Using DIA to inform the development of Cerebrospinal Fluid triple quad assays

Webinar #22: Using DIA Data To Create SRM Methods

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Strategies for building a new targeted triple quad assay:

• Analysis of recombinant target proteins

• Search for proteins in existing data

• Use prediction algorithms for selecting peptides
Using recombinant proteins to select peptides

Expand, Purify, & Sequence cDNA clone → In Vitro Transcription and Translation → Enrichment → digestion

detectable?

Measurement in a background matrix is important!
Strategies for building a new targeted triple quad assay:

- Analysis of recombinant target proteins
- Search for proteins in existing data
- Use prediction algorithms for selecting peptides
DDA is a poor indicator of SRM peptide performance

Bollinger, ASMS (2014)
Strategies for building a new targeted triple quad assay:

- Analysis of recombinant target proteins
- Search for proteins in existing data
- Use prediction algorithms for selecting peptides
DDA is a poor predictor of SRM peptide performance
DIA is a better predictor of SRM peptide performance
DIA provides evidence useful for SRM peptide selection

End goal: fragment ion quant

DIA more comprehensively measures fragment ions

MS1

MS2
Strategies for building a new targeted triple quad assay:

- Analysis of recombinant target proteins
- Search for proteins in existing data...now with DIA data
- Use prediction algorithms for selecting peptides
Gas-phase fractionated DIA: narrow isolation

51x “Staggered” DIA (12 m/z Effective Isolation)

GPF 51x Staggered DIA (x6 Injections, 2 m/z Effective Isolation)

Gas-phase fractionated DIA: narrow isolation

52 4 m/z-overlapping narrow windows

4 m/z

2 m/z

Replicate narrow DIA provide peptide knowledge base
Replicate narrow DIA provide peptide knowledge base

**Signal processing**

- Statistical priors
- Chromatogram extraction
- Peptide & transition filtering
- Top ranked transitions
- %CV cutoff
- Method scheduling

**EncyclopeDIA**

- Precursor-product ion pair detection
- Relative retention time
- Co-elution

*Searle et al., Nat. Comm (2018)*
Replicate narrow DIA provide peptide knowledge base

Signal processing

peptide knowledge base

%CV cutoff

top ranked transitions

method scheduling

quantitation of peptides
visualization of chromatography

Skyline
Replicate narrow DIA provide peptide knowledge base

Signal processing

peptide knowledge base

top ranked transitions

%CV cutoff

peptide & transition filtering

method scheduling

Filtering for well performing peptides & product ions

Day 1, Day 2, Day 3

gas phase fractionated narrow window DIA

statistical priors

chromatogram extraction
Replicate narrow DIA provide peptide knowledge base

- Pooled sample
  - Gas phase fractionated narrow window DIA
- Day 1
- Day 2
- Day 3

Signal processing
- Statistical priors
- Chromatogram extraction
- Peptide & transition filtering

Peptide knowledge base + %CV cutoff → Top ranked transitions

Method scheduling

Schedule new triple-quad method with spike-in iRT standard
Application to Alzheimer’s disease proteins in CSF

Cerebrospinal fluid:
- Parkinson’s Disease (n=30)
- Alzheimer’s Disease (n=11)
- Healthy Control (n=30)

Gas phase fractionated narrow window DIA

Day 1
Day 2
Day 3

Signal processing
- Statistical priors
- Chromatogram extraction

Peptide & transition filtering

%CV cutoff

Top ranked transitions

Method scheduling
Application to Alzheimer’s disease proteins in CSF

Cerebrospinal fluid:
- Parkinson’s Disease (n=30)
- Alzheimer’s Disease (n=11)
- Healthy Control (n=30)
Not all proteins and peptides measured are reproducible
In our DIA a majority of peptides have less than 20% CV
Reproducibility of peptides decreases with increasing sequence length.
Application to Alzheimer’s disease proteins in CSF

Cerebrospinal fluid:
- Parkinson’s Disease  (n=30)
- Alzheimer’s Disease  (n=11)
- Healthy Control   (n=30)
Building an assay for Alzheimer’s disease proteins in CSF

Proteins of interest: 100 from previous Alzheimer’s assay (Spellman et al., Proteomics Clin. Appl. 2016)

Peptide filtering criteria for 2 peptides/protein of interest:
- 5 interference-free product ions (top ranked intensity)
- <20 %CV
- Rank 1-2 based on total product intensity

Peptides from both previous assay and selected from DIA experiment measured in triplicate by SRM on Thermo Altis
Peptides selected from narrow window DIA results perform similarly to previously characterized Alzheimer’s disease assay peptide selections.
DIA provides useful information for expediting development of multiple triple quadrupole assays

- For precursor & product ion pairs:
  - Detectable in background
  - Retention time
  - Reproducibility
DIA provides useful information for expediting development of multiple triple quadrupole assays.
We can mine the same data for different targets

The human CSF pain proteome

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New assay: with 3.06 median %CV!
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Overview of our Skyline walk-through:

1. Importing GPF DIA search results into Skyline
2. Filtering peptides based on %CV
3. Filtering target proteins then filtering peptides based on intensity
4. Scheduling & exporting transition lists for SRM methods