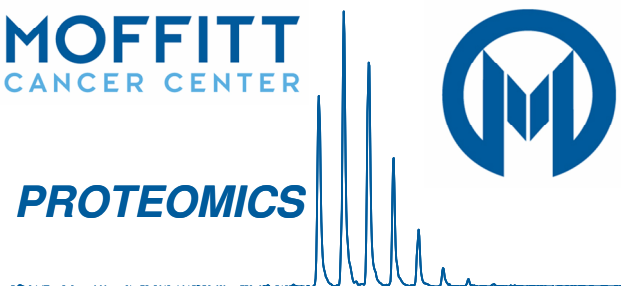


Peptide Synthesis Report

MOFFITT
CANCER CENTER

PROTEOMICS



Project Title: Internal standard for H2A1A_HUMAN

Submitter: Sayeef Mirza

PI: John Koomen

Design and Synthesis:

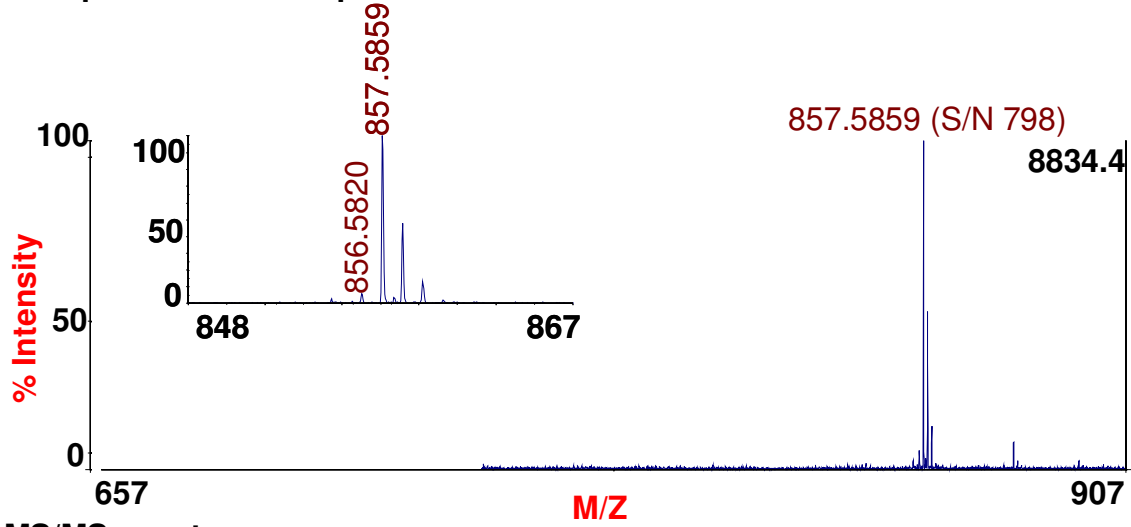
MPS#	Peptide Sequence	Residues	Modifications	MWT Monoisotopic
p-0401	HLQ <u>L</u> AIR		Stable Isotope on L4 (U-13C6, 15N1)	856.51851

Solid state peptide synthesis (Symphony, Protein Technologies, Tucson, AZ) is used to make standards at the 25 micromole scale using standard Fmoc chemistry. Briefly, Wang resin (Novabiochem, Germany) serves as the support to initiate peptide formation. Activator solvents for the amino acid coupling consist of 150 mM N-Methylmorpholine in N-methylpyrrolidone (NMP) with 100 mM of O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU). Deprotection between couplings is achieved using 20% piperidine in NMP with 2% 1,8-diazabicyclo [5,4,0] undec-7-ene. Upon sequence completion, cleavage from the resin is performed using 95% TFA, 2.5% water, and 2.5% triisopropylsilane. Peptides are then precipitated in ice cold ethyl ether, washed twice, and resuspended in water prior to lyophilization.

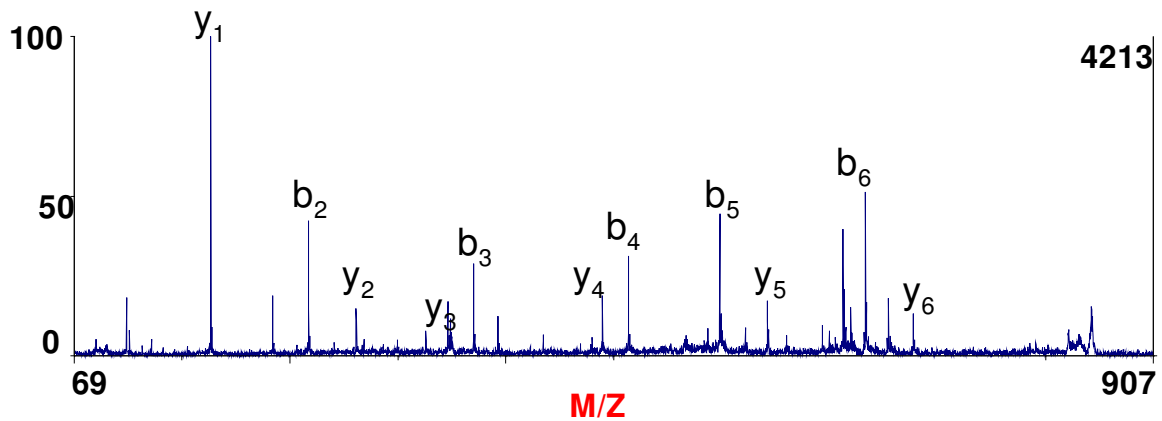
MALDI:

Purified peptides are analyzed with MALDI MS to verify purity and sequenced with MS/MS (4700, Applied Biosystems, Framingham, MA). Peptides in 2% ACN with 0.1% formic acid are mixed 1:1 with α -cyano-4-hydroxycinnamic acid (10 mg/ml) in 50% ACN and deposited in 1 microliter aliquots on the MALDI target.

MS Spectrum: ~100% pure



MS/MS spectrum:



Fragment Ion table with Identified peaks:

Seq	#	B	Y	# (+1)
H	1	138.06678	857.52634	7
L	2	251.15085	720.46743	6
Q	3	379.20942	607.38336	5
L	4	499.29349	479.32479	4
A	5	570.33060	359.24072	3
I	6	683.41466	288.20361	2
R	7	839.51577	175.11955	1