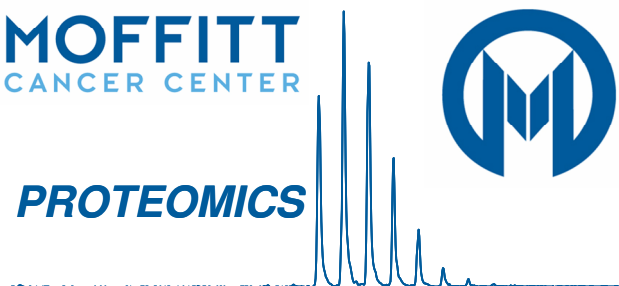


Peptide Synthesis Report

MOFFITT
CANCER CENTER

PROTEOMICS



Project Title: Cathepsin Z Internal standard

Submitter: Eric Thomas

PI: John Koomen

Design and Synthesis:

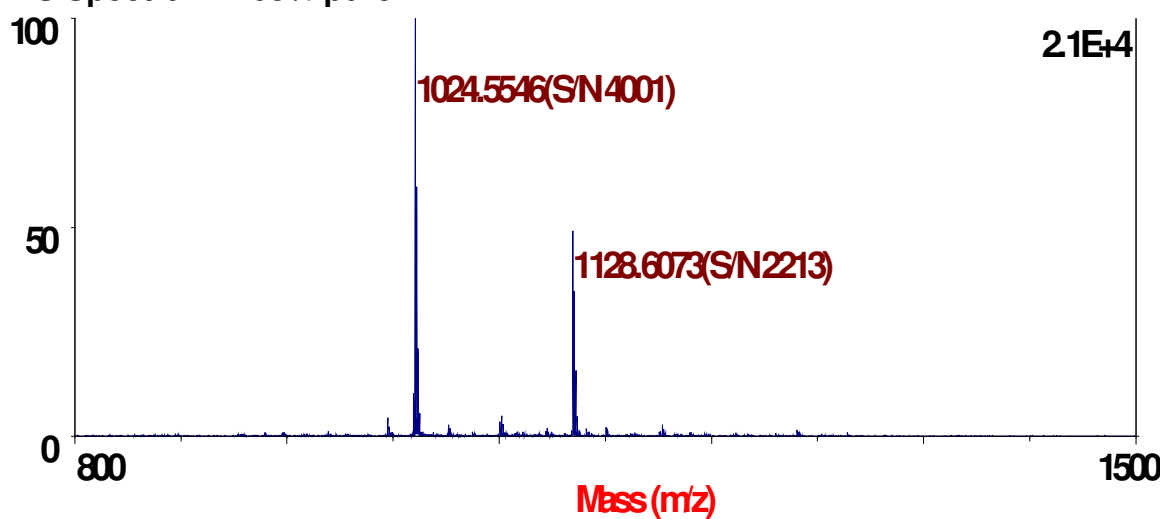
MPS#	Peptide Sequence	Residues	Modification	MWT Monoisotopic
p-304	VGDYGSLSAR		G9 to A	1023.49857

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.

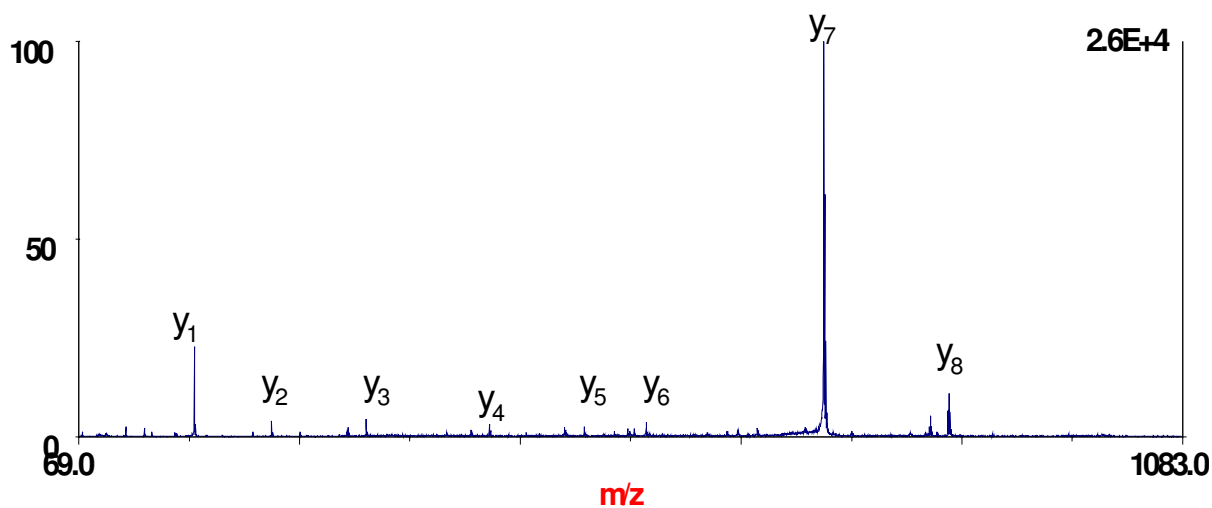
MALDI:

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: ~68% pure



MS/MS spectrum:



Fragment Ion table with Identified peaks:

Seq	#	B	Y	# (+1)
V	1	100.07628	1024.50639	10
G	2	157.09775	925.43798	9
D	3	272.12469	868.41651	8
Y	4	435.18802	753.38957	7
G	5	492.20948	590.32624	6
S	6	579.24151	533.30478	5
L	7	692.32557	446.27275	4
S	8	779.35760	333.18869	3
A	9	850.39472	246.15666	2
R	10	1006.49583	175.11955	1