USING AutoQC WITH SKYLINE FOR SYSTEM SUITABILITY

Quantitative mass spectrometry proteomics requires that we can attribute any variation in our peak area measurements to the samples themselves, rather than variation arising from the LC-MS system. To assess the performance of the LC-MS, we use system suitability testing* (SST) where we run a particular sample periodically (e.g. 1-2x day) and use it to measure LC-MS metrics like mass accuracy, peak area, TIC, retention time, etc.

Skyline can be downloaded from the skyline.ms website. Here, I'm using Skyline-daily.

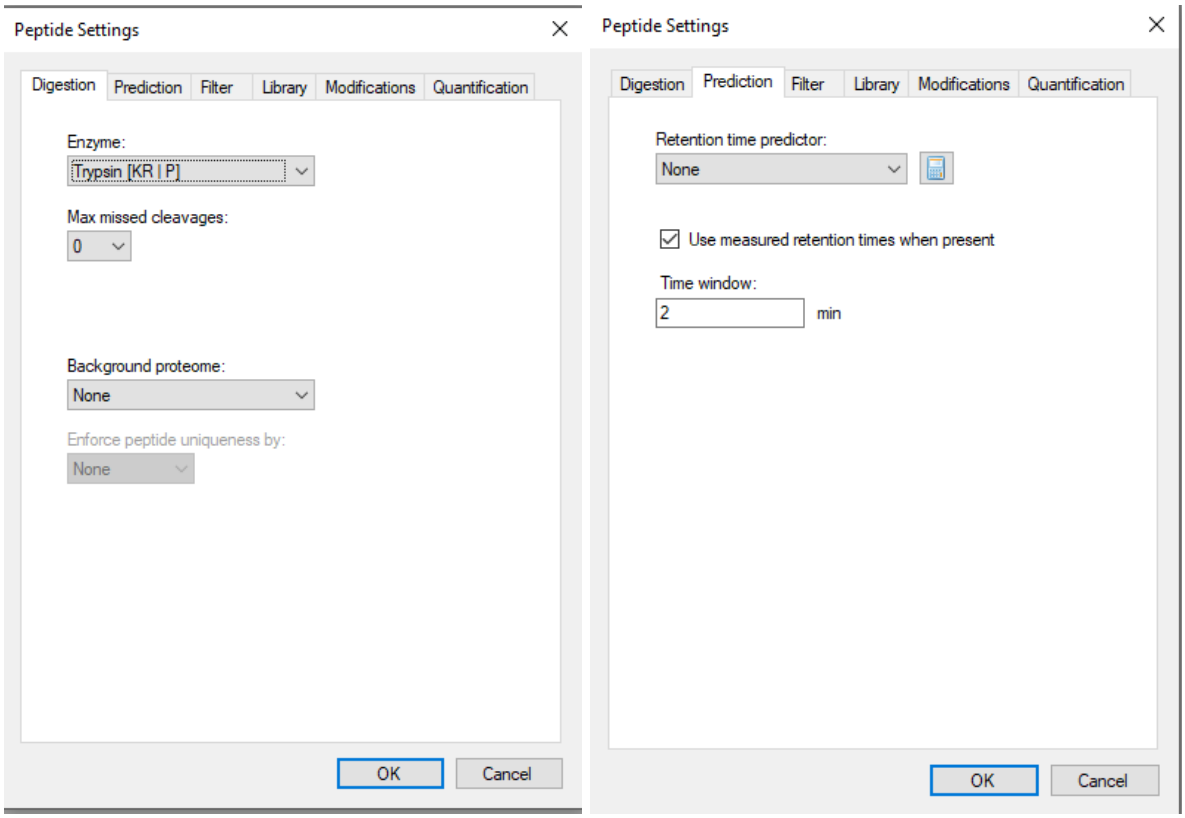
In this tutorial, we'll go over how to set up a Skyline document for use with AutoQC, including:

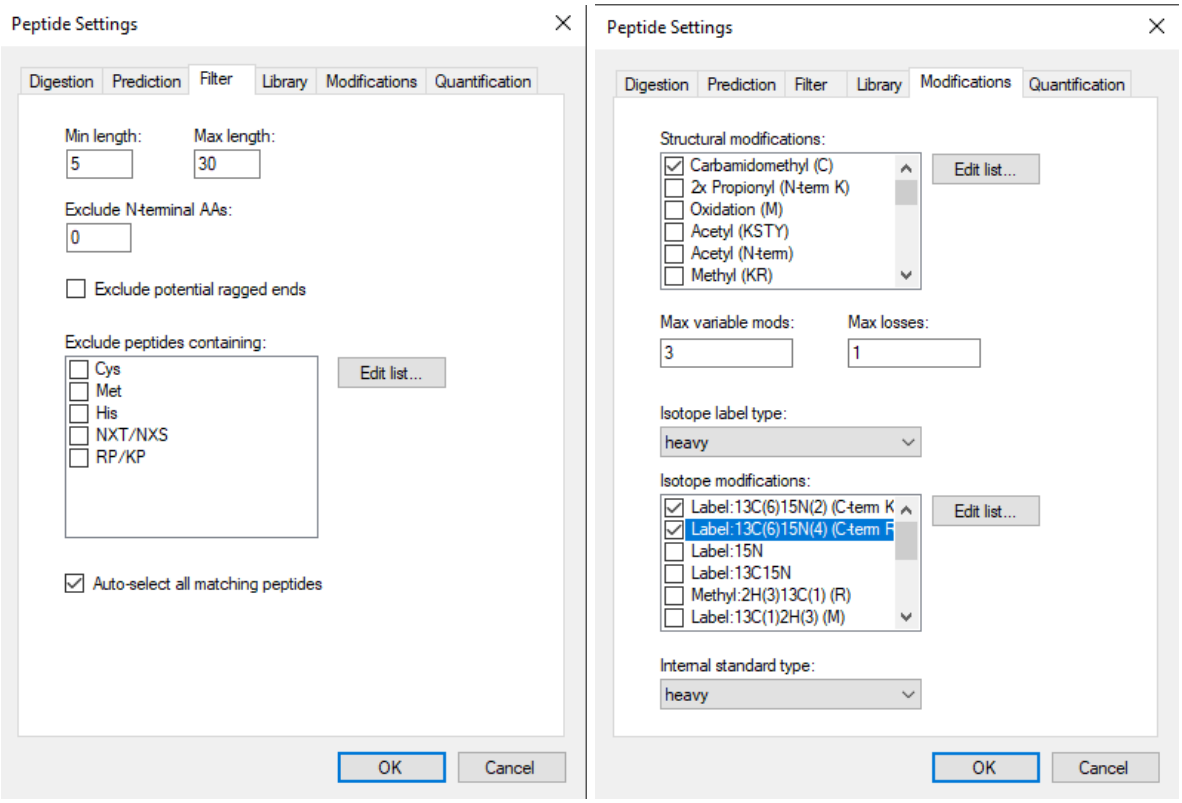
1. **Getting started with system suitability standards**
   Here, we're using the Pierce Retention Time Calibration Mixture standards (available online at https://www.thermofisher.com/order/catalog/product/88321#/88321). To get a list of these peptides, I went on the Thermo website and, under Documents, they list the peptide sequences for the PRTC peptides. I built a FASTA file from these sequences which is located in this tutorial folder (prtc.fasta). You can use any peptides you like, however!
2. **Setting up an instrument method**
   We'll be setting up a PRM method for Thermo Orbitrap mass spectrometers using these PRTC peptides, but you could build any type of method you want (SRM/MRM, DIA). We advise against DDA methods, as they do not provide quantitative metrics for system suitability!
3. **Setting up AutoQC on the instrument computer**
   This tutorial has been previously written by Vagisha Sharma -- available at https://panoramaweb.org/home/wiki-page.view?name=qc_with_panorama

**Nota Bene: SST is commonly misnomered as "quality control"! These are actually two different things. SST assesses the analytical system, i.e. the LC-MS, while quality control (QC) assesses the sample preparation method, i.e. the protocol. You can use Skyline+AutoQC to assess both, although it is maybe a bit advanced. Briefly, you would just set up a different Skyline document for each (e.g. for QC, you might spike a particular protein/peptide into each sample to assess digestion or enrichment efficiency) and the set up AutoQC monitoring for a different method or data folder with each Skyline document piping to a different Panorama folder.*

STEP 1. GETTING STARTED WITH SYSTEM SUITABILITY STANDARDS

1. Set up a Skyline document with the system suitability standards.
    - 1.1. Open a blank Skyline document.
    - 1.2. Navigate to Settings > Default to reset settings.
    - 1.3. Navigate to Settings > Peptide Settings and set the Digestion, Prediction, and Filter tabs as follows:

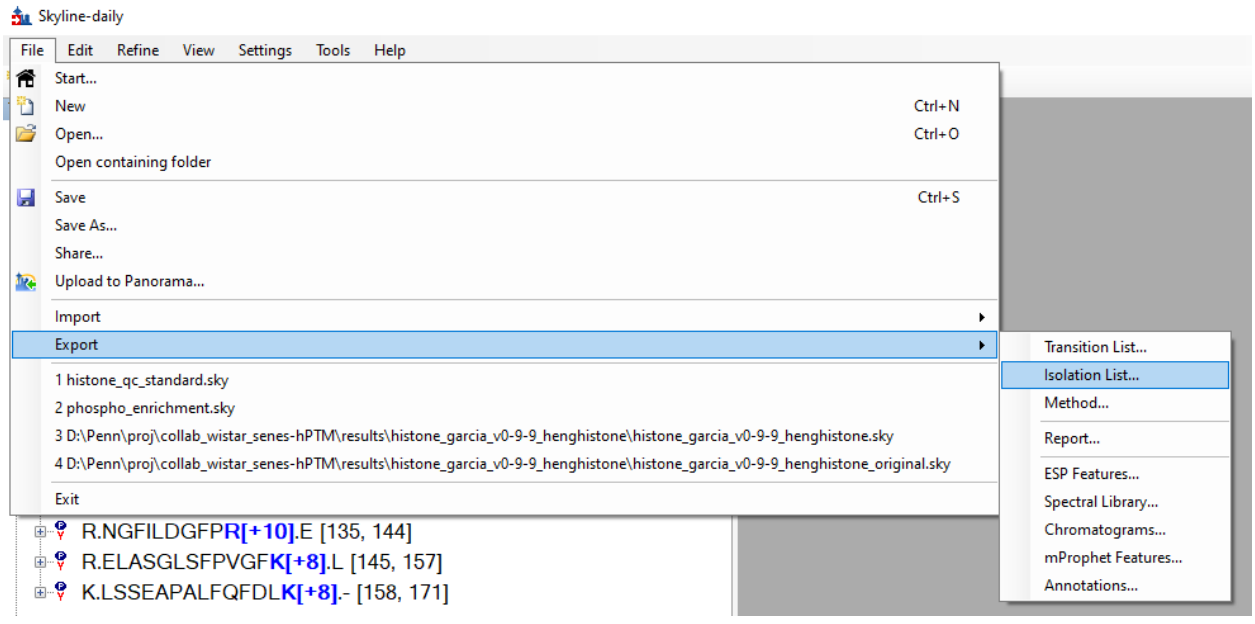




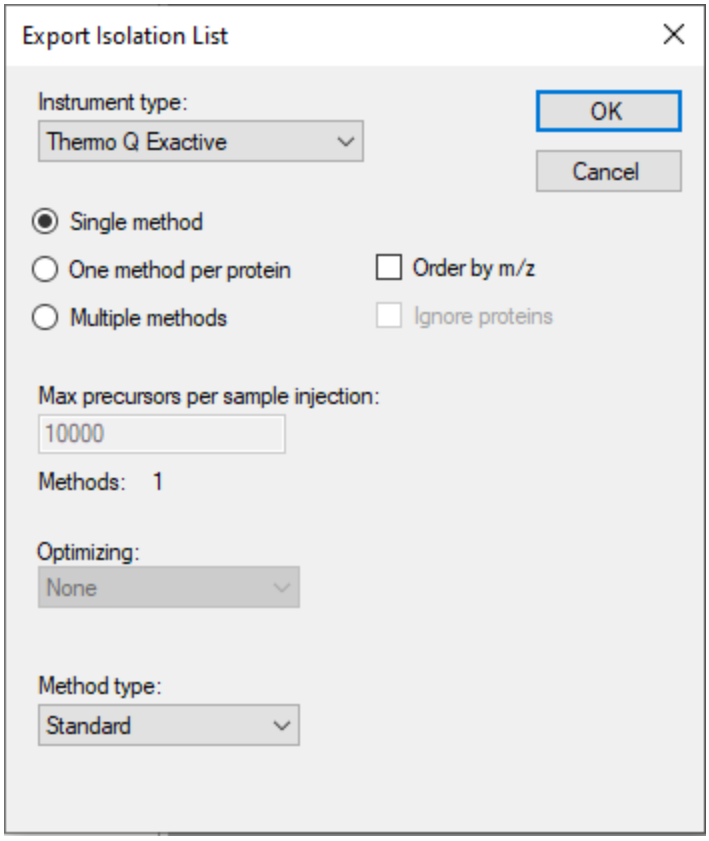
1.4. Click “OK”, then go to File > Import > FASTA

1.5. Navigate to this tutorial folder (autoqc2021) and select the prtc.fasta file. Click OK.

1.6. Navigate to File > Export > Isolation List ...



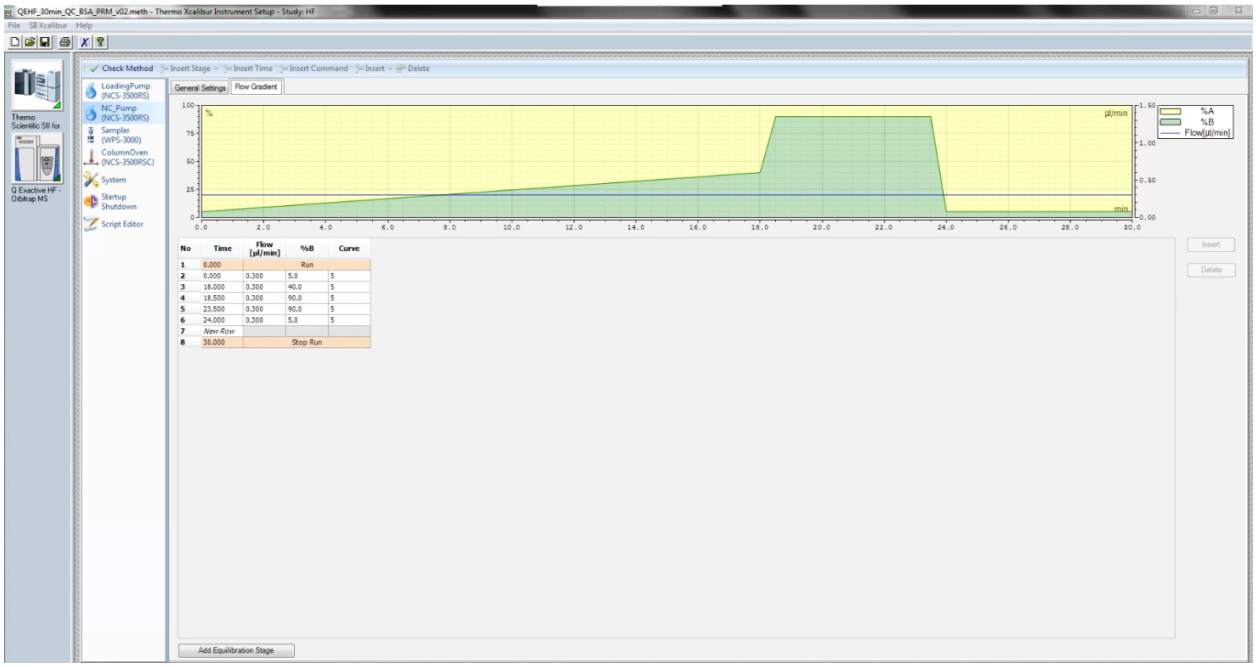
1.7. In the Export Isolation List pop-up window, select “Thermo Q Exactive” from the drop down



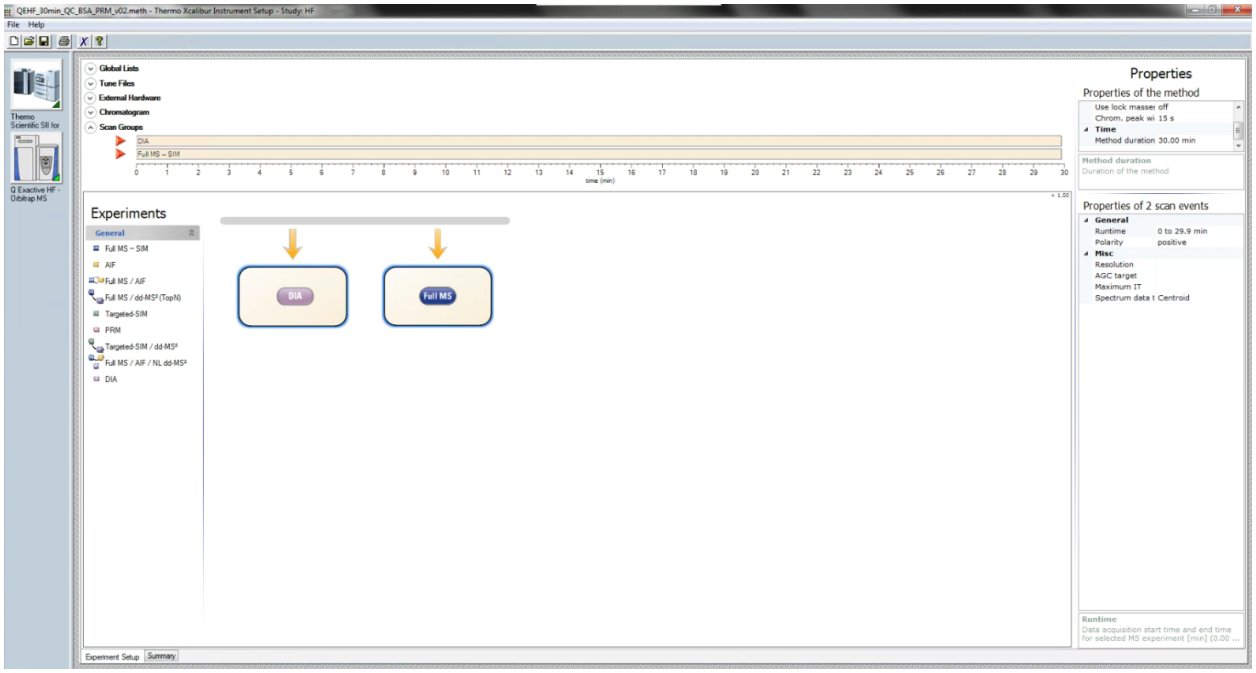
1.8. Save the Isolation List and move it to your instrument computer to build the PRM method in Part 2.

STEP 2. SETTING UP INSTRUMENT METHODS

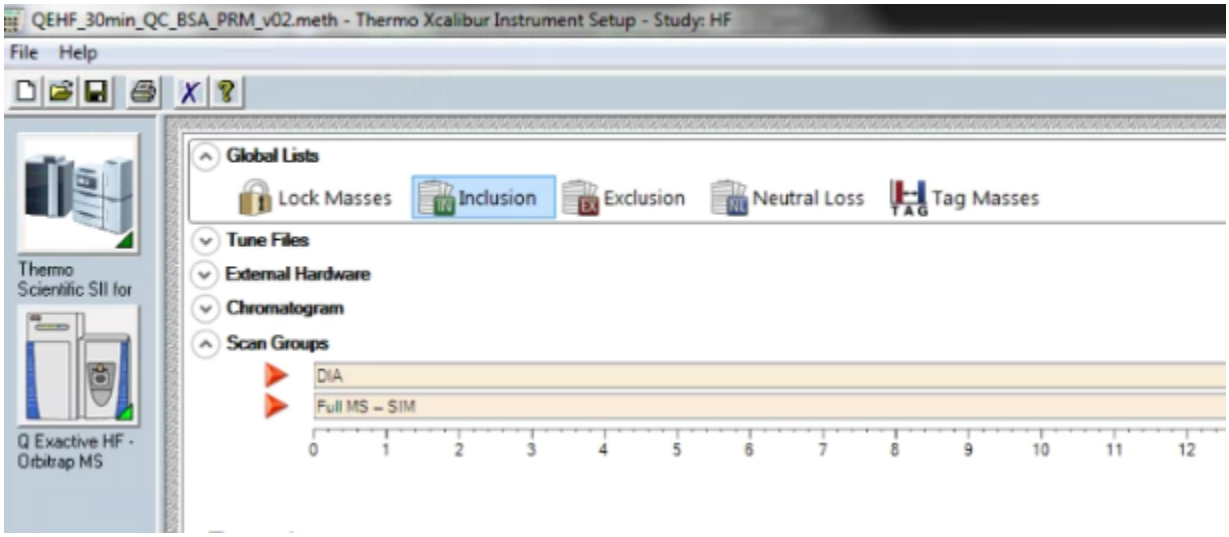
2. Set up the unscheduled PRM method. NOTE: this is just a suggestion! Feel free to set up an SRM/MRM, PRM, or other DIA method as fits your needs. This is just an example.
    2.1. Open Thermo Xcalibur Instrument Setup
    2.2. Enter a method spanning a typical gradient profile. This can be shorter than your typical experimental run. For example, my lab typically runs bottom-up proteomics samples using a separating gradient of 5% - 40% acetonitrile/B.



    2.3. Navigate to the Q Exactive HF and set up a DIA and a full scan in the acquisition method



2.4. At the top, open Global Lists and navigate to Inclusion



2.5. Navigate to File > Import > navigate to where you saved the Inclusion list exported from Skyline in Part I. Select OK and and click Done.

2.6. Set the DIA properties as follows. Note: the narrow isolation windows make this “DIA” method essentially a PRM method.

DIA	
Resolution	30,000
AGC target	1e5
Maximum IT	50 ms
Loop count	24
MSX count	1
MSX isochronous ITs	on
Isolation window	2.0 m/z
Fixed first mass	—
(N)CE / stepped (N)CE	nce: 28
Spectrum data type	Centroid

2.7. Acquire 1-3 runs using this method, importing into the Skyline document (File > Import > Results) in Part 1 to confirm that chromatograms look clean and method is working as intended.

STEP 3. SETTING UP AUTOQC ON THE INSTRUMENT COMPUTER

3. Please follow the tutorial written by Vagisha Sharma provided at
https://panoramaweb.org/home/wiki-page.view?name=qc_with_panorama