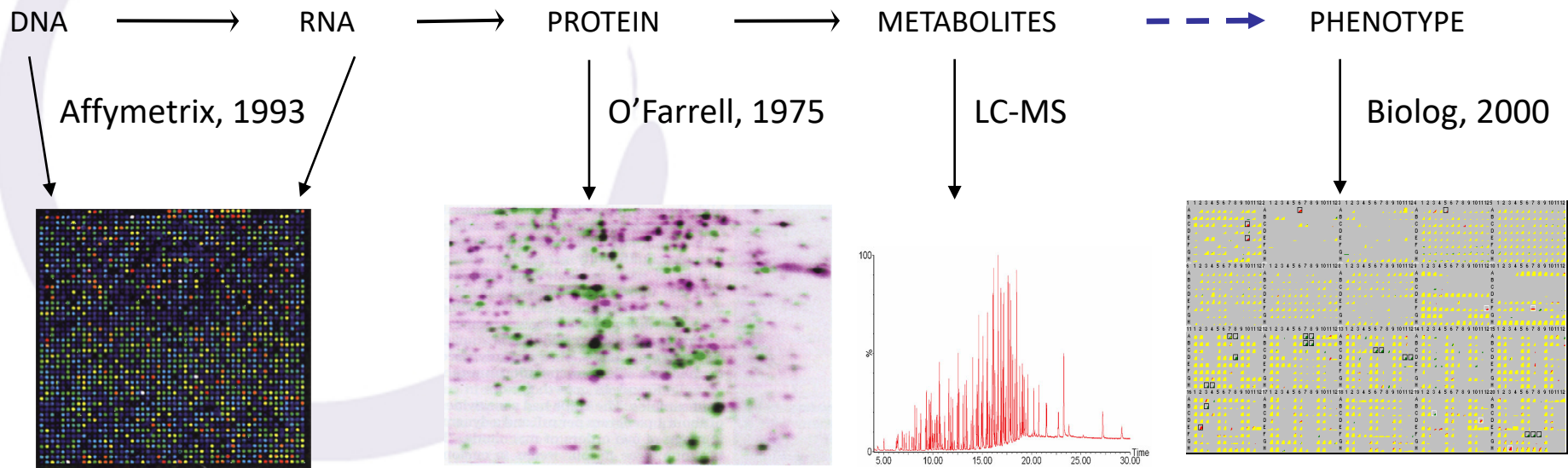# Biolog Phenotype MicroArray ™:
Harnessing High-Throughput Phenomics to Characterize Microbial Isolates in Culture Collections

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Biolog, Inc., etatti@biolog.com

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Tools Characterizing Cellular Traits:
Phenotype MicroArrays

DNA → RNA → PROTEIN → METABOLITES – – → PHENOTYPE

Affymetrix, 1993   O'Farrell, 1975   LC-MS   Biolog, 2000

Transcriptomics   **Proteomics**   **Metabolomics**   **Phenomics**

Molecular Analysis   **Cellular Analysis**

Snap shot - Characterization of cellular molecules in one growth state, in one instant in time

Motion picture – Record of cellular responses to hundreds of environments over many hours
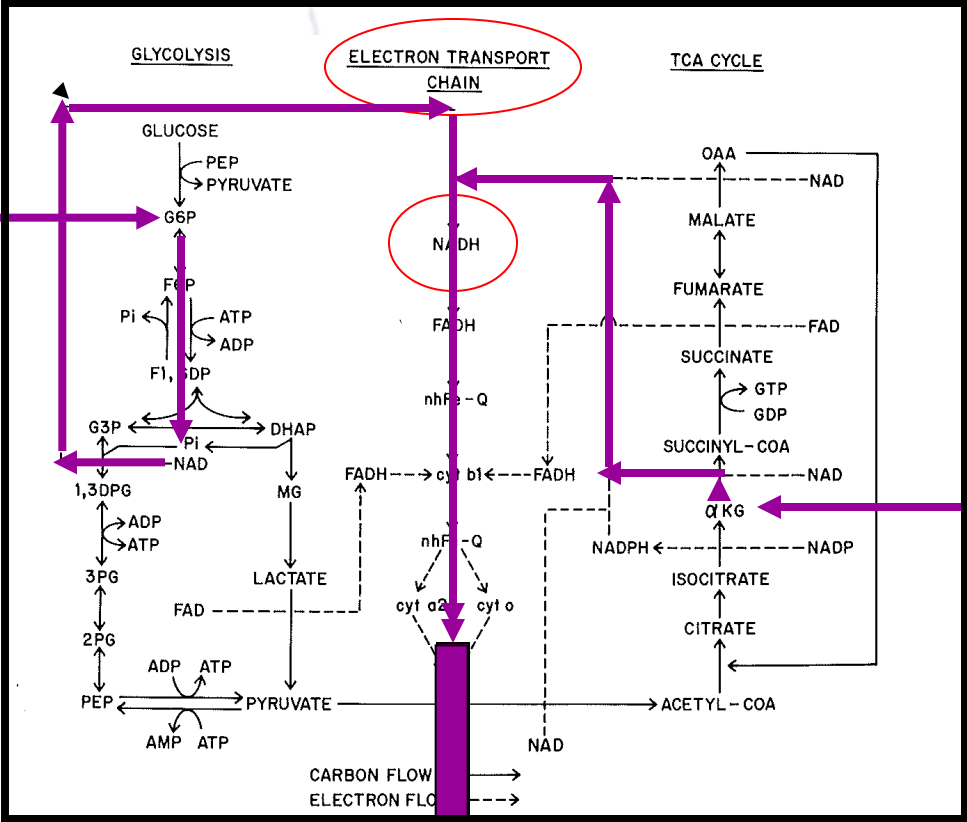
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Assay Principle:

Colorimetric Analysis of Cell Energetics

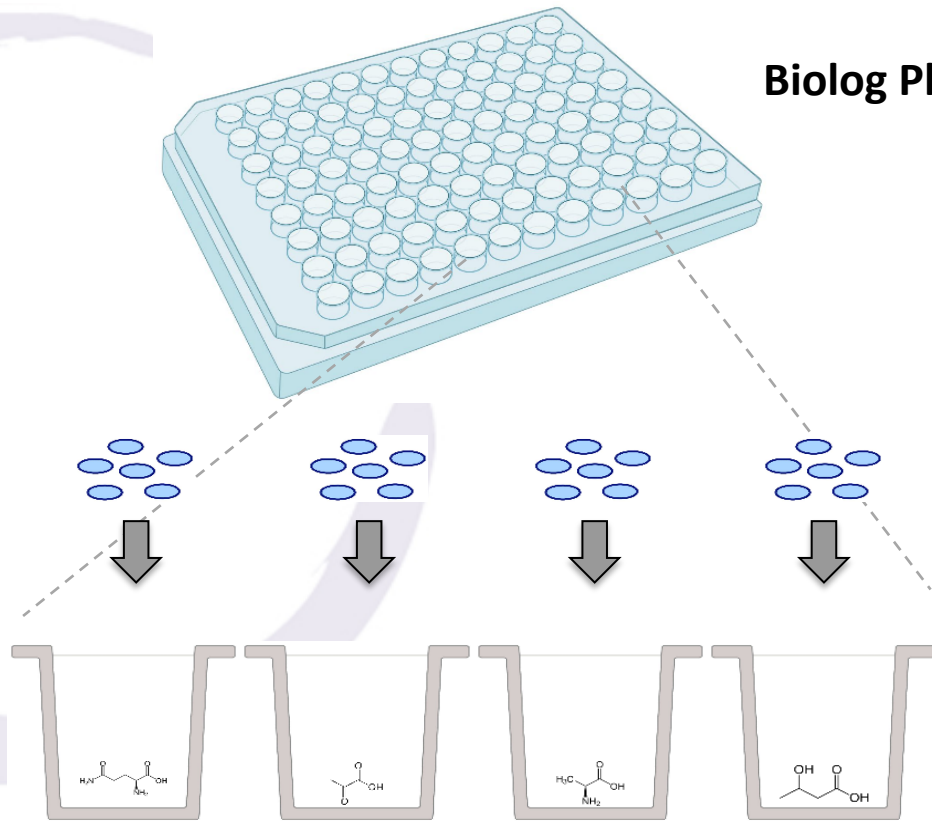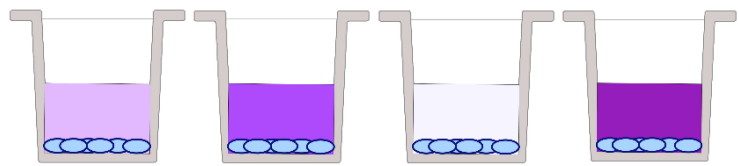# Metabolism of C-sources Produces Cellular Energy
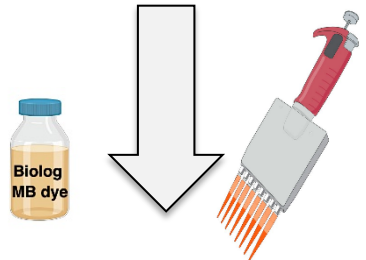
Biolog Phenotype MicroArray™

Chemical that inhibit the cell
OR
Chemical that stimulate the cell

Redox Dye addition

Biolog MB dye

Carbon Pathways

N

P

S

Biosynthetic Pathways

Nitrogen Pathways

Osmotic & Ion Effects

pH Effects

Sensitivity to 240 Chemicals

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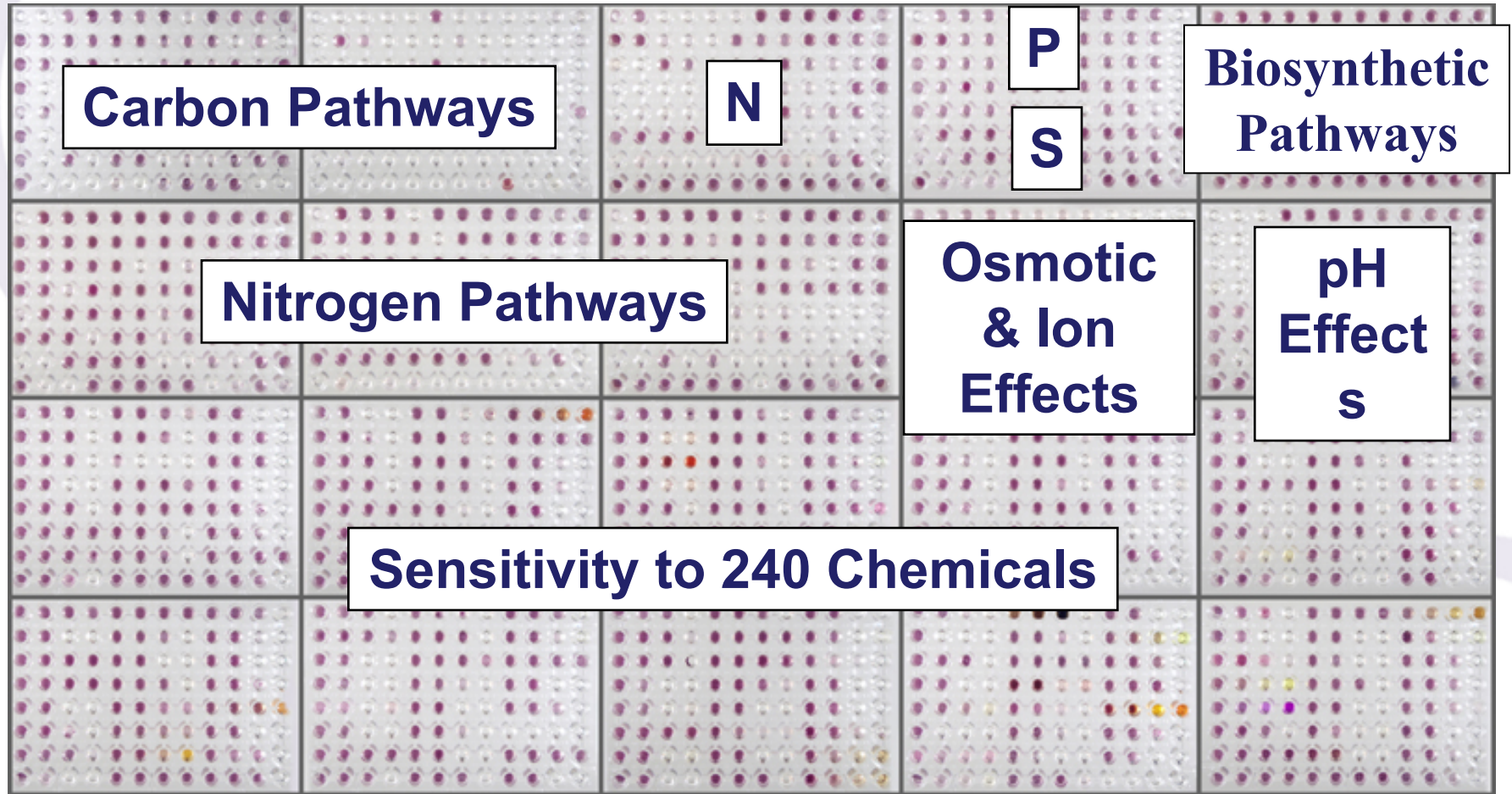
How can it be used?

1. **Identification**

2. **Characterization**

3. **Modeling/Annotation**
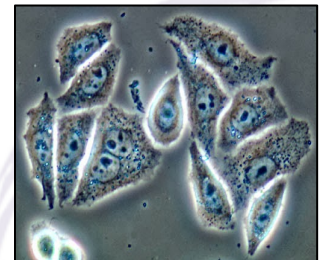
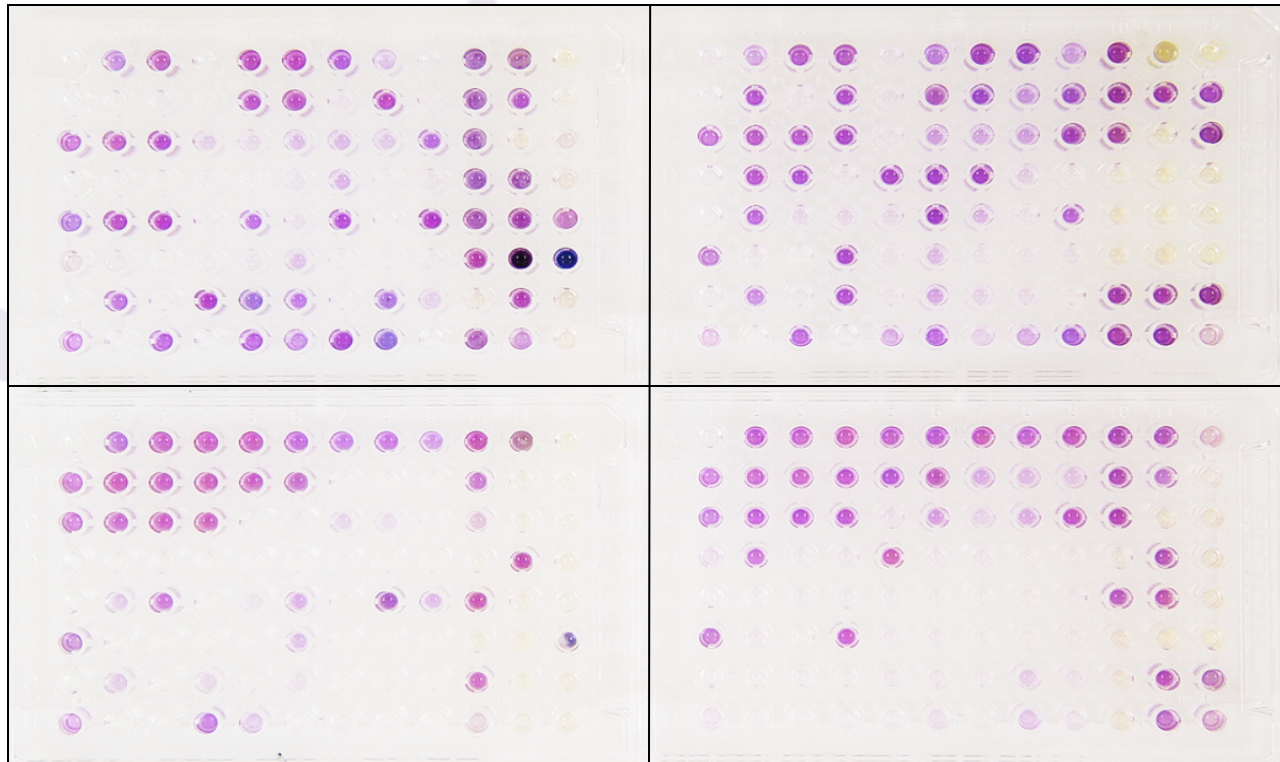4. **QC**

Mammalian

Bacteria Yeast Fungi
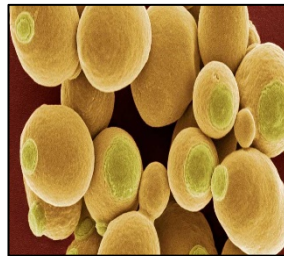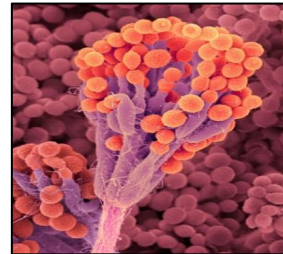
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Metabolic Fingerprinting for Identification of Cells
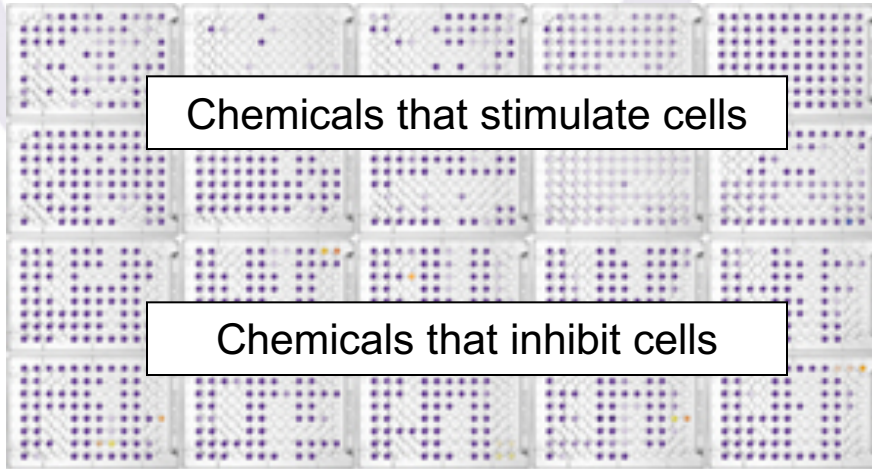
Stenotrophomonas maltophilia

Staphylococcus aureus

Sphingomonas paucimobilis

Paenibacillus polymyxa

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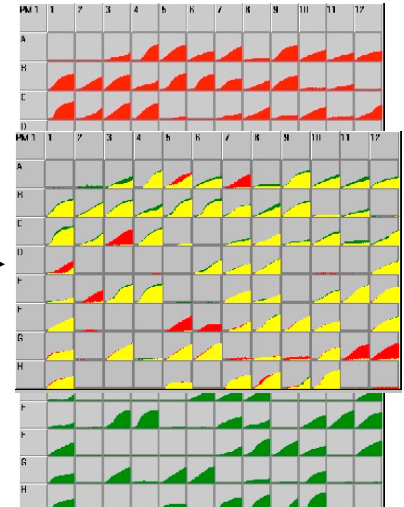# 2 Components of the Biolog Cell Assay Platform

OmniLog™ Incubator/Reader

Phenotype
MicroArrays™

Chemicals that stimulate cells

Chemicals that inhibit cells

colorimetric cell assays in 96-well microplates

incubation and recording of data in the OmniLog

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Phenotypic MicroArray Assays

Assay

- Grow on plate
- Add cells and dye
- Dispense cells into microplates
- Incubate for 24-48h

OmniLog PM Instrument

- Incubator
- Holds 50 Microplates
- Temperature can be set between 17ºC to 45ºC
- CCD camera records color density every 5 or 15 minutes

Dye Reduction Kinetics

- Measure Rate of dye reduction
- Negative controls to measure background
- Positive control to measure full response

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Unlocking Kinetic Information

- Each PM well will exhibit a different rate of dye formation, so single endpoint reads for an entire plate are not ideal

- OmniLog® PM software computes multiple parameters for phenotypic characterization and comparison
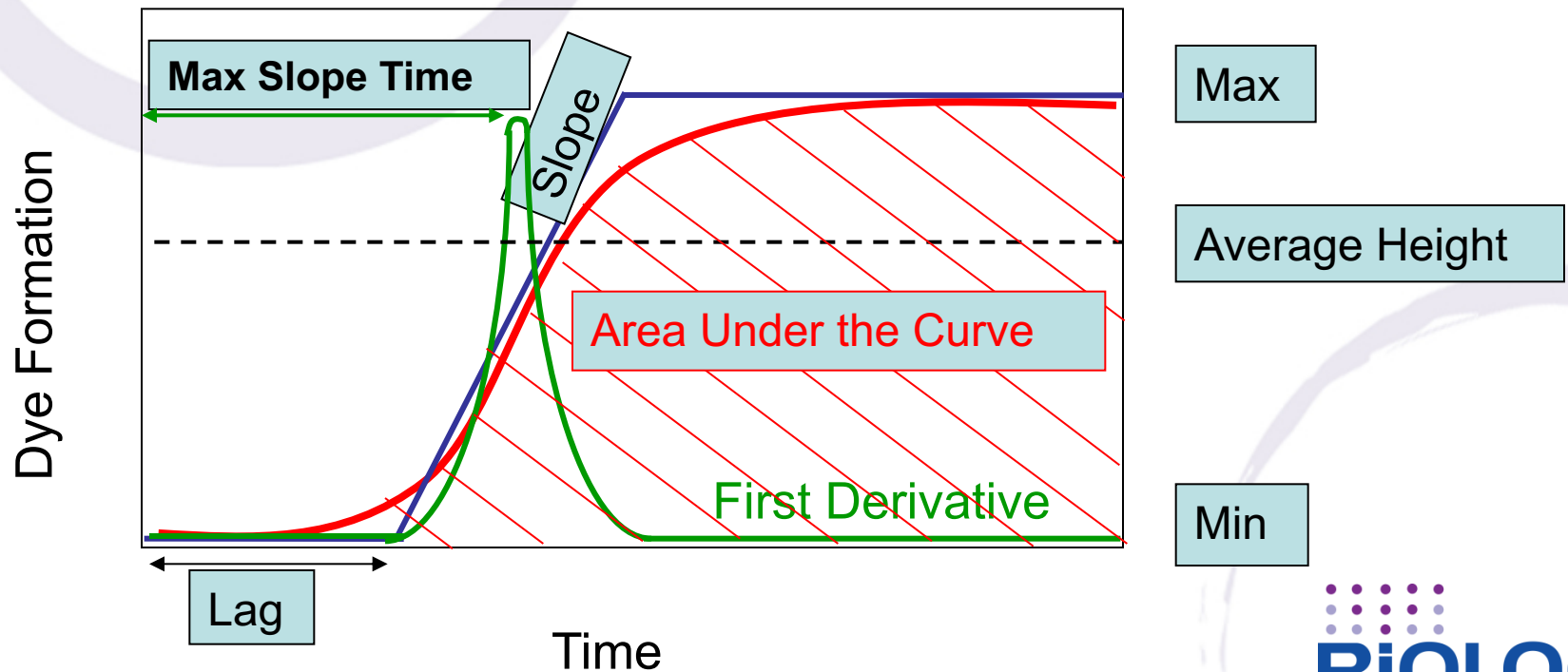
PM Analysis of *Streptomyces coelicolor*

succinate mannitol glutamate gelatin glycerol tweens lactose gentiobiose

nitrite, urea most amino acids (not met)
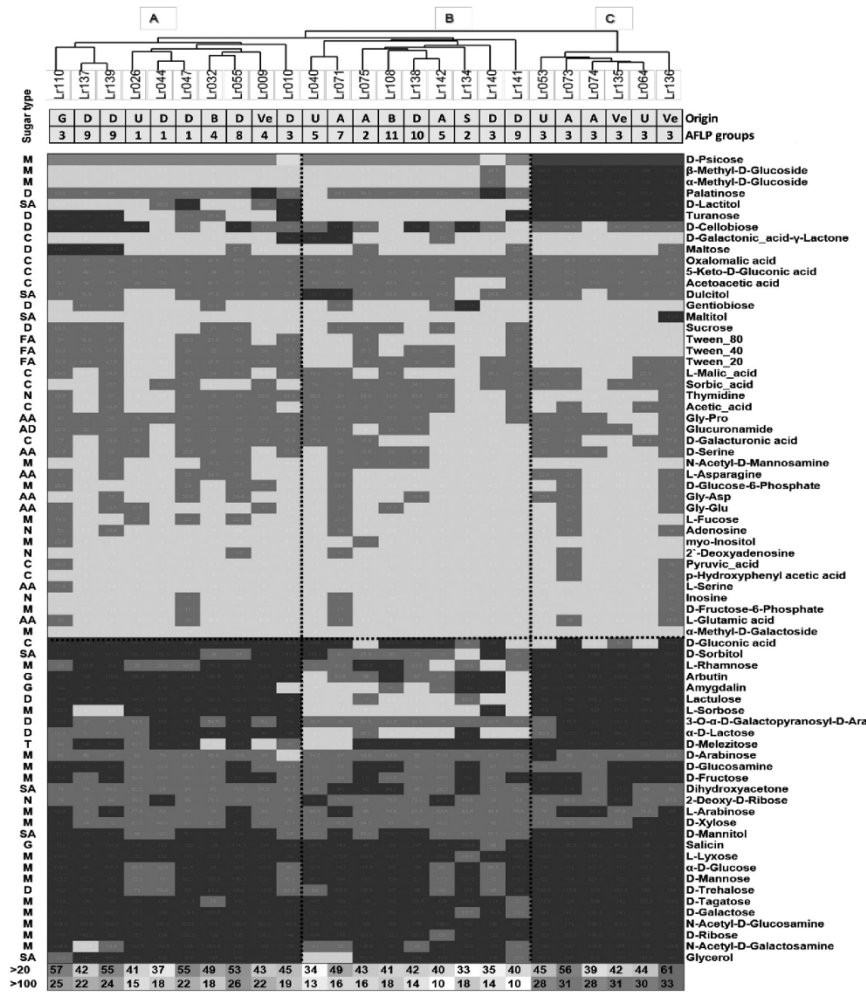
prototrophic

no met peptides

osmotically sensitive except to urea

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Danone Study of *Lactobacillus rhamnosus* strains

25 strains x 72 substrates

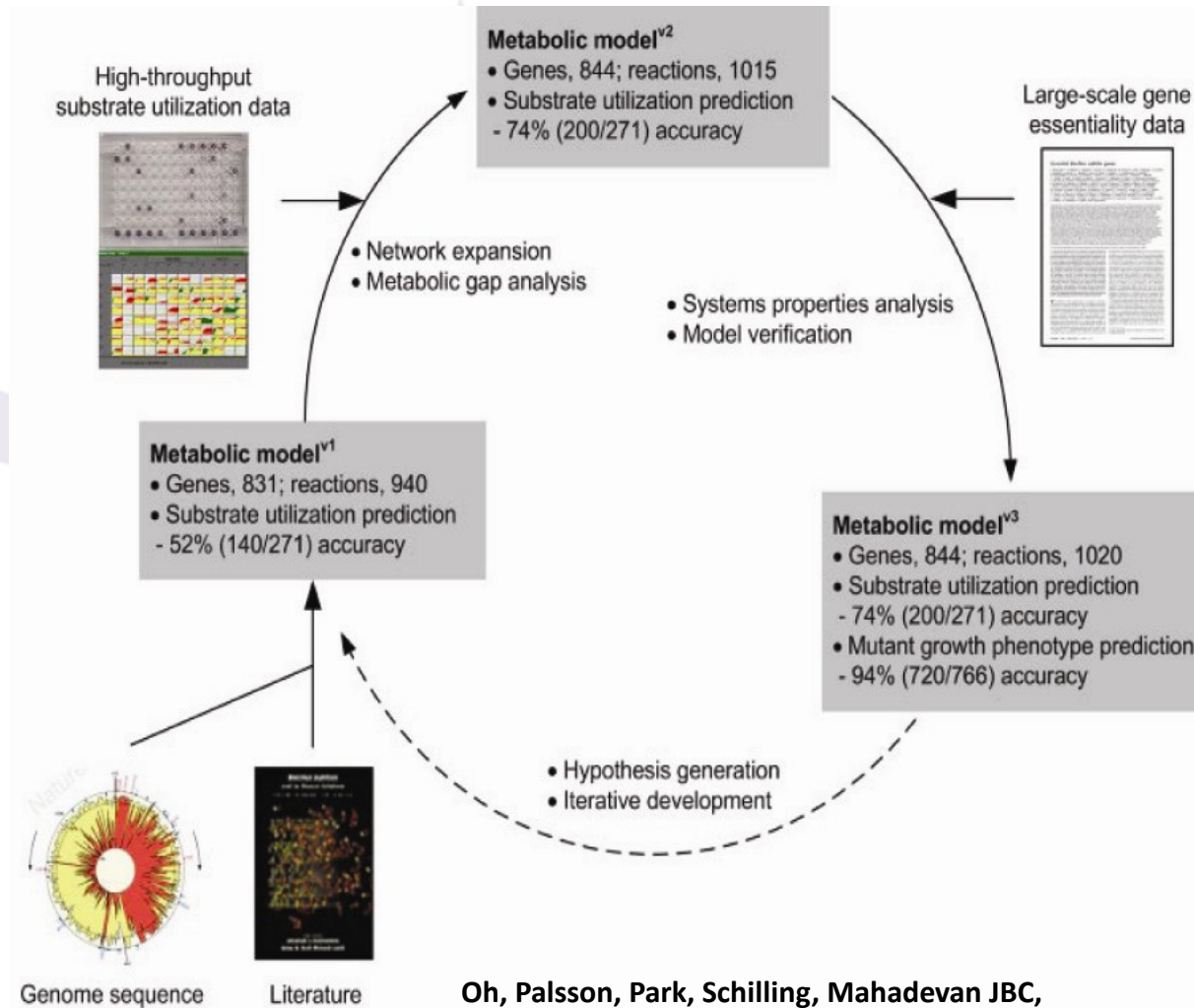Correlation of *Lactobacillus rhamnosus* genotypes and carbohydrate utilization signatures determined by phenotype profiling. AEM (2015) 81:5458
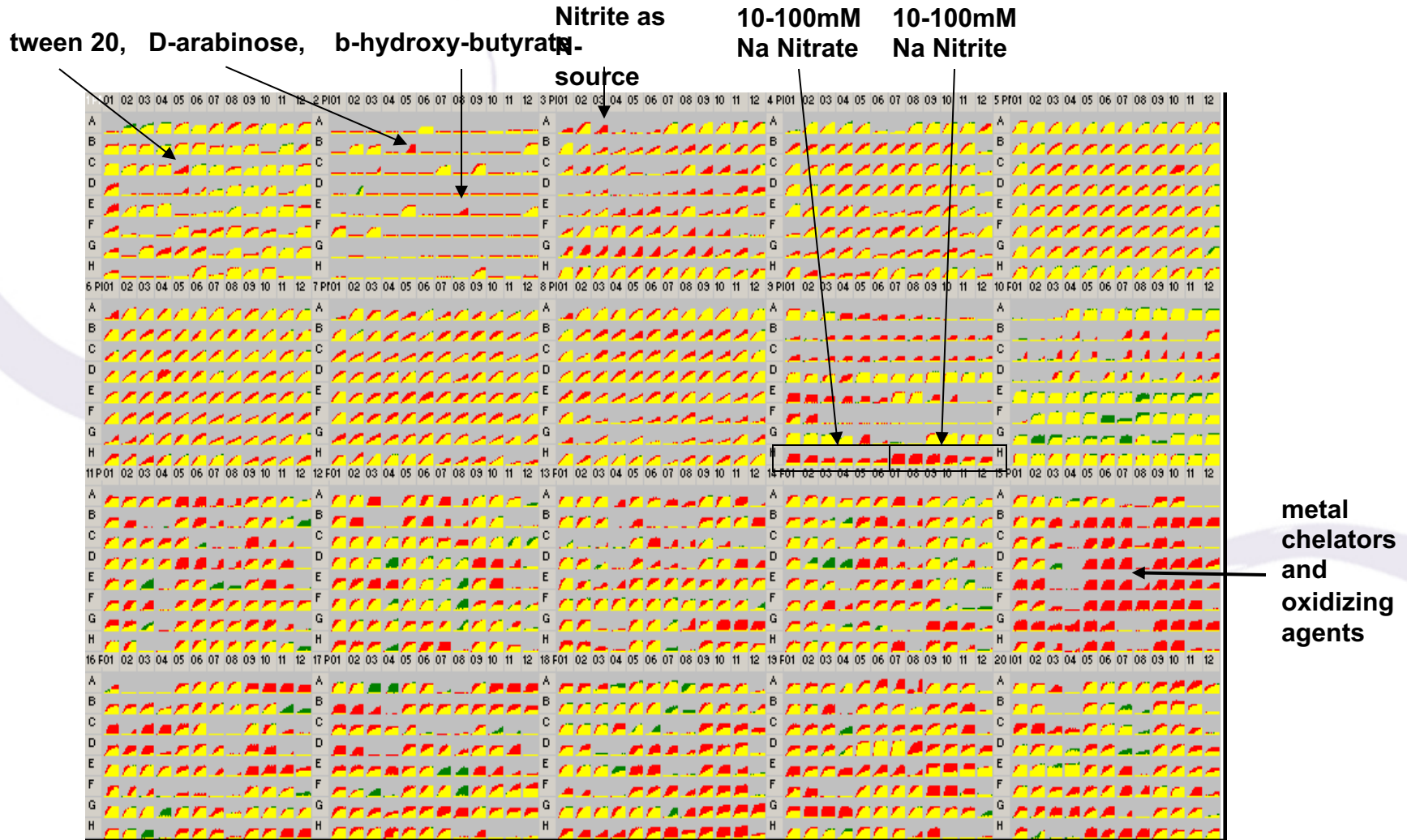
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Using PM to Improve Annotation and Modeling

Oh, Palsson, Park, Schilling, Mahadevan JBC,
2007, 39:28791-28799
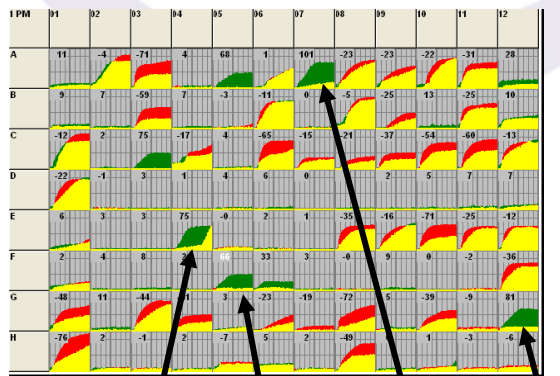
pH Regulation - Comparing *E. coli* at pH7 vs pH5

tween 20,   D-arabinose,   b-hydroxy-butyrate,   Nitrite as N-source   10-100mM Na Nitrate   10-100mM Na Nitrite   metal chelators and oxidizing agents

at acidic pH, $NO_3 \rightarrow NO_2 \rightarrow HNO_2$ (nitrous acid) and NO (nitric oxide)
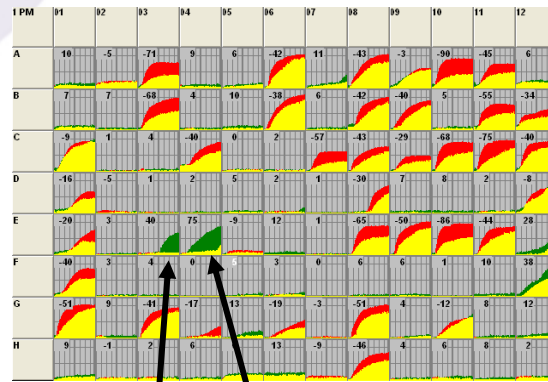
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Temperature Regulation of Carbon Metabolism

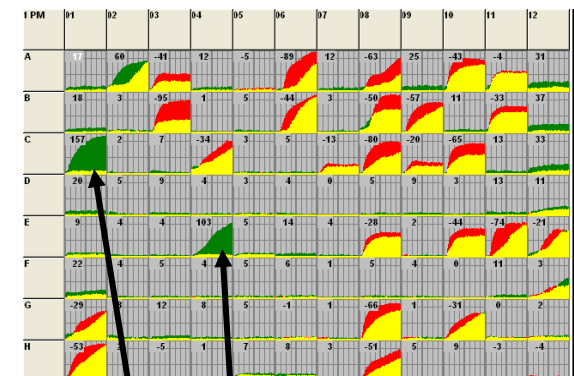*Yersinia pseudotuberculosis* at **26º C** vs **33º C**

1087 **15464 (type)** **15478**
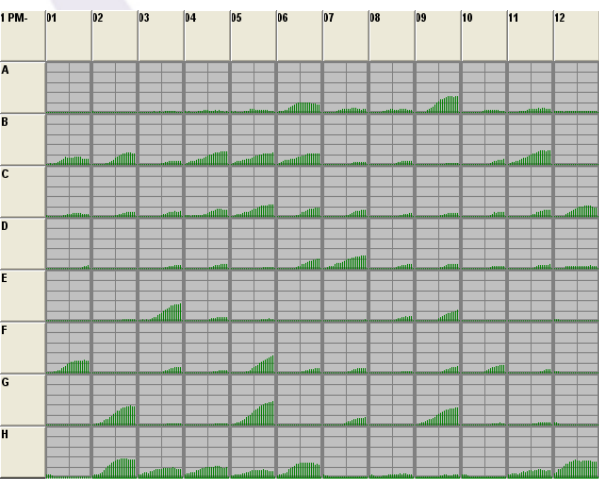
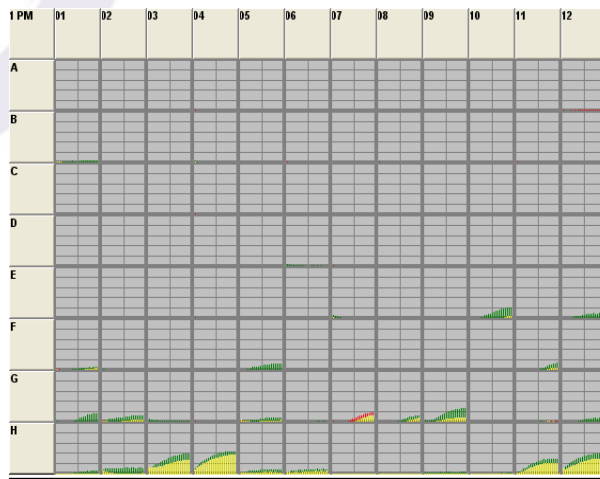F6P fumarate aspartate malate G1P F6P G6P F6P

Recent results show that *Yersinia* has a temperature sensing protein, RovA, that is an important regulator of pathogenicity
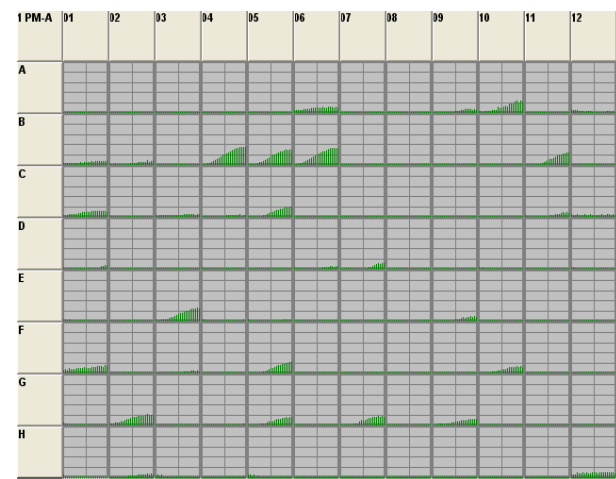
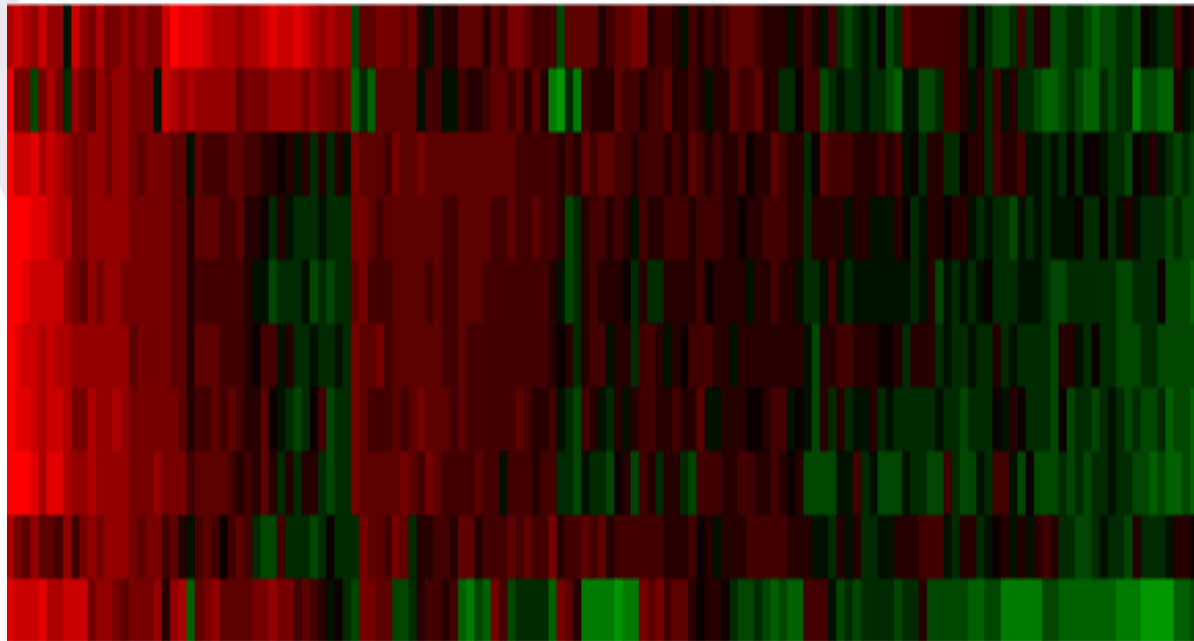# Hepatocyte Preps Show Lot-to-Lot Metabolic Differences
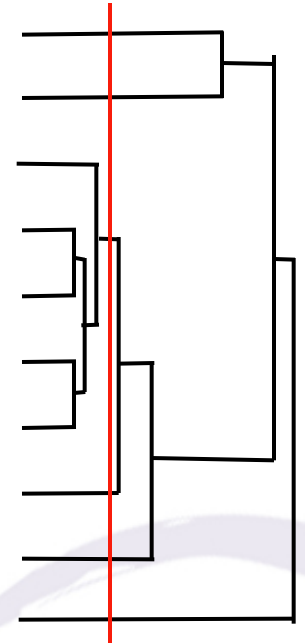
Lot 208   **Lot 212**   **Lot 256**

PMs as a Routine Cell Line QA and QC Tool
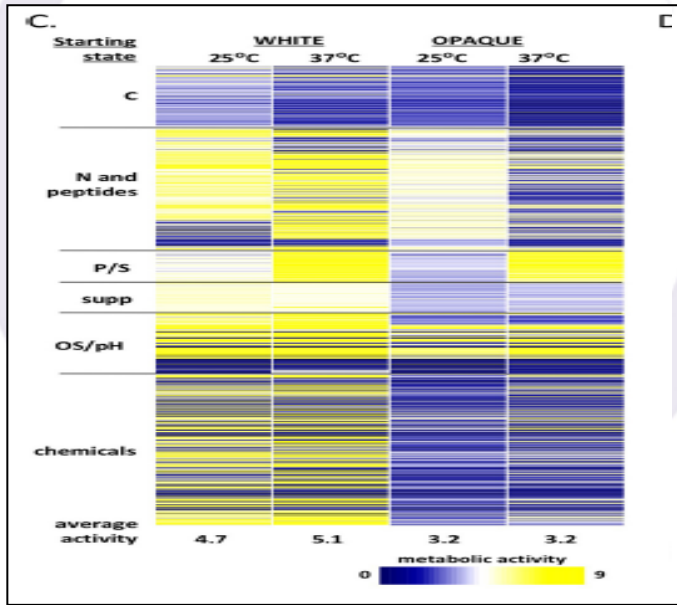
Various Substrates in PM-M1 through PM-M4

Cut Off

C3A_P13
HepG2_P17
A549_P21
A549_P17
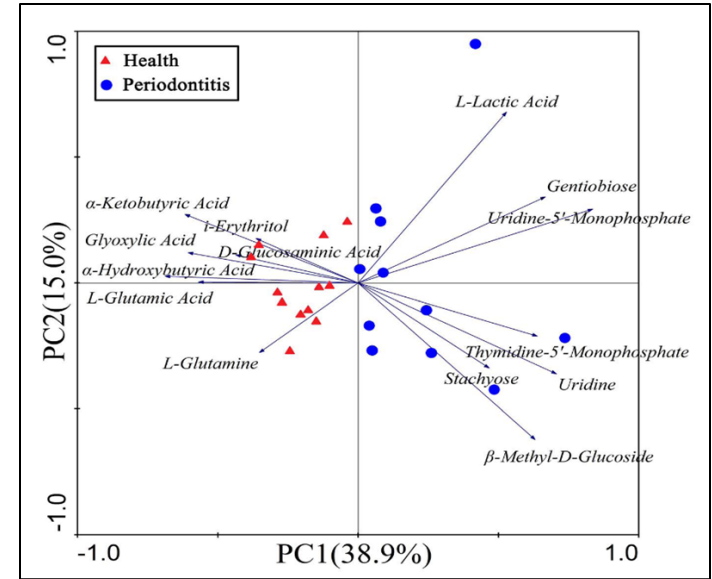A549_P19
A549_P50
A549_P52
A549_P54
PC3_P14
Colo205_P16

- QA criteria for cell line stability can be experimentally determined
- Cross-contamination can be easily detected

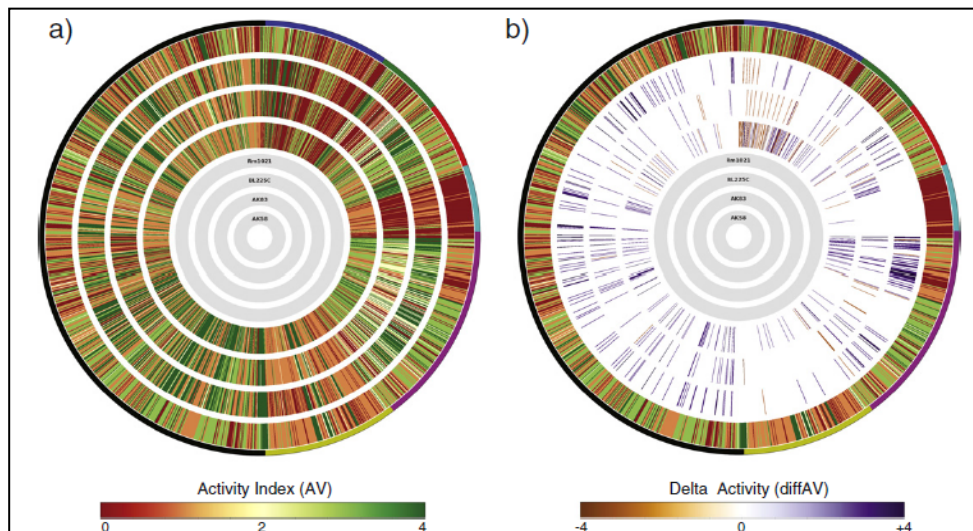**BiOLOG**

Possible outputs from Phenotype MicroArray

Multivariate analysis →

Heat maps (R_OMP)

Comparative analysis (DuctApe)

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Thank you.

Any Questions ?

Prefer to email? etatti@biolog.com

More information at biolog.com

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