Skyline-driven Targeted Data Extraction in diagonal-PASEF Workflows

Stephanie Kaspar-Schoenefeld[1], Daniel Hornburg[3], Tharan Srikumar[2] , Jonathan Krieger[2], Jason O'Neill[3]

[1]Bruker Daltonics GmbH & Co. KG, Bremen, Germany, [2]Bruker Ltd, Milton Ontario, Canada, [3]Bruker Daltonics Inc, Billerica Massachusetts, United States

Introduction

Combining Data-Independent Acquisition (DIA) with Trapped Ion Mobility Spectrometry (TIMS) reduces sample complexity by providing an additional dimension of separation, thereby increasing overall system peak capacity. This scan mode, termed dia-PASEF, employs fixed quadrupole isolation windows for ion selection in both the m/z and mobility planes. To further enhance the analytical performance, diagonal-PASEF has been developed, which optimizes the acquisition of the precursor ion cloud by continuously tracking its diagonal shape.

Although several data processing software tools currently support this scan mode, none specifically focuss on targeted data extraction. We present the implementation of diagonal-PASEF data processing in Skyline for targeted quantitation.

Material & Methods

Tryptic in-house generated protein digests (Homo sapiens (HeLa), Escherichia Coli (*E. coli*), and Saccharomyces cerevisiae (yeast)) mixed in the following mass ratios : Sample "A": HeLa:E. coli:Yeast 65:30:5; Sample "B": HeLa:E. coli:Yeast 65:15:20.

400 ng/µL were were loaded directly onto the analytical column (5cmx75µm, 1.7µm, Aurora Rapid, IonOpticks) and separated by a 5-minute gradient using a nanoElute 2 (Bruker) coupled to a timsTOF HT mass spectrometer (Bruker). For the diagonal-PASEF acquisition, the ion cloud was covered using 3 slices of 75 Th. The data files were processed in Aptila DIA-NN (v2.2). Targeted data extraction was done by loading diagonal-PASEF raw files into Skyline daily (v 25.1.1.271).

Results

Diagonal-PASEF uses a more efficient way to sample the mass-mobility range by scanning the quadrupole continuously and thereby following the ion cloud eluting from the TIMS. This enables very short cycle times which are particularly important for high-throughput proteomics. Whereas the application of diagonal-PASEF for discovery proteomics studies has been already shown, its applicability for targeted proteomics approaches has been hindered by the availability of targeted data processing software tools supporting this scan mode. Skyline, an open-source mass spectrometry proteomics software supports targeted quantitation workflows targeted data processing software tools supporting this scan mode. Skyline, an open-source mass spectrometry proteomics software supports targeted quantitation workflows for small molecules and proteomics applications, directly from raw data. dda-PASEF, dia-PASEF, and prm-PASEF workflows are already supported by Skyline, but due to its novelty, the diagonal-PASEF data format has not been supported until recently. Using DIA-NN, diagonal-PASEF data can be processed to create a spectral library that guides targeted data extraction and defines peak boundaries in Skyline. This integration provides a complete workflow for targeted proteomics based on diagonal-PASEF (Figure 1). Due to its generic implementation a wide range of different DIA workflows (e.g. synchro-PASEF, midia-PASEF and slice-PASEF) are supported. Here we showcase the usability of diagonal-PASEF for targeted proteomics (Figure 2).

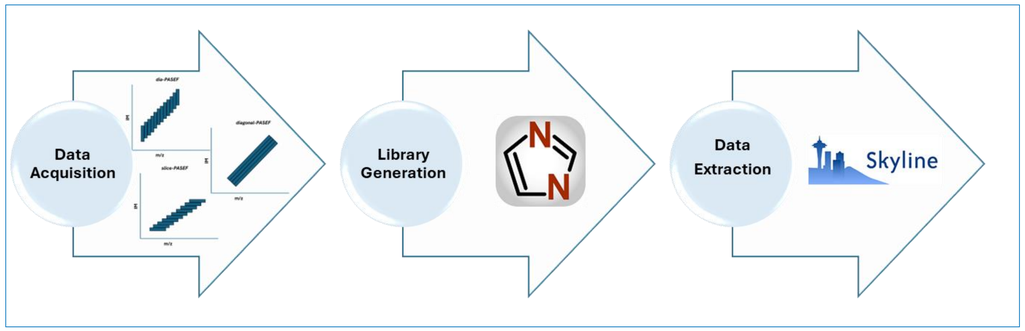

Figure 1: Overview about DIA-based data acquisition on timsTOF instruments and processing using targeted proteomics. Skyline daily (v25.1.271) provides a generic support for a variety of scan modes, including diagonal-PASEF.

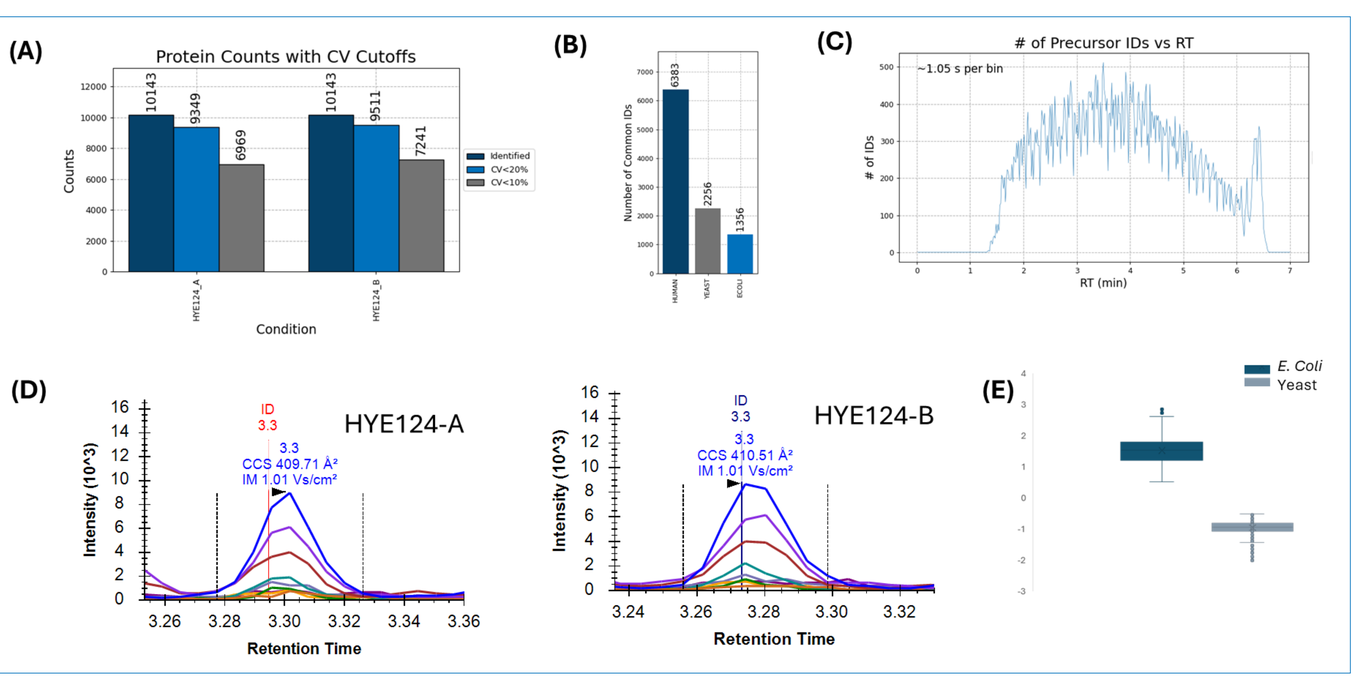

Figure 2: Results targeted data processing. In-depth protein identification using DIA-NN data processing results in the reproducible identification of more than 10,000 protein groups from a complex proteomics mixture using a 5-minute gradient. (A) Number of protein groups identified and quantified below 10% and 20% coefficient of variation from quintuplicate injections. (B) Common identifications across runs per organism (HeLa, Yeast, and *E. coli*). (C) Number of precursors identified per second across the complete gradient. (D) Data processing in Skyline. Shown are extracted chromatogram traces for sample A and B. (E) Accurate quantitation of regulated *E.coli* and Yeast proteins visualized as measured log2fold changes determined via quantitation using Skyline data processing.

- diagonal-PASEF uses a scanning quadrupole to cover the ion cloud of interest more efficiently, thereby resulting in very short cycle times, ideal for targeted proteomics in combination with high throughput analysis.
- Skyline in conjunction with DIA-NN enables fast and efficient targeted data extraction for diagonal-PASEF and other DIA window schemes.

nanoElute 2 + timsTOF HT

Conflict of interest: SK-S, DH,, TS, JK, & JO are employees of Bruker Daltonics.

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