

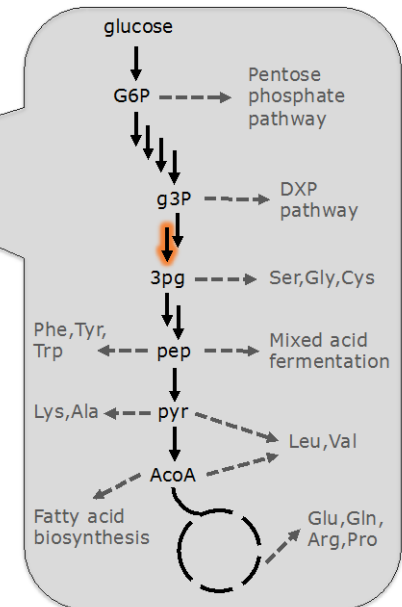
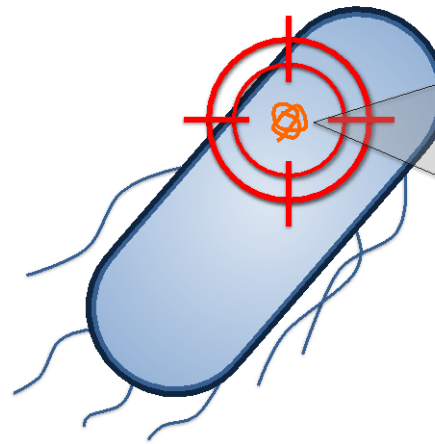
A SKYLINE-BASED WORKFLOW FOR RAPID DEVELOPMENT OF HIGH- THROUGHPUT QUANTITATIVE PROTEOMIC ASSAYS

Chris Petzold

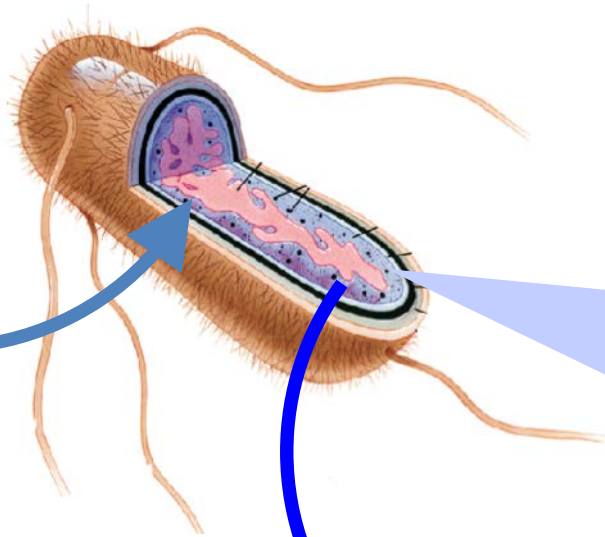
Joint BioEnergy Institute (JBEI)

Lawrence Berkeley National Laboratory
(LBNL)

cjpetzold@lbl.gov



ENGINEERING A CELL FACTORY

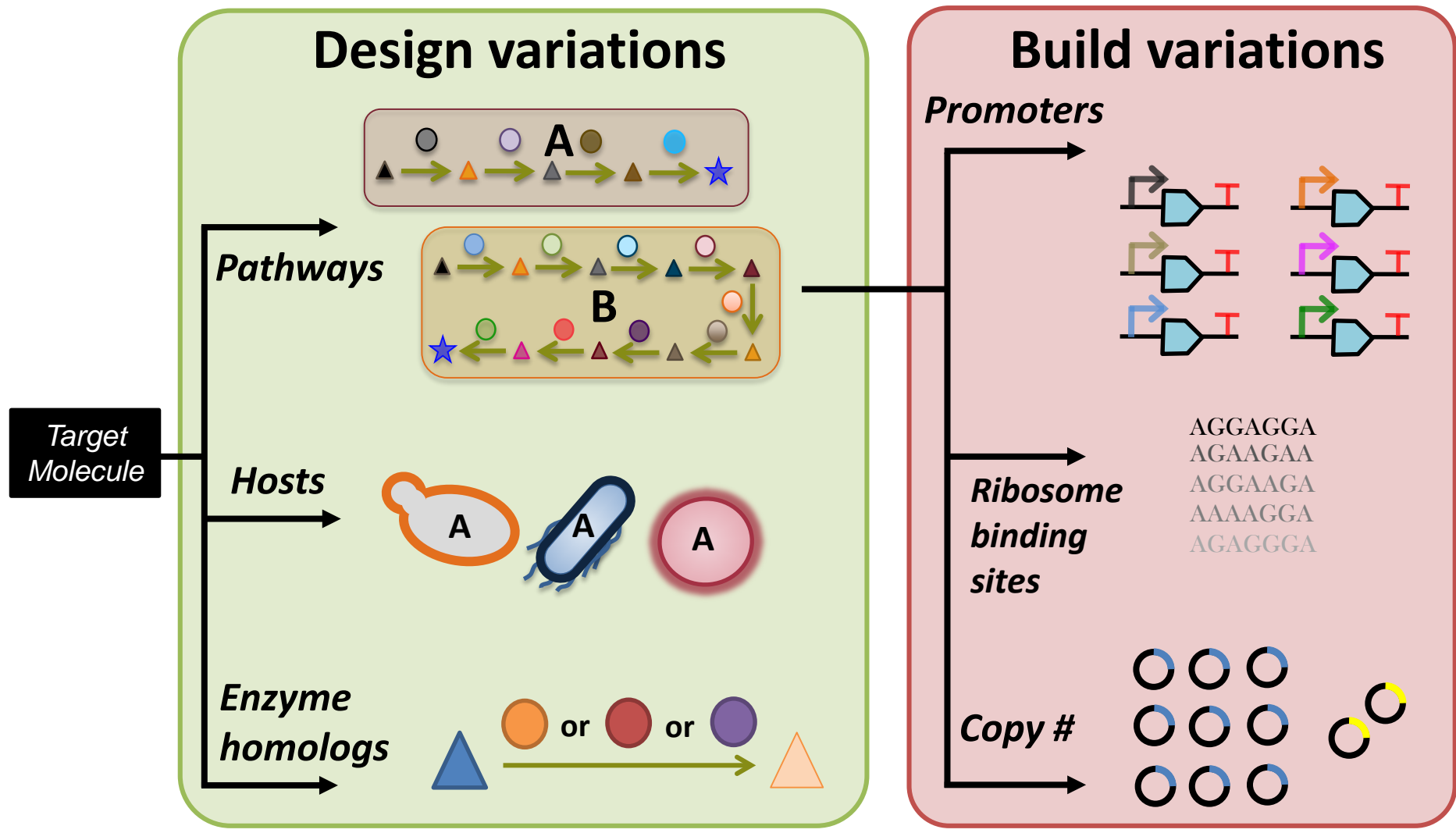


Transportation Fuels

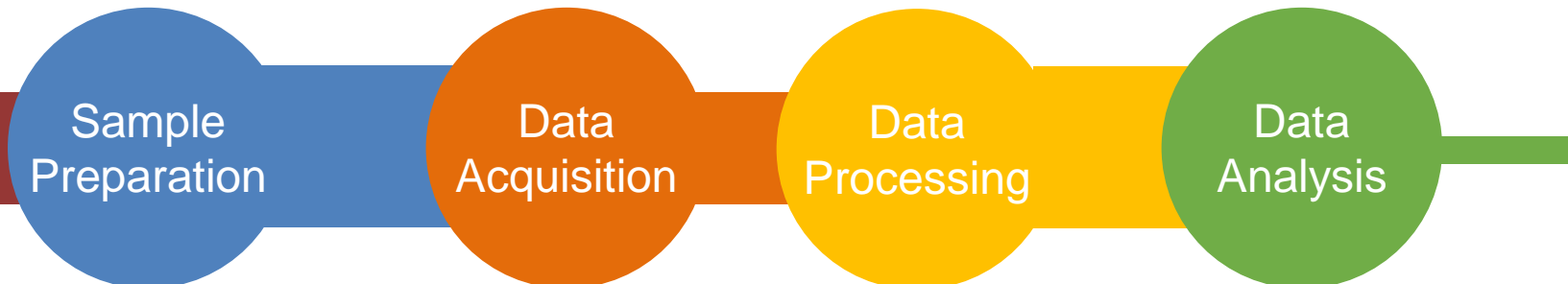
Jet Fuel
Diesel
Gasoline



MANY CHOICES FOR METABOLIC ENGINEERING



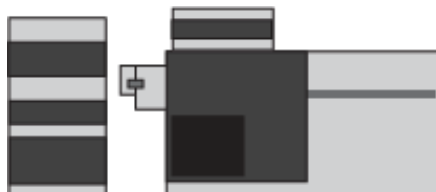
TEST: ANALYTICAL WORKFLOW



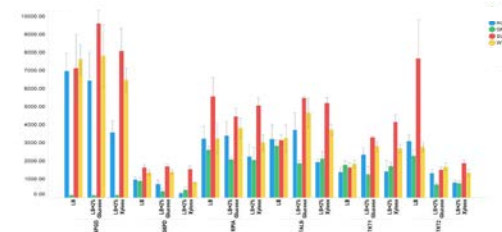
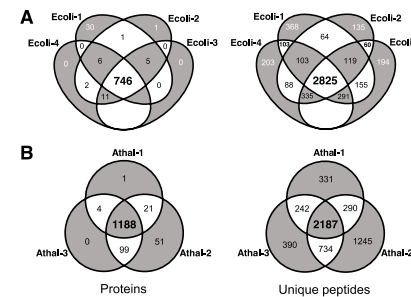
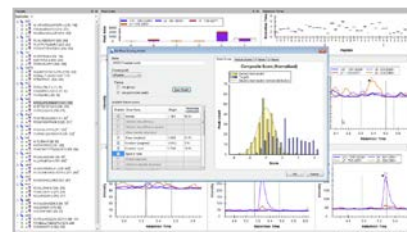
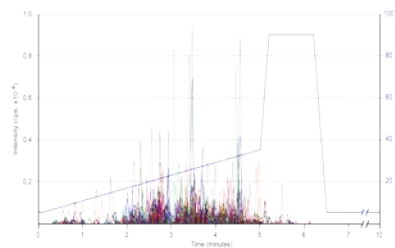
Automated sample preparation



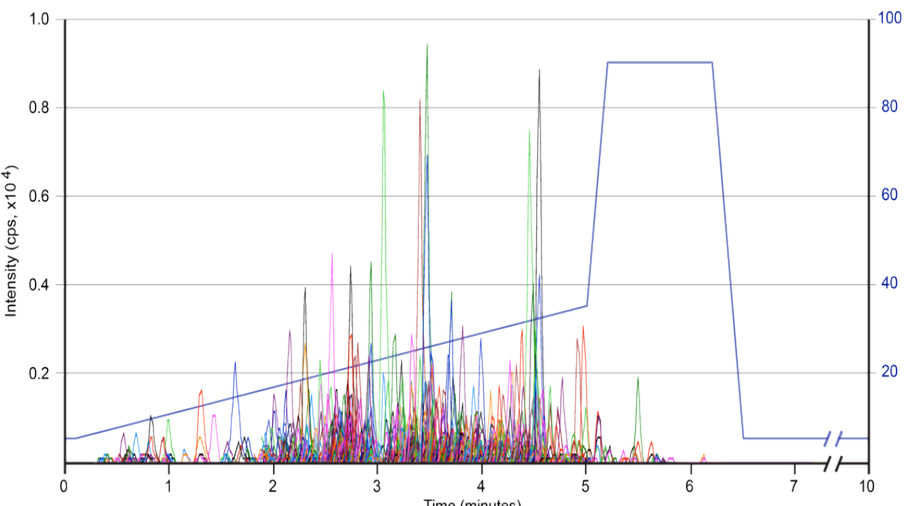
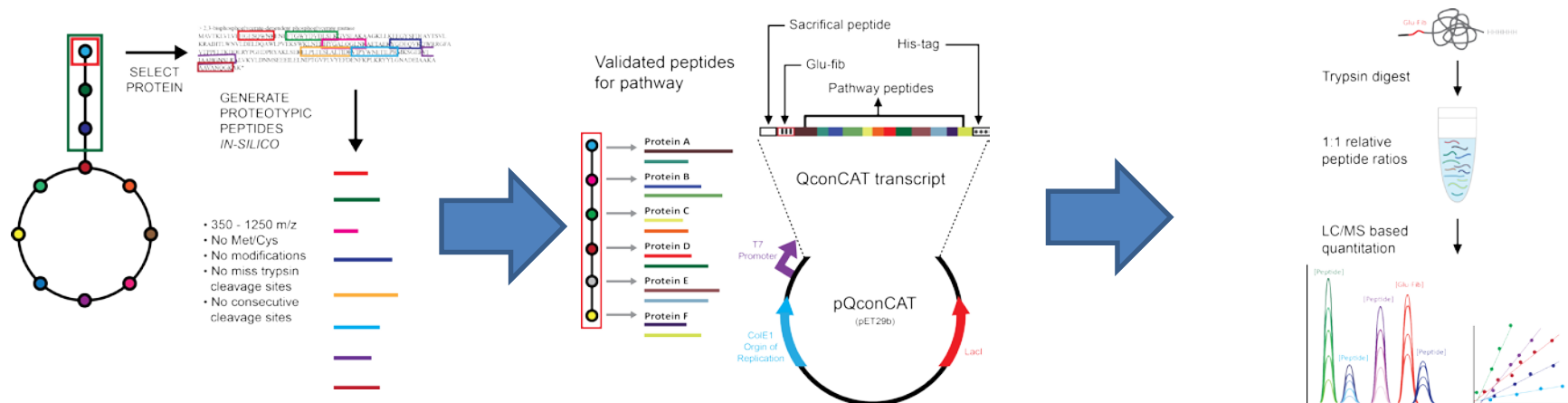
see Poster: ThP 632



HT data acquisition and processing



TARGETED PROTEOMIC METHODS DEVELOPMENT



Extracted Ion Chromatograms of >800 peptides

- Developed fast, robust LC-MS methods to increase throughput:
 - Quantify >800 peptides per sample
 - Sample Throughput = >100 per day

WHAT ABOUT METHODS DEVELOPMENT?

Sample Preparation

Methods Development

Data Acquisition

Data Processing

Data Analysis

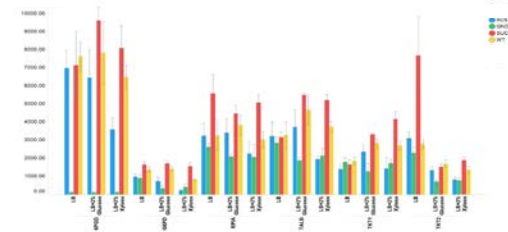
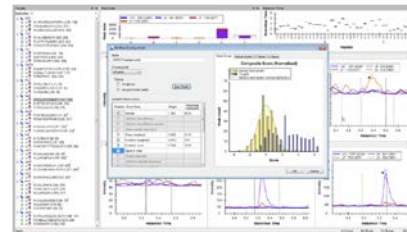
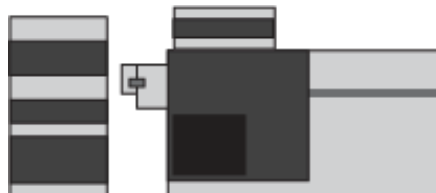
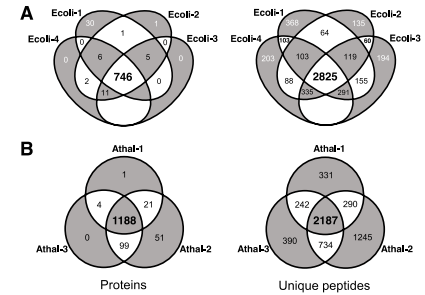
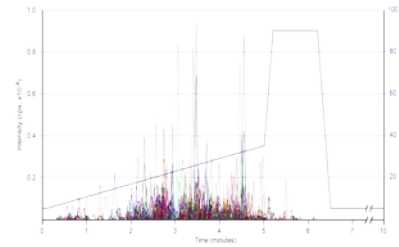
Automated sample preparation



see Poster: ThP 632

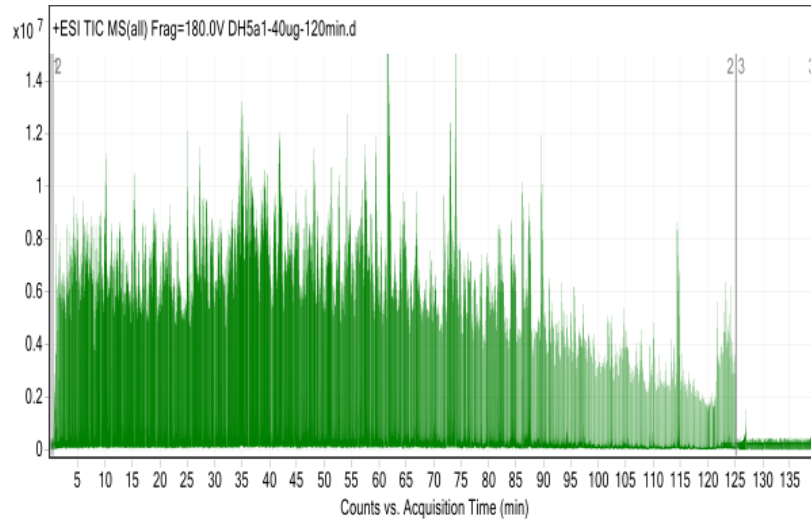


HT data acquisition and processing

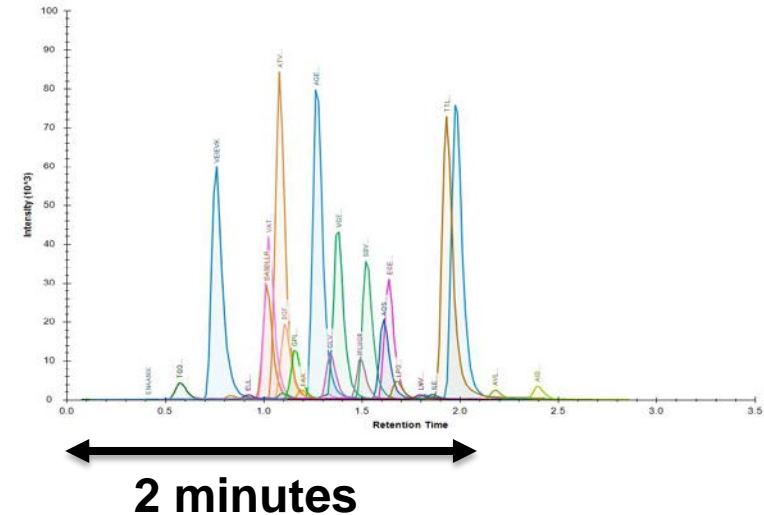


GAP BETWEEN IDENTIFYING AND QUANTIFYING YOUR TARGETS

Discovery Proteomics



Targeted Proteomics



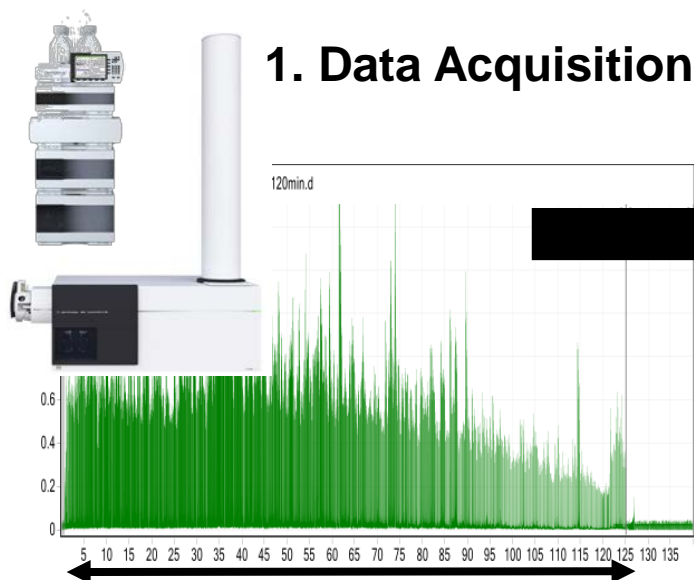
Challenges

- Different instrumentation
 - Fragmentation conditions
 - Sensitivity and resolution
- Different LC conditions
 - Peptide retention time shift
 - Competition for ionization efficiency



REDUCE METHODS DEVELOPMENT TIME

1. Data Acquisition



2. Process data to identify peptides

Proteins (453) Quantitation (662) Unassigned (1980)

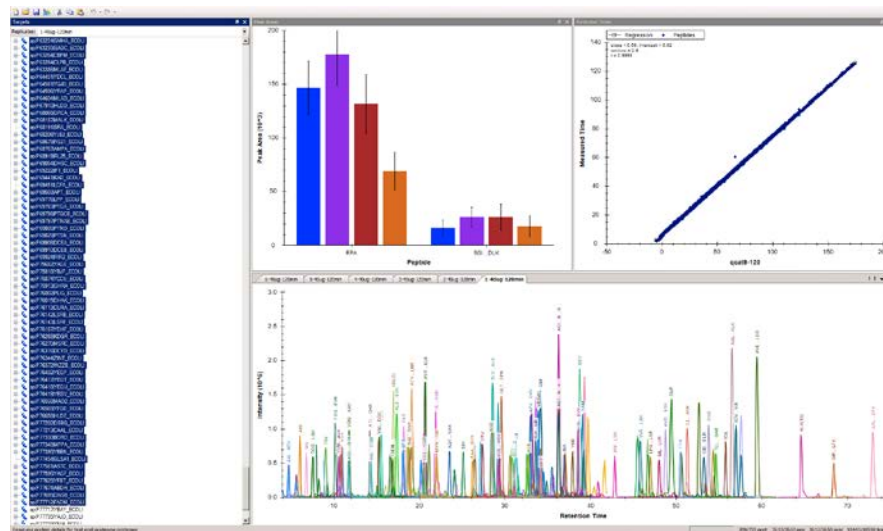
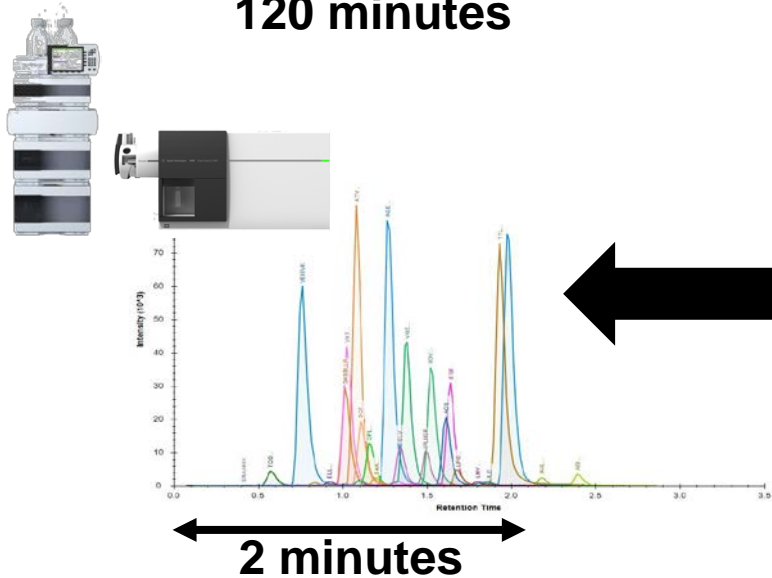
Protein families 1-10 (out of 453)

10 per page 1 2 3 4 5 6 65 Next Expand all Collapse all

Accession Find Match case

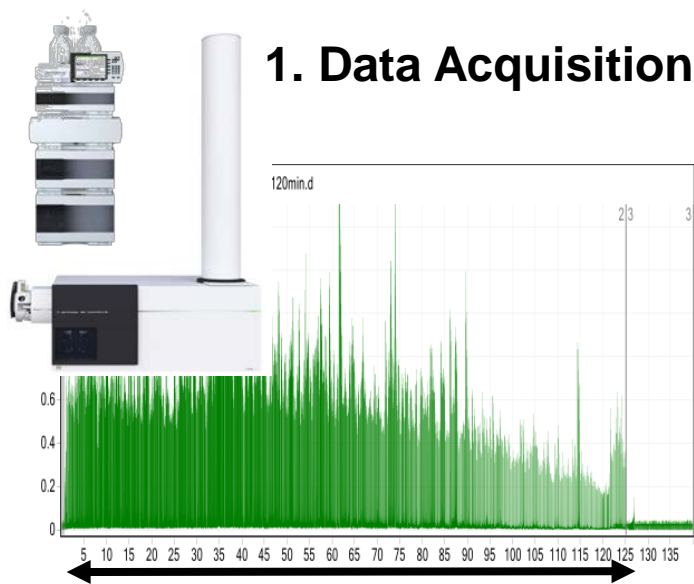
>1	CH60_ECOLI	2580	60 kDa chaperonin OS=Escherichia coli (strain K12) GN+prf PE=1 SV=2
>2	DNAK_ECOLI	2376	Chaperone protein DnaK OS=Escherichia coli (strain K12) GN+drax PE=1 SV=2
>3	ADHE_ECOLI	2126	Aldhyde-ethylol dehydrogenase OS=Escherichia coli (strain K12) GN+adh PE=1 SV=2
>4	PGK_ECOLI	2012	Phosphoglycerate kinase OS=Escherichia coli (strain K12) GN+pgk PE=1 SV=1
>5	ACEA_ECOLI	1986	Isocitrate lyase OS=Escherichia coli (strain K12) GN+ack PE=1 SV=1
>6	EFTU1_ECOLI	1967	Elongation factor Tu 1 OS=Escherichia coli (strain K12) GN+eaf PE=1 SV=1
>7	TMAA_ECOLI	1960	Thyrosinase OS=Escherichia coli (strain K12) GN+taa PE=1 SV=1
>8	RS1_ECOLI	1680	30S ribosomal protein S1 OS=Escherichia coli (strain K12) GN+rpsA PE=1 SV=1
>9	DGAL_ECOLI	1659	D-galactose-binding periplasmic protein OS=Escherichia coli (strain K12) GN+gpb PE=1 SV=1
>10	1 DCEA_ECOLI	1643	Glutamate decarboxylase alpha OS=Escherichia coli (strain K12) GN+gadA PE=1 SV=1
	2 DCEB_ECOLI	1620	Glutamate decarboxylase beta OS=Escherichia coli (strain K12) GN+gadB PE=1 SV=1
	3 METQ_ECOLI	212	D-methionine-binding lipoprotein MetQ OS=Escherichia coli (strain K12) GN+metQ PE=1 SV=2
	4 CLPB_ECOLI	805	Chaperone protein ClpB OS=Escherichia coli (strain K12) GN+clpB PE=1 SV=1
	5 YXC_F_ECOLI	41	YnfX protein YnfX OS=Escherichia coli (strain K12) GN+ynfX PE=1 SV=1

3. Build Spectral Library 4. iRT Prediction

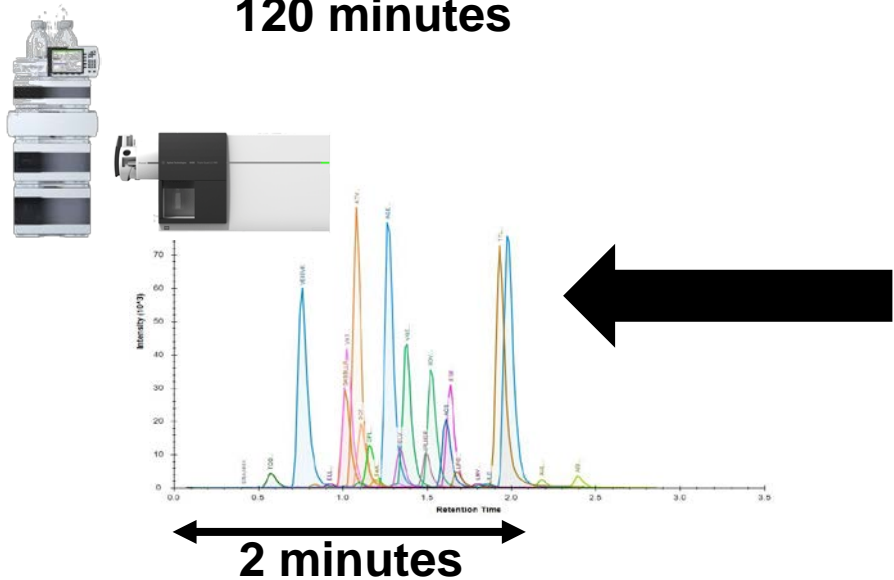


REDUCE METHODS DEVELOPMENT TIME

1. Data Acquisition



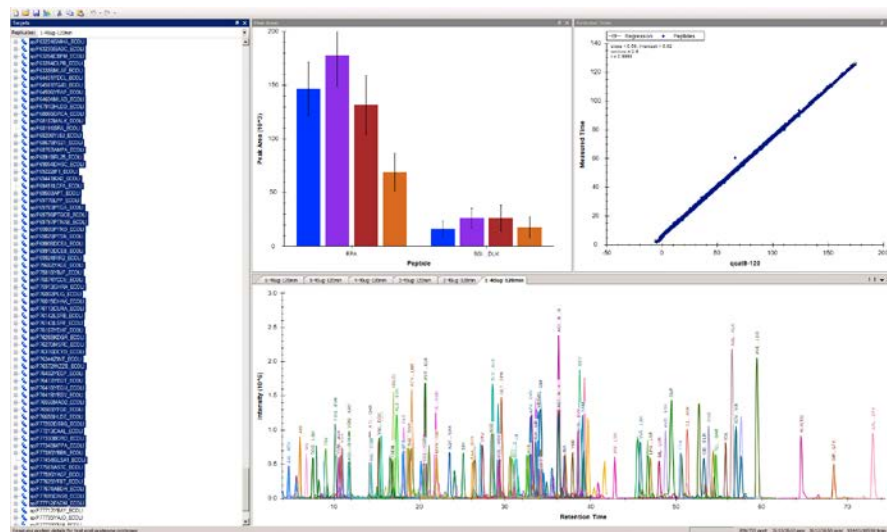
120 minutes



2 minutes

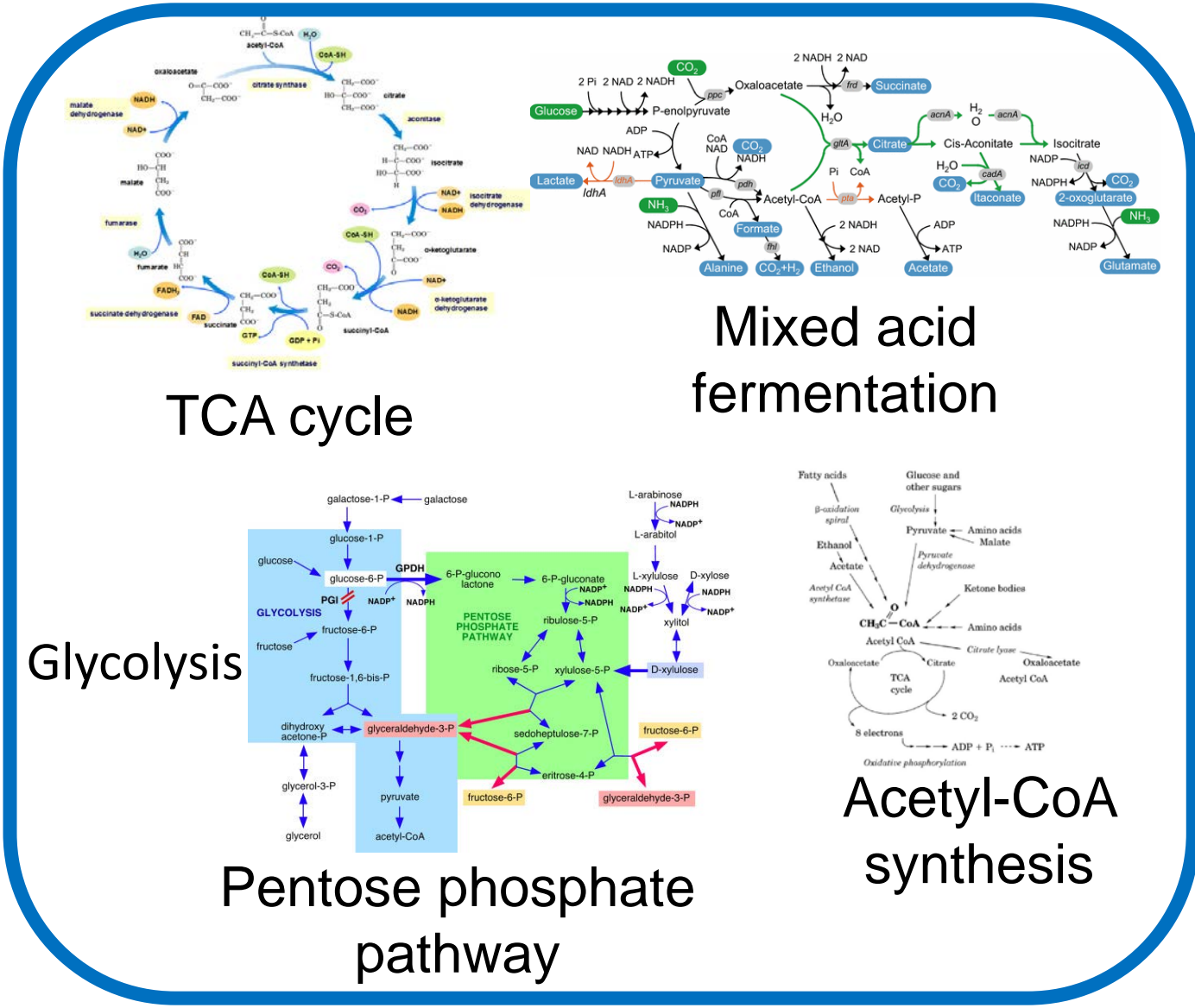
Advantages of spectral libraries:

- As more samples are analyzed, the library become more comprehensive
- Aids data management, transfer and sharing in the proteomic community
- Supports development of quantitative methods



QUANTIFICATION OF MAJOR METABOLIC PATHWAY PROTEINS IN *E. COLI* SINGLE GENE KO STRAINS

Utilize the *E. coli* KEIO single gene knock out mutant library



TCA cycle

Mixed acid fermentation

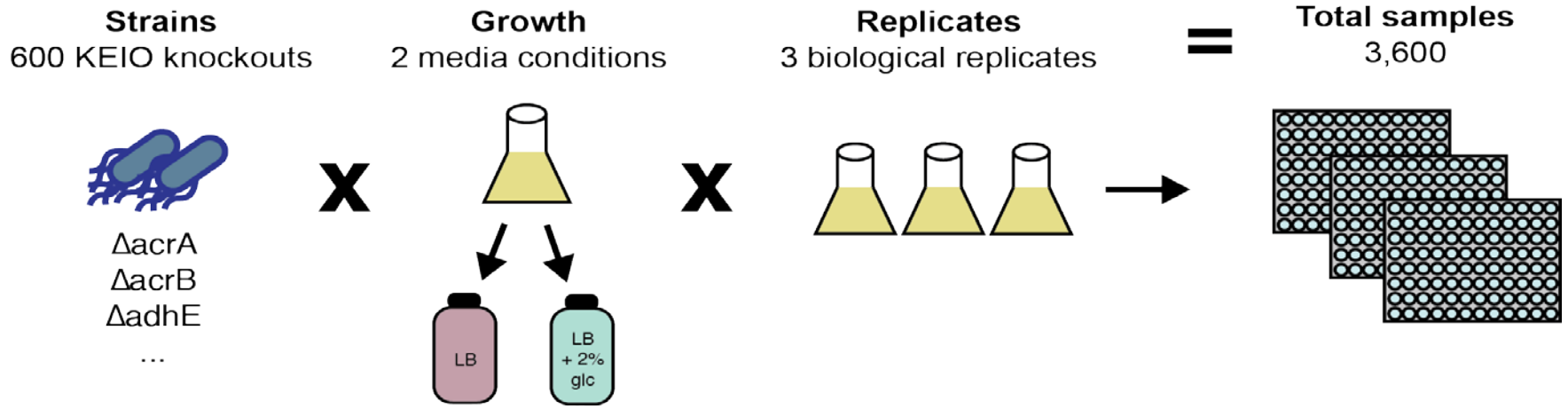
Glycolysis

Pentose phosphate pathway

Acetyl-CoA synthesis

see Poster: WP 698

APPLICATION OF THE HIGH THROUGHPUT WORKFLOW

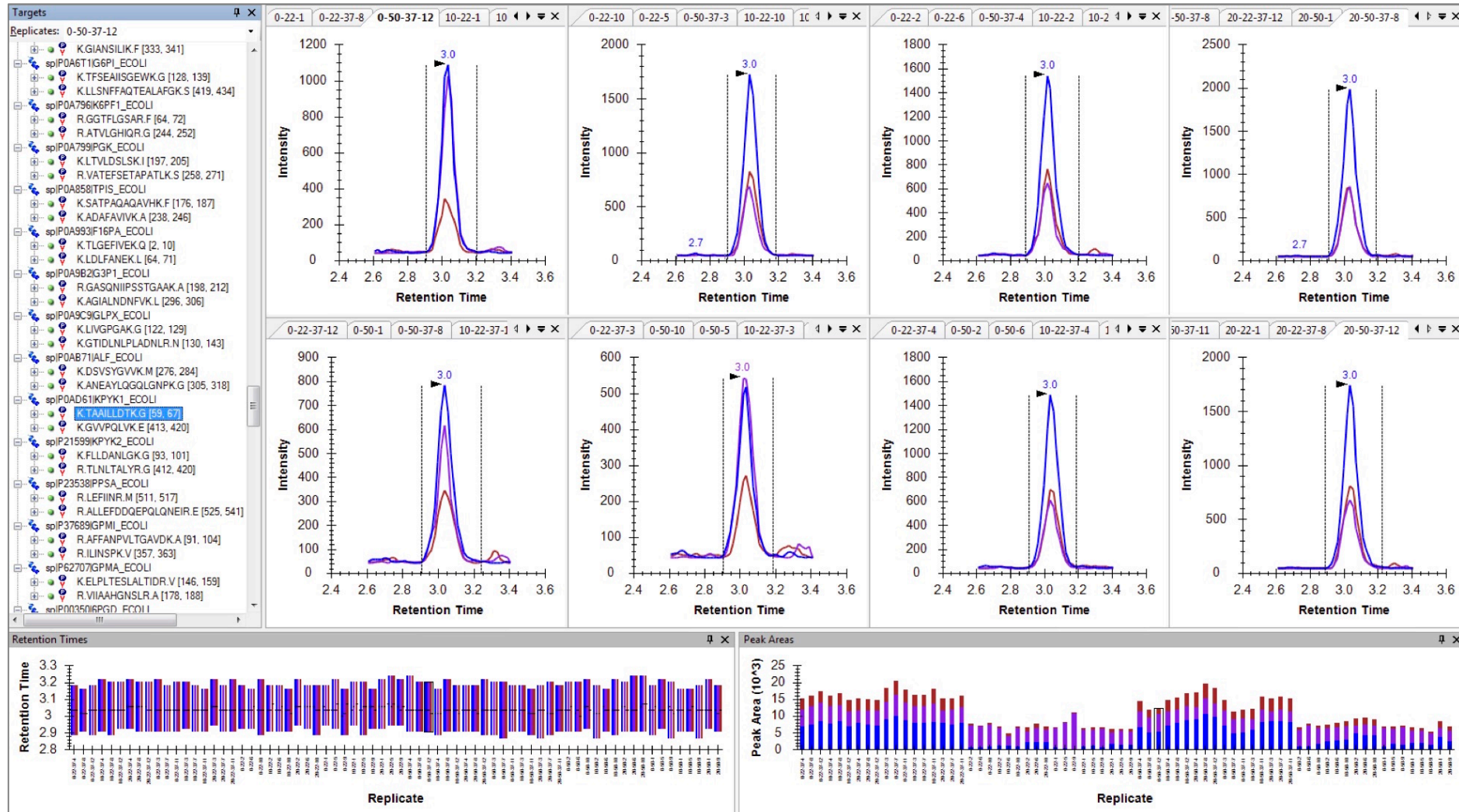


Automated sample preparation

LC-MS/MS

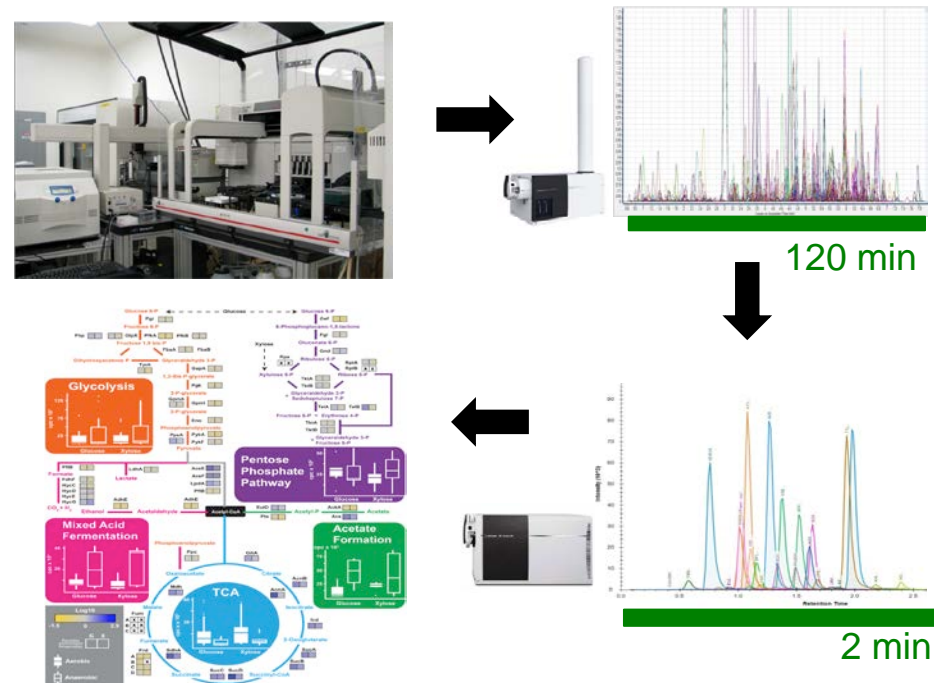
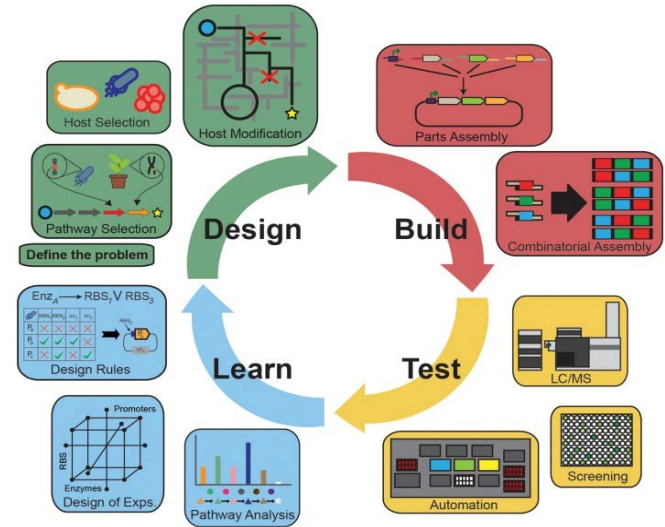


RESULTS PROCESSED IN SKYLINE FOR ANALYSIS



SUMMARY

- Skyline spectral libraries and iRT prediction enable fast methods development for quantifying novel protein targets
- This workflow aids robust, reproducible, and high-throughput proteomic assays for biofuel applications and more



ACKNOWLEDGMENTS

Jay Keasling

Paul Adams

Leanne Chan

Yan Chen

Tanveer Batth

Pragya Singh

Mirta de Sousa

Vikram Ramakrishnan

Jaron Mackey

Huu Tran

Nathan Hillson

Héctor García Martín

Josh Heazlewood

Susana M. González Fernández-Niño

Eva Pan

William Morrell

Nat Echols

Mark Kulawik

Arthur Panganiban

All of JBEI