

Within this section, we will provide some common usage examples of SWATH-Lib.

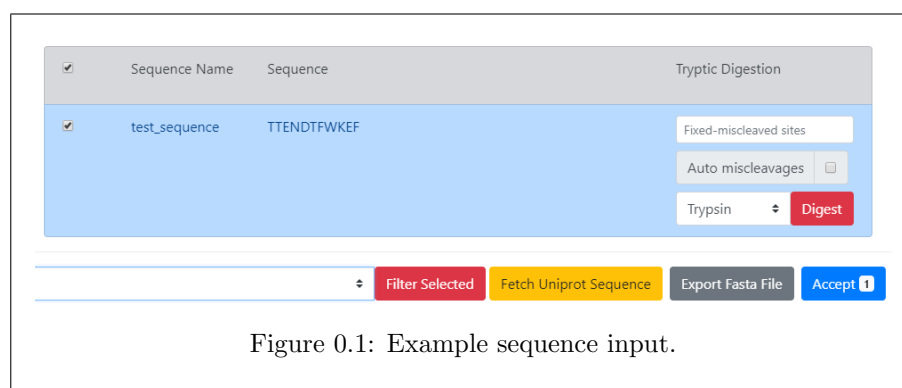
0.1 Creating A Y-library for N-glycosylated peptide

Here we have a precursor sequence with the id `test_sequence` and the aa sequence `TTENDTFWKEF`, or in fasta format

```
>test_sequence
TTENDTFWKEF
```

This sequence has an N-glycosylation sequon at position 4 and a tryptic digestion site right after position 9. With this library, we aimed to generate all HexNAc Y0, Y1, and Y2 transitions for the sequence at RT of 10 across all SWATH windows. The max charge allowed would be 2 and the annotated sequence at the modification position would include both RT and SWATH window value.

Upon input into the fasta content box and process, display similar to Figure ?? should appear.



After in-silico digestion with trypsin, we would obtain two sequences (Figure ??), one before and one after the digestion site. The name of the sequences have also been automatically modified to show the starting and stopping positions of each digested fragment. Using the filter rule for N-glycosylation sequon, the GUI would identify and select all sequences with a sequon within those already selected.

We currently have a tryptic digested sequence containing N-glycosylation sequon but without any modification and experiment settings applied to it. These settings can be selected within the **Queryset Input Settings** panel.

In Figure ??, we have selected Ytype variable modifications, HexNAc Y0, Y1, and Y2, RT time at 10, all SWATH windows, fragmentation iontype Y and output sequence format with retention time SWATH window.

Sequence Name Sequence Tryptic Digestion

<input checked="" type="checkbox"/>	test_sequence_1_9	TTENDTFWK	Fixed-miscleaved sites Auto miscleavages <input type="checkbox"/> ↓ Digest
<input type="checkbox"/>	test_sequence_1_0_11	EF	Fixed-miscleaved sites Auto miscleavages <input type="checkbox"/> ↓ Digest

N-Glycosylation Filter Selected Fetch Uniprot Sequence Export Fasta File Accept

Figure 0.2: Example sequence input after in-silico trypsin digestion and filtered for only those containing N-glycosylation sequon.

Queryset Input Settings

Static Modifications

- Propionamide (71.0371 Da)
- Carbamidomethyl (57.02 Da)

Non-Ytype Variable Modifications

- Phosphorylation (79.9663 Da)
- Sulfation (79.9568 Da)
- Carboxylation (43.9898 Da)
- Methylated Carboxylation (58.0055 Da)

Ytype Variable Modifications

- O-Mannose (Y1) (162.05 Da)
- HexNAc (Y0) (0 Da)
- HexNAc (Y1) (203.0794 Da)
- HexNAc (Y2) (406.1587 Da)

Retention Time

- 10
- 11
- 12
- 13

SWATH Windows

- 1149 - 1175
- 1174 - 1200
- 1199 - 1225
- 1224 - 1250

Extra Mass

0

Max Charge

2

Precursor Charge

2

Oxonium Ions

- Hex-2H2O (127.0389 m/z)
- HexNAc-(H2O)2-COH2 (138.055 m/z)
- Hex-H2O (145.0495 m/z)
- Hex (163.0601 m/z)

Fragmentation ion-type

Y

Output Sequence Format At Variable Modifications

Retention Time & SWATH

Apply Modifications

Figure 0.3: Example modification settings for N-glycosylation library with 3 HexNAc Ytype transitions at RT 10 and across all default SWATH windows.