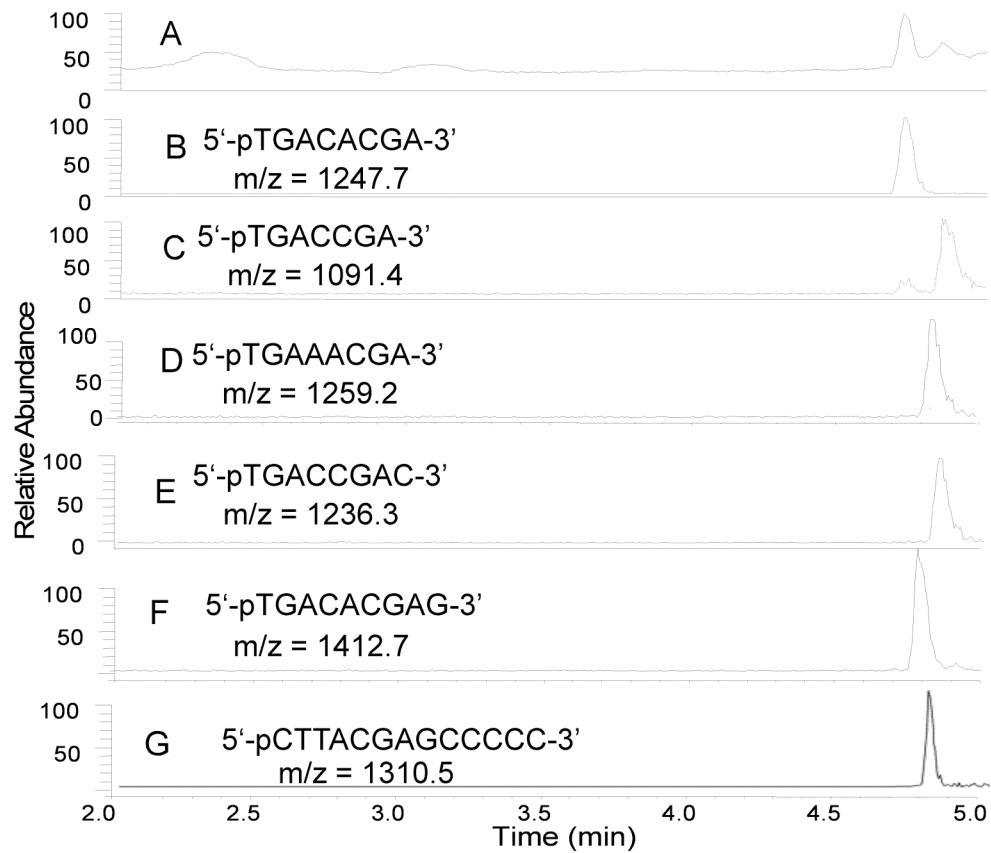


Figure S16. LC-ESI-MS/MS analysis of Kf^- extension products from oligonucleotide **2** : TIC (A) and select ion profiles of products $5'\text{-pTGACACGA-3}'$ (B), $5'\text{-pTGACCGA-3}'$ (C), $5'\text{-pTGAAACGA-3}'$ (D), $5'\text{-pTGACCGAC-3}'$ (E), and $5'\text{-pTGACACGAG-3}'$ (F) and standard $5'\text{-pCTTACGAGCCCC-3}'$ (G).

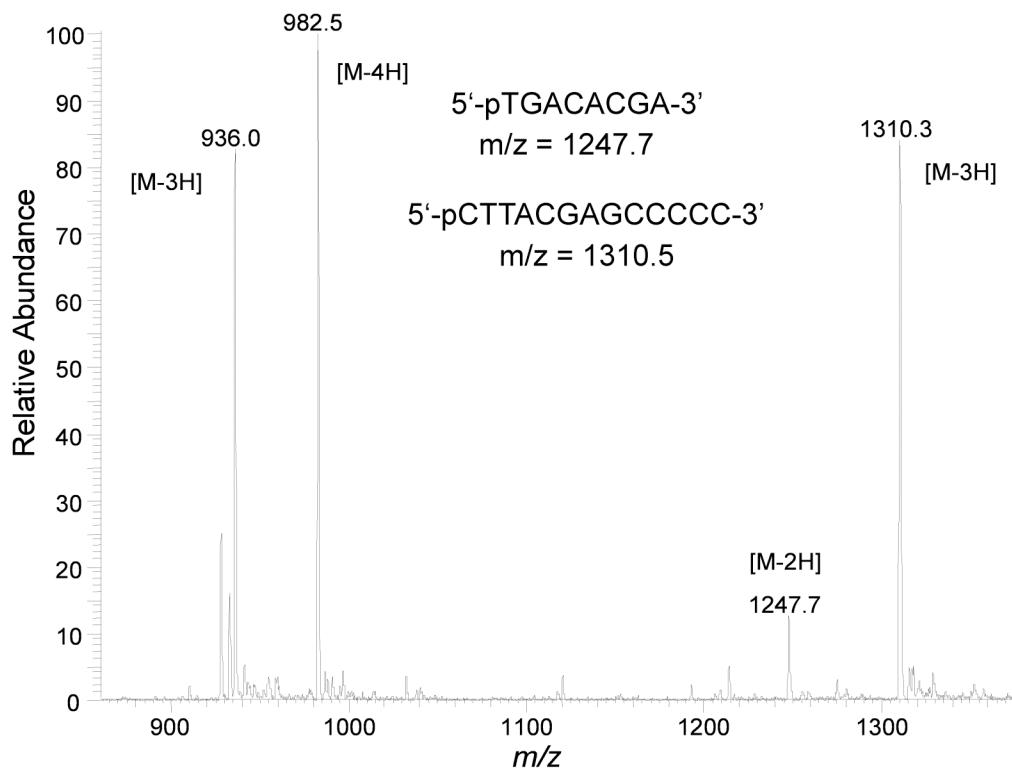


Figure S17. TIC spectrum of the Kf^- extension products from oligonucleotide **2**.

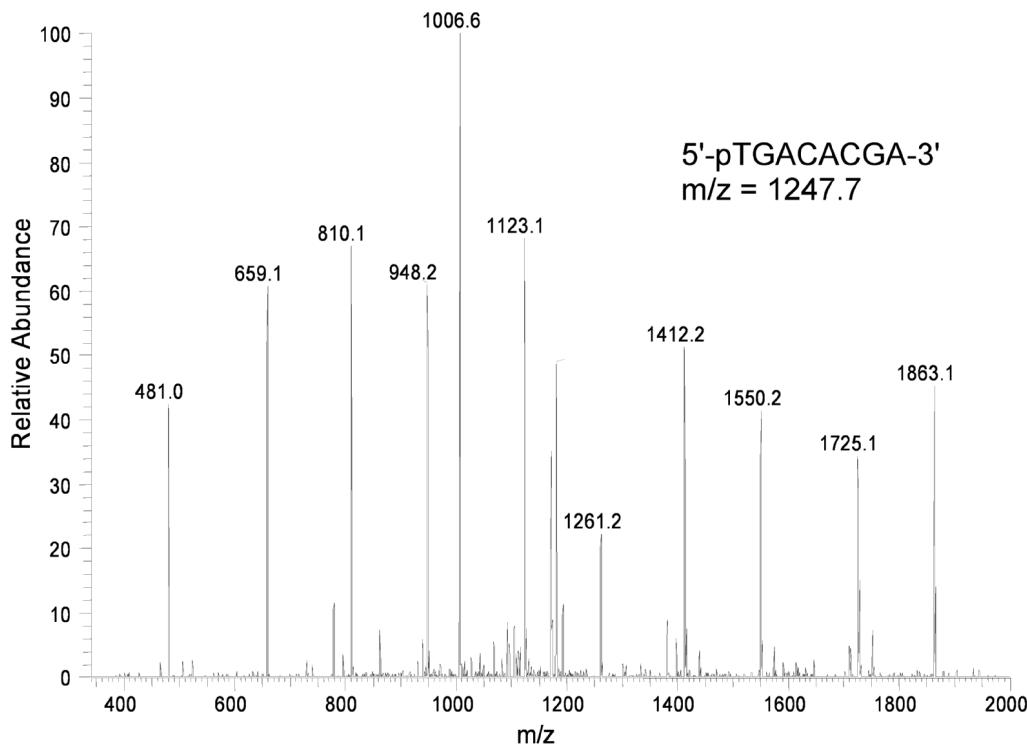


Figure S18. CID spectrum of the m/z 1247.7 product from the Kf^- extension of oligonucleotide **2**.

Table S1. Observed and calculated CID fragmentation of 5'-pTGACACGA-3'

Fragment assignment	Observed	Theoretical
5'-pT (a_2 -B)	481.1	481
5'-pTG (a_3 -B)	810.1	810
5'-pTGA (a_4 -B)	1123.1	1123.1
5'-pTGAC (a_5 -B)	1412.2	1412.1
5'-pTGACCA (a_6 -B)	1725.1	1725.2
5'-pTGACAC (a_7 -B, -2)	1006.6	1006.6
pACACGA-3' (w_6)	1863.1	1863.3
pCACGA-3' (w_5)	1550.2	1550.2
pACGA-3' (w_4)	1261.2	1261.2
pCGA-3' (w_3)	948.2	948.1
pGA-3' (w_2)	659.1	659.1

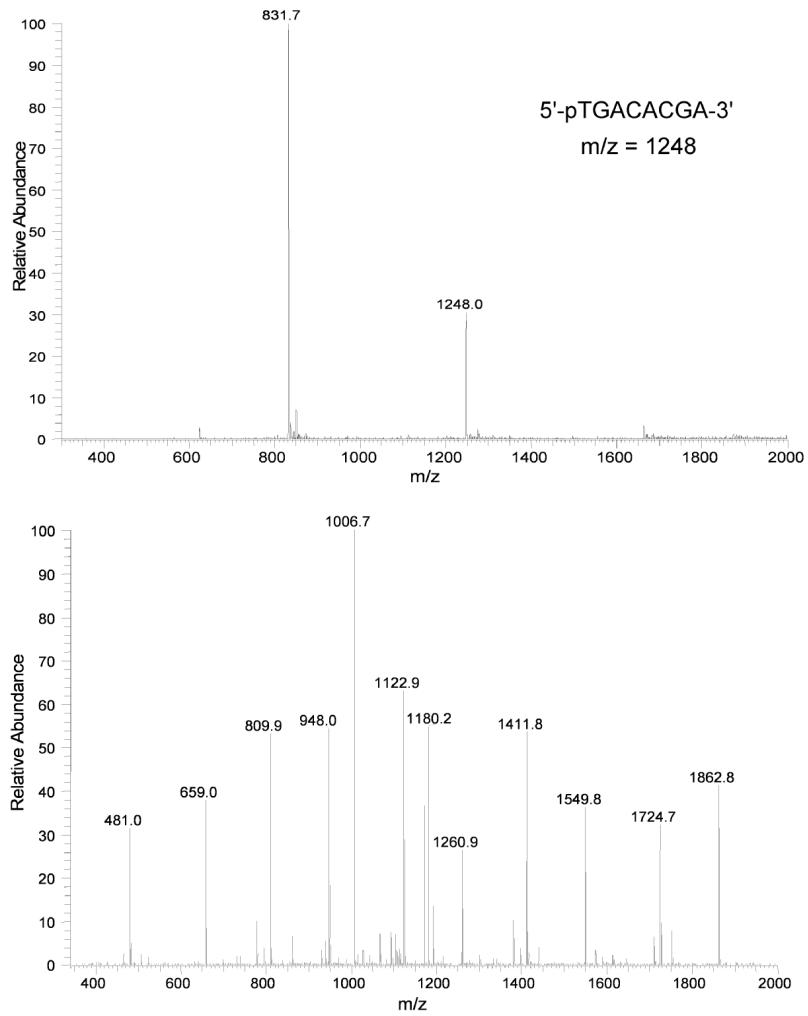


Figure S19. TIC (top) and CID (bottom) spectra of an authentic sample of 5'-pTGACACGA-3'

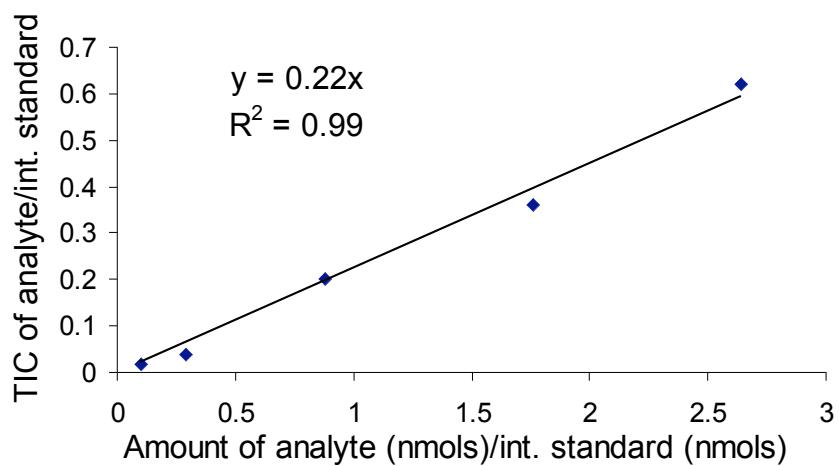


Figure S20. Calibration curve for 5'-pTGACACGA-3' vs. the internal standard 5'-pCTTACGAGCCCC-3'

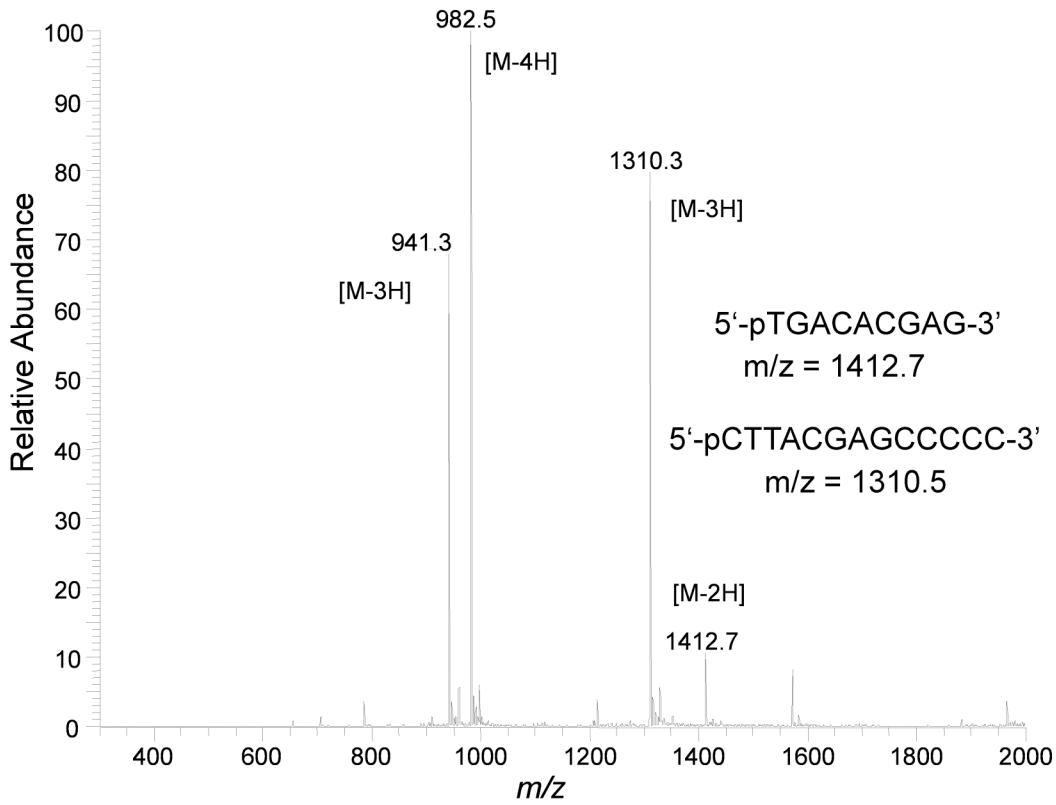


Figure S21. TIC spectrum of the Kf^- extension products from oligonucleotide **2**.

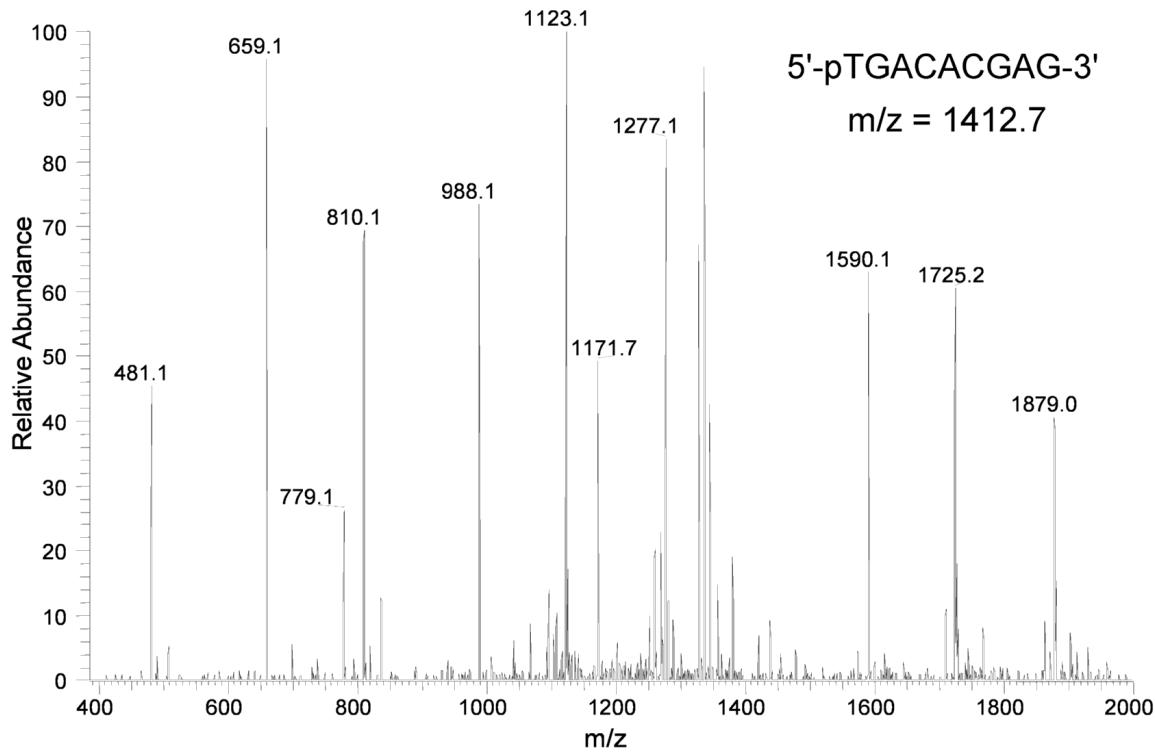


Figure S22. CID spectrum of the m/z 1412.8 product from the Kf^- extension of oligonucleotide **2**.

Table S2. Observed and calculated CID fragmentation of 5'-pTGACACGAG-3'

Fragment assignment	Observed	Theoretical
5'-pT (a ₂ -B)	480.1	481
5'-pTG (a ₃ -B)	810.1	810
5'-pTGA (a ₄ -B)	1123.1	1123.1
5'-pTGACA (a ₆ -B)	1725.2	1725.2
5'-pTGACACGA (a ₈ -B, -2)	1171.7	1171.1
5'-pTGACACGA (a ₈ -B, -3)	779.1	780.4
pCACGAG-3' (w ₆)	1879	1879.3
pACGAG-3' (w ₅)	1590.1	1590.2
pCGAG-3' (w ₄)	1277.1	1277.2
pGAG-3' (w ₃)	988.1	988.1
pAG-3' (w ₂)	659.1	659.1

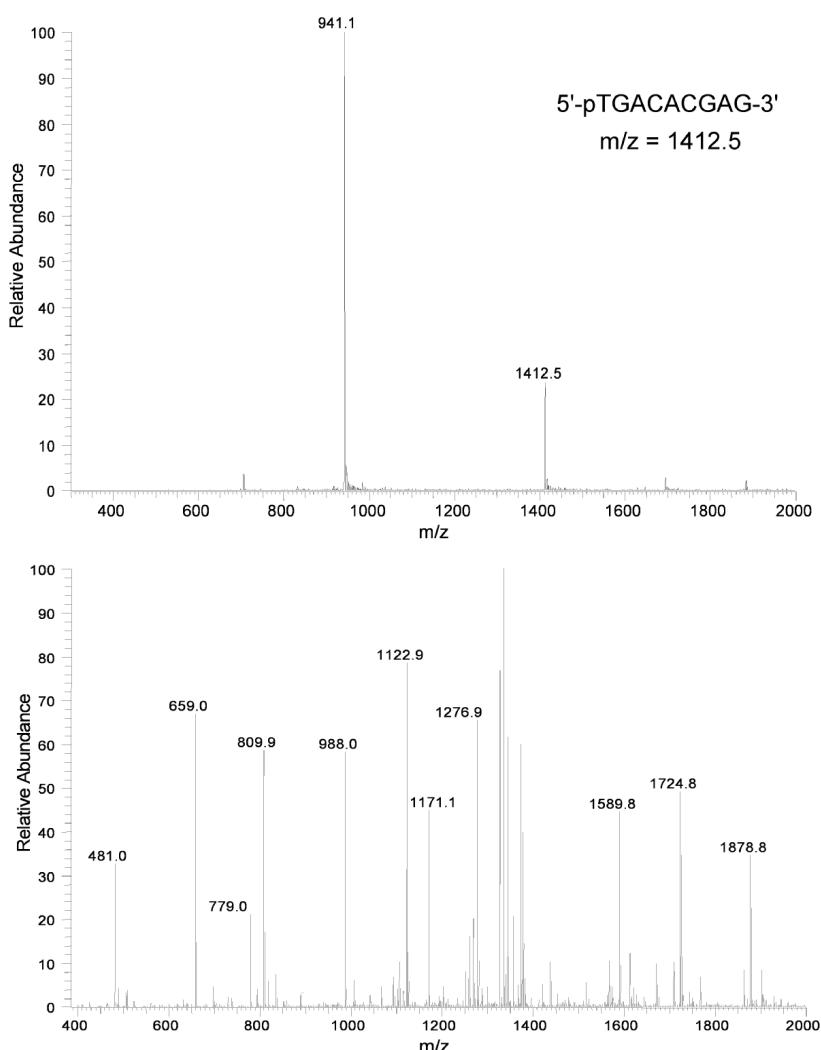


Figure S23. TIC (top) and CID (bottom) spectra of an authentic sample of 5'-pTGACACGAG-3'

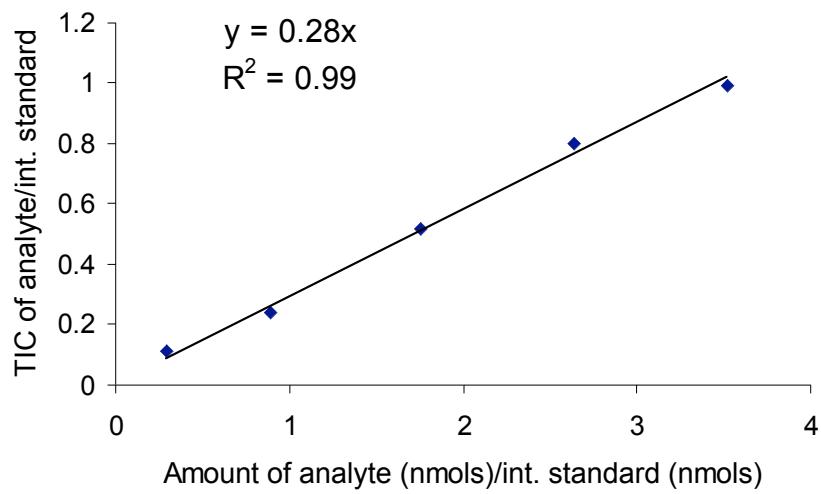


Figure S24. Calibration curve for 5'-pTGACACGAG-3' vs. the internal standard 5'-pCTTACGAGCCCCC-3'

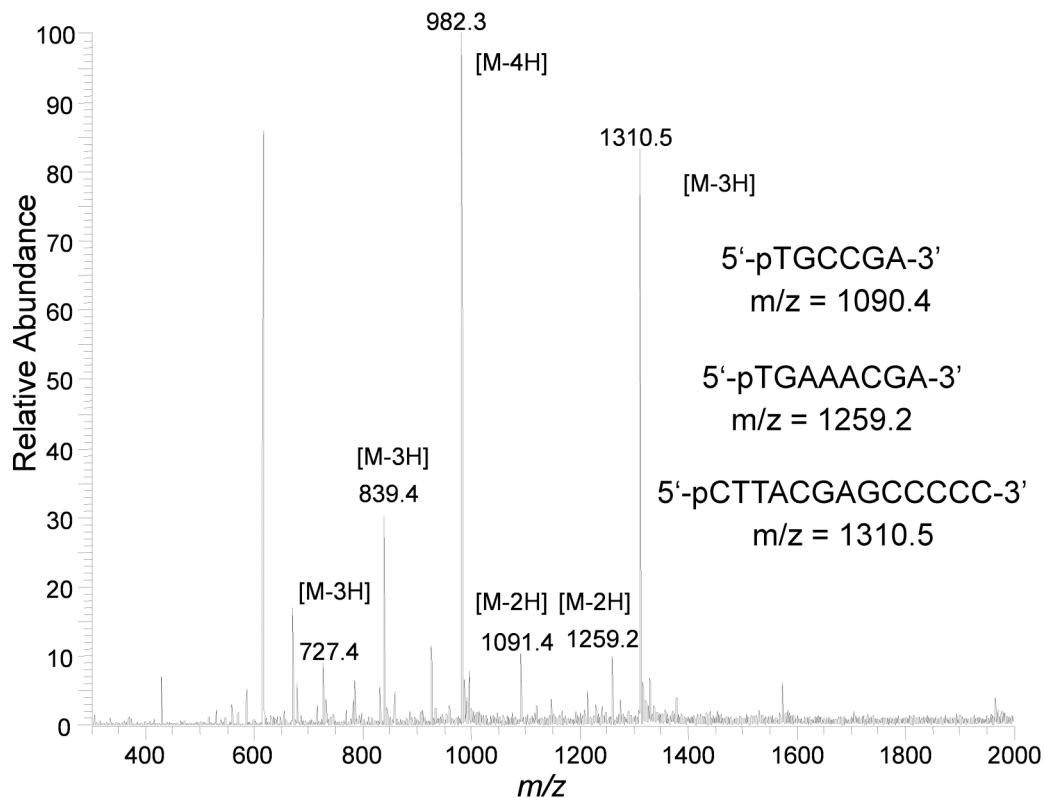


Figure S25. TIC spectrum of the Kf⁻ extension products from oligonucleotide 2.

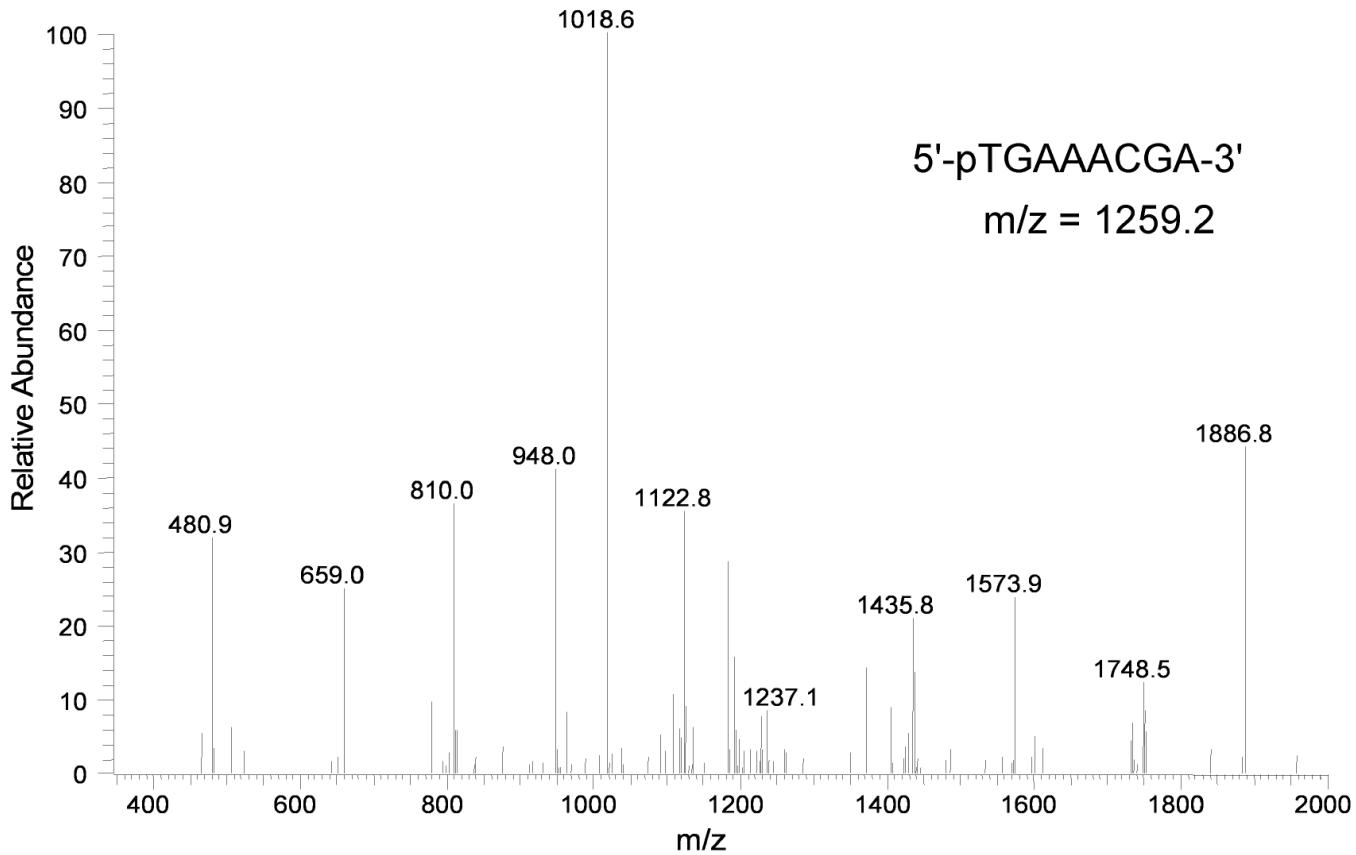


Figure S26. CID spectrum of the m/z 1259.2 product from the Kf^- extension of oligonucleotide 2.

Table S3. Observed and calculated CID fragmentation of 5'-pTGAAACGA-3

Fragment assignment	Observed	Theoretical
5'-pT ($a_2\text{-B}$)	480.9	481
5'-pTG ($a_3\text{-B}$)	810	810
5'-pTGA ($a_4\text{-B}$)	1122.8	1123.1
5'-pTGAA ($a_5\text{-B}$)	1435.8	1436.9
5'-pTGAAA ($a_6\text{-B}$)	1748.5	1750.11
5'-pTGAAA ($a_6\text{-B, -2}$)	874.4	874.55
5'-pTGAAAC ($a_7\text{-B, -2}$)	1018.6	1019.14
pAACGA-3' (w_5)	1886.8	1888.2
pAACGA-3' (w_5)	1573.9	1575.03
pCGA-3' (w_3)	948	948.61
pGA-3' (w_2)	659	659.42

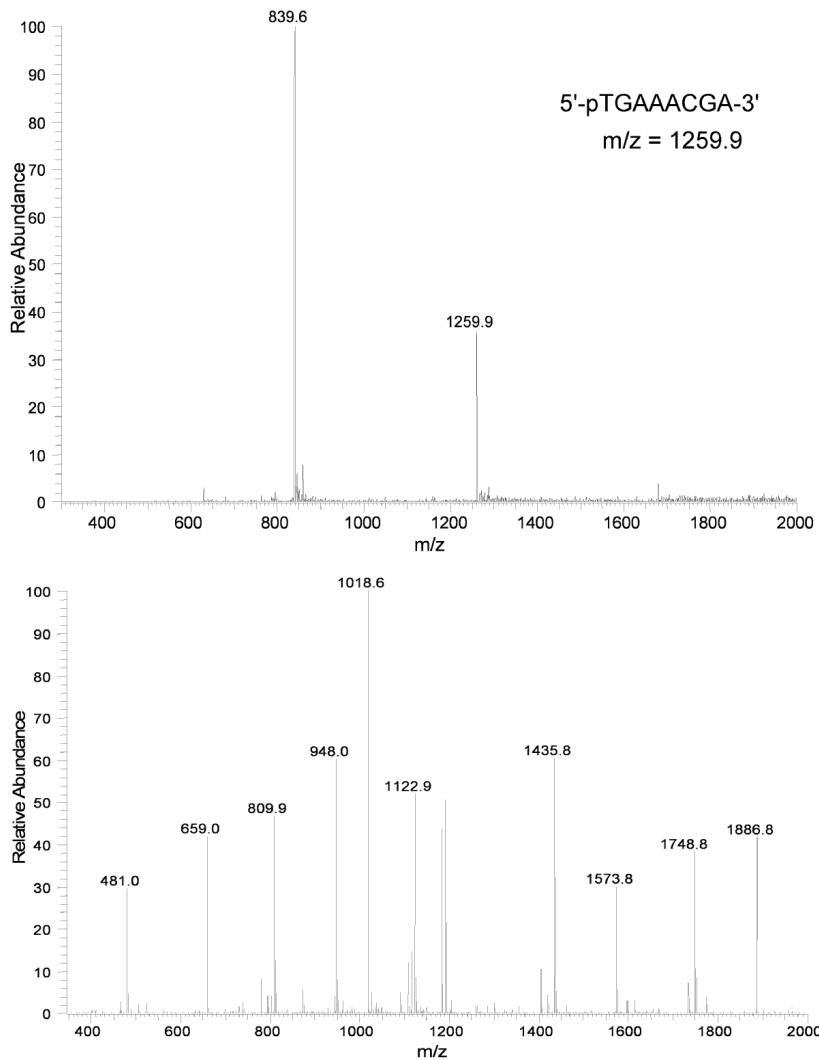


Figure S27. TIC (top) and CID (bottom) spectra of an authentic sample of 5'-pTGAAACGA-3'

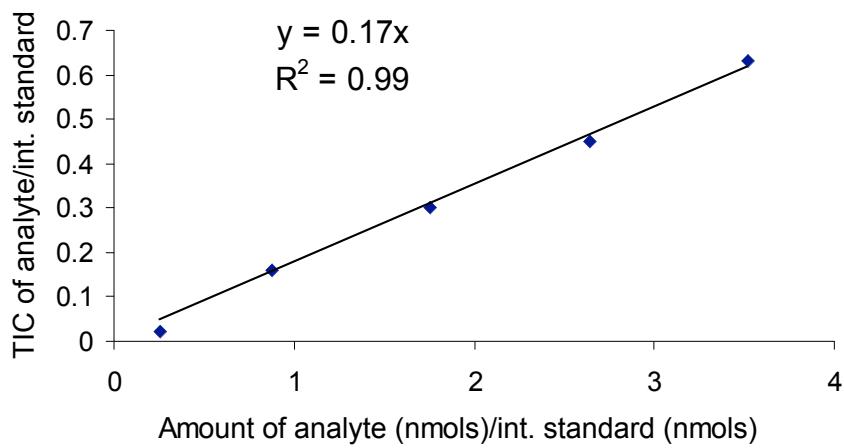


Figure S28. Calibration curve for 5'-pTGAAACGA-3' vs. the internal standard 5'-pCTTACGAGCCCC-3'

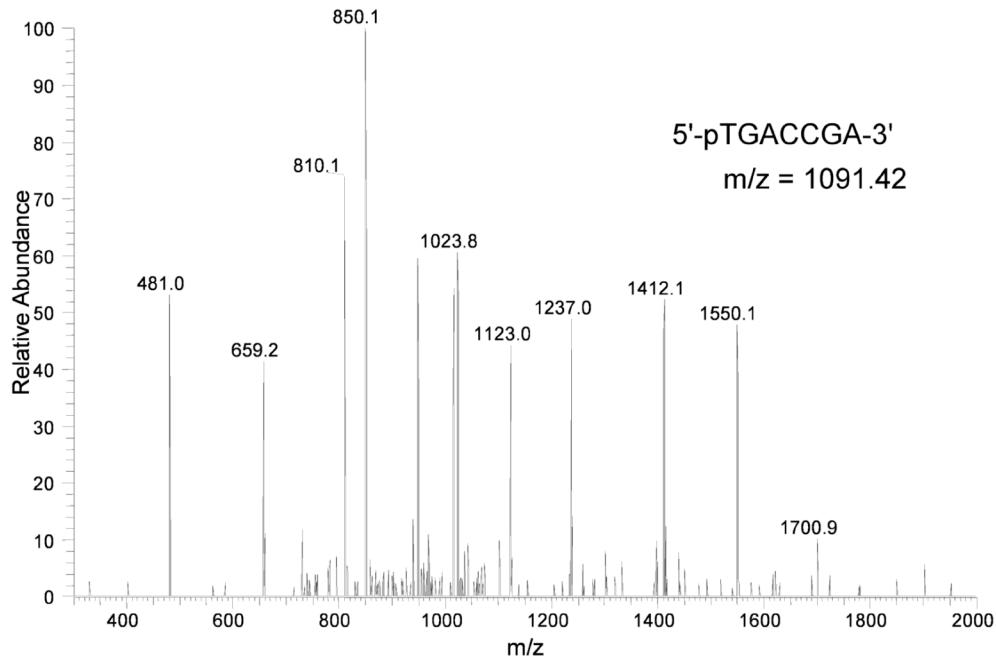


Figure S29. CID spectrum of the m/z 1091.42 product from the Kf^- extension of oligonucleotide 2. See Table 4 for fragment assignments.

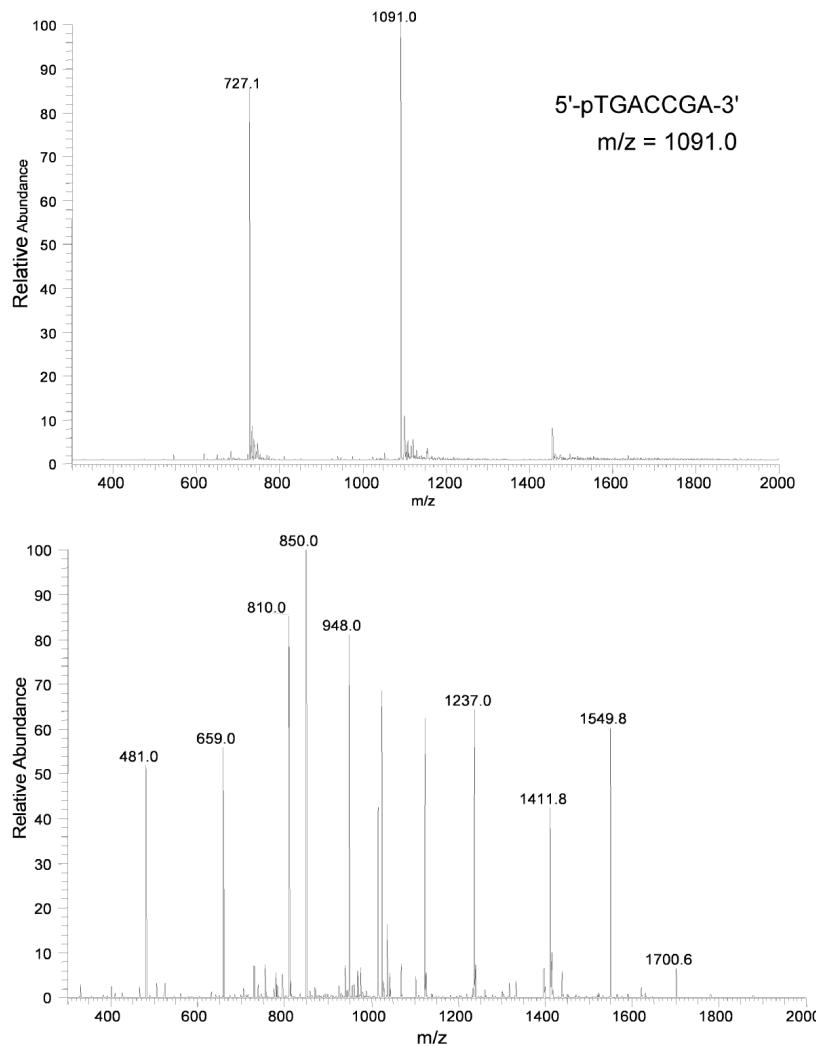


Figure S30. TIC (top) and CID (bottom) spectra of an authentic sample of $5'\text{-pTGACCGA-3'}$

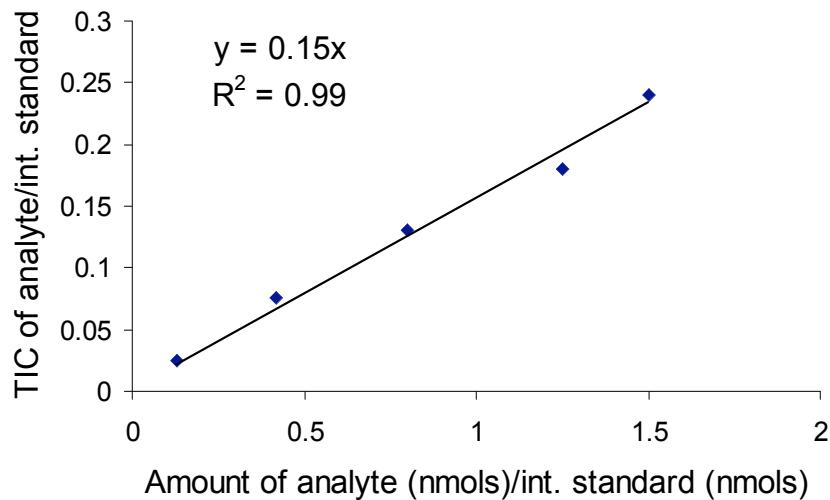


Figure S31. Calibration curve for 5'-pTGACCGA-3' vs. the internal standard 5'-pCTTACGAGCCCC-3'.

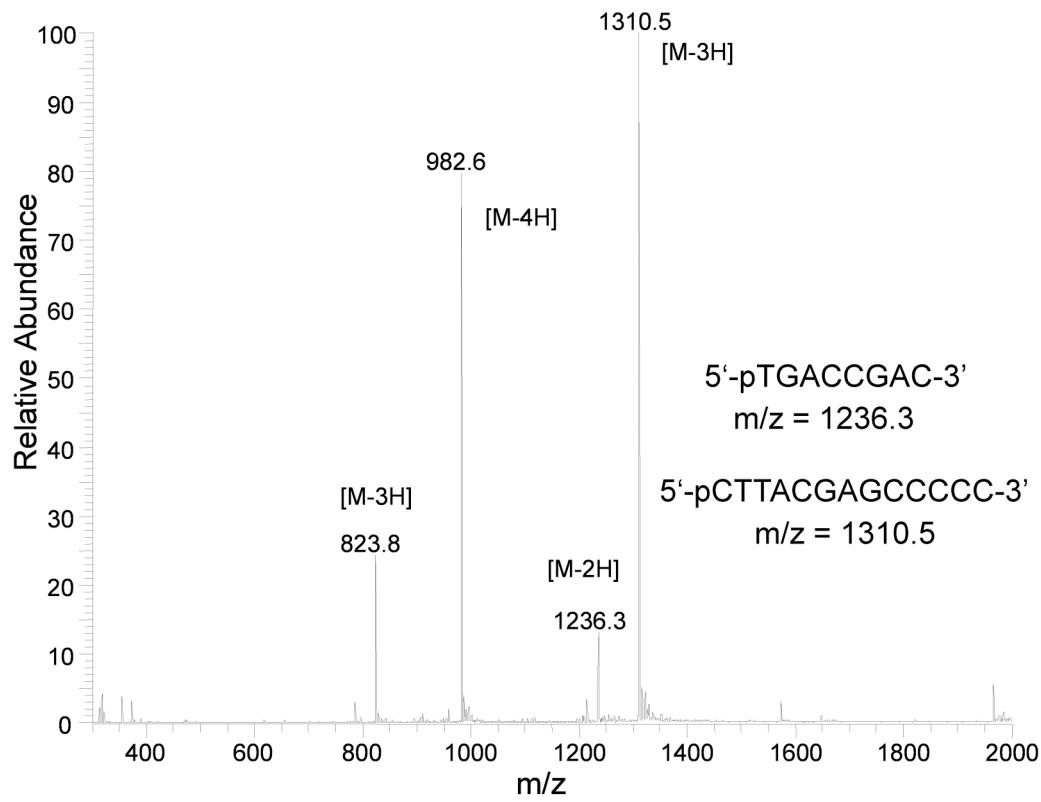


Figure S32. TIC spectrum of the Kf⁻ extension products from oligonucleotide 2.

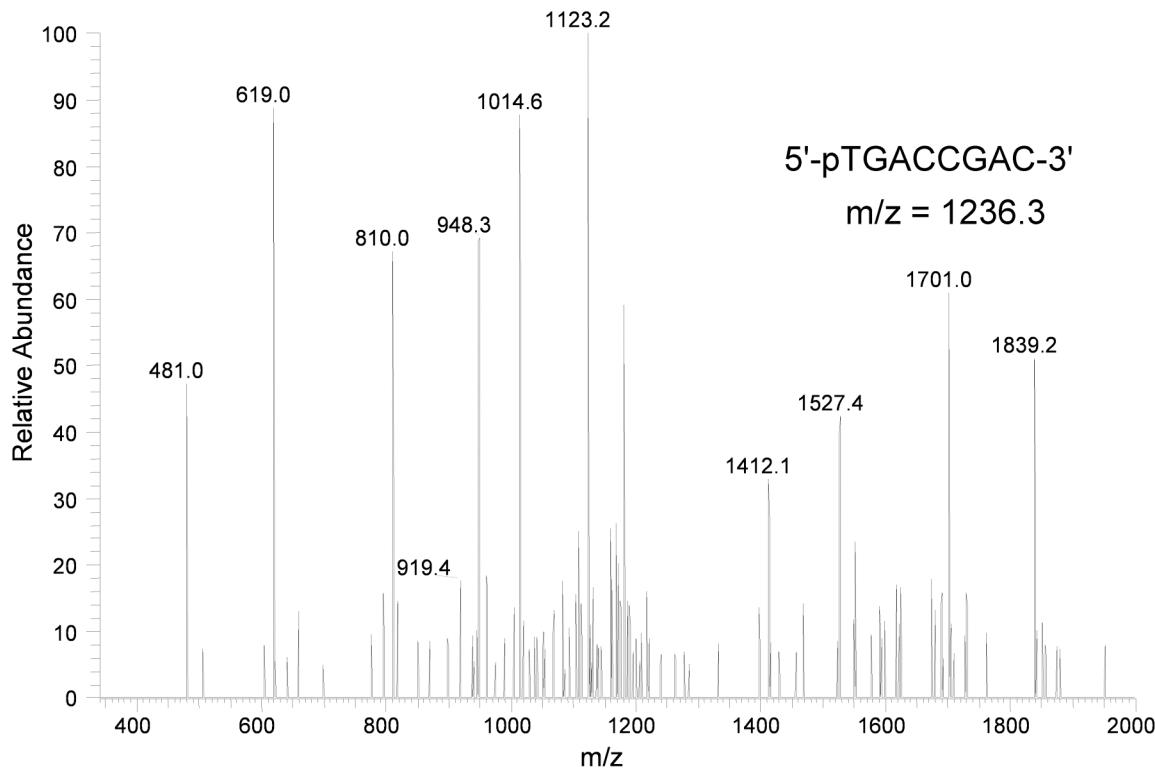


Figure S33. CID spectrum of the m/z 1236.3 product from the Kf⁻ extension of oligonucleotide **2**.

Table S4. Observed and calculated CID fragmentation of 5'-pTGACCGAC-3'

Fragment assignment	Observed	Theoretical
5'-pT (a ₂ -B)	481	481
5'-pTG (a ₃ -B)	810	810
5'-pTGA (a ₄ -B)	1123.2	1123.1
5'-pTGAC (a ₅ -B)	1412.1	1412.1
5'-pTGACC (a ₆ -B)	1701	1702
5'-pTGACCG (a ₇ -B, -2)	1014.6	1015.1
pACCGAC-3' (w ₆)	1839.2	1840.1
pACCGAC-3' (w ₆ , -2)	919.4	919.5
pCCGAC-3' (w ₅)	1527.4	1526.9
pCGAC-3' (w ₄)	619	618.3
pGAC-3' (w ₃)	948.3	948.6
pAC-3' (w ₂)	619	619.4

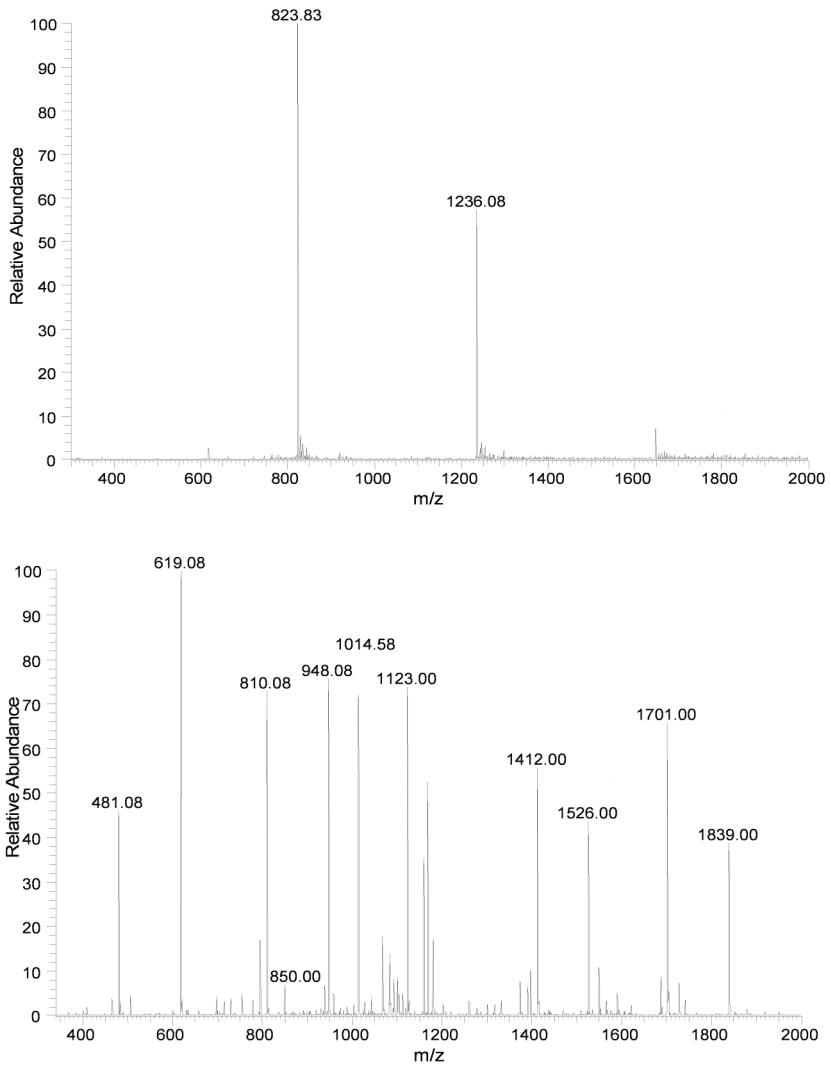


Figure S34. TIC (top) and CID (bottom) spectra of an authentic sample of 5'-pTGACCGAC-3'

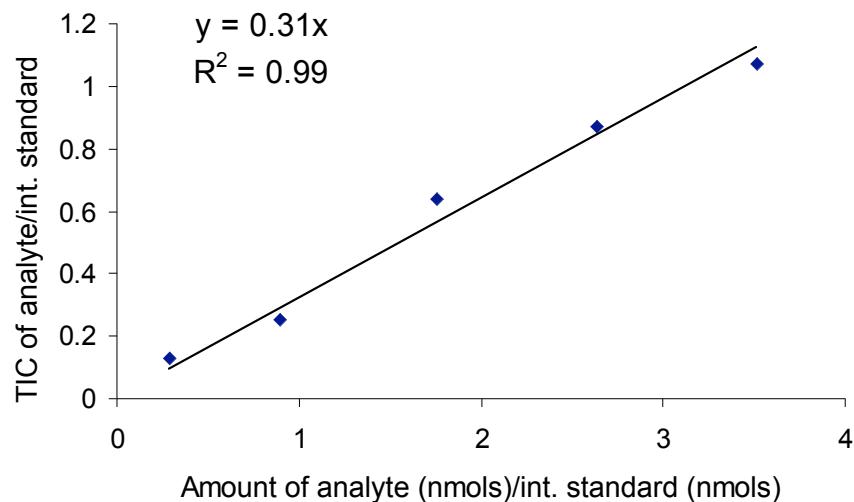


Figure S35. Calibration curve for 5'-pTGACCGAC-3' vs the internal standard 5'-pCTTACGAGCCCC-3'.

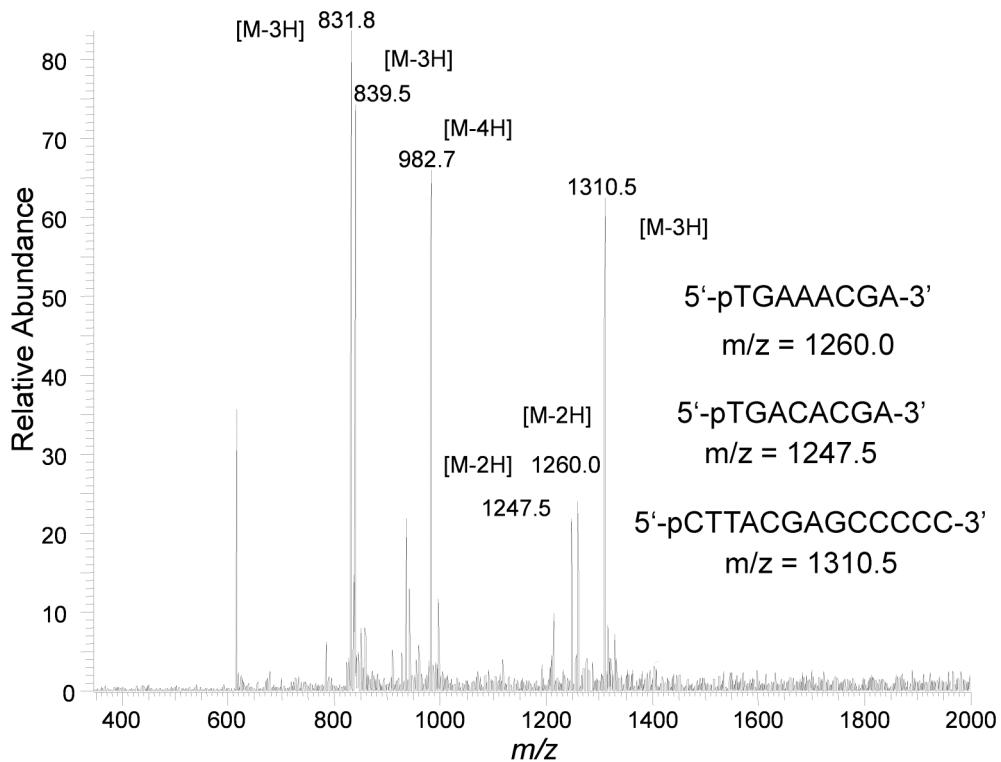


Figure S36. TIC spectrum of the Dpo4 extension products from oligonucleotide **2**.

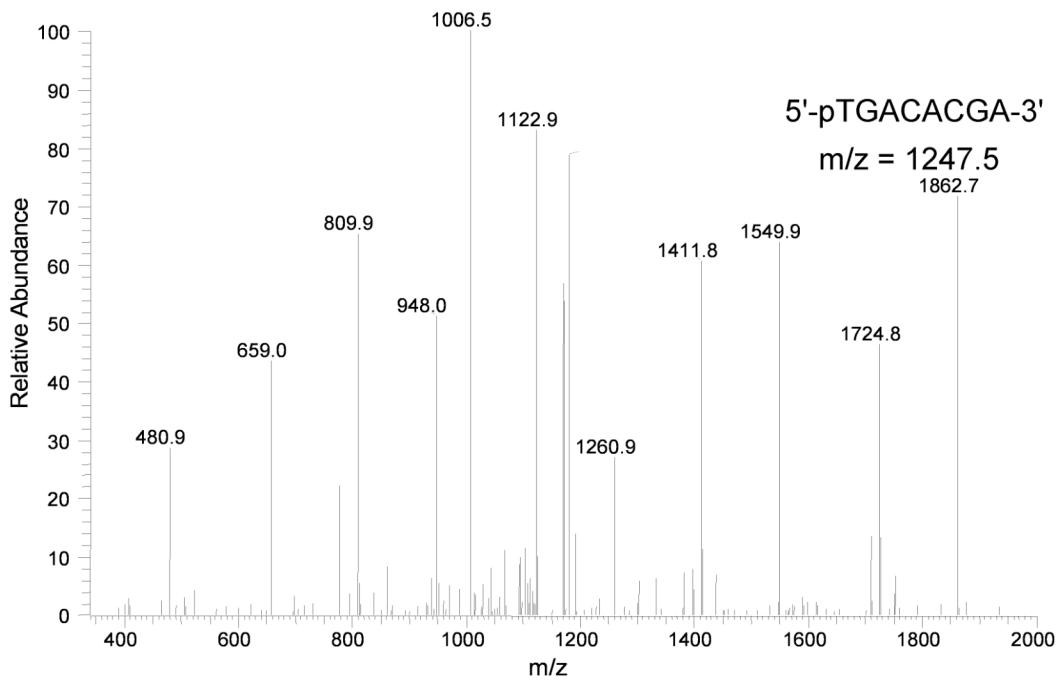


Figure S37. CID spectrum of the m/z 1247.5 product from the Dpo4 extension of oligonucleotide **2**. See Table S1 for fragment assignments.

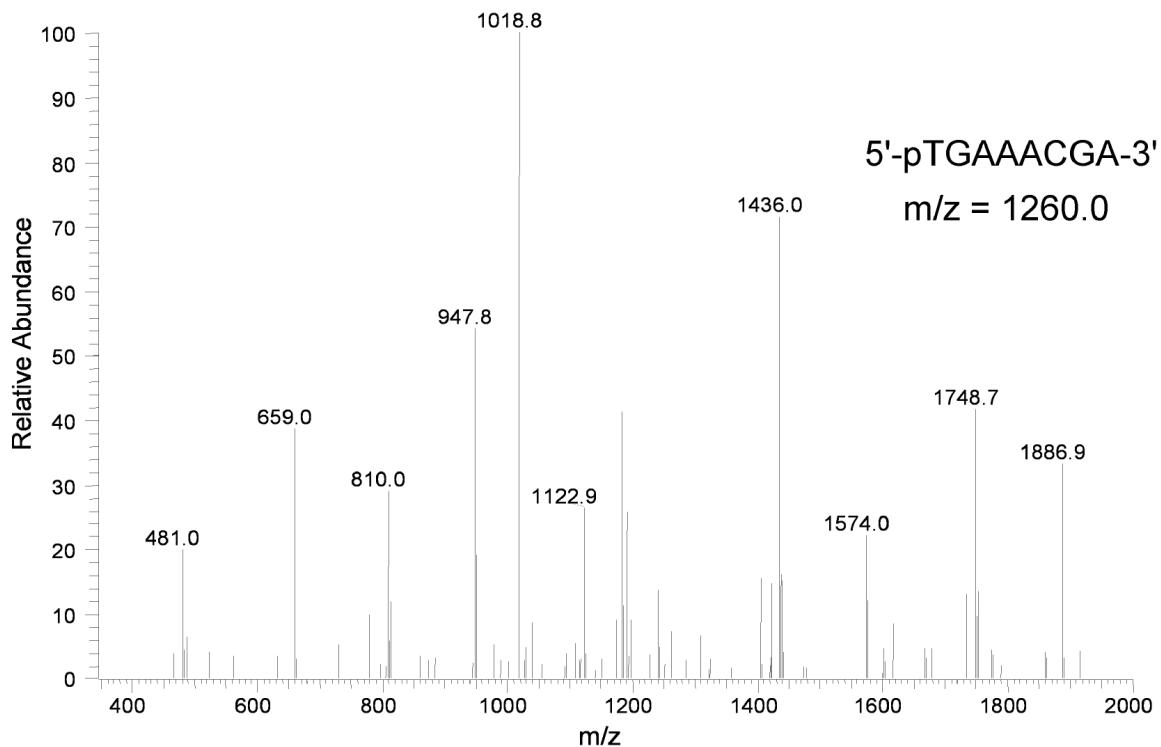


Figure S38. CID spectrum of the m/z 1260.0 product from the Dpo4 extension of oligonucleotide **2**.

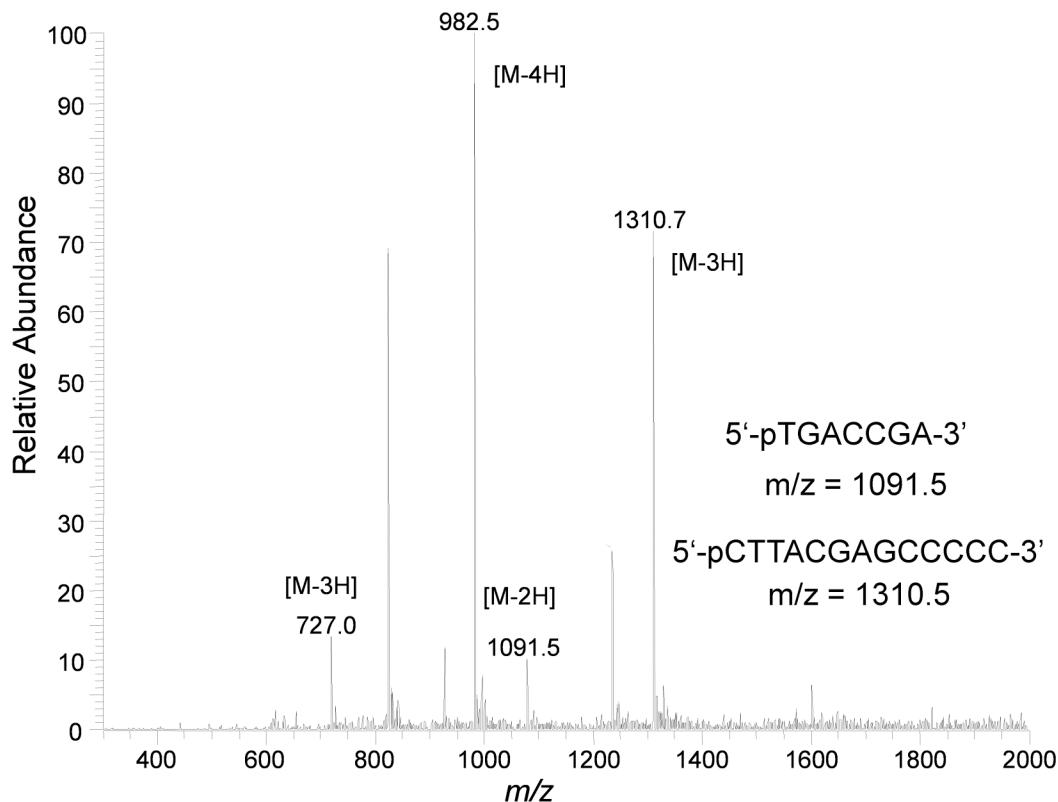


Figure S39. TIC spectrum of the Dpo4 extension products from oligonucleotide **2**.

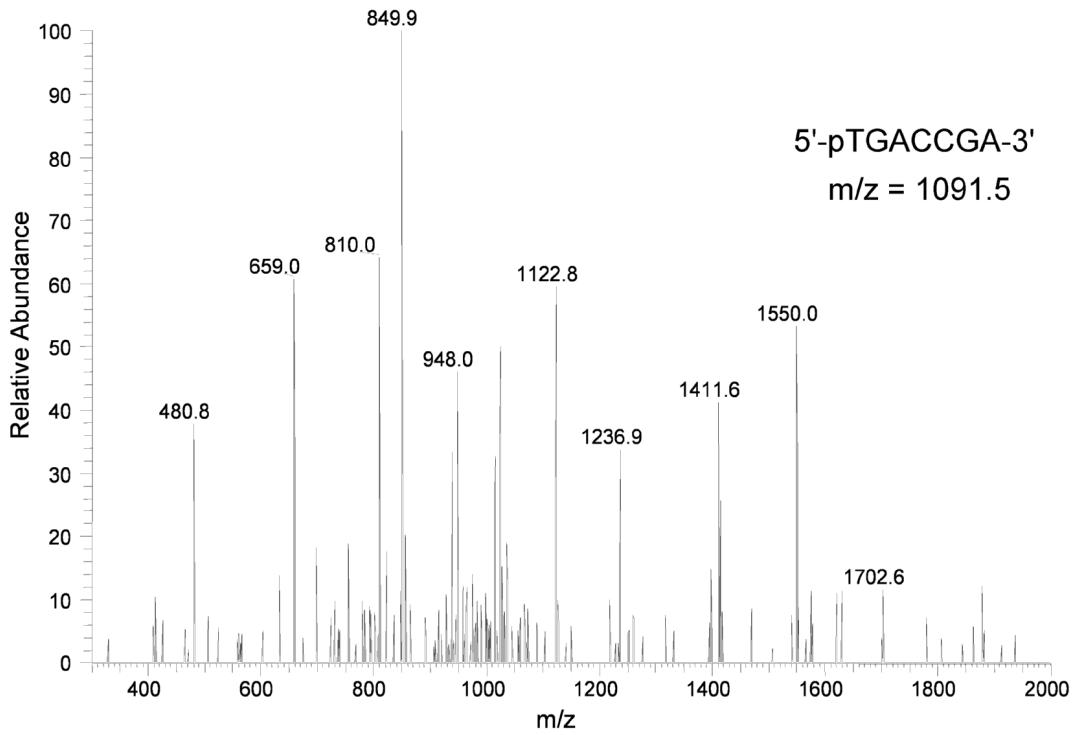


Figure S40. CID spectrum of the m/z 1091.5 product from the Dpo4 extension of oligonucleotide **2**. See Table 4 for fragment assignments.