# Comparative Differential Cuproproteomes of *Rhodobacter capsulatus* Reveal Novel Copper Homeostasis related Proteins

Nur Selamoglu[a], Özlem Önder[a,b], Yavuz Öztürk[a,e], Bahia Khalfaoui-Hassani[a,#], Crysten E. Blaby-Haas[c], Benjamin A. Garcia[d], Hans-Georg Koch[e] and Fevzi Daldal[a*]

[a]Department of Biology, University of Pennsylvania, Philadelphia, PA, 19104 USA; [b]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA; [c]Biology Department, Brookhaven National Laboratory, Upton, NY 11973, USA; [d]Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA, [e]Institute of Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine, Albert-Ludwigs University of Freiburg, 79104 Freiburg, Germany.

**Running Title:** *Rhodobacter capsulatus* Copper-Responsive Proteome

**Key words**: *Rhodobacter capsulatus proteome,* differential cuproproteomics, copper homeostasis, copper-responsive proteins, uncharacterized proteins.

[#]Present address: IPREM, UMR CNRS 5254, and Université de Pau et des Pays de l'Adour, BP1155 Pau, France

[*]Corresponding author: Fevzi Daldal

fdaldal@sas.upenn.edu; +1 215-898-4394

**Files in this Data Supplement:**

Supplemental Information and Table S3 (Excel file)

## SUPPLEMENTAL INFORMATION

### Experimental Procedures

**ProteaseMAX™ Extraction/Trypsin Digestion (protocol B)**. Each 100 mg aliquot from the resulting wet pellets was washed with ultrapure water, centrifuged and the supernatant discarded. Solubilization and extraction of proteins was performed using ProteaseMAX™ Surfactant Trypsin Enhancer (Promega Inc.), followed by trypsin digestion as follows (1). The pellet was resuspended in 0.5 ml of ProteaseMAX™ solution (1 mg/ml in 10% acetonitrile and 90% 50mM $NH_4HCO_3$), and 10 min of rigorous vortexing was followed by incubation in a 95 °C water bath for 10 min. Following 15 min of sonication at 4 °C (Bioruptor$^R$, Diagenode), the sample was vortexed at room temperature for 30 min. Estimates from separate BCA analysis of similar samples indicated that ~ 400-500 μg protein was solubilized at this stage. Further reduction/alkylation and digestions steps were completed without separating the insolubilized solid fraction. Proteins were reduced by adding dithiothreitol (DTT) to a final concentration of 5mM and incubating for 30 min at 37 °C, and alkylated by adding iodoacetamide to a final concentration of 15 mM and incubating for 30 min at room temperature in the dark. Trypsin (Promega, Sequencing Grade Modified Trypsin) was added to the mixture at a 1/100 w/w ratio of enzyme/protein, and the extract was digested overnight at 37 °C, pH 7. Digested mixtures were vortexed for 10 min and acidified with 10% formic acid to pH 3-4 to halt digestion, vortexed and placed in a 45 °C shaker for 30 min to degrade ProteaseMAX™. Following a 15 min centrifugation at 15,000 x g, the supernatants were removed and dried to completeness in a vacuum centrifuge at 45 °C. The digested peptide samples were resuspended in 50 μL 5% acetonitrile/0.1% formic acid solution, and desalted using ZipTips (Milipore U-C18 P10), eluted, lyophilized and stored at -80 °C. Samples were resuspended in 10 μL (5% acetonitrile/0.1% formic acid) prior to MS analysis.

The efficacy of the extraction/digestion protocols A (thiourea/urea/trypsin/LysC) and B (ProteaseMax/trypsin) was determined using the nano-LC-MS/MS Thermo Orbitrap Velos Pro system described below. Protocol A yielded ~40% more identified proteins than protocol B (1784 versus 1244 in triplicate samples), indicating that the former with multiple digestion steps was more effective than the latter. In addition, a further 15-20% increase in the number of identified proteins was observed when the samples prepared by protocol A were analyzed using the higher sensitivity Q-Exactive MS, rendering it the method of choice for the quantitative aspects of this study. The qualitative data for the protein identification included all datasets, including those treated by the procedure A or B, analyzed using Orbitrap Velos (*c.f.* below) as well as the Q-Exactive MS runs (*c.f.* main text).

**Data acquisition using nano-LC-MS/MS Thermo Orbitrap Velos Pro.** Prepared digests were loaded without pre-fractionation onto a 20 cm column (packed in-house, 75 μm ID, 3μm C18, RepoSil-Pur Q18-AQ (Dr.Maisch GmbH)) with buffer A (0.1% formic acid). Peptides were separated and eluted by nano-HPLC (EASY-nLC 1000, Thermo Inc.) using a 85 min linear gradient from 4% to 40% buffer B (0.1% formic acid in acetonitrile), followed by a 20 min linear gradient from 40% to 90% B, followed by 15 min washing with 90% B (flow rate 300 nL/min), and re-equilibration with buffer A. The nLC system was coupled to an Orbitrap Velos Pro mass spectrometer (Thermo Scientific), programmed to acquire spectra in data-dependent mode, with high resolution full survey scans followed by the top 15 most abundant ions fragmented for MS2 (Thermo Xcalibur 2.2 software). The resolution for full MS scans (300-2000 m/z) was 60,000 (at 400 m/z) and dual lock mass was enabled with 371.101233 m/z and 445.120024 m/z polysiloxane background ions. The 15 most intense ions were selected for fragmentation by collision induced dissociation (CID) with collision energy set at 35%, and with dynamic exclusion enabled to minimize repeat fragmentation (repeat count: 3, repeat duration: 30 sec, exclusion duration: 90 sec; exclusion list: 500). Unassigned charge states and singly charged species were rejected. The raw data were analyzed as described in the main text.

## REFERENCES

1. Pirmoradian, M., Budamgunta, H., Chingin, K., Zhang, B., Astorga-Wells, J., and Zubarev, R. A. (2013) Rapid and deep human proteome analysis by single-dimension shotgun proteomics. *Mol Cell Proteomics* **12**, 3330-3338
2. Sambrook, J., and Russell, D. W. (2001) *Molecular Cloning: a laboratory manual*, 3rd ed. ed., Cold Spring harbor Laboratory Press, Cold Spring harbor
3. Simon, R., Priefer, U., and Puhler, A. (1983) A Broad Host Range Mobilization System for Invivo Genetic-Engineering - Transposon Mutagenesis in Gram-Negative Bacteria. *Bio-Technology* **1**, 784-791
4. Zannoni, D., Prince, R. C., Dutton, P. L., and Marrs, B. L. (1980) Isolation and Characterization of a Cytochrome $c_2$-Deficient Mutant of *Rhodopseudomonas-capsulata*. *Febs Letters* **113**, 289-293
5. Yen, H. C., Hu, N. T., and Marrs, B. L. (1979) Characterization of the gene transfer agent made by an overproducer mutant of *Rhodopseudomonas capsulata*. *J Mol Biol* **131**, 157-168
6. Ditta, G., Schmidhauser, T., Yakobson, E., Lu, P., Liang, X. W., Finlay, D. R., Guiney, D., and Helinski, D. R. (1985) Plasmids related to the broad host range vector, pRK290, useful for gene cloning and for monitoring gene expression. *Plasmid* **13**, 149-153
7. Brimacombe, C. A., Stevens, A., Jun, D., Mercer, R., Lang, A. S., and Beatty, J. T. (2013) Quorum-sensing regulation of a capsular polysaccharide receptor for the *Rhodobacter capsulatus* gene transfer agent (RcGTA). *Mol Microbiol* **87**, 802-817
8. S. Ekici, S. Turkarslan, G. Pawlik, A. Dancis, N. S. Baliga, H. G. Koch and F. Daldal, Intracytoplasmic copper homeostasis controls cytochrome *c* oxidase production, *MBio*, 2014, **5**, e01055-01013.
9. Y. Zhang, C. E. Blaby-Haas, S. Steimle, A. F. Verissimo, V. A. Garcia-Angulo, H. G. Koch, F. Daldal and B. Khalfaoui-Hassani, Cu Transport by the Extended Family of CcoA-like Transporters (CalT) in Proteobacteria, *Sci Rep*, 2019, **9**, 1208.

**Table S1. Strains and plasmids used in this work**.

| **Strain or plasmid** | **Description** | **Phenotype** | **Reference** |
|---|---|---|---|
| ***Strains*** | | | |
| ***E. coli*** | | | |
| HB101 | F$^-$ Δ(*gpt-proA*)62 *leuB6 supE44 ara*-14 *galK2 lacY*1 Δ(*mcrC-mrr*) *rpsL20* (Str$^R$) *xyl-5 mtl-1recA*13 | Str$^r$ | (2) |
| S17-1 | *recA pro hsdR, RP4(Tc::Mu, Km::Tn7)* | | (3) |
| ***R. capsulatus*** | | | |
| [a]MT1131 | *crtD121* | wild type (Rif$^r$) | (4) |
| Y262 | GTA overproducer | | (5) |
| YO-Δ00098 | *Δ(rcc00098)* | In-frame, markerless | This work |
| YO-Δ00738 | *Δ(rcc00738::Gm)* | Gm$^r$ | This work |
| YO- Δ00885 | *Δ(rcc00891-rcc00885::Gm)* | Gm$^r$ | This work |
| YO-Δ01027 | *Δ(rcc01027-rcc01031::Gm)* | Gm$^r$, | This work |
| YO-Δ01423Su | *Δ(rcc01423)* | In-frame, markerless | This work |
| YO-Δ01423 | *Δ(rcc01423::Gm)* | Gm$^r$ | This work |
| YO-Δ01445 | *Δ(rcc01445::Gm)* | Gm$^r$ | This work |
| YO-Δ02109 | *Δ(rcc02109::Gm)* | Gm$^r$ | This work |

| | | | |
|---|---|---|---|
| YO-Δ02111 | *Δ ( rcc02111)* | In-frame, markerless | This work |
| YO-Δ03065 | *Δ(rcc03065-rcc03067::Gm)* | Gm$^r$ | This work |
| ***Plasmids*** | | | |
| pRK2013 | Conjugation helper | Kan$^r$ | (6) |
| pRK415 | Broad host-range vector | Tet$^r$ | (6) |
| pZDJ | Puc promoter replaced with *tetA* promoter on the suicide plasmid pZJD29a | *sacB*, Gm$^R$ | (7) |
| pYO98 | *Δ(rcc01423::Gm)* on pRK415 | Gm$^r$ | This work |
| pYO-Δ00738 | *Δ(rcc00738::Gm)* on pRK415 | Tet$^r$ | This work |
| pYO-Δ00885 | *Δ(rcc00891-rcc00885::Gm)* on pRK415 | Tet$^r$, Gm$^r$ | This work |
| pYO-Δ01027 | *Δ(rcc01027-rcc01031::Gm)* on pRK415 | Tet$^r$, Gm$^r$ | This work |
| pYO-Δ01445 | *Δ(rcc01445::Gm)* on pRK415 | Tet$^r$, Gm$^r$ | This work |
| pYO-Δ03065 | *Δ(rcc03065-rcc03067::Gm)* on pRK415 | Tet$^r$, Gm$^r$ | This work |
| pYO-Δ02109 | *Δ(rcc02109::Gm)* on pRK415 | Tet$^r$, Gm$^r$ | This work |
| pYO97 | *Δ(rcc01423)* on pZDJ; in-frame deletion of *rcc01423* without its first 4 and last 4 codons. | Gm$^r$ | This work |
| pYO-Δ00098 | *Δ(rcc00098)* on pZDJ; in-frame deletion of *rcc02111* without its first 4 and last 4 codons. | Gm$^r$ | This work |
| pYO-Δ02111Su | *Δ(rcc02111)* on pZDJ; in-frame deletion of *rcc02111* without its first 4 and last 4 codons. | Gm$^r$ | This work |

[a] *R.capsulatus* strain MT1131 is derived from SB1003 in multiple steps, as described in Zannoni *et al*. (1980): first a Ps-deficient mutant (TL1) was obtained using tetracycline suicide, then its *crtD* derivative was constructed by GTA cross to yield MT113, and then its Ps-proficient derivative was obtained via a second GTA cross.

**Table S2. Primers used in this work.**

| | |
|---|---|
| **Gm-F** | **5-A**AGCTTGTC**GACCCGATCTGAGC-5** |
| **Gm-R** | 5- TCTAGAACTAGTGGATCCCCCG-3 |
| **01423-F1** | 5-CGGCCAGTGAATTCGAGCTCGGTACATGCGCAGGGTATAGGTGACGCC-3 |
| **01423-R1** | 5-TCATTTTTCGAACTGCGGGTGGCTCCAGGCGTTTTCTTCTTCGAAGTGCC-3 |
| **01423-F2** | 5-GAGCCACCCGCAGTTCGAAAAATGATCCGACTTCGCAAGCCAGGTCC-3 |
| **01423-R2** | 5-AAGCTTGCATGCCTGCAGGTCGACTGCGATCGTCTTTGCCGATCTGGC-3 |
| **01423Gm-R1** | 5-GAAAATGGCTCAGATCGGGTCGACAAGCTTAAGTGGCAGACATGACATGTCTCC-3 |
| **01423Gm-F3** | 5-TTCCCGGCCGGGGGATCCACTAGTTCTAGAGAAGAAAACGCCTGATCCGACTTCG -3 |
| **01423Su-F1** | 5-TACGCCAAGCTTGCATGCCTGCAGGGTCTGTTGCGCCAGCACGGCTTTGC-3 |
| **01423Su-R1** | 5-AGTGGCAGACATGACATGTCTCCTC-3 |
| **01423Su-F2** | 5-GAGGAGACATGTCATGTCTGCCACTGAAGAAAACGCCTGATCCGACTTCG-3 |
| **01423Su-R2** | 5-AAACGACGGCCAGTGAATTCGAGCTGGCGACGGACAATTGCCGGGGCTG-3 |
| **00738-F1** | 5-CGGCCAGTGAATTCGAGCTCGGTACACTGCGCCAAGACCTGCCCCAAGG-3 |
| **00738-R1** | 5-AAAAATGGCTCAGATCGGGTCGACAAGCTTAGACGATGCGGAAGAAGCAGCCCG -3 |
| **00738-F2** | 5-TTCCCGGCCGGGGGATCCACTAGTTCTAGAGATCTGGGCGGCGCGCTCTCGGC-3 |
| **00738-R2** | 5-AAGCTTGCATGCCTGCAGGTCGACTTGACCATGCTGCTGGAAGACGAC-3 |
| **00891-F1** | 5-CGGCCAGTGAATTCGAGCTCGGTACAGATGGTGATCCGCGCCATCGCG-3 |
| **00891-R1** | 5-AAAAATGGCTCAGATCGGGTCGACAAGCTTCGCCGAGGCCGAGAGATGTTCGG -3 |
| **00885-F2** | 5-TTCCCGGCCGGGGGATCCACTAGTTCTAGAACCCGAACCATCACTGCCCCTTCTG-3 |
| **00885-R2** | 5-AAGCTTGCATGCCTGCAGGTCGACTACGCCGCCGGATGGCATTCAAAAGG-3 |
| **01027-F1** | 5-CGGCCAGTGAATTCGAGCTCGGTACCCGCCCGAAAGCGCCAGAAGCGC-3 |
| **01027-R1** | 5-AAAAATGGCTCAGATCGGGTCGACAAGCTTGCTGCCCGTAGGCGTCGGCAACC-3 |
| **01031-F2** | 5-TTCCCGGCCGGGGGATCCACTAGTTCTAGAGCCGACCGGGTTCTGGTGCTGGC-3 |
| **01031-R2** | 5-AAGCTTGCATGCCTGCAGGTCGACTGCGATCGCGCAATTCGGCCTTGGC-3 |
| **02111Su-F1** | 5-TACGCCAAGCTTGCATGCCTGCAGGATCGAGCTGTGGCAGGTGATGCGC-3 |
| **02111Su-R1** | 5-CAGAATCCGCATGGGAGGAGCATGGC-3 |
| **02111Su-F2** | 5-CCATGCTCCTCCCATGCGGATTCTGCCGCCCCGCCTCTGATCTTCGCC-3 |
| **02111Su-R2** | 5-AAACGACGGCCAGTGAATTCGAGCTGGCAGCGGCACCACCGGGTTGCC-3 |
| **02109-R1** | 5-AAAAATGGCTCAGATCGGGTCGACAAGCTTAAGGCACCCGGCGACAAGGGCC-3 |

| | |
|---|---|
| **02109-F2** | 5-TTCCCGGCCGGGGGATCCACTAGTTCTAGACTGATCACGGCGGACGGGCAAACC-3 |
| **02109-R2** | 5-AAGCTTGCATGCCTGCAGGTCGACTGTTCTAACCTTAGGCCGCCAGAC-3 |
| **00098Su-F1** | 5-TACGCCAAGCTTGCATGCCTGCAGGCGACGCTGGGCCTTGACCCGGAG-3 |
| **00098Su-R1** | 5-AAGGCGGCGCATGGGTGTCTCCAG-3 |
| **00098Su-F2** | 5-ACTGGAGACACCCATGCGCCGCCTTATCCGTTTCTGAGATCCGTTTCC-3 |
| **00098Su-R2** | 5-AAACGACGGCCAGTGAATTCGAGCTTCGTCCATGCGCAGATGCAGATC-3 |
| **01445-F1** | 5-CGGCCAGTGAATTCGAGCTCGGTACCGCTGGTGGCAGAACCGATCAGC-3 |
| **01445-R1** | 5-AAAAATGGCTCAGATCGGGTCGACAAGCTTGGTGATGACGATCGTGCCCAGCG-3 |
| **01445-F2** | 5-TTCCCGGCCGGGGGATCCACTAGTTCTAGAGACGGCGCTTTTGATCGACGCGC-3 |
| **01445-R2** | 5-AAGCTTGCATGCCTGCAGGTCGACTTGGCATGGATGCGATGCTGATCG-3 |
| **03065-F1** | 5-CGGCCAGTGAATTCGAGCTCGGTACTCCAGCGGCAGATGCGTGGCGGC-5 |
| **03065-R1** | 5-AAAAATGGCTCAGATCGGGTCGACAAGCTTCGGCGGCGCGCATCCAGAACACC-3 |
| **03067-F2** | 5-TTCCCGGCCGGGGGATCCACTAGTTCTAGATTCGGCCCGATGGTCGAGGACCGC-3 |
| **03067-R2** | 5-AAGCTTGCATGCCTGCAGGTCGACtCCAGCCGATGCGCGATGACATTCG-3 |

**Table S3**. **Excel spreadsheet compiled datasets.** Data related to the identification, annotations, quantitation and statistics of *R*. *capsulatus* proteins are cumulated in **Table S3**. **Sheet 1** (***R. capsulatus*** **Proteins**): Full genome listing of *R*. *capsulatus* (3632 entries, UniprotKB). Various annotations are included: PsortB localizations, TIGRFAM and TIGR roles, GO annotations, Interpro, and KEGG classifications, as well as protein identifiers. Those proteins experimentally identified in the current study are marked with asterisks (first column), and those unidentified are designated in gray colored rows. **Sheet 2 (Identified Proteins):** Proteins experimentally identified in the current study (2430 entries), with the number of unique peptides, MS/MS counts and sequence coverage listed along with other information. **Sheet 3 (Unidentified Proteins):** Proteins unidentified in the present study are listed along with functional annotations where available. **Sheet 4 (Single Peptide-ID):** List of proteins identified with only one unique peptide (59 entries). Those proteins identified with single MS/MS spectra are highlighted in yellow (13 entries). Annotated spectra for all single peptides are provided in **Figure S4**. **Sheet 5 (Quantified-Statistics):** The 1926 proteins quantified in this study are listed, along with T-test p-values and Fold-changes for +Cu vs control and for +BCS vs control (see Experimental Procedures). The 75 proteins that were significantly affected by changes in Cu levels (i.e., surpassed the criteria of $p<0.05$ and $FC>2.0$) are highlighted as noted in the table headings.

## Supplemental FIGURES

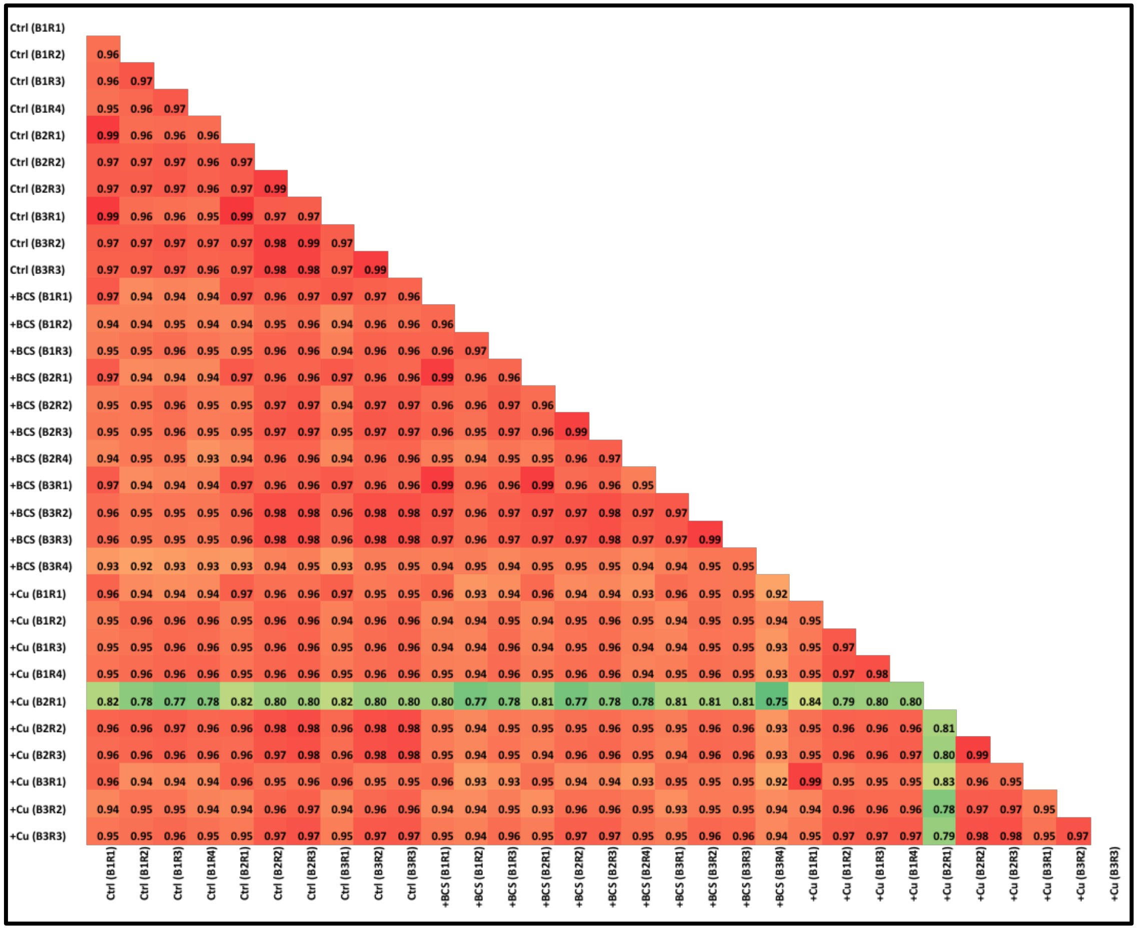
Cu-sufficient
(control)

Cu-excess
(+Cu)

Cu-depleted
(+BCS)

*Figure S1. Selamoglu et al.*

**Figure S1. Nadi staining of *R. capsulatus* colonies grown under different Cu concentrations.** Cu-sufficient (control), Cu-excess (+Cu) and Cu-depleted (+BCS) refer to MedA medium containing 0.3 µM Cu, 5 µM Cu and 5mM BCS, respectively.



| | Ctrl (B1R1) | Ctrl (B1R2) | Ctrl (B1R3) | Ctrl (B1R4) | Ctrl (B2R1) | Ctrl (B2R2) | Ctrl (B2R3) | Ctrl (B3R1) | Ctrl (B3R2) | Ctrl (B3R3) | +BCS (B1R1) | +BCS (B1R2) | +BCS (B1R3) | +BCS (B2R1) | +BCS (B2R2) | +BCS (B2R3) | +BCS (B2R4) | +BCS (B3R1) | +BCS (B3R2) | +BCS (B3R3) | +BCS (B3R4) | +Cu (B1R1) | +Cu (B1R2) | +Cu (B1R3) | +Cu (B1R4) | +Cu (B2R1) | +Cu (B2R2) | +Cu (B2R3) | +Cu (B3R1) | +Cu (B3R2) | +Cu (B3R3) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ctrl (B1R1) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ctrl (B1R2) | 0.96 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ctrl (B1R3) | 0.96 | 0.97 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ctrl (B1R4) | 0.95 | 0.96 | 0.97 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ctrl (B2R1) | 0.99 | 0.96 | 0.96 | 0.96 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ctrl (B2R2) | 0.97 | 0.97 | 0.97 | 0.96 | 0.97 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ctrl (B2R3) | 0.97 | 0.97 | 0.97 | 0.96 | 0.97 | 0.99 | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ctrl (B3R1) | 0.99 | 0.96 | 0.96 | 0.95 | 0.99 | 0.97 | 0.97 | | | | | | | | | | | | | | | | | | | | | | | | |
| Ctrl (B3R2) | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.98 | 0.99 | 0.97 | | | | | | | | | | | | | | | | | | | | | | | |
| Ctrl (B3R3) | 0.97 | 0.97 | 0.97 | 0.96 | 0.97 | 0.98 | 0.98 | 0.97 | 0.99 | | | | | | | | | | | | | | | | | | | | | | |
| +BCS (B1R1) | 0.97 | 0.94 | 0.94 | 0.94 | 0.97 | 0.96 | 0.97 | 0.97 | 0.97 | 0.96 | | | | | | | | | | | | | | | | | | | | | |
| +BCS (B1R2) | 0.94 | 0.94 | 0.95 | 0.94 | 0.94 | 0.95 | 0.96 | 0.94 | 0.96 | 0.96 | 0.96 | | | | | | | | | | | | | | | | | | | | |
| +BCS (B1R3) | 0.95 | 0.95 | 0.96 | 0.95 | 0.95 | 0.96 | 0.96 | 0.94 | 0.96 | 0.96 | 0.96 | 0.97 | | | | | | | | | | | | | | | | | | | |
| +BCS (B2R1) | 0.97 | 0.94 | 0.94 | 0.94 | 0.97 | 0.96 | 0.96 | 0.97 | 0.96 | 0.96 | 0.99 | 0.96 | 0.96 | | | | | | | | | | | | | | | | | | |
| +BCS (B2R2) | 0.95 | 0.95 | 0.96 | 0.95 | 0.95 | 0.97 | 0.97 | 0.94 | 0.97 | 0.97 | 0.96 | 0.96 | 0.97 | 0.96 | | | | | | | | | | | | | | | | | |
| +BCS (B2R3) | 0.95 | 0.95 | 0.96 | 0.95 | 0.95 | 0.97 | 0.97 | 0.95 | 0.97 | 0.97 | 0.96 | 0.95 | 0.97 | 0.96 | 0.99 | | | | | | | | | | | | | | | | |
| +BCS (B2R4) | 0.94 | 0.95 | 0.95 | 0.93 | 0.94 | 0.96 | 0.96 | 0.94 | 0.96 | 0.96 | 0.95 | 0.94 | 0.95 | 0.95 | 0.96 | 0.97 | | | | | | | | | | | | | | | |
| +BCS (B3R1) | 0.97 | 0.94 | 0.94 | 0.94 | 0.97 | 0.96 | 0.96 | 0.97 | 0.96 | 0.96 | 0.99 | 0.96 | 0.96 | 0.99 | 0.96 | 0.96 | 0.95 | | | | | | | | | | | | | | |
| +BCS (B3R2) | 0.96 | 0.95 | 0.95 | 0.95 | 0.96 | 0.98 | 0.98 | 0.96 | 0.98 | 0.98 | 0.97 | 0.96 | 0.97 | 0.97 | 0.97 | 0.98 | 0.97 | 0.97 | | | | | | | | | | | | | |
| +BCS (B3R3) | 0.96 | 0.95 | 0.95 | 0.95 | 0.96 | 0.98 | 0.98 | 0.96 | 0.98 | 0.98 | 0.97 | 0.96 | 0.97 | 0.97 | 0.97 | 0.98 | 0.97 | 0.97 | 0.99 | | | | | | | | | | | | |
| +BCS (B3R4) | 0.93 | 0.92 | 0.93 | 0.93 | 0.93 | 0.94 | 0.95 | 0.93 | 0.95 | 0.95 | 0.94 | 0.95 | 0.94 | 0.95 | 0.95 | 0.95 | 0.94 | 0.94 | 0.95 | 0.95 | | | | | | | | | | | |
| +Cu (B1R1) | 0.96 | 0.94 | 0.94 | 0.94 | 0.97 | 0.96 | 0.96 | 0.97 | 0.95 | 0.95 | 0.96 | 0.93 | 0.94 | 0.96 | 0.94 | 0.94 | 0.93 | 0.96 | 0.95 | 0.95 | 0.92 | | | | | | | | | | |
| +Cu (B1R2) | 0.95 | 0.96 | 0.96 | 0.96 | 0.95 | 0.96 | 0.96 | 0.94 | 0.96 | 0.96 | 0.94 | 0.94 | 0.95 | 0.94 | 0.95 | 0.96 | 0.95 | 0.94 | 0.95 | 0.95 | 0.94 | 0.95 | | | | | | | | | |
| +Cu (B1R3) | 0.95 | 0.95 | 0.96 | 0.96 | 0.95 | 0.96 | 0.96 | 0.95 | 0.96 | 0.96 | 0.94 | 0.94 | 0.96 | 0.94 | 0.95 | 0.96 | 0.94 | 0.94 | 0.95 | 0.95 | 0.93 | 0.95 | 0.97 | | | | | | | | |
| +Cu (B1R4) | 0.95 | 0.96 | 0.96 | 0.96 | 0.95 | 0.96 | 0.96 | 0.95 | 0.96 | 0.96 | 0.95 | 0.94 | 0.96 | 0.95 | 0.96 | 0.96 | 0.94 | 0.95 | 0.96 | 0.95 | 0.93 | 0.95 | 0.97 | 0.98 | | | | | | | |
| +Cu (B2R1) | 0.82 | 0.78 | 0.77 | 0.78 | 0.82 | 0.80 | 0.80 | 0.82 | 0.80 | 0.80 | 0.80 | 0.77 | 0.78 | 0.81 | 0.77 | 0.78 | 0.78 | 0.81 | 0.81 | 0.81 | 0.75 | 0.84 | 0.79 | 0.80 | 0.80 | | | | | | |
| +Cu (B2R2) | 0.96 | 0.96 | 0.97 | 0.96 | 0.96 | 0.98 | 0.98 | 0.96 | 0.98 | 0.98 | 0.95 | 0.94 | 0.95 | 0.95 | 0.95 | 0.96 | 0.95 | 0.95 | 0.96 | 0.96 | 0.93 | 0.95 | 0.96 | 0.96 | 0.96 | 0.81 | | | | | |
| +Cu (B2R3) | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 0.97 | 0.98 | 0.96 | 0.98 | 0.98 | 0.95 | 0.94 | 0.95 | 0.94 | 0.96 | 0.96 | 0.95 | 0.94 | 0.96 | 0.96 | 0.93 | 0.95 | 0.96 | 0.96 | 0.97 | 0.80 | 0.99 | | | | |
| +Cu (B3R1) | 0.96 | 0.94 | 0.94 | 0.94 | 0.96 | 0.95 | 0.96 | 0.96 | 0.95 | 0.95 | 0.96 | 0.93 | 0.93 | 0.95 | 0.94 | 0.94 | 0.93 | 0.95 | 0.95 | 0.95 | 0.92 | 0.99 | 0.95 | 0.95 | 0.95 | 0.83 | 0.96 | 0.95 | | | |
| +Cu (B3R2) | 0.94 | 0.95 | 0.95 | 0.94 | 0.94 | 0.96 | 0.97 | 0.94 | 0.96 | 0.96 | 0.94 | 0.94 | 0.95 | 0.93 | 0.96 | 0.96 | 0.95 | 0.93 | 0.95 | 0.95 | 0.94 | 0.94 | 0.96 | 0.96 | 0.96 | 0.78 | 0.97 | 0.97 | 0.95 | | |
| +Cu (B3R3) | 0.95 | 0.95 | 0.96 | 0.95 | 0.95 | 0.97 | 0.97 | 0.95 | 0.97 | 0.97 | 0.95 | 0.94 | 0.96 | 0.95 | 0.97 | 0.97 | 0.95 | 0.95 | 0.96 | 0.96 | 0.94 | 0.95 | 0.97 | 0.97 | 0.97 | 0.79 | 0.98 | 0.98 | 0.95 | 0.97 | |

**Figure S2. Pearson correlation for individual experimental runs.** Color-coded Pearson r-values are shown for the 31 individual runs using lysis/digestion protocol A and Q-Exactive mass spectrometer (Experimental Procedures). The samples are identified by Cu condition (Ctrl, +Cu and +BCS), biological repeat (B1, B2, B3) and technical repeat (R1, R2, R3, R4). All r values were consistently high, within 0.92 to 0.99, except the sample +Cu(B2R1) that had r-values between 0.75-0.83 and was subsequently discarded.

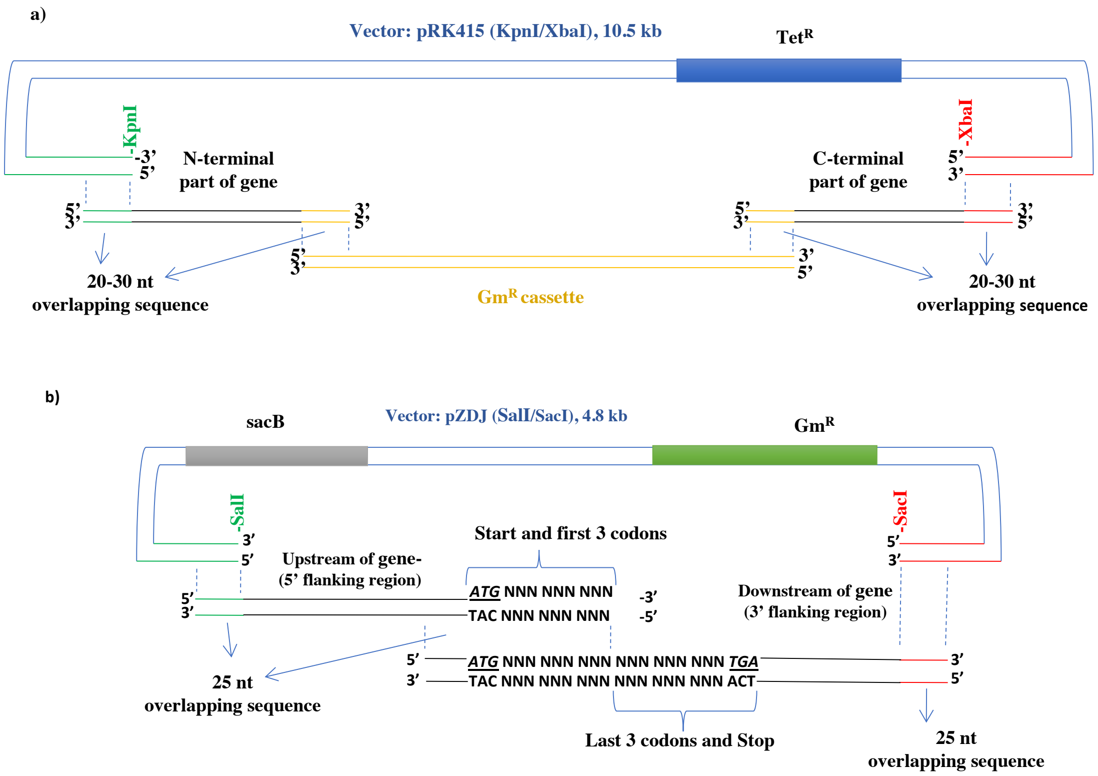
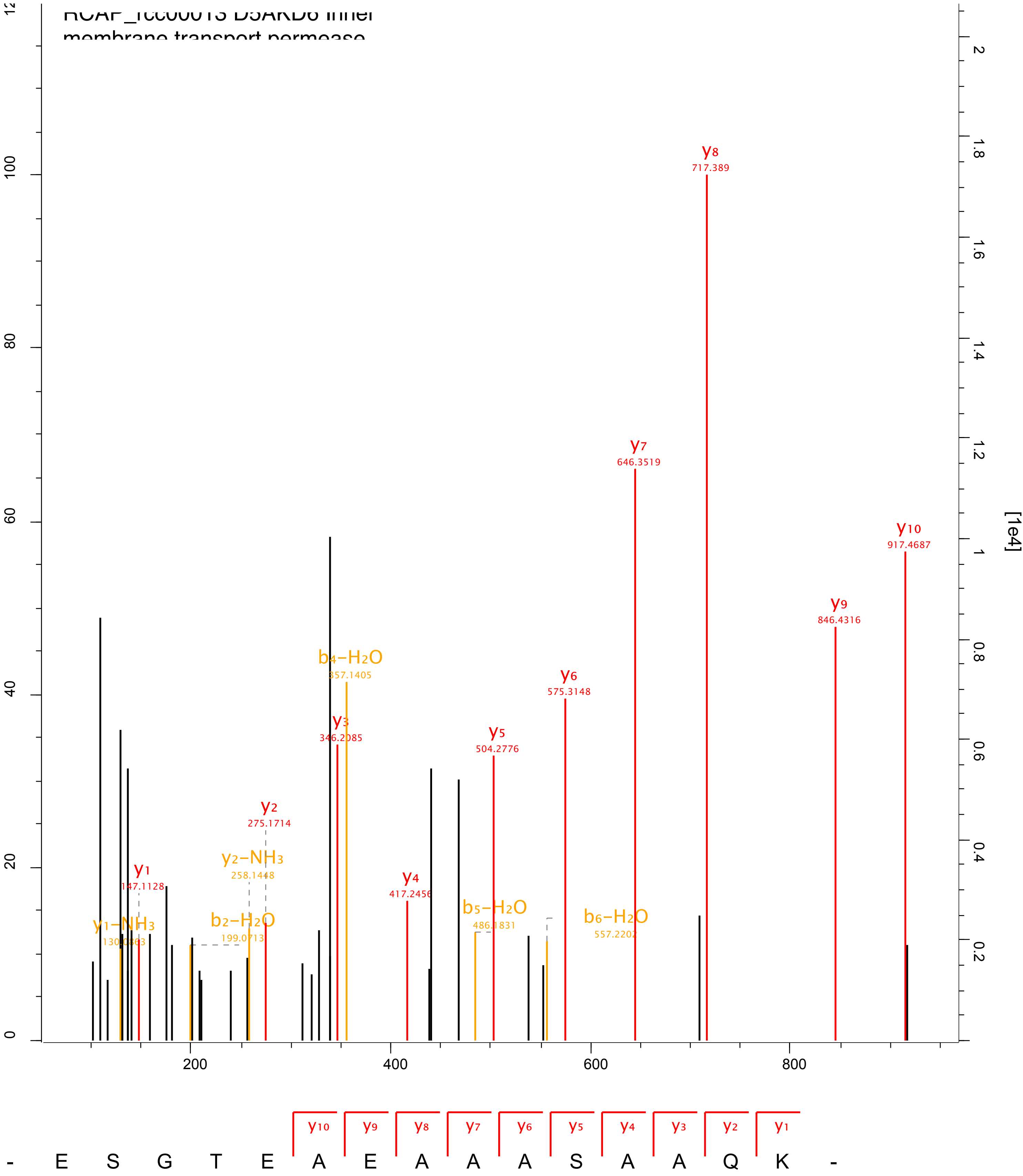


*Figure S3. Selamoglu et al.*

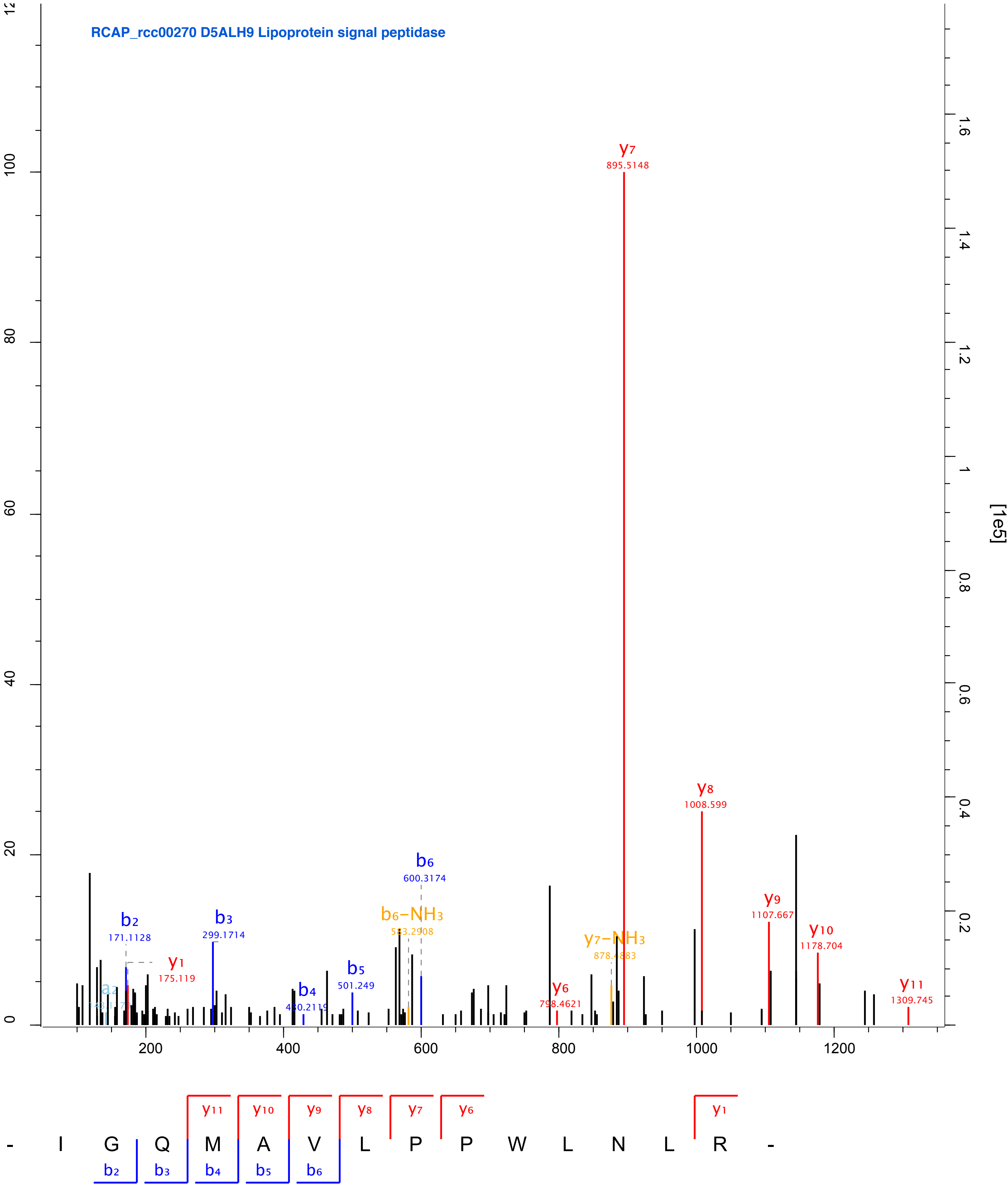
**Figure S3. The NEBuilder HiFi DNA Assembly Method.** (a) the version used for GTA mediated chromosomal inactivation, and (b) that used for construction of chromosomal in-frame seamless deletions of *R. capsulatus* chromosomal genes. The dotted lines indicate the overlapping regions between the vector arms and the DNA fragments being inserted.

**Figure S4.** Annotated spectra corresponding to proteins identified by a single unique peptide (see Sheet 4 (Single Peptide-ID) in **Table S3).**

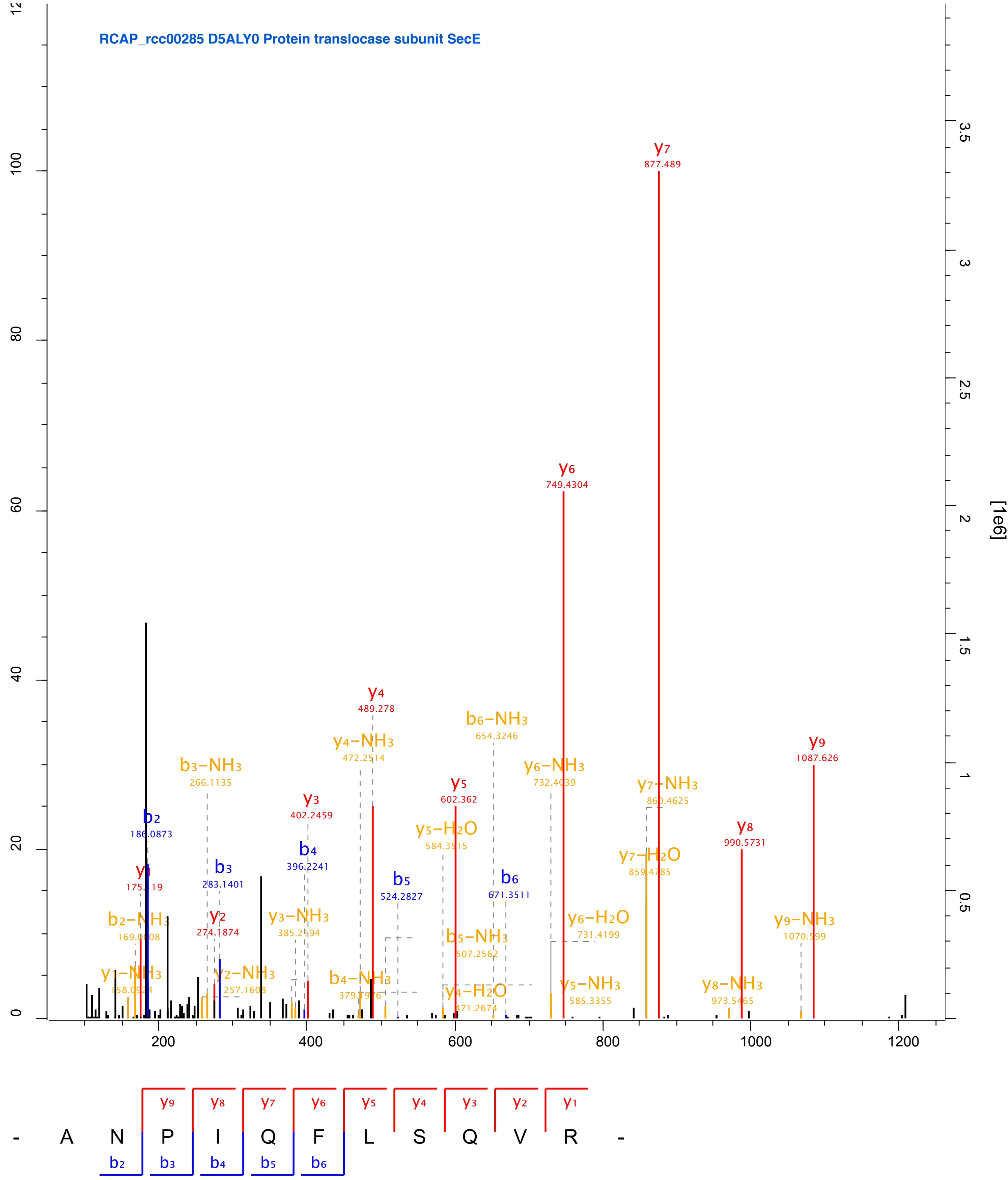
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-Set3-02 | 10728 | FTMS; HCD | 56.23 | 710.83 |



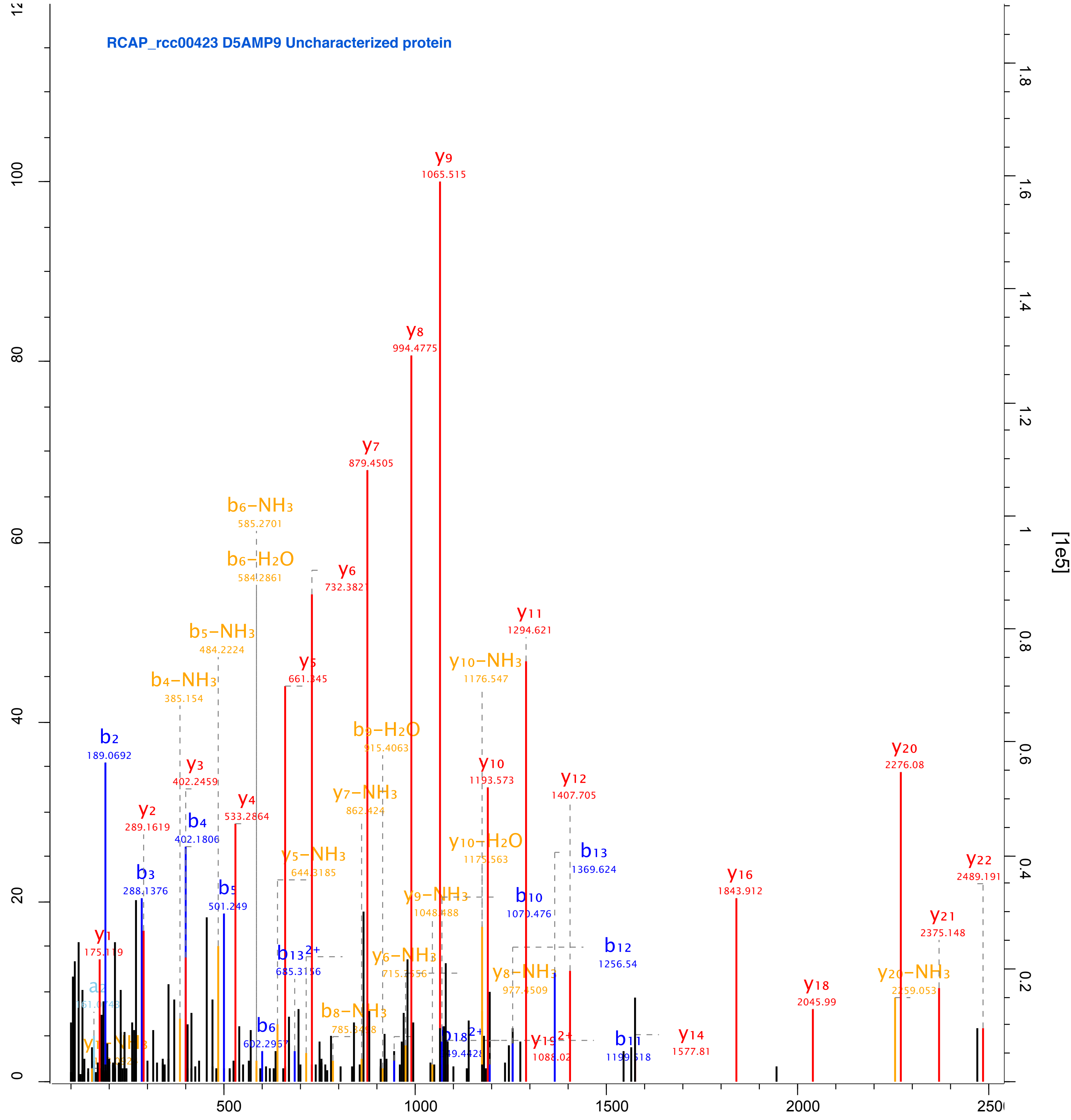
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-Set3-01 | 42715 | FTMS; HCD | 59.07 | 804.76 |

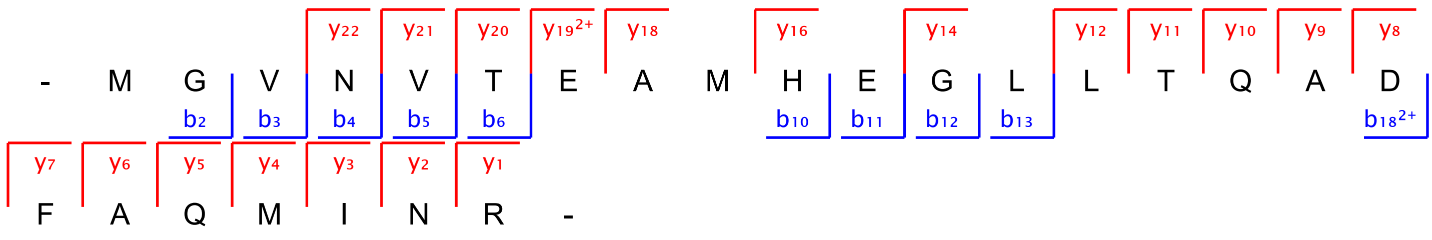


| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-Set2-02 | 33683 | FTMS; HCD | 215.14 | 636.86 |

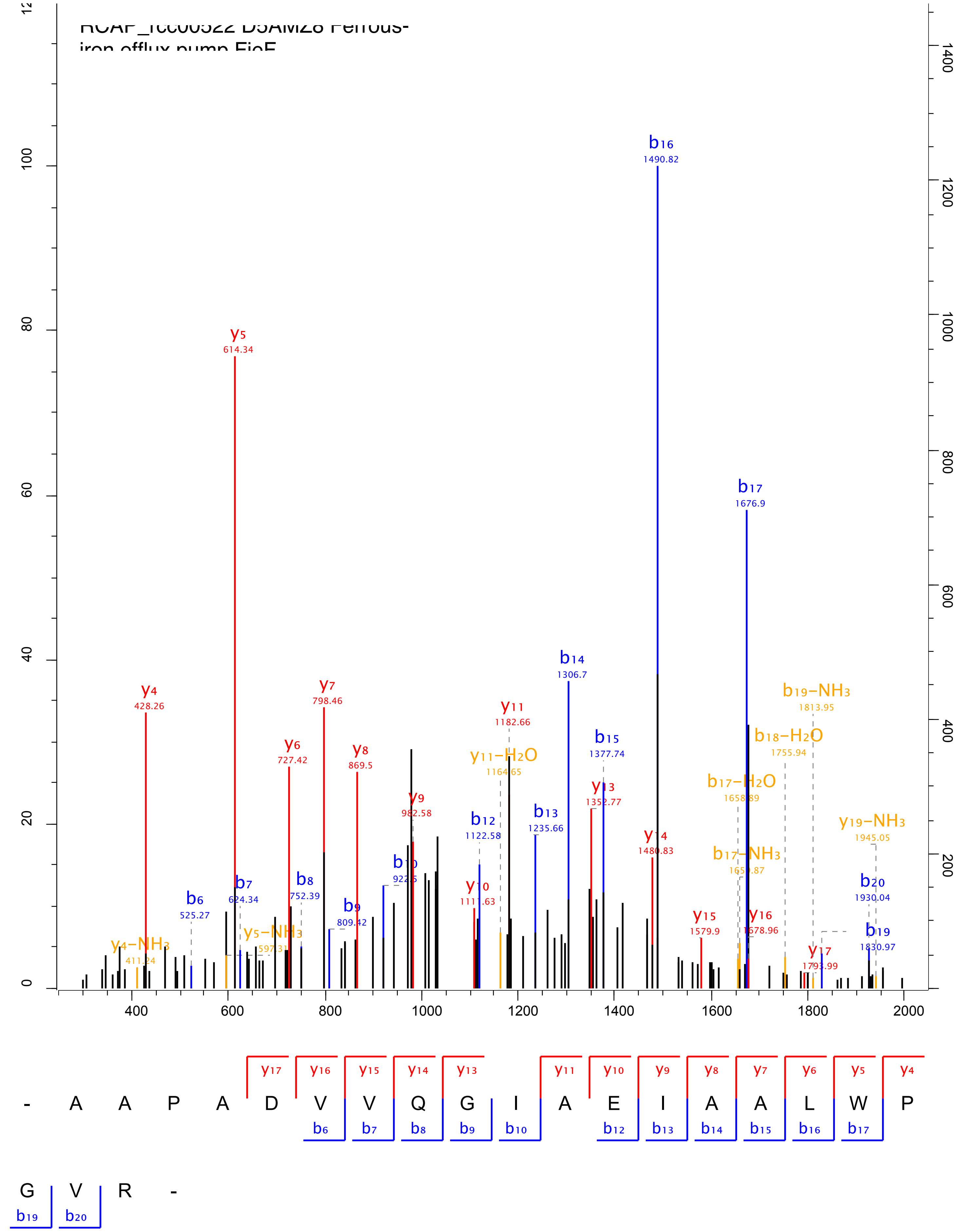


| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-Set2-01 | 41976 | FTMS; HCD | 148.95 | 926.45 |

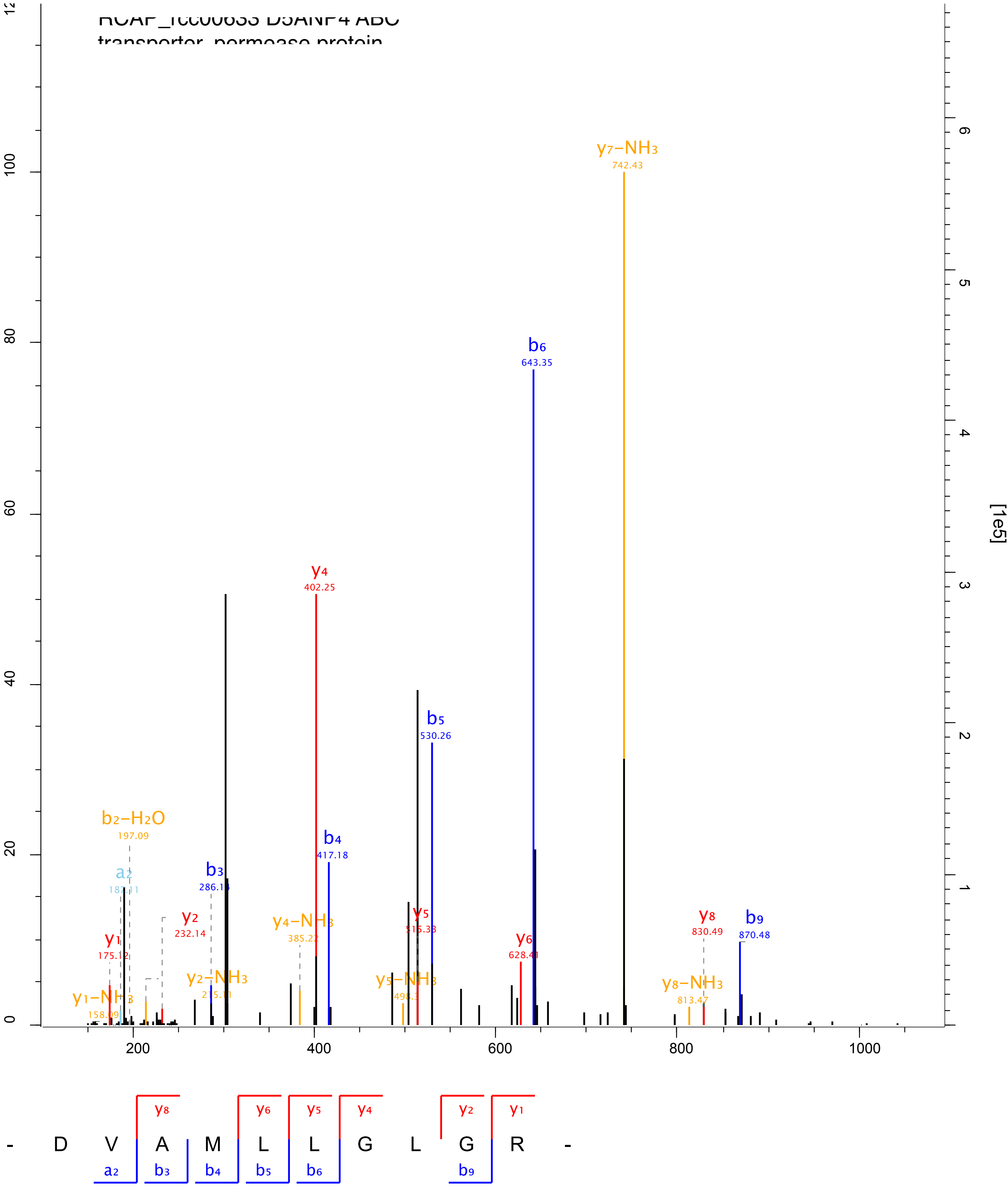




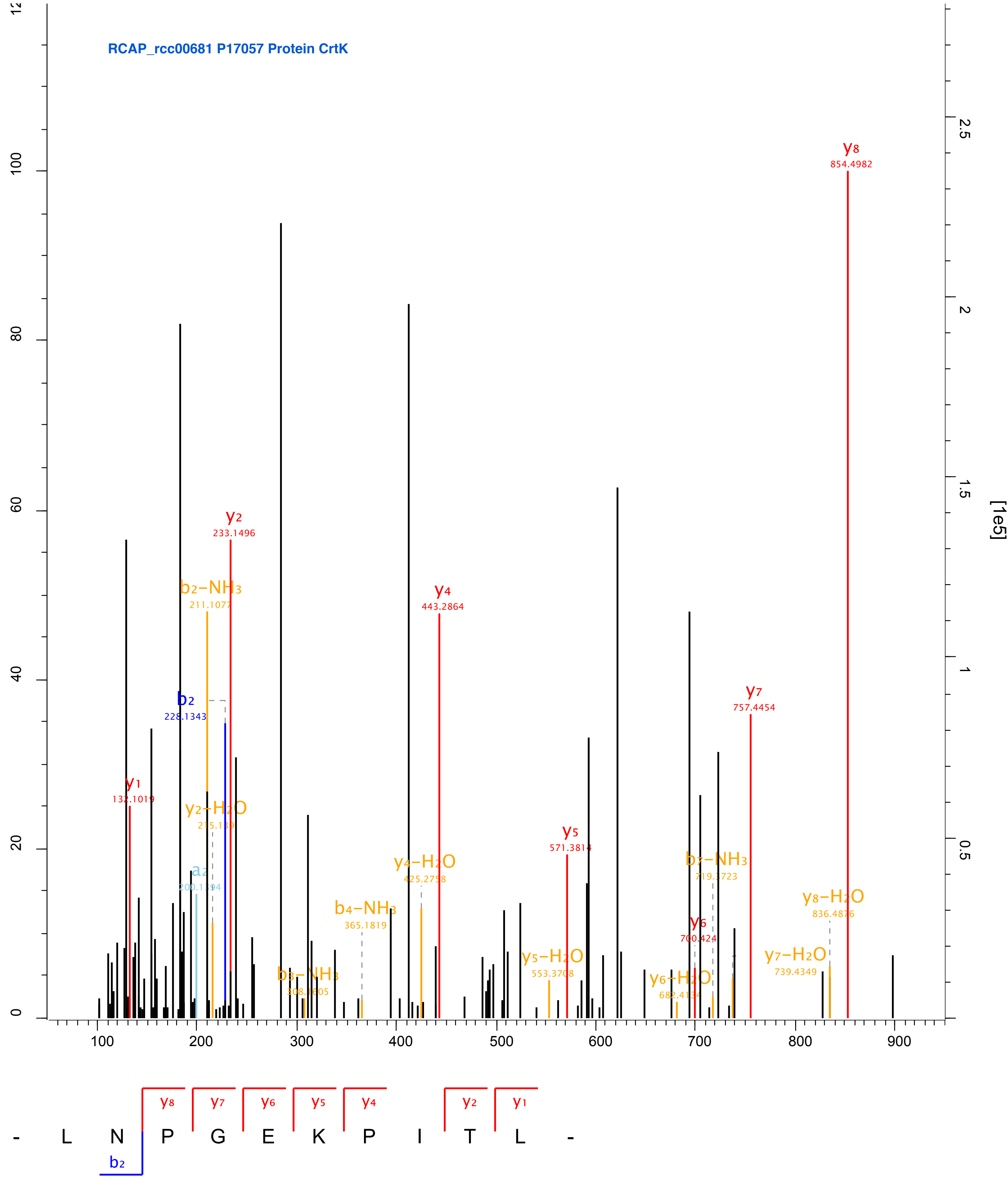
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| CTRL-minusCu-Biorep1-Run2 | 23785 | ITMS; CID | 154.46 | 1053.09 |



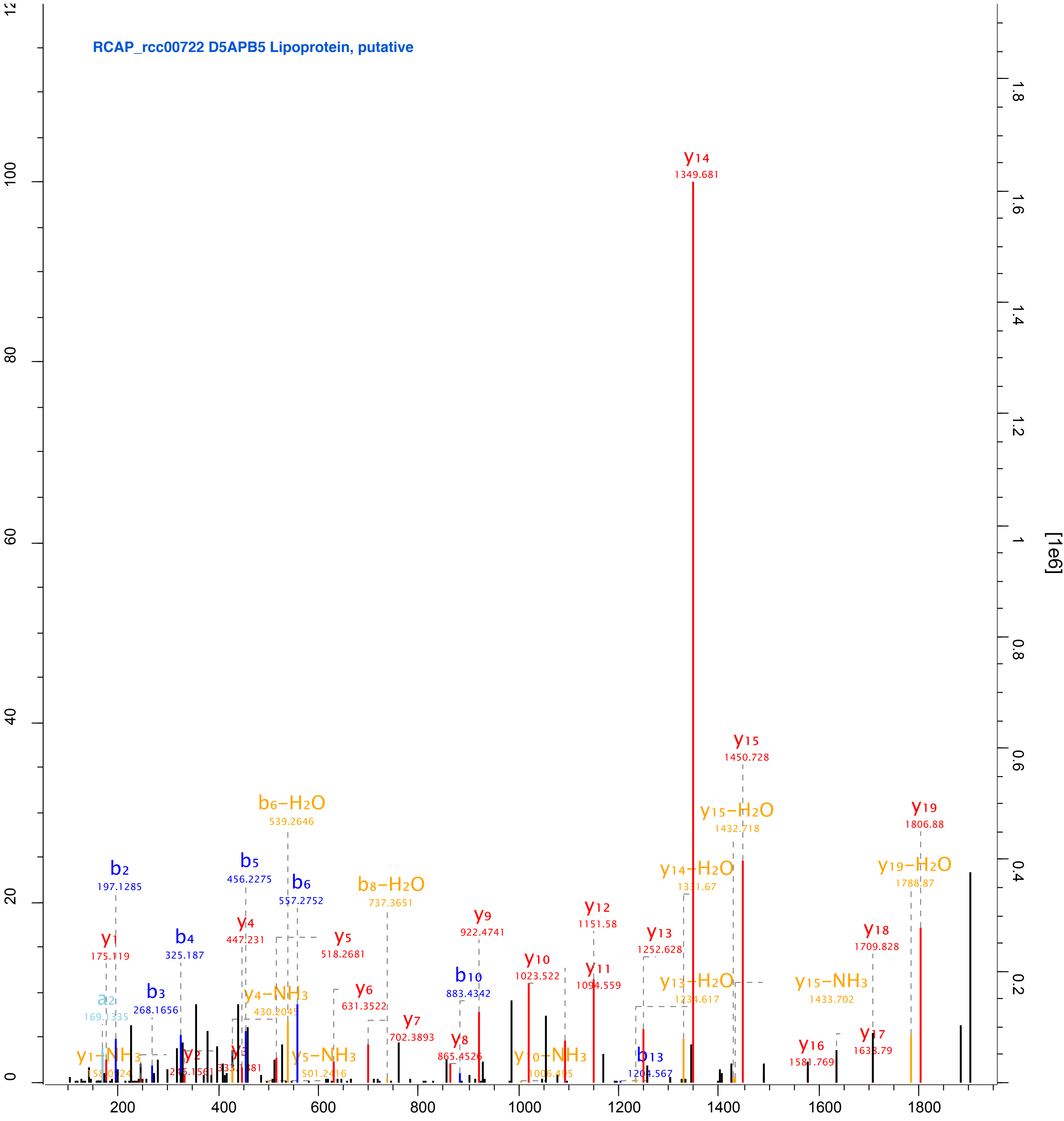
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| PlusBC-Biorep1-Run1 | 10767 | ITMS; CID | 114.5 | 523.3 |

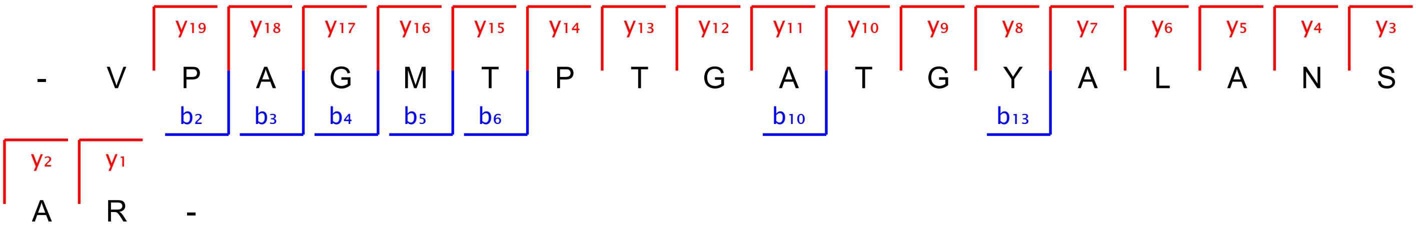


| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-Set3-02 | 21643 | FTMS; HCD | 83.31 | 541.32 |

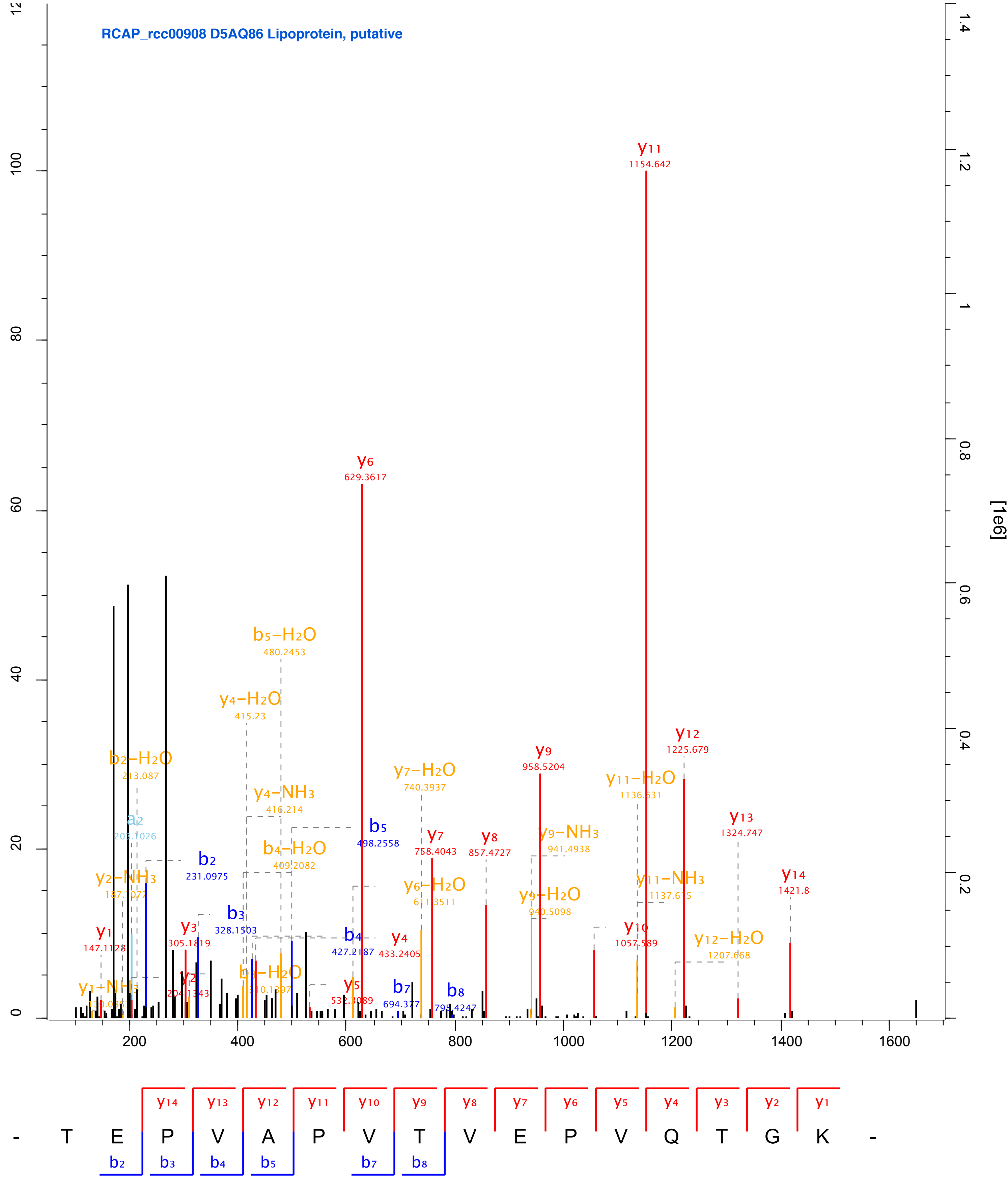


| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-01 | 26292 | FTMS; HCD | 150.12 | 953.98 |

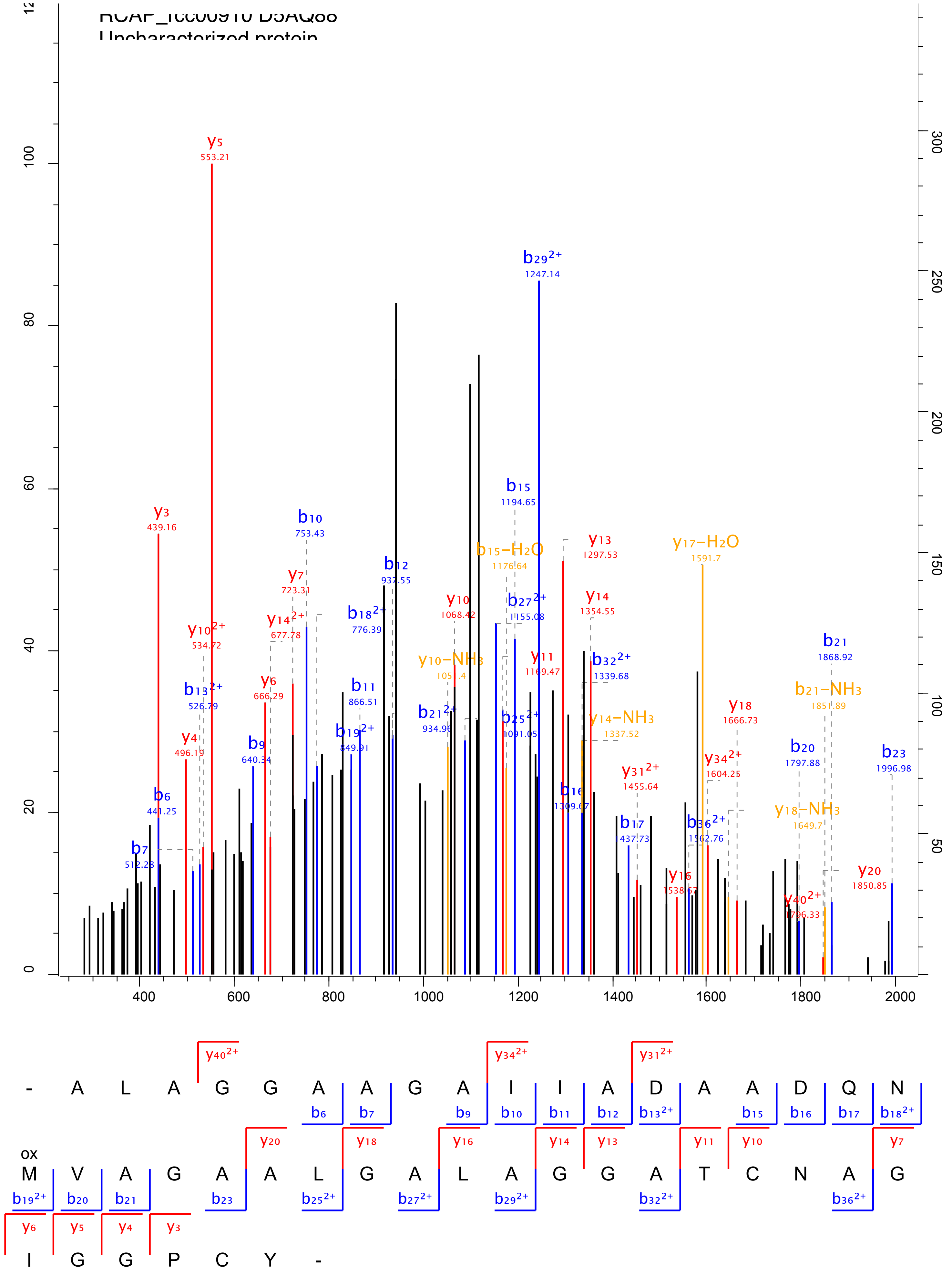




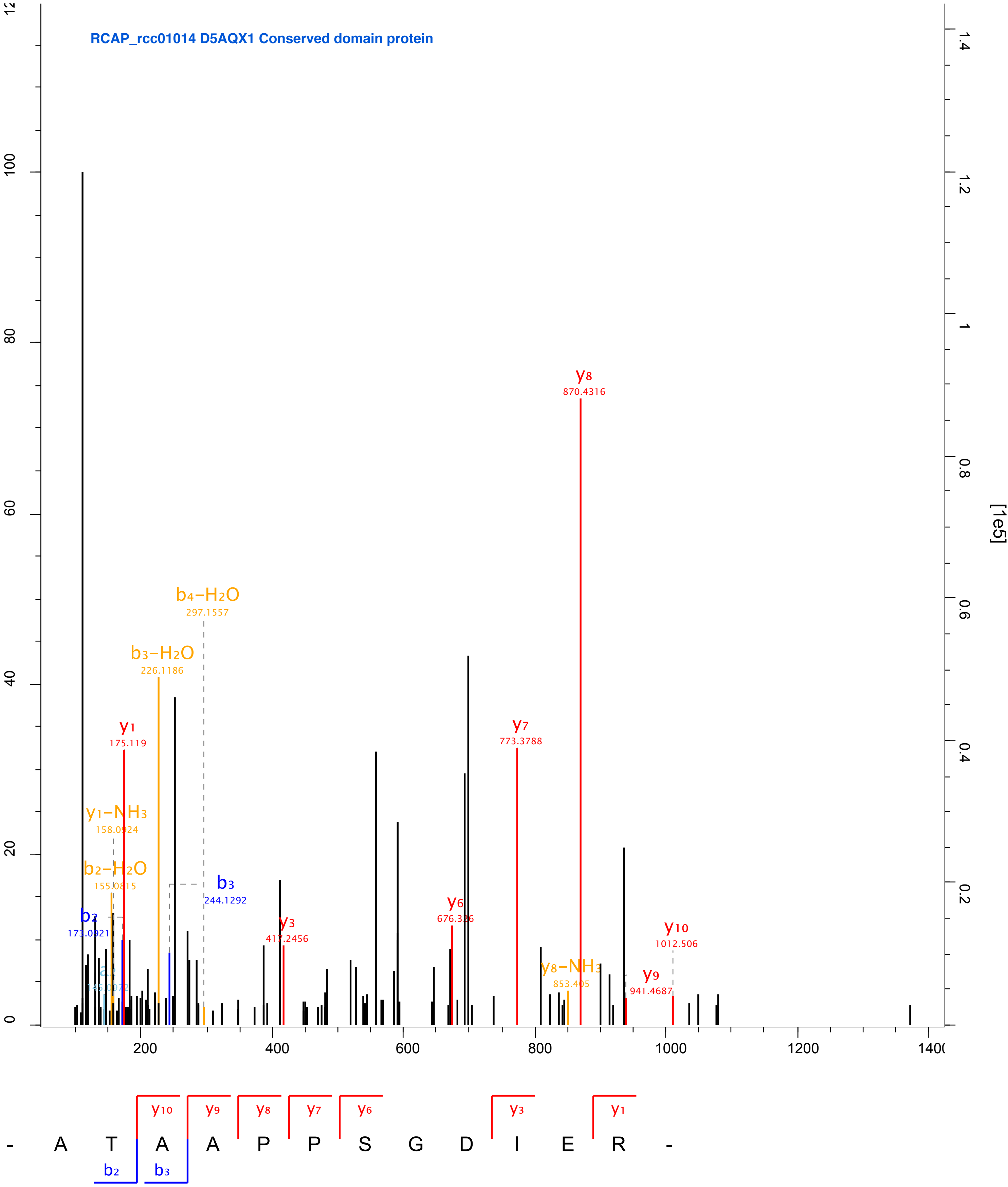
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-Set2-01 | 22555 | FTMS; HCD | 175.48 | 826.45 |



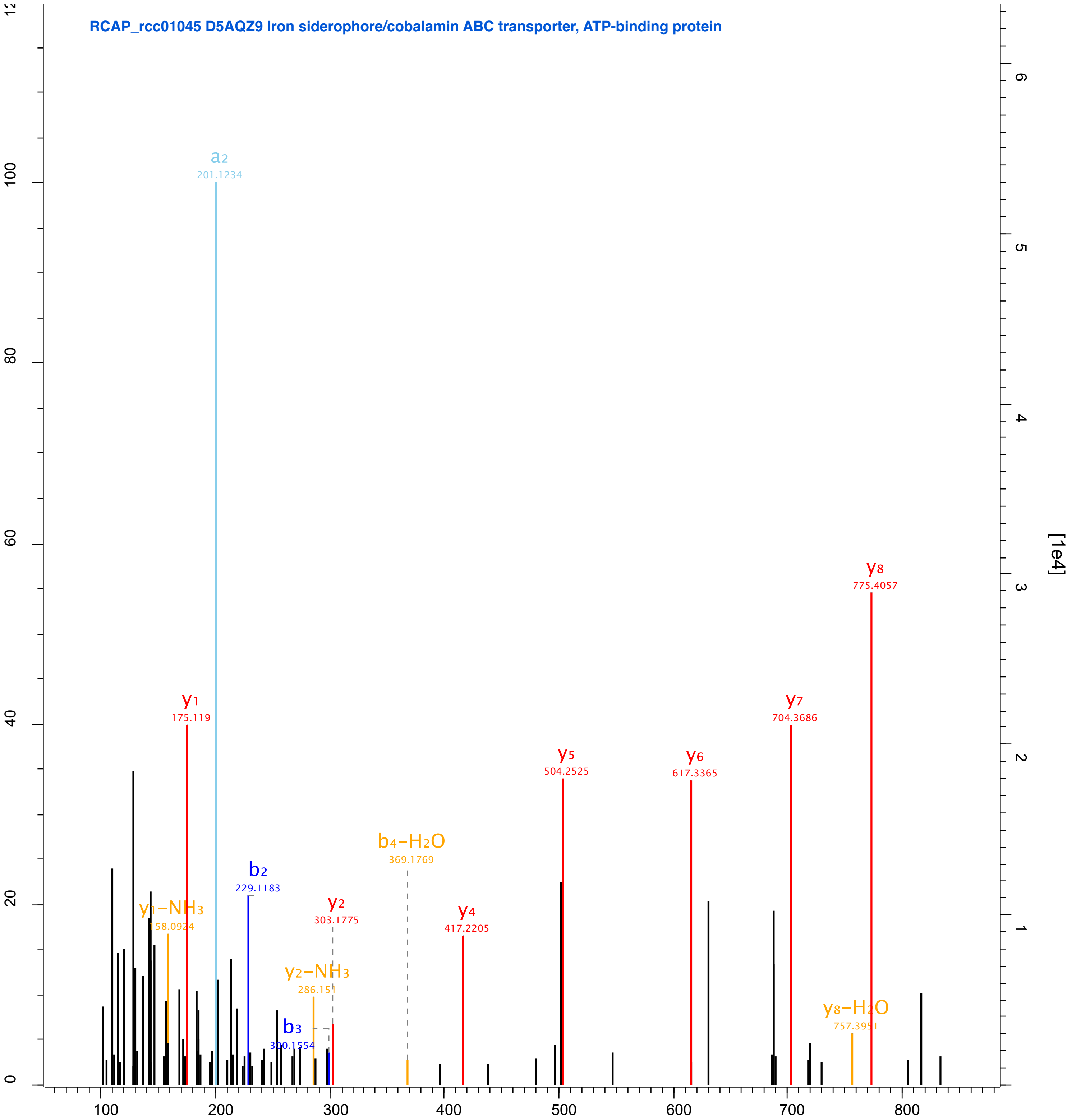
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| PlusBC-Biorep2-Run1 | 34218 | ITMS; CID | 74.87 | 962.96 |

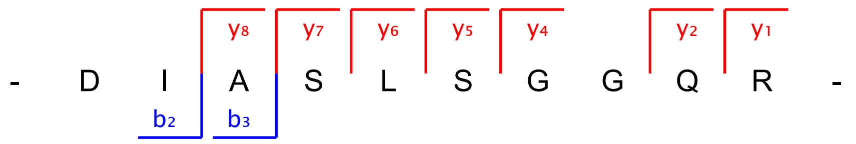


| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-01 | 9780 | FTMS; HCD | 60.1 | 592.32 |

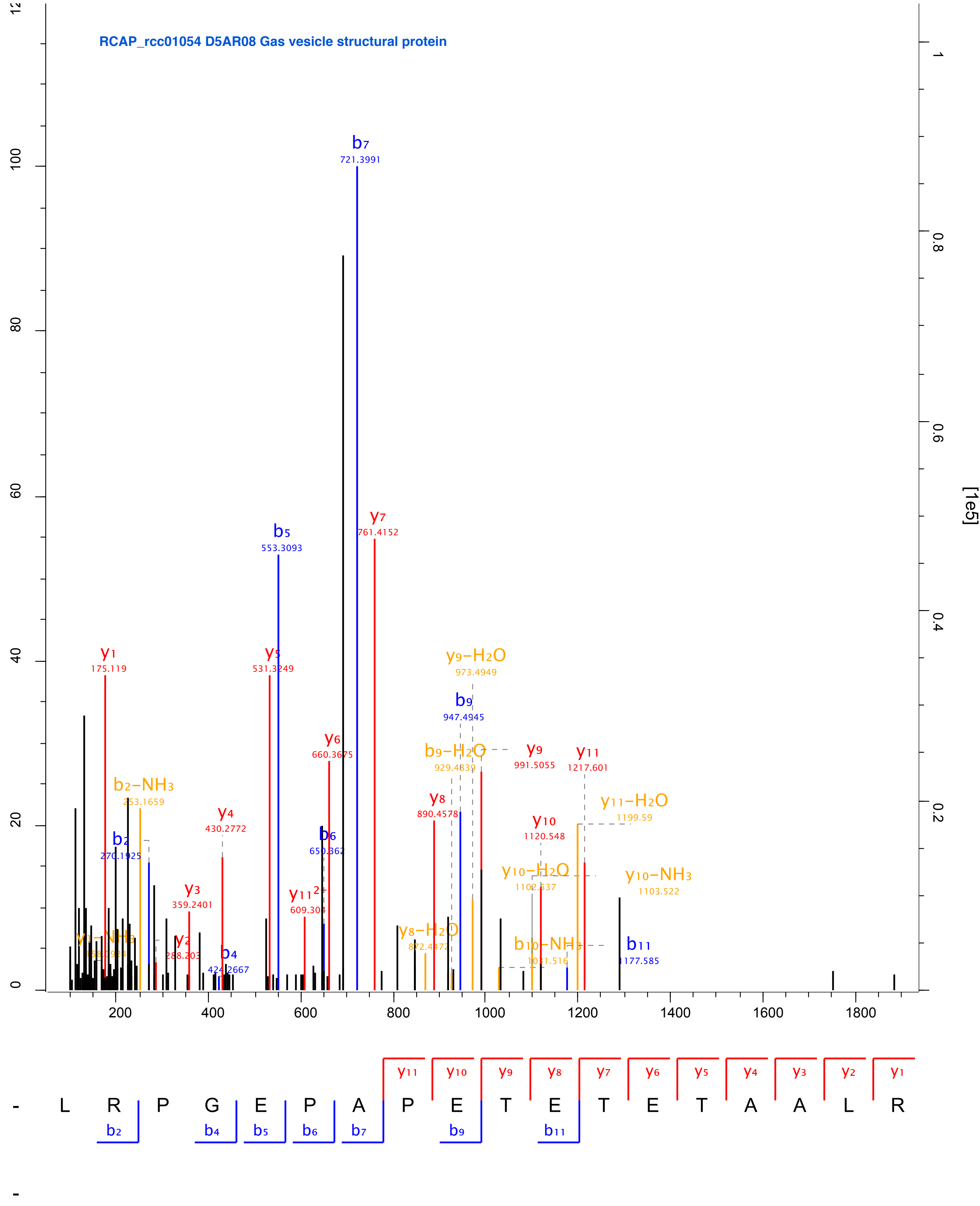


| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-Set3-02 | 14106 | FTMS; HCD | 66.69 | 502.26 |

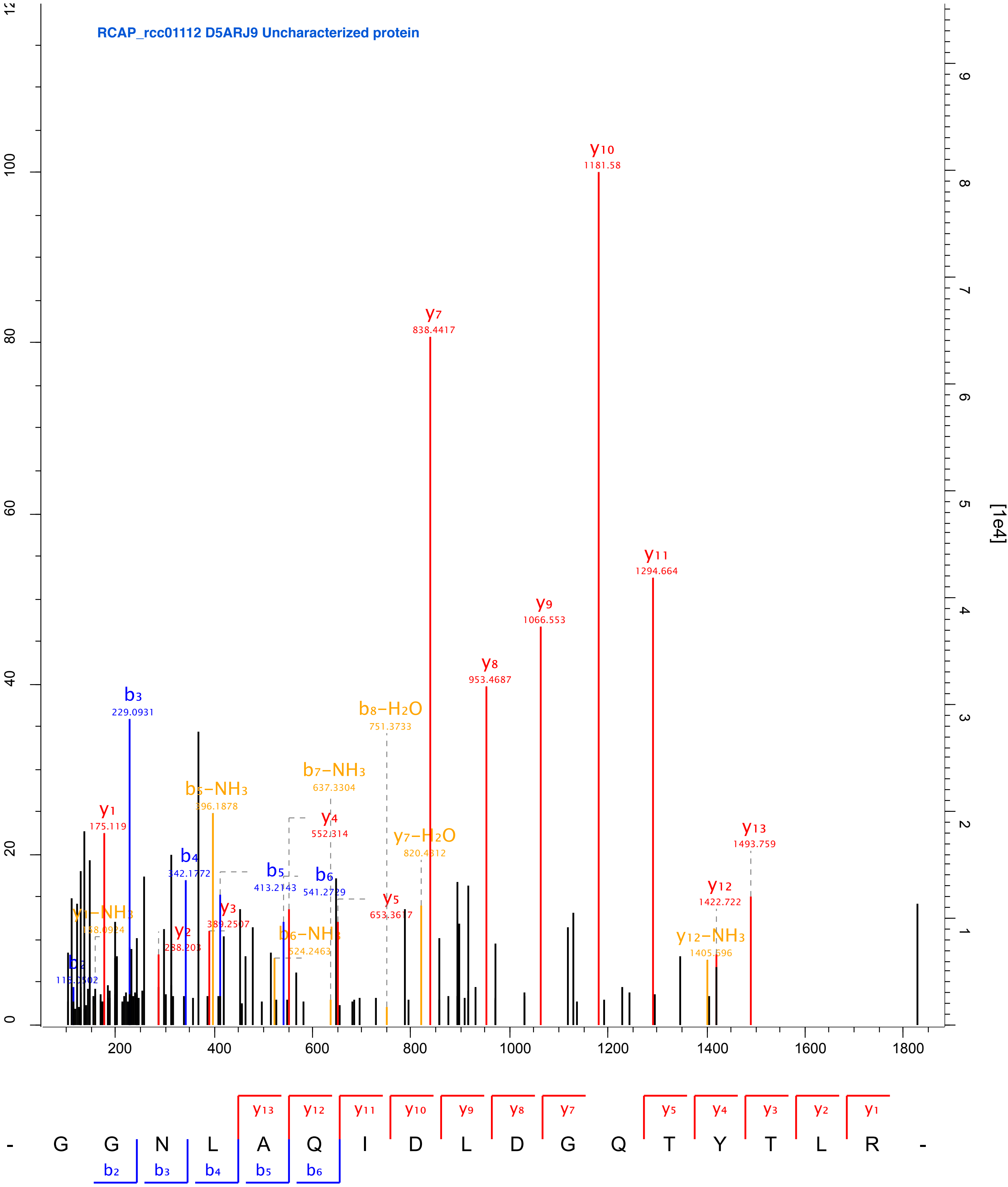




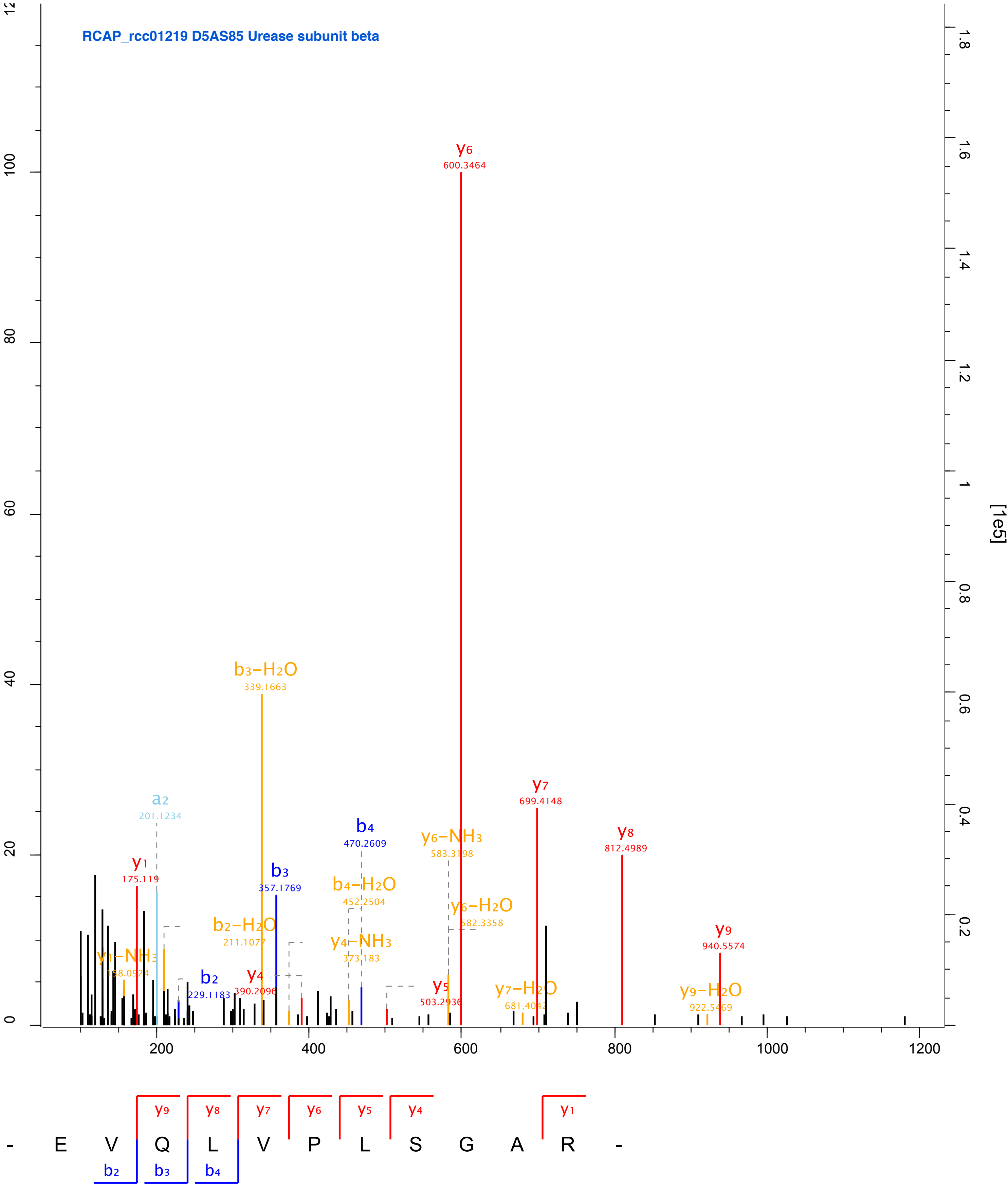
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-Set2-01 | 18462 | FTMS; HCD | 96.14 | 647 |



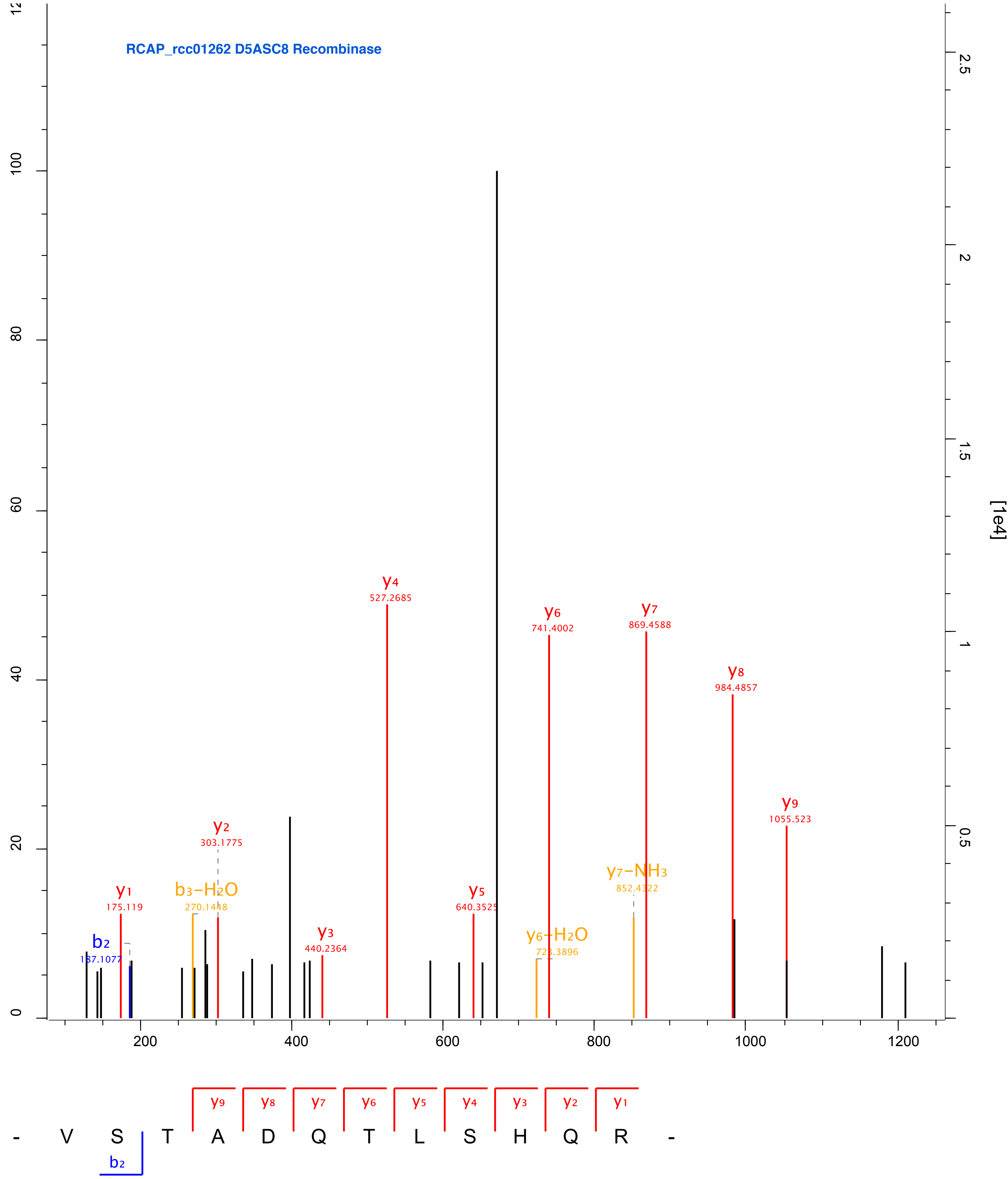
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-Set3-02 | 31927 | FTMS; HCD | 99.01 | 917.97 |



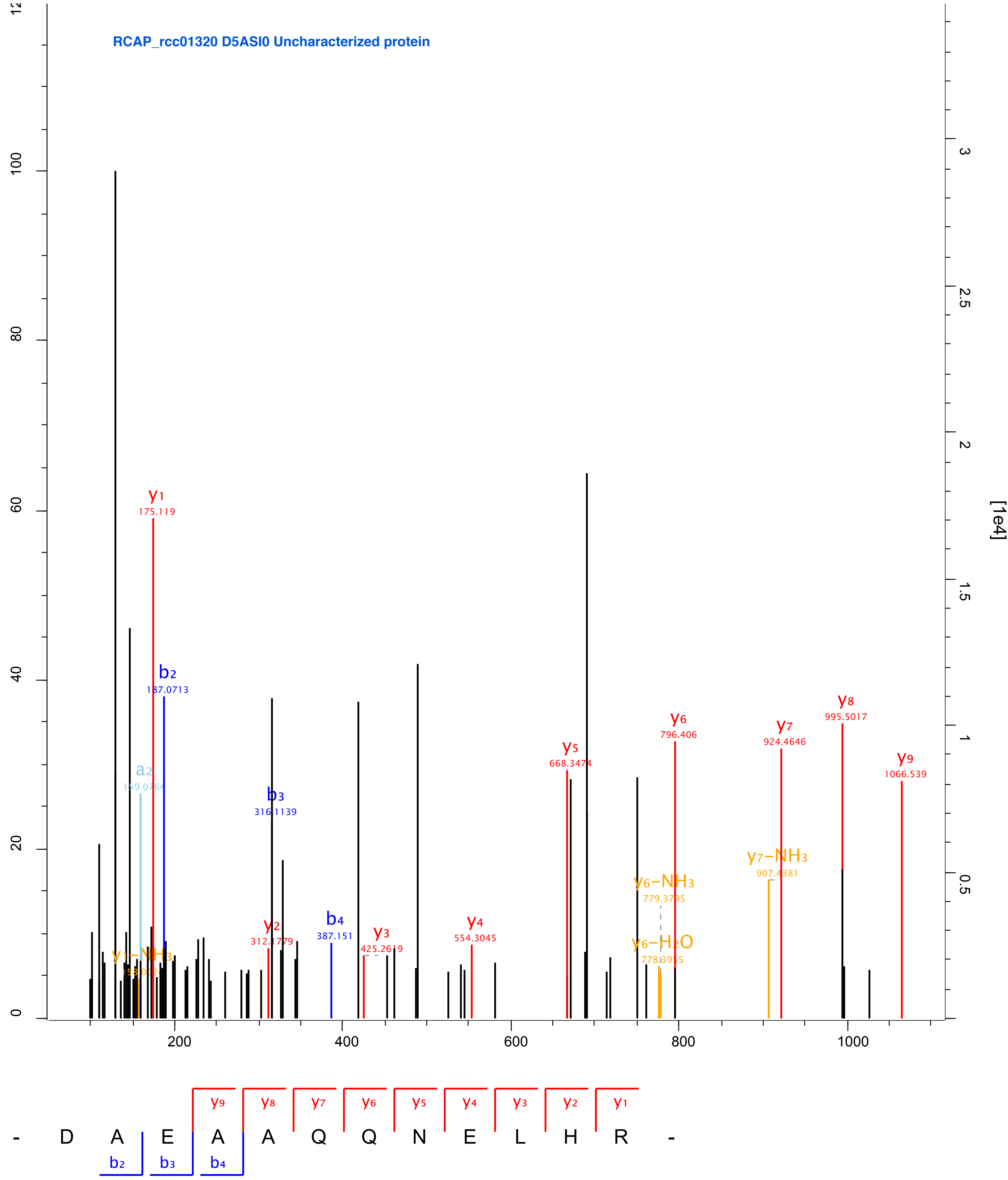
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-Set2-01 | 25381 | FTMS; HCD | 111.61 | 585.34 |



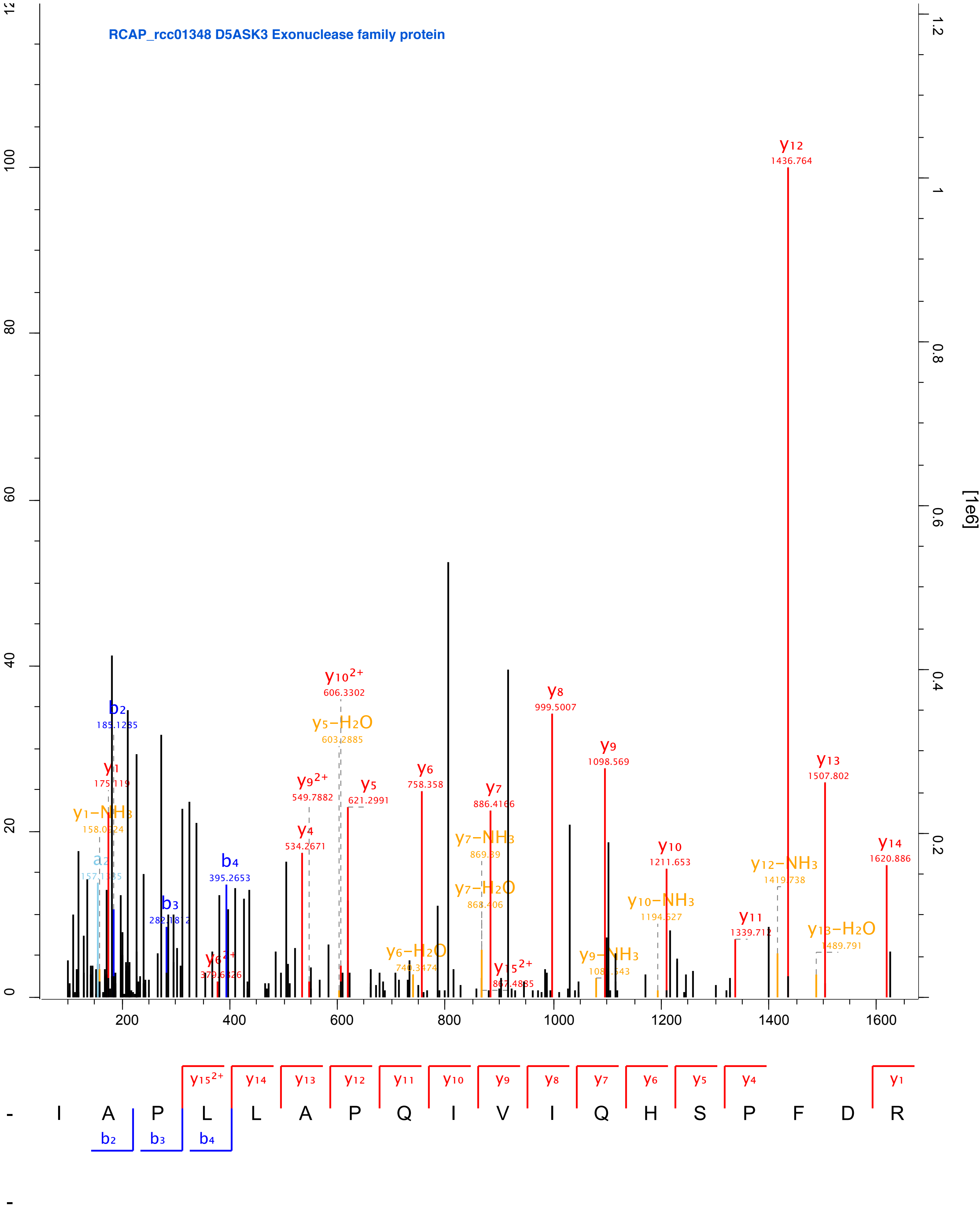
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-Set3-01 | 6420 | FTMS; HCD | 79.97 | 671.84 |



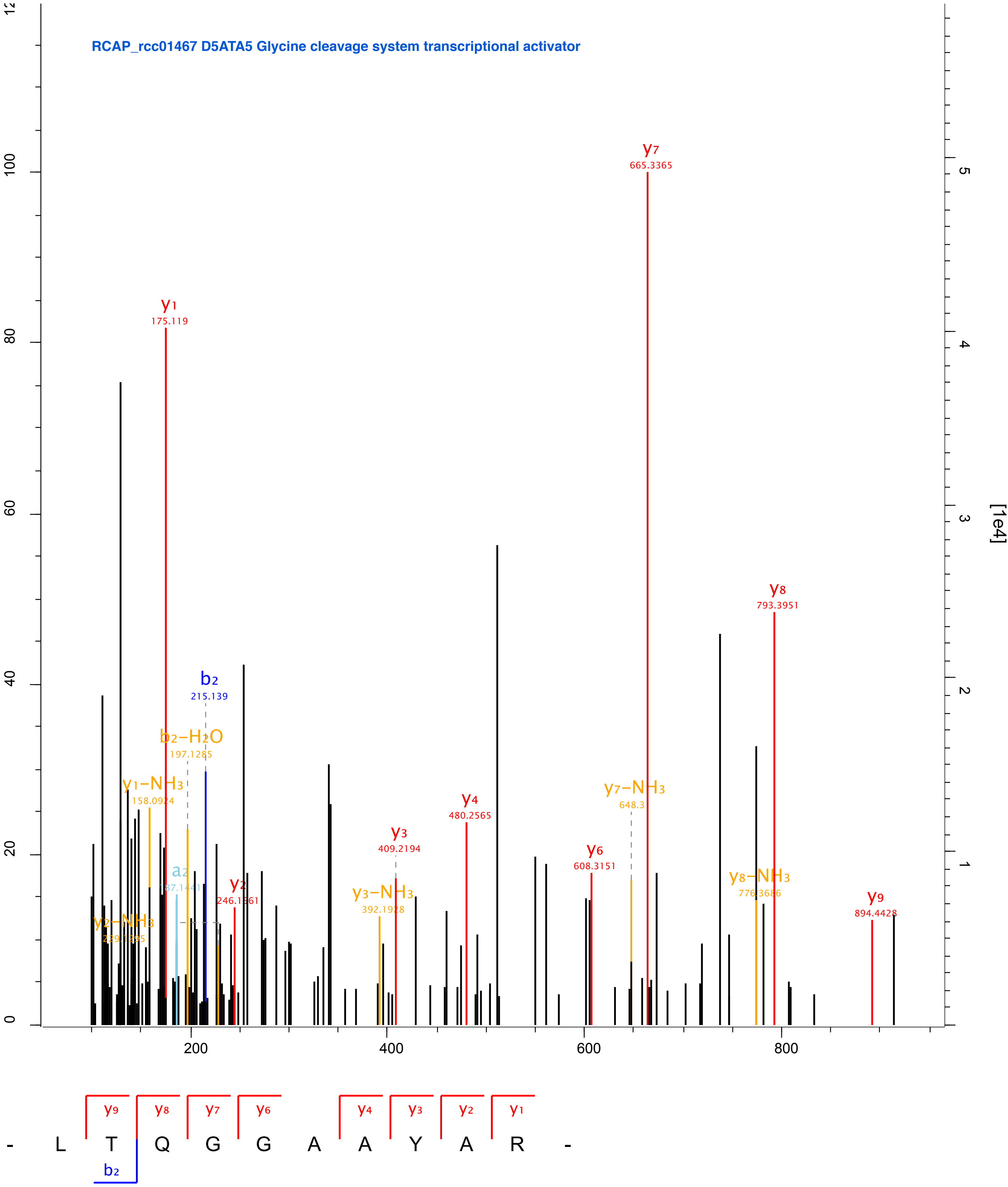
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-Set2-01 | 7984 | FTMS; HCD | 74.84 | 691.33 |



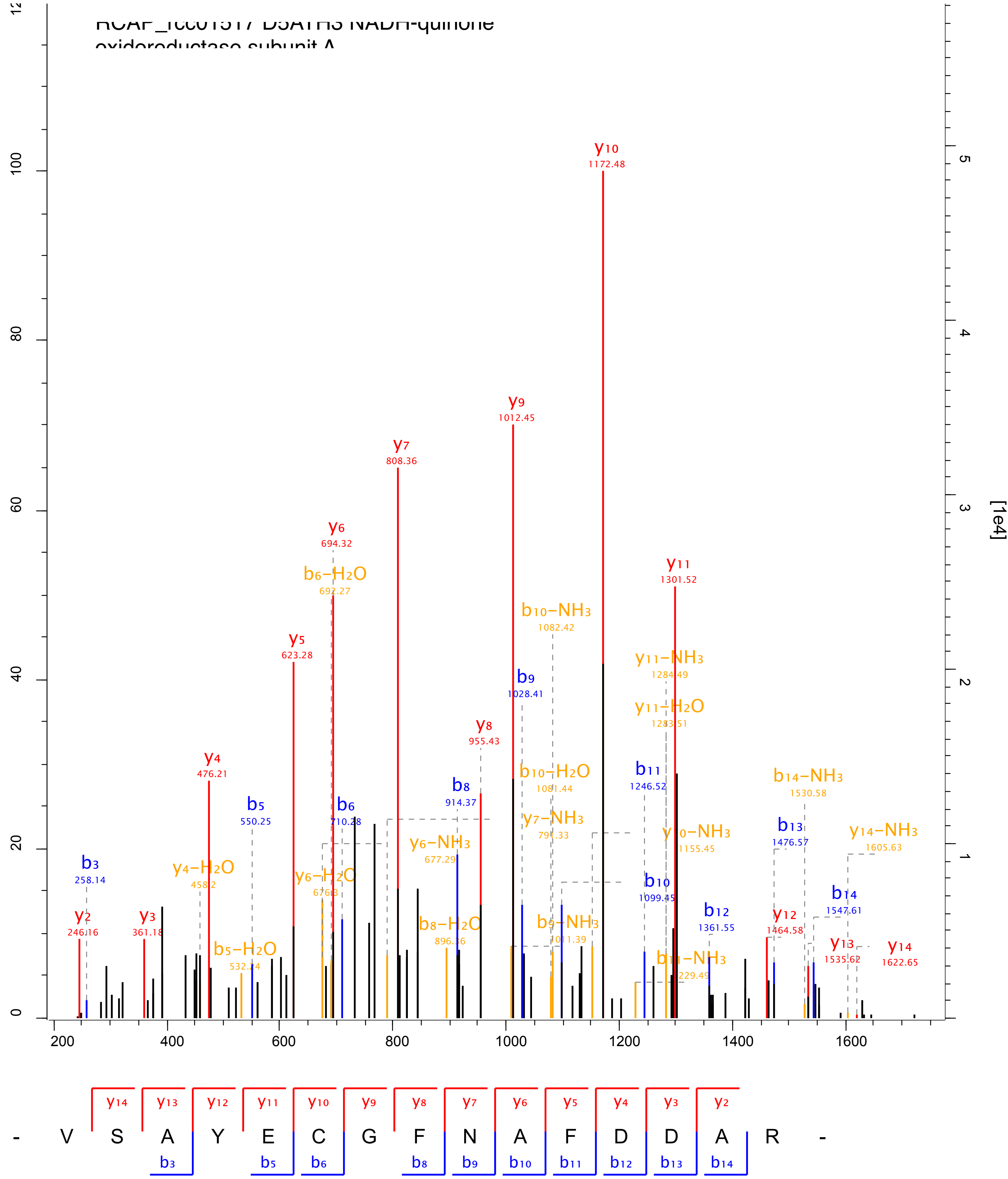
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-01_150512102711 | 44969 | FTMS; HCD | 73.17 | 672.72 |



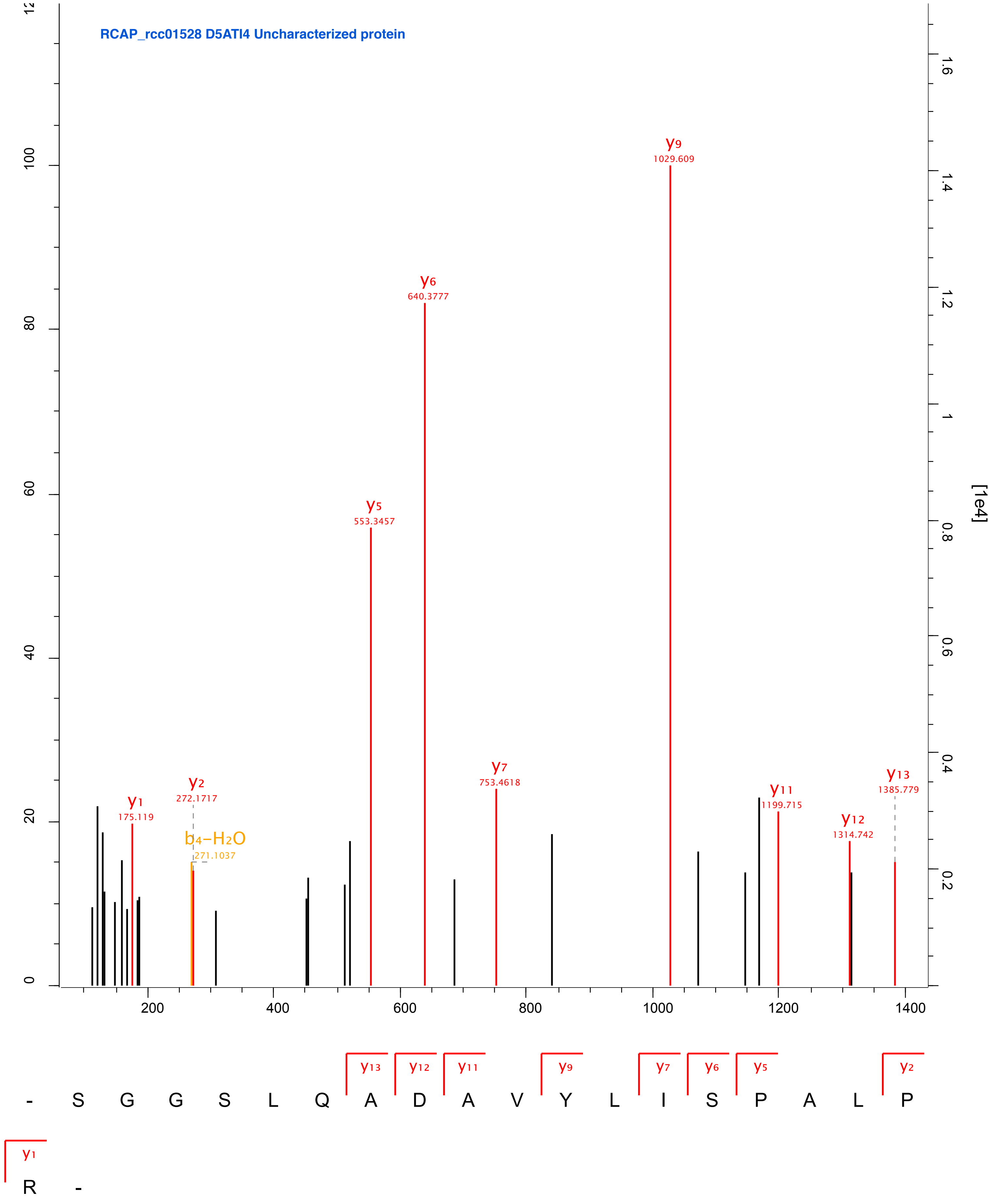
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-01 | 6607 | FTMS; HCD | 84.57 | 504.27 |



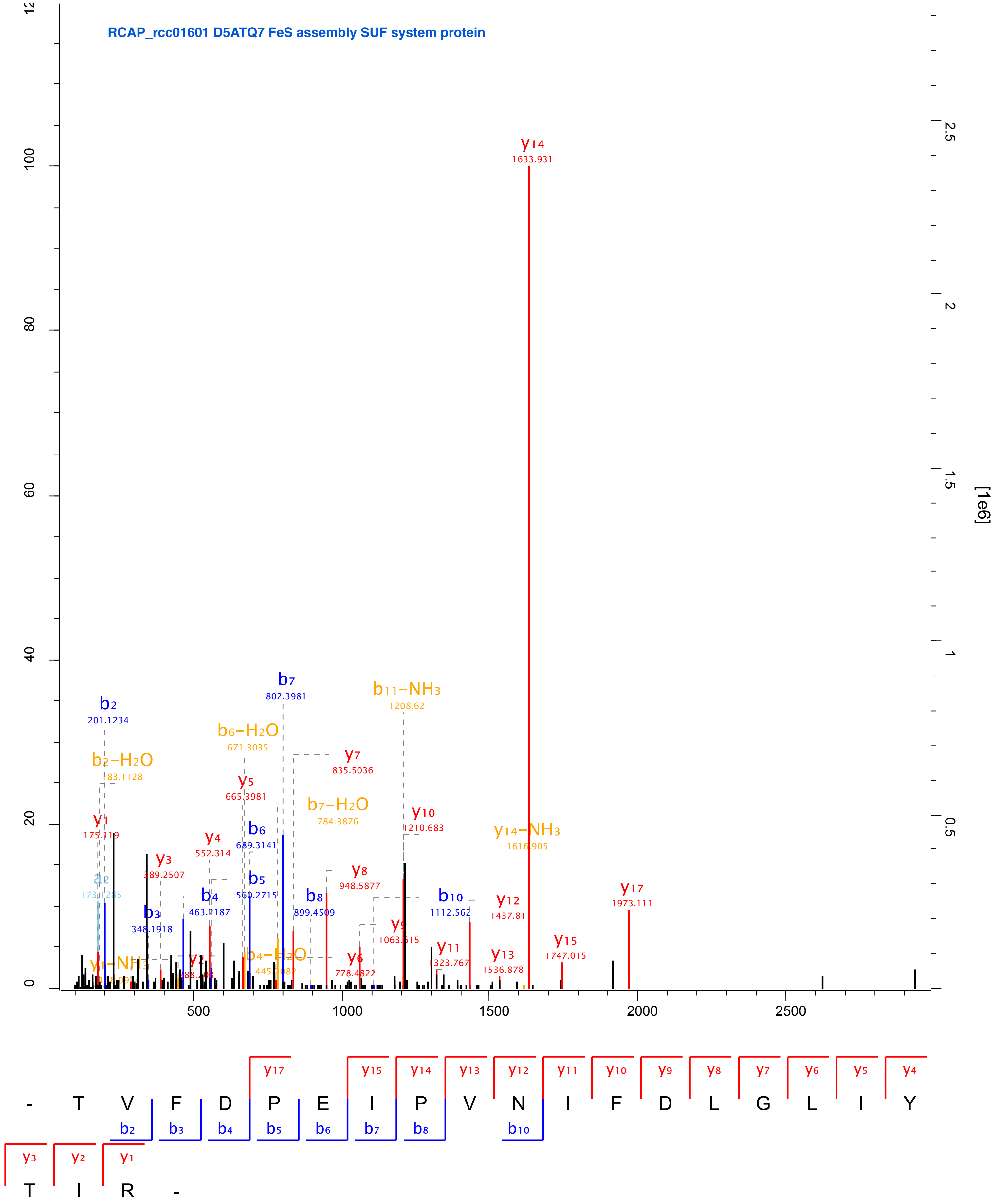
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| PlusCu-Biorep2-Run1 | 13938 | ITMS; CID | 220.37 | 861.87 |



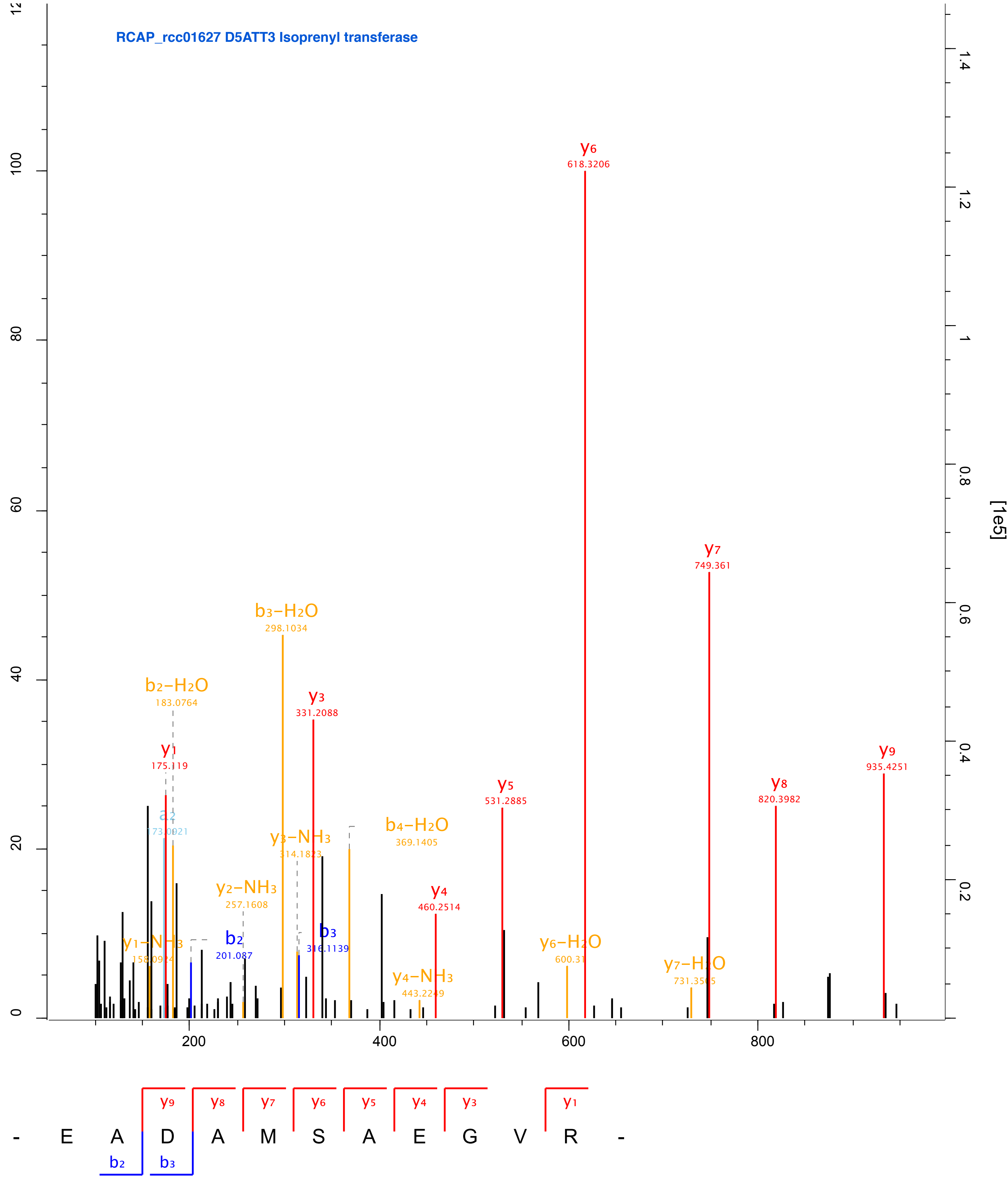
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-Set3-03 | 33767 | FTMS; HCD | 51.21 | 958.52 |



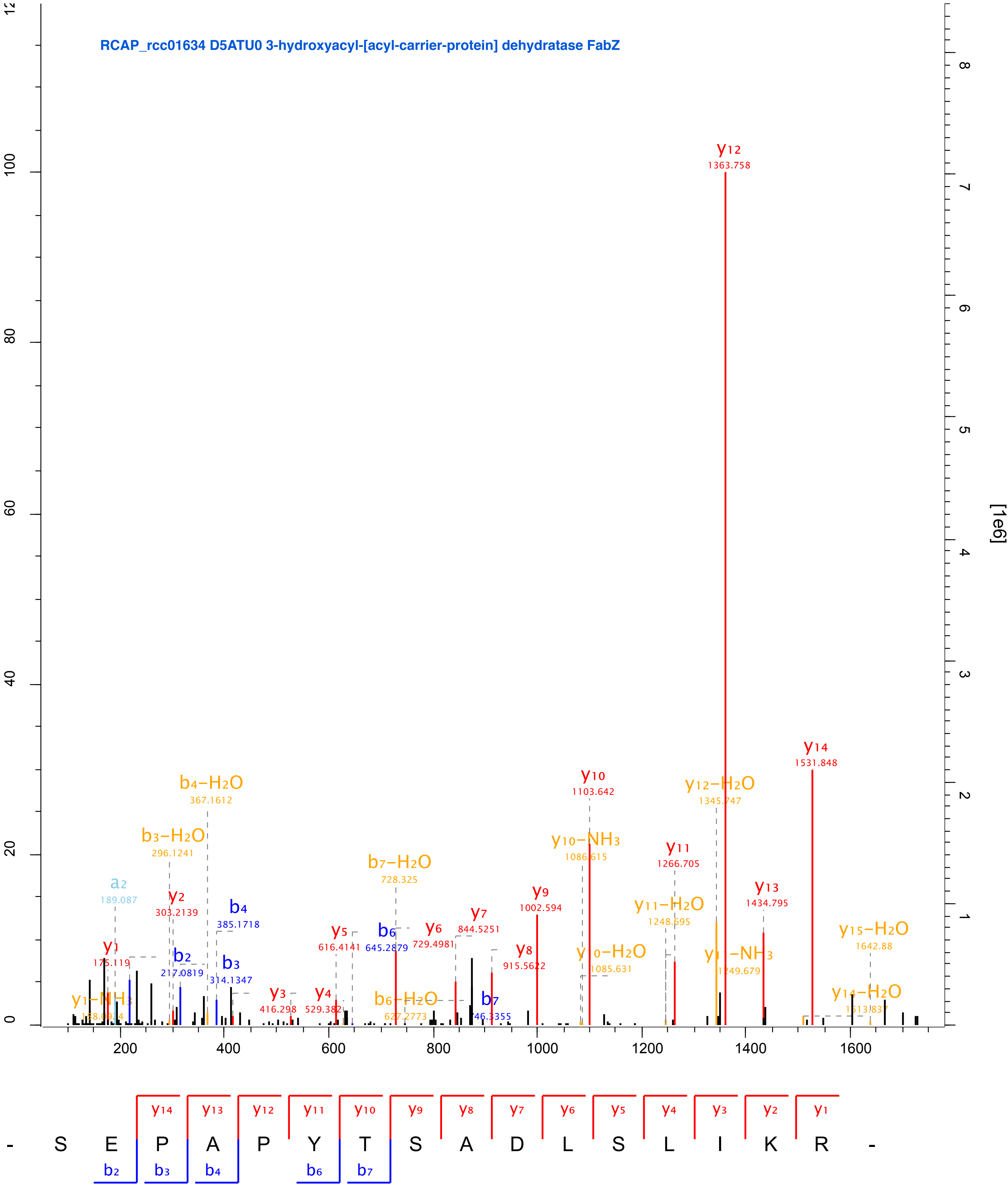
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-01_150512102711 | 67532 | FTMS; HCD | 124.92 | 1218.67 |



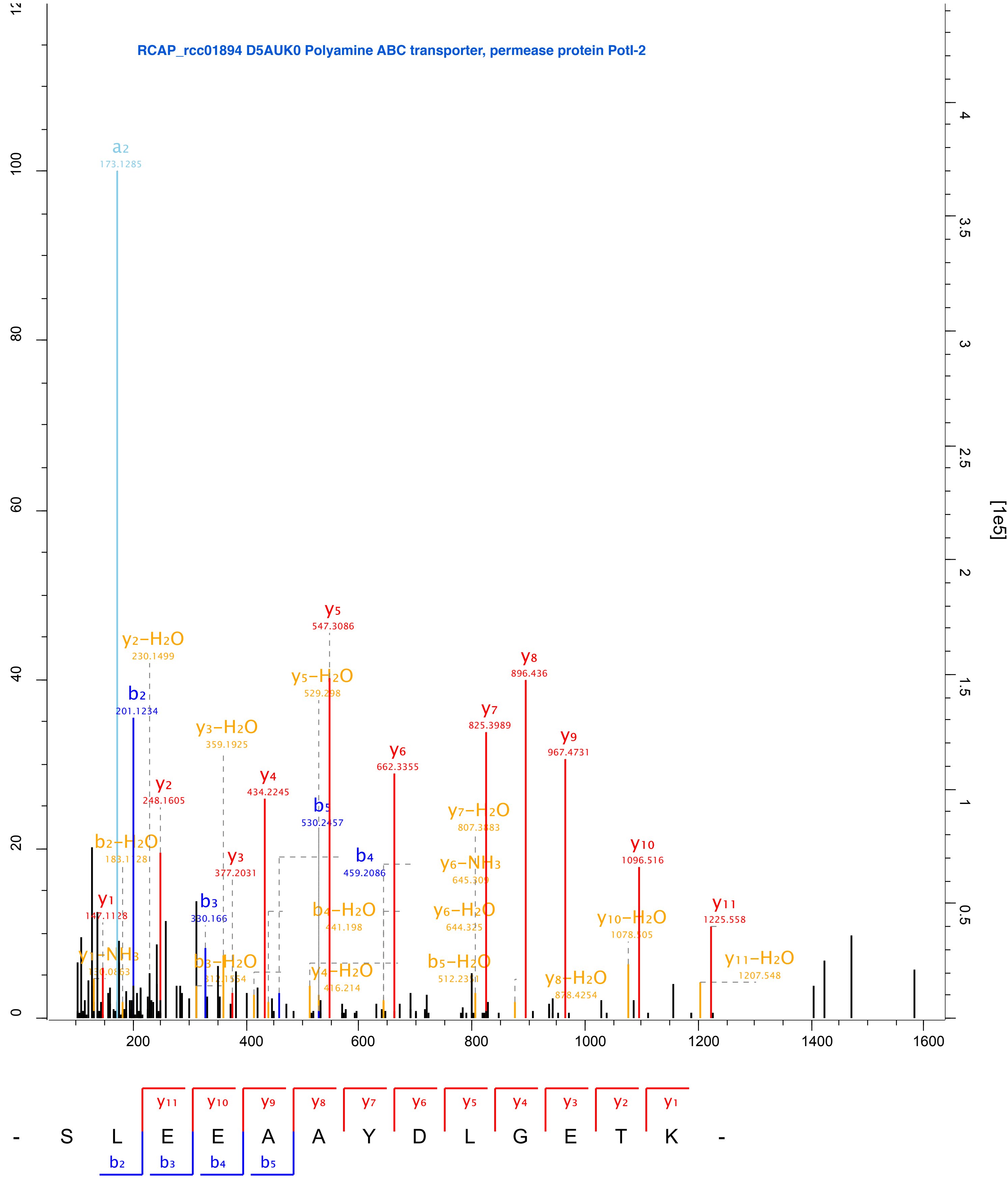
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-Set2-02 | 11862 | FTMS; HCD | 103.55 | 568.26 |



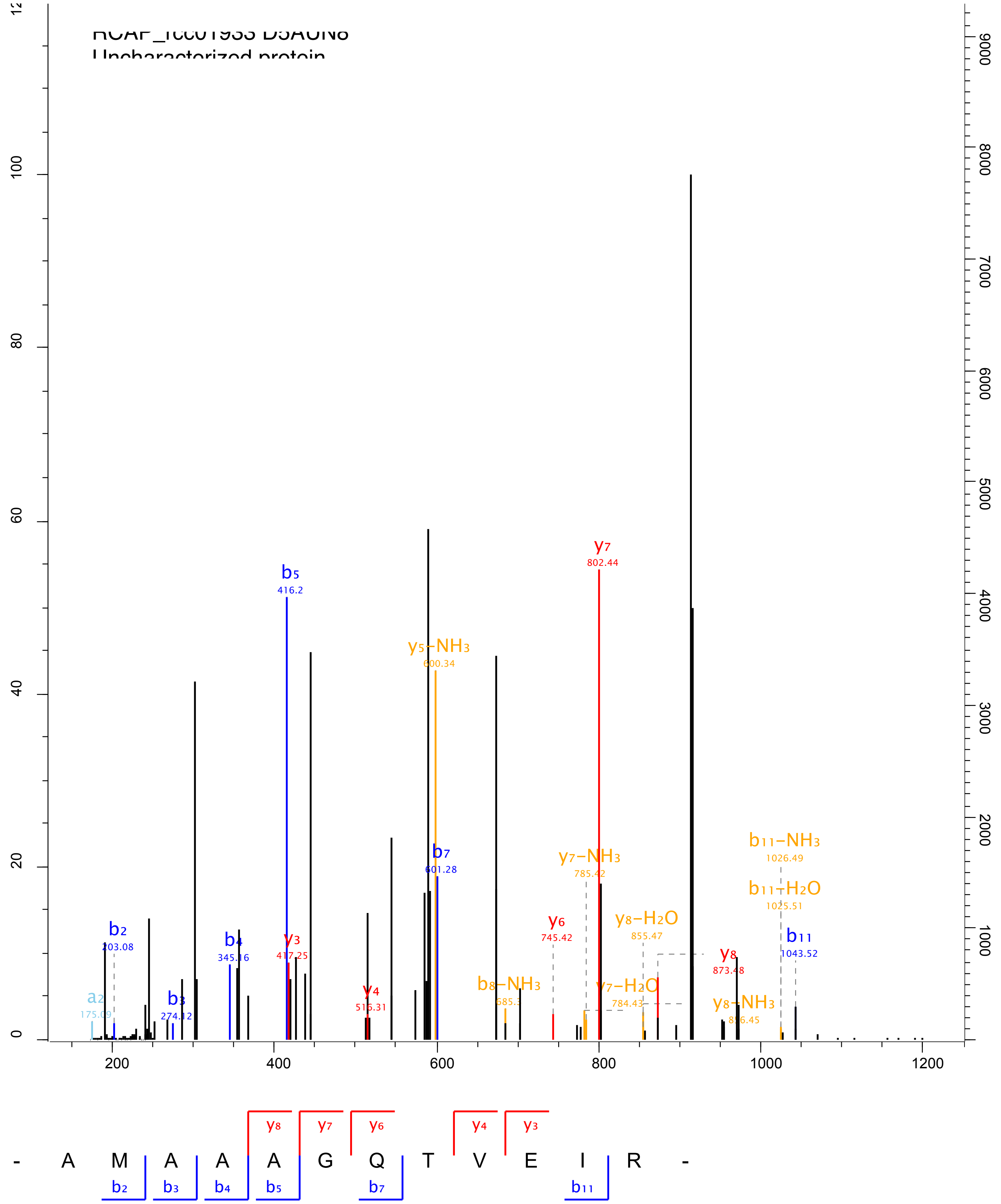
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-03 | 26579 | FTMS; HCD | 137.6 | 874.47 |



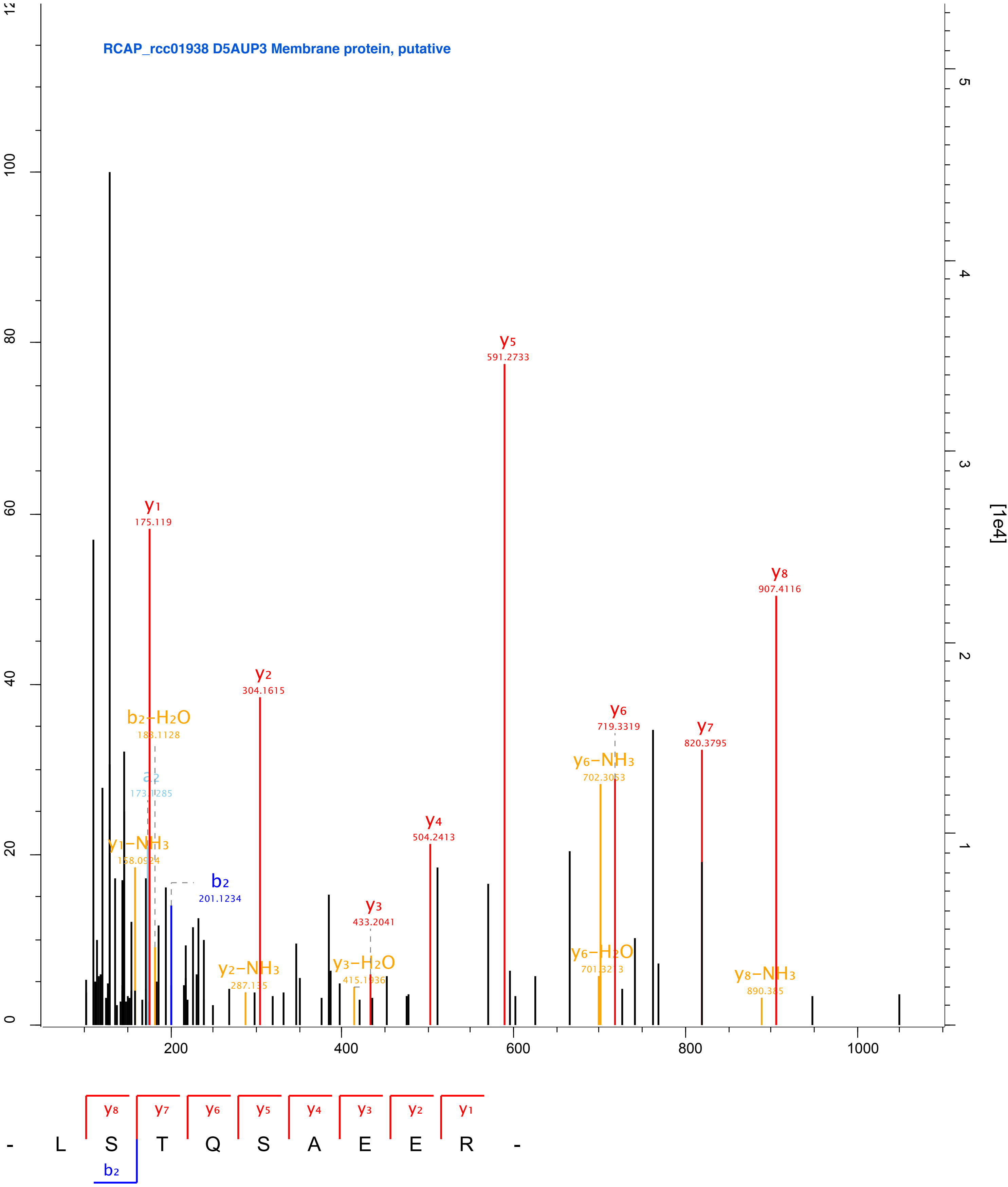
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-Set3-02 | 25432 | FTMS; HCD | 188.49 | 713.34 |


RCAP_rcc01894 D5AUK0 Polyamine ABC transporter, permease protein PotI-2

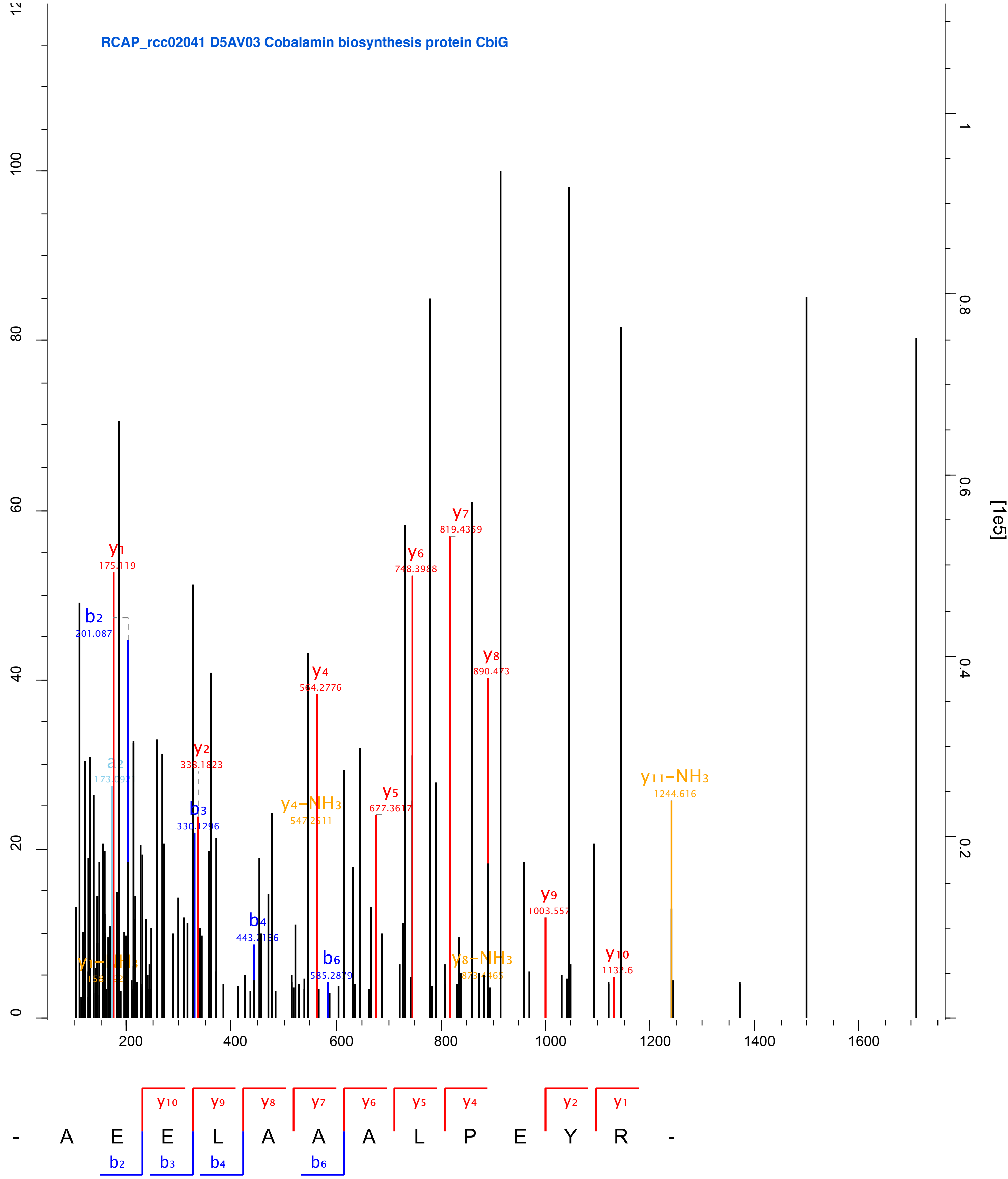
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| CTRL-minusCu-Biorep1-Run3 | 4792 | ITMS; CID | 83.65 | 609.82 |



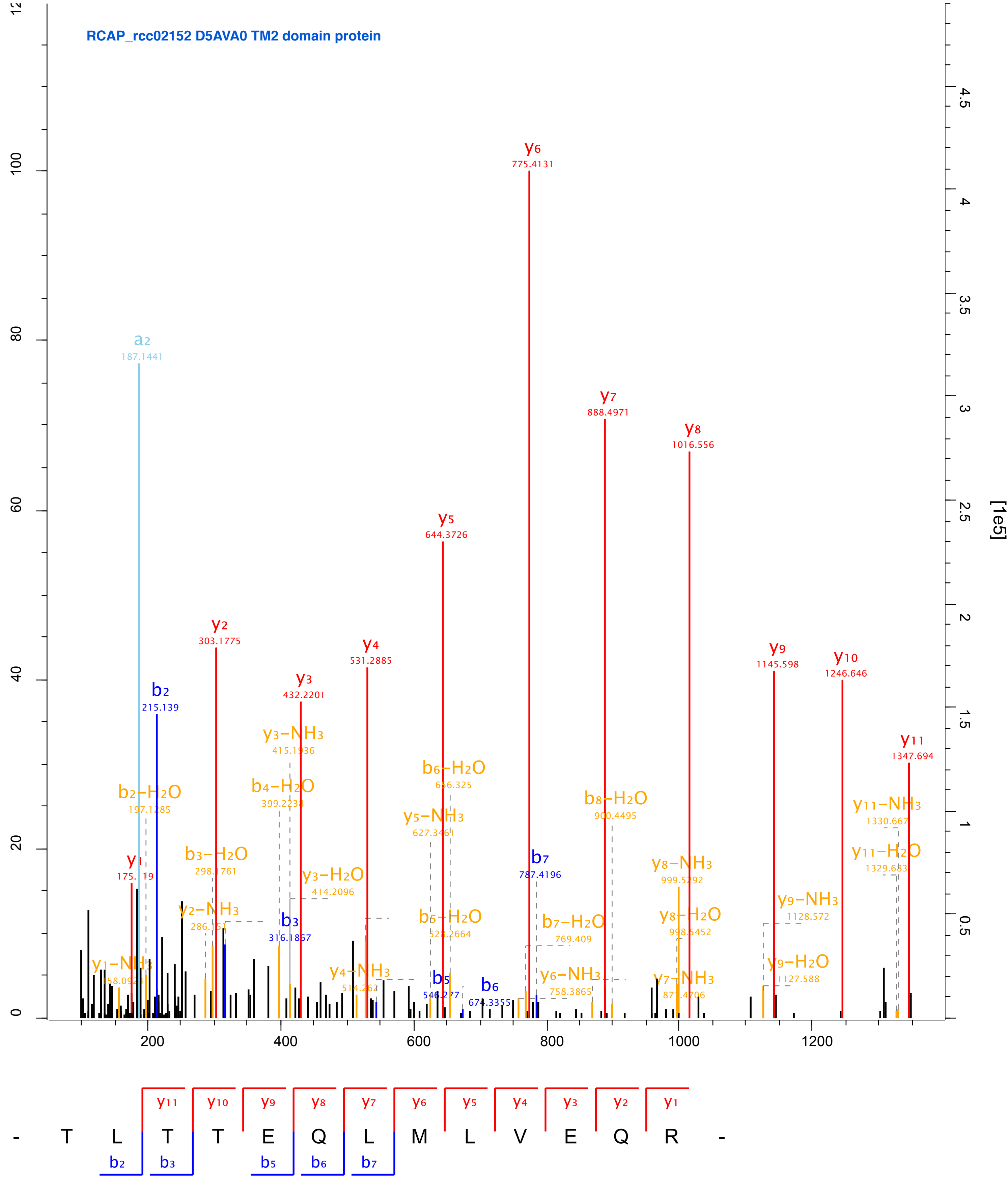
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-Set2-02 | 5857 | FTMS; HCD | 78.15 | 510.75 |



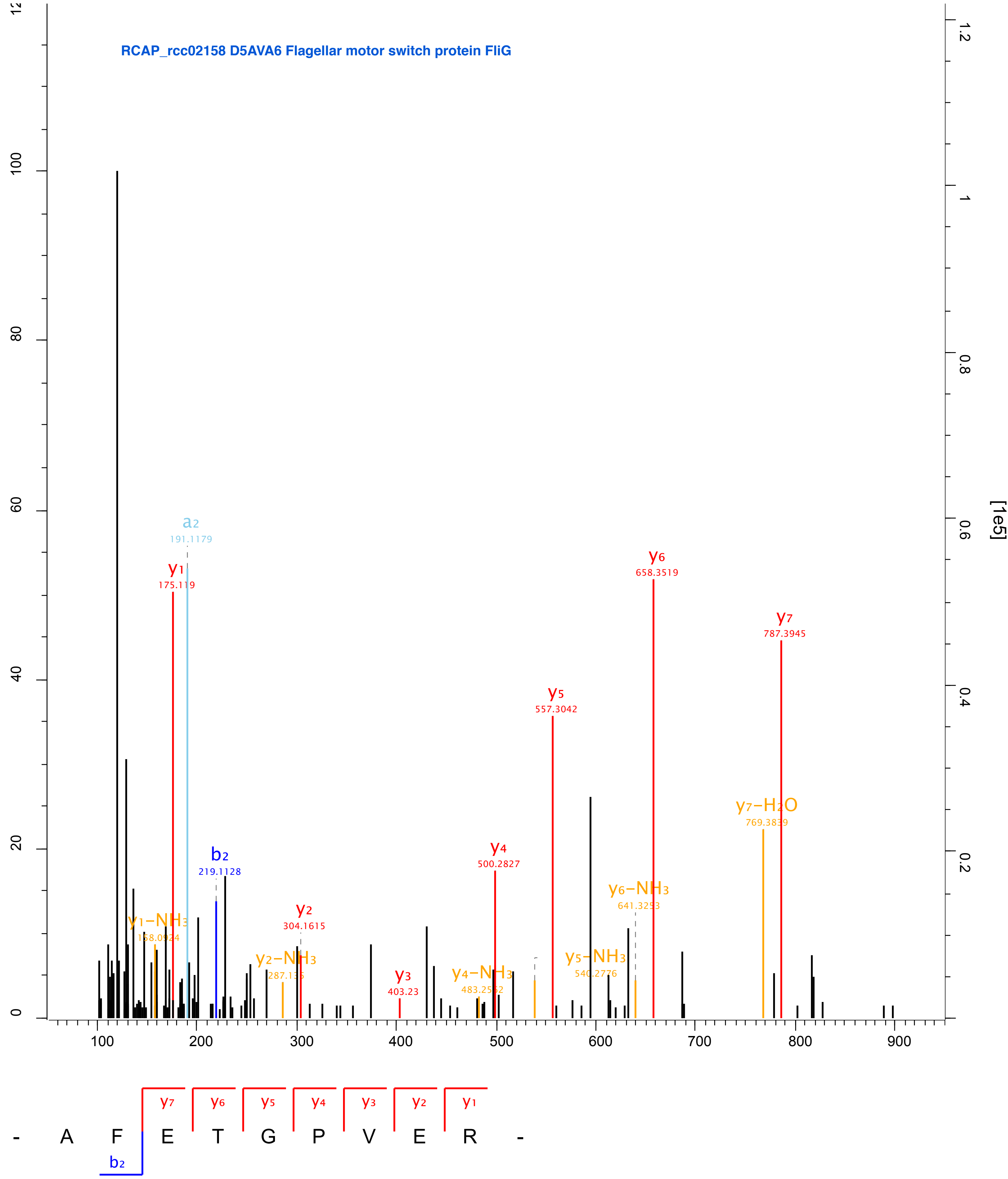
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-02 | 16394 | FTMS; HCD | 63.69 | 666.84 |



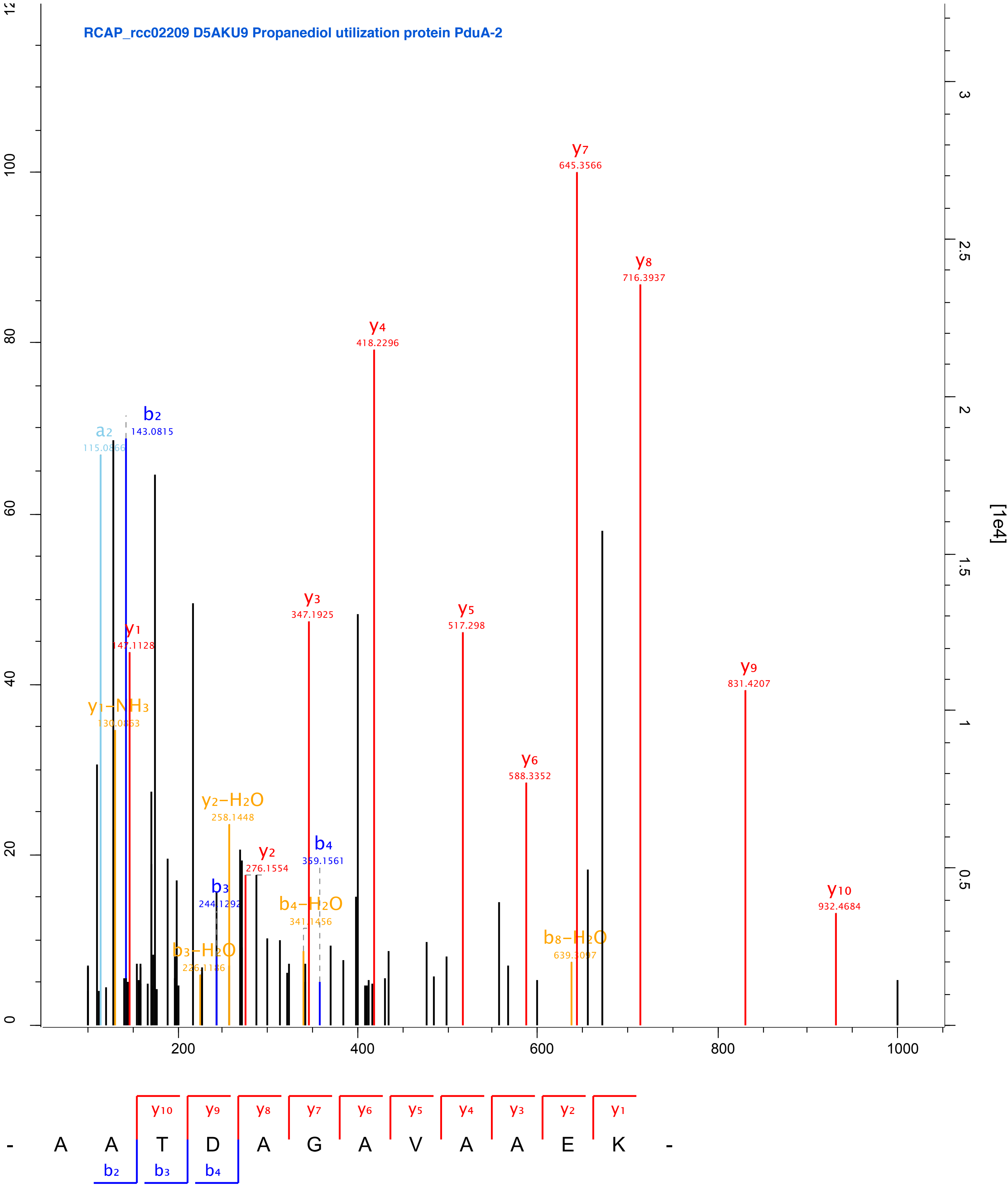
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-Set2-02 | 29313 | FTMS; HCD | 228.54 | 781.41 |



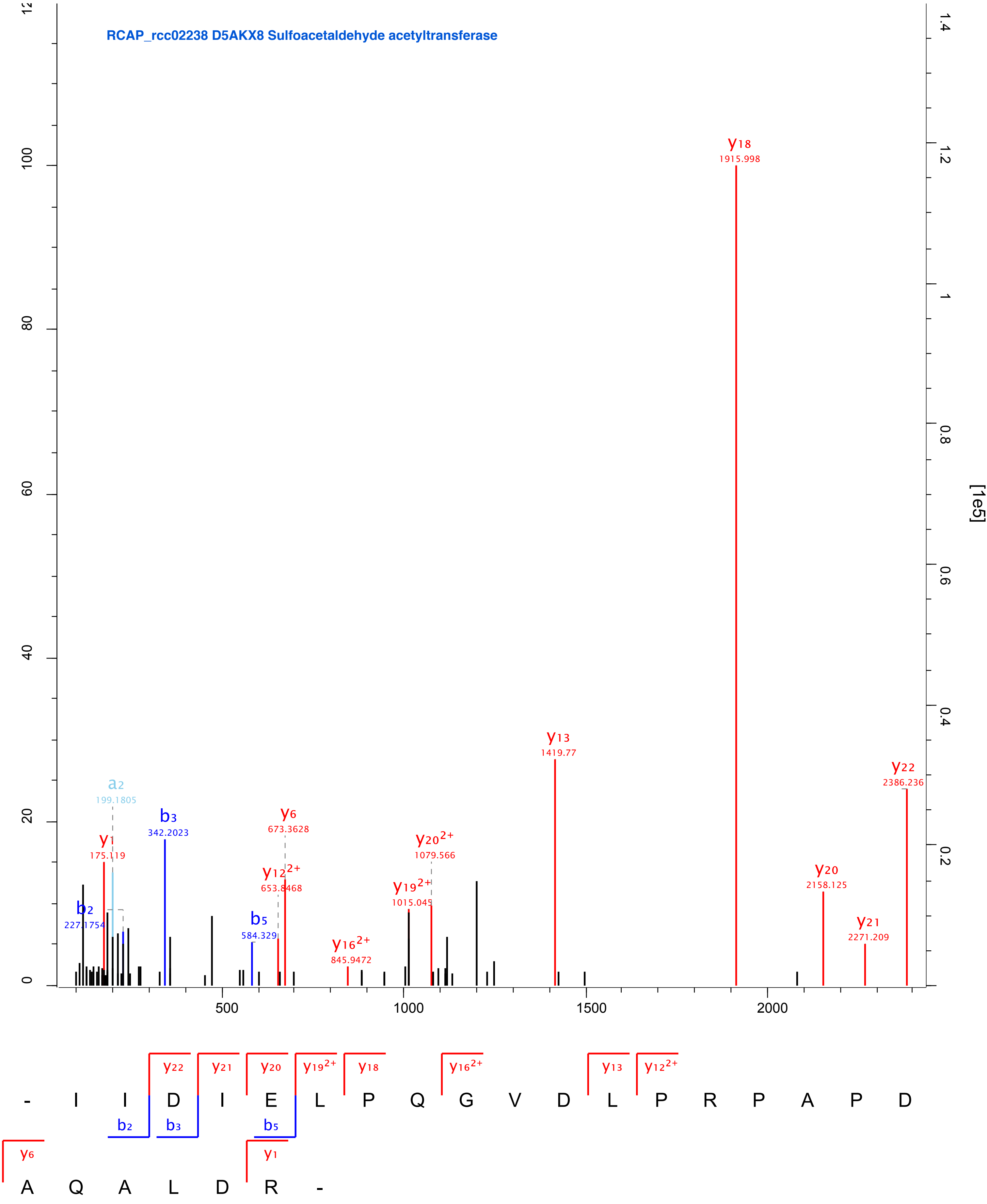
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-Set2-02 | 13402 | FTMS; HCD | 77.07 | 503.25 |



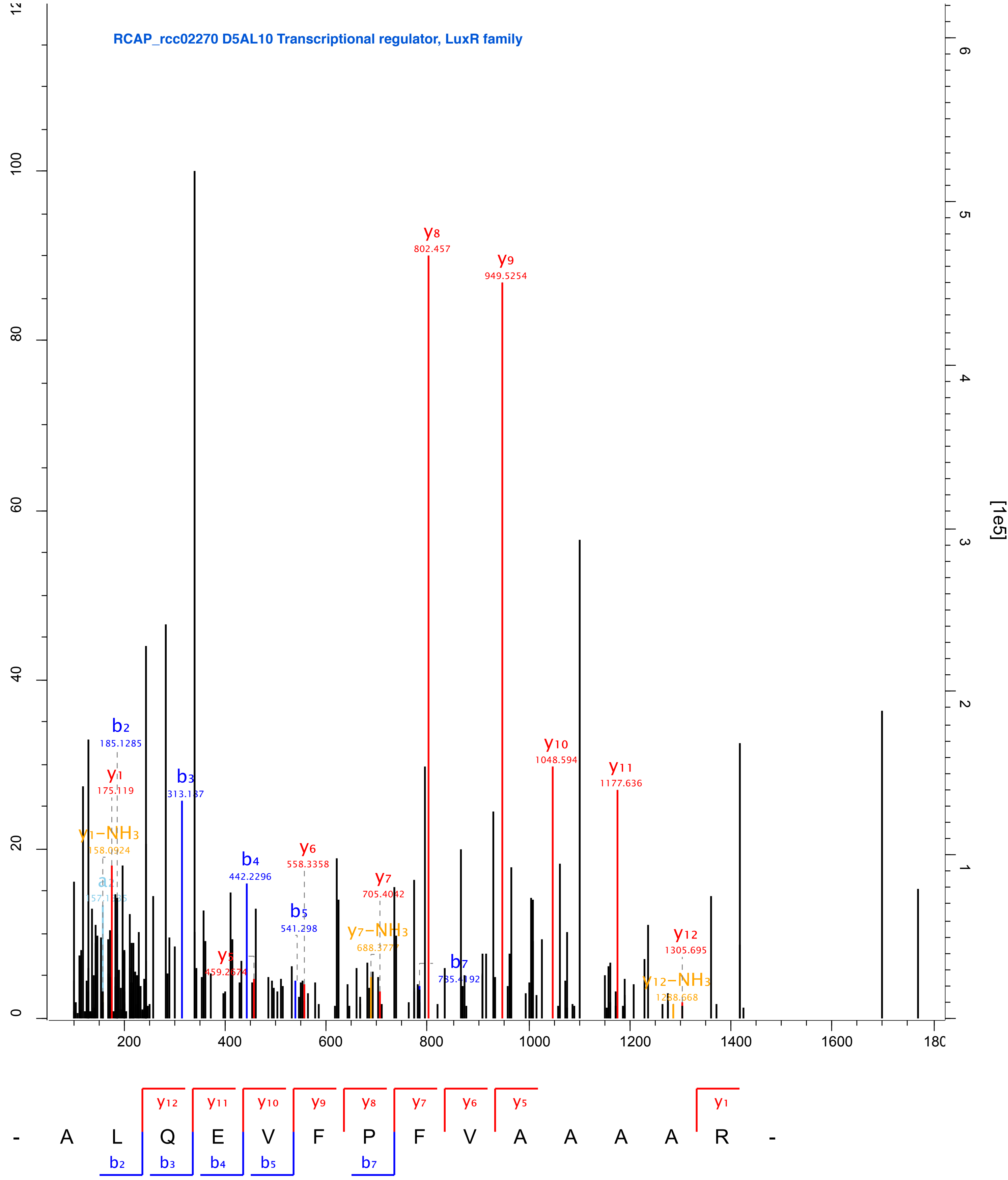
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-Set2-02 | 7165 | FTMS; HCD | 78.49 | 537.78 |



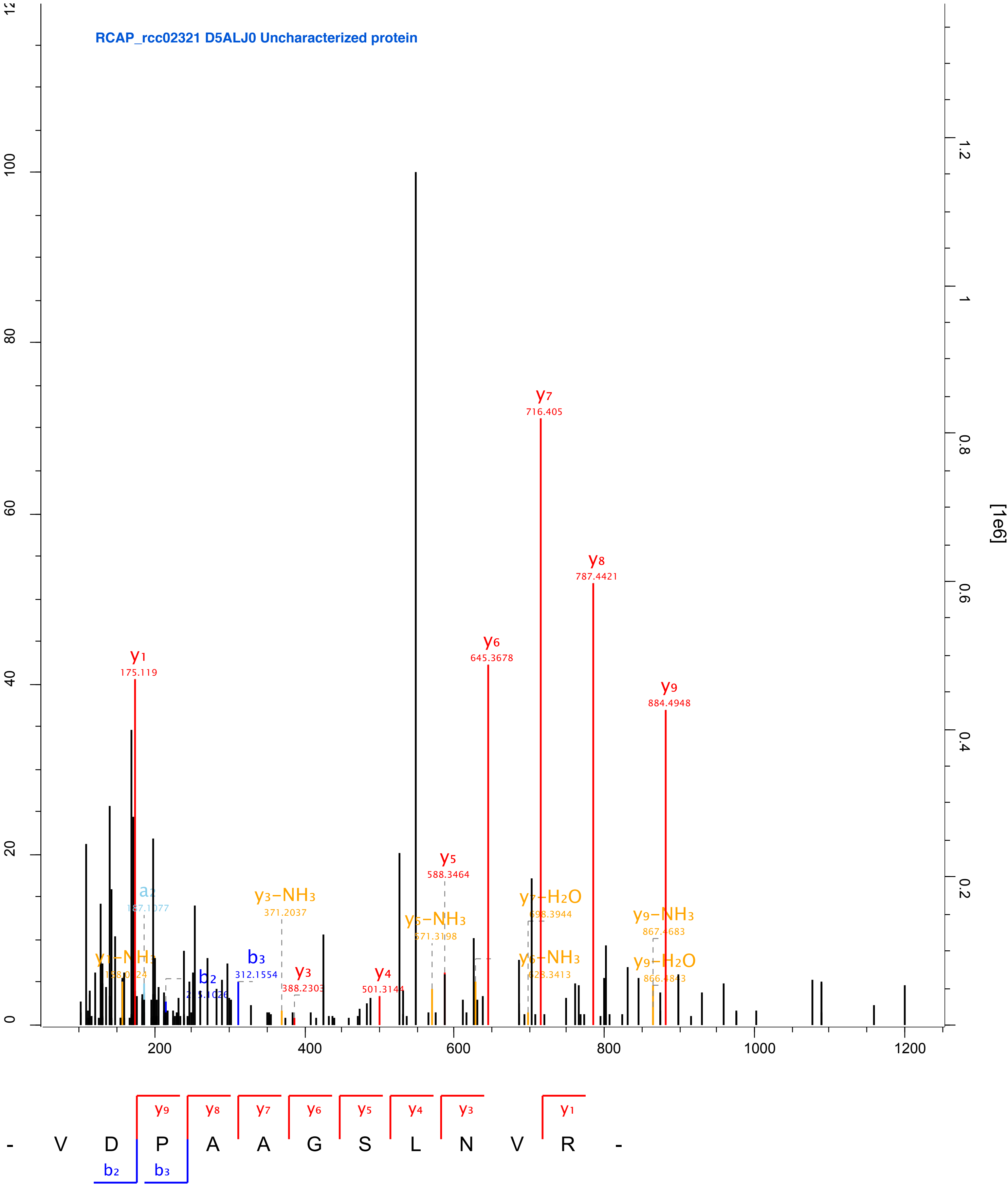
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-Set1-02 | 33696 | FTMS; HCD | 80.16 | 871.81 |



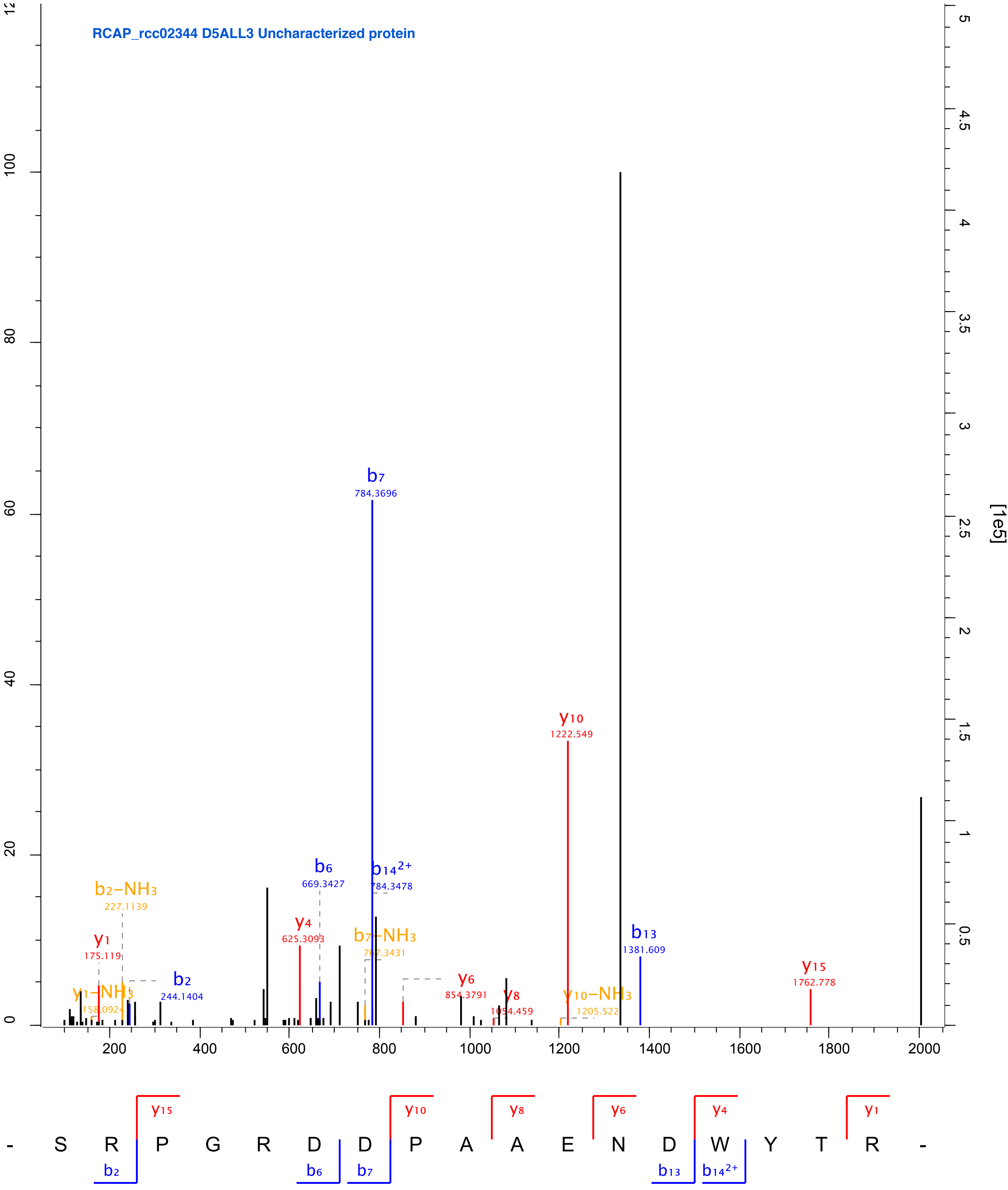
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-03 | 44457 | FTMS; HCD | 60 | 745.42 |



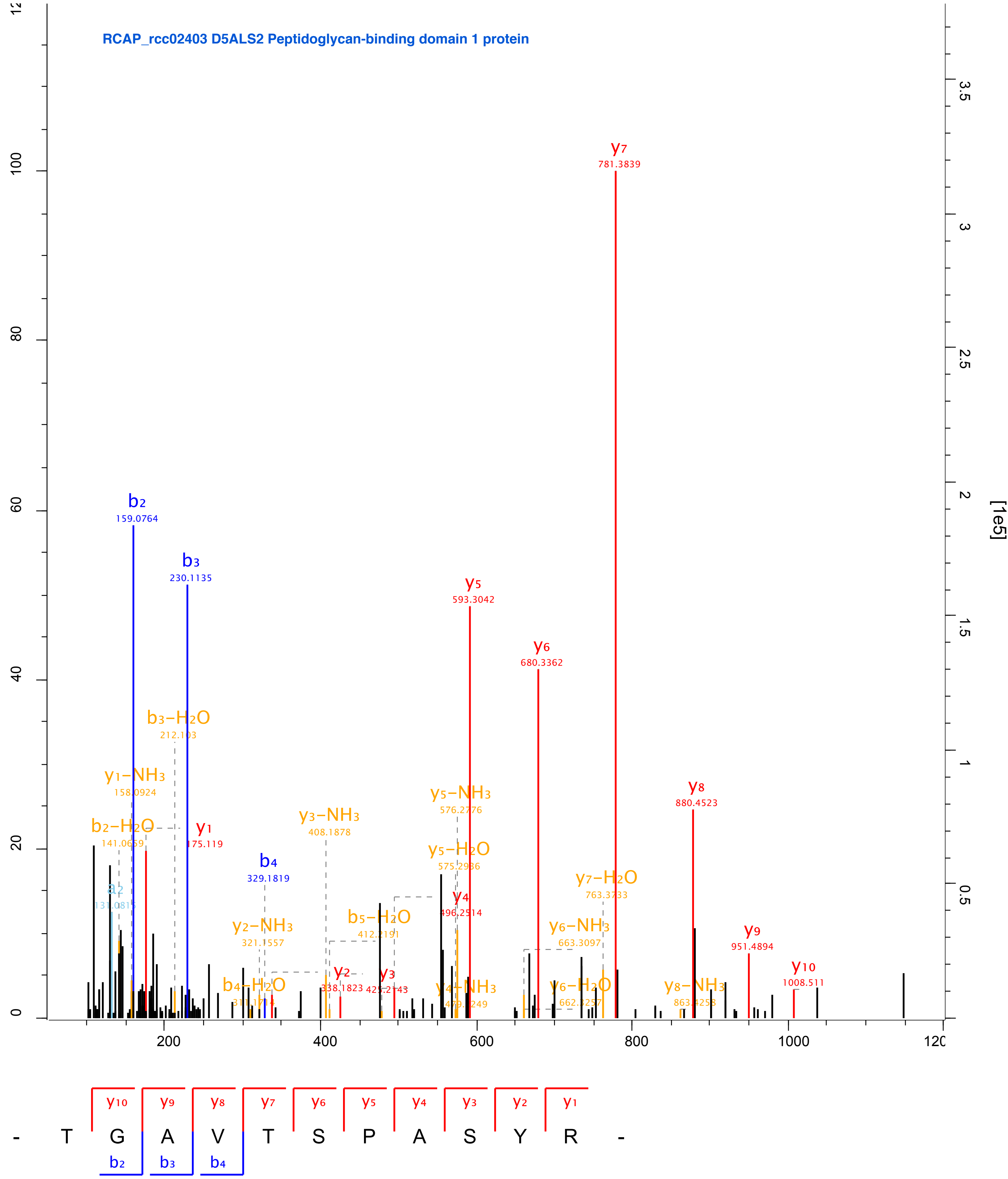
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-01_150512102711 | 14088 | FTMS; HCD | 66.96 | 549.8 |



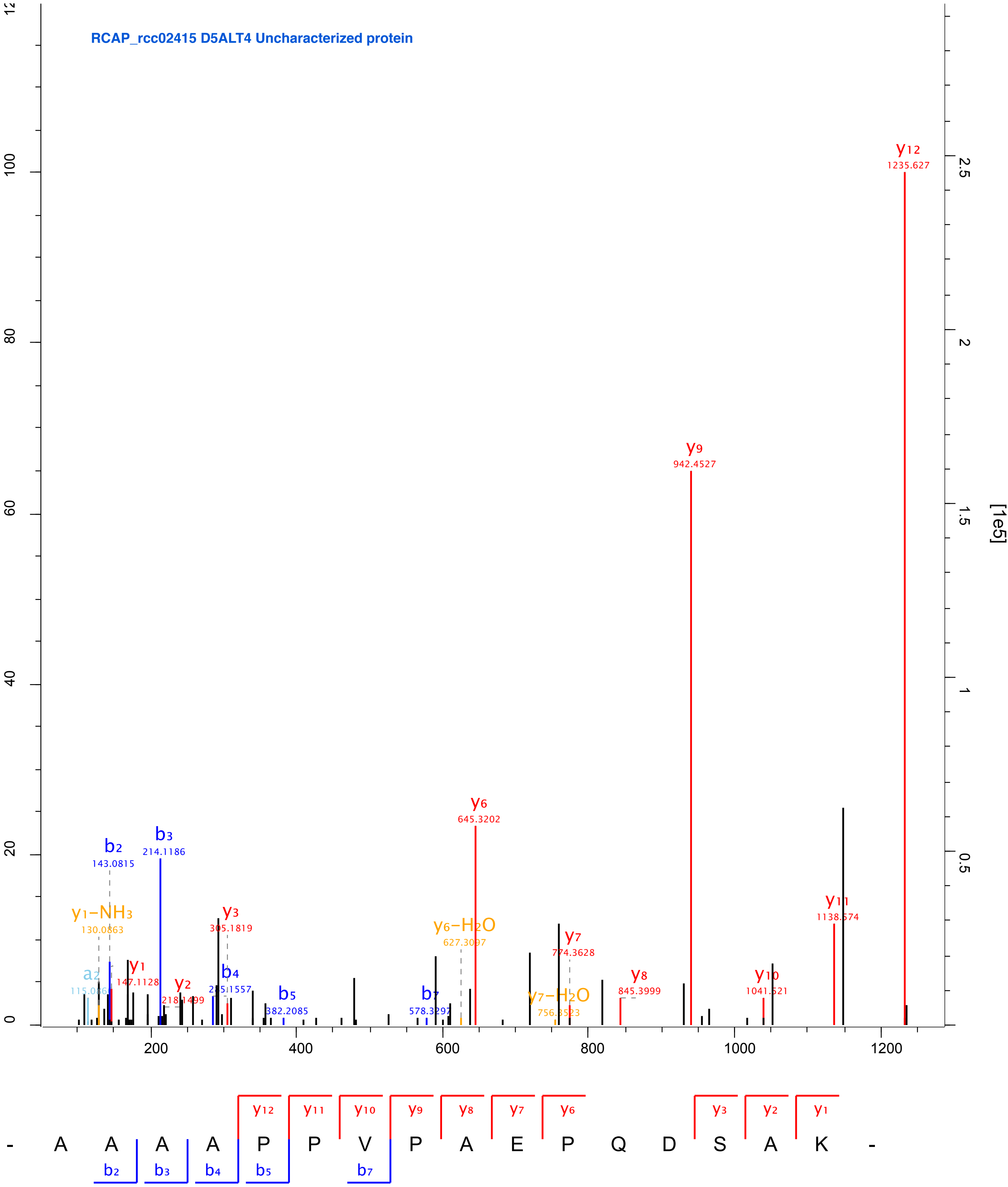
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-01 | 13431 | FTMS; HCD | 55.55 | 669.64 |



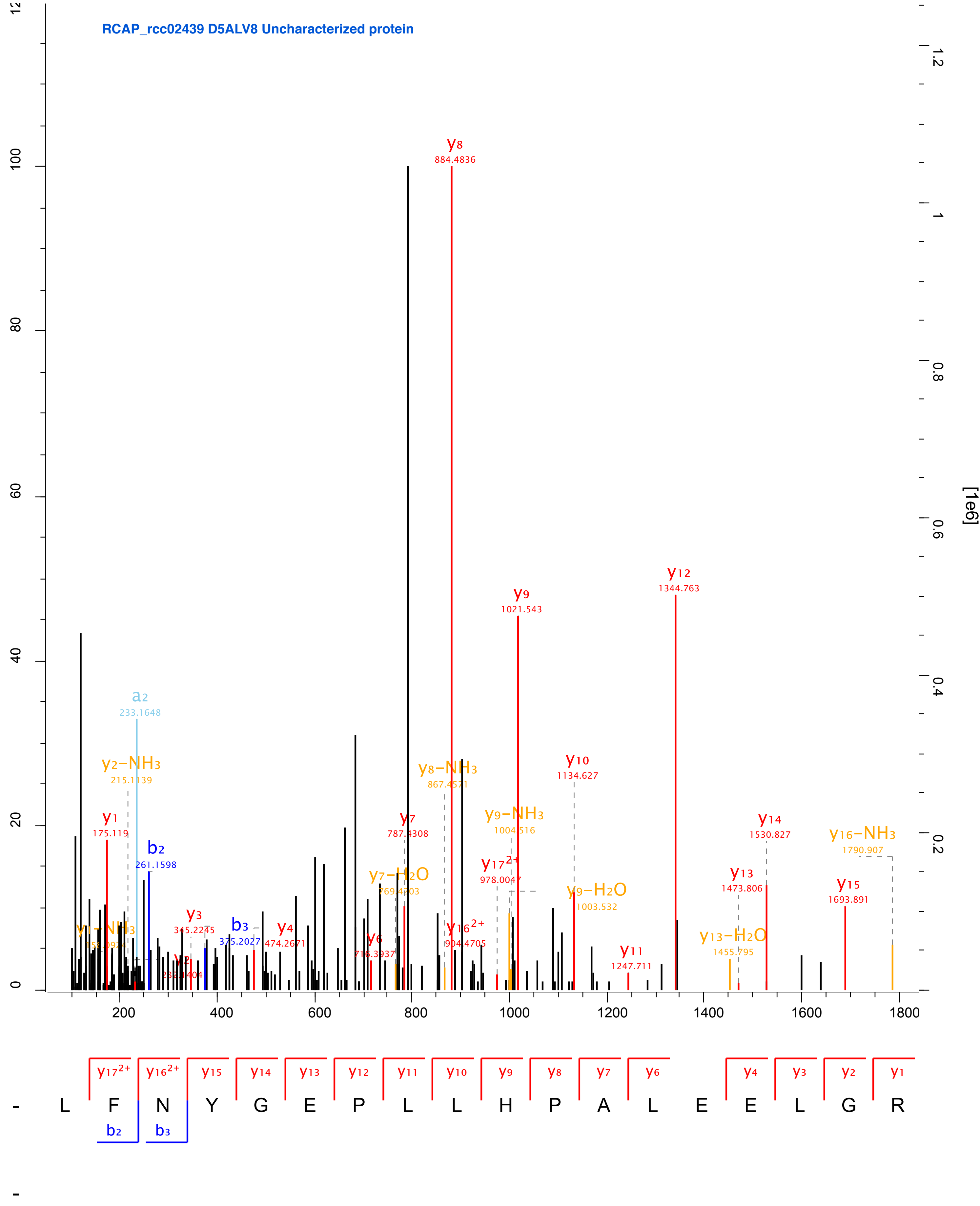
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-03 | 10695 | FTMS; HCD | 137.28 | 555.28 |



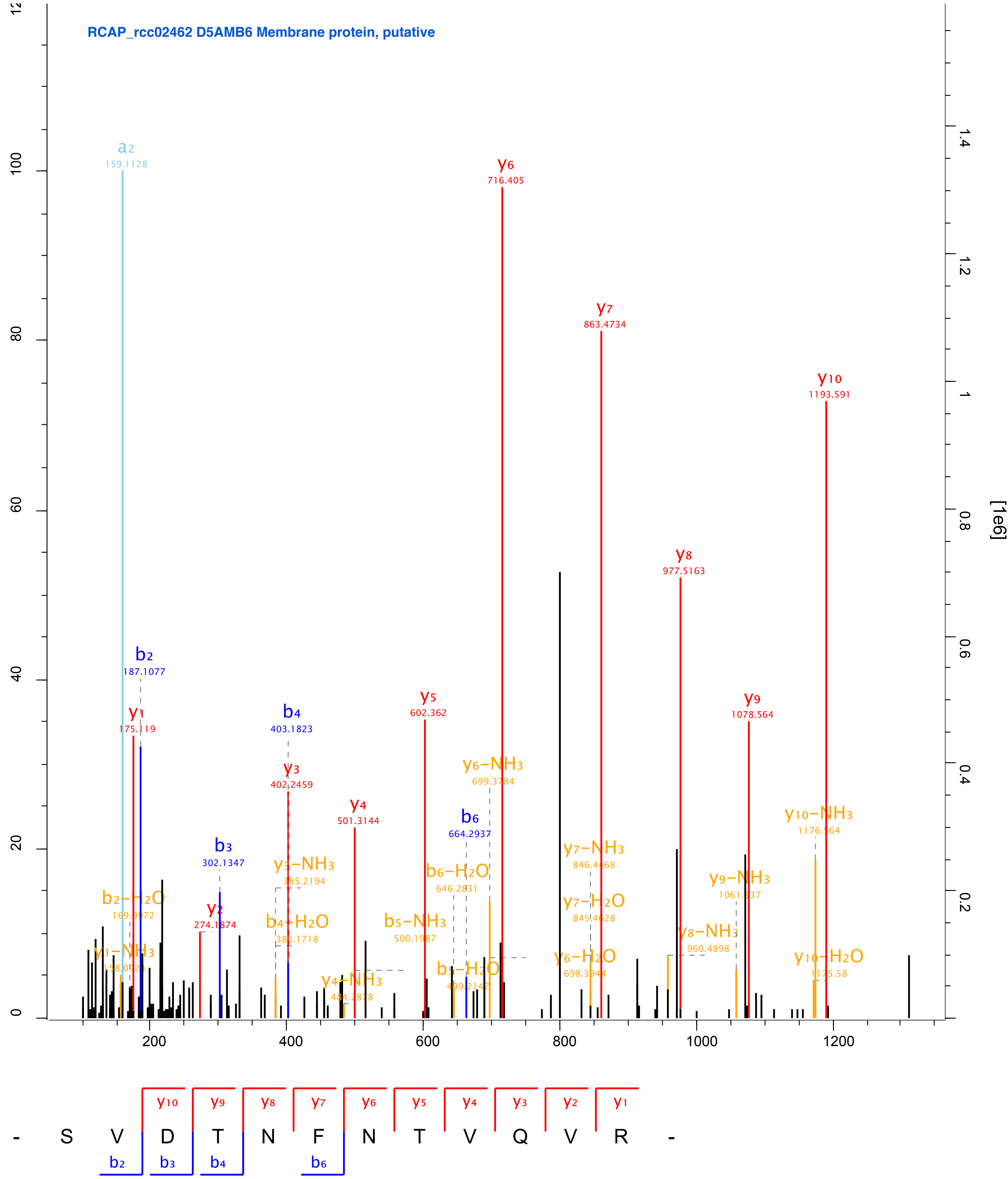
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-Set2-01 | 15756 | FTMS; HCD | 65.35 | 760.39 |



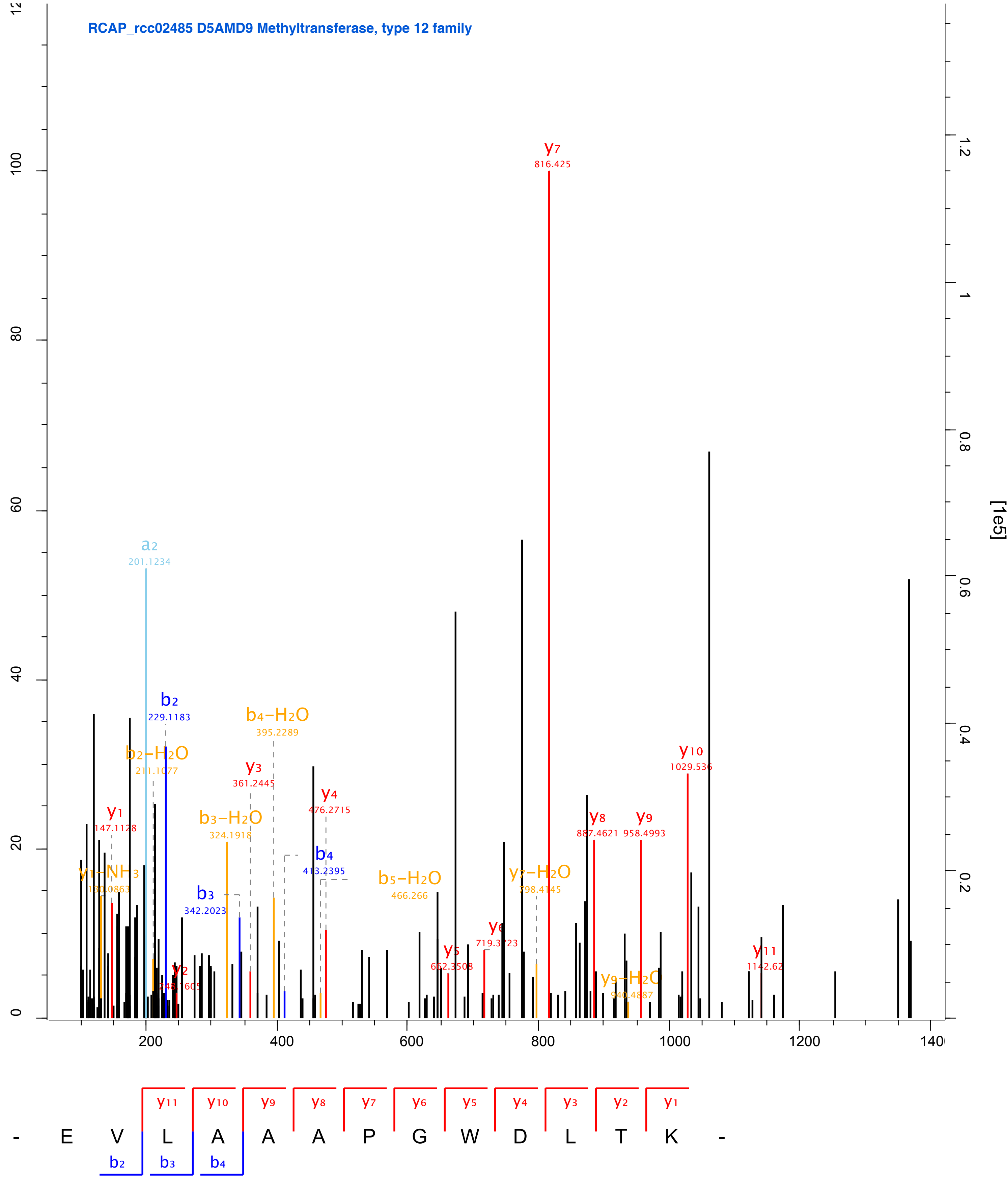
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-01_150512102711 | 44789 | FTMS; HCD | 66.89 | 690.03 |



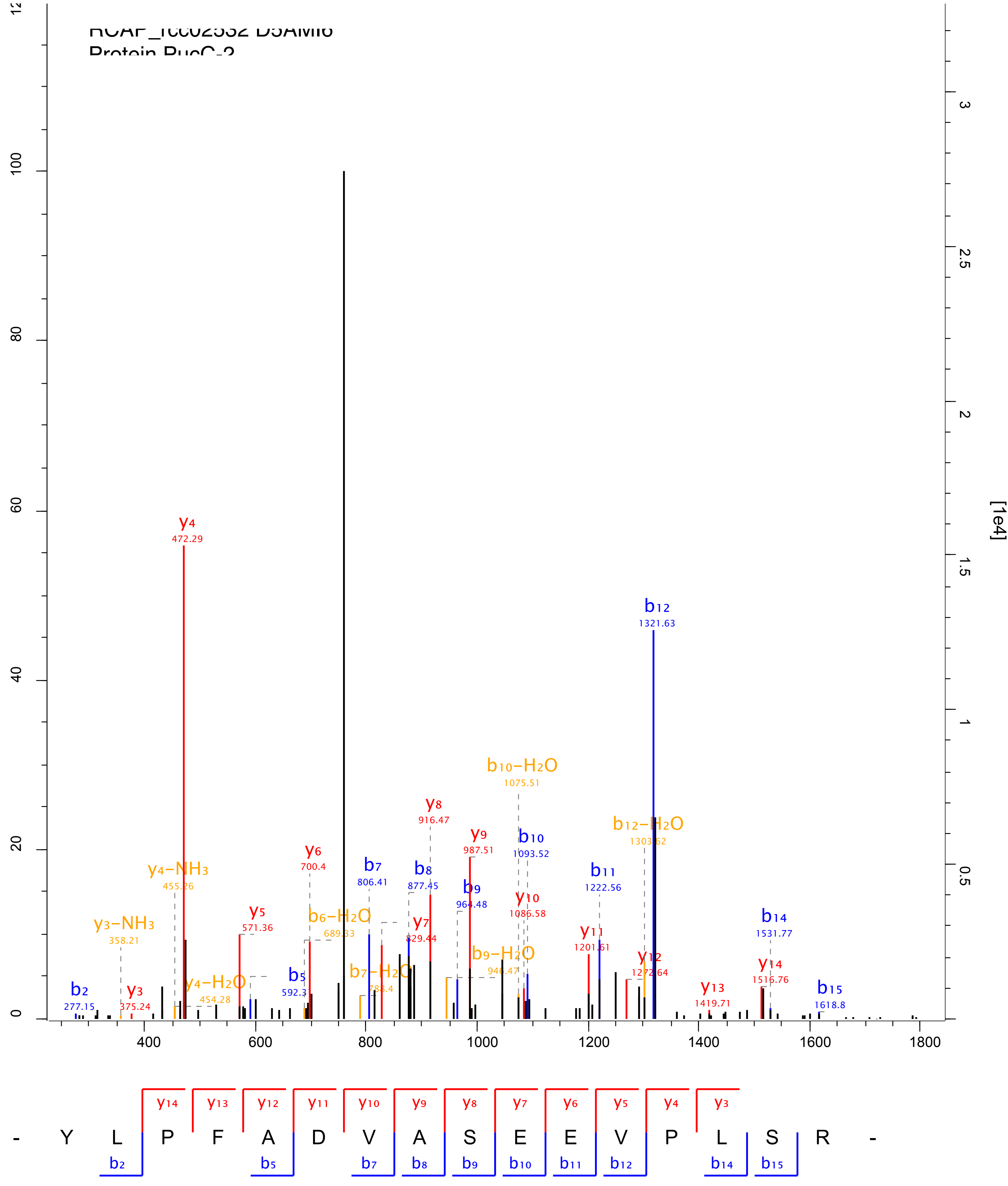
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-01_150512102711 | 19544 | FTMS; HCD | 192.26 | 690.35 |



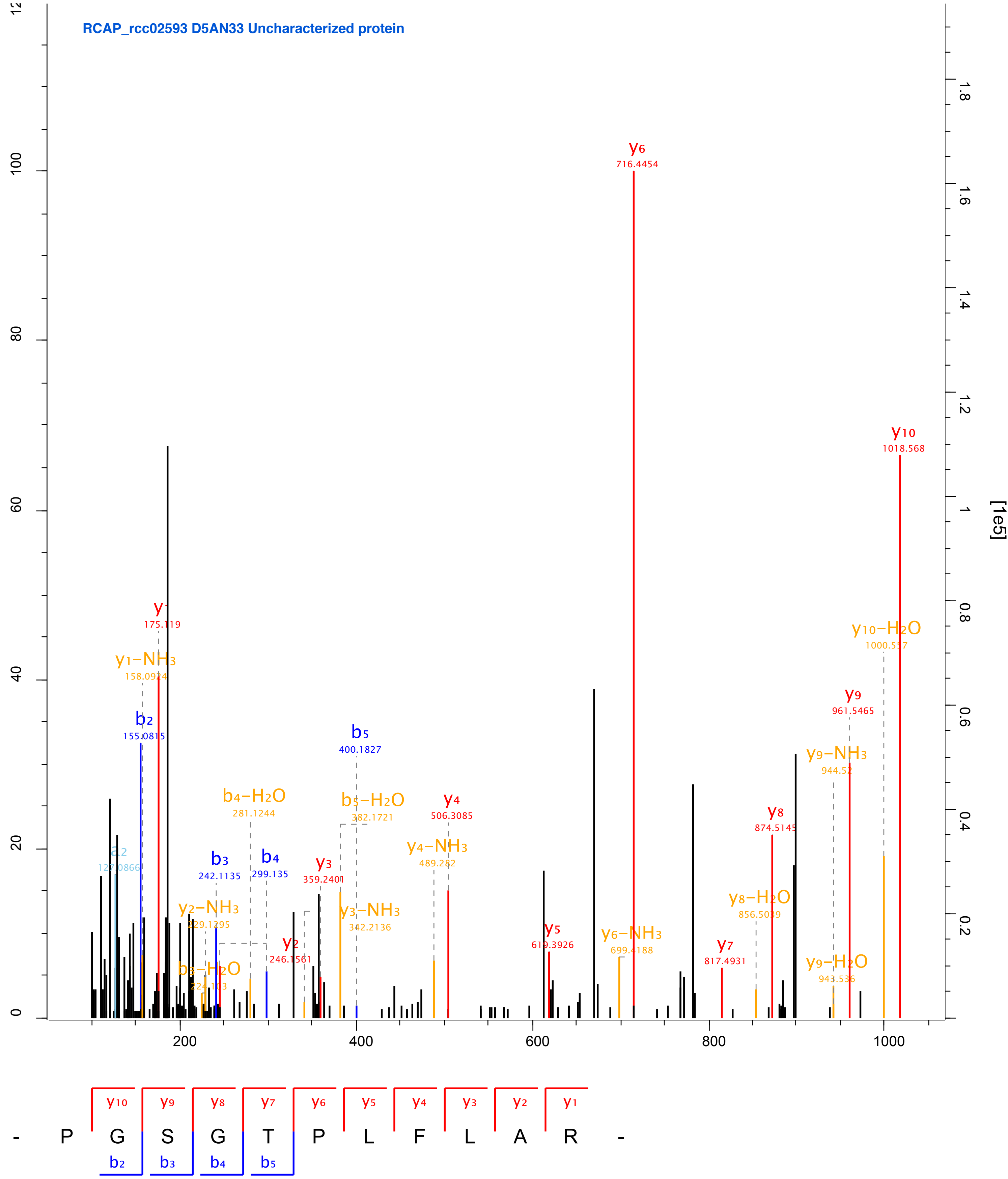
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-03 | 31175 | FTMS; HCD | 71.9 | 685.87 |



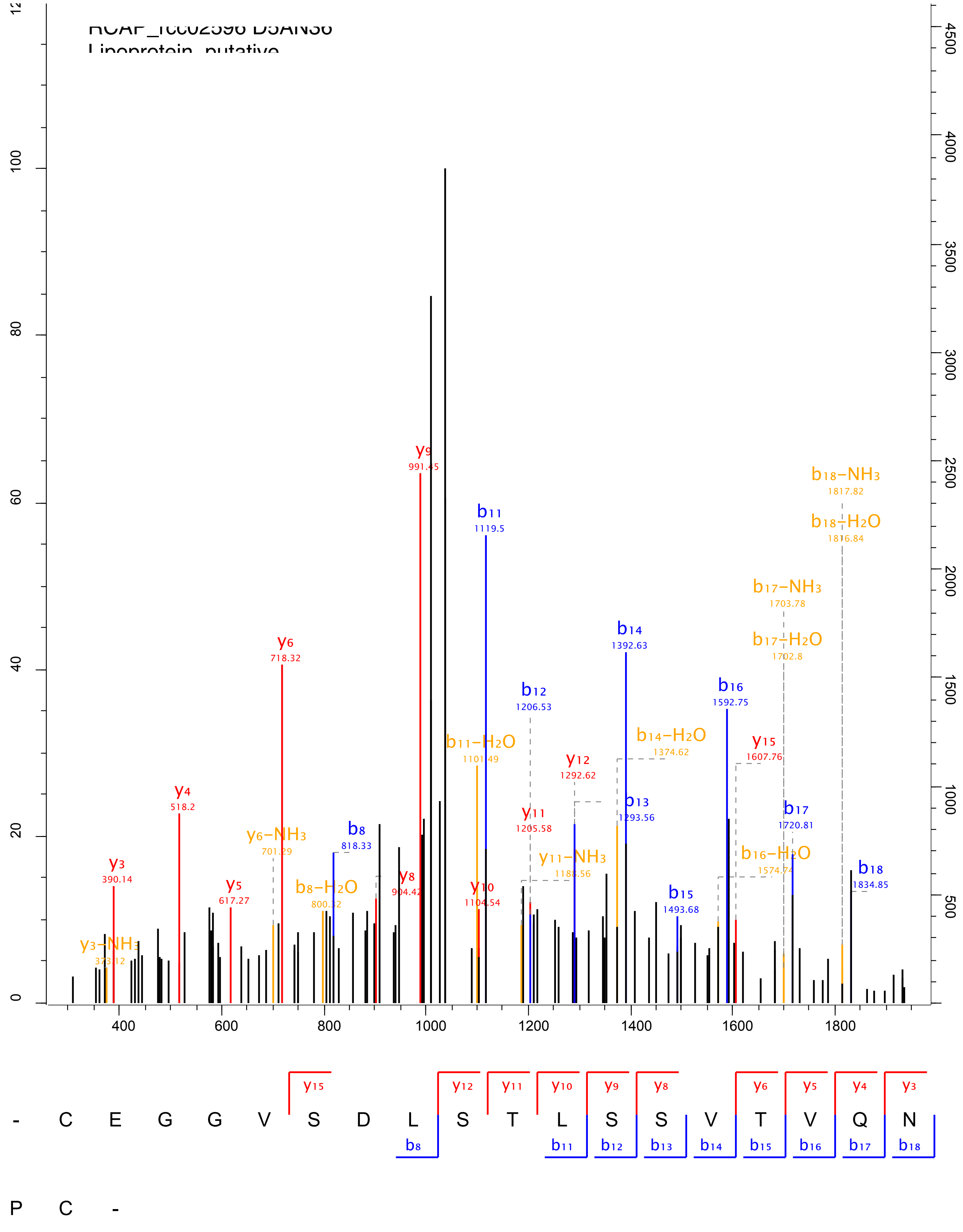
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| PlusCu-Biorep2-Run2 | 13179 | ITMS; CID | 164.48 | 897.46 |



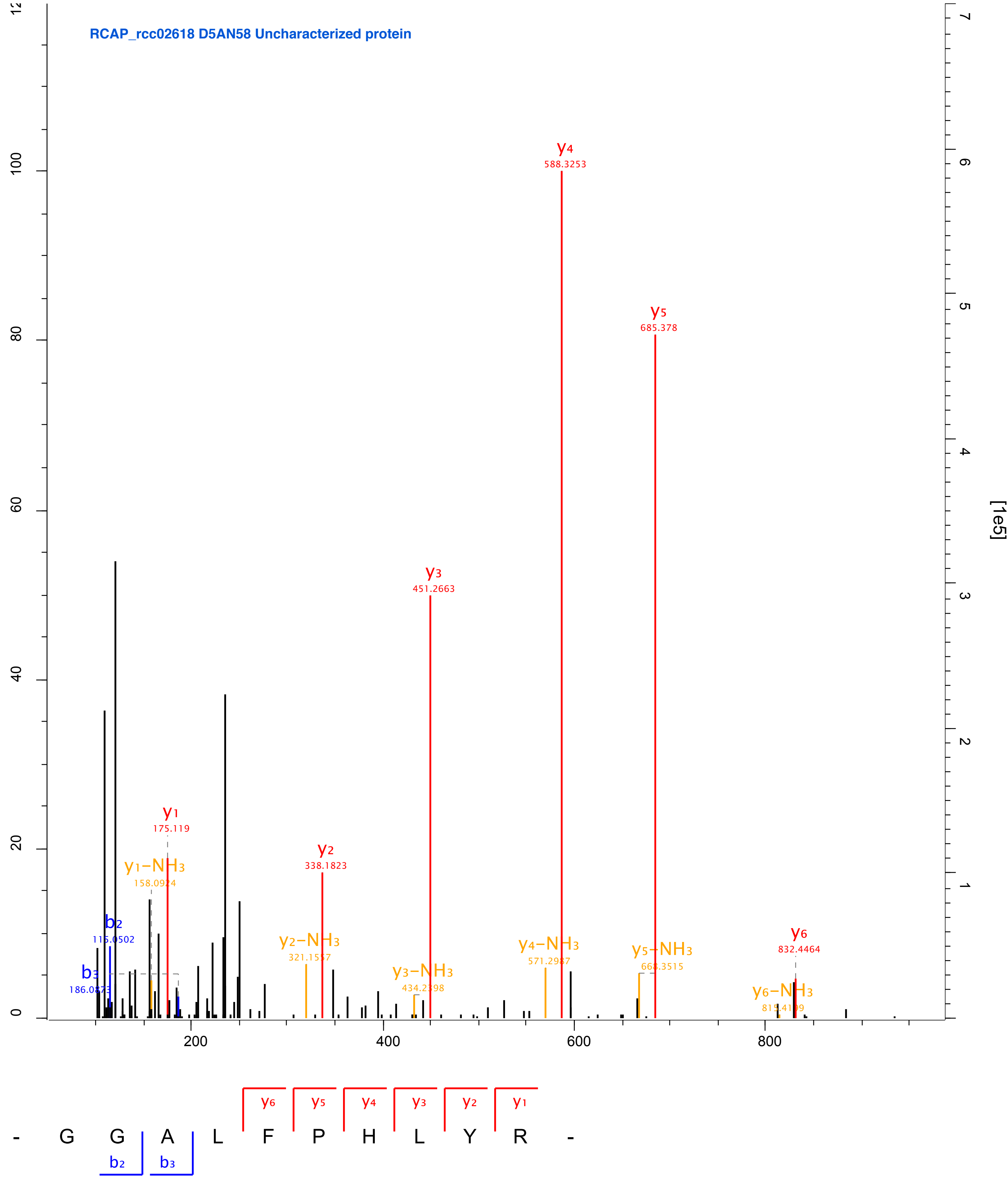
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-Set2-01 | 25672 | FTMS; HCD | 152.79 | 558.31 |



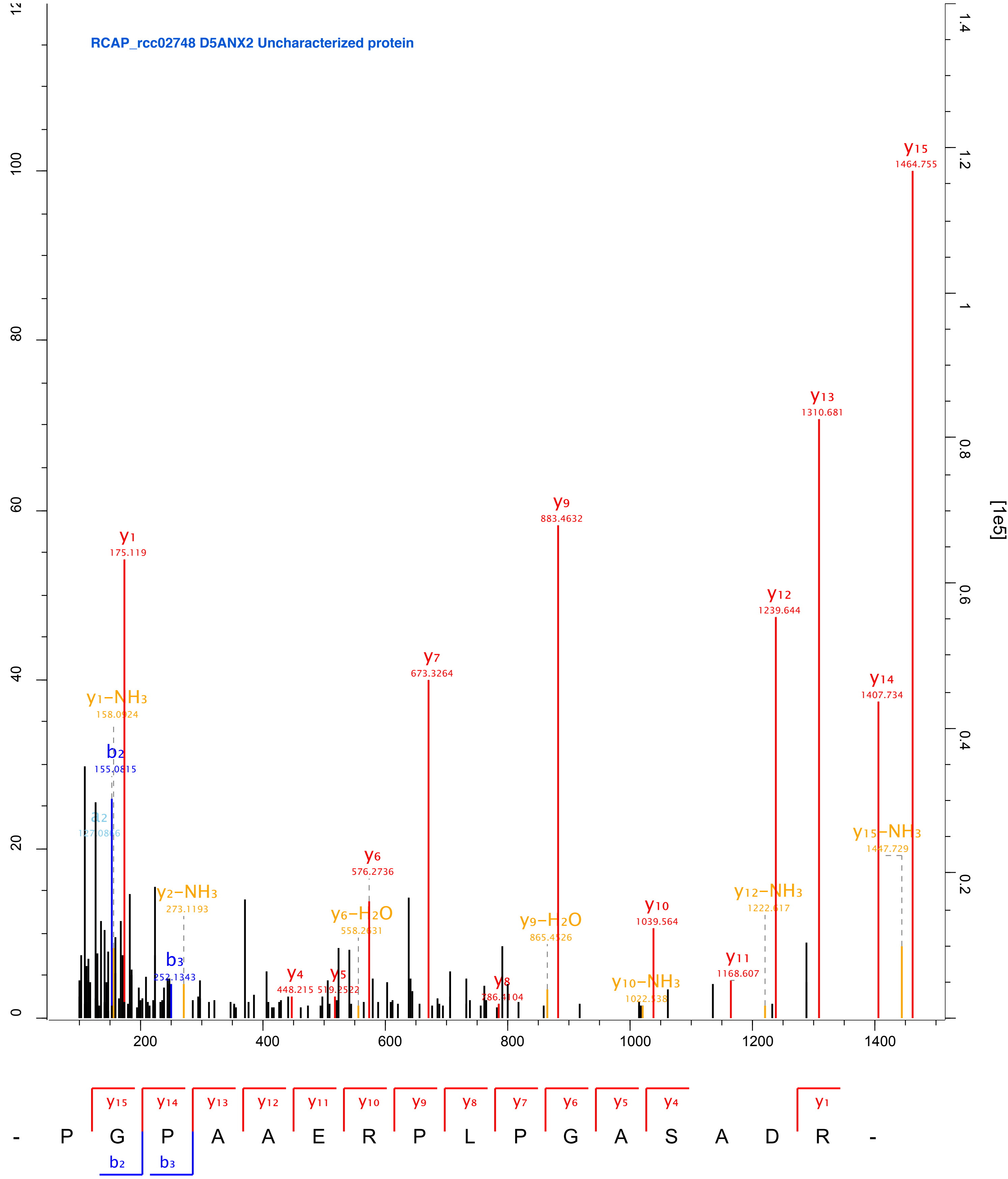
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| PlusCu-Biorep2-Run3 | 11287 | ITMS; CID | 170 | 1055.98 |



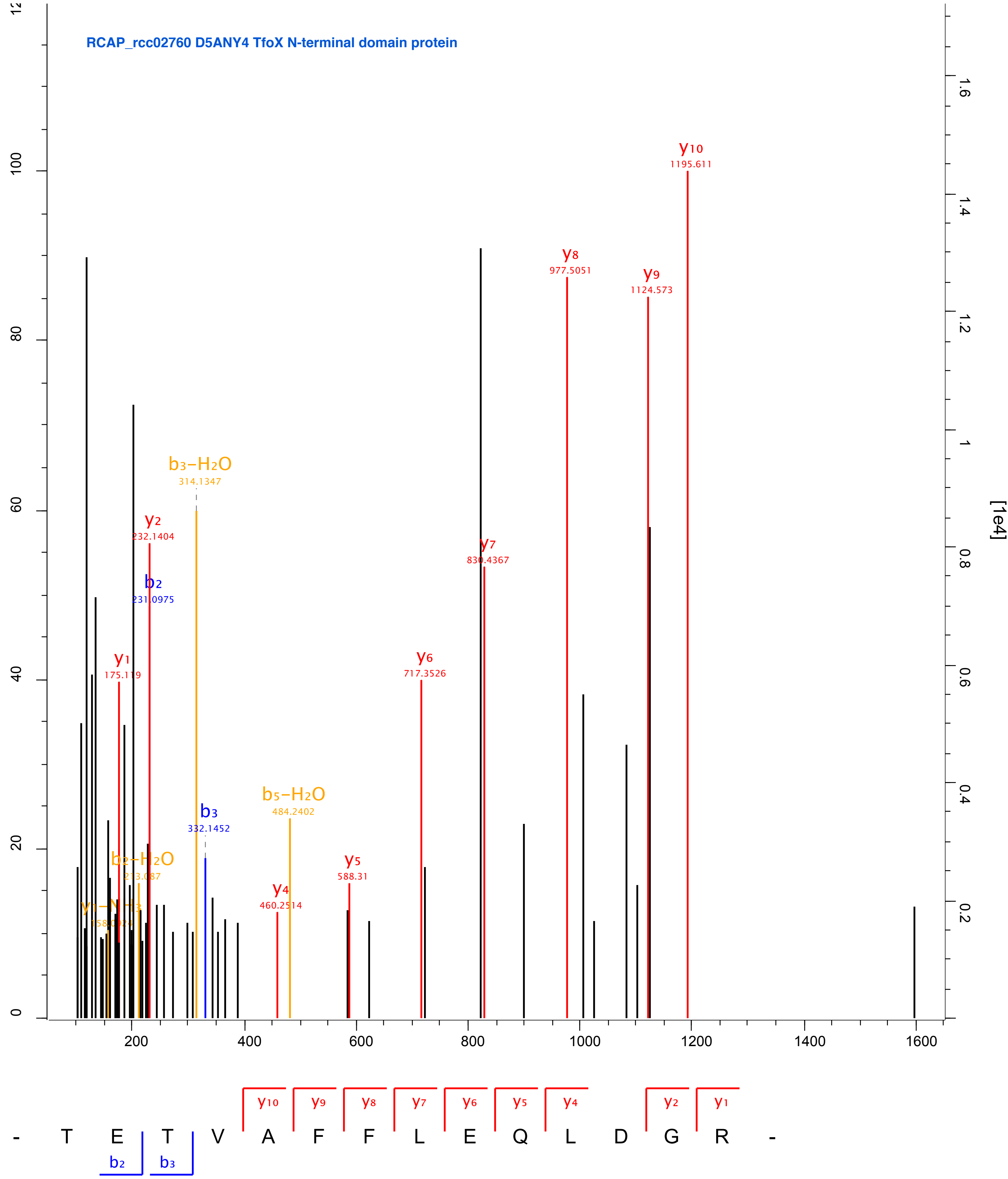
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-01 | 23501 | FTMS; HCD | 76.83 | 377.54 |



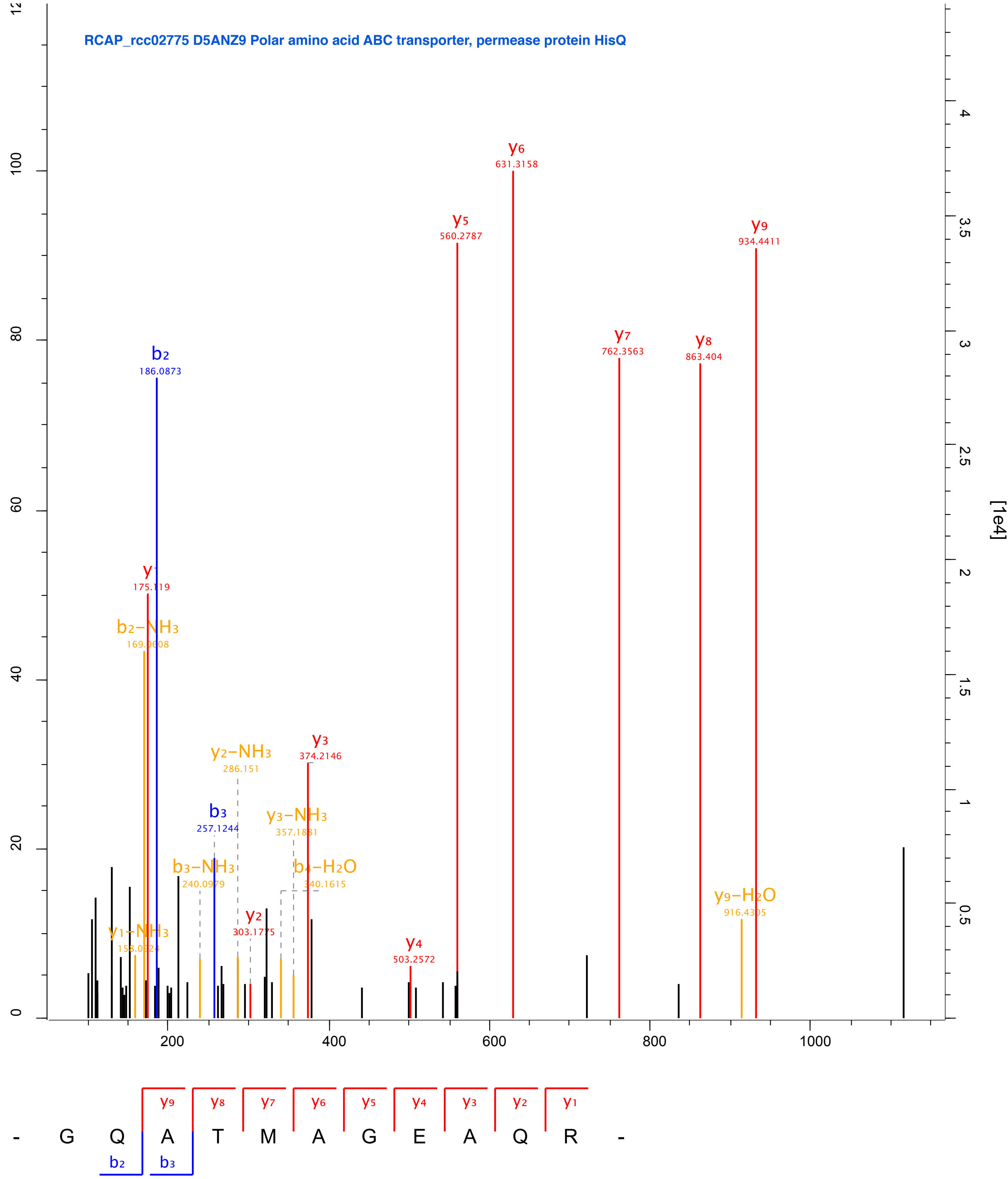
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-Set2-01 | 12688 | FTMS; HCD | 68.58 | 521.27 |



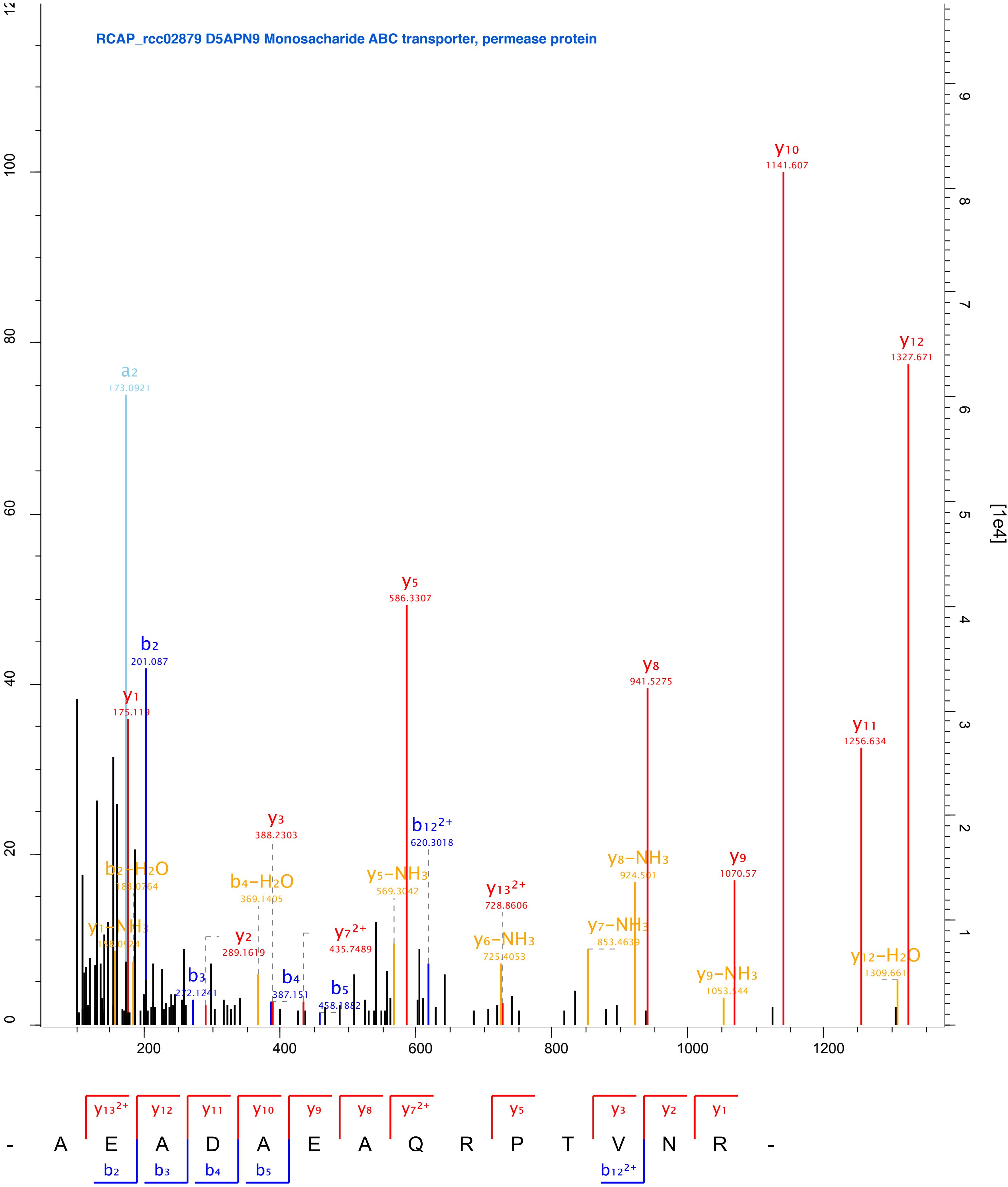
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-Set3-02 | 41150 | FTMS; HCD | 72.65 | 813.41 |



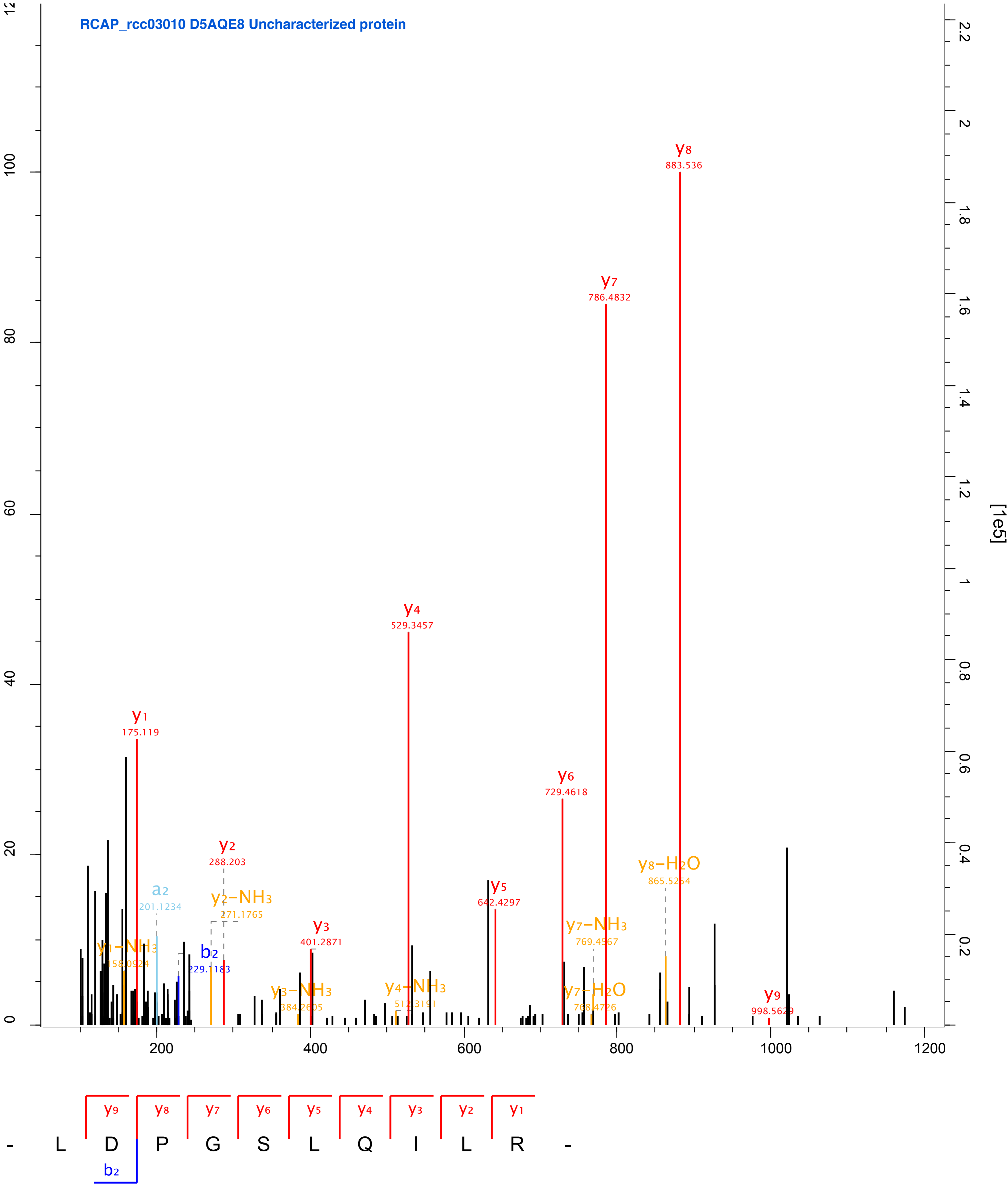
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-Set3-01 | 5779 | FTMS; HCD | 101.65 | 560.26 |



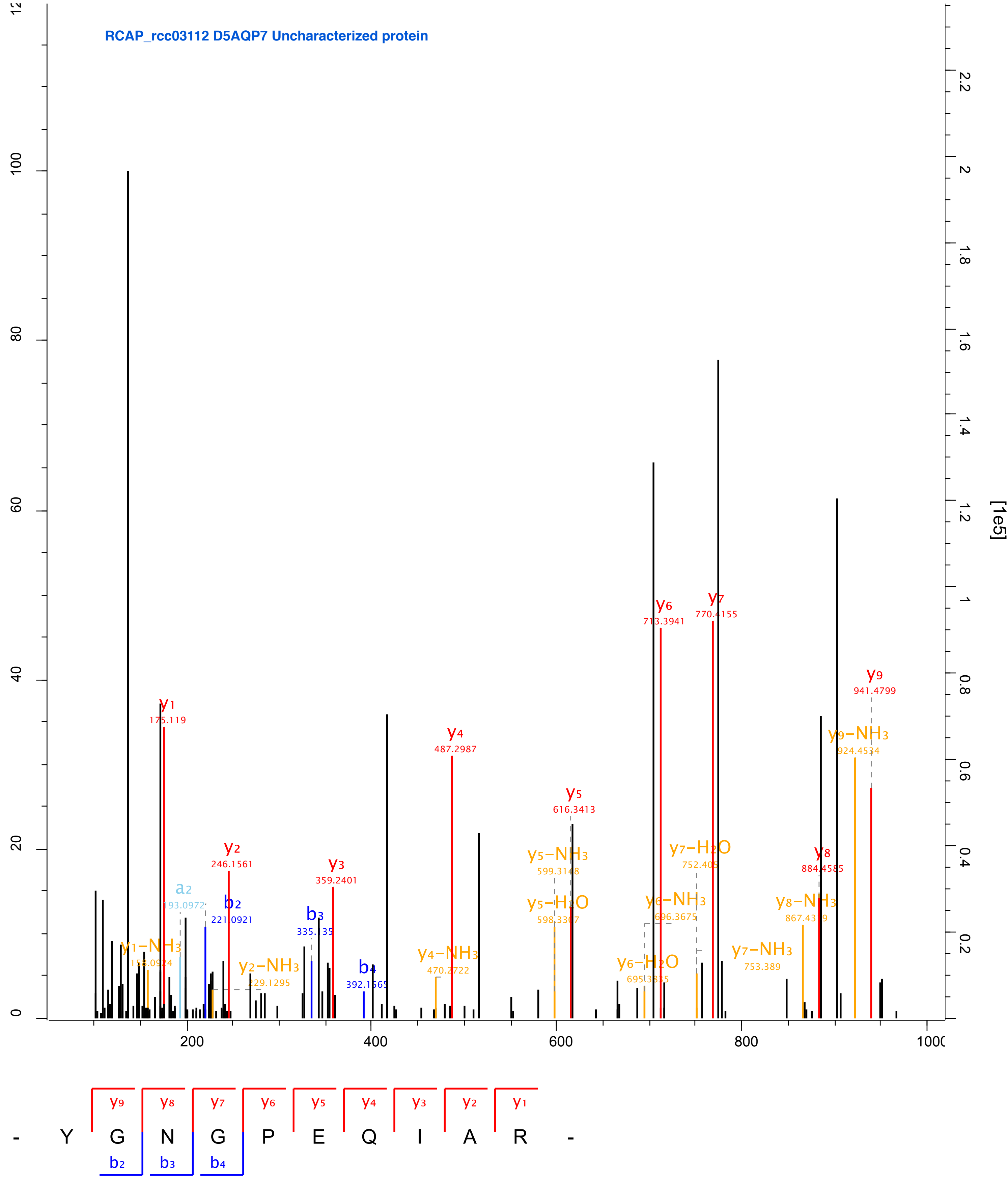
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-Set2-03 | 7237 | FTMS; HCD | 106.17 | 509.92 |



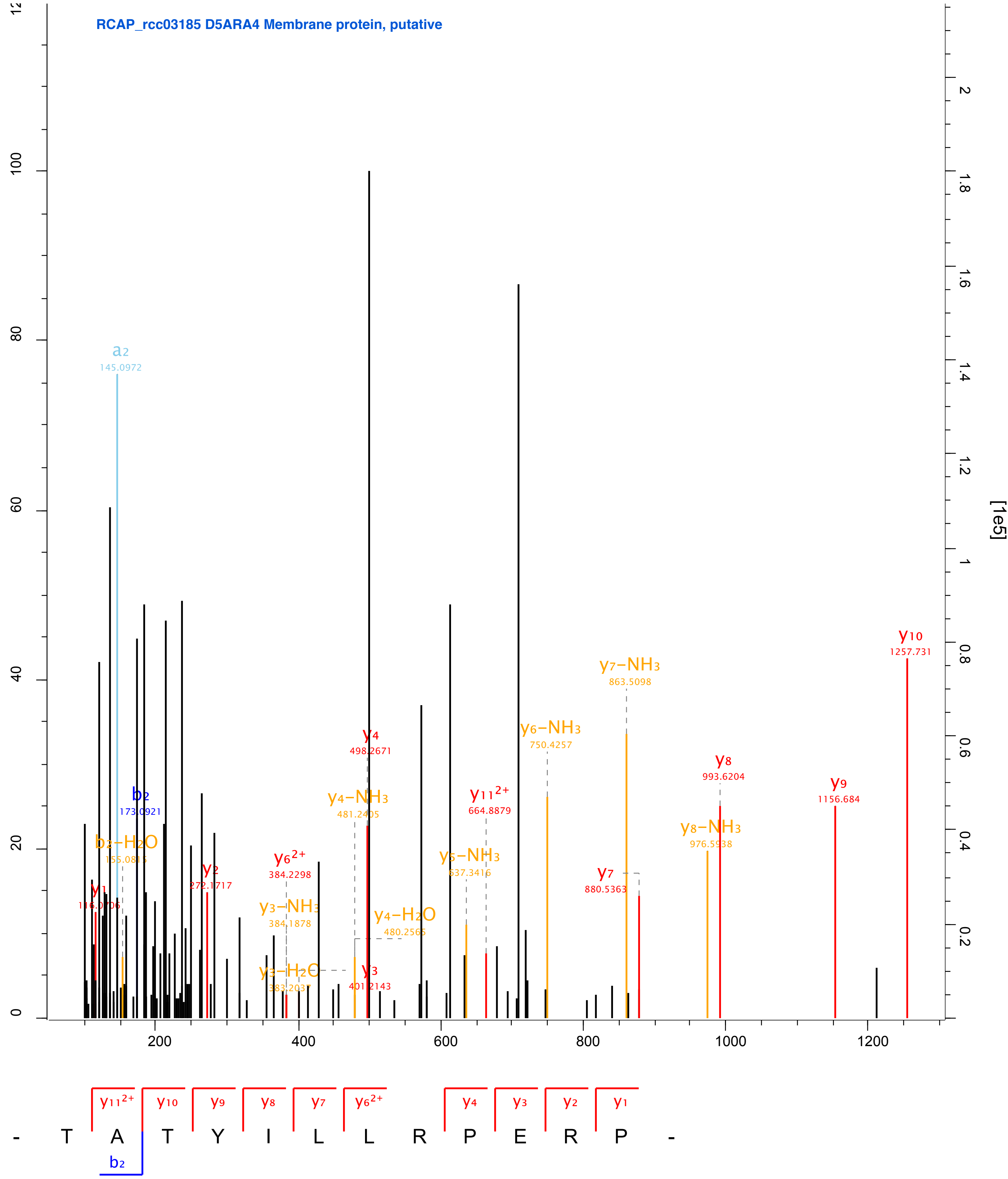
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-03 | 30374 | FTMS; HCD | 100.02 | 556.33 |



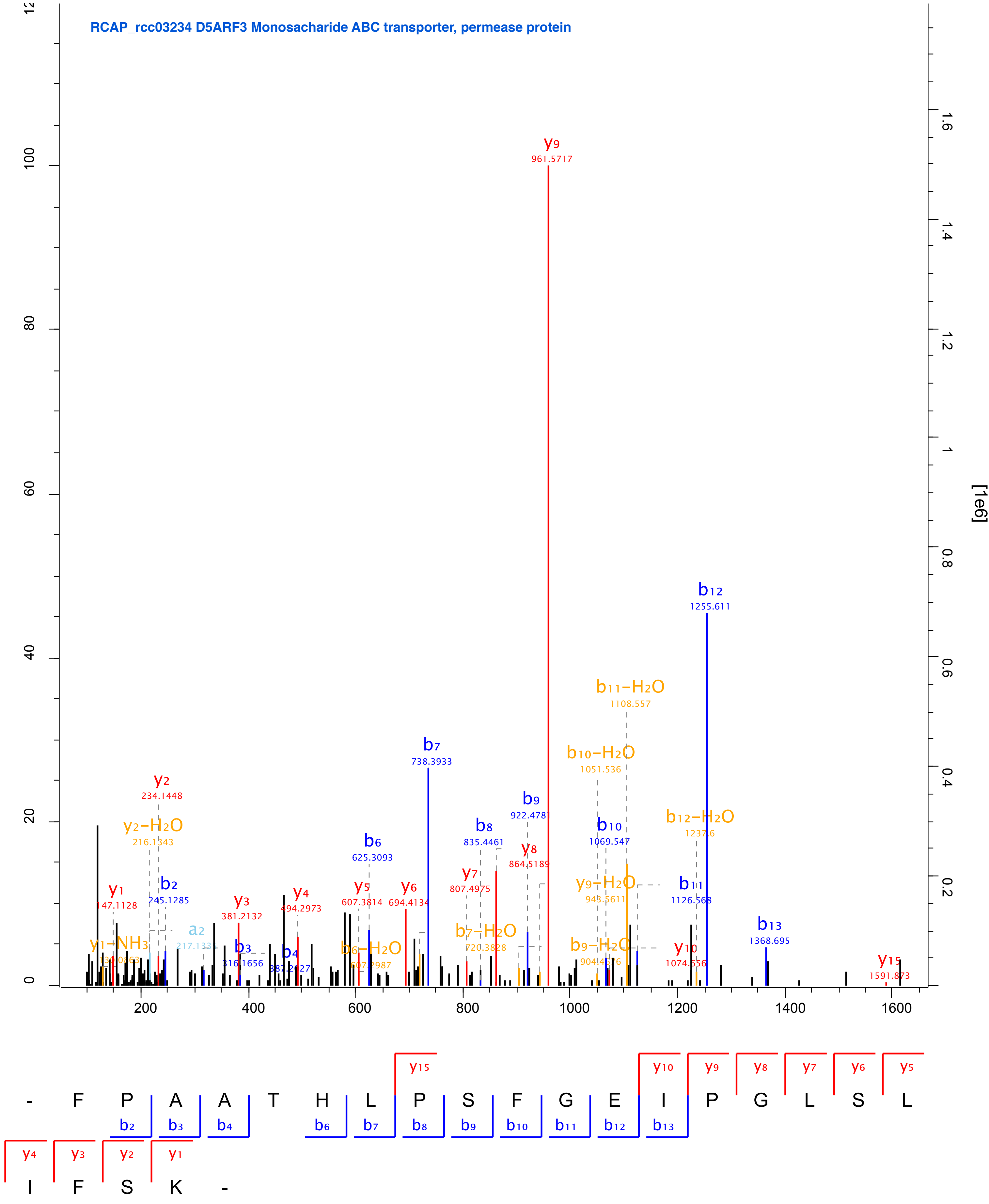
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-Set2-02 | 11575 | FTMS; HCD | 141.23 | 552.78 |



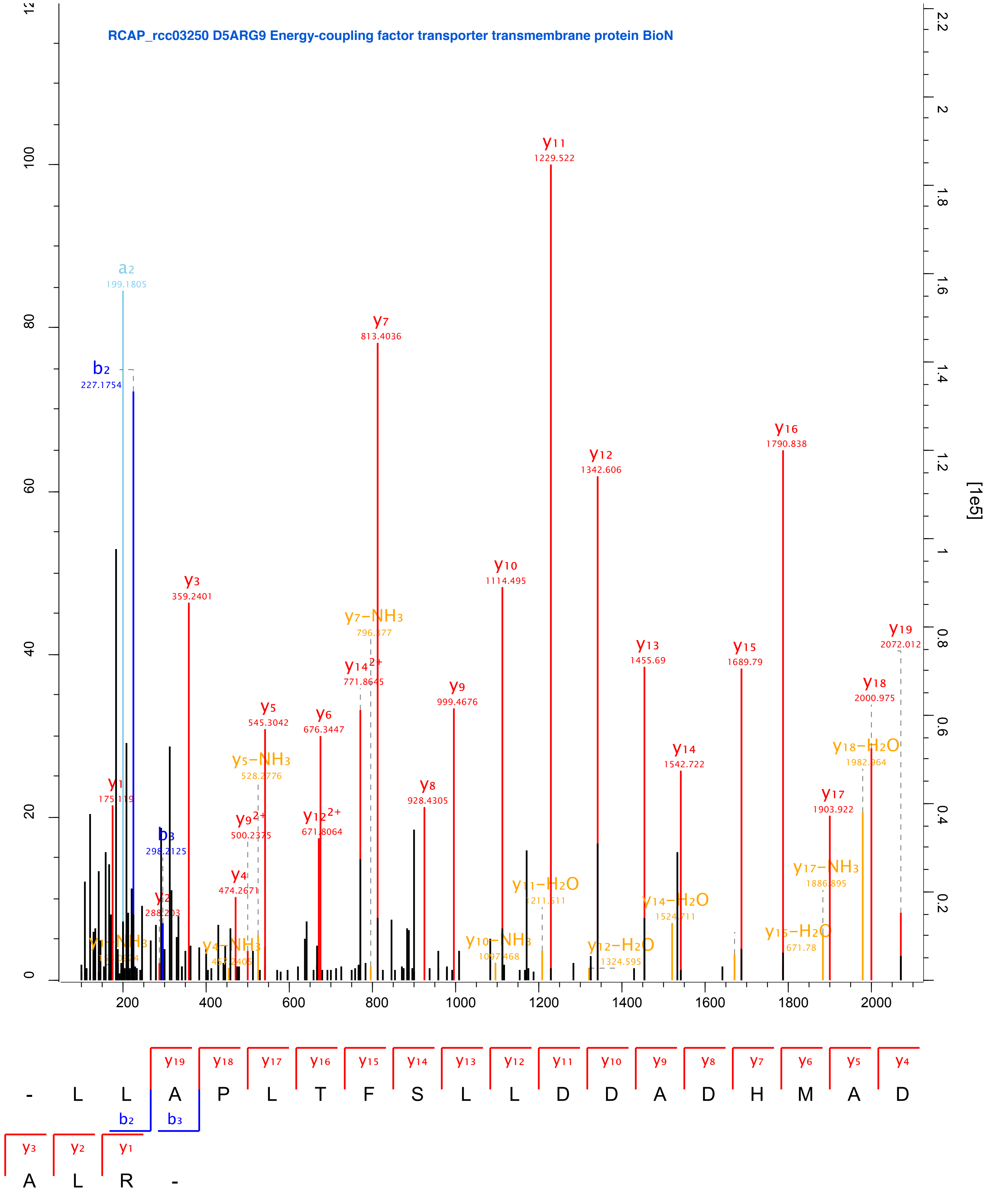
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-01 | 23330 | FTMS; HCD | 54.77 | 477.29 |



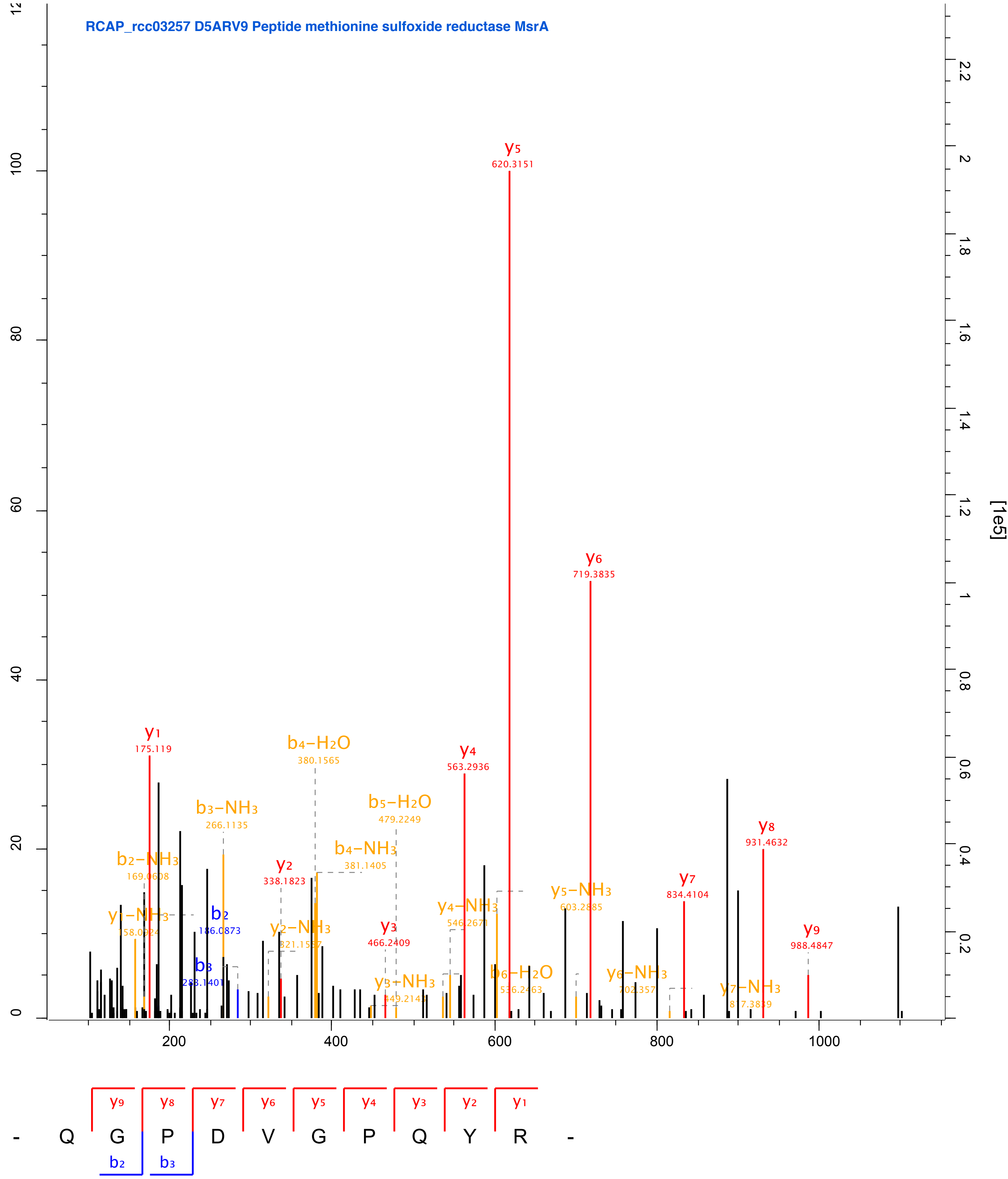
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-01_150512102711 | 61107 | FTMS; HCD | 91.28 | 777.43 |



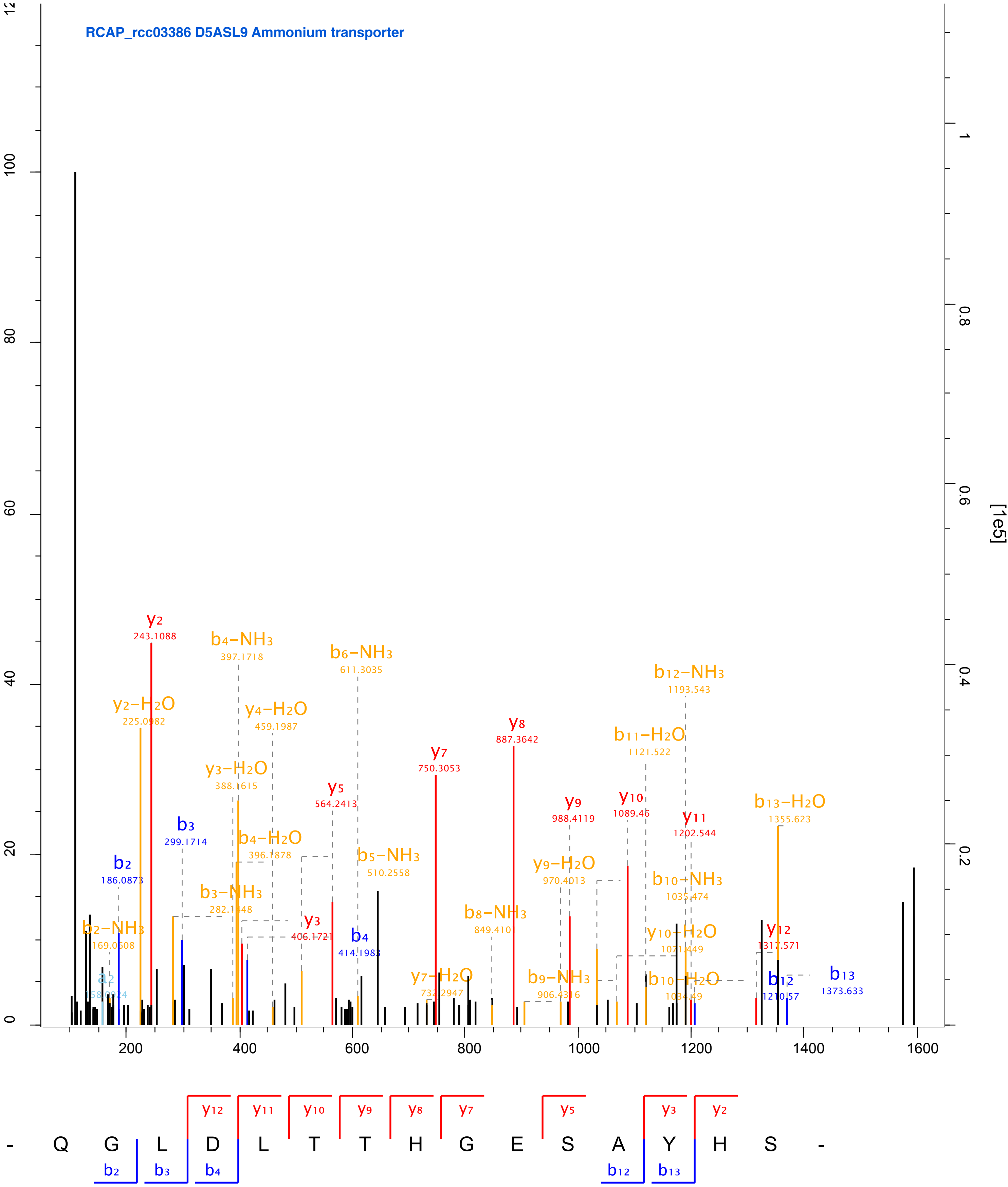
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-02 | 47093 | FTMS; HCD | 150.69 | 767.07 |



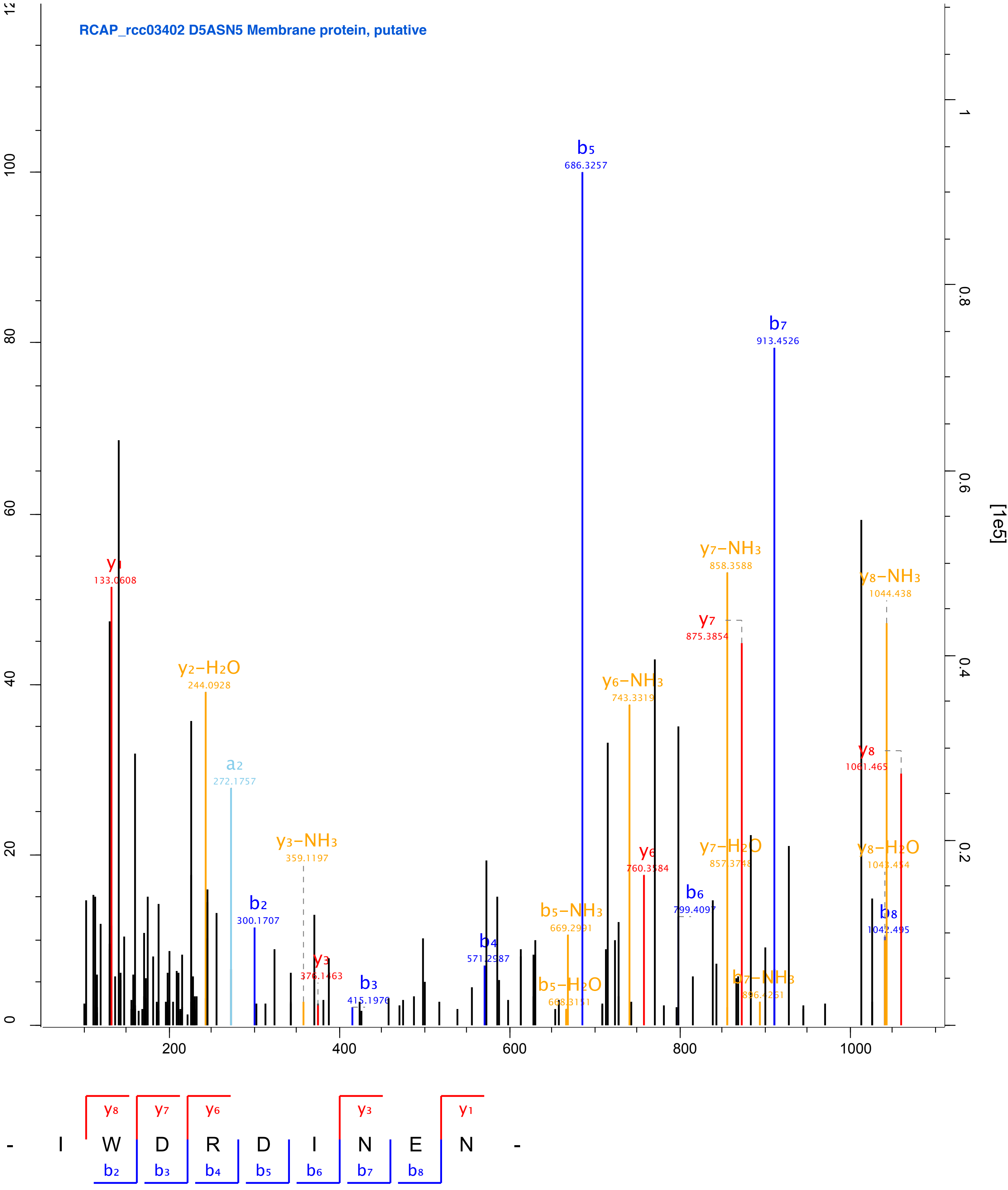
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-Set3-01 | 12049 | FTMS; HCD | 88.92 | 558.78 |



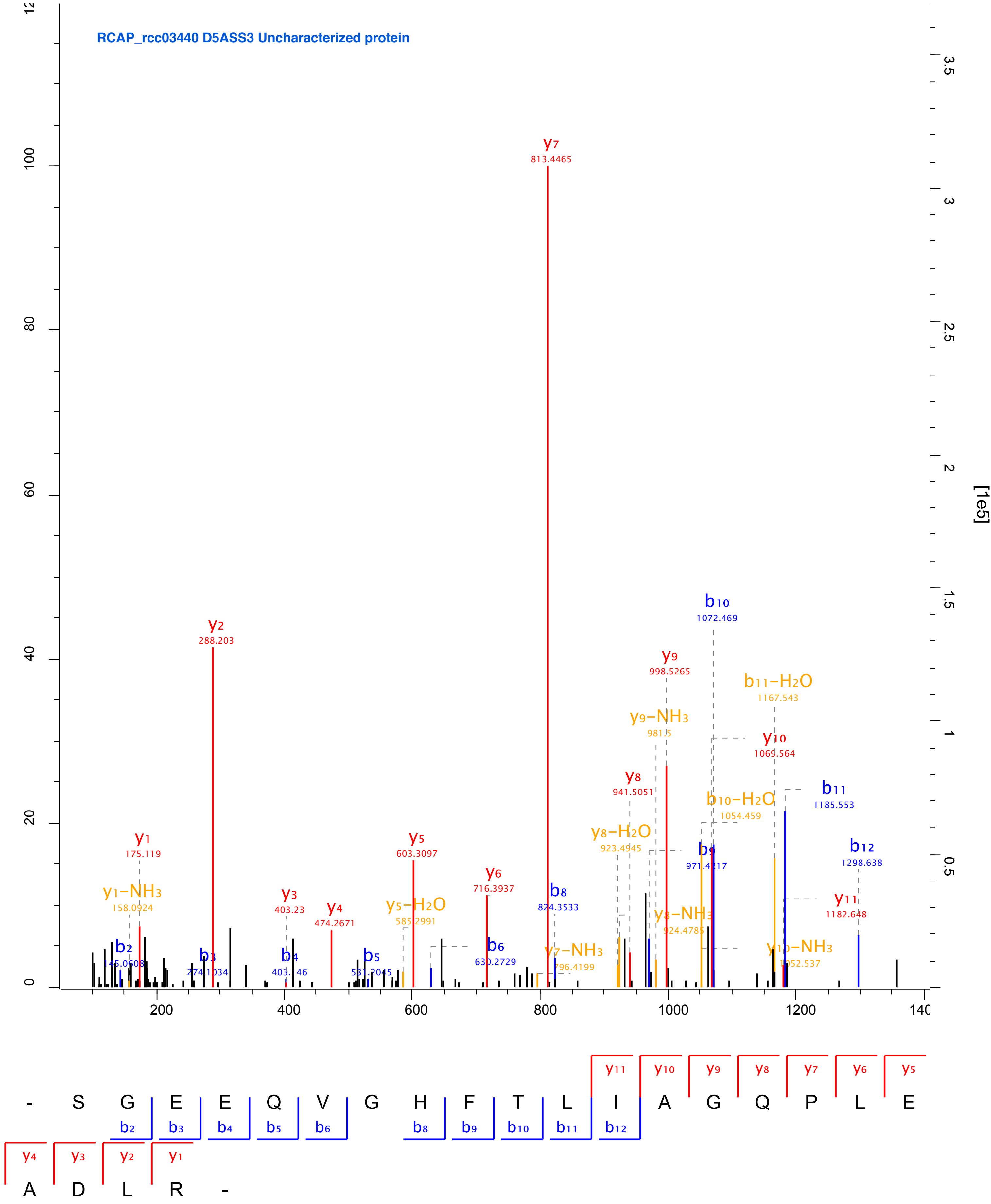
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-Set2-02 | 18113 | FTMS; HCD | 160.53 | 808.37 |



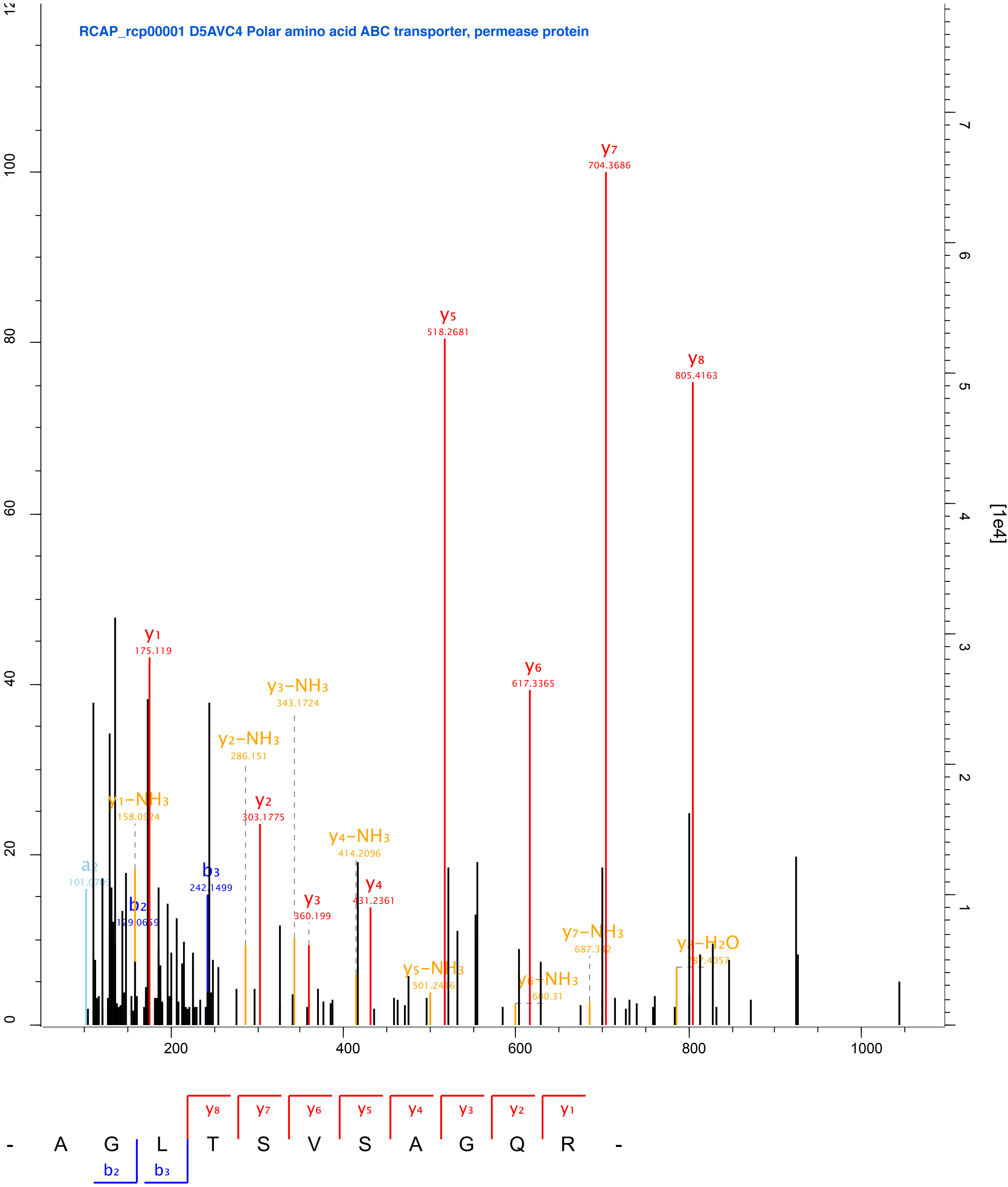
| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-Set2-02 | 16809 | FTMS; HCD | 104.01 | 587.78 |



| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-plusCu-Set2-01 | 33816 | FTMS; HCD | 80.31 | 790.07 |



| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-plusBC-02 | 9019 | FTMS; HCD | 104.06 | 523.78 |



| Raw File | Scan | Method | Score | m/z |
|---|---|---|---|---|
| MT1131-minusCu-03 | 44031 | FTMS; HCD | 86.13 | 928.79 |

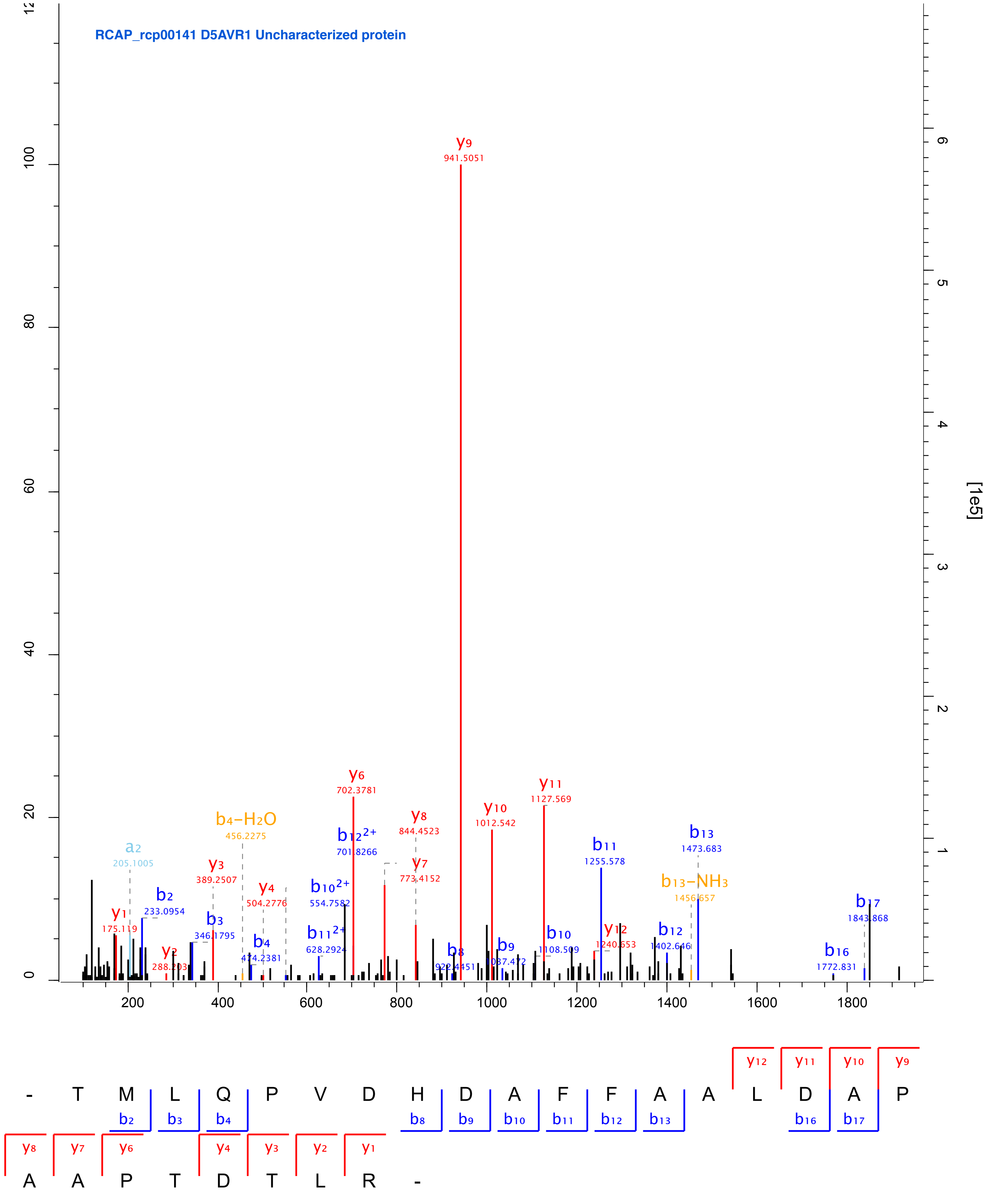


**Figure S5.** Total cellular metal contents of cells grown in Cu-sufficient control (-Cu), or + Cu (5 μM), or +BCS (5 mM) medium, determined by ICP-MS as described earlier (8, 9) (note the logarithmic scale of y axis).

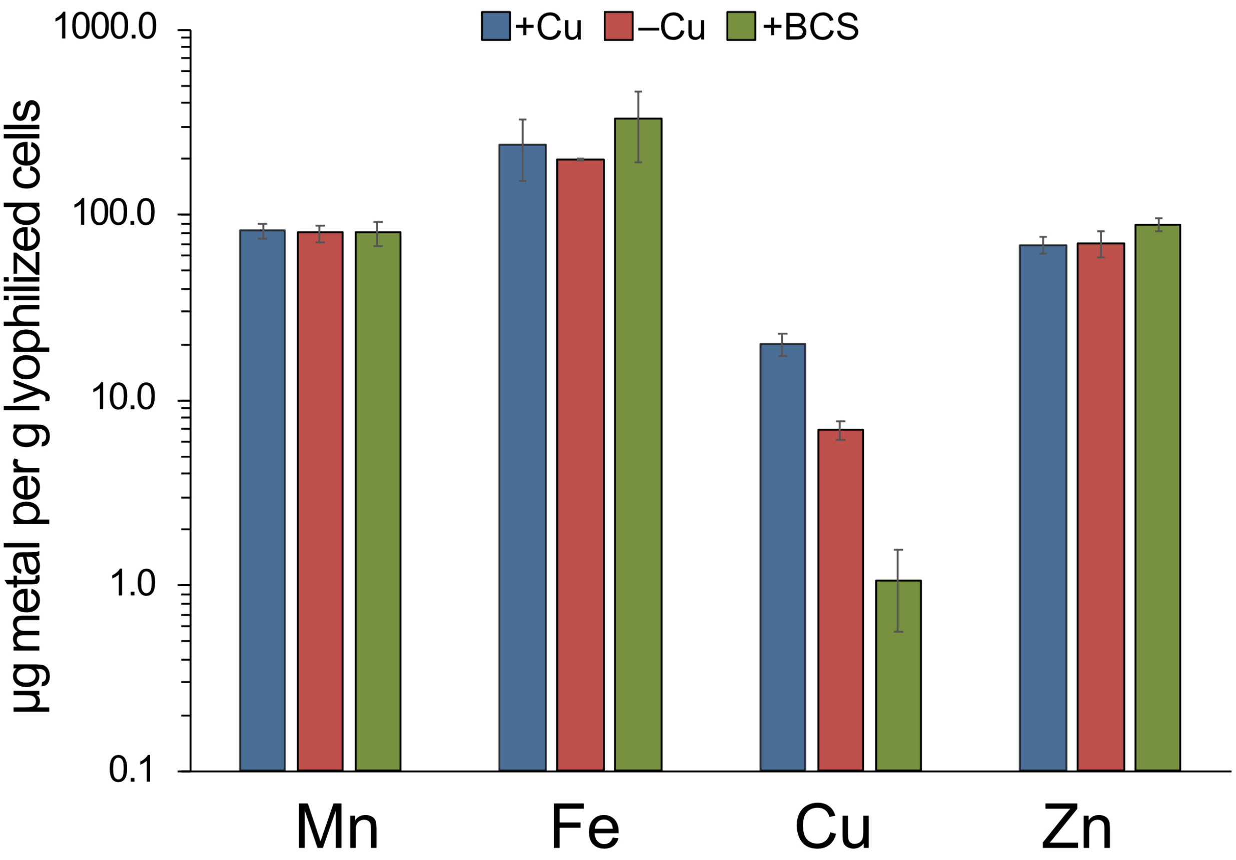


*Figure S5. Selamoglu et al.*