Supporting information: Nucleosome core particle catalyzed strand scission at abasic sites.

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General Methods.

Oligonucleotides were synthesized on an Applied Biosystems Incorporated 394 oligonucleotide synthesizer. Oligonucleotides containing **1** (Figures S1 and S2) were prepared as previously described (10). Expression and purification of all core histone proteins, as well as refolding and purification of the histone octamer, were done as previously described (19). T4 polynucleotide kinase, UDG, proteinase K, T4 DNA ligase, and DNase I were obtained from New England Biolabs (NEB). Nuclease P1 (from *Penicilium citrinum)* was from Sigma and was dissolved in water (1 U/μL). γ-^{32}P-ATP was from Perkin Elmer. C18-Sep-Pak cartridges were obtained from Waters. Quantification of radiolabeled oligonucleotides was carried out using a Molecular Dynamics Phosphorimager 840 equipped with ImageQuant TL software. All experiments were conducted in clear siliconized tubes (Bio Plas Incorporated). Photolyses of oligonucleotides were carried out in a Rayonet photoreactor (RPR-100) fitted with 16 lamps having an output maximum at 350 nm.

General procedure for preparation of 146 bp duplexes containing a modified nucleotide in the vicinity of SHL 1.5 and 4.7 (AP_{89}, AP_{124}, AP_{204}, and AP_{207}).

Chemically synthesized DNA oligonucleotides (Figure S1A) were enzymatically phosphorylated at their 5′-termini, each in a separate 100 μL reaction mixture containing 1.5 nmol DNA, 1 × T4 DNA ligase buffer (50 mM TRIS-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP), and 50 U of T4 polynucleotide kinase at 37 °C for 4 h. The phosphorylated oligonucleotides were combined with the appropriate complementary oligonucleotide (1.5 nmol, Figure S1B) and hybridized by heating at 90 °C for 5 min, followed by slow cooling to room temperature. The combined duplexes (~400 μL) were supplemented with additional ATP (0.5 mM final) and ligated using T4 DNA ligase (1200 U) at 16 °C for 10 h. The reaction was then phenol extracted (equal volume) and ethanol precipitated prior to purification by 8 % native PAGE. The product band was excised from the gel and the DNA was eluted overnight in 500 μL of elution buffer (0.1 M NaCl and 1 mM EDTA). The slurry was filtered using a 10 mL polyprep-column (BioRad) and the DNA was desalted using a Sephadex G25 spin column. The

concentrated DNA (Figure S1C) was stored at -20 °C until use. Typical yields were between 20 and 50%.

General procedure for the preparation of the 146 bp duplex containing a modified nucleotide at the nucleosome dyad (AP_{74}).

The DNA substrates containing **1** at the nucleosome dyad (AP_{74}) were prepared in an identical manner as described above. However, the oligonucleotides and ligated duplexes were those depicted in Figure S2.

Reconstitution of nucleosome core particles.

Salmon sperm DNA (10 μg) and 5′-^{32}P-labeled α-satellite DNA substrates containing **1** (~1 pmol) were combined in a small siliconized tube to a final volume of 10 μL containing 2 M NaCl. Several such reaction mixtures were prepared and to each was added a different amount of histone octamer (also in 2 M NaCl). Typically, between 0.8 and 1.6 equivalent (with respect to total DNA) of histone octamer were tested. The samples were incubated at room temperature for 30 min. The temperature then was increased to 30 °C and the samples were incubated for an additional 30 min before beginning a series of dilutions using nucleosome buffer (10 mM HEPES, 1 mM EDTA, and 0.1 mM PMSF, pH 7.5, 30 °C). Table S1 describes the volume and time duration of each dilution. After the final dilution, the samples were cooled to room temperature and any precipitate was pelleted via a brief (5 min) spin at ~ 15,000 g. The supernatant was then transferred to a fresh tube and a small aliquot was removed from each reaction mixture for analysis by 5 % native PAGE (acrylamide/bisacrylamide, 59:1) in order to determine the extent of reconstitution. Only those reactions with >85% reconstituted DNA were used in experiments. Following quantification, the data collected for all time course experiments was corrected to account for any remaining free DNA. The total volume of all bands in any given lane (volume here is equivalent to CPM, as determined by autoradiography) was multiplied by the fraction of remaining free

DNA. This amount was then subtracted from the volume of intact DNA for the same lane. All reconstituted nucleosome core particles were stored at 4 °C.

General procedure for the preparation of nucleosome core particles containing a 5′-^{32}P-AP site.

DNA duplexes were assembled as described above except that **1** was substituted with 5′-^{32}P-dU. The oligonucleotides containing 5′-^{32}P-dU at positions 89, 124, 204, and 207 (Figure S1) were prepared through ligation of two shorter oligonucleotides, one of which contained a 5′-terminal dU residue corresponding to the position of interest. The 5′-dU residue was phosphorylated using γ-^{32}P-ATP prior to ligation. DNA substrates containing dU were treated with UDG (5 U) in a 10 μL reaction mixture containing 20 mM TRIS-HCl (pH 8.0), 1 mM EDTA, and 1 mM DTT at 37 °C for 10 min. The DNA containing AP was then reconstituted as described above and used immediately.

Prepare the plasmid for expression of mutated histones. The plasmid used for expression of mutated H3 and H4 were prepared by site-directed mutagenesis. The wild type histone H4 (**pET3a-H4-WT**) and H3 (**pET3a-H3-WT**) plasmid was obtained as gift from Bowman's lab. The vector is pET3a and inset DNA sequence are listed below.

Sequence of pET3a-H4-WT:

-CTTTAAGAAGGAGATATACA|TATG(start codon)

1 **Nde I clone site**

tct ggt cgt ggt aaa ggt ggt aaa ggt ctg ggt aaa ggt ggt gct aaa cgt cac cgt

20

aaa gtt ctg cgt gac aac atc cag ggt atc acc aag ccg gct atc cgt cgt ctg gct

39

cgt cgt ggt ggt gtt aaa cgt atc tcc ggt ctg atc tac gaa gaa acc cgc ggt gtt

58

ctg aaa gtt ttc ctg gaa aac gtt atc cgt gac gct gtt acc tac acc gaa cac gct

77

aaa cgt aaa acc gtt acc gct atg gac gtt gtt tac gct ctg aaa cgt cag ggt cgt

96 102

acc ctg tac ggt ttc ggt ggt TAA(stop codon) AGA TCCGGCTGC-

Bam HI clone site

Preparation plasmid of H4 H18A (pET3a-H4-H18A)

Template: **pET3a-H4-WT**

Forward primer: 5′ GGTAAAGGTGGTGCTAAACGTGCTCGTAAAGTTCTGCGTGACAAC
Reverse primer: 5′ GTTGTCACGCAGAACTTTACGAGCACGTTTAGCACCACCTTTACC

Preparation plasmid of H4 K5R (pET3a-H4-K5R)

Template: **pET3a-H4-WT**

Forward primer: 5′ CATATGTCTGGTCGTGGTCGTGGTGGTAAAGGTCTGGG
Reverse primer: 5′ CCCAGACCTTTACCACCACGACCACGACCAGACATATG

Preparation plasmid of H4 K5,8R (pET3a-H4-K5,8R)

Template: **pET3a-H4-K5R**

Forward primer: 5′CATATGTCTGGTCGTGGTCGTGGTGGTCGTGGTCTGGGTAAAGGTGG

Reverse primer: 5′ CCACCTTTACCCAGACCACGACCACCACGACCACGACCAGACATATG

Preparation plasmid of H4 K5,8,12R (pET3a-H4-K5,8,12R)

Template: **pET3a-H4- K5,8R**

Forward primer: 5′GGTGGTCGTGGTCTGGGTCGTGGTGGTGCTAAACGTCACCGTAAAG

Reverse primer: 5′ CTTTACGGTGACGTTTAGCACCACCACGACCCAGACCACGACCACC

Preparation plasmid of H4 K5,8,12,16,20R (pET3a-H4-K5,8,12,16,20R)

Template: **pET3a-H4- K5,8,12R**

Forward primer: 5′ GGTCGTGGTGGTGCTCGTCGTCACCGTCGTGTTCTGCGTGACAACATC

Reverse primer: 5′ GATGTTGTCACGCAGAACACGACGGTGACGACGAGCACCACCACGACC

Preparation plasmid of H4 K5,8,12,16,20R, H18A (pET3a-H4-K5,8,12,16,20R, H18A)

Template: **pET3a-H4-K5,8,12,16,20R**

Forward primer: 5′ GGTCGTGGTGGTGCTCGTCGTGCTCGTCGTGTTCTGCGTGACAAC

Reverse primer: 5′ GTTGTCACGCAGAACACGACGAGCACGACGAGCACCACCACGACC

Sequence of pET3a-H3-WT:

-CTTTAAGAAGGAGATATACA|TATG(start codon)

Nde I clone site

1 10
gcc cgt acc aag cag acc gcc cgt aaa tcc acc gga ggg aag gct ccc cgc aag cag

20 30
ctg gcc acc aag gca gcc agg aag tcc gct cct gct acc ggc gga gtc aag aaa

40 50
cct cac cgt tac cgg ccc ggc aca gtc gct ctc cgc gag atc cgc cgc tac cag aaa

60 70
tcc acc gag ctg ctc atc cgc aaa ctg cct ttc cag cgc ctg gtc cgg gag atc gct

80 90
cag gac ttc aag acc gac ctg cgc ttc cag agc tcg gcc gtt atg gct ctg cag gag

100 110
gcc agc gag gct tat ctg gtc gct ctc ttt gag gac acc aac ctg tgc gcc atc cac

120 130
gcc aag agg gtc acc atc atg ccc aag gac atc cag ctg gcc cgc aga atc cga ggc

135
gag agg gct TAG| (stop codon) ATCCGGCTGC-

Bam HI clone site

The template and primer sequences used for mutagenesis are listed below:

Preparation plasmid of H3 del $_{1\text{-}37}$ (pET3a-H3-Del)

Template: **pET3a-H3-WT**

Forward primer: 5′ CTTTAAGAAGGAGATATACATATGCCTCACCGTTACCGGCCCGGCAC

Reverse primer: 5′ GTGCCGGGCCGGTAACGGTGAGGCATATGTATATCTCCTTCTTAAAG

Table S1. Dilutions for nucleosome reconstitution reactions.

Dilution #	Volume (µl)	Time (min)
1	12	60
2	6	60
3	6	30
4	10	30
5	10	30
6	20	30
7	50	30
8	100	30
9	100	30

A

5′–N_{74}TCAGCTGAACATGCN_{89}TTTTGATGGAG

5′–CAGTTTCCAAATACACTTTTGGTAGAATCTGCAGGTGGATATTGAT

5′–GCTGAACATGCCTTTTGATGGAGCAGTTTCCAAATACACTTTTGGTAGAATCTGCAGGTGGATATTGAT

5′–ACTGCTCCATCAAAAN_{204}GCN_{207}TGTTCAGCTGAATTCA

B

5′–N_1TCAATATCCACCTGCAGATTCTACCAAAAGTGTATTTGGAAACTGCTCCATCAAAAGGCATGTTCAGCTGAA
3′–T AGTTATAGGTGGACGTCTAAGATGGTTTTCACATAAACCTTTGACGAGGTAGTTTTCCGTACAAGTCG

5′–N_{74}TCAGCTGAACAT GCN_{89} TTTTGATGGAG
3′–ACTTA AGTCGACTTGTN_{207}CGN_{204}AAAACTACCTCGTCA

5′–CAGTTTCCAAATACACTTTTGGTAGAATCTGCAGGTGGATATTGAT
3′–AAGGTTTATGTGAAAACCATCTTAGACGTCCACCTATAACTN_{147}

C

5′–N_1TCAATATCCACCTGCAGATTCTACCAAAAGTGTATTTGGAAACTGCTCCATCAAAAGGCATGTTCAGCTGAA–
3′–T AGTTATAGGTGGACGTCTAAGATGGTTTTCACATAAACCTTTGACGAGGTAGTTTTCCGTACAAGTCGACTT–

–N_{74}TCAGCTGAACAT GCN_{89} TTTTGATGGAGCAGTTTCCAAATACACTTTTGGTAGAATCTGCAGGTGGATATTGAT–3′
–A AGTCGACTTGTN_{207}CGN_{204}AAAACTACCTCGTCAAAGGTTTATGTGAAAACCATCTTAGACGTCCACCTATAACTN_{147}–5′

	N_1	N_{74}	N_{89}	N_{124}	N_{147}	N_{204}	N_{207}
WT	A	T	C	A	A	G	A
AP_{89}	A	T	**1**	A	**CAP**	A	A
AP_{124}	A	T	C	**1**	**CAP**	G	A
AP_{204}	**CAP**	T	A	A	A	**1**	A
AP_{207}	**CAP**	T	T	A	A	A	**1**

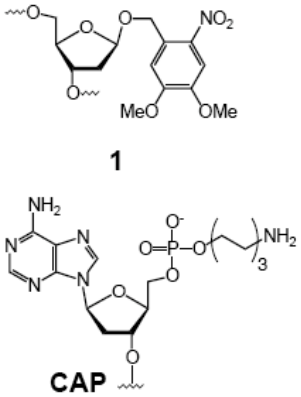


Figure S1. Oligonucleotides used in preparation of the 146 bp DNA duplexes containing **1** in the vicinity of SHL 1.5 and 4.7 (AP_{89}, AP_{124}, AP_{204}, and AP_{207}). The oligonucleotides (A) were 5'-phosphorylated and hybridized to the appropriate complementary strand. The resulting duplexes (B) were ligated to construct the 146 bp substrates (C).

A

5′-CTCCATCAAAAGGCATGTTCAGCTGAAN_{74}TCAG

5′-CTGAACATGCN_{89}TTTTGATGGAGCAGTTTCCAAATACACTTTTGGTAGAATCTGCAGGTGGATATTGAT

5′-GCTGAACATGCCTTTTGATGGAGCAGTTTCCAAATACACTTTTGGTAGAATCTGCAGGTGGATATTGAT

5′-ACTGCTCCATCAAAAN_{204}GCN_{207}TGTTCAGCTGAATTCA

B

5′-N_1TCAATATCCACCTGCAGATTCTACCAAAAGTGTATTTGGAAACTGCTCCATCAAAAGGCATGTTCAGCTGAAN_{74}TCAG
3′-T AGTTATAGGTGGACGTCTAAGATGGTTTTCACATAAACCTTTGACGAGGTAGTTTTCCGTACAAGTCG

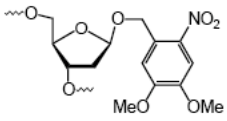
5′-CTGAACAT GCN_{89} TTTTGATGGAGCAGTTTCCAAATACACTTTTGGTAGAATCTGCAGGTGGATATTGAT
3′-ACTTAAGTCGACTTGTN_{207}CGN_{204}AAAACTACCTCGTCAAAGGTTTATGTGAAAACCATCTTAGACGTCCACCTATAACTN_{147}

C

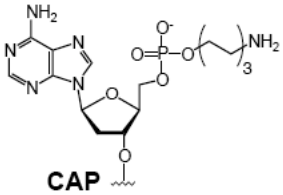
5′-N_1TCAATATCCACCTGCAGATTCTACCAAAAGTGTATTTGGAAACTGCTCCATCAAAAGGCATGTTCAGCTGAA-
3′-T AGTTATAGGTGGACGTCTAAGATGGTTTTCACATAAACCTTTGACGAGGTAGTTTTCCGTACAAGTCGACTT-

-N_{74}TCAGCTGAACAT GCN_{89} TTTTGATGGAGCAGTTTCCAAATACACTTTTGGTAGAATCTGCAGGTGGATATTGAT-3′
-A AGTCGACTTGTN_{207}CGN_{204}AAAACTACCTCGTCAAAGGTTTATGTGAAAACCATCTTAGACGTCCACCTATAACTN_{147}-5′

	N_1	N_{74}	N_{89}	N_{124}	N_{147}	N_{204}	N_{207}
WT	A	T	C	A	A	G	A
AP_{74}	A	**1**	C	A	**CAP**	G	A



1



CAP

Figure S2. Oligonucleotides used in preparation of the 146 bp DNA duplex containing a modified nucleotide at the nucleosome dyad (AP_{74}). The oligonucleotides (A) were 5'-phosphorylated and hybridized to the appropriate complementary strand. The resulting duplexes (B) were ligated to construct the 146 bp substrates (C).

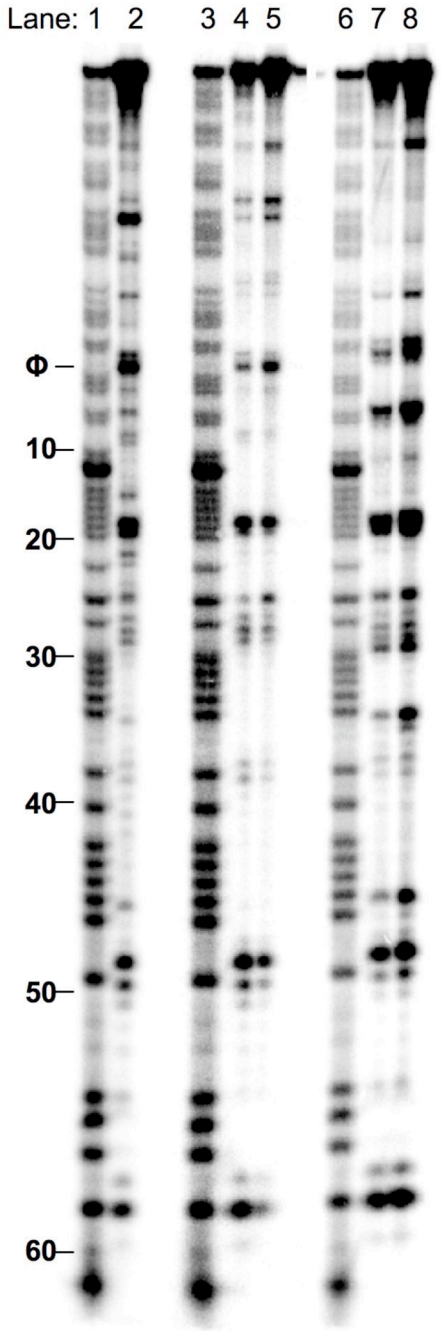


Figure S3. DNase footprinting of nucleosome core particles containing AP_{89} and AP_{207}. Lanes 1, 3, and 6 are A + G sequencing reactions. Lane 2 is the DNase I digested free (nonnucleosomal) α-satellite palindrome DNA containing no modifications. Lanes 4 and 5 (AP_{89}), 7 and 8 (AP_{207}).

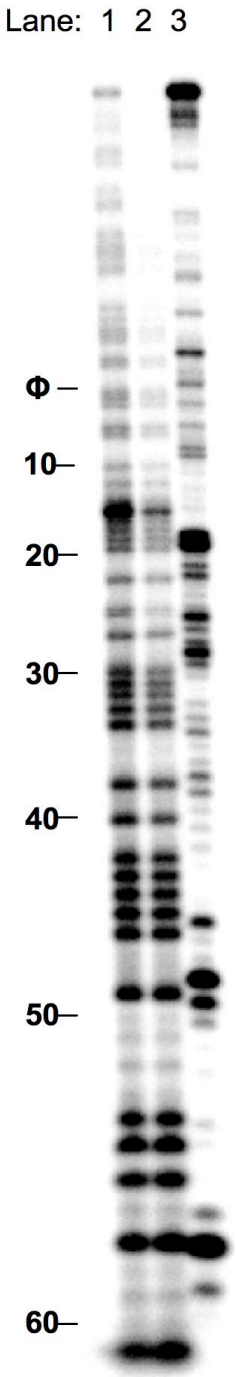


Figure S4. DNase footprinting of nucleosome core particle containing AP_{74}. Lanes 1 and 2 are A + G sequencing reactions. Lane 3 is the DNase I digested (0.1 U) nucleosome core particle containing AP_{74}.

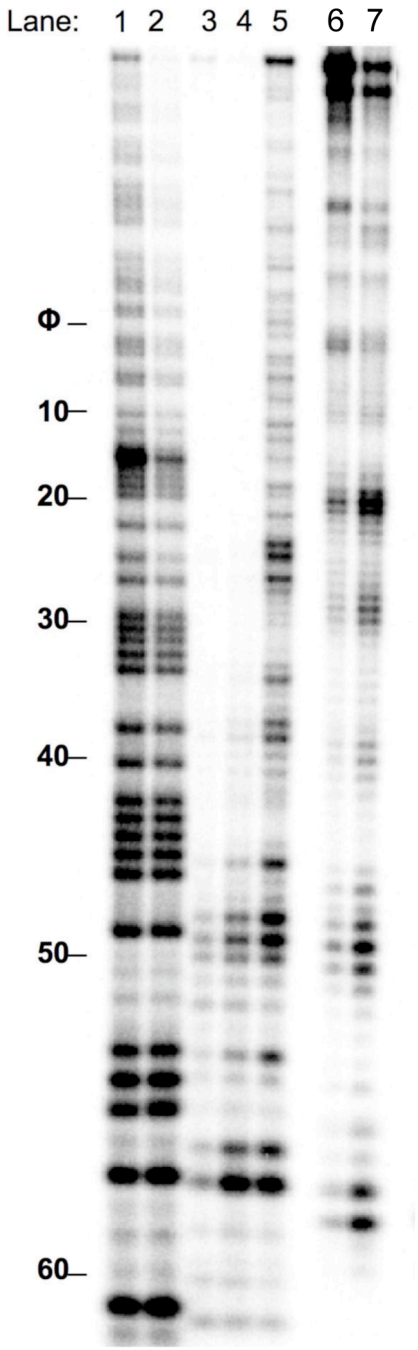


Figure S5. DNase footprinting of nucleosome core particles containing AP_{124}. Lanes 1 and 2 are A + G sequencing reactions. Lanes 3-5 are DNase I digests of naked DNA. Nucleosome core particles containing AP_{124} (lanes 6-7) were treated with DNase I in the following amounts: 0.1 U (lane 6) or 0.5 U (lane 7).

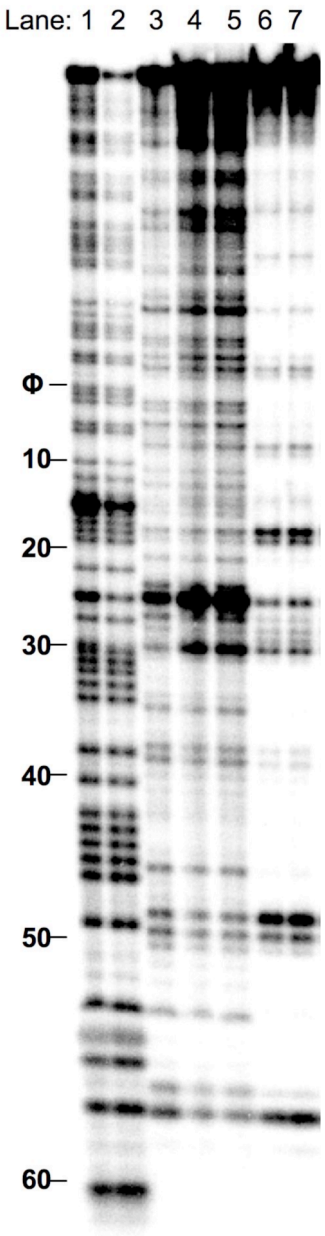


Figure S6. DNase footprinting of nucleosome core particles containing AP_{204}. A + G sequencing reactions are shown in lanes 1 and 2. Lanes 3-5 are DNase I digests of naked DNA. DNA samples in lanes 6 and 7 are DNase I digested nucleosome core particles containing AP_{204}. DNase I was added to each sample in the following amounts: lane 3, 0.01 U; lane 4, 0.05 U; lane 5, 0.1 U; lane 6, 0.1 U; lane 7, 0.5 U.

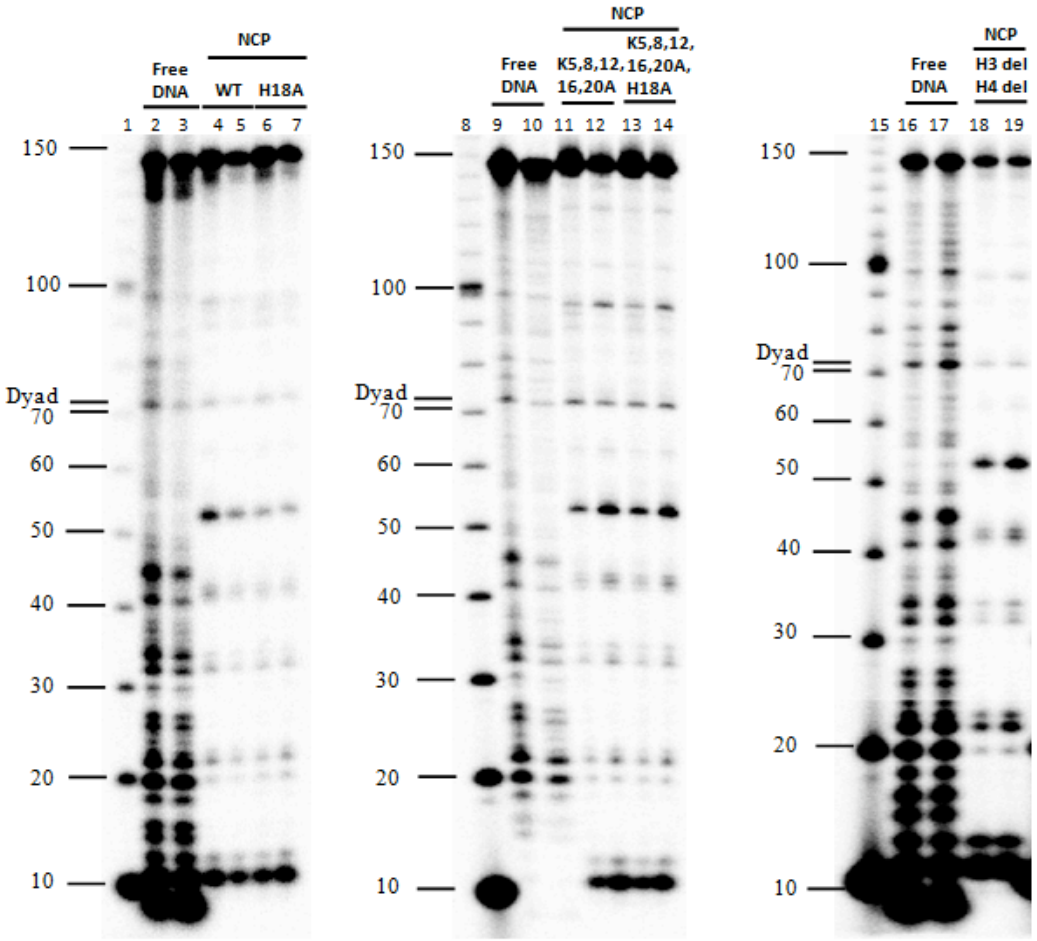


Figure S7. DNase I footprinting pattern of AP_{89} within 601 NCPs. (8% Denaturing PAGE) Lanes 1, 8 and 15, 10 bp DNA ladders. DNase I was added to each sample in the following amounts: 0.1 unit for lanes 3, 9, 16; 0.2 unit 4, 10, 17; 1 unit for lanes 4, 6, 11, 13, 18; 2 unit for lanes 5, 7, 12, 14, 19.

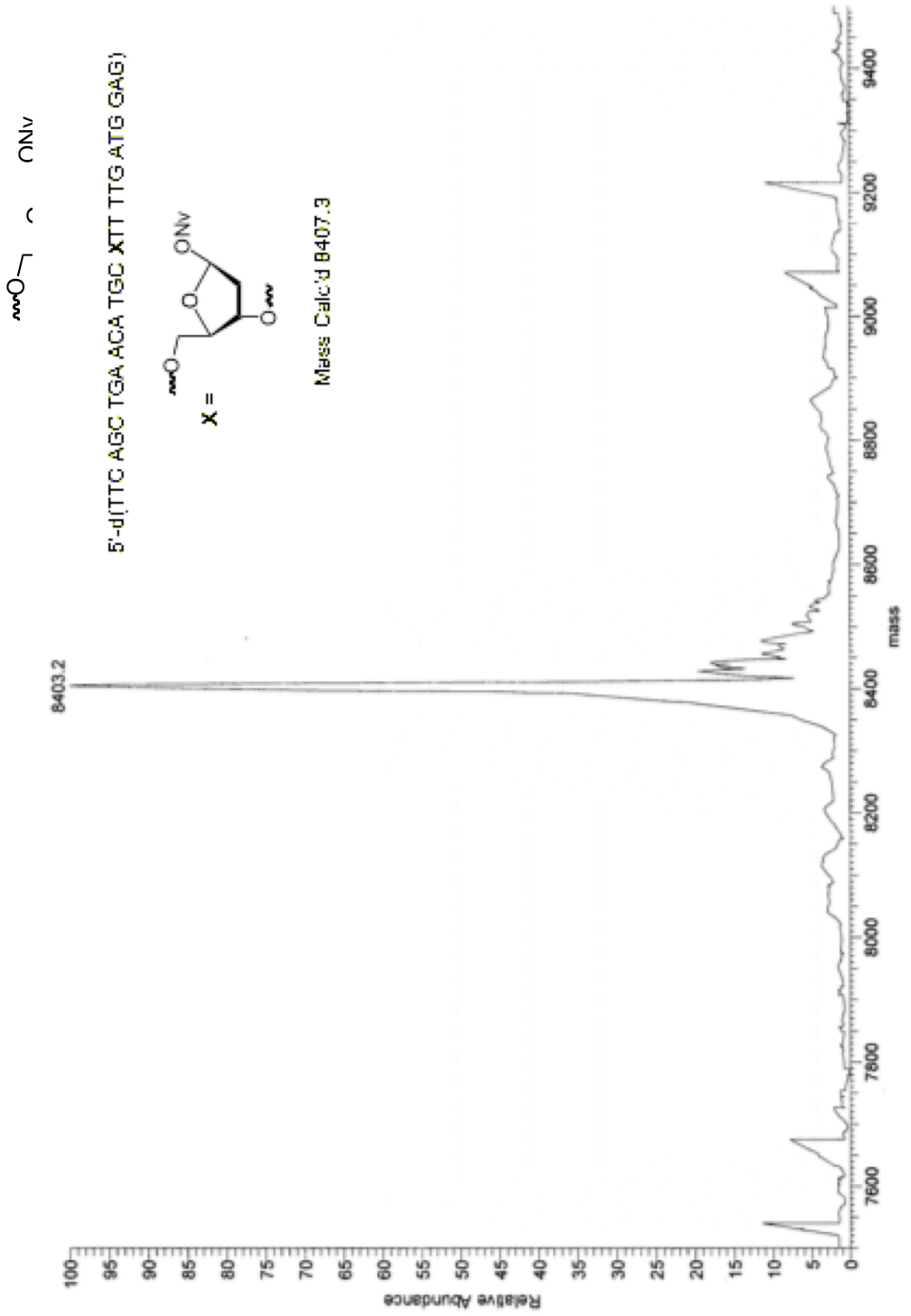


Figure S8. ESI-MS of oligonucleotide containing **1** used to prepare nucleosome core particles containing AP_{89}.

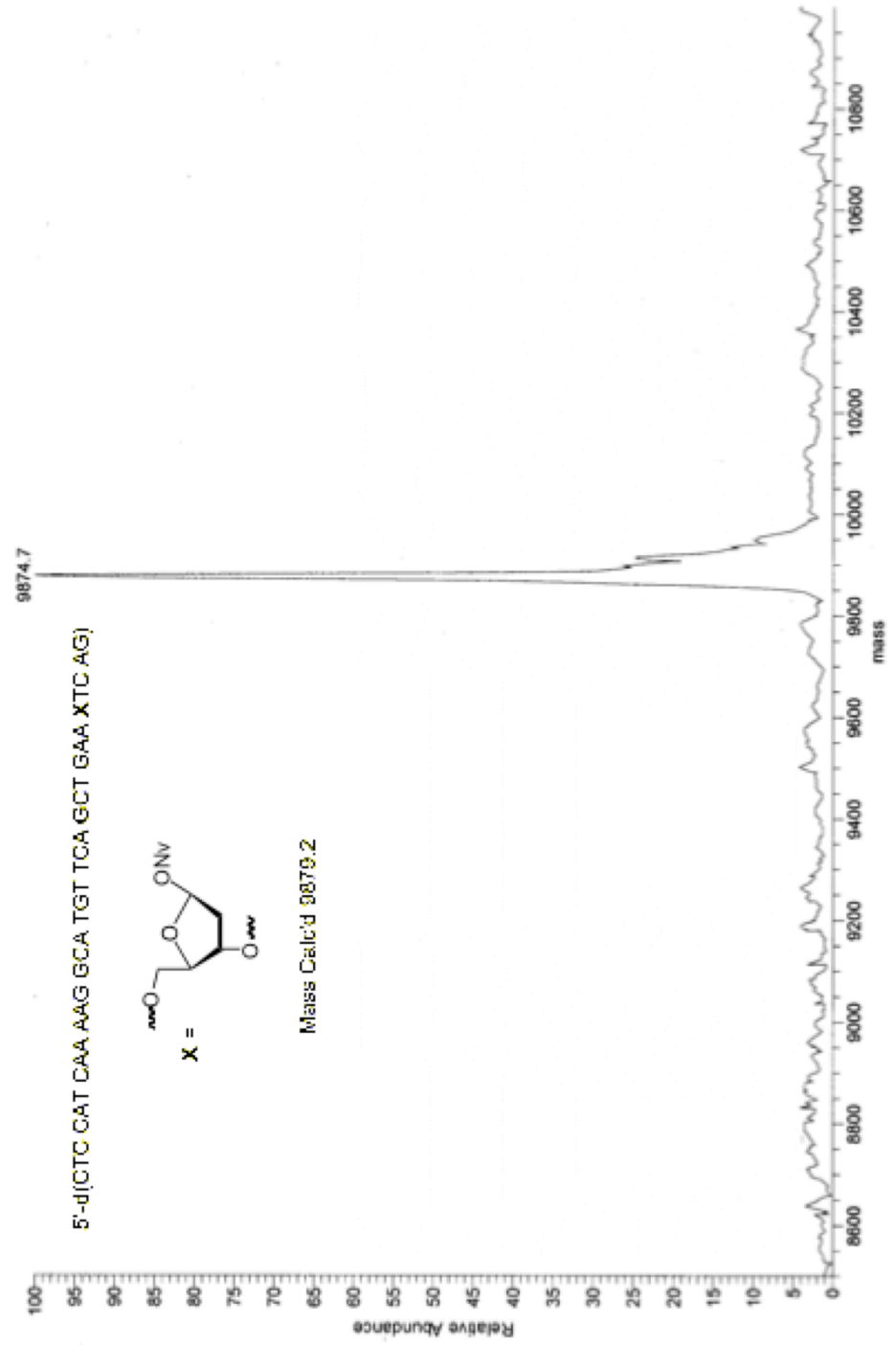


Figure S9. ESI-MS of oligonucleotide containing **1** used to prepare nucleosome core particles containing AP_{74}.

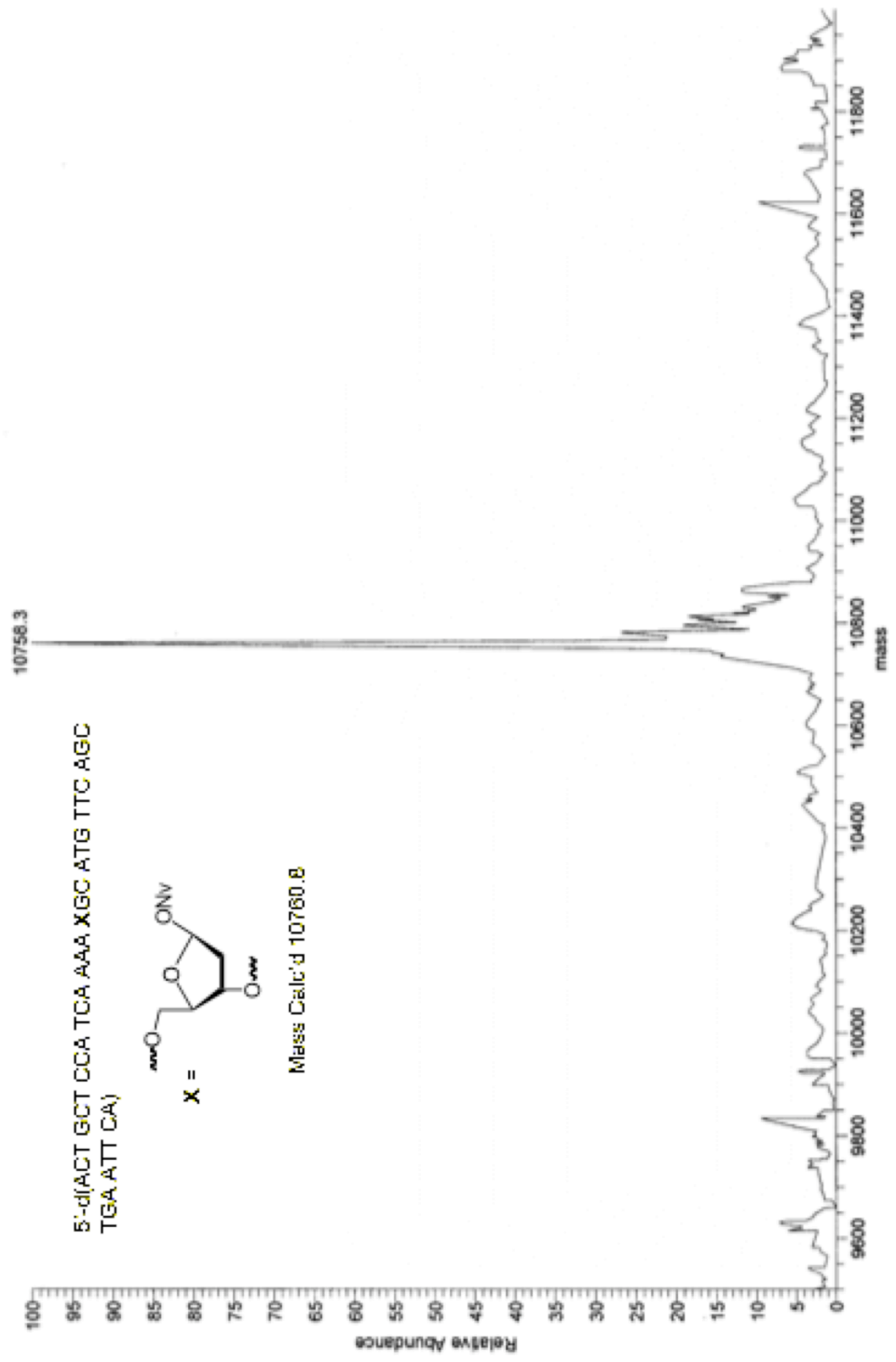


Figure S10. ESI-MS of oligonucleotide containing **1** used to prepare nucleosome core particles containing AP_{204}.

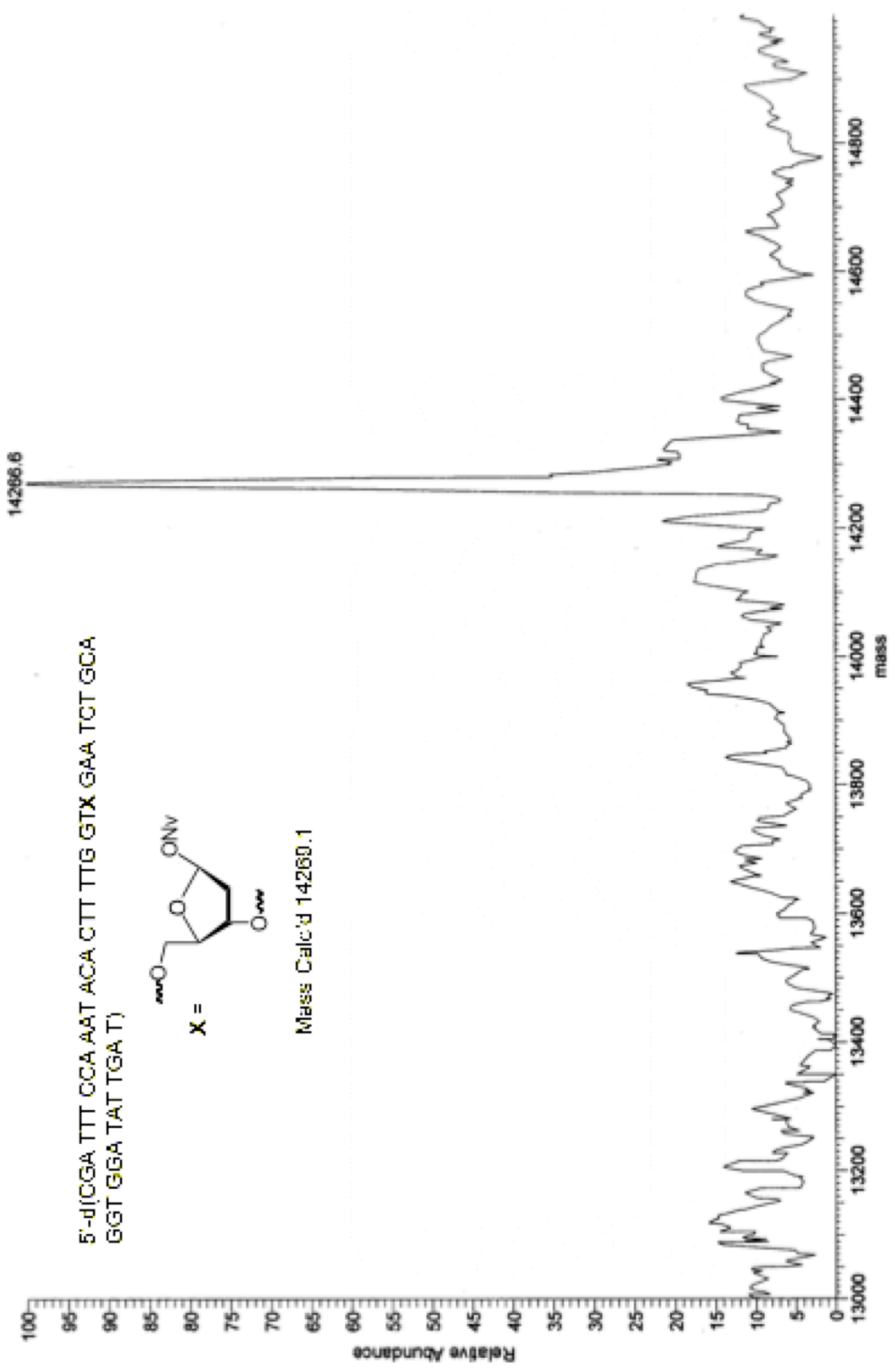


Figure S11. ESI-MS of oligonucleotide containing **1** used to prepare nucleosome core particles containing AP_{124}.

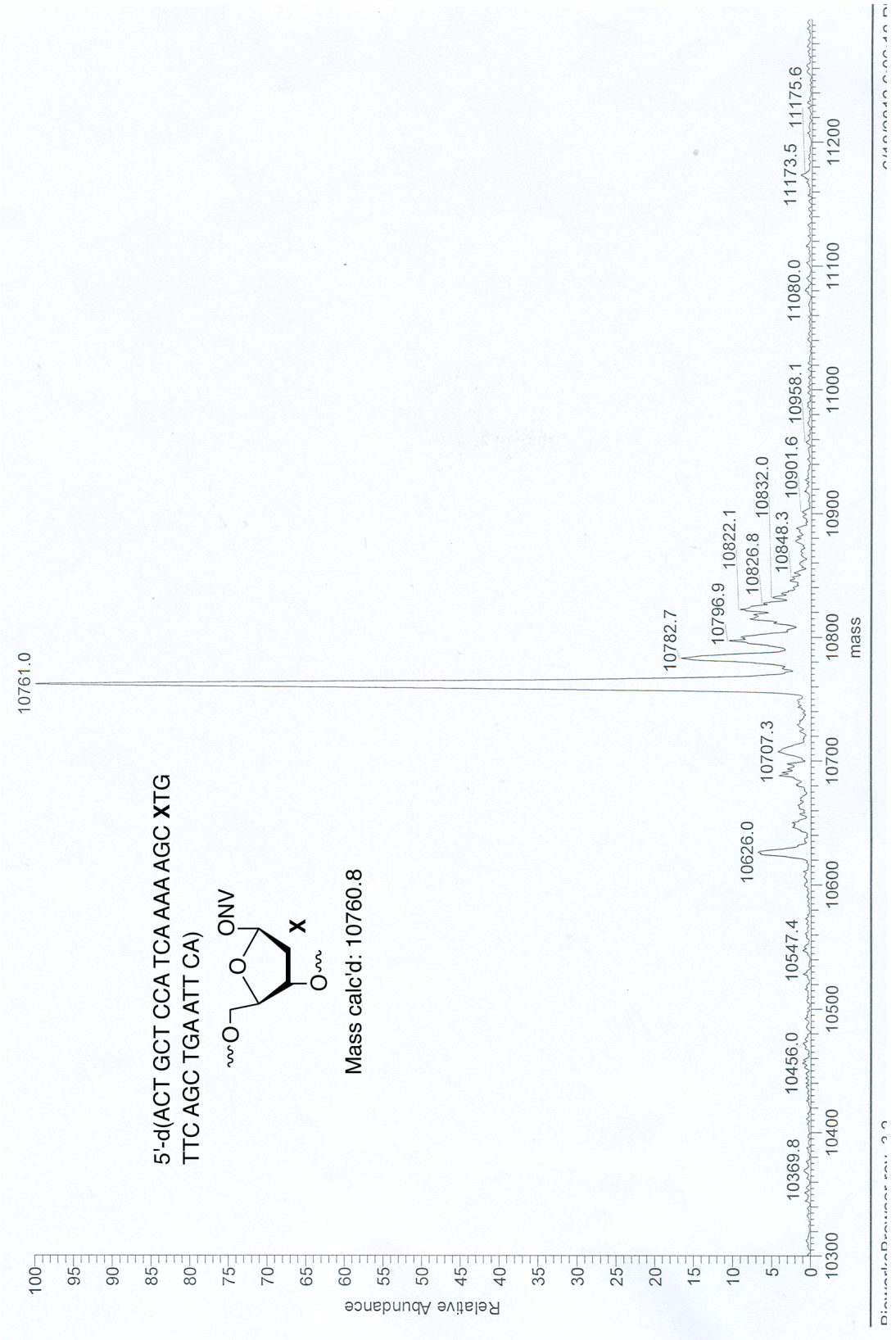
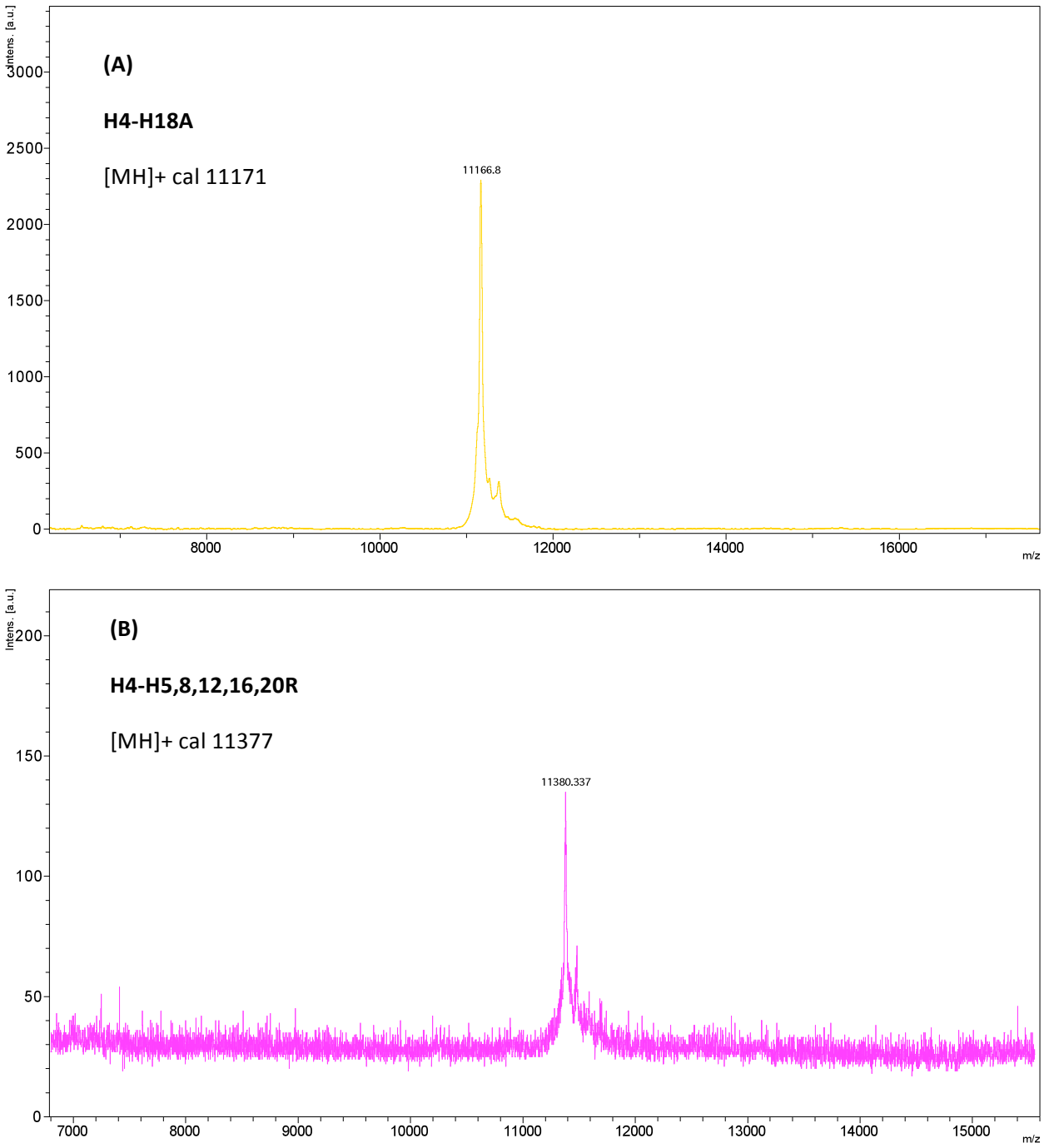
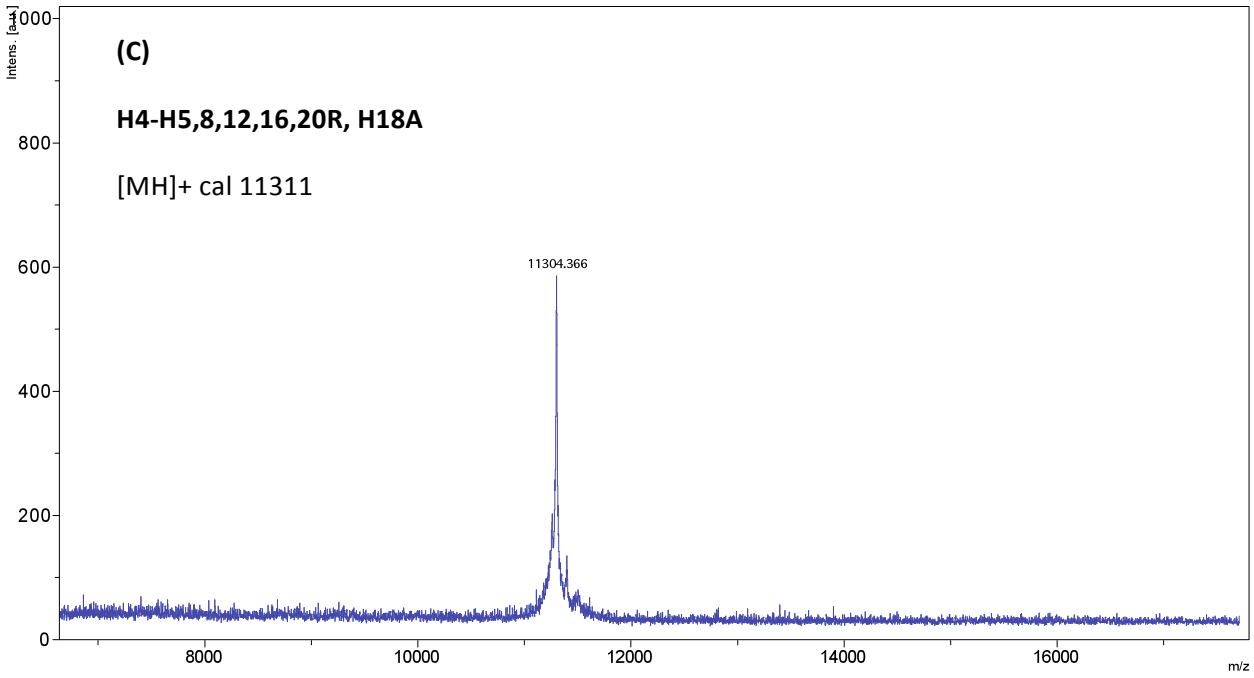
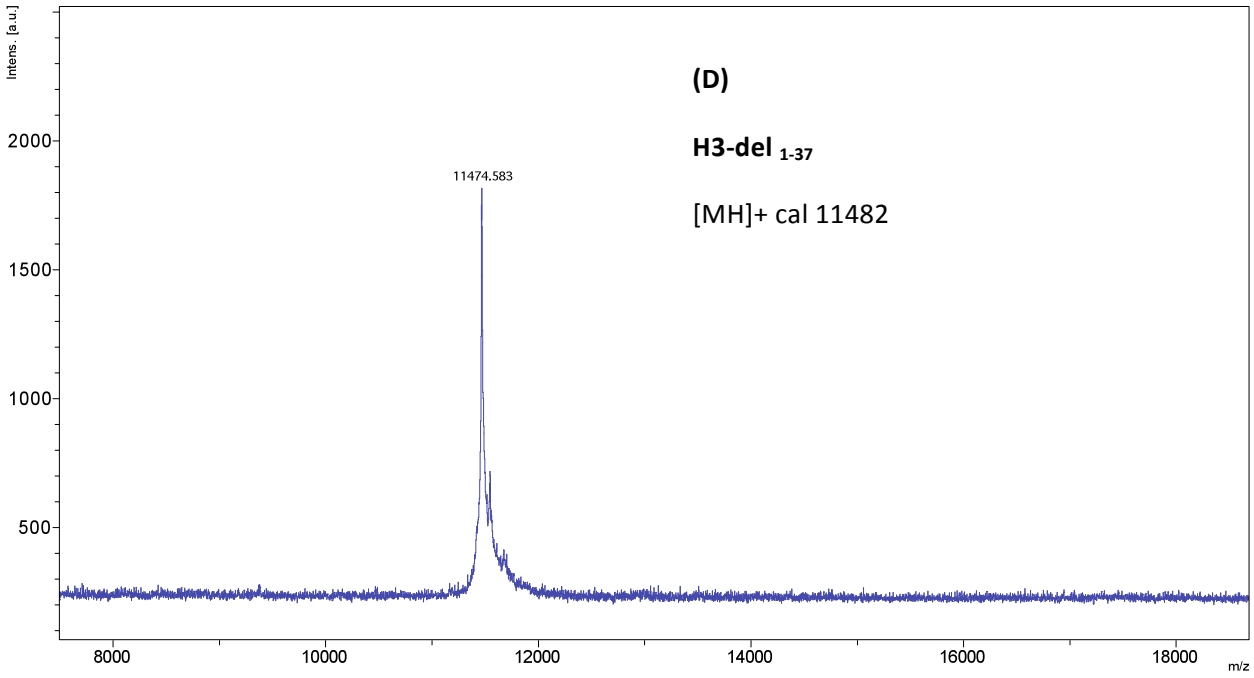


Figure S12. ESI-MS of oligonucleotide containing **1** used to prepare nucleosome core particles containing AP_{207}.

Figure S13. MALDI-TOF MS of histone variants.




(C)
H4-H5,8,12,16,20R, H18A
[MH]+ cal 11311
11304.366
Intens. [a.u.]
m/z


(D)
H3-del 1-37
[MH]+ cal 11482
11474.583
Intens. [a.u.]
m/z

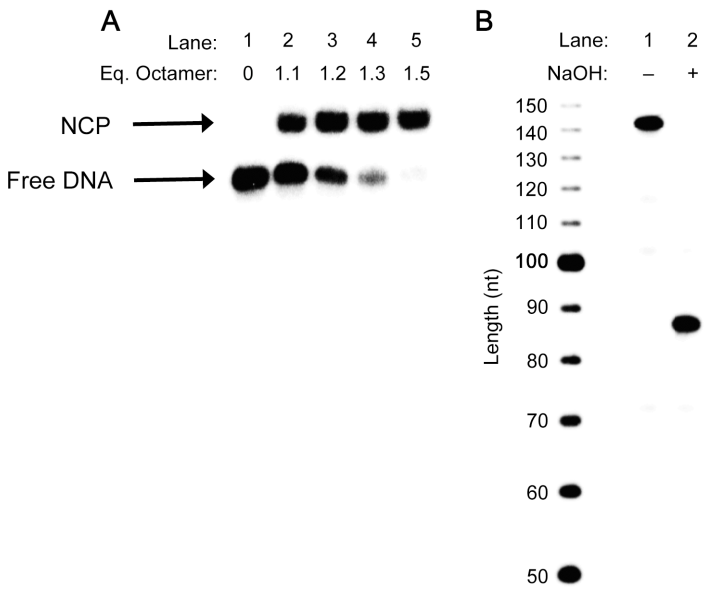


Figure S14. Representative gel illustrating reconstitution of nucleosome core particle containing precursor (**1**) to AP_{89} (A) Sample analysis of NCP following photolysis (20 % denaturing PAGE). NaOH (0.1 M). (B)

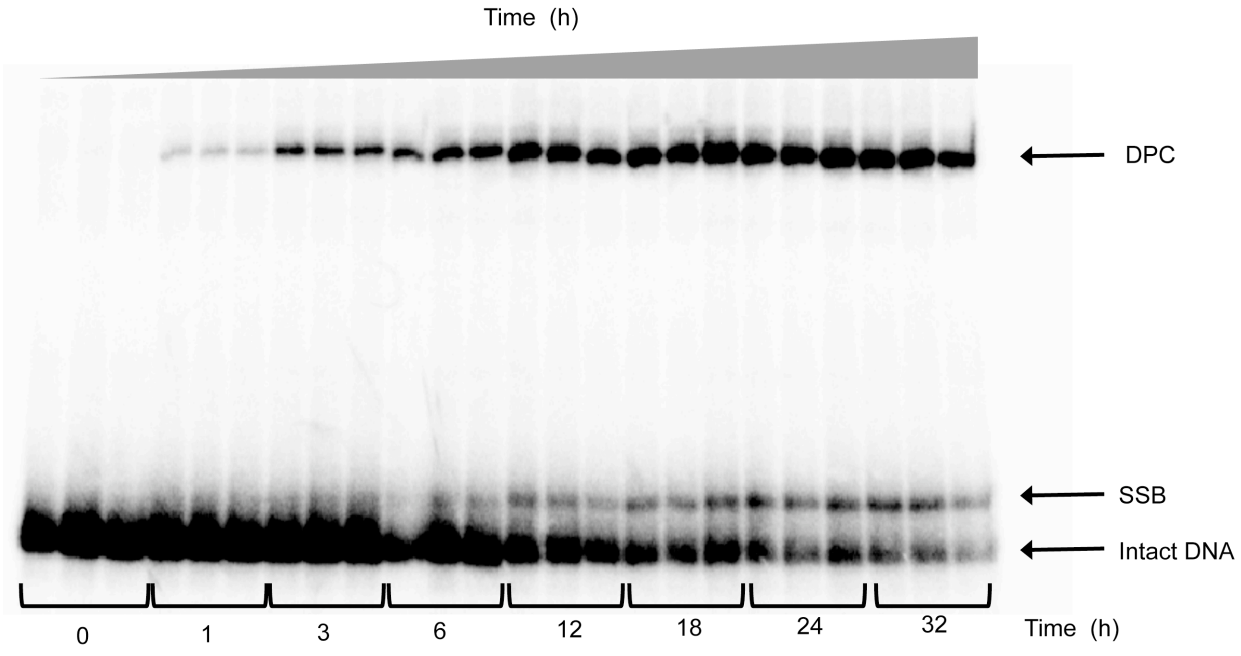


Figure S15. Representative SDS gel showing reactivity of AP_{89} over 24 hours. The experiment was run in triplicate and time points for 3 samples were loaded adjacent to one another.