

# Agenda

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- ▶ Welcome from the Skyline team!
- ▶ **Targeted Method Design**
- ▶ Introduction with Brendan MacLean
- ▶ Tutorial with Brendan MacLean
  - ▶ Targeted proteomics method design tutorial
- ▶ Sneak preview of small molecule targeting with Brian Pratt
- ▶ Audience Q&A – submit questions to Google Form:  
<http://tinyurl.com/QA4Skyline> (also in chat window)



# Chromatography-based Quantification

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- ▶ Hypothesis testing (Verification)
- ▶ SRM
- ▶ MSI chromatogram extraction
- ▶ PRM
- ▶ Data independent acquisition (DIA/SWATH)



Acquisition	Targeted	Survey
More Selective	PRM	DIA
Less Selective	SRM	MSI

**Got HYPOTHESIS??**

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# Where do I get a hypothesis?

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- ▶ **Discovery experiments**
  - ▶ DDA (MSI filtering, spectral counting, isobaric tags)
  - ▶ DIA (proteome-wide targeting or search)
- ▶ **Literature**
  - ▶ Studying a known pathway
  - ▶ Test or refine reported biomarkers
- ▶ **Studying a functional subset of proteins**
- ▶ **Studying PTM stoichiometry**
- ▶ **Measuring impact of genetic mutation**
- ▶ **Verifying protein knockdown**
- ▶ **Further reduce prior multiple hypothesis testing**



# On Error Rates

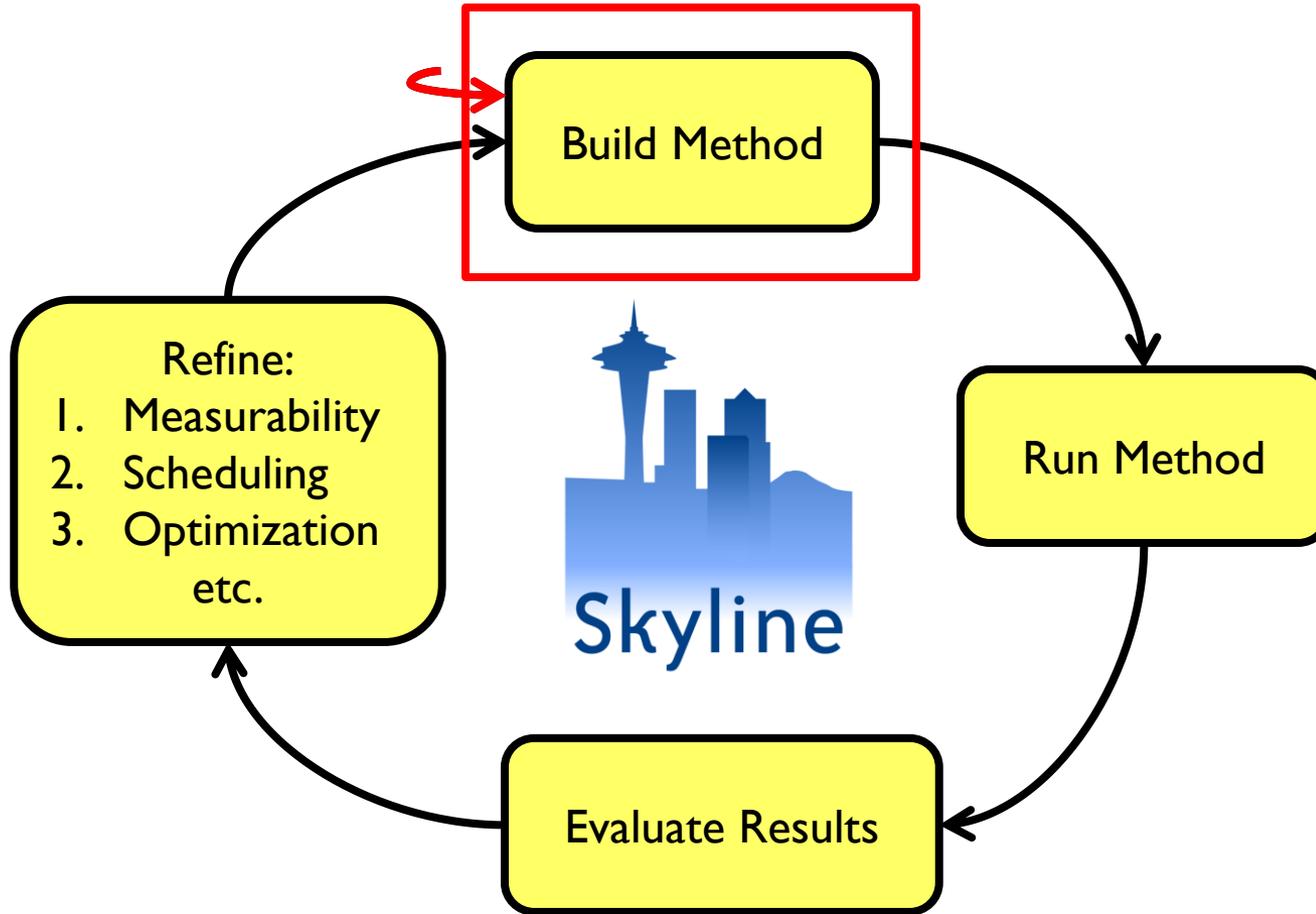
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- ▶ Discovery runs identify 4,500 peptides with 1% FDR
- ▶ Each is assigned a quantitative value from 10 runs
- ▶ 100 seem to be changing by at least 2-fold
- ▶ 90 are already explained by the literature
- ▶ 10 seem novel and exciting
  
- ▶ 1,000 labs have the same experience
  
- ▶ What is the error rate among the 10,000 novel peptides?



# Targeted Method Refinement

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# Sources of Targets

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- ▶ **Spectral libraries**
  - ▶ Personal (built from spectrum matching results)
  - ▶ Public (Peptide Atlas, Global Proteome Machine, NIST)
- ▶ **FASTA files**
  - ▶ Background proteome
  - ▶ Importing FASTA sequences
- ▶ **Protein lists**
- ▶ **Peptide lists**
- ▶ **Transition lists**
- ▶ **Assay libraries (transition list + iRT + intensities)**
- ▶ **Chromatogram libraries**



# Tutorial

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- ▶ Targeted Method Design in Skyline



# Learn More

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- ▶ **Webinar #5: Targeted Method Refinement**
  - ▶ Tuesday, March 10.
- ▶ **Weeklong Course at PRBB, Barcelona 2014 – videos**
  - ▶ <http://tinyurl.com/skyline-embo-2014>
- ▶ **Workshops at US HUPO and ASMS**
- ▶ **Weeklong Course in Boston**
  - ▶ May 18-22 (register by March 10<sup>th</sup>)
- ▶ **Weeklong Course at ETH, Zurich**
  - ▶ June 22-26 (register by February 28<sup>th</sup>)
- ▶ **Weeklong Course at PRBB, Barcelona**
  - ▶ November 15-20



# Questions?

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- ▶ Ask any questions you have on this month's topic, Targeted Method Design, at the following form:

<http://tinyurl.com/QA4Skyline>

- ▶ Take the post-webinar survey

<http://tinyurl.com/Survey4Webinar>

