Supplemental Information

Fig. S1 MALDI-TOF-MS of peptide ARTKQTARK before (A) and after (B) trypsin digestion, and peptide $(CH_3)_2$-ARTK$(CH_3)_2$QTARK$(CH_3)_2$ before (C) and after (D) trypsin digestion.

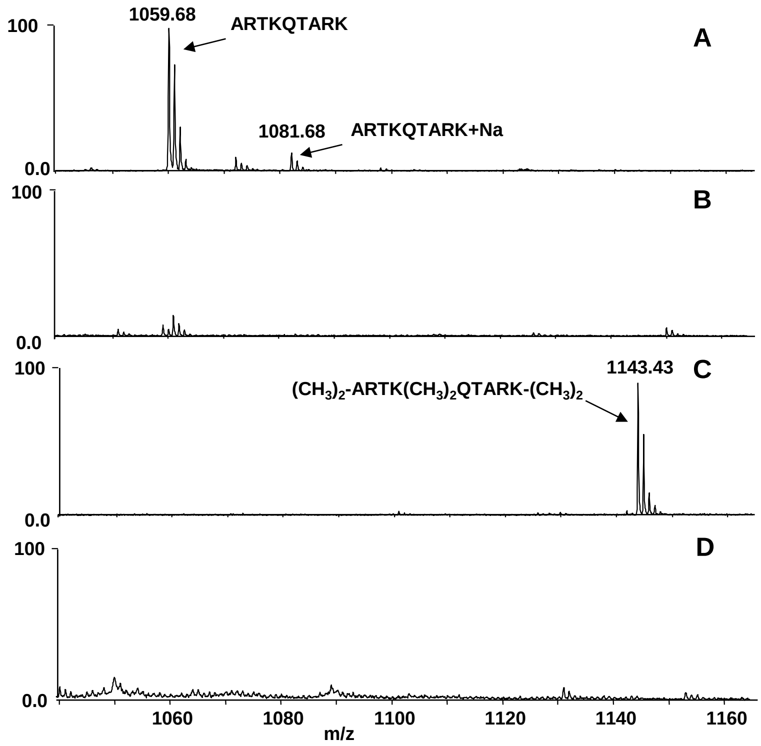


Fig. S2 MS/MS of QTAR (A) and TK$(CH_3)_2$QTAR (B). MALDI-TOF-MS of peptide TK$(CH_3)_2$QTAR without (C) or with (D) dimethyl labeling.

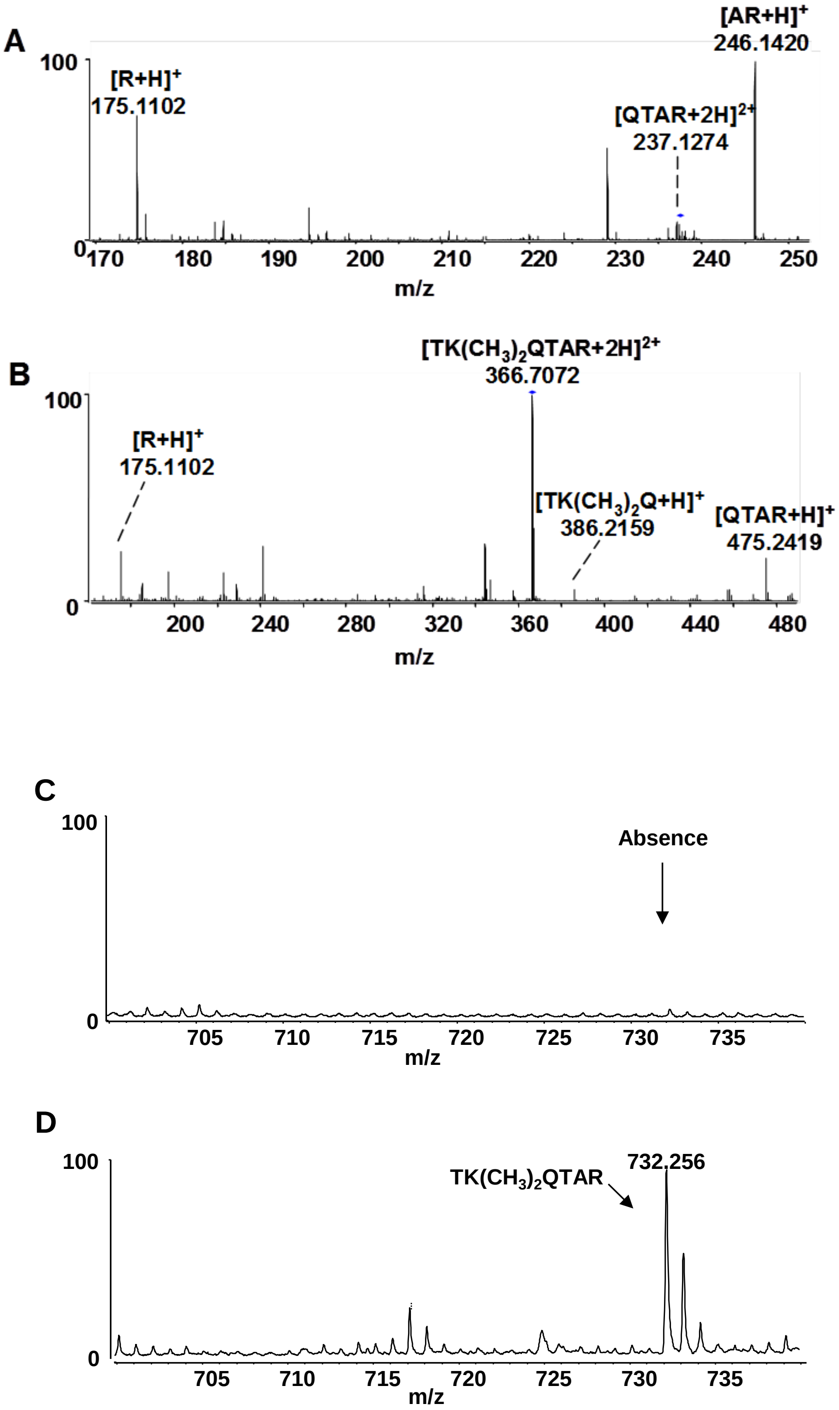
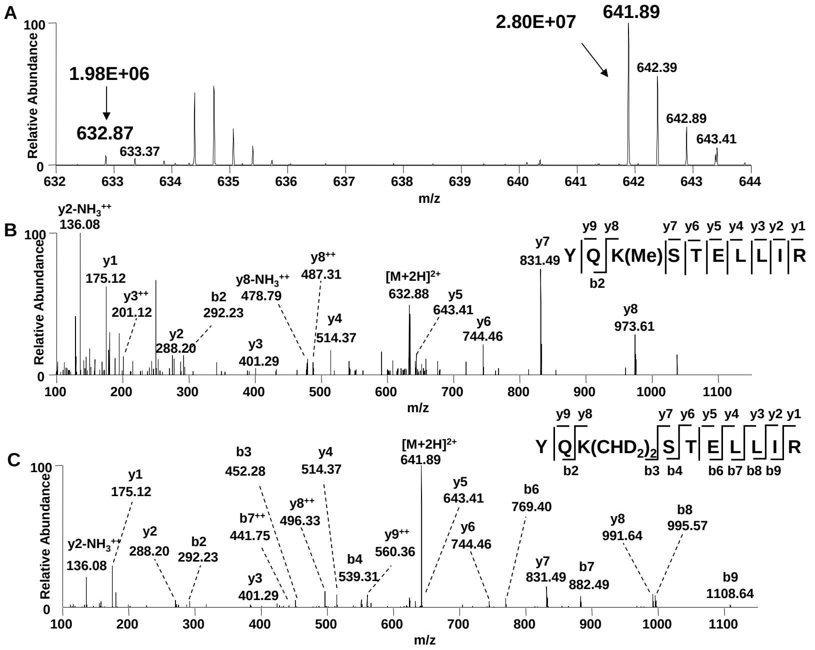


Fig. S3 The quantitative analysis of Lysine modification and non-modification at the same site.

(a) (A) A MS single-scan spectrum of YQK(Me)STELLIR (+632.87 Da, 2+) and YQK(CHD$_2$)$_2$STELLIR (+641.89Da, 2+) from H3 (residues 54-63). (B) MS/MS of YQK(Me)STELLIR. (C) MS/MS of YQK(CHD$_2$)$_2$STELLIR.

(b) (A) A MS single-scan spectrum of SAPATGGVK(Me$_2$)K(CHD$_2$)$_2$PHR (+683.42 Da, 2+) and SAPATGGVK(CHD$_2$)$_2$K(CHD$_2$)$_2$PHR (+685.43 Da, 2+) from H3 (residues 28-40). (B) MS/MS of SAPATGGVK(Me$_2$)K(CHD$_2$)$_2$PHR. (C) MS/MS of SAPATGGVK(CHD$_2$)$_2$K(CHD$_2$)$_2$PHR.

(a)



(b)

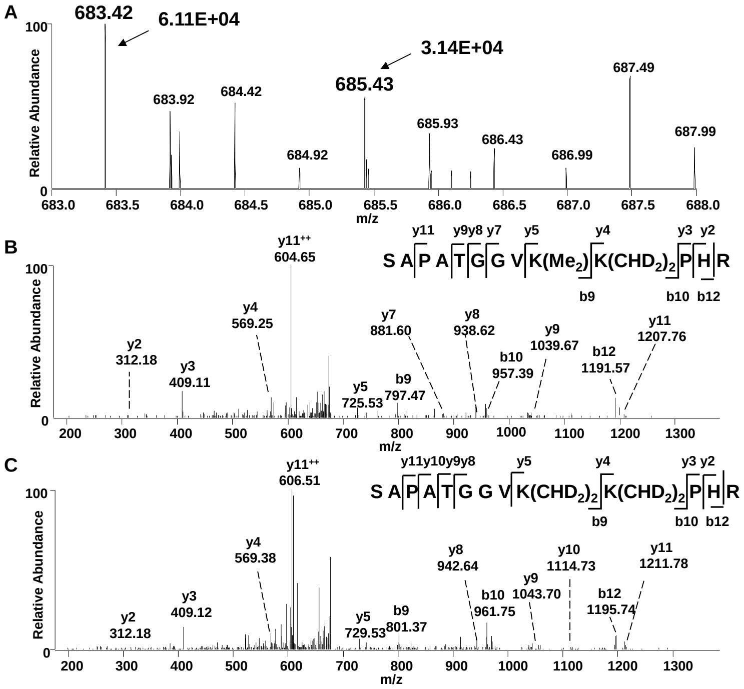


Fig.S4 MS/MS spectra for the modified peptides in Table 1.

Fig. S4A

GK$_{Ac}$GGK$_{Ac}$GLGK$_{Ac}$GGAK$_{Ac}$R

Monoisotopic mass of neutral peptide Mr(calc): 1437.81

Variable modifications:

K2: Acetyl (K); **K5:** Acetyl (K); **K9:** Acetyl (K); **K13:** Acetyl (K)

Ions Score: 71 **Expect:** 2.4e-006

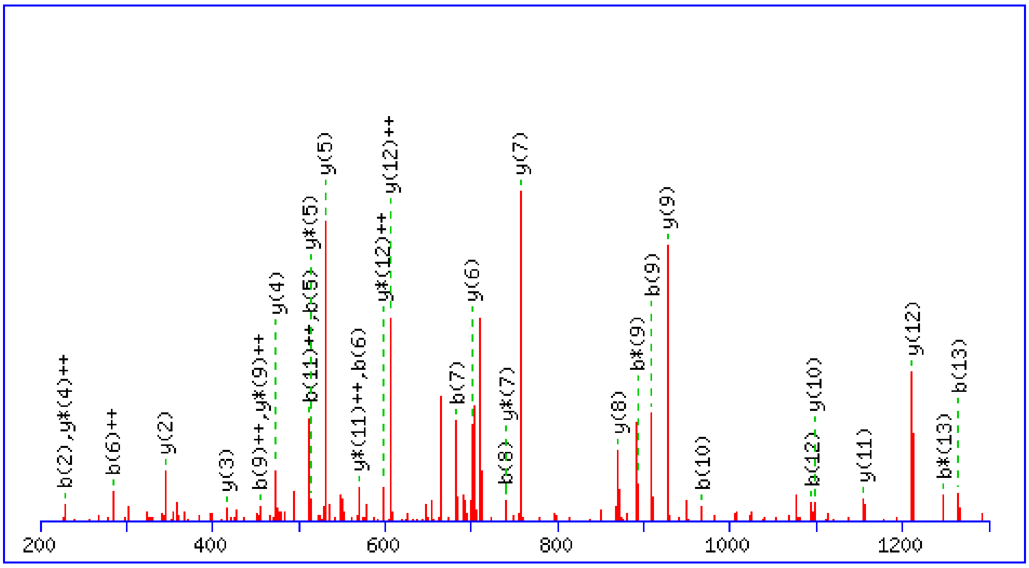


Fig. S4B

GK$_{(CHD2)2}$GGK$_{Ac}$GLGK$_{Ac}$GGAK$_{Ac}$R

Monoisotopic mass of neutral peptide Mr(calc): 1427.85

Variable modifications:

K2: Dimethyl:2H(4) (K); **K5:** Acetyl (K); **K9:** Acetyl (K); **K13:** Acetyl (K)

Ions Score: 64 **Expect:** 1.3e-005

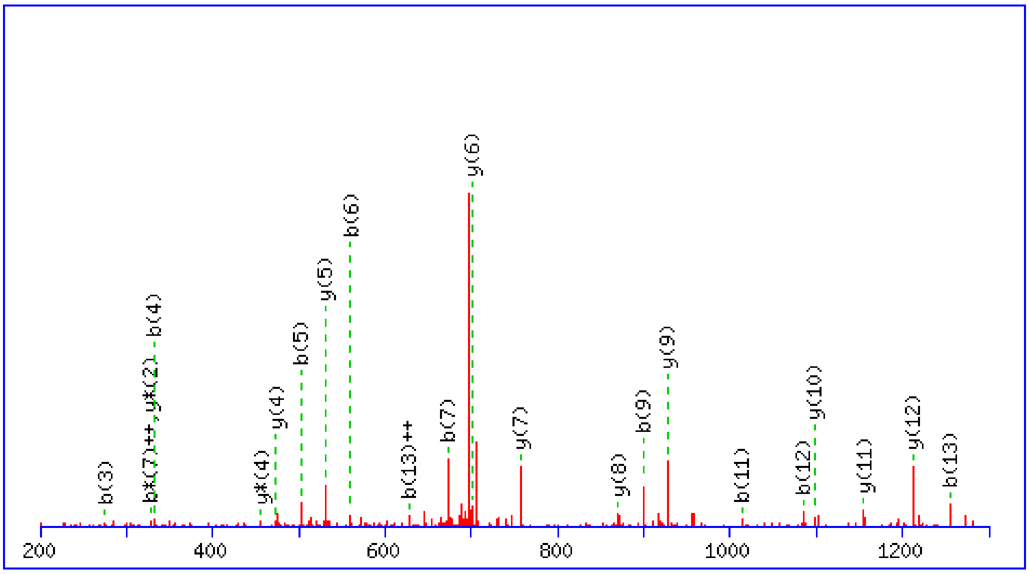


Fig. S4C

GK$_{Ac}$GGK$_{(CHD2)2}$GLGK$_{Ac}$GGAK$_{Ac}$R

Monoisotopic mass of neutral peptide Mr(calc): 1427.85

Variable modifications:

K2: Acetyl (K); **K5:** Dimethyl:2H(4) (K); **K9:** Acetyl (K); **K13:** Acetyl (K)

Ions Score: 54 **Expect:** 0.00012

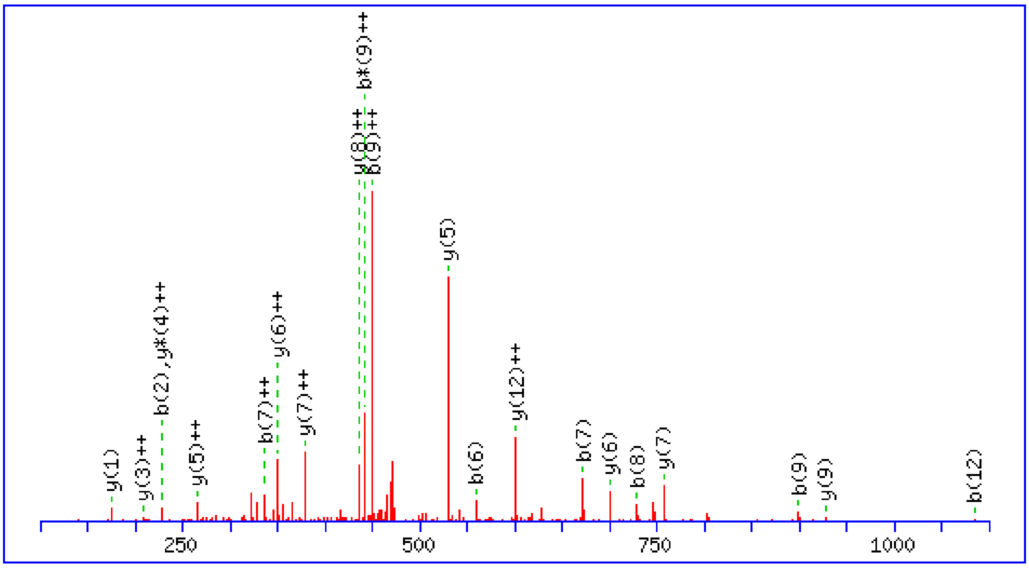


Fig. S4D

GK$_{Ac}$GGK$_{Ac}$GLGK$_{(CHD2)2}$GGAK$_{Ac}$R

Monoisotopic mass of neutral peptide Mr(calc): 1427.85

Variable modifications:

K2: Acetyl (K); **K5:** Acetyl (K); **K9:** Dimethyl:2H(4) (K); **K13:** Acetyl (K)

Ions Score: 51 **Expect:** 0.00016

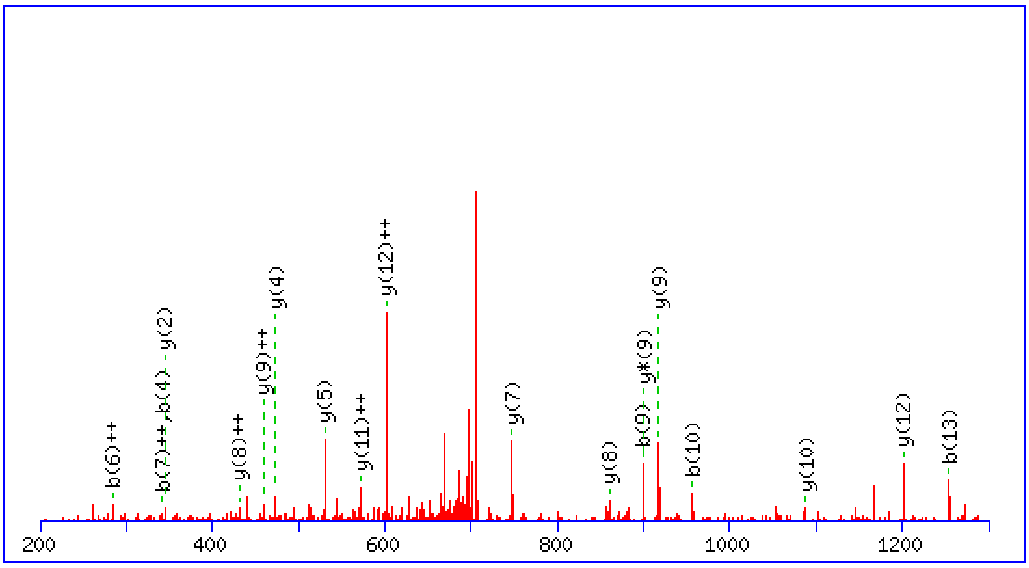


Fig. S4E

$\mathrm{GK_{(CHD2)2}GGK_{(CHD2)2}GLGK_{Ac}GGAK_{Ac}R}$

Monoisotopic mass of neutral peptide Mr(calc): 1417.90

Variable modifications:

K2: Dimethyl:2H(4) (K); **K5:** Dimethyl:2H(4) (K); **K9:** Acetyl (K); **K13:** Acetyl (K)

Ions Score: 55 **Expect:** 8.5e-005

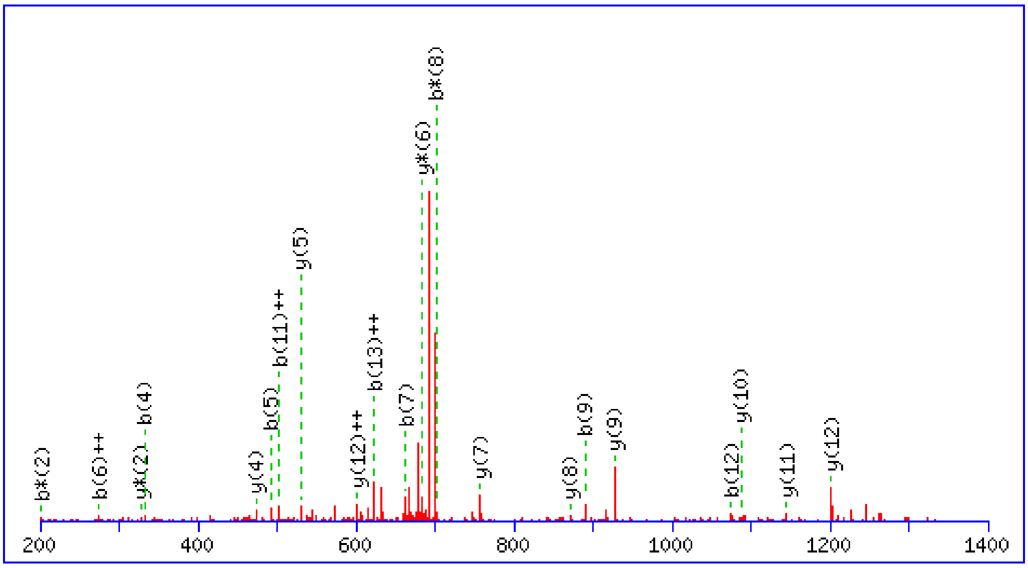


Fig. S4F

$\mathrm{GK_{(CHD2)2}GGK_{Ac}GLGK_{(CHD2)2}GGAK_{Ac}R}$

Monoisotopic mass of neutral peptide Mr(calc): 1417.90

Variable modifications:

K2: Dimethyl:2H(4) (K); **K5:** Acetyl (K); **K9:** Dimethyl:2H(4) (K); **K13:** Acetyl (K)

Ions Score: 53 **Expect:** 0.00012

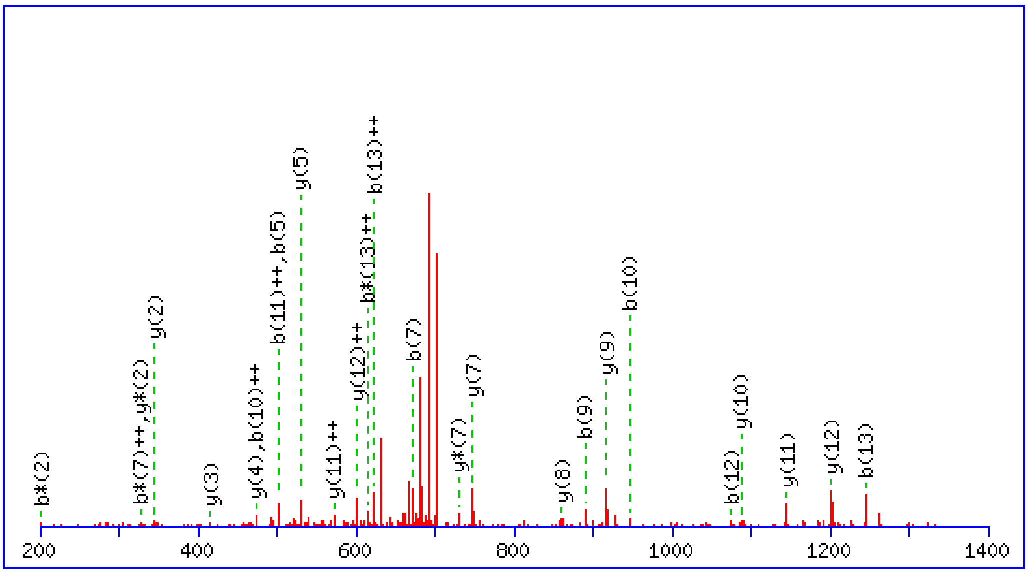


Fig. S4G

GK$_{Ac}$GGK$_{(CHD2)2}$GLGK$_{(CHD2)2}$GGAK$_{Ac}$R

Monoisotopic mass of neutral peptide Mr(calc): 1417.90

Variable modifications:

K2: Acetyl (K); **K5:** Dimethyl:2H(4) (K); **K9:** Dimethyl:2H(4) (K); **K13:** Acetyl (K)

Ions Score: 51 **Expect:** 0.0002

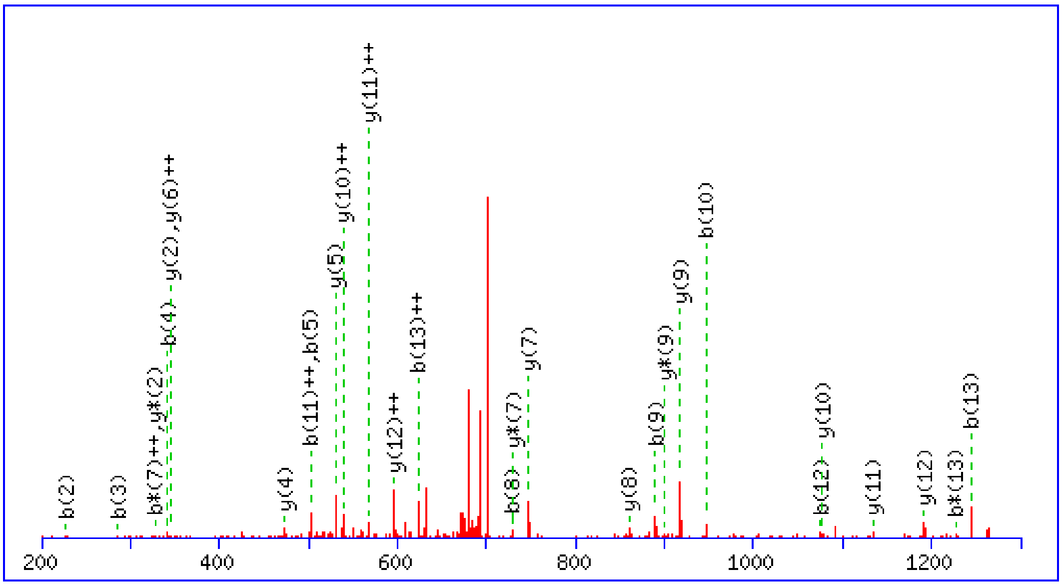


Fig. S4H

GK$_{(CHD2)2}$GGK$_{(CHD2)2}$GLGK$_{(CHD2)2}$GGAK$_{Ac}$R

Monoisotopic mass of neutral peptide Mr(calc): 1407.94

Variable modifications:

K2: Dimethyl:2H(4) (K); **K5:** Dimethyl:2H(4) (K); **K9:** Dimethyl:2H(4) (K); **K13:** Acetyl (K)

Ions Score: 49 **Expect:** 0.00042

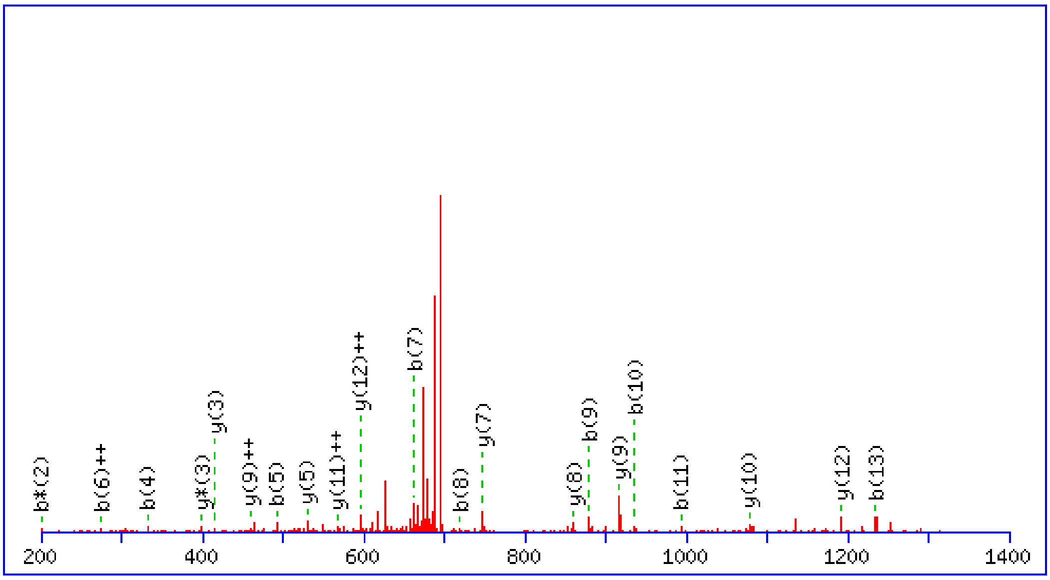


Fig. S4I

GK$_{(CHD2)2}$GGK$_{(CHD2)2}$GLGK$_{(CHD2)2}$GGAK$_{(CHD2)2}$R

Monoisotopic mass of neutral peptide Mr(calc): 1397.99

Variable modifications:

K2: Dimethyl:2H(4) (K); **K5:** Dimethyl:2H(4) (K); **K9:** Dimethyl:2H(4) (K); K13: Dimethyl:2H(4) (K)

Ions Score: 55 **Expect:** 8.1e-005

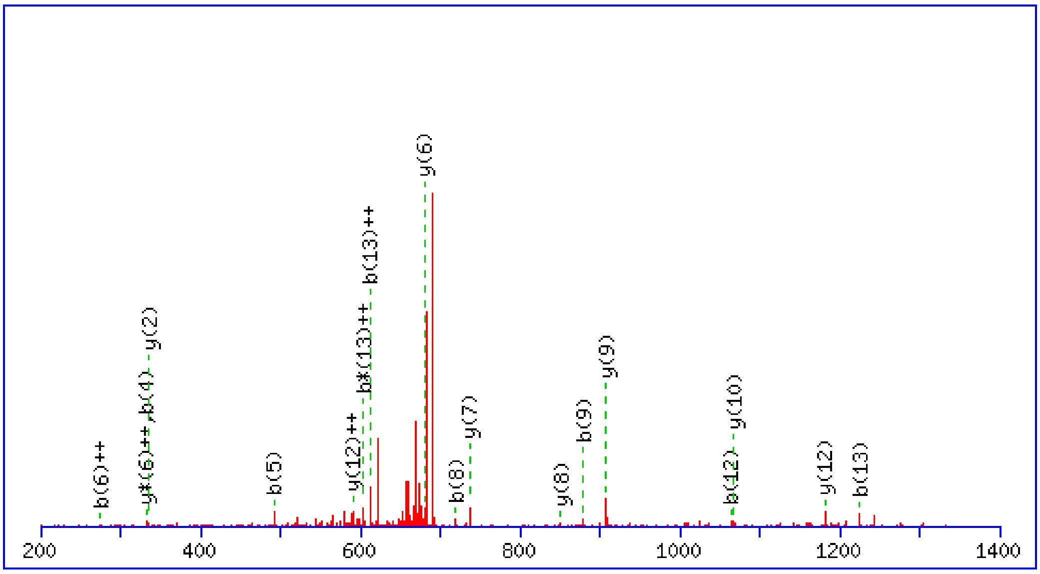


Fig. S4J

GK$_{(CHD2)2}$GGK$_{(CHD2)2}$GLGK$_{Ac}$GGAK$_{Me2}$R$_{Me}$

Monoisotopic mass of neutral peptide Mr(calc): 1417.93

Variable modifications:

K2: Dimethyl:2H(4) (K); **K5:** Dimethyl:2H(4) (K); **K9:** Acetyl (K); **K13:** Dimethyl (K); **R14:** Methyl (R)

Ions Score: 48 **Expect:** 0.00046

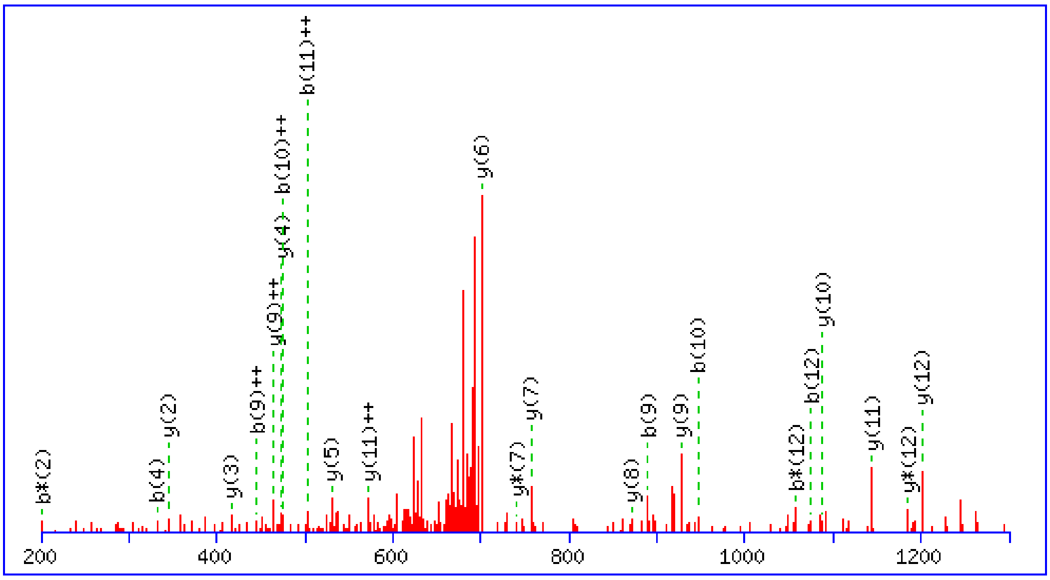


Table S1 In-vitro dimethylation of several modified peptides from histone H3.

		Peptide	**Abundance**
H3 9-17	Identified	K$(CHD_2)_2$STGGK$(CHD_2)_2$APR	4.23E+04
	Theoretical	K, STGGK, APR	
	Identified	K(Me2)STGGK$(CHD_2)_2$APR	8.76E+05
	Theoretical	K(Me2)STGGK, APR	
	Identified	K(Me2)STGGK(Ac)APR	1.13E+06
	Theoretical	K(Me2)STGGK(Ac)APR	
H3 18-26	Identified	K$(CHD_2)_2$QLATK$(CHD_2)_2$AAR	6.96E+06
	Theoretical	K, QLATK, AAR	
	Identified	K$(CHD_2)_2$QLATK(Ac)AAR	1.28E+06
	Theoretical	K, QLATK(Ac)AAR	
	Identified	K(Ac)QLATK(Ac)AAR	2.05E+07
	Theoretical	K(Ac)QLATK(Ac)AAR	
H3 27-40	Identified	K(Me2)SAPATGGVK(Me2)K$(CHD_2)_2$PHR	1.56E+07
	Theoretical	K(Me2)SAPATGGVK(Me2)K, PHR	
	Identified	K(Me2)SAPATGGVK$(CHD_2)_2$K$(CHD_2)_2$PHR	4.92E+04
	Theoretical	K(Me2)SAPATGGVK, K, PHR	

Table S2 Ratios of heavy/light dimethylation of H4 peptides (The highlighted N-terminal residue was heavy or light dimethylated).

Peptide	**Abundance(H)**	**Abundance(L)**	**Ratio(H/L)**
GK(Ac)GGK(Ac)GLGK(Ac)GGAK(Ac)R	7.10E+05	7.23E+05	0.9820
GK(CHD$_2$)$_2$GGK(Ac)GLGK(Ac)GGAK(Ac)R	3.03E+06	2.96E+06	1.0236
GK(Ac)GGK(CHD$_2$)$_2$GLGK(Ac)GGAK(Ac)R	1.50E+06	1.49E+06	1.0067
GK(Ac)GGK(Ac)GLGK(CHD$_2$)$_2$GGAK(Ac)R	2.83E+05	2.80E+05	1.0107
GK(CHD$_2$)$_2$GGK(CHD$_2$)$_2$GLGK(Ac)GGAK(Ac)R	1.97E+06	2.01E+06	0.9801
GK(CHD$_2$)$_2$GGK(Ac)GLGK (CHD$_2$)$_2$GGAK(Ac)R	1.27E+06	1.22E+06	1.0410
GK(Ac)GGK(CHD$_2$)$_2$GLGK(CHD$_2$)$_2$GGAK(Ac)R	1.04E+06	1.06E+06	0.9811
GK(CHD$_2$)$_2$GGK(CHD$_2$)$_2$GLGK(CHD$_2$)$_2$GGAK(Ac)R	6.52E+05	6.48E+05	1.0062
GK(CHD$_2$)$_2$GGK(CHD$_2$)$_2$GLGK(CHD$_2$)$_2$GGAK(CHD$_2$)$_2$R	6.40E+04	6.50E+04	0.9846
DAVTYTEHAK(CHD$_2$)$_2$R	2.10E+06	2.07E+06	1.0144
KTVTAMDVVYALK(CHD$_2$)$_2$R	2.94E+04	3.07E+04	0.9577
TLYGFGG	5.20E+07	5.26E+07	0.9886
DNIQGTK(CHD$_2$)$_2$PAIR	3.06E+05	2.81E+05	1.0890
ISGLIYEETR	7.02E+07	6.41E+07	1.0952
GVLK(CHD$_2$)$_2$VFLENVIR	7.72E+04	6.75E+04	1.1437
VFLENVIR	1.09E+07	1.25E+07	0.8720

Fig.S5 MS/MS spectra for the N-terminal heavy dimethylated peptides in Fig. 5.

Fig. S5a

(CHD2)2-GK$_{(CHD2)2}$GGK$_{(CHD2)2}$GLGK$_{(CHD2)2}$GGAK$_{Ac}$R

Monoisotopic mass of neutral peptide Mr(calc): 1440.00

Variable modifications:

N-term: Dimethyl:2H(4) (N-term)

K2: Dimethyl:2H(4) (K); **K5:** Dimethyl:2H(4) (K); **K9:** Dimethyl:2H(4) (K); **K13 :** Acetyl (K)

Ions Score: 54 **Expect:**0.00025

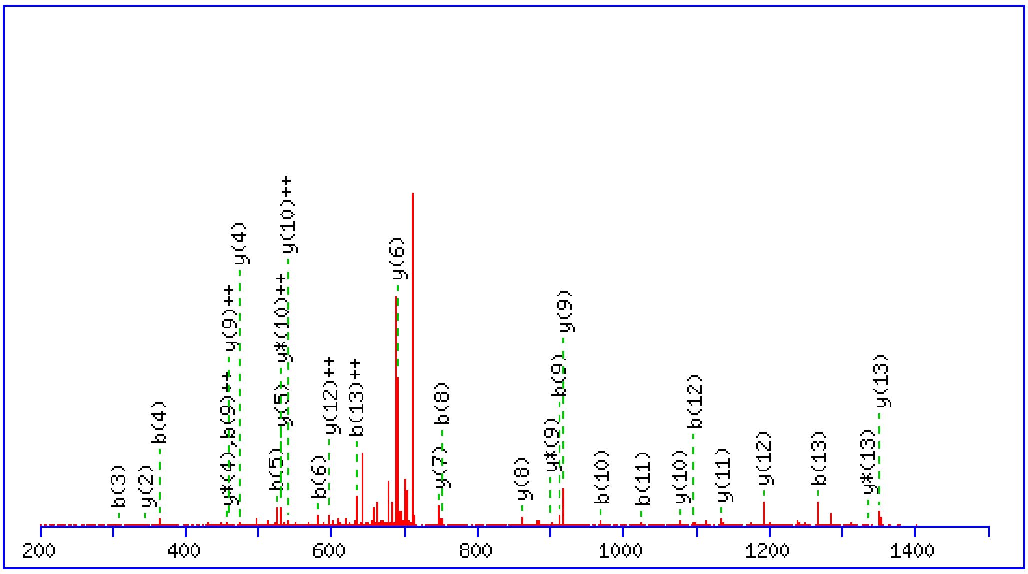


Fig. S5b

(CHD2)2-GK$_{Ac}$GGK$_{(CHD2)2}$GLGK$_{Ac}$GGAK$_{Ac}$R

Monoisotopic mass of neutral peptide Mr(calc): 1459.91

Variable modifications:

N-term: Dimethyl:2H(4) (N-term); **K2:**Acetyl (K); **K5:** Dimethyl:2H(4) (K); **K9:** Acetyl (K); **K13:** Acetyl (K)

Ions Score: 57 **Expect:** 0.00012

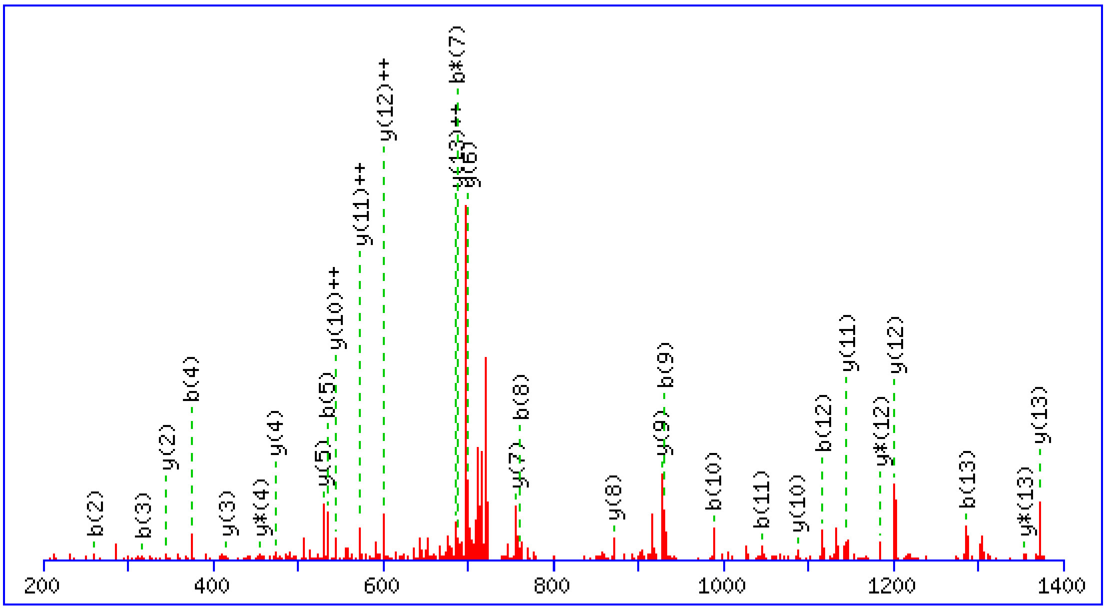


Fig. S5c

(CHD2)2-DAVTYTEHAK(CHD2)2R

Monoisotopic mass of neutral peptide Mr(calc): 1353.75

Variable modifications:

N-term: Dimethyl:2H(4) (N-term); **K10:** Dimethyl:2H(4) (K)

Ions Score: 42 **Expect:** 0.0038

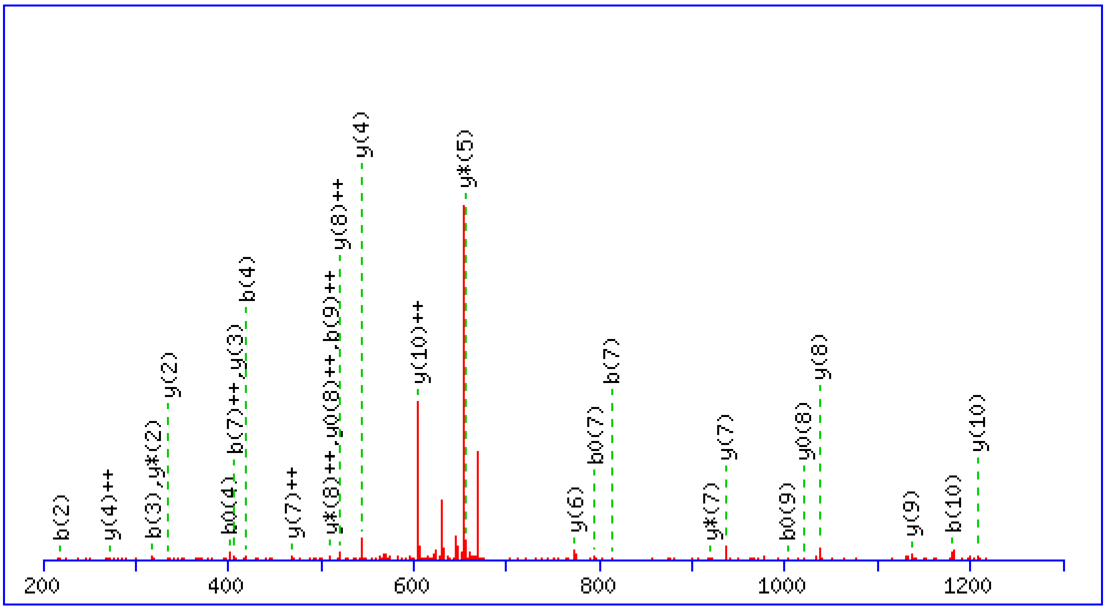
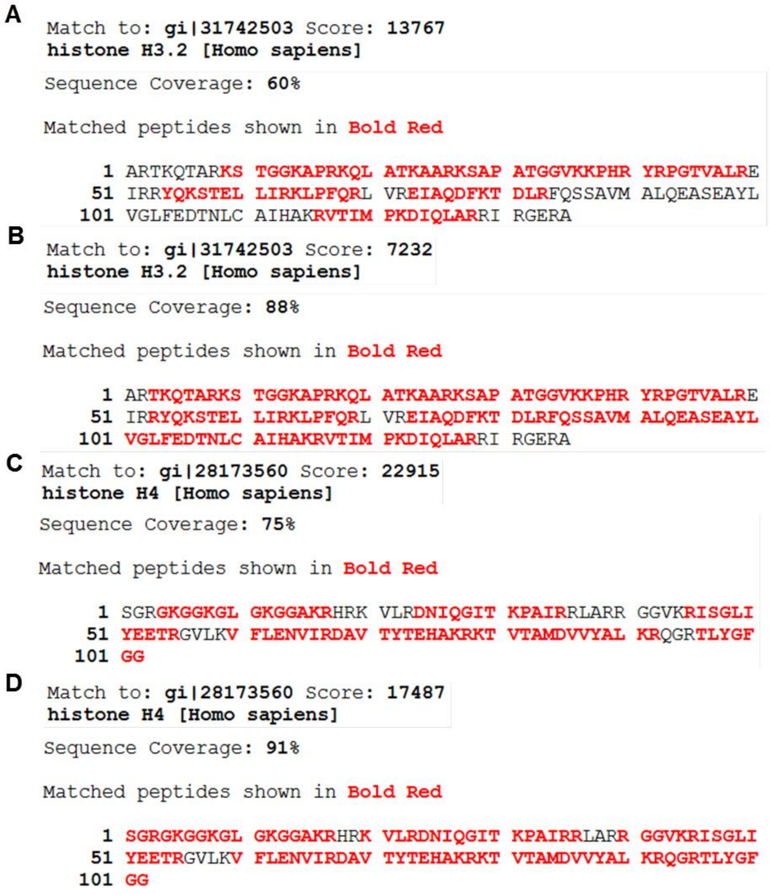


Fig.S6 Matched peptides of histone H3.2 (shown in bold red) before (A) and after (B) isotope dimethyl labeling. Matched peptides of histone H4 (shown in bold red) before (C) and after (D) isotope dimethyl labeling.



A

Match to: **gi|31742503** Score: **13767**
histone H3.2 [Homo sapiens]

Sequence Coverage: **60%**

Matched peptides shown in **Bold Red**

1 ARTKQTAR**KS TGGKAPRKQL ATKAARKSAP ATGGVKKPHR YRPGTVALR**E
51 IRR**YQKSTEL LIRKLPFQR**L VR**EIAQDFKT DLR**FQSSAVM ALQEASEAYL
101 VGLFEDTNLC AIHAK**RVTIM PKDIQLAR**RI RGERA

B

Match to: **gi|31742503** Score: **7232**
histone H3.2 [Homo sapiens]

Sequence Coverage: **88%**

Matched peptides shown in **Bold Red**

1 AR**TKQTARKS TGGKAPRKQL ATKAARKSAP ATGGVKKPHR YRPGTVALR**E
51 IR**RYQKSTEL LIRKLPFQR**L VR**EIAQDFKT DLRFQSSAVM ALQEASEAYL**
101 **VGLFEDTNLC AIHAKRVTIM PKDIQLAR**RI RGERA

C

Match to: **gi|28173560** Score: **22915**
histone H4 [Homo sapiens]

Sequence Coverage: **75%**

Matched peptides shown in **Bold Red**

1 SGR**GKGGKGL GKGGAKR**HRK VLR**DNIQGIT KPAIR**RLARR GGVK**RISGLI**
51 **YEETR**GVLK**V FLENVIRDAV TYTEHAKRKT VTAMDVVYAL KR**QGR**TLYGF**
101 **GG**

D

Match to: **gi|28173560** Score: **17487**
histone H4 [Homo sapiens]

Sequence Coverage: **91%**

Matched peptides shown in **Bold Red**

1 **SGRGKGGKGL GKGGAKR**HR**K VLRDNIQGIT KPAIRR**LA**R GGVKRISGLI**
51 **YEETR**GVLK**V FLENVIRDAV TYTEHAKRKT VTAMDVVYAL KRQGRTLYGF**
101 **GG**