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

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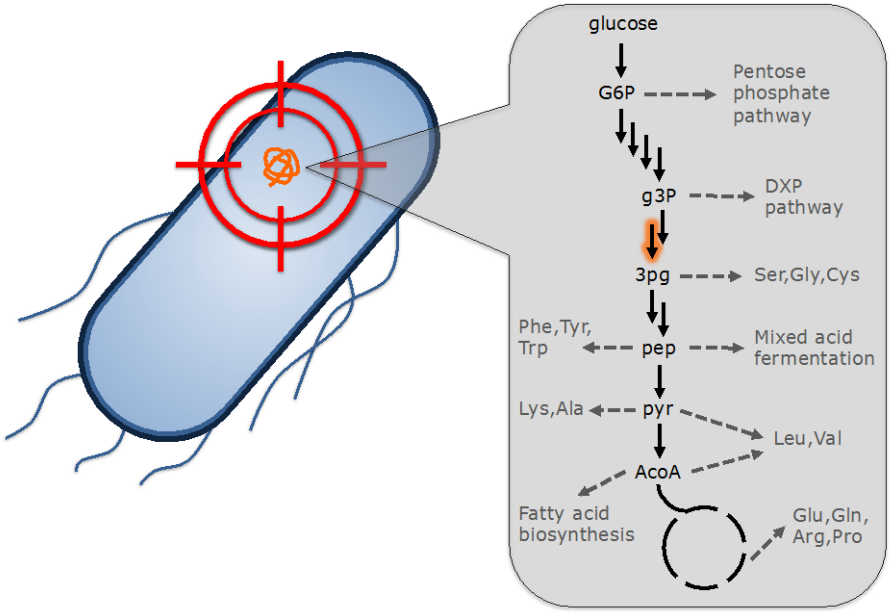
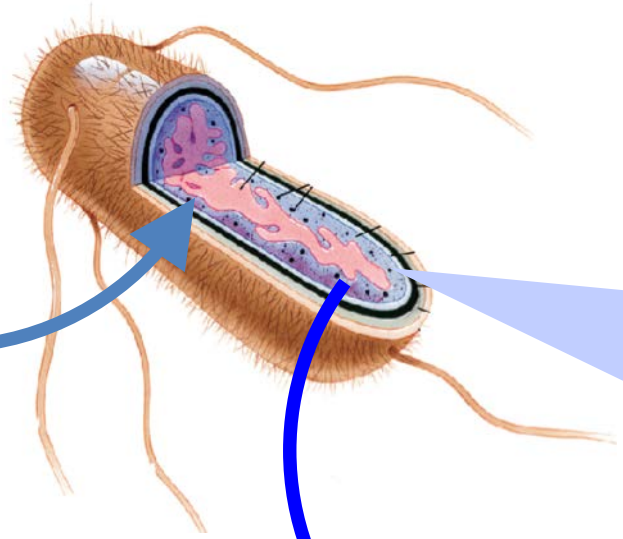


ENGINEERING A CELL FACTORY



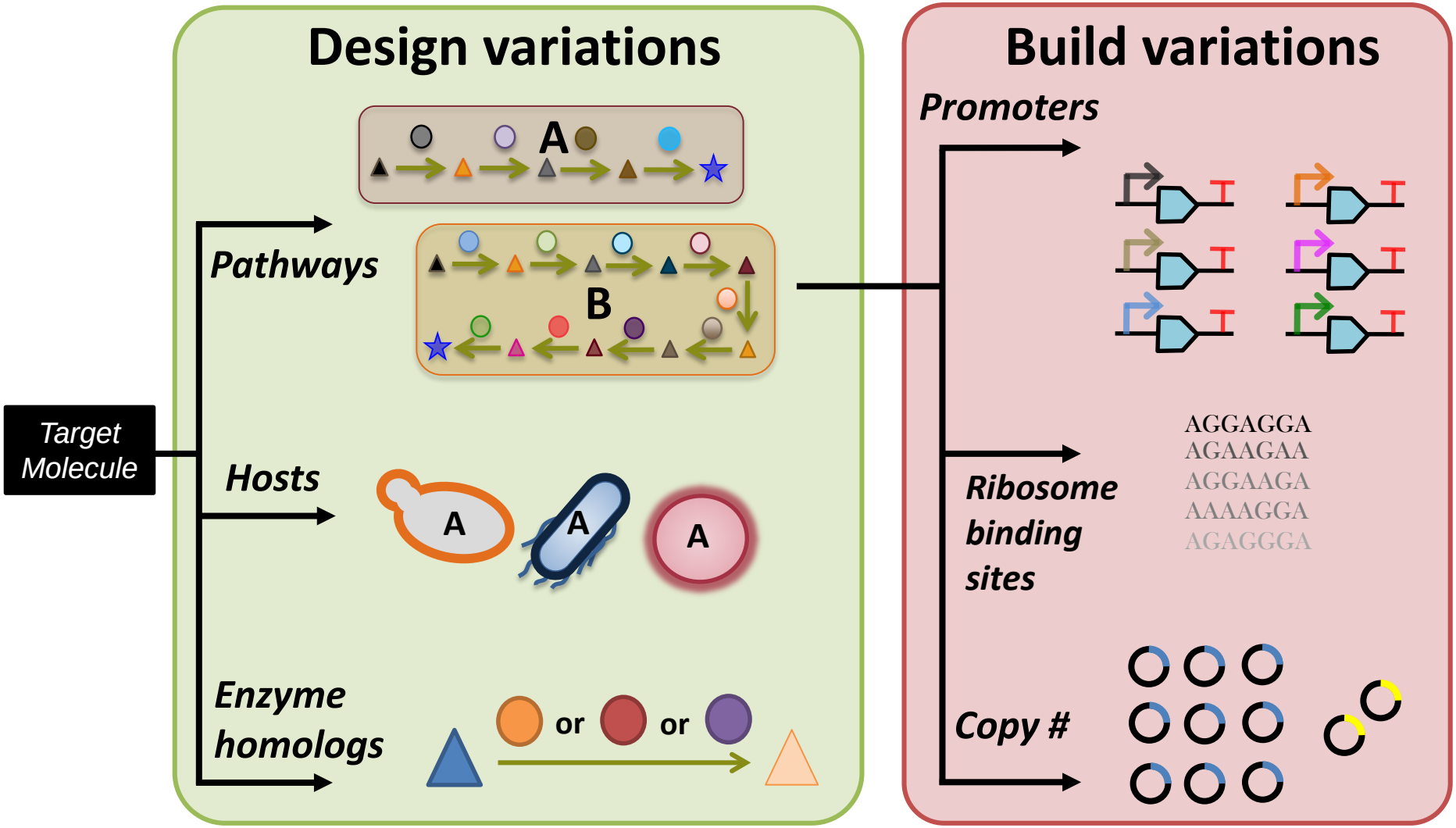
Transportation Fuels

Jet Fuel

Diesel

Gasoline

MANY CHOICES FOR METABOLIC ENGINEERING



TEST: ANALYTICAL WORKFLOW

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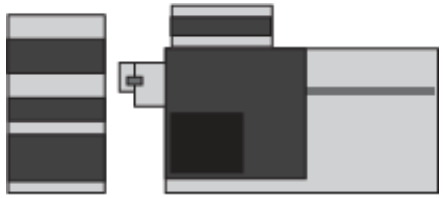
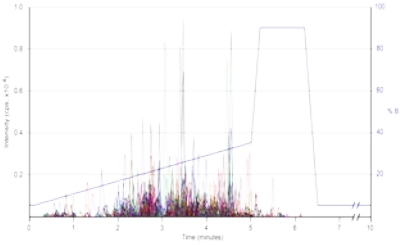
Sample Preparation → Data Acquisition → Data Processing → Data Analysis

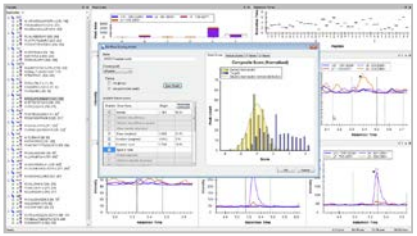
Automated sample preparation

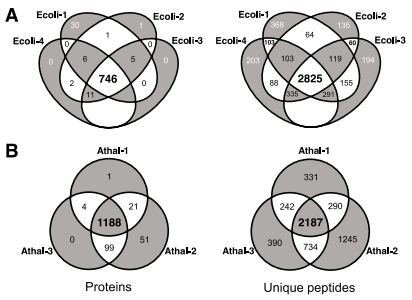
see Poster: ThP 632

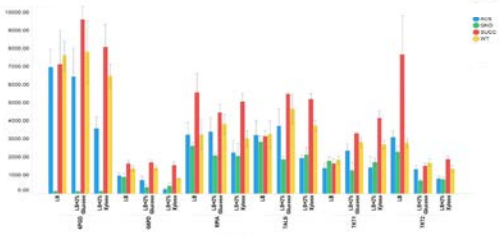


HT data acquisition and processing

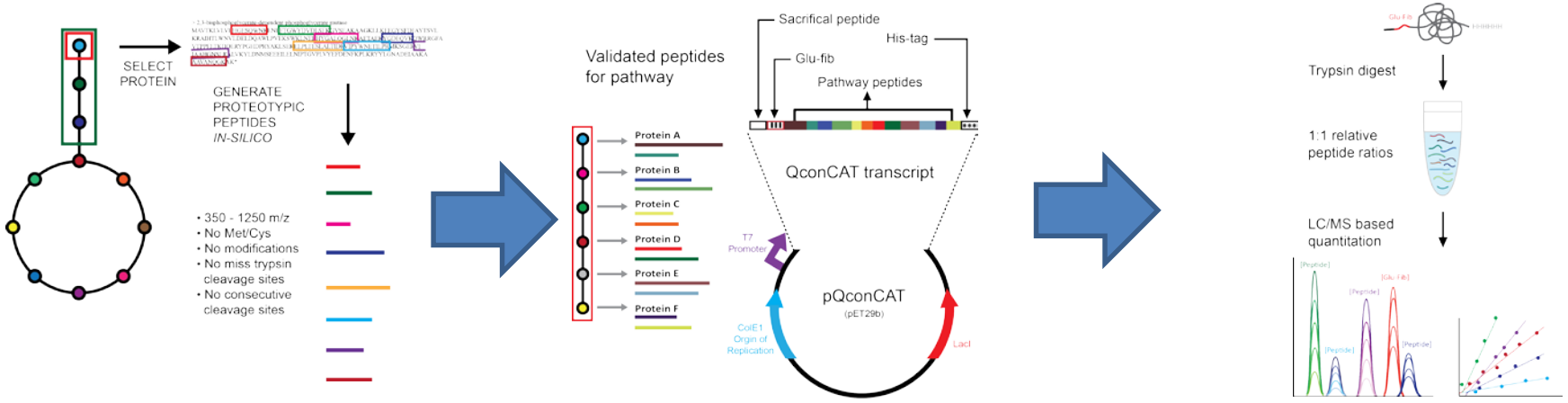


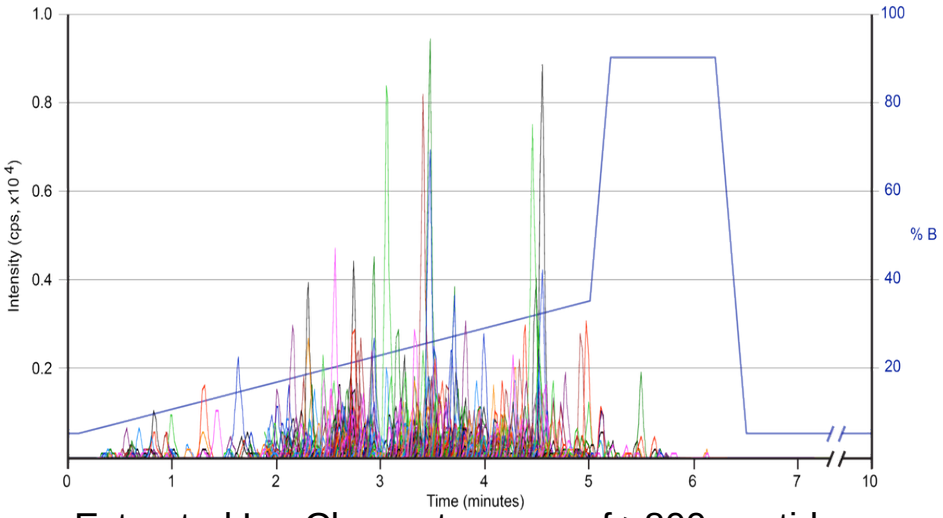






TARGETED PROTEOMIC METHODS DEVELOPMENT





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

T. Batth *et al.* Metab. Eng. 2014, 26, 48-56.

WHAT ABOUT METHODS DEVELOPMENT?

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Sample Preparation | Methods Development | Data Acquisition | Data Processing | Data Analysis

Automated sample preparation

see Poster: ThP 632

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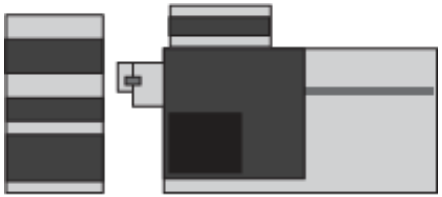
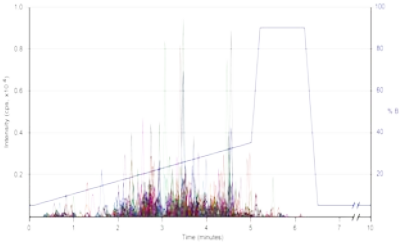
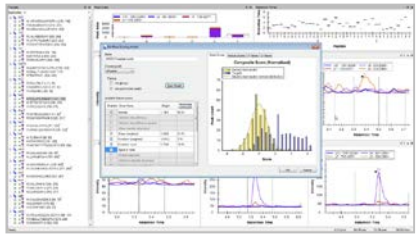
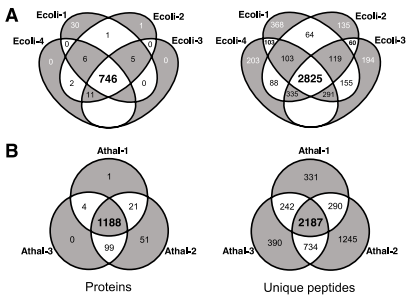
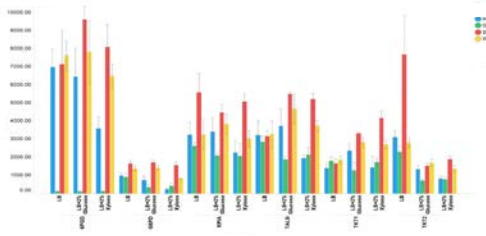


HT data acquisition and processing



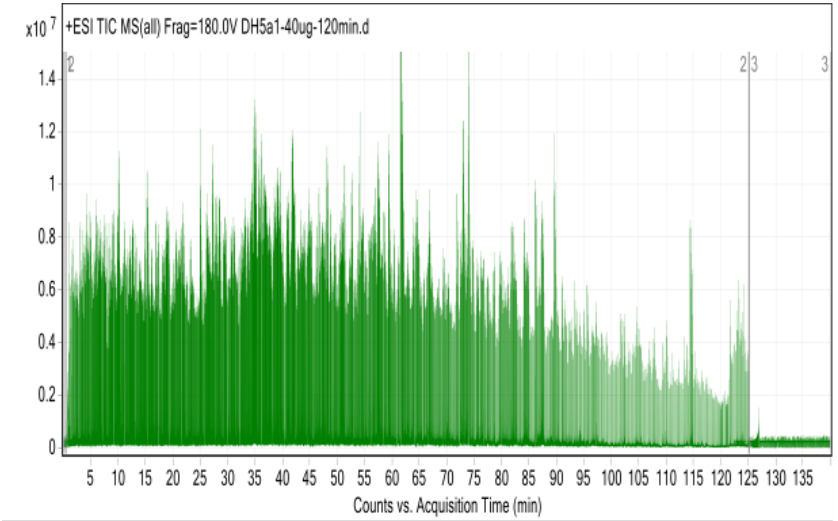




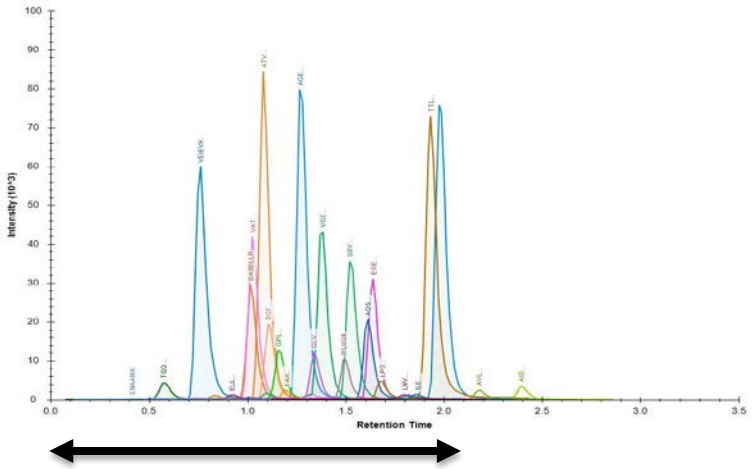


Gap between identifying and quantifying your targets

Discovery Proteomics



Targeted Proteomics

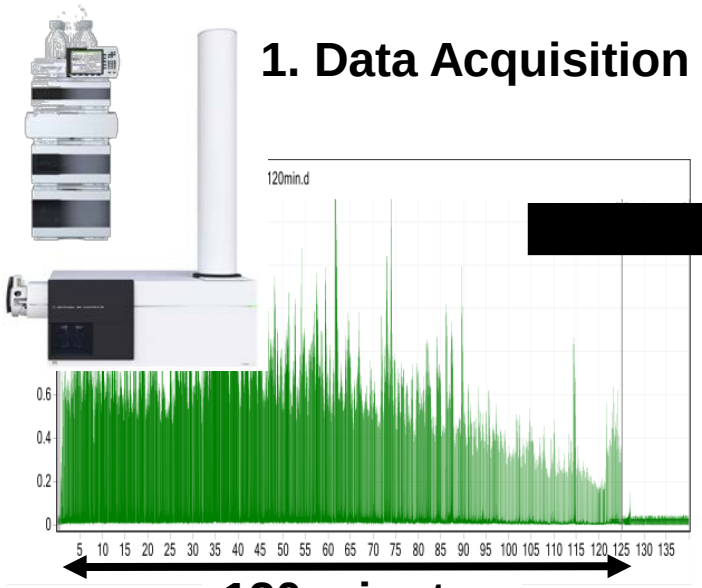


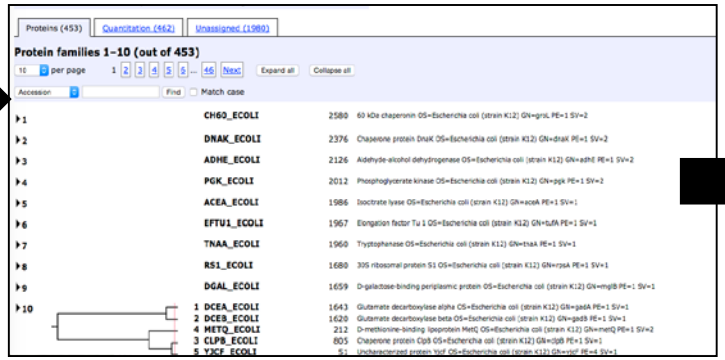
2 minutes

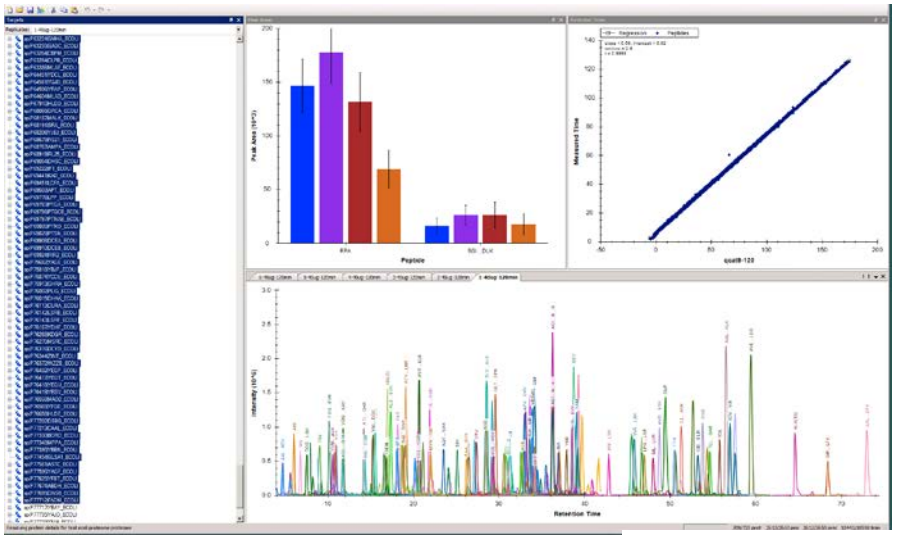
Challenges

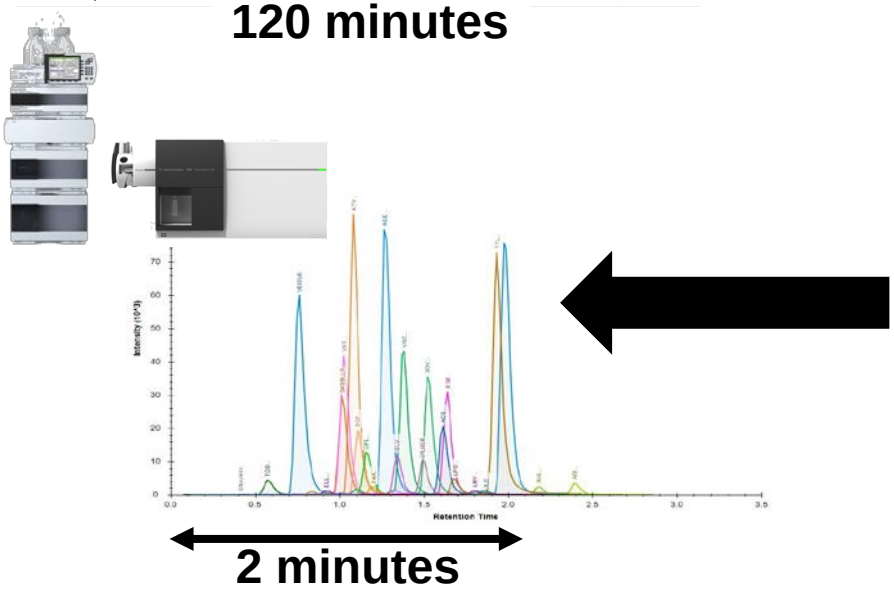
- Different instrumentation
  - Fragmentation conditions
  - Sensitivity and resolution
- Different LC conditions
  - Peptide retention time shift
  - Competition for ionization efficiency

REDUCE METHODS DEVELOPMENT TIME

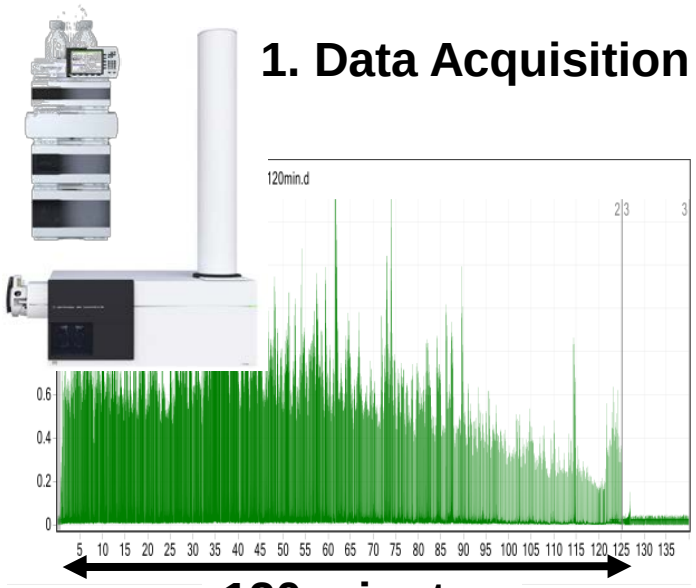






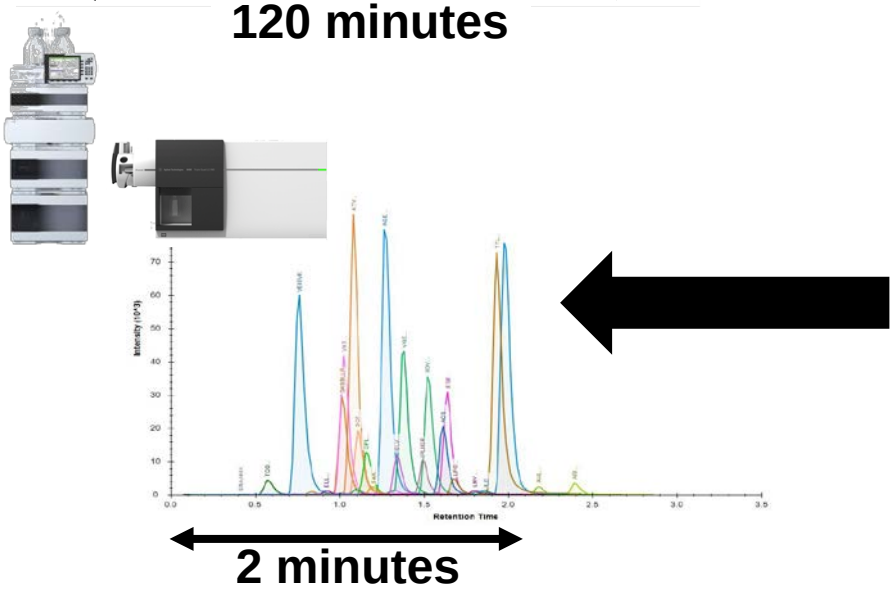


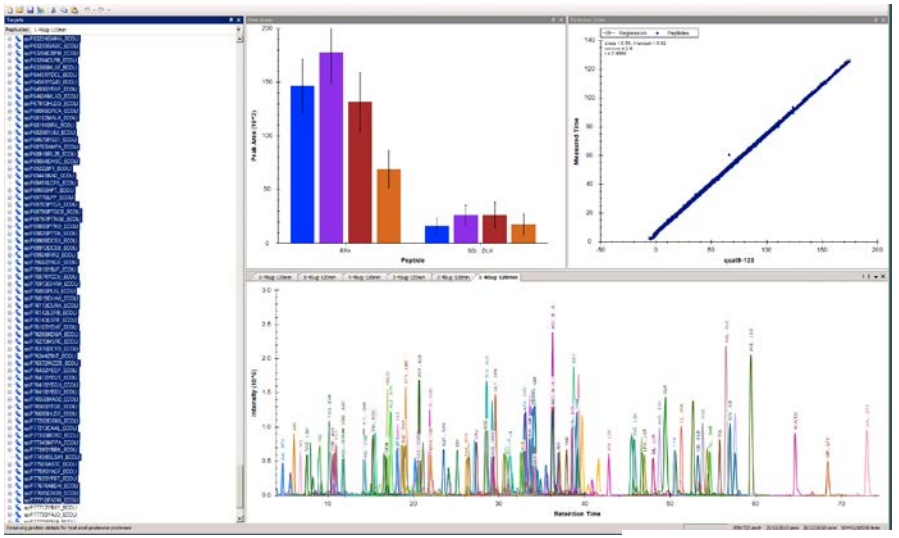
REDUCE METHODS DEVELOPMENT TIME



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

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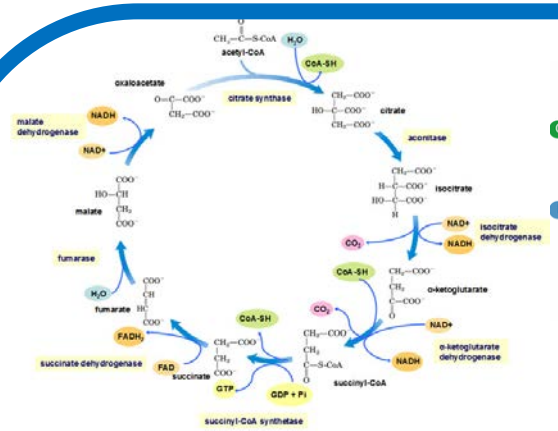
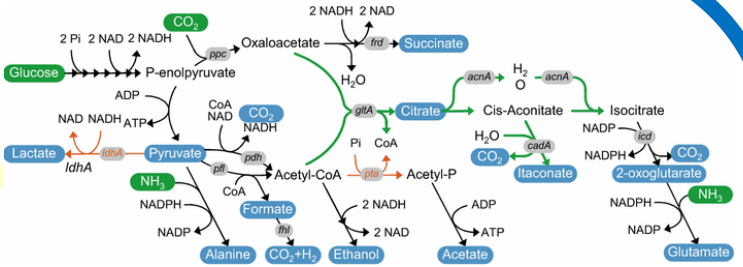
Utilize the *E. coli* KEIO single gene knock out mutant library

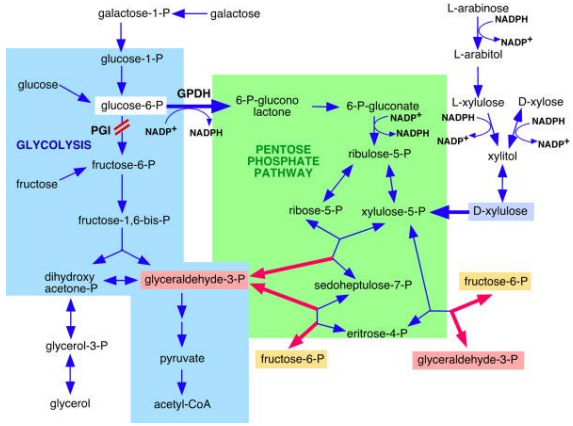


TCA cycle

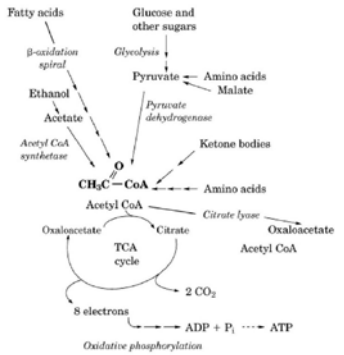


Mixed acid fermentation

Glycolysis



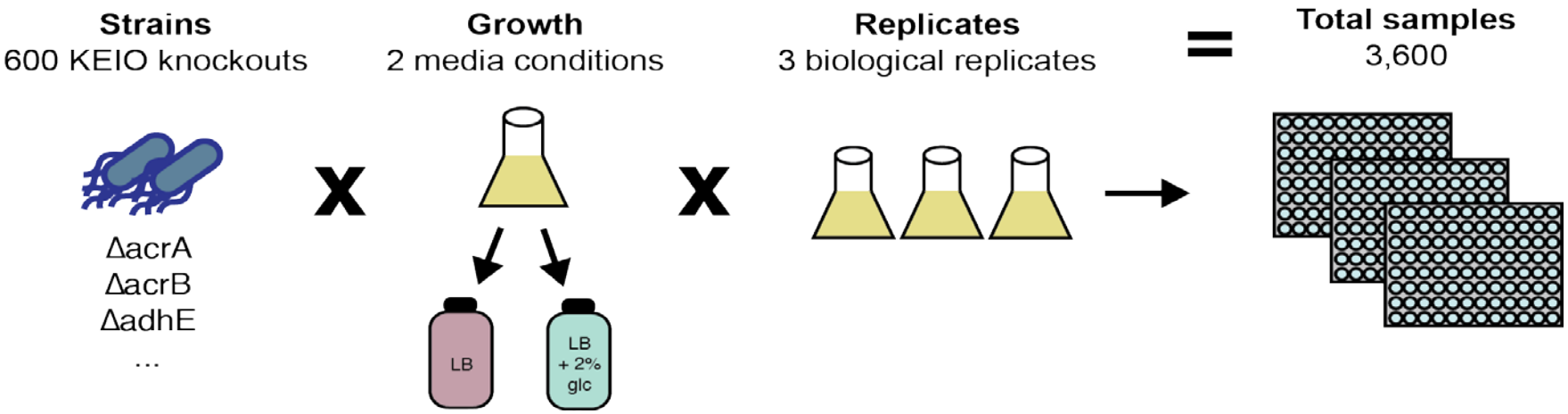
Pentose phosphate pathway



Acetyl-CoA synthesis

see Poster: WP 698

APPLICATION OF THE HIGH THROUGHPUT WORKFLOW



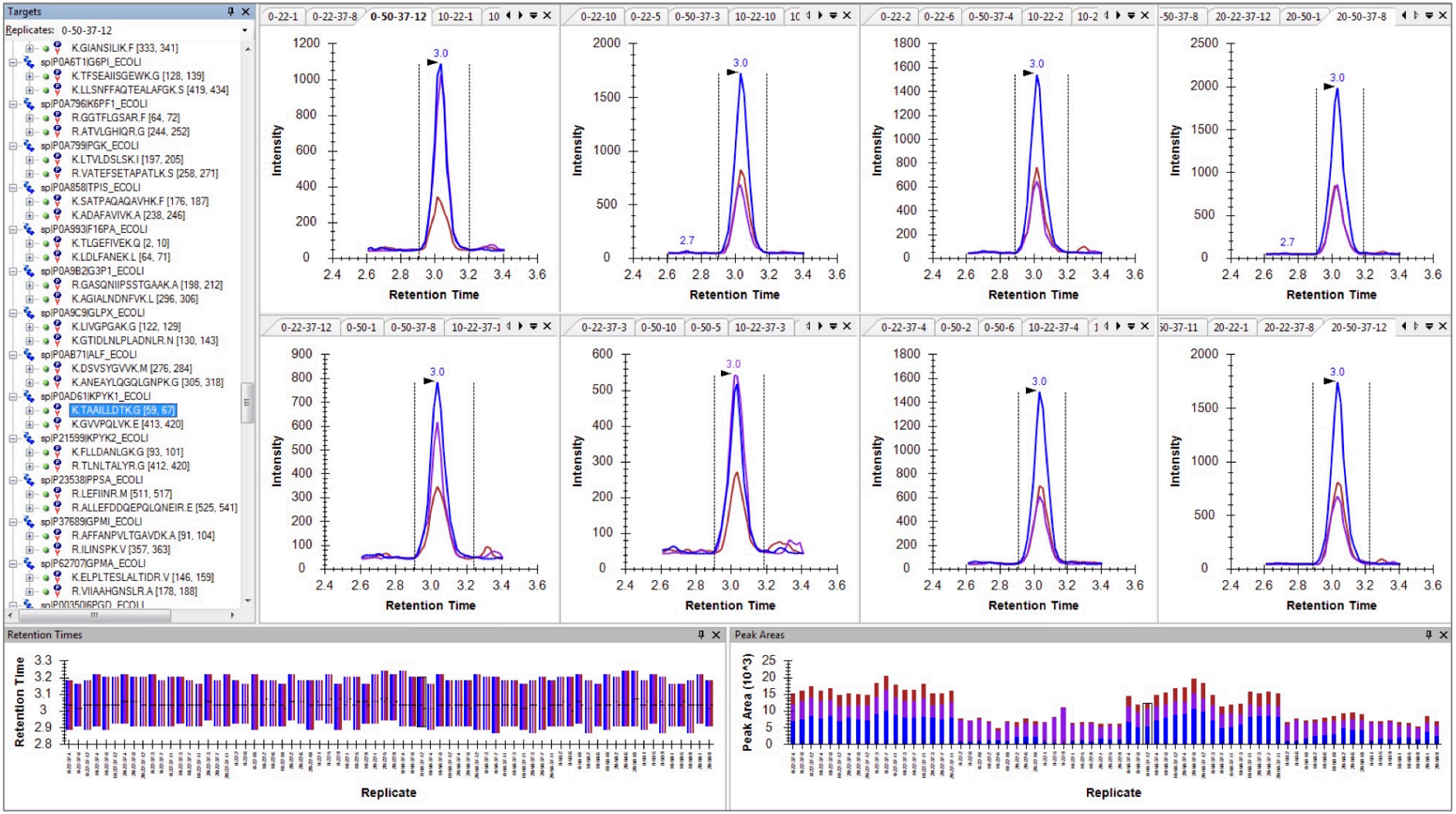
Automated sample preparation

LC-MS/MS

Agilent 1290 HPLC

Agilent 6460 QqQ

RESULTS PROCESSED IN SKYLINE FOR ANALYSIS



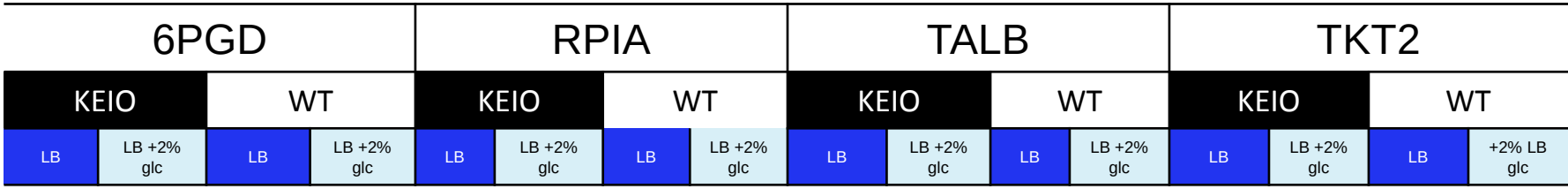
TCA CYCLE RESULTS

Normalized total area

DHSA				MDH				ODO2				SUCD			
KEIO		WT		KEIO		WT		KEIO		WT		KEIO		WT	
LB	LB +2% glc	LB	LB +2% glc	LB	LB +2% glc	LB	LB +2% glc	LB	LB +2% glc	LB	LB +2% glc	LB	LB +2% glc	LB	+2% LB glc

PENTOSE PHOSPHATE PATHWAY RESULTS

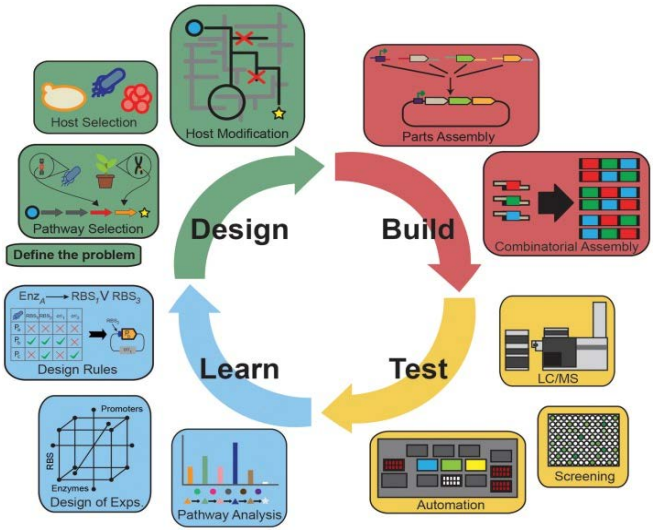
Normalized total area

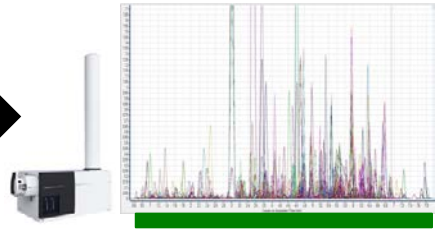


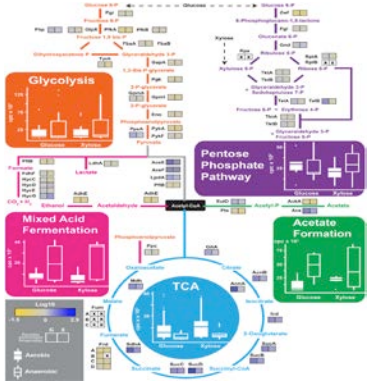
SUMMARY

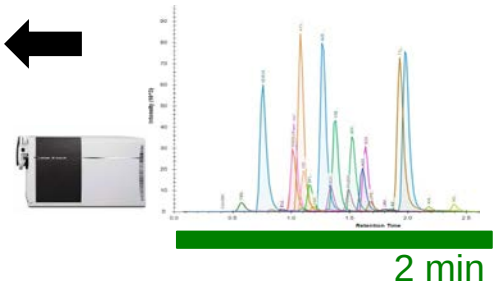
- Skyline spectral libraries and iRT prediction enable fast methods development for quantifying novel protein targets

- 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