Discovery to Targets for a Phosphoproteomic Signature Assay: One-stop shopping in Skyline

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Skyline Users Meeting

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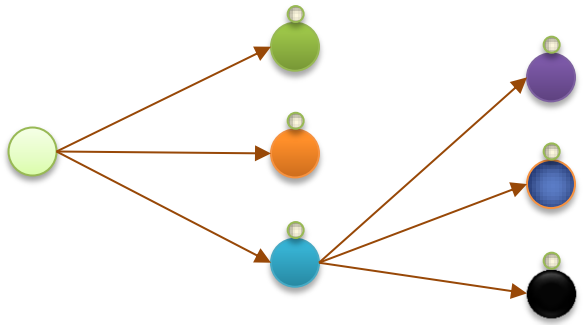
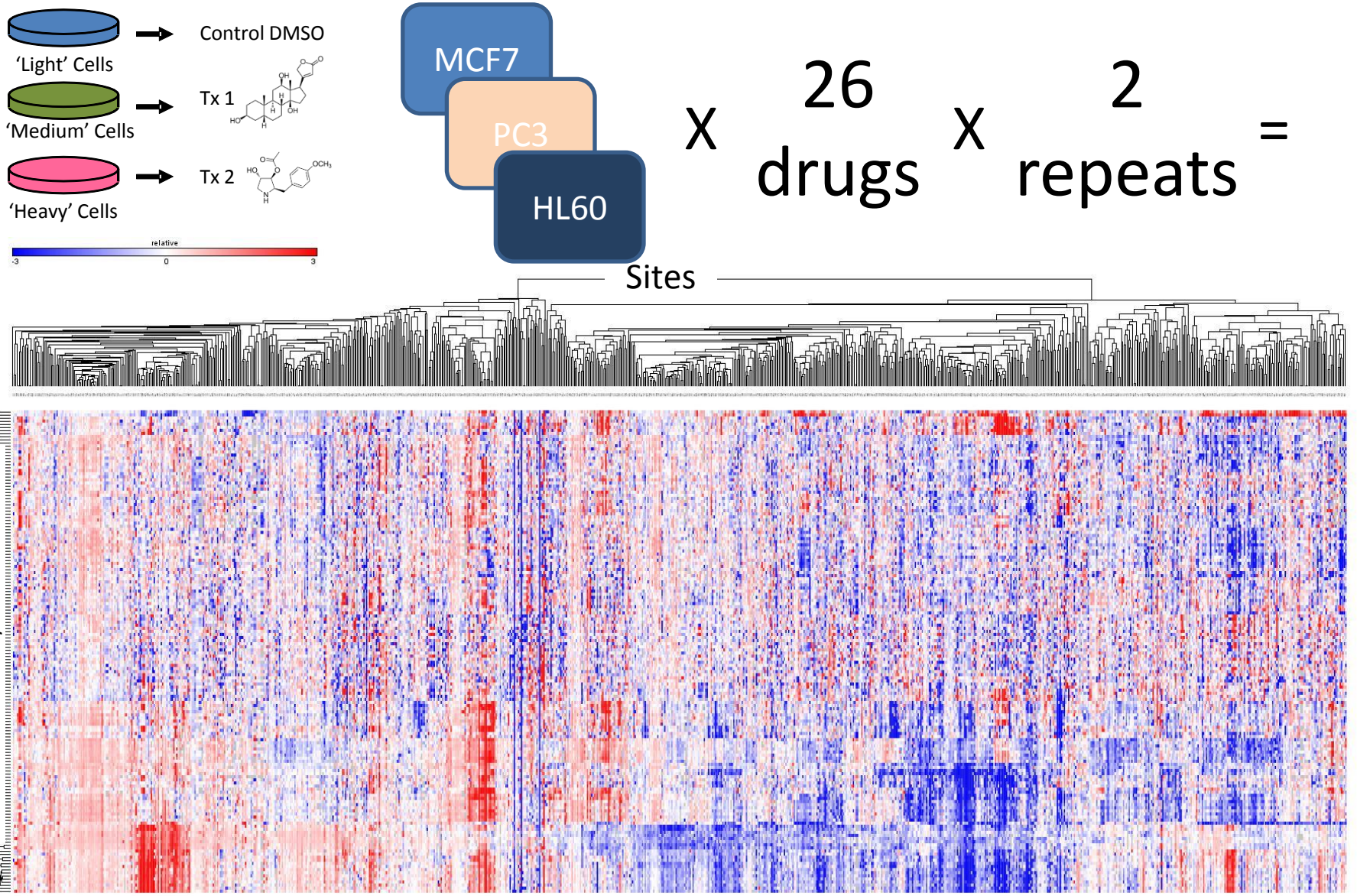
Idea: Phosphosignaling is coordinated!

- Phosphosignaling is likely coordinated
  - Kinase-substrate relationships tend to be 1-to-many
  - *A priori* expectation of coordinate regulation of sites



- Don’t need to monitor every phosphosite

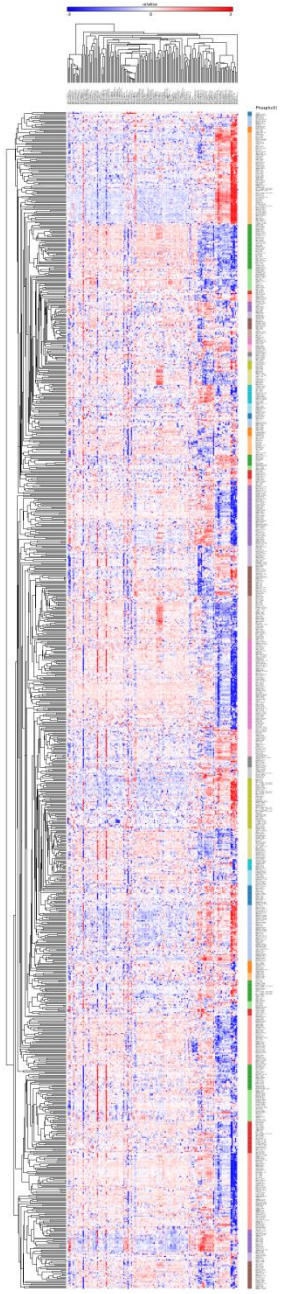
- Can we identify a few that we can reliably monitor?
  - Go ***wide*** instead of ***deep***



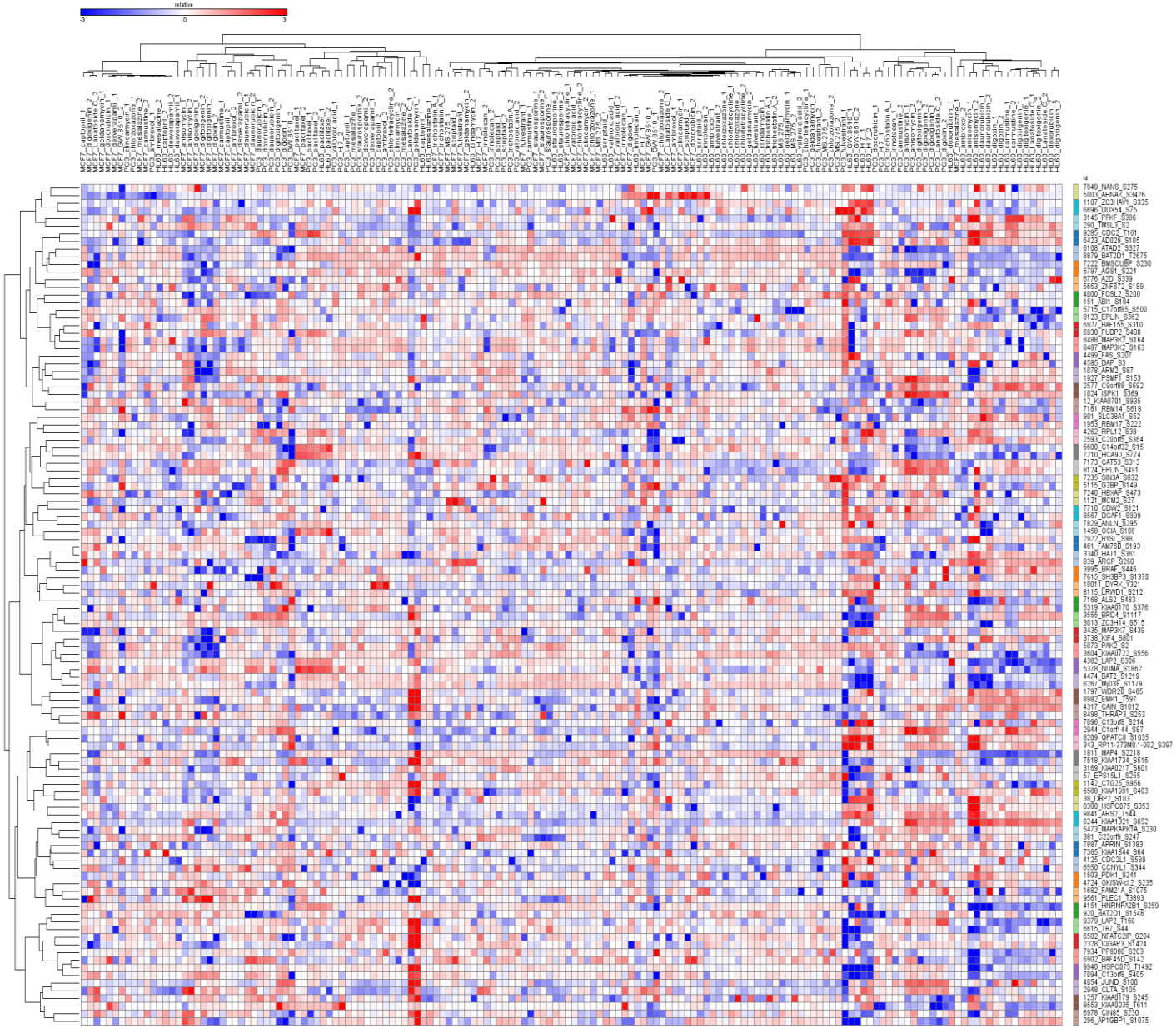
- Largest systematic set of perturbations with phosphoproteomics readout in existence
  - Over 10,000 phosphosites observed
- Over 1,200 sites present in >75% of all experiments

Jinal Patel, Xiaodong Lu

Dimensionality reduction: >1000 to 55 with 2x probe redundancy



Roger Hu



Now for the hard part…
Building the P100 Assay

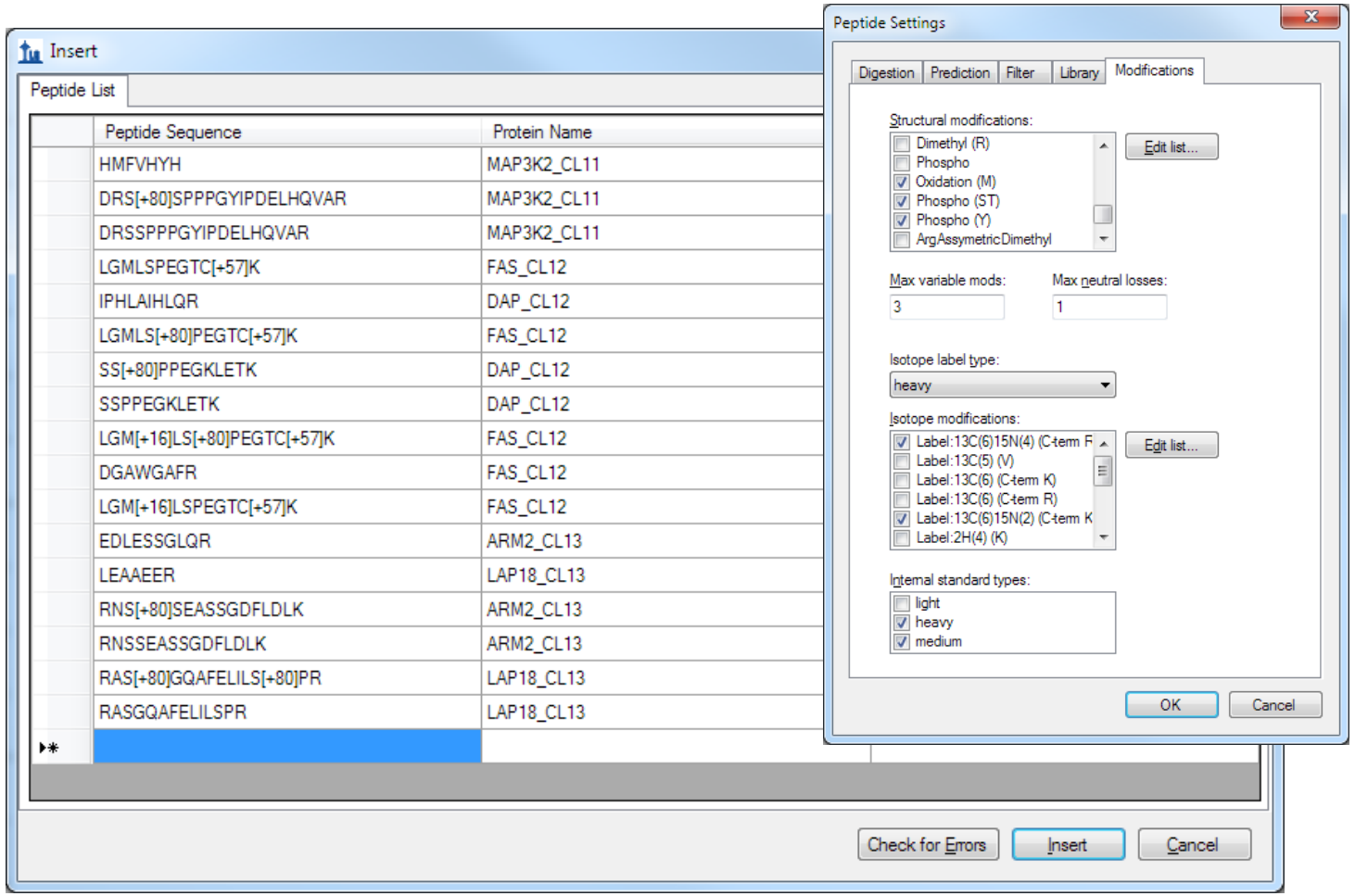
Optimal Phosphosite Quant Requires Multiple Inputs

- Level of phosphopeptide
  - Picked “best” representative peptides from each cluster
    - Observability, site localization, etc.
- Level of non-phosphophorylated cognate
  - Picked based on above
- Level of a non-involved peptide
  - Control for protein level
  - Gleaned from all in-house MaxQuant data

Assay Optimization Strategy

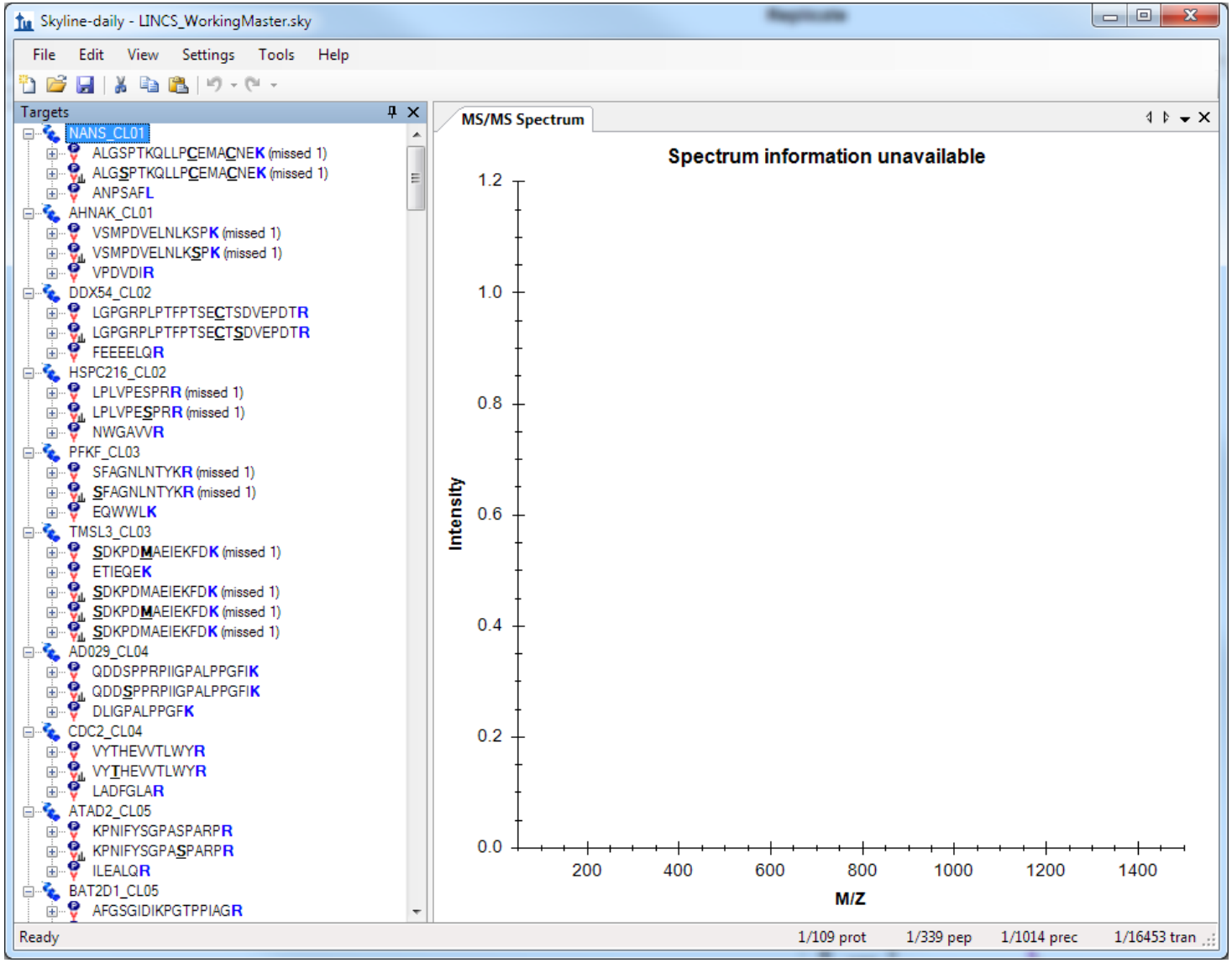
- ✓ Identify and order peptides
- Configure Skyline document
  - Sequence identities
  - Spectra libraries
- Characterize peptides
  - QC + establish RT properties
- Refine spectral libraries
- Configure final assay

Step 1: Get Sequences into Skyline

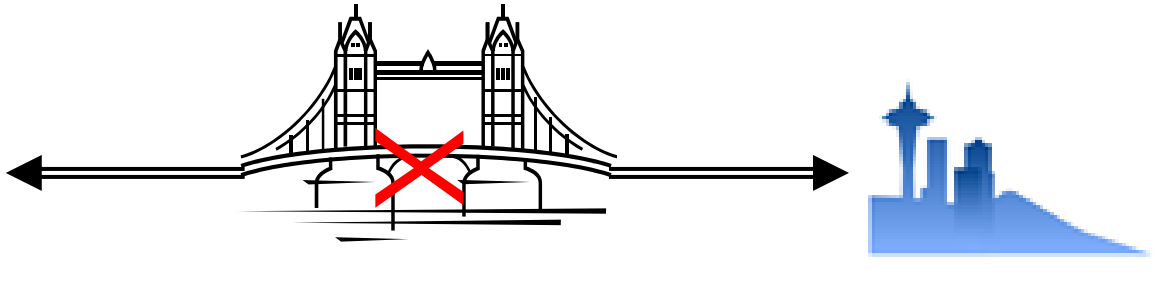


- Peptide shorthand makes it easy to insert modified sequences
  - Make sure modifications are present in document!

Entire assay document configured in one fell swoop

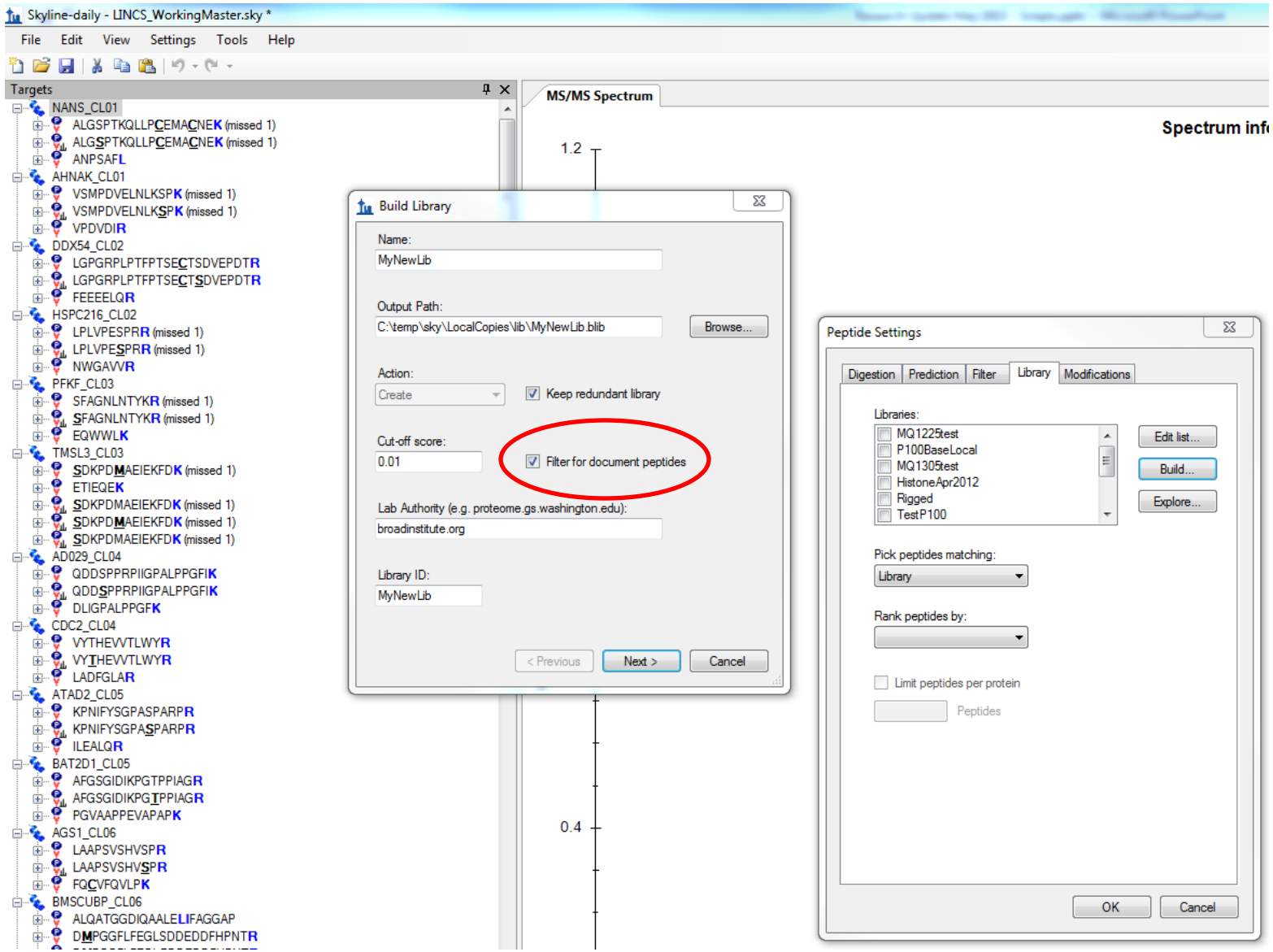


Discovery and Targeted Platforms are Different

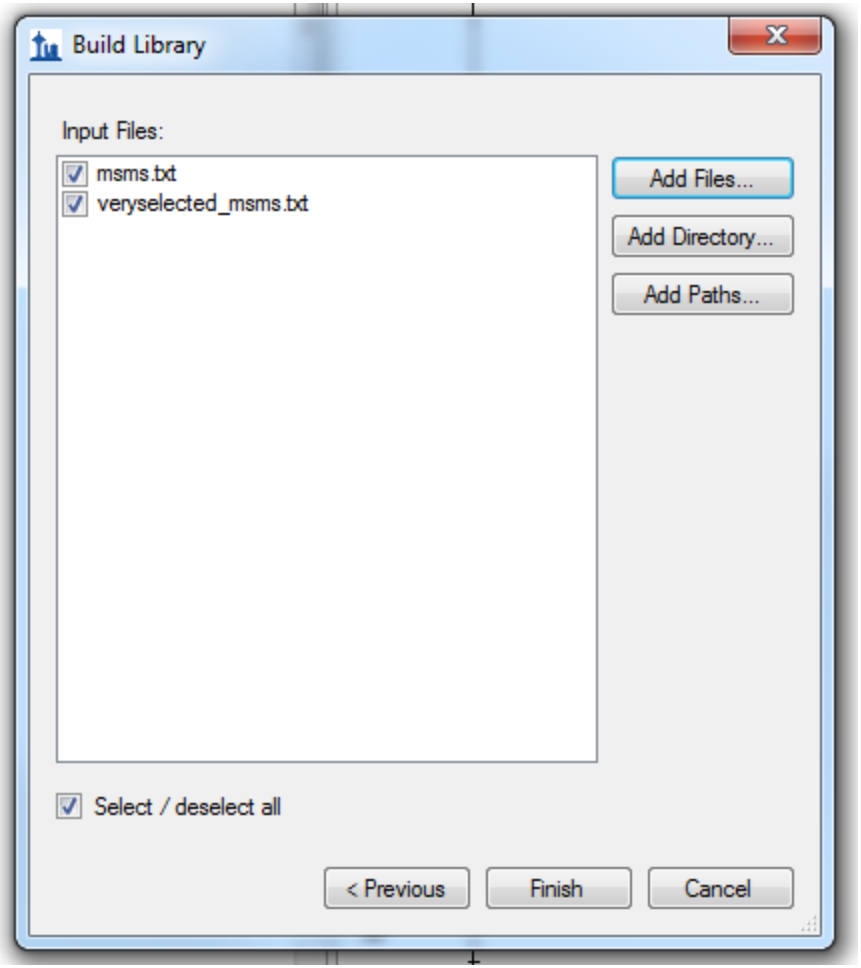


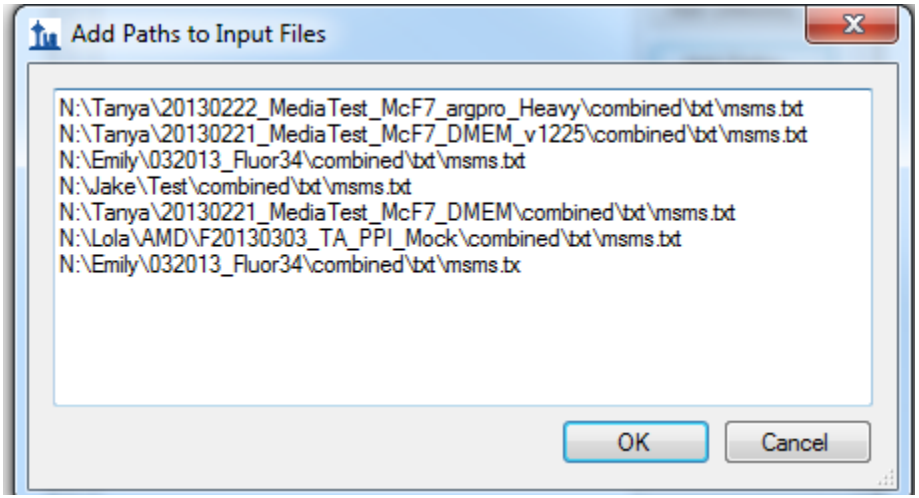
- MaxQuant
  - Used for all discovery data processing
  - Performed all SILAC quantification
  - MEGA result file
    - MS/MS table (txt)
    - 2.8 GB
- Skyline
  - Targeted proteomics environment of choice
  - ***Now supports MaxQuant / Andromeda formats***

Skyline Spectral Library Building

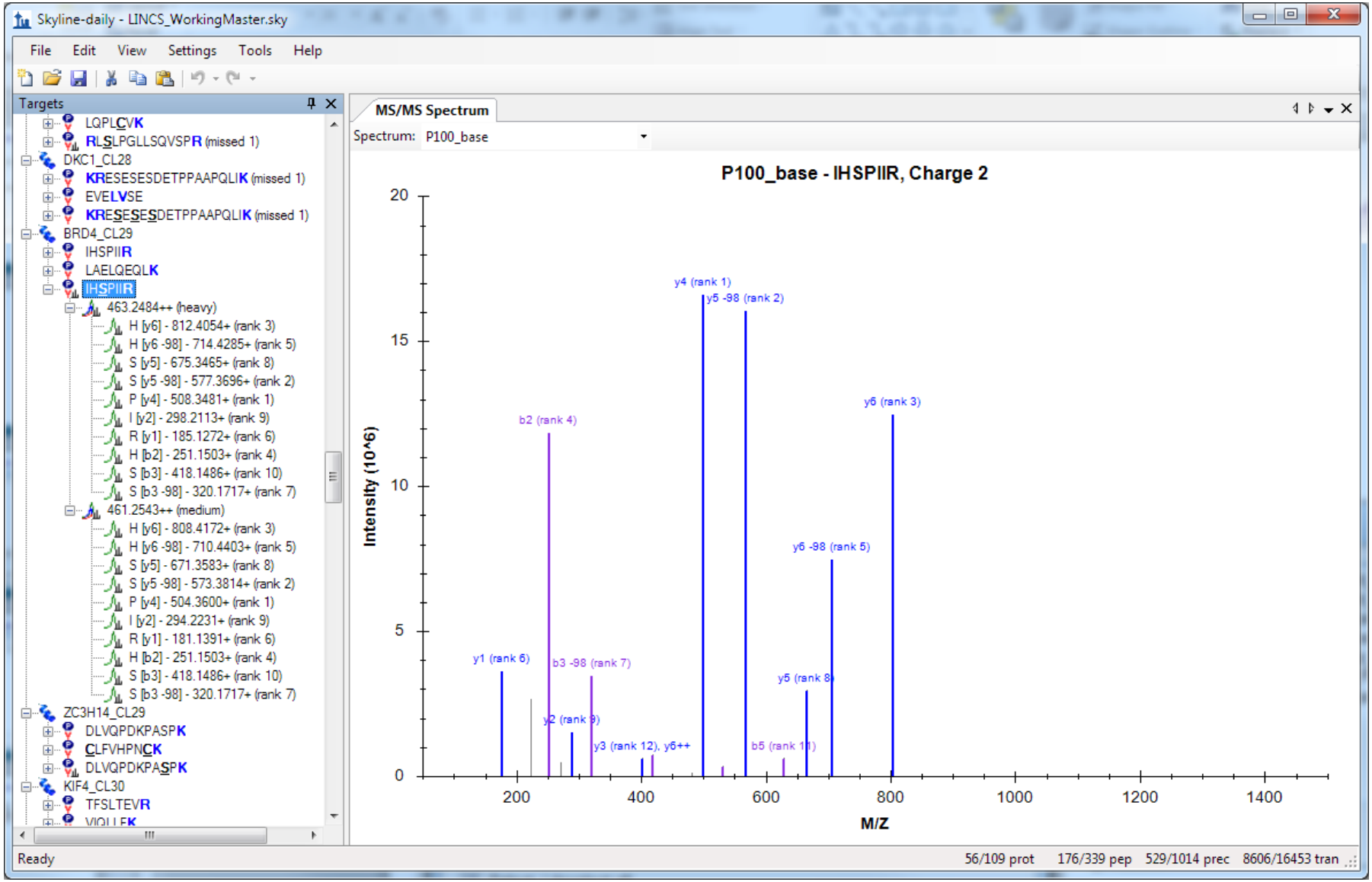


Easily done even when data are distributed





Instant Focused Spectral Library from > 2.8Gb Raw Spectra

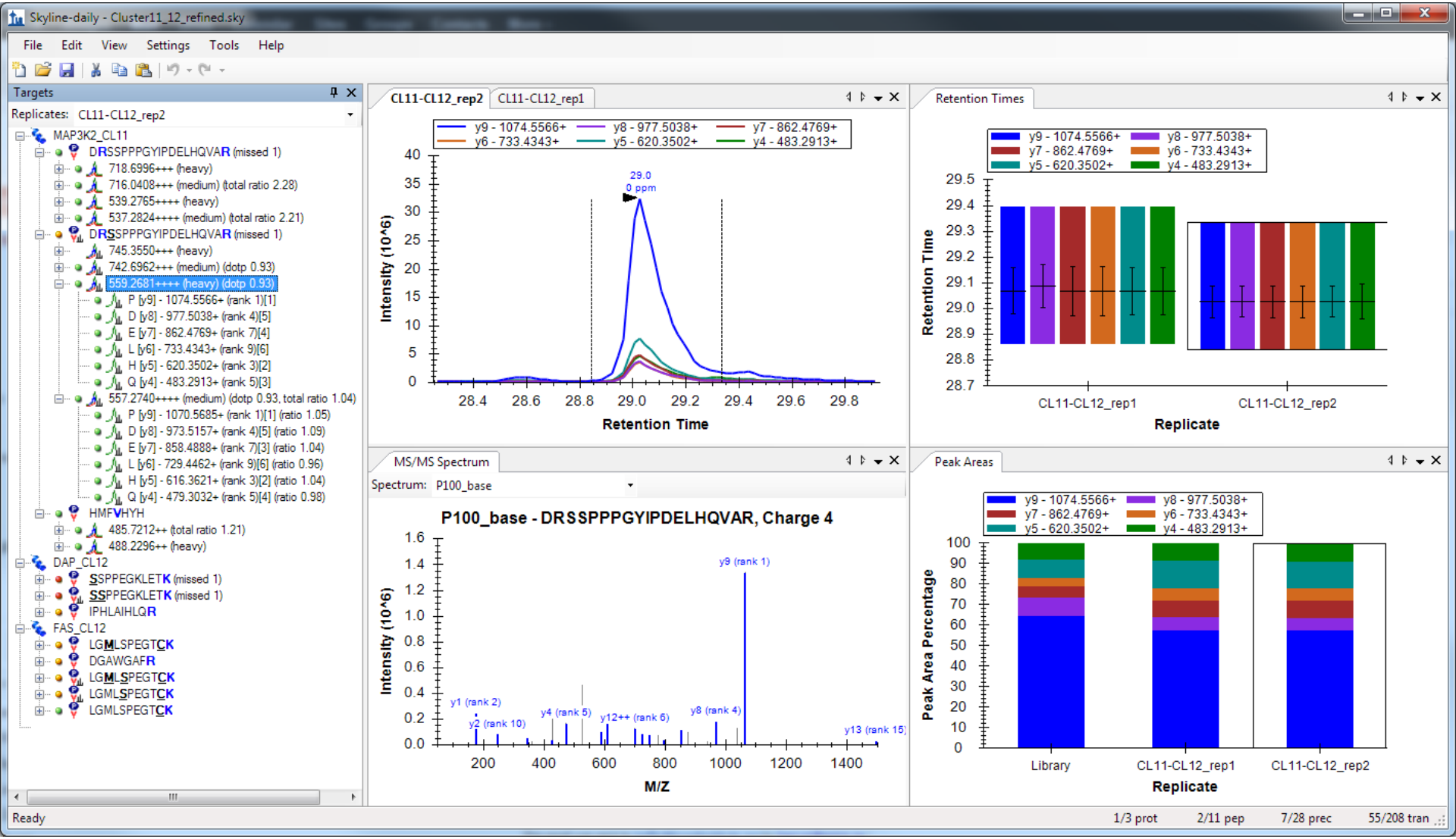


Peptide QC and RT Establishment

- Run mixtures of peptides conforming to analysis clusters
  - QExactive MS
  - Inclusion lists pulled from Skyline doc
  - Unscheduled…but fully targeted

- Full-scan hi-res MS enables proof positive ID
  - Allows comparison to library as a whole spectrum
  - Mass accuracy increases confidence
  - Can observe potential interferents when porting to triple quad

Full-scan QC allows for proof-positive refinement



Assay Optimization Strategy Revisited

- ✓ Identify and order peptides
- ✓ Configure Skyline document
  - Sequence identities
  - Spectra libraries
- ✓ Characterize peptides
  - QC + establish RT properties
  - Will later use iRT strategy for assay scheduling
- ✓ Refine spectral libraries
- ■ Configure final assay

Distribution of the assay with Panorama

- All of our work will be available to other labs
  - To implement the assay at multiple sites
- Ensure data analysis consistency
- Clearing house of assay data
- Communication with other project data infrastructures

Conclusions

- Skyline has contributed immensely to rapid configuration of our assay:
  - Easy sequence import – even for modified peptides
  - Easy spectral library creation based on discovery data
  - Helpful in validation of synthetic peptides
  - Sets groundwork for scheduling targeted assays
  - Makes assay portable
  - Panorama extension will make assay distributable

- Feedback / support cycle of Skyline development can drive projects in real time

Acknowledgements

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