Northeastern University

May Institute goes ONLINE!
Computation and statistics for mass spectrometry and proteomics

May 3 – May 14, 2021, Northeastern University, Boston MA
Organizer: Olga Vitek

May 5, 2021

11:00am-2:15pm **Hands-on, Brendan MacLean:** Part 2: Skyline-based comparison of refined versus proteome-wide queries of DIA experiments

Starting soon

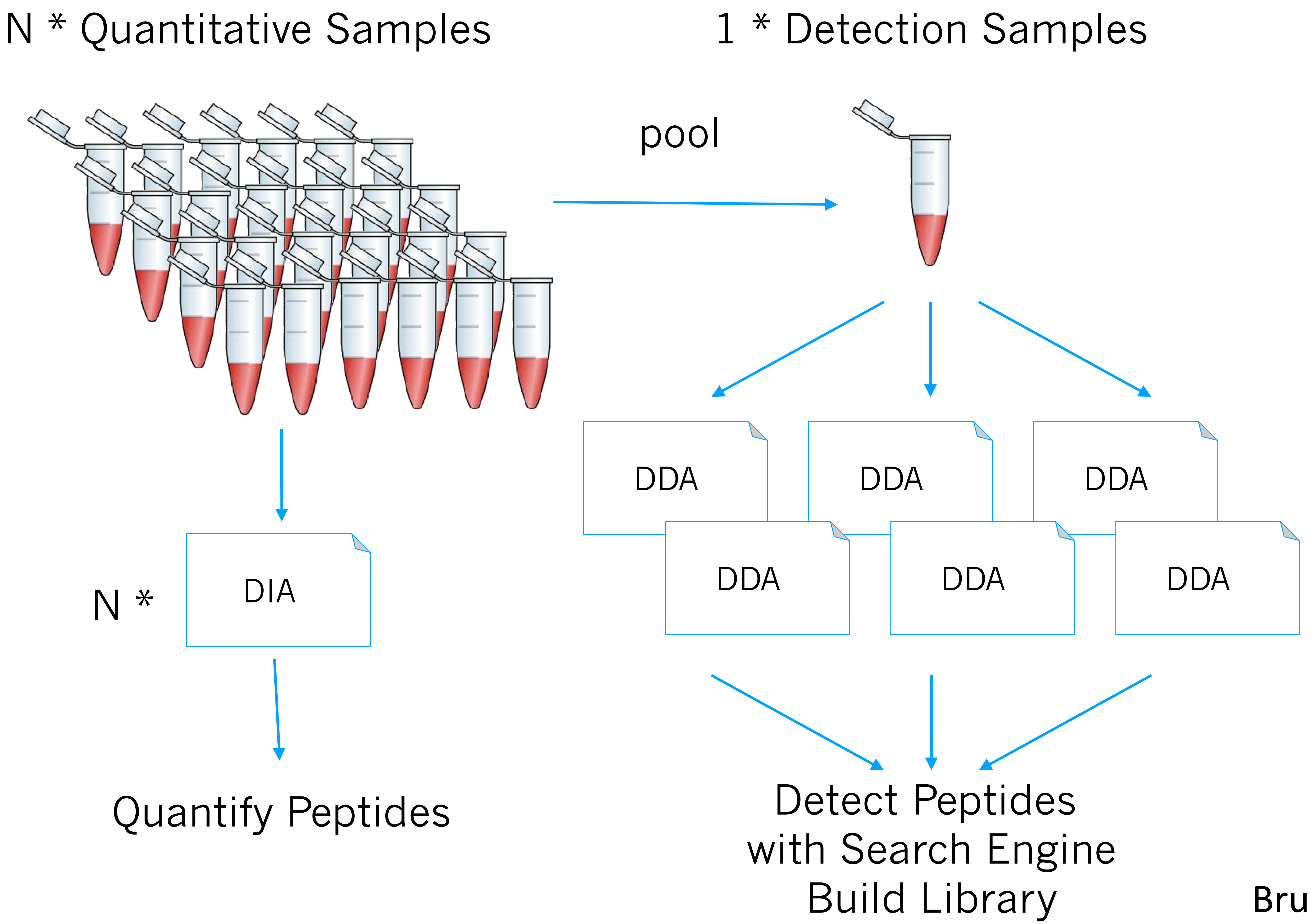
Materials at
https://computationalproteomics.khoury.northeastern.edu/

NIH National Institutes of Health

Chan Zuckerberg Initiative

ASA Boston Chapter

Two Phase Experiment



N * Quantitative Samples

1 * Detection Samples

pool

DDA

DDA

DDA

DDA

DDA

DDA

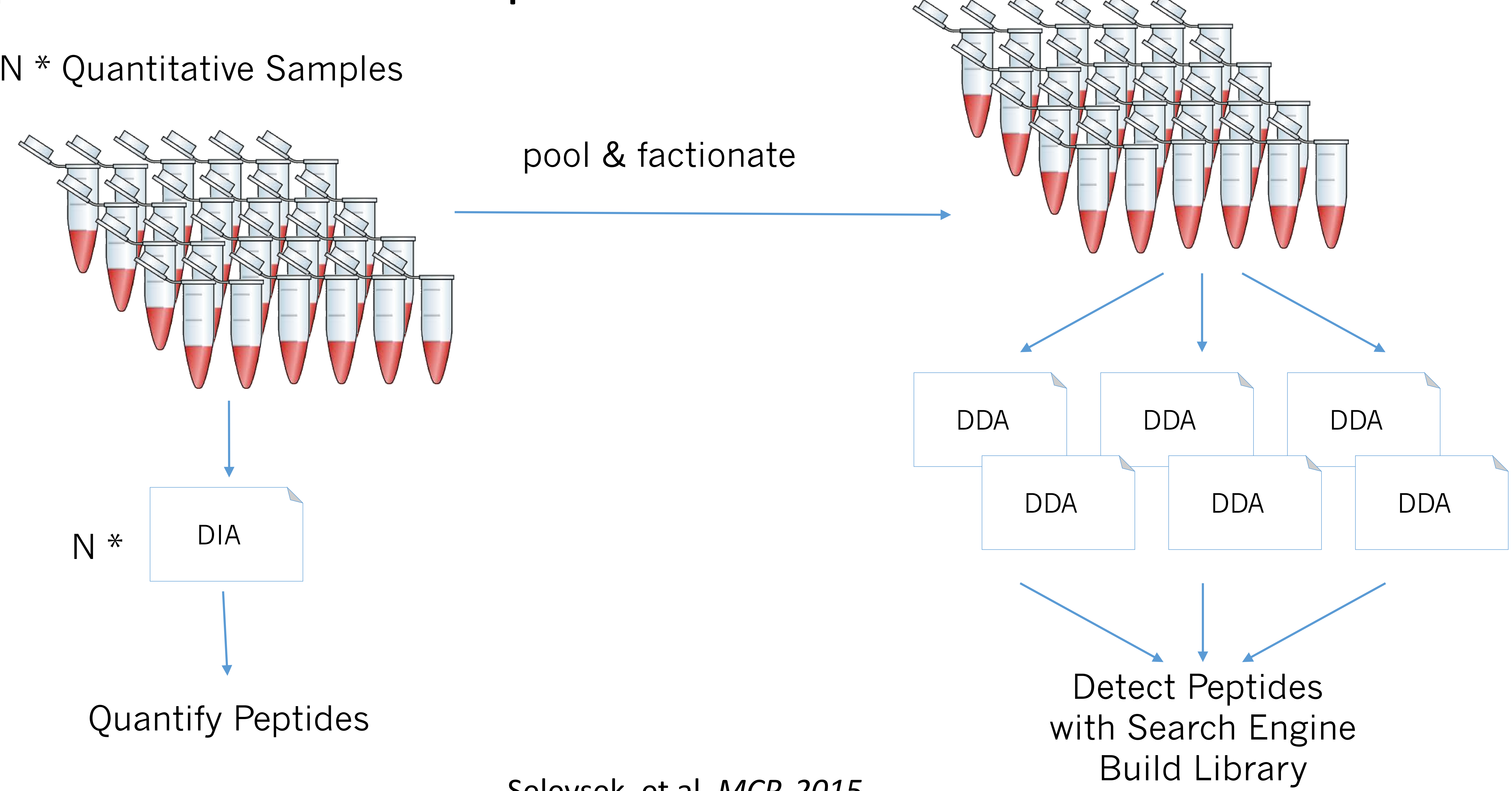
N * DIA

Quantify Peptides

Detect Peptides
with Search Engine
Build Library

Bruderer, et al. & Reiter, *MCP, 2015*

Deep Two-Phase Experiment



M * Detection Samples

N * Quantitative Samples

pool & factionate

DDA

DDA

DDA

DDA

DDA

DDA

N * DIA

Quantify Peptides

Detect Peptides
with Search Engine
Build Library

Selevsek, et al. *MCP, 2015*

Note on DDA Search Parameters

“For the search, we allowed for semi-tryptic digests and up to two missed cleavages per peptide, and we used carbamidomethylation as a fixed modification on cysteine and oxidation as variable modification on methionine residues. The Sequest and Mascot search results were converted to pep.xml and then combined using iProphet.”

Selevsek, MCP 2015

Note on FDR

“iProphet probability and filtered at 1% FDR by decoy counting (iProphet score cut-off 0.0242) at the peptide spectrum matches (PSM) level.”

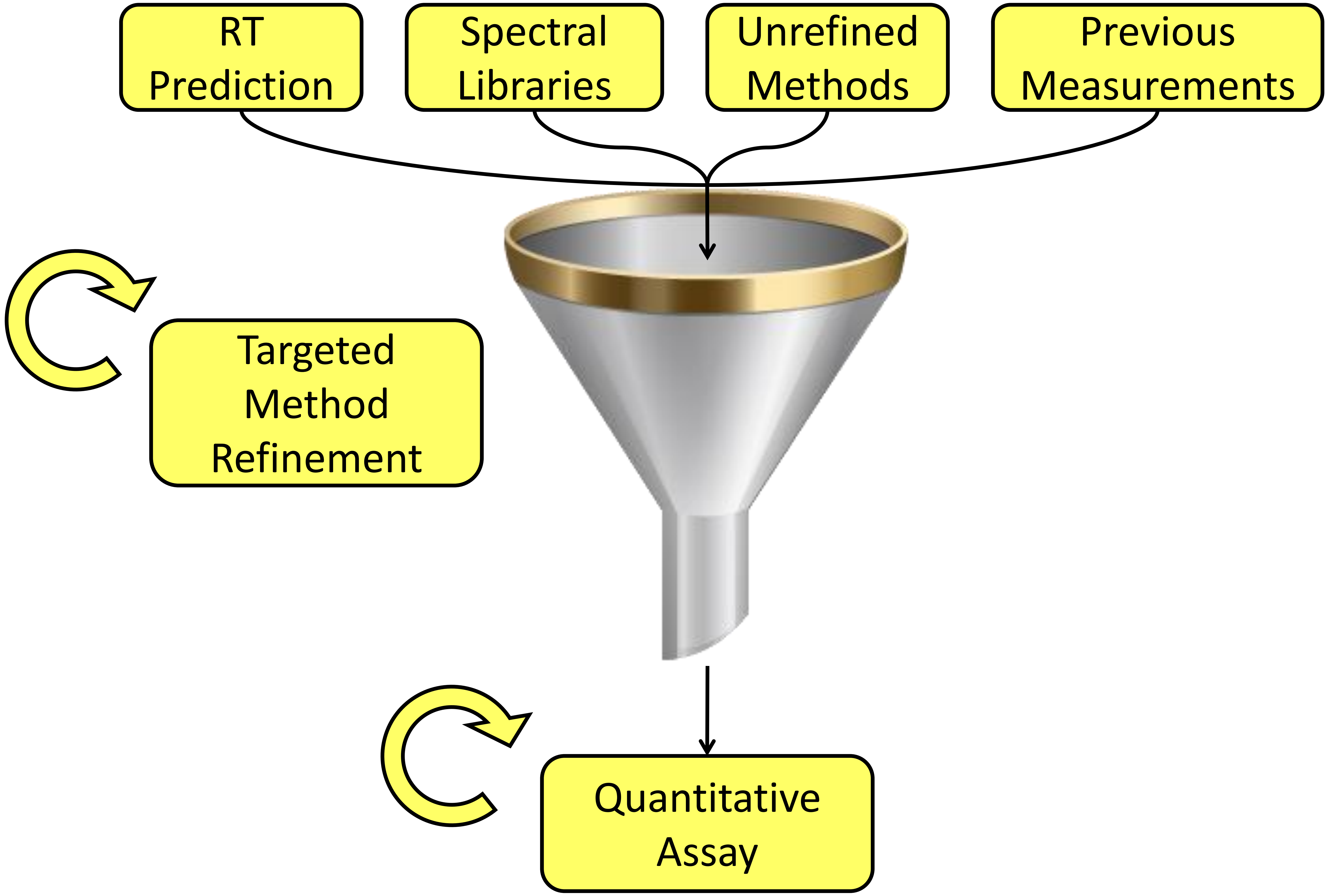
Selevsek, MCP 2015

Note on the Assay Library

“top 3–4 most intense (singly or doubly charged) y or b fragment ions … library comprised 331′449 transitions for 83′520 proteotypic precursors (66′007 unique modified peptides matching to 4′596 unique protein isoforms).”

Selevsek, MCP 2015

Supporting a Broader Hypothesis



RT Prediction

Spectral Libraries

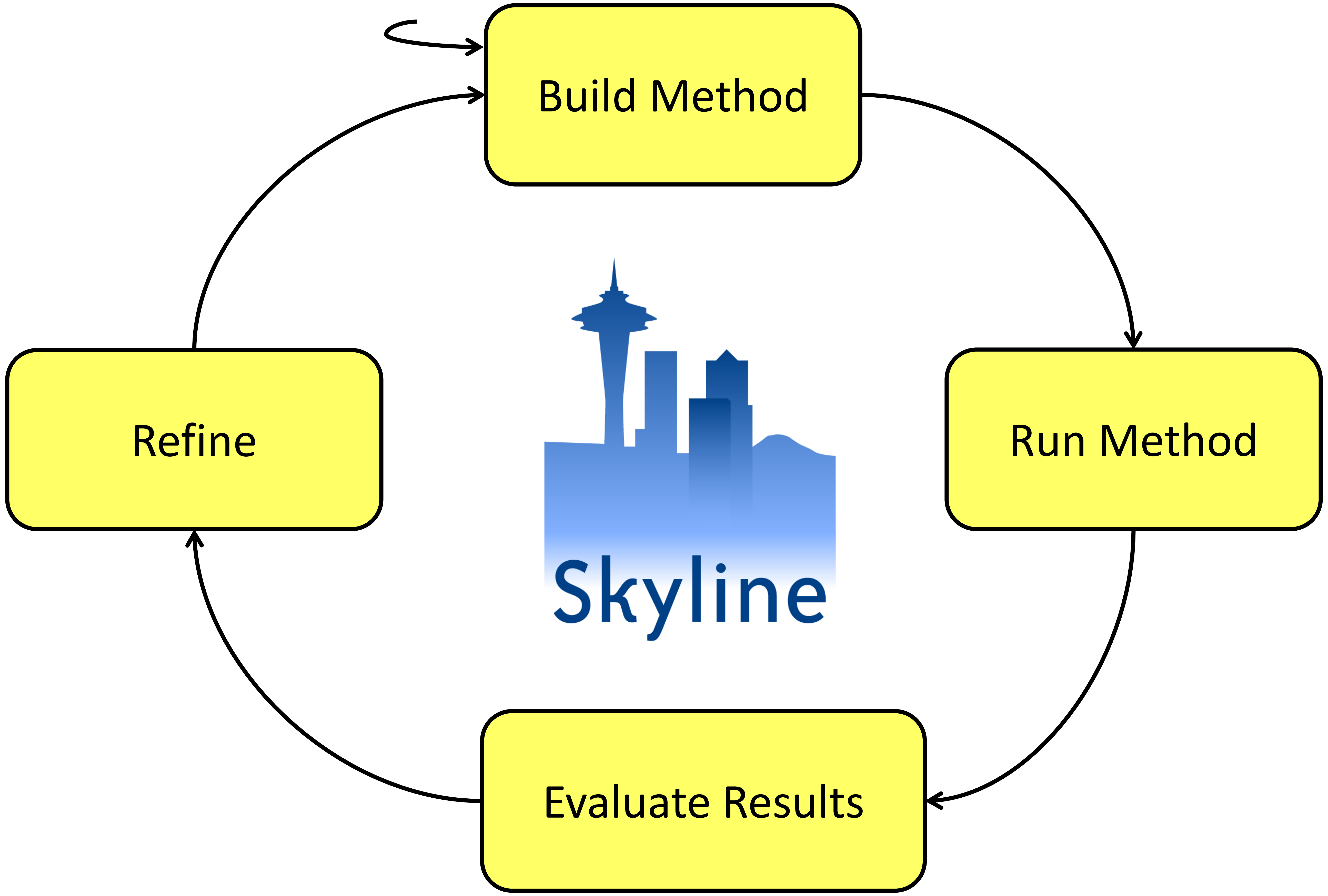
Unrefined Methods

Previous Measurements

Targeted Method Refinement

Quantitative Assay

Targeted Method Refinement

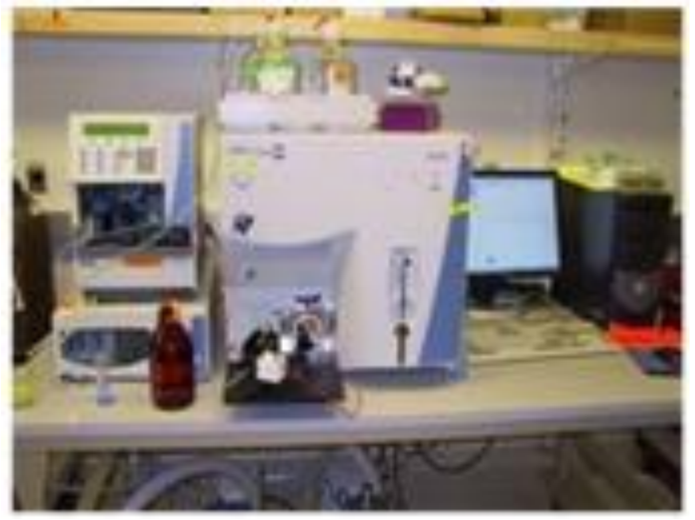


Build Method

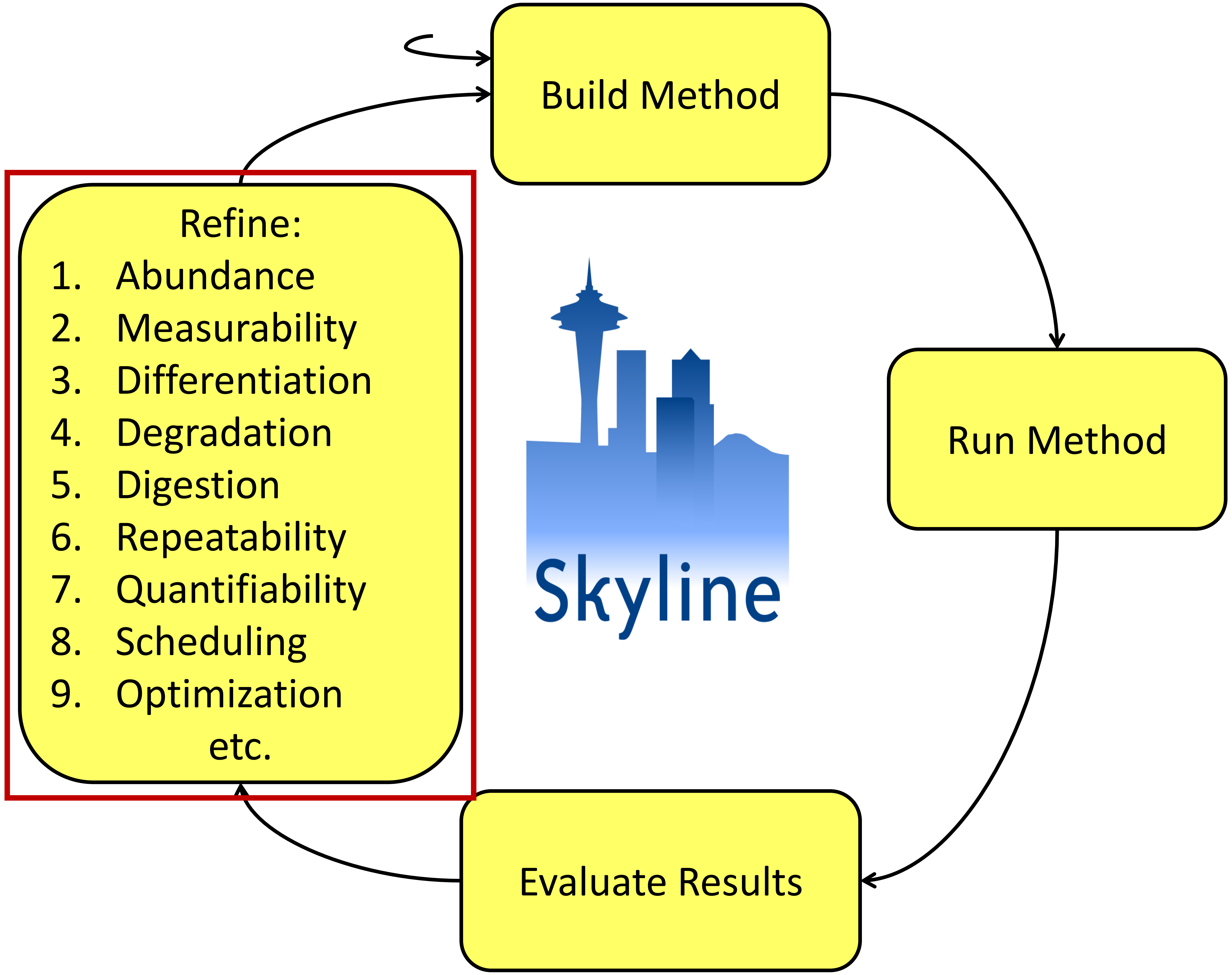
Run Method

Evaluate Results

Refine



Targeted Method Refinement



Build Method

Run Method

Evaluate Results

Refine:

1. Abundance
2. Measurability
3. Differentiation
4. Degradation
5. Digestion
6. Repeatability
7. Quantifiability
8. Scheduling
9. Optimization

etc.