012707 ABRF 2008 PRG Report

Summary:
Advanced glycosylation end product-specific receptor (Q15109 RAGE_HUMAN) was identified in both samples A and B. The N-terminal and C-terminal ends of the protein were different from the sequence in the database. There were forms of this protein in samples A and B that differed at the C-terminal end, which points to truncated versions being present in samples A and B. There was also a phosphorylated peptide present in sample B that was not identified in A.

Sample preparation:
Samples were dissolved in 50 mM ammonium bicarbonate. Trypsin was added directly to small aliquots of both samples A and B without reduction and alkylation to keep disulfide bonds intact for part of the analysis. The majority of the samples were mixed with a solution containing 50 mM ammonium bicarbonate and Rapigest prior to reduction with DTT and alkylation with iodoacetamide. TFA was added to the samples to hydrolyze the Rapigest prior to analysis.

Instrumental conditions:
Tryptic digests were run on a nanoACQUITY UPLC system configured with a 150 µm trapping column and a 100 µm by 100 mm analytical column packed with 1.7 µm BEH particles. A 90 minute gradient from 5% to 40% ACN was used with a flow rate of 530 nL/min. A Q-Tof Premier mass spectrometer was used to analyze the digests with MS^E. As peptides eluted from the column, the collision energy was alternated between a low- and elevated-energy state in order to obtain the mass information for both the precursor ions and their fragments.

Data analysis:
All data were processed with ProteinLynx Global Server version 2.3, with Identity^E informatics. Default processing and searching parameters were used with a database made up of the human proteins from the UniProt database. The tolerances for mass accuracy were 10 and 20 ppm for precursor and fragment ions, respectively. Manual *de novo* sequencing was performed to determine the N-terminal peptide. To look for truncated peptides, a search with no enzyme specificity was performed.

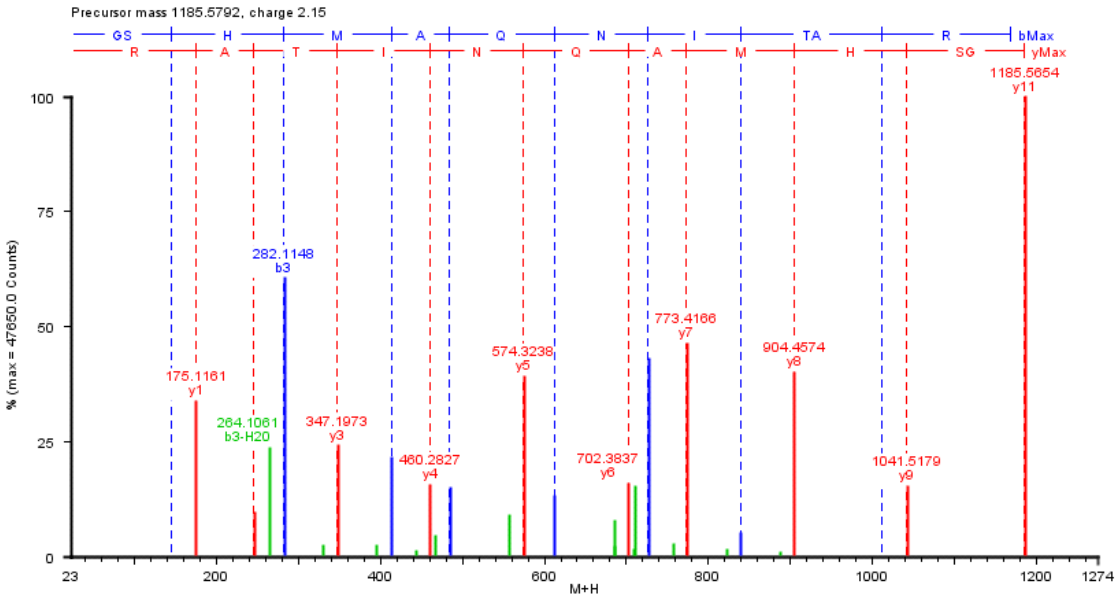

Figure 1. MS^E spectrum of N-terminal peptide GSHMAQNITAR.

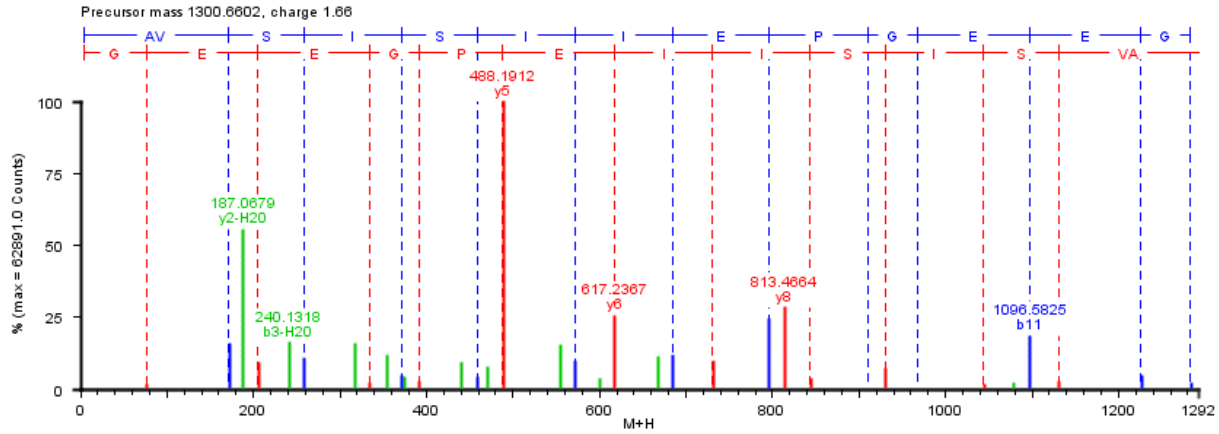

Figure 2. MS^E spectrum of truncated C-terminal peptide AVSISIIEPGEEG found in both A and B.

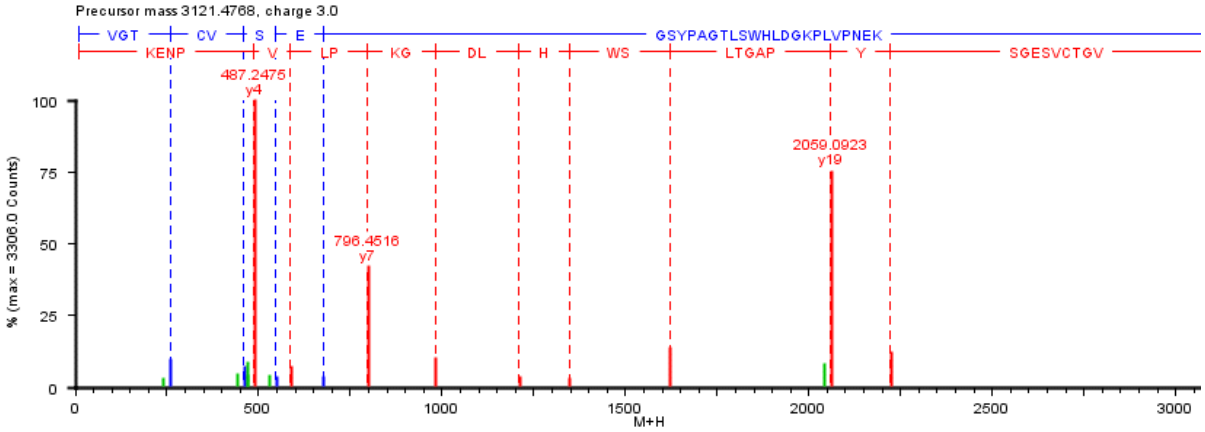

Figure 3. MS^E spectrum of VGTCVSEGpSYPAGTLSWHLDGKPLVPNEK found only in sample B.

GSHMAQNITA RIGEPLVLKC KGAPKKPPQR LEWKLNTGR**T EAWKVLSPQG**
GGPWDSVARV LPNGSLFLPA VGIQDEGIFR CQAMNRNGKE TKSNYRVR**VY**
QIPGKPEIVD SASE*LTAGVP NKVGTCVSEG SYPAGTLSWH LDGKPLVPNE
KGVSVK**EQTR RHPETGLFTL QSELMVTPAR GGDPRPTFSC SFSPGLPR**HR
ALR**TAPIQPR VWEPVPLEEV QLVVEPEGGA VAPGGTVTLT CEVPAQPSPQ**
IHWMKDGVPL PLPPSPVLIL PEIGPQDQGT Y#SCVATHSSH GPQESRAVSI
SIIEPGEEGG PTAGSVGGSG LGTLALALGI LGGLGTAALL IGVILWQRRQ
RRGEERKAPE NQEEEEERAE LNQSEEPEAG ESSTGGP

Figure 4. Tryptic map of Q15109 with 71% sequence coverage. The E* corresponds to a truncation site only observed in sample B and the Y# corresponds to a truncation site only observed in sample A.